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Minireview

Nuclear export and cytoplasmic maturation of ribosomal subunits

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Abstract **Based on the characterization of ribosome precursor particles and associated *trans*-acting factors, a biogenesis pathway for the 40S and 60S subunits has emerged. After nuclear synthesis and assembly steps, pre-ribosomal subunits are exported through the nuclear pore complex in a Crm1- and RanGTP-dependent manner. Subsequent cytoplasmic biogenesis steps of pre-60S particles include the facilitated release of several non-ribosomal proteins, yielding fully functional 60S subunits. Cytoplasmic maturation of 40S subunit precursors includes rRNA dimethylation and pre-rRNA cleavage, allowing 40S subunits to achieve translation competence. We review current knowledge of nuclear export and cytoplasmic maturation of ribosomal subunits.**

Keywords: Ribosome biogenesis; rRNA; Nuclear export; Crm1

1. Introduction

Ribosomes are among the most fundamental molecular machines in all cells as they are required for protein synthesis. In total, the two ribosomal subunits in eukaryotes consist of four rRNAs and about 80 proteins, and their biogenesis is a highly complex process that involves more than 150 non-ribosomal proteins, the so-called *trans*-acting factors. Following the application of tandem affinity purification and mass spectrometry techniques to characterize pre-ribosomal particles, a model for the maturation pathway of ribosomes emerged (reviewed in [1]). In recent years, ribosome biogenesis and *trans*-acting factors have been intensely studied, and many aspects of this process have been excellently reviewed [1–7].

The synthesis of ribosomes starts with the transcription of the rRNA from rDNA tandem repeats. RNA polymerase III synthesizes the 5S rRNA, whereas RNA polymerase I transcribes a long precursor rRNA in the nucleoli. This pre-rRNA contains the mature 18S, 5.8S and 25S/28S rRNAs (in yeast/higher eukaryotes), flanked and separated by interspersed spacer sequences. Concomitant with rRNA transcription, the rRNA is modified by methylation and pseudouridylation reactions, catalyzed by a large number of snoRNP particles. The nascent pre-rRNA assembles with *trans*-acting factors and ribosomal proteins, mostly of the small subunit, to a 90S pre-ribosome, the first ribosome precursor that can be isolated. In a series of endo- and exonucleolytic processing events, the pre-rRNA is then cleaved into the mature rRNAs. Cleavage in the spacer region between the sequences of the 18S and the 5.8S rRNAs leads to separation of the 90S pre-ribosome into a pre-40S and a pre-60S particle. Subsequent maturation of the two subunit precursors is mostly independent, as interference with the biogenesis of one subunit in most cases still allows maturation of the other subunit, and only few factors are involved in both pathways (reviewed in [7]).

Following nucleolar assembly and nucleolar and nucleoplasmic maturation, pre-ribosomes are exported to the cytoplasm through the nuclear pore complex (NPC). The two subunit precursors are translocated as independent entities, and once the pre-60S and pre-40S particles reach the cytoplasm, they undergo final maturation steps before achieving translation competence.

In this review, we will focus on factors involved in nuclear export and cytoplasmic maturation of ribosomal subunits. We will first discuss pre-60S and pre-40S biogenesis in the yeast *Saccharomyces cerevisiae*, followed by a review of late ribosome synthesis steps in vertebrate cells.

2. Late maturation of pre-60S particles

After separation of the 90S intermediate into a pre-60S and a pre-40S particle, the two subunit precursors have largely independent biogenesis pathways. In the case of pre-60S subunits, pre-rRNA processing is completed within the nucleus, and a pre-60S ribosome containing the mature 25S, 5.8S and 5S rRNAs is exported to the cytoplasm [8]. Notably, a lag phase is observed before exported 60S subunits are incorporated into polysomes, as the pre-60S particles have to undergo cytoplasmic maturation [9]. In these final subunit biogenesis steps, a number of non-ribosomal proteins associated with cytoplasmic pre-60S subunits have to be released before 60S subunits achieve translation competence (Fig. 1).

2.1. Nuclear export of the pre-60S subunit

Nuclear export of pre-60S subunits in *S. cerevisiae* has been studied monitoring the nuclear accumulation of ribosomal proteins fused to GFP or of 25S rRNA in a variety of mutant yeast strains. These studies showed that pre-60S export is dependent on several NPC components, such as the Nup159p-Nup82p-Nsp1p complex, Nup1p, Nup49p, Nup120p and Nic96p

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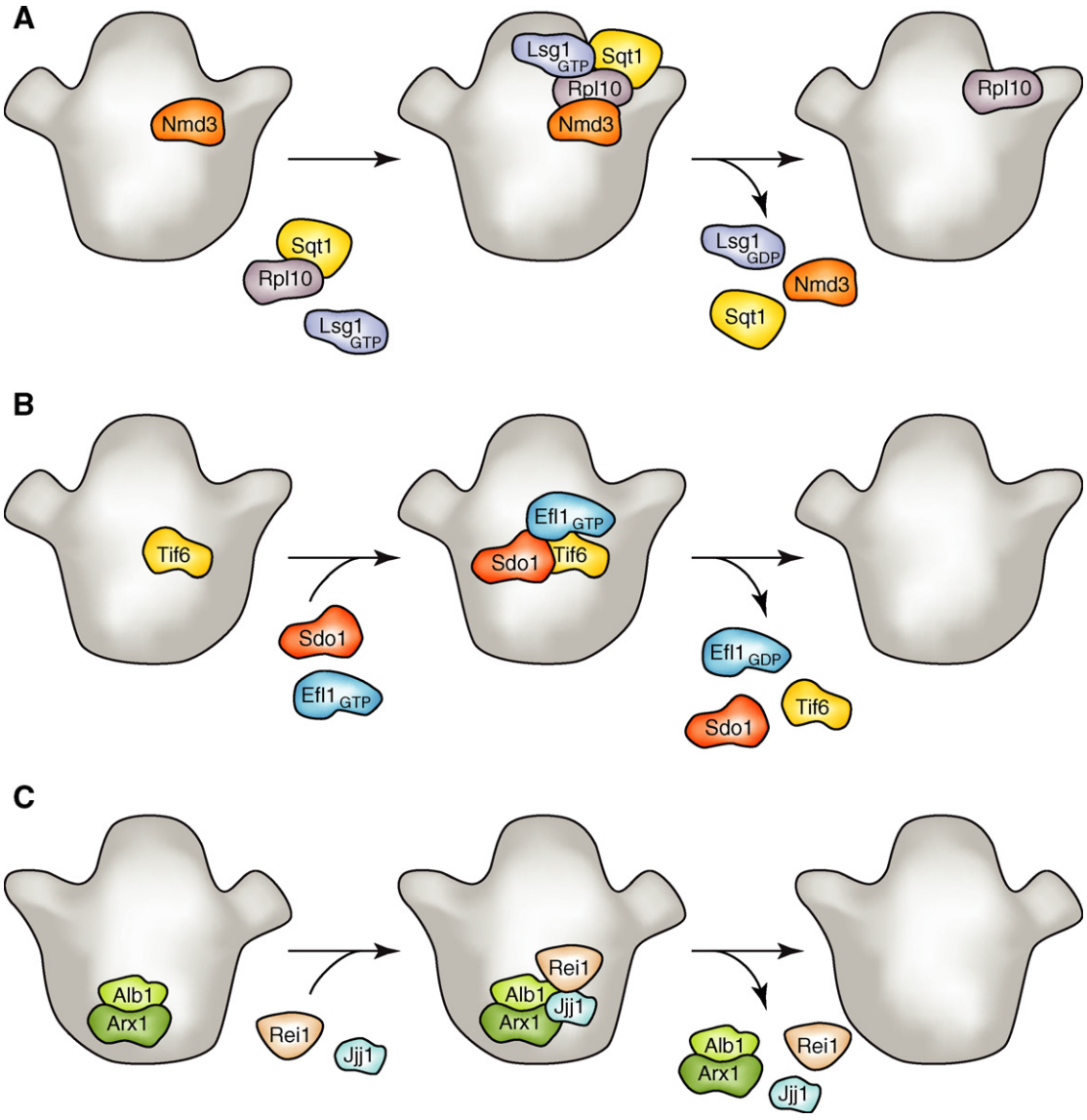

Fig. 1. Cytoplasmic maturation of 60S subunit precursors in yeast. (A) Release of Nmd3p from cytoplasmic pre-60S particles requires the GTPase Lsg1p and is coupled to loading of Rpl10p onto the subunit. GTP hydrolysis by Lsg1p might force the disassociation of both Nmd3p and the Rpl10p chaperone Sqt1p from 60S pre-ribosomes. (B) Tif6p recycling is mediated by Sdo1p and the GTPase Efl1p. Sdo1p might recruit Efl1p, whose GTPase activity triggers the dissociation of Tif6p from pre-60S particles. (C) Recycling of the heterodimer Arx1p/Alb1p from exported pre-60S subunits requires Rei1p and the J-protein Jjj1p.

