REVIEW

Ribosome Biogenesis in Plants: from Functional 45S Ribosomal DNA Organization to Ribosome Assembly Factors

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Short title: rRNA synthesis and ribosome assembly

One-sentence summary: The functional organization of ribosomal DNA and ribosome assembly in plants is described, along with recent findings concerning a plant-specific mechanism involved in ribosome stress responses.

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ABSTRACT

The transcription of 18S, 5.8S and 18S ribosomal RNA (rRNA) genes (45S rDNA), co-transcriptional processing of pre-rRNA, and assembly of mature rRNA with ribosomal proteins are the linchpins of ribosome biogenesis. In yeast (Saccharomyces cerevisiae) and animal cells, hundreds of pre-rRNA processing factors have been identified and their involvement in ribosome assembly determined. These studies, together with structural analyses, have yielded comprehensive models of the pre-40S and pre-60S ribosome subunits, as well as the largest co-transcriptionally assembled pre-ribosome particle: the 90S/SSU (Small SubUnit) processome. Here, we present our current knowledge of the functional organization of 45S rDNA, pre-rRNA transcription, rRNA processing activities, and ribosome assembly factors in plants, focusing on data from Arabidopsis thaliana. Based on yeast and mammalian cell studies, we describe the ribonucleoprotein complexes and RNA-associated activities and discuss how they might specifically affect production of 40S and 60S subunits. Finally, we review recent findings concerning pre-rRNA processing pathways and a novel mechanism involved in a ribosome stress response in plants.

INTRODUCTION

1 Eukaryotic ribosomes (80S) are made up of a large (60S) and a small (40S) ribosome subunit, with the S standing for the Svedberg unit sedimentation

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coefficient. 80S ribosome biogenesis involves the production and correct assembly of four ribosomal RNAs (rRNAs), 18S, 5.8S, 25S/28S and 5S, and approximately 80 ribosomal proteins (Figure 1).

The 18S, 5.8S, and 25S (or 28S in mammals) rRNAs are encoded by a single coding sequence or transcription unit known as 45S rDNA in plants, 47S rDNA in mammalian cells, and 35S rDNA in yeast. The 5S rRNA is encoded by transcriptionally separated 5S rDNA units. 45S, 47S and 35S rDNA are transcribed by RNA polymerase I (Pol I) in a process requiring a large number of General Transcription Factors (GTFs) (Russell and Zomerdijk, 2005; Saez-Vasquez and Echeverria, 2006; Schneider, 2012; Goodfellow and Zomerdijk, 2013). The resulting rRNA precursor transcripts (pre-rRNAs) are processed by numerous ribonucleoprotein (RNP) factors to obtain mature 18S, 5.8S and 25S/28S rRNAs (Fromont-Racine et al., 2003; Dez and Tollervey, 2004; Chedin et al., 2007; Ebersberger et al., 2014; Weis et al., 2015a). 5S rDNA is transcribed by RNA pol III via a process involving specific transcription factors TFIIIA, TFIIIB and TFIIIC (reviewed in (Ciganda and Williams, 2011; Layat et al., 2012; Orioli et al., 2012).

The 18S rRNAs assemble with ribosomal proteins of the small subunit (RPSs), while 5.8S and 25S/28S rRNAs assemble with ribosomal proteins of the large subunit (RPLs), as well as 5S rRNA, to form the small 40S and large 60S ribosome subunits, respectively. In mammalian, yeast, and plant cells, 40S ribosome subunits contain ~30 RPSs, and 60S ribosome subunits contain ~40 to 48 RPLs (Armache et al., 2010; Ben-Shem et al., 2011). Assembly of the ribosome subunits occurs co-translationally and requires more than 200 accessory factors known as ribosome biogenesis factors (RBFs; Supplemental Data Sets 1-4). Throughout transcription, the nascent pre-RNAs interact with RBFs, which are required for RNA processing and the assembly of RPSs and RPLs into pre-ribosomes (Figure 1).

Nearly 50 years ago, it was demonstrated that transcribed amphibian rRNA genes have a characteristic Christmas tree appearance when chromatin is spread and visualized by electron microscopy (Miller and Beaty, 1969). In these observations rDNA resembles the tree's trunk and nascent rRNA transcripts its "branches", whose tips are decorated with terminal "balls". Since that time, it has been shown that the terminal balls are large U3 snoRNP -associated complexes, named the 90S or SSU-processome (for small subunit), implicated in 45S pre-RNA processing and the
assembly of pre-40S ribosome particles (Mougey et al., 1993). Similarly, pre-60S ribosome particles can be observed at the ends of nascent pre-RNAs after cotranscriptional cleavage events in pre-rRNAs that release pre-40S particles (Phipps et al., 2011; Henras et al., 2015).

The processing of pre-rRNAs and the assembly of pre-ribosome subunit intermediates are relatively well understood in yeast and animals. In addition, the structures of several pre-40S complexes have been solved, including the 90S/SSU-processome and pre-60S complexes of yeast (Ben-Shem et al., 2011; Wu et al., 2016; Barandun et al., 2017; Chaker-Margot et al., 2017; Kater et al., 2017; Kressler et al., 2017; Sun et al., 2017; Ameismeier et al., 2018; Biedka et al., 2018; Sanghai et al., 2018).

rDNA transcription, pre-rRNA processing, and ribosome biogenesis have been studied for many years in plants. Initially, most of these studies addressed questions concerning rDNA organization in different plant species, including maize (Zea mays), common wheat (Triticum aestivum), rice (Oryza sativa), radish (Raphanus sativus), Brassica oleracea and rapa, Arabidopsis (Arabidopsis thaliana), tobacco (Nicotiana tabacum) and potato (Solanum tuberosum) (reviewed in (Saez-Vasquez and Echeverria, 2006)). Later, analysis of the A. thaliana genome allowed identification of all ribosomal proteins from the small and large ribosome subunits (Cooke et al., 1997; Barakat et al., 2001). In addition, biochemical approaches successfully identified and characterized RNA Pol I transcription and pre-rRNA processing complexes from Brassica species (Caparros-Ruiz et al., 1997; Saez-Vasquez and Pikaard, 1997, 2000; Saez-Vasquez et al., 2001; Saez-Vasquez et al., 2004a). Since then, genetic approaches combined with proteomic analysis of the nucleolus have led to the identification and characterization of RBFs and the mechanisms implicated in the functional organization of rDNA and/or the processing of rRNA precursors in plants.

Here we review these findings from plants and present an integrated picture of 45S rDNA organization and rRNA transcription in A. thaliana. Based on yeast and animal models, we discuss the events tied to pre-rRNA transcription that lead to the formation of the largest U3 snoRNP (small nucleolar Ribonucleoprotein) and/or SSU-processome complex. We also review current knowledge of the protein
complexes and biochemical activities required for the assembly of pre-60S particles in plants.

45S rDNA ORGANIZATION

Plant genomes contain hundreds to thousands of 45S rRNA genes, which are generally organized in a manner similar to the rDNA arrays of other organisms (Saez-Vasquez and Echeverria, 2006).

In the haploid A. thaliana (Col-0) genome, approximately 750 copies of the 45S rDNA repeat unit are localized in tandem arrays at the tops of chromosomes 2 and 4, corresponding to Nucleolus Organizer Regions 2 and 4 (NOR2 and 4) (Copenhaver and Pikaard, 1996a, b) (Figure 2). Under normal plant growth conditions, the rDNA of NOR4 is transcriptionally active, whereas the rDNA of NOR2 is transcriptionally silenced by repressive chromatin modifications (Chen and Pikaard, 1997; Fransz et al., 2002). Strikingly, the expression of rDNA within NOR2 is developmentally regulated (see below).

As depicted in Figure 2, the 45S rDNA transcribed sequence is a total of ~8.4 kilobase pairs (kb) in length and contains the 18S (1800 base pairs, bp); the 5.8S (161 bp) and 25S (3376 bp) rRNA sequences separated by Internal Transcribed Spacers (ITS1 and ITS2) and flanked by External Transcribed Spacers (5'ETS and 3'ETS) (Gruendler et al., 1989; Unfried et al., 1989; Unfried and Gruendler, 1990). Characterization of the 3'ETS revealed that 45S rDNA units are not all identical in A. thaliana (Col-0). There are four major classes or variants of 45S rDNA (VAR1-4), based on insertions/deletions (indels) in the 3'ETS (Figure 2A, (Earley et al., 2010; Pontvianne et al., 2010)). The 45S rDNA VAR1 class represents ~50% of the copies and maps to the inactive NOR2, while rDNA VAR2-4 primarily map to the active NOR4 (Chandrasekhara et al., 2016). Nonetheless, some rDNA VAR3 copies map to NOR2 (Chandrasekhara et al., 2016), while the low-abundance rDNA variant VAR4 also maps near the centromere of chromosome 3 (Abou-Ellail et al., 2011). A fifth rRNA gene variant (VAR5) was reported by (Havlova et al., 2016). The rDNA VAR5 was described as a VAR3 subtype based on the rDNA sequence length measured from the end of the 25S to the first Sal I restriction site in the 3'ETS (Figure 2B). Remarkably, the translocation of rRNA genes from inactive NOR2 to active NOR4 results in the expression of normally silenced rDNA variants.
This study demonstrated that the silencing of rRNA gene clusters in NOR2 depends on the chromosome on which they reside rather than on the sequences of these individual rRNA genes (Mohannath et al., 2016). In this context, the expression of rDNA VAR1 in *nucleolin2* (*nuc2*) plants is thought to result from the relocation of rDNA VAR1 copies from NOR2 to NOR4 (Durut et al., 2014).

The 5'ETS is 1.8 kb and contains the U3 snoRNP binding site A\textsuperscript{123}B and a 1.2 kb insertion found specifically in *A. thaliana* but not in other cruciferous plant genomes (Saez-Vasquez et al., 2004a). The 1.2 kb sequence contains five D (D1a, D1b, D2a, D12 and D2b) and two C motifs (C1 and C2) of unknown function (Figure 2A) (Gruendler et al., 1991).

Each 45S rDNA coding sequence is separated from the adjacent ones by ~2.2 kb InterGenic Spacer (IGS) regions, which contain the Sal I boxes (also called A motifs), two Spacer Promoters (SP1 and SP2) and the Gene Promoter (GP) (Figure 2A) (Gruendler et al., 1991).

The minimal GP sequence is from -55/-33 upstream to +6 downstream relative to the Transcription Initiation Site (+1, TIS) (Doelling and Pikaard, 1995). Remarkably, the sequence surrounding the TIS (TATATA\textsuperscript{GGGGG}, +1 is underlined) is highly similar among plant species ((Delcasso-Tremousaygue et al., 1988; Cordesse et al., 1993; Fan et al., 1995), reviewed in (Saez-Vasquez and Echeverria, 2006)).

Mutations in this consensus region abolish or inhibit rRNA transcription and perturb the position of transcription initiation (Doelling and Pikaard, 1995). The SP sequences share 90% similarity with the GP (from -92 to +6), but they are only ~10% active compared to the GP (Doelling et al., 1993). The SP and GP also contain multiple sites homologous to siRNA sequences, which are required for 45S rDNA silencing in hybrids (Preuss et al., 2008; Costa-Nunes et al., 2010).

