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REVIEW ARTICLE

Assembly and nuclear export of pre-ribosomal particles in budding yeast

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Abstract The ribosome is responsible for the final step of decoding genetic information into proteins. Therefore, correct assembly of ribosomes is a fundamental task for all living cells. In eukaryotes, the construction of the ribosome which begins in the nucleolus requires coordinated efforts of >350 specialized factors that associate with pre-ribosomal particles at distinct stages to perform specific assembly steps. On their way through the nucleus, diverse energy-consuming enzymes are thought to release assembly factors from maturing pre-ribosomal particles after accomplishing their task(s). Subsequently, recruitment of export factors prepares pre-ribosomal particles for transport through nuclear pore complexes. Pre-ribosomes are exported into the cytoplasm in a functionally inactive state, where they undergo final maturation before initiating translation. Accumulating evidence indicates a tight coupling between nuclear export, cytoplasmic maturation, and final proofreading of the ribosome. In this review, we summarize our current understanding of nuclear export of pre-ribosomal subunits and cytoplasmic maturation steps that render pre-ribosomal subunits translation-competent.

Introduction

Error-free protein synthesis is vital for optimal cellular growth and proliferation, a fundamental task carried out by the ribosome. This universal molecular machine consists of a large and small subunit comprising 60 % ribosomal RNA (rRNA) and 40 % ribosomal proteins (r-proteins) (Melnikov et al. 2012). The small subunit decodes the genetic information by bringing together the messenger RNA (mRNA) template and cognate transfer RNAs (tRNAs). The large subunit catalyzes the peptidyl transfer reaction to synthesize the nascent polypeptide chain. Despite a similar core, eukaryotic ribosomes are significantly larger than their prokaryotic counterparts. The large ribosomal subunit (60S) in yeast contains three rRNAs (25S, 5.8S, 5S) and 46 r-proteins, whereas the small subunit (40S) contains one single rRNA (18S) and 33 r-proteins (Ben-Shem et al. 2011; Klinge et al. 2011; Rabl et al. 2011). While the structure of the mature yeast ribosome is described at the molecular level, our knowledge regarding its assembly is emerging.

The assembly of the eukaryotic ribosome is a highly dynamic process that occurs in different cellular compartments: the nucleolus, the nucleoplasm, and the cytoplasm. In contrast to prokaryotes, eukaryotic ribosome assembly requires coordinated efforts of the intracellular transport machinery as well as numerous transiently interacting nonribosomal assembly factors. Pre-ribosomal particles released from the nucleolus undergo sequential maturation in the nucleoplasm and cytoplasm before they acquire translation competence.

Pioneering work from the Planta and Warner laboratories, in the early 1970s, led to the identification of the earliest pre-ribosome: the 90S that is the common precursor of the mature 60S and 40S subunits (Trapman et al. 1975; Udem and Warner 1972). The 90S was thought to contain pre-rRNAs, r-proteins, and numerous assembly factors. In the early 1990s, genetic screens in budding yeast permitted the identification of ~30 assembly factors. Analyses of these mutant strains primarily performed at the rRNA level led to the ordering and positioning of the cleavage sites within maturing pre-rRNA (Kressler et al. 1999; Venema and Tollervey 1995). In the late 1990s, visual approaches were developed to identify factors involved

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in the transport of pre-ribosomal subunits (Hurt et al. 1999; Milkereit et al. 2003; Moy and Silver 2002; Stage-Zimmermann et al. 2000). Screening of temperature-sensitive mutant libraries by visual approaches revealed factors involved in early ribosome maturation indicating a tight coupling between assembly and transport of pre-ribosomes (Gadal et al. 2001a, b; Milkereit et al. 2001). Despite these advances, the ribosome assembly pathway remained refractory to biochemical analyses. Only during the last decade, tandem affinity purification (TAP) protocols and sensitive mass spectrometry (MS) permitted the isolation and compositional analyses of pre-ribosomal particles (Bassler et al. 2001; Harnpicharnchai et al. 2001). These approaches have facilitated the general ordering of assembly events on the 60S and 40S maturation pathways and provided a framework to dissect this highly dynamic process (Grandi et al. 2002; Nissan et al. 2002; Schafer et al. 2003). Structural approaches are now beginning to facilitate high-resolution analyses of this dynamic pathway (Armache et al. 2010; Ben-Shem et al. 2011; Bradatsch et al. 2012; Greber et al. 2012; Klinge et al. 2011; Rabl et al. 2011). These studies are also guiding mechanistic analyses in higher eukaryotes (Tafforeau et al. 2013; Wild et al. 2010) and uncovering the molecular basis of ribosomopathies: diseases that are associated with impaired ribosome assembly and function.

Here, we summarize our current understanding of the eukaryotic ribosome assembly pathway in budding yeast with a focus on the nuclear export and cytoplasmic maturation of pre-ribosomal particles. For excellent reviews on bacterial ribosome assembly, the readers are referred to Shajani et al. (2011) and Britton (2009).

Assembly of the earliest ribosomal precursor, the 90S pre-ribosome

The process of ribosome assembly in budding yeast begins with RNA polymerase I-driven transcription of ribosomal DNA (rDNA) repeats on chromosome XII in the nucleolus to produce 35S pre-rRNAs (Fig. 1). Co-transcriptional association with small nucleolar RNAs (snoRNAs), assembly factors, and r-proteins mainly of the 40S drives the formation of the earliest ribosomal precursor, the 90S pre-ribosome (Grandi et al. 2002). The emerging 35S pre-rRNA can be cleaved co-transcriptionally in the internal transcribed spacer 1 (ITS1), thereby releasing the pre-40S subunit (Kos and Tollervey 2010; Osheim et al. 2004).

Two independent studies have revealed the composition of the 90S that remained refractory to biochemical analyses for nearly 30 years. By isolating Mpp10-TAP, a factor associated with the box C/D U3 snoRNA and a crucial component of the 90S, the Baserga group discovered 17 novel assembly factors, called UTPs (1–17) (Dragon et al. 2002) (Fig. 1). They designated this large >2.2 MDa U3 snoRNA-containing particle responsible for processing of the small subunit (SSU) processome. The composition of the Mpp10-TAP particle significantly overlaps with several nucleolar pre-ribosomal particles isolated and characterized by the Hurt laboratory (Grandi et al. 2002).

Assembly of the 90S appears to be a hierarchical addition of pre-formed protein sub-complexes (Fig. 1a). The stepwise assembly of UTP complexes and the U3 snoRNP is closely coupled to the pre-rRNA modification/folding and possibly drives compaction of the 90S (Perez-Fernandez et al. 2011). Several components of the 90S contain motifs involved in RNA-binding and/or protein-protein interaction (Table 1 and Fig. 1b). A protein-protein interaction map of the SSU processome, compiled by the Baserga group, provides an important framework for the elucidation of the architecture of the 90S (Lim et al. 2011). A detailed understanding of protein-protein and RNA-protein interactions will also be crucial to uncover the spatial-temporal assembly of the 90S and its subsequent disassembly.

More than 60 different snoRNPs mediate >100 covalent modifications of the 35S pre-rRNA during the assembly of the 90S (Decatur and Fournier 2002, 2003; Decatur et al. 2007; Hughes and Ares 1991; Hughes 1996). There are two types of modifications: methylation of the 2′-hydroxyl group of the ribose sugars (2′-*O*-methylation) carried out by C/D box containing snoRNAs and conversion of uridine to pseudouridine carried out by H/ACA snoRNAs (Kiss 2001). These pre-rRNA modifications by snoRNAs were suggested to be important for correct folding and possibly compaction of the 90S structure (Watkins and Bohnsack 2012). Modifications of the 35S pre-rRNA are accompanied by early pre-rRNA cleavages within the developing 90S pre-ribosome (Wehner et al. 2002). Binding of U3 snoRNP to pre-rRNA is necessary for the earliest pre-rRNA cleavages at the A_0 and A_1 sites and crucial for the assembly of the early pre-40S subunit (Borovjagin and Gerbi 1999, 2001; Marmier-Gourrier et al. 2011) (reviewed in Phipps et al. (2011), Watkins and Bohnsack (2012), and Yip et al. (2013)).

