E-content

M.Sc. Zoology (Semester IV)
Elective Paper: Cell and Molecular biology

Unit: 3.3

Nucleolar function: synthesis of rRNA, its processing and biogenesis of ribosomes

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SYNTHESIS OF RIBOSOMAL RNA

The eukaryotic ribosome consists of two subunits formed by the intricate association of 79 ribosomal proteins (RPs) with 4 distinct ribosomal RNAs (rRNAs).

- Small subunit (40S) comprises the 18S rRNA assembled to 33 RPs (RPSs)
- Large subunit (60S) contains the 5S, 5.8S, and 28S rRNAs associated with 46 RPs (RPLs).

The production of this huge molecular machine consumes the major part of the cellular energy and occupies vast nuclear domains before its final maturation in cytoplasm.

In eukaryotes, three ribosomal RNAs are processed from a long polycistronic pre-ribosomal RNA.

A complex sequence of processing steps is required to gradually release the mature RNAs from this precursor. A large set of transacting factors chaperone this process, including small nucleolar ribonucleoproteins.

Ribosomal RNA synthesis by RNA polymerase I

In eukaryotic cells, the task of transcribing nuclear genes is shared by Pols I, II and III.

Pol I is dedicated to the synthesis of rRNA, and this accounts for up to 60% of transcriptional activity in a eukaryotic cell.

Pol II produces messenger mRNAs, and many small nuclear (sn)RNAs, which are involved in mRNA processing.

Pol III synthesizes the transfer (t)RNAs, the 5S rRNA and a variety of other small, untranslated RNAs with essential roles in metabolism.

Site of rRNA synthesis by Pol I:

Ribosomal RNA synthesis by Pol I occurs in the nucleolus. The nucleolus forms around the nucleolar organizer regions (NORs) containing hundreds of rRNA genes organized head-to-tail into tandem arrays.

Ribosomal RNA synthesis by RNA polymerase I

Organization of rDNA repeats

In mammals, each rDNA repeat is around 43 kb and contains promoters, repetitive enhancers and terminators within an intergenic spacer (IGS) of approximately 30 kb, and a single transcribed region of approx 13 kb containing the 47S coding region.

Eukaryotic rRNA gene promoters contain two regulatory elements the core promoter (CP) and the upstream control element (UCE).

The CP is sufficient for basal transcription by Pol I. TheUCE lies further upstream of CP and is important for stimulating transcription from the core promoter.

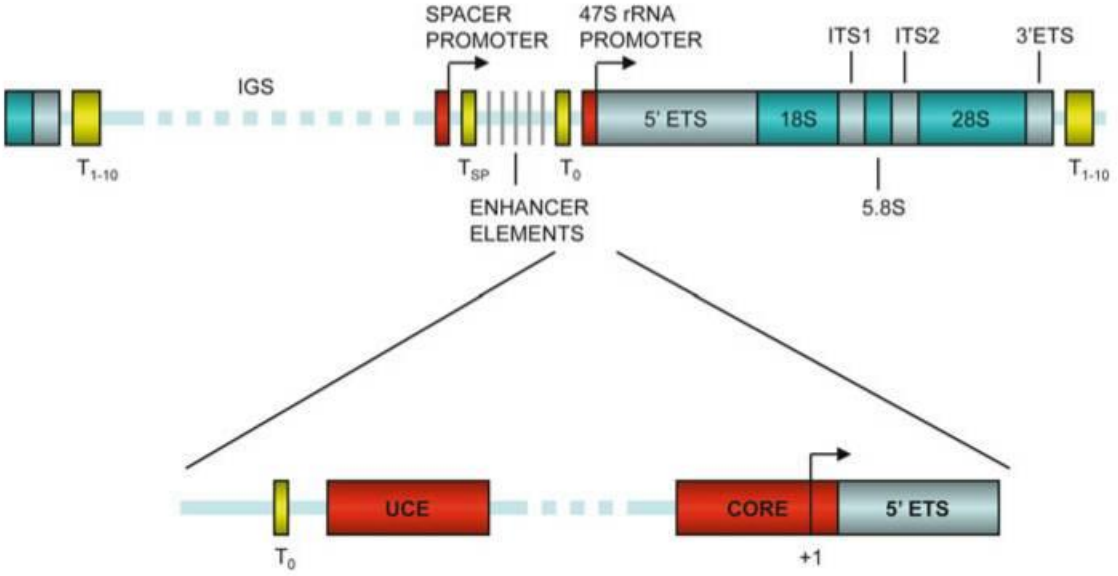


Fig1: Regulatory elements of rDNA repeats

In addition to the main rRNA gene promoter, related sequence elements known as spacer promoters have been identified within the IGS. The transcripts produced by Pol I from these promoters (known as promoter RNA (pRNA)) are involved in transcriptional silencing of the rRNA genes.

Transcription by RNA Polymerase I

Pol I catalyses the synthesis of rRNA from rDNA repeats. Transcription by Pol1 can be divided into following steps

1. Pre-initiation Complex (PIC) formation
2. Initiation and promoter escape
3. Elongation
4. Termination

1. Pre-initiation Complex (PIC) formation

RNA polymerases themselves have little affinity for promoter sequence elements and so rely upon specific transcription factors for accurate recruitment.

