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Minireview

Nuclear export and cytoplasmic maturation of ribosomal subunits

Ivo Zemp[a,b], Ulrike Kutay[a,*]

[a] *Institute of Biochemistry, HPM F11.1, Schafmattstr. 18, ETH Zurich, 8093 Zurich, Switzerland*
[b] *Molecular Life Science Ph.D. Program, Zurich, Switzerland*

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Abstract Based on the characterization of ribosome precursor particles and associated *trans*-acting factors, a biogenesis pathway for the 40S and 60S subunits has emerged. After nuclear synthesis and assembly steps, pre-ribosomal subunits are exported through the nuclear pore complex in a Crm1- and RanGTP-dependent manner. Subsequent cytoplasmic biogenesis steps of pre-60S particles include the facilitated release of several non-ribosomal proteins, yielding fully functional 60S subunits. Cytoplasmic maturation of 40S subunit precursors includes rRNA dimethylation and pre-rRNA cleavage, allowing 40S subunits to achieve translation competence. We review current knowledge of nuclear export and cytoplasmic maturation of ribosomal subunits.

Keywords: Ribosome biogenesis; rRNA; Nuclear export; Crm1

1. Introduction

Ribosomes are among the most fundamental molecular machines in all cells as they are required for protein synthesis. In total, the two ribosomal subunits in eukaryotes consist of four rRNAs and about 80 proteins, and their biogenesis is a highly complex process that involves more than 150 non-ribosomal proteins, the so-called *trans*-acting factors. Following the application of tandem affinity purification and mass spectrometry techniques to characterize pre-ribosomal particles, a model for the maturation pathway of ribosomes emerged (reviewed in [1]). In recent years, ribosome biogenesis and *trans*-acting factors have been intensely studied, and many aspects of this process have been excellently reviewed [1–7].

The synthesis of ribosomes starts with the transcription of the rRNA from rDNA tandem repeats. RNA polymerase III synthesizes the 5S rRNA, whereas RNA polymerase I transcribes a long precursor rRNA in the nucleoli. This pre-rRNA contains the mature 18S, 5.8S and 25S/28S rRNAs (in yeast/higher eukaryotes), flanked and separated by interspersed spacer sequences. Concomitant with rRNA transcription, the rRNA is modified by methylation and pseudouridylation reactions, catalyzed by a large number of snoRNP particles. The nascent pre-rRNA assembles with *trans*-acting factors and ribosomal proteins, mostly of the small subunit, to a 90S pre-ribosome, the first ribosome precursor that can be isolated. In a series of endo- and exonucleolytic processing events, the pre-rRNA is then cleaved into the mature rRNAs. Cleavage in the spacer region between the sequences of the 18S and the 5.8S rRNAs leads to separation of the 90S pre-ribosome into a pre-40S and a pre-60S particle. Subsequent maturation of the two subunit precursors is mostly independent, as interference with the biogenesis of one subunit in most cases still allows maturation of the other subunit, and only few factors are involved in both pathways (reviewed in [7]).

Following nucleolar assembly and nucleolar and nucleoplasmic maturation, pre-ribosomes are exported to the cytoplasm through the nuclear pore complex (NPC). The two subunit precursors are translocated as independent entities, and once the pre-60S and pre-40S particles reach the cytoplasm, they undergo final maturation steps before achieving translation competence.

In this review, we will focus on factors involved in nuclear export and cytoplasmic maturation of ribosomal subunits. We will first discuss pre-60S and pre-40S biogenesis in the yeast *Saccharomyces cerevisiae*, followed by a review of late ribosome synthesis steps in vertebrate cells.

2. Late maturation of pre-60S particles

After separation of the 90S intermediate into a pre-60S and a pre-40S particle, the two subunit precursors have largely independent biogenesis pathways. In the case of pre-60S subunits, pre-rRNA processing is completed within the nucleus, and a pre-60S ribosome containing the mature 25S, 5.8S and 5S rRNAs is exported to the cytoplasm [8]. Notably, a lag phase is observed before exported 60S subunits are incorporated into polysomes, as the pre-60S particles have to undergo cytoplasmic maturation [9]. In these final subunit biogenesis steps, a number of non-ribosomal proteins associated with cytoplasmic pre-60S subunits have to be released before 60S subunits achieve translation competence (Fig. 1).

2.1. Nuclear export of the pre-60S subunit

Nuclear export of pre-60S subunits in *S. cerevisiae* has been studied monitoring the nuclear accumulation of ribosomal proteins fused to GFP or of 25S rRNA in a variety of mutant yeast strains. These studies showed that pre-60S export is dependent on several NPC components, such as the Nup159p-Nup82p-Nsp1p complex, Nup1p, Nup49p, Nup120p and Nic96p

*Corresponding author. Fax: +41 44 632 1591.
E-mail address: ulrike.kutay@bc.biol.ethz.ch (U. Kutay).

