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The role of ribosomal proteins in the plant development

Role ribozomálních proteinů ve vývoji rostlin

Bachelor's thesis

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Declaration

I hereby declare that I have compiled this thesis independently, using the listed literature and resources only. Content of the thesis or any part of it has not been used to gain any other academic title.

Prohlášení

Prohlašuji, že jsem závěrečnou práci zpracovala samostatně a že jsem uvedla všechny použité informační zdroje a literaturu. Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu.

Prague, 4th May 2022

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Veronika Jirásková

Abstract

The translation is one of the fundamental cell processes, in which the protein is synthesized according to the sequence of the mRNA molecule. The foremost recognized element of the translation machinery is the ribosome, a molecule complex composed by rRNAs and ribosomal proteins. In plants, ribosomal proteins are encoded by more than one gene, which may lead to sub-functionalization and neo-functionalization of ribosomal protein paralogs in plant development or in the reaction to the contemporary environment. Assembly of ribosomal subunits from different ribosomal protein paralogs could lead to functionally distinct pools of ribosomes with specialized role in the translation and its regulation in plants. The aim of this work is to review current data regarding the individual ribosomal proteins function within the plant growth and development.

Keywords

translation, translation regulation, ribosome, ribosomal proteins

Abstrakt

Translace je jedním ze základních procesů probíhajícím v buňce, během translace dochází k syntéze proteinů podle informace zapsané v mRNA. Nejvíce prozkoumaným elementem v translaci je ribozom, molekulární komplex složený z rRNA a ribozomálních proteinů. V rostlinách je většina ribozomálních proteinů kodována více než jedním genem, což může vést k subfukcionalizaci a neofunkcionalizaci paralogů ribozomálních proteinů ve vývoji rostlin nebo k reakci na podmínky prostředí. Sestavení ribozomálních podjednotek ze zcela odlišných paralogů ribozomálních proteinů může vést k tvorbě funkčně odlišných populací ribozomů se specializovanou funkcí v translaci a její regulaci v rostlinách. Cílem této práce bude přehledně shrnout dosavadní data o funkcích jednotlivých ribozomálních proteinů během růstu a vývoje.

Klíčová slova

translace, regulace translace, ribozom, ribozomální proteiny

List of frequently used abbreviations

ABA - abscisic acid

ACL5 - acaulis5

amiRNA - artificial microRNA

AML1 - Arabidopsis Minute-like 1

API2 - APICULATA2

ARF3 - ADP Ribosylation Factor 3

AS1 - asymmetric leaves1

AS2 - asymmetric leaves2

AtHD2B - histone deacetylase 2B

atrzf1 - Arabidopsis thaliana ring zinc finger 1

CUC3 – cup-shaped cotyledon3

eEF - eukaryotic translation elongation factor

eIF - eukaryotic translation initiation factor

eRF - eukaryotic translation release factor

ES - expansion segment

ETS - external transcribed spacers

EVR1 – enhancer of variegation1

EVR1L1 - enhancer of variegation1-like1

GP - gene promoter

GTP - guanosine triphosphate

GTF - general transcription factor

ITS - internal transcribed spacers

LSU - large ribosomal subunit

MAX2 - more axillary branches2

NMD - nonsense mediated decay

NOR - nucleolar organizer region

OLI - oligucellula

PFL2 - pointed first leaf2

PGY - piggybak

PIC - pre-initiation complex

PID - pinoid

Pol I - RNA polymerase I

Pol II - RNA polymerase II

Pol III - RNA polymerase III

PTC - peptidyl transferase center

RAP - ribosome-associated proteins

RBF - ribosome biogenesis factor

REV - revoluta

RML1 - rice minute-like1

RP - ribosomal protein

SAC - suppresor of acaulis

snoRNP - sno-ribonucleoprotein

SP - spacer promoter

STV1 – short valve1

SSU - small ribosomal subunit

S6K - S6 kinase

TC - ternary complex

TOR - target of rapamycin

uORF - upstream open reading frame

VAR2 - yellow variegated2

VS - variable region

Table of contents

1. Introduction

The translation is a fundamental process for all living cells, involving many proteins and types of RNA; rRNAs, tRNAs and mRNA. The process of translation is considered a cycle divided into four-part; initiation, elongation, termination and recycling, wherein the genetic information encoded in the mRNA is translated into peptide chain and then folded into a functional protein. The foremost recognized element of the translational machinery is the ribosome.

Eukaryotic ribosomes are large ribonucleoprotein complexes responsible for the translation of mRNA transcripts into functional proteins. Ribosomes themselves are composed of four rRNAs (5S, 5.8S, 18S and 28S) and 79-80 ribosomal proteins (RPs), which is the case of plants ribosomes, with slight differences, as well. In *Arabidopsis thaliana*, the 60S large subunit consists of 5S, 5.8S, and 25-26S rRNAs and 48 RPs, while the small 40S subunit is comprised of 18S rRNA and 33 RPs. Ribosomes are one of the largest complexes in cells and their assembly is a multi-step process that starts in the nucleus or nucleolus, where 45S and 5S rDNA are transcribed. Then the pre-rRNA undergoes chemical modifications, cleavage event, folding, and assembly with RPs. The ribosomal pre-40S and pre-60S subunits are transported into the cytoplasm, where it undergoes the final maturation steps.

In the history of translational studies, ribosomes have been viewed as passive, indiscriminate machines and, only quite recently, have been recognized to play an essential role in the post-transcriptional regulation of gene expression. From each of the 81 RPs family, only one product of any ribosomal protein is included in one unit of the ribosome. However, the 81 RP families in plants are encoded by 254 genes. Each paralogue encoding a member of the RP family could then be expressed specifically at different developmental stages, in different tissues, or under particular stress conditions. This altogether could, in theory, lead to a distinct populations of ribosomes with different RP composition and specialized functions. This phenomenon is called ribosome heterogeneity and it could also arise from sequence variation of rRNAs, exchange of RP paralogs, or posttranslational modifications of RPs.

The aims of this work are to briefly overview the current knowledge about eukaryotic translation, ribosome structure and biogenesis, emphasizing on differences in plants. Then the concept of ribosome heterogeneity will be introduced with examples from plants and finally, all evidence of the effects of mutations in genes encoding ribosomal proteins will be listed with emphasis on developmental phenotypical changes.

2. Mechanism of eukaryotic translation

The translation is a fundamental process for all living cells wherein the genetic information encoded in the mRNA molecule is translated into a polypeptide chain and then folded into a functional protein. Translation process is preceded by transcription, pre-mRNA modification and splicing, which all occurs in the nucleus. The mRNA is then exported from the nucleus to the cytoplasm, where translation itself takes place. The translation is considered as a cycle that is divided into four steps; initiation, elongation, termination and ribosome recycling (**Fig. 1**). In plants the process of ribosome assembly, the mRNA, and tRNAs charging resemble those of other eukaryotes. Yet the unique lifestyle of plants has resulted in specific adaptations in their translation machinery (Browning & Bailey-Serres, 2015). This chapter will focus on cytoplasmic translation cycle in eukaryotes, emphasizing differences in plants.

2.1. Initiation

Initiation of translation is a process resulting in the assembly of the 80S ribosome loaded with Met-tRNAiMet, whose anticodon is base-paired with the start codon of mRNA. The main pathway in eukaryotes is cap-dependent and it is directed with the help of eukaryotic translation initiation factors (eIFs). The initiation starts with the eIF2 activation via the substitution of GDP to GTP, this exchange is mediated by the eIF2B. eIF2-GTP then recruits initiator Met-tRNA$_i^{Met}$. Initiator tRNA does not function in elongation and has a different sequence from Met-tRNA^{Met}, which helps recognize the correct initiation AUG codon (Wrede et. al., 1979). eIF2, GTP and initiator Met-tRNA$_i^{Met}$ form the ternary complex (TC). TC binds to the 40S subunit wit eIFs to form the 43S pre-initiation complex (43 PIC), the binding process is facilitated by initiation factors; eIF1, eIF1A, eIF5 and eIF3 (Jackson et al., 2010). In the meantime, the mRNA is activated by another set of initiation factors. The eIF4F protein complex binds to mRNA the 5'-m^{7}GpppN-cap of the mRNA, this eIF4F complex is composed of cap-binding protein eIF4E, scaffold protein eIF4G and RNA helicase eIF4A. To the scaffold protein eIF4G, is attached eIF4E recognizing the cap structure of the mRNA, also poly(A) tail is connected to eIF4G via poly(A)-binding protein (PABP) (Browning, & Bailey-Serres, 2015). This connection enables mRNA to form a closed loop. In the following step, eIF4A and eIF4B utilize the energy of ATP to promote the unwinding of the secondary structure of the mRNA prior to the interaction of the mRNA with the 43 PIC (Pestova & Kolupaeva, 2002). 43S PIC binds to circularized mRNA to form the 48S scanning complex in the open conformation, which scans 5′ UTR of mRNA until encounters the first AUG. The first AUG is usually selected as the start codon if the context of surrounding nucleotides complies with the

Kozak consensus sequence (Kozak, 1987). The GTP bound to eIF2 is hydrolysed to GDP and inorganic phosphate (P_i) (Hinnebusch, 2017). Successful AUG recognition triggers structural changes that lead to dissociation of eIF1 from the 40S subunit (Valášek, 2012). followed by dissociation of other initiation factors (eIF1A, eIF2-GDP, eIF3 and eIF5). eIF1 dissociation induces the irreversible transition of the 48S to the closed-form, which stalls the entire machinery at the AUG start codon (Hinnebusch, 2017). Eventually, the joining of the large ribosomal 60S subunit is stimulated by eIF5B to form the functional 80S ribosome. At this stage, the Met-$tRNA_i$ resides in the P-site of the 80S ribosome and the A-site of the ribosome is ready to recruit the second charged tRNA to start the first round of elongation (Browning, & Bailey-Serres, 2015).

