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Extra View

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ABSTRACT

Regulation of growth ultimately depends on the control of synthesis of new ribosomes. Ribosome biogenesis is thus a key element of cell biology, which is tightly regulated in response to environmental conditions. In eukaryotic cells, the supply of ribosomal components involves the activities of the three forms of nuclear RNA polymerase (Pol I, Pol II and Pol III). Recently, we demonstrated that upon rapamycin treatment, a partial derepression of Pol I transcription led to a concomitant derepression of Pol II transcription restricted to a small subset of class II genes encompassing the genes encoding all ribosomal proteins, and 19 additional genes.[1] The products of 14 of these 19 genes are principally involved in rDNA structure, ribosome biogenesis or translation, whereas the five remaining genes code for hypothetical proteins. We demonstrate that the proteins encoded by these five genes are required for optimal pre-rRNA processing. In addition, we show that cells in which regulation of Pol I transcription was specifically impaired are either resistant or hypersensitive to different stresses compared to wild-type cells. These results highlight the critical role of the regulation of Pol I activity for the physiology of the cells.

Cellular growth and division is tightly regulated by ribosome synthesis, a major cellular undertaking that is energetically very costly (reviewed in refs. 2 and 3). A striking feature of ribosome synthesis is a requirement for the coordinated activity of the three forms of RNA polymerase to produce the building blocks for ribosome construction. Pol I synthesizes the 35S rRNA which is processed into mature 25S, 18S and 5.8S rRNAs; Pol II synthesizes the mRNAs encoding the ribosomal proteins (RPs); and Pol III synthesizes 5S rRNA. How in this process coordination between the three forms of RNA polymerase is achieved is still unclear.

Recently, we showed in yeast that the accumulation of large ribosomal RNAs, as a result of deregulated Pol I transcription, led to the concomitant accumulation of 5S rRNA, of mRNAs encoding RPs, and of fully assembled ribosomes.[1] This observation points to a central role for Pol I activity in ribosome synthesis and suggests that Pol I transcription integrates the coordinated regulation of the two other forms of nuclear RNA polymerase.

THE CARA STRAIN, OR HOW TO MAKE Pol I TRANSCRIPTION CONSTITUTIVE?

In yeast, Pol I transcription initiation requires four general transcription factors: the upstream activating factor (UAF), the core factor (CF), the TATA binding protein (TBP) and the monomeric factor Rrn3. The two multimeric complexes UAF and CF bind to the rDNA promoter in conjonction with TBP to form the class I preinitiation complex,[4-6] whereas establishment of a transcription-competent initiation complex requires the interaction of Rrn3 with both Pol I and promoter-bound factors.[7,8] The essential and reversible interaction of the enzyme with Rrn3 is a critical event for Pol I transcription (reviewed in refs. 9–11), and has been shown to be a prime target for the regulation of the Pol I activity.[8,12,13] To interfere with these mechanisms, we constructed a yeast strain, named CARA (for Constitutive Association of Rrn3 and A43), in which the endogenous, essential Rrn3 factor and the A43 subunit of the Pol I that interacts with Rrn3,[14] were supplied as an Rrn3-A43 fusion protein.[1] The chimeric construct assembled properly within Pol I and formed a constitutively active, non dissociable Pol I-Rrn3 complex. Remarkably, under standard growth conditions, the CARA strain was not affected for growth and microarray analysis revealed that CARA cells have an mRNA expression profile indistinguishable from that of wild-type cells (Fig. 1, no rapamycin).

Ribosome synthesis is tightly regulated in response to a wide variety of intra and extra cellular stimuli.[2,15,16] In particular, the evolutionarily conserved TOR (target of rapamycin) signaling pathway regulates ribosome biogenesis and protein synthesis (in addition to nutrient import, autophagy and cell cycle progression). The TOR pathway is specifically inhibited by the antifungal and anticancer drug rapamycin. Upon rapamycin treatment, the level of all transcripts analyzed in our microarray experiments was similarly regulated in wild-type and CARA cells, with the notable exception of 147 mRNAs that were significantly over-represented in CARA cells (Fig. 1 and ref. 1). Remarkably, these account for only 2.5% of all the mRNAs analyzed. This striking observation demonstrates that upon rapamycin treatment, deregulation of Pol I transcription leads to a selective and concomitant deregulation of a highly specific subset of class II transcripts. The genes encoding these mRNAs can be grouped in four classes (see Table 1 for a summary):