[10–12]. Further, export of 60S subunits was shown to involve the RanGTPase system [10–12], which controls the directionality of nuclear transport pathways relying on RanGTP-binding transport receptors (reviewed in [13]). For instance, mutations in the RanGEF *PRP20* or the RanGAP *RNA1* lead to nucleoplasmic accumulation of pre-60S particles [10–12]. Based on these data, it was assumed that a RanGTP-binding exportin supports nuclear export of 60S subunits.

Of the export factors tested, the protein export receptor Crm1p was shown to be a major contributor to pre-60S export. Blocking Crm1p-mediated export in a strain carrying a leptomycin B (LMB)-sensitive allele of Crm1p (T539C) leads to nuclear accumulation of Rpl25-GFP [14,15]. Crm1p binds its export substrates by recognizing short leucine-rich export signals (reviewed in [16]). The nuclear export sequence (NES) for Crm1p-mediated pre-60S export is likely provided by the adapter protein Nmd3p, a *trans*-acting factor associated with late pre-60S particles [8,14,15]. Nmd3p was first identified in a screen for components of the non-sense-mediated mRNA decay (NMD) pathway [17], but further analysis revealed that it is involved in 60S ribosome biogenesis rather than in NMD [18,19]. Nmd3p is a cytosolic protein that constantly shuttles in and out of the nucleus [14,15]. Deletion of a C-terminal NES of Nmd3p yields a dominant negative mutant that induces accumulation of Rpl25-GFP in the nucleus. Addition of a heterologous NES to the dominant negative Nmd3p mutant rescues the 60S biogenesis defect, indicating that the Crm1p-dependent NES activity of Nmd3p is required for pre-60S export [14,15]. It is, however, not yet entirely clear if Nmd3p acts as a bona fide export adapter in 60S export, as Nmd3p-dependent recruitment of Crm1p and Gsp1p/Gsp2p (the yeast Ran proteins) to pre-60S particles has not been

demonstrated. It is possible that such an export competent particle is short-lived or not very stable, hindering its purification and characterization.

Since Crm1p inhibition leads to defective subunit export, the contribution of Crm1p to nuclear export of ribosomes is clearly important. Nevertheless, it is unlikely that a single export receptor tethered to one specific site on the surface of the subunit is sufficient to allow for translocation of the large and highly charged particle through the hydrophobic interior of the NPC. Additional export factors may chaperone the subunit during transport. One such factor could be Rrp12p, a HEAT repeat containing protein found in association with both pre-40S and pre-60S particles [20,21]. As HEAT repeats are the major secondary structure element in RanGTP-dependent nuclear transport receptors, Rrp12p was a likely candidate to serve NPC passage of ribosomal subunits. Indeed, lack of Rrp12p causes nucleoplasmic accumulation of Rpl11b-GFP as well as 20S pre-rRNA, the precursor to 18S rRNA [22]. Interestingly, Rrp12p was found to interact with Ran and FXFG repeats of nucleoporins. Therefore, it was suggested that Rrp12p is required for export of both subunit precursors, mediating interactions with the NPC during translocation to the cytoplasm. However, Rrp12p did not discriminate between the GDP-bound and GTP-bound form of Ran, a feature that is essential for the function of other exportins. Furthermore, it has not been proven that Rrp12p binds nucleoporins directly. As Rrp12p depletion leads to defects in pre-rRNA processing in both subunits, its involvement in subunit export can still be indirect.

Another mediator of nuclear export implicated in pre-60S export is Mtr2p, a protein known for its essential role in mRNA export as part of the mRNA export receptor Mex67p/Mtr2p heterodimer [23]. Mex67p/Mtr2p does not belong to the class of RanGTP-binding exportins and uses a distinct mechanism to bind to nucleoporins (for review see [24]). One particular mutant of *MTR2*, *mtr2-33*, allows mRNA export to occur but shows accumulation of Rpl25-EGFP in the nucleus [25]. Moreover, Mtr2p was detected in late pre-60S particles in proteomic analysis of pre-ribosomes [8], suggesting that Mtr2p assists in pre-60S nuclear export. Interestingly, the NPC-associated SUMO deconjugating enzyme Ulp1p shows a genetic interaction with the *mtr2-33* mutant, and the double mutant *ulp1-ts/mtr2-33* displays pre-60S export defects even at permissive temperature [26]. These results suggest that desumoylation of *trans*-acting factors at the NPC is important for efficient pre-60S export. Structural studies revealed that *mtr2-33* mutations are located in a loop region in Mtr2p that is conserved in different yeast but not in humans, indicating that its putative function in pre-ribosome export might not be conserved [27]. So far, no *MEX67* mutation is known to impair export of pre-60S particles. It will be interesting to see whether Mtr2p acts alone in pre-60S export or whether Mex67p is also involved.

It is generally difficult to distinguish whether a particular factor is required for the actual export process or whether it is needed for the pre-60S particle to achieve export competence. For instance, it has been suggested that late nucleoplasmic pre-60S maturation steps could be coupled to pre-60S export, in particular 3′-end processing of the 5.8S rRNA. Mutation or depletion of factors like Rlp7p [28], Sda1p [29], Rix1p complex members [30] and Rli1p [31,32] all lead to defective 5.8S rRNA formation and nuclear accumulation of pre-60S particles. However, there is no direct evidence for a coupling of pre-rRNA processing and pre-60S export and likely, 3′-end formation of 5.8S rRNA is crucial for achieving export competent pre-60S particles. Intriguingly, nuclear-restricted pre-ribosomes with failure in 5.8S rRNA processing caused by mutation of *SDA1* are detected by a surveillance mechanism that eliminates these particles [29].

2.2. Recycling of late trans-acting factors from the pre-60S subunit

Following nuclear export, the pre-60S subunits contain several *trans*-acting factors that have to be released from the particles before mature 60S subunits can participate in translation. These factors are then re-imported into the nucleus where they participate in the biogenesis of a next pre-60S complex. Several such recycling steps have been described in the literature and are summarized below. However, the order in which these release steps occur has not been defined in all cases. Further analysis is required to develop a model of cytoplasmic pre-60S maturation that includes the temporal aspects of these events.

The release of the Crm1p-dependent export adapter Nmd3p requires Lsg1p, a GTPase found on late pre-60S particles (see [8,33,34], Fig. 1A). Lsg1p does not accumulate in the nucleus of Crm1p-inhibited cells and is strictly cytoplasmic, yet its mutation causes nuclear accumulation of Rpl25-EGFP and thus a pre-60S export defect [33]. Therefore, Lsg1p was suggested to be required for recycling of an export factor from the cytoplasm to the nucleus. Indeed, release of Nmd3p from late cytoplasmic pre-60S particles is blocked by mutations in Lsg1p [34]. This defect is suppressed by high copy expression of Nmd3p or by expression of a dominant mutant of Nmd3p with reduced affinity for 60S subunits, indicating the functional relation between these two proteins. Release of Nmd3p further requires functional Rpl10p. Since *NMD3* and *RPL10* show a genetic interaction and Nmd3p can be co-purified with Rpl10p after co-expression in *E. coli* [14,35], it was suggested that Nmd3p is recruited to the pre-60S subunit via Rpl10p. However, high copy *NMD3* expression rescues pre-60S export defect in cells lacking Rpl10p [34]. Thus, Nmd3p can bind to pre-60S in the absence of Rpl10p, and Rpl10p might be recruited to pre-60S particles concomitant with or after release of Nmd3p (Fig. 1A).

Another protein involved in Nmd3p recycling is Sqt1p, which forms a complex with free Rpl10p and may serve as an Rpl10p chaperone required for loading of Rpl10p onto the subunit [36]. A dominant *LSG1* mutation leads to accumulation of Sqt1p on pre-60S particles, indicating that the presence of Lsg1p is necessary for correct loading of Rpl10p onto the subunit and subsequent release of Sqt1p. Thus, Nmd3p release seems to be coupled directly to loading of Rpl10p onto the maturing 60S subunit (Fig. 1A). It will be interesting to see in future how the GTPase cycle of Lsg1 is coupled to its function in remodeling 60S particles in the cytoplasm. Clearly, loading of Rpl10p is required for 60S subunits to be functional in translation [37,38]. This late step in subunit maturation occurs in the cytoplasm and may represent just one of several distinct mechanisms to ensure that subunits cannot prematurely interact during their nuclear biogenesis.

Another factor with a role in 60S subunit biogenesis that prevents precocious association of the two subunits is Tif6p. Tif6p, the yeast homolog of mammalian eIF6, is associated

with pre-60S particles but not found in translating ribosomes [8,39]. eIF6 was originally identified based on its ability to prevent 40S and 60S association in an *in vitro* assay [40–42]. However, studies on both mammalian and yeast homologs revealed that Tif6p is required for 60S ribosome biogenesis rather than translation initiation [39,43–46]. Removal of Tif6p from late pre-60S particles depends on the GTPase Efl1p and is necessary to allow 60S subunits to participate in translation (Fig. 1B). The deletion of Efl1p results in cytoplasmic accumulation of Tif6p, which is localized to the nucleus at steady state in wild type cells [45]. Recent data indicate that Sdo1p, the yeast ortholog of a human gene mutated in the Shwachman-Diamond syndrome, is another factor necessary for release of Tif6p [47]. Sdo1p and Efl1p were found to interact in a high-throughput study of protein complexes [48], and *SDO1* deletion leads to the same defect in Tif6p recycling as deletion of *EFL1* [47]. Thus Sdo1p and Efl1p appear to function together in Tif6p dissociation. Likely, a failure to release Tif6p from pre-60S particles in the cytoplasm causes a Tif6p deficiency in nuclear pre-60S particles, which are stalled in maturation and are no longer exported [45].

