Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical and Biophysical Research Communications 337 (2005) 89–94
BBRC
www.elsevier.com/locate/ybbrc

Sen34p depletion blocks tRNA splicing in vivo and delays rRNA processing

Viviana Volta [a], Marcello Ceci [a,1], Bertrand Emery [b], Angela Bachi [c], Elisabeth Petfalski [d], David Tollervey [d], Patrick Linder [c], Pier Carlo Marchisio [a], Simonetta Piatti [e], Stefano Biffo [a,f,*]

[a] *Molecular Histology and Cell Growth, DIBIT-HSR, 20132 Milan, Italy*
[b] *Department of Microbiology and Molecular Medicine, CMU, CH 1211 Genève, Switzerland*
[c] *Mass Spec Unit, DIBIT-HSR, 20132 Milan, Italy*
[d] *Wellcome Trust Centre for Cell Biology, University of Edinburgh, Edinburgh EH93JR, UK*
[e] *Department of Biotechnology and Biosciences, University of Bicocca, 20126 Milano, Italy*
[f] *DISAV, University of Eastern Piedmont, 15100 Alessandria, Italy*

Received 2 September 2005
Available online 13 September 2005

Abstract

Tif6p (eIF6) is necessary for 60S biogenesis, rRNA maturation and must be released from 60S to permit 80S assembly and translation. We characterized Tif6p interactors. Tif6p is mostly on 66S–60S pre-ribosomes, partly free. Tif6p complex(es) contain nucleo-ribosomal factors and Asc1p. Surprisingly, Tif6p particle contains the low-abundance endonuclease Sen34p. We analyzed Sen34p role on rRNA/tRNA synthesis, in vivo. Sen34p depletion impairs tRNA splicing and causes unexpected 80S accumulation. Accordingly, Sen34p overexpression causes 80S decrease and increased polysomes which suggest increased translational efficiency. With delayed kinetics, Sen34p depletion impairs rRNA processing. We conclude that Sen34p is absolutely required for tRNA splicing and that it is a rate-limiting element for efficient translation. Finally, we confirm that Tif6p accompanies 27S pre-rRNA maturation to 25S rRNA and we suggest that Sen34p endonuclease in Tif6p complex may affect also rRNA maturation.

Keywords: eIF6 (alias p27BBP); Pre-rRNA; 60S; eIF3; RACK1

Ribosomal biogenesis leads to synthesis of mature 60S and 40S subunits from a common 90S precursor. In *Saccharomyces cerevisiae*, the 60S ribosome is formed by 46 proteins, and 3 rRNAs, 5S, 5.8S, and 25S; the 40S subunit is formed by 32 proteins and the 18S rRNA. In the nucleolus, rDNA is transcribed into rRNA precursors (pre-rRNAs). RNA polymerase III transcribes 5S rRNA and tRNAs. RNA polymerase I transcribes 35S pre-rRNA which is further processed by endo- and exo-nucleases to three mature rRNAs: 5.8S, 25S (60S), and 18S rRNA (40S). In synthesis, ribosome maturation requires the dynamic assembly of pre-ribosomal particles containing trans-acting factors in ribosome biogenesis, nucleases, and ribosomal proteins forming complexes at different stages (reviewed in [1]). Endonucleases are still undefined. So far, affinity purification of pre-ribosomal particles has not led to endonuclease enrichment, possibly due to their rapid off-rate from pre-ribosomes.

Tif6p is the yeast homologue of eukaryotic initiation factor 6 (eIF6), originally described as a cytoplasmic factor preventing 60S subunit association with 40S [2]. Release of eIF6/Tif6p from 60S requires RACK1-PKC in mammals [3] and Efl1p in yeasts [4]. In mammals, eIF6 is mainly cytoplasmic (p27BBP, [5]) and approximately

* Corresponding author.
E-mail address: biffo.stefano@hsr.it (S. Biffo).
[1] Present address: IRCSS San Raffaele of Rome, 00128 Rome.

0006-291X/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2005.09.012

25% in the nucleolus [6]. Unequivocal evidence in yeast indicates that Tif6p is necessary for 60S biogenesis [6–8]. We decided to gain information on Tif6p nucleolar role, by analyzing its interactors. Besides ribosomal factors and four cytoplasmic proteins, we unexpectedly found Sen34p endonuclease [9]. Earlier biochemical work showed that Sen34p endonuclease is part of a tRNA splicing holoenzyme [9]. We characterized the role of Sen34p on RNA metabolism, in vivo.

Here we show that Sen34p depletion causes, in vivo, tRNA splicing impairment and 80S accumulation accompanied by flat polysomes, suggestive of a translational impairment. Accordingly, but rather surprisingly, Sen34p overexpression reduces 80S and increases polysomes, suggesting increased initiation of translation in the presence of excess Sen34p. Finally, Sen34p depletion leads also to a small impairment in rRNA processing, suggesting that its presence in the Tif6–66S complex may be associated to a role in rRNA maturation.