(1) Group A represents the vast majority (128 of 147) of the genes specifically deregulated in CARA cells and encompasses the RP genes. Among the 138 RP genes of the yeast *S. cerevisiae*, 131 are represented on the DNA array used in the experiment. Surprisingly, the level of only 3 RP mRNAs is similar in CARA and wild-type cells upon rapamycin treatment (Fig. 1). Each of the corresponding genes (*RPL1A*, *RPL7B* and *RPL33A*) belongs to a related pair, a general feature in *S. cerevisiae* that has retained many of the duplicated RP genes that were generated following the ancestral whole genome duplication.[17] In the experiment depicted in Figure 1, the level of mRNAs of the other member of each gene pair (namely *RPL1B*, *RPL7A* and *RPL33B*) however, was significantly overrepresented in CARA cells. Since for each pair of RP genes, the two corresponding mRNAs have a near identical nucleotide sequence and thus cannot be easily distinguished on the microarray, these data suggest that the absence of a specific rapamycin-dependent deregulation of the *RPL1A*, *RPL7B* and *RPL33A* genes in the CARA cells in fact likely reflects technical limitations.

(2) Group B (see Table 1) includes eight genes whose products are involved in ribosome synthesis, assembly and/or function: Utp22 is involved in the 35S primary transcript processing,[18] Emg1 is required for the maturation of the 18S rRNA and for 40S ribosome production,[19] Stm1 directly binds to mature 80S ribosomes and polysomes,[20] Rpg1 is a translation initiation factor,[21] Asc1 acts as a negative regulator of translation,[22] and Cdc60 aminoacylates leucyl-tRNA.[23] Finally, *SDC1* and *SPP1*, whose products are two subunits of the COMPASS (Set1C) complex which methylates lysine 4 of histone H3, and which is involved in rDNA silencing,[24,25] are also specifically deregulated in CARA cells during the rapamycin treatment.

(3) Group C (see Table 1) contains six genes encoding proteins whose function is apparently unrelated to cytoplasmic ribosome or translation. *LEA1* codes for a component of U2 snRNP.[26] Gip2 is a putative regulatory subunit of the protein phosphatase Glc7p, involved in glycogen metabolism.[27] *FET3* encodes an integral membrane multicopper oxidase, which mediates resistance to copper ion toxicity.[28,29] *BEM4* codes for a protein involved both in the establishment of cell polarity and bud emergence, in Rho protein signal transduction,[30] and in maintenance of proper telomere length.[31] Finally, the products of the last two genes are involved in mitochondrial metabolism: *CBP3* encodes a mitochondrial chaperone required for assembly of the cytochrome bc1 complex[32,33] whereas *MRLP24* codes for a mitochondrial RP of the large subunit.[34]

(4) The last class of genes (group D) encompasses five non-essential genes encoding hypothetical proteins (*YDR445C*, *YER039C-A*, *YOL047C*, *YOL048C* and *YPL216W*, see Table 1). To test whether these proteins are involved in ribosome synthesis, the pre-rRNA processing pathway was characterized by Northern blot hybridization in yeast strains deleted for each of these five genes (Fig. 2). All five strains analyzed were defective for pre-rRNA processing. The pathway leading to the synthesis of the small ribosomal subunit rRNA (18S rRNA) was most affected (cleavages at sites A_0-A_2). Since the aberrant 23S RNA that extends from the transcription start site to site A_3 was not detected in any of the strains tested, we concluded that cleavages at sites A_0-A_2 are delayed to various extent and that all five genes of class D are required for optimal pre-rRNA processing.

