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# Eukaryotic ribosome biogenesis at a glance

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## Summary

Ribosomes play a pivotal role in the molecular life of every cell. Moreover, synthesis of ribosomes is one of the most energetically demanding of all cellular processes. In eukaryotic cells, ribosome biogenesis requires the coordinated activity

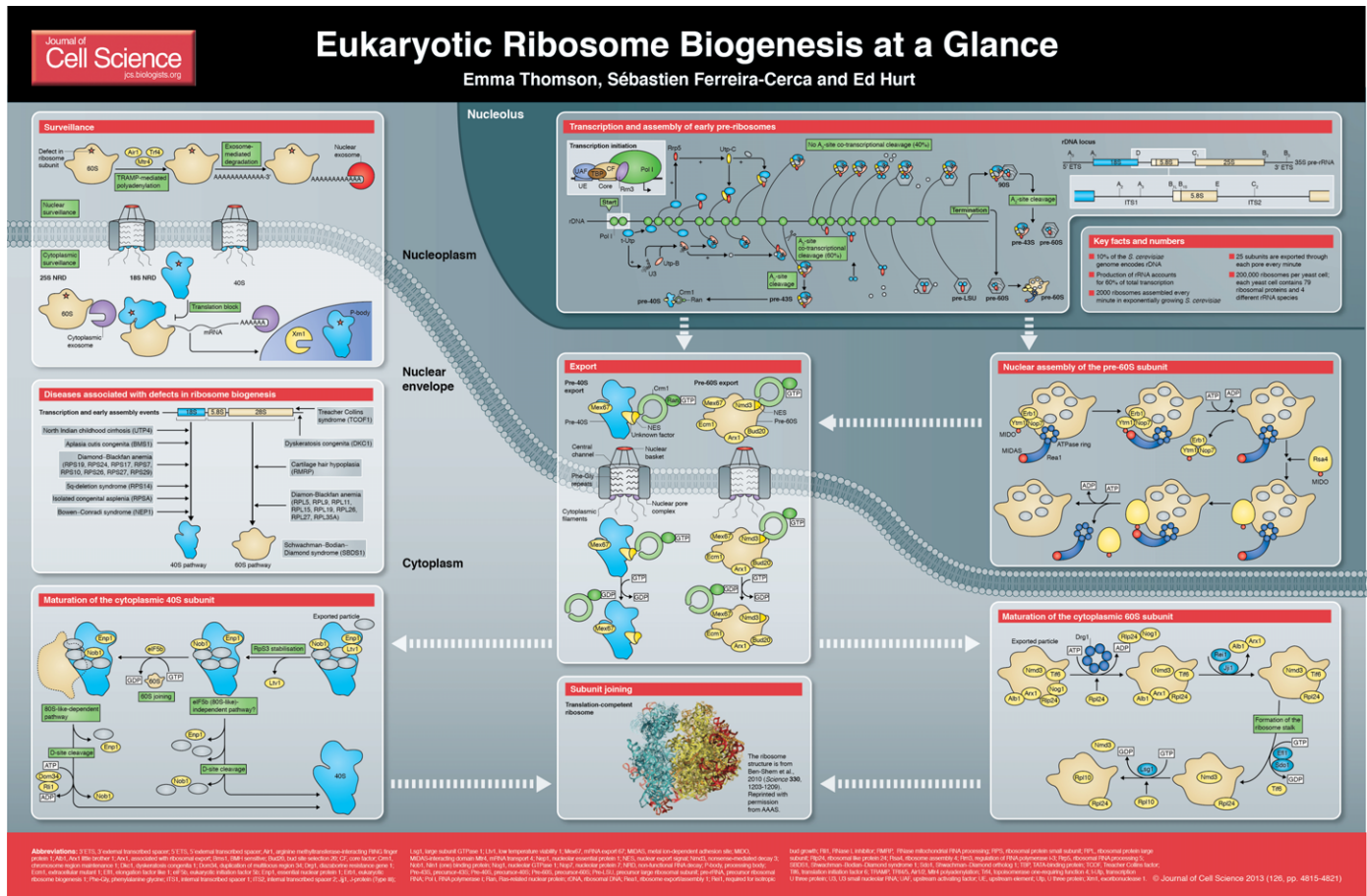
of all three RNA polymerases and the orchestrated work of many (>200) transiently associated ribosome assembly factors. The biogenesis of ribosomes is a tightly regulated activity and it is inextricably linked to other fundamental cellular processes, including growth and cell division. Furthermore, recent studies have demonstrated that defects in ribosome biogenesis are associated with several hereditary diseases. In this Cell Science at a Glance article and the accompanying poster, we summarise the current knowledge on eukaryotic ribosome biogenesis, with an emphasis on the yeast model system.