Materials and methods

Yeast manipulation. Standard genetic techniques were used to manipulate yeasts. All strains were derivatives of W303. *SEN34* deletion (*sen34::kanMX4*) was generated by one-step gene replacement [10] resulting in the diploid strain ySP2458. To generate a centromeric plasmid with a *GAL-SEN34* inducible fusion (pSP167), wild type *SEN34* was cloned in YCplac33. The ySP2458 strain was transformed with pSP167 and haploid segregants carrying both *sen34::kanMX4* and *GAL-SEN34* (ySP2539) were recovered upon sporulation and tetrad dissection. Obtained strains were called *GAL1::SEN34*. The TAP-tag cassette [11] was inserted in the TIF6 gene. The resulting strain had only tagged Tif6p.

The GST cassette was amplified from pFA6a-His3MX6 [12] and inserted downstream of GAL1 promoter. Oligonucleotides were designed to tag the N-terminus of *SEN34*. The resulting, viable, strain had no endogenous Sen34p, but only tagged Sen34p.

Antibodies. Nocs antibodies were from Dr. H. Tschochner [13]; Mpp10p antibodies from Dr. S. Baserga [14]. Antibodies against GST were from Massimiliano Pagani (Primm Biotech, Milan, Italy).

Polysome analysis and Western blotting. Yeast strains were grown until OD_{600} 0.6 was reached and cycloheximide was added to a final concentration of 100 μg/ml. Polysomal analysis and immunoblotting were performed as in [6]. To visualize ribosomal associations, fractions from polysomal profiles were analyzed with PAP (Tif6), anti-GST (Sen34p), and Mpp10. For Noc1p and Noc2p co-purification, extracts were purified by an immobilized calmodulin column. Eluted proteins were blotted with antibodies against Noc1 and Noc2. Detection was performed by chemiluminescence (ECL, Amersham).

Purification of complexes and protein identification. Complexes were purified according to [11], except for yeast grinding in liquid nitrogen and the lysis buffer (20 mM NaCl, 20 mM Tris–HCl, pH 7.5, 10% glycerol, 0.1% NP-40, and 1 mM PMSF). Eluted proteins were resolved on SDS–PAGE and visualized by silver staining. Bands were excised and digested as described elsewhere [15]. Proteins were unambiguously identified by searching a comprehensive non-redundant protein database using ProFound [16].

Pulse-chase and Northern blotting analysis of RNAs. Pulse-chase labelling of pre-rRNA was performed as in [17]. Northern blotting analysis was performed as in [18]. For Tif6p-associated pre-rRNA analysis, Tif6p-TAP was purified from extracts as above. Total RNA and Tif6p-associated RNAs were separated on agarose–formaldehyde gel and blotted. Oligonucleotides, 5′-labelled by polynucleotide kinase phosphorylation, were previously described [17,19].

Results

Tif6p complex characterization

Tif6p was fused to a TAP cassette at its C-terminus. The TIF6-TAP grew as the *wt*. Western blotting of polysomal fractions shows that Tif6p co-sediments with free 60S subunits and in the hydrosoluble phase. It was absent on polysomes (Fig. 1A), suggesting that it dissociates from mature 60S as described in mammals [3]. Next, we purified Tif6p and identified its interactors by MS. We consistently found (a) 60S ribosomal proteins, (b) trans-acting factors involved in 60S biogenesis, (c) Asc1p, (d) eIF3 subunits: Nip1p, Prt1p, and Rpg1p, and (e) Sen34p (Table 1). No 90S pre-ribosomal proteins were found. To define if 90S (40S/60S precursor) factors were sub-stoichiometrically associated with Tif6p, we performed Western blotting analysis of purified Tif6p with specific antibodies. Tif6p co-purified with Noc1p and Noc2p, markers of the transition 90S–66S [19], but not with Mpp10p, marker of 90S [14] (Fig. 1B). Consistently, Tif6p co-purified with 27S and 7S pre-rRNAs (Fig. 1C), but not with 35S. We unambiguously conclude that Tif6p is present on 66S pre-ribosomes throughout their maturation to 60S, absent from polysomes and 90S pre-ribosomal particle. Also, Tif6p can interact with cytoplasmic factors.

Tif6p is the first pre-ribosomal particle reported to contain an endonuclease (Sen34p). Sen34p was previously shown to be part of a tetrameric tRNA processing complex (composed by Sen54p and Sen15p as adaptors, and Sen2p and Sen34p as endonucleases) [9]. We did not detect any of the other three subunits in the Tif6p complex, due either to the detection limits of MS or to the presence of Sen34p as an active monomeric endonuclease. The in vivo global role of Sen34p in RNA maturation is not characterized. This fact, and its presence on the Tif6p complex led us to study the effects of Sen34p depletion on tRNA, rRNA maturation, and translation.