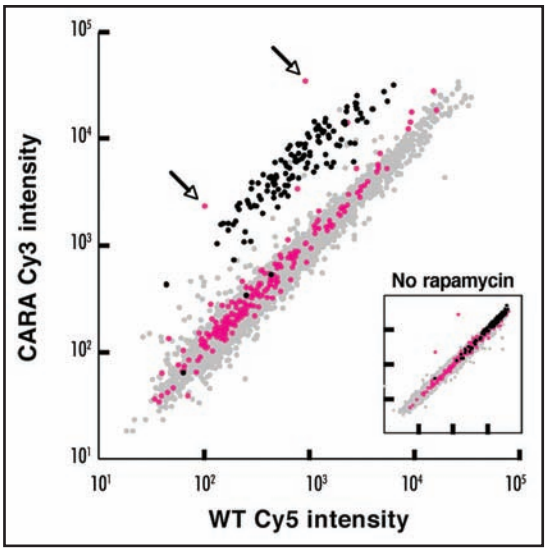

Figure 1. Members of the *Ribi* regulon are not deregulated in the CARA strain upon rapamycin treatment. Wild-type (WT) and CARA cells were grown in complete medium to OD_{600} = 1 (mid-log phase) and further incubated for 60 min with rapamycin or without rapamycin (inset). Cells were harvested and total RNAs were extracted. RNAs (20 µg) were labeled by reverse transcription in the presence of Cy5 dUTP (WT) or Cy3 dUTP (CARA) and used to probe a microarray harboring all yeast ORFs. Results were analyzed using the GeneSpring software (Silicon Genetics). A scatterplot representation of expression levels is displayed. Each individual spot corresponds to a gene, and its location on the diagonal indicates that the abundance of the corresponding mRNA is similar in both strains. mRNAs encoding RP (black spots) are present at the same level in untreated WT and CARA cells (inset) but are specifically overrepresented in CARA cells in the presence of rapamycin (from a threefold to a 13-fold factor, with an average factor of 7.7). In sharp contrast, after rapamycin treatment, the abundance of mRNAs from the *Ribi* regulon (pink spots) remains identical in both WT and CARA strain. The two spots, corresponding to *RRN3* and *RPA43* mRNAs (indicated by an arrow), which belongs to the *Ribi* regulon, are overexpressed in CARA cells because the Rrn3-A43 fusion is expressed from a multicopy plasmid.

Altogether, our microarray analysis underscores a very high specificity for the 147 mRNAs distinctively deregulated in CARA cells upon rapamycin treatment. The most prominent observation concerns the RP genes (group A). Although spread throughout the yeast genome, these genes are arguably the most coordinately regulated cluster of genes and are thus considered as a regulon (i.e., the *RP* regulon).[2,16,35] Remarkably, however, regulation of another regulon, the *Ribi* regulon (for ribosome biogenesis), which shows nearly identical transcriptional responses as RP genes to environmental or genetic perturbations,[16,36-39] is similar in CARA and wild-type cells upon rapamycin treatment (Fig. 1). The *Ribi* regulon encompasses a large number of genes (>200) encoding proteins involved in ribosome biogenesis, a complex process implicating accessory factors that

Table 1 **Class II transcripts deregulated in CARA cells upon rapamycin treatment[a]**

Group	Number of Genes	Systematic Name	Common Name	CARA / WT[b]		Biological Process[c]
				No Rapamycin	+ Rapamycin	
A	128		RPs	1.0[d]	7.7[d]	Ribosome Components
B	8	YBR079C	*RPG1*	0.9	5.5	
		YDR469W	*SDC1*	1.0	5.0	
		YGR090W	*UTP22*	0.7	3.6	rDNA structure, ribosome
		YLR150W	*STM1*	0.9	3.7	biogenesis or translation
		YLR186W	*EMG1*	0.9	4.5	
		YMR116C	*ASC1*	0.9	4.3	
		YPL160W	*CDC60*	0.7	6.0	
		YPL138C	*SPP1*	1.0	3.9	
C	6	YER054C	*GIP2*	1.1	3.3	
		YMR058W	*FET3*	1.1	3.0	
		YMR193W	*MRPL24*	1.0	4.4	Function unrelated
		YPL161C	*BEM4*	0.9	6.0	to ribosome biogenesis
		YPL213W	*LEA1*	0.9	3.4	
		YPL215W	*CBP3*	1.1	9.4	
D	5	YDR445C	-	1.0	3.2	
		YER039C-A	-	0.9	3.4	
		YOL047C	-	1.0	5.2	Uncharacterized function
		YOL048C	-	0.9	3.9	
		YPL216W	-	1.1	9.0	

[a]Genes specifically over-represented (>= 3-fold increase) in CARA cells versus WT cells in the presence of rapamycin. [b]Ratio of expression in CARA cells over WT cells. [c]According to the *Saccharomyces* Genome Database available at http://www.yeastgenome.org. [d]Average for the 128 RP genes (for details, see ref. 1)

assemble and modify rRNA and RPs[36-38,40] as well as additional functional categories including subunits of Pol I and Pol III, enzymes involved in ribonucleotide metabolism, tRNA synthetases, and translation factors.[16,37,38] The *Ribi* regulon thus consists of non-RP genes that enhance translational capacity.

