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Maturation of Eukaryotic Ribosomes: Acquisition of Functionality

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Abstract

In eukaryotic cells ribosomes are preassembled in the nucleus and exported to the cytoplasm where they undergo final maturation. This involves the release of *trans*-acting shuttling factors, transport factors, incorporation of the remaining ribosomal proteins and final rRNA processing steps. Recent work, especially on the large (60S) ribosomal subunit, has made it abundantly clear that the 60S subunit is exported from the nucleus in a functionally inactive state. Its arrival in the cytoplasm triggers events that that render it translationally competent. Here we focus on these cytoplasmic maturation events and speculate about why eukaryotic cells have evolved such an elaborate pathway of maturation.

The biogenesis of ribosomal subunits –“state of the art”

In all living cells, the ribosome is responsible for the final step of decoding genetic information into proteins. This universal “translating apparatus” comprises two subunits, each of which is a complex assemblage of RNA and proteins (Box 1). The two subunits display a distinct division of labour: the small 40S subunit (30S in prokaryotes) is responsible for decoding whereas the large 60S subunit (50S in prokaryotes) carries out the chemistry of polypeptide synthesis. Although structural analysis of prokaryotic ribosomes is providing detailed molecular insights into the mechanisms of ribosome function [1–3], our knowledge of the *in vivo* assembly of ribosomes remains rudimentary. How do cells assemble such an intricate machine and ensure that it functions faithfully in the critical role of decoding a cell’s genome? In this review we elaborate on the cytoplasmic maturation events that generate fully functional ribosomes and discuss why eukaryotic cells might have evolved these additional steps.

BOX 1

Prokaryotic and eukaryotic ribosome biogenesis

Ribosomes are universally constructed from two subunits. In *E. coli*, the large (50S) subunit contains two rRNAs (23S and 5S) and 34 r-proteins and the small (30S) subunit contains one rRNA (16S) and 21 r-proteins. Eukaryotic ribosomes are more complex: the large (60S) subunit contains three rRNAs (25S, 5.8S, 5S) and 49 r-proteins whereas the small (40S) subunit contains a single rRNA (18S) and 33 r-proteins.

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Bacterial 50S and 30S subunits can be assembled *in vitro* from purified rRNA and r-proteins, but this requires conditions that are non-physiological [65]. *In vivo* rRNA processing and modifying enzymes, and a small number of factors such as RNA helicases, DnaK/Hsp70 and rRNA chaperones systems, GTPases are required for the assembly process [66]. These additional factors are dispensable under optimal conditions, but their absence often leads to impaired ribosome synthesis under restrictive conditions [65].

Eukaryotic ribosome assembly is considerably more complicated and requires >200 non-ribosomal *trans*-acting factors, many of which are essential. The process begins with the RNA polymerase I transcription of the 35S pre-rRNA, the precursor that will give rise to the mature 18S, 5.8S and 25S rRNAs. The 35S pre-rRNA undergoes co-transcriptional methylation and pseudouridylation reactions catalyzed by snoRNPs and associates with a large number of *trans*-acting factors and ribosomal proteins, mostly of the small subunit, to a 90S pre-ribosome. Processing of the pre-rRNA involves a series of endo-and exonuclease events that remove the flanking and internal spacer regions. Cleavage in the spacer region between the 18S and the 5.8S rRNAs at site A2 leads to the formation of pre-40S and pre-60S particles [67, 68]. RNA polymerase III synthesizes the 5S rRNA, which is incorporated into the pre-60S subunit. After separation of the 90S intermediate into a pre-60S and a pre-40S particle, the two precursors follow largely independent biogenesis and export pathways.

Final maturation of the subunits occurs in the cytoplasm. A number of *trans*-acting factors and export factors associated with pre-60S and pre-40S particles are released before the subunits achieve translation competence. In addition, the final rRNA processing steps occur in the cytoplasm. These include the final trimming of the 3′-end of 5.8S rRNA in the 60S subunit [67–69], and dimethylation and cleavage of the 20S pre-rRNA to yield mature 18S rRNA in the 40S subunit.