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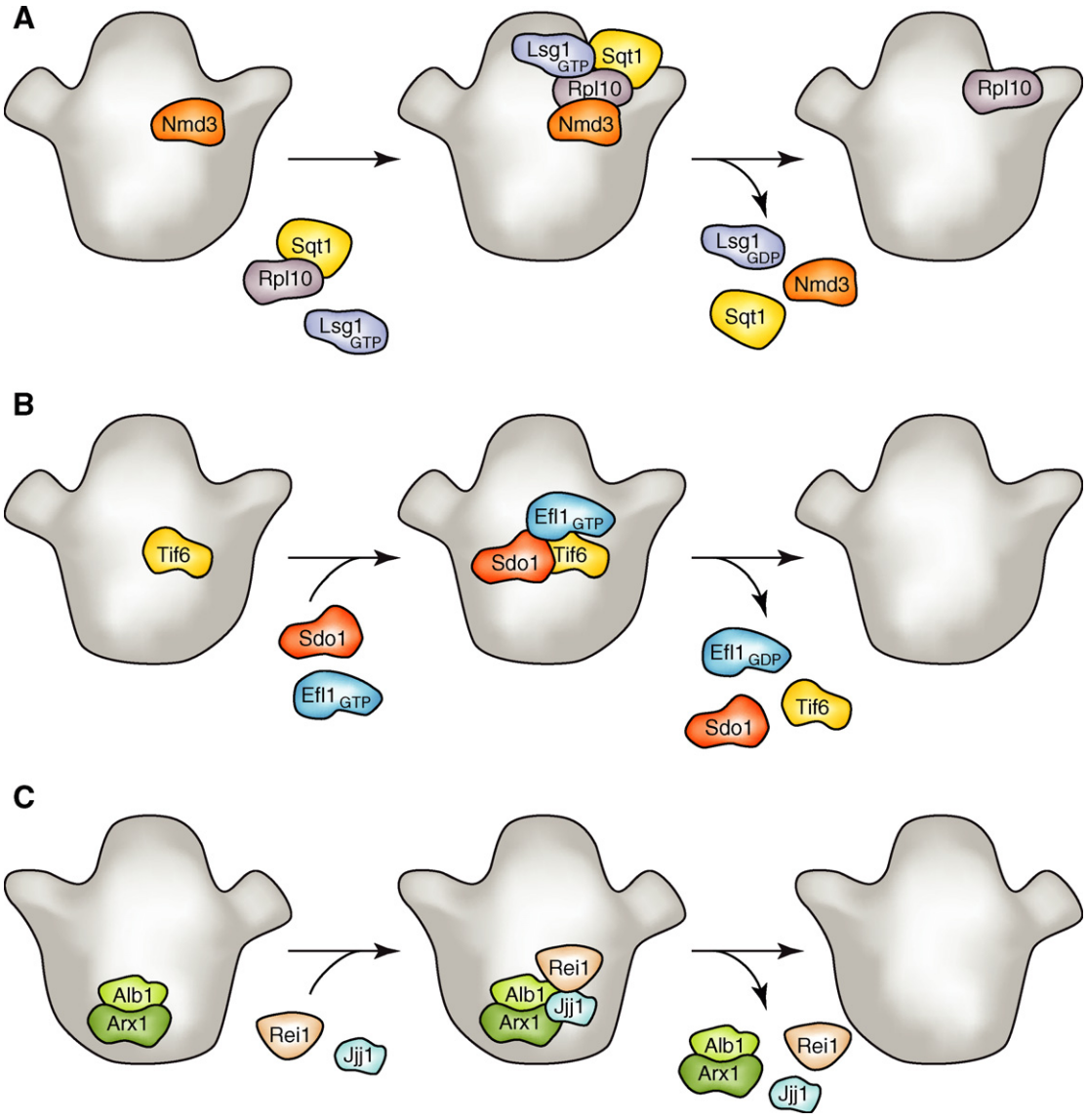

Fig. 1. Cytoplasmic maturation of 60S subunit precursors in yeast. (A) Release of Nmd3p from cytoplasmic pre-60S particles requires the GTPase Lsg1p and is coupled to loading of Rpl10p onto the subunit. GTP hydrolysis by Lsg1p might force the disassociation of both Nmd3p and the Rpl10p chaperone Sqt1p from 60S pre-ribosomes. (B) Tif6p recycling is mediated by Sdo1p and the GTPase Efl1p. Sdo1p might recruit Efl1p, whose GTPase activity triggers the dissociation of Tif6p from pre-60S particles. (C) Recycling of the heterodimer Arx1p/Alb1p from exported pre-60S subunits requires Rei1p and the J-protein Jjj1p.

[10–12]. Further, export of 60S subunits was shown to involve the RanGTPase system [10–12], which controls the directionality of nuclear transport pathways relying on RanGTP-binding transport receptors (reviewed in [13]). For instance, mutations in the RanGEF *PRP20* or the RanGAP *RNA1* lead to nucleoplasmic accumulation of pre-60S particles [10–12]. Based on these data, it was assumed that a RanGTP-binding exportin supports nuclear export of 60S subunits.

Of the export factors tested, the protein export receptor Crm1p was shown to be a major contributor to pre-60S export. Blocking Crm1p-mediated export in a strain carrying a leptomycin B (LMB)-sensitive allele of Crm1p (T539C) leads to nuclear accumulation of Rpl25-GFP [14,15]. Crm1p binds its export substrates by recognizing short leucine-rich export signals (reviewed in [16]). The nuclear export sequence (NES) for Crm1p-mediated pre-60S export is likely provided by the adapter protein Nmd3p, a *trans*-acting factor associated with late pre-60S particles [8,14,15]. Nmd3p was first identified in a screen for components of the non-sense-mediated mRNA decay (NMD) pathway [17], but further analysis revealed that it is involved in 60S ribosome biogenesis rather than in NMD [18,19]. Nmd3p is a cytosolic protein that constantly shuttles in and out of the nucleus [14,15]. Deletion of a C-terminal NES of Nmd3p yields a dominant negative mutant that induces accumulation of Rpl25-GFP in the nucleus. Addition of a heterologous NES to the dominant negative Nmd3p mutant rescues the 60S biogenesis defect, indicating that the Crm1p-dependent NES activity of Nmd3p is required for pre-60S export [14,15]. It is, however, not yet entirely clear if Nmd3p acts as a bona fide export adapter in 60S export, as Nmd3p-dependent recruitment of Crm1p and Gsp1p/Gsp2p (the yeast Ran proteins) to pre-60S particles has not been