In conclusion, the observation that the deregulation of Pol I activity specifically affects transcription of the *RP* regulon but not that of the *Ribi* regulon (Fig. 1) emphasizes the very high specificity of the cross-talk existing between the Pol I and Pol II transcriptional machineries.

WHAT ARE THE CONSEQUENCES OF RIBOSOME BIOGENESIS DEREGULATION?

Genome-wide analyses have clearly documented that most environmental alterations trigger important modifications of the yeast transcriptome. In particular, under stressful conditions, genes related to the ESR (Environmental Stress Response) exhibit either of two opposite responses: a cluster of around 600 genes, including all RP genes, is repressed, whereas a second cluster of approximately 300 genes is induced.[16] Even if Pol I and Pol III transcriptional activities were not the central focus of these systematic analyses, they are down regulated by most of the environmental stresses.[41-45] Altogether, these studies indicate that the concomitant down regulation of the synthesis of all ribosomal components is a general feature of yeast physiology in response to environmental changes. Since ribosome biogenesis is one of the most energy consuming cellular process,[3] it is tempting to attribute this phenomenon to a cellular "energy-saving" strategy but other explanations can be considered. For instance, it has been shown that signal-induced changes in the transcriptome are amplified at the translational level.[46] This effect, named potentiation, is characterized by a more efficient translation of mRNAs encoding genes that are induced in the transcriptome, and by lower translation efficiency for mRNAs encoding genes that are down regulated. Therefore, to modify rapidly the protein content of cells, one can imagine that a down regulation of ribosome biogenesis is required, while modifying the transcriptome profile. Little is known, however, on the effect on cell physiology of a deregulation of ribosome synthesis under stress conditions.

Taking advantage of the properties of the CARA strain, we investigated the effect of deregulating ribosome biogenesis in the presence of rapamycin.

In the absence of stress, we did not detect any growth differences between CARA and wild-type cells in complete medium (YPD), either liquid[1] or solid (Fig. 3A). In contrast, CARA cells were hypersensitive to rapamycin in plate assays for all drug concentrations tested (from 0.05 μg/ml to 1.6 μg/ml, Fig. 3B and data not shown). This hypersensitivity to rapamycin was observed for cells spotted from log-phase or from post-diauxic cultures (Fig. 3B). This result indicates that interfering with the rapamycin-dependent transcriptional repression of ribosomal components is deleterious for cell growth.

Next, we investigated how CARA cells responded to hydrogen peroxide, which, in contrary to rapamycin, induces a transient modification of the expression pattern of the ESR genes.[16,47] Wild-type and CARA cells, from either log-phase or post-diauxic culture, were spotted on plates containing different concentration of hydrogen peroxide. Wild-type and CARA cells from log-phase

