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Eukaryotic ribosome biogenesis at a glance

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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 A₂ 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 A₂ or 20S pre-rRNA (the two products of A₂ 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_3 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_3 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_2 , 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_3 factors) biogenesis factors drive ribosome assembly (see poster).

The A_3 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_3 pre-rRNA (termed the ' A_3 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_3 factors are required for processing at the 5' end of the 5.8S, RNA-protein crosslinking analyses show that most A_3 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. Individual poster panels are available as JPEG files at <http://jcs.biologists.org/lookup/suppl/doi:10.1242/jcs.111948/-/DC1>.

References

- Allmang, C., Mitchell, P., Petfalski, E. and Tollervey, D. (2000). Degradation of ribosomal RNA precursors by the exosome. *Nucleic Acids Res.* **28**, 1684-1691.
- Bassler, J., Kallas, M., Pertschy, B., Ulbrich, C., Thoms, M. and Hurt, E. (2010). The AAA-ATPase Real drives removal of biogenesis factors during multiple stages of 60S ribosome assembly. *Mol. Cell* **38**, 712-721.
- Bassler, J., Klein, I., Schmidt, C., Kallas, M., Thomson, E., Wagner, M. A., Bradatsch, B., Rechberger, G., Strohmaier, H., Hurt, E. et al. (2012). The conserved Bud20 zinc finger protein is a new component of the ribosomal 60S subunit export machinery. *Mol. Cell. Biol.* **32**, 4898-4912.
- Beltrame, M. and Tollervey, D. (1992). Identification and functional analysis of two U3 binding sites on yeast pre-ribosomal RNA. *EMBO J.* **11**, 1531-1542.
- Beltrame, M. and Tollervey, D. (1995). Base pairing between U3 and the pre-ribosomal RNA is required for 18S rRNA synthesis. *EMBO J.* **14**, 4350-4356.
- Beltrame, M., Henry, Y. and Tollervey, D. (1994). Mutational analysis of an essential binding site for the U3 snoRNA in the 5' external transcribed spacer of yeast pre-rRNA. *Nucleic Acids Res.* **22**, 4057-4065.
- Bernstein, K. A., Gallagher, J. E., Mitchell, B. M., Granneman, S. and Baserga, S. J. (2004). The small-subunit processome is a ribosome assembly intermediate. *Eukaryot. Cell* **3**, 1619-1626.
- Bolze, A., Mahlaoui, N., Byun, M., Turner, B., Trede, N., Ellis, S. R., Abhyankar, A., Itan, Y., Patin, E., Brebner, S. et al. (2013). Ribosomal protein SA haploinsufficiency in humans with isolated congenital asplenia. *Science* **340**, 976-978.
- Bradatsch, B., Katahira, J., Kowalinski, E., Bange, G., Yao, W., Sekimoto, T., Baumgärtel, V., Boese, G., Bassler, J., Wild, K. et al. (2007). Arx1 functions as an unorthodox nuclear export receptor for the 60S preribosomal subunit. *Mol. Cell* **27**, 767-779.
- Bradatsch, B., Leidig, C., Granneman, S., Gnädig, M., Tollervey, D., Böttcher, B., Beckmann, R. and Hurt, E. (2012). Structure of the pre-60S ribosomal subunit with nuclear export factor Arx1 bound at the exit tunnel. *Nat. Struct. Mol. Biol.* **19**, 1234-1241.
- Burger, K., Mühl, B., Rohmoser, M., Coordes, B., Heidemann, M., Kellner, M., Gruber-Eber, A., Heissmeyer, V., Strässer, K. and Eick, D. (2013). Cyclin-dependent kinase 9 links RNA Polymerase II transcription to processing of ribosomal RNA. *J. Biol. Chem.* **288**, 21173-21183.
- Carron, C., O'Donohue, M. F., Choemel, V., Faublader, M. and Gleizes, P. E. (2011). Analysis of two human pre-ribosomal factors, bystin and hTsr1, highlights differences in evolution of ribosome biogenesis between yeast and mammals. *Nucleic Acids Res.* **39**, 280-291.
- Cavaillé, J. and Bachellerie, J. P. (1998). SnoRNA-guided ribose methylation of rRNA: structural features of the guide RNA duplex influencing the extent of the reaction. *Nucleic Acids Res.* **26**, 1576-1587.