large 60S subunit [containing the 25S, 5.8S and 5S rRNA, and 46 ribosomal proteins (r-proteins)] and a small 40S subunit (containing the 18S rRNA and 33 r-proteins) (Fromont-Racine et al., 2003; Henras et al., 2008; Kressler et al., 2010).

Ribosome synthesis is one of the most energetically demanding of cellular activities and appears to be a process of extraordinary complexity (Warner, 1999; Fromont-Racine et al., 2003; Henras et al., 2008; Kressler et al., 2010). In eukaryotic cells, three of the mature rRNA species are co-transcribed as a single transcript that is matured through a series of nucleolytic processing steps. Maturation of the rRNAs and recruitment of the r-proteins occurs within a series of precursor ribosomal particles, or pre-ribosomes within the nucleolus, nucleoplasm and cytoplasm. The systematic purification of pre-ribosomes has allowed the protein and rRNA composition of multiple intermediates to be elucidated and ordered into a ribosome assembly map. The plethora of assembly

## Introduction

Ribosomes are fundamental macromolecular machines that function at the heart of the translation machinery, allowing the conversion of information encoded within mRNA into proteins. The 80S ribosome (named for its apparent sedimentation velocity) is a ribonucleoprotein complex that comprises two ribosomal subunits, a



(See poster insert)

factors, including those with predicted ATPase, GTPase, helicase, kinase or nuclease activity, orchestrate the ordered modification, folding and processing of rRNA, and the sequential recruitment of r-proteins (Fromont-Racine et al., 2003; Henras et al., 2008; Kressler et al., 2010).

Although the inventory of assembly factors is probably close to completion, the daunting task now is to understand the role that each of these factors play. With the help of selected examples, in this Cell Science at a Glance article and the accompanying poster, we highlight emerging concepts in the ribosome biogenesis field in yeast and higher eukaryotes, as well as diseases that are caused by mutations in associated factors (see Box 1).

#### Transcription and assembly of the earliest pre-ribosomes

Ribosome biogenesis begins in the nucleolus, where three of the rRNA species, the 18S, 5.8S and 25S, are co-transcribed by RNA polymerase I (Pol I)

as a single polycistronic transcript (see poster). In yeast, Pol I transcription starts with the recruitment of a Pol I initiation complex at the rDNA promoter. This step requires two basal transcription factor complexes, the upstream activating factor (UAF), which is associated with the TATA-box binding protein (TBP) and the core factor (CF), which bind the upstream and core promoter elements, respectively. This allows the recruitment of the initiation competent RNA Pol I that is associated with the Pol-I-specific initiation factor Rrn3 (see poster) (Drygin et al., 2010; Moss et al., 2007; Németh et al., 2013; Schneider, 2012). As the transcript emerges, many small nucleolar ribonucleoproteins (snoRNPs) (>60) mediate the co-transcriptional covalent modification of over 100 rRNA residues (Box 2) (Koř and Tollervey, 2010). Co-transcriptional assembly events are tightly linked to elongation of RNA Pol I, because mutations that affect the elongation activity of RNA Pol I lead

to rRNA maturation defects (reviewed by Schneider, 2012). As transcription ensues, the rRNA transcripts form ball-like structures on the 5' end of the nascent transcripts that emanate from the rDNA, as visualised by chromatin spreads (Miller and Beatty, 1969). It is believed that these structures are the earliest nascent pre-ribosomes, which probably correspond to the 90S or small subunit (SSU) 'processome' complexes that more recently have been described (Dragon et al., 2002; Grandi et al., 2002). Although the composition of the SSU processome and 90S pre-ribosomes differ subtly and are likely to correspond to different assembly or disassembly intermediates, both contain predominantly small subunit r-proteins and assembly factors (Bernstein et al., 2004; Dragon et al., 2002; Grandi et al., 2002).