Final maturation of pre-60S particles further includes the release of Arx1p. Arx1p was found as a component of late pre-60S particles in both nucleus and cytoplasm, indicating that it is exported together with the pre-60S subunit from the nucleus [8,25]. Arx1p is suggested to function in a heterodimer together with Alb1p [49], and both factors are not present in 80S ribosomes or polysomes [49,50], like Nmd3p or Tif6p. Recycling of Arx1p and Alb1p to the nucleus requires Rei1p, a cytoplasmic protein associated with pre-60S subunits (Fig. 1C). It is currently not clear if Rei1p is required for release of Arx1p from cytoplasmic 60S precursors [50] or for re-import of Arx1p into the nucleus subsequent to the release from pre-60S subunits [49]. In contrast to Lsg1p and Efl1p, the Zn-finger protein Rei1p is not a GTPase, and the molecular basis for its role in release and recycling of Arx1p/Alb1p is unknown. Energetic input to Arx1p/Alb1p release might come from another connection, namely the Hsp40 protein Jjj1p, a J protein recently implicated in late 60S maturation ([51], see Fig. 1C). Jjj1p is found in the cytoplasm, associated with pre-60S (or mature 60S) particles, but is not part of a nuclear 60S precursor. *JJJ1* deletion, however, leads to defects in 60S subunit maturation, and more specifically to a failure of Arx1p release from cytoplasmic pre-60S particles. As Jjj1p can stimulate the ATPase activity of the Hsp70 protein Ssa1p, it is tempting to speculate that ATPase activity of Ssa1p contributes to Arx1p release. Deletion of *REI1* or *JJJ1* both cause Arx1p recycling defects and it remains to be seen how they act together in Arx1p release.

Arx1p is related to methionine aminopeptidases, which act on polypeptides emerging from translating ribosomes in the cytoplasm. Binding of Arx1p to 60S subunits is compromised by the addition of large domains to ribosomal proteins that are located close to the polypeptide exit tunnel. Based on this finding, it has been speculated that Arx1 functions as a space holder on the subunit surface for cytoplasmic methionine aminopeptidases or other factors known to bind close to the protein exit tunnel [50]. Premature association of such factors might cause subunit assembly and export defects. Alternatively, binding of Arx1 to nuclear pre-60S could be part of a proofreading step to ensure that only those subunits with properly assembled exit sites gain access to the cytoplasm.

3. Late maturation of pre-40S particles

Following separation of the 90S pre-ribosome into a pre-60S and a pre-40S particle, the precursors to the small subunit have a less complex composition compared to large subunit precursors. Besides the 20S pre-rRNA and small ribosomal subunit proteins, only few *trans*-acting factors are present [21], such as Rio2p, Enp1p, Tsr1p, Dim1p, Dim2p, Nob1p, Rrp12p, Hrr25p, and Ltv1p. In contrast to pre-60S maturation, no series of defined nucleoplasmic intermediates of pre-40S particles is found. Rather, pre-40S subunits appear to be rapidly transported through the nucleoplasm and the NPC to the cytoplasm, where final maturation occurs. These last steps include dimethylation of the 20S pre-rRNA (Fig. 2B), and a pre-rRNA processing event to produce 18S rRNA from 20S pre-rRNA (Fig. 2C).

3.1. Nuclear export of the pre-40S subunit

Not only nuclear export of pre-60S but also of pre-40S particles depends on the export receptor Crm1p and a functional RanGTPase system. Cells expressing mutants of *PRP20* (RanGEF), *RNA1* (RanGAP) or *YRB1* (Ran binding protein 1), or strains carrying a deletion of *YRB2* (Ran binding protein 2) accumulate 20S pre-rRNA in their nuclei [52,53]. Inhibition of the export factor Crm1p using the leptomycin B (LMB)-sensitive allele Crm1p(T539C) also leads to nuclear accumulation of 20S pre-rRNA [53]. Finally, a number of nucleoporin mutants were tested for defects in pre-40S export, and mutation of the Nup159p-Nup82p-Nsp1p complex results in defective 20S pre-rRNA export as well [10,52,53].

In contrast to pre-60S export, where the adapter protein Nmd3p is suggested to mediate the binding of Crm1p to the pre-60S particle, no such adapter protein is known for 40S subunit precursors. A number of factors such as Rio2p, Dim2p or Ltv1p are associated with pre-40S complexes and show rapid nuclear accumulation upon Crm1 inhibition [21,54,55], two properties that are expected of an export adapter protein for pre-40S subunits. Yet, clear export defects were not observed upon mutation or deletion of these proteins, although conflicting results were obtained in studies on Rio2p (discussed below), and recent data indicate that Ltv1p might assist in pre-40S export [54]. However, Ltv1p is not an essential protein and thus cannot be the only adapter for Crm1p. It is conceivable that more than one adapter protein is involved in this process, and only simultaneous mutations or depletions of several factors would result in strong defects in pre-40S export.

As discussed above for pre-60S export, other factors than Crm1p might contribute to 40S precursor export as exemplified by Rrp12p, a component of pre-40S and pre-60S particles that interacts with Ran and FXFG repeats [22].

3.2. Phosphorylation-dependent pre-40S maturation steps

The presence of two protein kinases in the pre-40S particles, Hrr25p and Rio2p, suggests that phosphorylation reactions play a role in 40S biogenesis. Rio2p belongs to a conserved family of atypical protein kinases and the two yeast Rio family members Rio1p and Rio2p are required for 40S biogenesis [55–57]. Hrr25p is a yeast casein kinase 1 isoform that has been implicated in various processes (see [58] and references therein). Recent data add a novel function to Hrr25p, namely in late remodeling steps of the pre-40S subunit (Fig. 2A). Hurt and