Fewer studies have focused on the role of the IGS in rDNA organization or transcription. Two decades ago, the characterization of rRNA transgenes integrated into chromosomal regions outside NORs revealed that transgenic rDNA can associate with the nucleolus but that Sal I boxes do not enhance RNA Pol I transcription in this context (Wanzenbock et al., 1997). By contrast, IGS might play a role in rDNA recombination (Urawa et al., 2001). Recently, major differences were reported in the length and/or organization of the IGS in *A. thaliana*. However, all the
IGS rDNA sequences analyzed in this comprehensive study contain at least one SP and Sal I box clusters of variable sizes ranging from 294 to 1505 bp (Havlova et al., 2016). Importantly, there is no obvious structural or functional relationship between the 3'ETS and IGS, as rDNA VAR1-5 display similar variability in terms of the number and/or length of Sal I boxes and SP1/SP2 sequences (Havlova et al., 2016).

In animal cells, IGS transcripts are involved in rDNA repression (Bierhoff et al., 2010) and in nucleolar protein sequestering (Audas et al., 2012) in response to stress conditions. However, little is known about these mechanisms during plant development and/or in response to environmental stress.

**TRANSCRIPTION OF 45S rRNA GENES**

45S rDNA is transcribed by RNA Pol I in the nucleolus (Raska et al., 2004; Saez-Vasquez and Medina, 2008). RNA Pol I is a multisubunit protein enzyme that associates with additional GTFs to form larger RNA Pol I complexes or holoenzymes, which are competent to specifically initiate rDNA transcription (Saez-Vasquez and Pikaard, 1997; Seither et al., 1998; Albert et al., 1999).

In *A. thaliana*, RNA Pol I consists of 14 protein subunits: 12 are subunits homologous or common to those of all nuclear RNA polymerases (RNA Pol I-V), five of the RNA Pol I subunits are common to RNA Pol II and Pol III (subunits encoded by the same genes), and the two others are RNA Pol I-specific subunits (Ream et al., 2015). In plants, RNA Pol I holoenzyme purified from *B. oleracea* nuclear extracts was capable of binding specifically to the 45S rDNA promoter (GP) and initiating transcription *in vitro* (Saez-Vasquez and Pikaard, 1997, 2000). In addition, a CK2-like protein (CASEIN KINASE2) co-purifies with RNA Pol I holoenzyme activity (Saez-Vasquez et al., 2001). In mammalian cells, CK2 phosphorylates TIF-IA (TRANSCRIPTION INITIATOR FACTOR 1A), thereby increasing rDNA transcription (Bierhoff et al., 2008). The role of CK2 in plant rRNA gene expression has not been reported; however, CK2 has been implicated in multiple developmental and stress response pathways in plants (Mulekar and Huq, 2014).

Little is known about the GTFs and/or accessory factors required for RNA Pol I transcription initiation, elongation, or termination in plants (reviewed in (Saez-Vasquez and Echeverria, 2006)). However, the TFIIB-related protein, pBrp, is a plant-specific GTF for RNA Pol I. pBrp does not interact with the rDNA promoter but
forms a stable ternary complex with *A. thaliana* TBP2 (TATA BINDING PROTEIN2) and the rDNA promoter (Imamura et al., 2008).

More is known about the functional organization of rDNA chromatin, mostly thanks to studies involving rRNA gene silencing in interspecific hybrids called nucleolar dominance (Tucker et al., 2010; Ge et al., 2013; Michalak et al., 2015). For example, in the hybrid *Arabidopsis suecica*, rDNA derived from one parent (*A. thaliana*) is silenced by repressive chromatin modifications while the rDNA from the other parent (*A. arenosa*) remains active (Chen et al., 1998; Pikaard, 2000; Lawrence et al., 2004). Studies of nucleolar dominance also demonstrate that in transcriptionally active rDNA chromatin, cytosines are hypomethylated, and histone H3 is acetylated (H3Ac) and typically methylated at position lysine 4 (H3K4), whereas in inactive rDNA chromatin, cytosines are hypermethylated and histone H3 is typically methylated at position lysine 9 (H3K9) (Lawrence et al., 2004; Lawrence and Pikaard, 2004).

More recently, studies of rDNA variants in *A. thaliana* (Col-0) have allowed mechanisms controlling rDNA chromatin dynamics to be dissected in the context of non-hybrid plants. Together with the analysis of nucleolar dominance, this work addressed the question of how plants “select” one or another subset of 45S rDNA, because it is well known that not all rRNA gene copies are transcriptionally active in plants and other eukaryotic cells (Conconi et al., 1992; Gonzalez-Melendi et al., 2001; Fransz et al., 2002; French et al., 2003).

As mentioned above, in *A. thaliana* (Col-0), only a fraction of rRNA genes (those from NOR4) are transcribed throughout plant growth and development. NOR2 contains the rRNA VAR1 genes, which represent ~50% of rDNA and are silenced approximately 2 to 4 days after germination. Nevertheless, in globular embryos (Chen et al., 2016) and during the first few days after germination (Earley et al., 2006; Pontvianne et al., 2010), rRNA VAR1 transcripts can be detected, indicating that NOR2 is active during these stages of development (Figure 2B). The expression of rDNA VAR1 is correlated with the global decondensation of chromatin during germination (Layat et al., 2012).

Several protein factors have been described as transcriptional activators or repressors of NOR2 (Supplemental Data Set 1). For instance, rDNA VAR1 is
expressed in mutant plants with deficiencies of NUC1 or NUC2, HDA6 (HISTONE DEACETYLASE6), SUVH5/6 [SU(VAR)3-9HOMOLOG 5/6] or ATXR5/6 (ARABIDOPSIS TRITHORAX-RELATED PROTEINS5/6), FAS1 or FAS2 (FASCIATA1 or 2), CDC48A (Cell Division Cycle 48A), MAS2 (MORPHOLOGY OF AGO1-52 SUPPRESSED2), and ATRX (ALPHA THALASSEMIA-MENTAL RETARDATION X-LINKED), which indicates that NOR2 fail to be silenced in the absence of these protein factors (Earley et al., 2006; Mozgova et al., 2010; Pontvianne et al., 2010; Pontvianne et al., 2012; Pontvianne et al., 2013; Durut et al., 2014; Merai et al., 2014; Sanchez-Garcia et al., 2015; Duc et al., 2017). By contrast, embryonic expression of rDNA VAR1 was repressed in plants with mutations in THAL (THALLO) (Chen et al., 2016). Most of these proteins are histone chaperones (NUC1/2, FAS1/2 and ATRX), chromatin modifiers (SUVH5/6, ATXR5/6 and HDA6), and/or proteins involved in chromatin organization (CDC48 and MAS2). However, THAL is the homologue of Utp3, a protein that associates with the 90S/SSU-processome complex (Gallagher et al., 2004; Barandun et al., 2017) (see below). In A. thaliana, NUC1 and NUC2 play antagonistic roles in rRNA gene expression. While NUC1 appears to facilitate rDNA transcription (Pontvianne et al., 2010), NUC2 induces and/or maintains a repressive rDNA chromatin state (Durut et al., 2014). Nonetheless, the expression of NUC2 appears to rescue the absence of NUC1 in nuc1 plants ((Pontvianne et al., 2007) and reviewed in (Durut and Saez-Vasquez, 2015)).

Furthermore, rDNA VAR1 is expressed in mutant plants deficient for RRP7 (RNA RIBOSOMAL PROTEIN 7) or SMO4/Nop53 (SMALL ORGAN 4/NUCLEOLAR PROTEIN 53) (Micol-Ponce et al., 2018), which are proteins that associate with the 90S/SSU-processome and 60S pre-ribosome complexes (see below).

Additional factors affect or have been linked to rDNA functional organization and expression. For instance, RTEL1 (REGULATOR OF TELOMERE ELONGATION HELICASE1) is required for stabilizing 45S rDNA repeats, while simultaneous mutation of RTEL1 with RMI2 (RecQ-MEDIATED GENOME INSTABILITY PROTEIN 2) provokes the increased loss of 45S rDNA repeats (Rohrig et al., 2016), similar to fas mutants (Pontvianne et al., 2013). Overexpression of TOR (TARGET OF RAPAMYCIN) induces the accumulation of 45S rRNA transcripts, whereas overexpression of RPS6 (RIBOSOMAL PROTEIN SMALL SUBUNIT6) represses
this process (Ren et al., 2011; Kim et al., 2014). Moreover, 45S rRNA genes are overexpressed in plants with reduced FKBP53 (FK506 BINDING PROTEIN) gene expression (Li and Luan, 2010). In addition, the 3xHMG-box1 (HIGH MOBILITY GROUP) protein binds to 45S rDNA loci via its basic N-terminal domain (Antosch et al., 2015). It is not known whether 3xHMG-box1 is related to UBF (Upstream Binding Protein) and/or Hmo1 (High Mobility 1), two HMG proteins involved in rDNA transcription in animal and yeast cells, respectively (Sanij et al., 2008; Albert et al., 2013). The precise roles of RTEL1, RMI2, FKBP53, TOR, RPS6, and 3xHMG-box1 in regulating rDNA gene expression have not been determined. Notably, plants deficient in these factors (or overexpressing 3xHMG-box1) typically show plant growth and developmental defects (Supplemental Data Set 1).

EARLY CLEAVAGE EVENTS OF 45S PRE-rRNA

47S and 35S rRNAs are the longest pre-rRNA transcripts in animals and yeast, respectively (Venema and Tollervey, 1995; Fromont-Racine et al., 2003; Kressler et al., 2010; Henras et al., 2015; Tomecki et al., 2017). In A. thaliana, the longest pre-rRNA detected to date is referred to as 45S pre-rRNA (Figure 3). Based on data from yeast and mammalian systems (Henras et al., 2015), A. thaliana 45S pre-rRNA is likely subjected to similar cleavage events to those that generate 35S(P) pre-rRNA. This notion is supported by the experimental identification of cleavage sites and the characterization of plant RNP complexes and activities involved in the processing of the 5'ETS and 3'ETS (Saez-Vasquez et al., 2004a; Comella et al., 2008; Zakrzewska-Placzek et al., 2010).

The processing of pre-rRNA consists of exo- and endonucleolytic cleavages to remove external (5'ETS and 3'ETS) and internal (ITS1 and ITS2) transcribed sequences (Figure 3). The endonucleolytic cleavage sites in 45S pre-rRNA have been mapped in A. thaliana or predicted based on yeast and human pre-rRNA processing maps (Tomecki et al., 2017). The initial cleavage site of 45S pre-rRNA is at the P site (+1275) in the 5'ETS, just after the 1.2 kp insertion present in A. thaliana rRNA genes (Saez-Vasquez et al., 2004a). Further cleavages occur at the P' (+1757) and P2 (+1837) sites (Lange et al., 2008; Zakrzewska-Placzek et al., 2010). It appears that the reported P1 site is not an endonucleolytic cleavage site, because the expected P1-P' fragments have not been detected (Figure 3). Instead,
the accumulation of smaller and heterogeneous fragments mapping around \( P_1 \) suggest that fragments between \( P \) and \( P' \) are generated by exoribonucleolytic degradation (Lange et al., 2011; Sikorska et al., 2017).

