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Ribosome assembly coming into focus

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Abstract

In the past 25 years, genetic and biochemical analyses of ribosome assembly in yeast have identified most of the factors that participate in this complex pathway and have generated models for the mechanisms driving the assembly. More recently, the publication of numerous cryo-electron microscopy structures of yeast ribosome assembly intermediates has provided near-atomic resolution snapshots of ribosome precursor particles. Satisfyingly, these structural data support the genetic and biochemical models and provide additional mechanistic insight into ribosome assembly. In this Review, we discuss the mechanisms of assembly of the yeast small ribosomal subunit and large ribosomal subunit in the nucleolus, nucleus and cytoplasm. Particular emphasis is placed on concepts such as the mechanisms of RNA compaction, the functions of molecular switches and molecular mimicry, the irreversibility of assembly checkpoints and the roles of structural and functional proofreading of pre-ribosomal particles.

Protein synthesis in eukaryotes is carried out by the ribosome, which is a large RNA–protein complex consisting of a small and a large subunit. During protein synthesis, decoding of mRNA by the small subunit is coupled with peptide-bond formation by the large subunit. In the model organism *Saccharomyces cerevisiae*, the small subunit (40S) comprises 33 ribosomal proteins and the 18S ribosomal RNA (rRNA), whereas the large subunit (60S) comprises 46 ribosomal proteins and 3 rRNAs (25S, 5.8S and 5S rRNA). For a complete description of each ribosomal protein, please see table 1 in REF.[1].

Beginning with the first atomic structures of eukaryotic ribosomal complexes, the structural intricacy of this molecular machine has been revealed[2–4]. Whereas the ribosomal catalytic centres — the decoding site in the small subunit and the peptidyl transferase centre (PTC) in

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the large subunit — and other ribosomal functional modules and key architectural features, such as the central pseudoknot in the small subunit and the central protuberance, GTPase activating centre (GAC), P0 stalk and polypeptide exit tunnel (PET) in the large subunit, are evolutionarily conserved, eukaryotic ribosomes contain many additional RNA extensions and proteins[5,6] (Supplementary Figs 1,2).

The assembly and thus availability of eukaryotic ribosomal subunits is intimately linked to nutrient availability, stress and the cell cycle (reviewed in[7]). Approximately 200 non-ribosomal factors, including proteins, protein complexes and small nucleolar ribonucleoproteins (snoRNPs), are required for the assembly of the small and large ribosomal subunits (summarized in tables 3 and 4 in REF.[8]). The maturation of pre-rRNAs for both subunits requires endonucleolytic and exonucleolytic cleavage (FIG. 1). Distinct stages of this process take place first in the nucleolus, then in the nucleus and finally in the cytoplasm. Following a general overview of small-subunit assembly (FIG. 2), we provide a cryo-electron microscopy (cryo-EM) depiction of a nucleolar small-subunit intermediate (FIG. 3). Similarly to the illustration of the small subunit in FIG. 2, the arrival and departure of ribosome assembly factors for the large subunit are illustrated with structures of pre-ribosomal particles in FIG. 4 and serve as a general guide for ribosome assembly. We use the yeast nomenclature for ribosomal proteins throughout.

Nucleolar ribosome assembly is characterized by the co-transcriptional association of ribosome assembly factors with nascent pre-rRNA. Reduction of conformational freedom of the nascent pre-rRNA aids the formation of subdomains for both the small ribosomal subunit (FIG. 3) and the large ribosomal subunit (FIG. 5). During nuclear ribosome assembly, the relative orientations of these subdomains are already closer to the mature conformations in the small and large subunits, but the subdomains still undergo extensive remodelling (FIG. 6). Lastly, in the cytoplasm, final adjustments and steps of quality control are employed to test the functionality of both subunits for protein synthesis. Ribosome assembly factors associated with precursors of the small ribosomal subunit are chronologically listed in Supplementary Table 1, and those associated with precursors of the large ribosomal subunit are listed in Supplementary Table 2.

In this Review, we discuss emerging biochemical and structural concepts in nucleolar, nuclear and cytoplasmic assembly of the small and large ribosomal subunits in *S. cerevisiae*. A focus is placed on nucleolar stages for which considerable advances have been made in recent years. We draw the readers' attention to four key concepts of ribosome assembly: the systematic reduction of conformational freedom of pre-rRNA during early nucleolar stages; the chronology of assembly factor binding, which is enforced by molecular mimicry and molecular switches to prevent premature folding states or processing steps and enable timely progress of assembly; the irreversibility of key checkpoints, which is dependent on energy consumption and RNA-processing enzymes that can bring about structural changes; and the importance of structural and functional proofreading of functional centres of both ribosomal subunits.

Nucleolar assembly

In almost all eukaryotes, the small-subunit rRNA sequences are located close to the 5′ end and the large-subunit rRNA sequences are located close to the 3′ end of the precursor rRNA. In *S. cerevisiae*, RNA polymerase I (Pol I) transcribes a 35S rRNA precursor, which, in addition to external and internal transcribed spacers — the 5′ external transcribed spacer (5′ ETS), 3′ ETS, internal transcribed spacer 1 (ITS1) and ITS2 — contains the 18S rRNA for the small subunit and the 25S and 5.8S rRNAs for the large subunit (FIG. 1). Pol III separately transcribes the 5S rRNA, which is later integrated into the large subunit.

Co-transcriptional pre-rRNA processing.

Recent structural studies have elucidated how transcription is catalysed by Pol I, which is a 14-subunit complex that transcribes rRNA with the assistance of a dedicated set of initiation factors[9–14]. Although it is known that either Pol I or Pol II can be used to transcribe rRNA[15], Pol I is inactivated through dimerization following glucose depletion, thereby providing a direct link between nutrient availability and rRNA synthesis[16].

Processing of pre-rRNA can occur either co-transcriptionally or post-transcriptionally (reviewed in[17]). Early evidence for post-transcriptional processing was provided by experiments showing that a 35S pre-rRNA species can be synthesized first and that the entire precursor for both ribosomal subunits (then referred to as 37S rRNA) was present in 90S particles[18,19]. Later work showed that co-transcriptional processing of pre-rRNA can be visualized on Miller spreads[20] and that in rapidly growing cells, the majority of processing (~70%) occurs co-transcriptionally[21].

Co-incident with transcription, rRNA undergoes covalent modifications, most of which are clustered in functionally important domains and are thought to fine-tune rRNA structure or function[22]. Different classes of factors are associated with the modification of pre-rRNA. Among the RNA-containing classes, these include snoRNPs, which can catalyse either pseudouridylation (H/ACA snoRNPs) or 2′-*O*-ribose methylation (box C/D snoRNPs) of pre-rRNA. Both H/ACA snoRNPs and box C/D snoRNPs are guided by snoRNAs, which contain sequence elements that base-pair with the target rRNA, and structural motifs for binding dedicated protein cofactors. Box C/D-associated proteins include the methyltransferase Nop1, the Nop56–Nop58 heterodimer and Snu13; H/ACA-associated proteins include the pseudouridine synthase Cbf5 and Gar1, Nop10 and Nhp2 (REFS[23,24]).

Two other snoRNPs that have central roles in eukaryotic ribosome assembly are the U3 snoRNP and RNase MRP (mitochondrial RNA processing). The box C/D family U3 snoRNP is a key structural organizer for the assembly of the small subunit and for co-transcriptional cleavage of the small-subunit pre-rRNA at sites A_0, A_1 and A_2, as discussed below (FIG. 3c,d), but this does not result in 2′-*O*-ribose methylation. By contrast, RNase MRP catalyses the cleavage of site A_3 (REFS[25,26]), a site that is also associated with post-transcriptional cleavage (FIG. 1).

In addition to RNA-mediated RNA modifications, 19 RNA helicases, including DEAD-box and DEAH-box helicases, have been implicated in yeast ribosome assembly. Seven of these

factors (Dbp4, Dbp8, Dhr1, Dhr2, Fal1, Rok1 and Rrp3) are involved in small-subunit assembly, nine helicases (Dbp2, Dbp3, Dbp6, Dbp7, Dbp9, Dbp10, Drs1, Mak5 and Spb4) are involved in large-subunit assembly and three (Has1, Mtr4 and Prp43) have been implicated in the assembly of both subunits (reviewed in[27,28]). Additional energy-consuming enzymes include GTPases such as Bms1, Nog1, Nog2, Nug1, Lsg1 and Efl1; ATPases such as Rio1, Rio2 and Fap7; and AAA-ATPases (Mdn1, Drg1 and Rix7) (reviewed in[29]). The role of these factors is to drive ribosome assembly in a unidirectional manner. Many of the assembly factors have no predicted enzymatic functions but contain RNA-binding motifs and can be seen in the structures to bind to rRNA. Finally, the assembly of a number of ribosomal proteins into ribosome intermediates is aided by dedicated chaperones, which enable their co-translational folding, escort them into the nucleus or help insert them into pre-ribosomal particles undergoing assembly (reviewed in[30,31]).

The connection between the terminal structures observed in Miller spreads and the association of pre-rRNA with small-subunit ribosome assembly factors has long remained unclear. The terminal ball structures present in Miller spreads were originally hypothesized to contain pre-rRNA[32], but later studies identified them as rRNA-processing complexes that contain the 5′ ETS in *Xenopus laevis*[33] and require U3 snoRNA[34]. The identification of the small-subunit processome (SSU processome) as a U3 snoRNA-associated pre-ribosomal particle that contains many ribosome assembly factors provided the first evidence linking the previously observed terminal structures to a defined macromolecular complex[35]. In another study, a similar particle containing small-subunit, but lacking large-subunit, biogenesis factors was described, which was termed the 90S pre-ribosome as it was assumed to contain a 35S pre-rRNA[36]. As all current data suggest that both particles are indeed the SSU processome (as they do not contain the 35S pre-rRNA and lack large-subunit assembly factors)[37–40], we refer to this particle as the SSU processome.