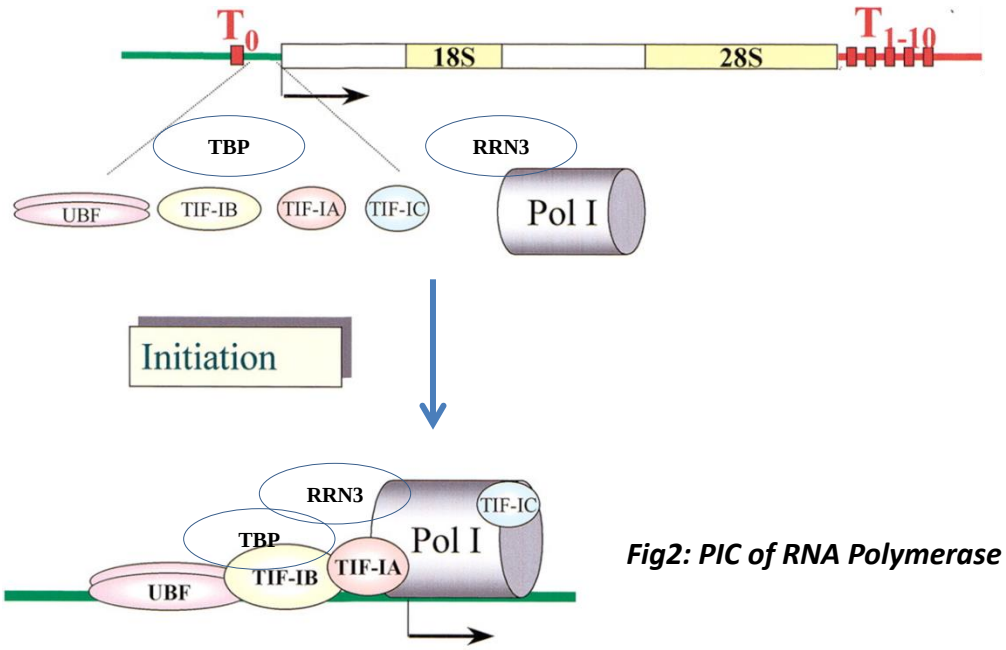


Fig2: PIC of RNA Polymerase I

The first stage of transcription by Pol I is the formation of a PIC at the gene promoter. Transcription by Pol I in mammalian cells is dependent upon selectivity factor 1 (SL1), which is a complex of TBP and various Pol I-specific TAFs including TAF1C, TAF1B, TAF1A and TAF1D.

1. Pre-initiation Complex (PIC)

Pol I has 14 polypeptide subunits in mammals. A catalytic core is formed by ten of these subunits. These specific Pol I subunits function at multiple stages in the Pol I transcription cycle, playing important roles in polymerase recruitment, promoter escape and elongation.

The multi-subunit Pol I complex exists as at least two distinct subpopulations , known as Pol Iα and Pol Iβ in mammalian cells . Both forms of Pol I are active and can catalyse the synthesis of RNA. RRN3 and the serine/threonine kinase CK2 specifically associated with Pol Iβ to regulates PIC assembly and stability.

SL1 and Pol Iβ alone are sufficient to support basal levels of Pol I transcription in vitro. However, to achieve activated transcription, UBF must also be incorporated into the Pol I PIC.

UBF interacts cooperatively with SL1 at the rDNA promoter, with SL1 binding the highly acidic C-terminus of UBF through its TAF1A and TBP subunits . This stabilises the association of UBF with the PIC, hence facilitating promoter-specific transcriptional activation.

2. Initiation and Promoter Escape

Following the assembly of a productive PIC at the rDNA promoter, promoter opening and transcription initiation by Pol I can commence.

Promoter escape following transcription initiation coincides with the release of RRN3 from polymerase I.

The interaction between RRN3 and Pol I is controlled at least in part by phosphorylation.

UBF is also required for promoter escape.

A network of interactions involving these factors could induce conformational changes in the PIC, triggering any post-translational modifications and the release of RRN3, converting initiation-competent Pol I into an elongating form.

3. Elongation

The transcription elongation by Pol I is highly efficient with, on average, 100 polymerases transcribing each active gene at a rate of approximately 95 nucleotides per second.

Pol I have RNA cleavage activity that helps in RNA proofreading and facilitates the elongation.

UBF, regulates Pol I elongation by phosphorylation-dependent remodelling of the rDNA chromatin.

The histone chaperones nucleolin, nucleophosmin and FACT assist in rDNA transcription. Further, various chromatin remodelling and modifying activities promotes transcription by Pol I including tip60, Williams syndrome transcription Factor (WSTF)-SNF2h and the histone methyltransferase G9a.

Given the high loading density of Pol I on rRNA genes, the physical obstructions encountered by a transcribing polymerase, caused by topological changes in the rDNA are efficiently resolved by Topoisomerase IIα. The topoisomerase promote transcriptional elongation by relieving the positive and negative supercoiling that occurs ahead of and behind transcribing Pol I.

4. Termination

Transcription termination by Pol I is a multistep process involving specific DNA sequence elements and regulatory proteins.

In mammals, transcription termination factor TTF-I binds terminator elements downstream of the rRNA gene (T1-T10), causing polymerase pausing.

Dissociation of the paused transcription complex is then mediated by Pol I and transcript release factor PTRF.

The termination of transcription by Pol I begins with cleavage of the nascent pre-rRNA by the endonuclease Rnt1, followed by the progressive digestion of the resulting Pol I-associated RNA cleavage product mediated by the cooperative actions of the 5′ to 3′ exonuclease Xrn2 and the RNA helicase Sen1.

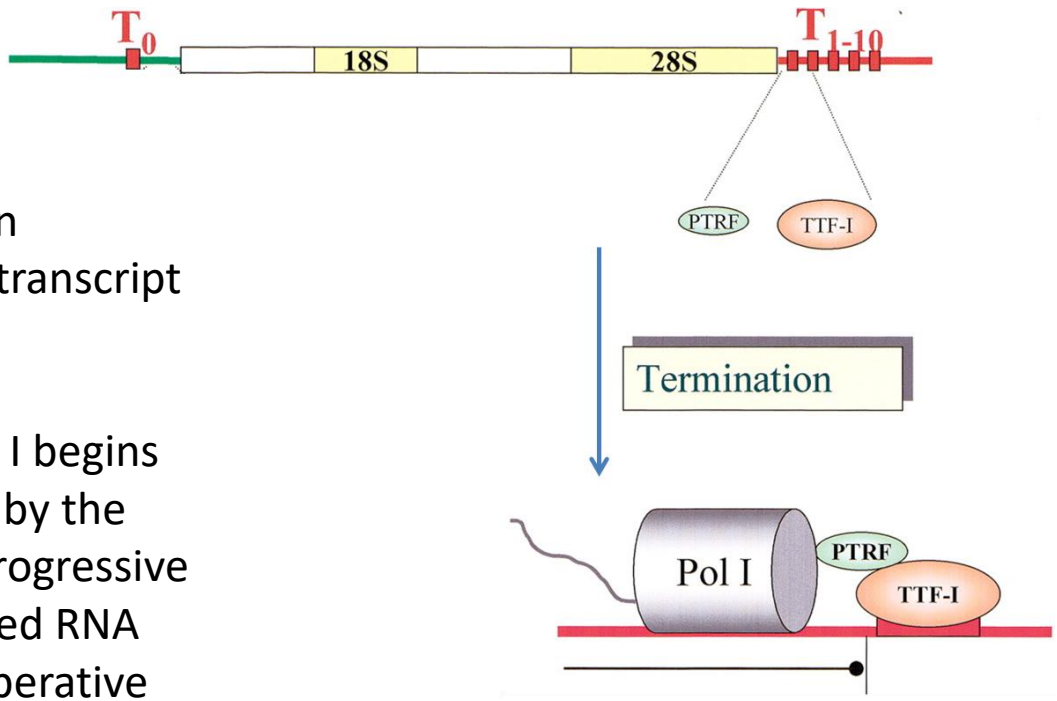


Fig3: Termination of transcription (Pol I)

Once Xrn2 reaches elongating Pol I, the transcription complex becomes unstable and dissociates from DNA, thus resulting in transcription termination.