The 268-bp ITS1 contains the \( D, A_2, A_3 \) and \( B_1 \) cleavage sites, whereas the 190-bp ITS2 contains the \( E, C_2 \) and \( C_1 \) cleavage sites. \( A_2, A_3 \) and \( C_2 \) are key cleavage sites that switch pre-rRNA processing to specific pathways: 5'ETS-first (minor) or ITS1-first (major) (Figure 3 and see below). The 3'ETS end and cleavage sites have not yet been precisely determined. However, two cleavage sites were detected upstream of the R repeat elements in \( nuc1 \) plants (Pontvianne et al., 2010), which might correspond to predicted \( B_0 \) cleavage sites (Tomecki et al., 2017).

In yeast, cleavage of the 3'ETS is required for RNA Pol I transcription termination by a mechanism similar to the torpedo mechanism (West et al., 2004), which involves the RNase III activity of Rnt1 (Rnase three 1) and Xrn2 (5'-3' Exoribonuclease 2) proteins (Kawauchi et al., 2008). In the torpedo mechanism, co-transcriptional cleavage by Rnt1 releases nascent pre-rRNA from the RNA Pol I elongation complexes, allowing Xrn2 to degrade nascent rRNA still associated with the elongating complex, ultimately provoking the dissociation of RNA Pol I from rDNA. In \( A. thaliana \), genetic studies have shown that cleavage of the 3'ETS requires RTL2 (RNASE THREE LIKE2) (Comella et al., 2008). The RTL2 cleavage sites in the 3'ETS have not yet been precisely mapped, and whether RTL2 is required for RNA Pol I termination remains unsettled. Nevertheless, RTL2 is required for 45S siRNA accumulation (Elvira-Matelot et al., 2016). Moreover, the transcription termination sequences (TTS) reported in \( B. oleracea \) (Yang et al., 2015) were not detected in the \( A. thaliana \) 3'ETS or IGS. This is in contrast to the evolutionary conservation among cruciferous plants of the rRNA gene promoter and 5'ETS A\(^{123}\)B boxes (Doelling and Pikaard, 1996; Saez-Vasquez et al., 2004a).

The 45S pre-rRNA of \( A. thaliana \) has a much longer 5'ETS than pre-rRNAs of yeast, mammalian cells, or even other crucifers (Tomecki et al., 2017). In \( B. oleracea \), biochemical studies have shown that a U3 snoRNP complex binds to the 5'ETS region of the rDNA and cleaves the pre-rRNA 5'ETS at the P site (Caparros-Ruiz et al., 1997; Saez-Vasquez et al., 2004a, b). In \( Brassica \) species and radish, the P site is located next to the A\(^{123}\)B binding site of the nucleolin-U3 snoRNP complex, whereas in \( A. thaliana \), the P site is located ~1.2 kb downstream of the A\(^{123}\)B motif.
In addition, the pre-rRNA 5'ETS in *A. thaliana* is shortened by the exonuclease XRN2 before being cleaved at the P site (Zakrzewska-Placzek et al., 2010). The biological relevance of this XRN2-mediated trimming remains unknown.

**PROCESSING OF PRE-rRNA: TWO ALTERNATIVE PATHWAYS**

In all eukaryotic cells, mature 18S, 5.8S and 25S/28S rRNAs are generated via distinct 40S (for 18S rRNA) and 60S (for 5.8S and 25S/28S rRNAs) pre-rRNA processing pathways (Eichler and Craig, 1994; Tomecki et al., 2017). Alternative processing steps have been described after co-transcriptional cleavages at A₀, A₁ and A₂ sites in yeast (equivalent to P, P₂ and A₂ sites in *A. thaliana*). In mammalian cells, cleavage of the 47S pre-rRNA can either start in the 5'ETS at A₀ and 1(A₁) sites (A₀ and A₁ in yeast) or in the ITS1 at site 2 (A₃ in *A. thaliana* and yeast), defining two distinct pathways: the minor 5'ETS-first pathway and the major ITS1-first pathway (Henras et al., 2015).

Based on the accumulation of specific pre-rRNA products and intermediates, two alternative pathways have also been described in *A. thaliana*: the minor 5'ETS-first pathway and the major ITS1-first pathway (Figure 3). This preferential ITS1-first processing was first observed in *xrn2* plants (Zakrzewska-Placzek et al., 2010), while the accumulation of 5'ETS-first transcripts was reported in *biogenesis of ribosomes in xenopus 1 (brx1)* plants (Weis et al., 2015b). These two pathways also co-exist in rice plants, with the ITS1-first mode being the major pathway (Hang et al., 2018). Environmental stimuli or stress conditions might prevent the accumulation of pre-rRNA from the ITS1-first pathway (Weis et al., 2015a).

In the 5'ETS-first pathway, the 35S(P) is cleaved at the P', P₂, A₂ sites and then at the B₁ site, while in the ITS1-first pathway, the 35S(P) is cleaved at the A₃ site and then at the P' and P₂ sites (Zakrzewska-Placzek et al., 2010; Weis et al., 2015a). Then, after the alternative A₂ or A₃ cleavage events, pre-rRNA precursors from both pathways are adenylated to be degraded and/or trimmed by the exosome. Uridylation of a small subset of intermediates might also occur, but the link between this modification and the exosome has not yet been established (Chekanova et al., 2000; Lange et al., 2008; Lange et al., 2011; Kumakura et al., 2013; Sikorski et al., 2015). Finally, 20S and 27SB₅ₛitura₂₅ losses from both alternative pathways are subjected to common processing events (at the C₂, D, E, E' and C₁ sites) to obtain...
the mature 18S, 5.8S and 25S rRNAs (reviewed in Shinozaki et al., 2003; Weis et al., 2015a; Tomecki et al., 2017)).

Alternative cleavages at either the A2 or A3 site were recently described in yeast (Choque et al., 2018). However, in contrast to A. thaliana or mammalian cells, only cleavage at A2 is productive in yeast. Indeed, cleavage at A2 by Utp24 leads to the production of 20S pre-rRNAs and then mature 18S rRNAs (productive A2 pathway). Cleavage at A3 by RNase MRP results in the production of 23S rRNAs. These 23S rRNAs are targeted by Upt24, generating 11S and 17S rRNAs, which are subsequently degraded by TRAMP/Exosome (non-productive A3 pathway) (Choque et al., 2018).

In yeast, cleavages at the A0, A1 and A2 sites occur co-transcriptionally and thus the larger 35S, 33S and 32S pre-rRNAs are barely detectable. In A. thaliana, these pre-rRNAs are easily detected (Zakrzewska-Placzek et al., 2010; Missbach et al., 2013; Weis et al., 2015b; Maekawa et al., 2018a), suggesting that cleavage at P, P' and A2 occurs mostly post-transcriptionally, as suggested for the A0, 1(A1) and 2 sites in mammalian cells (Henras et al., 2015). Thus, the major ITS1-first processing pathway in A. thaliana is analogous to the main processing pathway in mammalian cells, while the minor 5'ETS-first processing pathway is comparable to the pathway used in yeast.

A plant-specific ITS2 processing pathway was recently reported in A. thaliana (Palm et al., 2019). The ITS2 pathway involves a first cleavage of 35S pre-rRNAs at the C2 site in the ITS2 and the accumulation of specific pre-rRNAs (Figure 3). Remarkably, the ITS2 pathway becomes visible in A. thaliana irp7 plants (deficient for INVOLVED IN RNA PROCESSING7) after auxin treatment and in rapidly dividing cells. Moreover, IRP7, together with IRP8 and IRP9 (encoding INVOLVED IN RNA PROCESSING8 and 9, respectively), are required for the normal processing of plant-specific 5'-5.8S pre-rRNAs. In irp9 plants, only the processing of 5'-5.8S pre-rRNAs is affected, while in irp7 and irp8, the accumulation of 27S-A3, P-A3, P'-A3 and 18S-A3 pre-rRNAs is affected as well. The IRP7 and IRP8 proteins are predicted to contain ALBA (Acetylation Lower Binding Affinity) and RRM (RNA Recognition Motif) protein domains, respectively, whereas IRP9 contains a nucleotide hydrolase domain and appears to be crucial for the processing of the plant-specific 5'-5.8S pre-rRNA (Palm et al., 2019).
The exoribonuclease activities involved in pre-rRNA processing are relatively well-characterized in *A. thaliana*. For instance, XRN2 is required for the 5' trimming of 45S, 27S-A3, and 26S pre-rRNAs, while the exosome core complex Exo9 and the associated RRP44 and RRP6L2 proteins are necessary for the 3' trimming of P-A3, P'-A3, 18S-A2/20S, and/or P-P' and 5.8S pre-rRNAs. More precisely, the removal of 5.8S pre-rRNA, P-P', and fragments cleaved at A3 requires the exosome core complex (Exo9), the RNA helicase MTR4, and either RRP6L2 and DIS3 (DEFECTIVE IN SISTER CHROMATID DISJOINING3) or both, while the elimination of 18S-A2 fragments depends mostly on RRP6L2 alone (Lange et al., 2008; Lange et al., 2011; Kumakura et al., 2013; Lange et al., 2014; Sikorski et al., 2015) (Figure 3). Moreover, the nine-subunit exosome core has a distributive phosphorolytic activity involved in the degradation of P-P1 products (161, 168, 176 and 186-nt) and the processing of 5.8S pre-rRNA. Therefore, the plant RNA exosome harbors a unique combination of hydrolytic and phosphorolytic ribonucleolytic activities, in contrast to exosome complexes in yeast and animal cells (Sikorska et al., 2017).

Pre-rRNA trimming and the degradation of rRNA processing by-products involve the terminal nucleotidyltransferase TRL (TRF4/5-LIKE). Poly-A-tails added to these by-products are up to 18 nt long (Lange et al., 2008; Sikorski et al., 2015). Uridylation might also contribute to rRNA processing, because rRNA by-products can be uridylated in *A. thaliana* (Sikorski et al., 2015).

Much less is known about the RNase activities responsible for the endonucleolytic cleavage of plant pre-rRNAs. On the one hand, *A. thaliana* RTL2 is implicated in 3'ETS processing (Comella et al., 2008). Furthermore, *A. thaliana* NOB1, an homologue of the yeast protein Nob1, is required for P-A3 pre-rRNA processing (Missbach et al., 2013). This finding is unexpected, because yeast Nob1 is required for the cleavage of 20S pre-rRNA (Fatica et al., 2003). On the other hand, genomic and proteomic analyses have identified an *A. thaliana* homologue of the yeast protein Utp24 (Supplemental Data Set 2), which is likely involved in the cleavage of yeast A1 (equivalent to P2 in *A. thaliana*) and A2 in the 5'ETS and ITS1, respectively. Indeed, it was recently shown that Utp24 is essential for early cleavages at three pre-rRNA sites in yeast (A0, A1 and A2) and humans (A0, 1A1 and 2) (Wells et al., 2016). Similarly, *A. thaliana* encodes homologues of the yeast proteins RMRP, Las1, and Rcl1, which are endoribonucleases required for cleavage at the A3, C2,
and A₁/A₂ sites in yeast (Kiss et al., 1992; Gasse et al., 2015; Wells et al., 2016; Goldfarb and Cech, 2017; Maekawa et al., 2018a) (Supplemental Data Set 1 and 2). However, the predicted endonucleolytic activities of these A. thaliana homologues remain to be tested.

A more detailed picture of the current knowledge on the endo- and exoribonuclease activities required for the processing of diverse pre-rRNAs and the degradation of rRNA by-products has been published by (Tomecki et al., 2017).