Nucleolar assembly of the small subunit.

As early stages of nucleolar ribosome assembly occur co-transcriptionally, studying the temporal association of many ribosome assembly factors with pre-ribosomal particles has been a major hurdle because this process occurs dynamically at 100–200 ribosomal DNA (rDNA) loci. The use in yeast of pre-rRNA mimics tagged with RNA aptamers has enabled the study of these early steps of ribosome assembly for the small subunit[41,42] and for the large subunit[43] in relation to transcription. Together with the characterization of protein complexes[44–47] and depletion studies[48,49], these data have provided us with the current model for co-transcriptional assembly steps of the small ribosomal subunit (reviewed in[50]) (FIG. 2).

During the formation of the SSU processome, pre-rRNA undergoes chemical modifications and the four domains of the 18S rRNA (the 5′, central, 3′ major and 3′ minor domains) begin to be formed. These subdomains contain independent secondary structure elements, which in the mature small subunit form the 3D structure of the 18S rRNA (Supplementary Figure 2). Importantly, both RNA and protein factors are involved in the reduction of conformational freedom of pre-rRNA. This dual involvement is used to orient the four subdomains in a conformation that precludes their premature folding while allowing each

domain to be assembled separately in an encapsulated environment. This encapsulation is conceptually different from large-subunit maturation (discussed below), which occurs in a modular fashion, where subdomains are bound by more-isolated assembly factors.

The formation of the 5′ ETS pre-ribosomal particle[41] provides the first scaffold on which the 5′ ETS; the protein complexes UtpA, UtpB and Mpp10; the U3 snoRNP (an important RNA chaperone complex); and smaller individual proteins provide an architectural support for the SSU subdomains, which are formed subsequently[41,42] (FIG. 3a,b).

U3 snoRNA has a central role in the formation of the SSU processome, as it base-pairs with and therefore rigidifies regions in both the 5′ ETS (with its 5′ and 3′ hinges) and the pre-18S RNAs (with its Box A and Box A′)[39,50–56] (FIG. 3c,d). The close proximity of these binding sites within the 5′ region of U3 snoRNA provides a crucial spatial constraint that dictates the topology of the maturing particle. Similarly, several of the early multimodal binding proteins (Utp11, Sas10, Mpp10 and Fcf2) confine pre-rRNA domains within the particle by binding to either protein or RNA elements[57,58].

In addition, many factors have transient roles in the biogenesis of early small-subunit particles and may be present co-transcriptionally only before the cleavage at site A_2. These include both small RNAs, such as U14, snR10 and snR30 (REFS[59–61]), and proteins that have been associated with each of the 18S rRNA subdomains[62–64]. In comparison with the more stably associated assembly factors, which form part of the mature SSU processome, the roles of these more transiently bound factors are currently less well understood (FIG. 2).

RNA cleavage events represent irreversible steps that separate pre-rRNAs from each other and from ITSs or ETSs. During co-transcriptional pre-rRNA processing, cleavage occurs at sites A_0, A_1 and A_2, thereby liberating a 20S pre-rRNA precursor[21]; by contrast, during post-transcriptional processing, RNase MRP cleaves at site A_3 to generate a 23S precursor, which is processed further[25,26] (FIG. 1). The function of the 23S rRNA is not fully clear, as it has also been associated with aberrant pre-rRNA processing in response to depletion of small-subunit assembly factors and large-subunit ribosomal proteins[65,66]. Whereas recent evidence suggests that Utp24 is the nuclease responsible for cleavage at sites A_1 and A_2, the nuclease responsible for cleavage at site A_0 remains to be identified[67,68]

Recently, cryo-EM structures of the SSU processome have been determined that illustrate its architecture[37–39] and near-atomic structure[56,57]. The near-atomic structures together with crystallography data[4,69–77] and protein–protein interaction data[78–80] now provide a molecular snapshot of the earliest stable precursor of the small ribosomal subunit. Because highly similar particles were obtained using three different experimental conditions (cells in exponential growth[56], starved cells[57] and Mtr4-depleted cells[39]), it is likely that the SSU processome is an intermediate that forms under physiological conditions.

The yeast SSU processome provides an encapsulated environment for the rRNA subdomains of the small subunit. The base of the particle is formed around the 5′ ETS and the protein complexes UtpA and UtpB, which are evolutionarily related and act as molecular chaperones at the base and side of the particle, respectively (FIG. 3). The top of the particle contains the 5′, central and 3′ domains of the rRNA, each of which is housed in a separate

region of the SSU processome. The interconnectivity of many proteins containing long peptide extensions, such as Mpp10, Utp11 and Sas10 (FIG. 3b), and the base pairing of U3 snoRNA to both the 5′ ETS and 18S rRNA (FIG. 3c,d) provide further structural support for the particle. Ribosome assembly factors that are stably associated with a subdomain of the pre-18S rRNA predominantly act as local stabilizers of RNA elements[57].

Both helical-repeat-containing proteins (Nop14, Noc4, Rrp5, Utp10 and Utp20) and several enzymes, such as the methyltransferase Emg1 (REF.[75]), the acetyltransferase–helicase Kre33 (REF.[81]) and the GTPase Bms1 (REFS[82,83]), are located in the outer regions of the SSU processome. Whereas the roles of the helical-repeat-containing proteins are clearly structural, the temporal order in which the enzymes act on the encapsulated pre-18S rRNA remains to be determined.

A key structural role of the SSU processome is chaperoning the assembly of each of the subdomains of the 18S rRNA while preventing the premature formation of the central pseudoknot[57]. However, in all currently available structures, the SSU processome contains a highly intertwined pre-18S rRNA, which needs to be released from the SSU processome for further maturation (FIG. 2). To achieve this, RNA helicases such as Dhr1, which has a role in U3 snoRNA unwinding[84,85], are necessary to liberate the 18S precursor from U3 snoRNA. Similarly, cleavage at sites A_1 and A_2 by Utp24 is necessary but not possible in the available structures because the active site of Utp24 is inaccessible[57].

Lastly, the processing of the SSU processome, in particular the removal of the 5′ ETS, requires the exosome and exosome-interacting proteins such as Utp18, Sas10 and Lcp5 (REFS[57,86,87]).

Assembly of the large ribosomal subunit

The structure of the large subunit is more elaborate than that of the small subunit; consequently, its assembly follows a more complex pathway. The 25S rRNA in the large subunit consists of six conserved domains of secondary and tertiary structure, namely (5′ to 3′) domains I–VI, which are more intertwined with each other than are the 18S rRNA domains in the small subunit (Supplementary Figure 2). The solvent-exposed surface of the large sub unit includes domains I and II and the 5.8S rRNA, whereas the subunit interface contains functional centres that include domains IV and V. Domains III and IV bridge one edge of the solvent-exposed surface and subunit interfaces; domain III also links RNA domains at the bottom of the subunit. The 5.8S rRNA lies between domains I and III, and 5S rRNA is docked on top of domains II and V (Supplementary Figure 2).

Because we lack cryo-EM structures for the earliest stages of 60S subunit assembly in the nucleolus, our understanding is so far based only on biochemical and genetic studies of pre-rRNA processing and of the assembly factors that participate in initial packaging of pre-60S ribosomal ribonucleoproteins (rRNPs). By contrast, cryo-EM snapshots of a number of assembly intermediates following the earliest nucleolar stages of assembly support more detailed models of the later stages of large-subunit biogenesis (FIGS 4, 5). The assembly of the large subunit begins co-transcriptionally, with the covalent modification of pre-rRNA,

which is guided by snoRNPs. At the same time, folding of the long, flexible pre-rRNA into more compact and stable conformations and cleavage and processing of the ITS1 and 3′ ETS (FIG. 1) occur. The solvent-exposed surface of the large subunit is formed first by folding of the 5.8S rRNA with 25S domains I and II (seen in state 1 or state A (state 1/A)) and then with domain VI (state 2/B) into stable structures (FIG. 5a,d). The intervening domains III, IV and V are initially flexible and bound by assembly factors. As the 25S rRNA domains III, IV and V become structurally visible and join the already folded domains I, II and VI (FIG. 5b,e), initial stages of construction of the functional centres of the large subunit (the PTC and PET) become evident (state E). During the following transition from the nucleolus to the nucleoplasm (FIGS 4; 5c,f), numerous protein exchanges enable other major remodelling events, leading to export from the nucleus. Then, in the cytoplasm, pre-60S ribosomes undergo final stages of maturation, including the removal of remaining assembly factors, assembly of the last few ribosomal proteins and test-driving of functional centres.

Nucleolar stages of large-subunit assembly.

Among the assembly factors that participate in the earliest co-transcriptional stages of large-subunit assembly are several proteins that contain multiple RNA-binding domains or α-helical repeats, such as Rrp5, Mak21, Noc2 and Nop4. These proteins might enable rRNA compaction by forming rigid scaffolds to direct and stabilize RNA folding[88–95]. In Miller spreads, the terminal ball structures corresponding to pre-ribosomal particles[34] have a more loosened structure in mutants lacking these assembly factors[92]. The early assembly factors Npa1, Npa2, Rsa3 and Nop8 and the RNA helicase Dbp6 form a stable complex that also may serve a structural function[96–98]. Six other RNA helicases (Dbp2, Dbp3, Dbp7, Dbp9, Mak5 and Prp43) are also required for these very early assembly stages, most likely to remodel RNA or RNPs (reviewed in[28,60,99]). However, the binding sites and precise targets of these enzymes have not yet been defined.

