1998 - 2002	Ph.D. - European Molecular Biology Laboratory (EMBL) Heidelberg, Germany and Charles University in Prague, Czech Republic
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Ribosome biogenesis

Goal

Our aim is to understand how ribosomal RNAs are processed, correctly folded and assembled with proteins to form functional ribosomes.

Background

Ribosome biogenesis is a major energy consuming process in all organisms that is tightly regulated with cell growth. This highly conserved process begins with transcription of a large ribosomal RNA (rRNA) precursor that is subsequently covalently modified and processed into mature 18S, 5.8S and 25S rRNAs (Figure 1). Pre-rRNA processing takes place within very large particles (>2MDa) called pre-ribosomes, where the rRNA is properly processed, folded and assembled with ribosomal proteins. The process of ribosome maturation is very complex and highly dynamic; it takes only 6 minutes to make a functional mature ribosome. At least 180 non-ribosomal proteins and 70 small nucleolar RNAs (snoRNAs) have been implicated in ribosome biogenesis in yeast. However, the precise function of most of the factors remains unclear. The goal of the lab is to extend our understanding of the molecular mechanism underlying ribosome biogenesis and its regulation.

Research Highlights

RNA helicases in ribosome biogenesis

Mature rRNAs have a very complex structure that appears to be incompatible with their processing and assembly. Unsurprisingly, 19 RNA helicases were found to be essential for ribosome biogenesis in yeast. We investigate their role in rRNA processing. We could show that the RNA helicase Has1 has a dual and independent role in the synthesis of both small and large ribosomal subunits. Using RNA-protein crosslinking and affinity purifications, we have found that two molecules of Has1 bind co-transcriptionally to the pre-rRNA at two distinct sites and are required for pre-rRNA cleavage at the A2 site, which separates the small and large subunit pathways. After cleavage, the two Has1 molecules remain associated with their respective substrates (20S and 27S rRNAs) and likely facilitate recycling of associated ribosome biogenesis factors.

Role of rRNA modifications

In addition to the complex structure, rRNAs are also extensively modified by methylation and pseudouridylation at approximately 100 sites. The role of these modifications in either biogenesis or function of mature ribosomes remains largely unclear. We analyzed the role of a cluster of