2.1.1. Plant-specific initiation factors

Translational apparatus in plants is fundamentally similar to that of other eukaryotes, however, some distinctions between them are present. Best known example is that of plants possessing two cap-binding complexes; canonical eukaryotic eIF4F and plant-specific eIF4isoF (Merchante et al., 2017). eIFiso4F includes cap-binding protein eIFiso4E and scaffold protein eIFiso4G. Differences between eIF4F and eIF4isoF are also in the binding specificity to mRNA, with eIFiso4 better tolerating the presence of secondary structures in the 5′UTR eIFiso4 complex has also a higher affinity for hypermethylated 5′caps on the mRNA (Muench et al., 2012). Plants have also multiple paralogues of PABPs in contrast to eukaryotes most of which have only one or a small number of PABPs. Plants hold at least three ancient lineages of PABP genes and for example, *Arabidopsis* possess eight PABP genes and all of them are expressed (Belostotsky, 2003).

2.2. Elongation

Elongation factor eEF1A, which forms a ternary complex with GTP and charged tRNA, delivers charged aminoacyl-tRNA into the empty A-site of the assembled ribosome. Correct codon-anticodon interaction triggers GTP hydrolysis by eEF1A which stimulates peptide bond formation, leaving uncharged tRNA in the P-site. eEF1A-GDP is released and then reconstituted by eEF1B. Following is the translocation of mRNA by one codon which is facilitated by eEF2-GTP hydrolysis. During translocation the peptidyl-tRNA shifts to the P-site and the uncharged tRNA to the E-site. The A-site of the ribosome is at this point ready for the next round of elongation (Dever et al., 2018).

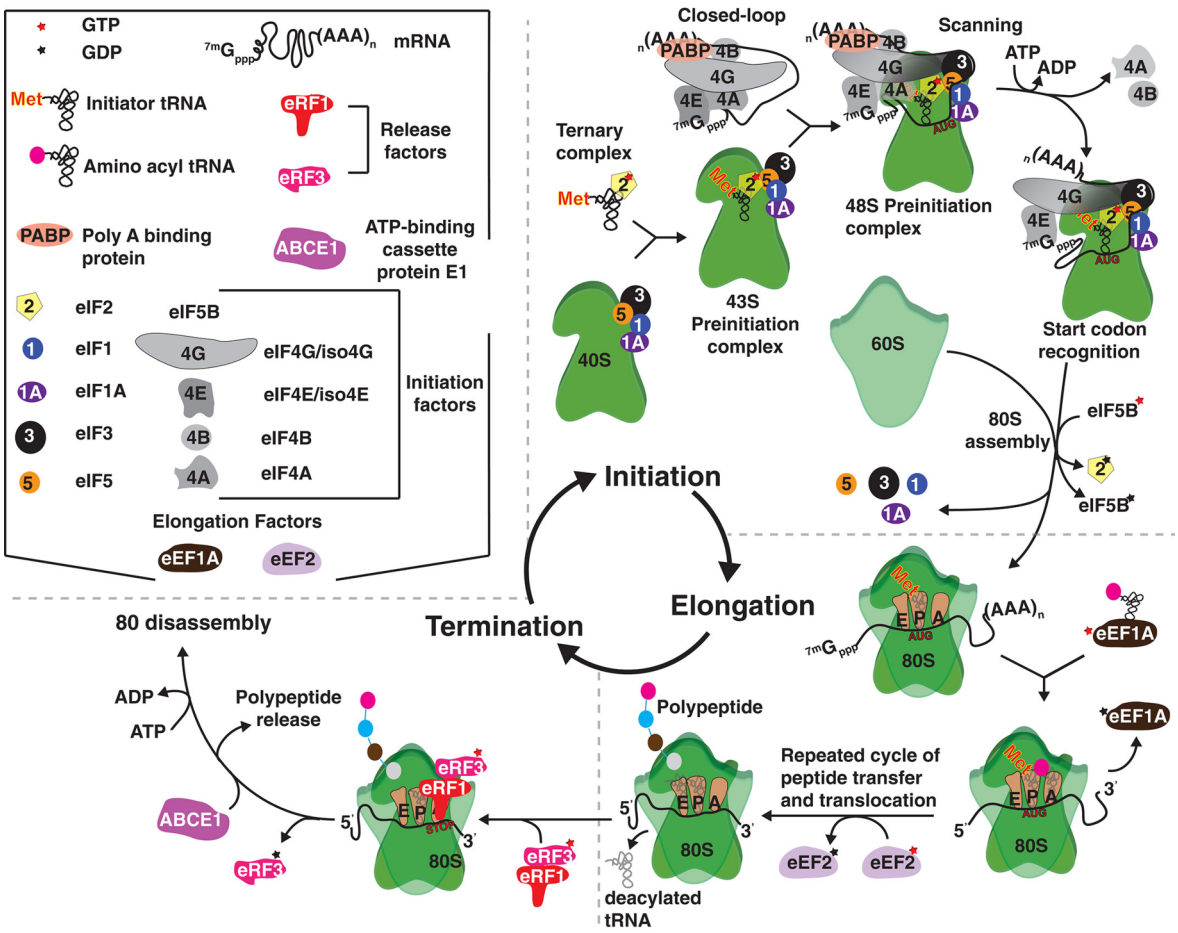

Figure 1: Cytoplasmic translation initiation, elongationa and termination cycles. First, cap-binding complex eIF4F binds to the 5′ cap. eIF4A and eIF4B then join the eIF4F complex. The 43 PIC is assembled of 40S subunit, associated initiation factors (eIF1, EIF1A, eIF3 and eIF5) and ternary complex (eIF2-GTP and Met-tRNAi). The 43 PIC interacts with the cap-binding complex to form 48S scanning complex which then locates the first AUG. After AUG recognition 40S subunit is joined by the 60S subunit and together form a functional 80S ribosome. Next, the elongation factor delivers charged aminoacyl-tRNA into the empty A-site of the assembled ribosome. Correct codon-anticodon interaction triggers GTP hydrolysis by eEF1A, which stimulates peptide bond formation, leaving uncharged tRNA in the P-site. The elongation factor eEF2 uses GTP hydrolysis to translocate the mRNA–tRNA complex by one codon, leaving the A-site empty and ready for the next round of elongation. Termination is mediated by the release factor complex eRF1-eRF3-GTP and occurs when one of the three stop codons (UAA, UGA, or UAG) enters the A-site of the ribosome. For details, see chapter 2 (adapted from Urquidi Camacho et al., 2020).

2.3. Termination and recycling

Termination is mediated by the release factor complex eRF1-eRF3-GTP and occurs when one of the three stop codons (UAA, UGA, or UAG) enters the A-site of the ribosome. The N-terminal domain of eRF1 recognizes the stop codon, whereas the middle domain is responsible for hydrolytic release of the polypeptide and the C-terminal domain binds to eRF3 and ABCE1. After hydrolysis of GTP bound to eRF3, eRF1 releases nascent peptide from the ribosome. The ribosome recycling, which takes after the termination, involves ATP-binding cassette protein ABCE1 that promotes dissociation of the 60S subunit, followed by the release of the tRNA and the mRNA from the 40S subunit, possibly mediated by initiation factors (Hellen, 2018).

3. Characterictics of the eukaryotic ribosome

Ribosomes are large macromolecular complexes of ribonucleoproteins found in prokaryotic and eukaryotic cells in mitochondria and chloroplast. Eukaryotic ribosomes are considered to be around 40% larger than prokaryotic (Ben-Shem et al., 2011) which results in additional expansion segments (ES), variable regions (VR) and extra RPs. Majority of these eukaryote-specific parts are localized on the surface of the ribosome, surrounding the evolutionarily conserved core (Ben-Shem et al., 2011). The eukaryotic 80S ribosome is composed of small ribosomal subunit 40S and large ribosomal subunit 60S that contain four rRNAs and approximately 80-81 RPs. The 60S subunit consists of 5S, 5.8S, 28S rRNAs (25-26S in plants) and around 47 RPs (48 RPs in plants). 40S subunit is comprised of 18S rRNA and around 33 RPs (33RPs in plants). Contact between large and small ribosomal subunits is mediated by intersubunit bridges which help to coordinate their activity (Spahn, et al., 2001). The assembled 80S ribosomes have only temporary existence, which ceases after synthesis of the polypeptide is terminated. Then subunits are split and either recycled or degraded. This chapter will focus on the structure of the eukaryotic small and large ribosomal subunits, and some differences unique to plant ribosomes will be mentioned as well.

3.1. Structure of eukaryotic 40S subunit

The 40S subunit forms parts known as the head, platform, body, beak, shoulder, right foot, and left foot that are specified by the 18S rRNA and 33 RPs. 18S rRNA is composed of a region homologous to the prokaryotic 16S rRNA and eukaryot-specific ESs, which, except for the one region, reside on the surface of the small subunit (**Fig. 2**) (Rabl et al., 2011). SSU is responsible for the decoding process of the mRNA. The major operational sites of the 40S are the mRNA path, mRNA decoding center and parts of the future tRNA binding sites. The mRNA path is used to guide mRNA during translation, where the mRNA enters through a tunnel found between the head and the shoulder and leaves through the exit site localized between the head and the platform of the small subunit (Melnikov et al., 2012).