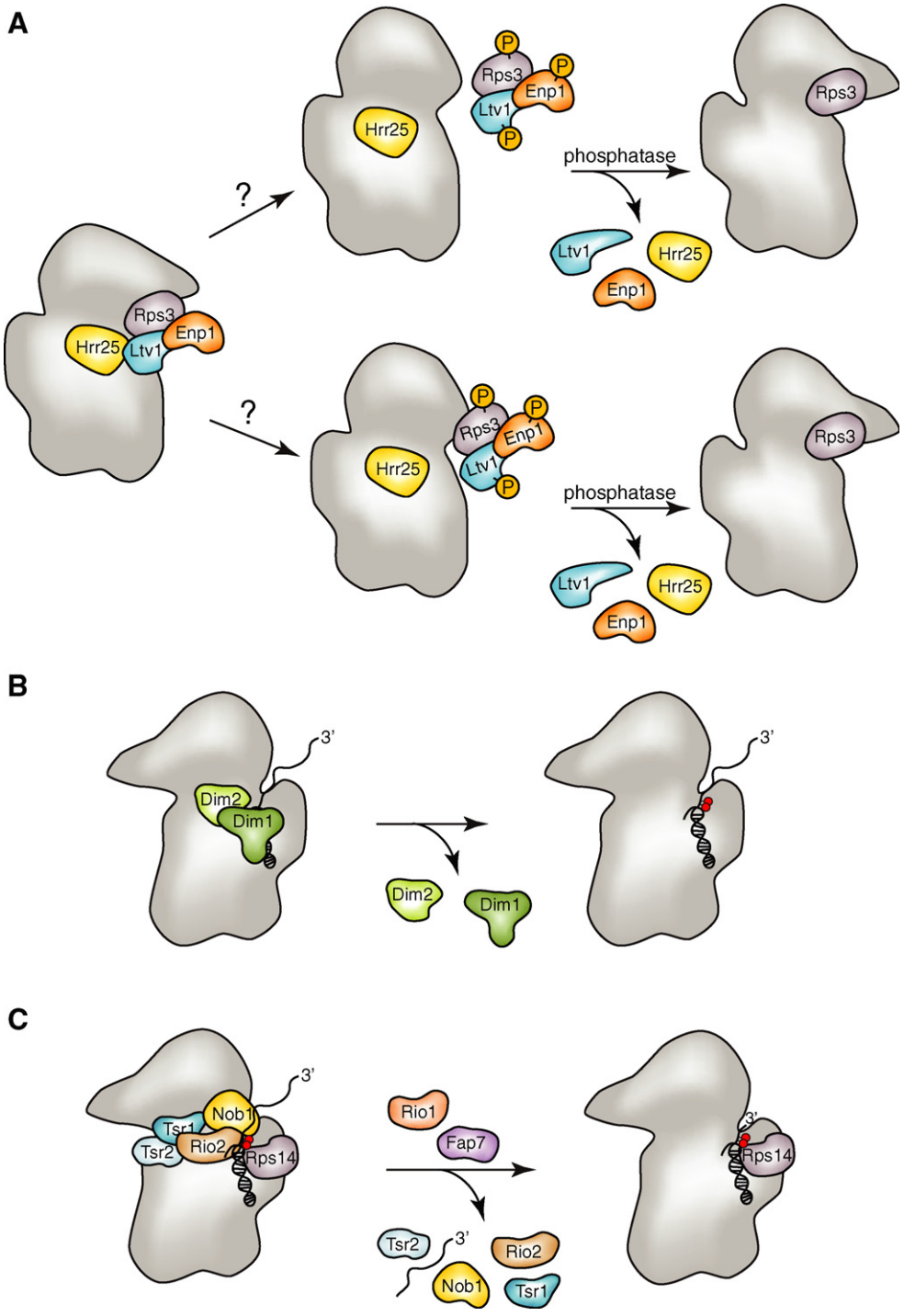

Fig. 2. Cytoplasmic maturation of 40S subunit precursors in yeast. (A) Hrr25p-dependent phosphorylation of an Enp1p/Ltv1p/Rps3p subcomplex, followed by dephosphorylation by an unknown phosphatase, is required for proper incorporation of Rps3p into the 40S subunit and correct formation of the beak structure. It is unknown whether the Enp1p/Ltv1p/Rps3p subcomplex is released from the subunit or whether there is no disassociation but an increase in conformational flexibility. (B) Dim1p-dependent dimethylation of 20S pre-rRNA at residues 1779 and 1780 of the 18S rRNA. (C) Endonucleolytic processing of 20S pre-rRNA to 18S rRNA, possibly catalyzed by Nob1p. Rio1p, Fap7p and the pre-40S components Tsr1p, Tsr2p, Rio2p and Rps14p are also involved in 3′-end formation of mature 18S rRNA.

colleagues observed that Hrr25p-dependent phosphorylation of a trimeric complex consisting of the *trans*-acting factors Enp1p/Ltv1p and the ribosomal protein Rps3p leads to the dissociation of this subcomplex from pre-40S subunits *in vitro* [59]. It is not clear whether Rps3p dissociates from and later re-associates to the 40S precursor *in vivo*. The authors suggest that an increase in conformational flexibility caused by Rps3p/Ltv1p/Enp1p phosphorylation could be necessary for efficient subunit export, as the final rigid beak structure close to the head of the 40S subunit might hinder passage through the NPC. A subsequent dephosphorylation step mediated by an unknown phosphatase allows for proper incorporation of

Rps3 into ribosomal subunits *in vitro*, and this maturation step appears to be crucial for correct formation of the beak in the 40S ribosomal structure (Fig. 2A). If the phosphorylation/dephosphorylation cycle were required for nuclear export of pre-40S particles, the phosphorylation reaction would be predicted to occur in the nucleus, whereas dephosphorylation should be a cytoplasmic event. It is currently not known where these reactions occur.

The second kinase found in pre-40S particles, Rio2p, appears not to be involved in phosphorylation of the Enp1/Ltv1/Rps3 complex [59], and the substrate(s) of Rio2p remain elusive to date. Nevertheless, it is clear that Rio2p is involved in late maturation of the 40S subunit, since its depletion or expression of a mutant with reduced kinase activity both lead to inhibition of 3′-end processing of 18S rRNA (see [55,56], Fig. 2C). Upon Rio2p depletion, dimethylated 20S pre-rRNA accumulates in the cytoplasm [55], indicating that Rio2p is neither required for Dim1p-dependent pre-rRNA dimethylation (see below) nor nuclear export of the pre-40S particle. However, in *rio2-1* mutant cells, the 40S subunit reporter protein Rps2-GFP was observed to accumulate in the nuclei, suggesting a role for Rio2p in 40S precursor export [21]. It is currently not clear how these conflicting results can be explained.

Like Rio2p, Rio1p is a protein kinase whose substrate(s) are not known to date. Both proteins are essential and thus do not have completely redundant functions. Although Rio1p was not found in pre-40S particles in TAP studies [21], there are indications that it is associated with 40S subunit precursors as Rio1p co-immunoprecipitates 20S pre-rRNA and co-migrates with 40S subunits. [57]. Depletion of Rio1p results in accumulation of dimethylated 20S pre-rRNA and reduced 18S rRNA levels, comparable to effects observed upon depletion of Rio2p [55,57]. The role of the two kinases in cytoplasmic pre-rRNA processing is currently not clear. One possible scenario would be that Rio1p and Rio2p are required for recruitment and/or activation of the endonuclease responsible for cleavage of 20S pre-rRNA (Fig. 2C), but no data are available to support this or any other model.

3.3. Dimethylation of 20S pre-rRNA

Another late maturation step in 40S biogenesis is the dimethylation of two adenine bases near the 3′-end of the 18S rRNA. The enzyme catalyzing this reaction is the essential protein Dim1p ([60], Fig. 2B). Dimethylation is first detected on the 20S rRNA precursor and suggested to take place on the 20S pre-rRNA once the pre-40S particle reaches the cytoplasm [61]. Although dimethylation occurs late during subunit maturation, Dim1p associates already with 90S pre-ribosomes and its presence is required for early nucleolar processing events [21,60,62]. Processing of 20S pre-rRNA to 18S rRNA can still occur in the absence of dimethylation, and dimethylation is not essential for ribosome biogenesis or function [62]. Therefore, the role of Dim1p in early processing steps must make *DIM1* essential. Dimethylation was suggested to play a role in fine-tuning of translation, as *in vitro* translation does not work with non-dimethylated 40S subunits, and the strain lacking 40S subunit dimethylation displays increased antibiotic sensitivity [62].

Dim2p, also known as Yor145p, Pno1p or Rrp20p, seems to be closely linked to Dim1p (Fig. 2B). Like Dim1p, it binds early to pre-90S particles and remains on the pre-40S particle until final maturation in the cytoplasm [21,63], and its depletion causes defects in early nucleolar cleavages [63–65]. The two proteins were found to interact [63], and it was suggested that Dim2p might recruit Dim1p to the 90S pre-ribosome. It is not clear though whether Dim1p or Dim2p binds first to the 90S precursor, or whether they join the pre-90S particle as a complex.

3.4. 20S to 18S rRNA processing

Two different factors have been proposed to act as the nuclease responsible for cytoplasmic processing of 20S pre-rRNA to 18S rRNA (Fig. 2C). The candidate enzyme with the stronger supporting evidence is Nob1p. Nob1p is found in late pre-ribosomal particles [21] and contains a PIN domain, which has sequence homology to 5′ exonuclease domains [66,67]. Moreover, an archaebacterial PIN domain displays exonuclease activity *in vitro* and has structural homology to flap endonucleases and to T4 phage RNase H exonuclease [68]. The importance of the PIN domain of Nob1p is demonstrated by the finding that mutation of a conserved residue within the PIN domain, like depletion of Nob1p, leads to strong inhibition of 20S to 18S processing [67,69]. Yet, the nuclease activity of Nob1p still needs to be shown directly, in particular its activity in cleaving 20S pre-rRNA.

Another candidate enzyme for 20S pre-rRNA processing is Fap7p (Fig. 2C). Depletion of Fap7p leads to inhibition of 20S to 18S processing, and mutations in putative catalytic residues in the NTPase motif of Fap7p lead to the same phenotype [64,70]. However, Fap7p does not contain a known endonuclease domain, and it could well be a regulator of endonuclease activity rather than the processing enzyme. Like for Nob1p, no nuclease activity has been proven so far for Fap7p.

Not only the two potential nucleases Nob1p and Fap7p, but also several other factors are required for efficient 20S to 18S processing. Besides Rio1p and Rio2p (discussed above), Tsr1p and Tsr2p are implicated in 20S pre-rRNA cleavage (Fig. 2C). Depletion of either protein results in accumulation of 20S pre-rRNA [64,71,72]. In the case of Tsr1p-depleted cells, 20S pre-rRNA accumulates in the cytoplasm, suggesting that 20S pre-rRNA processing is not compromised because of an export defect [72]. However, depletion of Tsr1p leads to nuclear accumulation of the small subunit reporter protein Rps2-GFP [21]. Thus, as for Rio2p, experiments addressing the role of Tsr1p in nuclear export of 40S precursors yield conflicting results using either 20S rRNA localization or the Rps2-GFP reporter as a read-out. As for many other factors involved in late 40S maturation steps, the molecular function of Tsr1 and Tsr2 remains to be investigated.

3.5. Roles of ribosomal proteins in pre-40S maturation

In the process of subunit maturation, *trans*-acting factors must be recruited to specific sites on the surface of the ribosomal subunit. Ribosomal proteins, together with the rRNA, must play an import role in this recruitment process by serving as landing pads for *trans*-acting factors, or by forming subcomplexes with *trans*-acting factors that are then recruited onto the maturing subunit. Furthermore, ribosomal proteins may play an active role in distinct steps of subunit biogenesis. Whereas the function of *trans*-acting factors in ribosome biogenesis has been studied intensely during the past years, information on the contribution of ribosomal proteins is still scarce (Fig. 2C).

