Trends in Plant Science

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Review

Plant-Specific Features of Ribosome Biogenesis

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The biogenesis of eukaryotic ribosomes is a fundamental process involving hundreds of ribosome biogenesis factors (RBFs) in three compartments of the cell, namely the nucleolus, nucleus, and cytoplasm. Many RBFs are involved in the processing of the primary ribosomal (r)RNA transcript, in which three of the four rRNAs are imbedded. While pre-rRNA processing is well described for yeast and mammals, a detailed processing scheme for plants is lacking. Here, we discuss the emerging scheme of pre-rRNA processing in *Arabidopsis thaliana* in comparison to other eukaryotes, with a focus on plant characteristics. In addition, we highlight the impact of the ribosome and its biogenesis on developmental processes because common phenotypes can be observed for ribosomal protein and RBF mutants.

Trends

Ribosome biogenesis is a central process in all eukaryotic cells, but is best studied in yeast. Although there is an overall conservation of ribosome biogenesis in eukaryotes, recent results have shown significant diversions between ribosome biogenesis in fungi, mammals, and plants.

Pre-ribosomal (r)RNA processing is a key process during ribosome biogenesis and has been elucidated in detail, mainly for *Arabidopsis thaliana*, leading to a complex scheme for plant pre-rRNA processing that follows two alternative routes, representing a hybrid between the yeast and mammalian pathways.

Eukaryotes have a distinct pool of ribosome biogenesis factors (RBFs) with respect to the number of genes encoding a RBF and the existence of species-specific factors.

With the analysis of an increasing number of RBFs in various plant species, general developmental abnormalities linked to defects in ribosome biogenesis have been observed.

The Eukaryotic Ribosome

Eukaryotic ribosomes comprise two ribosomal subunits that together form the 80S ribosome. The **small ribosomal subunit** (SSU, 40S; see Glossary) contains the 18S **ribosomal (r)RNA** together with approximately 33 **ribosomal proteins** (RPs) and functions in mRNA decoding. The **large ribosomal subunit** (LSU, 60S) comprises 25/28S, 5.8S, and 5S rRNAs together with approximately 47 RPs and carries out the peptidyl transferase reaction leading to peptide bonds. This process is thought to be mediated by the rRNA, making the ribosome a ribozyme [1]. Recent crystal structures of prokaryotic and eukaryotic ribosomes [2–4] and precursor- (pre-) ribosomes [5–7] improved our understanding of ribosome assembly and function. Yet, cryo-electron microscopy (cryo-EM) or crystal structures of plant (pre-)ribosomes are lacking. This might be due to the high heterogeneity of cytosolic ribosomes exemplified for *Arabidopsis thaliana* [8], in which each of the 80 RPs is encoded by two to seven paralogs [9]. Interestingly, the composition of plant ribosomes is dependent on the developmental stage [10] and the type of tissue [11], and is altered after environmental stimuli [12]. Similarly, post-translational modifications of ribosomal proteins depend on environmental stimuli, as exemplified best for Rps6 phosphorylation [13,14]. The impact of plant ribosomal proteins and the plant ribosome on development was nicely discussed in a previous review [15]. Here, we summarize current knowledge of ribosome biogenesis and **pre-rRNA processing** in plants (see Box 1 for a summary of the plant specifics in these processes) and its relevance for plant development.

Specifics of Plant Ribosome Biogenesis

The biogenesis of eukaryotic ribosomes is a fundamental process to all cells and starts with the transcription of a polycistronic primary rRNA transcript in a specialized nuclear compartment, the **nucleolus**. Although the nucleolus is not enclosed by a membrane and is very dynamic, because it is involved in the control of numerous cellular functions, it is tightly packed, which has enabled its purification for proteomic analysis in plant or human cells [16,17]. The bipartite nucleolus as it can be found in yeast and plant cells can be subdivided according to EM images into the fibrillar component (FC), where **ribosomal DNA** (rDNA) transcription and first steps of

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Box 1. Plant-Specific Features of Ribosome Biogenesis

Ribosome biogenesis is highly energy demanding and involves all RNA polymerases as well as a plethora of RBFs, which are best studied in yeast. For some yeast RBFs, multiple orthologs can be identified in the genome of various plant species, whereas many known yeast RBFs do not have an ortholog in plants [22,23]. Furthermore, some of the orthologs to yeast RBFs have a different function or have gained additional functions in plants [38,75]. This favors the existence of plant-specific RBFs and, thus, plant-specific events in ribosome biogenesis.

Recent studies of plant ribosome biogenesis mostly come from the model organism *Arabidopsis thaliana*. In this species, the ribosome itself is special because all 80 RPs are encoded by two to seven paralogs, leading to a high heterogeneity; this is because they are built into functional ribosomes depending on developmental stages or the tissue, or after certain environmental stimuli [15]. The primary rRNA transcript, which encodes three of the four mature rRNAs, contains a huge insertion that is specific for *A. thaliana* [33]. Although a function for this insertion is not yet established, it might have a regulatory role in transcript stability or efficiency of pre-rRNA processing.