It has been proposed that the co-transcriptional assembly of the 90S–SSU processome occurs in a modular, coordinated and hierarchical fashion (see poster). A first nucleating step is the assembly of the t-UTP (transcription U three protein) complex with the nascent rRNA (Gallagher et al., 2004; Granneman and Baserga, 2005). This is required for the subsequent downstream assembly of two independent (but not mutually exclusive) assembly lines that require the multi-component complexes U3 snoRNP::UTP-B and Rrp5::UTP-C, respectively, which are, in turn, required for completion of co-transcriptional assembly of the 90S/SSU processome (Pérez-Fernández et al., 2011; Pérez-Fernández et al., 2007). The precise role of these multi-component complexes is yet to be defined.

Within the 90S–SSU processome, cleavage of the rRNA at the site termed A<sub>2</sub> predominantly occurs co-transcriptionally (on two thirds of transcripts) (Koř and Tollervey, 2010; Osheim et al., 2004), but it can also be cleaved post-transcriptionally when the rDNA polycistron is transcribed 'en bloc', to produce the 35S pre-rRNA. This cleavage event effectively separates the maturation pathways of the two subunits by promoting the disassembly of the 90S–SSU processome and the emergence of pre-40S and pre-60S particles. The majority of factors involved in 90S–SSU processome biogenesis fail to precipitate with either the 27S A<sub>2</sub> or 20S pre-rRNA (the two products of A<sub>2</sub> cleavage),

#### Box 1. Ribosome biogenesis in higher eukaryotes and associated diseases

Although the 'core' of the eukaryotic ribosome biogenesis pathway is conserved, some aspects in higher eukaryotes differ from their yeast counterpart. These differences are accounted for by the acquisition of additional processing steps, the emergence of new factors or the acquisition of new regulatory pathways (see Burger et al., 2013; Carron et al., 2011; Drygin et al., 2010; Mullineux and Lafontaine, 2012; Preti et al., 2013; Rouquette et al., 2005; Sloan et al., 2013b; Widmann et al., 2012; Wild et al., 2010; Zemp et al., 2009; Tafforeau et al., 2013). Despite the central role of ribosomes, only a few rare inherited diseases have been specifically linked to defects in ribosome biogenesis (Bolze et al., 2013; Freed et al., 2010; Landowski et al., 2013; Marneros, 2013; Narla and Ebert, 2010; Teng et al., 2013). A rational explanation for this is that, as ribosome biogenesis is essential, the majority of defects that abrogate this process are lethal. The diseases identified, collectively termed ribosomopathies, result from mutation(s) in genes that encode either ribosomal proteins or ribosome biogenesis factors (see poster). One of the main phenotypes in patients with ribosomopathies is the failure to produce various cell types of the bone marrow, for example, red blood cells in Diamond–Blackfan anemia, or neutrophils in Shwachman–Bodian–Diamond syndrome (Narla and Ebert, 2010; Teng et al., 2013). It remains unclear how this tissue specificity is achieved. However, recent studies suggest that the cellular population of ribosomes is more heterogeneous in terms of individual components (e.g. complement of ribosomal proteins and post-translational modifications) than previously thought. This heterogeneity has been suggested to form the basis of a 'ribosome code' where ribosomes would act as 'mRNA filters' and enable selective translation of mRNA in a tissue- or condition-specific manner (Filipovska and Rackham, 2013; Komili et al., 2007; Kondrashov et al., 2011; Mauro and Edelman, 2002; Mauro and Edelman, 2007; McIntosh and Warner, 2007). Interestingly, links between the regulation of the tumor suppressor p53 and aberration in ribosome biogenesis have been the focus of several recent studies (reviewed by Teng et al., 2013). One current model proposes that the disruption of ribosome biogenesis leads to the accumulation of a free ribosomal RNP in which 5S rRNA is associated with the 5S-specific ribosomal proteins L5 and L11. In this scenario, the ubiquitin ligase Hdm2, which is responsible for p53 degradation, binds to the excess of free 5S RNP (Donati et al., 2013; Sloan et al., 2013a; Horn and Voudsen, 2008), thereby increasing cellular p53 levels and triggering p53-dependent cell cycle arrest. As p53 is not present in yeast, developing a more detailed understanding of ribosome biogenesis in higher eukaryotes is necessary to further elucidate the connection between p53 biology and ribosome biogenesis.



