TRACKING TRANSLATION FACTORS IN FISSION YEAST NUCLEUS

By

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ABSTRACT

Translation factors are essential components of the ribosome, yet it has been reported that many ribosomal proteins (RPs) and other translation factors are found at transcription sites of *Drosophila melanogaster* (*D. melanogaster*) polytene chromosomes. Whilst these findings might indicate the presence of ribosomal subunits at transcription sites, it has also been reported that these proteins associate with non-coding RNA in *Saccharomyces cerevisiae* (*S. cerevisiae*), suggesting that their localization with transcription sites reflects their non-ribosomal function. However, the functional significance of RPs and translation factors at transcription sites is unclear and may reflect excess protein synthesize unincorporated into ribosomes, leading to a large pool of free proteins able to interact non specifically with other proteins and nucleic acids. The following work investigates these issues further in *Schizosaccharomyces pombe* (*S. pombe*).

I tagged three RPs (RpL7, RpL11 and RpL25), by homologous recombination and used a Chromatin immunoprecipitation approach to investigate whether the association of RPs occurs across specific genes/transcripts or whether chromatin association is genome wide. In agreement with previous studies in *D. melanogaster* and *S. cerevisiae*, I found that RPs preferentially associate to transcriptionally active genes. ChIP followed by analysis on micro-arrays (ChIP-on-chip) revealed that RPs associate with several protein encoding genes.

Further analysis of the three RPs showed that they tend to bind a common subset of genes. Whilst RNase sensitivity suggests RPs association with nascent RNA, I found no correlation between the ChIP-on-chip signals of RPs with either Pol II occupancy or transcript level but did show that RPs associate with non-coding-RNA genes most notably with tRNA genes. ChIP of RpL7 in a strain carrying an exogenous wild-type $tDNA^{Tyr}$ gene or a mutant with a non-functional promoter confirmed that RpL7 associates only to the active tRNA gene. These results suggest a functional role of RPs in tRNAs biogenesis and perhaps in a role in Pol III transcription, as it was recently suggested by the finding that RPs copurify with TFIIIE in *S. cerevisiae*.

Nonsense-mediated mRNA decay (NMD) removes any mRNA containing premature termination codon (PTC), and requires Upf1. Phospho-Upf1 inhibits conversion of 40S/Met-tRNAiMet/mRNA to translationally competent 80S/Met-tRNAiMet/mRNA initiation complexes to repress continued translation initiation. Analysis with Upf1 showed Upf1 also associates with many transcription sites and has a role in DNA replication and/or repair. Notably, Upf1 binds to chromatin mostly during S phase; perhaps indicating a role for its helicase activity during DNA replication in *S. pombe*.

In summary, my data indicate that association of RPs, and at least one NMD factor, to chromatin is a general feature of eukaryotes. The main challenge for future studies is to identify the factors driving this association and the functional significance.

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THIS THESIS IS

DEDICATED TO MY

BELOVED PARENTS

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ABBREVATIONS AND ANNOTATIONS

Abbreviations in the thesis

BSA Bovine serum abulmin

CBC Cap binding complex

Cbp Cap binding protein

DAPI 4'-6-Diamidino-2-phenylindole

DMSO Dimethyl sulfoxide

DNA Deoxyribonucleic acid

DOC Deoxycholate

DTT Dithiothreitol

EDTA Ethylenediaminetetraacetic acid

EJC Exon junction complex

GFP Green fluorescent protein

HA Hemagglutinin

HRP Horseradish peroxidase

IGB Integrated genome browser

LB Luria Broth

MAT Model-based analysis of tiling array

NMD Nonsense-mediated mRNA decay

NP40 Nonidet P-40

NPC Nuclear pore complex

NTP Nucleotide tri-phosphate

PEG Polyethylene glycol

PTC Premature termination codon

RNA Ribonucleic acid

RP Ribosomal protein

RpL Ribosomal protein large subunit

RpS Ribosomal protein small subunit

SDS Sodium dodecyl sulfate

Tris Tris(hydroxymethyl) aminomethane

Upf Up-frameshift

UTR Untranslated region

Abbreviations names are usually given in full when they are first mentioned. This list only show the most frequently used and those for which full names were not given in the text.

1. INTRODUCTION

1.1 Eukaryotic gene expression and pre-mRNA processing

Gene expression involves transcription of the DNA into messenger RNA (mRNA) and translation of the mRNA into protein. In prokaryotes, mRNAs start to be translated at the transcription site, whereas in eukaryotes the primary transcripts (pre-mRNAs) undergo several post-transcriptional modifications (5′-end capping and 3′-end processing and splicing) before they are exported to the cytoplasm and translated (Moore and Proudfoot 2009). The first RNA processing event is 5' end capping which converts the pppN 5' terminus of the primary transcript to 7meGpppN (Shuman 2001). Almost all protein-coding genes in higher eukaryotes contain introns which split the pre-mRNA into two or more exons (Chow et al. 1977; Bratosin et al. 1978) and a macromolecular complex called a spliceosome removes the introns from the pre-mRNA via a splicing reaction (Fig. 1.1) (Zhou et al. 2002). Productive transcriptional elongation is tightly coupled to cotranscriptional splicing which is facilitated by the recruitment of splicing factors to the Pol II elongation complex, converting pre-mRNA into mature mRNA. In higher eukaryotes the pre-mRNA is generally alternatively spliced, giving rise to multiple mRNAs encoding different proteins (Stamm et al. 2006). This is the reason why humans, although having fewer genes than worms and flies, are more complex (Stamm et al. 2006). The 3’ end processing step involves pre-mRNA cleavage and poly (A) tail addition at the 3’end of the mRNA (Colgan and Manley 1997). The poly (A) site is specified by an evolutionary conserved flanking consensus sequence, AAUAAA which is located 30-40 nt before the polyadenylation site (Colgan and Manley 1997). Processed mRNAs are associated with several proteins forming an mRNP complex which is exported through the nuclear pore complexes (NPC) (Brodsky and Silver 2000). The composition and structure of the mRNP determines the

efficiency of nuclear export and cytoplasmic events such as mRNA localization, translation and stability (Moore and Proudfoot 2009).

1.2 Translation

Translation is the process that decodes the genetic code on the mRNA into proteins. Protein synthesis is accomplished by the ribosome, a large ribonucleoprotein assembly (~4 MDa) acting in concert with a number of translation factors (Fig. 1.1). The dynamic process of translation is usually divided into three phases: initiation, elongation and termination (Kapp and Lorsch 2004). Whereas elongation in prokaryotes and eukaryotes involves similar factors and proceeds by similar mechanisms, the initiation, termination, and ribosome recycling mechanisms appear quite different in the two lineages. The initiation phase includes all processes required for the assembly of a ribosome with an initiator-methionyl-transfer-RNA (Met-tRNAi Met) in the ribosome peptidyl (P-) site and the anticodon positioned at the start codon of the mRNA (Preiss and Hentze 2003). The initiation step is critical in determining translation yield. While most components of the initiation machinery are probably known, for many the biochemical function is not fully understood.

The elongation phase of translation is similar across all the species studied (Ramakrishnan 2002). The polypeptide synthesis takes place during the elongation phase and involves three steps: 1) binding of the cognate aminoacyl-tRNA in the ribosome aminoacyl A site, 2) peptide bond formation, and 3) translocation of the peptidyl-tRNA complex from the A site to the P site. The process of elongation requires two proteins that are conserved between prokaryotes and eukaryotes. In eukaryotes these are eEF1 and eEF2 (in yeast a third protein, eEF3, is required).

Both cognate and noncognate aminoacyl tRNAs can bind to the ribosomal A site, but several mechanisms involving codon-anticodon base pairing and conformational changes in the

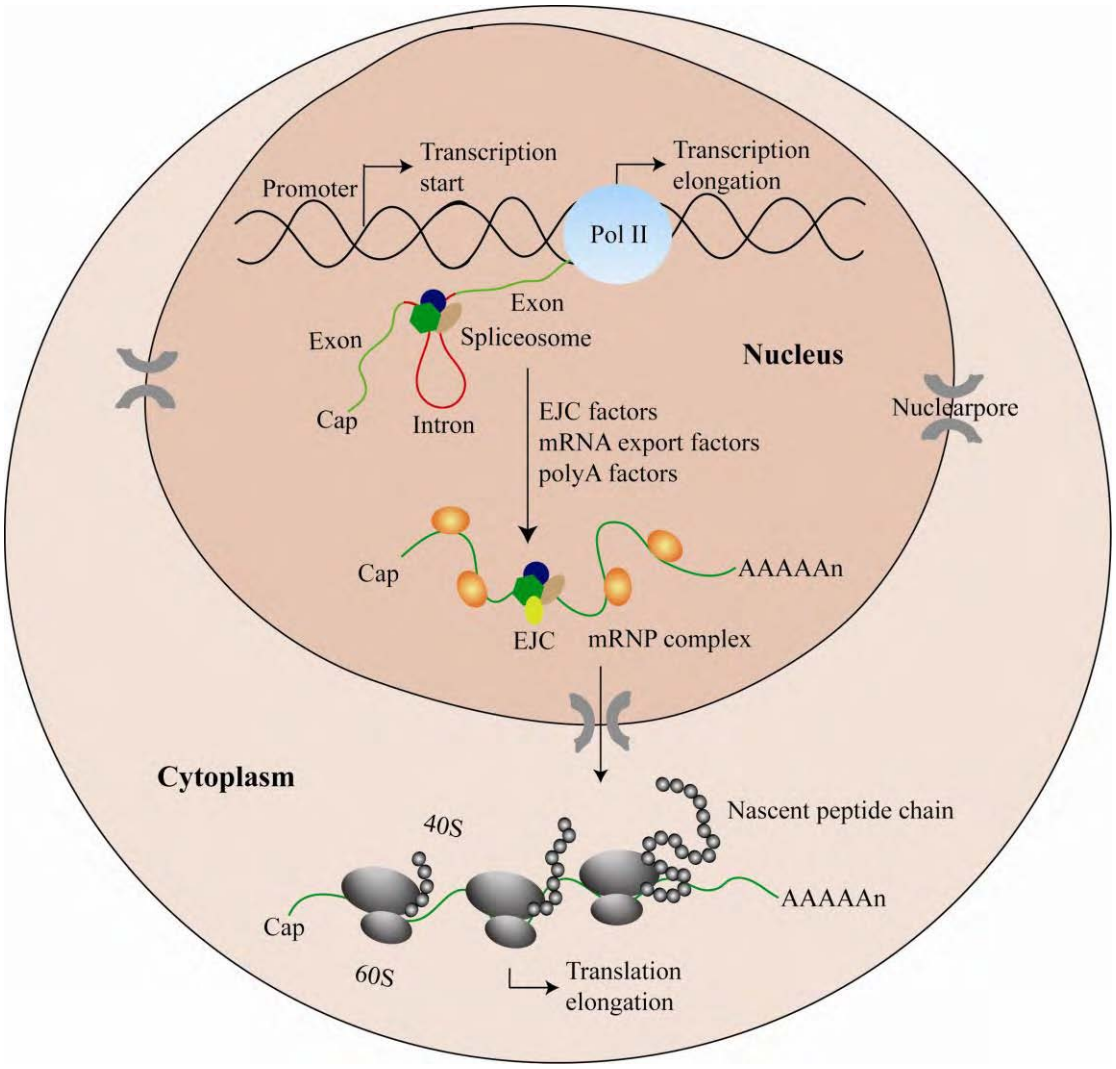


FIGURE 1.1 Schematic of eukaryotic gene expression. Protein coding genes are transcribed by RNA polymerase II (Pol II). The initial transcript is co-transcriptionally capped and, if introns are present, spliced. Mature mRNAs are packaged into mRNPs complexes and exported to the cytoplasm, where they associate with ribosomal subunits and undergo efficient translation producing nascent peptides. EJC- exon junction complex.

decoding center of the 40S ensure that only the cognate aa-tRNA is attached to the nascent peptide (Rodnina and Wintermeyer 2001). Peptide bond formation occurs on catalytic centers that are mostly formed by the ribosomal RNA (rRNA) of the large subunit (Doudna and Rath 2002). Following peptide bond formation, tRNAs and mRNA move through the A, P and exit (E) sites of the ribosome in a process called translocation (Beringer and Rodnina 2007). During translocation, which is catalyzed by eEF2, the ribosome repositions the A site over the next codon in the mRNA, the peptidyl-tRNA moves to the P site and the deacylated tRNA leaves the ribosome through the E site.

Elongation stops when the ribosome reaches a stop codon. There are no tRNAs to interact with the stop codon and instead the release factor eRF1 enters the A site and, together with eRF3, triggers the release of the nascent peptide (Pisareva et al. 2006). When the ribosome reaches the stop codon, translation terminates; this involves release of the nascent polypeptide and, presumably, release of the ribosome from the mRNA.

In eukaryotes there is cumulative evidence that translation occurs on mRNA that is kept in a closed-loop conformation whereby after termination the 40S subunit is recycled to the same mRNA and is not released into the cytosol (Wells et al. 1998). The 40S subunit may shuttle across or over the poly (A) tail back to the 5'-end of the mRNA via the 5'- and 3'-end-associated factors (Kapp and Lorsch 2004).

1.3 Ribosome

The ribosome is composed of two subunits, the large ribosomal subunit (50S in prokaryotes and 60S in eukaryotes) which, as mentioned above, catalyzes peptide bond formation, and the small ribosomal subunit (30S in prokaryotes and 40S in eukaryotes) which plays the critical role in decoding mRNA by scanning the codons on the mRNA using anticodons of the tRNA. Despite several decades of work, the molecular details of the process are not yet fully understood, but

rapid progress has been triggered by the recent determination of the high-resolution structure of the ribosomal subunits. The first high-resolution structure of 50S subunit from *Haloarcula marismortui* was reported in 2000 at the level of 2.4 Å resolution (Ban et al. 2000). Two high–resolution structures of the *Thermus thermophilus* 30S subunit at 3.3 Å and 3.0 Å resolution have also been reported (Schluenzen et al. 2000; Wimberly et al. 2000). The two subunits have a number of shared features. First, the interface side of both subunits is largely free of proteins and secondly, most of the subunit proteins have a globular domain, found generally on the solvent side and have long extensions which interact with ribosomal RNA (rRNA) and stabilise its tertiary structure.

The 16S and 23S are folded by interaction between RNA helices, which are connected by loops. There are striking differences between the two subunits in relation to the secondary structure of their rRNAs and overall morphology. The 16S rRNA forms distinct morphological components: the 5’ domain (body), central domain (platform), 3’ major domain (head) and the 3’ minor domain (located at the subunit interface) (Fig. 1.2). In contrast, the six secondary structural domains of 23S rRNA in the large ribosomal subunit are elaborately interlaced to form a compact structure. Most of what we know about ribosome structure is derived from prokaryotes; however, due to the evolutionary conservation of rRNA and many ribosomal proteins (RPs) it is assumed that the fundamental mechanism of protein biosynthesis is common to both and that they share a similar spatial arrangement of their components. However, the actual degree of similarity is still unknown and in fact some significant differences have been uncovered. For example in eukaryotic ribosome there are 20-30 extra proteins and the insertion of rRNA elements makes the eukaryotic ribosome much larger than its prokaryotic counterpart. The first 15Å resolution structural characterization of the translating ribosome from yeast was presented in 2001 (Spahn et al. 2001) (Fig. 1.2). This analysis revealed the positions of all the major rRNA expansion elements, additional proteins and inter-subunit bridges. The 18S rRNA

(1842 nt) of eukaryotes is 300 nucleotide (nt) longer than the 16S rRNA (1542 nt) of *E. coli*, and the 40S subunit of eukaryotes contains 11 more RPs than the 30S subunit of *E. coli*. Yeast 60S rRNA is built from 25S/28S rRNA (3485 nt), 5.8S (165 nt), 5S rRNA (119 nt) and contains 45 ribosomal proteins. The yeast large ribosomal subunit rRNA is 646 nt longer than its *E. coli* counterpart. These rRNA expansion segments are located at the surface of the subunits with 4 additional inter-subunit bridges identified in eukaryotes (Spahn et al. 2004).

Ribosomal proteins are present stoichiometrically in the ribosome, whereas translation factors are present generally with a copy number less than one per ribosome (Carter et al. 2001). About 30% of the *E. coli* RPs, especially those critical for ribosomal function and assembly, have orthologues in higher eukaryotic and archaeal ribosomes (Lecompte et al. 2002). Interestingly, archaeal ribosomes have an additional 30% of the r-proteins in common with eukaryotic ribosomes. In striking contrast, bacterial ribosomes do not share any RPs exclusively with either archaeal or eukaryotic ribosomes, thus supporting the view that the common ancestor of archaea and eukarya separated from the bacteria before the separation of the archaea and eukarya (Brodersen and Nissen 2005). Although in ribosome structure and function, the major role is accomplished by rRNA, RPs are nevertheless essential for these purposes, specifically in interacting with mRNA at the entrance pore, in translation factor binding site and ultimately in the exit tunnel.

1.4 Ribosome biogenesis in eukaryotes

Ribosomal RNA is transcribed by RNA polymerase I (Pol I) as a polycistronic pre-rRNA transcript, which is co-transcriptionally processed into the mature 18S, 5.8S and 28S (25S in yeast) rRNAs. The 5S rRNA is transcribed by RNA Pol III from independent loci. During ribosome biogenesis, the rRNA undergoes extensive nucleotide modifications which are guided

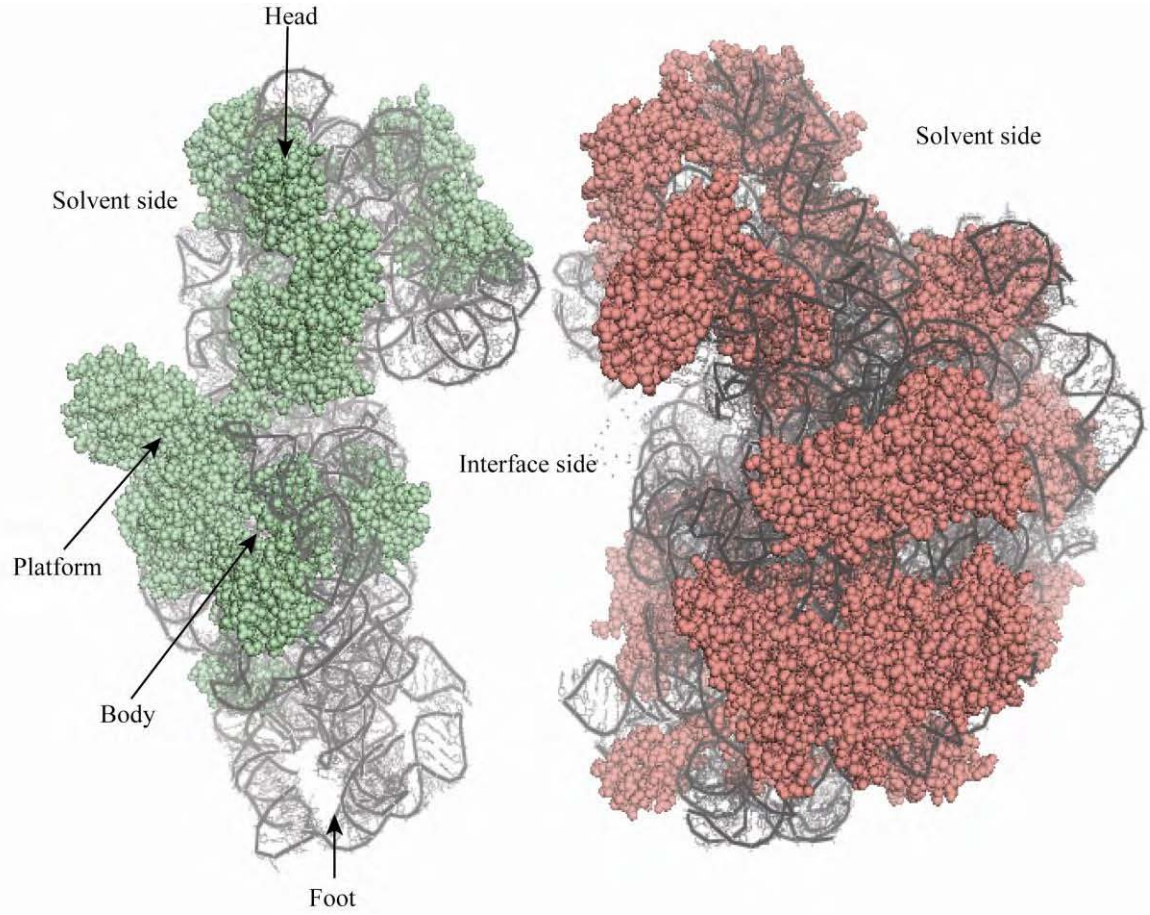


FIGURE 1.2 Cryo-EM structures of the yeast 40S and 60S ribosome subunits. The models are viewed from the right hand side, showing rRNA molecules (grey), ribosomal proteins (light green - small subunit protein, salmon red - large subunit protein) and the subunit interface cavity, with the 40S subunit on the left and the 60S subunit on the right. The distinct morphological structure of the small subunit (head, body, platform) are indicated by arrows. The structure was visualized with PyMol (www.pymol.org) using the pdb files deposited in the RCSB Protein Data Bank (Spahn et al., 2004); accession codes 1S1H and 1S1I, the corresponding prokaryotic PDB accession codes are 1J5E, 1FFK.

to specific sites by a large set of small nucleolar RNAs (snoRNAs) (Kiss 2001). The pre-rRNA processing pathway has been well characterised in *S. cerevisiae* (Fatica and Tollervey 2002) (Fig. 1.3A). Processing is initiated by the endonuclease Rnt1p at B_o site in the 3' external transcribed sequence (3'ETS) generating the 41S pre-rRNA, which undergoes three further successive cleavage reactions from A_0, A_1 and A_2 producing 20S and $27SA_2$ pre-rRNAs that act as intermediate precursors for the 40S and 60S subunits. 20S pre-rRNA is exported to the cytoplasm and cleaved near the 3' end at the D site, to produce mature 18S rRNA. Processing of $27SA_2$ is more complex, in the major pathway the $27SA_2$ pre-rRNA is cleaved at site A_3 followed by exonuclease digestion to site $B1_S$ by Rat1p and Xrn1p. In the alternative pathway the $27SA_2$ rRNA is processed to $B1_L$. The mechanism of the later pathway is not clear yet. The precursors to the $5.8S_S$ / $25S_S$ and $5.8S_L$/$25S_L$ are separated by endonuclease cleavage at C_2 site present in the internal transcribed spacers (ITS2). The short form of the 5.8S rRNA is seven nucleotides shorter at the 5' end than the longer ones and encompasses about 80% of the total. Finally the 3' end of the 25S rRNA is formed by the exonuclease Rex1p (Fatica and Tollervey 2002).

A similar rRNA processing pathway occurs in HeLa cells which showed that the completion of the maturation of the 18S rRNA occurred in the cytoplasm, a feature previously thought to be unique to yeast (Rouquette et al. 2005). This final cytoplasmic cleavage of the pre-18S RNA, conserved in yeast and mammals, may contribute to the unidirectionality of pre-ribosome translocation in eukaryotes by hampering their reassociation with transport factors. The rRNA processing in *S. pombe* appears similar to *S. cerevisiae* and other eukaryotes (Good et al. 1997). However, processing of the external spacers appears to be more complex than anticipated, transcript mapping indicate at least five extended, transient termini at 5' external transcribed spacers (ETS) (Good et al. 1997) (Fig. 1.3B).

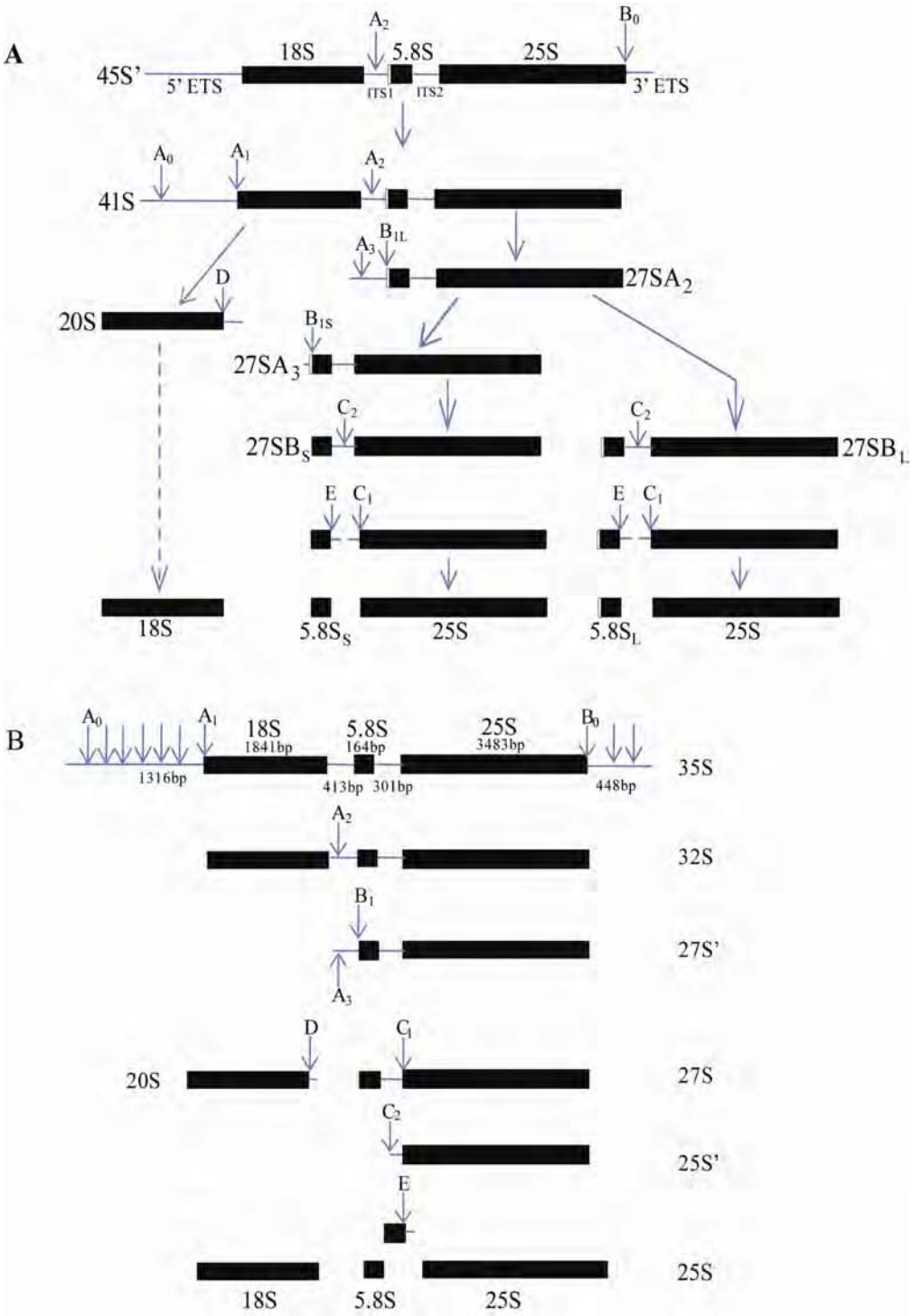


FIGURE 1.3 Structure and processing of pre-ribosomal RNA. (A) Pre-rRNA processing pathway in *S. cerevisiae*, the full length pre-rRNA is shown with solid black bars, and the spacers within blue lines. The cleavage sites are indicated by arrows, external transcribed spacers (ETS) and internal transcribed spacers (ITS). (B) Pre-rRNA processing pathway in fission yeast, very similar to budding yeast, however processing of the ETS is more complex: at least five extended transient termini are produced (indicated by arrows). [Figure modified from (Good et al. 1997)].

The processing and assembly of mature ribosomes involve a large number of RPs, along with 150 non-ribosomal proteins (Fatica and Tollervey 2002; Tschochner and Hurt 2003). However, very little is known about the particular functions of RPs in ribosome biogenesis, with most studies focusing on their function during translation. RPs were mainly ignored in ribosome biogenesis studies because they were thought to be non-specific contaminants in purified protein complexes. So far only the 40S ribosomal proteins have been investigated for a role in biogenesis. It has been reported that most 40S RPs have distinct roles in rRNA maturation, export and 40S biogenesis (Ferreira-Cerca et al. 2005). Only partial information is available about the function of 60S RPs in biogenesis of the subunit. Deletion of an isoleucine residue from a highly conserved hydrophobic domain present in the middle of RpL32 slows defects in the maturation of 35S and 27S rRNA (Vilardell and Warner 1997). Depletion of RpL25 or mutation in its pre r-RNA binding domain blocked the processing of the 27S ITS2 region. Mutations in the N and C terminal domains of RpL25 also cause defective 27S processing (van Beekvelt et al. 2001). Therefore, RPs, which are known to have function in the structural integrity of ribosomes and in translation, also appear to be involved in ribosome biogenesis.

1.5 Extraribosomal functions of RPs

All organisms need coordinated and regulated synthesis of RPs and this regulation differs in different organisms. The number of RPs varies from prokaryotes to eukaryotes and their extent of conservation also varies through evolution; 50 to 54 RPs for eubacteria, 57 to 68 for archaea and 79 to 81 for eukaryotes. Prokaryotes have a single gene coding for each protein (Nomura 1999). In contrast in yeast a quarter of the RPs are produced by a single gene and the rest RPs are encoded by two different genes either present in same or different chromosomes (Planta and Mager 1998). In mammals with few exceptions, ribosomal proteins are encoded by a single gene (Wool et al. 1995). Various studies have reported diverse phenotypic and genotypic

effects of ribosomal protein mutations suggesting they must have distinct extra-ribosomal functions. The first report of a RP with an extra ribosomal function is *E. coli* S1 (the nomenclature of bacterial RPs is either S or L followed by a numeral, corresponding to small or large subunit RPs respectively) which is one of the subunits of the bacteriophage Qβ replicase (Blumenthal and Carmichael 1979).

1.5.1 RPs in transcription

One of the classic examples of RPs involved in transcription is *E. coli* S10 which acts as a component of the NUS complex which is required for transcription of some λ phage genes by preventing premature transcription termination (Friedman et al. 1981). The interaction between S10 and the NusB subunit enables the NUS complex to interact with λ transcripts (Luo et al. 2008) (Fig. 1.4A). The ribosomal protein S10 has an extended loop penetrating the small subunit and a globular domain present on the surface of the subunit, the latter is important for NUS activity. There are several other reports of RPs that bind their own mRNAs or pre-mRNAs and negatively autoregulate their own expression by affecting translation, splicing or transcription (Wool 1996; Lindstrom 2009; Warner and McIntosh 2009). For example, RpS13 in mammalian cells represses its own gene expression by inhibiting splicing; RpS13 binds its own pre-mRNA close to the splice sites of the first intron and probably prevents spliceosome assembly (Fig. 1.4B) (Malygin et al. 2007). Similar regulatory mechanisms have been reported for other RPs in both human and yeast (Fewell and Woolford 1999; Vilardell et al. 2000; Ivanov et al. 2004; Cuccurese et al. 2005).

Notably, in mammalian cells there is also evidence of RPs that regulate genes other than their own, by binding directly to specific transcription factors. RpL11, for instance, associates with a defined domain of the oncoprotein cMyc and inhibits transcription activation of cMyc target genes (Fig. 1.4C); the effect appears specific for RpL11 since other RPs did not show similar

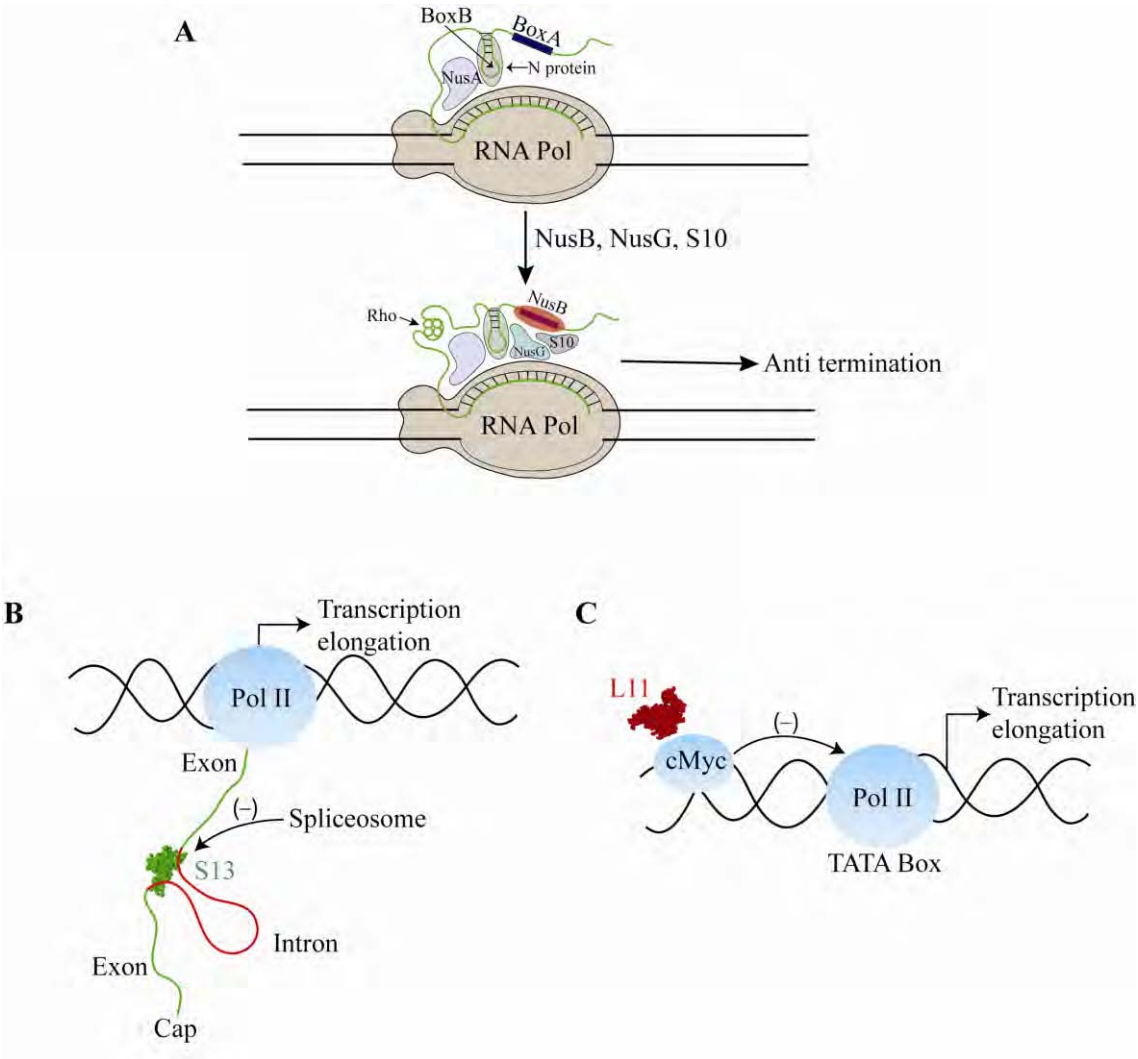


FIGURE 1.4 Examples of RPs functioning in transcription
(A) RpS10 is part of the NUS protein complex which promotes antitermination. NusG acts in concert with NusA, NusB, and S10 (NusE), binds boxA RNA, suppresses RNA polymerase (RNAP) pausing and promote read-through of terminators. (B) Schematic representation of possible autoregulatory role of RpS13. RpS13 in mammalian cells binds close to splice sites of intron 1 of its own mRNA, and inhibits splicing (De and Brogna). (C) Free RpL11 interacts with cMyc and represses transcription (De and Brogna).

activities (Dai et al. 2007; Dai et al. 2010). Similarly, it has also been reported that RpS3 specifically associates with the NF-κB DNA-binding protein complex and stabilizes the association of this transcription factor to its target sites (Wan et al. 2007).

1.5.2 RPs involved in DNA repair

Several RPs have been reported to be involved in DNA damage repair. An interesting example is mammalian RpS3 which cross reacts with a monoclonal antibody raised against AP endonuclease I and UV endonuclease III. Over-expression of RpS3 recovers the phenotype arising from the mutations in the two endonucleases (Kim et al. 1995). In *D. melanogaster* RpS3, apart from acting as AP endonuclease, also removes 8- oxoguanine residues efficiently (Yacoub et al. 1996). The phosphorylated human RpS3 has been co-localised with 8-oxoguanine implying that in human this protein is part of the cellular DNA damage response pathway (Hegde et al. 2007; Yadavilli et al. 2007).

1.5.3 RPs in translation regulation

Classical demonstrations of extra ribosomal functions in translation are RPs that bind a specific RNA structures in the 5'UTR and suppress their own translation - this mechanism is widespread in *E coli* but there are examples also in eukaryotes. These repressor functions create negative regulatory loops that synchronise synthesis of RPs with rRNA, preventing accumulation of free RPs in the cell (Nomura 1999). In prokaryotes, the RNA structure in the 5'UTR typically resembles that of the rRNA domain that RP binds to on the ribosome (Baughman and Nomura 1984; Zengel and Lindahl 1993). Another well documented case of a RP functioning as a translation repressor is RpL13a; when human cells are treated with IFNγ, RpL13a is phosphorylated at S77 and released from the ribosome, and is incorporated into the IFN-γ-activated inhibitor of translation (GAIT) complex (Mazumder et al. 2003; Mukhopadhyay et al. 2008). The GAIT complex, when activated, inhibits translation by binding

to a specific structure of the 3'UTR of the ceruloplasmin mRNA. Furthermore, it has been found that translation of mRNA of ZIPK kinase, involved in phosphorylation of L13a, (Mukhopadhyay, Ray et al. 2008) is inhibited in a similar manner (Fig. 1.5).

1.5.4 RPs in cancer, apoptosis and development

So far a number of RPs are reported to be involved in developmental roles of different organisms. Mutations in RPs cause a *minute* phenotype in *D. melanogaster* characterized by delayed larval development, reduced body size, decreased fertility, reduced viability and thin bristles (Marygold et al. 2007). In humans, RpS19 is a major gene involved in erythroid proliferation and differentiation and there is genetic evidence that this gene is involved in the pathogenesis of Diamond Blackfan Anemia (DBA) (Draptchinskaia et al. 1999). Missense mutations and a trinuclotide insertion in the RpS19 gene, are often found in DBA patients, which results in abnormal nucleolar localization and instability of the protein. The protein with these mutations cannot assemble into mature ribosome (Angelini et al. 2007; Kuramitsu et al. 2008). Several other ribosomal proteins have been implicated in DBA, e.g. RpS24, RpS17, RpL35a, RpL5 and RpL11.

RpL11 and RpL23 simultaneously but distinctly interact with Hdm2 protein (Hdm2 is an ubiquitin ligase responsible for ubiquitination of p53 and degradation of this protein) to form a ternary complex. When RpL11 binds to Hdm2 it confines this protein to the nucleolus of human cells, L11 inhibits the ability of Hdm2 to degrade p53 and expression of L11 induces a p53 response (Lohrum et al. 2003). L23 also binds to HDM2; over-expression of this protein can lead to cell-cycle arrest and apoptosis and si-RNA mediated suppression of L23 leads to p53 accumulation (Dai et al. 2004). In summary, there is abundant evidence for RPs that are not assembled into ribosome having additional functions unrelated to the role in translation.

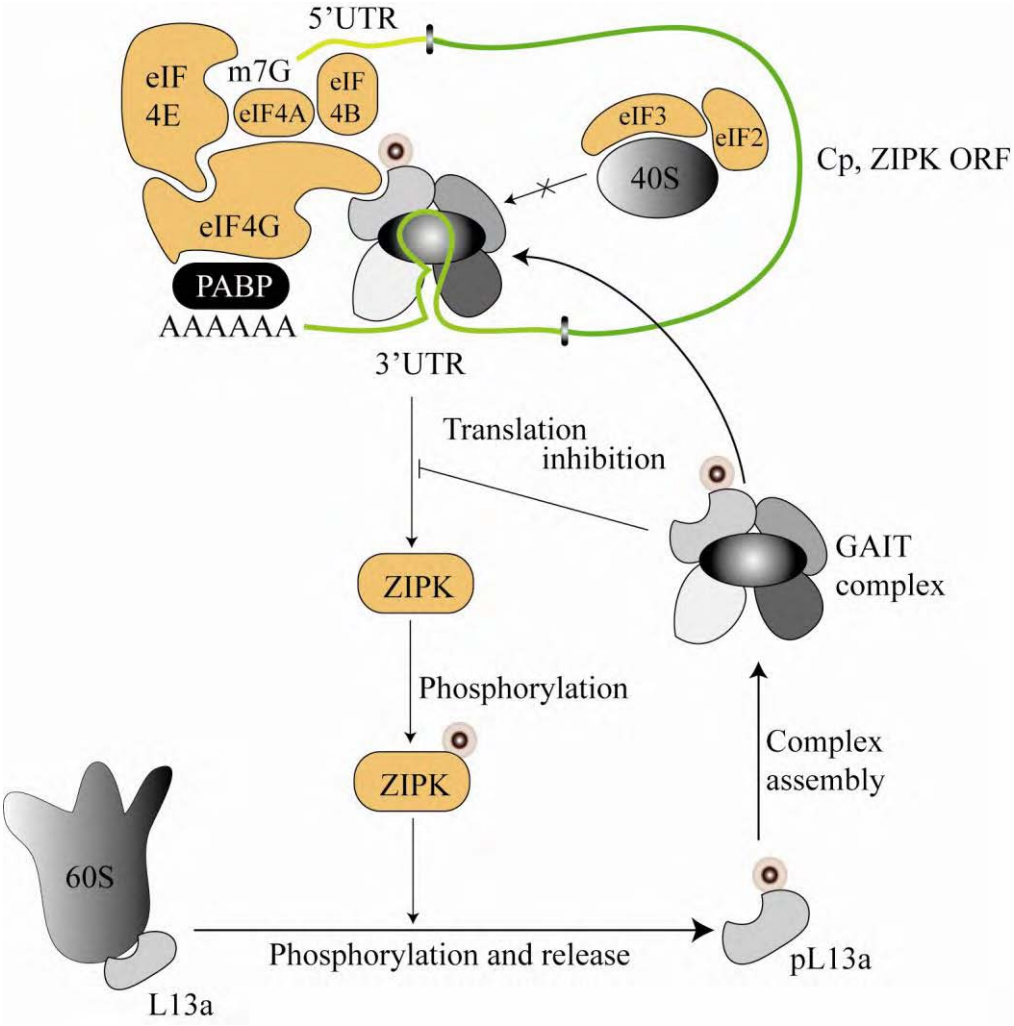


FIGURE 1.5 Schematic illustration of L13a involved in translation repression. IFN-γ causes transcriptional induction of inflammatory genes when include Ceruloplasmin (Cp) and zipper-interacting protein kinase (ZIPK). ZIPK phosphorylates RpL13a, when phosphorylated RpL13a is released from 60S subunit and binds to the GAIT complex. Activated GAIT protein complex binds to specific 3'UTR structure of target mRNAs, inhibiting the translation.

1.6 Eukaryotic cell compartmentalization and translation

In contrast to prokaryotic cells, eukaryotic cells are highly compartmentalized, with many steps of gene expression being restricted either to the nucleus or to the cytoplasm. It is commonly accepted that transcription and RNA processing takes place in the nucleus, but that translation occurs only in the cytoplasm. It was, therefore, believed that there was no direct link between nuclear events, (e.g. pre-mRNA splicing) and cytoplasmic events, (e.g. translation and mRNA destruction). This dogma has been challenged in recent years by reports that indicate that the nature of the nuclear mRNP also impinges on cytoplasmic events such as translation and NMD (Brogna 1999; Muhlemann et al. 2001; Moore and Proudfoot 2009). In addition, there are reports that translation, or a translation-like mechanism, may exist within the nucleus (Iborra et al. 2001; Brogna et al. 2002).

1.7 Nonsense mediated mRNA Decay

NMD is a general phenomenon that causes a reduction of abnormal mRNAs carrying nonsense codons (called also premature termination codons, PTCs), which arise from mutations or errors in gene expression. NMD has been observed in every eukaryotic organism tested and in particular has been investigated in *S. cerevisiae*, *D. melanogaster*, *C. elegans* and mammalian cells. NMD probably acts as a mechanism to protect the cell from the potentially disastrous effects of truncated proteins. NMD is not a passive mechanism. Specific proteins are required in the NMD process to destroy mRNAs that have not been translated properly. The best characterised factors required for NMD are the upstream frameshift proteins, Upf1, 2 and 3. These proteins were first identified in *S. cerevisiae* but later were found to be highly conserved and identified in multicellular organisms. These proteins localize predominantly in the cytoplasm (Perlick et al. 1996). According to the earlier model of yeast NMD, when a ribosome stops at the PTC, a surveillance complex composed of Upf proteins (1-3) and eukaryotic release

factors (eRF1 and eRF3) is formed and scans the mRNA in a 3' direction. Detection of a downstream destabilising sequence element (DSE) discriminates a PTC from a normal one prompting rapid deadenylation-independant decapping and 5'→3' decay. However, further analysis of DSE sequences from diverse yeast mRNAs showed that there was only a very weak sequence conservation and moreover that there was no evidence for a DSE sequences in mammals (Amrani et al. 2006).

A more recent model for how PTCs (premature termination codon) are distinguished from normal stop codons is called the "faux UTR". In this model the premature and normal termination signals are considered to be different biochemically. At a normal stop codon, the interactions between the terminating ribosome and a specific 3' mRNP structure or, specific RNA binding proteins localized at 3' of the stop codon, triggers proper termination of translation and the normal rate of mRNA decay (Fig. 1.6A). Instead at a PTC, the ribosome terminates in a sub-optimal mRNP environment and this triggers the recruitment of NMD factors such as Upf1 to the terminating ribosome resulting in rapid mRNA destruction**.**

According to the faux UTR model, normal termination requires the termination to occur in the proximity of Pab1 (PolyA binding protein), which is normally associated with the poly (A) tail. Interaction between Pab1, eRF3 and eRF1 results in efficient translation termination. Upf and other NMD factors can also interact with eRF3, but less well than Pab1 so that association of Upf factors to eRF3 is precluded during normal termination process. In contrast, termination at a PTC is characterized by the absence of Pab1, allowing instead the interaction of Upf and other NMD proteins to the terminating ribosome resulting in mRNA decay (Amrani et al. 2006) (Fig. 1.6B).

One peculiar characteristic that distinguishes mammalian from yeast NMD is the general requirement for at least one intron downstream of the PTC for NMD to occur

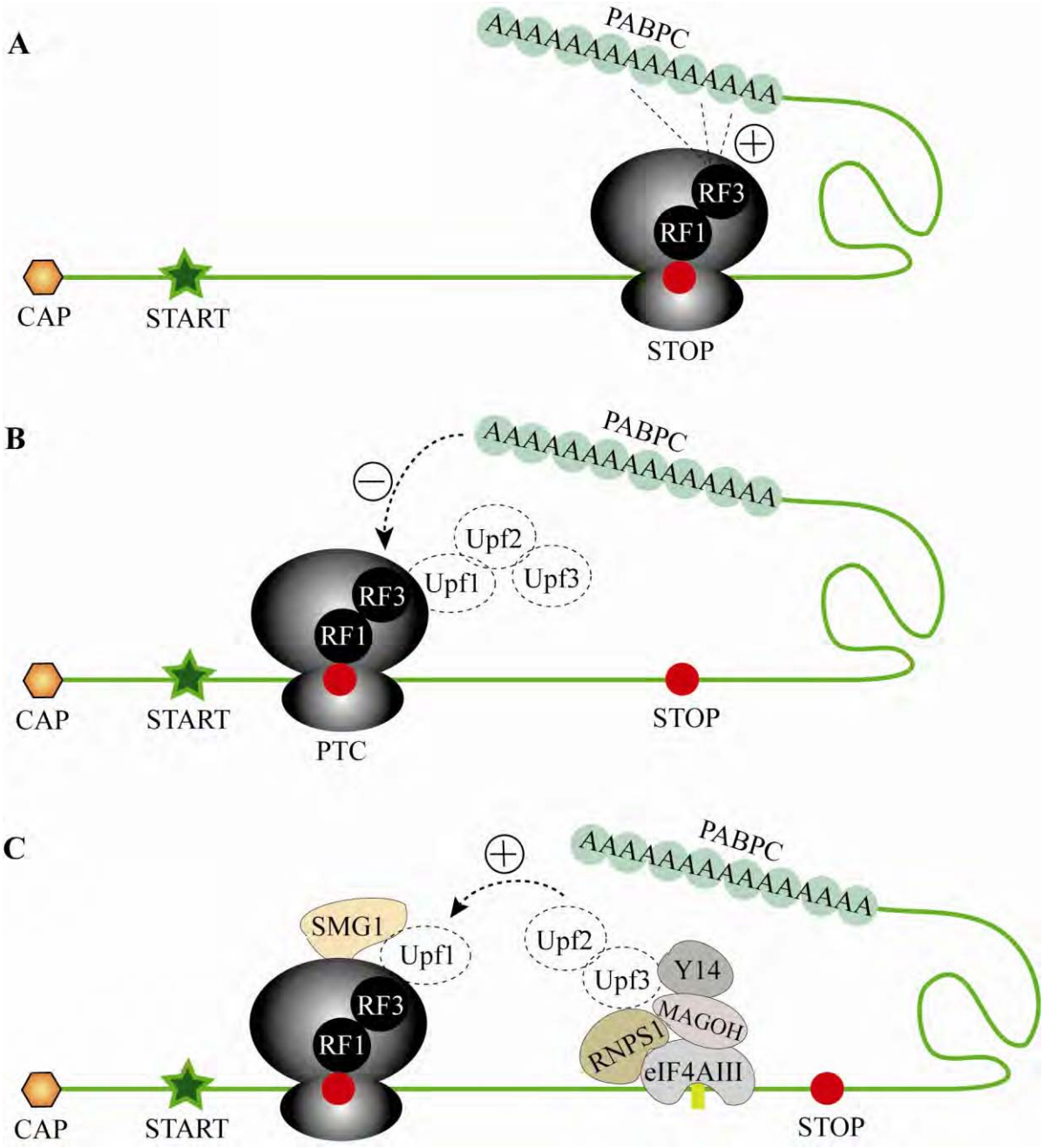


FIGURE 1.6 Current NMD models

(A) Normal termination. When the ribosome encounters the stop codon, eRF1 and eRF3 associate with cytoplasmic PABP and termination occurs efficiently. (B) A faux 3'-UTR-mediated premature termination. When the ribosome encounters an upstream termination codon, interaction between RF1/3 and PABPC1 is prevented, termination is not efficient and NMD is triggered. (C) EJC-dependent premature termination. Termination occurs upstream of an EJC, the EJC mediates the association of NMD factors with the terminating ribosome, and NMD is triggered. [modified from (Wen and Brogna 2008)].

(Carter et al. 1996; Zhang et al. 1998). The link with splicing is probably mediated by the exon junction complex (EJC), a protein complex which binds 20-24nts upstream of exon-exon junction prior to splicing (Maquat 2004; Kashima et al. 2006). The EJC core consists of eIF4AIII plus three interaction partners: MLN51, Y14, and Magoh. Magoh recruits Upf3 and the EJC is then exported with mRNA to the cytoplasm where it recruits the perinuclear protein Upf2 (Tange et al. 2005). The EJC is recognised by the post-termination ribosome and this interaction is thought to trigger NMD. The position of the PTC relative to the exon-exon junction is crucial i.e. 50-55nt upstream of the junction (Fig. 1.6C). A recent study in *S. pombe*, however, showed that EJC components are not required for NMD in this organism and proposed that the EJC model cannot properly explain the link between pre-mRNA splicing and NMD (Wen and Brogna 2010).

1.8 NMD in the nucleus

In *S. cerevisiae*, NMD occurs exclusively in the cytoplasm (Kuperwasser et al. 2004). However, in mammalian cells, there is data suggesting that NMD may take place in the nucleus (Cheng and Maquat 1993; Belgrader et al. 1994; Cheng et al. 1994). Translating ribosomes are the only known means of detecting termination codon and NMD requires translation: for example tRNA suppressors, antibiotics and hairpins in the 5'-UTR that inhibit translation also abolish NMD (Lim and Maquat 1992; Belgrader et al. 1993; Qian et al. 1993). Therefore, it is not easy to explain how NMD could take place in the nucleus while translation occurs only in the cytoplasm.

Other observations have also reported that premature termination codons (PTCs) are actually recognised during nuclear export by a ribosome present on the cytoplasmic side of the nuclear envelope (Maquat 1995). Some studies in human cells indicate that NMD occurs while the mRNA is still associated with the nuclear cap binding complex (CBC, formed by Cbp80 and

Cbp20), before the CBC is replaced by the cytoplasmic cap binding protein, eIF4E (Ishigaki et al. 2001). The EJC appears to associate with Cbp80-bound mRNA but not with eIF4E-bound mRNA (Lejeune et al. 2002). These studies, therefore, indicate that Cbp80 is required for NMD in mammalian cells. RNAi depletion of Cbp80 stabilizes NMD substrates (Hosoda et al. 2005). This first round of translation of CBC-associated mRNA was called the pioneer round of translation and may occur whilst the mRNA is still associated with the nuclear envelope. Therefore, it is feasible that nuclear NMD is simply due to a pioneer round of translation of mRNA not yet released from the nuclear envelope. However, it is also feasible that nonsense mutation recognition could occur while the transcript is still in the nucleus. In particular, it has been proposed that PTC can be recognised on nascent mRNA. This possibility is supported by a study showing that PTCs can affect pre-mRNA 3'end processing - the closer the premature stop codon is to the 5'end the longer the poly(A) tail is (Brogna 1999); and by another study showing that PTCs lead to an accumulation of pre-mRNA at the site of transcription (Muhlemann et al. 2001; Li et al. 2002). Observations that PTCs can affect pre-mRNA splicing are consistent with the suggestion that translation might occur also in the nucleus (Li et al. 2002).

The notion that translation might occur also in the nucleus was first proposed four decades ago (Goldstein 1970). Functional polyribosomes have been found in the nuclei of the slime mould *Dictyostelium discoideum* (Mangiarotti 1999). Furthermore, in *D. discoideum*, newly assembled 40S and 60S ribosomal subunits, that are still associated with pre-rRNA, appear to be fully active in protein synthesis and the pre-rRNA is often detected in 80S monosomes and even polyribosomes *in vivo* and *in vitro* (Mangiarotti et al. 1997). More recently, direct evidence for nuclear translation was provided by experiments that allow visualization of translation sites in mammalian cells (Iborra et al. 2001). It was reported that fluorescently labelled amino acids could be incorporated into nascent peptides in highly purified nuclei. In this assay, putative

translation sites were readily visible under a fluorescence microscope and appeared as distinct fluorescent foci. The occurrence of this nuclear fluorescence was prevented by translation inhibitor drugs. These observation were interpreted as evidence that, like in prokaryotes, translation might be coupled to transcription in the nucleus (Iborra et al. 2001). In agreement with this conclusion, a later study reported that several NMD, transcription and translation factors copurify in biochemical procedures and colocalize in electron microscopy (EM)-immunostaining assays (Iborra et al. 2004). Similar observations were also reported in *D. melanogaster*. It was found that [^{35}S] methionine/cysteine was rapidly incorporated into active transcription sites of polytene chromosomes and found in the nucleolus; this incorporation was once again sensitive to translation inhibitor drugs (Brogna et al. 2002). In this latter study, it was also reported that many RPs and some translation factors are found associated with transcription sites. In addition, it was also shown by *in situ* hybridization that rRNA is also present at these chromosomal sites (Brogna et al. 2002). Therefore, both of these studies support the view that translation also might be coupled to transcription in eukaryotes.

The view that translation might occur in the nucleus is controversial. It has been argued that the seemingly nuclear translation reported by Iborra et al. (2001) is due to contamination of the nuclei with endoplasmic reticulum (ER), as the ER is attached to the nuclear envelope and is difficult to strip away from the nuclei (Dahlberg et al. 2003). The nuclear signal could be an artifact of over-permeabilization of the nuclei, which might lead to entry of cytoplasmic ribosomes into the nucleus (Nathanson et al. 2003). Similarly to the Iborra study, the Brogna study was criticized; it was argued that the antibodies used were not specific for ribosomal proteins and that the immunostaining procedure allows artificial access of cytoplasmic materials into the nucleus (Dahlberg et al. 2003). However, support for the Brogna study come in 2002 in *S. cerevisiae*, where RPs were once again reported to be associate with transcription sites (Schroder and Moore 2005). Surprisingly, in the Schroder and Moore study RPs were

found to associate with both protein- and non-protein-encoding genes, and it was concluded that the presence of RPs at transcription sites might not be linked to translation.

In summary, it is apparent that ribosomal components and translation factors are present in the nucleus at active transcription sites; the issue is whether this localization reflects fully assembled ribosomes or merely free ribosomal proteins. In addition, it has been pointed out that the absence of a key translation factor would be sufficient to preclude translation in the nucleus (Bohnsack et al. 2002). A study with mammalian cells has concluded that key translation factors are actively excluded from the nucleus (Bohnsack et al. 2002). However, the issue is still open and we await further evidence before a conclusion can be drawn.

1.9 Additional functions of NMD factors

Core NMD factors appear also to have second functions unrelated to NMD. For example, Upf1, but not Upf2, is important for DNA replication in mammalian cells (Azzalin and Lingner 2006). In addition, chromosome breaks and rearrangements were observed upon caffeine mediated inhibition of checkpoint kinases in Upf1 depleted cells and that these cells showed accumulation of the damage DNA marker phosphorylated histone γH2AX in nuclear foci (Azzalin and Lingner 2006). According to present hypothesis chromatin bound Upf1 acts as helicase during DNA replication. Upf1 physically interacts with DNA polymerase delta and coimmunoprecipitates with the p66 and the p125 which are catalytic subunits of DNA polδ in HeLa nuclear extracts (Carastro et al. 2002; Azzalin and Lingner 2006) (Fig. 1.7).

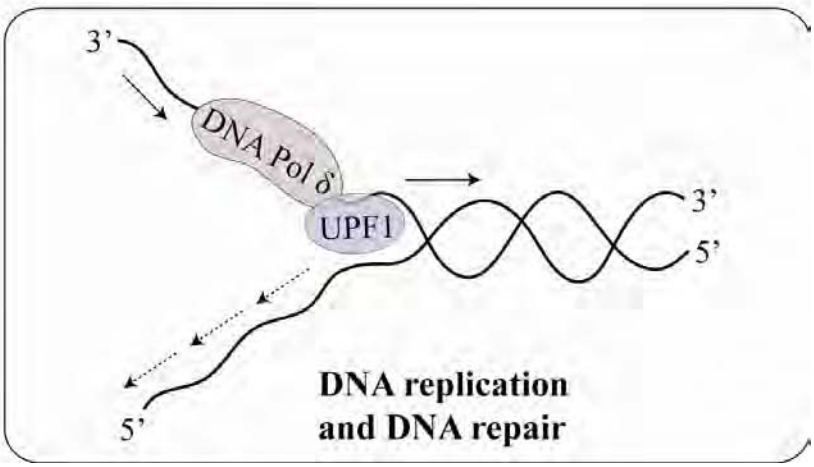


FIGURE 1.7 Extra NMD functions of Upf1 protein.
Upf1 associates with DNA polymerase δ during S phase acting as a major factor required for DNA replication.

1.10 Aims and objectives of this study

The main aim of the work in this thesis was to investigate the interaction of RPs with chromatin. To address this question I have used *S. pombe* as model organism and chromatin immunoprecipitation (ChIP) technique to assay chromatin interaction. For genome-wide identification of RP binding sites I have used ChIP followed by DNA hybridization to whole-genome tiling arrays (ChIP-on-chip).

I have also investigated whether NMD factors are involved in DNA metabolism also in *S. pombe.* In summary, the focus of my PhD research has been to investigate whether RPs and NMD factors have nuclear functions in *S. pombe*.

1.11 Why Fission yeast?

Fission yeast is the sixth model eukaryotic organism whose genome has been fully sequenced (Wood et al. 2002). This organism is a rod-shaped unicellular eukaryote. Cells typically measure 7 to 14 micrometres in length and 3 to 4 micrometres in diameter. Fission yeast was isolated by Lindner from East African millet beer in the year 1893 (http://en.wikipedia.org/wiki/Schizosaccharomyces_pombe). The species name derives from the Swahili word for beer (Pombe). Genome sequencing revealed gene density for the fission yeast genome is one gene every 2,508 base pairs, as compared to one gene every 2,088 base pairs for budding yeast and 43% of its genes have introns (a total of 4,793 introns), has very basic RNAi components and centromeric organisation is quite similar to that of mammals (Wood et al. 2002). Wild-type fission yeast grows relatively quickly: takes about 2.5–3 hr to complete a cell cycle at 30°C. A wild-type *S. pombe* cell grows by elongation, and divides by medial fission. These important characteristics allow telling at which stage of the cell cycle is the cell by simple visualization of the cell morphology. Because fission yeast has only three chromosomes, it is also a convenient organism for the cytological study of chromosome dynamics. Thus, fission yeast has become a popular system for studies of cell growth, division and basic biological pathways. This organism provides an excellent platform to study basic molecular biology pathways which is applicable to both lower and higher eukaryotes.

2. MATERIALS AND METHODS

2.1 Solutions and buffers

Solutions were prepared from analytical grade reagents supplied by Sigma-Aldrich, VWR or Fluka. All of the solutions and buffers were made in deionised water (Elix 5, Millipore) and sterilized by either autoclaving or filtration (0.22 μm, Millipore). All of the solutions used in RNA experiments were prepared in sterilized glassware and treated overnight with 0.1% (v/v) diethyl pyrocarbonate (DEPC), left overnight in a laminar flow hood and then autoclaved. Tris buffer solutions were not DEPC treated, but were prepared with DEPC treated water.

2.2 DNA cloning in *Escherichia coli*

Most standard protocols were as described in Molecular Cloning 2nd edition [Molecular cloning: a laboratory manual / J. Sambrook, E.F. Fritsch, T. Maniatis (1989)]. Typically XL1 blue (Stratagene) was used which has the following genotype: recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac [F´ proAB lacI^{q}ZΔM15 Tn10 (Tet^{r})]

2.2.1 Bacterial cultures

LB broth and LB agar recipes are shown in section 2.4.

E. coli coltures were routinely grown overnight at 37°C on inverted 9 cm LB agar plates. Liquid cultures were grown in a shaking incubator at 220 rpm.

2.2.2 Ligation and *E. coli* transformation

1. Ligation of DNA fragments was typically performed in a 20 μl reaction containing 100 ng of linearized plasmid and a four fold molar excess of the insert DNA, typically with 10 units of T4 DNA ligase (New England Biolabs, NEB).

2. The ligation reaction was kept at 18°C overnight or at room temperature for 2 hrs. 100 μl of *E. coli* competent cells were typically transformed with 5 μl of ligation mixture. Ligation mixture was mixed with competent cells and kept on ice for 20 min and then heat shocked at 42°C for 45 seconds and cooled on ice for 2 min.

3. The competent cells were mixed with 0.5 ml of SOC media and incubated at 37°C for 1 hour, with gentle shaking. The cells were briefly centrifuged and spread on an LB plate containing 100 μg/ml ampicillin.

Competent cells were typically made by the rubidium method:

1. XL1 blue were grown in 100 ml LB with 10 mM $MgCl_2$ and 10 mM $MgSO_4$ at 37°C until the cell density was about 0.5 OD_{650}.

2. The cells were harvested at 5000 rpm for 15 min at 4°C, resuspended in 30 ml filter sterilized Rb buffer 1 (100 mM RbCl, 50 mM $MnCl_2{\cdot}4H_2O$, 80 mM KAc, 10 mM $CaCl_2{\cdot}2H_2O$, 15% (v/v) glycerol and adjusting pH to 5.8 with 0.2 M acetic acid), and incubated on ice for 1 hour.

3. The cells were then pelleted (10,000 rpm, 10 min) and resuspended in 8ml filter sterilized Rb buffer 2 (10 mM RbCl, 10 mM MOPS, 75 nM $CaCl_2{\cdot}2H_2O$, 15% (v/v) glycerol and adjusting pH to 6.8 by NaOH).

4. Competent cells were aliquoted with 200 μl per tube, and kept at -80 °C for future use.

2.2.3 Small-scale preparation of plasmids

A single colony was inoculated into 5 ml of LB broth containing 100 μg/ml ampicillin and grown overnight. Plasmid DNA was typically purified from a 1 ml aliquot of this culture using the following boiling-prep method:

1. 1 ml of the cell culture was transferred into a fresh 1.5 ml tube and centrifuged briefly at 13000 rpm and the supernatant discarded.
2. 110 μl of ice cold STET buffer (8% sucrose, 50 mM Tris pH 8.0, 50 mM EDTA pH 8.0, 5% (v/v) Triton X-100) containing 5 μl of 20 mg/ml lyzozyme was added into each sample and the pellet was then completely resuspended by pipetting up and down.
3. The samples were placed in boiling water for 20 seconds and then centrifuged at 13000 rpm for 10 min. The pellets were removed by using sterile toothpicks.
4. 110 μl of isopropanol was added to the supernatant, mixed and centrifuged at 13000 rpm for 15 min.
5. The supernatant was discarded. The pellet was washed with 70% (v/v) ethanol, air-dried and resuspended in 40 μl TE (10 mM Tris·Cl, pH 8.0, 1 mM EDTA, pH 8.0) containing 1 μl of 1 mg/ml RNase A stock. The DNA samples were incubated at 65°C for 20 min to remove the RNA and stored at -20°C if required for future use.

When needed the remaining cell culture was used to prepare pure plasmids preparations for sequencing and yeast transformations (typically using Fermentas GeneJET Plasmid Miniprep Kit as recommended by the manufacturer).

2.2.4 Large-scale preparation of plasmid DNA

1. Typically a single colony was inoculated into 1 ml of LB broth containing 100 μg/ml ampicillin and grown overnight.
2. 200 μl of the overnight culture was incubated into 100 ml of LB broth containing 100 μg/ml ampicillin for 4-5 hours until OD_{650} 0.8-1.0.

3. Plasmid DNA was then prepared from the culture by using commercial kits (typically PureLink™ HiPure Plasmid Midiprep Kit, Invitrogen) following the manufacturer's instructions.

4. The extracted plasmid DNA was resuspended in 500 μl TE, pH 8.0, and the concentration of plasmid DNA was measured with a spectrometer (ND-1000, NanoDrop).

2.2.5 Restriction enzyme digestion

1. Restriction enzyme digestions were carried out in a 10-50 μl reaction. All restriction enzymes used in the study were obtained from New England Biolabs (NEB).

2. The conditions of the single-enzyme or double-enzyme digestion were followed according to the NEB enzyme instructions.

3. For a sequential digestion, the initial reaction contained the enzyme that is active in the buffer with the lowest salt concentration. After the reaction had proceeded for 2 hours, the second enzyme and the buffer with the higher salt concentration were added and the reaction continued to proceed for a further 1 hour.

2.2.6 Dephosphorylation of DNA

1. Antarctic phosphatase (NEB) was used to remove the 5' terminal phosphates of the DNA. This procedure was generally applied to prevent self-ligation of digested plasmid DNA. Following the restriction enzyme digestion, 1 μl of antarctic phosphatase (5 Units/μL) was added into the reaction and incubated at 37°C for 1 hour.

2. The antarctic phosphatase was then inactivated at 65°C for 15 min or purified by gel electrophoresis and gel extraction using a silica powder based technique (see below).

2.2.7 Purification of PCR product and restriction digested DNA fragments

Two methods were used to perform DNA purifications following PCR and restriction digestion. One is the polyethylene glycol (PEG) method, and the other is gel extraction.

2.2.7.1 PEG purification

1. An equal volume of the PEG solution (13% (w/v) PEG 8000, 0.6 M NaAc, and 6 mM $MgCl_2 \cdot 6H_2O$) was added to the DNA sample, mixed by vigorous vortexing. Keep at room temperature for 20 min. If the DNA fragment size was less than 300 bp, three volumes of the PEG solution was used instead.
2. The sample was centrifuged at 13000 rpm for 20 min and the supernatant was completely removed by a Pasteur pipette without touching the pellet.
3. The DNA pellet was washed by 1 ml 96% (v/v) ethanol and centrifuged at 13000 rpm for 3 min, and then washed again by 70% (v/v) ethanol.
4. The pellet was air-dried and dissolved in 20-30 μl TE buffer.

2.2.7.2 Gel purification

1. The portion of agarose gel containing DNA fragment was cut and placed into a 1.5 ml eppendorf tube.
2. The DNA was then purified using Silica Bead DNA Gel Extraction Kit, Fermentas, as described in the manufacturer's instructions.

2.2.8 Polymerase Chain Reaction (PCR)

All primers used in my study are shown in table 2.1. The primers were purchased from MWG. The PCR conditions varied, depending on the DNA polymerase, the melting temperature (T_m) of primer and the length of amplified DNA. PCR reactions were typycally run in a thermal

cycler (GeneAmp® PCR System 9700, Applied Biosystems) and the product analyzed by agarose gel electrophoresis.

2.2.8.1 *E. coli* and yeast PCR colony screening

For a bacteria colony PCR, the fresh colonies were picked using yellow tips (2-20 μl) and dipped into PCR tubes containing 10 μl PCR solutions which contained 1× PCR buffer, dNTP mixture (0.2 mM for each), 1.5 mM $MgCl_2$, 2 μM primers and 0.15 U Taq DNA polymerase (typically GoTaq, Promega).

The procedure for a yeast colony PCR is a slightly modification of a previously described protocol (Danilevich and Grishin 2002).

1. A fresh *S. pombe* colony was treated with 50 μl of lysis buffer D2 (4 M guanidine hydrothiocyanate, 50 mM Tris-HCl (pH 8.0), 5 mM EDTA, 0.1 M β-mercaptoethanol, and 0.5% (w/v) laurylsarcosine).
2. The sample was kept in boiling water for 5 min, and then centrifuged at 12000 rpm for 1 min.
3. The pellet was harvested, washed twice with 1 ml deionized water.
4. Finally, the pellet was dissolved in 20 μl water and 2-4 μl of the resuspended pellet was used for 25 μl PCR reaction.

2.2.8.2 Cloning of PCR fragments

1. Phusion DNA polymerase (NEB) was used to amplify the DNA fragments from yeast genomic DNA.

2. 20 ng of *S. pombe* genomic DNA was used as template in 50 μl PCR reactions which contained 1×HF buffer, dNTP mixture (0.2 mM for each), 2 μM primers and 1 U Phusion DNA polymerase.
3. The PCR amplification was as follow: 98°C denaturation for 1 min; 98°C for 5 sec., 55°C annealing for 20 sec, 72°C extension for 0.5 min/kb and running for 30 or 35 cycles; final 72°C extension for 5 min.

2.2.9 Reverse transcription of RNA

1. Reverse transcription was performed on 1 μg of total RNA using random hexamer primer and SuperScript III reverse transcriptase (Invitrogen).
2. The reverse transcription process was followed as described in the manufacturer's instructions. 1 μl of reverse transcription product (20 μl) was used in PCR amplification by centromere, telomere and gene specific primers.

2.2.10 Agarose gel electrophoresis of DNA

PCR or restriction enzyme digestion of DNA samples was run in the agarose gels to confirm and separate the correct bands by molecular weight. DNA samples and the loading control were mixed with DNA loading buffer (10× stock, 20% (v/v) glycerol, 0.1 M EDTA, pH 8.0, 1.0% (w/v) SDS, 0.25% (w/v) bromphenol blue and 0.25% (w/v) xylene cyanol), loaded onto the 0.8% -2% (w/v) horizontal agarose gel and run in TAE buffer (40 mM Tris base, 40 mM acetic acid and 2 mM EDTA) with 0.5 μg/ml ethidium bromide at a constant voltage of 80 V. The 1 kb DNA ladder and the 100 bp DNA ladder were used as the loading control (NEB). The DNA sizes in descending order correspond to 10000 bp, 8000 bp, 6000 bp, 5000 bp, 4000 bp, 3000 bp, 2000 bp, 1500 bp, 1000 bp and 500 bp for 1 kb ladder; 1500 bp, 1200 bp, 1000 bp, 900 bp,

800 bp, 700 bp, 600 bp, 500 bp, 400 bp, 300 bp, 200 bp and 100 bp for 100 bp DNA ladder respectively.

2.2.11 DNA sequencing

Sequencing of DNA samples was carried out by GATC biotech (Germany).

2.3 Plasmids construction

The plasmids used in this study are derived from pART1 (McLeod et al. 1987). pART1 is a *S. pombe E. coli* shuttle vector, derived from pUC118. It carries ars sequence (Losson and Lacroute 1983), the *LEU2* gene of *S. cerevisiae* (Beggs 1978) as selection marker which was used to screen the transformants by complementation the leucine auxotroph of *leu1d32* or *leu1*Δ strains and the constitutively expressed promoter of the fission yeast alcohol dehydrogenase gene (*ADH*) (Russell 1983). All of the primers used in the cloning are listed in table 2.1.

2.4 The recipe for E. coli growth media

2.4.1 LB broth

Dissolve 10 g Bacto-tryptone, 5 g yeast extract, and 10 g NaCl in 800 ml dH_2O. Adjust pH to 7.5 with NaOH, bring the volume to 1 L with dH_2O, transfer to a bottle and sterilize it by autoclaving at 121°C for 15 min. Add antibiotics to cold media at the required concentration just before using it.

2.4.2 Agar-LB plates

Dissolve 10 g Bacto-tryptone, 5 g yeast extract, 10 g NaCl and 10 g agar are dissolved in 800 ml of dH_2O. Adjust pH to 7.5 with NaOH, bring the volume to 1 L with dH_2O, transfer to a bottle and sterilize it by autoclaving at 121°C for 15 min. Cool the media to about 60°C, add the required antibiotic (for example, 1 ml of 100 mg/ml ampincillin) and pour 25-30 ml/plate (9 cm Petri dishes). Plates can be stored at 4°C.

2.4.3 SOC

Dissolve 20 g Bacto-tryptone, 5 g yeast extract, 0.6 g NaCl and 0.18 g KCl in 970 ml of dH_2O and sterilize by autoclaving at 121°C for 15 min (before autoclaving, the media can be aliquoted into 20 ml glass bottles for convenience). Before using it, add the following (for 20 ml aliquot): 200 µl of 1 M $MgCl_2$, 200 µl of 1 M $MgSO_4$ and 240 µl of 30% glucose.

2.5 *S. pombe*-specific methods

2.5.1 Strains

The *S. pombe* strains used in the project are shown in table 2.2. All strains were stored at -80°C in YES or EMM media plus 40% (w/v) sterile glycerol. Deletion strains were validated by PCR of genomic DNA, using the appropriate primer pair table 2.1.

2.5.2 *S. pombe* media and culturing

S. pombe cells were cultured in YES or EMM medium. The recipes of these media are shown in section 2.6. For cultures on Petri dishes, cells were streaked on YES or EMM plates and incubated at 37°C, 30°C and 25°C for 4-5 days.

1. Liquid cultures were typically inoculated with a single colony from streaked plate. First in small start-up cultures, 1-2 ml culture was grown overnight at 30°C or 25°C (ts strains) in shaking incubator.
2. Fresh small cultures were then used to inoculate 10-50 ml (1 to 50 dilution) and grown overnight.

2.5.3 *S. pombe* synchronization protocol

One of the most popular block-release methods is based on strains in which the key cell cycle protein CDC25 is mutated (*cdc25-22*) such that it is dysfunctional at high temperature (Russell

and Nurse 1986). Raising the temperature of such a culture thus arrests all cells at a specific stage in the cell cycle. A synchronized culture can then be obtained by lowering the temperature, whereby the protein is reactivated and the cells progress into the cell cycle from the same point (Park et al. 1995).

1. First in small start-up culture, 1-2 ml culture of *cdc25-22* was grown overnight at 25°C in shaking air incubator.
2. Next day *cdc25-22* was grown in 50 ml YES media in a 500 ml flask at 25°C to 2 x 10^6 cells/ml, three separate culture flasks started together for different cell cycle stages- G1/M, S and G2.
3. The cultures were transferred to a 37°C shaking air incubator for 3.5 hr to arrest.
4. 2 µl of the cultures were examined under the microscope for cell cycle arrest at 3 and 3.5 hr, more than 80% cells should be elongated and stuck in G2 phase after 3.5 hr.
5. Cell number was counted with a hemocytometer to 2 x 10^6 cells/ ml. One culture was fixed with formaldehyde for ChIP at G2 (for formaldehyde fixation process see section 2.5.12).
6. Other two cell cultures for G1/M and S phase were released from the *cdc25-22* arrest by transferring the flasks to a 25°C shaking air incubator.
7. Second culture was removed from 25°C incubator at 30 min after release and fixed with formaldehyde for ChIP at G1; third culture was removed from 25°C incubator at 60 min after release and fixed with formaldehyde for ChIP at S phase. Cell cycle synchrony was monitored every time by visualization under microscope before fixing the cells. Typically more than 80% of the cells should be in a specific cell cycle.

2.5.4 *S. pombe* DNA transformation

The strain to be transformed was cultured in 5-10 ml YES until the cell density reached 0.8-1.0 at OD_{650} (1×10^7 measured by haemocytometer). Cells were made competent for transformation using the LiAc method described below (Forsburg and Rhind 2006).

1. The cell culture was centrifuged at 3000 rpm for 5 min and the pellet was washed once with 10 ml of sterile water.
2. The cells were centrifuged again in the same condition and resuspended in 100-200 μl of sterilized water. An equal volume of LiAc buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA, 0.1 M lithium acetate) was added and thoroughly mixed with the resuspended cells (cell density should be above to 5×10^8).
3. For each transformation, 100 μl of LiAc-resuspended cells was transferred into a 1.5 ml sterile microtube and kept at room temperature (lower than 28°C) for 15-30 min.
4. Then 1 μg of plasmid DNA (episomal expression) or 4 μg linerized DNA for homologue recombination (gene knock-out or, tagging), and 2 μl of 10mg/ml ssDNA were mixed with 100 μl of LiAc-treated cells. The mixture was then kept at room temperature for 20-30 min.
5. After, 220 μl of 50% PEG solution and 40 μl of LiAc buffer were added to the transformation mixture and mixed gently. The sample was kept at 30°C (25°C for ts strain) for 1 hr.
6. Then the sample was heat shocked at 42°C for 15 min; briefly centrifuged at 3000 rpm and the pellet washed with 1ml of sterile water.

7. At the end, the cells were pelleted, and resuspended in 100 μl of sterilized deionized water and spread on the appropriate selection plate.

2.5.5 Homologue recombination method in *S. pombe*

1. The plasmid pFA6a-3HA-kanMX6 (a plasmid containing *Escherichia coli* Kan^r gene which confers resistance to G418/geneticin) was used for PCR-mediated single-step tagging of chromosomal genes with the hemagglutinin epitope (HA) (Bahler et al. 1998). For C teminal tagging, the gene specific portion (60 nts) of the forward primer correspond to the C-terminal codons of the target gene, ending just upstream of stop codon and the rest portion (20 nts) specific to transformation module. Similarly the reverse primer was designed, but the gene specific portion (60 nts) included the stop codon and the rest portion (20 nts) specific to transformation module (Fig. 2.1A).
2. 35 cycles of 94ºC for 1 min, 55ºC for 1 min and 72ºC for 2.5 min were executed followed by 10 min extension at 72ºC to amplify the plasmid cassette and *S. pombe* was transformed with the PCR product.
3. Recombinants were selected on G418 plates (which inhibits the elongation step of peptide synthesis in both pro- and eukaryotes). Individual HA-tagged insertions verified by PCR with one primer specific for the gene of interest and one for the antibiotic resistance gene.

For gene deletion in *S. pombe*, the plasmid pFA6a-hygMX6 (a plasmid containing *Escherichia coli* hyg^r gene which confers resistance to hygromycin) used for PCR-mediated single-step deletion of chromosomal genes was amplified using primers specific to 5' and 3' end of the target genes (Fig. 2.1B) (Hentges et al. 2005). Individual deletions were verified by PCR with primer pairs specific to upstream sequence and the antibiotic resistance gene.

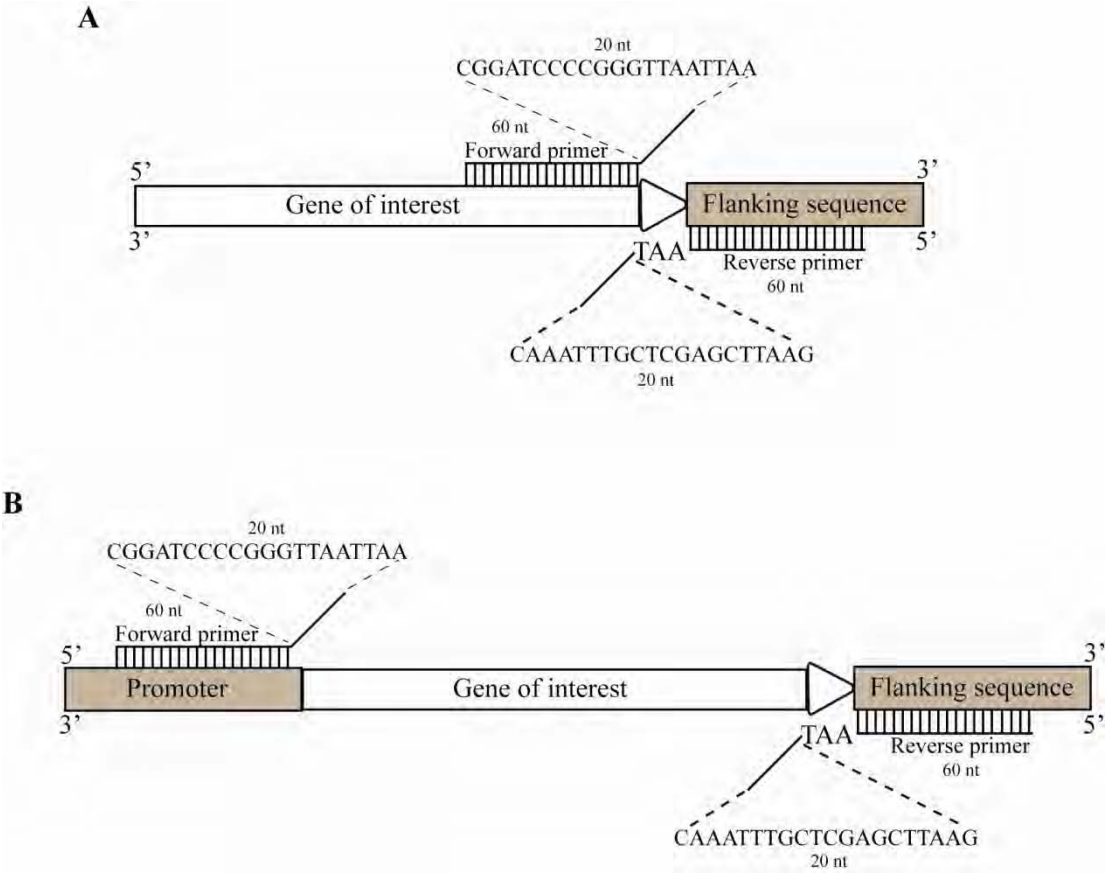


FIGURE 2.1 PCR primer design strategy for tagging or deletion of target chromosomal genes. (A) Tagging of 3' end of the gene. Primers of 80 nucleotide (nt) length were designed; forward primer- first 60 nt (5') upstream stop-codon (excluding stop) of the gene followed by 20 nt (3') complementary to the cassette which consists of a selection marker and sequences that encode for a tag (e.g. 3HA), reverse primer- first 60 nt (5') is the reverse complement of downstream of the stop-codon (including stop) of the gene followed by 20 nt (3') complementary to the cassette. White box represents gene of interest and brown box flanking sequence of the gene. (B) Deletion of a gene. Schematic as above.

2.5.6 Genomic DNA extraction

Single colonies were grown in 20ml cultures to stationary phase for 1.5-2 days, and centrifuged at 3000 rpm for 5min. The pellets were then briefly washed in sterile water and pelleted again. Finally genomic DNA was extracted using the CPES method described below.