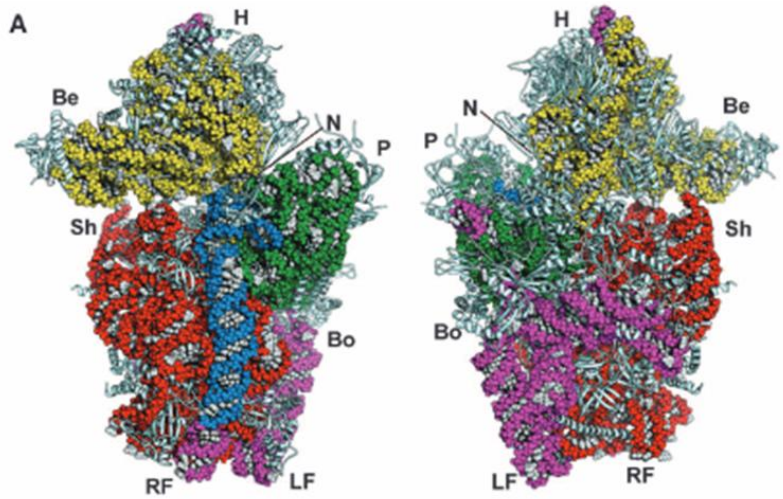

Figure 2: Front and back views of the structure of the 40S subunit. The tertiary structure is showing the 18S rRNA as spheres colored according to each domain: 5′domain is red, the central domain is green, 3′major domain is yellow, 3′minor domain is blue and ESs are magenta. The proteins are colored grey. Abbreviations: H - head, Be - beak, N - neck, P - platform, Sh - shoulder, Bo - body, RF - right foot, LF - left foot. (Adapted from Rabl et al., 2011)

3.2. Structure of eukaryotic 60S subunit

60S subunit is composed of three rRNAs (5S, 5.8S and 25-28S rRNA). Several rRNA ESs, extensions and insertions of conserved proteins and 27 eukaryote-specific proteins are concentrated on the periphery of the large subunit forming a ring-shaped assembly that surrounds the core. The ring-shaped assembly consists of two clusters of RNA expansion segments (Melnikov et al., 2012) to which eukaryotic-specific proteins or eukaryotic-specific extensions of conserved ribosomal proteins can bind (**Fig. 3**). The first cluster comprises ES7, divided into three parts (helices ES7A, ES7B and ES7C), ES39, ES9 and ES12. The second one includes ES5, ES19, ES31, ES20, ES26 and two helices H18 and H58 defining the external boundaries of this region (Klinge et al., 2011). 60S subunit is responsible for peptide bond formation. The major operational sites of the 60S subunit are the three tRNA binding sites (A, P and E) found on the interface side of the LSU. Then, te peptide exit tunnel, through which nascent proteins move, spreads through the body of the large subunit. The last functional site is the peptidyl transferase center (PTC). The peptide bond formation is catalysed by the PTC, which is localized at the tunnel entrance in a conserved region on the interface mostly formed of rRNA (Melnikov et al., 2012).

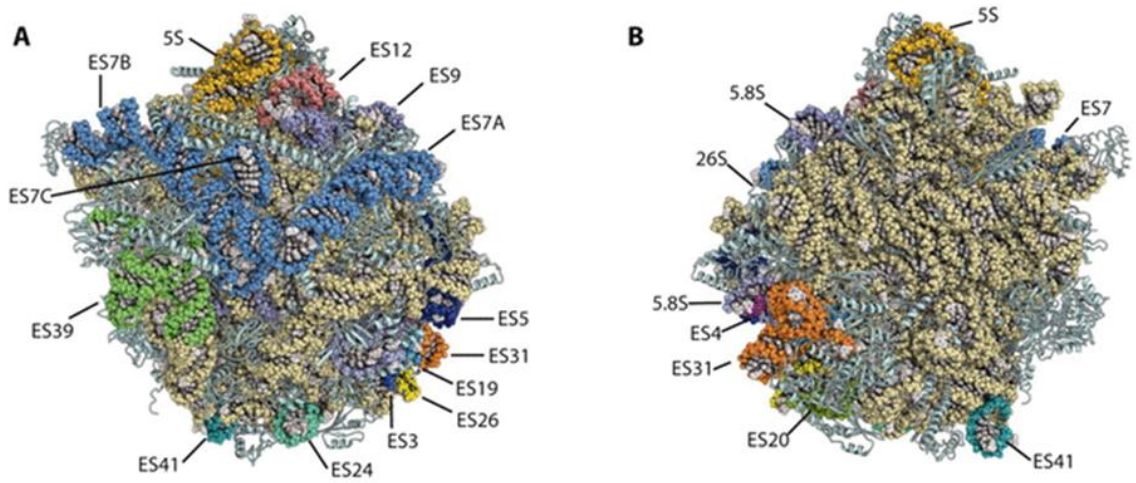

Figure 3: Structure of the 60S ribosomal subunit. A) View of the solvent-exposed and the 40S binding site of the 60S subunit. B) View of the solvent-exposet site with color-coded RNA expansion segments. (Adapted from Klinge et al., 2011).

3.3. Plant ribosome composition

Ribosomes in plants are similarly composed of 40S and 60S subunits. A difference found in plants is that the 60S large subunit comprises 5S, 5.8S, and 25-26S and approximately 48 RPs (Barakat et al., 2001). It was also reported that the plant 60S subunit is about 20% smaller than that of mammals (provided by three-dimensional reconstruction of rabbit and wheat ribosome) (Verschoor et al., 1996). In plants, there was also discovered plant-specific P-protein designated P3. Therefore plants possess three distinct groups of acidic phosphoproteins, P1, P2, and P3, whereas other eukaryotes have only two, P1 and P2 (Szick et al., 1998). In plants each RPs is encoded by multiple paralogs, and therefore is suggested that different members of the ribosomal protein family could be present in ribosomes at different points of development or under different growth conditions, (Schmid et al., 2005) which will be further discussed in the text below.

3.4. Ribosome biogenesis in plants

In plants, ribosome biogenesis is mainly studied in *Arabidopsis thaliana.* However these processes are overall best understood in yeast. The biogenesis of eukaryotic ribosomes includes the transcription of 45S rDNA and 5S rDNA, co-transcriptional processing of pre-rRNA and assembly of mature rRNA with RPs. It is a fundamental and complex process involving many ribosome biogenesis factors (RBFs). The ribosome biogenesis differs between fungi, mammals and plants. This part of the work will characterized described plant 5S and 45S rDNA and then will described processing of 45S pre-rRNA.

3.4.1. 5S rDNA

5S rRNA is encoded by 5S rDNA separated from 45S rDNA encoding 5.8S, 18S and 25-26S. Synthesis of 5S rRNA is maintained by RNA polymerase III (Pol III) and occurs in the nucleus. In the haploid genome of *Arabidopsis,* there is approximately 1000 copies of 5S rRNA genes, organized in tandem arrays and localized in the pericentromeric heterochromatin of chromosomes 3, 4 and 5 (Mathieu et al., 2002). Each unit of 5S rDNA is typically 0.5 kb long and is composed of 120bp transcribed sequence with an internal promoter and approximately 380bp non-transcribed spacer (**Fig. 4**) (Layat et al., 2012).

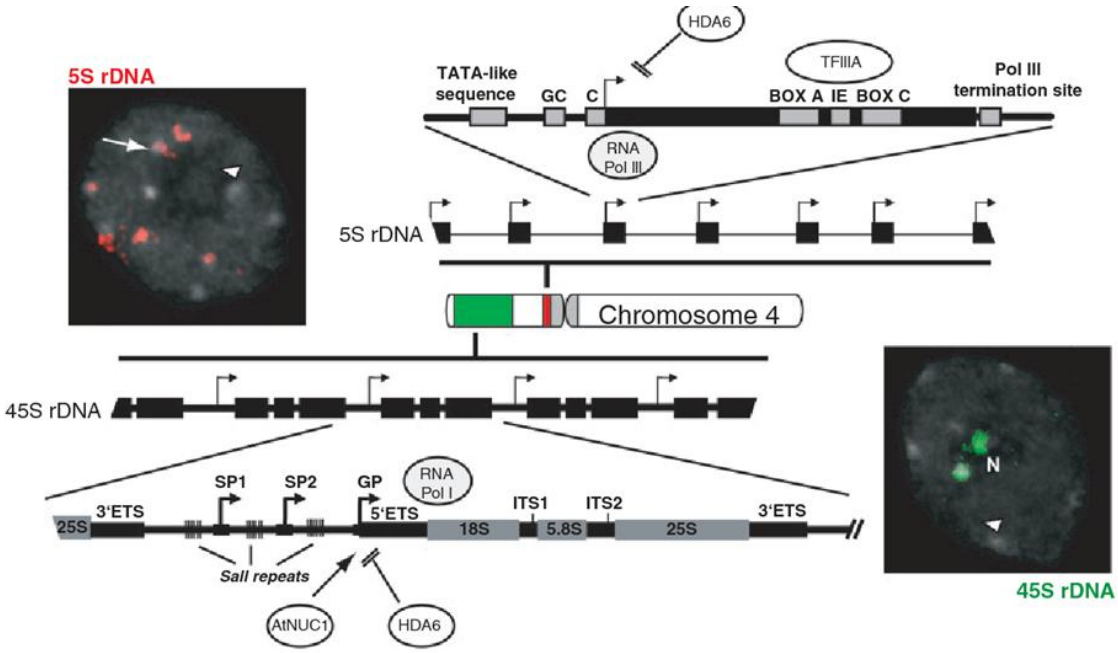

Figure 4: Organization of 45S and 5S rDNA in *Arabidopsis thaliana*. On chromosome 4 tandem repeats of 5S rDNA genes are colored red and 45S rDNA genes are colored green. Grey is used for rRNA genes at the centromere and NOR. Top: 5S rDNA unit composed of TATA-like motif, GC sequence, a C residue at1 and cluster of T residues. Bottom: 45S rDNA unit consists of the intergenic spacer (IGS) region containing SP1 and SP2 and gene promoter (GP). Transcribed region of 45S rDNA contains external (5´ETS and 3´ETS) and internal (ITS1 and ITS2) transcribed spacers and rRNAs (18S, 5.8S and 25S) (Adapted from Layat et al., 2012).

3.4.2. 45S rDNA

The 45S rDNA encoding 5.8S, 18S and 25S rRNAs is transcribed by RNA polymerase I (Pol I) in the nucleolus. Pol I is associated with a large number of GTFs that form larger RNA Pol complexes which then identify gene promoters and initiate transcription of rDNA. In *Arabidopsis* Pol I is formed by 14 subunits. (Saez-Vasouez & Pikaard, 1997).

In *Arabidopsis* haploid genome, 570-750 copies of the 45S genes are organized in tandem arrays at the top of the short arms of chromosomes 2 and chromosomes 4. Genes of the 45S rDNA are tandemly repeated at nucleolar organizer regions (NORs). The 45S rDNA transcribed sequence is 10 kb long and compose of 25S (3376 bp) 18S (1800 bp), 5.8S (161 bp) rRNA sequences Next,

there are the internal transcribed spacers (ITS1 and ITS2) that separate these rRNA sequences and the external transcribed spacers (5′ETS and 3′ETS) that define the 45S rDNA transcribed sequence (**Fig. 4**) (Sáez-Vásquez & Delseny, 2019). The 5′ETS consists of the $A_{123}B$ cluster and 1083 nucleotide insertion specific for plants (Weis et al., 2015). The 3′ETS is comprised of the four major variants (VAR1-VAR4). In 2016 fifth variant (VAR5) of 45S rDNA was reported by Havlová et. al. (Havlová et al., 2016) The rRNA sequences are separated from the adjacent gene in the array by an intergenic spacer (IGS). The IGS is composed of Shall repeats, spacer promoters (SP1 and SP2) and gene promotor (GP) where the transcription of the 45S rDNA starts (Layat et al., 2012).