### Box 2. Role of small nucleolar (sno)RNPs in rRNA modification and processing

Nucleoside modifications of rRNA were reported almost 50 years ago (Littlefield and Dunn, 1958a; Littlefield and Dunn, 1958b; Smith et al., 1992) and are found in all organisms. They occur mostly on the pre-rRNA during ribosome synthesis and essentially consist of two types: methylation of the 2'-hydroxyl group of sugar residues (2'-O-methylation) and conversion of uridine residues to pseudouridine by base rotation. Although the regions of the mature rRNA that undergo covalent modification are well conserved between species and are found to cluster in functionally important regions (e.g. A- and P-site) (Decatur and Fournier, 2002), the overall number of sites modified vary between organisms. The precise role of covalent modifications in mature ribosomes remains unclear. However, it is believed that modified nucleotides display altered steric properties and hydrogen bonding abilities that cumulatively act to stabilise the overall structure and conformation of the rRNAs and therefore the ribosome (King et al., 2003; Ofengand, 2002; Yoon et al., 2006). In eukaryotes, the vast majority of over 100 covalent modification reactions are mediated by more than 60 different snoRNP particles. Two major classes of snoRNPs exist that can be distinguished structurally and functionally. Box C/D snoRNPs are responsible for methylation reactions (Cavaillé and Bachellerie, 1998; Cavaillé et al., 1996; Kiss-László et al., 1996; Kiss-László et al., 1998), whereas pseudouridylation reactions are mediated by H/ACA-containing snoRNAs (Ganot et al., 1997a; Ganot et al., 1997b; Ni et al., 1997). The function of the snoRNA is to act as a guide sequence that base-pairs with the rRNA around the nucleotide to be modified and holds it in the correct position for modification. In addition, a few snoRNAs in yeast and higher eukaryotes have been shown to play a role in the processing of rRNA (e.g. Beltrame et al., 1994; Beltrame and Tollervey, 1992; Beltrame and Tollervey, 1995; Peculis and Steitz, 1993; Peculis and Steitz, 1994). Base-pairing between these snoRNPs and the pre-rRNA are thought to bring the processing sites into close proximity and promote a conformation that supports cleavage (Watkins and Bohnsack, 2012).

(ITS2). Because most of the A<sub>3</sub> factors are predicted to interact with each other (Sahasranaman et al., 2011; Tang et al., 2008), one outcome of their binding at distant sites would be to bring these regions of rRNA into close proximity. Furthermore, it has been predicted that the binding and subsequent release of the A<sub>3</sub> factors might trigger, or be triggered by, conformational switches within ITS2. This, in turn, could promote subsequent maturation events, including processing of the pre-rRNA and recruitment of ribosomal proteins (Granneman et al., 2011; Sahasranaman et al., 2011).

#### Rea1-mediated maturation events

Three AAA-type ATPases (ATPases associated with various cellular activities) act to strip factors from the maturing 60S subunit (Kressler et al., 2012). One of these, the dynein-like Rea1 is a large 560 kDa protein that is composed of six ATPase domains, which form a ring-like structure that is attached to a flexible tail containing a MIDAS (metal ion-dependent adhesion site) at its tip (Nissan et al., 2004). Negative-stain EM of pre-60S particles shows that Rea1 contacts the pre-ribosome through its ring structure, whereas its tail protrudes, thus giving the pre-ribosome a distinctive 'tadpole-like' appearance (Nissan et al., 2004; Ulbrich et al., 2009).

Two substrates of Rea1, Ytm1 and Rsa4, have been shown to interact with Rea1 through their MIDAS-interaction domain (MIDO) (Bassler et al., 2010; Ulbrich et al., 2009). Although the roles of Ytm1 and Rsa4 remain unclear, they are known to reside on distinct pre-ribosomes and are removed in two successive, ATP-dependent steps. Ytm1, along with its interactors, Nop7 and Erb1, are removed from nucleolar particles (Bassler et al., 2010), whereas Rsa4 together with Rea1 itself have been shown to dissociate from a later nucleoplasmic pre-ribosome (Ulbrich et al., 2009) (see poster).