Certain ribosomal proteins have been shown to affect cleavage of 20S pre-rRNA, such as Rps0p, Rps14p and Rps21ap [73–75]. A cryo electron microscopy study placed the C-terminus of Rps14p in proximity of the 3′-end of the 18S rRNA [76]. The deletion of the C-terminal tail of Rps14p leads to cytoplasmic accumulation of 20S pre-rRNA, supporting a role of this domain in processing ([74], Fig. 2C). The primary sequence of Rps14p does not show homology to known nuclease domains, but Rps14p might be required to induce a 20S pre-rRNA conformation that allows cleavage to occur, or for recruitment of the 20S pre-rRNA processing enzyme. Supporting the latter assumption, a direct interaction between Rps14p and Fab7p, one of the putative endonucleases mediating 20S pre-rRNA cleavage, has been observed [70].

Other ribosomal proteins are required earlier in 40S biogenesis, such as Rps18p and Rps15p [72]. Whereas Rps18p depletion leads to a nucleolar maturation defect, Rps15p was suggested to act in nuclear export of pre-40S, since its depletion causes nucleoplasmic accumulation of 20S pre-rRNA.

A recent study used a more systematic approach to investigate the role of nearly all small subunit ribosomal proteins in 40S biogenesis, and they could be classified into several groups with distinct roles in 40S maturation [77]. For instance, Rps0, Rps2, Rps3, Rps10, Rps15 and Rps26 appear to be required in nuclear export of pre-40S particles, since strains lacking any of these Rps proteins accumulate 20S pre-rRNA in the nucleus and export of 20S pre-rRNA is blocked or strongly reduced. In contrast, Rps20 depletion also inhibits 20S pre-rRNA processing but the 20S pre-rRNA is exported and accumulates in the cytoplasm, suggesting that Rps20 is not required for export but necessary for cytoplasmic pre-rRNA cleavage.

It is perhaps not surprising that many proteins are implicated in 20S pre-rRNA processing, since this process could well serve as quality control point for 40S biogenesis. According to this hypothesis, cleavage of 20S pre-rRNA would only occur if maturation up to this step were successful. Subunit maturation may be monitored at the level of particle composition and conformation. For instance, in case direct activators or binding partners of the 20S pre-RNA nuclease were missing, processing would be inhibited. Further, even if a factor has no direct interaction with the nuclease, its absence could cause structural aberrations of the pre-40S particle that lead to failure in recruitment or activation of the nuclease.

4. Late steps in vertebrate ribosome biogenesis

Ribosomal biogenesis appears to be conserved in many aspects from yeast to vertebrates. First, rRNA synthesis and processing is similar, albeit not identical, between yeast and metazoans. Second, for most *trans*-acting factors involved in yeast ribosome biogenesis, close homologs are found in higher eukaryotic cells. However, research in higher eukaryotes lags behind compared to *S. cerevisiae*, mostly because some of the experimental techniques are not applied as readily in metazoan systems as in yeast. As a consequence, the picture of nuclear export and cytoplasmic maturation of ribosome precursors in vertebrate cells is still fragmented, but some aspects known to date are discussed below.

4.1. Nuclear export of pre-ribosomes in vertebrate cells

Early *Xenopus* oocytes microinjection experiments showed that ribosome export is an energy-dependent and saturable process [78,79]. Later studies using the same experimental system revealed that the export of pre-ribosomes requires the RanGTPase system and the export factor Crm1: depletion of RanGTP or inhibition of Crm1 using LMB leads to nuclear accumulation of rRNAs [80], and export of both the pre-40S and the pre-60S subunit is efficiently competed by nuclear injection of an artificial Crm1 substrate, BSA-NES [80,81]. Furthermore, Crm1 inhibition in cultured human somatic cells also caused a defect in pre-ribosome export, as both ribosomal proteins and rRNAs accumulate in the nucleus following LMB treatment [81]. Thus, the Crm1-dependent nuclear export pathway is conserved from yeast to human cells.

The same conservation is observed for the function of the export adapter protein Nmd3. Expression of hNmd3 variants with mutations in or deletion of its NES in *Xenopus* oocytes resulted in nuclear accumulation of 28S and 5.8S rRNA precursors, indicating that the NES of Nmd3 contributes to pre-60S export [80]. Moreover, hNmd3 was shown to bind to Crm1 directly in a RanGTP-dependent manner [81]. As in yeast, however, no pre-60S export complex containing a 60S precursor, Nmd3, Crm1 and RanGTP has been described.

Interestingly, similar to *S. cerevisiae*, where the Nup159p–Nup82p–Nsp1p NPC subcomplex has been implicated in subunit export, a homologous nucleoporin complex composed of hNup214 and hNup88 is important for pre-60S export [82]. Depletion of hNup214 results in nuclear retention of GFP-Nmd3 and Rpl29-GFP, but not in general defects in Crm1-mediated nuclear export. The FG-repeat region of hNup214 forms a strong Crm1 binding site *in vitro* [83,84] but is likely dispensable for 60S subunit export *in vivo*. It has therefore been speculated that the hNup214-Nup88 subcomplex is required for a conformational change in the NPC that allows passage of the large pre-60S particle, rather than directly mediating pre-60S translocation across the NPC.

Little is known about the export of 40S precursors in higher eukaryotes, besides its dependence on Crm1 and RanGTP. Knockdown of Rps15 in HeLa cells by RNAi leads to nuclear accumulation of precursors of the 18S rRNA, thus Rps15 was suggested to act in nuclear export of pre-40S particles, similar to the role of its yeast homolog [72,77,85].

Additional factors, present only in higher eukaryotes, have been implicated in ribosome biogenesis and could be involved in nuclear export of subunit precursors. One of these factors, nucleophosmin (also known as B23, NPM, Numatrin or NO38), is an abundant nucleolar protein implicated in a variety of cellular processes (reviewed in [86]). Nucleophosmin was suggested to act as a molecular chaperone whose activity is regulated by phosphorylation [87,88]. *In vitro*, it also displays endoribonuclease activity at a specific site of the spacer region between the 5.8S and 28S rRNAs [89,90]. Furthermore, it might associate with pre-ribosomes in the nucleus and mature ribosomes in the cytoplasm [91]. Since nucleophosmin is a shuttling protein containing a Crm1-dependent NES, it might assist in pre-subunit export [91,92]. Indeed, expression of a non-shuttling mutant of nucleophosmin leads to nuclear accumulation of Rpl5, an interaction partner of nucleophosmin, and therefore pre-60S export seems to be perturbed [91]. However, since nucleophosmin might be required for pre-ribosome

assembly and pre-rRNA processing, nuclear accumulation of Rpl5 could be due to defective subunit assembly rather than a direct export defect.

4.2. Cytoplasmic maturation of preribosomal subunits

How Nmd3 is released from pre-60S particles after export to the cytoplasm has not been investigated in vertebrate systems. The proteins involved in Nmd3p release in yeast are conserved in human cells, but little data are available on these *trans*-acting factors. An initial characterization of hLsg1 has shown that it displays GTPase activity *in vitro* [93]. Interestingly, in contrast to the situation in yeast [33], hLsg1 is not strictly cytoplasmic in human cells and accumulates in the nucleus upon treatment with LMB. Nevertheless, loading of hRpl10 likely occurs in the cytoplasm, since hRpl10 is strictly excluded from nuclei at steady state [94] and does not accumulate in nuclei after LMB treatment (Thomas and Kutay, unpublished). These data are consistent with the current model for the role of Rpl10p in Nmd3p recycling in yeast.

As discussed above, eIF6, the homolog of yeast Tif6p, was initially described as a translation initiation factor [40–42], but later found to rather play a role in 60S subunit biogenesis [39,43–46]. Studies on eIF6 have shown that its phopshorylation plays a pivotal role during different steps of 60S subunit biogenesis. Casein kinase I is able to phosphorylate mammalian eIF6, and mutation of the conserved phosphorylation sites to alanine in the yeast homolog lead to a mutant with slow growth, defective 25S rRNA processing and nuclear accumulation of the yeast mutant protein [95]. Phosphorylation of eIF6 at a distinct site is important for a late step in 60S maturation, namely the release of eIF6 from exported 60S subunits [96]. This phosphorylation-dependent release of eIF6 from 60S subunits is stimulated by the activation of the PKC pathway, which reduces the translation inhibition activity of eIF6. A complex between the PKC adapter protein RACK1 and PKC βII was able to phosphorylate eIF6 and to promote subunit joining *in vitro*. Whether this phosphorylation-dependent release of eIF6 from 60S subunits is conserved in yeast is not known. On the other hand, eIF6 release in yeast involves Sdo1p and the GTPase Efl1p, and it remains to be investigated whether their human homologs are needed for eIF6 release in human cells, in addition to the RACK1-PKC βII-dependent phosphorylation.