Ribosome biogenesis begins with transcription of the pre-rRNA, which undergoes co-transcriptional folding, modification and assembly with ribosomal proteins (r-proteins) to form the two subunits. The assembly of ribosomal subunits in bacteria appears to require few (<25) trans-acting factors. By contrast, eukaryotic ribosome assembly is a complicated process that requires the concerted efforts of all three RNA polymerases and >200 trans-acting factors, that aid the assembly, maturation and intracellular transport of ribosomal subunits.

Eukaryotic ribosomes are initially assembled in the nucleolus, the site of rRNA transcription. Although the nascent pre-ribosomal particles released from the nucleolus appear to be largely preassembled, they require additional maturation steps in the nucleoplasm and/or cytoplasm. Pioneering work in the early 1970s by the Planta and Warner laboratories led to the identification of the first pre-ribosome, the 90S particle [4, 5], that is subsequently processed to yield smaller 66S and 43S particles, the precursors to the mature 60S and 40S subunits, respectively. These particles contain pre-rRNA, r-proteins and numerous *trans*-acting factors. In the early 1990s, the application of genetic approaches in budding yeast permitted the identification of multiple *trans*-acting factors and led to a better understanding of the highly ordered rRNA processing steps [6, 7]. Despite these fundamental advances, the composition of pre-ribosomal particles remained largely unknown until this past decade, when the advent of tandem affinity purification (TAP) protocols, combined with sensitive mass spectrometry, allowed the isolation and compositional analysis of maturing pre-60S and pre-40S particles in budding yeast. These analyses have aided the sequential ordering of evolving pre-ribosomal particles along the 60S and 40S pathways, giving us ‘biochemical snapshots’ of the highly complex and dynamic assembly process [8, 9].

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In yeast the precursor 35S rRNA is transcribed by RNA polymerase I in the nucleolus [4, 5]. The emerging rRNA is co-transcriptionally methylated, pseudouridylated, and loaded with r-proteins and trans-acting factors, to form the 90S particle [10, 11]. Strikingly, the 90S contains r-proteins and *trans*-acting factors primarily devoted to the 40S biogenesis pathway. Cleavage of the rRNA at the A2 site (Box 1) releases the pre-40S particle whose subsequent biogenesis and maturation is independent of the 60S. Following release of the pre-40S the remaining pre-rRNA assembles with large subunit r-proteins and biogenesis factors to form pre-60S particles.

Pre-40S particles undergo relatively few compositional changes as they travel through the nucleoplasm and, compared to pre-60S particles, are rapidly exported to the cytoplasm [4, 5, 12]. By contrast, pre-60S particles associate with ~100 *trans*-acting factors along its biogenesis pathway and undergo dynamic changes in compositional as they travel through the nucleoplasm towards the nuclear pore complex (NPC) [8, 13, 14]. At distinct stages of biogenesis in the nucleus and in the cytoplasm, *trans*-acting factors are released from the pre-ribosomal particles to be recycled for new rounds of biogenesis. These remodeling events are likely triggered by energy-consuming enzymes (e.g., ATPases and GTPases) that associate with maturing pre-ribosomal particles (for recent reviews see [14, 15]). The site(s) of action of these enzymes and their precise function in ribosome maturation are largely unknown. Recent work implicates two large AAA-ATPases Rix7 and Rea1 in the maturation of the pre-60S subunit. Rix7 appears to strip Nsa1 from the subunit at the nucleolar/nucleoplasmic transition [14], whereas Rea1 is thought to drive pre-60S particles towards export competence by removing Rsa4 [16]. Thus, these AAA-ATPases contribute directly to the sequential reduction of complexity of pre-ribosomal particles prior to their export from the nucleus. We refer the reader to other recent reviews for aspects of ribosome biogenesis, export and maturation not covered herein [9, 15, 17, 18].

