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Review

# The power of AAA-ATPases on the road of pre-60S ribosome maturation — Molecular machines that strip pre-ribosomal particles ☆

Dieter Kressler [a], Ed Hurt [b], Helmut Bergler [c,*], Jochen Baßler [b,**]

[a] University of Fribourg, Department of Biology, Unit of Biochemistry, Chemin du Musée 10, 1700 Fribourg, Switzerland
[b] Biochemie-Zentrum der Universität Heidelberg, Im Neuenheimer Feld 328, 69120 Heidelberg, Germany
[c] Institut für Molekulare Biowissenschaften, Karl-Franzens Universität Graz, Humboldtstrasse 50, A-8010 Graz, Austria

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ABSTRACT

The biogenesis of ribosomes is a fundamental cellular process, which provides the molecular machines that synthesize all cellular proteins. The assembly of eukaryotic ribosomes is a highly complex multi-step process that requires more than 200 ribosome biogenesis factors, which mediate a broad spectrum of maturation reactions. The participation of many energy-consuming enzymes (e.g. AAA-type ATPases, RNA helicases, and GTPases) in this process indicates that the expenditure of energy is required to drive ribosome assembly. While the precise function of many of these enzymes remains elusive, recent progress has revealed that the three AAA-type ATPases involved in 60S subunit biogenesis are specifically dedicated to the release and recycling of distinct biogenesis factors. In this review, we will highlight how the molecular power of yeast Drg1, Rix7, and Rea1 is harnessed to promote the release of their substrate proteins from evolving pre-60S particles and, where appropriate, discuss possible catalytic mechanisms. This article is part of a Special Issue entitled: AAA ATPases: structure and function.



## 1. Introduction

Ribosomes are the molecular machines that translate the genetic information contained within messenger RNAs into proteins. While there is a detailed understanding of eukaryotic ribosomes at both a structural and functional level [1–3], much less is known about the intricate assembly process of the ribosomal subunits. The biogenesis of ribosomes is initiated in the nucleolus by the transcription of a common large precursor of mature ribosomal RNAs. The nascent precursor rRNA (pre-rRNA), which undergoes snoRNP-mediated modification of nucleotides, assembles with some ribosomal proteins and early biogenesis factors to form the first pre-ribosomal particles. Concomitant to or shortly after completion of transcription, the pre-rRNA undergoes endonucleolytic cleavages that separate the precursor particles to the mature 40S and 60S ribosomal subunits. These pre-40S and pre-60S particles mature further in the nucleolus and nucleoplasm before being exported to the cytoplasm, where final maturation events yield the translation-competent ribosomal subunits [4–10]. Proteomic analyses have identified many distinct pre-ribosomal particles that can be chronologically ordered along the maturation pathway from the nucleolus to the cytoplasm (Fig. 1). These landmark pre-ribosomal particles significantly differ in protein and (pre-)rRNA composition, thus highlighting the remarkable dynamics and complexity of shaping the rRNA and its associated ribosomal proteins into the correct structure.

The combination of genetic, cell biological, and proteomic methods has revealed that more than 200, mostly essential, non-ribosomal factors (also called biogenesis factors or protein *trans*-acting factors) contribute to eukaryotic ribosome biogenesis. While some of these factors are directly involved in the modification and processing of the pre-rRNA, others stabilize the pre-ribosomal particles, promote formation of productive RNA folding intermediates, or act as placeholders for selected ribosomal proteins that are recruited at a later time point in biogenesis. A further set of factors are essential for, or facilitate, the export of pre-ribosomes through the nuclear pore complex (NPC). A prominent number of biogenesis factors belong to different classes of energy-consuming enzymes, including ATP-dependent RNA helicases, GTPases, protein kinases, and three AAA-type ATPases [4,7]. Nucleotide binding or hydrolysis by these enzymes is believed to be instrumental for the promotion or regulation of key biogenesis steps, thus conferring directionality and accuracy to the assembly process. For recent reviews on non-ribosomal factors and their functions during ribosome biogenesis, see [4,5,7].