Cleavage at the A_2 site, by a yet unidentified endonuclease, releases the earliest pre-40S and pre-60S particles (Fig. 2). Pre-40S subunits containing immature 20S pre-rRNAs are rapidly exported into the cytoplasm where they undergo final pre-rRNA processing to 18S rRNA. In contrast, the $27SA_2$ pre-rRNA precursor within a pre-60S subunit undergoes a series of sequential processing steps to yield the mature 25S rRNA and 5.8S rRNA (Fig. 2b). A third rRNA species of the 60S subunit, the 5S rRNA, is generated by RNA polymerase III (Fig. 2). Processing of the 5S rRNA 3′ end can be carried out by multiple redundant exonucleases Rex1, Rex2, and Rex3 (van Hoof et al. 2000). A rationally guided screen for ribosome biogenesis factors uncovered a requirement of Snu66, a component of the splicing machinery, for proper

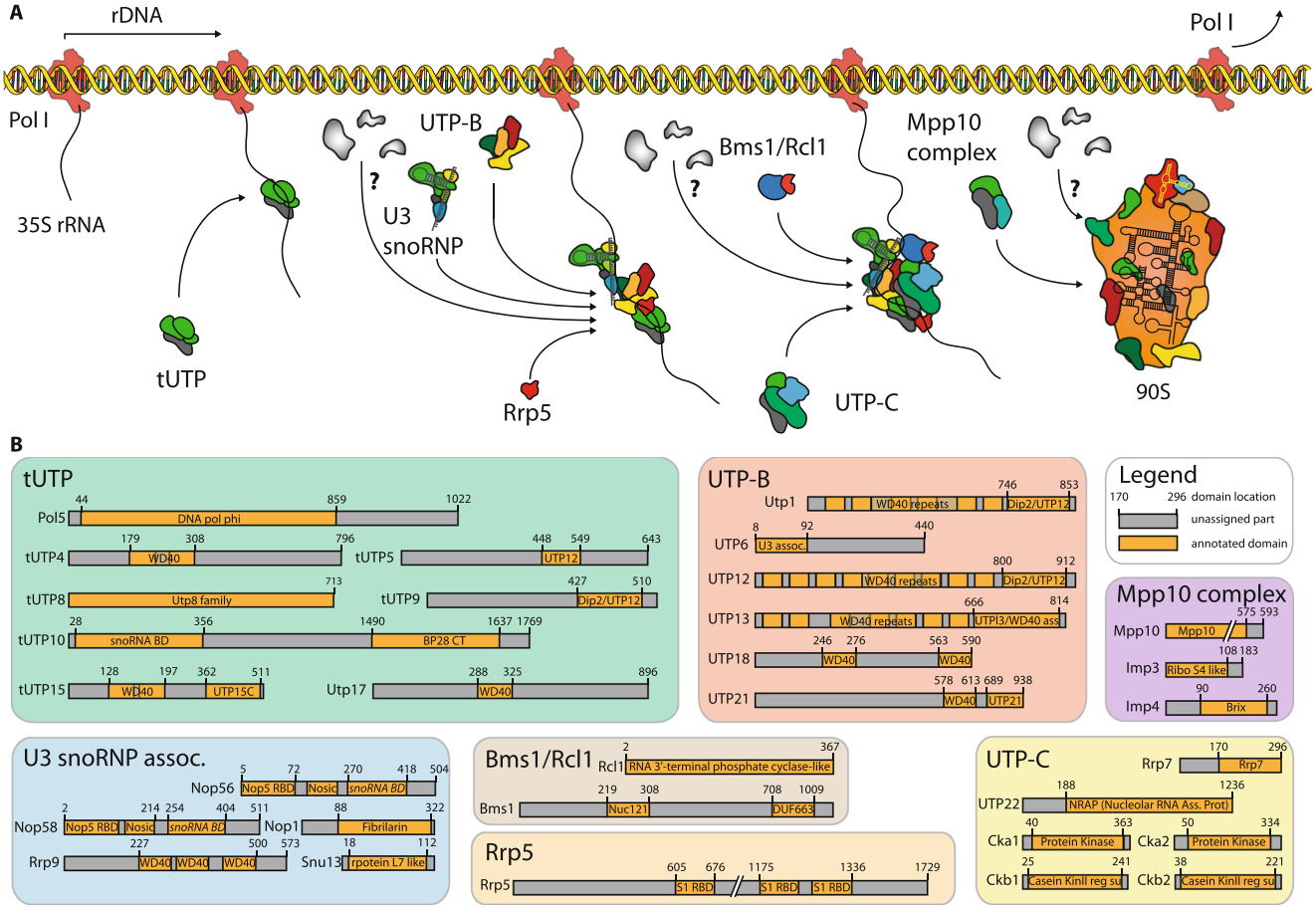


Fig. 1 Model for the hierarchical assembly of the 90S pre-ribosome. **a** The assembly of the tUTP sub-complex is responsible for the initial formation of the 90S. This step allows subsequent incorporation of the indicated sub-complexes. Two independent assembly steps guide 90S formation: recruitment of the U3 snoRNP and UTP-B sub-complexes (*top*). These primary steps are necessary for the assembly of at least 20 components of the particle (Dunbar et al. 1997; Granneman et al. 2003; Lee and Baserga 1999; Wehner et al. 2002). GTPase Bms1 is necessary for a secondary assembly step that promotes the subsequent incorporation of numerous proteins and the Mpp10 sub-complex. A second assembly step involves the incorporation of Rrp5 (*bottom*), which is crucial for the recruitment of the UTP-C sub-complex but not the U3 snoRNP, the tUTP, or the UTP-B complexes (Perez-Fernandez et al. 2007; Vos et al. 2004). **b** Schematic representation of the protein constituents of the 90S sub-complexes. The domain identification was performed using the online tool Pfam, as described by Punta et al. (2012)

processing of the 5S rRNA precursor (Li et al. 2009). However, its precise contribution to this processing step remains unclear.

Compositional dynamics of pre-ribosomal particles before nuclear export

After separation of the 90S into pre-40S and pre-60S particles, the two precursors follow independent maturation pathways. Pre-40S subunits undergo few compositional changes as they travel through the nucleoplasm and are rapidly exported into the cytoplasm. During their transit through the nucleoplasm, pre-40S subunits associate with protein kinases (Rio1 and Hrr25) and the ATPase Rio2. These energy-consuming steps possibly prepare the pre-40S subunit for nuclear export and/or final cytoplasmic maturation (Geerlings et al. 2003; Vanrobays et al. 2003). However, the relevant substrates for Rio1, Rio2, and Hrr25 remain to be uncovered. The assembly factors Enp1, Ltv1, and the r-protein uS3 (Rps3, new nomenclature as proposed by Ban et al. (2014)) form a sub-complex at the landmark beak structure of the 40S subunit (Schafer et al. 2006). This complex can be dissociated in vitro from pre-40S subunits by the activity of the conserved kinase Hrr25. It was proposed that phosphorylation of the Enp1-Ltv1-uS3 (Enp1-Ltv1-Rps3) sub-complex increases the conformational flexibility in the head region of the pre-40S subunit in vivo. Hrr25 depletion impairs nuclear export of pre-40S subunits supporting the notion that its kinase activity makes the rigid head region more flexible during transport through the nuclear pore complex (NPC). Dephosphorylation by an unknown phosphatase in the cytoplasm could permit the stable

Table 1 Composition of sub-complexes within the earliest ribosome precursor, the 90S. The domain identification was performed using the online tool Pfam, as described by Punta et al. (2012)