Sen34p-depleted cells show impairment of tRNA synthesis and delay of pre-rRNA maturation

We constructed a conditional GAL1::SEN34 strain expressing a galactose inducible *SEN34*. In non-permissive conditions (absence of galactose) this strain is not viable. Upon partial, progressive Sen34p depletion yeast cells showed a growth defect (Fig. 2A). FACS analysis showed that Sen34p depletion was associated with a cell cycle delay in G1 (Fig. 2B).

Next, we analyzed polysomal profiles in depletion conditions. After Sen34p depletion, cells presented a strong 80S accumulation (up to fivefold) and a decrease of polysomal peaks (Fig. 2C, bottom right). These results indicate that in the absence of Sen34p a block in initiation of translation occurs (see Discussion).

To analyze the role of Sen34p in tRNA and pre-rRNA processing, we performed pulse-chase experiments and

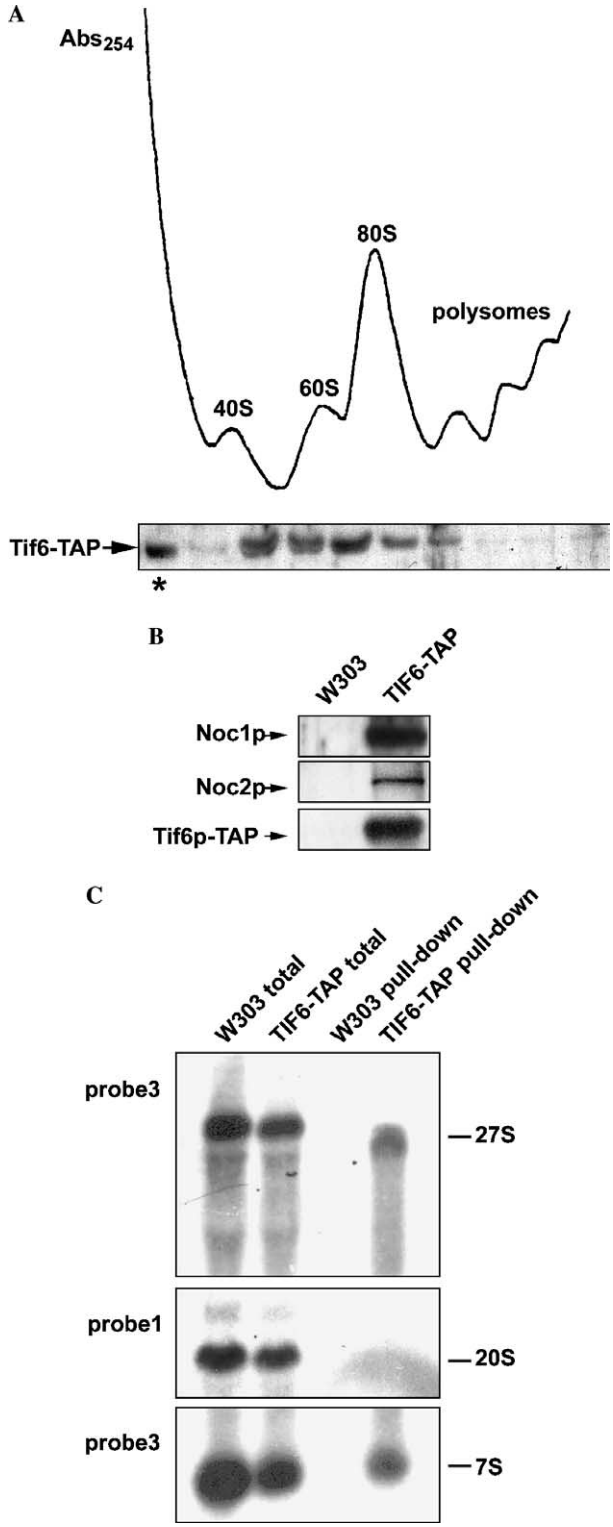

Fig. 1. Tif6p characterization. (A) Polysomal profile of TIF6-TAP strain. Immunoblotting for Tif6p performed on collected fractions shows its presence on 60S ribosomal subunits and in the hydrosoluble (*) fraction at the top of the gradient. (B,C) Tif6p interacts with Noc1p and Noc2p, and is on early 66S. (B) Tif6p pulldowns from the TIF6-TAP strain and parental W303 strain (negative control) were immunoblotted for Noc1p and Noc2p. (C) Tif6p associates with 27S and 7S rRNA, but not with 20S. Total RNA and TAP-purified RNA from W303 parental strain and TIF6-TAP were probed for 27S and 7S pre-rRNA intermediates (probe 3) and 20S intermediate (probe 1).

