Acta Biochimica Polonica

Vol. 51 No. 1/2004

173–187

QUARTERLY

Functional and physical interactions of Krr1p, a *Saccharomyces cerevisiae* nucleolar protein✪

Robert Gromadka, Iwona Karkusiewicz, Bożenna Rempoła and Joanna Rytka✉

Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warszawa, Poland

Received: 06 November, 2003; revised: 23 January, 2004; accepted: 28 January, 2004

Key words: *S. cerevisiae, S. pombe,* suppressors, transcription, ribosomal proteins

The Krr1 protein of *Saccharomyces cerevisiae* is involved in processing of pre-rRNA and assembly of pre-ribosomal 40S subunits. To further investigate the function of Krr1p we constructed a conditional cold sensitive mutant *krr1-21,* and isolated seven genes from *Schizosaccharomyces pombe* whose products suppressed the cold sensitive phenotype of *krr1-21* cells. Among the multicopy suppressors we found genes coding for translation elongation factor EF-1α, a putative ribose methyltransferase and five genes encoding ribosomal proteins. Using the tandem affinity purification (TAP) method we identified thirteen *S. cerevisiae* ribosomal proteins interacting with Krr1p. Taken together, these results indicate that Krr1p interacts functionally as well as physically with ribosomal proteins. Northern blot analysis revealed that changes in the level of *krr1-21* mRNA were accompanied by similar changes in the level of mRNAs of genes encoding ribosomal proteins. Thus, Krr1p and the genes encoding ribosomal proteins it interacts with seem to be coordinately regulated at the level of transcription.

All cellular life forms contain ribosomes, and the only known function of this sophisticated molecular machine is protein synthesis. Although ribosomes carry out their function in the cytoplasm, eukaryotic ribosomes are assembled as pre-ribosomes in the nucleolus, where ribosomal RNA (rRNA) is transcribed and processed. This process requires coordinated synthesis of rRNA and ribosomal proteins. Cells must synthesize enough rRNA to assemble a very large number of ribosomes (1.7×10^5, 1.5×10^4, 10^6 per generation in a growing bacterial, yeast and mammalian cell, respectively). Adequate quantities of rRNA

✪This work was supported by the State Committee for Scientific Research (KBN, Poland) grant 6 P04A 00919, and by the French-Polish Centre of Plant Biotechnology.

✉Corresponding author: Joanna Rytka: Institute of Biochemistry and Biophysics, Polish Academy of Sciences, A. Pawińskiego 5a, 02-106 Warszawa, Poland; phone: (48 22) 658 4701; fax: (48 22) 658 4636; e-mail rytka@psd.ibb.waw.pl

Abbreviations:HA, hemagglutinin; NLS, nuclear localization signal; RP, ribosomal protein; TAP, tandem affinity purification.

can be produced because the cell contains multiple copies of the rDNA genes, which encode the pre-rRNA. The *Saccharomyces cerevisiae* genome contains, depending on the genetic background, 100–220 copies of the rDNA on chromosome XII. A single rDNA copy consists of two transcription units: 35S rDNA encoding precursor rRNA transcribed by RNA polymerase I and 5S rDNA transcribed in the opposite direction by RNA polymerase III. Transcription of a single rRNA precursor molecule provides equimolar amounts of the three species of rRNA for assembly into ribosomes. The newly synthesized 35S pre-rRNA is processed into three mature species: 18S, 25S and 5.8S rRNA. The maturation of 35S pre-rRNA involves numerous processing steps extensively reviewed in (Kressler *et al.*, 1999; Venema & Tollervey, 1999; Geerlings *et al.*, 2000). The precursor's life time is very short, approximately 10 s, and assembly of rRNA and almost all of the ribosomal proteins begins while the pre-rRNA emerges from the transcriptional machinery and its association with proteins in ribonucleoprotein complexes (RNP) persists during processing and throughout the assembly of ribosomal subunits (Warner, 1989; 1999; Venema & Tollervey, 1999; Verschoor *et al.*, 1998; Gromadka & Rytka, 2000a; Grandi *et al.*, 2002 and references therein). The ribosomal precursors also contain a number of snoRNAs (Samarsky & Fournier, 1999; Smith & Steitz, 1997) and nonribosomal proteins required for the proper maturation and assembly of pre-rRNAs with ribosomal proteins, and export of ribosomal subunits into the cytoplasm. The auxiliary factors are then selectively dissociated to yield mature ribosomal subunits.

In *S. cerevisiae* the synthesis of the ribosome components is coordinately responsive to changes in environmental conditions (Warner, 1989; Planta, 1997; Li *et al.*, 1999). Although it is known that the primary level of regulation is transcription, comparatively little is known about the mechanisms of the concerted action of all the components involved in the biogenesis of the ribosomes.

KRR1 is an *S. cerevisiae* gene discovered during the functional analysis of open reading frames of unknown function (Gromadka *et al.*, 1996). Experiments with a HA-Krr1p fusion showed that the protein is localized to the nucleolus. Krr1p is essential for cell viability and reduced levels of this protein led to an impairment in 18S rRNA synthesis which in turn caused a deficiency in 40S ribosomal subunits (Gromadka & Rytka, 2000b; Sasaki *et al.*, 2000). Recent studies of Grandi *et al.* (2002) showed that Krr1p is associated with early pre-ribosomal particles involved in 40S subunit assembly. It may be involved in nuclear transport of ribosomes (Grandi *et al.*, 2002) and in the S phase check point (Kondoh *et al.*, 2000).

In this study, to integrate the function of Krr1p into the nucleolar network, we created a conditional cold sensitive mutant *krr1-21,* which enabled a search for genetic interactions by isolation of suppressors that correct the mutant phenotype. We show that Krr1p interacts both genetically and physically with ribosomal proteins and that the transcription of *KRR1* is tightly coordinated with the transcription of some ribosomal protein genes (RPs).

MATERIAL AND METHODS

Media and genetic analysis. The *S. cerevisiae* strains and plasmids used in this study are described in Table 1 and Table 2, respectively. *Escherichia coli* DH5α was used for plasmid preparation (Sambrook *et al.*, 1989). Standard complete YPD (1% yeast extract, 1% bactopeptone, 2% dextrose), minimal SD (0.67% yeast nitrogen base w/o amino acids, 2% dextrose) and SC-dropout media were used (Rose *et al.*, 1990). For biochemical analysis cells were grown in liquid media at 28°C or 23°C with vigorous aeration. Growth rate was followed by counting

cells in a Thoma's camera or measurement of A_{600}.

Standard media and procedures were used for crossing, sporulation and tetrad analysis (Sherman, 1991). Diploid strains were obtained by separating zygotes from a mixture of mating cells by micromanipulation.

DNA manipulations. Routine DNA manipulations: plasmid preparation, subcloning, transformation of *E. coli,* and agarose gel electrophoresis were carried out as described in Sambrook *et al.* (1989). Rapid plasmid isolation from 1.5 ml bacterial cultures was performed as described by Le Gouill and Dery (1991). To release plasmid DNA from yeast cells, for transformation of *E. coli* and to prepare chromosomal DNA for PCR, the procedures described by Hoffman and Winston (1987) were used.