Sub-complex	Component	Activity/function/[predicted domain composition]	References
tUTP/UTP-A	tUTP4	[WD40 repeats]	Dragon et al. (2002), Gallagher et al. (2004), Krogan et al. (2002)
	tUTP5	[UTP12 domain]	Dragon et al. (2002), Gallagher et al. (2004)
	tUTP8	Participates also in nuclear export of tRNA; [UTP8 family domain]	Dragon et al. (2002), Gallagher et al. (2004), Strub et al. (2007)
	tUTP9	[Dip2/UTP12 domain]	Dragon et al. (2002), Gallagher et al. (2004), Krogan et al. (2002)
	tUTP10	HEAT repeats; [snoRNA BD]; [BP28CT domain]	Dragon et al. (2002), Gallagher et al. (2004), Krogan et al. (2002)
	tUTP15	[WD40 repeats]; [UTP15C domain]	Dragon et al. (2002), Gallagher et al. (2004), Krogan et al. (2002)
	tUTP17 (Nan1)	Member of the RENT complex; [WD40 repeats]	Dragon et al. (2002), Gallagher et al. (2004), Krogan et al. (2002)
	Pol5	Required for rRNA synthesis; [DNA pol phi domain]	Gallagher et al. (2004), Krogan et al. (2002), Shimizu et al. (2002)
U3 snoRNP associated	Nop1	2′-*O*-methyltransferase; [Fibrilarin domain]	Lischwe et al. (1985), Tollervey et al. (1991)
	Nop56	KKE/D repeats; coiled coils; structural scaffold for the snoRNP complex; [Nop5 RBD]; [Nosic domain]; [snoRNA BD]	Lafontaine and Tollervey (2000), Watkins and Bohnsack (2012)
	Nop58	KKE/D repeats; [Nop5 RBD]; [Nosic domain]; [snoRNA BD]	Wu et al. (1998)
	Snu13	Binds to K-turn motifs of U3 snoRNA; member of the U4/U6-U5 tri-snRNP; [r-protein L7-like domain]	Watkins et al. (2000)
	Rrp9	Binds to the B/C motif of U3 snoRNA; essential for recruitment of U3 snoRNP to SSU processome; [WD40 repeats]	Lukowiak et al. (2000), Venema et al. (2000)
UTP-B	UTP1	β Transducin family; [WD40 repeats]; [Dip2/UTP12 domain]	Champion et al. (2008), Dosil and Bustelo (2004), Dragon et al. (2002), Krogan et al. (2002)
	UTP6	HAT motif; [U3 assoc. domain]	Champion et al. (2008), Dosil and Bustelo (2004), Dragon et al. (2002), Krogan et al. (2002)
	UTP12	[WD40 repeats]; [Dip2/UTP12 domain]	Champion et al. (2008), Dosil and Bustelo (2004), Dragon et al. (2002), Krogan et al. (2002)
	UTP13	[WD40 repeats]; [UTP-like3/WD40 assoc. domain]	Champion et al. (2008), Dosil and Bustelo (2004), Dragon et al. (2002), Krogan et al. (2002)
	UTP18	[WD repeats]	Bernstein et al. (2004), Champion et al. (2008), Dosil and Bustelo (2004), Krogan et al. (2002)
	UTP21	Coiled coils; [WD40 repeats]; [UTP21 domain]	Bernstein et al. (2004), Champion et al. (2008), Dosil and Bustelo (2004), Krogan et al. (2002), Samanta and Liang (2003)
Bms1/Rcl1	Bms1	GTPase stimulated by Rcl1; [Nuc121 domain]; [DUF633 domain]	Karbstein et al. (2005), Wegierski et al. (2001)
	Rcl1	Stimulates Bms1; [RNA 3′-terminal phosphate cyclase-like domain]	Billy et al. (2000), Tanaka et al. (2011), Wegierski et al. (2001)
UTP-C	Rrp7	Also part of the CURI complex; [Rrp7 domain]	Baudin-Baillieu et al. (1997), Dalley et al. (2008), Krogan et al. (2002), Rudra et al. (2007)
	UTP22	Also part of the CURI complex; [nucleolar RNA assoc. protein domain]	Bernstein et al. (2004), Krogan et al. (2002), Peng et al. (2003), Rudra et al. (2007), Samanta and Liang (2003)
	Cka1	α Catalytic subunit casein kinase 2; nonessential; also part of the CURI complex; [protein kinase domain]	Krogan et al. (2002), Rudra et al. (2007)
	Cka2	α′ Catalytic subunit casein kinase 2; nonessential; also part of the CURI complex; [protein kinase domain]	Krogan et al. (2002), Rudra et al. (2007)
	Ckb1	β Regulatory subunit casein kinase 2; nonessential; also part of the CURI complex; [casein kinase II reg. subunit domain]	Krogan et al. (2002), Rudra et al. (2007)
	Ckb2		Krogan et al. (2002), Rudra et al. (2007)

(continued)

Sub-complex	Component	Activity/function/[predicted domain composition]	References
		β′ Regulatory subunit casein kinase 2; nonessential; also part of the CURI complex; [casein kinase II reg. subunit domain]	
Mpp10	Imp3	[S4 RNA-binding domain]	Lee and Baserga (1999)
	Imp4	[Brix domain]	Ng et al. (2005)
	Mpp10	Coiled coils; interacts with the hinge region of U3 snoRNA; [Mpp10 domain]	Dunbar et al. (1997)

incorporation of uS3 (Rps3) into the mature 40S subunit and formation of the final beak structure (Schafer et al. 2006).

In the 60S assembly pathway, incorporation of 5S rRNA in complex with r-proteins uL18 (Rpl5) and uL5 (Rpl11) is an important early nucleolar/nuclear event. Recent work from the Hurt laboratory suggested that the r-proteins uL18 (Rpl5) and uL5 (Rpl11) are co-imported by Syo1, an adaptor for the importin Kap104 (Kressler et al. 2012a). In the nucleus, the uL18-uL5-Syo1 (Rpl5-Rpl11-Syo1) complex is released from Kap104 in a RanGTP-dependent manner and then loaded on the 5S rRNA (Kressler et al. 2012a). Incorporation of uL18-uL5-5S rRNA complex into the pre-60S particle is facilitated by the assembly factors Rpf2 and Rpf1 (Ciganda and Williams 2011; Zhang et al. 2007). However, the exact timing of incorporation into pre-60S subunits remains to be investigated.

During the journey through the nucleoplasm, pre-60S particles associate with >150 assembly factors as they travel through the nucleoplasm toward the nuclear periphery. At distinct maturation stages, assembly factors are released from pre-ribosomal particles and recycled back to participate in new rounds of biogenesis steps. This sequential reduction in complexity of the pre-60S subunits is very likely triggered by a multitude of energy-consuming enzymes. ATP-dependent RNA helicases, AAA-ATPases, ABC-ATPases, and GTPases associate with maturing pre-ribosomal particles and confer directionality to the assembly and maturation process. The binding site(s) of these energy-consuming enzymes on maturing pre-60S particles are beginning to be uncovered (Kressler et al. 2012b; Strunk et al. 2012).

Among the diverse energy-consuming enzymes that drive ribosome assembly, the molecular roles of AAA-ATPases are better understood. Three essential AAA-ATPases contribute to pre-60S subunit maturation. The AAA-ATPases Rix7 and Drg1 are closely related to the well-characterized Cdc48 (p97 in mammals) and contribute to early nucleolar and cytoplasmic pre-60S subunit maturation, respectively (Kappel et al. 2012; Pertschy et al. 2007). Rea1, which is the largest protein in yeast, shares similarity to the microtubule motor protein dynein heavy chain and functions at different nuclear steps during 60S maturation (Bassler et al. 2010; Kressler et al. 2012b; Ulbrich et al. 2009).

Rix7 is the first AAA-ATPase that is involved in the maturation of the pre-60S subunit. Like the archetypical AAA-ATPase Cdc48, which uses cycles of ATP hydrolysis to remodel protein complexes (Meyer et al. 2012), Rix7 was suggested to strip off the assembly factor Nsa1 and facilitate the nucleolar to nucleoplasmic transition of pre-60S subunits along the assembly pathway (Kressler et al. 2008). How Rix7 recognizes Nsa1 and extracts it from maturing pre-60S subunits remains to be investigated. It was suggested that Rix7 might recognize posttranslational modifications like ubiquitin and SUMO, either directly or via adaptor proteins, to release Nsa1 and further assembly factors (Kressler et al. 2008; Panse et al. 2006).

Rea1 consists of six ATPase modules, forming a binding platform on the ribosome and binds in close proximity to the Rix1-Ipi3-Ipi1 sub-complex. A long α-helical linker domain, a D/E-rich region, and a functionally important metal-ion-dependent adhesion site (MIDAS) domain follow the ATPase modules. By employing the MIDAS domain, Rea1 can directly contact the MIDAS interacting domains (MIDO) of Ytm1 and Rsa4 and triggers the release of both assembly factors via ATP hydrolysis cycles (Bassler et al. 2010; Ulbrich et al. 2009). Removal of the Ytm1-Erb1-Nop7 sub-complex may trigger the release of neighboring biogenesis factors on the pre-ribosomal particles, as well as recruitment of further assembly factors involved in later biogenesis steps. The release of Rsa4 occurs at a later stage of the assembly pathway and signals the progression toward export competence (Matsuo et al. 2014; Nissan et al. 2002; Ulbrich et al. 2009).

Recently, Rea1 and the GTPase Nug2 were implicated in a nuclear checkpoint step that prevents premature formation of an export competent pre-60S subunit (Matsuo et al. 2014). The GTPase Nug2 (also known as Nog2) binds the pre-60S subunits in the nucleus at a site, which clashes with the binding site for the export adaptor Nmd3 (Sengupta et al. 2010). Nug2 bound to the pre-60S subunit allows nucleoplasmic maturation to occur. Release of Nug2 depends on its GTPase activity as well as the ATPase activity of Rea1. Only after the release of Nug2, Nmd3 can bind pre-60S subunits and they can be exported. This mechanism provides a timed acquisition of export competence for the large ribosomal subunit (Matsuo et al. 2014).