Transcription of 5S rRNA by RNA Polymerase III

Unlike the 47S pre-rRNA, the precursor to the 5S rRNA is transcribed by RNA polymerase III. In humans, the RNA5S genes encoding 5S rRNA are tandemly repeated on chromosome 1.

While this chromosome is distinct from ribosomal DNA, the RNA 5S genes are localized in close proximity to nucleoli.

Synthesis of the 5S rRNA requires a specific regulatory factor called transcription factor IIIA (TFIIIA).

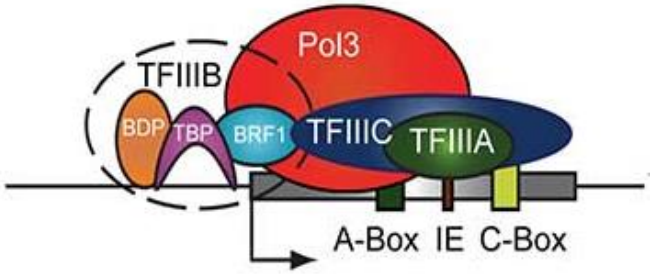


Fig 5: PIC at 5S rRNA gene

TFIIIA associates with the general class III initiation factors TFIIIB and TFIIIC on the 5S gene promoter and stimulates transcription.

Notably, the basal promoter element necessary for 5S rRNA gene transcription is located in the transcribed region.

Transcription starts directly at the 5’ end of the 5S rRNA, but the primary transcript bears a uridine-rich 3’ extension.

PROCESSING OF RIBOSOMAL RNA

As elongation by Pol I proceeds, the nascent pre-rRNA associates with components of the processing machinery, allowing co-transcriptional maturation of the rRNA and assembly of ribosomal particles. As a result, pre-rRNA synthesis and processing are closely coordinated.

The nascent primary transcripts associate co-transcriptionally with some RPs, numerous pre-ribosomal factors (PRFs), and small nucleolar ribonucleoprotein particles (snoRNPs) to form a series of large RNPs in which pre-rRNA folding and modification take place together with RP assembly.

Along this process, the transcribed spacers are sequentially eliminated through a complex series of endonucleolytic and exonucleolytic cleavages.

Within the eukaryotic primary rRNA transcript (47S), the mature 18S, 5.8S, and 28S rRNAs are separated by the internal transcribed spacers 1 (ITS1) and 2 (ITS2) and flanked by the 5′ and 3′ external transcribed spacers (5′-ETS and 3′-ETS).

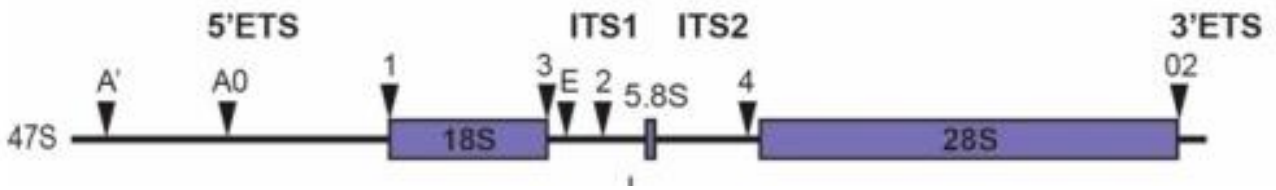


Fig 6: 47S primary rRNA transcript; arrowheads represents the endonucleolytic sites

Processing of 47S pre-rRNA

The production pathways of the two ribosomal subunits diverge after cleavage in the ITS1. The maturating pre-60S particles form the so-called granular component of the nucleolus, whereas pre-40S particles are more rapidly exported to the cytoplasm.

The transcribed spacers contain several cleavage sites targeted by endonucleases that act sequentially to free the rRNAs. While the rRNA sequences are conserved among eukaryotes, the sequence and the length of the transcribed spacers strongly diverge.

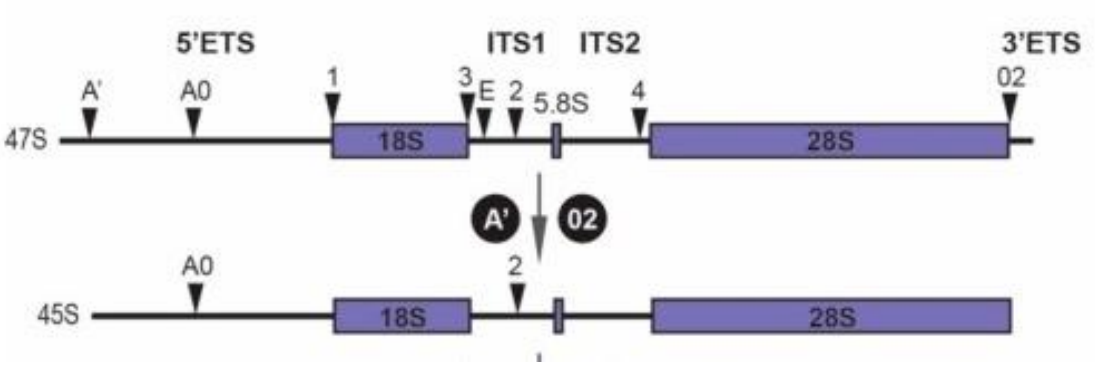


Fig 7: Processing of 47S rRNA to 45S rRNA

The cleavage at A' and 02 sites on 47S rRNA yield a shorter RNA called 45S rRNA. This RNA is processed further to generate 18S, 5.8S and 28S rRNA as summarized in figure 8.

Figure 8: Overview of Pre-ribosomal RNA processing in human cells.

Three of the four ribosomal RNAs arise from a long primary transcript (47S pre-rRNA) synthesized by RNA polymerase I . The sequences of 18S, 5.8S, and 28S rRNAs are flanked by external (5'-ETS, 3' ETS) and internal transcribed spacers (ITS1, ITS2), which are gradually removed by endo- and exonucleases.