Oligonucleotide primers were prepared using a Beckman Oligo 1000M DNA Synthesizer according to manufacturer's instructions. The primers used are listed in Table 3. The nucleotide sequences were determined using ABI310 Perkin-Elmer. Routine sequence analysis was performed using the UWGCG programs (Devereux *et al.*, 1984) and DNA Strider (Marck, 1988).

PCR was performed in a Perkin Elmer GeneAmp PCR System 9600 with the Boehringer Mannheim kit "Expanded High Fidelity PCR System" according to the manufacturer's instruction

Transformation of yeast cells. The rapid and high efficiency transformation of yeast cells was performed by the lithium acetate/single-stranded carrier DNA/PEG method described by Gietz and Woods (1998).

Construction of vectors. The plasmid pRG42 was constructed by gap-repair using a PCR generated cassette. Yeast cells were co-transformed with plasmid pRG14 digested with *Bal*I/*Sty*I and a PCR generated fragment of *KRR1* with deletion of the nuclear localization signal (RKKPKK) at pos. 234–239 (primers RG30, RG112) and were selected for Trp^+. The plasmid pRG47 was constructed by cloning the *Nco*I/*Eco*RI fragment of pRG42 into pRG45. The plasmid pRG51 was constructed by gap-repair using a PCR generated cassette. Yeast cells were co-transformed with plasmid pRG44 digested with *Afl*II/*Msc*I and PCR-amplified gene *MIS3* (primers RG137 and RG138, total DNA from *Schizosaccharomyces pombe* was used as a template) flanked by 40 nucleotides identical to plasmid sequence (promoter and terminator of *KRR1*) and selected for Trp^+.

Tagging Krr1p with TAP Tag. To tag Krr1p at the C-terminus, a construct containing TAP (Rigaut *et al.*, 1999) in-frame with the *KRR1* ORF, together with the *Kluyveromyces lactis URA3* marker was inserted into the *S. cerevisiae* genome by transformation with a PCR fragment. This fragment was generated by amplification from plasmid pBS1539 with oligonucleotides RG121 and RG122. The PCR product was integrated into the *KRR1* locus by transformation of the strain FA29/1 to give GR31/15. Tetrad analysis of the heterozygous diploid *MAT*a/*MAT*α *KRR1-TAP::URA3/krr1*Δ*::HIS3* revealed two viable Ura^+ spores.

Protein purification and identification. TAP-tagged Krr1p was isolated from 4 litres culture grown to A_{600} 1 by two step purification (Rigaut *et al.*, 1999). The purified proteins were separated on a 12% polyacrylamide SDS gel and visualized by silver staining. Bands were excised from the gel. Samples were reduced, alkylated and digested with trypsin (sequencing grade – Promega) following standard protocol. Samples were applied to an RP-18 pre-column (LC Packings) using 0.1% trifluoroacetic acid mobile phase and transferred to a nano-HPLC RP-18 column (LC-Packings) eluted using an acetonitrile gradient in the presence of 0.05% formic acid at a flow rate of 200 nl/min. The column outlet was directly coupled to a nano-Z-spray ion source of a Q-Tof electrospray mass spectrometer (Micromass) working in the regime of data dependent MS to MS/MS switch, allowing a 3 s sequencing scan for each detected

peptide. The peptide sequence tags obtained from the analysis were used to search a protein sequence database with the MASCOT search engine. When necessary manual data analysis was applied (MassLynx software).

RNA extraction and Northern blot analysis. Total RNA was extracted from cells grown in 100 ml of YPD medium to A_{600} of 0.2–0.3 using the acid-phenol method (Ausubel *et al.*, 1987). RNA was separated by electrophoresis in vertical 1.2% agarose formaldehyde gels (Ausubel *et al.*, 1987). RNA was transferred to Hybond-N (Amersham) according to the manufacturer's instruction and was hybridized with end-labelled oligonucleotides. Ten picomoles of dephosphorylated oligonucleotides were 5′-end labelled or 60 min at 37°C in the following reaction mixture: 1.5 μl 10 $\times$ Kinase Buffer (Promega), 1.0 μl [10 units/ml] of T4 Polynucleotide Kinase (Promega), 1.0 μl [γ-^{32}P]ATP (>7000 Ci/mmol, >100 mCi/ml, end-labelling grade, ICN Biomedical), water up to 15 μl.

Inhibition of transcription with 1,10-phenantroline. The method described by Parker *et al.* (1991) was used. Cells were grown at 23°C in 100 ml of YPD medium to A_{600} of 0.8–1.0. Cultures were concentrated to 7.2 ml of the YPD medium and 1,10-phenantroline was added to a final concentration of 100 μg/ml. Samples of 2.4 ml were harvested immediately after inhibitor addition (time 0) and after 10 and 30 min of incubation at 23°C. Pellets were rapidly frozen in liquid nitrogen and stored at –80°C. Total RNA was isolated from the frozen cell pellets.

Western blotting. Proteins were isolated as previously described (Gromadka & Rytka, 2000b). Proteins from 7×10^6 cells were separated on 12% polyacrylamide/SDS gel and transferred to a nitrocellulose membrane by electroblotting. Mouse monoclonal anti-HA antibody (clone 16B12, BabCo) was used as the primary antibody at a 1:1000 dilution, and goat anti-mouse alkaline phosphatase conjugated antibody was used as the secondary antibody at 1:2500 dilution, and detected with the Amersham CDP-*Star* detection system according to the manufacturer's instruction.

RESULTS AND DISCUSSION

Construction of strains mutated in the predicted nuclear localization signal of Krr1p

Modulation of *KRR1* gene expression by the use of a conditional promoter gave a wealth of information concerning the function of Krr1p (Gromadka & Rytka, 2000b; Sasaki *et al.*, 2000). However, this system is poorly adapted to searching for genetic interactions by suppression, because of the high probability of isolating mutations, which affect the regulation of the promoter. To overcome this difficulty we constructed a conditional mutant partially defective in Krr1p function. The approach we used was to create a mutation in the predicted nuclear localization signal (NLS). The *krr1-21* mutation is a deletion of 18 nucleotides at position +702→+719 coding for the cluster of basic amino acids RKKPKK highly conserved in evolutionarily distant species from yeast to human (Gromadka *et al.*, 1996).

An integrative plasmid, pRG47, bearing *krr1-21* was constructed in which the coding sequence is under the control of the native promoter and the 9-amino acid hemagglutinin (HA) epitope is inserted in frame in front of the first ATG of the coding sequence. *KRR1/krr1*Δ*::HIS3* cells were transformed with the plasmid and correct integration into the *TRP1* locus was verified by Southern analysis. After selection for tryptophan prototrophy diploids were sporulated and tetrad analysis was performed. The Trp$^+$ His$^+$ spore clones bearing the *HA-krr1-21* allele co-segregating with *krr1*Δ*::HIS3* were viable but germinated poorly and formed small colonies (Fig. 1B). The mutant clones grew poorly already at 28°C and at 15°C they showed the cold sensitive, *cs,* phenotype (Fig. 1A).