Interestingly, cleavage at both the A_2 and A_3 sites in the ITS1 is linked to transcription or processing of sequences several kilobases away from those sites. Co-transcriptional cleavage at the A_2 site occurs only once Pol I reaches ~1.2–1.5 kb downstream of it, in the domain I and domain II portion of 25S rDNA sequences[21,100], and cleavage at the A_3 site (near what will become the 5′ end of 27SB pre-rRNA) occurs only upon termination of transcription and processing of the 3′ ETS several kilobases downstream[101]. Exactly how these cleavages are coupled to downstream events is unclear, but large-scale as well as local folding of RNA mediated by protein binding might be necessary to create proper substrates for cleavage. For example, binding of Rrp5 near pre-rRNA processing sites in both the SSU processome (the A_2 site)[41,42,57] and pre-60S particles (the A_3 site) may regulate the timing of cleavage at these sites and coordinate assembly of both subunits[88,91,92,102]. The amino-terminal domain half of Rrp5, which contains multiple RNA-binding motifs that bind close to the A_3 cleavage site in ITS1, is necessary for A_3 cleavage. The carboxy-terminal half of Rrp5, which contains additional RNA-binding motifs, crosslinks near the A_2 cleavage site in ITS1 and is necessary for A_2 cleavage. Thus, proper folding of ITS1 sequences as well as domain I and domain II bound to the amino-terminal domain of Rrp5 may be necessary to signal successful initiation of assembly of the large subunit before the SSU processome can be

separated from pre-60S particles by cleavage at the A_2 site. Likewise, 3D proximity of the 5′ end and 3′ end of the 27SB pre-rRNA suggests that their processing may be coordinated, perhaps through protein–protein or protein–RNA interactions of Rrp5 and ribosomal protein L3, which bind to these termini[66,92].

Cryo-EM structures[103–105] begin to reveal how the rRNA domains of early nucleolar pre-60S subunits are held together: the assembly factors and ribosomal proteins form bridges within and between each rRNA domain. For example, in state 2/B particles, three assembly factors (Nop15, Cic1 and Rlp7) bind to the ITS2 and five others (Nop7, the Erb1–Ytm1 complex, the RNA helicase Has1 and Nop16) assemble with domain I surrounding ITS2 (FIG. 5a,d). These eight factors, together with several ribosomal proteins bound nearby, may help stabilize the very early folding achieved by base pairing of 5.8S rRNA with sequences in domain I (Supplementary Figure 2). Nine other assembly factors (Mak16, Rpf1, Nsa1, Rrp1, Ssf1 in complex with Rrp15, Rrp14 and Ebp2 in complex with Brx1) form a ring around the centre of particles in state 2/B to bridge domains I, II and VI to each other[103] (FIG. 5a). Four other assembly factors (the GTPase Nog1, eIF6, Rlp24 and Mak11) bind to domain VI near the interface with domain V (FIG. 5a).

Although most ribosomal proteins in rRNA domain I and domain II are already in their mature conformation in nucleolar particles, the assembly of these domains is finished only once the remaining assembly factors bound to them are released from pre-ribosomal particles. For example, assembly factors such as Ebp2–Brx1 are situated to prevent the premature formation of RNA-RNA interactions between domains I and V (FIG. 5a) and must be removed to enable downstream maturation steps[103]. This transition nicely illustrates an important principle of ribosome assembly — the exit of assembly factors from pre-ribosomal particles is as important as their entry or their presence.

In the nucleolus, functional centres of the large subunit (the GAC, PTC and PET) begin to emerge during the transition from state 2/B particles to state C, D and E particles[104] (FIG. 5a,b,d,e). For example, assembly factors Nop2, Nip7, Noc3 and the methyltransferase Spb1 bind to rRNA domains IV and V to aid in the formation of the PTC[104] (FIG. 5b). Assembly of the rim around the exit of the PET is completed by release of assembly factors Ssf1–Rrp15 and Rrp14 and by stable association of ribosomal proteins L19, L25 and L31 (FIG. 4). Ssf1 is located in the same position in early nucleolar intermediates (FIG. 5a) as that of L31 in mature ribosomes[103]. Thus, removal of Ssf1 along with Rrp15 and Rrp14 might allow the subsequent association of L31, L19 and L25 to complete the PET rim. However, it is not known what drives the release of these assembly factors to enable this molecular switch.

During the transition from state D to state E, formation of the vestibule — the outer, wider portion of the PET — is enabled by exit of the assembly factor Rpf1. The amino-terminal portion of Rpf1 lies in what will become the vestibule, which is formed once Rpf1 departs[103–105]. Rpf1 disassembles from early pre-rRNPs together with the adjacent proteins Mak16, Rrp1 and Nsa1 (FIG. 4). Most likely, release of these assembly factors is enabled by the direct removal of Nsa1 by the AAA-ATPase Rix7 (REF.[106]).

Considerable remodelling upon transit of pre-60S ribosomes from the nucleolus to the nucleoplasm.

At least 9 assembly factors exit and 11 factors enter pre-60S particles during their transition from the nucleolus to the nucleus. This is evident in the differences between state E particles and Nog2 or Rix1–Mdn1 particles[104,107–109] (FIGS 4; 5b,c,e,f; 6c–e). These and the above-described protein exchanges trigger numerous downstream events, including further construction of functional centres such as the PTC and PET as well as the stable docking of the previously flexible central protuberance and cleavage of the ITS2 to initiate its removal.

The PET undergoes additional steps of construction during the late nucleolar stage of large-subunit assembly, as Ebp2, Brx1, Noc3, Spb1, Nop2 and Nip7 exit and Nog2 and Rsa4 bind to domain V (FIGS 5b,c; 6c,d). Comparison of the nucleolar state E particles and Nog2 reveals that in state E particles, assembly factors Spb1, Ebp2 and Noc3 block the formation of the mature structure of rRNA helices in domains II and V, which form a portion of the RNA walls of the mature PET. Thus, release of these factors during the transition from nucleolar particles in state E to Nog2 particles may enable the formation of a functional PET. The surroundings of Nog1 are also subjected to major remodelling during the transition from nucleolar pre-60S particles (FIG. 6c) through the late nucleolar Nog2 particle (FIG. 6d) to the nuclear Rix1–Mdn1-containing pre-60S particle (FIG. 6e). Especially noteworthy is the insertion of the carboxy-terminal extension of Nog1 into the PET, almost back through to the PTC[108]. This observation may enable continued assembly of the tunnel, as during this interval, helices that form the walls of the tunnel transition from an immature unfolded state to the mature conformation. Alternatively, or in addition, the Nog1 extension might control proper assembly of the tunnel or prevent entry of other molecules.

The central protuberance, atop the mature 60S subunit, includes the 5S RNP (ribosomal proteins L5 and L11 bound to 5S rRNA) and domain V helices 80 and 82–88 (REFS[3,4]). Because 5S rRNA is transcribed from different genes from the other three rRNAs, it must separately assemble into pre-ribosomes. The dedicated chaperone Syo1 facilitates assembly of 5S rRNA with L5 and L11 in the cytoplasm and nucleoplasm, and the assembly factors Rpf2 and Rrs1 are necessary for association of this 5S rRNP with pre-ribosomal particles[110–112]. Although the 5S RNP associates with the earliest pre-60S particles, presumably bound to central protuberance helices, it is not visible by cryo-EM until Nog2 particles are formed, most likely owing to the flexibility of the undocked central protuberance in the earlier assembly intermediates[108]. Because the binding sites of Nip7 and Nop2 in early nucleolar particles are quite near or overlap those for Rpf2 and Rrs1 in later Nog2 particles[104], the transition of the 5S rRNP to a more stable conformation in pre-ribosomes could depend in part on the displacement of the Nop2–Nip7 complex from pre-ribosomal particles before formation of Nog2 particles (FIGS 5b,c; 6c,d). Rpf2, Rrs1, L5 and L11 are also required for assembly of Nog2 with domain V of rRNA[113], suggesting that stable docking of the central protuberance may influence further construction of the PTC in domain V.

Cleavage of the ITS2 is coupled with formation of the PET and the PTC and with docking of the central protuberance. The primary trigger for this cleavage seems to be the release of nearby assembly factors[109,114–118]. The AAA-ATPase Mdn1 binds to the assembly factor

Ytm1 and releases Ytm1 and the associated protein Erb1, the amino-terminal extension of which winds around pre-25S rRNA domain I proximally to ITS2 (REFS[103,104]). Assembly factors that interact with this long extension of Erb1 also exit at this time, and their exit presumably is coupled to the release of Erb1. Although we do not know how the endonuclease Las1 is specifically targeted to pre-ribosomal particles to cleave ITS2, mutations in ribosomal proteins or assembly factors (including Mdn1) that prevent release of these proteins, which are proximal to ITS2, also block its cleavage by Las1. This observation suggests that remodelling of rRNA domain I near ITS2 in pre-ribosomal particles is necessary to create a proper substrate for Las1 (REFS[109,113,114,118,119]).

Compared with Las1, it is clearer how the exosome is recruited to pre-ribosomal particles to process ITS2 once it is cleaved[120]. A mechanism that combines a molecular switch and molecular mimicry is employed, enabled by the remodelling of the domain around ITS2. Mtr4, which is the helicase component of the exosome, binds to the adaptor protein Nop53 in pre-ribosomal particles[86,121]. Because both Erb1 and Nop53 use the same motifs to bind to assembly factor Nop7 in pre-ribosomal particles[86,103,121], release of Erb1 is necessary for binding of Nop53 and thus docking of the exosome.