- Cavaillé, J., Nicoloso, M. and Bachellerie, J. P. (1996). Targeted ribose methylation of RNA *in vivo* directed by tailored antisense RNA guides. *Nature* **383**, 732-735.
- Cole, S. E., LaRiviere, F. J., Merrikh, C. N. and Moore, M. J. (2009). A convergence of rRNA and mRNA quality control pathways revealed by mechanistic analysis of nonfunctional rRNA decay. *Mol. Cell* **34**, 440-450.
- Decatur, W. A. and Fournier, M. J. (2002). rRNA modifications and ribosome function. *Trends Biochem. Sci.* **27**, 344-351.
- Demoinet, E., Jacquier, A., Lutfalla, G. and Fromont-Racine, M. (2007). The Hsp40 chaperone Jjj1 is required for the nucleo-cytoplasmic recycling of

- preribosomal factors in *Saccharomyces cerevisiae*. *RNA* **13**, 1570-1581.
- Dez, C., Houseley, J. and Tollervey, D. (2006). Surveillance of nuclear-restricted pre-ribosomes within a subnuclear region of *Saccharomyces cerevisiae*. *EMBO J.* **25**, 1534-1546.
- Donati, G., Peddigari, S., Mercer, C. A. and Thomas, G. (2013). 5S ribosomal RNA is an essential component of a nascent ribosomal precursor complex that regulates the Hdm2-p53 checkpoint. *Cell Rep.* **4**, 87-98.
- Dragon, F., Gallagher, J. E., Compagnone-Post, P. A., Mitchell, B. M., Porwancher, K. A., Wehner, K. A., Wormsley, S., Settlage, R. E., Shabanowitz, J., Osheim, Y. et al. (2002). A large nucleolar U3 ribonucleoprotein required for 18S ribosomal RNA biogenesis. *Nature* **417**, 967-970.
- Drygin, D., Rice, W. G. and Grummt, I. (2010). The RNA polymerase I transcription machinery: an emerging target for the treatment of cancer. *Annu. Rev. Pharmacol. Toxicol.* **50**, 131-156.
- Dunbar, D. A., Dragon, F., Lee, S. J. and Baserga, S. J. (2000). A nucleolar protein related to ribosomal protein L7 is required for an early step in large ribosomal subunit biogenesis. *Proc. Natl. Acad. Sci. USA* **97**, 13027-13032.
- Fatica, A., Oeffinger, M., Tollervey, D. and Bozzoni, I. (2003). Ciplp/Nsa3p is required for synthesis and nuclear export of 60S ribosomal subunits. *RNA* **9**, 1431-1436.
- Faza, M. B., Chang, Y., Occhipinti, L., Kemmler, S. and Panse, V. G. (2012). Role of Mex67-Mtr2 in the nuclear export of 40S pre-ribosomes. *PLoS Genet.* **8**, e1002915.
- Ferreira-Cerca, S., Pöll, G., Gleizes, P. E., Tschochner, H. and Milkereit, P. (2005). Roles of eukaryotic ribosomal proteins in maturation and export of pre-18S rRNA and ribosome function. *Mol. Cell* **20**, 263-275.
- Ferreira-Cerca, S., Pöll, G., Kühn, H., Neueder, A., Jakob, S., Tschochner, H. and Milkereit, P. (2007). Analysis of the in vivo assembly pathway of eukaryotic 40S ribosomal proteins. *Mol. Cell* **28**, 446-457.
- Filipovska, A. and Rackham, O. (2013). Specialization from synthesis: how ribosome diversity can customize protein function. *FEBS Lett.* **587**, 1189-1197.
- Finch, A. J., Hilcenko, C., Basse, N., Drynan, L. F., Goyenechea, B., Menne, T. F., González Fernández, A., Simpson, P., D'Santos, C. S., Arends, M. J. et al. (2011). Uncoupling of GTP hydrolysis from eIF6 release on the ribosome causes Shwachman-Diamond syndrome. *Genes Dev.* **25**, 917-929.
- Freed, E. F., Bleichert, F., Dutca, L. M. and Baserga, S. J. (2010). When ribosomes go bad: diseases of ribosome biogenesis. *Mol. Biosyst.* **6**, 481-493.
- Fromont-Racine, M., Senger, B., Saveanu, C. and Fasiolo, F. (2003). Ribosome assembly in eukaryotes. *Gene* **313**, 17-42.
- 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.
- 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**, 941-952.