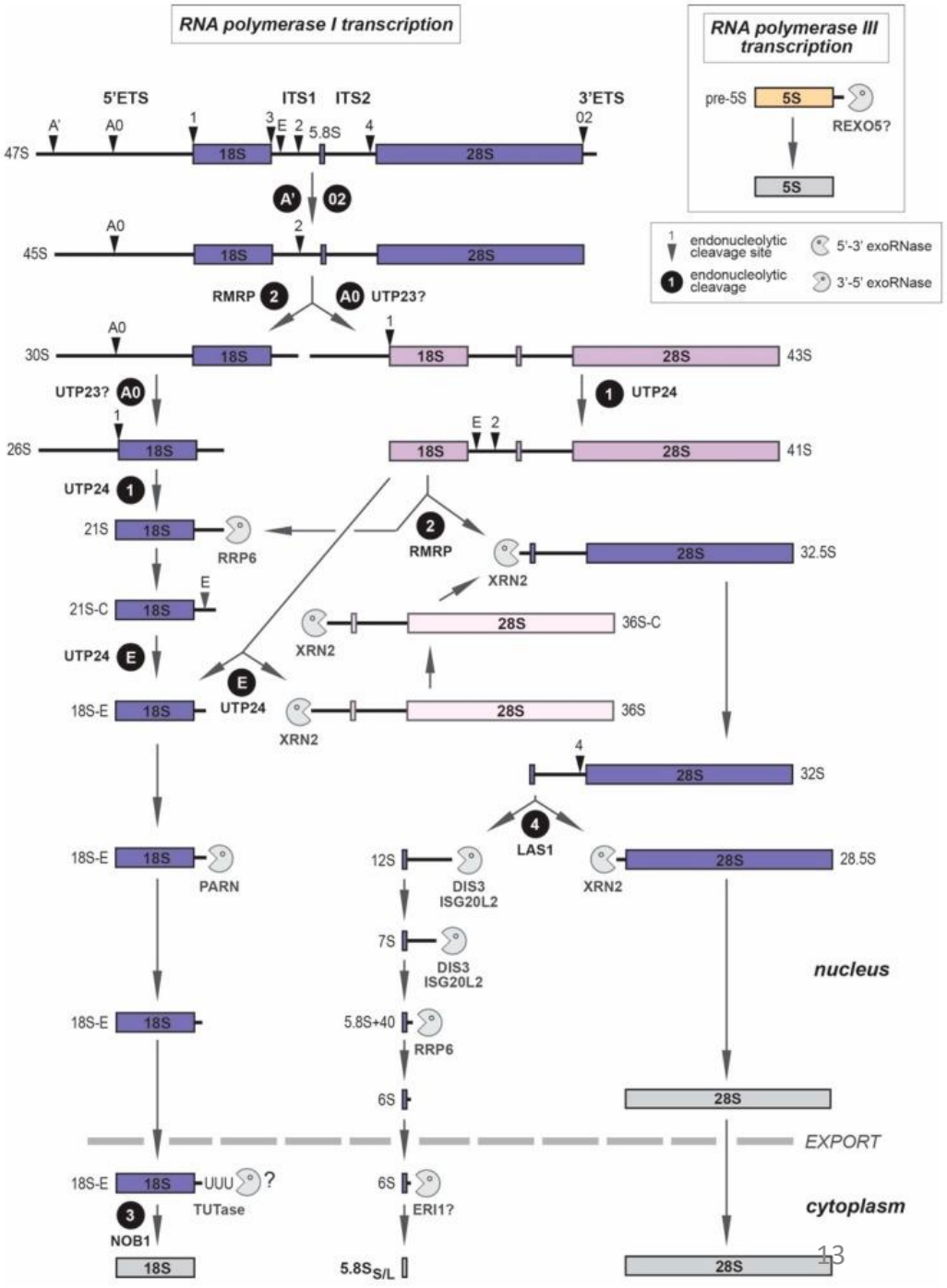
In the main maturation pathway (pre-rRNAs colored in violet), cleavage at site 2 occurs prior to cleavage at site A0. Less abundant precursors are characteristic of alternative (pale lavender) or minor (pink) routes.

The 5S rRNA is transcribed by RNA polymerase III.

Endoribonucleases are quoted in black and 5'-3' or 3'-5' exoribonucleases in grey.

Question marks refer to uncertain enzymatic activities. Site E is also known as 2a.

Biomolecules,2018 Dec; 8(4): 123.



18S rRNA processing

The cleavage of the 45S pre-rRNA at site 2 separates the 30S pre-rRNA, precursor to the 18S rRNA, from the large subunit RNAs (figure 8).

This cleavage is performed by RNase MRP (mitochondrial RNA processing) or RMRP.

Formation of the 18S rRNA then requires elimination of the 5'ETS and the ITS1 at the 3' end. The 5'-ETS contains three cleavage sites: A' (also called 01) and A0 located within the 5'-ETS, and site 1, which defines the 5' end of the 18S rRNA.

As indicated above, cleavage at A' takes place very early, before processing at site 2 and independently from cleavage at A0 and 1.

Unlike A', cleavages at sites A0 and 1 are coordinated with one another.

In addition, cleavage of the ITS1 at site E is subordinated to cleavage at site 1.

hUTP24 is the endonuclease for that cleave at sites 1 and E.

18S rRNA processing

Removal of the 5′-ETS and ITS1 cleavage at site 2 yields the 21S pre-rRNA (figure 8). This precursor is processed at its 3′ end through the sequential action of endo- and exonucleases.

First, the 3′ end of the ITS1 is trimmed by the exosome, RRP6, which yields the 21S-C (figure 8).

Next, endonucleolytic cleavage of the 21S-C pre-rRNA at site E generates the 18S-E precursor, which are gradually shortened by a 3′–5′ exonuclease by Poly(A)-specific ribonuclease (PARN) (figure 8).

The pre-40S particles then leave the nucleus and are exported to the cytoplasm.

In the cytoplasm, the 18S-E pre-rRNA is trimmed at its 3′ end by an exonuclease.

the 18S-E pre-rRNA is then cleaved by the endonuclease NOB1 to generate the 18S mature rRNA (figure 8).

Processing of 5.8 and 28S rRNAs

ITS1 cleavage at site 2 generates the 32.5S pre-rRNA, which contains the 5.8S and 28S rRNAs (figure 8).