Interestingly, although cleavage of the C_2 site in ITS2 is linked to earlier remodelling events, failure to cleave or process ITS2 does not appear to affect downstream steps of 60S subunit assembly until late cytoplasmic stages[109,122,123]. Following depletion or inactivation of Las1 or Mtr4, the ITS2 is not removed. However, subunit maturation continues, although the resulting ribosomes retain the assembly factors Nop15, Cic1 and Rlp7 bound to ITS2. The ITS2-containing 60S subunits somehow bypass nuclear surveillance mechanisms and are exported to the cytoplasm but are defective in translation and are targeted for turnover by the ribosome quality control complex and the cytoplasmic exosome[123].

Nucleoplasmic stages of large-subunit assembly.

On reaching the nucleoplasm, pre-60S particles undergo additional remodelling steps as they become competent for export to the cytoplasm. These include removal of ITS2 and restructuring of the central protuberance as well as structural proofreading of functional centres.

The ITS2 is removed sequentially by three nuclease complexes: the Las1–Grc3–Rat1–Rai1 complex, the exosome and Ngl2–Rex1–3 (REFS[17,86,121,124–130]). The endonuclease Las1 cleaves the C_2 site in ITS2 to generate the 7S and 25.5S pre-rRNAs (FIG. 1). Trimming of the 25.5S pre-rRNA to 25S rRNA is enabled by the kinase Grc3 and is carried out by the nucleases Rat1 and Rai1. The 14-subunit exosome[120] removes from the 3′ end of 7S pre-rRNA all but the most 5′ 30 nucleotides of the ITS2 and then in a second step all but the last 8 nucleotides to form the 6S pre-rRNA[130]. In the cytoplasm, Ngl2 and Rex1–3 catalyse processing of the 6S precursor to mature 5.8S rRNA[131].

Formation of the mature central protuberance structure requires a large-scale structural rearrangement, which serves as a checkpoint in the nucleoplasm before nuclear export. The 5S RNP initially docks ‘backwards’ onto the top of pre-60S particles, then rotates ~180° to assume its mature configuration. At the same time, the accompanying central protuberance

helices 80 and 82–88 also undergo a large-scale structural rearrangement. These remodelling events involve alterations of numerous protein–RNA contacts, yet exactly what empowers them remains unclear[108,132]. Immediately before rotation of the central protuberance, Rpf2 and Rrs1 are released. These two assembly factors link the 5S RNP to the central protuberance helices in rRNA domain V at the top of the pre-60S ribosome[108,133–135]. Thus, their exit might trigger destabilization of the pre-rotated state. Release of Rpf2 and Rrs1 may be induced by binding of the assembly factor Sda1 with pre-ribosomal particles and by its competition with Rpf2 for partially overlapping binding sites (FIG. 6d,e). Following rotation, the Rix1–Ipi1–Ipi3 complex and the AAA-ATPase Mdn1 establish multiple contacts with the pre-ribosomal particle to stabilize the rotated state[119] (FIG. 6e). ATP hydrolysis by Mdn1 activates the Nog2 GTPase, resulting in its release as well as the release of Sda1, Rix1–Ipi1–Ipi3, Mdn1 and Rsa4 (FIG. 4).

To minimize production of dysfunctional ribosomes, yeast employs structural proofreading of ribosome functional centres during nuclear steps of assembly, which is coupled with export from the nucleus (reviewed in[136]). The export factor Arx1 and its partner Alb1 assemble onto the rim around the PET by binding to ribosomal proteins L19, L25, L26 and L35 (REFS[107,137]). Thus, before export of pre-ribosomal particles, Arx1 binding may serve to proofread the proper accommodation of these ribosomal proteins into the tunnel exit, where a number of factors chaperone and target emerging nascent polypeptides during protein synthesis[138,139]. The GTPase Nog2 (REF.[140]) and the adjacent GTPases Nog1 and Nug1 and RNA helicase Dbp10 (REFS[108,141]) may influence or scrutinize the proper formation of the PTC: following release of Nog2, this RNA neighbourhood looks much more like the mature PTC[142]. The departure of Nog2 enables assembly of the essential export factor Nmd3, the binding site of which stretches from the L1 stalk through the E site and P site towards the sarcin–ricin loop[140,142,143]. Thus, the formation of an almost-mature PTC may signify export competence of pre-ribosomal particles. Maturation of the P0 stalk is also coupled with nuclear export: the assembly factor Yvh1 enables release of assembly factor Mrt4 from the premature stalk to enable binding of the export factors Mex67 and Mtr2 (REFS[144,145]).

Cytoplasmic stages of large-subunit assembly.

The final stages of large-subunit maturation occur in the cytoplasm, where remaining assembly factors are dissociated from pre-ribosomal particles and recycled back to the nucleus, and the last nine ribosomal proteins are incorporated (reviewed in[136,146]). The release of each of these proteins is driven by a GTPase or ATPase and is coupled with upstream and downstream steps in a hierarchical pathway[147]. Importantly, each of the functional centres of the large subunit is associated with assembly factors until these last stages, and each functional centre undergoes functional proofreading.

The assembly factor Rlp24 recruits and activates the AAA-ATPase Drg1, which catalyses the replacement of Rlp24 with its homologous ribosomal protein L24 (REFS[147–149]). Because L24 lacks the motif in Rlp24 that is recognized by Drg1, this replacement is a unidirectional molecular switch. Inhibition of Drg1 with diazaborine blocks Rlp24 release in

the cytoplasm but also (directly or indirectly) release of the Rlp24-adjacent assembly factors Nog1, Nug1 and Nsa2 (REFS[148,150]).

The assembly factors Rei1 and Jjj1 and the Hsp70 (chaperone)-like ATPase Ssa are required for the release of Arx1 and Alb1 from the rim around the exit of the PET[151–154]. Rei1 binds adjacently to Arx1 and inserts its carboxy-terminal domain into the PET, extending almost back to the PTC[137,154]. Upon removal of Rei1 from the PET, it is replaced by Reh1. How release of Arx1 and Rei1 is coupled with the subsequent exit of the assembly factors eIF6 and Nmd3 is not known but might involve communication with the PTC through the 'tail' of Rei1 located inside the PET.

Nmd3 and eIF6 are released in the final steps of large-subunit biogenesis, perhaps in a concerted fashion mediated by a complicated dance of assembly factors[143,147,155,156]. These remodellers include the GTPases Lsg1 and Efl1, the ribosome recycling factor-like protein Sdo1 and the ribosomal protein L10. Nmd3 helps L10 assembly, while the assembly of Efl1 onto the GAC, which is accompanied by a rotation within Sdo1, enables eIF6 release by Efl1. The P loop domain of L10 is also required for eIF6 release by Efl1. Lsg1 is required for the exit of Nmd3.

Thus, the structure and function of the A site, P site, E site and the GAC appear to be proofread by Nmd3, Sdo1, L10 and Efl1 to enable late steps of assembly. Meanwhile, the PET may undergo successive proofreading by Nog1, Rei1 and Reh1 throughout assembly. Successful completion of these cytoplasmic steps releases assembly factors bound to the functional centres of 60S subunits, enables assembly of ribosomal proteins and licenses each domain to participate in protein synthesis.

SSU assembly: nucleocytoplasmic stages

The 3′ domain of 18S rRNA, which later forms the head of the mature small subunit, is subject to major remodelling steps between the nucleolar SSU processome and the cytoplasmic pre-40S particle[57,157,158] (FIG. 6a,b). Whereas factors such as Enp1, Pno1 and Rrp12 remain associated with the 3′ domain, all other SSU processome factors are separated from the pre-40S subunit by a mechanism that is still poorly understood. Nuclear export of small-subunit precursors occurs in a RanGTP-dependent and Crm1-dependent manner (reviewed in[31]). In addition, nuclear pre-40S particles require Rrp12 for efficient export into the cytoplasm[159], and Rrp12 is absent from the cytoplasmic yeast intermediates that have recently been determined[157,158,160].

During the transition from the nucleolus to the cytoplasm, pre-40S ribosomes also acquire a series of distinct nucleocytoplasmic assembly factors, including Tsr1, Rio2, Dim1 and Ltv1. Ltv1 replaces a peptide of Nop14 on the surface of Enp1 (REFS[39,57]), and the catalytically inactive Tsr1 (REF.[161]) replaces the structurally related GTPase Bms1 (REFS[82,83]) near the 5′ domain of the pre-40S particle (FIG. 2). Another important step is the formation of the beak structure of the small subunit, which in the mature structure is formed by rpS31, rpS12, rpS10, rpS3 and rpS20. In late pre-40S particles, Enp1 and the associated Ltv1 peptide block the access of rpS10; likewise, only the amino-terminal domain of rpS3 is incorporated into

these structures. Pno1 not only binds to the 3′ end of the 20S pre-rRNA to assist in the cleavage at the D site by the flexibly attached Nob1 but also prevents the premature association of rpS26, which is a protein that is integrated with the help of its dedicated chaperone Tsr2 (REFS[157,158,162]).

Very late steps of pre-40S assembly include test-driving the function of the subunit. Here, the dissociation of factors such as Tsr1 and Rio2 is thought to precede the association of Rio1 with the pre-40S particle[163,164]. Together with a mature 60S subunit, eukaryotic translation initiation factor 5B and the ATPase Fap7 promote the formation of an 80S-like particle, which is used to functionally proofread the pre-40S particle, activate Nob1 for cleavage at the D site and remove late assembly factors such as Nob1 and Pno1 (REFS[165–167]) (FIG. 2).

Ribosome assembly and human diseases

In comparison with ribosome assembly in yeast, much less is known about how ribosomes are assembled in mammalian cells[168]. A wide range of assembly factors involved in the synthesis of the human ribosome have been identified by large-scale screens[169–173], which have identified many homologues to proteins that were previously studied in yeast as well as new potential ribosome assembly factors.