- Gallagher, J. E., Dunbar, D. A., Granneman, S., Mitchell, B. M., Osheim, Y., Beyer, A. L. and Baserga, S. J. (2004). RNA polymerase I transcription and pre-rRNA processing are linked by specific SSU processome components. *Genes Dev.* **18**, 2506-2517.
- Ganot, P., Bortolin, M. L. and Kiss, T. (1997a). Site-specific pseudouridine formation in preribosomal RNA is guided by small nucleolar RNAs. *Cell* **89**, 799-809.
- Ganot, P., Caizergues-Ferrer, M. and Kiss, T. (1997b). The family of box ACA small nucleolar RNAs is defined by an evolutionarily conserved secondary structure and ubiquitous sequence elements essential for RNA accumulation. *Genes Dev.* **11**, 941-956.
- Grandi, P., Rybin, V., Bassler, J., Petfalski, E., Strauss, D., Marzioch, M., Schäfer, T., Kuster, B., Tschochner, H., Tollervey, D. et al. (2002). 90S pre-ribosomes include the 35S pre-rRNA, the U3 snoRNP, and 40S subunit processing factors but predominantly lack 60S synthesis factors. *Mol. Cell* **10**, 105-115.
- Granneman, S. and Baserga, S. J. (2005). Crosstalk in gene expression: coupling and co-regulation of rDNA transcription, pre-ribosome assembly and pre-rRNA processing. *Curr. Opin. Cell Biol.* **17**, 281-286.
- Granneman, S., Petfalski, E., Swiatkowska, A. and Tollervey, D. (2010). Cracking pre-40S ribosomal subunit structure by systematic analyses of RNA-protein cross-linking. *EMBO J.* **29**, 2026-2036.
- Granneman, S., Petfalski, E. and Tollervey, D. (2011). A cluster of ribosome synthesis factors regulate pre-rRNA folding and 5.8S rRNA maturation by the Rat1 exonuclease. *EMBO J.* **30**, 4006-4019.
- Greber, B. J., Boehringer, D., Montellese, C. and Ban, N. (2012). Cryo-EM structures of Arx1 and maturation factors Reil and Jjj1 bound to the 60S ribosomal subunit. *Nat. Struct. Mol. Biol.* **19**, 1228-1233.
- Green, R. and Noller, H. F. (1997). Ribosomes and translation. *Annu. Rev. Biochem.* **66**, 679-716.
- Hackmann, A., Gross, T., Baierlein, C. and Krebber, H. (2011). The mRNA export factor Npl3 mediates the nuclear export of large ribosomal subunits. *EMBO Rep.* **12**, 1024-1031.
- Harpicharnchai, P., Jakovljevic, J., Horsey, E., Miles, T., Roman, J., Rout, M., Meagher, D., Imai, B., Guo, Y., Brame, C. J. et al. (2001). Composition and functional characterization of yeast 66S ribosome assembly intermediates. *Mol. Cell* **8**, 505-515.
- 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.
- Henras, A. K., Soudet, J., Gérés, M., Lebaron, S., Caizergues-Ferrer, M., Mougín, A. and Henry, Y. (2008). The post-transcriptional steps of eukaryotic ribosome biogenesis. *Cell. Mol. Life Sci.* **65**, 2334-2359.
- Ho, J. H., Kallstrom, G. and Johnson, A. W. (2000). Nmd3p is a Crmlp-dependent adapter protein for nuclear export of the large ribosomal subunit. *J. Cell Biol.* **151**, 1057-1066.
- Horn, H. F. and Vowsden, K. H. (2008). Cooperation between the ribosomal proteins L5 and L11 in the p53 pathway. *Oncogene* **27**, 5774-5784.
- Kappel, L., Loibl, M., Zisser, G., Klein, I., Fruhmann, G., Gruber, C., Unterwiesing, S., Rechberger, G., Pertschy, B. and Bergler, H. (2012). Rlp24 activates the AAA-ATPase Drg1 to initiate cytoplasmic pre-60S maturation. *J. Cell Biol.* **199**, 771-782.
- Kemmler, S., Occhipinti, L., Veisu, M. and Panse, V. G. (2009). Yvh1 is required for a late maturation step in the 60S biogenesis pathway. *J. Cell Biol.* **186**, 863-880.
- King, T. H., Liu, B., McCully, R. R. and Fournier, M. J. (2003). Ribosome structure and activity are altered in cells lacking snoRNPs that form pseudouridines in the peptidyl transferase center. *Mol. Cell* **11**, 425-435.