The ITS1 is rapidly removed by the 5′–3′ exoribonuclease XRN2, which forms the 5′ ends of the long and short forms of the 5.8S rRNA.

Cleavage of the ITS2 at site 4 in the 32S pre-rRNA by endonuclease Las1 then gives rise of the 12S and the 28.5S pre-rRNA, the precursors to the 5.8S and the 28S rRNAs, respectively.

Cleavage at site 4 is then followed by exonucleolytic processing of the resulting precursors.

The ITS2 domain forming the 5′ end of the 28.5S pre-rRNA is trimmed by XRN2 to form the 28S rRNA (figure 8).

The 12S pre-rRNA in turn is sequentially digested by several 3′–5′ exonucleases to form the mature 3′ end of the 5.8S rRNA (figure 8).

The last nucleotides in 3′ of the 5.8S rRNA precursor are removed by exonuclease ERI1 and transported to cytoplasm where final maturation takes place (figure 8).

Processing of 5S rRNA

This 5S precursor, called 5S*, is recognized by the La protein, which associates with diverse RNA polymerase III transcripts.

La has affinity for uridylates in 3′ and acts as a chaperone.

The 5S* RNA 3′ end is processed by a 3′–5′ exonuclease.

After processing of the 3′ end, the 5S rRNA is associated with the ribosomal protein L5 (RPL5).

In mammalian cells, the 5S-RPL5 complex helps in its incorporation into the 60S particles.

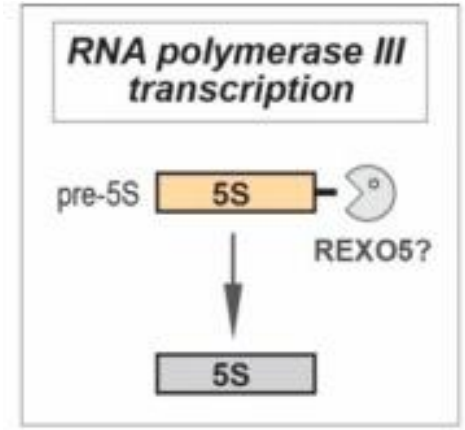


Fig 9: Processing of 5S rRNA

RIBOSOMAL RNA MODIFICATIONS

Cleavage of pre-rRNAs is paralleled by chemical modification of around 200 nucleotides within the emerging rRNA sequences.

Most of these modifications are pseudouridylations and 2'-O-ribose methylations that are guided and catalyzed by two families of small nucleolar RNPs, respectively called H/ACA box and C/D box snoRNPs.

Each modification is performed by a particular snoRNP that combines a set of core proteins with a specific small guide RNA (snoRNA) hybridizing around the position to modify. Each complex includes either the pseudouridyl synthase dyskerin (H/ACA box snoRNPs), or the methyltransferase fibrillarin (C/D box snoRNPs).

These modifications take place early in the maturation process. They target nucleotides located in functionally important regions of the ribosome, including the peptidyl transferase center or the decoding center.

BIOGENESIS OF RIBOSOMES

The biogenesis of eukaryotic ribosomes is a complicated process during which the transcription, modification, folding, and processing of the rRNA is coupled with the ordered assembly of approx. 80 ribosomal proteins (r-proteins).

Basic Steps of Eukaryotic Ribosome Assembly

- Transcription of rDNA into pre-RNA and its modification in the nucleolus.
- Cytoplasmic synthesis of r-proteins and biogenesis factors and, subsequent transport to the nucleolus.
- Spatiotemporal association of biogenesis factors and r-proteins with the pre-rRNA results in the formation of pre-ribosomal particles.
- Processing of the pre-rRNA (endo- and exonucleolytic removal of ETS and ITS sequences) separates the maturation pathways of the two subunits and is coordinated with the progression of ribosome assembly.
- Export of pre-ribosomes from the nucleus across the nuclear pore complex to the cytoplasm.
- Final maturation of both pre-ribosomal subunits occurs in the cytoplasm and release and recycling of biogenesis factors.