3.4.2.1. Pre-rRNA processing in plants

Several (sno)RNAs are involved in pre-rRNA processing and rRNA modification. snoRNAs are noncoding RNAs, usually packed in sno-ribonucleoproteins (snoRNPs). Immature (sno)RNAs are localized in the nucleolus in the nucleolar cavity (or nucleolar vacuole) specific for plants (Weis, et al., 2015). Several modifications occur before removing ETS and ITS segments from rRNAs precursors. Two of the most common modifications of rRNA are 2′-O ribose methylation and isomerization of uridine to pseudouridine. 2′-O-methylation of the ribose is directed by snoRNAs of the C/D box type. (Azevedo-Favory et al., 2021). The C/D snoRNA associates with four nucleolar proteins known as Fibrillarin/Nop1p, Snu13 and two scaffold proteins Nop56 and Nop58. For 2′-O-Methylation activity, a characteristic SAM-binding methyltransferase motif localized on Fibrillarin is required (Azevedo-Favory et al., 2021). Two functional Fibrillarin proteins (FIB1 and FIB2) are encoded in the *Arabidopsis* genome (Sáez-Vásquez & Delseny, 2019). Pseudouridylation is guided by H/ACA box snoRNAs and associated with core proteins Nhp2, Nop10, Gar1 and pseudouridine synthetase Dyskerin/Cbf5p. snoRNAs temporarily bind to rRNA precursors, thereby determining bases for nucleotide modification. snoRNAs are assembled with core proteins to form a stable and functional snoRNP particle, and these functional snoRNPs secure nucleotide modification. The structure of both types of snoRNP is bipartite, which means each snoRNP could modify two positions in rRNA (Weis et al., 2015).

The processing of 45S pre-rRNA (**Fig. 5**) includes exonucleolytic and endonucleolytic cleavages to remove external and internal transcribed sequences. First, the 5′ETS is shortened by the exonuclease XRN2 to expose the P site, then the 5′EST of the pre-RNA is cleaved by a U3 snoRNP complex at the exposed P site, which is the initial cleavage site (Sáez-Vásquez & Delseny, 2019). RNase III mediates cleavage of 3′EST, and it helps to stimulate the termination of transcription. (Weis et al., 2015).

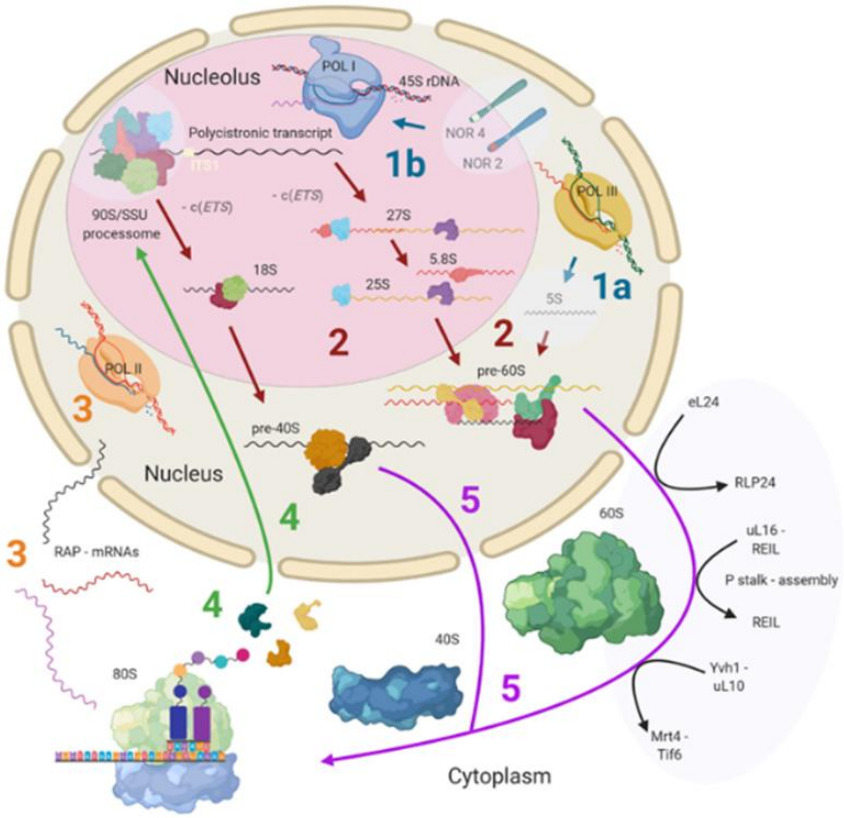

Figure 5: Scheme of plant ribosome biogenesis. Ribosome biogenesis involves three cell compartments, the nucleolus, the nucleus and the cytoplasm. The linchpins of biogenesis are the transcription of 45S rDNA, co-transcriptional processing of pre-RNA and assembly of mature rRNA together with RPs. The 5S rRNA is transcribed in the nucleus by RNA polymerase III. The 5.8S, 18S and 25S rRNAs are encoded by 45S rDNA and transcribed by RNA polymerase I in the nucleolus. The large pre-rRNA transcript composes the 90S pre-ribosome, then split into pre-40S and pre-60S subunits. The RAPs are transcribed by RNA polymerase II. RPs and other RAPs are translated in the cytoplasm and imported into the nucleus, where they are assembled or help the assembly process. The ribosomal pre-40S and pre-60S subunits are exported to the cytoplasm, where the final maturation steps are undergone. (Adapted from Martinez-Seidel et al., 2020).

Removal of ETS and ITS leads to 35S pre-rRNA, which can be processed in two alternative ways: the minor 5´ETS-first pathway and the major ITS1-first pathway. In 2019, another plant-specific ITS2 processing pathway was reported in *Arabidopsis* by Palm et al. The ITS2 pathway is initiated by cleavage at C2 within ITS2 stimulated by endonuclease Las1 (Pillon et al., 2019). The 35S pre-rRNA is cleaved at the P´, P_2 and A_2 sites, followed by the cleavage at the B_1 site in the 5´ ETS-first pathway. In *Arabidopsis*, 33S and 32S pre-rRNAs were identified in this pathway (Weis et al., 2015). In the major ITS1-first pathway, the 35S is cleaved at the A_3 site, followed by the cleavage at the P´ and P_2 sites. In *Arabidopsis*, the major ITS1-first pathway is similar to the pathway used in mammals while the minor 5´ETS-firts pathway is comparable to the pathway in yeast (Sáez-Vásquez & Delseny, 2019). Separation of the precursors of the small and large ribosome subunits is especially conducted by A_2 cleavage in ITS1 (Weis et al., 2015), but with the identification of $27SA_2$ in *Arabidopsis* that overlap with the P-A_3 fragment, it was suggested that

also cleavage at the A_3 site is used for separation of the 90S into pre-40S and pre-60S particles (Weis et al., 2015). The convergence point of both alternative paths is the 20S and $27SB_{S/L}$ rRNA. These precursors of rRNA are subjected to standard processing events to receive the mature 25S, 18S and 5.8S rRNAs. Ribosome-associated proteins (RAPs), which includes also the RPs, are transcribed by RNA polymerase II in the nucleus, then their transcripts are spliced and exported to the cytoplasm, where translation of the protein takes place. In the next step, RPs are imported to the nucleus or nucleolus. This transfer is mediated by chaperones which protect RPs from degradation. Finally, in the nucleus or nucleolus, RPs are associated with nascent ribosomal subunits (Lindah et al., 2019). Following nucleolar and nuclear processing, pre-60S and pre-40S subunits are exported into the cytoplasm to undergo final maturation (Martinez-Seidel et al., 2020).

4. The concept of ribosomal heterogeneity

Historically, the ribosome has been viewed as a static, indiscriminate protein synthesis machine that only reads the mRNA and creates polypeptides. With new and increasing evidence, ribosome transitioned to dynamic macromolecular complex and it´s heterogeneous nature was demonstrated on various model organisms. A prominent model of ribosome heterogeneity is seen in plants, namely *Arabidopsis* where each of the 81 RPs is encoded by two to seven paralogs (Xiong et al., 2021). Multiple paralogs of each RPs gene are typically present due to genome duplication. The ribosome heterogeneity is described as any altered formation of the translation machinery at any level of the ribosome, including sequence variation of rRNAs, exchange of RP paralogs, absence of specific RPs from the canonical ribosome structure, and posttranscriptional or posttranslational modifications of RPs or rRNAs (**Fig. 6**) (Martinez-Seidel et al.,2020). Plant ribosomes are heterogeneous at multiple levels, but the functional impact of ribosome heterogeneity is currently, in most cases a matter of debate. Overall, it´s hypothesized that ribosome functions (translation, ribosome recycling, ribosome biogenesis) could be affected by ribosome heterogeneity (Martinez-Seidel et al., 2020). Several studies indicated deviations from the canonical 80S structure of the ribosome in plants. For example, the RPL4 family contains two expressed members, RPL4A and RPL4D, which are almost identical and share 95,1% of amino acid sequences. Subpopulations of heterogeneous ribosomes containing RPL4 paralog A or D members co-exist in plants (Rosado et al., 2010).