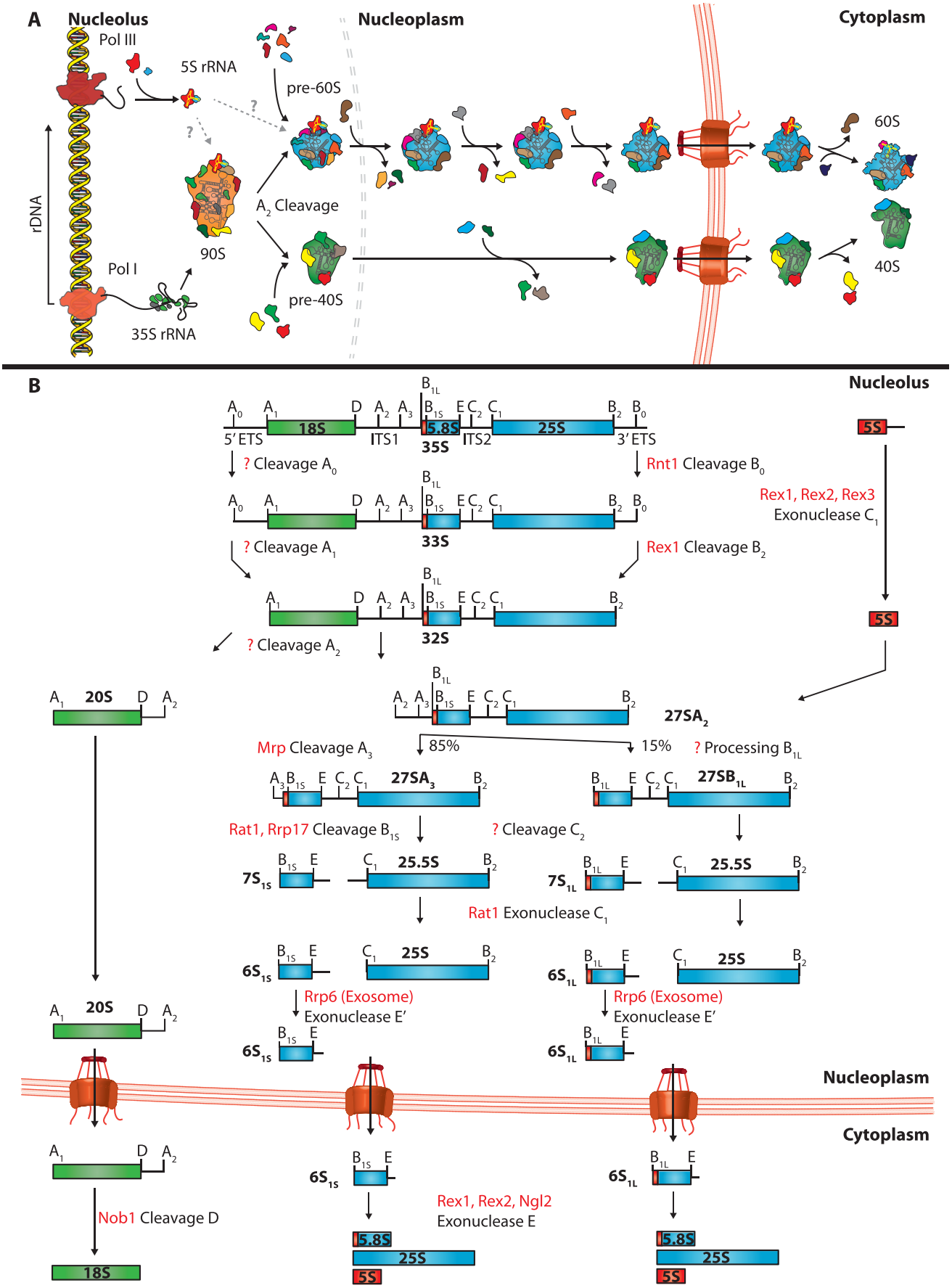
Nuclear export of pre-ribosomal particles

Assembly of eukaryotic ribosomes begins in the nucleolus, but translation of mRNA into proteins by the mature ribosome occurs in the cytoplasm. Inevitably, pre-ribosomal particles need to be transported through the NPCs into the cytoplasm. NPCs are huge protein assemblies embedded within the double lipid bilayer of the nuclear envelope and serve as ports to exchange macromolecules between the nucleus and cytoplasm. For excellent reviews on the NPC structure and assembly, readers are referred to Hoelz et al. (2011), Wente and Rout (2010), and Zwerger and Medalia (2012). The NPC transport channel permits free diffusion of molecules <40 kDa (Raices and D'Angelo 2012). Translocation of complex cargos such as the charged >2 MDa pre-ribosome through the hydrophobic phenylalanine-glycine (FG)-repeat meshwork of the NPC channel poses a major challenge. Transport of pre-ribosomal particles is facilitated by multiple factors that interact with the FG-meshwork of the NPC channel (Fig. 3). In actively growing budding yeast cells, it is estimated that each NPC contributes to the export of ~25 pre-ribosomal particles per minute (Warner 1999). Such a rapid process requires an efficient transport machinery that ensures rapid translocation of pre-ribosomal cargos through the NPC channel. Cell biological tools that monitored the intracellular localization of ribosomal subunits revealed the requirement of several components of the NPC, the Ran GTPase cycle, and the export receptor Xpo1, in the nuclear export of pre-ribosomes (Hurt et al. 1999; Stage-Zimmermann et al. 2000). Subsequently, a visual screen and an independent genetic approach uncovered an essential nuclear export signal (NES) containing adaptor Nmd3. Nmd3 forms a complex with Xpo1 in the presence of the GTPase Ran and facilitates export of the bound pre-60S subunit (Gadal et al. 2001a, b; Ho et al. 2000).

Efficient translocation of pre-ribosomes through the NPC requires multiple export factors (Oeffinger et al. 2004). Notably, the essential general mRNA transport receptor Mex67-Mtr2 (Santos-Rosa et al. 1998; Segref et al. 1997) and the HEAT-repeat containing protein Rrp12 contribute to export of both pre-ribosomal subunits (Faza et al. 2012; Oeffinger et al. 2004; Yao et al. 2007) (Fig. 3). Mex67, the large subunit of the heterodimer, is composed of an amino-terminal (N) domain, a leucine-rich repeat (LRR) domain, a nuclear transport factor 2 (NTF2)-like middle domain, and a C-terminal ubiquitin associated (UBA)-like domain (Strasser et al. 2000). Mtr2 shares structural features with NTF2 (Bayliss et al. 2002) and heterodimerizes with the NTF2-like middle domain of Mex67 (Santos-Rosa et al. 1998; Strasser et al. 2000). Loops emanating from the NTF2-like domains contribute to pre-60S and pre-40S subunit binding (Fribourg and Conti 2003; Senay et al. 2003; Yao et al. 2007) suggesting a versatile common interaction platform on Mex67-Mtr2. A recent study from the Tollervey laboratory revealed crosslinks between Mex67 and the 3′ end of 20S pre-rRNA transcript as well as 5.8S rRNA and multiple regions along the 25S rRNA in the vicinity of 5.8S rRNA supporting the idea that Mex67 interacts with both pre-40S and pre-60S subunits (Tuck and Tollervey 2013). The NTF2-like domains of Mex67-Mtr2 and the UBA-like domain of Mex67 interact directly with the FG-meshwork and therefore facilitate nuclear export of the bound cargo (Strasser et al. 2000; Strawn et al. 2001). Mex67-Mtr2 does not directly rely on the Ran cycle for export (Yao et al. 2007) and is the only known transport factor that contributes to the export of three major cargos: mRNA, pre-60S, and pre-40S subunits. It is not understood how the Mex67-Mtr2-pools are split to export all three substrates. Understanding the molecular basis of this allocation will reveal how the three export pathways cross talk to deliver required levels of mRNA and ribosomal subunits in the cytoplasm.