Basic Steps of Eukaryotic Ribosome Assembly

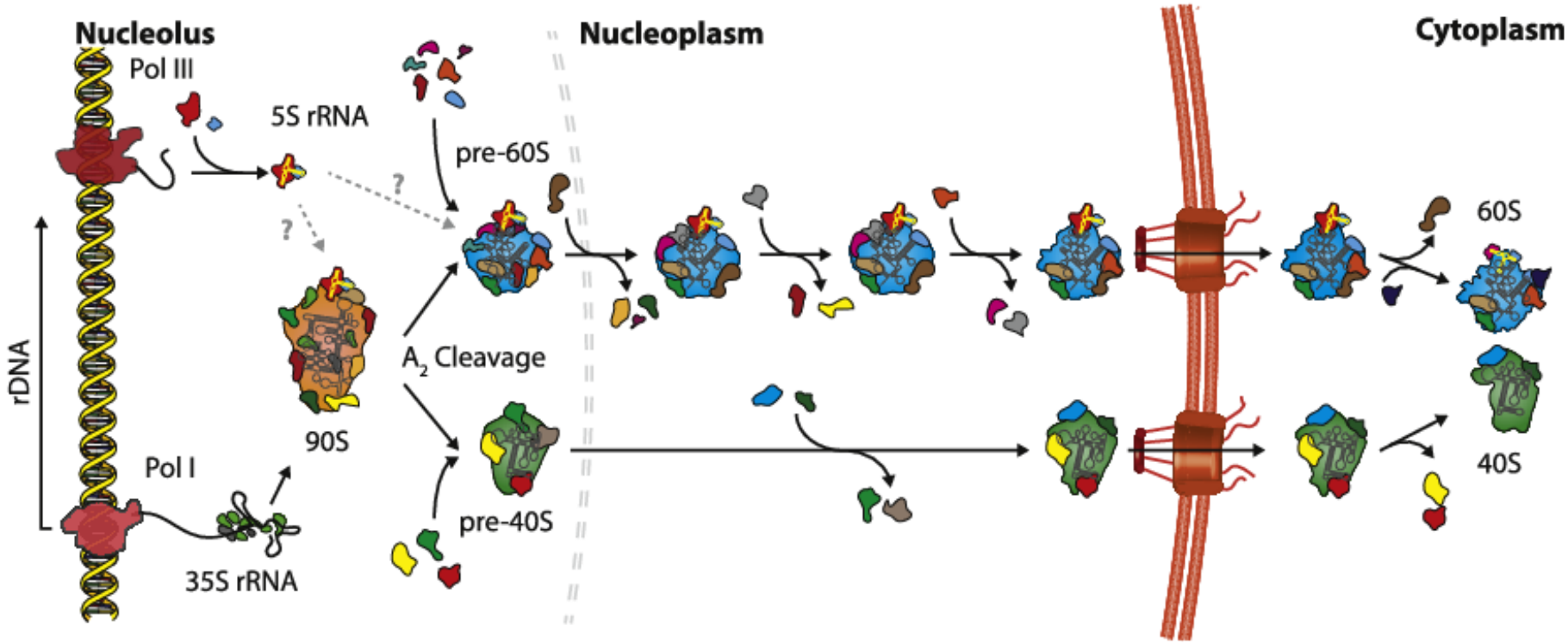


Fig 10: Overview of eukaryotic ribosome maturation. Cotranscriptional binding of early trans-acting factors to the 35S rRNA forms the 90S preribosome. Cleavage at the A2 site separates the pre-60S subunit and the pre-40S subunit, which undergo independent maturation pathways in the nucleoplasm and, eventually, in the cytoplasm. Final maturation in the cytoplasm allows joining of both subunits to form the functional 80S ribosome. ***Adapted from Gerhardy et al. (2014)***

BIOGENESIS OF RIBOSOMES

Ribosome biogenesis begins in the nucleolus, where three of the rRNA species, the 18S, 5.8S and 25S, are cotranscribed by RNA polymerase I (Pol I) as a single polycistronic transcript.

As the transcript emerges, many small nucleolar ribonucleoparticles (snoRNPs) (>60) mediate the co-transcriptional covalent modification of over 100 rRNA residues.

The ribosome biogenesis includes following steps

1. Formation of SSU processome or 90S pre-ribosomes
2. Maturation of pre-40S subunit
3. Nuclear maturation of pre-60S subunit
4. Export of pre-ribosome particles to cytosol
5. Cytosolic maturation of pre-60S subunit

1. SSU processome and 90S pre-ribosomes

As transcription ensues, the rRNA transcripts form ball like structures on the 5’ end of the nascent transcripts that emanate from the rDNA to yield the earliest nascent pre-ribosomes, which correspond to the 90S or small subunit (SSU) ‘processome’ complexes.

A first nucleating step is the assembly of the t-UTP (transcription U three protein) complex with the nascent pre-rRNA. This complex have U3 snoRNP and the UTP-A, UTP-B, and UTP-C modules, which assemble in a stepwise manner with the pre-rRNA.

All of these proteins associate with the 5’-external transcribed spacer (ETS) region of the nascent 35S pre-rRNA to generate 90S-SSU processome.

Within the 90S–SSU processome, cleavage of the rRNA at the site termed A2 predominantly occurs cotranscriptionally (figure 10).

This cleavage event effectively separates the maturation pathways of the two subunits by promoting the disassembly of the 90S– SSU processome and the emergence of pre-40S and pre-60S particles.

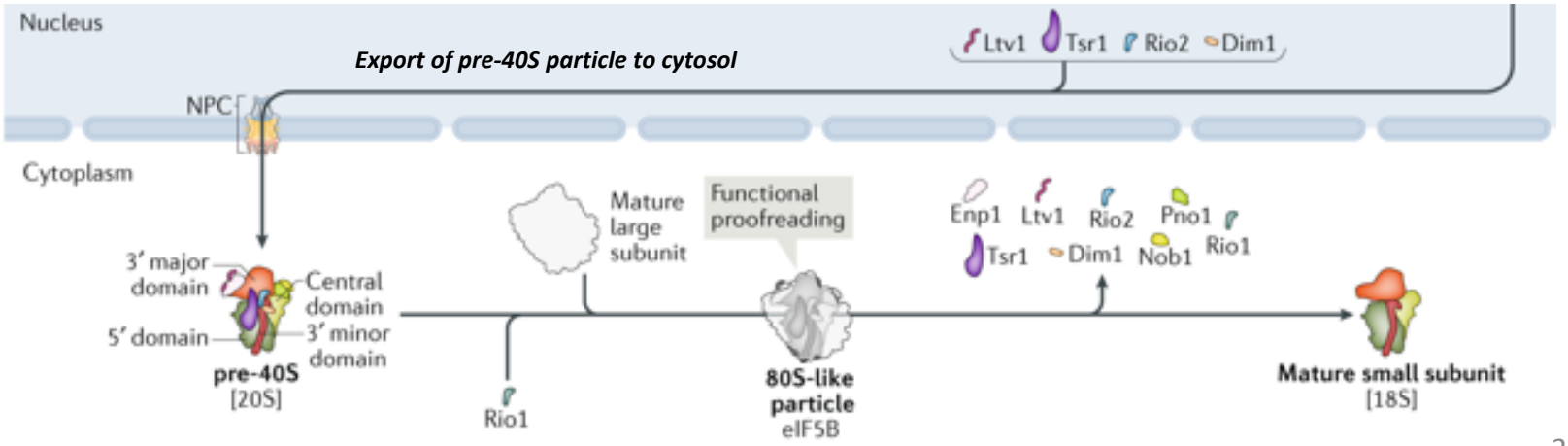
2. Maturation of pre-40S subunit

The pre-40S particles, which have most SSU r-proteins and a few newly recruited 40S biogenesis factors, are exported to the cytoplasm, for maturation events.

During the transport into the cytoplasm, the pre-40S particle associates with Enp1, Ltv1, Rio2, Dim1 and others.

The cleavage of 20S pre-rRNA to 18S rRNA takes place, which is mediated by the endonuclease Nob1. This reaction is stimulated by the translation initiation factor eIF5b, which promotes the formation of an 80S-like complex through the recruitment of the 60S subunit.

Maturation also include a translation-like event that could serve to check the integrity of the newly synthesised 40S. Once fully matured, both cytosolic ribosomal subunits are competent to engage in the translation of mRNA.



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3. Nuclear maturation of pre-60S subunit

Maturation of the 60S subunit requires extensive rearrangements within the nucleolar and nucleoplasmic compartments before its export and final maturation in the cytoplasm

The initial steps of 60S subunit assembly is the formation of the first pre-60S particle and the association of early-binding large subunit (LSU) r-proteins that connect the 5' and 3' regions of the 27S pre-rRNA.