In this review, we focus on the molecular roles of the AAA-ATPases (ATPases associated with various cellular activities) involved in

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* Corresponding author. Tel.: +43 3163805629; fax: +43 3163809898.
** Corresponding author. Tel.: +49 6221 54 67 57; fax: +49 6221 54 43 69.
*E-mail addresses:* helmut.bergler@uni-graz.at (H. Bergler), jochen.bassler@bzh.uni-heidelberg.de (J. Baßler).

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ribosome biogenesis. To date, a role in this process could be attributed to three essential AAA-ATPases, namely Rix7 (ribosome export), Rea1/Mdn1 (ribosome export associated/midasin), and Drg1/Afg2 (diazaborine resistance gene/ATPase family gene), which act at distinct steps during 60S subunit biogenesis in the yeast *Saccharomyces cerevisiae*. AAA-ATPases contain at least one structurally conserved ATPase module that assembles into functionally active ring structures. In addition to the conserved Walker A and Walker B motifs, they are characterized by class-specific elements that also contribute to ATP hydrolysis, such as the sensor-I, sensor-II and the arginine finger [11]. Within these molecular machines, cycles of nucleotide binding and hydrolysis induce conformational changes that affect a broad range of substrate proteins [11,12].

While the AAA-ATPases Rix7 and Drg1 are closely related to the well-characterized Cdc48 (p97 in mammals), Rea1, which is the largest yeast protein, shares similarity to the microtubule motor protein dynein heavy chain (Dyn1). Interestingly, all three AAA-ATPases promote the release of distinct biogenesis factors from nucleolar (Nsa1 by Rix7), nucleolar and nucleoplasmic (Ytm1-Erb1-Nop7 and Rsa4 by Rea1) and cytoplasmic (several shuttling factors by Drg1) pre-60S intermediates (Fig. 1) [13–16]. The release of these factors from pre-60S particles ensures their recycling and likely triggers conformational changes that are critical determinants for the progression of ribosome assembly, e.g. promoting export or subunit-joining competence.

## 2. The type II AAA-ATPases Drg1 and Rix7 are closely related to Cdc48/p97

Drg1 and Rix7 are essential, eukaryote-specific AAA-ATPases that belong to the classical AAA clade [12,17–19]. Proteins of the classical clade are structurally defined by the presence of a short additional α-helix between β-strand 2 and α-helix 2 within their AAA domains (see Fig. S1). The high sequence similarity of their AAA domains clearly separates this classical clade from the other AAA+ clades; i.e. (i) the generally conserved sensor-II arginine is replaced by an alanine, (ii) there is a short insertion within the arginine finger region leading to the occurrence of two conserved arginines (Fig. S1) [12,17]. Analysis of the improved structures of p97 suggests that the first of the two arginines, and not the conventional arginine, may be involved in catalysis [20].

Due to the strong sequence similarity of Drg1 and Rix7 to Cdc48/p97/VCP – they are in fact the closest relatives of Cdc48 – Drg1 and Rix7 can be further classified as members of the NSF/Cdc48/Pex family of AAA proteins [12]. These type II AAA-ATPases [21] notably contain two consecutive AAA domains (designated D1 and D2) that are preceded by a specific N-terminal domain, which is generally involved in substrate recognition (see below, Fig. 2). While the sequence conservation between Rix7 and Cdc48 is restricted to the two AAA domains, the similarity between Drg1 and Cdc48 extends to the N-terminal domain (see below, Fig. S2). Secondary and tertiary structure prediction suggests that the N-terminal domain of Drg1, like the N-terminal extensions of p97, NSF, PEX1, and the archaeal VCP-like AAA-ATPase VAT [22–28], folds into two sub-domains: a double-psi (ψ) beta (β) barrel Nn-domain and a four-stranded β-barrel Nc-domain (see Fig. S2).