Rrp12 is the second factor that binds both pre-60S and pre-40S subunits and is required for their nuclear export (Oeffinger et al. 2004). Rrp12 contains secondary structural elements called HEAT (Huntingtin, elongation factor 3, protein phosphatase 2A, and TOR1) repeats, which are found in other RanGTP-dependent export factors, and shown to interact with the FG-meshwork. How Rrp12 binds FXFG-repeat nucleoporins remains to be investigated. Whether RanGTP regulates the interaction of Rrp12 with pre-ribosomal subunits and/or FG-nucleoporins remains to be determined. Identifying mutants of Rrp12 that are impaired in binding to pre-

Fig. 2 The assembly pathway of the eukaryotic ribosome. **a** Co-transcriptional recruitment of small subunit r-proteins and assembly factors to the 35S pre-rRNA yields the earliest ribosomal precursor, the 90S (*orange*). Cleavage at the A_2 site separates the 90S into a pre-40S subunit (*green*) and a pre-60S subunit (*blue*), which undergo independent maturation. Transiently associating assembly factors drive maturation of the pre-ribosomal subunits as they travel through the nucleoplasm toward the NPCs. Final maturation in the cytoplasm yields translation competent ribosomal subunits. **b** The pre-rRNA processing pathway of pre-ribosomal particles. The common 35S rRNA precursor is trimmed at both ends (Kufel et al. 1999) and cleaved at the A_2 site. The cleavage yields 20S and $27SA_2$ rRNAs which mature independently: The 20S rRNA is processed to 18S rRNA in the cytoplasm (Lamanna and Karbstein 2009; Pertschy et al. 2009). The $27SA_2$ pre-rRNA is processed in two ways generating two different 5.8S rRNAs (Lygerou et al. 1996). Following the cleavage at the A_3 site, the 5′ end of 5.8S is rapidly processed to site B_{1S}, by exonucleases Rrp17 (Oeffinger et al. 2009) and the Rat1-Rai1 heterodimer (Henry et al. 1994). $27SB_{1L}$ and the $27SB_{1S}$ pre-RNAs are cleaved at the C_2 site, to produce the $7S_{L/S}$ and the 25.5S pre-rRNA. The latter is converted into the 25S rRNA by Rat1-Rai1 in the nucleus (Geerlings et al. 2000). The 7S pre-rRNAs are processed at the 3′ ends to shorter intermediates (Allmang et al. 1999; Mitchell et al. 1997) and then to $6S_{1S/1L}$ by the nuclear exosome (Briggs et al. 1998). Processing of 6S pre-rRNA to mature 5.8S rRNA takes place in the cytoplasm and requires the nucleases Rex1, Rex2, and Ngl2 (Faber et al. 2002; Thomson and Tollervey 2010; van Hoof et al. 2000). The 5S rRNA is processed in by the nucleases Rex1, Rex2, and Rex3 (van Hoof et al. 2000). For detailed information on pre-rRNA processing, readers are referred to Woolford and Baserga (2013) and Fromont-Racine et al. (2003)


A
Nucleolus
Nucleoplasm
Cytoplasm
Pol III
5S rRNA
pre-60S
60S
rDNA
90S
A2 Cleavage
Pol I
pre-40S
40S
35S rRNA
B
Nucleolus
5′ ETS
18S
ITS1
5.8S
ITS2
25S
3′ ETS
35S
? Cleavage A0
Rnt1 Cleavage B0
Rex1, Rex2, Rex3
Exonuclease C1
? Cleavage A1
Rex1 Cleavage B2
33S
32S
? Cleavage A2
5S
20S
27SA2
Mrp Cleavage A3
85%
15%
? Processing B1L
27SA3
27SB1L
Rat1, Rrp17 Cleavage B1S
? Cleavage C2
7S1S
25.5S
7S1L
Rat1 Exonuclease C1
6S1S
25S
6S1L
Rrp6 (Exosome)
Exonuclease E′
Nucleoplasm
Cytoplasm
Nob1 Cleavage D
Rex1, Rex2, Ngl2
Exonuclease E

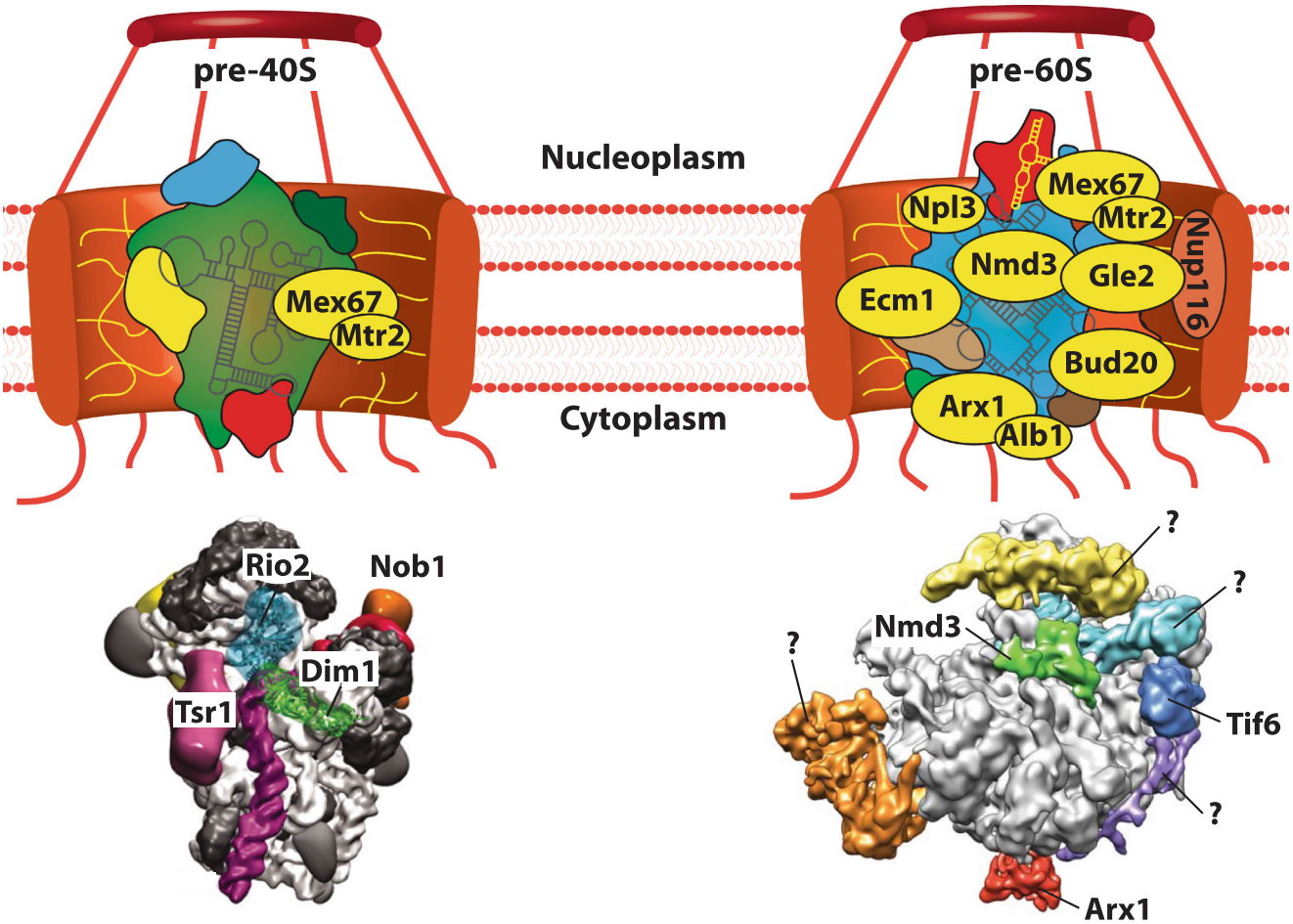


Fig. 3 Transport of pre-ribosomal particles through the NPC channel. Export of the pre-ribosomal particles is facilitated by indicated export factors (*yellow*) that interact with the FG meshwork of the NPC channel. Cryo-EM maps of late pre-ribosomal particles isolated by Rio2-TAP and Alb1-TAP. The pre-40S model is adapted from Figure 2 from Strunk et al. (2011) and the pre-60S model is adapted from Figure 2 from Bradatsch et al. (2012)

ribosomal particles, FXFG-repeat nucleoporins and RanGTP will clarify the contribution of Rrp12 to pre-ribosome export.

Additionally, pre-ribosomal particles employ multiple non-essential, auxiliary factors that can directly bind FG-rich nucleoporins and directly facilitate translocation of pre-ribosomal particles through the NPC channel (Fig. 3). The *trans*-acting factor Arx1 that localizes to the exit tunnel plays an auxiliary role in pre-60S subunit nuclear export (Bradatsch et al. 2007; Hung et al. 2008). Arx1 contains a methionine aminopeptidase (MetAP)-like fold, which is present in a family of proteins that remove the N-terminal methionine from nascent polypeptides as they emerge from the ribosome. However, Arx1 lacks a methionine aminopeptidase activity. Mutations in the methionine-binding pocket impair the function of Arx1 in pre-60S subunit export, which led to the proposal that this fold has evolved to interact with FG-nucleoporins (Bradatsch et al. 2007). Two recent studies revealed that the proposed FG-interacting pocket of Arx1 points toward the exit tunnel of the 60S subunit and hence does not appear to be exposed to the solvent. Further, Arx1 not only covers the exit tunnel of the 60S subunit, but it also arrests a conserved rRNA expansion segment 27 (ES27) in a so-called tunnel conformation (Bradatsch et al. 2012; Greber et al. 2012). It could be that Arx1 gets detached from the main body of the pre-60S subunit, while it remains bound to the pre-60S subunit via its interaction with ES27, during translocation of pre-60S subunits through the NPC. Such a scenario may reconcile the apparent paradox, as to how Arx1 simultaneously interacts with the pre-60S subunit as well as FG-rich nucleoporins. Nevertheless, the functional significance of the interaction between ES27 and Arx1 remains to be determined.