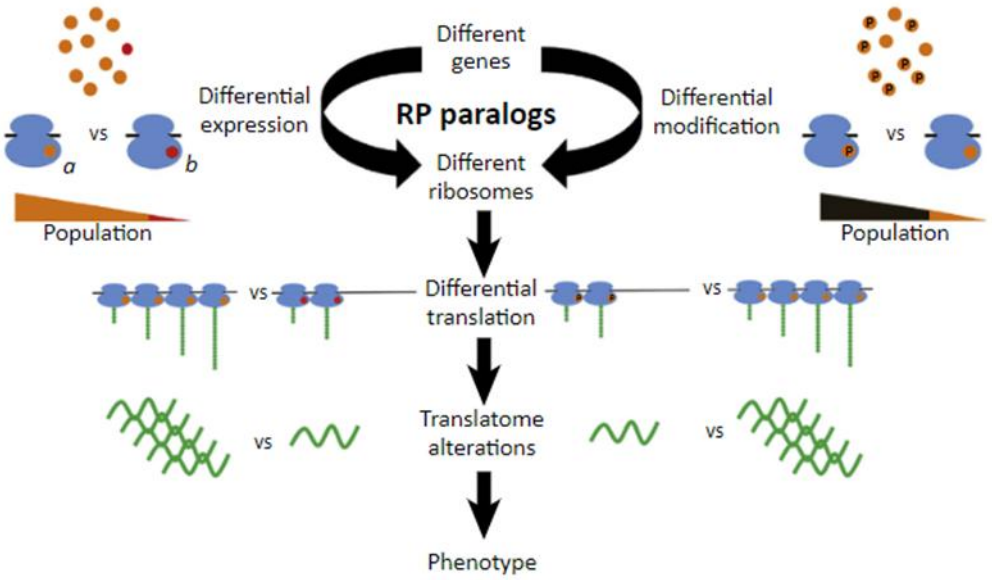

Figure 6: Ribosome heterogenity. Ribosome heterogenity can occur via incorporating different a or b RP paralogs or by incorporating post-translationally modified RPs into ribosomes. This could lead to the formation of distinct ribosomes that vary in the intrinsic ability to identify mRNA substrates and therefore affect changes in translation as well as in phenotype. (Adapted from Gerst, 2018).

A fast and efficient way to create heterogeneity is also post-translational modification of RPs where *de novo* synthesis of complete ribosome or RPs is not required. RPs can undergo a variety of covalent modifications (for example, phosphorylation, N-terminal methylation, initiator methionine removal, phosphorylation and so on)(Martinez-Seidel et al., 2020). One of the best known post-translational modifications is phosphorylation of the C-terminus of RPS6 protein at multiple serine residues mediated by the TOR kinase pathway (Turkina et al., 2011) and by this phosphorylation, the RPS6 is thought to regulate the translation of specific proteins (Beltran-Penaet et al., 2002). TOR kinase stimulates cell growth and development by translational activation of specific mRNAs. Multiple external stimuli (light, hormones, stress) can trigger TOR kinase activation. Activated TOR kinase then phosphorylate the N-terminus of S6K kinase, which contains TOR signalling motif essential for S6K regulation. Activated S6K then phosphorylate RPS6 (Dobrenel et al., 2016; Schepetilnikov et al., 2017).

The translated proteome must be shaped by selective transcript recruiting according to external stimuli for heterogeneous ribosomes to be functional. For instance, in plants, cis-regulatory elements of mRNAs are used as one of the regulatory mechanisms to select subsets of transcripts for translation (Martinez-Seidel et al., 2020). The upstream open reading frame (uORF), as a cis-regulatory element, begins with AUG in the 5´untranslated region of an mRNA. uORFs can be translated, resulting in supression of initiation event repress of the main ORF (Urquidi Camacho et al., 2020). External stimuli (for example, the presence of phytohormone auxin) trigger activation of

TOR kinase, which phosphorylates the S6K, which then phosphorylates eIF3h. Phosphorylated eIF3h is known for promoting reinitiation event on the main ORF after translation termination at the uORF (Schepetilnikov et al., 2013).

5. Characterization of plant ribosomal protein mutants

The cytoplasmatic ribosomes in *Arabidopsis thaliana* are composed of four rRNAs (5.8S, 5S, 18S and 25-26S) and 81 RPs, as mentioned above. The 81 ribosomal proteins are encoded by 254 genes. RP genes in plants exist as families of multiple expressed members; (Barakat et al., 2001) however, from each family, only one RP (with the exception of ribosomal acidic phosphoproteins P1-P3) is included in any ribosome, which indicates that many RP genes could be development-specific, tissue-specific or stress-specific. (Sormani et al., 2011; Horiguchi et al., 2012). Interestingly, was found that the peak of translation of RP occurs at night. The translation of RP was high from midnight to dawn. Synthesis of RPs occupies a large part of the translational capacity of plant cells therefore it´s logical the RPs translation peaked at night because the cell has spare capacity at that time (Missra et al., 2015). Historically RPs were considered as purely housekeeping proteins, needed only for primary architectural roles in the ribosome; however, it was revealed that during cold treatment, UV treatment, and over a variety of developmental stages, paralogs are differently expressed, which indicated that RPs have a more significant role in plant growth and development. Evidence that RPs partake in plant development provides the phenotypes resulting from a mutation in several RP genes. In addition, RPs also took part in response to stress (Sormani et al., 2011; Horiguchi et al., 2012). This chapter will be focused on ribosomal protein mutants and their role in plant development their importance is demonstrated through mutants.

5.1. Nomenclature of plant ribosomal proteins

With the characterization of constantly new ribosomal proteins, a unified naming system has become a necessity. Ribosomal protein families are marked with S if they are components of a small ribosomal subunit and L if they belong to a large ribosomal subunit. Members of each ribosomal family are distinguished by letter (starting with A and continuing). There are also some exceptions to this naming system; acidic ribosomal phosphoproteins (RPP1-RPP3) components of the 60S subunit and the receptor for activated C kinase 1 (RACK1) component of the 40S subunit (Barakat et al., 2001). This nomenclature was later changed, and new names were assigned for ribosomal proteins. Proteins get assigned E.coli name (because RPs from E. coli were the first to be isolated and fully sequenced) and marked by the prefix ‘u’ (universal) for proteins found in all three domains of life, ‘b’ (bacterial) for proteins found only in bacteria, ‘e’ (eukaryotic) for proteins found

only in eukaryotes and ‘a’ (archeal) for archaeal ribosomal proteins (Ban et al., 2014). In this chapter the older nomenclature will be used older nomenclature, which was adapted from Browning et al., 2015.

5.2. Cytoplasmic ribosomal proteins of the 60S large subunit

RPs deficiency in ribosomal mutants can cause a variety of defects in developmental phenotypes and in extreme cases, it can result in embryo lethality. RPs deficiency mutants generally share some similar defects; reduced growth, altered leaf morphology, and reduced cell proliferation, which will be demonstrated below. Members from the same RP family can have non-equivalent functions and can act differently under specific stress conditions or in different developmental stages. Here will be summarized observed phenotypes in RPs mutants of 60S subunits.

P3 protein designated AtP3B was initially isolated from heat-treated *Arabidopsis* suspension culture cells (Kang et al., 2016). P3 proteins are plant-specific ribosomal P-proteins that act as both protein and RNA chaperones to enhance heat and cold stress tolerance in *Arabidopsis thaliana*. Both overexpression and knockdown of the *AtP3B* gene were examined in plants. Under heat stress, overexpressed plants showed significantly increased thermotolerance, whereas knockdown mutants were more sensitive to heat stress. Also, under low temperatures, plants with *AtP3B* overexpression exhibit better tolerance to cold and knockdown mutants were more sensitive to cold stress. In addition, the effect of low temperature on plant growth was examined and it was shown that knockdown mutants were about 30% shorter and overexpressed plants were about 35% longer than wild-type plants (Kang et al., 2016). In *Ipomoea batatas* (sweet potato) plants with the *Arabidopsis* ribosomal gene *AtP3B* overexpression were generated and exposed to low and high temperatures. These transgenic plants exhibited better tolerance to heat and cold, consistent with previous results in *Arabidopsis*. Further, increased storage ability under long-term exposure to low temperature was reported in sweet potato (Ji et al., 2017).

In *Arabidopsis thaliana*, *Oryza sativa* and *Nicotiana tabacum* the RPL3 family is composed of two genes *RPL3A* and *RPL3B*. In *Arabidopsis*, T-DNA insertion of *RPL3A* resulted in embryo lethality (Tzafrir et al., 2004). Silencing of both genes of RPL3 in *Nicotiana tabacum* affects growth, in silenced plants retarded development, inhibition of lateral root growth, and also reduction in the accumulation of pre-RNA were observed. These analyses demonstrated that cell division is positively regulated by RPL3 (Popescu & Tumer, 2004). In (rice), it was reported that the *RICE MINUTE-LIKE1 (RML1)* gene encodes *RPL3B*. *rml1* mutants were characterized by narrow leaves, irregular leaf edges, reduced seed size, reduced number of large and small veins in the leaf

blades, inhibited growth of lateral root, and delayed flowering. The abnormal vascular pattern and leaf shape observed in mutants are thought to be due to defects in auxin distribution. *rml1* mutant plants were also significantly shorter (by 72%) than wild-type plants. It was shown that *RPL3A* expression levels in mutants are comparable to that in control plants and, therefore, cannot compensate for the mutation of *RPL3B*. Reduced accumulation of polysomes examined in mutants suggested that *RPL3B* has an essential role in translating specific transcripts and that developmental abnormalities observed in rml1 mutants might be caused by defective translation initiation. (Zheng et al., 2016).