1. The cell pellet was resuspended in 1ml of Citrate/ Phosphate buffer (50 mM citric acid and phosphate pH 5.6, 40 mM EDTA pH 8.0, and 1 M sorbitol).
2. 50 µl of Zymolyase 20T (Europa Bioproducts Ltd) was added and then the mixture was incubated at 37°C for 60 min. Cells are ready for next step if more than 50% cells show a loss in refractivity under the microscope (the change in refraction is apparent by adding 1% (w/v) SDS to the aliquot of culture to be examined).
3. Cells were pelleted at 8000 rpm and resuspended in 0.55 ml TE and 1% (w/v) SDS and incubated at 65°C for 1 hour for lysis. Then 300 µl of 3 M potassium acetate (pH 5.0) was added and the tubes were kept on ice for 15 min.
4. After the samples were centrifuged at 13000 rpm for 15 min at 4°C, 600 µl of supernatant was transferred into a fresh tube, and an equal volume of ice-cold isopropanol was mixed completely with the supernatants. The mixture was incubated at -20°C for 10 min and centrifuged at 13000 rpm for 15 min at 4°C.
5. After washed by 70% (v/v) cold ethanol, the pellet was resuspended in 300 µl TE buffer with 50 µg/ml RNase A, and incubated at 65°C for 15 min.
6. The sample was mixed with 300 µl of buffer saturated phenol (pH 7.9): chloroform: isoamyl alcohol (25:24:1), vortexed vigorously and centrifuged. The supernatant was then transferred into a 1.5 ml sterile tube. The phenol: chloroform extraction was repeated several times (typically 2 times) until the interface turned clear.

7. The aqueous phase was transferred into a fresh tube and then 30 µl of 3 M sodium acetate (pH 7.5) and 700 µl of cold absolute ethanol were added to precipitate the DNA, the tubes were kept at -20°C for 5-10 min. After precipitation to pellet the DNA tubes were centrifuged at 13000 rpm for 10 min at 4°C and supernatant was removed.
8. The pellet was finally washed with 70% (v/v) ethanol and centrifuged at 13000 rpm for 10 min. After removal of the 70% (v/v) ethanol by pipetting the pellet was air-dried. Dry pellet was dissolved in 50 µl TE and 1 µl of the solution was run in agarose gel to estimate the quality and quantity of the genomic DNA (there should be a band at proximate 20 kb).

Fermentas genomic DNA extraction kit (Fermentas, K0512) was also used for genomic DNA extraction in some experiments.

2.5.7 RNA extraction

Total-RNA was purified from either 10 ml (plasmid transformed cells) or 20 ml cultures (strains with integrated constructs). The RNA was extracted with the hot-acid phenol (pH 4.5) method, with slight modifications (Ausubel et al. 1996). Cells were centrifuged at 3000 rpm for 3 min at 4°C, washed once with 10ml of sterile water.

1. Cells were centrifuged again and the pellet was resuspended in 600 µl of TES buffer (10 mM Tris pH 7.5; 10 mM EDTA pH 8; 0.5% (w/v) SDS). Then the resuspended cells were mixed with 600 µl of acid phenol pre-heated at 65°C.
2. The sample was incubated at 65°C for 1 hr and vortexed for 10 sec every 10 min. At the end the sample was cooled on ice briefly.

3. The sample was centrifuged at 13000 rpm for 20 min at 4°C; 550 μl of the aqueous phase was transferred into a clean sterile tube and mixed (by vortexing) with 500 μl acid phenol:chloroform:isoamyl alcohol (25:24:1) .
4. The mixture was centrifuged at 13000 rpm for 15 min at 4°C and 500 μl of the aqueous phase transferred into a clean sterile tube. The aqueous phase was re-extracted with 500 μl chloroform: isoamyl alcohol (24:1) as above.
5. The final aqueous phase was transferred into a nuclease free sterilized tube and the RNA precipitated by adding 50 μl of 3 M NaAc (pH 5.2) and 1.2 ml of pre-chilled 100% ethanol.
6. The tube was kept at -20°C for 20 min, and then centrifuged at 13000 rpm for 15 min at 4°C; the pellet was washed once with 70% (v/v) ethanol, dried and dissolved in 50 μl DEPC-treated water.

2.5.8 Protein extraction from *S. pombe* cells

Two different methods were used to extract proteins from yeast cells. One method makes use of glass beads.

1. A 5-15 ml aliquot of a fresh cell culture (cell number > 10^8) was centrifuged at 3000 rpm for 5 min, and the pellet was washed with 1.2 ml of cold distilled water, and then transferred into a 1.5 ml fresh screw cap tube and centrifuged again.
2. The pellets were resuspended in 200 μl of protein lysis buffer [50 mM Tris, pH 7.5, 150 mM NaCl, 5 mM EDTA, 10% (v/v) glycerol, 1 mM phenylmethylsulphonyl-fluoride (PMSF)] and about 400 μl of 0.5 mm acid-washed glass beads (Sigma) were added into each tube.

3. Each sample was vortexed at 5500 rpm for 15 seconds in Precellys 24 homogenizer (Bertin Technologies) and kept at 4°C for 2 min. The process was repeated 2-3 times until more than 70% of cells were broken as observed under the microscope.

4. To recover the cell lysate, the bottom of the tube was pierced with a needle (the needle can be heated over a flame to facilitate piercing). The punctured tube was placed in a fresh 1.5 ml tube and centrifuged at 13000 rpm for 2 min at 4°C. The accumulated cell lysate present in the fresh 1.5 ml tube was further centrifuged at 13000 rpm for 10 min at 4°C; supernatant was transferred to a new 1.5 ml tube.

5. 30 µl of supernatant was taken and mixed with 30µl of 2× SDS loading buffer (120 mM Tris-HCl, pH 6.8, 8% (w/v) SDS, 8% (w/v) β-mercapto-ethanol, 25 mM EDTA, 0.04% (w/v) bromophenol blue, 20% glycerol). The extract was boiled for 5 min and either loaded on a SDS-PAGE gel or stored at -20°C for future use.

The second protein extraction method involves sodium hydroxide mediated lysis of the cells (Matsuo et al. 2006).

1. A 2-5 ml aliquot of cell culture was centrifuged down and washed once with 0.5 ml of water. The pellet was completely resuspended in 300 µl water, and then 130 µl of 1 M NaOH was added and into the sample and mixed gently by flipping the tube. The tube was incubated at room temperature for 10 min.

2. The sample was then centrifuged at 8000 rpm for 3 min at 4^{o}C and the supernatant was fully removed.

3. 50-100 µl of 1×SDS loading buffer was used to resuspend the cells completely, and then boiled for 5 min and centrifuged at 13000 rpm for 2 min. Typically 20 µl of the sample were loaded on the gel or stored at -20^{o}C for future use.

2.5.9 SDS-PAGE and Western blot

SDS-PAGE gels (10%) were prepared as described in Molecular Cloning 2nd edition [Molecular cloning: a laboratory manual / J. Sambrook, E.F. Fritsch, T. Maniatis (1989)].

1. SDS-PAGE gels were run at 120 V for 60-100 min at room temperature by using 1× running buffer (10× stock buffer: 0.25 M Tris-HCl, 1.92 M Glycine, 1.0% (w/v) SDS, pH 8.3).
2. The proteins were transferred onto nitrocellulose membranes (Schleicher & Schuell) using Mini PROTEAN II electrophoresis chamber (BIORAD) at 100 V for 60-90 min in cold room in transfer buffer (25 mM Tris-HCl, 190 mM Glycine, 20% (v/v) Methanol).
3. Membranes were blocked with 30 ml of 5% (w/v) skimmed milk-TBS (25 mM Tris-HCl, 137 mM NaCl) at room temperature for 1-2 hr and then washed with TBS 0.1% (w/v) Tween20 (TBST) three times, each for 10 min.
4. The primary antibody was diluted 1:3000 directly in TBST and incubated with the membrane overnight in the cold room. The following day, the membrane was washed with TBST three times, each for 10 min.
5. The secondary antibody, horseradish peroxidase (HRP) conjugated anti-mouse IgG (Sigma), was diluted 1:10000 in TBST and incubated with the membrane for 40-60 min. The membrane was washed with TBST three times, each for 10 min.
6. Supersignal West Pico Chemiluminescent Substrate kit (PIERCE) [contains an enhanced chemilumescent (ECL) substrate for HRP] was used to detect our target protein. 0.5 ml of Supersignal WestPico Luminol/Enhance solution was mixed with 0.5 ml Stable Peroxide solution and the mix spread uniformly onto the membrane and incubated for 5 min. The solution was drained away and the membrane wrapped in cling

film. The signal was detected with X-ray films or visualized with a ChemiDoc imaging system (Bio-Rad).

2.5.10 Polysome profile

2.5.10.1 Preparation of sucrose gradients

Tubes with 10-50% (w/v) sucrose gradients were prepared with DEPC-treated water and polysome buffer (100 mM Tris acetate pH 7.4, 700 mM ammonium acetate, 40 mM magnesium acetate). Sucrose solutions of 10% and 50% were prepared; polysome gradient was prepared using gradient maker (SG15, Hoefer) into SW41 polyallomer centrifuge tubes (Beckman Coulter).

2.5.10.2 Cell extracts preparation

1. *S. pombe* cell culture (50 ml) was grown at 30^{o}C until OD_{650} 0.3-0.4, mixed with 500 μl of 10 mg/ml cycloheximide and incubated for further 15 min. The following steps were all performed on ice or in a cold room.
2. After the cycloheximide treatment, the cells were harvested by centrifuging at 3000 rpm for 10 min and washed by 10 ml of lysis buffer (20 mM HEPES pH 7.4, 2 mM magnesium acetate, 100 mM potassium acetate, 100 μg/ml cycloheximide, 0.5 mM dithiothreitol).
3. The cells were pelleted again and resuspended in 300μl of lysis buffer with RNase inhibitor (40 U/ml, Invitrogen) and protease inhibitor cocktail tablet, EDTA free (Roche, 1/4th of the tablet in 10 ml of lysis buffer). The suspension was transferred into a fresh 2 ml screw-cap tube and filled with acid-washed glass beads (0.5 mm) until the beads reached the meniscus of the solution.

4. Cells were lysed by vortexing for three rounds of 15 seconds at 5500 rpm in Precellys 24 homogenizer (Bertin Technologies) with 2 min cooling in between. Typically more than 70% of cells were broken after 2-3 rounds. An additional 300 µl of lysis buffer with 40 U/ml RNase inhibitor and protease inhibitor cocktail was added to dilute the sample.

5. The 2 ml screw-cap tube was pierced at the bottom with a needle, placed through a hole cut through the cap of a 15 ml screw cap tube, and centrifuged at 5000 rpm for 5 min at 4°C to recover the cell lysate.

6. The lysate was transferred into a fresh 1.5 ml tube and cleared by centrifugation at 13000 rpm for 15 min at 4°C. The supernatant was transferred into a fresh 1.5 ml tube and kept on ice.

7. A 1:10 dilution of the extract was used to measure the absorbance at 260 nm with a NanoDrop spectrometer (ND-1000, NanoDrop). The absorbance at 260 nm is proportional to the RNA concentration and therefore can be used to normalize loading of the polysome extracts.

2.5.10.3 Sucrose gradient separation

1. The polysome extracts were adjusted to 10 Abs_{260} units/ml and carefully loaded onto the top of the 10-50% sucrose gradients. The tubes were carefully balanced, loaded into the buckets of a Beckman SW41 rotor and centrifuged at 38000 rpm for 160 min.

2. The gradients were pumped (from the bottom, using a steel capillary) through a flow-through UV spectrophotometer (Pharmacia LKB-Optical Unit UV-1) by using a peristaltic pump (P-1, Pharmacia) with the speed at 1.2 ml/min. The absorbance was measured at 254 nm and recorded on chart recorder (Pharmacia LKB REC 102, speed

setting- 1 cm/min). Fractions (0.8 ml per tube, 15 fractions) were collected with a fraction collector (FRAC100, Pharmacia).

2.5.10.4 RNA and protein purification from sucrose fractions

1. To extract the RNA from the fractions, the acid phenol (pH 4.5): chloroform (5:1) method was adopted. A 400 μl aliquot of every fraction was mixed with 600μl of phenol: chloroform and centrifuged at 13000 rpm at 4°C for 10 min.
2. The aqueous phase was transferred into a new eppendorf tube and extracted with 400 μl of chloroform: isoamyl alcohol (24:1) and centrifuged at 13000 rpm at 4°C for 10 min.
3. The aqueous phase was transferred into a new eppendorf tube, and then mixed with 40 μl of 3 M NaAc (pH 5.2) plus 1 ml of 100% ethanol (pre-chilled) and kept at -20°C for 30 min.
4. RNA was pelleted by centrifugation of the sample at 13000 rpm for 15 min. Finally RNA was washed once with 70% (v/v) ethanol (RNase free) and pelleted again by centrifugation at 13000 rpm for 10 min. The RNA sample was then dried and dissolved in 20 μl of DEPC water with 60% (v/v) of formamide for future use.
5. To precipitate the proteins from the fractions, a 400 μl aliquot was mixed with 70 μl of 100% (w/v) trichloroacetic acid and kept at 4°C overnight.
6. The sample was centrifuged at 13000 rpm for 20 min to pellet proteins from the sucrose solution. The pellet was washed with 300 μl of acetone three times, dried in a heat block at 95°C, resuspended in 30 μl of 1×SDS loading buffer and boiled for 5 min. Protein samples were run in 10% SDS-PAGE and analyzed by Western blot.

2.5.11 4'-6-Diamidino-2-phenylindole (DAPI) staining of *S. pombe* cells

4'-6-Diamidino-2-phenylindole (DAPI) is known to form fluorescent complexes with natural double-stranded DNA, showing fluorescence specificity for AT, AU and IC clusters. Because of this property DAPI is a useful tool in various cytochemical investigations. There is also evidence that DAPI binds to the minor groove, stabilized by hydrogen bonds beween DAPI and acceptor groups of AT, AU and IC base pairs (Kubista et al. 1987; Barcellona et al. 1990). The DAPI staining method I adopted here enabled me detection of nucleus, cell wall, and septum of *S. pombe* cells.

1. A 2.0 ml cell culture was grown overnight and pelleted in a microfuge tube by centrifugation at 3000 rpm for 5 min.
2. Pelleted cells were washed by resuspending the pellet in ice-cold 1xPBS once and pelleted by centrifugation at 3000 rpm for 5 min, supernatant was removed by pipetting.
3. Cells were again suspended in 100 µl of 1xPBS and pelleted by centrifugation as above and kept on ice.
4. 1µl of cells (from the bottom) and 1µl of DAPI (50 µg/ml) were mixed on a glass slide (in the microscope room) and a cover slip was placed (22x22) over the cells; micropictures were taken within 30 min with a epifluorescence microscope.

2.5.12 Chromatin immunoprecipitation

2.5.12.1 Crosslinking and sonication

1. 2 ml pre-culture of tested strain was grown over night. Next day, appropriate amount of over night grown pre-culture was added to 100 ml of YES media, the volume of cells inoculated should be such that the culture reaches the desired cell density next morning.

2. To the 100 ml culture, 10 ml of 11% (v/v) formaldehyde (freshly made from commercial 37% solution) was added so that the final formaldehyde concentration was 1%. 11% solution was made by adding 7.5ml of 37% formaldehyde into 17.5 ml diluent (final concentration 0. 0.143 M NaCl, 1.43 mM EDTA and 71.43 mM HEPES-KOH, pH 7.5). Incubated 20 min at RT, every 5 min the culture was swirled briefly (For RNase treatment only 5 min fixation was done).

3. 15 ml of 3 M Glycine, 20 mM Tris added and incubated for another 5 min.

4. Cells were pelleted in plastic Nalgene bottles (500 ml) in the J2-MC Beckman centrifuge (3000 rpm; 10 min), washed 2 times with 200 ml cold TBS (20 mM Tris-HCl, pH 7.5, 150 mM NaCl), once with 10 ml ice cold FA lysis buffer [50 mM Hepes-KOH (pH 7.5), 150 mM NaCl, 1 mM EDTA, 0.4 ml 0.5M EDTA, 1% Triton X-100,0.1% Na Deoxycholate] with 0.1% (w/v) SDS then transferred to a 14 ml Falcon tube. At this point pelleted cells can be frozen at –80°C. Quick freeze in liquid nitrogen first.

5. Pellet was resuspended in 0.5 ml ice cold FA lysis buffer/0.1% (w/v) SDS plus PMSF/Protease inhibitor cocktail [25 µl of 0.2 M PMSF and half tablet of protease inhibitor cocktail (Roche) was added to 5 ml buffer before using].

6. 1.5 ml small glass beads was added and vortexed in 2 ml screw capped plastic tube in the Precellys cell lysis machine at maximum setting for 2 min in the cold room.

7. Bottom of the screw capped tube was punctured with a 22 gauge 1/2 inch needle and inserted into a plastic 15 ml falcon tube. Lystate was collected by centrifugation at 1000 rpm for 2 min. 3 ml ice cold FA lysis buffer/0.1% SDS (w/v) was added to the glass bead containing tube to wash the remaining lysate out.

8. Lysate was transferred to two 2 ml eppendorf tubes and spinned in a refrigerated microfuge at 15000 rpm for 10 min.
9. Supernatant was removed and resuspended in 2 ml FA Lysis buffer/0.1% (w/v) SDS and respined at 15000 rpm for 10 min, step was repeated again.
10. Supernatant was decanted, each pellet was resuspended in 0.75 ml of ice cold FA lysis buffer/ 0.1% (w/v) SDS, combined like samples and transferred to a 2 ml plastic screw capped tube.
11. Sonicated in the CellBio sonicator—6 pulses, 20" on, 20"off at setting 2.5. This gives fragments between 500-1000 bp.
12. Transferred to 0.75 ml of each sample to two 2 ml eppendorf tubes. 0.5 ml of ice cold FA lysis buffer/0.1% (w/v) SDS was added to each tube and centrifuged in the refrigerated microfuge at 15000 rpm for 10 min.
13. Sheared chromatin (100-1000bp pieces) was in the supernatant which was aliquoted in 800µl and stored at -80°C.
14. To test chromatin sonication yield and quality, 100 µl of the chromatin supernatant was taken and de-crosslinking was performed.

2.5.12.2 Immunoprecipitation

1. Chromatin solution was thawed and 4 M NaCl (Filter sterilized; this is critical) was added until the final concentration is 275 mM NaCl; this corresponds to 25 µl of 4 M NaCl for 800 µl of chromatin.
2. 25 µl of protein A beads + 25 µl of protein G beads was pre-washed per reaction with 1 ml FA Lysis buffer/0.1% (w/v) SDS at RT for 4 min on a rotator. 75 µl of anti-HA antibody (1 µg/µl, CRUK) [for Pol II 10µl, 7.5 µg/µl, Covance] was added to the beads.

125 µl of TE pH 8.0 was added and rotated at 4°C for 45 min. Washed in 1 ml TE and then added 750 µl of chromatin (Rest was saved as an INPUT sample).

3. Incubated on rotator for overnight at 4°C (some protocols do it for 90 min at room temp).

4. Beads were pelleted next day and washed once with 1.4 ml of each of the following:

A. WASH BUFFER- 1
FA lysis buffer/ 0.1% (w/v) SDS/ 275 mM NaCl 4 min at RT on rotator
(Add 312 µl of 4 M NaCl to 10 ml 0.1% (w/v) SDS/ FA Buffer)
B. WASH BUFFER- 2
FA lysis buffer/ 0.1% (w/v) SDS/ 500 mM NaCl 4 min at RT on rotator
(Add 875 µl 4 M NaCl to 10 ml 0.1% (w/v) SDS/ FA Buffer)
C. WASH BUFFER- 3
(10mM Tris-HCl, pH 8.0, 0.25 mM LiCl, 4 min at RT on rotator
1 mM EDTA, 0.5% NP-40, 0.5% DOC)
D. TE (10 mM Tris-HCl, pH 8.0, 1 mM EDTA) 4 min at RT on rotator

* For the RNase sensitivity test, samples were treated with 7.5 U of RNase A (Sigma) and 300 U of RNase T1 (Sigma) for 30 min (Abruzzi et al. 2004). To improve RNase activity, the sheered chromatin sample was purified by centrifugal filtration before RNase treatment using an YM-10 Microcon cartridge (Millipore); this step removes SDS and other chemicals that block RNase enzymes.

2.5.12.3 Removal of proteins from cross-linked material

1. To reverse crosslinks, immunoprecipitated DNA was heated for 10min at 65°C in Elution Buffer (250 µl of 50 mM Tris-HCl, pH 7.5, 10 mM EDTA, and 1% SDS).

2. Beads were pelleted and supernatant was transferred to a fresh tube then beads were washed with 250 µl TE and added that to the supernatant.

3. To remove protein from the immunoprecipitated DNA 15 µl of 20 mg/ml Pronase (Sigma) was added per 250 µl of solution and incubated for 1 hour at 42°C, then 4-5 hours at 65°C.
4. 50 µl of 4 M LiCl was added.
5. Chromatin was extracted with 400 µl Phenol-Chloroform-Isoamyl alcohol and then with 300 µl choloroform. In each case vortexed well and spined for 6 min.
6. DNA was precipitated by adding 1 µl of 20 mg/ml Glycogen, 1 ml 100% EtOH. Incubate for 1 hour or O/N at -20°C.
7. Resuspended in 30 µl of TE.
8. Treated with RNase and ran on a 1.0% (w/v) agarose gel.

2.5.13 ChIP optimization with *S. cerevisiae*

There are four critical steps which must be optimised in the ChIP procedure: sonication, immunoprecipitation, primer pairs and PCR conditions.

2.5.13.1 Sonication condition generates chromatin fragments with an average size of 500bp to 1kb

The greatest advantage of formaldehyde crosslinked chromatin immunoprecipitation (X-ChIP) is the ability to assign the location of the target protein to a specific site on the DNA *in vivo*. The resolution of X-ChIP is ultimately determined by the size of the sonicated DNA fragment which is co-immunoprecipitated. Therefore, it is essential to shear the DNA prior to the IP to generate sufficient small fragments: 0.5-1.0 kb. Recent experiments show that the shorter the fragment size the better the immunoprecipitation efficiency and the resolution of the protein binding site (Fan et al. 2008). The specific sonication conditions depend on the sonicator and the size of the tip being used (see above).

First the sonication conditions were optimised using *S. cerevisiae* chromatin preps and a MSE Soniprep 150 machine (Sanyo Gallenkamp plc.) with 'Exponential microprobe' and a pulse of 20 seconds on and 20 seconds off, three times, produce DNA fragments ranging in the size of 0.5-1.0 kb as visualized by agarose gel electrophoresis and ethidium bromide (EtBr) staining (Fig. 2.2A).

2.5.13.2 Antibodies can immunoprecipitate HA-tagged proteins efficiently

The success of any chromatin immunoprecipitation procedure ultimately depends on the reactivity and specificity of the antibody used to bind the protein of interest. Two approaches are generally used: firstly, using antibodies against native proteins and secondly, tagging the endogenous proteins with epitope tags (eg. HA). With the advancement of rapid gene tagging techniques in yeast it has rapidly accelerated the application of ChIP since any protein can theoretically be studied quickly. Moreover a standard set of antibodies and tags can be developed in such a way that once optimized numerous target proteins can be assayed quickly. The major drawback of endogenous tagging of any protein is that it might alter the function of the target protein. So it is critical to ensure that when employing the tagging method, it does not interfere substantially with the functional characteristics of the protein. It is also very important to optimize the amount of antibody used for immunoprecipitation.

To optimise the ChIP assay in our laboratory, initially *S. cerevisiae* expressing Yra1-HA (a gift from Dr. Michael Rosbash) was used as a positive control (Abruzzi et al. 2004). Yra1 is a nuclear protein required for the export of mRNAs containing a polyA tail from the nucleus to the cytoplasm. Yra1-HA was used as a positive control. Previous ChIP experiments show that Yra1 associates with transcriptionally active genes during transcriptional elongation (Abruzzi et al. 2004). We tested two primer sets specific to promoter and coding region of the *PMA1* gene, and a pair of primers to an intergenic region where Yra1 does not bind (Abruzzi et al. 2004).

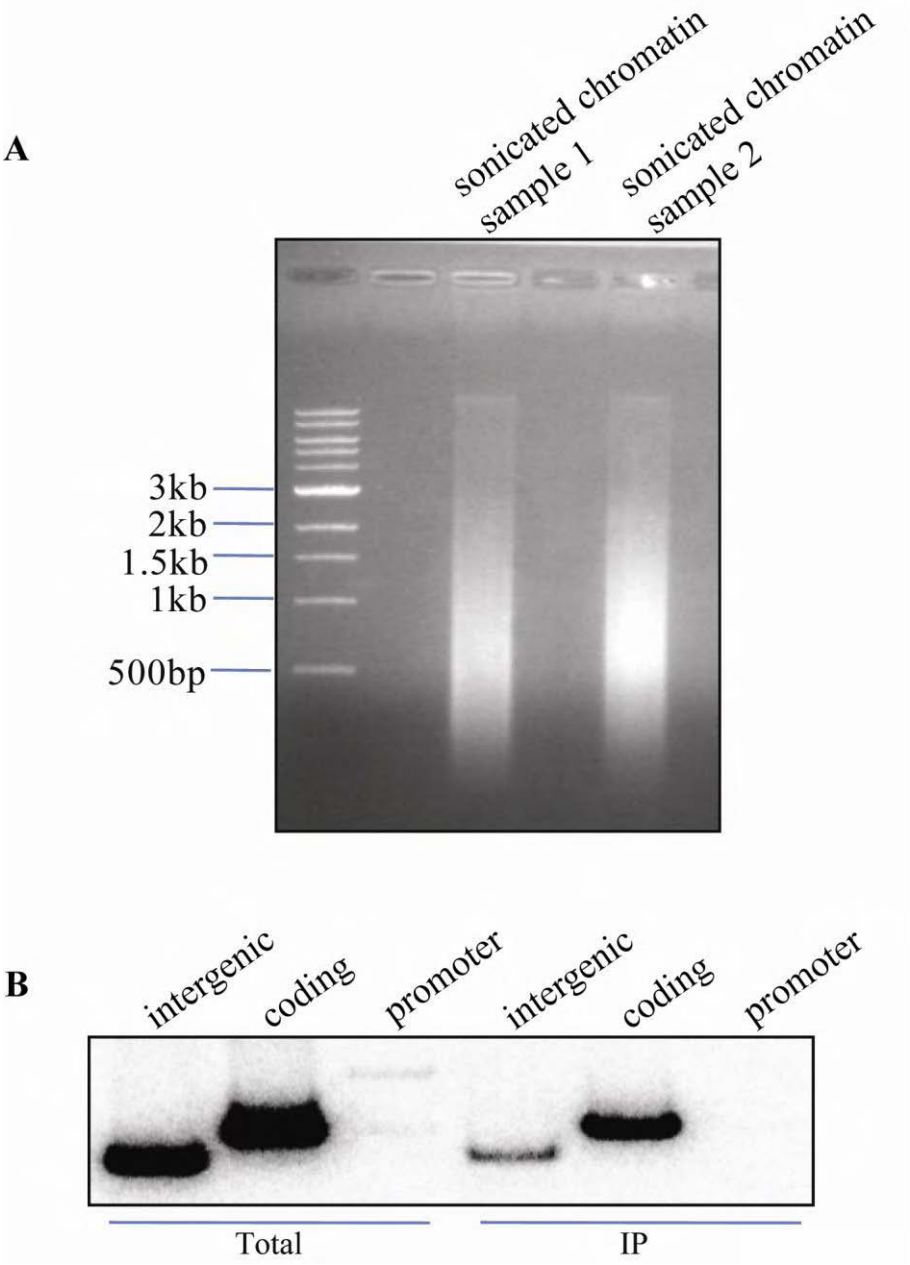


FIGURE 2.2. ChIP standardization in *S. cerevisiae*
(A) Duplicate sonicated *S. cerevisiae* chromatin samples (lane 1, 2) using a three pulses of 20 sec. on and 20 sec. off (MSE Soniprep 150); 1µg DNA was separeted on a 1% agarose gel and stained with ethidium bromide. (B) XChIP was performed from the cross-linked sonicated chromatin sample using anti-HA antibody against YRA1-HA and immunoprecipitated chromatin sample was amplified for the *PMA1* gene in presence of ^{32}P radioactive isotope and was analyzed using 10% polyacrylamide gel. Total- input DNA sample, IP- immunoprecipitated sample.

As expected following PCR of the specific immunoprecipitated Yra1 sample we found Yra1 is bound in the coding region of *PMA1* gene but did not bind at the promoter (Fig. 2.2B).

2.5.13.3 Multiplex PCR analysis

After the purification of immunoprecipitated DNA, the enrichment of immunoprecipitated DNA can be analyzed in number of ways. For the analysis of immunoprecipitated DNA a PCR based strategy was used. To analyze the ChIP DNA by standard PCR, it is very important to select primer pairs that amplify their target sequences efficiently and should be tested on genomic DNA before performing any experiment. Optimum fragment size for amplification following immunoprecipitation is around 200-300 bp. If the specific binding site of the protein is known then the primers can be designed targeting this site and if the binding site is unknown then it is useful to amplify a series of target sites to empirically determine the DNA fragment that gives the most robust ChIP signal.

The most common approach that economizes the use of limiting ChIP DNA is to use multiplex PCR in which the reference and the gene specific primers (which have same melting temperature and have a slightly different amplification size) are amplified at the same time so that the two fragments can be identified together. Preliminary experiments can be performed using genomic DNA sample. It is very important in any ChIP experiment that the PCR condition is quantitative. In practice it is difficult to balance linearity (which decreases with PCR cycle number) with signal intensity (which increases with PCR cycle number).

The most useful way to determine whether the ChIP procedure is working is to design a ChIP experiment with a robust positive control. Here we used a commercially available monoclonal antibody against RNA polymerase II (8WG16: Covance) that consistently performs well in ChIP and yields a strong signal with most trancriptionally active mRNA encoding gene. We also used small subunit of nuclear cap binding protein complex (Cbp20) as a positive control

which binds to chromatin via RNA (Komarnitsky et al. 2000). To prevent the problems of antibody cross reactivity, a no-antibody control was used. Finally it is crucial to include appropriate controls for analysis of co-immunoprecipitated DNAs. Even if the antibody used is highly specific, due to non-specific binding to the antibody, to the sepharose beads or to the plastic of the eppendorf tubes, inappropriate DNA molecules may be brought down during the ChIP assay. To control for non-specific DNA recovery, we included in our PCR analysis a set of primers designed to amplify a DNA fragment at which the protein of interest is not expected to bind.

2.5.14 Bulk amplification of immunoprecipitated DNA (for microarray hybridization)

The amount of DNA recovered by immunoprecipitation is generally not sufficient for labelling and hybridization to DNA microarrays. Thus, a DNA amplification procedure by PCR is required for bulk production of ChIP DNA. The approach adopted here involves a two steps amplification in which in the first step, two rounds of denaturation–annealing–elongation are performed with primers with degenerate 3' ends and Sequenase 2.0 DNA polymerase (USB Corporation) (Robyr and Grunstein 2003). The second step of the DNA amplification procedure is a standard PCR with primers corresponding to the fixed flanking sequence of the primers used in the first step. A relatively small number of PCR cycles were used (25 cycles) to ensure amplification linearity. The PCR product was subsequently purified with columns (QIAquick PCR Purification Kit, Qiagen Inc.) or, PEG method. This purification step is crucial since the presence of non-incorporated oligonucleotides and dNTPs interferes with the labelling reaction described below. Due to the random nature of the amplification procedure, the average size of DNA is reduced from about 500-1000 bp after sonication down to 200-500 bp as tested on 1.0% (w/v) agarose gel (Fig. 2.3A).

Maintenance of immunoprecipitation enrichment post-amplification is crucial for obtaining

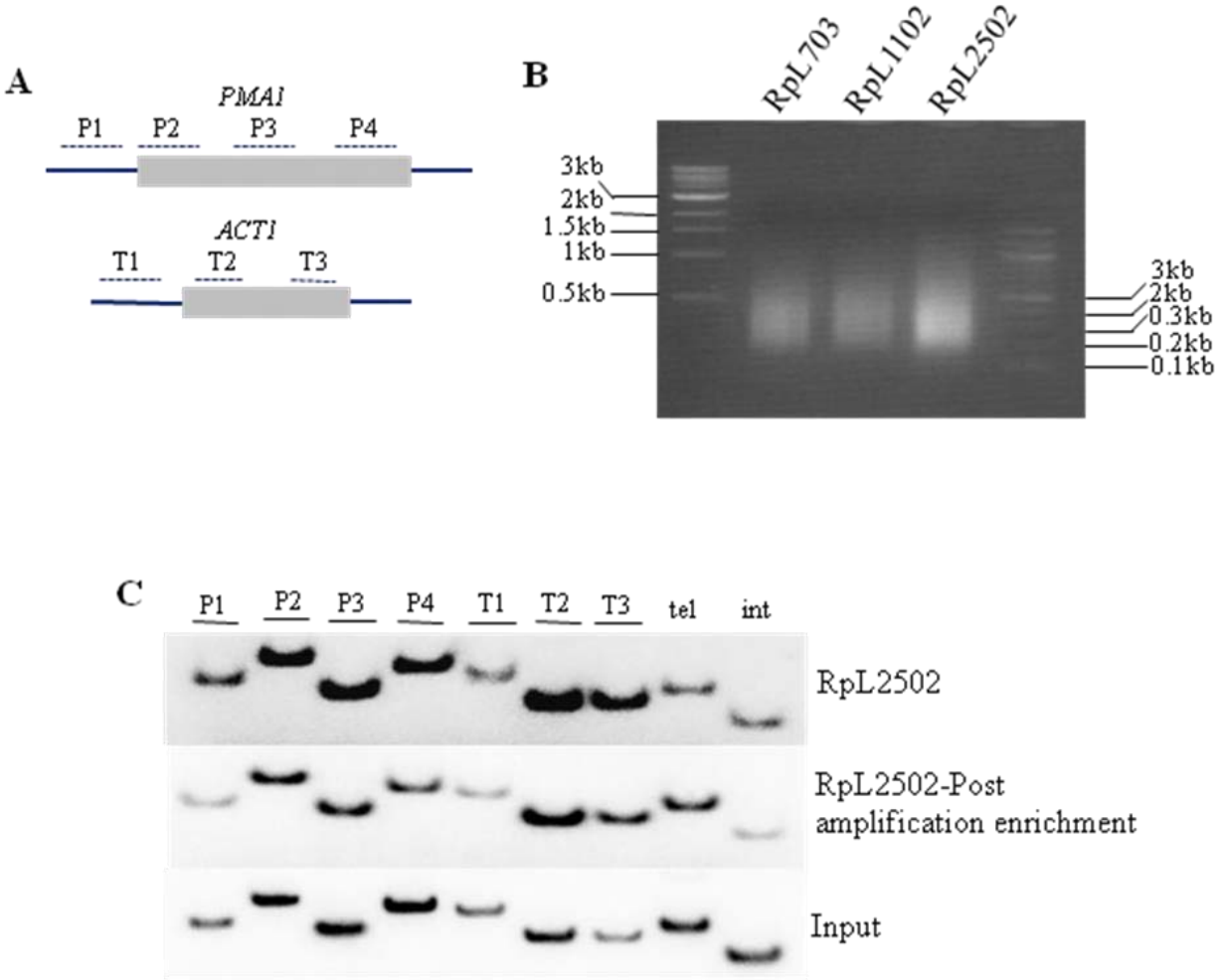


FIGURE 2.3 Post-amplification maintenance of immunoprecipitation enrichment is achieved. (A) Schematic diagram of the *PMA1* and *ACT1* gene, grey bars represent the gene ORF; the PCR amplicons are indicated by dotted lines above (numbers correspond to the primer position relative to start codon) **(**B) 1% Agarose gel showing post-amplified DNA fragments, strain names are indicated above each lane. (C) Polyacrylamide gel with radiolabelled PCR products produced by the *PMA1*, *ACT1*, telomere (tel) and intergenic region (int)-specific primer pairs, using input DNA before ChIP, DNA after ChIP and post-amplification ChIPed DNA from RpL2502-HA strain. Name of the primers are indicated above each lane.

good array results. Radioactive or, qPCR should be performed with post-amplified samples to ensure that differences between the immunoprecipitated and control- samples are as in the initial immunoprecipitated DNA. In present experiment the same primer sets were used as for assaying the *PMA1* and *ACT1* genes. The results show the post amplification level of enrichment of immunoprecipitated sample was comparable to the original immunoprecipitated sample (Fig. 2.3B). For hybridization to probe array, amplified ChIP DNA was fragmented and labelled using the GeneChip® WT Double-Stranded DNA Terminal Labeling Kit (P/N 900812).

2.5.14.1 Round A

1) Total 7 µl DNA (conc. 1 ng/µl) was denatured in the presence of 1x Sequenase buffer and the oligo (see below) for 2 min at 94°C and incubated for 2 min at 8°C.

Reagents	**Amount**	**Concentration**
DNA template	7 µl	
5x Sequenase Buffer	2 µl	1x
40 µM oligo T-PCRA	1 µl	

2) The 5 µl of Reaction Mix containing enzyme and nucleotides was added to the denatured DNA (see below).

Reaction Mix enough for 5 round A reactions:

Reagents	**Amount**	**Concentration**
5X Sequenase Buffer	5 µl	
25 mM dNTP	0.9 µl	40 pmole
100 mM DTT	3.75 µl	
500 µg/ml BSA	7.5 µl	
Sequenase (13 U/µl)	1.5 µl	4 U
Water	6.35 µl	

Total	25 µl

3) Temperature was increased slowly from 8°C to 37°C over a period of 8 min in a thermal cycler.
4) Incubated another 8 min at 37°C.
5) Again denatured for 2 min at 94°C and incubated for 2 min at 8°C
6) Then added 1 µl Sequenase (diluted, 4 Units/µl with the dilution buffer provided with the enzyme).
7) Temperature increased slowly from 8°C to 37°C over a period of 8 min and incubated for additional 8 min as before.
8) Stop the reaction by adding 35 µl of TE 1x (Tris-HCl 10mM, pH 8.0; 1mEDTA).
9) Proceeded right away to Round B, or store at -20°C.

2.5.14.2 Round B PCR

For AFFYMETRIX PLATFORM and dUTP incorporation - a mix of 20 mM + 5 mM dUTP was used.

1) Started with 15 µl of Round A DNA and 85 µl of Round B Mix was added to it (see below).

Reaction mix for 5 round B reactions	**µl**	**Final**
Template from Round A	75	
10x PCR Buffer	50	1x
25 mM dNTP + dUTP	5	0.25 mM
364 mM oligo T-PCRB	17	1.25 nmole/reaction
25 mM MgCl2	40	2 mM

GoTaq pol (5 U/µl)	5	5 U/ reaction
Water	308	
Total	500	

2) PCR amplified, cycles: 24x (92°C 30"/40°C 30"/ 50°C 30"/72°C 1') and 72°C 10'.

3) PCR product purified (Qiagen PCR purification Kit) using PB buffer (not PBI) and eluted in 50 µl 10mM Tris pH (elution buffer from Kit). Column can take up to 10 µl of DNA.

4) 2-3 µl of the purified DNA was loaded on a 1.0 % agarose gel and the concentration measured with Nanodrop. Normally, greater than 9 µg of amplified DNA was obtained from each reaction.

Primers used: Round A- T-PCRA 5'-GTTTCCCAGTCACGATCNNNNNNNNN-3'
Round B- T-PCRB 5'-GTTTCCCAGTCACGATC-3'

2.5.15 Fragmentation and labelling of amplified DNA

Independently amplified immunoprecipitated and input DNA samples were fragmented according to the protocol provided with the GeneChip® WT Double-Stranded DNA Terminal Labeling Kit (Affymetrix, P/N 900812). The fragmentation reaction mixture contains Uracil-DNA Glycosylase (UDG) [UDG catalyses the release of free uracil from uracil-containing DNA] and apurinic/apyrimidinic (AP) endonuclease 1 (APE 1) [APE 1 cleaves the phosphodiester backbone immediately 5´ to an AP site, via hydrolytic mechanism], after treatment typically ~66 nt long DNA fragments are produced. Fragmented products were analyzed on an Agilent Bioanalyzer with the RNA 6000 Nano LabChip Kit. Analyzing fragmented DNA on the RNA 6000 LabChip is recommended because it quickly assesses the degree and uniformity of the fragmented products. DNA fragments are later labelled using

again the GeneChip® WT Double-Stranded DNA Terminal Labeling Kit (P/N 900812) as recommended by the manufacturer.

2.5.16 Hybridization and Array Processing

Fragmented and labelled DNA samples were hybridized according to the protocol described by the GeneChip® Hybridization, Wash, and Stain Kit (Affymetrix). The hybridized GeneChip® Tiling Arrays were washed and stained using GeneChip® Fluidics Station 450/250 or 400 (Affymetrix), which is operated by the GeneChip® Operating Software (GCOS) (Affymetrix).

Finally, the hybridization signal produced from tiling arrays was detected with a GeneChip® Scanner 3000 7G and .CEL files (The CEL file stores the results of the intensity calculations on the pixel values of the DAT file. This includes an intensity value, standard deviation of the intensity, the number of pixels used to calculate the intensity value, a flag to indicate an outlier as calculated by the algorithm and a user defined flag indicating the feature should be excluded from future analysis. The file stores the previously stated data for each feature on the probe array) generated.

2.5.17 Chip-on-chip analysis

We used the Model-based Analysis of Tiling Array (MAT) software (Johnson et al. 2006) for analysis of the Affymetrix hybridization data. ChIP input DNA was used as a control for the analysis. Enrichment scores were assigned to genomic positions using the *S. pombe* genome coordinates and a bpmap file for the Affymetrix array (ftp://ftp.sanger.ac.uk/pub/yeast/pombe/GFF; and *S.pombe* 8/23/07 library). Enriched regions were initially defined at different p-value thresholds; the p-value of 10^{-4} was chosen because this was the lowest p-value at which both the experimentally validated *PMA1* (all but one experiment) and *ACT1* genes were flagged as enriched. Given genes were classified as positive hits only if the enrichment was at least 50% or more of the gene sequence, therefore excluding

regions with minimal levels of enrichment. Other than the p-value, default parameters were used with the MAT software. The results of the MAT software were visualised with the Affymetrix Integrated Genome Browser (IGB). In order to test the statistical significance of the overlap between the enriched regions identified with the three proteins, a program was written which randomly sampled the observed number of enriched regions, from the total number of unique features of the *S. pombe* genome (total size of 10694 - including all genes and unknown repeat regions), for each of the proteins, and determines what is the overlap between the three samples. We never observed an overlap larger than 6 in 4.5×10^{6} simulations, implying a p-value $<10^{-6}$.

2.6 The recipe for S. pombe growth media

2.6.1 YES

Dissolve 5 g yeast extract, 225 mg each of the amino acid and nucleotides supplements (adenine, histidine, leucine, uracil and lysine hydrochloride), in 900 ml of dH2O, transfer to a bottle and sterilize by autoclaving at 121 °C for 15 minutes. After autoclaving add 30 ml of sterile 30% glucose stock). The desired antibiotic is added to media just before use (for example the 100 µg/ml G418 for the screening of knockout strain by KanMX6 cassette). Agar YES media was made by adding extra 2% agar, adding 3% glucose and desired antibiotics after autoclaving.

2.6.2 EMM without leucine

Dissolve 3 g potassium hydrogen phthalate, 2.2 g Na_2HPO_4, 5 g NH_4Cl and 225 mg of each of the amino acid and nucleotide supplements (adenine, histidine, uracil and lysine hydrochloride), in 875 ml of dH2O and then sterilized by autoclaving at 121 °C for 15 minutes. After autoclaving, to cold media add 2% glucose, 1x salt stock, vitamin stock and mineral stock

(see recipes below) (EMM agar media is made by adding 2% agar. After autoclaving, add 2% glucose.)

2.6.3 Stocks in media

1. 10x Glucose stock

300 g glucose dissolved in 1 L dH2O, autoclaved and stored at RT.

2. 50x Salt stock

52.5 g $MgCl_2.6H_2O$, 0.735 g $CaCl_2.2H_2O$, 50 g KCl and 2 g Na_2SO_4 in 1 L dH_2O. Autoclaved and stored at RT.

3. 1000x Vitamin stock

0.1 g pantothenic acid, 1 g nicotinic acid, 1 g inositol and 1 mg biotin in 100 ml dH2O. Autoclaved and stored in dark environment.

4. 10000x Mineral stock

0.5 g boric acid, 0.4 g $MnSO_4$, 0.4 g $ZnSO_4.7H_2O$, 0.2 g $FeCl_2.6H_2O$, 40 mg molybdic acid, 0.1 g KI, 40 mg $CuSO_4.5H_2O$ and 1 g citric acid in 100 ml dH_2O. Autoclaved and stored in dark environment.

5. 1000x antibiotics stocks

100 mg/ml ampicillin and 50 mg/ml kanamycin for LB and 200 mg/L G418 and 100 mg/L hygromycin for YES. Filtration steriled and stored in -20°C.

Table 2.1- Primers used in the study-

Primers for Result I

RpL7	RpL7-F	GAGGGTCGAAAGGCTGGCTATTGTGGAGAAGAAATTAACG	55 °C
		AATTGATTAAGAAACAAGTTCGGATCCCCGGGTTAATTAA	
	RpL7-R	TTTTGCATTTTCATCTGAATGCAAAATTTAAGTCATTACACG	
		AACTCAAAGAAAGTTTTAGAATTCGAGCTCGTTTAAAC	48 °C
	RpL7-con	GCCAACGAGAAAGAGAAC	
RpL11	RpL11-F	AATGCTGAGGACACCATCAACTGGTTCAAGCAAAAGTAT	55 °C
		GATGCCGTCGTTTTAGGAAAGCGGATCCCCGGGTTAATTAA	
	RpL11-R	ACAAGATGAGGAATTAATTTTCAATTGAATGTAAGCACAGT	
		AGTACCAAATGAAGTTTAGAATTCGAGCTCGTTTAAAC	
	RpL11-con	CATCAAGTACGATCCTTC	48 °C
RpL25	RpL25-F	TTTGTCAAGCTTTCTGCCGATGCCGATGCCCTTGATGTAG	55 °C
		CCAACCGCATTGGCTTCCTTCGGATCCCCGGGTTAATTAA	
	RpL25-R	GAAAGTACATGATGCAATTTCGGAGCTCAAAAGTGAATT	
		ATTAAACTAGTTTTTAAGCTAGAATTCGAGCTCGTTTAAAC	
	RpL25-con	TCCCGTCTTGATGAATAC	48 °C
Cbp20	Cbp20-F	CCTTTAGGATCTTCTCTAGAACTTCAAAGTAATCCAAGATA	55 °C
		CAATCGTTGGAAAAAAAATCGGATCCCCGGGTTAATTAA	
	Cbp20-R	AGTCTTATGCCAAAAAAATATATCGCAAGCTGGTCTTACAG	
		ATTTCTTTGTAAAGCATTAGAATTCGAGCTCGTTTAAAC	
	Cbp20-con	GTCAGAGATGAAATGCGGGAAG	48 °C
Cassette	Kan-1R	TATTCTGGGCCTCCATGTC	48 °C
PMA1	P1-PMAP-F	GAAACTATAGGTTAATGGAAG	55 °C
	P1-PMAP-R	GTTTCCTGCCGGCTTGTC	
	P2-PMAC1-F	GTCTTCGTGATTGGGTCGAT	55 °C
	P2-PMAC1-R	GGGGTCACCATAGTGCTTGT	
	P3-PMAC2-F	ATCCCGTTTCCAAGAAGGTT	55 °C
	P3-PMAC2-R	GAGGATCGGAACAAGGCATA	
	P4-PMAC3-F	GTCTTTCCACCGTCATTGGT	55 °C
	P4-PMAC3-R	ACGGAGAACGGCAACAATAG	
ACT1	T1-ACTP-F	GCTCAATGTTATCCGTTTCCG	55 °C
	T1-ACTP-R	GTAGTTGGTAAACGGTAAGTTATAACAC	
	T2-ACTC1-F	GGAAGAAGAAATCGCAGCGT	55 °C
	T2-ACTC1-R	CATATCATCCCAGTTGTTGACAATAC	
	T3-ACTC2-F	GAAATGTGATGTTGATATTCGTAAAG	55 °C
	T3-ACTC2-R	GCTCTCATCATACTCTTGCTTGG	
ADE6	E1-ADEP-F	CAGTATAAGGTATAACGACAACAAACG	55 °C
	E1-ADEP-R	GCTTAATGAGATGGTAAATGTTGA	
	E2-ADEC1-F	GGGATCCTTGGAGGTGGTCAA	55 °C
	E2-ADEC1-R	GAGGGTTCAACAGCAACAGATTTC	
	E3-ADEC2-F	GGCTATTATTGATAGCAACAGTGGC	55 °C
	E3-ADEC2-R	CGAACTTACTTACTATTGTTTCAGCTCACC	
Intergenic	Int F	GCGAAACCAGTATGGACGAT	55 °C
	Int R	AACGGGCAAATGTAAAGACG	
Cnt	Cnt-F	GTATTAGTGGTCGGTTTTCTTTTTGTT	55 °C
	Cnt-R	TGATCCTTTTGTTACCGGCG	
Imr	Imr-F	CCTTTACTGGAAAATTGTCGATATTACTAC	55 °C

	Imr-R	CAAACACTAAACAAGCAAATTTCAC	
Dh	Dh-F	GCACCGTATTGTTTCAGTCTCG	55 °C
	Dh-R	GAAAACACATCGTTGTCTTCAGAG	
Dg	Dg-F	CGGTCTTTGCAGGACTCTTGA	55 °C
	Dg-R	GGTGATTGATGAAAACATCAAC	
tDNA	tDNA-F	GGTCGACGGTATCGATAAGCTTGAT	55 °C
	tDNA-R	ACCCGCTGTCTTACCCTAAAATGAT	
ACT1	ACT1-F	GGCATCACACTTTCTACAACG	55 °C
	ACT1-R	GAGTCCAAGACGATACCAGTG	

Primers for Result II

Upf1	Upf1-F	TGTTACAATTATTTACACTTTGCAAATTGACGGCTTAATA ACATATCAAGTTGTCTTTCCCGGATCCCCGGGTTAATTAA	55 °C
	Upf1-R	ATATCAACAAATAAAAGATATGTTGGCATTCGTAATTAC AAGTAAGCAAATACTTATTAGAATTCGAGCTCGTTTAAAC	
	Upf1-con	CGAGTAGATACAACTCTCATG	55 °C
Upf2	Upf2-F	CAGCAAACTGAAGAAATAATGGAACGGAAACGTGTTAAA GAAATGGTTTTGAACTTCGAACGGATCCCCGGGTTAATTAA	55 °C
	Upf2-R	TACCTAATTAGCTTGCATATTTTAGAATGTAAATAATTAAA GAAGGCTACCATAGTAAAT GAATTCGAGCTCGTTTAAAC	
	Upf2-con	GTAGAGAGCTTGATGAAGAG	55 °C
GFP	GFP-F-smaI	GGGCCCGGGAATGGCTAGCAAAGGAGAAG	55 °C
	GFP-R-smaI	GGGCCCGGGTTAGCAGCCAGATCCTTTG	
Upf1	Upf1-F	GGGGGATCCATGTCTTTAGGGCTACAAC	55 °C
	Upf1-R	GGGGGATCCGAACCTAGTAGGTTCGTC	

Table 2.2- List of strains used in the study

Name	Strain	Genotype	Source
DB1	RpL703-HA	*h⁻ rpl703-3HA::kanMx6*	This study
DB2	RpL1102-HA	*h⁻ rpl1102-3HA::kanMx6*	This study
DB3	RpL2502-HA	*h⁻ rpl2502-3HA::kanMx6*	This study
DB4	Cbp20-HA	*h⁻ cbp20-3HA::kanMx6 ade6-M216 leu1-32 ura4-D18*	This study
SAL424	Cdc25-22	*h? cdc25-22 ade6-704 leu1-32 ura4-D1*	Tony Carr
DB5	RpL703-HA-tRNA^{Tyr}	*h? rpl703-3HA::kanMx6 pJK148-tDNA^{Tyr}::leu1-32 ade6-704 ura4-D18 cdc25-22*	This study
DB6	RpL703-HA-mutB BoxtRNA^{Tyr}	*h? rpl703-3HA::kanMx6 pJK148-tDNA^{Tyr}-mutBBox::leu1-32 ade6-704 ura4-D18 cdc25-22*	This study
DB7	RpL703-HA-ΔtRNA^{Tyr}	*h? rpl703-3HA::kanMx6 pJK148-ΔtDNA^{Tyr}::leu1-32 ade6-704 ura4-D18 cdc25-22*	This study
DB8	ΔRpS1501	*h- rps1501Δ::kanMx6 ade6-M216 leu1-32 ura4-D18*	This study
DB9	ΔRpS1501/nmt1-RpS1502	*h- rps1501Δ::kanMx6 rps1502-nmt1::kanMx6 ade6-M216 leu1-32 ura4 D18*	This study
GP937	WT	*h- ade6-M216 leu1-32 ura4-D18*	(Szankasi and Smith 1996)
MR3567	ΔUpf1	*h- upf1 Δ::KanMX6, leu1-32; ura4D18;h-*	(Rodriguez-Gabriel et al. 2006)
MR3569	ΔUpf2	*h- upf2 Δ::KanMX6, leu1-32; ura4D18;h-*	(Rodriguez-Gabriel et al. 2006)
MR3570	ΔUpf1/ ΔUpf2	*h- upf1 Δ::kanMX6/upf2 Δ::KanMX6, leu1-32; ura4D18*	(Rodriguez-Gabriel et al.

			2006)
FY7095	WT-otr1-*ade6*	*h+ otr1Rsph1::ade6 lys1::Nat ade6Δ::kan ura4-D18 leu1-32*	(Bayne et al. 2008)
FY7341	ΔClr4-otr1-*ade6*	*h+ clr4Δ::ura4 otr1Rsph1::ade6 lys1::Nat ade6Δ::kan ura4-D18 leu1-32*	(Bayne et al. 2008)
FY7005	ΔDcr1-otr1-*ade6*	*h+ dcr1Δ::Nat otr1Rsph1::ade6 ade6-210 leu1-32 ura4-D18 ura4-D18 leu1-3*	(Bayne et al. 2008)
FY7343	ΔSwi6-otr1-*ade6*	*h+ swi6Δ::ura4 otr1Rsph1::ade6 lys1::Nat ade6Δ::kan ura4-D18 leu1-32*	(Bayne et al. 2008)
DB10	ΔUpf1-otr1-*ade6*	*h+ upf1Δ::ura4 otr1Rsph1::ade6 lys1::Nat ade6Δ::kan ura4-D18 leu1-32*	This study

3. RESULT- I - Associaiton of RPs to transcription sites peaks at tRNA genes

3.1 HA-tagging of ribosomal proteins

To investigate whether RPs associate with actively transcribed genes I assayed chromatin association of three RPs by chromatin immunoprecipitation (ChIP). I analysed three 60S RPs: RpL7, RpL11 and RpL25. These evolutionarily conserved proteins were previously reported to be associated with chromosomal sites in *D. melanogaster* and *S. cerevisiae* (Brogna et al. 2002; Schroder and Moore 2005; Ni et al. 2006). Like other RPs in yeasts – both *S. pombe* and *S. cerevisiae* – these are encoded by duplicated genes called a or b isoform in *S. cerevisiae* or identified by a numeral suffix, typically, 01, 02 or 03 in *S. pombe* that encode identical or very similar proteins (Komili et al. 2007). The recombinant strains were generated by homologous recombination with the endogenous genes with a 3HA hemagglutinin (HA) tagging cassette and verified by PCR using a set of primers, forward primer specific to the 3' end of gene and a reverse primer specific to the tagging cassette (Fig. 3.1A, details in Material and Methods).

Western blot analysis of the total protein extracted from HA tagged RpL703, RpL1102 and RpL2502 strains identified 3HA tagged RpL703, RpL1102 and RpL2502 with the expected sizes of 32.37 kD, 23.07 kD and 18.97 kD (Fig. 3.1B). To test the functionality of HA-tagged RPs, I performed polysome analysis and all three tagged RPs could be incorporated in polysomes (Fig. 3.1C). Possible adverse effect of the HA tag on the RPs was assessed by comparing the growth of the strains using a spot dilution test. I found that there is negligible effect of HA tagging on the growth of the cells (Fig. 3.1D). We didn't check the functionality of tagged RPs in a null background which is more confirmatory experiment for functionality analysis. As a positive control, I performed ChIP experiments with RNA Pol II CTD and the small subunit of the nuclear cap binding protein complex (Cbp20); Pol II binds the DNA

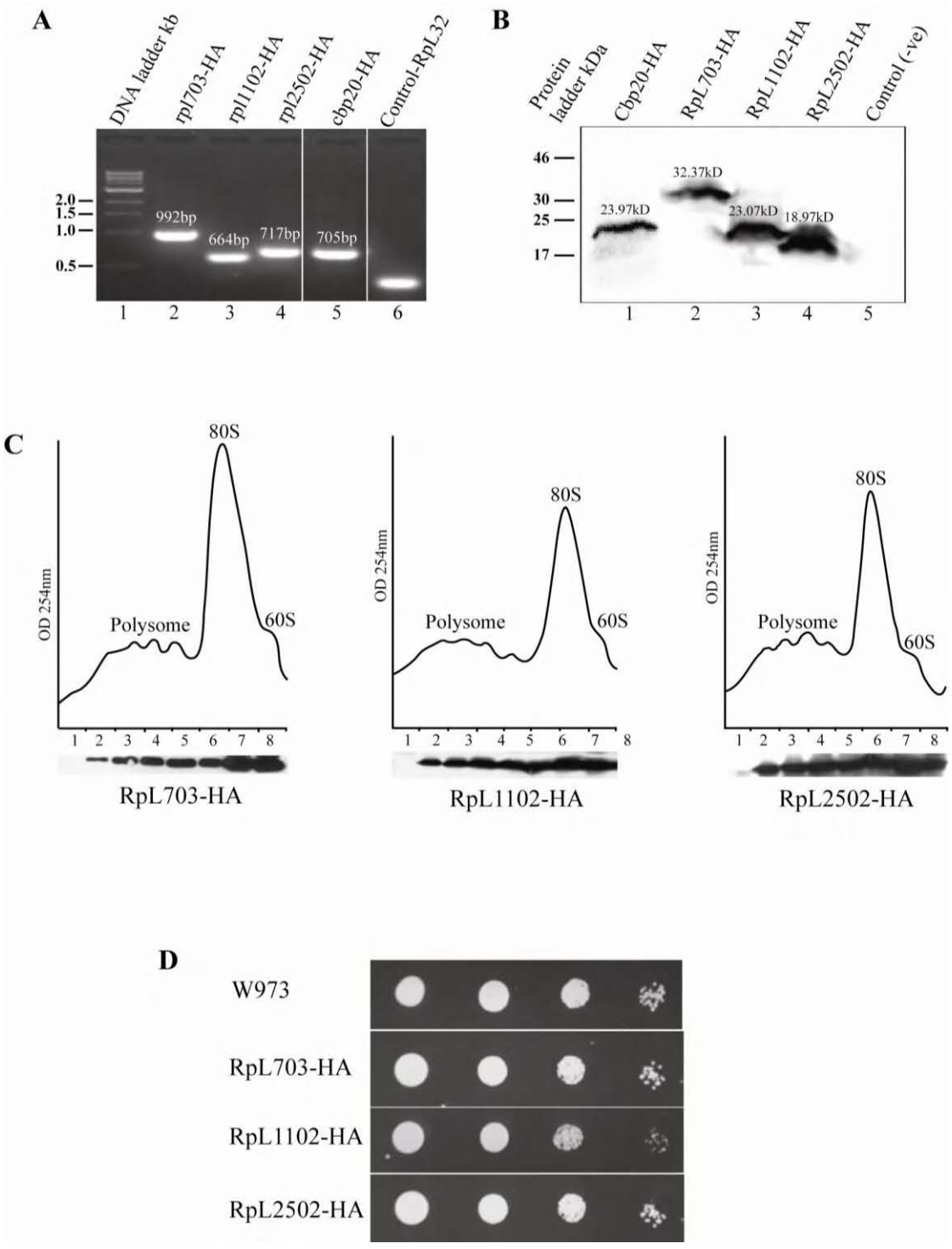


FIGURE 3.1 HA-tagged RPs are functional. (A) Agarose gel showing the expected PCR products from correct HA tagging of RP genes indicated. *CBP20* gene was also tagged with HA. Control- PCR product from *RpL32* gene (B) Western blotting analysis of whole-cell protein extracts of cells expressing the HA-tagged RPs and cbp20 indicated. (C) Representative polysome profile and Western analysis of HA-tagged RPs TCA precipitated from 10% to 50% sucrose gradient fractions. (D) Serial dilution colony spot assay of the tagged strains (from left to right, approximately 10^6, 10^5, 10^4 and 10^3 cells/ml, 4 µl were spotted onto YES plate).

directly while Cbp20 is tethered via nascent RNA. Cbp20 gene was tagged with HA using similar homologous recombination technique with primers specific to Cbp20 gene and confirmed by both PCR and Western blot (Fig. 3.1A, B).

3.2 Standardization of chromatin immunoprecipitation (ChIP) protocol

The chromatin immunoprecipitated (ChIPed) DNA can be assayed by PCR of specific sequences or by hybridization to genomic microarray chips (ChIP-on-chip) which allows genome-wide detection of the binding sites. In order to efficiently utilise the ChIP assay, the technique must be optimised for each protein and cell type. Therefore, first I standardized the ChIP technique with available *S. cerevisiae* strains expressing HA-tagged proteins and then I optimised it for *S. pombe*. The details of ChIP protocol and optimization in *S. cerevisiae* are described in Materials and Methods.

3.3 Validation of ChIP conditions in *S. pombe*

To validate whether the ChIP procedure I optimised in *S. cerevisiae* works in *S. pombe*, I have selected three different gene loci. I have chosen these genes based on three criteria: (a) genes that are constitutively transcribed (b) genes that are quite apart from neighbouring genes and (c) that have relatively long coding sequences to facilitate distinction of promoter and ORF association. Three genes were selected based on these criteria: *PMA1* (SPAC1071.10c) which encodes plasma membrane ATPase 1, *ACT1* (SPBC32H8.12c) which encodes cytoplasmic actin and *ADE6* (SPCC1322.13) which encodes phosphoribosylaminoimidazole carboxylase. These genes are constitutively active with estimated transcriptional rates of 80, 45 and 13 mRNAs per hour for *PMA1*, *ACT1* and *ADE6* respectively (Holstege et al. 1998; Komarnitsky et al. 2000). Four primer pairs for *PMA1* and three for *ACT1* and *ADE6* were designed, targeting the promoters and coding regions; an additional primer pair was

designed to an intergenic region of chromosome III, representing a control for nontranscribed DNA (the relative primer positions for each gene are shown in Fig. 3.2A). Chromatin preparations were sonicated as described in Materials and Methods, on average the DNA was sheared into 500-1000 bp fragments (Fig. 3.2B). Initially to assess the efficiency of the PCR primers and the efficiency of the antibodies in *S. pombe* - 8WG16 against Pol II (COVANCE) and 12CA5 against HA (CRUK) - I performed ChIP for Pol II and for RPs with chromatin preps from all three strains expressing HA-tagged RPs. For single PCR reactions, *PMA1* and *ACT1* primers were used for amplification. Both antibodies could pull down transcribed DNA regions (Fig. 3.2C). As expected, Pol II enrichment is highest at promoters and coding regions in both the *PMA1* and *ACT1* genes. The relative enrichment of *PMA1* and *ACT1* DNA relative to intergenic sequence was calculated as ratio of ratio of the intensitity of the same fragments produced with the input DNA. In comparison to Pol II, the RP ChIPs showed less enrichment at both promoters and coding regions (Fig. 3.2C). I tested two different amounts of antibody in the assay, either 75 µg (as described in the original protocol, see Materials and Methods) and 100 µg; but I did not find any significant difference in the enrichment level (Fig. 3.2C). As a negative control, I performed a no antibody immunoprecipitation reaction with just Sepharose A beads; result shows a much lower amplification, which was considered background noise (Fig. 3.2C). Once the efficiency of the individual primer sets was verified I performed multiplex PCR in which gene-specific primer pairs were added together with the control intergenic pair. It was important that each primer pair did not interfere with the other. Several pilot experiments were carried out using purified sonicated chromatin with different PCR conditions. Once the PCR was optimised, I assayed Pol II and Cbp20.

Cbp20 is part of the nuclear complex that binds the 7- methylguanosine cap added to all Pol II transcripts. Previous ChIP studies with Pol II and Cbp20-HA in *S. cerevisiae* have revealed

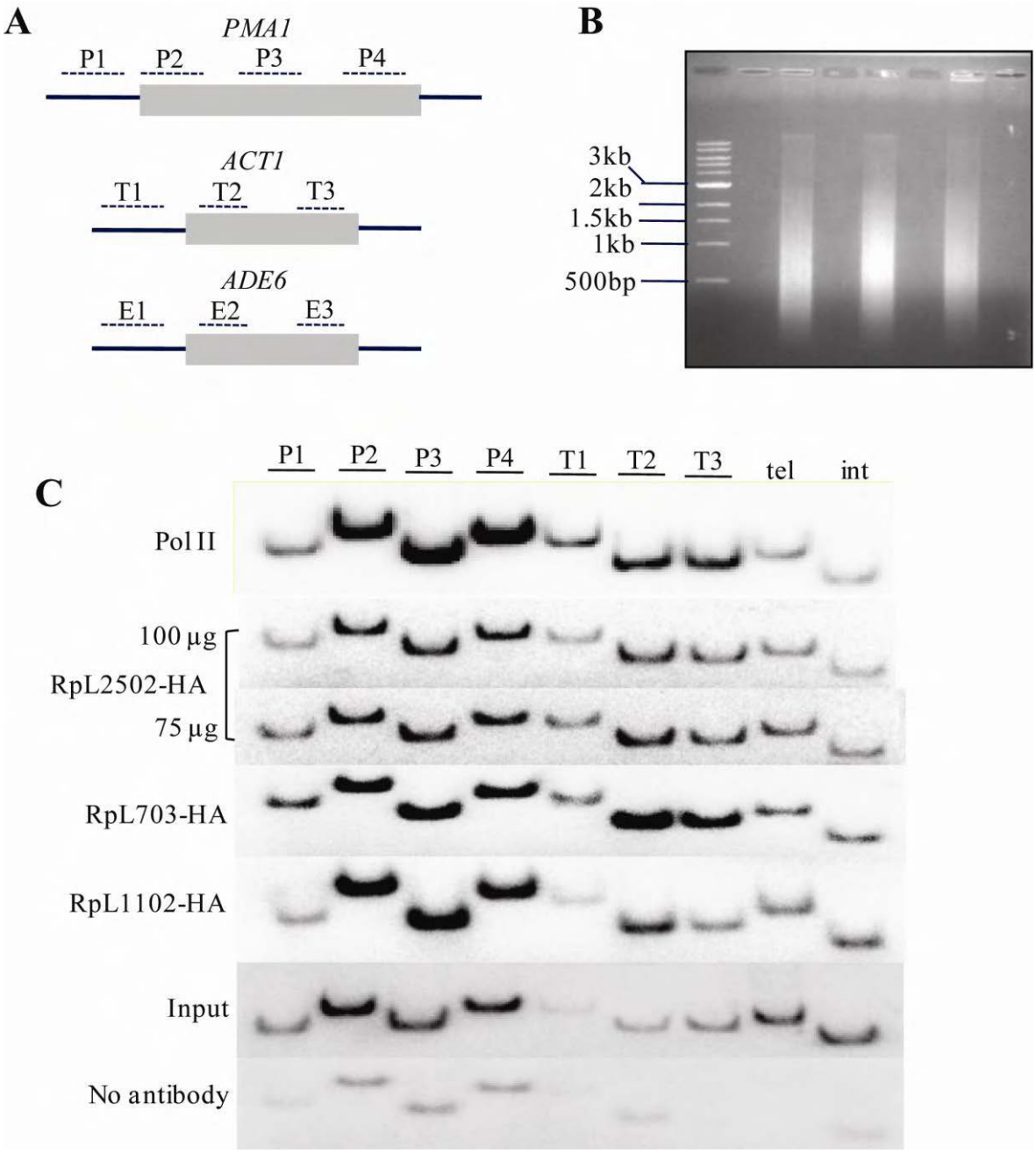


FIGURE 3.2 ChIP standardization in *S. pombe*
(A) Schematic diagram of the *PMA1, ACT1* and *ADE6* gene; grey bars represent the gene ORF; the PCR amplicons are indicated by dotted lines above (numbers correspond to the primer position relative to start codon). (B) Sonicated chromatin sample using 20 sec. on 20 sec. off (MSE Sonicator 150) was prepared from *S. pombe* cells RpL703, RpL1102 and RpL2502 which were all HA tagged, and 1µg was electrophoresed using 1% agarose gel and stained with ethidium bromide. (C) ChIP performed using HA antibody and the input and precipitated material amplified using semi-quantitative radioactive PCR against *PMA1* (P1 to P4*)* and *ACT1* (T1 to T3) primers [name of primers are indicated above each lane]. Intergenic (int) and telomeric (tel) primer pairs serve as non-transcribed controls.

strong association of both proteins across active genes (Komarnitsky et al. 2000; Schroder and Moore 2005). Previous reports showed that both Pol II and the HA tagged version of Pol II show a similar association pattern across gene regions, indicating that HA-Pol II is functional and that the HA antibody is very specific (Abruzzi et al. 2004).

Following IP with Pol II and multiplex PCR, high reproducible strong ChIP signals were observed both at promoters and throughout coding regions of the highly transcribed *PMA1* and *ACT1* genes as well as less transcribed *ADE6* gene (Fig. 3.3 & 3.4). In order to quantify the enrichment of Pol II at any given site, the intensity of signal of the Pol II amplicon was normalized to the intergenic control and then enrichment was expressed as the ratio of the intensity of the PCR fragments produced with immunoprecipitated vs. input DNA. If Pol II was enriched at any given site then the immunoprecipitated/input ratio would be greater than one and less than one if depleted or absent. Enrichment of Pol II at the *PMA1* promoter was clearly observed (15.4 fold, Fig. 3.3, Pol II lane P1); enrichment was apparent also in the coding region but to a lesser degree (5-8 fold, Fig. 3.3, Pol II lane P2-P4). In comparison, Pol II was present across both promoter and coding region of the *ACT1* gene, showing enrichment in the range of 8-10 fold (Fig. 3.3, Pol II lane T1-T3). However, Pol II enrichment across the *ADE6* gene was generally lower in comparison to the other two genes (1.4-5.4 fold, Fig. 3.3, Pol II lane E1-E3). This may be due to ADE6 being less transcribed than the other two genes or for other not obvious technical problems.

When the ChIP was performed with Cbp20-HA, it also exhibited strong association throughout the coding regions similar to the Pol II. However, Cbp20 showed somewhat lower association with the promoter regions in comparison to Pol II (Fig. 3.3; e.g. 5 fold vs. 9 fold enrichment on *ACT1*). There was a drastic signal loss for Cbp20 at the 3' end of the *ACT1* gene (Fig. 3.3, Cbp20 lane T3). However, this effect was not observed on either the *PMA1* or *ADE6* gene

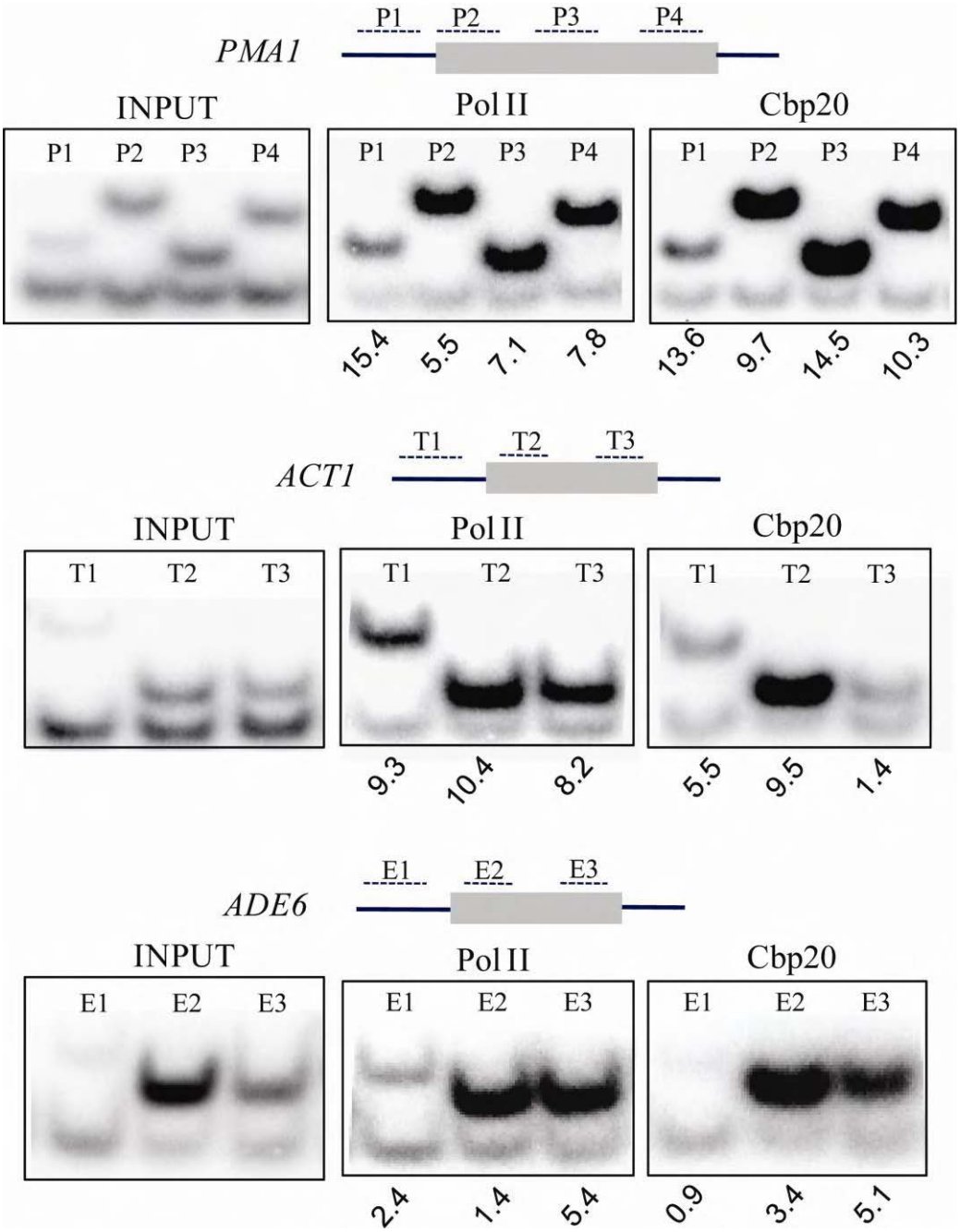


FIGURE 3.3 Pol II and Cbp20 association with the three test genes.
Polyacrylamide gels showing radiolabelled PCR products produced by the *PMA1*, *ACT1* and *ADE6* gene specific primer pairs (top band) and by the pair specific for the intergenic region (bottom band), amplified from input and ChIP DNA samples of Pol II and Cbp20-HA, and quantified using Phosphorimager. The relative enrichment of a specific DNA fragment relative to the intergenic sequence is expressed as ratio of ratio of the intensity of the same fragments produced with the input DNA and is shown just below each lane. Diagrams above represent locations of individual primer sets within the tested genes, and name of the primers are indicated above each lane.

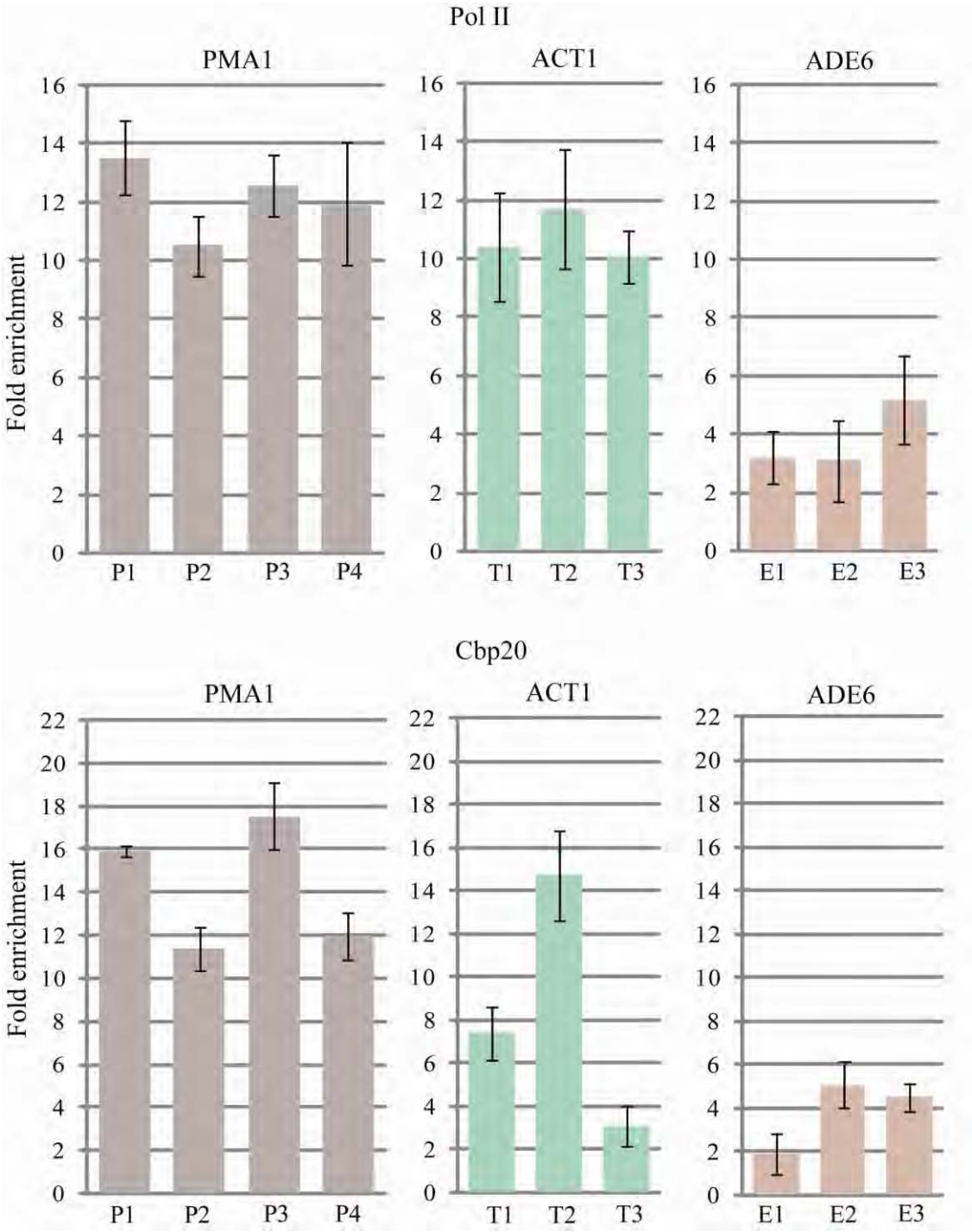


FIGURE 3.4 Real-time PCR quantification of the association of Pol II and Cbp20 with test genes.

Real-time PCR quantification using same set of primers for the *PMA1* and *ACT1* genes (indicated in the X-axis). Fold enrichment represented by coloured bars (Y-axis) are relative to the intergenic control fragment and are calculated as ratio of ratios as in Fig. 3.3. Error bars represent standard deviation of three repeats.

(Fig. 3.3, CBC20 lane P4 & E3). Radioactive PCR allows a semi-quantitative analysis of the ChIP data. In order to confirm these results real-time PCR (qPCR) was performed using the same ChIP DNA and primers (Fig. 3.4). The results are similar: Pol II showed a high degree of enrichment across all the regions tested for *PMA1* (~11 to 13 fold), *ACT1* (~10 to 12 fold) and moderate enrichment with *ADE6* gene (~3 to 5 fold). Cbp20 showed association at both promoter and coding region of the *PMA1* (~12 to 18 fold), *ACT1* (~3 to 15 fold) and *ADE6* (~2 to 5 fold). As with the radioactive PCR assay, the real-time also shows relative low association of Cbp20 at the 3' end of the *ACT1* gene.

3.4 Differential association of RPs with actively transcribing genes

To quantify the association of RpL703, RpL1102 and RpL2502 with the *PMA1*, *ACT1* and *ADE6* genes, the chromatin immunoprecipitated DNA was assayed with the same primer sets used above for the Pol II and Cbp20 ChIP (Fig. 3.5). The results show strong chromatin association with both *PMA1* and *ACT1*, but the level of enrichment is lower than with Pol II and Cbp20 (Fig. 3.5). Although the magnitude of the RP ChIP signals varied between experiments, they consistently showed the same pattern of association across genes. The RP association is higher within the coding regions (2-7.5 fold, Fig. 3.5) than at the promoter on both genes. However, RpL2502 showed comparatively higher promoter association than the other two ribosomal proteins (Fig. 3.5- RpL2502 lane P1, T1). Surprisingly, none of the RPs were enriched across *ADE6* gene (Fig. 3.5). As before, enrichment was also assessed by q-PCR using the same set of primers. Similar to the radioactive PCR data, I found that RPs tend to bind more to the coding region of genes than the promoter region, 2-6 fold enrichment (Fig. 3.6).

3.5 Standardization of RNase method

A key question I wanted to address was whether the association of RPs with genes is RNA

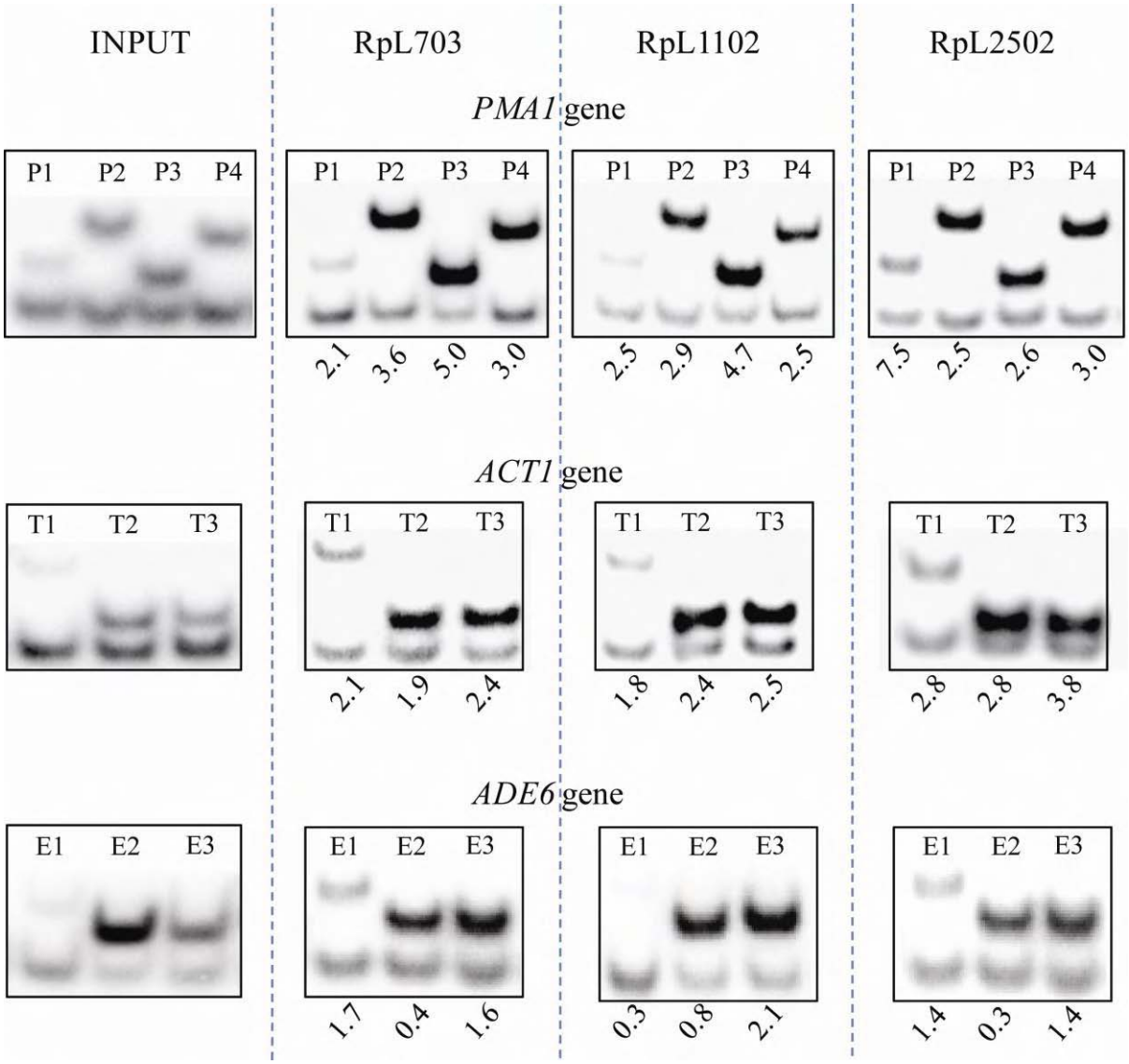


FIGURE 3.5 RPs are at the *PMA1* and *ACT1* genes.
Polyacrylamide gels showing radiolabelled PCR products produced by the *PMA1*, *ACT1* and *ADE6* gene specific primer pairs (top band) and by the pair specific for the intergenic region (bottom band), amplified from input and ChIP DNA samples of HA-tagged RPs, and quantified using Phosphorimager. The relative enrichment of a specific DNA fragment relative to the intergenic sequence is expressed as ratio of ratio of the intensity of the same fragments produced with the input DNA and is shown just below each lane. Name of the primers are indicated above each lane.