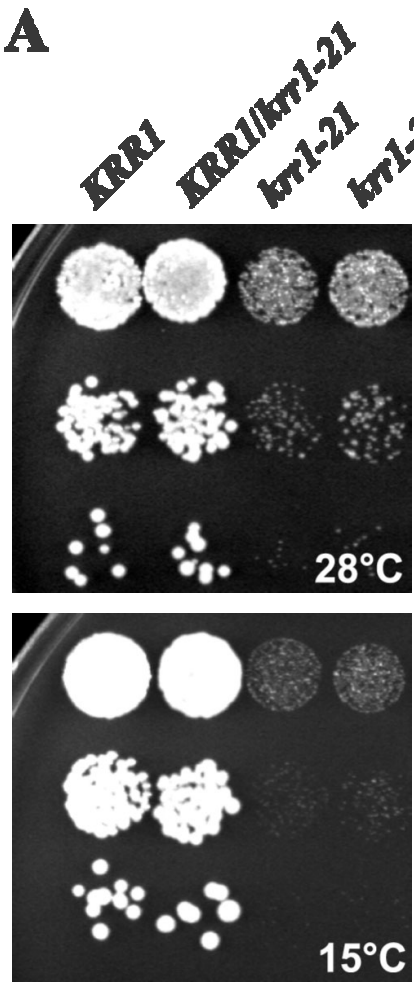

Figure 1. *krr1-21* displays slow growth and cold-sensitive phenotype.

A. Precultures grown in liquid YPD at 28°C were collected at 10^8 cells/ml. Five-microliter drops of undiluted culture and serial 33-fold dilutions were spotted onto YPD media and incubated at indicated temperatures.

B. Tetrads derivative of GR26/7 diploid. Heterozygous diploid *KRR1/trp1-1::TRP1::*P_{KRR1}*-HA-krr1-21 krr1*Δ*::HIS3* was sporulated and dissected onto YPD medium.

Small colonies were *krr1-21* as determined by their growth on minimal medium lacking histidine and tryptophan.

Decreased expression of *krr1-21*

Previously we demonstrated that Krr1p synthesis depends on the growth phase. Synthesis of Krr1p increases before the entrance of the cells into the logarithmic phase of growth, is highest in the early log phase, decreases in cells entering the stationary phase and almost completely ceases in the late stationary phase. The observed decrease of the Krr1p level during growth resulted from the low level of transcription of *KRR1* (Gromadka & Rytka, 2000b). The results presented in Fig. 2 demonstrate that the pattern of variation of the mutated Krr1-21p synthesis depending on the phase of growth was the same as that observed

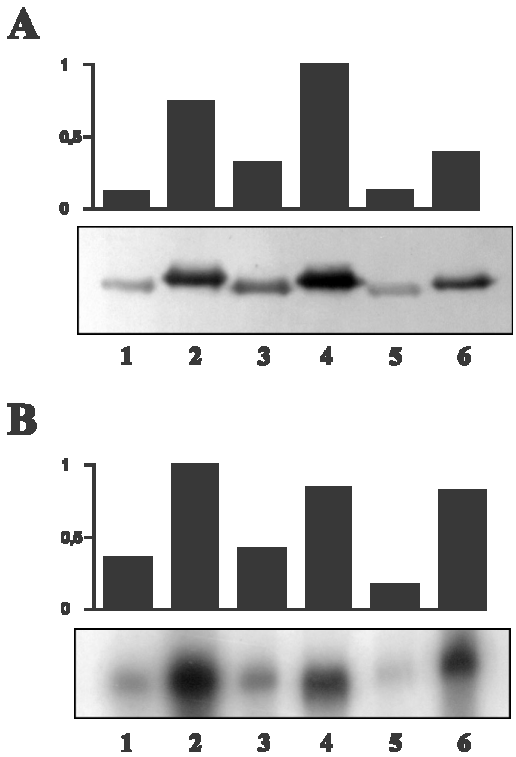

Figure 2. Decreased expression of *krr1-21* allele during growth compared with the *KRR1* allele.

A. Western blot, proteins were separated by SDS/PAGE and analyzed by immunoblotting using anti-HA antibodies. **B**. Northern blot, extracted RNA was hybridized with RG–158 probe. Samples were taken from mutant culture (lanes 1, 3 and 5) and wild type culture (lanes 2, 4 and 6). Cells were grown at 23°C for 2 h (lanes 1 and 2), 6 h (lanes 3 and 4), and 24 h (lanes 5 and 6). Bars are proportional to the band area. Band intensity is expressed in arbitrary unit by GelScan XL v. 2.1 from Pharmacia LKB.

for Krr1p. However, the content of the mutated protein was substantially lower than of the wild type protein (Fig. 2A). As for the wild type, the lowered amount of the mutated protein is correlated with a decreased level of the *krr1-21* mRNA (Fig. 2B). We have also tested the effect of the mutation on rRNA processing. Already in cells grown at 28°C a low rate of processing of 23S rRNA was observed (not shown), analogous with that found for Krr1p-depleted cells in a promoter "shut-off" experiment (Gromadka & Rytka, 2000b).

Isolation and *in silico* analysis of functional heterologous suppressors of *krr1-21* from the yeast *Schizosaccharomyces pombe*

As shown in Fig. 1, the *krr1-21* mutation led to a clear phenotype. Therefore, it was possible to use this phenotype to search for the correction or alleviation of the growth defect and select functional suppressors. Sasaki *et al.* (2000) selected two temperature sensitive *krr1* mutants, which were used subsequently for isolation of multicopy suppressors from an *S. cerevisiae* genomic library. As *ts* suppressors they isolated two genes: *RPA14A,* a gene encoding a 40S ribosomal protein, and a novel gene, *YNL308c,* which they named *KRI1*. Therefore, to search for new classes of functional suppressors, we used a heterologous cDNA bank of *S. pombe* to suppress the *cs* phenotype of the *krr1-21* strain. This approach is additionally justified by fact that the *MIS3* gene, an *S. pombe* ortholog of *KRR1* (Kondoh *et al.*, 2000), when cloned under *KRR1* promoter complemented the lethal phenotype of the *KRR1* deletion. The growth rate of the *krr1Δ::HIS3* strain complemented by the *S. pombe MIS* gene was similar to that of the parental *KRR1* strain, regardless of the culture conditions (not shown).

The *S. cerevisiae* strain GR26/7 bearing the *krr1-21* mutation in a *krr1Δ* background was transformed with a cDNA library of *S. pombe* (kindly provided by F. Lacroute, CGM CNRS, Gif-sur-Yvette, France) constructed in the vector pFL61. The cDNAs were cloned under the control of the strong, constitutive *PGK* promoter. The gene *URA3* was a selection

Table 1. Yeast strains used

Strain	Genotype
W303-1B	*MATα ade2-1 leu2-3,112 trp1-1 his3-11,15 ura3-1 can1-100*
W303	*MAT***a**/*MATα*
GR19/2*	*MATα, krr1Δ::HIS3*[pRG38]
FA29/1*	*MAT***a**/*MATα KRR1/krr1Δ::HIS3*
GR24/2*	*MAT***a**/*MATα trp1-1/ TRP1::*P_{KRR1}*-HA-KRR1 KRR1/krr1Δ::HIS3*
GR24/2-2A*	*MAT***a** *trp1-1::TRP1::*P_{KRR1}*-HA-KRR1 krr1Δ::HIS3*
GR24/2-3A*	*MATα, trp1-1::TRP1::*P_{KRR1}*-HA-KRR1, krr1Δ::HIS3*
GR26/7#	*MAT***a**/*MATα, trp1-1/trp1-1::TRP1::*P_{KRR1}*-HA-krr1-21 KRR1//krr1Δ::HIS3*
GR26/7-A#	*MATα trp1-1::TRP1::*P_{KRR1}*-HA-krr1-21 krr1Δ::HIS3*
GR26/7-C#	*MAT***a** *trp1-1::TRP1::*P_{KRR1}*-HA-krr1-21 krr1Δ::HIS3*
GR27/3#	*MAT***a**/*MATα krr1Δ::HIS3/ krr1Δ::HIS3trp1-1::TRP1::*P_{KRR1}*-HA-krr1-21 /trp1-1::TRP1::*P_{KRR1}*-HA-krr1-21*
GR31/15#	*MAT***a**/*MATα KRR1-TAP::URA3/krr1Δ::HIS3*
GR32/1	*MAT***a**/*MATα, KRR1-TAP::URA3/KRR1-TAP::URA3*