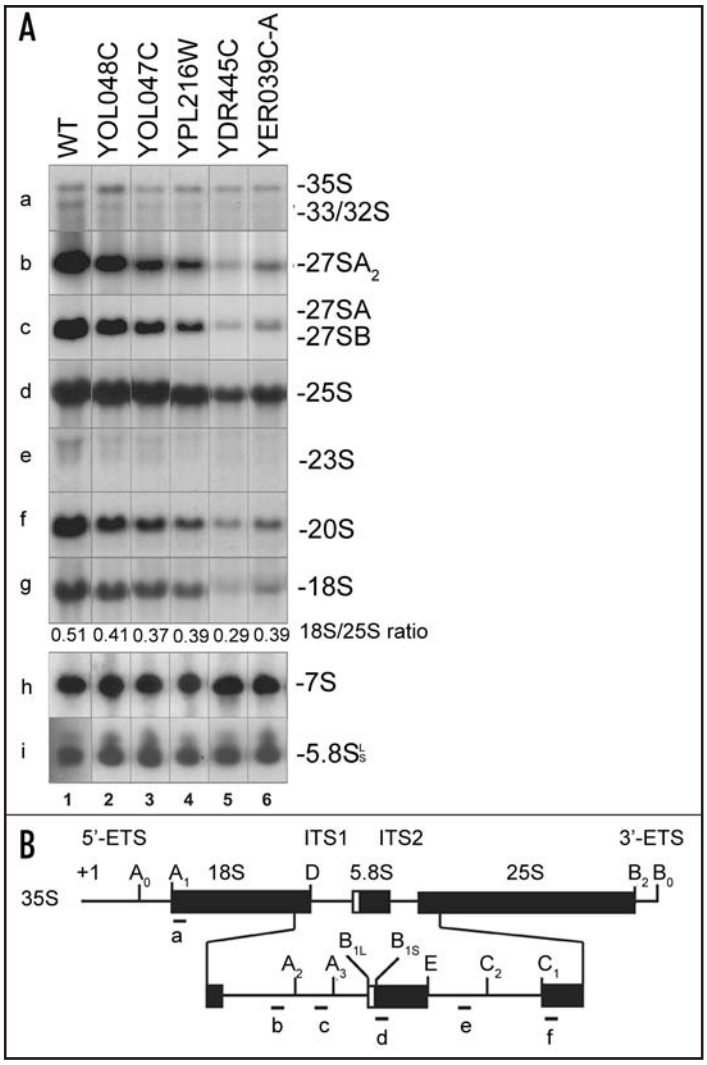

Figure 2. Group D genes are required for ribosomes synthesis. (A) Cells deleted for each of the five genes listed in Group D were grown to early log phase in complete medium. Total RNAs were extracted, separated by electrophoresis on denaturing 1.2% agarose/formaldehyde (panels a–g) or acrylamide gels (panels h and i), transferred to nylon membranes and analyzed by Northen-blot hybridization. RNA species detected to the right. The 18S rRNA to 25S rRNA ratio was established using Phosphor Imager quantitation (Typhoon and Image Quant, GE Healthcare). Oligonucleotides used in the hybridizations are as described in reference 52. (B) Schematic representation of the structure of the 35S rRNA primary Pol I transcript. The coding sequences for three out of the four mature rRNAs (25S, 18S, and 5.8S) are embedded within external (5'- and 3'-ETS) and internal (ITS1 and ITS2) transcribed spacer. Cleavage sites A_0 to E are indicated. +1, transcription start site. The fourth rRNA (5S) is independently transcribed by Pol III (not represented). For a full description of the pre-rRNA processing pathway, (see ref. 53).

cultures exhibited the same sensitivity to hydrogen peroxide (Fig. 3C and D). Surprisingly, CARA cells were significantly more resistant to hydrogen peroxide compared to wild-type cells when spotted from post-diauxic cultures (Fig. 3C and D). The reasons why post-diauxic culture cells show a greater resistance to hydrogen peroxide when containing a larger amount of assembled ribosome[1] are unclear, but may indicate that control of the protein biosynthesis machinery is important for the oxidative equilibrium of the cell, in agreement with recent data showing that changes in translational fidelity affect this balance.[48]

In conclusion, the recent characterization of the CARA strain strongly supports the emerging concept that Pol I activity is a key element for the coordinated synthesis of ribosome components. To dissect the molecular mechanisms involved, and more specifically to understand how the level of Pol I transcription impacts on Pol II transcription will certainly represent a major breakthrough. Another important issue is to elucidate how Pol I activity influence the level of the 5S rRNA synthesized by the Pol III. In CARA cells, upon rapamycin treatment, we observed an attenuated decrease of the level of this transcript concomitant to the attenuated down regulation of Pol I transcription.[1] Whether this deregulation of 5S rRNA is transcriptional and/or post-transcriptional is an important question that remains to be addressed.

Finally, a crucial question is to determine whether the central role of Pol I activity in the control of ribosome biogenesis has been evolutionarily conserved. To unravel how human cells regulate ribosome biogenesis is essential, as exemplified by numerous data suggesting that altering the protein synthesis machinery may promote malignant progression (see Ref. 49 for a review). The possibility that Pol I activity plays in mammalian cells a predominant role for the supply of ribosome components rationalizes the abundant evidence that Pol I transcription is altered in cancer cells. It may also explain why changes in nucleolar structure are recognized as a reliable marker of cellular transformation.[50] Whether deregulation of Pol I transcription might be an initiating step in tumorigenesis (see ref. 51 for a discussion of this hypothesis) by promoting increased cellular growth, proliferation, and transformation is thus a critical question that needs to be addressed in the near future.