Cytoplasmic maturation steps of the pre-40S subunit in yeast include dimethylation of the 20S pre-rRNA and final pre-rRNA cleavage to yield 18S rRNA. It was long thought that pre-rRNA processing in higher eukaryotes is completed within the nucleus, and pre-40S particles containing mature 18S rRNA are translocated to the cytoplasm. However, recent results indicate that a 3′ extended precursor of 18S rRNA, the 18S-E pre-rRNA, is exported to the cytoplasm in human cells, where the final cleavage reaction occurs [85]. In analogy to yeast 20S to 18S rRNA processing, hRio2 kinase, the homolog of yeast Rio2p, is required for this step. The nuclease responsible for 18S-E processing is unknown.

Dimethylation of the small subunit rRNA near its 3′-end is conserved from bacteria to humans, and occurs late in 40S biogenesis but not on the early 45S pre-rRNA as most other pre-rRNA modifications [97]. As the dimethylase of yeast, Dim1p, has a human homolog, this is the likely candidate to perform this function.

5. Conclusions and perspectives

Our understanding of ribosome biogenesis has greatly improved in recent years. Yeast genetics has been a very powerful tool in the characterization of the processes and factors involved in ribosomal subunit assembly. Furthermore, the description of pre-ribosomal particles has allowed to develop a map of the ribosome biogenesis pathway with intermediate particles for the 40S and 60S subunits. As discussed in this and other reviews, many maturation steps have been described along this assembly pathway. Particularly for late pre-60S maturation, key players have been identified that are required for release of *trans*-acting factors to allow 60S subunits to fully mature and become functional in translation. However, the molecular details of the role of these proteins are not yet fully understood.

Structural investigations of how *trans*-acting factors bind to pre-ribosomal particles and which conformational transitions they induce on subunit precursors are required to improve our understanding of these processes. The recent description of the phosphorylation-dependent maturation step in formation of the beak structure of the 40S subunit exemplifies the value of this type of experiments [59].

Further, the mechanistic aspects of nuclear export of ribosomes remain somewhat of a mistery to date. How translocation of this huge and highly charged ribonucleoprotein particle through the nuclear pore is achieved is uncertain. Although the role of Crm1 seems very important in ribosome export, it remains to be seen whether its contribution is sufficient or whether other factors are required, for instance to shield the ribosome precursor surface during transport through the hydrophobic interior of the NPC.

Last but not least, an important question is how the final steps of pre-ribosome maturation are controlled and whether they contribute to 'proofreading' of the subunits. For instance, 20S pre-rRNA processing might serve as a quality control point for 40S subunit biogenesis. It is striking that a large number of factors are required for late pre-40S maturation and in particular late processing of 18S rRNA precursors. Some of these factors could be involved in monitoring particle composition and conformation, and make certain that only correctly assembled ribosomal subunits are generated. Thereby, the cytoplasmic biogenesis steps of pre-ribosomes might possess a decisive role in ensuring that the ribosome, the molecular machine for protein synthesis, is built from functional subunits.

Note added in proof:

After acceptance of this manuscript, Yao *et al.* provided evidence for a role of the general mRNA export receptor Mex67p/Mtr2p in export of pre-60S particles [98]. Mex67p/Mtr2p were shown to bind pre-60S particles via loop insertions in both Mtr2p and Mex67p that are present in the yeast factors but absent from mammalian TAP-p15. Mutations in these loops caused defects in export of 60S pre-ribosomes, whereas mRNA export was not affected.

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References

[1] Tschochner, H. and Hurt, E. (2003) Pre-ribosomes on the road from the nucleolus to the cytoplasm. Trends Cell Biol. 13, 255–263.

[2] Fromont-Racine, M., Senger, B., Saveanu, C. and Fasiolo, F. (2003) Ribosome assembly in eukaryotes. Gene 313, 17–42.

[3] Granneman, S. and Baserga, S.J. (2004) Ribosome biogenesis: of knobs and RNA processing. Exp. Cell Res. 296, 43–50.

[4] Johnson, A.W., Lund, E. and Dahlberg, J. (2002) Nuclear export of ribosomal subunits. Trends Biochem. Sci. 27, 580–585.

[5] Kressler, D., Linder, P. and de La Cruz, J. (1999) Protein *trans*-acting factors involved in ribosome biogenesis in *Saccharomyces cerevisiae*. Mol. Cell. Biol. 19, 7897–7912.

[6] Nazar, R.N. (2004) Ribosomal RNA processing and ribosome biogenesis in eukaryotes. IUBMB Life 56, 457–465.

[7] Venema, J. and Tollervey, D. (1999) Ribosome synthesis in *Saccharomyces cerevisiae*. Annu. Rev. Genet. 33, 261–311.

[8] Nissan, T.A., Bassler, J., Petfalski, E., Tollervey, D. and Hurt, E. (2002) 60S pre-ribosome formation viewed from assembly in the nucleolus until export to the cytoplasm. EMBO J. 21, 5539–5547.

[9] Warner, J.R. (1971) The assembly of ribosomes in yeast. J. Biol. Chem. 246, 447–454.

[10] Gleizes, P.E., Noaillac-Depeyre, J., Leger-Silvestre, I., Teulieres, F., Dauxois, J.Y., Pommet, D., Azum-Gelade, M.C. and Gas, N. (2001) Ultrastructural localization of rRNA shows defective nuclear export of preribosomes in mutants of the Nup82p complex. J. Cell Biol. 155, 923–936.

[11] Hurt, E., Hannus, S., Schmelzl, B., Lau, D., Tollervey, D. and Simos, G. (1999) A novel in vivo assay reveals inhibition of ribosomal nuclear export in ran-cycle and nucleoporin mutants. J. Cell Biol. 144, 389–401.

[12] Stage-Zimmermann, T., Schmidt, U. and Silver, P.A. (2000) Factors affecting nuclear export of the 60S ribosomal subunit in vivo. Mol. Biol. Cell 11, 3777–3789.

[13] Fried, H. and Kutay, U. (2003) Nucleocytoplasmic transport: taking an inventory. Cell. Mol. Life Sci. 60, 1659–1688.

[14] Gadal, O., Strauss, D., Kessl, J., Trumpower, B., Tollervey, D. and Hurt, E. (2001) 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, 3405–3415.

[15] Ho, J.H., Kallstrom, G. and Johnson, A.W. (2000) Nmd3p is a Crm1p-dependent adapter protein for nuclear export of the large ribosomal subunit. J. Cell Biol. 151, 1057–1066.

[16] Kutay, U. and Guttinger, S. (2005) Leucine-rich nuclear-export signals: born to be weak. Trends Cell Biol. 15, 121–124.

[17] He, F. and Jacobson, A. (1995) Identification of a novel component of the nonsense-mediated mRNA decay pathway by use of an interacting protein screen. Genes Dev. 9, 437–454.

[18] Belk, J.P., He, F. and Jacobson, A. (1999) Overexpression of truncated Nmd3p inhibits protein synthesis in yeast. RNA 5, 1055–1070.

[19] Ho, J.H. and Johnson, A.W. (1999) NMD3 encodes an essential cytoplasmic protein required for stable 60S ribosomal subunits in *Saccharomyces cerevisiae*. Mol. Cell. Biol. 19, 2389–2399.

[20] Gavin, A.C. et al. (2002) Functional organization of the yeast proteome by systematic analysis of protein complexes. Nature 415, 141–147.

[21] Schafer, T., Strauss, D., Petfalski, E., Tollervey, D. and Hurt, E. (2003) The path from nucleolar 90S to cytoplasmic 40S pre-ribosomes. EMBO J. 22, 1370–1380.

[22] Oeffinger, M., Dlakic, M. and Tollervey, D. (2004) A pre-ribosome-associated HEAT-repeat protein is required for export of both ribosomal subunits. Genes Dev. 18, 196–209.

[23] Santos-Rosa, H., Moreno, H., Simos, G., Segref, A., Fahrenkrog, B., Pante, N. and Hurt, E. (1998) Nuclear mRNA export requires complex formation between Mex67p and Mtr2p at the nuclear pores. Mol. Cell. Biol. 18, 6826–6838.

[24] Stutz, F. and Izaurralde, E. (2003) The interplay of nuclear mRNP assembly, mRNA surveillance and export. Trends Cell Biol. 13, 319–327.

[25] Bassler, J., Grandi, P., Gadal, O., Lessmann, T., Petfalski, E., Tollervey, D., Lechner, J. and Hurt, E. (2001) Identification of a 60S preribosomal particle that is closely linked to nuclear export. Mol. Cell 8, 517–529.

[26] Panse, V.G., Kressler, D., Pauli, A., Petfalski, E., Gnadig, M., Tollervey, D. and Hurt, E. (2006) Formation and nuclear export of preribosomes are functionally linked to the small-ubiquitin-related modifier pathway. Traffic 7, 1311–1321.