All strains are derivatives of W303 (Thomas & Rothstein, 1989) and harbour the following additional mutations: *ade2-1 leu2-3,112 trp1-1 his3-11,15 ura3-1 can1-100;* * described in Gromadka *et al.* (2000b); # this study; *krr1-21,* deletion of nuclear localization signal RKKPKK (aa position 234–239).

marker. Thirty-four transformants able to grow at 15°C were isolated from 3×10^6 Ura^+ colonies. On the basis of their restriction profiles the recovered plasmids were divided into seven groups and one representative of each group was sequenced and compared with the sequences deposited in the database of the *S. pombe* Genome Sequencing Project under the address http://www.ncbi.nlm.nih.gov/mapview/map_search.cgi. The isolated *S. pombe* genes, which functionally complemented the *krr1-21* mutation, are listed in Table 4. Control experiments demonstrated that the isolated suppressors did not complement the *KRR1* deletion (not shown). Microscopic immunofluorescence observation showed an increased level of Krr1-21p and its correct localization in the *krr1-21* mutant bearing the suppressor genes (Fig. 3).

As shown in Table 4 the search led mainly to isolation of genes encoding ribosomal proteins. *rps7, rps19-2, rps3,* and *rps9-1* encode 40S ribosomal proteins. All these proteins have their counterparts in the *S. cerevisiae* genome. The *rpl36-1* gene was classified as a gene encoding a 60S ribosomal protein; its *S. cerevisiae* ortholog RPL36A is an RNA-binding protein, a component of 60S subunit. Genetic interaction of Krr1p with ribosomal proteins is in agreement with the finding of Grandi *et al.* (2002) who identified Krr1p as a component of 90S pre-ribosomes.

Table 2. Vectors used

Plasmid	Properties	Source
pFL61	2 μm *URA3*	Lacroute collection
PRS426	2 μm *URA3*	Sikorski & Hieter (1989)
pRS304	integrative *TRP1*	Sikorski & Hieter (1989)
pRS314	*CEN6 TRP1*	Sikorski & Hieter (1989)
pBS1539	C-terminal TAP *K. lactis-URA3*	Rigaut *et al.* (1999)
pRG14	2 μm *URA3 KRR1*	Gromadka *et al.* (1996)
PRG38	*CEN6 URA3* UAS_{GAL10}-P_{CYC1}-*UBI-HA-KRR1*	Gromadka & Rytka (2000b)
pRG42	*CEN6 TRP1* P_{KRR1}*HA-krr1-21*	This study
pRG44	*CEN6 TRP1* P_{KRR1}*-HA-KRR1*	Gromadka & Rytka (2000b)
pRG45	integrative *TRP1* P_{KRR1}*-HA-KRR1*	Gromadka & Rytka (2000b)
pRG47	integrative *TRP1* P_{KRR1}*-HA- krr1-21*	This study
pRG51/6	*CEN6* P_{KRR1}*-HA- TRP1MIS3*	This study

CEN-centromeric, 2 μm-multicopy, *HA* influenza virus hemagglutinin epitope HA (the sequence codes for the 9-aa epitope YPYDVPDYA); P_{CYC1} promoter of cytochrome *c* isoform 1, regulated by glucose repression (Guarente *et al.*, 1984); *krr1-21* deletion of nuclear localization signal RKKPKK (aa position 234–239); TAP tag consists of two IgG binding domains of *Staphylococcus aureus* protein A and a calmodulin binding peptide separated by a TEV protease cleavage site.

The product of the ORF SPCC794.09c was classified as translation elongation factor EF-1α, the catalytic subunit of a guanine nucleotide exchange factor, on the basis of amino-acid sequence homology to *S. cerevisiae* eEF1A (87% identity). In the *S. pombe* genome, three distinct *ef1a-*α^+genes that encode proteins which are 99.5% identical, were found (ORFs SPAC24H6.07, SPBC839.15c and SPAC23A1.10). Although this protein associates with ribosomes and is an essential component of the translational machinery, it also performs other biological functions. Munshi *et al.* (2001) demonstrated that in *S. cerevisiae* excess eEF1A caused reduced budding and changed cellular morphology due to altered actin distribution. In *S. pombe* over-expression of EF1α-encoding genes also caused aberrant cell morphology, growth defects and supersensitivity to actin and tubulin inhibitors (Suda *et al.*, 1999). Our data seem to be in contradiction with those results, since the *ef1a-*α gene expressed from the strong *PGK* promoter suppressed the slow

Table 3. Primers used

Primer	Sequence	Description
RG30	CTT GCT AAA TGC CCT TGA GT	358 nt upstream of the ATG codon of *KRR1*
RG61	GAT TTC TCT TTC CAT TTG C	lower primer for *KRR1*
RG112	GGC AAT TGG GCA GGA GGA AAT GGA GTA TAG ACC TTC TTT TCG ACG TTT CTG ATG GCC ACA TTC CTC TTC TTA AAC	upper primer with deletion of nuclear localization signal RKKPKK
RG121	GAT TTC ATA GCT CCG GAA GAA GAA GCA TAC AAG CCA AAC CAA AAT TCC ATG GAA AAG AGA AG	upper primer for *KRR1*-TAPtag fusion
RG122	TGT AGG TGG TAG TTC TCT TCT TTG CAG TCA ACG AGG ACA AAG CAT TAC GAC TCA CTA TAG GG	lower primer for *KRR1*-TAPtag fusion
RG137	GAT GGG CGG CCG CTA CCC ATA CGA CGT TCC AGA CTA CGC T	upper primer for *MIS3* synthesis and cloning into vector pRG44
RG138	TAT ATA TAG ACA TAT ATG AAG GAT TCC GTA GCG GTG TAA ACT AAT CCC TTT TAC GCT	lower primer for *MIS3* synthesis and cloning into vector pRG44
RG-158	TCA TAA AAC TGG ACT CTT CAG CAA AAG GTT GAC CGG ATG C	probe to *KRR1*mRNA
RG-159	GTA TTC TTG TTT TGA GAT CCA CAT TTG TTG GAA GGT AGT C	probe to *ACT1*mRNA
RG-160	AAC CTT TCT AGG CAA TTG GGC AGG AGG AAA TGG AGT ATA G	probe to *KRR1*mRNA
001	CCA GTT ACG AAA ATT CTT G	probe to A3-B1L site in 35S rRNA
002	GCT CTT TGC TCT TGC C	probe to D-A2 site in 35S rRNA
003	TGT TAC CTC TGG GCC C	probe to A2-A3 site in 35S rRNA
007	CTC CGC TTA TTG ATA TGC	probe to mature 25S rRNA
008	CTC CGC TTA TTG ATA TGC	probe to mature 18S rRNA
013	GGC CAG CAA T TT CAA GTT A	probe to E-C2 site in 35S rRNA
911	CCA GAT AAC TAT CTT AAA A	probe to A0-A1 site in 35S rRNA
antiRPS11A	CTT GCT GGT TGC TTA ATT T	probe to *RPS11A* mRNA
antiRPS11B	TCC CTG GCT TGA TAC GTT	probe to *RPS11B* mRNA
antiRPS3	GAC ACC GTC AGC GAC TAG	probe to *RPS3* mRNA
antiRPL10	CTG TAA CAT CTA GCT GGT C	probe to *RPL10* mRNA

growth of the *krr1-21* mutant. In a control experiment we transformed the parental *KRR1* strain with empty vector and plasmid bearing this gene and it appeared that the growth of both transformants was comparable (not shown).