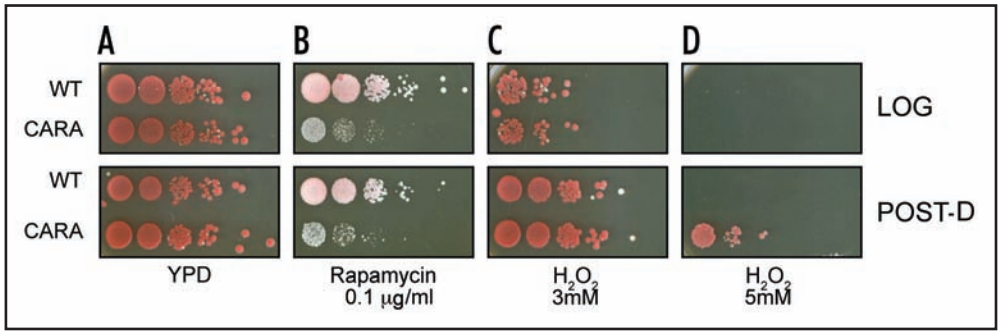

Figure 3. CARA cells are hypersensitive to rapamycin and resistant to H_2O_2. Wild-type (WT) or CARA cells were harvested either in log-phase (OD_{600} = 1, Log) or after the post-diauxic transition (two days of culture, Post-D). Serial dilutions from the same number of cells were spotted on YPD plates (A), either supplemented with rapamycin (0.1 µg/ml) (B) or with H_2O_2 (3 and 5 mM) (C and D). Growth of WT and CARA colonies was analyzed after seven days of incubation at 30°C.

References

1. Laferté A, Favry E, Sentenac A, Riva M, Carles C, Chédin S. The transcriptional activity of RNA polymerase I is a key determinant for the level of all ribosome components. Genes and Dev 2006; 20:2030-40.
2. Warner JR. The economics of ribosome biosynthesis in yeast. Trends Biochem Sci 1999; 24:437-40.
3. Warner JR, Vilardell J, Sohn JH. Economics of ribosome biosynthesis. Cold Spring Harb Symp Quant Biol 2001; 66:567-74.