- Kiss-László, Z., Henry, Y., Bachellerie, J.-P., Caizergues-Ferrer, M. and Kiss, T. (1996). Site-specific ribose methylation of preribosomal RNA: a novel function for small nucleolar RNAs. *Cell* **85**, 1077-1088.
- Kiss-László, Z., Henry, Y. and Kiss, T. (1998). Sequence and structural elements of methylation guide snoRNAs essential for site-specific ribose methylation of pre-rRNA. *EMBO J.* **17**, 797-807.
- Komili, S., Farny, N. G., Roth, F. P. and Silver, P. A. (2007). Functional specificity among ribosomal proteins regulates gene expression. *Cell* **131**, 557-571.
- Kondrashov, N., Pusic, A., Stumpf, C. R., Shimizu, K., Hsieh, A. C., Xue, S., Ishijima, J., Shiroishi, T. and Barna, M. (2011). Ribosome-mediated specificity in Hox mRNA translation and vertebrate tissue patterning. *Cell* **145**, 383-397.
- Koš, M. and Tollervey, D. (2010). Yeast pre-rRNA processing and modification occur cotranscriptionally. *Mol. Cell* **37**, 809-820.
- Kressler, D., Roser, D., Pertschy, B. and Hurt, E. (2008). The AAA-ATPase Rix7 powers progression of ribosome biogenesis by stripping Nsa1 from pre-60S particles. *J. Cell Biol.* **181**, 935-944.
- Kressler, D., Hurt, E. and Bassler, J. (2010). Driving ribosome assembly. *Biochim. Biophys. Acta* **1803**, 673-683.
- Kressler, D., Hurt, E., Bergler, H. and Bassler, J. (2012). The power of AAA-ATPases on the road of pre-60S ribosome maturation – molecular machines that strip pre-ribosomal particles. *Biochim. Biophys. Acta* **1823**, 92-100.
- Lamanna, A. C. and Karbstein, K. (2009). Nob1 binds the single-stranded cleavage site D at the 3'-end of 18S rRNA with its PIN domain. *Proc. Natl. Acad. Sci. USA* **106**, 14259-14264.
- Landowski, M., O'Donohue, M. F., Buros, C., Ghazvinian, R., Montel-Lehry, N., Vlachos, A., Sieff, C. A., Newburger, P. E., Niewiadomska, E., Matysiak, M. et al. (2013). Novel deletion of RPL15 identified by array-comparative genomic hybridization in Diamond-Blackfan anemia. *Hum. Genet.* [Epub ahead of print] doi:10.1007/s00439-013-1326-z/T.
- LaRiviere, F. J., Cole, S. E., Ferullo, D. J. and Moore, M. J. (2006). A late-acting quality control process for mature eukaryotic rRNAs. *Mol. Cell* **24**, 619-626.
- Lebaron, S., Schneider, C., van Nüss, R. W., Swiatkowska, A., Walsh, D., Böttcher, B., Granneman, S., Watkins, N. J. and Tollervey, D. (2012). Proofreading of pre-40S ribosome maturation by a translation initiation factor and 60S subunits. *Nat. Struct. Mol. Biol.* **19**, 744-753.
- 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.
- Léger-Silvestre, I., Milkereit, P., Ferreira-Cerca, S., Saveanu, C., Rousselle, J. C., Choemel, V., Guinefouleau, C., Gas, N. and Gleizes, P. E. (2004). The ribosomal protein Rps15p is required for nuclear exit of the 40S subunit precursors in yeast. *EMBO J.* **23**, 2336-2347.
- Littlefield, J. W. and Dunn, D. B. (1958a). Natural occurrence of thymine and three methylated adenine bases in several ribonucleic acids. *Nature* **181**, 254-255.
- Littlefield, J. W. and Dunn, D. B. (1958b). The occurrence and distribution of thymine and three methylated-adenine bases in ribonucleic acids from several sources. *Biochem. J.* **70**, 642-651.
- Lo, K. Y., Li, Z., Wang, F., Marcotte, E. M. and Johnson, A. W. (2009). Ribosome stalk assembly requires the dual-specificity phosphatase Yvh1 for the exchange of Mrt4 with P0. *J. Cell Biol.* **186**, 849-862.