**RIBOSOMAL RNA PROCESSING FACTORS**

Before the cleavage and removal of ETS and ITS segments from pre-rRNAs, the 18S, 5.8S, and 25S rRNA sequence regions of the precursor are subjected to a number of co-transcriptional sugar and base modifications (Sharma and Lafontaine, 2015). The most common rRNA modifications are 2'-0-ribose methylation and uridine isomerization (pseudouridylation). 2'-0-methylation is guided by small nucleolar RNAs (snoRNAs) of the C/D box type and is accomplished by the protein FIBRILLARIN/Nop1, whereas pseudouridylation is guided by H/ACA box snoRNAs and is accomplished by the protein DYSKERIN/Cbf5p. The distinct snoRNA guides and FIBRILLARIN or DYSKERIN associate with additional proteins to form C/D box or H/ACA box snoRNPs (Massenet et al., 2017), respectively, which have also been identified in plants (Supplemental Data Set 1 and 2).

In A. thaliana, hundreds of C/D box snoRNAs have been identified, and the expected methylation sites have been mapped on the 18S, 5.8S and 25S rRNA sequences (Barneche et al., 2001; Brown et al., 2001; Qu et al., 2001; Brown et al., 2003a; Brown et al., 2003b; Chen and Wu, 2009). Only a few snoRNAs have been associated with the cleavage of pre-rRNAs. For instance, the U3 and U14 C/D box and the E3 H/ACA box snoRNAs facilitate cleavages within the 5'ETS in yeast and animal cells (Hughes and Ares, 1991; Enright et al., 1996; Borovjagin and Gerbi, 1999). In plants, the U3snoRNP is involved in processing at the P-site in the 5'ETS (Saez-Vasquez et al., 2004a), but a direct role for U3snoRNA in pre-rRNA cleavage has not been demonstrated. By contrast, the base pairing of the HID2 (HIDDEN TREASURE2) C/D box snoRNA with rRNA is required for 25S pre-rRNA processing. Intriguingly, the predicted HID2 methylation target site in the 25S rRNA sequence is not affected in the hid2 mutant. HID2 is thought to play a role in promoting pre-rRNA
processing and minimizing the production of aberrant rRNA species (Zhu et al., 2016).

The *A. thaliana* genome encodes two functional FIBRILLARIN proteins, FIB1 and FIB2 (Barneche et al., 2000; Pih et al., 2000) (Supplemental Data Set 2). Remarkably, it appears that the role of FIBRILLARIN is not limited to the methylation of rRNA. Indeed, FIBRILLARIN methylates histone H2A on rRNA gene promoters in both humans and *Brassica* (Tessarz et al., 2014; Loza-Muller et al., 2015). Furthermore, a ribonuclease activity was demonstrated for the GAR domain of *A. thaliana* FIB2, but not for that of FIB1 (Rodriguez-Corona et al., 2017). Because FIBRILLARIN co-purifies with the NUCLEOLIN-U3snoRNP complex, it was suggested that FIB2 might be involved in the cleavage of the P site in the 5'ETS (Rodriguez-Corona et al., 2017). More recently, analysis of *fib2* knock-out plants demonstrated that FIB2 binds to and negatively regulates the expression of innate immune response genes in *A. thaliana*. Upon *Pseudomonas syringae* inoculation, FIB2 interacts with ELF18-INDUCED LONG-NONCODING RNA1 (ELENA1) and is then evicted from gene promoters to activate innate immune response genes (Seo et al., 2018). Taken together, these findings suggest that FIB1 and FIB2 play distinct roles in the modification of and interaction with RNA, DNA, and/or histone proteins in *A. thaliana*.

In contrast to FIBRILLARIN, neither DYSKERIN/Cbf5p nor other H/ACA box snoRNP proteins appear to be associated with the processome. In yeast and mammalian cells, the functions of DYSKERIN/Cbf5p and H/ACA box snoRNPs are essential for ribosome biogenesis, pre-mRNA splicing, and telomere maintenance (Caton et al., 2018), but relatively little is known about these factors in plants. Several H/ACA box snoRNAs and a DYSKERIN/Cbf5p orthologue, NAP57, have been reported in *A. thaliana* (Maceluch et al., 2001; Brown et al., 2003b) (Supplemental Data Set 1). Characterization of a T-DNA insertion mutant in NAP57 showed that DYSKERIN/NAP57 is an essential protein in *A. thaliana* (Lermontova et al., 2007), but the impact of rRNA pseudouridylation in plant growth and development remains unclear.

It was recently demonstrated that the histone deacetylase HD2A/C negatively regulates rRNA methylation and pre-rRNA processing in *A. thaliana* (Chen et al., 2018) (Supplemental Data Set 1). Remarkably, HD2C interacts with both pre-rRNAs
and snoRNAs. Precisely how HD2A/C inhibits the methylation activity of C/D box snoRNPs remains an open question. HD2A/C likely does not affect rRNA pseudouridylation because most of the HD2A/C-interacting snoRNAs are C/D box snoRNAs.

The impact of rRNA modifications is not well understood in plants. However, rRNA modifications are thought to play roles in ribosome assembly and protein translation (Song and Nazar, 2002; Sloan et al., 2017). For example, ribose 2'-0-methylation and rRNA pseudouridylation can influence translational initiation, translational fidelity, and the ribosome’s affinity for specific (tRNA/IRES) ligands in yeast and mammalian cells (Jack et al., 2011; Marcel et al., 2013; Erales et al., 2017).

In yeast, several additional rRNA modifications have been described. These include the methylation of purine rings (m^6^A, m^1^A and m^7^G) and pyrimidine rings (m^5^C, m^3^U and 1-methyl-3-(3-amino-3-carboxypropyl)pseudouridine). The latter, hypermodified uridine base contains an aminocarboxylat, and cytosine acetylation has also been detected (ac^4^C) (Sharma and Lafontaine, 2015). However, the exact timing of these modifications remains unknown in most cases. In A. thaliana, the RNA methyltransferase NSUN5/Rcm1 (Nop2/Sun-like domain containing protein 5/RNA (cytosine-5)-methyltransferase 1) is required for N^5^-methylation at position C2268 of the 25S rRNA (Burgess et al., 2015), while a DIM1A/Dim1 (ADENOSINE DIMETHYL TRANSFERASE 1A) homologue is required for N^6^-dimethylation at positions A1785 and A1786 of 18S rRNA (Wieckowski and Schiebel, 2012). In addition, m^5^C and m^7^G RNA methyltransferase homologues OLI2 (OLIGOCELLULA2) and RID2 (ROOT INITIATION DEFECTIVE2), respectively, have been characterized in A. thaliana (Fujikura et al., 2009; Ohbayashi et al., 2011). The roles of these proteins in rRNA methylation have not yet been demonstrated, but pre-rRNA accumulates in oli2 and rid2 plants (Ohbayashi et al., 2011; Kojima et al., 2017). The A. thaliana genome also encodes homologues of yeast Emg1/Nep1 (Essential for Mitotic Growth/Nucleolar Essential Protein 1) and Kre33/Rra1 (Killer toxin Resistant/Ribosomal RNA cytidine Acetyltransferase 1) (Supplemental Data Set 2). In yeast, these proteins are required for N^1^-methyltransferase and C-acetylation activities, respectively (Sharma and Lafontaine, 2015). Importantly, Emg1/Nep1 and Kre33/Rra1 associate with the processome in yeast (Thomas et al., 2011; Sondalle and Baserga, 2014; Sharma et al., 2015; Sharma et al., 2017). Whether the plant
homologues of Emg1/Nep1 and Kre33/Rra1 associate with 90S/SSU-processome complexes or have RNA methyltransferase or acetylation activity in A. thaliana is currently unknown. Finally, the A. thaliana enzyme PRMT3 (PROTEIN ARGinine METHYLTRANSFERASE3) is required for proper accumulation of the P-A3 fragment and promotes the major pre-rRNA processing pathway (Hang et al., 2014).

90S/SSU-PROCESSOME AND MATURATION OF PRE-40S PARTICLES

Ribosome assembly is initiated on nascent pre-rRNA transcripts, as was observed almost 50 years ago (Miller and Beaty, 1969). The largest pre-ribosome particle identified to date in yeast and mammalian cells is the 90S or the SSU processome complex (Gallagher et al., 2004), a large U3 snoRNP complex from which the 40S ribosome particle is formed. The 90S/SSU processome is assembled co-transcriptionally in the nucleolus (reviewed in (Phipps et al., 2011; Kressler et al., 2017)). The genomes of A. thaliana and rice contain orthologous genes for most yeast and human processome proteins (Supplemental Data Set 2), pointing to the existence of a 90S/SSU processome complex in plants and suggesting that animals, yeast, and plants share conserved mechanisms for 40S assembly (Figure 4).

U3 is a C/D box small nucleolar RNA (U3 snoRNA) required for nucleolar processing (cleavage, not methylation) of pre-18S rRNA in all eukaryotes (Rothe et al., 2017). U3 snoRNA associates with proteins common to all C/D box snoRNAs, including Nop56, Nop58 (Nucleolar proteins 56 and 58), Nop1/FIBRILLARIN, and Snu13 (Small nuclear protein 13), and with the U3-specific proteins Rrp9/U3-55k/Sof1 (Ribosomal RNA Processing 9/Supressor of fibrillarin 1), Mpp10p (M phase phosphoprotein 10), Lcp5p (Lethal conditional pap1 [poly(A) polymerase] allele), Imp3 and Imp4 (Interacting with Mpp10) and Rrp9/U3-55k to form U3 snoRNP (Rothe et al., 2017). Protein-based affinity purification of the U3 snoRNP complex identified the Utp proteins (U three) and demonstrated that U3 and Utp proteins are the main components of the 90S/SSU processome (Gallagher et al., 2004; Phipps et al., 2011) (Supplemental Data Set 2).

In yeast, the 90S/SSU processome contains the U3 snoRNP complex and more than 70 proteins, including 17 Utp proteins (Utp1 to Utp17) and Rrp5 (for Ribosomal RNA processing 5) (Dragon et al., 2002). The Utp proteins form the UtpA (Utp4, 8, 9, 10, 15, Pol5 and Nan1), UtpB (Utp1, 6, 12, 13, 18 and 21), and UtpC complexes
The Utp complexes associate with the U3 snoRNP, Mpp10 (Mpp10-Imp3-Imp4), and Dbp4 (DEAD box protein 4)/Enp2 (Essential nuclear protein 2)/Brf2 (TFIIB-related factor 2) complexes in a sequential manner on the nascent pre-rRNA transcript to form 90S particles (Dragon et al., 2002; Grandi et al., 2002; Gallagher et al., 2004). Although the 90S/SSU processome has not yet been directly observed in plants, some evidence indicates that a processome complex might exist (Figure 4). First, a complex of NUCLEOLIN and U3 snoRNP was isolated and characterized in B. oleracea (Saez-Vasquez et al., 2004a; Samaha et al., 2010). Second, the A. thaliana genome encodes most of the Utps (Matsumura et al., 2016) and U3snoRNP (Kojima et al., 2007) homologues (Supplemental Data Set 2). Third, proteomic analysis identified most of the processome homologue proteins in purified A. thaliana nucleolar fractions (Palm et al., 2016; Montacie et al., 2017).