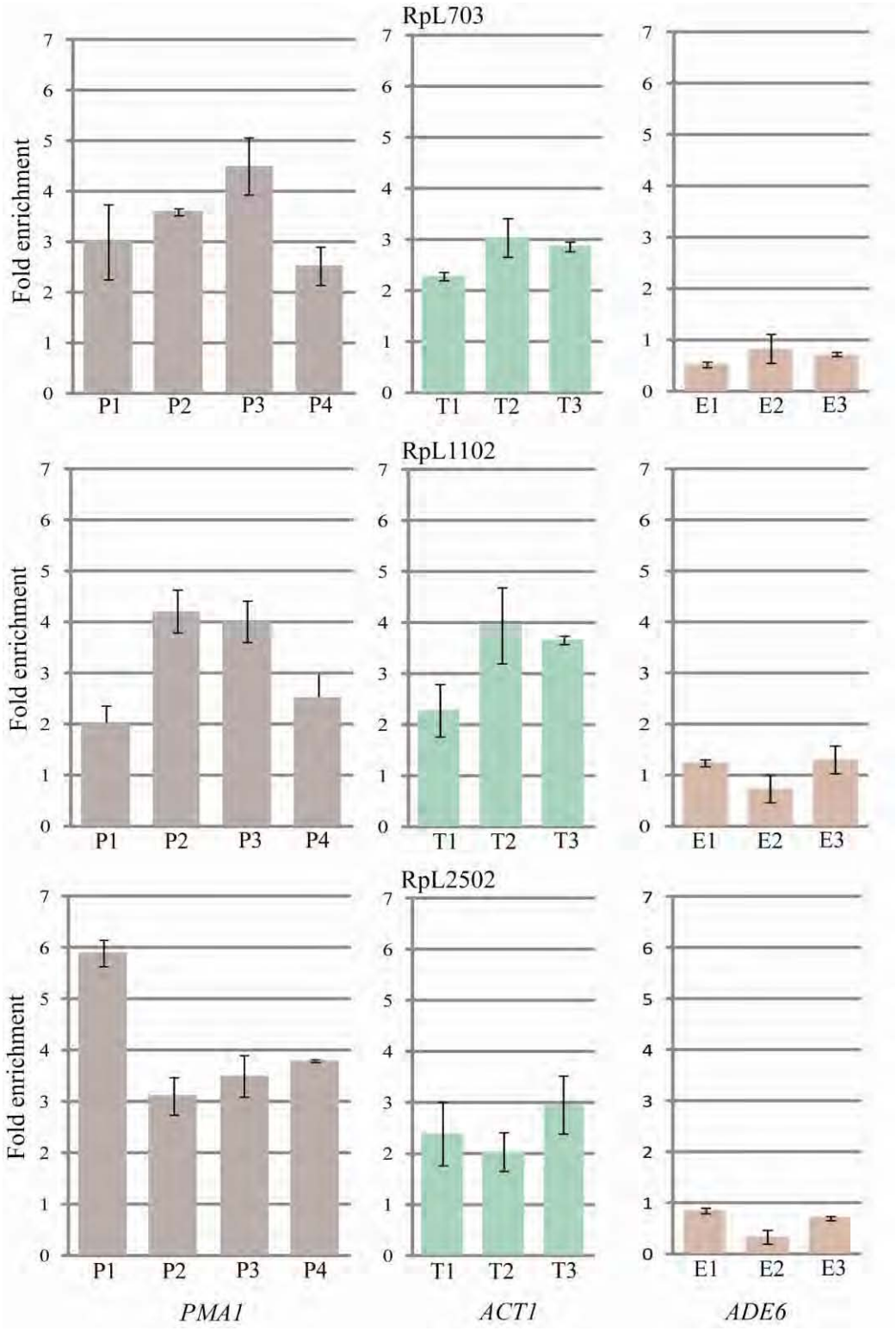


FIGURE 3.6 Real-time- PCR quantification of RPs association at the test genes.
Real-time PCR quantification using same set of primers from *PMA1*, *ACT1* and *ADE6* gene (indicated in the X-axis). Fold enrichment represented by grey bars (Y-axis) are relative to the intergenic control fragment and are calculated as ratio of ratios as in Fig. 3.6. Error bars represent standard deviation of three repeats.

dependent. To assess this, I tested whether the association of RpL03 across *PMA1* and *ACT1* is lost upon RNase digestion of the chromatin sample. Initially, I essentially followed a protocol described earlier (Abruzzi et al. 2004) which requires a reduction the crosslinking time from 20 min to 5 min and incubation with RNase A and RNase T1 after the crosslinking, just before the immunoprecipitation. However, using this protocol, I did not see any apparent RNase sensitivity for the RPs tested or for Cbp20 (Fig. 3.7D). We reasoned that the reason might be the presence of detergents (e.g. SDS) in the ChIP buffer that can inhibit the RNases. To improve the RNase-ChIP procedure I included a purification step which removes detergents and other small molecular weight compounds from the chromatin prep. The purification consists of ultrafiltration using a Microcon centrifugal filter- YM10 (Millipore), see Materials and Methods. After removal of SDS the samples were incubated with RNase A and RNase T1 for 30 min before ChIP. Our data show that the purification step dramatically improves the RNase digestion step: now RpL703 association is RNase sensitive (Fig. 3.7E).

To further validate this protocol, Cbp20 protein was used as a positive control and the RNase sensitivity tested for binding across primers for *PMA1* and *ACT1*. Quantification by radioactive PCR showed a reduction of Cbp20 binding across both genes which was confirmed by real-time quantification: after RNase treatment Cbp20 show <1 fold enrichment (Fig. 3.8A & B).

3.6 Ribosomal proteins are probably associated with nascent RNA

To determine whether chromatin association of the other RPs is RNA dependent, I assessed the effect of RNase treatment on RP binding using the same procedure described above. RNase treatment reduced the ChIP signal strength of the tested RPs. For RpL703 and RpL1102 considerably lower ChIP signal was detectable after RNase treatment with both the *PMA1* and *ACT1* gene by radioactive PCR (Fig. 3.9A). RpL2502 showed moderate sensitivity to RNase (Fig. 3.9A). RNase sensitivity data was further validated by real-time PCR using the same

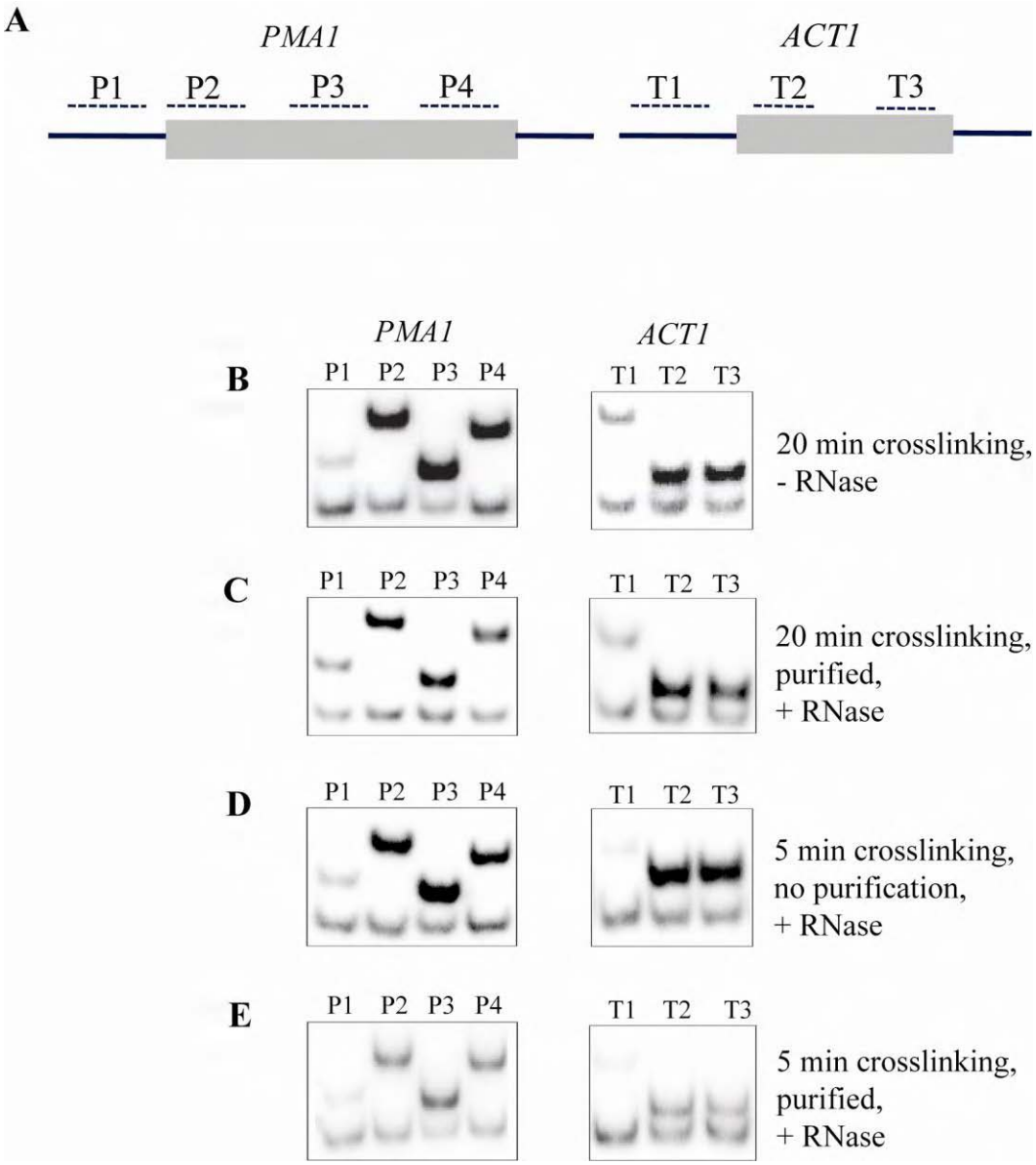


FIGURE 3.7 Chromatin filtration improves RNase digestion. (A) Schematics of the *PMA1*and *ACT1* genes with indicated the relative positions of the primers used for the PCR. (B) Polyacrylamide gels showing radiolabelled DNA fragments, PCR amplified from ChIP DNA (RpL703-HA strain), with *PMA1* or *ACT1* specific primer pairs (top bands), and by the pair specific for the intergenic region (bottom bands). Formaldehyde crosslinking was done for 20 min. (C) ChIP results as in B but the chromatin was filter purified (see Material and Methods) and RNases A and T1 treated prior ChIP (30 min, RT), formaldehyde crosslinking was for 20 min as in B. (D) ChIP results as in B but the chromatin was RNases A and T1 treated (30 min, RT), formaldehyde crosslinking was done for 5 min. (E) ChIP results as in B but the chromatin was filter purified (see Material and Methods) and RNase treated (30 min, RT), formaldehyde crosslinking was for 5 min as in D.

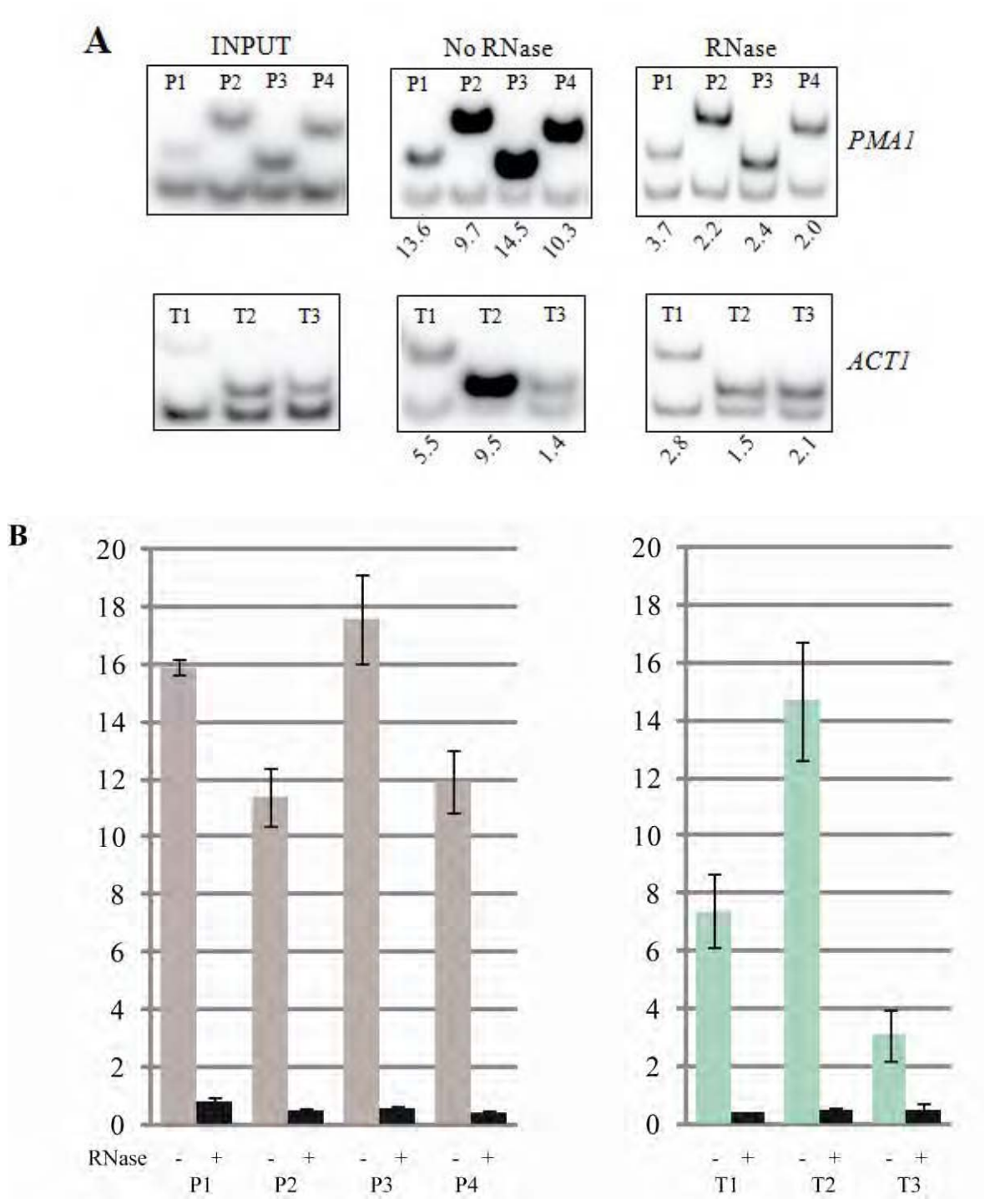


FIGURE 3.8 Cbp20 is associated to DNA via RNA. (A) Polyacrylamide gel with radiolabelled PCR products produced by the *PMA1* and *ACT1*-specific primer pairs (top bands) and the pair corresponding to the intergenic region (bottom bands); using input DNA before ChIP, DNA after ChIP (as in Fig. 3.3) and ChIPed DNA after RNases A and T1 treatment. The relative enrichment is shown just below each lane, name of the primers are indicated above each lane. (B) Quantification of ChIP-enriched DNA using real-time PCR with the primers indicated in the X-axis.

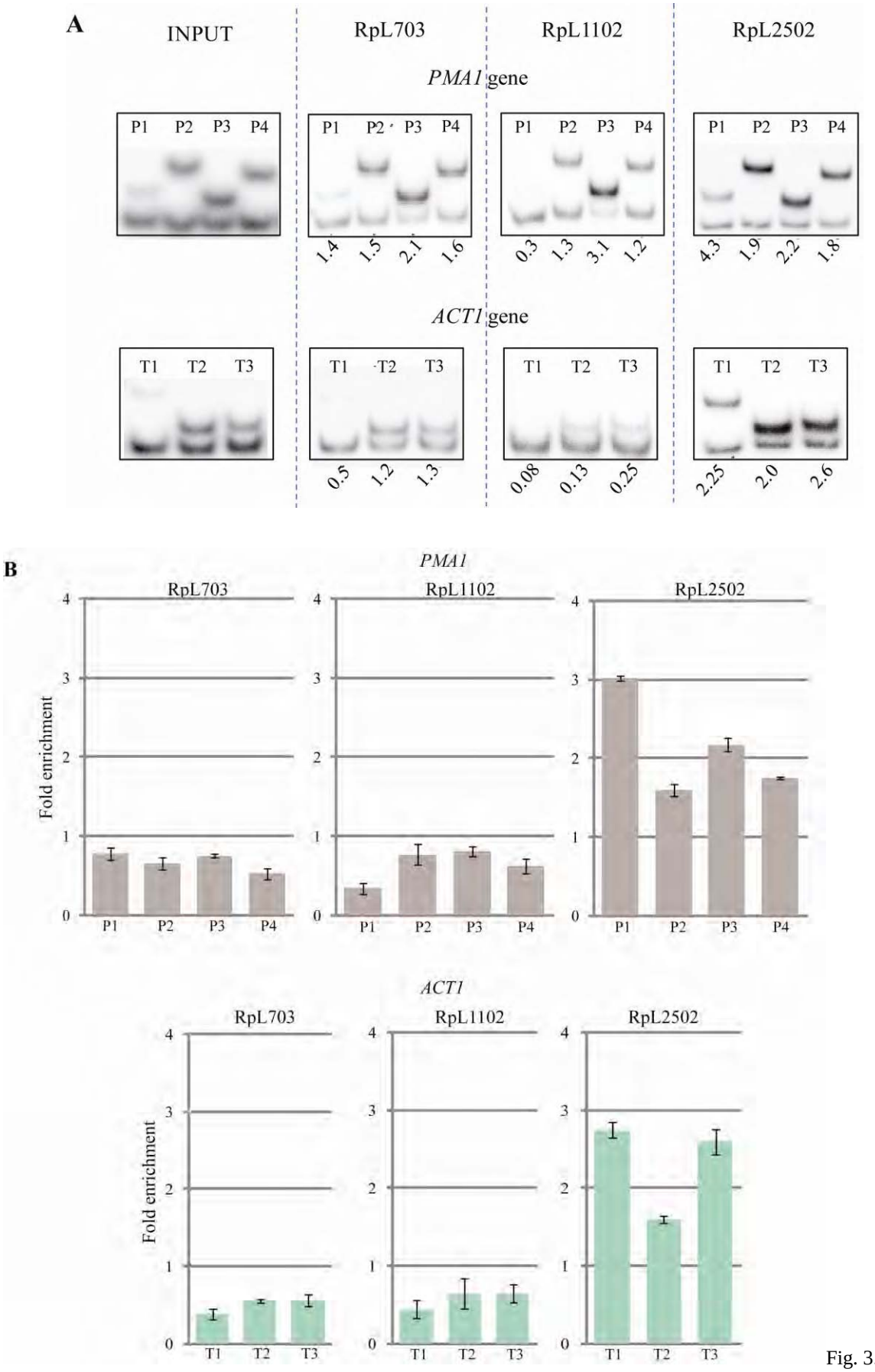

A
INPUT
RpL703
RpL1102
RpL2502
PMA1 gene
P1 P2 P3 P4
1.4 1.5 2.1 1.6
0.3 1.3 3.1 1.2
4.3 1.9 2.2 1.8
ACT1 gene
T1 T2 T3
0.5 1.2 1.3
0.08 0.13 0.25
2.25 2.0 2.6
B
PMA1
RpL703
RpL1102
RpL2502
Fold enrichment
0 1 2 3 4
P1 P2 P3 P4
ACT1
RpL703
RpL1102
RpL2502
Fold enrichment
0 1 2 3 4
T1 T2 T3

Fig. 3.9A, B

FIGURE 3.9 RPs are associated to transcriptionally active chromatin in RNA dependent manner. (A) Polyacrylamide gel with radiolabelled PCR products produced by the *PMA1* and *ACT1*-specific primer pairs (top bands) and the pair corresponding to the intergenic region (bottom bands); using input DNA before ChIP and ChIPed DNA after RNases A and T1 treatment, strains used are indicated above. The relative enrichment is shown just below each lane, name of the primers are indicated above each lane. (B) Quantification of ChIP-enriched DNA using real-time PCR with the primers indicated in the X-axis.

RNase treated chromatin; this showed similar results (Fig. 3.9B). The RNase treatment reduced the chromatin enrichment from ~2 to ~5 fold (Fig. 3.5 & 3.7) to ~0 to ~1 fold (Fig. 3.9B). The above experiments therefore indicate that two of our tested RPs (RpL703 and RpL1102) are primarily associated to chromatin via RNA whereas some RpL2502 proteins are directly associated to chromatin.

3.7 RPs bind at many sites on fission yeast chromosomes

To map the sites with which RPs associate across the genome, I performed ChIP-on-chip assays: chromatin-immunoprecipitated DNA was hybridised to genomic tiling arrays (technical details of the optimization procedure are in Materials and Methods, section 2.5.14). As for the single-gene ChIP, I analysed the three strains encoding HA-tagged RpL7, RpL11 and RpL25. Following immunoprecipitation the DNA as was hybridized to a *S. pombe* DNA tiling array (GeneChip® *S. pombe* Tiling 1.0FR Array); this array is comprised of over 1.2 million perfect match/mismatch probe pairs tiled through the complete *S. pombe* genome (except repetitive regions which are underrepresented). Probes are tiled for both strands of the genome at an average of 20 base pair resolution, as measured from the central position of adjacent 25-mer oligos, creating an overlap of approximately 5 base pairs for adjacent probes and used for 'ChIP-on-chip' analysis. The GeneChip® Scanner 3000 7G which is controlled by GeneChip® Operating Software (GCOS), is used for scanning the hybridization signal produced from probe array and produces .CEL files by assigning hybridization data therein to specific probes. Due to the stochastic nature of gene expression and experimental error, it is now widely accepted that ChIP-on-chip experiments need to be replicated. The question is how to compare the ChIP enrichment across these replicates, since besides biological variations; they are also subject to variation from the manufacturing and hybridization process. This creates the need for normalization across arrays. Therefore, I performed two biological replicas (independent ChIPs,

performed on different days) for both RpL703 and RpL2502 ChIP DNA, with an input DNA to allow normalization of the signal. Due to financial restrictions, I was able to perform only a single hybridization for the RpL1102 sample. Correlation analysis between two replicates was performed using intensities of all probes extracted from the tiling array by our bioinformatics collaborators (Wazeer Varsally and Dr. Francesco Falciani). The correlation was visualised with a scatter plot and quantified by calculating the Pearson's correlation coefficient, which is a measure of the strength of the association between the two variables. Pearson correlation coefficients (r) were highly significant: 0.76 for RpL703 (Fig. 3.10A) and 0.78 for RpL2502 (Fig. 3.10B).

To identify regions enriched by RPs on ChIP-chip Affymetrix tiling arrays, I used a fast and powerful analysis algorithm, Model-based Analysis of Tiling-arrays (MAT). MAT can standardize the signals of each probe in each array individually, and detect ChIP regions from a single ChIP sample or multiple ChIP samples. For detection of enriched regions in our ChIP-on-chip data, the MAT parameters used were: Bandwith=300; MaxGap=300; MinProbe=10; p-value cutoff=10^{-4} (BandWidth: the number of bases to extended from the position being analyzed. The result is that 2*Bandwidth probe positions are included in the signal and p-value analysis. MaxGap: maximum gap between positive probes; All regions separated by < MaxGap will be mergered into one. MinProbe: minimum number of probes for MAT score analysis). Enriched regions were initially defined at different p-value thresholds; the p-value of 10^{-4} was chosen because this was the lowest p-value at which both the experimentally validated *PMA1* (all but one experiment) and *ACT1* genes were flagged as enriched. Given genes were classified as positive hits only if the enrichment was at least 50% or more of the gene sequence, therefore excluding regions with minimal levels of enrichment. Given genes were classified as positive hits only if the enrichment was at least over 50% of the gene sequence, thereby discarding those regions that were only partially enriched. The enrichment scores were assigned to *S. pombe*

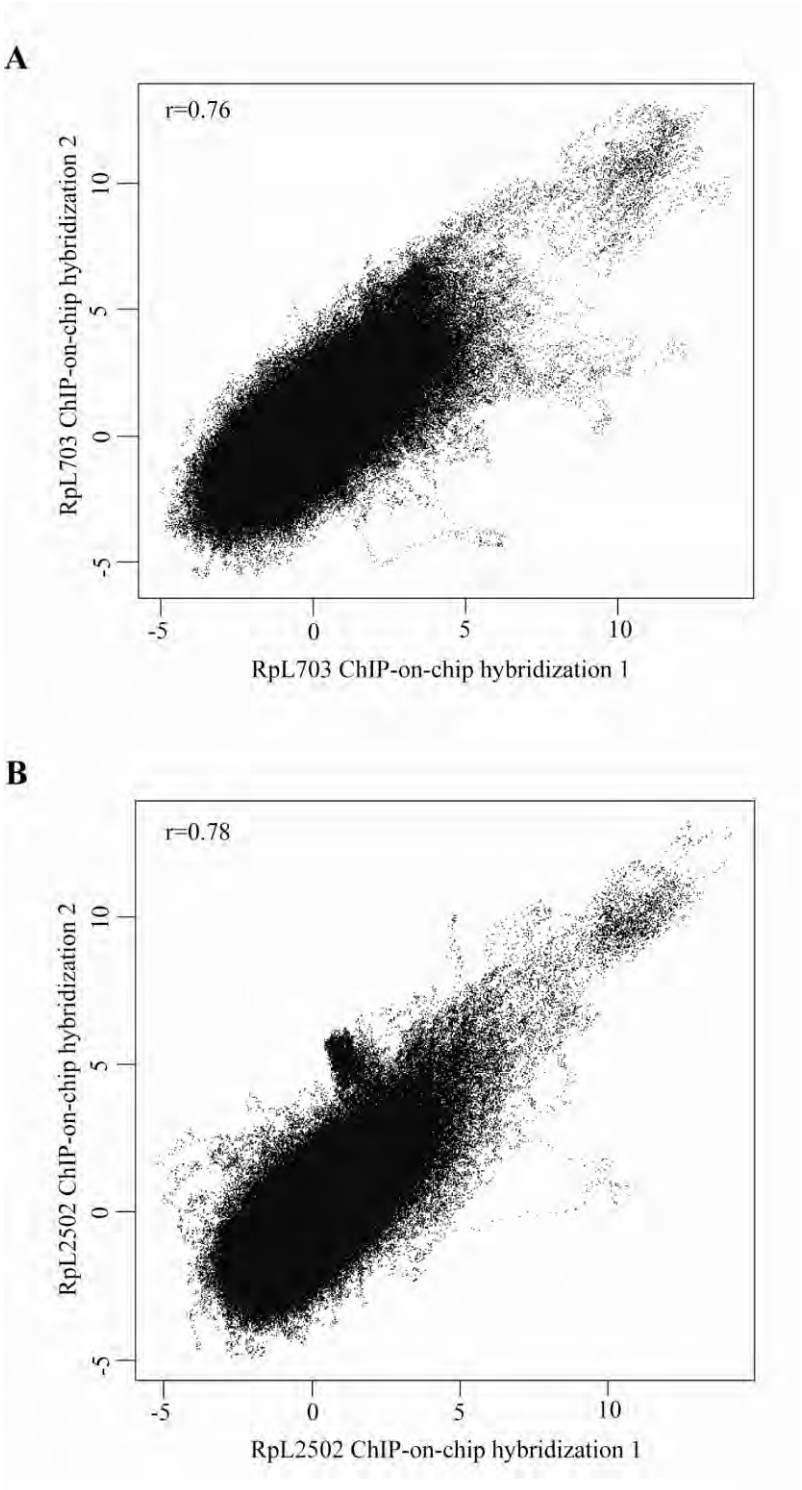


FIGURE 3.10 RPs chromosomal association shows high correlation between biological replicas. Scatter plot of the intensities of all probes in the tilling array: ChIP-on-chip experiment 1 (X axis) versus ChIP-on-chip experiment 2 (Y axis). (A) Correlation between the replicas of the RpL703 experiment; (B) that of RpL2502. Pearson correlation is shown at the top left of each panel.

genomic positions using the *S. pombe* coordinates and a bpmap file for the Affymetrix array (ftp://ftp.sanger.ac.uk/pub/yeast/pombe/GFF, Sanger 8/23/07 library). Finally the output of the MAT software were visualised with the Affymetrix Integrated Genome Browser (IGB) software.

3.8 RPs associate with both protein- and non protein-coding genes

The ChIP-on-chip analysis revealed that the three RPs associate with many loci throughout the three chromosomes of *S. pombe*. There are several high enrichment peaks on all chromosomes which are easily detectable and are common to all three RP enrichment profile (Fig. 3.11A). The highest and broadest peaks were at all three centromeres with a second RP binding hotspot at the telomeres. Generally however RPs appeared to bind across the whole length of the chromosomes. I identified a total of 507 genes/genomic regions which were either shared by all three or two of the RPs or enriched with at least one; 239 with RpL703, 276 with RpL1102 and 376 with RpL2502; 130 genes/genomic regions are associated with all of the three proteins (Fig. 3.11B, table 3.1). RpL2502 associates with more sites than other two proteins, which might be because of its histone-like domain which may contact DNA directly. The hits are clearly shared between the three proteins. Both coding and non-coding genes were identified as enriched. This tendency for the three RPs to bind to the same genes is highly statistically significant (p-value < 10^{-6}). The hits that are not shared have lower enrichment scores; however, at the p= 10^{-3} cutoff, there are twice as many enriched regions. Although many regions were not included because their score was below the stringent p= 10^{-4} cutoff, enrichment peaks are visually apparent in close-up views of genomic regions. For example a visual inspection of the enrichment profile over the 80 kb region around the *ACT1* locus shows the clear enrichment of all three RPs at the *ACT1* and SPBC110210.08 loci. However, both upstream and downstream peaks could also be observed but these were undetected due to the stringent cutoff selected

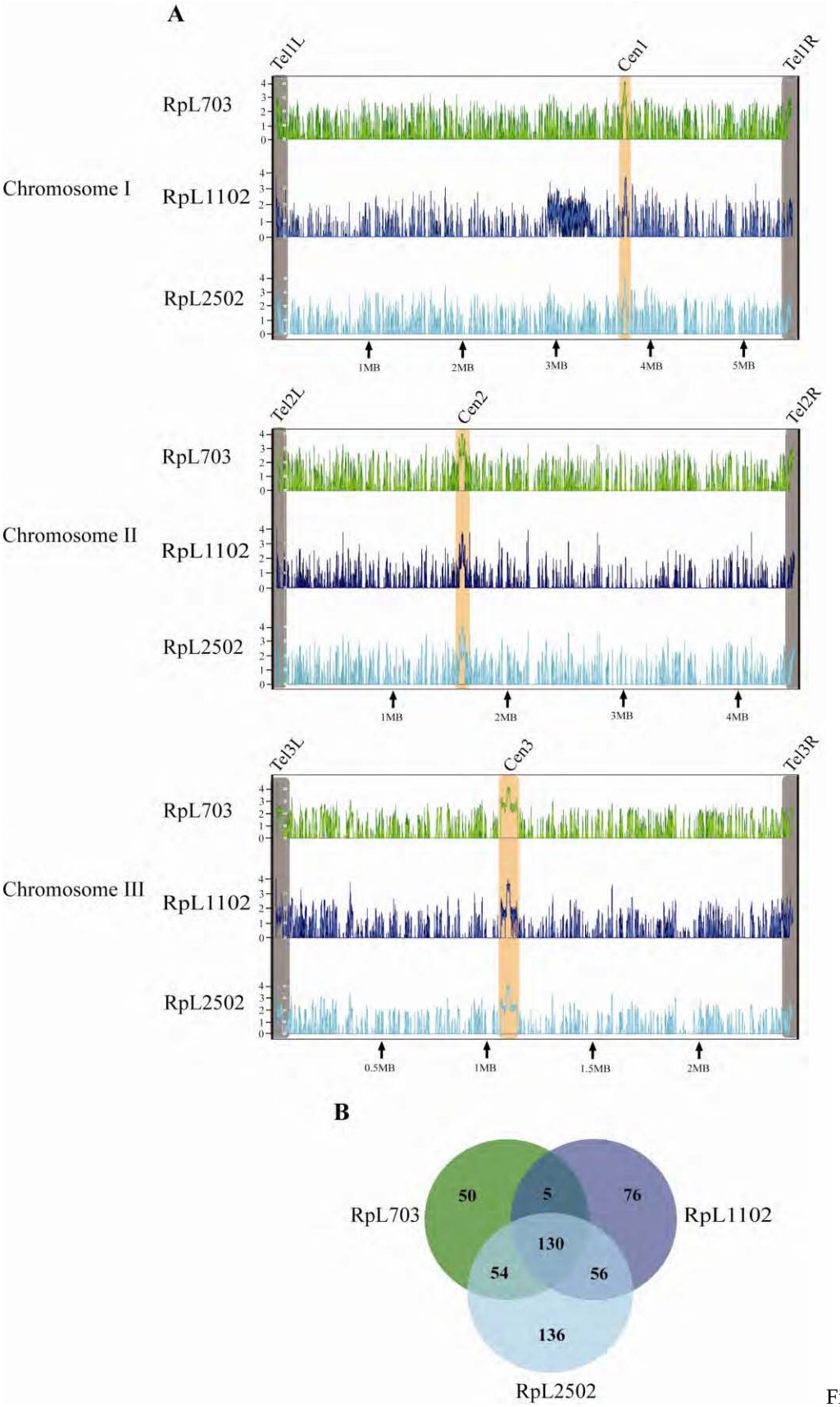

A
Tel1L
Cen1
Tel1R
RpL703
RpL1102
RpL2502
Chromosome I
1MB
2MB
3MB
4MB
5MB
Tel2L
Cen2
Tel2R
RpL703
RpL1102
RpL2502
Chromosome II
1MB
2MB
3MB
4MB
Tel3L
Cen3
Tel3R
RpL703
RpL1102
RpL2502
Chromosome III
0.5MB
1MB
1.5MB
2MB
B
RpL703
RpL1102
RpL2502
50
5
76
130
54
56
136

Fig. 3.11A, B

FIGURE 3.11 Genome-wide association of RPs. (A) Chromosomal binding profiles of the RPs on all three chromosomes, viewed with the Integrated Genome Browser (IGB) software. Each RP is shown with a different colour on the graph: green (RpL703), navy blue (RpL1102) and sky blue (RpL2502); X-axis show the distance from the left chromosome end, in mega bases (Mb), the Y-axis log2 MAT enrichment score (0 to 4). Position of centromeres (*cen*) and telomeres (*tel*) are highlighted with orange and grey boxes respectively. (B) Venn diagram showing the number of genes/genomic regions associated with the three RPs.

Table 3.1- List of most enriched genes/genomic regions with MAT enrichment score

RpL7	Enrichment Score	RpL11	Enrichment Score	RpL25	Enrichment Score
SPAC1A6.04c	5.6707237	SPAC1A6.04c	4.85270	SPAC1A6.04c	7.37459
SPAC9.09	5.13950211	SPAC9.09	5.60843	SPAC9.09	6.97936
SPAC1002.13c	6.3690838	SPAC1002.13c	4.34991	SPAC1002.13c	7.00958
unknown_1907	4.5961623	unknown_1907	1.3583	unknown_1907	3.01021
SPAPB24D3.07c	8.70073523	SPAPB24D3.07c	10.3737	SPAPB24D3.07c	11.9857
SPAC31G5.10	7.6994574	SPAC31G5.10	6.18536	SPAC31G5.10	6.38418
SPNCRNA.01	7.36133802	SPNCRNA.01	5.81211	SPNCRNA.01	6.1569
unknown_2876	7.58990	unknown_2876	5.93955	unknown_2876	6.8068
SPAC17A2.09c	5.23254107	SPAC17A2.09c	6.14914	SPAC17A2.09c	6.6594
SPAC17A2.11	6.12045035	SPAC17A2.11	3.53956	SPAC17A2.11	5.33600
unknown_3603	7.0141158	unknown_3603	4.07645	unknown_3603	4.55683
unknown_3606	11.875976	unknown_3606	8.09861	unknown_3606	10.0634
SPATRNAGLU.03	9.742357	SPATRNAGLU.03	5.31116	SPATRNAGLU.03	7.80297
unknown_3608	14.7660544	unknown_3608	10.7875	unknown_3608	13.3248
SPATRNAILE.03	16.41987	SPATRNAILE.03	11.7999	SPATRNAILE.03	14.2211
unknown_3609	15.2239263	unknown_3609	11.6047	unknown_3609	14.7677
unknown_3610	14.974641	unknown_3610	10.7116	unknown_3610	13.2903
SPATRNAILE.04	14.0784857	SPATRNAILE.04	11.2282	SPATRNAILE.04	13.0098
SPATRNAGLU.04	16.3021285	SPATRNAGLU.04	12.3639	SPATRNAGLU.04	14.6378
unknown_3611	12.5667646	unknown_3611	8.73736	unknown_3611	10.9240
SPATRNAALA.05	15.9060333	SPATRNAALA.05	11.1695	SPATRNAALA.05	14.9808
unknown_3612	9.64908729	unknown_3612	5.47497	unknown_3612	6.99095
prl49	7.69419434	prl49	5.55086	prl49	9.91585
SPNCRNA.53	7.72542454	SPNCRNA.53	5.18752	SPNCRNA.53	9.46302
SPNCRNA.63	7.74737210	SPNCRNA.63	5.20098	SPNCRNA.63	9.25174
SPAC27E2.13	7.68	SPAC27E2.13	5.08163	SPAC27E2.13	9.6573
SPAC27E2.11c	8.05352652	SPAC27E2.11c	7.49469	SPAC27E2.11c	11.582
SPAC19G12.10c	7.56849083	SPAC19G12.10c	5.19099	SPAC19G12.10c	7.8832
SPNCRNA.99	7.4578	SPNCRNA.99	5.39613	SPNCRNA.99	8.48333
unknown_4190	5.77031666	unknown_4190	4.12473	unknown_4190	6.51279
SPAC19B12.02c	6.52861421	SPAC19B12.02c	4.06382	SPAC19B12.02c	6.79611
SPAC26F1.06	5.99229777	SPAC26F1.06	6.44025	SPAC26F1.06	5.95129
SPAC922.04	6.40177	SPAC922.04	4.02800	SPAC922.04	6.45203
SPNCRNA.61	6.12849452	SPNCRNA.61	4.70843	SPNCRNA.61	4.7690
SPBPB21E7.04c	6.407112	SPBPB21E7.04c	5.15733	SPBPB21E7.04c	8.64883
SPBC1198.14c	7.59939389	SPBC1198.14c	5.27700	SPBC1198.14c	6.3218
SPBC660.04c	6.97319045	SPBC660.04c	3.8281	SPBC660.04c	5.82963
SPBC660.06	5.63605447	SPBC660.06	5.44349	SPBC660.06	5.80753
SPBTRNAGLY.04	6.58625	SPBTRNAGLY.04	4.02507	SPBTRNAGLY.04	5.3742
SPBTRNAALA.07	5.69045	SPBTRNAALA.07	3.85848	SPBTRNAALA.07	5.07949
SPBTRNAGLY.05	9.25805	SPBTRNAGLY.05	4.58084	SPBTRNAGLY.05	7.0745
SPBTRNAARG.04	6.62778	SPBTRNAARG.04	5.17859	SPBTRNAARG.04	6.47199
SPBC1685.12c	7.36805387	SPBC1685.12c	4.32008	SPBC1685.12c	4.25167
unknown_414	5.17312705	unknown_414	3.92813	unknown_414	3.93105
SPBC354.12	7.14146301	SPBC354.12	9.98031	SPBC354.12	8.7694
unknown_1138	9.25282	unknown_1138	5.6020	unknown_1138	9.8902
SPSNORNA.21	9.64748333	SPSNORNA.21	5.89565	SPSNORNA.21	10.2538
SPBC32H8.12c	5.37638653	SPBC32H8.12c	3.47680	SPBC32H8.12c	6.41313
SPBC11B10.08	4.08943557	SPBC11B10.08	2.81825	SPBC11B10.08	3.03295

SPBTRNALEU.06	6.53220285	SPBTRNALEU.06	4.06854	SPBTRNALEU.06	5.35556
SPBTRNAGLY.07	11.3686142	SPBTRNAGLY.07	7.84357	SPBTRNAGLY.07	11.1871
SPBTRNALYS.07	10.7699	SPBTRNALYS.07	7.41999	SPBTRNALYS.07	9.8350
SPBTRNAILE.05	10.639	SPBTRNAILE.05	6.05736	SPBTRNAILE.05	9.35708
SPBTRNAALA.08	11.004	SPBTRNAALA.08	5.94131	SPBTRNAALA.08	9.72396
SPBTRNAVAL.05	12.551912	SPBTRNAVAL.05	7.16543	SPBTRNAVAL.05	10.9981
SPBTRNAGLU.06	9.31454333	SPBTRNAGLU.06	4.99456	SPBTRNAGLU.06	6.01308
SPBTRNAARG.06	8.09637428	SPBTRNAARG.06	5.84922	SPBTRNAARG.06	7.3900
unknown_1440	8.57681988	unknown_1440	6.29782	unknown_1440	8.49855
SPBTRNAASP.03	7.6746	SPBTRNAASP.03	5.86830	SPBTRNAASP.03	7.3401
unknown_1448	7.55736542	unknown_1448	4.35464	unknown_1448	4.79289
SPBTRNAVAL.06	7.68142285	SPBTRNAVAL.06	4.77289	SPBTRNAVAL.06	5.8971
unknown_1450	13.834935	unknown_1450	10.5730	unknown_1450	13.0255
unknown_1452	13.8728882	unknown_1452	11.0039	unknown_1452	13.4594
unknown_1453	13.7303698	unknown_1453	10.2457	unknown_1453	12.9981
SPBTRNAVAL.07	13.516412	SPBTRNAVAL.07	10.6453	SPBTRNAVAL.07	14.073
SPBTRNAALA.10	14.16662	SPBTRNAALA.10	10.9915	SPBTRNAALA.10	13.4780
SPBTRNAILE.07	13.64507	SPBTRNAILE.07	11.0017	SPBTRNAILE.07	13.525
SPBTRNALYS.08	5.96376555	SPBTRNALYS.08	3.64581	SPBTRNALYS.08	6.28916
SPBTRNAILE.08	10.0225	SPBTRNAILE.08	5.71675	SPBTRNAILE.08	9.6609
SPBTRNAALA.11	10.835	SPBTRNAALA.11	6.82359	SPBTRNAALA.11	10.7388
SPBTRNAVAL.08	11.4111666	SPBTRNAVAL.08	5.97841	SPBTRNAVAL.08	9.34340
SPBTRNAGLU.07	12.0005	SPBTRNAGLU.07	6.42640	SPBTRNAGLU.07	10.7460
SPBTRNAARG.07	12.59	SPBTRNAARG.07	7.13216	SPBTRNAARG.07	10.4414
SPBC21B10.13c	8.5066987	SPBC21B10.13c	5.4939	SPBC21B10.13c	7.88348
SPBC21B10.12	2.71328208	SPBC21B10.12	1.76827	SPBC21B10.12	3.22113
SPBC19C2.07	7.4280	SPBC19C2.07	8.7918	SPBC19C2.07	8.5618
SPBC1E8.05	6.7210059	SPBC1E8.05	5.82219	SPBC1E8.05	8.85583
SPBC1815.01	8.56854754	SPBC1815.01	12.5037	SPBC1815.01	9.93707
SPBC29A10.08	9.52208	SPBC29A10.08	7.25632	SPBC29A10.08	11.3995
SPBC32F12.11	8.2298	SPBC32F12.11	8.67217	SPBC32F12.11	9.6756
SPBC19C7.04c	8.18105176	SPBC19C7.04c	5.99700	SPBC19C7.04c	10.5979
SPNCRNA.26	6.82751	SPNCRNA.26	2.08822	SPNCRNA.26	5.57927
SPBCPT2R1.08c	4.10886	SPBCPT2R1.08c	2.06293	SPBCPT2R1.08c	2.81455
SPCTRNAHIS.03	9.1306	SPCTRNAHIS.03	6.3800	SPCTRNAHIS.03	9.2718
SPCTRNAGLY.10	6.46035333	SPCTRNAGLY.10	5.1351	SPCTRNAGLY.10	4.94748
SPCC1235.14	5.02442083	SPCC1235.14	4.52904	SPCC1235.14	7.26229
SPCC548.06c	6.37869939	SPCC548.06c	5.51987	SPCC548.06c	7.08004
unknown_192	4.66069575	unknown_192	3.17112	unknown_192	4.51310
unknown_294	6.41998918	unknown_294	3.57490	unknown_294	4.73617
SPCC1393.08	6.03907789	SPCC1393.08	6.30000	SPCC1393.08	7.71537
SPCC24B10.21	5.70742043	SPCC24B10.21	7.23337	SPCC24B10.21	7.60203
SPCC1795.11	5.93617975	SPCC1795.11	5.22744	SPCC1795.11	7.23353
SPCTRNAALA.12	6.52759166	SPCTRNAALA.12	3.2434	SPCTRNAALA.12	6.24718
tRNA_pseudo	9.99327833	tRNA_pseudo	5.0993	tRNA_pseudo	6.9446
SPCTRNASER.09	7.4159162	SPCTRNASER.09	4.99932	SPCTRNASER.09	7.53290
SPCTRNAARG.10	8.04457571	SPCTRNAARG.10	4.31552	SPCTRNAARG.10	6.77475
SPCTRNAASP.05	7.84614666	SPCTRNAASP.05	6.3808	SPCTRNAASP.05	8.12602
SPCTRNAARG.11	7.15712714	SPCTRNAARG.11	6.33694	SPCTRNAARG.11	8.04280
unknown_1068	8.84819	unknown_1068	4.96345	unknown_1068	6.45070
SPCTRNALYS.10	9.55082	SPCTRNALYS.10	5.20770	SPCTRNALYS.10	6.75928
unknown_1083	6.90254488	unknown_1083	4.49362	unknown_1083	5.21615
SPCTRNAASP.06	6.99743571	SPCTRNAASP.06	4.91932	SPCTRNAASP.06	5.79591

SPCTRNAARG.12	8.02921166	SPCTRNAARG.12	5.20523	SPCTRNAARG.12	6.48411
SPCTRNAVAL.09	10.1133714	SPCTRNAVAL.09	5.93735	SPCTRNAVAL.09	7.71242
SPCTRNATHR.08	10.09908	SPCTRNATHR.08	6.86723	SPCTRNATHR.08	8.56719
SPCTRNALEU.12	16.813637	SPCTRNALEU.12	10.5580	SPCTRNALEU.12	14.2831
unknown_1088	15.2108342	unknown_1088	11.8086	unknown_1088	14.7092
SPCTRNAGLU.10	16.4327714	SPCTRNAGLU.10	11.407	SPCTRNAGLU.10	13.8643
unknown_1090	15.4573570	unknown_1090	11.1284	unknown_1090	14.5009
SPCTRNALEU.13	14.9474222	SPCTRNALEU.13	10.5083	SPCTRNALEU.13	13.3700
SPCTRNATHR.09	17.2531	SPCTRNATHR.09	12.7677	SPCTRNATHR.09	15.6648
SPCTRNAVAL.10	15.3731857	SPCTRNAVAL.10	12.1528	SPCTRNAVAL.10	14.0966
SPCTRNAARG.13	14.9404	SPCTRNAARG.13	12.202	SPCTRNAARG.13	14.5821
SPCTRNAASP.07	15.0790714	SPCTRNAASP.07	11.891	SPCTRNAASP.07	15.0995
unknown_1093	10.74339	unknown_1093	7.94093	unknown_1093	10.2086
SPCC1322.10	7.53142777	SPCC1322.10	6.45713	SPCC1322.10	9.29038
SPCC13B11.01	6.66749755	SPCC13B11.01	10.725	SPCC13B11.01	9.12272
SPCC737.04	6.52694775	SPCC737.04	5.46221	SPCC737.04	8.21523
SPAC1F8.07c	5.96218040	SPAC1F8.07c	6.56338	SPAC1F8.07c	7.88858
SPAC19E9.03	5.26577391	SPAC19E9.03	4.39271	SPAC19E9.03	7.06675
SPAC23C11.06c	5.04500048	SPAC23C11.06c	4.54176	SPAC23C11.06c	5.88658
SPAC4H3.10c	5.94711742	SPAC4H3.10c	7.61141	SPAC4H3.10c	7.93072
SPAC19G12.09	4.54233827	SPAC19G12.09	4.11602	SPAC19G12.09	5.62805
SPAC16.05c	5.49504704	SPAC16.05c	4.53567	SPAC16.05c	7.32661
SPAC9E9.01	5.41296709	SPAC9E9.01	3.11240	SPAC9E9.01	4.31917
SPBC119.05c	5.73064073	SPBC119.05c	5.42005	SPBC119.05c	6.53816
SPBC19G7.06	5.53343878	SPBC19G7.06	4.50366	SPBC19G7.06	7.45538
SPCC736.15	7.03249261	SPCC736.15	8.17003	SPCC736.15	8.73958
SPBC1815.01	8.56854754	SPBC1815.01	12.5037	SPBC1815.01	9.93707
unknown_1101	5.09936	unknown_1101	3.79389	unknown_1101	6.33052

(Fig. 3. 12A). Indeed, even the *PMA1* gene, which the gene-specific ChIP experiments clearly indicated as enriched with all three RPs, at the stringent threshold I have selected the MAT software flags this gene as enriched only for RpL1102 and RpL2502 (Fig. 3. 12B). For these reasons, the number of regions which are associated with RPs is probably underestimated.

To gain further insight, I examined more closely the regions which overlapped between the three RPs and classified the shared 130 hits according to whether the genes encoded proteins or non-protein coding RNAs. Surprisingly, only ~38% (50 loci) are protein-coding genes. Of the others, ~36% (48 loci) are tRNA genes, ~19% are in repeat regions of the genome, and the other ~5% correspond to miscellaneous non-protein coding loci, including 1 snoRNA gene and 6 snRNA genes (Fig. 3. 12C) (Table 3.1).

3.9 Functional classification of the genes associated with RPs

Next I was interested to know whether the genes which are associated with RPs are involved in particular biological functions. In order to perform this analysis, all enriched genes (coding and non-coding) were grouped into Gene Ontology (GO) categories. There are many tools available for performing GO analysis - for example, EasyGO, GOminer, DAVID, GENECODIS etc. In collaboration with W. Varsally I used the GENECODIS2.0 software because this software includes the *S. pombe* genome (Carmona-Saez et al. 2007). Analysis for 'GO biological process' with the enriched genes for the three RPs, identified that RpL1102 is associated with the maximum number of gene classes (8 GO-BP classes) whereas RpL2502 and RpL703 associate with 6 and 3 GO-BP classes respectively. Further analysis showed that most of the enriched genes are involved in the translation elongation process in *S. pombe* (Fig. 3.13). This probably reflects the large fraction of hits (48) which are tRNA genes. The other two major classes, which were also shared by the three RPs, are genes involved in glycolysis and genes involved in gluconeogenesis, glycolysis (Fig. 3.13).

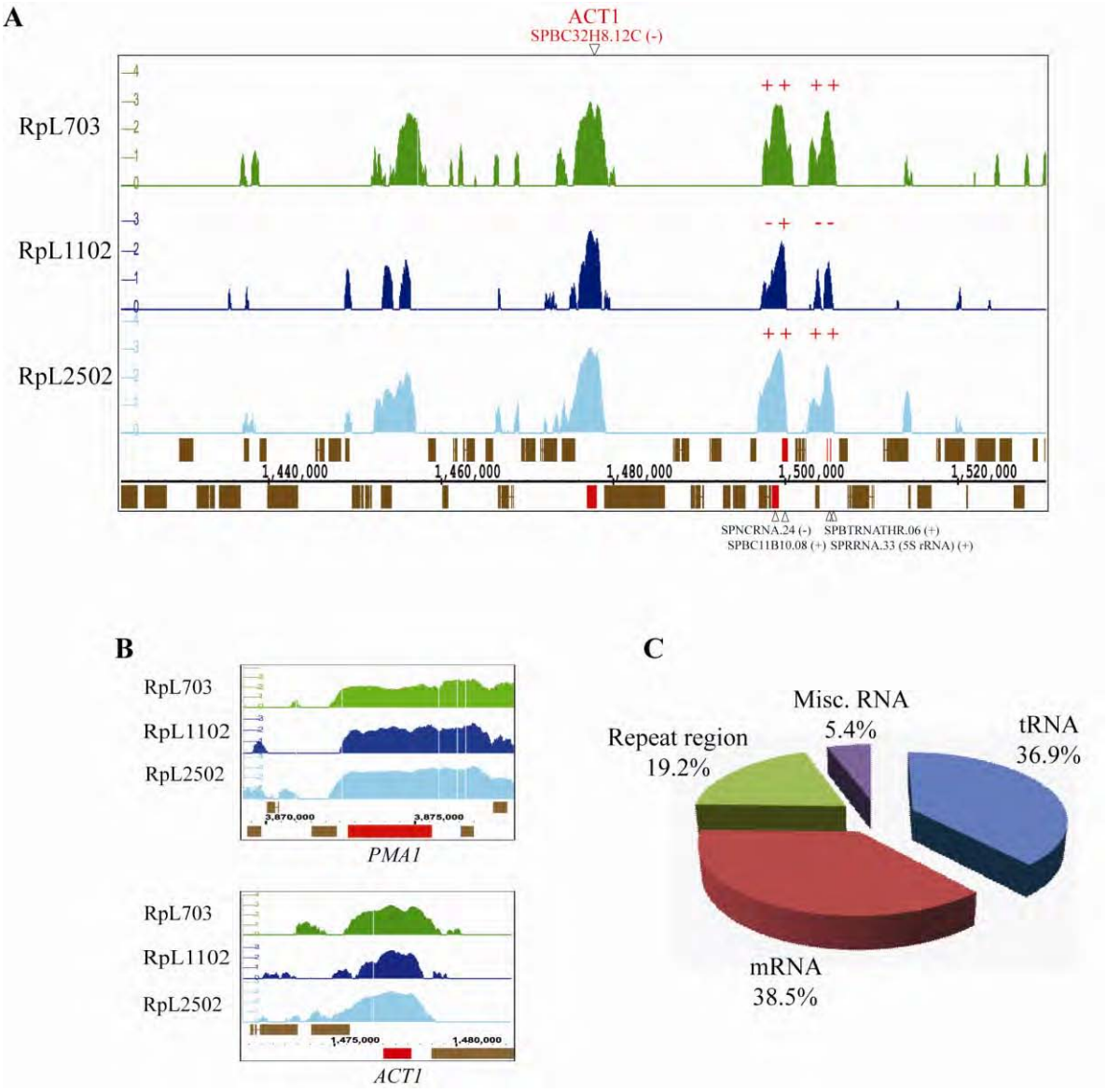


FIGURE 3.12 RPs are associated to both coding and non-coding genes. (A) Enrichment at loci flanking the *ACT1* gene. IGB generated enrichment profiles flanking the *ACT1* gene (SPBC32H8.12C). Genes on both DNA strands are represented by either brown or, if enriched by at least one protein, red boxes. The systematic names of the enriched genes are indicated (+ refers to genes in the DNA strand above, - to genes in the other strand). The red - or + sign above the peak indicates whether the region was enriched with the RP indicated on the left, at the stringent p-value of $1e^{-4}$ (see Material and Methods). X-axis show genome position in base pairs, the Y-axis log2 MAT enrichment score (0 to 4). (B) Two of our tested genes (*PMA1* and *ACT1*) used for standardization are highly enriched on ChIP-on-chip microarray. Target genes are indicated by red colour and other ORFs are presented in brown colour, enrichment profile is represented from IGB viewer. (C) Pie-chart showing the proportions of the genes which are associated with all three RPs that fall within various gene classes.

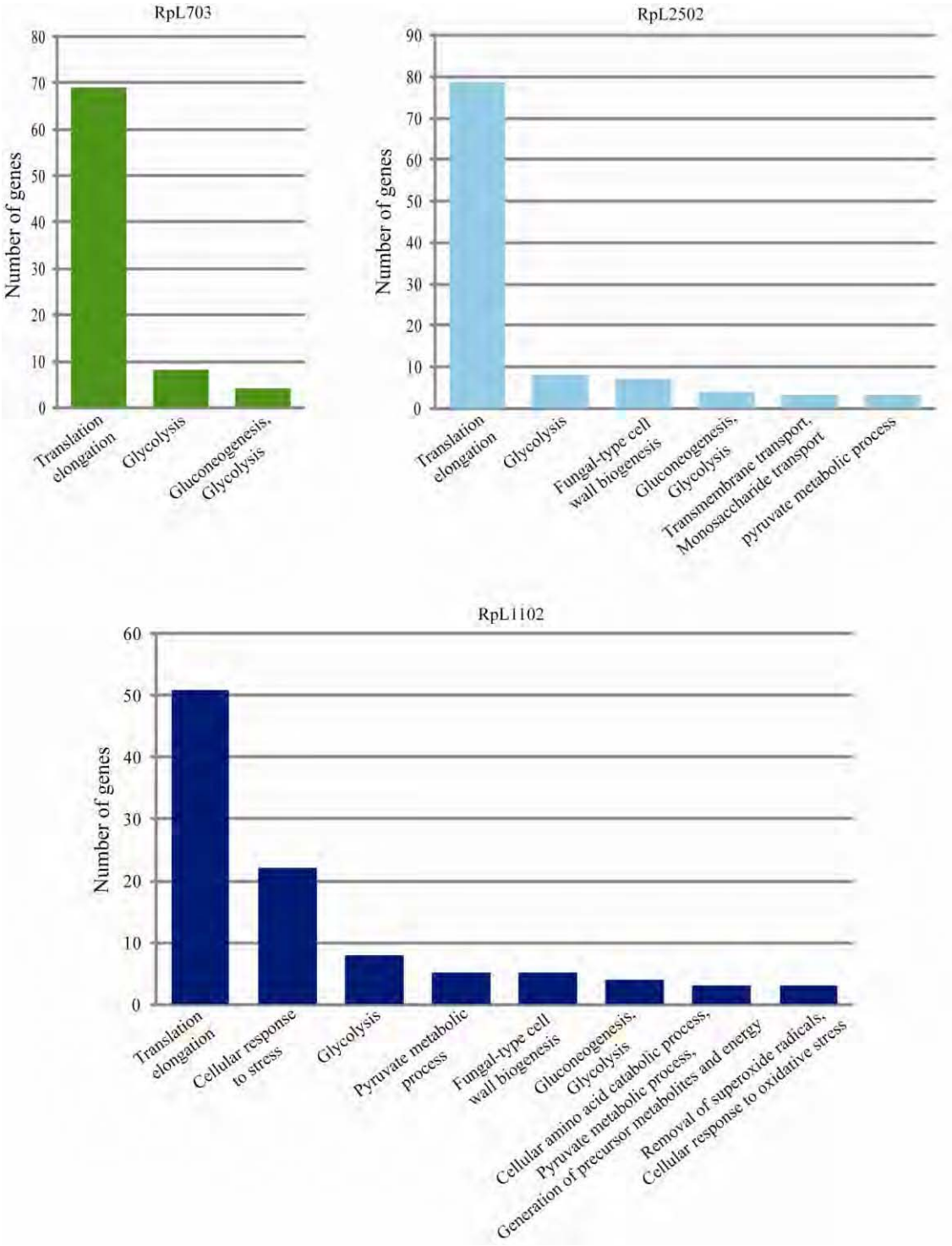


FIGURE 3.13 Functional clasisfication of RP binding genes. All *S. pombe* genes identified as binding sites for the three tested RPs are annotated according to the Gene Ontology-biological processes (GO) using GENECODIS2.0. Three RPs indicated above each histogram are coded with three different colours, number of genes shared by each GO category are on X-axis.

3.10 Association of RPs to the transcription sites does not correlate with transcription rate or Pol II occupancy

Previous reports in *D. melanogaster* and *S. cerevisiae* identified different RPs from both ribosomal subunits are present at transcription sites (Brogna et al. 2002; Schroder and Moore 2005). A possible explanation for these findings was that RPs bind to these sites unspecifically because they are abundant and basic (pI>10) proteins, and may bind transcribed regions because the RNA or exposed DNA is acidic. To test this assumption I, in collaboration with W. Varsally, calculated the Pearson correlation between RP association and Pol II occupancy. To do this, .CEL files of Pol II ChIP-on-chip data were obtained from a published study (Wilhelm et al. 2008), and analysed with the MAT software. After removal of all the genes which did not show any association with either RPs or Pol II, a total of 1110, 1192 and 1153 genes RP occupancy versus Pol II correlation were plotted for RpL703, RpL1102 and RpL2502 respectively. Correlation analysis showed a very low degree of correlation existed between RP and Pol II occupancy with the Pearson correlation coefficient of 0.12 for RpL703 (Fig. 3.14A), 0.16 for RpL1102 (Fig. 3.14B) and 0.15 for RpL2502 (Fig. 3.14C). I found a correlation with Pol II for RpL703, RpL1102 and RpL2502 for a very small number of genes 6, 18 and 12 genes respectively (list of genes is shown in table 3.2). As transcript levels are a proxy of the transcription rate of the corresponding genes, our next target was to find whether there is correlation between the transcript level and RP occupancy in *S. pombe*. To do this, high-throughput sequencing data of complementary DNAs (RNA-Seq) and data from high-density Affymetrix tiling arrays of global transcripts of the *S. pombe* were obtained from a previous work (Wilhelm et al. 2008). A Pearson correlation analysis was carried out between the Affymetrix global transcripts tiling arrays data and RPs ChIP-on-chip data (Fig. 3.15 left panel). Similar correlation analysis was performed between RNA-Seq data and RPs

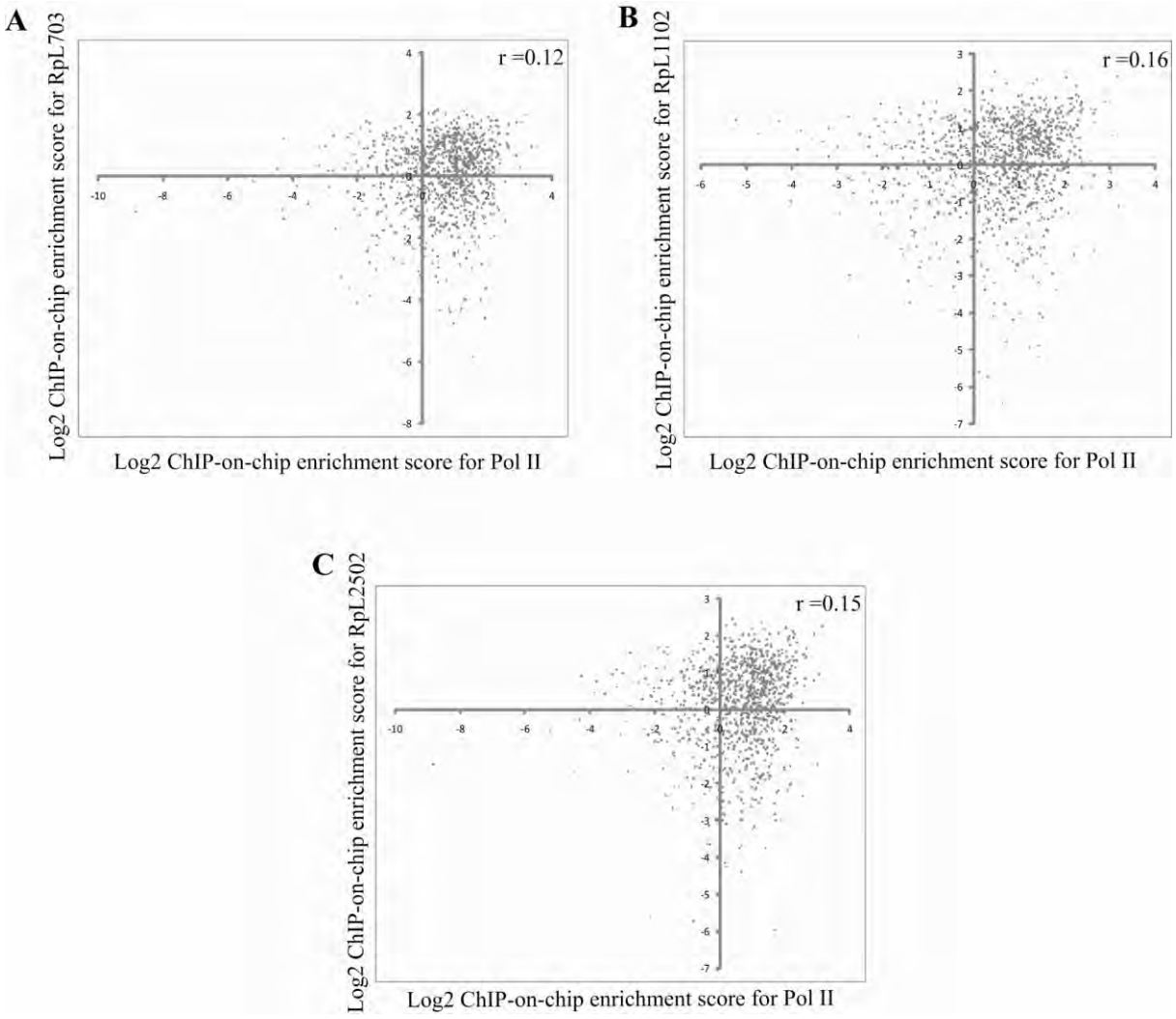


FIGURE 3.14 RPs chromosomal association shows little correlation to Pol II occupancy. Scatter plot showing RPs versus Pol II occupancy; based on published genome wide enrichment scores for Pol II (X axis) (Wilhelm et al. 2008) and RPs enrichment data from this study (Y axis). A shows correlation with RpL703; B with RpL1102, and C with RpL2502. Pearson correlation is shown at the top right of each panel.

Table 3.2- List of genes showing correlation between Pol II and RPs occupancy

List of genes showing correlation between Pol II and RpL703 occupancy

SPAC4H3.10C	PYRUVATE KINASE
SPAC26F1.05	SEQUENCE ORPHAN
SPCC736.15	PROTEIN KINASE INHIBITOR
SPCC1393.08	TRANSCRIPTION FACTOR, ZINC FINGER PROTEIN
SPCC24B10.21	TRIOSEPHOSPHATE ISOMERASE
SPCC13B11.01	ALCOHOL DEHYDROGENASE

List of genes showing correlation between Pol II and RpL1102 occupancy

SPAC9.09	HOMOCYSTEINE METHYLTRANSFERASE
SPAC343.12	CONSERVED FUNGAL PROTEIN
SPAC4H3.10C	PYRUVATE KINASE
SPAC16.02C	RNA EXPORT FACTOR SRP2
SPAC4F10.15C	WASP HOMOLOG
SPBC16E9.16C	LSD90
SPCC613.05C	60S RIBOSOMAL PROTEIN L35
SPCC330.06C	THIOREDOXIN PEROXIDASE
SPCC736.15	PROTEIN KINASE INHIBITOR
SPCC594.01	DUF1769 FAMILY PROTEIN
SPCC1393.08	TRANSCRIPTION FACTOR
SPCC63.14	CONSERVED FUNGAL PROTEIN
SPCC24B10.21	TRIOSEPHOSPHATE ISOMERASE
SPCC13B11.01	ALCOHOL DEHYDROGENASE ADH1
SPCC417.08	TRANSLATION ELONGATION FACTOR EEF3
SPCC297.03	SERINE/THREONINE PROTEIN KINASE

SPCC1739.13	HEAT SHOCK PROTEIN SSA2
SPCP1E11.04C	MEMBRANE ASSOCIATED PROTEIN PAL1

List of genes showing correlation between Pol II and RpL2502 occupancy

SPAC9.09	HOMOCYSTEINE METHYLTRANSFERASE
SPAC343.12	CONSERVED FUNGAL PROTEIN
SPAC4H3.10C	PYRUVATE KINASE
SPAC16.02C	RNA EXPORT FACTOR SRP2
SPAC4F10.15C	WASP HOMOLOG
SPCC613.05C	60S RIBOSOMAL PROTEIN L35
SPCC736.15	PROTEIN KINASE INHIBITOR
SPCC594.01	DUF1769 FAMILY PROTEIN
SPCC1393.08	TRANSCRIPTION FACTOR
SPCC24B10.21	TRIOSEPHOSPHATE ISOMERASE
SPCC13B11.01	ALCOHOL DEHYDROGENASE ADH1
SPCC417.08	TRANSLATION ELONGATION FACTOR EEF3

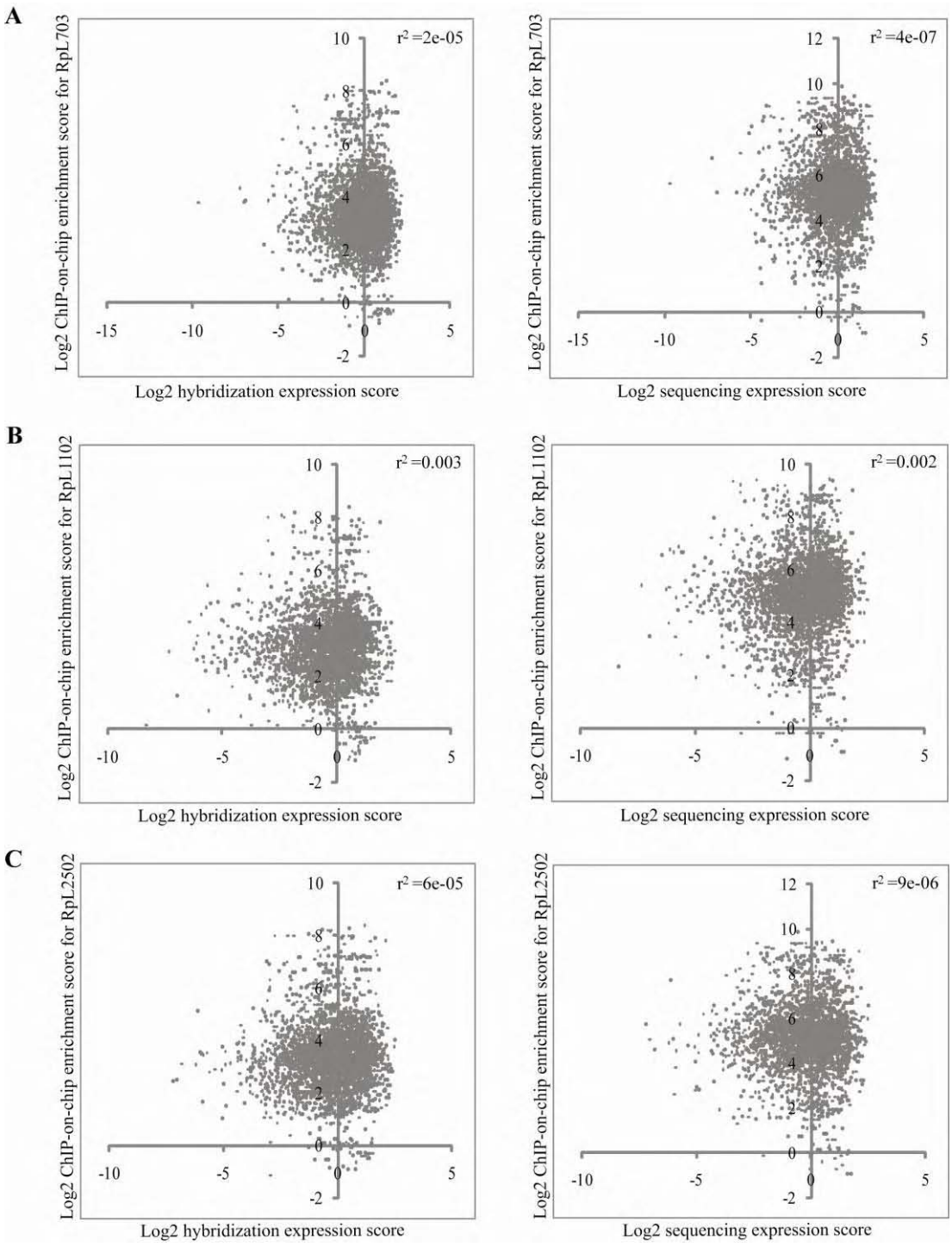


FIGURE 3.15 RPs chromosomal association shows no correlation to transcription rate. Left panel: scatter plot showing correlation between RPs ChIP-on-chip enrichment signals (Y axis) versus Affymetrix expression-chip hybridization signals (X axis) (Wilhelm et al. 2008). Right panel: scatter plot showing correlation between RPs ChIP-on-chip enrichment signals (Y axis) versus gene-expression scores based on high-throughput sequencing (X axis) (Wilhelm et al. 2008). A shows correlation with RpL703; B with RpL1102, and C with RpL2502. Pearson correlation is shown at the top right of each panel.

ChIP-on-chip data (Fig. 3.15 right panel). This analysis did not show any correlation between transcript level and RP association to the corresponding chromatin region (Fig. 3.15).

3.11 RPs do not bind transcriptionally active H2A beta gene during S phase

In contrast to the earlier findings that RPs always bind to highly transcribing regions, our RP-Pol II correlation data clearly shows that only a very small subset of genes show a high correlation for assiociation of both RPs and Pol II (Table 3.2). To further validate our bioinformatics finding, I performed cell cycle specific ChIP to see whether RPs bind to the histone H2A beta gene during S phase when this gene is activly transcribed. For this purpose ChIP analysis was performed on cells syncronysed in S-phase; this was achieved by using the temperature-sensitive *cdc25-22* mutation which allows synchronization cells at a particular stage of the cell cycle - *cdc25-22* is a temperature sensitive allele of $cdc25^{+}$ and when incubated at the restrictive temperature of 36.5 °C, the *cdc25-22* cells accumulate in late G2 prior to mitosis. Once synchronized, the cells can be released to the permissive temperature of 25 °C for normal growth (Russell and Nurse 1986). RpL703 was tagged with 3HA into the *cdc25-22* mutant strain by homologous recombination using the same set of primers used earlier for WT strain. Tagging was confirmed both by PCR and Western blot analysis (Fig. 3.16A & B). Cultures corresponding to S-, G1/M- and G2-phase were analysed by ChIP using a set of primers for the histone H2A betagene (SPAC19G12.06c). Surprisingly, the realtime PCR showed no enrichment of RpL703 in S-phase, G1/M or G2 phase (Fig. 3.16C).

3.12 RPs accumulates at heterochromatic regions

Interestingly, our genome-wide analysis revealed prominent RPs peaks in all heterochromatic loci, including centromeres, telomeres and mating type locus (Fig. 3.11A). The highest enrichment was at centromeres. The three fission yeast centromeres vary in size with

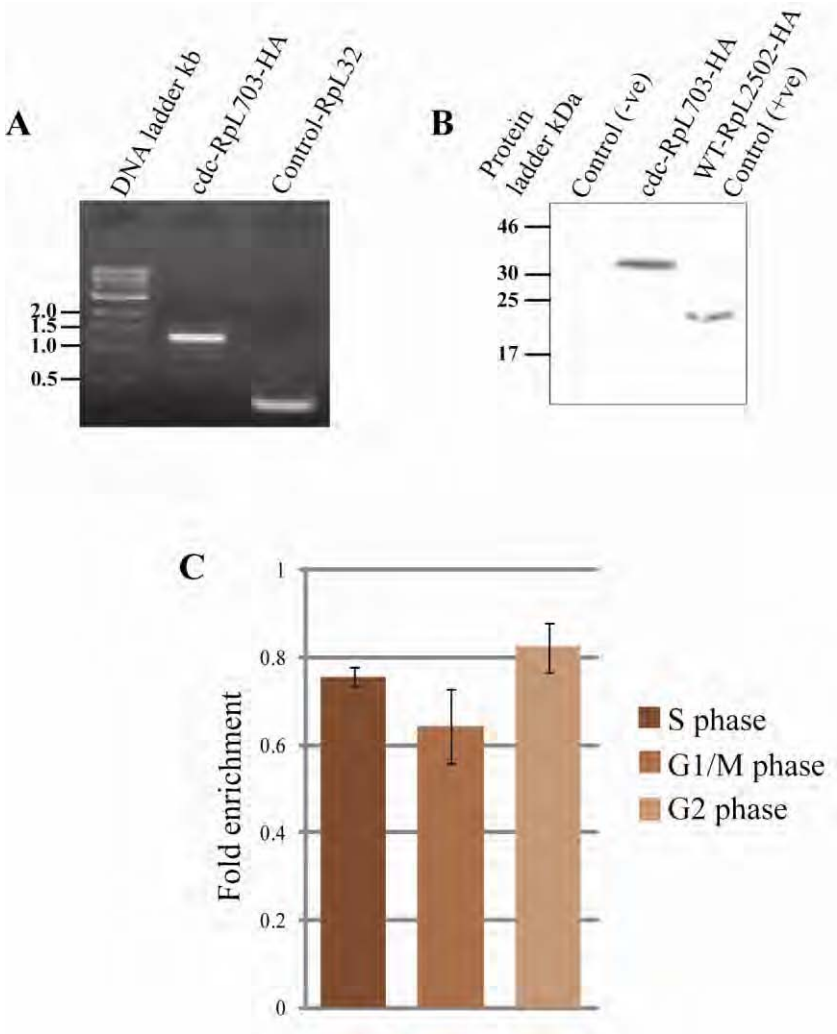
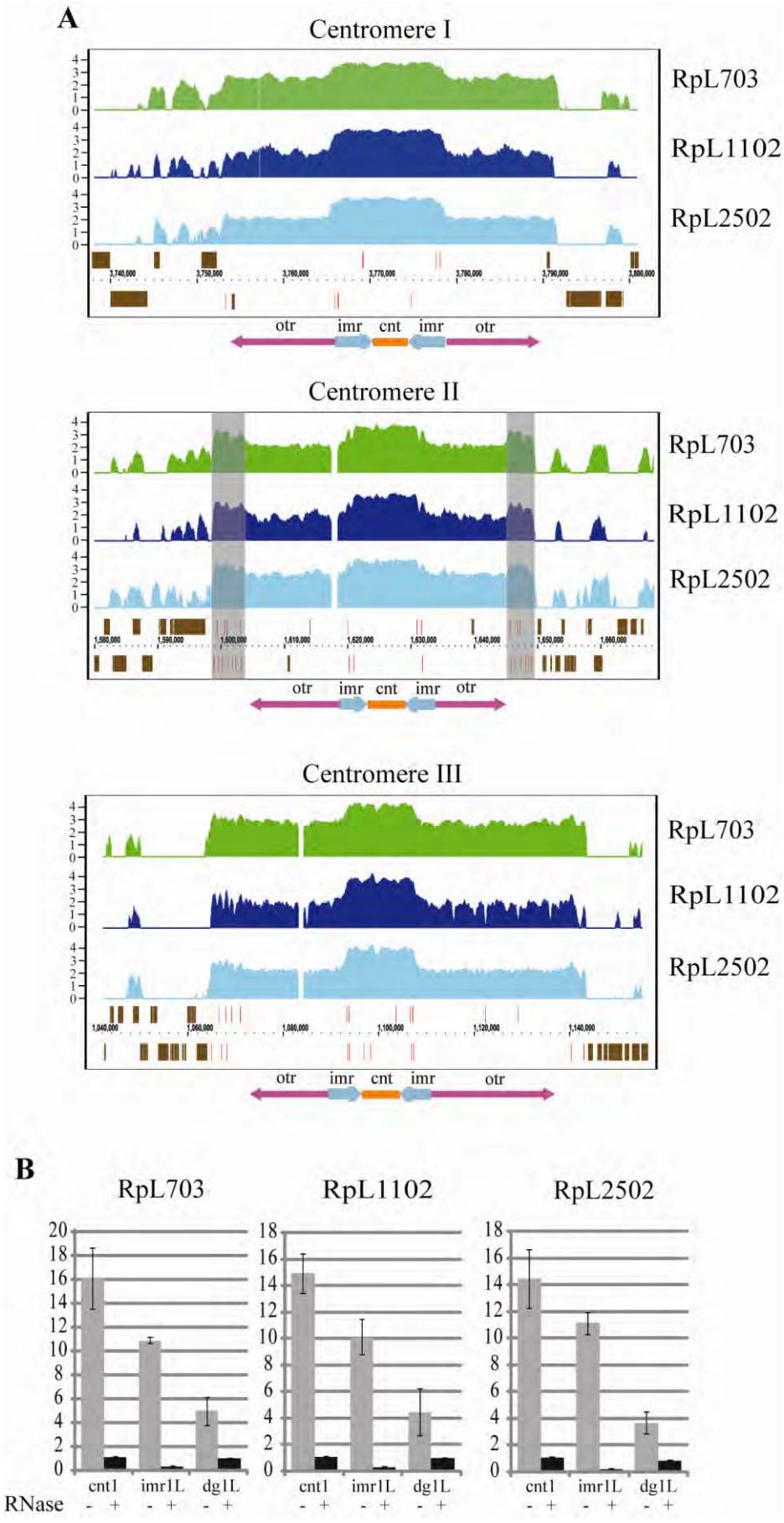


FIGURE 3.16 RPs association to chromatin is transcription rate independent. (A) Agarose gel showing the expected PCR products from correct HA tagging of RP gene indicated. Control- PCR product from RpL32 gene. (B) Western blotting analysis of whole-cell protein extracts of cells expressing the HA-tagged RPs indicated. Positive control- WT-RpL2502-HA (C) Quantification of ChIP-enriched DNA from cdc-RpL703-HA strain using real-time PCR with the primers of histone H2A gene; coloured bars represent ChIPed DNA from different cell cycle stages indicated on right. Error bars represent strandard deviation of three repeats.

centromere I (cen1) occupying 35 kb (5.6 kb), centromere II (cen2) covering 65 kb (4.4 Mb) and centromere III (cen3) 110 kb (2.5 Mb) (Takahashi et al. 1992; Wood et al. 2002). The three centromeres have a central core (cnt) of 4-7 kb of non-repetitive sequence. Each *cnt* element is surrounded by inverted 'innermost' (imr) repeats which are unique to each centromere. The outer repeat regions that flank cnt/imr on each side are composed of 'dg' and 'dh' elements. Parts of the outer repeat elements are highly homologous between the different centromeres (dg is 97% identical, whilst dh is 48% identical) and there is also some homology with elements at the mating type locus and telomeres. The main difference in size between the three centromeres results from the variation in number of outer repeat elements. In addition, there are differences in the organization and orientation of these elements relative to the central core at each centromere (Grewal and Klar 1997; Pidoux and Allshire 2004). Additionally, dg/dh repeats sequences are transcribed into small interfering RNA (siRNA) to promote heterochromatin formation during S-phase (Volpe et al. 2002; Chen et al. 2008). Detailed analysis revealed that RPs were enriched at all centromeric regions in all three centromeres (Fig. 3.17A), highest enrichment was visible at cnt and imr. To investigate this further, I examined segments of the cnt, imr and dg domains by ChIP and real-time.