Defects in ribosome assembly in humans have been associated with cancer[174] and with ribosomopathies, which comprise a heterogeneous set of diseases in which mutations of ribosomal proteins or assembly factors cause a variety of phenotypes[175,176]. Ribosomopathies include Diamond–Blackfan anaemia (DBA)[177,178], isolated congenital asplenia[179], North American Indian child cirrhosis[180], chromosome 5q$^-$ syndrome[181], Treacher Collins syndrome[182] and Shwachman–Bodian–Diamond syndrome[176,183], among others. Despite the differences in their phenotypes, the biological underpinnings of these diseases can be subdivided broadly into two groups. The first is the triggering of a p53-dependent stress response and the second is the tissue-specific effect that has been observed in bone-marrow-derived cell lineages or skeletal tissues[184].

The activation of p53 due to defective ribosome assembly occurs through a complex containing the ribosomal 5S RNP[185–188]. Importantly, the p53 response is a key cause of the phenotype of Treacher Collins syndrome[189], chromosome 5q$^-$ syndrome[190] and North American Indian child cirrhosis[191].

Mutations in ribosomal proteins cause their haploinsufficiency. In human cell lines, the depletion of ribosomal proteins can result in the accumulation of distinct ribosomal precursors[192]. Although the largest number of mutations in DBA has been associated with ribosomal proteins[193], additional mutations were found in the rpS26 chaperone Tsr2 (REF. [194]).

A more puzzling observation is the tissue-specific phenotype of DBA and other ribosomopathies. Early hypotheses included the presence of specialized ribosomes in these tissues, which would translate specific mRNAs[195]; a more recent model proposed that limiting the number of ribosomes may affect particular sets of mRNAs, which are required

for differentiation of particular tissues[184]. Key pieces of evidence for this model include the identification of mutations in the haematopoiesis-specific transcription factor GATA1 in individuals with DBA and the demonstration that expression of GATA1 can at least partially compensate for haploinsufficiency of rpS19 (REFS[196,197]). More recent data show that mutations of ribosomal proteins reduce the pool of available ribosomes in DBA, which specifically affects the translation of a pool of transcripts that are necessary for erythroid lineage commitment[198]. We believe that this may be a general mechanism, applicable to other ribosomopathies such as isolated congenital asplenia[179], in which the spleen does not form as a result of rpSA haploinsufficiency.

Conclusions and future perspective

Eukaryotic ribosome assembly is a highly complex and tightly regulated process, the basic principles of which we are beginning to understand. Recurring themes in ribosome assembly include the systematic compaction of pre-rRNA; an enforced chronology of assembly factor binding, which is mediated by molecular mimicry and steric hindrance; and the irreversibility of key checkpoints and proofreading of functional centres. Recent advances in cryo-EM have provided detailed molecular snapshots of pre-ribosomal particles at near-atomic resolution[56,57,103–105,108,142,157,158]. The combination of cryo-EM with crosslinking and mass spectrometry[57,103,199] has enabled many protein factors to be assigned. Current models of ribosome assembly are attempts to rationalize structural, biochemical and genetic data but will require regular revision in the near future as further data become available.

The dynamic and flexible nature of early ribosome assembly events together with the poorly understood roles of DEAD-box and DEAH-box helicases, GTPases, AAA-ATPases and other ATPases highlight areas in which work and the implementation of new technologies will be essential. The use of small-molecule inhibitors has already facilitated the study of key enzymatic steps in eukaryotic ribosome assembly[200–202], and the identification and development of more inhibitors will be essential to ‘trap’ and study particular particles.

Beyond the assembly mechanism, the interplay between ribosome assembly, nutrient availability and cell proliferation is only beginning to emerge[203–206]. Similarly, fairly little is known about the interdependence of ribosome assembly and nucleolar morphology[207]. We anticipate that with more molecular snapshots of pre-ribosomal particles in combination with cell biology, super-resolution microscopy and cryo-electron tomography, the regulation and fine-tuning of ribosome assembly will come further into focus.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Ribosomal proteins

Seventy-nine protein components of mature ribosomal subunits in yeast. Most are essential for assembly of their respective subunits.

Decoding site

The functional centre of the small subunit, where the anticodon of an amino-acyl tRNA undergoes base pairing with the respective codon in the mRNA.

Peptidyl transferase centre (PTC).

The active site of the large subunit, located in its interface, where ribosomal RNA catalyses the formation of peptide bonds and the hydrolysis of peptidyl-tRNA bonds.

Central pseudoknot

An architectural motif within the small ribosomal subunit ribosomal RNA, at the interface of the four subdomains of the small ribosomal subunit.

Central protuberance

A module that includes the 5S ribonucleoprotein and helices 80 and 82–88 of 25S ribosomal RNA, located at the top of the mature large ribosomal subunit.

GTPase activating centre

(GAC). Within mature, large ribosomal subunits, the site that binds to and activates GTPases that participate in translation initiation and elongation.

P0 stalk

A complex of three ribosomal proteins bound to helices 43 and 44 in 25S ribosomal RNA, which forms a stalk structure in mature large ribosomal subunits, to which translation factors bind.

Polypeptide exit tunnel

(PET). A tunnel through which nascent polypeptides traverse the large ribosomal subunit. It comprises mostly ribosomal RNA and extends from the peptidyl transferase centre to the solvent side of the large subunit.

Small nucleolar ribonucleoproteins

(snoRNPs). Complexes of small nucleolar RNAs (snoRNAs) and proteins. Base pairing of specific snoRNAs with target sequences in ribosomal RNA (rRNA) directs either methylation or pseudouridylation of the rRNA.

Assembly factors

Proteins or protein complexes with roles in ribosome assembly. Most are present in ribosome assembly intermediates but none are components of mature ribosomes.

Molecular mimicry

Refers to the activity of proteins for which the structure mimics that of other proteins (or of RNA). In ribosome assembly, molecular mimicry is used to block distinct sites by steric hindrance and prevent early binding of a structurally related protein.

Molecular switches

Refers to factors that can switch between two states (for example, an assembly factor present or absent in a pre-ribosome) in response to internal hardwiring or an external cue.

External and internal transcribed spacers

RNA sequences in the primary ribosomal RNA (rRNA) transcript, which are removed during ribosome biogenesis by a series of endonucleolytic or exonucleolytic reactions. They are thought to aid in proper folding of nascent rRNA.

90S particles

The very early pre-ribosomal particles containing the 35S pre-ribosomal RNA, which includes transcripts for both small and large ribosomal subunits.

Miller spreads

Visualization of actively transcribed chromatin by electron microscopy of fixed, gently lysed nuclei, first used to look at transcription of ribosomal DNA repeats in *Xenopus laevis*.

AAA-ATPases

(ATPases associated with diverse cellular activities). There are three different AAA-ATPases that function as ribosome assembly factors to remove other assembly factors from pre-ribosomes.

Aptamers

RNA oligonucleotides that are able to adopt a distinct shape and bind to a specific target with high specificity and affinity.

Multimodal binding

Binding of a single protein to multiple ligands through separate binding sites in the protein.

Exosome

A protein complex with 3′ to 5′ exonuclease activity, which processes cleaved pre-ribosomal RNA spacer sequences as well as other RNA species.

Subunit interface

The surfaces of the small and large ribosomal subunits that face each other in functioning ribosomes.

Ribosome quality control complex

A complex that extracts nascent polypeptides from ribosomes that have stalled in translation.

E site

The specific binding site in ribosomes from which the deacylated (empty) tRNA exits from the ribosomes.

P site

The specific binding site in ribosomes for peptidyl-tRNA.

A site

The specific binding site in ribosomes for acceptor aminoacylated tRNA.

Beak structure

A structure in the small ribosomal subunit consisting of a protrusion of helix 33 of the 18S ribosomal bound by ribosomal proteins.