Most genes encoding proteins in the U3 snoRNP and the UtpA, UtpB, and UtpC classes in yeast have corresponding gene homologues in the human, A. thaliana and rice genomes. Exceptions include Utp8, 9, and 16, which appear to be yeast-specific, and Pol5, Rrp36, Lcp5, Ltv1, Pfa1, Slx9 and Nop19, which are present in the yeast and human genomes but have not been detected in the A. thaliana or rice genomes (Supplemental Data Set 2). These genes might have evolved to such an extent that they cannot be identified based on sequence homology. Among the A. thaliana factors without a yeast or human RBF homologue, the following protein-protein interactions were demonstrated: PCP1 and PCP2 proteins (PLANT–SPECIFIC COMPONENT OF THE PRE-rRNA PROCESSING COMPLEX1 and 2) interact with UtpA and/or UtpB (Figure 4) (Ishida et al., 2016).

Genes encoding components of U3 snoRNPs, which are single genes in yeast and human, are typically duplicated in A. thaliana and rice, whereas genes in other processome categories are usually present in single copies in A. thaliana, with some exceptions (Utp12, Utp3, Utp24, Kre33, Mrd1, Nsr1, Fal1 and Prp43). Interestingly, there are also differences in copy number between A. thaliana and rice, and sometimes it is difficult to determine which rice gene is the homologue of a particular A. thaliana gene (Supplemental Data Set 2).
In *B. oleracea*, the U3 snoRNP complex interacts with NUCLEOLIN and plays a key role coupling the transcription and processing of nascent pre-rRNAs. Indeed, the *Brassica* NUCLEOLIN-U3 snoRNP complex binds 5'ETS rDNA sequences and cleaves the pre-rRNA specifically at the primary cleavage site P in the 5'ETS rRNA (Saez-Vasquez et al., 2004a). The NUCLEOLIN-U3 snoRNP complex from *Brassica* contains more than 80 proteins, including FIBRILLARIN, NOP56, NOP58 and ribosomal proteins from the small ribosome subunit (Samaha et al., 2010). Utp homologues have not been identified in the NUCLEOLIN-U3 snoRNP fraction. Importantly, the nucleolin-U3 snoRNP complex was purified based on the interactions with rDNA (Caparros-Ruiz et al., 1997; Saez-Vasquez et al., 2004a; Samaha et al., 2010), in contrast to the yeast processome complex. It is likely that specific forms of NUCLEOLIN-U3 snoRNP bind rDNA while others associate with the SSU-processome. During transcription, the NUCLEOLIN-U3 snoRNP complex bound to rDNA might translocate to the pre-rRNA and/or processome (Saez-Vasquez et al., 2004b, a).

Only a few *A. thaliana* homologues of yeast Utp-encoding genes have been functionally characterized (Supplemental Data Set 3). Several putative Utp homologues are essential for gametogenesis and/or embryogenesis, including SWA1, PWP2, NOC4, TORMOZ, and NOF1 (Shi et al., 2005; Griffith et al., 2007; Harscoet et al., 2010; Missbach et al., 2013; Ishida et al., 2016). Similarly, studies involving disrupting the genes encoding THAL1, RRP5, ENP1, SW2 or PUM24 (Li et al., 2009; Missbach et al., 2013; Chen et al., 2016; Shanmugam et al., 2017; Maekawa et al., 2018a) indicated that these factors are essential for gametogenesis and/or embryogenesis. By contrast, disrupting *RRP7* affects *A. thaliana* plant growth and development, as well as abscisic acid sensitivity. Furthermore, *A. thaliana* *RRP7* is required for 18S rRNA maturation, and *rrp7* deficiency mutants genetically interact with *mas2*, *nuc1* and *hda6* deficiency mutants (Micol-Ponce et al., 2018).

*nuc1* plants show altered growth and leaf development (Pontvianne et al., 2007), while *nuc2* plants display delayed flowering time (Durut et al., 2014). Plants with disrupted APUM23 (*A. thaliana* PUMILIO23) or GDP1 (G-PATCH DOMAIN PROTEIN1) display growth and developmental defects reminiscent of *nuc1* mutants (Abbasi et al., 2010; Zhang and Muench, 2015; Kojima et al., 2017). In addition, *apum23/salt hypersensitive*9 (*sahy9*) plants exhibit hypersensitivity to salt mediated
by abscisic acid (Huang et al., 2018). By contrast, *A. thaliana* glucose hypersensitive40 (*ghs40*) plants exhibit hypersensitivity to glucose and abscisic acid (Hsiao et al., 2016). Moreover, the GHS40 protein co-immunoprecipitates with SWA1 and other Utp protein homologs (Ishida et al., 2016).

The sub-complexes Mpp10/Imp3/Imp4 and Brf2/Dbp4/Enp2 are the last to assemble into the processome (Granneman et al., 2003; Soltanieh et al., 2014) (Figure 4). *A. thaliana* homologues of yeast Mpp10/Imp3/Imp4 and Dbp4 have been identified, but none have been functionally characterized. However, it has been demonstrated that an MPP10-like protein interacts with *A. thaliana* homologues of THAL1 and NOF1 in a presumed processome complex (Chen et al., 2016).

Most enzymatic activities associated with the processome in yeast have functional homologues in *A. thaliana*. However, only a few of these activities have been characterized (Supplemental Data Set 3). RNA helicases of the DEAE-, DEAH- and DExH-box families play key roles in pre-rRNA processing and ribosome biogenesis (Fatica and Tollervey, 2002; Kressler et al., 2010; Henras et al., 2015). Several plant helicases that are homologues of yeast and human proteins associated with the SSU-processome or involved in the 40S/60S pathways have been reported (Supplemental Data Sets 2 and 4). Among these are the RNA Helicases SW3/RH36, RH57 and RH10, which are *A. thaliana* homologues of yeast Dbp8, Rok1, and Rrp3, respectively (Liu et al., 2010; Hsu et al., 2014; Matsumura et al., 2016) (Figure 4).

In *A. thaliana*, SW3/RH36 gene expression is essential for gametogenesis, whereas the SW3/RH36 protein interacts with ESF2 (EIGHTEEN S FACTOR2). The yeast Esf2 protein is a *bona fide* component of the SSU processome required for pre-rRNA binding and enhancing Dbp8 ATPase activity (Liu et al., 2010). Moreover, *rh57* plants are impaired in seedling growth and hypersensitive to glucose. In *rh57* plants, 45S/35S pre-rRNA accumulates and 40S subunit levels decrease (Hsu et al., 2014). Finally, thermosensitive *rh10* plants display a weak pointed leaf phenotype at 22°C, and their leaves become narrower at 26°C. The accumulation of 35S/33S pre-rRNA is even more evident at 26°C (Matsumura et al., 2016).

The *A. thaliana* protein RH7/PRH75 is required for processing of 35S and 18S pre-rRNAs and likely associates with the SSU-processome. RH7/PRH75 participates in cold tolerance, but the accumulation of 35S/18S pre-rRNAs is not further affected in
rh7 plants under cold conditions (Huang et al., 2016; Liu et al., 2016). RH7/PRH75 is closely related to human DDX21, which might coordinate rRNA transcription and processing (Calo et al., 2015).

Homologues of the yeast proteins Dhr1 (DEAH-box RNA helicases), Dbp4, Fal1 (eukaryotic translation initiation factor Four A Like), and Has1 (Helicase Associated with Set1 helicases) have been identified in A. thaliana and rice (Supplemental Data Set 2). These homologues were also detected in the proteome of the A. thaliana nucleolus and thus might be associated with the plant processome (Pendle et al., 2005; Palm et al., 2016; Montacie et al., 2017). For example, STRS1 and STRS2 (STRESS RESPONSE SUPPRESSOR1 and 2) are two A. thaliana homologues of yeast Has1 that show nucleolar localization. STRS proteins are involved in heat stress tolerance and epigenetic gene silencing (Khan et al., 2014). However, their role in pre-rRNA transcription or processing has not been determined.

Among other processome-associated factors are the A. thaliana homologues of Utp11, Rrp20 (Ribosomal RNA processing 20), and the U3 snoRNP-specific protein U3-55K (Senapin et al., 2003; Pagnussat et al., 2005; Urbanek et al., 2005).

By combining the above information with current models of SSU processome organization and assembly in yeast (Phipps et al., 2011; Soltanieh et al., 2014; Barandun et al., 2017; Chaker-Margot et al., 2017), we developed a model for 90S/SSU-processome assembly in A. thaliana (Figure 4). According to this model, the UtpA, UtpB, U3 snoRNP, UtpC subcomplexes and related factors undergo stepwise assembly on the nascent 40S pre-rRNA while it is being transcribed by RNA Pol I. The A. thaliana SSU-processome appears to exhibit some plant-specific attributes, including the interactions of PCP1/2 with UtpA and UtpB class proteins.

In yeast and animal cells, the first pre-40S particle is obtained following disassembly of the SSU processome. In addition to 20S pre-rRNA and 40S RPS, this pre-40S particle contains the assembly factor Enp1/Bystin, the endonuclease Nob1, its interacting partner Pno1/Dim2, the methyltransferase Dim1, and the RNA helicase Prp43/DHX15. In yeast, Dim1 dissociates from the pre-40S particle in the cytoplasm, while in mammals, DIM1 appears to dissociate in the nucleus. Subsequently, Ltv1, the kinase Hrr25/CKI, and the ATPase/kinase Rio2 are incorporated into the pre-40S particle in the nucleus. The cytoplasmic export of the pre-40S particle requires the
module Noc4/Utp19-Nop14/Utp2 and Crm1, which recognizes nuclear export signals (Schafer et al., 2003; Kuhn et al., 2009). In the cytoplasm, Enp1p/Bystin, Nob1, Ltv1, and other proteins dissociate from pre-40S particles while RACK1, and later, RPS are incorporated to form mature 40S particles (Figure 4; recently reviewed in (Cerézo et al., 2018)).

*A. thaliana* homologues of yeast Nob1, Noc4, and Enp1 are required pre-rRNA processing (Missbach et al., 2013), and Dim1 is required for N6-dimethylation of 18S rRNA (Wieckowski and Schiefelbein, 2012). *A. thaliana* NOB1 and ENP1 are nuclear/nucleolar and nuclear/cytoplasmic proteins, respectively, like their yeast homologues, while *A. thaliana* NOC4 and DIM1A strongly localize to the nucleolus (Wieckowski and Schiefelbein, 2012; Missbach et al., 2013). Finally, RACK1 co-purifies with U3 snoRNP in *Brassica* (Samaha et al., 2010) and with the 80S ribosome in *A. thaliana* (Giavalisco et al., 2005). Plant genomes also encode homologues of Prp43/DHX15 and Nop14/Utp2, which remain uncharacterized (Supplemental Data Set 2 and Figure 4).

Although the roles of many *A. thaliana* homologues of the yeast and mammalian 90S/SSU processome and pre-40S particles remain unknown, current data indicate that they are evolutionarily conserved in plants and that their expression is required for plant growth and development. At the molecular level, disruption of these genes also affects pre-rRNA transcription and/or processing and, in some cases, provokes changes in the morphology and structure of the nucleolus (Supplemental Data Set 3).