Nuclear export of pre-ribosomal subunits

Pre-ribosomal subunits are transported through the NPC to be released into the cytoplasm. In the late 1990s *in vivo* transport/export assays were developed employing both large-subunit (Rpl25–GFP and Rpl11–GFP) and small-subunit reporters (Rps2–GFP) to identify factors involved in the nuclear export of r-subunits [19–21]. This work revealed that specific nucleoporins, the proteins of the NPC, and the Ran GTP-GDP cycle are required for nuclear export of both subunits [19, 21–23]. Although pre-40S and pre-60S particles are exported independently of each other, both require the general nuclear export factor Xpo1 (hereafter referred to as Crm1) that directly recognizes nuclear export sequences (NES) on cargo molecules. Whereas Nmd3 is the only known Crm1 adapter for the pre-60S particle, at least three NES-containing *trans*-acting factors, Ltv1, hDim2 and hRio2, have been reported to serve as Crm1 adapters in pre-40S export [24, 25]. The non-essential nature of Ltv1 suggests that there is redundancy in 40S export adapters. Indeed, efficient transport of large cargoes requires multiple receptors [26]. In budding yeast, pre-60S particles employ additional factors that interact directly with the NPC to facilitate export. These include the general mRNA export factor Mtr2–Mex67 [27] and the shuttling *trans*-acting factor Arx1 [28, 29]. However, unlike Nmd3, the ribosome export function of Arx1 and Mtr2–Mex67 does not appear to be conserved in their metazoan orthologs [27, 28].

Cytoplasmic maturation of pre-ribosomal subunits

The majority of *trans*-acting factors that associate with pre-ribosomal particles during early biogenesis are released and recycled back to the nucleolus prior to nuclear export. However, a few factors remain associated with the particles as they enter the cytoplasm. The release and recycling of these factors, along with the assembly of the few remaining r-proteins, and final RNA processing events constitute “cytoplasmic maturation steps” in the ribosome biogenesis

pathway. These steps are crucial not only for completing maturation of the subunit to which they are bound, but also because a failure to recycle a factor to the nucleus leads to its depletion from its nucleolar/nuclear sites of action, inducing delays in pre-rRNA processing, defects in assembly and impaired nuclear export.

Maturation of the pre-60S subunit

Pre-60S subunits are accompanied to the cytoplasm by a small entourage of non-ribosomal factors that must be released by specialized factors in the cytoplasm (Table 1). The subunits also require the assembly of several ribosomal proteins to add functionality (Fig 1). Upon arrival in the cytoplasm, pre-60S particles encounter a third essential AAA-ATPase, Drg1 [30]. *drg1* mutants accumulate Rlp24, Nog1, Arx1 and, to a lesser extent, the translation initiation factor Tif6 in the cytoplasm where they remain bound to pre-60S subunits. AAA-ATPases typically have discrete substrate specificities [31]; thus it is unlikely that Drg1 acts directly to release each of these proteins. Indeed, Drg1 appears to target Rlp24 and/or Nog1, with the effect on Arx1 and Tif6 being secondary. The ATPase activity of Drg1 is essential for its function, suggesting that a mechano-chemical activity of Drg1 is required for its role in early cytoplasmic maturation of pre-60S subunits.

Rlp24 is closely related to the r-protein Rpl24. Their sequence similarity and apparent mutually exclusive binding to the 60S subunit [32] suggest that the two proteins bind sequentially to the same site. This implies that the release of Rlp24 is necessary for Rpl24 to assemble into the subunit.