coordination between protein translation and nuclear tRNA processing and transport machinery (Grosshans *et al.*, 2000a; 2000b). Therefore the suppression of the *krr1-21* phenotype by the *ef1a-*α^+ gene might indicate an unforeseen role of Krr1p in nuclear trans-

Table 4. *S. pombe* genes alleviating the effect of *krr1-21* mutation

Number of clones	ORF	Gene		Function
		S. pombe	*S. cerevisiae*	
9	SPAC18G6.13C	*rps7*	*RPS7A/B*	40S ribosomal protein S7
7	SPBC649.02	*rps19-2*	*RPS19A/B*	40S ribosomal protein S19
5	SPBC16G5.14C	*rps3*	*RPS3*	40S ribosomal protein S3
5	SPAC24H6.07	*rps9-1*	*RPS9A/B*	40S ribosomal protein S9
4	SPCC794.09c	*ef1a-*α	*TEF1/2*	Translation elongation factor, alpha subunit
3	SPBC1347.13c		*PET56*	Putative ribose methyltransferase
1	SPCC970.05	*rpl36-1*	*RPL36A/B*	60S ribosomal protein L36

The cDNAs of *S. pombe* were cloned under the *S. cerevisiae PGK* promoter in the pFL61 vector. The library was kindly provided by F. Lacroute (CGM CNRS, Gif-sur-Yvette, France).

Results have been published showing that eEF1A is required for efficient nuclear tRNA export in *S. cerevisiae* cells, which suggests a

port. This is supported by the observation that Krr1p was found in a highly enriched yeast nucleopore fraction (Rout *et al.*, 2000);

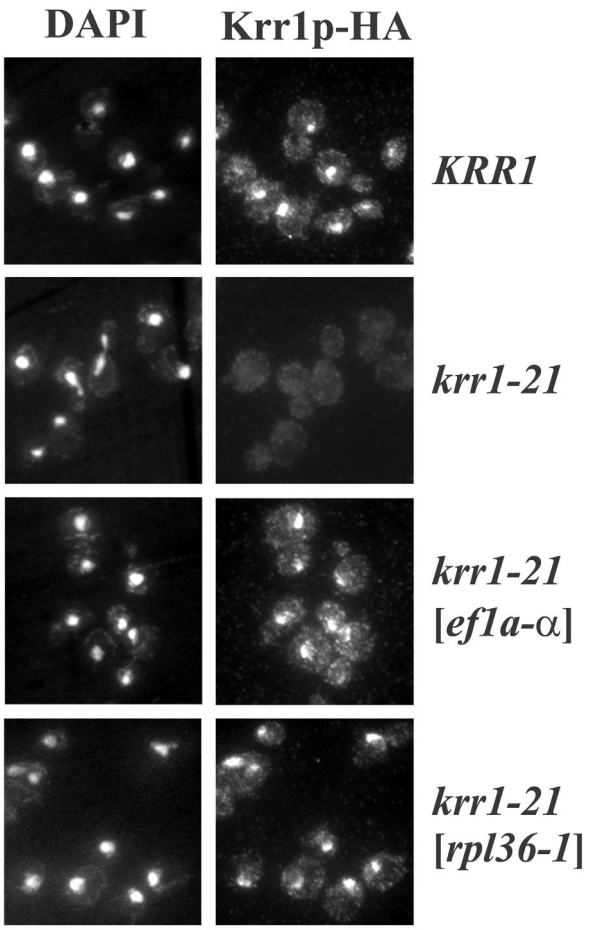

Figure 3. Nuclear localization of Krr1-21p in strains bearing suppressor genes.

The same fields were viewed for DNA by DAPI staining (left) and for HA-Krr1-21p by anti-HA antibody fluorescence (right). Cells of strain *trp1-1::TRP1::* P_{KRR1}*-HA-krr1-21 krr1*Δ*::HIS3* bearing indicated suppressor genes were incubated with mouse anti-HA monoclonal antibody (clone 16B12, BabCo, at 1:750 dilution) followed by $Cy3^{-}$ conjugated goat anti-mouse antibody (Jackson ImmunoResearch Lab, at 1:250 dilution). The samples were taken at early log phase of growth and were viewed at 600 × magnification in a Mikrophot-SA microscope equipped with filters for epifluorescence.

also, using the two hybrid system we found an interaction between Krr1p and Kap95p, which is a protein involved in nuclear import (Gromadka, unpublished).

As shown in Table 4, three clones contained the ORF SPBC1347.13c. There are no experimental data concerning its function. However, on the basis of a rather weak similarity of the ORF's product (37%) to Pet56p of *S. cerevisiae* encoding methyl transferase required for mitochondrial ribosome assembly (Sirum-Connolly & Mason, 1995), it was proposed that ORF SPBC1347.13c encodes a putative ribose methyltransferase. In rRNAs methylation of the ribose moiety at the 2′-hydroxyl group and base methylation are the prevalent nucleotide modifications. After transcription is completed, approximately 65 methyl groups are added to the 35S rRNA (Kiss-Laszlo *et al.*, 1996; Lafontaine *et al.*, 1998; Kressler *et al.*, 1999). Although it is assumed that C/D-box snoRNPs are required for 2′-O-ribose methylation, the enzyme(s) catalyzing this reaction in yeast have not been identified yet. So far, from all the proteins identified on the basis of amino-acid sequence as putative methyltransferases only Nop1p (*YDL014w*) has been described as required for overall 2′-O-methylation (Tollervey *et al.*, 1993). Finding a gene encoding a putative ribose methyltransferase as a suppressor of *krr1-21* mutation may indicate an involvement of the protein encoded by the ORF SPBC1347 in the process of rRNA maturation.

Efficiency of transcription is affected in *krr1-21* mutant

The most likely interpretation of the low level of Krr1-21p is that RKKPKK deletion led to a defect in the transport of the protein into the nucleus and to consequent degradation of the synthesized but incorrectly localized protein. If such were the case, the levels of the *KRR1* transcript should not differ between the wild type and mutant cells. As shown in Fig. 2 the decreased amount of Krr1-21p correlated with a decreased level of *krr1-21* transcript. Therefore it was important to determine whether the mutation led to a defective, unstable mRNA, as the process of mRNA decay is considered an important point in gene expression.