**MATURATION OF PRE-60S PARTICLES**

The assembly of pre-60S particles begins on nascent pre-60S rRNA sequences after co-transcriptional cleavage of the pre-rRNA, leading to the release of pre-40S particles. A vast number of protein factors and complexes have been identified that are involved in 60S assembly (Phipps et al., 2011; Henras et al., 2015; Espinar-Marchena et al., 2017; Konikkat and Woolford, 2017; Bassler and Hurt, 2018; Klinge and Woolford, 2019).

The *A. thaliana* genome contains homologues for most 60S RBFs, indicating that the mechanisms of 60S assembly are conserved in animals, yeast, and plants (Supplemental Data Set 4 and Figure 5). With a few exceptions, most of these
protein homologues of yeast and human 60S RBFs are encoded by single copy genes in *A. thaliana*. The disruption or inhibition of these orthologous genes thus causes pre-60S processing deficiencies along with plant growth and developmental defects, or lethality during gametogenesis or embryogenesis (Supplemental Data Set 4).

GTPase homologues of the yeast and human proteins Nug1/NUCLEOSTEMIN (NS1), Nug2/Nog2 (Nuclear GTP-binding protein 1 and 2, Nucleolar GTP-binding protein 2), Nog1 (Nucleolar GTP-binding protein 1) and Lsg1/LSG1 (Large Subunit GTPase 1) are required for maturation of the pre-60S subunit in *A. thaliana* (Im et al., 2011; Weis et al., 2014; Jeon et al., 2015; Zhao et al., 2015; Lee et al., 2017). However, no GTPase homologues have thus far been shown to mediate pre-40S subunit maturation.

Nucleostemin forms a large protein complex (>700 kDa) that co-fractionates with the pre-60S ribosomal subunit in yeast (Romanova et al., 2009). This appears to be the case in plants as well. Indeed, *A. thaliana* NSN1 interacts with PES (PESCADILLO) and EBP2 (Epstein-Barr virus nuclear antigen-binding protein 2) orthologs (Jeon et al., 2015).

In yeast, Brx1 also interacts with Ebp2 and is required for the assembly of 60S ribosomal subunits (Shimoji et al., 2012). The *A. thaliana* genome encodes two homologues of yeast Brx1: BRX1-1 and BRX1-2. brx1-1 and brx1-2 single mutants grow slowly and display defects in pre-rRNA processing. BRX1-1 and BRX1-2 likely play redundant roles in *A. thaliana*, as genetic crosses between brx1-1 and brx1-2 did not yield any double homozygous plants (Weis et al., 2015b).

*A. thaliana* PES interacts with PEIP1 and PEIP2 (PESCADILLO-INTERACTING PROTEIN1 and 2), the homologues of Erb1/BOP1 (Eukaryotic Ribosome Biogenesis 1/ Block of proliferation 1) and Ytm1/WDR2 (WD40 repeat 2 protein) (Cho et al., 2013; Zografidis et al., 2014). Hence, plants might also express the trimeric PES-BOP1-WDR12 complex (Ahn et al., 2016), which is essential for the processing and maturation of mammalian 5.8S and 28S rRNAs. This complex is thought to associate with other proteins to form a much larger complex (Rohrmoser et al., 2007). Yeast Nug2 (nucleostemin 2 in human) is a regulatory GTPase that monitors pre-60S maturation. Nug2 associates with two pre-60S remodeling factors, Rea1
(dynein-related AAA-type ATPase) and its co-substrate Rsa4 (RiboSome Assembly 4) (Matsuo et al., 2014). In *A. thaliana* and rice, the Nug2 homologues exhibit GTPase activity and are required for processing 27S pre-rRNA into 25S rRNA (Im et al., 2011).

No plant homologues of yeast Rsa4 have thus far been reported. However, characterization of the *dsr1* (*dwarf and short root 1*) mutant revealed that DSR1 is the *A. thaliana* homologue of the yeast Rea1 and human MDN1 proteins (Li et al., 2016). Mutating a key amino acid position in *A. thaliana* MDN1 (glutamic acid to lysine at position 3838) induces pleiotropic developmental phenotypes, including slow germination, short roots, dwarf shoots, and reduced seed set (Li et al., 2016).

Moreover, homologues of the yeast Nog1 protein have been reported in *Nicotiana benthamiana* and *A. thaliana* plants. The *N. benthamiana* homologue of Nog1 is required for processing of 35S, 33S, and 27S intermediates (Guo et al., 2018), while *A. thaliana* NOG1-1 and NOG1-2 are involved in biotic and abiotic stress responses (Lee et al., 2017).

In *A. thaliana*, the PES, NSN1, and NUG2/NOG2 homologues are encoded by single genes, and their expression is required for plant growth and/or development. Indeed, *PES* gene silencing causes growth arrest and cell death (Cho et al., 2013), *NSN1* gene expression is required for embryogenesis and shoot apical meristem development (Wang et al., 2012; Jeon et al., 2015), while knockdown of *NUG2/NOG2* leads to defective growth on medium containing cycloheximide (Im et al., 2011).

The *A. thaliana* genome encodes two homologues of yeast Lsg1 and Rei1 (required for isotropic bud growth 1). Yeast Lsg1 and Rei1 proteins are involved in the recycling of the pre-60S subunit export adaptor Nmd3 (Nonsense-Mediated mRNA Decay 3) and Arx1 (Associated with Ribosomal eXport complex) (Hedges et al., 2005; Hung and Johnson, 2006). In *A. thaliana*, LSG1-2 physically associates with 60S/pre-60S particles, and in *lsg1-2* plants, 18S pre-rRNA accumulates and monosome levels decrease, indicating that LSG1-2 is the functional homologue of yeast Lsg1 (Weis et al., 2014; Zhao et al., 2015). *A. thaliana* LSG1-1 does not appear to be involved in ribosome biogenesis. The *lsg1-2* mutants show severe defects in plant development and auxin responses, while *lsg1-1* mutants grow more
slowly than wild-type plants but do not show other defects (Weis et al., 2014; Zhao et al., 2015). Nevertheless, *A. thaliana* plants in which both *LSG1* genes are disrupted cannot be recovered, suggesting that *LSG1*-1 and *LSG1*-2 play partially redundant roles (Weis et al., 2014). By contrast, *A. thaliana* plants with the simultaneous mutation of *REIL1* and *REIL2* are fully viable under optimal growth conditions. However, under cold conditions, the growth of *reil1 reil2* double mutants is arrested, which is correlated with delayed accumulation of 60S particles (Schmidt et al., 2013). *A. thaliana* *REIL1*, but not *REIL2*, complemented the temperature-dependent growth defect of the yeast ∆rei1 mutant. However, in contrast to yeast Rei1, *A. thaliana* *REIL1* is also present in translating ribosomes, pointing to the functional divergence of *REIL* proteins during plant evolution (Beine-Golovchuk et al., 2018).

The *A. thaliana* genome also encodes homologues of the yeast export factors Nmd3 and Arx1 (Supplemental Data Set 4). *A. thaliana* NMD3 plays a role in the nuclear export of 60S ribosomal subunits (Chen et al., 2012), but its functional interactions with *LSG1*-2, *REIL1*/2, or BRX1 have not been determined. In yeast, transport of 60S ribosome subunits from the nucleolus to cytoplasm also requires the nucleolar proteins Noc1 to Noc3. The Noc1-Noc2 complex is likely required for the early transport of ribosome particles from the nucleolus to nucleoplasm, while the Noc3-Noc2 complex appears to be involved in their subsequent transport from the nucleoplasm to cytoplasm (Milkereit et al., 2001). In *A. thaliana*, SWA2 (*SLOW WALKER2*) encodes an Noc1/Mak21 homologue that is essential for female gametophyte development, while *RBL* (*REBELOTE*) encodes a Noc2 homologue that is required for floral meristem termination (Prunet et al., 2008; Li et al., 2009).

The *A. thaliana* genome also contains uncharacterized second copies of genes encoding homologues of yeast Noc2 and Noc3p (Prunet et al., 2008). The *A. thaliana* proteins ARPF2 and ARRS1 are homologues of yeast Rpf2 and Rrs1 (Maekawa et al., 2018b). In yeast, Rpf2 and Rrs1 form a protein complex that binds to 5S rRNA and assembles 5S RNP into pre-60S particles (Madru et al., 2015). *A. thaliana* ARPF2 and ARRS1 are encoded by single-copy genes. Mutating ARPF2 results in aborted embryos, while the overexpression of *ARPF2* or *ARRS1* results in shorter stems and abnormal leaf morphology.
The *A. thaliana* genome also encodes homologues of the yeast/human exonucleases Rrp17 and Nop53/SMO4, as well as two Tif6 homologues (Oeffinger et al., 2009) (Kato et al., 2010; Zhang et al., 2015). In *A. thaliana*, smo4 plants exhibit reduced organ size (Zhang et al., 2015), while mutation of the Tif6 homologue gene *At-eIF6;1* causes embryo lethality (Kato et al., 2010). The precise roles of these protein factors in 60S rRNA processing, assembly, and/or the export of ribosome particles remain unsettled.

Little is known about the RNA helicases involved in pre-60S ribosomal subunit processing and assembly in plants. For instance, *A. thaliana* IRP6 (also named DRH1 (Okanami et al., 1998)) is involved in the processing of 27SB pre-rRNA at the C2 site (Palm et al., 2019). Remarkably, RNA helicases from *A. thaliana* might affect the processing of different pre-rRNA substrates from their yeast counterparts. This is the case for MTR4 (mRNA transport regulator 4), which is required for the processing of 18S pre-rRNA and degradation of rRNA processing by-products, but not for 5.8S pre-rRNA processing, as observed in yeast (Lange et al., 2011).

Of the ~70 yeast LSU-60S RBF proteins listed in Supplemental Data Set 4 (constructed from (Schmidt et al., 2014; Biedka et al., 2018; Zisser et al., 2018)), ~50 have *A. thaliana* homologues. The *A. thaliana* genome also encodes homologues of human RBFs and proteins with homology to bacterial sequences. For instance, GTS1 (GIGANTUS1), the *A. thaliana* homologue of human WDR39 (WD40-REPEAT protein), interacts with L19e and NOP16 and regulates plant growth and development (Gachomo et al., 2014). *A. thaliana* CSP3 (COLD SHOCK DOMAIN PROTEIN3), which has an RNA chaperone function like that of *E. coli* cold shock proteins, interacts with RPL26A, RPL40A, GAR1, and NUC1 (Kim et al., 2013), and also with the cold tolerance-related RNA helicase RH7/PRH75 (Huang et al., 2016).

Taken together, the data indicate that the overall assembly of 60S ribosome particles occurs in a similar manner in plants, yeast, and mammalian cells (Figure 5). However, differences in the timing of events (transcriptional or post transcriptional) and pre-rRNA cleavage patterns (5'ETS-first or ITS-first) suggest that there are differences in the assembly of 40S and 60S particles on nascent pre-rRNA transcripts. As observed for 40S-related RBF genes, it appears that some 60S homologue proteins are also encoded by two *A. thaliana* genes. In some cases,
these duplicated genes have redundant ribosomal functions (REIL1/REIL2 and BRX1-1/BRX1-2), whereas in other cases, the two genes have diverged, leaving only one copy encoding the ancestral function in ribosome biogenesis (LSG1-1/LSG1-2). Plant RBFs might also have acquired some unique functions in ribosome biogenesis. For instance, A. thaliana REIL1 is present in the translating ribosome, in contrast to its yeast homologue, which only interacts with pre-60S particles (Beine-Golovchuk et al., 2018). Likewise, while in yeast, a single Noc2 protein might interact with Noc1 and Noc3, in A. thaliana, both NOC2 and RBL interact with SWA2/NOC1 and NOC3 (de Bossoreille et al., 2018). The functional redundancy and specificity of these protein-protein interactions in ribosome biogenesis have not been reported. Finally, studies about the nucleolar, nucleoplasmic, and cytosolic distribution of ribosomal proteins (Montacie et al., 2017; Palm et al., 2018) suggest that the assembly and maturation of 60S particles differ between plants and yeast.