- Lo, K. Y., Li, Z., Bussiere, C., Bresson, S., Marcotte, E. M. and Johnson, A. W. (2010). Defining the pathway of cytoplasmic maturation of the 60S ribosomal subunit. *Mol. Cell* **39**, 196-208.
- Marneros, A. G. (2013). BMS1 is mutated in aplasia cutis congenita. *PLoS Genet.* **9**, e1003573.
- Mauro, V. P. and Edelman, G. M. (2002). The ribosome filter hypothesis. *Proc. Natl. Acad. Sci. USA* **99**, 12031-12036.
- Mauro, V. P. and Edelman, G. M. (2007). The ribosome filter redux. *Cell Cycle* **6**, 2246-2251.
- McIntosh, K. B. and Warner, J. R. (2007). Yeast ribosomes: variety is the spice of life. *Cell* **131**, 450-451.
- Menne, T. F., Goyenechea, B., Sánchez-Puig, N., Wong, C. C., Tonkin, L. M., Ancliff, P. J., Brost, R. L., Costanzo, M., Boone, C. and Warren, A. J. (2007). The Shwachman-Bodian-Diamond syndrome protein mediates translational activation of ribosomes in yeast. *Nat. Genet.* **39**, 486-495.
- Meyer, A. E., Hoover, L. A. and Craig, E. A. (2010). The cytosolic J-protein, Jjj1, and Reil function in the removal of the pre-60 S subunit factor Arx1. *J. Biol. Chem.* **285**, 961-968.
- Miles, T. D., Jakovljevic, J., Horsey, E. W., Harpicharnchai, P., Tang, L. and Woolford, J. L., Jr (2005). Ytm1, Nop7, and Erbl1 form a complex necessary for maturation of yeast 66S preribosomes. *Mol. Cell Biol.* **25**, 10419-10432.

- Miller, O. L., Jr and Beatty, B. R. (1969). Visualization of nucleolar genes. *Science* **164**, 955-957.
- Mitchell, P., Petfalski, E., Shevchenko, A., Mann, M. and Tollervey, D. (1997). The exosome: a conserved eukaryotic RNA processing complex containing multiple 3'→5' exoribonucleases. *Cell* **91**, 457-466.
- Moss, T., Langlois, F., Gagnon-Kugler, T. and Stefanovsky, V. (2007). A housekeeper with power of attorney: the rRNA genes in ribosome biogenesis. *Cell. Mol. Life Sci.* **64**, 29-49.
- Moy, T. I. and Silver, P. A. (2002). Requirements for the nuclear export of the small ribosomal subunit. *J. Cell Sci.* **115**, 2985-2995.
- Mullineux, S. T. and Lafontaine, D. L. (2012). Mapping the cleavage sites on mammalian pre-rRNAs: where do we stand? *Biochimie* **94**, 1521-1532.
- Narla, A. and Ebert, B. L. (2010). Ribosomopathies: human disorders of ribosome dysfunction. *Blood* **115**, 3196-3205.
- Németh, A., Perez-Fernandez, J., Merkl, P., Hamperl, S., Gerber, J., Griesenbeck, J. and Tschochner, H. (2013). RNA polymerase I termination: Where is the end? *Biochim. Biophys. Acta* **1829**, 306-317.
- Ni, J., Tien, A. L. and Fournier, M. J. (1997). Small nucleolar RNAs direct site-specific synthesis of pseudouridine in ribosomal RNA. *Cell* **89**, 565-573.
- 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.
- Nissan, T. A., Galani, K., Maco, B., Tollervey, D., Aebi, U. and Hurt, E. (2004). A pre-ribosome with a tadpole-like structure functions in ATP-dependent maturation of 60S subunits. *Mol. Cell* **15**, 295-301.
- Oeffinger, M. and Tollervey, D. (2003). Yeast Nop15p is an RNA-binding protein required for pre-rRNA processing and cytokinesis. *EMBO J.* **22**, 6573-6583.
- Oeffinger, M., Leung, A., Lamond, A. and Tollervey, D. (2002). Yeast Pescadillo is required for multiple activities during 60S ribosomal subunit synthesis. *RNA* **8**, 626-636.
- 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.
- Ofengand, J. (2002). Ribosomal RNA pseudouridines and pseudouridine synthases. *FEBS Lett.* **514**, 17-25.