The *Arabidopsis* RPL4 family is a highly conserved component of the 60S ribosomal subunit and is composed of two transcriptionally active genes, *RPL4A* and *RPL4D* and two pseudogenes, *RPL4B* and *RPL4C* (Barakat et al., 2001). In *rpl4a-1* mutants, narrow pointed leaves, retarded growth, altered cotyledon architecture, reduced venation, few or no tertiary or quaternary veins, reduced root elongation, altered root gravitropic responses, delayed transition to the reproductive phase, and decreased hypocotyl elongation were observed. In addition, the *rpl4a-1* mutants exhibited more chaotic nucleolar structures with an abundance of large nucleolar vacuoles in root meristem cells (Rosado et al., 2010). A similar phenotype to that of *rpl4a* was also observed in *rpl4d* mutants. Both mutants had also delayed delivery of vacuolar proteins, therefore it was suggested that both *RPL4A* and *RPL4D* genes have similar functions. Various developmental phenotypes reported in both mutants are possible consequences of impaired auxin responses (Rosado et al., 2010). In 2012, it was reported that RPL4A and RPL4D (as well as RPL5A) could modulate auxin responses through the translational regulation of multiple auxin response factors (ARFs). ARFs are regulated through upstream open reading frames (uORFs) (Urquidi Camacho, et al., 2020). Ribosomal mutants were used to reveal the unique functions of ARFs in plant development. The double mutant with ribosomal proteins *rpl4d*, *rpl4a* (and *rpl5a*) and auxin responsible factors *arf2*, *arf3*, and *arf6* were examined. The *arf2 rpl4d* double mutants exhibited a higher number of distorted and spiral rosette leaves, and also distorted stems were observed in these mutants, indicating that ARF2 plays a role in proper leaf development and stem development. Further was found that *arf6 rpl4d* exhibited the same phenotype as *arf2 rpl4d* mutant plants, which showed that also ARF6 is needed for proper leaf development. *arf3 rpl4* double mutants displayed terminal inflorescences, organ fusion and enhanced branching, meaning that ARF3 is essential for the development of shoot apical meristem (SAM) and boundary establishment in organs. In addition, in *arf3 rpl4d* terminal floral meristem was observed (Rosado et al., 2012). In 2015, in *rpl4d* mutants, reduced lipid accumulation was demonstrated (Li et al., 2015). By Kakhei et al., mutation in *RPL4A* was reported as the *suppressor of acaulis 56* (*sac56*) which restored the

phenotype of *acaulis5* (*acl5*). Interestingly was found that even though *rpl4a-2* mutants (previously reported by Rosado et al., 2010) did not restore the *acl5* phenotype, the *sac56-d/+ rpl4a-2/+* double mutants did (Kakehi et al., 2015). In 2016 silencing of RPL4 in *Nicotiana benthamiana* showed that these mutant plants have the tolerance to high-temperature stress (Gangadhar et al., 2016).

The RPL5 family consists of two members, *RPL5A* and *RPL5B*. Mutations in *RPL5A* were independently reported as *enhancer6* (*ae6*) (Yao et al., 2008) and *piggyback3* (*pgy3*); (Pinon et al., 2008) these mutations enhance defects in adaxial patterning in *asymmetric leaves1* (*as1*) and asy*mmetric leaves2* (*as2*). *ae6 as2* double mutants exhibited abaxialized leaves, and three types of leaves were observed expanded, lotus-like and needle-like (Yao et al., 2008). In *rpl5b as2* double mutants, rosette needle-like leaves were observed. Additionally, it was also shown that double heterozygote (*ae6-2 rpl5b*) demonstrated a phenotype similar to single mutants of *ae6-2 or rpl5b* (Yao et al., 2008). In *pgy3* single mutants, serrated-pointed rosette leaves were observed. And the double mutants *as1 pgy3* were characterized by narrower, more elongated leaves with adaxial ectopic lamina (Pinon et al., 2008). The *ang3* mutant identifies the *RPL5B* gene. The *ang3* mutants are characterized by significantly reduced leaf lamina area from the third rosette leaf, cell number in the epidermal layer of the third leaf was equal to that of control plants, however, the cell size in mutants was significantly decreased, suggesting that the reduced width and area of the leaf is a result of decreased cell expansion. In addition, hypocotyl and primary root length were reduced in *ang3* mutants (van Minnebruggen et al., 2010). In 2009, it was reported that *oligucellula 5* and *7* (*oli5* and *oli7*) encode *RPL5A* and *RPL5B,* respectively. Both *oli5* and *oli7* mutants were characterized by a reduced number of palisade mesophyll cells consistent with double mutants of both *oli5* and *oli7* with *oli2,* where extremely reduced cell number in leaves was observed (Fujikura et al., 2009). As mentioned above, RPL5A also modulates auxin response through the translational regulation of ARFs (Rosado et al., 2012).

Ribosomal protein large subunit gene, *RPL6*, was functionally characterized for its role in salt stress tolerance in rice. In the promoter region of RPL6, various cis-regulatory elements are present, these elements likely respond to stress or hormone-specific signals. (Moin et al.,2016a). Additionally, enhanced water-use efficiency was reported in plants with *RPL6* overexpression. (Moin et al., 2016b). Further, the transgenic plants with RPL6 overexpression showed better salt stress tolerance and could tolerate moderate (150mM) to high (200mM) salt levels. Further, transgenic plants exhibited increased chlorophyll and proline accumulation and higher seed yield. It was shown that *RPL6* enhances the expression of growth, development and stress associated proteins, which helps to balance the growth and yield and leads to salt tolerance in rice (Moin et al., 2021).

The RPL9 family in *Arabidopsis* consists of three genes *RPL9B*, *RPL9C* and *RPL9D* (Barakat et al., 2001). *RPL9B* and *RPL9C* genes share 100% identity in amino acid sequences. *RPL9D* is a more divergent member, sharing 89% identity in amino acid sequences with the other two genes (Devis et al., 2015). In 2008, *piggyback2* (*pgy2*) mutation in *RPL9C* was reported. In *pgy2* single mutants, serrated-pointed leaves were observed and in *as1 pgy2* double mutants, narrower, more elongate leaves with adaxial ectopic lamina were formed (Pinon et al., 2008). T-DNA mutants, *rpl9d*, showed no distinction in a leaf shape in contrast to rpl9c (named *pgy2*) that was previously reported by Pinon et al., in 2008. Further, in r*pl9c/+ rpl9d* mutant plants, mildly serrated leaves were observed, whereas *rpl9c rpl9d/+* double mutants exhibited a more severely altered leaf phenotype, leaves were more pointed, and their size was reduced. These results suggested, that *RPL9D* also plays a role in leaf growth but acts redundantly with *RPL9C* in leaf development. The *rpl9c rpl9d* double homozygous mutants were embryo-lethal, arrested at the globular stage of development. These examinations indicate *RPL9C* and *RPL9D* are redundant (Devis et al., 2015).

In the genome of *Arabidopsis thaliana* were identified three RPL10 genes *RPL10A*, *RPL10B*, and *RPL10C* (Barakat et al., 2001). Amino acid sequences of these three RPL10 genes show 94-97% identity. In 2008, a semi-dominant mutation in *RPL10A* was reported and named as *sac52*. It was demonstrated that *suppressor of acaulis52* (*sac52)* mutation can restore the ability of the stem elongation and so suppresses the dwarf phenotype of the *acaulis* (*acl5)* mutants. Additionally, T-DNA heterozygous mutant in RPL10A, *sac52-t1/+*, exhibited siliques with a 1:1 ratio of normal fertilized seeds and aborted ovules, indicating the essential role of *RPL10A* in female gametophyte development. (Imai et al., 2008). Further, in the *RPL10A* gene, two T-DNA insertional lines (*rpl10A-1* with insertion in the second exon and *rpl10A-2* with insertion in the third exon) were observed and in both, only heterozygous plants were viable. No visible phenotypes were present in either mutant line compared to control plants. In the *RPL10B* gene T-DNA insertion mutant, both heterozygous and homozygous plants were identified. Homozygous *rpl10B* mutants were characterized by narrow and pointed first leaves, reduced width and length of the leaves, shorter roots, shorter inflorescence shoot, and reduced length of the siliques. Two homozygous lines with T-DNA insertion were found for the *RPL10C* gene, but neither exhibited an abnormal phenotype (Ferreyra et al., 2010a). The influence of UV-B on RPL10 regulation was described in 2010. It was observed that the *RPL10C* transcript level was up-regulated when irradiated by UV-B at 0,5, 1 and 2 Wm^{-2}, the highest increase of RPL10C expression occurred at 2 Wm^{-2} (watt per square metre) irradiation. *RPL10B* expression was down-regulated, but only at higher (2 Wm^{-2}) Transcript levels of *RPL10A* did not change under any UV-B irradiation (Ferreyra et al., 2010b). In

2020 the effect of abscisic acid (ABA) on RPL10A was investigated. In presence of ABA *rpl10A/+* mutants exhibited earlier germination of seeds, whereas mutants with *RPL10A* overexpression showed reduced germination pattern, suggesting RPL10A is essential for the ABA-mediated inhibition of seed germination. In addition, during control conditions without the presence of ABA, *rpl10A/+* mutants exhibited reduced cotyledon greening and decreased early seedling development in contrast, overexpressed mutants did not show any differences. Further, *rpl10A/+* showed defects in root growth with short primary roots and impaired lateral root formation, whereas overexpressed showed longer primary roots with higher lateral root density than control plants. (Ramos et al., 2020).

RPL10aB gene is a member of the *Arabidopsis* RPL10a family (Barakat et al., 2001). RPL10aB is encoded by *PIGGYBACK1* (*PGY1)* gene. *pgy1* single mutants exhibited slightly pointed leaves and more prominent marginal serrations. In double mutant *pgy1 as1*, again narrower, more elongated leaves with adaxial ectopic lamina were observed. In triple (*as1 pgy1 pgy2* and *as1 pgy1 pgy3*) and quadruple (*as1 pgy1 pgy2 pgy3*) mutants, a slightly increased frequency of outgrowths was observed. Additionally, it was reported that *pgy1* enhanced *revoluta* (*rev*) inflorescence defect, inflorescence formed a terminal filamentous organ or did not produce organs at all (Pinon et al., 2008).

RPL12 and RPL19 protein families are involved in nonhost resistance in Nicotiana benthamiana and *Arabidopsis thaliana*. Silencing of both genes *NbRPL12* and *NbRPL19* showed a delay in nonhost bacteria-induced hypersensitive response with a concurrent increase in nonhost bacterial accumulation. However, a more severe decrease in resistance was observed in *NbRPL12*-silenced plants than in *NbRPL19*-silenced plants. *Arabidopsis* mutants *rpl12c* and *rpl19b* also compromised nonhost resistance (Nagaraj et al., 2016).