Functional screens in yeast have identified factors that directly promote nuclear export of pre-ribosomes. These approaches uncovered the shuttling assembly factors (Ecm1 and Bud20) and mRNA export factor (Npl3) involved in pre-60S subunit nuclear export (Altvater et al. 2012; Bassler et al. 2001, 2012; Hackmann et al. 2011; Yao et al. 2010). Whether pre-ribosomal subunits employ all export factors for their translocation through the NPC is currently unclear. It could be that a minimal set might be sufficient to facilitate

rapid export. A particular challenge is to localize export factors on pre-ribosomal particles. These analyses are expected to provide insight into how pre-ribosomal particles are oriented during the translocation through the NPC channel.

All export factors described to date utilize the FG-meshwork to translocate pre-ribosomal subunits through the NPCs. Recently, we have uncovered a role for the non-FG-interacting transport factor Gle2 in pre-60S subunit export (Occhipinti et al. 2013). Gle2 interacts with pre-60S through a conserved basic patch and utilizes a second interaction surface to simultaneously bind the GLEBS (Gle2-binding sequence) motif of Nup116 (Fig. 3). These interactions together facilitate the transit of the pre-60S particle through the NPC. Mutations that impair the function of Gle2 in pre-60S nuclear export do not affect mRNA export (Occhipinti et al. 2013). Curiously, the recruitment of Gle2 to pre-60S subunits requires its prior tethering to the GLEBS motif of nucleoporin Nup116. Thus, Gle2 could utilize distinct interaction surfaces to prevent kinetic delays experienced by mRNPs and pre-60S subunits during translocation through the NPC channel, especially in the case when cargos have failed to recruit its optimal complement of export factors.

In contrast to the pre-60S, fewer export factors are described for the pre-40S subunit. Studies in mammalian cells have revealed multiple NES containing assembly factors (Ltv1, Dim2, and Rio2) that recruit the export factor Crm1 (Xpo1) (Zemp et al. 2009). An essential NES containing adaptor for pre-40S subunits that recruits Xpo1 remains elusive. One possibility could be that multiple NES containing adaptors play redundant roles to recruit the essential export receptor Xpo1 and guarantee efficient nuclear export of pre-40S subunits.

Another conserved factor that specifically functions in the nuclear export of pre-40S subunits is the conserved RanGTP-binding protein Yrb2 (Moy and Silver 2002; Taura et al. 1998). The *yrb2*Δ mutant exhibits a marked decrease in the 40S subunit levels and strong nucleoplasmic accumulation of the small subunit reporters (Faza et al. 2012; Moy and Silver 2002). Notably, in vitro studies showed that the human homolog of Yrb2 (RanBP3) triggers the loading of Xpo1 (Crm1) and RanGTP on certain cargoes that are exported into the cytoplasm (Englmeier et al. 2001). Therefore, one possibility could be that Yrb2 delivers Xpo1 and RanGTP to certain yet unknown NES containing adaptor(s) to promote nuclear export of pre-40S subunits.

Cytoplasmic maturation pathway of pre-ribosomes

Prior to nuclear export, the majority of assembly factors, which associate with pre-ribosomal particles during early biogenesis, are released after fulfilling their function. Only a handful of assembly factors travel with pre-ribosomal particles to the cytoplasm. The release of these factors, the incorporation of the remaining r-proteins, and final pre-rRNA processing events constitute cytoplasmic maturation in the ribosome biogenesis pathway (Fig. 4). These steps are not only crucial for completing ribosome maturation, but are also crucial for new rounds of ribosome biogenesis. A failure to release and recycle assembly and export factors back to the nucleus induces pre-rRNA processing delays, assembly defects, and impaired nuclear export.

Cytoplasmic maturation of pre-40S subunits

The pre-40S subunits are accompanied to the cytoplasm by a handful of proteins (Enp1, Tsr1, Ltv1, Dim1, Dim2, Nob1, Rio2, and Hrr25) that contribute to their export as well as subsequent pre-rRNA processing (Strunk et al. 2011). How these shuttling assembly factors are released from pre-40S subunits remains unclear. Cytoplasmic processing of immature 20S pre-rRNA within pre-40S subunits involves two conserved events: dimethylation of the 20S pre-rRNA and the endonucleolytic cleavage (site D in Fig. 2b) of 20S pre-rRNA to 18S rRNA.

A late pre-rRNA modification step in 40S biogenesis is the dimethylation of two adenine bases near the 3′ end of the 18S rRNA. The enzyme responsible for catalyzing this modification is the essential factor Dim1 that is loaded on the pre-40S subunits (Lafontaine et al. 1994). Dimethylation is first detected on the 20S rRNA precursor and was suggested to take place once the pre-40S particle reaches the cytoplasm. Although dimethylation occurs late during subunit maturation, Dim1 associates already with the 90S. Dim1 depletion causes an early nucleolar pre-rRNA processing defect, which can be rescued by a catalytically inactive *dim1* mutant. Intriguingly, the catalytically inactive *dim1* mutant rescues the lethality of the *dim1*Δ strain suggesting that the dimethylating activity of Dim1 is not essential and can be separated from its essential role in early pre-rRNA processing. Dimethylation was suggested to play a role in fine-tuning translation as the *dim1* mutant displays increased antibiotic sensitivity (Lafontaine et al. 1998).

An essential cytoplasmic maturation event that renders pre-40S particles translation-competent is the endonucleolytic cleavage of the immature 20S rRNA into mature 18S rRNA. Multiple energy-consuming ATPases (Prp43, Rio2, and Fap7) and the PIN-domain endonuclease Nob1 were implicated in this late maturation step (Clissold and Ponting 2000; Geerlings et al. 2003; Jakovljevic et al. 2004; Lamanna and Karbstein 2009; Pertschy et al. 2009; Vanrobays et al. 2003). Nob1 is recruited to 40S pre-ribosomes already in the nucleus, suggesting that there must be an activating mechanism for Nob1 in the cytoplasmic compartment. Studies from the Tollervey and Karbstein laboratories revealed that pre-40S subunits interact with mature 60S subunits to form an 80S-like particle in vitro

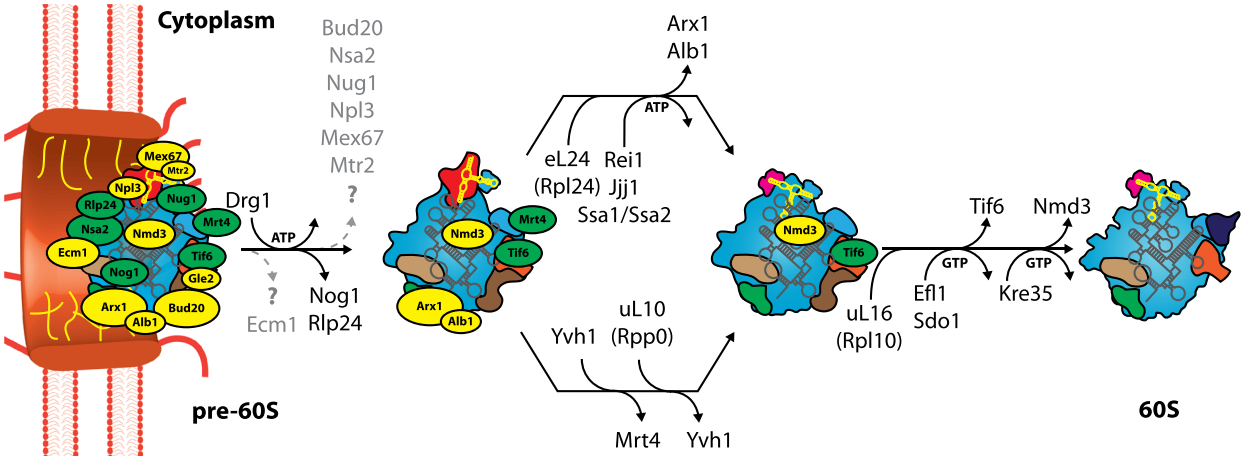


Fig. 4 Cytoplasmic maturation of a large pre-ribosomal subunit prior to initiating translation. Exported pre-60S subunits are bound by export factors (*yellow*) and shuttling factors (*green*) which are released in the cytoplasm. The ATPase Drg1 releases Rlp24 from the pre-ribosomal particles. This event triggers the subsequent maturation events. Arx1 and Alb1 require Rei1, Jjj1, and Ssa1/Ssa2 for their release, whereas the stalk assembly can only take place after the release of the shuttling ribosomal-like protein Mrt4 by the cytoplasmic release factor Yvh1. Recruitment of uL10 (Rpp0) releases Yvh1, which allows further assembly of the P1 (Rpp1) and P2 (Rpp2) heterodimer onto the stalk. Loading of uL16 (Rpl10) triggers the final maturation steps. The GTPase Efl1 and its co-factor Sdo1 release Tif6 and another GTPase Kre35 (Lsg1) removes Nmd3. The release mechanisms/factors of shuttling assembly factors like Nog1, Nug1, and Nsa2 and the transport factors Mex67-Mtr2, Bud20, Ecm1, and Gle2 (depicted in *gray*) remain to be uncovered