Rpl24 recruits the zinc-finger protein Rei1 by virtue of their direct interaction [33], thereby establishing an order action of Rei1 after Drg1. Rei1 is not essential, but it is required for the recycling of Arx1, the Arx1 binding partner Alb1 and, to a lesser extent, Tif6 to the nucleus [33, 34]. Rei1 works in conjunction with the Hsp40 J protein Jjj1 and Ssa1/Ssa2, an Hsp70 family ATPase [35, 36]. *rei1* and *jjj1* mutants accumulate Arx1 and Alb1 in the cytoplasm [33, 35], where they remain bound to pre-60S subunits [34, 36]. Deletion of, or mutations in, *ARX1* suppress the growth defect of a *rei1* mutant [33, 34], suggesting that Arx1 is the direct target of Rei1 (Box 2). Although the mechanism of Rei1 function is not known, 60S subunits from *rei1* mutant cells are salt-labile [37], suggesting an additional role for Rei1 in the proper folding or assembly of the subunit.

BOX 2

Genetic analysis of ribosome maturation

Maturation of the 60S subunit involves the release of a handful of shuttling factors by cytoplasmic ATPases and GTPases. Mutation in a given releasing factor often results in the cytoplasmic accumulation of multiple shuttling factors that remain associated with the pre-60S particles [30, 33–36, 44–46, 48, 49]. Identifying the direct targets of these factors is important for understanding their molecular function. Bypass suppression is the litmus test to identify such a relationship. To illustrate, let us consider Mrt4 and Yvh1. Mrt4 assembles into pre-60S particles in the place of the ribosomal stalk protein P0 [51] and Yvh1 is required for its release [48, 49]. *YVH1* deletion leads to the cytoplasmic accumulation of Mrt4 as well as Tif6 on pre-60S particles. A screen for mutations that suppress the growth defect of *yvh1Δ* mutants identified mutations in *MRT4*, but not *TIF6*. Suppression is specific as these *mrt4* mutations do not suppress *efl1* mutants, nor do mutations in *TIF6* that bypass *efl1Δ* mutants suppress *yvh1Δ* mutants. The amino acid substitutions in Mrt4 are predicted to map to its RNA binding face where they would be expected to impair binding to 25S rRNA. Indeed, the suppressor Mrt4-G68D shows reduced affinity for pre-60S particles. Thus, these mutations appear to suppress *yvh1Δ* by allowing the protein to be recycled without the need

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for its specialized releasing factor. Such mutations in a target factor that specifically suppress mutations in a releasing factor provide compelling genetic evidence that the target is the direct substrate. Similar genetic relationships exist between *ARX1* and *REI1* [33, 34], *TIF6* and *EFL1* [43, 44] and *NMD3* and *LSG1* [46].

Arx1 has evolved from methionyl amino peptidases (MetAPs), a family of proteins that remove N-terminal methionine from nascent polypeptides as they emerge from the exit tunnel of the ribosome [38]. Based on sequence and structural similarity of Arx1 to MetAPs, one would predict that they bind to the same site on the ribosome and that Arx1. Genetic evidence suggests that Arx1 binds in the vicinity of Rpl25 at the polypeptide exit tunnel [34]. This is an important functional site on the ribosome as Rpl25 interacts with the signal recognition particle as well as the translocon in the endoplasmic reticulum (ER) [39].

The pre-60S particle entering the cytoplasm also contains Tif6 (Fig 1). Its mammalian ortholog eIF6 was identified many years ago as a protein that prevented the joining of the 60S and 40S subunits [40, 41]. Biochemical analysis of the archaeal ortholog aIF6 suggests that this protein binds the joining face of the 60S subunit [42]. The GTPase Efl1 and the Swachman-Bodian Syndrome protein ortholog Sdo1 are required to release Tif6 [43–45]. Mutations in either of these factors result in retention of Tif6 on nascent subunits and a bulk redistribution of Tif6 to the cytoplasm. Amino acid substitutions in Tif6 that weaken its affinity for the subunit suppress the growth defects of *efl1* and *sdo1* [43–45] mutants, providing strong genetic evidence that Tif6 is the primary substrate of Efl1 and Sdo1 (Box 2). Efl1 bears strong sequence similarity to translation elongation factor 2, a protein that facilitates translocation of the ribosome following peptidyl transfer. How the function of Efl1 is related to eEF2 in facilitating the release of Tif6 is an intriguing question.