4. Keys DA, Lee BS, Dodd JA, Nguyen TT, Vu L, Fantino E, Burson LM, Nogi Y, Nomura M. Multiprotein transcription factor UAF interacts with the upstream element of the yeast RNA polymerase I promoter and forms a stable preinitiation complex. Genes and Dev 1996; 10:887-903.
5. Lalo D, Steffan JS, Dodd JA, Nomura M. RRN11 encodes the third subunit of the complex containing Rrn6p and Rrn7p that is essential for the initiation of rDNA transcription by yeast RNA polymerase I. J Biol Chem 1996; 271:21062-7.
6. Steffan J, Keys D, Dodd J, Nomura M. The role of TBP in rDNA transcription by RNA polymerase I in *Saccharomyces cerevisiae*: TBP is required for upstream activation factor dependent recruitment of core factor. Genes and Dev 1996; 10:2551-63.
7. Yamamoto RT, Nogi Y, Dodd JA, Nomura M. *RRN3* gene of *Saccharomyces cerevisiae* encodes an essential RNA polymerase I transcription factor which interacts with the polymerase independently of DNA template. EMBO J 1996; 15:3964-73.
8. Milkereit P, Tschochner H. A specialized form of RNA polymerase I, essential for initiation and growth-dependent regulation of rRNA synthesis, is disrupted during transcription. EMBO J 1998; 17:3692-705.
9. Moss T, Stefanovsky VY. At the center of eukaryotic life. Cell 2002; 109:545-8.
10. Grummt I. Life on a planet of its own: Regulation of RNA polymerase I transcription in the nucleolus. Genes and Dev 2003; 17:1691-702.
11. Russell J, Zomerdijk JC. The RNA polymerase I transcription machinery. Biochem Soc Symp 2006; 203-16.
12. Miller G, Panov KI, Friedrich JK, Trinkle-Mulcahy L, Lamond AI, Zomerdijk JC. hRRN3 is essential in the SL1-mediated recruitment of RNA Polymerase I to *rRNA* gene promoters. EMBO J 2001; 20:1373-82.
13. Mayer C, Zhao J, Yuan X, Grummt I. mTOR-dependent activation of the transcription factor TIF-IA links rRNA synthesis to nutrient availability. Genes and Dev 2004; 18:423-34.
14. Peyroche G, Milkereit P, Bischler N, Tschochner H, Schultz P, Sentenac A, Carles C, Riva M. The recruitment of RNA polymerase I on rDNA is mediated by the interaction of the A43 subunit with Rrn3. EMBO J 2000; 19:5473-82.
15. Ju Q, Warner JR. Ribosome synthesis during the growth cycle of *Saccharomyces cerevisiae*. Yeast 1994; 10:151-7.
16. Gasch AP, Spellman PT, Kao CM, Carmel-Harel O, Eisen MB, Storz G, Botstein D, Brown PO. Genomic expression programs in the response of yeast cells to environmental changes. Mol Biol Cell 2000; 11:4241-57.
17. Lin YS, Hwang JK, Li WH. Protein complexity, gene duplicability and gene dispensability in the yeast genome. Gene 2006; In press.
18. Bernstein KA, Gallagher JE, Mitchell BM, Granneman S, Baserga SJ. The small-subunit processome is a ribosome assembly intermediate. Eukaryot Cell 2004; 3:1619-26.
19. Liu PC, Thiele DJ. Novel stress-responsive genes *EMG1* and *NOP14* encode conserved, interacting proteins required for 40S ribosome biogenesis. Mol Biol Cell 2001; 12:3644-57.
20. Van Dyke MW, Nelson LD, Weilbaecher RG, Mehta DV. Stm1p, a G_4 quadruplex and purine motif triplex nucleic acid-binding protein, interacts with ribosomes and subtelomeric Y' DNA in *Saccharomyces cerevisiae*. J Biol Chem 2004; 279:24323-33.
21. Phan L, Zhang X, Asano K, Anderson J, Vornlocher HP, Greenberg JR, Qin J, Hinnebusch AG. Identification of a translation initiation factor 3 (eIF3) core complex, conserved in yeast and mammals, that interacts with eIF5. Mol Cell Biol 1998; 18:4935-46.
22. Baum S, Bittins M, Frey S, Seedorf M. Asc1p, a WD40-domain containing adaptor protein, is required for the interaction of the RNA-binding protein Scp160p with polysomes. Biochem J 2004; 380:823-30.
23. Hohmann S, Thevelein JM. The cell division cycle gene *CDC60* encodes cytosolic leucyl-tRNA synthetase in *Saccharomyces cerevisiae*. Gene 1992; 120:43-9.
24. Miller T, Krogan NJ, Dover J, Erdjument-Bromage H, Tempst P, Johnston M, Greenblatt JF, Shilatifard A. COMPASS: A complex of proteins associated with a trithorax-related SET domain protein. Proc Natl Acad Sci USA 2001; 98:12902-7.
25. Roguev A, Schaft D, Shevchenko A, Pijnappel WW, Wilm M, Aasland R, Stewart AF. The *Saccharomyces cerevisiae* Set1 complex includes an Ash2 homologue and methylates histone 3 lysine 4. EMBO J 2001; 20:7137-48.
26. Caspary F, Séraphin B. The yeast U2A'/U2B complex is required for prespliceosome formation. EMBO J 1998; 17:6348-58.
27. Cheng C, Huang D, Roach PJ. Yeast PIG genes: *PIG1* encodes a putative type 1 phosphatase subunit that interacts with the yeast glycogen synthase Gsy2p. Yeast 1997; 13:1-8.
28. Hassett RF, Yuan DS, Kosman DJ. Spectral and kinetic properties of the Fet3 protein from *Saccharomyces cerevisiae*, a multinuclear copper ferroxidase enzyme. J Biol Chem 1998; 273:23274-82.