All three RPs are most enriched in association with the cnt region, and RNase treatment destroys this association (Fig. 3.17B). The telomere sequence coverage on the *S. pombe* microarray is incomplete and the best coverage is of the subtelomere located on the left arm of chromosome 1 (Cam et al. 2005). We identified association of RPs to this region of the telomere. A medium domain of RP association was also detected in the rRNA present on the left and right arm of chromosome 3 (Fig. 3.17C). The rDNA consists of ~150 tandem repeats, but the microarray probes represented only a few repeat units, the gaps between RPs ChIP-on-chip peaks reflect the absence of microarray probes at repetitive DNA sequence (Fig. 3.17C).


A
Centromere I
RpL703
RpL1102
RpL2502
otr imr cnt imr otr
Centromere II
RpL703
RpL1102
RpL2502
otr imr cnt imr otr
Centromere III
RpL703
RpL1102
RpL2502
otr imr cnt imr otr
B
RpL703
RpL1102
RpL2502
cnt1 imr1L dg1L
RNase - + - + - +

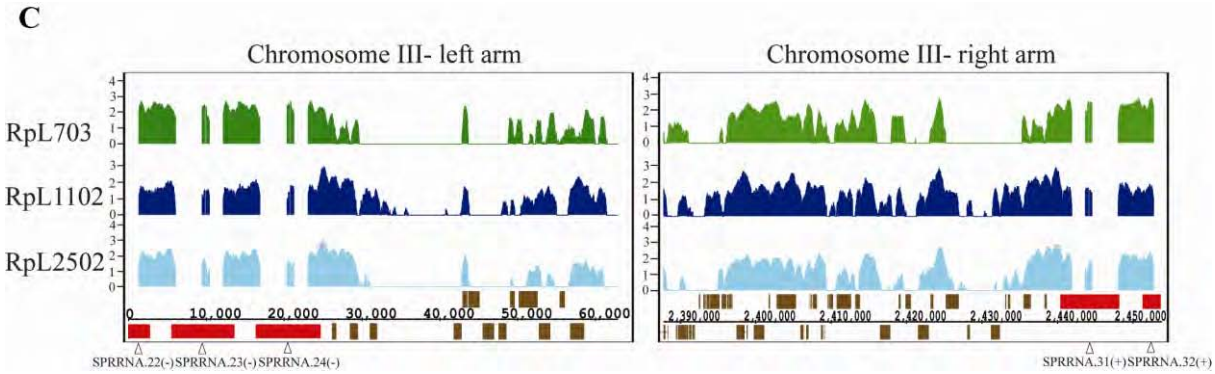


FIGURE 3.17 RPs are highly enriched at centromeric regions as well as at rDNAs. (A) ChIP-on-chip enrichment graphs for RPs around the centromeric region of each chromosome, generated with the IGB software. The map below each panel shows a schematic of fission yeast centromeres, with the three major domains labelled otr, imr and cnt (see text for more details). Centromeric tRNA gene loci are indicated by red lines, and the other genes by brown boxes. Two tDNA clusters in centromere 2 are highlighted by gray boxes. (B) real-time PCR quantification of RPs enrichment at three specific centromeric repeats regions, with or without RNase digestion prior to ChIP. (C) Detailed ChIP-on-chip distribution of RPs in the rDNA on the left and right arm of chromosome 3. Telomeric 35S rDNA gene loci are indicated by red boxes, and the other genes by brown boxes.

3.13 The association of RPs with tRNA genes requires their transcription

Within centromeric regions RPs peaks correspond to tRNA genes (Fig. 3.17A). Notably, many of the tRNA genes (53) are located in the centromere at *S. pombe* (Wood et al. 2002) (Fig. 3.17A). For example on chromosome II, there are two obvious peaks of enrichment at the edges of the centromere which coincide with two clusters of tRNA genes (Fig. 3. 17A; centromere II, tRNA clusters highlighted). However, tRNA genes positioned outside the centromere are also clearly enriched (Fig. 3.18A, tRNA genes are indicated by arrows).

In addition, I systematically analysed the RP enrichment score at all known tRNA loci and classified them into 6 classes depending on their enrichment, ranging from <1 to >5 fold enrichment. RPs are essentially present at all tRNA genes (Fig. 3.18B).

To further investigate the association of RPs with tRNA genes, I assessed the recruitment of RpL703 to an ectopic $tDNA^{Tyr}$ construct integrated at the *leu1*$^{+}$ locus (Pebernard et al. 2008). I found that RpL703 associates with a wild-type copy of the gene construct but not with two mutant derivatives (Fig. 3.18C). One mutant (mutB Box-$tDNA^{Tyr}$) carries a C→G mutation in the B Box of the Pol III promoter, inhibiting TFIIIC binding and transcription (Kurjan and Hall 1982; Baker et al. 1986; Pebernard et al. 2008), and the other ($\Delta tDNA^{Tyr}$) lacks the tRNA sequence (Pebernard et al. 2008). It appears that RpL703 binds to this tRNA gene only if it is actively transcribed.

3.14 RPs association with transcriptionally active chromatin is minimally affected by translation inhibitory drugs

Next, I examined the effects of different translation inhibitory drugs on the RPs ChIP signals. I used 3-Amino-1,2,4-Triazole (3-AT) – 3-AT is a competitive inhibitor of imidazoleglycerol-phosphate dehydratase, which is an enzyme catalysing the sixth step of histidine production and

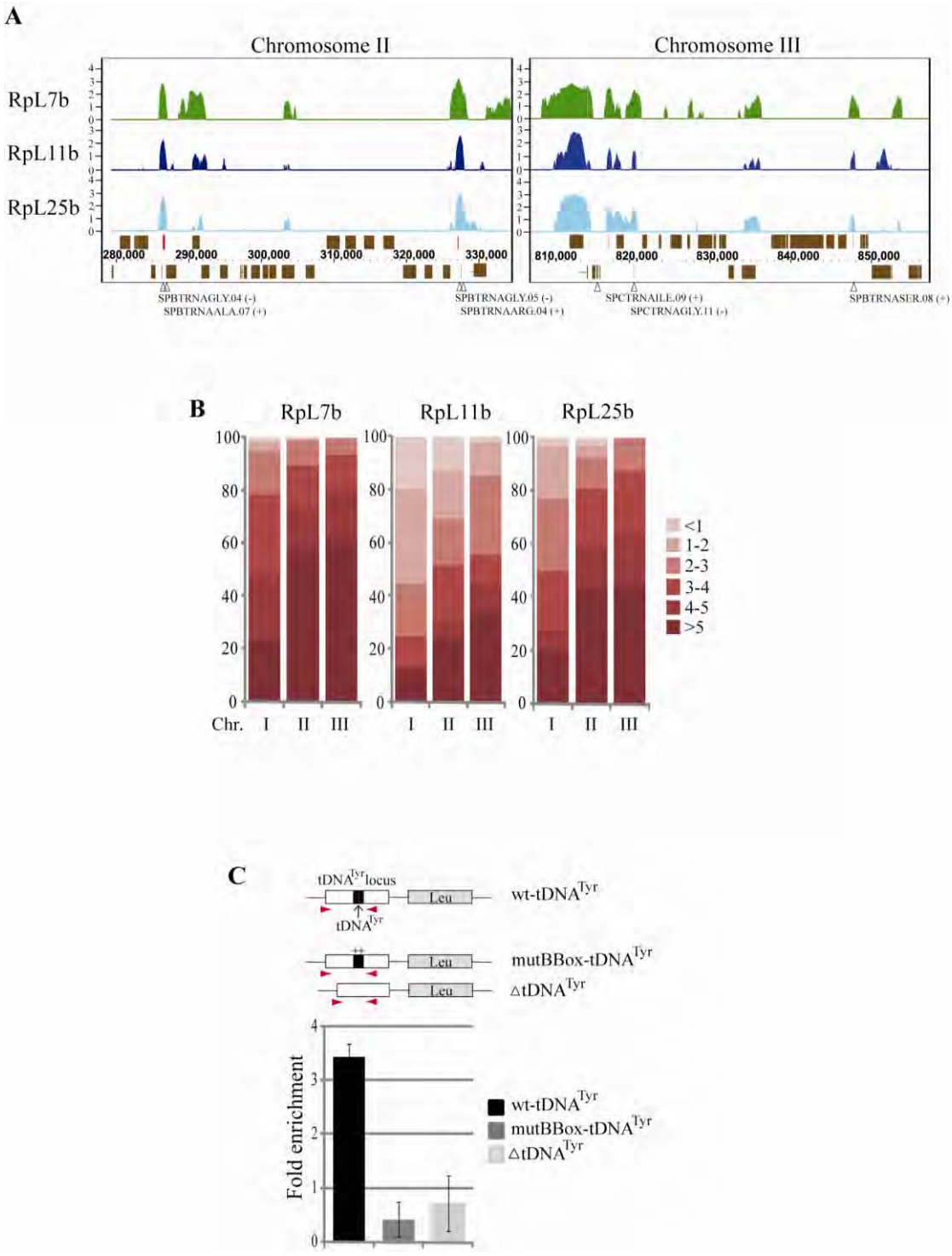


FIGURE 3.18 RPs associate with most tRNA genes (A) Non-centromeric regions of the chromosomes II and III showing RPs associated with tRNA genes. There are peaks of enrichment at tRNA loci (highlighted by red lines and by arrows at the bottom); individual tRNA are indicated (+ and - refer to genes in the upper and lower DNA strands, respectively). (B) Histogram displaying the association of the RPs with all known 171 tRNA genes: all tRNA genes were classified in six classes based on the increasing degree to which they were RPs-enriched (from 0 to 6 fold, indicated by the colour legend on the right). The heights of the bars represent cumulative percentages of the tRNAs encoded by each chromosome. (C) Schematic

of the $tRNA^{Tyr}$ constructs. Upper panel shows the wild-type construct, below a derivative carrying a mutation in the promoter (B Box deletion) and the bottom a derivative carrying a deletion of the entire tRNA sequence. Red arrow heads represent the location of primers used for real-time PCR. Graph on the bottom show real-time PCR quantification of ChIP enrichment for RpL703-HA at the different tRNA constructs. Error bars represent standard deviation from three repeats.

thereby stops translation. I also used cycloheximide (CHX), which inhibits the transfer of the amino acid from the aminoacyl-tRNA to the peptide chain and therefore blocks elongation. Our hypothesis was that when translation is stopped in the presence of 3-AT, if there is nuclear translation occuring at transcription sites, the signal of ChIP against any tested RP will be reduced because there will be fewer ribosomes present as transcription sites. The opposite trend should be observed in the case of CHX, since the ribosomal subunits should be stalled onto the mRNA. For both drugs cells were treated for 30 min before fixation (3AT, 40 mM; CHX, 100 µg/ml) (Dickinson 1983; Erickson and Hannig 1995). We tested this hypothesis with RpL703. I observed only a small reduction of ChIP signal with either the 3-AT or CHX treatment (Fig. 3.19). The signal reduction was more prominent with 3-AT particularly at the promoter of both *PMA1* and *ACT1*. Further experiments will be required to reach a conclusion on whether the association of RPs to genes could be affected by translation.

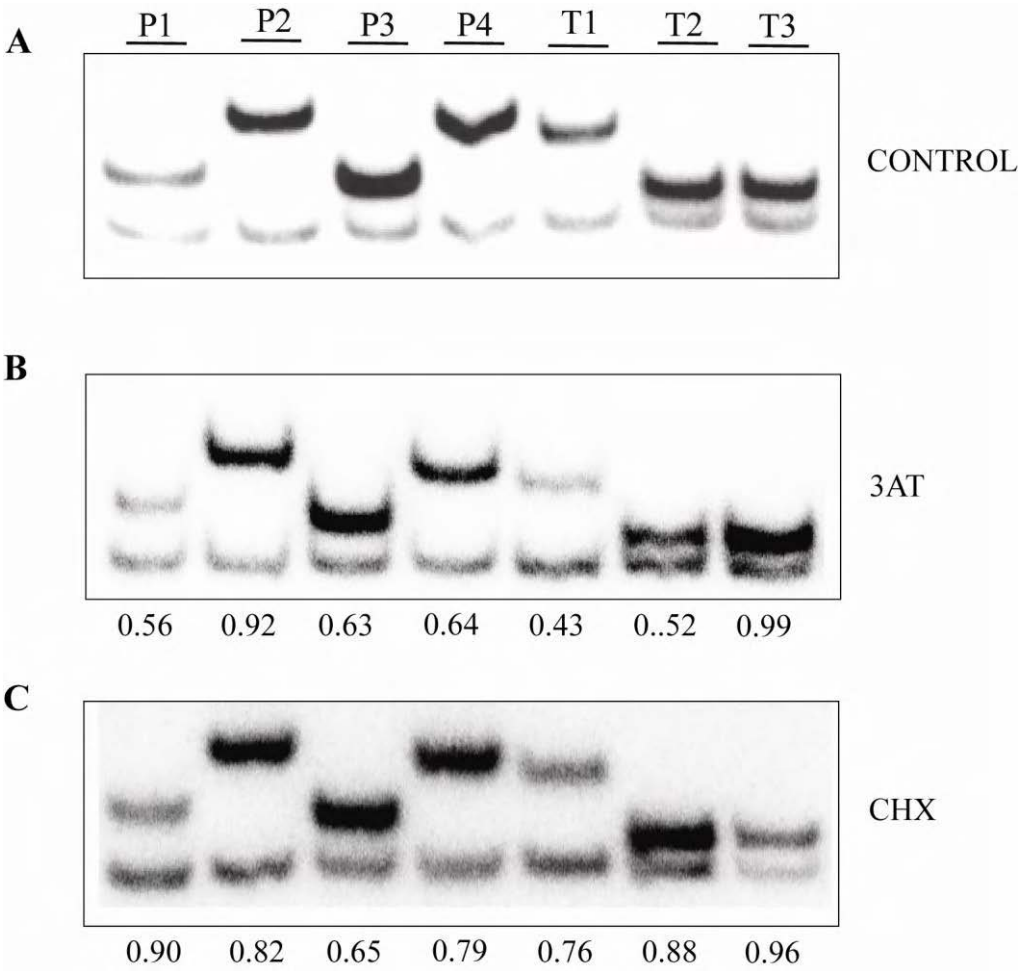


FIGURE 3.19 Treatment of cells with translation inhibitory drugs show some reduction in ChIP signal. (A) Polyacrylamide gel with radiolabelled PCR products produced by the *PMA1* and *ACT1*-specific primer pairs (top bands) and the pair corresponding to the intergenic region (bottom bands), using ChIP enriched DNA without drug treatment. (B) PCR products as in A using samples treated with 3-AT. (C) PCR products as in A using samples treated with CHX. The relative enrichment of each DNA fragment is expressed as ratio of ratio of the intensity of the same fragment produced without drug treatment and indicated below each lane.

4. DISCUSSION- I

The results we have presented here indicate that RpL703, RpL1102 and RpL2502 – chosen as representative of all RPs – are present at many transcription sites on *S. pombe* chromosomes. This finding confirms previous observations on the polytene chromosomes of *D. melanogaster* (Brogna et al. 2002) and indicates that the physical association of RPs with transcription sites may be a general feature of eukaryote cells. As in a previous study in *S. cerevisiae* (Schroder and Moore 2005), we found RPs both at protein-coding and at non protein-coding genes in *S. pombe*. The chromosomal association of RPs is RNA-dependent: RNase treatment eliminates the ChIP signal for RpL7 and RpL11 and reduces that of RpL25. Maybe RpL25 can also contact DNA directly – it belongs to the RpL23 family of RPs, which in higher eukaryotes contains a histone-H1-like domain in the N-terminus which could bind DNA (Ross et al. 2007).

The observed RNase sensitivity suggests that RPs associate with genes that have nascent RNA, but there was little correlation between RP enrichment at genes and either Pol II occupancy or the presence of a steady-state transcript at the same genes. For example, there is not even a visual sign of enrichment at some highly expressed Pol II genes like RpS1702 (SPCC24B10.09) and Sec26 (SPBC146.14c); we also assayed the histone hta2 (SPAC19G12.06c) by ChIP during S phase and found no enrichment. Highly transcribed genes typically have more Pol II molecules engaged at the transcription unit (Wilhelm et al. 2008), so their DNA should be more accessible and replete with nascent RNA (Jackson et al. 1993; Wansink et al. 1996). We do not fully understand these apparently contradictory observations, but the fact that RPs are not associated with some highly transcribed genes at least suggests that recruitment is not primarily driven either by the passive affinity of RPs for RNA or DNA or by their association to the Pol II C-terminal domain.

The unexpected finding that the three representive RPs are most enriched at the centromeres of all chromosomes in an RNase-sensitive manner indicates that RNA is essential for the association – the centromeric regions are highly transcribed in *S. pombe* (Djupedal et al. 2005). And even the association of RpL25 with centromeric regions is very sensitive to RNase treatment – possibly the histone-like domain of RpL25 cannot contact the DNA at centromeres. RPs association within the centromeres is most apparent at tRNA genes, which cluster in the centromeres in *S. pombe* (Wood et al. 2002), but RPs also associate with tRNA genes located outside centromeres. We have also considered whether the strong association with centromeric regions might reflect spatial proximity of the centromeres to the nucleolus. This seems unlikely: in *S. pombe* the rDNA repeats are in both telomeres of chromosome III (Wood et al. 2002), yet the enrichment profiles at these regions is not significantly different from that at the telomeres of the other two chromosomes. Furthermore, rDNAs regions show only peaks of moderate enrichment and the flanking regions are not significantly more enriched. Association at tRNA loci appears to require transcription: RpL7 associates with an active tRNA gene but not at a locus without a functional promoter. These results suggest that RPs might influence the biogenesis of tRNAs, maybe through some role in Pol III transcription. The recent findings that three other RPs – RpL6, RpL26 and RpL14 – copurify with TFIIIE in *S. cerevisiae* (Dieci et al. 2009) and that RpL11 represses Pol III transcription in mammalian cells (Dai et al. 2010) support this view.

Individual RPs might also have specific non ribosomal functions in S. *pombe*. For example, RpL11 might both bind specific transcription factors at the promoters of a subset of genes involved in growth as it does in mammalian cells (Dai et al. 2007) and also be recruited at other genes as a complex with other RPs. It is communally understood that to carry out the extra ribosomal functions RPs need to be free from the ribosomal subunits, however future studies might change this view; in *E. coli* for example ribosomal protein S10 (a classic example of RP

moonlighting, see Warner and McIntosh, 2009) can bind the transcription factor NusG while still associated to the small ribosomal subunit (Burmann et al. 2010) – this interaction couples bacterial transcription to translation (Proshkin et al. 2010).

In summary, our results demonstrate that RPs can associate with transcription sites in *S. pombe*, strengthening the view that the association of RPs with transcription sites is a general feature of eukaryotic cells. The tendency for several RPs simultaneously to associate with some genes and not with others suggests that the RPs may well be recruited together, most likely as parts of pre-assembled complexes, perhaps even as entire ribosomal subunits. The observed RNAase sensitivity could, particularly at promoter regions, also be due to the RPs proteins being bound to rRNA. Many of the genes with which RPs associate do not encode proteins, so should not be associated with fully assembled ribosomes or with translation. Future work shall unveil the mechanism and function of RPs at transcription sites and address the important issue of whether ribosomal subunits are present.

5. RESULT- II- S phase association of Upf1 to chromatin is required for genome stability

As reviewed in the Introduction, NMD degrades mRNAs containing premature stop codons (PTCs). NMD requires several protein factors of which Upf1, 2 and 3 are the most studied across organisms. Upf1, however, may have other functions apart from NMD as it has been showed to be required for genome stability in mammalian cells (Lew et al. 1998; Azzalin and Lingner 2006) and telomere maintenance in both *S. cerevisiae* and mammalian cells (Lew et al. 1998). We were interested to investigate whether Upf1 and other NMD factors have additional functions that do not relate to NMD in *S. pombe*. In this chapter I describe the results of experiments which I have performed to search for novel phenotypes of NMD mutants and to investigate the association of Upf1 with chromatin.

5.1 Upf1 mutant shows morphological defects

By inspecting wild type, *upf1* and *upf2* knockout strains, I observed a difference in cell size amongst these strains: it seemed that the size of *upf1* and *upf2* cells was larger than the WT (Fig. 5.1A). This size difference was unexpected because previous studies have not described any change in the growth of these mutant strains (Rodriguez-Gabriel et al. 2006). To test for a possible difference in growth, I performed a dilutions spot assay in which I plated the strains on YES agar medium along with two other strains, *cdc17* and *swi6*, that have a well-characterised slow-growth phenotype - Cdc17 is an ATP-dependent DNA ligase (Johnston et al. 1986); Swi6 is a chromodomain protein with multiple chromatin functions (Ekwall et al. 1995). The growth assay was conducted at two different temperatures (30°C and 37°C) (Fig. 5.1B). I found that both *upf1* and the double knockout *upf1*/*upf2* mutants grow slowly at the lower temperature; and as expected both *cdc17* and *swi6* showed growth defect at 30°C while *swi6* could not grow

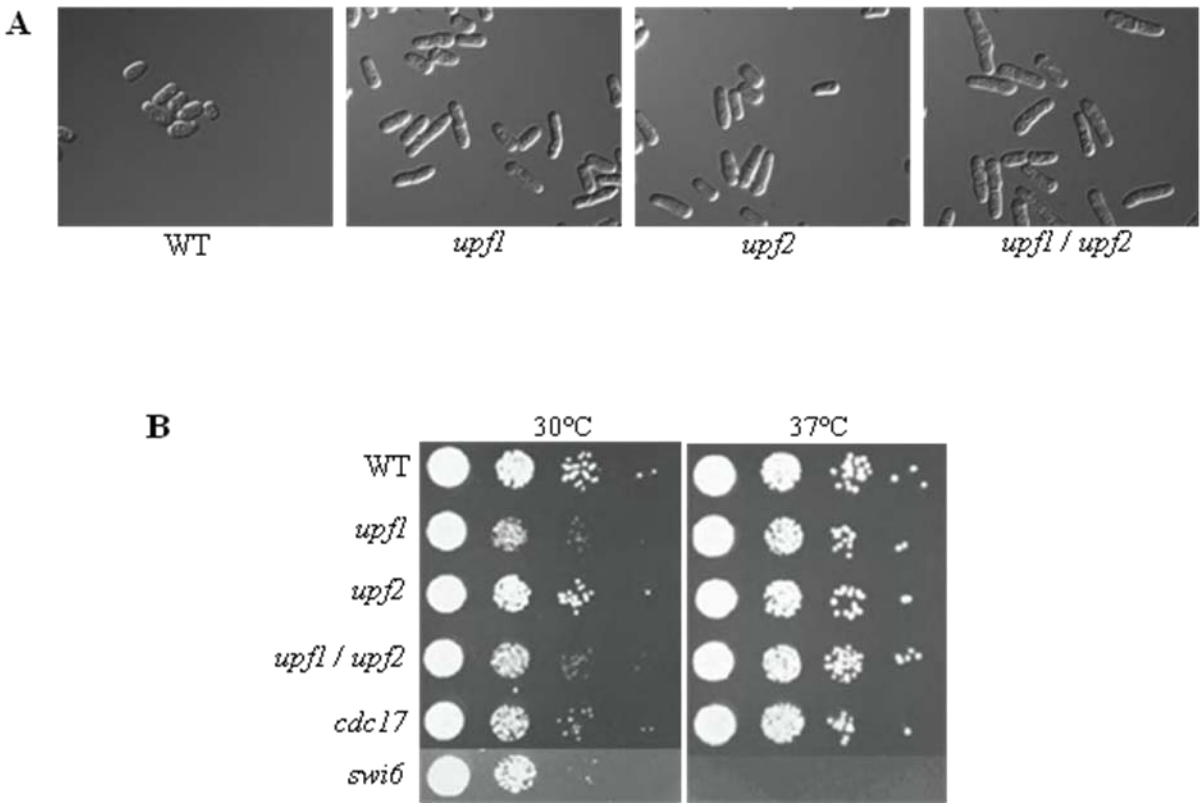
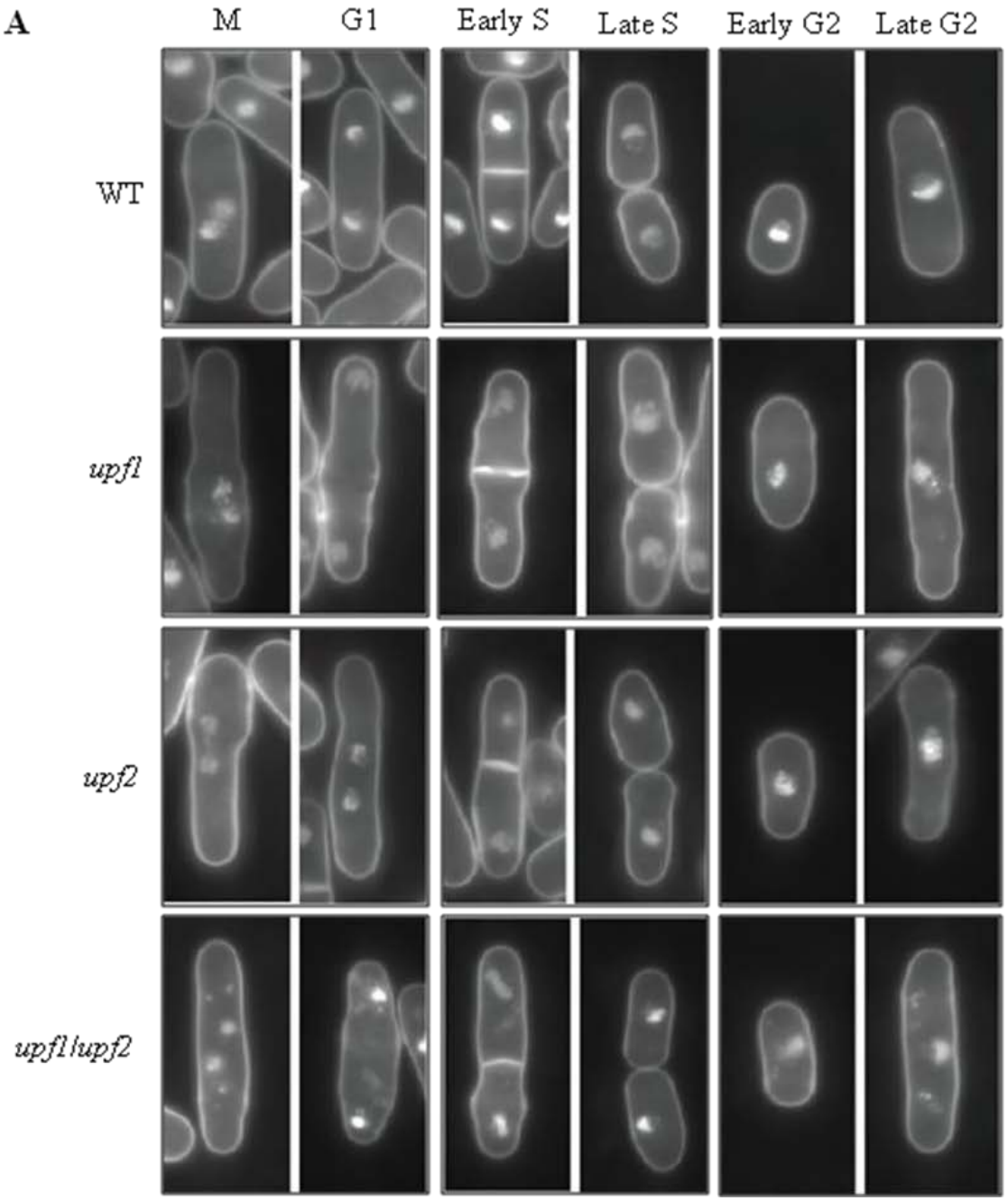


FIGURE 5.1 Upf1 mutants have an abnormal cell morphology and grow slowly. (A) Cell morphology of the wild type WT, *upf1*, *upf2* and *upf1*/ *upf2* grown at 30°C. Low light DIC images of logarithmic phase cultures (5 x 10^6 – 1 x 10^7 cells/ml) of the strains named below. These images are representative of three replicate cultures. (B) Serial dilutions growth assay of WT, *upf1*, *upf2*, *upf1*/ *upf2*, *cdc17* and *swi6* strains on YES plates. All strains were grown to exponential phase (~1 x 10^7 cells/ml) in YES + 3% glucose media. A dilution assay was then carried out as described in Materials and Methods. Aliquotes of diluted culture (from left to right, approximately 10^6, 10^5, 10^4 and 10^3) were grown on YES agar plates (3% glucose) at 30^{o}C and 37^{o}C for 4 days and at 30^{o}C for 6 days.

at higher temperature 37°C (Fig. 5.1).

5.2 Deletion of NMD factors disrupts chromosomal condensation and morphology

Next, I assessed whether the NMD mutants have abnormal nuclear morphology. The cell nucleus morphology was evaluated by fluorescence microscopy after staining with DAPI (4', 6-diamidino-2-phenylindole), a fluorescent stain that intercalates into DNA. Since DAPI will pass through the intact cell membrane, it may be used to stain both live and fixed cells; this examination was carried out with live cells in different cell cycle stages. Fission yeast is rod shaped cells that grow by elongation, after mitosis, cytokinesis follows by cell cleavage at a septum, or midpoint of the cell. Cytokinesis in fission yeast differs from the higher metazoans, instead of occurring after M phase, it starts when the cell enters the S phase. In *S. pombe*, DAPI-stained nuclei typically show a half moon structure (Fig. 5.2A, indicated with an arrow in WT). I found that in all the mutants, such a structure was not clearly defined in any of the cell cycle stages (Fig 5.2A). In fact DAPI stained DNA fragments were observed (Fig. 5.2A & B) and the extent of DNA fragments was particularly high in the *upf1*/*upf2* mutant (Fig. 5.2B), the possibility of these fragments are generated from chromosomes than of mitochondria because in most of the cells pieces of DNA are close to the nucleus. In all NMD mutants chromosomal domain was extended and often smeared; in the mutants chromosomal bridges between nuclei can also be observed in M phase cells (Fig. 5.2C). DAPI also stains the cell wall. Compared to wild type, we found a very uneven cell wall with bulges in the NMD mutants (Fig. 5.2A). We also observed cells with abnormally positioned nuclei (Fig. 5.2D), which typically occurs in 'CUT' mutants, where the septum forms without complete chromosomal segregation (Hirano et al. 1986). Therefore, in NMD mutants it seems that the septum can form when DNA replication or chromosomal segregation is incomplete.


A
M
G1
Early S
Late S
Early G2
Late G2
WT
upf1
upf2
upf1/upf2

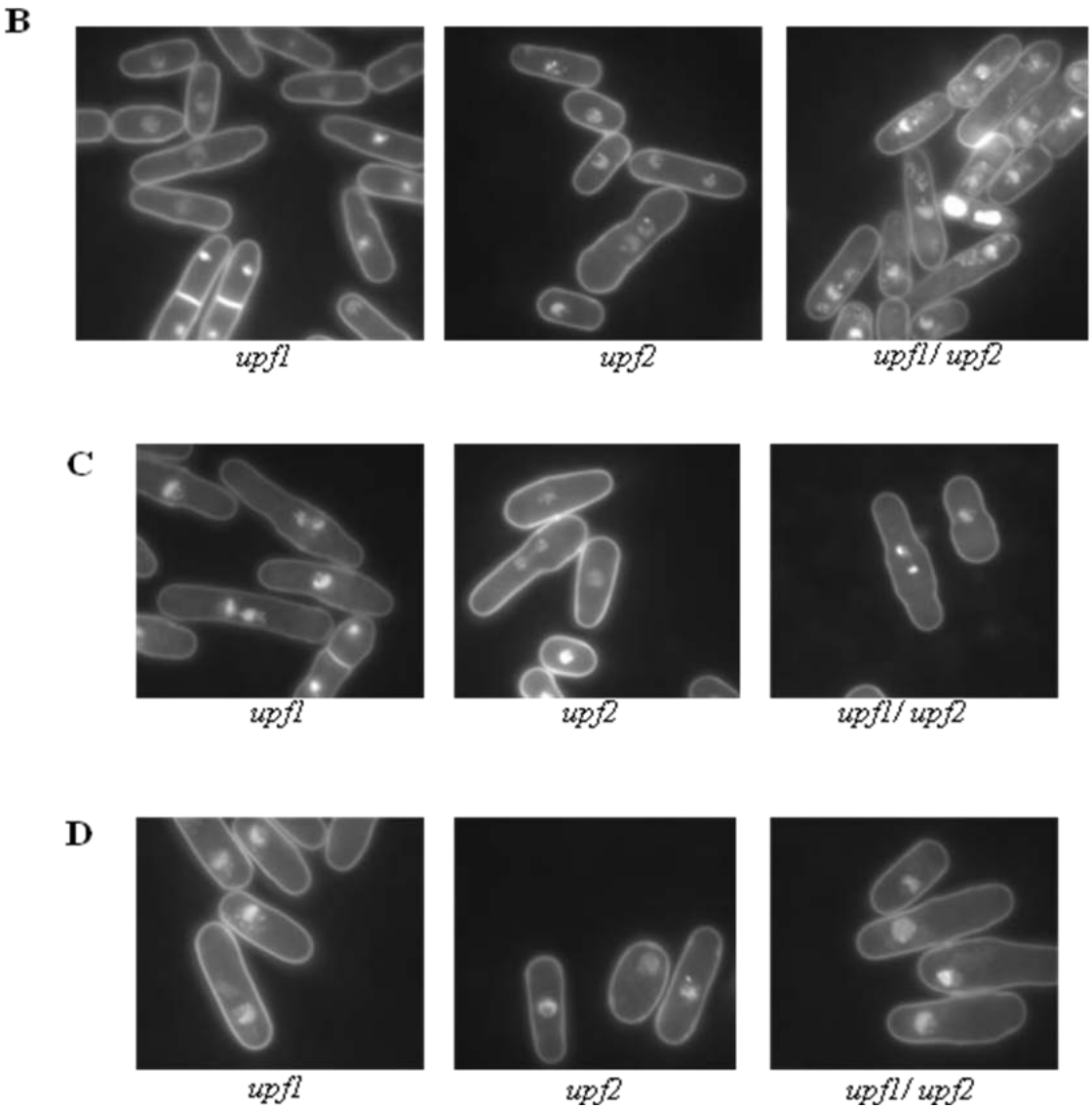


FIGURE 5.2 NMD mutants show signs of abnormal nuclear morphology. (A) Nuclear morphology of wild type, *upf1*, *upf2* and *upf1*/*upf2* cells at 30°C. Cells were grown to exponential phase (~1 x 10^7 cells/ml) in YES + 3% glucose media at 30°C, live stained with 1 µg of DAPI/ml to visualize nuclear DNA. Fluorescence images were obtained with an epifluorescence microscope (100X objective). Cell at different cycle stages, indicated above; strain names are indicated on left. (B) Example of cells with fragmented DNA; most apparent in double mutant. (C) Examples of cells with chromosomal bridges, all pictures were taken from dividing asynchronous cell cultures. (D) Examples of cells with abnormally positioned nucleus. In panel B, C and D strain names are indicated below.

5. 3 NMD mutants are highly sensitive to drugs that inhibit S-phase

I reasoned that nuclear morphology is abnormal in the mutants because *UPF1* and *UPF2* might be required for DNA replication or repair. To investigate further this hypothesis I assessed whether the mutants are hypersensitive to either hydroxyurea (HU) or methyl methanesulphonate (MMS). HU inhibits ribonucleotide reductase involved in the nucleotide biosynthesis pathway, MMS methylates DNA on N^7-deoxyguanine and N^3-deoxyadenine causing a stall to replication forks (Yarbro 1992; Lundin et al. 2005). Growth assays were performed at 30°C and 37°C. All three NMD mutants (*upf1*, *upf2* and *upf1*/ *upf2*) along with the DNA ligase mutant (*cdc17*) showed hypersensitivity to HU at both low and high temperatures (Fig. 5.3A). At 30°C the NMD mutants appear more sensitive than *cdc17* whereas at 37°C the *cdc17* mutation is lethal. As for MMS, the sensitivity of the NMD mutants was apparent only at 37°C whereas *cdc17* showed sensitivity to MMS at both temperatures (Fig. 5.3B). To test whether chromosomal segregation defects causes these strains to grow slow, parallel growth assays were carried out on a YES plate containing thiabendazole (TBZ) which inhibits microtubule polymerization (Davidse and Flach 1978). As positive control we used a *swi6* mutant, which has been reported to show hypersensitivity to thiabendazole (Yamagishi et al. 2008). *swi6* is a chromodomain protein involved in heterochromatinization of the silent mating-type loci, centromeres, and telomeres (Ekwall et al. 1995). In cells lacking *swi6*, centromeres lag on the spindle during anaphase and chromosomes are lost at high rate (Ekwall et al. 1995). *upf1* and *upf1*/*upf2* strains showed low sensitivity to TBZ at both temperatures, with *upf2* being insensitive to TBZ (Fig. 5.3C).

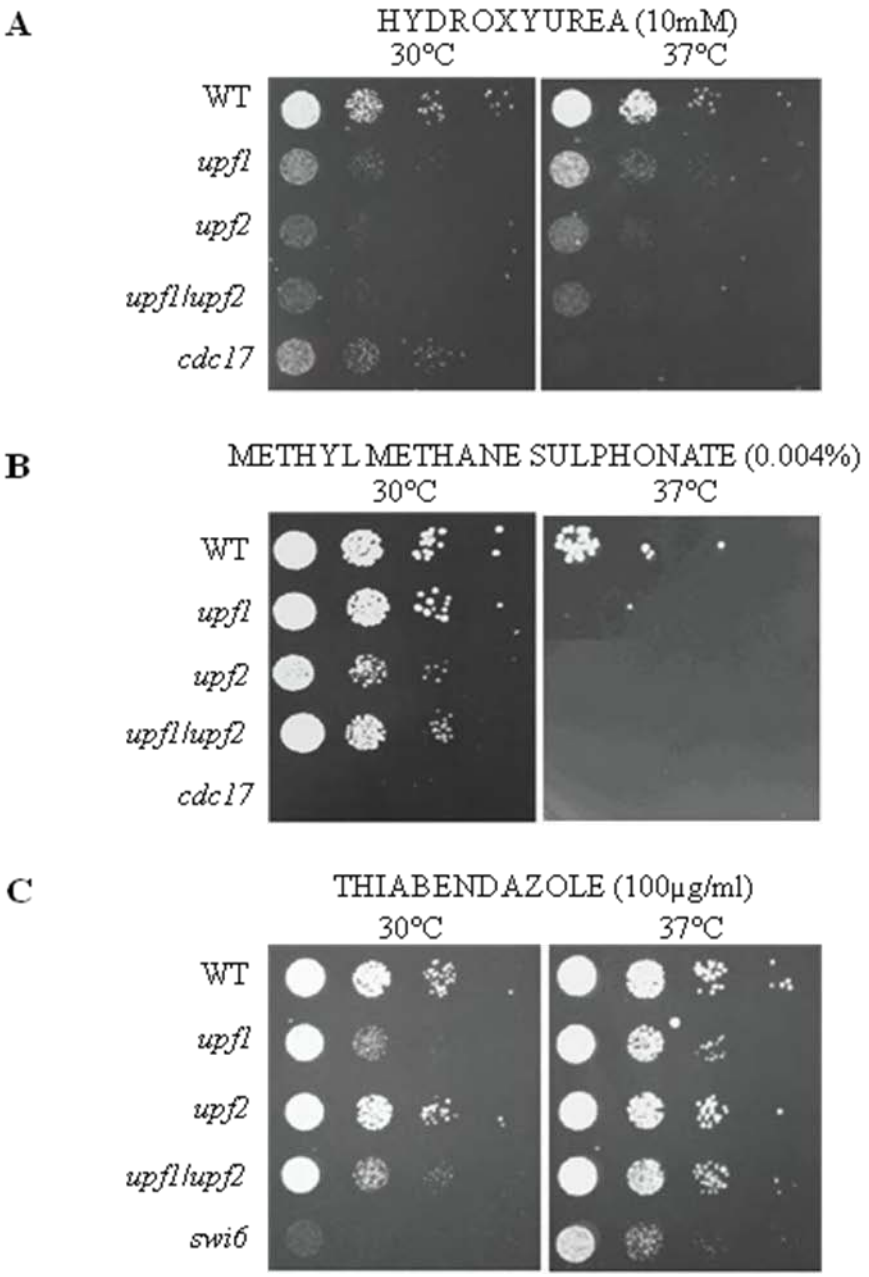


FIGURE 5.3 NMD mutants are sensitive to S phase drugs. (A) Serial dilutions growth assay of WT, *upf1*, *upf2*, *upf1*/*upf2* and *cdc17* on YES plates containing hydroxyurea (10 µg/ml). (B) Serial dilutions growth assay of strains mentioned above on YES plates containing methyl methanesulphonate [0.004% (w/v)]. (C) Serial dilutions growth assay of WT, *upf1*, *upf2*, *upf1*/*upf2* and *swi6* on YES plates containing thiabendazole (100 µg/ml). All strains were grown to exponential phase (~1 x 10^7 cells/ml) in YES + 3% glucose media. A dilution assay was then carried out as described in Materials and Methods. Cells were serially diluted ten-fold (from left to right, approximately 10^6, 10^5, 10^4 and 10^3) then grown on YES agar plates (3% glucose) at 30^{o}C for 4 days and 30^{o}C for 6 days.

5.4 Deletion of *UPF* genes induces a DNA damage response

Since our data indicated hypersensitivity of NMD mutants to drugs that affect S phase DNA replication, I wanted to investigate further these phenotypes. First I assessed whether there is an increase in Proliferating Cell Nuclear Antigen protein (PCNA), a marker of DNA damage and repair (Frampton et al. 2006). Whole cell protein extracts were assayed by Western blotting with an anti-PCNA antibody (PC10, Merck). We detected both unmodified and modified forms of PCNA, which migrated as distinct bands on the gel; these represent mono- and polyubiquinated PCNA species (Fig. 5.4A, top panel). The accumulation of ubiquitinated PCNA was higher in *upf1* and the double mutant compared to *upf2* knockout (Fig. 5.4A, top panel). The WT and *cdc17* mutant showed minimal accumulation of ubiquitinated PCNA (Fig. 5.4A, lane 1 and 5). To further investigate this data we performed a Western blot for γ-H2AX, a histone variant that is typically enriched at DNA double-strand breaks (DSBs) (Bouquet et al. 2006). As expected from the PCNA results, γ-H2AX was clearly increased in the *upf1* and *cdc17* mutants (Fig. 5.4A, middle panel, lane 2 and 5), while I detected only a minor increase in *upf2* mutant cells. The above observations suggested that depletion of *upf1* might increase DNA damage in *S. pombe* cells. To test this possibility further, I used the comet assay to compare levels of DNA damage in wild type and mutant *upf1* single-cells. In this assay, cells were embedded in agarose plugs, and subjected to alkaline electrophoresis (Miloshev et al. 2002). Intact genomic DNA shows very poor mobility under these conditions; DNA DSBs increase the migration of the DNA, generating "comets" of DNA. The relative size of the comet tail is used to assess levels of DSBs in individual cells. With the help of Dr. Nick Hodges I performed a comet assay, in the absence of any external DNA damaging agent, control WT cells did not show any sign of comets but the absence of Upf1 protein led to comet tail formation in *upf1* strains (Fig. 5.4B). Together, these data provide evidence that deletion of *UPF1* leads to increased accumulation of broken DNA.

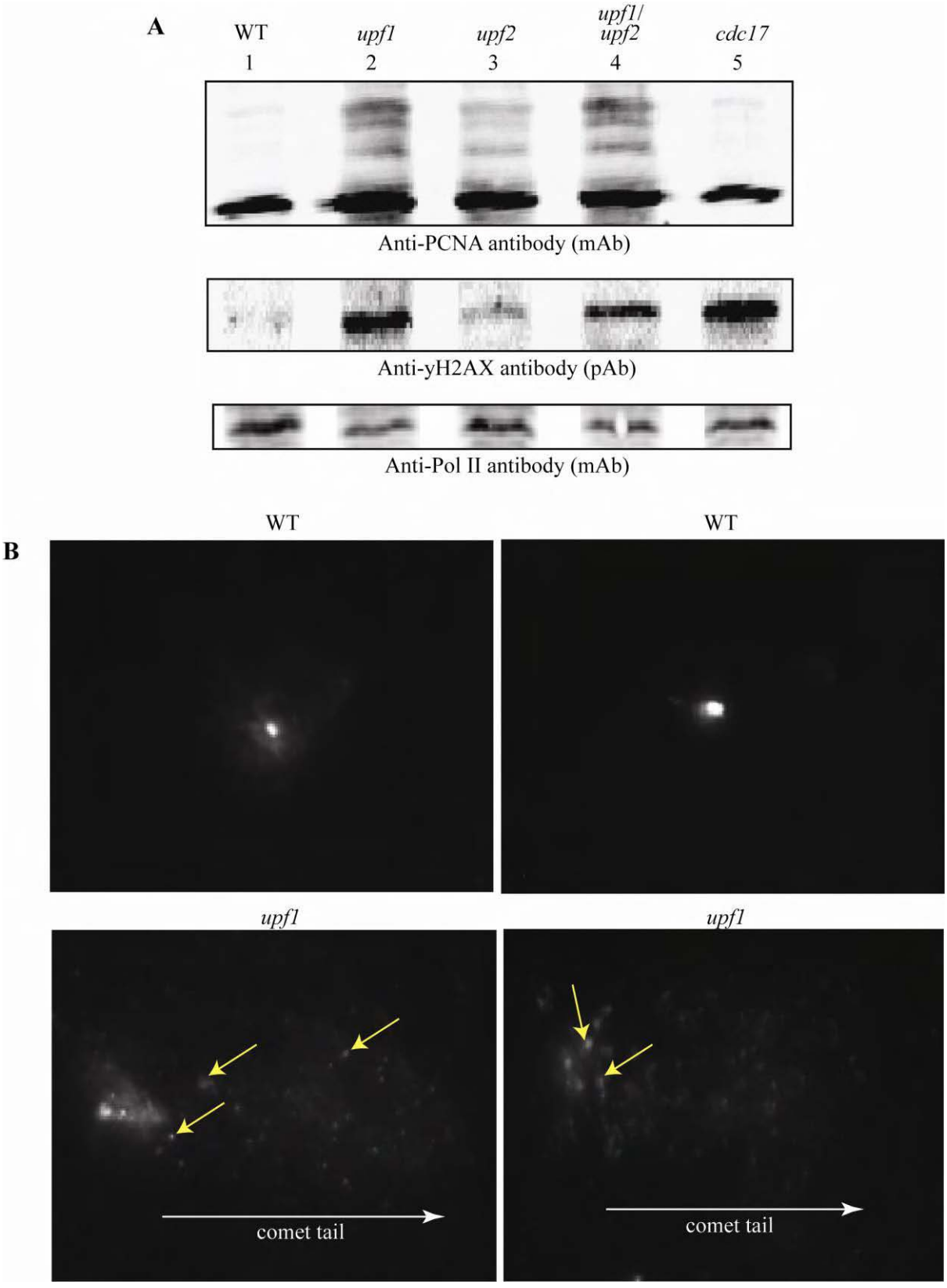


FIGURE 5.4 DNA damage accumulates in ΔUpf1 cells.
(A) Western blot analysis of whole-cell protein extracts of wild type and NMD mutant cells. The top panel shows a blot probed with a mouse monoclonal anti-PCNA antibody (AbD

Serotec), middle panel shows a blot probed with goat-polyclonal anti-yH2AX (A gift from Dr. Laura O'Neill) antibody and lower panel shows a blot of equally loaded gel probed with monoclonal 8WG16 (Covance) anti-Pol II antibody, as loading control. (B) Alkaline single-cell electrophoresis (comet assay) of wild-type and mutant *upf1* cells. The head is composed of intact DNA, whereas the comet tail (indicated by arrow) consists of DNA with DSB. DNA fragments are shown with yellow arrow.

5.5 UPF1 mutants shows defect in S phase progression

Given the evidence of DNA damage in the NMD mutants, I assessed whether there is any delay in the cell cycle. As shown above (Fig. 5.2A) fission yeast cells at different stages of the cell cycle are clearly distinguishable by their morphology under a phase contrast microscropy. I inspected mutant and wild-type cultures and found that Upf1-depleted cells are more likely to be arrested in S phase (Table 5.1).

5.6 Upf1 but not Upf2 is associated with chromatin

As reviewed in the Introduction, previous studies indicate that Upf1 might be involved in DNA replication by contacting directly one of the DNA polymerases (Azzalin and Lingner 2006). It was interesting to test whether Upf1 interacts with chromatin in *S. pombe*. Using HA-tagged Upf1 and Upf2 strains ChIP assays were performed as detailed previously (chapter 3). I initially assayed three genes *PMA1*, *ACT1* and *ADE6* with both Upf1 and Upf2 proteins. I found strong Upf1 ChIP enrichment at promoters and coding regions of both the *PMA1* (2.5-6.5 fold) and *ACT1* (3.8-4.3 fold) genes, but not with the *ADE6* gene (Fig. 5.5). In contrast to Upf1, Upf2 showed a very low level association with all three genes mentioned above (Fig. 5.5); further studies would be necessary to evaluate whether the Upf2 enrichment is significant.

5.7 Upf1 bind to chromatin in a cell cycle dependent manner

To test for a direct role of Upf1 in DNA metabolism, we then tested whether Upf1 bind to chromatin better at specific cell cycles. For this experiment, Upf1 was HA-tagged in the *cdc25-22* mutant strain. ChIP was performed with anti-HA antibody as before. I found that, Upf1 association with chromatin is most apparent in S-phase (Fig. 5.6). The association of Upf1 varied with the cell cycle stages, i.e. ChIP signal of Upf1 at *PMA1* was comparatively higher than at *ACT1* and *ADE6* in all stages of cell cycle (Fig. 5.6). These data strongly indicate that

Cell cycle	**WT (%)**	***upf1* (%)**	***upf2* (%)**	***upf1/ upf2* (%)**
early S/ late S	16.65	23.6	15.7	17.74
early G2/ late G2	68.5	74.8	84.3	80.5
M/ G1	6.02	1.52	1.35	1.73

Table 5.1 In *upf1* there is an accumulation of cells at S phase.
Distribution of NMD mutant strains in different cell cycle. Strain names are shown on top panel and different cell cycle stages are indicted on left panel. Percentage of cells present in each cell cycle stages for different strains is shown. A total of 700 cells were counted.

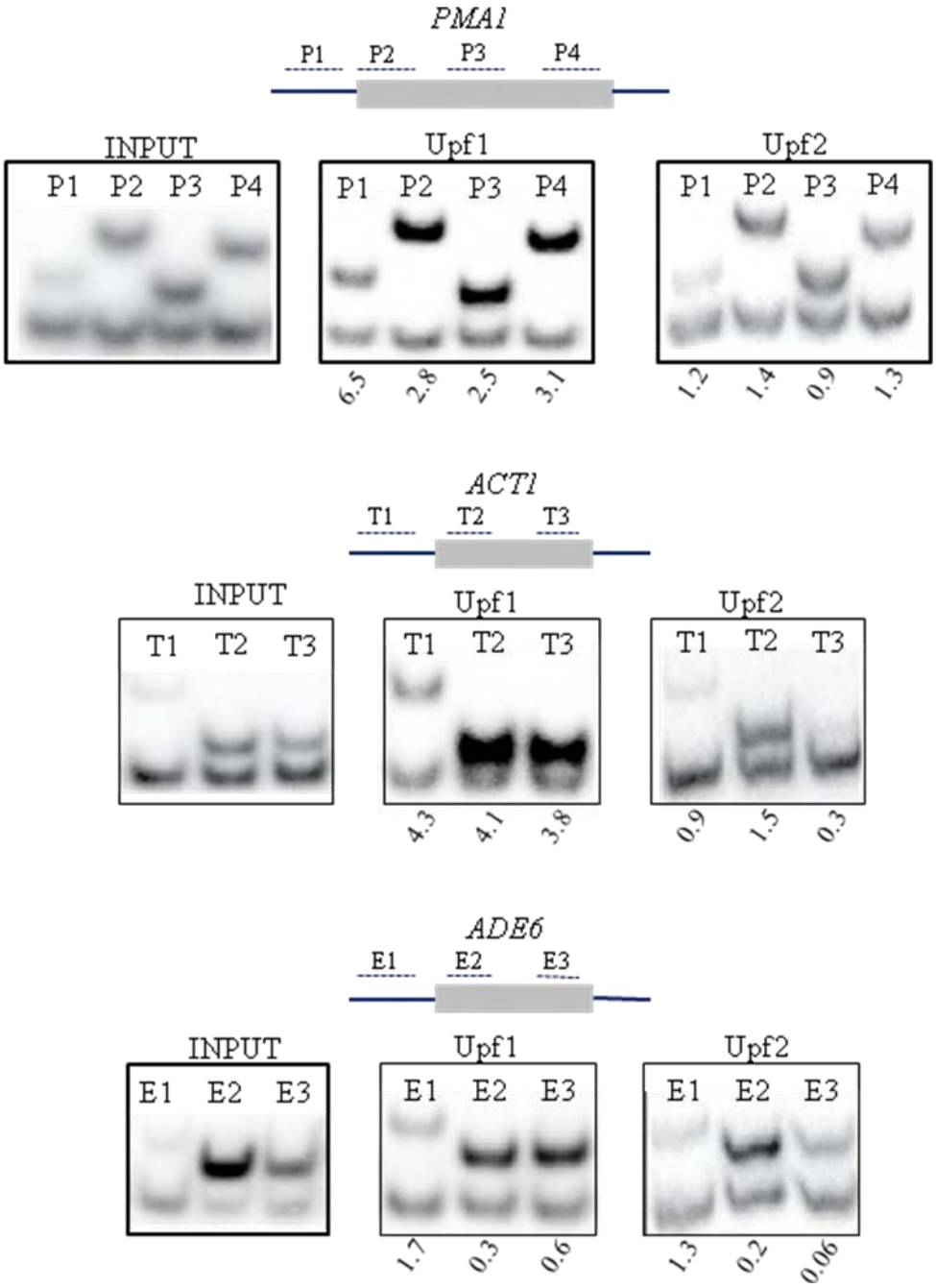


FIGURE 5.5 Upf1 is associated with chromatin. Polyacrylamide gels showing radiolabelled PCR products produced by the *PMA1*, *ACT1* and *ADE6* gene specific primer pairs (top band) and by the pair specific for the intergenic region (bottom band), amplified from input and ChIP DNA samples of HA-tagged Upf1 and Upf2, and quantified by phosphorimager. The relative enrichment of a specific DNA fragment relative to the intergenic sequence is expressed as ratio of ratio of the intensity of the same fragments produced with the input DNA and is shown just below each lane. Name of the primers are indicated above each lane.

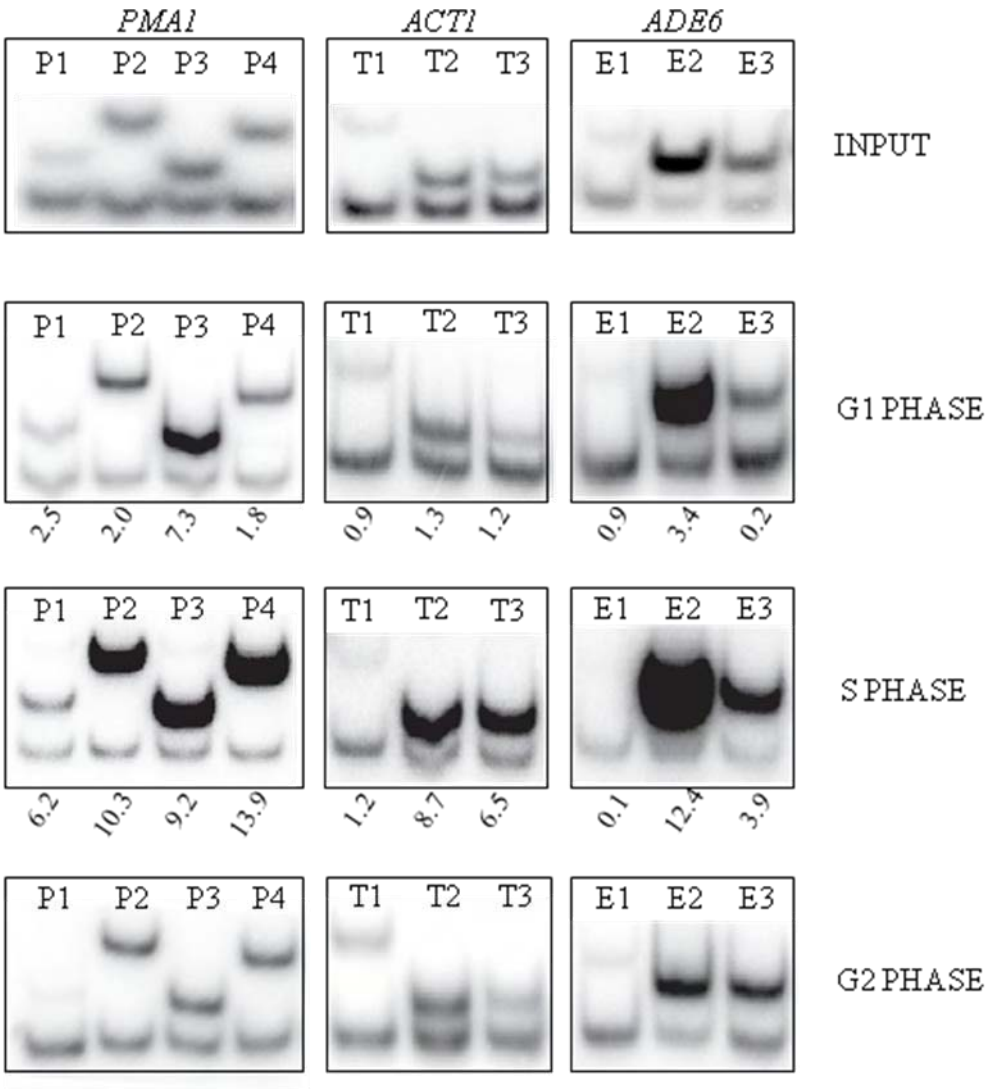


FIGURE 5.6 Upf1 associates with the chromatin in cell cycle dependent manner.
Polyacrylamide gels showing radiolabelled PCR products produced by the *PMA1*, *ACT1* and *ADE6* gene specific primer pairs (top band) and by the pair specific for the intergenic region (bottom band), amplified from input and ChIP DNA samples from three different cell cycle stages (G1 [30 min after release], S [60 min after release] and G2 [0 min after release], indicated on right) of HA-tagged Upf1, and quantified by phosphorimager. The relative enrichment of a specific DNA fragment relative to the intergenic sequence is expressed as ratio of ratio of the intensity of the same fragments produced with the input DNA and is shown just below each lane. Primers used are indicated above each lane.

fraction of Upf1 is present in the nucleus and in view of the phenotypes of the deletion strains, is likely to be involved in DNA metabolism.

To identify nuclear localization of Upf1, I GFP tagged Upf1 and expressed with a plasmid from a constitutively expressed promoter (*ADH* gene promoter). As expected Upf1-GFP is mainly distributed in the cytoplasm, but a low level of Upf1-GFP is detected within nucleus (Fig. 5.7).

5.8 Mapping of Upf1 binding sites on fission yeast chromosomes

To map Upf1-binding sites across all *S. pombe* genome, I hybridized the amplified Upf1 ChIP DNA samples with *S. pombe* genomic DNA tiling array (GeneChip® *S. pombe* Tiling 1.0FR Array). As previously described in Chapter 2, the chromatin samples were prepared from asynchronous cultures of active dividing cells. The ChIP-on-chip experiments revealed that Upf1 binds to many sites, including both coding and non-coding genes (Fig. 5.8). Surprisingly, similar to RPs, Upf1 appears also to bind to heterochromatin regions, including centromeres and telomeres (Fig. 5.9A & B) and with rDNA loci (Fig. 5.9B). As expected the *PMA1* and *ACT1* were flagged as enriched in the ChIP-on-Chip analysis, but not the *ADE6* gene. As mentioned in Chapter 4, fission yeast centromeres span 35-110 kb and are composed of a central core region of non-repititive DNA (cnt), flanked by inverted repeat regions- the innermost repeats (imr) and the outer repeats (otr), the latter of which contains multiple copies of dh/dg repeats (Pidoux and Allshire 2004). The ChIP-on-chip data show that Upf1 protein associates with the centromeric region in all three chromosomes. Similar to the RPs ChIP-on-chip profile, Upf1 peaked at the cnt region. Upf1 showed differential association to the otr repeats. It is quite clear that Upf1 associate to two distinct regions in the otr domain of chromosome I, one being close to imr domain and another in the boundary of the centromere (Fig. 5.9A). In centromere II Upf1 was associated with cnt2 and otr2 domains but showed no

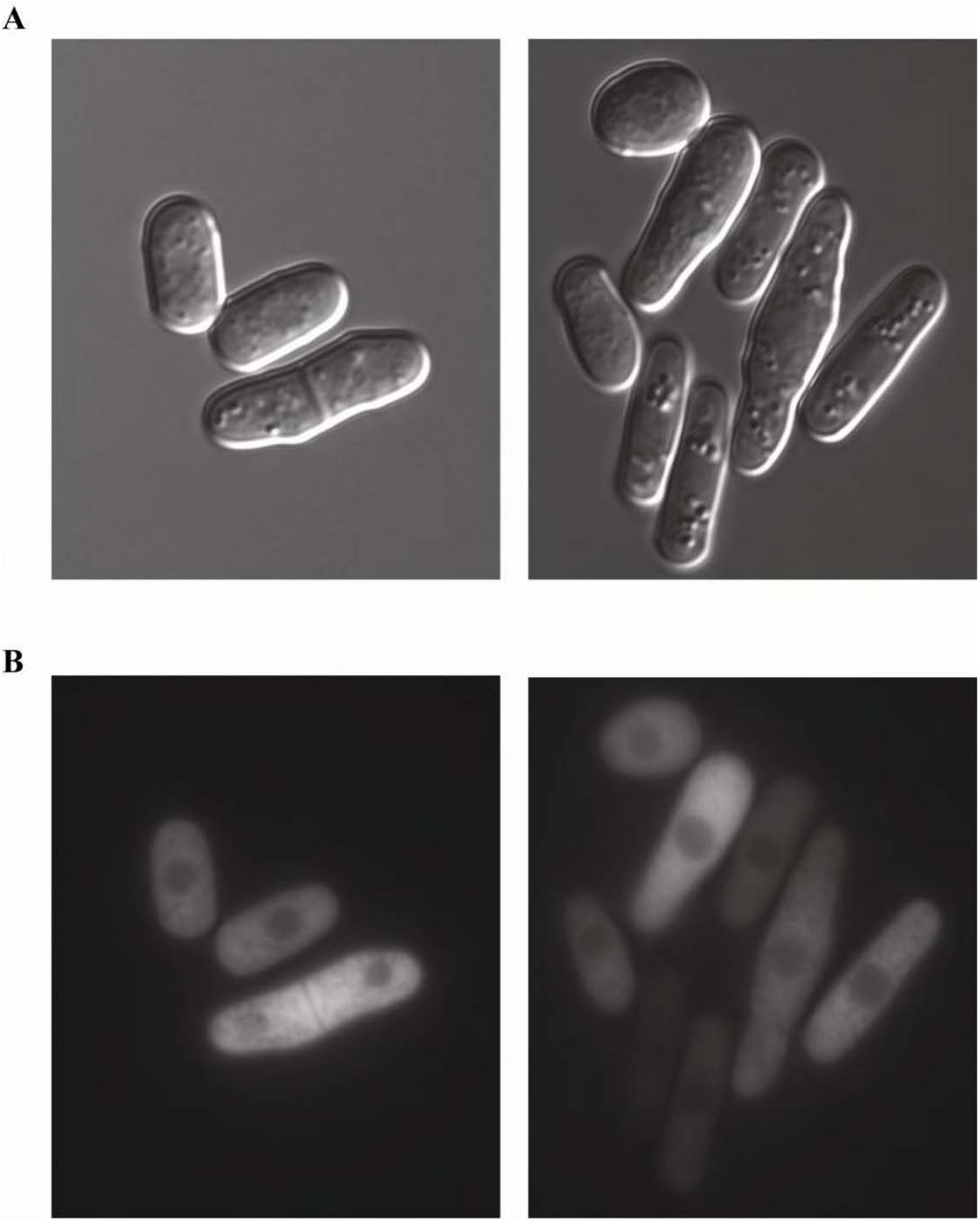


FIGURE 5.7 Upf1 is primarily localized in the cytoplasm.
Micrographs of wild type cells transformed with pART1-Upf1-GFP constructs. (A) DIC images. (B) GFP fluorescence showing the subcellular localization of constitutively expressed Upf1-GFP proteins. Images were captured with a CCD camera (Hamamatsu).

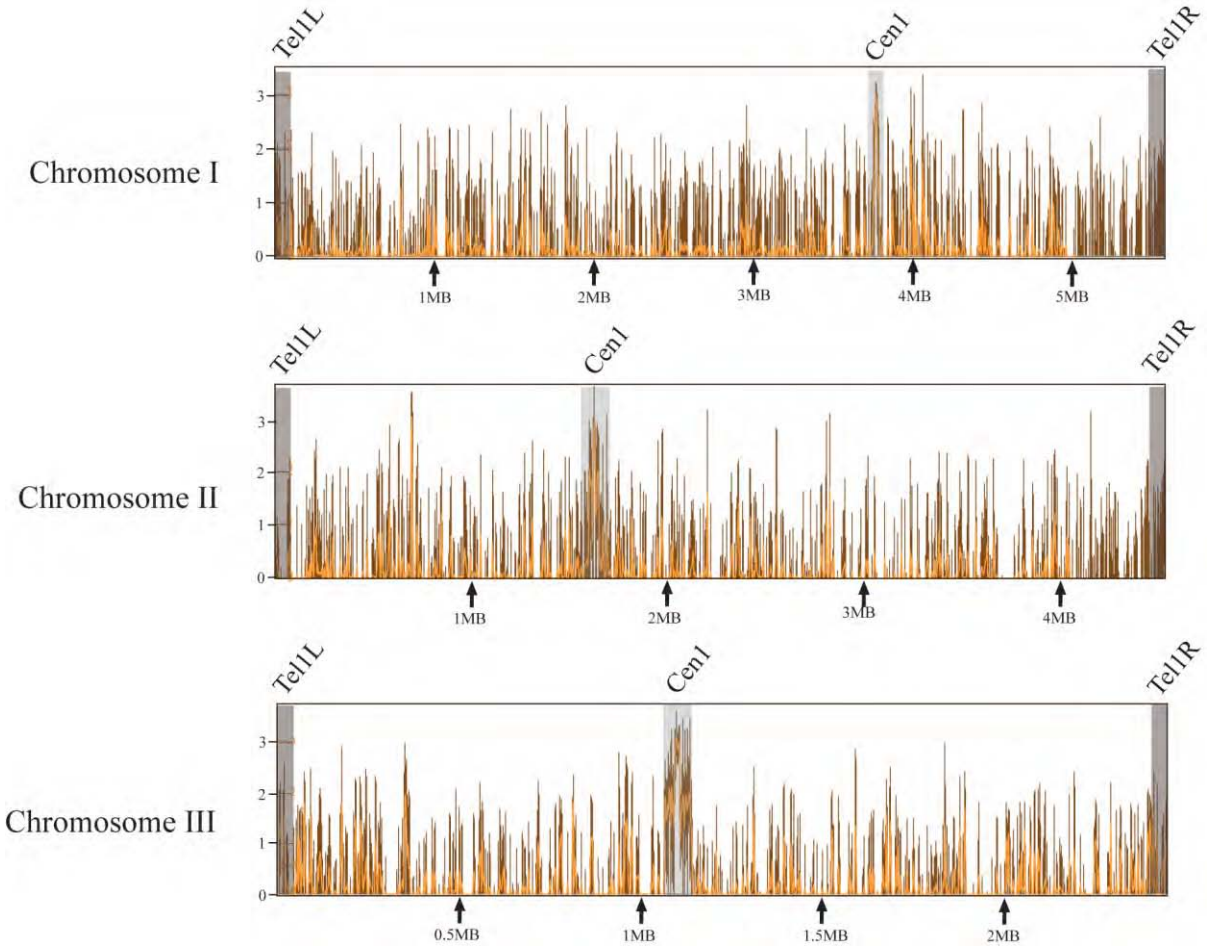


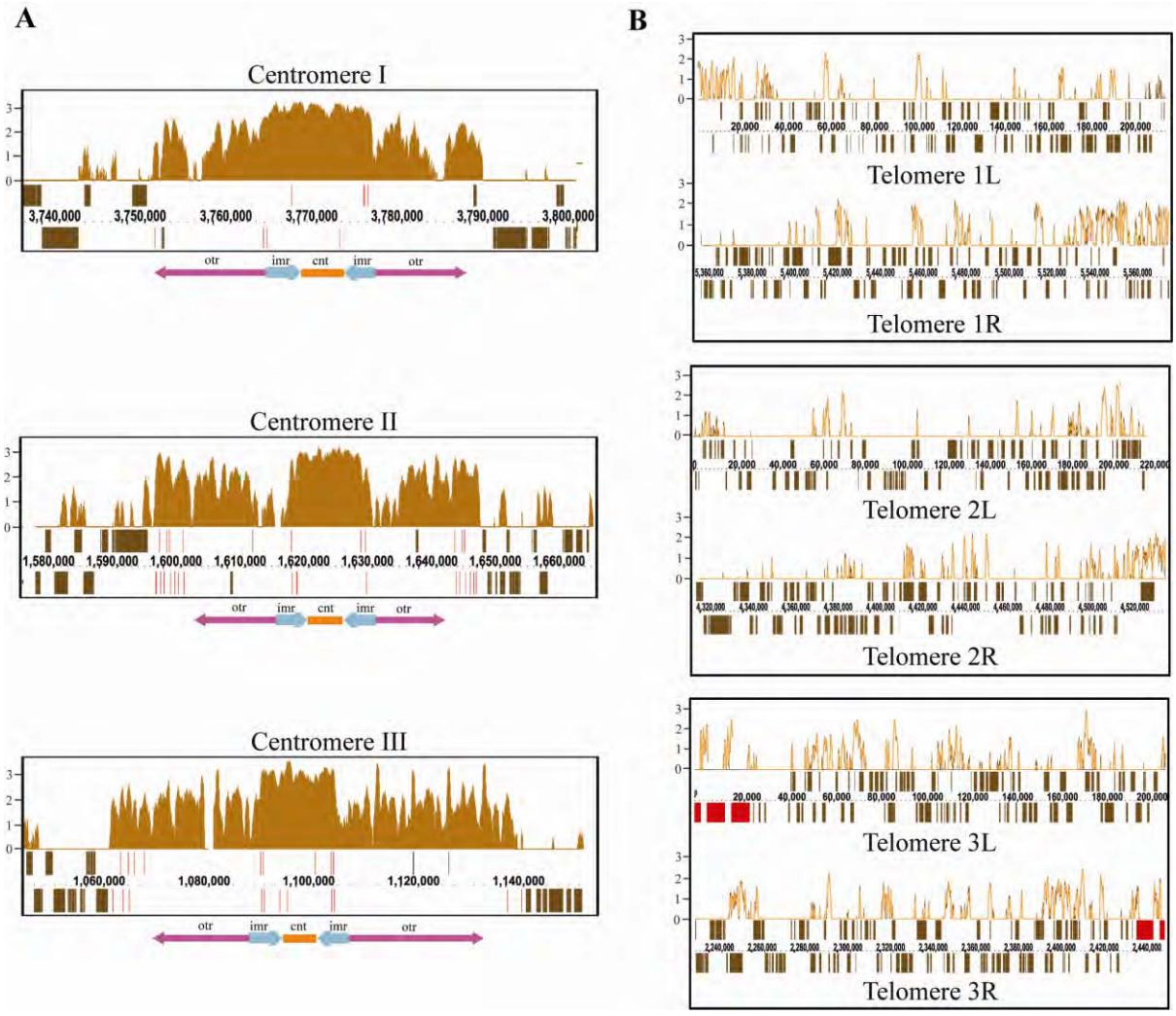
FIGURE 5.8 Genome-wide localization of Upf1 protein.
Genome-wide mapping (ChIP-on-chip) of Upf1 protein binding sites in the Upf1-HA strain. Positions of centromeres (cen) and subtelomeres (tel) are indicated with gray boxes. X-axis: distance from the left chromosome end, in kilobases (kb) Y-axis: log2 (MAT score) from 0 to 3.

enrichment in imr2 domains, in centromere III this protein is distributed in all three domains with some abrupt enrichment peaks (Fig. 5.9A). The current publicly available *S. pombe* genome sequence lacks sequences near the ends of three *pombe* chromosomes, so the telomere sequence coverage on the *S. pombe* microarray is incomplete (Cam et al. 2005). Detailed analysis showed that two large non-uniforms Upf1 enrichment domains are extended up to 40kb away from the two subtelomeres in chromosome I (5.9B), we observed a 20kb domain of Upf1 association on the right subtelomere of chromosome II. Upf1 is also distributed as well on the rDNA present in the chromosome III telomeres (Fig. 5.9B, rRNA loci indicated in red). However, unlike RPs, Upf1 is not associated with tDNA genes outside the centromeres (Fig. 5.9C).

5.9 Upf1 is not involved in heterochromatic gene silencing

Since we have found Upf1 at the centromere and since it has been reported that this protein might be involved in the RNAi pathway in *Arabidopsis* (Arciga-Reyes et al. 2006), we tested whether Upf1 is involved in heterochromatinization. To test this hypothesis we used a fission yeast reporter system where a marker gene *ade6*$^{+}$ cassette has been inserted into the dg region of the otr domain in the chromosome I centromere. When this gene is silenced colonies have a dark red colour, whereas the colonies whichare defective in chromatin silencing are white or pink in the mutant strains because ade6 is expressed. I deleted *UPF1* from this strain and compared the colour of the colonies with that of WT and known RNAi/silencing mutants. I found that *upf1* mutants are dark red and there was no obvious difference in colour intensity with the WT at any of the three different temperatures (25°C, 30°C and 37°C). Instead RNAi (*dcr1*) and silencing (*clr4*) mutants were either white or pink as expected (Hansen et al. 2005) (Fig. 5.10B).

To further validate the result of the red-white colony assay, I assessed whether *upf1* mutants


A
Centromere I
3,740,000
3,750,000
3,760,000
3,770,000
3,780,000
3,790,000
3,800,000
otr
imr
cnt
imr
otr
Centromere II
1,580,000
1,590,000
1,600,000
1,610,000
1,620,000
1,630,000
1,640,000
1,650,000
1,660,000
otr
imr
cnt
imr
otr
Centromere III
1,060,000
1,080,000
1,100,000
1,120,000
1,140,000
otr
imr
cnt
imr
otr
B
Telomere 1L
Telomere 1R
Telomere 2L
Telomere 2R
Telomere 3L
Telomere 3R

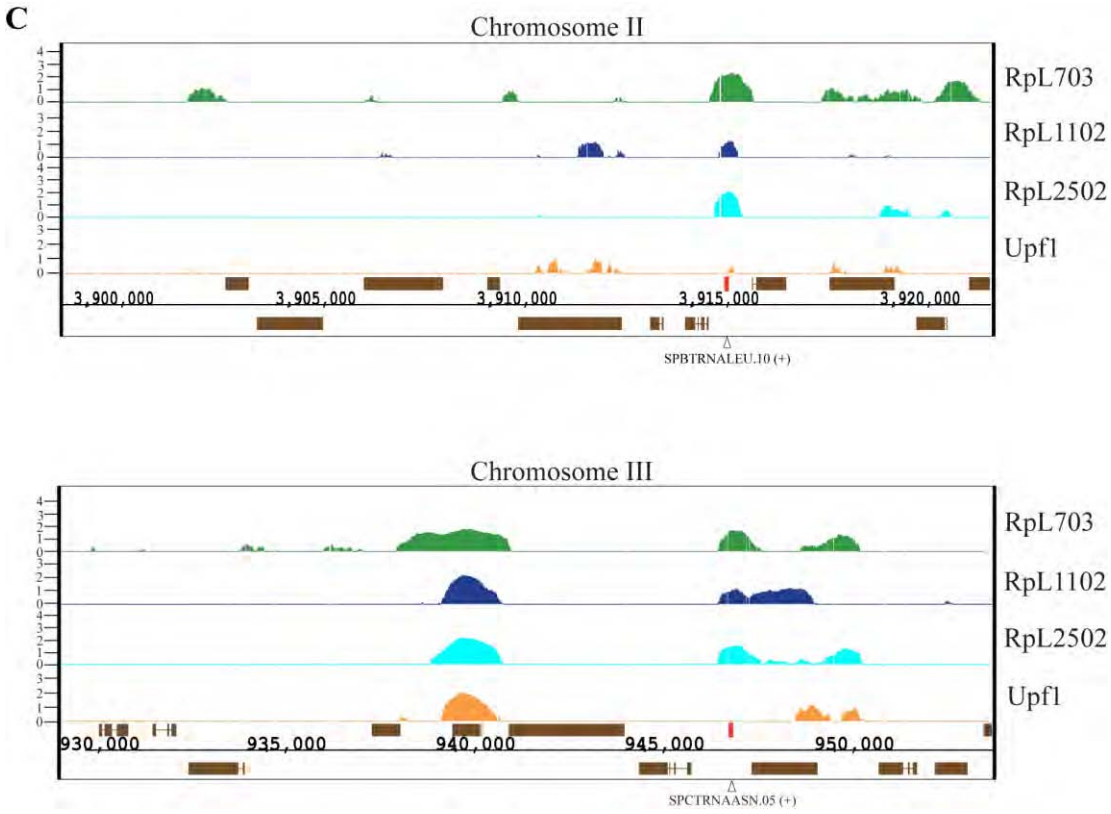


FIGURE 5.9 Upf1 is at centromeric and subtelomeric regions.
(A) ChIP-on-chip enrichment graphs for Upf1 protein around the centromeric region of each chromosome, generated with the IGB software. The map below each panel shows a schematic of fission yeast centromeres, with the three major domains labelled otr, imr and cnt. Centromeric tDNA gene loci are indicated by red lines, and the other genes by brown boxes. (B) ChIP-on-chip enrichment graphs for Upf1 protein around the subtelomeric region of each chromosome, generated with the IGB software. Telomeric 35S rDNA gene loci are indicated by red boxes, and the other genes by grey boxes. Y-axis shows log2 MAT enrichment score (0 to 3). (C) non-centromeric regions of the II and III chromosome showing RPs association at tRNA genes. the graph shows peaks of enrichment at individual tRNA loci (highlighted by red lines and by arrows at the bottom, + refer to genes in the DNA strand above).

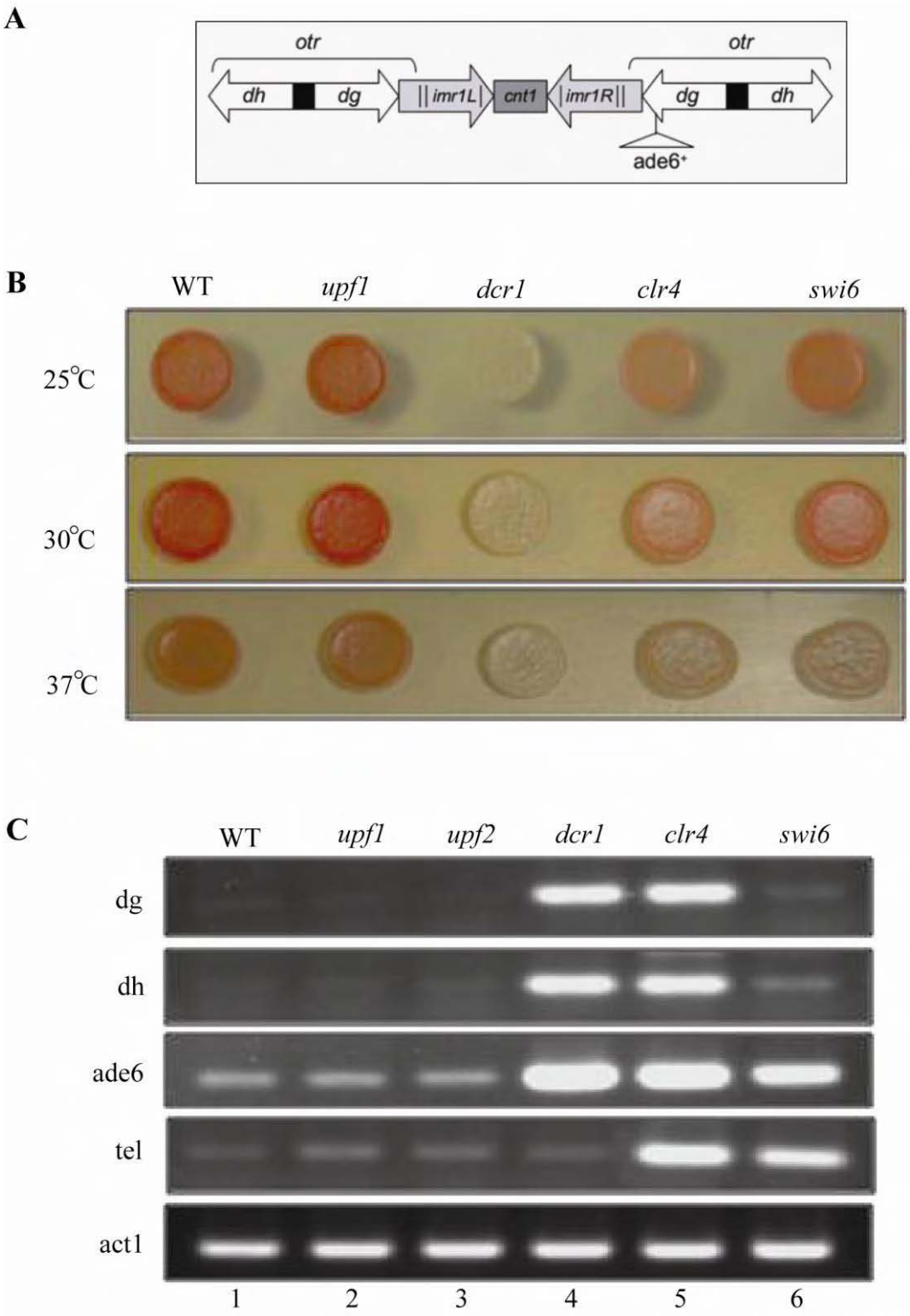


FIGURE 5.10 Upf1 is not involved in heterochromatic gene silencing
(A) Schematic of fission yeast centromere I, indicating site of integration of cen1:*ade6*$^{+}$ marker, outer repeat (otr) dg and dh elements, inner repeats (imr), and central core (cnt). (B) Silencing assay on strains bearing cen1:*ade6*$^{+}$ (red, silent; pink/white, alleviated). (C) RT-PCR analysis of transcripts from *ade6*, cen-otr (dg and dh), tel and act1 control.

affect the level of transcripts generated from repeats of centromeric and telomeric regions; these transcripts are affected in known RNAi mutants. I assayed these transcripts by RT-PCR with primers specific to dg and dh repeats, as well as primers specific to the *ADE6*$^{+}$ gene inserted into the centromeric dg repeat of chromosome I. Consistent with earlier observations (Hansen et al. 2005) the signal intensities for all centromeric and telomeric transcripts were low in wild type cells in comparison to silencing mutant (*clr4*) (Fig. 5.10C, lane 4 & 5). The intensity of dg, dh and ade6^{+} transcripts in the RNAi mutant (*dcr1*) were similar to those in a silencing mutant (*clr4*) (Fig. 5.10C, lane 4 & 5). Telomeric transcripts were very low in the RNAi mutant but high in RNAi mutant (*dcr1*) (Fig. 5.10C, lane 4) (Hansen et al. 2005). However, the transcript levels of any tested region were not changed in the *upf1* and *upf2* (Fig. 5.10C): In both mutants the transcript levels are comparable to that in wild type strain (Fig. 5.10C, lane 2 & 3). This indicates that though Upf1 is present at the centromere and telomere, it is not involved in the degradation of associated transcripts.