Table 1
Tif6p associates with pre-ribosomal particle and with cytoplasmic factors

Proteins	References
Ribosomal proteins	
Rpl11b	
Rpl18	
Rpl20	
Rpl9	
Rpl16	
Rpl13	
Rpl7b	
Rpl4b	
Rpl5	
Rpl4a	
Rpl3	
Ribosome biogenesis factors	
Loc1p	Long et al. [23], Harnpicharnchai et al. [24]
Mrt4p	Zuk et al. [25], Bassler et al. [26]
Tif6p	Basu et al. [8]
Rlp7p	Dunbar et al. [27], Gadal et al. [28]
Mak11p	Ohtake et al. [29]
Ytm1p	Harnpicharnchai et al. [24]
Has1p	Harnpicharnchai et al. [24], Fatica et al. [1]
Nug1p	Bassler et al. [26]
Dbp9p	Daugeron et al. [30]
Nop7p	Harnpicharnchai et al. [24]
Nog1p	Bassler et al. [26]
Noc3p	Milkereit et al. [13]
Erb1p	Pestov et al. [31]
Cytoplasmic proteins	
Asc1p	Chantrel et al. [21]
Nip1p	Phan et al. [32]
Prt1p	Phan et al. [32]
Rpg1p	Phan et al. [32]
Other proteins	
Ssa2p	Bush et al. [33]
Sen34p	Trotta et al. [9]

Northern blotting. In pulse-chase (Fig. 3A) Sen34p-depleted cells showed a strong delay and reduction in the processing of tRNA (Fig. 3A, lower panel). We also observed a delay in the processing of 35S and 27S rRNA that caused a reduction of mature 18S and 25S rRNA (Fig. 3A, upper panel). These data indicate that Sen34p depletion decreases the kinetics of tRNA and rRNA maturation.

Steady-state levels of rRNAs and $tRNA^{Leu}$ were assessed by Northern hybridization. Data show that after Sen34p depletion unspliced pre-$tRNA^{Leu}$ accumulates. With a delayed kinetics, accumulation of rRNA mature forms is also impaired, albeit to a minor extent (Fig. 3B). In agreement with pulse-chase experiments, these data indicate that $tRNA^{Leu}$ production is rapidly blocked upon Sen34p depletion and that with delayed timing also rRNA maturation is impaired.

To analyze if Sen34p cosedimented with ribosomes we constructed a yeast strain carrying an N-terminal tagged Sen34p protein integrated in the disrupted endogenous SEN34 gene. Yeasts were viable and no signs of degradation of tagged protein were found. Polysomal profiles show that in isotonic buffer conditions Sen34p cosediments with ribosomes (Fig. 4). Note that Sen34p overexpression alters