The maturing pre-60S is characterized by a gradual reduction in complexity of associated trans-acting factors as it moves from the nucleolus to the cytoplasm. Two proteins plays important role in maturation of pre-60S subunit are A3 factor and ATPases

a) The A3 factors

Approximately eight biogenesis factors start the processing of the 27S A3 pre-rRNA (termed the 'A3 cluster'), which act to complete the maturation of the 5' end of the 5.8S rRNA.

The binding of the A3 factors trigger, conformational switches within ITS2. This, in turn, promote subsequent maturation events, including processing of the pre-rRNA and recruitment of ribosomal proteins.

b) ATPase-mediated maturation events

Three AAA-type ATPases (Rix1, Rea1 and Drg1) act to remove biogenesis factors from the maturing pre-60S subunit .

One of these, the Rea1 protein that is composed of six ATPase domains, which form a ring-like structure through which it contacts the pre-ribosome, whereas its tail protrudes, thus giving the pre-ribosome a distinctive 'tadpolelike' appearance.

The ATPase activity of Rea1, Drg1 and Rix1 on their substrates triggers rearrangements in pre-60S ribosome that drive maturation process.

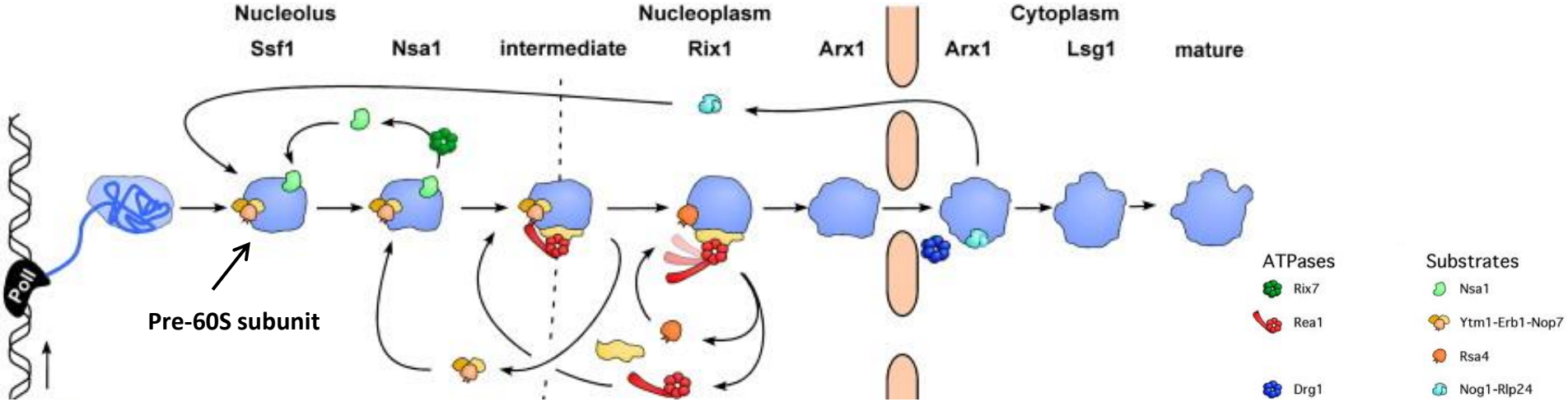


Fig 11: Rix7, Rea1, and Drg1 are dedicated to the release and recycling of distinct biogenesis factors from different pre-60S particles.
BBA - Molecular Cell Research, Volume 1823, Issue 1, January 2012, Pages 92-100

4. Export of pre-ribosome particles to cytosol

Ribosomal subunits must be transported to the cytoplasm for their final maturation.

In order for the subunits to be exported efficiently they interact, through export receptors, with the hydrophobic central channel of the nuclear pore complex (NPC).

The karyopherin Crm1 acts as the receptor for both ribosomal subunits and was found to mediate export in a Ran-GTP-dependent manner.

Several r-proteins and trans-acting factors, Mex67 and Rrp12 have been suggested to facilitate the export of both subunits.

The factors Arx1, Ecm1, Bud20 and Npl3 have been implicated in the export of the large pre-60S subunit (figure 12).

5. Cytoplasmic maturation of 60S

Once the pre-60S has been exported into the cytoplasm, substantial structural rearrangements convert the inactive pre-60S into a functional 60S.

The large subunit ribosomal proteins remains associated with pre-60S as the maturation proceeds, whereas the non-ribosomal assembly factors dissociate and are recycled to the nucleus.

The release of the remaining biogenesis factors appears to follow a hierarchical process that is mediated predominantly by GTPases such as Lsg1 and ATPases such as Drg1 (figure 12).

Following export, the AAA-type ATPase Drg1 has been shown to mediate removal of the predicted GTPase Nog1 and ribosomal-like protein Rlp24 (figure 12).

Dissociation of Rlp24 allows the stable incorporation of ribosomal protein L24, into the pre60S particle. The presence of L24 then allows for the recruitment of Rei1, which along with Jjj1 promotes the release of the shuttling factor Arx1 and of its binding partner Alb1 (figure 12).

The presence of the biogenesis factor Tif6 on the pre-60S inhibit the joining of the small subunit. Tif6 is removed from the 60S subunit by the GTPase Efl1 followed by sequent release of the export adapter Nmd3(figure 12).