1,10-Phenanthroline was used to block transcription in order to compare the stability of *KRR1* mRNA in wild type and in *krr1-21* cells and in transformants bearing the *S. pombe* suppressor genes *rpl36-1* and *ef1a-a* encoding the 60S ribosomal protein L36A and EF1-α translation elongation factor, respectively (Table 4). The amount of the transcript present as a function of time after inhibitor addition was determined by Northern blotting. Since 1,10-phenanthroline inhibits transcription of most genes (Parker *et al.*, 1991), blots were probed for *KRR1* mRNA and mRNAs of the ribosomal genes *RPS3, RPS11* and *RPL10*. The 40S ribosomal protein Rps11 was found associated with TAP-tagged Krr1p (see below). Rps3p has been identified in this system by Grandi *et al.* (2002) and Rpl10p represents a 60S ribosomal protein not found in association with Krr1p (negative control). *ACT1* mRNA (actin) was used as a control. As shown in Fig. 4 the *krr1-21* mRNA is barely visible. To detect this transcript the amount of total RNA loaded into the gel was four times higher than in other samples. Despite overloaded samples the kinetics of *krr1-21* mRNA decay could not be followed. Interestingly, the decreased level of *krr1-21* mRNA was accompanied by some decrease in the time 0 level of the ribosomal mRNAs tested. However, their decay kinetics seemed to be unaffected. The suppressors restored simultaneously the wild type levels of *krr1-21*and *RPS11, RPS3*, and *RPL10* mRNAs. From the presented data it appears that the suppressors isolated increased the efficiency of transcription of *krr1-21* and of the tested genes encoding ribosomal proteins.

Table 5. Ribosomal proteins associated with TAP-tagged Krr1p

	Ribosomal proteins																					Ref.
	Rps – 40S submit													Rpl – 60S submit								
	0A	SOA	Ia	3	4a/b	5	7a	8a/b	11a/b	13	24	25	4a	7b	8	11a	23	25	28			
A			+		+			+	+	+	+	+	+	+	+		+	+	+	This study		
B	+	+		+	+	+	+	+					+			+				Grandi *et al.* (2002).		

A. This work; Krr1p was tagged by insertion of in frame fusion of *KRR1* and TAP tag into the *KRR1* locus and expressed from the endogenous promoter. Purified proteins obtained from TAPs were resolved on 12% SDS/PAGE gels. Eluted proteins were analyzed by mass spectroscopy (see Material and Methods). **B.** data from Grandi *et al.* (2002).

An additional genetic experiment was performed to confirm that the suppressors isolated did not act by stabilization of *krr1-21* mRNA. A strain was constructed in which *KRR1* coding sequence was put under the control of the conditional UAS_{GAL10}-P_{CYC1}

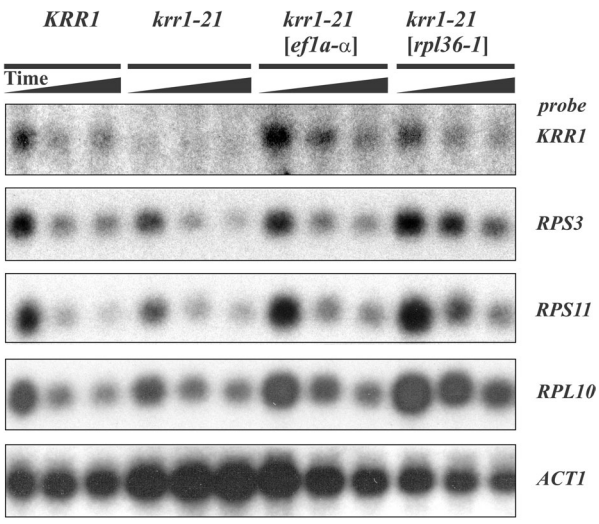

Figure 4. The suppressors increase the efficiency of transcription of *krr1-21* and the tested genes encoding ribosomal proteins.

Northern blot of RNA isolated from strains: *KRR1*; *krr1-21*; *krr1-21* p[P_{PGK} *ef1a-*α^+]; *krr1-21* p[P_{PGK} *rpl3601*$^+$]. After addition of 1,10-phenantroline samples were drawn at time 0, 10 and 30 min. Total RNAs (20 μg of *krr1-21* samples and 5 μg of the remaining samples) were separated on 1.2% agarose gel and analyzed by hybridization with indicated probes specific for mRNA coded by *KRR1, RPS3, RPS11* and *RPL10*.

promoter combined with a ubiquitin-dependent degradation signal [pRG38, UAS-$_{GAL10}$-P$_{CYC1}$-*UBI-HA-KRR1*]. In this system expression of *KRR1* is induced by galactose and repressed by glucose. The *krr1*Δ::*HIS3* strain bearing the UAS$_{GAL10}$-P$_{CYC1}$-UBI-HA-KRR1 construct grew normally compared with isogenic *KRR1* strain in galactose medium, whereas it did not grow in glucose medium (Gromadka & Rytka, 2000b). The GR19/2 strain (relevant genotype *krr1*Δ::*HIS3* [pRG38]) was transformed with the *S. pombe* suppressors SPAC24H6.07, SPCC970.05, or SPCC794.09c, respectively (Table 4). The transformants were selected on galactose medium. When transferred onto glucose plates, similarly to their untransformed parent, they did not grow (not shown). In this experiment the suppressors would have alleviated the growth defect if they had stabilized the *KRR1* mRNA. This result is in agreement with the measurement of *KRR1* and *krr1-21* mRNAs decay rates.

The synthesis of ribosomal proteins and of proteins involved in the processing and assembly of ribosomes is precisely coordinated and balanced with the synthesis of rRNA. In *S. cerevisiae*, cells change the intensity of production of new ribosomes in response to changes of growth conditions. There is a tight coupling between ribosome content and growth rate. Under conditions in which the demand for protein synthesis is reduced, the concentration of ribosomes within the cell can decrease several fold (Warner, 1999; Planta, 1997; Li *et al.*, 1999). A growing body of evidence indicates that the synthesis of ribosomal components is primarily regulated at the level of transcription. The efficient transcription of most of the RP genes requires Rap1p, a multifunctional, sequence specific DNA binding protein (Warner, 1999; Planta, 1997; Lieb *et al.*, 2001). According to data presented by Lieb *et al.* (2001) Rap1p binds to the promoters of 362 ORFs and its main targets are the RP genes (122 of 137). However, that genome-wide search for the promoters of genes binding Rap1p failed to identify *KRR1* (Lieb *et al.*, 2001). We have inspected the promoter region of the *KRR1* gene and searched for homology with the MR2 consensus sequence (Lascaris *et al.*, 1999) by the GAP program, part of GCG package. At position –258 of the *KRR1* promoter region a putative Rap1p binding site was found showing 64.3% identity to the MR2 consensus sequence. A comparison of the promoter regions of *KRR1* and the studied genes (encoding ribosomal proteins and translation elongation factor), presented in Fig. 5, suggests that the observed transcriptional coregulation of *KRR1* and ribosomal proteins might be mediated by the Rap1 protein. Although the main transcription factors, Rap1p and Abf1p, involved in the expression of RP genes have been identified, the mechanism of the signal transduction pathways that underlie the regulation in response to varying growth conditions

RPL10	-274	TTT **TACACC***T***GTACATC** TTT	92.8% identity
RPL36A	-168	TTT **TACACCCGTACATT** TCA	100% identity
RPS11A	-388	TAT **TACACCCA***AT***CATT** CAG	85.7% identity
RPS11B	-294	TTC **TAAACCCA***A***ACAT***G* TTT	85.7% identity
RPS3	-263	CGT **AACATCCATAC***C***TT** TCC	92.8% identity
RPS9A	-248	CTG ***C*ACACCCAT***G*CATC** ATT	85.7% identity
TEF1	-338	CAA **AACACCCA***AG***CA***CA* GCA	71.4% identity
KRR1	-258	GCC **AACACC***A***A***C***A***TTC***T** TGC	64.3% identity
MR2 consensus		**WACAYCCRTACATY**	

Figure 5. Comparison of promoter regions of studied genes to MR2 consensus sequence.