**RBFs IN CULTIVATED PLANT SPECIES**

Most of our knowledge concerning the functional organization of plant rRNA genes, plant ribosome biogenesis, and related regulatory pathways comes from A. thaliana plants of the Col-0 ecotype. However, RBFs have been described and characterized in several plant species of agronomic interest, providing important insights into rRNAs, ribosome biogenesis, and stress response pathways in plants.

In rice, genes for NUCLEOLIN and the DEAD-Box helicase TOGR1 (THERMOTOLERAN GROWTH REQUIRED1) have been reported (Sripinyowanich et al., 2013; Wang et al., 2016). Interestingly, overexpression of NUCLEOLIN enhances salt adaptation, while TOGR1 is required for thermo-tolerance in rice. Furthermore, the characterization of SAD1 (SUPER APICAL DORMANT1) revealed an interaction between the Mediator complex and the RNA Pol I machinery in rice. SAD1 is an RNA Pol I subunit orthologous to yeast RPA34.5 and A. thaliana NRPA14, and Mediator is a multifunctional complex involved in RNA transcription, elongation, termination, processing, and chromatin looping (Li et al., 2015; Ream et al., 2015). Other rice proteins showing homology to RBFs are listed in Supplemental Data Set 2. In some cases, however, there are several copy genes of particular RBF in rice, and it is therefore difficult to determine which copies are the homologues in A. thaliana.
In maize, three protein-coding genes related to rRNA and ribosome biosynthesis have been characterized. The first, ZmDRH1 encodes a DEAE box RNA Helicase homologous to RH14/DRH1 in A. thaliana. The ZmDRH1 protein localizes to the nucleolus and interacts with the RNA-binding protein MA6 and FIBRILLARIN (Gendra et al., 2004). The others, ZmURB2 (UNHEALTHY RIBOSOME BIOGENESIS2) and ZmREAS1 (RIBOSOME EXPORT ASSOCIATED1) are required for kernel development and are involved in pre-rRNA processing and ribosome assembly (Qi et al., 2016; Wang et al., 2018).

In barley (Hordeum vulgare), a functional homologue of yeast RRP46 (rRNA PROCESSING PROTEIN46) was described. Yeast RRP46 is a critical component of the RNA exosome complex. Silencing of barley RRP46 provoked tip cell death in leaves and induced the accumulation of pre-rRNAs (Xi et al., 2009).

Finally, Simm and colleagues reported the identification of putative RBFs and their expression in tomato (Solanum lycopersicum). This analysis revealed that 25% of all predicted RBFs are encoded by more than one gene and that clusters of RBFs and RPs are co-regulated (Simm et al., 2015).

**RIBOSOME RESPONSE PATHWAY**

In both animal and plant cells, rRNA and ribosome biogenesis can be disturbed by various stress conditions. These include nutrient starvation, changes in energy status, hypoxia, temperature changes, DNA damage, genotoxic stress, and other alterations to ribosome biogenesis factors. Disturbed ribosome biogenesis is typically accompanied by changes in nucleolar morphology and the activation of pathways leading to cell cycle arrest or apoptosis, which is commonly referred to as the ribosomal or nucleolar stress response (Boulon et al., 2010; Tsai and Pederson, 2014; Stepinski, 2016; Kalinina et al., 2018).

In animals, the ribosomal stress response involves the p53/MDM2 (tumor protein53/Murine Double Minute 2) pathway (Boulon et al., 2010; van Sluis and McStay, 2017). MDM2 is an E3 ubiquitin ligase and p53 is an antitumor transcriptional regulator that drives cell cycle arrest, senescence, and apoptosis. Under normal conditions, MDM2 targets, ubiquitinates, and programs the degradation of p53. By contrast, under stress conditions, MDM2 interacts with ribosomal proteins released from the nucleolus, which prevents p53 degradation.
The ribosomal stress response also involves eNoSC (energy-dependent Nucleolar Silencing Complex) and MYBB1A (Myb-binding protein 1A). eNoSC is composed of the proteins NML (NUCLEOMETHYLIN), the histone deacetylase SIRT1 (Sirtuin-1), and the histone methyltransferase SUV39H1 (Suppressor of Variegation 39 Homolog 1). In this pathway, eNoSC senses the energy status of the cell and represses rRNA transcription under glucose deprivation. As a result, MYBB1A translocates from the nucleolus to the nucleoplasm, inducing the activation of p53 by enhancing its acetylation by p300 (Murayama et al., 2008; Kumazawa et al., 2011).

Plants do not have homologs of the mammalian MDM2, p53, or NML proteins. However, the characterization of ANAC082, a NAC family transcriptional activator, suggests that plants have a specific ribosomal or nucleolar stress response pathway (Ohbayashi et al., 2017). ANAC082 was identified genetically in a mutagenized population of rid2 plants as the suppressor mutant sriw1 (suppressor of root initiation defective two 1), which is able to rescue the impaired developmental abnormalities of rid2, including root length, leaf blade size, and venation patterns, but not the accumulation of rRNA precursors. Similarly, the sriw1 mutation also alleviates the developmental abnormalities of rid3, oligocellula5, and rh10 mutants, but not their impaired pre-rRNA processing. Also, ANAC082 expression is induced in temperature-sensitive RBF mutants, and ANAC082 appears to function downstream of ribosome biogenesis and nucleolar perturbations. Interestingly, the 5'UTR of ANAC082 mRNA contains a uORF (upstream ORF) encoding a 37 aa peptide that might negatively regulate ANAC082 protein translation. Ribosomal stress or nucleolar perturbations might inhibit the expression of this uORF, allowing increased ANAC082 protein expression ((Ohbayashi et al., 2017) and reviewed in (Ohbayashi and Sugiyama, 2018)).

In *A. thaliana*, the inhibition of RBF or RP gene expression induces a wide variety of plant growth and developmental phenotypes, including embryo lethality, delayed flowering time, and abnormal leaf and root phenotypes (Supplemental Data Set 3 and 4). Cytological investigations have shown that the nucleolus becomes larger in *tormoz* (Griffith et al., 2007), *nof1* (Harscoet et al., 2010), *apum23* (Abbasi et al., 2010), *rid2* (Ohbayashi et al., 2011; Shinozohara et al., 2014), *ren1* (Renak et al., 2014), *la1* (Fleurdepine et al., 2007), and *domino1* (Lahmy et al., 2004) compared to
wild type plants. By contrast, the nucleolus is small in fem111 (Portereiko et al., 2006) and disorganized in nuc1 (Pontvianne et al., 2007) plants. The accumulation of pre-rRNA is affected in nof1, apum23, brx1, lsg1, and other mutants as well (Supplemental Data Set 1 and 3).

Remarkably, some molecular phenotypes only become evident under specific growth conditions (Supplemental Data Sets 3 and 4). For instance, in response to high sugar concentrations, increased accumulation of pre-rRNA is observed in rh57 (Hsu et al., 2014) and apum24 (Maekawa et al., 2018a). At high temperatures, pre-rRNA accumulates in rh10 (Matsumura et al., 2016) and rid3 (Shinohara et al., 2014). At low temperatures, reil1 reil2 double mutants accumulate less ribosome subunits compared to wild-type plants (Beine-Golovchuk et al., 2018). Furthermore, rh10 nucleoli are enlarged at 28°C (Matsumura et al., 2016), whereas apum24 nucleoli become smaller in the presence of 50 mM glucose (Maekawa et al., 2018a).

Notably, APUM24 interacts with other pre-rRNA processing factors and recruits LAS1, the endonuclease involved in the processing of ITS2 (Figure 3). Perhaps A. thaliana APUM24 links the nucleolar stress response pathway to altered sugar responses (Maekawa et al., 2018a). How APUM24 might sense the energy status of the cell remains unclear (reviewed in (Maekawa and Yanagisawa, 2018)).

We surveyed the expression of the genes listed in Supplemental Data Sets 3 and 4 using the Bio-Analytical Resource for Plant Biology (bar.utoronto.ca, (Winter et al., 2007)), which is primarily based on Affymetrix microarray experiments. We investigated developmental stage-specific effects and abiotic stress responses. In Supplemental Data Sets 3 and 4, we report the developmental stages at which the expression level of each gene is the highest. Several developmental stages are reported, such as dry seeds, seeds 24 hours after imbibition, rosette leaves, and the apical meristem (including three situations: vegetative, transition, and inflorescence).

For abiotic stress, the analyzed experiments deal with cold, osmotic stress, salt, drought, genotoxic stress, oxidative stress, UVB exposure, wounding, and heat treatment, as described in (Winter et al., 2007)).

The most striking result is that most of the genes surveyed show a remarkably homogeneous pattern of expression (Supplemental Data Sets 3 and 4). Not surprisingly, all these genes involved in the biosynthesis of rRNA and ribosomes are...
highly expressed in the most active tissues or organs, such as imbibed seeds, meristems, floral bud, and developing siliques and embryos. In some cases, there are small differences in the relative expression between the different stages of the apical meristem, suggesting that the transition from the vegetative to inflorescence meristem is associated with fine-tuning of the expression of genes involved in ribosome biogenesis, although this needs to be confirmed by studies at the protein level. An interesting observation is that many of the genes are expressed at unexpectedly high levels in dry seeds, which suggests that dry seeds contain all the mRNA necessary to immediately resume ribosome biogenesis as soon as the seeds are imbibed.

Finally, of the genes involved in stress responses, it is striking that most of the genes listed in Supplemental Data Sets 3 and 4 are induced by three major stresses: cold, heat, and UVB. This finding suggests that ribosome biogenesis can adapt to respond to these stresses by increasing the transcript levels of these key genes. Some of the genes are equally responsive to these various stresses, whereas others selectively respond to one or two of the stresses. The physiological significance of these differences is not yet clear, but the observed responses again suggest a fine-tuning of the regulation of ribosomal RNA and ribosomes biogenesis. Surprisingly, very few of these genes respond to osmotic stress, drought, or wounding at the transcriptional level. An intriguing possibility is that the responses to these stresses might occur at the post-transcriptional level. In the future, it would be interesting to determine whether this is a general situation in plants or a situation restricted to A. thaliana and several related species.