6. DISCUSSION- II

NMD factors appear to have other functions unrelated to NMD. In particular, it has been reported that Upf1 is important for DNA replication during S-phase in mammalian cells (Azzalin and Lingner 2006). In mammalian cells depletion of Upf1, but not Upf2, impairs growth. In agreement with this report, I found that *upf1* mutants grow slower also in fission yeast, but *upf2* had no effect. However, both *upf1* and *upf2* mutant cells showed longer body morphology, suggesting that both could have problems in cell division. Furthermore, I noticed that the chromatin of both mutants appear decondensed, with fragments of DNA scattered throughout the cell (this was most apparent in the *upf1*/ *upf2* double mutant). Perhaps Upf1 and Upf2 proteins might have separate functions in DNA replication or, that contrary to what has been proposed the effect on growth is in fact an indirect effect of lack of NMD. Inability of *upf1* and *upf2* mutants as well as the double mutant to grow on HU plates and MMS plates (at higher temperature) indicates that these strains are defective in DNA replication and/or, repair. The appearance of CUT-like phenotypes and chromosomal bridges also suggests that chromosomal segregation is impaired in the mutants. That *upf1* and *upf2* mutants have problems with DNA replication, is also supported by my observation that PCNA- a protein required for DNA replication during S phase becomes ubiquitinated (Hoege et al. 2002). The accumulation of poly-ubiquitinated PCNA is a marker of DNA damage and is proposed to control template switching during DNA damage replication in *S. cerevisiae* (Xiao et al. 2000; Ulrich 2005; Frampton et al. 2006). I found that the amount of poly-ubiquitinated PCNA in *upf1* is twice of that in *upf2* mutant, indicating higher degree of DNA replication defects in *upf1*. My data also indicate that γH2AX, a second marker of DNA damage, accumulates more in the *upf1* than *upf2* mutant. Perhaps, the most convincing indication that damaged DNA accumulates in *upf1* mutants, is provided by the comet assay,

which clearly show that DNA breaks accumulate in the mutant. Future studies should address whether the fragmentation is caused by double or single strand breaks. Consistent with an increase in DNA damage, it appears that a higher fraction of cells are arrested at S phase in *upf1* mutant compared to the wild-type and *upf2* mutant, this might have resulted from DNA replication problem in the *upf1* mutant.

My data indicate that DNA damage accumulates in cells lacking Upf1 and to a lesser extent in *upf2* strain. The important issue is whether this is due to these proteins having a second function, unrelated to NMD, or simply that the DNA damage is the consequence of lack of NMD. The finding that Upf1 is directly associated to replicating DNA in *S. pombe*, would support the view that Upf1 is directly involved in DNA replication, as previously suggested in mammalian cells. Upf1 associates with many sites on all three chromosomes. Enrichment of Upf1 in heterochromatin regions is the most intriguing outcome of our ChIP-on-chip analysis. Similar to the RPs distribution (Chapter 4), Upf1 is highly enriched in centromeres and telomeres. Heterochromatin establishment in *S. pombe* occurs during DNA replication and involves assembly of multiple protein-DNA complexes, which could impede replisome progression (de la Serna and Imbalzano 2002; Chen et al. 2008; Kloc et al. 2008). Additionally, transcription of siRNAs at the centromeric and subtelomeric dg/dh repeats could induce collisions between the replisome and transcription machinery (Chen et al. 2008; Kloc et al. 2008). How replication fork progression is coordinated with heterochromatin formation is poorly understood. It is possible that in *S. pombe* Upf1 plays a major role in centromeric DNA replication during S-phase either acting as a helicase or, as a part of chromatin remodelling complex. Cell cycle specific ChIP-on-chip needs to be performed to see the differences in Upf1 distribution pattern on whole genome which can give a lot of information. In addition the high enrichment of Upf1 at the cen and imr domains and the moderate TBZ sensitivity of *upf1* might indicate some functional role of Upf1in kinetochore

formation during mitosis (Lew et al. 1998; Azzalin et al. 2007). However, according to our ChIP results, Upf2 is not associated to chromatin, yet similar to *upf1* mutants, *upf2* mutants have phenotypes suggestive of problems with DNA replication and chromosome segregation.

Finally, in *S. cerevisiae* and mammalian cells Upf1 and Upf2 are also involved in telomere maintenance. It is possible that in *S. pombe* that these proteins also have telomere functions and some of the chromosomal fragments detected in DAPI staining might be generated from telomere damage. Overall, our data indicate that NMD factors are involved directly or, indirectly in DNA replication/repair pathways.

7. DISCUSSION AND CONCLUSION

7.1 RPs interact with chromatin in S. pombe

Although the rRNA contributes mostly to the structure and function of the ribosome, RPs are also important for viability in all cell types. RPs are mostly located on the surface of the ribosome- typically they contain an exposed N-terminal globular domain and a long C-terminal domain extending inwards the rRNA (Ban et al. 2000; Wimberly et al. 2000; Brodersen et al. 2002; Klein et al. 2004). Ribosomal subunits are assembled in the nucleolus, a recent study shows that RPs accumulate in nucleolus much faster than other nucleolar proteins (Lam et al. 2007). However, the rate at which RPs are imported in the nucleus is higher than the rate at which they are exported back to the cytoplasm (presumably as ribosomal subunits) (Lam et al. 2007); it appears that a large fraction of RPs is destroyed by the proteasome in the nucleus (Lam et al. 2007). Yet some RPs must escape degradation because many RPs are found associated with pre-mRNAs and proteins in the nucleus.

Here we provide evidence that the RPs and NMD factor are associated with sites of active transcription in S. *pombe*. These results are consistent with previous observations (Brogna et al. 2002; Schroder and Moore 2005). As reported in *S. cerevisiae*, we found RPs associate to both coding and non-coding genes: rRNA, tRNA and other ncRNAs, so there may not be a link between RPs association and translation potential of the transcripts (Schroder and Moore 2005). The RPs association is RNA dependent: RNase treatment abolishes the ChIP signal. While RNase sensitivity suggests RPs association with nascent RNA, we found a high level of correlation between ChIP enrichment of RPs and Pol II occupancy only for a small set of genes, suggesting that association of RPs to the transcription sites is not due unspecific electrostatic interactions between RPs and nucleic acids.

7.2 Are RPs recruited as a multiprotein complex?

As reviewed in the introduction, the current understanding is that free proteins not assembled into ribosomal subunits mediate extra ribosomal functions of RPs in the nucleus. RPs are often found together in biochemical preparations of complexes involved in transcription and pre-mRNA processing (Gavin et al. 2002; Jurica and Moore 2003; Shi et al. 2009; Ohta et al. 2010); however, the presence of RPs in these complexes is typically dismissed as a contamination with proteins that associate after cell lysis (Gavin et al. 2002). The observation that the full complement of 40S or 60S RPs is never found in these complexes also suggest that the RPs are contaminants. The caveat of this argument is RPs or whole complexes might have been lost during the purification or experimental processing.

In our data we have provided strong evidence that three of our tested RPs bind to specific regions of chromatin and this binding is non-random as there is very high correlation between independent replicates. This association is not localized to few loci but is global phenomenon. It appears that the three representatives RPs has a tendency to bind the same locus of the genome irrespective of the transcription rate. While it is not clear why these RPs tend to bind together, it is feasible that these proteins are recruited as multiprotein complex most probably as a ribosomal subunit. However, given that we did not find convincing evidence of translation at these sites, the issue of whether translation is occurring remains to be further investigated. Alternative explanations as to why RPs associate with chromatin need also to be explored; for example, the chromosome association might be due to some non-ribosomal function of these RPs**.**

The data originating from this work demonstrate that RPs are associated with transcription sites. The challenge is to identify those interactions which are physiologically relevant. This might be

a difficult task given that most RPs and translation factors are essential, but conditional knock-out models and RNA interference could be very useful tools.

7.3 Do ribosomal subunits interact in the nucleus?

Most steps in the assembly of the ribosomal subunits take place in a specialized sub-nuclear compartment called nucleolus. Here, the ribosomal RNAs (rRNAs) are transcribed as large precursors (pre-rRNA), which undergo extensive nucleotide modification and a complex maturation pathway. A large number of non-ribosomal, resident nucleolar proteins, (more than 200) are required to process and modify the rRNAs and to aid their assembly with the ~80 RPs. Analyses in yeast have underlined the enormous metabolic cost of synthesis of the rRNAs and ribosomal proteins (Warner 1999), which absorbs some 70% of all transcription. Because of the extremely high energy cost of ribosome synthesis for the cell, the various activities are coordinated spatio-temporally for efficiency. A recent further proof of such coordination is the finding that rRNA transcription and rRNA processing are coordinated through a subset of proteins shared by the two processes (Gallagher et al. 2004). In addition, a recent electron microscopy study has shown that 40S-subunit processing proteins associate with and compact the rRNA within seconds of completion of rRNA transcription (Osheim et al. 2004). The 60S-subunit processing machinery is recruited later, after the release of the 40S precursor from the 90S particle and the completion of rRNA transcription. Although we are still far from understanding the precise roles of the participating factors and obtaining a comprehensive overview of the composition and dynamics of the multiple pre-ribosomal particles, studies over the past 10 years have revealed a fascinating insight into the complexity of pre-ribosome formation. The 60S-subunit is fully assembled in the nucleus whereas the very last step of 40S-subunit maturation i.e. processing of 20S rRNA to 18S rRNA occurs in the cytoplasm (Leger-Silvestre et al. 2004). The nuclear dwelling time for

pre-40S and pre-60S subunits is different, with pre-40S subunits reaching the cytoplasm much faster than pre-60S (Lafontaine 2010). Translocation through the various nucleolar sub-compartments is facilitated by specific trans-acting factors. Nuclear export involves redundant pathways. About 2000 ribosomes are exported to the cytoplasm every minute in exponentially growing yeast cells, and the rate depends on nutrients availability (Honma et al. 2006; Vanrobays et al. 2008). There appears to be good timing between nucleo-cytoplasmic partitioning and the acquisition of prominent ribosomal structural features. For example, final shaping of the 'beak', a protruding structure of the small subunit, is thought to occur only once pre-40S has reached the cytoplasm (Schafer et al. 2006). A recent report, however, has shown that the immature pre-40S can be used in translation initiation in *S. cerevisiae* (Soudet et al. 2010). So it is quite possible that pre-40S and pre-60S particles can interact within the nucleus before of while being transported to the cytoplasm. Recent work by one of my colleagues shows that the two subunits might interact prior to nuclear export in Salivary glands and under certain conditions also in S2 cells. The pre-ribosomal subunits (pre-40S and pre-60S) can interact in the nucleolus for translation or for some yet unknown function, this important issue will need to be addressed by future studies.

7.4 Upf1 in genome stability

NMD factors can function in cellular mechanisms other than NMD or other mRNA-decay pathways. Several lines of evidence implicate NMD factors such as Upf1 in the maintenance of genome stability. Genome integrity is essential to maintain genome function; the maintenance of genomic integrity is achieved by a combination of processes, including DNA repair and telomere maintenance. Upf1 is a 5' to 3' DNA and RNA helicase with nucleic acid-dependent ATPase activity (Czaplinski et al. 1995). A straight forward hypothesis is that chromatin-bound Upf1 works as a replicative helicase unwinding the DNA in front of the

replication fork. Therefore, the S-phase arrest I observed in *S. pombe*, and that others have seen in mammalian cells, might be due to physical arrest of the replication forks, which when collapsed lead to genome rearrangement events. Supporting the hypothesis that Upf1 might be a helicase, Upf1 is found to physically interact with DNA polymerase delta (polδ), one of the major DNA polymerases involved in genome replication (Azzalin and Lingner 2006). Importantly, while Upf1 also bind to Upf2, Upf2 does not coimmunoprecipitate with polδ. But our data suggest that during S-phase only Upf1 binds. Notably, in the genes tested Upf1 bind the coding sequence and not the promoter or intergenic region. It is possible that Upf1 might be a specialized helicase essential for the replication of particular regions of the genome. Upf1 might alone or in concert with other proteins engage resolving conflict between transcription and DNA peplication complexes, especially when there is a demand for rapid chromosome duplication and high levels of gene expression. In my study, however, DNA damage was observed in both Upf1 and Upf2 knockout strains, suggesting that DNA damage is an indirect effect of lacking NMD; NMD might regulate specific genes involved in DNA metabolism. These possibilities need also to be addressed by future studies.

7.5 Translation factors that function outside translation

As reviewed in the Introduction, it is well established that RPs apart from being essential components of ribosomal subunit, they are also involved in several extra-ribosomal functions. There are also evidences of translation factors participate in non-translation related functions. For example microscopic analysis has demonstrated that a number of translational factors co-localize with cytoskeletal structures (Shestakova et al. 1993; Bassell et al. 1994). The physiological significance of interactions between components of the translation apparatus and the cytoskeleton remains unknown, although models have been proposed that link such associations reflect compartmentalization of the translation apparatus within the cell

cytoskeleton. In agreement with this view, it has been shown that polyribosomes (Dang et al. 1983; Zambetti et al. 1990; Hesketh 1996), aminoacyl tRNA synthetases (Dang et al. 1983; Mirande et al. 1985), initiation factors, and elongation factors (Yang et al. 1990; Bektas et al. 1994) can all associate with the cytoskeleton. However, it is possible that the function of translation factors at the cytoskeletal is independent of translation (Edmonds et al. 1995; Stapulionis and Deutscher 1995; Tikhomirova and Inge-Vechtomov 1996).

In mammalian cells, during Notch signalling, the activated form of Notch needs to be deubiquitinated before entering the nucleus, where it fulfils its transcriptional function. The enzyme accounting for this deubiquitinase activity is eIF3f (Moretti et al. 2010). A clear example of a translation factors moonlighting, are EF-Tu and EF-Ts, which play a role in (+) RNA virus replication of the bacteriophage Qβ (Blumenthal et al. 1976). The eukaryotic homolog of EF-Tu, eEF1A was found to bind to many viral RNAs (Dreher 1999; De Nova-Ocampo et al. 2002; Zeenko et al. 2002; Thivierge et al. 2008). It is proposed that eEF1A facilitates the assembly of the viral replicase (Li et al. 2010).

It seems clear that translation factors can act off the ribosome to control several cellular processes. In my data, indicate that Upf1 has a role beyond NMD and translation.

7.6 Is there any nuclear translation going on?

As reviewed in the Introduction, the case for nuclear translation rests on three lines of evidence. The first is indirect. Some NMD occurs within the `nuclear' fraction and, because translating ribosomes are the only known means of detecting termination codons, it is possible that nuclear scanning is done nuclear ribosomes. This view contrasts with the current understanding translation occurs only in the cytoplasm. The seemingly nuclear NMD maybe caused by cytoplasmic ribosomes which scan the mRNA as it emerges from nuclear pores. However, there is little evidence for this model, and it has been argued that too few

transcripts pass through pores at any moment to account for the degradation levels seen (Iborra et al. 2004). If translation does occur in nuclei, then nuclear translation/scanning provide the natural explanation of why NMD could occur in the nucleus.

The second line of evidence is again indirect. And that is translation components (e.g. ribosomal proteins and rRNA, initiation and elongation factors) and NMD (e.g. Upf1, Upf2 and Upf3) colocalize, co-immunoprecipitate and co-purify with the transcription machinery (Brogna et al. 2002; Iborra et al. 2004). The third line of evidence is more direct and relies on the observation that amino acid are incorporated at nuclear sites (Iborra et al. 2001; Brogna et al. 2002). In the Iborra et al study cells were permeabilized and the criticisms have been on whether the nuclear signal seen in such experiments is an artefact resulting from the permeabilization. But none of these criticisms addresses what I consider to be the strongest evidence – the dependence of the nuclear signal on ongoing transcription. Furthermore, in the Brogna et al study amino chromosomal amimo acid incorporation was seen in intact cells incubated with S^{35}-labelled methionine.

But it has been said that there is as yet no smoking gun: decisive evidence for the coupling of transcription and translation is lacking. Better evidence would include the demonstrating that nascent peptides accumulate at the transcription site on the corresponding gene: translation-dependent detection of the gene product at the gene. Future studies should solve these important issues.

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9. APPENDIX

Appendix I- List of genes or genomic regions enriched by RpL703

RpL7

mRNA SPAC1F8.07c ; p_c pyruvate decarboxylase (predicted) ||| similar to S. pombe SPAC186.09 (paralog) ||| no apparent S. cerevisiae ortholog ; colour 7 ; gene SPAC1F8.07c ; product pyruvate decarboxylase (predicted) ; EC_number 4.1.1.1 ;

mRNA SPAC1F8.08 ; p_c sequence orphan ||| 2 predicted transmembrane helices ; colour 8 ; gene SPAC1F8.08 ; product sequence orphan ;

misc_feature unknown_79 ; note nominal overlap with cosmid SPAC806, EM:AL117212 S. pombe chromosome 1 ;

misc_feature unknown_80 ; note nominal overlap with cosmid SPAC24B11, EM:Z67757 S. pombe chromosome 1 ;

mRNA SPAC24B11.14 ; p_c sequence orphan ; colour 8 ; gene SPAC24B11.14 ||| SPAC806.10 ; product sequence orphan ; note added 3-9-99 ;

tRNA SPA**TRNA**ALA.01 ; evidence not_experimental ; gene SPA**TRNA**ALA.01 ; product **tRNA** Alanine ; note **tRNA** Ala anticodon AGC, Cove score 67.24 ;
snRNA SP**SNRNA**.02 ; Alias snu2 ; db_xref EMBL:X55772 ||| Rfam:RF00004 ; synonym U2 ; systematic_id SP**SNRNA**.02 ; gene snu2 ||| U2 ||| SP**SNRNA**.02 ; primary_name snu2 ; product small nuclear RNA U2 ;

mRNA SPAC1A6.11 ; p_c dubious ||| compositionally biased ORF ; colour 6 ; gene SPAC1A6.11 ; product dubious ;

mRNA SPAC1A6.04c ; temporary_systematic_id SPAC1A6.04c ; note **mRNA** from AU010125 ;

mRNA SPAC19E9.03 ; temporary_systematic_id SPAC19E9.03 ; note **mRNA** from AB045126 ;
mRNA SPAC9.09 ; p_c homocysteine methy**LTR**ansferase ||| similar to S. cerevisiae YER091C ; colour 2 ; gene met26 ||| SPAC9.09 ;
note accummulation of homocysteine causes a defect in purine biosynthesis (PMID 16436428) ; Alias met26 ; GO GO:0009086; methionine biosynthesis
 GO:0003871; 5-methyltetrahydropteroy**LTR**iglutamate- homocysteine S-methy**LTR**ansferase activity
 GO:0005737; cytoplasm
 GO:0046084; adenine biosynthesis ; primary_name met26 ; product homocysteine methy**LTR**ansferase ; EC_number 2.1.1.14 ;

mRNA SPAC1002.13c ; temporary_systematic_id SPAC1002.13c ; note **mRNA** from AU013582 ;

mRNA SPAC1002.20 ; p_c sequence orphan ; colour 8 ; gene SPAC1002.20 ; product sequence orphan ;

misc_feature unknown_1907 ; note gene free region ;

misc_feature unknown_1908 ; note slightly palindromic region at the middle of gene free region; causing self match ;

real_mRNA unknown_1909 ; colour 3 ; note **mRNA** from spc09196 104 1 ;

mRNA SPAC23C11.06c ; temporary_systematic_id SPAC23C11.06c ; note **mRNA** from spc01094 ;

LTR unknown_2139 ; note Tf2 type **LTR** ;

mRNA SPAC22H10.13 ; temporary_systematic_id SPAC22H10.13 ; note **mRNA** from AU009741 ;

mRNA SPAPB24D3.07c ; p_c sequence orphan ||| predicted N-terminal signal sequence ; colour 8 ; gene SPAPB24D3.07c ; product sequence orphan ;

mRNA SPAC31G5.10 ; temporary_systematic_id SPAC31G5.10 ; note AB084881 ;
misc_RNA SPNCRNA.01 ; systematic_id SPNCRNA.01 ; gene prl01 ||| prl1 ; note possibly part of the UTR of eta2 ; Alias prl1 ; db_xref PMID:12597277 ||| EMBL:AB084813 ; synonym prl01 ; controlled_curation term=non-coding RNA; qualifier=predicted; db_xref=PMID:12597277; date=20050412 ||| term=poly(A)-bearing RNA; qualifier=predicted; db_xref=PMID:12597277; date=20050412 ||| term=no detectable long open reading frame; qualifier=predicted; db_xref=PMID:12597277; date=20050412 ; primary_name prl1 ; product non-coding RNA (predicted) ;
mRNA unknown_2876 ; note AB084881 ;
misc_feature unknown_2908 ; note low-complexity gene-free region ;
mRNA SPAC17A2.09c ; p_c RNA-binding protein Csx1 ||| rrm RNA recognition motif ||| similar to S. pombe SPBC23E6.01c ||| similar to S. cerevisiae YHR086W and YBR212W ; colour 2 ; gene csx1 ||| SPAC17A2.09c ; Alias csx1 ; GO GO:0006979; response to oxidative stress
 GO:0003723; RNA binding
 GO:0005737; cytoplasm ; primary_name csx1 ; product RNA-binding protein Csx1 ;
mRNA SPAC17A2.11 ; p_c sequence orphan ; colour 8 ; gene SPAC17A2.11 ; product sequence orphan ; note previously annotated as dubious, but has localization signal ||| see comment on SPAC17A2.10, this region is the same but inverted orientation ||| largish ORF in compositionally biased region, probably not real. has odd translation. this is in region where botton strand is T rich, so lots of LLLFFFFLLFFLSFSFSFS ;
mRNA SPAC8C9.20 ; p_c dubious ; colour 6 ; gene SPAC8C9.20 ; product dubious ; note non consensus branch sites ;
misc_feature unknown_3598 ; note low complexity gene free region ;
misc_RNA SPNCRNA.84 ; db_xref EMBL:AU010014 ; systematic_id SPNCRNA.84 ; controlled_curation term=non-coding RNA; qualifier=predicted; date=20050412 ||| term=no detectable long open reading frame; qualifier=predicted; date=20050412 ; gene SPNCRNA.84 ; product non-coding RNA (predicted) ;
repeat_unit unknown_3599 ; colour 12 ; note region between dg and dh repeat ;
repeat_unit unknown_3600 ; colour 2 ; note dhI repeat ;
repeat_unit unknown_3603 ; colour 2 ; note dh1 repeat ;
repeat_unit unknown_3605 ; colour 3 ; note dgI repeat ;
repeat_unit unknown_3606 ; colour 1 ; note imr1L ;
tRNA SPA**TRNA**ALA.04 ; gene SPA**TRNA**ALA.04 ; product **tRNA** Alanine ; note anticodon AGC, Cove score 67.24 ;
tRNA SPA**TRNA**GLU.03 ; gene SPA**TRNA**GLU.03 ; product **tRNA** Glutamic acid ; note **tRNA** Glu anticodon CTC, Cove score 74.15 ;
misc_feature unknown_3608 ;
tRNA SPA**TRNA**ILE.03 ; gene SPA**TRNA**ILE.03 ; product **tRNA** Isoleucine ; note **tRNA** Ile anticodon AAT, Cove score 66.44 ;
repeat_unit unknown_3609 ; colour 4 ; note cnt1 ;
repeat_unit unknown_3610 ; colour 1 ; note imr1R ;
tRNA SPA**TRNA**ILE.04 ; gene SPA**TRNA**ILE.04 ; product **tRNA** Isoleucine ; note **tRNA** Ile anticodon AAT, Cove score 66.44 ;
tRNA SPA**TRNA**GLU.04 ; gene SPA**TRNA**GLU.04 ; product **tRNA** Glutamic acid ; note **tRNA** Glu anticodon CTC, Cove score 74.15 ;

repeat_unit unknown_3611 ; colour 3 ; partial _no_value ; note dg1 repeat ;

tRNA SPA**TRNA**ALA.05 ; gene SPA**TRNA**ALA.05 ; product **tRNA** Alanine ; note **tRNA** Ala anticodon AGC, Cove score 67.24 ;

misc_feature unknown_3612 ; note nominal overlap with SPAC1856 S. pombe chromosome 1 ;

repeat_unit unknown_3613 ; colour 2 ; note dhI repeat, first 897 bp of SPDHI entry missing ;

repeat_unit unknown_3615 ; colour 12 ; note region between dg and dh repeat ;

misc_RNA SPNCRNA.95 ; systematic_id SPNCRNA.95 ; controlled_curation term=non-coding RNA; qualifier=predicted; date=20050412 ||| term=no detectable long open reading frame; qualifier=predicted; date=20050412 ; gene SPNCRNA.95 ; product non-coding RNA (predicted) ; note from spc00798 133 368; not attached to CDS ||| possibly spurious, expression low on both strands (pers comm. Jurg Bahler) ;

mRNA SPAC4H3.10c ; p_c pyruvate kinase (predicted) ||| similar to S. cerevisiae YAL038W and YOR347C ; colour 7 ; gene pyk1 ||| SPAC4H3.10c ; Alias pyk1 ; primary_name pyk1 ; product pyruvate kinase (predicted) ; EC_number 2.7.1.40 ;

misc_RNA SPNCRNA.92 ; db_xref EMBL:AU008923 ; systematic_id SPNCRNA.92 ; controlled_curation term=non-coding RNA; qualifier=predicted; db_xref=EMBL:AU008923; date=20050412 ||| term=no detectable long open reading frame; qualifier=predicted; db_xref=EMBL:AU008923; date=20050412 ||| term=low complexity gene free region; qualifier=predicted; db_xref=EMBL:AU008923; date=20050412 ; gene SPNCRNA.92 ; product non-coding RNA (predicted) ; note **mRNA** from AU008923. **mRNA** not associated with an ORF ||| strand altered 19.6.2003, evidence from microarray profile (pers comm. Jurg Bahler) ;

mRNA SPAPB15E9.02c ; p_c sequence orphan ||| predicted N-terminal signal sequence ||| 3 predicted transmembrane helices ; colour 8 ; gene SPAPB15E9.02c ; product sequence orphan ; note has transcript on microarray ;

misc_feature unknown_3865 ; note low complexity gene free region ;

misc_feature prl49 ; Alias prl53 ; primary_name prl53 ; gene prl53 ||| prl63 ||| prl49 ; note covered by abundant ESTs; prl53 prl63 and prl49 all map to within this highly transcribed region (PMID 12597277) ;

misc_RNA SPNCRNA.53 ; Alias prl53 ; db_xref PMID:12597277 ||| EMBL:AB084865 ; systematic_id SPNCRNA.53 ; controlled_curation term=non-coding RNA; qualifier=predicted; db_xref=PMID:12597277; date=20050412 ||| term=poly(A)-bearing RNA; qualifier=predicted; db_xref=PMID:12597277; date=20050412 ||| term=no detectable long open reading frame; qualifier=predicted; db_xref=PMID:12597277; date=20050412 ; gene prl53 ; primary_name prl53 ; product non-coding RNA (predicted) ;

misc_RNA SPNCRNA.63 ; systematic_id SPNCRNA.63 ; gene prl63 ; Alias prl63 ; obsolete_name SPNCRNA.49 ; db_xref PMID:12597277 ||| EMBL:AB084875 ||| EMBL:AB084861 ; synonym prl49 ; controlled_curation term=non-coding RNA; qualifier=predicted; db_xref=PMID:12597277; date=20050412 ||| term=poly(A)-bearing RNA; qualifier=predicted; db_xref=PMID:12597277; date=20050412 ||| term=no detectable long open reading frame; qualifier=predicted; db_xref=PMID:12597277; date=20050412 ; primary_name prl63 ; product non-coding RNA (predicted) ;

mRNA SPAC27E2.13 ; p_c dubious ; colour 6 ; controlled_curation term=longest ORF in prl53 (60AA), possibly be protein coding; date=20060721 ; gene SPAC27E2.13 ; product dubious ;

mRNA SPAC27E2.11c ; p_c glycoprotein (predicted) ||| possibly S. pombe specific ||| GPI anchored protein (predicted) (PMID 12845604) (pers. comm. Birgit Eisenhaber) ||| predicted N-terminal signal sequence ; GO GO:0009986; cell surface ; colour 12 ; gene SPAC27E2.11c ; product glycoprotein (predicted) ; note possibly not coding ;

mRNA SPAC19G12.09 ; temporary_systematic_id SPAC19G12.09 ; note **mRNA** from AU009204 1 137 ;

mRNA SPAC19G12.10c ; temporary_systematic_id SPAC19G12.10c ; note **mRNA** from spc11281 231 187 ;
mRNA SPAC16.05c ; p_c transcription factor Sfp1 (predicted) ||| zinc finger protein ||| zf-C2H2 type ||| similar to S. cerevisiae YLR403W ; colour 7 ; gene sfp1 ||| SPAC16.05c ; Alias sfp1 ; GO GO:0006355; regulation of transcription, DNA- dependent
 GO:0003700; transcription factor activity
 GO:0005634; nucleus
 GO:0005737; cytoplasm
 GO:0006950; response to stress ; primary_name sfp1 ; product transcription factor Sfp1 (predicted) ;
misc_RNA SPNCRNA.99 ; systematic_id SPNCRNA.99 ; controlled_curation term=non-coding RNA; qualifier=predicted; date=20050412 ||| term=no detectable long open reading frame; qualifier=predicted; date=20050412 ; gene SPNCRNA.99 ; product non-coding RNA ; note **mRNA** not associated with an ORF ;

misc_feature unknown_4190 ; note nominal overlap with cosmid SPAC9E9, EM:Z99262 S. pombe chromosome 1 ;

mRNA SPAC9E9.01 ; colour 8 ;

misc_feature unknown_4192 ; colour 6 ; note gtacgc, splice donor sequence ;

misc_feature unknown_4193 ; colour 6 ; note ctaatatacggtcaag, splice branch and acceptor ;
misc_RNA SPNCRNA.12 ; Alias prl12 ; db_xref PMID:12597277 ||| EMBL:AB084824 ; systematic_id SPNCRNA.12 ; controlled_curation term=non-coding RNA; qualifier=predicted; db_xref=PMID:12597277; date=20050412 ||| term=poly(A)-bearing RNA; qualifier=predicted; db_xref=PMID:12597277; date=20050412 ||| term=no detectable long open reading frame; qualifier=predicted; db_xref=PMID:12597277; date=20050412 ; gene prl12 ; primary_name prl12 ; product non-coding RNA (predicted) ;

tRNA SPA**TRNA**THR.05 ; gene SPA**TRNA**THR.05 ; product **tRNA** Threonine ; note **tRNA** Thr anticodon AGT, Cove score 75.01 ;

mRNA SPAC19B12.02c ; temporary_systematic_id SPAC19B12.02c ; note **mRNA** from AU010793 ;

mRNA SPAC26F1.06 ; temporary_systematic_id SPAC26F1.06 ; note **mRNA** from AU010092 ;

mRNA SPAC26F1.05 ; p_c sequence orphan ; colour 8 ; gene SPAC26F1.05 ; product sequence orphan ; note has transcript profile on microarray ;

mRNA SPAC922.04 ; temporary_systematic_id SPAC922.04 ; note **mRNA** from AU006605 ;

LTR unknown_5196 ; note 719 (-1) 44 349 Tf2-type **LTR** ;
misc_RNA SPNCRNA.61 ; Alias prl61 ; db_xref EMBL:AB084873 ||| PMID:12597277 ; systematic_id SPNCRNA.61 ; controlled_curation term=non-coding RNA; qualifier=predicted; db_xref=PMID:12597277; date=20050412 ||| term=antisense to SPAC186.07c; qualifier=predicted; db_xref=PMID:12597277; date=20050412 ||| term=poly(A)-bearing RNA; qualifier=predicted; db_xref=PMID:12597277; date=20050412 ||| term=no detectable long open reading frame; qualifier=predicted; db_xref=PMID:12597277; date=20050412 ; gene prl61 ; primary_name prl61 ; product non-coding RNA (predicted) ;

repeat_region unknown_5197 ; note region duplicated in c16A3; c5G8; c569; putative telomere associated fragment ;
mRNA SPBPB21E7.04c ; p_c S-adenosylmethionine-dependent methy**LTR**ansferase (predicted) ||| O-methy**LTR**ansferase (predicted) ||| conserved eukaryotic protein ||| no apparent S. cerevisiae ortholog ||| similar to S. pombe SPBC119.03 ; synonym SPAPB21E7.04c ; colour 10 ; gene SPBPB21E7.04c ; product S-adenosylmethionine-dependent methy**LTR**ansferase (predicted) ;

real_mRNA unknown_33 ; colour 3 ; note **mRNA** from spc08587 1 193 ;
mRNA SPBC1198.14c ; p_c fructose-1,6-bisphosphatase Fbp1 (PMID 2157626) ||| similar to S. cerevisiae YLR377C ; colour 2 ; gene fbp1 ||| SPBC1198.14c ||| SPBC660.04c ; curation transcriptional repression occurs by a cAMP signaling pathway (PMID 1849107) ||| transcriptionnally regulated by adenylate cyclase

activation by a G protein alpha subunit encoded by Gpa2 (PMID 8001792) ||| transcriptional regulators include two redundant Tup1p-like corepressors and the CCAAT binding factor activation complex (PMID 11238405) ; Alias fbp1 ; primary_name fbp1 ; product fructose-1,6-bisphosphatase Fbp1 (PMID 2157626) ;

mRNA SPBC660.04c ; gene SPBC660.04c ||| fbp1 ; note Charlie Hofmann, pers comm ;

mRNA unknown_4363 ;

mRNA SPBC660.06 ; p_c conserved fungal protein ||| glycine-rich ||| WW domain ||| similar to S. pombe SPBC11B10.08 and SPBC660.05 ||| similar to S. cerevisiae YFL010C ; colour 10 ; gene SPBC660.06 ; product conserved fungal protein ;

tRNA SPB**TRNA**GLN.02 ; gene SPB**TRNA**GLN.02 ; product **tRNA** Glutamine ; note **tRNA** Gln anticodon TTG, Cove score 71.12 ;

tRNA SPB**TRNA**GLY.04 ; gene SPB**TRNA**GLY.04 ; product **tRNA** Glycine ; note **tRNA** Gly anticodon TCC, Cove score 54.33 ;

tRNA SPB**TRNA**ALA.07 ; gene SPB**TRNA**ALA.07 ; product **tRNA** Alanine ; note **tRNA** Ala anticodon AGC, Cove score 56.39 ;

tRNA SPB**TRNA**GLY.05 ; gene SPB**TRNA**GLY.05 ; product **tRNA** Glycine ; note **tRNA** Gly anticodon TCC, Cove score 60.52 ;

tRNA SPB**TRNA**ARG.04 ; gene SPB**TRNA**ARG.04 ; product **tRNA** Arginine ; note **tRNA** Arg anticodon TCT, Cove score 69.29 ;

tRNA SPB**TRNA**HIS.01 ; gene SPB**TRNA**HIS.01 ; product **tRNA** Histidine ; note **tRNA** His anticodon GTG ;

rRNA SPRRNA.28 ; systematic_id SPRRNA.28 ; product 5S rRNA ; note SPA Yeast (S.pombe) 5S ribosomal RNA. ;

misc_feature unknown_413 ; note putative gene-free region ;

mRNA SPBC1685.12c ; p_c dubious ||| ORF in compositionally biased region ; colour 6 ; gene SPBC1685.12c ; product dubious ;

misc_feature unknown_414 ; note gt repeat region similar to human/mouse repeated reg ion ;

mRNA SPBC354.12 ; p_c glyceraldehyde 3-phosphate dehydrogenase Gpd3 ||| similar to S. cerevisiae YJL052W and YGR192C and YJR009C ; colour 2 ; gene gpd3 ||| SPBC354.12 ; Alias gpd3 ; GO GO:0006096; glycolysis
 GO:0004365; glyceraldehyde-3-phosphate dehydrogenase (phosphorylating) activity ; primary_name gpd3 ; product glyceraldehyde 3-phosphate dehydrogenase Gpd3 ; EC_number 1.2.1.12 ;

tRNA SPB**TRNA**LYS.06 ; gene SPB**TRNA**LYS.06 ; product **tRNA** Lysine ; note **tRNA** Lys anticodon TTT, Cove score 77.52 ;

mRNA SPBC839.15c ; temporary_systematic_id SPBC839.15c ; note **mRNA** from AU008702 ;

mRNA SPBC119.03 ; temporary_systematic_id SPBC119.03 ; note **mRNA** from spc05940 2 33 ;

mRNA SPBC119.05c ; obsolete_name csh3 ; p_c Wiskott-Aldrich syndrome homolog binding protein Lsb1 (predicted) ||| src (SH3) homology domain ||| similar to S. cerevisiae YGR136W and YPR154W ; colour 7 ; gene SPBC119.05c ; product Wiskott-Aldrich syndrome homolog binding protein Lsb1 (predicted) ;

mRNA SPBC119.05c ; obsolete_name csh3 ; p_c Wiskott-Aldrich syndrome homolog binding protein Lsb1 (predicted) ||| src (SH3) homology domain ||| similar to S. cerevisiae YGR136W and YPR154W ; colour 7 ; gene SPBC119.05c ; product Wiskott-Aldrich syndrome homolog binding protein Lsb1 (predicted) ;

misc_feature unknown_663 ; colour 3 ; note low complexity gene-free region ;

LTR unknown_664 ; colour 4 ; note TF2 type lone **LTR** ;

misc_feature unknown_760 ; note nominal overlap with cosmid p35G2 S. pombe chromosome 2 ;

tRNA SPB**TRNA**HIS.02 ; gene SPB**TRNA**HIS.02 ; product **tRNA** Histidine ; note **tRNA** His anticodon GTG, Cove score 69.87 ;

misc_feature unknown_1138 ; note dicrepancy: with published U14 small nuclear RNA gen e and with cosmid c1268 sequence - additional T residue ins ertion at base 9128 ;
snoRNA SP**SNORNA**.21 ; Alias snoU14 ; db_xref RFAM:RF00016 ; systematic_id SP**SNORNA**.21 ; gene snoU14 ||| SP**SNORNA**.21 ; primary_name snoU14 ; product small nucleolar RNA U14 ;
misc_feature unknown_1287 ; note low complexity gene free region ;
mRNA SPBC32H8.12c ; temporary_systematic_id SPBC32H8.12c ; note **mRNA** from AU013563 ;
misc_feature unknown_1333 ; colour 6 ; note gtaagt, splice donor sequence ;
mRNA SPBC11B10.07c ; colour 7 ;
misc_RNA SPNCRNA.24 ; Alias prl24 ; db_xref PMID:12597277 ||| EMBL:AB084836 ; systematic_id SPNCRNA.24 ; controlled_curation term=non-coding RNA; qualifier=predicted; db_xref=PMID:12597277; date=20050412 ||| term=poly(A)-bearing RNA; qualifier=predicted; db_xref=PMID:12597277; date=20050412 ||| term=no detectable long open reading frame; qualifier=predicted; db_xref=PMID:12597277; date=20050412 ; gene prl24 ; primary_name prl24 ; product non-coding RNA (predicted) ;
mRNA SPBC11B10.08 ; obsolete_name pi003 ||| SPACTOKYO_453.33c ; p_c conserved fungal protein ||| similar to S. cerevisiae YFL010C ||| WW domain ; colour 10 ; gene SPBC11B10.08 ; product conserved fungal protein ;
mRNA SPBC83.19c ; p_c sequence orphan ; colour 8 ; gene SPBC83.19c ; product sequence orphan ; note previously annotated as dubious, but has localization signal ;
tRNA SPB**TRNA**TYR.02 ; gene SPB**TRNA**TYR.02 ; product **tRNA** Tyrosine ; note **tRNA** Tyr anticodon GTA, Cove score 70.26 ;
tRNA SPB**TRNA**LEU.06 ; gene SPB**TRNA**LEU.06 ; product **tRNA** Leucine ; note **tRNA** Leu anticodon CAA, Cove score 56.07 ;
tRNA SPB**TRNA**GLY.07 ; gene SPB**TRNA**GLY.07 ; product **tRNA** Glycine ; note **tRNA** Gly anticodon GCC, Cove score 72.94 ;
tRNA SPB**TRNA**LYS.07 ; gene SPB**TRNA**LYS.07 ; product **tRNA** Lysine ; note **tRNA** Lys anticodon CTT, Cove score 74.83 ;
tRNA SPB**TRNA**ILE.05 ; gene SPB**TRNA**ILE.05 ; product **tRNA** Isoleucine ; note **tRNA** Ile anticodon AAT, Cove score 66.44 ;
tRNA SPB**TRNA**ALA.08 ; gene SPB**TRNA**ALA.08 ; product **tRNA** Alanine ; note **tRNA** Ala anticodon AGC, Cove score 67.24 ;
tRNA SPB**TRNA**VAL.05 ; gene SPB**TRNA**VAL.05 ; product **tRNA** Valine ; note **tRNA** Val anticodon AAC, Cove score 64.30 ;
tRNA SPB**TRNA**GLU.06 ; gene SPB**TRNA**GLU.06 ; product **tRNA** Glutamic acid ; note **tRNA** Glu anticodon TTC, Cove score 62.18 ;
tRNA SPB**TRNA**ARG.06 ; gene SPB**TRNA**ARG.06 ; product **tRNA** Arginine ; note **tRNA** Arg anticodon ACG, Cove score 75.46 ;
repeat_unit unknown_1440 ; colour 1 ; note similar to IMR repeat, not marked on Nature publication map, added May 2002 VW ;
tRNA SPB**TRNA**ASP.03 ; gene SPB**TRNA**ASP.03 ; product **tRNA** Aspartic acid ; note **tRNA** Asp anticodon GTC, Cove score 70.49 ;
repeat_unit unknown_1441 ; colour 1 ; note similar to IMR repeat, not marked on Nature publication map, added May 2002 VW ;
repeat_unit unknown_1442 ; colour 3 ; note dgII repeat ;
repeat_unit unknown_1443 ; colour 7 ; note cen253 Yeast centromere CEN2 repetitive DNA PSS253, between dhIIa and dgIIa ;
repeat_unit unknown_1445 ; colour 2 ; note dhII repeat ;

repeat_unit unknown_1447 ; colour 1 ; note distal part of imr repeated ;
repeat_unit unknown_1448 ; colour 2 ; note dg/dh repeat? ;
repeat_unit unknown_1449 ; colour 1 ; note imr2r ;
tRNA SPB**TRNA**ALA.09 ; gene SPB**TRNA**ALA.09 ; product **tRNA** Alanine ; note **tRNA** Ala anticodon AGC, Cove score 56.39 ;
tRNA SPB**TRNA**VAL.06 ; gene SPB**TRNA**VAL.06 ; product **tRNA** Valine ; note **tRNA** Pseudo VAL anticodon AAC, Cove score 40.82 ;
repeat_unit unknown_1450 ; colour 4 ; note cnt2 ;
repeat_unit unknown_1452 ; colour 4 ; note cnt2 ;
repeat_unit unknown_1453 ; colour 1 ; note imr2L ;
tRNA SPB**TRNA**VAL.07 ; gene SPB**TRNA**VAL.07 ; product **tRNA** Valine ; note **tRNA** Val anticodon AAC, Cove score 64.30 ;
tRNA SPB**TRNA**ALA.10 ; gene SPB**TRNA**ALA.10 ; product **tRNA** Alanine ; note **tRNA** Ala anticodon AGC, Cove score 67.24 ;
tRNA SPB**TRNA**ILE.07 ; gene SPB**TRNA**ILE.07 ; product **tRNA** Isoleucine ; note **tRNA** Ile anticodon AAT, Cove score 66.44 ;
repeat_unit unknown_1455 ; colour 2 ; note dhII repeat partial ;
repeat_unit unknown_1457 ; colour 2 ; note dhII repeat partial ;
repeat_unit unknown_1458 ; colour 12 ; note cen253 Yeast centromere CEN2 repetitive DNA PSS253, between dhIIa and dgIIa ;
repeat_unit unknown_1459 ; colour 3 ; note dgII repeat ;
tRNA SPB**TRNA**TYR.03 ; gene SPB**TRNA**TYR.03 ; product **tRNA** Tyrosine ; note **tRNA** Tyr anticodon GTA, Cove score 70.26 ;
tRNA SPB**TRNA**LEU.07 ; gene SPB**TRNA**LEU.07 ; product **tRNA** Leucine ; note **tRNA** Leu anticodon CAA, Cove score 56.07 ;
tRNA SPB**TRNA**GLY.08 ; gene SPB**TRNA**GLY.08 ; product **tRNA** Glycine ; note **tRNA** Gly anticodon GCC, Cove score 72.94 ;
tRNA SPB**TRNA**LYS.08 ; gene SPB**TRNA**LYS.08 ; product **tRNA** Lysine ; note **tRNA** Lys anticodon CTT, Cove score 74.83 ;
tRNA SPB**TRNA**ILE.08 ; gene SPB**TRNA**ILE.08 ; product **tRNA** Isoleucine ; note **tRNA** Ile anticodon AAT, Cove score 66.44 ;
tRNA SPB**TRNA**ALA.11 ; gene SPB**TRNA**ALA.11 ; product **tRNA** Alanine ; note **tRNA** Ala anticodon AGC, Cove score 67.24 ;
tRNA SPB**TRNA**VAL.08 ; gene SPB**TRNA**VAL.08 ; product **tRNA** Valine ; note **tRNA** Val anticodon AAC, Cove score 64.30 ;
tRNA SPB**TRNA**GLU.07 ; gene SPB**TRNA**GLU.07 ; product **tRNA** Glutamic acid ; note **tRNA** Glu anticodon TTC, Cove score 62.18 ;
tRNA SPB**TRNA**ARG.07 ; gene SPB**TRNA**ARG.07 ; product **tRNA** Arginine ; note **tRNA** Arg anticodon ACG, Cove score 75.46 ;
mRNA SPBC21B10.13c ; p_c transcription factor (predicted) ||| homeobox domain ||| no apparent orthologs, cannot be distinguished ; GO GO:0005634; nucleus
 GO:0003700; transcription factor activity
 GO:0003677; DNA binding
 GO:0006355; regulation of transcription, DNA- dependent ; colour 7 ; gene SPBC21B10.13c ; product transcription factor (predicted) ;
mRNA SPBC21B10.12 ; p_c meiotic recombination protein Rec6 ||| no apparent orthologs ; colour 2 ; gene rec6 ||| SPBC21B10.12 ; Alias rec6 ; GO GO:0007131; meiotic recombination ; primary_name rec6 ; product meiotic recombination protein Rec6 ;

mRNA SPBC19C2.07 ; temporary_systematic_id SPBC19C2.07 ; note **mRNA** from AU011079 ;
mRNA SPBC1E8.05 ; p_c conserved fungal protein ||| no apparent S. cerevisiae ortholog ; GO GO:0009986; cell surface ; colour 10 ; controlled_curation term=glycoprotein; qualifier=predicted; date=20061206 ||| term=serine-rich protein; date=20061206 ||| term=GPI anchored protein; qualifier=RCA; db_xref=PMID: 12845604; date=20061206 ||| term=predicted N-terminal signal sequence; date=20061206 ; gene SPBC1E8.05 ; product conserved fungal protein ;
repeat_region unknown_1954 ; note (taacc)8 ;
mRNA SPBC1815.01 ; p_c enolase ||| similar to S. cerevisiae YGR254W and YHR174W and YOR393W and YPL281C and YMR323W ||| similar to S. pombe eno102 (paralog) ; colour 2 ; gene eno101 ||| eno1 ||| SPBC1815.01 ; Alias eno101 ; primary_name eno101 ; product enolase ; EC_number 4.2.1.11 ;
mRNA SPBC19G7.06 ; colour 2 ;
mRNA SPBC29A10.08 ; temporary_systematic_id SPBC29A10.08 ; note **mRNA** from SPD134 ;
mRNA SPBC32F12.11 ; db_xref EMBL:X85332 ; systematic_id SPBC32F12.11 ;
mRNA SPBC19C7.04c ; p_c conserved fungal protein ||| similar to S. cerevisiae YMR295C ; colour 10 ; gene SPBC19C7.04c ; product conserved fungal protein ;
misc_feature unknown_2607 ; note gene-free region; no gene predictions putative in this region, possibility of small or spliced orfs? ;
misc_RNA SPNCRNA.26 ; Alias prl26 ; db_xref PMID:12597277 ||| EMBL:AB084838 ; systematic_id SPNCRNA.26 ; controlled_curation term=non-coding RNA; qualifier=predicted; db_xref=PMID:12597277; date=20050412 ||| term=poly(A)-bearing RNA; qualifier=predicted; db_xref=PMID:12597277; date=20050412 ||| term=no detectable long open reading frame; qualifier=predicted; db_xref=PMID:12597277; date=20050412 ||| term=low complexity gene free region; qualifier=predicted; date=20050412 ; gene prl26 ; primary_name prl26 ; product non-coding RNA (predicted) ;
misc_feature unknown_2809 ; note low complexity gene-free region ;
misc_feature unknown_2839 ; colour 6 ; note gtaagt, splice donor sequence ;
mRNA SPBC13E7.08c ; colour 7 ;
LTR unknown_3466 ; note Tf2-type **LTR** ;
LTR unknown_3467 ; note Tf1-type **LTR** ;
tRNA SPB**TRNA**LYS.09 ; gene SPB**TRNA**LYS.09 ; product **tRNA** Lysine ; note **tRNA** Lys anticodon CTT ;
tRNA SPB**TRNA**TYR.04 ; gene SPB**TRNA**TYR.04 ; product **tRNA** Tyrosine ; note **tRNA** Tyr anticodon GTA ;
misc_feature unknown_3802 ; note low-complexity gene free region ;
snRNA SP**SNRNA**.07 ; Alias snu32 ; db_xref EMBL:X56189 ||| RFAM:RF00012 ; systematic_id SP**SNRNA**.07 ; gene snu32 ||| SP**SNRNA**.07 ; primary_name snu32 ; product small nuclear RNA U3B ;
mRNA SPBC26H8.11c ; temporary_systematic_id SPBC26H8.11c ; note **mRNA** from AU011994 ;
misc_feature unknown_3873 ; note nominal overlap with cosmid SPBC26H8, EM:AL031743 S. pombe chromosome 2 ;
misc_RNA SPNCRNA.111 ; db_xref EMBL:AU009915 ; systematic_id SPNCRNA.111 ; controlled_curation term=non-coding RNA; qualifier=predicted; db_xref=EMBL:AU009915; date=20050412 ||| term=no detectable long open reading frame; qualifier=predicted; db_xref=EMBL:AU009915; date=20050412 ; gene SPNCRNA.111 ; product non-coding RNA ; note identical to **mRNA** from spc00524 and AU009915 231 1 but not associated with an ORF ;

tRNA SPB**TRNA**GLU.08 ; gene SPB**TRNA**GLU.08 ; product **tRNA** Glutamic acid ; note **tRNA** Glu anticodon CTC, Cove score 74.15 ;
mRNA SPBC14F5.03c ; temporary_systematic_id SPBC14F5.03c ; note **mRNA** from AU007817 ;
real_mRNA unknown_4327 ; note **mRNA** from AU012671 ;
mRNA SPBCPT2R1.08c ; db_xref EMBL:BK005597 ; gene SPBCPT2R1.08c ;
repeat_region unknown_4362 ; note telomeric repeats ;
tRNA SPC**TRNA**HIS.03 ; gene SPC**TRNA**HIS.03 ; product **tRNA** Histidine ; note **tRNA** His anticodon GTG ;
tRNA SPC**TRNA**GLY.10 ; gene SPC**TRNA**GLY.10 ; product **tRNA** Glycine ; note **tRNA** Gly anticodon GCC ;
mRNA SPCC1235.14 ; Alias ght5 ; primary_name ght5 ; gene ght5 ||| SPCC1235.14 ; note **mRNA** from AF017180 ;
mRNA SPCC548.06c ; temporary_systematic_id SPCC548.06c ; note **mRNA** from spc05276 ;
misc_feature unknown_192 ; note gene free region ;
mRNA SPCC736.15 ; p_c protein kinase inhibitor (predicted) ||| similar to S. cerevisiae YGR086C and YPL004C ||| similar to S. pombe SPAC3C7.02c ; GO GO:0005739; mitochondrion
 GO:0005737; cytoplasm
 GO:0005739; mitochondrion
 GO:0004860; protein kinase inhibitor activity
 GO:0009408; response to heat
 GO:0007165; signal transduction ; colour 7 ; gene SPCC736.15 ; product protein kinase inhibitor (predicted) ;
misc_feature unknown_294 ; note low-complexity gene-free region ;
mRNA SPCC1393.08 ; p_c transcription factor (predicted) ||| zinc finger protein ||| zf-GATA type ||| no apparent orthologs, cannot be distinguished ; colour 7 ; gene SPCC1393.08 ; product transcription factor (predicted) ;
mRNA SPCC24B10.21 ; p_c triosephosphate isomerase ||| similar to S. cerevisiae YDR050C ; colour 2 ; gene tpi1 ||| tpi ||| SPCC24B10.21 ; Alias tpi1 ; GO GO:0004807; triose-phosphate isomerase activity
 GO:0006096; glycolysis
 GO:0006094; gluconeogenesis
 GO:0005829; cytosol ; controlled_curation term=disease associated, hemolytic anemia; date=20060920 ||| term=conserved eukaryotic protein; date=20060920 ; primary_name tpi1 ; product triosephosphate isomerase ; EC_number 5.3.1.1 ;
mRNA SPCC1795.11 ; p_c ATP-dependent RNA helicase Sum3 ||| DEAD/DEAH box helicase ||| essential (PMID 9832516) ||| similar to S. cerevisiae YOR204W and YPL119C ; colour 2 ; gene sum3 ||| ded1 ||| slh3 ||| moc2 ||| SPCC1795.11 ; note suppressor of uncontrolled mitosis ||| Multicopy supressor of Overexpressed Cyr1 ; Alias sum3 ; GO GO:0004004; ATP-dependent RNA helicase activity
 GO:0005737; cytoplasm
 GO:0005515; protein binding
 GO:0005515; protein binding
 GO:0006412; translation
 GO:0000086; G2/M transition of mitotic cell cycle
 GO:0000076; DNA replication checkpoint
 GO:0006970; response to osmotic stress
 GO:0031137; regulation of conjugation with cellular fusion ; primary_name sum3 ; product ATP-dependent RNA helicase Sum3 ;
tRNA SPC**TRNA**ALA.12 ; gene SPC**TRNA**ALA.12 ; product **tRNA** Alanine ; note **tRNA** Ala anticodon AGC, Cove score 56.39 ;
tRNA **tRNA**_pseudo anticodon AAC ; pseudo _no_value ; gene **tRNA**_pseudo anticodon AAC ; note **tRNA** Pseudo anticodon AAC, Cove score 40.82 ;
tRNA SPC**TRNA**SER.09 ; gene SPC**TRNA**SER.09 ; product **tRNA** Serine ; note **tRNA** Ser anticodon AGA, Cove score 59.19 ;
tRNA SPC**TRNA**ARG.10 ; gene SPC**TRNA**ARG.10 ; product **tRNA** Arginine ; note **tRNA** Arg anticodon TCG, Cove score 59.66 ;
tRNA SPC**TRNA**ASP.05 ; gene SPC**TRNA**ASP.05 ; product **tRNA** Asparagine ; note **tRNA** Asp anticodon GTC, Cove score 57.96 ;

tRNA SPC**TRNA**ARG.11 ; gene SPC**TRNA**ARG.11 ; product **tRNA** Arginine ; note **tRNA** Arg anticodon ACG, Cove score 54.83 ;
tRNA SPC**TRNA**LEU.11 ; gene SPC**TRNA**LEU.11 ; product **tRNA** Leucine ; note **tRNA** Leu anticodon AAG, Cove score 46.56 ;
misc_feature unknown_1068 ; note nominal overlap with cosmid c1259 ;
repeat_unit unknown_1069 ; colour 1 ; note centromeric region duplicated in SPCC4B3 S. pombe chromosome 3 ;
tRNA SPC**TRNA**LYS.10 ; gene SPC**TRNA**LYS.10 ; product **tRNA** Lysine ; note **tRNA** Lys anticodon CTT, Cove score 57.56 ;
repeat_unit unknown_1070 ; colour 2 ; note dh repeat ;
repeat_unit unknown_1071 ; colour 3 ; note cen3b dgIII repeat ;
repeat_unit unknown_1072 ; colour 1 ; note region between dg and dh repeat ;
repeat_unit unknown_1073 ; colour 2 ; note cen3a dhIII repeat ;
repeat_unit unknown_1076 ; colour 3 ; note dgIII repeat cen3b 250bp ;
repeat_unit unknown_1077 ; colour 12 ; note region between dg and dh repeat ;
repeat_unit unknown_1078 ; colour 2 ; note cen3a dhIII repeat ;
repeat_unit unknown_1080 ; colour 2 ; note dhIII repeat ;
misc_feature unknown_1079 ; note nominal overlap with cosmid c1676 ;
repeat_unit unknown_1081 ; colour 11 ; note cen3xc central region ;
repeat_unit unknown_1083 ;
repeat_unit unknown_1085 ; colour 1 ; note imr3L ;
tRNA SPC**TRNA**ASP.06 ; gene SPC**TRNA**ASP.06 ; product **tRNA** Asparagine ; note **tRNA** Asp anticodon GTC, Cove score 70.49 ;
tRNA SPC**TRNA**ARG.12 ; gene SPC**TRNA**ARG.12 ; product **tRNA** Arginine ; note **tRNA** Arg anticodon ACG, Cove score 75.46 ;
tRNA SPC**TRNA**VAL.09 ; gene SPC**TRNA**VAL.09 ; product **tRNA** Valine ; note **tRNA** Val anticodon AAC, Cove score 64.30 ;
tRNA SPC**TRNA**THR.08 ; gene SPC**TRNA**THR.08 ; product **tRNA** Threonine ; note **tRNA** Thr anticodon AGT, Cove score 75.01 ;
tRNA SPC**TRNA**LEU.12 ; gene SPC**TRNA**LEU.12 ; product **tRNA** Leucine ; note **tRNA** Leu anticodon CAA, Cove score 56.07 ;
repeat_unit unknown_1088 ; colour 4 ; note cnt3 ;
repeat_unit unknown_1089 ; colour 4 ; note cnt3 partial ;
tRNA SPC**TRNA**GLU.10 ; gene SPC**TRNA**GLU.10 ; product **tRNA** Glutamic acid ; note **tRNA** Glu anticodon CTC, Cove score 74.15 ;
repeat_unit unknown_1090 ; colour 1 ; note imr3R ;
tRNA SPC**TRNA**LEU.13 ; gene SPC**TRNA**LEU.13 ; product **tRNA** Leucine ; note **tRNA** Leu anticodon CAA, Cove score 56.07 ;
tRNA SPC**TRNA**THR.09 ; gene SPC**TRNA**THR.09 ; product **tRNA** Threonine ; note **tRNA** Thr anticodon AGT, Cove score 75.01 ;
tRNA SPC**TRNA**VAL.10 ; gene SPC**TRNA**VAL.10 ; product **tRNA** Valine ; note **tRNA** Val anticodon AAC, Cove score 64.30 ;

tRNA SPC**TRNA**ARG.13 ; gene SPC**TRNA**ARG.13 ; product **tRNA** Arginine ; note **tRNA** Arg anticodon ACG, Cove score 75.46 ;
tRNA SPC**TRNA**ASP.07 ; gene SPC**TRNA**ASP.07 ; product **tRNA** Asparagine ; note **tRNA** Asp anticodon GTC, Cove score 70.49 ;
repeat_unit unknown_1093 ; colour 11 ; note cen3xc central region ;
repeat_unit unknown_1097 ; colour 2 ; note cen3a dhIII repeat ;
repeat_unit unknown_1101 ; colour 12 ; note region between dg and dh repeat ;
repeat_unit unknown_1102 ; colour 3 ; note cen3b dgIII repeat ;
repeat_unit unknown_1104 ; colour 12 ; note region between dg and dh repeat ;
repeat_unit unknown_1105 ; colour 3 ; note cen3b dgIII repeat ;
repeat_unit unknown_1106 ; colour 2 ; note dh repeat ;
misc_feature unknown_1107 ; note nominal overlap with pB5A12 S. pombe chromosome 3 ;
tRNA SPC**TRNA**LYS.11 ; gene SPC**TRNA**LYS.11 ; product **tRNA** Lysine ; note **tRNA** Lys anticodon CTT, Cove score 74.69 ;
tRNA SPC**TRNA**VAL.11 ; gene SPC**TRNA**VAL.11 ; product **tRNA** Valine ; note **tRNA** Val anticodon TAC, Cove score 76.36 ;
mRNA SPCC1322.10 ; p_c glycoprotein (predicted) ||| possibly S. pombe specific ||| serine-rich protein ||| GPI anchored protein (predicted) (PMID 12845604) (pers. comm. Birgit Eisenhaber) ||| predicted N-terminal signal sequence ; GO GO:0009986; cell surface ; colour 12 ; gene SPCC1322.10 ; product glycoprotein (predicted) ;
mRNA SPCC13B11.01 ; p_c alcohol dehydrogenase Adh1 ||| similar to S. cerevisiae YOL086C and YMR303C and YMR083W and YBR145W ; colour 2 ; gene adh1 ||| adh ||| SPCC13B11.01 ; Alias adh1 ; GO GO:0006066; alcohol metabolism
 GO:0005759; mitochondrial matrix ; primary_name adh1 ; product alcohol dehydrogenase Adh1 ; EC_number 1.1.1.1 ;
LTR unknown_1643 ; colour 4 ; note lone tf1-type **LTR** ;
tRNA SPC**TRNA**GLN.05 ; gene SPC**TRNA**GLN.05 ; product **tRNA** Glutamine ; note **tRNA** Gln anticodon TTG, Cove score 54.89 ;
mRNA SPCC737.04 ; p_c UPF0300 family ||| possibly S. pombe specific ; colour 12 ; gene SPCC737.04 ; product UPF0300 family ;
tRNA SPC**TRNA**GLY.12 ; gene SPC**TRNA**GLY.12 ; product **tRNA** Glycine ; note **tRNA** Gly anticodon TCC, Cove score 55.00 ;
tRNA SPC**TRNA**SER.13 ; gene SPC**TRNA**SER.13 ; product **tRNA** Serine ; note **tRNA** Ser anticodon GCT, Cove score 74.09 ;
LTR unknown_1954 ; note TF1 **LTR** ;

Appendix II- List of genes or genomic regions enriched by RpL1102

RpL11

mRNA SPAC1F8.07c ; temporary_systematic_id SPAC1F8.07c ; note **mRNA** from AU011749 ;
mRNA SPAC13G6.10c ; p_c O-glucosyl hydrolase (predicted) ||| conserved fungal protein ||| serine-rich protein ||| glycoprotein (predicted) ||| predicted N-terminal signal sequence ||| similar to N. crassa B24p11.210 ||| no apparent S. cerevisiae ortholog ; GO GO:0009986; cell surface
 GO:0004553; hydrolase activity, hydrolyzing O- glycosyl compounds ; colour 10 ; gene SPAC13G6.10c ; product O-glucosyl hydrolase (predicted) ;
mRNA SPAC222.11 ; p_c coproporphyrinogen III oxidase (predicted) ||| similar to S. cerevisiae YDR044W ; systematic_id SPAC222.11 ; colour 7 ; gene hem13 ||| SPAC222.11 ; Alias hem13 ; GO GO:0004109; coproporphyrinogen oxidase activity
 GO:0006783; heme biosynthesis
 GO:0005743; mitochondrial inner membrane ; primary_name hem13 ; product coproporphyrinogen III oxidase (predicted) ; EC_number 1.3.3.3 ; reserved_name hem13 ;
mRNA SPAC1A6.04c ; temporary_systematic_id SPAC1A6.04c ; note **mRNA** from AU010125 ;
mRNA SPAC10F6.06 ; db_xref EMBL:Y13635 ; systematic_id SPAC10F6.06 ;
mRNA SPAC1565.01 ; p_c conserved fungal protein ||| 4 predicted transmembrane helices ||| similar to S. cerevisiae YNR018W ; GO GO:0005739; mitochondrion ; colour 10 ; gene SPAC1565.01 ; product conserved fungal protein ;
mRNA SPAC19E9.03 ; p_c cyclin (PMID 10982385) ||| similar to S. cerevisiae YHR071W ; colour 2 ; gene pas1 ||| SPAC57A10.01 ||| SPAC19E9.03 ; Alias pas1 ; GO GO:0016538; cyclin-dependent protein kinase regulator activity
 GO:0005515; protein binding
 GO:0000082; G1/S transition of mitotic cell cycle
 GO:0000083; G1/S-specific transcription in mitotic cell cycle
 GO:0043091; L-arginine import
 GO:0043091; L-arginine import
 GO:0000079; regulation of cyclin-dependent protein kinase activity
 GO:0007089; traversing start control point of mitotic cell cycle ; primary_name pas1 ; product cyclin (PMID 10982385) ;
mRNA SPAC9.09 ; p_c homocysteine methy**LTR**ansferase ||| similar to S. cerevisiae YER091C ; colour 2 ; gene met26 ||| SPAC9.09 ; note accummulation of homocysteine causes a defect in purine biosynthesis (PMID 16436428) ; Alias met26 ; GO GO:0009086; methionine biosynthesis
 GO:0003871; 5-methyltetrahydropteroy**LTR**iglutamate- homocysteine S-methy**LTR**ansferase activity
 GO:0005737; cytoplasm
 GO:0046084; adenine biosynthesis ; primary_name met26 ; product homocysteine methy**LTR**ansferase ; EC_number 2.1.1.14 ;
mRNA SPAC57A7.04c ; p_c **mRNA** export shuttling protein (PMID 12112233) ||| rrm RNA recognition motif (4) ||| non-essential (PMID 12112233) ||| similar to S. cerevisiae YER165W ; colour 2 ; gene SPAC57A7.04c ||| pabp ; note pab1 used previously by SPAC227.07c ; obsolete_name pab1 ; GO GO:0008143; poly(A) binding
 GO:0006406; **mRNA** export from nucleus
 GO:0005737; cytoplasm
 GO:0005634; nucleus ; product **mRNA** export shuttling protein (PMID 12112233) ;
mRNA SPAC1705.03c ; obsolete_name SPAC1F2.01 ; p_c GPI anchored protein (predicted) (PMID 12845604) (pers. comm. Birgit Eisenhaber) ||| predicted N-terminal signal sequence ||| similar to S. cerevisiae YBR078W and YDR055W and YDR522C and YCL048W ||| similar to S. pombe SPCC1223.12c ; GO GO:0007047; cell wall organization and biogenesis
 GO:0009986; cell surface
 GO:0009897; external side of plasma membrane
 GO:0009277; cell wall (sensu Fungi) ; colour 7 ; gene SPAC1705.03c ||| SPAC23H4.19 ; product GPI anchored protein (predicted) (PMID 12845604)

(pers. comm. Birgit Eisenhaber) ;

mRNA SPAC23H4.06 ; p_c glutamate-ammonia ligase Gln1 ||| similar to S. cerevisiae YPR035W ; colour 2 ; gene gln1 ||| SPAC23H4.06 ; Alias gln1 ; GO GO:0006542; glutamine biosynthesis
 GO:0019740; nitrogen utilization
 GO:0004356; glutamate-ammonia ligase activity
 GO:0005737; cytoplasm ; primary_name gln1 ; product glutamate-ammonia ligase Gln1 ; EC_number 6.3.1.2 ;

mRNA SPAC343.12 ; p_c conserved fungal protein ||| regulated by glucose, ammonium, phosphate, carbon dioxide and temperature (PMID 7565608) ||| predicted N-terminal signal sequence ||| no apparent S. cerevisiae ortholog ||| similar to N. crassa b19a17.210 ; colour 2 ; gene rds1 ||| SPAC343.12 ; Alias rds1 ; GO GO:0006950; response to stress ; primary_name rds1 ; product conserved fungal protein ;

mRNA SPAC664.05 ; p_c 60S ribosomal protein L13 ||| similar to S. cerevisiae YDL082W and YMR142C ; colour 7 ; gene rpl13 ||| SPAC664.05 ; Alias rpl13 ; GO GO:0003735; structural constituent of ribosome
 GO:0006412; translation
 GO:0005842; cytosolic large ribosomal subunit (sensu Eukaryota) ; primary_name rpl13 ; product 60S ribosomal protein L13 ;

mRNA SPAC1002.13c ; temporary_systematic_id SPAC1002.13c ; note **mRNA** from AU013582 ;

misc_feature unknown_1907 ; note gene free region ;

mRNA SPAC23C11.06c ; p_c hydrolase (inferred from context) ||| conserved fungal protein ||| similar to S. cerevisiae YNL115C ||| 5 predicted transmembrane helices ; colour 10 ; gene SPAC23C11.06c ; product hydrolase (inferred from context) ;

mRNA SPAC13F5.03c ; p_c glycerol dehydrogenase (Phlippen, Stevens, Wolf, Zimmermann manuscript in preparation) ||| conserved protein (broad species distribution) ||| predicted N-terminal signal sequence ||| no apparent S. cerevisiae ortholog ; GO GO:0005739; mitochondrion
 GO:0008270; zinc ion binding
 GO:0008888; glycerol dehydrogenase activity
 GO:0019563; glycerol catabolism ; colour 7 ; gene SPAC13F5.03c ; product glycerol dehydrogenase (Phlippen, Stevens, Wolf, Zimmermann manuscript in preparation) ; EC_number 1.1.1.6 ;

mRNA SPAC4A8.04 ; p_c vacuolar serine protease Isp6 ||| similar to S. cerevisiae YEL060C ||| similar to S. pombe SPAC1006.01 ; colour 2 ; gene isp6 ||| prb1 ||| SPAC4A8.04 ; Alias isp6 ; GO GO:0008236; serine-type peptidase activity
 GO:0006401; RNA catabolism
 GO:0006914; autophagy
 GO:0006995; cellular response to nitrogen starvation
 GO:0006995; cellular response to nitrogen starvation
 GO:0000747; conjugation with cellular fusion
 GO:0051603; proteolysis during cellular protein catabolism
 GO:0006508; proteolysis
 GO:0051171; regulation of nitrogen metabolism
 GO:0007033; vacuole organization and biogenesis
 GO:0005773; vacuole ; controlled_curation term=expressed during nitrogen starvation; db_xref=PMID:11872168; date=20060516 ; primary_name isp6 ; product vacuolar serine protease Isp6 ; EC_number 3.4.24.- ||| 3.4.21.- ;

repeat_region unknown_2852 ; note (at)12 ;

misc_feature unknown_2853 ; colour 8 ; note PS01164 Copper amine oxidase topaquinone signature ;

misc_feature unknown_2854 ; colour 8 ; note PS01165 Copper amine oxidase copper-binding site signature ;

mRNA SPAPB24D3.07c ; p_c sequence orphan ||| predicted N-terminal signal sequence ; colour 8 ; gene SPAPB24D3.07c ; product sequence orphan ;

mRNA SPAPB24D3.10c ; temporary_systematic_id SPAPB24D3.10c ; note **mRNA** from AB045751 ;

misc_RNA SPNCRNA.31 ; Alias prl31 ; db_xref PMID:12597277 ||| EMBL:AB084843 ; systematic_id SPNCRNA.31 ; controlled_curation term=non-coding RNA; qualifier=predicted; db_xref=PMID:12597277; date=20050412 ||| term=poly(A)-bearing RNA; qualifier=predicted; db_xref=PMID:12597277; date=20050412 ||| term=no detectable long open reading frame; qualifier=predicted; db_xref=PMID:12597277; date=20050412 ; gene prl31 ; primary_name prl31 ; product non-coding RNA (predicted) ;

rRNA SPRRNA.13 ; systematic_id SPRRNA.13 ; product 5S rRNA ; note 784 (+1) 22 210 SPRG5S yeast (s.pombe) 5s rRNA gene, clone pym3 ;
mRNA SPAPB1A11.03 ; p_c FMN dependent dehydrogenase ||| similar to S. cerevisiae YML054C (C-term) ||| SPAPB1A11.03 lacks S. cerevisiae CYB2 the N-terminal heme binding domain ; colour 7 ; gene SPAPB1A11.03 ; GO GO:0005746; mitochondrial electron transport chain
 GO:0004460; L-lactate dehydrogenase (cytochrome) activity
 GO:0006118; electron transport ; controlled_curation term=cytochrome b2 (l-lactate dehydrogenase); date=20061218 ; product FMN dependent dehydrogenase ; EC_number 1.1.2.3 ;

promoter unknown_2868 ; colour 4 ; note Homol E box ;

promoter unknown_2869 ; colour 4 ; note Homol D box ;
mRNA SPAC31G5.03 ; p_c 40S ribosomal protein S11 ||| similar to S. cerevisiae YDR025W and YBR048W ; colour 2 ; gene rps1101 ||| rps11-1 ||| SPAC31G5.03 ; Alias rps1101 ; GO GO:0003735; structural constituent of ribosome
 GO:0005843; cytosolic small ribosomal subunit (sensu Eukaryota)
 GO:0006412; translation ; primary_name rps1101 ; product 40S ribosomal protein S11 ;
misc_RNA SPNCRNA.71 ; db_xref EMBL:AB084882 ; systematic_id SPNCRNA.71 ; controlled_curation term=non-coding RNA; qualifier=predicted; db_xref=EMBL:AB084882; date=20050412 ||| term=no detectable long open reading frame; qualifier=predicted; db_xref=EMBL:AB084882; date=20050412 ; gene SPNCRNA.71 ; product non-coding RNA (predicted) ;
misc_RNA SPNCRNA.72 ; db_xref EMBL:AB084883 ; systematic_id SPNCRNA.72 ; controlled_curation term=non-coding RNA; qualifier=predicted; db_xref=EMBL:AB084883; date=20050412 ||| term=antisense to SPAC31G5.10; qualifier=predicted; db_xref=EMBL:AB084883; date=20050412 ||| term=no detectable long open reading frame; qualifier=predicted; db_xref=EMBL:AB084883; date=20050412 ; gene SPNCRNA.72 ; product non-coding RNA (predicted) ; note multiplex transcript ||| polyA plus antisense RNA ;

mRNA unknown_2873 ; note AB084882 ;
mRNA SPAC31G5.10 ; p_c Myb family transcriptional regulator Eta2 ||| Myb family ||| expression induced by nitrogen starvation (AB084881) ||| no apparent orthologs, cannot be distinguished ||| antisense transcripts ||| most similar to Reb1 ; colour 7 ; gene eta2 ||| SPAC31G5.10 ; Alias eta2 ; GO GO:0003677; DNA binding
 GO:0045449; regulation of transcription
 GO:0030528; transcription regulator activity ; primary_name eta2 ; product Myb family transcriptional regulator Eta2 ;

mRNA unknown_2874 ; note AB084884 ;
misc_RNA SPNCRNA.73 ; db_xref EMBL:AB084884 ; systematic_id SPNCRNA.73 ; controlled_curation term=non-coding RNA; qualifier=predicted; db_xref=EMBL:AB084884; date=20050412 ||| term=antisense to SPAC31G5.10; qualifier=predicted; db_xref=EMBL:AB084884; date=20050412 ||| term= no detectable long open reading frame; qualifier=predicted; db_xref=EMBL:AB084884; date=20050412 ; gene SPNCRNA.73 ; product non-coding RNA (predicted) ; note multiplex transcript ||| polyA plus antisense RNA ;
misc_RNA SPNCRNA.74 ; db_xref EMBL:AB084885 ; systematic_id SPNCRNA.74 ; controlled_curation term=non-coding RNA; qualifier=predicted; db_xref=EMBL:AB084885; date=20050412 ||| term=antisense to SPAC31G5.10; qualifier=predicted; db_xref=EMBL:AB084885; date=20050412 ||| term=no detectable long open reading frame; qualifier=predicted; db_xref=EMBL:AB084885; date=20050412 ; gene SPNCRNA.74 ; product non-coding RNA (predicted) ; note multiplex transcript ||| polyA plus antisense RNA ;

polyA_signal unknown_2875 ; note AB084885 ;

mRNA SPAC31G5.10 ; temporary_systematic_id SPAC31G5.10 ; note AB084881 ;

misc_RNA SPNCRNA.01 ; systematic_id SPNCRNA.01 ; gene prl01 ||| prl1 ; note possibly part of the UTR of eta2 ; Alias prl1 ; db_xref PMID:12597277 ||| EMBL:AB084813 ; synonym prl01 ; controlled_curation term=non-coding RNA; qualifier=predicted; db_xref=PMID:12597277; date=20050412 ||| term=poly(A)-bearing RNA; qualifier=predicted; db_xref=PMID:12597277; date=20050412 ||| term=no detectable long open reading frame; qualifier=predicted; db_xref=PMID:12597277; date=20050412 ; primary_name prl1 ; product non-coding RNA (predicted) ;

mRNA unknown_2876 ; note AB084881 ;

mRNA SPAC31G5.11 ; p_c cAMP-independent regulatory protein Pac2 (PMID 8536311) ||| similar to S. pombe gti1 ||| similar to S. cerevisiae YEL007W and YHR177W ; colour 2 ; gene pac2 ||| SPAC31G5.11 ; Alias pac2 ; GO GO:0031139; positive regulation of conjugation with cellular fusion ; primary_name pac2 ; product cAMP-independent regulatory protein Pac2 (PMID 8536311) ;

mRNA SPAC31G5.17c ; p_c 40S ribosomal protein S10 ||| similar to S. cerevisiae YOR293W and YMR230W ; colour 2 ; gene rps1001 ||| rps10-1 ||| SPAC31G5.17c ; Alias rps1001 ; GO GO:0003735; structural constituent of ribosome
 GO:0005843; cytosolic small ribosomal subunit (sensu Eukaryota)
 GO:0006412; translation ; primary_name rps1001 ; product 40S ribosomal protein S10 ;

misc_feature unknown_2908 ; note low-complexity gene-free region ;

mRNA SPAC1786.04 ; p_c sequence orphan ; colour 8 ; gene SPAC1786.04 ; product sequence orphan ; note previously annotated as dubious, but has localization signal ;

mRNA SPAC1786.02 ; p_c phospholipase (predicted) ||| similar to S. pombe SPAC977.09c and SPAC1A6.03c and SPCC1450.09c and SPBC1348.10c and SPAC1A6.04c ||| GPI anchored protein (predicted) (PMID 12845604) (pers. comm. Birgit Eisenhaber) ||| glycoprotein (predicted) ||| similar to S. cerevisiae YMR008C and YMR006C and YOL011W ; GO GO:0004620; phospholipase activity
 GO:0046475; glycerophospholipid catabolism
 GO:0009277; cell wall (sensu Fungi)
 GO:0009897; external side of plasma membrane
 GO:0005576; extracellular region ; colour 7 ; gene SPAC1786.02 ; product phospholipase (predicted) ;

mRNA SPAC24C9.05c ; p_c conserved protein (fungal and plant) ||| CBS domain protein ||| octicosapeptide repeat ||| no apparent S. cerevisiae ortholog ; GO GO:0005634; nucleus
 GO:0005737; cytoplasm ; colour 10 ; gene SPAC24C9.05c ; product conserved protein (fungal and plant) ;

tRNA SPA**TRNA**SER.02 ; evidence not_experimental ; gene SPA**TRNA**SER.02 ; product **tRNA** Serine ; note **tRNA** Ser anticodon AGA, Cove score 80.47 ;

mRNA SPAC24C9.06c ; temporary_systematic_id SPAC24C9.06c ; note **mRNA** from AU014505 ;

intron unknown_2927 ; note confirmed intron ;

mRNA SPAC24C9.12c ; p_c glycine hydroxymethy**LTR**ansferase (predicted) ||| similar to S. cerevisiae YLR058C ; colour 7 ; gene SPAC24C9.12c ; GO GO:0006544; glycine metabolism
 GO:0006563; L-serine metabolism
 GO:0006730; one-carbon compound metabolism
 GO:0004372; glycine hydroxymethy**LTR**ansferase activity
 GO:0005737; cytoplasm ; product glycine hydroxymethy**LTR**ansferase (predicted) ; psu_db_xref PATH:MAP00260; ||| PATH:MAP00310; ||| PATH:MAP00460; ||| PATH:MAP00670; ||| PATH:MAP00680; ; EC_number 2.1.2.1 ;

real_mRNA unknown_2946 ; note **mRNA** from AU008838 ;

mRNA SPAC16A10.01 ; p_c conserved protein (broad species distribution) ||| DUF1212 ||| similar to S. cerevisiae YJL107C ||| S. cerevisiae YJL108C is regulated by pheromone ||| 9 predicted transmembrane helices ; colour 10 ; gene SPAC16A10.01 ; product conserved protein (broad species distribution) ;

mRNA SPAC589.06c ; p_c pho88 family protein ||| 3 predicted transmembrane helices ||| similar to S. cerevisiae YBR106W ; GO GO:0016020; membrane
 GO:0006817; phosphate transport ; colour 7 ; gene SPAC589.06c ; product pho88 family protein ;

mRNA SPAC688.03c ; colour 10 ;
mRNA SPAC688.04c ; p_c glutathione S-transferase (PMID 12151111) ||| similar to S. cerevisiae YIR038C ; colour 2 ; gene gst3 ||| SPAC688.04c ; Alias gst3 ; GO GO:0006979; response to oxidative stress
 GO:0042493; response to drug
 GO:0004364; glutathione transferase activity
 GO:0005737; cytoplasm
 GO:0004602; glutathione peroxidase activity
 GO:0042803; protein homodimerization activity ; primary_name gst3 ; product glutathione S-transferase (PMID 12151111) ; EC_number 2.5.1.18 ;
misc_feature SPAC688.11 ; gene SPAC688.11 ; psu_db_xref PO:phos_site ||| PMID: ; note effector Ark1/Prk1 family protein kinase ||| evidence ISS ;
mRNA SPAC3G9.11c ; p_c pyruvate decarboxylase (predicted) ||| similar to S. pombe SPAC13A11.06 ||| similar to S. cerevisiae YGR087C and YLR134W and YLR044C and YDL080C ; colour 7 ; gene SPAC3G9.11c ; product pyruvate decarboxylase (predicted) ; EC_number 4.1.1.1 ;
mRNA SPAC3G9.10c ; temporary_systematic_id SPAC3G9.10c ; note **mRNA** from SPD141 ;
tRNA SPA**TRNA**THR.04 ; gene SPA**TRNA**THR.04 ; product **tRNA** Threonine ; note **tRNA** Thr anticodon CGT, Cove score 76.00 ;
mRNA SPAC3G9.03 ; temporary_systematic_id SPAC3G9.03 ; note **mRNA** from AU009010 293 10 ;
mRNA SPAC1486.05 ; colour 2 ;
mRNA SPAC6G10.03c ; p_c abhydrolase family protein, unknown biological role ||| abhydrolase family ||| conserved eukaryotic protein ||| similar to S. cerevisiae YGR110W ; colour 10 ; gene SPAC6G10.03c ; product abhydrolase family protein, unknown biological role ;
mRNA SPAC6G10.11c ; colour 7 ;
mRNA SPAC6G9.09c ; p_c 60S ribosomal protein L24 ||| similar to S. cerevisiae YGL031C and YGR148C and YLR009W ; colour 7 ; gene rpl24 ||| SPAC6G9.09c ; Alias rpl24 ; GO GO:0003723; RNA binding
 GO:0003735; structural constituent of ribosome
 GO:0005842; cytosolic large ribosomal subunit (sensu Eukaryota)
 GO:0006412; translation ; primary_name rpl24 ; product 60S ribosomal protein L24 ;
mRNA SPAC6G9.10c ; p_c splicing endonuclease Sen1 ||| DNA2/NAM7 family ||| DEAD/DEAH box helicase ||| similar to S. pombe SPBC29A10.10c ||| similar to S. cerevisiae YLR430W ||| disease associated, Ataxia-ocular apraxia 2 ; colour 2 ; gene sen1 ||| SPAC6G9.10c ; Alias sen1 ; controlled_curation term=conserved eukaryotic protein; date=20070115 ; GO GO:0008033; **tRNA** processing
 GO:0004004; ATP-dependent RNA helicase activity
 GO:0005634; nucleus
 GO:0006365; 35S primary transcript processing
 GO:0016180; **snRNA** processing
 GO:0005524; ATP binding ; primary_name sen1 ; product splicing endonuclease Sen1 ;
mRNA SPAC6G9.12 ; p_c Chs five related protein Cfr1 ||| similar to S. cerevisiae YLR330W ; systematic_id SPAC6G9.12 ; colour 2 ; gene cfr1 ||| SPAC6G9.12 ; Alias cfr1 ; GO GO:0000747; conjugation with cellular fusion
 GO:0009250; glucan biosynthesis
 GO:0006031; chitin biosynthesis
 GO:0032219; cell wall catabolism during cytogamy
 GO:0032219; cell wall catabolism during cytogamy
 GO:0000755; cytogamy
 GO:0006893; Golgi to plasma membrane transport
 GO:0005794; Golgi apparatus ; controlled_curation term=BRCT domain; date=20060519 ||| term=fibronectin type III domain; date=20060519 ; primary_name cfr1 ; product Chs five related protein Cfr1 ;
mRNA SPAPB1E7.12 ; p_c 40S ribosomal protein S6 ||| similar to S. cerevisiae YPL090C and YBR181C ; colour 7 ; gene rps602 ||| rps6-2 ||| rps6 ||| SPAPB1E7.12 ; Alias rps602 ; GO GO:0005843; cytosolic small ribosomal subunit (sensu Eukaryota)
 GO:0006412; translation
 GO:0003735; structural constituent of ribosome ; primary_name rps602 ; product 40S ribosomal protein S6 ;
mRNA SPAC26A3.01 ; p_c aspartic protease Sxa1 (PMID 1549128) ||| predicted N-terminal signal sequence ||| GPI anchored protein (predicted) (PMID 12845604) (pers. comm. Birgit Eisenhaber) ||| glycoprotein (predicted) ||| similar to S. cerevisiae YLR120C and YDR144C and YLR121C and YIR039C and YDR349C ;