Summary of cytoplasmic maturation of 60S

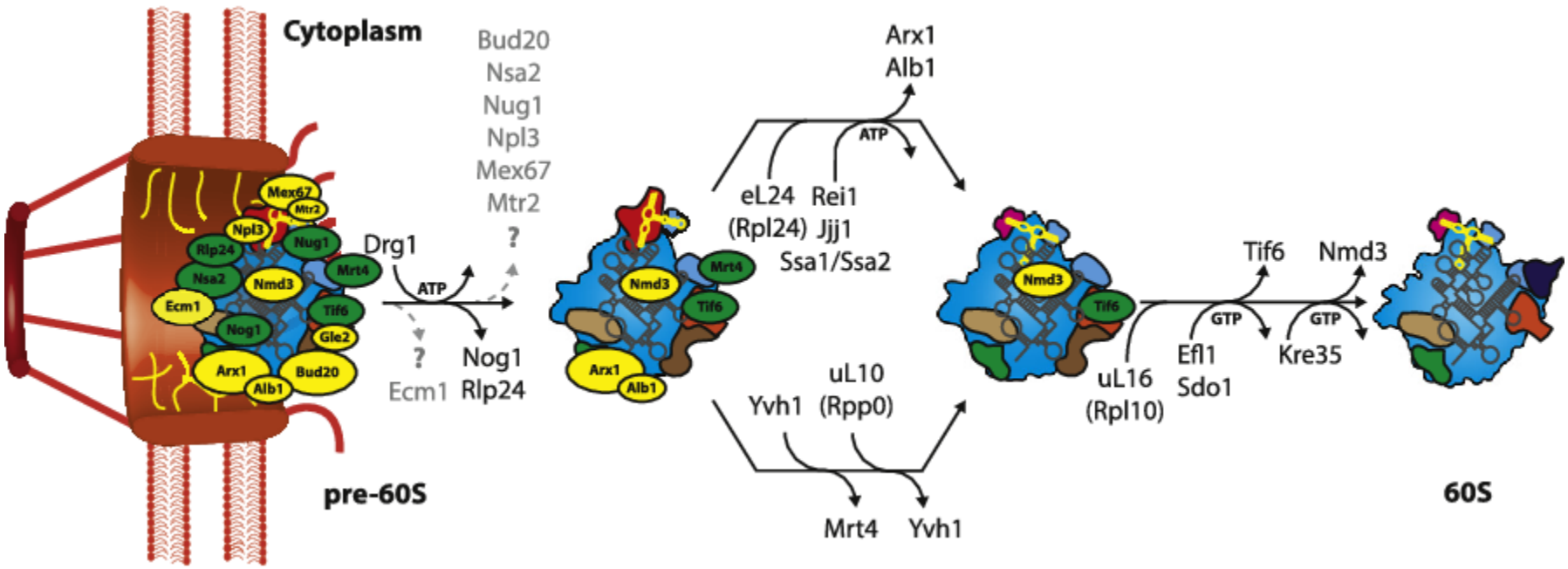
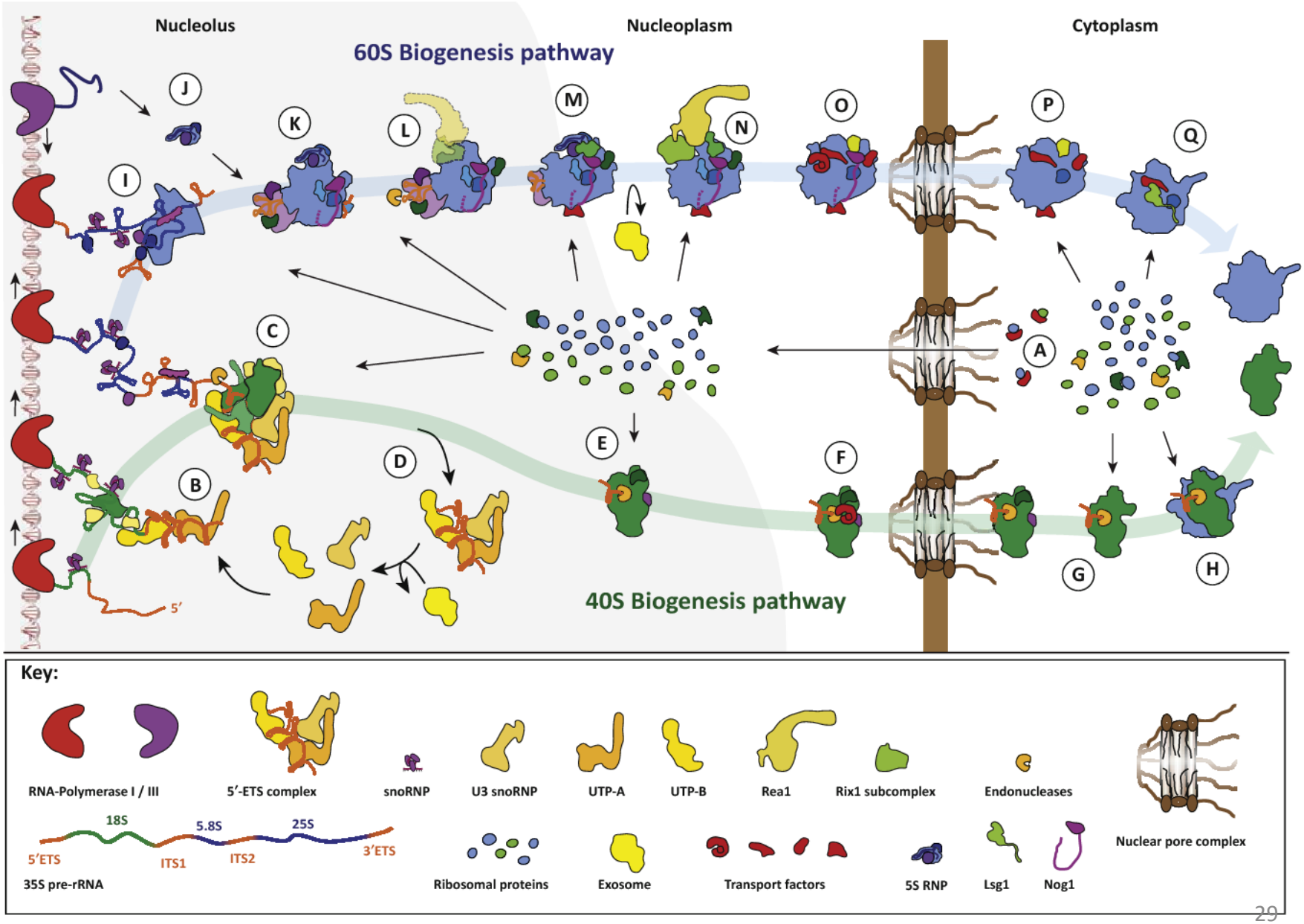


Fig 12: Exported pre-60S subunits are bound by export factors (yellow) and shuttling factors (green), which are released in the cytoplasm. The ATPase Drg1 releases Rlp24 from the pre-ribosomal particles, which triggers subsequent maturation steps. Arx1 and Alb1 require Rei1, Jjj1, and Ssa1/Ssa2 for their release, whereas stalk assembly can only occur after the release of Mrt4 by Yvh1. Recruitment of uL10 (Rpp0) releases Yvh1, which allows further assembly of the P1 (Rpp1) and P2 (Rpp2) heterodimer onto the stalk. Loading of uL16 (Rpl10) triggers the final maturation steps. The GTPase Efl1 and its cofactor Sdo1 release Tif6, and the GTPase Kre35 (Lsg1) removes Nmd3.

Adapted from Gerhardy et al. (2014)

Summary Of Ribosome Biogenesis



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