- Osheim, Y. N., French, S. L., Keck, K. M., Champion, E. A., Spasov, K., Dragon, F., Baserga, S. J. and Beyer, A. L. (2004). Pre-18S ribosomal RNA is structurally compacted into the SSU processome prior to being cleaved from nascent transcripts in *Saccharomyces cerevisiae*. *Mol. Cell* **16**, 943-954.
- Peculis, B. A. and Steitz, J. A. (1993). Disruption of U8 nucleolar snRNA inhibits 5.8S and 28S rRNA processing in the *Xenopus* oocyte. *Cell* **73**, 1233-1245.
- Peculis, B. A. and Steitz, J. A. (1994). Sequence and structural elements critical for U8 snRNP function in *Xenopus* oocytes are evolutionarily conserved. *Genes Dev.* **8**, 2241-2255.
- Pérez-Fernández, J., Román, A., De Las Rivas, J., Bustelo, X. R. and Dosil, M. (2007). The 90S pre-ribosome is a multimodular structure that is assembled through a hierarchical mechanism. *Mol. Cell. Biol.* **27**, 5414-5429.
- Pérez-Fernández, J., Martín-Marcos, P. and Dosil, M. (2011). Elucidation of the assembly events required for the recruitment of Utp20, Imp4 and Bms1 onto nascent pre-ribosomes. *Nucleic Acids Res.* **39**, 8105-8121.
- Pertschy, B., Saveanu, C., Zisser, G., Lebreton, A., Tengg, M., Jacquier, A., Liebming, E., Nobis, B., Kappel, L., van der Klei, I. et al. (2007). Cytoplasmic recycling of 60S preribosomal factors depends on the AAA protein Drg1. *Mol. Cell. Biol.* **27**, 6581-6592.
- Pertschy, B., Schneider, C., Gnädig, M., Schäfer, T., Tollervey, D. and Hurt, E. (2009). RNA helicase Prp43 and its co-factor Pfa1 promote 20 to 18 S rRNA processing catalyzed by the endonuclease Nob1. *J. Biol. Chem.* **284**, 35079-35091.
- Pestov, D. G., Stockelman, M. G., Strezoska, Z. and Lau, L. F. (2001). ERB1, the yeast homolog of mammalian Bop1, is an essential gene required for maturation of the 25S and 5.8S ribosomal RNAs. *Nucleic Acids Res.* **29**, 3621-3630.
- Preti, M., O'Donohue, M. F., Montel-Lehry, N., Bortolin-Cavaillé, M. L., Choemel, V. and Gleizes, P. E. (2013). Gradual processing of the ITS1 from the nucleolus to the cytoplasm during synthesis of the human 18S rRNA. *Nucleic Acids Res.* **41**, 4709-4723.
- 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.
- Rodríguez-Mateos, M., Abia, D., García-Gómez, J. J., Morreale, A., de la Cruz, J., Santos, C., Remacha, M. and Ballesta, J. P. (2009a). The amino terminal domain from Mrt4 protein can functionally replace the RNA binding domain of the ribosomal P0 protein. *Nucleic Acids Res.* **37**, 3514-3521.
- Rodríguez-Mateos, M., García-Gómez, J. J., Francisco-Velilla, R., Remacha, M., de la Cruz, J. and Ballesta, J. P. (2009b). Role and dynamics of the ribosomal protein P0 and its related trans-acting factor Mrt4 during ribosome assembly in *Saccharomyces cerevisiae*. *Nucleic Acids Res.* **37**, 7519-7532.
- Rouquette, J., Choemel, 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.
- Sahasranaman, A., Dembowski, J., Strahler, J., Andrews, P., Maddock, J. and Woolford, J. L., Jr (2011). Assembly of *Saccharomyces cerevisiae* 60S ribosomal subunits: role of factors required for 27S pre-rRNA processing. *EMBO J.* **30**, 4020-4032.
- Schäfer, 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.
- Schäfer, T., Maco, B., Petfalski, E., Tollervey, D., Böttcher, B., Aebi, U. and Hurt, E. (2006). Hrr25-dependent phosphorylation state regulates organization of the pre-40S subunit. *Nature* **441**, 651-655.
- Schneider, D. A. (2012). RNA polymerase I activity is regulated at multiple steps in the transcription cycle: recent insights into factors that influence transcription elongation. *Gene* **493**, 176-184.
- 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.
- Sloan, K. E., Bohnsack, M. T. and Watkins, N. J. (2013a). The 5S RNP couples p53 homeostasis to ribosome biogenesis and nucleolar stress. *Cell Rep.* **5**, 237-247.