MR2 – DNA-binding sequence for Rap1p (Lascaris *et al.*, 1999); in bold – matches to the MR2 consensus; in italics – mismatches with the MR2 consensus.

remains largely obscure (references in Fourel *et al.*, 2002).

Krr1p physically interacts with ribosomal proteins

To further investigate the possible interactions of Krr1p we attempted to identify proteins that physically interact with Krr1p. For this purpose we employed the stringent tandem affinity purification (TAP) method. In order to purify and characterize a Krr1p-containing complex, we used the homozygous diploid *KRR1*-TAP/*KRR1*-TAP strain in which a TAP tag was introduced in-frame at the 3' end of *KRR1* under the control of its own promoter. The tagged strain grew at a rate identical to the wild-type strain, indicating that the tag does not measurably affect the function of Krr1p. The TAP-tagged version of Krr1p was purified under native conditions by two successive affinity purification steps according to Rigaut *et al.* (1999). The proteins obtained were resolved on 12% SDS/PAGE gels and identified by mass spectroscopy. As shown in Table 5 A, among the proteins reproducibly copurifying with Krr1p seven ribosomal proteins of the 40S subunit (Rps 1a, 4a/b, 8a/b, 11a/b, 24, 25) and six of the 60S subunit (Rpl 4a, 7b, 8, 23, 25, 28) were identified. In addition to the ribosomal proteins we found Kri1p as physically interacting with Krr1p. This finding is consistent with the data of Sasaki *et al.* (2000) indicating that Krr1p and Kri1p form a complex. The proteins detected in the absence of TAP tagged Krr1p and when TAP tagged Ccz1p was used as a bait were classified as non-specific contaminants. As shown in Table 5, in our experiments, except for three proteins (Rps 4 a/b and 8a/b and Rpl 4a), Krr1p copurified with a different set of ribosomal proteins than that identified by Grandi *et al.* (2002). Interestingly, among the proteins found as copurifing with Krr1p were two proteins, Rps 3 and Rps7 , orthologs of *S. pombe* proteins rps 3 and rps 7 that we identified as suppressors of the *krr1-21* mutation.

In a global analysis of 90S pre-ribosomes (Grandi *et al.*, 2002) eight nonribosomal proteins coprecipitated with Krr1p. Besides two completely unknown ORFs, according to the data presented in SGD the others are components of small nucleolar U3 ribonucleoprotein complex, a part of small ribosomal subunit processome (SSU). In contrast, in the data presented by Dragon *et al.* (2002) among the 28 proteins identified in the SSU complex, essential for growth and required for 18S rRNA maturations, these authors did not find Krr1p. The diversity in proteins found as interacting genetically or physically with Krr1p may result from the differences in the experimental design.

In conclusion, the essential and evolutionarily conserved Krr1p must be considered a multifunctional protein. Physically associated with pre-ribosomes, it appears as an element of the protein complex involved in rRNA processing and regulation of ribosome biogenesis. In our previous work we documented that the pattern of *KRR1* expression mirrors that of the RP genes during cell growth and is characterized by high transcription rate during exponential growth (Gromadka & Rytka, 2000b), which is a common characteristic of genes identified as targets of the transcription factor Rap1p (Fourel *et al.*, 2002). From the present data it appears that depletion of the Krr1 protein leads to the cessation of cell division not only due to defective rRNA processing causing severe reduction of 40S ribosomal subunits but also to declined transcription of ribosomal genes. Grandi *et al.* (2002) reported that in *krr1 ts* mutants nuclear export of 40S subunit was impaired. The nuclear localization of Krr1p together with the detected physical association with ribosomal proteins indicates that Krr1p may accompany pre-ribosomes to the nuclear pore. At the present stage we search for Krr1p interactions using two hybrid sys-

tem. Although the results are of a preliminary character, two proteins interacting with Krr1, Mtr10 and Kap95, involved in nuclear transport (Pemberton *et al.*, 1997; Iovine & Wente, 1997) were found by two-hybrid screening.

We thank Dr. Chris Herbert for critical reading of the manuscript and Krzysztof Flis for help in preparation of figures.

REFERENCES

Ausubel FA, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA, Struhl K. (1987) *Current Protocols in Molecular Biology*, vols 1–3, Green Publishing and Wiley-Interscience, New York.

Devereux J, Haeberli P, Smithies O. (1984) A comprehensive set of sequence analysis programs for the VAX. *Nucleic Acids Res.*; **12**: 387–95.

Dragon F, Gallagher JE, Compagnone-Post PA, Mitchell BM, Porwancher KA, Wehner KA, Wormsley S, Settlage RE, Shabanowitz J, Osheim Y, Beyer AL, Hunt DF, Baserga SJ. (2002) A large nucleolar U3 ribonucleoprotein required for 18S ribosomal RNA biogenesis. *Nature.*; **417**: 967–70.

Fourel G, Miyake T, Defossez P-A, Li R, Gilson E. (2002) General regulatory factors (GRFs) as genome partitioners. *J Biol Chem.*; **277**: 41736–43.

Geerlings TH, Vos JC, Raue HA. (2000) The final step in the formation of 25S rRNA in *Saccharomyces cerevisiae* is performed by 5′→3′ exonucleases. *RNA.*; **6**: 1698–703.

Gietz RD, Woods RA. (1998) Transformation of yeast by the lithium acetate/single-stranded carrier DNA/PEG method. *Methods Microbiol.*; **26**: 53–66.

Grandi P, Rybin V, Bassler J, Petfalski E, Strauss D, Marzioch M, Schafer T, Kuster B, Tschochner H, Tollervey D, Gavin AC, Hurt E. (2002) 90S pre-ribosomes include the 35S pre-rRNA, the U3 snoRNP, and 40S subunit processing factors but predominantly lack 60S synthesis factors. *Mol Cell.*; **10**: 105–15.

Gromadka R, Kaniak A, Slonimski PP, Rytka J. (1996) A novel cross-phylum family of proteins comprises a *KRR1* (*YCL059c*) gene which is essential for viability of *Saccharomyces cerevisiae* cells. *Gene.*; **171**: 27–32.

Gromadka R, Rytka J. (2000a) Ribosome biogenesis and nucleolar function in yeast. *Cell Mol Biol Lett.*; **5**: 191–216.

Gromadka R, Rytka J. (2000b) The *KRR1* gene encodes a protein required for 18S rRNA synthesis and 40S ribosomal subunit assembly in *Saccharomyces cerevisiae*. *Acta Biochim Polon.*; **47**: 993–1005.

Grosshans H, Hurt E, Simos G. (2000a) An aminoacylation-dependent nuclear tRNA export pathway in yeast. *Genes Dev.*; **14**: 830–40.