The nuclear export adapter Nmd3 must also be recycled to the nucleus, and two proteins, Rpl10 and the GTPase Lsg1, have been implicated in its release [46]. Depletion of, or mutations in, *RPL10* prevents Nmd3 nuclear recycling [46]. Similarly, amino acid substitutions in the P-loop of Lsg1 that are predicted to disrupt its GTPase activity also block Nmd3 recycling [46, 47]. These results suggest that Lsg1 induces a conformational change upon Rpl10 loading that stabilizes Rpl10 in the subunit, thus triggering Nmd3 release.

More recently, our laboratories have identified the assembly of the ribosome stalk as an additional event in the cytoplasm, [48, 49] (Fig 1). The stalk is essential for recruitment and activation of translation factors, in particular the elongation factors [50] and its assembly is a critical step in adding functionality to the ribosome. In yeast, the stalk is composed of P0 and two heterodimers of P1 and P2. P0 anchors the stalk to the ribosome by binding to the rRNA of helices 43 and 44. However, ribosomes are first assembled in the nucleus with Mrt4 functioning in place of P0 [51]. Mrt4 is a nuclear paralog of P0, but it lacks the domains that recruit translation factors, thus necessitating an additional step in the maturation pathway, the exchange of P0 for Mrt4 [51]. We have shown that the dual specificity phosphatase Yvh1 is required for the removal of Mrt4. However, the ability of Yvh1 to release Mrt4 depends on a conserved Zn^{+2} binding domain, not its phosphatase domain.

Is there a "Pathway" of cytoplasmic maturation?

How are these various release and assembly events coordinated with each other? The ATPases and GTPases driving these events could work independently of one another without a defined order of events. By contrast, some or all of these events could be coupled, either in series or as interdependent events. Some evidence for coupling already exists. As mentioned above, *drg1* mutants prevent the recycling of Rlp24, Nog1 and Arx1 and partially block Tif6 recycling [30]. Here, we can begin to order these events. Following the release of Rlp24 by Drg1, the

loading of Rpl24 into the subunit recruits Rei1 [33]. In conjunction with Jjj1 and Ssa1/Ssa2, these proteins then release Arx1 [33–36], whose persistence on the subunit impedes the release of Tif6. This scenario suggests a linear pathway from Drg1 release of Rlp24 to Efl1 release of Tif6. However, Tif6 is also mislocalized in *yvh1* mutants in which stalk assembly is blocked [48]. Considering that the function of the stalk in translation is to recruit and activate GTPases, and that Efl1 is closely related to eEF2, we suggest that stalk assembly plays a similar role in biogenesis, to recruit the related GTPase Efl1 for the release of Tif6. How the Yvh1 and Efl1 events are related to Rei1 and Lsg1, however, remain to be addressed.

Maturation of the 40S subunit

Like the large subunit, the small subunit is also accompanied to the cytoplasm by a handful of proteins that mediate its export as well as subsequent rRNA processing. These include, Enp1, Tsr1, Ltv1, Dim1, Dim2, Nob1, Rio2, Hrr25 and possibly Prp43 [52, 53]. However, unlike maturation of the pre-60S particle, the detection of distinct intermediates of pre-40S maturation has been challenging. Early work on the kinetics of subunit maturation indicated that the pre-40S subunit engages in translation faster than the 60S subunit [5], suggesting a more rapid conversion into a functional subunit.