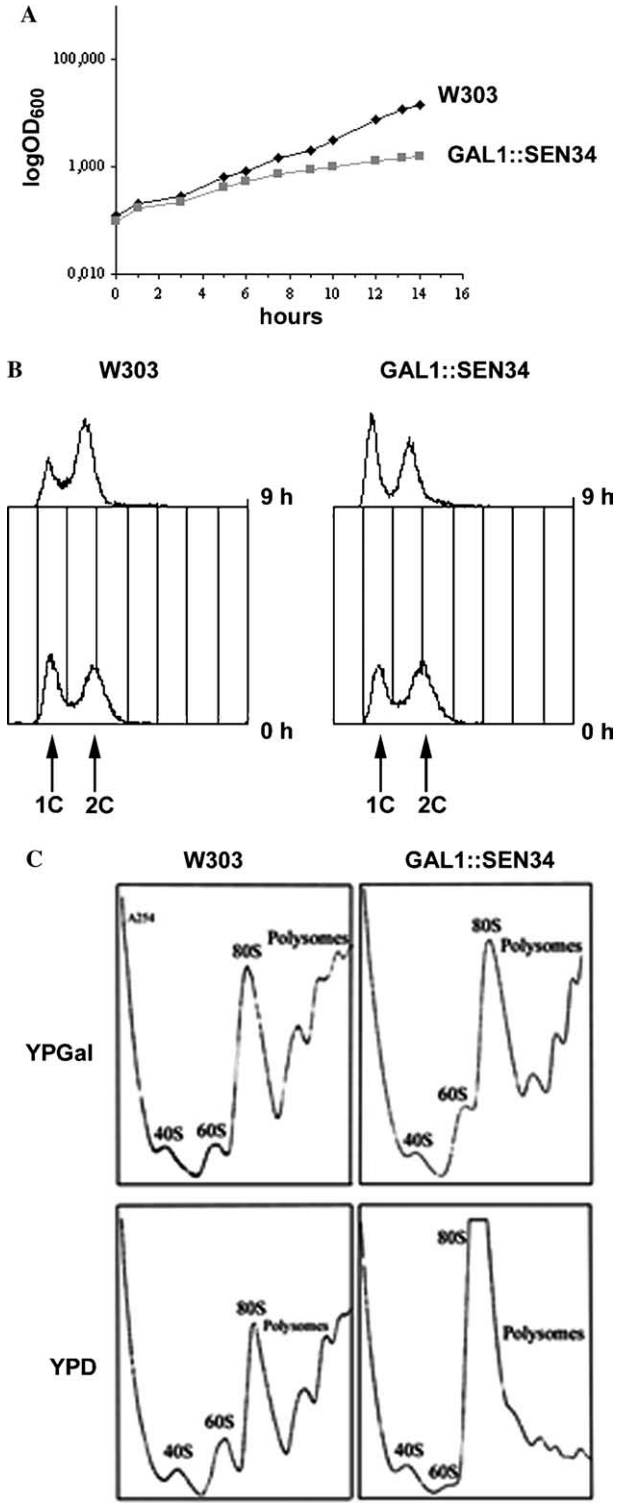

Fig. 2. Sen34p-depleted cells arrest in G1 and accumulate 80S. (A) GAL1::SEN34 strain carrying *SEN34* gene under the control of an inducible promoter, and W303 parental strain were grown in permissive medium and then shifted in non-permissive medium for the indicated hours. Growth in arbitrary units. (B) Sen34p-depleted cells arrest in G1. FACS analysis performed on Sen34p-depleted cells and on W303 parental strain before and after shift in non-permissive medium. (C) Translational block after Sen34p depletion. Polysomal profiles from GAL1::SEN34 and W303 parental strain in permissive medium (YPGal) and after 6 h shift in non-permissive medium (YPD). Accumulation of 80S and decreased polysomal area are observed upon Sen34p depletion.

80S/polysome ratio (lowering 80S peak as compared to polysomal area).

Discussion

We show for the first time the effect of Sen34p depletion on polysomal peaks. We unveiled that Sen34p depletion leads to 80S accumulation, which in turn is indicative of an initiation block. Initiation is a rate-limiting step of translation, strictly regulated by eIFs. An easy interpretation is that reduction in translation is due to a reduction in mature tRNA levels which somewhat signals to eIFs. However, most tRNAs including initiator $tRNA^{Met}$ are intronless and they are not directly affected by Sen34p depletion. Only three classes of tRNAs (Tyr, Trp, and Phe) are transcribed exclusively from intron-containing genes and require splicing for maturation. Thus, 80S accumulation would suggest that a block in tRNA splicing of a subset of tRNAs results in an active pathway depressing initiation. More polysomes in GST-Sen34p overexpressors indirectly confirm that Sen34p levels are important in regulating initiation of translation. A pathway linking tRNA maturation to control of initiation has not been described and deserves further characterization.

Tif6p is a conserved trans-acting factor in ribosome biogenesis. Its depletion leads to a delay in rRNA maturation [8], indicating that Tif6p complex hosts endonucleases. The presence of low-abundance (estimated 100 molecules/cell) Sen34p endonuclease in the Tif6p particle was intriguing. So far, trans-acting factors in ribosome biogenesis have been employed to purify ribosomal particles [1], but no endonucleases were co-purified, in spite of their (theoretical) expected presence. The copurification of Sen34p with Tif6p may be due to the unique structure of this complex or, alternatively, to the rapid, low temperature purification procedure we used. We failed to detect Tif6p in pull down of Sen34p, confirming the lability of the presence of Sen34p (and other endonucleases) in pre-ribosomal particles. Since we observed Sen34p in pre-ribosomal Tif6p particle, we analyzed the effects of its depletion in rRNA synthesis. Here, we observe, in the absence of Sen34p, a delay in rRNA processing, consistent with its detection in the Tif6p complex. The small effect on rRNA synthesis (compared to tRNA synthesis) of Sen34p depletion can be due to the fact that, in vivo, Sen34p steady-state levels are more critical for tRNA synthesis than rRNA synthesis. Alternatively, we cannot rule out that the rRNA deficit is an indirect effect of tRNA block (although at the time-points here used tRNA is still partly formed). We should however note that in human cells, downregulation of Sen2 and Sen34 impaired mRNA splicing [20], demonstrating that this endonuclease is not tRNA specific. In this context, the absence of reliable in vitro assays for rRNA processing (compared to tRNA or splicing) is a gap.

Among the interactors of Tif6, we found Asc1p and three eIF3 subunits. Asc1p is the yeast homolog [21] of