Grosshans H, Simos G, Hurt E. (2000b) Transport of tRNA out of the nucleus-direct channeling to the ribosome? *J Struct Biol.*; **129**: 288–94.

Guarente L, Lalonde B, Gifford P, Alani E. (1984) Distinctly regulated tandem upstream activation sites mediate catabolite repression of the *CYC1* gene of *S. cerevisiae*. *Cell.*; **36**: 503–11.

Hoffman ChS, Winston F. (1987) A ten minutes DNA preparation from yeast efficiently releases autonomous plasmids for transformation of *Escherichia coli*. *Gene.*; **57**: 267–72.

Iovine MK, Wente SR. (1997) A nuclear export signal in Kap95p is required for both recycling the import factor and interaction with the nucleoporin GLFG repeat regions of Nup16p and Nup100p. *J Cell Biol.*; **137**: 797–811.

Kiss-Laszlo Z, Henry Y, Bachellerie JP, Caizergues-Ferrer M, Kiss T. (1996) Site-specific ribose methylation of preribosomal RNA: a novel function for small nucleolar RNAs. *Cell.*; **85**: 1077–88.

Kondoh H, Yuasa T, Yanagida M. (2000) Mis3 with a conserved RNA binding motif is essential for ribosome biogenesis and implicated in the start of cell growth and S phase checkpoint. *Genes Cells.*; **5**: 525–41.

Kressler D, Linder P, de La Cruz J. (1999) Protein trans-acting factors involved in ribosome biogenesis in *Saccharomyces cerevisiae*. *Mol Cell Biol.*; **19**: 7897–912.

Kressler D, Rojo M, Linder P, de la Cruz J. (1999) Spb1 is a putative methyltransferase required for 60S ribosomal subunit biogenesis in *Saccharomyces cerevisiae*. *Nucleic Acids Res.*; **27**: 4598–608.

Lafontaine DL, Preiss T, Tollervey D. (1998) Yeast 18S rRNA dimethylase Dim1p: a quality control mechanism in ribosome synthesis? *Mol Cell Biol.*; **18**: 2360–70.

Lascaris RF, Mager WH, Planta RJ. (1999) DNA-binding requirements of the yeast protein Rap1p as selected *in silico* from ribosomal protein gene promoter sequences. *Bioinformatics.*; **15**: 267–77.

Le Gouill C, Dery CV. (1991) A rapid procedure for the screening of recombinant plasmids. *Nucleic Acids Res.*; **19**: 6655.

Li B, Nierras CR, Warner JR. (1999) Transcriptional elements involved in the repression of ribosomal protein synthesis. *Mol Cell Biol.*; **19**: 5393–404.

Lieb JD, Liu X, Botstein D, Brown PO. (2001) Promoter-specific binding of Rap1 revealed by genome-wide maps of protein-DNA association. *Nat Genet.*; **28**: 327–34.

Marck C. (1988) 'DNA Strider': a 'C' program for the fast analysis of DNA and protein sequences on the Apple Macintosh family of computers. *Nucleic Acids Res.*; **16**: 1829–36.

Munshi R, Kandl KA, Carr-Schmi A, Whitacre JL, Adams AEM, Kinzy TG. (2001) Overexpression of translation elongation factor 1A affects the organization and function of the actin cytoskeleton in yeast. *Genetics.*; **157**: 1425–36.

Parker R, Herrick D, Peltz SW, Jacobson A. (1991) A measurement of mRNA decay rates in *Saccharomyces cerevisiae*. *Methods Enzymol.*; **194**: 415–23.

Pederson T. (1998) The plurifunctional nucleolus. *Nucleic Acids Res.*; **26**: 3871–6.

Pemberton LF, Rosenblum JS, Blobel G. (1997) A distinct and parallel pathway for the nuclear import of an mRNA-binding protein. *J Cell Biol.*; **139**: 1645–53.

Planta RJ. (1997) Regulation of ribosome synthesis in yeast. *Yeast.*; **13**: 1505–18.

Rigaut G, Shevchenko A, Rutz B, Wilm M, Mann M, Seraphin B. (1999) A generic protein purification method for protein complex characterization and proteome exploration. *Nat Biotechnol.*; **17**: 1030–2.

Rose MD, Winston F, Hieter P. (1990) *Methods in Yeast Genetics: A Laboratory Course Manual*. Cold Spring Harbour Laboratory, CSH NY.

Rout MP, Aitchinson JD, Suprapto A, Hjertaas K, Zhao Y, Chait BT. (2000) The yeast nuclear pore complex: composition, architecture, and transport mechanism. *J Cell Biol.*; **148**: 635–51.

Samarsky DA, Fournier MJ. (1999) A comprehensive database for the small nucleolar RNAs from *Saccharomyces cerevisiae*. *Nucleic Acids Res.*; **27**: 161–4.

Sambrook J, Fritsch EF, Maniatis T. (1989) *Molecular Cloning: A Laboratory Manual*, 2nd edn. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Sasaki T, Toh-E A, Kikuchi Y. (2000) Yeast Krr1p physically and functionally interacts with a novel essential Kri1p, and both proteins are required for 40S ribosome biogenesis in the nucleolus. *Mol Cell Biol.*; **20**: 7971–9.

Sherman F. (1991) Getting started with yeast. *Methods Enzymol.*; **194**: 3–21.

Sikorski RS, Hieter P. (1989) A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. *Genetics.*; **122**: 19–27.

Sirum-Connolly K, Mason TL. (1995) The role of nucleotide modifications in the yeast mitochondrial ribosome. *Nucleic Acids Symp Ser.*; **33**: 73–5.

Smith CM, Steitz JA. (1997) Sno storm in the nucleolus: new roles for myriad small RNPs. *Cell.*; **89**: 669–72.

Suda M, Fukui M, Sogabe Y, Sato K, Morimatsu A, Arai R, Motegi F, Miyakawa T, Mabuchi I, Hirata D. (1999) Overproduction of elongation factor 1alpha, an essential translational component, causes aberrant cell morphology by affecting the control of growth polarity in fission yeast. *Genes Cells.*; **4**: 517–27.

Thomas BJ, Rothstein R. (1989) The genetic control of direct-repeat recombination in *Saccharomyces*: the effect of *rad52* and *rad1* on mitotic recombination at *GAL10,* a transcriptionally regulated gene. *Genetics.*; **123**: 725–38.

Tollervey D, Lehtonen H, Jansen R, Kern H, Hur E. (1993) Temperature-sensitive mutations demonstrate roles for yeast fibrillarin in pre-rRNA processing, pre-rRNA methylation, and ribosome assembly. *Cell.*; **72**: 443–57.

Venema J, Tollervey D. (1999) Ribosome synthesis in *Saccharomyces cerevisiae. Annu Rev Genet.*; **33**: 261–311.

Verschoor A, Warner JR, Srivastava S, Grassucci RA, Frank J. (1998) Three-dimensional structure of the yeast ribosome. *Nucleic Acids Res.*; **26**: 655–61.

Warner JR. (1989) Synthesis of ribosomes in *Saccharomyces cerevisiae. Microbiol Rev.*; **53**: 256–71.

Warner JR. (1999) The economics of ribosome biosynthesis in yeast. *Trends Biochem Sci.*; **24**: 437–40.