29. Shi X, Stoj C, Romeo A, Kosman DJ, Zhu Z. Fre1p Cu2+ reduction and Fet3p Cu1+ oxidation modulate copper toxicity in *Saccharomyces cerevisiae*. J Biol Chem 2003; 278:50309-15.
30. Mack D, Nishimura K, Dennehey BK, Arbogast T, Parkinson J, Toh-e A, Pringle JR, Bender A, Matsui Y. Identification of the bud emergence gene *BEM4* and its interactions with rho-type GTPases in *Saccharomyces cerevisiae*. Mol Cell Biol 1996; 16:4387-95.
31. Askree SH, Yehuda T, Smolikov S, Gurevich R, Hawk J, Coker C, Krauskopf A, Kupiec M, McEachern MJ. A genome-wide screen for *Saccharomyces cerevisiae* deletion mutants that affect telomere length. Proc Natl Acad Sci USA 2004; 101:8658-63.
32. Wu M, Tzagoloff A. Identification and characterization of a new gene (*CBP3*) required for the expression of yeast coenzyme QH2-cytochrome c reductase. J Biol Chem 1989; 264:11122-30.
33. Shi G, Crivellone MD, Edderkaoui B. Identification of functional regions of Cbp3p, an enzyme-specific chaperone required for the assembly of ubiquinol-cytochrome c reductase in yeast mitochondria. Biochim Biophys Acta 2001; 1506:103-16.
34. Graack HR, Wittmann-Liebold B. Mitochondrial ribosomal proteins (MRPs) of yeast. Biochem J 1998; 329(Pt 3):433-48.
35. Jorgensen P, Rupes I, Sharom JR, Schneper L, Broach JR, Tyers M. A dynamic transcriptional network communicates growth potential to ribosome synthesis and critical cell size. Genes and Dev 2004; 18:2491-505.
36. Hughes JD, Estep PW, Tavazoie S, Church GM. Computational identification of cis-regulatory elements associated with groups of functionally related genes in *Saccharomyces cerevisiae*. J Mol Biol 2000; 296:1205-14.
37. Wade C, Shea KA, Jensen RV, McAlear MA. EBP2 is a member of the yeast RRB regulon, a transcriptionally coregulated set of genes that are required for ribosome and rRNA biosynthesis. Mol Cell Biol 2001; 21:8638-50.
38. Jorgensen P, Nishikawa JL, Breitkreutz BJ, Tyers M. Systematic identification of pathways that couple cell growth and division in yeast. Science 2002; 297:395-400.
39. Miyoshi K, Shirai C, Mizuta K. Transcription of genes encoding trans-acting factors required for rRNA maturation/ribosomal subunit assembly is coordinately regulated with ribosomal protein genes and involves Rap1 in *Saccharomyces cerevisiae*. Nucleic Acids Res 2003; 31:1969-73.
40. Fatica A, Tollervey D. Making ribosomes. Curr Opin Cell Biol 2002; 14:313-8.
41. Mizuta K, Warner JR. Continued functioning of the secretory pathway is essential for ribosome synthesis. Mol Cell Biol 1994; 14:2493-502.
42. Zaragoza D, Ghavidel A, Heitman J, Schultz MC. Rapamycin induces the G_0 program of transcriptional repression in yeast by interfering with the TOR signaling pathway. Mol Cell Biol 1998; 18:4463-70.
43. Powers T, Walter P. Regulation of ribosome biogenesis by the rapamycin-sensitive TOR-signaling pathway in *Saccharomyces cerevisiae*. Mol Biol Cell 1999; 10:987-1000.
44. Li Y, Moir RD, Sethy-Coraci IK, Warner JR, Willis IM. Repression of ribosome and tRNA synthesis in secretion-defective cells is signaled by a novel branch of the cell integrity pathway. Mol Cell Biol 2000; 20:3843-51.
45. Upadhya R, Lee J, Willis IM. Maf1 is an essential mediator of diverse signals that repress RNA polymerase III transcription. Mol Cell 2002; 10:1489-94.
46. Preiss T, Baron-Benhamou J, Ansorge W, Hentze MW. Homodirectional changes in transcriptome composition and mRNA translation induced by rapamycin and heat shock. Nat Struct Biol 2003; 10:1039-47.
47. Causton HC, Ren B, Koh SS, Harbison CT, Kanin E, Jennings EG, Lee TI, True HL, Lander ES, Young RA. Remodeling of yeast genome expression in response to environmental changes. Mol Biol Cell 2001; 12:323-37.
48. Konstantinidis TC, Patsoukis N, Georgiou CD, Synetos D. Translational fidelity mutations in 18S *rRNA* affect the catalytic activity of ribosomes and the oxidative balance of yeast cells. Biochemistry 2006; 45:3525-33.
49. Ruggero D, Pandolfi PP. Does the ribosome translate cancer? Nat Rev Cancer 2003; 3:179-92.
50. Gani R. The nucleoli of cultured human lymphocytes. I. Nucleolar morphology in relation to transformation and the DNA cycle. Exp Cell Res 1976; 97:249-58.
51. Rosenwald IB. Deregulation of protein synthesis as a mechanism of neoplastic transformation. BioEssays 1996; 18:243-50.
52. Hoang T, Peng WT, Vanrobays E, Krogan N, Hiley S, Beyer AL, Osheim YN, Greenblatt J, Hughes TR, Lafontaine DL. Esf2p, a U3-associated factor required for small-subunit processome assembly and compaction. Mol Cell Biol 2005; 25:5523-34.
53. Lafontaine DL, Tollervey D. The function and synthesis of ribosomes. Nat Rev Mol Cell Biol 2001; 2:514-20.