CONCLUDING REMARKS

Based on studies in A. thaliana and other plant species, one can reasonably conclude that rRNA gene expression and ribosome assembly in plants resemble the corresponding processes in yeast and/or mammalian cells. However, these studies also revealed features of ribosome biogenesis particular to plants, i.e., features specific to plant growth and development and to the sessile lifestyle of plants, requiring them to adapt to local environmental conditions. With a few exceptions, all RBF genes detected in the yeast and human genomes are also present in A. thaliana, rice, and presumably in other plant species. However, there are significant differences in gene copy number. For instance, genes encoding the
U3 snoRNP complex are duplicated in plants, whereas they are single copy genes in yeast and animals. The possibility that duplicated genes for U3 snoRNP proteins could be subfunctionalized in A. thaliana or rice is intriguing and should be investigated in future studies. This, together with the finding that the NUCLEOLIN-U3 snoRNP complex binds to rDNA in Brassica (Saez-Vasquez et al., 2004b, a), suggests that the biosynthesis of rRNA and ribosomes might be more complex processes in plants than in yeast and animals. The combination of these processes could produce slightly different ribosomes depending on the developmental phase or stress conditions. Notably, in addition to the genes directly involved in transcription and assembly of the processome/pre-40S and pre-60S particles, all of the ribosomal protein genes belong to small multigene families (Barakat et al., 2001). Few situations are well-documented concerning the respective functions of these duplicated genes. The case of the two nucleolin genes illustrates that duplicated genes can acquire distinct and sometimes antagonistic functions, allowing them to induce or silence distinct variants of ribosomal RNA genes.

It appears that some of RBFs in yeast lack homologues in A. thaliana, which suggests that either there is high sequence divergence between the species or that some of these factors simply do not exist in plant species. In turn, genetic, genomic, and proteomic analyses have revealed several nucleolar proteins that lack homology with yeast and/or mammalian RBFs and that might thus be considered plant-specific (such as PCP1 and PCP2). Functional characterization of these factors would provide novel insight into the impact of rRNA transcription and processing as well as ribosome assembly on plant growth, development, and adaptation to environmental conditions.

Studies concerning the functional organization of rDNA in A. thaliana have also raised new questions. Rabanal and colleagues recently demonstrated that rRNA variants can be expressed from NOR2, from NOR4, or from both NORs in different A. thaliana ecotypes (Rabanal et al., 2017). The functional or structural significance of sequence variation in the 3'ETS of A. thaliana rRNA genes and in those of other plant species is also unclear. Variation in the 3'ETS likely does not result in ribosome heterogeneity, because these sequences are removed from mature rRNAs, although it cannot be completely excluded that these variants are genetically linked to discrete SNPs (single-nucleotide polymorphisms) that have yet to be
detected in the mature RNAs. High-throughput DNA sequencing technologies that generate ultra-long reads (well over 10,000 bp) should begin to paint a better picture of 45S rDNA organization.

The modification of structural and catalytic rRNAs (18S, 5.8S, and 25S) might have a major impact on the fine-tuning of ribosome translational efficiency and capacity, as demonstrated in yeast and mammalian cells. How plant growth, development, or environmental conditions affect rRNA processing in plants is almost completely unknown. It would also be interesting to determine if plant IGS transcripts are involved in rDNA silencing and/or protein sequestering, as reported in mammalian cells in response to heat stress and acidosis.

The existence of two distinct pre-rRNA processing pathways remains controversial and raises many questions concerning the factors and conditions (developmental or environmental) that govern either the ITS1-first or 5'ETS-first processing pathways. The recent report of a third, plant-specific ITS2 processing pathway is intriguing and adds a new level of complexity to pre-rRNA processing in plants. Clearly, energy status and temperature affect RBF gene expression and pre-rRNA processing. However, the functional crosstalk between this processing and cellular and/or external signals remains unknown.

ANAC082 plays a role as a ribosomal stress response mediator in plants and is likely the plant functional homologue of mammalian p53. However, p53 is structurally different from ANAC082. Furthermore, other key players of the animal ribosomal stress response pathway are absent in plants. This suggests that the molecular mechanisms of ribosomal stress responses are different in plant cells compared to animal cells. The identification and characterization of ANAC082-related factors should help us to better understand ribosomal stress responses in plants.

Thus, although it is clear that plant growth and development are directly linked to ribosome synthesis and protein translation, many of the molecular mechanisms and signals that connect cellular and environmental conditions with rRNA synthesis and ribosome assembly remain to be characterized in plants. The question of the heterogeneity of ribosomes and their fine-tuned regulation is a challenge for the future. An associated issue is the protein synthesis capacity and specificity of slightly different ribosomes: can they preferentially translate different sets of mRNAs?
ACCESSION NUMBERS

Sequence numbers from this article can be found in Supplemental Data Set 1–4.

SUPPLEMENTAL DATA

Supplemental Data Set 1. Ribosome Biogenesis Factors (RBF) from S. cerevisiae and H. sapiens identified and/or characterized in A. thaliana plants.

Supplemental Data Set 2. Components of the SSU processome and pre-40S particles from S. cerevisiae and H. sapiens in A. thaliana and O. sativa plants.

Supplemental Data Set 3. Characterization studies of the A. thaliana SSU processome and pre-40S orthologues.

Supplemental Data Set 4. Ribosome biogenesis factors (RBF) from S. cerevisiae and H. sapiens identified and/or characterized in A. thaliana plants.

ACKNOWLEDGEMENTS

The authors thank D. Gagliardi, H. Lange and T. Blevins for the detailed critical reading of the manuscript, and C. S. Pikaard for comments and correction of the English. We also thank J. Salses and C. Pont for the identification of rice homologues of known RBFs and all the team members who have contributed in our research on rRNA dynamics and ribosome biogenesis in plants. We apologize for any papers we forgot to mention or that could not be included due to space restrictions. This work was supported by the CNRS and by a grant from the ANR (Agence Nationale de la Recherche): RiboStress 17-CE12-0026-01 to JSV.

AUTHOR CONTRIBUTIONS

J.S-V. and M.D. wrote the article.

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include the 35S pre-rRNA, the U3 snoRNP, and 40S subunit processing factors but predominantly lack 60S synthesis factors. Mol Cell. 10, 105-115.


RNA precursors are targets of the exosome complex, the


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exoribonuclease RRP6L2 and the terminal nucleotidytranferase TRL in Arabidopsis thaliana. Plant J 83, 991-1004.


Figure 1. Graphic representation of ribosome biogenesis in eukaryotic cells. Transcription of rDNA (45S, 47S and 35S in Arabidopsis, mammals, and yeast, respectively) requires RNA Polymerase I (RNA Pol I) activity and a subset of General Transcription Factors (GTFs). Transcribed transcript contains the 18S, 5.8S and 25S (in Arabidopsis and yeast) /28S (in mammals) rRNAs and is first co-transcriptionally processed into pre-rRNAs precursor (35S in Arabidopsis and yeast or 45S in mammalians). Processing of pre-rRNAs into mature 18S, 5.8S, and 25S/28S rRNA involve multiple endo- and exonucleolytic cleavages and the modification of numerous rRNA residues, mainly pseudouridylation and 2'-O-ribose methylation, by small nucleolar Ribonucleoprotein (snoRNP) complexes (see Figure 3). 18S rRNAs assemble with ribosomal proteins RPSs (S for Small) to form 40S ribosome subunits, while 5.8S and 25S/28S assemble with ribosomal proteins RPLs (L for Large) and 5S rRNA transcribed by RNA Pol III. Assembly and transport of ribosomal particles from the nucleolus to cytoplasm requires hundreds of specific 40S and 60S Ribosome Biogenesis Factors (RBFs) (see Supplemental Data Sets 3 and 4 and Figures 4 and 5). The 40S and 60S ribosomal subunits join to form translationally competent ribosomes.
Figure 2. 45S rDNA organization in *A. thaliana* accession Col-0. A) The figure illustrates the localization of 45S rDNA tandem repeat units in the Nucleolus Organizer Regions (NORs) immediately adjacent to telomeres (TELs) on chromosomes 2 and 4. 45S rDNA units are separated by InterGenic Spacers (IGS), which each contain repeated Sal I box elements, the Spacer Promoters (SP1 and SP2), and the Gene Promoter (GP). The 45S rDNA transcribed sequence contains structural rRNAs (18S, 5.8S, and 25S) separated by External (5’ETS and 3’ETS) and Internal (ITS1 and ITS2) Transcribed Spacers. Within the 5’ETS is the A123B motif and a 1.2-kb insertion, which contain D and C motifs. In the 3’ETS are polymorphic R1-R5 elements that allow the 45S rDNA variants VAR1 through VAR5 to be discriminated. B) The image illustrates the distribution of 45S rDNA variants in NOR2 (VAR1 and VAR3a) and NOR4 (VAR2a/b, VAR3b/c and VAR4) (Chandrasekhara et al., 2016) and the repression of NOR2 during plant development.
Figure 3. 35S pre-rRNA processing pathways. The figure illustrates the 45S pre-rRNA and cleavage sites in the ETS and ITS sequences. In the current models, the 5’ETS of 45S pre-rRNAs is trimmed by XRN2 before being cleaved at the P site by a nucleolin-U3 snoRNP complex. The 3’ETS is cleaved by the RNase III endonuclease, RTL2. Structural rRNAs are methylated by C/D snoRNP (2-O-ribose methylation) or pseudouridylated by H/ACA snoRNP complexes. The resulting 35S(P) pre-rRNA follows either Pathway 1: 5’ETS-first (minor), Pathway 2: ITS1-first (major), or Pathway 3: ITS2-first. Specific processing events in Pathway 1, 2, or 3 are shown in the boxes. The characterized protein factors required for RNA modification (C/D and H/ACA snoRNP, and TRL), endonucleolytic cleavages (Nucleolin-U3snoRNP, RTL2, NOB1), or exonucleolytic cleavages (XRN2, AtRRP6L2 and AtRRP41) are indicated.
Figure 4. Model for processome assembly and the maturation of pre-40S particles in Arabidopsis. The picture illustrates UtpA (green), UtpB (blue), UtpC (yellow), U3snoRNP (violet), Dbp4/Enp2/Brf2 (pink), and Mpp10/Imp3/Imp4 (brown) sub-complexes. Arabidopsis thaliana homologues (NuGWD1/Utp5; Nucleolin/Nrs1, Utp25, THAL1/Utp3; RH57/Rock1, RH10/Rrp3 and SW3/Dbp8) or specific (PCP1/2) factors that interact with processome factors or subunits are represented in dark blue. A. thaliana homologues that interact with the processome and are required for pre-40S maturation and transport are represented in red. Known or predicted A. thaliana homologous are labeled in white and those not yet identified in black. Anticipated assembly of pre-60S particles on nascent pre-RNA is shown. Assembly might initiate on full-length rRNA precursor or after cleavage at A2/A3 sites that release processome factors precursors and pre-40S particles. The figure was adapted and drawn from models of the assembly steps of the 90S/SSU-processome and pre-40S in yeast and mammalian cells (Soltanieh et al., 2014; Cerezo et al., 2018). Accession numbers for all the A. thaliana factors are provided in Supplemental Data Set 2.
Figure 5. Model for the assembly and maturation of pre-60S particles in
Arabidopsis. The picture illustrates the so-called yeast 60S particle in the nucleolus (Ssf1 and Nsa), nucleoplasm (Arx1/Rsa4, Rix1/Rea1 and Arx1/Nmd3/Mex67), and cytoplasm (Lsg1). Arabidopsis thaliana homologues of yeast processome factors and sub-complexes (blue and violet), protein export (red), and GTPase (orange) activities are represented. Those not yet identified are shown in gray. Plant GTS1, orthologous to human WDR39, is represented in dark green. The figure was adapted and drawn from simplified models of the assembly steps of the 60S particles in yeast (Shchepachev and Tollervey, 2016; Kressler et al., 2017). Accession numbers for all the A. thaliana factors are provided in Supplemental Data Set 4.