Cytoplasmic maturation of the pre-40S particle seems to be devoted to two major events: a structural rearrangement to generate the “beak” structure of the mature 40S subunit, and the final endonucleolytic cleavage of the pre-rRNA to yield mature 18S rRNA. There is compelling evidence that this conformational change is regulated by phosphorylation. The casein kinase isoform Hrr25 phosphorylates Rps3 as well as Ltv1 and Enp1 [54]. When phosphorylated, Rps3 is weakly associated with the subunit, and a subcomplex comprising Rps3, Ltv1 and Enp1 can be isolated. Subsequent dephosphorylation is required for the stable incorporation of Rps3 into the small subunit and the corresponding production of the “beak” within the head domain of the small subunit [54]. Although a cycle of phosphorylation and dephosphorylation appears to be critical for the stable association of Rps3 with the small subunit and for maturation of the “beak” domain, we do not yet know if Ltv1 and/or Enp1 are the critical targets for Hrr25 phosphorylation.

The second major event required for the maturation of the 40S subunit in the cytoplasm is cleavage of the pre-rRNA to generate mature 18S rRNA. This step appears to be universally conserved in eukaryotes [55], and mounting evidence suggests that Nob1 is the nuclease responsible for this cleavage [52, 56]. Surprisingly, Nob1 is loaded into the pre-40S complex in the nucleus. What then prevents it from cleaving its rRNA substrate prematurely? Recent work describes a synthetic lethal interaction between Ltv1 and the RNA helicase Prp43 that is suppressed by Nob1 overexpression [52]. This finding led to the proposal that Prp43 drives a conformational change in the pre-40S particle that allows Nob1 access to its RNA substrate. Cleavage also requires the essential kinase Rio2 [25, 57, 58]. Because hRio2 is also required for the recycling of hLtv1, hEnp1, hNob1 and hDim2 [25], it is not yet clear if Rio2 promotes cleavage of 20S and thereby allows the recycling of associated proteins, or if it promotes a conformational change that coordinately releases the pre-40S factors, thus allowing cleavage by Nob1.

Ribosomes are exported from the nucleus in an inactive state

Ribosomes are assembled in the nucleus in an environment that is physically separated from translation, and presumably provides an environment for rRNA processing and particle assembly in which translation and other ribosome-associated factors do not interfere. However, the general picture that is emerging from studies of pre-ribosome maturation is that in addition to this physical barrier, assembling subunits are also packaged in a functionally inactive state, lacking critical r-proteins that provide functionality to the ribosome and containing trans-acting

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factors that prevent their function. Thus, in addition to physical compartmentalization, there is functional compartmentalization of the nascent subunits. Two examples are illustrative. First, Tif6 binds to the joining face of the subunit [42], preventing its association [40] with the small subunit and thereby holding the subunit inactive until it is released. Second, the nascent subunit in the nucleus is assembled with Mrt4 instead of the P protein stalk and consequently is incapable of supporting translation until the stalk is assembled [48, 49, 51]. In addition, Arx1 binds in the vicinity of Rpl25 [34] where it might prevent the association of exit tunnel-associated factors. Other yet unknown trans-acting factors on the subunit might also block its entry into the translating pool.

Why synthesize and export functionally inactive subunits? One possibility is that ribosomes are held in a functionally inactive state to facilitate their transport in the cytoplasm. Analogous to mRNAs that are translationally repressed during transport, a similar phenomenon might occur with ribosomes. This could provide a means of targeting newly made ribosomes to specific sites in a cell, and for avoiding premature engagement with mRNAs and translation factors along the way. For instance, ribosomes might be directed to a region of cell growth, such as growing bud in yeast, a growth cone in a neuron, or to a site of active translation, such as the ER, in a cell devoted to secretion.