[27] Senay, C., Ferrari, P., Rocher, C., Rieger, K.J., Winter, J., Platel, D. and Bourne, Y. (2003) The Mtr2-Mex67 NTF2-like domain complex. Structural insights into a dual role of Mtr2 for yeast nuclear export. J. Biol. Chem. 278, 48395–48403.

[28] Gadal, O., Strauss, D., Petfalski, E., Gleizes, P.E., Gas, N., Tollervey, D. and Hurt, E. (2002) Rlp7p is associated with 60S preribosomes, restricted to the granular component of the nucleolus, and required for pre-rRNA processing. J. Cell Biol. 157, 941–951.

[29] Dez, C., Houseley, J. and Tollervey, D. (2006) Surveillance of nuclear-restricted pre-ribosomes within a subnucleolar region of *Saccharomyces cerevisiae*. EMBO J. 25, 1534–1546.

[30] Galani, K., Nissan, T.A., Petfalski, E., Tollervey, D. and Hurt, E. (2004) Rea1, a dynein-related nuclear AAA-ATPase, is involved in late rRNA processing and nuclear export of 60 S subunits. J. Biol. Chem. 279, 55411–55418.

[31] Kispal, G. et al. (2005) Biogenesis of cytosolic ribosomes requires the essential iron–sulphur protein Rli1p and mitochondria. EMBO J. 24, 589–598.

[32] Yarunin, A., Panse, V.G., Petfalski, E., Dez, C., Tollervey, D. and Hurt, E.C. (2005) Functional link between ribosome formation and biogenesis of iron–sulfur proteins. EMBO J. 24, 580–588.

[33] Kallstrom, G., Hedges, J. and Johnson, A. (2003) The putative GTPases Nog1p and Lsg1p are required for 60S ribosomal subunit biogenesis and are localized to the nucleus and cytoplasm, respectively. Mol. Cell. Biol. 23, 4344–4355.

[34] Hedges, J., West, M. and Johnson, A.W. (2005) Release of the export adapter, Nmd3p, from the 60S ribosomal subunit requires Rpl10p and the cytoplasmic GTPase Lsg1p. EMBO J. 24, 567–579.

[35] Karl, T. et al. (1999) GRC5 and NMD3 function in translational control of gene expression and interact genetically. Curr. Genet. 34, 419–429.

[36] West, M., Hedges, J.B., Chen, A. and Johnson, A.W. (2005) Defining the order in which Nmd3p and Rpl10p load onto nascent 60S ribosomal subunits. Mol. Cell. Biol. 25, 3802–3813.

[37] Dick, F.A., Eisinger, D.P. and Trumpower, B.L. (1997) Exchangeability of Qsr1p, a large ribosomal subunit protein required for subunit joining, suggests a novel translational regulatory mechanism. FEBS Lett. 419, 1–3.

[38] Eisinger, D.P., Dick, F.A. and Trumpower, B.L. (1997) Qsr1p, a 60S ribosomal subunit protein, is required for joining of 40S and 60S subunits. Mol. Cell. Biol. 17, 5136–5145.

[39] Si, K. and Maitra, U. (1999) The *Saccharomyces cerevisiae* homologue of mammalian translation initiation factor 6 does not function as a translation initiation factor. Mol. Cell. Biol. 19, 1416–1426.

[40] Raychaudhuri, P., Stringer, E.A., Valenzuela, D.M. and Maitra, U. (1984) Ribosomal subunit antiassociation activity in rabbit reticulocyte lysates. Evidence for a low molecular weight ribosomal subunit antiassociation protein factor (Mr = 25,000). J. Biol. Chem. 259, 11930–11935.

[41] Russell, D.W. and Spremulli, L.L. (1979) Purification and characterization of a ribosome dissociation factor (eukaryotic initiation factor 6) from wheat germ. J. Biol. Chem. 254, 8796–8800.

[42] Valenzuela, D.M., Chaudhuri, A. and Maitra, U. (1982) Eukaryotic ribosomal subunit anti-association activity of calf liver is contained in a single polypeptide chain protein of Mr = 25,500 (eukaryotic initiation factor 6). J. Biol. Chem. 257, 7712–7719.

[43] Basu, U., Si, K., Warner, J.R. and Maitra, U. (2001) The *Saccharomyces cerevisiae* TIF6 gene encoding translation initiation factor 6 is required for 60S ribosomal subunit biogenesis. Mol. Cell. Biol. 21, 1453–1462.