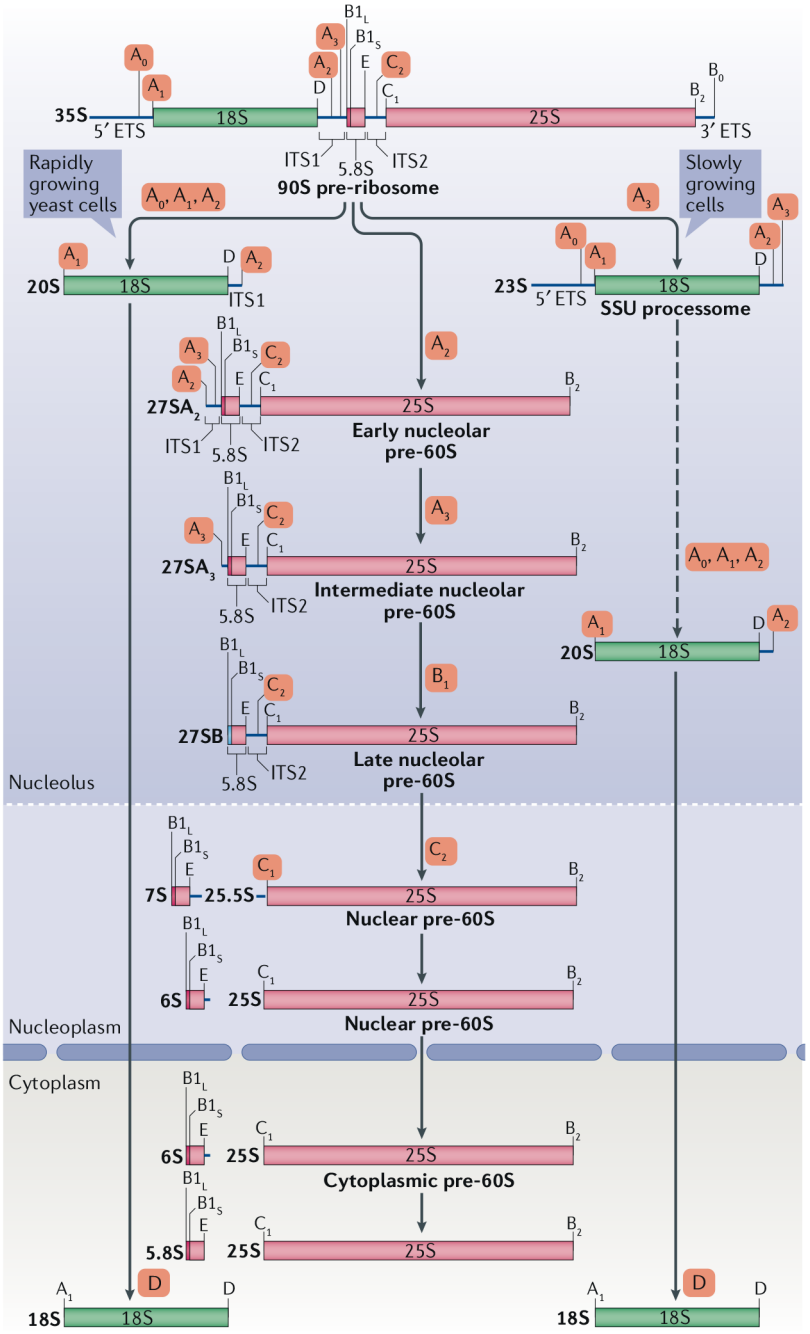

Fig. 1 |. Pre-rRNA processing in yeast.

Consecutive pre-ribosomal RNA (pre-rRNA) processing stages produce rRNA intermediates through endonucleolytic and exonucleolytic removal of internal transcribed spacers (ITSs) and external transcribed spacers (ETSs). Precursors of mature rRNAs destined for the small ribosomal subunit are shown in green and those for the large ribosomal subunit in pink. Processing and assembly begin in the nucleolus, continue in the nucleoplasm and are completed in the cytoplasm. Sites in pre-rRNAs at which endonucleases cleave (A_0, A_1, A_2, A_3 and C_2) or exonucleases halt (B_1) are highlighted in red at the step at which they occur. Each precursor RNA is designated by its size (assayed by velocity sedimentation on sucrose

gradients) and by the site processed to generate that intermediate (for example, $27SA_2$). Each of these processing steps occurs within the indicated pre-ribosomal ribonucleoprotein particle. In rapidly growing yeast cells, processing and assembly occur mostly co-transcriptionally, with cleavage at the A_0, A_1 and A_2 sites occurring in nascent pre-rRNAs, indicated by production of the 20S pre-rRNA (shown on the left). By contrast, in slowly growing yeast cells, processing occurs post-transcriptionally, with processing of the full-length 35S pre-rRNA (top) by cleavage at the A_3 site to generate the 23S pre-rRNA and then at the A_0 A_1 and A_2 sites (dashed arrow on the right). SSU processome, small-subunit processome.

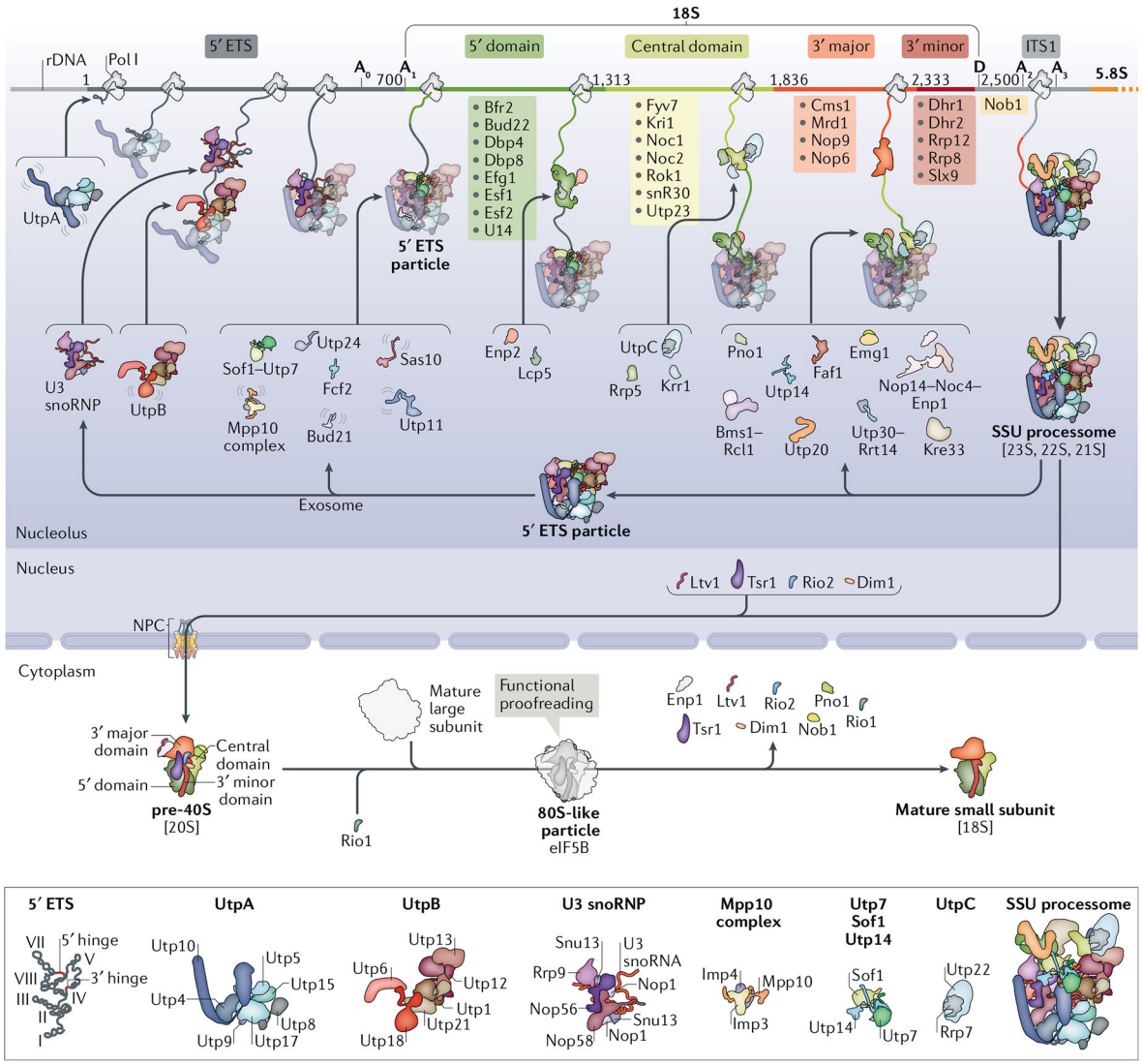

Fig. 2 |. Assembly of the small ribosomal subunit.

Consecutive stages in the maturation of the small ribosomal subunit (40S) are shown, beginning with the earliest co-transcriptional steps in the nucleolus, the formation of the small-subunit (SSU) processome, nuclear export and final assembly in the cytoplasm. Small-subunit-specific portions of ribosomal DNA (rDNA) are depicted with colour-coding of the 5′ external transcribed spacer (5′ ETS); the 5′, central, ′ major and 3′ minor domains of the 18S ribosomal RNA (rRNA); and the internal transcribed spacer 1 (ITS1). The pre-rRNA cleavage sites A_0, A_1, D and A_2 are indicated. Three ribosome particle intermediates are shown: the 5′ ETS particle, SSU processome and the pre-40S particle. Pre-rRNA intermediates present in each particle are indicated in square parentheses beneath each particle. There are likely additional assembly intermediates not yet identified. Sequential association and dissociation of assembly factors and complexes of assembly factors are shown. Assembly factors and complexes for which structures have been determined are depicted in cartoon form, whereas those for which no structures are known are indicated only with text. The 5′ ETS particle was inferred from purification of complexes assembled

on 3′ truncated pre-rRNAs. The earliest assembly intermediate for which cryo-electron microscopy structures were obtained is the SSU processome. Endonucleolytic cleavage at the A_0, A_1 and A_2 sites and major structural remodelling (not shown) result in the release of assembly factors and the 5′ ETS particle from the SSU processome. The resulting pre-40S particle assembles in the nucleus with a set of export factors and is rapidly exported to the cytoplasm, where the pre-40S particles engage in functional proofreading by joining with mature 60S subunits. The last assembly factors are released and the D site is cleaved to generate mature subunits containing 18S rRNA. Proteins that joined the growing SSU processome at an earlier stage are shown as transparent to highlight new components (not transparent). The ‘wiggling’ signs highlight components that are flexible in isolation. NPC, nuclear pore complex; Pol I, RNA polymerase I; snoRNP, small nucleolar ribonucleoprotein. Adapted with permission from REF.[50], Elsevier.