RPL14B, a member of the *Arabidopsis* RPL14 family, has an essential role in fertilization, especially in the growth and guidance of the pollen tube. Homozygous *rpl14b* mutant is embryo lethal, and reduced male and female fertility were observed in heterozygous mutants (*rpl14b-1/+* and *rpl14b-2/+*) Further, defects in *rpl14b-1* male gametophyte were examined. Affected pollen size and pollen tube competitiveness were observed in mutants, but pollen viability and mitosis were normal. Additionally, in heterozygous mutants, cell fate in female gametophytes is impaired, affecting ovule fertilization (Luo et al., 2020).

Protein family RPL18 comprises three members *RPL18A*, *RPL18B* and *RPL18C* in *Arabidopsis thaliana*. Mutants with T-DNA insertion in *RPL18C* (*rpl18c-1*) are characterized by smaller-pointed leaves (Horiguchi et al., 2011). Double mutants *rpl18c-2 var2* showed an increased proportion of white leaf sectors (Wang et al., 2018). *var2* mutants are characterized by yellow true

leaves that during their development turn green- or white sectors (Martínez-Zapater, 1993). Single mutants *rpl18c-2* exhibited delayed leaf development (Wang et al., 2018).

RPL18aB gene, together with the *RPL18aC* gene, is a member of the RPL18a family. In *Arabidopsis*, *rpl18aB* mutant embryos showed delayed or failed pattern formation and irregular cell division orientations in the early stages of development, resulting in abnormal shapes. Additionally, embryo development was arrested at the globular stage and thus never went through the apical-basal pattern formation stage, which resulted in the abortion of embryogenesis and seed formation. Further, *rpl18aB* mutant plants exhibited reduced competitiveness of pollen grains in growing in the style, but their ability to germinate wasn´t affected. It was determined that the *RPL18aB* gene is required for both male gametophyte function and embryo development (Yan et al., 2016).

The *RPL23A* is a member of the RPL23 family. The promoter region of RPL23A has several cis-regulatory elements that possibly respond to stress/hormone-specific signals (Moin et al., 2016). In rice, transgenic plants with *RPL23A* overexpression had high water-use efficiency, increased fresh weight, root length, accumulation of proline and chlorophyll contents under simulated drought and salt stresses (Moin et al., 2017).

Arabidopsis RPL23a family includes two membres; *RPL23aA* and *RPL23aB*. The amino acid sequences of the RPL23a genes share 94.8% identity. Silencing of *RPL23aA* gene in *Arabidopsis* resulted in retarded plant growth, delayed transition to reproductive growth and morphological abnormalities such as irregularly shaped or fused older leaves, loss of apical dominance, increased rosette branching, reduced root growth and abnormal root phenotype (short root hairs and malformed lateral roots). *RPL23aB* silencing in Arabidopsis did not affect plant development. Further, was demonstrated, that coordinated silencing of both genes *RPL23aA* and *RPL23aB* is lethal (Degenhardt & Bonham-Smith, 2008). In 2020, T-DNA insertion mutants of *RPL23aA* (*rpl23aa*) and *RPL23aB* (*rpl23ab*) were reported. *rpl23aa* mutants showed pointed leaves, retarded root growth, and reduced plant size, whereas in *rpl23ab* no phenotype was detected. In addition, in 5% of the population of *rpl23aa* mutants, an incompletely penetrant tricotyledon phenotype was present. Interestingly, it was demonstrated that the phenotype of *rpl23aa* can be recovered by the expression of *RPL23aB* driven by the *RPL23aA*. This result suggested that RPL23aA and RPL23aB proteints are equivavalent in function. (Xiong et al., 2020).

RPL24 family is encoded by three genes *RPL24A*, *RPL24B* and *RPL24C* in *Arabidopsis*. *STV1* encodes the RPL24B gene in *Arabidopsis*. In *stv1-1* mutants, disrupted apical-basal development of the gynoecium was demonstrated, the gynoecium possessed a longer gynophore, shorter ovary and in some cases, only one side of the ovary was present. Defects in gynoecium

patterning in *stv1* mutants are thought to be due to impaired translation reinitiation of ARF5 and ARF3 containing uORFs in 5´UTR and therefore affected auxin response. (Nishimura et al., 2005). In addition, in *stv1-1* mutants, pale green leaves were observed. Double mutants *stv1-1 as2-1* were characterized by needle-like rosette leaves (Yao et al., 2008). Further, in 2010 was shown that *eif3h* displayed a similar valve defect to *stv1* mutants. In addition, it was also found that both *stv1-1* and eif3h-1 mutants had decreased translational efficiency on the uORF containing mRNA of *Arabidopsis* leucine zipper transcription factor 11 (bZIP11), suggesting that both RPL24B and eIF3h similarly promote translation reinitiation (Zhou et al., 2010). The *PCA21* gene encodes RPL24A. In general, the *pca21* mutant partially suppressed the insensitivity of *Arabidopsis thaliana ring zinc finger1* (*atrzf1)* to osmotic stress and abscisic acid during seed germination and the early seedling stage (Park et al., 2017). In 2015 study was carried out on Cucurbitaceae family members, and in melon, the response of the *RPL24* (*CmRPL24-01*) gene to drought stress was examined. Gene expression was up-regulated during the early response to drought stress, but after 12 hours of stress condition, expression levels of the RPL24 gene significantly decreased (Baloglu et al., 2015).

The RPL27a family in the *Arabidopsis* genome is encoded by three genes, *RPL27aA*, *RPL27aB* and *RPL27aC*. *RPL27aA* gene is not expressed and is assumed to be a pseudogene (Barakat et al., 2001). *RPL27aB* and *RPL27aC* are transcriptionally active and their encoded proteins are highly conserved, varying in just two of 146 amino acids (Zsögön et al., 2014). Semidominant mutants of the *RPL27aC* gene (*rpl27ac-1d*) have a broad range of defects, slow embryo growth during embryogenesis, delayed patterning of the apical domain of the embryo and tissue-specific misexpression of meristem and organ-patterning genes. *RPL27aC* is also needed for female gametophyte viability. In heterozygous *rpl27ac-1d/+* mutants, serrated-pointed leaves and a weak reduction in fertility was observed. Homozygous *rpl27ac-1d* mutants exhibited more severe defects, they were predominantly female sterile, their siliques had increased number of aborted ovules and extremely reduced seed set. In addition, *rpl27ac-1d* homozygotes had smaller leaves with asymmetric lamina, unfused cotyledon petioles, and reduced petal and stamen numbers. (Szakonyi & Byrne, 2011). Both homozygous insertion mutants of the *RPL27aC* gene (*rpl27ac-2* and *rpl27ac-3*) and the *RPL27aB* gene (r*pl27ab-1* and *rpl27ab-2*) had serrated-pointed leaves and shorter siliques with reduced seed number and with aborted or unfertilized ovules. However, *rpl27ab* mutants had a less severe phenotype than *rpl27ac* mutants, suggesting that the RPL27aB gene has a less important role in leaf development than the *RPL27aC* gene (Zsögön et al., 2014).

In *Arabidopsis*, RPL36a family includes two genes, *RPL36aA* and *RPL36aB*, which are identical in amino acid sequences. Mutation in *RPL36aB* was reported as allele *apiculata2* (*api2*). Homozygous *api2* and *rpl36aa* mutants showed pointed leaf phenotype. It was also demonstrated

that both api2 and rpl36aa enhanced the phenotype of the *as2* mutants. In double mutants *api2 as2*, trumpet-shaped leaves were observed. In contrast, in double mutants, *rpl36aa as2* were present not only trumpet-shaped leaves but also radial leaves, indicating a more severe phenotype than in api2 as2 mutants. Further in *api2/+ rpl36/+* double heterozygotes pointed leaves were found. (Casanova-Sáez et al., 2014).

5.3. Cytoplasmic ribosomal proteins of the 40S small subunit

As mentioned above, mutation of specific RPs of the 60s subunit can result in various developmental phenotypes, similar phenotypes are observed also in mutants of RPs of the 40S subunit, which will be characterized in this part.

Arabidopsis RPS5 family consists of two genes, *RPL5A* and *RPL5B*, which are 94% identical in amino acid sequences. The T-DNA insertion in the fifth exon of the RPS5A was reported as Arabidopsis minute-like 1 (aml1). The T-DNA insertion caused semi-dominant developmental phenotypes in the mutant plants. In *aml1* mutants, growth retardation, floral defects, and vascular defects were observed. In addition, heterozygous *aml1* embryos exhibited delayed cell division, whereas homozygous *aml1* embryos were lethal (Weijers et al., 2001).

Arabidopsis RPS6 family includes two members, *RPS6A* and *RPS6B*. It was shown that these two genes have redundant and interchangeable functions. In 2002 study, it was demonstrated that the partial suppression of RPS6 expression with antisense construct affected shoot development and resulted in reduced apical dominance and irregular positioning of leaves (Morimoto et al., 2002). Knockout rps6a and *rps6b* single mutants demonstrated delayed root growth, reduced root meristem, smaller more elongated and pointed leaves and slightly altered dorsoventral leaf patterning. Interestingly double heterozygous *rps6a/+ rps6b/+* mutants exhibited a similar phenotype as observed in single *rps6* mutants (Creff et al., 2010). Also, it was reported by Horiguchi, et al. that *rps6a-1* mutants exhibited pointed leaves and parallel veins on the proximal lamina extended into the petiole. Additionally, in *rps6a-1* and *rps6a-3* mutants, reduced size and number of adaxial epidermal cells were observed (Horiguchi et al., 2011). Further, it was demonstrated that the interaction of RPS6 with AtHD2B (plants specific histone deacetylase) resulted in suppression of the pre-18S rRNA transcript level, suggesting an extra-ribosomal function of the RPS6 (Kim et al., 2014). In addition, double mutants *rps6a var2* exhibited an increased proportion of white leaf sectors (Wang et al., 2018). Finally, in 2018 was reported TOR-S6K-RPS6 pathway, which is dependent on auxin, triggered by light and needed for de novo protein synthesis and cotyledon opening in *Arabidopsis*. Mutant plants in either RPS6A, RPS6B or TOR showed a delay in cotyledon opening. (Chen et al., 2018).