Although the mechanism of Rea1-mediated release of biogenesis factors might be unique owing to its molecular architecture, it is anticipated that the other two AAA-type ATPases, Rix7 and Drg1, that are required for ribosome biogenesis, also stimulate the removal of assembly factors (Kappel et al., 2012; Kressler et al., 2008; Pertschy et al., 2007). Similarly, other enzymes that have been

suggesting that following rRNA cleavage at A<sub>2</sub>, the majority, although not all, of the trans-acting factors dissociate. It is believed that pre-40S particles, which already contain most SSU r-proteins (Ferreira-Cerca et al., 2007) as well as a few newly recruited 40S biogenesis factors, are exported relatively rapidly to the cytoplasm, where their maturation is completed (Schäfer et al., 2003). By contrast, maturation of the 60S subunit requires far more extensive rearrangements within the nucleolar and nucleoplasmic compartments before its export and final maturation in the cytoplasm (Nissan et al., 2002). Consequently, a greater number of discrete nuclear pre-60S intermediates have been identified (Harnpicharnchai et al., 2001; Nissan et al., 2002).

#### Nuclear maturation of 60S

Maturation of the 60S subunit requires a large inventory of biogenesis factors, which associate and dissociate throughout the maturation process, (Harnpicharnchai et al., 2001; Nissan et al., 2002). Some assembly factors associate with multiple 60S particles, whereas others interact only transiently and associate with discrete 60S intermediates. Generally, the maturing

pre-60S is characterised by a gradual reduction in complexity of associated trans-acting factors as it moves from the nucleolus to the cytoplasm (Nissan et al., 2002). Below, we will highlight two examples that illustrate emerging concepts for how both catalytic (e.g. Rea1) and non-catalytic (e.g. the A<sub>3</sub> factors) biogenesis factors drive ribosome assembly (see poster).

#### The A<sub>3</sub> factors

Approximately 80 factors have been implicated in the maturation of the 60S subunit, and eight of these have been linked specifically to processing of the 27S A<sub>3</sub> pre-rRNA (termed the 'A<sub>3</sub> cluster'), which act to complete the maturation of the 5' end of the 5.8S rRNA (Dunbar et al., 2000; Fatica et al., 2003; Gadal et al., 2002; Miles et al., 2005; Oeffinger et al., 2002; Oeffinger and Tollervey, 2003; Pestov et al., 2001). Although the A<sub>3</sub> factors are required for processing at the 5' end of the 5.8S, RNA-protein crosslinking analyses show that most A<sub>3</sub> factors bind within the pre-rRNA at sites close to either the 3' end of the 5.8S rRNA, or the 5' region of 25S rRNA (Granneman et al., 2011), which is separated from the 5.8S rRNA by an internally transcribed spacer sequence

implicated in biogenesis, such as GTPases and helicases (Kressler et al., 2010; Kressler et al., 2012), have been predicted to remodel pre-ribosomes through the removal of biogenesis factors and the rearrangement of RNA, thus helping to drive the maturation process.

### Export

Ribosomal subunits must be transported to the cytoplasm for their final maturation. Export appears tightly regulated, because insufficiently mature ribosomal subunits are excluded from export. However, the precise events that underlie the acquisition of export competence remain obscure. In order for the subunits to be exported efficiently they must interact, through export receptors, with the hydrophobic central channel of the nuclear pore complex (NPC) (see poster). The karyopherin Crm1 was identified as the receptor for both ribosomal subunits and was found to mediate export in a Ran-GTP-dependent manner (Gadal et al., 2001; Ho et al., 2000; Moy and Silver, 2002). Crm1 recognises cargo molecules that contain a leucine-rich nuclear export signal (NES) and is recruited to the large 60S subunit by the NES-containing adapter protein Nmd3, (Gadal et al., 2001; Ho et al., 2000; Moy and Silver, 2002). However, the source(s) of NES within the pre-40S remains elusive. A number of candidates have been suggested, including several r-proteins (Ferreira-Cerca et al., 2005; Léger-Silvestre et al., 2004) and trans-acting factors (Schäfer et al., 2003; Seiser et al., 2006), but no single factor has been shown to directly mediate export of the pre-40S. Although screens have been performed to identify components of the export machinery (Gadal et al., 2001; Yao et al., 2007), there is an inherent difficulty in distinguishing factors that are directly required for export from those that make pre-ribosomes competent for export.