Intriguingly, the N-terminal domain of Rix7 differs structurally from the double-ψ β barrel/four-stranded β-barrel sub-domain organization that is prevalent among NSF/Cdc48/Pex family members. Secondary structure predictions suggest a primarily α-helical organization that is followed by a short linker region and a bipartite nuclear localization signal (NLS) (see Fig. S3A and B). A recently released NMR structure reveals that the first 74 amino acids of the Rix7 mouse orthologue NVL (nuclear VCP-like protein) fold into a three-helix bundle [29]. Even

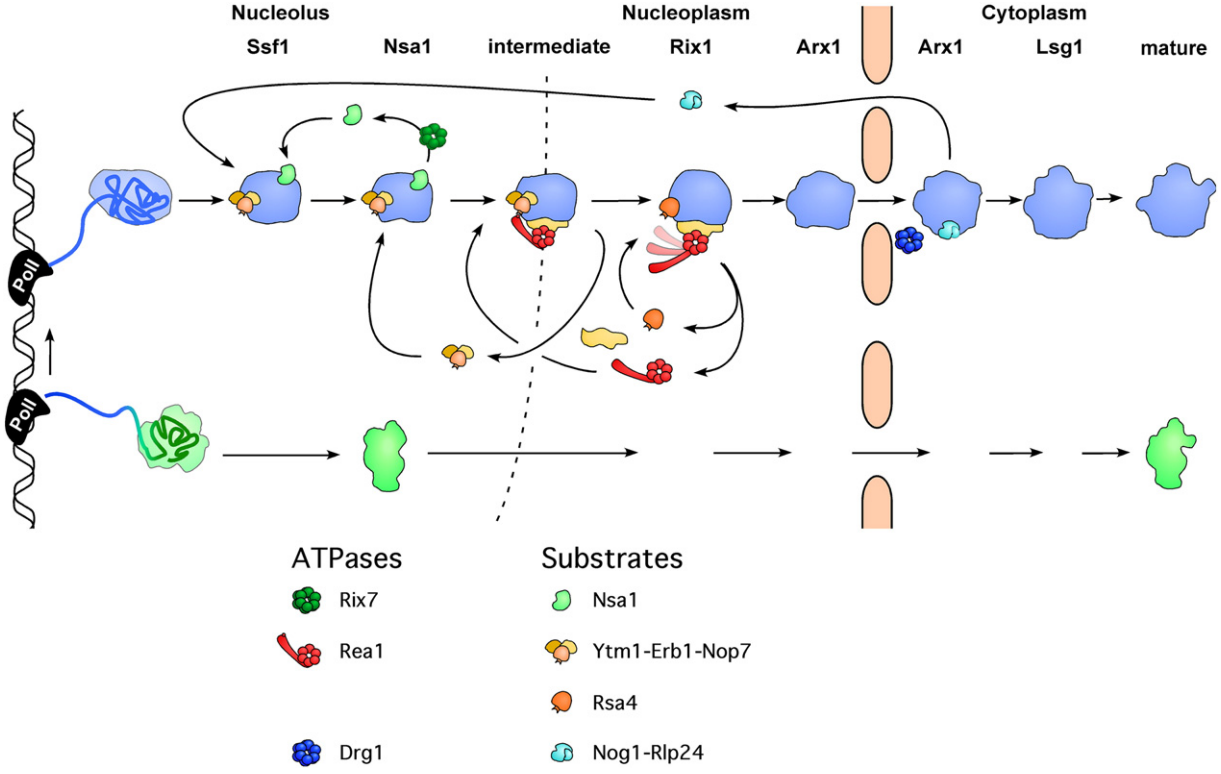


**Fig. 1.** Biogenesis of the large (blue) and small (green) ribosomal subunit and the contribution of AAA-ATPases. Rix7, Rea1, and Drg1 are dedicated to the release and recycling of distinct biogenesis factors from different pre-60S particles. Major landmark of pre-60S ribosomes are depicted together with their corresponding bait proteins. These factors, namely Ssf1, Nsa1, Rix1, Arx1, and Lsg1, are only associated during a short time window with pre-60S particles and thus co-purify rather distinct pre-60S ribosomes. Ssf1 and Nsa1 purify nucleolar particles, whereas Rix1 purifies a nucleoplasmic intermediate. Arx1 represents an export-competent particle that carries export factors. Finally, Lsg1 is associated with an almost mature, cytoplasmic 60S particle. The three AAA-ATPases and their potential substrates are indicated.