colour 2 ; gene sxa1 ||| SPAC2E1P5.06 ||| SPAC26A3.01 ; note does not appear to be a yapsin (PMID 11115118) ; Alias sxa1 ; GO GO:0000747; conjugation with cellular fusion
 GO:0005886; plasma membrane
 GO:0009986; cell surface
 GO:0004190; aspartic-type endopeptidase activity ; primary_name sxa1 ; product aspartic protease Sxa1 (PMID 1549128) ; EC_number 3.4.23.- ;
mRNA SPAC26A3.04 ; p_c 60S ribosomal protein L20 ||| similar to S. cerevisiae YMR242C and YOR312C ; colour 7 ; gene rpl2002 ||| rpl20 ||| rpl20-2 ||| SPAC26A3.04 ; Alias rpl2002 ; GO GO:0005842; cytosolic large ribosomal subunit (sensu Eukaryota)
 GO:0006412; translation
 GO:0003735; structural constituent of ribosome ; primary_name rpl2002 ; product 60S ribosomal protein L20 ;
mRNA SPAC17A2.09c ; p_c RNA-binding protein Csx1 ||| rrm RNA recognition motif ||| similar to S. pombe SPBC23E6.01c ||| similar to S. cerevisiae YHR086W and YBR212W ; colour 2 ; gene csx1 ||| SPAC17A2.09c ; Alias csx1 ; GO GO:0006979; response to oxidative stress
 GO:0003723; RNA binding
 GO:0005737; cytoplasm ; primary_name csx1 ; product RNA-binding protein Csx1 ;
mRNA SPAC17A2.11 ; p_c sequence orphan ; colour 8 ; gene SPAC17A2.11 ; product sequence orphan ; note previously annotated as dubious, but has localization signal ||| see comment on SPAC17A2.10, this region is the same but inverted orientation ||| largish ORF in compositionally biased region, probably not real. has odd translation. this is in region where botton strand is T rich, so lots of LLLFFFFLLFFLSFSFSFS ;
mRNA SPAC8C9.04 ; p_c sequence orphan ; GO GO:0007126; meiosis
 GO:0007126; meiosis ; colour 8 ; gene SPAC8C9.04 ; product sequence orphan ;
repeat_unit unknown_3603 ; colour 2 ; note dh1 repeat ;
repeat_unit unknown_3606 ; colour 1 ; note imr1L ;
tRNA SPA**TRNA**GLU.03 ; gene SPA**TRNA**GLU.03 ; product **tRNA** Glutamic acid ; note **tRNA** Glu anticodon CTC, Cove score 74.15 ;
misc_feature unknown_3608 ;
tRNA SPA**TRNA**ILE.03 ; gene SPA**TRNA**ILE.03 ; product **tRNA** Isoleucine ; note **tRNA** Ile anticodon AAT, Cove score 66.44 ;
repeat_unit unknown_3609 ; colour 4 ; note cnt1 ;
repeat_unit unknown_3610 ; colour 1 ; note imr1R ;
tRNA SPA**TRNA**ILE.04 ; gene SPA**TRNA**ILE.04 ; product **tRNA** Isoleucine ; note **tRNA** Ile anticodon AAT, Cove score 66.44 ;
tRNA SPA**TRNA**GLU.04 ; gene SPA**TRNA**GLU.04 ; product **tRNA** Glutamic acid ; note **tRNA** Glu anticodon CTC, Cove score 74.15 ;
repeat_unit unknown_3611 ; colour 3 ; partial _no_value ; note dg1 repeat ;
tRNA SPA**TRNA**ALA.05 ; gene SPA**TRNA**ALA.05 ; product **tRNA** Alanine ; note **tRNA** Ala anticodon AGC, Cove score 67.24 ;
misc_feature unknown_3612 ; note nominal overlap with SPAC1856 S. pombe chromosome 1 ;
mRNA SPAC4H3.10c ; Alias pyk1 ; primary_name pyk1 ; gene pyk1 ||| SPAC4H3.10c ; note **mRNA** from AU012784 ;
mRNA SPAC1071.10c ; p_c P-type proton ATPase Pma1 (PMID 12707717) ||| P3 type (PMID 12707717) ||| 9 predicted transmembrane helices ||| similar to S. cerevisiae YGL008C ; colour 2 ; gene pma1 ||| SPAC1071.10c ; Alias pma1 ; GO GO:0005886; plasma membrane
 GO:0008553; hydrogen-exporting ATPase activity, phosphorylative mechanism
 GO:0015992; proton transport
 GO:0006885; regulation of pH
 GO:0005887; integral to plasma membrane ; primary_name pma1 ; product P-type proton ATPase Pma1 (PMID 12707717) ; EC_number 3.6.3.6 ;
misc_feature prl49 ; Alias prl53 ; primary_name prl53 ; gene prl53 ||| prl63 ||| prl49 ; note covered by abundant ESTs; prl53 prl63 and prl49 all map to within this highly transcribed region (PMID 12597277) ;

misc_RNA SPNCRNA.53 ; Alias prl53 ; db_xref PMID:12597277 ||| EMBL:AB084865 ; systematic_id SPNCRNA.53 ; controlled_curation term=non-coding RNA; qualifier=predicted; db_xref=PMID:12597277; date=20050412 ||| term=poly(A)-bearing RNA; qualifier=predicted; db_xref=PMID:12597277; date=20050412 ||| term=no detectable long open reading frame; qualifier=predicted; db_xref=PMID:12597277; date=20050412 ; gene prl53 ; primary_name prl53 ; product non-coding RNA (predicted) ;
misc_RNA SPNCRNA.63 ; systematic_id SPNCRNA.63 ; gene prl63 ; Alias prl63 ; obsolete_name SPNCRNA.49 ; db_xref PMID:12597277 ||| EMBL:AB084875 ||| EMBL:AB084861 ; synonym prl49 ; controlled_curation term=non-coding RNA; qualifier=predicted; db_xref=PMID:12597277; date=20050412 ||| term=poly(A)-bearing RNA; qualifier=predicted; db_xref=PMID:12597277; date=20050412 ||| term=no detectable long open reading frame; qualifier=predicted; db_xref=PMID:12597277; date=20050412 ; primary_name prl63 ; product non-coding RNA (predicted) ;
mRNA SPAC27E2.13 ; p_c dubious ; colour 6 ; controlled_curation term=longest ORF in prl53 (60AA), possibly be protein coding; date=20060721 ; gene SPAC27E2.13 ; product dubious ;
mRNA SPAC27E2.11c ; p_c glycoprotein (predicted) ||| possibly S. pombe specific ||| GPI anchored protein (predicted) (PMID 12845604) (pers. comm. Birgit Eisenhaber) ||| predicted N-terminal signal sequence ; GO GO:0009986; cell surface ; colour 12 ; gene SPAC27E2.11c ; product glycoprotein (predicted) ; note possibly not coding ;
mRNA SPAC19G12.09 ; p_c NADH/NADPH dependent indole-3-acetaldehyde reductase AKR3C2 ||| aldo/keto reductase ||| similar to S. cerevisiae YDL124W ; GO GO:0047018; indole-3-acetaldehyde reductase (NADH) activity
 GO:0047019; indole-3-acetaldehyde reductase (NADPH) activity
 GO:0008152; metabolism
 GO:0051268; alpha-keto amide reductase activity
 GO:0051269; alpha-keto ester reductase activity
 GO:0005737; cytoplasm
 GO:0005634; nucleus
 GO:0016652; oxidoreductase activity, acting on NADH or NADPH, NAD or NADP as acceptor ; colour 2 ; gene SPAC19G12.09 ; product NADH/NADPH dependent indole-3-acetaldehyde reductase AKR3C2 ;
mRNA SPAC19G12.10c ; temporary_systematic_id SPAC19G12.10c ; note **mRNA** from spc11281 231 187 ;
mRNA SPAC26H5.08c ; p_c glucan 1,3-beta-glucosidase Bgl2 ||| glycosyl hydrolase family 17 ||| glycoprotein (predicted) ||| similar to S. cerevisiae YGR282C ; colour 7 ; gene bgl2 ||| SPAC26H5.08c ; Alias bgl2 ; GO GO:0009277; cell wall (sensu Fungi)
 GO:0004338; glucan 1,3-beta-glucosidase activity
 GO:0007047; cell wall organization and biogenesis
 GO:0006076; 1,3-beta-glucan catabolism ; primary_name bgl2 ; product glucan 1,3-beta-glucosidase Bgl2 ; EC_number 3.2.1.58 ;
mRNA SPAC26H5.09c ; p_c GFO_IDH_MocA family oxidoreductase ||| conserved eukaryotic protein ||| similar to S. cerevisiae YMR315W ||| similar to S. pombe SPBC115.03 (paralog) ; colour 10 ; gene SPAC26H5.09c ; product GFO_IDH_MocA family oxidoreductase ;
mRNA SPAC16.02c ; db_xref EMBL:D89163 ; systematic_id SPAC16.02c ;
mRNA SPAC16.05c ; p_c transcription factor Sfp1 (predicted) ||| zinc finger protein ||| zf-C2H2 type ||| similar to S. cerevisiae YLR403W ; colour 7 ; gene sfp1 ||| SPAC16.05c ; Alias sfp1 ; GO GO:0006355; regulation of transcription, DNA- dependent
 GO:0003700; transcription factor activity
 GO:0005634; nucleus
 GO:0005737; cytoplasm
 GO:0006950; response to stress ; primary_name sfp1 ; product transcription factor Sfp1 (predicted) ;
misc_RNA SPNCRNA.99 ; systematic_id SPNCRNA.99 ; controlled_curation term=non-coding RNA; qualifier=predicted; date=20050412 ||| term=no detectable long open reading frame; qualifier=predicted; date=20050412 ; gene SPNCRNA.99 ; product non-coding RNA ; note **mRNA** not associated with an ORF ;
misc_feature unknown_4190 ; note nominal overlap with cosmid SPAC9E9, EM:Z99262 S. pombe chromosome 1 ;
mRNA SPAC9E9.01 ; p_c sequence orphan ; colour 8 ; gene SPAC9E9.01 ; product sequence orphan ;

mRNA SPAC9E9.03 ; p_c 3-isopropylmalate dehydratase Leu2 (predicted) ||| similar to S. cerevisiae YGL009C ; colour 7 ; gene leu2 ||| SPAC9E9.03 ; Alias leu2 ; GO GO:0003861; 3-isopropylmalate dehydratase activity
 GO:0009098; leucine biosynthesis
 GO:0005829; cytosol ; primary_name leu2 ; product 3-isopropylmalate dehydratase Leu2 (predicted) ; EC_number 4.2.1.33 ;
mRNA SPAPYUG7.03c ; p_c anillin homologue Mid2 ||| pleckstrin homology domain ||| transcriptionally regulated by Ace2 (PMID 16317047) ||| similar to S. cerevisiae YJR092W ; colour 2 ; gene mid2 ||| SPAPYUG7.03c ; Alias mid2 ; GO GO:0000920; cell separation during cytokinesis
 GO:0000910; cytokinesis
 GO:0005525; GTP binding
 GO:0005940; septin ring
 GO:0031106; septin ring organization
 GO:0031107; septin ring disassembly
 GO:0031097; medial ring ; primary_name mid2 ; product anillin homologue Mid2 ;
mRNA SPAC4F10.15c ; p_c WASp homolog ||| disease associated, Wiskott- Aldrich syndrome ||| conserved eukaryotic protein ||| similar to S. cerevisiae YOR181W ; colour 2 ; gene wsp1 ||| SPAC4F10.15c ; curation promoter homolD box ; Alias wsp1 ; GO GO:0030479; actin cortical patch
 GO:0051286; cell tip
 GO:0005826; contractile ring
 GO:0005515; protein binding
 GO:0000147; actin cortical patch assembly
 GO:0000147; actin cortical patch assembly
 GO:0045010; actin nucleation
 GO:0045010; actin nucleation
 GO:0009272; cell wall biosynthesis (sensu Fungi)
 GO:0030467; establishment and/or maintenance of cell polarity (sensu Fungi)
 GO:0030833; regulation of actin filament polymerization
 GO:0000915; cytokinesis, contractile ring formation
 GO:0000916; cytokinesis, contractile ring contraction ; primary_name wsp1 ; product WASp homolog ;
mRNA SPAC19B12.02c ; temporary_systematic_id SPAC19B12.02c ; note **mRNA** from AU010793 ;
mRNA SPAC1B3.16c ; p_c vitamin H transporter Vth1 ||| similar to S. pombe SPAC1B3.15c ||| similar to S. cerevisiae YGR065C ; colour 2 ; gene vht1 ||| SPAC1B3.16c ; Alias vht1 ; controlled_curation term=tandem duplication; date=20061218 ||| term=allantoate permease family; cv=protein_family; date=20061218 ||| term=conserved eukaryotic family; cv=species_dist; date=20061218 ; GO GO:0015225; biotin transporter activity
 GO:0005886; plasma membrane
 GO:0016021; integral to membrane
 GO:0015878; biotin transport
 GO:0005386; carrier activity ; primary_name vht1 ; product vitamin H transporter Vth1 ;
mRNA SPAC26F1.06 ; temporary_systematic_id SPAC26F1.06 ; note **mRNA** from AU010092 ;
mRNA SPAP8A3.04c ; p_c heat shock protein Hsp9 (PMID 8679693) ||| similar to S. cerevisiae YFL014W ; colour 2 ; gene hsp9 ||| scf1 ||| SPAP8A3.04c ; note possible sequencing error, 98 aa version reported (PMID:8654972) ; Alias hsp9 ; GO GO:0000915; cytokinesis, contractile ring formation ; primary_name hsp9 ; product heat shock protein Hsp9 (PMID 8679693) ;
mRNA SPAC29B12.04 ; p_c pyridoxine biosynthesis protein ||| similar to S. cerevisiae YMR096W and YFL059W and YNL333W ; colour 2 ; gene snz1 ||| SPAC29B12.04 ; Alias snz1 ; GO GO:0008615; pyridoxine biosynthesis
 GO:0008615; pyridoxine biosynthesis ; primary_name snz1 ; product pyridoxine biosynthesis protein ;
mRNA SPAC922.04 ; temporary_systematic_id SPAC922.04 ; note **mRNA** from AU006605 ;
LTR unknown_5196 ; note 719 (-1) 44 349 Tf2-type **LTR** ;
mRNA SPAC186.07c ; p_c hydroxyacid dehydrogenase (predicted) ||| D isomer specific ||| no apparent S. cerevisiae ortholog ||| similar to S. pombe SPAC186.02C ; colour 10 ; gene SPAC186.07c ; product hydroxyacid dehydrogenase (predicted) ;
misc_RNA SPNCRNA.61 ; Alias prl61 ; db_xref EMBL:AB084873 ||| PMID:12597277 ; systematic_id SPNCRNA.61 ; controlled_curation term=non-coding RNA; qualifier=predicted; db_xref=PMID:12597277; date=20050412 ||| term=antisense to SPAC186.07c; qualifier=predicted; db_xref=PMID:12597277; date=20050412 ||| term=poly(A)-bearing RNA; qualifier=predicted; db_xref=PMID:12597277; date=20050412 ||| term=no detectable long open reading frame; qualifier=predicted;

db_xref=PMID:12597277; date=20050412 ; gene prl61 ; primary_name prl61 ; product non-coding RNA (predicted) ;

mRNA SPAC186.09 ; p_c pyruvate decarboxylase (predicted) ||| similar to S. pombe SPAC1F8.07C ||| no apparent S. cerevisiae ortholog ; colour 7 ; gene SPAC186.09 ; product pyruvate decarboxylase (predicted) ;

mRNA SPBPB21E7.04c ; p_c S-adenosylmethionine-dependent methy**LTR**ansferase (predicted) ||| O-methy**LTR**ansferase (predicted) ||| conserved eukaryotic protein ||| no apparent S. cerevisiae ortholog ||| similar to S. pombe SPBC119.03 ; synonym SPAPB21E7.04c ; colour 10 ; gene SPBPB21E7.04c ; product S-adenosylmethionine-dependent methy**LTR**ansferase (predicted) ;

mRNA SPBC1198.01 ; p_c glutathione-dependent formaldehyde dehydrogenase (predicted) ||| no apparent S. cerevisiae ortholog ||| conserved protein (mainly bacterial) ; colour 7 ; gene SPBC1198.01 ; product glutathione-dependent formaldehyde dehydrogenase (predicted) ;

mRNA SPBC1198.14c ; p_c fructose-1,6-bisphosphatase Fbp1 (PMID 2157626) ||| similar to S. cerevisiae YLR377C ; colour 2 ; gene fbp1 ||| SPBC1198.14c ||| SPBC660.04c ; curation transcriptional repression occurs by a cAMP signaling pathway (PMID 1849107) ||| transcriptionnally regulated by adenylate cyclase activation by a G protein alpha subunit encoded by Gpa2 (PMID 8001792) ||| transcriptional regulators include two redundant Tup1p-like corepressors and the CCAAT binding factor activation complex (PMID 11238405) ; Alias fbp1 ; primary_name fbp1 ; product fructose-1,6-bisphosphatase Fbp1 (PMID 2157626) ;

mRNA SPBC660.04c ; gene SPBC660.04c ||| fbp1 ; note Charlie Hofmann, pers comm ;

mRNA unknown_4363 ;

mRNA SPBC660.06 ; p_c conserved fungal protein ||| glycine-rich ||| WW domain ||| similar to S. pombe SPBC11B10.08 and SPBC660.05 ||| similar to S. cerevisiae YFL010C ; colour 10 ; gene SPBC660.06 ; product conserved fungal protein ;

tRNA SPB**TRNA**GLY.04 ; gene SPB**TRNA**GLY.04 ; product **tRNA** Glycine ; note **tRNA** Gly anticodon TCC, Cove score 54.33 ;

tRNA SPB**TRNA**ALA.07 ; gene SPB**TRNA**ALA.07 ; product **tRNA** Alanine ; note **tRNA** Ala anticodon AGC, Cove score 56.39 ;

tRNA SPB**TRNA**GLY.05 ; gene SPB**TRNA**GLY.05 ; product **tRNA** Glycine ; note **tRNA** Gly anticodon TCC, Cove score 60.52 ;

tRNA SPB**TRNA**ARG.04 ; gene SPB**TRNA**ARG.04 ; product **tRNA** Arginine ; note **tRNA** Arg anticodon TCT, Cove score 69.29 ;

mRNA SPBC1685.12c ; p_c dubious ||| ORF in compositionally biased region ; colour 6 ; gene SPBC1685.12c ; product dubious ;

mRNA SPBC1685.13 ; p_c non classical export pathway protein (predicted) ||| predicted N-terminal signal sequence ||| 3 predicted transmembrane helices ||| non-essential (PMID 12618370) ||| similar to S. cerevisiae YPR149W and YGR131W ; GO GO:0005737; cytoplasm
 GO:0005739; mitochondrion
 GO:0009306; protein secretion
 GO:0005783; endoplasmic reticulum ; colour 7 ; gene SPBC1685.13 ; product non classical export pathway protein (predicted) ;

misc_feature unknown_414 ; note gt repeat region similar to human/mouse repeated reg ion ;

mRNA SPBC649.04 ; p_c UV-induced protein Uvi15 ||| fibrillarin binds to a 3' cis-regulatory element in pre-**mRNA** of Uvi15 (PMID 1207460) ||| non-essential (PMID 12618370) ||| similar to S. cerevisiae YDL012C and YDR210W and YBR016W ; colour 2 ; gene uvi15 ||| SPBC649.04 ; Alias uvi15 ; GO GO:0009408; response to heat
 GO:0006974; response to DNA damage stimulus
 GO:0007584; response to nutrient ; primary_name uvi15 ; product UV-induced protein Uvi15 ;

mRNA SPBC649.04 ; colour 2 ;

mRNA SPBC354.12 ; p_c glyceraldehyde 3-phosphate dehydrogenase Gpd3 ||| similar to S. cerevisiae YJL052W and YGR192C and YJR009C ; colour 2 ; gene gpd3 ||| SPBC354.12 ; Alias gpd3 ; GO GO:0006096; glycolysis
 GO:0004365; glyceraldehyde-3-phosphate dehydrogenase (phosphorylating) activity ;

primary_name gpd3 ; product glyceraldehyde 3-phosphate dehydrogenase Gpd3 ; EC_number 1.2.1.12 ;

mRNA SPBC119.03 ; p_c S-adenosylmethionine-dependent methy**LTR**ansferase (predicted) ||| O-methy**LTR**ansferase (predicted) ||| conserved eukaryotic protein ||| no apparent S. cerevisiae ortholog ||| similar to S. pombe SPBPB21E7.03c (paralog) ; colour 10 ; gene SPBC119.03 ; product S-adenosylmethionine-dependent methy**LTR**ansferase (predicted) ;

mRNA SPBC119.05c ; obsolete_name csh3 ; p_c Wiskott-Aldrich syndrome homolog binding protein Lsb1 (predicted) ||| src (SH3) homology domain ||| similar to S. cerevisiae YGR136W and YPR154W ; colour 7 ; gene SPBC119.05c ; product Wiskott-Aldrich syndrome homolog binding protein Lsb1 (predicted) ;

mRNA SPBC577.14c ; colour 2 ;

mRNA SPBC713.12 ; p_c squalene monooxygenase Erg1 (predicted) ||| similar to S. cerevisiae YGR175C ; systematic_id SPBC713.12 ; colour 7 ; gene erg1 ||| SPBC713.12 ; Alias erg1 ; GO GO:0006696; ergosterol biosynthesis
 GO:0004506; squalene monooxygenase activity
 GO:0005789; endoplasmic reticulum membrane ; primary_name erg1 ; product squalene monooxygenase Erg1 (predicted) ; EC_number 1.14.99.7 ; reserved_name erg1 ;

mRNA SPBC1709.05 ; p_c heat shock protein Sks2 ||| heat shock protein 70 family (PMID 8973306) ||| confers K-252a resistance (PMID 9161410) ||| similar to S. cerevisiae YDL229W and YNL209W ; colour 2 ; gene sks2 ||| hsc1 ||| SPBC1709.05 ; note ask2 is the name which was given to the spurious orf on the opposite strand ; Alias sks2 ; primary_name sks2 ; product heat shock protein Sks2 ;

mRNA SPBC31A8.02 ; p_c pseudo ; colour 13 ; gene SPBC3D6.01 ||| SPBC31A8.02 ; note previously annotated as very hypothetical protein but has no met so assuming dubious or pseudo ; Alias SPBC3D6.01 ; pseudo _no_value ; primary_name SPBC3D6.01 ; product pseudo ;

misc_feature unknown_1101 ; note putative gene free region ;

mRNA SPBC31A8.02 ; p_c pseudo ; colour 13 ; gene SPBC3D6.01 ||| SPBC31A8.02 ; note previously annotated as very hypothetical protein but has no met so assuming dubious or pseudo ; Alias SPBC3D6.01 ; pseudo _no_value ; primary_name SPBC3D6.01 ; product pseudo ;

mRNA SPBC3D6.02 ; p_c neddylation pathway protein But2 (PMID 14623327) ||| But2 family protein ||| possibly S. pombe specific ||| predicted N- terminal signal sequence ||| similar to S. pombe SPAC27D7.09c and SPAC27D7.10c and SPAC27D7.11c (paralogs) ; colour 2 ; gene but2 ||| SPBC3D6.02 ; Alias but2 ; GO GO:0005515; protein binding
 GO:0005515; protein binding ; primary_name but2 ; product neddylation pathway protein But2 (PMID 14623327) ;

misc_feature unknown_1138 ; note dicrepancy: with published U14 small nuclear RNA gen e and with cosmid c1268 sequence - additional T residue ins ertion at base 9128 ;

snoRNA SP**SNORNA**.21 ; Alias snoU14 ; db_xref RFAM:RF00016 ; systematic_id SP**SNORNA**.21 ; gene snoU14 ||| SP**SNORNA**.21 ; primary_name snoU14 ; product small nucleolar RNA U14 ;

mRNA SPBC8D2.04 ; p_c histone H3 ||| histone fold ||| similar to S. pombe hht1 and hht3 ||| amino terminus K9, K14, S8 are involved in centomeric silencing (PMID 14561399) ||| similar to S. cerevisiae YBR010W and YNL031C ; colour 2 ; gene hht2 ||| h3.2 ||| SPBC8D2.04 ; Alias hht2 ; GO GO:0030702; chromatin silencing at centromere
 GO:0003677; DNA binding
 GO:0006333; chromatin assembly or disassembly
 GO:0000788; nuclear nucleosome ; primary_name hht2 ; product histone H3 ;

mRNA SPBC32H8.12c ; temporary_systematic_id SPBC32H8.12c ; note **mRNA** from AU013563 ;

mRNA SPBC11B10.08 ; obsolete_name pi003 ||| SPACTOKYO_453.33c ; p_c conserved fungal protein ||| similar to S. cerevisiae YFL010C ||| WW domain ; colour 10 ; gene SPBC11B10.08 ; product conserved fungal protein ;

tRNA SPB**TRNA**LEU.06 ; gene SPB**TRNA**LEU.06 ; product **tRNA** Leucine ; note **tRNA** Leu anticodon CAA, Cove score 56.07 ;

tRNA SPB**TRNA**GLY.07 ; gene SPB**TRNA**GLY.07 ; product **tRNA** Glycine ; note **tRNA** Gly anticodon GCC, Cove score 72.94 ;
tRNA SPB**TRNA**LYS.07 ; gene SPB**TRNA**LYS.07 ; product **tRNA** Lysine ; note **tRNA** Lys anticodon CTT, Cove score 74.83 ;
tRNA SPB**TRNA**ILE.05 ; gene SPB**TRNA**ILE.05 ; product **tRNA** Isoleucine ; note **tRNA** Ile anticodon AAT, Cove score 66.44 ;
tRNA SPB**TRNA**ALA.08 ; gene SPB**TRNA**ALA.08 ; product **tRNA** Alanine ; note **tRNA** Ala anticodon AGC, Cove score 67.24 ;
tRNA SPB**TRNA**VAL.05 ; gene SPB**TRNA**VAL.05 ; product **tRNA** Valine ; note **tRNA** Val anticodon AAC, Cove score 64.30 ;
tRNA SPB**TRNA**GLU.06 ; gene SPB**TRNA**GLU.06 ; product **tRNA** Glutamic acid ; note **tRNA** Glu anticodon TTC, Cove score 62.18 ;
tRNA SPB**TRNA**ARG.06 ; gene SPB**TRNA**ARG.06 ; product **tRNA** Arginine ; note **tRNA** Arg anticodon ACG, Cove score 75.46 ;
repeat_unit unknown_1440 ; colour 1 ; note similar to IMR repeat, not marked on Nature publication map, added May 2002 VW ;
tRNA SPB**TRNA**ASP.03 ; gene SPB**TRNA**ASP.03 ; product **tRNA** Aspartic acid ; note **tRNA** Asp anticodon GTC, Cove score 70.49 ;
repeat_unit unknown_1441 ; colour 1 ; note similar to IMR repeat, not marked on Nature publication map, added May 2002 VW ;
repeat_unit unknown_1448 ; colour 2 ; note dg/dh repeat? ;
tRNA SPB**TRNA**VAL.06 ; gene SPB**TRNA**VAL.06 ; product **tRNA** Valine ; note **tRNA** Pseudo VAL anticodon AAC, Cove score 40.82 ;
repeat_unit unknown_1450 ; colour 4 ; note cnt2 ;
repeat_unit unknown_1452 ; colour 4 ; note cnt2 ;
repeat_unit unknown_1453 ; colour 1 ; note imr2L ;
tRNA SPB**TRNA**VAL.07 ; gene SPB**TRNA**VAL.07 ; product **tRNA** Valine ; note **tRNA** Val anticodon AAC, Cove score 64.30 ;
tRNA SPB**TRNA**ALA.10 ; gene SPB**TRNA**ALA.10 ; product **tRNA** Alanine ; note **tRNA** Ala anticodon AGC, Cove score 67.24 ;
tRNA SPB**TRNA**ILE.07 ; gene SPB**TRNA**ILE.07 ; product **tRNA** Isoleucine ; note **tRNA** Ile anticodon AAT, Cove score 66.44 ;
tRNA SPB**TRNA**LYS.08 ; gene SPB**TRNA**LYS.08 ; product **tRNA** Lysine ; note **tRNA** Lys anticodon CTT, Cove score 74.83 ;
tRNA SPB**TRNA**ILE.08 ; gene SPB**TRNA**ILE.08 ; product **tRNA** Isoleucine ; note **tRNA** Ile anticodon AAT, Cove score 66.44 ;
tRNA SPB**TRNA**ALA.11 ; gene SPB**TRNA**ALA.11 ; product **tRNA** Alanine ; note **tRNA** Ala anticodon AGC, Cove score 67.24 ;
tRNA SPB**TRNA**VAL.08 ; gene SPB**TRNA**VAL.08 ; product **tRNA** Valine ; note **tRNA** Val anticodon AAC, Cove score 64.30 ;
tRNA SPB**TRNA**GLU.07 ; gene SPB**TRNA**GLU.07 ; product **tRNA** Glutamic acid ; note **tRNA** Glu anticodon TTC, Cove score 62.18 ;
tRNA SPB**TRNA**ARG.07 ; gene SPB**TRNA**ARG.07 ; product **tRNA** Arginine ; note **tRNA** Arg anticodon ACG, Cove score 75.46 ;
mRNA SPBC21B10.13c ; p_c transcription factor (predicted) ||| homeobox domain ||| no apparent orthologs, cannot be distinguished ; GO GO:0005634; nucleus
 GO:0003700; transcription factor activity
 GO:0003677; DNA binding
 GO:0006355; regulation of transcription, DNA- dependent ; colour 7 ; gene SPBC21B10.13c ; product transcription factor (predicted) ;
mRNA SPBC21B10.12 ; p_c meiotic recombination protein Rec6 ||| no apparent orthologs ; colour 2 ; gene rec6 ||| SPBC21B10.12 ; Alias rec6 ; GO GO:0007131; meiotic recombination ; primary_name rec6 ; product meiotic recombination protein Rec6 ;

mRNA SPBC19C2.07 ; temporary_systematic_id SPBC19C2.07 ; note **mRNA** from AU011079 ;
mRNA SPBC16E9.16c ; p_c sequence orphan ; colour 8 ; gene SPBC16E9.16c ; product sequence orphan ; note was previously annotated as pseudo however has peptide fragments two peptides in our mass spec analysis (pers. comm. Dieter Wolf), and is constantly expressed under stress conditions from microarray data; updated gene prediction to give valid translation but splice consensus isn't great for either intron 8-11-05 ;
mRNA SPBC1E8.05 ; p_c conserved fungal protein ||| no apparent S. cerevisiae ortholog ; GO GO:0009986; cell surface ; colour 10 ; controlled_curation term=glycoprotein; qualifier=predicted; date=20061206 ||| term=serine-rich protein; date=20061206 ||| term=GPI anchored protein; qualifier=RCA; db_xref= PMID:12845604; date=20061206 ||| term=predicted N-terminal signal sequence; date=20061206 ; gene SPBC1E8.05 ; product conserved fungal protein ;
mRNA SPBP23A10.11c ; p_c glycoprotein ||| predicted N-terminal signal sequence ||| serine-rich protein ||| conserved fungal protein ||| similar to S. cerevisiae YBR162C and YJL171C ; GO GO:0009277; cell wall (sensu Fungi) ; colour 10 ; gene SPBP23A10.11c ; product glycoprotein ;
mRNA SPBC17G9.10 ; p_c 60S ribosomal protein L11 ||| similar to S. cerevisiae YPR102C and YGR085C ; colour 7 ; gene rpl1102 ||| rpl11-2 ||| SPBC17G9.10 ; Alias rpl1102 ; GO GO:0005843; cytosolic small ribosomal subunit (sensu Eukaryota)
 GO:0006412; translation
 GO:0003735; structural constituent of ribosome ; primary_name rpl1102 ; product 60S ribosomal protein L11 ;
mRNA SPBC1815.01 ; p_c enolase ||| similar to S. cerevisiae YGR254W and YHR174W and YOR393W and YPL281C and YMR323W ||| similar to S. pombe eno102 (paralog) ; colour 2 ; gene eno101 ||| eno1 ||| SPBC1815.01 ; Alias eno101 ; primary_name eno101 ; product enolase ; EC_number 4.2.1.11 ;
mRNA SPBC19G7.06 ; p_c MADS-box transcription factor Mbx1 ||| MADS-box ||| similar to S. pombe SPAC11E3.06 ||| similar to S. cerevisiae YMR043W and YMR042W ||| not required for periodic transcription in M phase ||| PBF transcription factor complex (PMID 15509866) ||| possibly functional ortholog of YMR043W (PMID 15509866) ; systematic_id SPBC19G7.06 ; colour 2 ; gene mbx1 ||| SPBC19G7.06 ; note Mads BoX protrein 1 ; Alias mbx1 ; GO GO:0045896; regulation of transcription, mitotic
 GO:0000086; G2/M transition of mitotic cell cycle
 GO:0000910; cytokinesis
 GO:0005667; transcription factor complex
 GO:0003702; RNA polymerase II transcription factor activity
 GO:0003677; DNA binding ; controlled_curation term=phosphorylated; cv=[pt_mod; db_xref=PMID:15509866; date=20061127 ; primary_name mbx1 ; product MADS-box transcription factor Mbx1 ;
mRNA SPBC29A10.08 ; temporary_systematic_id SPBC29A10.08 ; note **mRNA** from SPD134 ;
mRNA SPBC32F12.11 ; db_xref EMBL:X85332 ; systematic_id SPBC32F12.11 ;
mRNA SPBC19C7.04c ; p_c conserved fungal protein ||| similar to S. cerevisiae YMR295C ; colour 10 ; gene SPBC19C7.04c ; product conserved fungal protein ;
misc_RNA SPNCRNA.26 ; Alias prl26 ; db_xref PMID:12597277 ||| EMBL:AB084838 ; systematic_id SPNCRNA.26 ; controlled_curation term=non-coding RNA; qualifier=predicted; db_xref=PMID:12597277; date=20050412 ||| term=poly(A)-bearing RNA; qualifier=predicted; db_xref=PMID:12597277; date=20050412 ||| term=no detectable long open reading frame; qualifier=predicted; db_xref=PMID:12597277; date=20050412 ||| term=low complexity gene free region; qualifier= predicted; date=20050412 ; gene prl26 ; primary_name prl26 ; product non-coding RNA (predicted) ;
mRNA SPBC887.15c ; p_c sphingosine hydroxylase (predicted) ||| sterol desaturase (predicted) ||| 3 predicted transmembrane helices ||| similar to S. cerevisiae YDR297W ; GO GO:0000170; sphingosine hydroxylase activity
 GO:0030148; sphingolipid biosynthesis
 GO:0005789; endoplasmic reticulum membrane ; colour 7 ; gene SPBC887.15c ; product sphingosine hydroxylase (predicted) ;
mRNA SPBC26H8.10 ; p_c 3'-5' exoribonuclease subunit Dis3 (predicted) ||| essential (PMID 1944266) ||| RNB domain ||| similar to S. cerevisiae YOL021C ; colour 2 ; gene dis3 ||| SPBC26H8.10 ; note does not bind GeneDB_Spombe:SPBC776.02c PMID 1944266 ; Alias dis3 ; GO GO:0000176; nuclear exosome (RNase complex)
 GO:0005739; mitochondrion
 GO:0000177; cytoplasmic exosome (RNase complex)
 GO:0006365; 35S primary transcript

processing
 GO:0006402; **mRNA** catabolism
 GO:0000175; 3'-5'-exoribonuclease activity
 GO:0031125; rRNA 3'-end processing
 GO:0005730; nucleolus
 GO:0000070; mitotic sister chromatid segregation
 GO:0005634; nucleus
 GO:0005515; protein binding
 GO:0007346; regulation of progression through mitotic cell cycle
 GO:0005515; protein binding
 GO:0005515; protein binding
 GO:0008536; Ran GTPase binding
 GO:0016075; rRNA catabolism ; primary_name dis3 ; product 3'-5' exoribonuclease subunit Dis3 (predicted) ;
mRNA SPBC32C12.02 ; p_c transcription factor Ste11 ||| HMG box ||| NLS ||| conserved fungal protein ||| no apparent S. cerevisiae ortholog ||| similar to P. Carinii STE11 ; colour 2 ; gene ste11 ||| aff1 ||| stex ||| SPBC32C12.02 ; note the equivalent function in S. cerevisiae is performed by STE12, but this is STE-like not HMG ; Alias ste11 ; GO GO:0000747; conjugation with cellular fusion
 GO:0003700; transcription factor activity
 GO:0051039; positive regulation of transcription, meiotic
 GO:0005634; nucleus ; controlled_curation term=transcriptionally regulates Mei2; date=20060818 ; primary_name ste11 ; product transcription factor Ste11 ;

mRNA SPBC215.05 ; temporary_systematic_id SPBC215.05 ; note **mRNA** from AU013069 ;
mRNA SPBC56F2.12 ; p_c acetohydroxyacid reductoisomerase ||| ketol-acid reductoisomerase family ||| similar to S. cerevisiae YLR355C ; GO GO:0004455; ketol-acid reductoisomerase activity
 GO:0000002; mitochondrial genome maintenance
 GO:0005739; mitochondrion
 GO:0009099; valine biosynthesis
 GO:0009097; isoleucine biosynthesis
 GO:0006551; leucine metabolism ; colour 7 ; gene SPBC56F2.12 ||| ilv5 ; product acetohydroxyacid reductoisomerase ; EC_number 1.1.1.86 ;

mRNA SPBC14F5.04c ; temporary_systematic_id SPBC14F5.04c ; note **mRNA** from AU014126 ;
mRNA SPBC1652.01 ; p_c conserved fungal protein ||| similar to S. cerevisiae YDR169C ||| possibly binds Pst1 histone deacytylase B ||| ; colour 10 ; gene SPBC1652.01 ; product conserved fungal protein ;

real_mRNA unknown_4327 ; note **mRNA** from AU012671 ;

mRNA SPBC8E4.02c ; p_c sequence orphan ; colour 8 ; gene SPBC8E4.02c ; product sequence orphan ;

mRNA SPBCPT2R1.08c ; db_xref EMBL:BK005597 ; gene SPBCPT2R1.08c ;

repeat_region unknown_4360 ; note dupliated region in c212 ;
mRNA SPCC757.07c ; p_c catalase ||| similar to S. cerevisiae YDR256C and YGR088W ; colour 2 ; gene ctt1 ||| cta1 ||| SPCC757.07c ; Alias ctt1 ; GO GO:0004096; catalase activity
 GO:0005739; mitochondrion
 GO:0005777; peroxisome
 GO:0006979; response to oxidative stress ; controlled_curation term=disease associated, acatalasia; date=20060725 ||| term=conserved eukaryotic protein; date=20070115 ; primary_name ctt1 ; product catalase ; EC_number 1.11.1.6 ;
mRNA SPCC757.09c ; p_c RNA-binding protein that suppresses calcineurin deletion Rnc1 ||| KH domain ||| similar to S. cerevisiae YBR233W ; colour 2 ; gene rnc1 ||| SPCC757.09c ; Alias rnc1 ; GO GO:0000165; MAPKKK cascade
 GO:0006469; negative regulation of protein kinase activity
 GO:0043409; negative regulation of MAPKKK cascade
 GO:0003729; **mRNA** binding
 GO:0048255; **mRNA** stabilization
 GO:0005515; protein binding ; primary_name rnc1 ; product RNA-binding protein that suppresses calcineurin deletion Rnc1 ;

tRNA SPC**TRNA**HIS.03 ; gene SPC**TRNA**HIS.03 ; product **tRNA** Histidine ; note **tRNA** His anticodon GTG ;
mRNA SPCC757.12 ; p_c alpha-amylase homolog (predicted) ||| no apparent orthologs, cannot be distinguished ; colour 7 ; gene SPCC757.12 ; GO GO:0009986; cell surface
 GO:0004556; alpha-amylase activity
 GO:0044247; cellular polysaccharide catabolism
 GO:0009897; external side of plasma membrane
 GO:0005618; cell wall
 GO:0048503; GPI anchor binding
 GO:0009272; cell wall biosynthesis (sensu Fungi)
 GO:0000902;

cell morphogenesis ; controlled_curation term=glycoprotein; cv=pt_mod; date=20060611 ||| term=N-glycosylated; evidence=IDA; cv=pt_mod; date=20060611 ||| term=N-glycosylation site; cv=pt_mod; date=20060611 ||| term=glycosyl hydrolase family 13; date=20060611 ||| term=predicted N-terminal signal sequence; date=20060611 ||| term=GPI anchored protein; evidence=ISS; db_xref=PMID:12845604; date=20060611 ; product alpha-amylase homolog (predicted) ; EC_number 3.2.1.1 ;

mRNA SPCC613.05c ; p_c 60S ribosomal protein L35 ||| similar to S. cerevisiae YDL191W and YDL136W ; colour 2 ; gene rpl35 ||| SPCC613.05c ; Alias rpl35 ; GO GO:0005842; cytosolic large ribosomal subunit (sensu Eukaryota)
 GO:0006412; translation
 GO:0003735; structural constituent of ribosome ; primary_name rpl35 ; product 60S ribosomal protein L35 ;

tRNA SPC**TRNA**GLY.10 ; gene SPC**TRNA**GLY.10 ; product **tRNA** Glycine ; note **tRNA** Gly anticodon GCC ;

misc_RNA SPNCRNA.129 ; db_xref EMBL:AU014447 ; systematic_id SPNCRNA.129 ; controlled_curation term=non-coding RNA; qualifier=predicted; db_xref=EMBL:AU014447; date=20050412 ||| term=no detectable long open reading frame; qualifier=predicted; db_xref=EMBL:AU014447; date=20050412 ; gene SPNCRNA.129 ; product non-coding RNA (predicted) ; note from AU014447 spc09864; RNA not associated with an ORF ;

mRNA SPCC330.05c ; temporary_systematic_id SPCC330.05c ; note **mRNA** from S60039 ;

mRNA SPCC330.06c ; temporary_systematic_id SPCC330.06c ; note **mRNA** from AU010632 ;

mRNA SPCC330.14c ; p_c 60S ribosomal protein L24 ||| similar to S. cerevisiae YGL031C and YGR148C and YLR009W ; colour 7 ; gene rpl2402 ||| rpl24-2 ||| SPCC330.14c ; Alias rpl2402 ; GO GO:0006412; protein biosynthesis
 GO:0003735; structural constituent of ribosome
 GO:0005842; cytosolic large ribosomal subunit (sensu the Eukaryota research community) ; primary_name rpl2402 ; product 60S ribosomal protein L24 ;

mRNA SPCC1235.01 ; p_c glycoprotein (predicted) ||| sequence orphan ; colour 8 ; gene SPCC320.02c ||| SPCC1235.01 ; note ~37 copies of a 7-10 repeat consensus 'PMEEITTMTI' and a S/N rich C terminal region ; Alias SPCC320.02c ; primary_name SPCC320.02c ; product glycoprotein (predicted) ;

mRNA SPCC1235.14 ; Alias ght5 ; primary_name ght5 ; gene ght5 ||| SPCC1235.14 ; note **mRNA** from AF017180 ;

mRNA SPCC548.06c ; temporary_systematic_id SPCC548.06c ; note **mRNA** from spc05276 ;

misc_feature unknown_192 ; note gene free region ;

misc_feature unknown_212 ; note low complexity gene-free region ;

mRNA SPCC794.09c ; db_xref EMBL:D82571 ; gene SPCC794.09c ;

mRNA SPCC794.12c ; p_c malic enzyme ||| malate dehydrogenase (oxaloacetate decarboxylating) ||| similar to S. cerevisiae YKL029C ; colour 2 ; gene mae2 ||| SPCC794.12c ; Alias mae2 ; GO GO:0016619; malate dehydrogenase (oxaloacetate- decarboxylating) activity
 GO:0006520; amino acid metabolism
 GO:0006090; pyruvate metabolism
 GO:0005739; mitochondrion ; primary_name mae2 ; product malic enzyme ; EC_number 1.1.1.38 ;

misc_feature unknown_293 ; note low complexity gene free region ;

mRNA SPCC736.15 ; temporary_systematic_id SPCC736.15 ; note **mRNA** from AU012604 ;

misc_feature unknown_294 ; note low-complexity gene-free region ;

mRNA SPCC594.01 ; colour 10 ;

mRNA SPCC962.06c ; p_c zinc finger protein ||| zf-CCHC type (zinc knuckle) ||| KH domain ||| similar to S. cerevisiae YLR116W ; colour 2 ; gene bpb1 ||| sf1 ||| SPCC962.06c ; Alias bpb1 ; GO GO:0000243; commitment complex
 GO:0000356; U2-type catalytic spliceosome formation for first transesterification

step
 GO:0003723; RNA binding ; primary_name bpb1 ; product zinc finger protein ;

mRNA SPCC1672.02c ; p_c switch-activating protein Sap1 ||| essential (PMID 8114737) ||| N-terminal DNA-binding domain (PMID 8065904) ||| C- terminal dimerization domain (PMID 8065904) ||| no apparent orthologs ; colour 2 ; gene sap1 ||| SPCC1672.02c ; Alias sap1 ; GO GO:0000790; nuclear chromatin
 GO:0008301; DNA bending activity
 GO:0003677; DNA binding
 GO:0003677; DNA binding
 GO:0042803; protein homodimerization activity
 GO:0043110; rDNA spacer replication fork barrier binding
 GO:0043110; rDNA spacer replication fork barrier binding
 GO:0007001; chromosome organization and biogenesis (sensu Eukaryota)
 GO:0007059; chromosome segregation
 GO:0000728; gene conversion at mating-type locus, DNA double-strand break formation
 GO:0031582; replication fork blocking at rDNA repeats
 GO:0031582; replication fork blocking at rDNA repeats ; primary_name sap1 ; product switch-activating protein Sap1 ;

mRNA SPCC1393.08 ; p_c transcription factor (predicted) ||| zinc finger protein ||| zf-GATA type ||| no apparent orthologs, cannot be distinguished ; colour 7 ; gene SPCC1393.08 ; product transcription factor (predicted) ;

mRNA SPCC63.13 ; p_c DNAJ domain protein ||| no apparent orthologs, cannot be distinguished ||| 1 predicted transmembrane helix ; GO GO:0030544; Hsp70 protein binding ; colour 10 ; gene SPCC63.13 ; product DNAJ domain protein ; note YMR161W same domain organization and single predicted tmm helix ;

mRNA SPCC63.13 ; colour 10 ;

mRNA SPCC63.14 ; p_c conserved fungal protein ||| coiled-coil (region) (predicted) ||| similar to S. cerevisiae YMR031C and YKL050C ; colour 10 ; gene SPCC63.14 ; product conserved fungal protein ;

mRNA SPCC24B10.21 ; p_c triosephosphate isomerase ||| similar to S. cerevisiae YDR050C ; colour 2 ; gene tpi1 ||| tpi ||| SPCC24B10.21 ; Alias tpi1 ; GO GO:0004807; triose-phosphate isomerase activity
 GO:0006096; glycolysis
 GO:0006094; gluconeogenesis
 GO:0005829; cytosol ; controlled_curation term=disease associated, hemolytic anemia; date=20060920 ||| term=conserved eukaryotic protein; date=20060920 ; primary_name tpi1 ; product triosephosphate isomerase ; EC_number 5.3.1.1 ;

mRNA SPCC1795.11 ; p_c ATP-dependent RNA helicase Sum3 ||| DEAD/DEAH box helicase ||| essential (PMID 9832516) ||| similar to S. cerevisiae YOR204W and YPL119C ; colour 2 ; gene sum3 ||| ded1 ||| slh3 ||| moc2 ||| SPCC1795.11 ; note suppressor of uncontrolled mitosis ||| Multicopy supressor of Overexpressed Cyr1 ; Alias sum3 ; GO GO:0004004; ATP-dependent RNA helicase activity
 GO:0005737; cytoplasm
 GO:0005515; protein binding
 GO:0005515; protein binding
 GO:0006412; translation
 GO:0000086; G2/M transition of mitotic cell cycle
 GO:0000076; DNA replication checkpoint
 GO:0006970; response to osmotic stress
 GO:0031137; regulation of conjugation with cellular fusion ; primary_name sum3 ; product ATP-dependent RNA helicase Sum3 ;

tRNA SPC**TRNA**ALA.12 ; gene SPC**TRNA**ALA.12 ; product **tRNA** Alanine ; note **tRNA** Ala anticodon AGC, Cove score 56.39 ;

tRNA **tRNA**_pseudo anticodon AAC ; pseudo _no_value ; gene **tRNA**_pseudo anticodon AAC ; note **tRNA** Pseudo anticodon AAC, Cove score 40.82 ;

tRNA SPC**TRNA**SER.09 ; gene SPC**TRNA**SER.09 ; product **tRNA** Serine ; note **tRNA** Ser anticodon AGA, Cove score 59.19 ;

tRNA SPC**TRNA**ARG.10 ; gene SPC**TRNA**ARG.10 ; product **tRNA** Arginine ; note **tRNA** Arg anticodon TCG, Cove score 59.66 ;

tRNA SPC**TRNA**ASP.05 ; gene SPC**TRNA**ASP.05 ; product **tRNA** Asparagine ; note **tRNA** Asp anticodon GTC, Cove score 57.96 ;

tRNA SPC**TRNA**ARG.11 ; gene SPC**TRNA**ARG.11 ; product **tRNA** Arginine ; note **tRNA** Arg anticodon ACG, Cove score 54.83 ;

misc_feature unknown_1068 ; note nominal overlap with cosmid c1259 ;

tRNA SPC**TRNA**LYS.10 ; gene SPC**TRNA**LYS.10 ; product **tRNA** Lysine ; note **tRNA** Lys anticodon CTT, Cove score 57.56 ;
repeat_unit unknown_1083 ;
repeat_unit unknown_1084 ; colour 1 ; note imr3L ;
tRNA SPC**TRNA**ASP.06 ; gene SPC**TRNA**ASP.06 ; product **tRNA** Asparagine ; note **tRNA** Asp anticodon GTC, Cove score 70.49 ;
tRNA SPC**TRNA**ARG.12 ; gene SPC**TRNA**ARG.12 ; product **tRNA** Arginine ; note **tRNA** Arg anticodon ACG, Cove score 75.46 ;
tRNA SPC**TRNA**VAL.09 ; gene SPC**TRNA**VAL.09 ; product **tRNA** Valine ; note **tRNA** Val anticodon AAC, Cove score 64.30 ;
tRNA SPC**TRNA**THR.08 ; gene SPC**TRNA**THR.08 ; product **tRNA** Threonine ; note **tRNA** Thr anticodon AGT, Cove score 75.01 ;
tRNA SPC**TRNA**LEU.12 ; gene SPC**TRNA**LEU.12 ; product **tRNA** Leucine ; note **tRNA** Leu anticodon CAA, Cove score 56.07 ;
repeat_unit unknown_1088 ; colour 4 ; note cnt3 ;
tRNA SPC**TRNA**GLU.10 ; gene SPC**TRNA**GLU.10 ; product **tRNA** Glutamic acid ; note **tRNA** Glu anticodon CTC, Cove score 74.15 ;
repeat_unit unknown_1090 ; colour 1 ; note imr3R ;
tRNA SPC**TRNA**LEU.13 ; gene SPC**TRNA**LEU.13 ; product **tRNA** Leucine ; note **tRNA** Leu anticodon CAA, Cove score 56.07 ;
tRNA SPC**TRNA**THR.09 ; gene SPC**TRNA**THR.09 ; product **tRNA** Threonine ; note **tRNA** Thr anticodon AGT, Cove score 75.01 ;
tRNA SPC**TRNA**VAL.10 ; gene SPC**TRNA**VAL.10 ; product **tRNA** Valine ; note **tRNA** Val anticodon AAC, Cove score 64.30 ;
tRNA SPC**TRNA**ARG.13 ; gene SPC**TRNA**ARG.13 ; product **tRNA** Arginine ; note **tRNA** Arg anticodon ACG, Cove score 75.46 ;
tRNA SPC**TRNA**ASP.07 ; gene SPC**TRNA**ASP.07 ; product **tRNA** Asparagine ; note **tRNA** Asp anticodon GTC, Cove score 70.49 ;
repeat_unit unknown_1093 ; colour 11 ; note cen3xc central region ;
mRNA SPCC1322.10 ; p_c glycoprotein (predicted) ||| possibly S. pombe specific ||| serine-rich protein ||| GPI anchored protein (predicted) (PMID 12845604) (pers. comm. Birgit Eisenhaber) ||| predicted N-terminal signal sequence ; GO GO:0009986; cell surface ; colour 12 ; gene SPCC1322.10 ; product glycoprotein (predicted) ;
mRNA SPCC1281.06c ; p_c acyl-coA desaturase (predicted) ||| similar to S. cerevisiae YGL055W ; GO GO:0006633; fatty acid biosynthesis
 GO:0004768; stearoyl-CoA 9-desaturase activity
 GO:0005789; endoplasmic reticulum membrane
 GO:0006636; fatty acid desaturation
 GO:0031227; intrinsic to endoplasmic reticulum membrane ; colour 7 ; gene SPCC1281.06c ; product acyl-coA desaturase (predicted) ;
mRNA SPCC622.09 ; temporary_systematic_id SPCC622.09 ; note **mRNA** from AU010164 ;
mRNA SPCC622.12c ; p_c NADP-specific glutamate dehydrogenase (predicted) ||| similar to S. cerevisiae YOR375C and YAL062W ; colour 7 ; gene SPCC622.12c ; GO GO:0006537; glutamate biosynthesis
 GO:0006807; nitrogen compound metabolism
 GO:0004354; glutamate dehydrogenase (NADP+) activity
 GO:0005634; nucleus
 GO:0005737; cytoplasm
 GO:0005739; mitochondrion ; product NADP-specific glutamate dehydrogenase (predicted) ; psu_db_xref PATH:MAP00251; ||| PATH:MAP00910; ; EC_number 1.4.1.4 ;
mRNA SPCC162.07 ; p_c epsin ||| essential (PMID 15659877) ||| ENTH domain protein ||| similar to S. cerevisiae YDL161W and YLR206W and YLL038C ; systematic_id SPCC162.07 ; colour 2 ; gene ent1 ||| SPCC162.07 ; Alias ent1 ; GO GO:0030479; actin cortical patch
 GO:0006897; endocytosis
 GO:0000147; actin cortical patch assembly
 GO:0030276; clathrin binding
 GO:0043130; ubiquitin binding
 GO:0005546;

phosphatidylinositol-4,5-bisphosphate binding
 GO:0007015; actin filament organization
 GO:0005096; GTPase activator activity ; primary_name ent1 ; product epsin ;
mRNA SPCC13B11.01 ; p_c alcohol dehydrogenase Adh1 ||| similar to S. cerevisiae YOL086C and YMR303C and YMR083W and YBR145W ; colour 2 ; gene adh1 ||| adh ||| SPCC13B11.01 ; Alias adh1 ; GO GO:0006066; alcohol metabolism
 GO:0005759; mitochondrial matrix ; primary_name adh1 ; product alcohol dehydrogenase Adh1 ; EC_number 1.1.1.1 ;
mRNA SPCC417.08 ; p_c translation elongation factor eEF3 ||| AAA family ATPase ||| HEAT repeat (inferred from context) ||| similar to S. cerevisiae YNL014W and YLR249W ; colour 2 ; gene tef3 ||| SPCC417.08 ; Alias tef3 ; GO GO:0003746; translation elongation factor activity
 GO:0016887; ATPase activity
 GO:0005830; cytosolic ribosome (sensu Eukaryota)
 GO:0006414; translational elongation
 GO:0005524; ATP binding ; primary_name tef3 ; product translation elongation factor eEF3 ;
misc_RNA SPNCRNA.10 ; Alias prl10 ; db_xref PMID:12597277 ||| EMBL:AB084822 ; systematic_id SPNCRNA.10 ; controlled_curation term=non-coding RNA; qualifier=predicted; db_xref=PMID:12597277; date=20050412 ||| term=poly(A)-bearing RNA; qualifier=predicted; db_xref=PMID:12597277; date=20050412 ||| term=no detectable long open reading frame; qualifier=predicted; db_xref=PMID:12597277; date=20050412 ; gene prl10 ; primary_name prl10 ; product non-coding RNA (predicted) ;
misc_feature SPCC297.02 ; colour 6 ; gene SPCC297.02 ; product dubious ;
mRNA SPCC297.03 ; p_c serine/threonine protein kinase Ssp1 (PMID 7628434) ||| ELM kinase family ||| functions in a different pathway to Spc1 (PMID 10233158) ||| similar to S. cerevisiae YGL179C and YER129W ||| non-essential (PMID 15821139) ; colour 2 ; gene ssp1 ||| SPCC297.03 ; Alias ssp1 ; GO GO:0030467; establishment and/or maintenance of cell polarity (sensu Fungi)
 GO:0006950; response to stress
 GO:0005737; cytoplasm
 GO:0030950; establishment and/or maintenance of actin cytoskeleton polarity
 GO:0008104; protein localization
 GO:0030042; actin filament depolymerization
 GO:0051285; cell cortex of cell tip
 GO:0030428; cell septum
 GO:0004674; protein serine/threonine kinase activity
 GO:0051519; activation of bipolar cell growth
 GO:0006468; protein amino acid phosphorylation
 GO:0005524; ATP binding ; primary_name ssp1 ; product serine/threonine protein kinase Ssp1 (PMID 7628434) ;
mRNA SPCC737.04 ; p_c UPF0300 family ||| possibly S. pombe specific ; colour 12 ; gene SPCC737.04 ; product UPF0300 family ;
mRNA SPCC1906.01 ; p_c mannose-1-phosphate guany**LTR**ansferase Mpg1 ||| essential (PMID 16049679) ||| similar to S. cerevisiae YDL055C ; colour 2 ; gene mpg1 ||| SPCC1906.01 ; note deletion mutant results in G2/M arrest (cell size checkpoint) activation mediated be Wee1 ; Alias mpg1 ; GO GO:0004475; mannose-1-phosphate guanyly**LTR**ansferase activity
 GO:0009298; GDP-mannose biosynthesis
 GO:0000032; cell wall mannoprotein biosynthesis
 GO:0006486; protein amino acid glycosylation
 GO:0005737; cytoplasm
 GO:0051286; cell tip
 GO:0009272; cell wall biosynthesis (sensu Fungi)
 GO:0000917; barrier septum formation
 GO:0031567; cell size control checkpoint ; primary_name mpg1 ; product mannose-1-phosphate guany**LTR**ansferase Mpg1 ; EC_number 2.7.7.13 ;
mRNA SPCC1739.10 ; p_c conserved fungal protein ||| similar to S. pombe SPAC13G7.04c (paralog) ||| 3 predicted transmembrane helices ||| similar to S. cerevisiae YOL019W and YFR012W and YMR063W ||| predicted N-terminal signal sequence ; GO GO:0005886; plasma membrane ; colour 10 ; gene SPCC1739.10 ; product conserved fungal protein ;
mRNA SPCC1739.13 ; temporary_systematic_id SPCC1739.13 ; note **mRNA** from AB012387 ;
mRNA SPCC576.03c ; temporary_systematic_id SPCC576.03c ; note **mRNA** from AF083335 ;

mRNA SPCC576.08c ; temporary_systematic_id SPCC576.08c ; note **mRNA** from AU012777 ;
mRNA SPCC576.11 ; p_c 60S ribosomal protein L15 ||| similar to S. cerevisiae YLR029C and YMR121C ; colour 7 ; gene rpl15 ||| SPCC576.11 ; Alias rpl15 ; GO GO:0005842; cytosolic large ribosomal subunit (sensu Eukaryota)
 GO:0006412; translation
 GO:0003735; structural constituent of ribosome ; primary_name rpl15 ; product 60S ribosomal protein L15 ;
mRNA SPCC830.07c ; p_c DNAJ domain protein ||| similar to S. cerevisiae YNL007C ; colour 2 ; gene psi1 ||| psi ||| SPCC830.07c ; Alias psi1 ; GO GO:0030544; Hsp70 protein binding
 GO:0051082; unfolded protein binding
 GO:0006413; translational initiation
 GO:0005843; cytosolic small ribosomal subunit (sensu Eukaryota) ; primary_name psi1 ; product DNAJ domain protein ;
mRNA SPCC70.03c ; p_c proline dehydrogenase ||| similar to S. cerevisiae YLR142W ; colour 7 ; gene SPCC70.03c ; GO GO:0006562; proline catabolism
 GO:0006537; glutamate biosynthesis
 GO:0004657; proline dehydrogenase activity
 GO:0005759; mitochondrial matrix ; product proline dehydrogenase ; psu_db_xref PATH:MAP00330; ; EC_number 1.5.99.8 ;
mRNA SPCC70.10 ; temporary_systematic_id SPCC70.10 ; note **mRNA** from SPD146 ;
mRNA SPCC1827.03c ; p_c acetyl-CoA ligase (predicted) ||| similar to S. cerevisiae YBR222C ; GO GO:0005737; cytoplasm
 GO:0005778; peroxisomal membrane
 GO:0006631; fatty acid metabolism
 GO:0006084; acetyl-CoA metabolism ; colour 7 ; gene SPCC1827.03c ; product acetyl-CoA ligase (predicted) ;
mRNA SPCP1E11.04c ; p_c membrane associated protein Pal1 (PMID 15975911) ||| Pal1 family protein ||| conserved fungal protein ||| similar to S. cerevisiae YDR348C ; systematic_id SPCP1E11.04c ; colour 2 ; gene pal1 ||| SPCP1E11.04c ; note pears and lemons (PMID 15975911) ; Alias pal1 ; GO GO:0032153; cell division site
 GO:0030427; site of polarized growth
 GO:0051286; cell tip
 GO:0031097; medial ring
 GO:0005515; protein binding
 GO:0030467; establishment and/or maintenance of cell polarity (sensu Fungi)
 GO:0031505; cell wall organization and biogenesis (sensu Fungi) ; controlled_curation term=localization at the cell division site is independent of F-actin and microtubule function; db_xref=PMID:15975911; date=20060207 ; primary_name pal1 ; product membrane associated protein Pal1 (PMID 15975911) ;
mRNA SPCP1E11.06 ; p_c AP-1 adaptor complex gamma subunit Apl4 (predicted) ||| adaptin family ||| similar to S. cerevisiae YPR029C ||| HEAT repeat ; colour 7 ; gene apl4 ||| SPCP1E11.06 ; note May use internal initiator MET ; Alias apl4 ; GO GO:0016192; vesicle-mediated transport
 GO:0030121; AP-1 adaptor complex
 GO:0030276; clathrin binding ; primary_name apl4 ; product AP-1 adaptor complex gamma subunit Apl4 (predicted) ;
LTR unknown_2204 ; note TF1 **LTR** ;
mRNA SPCP1E11.08 ; colour 7 ;
LTR unknown_2224 ; note Tf1-type **LTR** ;

Appendix I- List of genes or genomic regions enriched by RpL2502

RpL25

repeat_region unknown_31 ; note (ttattttaagttttgtc)3 ;
repeat_region unknown_32 ; note region duplicated in SPAC750 S. pombe chromosome 1; appears to be pseudo in c750 as CDS has no initiator methionine ;
mRNA SPAC977.14c ; temporary_systematic_id SPAC977.14c ; note **mRNA** from AU007498 ;
mRNA SPAC1F8.07c ; temporary_systematic_id SPAC1F8.07c ; note **mRNA** from AU011749 ;
mRNA SPAC1F8.08 ; p_c sequence orphan ||| 2 predicted transmembrane helices ; colour 8 ; gene SPAC1F8.08 ; product sequence orphan ;
mRNA SPAC5H10.13c ; p_c alpha-1,2-galactosy**LTR**ansferase Gmh2 (predicted) ||| GMA12/MNN10 family ||| predicted N-terminal signal sequence ||| similar to S. pombe gmh3 and gmh1 and gma12 and SPBC1289.13C and SPAC5H10.13C and SPAC637.06 and SPBC8D2.17 (paralogs) ||| similar to S. cerevisiae YDR245W and YJL183W ; colour 7 ; gene gmh2 ||| SPAC5H10.13c ; Alias gmh2 ; GO GO:0042125; protein amino acid galactosylation
 GO:0009272; cell wall biosynthesis (sensu Fungi)
 GO:0000139; Golgi membrane
 GO:0008378; galactosy**LTR**ansferase activity
 GO:0031278; alpha-1,2-galactosy**LTR**ansferase activity ; primary_name gmh2 ; product alpha-1,2-galactosy**LTR**ansferase Gmh2 (predicted) ; EC_number 2.4.1.- ;
mRNA SPAC13G6.10c ; temporary_systematic_id SPAC13G6.10c ; note **mRNA** from AU011742 ;
mRNA SPAC630.08c ; p_c C-4 methylsterol oxidase ||| similar to S. cerevisiae YGR060W ||| sterol desaturase domain ; systematic_id SPAC630.08c ; colour 7 ; gene SPAC630.08c ||| erg25 ; Alias erg25 ; GO GO:0000254; C-4 methylsterol oxidase activity
 GO:0006696; ergosterol biosynthesis
 GO:0005789; endoplasmic reticulum membrane ; primary_name erg25 ; product C-4 methylsterol oxidase ; EC_number 1.-.-.- ; reserved_name erg25 ;
mRNA SPAC2F7.05c ; p_c translation initiation factor eIF5 (predicted) ||| similar to S. cerevisiae YPR041W ; GO GO:0003743; translation initiation factor activity
 GO:0005096; GTPase activator activity
 GO:0042256; mature ribosome assembly
 GO:0006446; regulation of translational initiation
 GO:0005843; cytosolic small ribosomal subunit (sensu Eukaryota) ; colour 7 ; gene SPAC2F7.05c ; product translation initiation factor eIF5 (predicted) ;
mRNA SPAC13A11.02c ; p_c sterol 14-demethylase ||| cytochrome p450 family (predicted) ||| 2 predicted transmembrane helices ||| similar to S. cerevisiae YHR007C ; systematic_id SPAC13A11.02c ; colour 7 ; gene erg11 ||| SPAC13A11.02c ; note source of the name cyp51 unknown ; Alias erg11 ; obsolete_name cyp51 ; GO GO:0008398 ; sterol 14-demethylase activity
 GO:0006696; ergosterol biosynthesis
 GO:0005789; endoplasmic reticulum membrane ; controlled_curation term=conserved eukaryotic protein; date=20060920 ||| term=disease associated, cholesterinosis; date=20060920 ; primary_name erg11 ; product sterol 14-demethylase ; EC_number 1.14.13.70 ; reserved_name erg11 ;
mRNA SPAC4G8.13c ; p_c transcription factor Prz1 (PMID 12637524) ||| zinc finger protein (PMID 12637524) ||| zf-C2H2 type (3) ||| EGR family ||| dephosphorylated by calcineurin (PMID 12637524) ||| transcriptionally regulates pmc1 (PMID 12637524) ||| undergoes nucleocytoplasmic shuttling (PMID 12637524) ||| similar to S. cerevisiae YNL027W ; colour 2 ; gene prz1 ||| SPAC4G8.13c ; Alias prz1 ; GO GO:0005737; cytoplasm
 GO:0005634; nucleus
 GO:0003700; transcription factor activity
 GO:0006874; calcium ion homeostasis
 GO:0019722; calcium-mediated signaling
 GO:0030644; chloride ion homeostasis ; primary_name prz1 ; product transcription factor Prz1 (PMID 12637524) ;
mRNA SPAC1687.16c ; p_c lathosterol oxidase (predicted) ||| sterol desaturase (ISS) ||| similar to S. cerevisiae YLR056W ; GO GO:0050046; lathosterol oxidase activity
 GO:0006696; ergosterol biosynthesis
 GO:0005789; endoplasmic reticulum membrane ; colour 7 ; gene SPAC1687.16c ; product lathosterol

oxidase (predicted) ; EC_number 1.3.3.2 ;

snRNA SP**SNRNA**.02 ; Alias snu2 ; db_xref EMBL:X55772 ||| Rfam:RF00004 ; synonym U2 ; systematic_id SP**SNRNA**.02 ; gene snu2 ||| U2 ||| SP**SNRNA**.02 ; primary_name snu2 ; product small nuclear RNA U2 ;

mRNA SPAC222.09 ; colour 2 ;

mRNA SPAC222.11 ; p_c coproporphyrinogen III oxidase (predicted) ||| similar to S. cerevisiae YDR044W ; systematic_id SPAC222.11 ; colour 7 ; gene hem13 ||| SPAC222.11 ; Alias hem13 ; GO GO:0004109; coproporphyrinogen oxidase activity
 GO:0006783; heme biosynthesis
 GO:0005743; mitochondrial inner membrane ; primary_name hem13 ; product coproporphyrinogen III oxidase (predicted) ; EC_number 1.3.3.3 ; reserved_name hem13 ;

snRNA SP**SNRNA**.03 ; Alias snu3 ; db_xref RFAM:RF00012 ; synonym U3 ||| U3**snRNA** ; systematic_id SP**SNRNA**.03 ; gene U3**snRNA** ||| snu3 ||| U3 ||| SP**SNRNA**.03 ; primary_name snu3 ; product small nuclear RNA U3 ;

mRNA SPAC821.10c ; temporary_systematic_id SPAC821.10c ; note **mRNA** from AU011168 ;

mRNA SPAC1A6.11 ; p_c dubious ||| compositionally biased ORF ; colour 6 ; gene SPAC1A6.11 ; product dubious ;

mRNA SPAC1A6.04c ; temporary_systematic_id SPAC1A6.04c ; note **mRNA** from AU010125 ;

mRNA SPAC30D11.13 ; colour 2 ;

rep_origin unknown_1050 ; note ars1, minimal sequence (Clyne & Kelly) ;

tRNA SPA**TRNA**SER.01 ; db_xref EMBL:V01360 ; evidence experimental ; gene SPA**TRNA**SER.01 ||| sup12 ; product **tRNA** Serine ; note **tRNA** Ser anticodon CGA, Cove score 77.58 ;

tRNA SPA**TRNA**MET.01 ; db_xref EMBL:V01360 ; evidence experimental ; gene SPA**TRNA**MET.01 ; product **tRNA** Methionine ; note **tRNA** Met anticodon CAT, Cove score 69.52: EMBL:V01360 ;

mRNA SPAC30D11.12 ; colour 7 ;

misc_feature unknown_1108 ; note gene free region (predicted) ;

mRNA SPAC56F8.13 ; p_c dubious ||| ORF in predicted gene free region ; colour 6 ; gene SPAC56F8.13 ; product dubious ;

mRNA SPAC56F8.14c ; p_c sequence orphan ; colour 8 ; gene SPAC56F8.14c ; product sequence orphan ; note has expression profile on microarray in otherwise apparently gene free region ;

mRNA SPAC10F6.06 ; db_xref EMBL:Y13635 ; systematic_id SPAC10F6.06 ;

mRNA SPAC1565.01 ; p_c conserved fungal protein ||| 4 predicted transmembrane helices ||| similar to S. cerevisiae YNR018W ; GO GO:0005739; mitochondrion ; colour 10 ; gene SPAC1565.01 ; product conserved fungal protein ;

mRNA SPAC6F12.04 ; temporary_systematic_id SPAC6F12.04 ; note **mRNA** from spc08792 340 482 ;

misc_RNA SPNCRNA.86 ; db_xref EMBL:AU010322 ; systematic_id SPNCRNA.86 ; controlled_curation term=non-coding RNA; qualifier=predicted; db_xref= EMBL:AU010322; date=20050412 ||| term=no detectable long open reading frame; qualifier=predicted; db_xref=EMBL:AU010322; date=20050412 ||| term=low complexity gene free region; qualifier=predicted; db_xref=EMBL:AU010322; date=20050412 ; gene SPNCRNA.86 ; product non-coding RNA (predicted) ; note **mRNA** not associated with an ORF; **mRNA** from AU010322; may be the 3'UTR of fin1 ||| strand altered 19.06.2003, evidence from microarray expression profile (pers comm. Jurg Bahler) ;

mRNA SPAC19E9.03 ; temporary_systematic_id SPAC19E9.03 ; note **mRNA** from AB045126 ;
mRNA SPAC9.09 ; p_c homocysteine methy**LTR**ansferase ||| similar to S. cerevisiae YER091C ; colour 2 ; gene met26 ||| SPAC9.09 ; note accummulation of homocysteine causes a defect in purine biosynthesis (PMID 16436428) ; Alias met26 ; GO GO:0009086; methionine biosynthesis
 GO:0003871; 5-methyltetrahydropteroy**LTR**iglutamate- homocysteine S-methy**LTR**ansferase activity
 GO:0005737; cytoplasm
 GO:0046084; adenine biosynthesis ; primary_name met26 ; product homocysteine methy**LTR**ansferase ; EC_number 2.1.1.14 ;
mRNA SPAC57A7.04c ; p_c **mRNA** export shuttling protein (PMID 12112233) ||| rrm RNA recognition motif (4) ||| non-essential (PMID 12112233) ||| similar to S. cerevisiae YER165W ; colour 2 ; gene SPAC57A7.04c ||| pabp ; note pab1 used previously by SPAC227.07c ; obsolete_name pab1 ; GO GO:0008143; poly(A) binding
 GO:0006406; **mRNA** export from nucleus
 GO:0005737; cytoplasm
 GO:0005634; nucleus ; product **mRNA** export shuttling protein (PMID 12112233) ;

mRNA SPAC1705.02 ; colour 10 ;

mRNA SPAC1705.03c ; temporary_systematic_id SPAC1705.03c ; note **mRNA** from AU010387 ;
mRNA SPAC23H4.06 ; p_c glutamate-ammonia ligase Gln1 ||| similar to S. cerevisiae YPR035W ; colour 2 ; gene gln1 ||| SPAC23H4.06 ; Alias gln1 ; GO GO:0006542; glutamine biosynthesis
 GO:0019740; nitrogen utilization
 GO:0004356; glutamate-ammonia ligase activity
 GO:0005737; cytoplasm ; primary_name gln1 ; product glutamate-ammonia ligase Gln1 ; EC_number 6.3.1.2 ;
mRNA SPAC343.12 ; p_c conserved fungal protein ||| regulated by glucose, ammonium, phosphate, carbon dioxide and temperature (PMID 7565608) ||| predicted N-terminal signal sequence ||| no apparent S. cerevisiae ortholog ||| similar to N. crassa b19a17.210 ; colour 2 ; gene rds1 ||| SPAC343.12 ; Alias rds1 ; GO GO:0006950; response to stress ; primary_name rds1 ; product conserved fungal protein ;

mRNA SPAC343.20 ; p_c sequence orphan ; colour 8 ; gene SPAC343.20 ; product sequence orphan ; note has transcript profile on microarray ;

mRNA SPAC1002.13c ; temporary_systematic_id SPAC1002.13c ; note **mRNA** from AU013582 ;

mRNA SPAC1002.20 ; p_c sequence orphan ; colour 8 ; gene SPAC1002.20 ; product sequence orphan ;
mRNA SPAPB1A10.14 ; p_c F-box protein, unnamed ||| F-box protein (context dependent) ||| no apparent orthologs, cannot be distinguished ; GO GO:0000151; ubiquitin ligase complex
 GO:0030674; protein binding, bridging
 GO:0031146; SCF-dependent proteasomal ubiquitin- dependent protein catabolism ; colour 7 ; gene SPAPB1A10.14 ; product F-box protein, unnamed ;

misc_feature unknown_1907 ; note gene free region ;

mRNA SPAC23C11.05 ; temporary_systematic_id SPAC23C11.05 ; note **mRNA** from SPPPAG X54301 ;
mRNA SPAC23C11.06c ; p_c hydrolase (inferred from context) ||| conserved fungal protein ||| similar to S. cerevisiae YNL115C ||| 5 predicted transmembrane helices ; colour 10 ; gene SPAC23C11.06c ; product hydrolase (inferred from context) ;
mRNA SPAC13F5.03c ; p_c glycerol dehydrogenase (Phlippen, Stevens, Wolf, Zimmermann manuscript in preparation) ||| conserved protein (broad species distribution) ||| predicted N-terminal signal sequence ||| no apparent S. cerevisiae ortholog ; GO GO:0005739; mitochondrion
 GO:0008270; zinc ion binding
 GO:0008888; glycerol dehydrogenase activity
 GO:0019563; glycerol catabolism ; colour 7 ; gene SPAC13F5.03c ; product glycerol dehydrogenase (Phlippen, Stevens, Wolf, Zimmermann manuscript in preparation) ; EC_number 1.1.1.6 ;

mRNA SPAC22H10.06c ; p_c dubious ||| under 100 amino acid threshold ; colour 6 ; gene SPAC22H10.06c ; product dubious ;

mRNA SPAC22H10.13 ; temporary_systematic_id SPAC22H10.13 ; note **mRNA** from AU009741 ;
mRNA SPAC4A8.04 ; p_c vacuolar serine protease Isp6 ||| similar to S. cerevisiae YEL060C ||| similar to S. pombe SPAC1006.01 ; colour 2 ; gene isp6 ||| prb1 ||| SPAC4A8.04 ; Alias isp6 ; GO GO:0008236; serine-type peptidase activity
 GO:0006401; RNA catabolism
 GO:0006914; autophagy
 GO:0006995; cellular response to nitrogen starvation
 GO:0006995; cellular response to nitrogen starvation
 GO:0000747; conjugation with cellular fusion
 GO:0051603; proteolysis during cellular protein catabolism
 GO:0006508; proteolysis
 GO:0051171; regulation of nitrogen metabolism
 GO:0007033; vacuole organization and biogenesis
 GO:0005773; vacuole ; controlled_curation term=expressed during nitrogen starvation; db_xref= PMID:11872168; date=20060516 ; primary_name isp6 ; product vacuolar serine protease Isp6 ; EC_number 3.4.24.- ||| 3.4.21.- ;
tRNA SPA**TRNA**ILE.02 ; evidence not_experimental ; gene SPA**TRNA**ILE.02 ; product **tRNA** Isoleucine ; note **tRNA** Ile anticodon TAT, Cove score 59.62 ;
mRNA SPAC4A8.15c ; temporary_systematic_id SPAC4A8.15c ; note **mRNA** from AU008190 ;
mRNA SPAC4F8.08 ; p_c sequence orphan ; colour 8 ; gene SPAC4F8.08 ; product sequence orphan ;
mRNA SPAC3F10.18c ; Alias rpl4102 ; primary_name rpl4102 ; gene rpl4102 ||| rpl41-2 ||| rpl41 ||| SPAC8F11.01c ||| SPAC3F10.18c ; note **mRNA** from AU007741
mRNA SPAPB24D3.07c ; p_c sequence orphan ||| predicted N-terminal signal sequence ; colour 8 ; gene SPAPB24D3.07c ; product sequence orphan ;
misc_RNA SPNCRNA.31 ; Alias prl31 ; db_xref PMID:12597277 ||| EMBL:AB084843 ; systematic_id SPNCRNA.31 ; controlled_curation term=non-coding RNA; qualifier=predicted; db_xref=PMID:12597277; date=20050412 ||| term=poly(A)-bearing RNA; qualifier=predicted; db_xref=PMID:12597277; date=20050412 ||| term=no detectable long open reading frame; qualifier=predicted; db_xref=PMID:12597277; date=20050412 ; gene prl31 ; primary_name prl31 ; product non-coding RNA (predicted) ;
mRNA SPAC31G5.10 ; temporary_systematic_id SPAC31G5.10 ; note AB084881 ;
misc_RNA SPNCRNA.01 ; systematic_id SPNCRNA.01 ; gene prl01 ||| prl1 ; note possibly part of the UTR of eta2 ; Alias prl1 ; db_xref PMID:12597277 ||| EMBL:AB084813 ; synonym prl01 ; controlled_curation term=non-coding RNA; qualifier=predicted; db_xref=PMID:12597277; date=20050412 ||| term=poly(A)-bearing RNA; qualifier=predicted; db_xref=PMID:12597277; date=20050412 ||| term=no detectable long open reading frame; qualifier=predicted; db_xref= PMID:12597277; date=20050412 ; primary_name prl1 ; product non-coding RNA (predicted) ;
mRNA unknown_2876 ; note AB084881 ;
mRNA SPAC31G5.11 ; p_c cAMP-independent regulatory protein Pac2 (PMID 8536311) ||| similar to S. pombe gti1 ||| similar to S. cerevisiae YEL007W and YHR177W ; colour 2 ; gene pac2 ||| SPAC31G5.11 ; Alias pac2 ; GO GO:0031139; positive regulation of conjugation with cellular fusion ; primary_name pac2 ; product cAMP-independent regulatory protein Pac2 (PMID 8536311) ;
mRNA SPAC26A3.01 ; p_c aspartic protease Sxa1 (PMID 1549128) ||| predicted N-terminal signal sequence ||| GPI anchored protein (predicted) (PMID 12845604) (pers. comm. Birgit Eisenhaber) ||| glycoprotein (predicted) ||| similar to S. cerevisiae YLR120C and YDR144C and YLR121C and YIR039C and YDR349C ; colour 2 ; gene sxa1 ||| SPAC2E1P5.06 ||| SPAC26A3.01 ; note does not appear to be a yapsin (PMID 11115118) ; Alias sxa1 ; GO GO:0000747; conjugation with cellular fusion
 GO:0005886; plasma membrane
 GO:0009986; cell surface
 GO:0004190; aspartic-type endopeptidase activity ; primary_name sxa1 ; product aspartic protease Sxa1 (PMID 1549128) ; EC_number 3.4.23.- ;
mRNA SPAC8E11.02c ; p_c 14-3-3 protein Rad24 ||| similar to S. cerevisiae YDR099W and YER177W ||| similar to S. pombe rad25 ||| non- essential ||| negative regulator of the Ras1-Byr2 signalling pathway, acting downstream of Ras1 and upstream of Byr2 (PMID 12242289) ; colour 2 ; gene rad24 ||| SPAC8E11.02c ; Alias rad24 ; GO GO:0005826; contractile ring
 GO:0005737; cytoplasm
 GO:0051233; spindle midzone
 GO:0005515; protein binding