Although Crm1 is the main mediator of export, additional factors have been shown to play a role. The general mRNA export receptor Mex67 (Faza et al., 2012; Yao et al., 2007) and the HEAT-repeat-containing protein Rrp12 (Oeffinger et al., 2004) have been suggested to facilitate the export of both subunits, and several other factors such as Arx1, Ecm1, Bud20 and Npl3 have been implicated in the export of the large

pre-60S subunit (Bassler et al., 2012; Bradatsch et al., 2007; Hackmann et al., 2011; Yao et al., 2010). Although these additional factors are tightly linked to the export process, most are non-essential proteins and it is likely that they function to optimise the export of these gigantic molecules.

### Cytoplasmic maturation of 60S

Once the pre-60S has been exported into the cytoplasm, substantial structural rearrangements are likely to be required to convert the inactive pre-60S into a functional 60S. The remaining large subunit ribosomal proteins associate and the maturation of rRNA is completed, whereas the remaining non-ribosomal assembly factors dissociate and are recycled to the nucleus (see poster). The release of the remaining biogenesis factors appears to follow a hierarchical process that is mediated predominantly by GTPases such as Lsg1 (Hedges et al., 2005) and ATPases such as Drg1 (Pertschy et al., 2007). These steps have been temporally ordered but it remains unclear how each of these events are linked to those occurring before or after (Lo et al., 2010).

Following export, the AAA-type ATPase Drg1 has been shown to mediate removal of the predicted GTPase Nog1 and ribosomal-like protein Rlp24 (Kappel et al., 2012; Pertschy et al., 2007). Dissociation of Rlp24 allows the stable incorporation of ribosomal protein L24, a protein to which it shares sequence similarity, into the pre-60S particle. The presence of L24 then allows for the recruitment of Rei1, which along with Jjj1 promotes the release of the shuttling factor Arx1 and of its binding partner Alb1 (Demoinet et al., 2007; Greber et al., 2012; Lebreton et al., 2006; Meyer et al., 2010). Arx1 binds at the ribosome exit tunnel, where the polypeptide will emerge during translation, and while bound, it has been suggested to inhibit the association of translation factor(s) (Bradatsch et al., 2012; Greber et al., 2012). Similarly, the presence of the biogenesis factor Tif6 on the pre-60S has been proposed to inhibit the joining of the small subunit (Raychaudhuri et al., 1984). Tif6 is removed from the 60S subunit by the GTPase Efl1, along with Sdo1 and requires the prior dissociation of Arx1 (Finch et al., 2011; Menne et al., 2007). Additionally, the 60S 'stalk' structure,

to which GTPases associate during translation, must be formed for Efl1 to act. Stalk formation requires the incorporation of the ribosomal protein P0; however, the timing of this association continues to be debated (Kemmler et al., 2009; Lo et al., 2009; Rodríguez-Mateos et al., 2009a; Rodríguez-Mateos et al., 2009b). The removal of Tif6 appears to be a prerequisite for the subsequent release of the export adapter Nmd3, which requires another GTPase, Lsg1 (Hedges et al., 2005). Although it is not yet complete, the pathway of cytoplasmic maturation presents one example of just how interrelated the events involved in ribosome maturation are.

### Maturation of cytoplasmic 40S

Similar to pre-60S subunits, pre-40S particles undergo key maturation events following their export to the cytoplasm. Pioneering work from the Warner laboratory in the 1970s showed that the 20S pre-rRNA component of the 40S subunit is matured in the cytoplasm (Udem and Warner, 1973); however, it is only in light of more recent results that the complex dynamics of cytoplasmic 40S maturation can be fully appreciated. Recent work from our laboratory suggests that the 'beak' structure, a distinctive structural landmark of the mature 40S subunit, is not fully assembled in the pre-40S particle before its export. Once in the cytoplasm, the ribosomal protein S3 is incorporated in close proximity to the beak structure and the biogenesis factors Ltv1 and Enp1 dissociate (Schäfer et al., 2006) (see poster). Interestingly, RNA-protein crosslinking and EM analysis have shown that Enp1 and Ltv1 bind proximal to S3 (Granneman et al., 2010; Strunk et al., 2011), suggesting that the stabilisation of S3 would not be possible while they are bound.