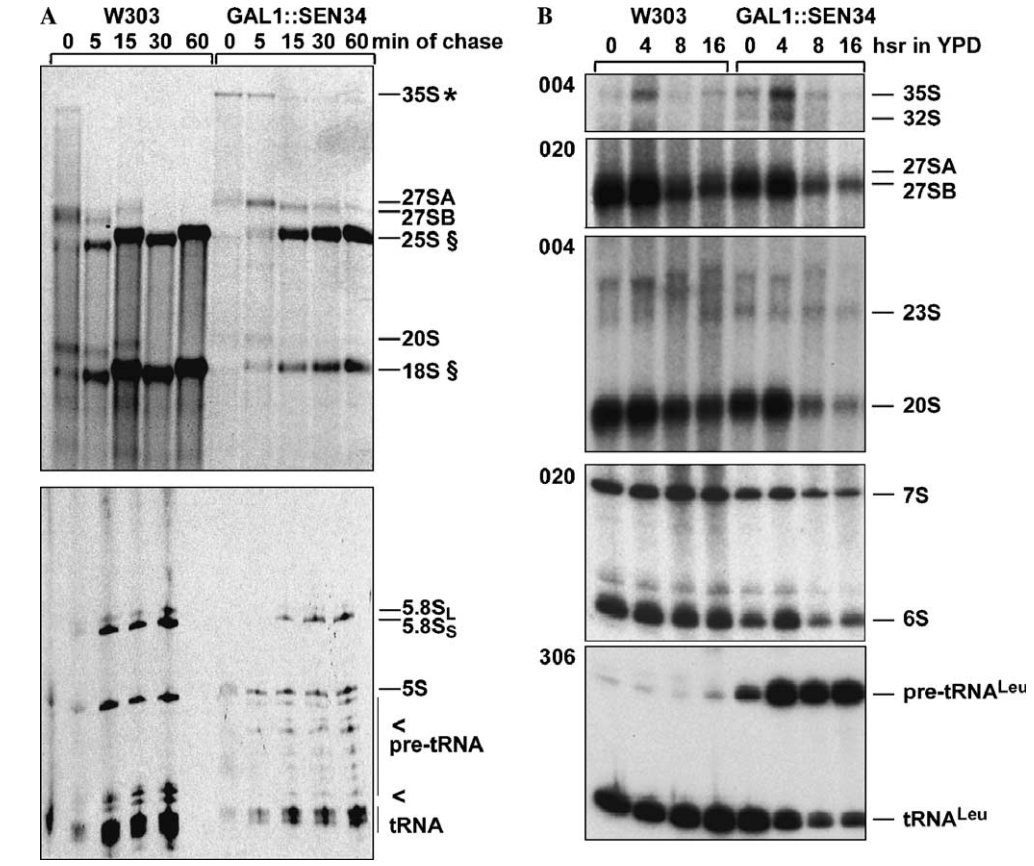

Fig. 3. Sen34p depletion blocks tRNA processing and delays rRNA maturation. (A) RNA pulse-chase on GAL1::SEN34 and W303 parental strain. Cells were pulse-labelled for 2 min and then chased for 5, 15, 30, and 60 min. RNA was separated either on agarose–formaldehyde gel (high molecular weight RNAs, top) or polyacrylamide–8 M urea gel (low molecular weight RNAs, bottom). Equal c.p.m. loaded in each lane. *Note.* delay of 35S processing (*), delay of mature rRNAs (§), and accumulation of unspliced tRNA (<) (B) Steady-state RNAs in Sen34p-depleted cells. Cells were grown in permissive medium and then shifted in non-permissive medium for 4, 8, and 16 h. RNA separated as in (A) and probed for pre-rRNA processing intermediates and pre-tRNA^{Leu} (indicated on the left of the panel). Note the rapid accumulation of unspliced pre-tRNA^{Leu} and the reduction of mature rRNA.

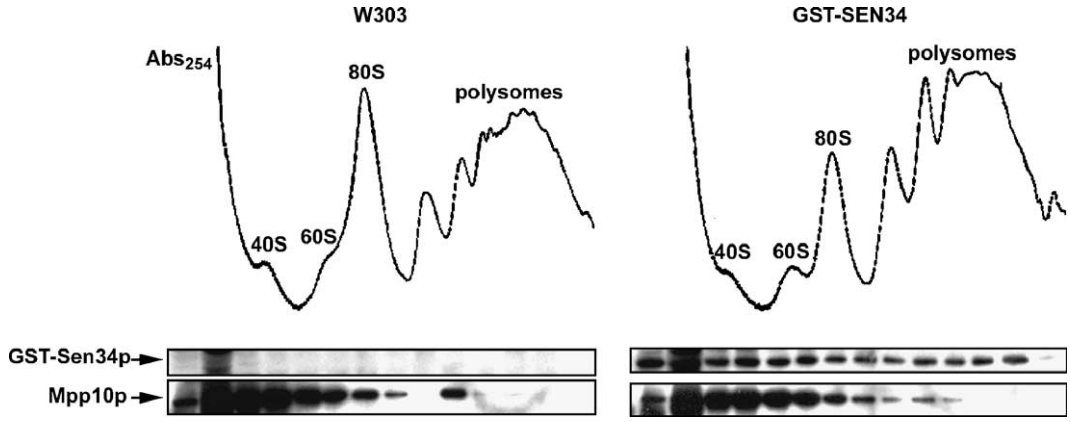

Fig. 4. Sen34p overexpression increases polysomal peaks. Polysomal profiles from wild type (left) and GST-SEN34 strain expressing inducible tagged Sen34p but not the endogenous protein (right) are shown. Fractions were collected and analyzed for GST-Sen34p (top) and Mpp10p (bottom). Representative results from multiple experiments. Note the localization of GST-Sen34 in polysomal fraction. Sen34p overexpression alters also 80S/polysome ratio (lowering 80S peak as compared to polysomal area).