 GO:0005515; protein binding
 GO:0005515; protein binding
 GO:0005515; protein binding
 GO:0005515; protein binding
 GO:0005515; protein binding
 GO:0005515; protein binding
 GO:0046982; protein heterodimerization activity
 GO:0004864; protein phosphatase inhibitor activity
 GO:0000077; DNA damage checkpoint
 GO:0031565; cytokinesis checkpoint
 GO:0007126; meiosis
 GO:0031031; positive regulation of septation initiation signaling
 GO:0008104; protein localization
 GO:0008104; protein localization
 GO:0000074; regulation of progression through cell cycle ; primary_name rad24 ; product 14-3-3 protein Rad24 ;
snoRNA SP**SNORNA**.20 ; Alias snoU17 ; db_xref EMBL:AJ544685 ; systematic_id SP**SNORNA**.20 ; controlled_curation term=18S rRNA production; date=20070104 ; evidence EXPERIMENTAL ; gene snoU17 ||| SP**SNORNA**.20 ; primary_name snoU17 ; product small nucleolar RNA U17 ;
mRNA SPAC17A2.09c ; p_c RNA-binding protein Csx1 ||| rrm RNA recognition motif ||| similar to S. pombe SPBC23E6.01c ||| similar to S. cerevisiae YHR086W and YBR212W ; colour 2 ; gene csx1 ||| SPAC17A2.09c ; Alias csx1 ; GO GO:0006979; response to oxidative stress
 GO:0003723; RNA binding
 GO:0005737; cytoplasm ; primary_name csx1 ; product RNA-binding protein Csx1 ;
misc_feature unknown_3422 ; note gene free region, SPAC17A2.10 and SPAC17A2.11 were marked in this region, because of their size, but have very low correlation scores, and are in a region of unusual base composition. This region also has GC/AT deviation, GC rich on the top strand for the first 4 kb, then GC poor for the next 4 kb., similar to the composition of the mat mc loci ;
mRNA SPAC17A2.10c ; p_c sequence orphan ; colour 8 ; gene SPAC17A2.10c ; product sequence orphan ; note previously annotated as dubious, but has localization signal ||| largish ORF in compositionally biased region, probably not real. has odd translation. this is in region where botton strand is T rich, so lots of LLLFFFFLLFFLSFSFSFS ||| see comment on SPAC17A2.11, this region is the same but inverted orientation ;
mRNA SPAC17A2.11 ; p_c sequence orphan ; colour 8 ; gene SPAC17A2.11 ; product sequence orphan ; note previously annotated as dubious, but has localization signal ||| see comment on SPAC17A2.10, this region is the same but inverted orientation ||| largish ORF in compositionally biased region, probably not real. has odd translation. this is in region where botton strand is T rich, so lots of LLLFFFFLLFFLSFSFSFS ;
tRNA SPA**TRNA**LEU.03 ; gene SPA**TRNA**LEU.03 ; product **tRNA** Leucine ; note **tRNA** Leu anticodon TAG, Cove score 53.30 ;
mRNA SPAC8C9.04 ; p_c sequence orphan ; GO GO:0007126; meiosis
 GO:0007126; meiosis ; colour 8 ; gene SPAC8C9.04 ; product sequence orphan ;
mRNA SPAC15A10.09c ; p_c conserved fungal protein ||| 4 predicted transmembrane helices ||| similar to S. cerevisiae YLR414C ; colour 10 ; gene SPAC15A10.09c ; product conserved fungal protein ;
tRNA SPA**TRNA**PHE.02 ; gene SPA**TRNA**PHE.02 ; product **tRNA** Phenylalanine ; note **tRNA** Phe anticodon GAA, Cove score 69.56 ;
misc_RNA SPNCRNA.84 ; db_xref EMBL:AU010014 ; systematic_id SPNCRNA.84 ; controlled_curation term=non-coding RNA; qualifier=predicted; date=20050412 ||| term=no detectable long open reading frame; qualifier=predicted; date=20050412 ; gene SPNCRNA.84 ; product non-coding RNA (predicted) ;
repeat_unit unknown_3599 ; colour 12 ; note region between dg and dh repeat ;
repeat_unit unknown_3600 ; colour 2 ; note dhI repeat ;
repeat_unit unknown_3603 ; colour 2 ; note dh1 repeat ;
repeat_unit unknown_3606 ; colour 1 ; note imr1L ;
tRNA SPA**TRNA**ALA.04 ; gene SPA**TRNA**ALA.04 ; product **tRNA** Alanine ; note anticodon AGC, Cove score 67.24 ;
tRNA SPA**TRNA**GLU.03 ; gene SPA**TRNA**GLU.03 ; product **tRNA** Glutamic acid ; note **tRNA** Glu anticodon CTC, Cove score 74.15 ;

misc_feature unknown_3608 ;
tRNA SPA**TRNA**ILE.03 ; gene SPA**TRNA**ILE.03 ; product **tRNA** Isoleucine ; note **tRNA** Ile anticodon AAT, Cove score 66.44 ;
repeat_unit unknown_3609 ; colour 4 ; note cnt1 ;
repeat_unit unknown_3610 ; colour 1 ; note imr1R ;
tRNA SPA**TRNA**ILE.04 ; gene SPA**TRNA**ILE.04 ; product **tRNA** Isoleucine ; note **tRNA** Ile anticodon AAT, Cove score 66.44 ;
tRNA SPA**TRNA**GLU.04 ; gene SPA**TRNA**GLU.04 ; product **tRNA** Glutamic acid ; note **tRNA** Glu anticodon CTC, Cove score 74.15 ;
repeat_unit unknown_3611 ; colour 3 ; partial _no_value ; note dg1 repeat ;
tRNA SPA**TRNA**ALA.05 ; gene SPA**TRNA**ALA.05 ; product **tRNA** Alanine ; note **tRNA** Ala anticodon AGC, Cove score 67.24 ;
misc_feature unknown_3612 ; note nominal overlap with SPAC1856 S. pombe chromosome 1 ;
misc_feature unknown_3614 ; note nominal overlap with SPAP4C9 S. pombe chromosome 1 ;
repeat_unit unknown_3613 ; colour 2 ; note dhI repeat, first 897 bp of SPDHI entry missing ;
repeat_unit unknown_3615 ; colour 12 ; note region between dg and dh repeat ;
misc_RNA SPNCRNA.95 ; systematic_id SPNCRNA.95 ; controlled_curation term=non-coding RNA; qualifier=predicted; date=20050412 ||| term=no detectable long open reading frame; qualifier=predicted; date=20050412 ; gene SPNCRNA.95 ; product non-coding RNA (predicted) ; note from spc00798 133 368; not attached to CDS ||| possibly spurious, expression low on both strands (pers comm. Jurg Bahler) ;
mRNA SPAC4H3.10c ; Alias pyk1 ; primary_name pyk1 ; gene pyk1 ||| SPAC4H3.10c ; note **mRNA** from AU012784 ;
mRNA SPAC4H3.10c ; p_c pyruvate kinase (predicted) ||| similar to S. cerevisiae YAL038W and YOR347C ; colour 7 ; gene pyk1 ||| SPAC4H3.10c ; Alias pyk1 ; primary_name pyk1 ; product pyruvate kinase (predicted) ; EC_number 2.7.1.40 ;
mRNA SPAC1071.10c ; temporary_systematic_id SPAC1071.10c ; note **mRNA** from AU007984 ;
misc_RNA SPNCRNA.92 ; db_xref EMBL:AU008923 ; systematic_id SPNCRNA.92 ; controlled_curation term=non-coding RNA; qualifier=predicted; db_xref=EMBL:AU008923; date=20050412 ||| term=no detectable long open reading frame; qualifier=predicted; db_xref=EMBL:AU008923; date=20050412 ||| term=low complexity gene free region; qualifier=predicted; db_xref=EMBL:AU008923; date=20050412 ; gene SPNCRNA.92 ; product non-coding RNA (predicted) ; note **mRNA** from AU008923. **mRNA** not associated with an ORF ||| strand altered 19.6.2003, evidence from microarray profile (pers comm. Jurg Bahler) ;
mRNA SPAPB18E9.05c ; p_c dubious ||| below 100 amino acid size threshold ; colour 6 ; gene SPAPB18E9.05c ; product dubious ;
mRNA SPAPB15E9.01c ; temporary_systematic_id SPAPB15E9.01c ; note **mRNA** from AU009814 ;
mRNA SPAPB15E9.02c ; p_c sequence orphan ||| predicted N-terminal signal sequence ||| 3 predicted transmembrane helices ; colour 8 ; gene SPAPB15E9.02c ; product sequence orphan ; note has transcript on microarray ;
misc_feature prl49 ; Alias prl53 ; primary_name prl53 ; gene prl53 ||| prl63 ||| prl49 ; note covered by abundant ESTs; prl53 prl63 and prl49 all map to within this highly transcribed region (PMID 12597277) ;
misc_RNA SPNCRNA.53 ; Alias prl53 ; db_xref PMID:12597277 ||| EMBL:AB084865 ; systematic_id SPNCRNA.53 ; controlled_curation term=non-coding RNA; qualifier=predicted; db_xref=PMID:12597277; date=20050412 ||| term=poly(A)-bearing RNA; qualifier=predicted; db_xref=PMID:12597277; date=20050412 |||

term=no detectable long open reading frame; qualifier=predicted; db_xref=PMID:12597277; date=20050412 ; gene prl53 ; primary_name prl53 ; product non-coding RNA (predicted) ;
misc_RNA SPNCRNA.63 ; systematic_id SPNCRNA.63 ; gene prl63 ; Alias prl63 ; obsolete_name SPNCRNA.49 ; db_xref PMID:12597277 ||| EMBL:AB084875 ||| EMBL:AB084861 ; synonym prl49 ; controlled_curation term=non-coding RNA; qualifier=predicted; db_xref=PMID:12597277; date=20050412 ||| term=poly(A)-bearing RNA; qualifier=predicted; db_xref=PMID:12597277; date=20050412 ||| term=no detectable long open reading frame; qualifier=predicted; db_xref= PMID:12597277; date=20050412 ; primary_name prl63 ; product non-coding RNA (predicted) ;
mRNA SPAC27E2.13 ; p_c dubious ; colour 6 ; controlled_curation term=longest ORF in prl53 (60AA), possibly be protein coding; date=20060721 ; gene SPAC27E2.13 ; product dubious ;
mRNA SPAC27E2.11c ; p_c glycoprotein (predicted) ||| possibly S. pombe specific ||| GPI anchored protein (predicted) (PMID 12845604) (pers. comm. Birgit Eisenhaber) ||| predicted N-terminal signal sequence ; GO GO:0009986; cell surface ; colour 12 ; gene SPAC27E2.11c ; product glycoprotein (predicted) ; note possibly not coding ;
mRNA SPAC19G12.08 ; temporary_systematic_id SPAC19G12.08 ; note **mRNA** from spc10699 1 222 ;
tRNA SPA**TRNA**LEU.04 ; gene SPA**TRNA**LEU.04 ; product **tRNA** Leucine ; note **tRNA** Leu anticodon AAG, Cove score 59.59 ;
mRNA SPAC19G12.09 ; p_c NADH/NADPH dependent indole-3-acetaldehyde reductase AKR3C2 ||| aldo/keto reductase ||| similar to S. cerevisiae YDL124W ; GO GO:0047018; indole-3-acetaldehyde reductase (NADH) activity
 GO:0047019; indole-3-acetaldehyde reductase (NADPH) activity
 GO:0008152; metabolism
 GO:0051268; alpha-keto amide reductase activity
 GO:0051269; alpha-keto ester reductase activity
 GO:0005737; cytoplasm
 GO:0005634; nucleus
 GO:0016652; oxidoreductase activity, acting on NADH or NADPH, NAD or NADP as acceptor ; colour 2 ; gene SPAC19G12.09 ; product NADH/NADPH dependent indole-3-acetaldehyde reductase AKR3C2 ;
mRNA SPAC19G12.10c ; temporary_systematic_id SPAC19G12.10c ; note **mRNA** from spc11281 231 187 ;
mRNA SPAC26H5.08c ; temporary_systematic_id SPAC26H5.08c ; note **mRNA** from SPAB539 ;
mRNA SPAC1F7.04 ; db_xref EMBL:D38180 ; systematic_id SPAC1F7.04 ;
mRNA SPAC29E6.07 ; p_c sequence orphan ; colour 8 ; gene SPAC30.11 ||| SPAC29E6.07 ; note previously annotated as dubious ; Alias SPAC30.11 ; GO GO:0007126; meiosis
 GO:0007126; meiosis
 GO:0009897; external side of plasma membrane ; primary_name SPAC30.11 ; product sequence orphan ;
mRNA SPAC29E6.08 ; p_c TATA-binding protein (TBP) ||| similar to S. cerevisiae YER148W ; colour 2 ; gene tbp1 ||| tdf1 ||| tbp ||| SPAC29E6.08 ||| SPAC30.12 ; Alias tbp1 ; GO GO:0005669; transcription factor TFIID complex
 GO:0000126; transcription factor TFIIIB complex
 GO:0016251; general RNA polymerase II transcription factor activity
 GO:0003709; RNA polymerase III transcription factor activity
 GO:0003701; RNA polymerase I transcription factor activity
 GO:0006360; transcription from RNA polymerase I promoter
 GO:0006367; transcription initiation from RNA polymerase II promoter
 GO:0006384; transcription initiation from RNA polymerase III promoter
 GO:0000120; RNA polymerase I transcription factor complex ; primary_name tbp1 ; product TATA-binding protein (TBP) ;
mRNA SPAC16.01 ; db_xref EMBL:D38181 ; systematic_id SPAC16.01 ;
mRNA SPAC16.02c ; db_xref EMBL:D89163 ; systematic_id SPAC16.02c ;
mRNA SPAC16.05c ; temporary_systematic_id SPAC16.05c ; note **mRNA** from spc06207 ;

misc_RNA SPNCRNA.99 ; systematic_id SPNCRNA.99 ; controlled_curation term=non-coding RNA; qualifier=predicted; date=20050412 ||| term=no detectable long open reading frame; qualifier=predicted; date=20050412 ; gene SPNCRNA.99 ; product non-coding RNA ; note **mRNA** not associated with an ORF ;

misc_feature unknown_4190 ; note nominal overlap with cosmid SPAC9E9, EM:Z99262 S. pombe chromosome 1 ;

mRNA SPAC9E9.01 ; p_c sequence orphan ; colour 8 ; gene SPAC9E9.01 ; product sequence orphan ;
mRNA SPAC9E9.03 ; p_c 3-isopropylmalate dehydratase Leu2 (predicted) ||| similar to S. cerevisiae YGL009C ; colour 7 ; gene leu2 ||| SPAC9E9.03 ; Alias leu2 ; GO GO:0003861; 3-isopropylmalate dehydratase activity
 GO:0009098; leucine biosynthesis
 GO:0005829; cytosol ; primary_name leu2 ; product 3-isopropylmalate dehydratase Leu2 (predicted) ; EC_number 4.2.1.33 ;

tRNA SPA**TRNA**THR.05 ; gene SPA**TRNA**THR.05 ; product **tRNA** Threonine ; note **tRNA** Thr anticodon AGT, Cove score 75.01 ;
mRNA SPAPYUG7.03c ; p_c anillin homologue Mid2 ||| pleckstrin homology domain ||| transcriptionally regulated by Ace2 (PMID 16317047) ||| similar to S. cerevisiae YJR092W ; colour 2 ; gene mid2 ||| SPAPYUG7.03c ; Alias mid2 ; GO GO:0000920; cell separation during cytokinesis
 GO:0000910; cytokinesis
 GO:0005525; GTP binding
 GO:0005940; septin ring
 GO:0031106; septin ring organization
 GO:0031107; septin ring disassembly
 GO:0031097; medial ring ; primary_name mid2 ; product anillin homologue Mid2 ;

mRNA SPAPYUG7.04c ; colour 2 ;
mRNA SPAC4F10.14c ; p_c nascent polypeptide-associated complex subunit (predicted) (PMID 8809106) ||| similar to S. cerevisiae YDR252W and YPL037C ; colour 2 ; gene btf3 ||| SPAC4F10.14c ; note not a chaperone in the GO definition sense? check ; Alias btf3 ; synonym egd1 ||| btt1 ; GO GO:0005854; nascent polypeptide-associated complex
 GO:0051083; cotranslational protein folding
 GO:0051082; unfolded protein binding ; primary_name btf3 ; product nascent polypeptide-associated complex subunit (predicted) (PMID 8809106) ;
mRNA SPAC4F10.15c ; p_c WASp homolog ||| disease associated, Wiskott- Aldrich syndrome ||| conserved eukaryotic protein ||| similar to S. cerevisiae YOR181W ; colour 2 ; gene wsp1 ||| SPAC4F10.15c ; curation promoter homolD box ; Alias wsp1 ; GO GO:0030479; actin cortical patch
 GO:0051286; cell tip
 GO:0005826; contractile ring
 GO:0005515; protein binding
 GO:0000147; actin cortical patch assembly
 GO:0000147; actin cortical patch assembly
 GO:0045010; actin nucleation
 GO:0045010; actin nucleation
 GO:0009272; cell wall biosynthesis (sensu Fungi)
 GO:0030467; establishment and/or maintenance of cell polarity (sensu Fungi)
 GO:0030833; regulation of actin filament polymerization
 GO:0000915; cytokinesis, contractile ring formation
 GO:0000916; cytokinesis, contractile ring contraction ; primary_name wsp1 ; product WASp homolog ;

mRNA SPAC19B12.01 ; temporary_systematic_id SPAC19B12.01 ; note **mRNA** from AU012438 ;

mRNA SPAC19B12.02c ; temporary_systematic_id SPAC19B12.02c ; note **mRNA** from AU010793 ;

LTR unknown_4683 ; note fragment Tf1-type **LTR** ;

repeat_region unknown_4684 ; note polyT 35 ;

repeat_region unknown_4685 ; note duplicated region in cosmid c212 ;

intron unknown_4686 ; note Intron predicted by HMM_intron, Score=10.95 ;
mRNA SPAPB8E5.04c ; p_c phosphatidylglycerol/phosphatidylinositol transfer protein (predicted) ||| similar to S. cerevisiae YDL046W ; colour 7 ; gene SPAPB8E5.04c ; product phosphatidylglycerol/phosphatidylinositol transfer protein (predicted) ;

mRNA SPAC1006.01 ; p_c serine protease Psp3 (predicted) ||| similar to S. pombe SPAC4A8.04 ||| similar to S. cerevisiae YEL060C ; colour 2 ; gene psp3 ||| SPAC1006.01 ; note the third S. pombe serine protease (PMID 11115118) ; Alias psp3 ; primary_name psp3 ; product serine protease Psp3 (predicted) ; EC_number 3.4.24.- ;
mRNA SPAC29A4.16 ; p_c halotolerence protein 4 ||| non-essential (PMID 15821139) ||| similar to S. cerevisiae YCR008W ; systematic_id SPAC29A4.16 ; colour 2 ; gene hal4 ||| sat4 ||| ppk10 ||| SPAC29A4.16 ; Alias hal4 ; synonym sat4 ||| ppk10 ; GO GO:0006468; protein amino acid phosphorylation
 GO:0004674; protein serine/threonine kinase activity
 GO:0005524; ATP binding
 GO:0042391; regulation of membrane potential
 GO:0030003; cation homeostasis
 GO:0042493; response to drug
 GO:0043157; response to cation stress
 GO:0005515; protein binding
 GO:0043157; response to cation stress
 GO:0030003; cation homeostasis ; primary_name hal4 ; product halotolerence protein 4 ;
mRNA SPAC26F1.07 ; p_c 2-methylbutyraldehyde reductase (predicted) ||| aldo/keto reductase ||| similar to S. cerevisiae YDR368W and YOR120W ||| similar to S. pombe SPBC8E4.04 (paralog) ; GO GO:0042843; D-xylose catabolism
 GO:0019568; arabinose catabolism
 GO:0005737; cytoplasm
 GO:0005634; nucleus
 GO:0032018; 2-methylbutanal reductase activity ; colour 7 ; gene SPAC26F1.07 ; product 2-methylbutyraldehyde reductase (predicted) ; EC_number 1.1.1.21 ;

mRNA SPAC26F1.06 ; temporary_systematic_id SPAC26F1.06 ; note **mRNA** from AU010092 ;

misc_feature unknown_5041 ; note nominal overlap with plasmid SPAP8A3, EM:AL117210 S. pombe chromosome 1 ;
mRNA SPAC11E3.15 ; p_c 60S ribosomal protein L22 ||| similar to S. cerevisiae YLR061W and YFL034C-A ; colour 7 ; gene rpl22 ||| SPAC11E3.15 ||| SPAP8A3.01 ; Alias rpl22 ; GO GO:0005842; cytosolic large ribosomal subunit (sensu Eukaryota)
 GO:0003735; structural constituent of ribosome
 GO:0006412; translation ; primary_name rpl22 ; product 60S ribosomal protein L22 ;

mRNA SPAC11E3.15 ; colour 7 ;
mRNA SPAP8A3.04c ; p_c heat shock protein Hsp9 (PMID 8679693) ||| similar to S. cerevisiae YFL014W ; colour 2 ; gene hsp9 ||| scf1 ||| SPAP8A3.04c ; note possible sequencing error, 98 aa version reported (PMID:8654972) ; Alias hsp9 ; GO GO:0000915; cytokinesis, contractile ring formation ; primary_name hsp9 ; product heat shock protein Hsp9 (PMID 8679693) ;

mRNA SPAC922.04 ; temporary_systematic_id SPAC922.04 ; note **mRNA** from AU006605 ;

mRNA SPAC869.02c ; temporary_systematic_id SPAC869.02c ; note **mRNA** from AU010057 ;
misc_RNA SPNCRNA.61 ; Alias prl61 ; db_xref EMBL:AB084873 ||| PMID:12597277 ; systematic_id SPNCRNA.61 ; controlled_curation term=non-coding RNA; qualifier=predicted; db_xref=PMID:12597277; date=20050412 ||| term=antisense to SPAC186.07c; qualifier=predicted; db_xref=PMID:12597277; date=20050412 ||| term=poly(A)-bearing RNA; qualifier=predicted; db_xref=PMID:12597277; date=20050412 ||| term=no detectable long open reading frame; qualifier=predicted; db_xref=PMID:12597277; date=20050412 ; gene prl61 ; primary_name prl61 ; product non-coding RNA (predicted) ;
mRNA SPAC186.09 ; p_c pyruvate decarboxylase (predicted) ||| similar to S. pombe SPAC1F8.07C ||| no apparent S. cerevisiae ortholog ; colour 7 ; gene SPAC186.09 ; product pyruvate decarboxylase (predicted) ;

repeat_region unknown_5201 ; note duplicated region in 977 ;

mRNA SPAC750.01 ; p_c pseudogene ||| oxidoreductase ||| no initiator Methionine ; pseudo _no_value ; colour 13 ; gene SPAC750.01 ; product pseudogene ;
mRNA SPBPB21E7.04c ; p_c S-adenosylmethionine-dependent methy**LTR**ansferase (predicted) ||| O-methy**LTR**ansferase (predicted) ||| conserved eukaryotic protein ||| no apparent S. cerevisiae ortholog ||| similar to S. pombe SPBC119.03 ; synonym SPAPB21E7.04c ; colour 10 ; gene SPBPB21E7.04c ; product

S-adenosylmethionine-dependent methy**LTR**ansferase (predicted) ;

misc_feature unknown_26 ; note possible rearrangement here. Possible N terminal Adenine deaminase fragment ;
mRNA SPBPB21E7.08 ; p_c pseudogene ||| similar to S. cerevisiae YJL213W ; colour 13 ; gene SPBPB21E7.08 ; note 2 frameshifts ; synonym SPAPB21E7.08; pseudo _no_value ; product pseudogene ;
mRNA SPBC1198.14c ; p_c fructose-1,6-bisphosphatase Fbp1 (PMID 2157626) ||| similar to S. cerevisiae YLR377C ; colour 2 ; gene fbp1 ||| SPBC1198.14c ||| SPBC660.04c ; curation transcriptional repression occurs by a cAMP signaling pathway (PMID 1849107) ||| transcriptionnally regulated by adenylate cyclase activation by a G protein alpha subunit encoded by Gpa2 (PMID 8001792) ||| transcriptional regulators include two redundant Tup1p-like corepressors and the CCAAT binding factor activation complex (PMID 11238405) ; Alias fbp1 ; primary_name fbp1 ; product fructose-1,6-bisphosphatase Fbp1 (PMID 2157626) ;
mRNA SPBC660.04c ; gene SPBC660.04c ||| fbp1 ; note Charlie Hofmann, pers comm ;
mRNA SPBC660.06 ; p_c conserved fungal protein ||| glycine-rich ||| WW domain ||| similar to S. pombe SPBC11B10.08 and SPBC660.05 ||| similar to S. cerevisiae YFL010C ; colour 10 ; gene SPBC660.06 ; product conserved fungal protein ;
tRNA SPB**TRNA**GLN.01 ; gene SPB**TRNA**GLN.01 ; product **tRNA** Glutamine ; note **tRNA** Gln anticodon CTG, Cove score 71.48 ;
tRNA SPB**TRNA**GLN.03 ; gene SPB**TRNA**GLN.03 ; product **tRNA** Glutamine ; note **tRNA** Gln anticodon CTG, Cove score 71.48 ;
mRNA SPBC800.04c ; temporary_systematic_id SPBC800.04c ; note **mRNA** from spc03862 ;
tRNA SPB**TRNA**GLY.04 ; gene SPB**TRNA**GLY.04 ; product **tRNA** Glycine ; note **tRNA** Gly anticodon TCC, Cove score 54.33 ;
tRNA SPB**TRNA**ALA.07 ; gene SPB**TRNA**ALA.07 ; product **tRNA** Alanine ; note **tRNA** Ala anticodon AGC, Cove score 56.39 ;
mRNA SPBC1773.03c ; temporary_systematic_id SPBC1773.03c ; note **mRNA** from AU007673 ;
tRNA SPB**TRNA**GLY.05 ; gene SPB**TRNA**GLY.05 ; product **tRNA** Glycine ; note **tRNA** Gly anticodon TCC, Cove score 60.52 ;
tRNA SPB**TRNA**ARG.04 ; gene SPB**TRNA**ARG.04 ; product **tRNA** Arginine ; note **tRNA** Arg anticodon TCT, Cove score 69.29 ;
mRNA SPBC1271.03c ; p_c phosphoprotein phosphatase ||| NLI interacting factor family ||| non-essential (PMID 12618370) ||| similar to S. cerevisiae YLL010C and YLR019W ||| similar to S. pombe SPAC2F7.02c ; GO GO:0004721; phosphoprotein phosphatase activity
 GO:0005886; plasma membrane
 GO:0006470; protein amino acid dephosphorylation
 GO:0006950; response to stress ; colour 7 ; gene SPBC1271.03c ; product phosphoprotein phosphatase
tRNA SPB**TRNA**HIS.01 ; gene SPB**TRNA**HIS.01 ; product **tRNA** Histidine ; note **tRNA** His anticodon GTG ;
rRNA SPRRNA.28 ; systematic_id SPRRNA.28 ; product 5S rRNA ; note SPA Yeast (S.pombe) 5S ribosomal RNA. ;
snRNA SP**SNRNA**.04 ; Alias snu4 ; db_xref RFAM:RF00015 ; synonym U4 ; systematic_id SP**SNRNA**.04 ; gene U4 ||| snu4 ||| SP**SNRNA**.04 ; primary_name snu4 ; product small nuclear RNA U4 ;
mRNA SPBC1685.09 ; p_c 40S ribosomal protein S29 ||| non-essential (PMID 12618370) ||| similar to S. cerevisiae YLR388W and YDL061C ; colour 2 ; gene rps29 ||| SPBC1685.09 ; Alias rps29 ; GO GO:0005843; cytosolic small ribosomal subunit (sensu Eukaryota)
 GO:0006412; translation
 GO:0003735; structural constituent of ribosome ; primary_name rps29 ; product 40S ribosomal protein S29 ;
mRNA SPBC1685.10 ; p_c 40S ribosomal protein S27 ||| similar to S. cerevisiae YKL156W and YHR021c ; colour 2 ; gene rps27 ||| SPBC1685.10 ; Alias rps27 ; GO GO:0005843; cytosolic small ribosomal subunit (sensu Eukaryota)
 GO:0006412; translation
 GO:0003735; structural constituent of ribosome ; primary_name rps27 ; product 40S ribosomal protein S27 ;

mRNA SPBC1685.12c ; p_c dubious ||| ORF in compositionally biased region ; colour 6 ; gene SPBC1685.12c ; product dubious ;

misc_feature unknown_414 ; note gt repeat region similar to human/mouse repeated reg ion ;

misc_RNA SPNCRNA.133 ; db_xref EMBL:AU007212 ||| EMBL:AU011804 ; systematic_id SPNCRNA.133 ; controlled_curation term=non-coding RNA; qualifier=predicted; db_xref=EMBL:AU012817; date=20050412 ||| term=no detectable long open reading frame; qualifier=predicted; db_xref=EMBL:AU012817; date=20050412 ; product non-coding RNA (predicted) ; note RNA from AU011804 and AU007212 ;

misc_RNA SPNCRNA.134 ; db_xref EMBL:AU007844 ||| EMBL:AU008659 ; systematic_id SPNCRNA.134 ; controlled_curation term=non-coding RNA; qualifier=predicted; db_xref=EMBL:AU012817; date=20050412 ||| term=no detectable long open reading frame; qualifier=predicted; db_xref=EMBL:AU012817; date=20050412 ; product non-coding RNA (predicted) ; note RNA from AU007844 and AU008659 ;

mRNA SPBC649.04 ; p_c UV-induced protein Uvi15 ||| fibrillarin binds to a 3' cis-regulatory element in pre-**mRNA** of Uvi15 (PMID 1207460) ||| non-essential (PMID 12618370) ||| similar to S. cerevisiae YDL012C and YDR210W and YBR016W ; colour 2 ; gene uvi15 ||| SPBC649.04 ; Alias uvi15 ; GO GO:0009408; response to heat
 GO:0006974; response to DNA damage stimulus
 GO:0007584; response to nutrient ; primary_name uvi15 ; product UV-induced protein Uvi15 ;

mRNA SPBC649.05 ; colour 2 ;

mRNA SPBC354.12 ; p_c glyceraldehyde 3-phosphate dehydrogenase Gpd3 ||| similar to S. cerevisiae YJL052W and YGR192C and YJR009C ; colour 2 ; gene gpd3 ||| SPBC354.12 ; Alias gpd3 ; GO GO:0006096; glycolysis
 GO:0004365; glyceraldehyde-3-phosphate dehydrogenase (phosphorylating) activity ; primary_name gpd3 ; product glyceraldehyde 3-phosphate dehydrogenase Gpd3 ; EC_number 1.2.1.12 ;

mRNA SPBC839.15c ; temporary_systematic_id SPBC839.15c ; note **mRNA** from AU008702 ;

mRNA SPBC119.02 ; p_c ubiquitin conjugating enzyme (PMID 7698660) ||| similar to S. cerevisiae YDR059C and YBR082C ; colour 2 ; gene ubc4 ||| SPBC119.02 ; Alias ubc4 ; GO GO:0000070; mitotic sister chromatid segregation
 GO:0016567; protein ubiquitination
 GO:0008054; cyclin catabolism
 GO:0004840; ubiquitin conjugating enzyme activity ; primary_name ubc4 ; product ubiquitin conjugating enzyme (PMID 7698660) ; EC_number 6.3.2.19 ;

mRNA SPBC119.03 ; temporary_systematic_id SPBC119.03 ; note **mRNA** from spc05940 2 33 ;

mRNA SPBC119.05c ; temporary_systematic_id SPBC119.05c ; note **mRNA** from AB011825 ;

mRNA SPBC530.10c ; db_xref EMBL:D89102 ; systematic_id SPBC530.10c ;

mRNA SPBC36.03c ; p_c spermidine family transporter (predicted) ||| MFS family membrane transporter ||| similar to S. pombe SPBC530.15c and SPBC36.01C and SPCC569.05c and SPBC36.02C and SPBC947.06 ||| similar to S. cerevisiae YLL028W ||| 12 predicted transmembrane helices ||| tandem duplication ; GO GO:0015606; spermidine transporter activity
 GO:0000297; spermine transporter activity
 GO:0015846; polyamine transport
 GO:0005886; plasma membrane
 GO:0015848; spermidine transport
 GO:0000296; spermine transport ; colour 7 ; gene SPBC36.03c ; product spermidine family transporter (predicted) ;

mRNA SPBC713.11c ; p_c plasma membrane proteolipid Pmp3 ||| similar to S. cerevisiae YDR276C ; colour 2 ; gene pmp3 ||| SPBC713.11c ; Alias pmp3 ; GO GO:0006812; cation transport
 GO:0005886; plasma membrane
 GO:0042391; regulation of membrane potential
 GO:0030003; cation homeostasis
 GO:0043157; response to cation stress ; controlled_curation term=predicted N-terminal signal sequence; date=20060412 ||| term=UPF0057 family; db_xref=Pfam:PF01679; date=20060412 ||| term=non-essential; db_xref=PMID:16603158; date=20060412 ||| term=induced by Spc1 SAPK pathway; db_xref=PMID:16603158; date=20060412 ; primary_name pmp3 ; product plasma membrane proteolipid Pmp3 ; reserved_name pmp3 ;

mRNA SPBC713.12 ; p_c squalene monooxygenase Erg1 (predicted) ||| similar to S. cerevisiae YGR175C ; systematic_id SPBC713.12 ; colour 7 ; gene erg1 ||| SPBC713.12 ; Alias erg1 ; GO GO:0006696; ergosterol biosynthesis
 GO:0004506; squalene monooxygenase activity
 GO:0005789; endoplasmic reticulum membrane ; primary_name erg1 ; product squalene monooxygenase Erg1 (predicted) ; EC_number 1.14.99.7 ; reserved_name erg1 ;

misc_feature unknown_760 ; note nominal overlap with cosmid p35G2 S. pombe chromosome 2 ;

mRNA SPBC646.17c ; colour 2 ;

tRNA SPB**TRNA**HIS.02 ; gene SPB**TRNA**HIS.02 ; product **tRNA** Histidine ; note **tRNA** His anticodon GTG, Cove score 69.87 ;

mRNA SPBC1709.05 ; p_c heat shock protein Sks2 ||| heat shock protein 70 family (PMID 8973306) ||| confers K-252a resistance (PMID 9161410) ||| similar to S. cerevisiae YDL229W and YNL209W ; colour 2 ; gene sks2 ||| hsc1 ||| SPBC1709.05 ; note ask2 is the name which was given to the spurious orf on the opposite strand ; Alias sks2 ; primary_name sks2 ; product heat shock protein Sks2 ;

mRNA SPBC31A8.02 ; p_c pseudo ; colour 13 ; gene SPBC3D6.01 ||| SPBC31A8.02 ; note previously annotated as very hypothetical protein but has no met so assuming dubious or pseudo ; Alias SPBC3D6.01 ; pseudo _no_value ; primary_name SPBC3D6.01 ; product pseudo ;

misc_feature unknown_1101 ; note putative gene free region ;

mRNA SPBC3D6.02 ; p_c neddylation pathway protein But2 (PMID 14623327) ||| But2 family protein ||| possibly S. pombe specific ||| predicted N- terminal signal sequence ||| similar to S. pombe SPAC27D7.09c and SPAC27D7.10c and SPAC27D7.11c (paralogs) ; colour 2 ; gene but2 ||| SPBC3D6.02 ; Alias but2 ; GO GO:0005515; protein binding
 GO:0005515; protein binding ; primary_name but2 ; product neddylation pathway protein But2 (PMID 14623327) ;

misc_feature unknown_1138 ; note dicrepancy: with published U14 small nuclear RNA gen e and with cosmid c1268 sequence - additional T residue ins ertion at base 9128 ;

snoRNA SP**SNORNA**.21 ; Alias snoU14 ; db_xref RFAM:RF00016 ; systematic_id SP**SNORNA**.21 ; gene snoU14 ||| SP**SNORNA**.21 ; primary_name snoU14 ; product small nucleolar RNA U14 ;

mRNA SPBC8D2.03c ; p_c histone H4 ||| histone fold ||| similar to S. pombe hhf1 and hhf3 ||| similar to S. cerevisiae YBR009C and YNL030W ; colour 2 ; gene hhf2 ||| ams3 ||| h4.2 ||| SPBC8D2.03c ; Alias hhf2 ; GO GO:0003677; DNA binding
 GO:0006333; chromatin assembly or disassembly
 GO:0000788; nuclear nucleosome ; primary_name hhf2 ; product histone H4 ;

mRNA SPBC8D2.04 ; p_c histone H3 ||| histone fold ||| similar to S. pombe hht1 and hht3 ||| amino terminus K9, K14, S8 are involved in centomeric silencing (PMID 14561399) ||| similar to S. cerevisiae YBR010W and YNL031C ; colour 2 ; gene hht2 ||| h3.2 ||| SPBC8D2.04 ; Alias hht2 ; GO GO:0030702; chromatin silencing at centromere
 GO:0003677; DNA binding
 GO:0006333; chromatin assembly or disassembly
 GO:0000788; nuclear nucleosome ; primary_name hht2 ; product histone H3 ;

misc_feature unknown_1287 ; note low complexity gene free region ;

mRNA SPBC32H8.12c ; temporary_systematic_id SPBC32H8.12c ; note **mRNA** from AU013563 ;

mRNA SPBC11B10.07c ; colour 7 ;

misc_RNA SPNCRNA.24 ; Alias prl24 ; db_xref PMID:12597277 ||| EMBL:AB084836 ; systematic_id SPNCRNA.24 ; controlled_curation term=non-coding RNA; qualifier=predicted; db_xref=PMID:12597277; date=20050412 ||| term=poly(A)-bearing RNA; qualifier=predicted; db_xref=PMID:12597277; date=20050412 ||| term=no detectable long open reading frame; qualifier=predicted; db_xref=PMID:12597277; date=20050412 ; gene prl24 ; primary_name prl24 ; product non-coding RNA (predicted) ;

mRNA SPBC11B10.08 ; obsolete_name pi003 ||| SPACTOKYO_453.33c ; p_c conserved fungal protein ||| similar to S. cerevisiae YFL010C ||| WW domain ; colour 10 ; gene SPBC11B10.08 ; product conserved fungal protein ;

misc_feature unknown_1346 ; note nominal overlap with cosmid SPAB4534 (c1750), EM:AB004534 S. pombe chromosome 2 ;

tRNA SPB**TRNA**THR.06 ; gene SPB**TRNA**THR.06 ; product **tRNA** Threonine ; note **tRNA** Thr anticodon TGT, Cove score 78.51 ;

rRNA SPRRNA.33 ; systematic_id SPRRNA.33 ; product 5S rRNA ; note SPRG5SD K00771 Yeast (s.pombe) 5S rRNA gene and flanks ;

mRNA SPBC83.17 ; p_c transcriptional coactivator, multiprotein bridging factor Mbf1 (predicted) ||| similar to S. cerevisiae YOR298C-A ; GO GO:0003713; transcription coactivator activity
 GO:0005634; nucleus
 GO:0045944; positive regulation of transcription from RNA polymerase II promoter
 GO:0003677; DNA binding
 GO:0003700; transcription factor activity ; colour 7 ; gene SPBC83.17 ; product transcriptional coactivator, multiprotein bridging factor Mbf1 (predicted) ;

mRNA SPBC29B5.03c ; Alias rpl26 ; primary_name rpl26 ; gene rpl26 ||| SPBC29B5.03c ; note **mRNA** from AU008061 ;

tRNA SPB**TRNA**TYR.02 ; gene SPB**TRNA**TYR.02 ; product **tRNA** Tyrosine ; note **tRNA** Tyr anticodon GTA, Cove score 70.26 ;

tRNA SPB**TRNA**LEU.06 ; gene SPB**TRNA**LEU.06 ; product **tRNA** Leucine ; note **tRNA** Leu anticodon CAA, Cove score 56.07 ;

tRNA SPB**TRNA**GLY.07 ; gene SPB**TRNA**GLY.07 ; product **tRNA** Glycine ; note **tRNA** Gly anticodon GCC, Cove score 72.94 ;

tRNA SPB**TRNA**LYS.07 ; gene SPB**TRNA**LYS.07 ; product **tRNA** Lysine ; note **tRNA** Lys anticodon CTT, Cove score 74.83 ;

tRNA SPB**TRNA**ILE.05 ; gene SPB**TRNA**ILE.05 ; product **tRNA** Isoleucine ; note **tRNA** Ile anticodon AAT, Cove score 66.44 ;

tRNA SPB**TRNA**ALA.08 ; gene SPB**TRNA**ALA.08 ; product **tRNA** Alanine ; note **tRNA** Ala anticodon AGC, Cove score 67.24 ;

tRNA SPB**TRNA**VAL.05 ; gene SPB**TRNA**VAL.05 ; product **tRNA** Valine ; note **tRNA** Val anticodon AAC, Cove score 64.30 ;

tRNA SPB**TRNA**GLU.06 ; gene SPB**TRNA**GLU.06 ; product **tRNA** Glutamic acid ; note **tRNA** Glu anticodon TTC, Cove score 62.18 ;

tRNA SPB**TRNA**ARG.06 ; gene SPB**TRNA**ARG.06 ; product **tRNA** Arginine ; note **tRNA** Arg anticodon ACG, Cove score 75.46 ;

repeat_unit unknown_1440 ; colour 1 ; note similar to IMR repeat, not marked on Nature publication map, added May 2002 VW ;

tRNA SPB**TRNA**ASP.03 ; gene SPB**TRNA**ASP.03 ; product **tRNA** Aspartic acid ; note **tRNA** Asp anticodon GTC, Cove score 70.49 ;

repeat_unit unknown_1444 ; colour 2 ; note dhII repeat partial ;

repeat_unit unknown_1445 ; colour 2 ; note dhII repeat ;

repeat_unit unknown_1447 ; colour 1 ; note distal part of imr repeated ;

repeat_unit unknown_1448 ; colour 2 ; note dg/dh repeat? ;

repeat_unit unknown_1449 ; colour 1 ; note imr2r ;

tRNA SPB**TRNA**ALA.09 ; gene SPB**TRNA**ALA.09 ; product **tRNA** Alanine ; note **tRNA** Ala anticodon AGC, Cove score 56.39 ;

tRNA SPB**TRNA**VAL.06 ; gene SPB**TRNA**VAL.06 ; product **tRNA** Valine ; note **tRNA** Pseudo VAL anticodon AAC, Cove score 40.82 ;

repeat_unit unknown_1450 ; colour 4 ; note cnt2 ;

misc_feature unknown_1451 ; note nominal overlap with SPBC633 S. pombe chromosome 2 ;

repeat_unit unknown_1452 ; colour 4 ; note cnt2 ;
repeat_unit unknown_1453 ; colour 1 ; note imr2L ;
tRNA SPB**TRNA**VAL.07 ; gene SPB**TRNA**VAL.07 ; product **tRNA** Valine ; note **tRNA** Val anticodon AAC, Cove score 64.30 ;
tRNA SPB**TRNA**ALA.10 ; gene SPB**TRNA**ALA.10 ; product **tRNA** Alanine ; note **tRNA** Ala anticodon AGC, Cove score 67.24 ;
tRNA SPB**TRNA**ILE.07 ; gene SPB**TRNA**ILE.07 ; product **tRNA** Isoleucine ; note **tRNA** Ile anticodon AAT, Cove score 66.44 ;
repeat_unit unknown_1455 ; colour 2 ; note dhII repeat partial ;
repeat_unit unknown_1457 ; colour 2 ; note dhII repeat partial ;
repeat_unit unknown_1458 ; colour 12 ; note cen253 Yeast centromere CEN2 repetitive DNA PSS253, between dhIIa and dgIIa ;
tRNA SPB**TRNA**GLY.08 ; gene SPB**TRNA**GLY.08 ; product **tRNA** Glycine ; note **tRNA** Gly anticodon GCC, Cove score 72.94 ;
tRNA SPB**TRNA**LYS.08 ; gene SPB**TRNA**LYS.08 ; product **tRNA** Lysine ; note **tRNA** Lys anticodon CTT, Cove score 74.83 ;
tRNA SPB**TRNA**ILE.08 ; gene SPB**TRNA**ILE.08 ; product **tRNA** Isoleucine ; note **tRNA** Ile anticodon AAT, Cove score 66.44 ;
tRNA SPB**TRNA**ALA.11 ; gene SPB**TRNA**ALA.11 ; product **tRNA** Alanine ; note **tRNA** Ala anticodon AGC, Cove score 67.24 ;
tRNA SPB**TRNA**VAL.08 ; gene SPB**TRNA**VAL.08 ; product **tRNA** Valine ; note **tRNA** Val anticodon AAC, Cove score 64.30 ;
tRNA SPB**TRNA**GLU.07 ; gene SPB**TRNA**GLU.07 ; product **tRNA** Glutamic acid ; note **tRNA** Glu anticodon TTC, Cove score 62.18 ;
tRNA SPB**TRNA**ARG.07 ; gene SPB**TRNA**ARG.07 ; product **tRNA** Arginine ; note **tRNA** Arg anticodon ACG, Cove score 75.46 ;
mRNA SPBC21B10.13c ; p_c transcription factor (predicted) ||| homeobox domain ||| no apparent orthologs, cannot be distinguished ; GO GO:0005634; nucleus
 GO:0003700; transcription factor activity
 GO:0003677; DNA binding
 GO:0006355; regulation of transcription, DNA- dependent ; colour 7 ; gene SPBC21B10.13c ; product transcription factor (predicted) ;
mRNA SPBC21B10.12 ; p_c meiotic recombination protein Rec6 ||| no apparent orthologs ; colour 2 ; gene rec6 ||| SPBC21B10.12 ; Alias rec6 ; GO GO:0007131; meiotic recombination ; primary_name rec6 ; product meiotic recombination protein Rec6 ;
mRNA SPBC21B10.07 ; p_c glycosyl hydrolase family 16 ||| similar to S. cerevisiae YGR189C and YLR213C and YEL040W ; GO GO:0009277; cell wall (sensu Fungi) ; colour 7 ; gene SPBC21B10.07 ; product glycosyl hydrolase family 16 ;
mRNA SPBC19C2.07 ; temporary_systematic_id SPBC19C2.07 ; note **mRNA** from AU011079 ;
mRNA SPBC1D7.04 ; temporary_systematic_id SPBC1D7.04 ; note **mRNA** from AU009389 ;
mRNA SPBC1D7.03 ; temporary_systematic_id SPBC1D7.03 ; note **mRNA** from AU012097 ;
tRNA SPB**TRNA**PRO.04 ; gene SPB**TRNA**PRO.04 ; product **tRNA** Proline ; note **tRNA** Pro anticodon AGG ;
mRNA SPBC16E9.16c ; p_c sequence orphan ; colour 8 ; gene SPBC16E9.16c ; product sequence orphan ; note was previously annotated as pseudo however has peptide fragments two peptides in our mass spec analysis (pers. comm. Dieter Wolf), and is constantly expressed under stress conditions from microarray data; updated gene prediction to give valid translation but splice consensus isn't great for either intron 8-11-05 ;
mRNA SPBC1E8.05 ; p_c conserved fungal protein ||| no apparent S. cerevisiae ortholog ; GO GO:0009986; cell surface ; colour 10 ; controlled_curation term= glycoprotein; qualifier=predicted; date=20061206 ||| term=serine-rich protein; date=20061206 ||| term=GPI anchored protein; qualifier=RCA; db_xref=PMID:

12845604; date=20061206 ||| term=predicted N-terminal signal sequence; date=20061206 ; gene SPBC1E8.05 ; product conserved fungal protein ;

mRNA SPBP23A10.11c ; p_c glycoprotein ||| predicted N-terminal signal sequence ||| serine-rich protein ||| conserved fungal protein ||| similar to S. cerevisiae YBR162C and YJL171C ; GO GO:0009277; cell wall (sensu Fungi) ; colour 10 ; gene SPBP23A10.11c ; product glycoprotein ;

mRNA SPBC17G9.10 ; p_c 60S ribosomal protein L11 ||| similar to S. cerevisiae YPR102C and YGR085C ; colour 7 ; gene rpl1102 ||| rpl11-2 ||| SPBC17G9.10 ; Alias rpl1102 ; GO GO:0005843; cytosolic small ribosomal subunit (sensu Eukaryota)
 GO:0006412; translation
 GO:0003735; structural constituent of ribosome ; primary_name rpl1102 ; product 60S ribosomal protein L11 ;

mRNA SPBC1815.01 ; p_c enolase ||| similar to S. cerevisiae YGR254W and YHR174W and YOR393W and YPL281C and YMR323W ||| similar to S. pombe eno102 (paralog) ; colour 2 ; gene eno101 ||| eno1 ||| SPBC1815.01 ; Alias eno101 ; primary_name eno101 ; product enolase ; EC_number 4.2.1.11 ;

mRNA SPBC19G7.06 ; p_c MADS-box transcription factor Mbx1 ||| MADS-box ||| similar to S. pombe SPAC11E3.06 ||| similar to S. cerevisiae YMR043W and YMR042W ||| not required for periodic transcription in M phase ||| PBF transcription factor complex (PMID 15509866) ||| possibly functional ortholog of YMR043W (PMID 15509866) ; systematic_id SPBC19G7.06 ; colour 2 ; gene mbx1 ||| SPBC19G7.06 ; note Mads BoX protrein 1 ; Alias mbx1 ; GO GO:0045896; regulation of transcription, mitotic
 GO:0000086; G2/M transition of mitotic cell cycle
 GO:0000910; cytokinesis
 GO:0005667; transcription factor complex
 GO:0003702; RNA polymerase II transcription factor activity
 GO:0003677; DNA binding ; controlled_curation term=phosphorylated; cv=[pt_mod; db_xref=PMID:15509866; date=20061127 ; primary_name mbx1 ; product MADS-box transcription factor Mbx1 ;

mRNA SPBC12C2.11 ; colour 7 ;

mRNA SPBC17F3.01c ; p_c GTPase activating protein Rga5 ||| RhoGAP domain ||| non-essential (PMID 12519200) ||| deletion mutant results in multiseptation at high temperatures (PMID 12519200) ||| double mutant rga5delta/rga1delta unable to germinate (possibly) (PMID 12519200) ||| double mutant rga5delta/rga2,3,4,and 6delta no apparent genetic interaction (Nakano et. al) ||| regulates the interaction of Rho1p with Pck1p and Pck2p (PMID 12519200) ||| similar to S. cerevisiae YOR134W and YDR389W ; colour 2 ; gene rga5 ||| SPBC17F3.01c ||| SPBC557.01 ; Alias rga5 ; GO GO:0031097; medial ring
 GO:0051286; cell tip
 GO:0006075; 1,3-beta-glucan biosynthesis
 GO:0000902; cell morphogenesis
 GO::0005100; Rho GTPase activator activity
 GO:0032320; positive regulation of Ras GTPase activity
 GO:0007264; small GTPase mediated signal transduction ; primary_name rga5 ; product GTPase activating protein Rga5 ;

mRNA SPBC29A10.08 ; temporary_systematic_id SPBC29A10.08 ; note **mRNA** from SPD134 ;

mRNA SPBC4F6.04 ; p_c 60S ribosomal protein L25 ||| similar to S. cerevisiae YOL127W ||| similar to S. pombe rpl2501 ; colour 7 ; gene rpl2502 ||| rpl25b ||| rpl23a-2 ||| SPBC4F6.04 ; Alias rpl2502 ; GO GO:0005842; cytosolic large ribosomal subunit (sensu Eukaryota)
 GO:0006412; translation
 GO:0003735; structural constituent of ribosome ; primary_name rpl2502 ; product 60S ribosomal protein L25 ;

mRNA SPBC4F6.05c ; temporary_systematic_id SPBC4F6.05c ; note **mRNA** from AU010970 ;

mRNA SPBC32F12.11 ; db_xref EMBL:X85332 ; systematic_id SPBC32F12.11 ;

mRNA SPBC19C7.04c ; p_c conserved fungal protein ||| similar to S. cerevisiae YMR295C ; colour 10 ; gene SPBC19C7.04c ; product conserved fungal protein ;

misc_RNA SPNCRNA.26 ; Alias prl26 ; db_xref PMID:12597277 ||| EMBL:AB084838 ; systematic_id SPNCRNA.26 ; controlled_curation term=non-coding RNA; qualifier=predicted; db_xref=PMID:12597277; date=20050412 ||| term=poly(A)-bearing RNA; qualifier=predicted; db_xref=PMID:12597277; date=20050412 ||| term=no detectable long open reading frame; qualifier=predicted; db_xref=PMID:12597277; date=20050412 ||| term=low complexity gene free region; qualifier=predicted; date=20050412 ; gene prl26 ; primary_name prl26 ; product non-coding RNA (predicted) ;

tRNA SPB**TRNA**PRO.07 ; gene SPB**TRNA**PRO.07 ; product **tRNA** Proline ; note **tRNA** Pro anticodon AGG ;
snRNA SP**SNRNA**.01 ; Alias snu1 ; db_xref RFAM:RF00003 ; systematic_id SP**SNRNA**.01 ; gene snu1 ||| SP**SNRNA**.01 ; primary_name snu1 ; product small nuclear RNA U1 ;
snRNA SP**SNRNA**.05 ; Alias snu5 ; db_xref RFAM:RF00020 ; systematic_id SP**SNRNA**.05 ; gene snu5 ||| SP**SNRNA**.05 ; primary_name snu5 ; product small nuclear RNA U5 ;
mRNA SPBC3B8.07c ; p_c dihydroceramide delta-4 desaturase ||| conserved eukaryotic protein ||| no apparent S. cerevisiae ortholog ; systematic_id SPBC3B8.07c ; colour 2 ; gene dsd1 ||| SPBC3B8.07c ; Alias dsd1 ; synonym SDCB3B8.07c ; GO GO:0042284; sphingolipid delta-4 desaturase activity
 GO:0046512; sphingosine biosynthesis
 GO:0009966; regulation of signal transduction
 GO:0040020; regulation of meiosis
 GO:0016021; integral to membrane ; controlled_curation term=5 predicted transmembrane helices; date=20060411 ||| term=non-essential; db_xref=PMID:12633877; date=20060411 ; primary_name dsd1 ; product dihydroceramide delta-4 desaturase ; EC_number 1.14.-.- ;
snoRNA SP**SNORNA**.33 ; Alias sno16 ; db_xref EMBL:AJ577640 ||| EMBL:AJ632018 ||| PMID:15716270 ; synonym snR100 ; systematic_id SP**SNORNA**.33 ; evidence EXPERIMENTAL ; gene SP**SNORNA**.33 ||| sno16 ; primary_name sno16 ; product 16 small nucleolar RNA ;
mRNA SPBC3B8.02 ; db_xref EMBL:SPU88525 ; systematic_id SPBC3B8.02 ;
mRNA SPBC4B4.08 ; temporary_systematic_id SPBC4B4.08 ; note **mRNA** from AF017180 ;
mRNA SPBC887.15c ; p_c sphingosine hydroxylase (predicted) ||| sterol desaturase (predicted) ||| 3 predicted transmembrane helices ||| similar to S. cerevisiae YDR297W ; GO GO:0000170; sphingosine hydroxylase activity
 GO:0030148; sphingolipid biosynthesis
 GO:0005789; endoplasmic reticulum membrane ; colour 7 ; gene SPBC887.15c ; product sphingosine hydroxylase (predicted) ;
tRNA SPB**TRNA**LYS.09 ; gene SPB**TRNA**LYS.09 ; product **tRNA** Lysine ; note **tRNA** Lys anticodon CTT ;
tRNA SPB**TRNA**TYR.04 ; gene SPB**TRNA**TYR.04 ; product **tRNA** Tyrosine ; note **tRNA** Tyr anticodon GTA ;
mRNA SPBC21C3.13 ; p_c 40S ribosomal protein S19 ||| similar to S. cerevisiae YOL121C and YNL302C ||| disease associated, Diamond Blackfan Anemia ; colour 2 ; gene rps1901 ||| rps19-1 ||| SPBC21C3.13 ; Alias rps1901 ; controlled_curation term=conserved eukaryotic protein; date=20070115 ; GO GO:0005843; cytosolic small ribosomal subunit (sensu Eukaryota)
 GO:0006412; translation
 GO:0003735; structural constituent of ribosome ; primary_name rps1901 ; product 40S ribosomal protein S19 ;
rRNA SPRRNA.36 ; systematic_id SPRRNA.36 ; product 5S rRNA ; note 5S rRNA, experimental EM:K00570 ;
tRNA SPB**TRNA**ASP.04 ; gene SPB**TRNA**ASP.04 ; product **tRNA** Aspartic acid ; note **tRNA** Asp anticodon GTC, Cove score 70.49 ;
mRNA SPBC26H8.06 ; p_c glutaredoxin Grx4 ||| glutaredoxin ||| monothiol glutaredoxin ||| PICOT domain ||| essential (PMID 15796926) ||| similar to S. cerevisiae YDR098C and YER174C ; colour 2 ; gene grx4 ||| SPBC26H8.06 ; Alias grx4 ; GO GO:0008794; arsenate reductase (glutaredoxin) activity
 GO:0030508; thiol-disulfide exchange intermediate activity
 GO:0005634; nucleus
 GO:0005737; cytoplasm
 GO:0006979; response to oxidative stress ; primary_name grx4 ; product glutaredoxin Grx4 ;
snRNA SP**SNRNA**.07 ; Alias snu32 ; db_xref EMBL:X56189 ||| RFAM:RF00012 ; systematic_id SP**SNRNA**.07 ; gene snu32 ||| SP**SNRNA**.07 ; primary_name snu32 ; product small nuclear RNA U3B ;
mRNA SPBC26H8.10 ; p_c 3'-5' exoribonuclease subunit Dis3 (predicted) ||| essential (PMID 1944266) ||| RNB domain ||| similar to S. cerevisiae YOL021C ; colour 2 ; gene dis3 ||| SPBC26H8.10 ; note does not bind GeneDB_Spombe:SPBC776.02c PMID 1944266 ; Alias dis3 ; GO GO:0000176; nuclear exosome

(RNase complex)
 GO:0005739; mitochondrion
 GO:0000177; cytoplasmic exosome (RNase complex)
 GO:0006365; 35S primary transcript processing
 GO:0006402; **mRNA** catabolism
 GO:0000175; 3'-5'-exoribonuclease activity
 GO:0031125; rRNA 3'-end processing
 GO:0005730; nucleolus
 GO:0000070; mitotic sister chromatid segregation
 GO:0005634; nucleus
 GO:0005515; protein binding
 GO:0007346; regulation of progression through mitotic cell cycle
 GO:0005515; protein binding
 GO:0005515; protein binding
 GO:0008536; Ran GTPase binding
 GO:0016075; rRNA catabolism ; primary_name dis3 ; product 3'-5' exoribonuclease subunit Dis3 (predicted) ;

mRNA SPBC26H8.11c ; temporary_systematic_id SPBC26H8.11c ; note **mRNA** from AU011994 ;
mRNA SPBC32C12.02 ; p_c transcription factor Ste11 ||| HMG box ||| NLS ||| conserved fungal protein ||| no apparent S. cerevisiae ortholog ||| similar to P. Carinii STE11 ; colour 2 ; gene ste11 ||| aff1 ||| stex ||| SPBC32C12.02 ; note the equivalent function in S. cerevisiae is performed by STE12, but this is STE-like not HMG ; Alias ste11 ; GO GO:0000747; conjugation with cellular fusion
 GO:0003700; transcription factor activity
 GO:0051039; positive regulation of transcription, meiotic
 GO:0005634; nucleus ; controlled_curation term=transcriptionally regulates Mei2; date=20060818 ; primary_name ste11 ; product transcription factor Ste11 ;

mRNA SPBC215.05 ; temporary_systematic_id SPBC215.05 ; note **mRNA** from AU013069 ;
mRNA SPBC56F2.12 ; p_c acetohydroxyacid reductoisomerase ||| ketol-acid reductoisomerase family ||| similar to S. cerevisiae YLR355C ; GO GO:0004455; ketol-acid reductoisomerase activity
 GO:0000002; mitochondrial genome maintenance
 GO:0005739; mitochondrion
 GO:0009099; valine biosynthesis
 GO:0009097; isoleucine biosynthesis
 GO:0006551; leucine metabolism ; colour 7 ; gene SPBC56F2.12 ||| ilv5 ; product acetohydroxyacid reductoisomerase ; EC_number 1.1.1.86 ;

tRNA SPB**TRNA**GLU.08 ; gene SPB**TRNA**GLU.08 ; product **tRNA** Glutamic acid ; note **tRNA** Glu anticodon CTC, Cove score 74.15 ;
mRNA SPBC56F2.02 ; p_c 60S ribosomal protein L19 ||| similar to S. cerevisiae YBR084C-A and YBL027W ; colour 2 ; gene rpl1901 ||| rpl19-1 ||| SPBC56F2.02 ; Alias rpl1901 ; GO GO:0005842; cytosolic large ribosomal subunit (sensu Eukaryota)
 GO:0006412; translation
 GO:0003735; structural constituent of ribosome ; primary_name rpl1901 ; product 60S ribosomal protein L19 ;

mRNA SPBC14F5.04c ; temporary_systematic_id SPBC14F5.04c ; note **mRNA** from AU014126 ;
mRNA SPBC16G5.14c ; p_c 40S ribosomal protein S3 ||| similar to S. cerevisiae YNL178W ; colour 2 ; gene rps3 ||| SPBC16G5.14c ; Alias rps3 ; GO GO:0003735; structural constituent of ribosome
 GO:0005843; cytosolic small ribosomal subunit (sensu the Eukaryota research community)
 GO:0006412; protein biosynthesis ; primary_name rps3 ; product 40S ribosomal protein S3 ;

mRNA SPBC8E4.02c ; p_c sequence orphan ; colour 8 ; gene SPBC8E4.02c ; product sequence orphan ;

mRNA SPBC8E4.01c ; Alias SPBP4G3.01 ; primary_name SPBP4G3.01 ; gene SPBP4G3.01 ||| SPBC8E4.01c ; note **mRNA** from AU009778 ;
mRNA SPBP4G3.03 ; p_c PI31 proteasome regulator related ||| no apparent orthologs, S. pombe variant ||| related to S. pombe SPAC15E1.10 ; colour 10 ; gene SPBP4G3.03 ; product PI31 proteasome regulator related ;

mRNA SPBCPT2R1.08c ; db_xref EMBL:BK005597 ; gene SPBCPT2R1.08c ;

repeat_region unknown_4360 ; note dupliated region in c212 ;

repeat_region unknown_4362 ; note telomeric repeats ;

tRNA SPC**TRNA**HIS.03 ; gene SPC**TRNA**HIS.03 ; product **tRNA** Histidine ; note **tRNA** His anticodon GTG ;

mRNA SPCC757.12 ; p_c alpha-amylase homolog (predicted) ||| no apparent orthologs, cannot be distinguished ; colour 7 ; gene SPCC757.12 ; GO GO:0009986; cell surface
 GO:0004556; alpha-amylase activity
 GO:0044247; cellular polysaccharide catabolism
 GO:0009897; external side of plasma membrane
 GO:0005618; cell wall
 GO:0048503; GPI anchor binding
 GO:0009272; cell wall biosynthesis (sensu Fungi)
 GO:0000902; cell morphogenesis ; controlled_curation term=glycoprotein; cv=pt_mod; date=20060611 ||| term=N-glycosylated; evidence=IDA; cv=pt_mod; date=20060611 ||| term=N-glycosylation site; cv=pt_mod; date=20060611 ||| term=glycosyl hydrolase family 13; date=20060611 ||| term=predicted N-terminal signal sequence; date=20060611 ||| term=GPI anchored protein; evidence=ISS; db_xref=PMID:12845604; date=20060611 ; product alpha-amylase homolog (predicted) ; EC_number 3.2.1.1 ;
mRNA SPCC613.05c ; p_c 60S ribosomal protein L35 ||| similar to S. cerevisiae YDL191W and YDL136W ; colour 2 ; gene rpl35 ||| SPCC613.05c ; Alias rpl35 ; GO GO:0005842; cytosolic large ribosomal subunit (sensu Eukaryota)
 GO:0006412; translation
 GO:0003735; structural constituent of ribosome ; primary_name rpl35 ; product 60S ribosomal protein L35 ;
mRNA SPCC330.03c ; p_c NADPH-hemoprotein reductase ||| cytochrome b5 family ||| similar to S. cerevisiae YMR073C ||| similar to S. pombe SPAC1F12.10C (paralog) ; GO GO:0020037; heme binding
 GO:0042168; heme metabolism
 GO:0003958; NADPH-hemoprotein reductase activity ; colour 7 ; gene SPCC330.03c ; product NADPH-hemoprotein reductase ;
tRNA SPC**TRNA**GLY.10 ; gene SPC**TRNA**GLY.10 ; product **tRNA** Glycine ; note **tRNA** Gly anticodon GCC ;
mRNA SPCC1235.01 ; p_c glycoprotein (predicted) ||| sequence orphan ; colour 8 ; gene SPCC320.02c ||| SPCC1235.01 ; note ~37 copies of a 7-10 repeat consensus 'PMEEITTMTI' and a S/N rich C terminal region ; Alias SPCC320.02c ; primary_name SPCC320.02c ; product glycoprotein (predicted) ;
mRNA SPCC1235.14 ; Alias ght5 ; primary_name ght5 ; gene ght5 ||| SPCC1235.14 ; note **mRNA** from AF017180 ;
mRNA SPCC548.06c ; temporary_systematic_id SPCC548.06c ; note **mRNA** from spc05276 ;
misc_feature unknown_192 ; note gene free region ;
mRNA SPCC794.09c ; db_xref EMBL:D82571 ; gene SPCC794.09c ;
mRNA SPCC553.10 ; temporary_systematic_id SPCC553.10 ; note **mRNA** from AU010226 ;
mRNA SPCC736.15 ; temporary_systematic_id SPCC736.15 ; note **mRNA** from AU012604 ;
misc_feature unknown_294 ; note low-complexity gene-free region ;
mRNA SPCC594.01 ; colour 10 ;
mRNA SPCC306.11 ; p_c sequence orphan ||| predicted N-terminal signal sequence ; colour 8 ; gene SPCC306.11 ; product sequence orphan ;
mRNA SPCC1393.08 ; p_c transcription factor (predicted) ||| zinc finger protein ||| zf-GATA type ||| no apparent orthologs, cannot be distinguished ; colour 7 ; gene SPCC1393.08 ; product transcription factor (predicted) ;
rRNA SPRRNA.25 ; db_xref EMBL:Z19136 ; systematic_id SPRRNA.25 ; product 25S rRNA ; note fragment ;
mRNA SPCC24B10.21 ; p_c triosephosphate isomerase ||| similar to S. cerevisiae YDR050C ; colour 2 ; gene tpi1 ||| tpi ||| SPCC24B10.21 ; Alias tpi1 ; GO GO:0004807; triose-phosphate isomerase activity
 GO:0006096; glycolysis
 GO:0006094; gluconeogenesis
 GO:0005829; cytosol ; controlled_curation term=disease associated, hemolytic anemia; date=20060920 ||| term=conserved eukaryotic protein; date=20060920 ; primary_name tpi1 ; product triosephosphate isomerase ; EC_number 5.3.1.1 ;

mRNA SPCC1795.11 ; p_c ATP-dependent RNA helicase Sum3 ||| DEAD/DEAH box helicase ||| essential (PMID 9832516) ||| similar to S. cerevisiae YOR204W and YPL119C ; colour 2 ; gene sum3 ||| ded1 ||| slh3 ||| moc2 ||| SPCC1795.11 ; note suppressor of uncontrolled mitosis ||| Multicopy supressor of Overexpressed Cyr1 ; Alias sum3 ; GO GO:0004004; ATP-dependent RNA helicase activity
 GO:0005737; cytoplasm
 GO:0005515; protein binding
 GO:0005515; protein binding
 GO:0006412; translation
 GO:0000086; G2/M transition of mitotic cell cycle
 GO:0000076; DNA replication checkpoint
 GO:0006970; response to osmotic stress
 GO:0031137; regulation of conjugation with cellular fusion ; primary_name sum3 ; product ATP-dependent RNA helicase Sum3 ;

misc_feature unknown_1009 ; note nominal overlap with cosmid SPCC825, EM:AL122011 S. pombe chromosome 3 ;

mRNA SPCC1259.01c ; p_c 40S ribosomal protein S18 ||| similar to S. cerevisiae YDR450W and YML026C ; colour 2 ; gene rps1802 ||| rps18-2 ||| SPCC1259.01c ||| SPCC825.06c ; Alias rps1802 ; GO GO:0005843; cytosolic small ribosomal subunit (sensu Eukaryota)
 GO:0006412; translation
 GO:0003735; structural constituent of ribosome
 GO:0019843; rRNA binding ; primary_name rps1802 ; product 40S ribosomal protein S18 ;

tRNA SPC**TRNA**ALA.12 ; gene SPC**TRNA**ALA.12 ; product **tRNA** Alanine ; note **tRNA** Ala anticodon AGC, Cove score 56.39 ;

tRNA **tRNA**_pseudo anticodon AAC ; pseudo _no_value ; gene **tRNA**_pseudo anticodon AAC ; note **tRNA** Pseudo anticodon AAC, Cove score 40.82 ;

tRNA SPC**TRNA**SER.09 ; gene SPC**TRNA**SER.09 ; product **tRNA** Serine ; note **tRNA** Ser anticodon AGA, Cove score 59.19 ;

tRNA SPC**TRNA**ARG.10 ; gene SPC**TRNA**ARG.10 ; product **tRNA** Arginine ; note **tRNA** Arg anticodon TCG, Cove score 59.66 ;

tRNA SPC**TRNA**ASP.05 ; gene SPC**TRNA**ASP.05 ; product **tRNA** Asparagine ; note **tRNA** Asp anticodon GTC, Cove score 57.96 ;

tRNA SPC**TRNA**ARG.11 ; gene SPC**TRNA**ARG.11 ; product **tRNA** Arginine ; note **tRNA** Arg anticodon ACG, Cove score 54.83 ;

tRNA SPC**TRNA**LEU.11 ; gene SPC**TRNA**LEU.11 ; product **tRNA** Leucine ; note **tRNA** Leu anticodon AAG, Cove score 46.56 ;

misc_feature unknown_1068 ; note nominal overlap with cosmid c1259 ;

repeat_unit unknown_1069 ; colour 1 ; note centromeric region duplicated in SPCC4B3 S. pombe chromosome 3 ;

tRNA SPC**TRNA**LYS.10 ; gene SPC**TRNA**LYS.10 ; product **tRNA** Lysine ; note **tRNA** Lys anticodon CTT, Cove score 57.56 ;

repeat_unit unknown_1072 ; colour 1 ; note region between dg and dh repeat ;

repeat_unit unknown_1076 ; colour 3 ; note dgIII repeat cen3b 250bp ;

repeat_unit unknown_1078 ; colour 2 ; note cen3a dhIII repeat ;

misc_feature unknown_1079 ; note nominal overlap with cosmid c1676 ;

repeat_unit unknown_1080 ; colour 2 ; note dhIII repeat ;

repeat_unit unknown_1081 ; colour 11 ; note cen3xc central region ;

repeat_unit unknown_1083 ;

repeat_unit unknown_1084 ; colour 1 ; note imr3L ;

tRNA SPC**TRNA**ASP.06 ; gene SPC**TRNA**ASP.06 ; product **tRNA** Asparagine ; note **tRNA** Asp anticodon GTC, Cove score 70.49 ;

tRNA SPC**TRNA**ARG.12 ; gene SPC**TRNA**ARG.12 ; product **tRNA** Arginine ; note **tRNA** Arg anticodon ACG, Cove score 75.46 ;

tRNA SPC**TRNA**VAL.09 ; gene SPC**TRNA**VAL.09 ; product **tRNA** Valine ; note **tRNA** Val anticodon AAC, Cove score 64.30 ;
tRNA SPC**TRNA**THR.08 ; gene SPC**TRNA**THR.08 ; product **tRNA** Threonine ; note **tRNA** Thr anticodon AGT, Cove score 75.01 ;
tRNA SPC**TRNA**LEU.12 ; gene SPC**TRNA**LEU.12 ; product **tRNA** Leucine ; note **tRNA** Leu anticodon CAA, Cove score 56.07 ;
repeat_unit unknown_1088 ; colour 4 ; note cnt3 ;
repeat_unit unknown_1089 ; colour 4 ; note cnt3 partial ;
tRNA SPC**TRNA**GLU.10 ; gene SPC**TRNA**GLU.10 ; product **tRNA** Glutamic acid ; note **tRNA** Glu anticodon CTC, Cove score 74.15 ;
repeat_unit unknown_1090 ; colour 1 ; note imr3R ;
tRNA SPC**TRNA**LEU.13 ; gene SPC**TRNA**LEU.13 ; product **tRNA** Leucine ; note **tRNA** Leu anticodon CAA, Cove score 56.07 ;
tRNA SPC**TRNA**THR.09 ; gene SPC**TRNA**THR.09 ; product **tRNA** Threonine ; note **tRNA** Thr anticodon AGT, Cove score 75.01 ;
tRNA SPC**TRNA**VAL.10 ; gene SPC**TRNA**VAL.10 ; product **tRNA** Valine ; note **tRNA** Val anticodon AAC, Cove score 64.30 ;
tRNA SPC**TRNA**ARG.13 ; gene SPC**TRNA**ARG.13 ; product **tRNA** Arginine ; note **tRNA** Arg anticodon ACG, Cove score 75.46 ;
tRNA SPC**TRNA**ASP.07 ; gene SPC**TRNA**ASP.07 ; product **tRNA** Asparagine ; note **tRNA** Asp anticodon GTC, Cove score 70.49 ;
repeat_unit unknown_1093 ; colour 11 ; note cen3xc central region ;
repeat_unit unknown_1094 ; colour 2 ; note cen3a dhIII repeat ;
repeat_unit unknown_1095 ; colour 12 ; note region between dh and dg repeat ;
repeat_unit unknown_1098 ; colour 12 ; note region between dg and dh repeat ;
repeat_unit unknown_1101 ; colour 12 ; note region between dg and dh repeat ;
repeat_unit unknown_1102 ; colour 3 ; note cen3b dgIII repeat ;
repeat_unit unknown_1103 ; colour 2 ; note cen3a dhIII repeat ;
repeat_unit unknown_1104 ; colour 12 ; note region between dg and dh repeat ;
repeat_unit unknown_1105 ; colour 3 ; note cen3b dgIII repeat ;
misc_feature unknown_1107 ; note nominal overlap with pB5A12 S. pombe chromosome 3 ;
tRNA SPC**TRNA**LYS.11 ; gene SPC**TRNA**LYS.11 ; product **tRNA** Lysine ; note **tRNA** Lys anticodon CTT, Cove score 74.69 ;
mRNA SPCC1322.10 ; p_c glycoprotein (predicted) ||| possibly S. pombe specific ||| serine-rich protein ||| GPI anchored protein (predicted) (PMID 12845604) (pers. comm. Birgit Eisenhaber) ||| predicted N-terminal signal sequence ; GO GO:0009986; cell surface ; colour 12 ; gene SPCC1322.10 ; product glycoprotein (predicted) ;
tRNA SPC**TRNA**THR.10 ; gene SPC**TRNA**THR.10 ; product **tRNA** Threonine ; note **tRNA** Thr anticodon TGT, Cove score 68.01 ;
mRNA SPCC1281.06c ; p_c acyl-coA desaturase (predicted) ||| similar to S. cerevisiae YGL055W ; GO GO:0006633; fatty acid biosynthesis
 GO:0004768; stearoyl-CoA 9-desaturase activity
 GO:0005789; endoplasmic reticulum membrane
 GO:0006636; fatty acid desaturation
 GO:0031227; intrinsic to endoplasmic reticulum membrane ; colour 7 ; gene SPCC1281.06c ; product acyl-coA desaturase (predicted) ;

mRNA SPCC622.09 ; temporary_systematic_id SPCC622.09 ; note **mRNA** from AU010164 ;
mRNA SPCC13B11.01 ; p_c alcohol dehydrogenase Adh1 ||| similar to S. cerevisiae YOL086C and YMR303C and YMR083W and YBR145W ; colour 2 ; gene adh1 ||| adh ||| SPCC13B11.01 ; Alias adh1 ; GO GO:0006066; alcohol metabolism
 GO:0005759; mitochondrial matrix ; primary_name adh1 ; product alcohol dehydrogenase Adh1 ; EC_number 1.1.1.1 ;
mRNA SPCC663.04 ; p_c 60S ribosomal protein L39 ||| similar to S. cerevisiae YJL189W ; colour 2 ; gene rpl39 ||| SPCC663.04 ; Alias rpl39 ; GO GO:0005842; cytosolic large ribosomal subunit (sensu Eukaryota)
 GO:0006412; translation
 GO:0003735; structural constituent of ribosome ; primary_name rpl39 ; product 60S ribosomal protein L39 ;
mRNA SPCC417.05c ; p_c chitin synthase regulatory factor (putative) Chr2 (PMID 15449309) ||| SEL1 repeat protein ||| non-essential (PMID 15449309) ||| similar to S. cerevisiae YBL061C and YDL203C and YER096W ; systematic_id SPCC417.05c ; colour 2 ; gene chr2 ||| SPCC417.05c ||| cfh2 ; Alias chr2 ; synonym cfh2 ; GO GO:0006038; cell wall chitin biosynthesis ; controlled_curation term=Chs Four Homologue; qualifier=pers. comm. Henar Montero; date= 20060106 ; primary_name chr2 ; product chitin synthase regulatory factor (putative) Chr2 (PMID 15449309) ;
mRNA SPCC417.08 ; p_c translation elongation factor eEF3 ||| AAA family ATPase ||| HEAT repeat (inferred from context) ||| similar to S. cerevisiae YNL014W and YLR249W ; colour 2 ; gene tef3 ||| SPCC417.08 ; Alias tef3 ; GO GO:0003746; translation elongation factor activity
 GO:0016887; ATPase activity
 GO:0005830; cytosolic ribosome (sensu Eukaryota)
 GO:0006414; translational elongation
 GO:0005524; ATP binding ; primary_ name tef3 ; product translation elongation factor eEF3 ;
tRNA SPC**TRNA**ASN.06 ; gene SPC**TRNA**ASN.06 ; product **tRNA** Asparagine ; note **tRNA** Asn anticodon GTT ;
rRNA SPRRNA.06 ; Alias 5S ; systematic_id SPRRNA.06 ; primary_name 5S ; gene 5S ; product 5S rRNA ; note 5s rRNA gene and flanks ;
mRNA SPCC191.07 ; temporary_systematic_id SPCC191.07 ; note **mRNA** from AU010442 ;
tRNA SPC**TRNA**GLN.05 ; gene SPC**TRNA**GLN.05 ; product **tRNA** Glutamine ; note **tRNA** Gln anticodon TTG, Cove score 54.89 ;
misc_feature SPCC297.02 ; colour 6 ; gene SPCC297.02 ; product dubious ;
mRNA SPCC737.04 ; p_c UPF0300 family ||| possibly S. pombe specific ; colour 12 ; gene SPCC737.04 ; product UPF0300 family ;
LTR unknown_1652 ; colour 4 ; note Tf2-type **LTR** ;
mRNA SPCC4F11.03c ; temporary_systematic_id SPCC4F11.03c ; note **mRNA** from SPD233 ;
mRNA SPCC1906.01 ; temporary_systematic_id SPCC1906.01 ; note **mRNA** from SPD128 ;
tRNA SPC**TRNA**GLY.12 ; gene SPC**TRNA**GLY.12 ; product **tRNA** Glycine ; note **tRNA** Gly anticodon TCC, Cove score 55.00 ;
mRNA SPCC1739.08c ; p_c short chain dehydrogenase ||| similar to S. pombe SPAC22A12.17C and SPAC8E11.10 ||| no apparent orthologs, cannot be distinguished ; colour 10 ; gene SPCC1739.08c ; product short chain dehydrogenase ;
mRNA SPCC1739.10 ; p_c conserved fungal protein ||| similar to S. pombe SPAC13G7.04c (paralog) ||| 3 predicted transmembrane helices ||| similar to S. cerevisiae YOL019W and YFR012W and YMR063W ||| predicted N-terminal signal sequence ; GO GO:0005886; plasma membrane ; colour 10 ; gene SPCC1739.10 ; product conserved fungal protein ;
mRNA SPCPB1C11.02 ; p_c amino acid permease family (predicted) ||| similar to S. cerevisiae YNL268W ||| 11 predicted transmembrane helices ||| no apparent orthologs, cannot be distinguished ; colour 7 ; gene SPCPB1C11.02 ; product amino acid permease family (predicted) ;

tRNA SPC**TRNA**GLN.06 ; gene SPC**TRNA**GLN.06 ; product **tRNA** Glutamine ; note **tRNA** Gln anticodon TTG, Cove score 69.22 ;

mRNA SPCC576.03c ; temporary_systematic_id SPCC576.03c ; note **mRNA** from AF083335 ;

mRNA SPCC576.08c ; temporary_systematic_id SPCC576.08c ; note **mRNA** from AU012777 ;

mRNA SPCC576.09 ; p_c 40S ribosomal protein S20 ||| similar to S. cerevisiae YHL015W ; colour 7 ; gene rps20 ||| SPCC576.09 ; Alias rps20 ; GO GO:0005843; cytosolic small ribosomal subunit (sensu Eukaryota)
 GO:0006412; translation
 GO:0003735; structural constituent of ribosome ; primary_name rps20 ; product 40S ribosomal protein S20 ;

mRNA SPCC1840.02c ; temporary_systematic_id SPCC1840.02c ; note **mRNA** from AU008494 ;

mRNA SPCC70.03c ; p_c proline dehydrogenase ||| similar to S. cerevisiae YLR142W ; colour 7 ; gene SPCC70.03c ; GO GO:0006562; proline catabolism
 GO:0006537; glutamate biosynthesis
 GO:0004657; proline dehydrogenase activity
 GO:0005759; mitochondrial matrix ; product proline dehydrogenase ; psu_db_xref PATH:MAP00330; ; EC_number 1.5.99.8 ;

mRNA SPCC70.10 ; temporary_systematic_id SPCC70.10 ; note **mRNA** from SPD146; putative **mRNA** for SPCC70.10 ;

mRNA SPCP1E11.04c ; colour 2 ;

mRNA SPCP1E11.09c ; colour 2 ;