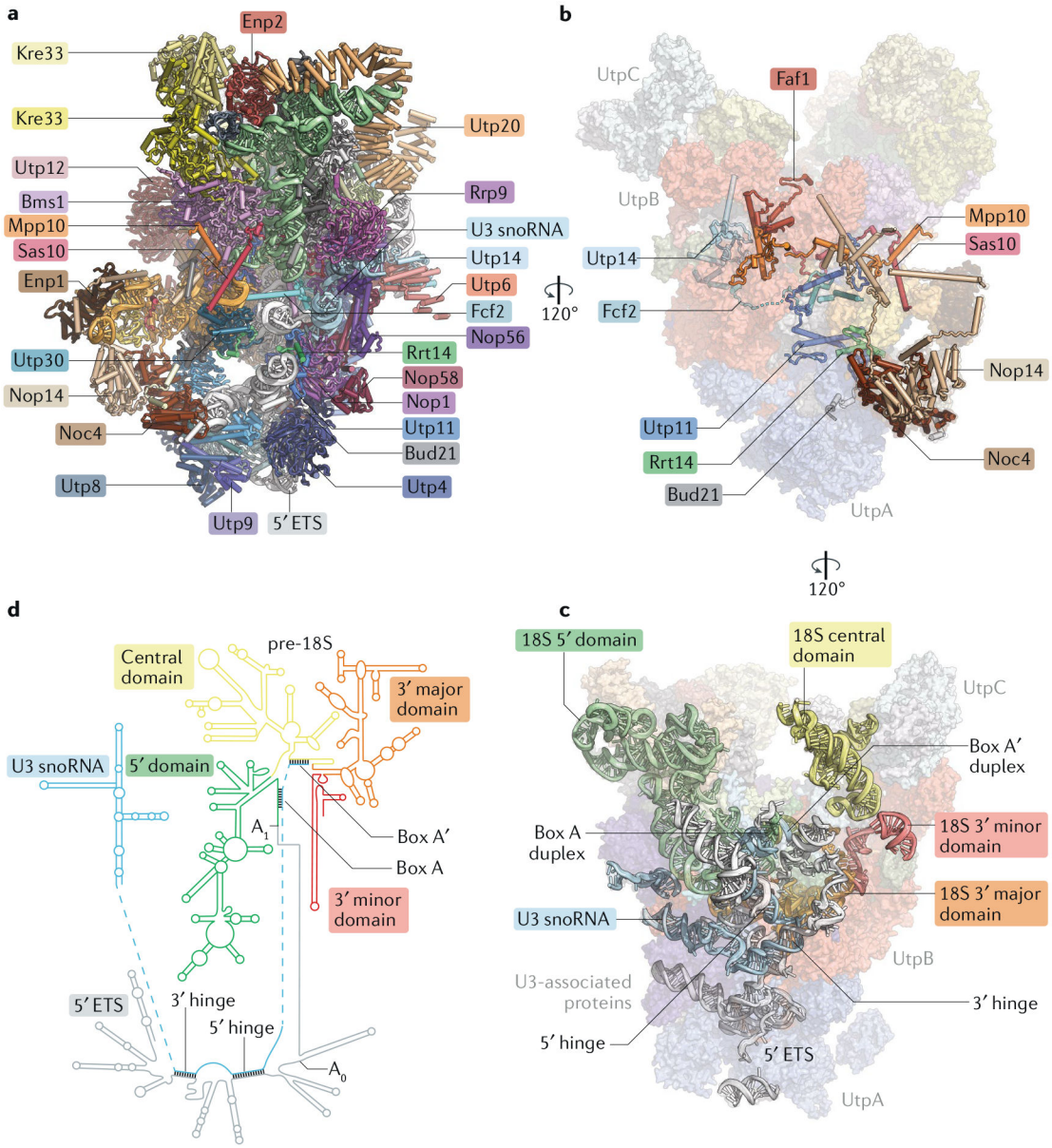

Fig. 3 |. Cryo-electron microscopy structure of a nucleolar small-subunit precursor.

a–c | Three views of the small-subunit processome (PDB ID: 5WLC), highlighting the overall architecture (part **a**), organization of long peptide extensions that bridge distant regions in the particle (part **b**) and the interaction between pre-18S ribosomal RNA (rRNA), 5′ external transcribed spacer (ETS) and U3 small nucleolar RNA (U3 snoRNA) (part **c**). **d** | Schematic representation of secondary structures of pre-18S rRNA and U3 snoRNA. The four domains of the pre-18S rRNA (5′, central, 3′ major and 3′ minor) are colour-coded. Base pairing of the U3 snoRNA 3′ hinge and 5′ hinge with the 5′ ETS, and of the U3 snoRNA Box A and Box A′ sequences with pre-18S rRNA are indicated. Protein complexes are outlined as transparent surfaces in parts **b** and **c**.

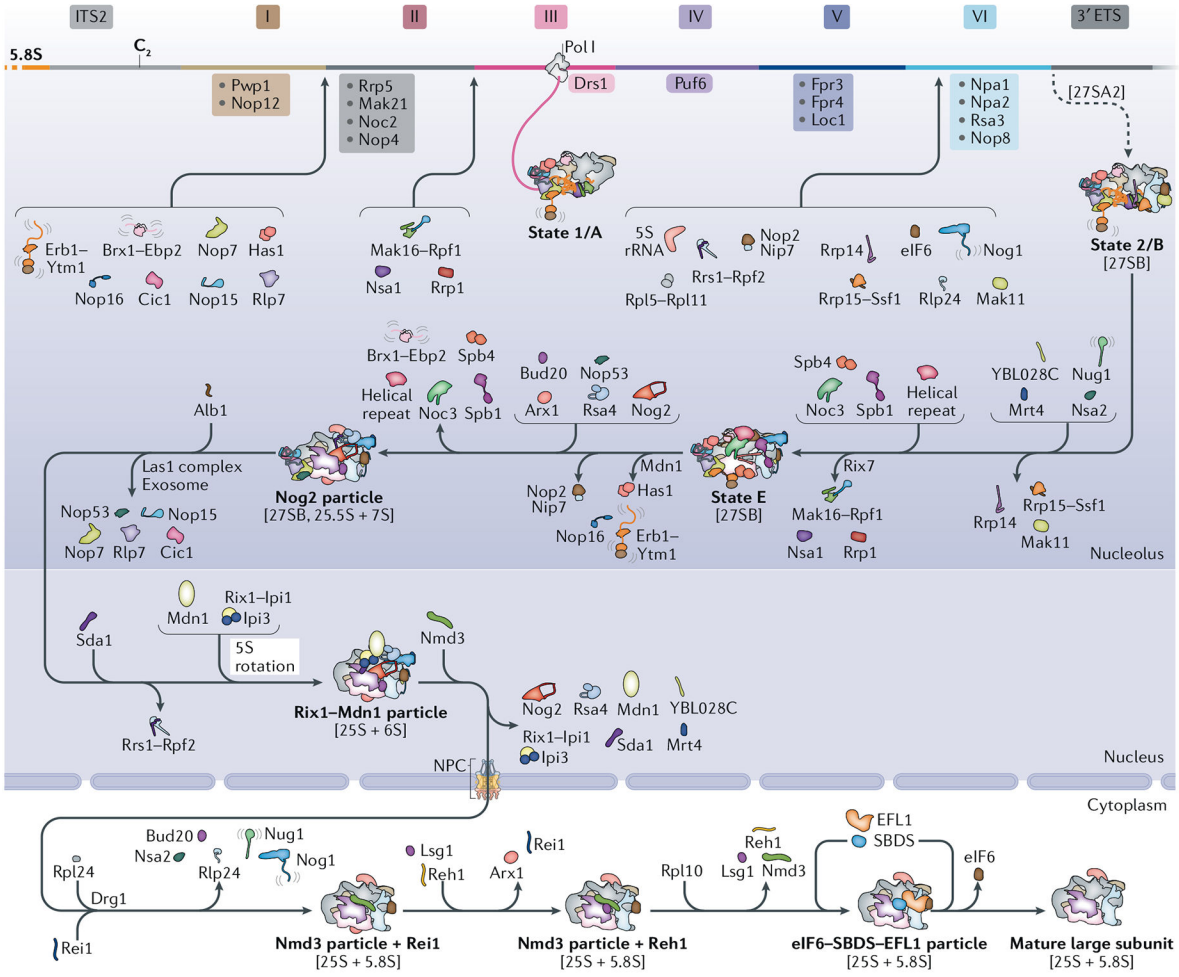

Fig. 4 |. Assembly of the large ribosomal subunit.

Consecutive stages in the maturation of the large ribosomal subunit (60S) are shown, from the earliest stages in the nucleolus, through stages in the nucleoplasm and finally in the cytoplasm. Large-subunit-specific portions of ribosomal DNA (rDNA) are depicted with colour-coding of the 5.8S ribosomal RNA (rRNA), the internal transcribed spacer 2 (ITS2), the 25S rRNA domains I–VI and the 3′ external transcribed spacer (3′ ETS). Six assembly intermediates for which cryo-electron microscopy (cryo-EM) structures have been determined are shown: state 1 or state A (state 1/A), state 2/B, state E, Nog2, Rix1–Mdn1 and Nmd3 particles. Pre-rRNA intermediates present in each particle are indicated in square brackets, and rRNA domains that have assembled into stable visible domains are depicted using the same colours of the rDNA. Note that some of the different particles contain the same pre-rRNAs but differ in structure and protein content (for example, state 1/A and state 2/B). There are likely additional assembly intermediates to be discovered. The association and dissociation of assembly factors is shown. Assembly factors for which structural information is available are depicted in cartoon form; those for which no structures are known are indicated with text only. The earliest pre-ribosomal particles present before state 1/A particles are formed cotranscriptionally and have not been visualized by electron microscopy. In the state 1/A and state 2/B particles, 25S rRNA domains I, II and VI and the

5.8S rRNA and ITS2 have begun to form and become stable, visible conformations. The transition from state 2/B to states C and D (which are not shown as particles), and then to state E, involves assembly of domains III, IV and V and includes early steps in the formation of the peptidyl transferase centre and polypeptide exit tunnel functional centres. Major structural remodelling occurs to form Nog2 particles, which translocate from the nucleolus to the nucleoplasm, where additional restructuring as well as quality control checkpoints are carried out to prepare particles for nuclear export. Upon entry into the cytoplasm, the remaining assembly factors are released, as the assembly and surveillance of functional centres is completed. The ‘wiggling’ signs highlight components that are flexible. NPC, nuclear pore complex.