[44] Sanvito, F., Piatti, S., Villa, A., Bossi, M., Lucchini, G., Marchisio, P.C. and Biffo, S. (1999) The beta4 integrin interactor p27(BBP/eIF6) is an essential nuclear matrix protein involved in 60S ribosomal subunit assembly. J. Cell Biol. 144, 823–837.
[45] Senger, B. et al. (2001) The nucle(ol)ar Tif6p and Efl1p are required for a late cytoplasmic step of ribosome synthesis. Mol. Cell 8, 1363–1373.
[46] Wood, L.C., Ashby, M.N., Grunfeld, C. and Feingsold, K.R. (1999) Cloning of murine translation initiation factor 6 and functional analysis of the homologous sequence YPR016c in *Saccharomyces cerevisiae*. J. Biol. Chem. 274, 11653–11659.
[47] Menne, T.F. et al. (2007) The Shwachman–Bodian–Diamond syndrome protein mediates translational activation of ribosomes in yeast. Nat. Genet. 39, 486–495.
[48] Krogan, N.J. et al. (2006) Global landscape of protein complexes in the yeast *Saccharomyces cerevisiae*. Nature 440, 637–643.
[49] Lebreton, A., Saveanu, C., Decourty, L., Rain, J.C., Jacquier, A. and Fromont-Racine, M. (2006) A functional network involved in the recycling of nucleocytoplasmic pre-60S factors. J. Cell Biol. 173, 349–360.
[50] Hung, N.J. and Johnson, A.W. (2006) Nuclear recycling of the pre-60S ribosomal subunit-associated factor Arx1 depends on Rei1 in *Saccharomyces cerevisiae*. Mol. Cell. Biol. 26, 3718–3727.
[51] Meyer, A.E., Hung, N.J., Yang, P., Johnson, A.W. and Craig, E.A. (2007) The specialized cytosolic J-protein, Jjj1, functions in 60S ribosomal subunit biogenesis. Proc. Natl. Acad. Sci. USA 104, 1558–1563.
[52] Moy, T.I. and Silver, P.A. (1999) Nuclear export of the small ribosomal subunit requires the ran-GTPase cycle and certain nucleoporins. Genes Dev. 13, 2118–2133.
[53] Moy, T.I. and Silver, P.A. (2002) Requirements for the nuclear export of the small ribosomal subunit. J. Cell Sci. 115, 2985–2995.
[54] Seiser, R.M., Sundberg, A.E., Wollam, B.J., Zobel-Thropp, P., Baldwin, K., Spector, M.D. and Lycan, D.E. (2006) Ltv1 is required for efficient nuclear export of the ribosomal small subunit in *Saccharomyces cerevisiae*. Genetics 174, 679–691.
[55] Vanrobays, E., Gelugne, J.P., Gleizes, P.E. and Caizergues-Ferrer, M. (2003) Late cytoplasmic maturation of the small ribosomal subunit requires RIO proteins in *Saccharomyces cerevisiae*. Mol. Cell. Biol. 23, 2083–2095.
[56] Geerlings, T.H., Faber, A.W., Bister, M.D., Vos, J.C. and Raue, H.A. (2003) Rio2p, an evolutionarily conserved, low abundant protein kinase essential for processing of 20 S Pre-rRNA in *Saccharomyces cerevisiae*. J. Biol. Chem. 278, 22537–22545.
[57] Vanrobays, E., Gleizes, P.E., Bousquet-Antonelli, C., Noaillac-Depeyre, J., Caizergues-Ferrer, M. and Gelugne, J.P. (2001) Processing of 20S pre-rRNA to 18S ribosomal RNA in yeast requires Rrp10p, an essential non-ribosomal cytoplasmic protein. EMBO J. 20, 4204–4213.
[58] Kafadar, K.A., Zhu, H., Snyder, M. and Cyert, M.S. (2003) Negative regulation of calcineurin signaling by Hrr25p, a yeast homolog of casein kinase I. Genes Dev. 17, 2698–2708.
[59] Schafer, T., Maco, B., Petfalski, E., Tollervey, D., Bottcher, B., Aebi, U. and Hurt, E. (2006) Hrr25-dependent phosphorylation state regulates organization of the pre-40S subunit. Nature 441, 651–655.
[60] Lafontaine, D., Vandenhaute, J. and Tollervey, D. (1995) The 18S rRNA dimethylase Dim1p is required for pre-ribosomal RNA processing in yeast. Genes Dev. 9, 2470–2481.
[61] Brand, R.C., Klootwijk, J., Van Steenbergen, T.J., De Kok, A.J. and Planta, R.J. (1977) Secondary methylation of yeast ribosomal precursor RNA. Eur. J. Biochem. 75, 311–318.
[62] Lafontaine, D.L., Preiss, T. and Tollervey, D. (1998) Yeast 18S rRNA dimethylase Dim1p: a quality control mechanism in ribosome synthesis? Mol. Cell. Biol. 18, 2360–2370.
[63] Vanrobays, E., Gelugne, J.P., Caizergues-Ferrer, M. and Lafontaine, D.L. (2004) Dim2p, a KH-domain protein required for small ribosomal subunit synthesis. RNA 10, 645–656.
[64] Peng, W.T. et al. (2003) A panoramic view of yeast noncoding RNA processing. Cell 113, 919–933.
[65] Senapin, S., Clark-Walker, G.D., Chen, X.J., Seraphin, B. and Daugeron, M.C. (2003) RRP20, a component of the 90S preribosome, is required for pre-18S rRNA processing in *Saccharomyces cerevisiae*. Nucleic Acids Res. 31, 2524–2533.
[66] Clissold, P.M. and Ponting, C.P. (2000) PIN domains in nonsense-mediated mRNA decay and RNAi. Curr. Biol. 10, R888–R890.
[67] Fatica, A., Oeffinger, M., Dlakic, M. and Tollervey, D. (2003) Nob1p is required for cleavage of the 3′ end of 18S rRNA. Mol. Cell. Biol. 23, 1798–1807.
[68] Arcus, V.L., Backbro, K., Roos, A., Daniel, E.L. and Baker, E.N. (2004) Distant structural homology leads to the functional characterization of an archaeal PIN domain as an exonuclease. J. Biol. Chem. 279, 16471–16478.
[69] Fatica, A., Tollervey, D. and Dlakic, M. (2004) PIN domain of Nob1p is required for D-site cleavage in 20S pre-rRNA. RNA 10, 1698–1701.
[70] Granneman, S., Nandineni, M.R. and Baserga, S.J. (2005) The putative NTPase Fap7 mediates cytoplasmic 20S pre-rRNA processing through a direct interaction with Rps14. Mol. Cell. Biol. 25, 10352–10364.
[71] Gelperin, D., Horton, L., Beckman, J., Hensold, J. and Lemmon, S.K. (2001) Bms1p, a novel GTP-binding protein, and the related Tsr1p are required for distinct steps of 40S ribosome biogenesis in yeast. RNA 7, 1268–1283.
[72] Leger-Silvestre, I. et al. (2004) The ribosomal protein Rps15p is required for nuclear exit of the 40S subunit precursors in yeast. EMBO J. 23, 2336–2347.
[73] Ford, C.L., Randal-Whitis, L. and Ellis, S.R. (1999) Yeast proteins related to the p40/laminin receptor precursor are required for 20S ribosomal RNA processing and the maturation of 40S ribosomal subunits. Cancer Res. 59, 704–710.
[74] Jakovljevic, J., de Mayolo, P.A., Miles, T.D., Nguyen, T.M., Leger-Silvestre, I., Gas, N. and Woolford Jr., J.L. (2004) The carboxy-terminal extension of yeast ribosomal protein S14 is necessary for maturation of 43S preribosomes. Mol. Cell 14, 331–342.
[75] Tabb-Massey, A., Caffrey, J.M., Logsden, P., Taylor, S., Trent, J.O. and Ellis, S.R. (2003) Ribosomal proteins Rps0 and Rps21 of *Saccharomyces cerevisiae* have overlapping functions in the maturation of the 3′ end of 18S rRNA. Nucleic Acids Res. 31, 6798–6805.
[76] Spahn, C.M., Beckmann, R., Eswar, N., Penczek, P.A., Sali, A., Blobel, G. and Frank, J. (2001) Structure of the 80S ribosome from *Saccharomyces cerevisiae*–tRNA-ribosome and subunit–subunit interactions. Cell 107, 373–386.
[77] Ferreira-Cerca, S., Poll, G., Gleizes, P.E., Tschochner, H. and Milkereit, P. (2005) Roles of eukaryotic ribosomal proteins in maturation and transport of pre-18S rRNA and ribosome function. Mol. Cell 20, 263–275.
[78] Bataille, N., Helser, T. and Fried, H.M. (1990) Cytoplasmic transport of ribosomal subunits microinjected into the *Xenopus laevis* oocyte nucleus: a generalized, facilitated process. J. Cell Biol. 111, 1571–1582.
[79] Khanna-Gupta, A. and Ware, V.C. (1989) Nucleocytoplasmic transport of ribosomes in a eukaryotic system: is there a facilitated transport process? Proc. Natl. Acad. Sci. USA 86, 1791–1795.
[80] Trotta, C.R., Lund, E., Kahan, L., Johnson, A.W. and Dahlberg, J.E. (2003) Coordinated nuclear export of 60S ribosomal subunits and NMD3 in vertebrates. EMBO J. 22, 2841–2851.
[81] Thomas, F. and Kutay, U. (2003) Biogenesis and nuclear export of ribosomal subunits in higher eukaryotes depend on the CRM1 export pathway. J. Cell Sci. 116, 2409–2419.
[82] Bernad, R., Engelsma, D., Sanderson, H., Pickersgill, H. and Fornerod, M. (2006) Nup214–Nup88 nucleoporin subcomplex is required for CRM1-mediated 60 S preribosomal nuclear export. J. Biol. Chem. 281, 19378–19386.
[83] Fornerod, M., van Deursen, J., van Baal, S., Reynolds, A., Davis, D., Murti, K.G., Fransen, J. and Grosveld, G. (1997) The human homologue of yeast CRM1 is in a dynamic subcomplex with CAN/Nup214 and a novel nuclear pore component Nup88. EMBO J. 16, 807–816.
[84] Kehlenbach, R.H., Dickmanns, A., Kehlenbach, A., Guan, T. and Gerace, L. (1999) A role for RanBP1 in the release of CRM1 from the nuclear pore complex in a terminal step of nuclear export. J. Cell Biol. 145, 645–657.
[85] Rouquette, J., Choesmel, V. and Gleizes, P.E. (2005) Nuclear export and cytoplasmic processing of precursors to the 40S ribosomal subunits in mammalian cells. EMBO J. 24, 2862–2872.

[86] Frehlick, L.J., Eirin-Lopez, J.M. and Ausio, J. (2007) New insights into the nucleophosmin/nucleoplasmin family of nuclear chaperones. BioEssays 29, 49–59.
[87] Szebeni, A., Hingorani, K., Negi, S. and Olson, M.O. (2003) Role of protein kinase CK2 phosphorylation in the molecular chaperone activity of nucleolar protein b23. J. Biol. Chem. 278, 9107–9115.
[88] Szebeni, A. and Olson, M.O. (1999) Nucleolar protein B23 has molecular chaperone activities. Protein Sci. 8, 905–912.
[89] Herrera, J.E., Savkur, R. and Olson, M.O. (1995) The ribonuclease activity of nucleolar protein B23. Nucleic Acids Res. 23, 3974–3979.
[90] Savkur, R.S. and Olson, M.O. (1998) Preferential cleavage in pre-ribosomal RNA byprotein B23 endoribonuclease. Nucleic Acids Res. 26, 4508–4515.
[91] Yu, Y., Maggi Jr., L.B., Brady, S.N., Apicelli, A.J., Dai, M.S., Lu, H. and Weber, J.D. (2006) Nucleophosmin is essential for ribosomal protein L5 nuclear export. Mol. Cell. Biol. 26, 3798–3809.
[92] Borer, R.A., Lehner, C.F., Eppenberger, H.M. and Nigg, E.A. (1989) Major nucleolar proteins shuttle between nucleus and cytoplasm. Cell 56, 379–390.
[93] Reynaud, E.G., Andrade, M.A., Bonneau, F., Ly, T.B., Knop, M., Scheffzek, K. and Pepperkok, R. (2005) Human Lsg1 defines a family of essential GTPases that correlates with the evolution of compartmentalization. BMC Biol 3, 21.
[94] Nguyen, Y.H., Mills, A.A. and Stanbridge, E.J. (1998) Assembly of the QM protein onto the 60S ribosomal subunit occurs in the cytoplasm. J. Cell. Biochem. 68, 281–285.
[95] Basu, U., Si, K., Deng, H. and Maitra, U. (2003) Phosphorylation of mammalian eukaryotic translation initiation factor 6 and its *Saccharomyces cerevisiae* homologue Tif6p: evidence that phosphorylation of Tif6p regulates its nucleocytoplasmic distribution and is required for yeast cell growth. Mol. Cell. Biol. 23, 6187–6199.
[96] Ceci, M., Gaviraghi, C., Gorrini, C., Sala, L.A., Offenhauser, N., Marchisio, P.C. and Biffo, S. (2003) Release of eIF6 (p27BBP) from the 60S subunit allows 80S ribosome assembly. Nature 426, 579–584.
[97] Salim, M. and Maden, B.E. (1973) Early and late methylations in HeLa cell ribosome maturation. Nature 244, 334–336.
[98] Yao, W., Roser, D., Kohler, A., Bradatsch, B., Bassler, J. and Hurt, E. (2007) Nuclear export of ribosomal 60S subunits by the general mRNA export receptor Mex67-Mtr2. Mol. Cell. 26, 51–62.