RACK1 that in mammals contribute to eIF6 release from 60S [3]. Whether Asc1p interaction cooperates with Efl1p [4] to the release of Tif6p from yeast 60S remains to be studied.

eIF3 subunits Prt1p-Rpg1p-Nip1p are part of a subcomplex [22] stimulating binding of mRNA and tRNA(i)Met to ribosomes. The role of eIF6 in translation should be experimentally re-evaluated.

Acknowledgments

We acknowledge Susan Baserga, Mark Longtine, Massimilano Pagani, Herbert Tschochner, Alessandro Fatica, Annamaria Barbieri, and Leonardo Sala for reagents and advice. Work in the Linder lab is supported by Swiss National Science Foundation (PL). S.B. is supported by AIRC (Associazione Italiana Ricerca contro il Cancro).

References

[1] A. Fatica, D. Tollervey, Making ribosomes, Curr. Opin. Cell Biol. 14 (2002) 313–318.
[2] D.W. Russell, L. Spremulli, Mechanism of action of the wheat germ ribosome dissociation factor: interaction with the 60S subunit, Arch. Biochem. Biophys. 201 (1980) 518–526.
[3] M. Ceci, C. Gaviraghi, C. Gorrini, L.A. Sala, N. Offenhauser, P.C. Marchisio, S. Biffo, Release of eIF6 ($p27^{BBP}$) from the 60S subunit allows 80S ribosome assembly, Nature 426 (2003) 579–584.
[4] B. Senger, D.L.J. Lafontaine, J.-S. Graindorge, O. Gadal, A. Camasses, A. Sanni, J.-M. Garnier, M. Breitenbach, E. Hurt, F. Fasiolo, The nucle(ol)ar Tif6p and Efl1p are required for late cytoplasmic step of ribosome synthesis, Mol. Cell 8 (2001) 1363–1373.
[5] S. Biffo, F. Sanvito, S. Costa, L. Preve, R. Pignatelli, L. Spinardi, P.C. Marchisio, Isolation of a novel β4 integrin-binding protein ($p27^{BBP}$) highly expressed in epithelial cells, J. Biol. Chem. 272 (1997) 30314–30321.
[6] F. Sanvito, S. Dichieri, S. Piatti, A. Villa, M. Bossi, G. Lucchini, P.C. Marchisio, S. Biffo, The β4 integrin interactor $p27^{BBP/eIF6}$ is an essential nuclear matrix protein involved in 60S ribosomal subunit assembly, J. Cell Biol. 144 (1999) 823–837.
[7] K. Si, U. Maitra, The *Saccharomyces cerevisiae* homologue of mammalian translation initiation factor 6 does not function as a translation initiation factor, Mol. Cell. Biol. 19 (1999) 1416–1426.
[8] U. Basu, K. Si, J. Warner, U. Maitra, The *Saccharomyces cerevisiae* TIF6 gene encoding translation initiation factor 6 is required for 60S ribosomal subunit biogenesis, Mol. Cell. Biol. 21 (2001) 1453–1462.
[9] C.R. Trotta, F. Miao, E.A. Arn, S.W. Stevens, C.K. Ho, R. Rauhut, J.N. Abelson, The yeast tRNA splicing endonuclease: a tetrameric enzyme with two active site subunits homologous to the archaeal tRNA endonucleases, Cell 89 (1997) 849–858.
[10] A. Wach, A. Brachat, R. Pohlmann, P. Philippsen, New heterologous modules for classical or PCR-based gene disruptions in *Saccharomyces cerevisiae*, Yeast 10 (1994) 1793–1808.
[11] G. Rigaut, A. Shevchenko, B. Rutz, M. Wilm, M. Mann, B. Séraphin, A generic protein purification method for protein complex characterization and proteome exploration, Nat. Biotechnol. 17 (1999) 1030–1032.
[12] M.S. Longtine, A. McKenzie III, D.J. Demarini, N.G. Shah, A. Wach, A. Brachat, P. Philippsen, J.R. Pringle, Additional modules for versatile and economical PCR-based gene deletion and modification in *Saccharomyces cerevisiae*, Yeast 14 (1998) 953–961.
[13] P. Milkereit, O. Gadal, A. Podtelejnikov, S. Trumtel, N. Gas, E. Petfalski, D. Tollervey, M. Mann, E. Hurt, H. Tschochner, Maturation and intranuclear transport of pre-ribosome requires Noc proteins, Cell 105 (2001) 499–509.
[14] K.A. Wehner, J.E. Gallagher, S.J. Baserga, Components of an interdependent unit within the SSU processome regulate and mediate its activity, Mol. Cell. Biol. 22 (2002) 7258–7267.
[15] A. Shevchenko, M. Wilm, O. Vorm, M. Mann, Mass spectrometric sequencing of proteins from silver stained polyacrylamide gels, Anal. Chem. 68 (1996) 850–858.