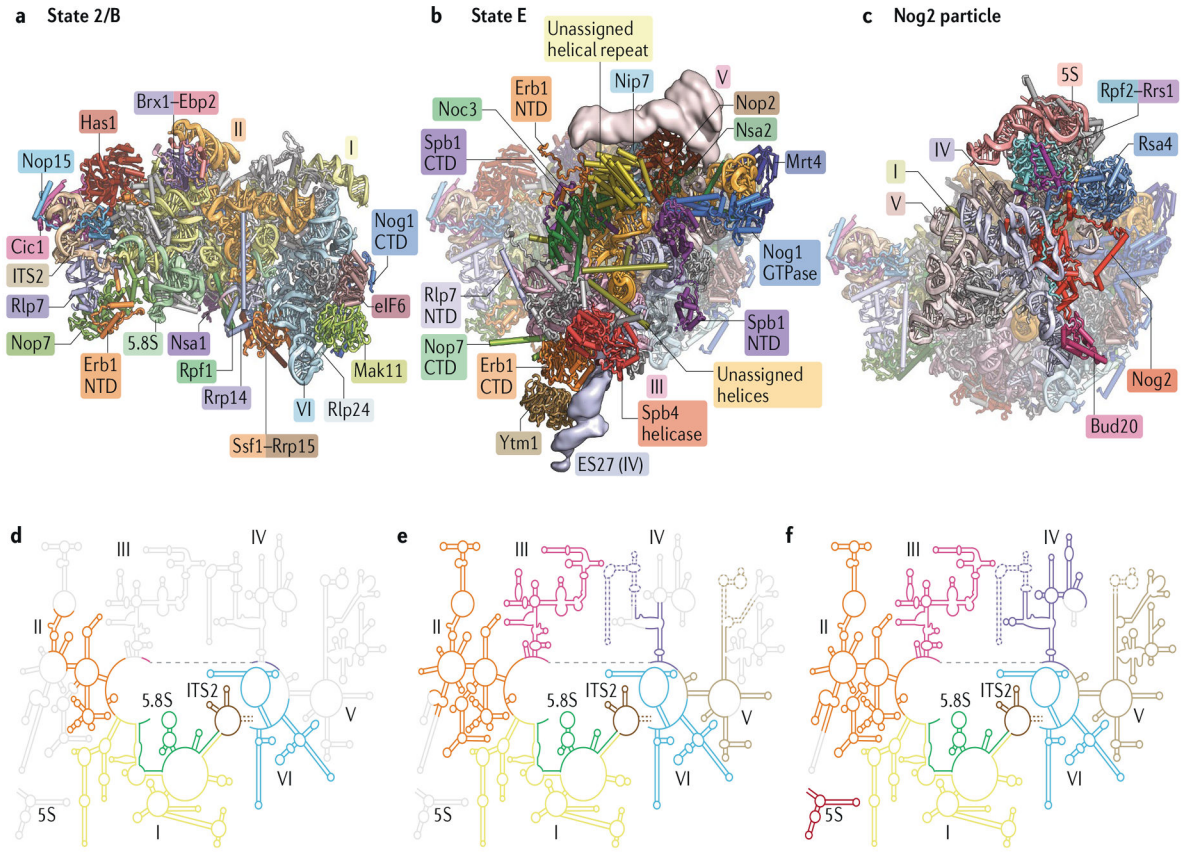

Fig. 5 |. Cryo-electron microscopy structures of large-subunit precursors.

a | Architecture of an early nucleolar pre-60S particle known as state 2 or state B (state 2/B; PDB ID: 6C0F), which includes the folded domains I, II and VI of pre-25S ribosomal RNA (rRNA) and the pre-5.8S rRNA. **b** | Architecture of a nucleolar pre-60S particle known as state E (PDB ID: 6ELZ, EMD 3891 and manual building). Note that domains III and V, and expansion segment 27 (ES27) of domain IV of the pre-25S rRNA, are now folded and visible. **c** | Architecture of the late nucleolar Nog2 particle (PDB ID: 3JCT). Here, domain IV is folded and visible with the 5S rRNA. Components already present in the previous particle are shown as transparent. **d–f** | Diagrams of the large-subunit rRNA secondary structure, showing the successive folding and stabilization of subdomains. Parts **d–f** depict rRNA organization of state 2/B, state E and Nog2 particles, respectively. Stably ordered RNAs (solid lines), largely ordered RNAs (dashed lines) and disordered RNAs (in light grey) are indicated with the same colour-coding as in parts **a–c**. CTD, carboxy-terminal domain; ITS2, internal transcribed spacer 2; NTD, amino-terminal domain.

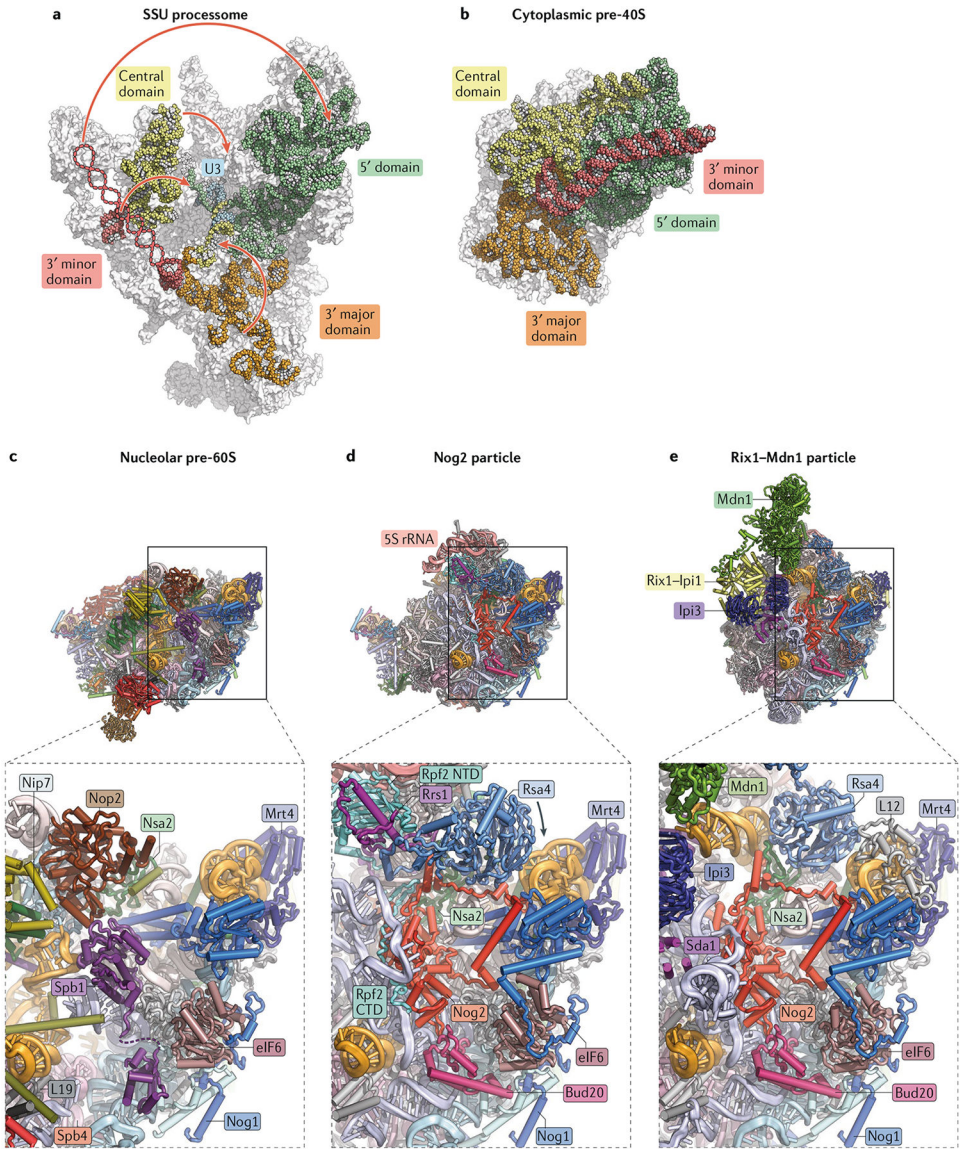

Fig. 6 |. Structural changes occurring in nucleolar and nuclear ribosomal precursors.

a,b | Rearrangement of the relative orientations of pre-18S rRNA domains during the transition from the small-subunit processome (SSU processome; PDB ID: 5WLC) to the cytoplasmic pre-40S particle (PDB ID: 6FAI). The 5′, central, 3′ major and 3′ minor domains and the U3 small nucleolar RNA (U3) are indicated. These conformational changes are indicated by arrows in part **a. c–e** | Maturation of large-subunit particles near the Nog1 binding site in the nucleolar pre-60S particle (also known as state E; PDB ID: 6ELZ, manual building), the nucleolar Nog2 particle (PDB ID: 3JCT) and the nuclear Rix1–Mdn1 particle (PDB ID: 5JCS, manual building). In the transition from state E to Nog2 particles, the release of assembly factors Spb1, Noc3, Nip7 and Nop2 enables maturation of the polypeptide exit tunnel and stable docking of the 5S ribonucleoprotein (RNP) through Rpf2 and Rrs1. Subsequent formation of the nucleoplasmic Rix1–Mdn1 particle involves release of Rpf2 and Rrs1 to destabilize the pre-rotated state of the central protuberance and

assembly of Sda1, the Ipi1–Rix1–Ipi3 complex and Mdn1 to stabilize the rotated state of the central prot.