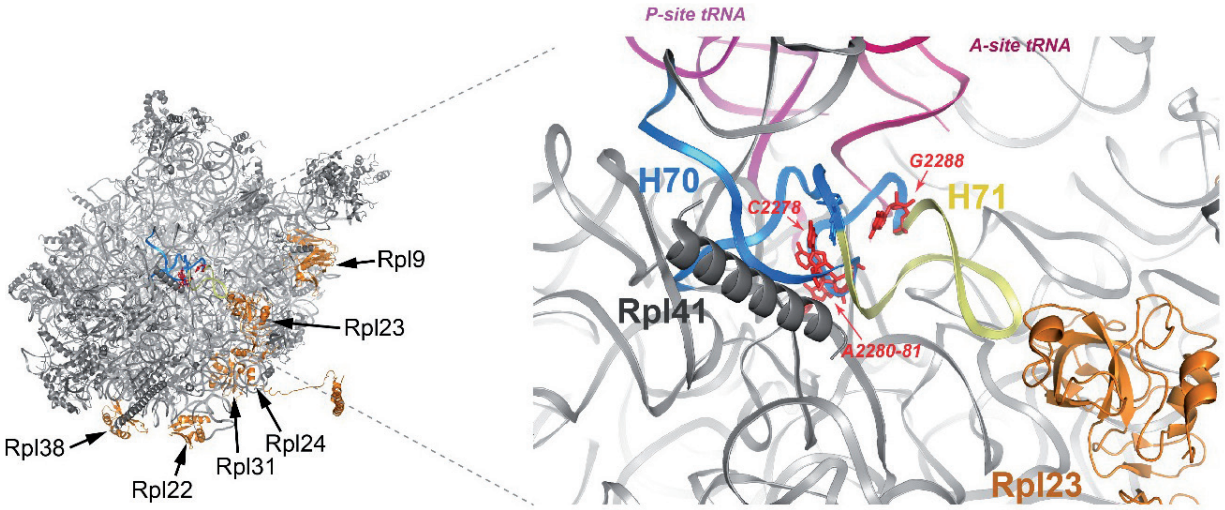

Fig. 1. Proteins affected in ribosomes lacking methylations of nucleotides C2278 and G2288. *Left: crystal structure of the 60S ribosomal subunit. Proteins that are lost from ribosomes lacking rRNA methylation are highlighted in orange. Right: Detailed view of the domain IV of 25S rRNA. The methylated nucleotides are in red, the helix H71, which participates in the intersubunit bridge B3, is highlighted in yellow.*

rRNA methylations located near the inter-subunit bridge B3 in 25S rRNA. We identified Rcm1 as an enzyme required for highly conserved cytosine-5 methylation of C2278 in the 25S rRNA. We could then show that methylation at two sites, C2278 and G2288 is required for stability of the mature 60S ribosomal subunit. Ribosomes lacking these two methylations exhibited changes in the structure of 25S rRNA and the loss of several ribosomal proteins (Fig. 1).

Timing of ribosome biogenesis events

At least 180 biogenesis factors and 82 ribosomal proteins are known to be required to build a mature ribosome. Affinity purification of various proteins led to the identification of a large number of intermediate pre-ribosomal complexes. Their overlapping composition allowed the various factors to be roughly assigned different stages of ribosome biogenesis. However, more precise information about when they join and leave the pathway is lacking for most of these factors. We attempted to tackle this problem from another direction, to purify pre-ribosomes directly through the pre-rRNA. We created strains with truncated rRNA genes (native promoter and terminator context), each with an RNA affinity tag at its 3'end. This allows us to express pre-RNAs truncated at a chosen position and thus purify "arrested" pre-ribosomes with a theoretical nucleotide resolution. Our system faithfully reproduces pre-rRNA processing and provides information about timing of all cleavages. We can also analyze timing of rRNA modifications and joining of individual processing factors. We are currently optimizing purification of the associated complexes.

Selected Publications

Gigova, A., Duggimpudi S., Polex T., Schaefer M. and Koš M. (2013). A cluster of methylations in 25S rRNA is required for ribosome stability. (submitted)

Gnanasundram S. and Koš M. (2013). Has1 RNA helicase is required for two distinct processing events during ribosome biogenesis. (submitted)

Kos-Braun,I.C., Jung, I. and Koš M. (2013). Timing of ribosome biogenesis with nucleotide resolution using truncated rDNA genes. (submitted)

Ross,D.A., Barrass, J.D., Dichtl,B., Koš,M., Obtulowicz,T., Robert,M.-C., Koper,M., Karkusiewicz,I., Mariconti,L., Tollervey,D., Dichtl,B., Kufel,J., Bertrand,E. and Beggs,J.D. (2010). RiboSys, a high-resolution, quantitative approach to measure the *in vivo* kinetics of pre-mRNA splicing and 3'-end processing in *Saccharomyces cerevisiae*. *RNA* 16, 2570-2580.

Boon,K.-L. and Koš,M. (2010). Deletion of Swm2p selectively impairs trimethylation of snRNAs by Trimethylguanosine synthase (Tgs1p). *FEBS Lett.* 584, 3299-3304.

Koš,M. and Tollervey,D. (2010). Yeast Pre-rRNA Processing and Modification Occur Cotranscriptionally. *Mol. Cell* 37, 809-820.

Bohnsack, M.T., Koš,M. and Tollervey,D. (2008). Quantitative analysis of snoRNA association with pre-ribosomes and release of snR30 by Rok1 helicase. *EMBO Rep.* 9, 1230-1236.

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