The most conspicuous feature of the pre-40S subunit is the 3′-extension of its pre-rRNA that is cleaved in the cytoplasm. Might this feature regulate the function of the small subunit? Indeed, there is a close correlation between cytoplasmic cleavage to generate mature 18S rRNA and its incorporation into active 80S ribosomes [5]. Furthermore, this extension emanates from a position close to the decoding center of the small subunit where it would seem likely to interfere with the function of the small subunit. Nevertheless, in certain mutant backgrounds, 20S rRNA is observed in polysomes [59,52], suggesting that ribosomes can function with this remnant pre-rRNA. Considering the cluster of biogenesis factors that accompany the small subunit to the cytoplasm, one or more of these might also block its function. In fact, the bacterial dimethylase KsgA has been mapped to the joining face of the small subunit, in a position that overlaps the binding site for initiation factor 3 [60]. Dim1, the yeast ortholog of KsgA, might play a similar role in masking the function of the pre-40S subunit.

Why have eukaryotic cells evolved such an elaborate system for assembly and maturation of subunits?

The release of each shuttling factor in the cytoplasm requires a specialized set of releasing factors. However, in nearly every case, there are alleles of the target protein that suppress mutations within the releasing factor. For example, in Mrt4 Gly68 is at the protein–RNA interface. Substitutions of acidic residues at this position reduce its affinity for the 60S subunit, allowing it to recycle in the absence of its release factor Yvh1 and suppress the growth defect of a *yvh1* mutant [48, 49]. In fact, these double mutants grow at nearly wild-type rates. Similarly, in the absence of Efl1 or Sdo1, spontaneous mutations arise in *TIF6* that bypass the requirement for Efl1 or Sdo1 [43–45], and mutations in *NMD3* suppress *lsg1* mutants [46]. If the system can be simplified by eliminating the releasing factors, one might wonder why they evolved in the first place.

Perhaps these factors provide a mechanism for quality control in ribosome synthesis. We previously proposed that nuclear ribosome assembly utilizes “structural proofreading” [61] in which the recruitment of an export factor depends on the correct assembly of a binding surface that is achieved only if the proper folding and assembly pathway has been followed. However, in the cytoplasm, where the ribosome acquires functionality and where translation takes place, the opportunity might exist for functional proofreading to ensure that only active ribosomes are released for translation. Efl1 could act in such a process. Efl1 closely resembles elongation

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factor 2 in sequence and therefore likely interacts with the subunit in a manner similar to eEF2. Following stalk assembly, Efl1 recruitment could "test" the GTPase activating center of the ribosome, releasing Tif6 in the process. The utilization of a translation-like factor in biogenesis would be a clever evolutionary strategy to test the ribosome for its competence in "translation" prior to its first round in bona fide translation.

Are any of these steps reversible, providing a means to arrest maturation or perhaps store ribosomes under stress conditions? This is a largely unexplored topic. There is evidence that Nmd3 and Lsg1 can bind to mature recycling subunits as well as nascent subunits [62, 63], but it is not known what conditions might promote the reassociation of these factors with ribosomes. However, Nmd3 depends on the GTPase Lsg1 for its release [46] and it has been noted that the bacterial GTPases involved in ribosome assembly have low affinity for guanine nucleotides leading to the suggestion that they might respond directly to cellular GTP levels [64]. Thus, Lsg1 could similarly be regulated by GTP levels, controlling the release, and possibly binding, of Nmd3.

Concluding remarks

Given the importance for correctly translating the genetic code, we can expect that eukaryotic cells have evolved quality control mechanisms to monitor ribosome biogenesis. Recent work from several laboratories has uncovered cytoplasmic maturation steps in both the 40S and 60S biogenesis pathways that appear to activate subunits by removing inhibitory factors and adding functionality. The control of these steps could ensure funnelling of only functional ribosomal subunits into the translation pathway. How would such a sensing mechanism work, and how does biogenesis interface with translation? Clearly, a more detailed molecular understanding of these maturation steps is needed.