In addition to Ltv1 and Enp1, the binding sites of most of the factors that are involved in late events of 40S biogenesis have been identified (Granneman et al., 2010; Strunk et al., 2011). Many factors bind at regions that will ultimately form the catalytic centre of the small subunit (e.g. the A- and P-sites). Accordingly, it has been proposed that these late biogenesis factors could prevent the premature association of the translation machinery and subunit joining. An alternative, although not mutually exclusive, interpretation is that

late biogenesis factors are required for the maturation of the essential functional sites. Although biogenesis factors from eubacteria and eukaryotes are poorly conserved, some factors bind to similar sites at the functional centres of the ribosome (Granneman et al., 2010; Strunk et al., 2011). This similarity supports the idea that ribosome biogenesis has evolved to use different factors to ensure proper maturation and/or protection of the active centres.

Recent studies suggest that one of the final steps in the maturation of the small subunit is the final cleavage of 20S pre-rRNA to 18S rRNA, which is mediated by the endonuclease Nob1 (Lamanna and Karbstein, 2009; Pertschy et al., 2009). This reaction is stimulated by the translation initiation factor eIF5b, which promotes the formation of an 80S-like complex through the recruitment of the 60S subunit (Lebaron et al., 2012; Strunk et al., 2012) (see poster). Furthermore, the maturation of 20S in the resulting 80S-like complex involves the general translation termination factors Rli1 and Dom34 (Strunk et al., 2012). Maturation might therefore include a translation-like event that could serve to check the integrity of the newly synthesised 40S. However, it remains to be established whether this translation-like cycle is essential for both the maturation of the 20S into 18S rRNA and the acquisition of translation competence.

Once fully matured, both cytosolic ribosomal subunits are competent to engage in the translation of mRNA (Green and Noller, 1997).

### Surveillance

To ensure that ribosomes are synthesised correctly and function accurately, an active surveillance system exists that recognises aberrant or stalled pre-ribosomes and targets them for degradation (see poster). Pre-ribosomes that accumulate in the nucleus are degraded by the exosome, a multisubunit complex that exhibits exonuclease activity (Allmang et al., 2000; Mitchell et al., 1997). A co-factor of the exosome, the TRAMP complex, specifically targets the exosome to substrates that are destined for degradation, including nuclear pre-rRNAs, through the addition of a 3' oligo-A tail (Dez et al., 2006). Defective ribosomal subunits that escape nuclear surveillance can be targeted for

degradation in the cytoplasm. It has been shown that mutation of residues within either the peptidyl transfer centre (PTC) of the large subunit or the decoding centre of the small subunit results in the degradation of the RNA components by non-functional RNA decay (NRD) (Cole et al., 2009; LaRiviere et al., 2006).

The number of potential defects that can arise during ribosome assembly is enormous, and it remains unclear how the surveillance system can identify all possible defects. One potential mechanism of recognition could be that the surveillance system does not identify specific defects, but the consequence of them, such as a delay in assembly. In this scenario, if maturation of pre-ribosomes proceeds normally, surveillance would be circumvented. However, if there was any disruption in ribosome biogenesis that results in the delay of maturation, the surveillance machinery could act. Finally, although it is clear that the RNA of defective ribosomal particles is targeted for degradation, the fate of the protein components remains unclear.

### Concluding remarks

The study of ribosome biogenesis stands at an exciting crossroads. The field is entering a new era where comprehensive systematic approaches must be combined with a factor-by-factor analysis to understand the exact roles of biogenesis factors and integrate them into the ribosome synthesis pathway. The combination of structural-based functional analysis (RNA folding, X-ray crystallography and high-resolution electron microscopy) together with *in vitro* reconstitution of distinct biogenesis steps will provide the means to expand our current view on the dynamic ribosome assembly process.

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A high-resolution version of the poster is available for downloading in the online version of this article at [jcs.biologists.org](http://jcs.biologists.org). Individual poster panels are available as JPEG files at <http://jcs.biologists.org/lookup/suppl/doi:10.1242/jcs.111948/-/DC1>.

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