[16] W. Zhang, B.T. Chait, ProFound: an expert system for protein identification using mass spectrometric peptide mapping information, Anal. Chem. 72 (2000) 2482–2489.
[17] D. Kressler, J. de la Cruz, M. Rojo, P. Linder, Fal1p is an essential DEAD-box protein involved in 40S-ribosomal-subunit biogenesis in *Saccharomyces cerevisiae*, Mol. Cell. Biol. 17 (1997) 7283–7294.
[18] M. Oeffinger, M. Dlakic, D. Tollervey, A pre-ribosome-associated HAET-repeat protein is required for export of both ribosomal subunits, Genes Dev. 18 (2004) 196–209.
[19] L. Pintard, D. Kressler, B. Lapeyre, Spb1p is a yeast nucleolar protein associated with Nop1p and Nop58p able to bind *S*-adenosyl-L-methionine in vitro, Mol. Cell. Biol. 20 (2000) 1370–1381.
[20] S.V. Paushkin, M. Patel, B.S. Furia, S.W. Peltz, C.R. Trotta, Identification of a human endonuclease complex reveals a link between tRNA splicing and pre-mRNA 3′ end formation, Cell 117 (2004) 311–321.
[21] Y. Chantrel, M. Gaisne, C. Lions, J. Verdiere, The transcriptional regulator Hap1p (Cyp1p) is essential for anaerobic or heme-deficient growth of *Saccharomyces cerevisiae*: genetic and molecular characterization of an extragenic suppressor that encodes a WD repeat protein, Genetics 148 (1998) 559–569.
[22] L. Phan, L.W. Schoenfeld, L. Valasek, K.H. Nielsen, A.G. Hinnebusch, A subcomplex of three eIF3 subunits binds eIF1 and eIF5 and stimulates ribosome binding of mRNA and $tRNA_i^{Met}$, EMBO J. 20 (2001) 2954–2965.
[23] R.M. Long, W. Gu, X. Meng, G. Gonsalvez, R.H. Singer, P. Chartrand, An exclusively nuclear RNA-binding protein affects asymmetric localization of ASH1 mRNA and Ash1p in yeast, J. Cell Biol. 153 (2001) 307–318.
[24] P. Harnpicharnchai, J. Jakovljevic, E. Horsey, T. Miles, J. Roman, M. Rout, D. Meagher, B. Imai, Y. Guo, C.J. Brame, J. Shabanowitz, D.F. Hunt, J.L. Woolford Jr., Composition and functional characterization of yeast 66S ribosome assembly intermediates, Mol. Cell. 8 (2001) 505–515.
[25] D. Zuk, J.P. Belk, A. Jacobson, Temperature-sensitive mutations in the *Saccharomyces cerevisiae* MRT4, GRC5, SLA2 and THS1 genes result in defects in mRNA turnover, Genetics 153 (1999) 35–47.
[26] J. Bassler, P. Grandi, O. Gadal, T. Lessmann, E. Petfalski, D. Tollervey, J. Lechner, E. Hurt, Identification of a 60S pre-ribosomal particle that is closely linked to nuclear export, Mol. Cell 8 (2001) 517–529.
[27] D.A. Dunbar, F. Dragon, S.J. Lee, S.J. Baserga, A nucleolar protein related to ribosomal protein L7 is required for an early step in large ribosomal subunit biogenesis, Proc. Natl. Acad. Sci. USA 97 (2000) 13027–13032.
[28] O. Gadal, D. Strauss, J. Kessl, B. Trumpower, D. Tollervey, E. Hurt, Nuclear export of 60s ribosomal subunits depends on Xpo1p and requires a nuclear export sequence-containing factor, Nmd3p, that associates with the large subunit protein Rpl10p, Mol. Cell. Biol. 21 (2001) 3410–3415.
[29] Y. Ohtake, R.B. Wickner, Yeast virus propagation depends critically on free 60S ribosomal subunit concentration, Mol. Cell Biol. 15 (1995) 2772–2781.
[30] M.C. Daugeron, D. Kressler, P. Linder, Dbp9p, a putative ATP-dependent RNA helicase involved in 60S-ribosomal-subunit biogenesis, functionally interacts with Dbp6p, RNA 7 (2001) 1317–1334.
[31] D.G. Pestov, M.G. Stockelman, Z. Strezoska, L.F. Lau, ERB1, the yeast homolog of mammalian Bop1, is an essential gene required for maturation of the 25S and 5.8S ribosomal RNAs, Nucleic Acids Res. 29 (2001) 3621–3630.
[32] L. Phan,, X. Zhang, K. Asano, J. Anderson, H.P. Vornlocher, J.R. Greenberg, J. Qin, A.G. Hinnebusch, Identification of a translation initiation factor 3 (eIF3) core complex, conserved in yeast and mammals, that interacts with eIF5, Mol. Cell Biol. 18 (1998) 4935–4946.
[33] G.L. Bush, D.I. Meyer, The refolding activity of the yeast heat shock proteins Ssa1 and Ssa2 defines their role in protein translocation, J. Cell Biol. 135 (1996) 1229–1237.