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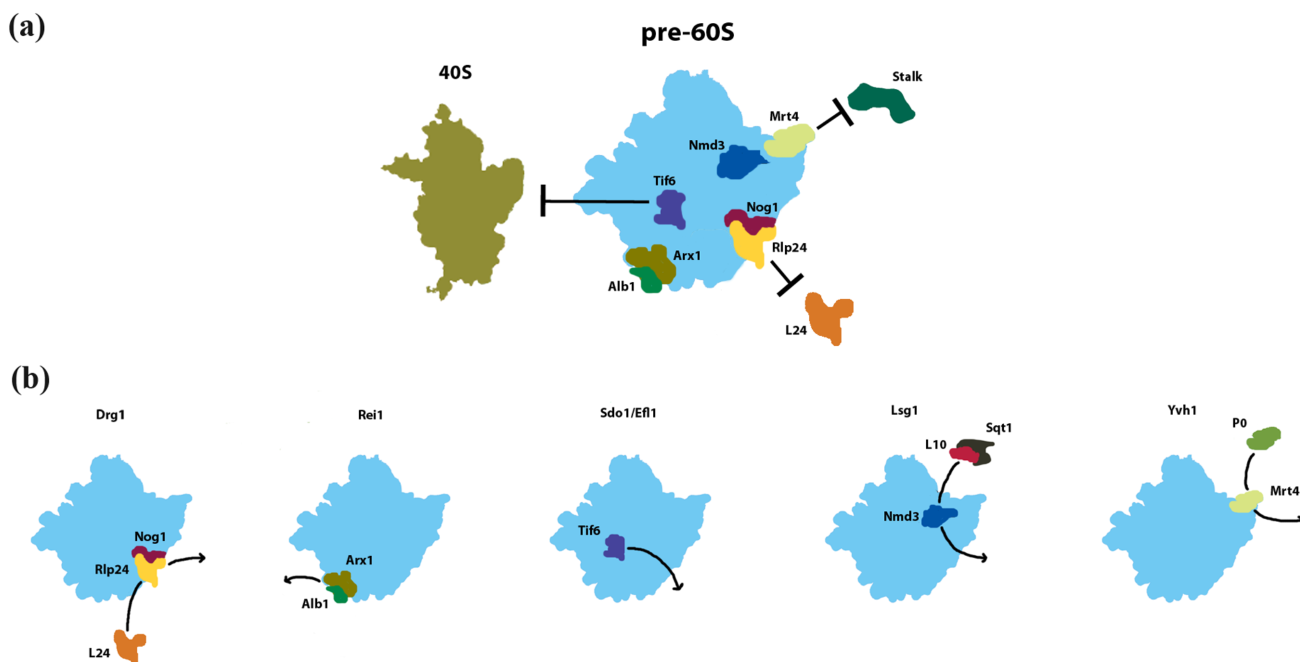

Figure 1. Cytoplasmic maturation events in the 60S biogenesis pathway

(a) Summary of the shuttling trans-acting factors (Rlp24, Mrt4, Tif6, Alb1) and transport factors (Nmd3, Arx1) that are present on pre-60S particles as they arrive in the cytoplasm. Black bars indicate that these pre-60S factors block the association of factors important for function or maturation of the 60S subunit, including Rpl24, the stalk, composed of the P-proteins and the 40S subunit. Until the factors are removed, the pre-60S is not translationally active.

(b) Summarizes the cytoplasmic factors (Drg1, Rei1, Sdo1, Efl1, Lsg1 and Yvh1) that are required for the release of the depicted shuttling trans-acting factors and transport factors.

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Table 1

Summary of non-ribosomal factors required for late cytoplasmic maturation and their respective protein targets on late pre-60S particles

Non-ribosomal Factor	Activity	Target	References
Drg1	AAA-ATPase	Rlp24/Nog1	30
Rei1	Zn^{2+} finger protein	Arx1/Alb1	33, 34, 37
Jjj1–Ssa1/Ssa2	Hsp40-Hsp70 ATPase	Arx1/Alb1	35, 36
Yvh1	Dual-Specificity phosphatase	Mrt4	48, 49
Lsg1/Kre35	GTPase	Nmd3	46
Efl1	GTPase	Tif6	43, 44
Sdo1	-	Tif6	45