- Sloan, K. E., Mattijssen, S., Lebaron, S., Tollervey, D., Puijig, G. J. and Watkins, N. J. (2013b). Both endonucleolytic and exonucleolytic cleavage mediate ITS1 removal during human ribosomal RNA processing. *J. Cell Biol.* **200**, 577-588.
- Smith, J. E., Cooperman, B. S. and Mitchell, P. (1992). Methylation sites in *Escherichia coli* ribosomal RNA: localization and identification of four new sites of methylation in 23S rRNA. *Biochemistry* **31**, 10825-10834.
- Strunk, B. S., Loucks, C. R., Su, M., Vashisth, H., Cheng, S., Schilling, J., Brooks, C. L., III, Karbstein, K. and Skiniotis, G. (2011). Ribosome assembly factors prevent premature translation initiation by 40S assembly intermediates. *Science* **333**, 1449-1453.
- Strunk, B. S., Novak, M. N., Young, C. L. and Karbstein, K. (2012). A translation-like cycle is a quality control checkpoint for maturing 40S ribosome subunits. *Cell* **150**, 111-121.
- Tafforeau, L., Zorbas, C., Langhendries, J. L., Mullineux, S. T., Stamatopoulou, V., Mullier, R., Wacheul, L. and Lafontaine, D. L. (2013). The complexity of human ribosome biogenesis revealed by systematic nucleolar screening of pre-rRNA processing factors. *Mol. Cell.* **51**, 539-551.
- Tang, L., Sahasranaman, A., Jakovljevic, J., Schleifman, E. and Woolford, J. L., Jr (2008). Interactions among Ytm1, Erb1, and Nop7 required for assembly of the Nop7-subcomplex in yeast pre-ribosomes. *Mol. Biol. Cell* **19**, 2844-2856.
- Teng, T., Thomas, G. and Mercer, C. A. (2013). Growth control and ribosomopathies. *Curr. Opin. Genet. Dev.* **23**, 63-71.
- Udem, S. A. and Warner, J. R. (1973). The cytoplasmic maturation of a ribosomal precursor ribonucleic acid in yeast. *J. Biol. Chem.* **248**, 1412-1416.
- Ulbrich, C., Diepholz, M., Bassler, J., Kressler, D., Pertschy, B., Galani, K., Böttcher, B. and Hurt, E. (2009). Mechanochemical removal of ribosome biogenesis factors from nascent 60S ribosomal subunits. *Cell* **138**, 911-922.
- Warner, J. R. (1999). The economics of ribosome biosynthesis in yeast. *Trends Biochem. Sci.* **24**, 437-440.
- Watkins, N. J. and Bohnsack, M. T. (2012). The box C/D and H/ACA snoRNPs: key players in the modification, processing and the dynamic folding of ribosomal RNA. *Wiley Interdiscip. Rev. RNA* **3**, 397-414.
- Widmann, B., Wandrey, F., Badertscher, L., Wyler, E., Pfannstiel, J., Zemp, I. and Kutay, U. (2012). The kinase activity of human Rio1 is required for final steps of cytoplasmic maturation of 40S subunits. *Mol. Biol. Cell* **23**, 22-35.
- Wild, T., Horvath, P., Wyler, E., Widmann, B., Badertscher, L., Zemp, I., Kozak, K., Csucs, G., Lund, E. and Kutay, U. (2010). A protein inventory of human ribosome biogenesis reveals an essential function of exportin 5 in 60S subunit export. *PLoS Biol.* **8**, e1000522.
- Yao, W., Roser, D., Köhler, 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.
- Yao, Y., Demoinet, E., Saveanu, C., Lenormand, P., Jacquier, A. and Fromont-Racine, M. (2010). Ecm1 is a new pre-ribosomal factor involved in pre-60S particle export. *RNA* **16**, 1007-1017.
- Yoon, A., Peng, G., Brandenburger, Y., Zollo, O., Xu, W., Rego, E. and Ruggero, D. (2006). Impaired control of IRES-mediated translation in X-linked dyskeratosis congenita. *Science* **312**, 902-906.
- Zemp, I., Wild, T., O'Donohue, M. F., Wandrey, F., Widmann, B., Gleizes, P. E. and Kutay, U. (2009). Distinct cytoplasmic maturation steps of 40S ribosomal subunit precursors require hRio2. *J. Cell Biol.* **185**, 1167-1180.