repeat_region unknown_31 ; note (ttattttaagttttgtc)3 ;
mRNA SPAC977.14c ; temporary_systematic_id SPAC977.14c ; note **mRNA** from AU007498 ;
mRNA SPAC1F8.07c ; p_c pyruvate decarboxylase (predicted) ||| similar to S. pombe SPAC186.09 (paralog) ||| no apparent S. cerevisiae ortholog ; colour 7 ; gene SPAC1F8.07c ; product pyruvate decarboxylase (predicted) ; EC_number 4.1.1.1 ;
misc_feature unknown_79 ; note nominal overlap with cosmid SPAC806, EM:AL117212 S. pombe chromosome 1 ;
misc_feature unknown_80 ; note nominal overlap with cosmid SPAC24B11, EM:Z67757 S. pombe chromosome 1 ;
mRNA SPAC24B11.14 ; p_c sequence orphan ; colour 8 ; gene SPAC24B11.14 ||| SPAC806.10 ; product sequence orphan ; note added 3-9-99 ;
mRNA SPAC630.08c ; temporary_systematic_id SPAC630.08c ; note AU010457 clone spc05912 ;
mRNA SPAC630.15 ; p_c sequence orphan ; colour 8 ; gene SPAC630.15 ; product sequence orphan ; note has transcript profile on microarray ||| poor correlation score ;
mRNA SPAC2F7.05c ; p_c translation initiation factor eIF5 (predicted) ||| similar to S. cerevisiae YPR041W ; GO GO:0003743; translation initiation factor activity
 GO:0005096; GTPase activator activity
 GO:0042256; mature ribosome assembly
 GO:0006446; regulation of translational initiation
 GO:0005843; cytosolic small ribosomal subunit (sensu Eukaryota) ; colour 7 ; gene SPAC2F7.05c ; product translation initiation factor eIF5 (predicted) ;
mRNA SPAC22H12.04c ; p_c 40S ribosomal protein S3a ||| similar to S. cerevisiae YLR441C and YML063W ; colour 7 ; gene rps102 ||| rps1-2 ||| rps3a-2 ||| SPAC22H12.04c ; Alias rps102 ; GO GO:0005843; cytosolic small ribosomal subunit (sensu Eukaryota)
 GO:0006412; translation
 GO:0003735; structural constituent of ribosome ; primary_name rps102 ; product 40S ribosomal protein S3a ;
snRNA SP**SNRNA**.02 ; Alias snu2 ; db_xref EMBL:X55772 ||| Rfam:RF00004 ; synonym U2 ; systematic_id SP**SNRNA**.02 ; gene snu2 ||| U2 ||| SP**SNRNA**.02 ; primary_name snu2 ; product small nuclear RNA U2 ;
mRNA SPAC222.09 ; colour 2 ;
mRNA SPAC222.11 ; p_c coproporphyrinogen III oxidase (predicted) ||| similar to S. cerevisiae YDR044W ; systematic_id SPAC222.11 ; colour 7 ; gene hem13 ||| SPAC222.11 ; Alias hem13 ; GO GO:0004109; coproporphyrinogen oxidase activity
 GO:0006783; heme biosynthesis
 GO:0005743; mitochondrial inner membrane ; primary_name hem13 ; product coproporphyrinogen III oxidase (predicted) ; EC_number 1.3.3.3 ; reserved_name hem13 ;
mRNA SPAC821.10c ; p_c superoxide dismutase Sod1 ||| transcriptionally regulated by Pap1 (PMID 12073089) ||| essential ||| similar to S. cerevisiae YJR104C ; colour 2 ; gene sod1 ||| SPAC821.10c ; Alias sod1 ; GO GO:0004785; copper, zinc superoxide dismutase activity
 GO:0006878; copper ion homeostasis
 GO:0006801; superoxide metabolism
 GO:0006882; zinc ion homeostasis
 GO:0005829; cytosol
 GO:0005758; mitochondrial intermembrane space
 GO:0006979; response to oxidative stress ; controlled_curation term=conserved eukaryotic protein; date=20060920 ||| term=disease associated, amyotrophic lateral sclerosis; date=20060920 ; primary_name sod1 ; product superoxide dismutase Sod1 ; EC_number 1.15.1.1 ;
mRNA SPAC30D11.13 ; colour 2 ;
rep_origin unknown_1050 ; note ars1, minimal sequence (Clyne & Kelly) ;
rep_origin unknown_1051 ; colour 2 ; note ars1, segment 1 (Clyne & Kelly) ;

rep_origin unknown_1052 ; colour 4 ; note protected region F (PMID 11717425), protected region D (PMID 12374757) ;

rep_origin unknown_1053 ; colour 7 ; note Initiation site in ars1 determined by RIP mapping (PMID: 10523311) ;

rep_origin unknown_1054 ; colour 4 ; note protected region E (PMID 11717425) ;

rep_origin unknown_1055 ; colour 4 ; note protected region D (PMID 11717425); protected region C (PMID 12374757) ;

rep_origin unknown_1056 ; colour 2 ; note segment 9 (Clyne and Kelly) ;

rep_origin unknown_1057 ; colour 4 ; note protected region C (PMID 11717425) ;

rep_origin unknown_1058 ; colour 4 ; note protected region B (PMID 11717425) ;

misc_feature unknown_1059 ; colour 9 ; note S. pombe ARS element (pFL201.2) consensus EMBL:SPARS201 X0789 4 ;

misc_feature unknown_1060 ; colour 4 ; note protected region A (PMID 11717425) ;

tRNA SPA**TRNA**SER.01 ; db_xref EMBL:V01360 ; evidence experimental ; gene SPA**TRNA**SER.01 ||| sup12 ; product **tRNA** Serine ; note **tRNA** Ser anticodon CGA, Cove score 77.58 ;

intron unknown_1061 ; note intron **tRNA** Ser anticodon CGA ;

tRNA SPA**TRNA**MET.01 ; db_xref EMBL:V01360 ; evidence experimental ; gene SPA**TRNA**MET.01 ; product **tRNA** Methionine ; note **tRNA** Met anticodon CAT, Cove score 69.52: EMBL:V01360 ;

mRNA SPAC56F8.13 ; p_c dubious ||| ORF in predicted gene free region ; colour 6 ; gene SPAC56F8.13 ; product dubious ;

mRNA SPAC10F6.06 ; db_xref EMBL:Y13635 ; systematic_id SPAC10F6.06 ;

mRNA SPAC9.09 ; p_c homocysteine methy**LTR**ansferase ||| similar to S. cerevisiae YER091C ; colour 2 ; gene met26 ||| SPAC9.09 ; note accummulation of homocysteine causes a defect in purine biosynthesis (PMID 16436428) ; Alias met26 ; GO GO:0009086; methionine biosynthesis
 GO:0003871; 5-methyltetrahydropteroy**LTR**iglutamate- homocysteine S-methy**LTR**ansferase activity
 GO:0005737; cytoplasm
 GO:0046084; adenine biosynthesis ; primary_name met26 ; product homocysteine methy**LTR**ansferase ; EC_number 2.1.1.14 ;

mRNA SPAC57A7.04c ; p_c **mRNA** export shuttling protein (PMID 12112233) ||| rrm RNA recognition motif (4) ||| non-essential (PMID 12112233) ||| similar to S. cerevisiae YER165W ; colour 2 ; gene SPAC57A7.04c ||| pabp ; note pab1 used previously by SPAC227.07c ; obsolete_name pab1 ; GO GO:0008143; poly(A) binding
 GO:0006406; **mRNA** export from nucleus
 GO:0005737; cytoplasm
 GO:0005634; nucleus ; product **mRNA** export shuttling protein (PMID 12112233) ;

mRNA SPAC1705.03c ; temporary_systematic_id SPAC1705.03c ; note **mRNA** from AU010387 ;

misc_feature unknown_1532 ; note nominal overlap with cosmid SPAC23H4, EM:Z98977 S.pombe chromosome 1 ;

misc_feature unknown_1533 ; note nominal overlap with cosmid SPAC1705, S. pombe chromosome 1 ;

misc_feature unknown_1534 ; note nominal overlap with cosmid SPAC1F2, EM:Z98976 S. pombe chromosome 1 ;

mRNA SPAC1705.03c ; temporary_systematic_id SPAC1705.03c ; note **mRNA** from AU009794 ;

misc_RNA SPNCRNA.80 ; db_xref EMBL:AU012652 ; systematic_id SPNCRNA.80 ; controlled_curation term=non-coding RNA; qualifier=predicted; db_xref=EMBL:AU012652; date=20050412 ||| term=no detectable long open reading frame; qualifier=predicted; db_xref=EMBL:AU012652; date=20050412 ;

gene SPNCRNA.80 ; product non-coding RNA (predicted) ; note **mRNA** from AU012652 1 299 ||| not associated with an ORF ;

mRNA SPAC23H4.06 ; p_c glutamate-ammonia ligase Gln1 ||| similar to S. cerevisiae YPR035W ; colour 2 ; gene gln1 ||| SPAC23H4.06 ; Alias gln1 ; GO GO:0006542; glutamine biosynthesis
 GO:0019740; nitrogen utilization
 GO:0004356; glutamate-ammonia ligase activity
 GO:0005737; cytoplasm ; primary_name gln1 ; product glutamate-ammonia ligase Gln1 ; EC_number 6.3.1.2 ;
mRNA SPAC343.12 ; p_c conserved fungal protein ||| regulated by glucose, ammonium, phosphate, carbon dioxide and temperature (PMID 7565608) ||| predicted N-terminal signal sequence ||| no apparent S. cerevisiae ortholog ||| similar to N. crassa b19a17.210 ; colour 2 ; gene rds1 ||| SPAC343.12 ; Alias rds1 ; GO GO:0006950; response to stress ; primary_name rds1 ; product conserved fungal protein ;

mRNA SPAC664.04c ; colour 7 ;
mRNA SPAC664.05 ; p_c 60S ribosomal protein L13 ||| similar to S. cerevisiae YDL082W and YMR142C ; colour 7 ; gene rpl13 ||| SPAC664.05 ; Alias rpl13 ; GO GO:0003735; structural constituent of ribosome
 GO:0006412; translation
 GO:0005842; cytosolic large ribosomal subunit (sensu Eukaryota) ; primary_name rpl13 ; product 60S ribosomal protein L13 ;

misc_feature unknown_1699 ; colour 8 ; note PS01104 Ribosomal protein L13e signature ;

mRNA SPAC1002.13c ; temporary_systematic_id SPAC1002.13c ; note **mRNA** from AU013582 ;
mRNA SPAC1002.21 ; p_c sequence orphan ; colour 8 ; gene SPAC1002.21 ; product sequence orphan ; note previously annotated as dubious, but has localization signal ;
mRNA SPAC140.02 ; p_c GAR family ||| rrm RNA recognition motif ||| similar to S. cerevisiae YGR159C ||| phosphoprotein (mitosis) (PMID 9211981) ||| NLS binding (predicted) (ISS) ; colour 2 ; gene gar2 ||| SPAC140.02 ; Alias gar2 ; GO GO:0042254; ribosome biogenesis and assembly
 GO:0005730; nucleolus
 GO:0006364; rRNA processing
 GO:0005732; small nucleolar ribonucleoprotein complex
 GO:0003723; RNA binding
 GO:0007000; nucleolus organization and biogenesis ; primary_name gar2 ; product GAR family ;

misc_feature unknown_1907 ; note gene free region ;

misc_feature unknown_1908 ; note slightly palindromic region at the middle of gene free region; causing self match ;

real_mRNA unknown_1909 ; colour 3 ; note **mRNA** from spc09196 104 1 ;
mRNA SPAC23C11.06c ; p_c hydrolase (inferred from context) ||| conserved fungal protein ||| similar to S. cerevisiae YNL115C ||| 5 predicted transmembrane helices ; colour 10 ; gene SPAC23C11.06c ; product hydrolase (inferred from context) ;
mRNA SPAC13F5.03c ; p_c glycerol dehydrogenase (Phlippen, Stevens, Wolf, Zimmermann manuscript in preparation) ||| conserved protein (broad species distribution) ||| predicted N-terminal signal sequence ||| no apparent S. cerevisiae ortholog ; GO GO:0005739; mitochondrion
 GO:0008270; zinc ion binding
 GO:0008888; glycerol dehydrogenase activity
 GO:0019563; glycerol catabolism ; colour 7 ; gene SPAC13F5.03c ; product glycerol dehydrogenase (Phlippen, Stevens, Wolf, Zimmermann manuscript in preparation) ; EC_number 1.1.1.6 ;
mRNA SPAC18G6.09c ; p_c sequence orphan ||| serine-rich protein ; GO GO:0007126; meiosis
 GO:0007126; meiosis ; colour 8 ; gene SPAC18G6.09c ; product sequence orphan ;

mRNA SPAC22H10.06c ; p_c dubious ||| under 100 amino acid threshold ; colour 6 ; gene SPAC22H10.06c ; product dubious ;

mRNA SPAC22H10.13 ; temporary_systematic_id SPAC22H10.13 ; note **mRNA** from AU009741 ;

mRNA SPAC6B12.07c ; p_c ubiquitin-protein ligase E3 (predicted) ||| conserved fungal protein ||| similar to P. anserina Pa5D0011 ||| no apparent S. cerevisiae ortholog ; GO GO:0007186; G-protein coupled receptor protein signaling pathway
 GO:0004842; ubiquitin-protein ligase activity
 GO:0016567; protein ubiquitination
 GO:0008270; zinc ion binding ; colour 7 ; controlled_curation term=ubiquitin-protein ligase E3; date=20060726 ||| term=zinc finger protein; date=20060726 ||| term=zf-C3HC4 type (RING finger); date=20060726 ||| term=SPEX domain protein; date=20060726 ; gene SPAC6B12.07c ; product ubiquitin-protein ligase E3 (predicted) ;

mRNA SPAPB24D3.07c ; p_c sequence orphan ||| predicted N-terminal signal sequence ; colour 8 ; gene SPAPB24D3.07c ; product sequence orphan ;
misc_RNA SPNCRNA.31 ; Alias prl31 ; db_xref PMID:12597277 ||| EMBL:AB084843 ; systematic_id SPNCRNA.31 ; controlled_curation term=non-coding RNA; qualifier=predicted; db_xref=PMID:12597277; date=20050412 ||| term=poly(A)-bearing RNA; qualifier=predicted; db_xref=PMID:12597277; date=20050412 ||| term=no detectable long open reading frame; qualifier=predicted; db_xref=PMID:12597277; date=20050412 ; gene prl31 ; primary_name prl31 ; product non-coding RNA (predicted) ;
mRNA SPAC3G9.11c ; p_c pyruvate decarboxylase (predicted) ||| similar to S. pombe SPAC13A11.06 ||| similar to S. cerevisiae YGR087C and YLR134W and YLR044C and YDL080C ; colour 7 ; gene SPAC3G9.11c ; product pyruvate decarboxylase (predicted) ; EC_number 4.1.1.1 ;

tRNA SPA**TRNA**THR.04 ; gene SPA**TRNA**THR.04 ; product **tRNA** Threonine ; note **tRNA** Thr anticodon CGT, Cove score 76.00 ;

mRNA SPAC3G9.03 ; temporary_systematic_id SPAC3G9.03 ; note **mRNA** from AU009010 293 10 ;

mRNA SPAC3G9.03 ; colour 2 ;
mRNA SPAC6G10.11c ; p_c ribosomal-ubiquitin fusion protein ||| ubiquitin family protein ||| Ribosomal S37 family ||| similar to S. pombe SPAC589.10c (identical) ||| similar to S. cerevisiae YLR167W ; colour 7 ; gene ubi3 ||| SPAC6G10.11c ; Alias ubi3 ; GO GO:0003735; structural constituent of ribosome
 GO:0042254; ribosome biogenesis and assembly
 GO:0005737; cytoplasm
 GO:0000074; regulation of progression through cell cycle ; primary_name ubi3 ; product ribosomal-ubiquitin fusion protein ;

mRNA SPAC3A11.09 ; colour 7 ;
misc_feature unknown_3422 ; note gene free region, SPAC17A2.10 and SPAC17A2.11 were marked in this region, because of their size, but have very low correlation scores, and are in a region of unusual base composition. This region also has GC/AT deviation, GC rich on the top strand for the first 4 kb, then GC poor for the next 4 kb., similar to the composition of the mat mc loci ;
mRNA SPAC17A2.11 ; p_c sequence orphan ; colour 8 ; gene SPAC17A2.11 ; product sequence orphan ; note previously annotated as dubious, but has localization signal ||| see comment on SPAC17A2.10, this region is the same but inverted orientation ||| largish ORF in compositionally biased region, probably not real. has odd translation. this is in region where botton strand is T rich, so lots of LLLFFFFLLFFLSFSFSFS ;

mRNA SPAC8C9.04 ; p_c sequence orphan ; GO GO:0007126; meiosis
 GO:0007126; meiosis ; colour 8 ; gene SPAC8C9.04 ; product sequence orphan ;

mRNA SPAC8C9.14 ; colour 2 ;

repeat_unit unknown_3600 ; colour 2 ; note dhI repeat ;

misc_feature unknown_3601 ; note nominal overlap with SPAP7G5 S. pombe chromosome 1 ;

repeat_unit unknown_3602 ; colour 2 ; note dh1 repeat ;

misc_feature unknown_3604 ; note nominal overlap with plasmid p7G5 ;

repeat_unit unknown_3606 ; colour 1 ; note imr1L ;
repeat_unit unknown_3607 ;
tRNA SPA**TRNA**ALA.04 ; gene SPA**TRNA**ALA.04 ; product **tRNA** Alanine ; note anticodon AGC, Cove score 67.24 ;
tRNA SPA**TRNA**GLU.03 ; gene SPA**TRNA**GLU.03 ; product **tRNA** Glutamic acid ; note **tRNA** Glu anticodon CTC, Cove score 74.15 ;
misc_feature unknown_3608 ;
tRNA SPA**TRNA**ILE.03 ; gene SPA**TRNA**ILE.03 ; product **tRNA** Isoleucine ; note **tRNA** Ile anticodon AAT, Cove score 66.44 ;
repeat_unit unknown_3609 ; colour 4 ; note cnt1 ;
repeat_unit unknown_3610 ; colour 1 ; note imr1R ;
tRNA SPA**TRNA**ILE.04 ; gene SPA**TRNA**ILE.04 ; product **tRNA** Isoleucine ; note **tRNA** Ile anticodon AAT, Cove score 66.44 ;
tRNA SPA**TRNA**GLU.04 ; gene SPA**TRNA**GLU.04 ; product **tRNA** Glutamic acid ; note **tRNA** Glu anticodon CTC, Cove score 74.15 ;
repeat_unit unknown_3611 ; colour 3 ; partial _no_value ; note dg1 repeat ;
tRNA SPA**TRNA**ALA.05 ; gene SPA**TRNA**ALA.05 ; product **tRNA** Alanine ; note **tRNA** Ala anticodon AGC, Cove score 67.24 ;
misc_feature unknown_3612 ; note nominal overlap with SPAC1856 S. pombe chromosome 1 ;
repeat_unit unknown_3615 ; colour 12 ; note region between dg and dh repeat ;
misc_RNA SPNCRNA.95 ; systematic_id SPNCRNA.95 ; controlled_curation term=non-coding RNA; qualifier=predicted; date=20050412 ||| term=no detectable long open reading frame; qualifier=predicted; date=20050412 ; gene SPNCRNA.95 ; product non-coding RNA (predicted) ; note from spc00798 133 368; not attached to CDS ||| possibly spurious, expression low on both strands (pers comm. Jurg Bahler) ;
mRNA SPAC4H3.10c ; Alias pyk1 ; primary_name pyk1 ; gene pyk1 ||| SPAC4H3.10c ; note **mRNA** from AU012784 ;
mRNA SPAC4H3.10c ; Alias pyk1 ; primary_name pyk1 ; gene pyk1 ||| SPAC4H3.10c ; note **mRNA** from AU014480 ;
misc_RNA SPNCRNA.92 ; db_xref EMBL:AU008923 ; systematic_id SPNCRNA.92 ; controlled_curation term=non-coding RNA; qualifier=predicted; db_xref=EMBL:AU008923; date=20050412 ||| term=no detectable long open reading frame; qualifier=predicted; db_xref=EMBL:AU008923; date=20050412 ||| term=low complexity gene free region; qualifier=predicted; db_xref=EMBL:AU008923; date=20050412 ; gene SPNCRNA.92 ; product non-coding RNA (predicted) ; note **mRNA** from AU008923. **mRNA** not associated with an ORF ||| strand altered 19.6.2003, evidence from microarray profile
misc_RNA SPNCRNA.93 ; db_xref EMBL:AU010570 ; systematic_id SPNCRNA.93 ; controlled_curation term=non-coding RNA; qualifier=predicted; db_xref=EMBL:AU010570; date=20050412 ||| term=no detectable long open reading frame; qualifier=predicted; db_xref=EMBL:AU010570; date=20050412 ||| term=low complexity gene free region; qualifier=predicted; db_xref=EMBL:AU010570; date=20050412 ; gene SPNCRNA.93 ; product non-coding RNA (predicted) ; note **mRNA** from AU010570. **mRNA** not associated with an ORF ||| possibly spurious, expression low on both strands (pers comm. Jurg Bahler) ;
misc_feature unknown_3807 ; note The overlapping cosmid c11G7 has an extra base A at this position. This does not appear it the EMBL entry of c11G7, as c11G7 was cut to a nominal overlap ;
mRNA SPAPB15E9.01c ; Alias SPAPB18E9.06c ; p_c glycoprotein (predicted) ||| possibly S. pombe specific ||| localization cell surface (predicted) ||| GPI anchored protein (predicted) (PMID 12845604) ||| predicted N- terminal signal sequence ; colour 12 ; primary_name SPAPB18E9.06c ; gene SPAPB18E9.06c

||| SPAPB15E9.01c ; product glycoprotein (predicted) ;

misc_feature unknown_3863 ; note nominal overlap with pB15E9 S. pombe chromosome 1 ;

LTR unknown_3866 ; note Tf1-type **LTR** ;

LTR unknown_3868 ; note Tf1-type **LTR** ;

misc_feature prl49 ; Alias prl53 ; primary_name prl53 ; gene prl53 ||| prl63 ||| prl49 ; note covered by abundant ESTs; prl53 prl63 and prl49 all map to within this highly transcribed region (PMID 12597277) ;

misc_RNA SPNCRNA.53 ; Alias prl53 ; db_xref PMID:12597277 ||| EMBL:AB084865 ; systematic_id SPNCRNA.53 ; controlled_curation term=non-coding RNA; qualifier=predicted; db_xref=PMID:12597277; date=20050412 ||| term=poly(A)-bearing RNA; qualifier=predicted; db_xref=PMID:12597277; date=20050412 ||| term=no detectable long open reading frame; qualifier=predicted; db_xref=PMID:12597277; date=20050412 ; gene prl53 ; primary_name prl53 ; product non-coding RNA (predicted) ;

misc_RNA SPNCRNA.63 ; systematic_id SPNCRNA.63 ; gene prl63 ; Alias prl63 ; obsolete_name SPNCRNA.49 ; db_xref PMID:12597277 ||| EMBL:AB084875 ||| EMBL:AB084861 ; synonym prl49 ; controlled_curation term=non-coding RNA; qualifier=predicted; db_xref=PMID:12597277; date=20050412 ||| term=poly(A)-bearing RNA; qualifier=predicted; db_xref=PMID:12597277; date=20050412 ||| term=no detectable long open reading frame; qualifier=predicted; db_xref=PMID:12597277; date=20050412 ; primary_name prl63 ; product non-coding RNA (predicted) ;

mRNA SPAC27E2.13 ; p_c dubious ; colour 6 ; controlled_curation term=longest ORF in prl53 (60AA), possibly be protein coding; date=20060721 ; gene SPAC27E2.13 ; product dubious ;

mRNA SPAC27E2.11c ; p_c glycoprotein (predicted) ||| possibly S. pombe specific ||| GPI anchored protein (predicted) (PMID 12845604) (pers. comm. Birgit Eisenhaber) ||| predicted N-terminal signal sequence ; GO GO:0009986; cell surface ; colour 12 ; gene SPAC27E2.11c ; product glycoprotein (predicted) ; note possibly not coding ;

tRNA SPA**TRNA**LEU.04 ; gene SPA**TRNA**LEU.04 ; product **tRNA** Leucine ; note **tRNA** Leu anticodon AAG, Cove score 59.59 ;

mRNA SPAC19G12.09 ; p_c NADH/NADPH dependent indole-3-acetaldehyde reductase AKR3C2 ||| aldo/keto reductase ||| similar to S. cerevisiae YDL124W ; GO GO:0047018; indole-3-acetaldehyde reductase (NADH) activity
 GO:0047019; indole-3-acetaldehyde reductase (NADPH) activity
 GO:0008152; metabolism
 GO:0051268; alpha-keto amide reductase activity
 GO:0051269; alpha-keto ester reductase activity
 GO:0005737; cytoplasm
 GO:0005634; nucleus
 GO:0016652; oxidoreductase activity, acting on NADH or NADPH, NAD or NADP as acceptor ; colour 2 ; gene SPAC19G12.09 ; product NADH/NADPH dependent indole-3-acetaldehyde reductase AKR3C2 ;

mRNA SPAC19G12.10c ; temporary_systematic_id SPAC19G12.10c ; note **mRNA** from spc11281 231 187 ;

repeat_region unknown_3929 ; note duplicated region in c25G10, possible ncRNA? ;

mRNA SPAC26H5.08c ; p_c glucan 1,3-beta-glucosidase Bgl2 ||| glycosyl hydrolase family 17 ||| glycoprotein (predicted) ||| similar to S. cerevisiae YGR282C ; colour 7 ; gene bgl2 ||| SPAC26H5.08c ; Alias bgl2 ; GO GO:0009277; cell wall (sensu Fungi)
 GO:0004338; glucan 1,3-beta-glucosidase activity
 GO:0007047; cell wall organization and biogenesis
 GO:0006076; 1,3-beta-glucan catabolism ; primary_name bgl2 ; product glucan 1,3-beta-glucosidase Bgl2 ; EC_number 3.2.1.58 ;

mRNA SPAC26H5.10c ; temporary_systematic_id SPAC26H5.10c ; note **mRNA** from AU009021 ;

mRNA SPAC1F7.13c ; p_c 60S ribosomal protein L8 ||| similar to S. cerevisiae YFR031C-A and YIL018W ; colour 7 ; gene rpl801 ||| rpl8-1 ||| rpl18 ||| rpk5a ||| rpl2-1 ||| SPAC1F7.13c ||| SPAC21E11.02c ; Alias rpl801 ; GO GO:0003735; structural constituent of ribosome
 GO:0006412; translation
 GO:0005842; cytosolic large ribosomal subunit (sensu Eukaryota)
 GO:0019843; rRNA binding ; primary_name rpl801 ; product 60S ribosomal protein L8 ;
misc_feature SPAC25G10.09c ; gene SPAC25G10.09c ; psu_db_xref PO:phos_site ||| PMID: ; note 31 copies of putative phosphorylation signal G[FML]Q [1-4 X QPMIVA] PQ [MR]T ||| effector Ark1/Prk1 family protein kinase ||| evidence ISS ;
mRNA SPAC29E6.07 ; p_c sequence orphan ; colour 8 ; gene SPAC30.11 ||| SPAC29E6.07 ; note previously annotated as dubious ; Alias SPAC30.11 ; GO GO:0007126; meiosis
 GO:0007126; meiosis
 GO:0009897; external side of plasma membrane ; primary_name SPAC30.11 ; product sequence orphan ;
mRNA SPAC29E6.08 ; colour 2 ;
mRNA SPAC16.02c ; db_xref EMBL:D89163 ; systematic_id SPAC16.02c ;
mRNA SPAC16.05c ; p_c transcription factor Sfp1 (predicted) ||| zinc finger protein ||| zf-C2H2 type ||| similar to S. cerevisiae YLR403W ; colour 7 ; gene sfp1 ||| SPAC16.05c ; Alias sfp1 ; GO GO:0006355; regulation of transcription, DNA- dependent
 GO:0003700; transcription factor activity
 GO:0005634; nucleus
 GO:0005737; cytoplasm
 GO:0006950; response to stress ; primary_name sfp1 ; product transcription factor Sfp1 (predicted) ;
misc_RNA SPNCRNA.99 ; systematic_id SPNCRNA.99 ; controlled_curation term=non-coding RNA; qualifier=predicted; date=20050412 ||| term=no detectable long open reading frame; qualifier=predicted; date=20050412 ; gene SPNCRNA.99 ; product non-coding RNA ; note **mRNA** not associated with an ORF ;
misc_feature unknown_4190 ; note nominal overlap with cosmid SPAC9E9, EM:Z99262 S. pombe chromosome 1 ;
misc_feature unknown_4191 ; note nominal overlap with cosmid SPAC16, EM:AL121745 S. pombe chromosome 1 ;
mRNA SPAC9E9.01 ; p_c sequence orphan ; colour 8 ; gene SPAC9E9.01 ; product sequence orphan ;
mRNA SPAC1093.01 ; Alias SPAC12B10.18 ; p_c PPR repeat protein ||| PPR domains ||| predicted N-terminal signal sequence ||| no apparent orthologs, cannot be distinguished ; colour 10 ; primary_name SPAC12B10.18 ; gene SPAC12B10.18 ||| SPAC1093.01 ; product PPR repeat protein ;
misc_feature unknown_4369 ; note nominal overlap with cosmid SPAC1093, EM:AL132839 S. pombe chromosome 1 ;
misc_feature unknown_4370 ; note nominal overlap with cosmid SPAC12B10, EM:Z70721 S. pombe chromosome 1 ;
mRNA SPAC4F10.14c ; p_c nascent polypeptide-associated complex subunit (predicted) (PMID 8809106) ||| similar to S. cerevisiae YDR252W and YPL037C ; colour 2 ; gene btf3 ||| SPAC4F10.14c ; note not a chaperone in the GO definition sense? check ; Alias btf3 ; synonym egd1 ||| btt1 ; GO GO:0005854; nascent polypeptide-associated complex
 GO:0051083; cotranslational protein folding
 GO:0051082; unfolded protein binding ; primary_name btf3 ; product nascent polypeptide-associated complex subunit (predicted) (PMID 8809106) ;
mRNA SPAC4F10.15c ; p_c WASp homolog ||| disease associated, Wiskott- Aldrich syndrome ||| conserved eukaryotic protein ||| similar to S. cerevisiae YOR181W ; colour 2 ; gene wsp1 ||| SPAC4F10.15c ; curation promoter homolD box ; Alias wsp1 ; GO GO:0030479; actin cortical patch
 GO:0051286; cell tip
 GO:0005826; contractile ring
 GO:0005515; protein binding
 GO:0000147; actin cortical patch assembly
 GO:0000147; actin cortical patch assembly
 GO:0045010; actin nucleation
 GO:0045010; actin nucleation
 GO:0009272; cell wall biosynthesis

(sensu Fungi)
 GO:0030467; establishment and/or maintenance of cell polarity (sensu Fungi)
 GO:0030833; regulation of actin filament polymerization
 GO:0000915; cytokinesis, contractile ring formation
 GO:0000916; cytokinesis, contractile ring contraction ; primary_name wsp1 ; product WASp homolog ;
mRNA SPAC29A4.16 ; p_c halotolerence protein 4 ||| non-essential (PMID 15821139) ||| similar to S. cerevisiae YCR008W ; systematic_id SPAC29A4.16 ; colour 2 ; gene hal4 ||| sat4 ||| ppk10 ||| SPAC29A4.16 ; Alias hal4 ; synonym sat4 ||| ppk10 ; GO GO:0006468; protein amino acid phosphorylation
 GO:0004674; protein serine/threonine kinase activity
 GO:0005524; ATP binding
 GO:0042391; regulation of membrane potential
 GO:0030003; cation homeostasis
 GO:0042493; response to drug
 GO:0043157; response to cation stress
 GO:0005515; protein binding
 GO:0043157; response to cation stress
 GO:0030003; cation homeostasis ; primary_name hal4 ; product halotolerence protein 4 ;
mRNA SPAC29A4.12c ; p_c sequence orphan ; colour 8 ; gene SPAC29A4.12c ; product sequence orphan ; note has transcript profile on microarray ;
mRNA SPAC26F1.06 ; p_c monomeric 2,3-bisphosphoglycerate (BPG)- dependent phosphoglycerate mutase (PGAM) (PMID 8110200) ||| similar to S. cerevisiae YKL152C and YDL021W and YOL056W ; colour 2 ; gene gpm1 ||| SPAC26F1.06 ; Alias gpm1 ; GO GO:0006096; glycolysis
 GO:0005829; cytosol
 GO:0004619; phosphoglycerate mutase activity
 GO:0006094; gluconeogenesis ; primary_name gpm1 ; product monomeric 2,3-bisphosphoglycerate (BPG)- dependent phosphoglycerate mutase (PGAM) (PMID 8110200) ; EC_number 5.4.2.1 ;
mRNA SPAP8A3.04c ; p_c heat shock protein Hsp9 (PMID 8679693) ||| similar to S. cerevisiae YFL014W ; colour 2 ; gene hsp9 ||| scf1 ||| SPAP8A3.04c ; note possible sequencing error, 98 aa version reported (PMID:8654972) ; Alias hsp9 ; GO GO:0000915; cytokinesis, contractile ring formation ; primary_name hsp9 ; product heat shock protein Hsp9 (PMID 8679693) ;
mRNA SPAC922.04 ; temporary_systematic_id SPAC922.04 ; note **mRNA** from AU006605 ;
mRNA SPAC869.02c ; temporary_systematic_id SPAC869.02c ; note **mRNA** from AU010057 ;
LTR unknown_5196 ; note 719 (-1) 44 349 Tf2-type **LTR** ;
misc_RNA SPNCRNA.61 ; Alias prl61 ; db_xref EMBL:AB084873 ||| PMID:12597277 ; systematic_id SPNCRNA.61 ; controlled_curation term=non-coding RNA; qualifier=predicted; db_xref=PMID:12597277; date=20050412 ||| term=antisense to SPAC186.07c; qualifier=predicted; db_xref=PMID:12597277; date=20050412 ||| term=poly(A)-bearing RNA; qualifier=predicted; db_xref=PMID:12597277; date=20050412 ||| term=no detectable long open reading frame; qualifier=predicted; db_xref=PMID:12597277; date=20050412 ; gene prl61 ; primary_name prl61 ; product non-coding RNA (predicted) ;
LTR unknown_5202 ; note 251 (-1) 56 275 Tf2-type **LTR** ;
mRNA SPBPB21E7.04c ; p_c S-adenosylmethionine-dependent methy**LTR**ansferase (predicted) ||| O-methy**LTR**ansferase (predicted) ||| conserved eukaryotic protein ||| no apparent S. cerevisiae ortholog ||| similar to S. pombe SPBC119.03 ; synonym SPAPB21E7.04c ; colour 10 ; gene SPBPB21E7.04c ; product S-adenosylmethionine-dependent methy**LTR**ansferase (predicted) ;
misc_feature unknown_26 ; note possible rearrangement here. Possible N terminal Adenine deaminase fragment ;
mRNA SPBPB21E7.08 ; p_c pseudogene ||| similar to S. cerevisiae YJL213W ; colour 13 ; gene SPBPB21E7.08 ; note 2 frameshifts ; synonym SPAPB21E7.08 ; pseudo _no_value ; product pseudogene ;
mRNA SPBC1198.14c ; p_c fructose-1,6-bisphosphatase Fbp1 (PMID 2157626) ||| similar to S. cerevisiae YLR377C ; colour 2 ; gene fbp1 ||| SPBC1198.14c ||| SPBC660.04c ; curation transcriptional repression occurs by a cAMP signaling pathway (PMID 1849107) ||| transcriptionnally regulated by adenylate cyclase activation by a G protein alpha subunit encoded by Gpa2 (PMID 8001792) ||| transcriptional regulators include two redundant Tup1p-like corepressors and the

CCAAT binding factor activation complex (PMID 11238405) ; Alias fbp1 ; primary_name fbp1 ; product fructose-1,6-bisphosphatase Fbp1 (PMID 2157626) ;

mRNA SPBC660.04c ; gene SPBC660.04c ||| fbp1 ; note Charlie Hofmann, pers comm ;

mRNA unknown_4363 ;
mRNA SPBC660.06 ; p_c conserved fungal protein ||| glycine-rich ||| WW domain ||| similar to S. pombe SPBC11B10.08 and SPBC660.05 ||| similar to S. cerevisiae YFL010C ; colour 10 ; gene SPBC660.06 ; product conserved fungal protein ;

mRNA SPBC800.04c ; temporary_systematic_id SPBC800.04c ; note **mRNA** from spc03862 ;
mRNA SPBC1773.04 ; p_c flavonol reductase/cinnamoyl-CoA reductase family ||| similar to S. pombe SPAC513.07 ||| similar to S. cerevisiae YGL157W and YOL151W and YGL039W and YDR541C ; colour 10 ; gene SPBC1773.04 ; product flavonol reductase/cinnamoyl-CoA reductase family ;

tRNA SPB**TRNA**GLY.05 ; gene SPB**TRNA**GLY.05 ; product **tRNA** Glycine ; note **tRNA** Gly anticodon TCC, Cove score 60.52 ;

tRNA SPB**TRNA**ARG.04 ; gene SPB**TRNA**ARG.04 ; product **tRNA** Arginine ; note **tRNA** Arg anticodon TCT, Cove score 69.29 ;
mRNA SPBC1271.03c ; p_c phosphoprotein phosphatase ||| NLI interacting factor family ||| non-essential (PMID 12618370) ||| similar to S. cerevisiae YLL010C and YLR019W ||| similar to S. pombe SPAC2F7.02c ; GO GO:0004721; phosphoprotein phosphatase activity
 GO:0005886; plasma membrane
 GO:0006470; protein amino acid dephosphorylation
 GO:0006950; response to stress ; colour 7 ; gene SPBC1271.03c ; product phosphoprotein phosphatase ;
mRNA SPBC1685.09 ; p_c 40S ribosomal protein S29 ||| non-essential (PMID 12618370) ||| similar to S. cerevisiae YLR388W and YDL061C ; colour 2 ; gene rps29 ||| SPBC1685.09 ; Alias rps29 ; GO GO:0005843; cytosolic small ribosomal subunit (sensu Eukaryota)
 GO:0006412; translation
 GO:0003735; structural constituent of ribosome ; primary_name rps29 ; product 40S ribosomal protein S29 ;

mRNA SPBC1685.12c ; p_c dubious ||| ORF in compositionally biased region ; colour 6 ; gene SPBC1685.12c ; product dubious ;
mRNA SPBC1685.13 ; p_c non classical export pathway protein (predicted) ||| predicted N-terminal signal sequence ||| 3 predicted transmembrane helices ||| non-essential (PMID 12618370) ||| similar to S. cerevisiae YPR149W and YGR131W ; GO GO:0005737; cytoplasm
 GO:0005739; mitochondrion
 GO:0009306; protein secretion
 GO:0005783; endoplasmic reticulum ; colour 7 ; gene SPBC1685.13 ; product non classical export pathway protein (predicted) ;

misc_feature unknown_414 ; note gt repeat region similar to human/mouse repeated reg ion ;
mRNA SPBC649.04 ; p_c UV-induced protein Uvi15 ||| fibrillarin binds to a 3' cis-regulatory element in pre-**mRNA** of Uvi15 (PMID 1207460) ||| non-essential (PMID 12618370) ||| similar to S. cerevisiae YDL012C and YDR210W and YBR016W ; colour 2 ; gene uvi15 ||| SPBC649.04 ; Alias uvi15 ; GO GO:0009408; response to heat
 GO:0006974; response to DNA damage stimulus
 GO:0007584; response to nutrient ; primary_name uvi15 ; product UV-induced protein Uvi15 ;
mRNA SPBC649.05 ; p_c spindle pole body protein Cut12 (PMID 9531532) ||| essential (PMID 12618370) ||| activator of Plo1p (PMID 12815070) ||| no apparent orthologs ; colour 2 ; gene cut12 ||| stf1 ||| SPBC649.05 ; Alias cut12 ; GO GO:0007088; regulation of mitosis
 GO:0005816; spindle pole body
 GO:0051227; mitotic spindle assembly
 GO:0007094; mitotic spindle checkpoint
 GO:0005515; protein binding ; primary_name cut12 ; product spindle pole body protein Cut12 (PMID 9531532) ;
mRNA SPBC354.12 ; p_c glyceraldehyde 3-phosphate dehydrogenase Gpd3 ||| similar to S. cerevisiae YJL052W and YGR192C and YJR009C ; colour 2 ; gene gpd3 ||| SPBC354.12 ; Alias gpd3 ; GO GO:0006096; glycolysis
 GO:0004365; glyceraldehyde-3-phosphate dehydrogenase (phosphorylating)

activity ; primary_name gpd3 ; product glyceraldehyde 3-phosphate dehydrogenase Gpd3 ; EC_number 1.2.1.12 ;

mRNA SPBC839.15c ; temporary_systematic_id SPBC839.15c ; note **mRNA** from AU008702 ;

mRNA SPBC119.02 ; colour 2 ;
mRNA SPBC119.03 ; p_c S-adenosylmethionine-dependent methy**LTR**ansferase (predicted) ||| O-methy**LTR**ansferase (predicted) ||| conserved eukaryotic protein ||| no apparent S. cerevisiae ortholog ||| similar to S. pombe SPBPB21E7.03c (paralog) ; colour 10 ; gene SPBC119.03 ; product S-adenosylmethionine-dependent methy**LTR**ansferase (predicted) ;
mRNA SPBC119.05c ; obsolete_name csh3 ; p_c Wiskott-Aldrich syndrome homolog binding protein Lsb1 (predicted) ||| src (SH3) homology domain ||| similar to S. cerevisiae YGR136W and YPR154W ; colour 7 ; gene SPBC119.05c ; product Wiskott-Aldrich syndrome homolog binding protein Lsb1 (predicted) ;
mRNA SPBC36.03c ; p_c spermidine family transporter (predicted) ||| MFS family membrane transporter ||| similar to S. pombe SPBC530.15c and SPBC36.01C and SPCC569.05c and SPBC36.02C and SPBC947.06 ||| similar to S. cerevisiae YLL028W ||| 12 predicted transmembrane helices ||| tandem duplication ; GO GO:0015606; spermidine transporter activity
 GO:0000297; spermine transporter activity
 GO:0015846; polyamine transport
 GO:0005886; plasma membrane
 GO:0015848; spermidine transport
 GO:0000296; spermine transport ; colour 7 ; gene SPBC36.03c ; product spermidine family transporter (predicted) ;

misc_feature unknown_1099 ; note nominal overlap with cosmid SPBC3D6, EM:Z95620 S.pombe chromosome 2 ;
mRNA SPBC31A8.02 ; p_c pseudo ; colour 13 ; gene SPBC3D6.01 ||| SPBC31A8.02 ; note previously annotated as very hypothetical protein but has no met so assuming dubious or pseudo ; Alias SPBC3D6.01 ; pseudo _no_value ; primary_name SPBC3D6.01 ; product pseudo ;
mRNA SPBC3D6.02 ; p_c neddylation pathway protein But2 (PMID 14623327) ||| But2 family protein ||| possibly S. pombe specific ||| predicted N- terminal signal sequence ||| similar to S. pombe SPAC27D7.09c and SPAC27D7.10c and SPAC27D7.11c (paralogs) ; colour 2 ; gene but2 ||| SPBC3D6.02 ; Alias but2 ; GO GO:0005515; protein binding
 GO:0005515; protein binding ; primary_name but2 ; product neddylation pathway protein But2 (PMID 14623327) ;
misc_feature unknown_1138 ; note dicrepancy: with published U14 small nuclear RNA gen e and with cosmid c1268 sequence - additional T residue insertion at base 9128 ;
snoRNA SP**SNORNA**.21 ; Alias snoU14 ; db_xref RFAM:RF00016 ; systematic_id SP**SNORNA**.21 ; gene snoU14 ||| SP**SNORNA**.21 ; primary_name snoU14 ; product small nucleolar RNA U14 ;
mRNA SPBC8D2.04 ; p_c histone H3 ||| histone fold ||| similar to S. pombe hht1 and hht3 ||| amino terminus K9, K14, S8 are involved in centomeric silencing (PMID 14561399) ||| similar to S. cerevisiae YBR010W and YNL031C ; colour 2 ; gene hht2 ||| h3.2 ||| SPBC8D2.04 ; Alias hht2 ; GO GO:0030702; chromatin silencing at centromere
 GO:0003677; DNA binding
 GO:0006333; chromatin assembly or disassembly
 GO:0000788; nuclear nucleosome ; primary_name hht2 ; product histone H3 ;
mRNA SPBC8D2.18c ; p_c adenosylhomocysteinase (predicted) ||| similar to S. cerevisiae YER043C ; GO GO:0004013; adenosylhomocysteinase activity
 GO:0006555; methionine metabolism
 GO:0016259; selenocysteine metabolism ; colour 7 ; gene SPBC8D2.18c ; product adenosylhomocysteinase (predicted) ; EC_number 3.3.1.1 ; note This gene is not the gene described in Abe and Shimoda (2000), Genetics 154 1497-1508. They describe it as ORF SPBC8D2.18c, and as a kinase homologous to S. cerevisiae IME2 and S. pombe pit1.mde3 but the gene described in this paper

corresponds to SPC8D2.19 ;

mRNA SPBC32H8.12c ; Alias act1 ; obsolete_name pi012 ; p_c actin (PMID 10547441) ||| essential (PMID 1524835) ||| similar to S. cerevisiae YFL039C ; GO GO:0005884; actin filament
 GO:0000915; cytokinesis, contractile ring formation
 GO:0030479; actin cortical patch
 GO:0030467; establishment and/or maintenance of cell polarity (sensu Fungi)
 GO:0030036; actin cytoskeleton organization and biogenesis
 GO:0005826; contractile ring
 GO:0009272; cell wall biosynthesis (sensu Fungi)
 GO:0030100; regulation of endocytosis
 GO:0005628; forespore membrane ; colour 2 ; primary_name act1 ; gene act1 ||| cps8 ||| SPBC32H8.12c ; product actin (PMID 10547441) ;

mRNA SPBC11B10.07c ; colour 7 ;

misc_RNA SPNCRNA.24 ; Alias prl24 ; db_xref PMID:12597277 ||| EMBL:AB084836 ; systematic_id SPNCRNA.24 ; controlled_curation term=non-coding RNA; qualifier=predicted; db_xref=PMID:12597277; date=20050412 ||| term=poly(A)-bearing RNA; qualifier=predicted; db_xref=PMID:12597277; date= 20050412 ||| term=no detectable long open reading frame; qualifier=predicted; db_xref=PMID:12597277; date=20050412 ; gene prl24 ; primary_name prl24 ; product non-coding RNA (predicted) ;

mRNA SPBC11B10.08 ; obsolete_name pi003 ||| SPACTOKYO_453.33c ; p_c conserved fungal protein ||| similar to S. cerevisiae YFL010C ||| WW domain ; colour 10 ; gene SPBC11B10.08 ; product conserved fungal protein ;

mRNA SPBC29B5.03c ; Alias rpl26 ; primary_name rpl26 ; gene rpl26 ||| SPBC29B5.03c ; note **mRNA** from AU008061 ;

mRNA SPBC28F2.03 ; p_c cyclophilin family peptidyl-prolyl cis-trans isomerase ||| cyclophilin ||| similar to S. cerevisiae YDR155C ; colour 2 ; gene ppi1 ||| cyp2 ||| SPBC28F2.03 ; note cyp2 in PMID 11690648 is SPAC57A10.03 cyp1 ; Alias ppi1 ; primary_name ppi1 ; product cyclophilin family peptidyl-prolyl cis-trans isomerase ; EC_number 5.2.1.8 ;

tRNA SPB**TRNA**TYR.02 ; gene SPB**TRNA**TYR.02 ; product **tRNA** Tyrosine ; note **tRNA** Tyr anticodon GTA, Cove score 70.26 ;

tRNA SPB**TRNA**LEU.06 ; gene SPB**TRNA**LEU.06 ; product **tRNA** Leucine ; note **tRNA** Leu anticodon CAA, Cove score 56.07 ;

tRNA SPB**TRNA**GLY.07 ; gene SPB**TRNA**GLY.07 ; product **tRNA** Glycine ; note **tRNA** Gly anticodon GCC, Cove score 72.94 ;

tRNA SPB**TRNA**LYS.07 ; gene SPB**TRNA**LYS.07 ; product **tRNA** Lysine ; note **tRNA** Lys anticodon CTT, Cove score 74.83 ;

tRNA SPB**TRNA**ILE.05 ; gene SPB**TRNA**ILE.05 ; product **tRNA** Isoleucine ; note **tRNA** Ile anticodon AAT, Cove score 66.44 ;

tRNA SPB**TRNA**ALA.08 ; gene SPB**TRNA**ALA.08 ; product **tRNA** Alanine ; note **tRNA** Ala anticodon AGC, Cove score 67.24 ;

tRNA SPB**TRNA**VAL.05 ; gene SPB**TRNA**VAL.05 ; product **tRNA** Valine ; note **tRNA** Val anticodon AAC, Cove score 64.30 ;

tRNA SPB**TRNA**GLU.06 ; gene SPB**TRNA**GLU.06 ; product **tRNA** Glutamic acid ; note **tRNA** Glu anticodon TTC, Cove score 62.18 ;

tRNA SPB**TRNA**ARG.06 ; gene SPB**TRNA**ARG.06 ; product **tRNA** Arginine ; note **tRNA** Arg anticodon ACG, Cove score 75.46 ;

repeat_unit unknown_1440 ; colour 1 ; note similar to IMR repeat, not marked on Nature publication map, added May 2002 VW ;

tRNA SPB**TRNA**ASP.03 ; gene SPB**TRNA**ASP.03 ; product **tRNA** Aspartic acid ; note **tRNA** Asp anticodon GTC, Cove score 70.49 ;

repeat_unit unknown_1442 ; colour 3 ; note dgII repeat ;

repeat_unit unknown_1443 ; colour 7 ; note cen253 Yeast centromere CEN2 repetitive DNA PSS253, between dhIIa and dgIIa ;

repeat_unit unknown_1444 ; colour 2 ; note dhII repeat partial ;

conflict unknown_1446 ; citation [1] ; note In the consensus sequence pB36C4.con between positions 1668 and 1687 (inclusive) there are 10 copies of a TA repeat. This number of repeats was seen in 4 x pUC subclones, in 1 x pUC subclone there were 13 copies ;
repeat_unit unknown_1450 ; colour 4 ; note cnt2 ;
misc_feature unknown_1451 ; note nominal overlap with SPBC633 S. pombe chromosome 2 ;
repeat_unit unknown_1452 ; colour 4 ; note cnt2 ;
repeat_unit unknown_1453 ; colour 1 ; note imr2L ;
tRNA SPB**TRNA**VAL.07 ; gene SPB**TRNA**VAL.07 ; product **tRNA** Valine ; note **tRNA** Val anticodon AAC, Cove score 64.30 ;
tRNA SPB**TRNA**ALA.10 ; gene SPB**TRNA**ALA.10 ; product **tRNA** Alanine ; note **tRNA** Ala anticodon AGC, Cove score 67.24 ;
tRNA SPB**TRNA**ILE.07 ; gene SPB**TRNA**ILE.07 ; product **tRNA** Isoleucine ; note **tRNA** Ile anticodon AAT, Cove score 66.44 ;
repeat_unit unknown_1458 ; colour 12 ; note cen253 Yeast centromere CEN2 repetitive DNA PSS253, between dhIIa and dgIIa ;
repeat_unit unknown_1459 ; colour 3 ; note dgII repeat ;
tRNA SPB**TRNA**TYR.03 ; gene SPB**TRNA**TYR.03 ; product **tRNA** Tyrosine ; note **tRNA** Tyr anticodon GTA, Cove score 70.26 ;
tRNA SPB**TRNA**LEU.07 ; gene SPB**TRNA**LEU.07 ; product **tRNA** Leucine ; note **tRNA** Leu anticodon CAA, Cove score 56.07 ;
tRNA SPB**TRNA**GLY.08 ; gene SPB**TRNA**GLY.08 ; product **tRNA** Glycine ; note **tRNA** Gly anticodon GCC, Cove score 72.94 ;
tRNA SPB**TRNA**LYS.08 ; gene SPB**TRNA**LYS.08 ; product **tRNA** Lysine ; note **tRNA** Lys anticodon CTT, Cove score 74.83 ;
tRNA SPB**TRNA**ILE.08 ; gene SPB**TRNA**ILE.08 ; product **tRNA** Isoleucine ; note **tRNA** Ile anticodon AAT, Cove score 66.44 ;
tRNA SPB**TRNA**ALA.11 ; gene SPB**TRNA**ALA.11 ; product **tRNA** Alanine ; note **tRNA** Ala anticodon AGC, Cove score 67.24 ;
tRNA SPB**TRNA**VAL.08 ; gene SPB**TRNA**VAL.08 ; product **tRNA** Valine ; note **tRNA** Val anticodon AAC, Cove score 64.30 ;
tRNA SPB**TRNA**GLU.07 ; gene SPB**TRNA**GLU.07 ; product **tRNA** Glutamic acid ; note **tRNA** Glu anticodon TTC, Cove score 62.18 ;
tRNA SPB**TRNA**ARG.07 ; gene SPB**TRNA**ARG.07 ; product **tRNA** Arginine ; note **tRNA** Arg anticodon ACG, Cove score 75.46 ;
mRNA SPBC21B10.13c ; p_c transcription factor (predicted) ||| homeobox domain ||| no apparent orthologs, cannot be distinguished ; GO GO:0005634; nucleus
 GO:0003700; transcription factor activity
 GO:0003677; DNA binding
 GO:0006355; regulation of transcription, DNA- dependent ; colour 7 ; gene SPBC21B10.13c ; product transcription factor (predicted) ;
mRNA SPBC21B10.12 ; p_c meiotic recombination protein Rec6 ||| no apparent orthologs ; colour 2 ; gene rec6 ||| SPBC21B10.12 ; Alias rec6 ; GO GO:0007131; meiotic recombination ; primary_name rec6 ; product meiotic recombination protein Rec6 ;
mRNA SPBC19C2.07 ; temporary_systematic_id SPBC19C2.07 ; note **mRNA** from AU011079 ;
mRNA SPBC2F12.04 ; Alias rpl1701 ; obsolete_name SPCC2F12.04 ; p_c 60S ribosomal protein L17 ||| similar to S. cerevisiae YKL180W and YJL177W ||| similar to S. pombe rpl1702 ; GO GO:0005842; cytosolic large ribosomal subunit (sensu Eukaryota)
 GO:0006412; translation
 GO:0003735; structural constituent of ribosome ; colour 2 ; primary_name rpl1701 ; gene rpl1701 ||| rpl17 ||| rpl17-1 ||| SPBC2F12.04 ; product 60S ribosomal protein L17 ;
mRNA SPBC16E9.16c ; p_c sequence orphan ; colour 8 ; gene SPBC16E9.16c ; product sequence orphan ; note was previously annotated as pseudo however has peptide fragments two peptides in our mass spec analysis (pers. comm. Dieter Wolf), and is constantly expressed under stress conditions from microarray

data; updated gene prediction to give valid translation but splice consensus isn't great for either intron 8-11-05 ;

mRNA SPBC1E8.05 ; p_c conserved fungal protein ||| no apparent S. cerevisiae ortholog ; GO GO:0009986; cell surface ; colour 10 ; controlled_curation term =glycoprotein; qualifier=predicted; date=20061206 ||| term=serine-rich protein; date=20061206 ||| term=GPI anchored protein; qualifier=RCA; db_xref =PMID:12845604; date=20061206 ||| term=predicted N-terminal signal sequence; date=20061206 ; gene SPBC1E8.05 ; product conserved fungal protein ;
mRNA SPBC29A3.04 ; p_c 60S ribosomal protein L7a (L8) ||| similar to S. cerevisiae YHL033C and YLL045C ; colour 2 ; gene rpl8 ||| SPBC29A3.04 ; note named incorrectly, updated 20051010 ; Alias rpl8 ; obsolete_name rpl701 ||| rpl7a ; GO GO:0005842; cytosolic large ribosomal subunit (sensu Eukaryota)
 GO:0006412; translation
 GO:0003735; structural constituent of ribosome ; primary_name rpl8 ; product 60S ribosomal protein L7a (L8) ;

real_mRNA unknown_1783 ; colour 3 ; note **mRNA** from AU007686 117 4 ;

repeat_region unknown_1954 ; note (taacc)8 ;
mRNA SPBC1815.01 ; p_c enolase ||| similar to S. cerevisiae YGR254W and YHR174W and YOR393W and YPL281C and YMR323W ||| similar to S. pombe eno102 (paralog) ; colour 2 ; gene eno101 ||| eno1 ||| SPBC1815.01 ; Alias eno101 ; primary_name eno101 ; product enolase ; EC_number 4.2.1.11 ;
mRNA SPBC19G7.06 ; p_c MADS-box transcription factor Mbx1 ||| MADS-box ||| similar to S. pombe SPAC11E3.06 ||| similar to S. cerevisiae YMR043W and YMR042W ||| not required for periodic transcription in M phase ||| PBF transcription factor complex (PMID 15509866) ||| possibly functional ortholog of YMR043W (PMID 15509866) ; systematic_id SPBC19G7.06 ; colour 2 ; gene mbx1 ||| SPBC19G7.06 ; note Mads BoX protrein 1 ; Alias mbx1 ; GO GO:0045896; regulation of transcription, mitotic
 GO:0000086; G2/M transition of mitotic cell cycle
 GO:0000910; cytokinesis
 GO:0005667; transcription factor complex
 GO:0003702; RNA polymerase II transcription factor activity
 GO:0003677; DNA binding ; controlled_curation term=phosphorylated; cv=[pt_mod; db_xref=PMID:15509866; date=20061127 ; primary_name mbx1 ; product MADS-box transcription factor Mbx1 ;
mRNA SPBC21D10.12 ; p_c BAR adaptor protein Hob1 ||| GTPase-binding (predicted) ||| functionally complemented by human BIN1 (PMID 12569356) ||| similar to S. cerevisiae YDR388W ; colour 2 ; gene hob1 ||| SPBC21D10.12 ; Alias hob1 ; controlled_curation term=BAR domain; cv=protein_family; date= 20060913 ||| term=src (SH3) homology domain; cv=protein_family; date=20060913 ||| term=non-essential; db_xref=PMID:12569356; date=20060913 ; GO GO:0030479; actin cortical patch
 GO:0008092; cytoskeletal protein binding
 GO:0006950; response to stress
 GO:0000074; regulation of progression through cell cycle
 GO:0005515; protein binding
 GO:0005515; protein binding
 GO:0030467; establishment and/or maintenance of cell polarity (sensu Fungi)
 GO:0051285; cell cortex of cell tip
 GO:0031097; medial ring ; primary_name hob1 ; product BAR adaptor protein Hob1 ;

mRNA SPBC29A10.08 ; temporary_systematic_id SPBC29A10.08 ; note **mRNA** from SPD134 ;

mRNA SPBC32F12.11 ; db_xref EMBL:X85332 ; systematic_id SPBC32F12.11 ;

mRNA SPBC19C7.04c ; p_c conserved fungal protein ||| similar to S. cerevisiae YMR295C ; colour 10 ; gene SPBC19C7.04c ; product conserved fungal protein ;
misc_RNA SPNCRNA.26 ; Alias prl26 ; db_xref PMID:12597277 ||| EMBL:AB084838 ; systematic_id SPNCRNA.26 ; controlled_curation term=non-coding RNA; qualifier=predicted; db_xref=PMID:12597277; date=20050412 ||| term=poly(A)-bearing RNA; qualifier=predicted; db_xref=PMID:12597277; date= 20050412 ||| term=no detectable long open reading frame; qualifier=predicted; db_xref=PMID:12597277; date=20050412 ||| term=low complexity gene free region; qualifier=predicted; date=20050412 ; gene prl26 ; primary_name prl26 ; product non-coding RNA (predicted) ;

snRNA SP**SNRNA**.01 ; Alias snu1 ; db_xref RFAM:RF00003 ; systematic_id SP**SNRNA**.01 ; gene snu1 ||| SP**SNRNA**.01 ; primary_name snu1 ; product small

nuclear RNA U1 ;

mRNA SPBC13E7.09 ; p_c verprolin ||| proline-rich protein ||| similar to S. cerevisiae YLR337C ||| conserved eukaryotic protein ||| Wiskott- Aldrich homolog ||| WH2 motif ; colour 2 ; gene vrp1 ||| SPBC13E7.09 ; Alias vrp1 ; controlled_curation term=disease associated, Wiskott- Aldrich syndrome; date=20060809 ; GO GO:0007015; actin filament organization
 GO:0030479; actin cortical patch
 GO:0051286; cell tip
 GO:0006897; endocytosis
 GO:0003779; actin binding
 GO:0005515; protein binding
 GO:0031097; medial ring
 GO:0045010; actin nucleation
 GO:0045010; actin nucleation
 GO:0045010; actin nucleation
 GO:0000147; actin cortical patch assembly ; primary_name vrp1 ; product verprolin ;
snRNA SP**SNRNA**.05 ; Alias snu5 ; db_xref RFAM:RF00020 ; systematic_id SP**SNRNA**.05 ; gene snu5 ||| SP**SNRNA**.05 ; primary_name snu5 ; product small nuclear RNA U5 ;

mRNA SPBP8B7.16c ; temporary_systematic_id SPBP8B7.16c ; note **mRNA** from SPP68DBP ;

tRNA SPB**TRNA**LYS.09 ; gene SPB**TRNA**LYS.09 ; product **tRNA** Lysine ; note **tRNA** Lys anticodon CTT ;

tRNA SPB**TRNA**TYR.04 ; gene SPB**TRNA**TYR.04 ; product **tRNA** Tyrosine ; note **tRNA** Tyr anticodon GTA ;

tRNA SPB**TRNA**ASP.04 ; gene SPB**TRNA**ASP.04 ; product **tRNA** Aspartic acid ; note **tRNA** Asp anticodon GTC, Cove score 70.49 ;
mRNA SPBC26H8.06 ; p_c glutaredoxin Grx4 ||| glutaredoxin ||| monothiol glutaredoxin ||| PICOT domain ||| essential (PMID 15796926) ||| similar to S. cerevisiae YDR098C and YER174C ; colour 2 ; gene grx4 ||| SPBC26H8.06 ; Alias grx4 ; GO GO:0008794; arsenate reductase (glutaredoxin) activity
 GO:0030508; thiol-disulfide exchange intermediate activity
 GO:0005634; nucleus
 GO:0005737; cytoplasm
 GO:0006979; response to oxidative stress ; primary_name grx4 ; product glutaredoxin Grx4 ;
mRNA SPBC26H8.10 ; p_c 3'-5' exoribonuclease subunit Dis3 (predicted) ||| essential (PMID 1944266) ||| RNB domain ||| similar to S. cerevisiae YOL021C ; colour 2 ; gene dis3 ||| SPBC26H8.10 ; note does not bind GeneDB_Spombe:SPBC776.02c PMID 1944266 ; Alias dis3 ; GO GO:0000176; nuclear exosome (RNase complex)
 GO:0005739; mitochondrion
 GO:0000177; cytoplasmic exosome (RNase complex)
 GO:0006365; 35S primary transcript processing
 GO:0006402; **mRNA** catabolism
 GO:0000175; 3'-5'-exoribonuclease activity
 GO:0031125; rRNA 3'-end processing
 GO:0005730; nucleolus
 GO:0000070; mitotic sister chromatid segregation
 GO:0005634; nucleus
 GO:0005515; protein binding
 GO:0007346; regulation of progression through mitotic cell cycle
 GO:0005515; protein binding
 GO:0005515; protein binding
 GO:0008536; Ran GTPase binding
 GO:0016075; rRNA catabolism ; primary_name dis3 ; product 3'-5' exoribonuclease subunit Dis3 (predicted) ;

misc_feature unknown_3872 ; colour 8 ; note PS01175 Ribonuclease II family signature ;
mRNA SPBC26H8.11c ; p_c conserved fungal protein ||| similar to S. pombe SPAPB2B4.06 (paralog) ||| similar to S. cerevisiae YBL095W ; GO GO:0005739; mitochondrion ; colour 10 ; gene SPBC26H8.11c ; product conserved fungal protein ;

misc_feature unknown_3873 ; note nominal overlap with cosmid SPBC26H8, EM:AL031743 S. pombe chromosome 2 ;

misc_feature unknown_3874 ; note nominal overlap with cosmid SPBC604, EM:AL132720 S. pombe chromosome 2 ;
mRNA SPBC32C12.02 ; p_c transcription factor Ste11 ||| HMG box ||| NLS ||| conserved fungal protein ||| no apparent S. cerevisiae ortholog ||| similar to P. Carinii STE11 ; colour 2 ; gene ste11 ||| aff1 ||| stex ||| SPBC32C12.02 ; note the equivalent function in S. cerevisiae is performed by STE12, but this is STE-like not HMG ; Alias ste11 ; GO GO:0000747; conjugation with cellular fusion
 GO:0003700; transcription factor activity
 GO:0051039; positive regulation of transcription, meiotic
 GO:0005634; nucleus ; controlled_curation term=transcriptionally regulates Mei2; date=20060818 ; primary_name ste11 ; product transcription factor Ste11 ;

mRNA SPBC215.05 ; temporary_systematic_id SPBC215.05 ; note **mRNA** from AU013069 ;

misc_feature unknown_3957 ; note dinucleotide repeat GT ;

mRNA SPBC14F5.04c ; p_c phosphoglycerate kinase ||| similar to S. cerevisiae YCR012W ; colour 7 ; gene pgk1 ||| SPBC14F5.04c ; Alias pgk1 ; GO GO:0005829; cytosol
 GO:0006094; gluconeogenesis
 GO:0006096; glycolysis
 GO:0004618; phosphoglycerate kinase activity
 GO:0005524; ATP binding ; controlled_curation term=monomer; date=20060106 ; primary_name pgk1 ; product phosphoglycerate kinase ; EC_number 2.7.2.3 ;

real_mRNA unknown_4327 ; note **mRNA** from AU012671 ;

mRNA SPBC8E4.02c ; p_c sequence orphan ; colour 8 ; gene SPBC8E4.02c ; product sequence orphan ;

mRNA SPBP4G3.03 ; p_c PI31 proteasome regulator related ||| no apparent orthologs, S. pombe variant ||| related to S. pombe SPAC15E1.10 ; colour 10 ; gene SPBP4G3.03 ; product PI31 proteasome regulator related ;

mRNA SPBCPT2R1.08c ; db_xref EMBL:BK005597 ; gene SPBCPT2R1.08c ;

repeat_region unknown_4361 ; note duplicated region in c212 ;

tRNA SPC**TRNA**HIS.03 ; gene SPC**TRNA**HIS.03 ; product **tRNA** Histidine ; note **tRNA** His anticodon GTG ;

mRNA SPCC613.05c ; p_c 60S ribosomal protein L35 ||| similar to S. cerevisiae YDL191W and YDL136W ; colour 2 ; gene rpl35 ||| SPCC613.05c ; Alias rpl35 ; GO GO:0005842; cytosolic large ribosomal subunit (sensu Eukaryota)
 GO:0006412; translation
 GO:0003735; structural constituent of ribosome ; primary_name rpl35 ; product 60S ribosomal protein L35 ;

mRNA SPCC1235.01 ; p_c glycoprotein (predicted) ||| sequence orphan ; colour 8 ; gene SPCC320.02c ||| SPCC1235.01 ; note ~37 copies of a 7-10 repeat consensus 'PMEEITTMTI' and a S/N rich C terminal region ; Alias SPCC320.02c ; primary_name SPCC320.02c ; product glycoprotein (predicted) ;

mRNA SPCC1235.14 ; Alias ght5 ; primary_name ght5 ; gene ght5 ||| SPCC1235.14 ; note **mRNA** from AF017180 ;

mRNA SPCC548.05c ; temporary_systematic_id SPCC548.05c ; note **mRNA** from spc10274 ;

mRNA SPCC548.06c ; temporary_systematic_id SPCC548.06c ; note **mRNA** from spc05276 ;

misc_feature unknown_293 ; note low complexity gene free region ;

mRNA SPCC736.15 ; temporary_systematic_id SPCC736.15 ; note **mRNA** from AU012604 ;

misc_feature unknown_294 ; note low-complexity gene-free region ;

mRNA SPCC594.01 ; colour 10 ;

mRNA SPCC594.03 ; p_c dubious ; colour 6 ; gene SPCC594.03 ; product dubious ;

mRNA SPCC962.06c ; p_c zinc finger protein ||| zf-CCHC type (zinc knuckle) ||| KH domain ||| similar to S. cerevisiae YLR116W ; colour 2 ; gene bpb1 ||| sf1 ||| SPCC962.06c ; Alias bpb1 ; GO GO:0000243; commitment complex
 GO:0000356; U2-type catalytic spliceosome formation for first transesterification step
 GO:0003723; RNA binding ; primary_name bpb1 ; product zinc finger protein ;

mRNA SPCC1672.02c ; p_c switch-activating protein Sap1 ||| essential (PMID 8114737) ||| N-terminal DNA-binding domain (PMID 8065904) ||| C- terminal dimerization domain (PMID 8065904) ||| no apparent orthologs ; colour 2 ; gene sap1 ||| SPCC1672.02c ; Alias sap1 ; GO GO:0000790; nuclear chromatin
 GO:0008301; DNA bending activity
 GO:0003677; DNA binding
 GO:0003677; DNA binding
 GO:0042803; protein

homodimerization activity
 GO:0043110; rDNA spacer replication fork barrier binding
 GO:0043110; rDNA spacer replication fork barrier binding
 GO:0007001; chromosome organization and biogenesis (sensu Eukaryota)
 GO:0007059; chromosome segregation
 GO:0000728; gene conversion at mating-type locus, DNA double-strand break formation
 GO:0031582; replication fork blocking at rDNA repeats
 GO:0031582; replication fork blocking at rDNA repeats ; primary_name sap1 ; product switch-activating protein Sap1 ;
mRNA SPCC1393.08 ; p_c transcription factor (predicted) ||| zinc finger protein ||| zf-GATA type ||| no apparent orthologs, cannot be distinguished ; colour 7 ; gene SPCC1393.08 ; product transcription factor (predicted) ;
mRNA SPCC63.13 ; p_c DNAJ domain protein ||| no apparent orthologs, cannot be distinguished ||| 1 predicted transmembrane helix ; GO GO:0030544; Hsp70 protein binding ; colour 10 ; gene SPCC63.13 ; product DNAJ domain protein ; note YMR161W same domain organization and single predicted tmm helix ;
mRNA SPCC63.14 ; p_c conserved fungal protein ||| coiled-coil (region) (predicted) ||| similar to S. cerevisiae YMR031C and YKL050C ; colour 10 ; gene SPCC63.14 ; product conserved fungal protein ;
mRNA SPCC24B10.21 ; p_c triosephosphate isomerase ||| similar to S. cerevisiae YDR050C ; colour 2 ; gene tpi1 ||| tpi ||| SPCC24B10.21 ; Alias tpi1 ; GO GO:0004807; triose-phosphate isomerase activity
 GO:0006096; glycolysis
 GO:0006094; gluconeogenesis
 GO:0005829; cytosol ; controlled_curation term=disease associated, hemolytic anemia; date=20060920 ||| term=conserved eukaryotic protein; date=20060920 ; primary_name tpi1 ; product triosephosphate isomerase ; EC_number 5.3.1.1 ;
mRNA SPCPB16A4.06c ; p_c sequence orphan ; colour 8 ; gene SPCPB16A4.06c ; product sequence orphan ; note compositionally biased region ||| previously annotated as dubious, but has localization signal ;
mRNA SPCC1795.11 ; p_c ATP-dependent RNA helicase Sum3 ||| DEAD/DEAH box helicase ||| essential (PMID 9832516) ||| similar to S. cerevisiae YOR204W and YPL119C ; colour 2 ; gene sum3 ||| ded1 ||| slh3 ||| moc2 ||| SPCC1795.11 ; note suppressor of uncontrolled mitosis ||| Multicopy supressor of Overexpressed Cyr1 ; Alias sum3 ; GO GO:0004004; ATP-dependent RNA helicase activity
 GO:0005737; cytoplasm
 GO:0005515; protein binding
 GO:0005515; protein binding
 GO:0006412; translation
 GO:0000086; G2/M transition of mitotic cell cycle
 GO:0000076; DNA replication checkpoint
 GO:0006970; response to osmotic stress
 GO:0031137; regulation of conjugation with cellular fusion ; primary_name sum3 ; product ATP-dependent RNA helicase Sum3 ;
misc_feature unknown_1009 ; note nominal overlap with cosmid SPCC825, EM:AL122011 S. pombe chromosome 3 ;
misc_feature unknown_1010 ; note nominal overlap with cosmid SPCC1259, EM: AL034564 S. pombe chromosome 3 ;
mRNA SPCC1259.01c ; p_c 40S ribosomal protein S18 ||| similar to S. cerevisiae YDR450W and YML026C ; colour 2 ; gene rps1802 ||| rps18-2 ||| SPCC1259.01c ||| SPCC825.06c ; Alias rps1802 ; GO GO:0005843; cytosolic small ribosomal subunit (sensu Eukaryota)
 GO:0006412; translation
 GO:0003735; structural constituent of ribosome
 GO:0019843; rRNA binding ; primary_name rps1802 ; product 40S ribosomal protein S18 ;
tRNA SPC**TRNA**ALA.12 ; gene SPC**TRNA**ALA.12 ; product **tRNA** Alanine ; note **tRNA** Ala anticodon AGC, Cove score 56.39 ;
tRNA SPC**TRNA**SER.09 ; gene SPC**TRNA**SER.09 ; product **tRNA** Serine ; note **tRNA** Ser anticodon AGA, Cove score 59.19 ;
tRNA SPC**TRNA**ARG.10 ; gene SPC**TRNA**ARG.10 ; product **tRNA** Arginine ; note **tRNA** Arg anticodon TCG, Cove score 59.66 ;
tRNA SPC**TRNA**ASP.05 ; gene SPC**TRNA**ASP.05 ; product **tRNA** Asparagine ; note **tRNA** Asp anticodon GTC, Cove score 57.96 ;
tRNA SPC**TRNA**ARG.11 ; gene SPC**TRNA**ARG.11 ; product **tRNA** Arginine ; note **tRNA** Arg anticodon ACG, Cove score 54.83 ;

tRNA SPC**TRNA**LEU.11 ; gene SPC**TRNA**LEU.11 ; product **tRNA** Leucine ; note **tRNA** Leu anticodon AAG, Cove score 46.56 ;
misc_feature unknown_1068 ; note nominal overlap with cosmid c1259 ;
repeat_unit unknown_1069 ; colour 1 ; note centromeric region duplicated in SPCC4B3 S. pombe chromosome 3 ;
tRNA SPC**TRNA**LYS.10 ; gene SPC**TRNA**LYS.10 ; product **tRNA** Lysine ; note **tRNA** Lys anticodon CTT, Cove score 57.56 ;
repeat_unit unknown_1070 ; colour 2 ; note dh repeat ;
repeat_unit unknown_1071 ; colour 3 ; note cen3b dgIII repeat ;
repeat_unit unknown_1072 ; colour 1 ; note region between dg and dh repeat ;
repeat_unit unknown_1073 ; colour 2 ; note cen3a dhIII repeat ;
repeat_unit unknown_1077 ; colour 12 ; note region between dg and dh repeat ;
repeat_unit unknown_1078 ; colour 2 ; note cen3a dhIII repeat ;
misc_feature unknown_1079 ; note nominal overlap with cosmid c1676 ;
repeat_unit unknown_1083 ;
repeat_unit unknown_1084 ; colour 1 ; note imr3L ;
repeat_unit unknown_1085 ; colour 1 ; note imr3L ;
tRNA SPC**TRNA**ASP.06 ; gene SPC**TRNA**ASP.06 ; product **tRNA** Asparagine ; note **tRNA** Asp anticodon GTC, Cove score 70.49 ;
tRNA SPC**TRNA**ARG.12 ; gene SPC**TRNA**ARG.12 ; product **tRNA** Arginine ; note **tRNA** Arg anticodon ACG, Cove score 75.46 ;
tRNA SPC**TRNA**VAL.09 ; gene SPC**TRNA**VAL.09 ; product **tRNA** Valine ; note **tRNA** Val anticodon AAC, Cove score 64.30 ;
tRNA SPC**TRNA**THR.08 ; gene SPC**TRNA**THR.08 ; product **tRNA** Threonine ; note **tRNA** Thr anticodon AGT, Cove score 75.01 ;
tRNA SPC**TRNA**LEU.12 ; gene SPC**TRNA**LEU.12 ; product **tRNA** Leucine ; note **tRNA** Leu anticodon CAA, Cove score 56.07 ;
repeat_unit unknown_1088 ; colour 4 ; note cnt3 ;
repeat_unit unknown_1089 ; colour 4 ; note cnt3 partial ;
tRNA SPC**TRNA**GLU.10 ; gene SPC**TRNA**GLU.10 ; product **tRNA** Glutamic acid ; note **tRNA** Glu anticodon CTC, Cove score 74.15 ;
repeat_unit unknown_1090 ; colour 1 ; note imr3R ;
tRNA SPC**TRNA**LEU.13 ; gene SPC**TRNA**LEU.13 ; product **tRNA** Leucine ; note **tRNA** Leu anticodon CAA, Cove score 56.07 ;
tRNA SPC**TRNA**THR.09 ; gene SPC**TRNA**THR.09 ; product **tRNA** Threonine ; note **tRNA** Thr anticodon AGT, Cove score 75.01 ;
tRNA SPC**TRNA**VAL.10 ; gene SPC**TRNA**VAL.10 ; product **tRNA** Valine ; note **tRNA** Val anticodon AAC, Cove score 64.30 ;
intron unknown_1092 ; note intron **tRNA** Val anticodon AAC ;
tRNA SPC**TRNA**ARG.13 ; gene SPC**TRNA**ARG.13 ; product **tRNA** Arginine ; note **tRNA** Arg anticodon ACG, Cove score 75.46 ;
tRNA SPC**TRNA**ASP.07 ; gene SPC**TRNA**ASP.07 ; product **tRNA** Asparagine ; note **tRNA** Asp anticodon GTC, Cove score 70.49 ;

repeat_unit unknown_1093 ; colour 11 ; note cen3xc central region ;
repeat_unit unknown_1095 ; colour 12 ; note region between dh and dg repeat ;
misc_feature unknown_1096 ; colour 3 ; note cen3b dgIII repeat ;
repeat_unit unknown_1097 ; colour 2 ; note cen3a dhIII repeat ;
repeat_unit unknown_1098 ; colour 12 ; note region between dg and dh repeat ;
repeat_unit unknown_1100 ; colour 2 ; note cen3a dhIII repeat ;
repeat_unit unknown_1101 ; colour 12 ; note region between dg and dh repeat ;
repeat_unit unknown_1102 ; colour 3 ; note cen3b dgIII repeat ;
repeat_unit unknown_1103 ; colour 2 ; note cen3a dhIII repeat ;
repeat_unit unknown_1104 ; colour 12 ; note region between dg and dh repeat ;
repeat_unit unknown_1105 ; colour 3 ; note cen3b dgIII repeat ;
repeat_unit unknown_1106 ; colour 2 ; note dh repeat ;
mRNA SPCC1322.10 ; p_c glycoprotein (predicted) ||| possibly S. pombe specific ||| serine-rich protein ||| GPI anchored protein (predicted) (PMID 12845604) (pers. comm. Birgit Eisenhaber) ||| predicted N-terminal signal sequence ; GO GO:0009986; cell surface ; colour 12 ; gene SPCC1322.10 ; product glycoprotein (predicted) ;
mRNA SPCC1281.06c ; p_c acyl-coA desaturase (predicted) ||| similar to S. cerevisiae YGL055W ; GO GO:0006633; fatty acid biosynthesis
 GO:0004768; stearoyl-CoA 9-desaturase activity
 GO:0005789; endoplasmic reticulum membrane
 GO:0006636; fatty acid desaturation
 GO:0031227; intrinsic to endoplasmic reticulum membrane ; colour 7 ; gene SPCC1281.06c ; product acyl-coA desaturase (predicted) ;
mRNA SPCC622.09 ; temporary_systematic_id SPCC622.09 ; note **mRNA** from AU010164 ;
mRNA SPCC622.12c ; p_c NADP-specific glutamate dehydrogenase (predicted) ||| similar to S. cerevisiae YOR375C and YAL062W ; colour 7 ; gene SPCC622.12c ; GO GO:0006537; glutamate biosynthesis
 GO:0006807; nitrogen compound metabolism
 GO:0004354; glutamate dehydrogenase (NADP+) activity
 GO:0005634; nucleus
 GO:0005737; cytoplasm
 GO:0005739; mitochondrion ; product NADP-specific glutamate dehydrogenase (predicted) ; psu_db_xref PATH:MAP00251; ||| PATH:MAP00910; ; EC_number 1.4.1.4 ;
mRNA SPCC13B11.01 ; p_c alcohol dehydrogenase Adh1 ||| similar to S. cerevisiae YOL086C and YMR303C and YMR083W and YBR145W ; colour 2 ; gene adh1 ||| adh ||| SPCC13B11.01 ; Alias adh1 ; GO GO:0006066; alcohol metabolism
 GO:0005759; mitochondrial matrix ; primary_name adh1 ; product alcohol dehydrogenase Adh1 ; EC_number 1.1.1.1 ;
mRNA SPCC417.08 ; p_c translation elongation factor eEF3 ||| AAA family ATPase ||| HEAT repeat (inferred from context) ||| similar to S. cerevisiae YNL014W and YLR249W ; colour 2 ; gene tef3 ||| SPCC417.08 ; Alias tef3 ; GO GO:0003746; translation elongation factor activity
 GO:0016887; ATPase activity
 GO:0005830; cytosolic ribosome (sensu Eukaryota)
 GO:0006414; translational elongation
 GO:0005524; ATP binding ; primary_name tef3 ; product translation elongation factor eEF3 ;
misc_RNA SPNCRNA.10 ; Alias prl10 ; db_xref PMID:12597277 ||| EMBL:AB084822 ; systematic_id SPNCRNA.10 ; controlled_curation term=non-coding RNA; qualifier=predicted; db_xref=PMID:12597277; date=20050412 ||| term=poly(A)-bearing RNA; qualifier=predicted; db_xref=PMID:12597277; date=

20050412 ||| term=no detectable long open reading frame; qualifier=predicted; db_xref=PMID:12597277; date=20050412 ; gene prl10 ; primary_name prl10 ; product non-coding RNA (predicted) ;

misc_feature SPCC297.02 ; colour 6 ; gene SPCC297.02 ; product dubious ;

mRNA SPCC737.04 ; p_c UPF0300 family ||| possibly S. pombe specific ; colour 12 ; gene SPCC737.04 ; product UPF0300 family ;

mRNA SPCC576.03c ; temporary_systematic_id SPCC576.03c ; note **mRNA** from AF083335 ;

mRNA SPCC576.08c ; p_c 40S ribosomal protein S2 ||| similar to S. cerevisiae YGL123W ; colour 7 ; gene rps2 ||| SPCC576.08c ; Alias rps2 ; GO GO:0005843; cytosolic small ribosomal subunit (sensu Eukaryota)
 GO:0006412; translation
 GO:0003735; structural constituent of ribosome ; primary_name rps2 ; product 40S ribosomal protein S2 ;

mRNA SPCC576.11 ; p_c 60S ribosomal protein L15 ||| similar to S. cerevisiae YLR029C and YMR121C ; colour 7 ; gene rpl15 ||| SPCC576.11 ; Alias rpl15 ; GO GO:0005842; cytosolic large ribosomal subunit (sensu Eukaryota)
 GO:0006412; translation
 GO:0003735; structural constituent of ribosome ; primary_name rpl15 ; product 60S ribosomal protein L15 ;

misc_feature unknown_1817 ; note PS01194 Ribosomal protein L15e signature ;

mRNA SPCC576.17c ; colour 7 ;

mRNA SPCP1E11.04c ; p_c membrane associated protein Pal1 (PMID 15975911) ||| Pal1 family protein ||| conserved fungal protein ||| similar to S. cerevisiae YDR348C ; systematic_id SPCP1E11.04c ; colour 2 ; gene pal1 ||| SPCP1E11.04c ; note pears and lemons (PMID 15975911) ; Alias pal1 ; GO GO:0032153; cell division site
 GO:0030427; site of polarized growth
 GO:0051286; cell tip
 GO:0031097; medial ring
 GO:0005515; protein binding
 GO:0030467; establishment and/or maintenance of cell polarity (sensu Fungi)
 GO:0031505; cell wall organization and biogenesis (sensu Fungi) ; controlled_curation term=localization at the cell division site is independent of F-actin and microtubule function; db_xref=PMID:15975911; date=20060207 ; primary_name pal1 ; product membrane associated protein Pal1 (PMID 15975911) ;

mRNA SPCP1E11.06 ; p_c AP-1 adaptor complex gamma subunit Apl4 (predicted) ||| adaptin family ||| similar to S. cerevisiae YPR029C ||| HEAT repeat ; colour 7 ; gene apl4 ||| SPCP1E11.06 ; note May use internal initiator MET ; Alias apl4 ; GO GO:0016192; vesicle-mediated transport
 GO:0030121; AP-1 adaptor complex
 GO:0030276; clathrin binding ; primary_name apl4 ; product AP-1 adaptor complex gamma subunit Apl4 (predicted) ;

LTR unknown_2204 ; note TF1 **LTR** ;

LTR unknown_2224 ; note Tf1-type **LTR** ;