Pre-rRNA processing in *A. thaliana*, and likely also in other plant species, follows at least two alternative routes (Figure 1, main text), and these appear to be a mixture between the mammalian and yeast pre-rRNA processing pathways [49,51]. However, these alternative routes are more independent of each other compared with mammalian cells and one independent processing site has also been identified [42,49]. The coexistence of alternative routes is thought to secure the generation of sufficient amounts of ribosomes or gives rise to regulation under certain stress situations [49–51]. Consistent with this notion, the analysis of RBFs in *A. thaliana* and other plant species revealed a tight connection between ribosome biogenesis and development of plant organs such as cotyledons and young leaves [38,59,75,82]; in addition, and many genes encoding plant RPs are under the control of TOR, a master regulator of cell cycle control [69,70].

ribosome assembly take place, and the granular component (GC), where further ribosome biogenesis steps occur [18,19]. The plant nucleolus is specific because it contains a unique feature in the center called the nucleolar cavity, also termed the 'nucleolar vacuole' [19]. The nucleolar cavity contains several immature **small nucleolar (sno)RNAs**, which are usually packed in **sno-ribonucleoproteins** (snoRNPs) and are key players in rRNA modification and pre-rRNA processing (Box 2) [20]. Thus, this region might be a place for the storage or preprocessing of snoRNAs [20]. However, comprehensive studies of the role of the nucleolar cavity are lacking.

In yeast, approximately 250 so-called '**RBFs**' of several protein classes are involved in the pre-rRNA transcription, processing, modification, folding, and incorporation of RPs [21]. This

Box 2. Plant snoRNAs and rRNA Modifications

SnoRNAs, as their name implicates, are noncoding RNAs (ncRNAs) found in the nucleolus, but they share sequence motifs with other ncRNAs, such as the snRNAs involved in pre-mRNA splicing [83]. Most snoRNAs have a function in rRNA modification, whereas a few are also essential for pre-rRNA processing [83]. In general, snoRNAs can be divided into two classes according to conserved sequence boxes: box C/D snoRNAs direct 2′-*O*-methylation of the ribose, whereas box H/ACA snoRNAs direct the conversion of uridine to pseudouridine (ψ). *In vivo*, snoRNAs are associated with a conserved set of proteins to form functional snoRNPs. In yeast, box C/D snoRNAs are bound by the kink-turn binding protein Snu13, the scaffold proteins Nop56 and Nop58, and the methyltransferase Nop1 (fibrillarin in plants and humans). Box H/ACA snoRNAs assemble with Nhp2, Gar1, Nop1, and the ψ-synthase Cbf5 (dyskerin in humans and NOP57 in plants). The architecture of both classes of snoRNP is bipartite and, thus, each snoRNP could modify two positions in rRNA. Even though the modifications introduced by many snoRNPs are not essential for ribosome function, they could lead to the translation of specific mRNAs or might enhance efficiency and accuracy of translation. Besides snoRNA-mediated rRNA modification, lone-standing enzymes are involved in base methylation or acetylation (e.g., [84,85]). In plants, the cytoplasmic dimethylase DIM1A, responsible for adenosine dimethylation, as well as the homolog of the methyltransferase Bud23/WBSCR22, have recently been characterized [79,84].

The organization of snoRNAs in the genome as well as their number in plants is different compared with yeast or mammals. While most snoRNAs in yeast are independently transcribed and most human snoRNAs are imbedded in introns, plant snoRNAs are often transcribed as a polycistronic transcript, which comprises both homologous or heterologous snoRNAs [86,87]. Furthermore, plants contain a huge amount of 2′-*O*-methylations and ψ residues in their rRNAs and, accordingly, the largest number of snoRNAs in eukaryotes known so far, but the list is far from being complete, especially because H/ACA snoRNAs are hard to predict [87–90]. In yeast, 75 snoRNAs are known to be involved in ribosome biogenesis [83], whereas more than 140 snoRNAs haven been proposed for *A. thaliana*. Among these are many orphan snoRNAs without complementary sites to other RNAs [91,92].

Glossary

$A_{123}B$ cluster: a conserved sequence containing three repeats of eight nucleotides, located in the 5′-ETS in the primary rRNA transcript in Brassicaceae.
External transcribed spacer (ETS): noncoding RNA sequences surrounding the mature rRNAs in the primary rRNA transcript.
Large subunit (LSU): 50S in prokaryotes and 60S in eukaryotes, responsible for peptide bond formation.
Internal transcribed spacer (ITS): noncoding RNA sequences in between the mature rRNAs in the primary rRNA transcript.
Nucleolus: compartment within the nucleus that is not enclosed by a membrane; place for rDNA transcription and first ribosome biogenesis steps.
Pre-rRNA processing: a coordinated process in all cells to excise mature rRNAs from a primary rRNA transcript (Figure 1, main text).
Ribosome biogenesis factor (RBF): a protein or RNA involved in ribosome biogenesis.
Ribosomal DNA (rDNA): the gene encoding the primary rRNA transcript as well as the 5S rRNA that is found with hundreds to thousands of copies on the genomic DNA.
Ribosomopathies: severe developmental abnormalities caused by mutations in RBFs or RPs (see Figure 2 for developmental defects in plants caused by RBF mutants, main text).
Ribosomal protein (RP): a structural constituent of the mature ribosome.
Ribosomal RNA (rRNA): a constituent of the ribosome that gives the characteristic structure and has catalytic activity.
Small nucleolar RNA (snoRNA): a constituent of snoRNPs.
Small nucleolar ribonucleoprotein (snoRNP): comprises snoRNA and a core set of proteins; its main function is the modification of rRNA.
Small subunit (SSU): 30S in prokaryotes and 40S in eukaryotes, involved in decoding mRNA.
Small subunit processome (SSU processome): the first visible particle on the nascent pre-rRNA that mostly contains SSU RBFs and SSU RPs.
Target of rapamycin (TOR): master regulator of cell growth and cell cycle.