and in vivo (Lebaron et al. 2012; Strunk et al. 2012). The interaction with the 60S subunit triggers the activity of Nob1 to cleave 20S pre-RNA to mature 18S rRNA in vitro. Both studies implicated that the conserved cytoplasmic GTPase and translation initiation factor eIF5b/Fun12 might be important for the formation of the 80S-like particle, which triggers this final cleavage step in the cytoplasm. Subsequently, Nob1 is released and recycled due to the action of the ABC-ATPase Rli1 that dissociates the pre-40S subunit from the 60S subunit, after 20S pre-rRNA cleavage (Strunk et al. 2012). Fun12 is not an essential gene in budding yeast, whereas the endonucleolytic cleavage of 20S pre-rRNA is essential. Considering the high abundance of pre-40S subunits, it is intriguing that the *fun12*Δ mutant shows only minor accumulation of 20S pre-rRNAs in the cytoplasm (Lebaron et al. 2012). It could be that in a *fun12*Δ mutant, pre-40S subunits containing immature 20S pre-rRNA fail to undergo cytoplasmic maturation and are then rapidly degraded. In support of an 80S-like translation cycle, mutations in the 60S subunit r-protein uL3 (Rpl3), which affect the affinity to translation elongation factors, were shown to specifically impair cytoplasmic 20S pre-rRNA processing (Garcia-Gomez et al. 2014).

Cytoplasmic maturation of pre-60S subunits

In addition to shuttling export factors (Nmd3, Arx1, Ecm1, and Mex67-Mtr2), genetic approaches identified few assembly factors (Rlp24, Tif6, Nog1, and Alb1) that associate with pre-60S particles in the nucleus and are transported to the cytoplasm. Release of these factors is catalyzed by conserved energy-consuming GTPases (Kre35, Efl1), ATPases (Drg1, Hsp70), and their cofactors (Sdo1, Rei1, Jjj1) that transiently associate with pre-60S particles exclusively in the cytoplasm (Panse and Johnson 2010).

The Johnson laboratory established the order of known release events that provided an initial framework of the cytoplasmic maturation pathway (Fig. 4) (Lo et al. 2010). A characteristic feature of the pre-60S cytoplasmic maturation is the sequential release of assembly factors and transport factors (Fig. 4 and Table 2). The AAA-ATPase Drg1 triggers the earliest cytoplasmic maturation step on pre-60S particles, which is a prerequisite for subsequent cytoplasmic maturation steps of 60S pre-ribosomes. Catalytically impaired *drg1* mutants accumulate the nuclear assembly factors Rlp24, Nog1, Arx1, and Tif6 in the cytoplasm where they remain bound to pre-60S subunits (Pertschy et al. 2007). Thus, the activity of Drg1 is required for their release from pre-60S particles, thereby also allowing their re-import into the nucleus. AAA-ATPases typically do not exhibit broad substrate specificities (Lupas and Martin 2002), and therefore, it is unlikely that Drg1 releases each of these factors from pre-60S subunits. The Bergler laboratory demonstrated that Drg1 catalyzes the release of Rlp24 that is essential for subsequent release events (Kappel et al. 2012). A C-terminal region within Rlp24 acts as a recruiting site for Drg1 to stimulate its ATPase activity. The release of Rlp24 from the pre-60S subunit appears to require the nonessential FG-nucleoporin Nup116. Thus, the mechanochemical activity of Drg1 makes the export step irreversible and simultaneously initiates the cytoplasmic maturation cascade.

Table 2 Cytoplasmic release of shuttling assembly and transport factors in the 60S biogenesis pathway

Release factor/co-factor	Activity/domain	Target	References
Drg1	AAA-ATPase	Rlp24	Kappel et al. (2012), Pertschy et al. (2007)
Rei1	Zn-finger protein	Arx1/Alb1	Hung and Johnson (2006), Lebreton et al. (2006), Meyer et al. (2007)
Jjj1-Ssa1/Ssa2	DnaJ domain stimulates the ATPase Ssa1/Ssa2	Arx1/Alb1	Demoinet et al. (2007), Meyer et al. (2007, 2010)
Yvh1	Dual specificity phosphatase	Mrt4	Kemmler et al. (2009), Lo et al. (2009)
Efl1	GTPase	Tif6	Becam et al. (2001), Senger et al. (2001)
Sdo1	–	Tif6	Menne et al. (2007)
Kre35	GTPase	Nmd3	Hedges et al. (2005)
–	–	Bud20	
–	–	Nsa2	
–	–	Nug1	
–	–	Npl3	
–	–	Mex67/Mtr2	
–	–	Nog1	
–	–	Gle2	
–	–	Ecm1	

Release of the ribosomal-like protein Rlp24 is necessary to allow the r-protein eL24 (Rpl24) to assemble into the pre-60S subunit. This exchange event triggers recruitment of the zinc-finger proteins Rei1 and Yvh1 (Altvater et al. 2012; Lo et al. 2010). Rei1 is nonessential, but needed for the recycling of Arx1 and its interacting partner Alb1 (Hung and Johnson 2006; Lebreton et al. 2006). Rei1 works in conjunction with the DnaJ domain-containing Jjj1 and the ATPase Ssa1/Ssa2 (Hsp70) to release Arx1 (Demoinet et al. 2007; Lo et al. 2010; Meyer et al. 2007, 2010). This data also indicates that Arx1 appears to have an inhibitory role in driving cytoplasmic maturation pathway of pre-60S subunits. Based on similarity of Arx1 to MetAPs, a prediction is that they bind to the same site on the ribosome and that Arx1 prevents the binding of MetAP. Further, Arx1 binds in the vicinity of the r-protein uL23 (Rpl25) at the polypeptide exit tunnel, which is an important functional site on the ribosome, as uL23 (Rpl25) interacts with the signal recognition particle (SRP) as well as the translocon in the endoplasmic reticulum (ER) (Bradatsch et al. 2012; Dalley et al. 2008; Greber et al. 2012).

The Johnson and Panse laboratories have uncovered a cytoplasmic maturation event that is crucial for assembly of the ribosome stalk, a structural landmark of the 60S subunit (Kemmler et al. 2009; Lo et al. 2009). Assembly of the stalk is a major step in acquisition of functionality of the ribosome, since it is essential for recruitment and activation of translation factors, in particular the elongation factors. In yeast, the stalk is composed of uL10 (Rpp0) and two heterodimers of P1 and P2 (Rpp1/Rpp2). uL10 (Rpp0) anchors the stalk to the ribosome by binding to the rRNA of helices 43 and 44. However, pre-60S subunits are first assembled in the nucleus with the ribosomal-like protein Mrt4 in place of uL10 (Rpp0). Mrt4 lacks the domains that recruit translation factors, necessitating the exchange of Mrt4 for uL10 (Rpp0). The dual specificity phosphatase Yvh1 is required for the removal of Mrt4 and uses its zinc-binding domain but not its phosphatase activity to release of Mrt4 from pre-60S subunits. While the key players are identified, the precise mechanism of Mrt4 release and the molecular events that lead to the assembly of the stalk remain elusive.

Following the assembly of the stalk and the removal of Arx1, Tif6 is released. Tif6 is a shuttling assembly factor that prevents the joining of immature 60S to 40S subunits (Russell and Spremulli 1979; Valenzuela et al. 1982). The release of Tif6 depends on previous events as it mislocalizes in *yvh1* mutants in which stalk assembly is blocked (Kemmler et al. 2009). During translation, the stalk functions in recruitment and activation of the GTPase eEF2 (Bargis-Surgey et al. 1999). Given that Efl1 is closely related to eEF2, stalk assembly might play a similar role in biogenesis, thus recruiting Efl1 for the release of Tif6. This also suggests that the cytoplasmic maturation events in the 60S biogenesis are coupled and ordered sequentially from Drg1-dependent release of Rlp24 to Efl1-mediated release of Tif6 (Fig. 4).

The GTPase Efl1 and the Shwachman-Bodian-Diamond syndrome protein Sdo1 are required to release Tif6 (Becam et al. 2001; Senger et al. 2001). In *efl1* and *sdo1* mutants, Tif6 accumulates on late pre-60S subunits and is mislocalized to the cytoplasm (Menne et al. 2007; Senger et al. 2001). Mutations in Tif6 that weaken its affinity for the 60S subunit suppress the growth defects of *efl1* and *sdo1* mutants, providing strong genetic evidence that Tif6 is the primary substrate of Efl1 and Sdo1. Efl1 bears a significant sequence similarity to the translation elongation factor 2, a GTPase that facilitates

translocation of the ribosome following the action of the peptidyl transferase.

The essential NES containing adaptor Nmd3 must be released from pre-60S subunits and recycled back to the nucleus. The r-protein uL16 (Rpl10) and the GTPase Kre35 (Lsg1) were implicated in the release of Nmd3 (Hedges et al. 2005; West et al. 2005). Mutations in uL16 (Rpl10) prevent the release of Nmd3 from pre-60S subunits. Moreover, mutations in Kre35 that are predicted to disrupt its GTPase activity also block Nmd3 release in the cytoplasm (Hedges et al. 2005). These results suggest that Kre35 triggers the binding of uL16 (Rpl10) to the 60S, an event that is coupled to the release of Nmd3 (Hedges et al. 2005; Karl et al. 1999; West et al. 2005). Interestingly, recent work from the Johnson laboratory indicates that loading of uL16 (Rpl10) on late pre-60S subunits is a prerequisite for the release of Tif6 (Bussiere et al. 2012).

Recently, we have employed a combination of genetic trapping, affinity purification, and a targeted proteomic approach based on selected reaction monitoring mass spectrometry (SRM-MS) to interrogate the proteome of 60S preribosomes after nuclear export (Altvater et al. 2012). Using a resource of SRM assays, we uncovered unanticipated assembly factors (Bud20, Nug1, Nsa2, and Rli1). They are exported to the cytoplasm and are only released after Drg1-mediated release of Rlp24. The functional significance of shuttling behavior of the identified assembly factors is unknown. It could be that they participate directly in their transport and/or final functional proofreading of pre-60S subunits. Mechanisms that trigger the release of these shuttling assembly factors from pre-60S particles in the cytoplasm remain to be discovered.

Cytoplasmic proofreading systems for the ribosome

Given the importance to correctly translate proteins, an efficient quality control system must ensure that only functional ribosomes enter translation. In the nucleus, the TRAMP complex marks and targets aberrant pre-rRNAs for degradation by the nuclear exosome (Allmang et al. 2000; Dez et al. 2006; LaCava et al. 2005; Mitchell et al. 1997). The precise mechanism(s) of recognition of incorrectly assembled preribosomal particles by the TRAMP complex in the nucleus remains unclear. Improperly assembled pre-ribosomal subunits that escape nuclear surveillance mechanisms are segregated and targeted for degradation in the cytoplasm by nonfunctional RNA decay (NRD) (Cole et al. 2009; Fujii et al. 2009, 2012; Graille and Seraphin 2012; LaRiviere et al. 2006). For a more detailed review on nuclear and cytoplasmic surveillance mechanisms for eukaryotic ribosome assembly, the readers are referred to Lafontaine (2010).

In the cytoplasm, two antagonistic mechanisms appear to proofread pre-ribosomes: (1) Assembly factors might either prevent and/or delay pre-ribosomes from prematurely interacting or initiating translation. (2) They could actively check pre-ribosomal subunits for functionality. The ribosomal-like proteins (Rlp24 and Mrt4) act as placeholders for r-proteins. Therefore they delay maturation of the subunit and perhaps provide a time window for the functional proofreading of the 60S subunits. The assembly factor Tif6 is another example, whose binding to the 60S subunit interface prevents premature interactions with the 40S subunit. Tif6 is released from pre-60S subunits in the cytoplasm only after the formation of the acidic ribosomal stalk. Efl1 and Sdo1 promote the release of Tif6 from pre-60S ribosomes (Lo et al. 2010; Menne et al. 2007; Senger et al. 2001). Efl1 shares sequence similarity with the GTPase elongation factor eEF2. Additionally, Efl1 was proposed to check the integrity of the GTPase activating center, the P-site of the ribosome for functionality (Bussiere et al. 2012). Thus, cytoplasmic release factors may couple the recycling of shuttling assembly factors and simultaneously check ribosome function.

In the case of the pre-40S subunit, a number of assembly factors were proposed to block premature binding of initiation factors, mRNA, tRNA, and the 60S subunit. The cryo-EM structure of a late cytoplasmic pre-40S particle assigned the binding sites for Ltv1, Enp1, Rio2, Tsr1, Dim1, Pno1, and Nob1 on the small subunit (Strunk et al. 2011, Fig. 3). Their binding sites imply possible functions in preventing premature translation initiation by blocking access of translation factors. Ltv1 and Enp1 directly bind uS3 (Rps3) on its solvent exposed side thereby blocking the mRNA channel opening. Rio2, Tsr1, and Dim1 bind the subunit interface, thus preventing joining of the mature 60S subunit and translation initiation factor eIF1A. Nob1 and Pno1 block the binding of eIF3 and thereby interfere with translation initiation. After the release of Rio2, Tsr1, and Dim1, such a pre-40S particle may structurally mimic the translation initiation surface of a mature 40S subunit and hence can interact with a mature 60S subunit to form an 80S-like particle (Lebaron et al. 2012). This translation-like interaction could test the ability of a pre-40S subunit to bind 60S subunits. Additionally, by constantly interacting with each other in the cytoplasm, ribosomal subunits may sense their decoding ability to segregate and target aberrant particles for disassembly and degradation. Interestingly, Nob1 activity is stimulated by the translation initiation factor Fun12 (eIF5B) (Lebaron et al. 2012; Strunk et al. 2012). Thus, processing of the 20S pre-rRNA may represent a quality control mechanism that simultaneously triggers subunit maturation and senses translation competence.

Concluding remarks

Despite the initial description of eukaryotic ribosome assembly, which now dates back nearly 40 years, our understanding of this fundamental pathway still remains rudimentary. During

the last decades genetic screens, the development of visual reporters and proteomic approaches in budding yeast has provided crucial breakthroughs to further dissect eukaryotic ribosome biogenesis. These methodologies have expanded the inventory of assembly and transport factors that aid ribosome production. Deciphering the contribution of assembly factors during ribosome formation remains an important challenge. Structural approaches are beginning to facilitate these functional analyses of this highly dynamic pathway (Armache et al. 2010; Ben-Shem et al. 2011; Bradatsch et al. 2012; Greber et al. 2012; Klinge et al. 2011; Rabl et al. 2011). Moreover, these studies are guiding mechanistic analyses of this conserved pathway in higher eukaryotes (Tafforeau et al. 2013; Wild et al. 2010). Large-scale genome-wide visual screens and advanced imaging technologies will provide new insights into the assembly pathway of the human ribosome.

The importance of producing translation-competent ribosomes is reflected by the growing list of diseases that are linked to defects in ribosome assembly. Diamond-Blackfan anemia (DBA) is a rare human genetic disease. It causes bone marrow failure and severe anemia (Ellis and Lipton 2008; Ellis and Gleizes 2011). DBA is characterized by mutations in multiple proteins of the small and the large subunit and results in reduced translation capacity of the cell. Recently, mutations in the r-protein uL16 (Rpl10) and uL18 (Rpl5) were associated with T-cell acute lymphoblastic leukemia (De Keersmaecker et al. 2013). Mutations in the SSU component hUTP4 might be the basis of North American Indian childhood cirrhosis (Freed et al. 2012). The Shwachman-Bodian-Diamond syndrome arises from the inability to release Tif6 from pre-60S subunits (Finch et al. 2011; Menne et al. 2007; Wong et al. 2011).

Robust ribosome production allows cells to increase their mass and to proliferate, thus making ribosome assembly an important target for cancer treatments. Increased transcription of rDNA by RNA polymerase I is a characteristic feature of malignant cancers and to boost ribosome synthesis, emphasizing the importance of targeting the ribosome biogenesis pathway. Indeed, a recently developed small molecule inhibitor CX-5461 that targets rDNA transcription is able to selectively kill B-lymphoma cells (Bywater et al. 2012; Hannan et al. 2013). Unraveling the pathways and mechanisms by which eukaryotes build ribosomes will provide fundamental knowledge that will facilitate the rational design of therapeutics in the treatment of malignant cancers.

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