RPS10 family of *Arabidopsis* is encoded by three genes *RPS10A*, *RPS10B* and *RPS10C*. *RPS10B* share 78% amino acid identity with the *RPS10A* gene and 74% with the *RPS10C* gene. *rps10b-1* mutants exhibited an increased number of petals and carpels, affected formation and separation of shoot lateral organs, including the shoot axillary meristems, defects in patterning of cauline node and fusion between organs (primary stamens). Also, in rps10b-1 mutant plants cauline nodes leaf was rudimentary or absent. Additionally, combining *rps10b-1* and *cuc3* enhanced organ separation defects, most significantly at cauline nodes, which was rare in the single mutants,. It was found that *rps10b-1* suppresses excessive shoot branching in the *max2-1* mutant background (Stirnberg et al., 2012). Finally, it was also reported that *rps10b-1*enhanced the floral meristem defects of *revoluta* (*rev*) and *pinoid* (*pid*) (Stirnberg et al., 2012). *REV* gene is essential for normal growth of apical meristem and in leaves and stems limit the cell division (Talbert et al., 1995). PINOID protein kinase is required in the early stages of meristem development and for auxin transport direction (Friml et al., 2004; Bennett et al., 1995).

The RPS13 family of *Arabidopsis thaliana* comprises *RPS13A* gene and *RPS13B* gene. Mutation in RPS13A was reported as *pointed first leaf2* (*pfl2*). *pfl2* mutant has a similar phenotype as previously reported *pfl1* mutant of the *RPS18A* gene. *pfl2* mutants exhibited inhibited root growth, late flowering, altered vascular system, and aberrant leaf morphology such as narrow pointed first and second leaves, leaf blade with many enlarged cells and intercellular spaces. Also, trichome morphology was affected in *pfl2* mutants, it was demonstrated that trichome defects were different for the first, fifth and the tenth leaf (Ito et al., 2000).

In the *Arabidopsis* genome, S15a is encoded by six genes (Barakat et al., 2001) which are divided into two types. Type I includes *RPS15aA*, *RPS15aC*, *RPS15aD*, and *RPS15aF*. These genes are universally conserved and remain in the cytoplasm. Type II includes *RPS15aB* and *RPS15aE*, which are plant-specific and are translocated to the mitochondria (Adams et al., 2002; Szick-Miranda et al., 2010). Knockdown mutants of the *RPS15aE* gene (*rps15aE-mut1*) were larger than wild-type plants, produced larger leaves, grew longer roots and possessed larger cells (Szick-Miranda et al., 2010). In *RPS15aB* double mutant with *RPL28A* (*rps15ab-1 rpl28a-3*) pointed leaves and parallel veins on the proximal lamina expanding into the petiole were observed (Horiguchi et al., 2011).

The RPS18 family includes *RPS18A*, *RPS18B* and *RPS18C* genes. All three genes share 100% identity in amino acid sequences. Mutations in RPS18A were reported as pointed *first leaf1* (*pfl1*). In *Arabidopsis*, in *pfl1* mutants pointed first rosette leaves, delayed root growth, extended life cycle and 20% reduction in vegetative fresh weight were observed (van Lijsebettens et al., 1994).

The *Arabidopsis* RPS21 family comprises *RPS21A*, *RPS21B*, and *RPS21C*, where *RPS21A* represents a pseudogene (Barakat et al., 2001). *RPS21B* and *RPL21C* genes are encoded by *ENHANCER OF VARIEGATION1* (*EVR1*) and *ENHANCER OF VARIEGATION1-LIKE1* (*EVR1L1*), respectively. Amino acid sequences of these two genes share 93% identity. Both homozygous *evr1* and *evr1l1* mutants exhibited delayed leaf initiation and expansion. In addition, in double mutants, *evr1 var2* and *evr1l1 var2* was demonstrated increased leaf variegation, which showed that mutation in either *EVR1* or *EVR1L1* enhances *var2* leaf variegation (Wang et al., 2018). Further, *rps21b-1* mutants exhibited small leaves with reduced cells and finally, *rps21b-2* mutants showed a reduced number of leaf palisade cells (Horiguchi et al., 2011).

The *RPS28B* gene is a member of the *Arabidopsis* RPS28 family. rps28b-1 mutants showed reduced leaf area, reduced number of mesophyll cell and decreased proliferation of cells. Further, was demonstrated that *rps28b* slightly enhanced leaf abaxialization of *as2*, also *as1* phenotype was weakly enhanced by *rps28b* (Horiguchi et al., 2011).

In the *Arabidopsis*, in RACK1 family are three genes, *RACK1A*, *RACK1B* and *RACK1C*. All three genes have similar expression pattern but are expressed at different levels, with the highest expression is the gene for *RACK1A*. It was demonstrated that *RACK1A* could regulate plant growth and development in response to hormones including auxin, gibberellin, ABA, brassinosteroids (Chen et al., 2006). Also, it was recently found in the regulation of the response to ethylene (Wang et al., 2019). *rack1a* mutants showed reduced rosette size, delayed flowering, shorter hypocotyls, and slightly narrow and epinastic (downward curving) rosette leaves. Further, *rack1a* mutants displayed altered sensitivities to several plant hormones. Sensitivity to gibberellin and brassinosteroid in response to seed germination was reduced, also response to auxin in adventitious and lateral root formation was decreased in mutants whereas hypersensitive response was found to ABA in seed germination and early seedling development (Chen et al., 2006). Further, it was shown that *rack1b* and *rack1c* mutants did not display any apparent defects in plant development but could substantially enhance the phenotype of rack1a single mutants. Both rack1a rack1b and rack1a rack1c mutants exhibited a reduced number of rosette leaves (Guo & Chen, 2008). Additionally, was shown that *SUPPRESSOR OF ACAULIS 53* (*SAC53*) encodes RACK1A. It was reported that *sac53* can restore the phenotype of *acl5*. *ACAULIS5* gene is needed for elongation of internodes and it´s mutation can cause severe dwarfism in plants (Hanzawa et al., 1997). Double mutants *rack1a-1 acl5-1* and *rack1a-2 acl5-1* were able to partially restored the stem growth (Kakehi et al., 2015). In 2019, was reported that response to ethylene in the rack1a mutants was largely unaffected and only slightly shorter hypocotyl indicated some regulation in response to ethylene. However, in double

mutants (*rack1a rack1b* and *rack1a rack1c*), it was demonstrated that sensitivity in response to ethylene is reduced, together with significantly reduced hypocotyl length (Wang et al., 2019).

6. Summary

This work overviewed basic knowledge about translation, eukaryotic ribosome and its biogenesis and, where possible, supplemented with plant-specific information to these chapters. Then, the concept of ribosome heterogeneity was introduced and finally, current data about ribosomal proteins in plants were summarized and mutants characterized in literature were listed, which highlighted their role in plant development and under environmental stress.

In conclusion, RPs have similar expression patterns, but some divergences from the co-expression pattern are also present, indicating that under different stress conditions and at different stages of growth and development, different RP paralogs from the same family are present in the ribosome population. For instance, RPL23aA and RPL23aB share 94.8% amino acid sequences and the silencing of *RPL23aA* leads to a severe developmental phenotype, whereas the mutation of *RPL23aB* does not affect development at all.

This work also showed that RP mutants share some similar developmental phenotypes, but individual mutant lines have also rare developmental phenotypes such as defect in trichome formation in *rps13a* (*pfl2*) mutants. But generally, RP mutants share developmental defects such as delayed growth, altered leaf morphology, and auxin-related phenotypes. One of the characteristic phenotypes of RP mutant is the pointed leaf phenotype observed in mutants of ribosomal proteins RPL4A, RPL10B, RPL10aB (*pgy1*), RPL18C, RPL23aA, RPL27aC, RPL27aB, RPL36aA (*api2*), RPL36aB, RPS5A, RPS5b, RPS13A (*pfl2*) and RPS18A (*pfl1*). Pointed leaves were also exhibited in *rps15ab rpl28a* double mutants. Additionally, some RPs also enhanced leaf defects in adaxial-abaxial polarity establishment of *as1* or *as2* mutants. *as2* phenotype was enhanced in double mutants with *RPL24B* or *RPL5A* as these mutants exhibited needle-like leaves. Mutation in *RPL5A* also enhanced the *as1* phenotype, as in these double mutants, narrower and more elongated leaves were present. In extreme cases, mutation of some RPs leads to embryo lethality, which was the case of mutants in *RPL10A* and *RPL14B*, which is interesting because mutation in almost identical paralogs of these RPs family did not affected embryo viability. Further, *rpl23aa rpl23ab* and *rpl9c rpl9d* double homozygous mutants were also embryo lethal. In several RP mutants, auxin-related phenotypes were observed, specifically in *rpl3b* (*rml1*), *rpl4a* and *rps5* mutants, which showed an abnormal vascular pattern, or *rpl24b* (*stv1*) mutants that exhibited disrupted apical-basal development of the gynoecium caused by impaired translation reinitiation of ARFs containing

uORFs in the 5′UTR. Reinitiation is enhanced by RPL24B and also eIF3h, whose mutant has a very similar phenotype to stv1 mutant. In addition, it was also described that RPL4A, RPL4D, and RPL5A can modulate auxin response through translational regulation of multiple auxin response factors (ARF2, ARF3, and ARF6).

RPs also react differently under stress conditions (cold, heat or drought). Generally, plants with overexpression of one specific RP interestingly showed better tolerance to environmental stress conditions, whereas mutation of individual RP led to greater sensitivity to stress conditions. For example overexpression of P3 led to enhanced tolerance to heat and cold, whereas mutant plants were more sensitive to high and low temperatures. Overexpression of RPL6 and RPL23 in rice enhanced water-use efficiency and therefore plants -tolerated drought and salt stress better than control plants. rack1 mutants had decreased sensitivity to gibberellin and brassinosteroids in response to seed germination whereas the response to ABA had increased sensitivity. These data indicate that overexpression of some RPs could be used to develop transgenic plants with better tolerance to stress conditions which could help to grow agricultural crops in an ever-changing environment.

Altogether, this work summarized current state of knowledge about RPs and their mutants not only in plant development. The majority of ribosomal proteins in plants are still largely unexplored and the major part of the research on mutation in ribosomal proteins was obtained from model plant *Arabidopsis thaliana*, but also from *Nicotiana tabacum*, *Nicotiana benthamiana* and *Oryza sativa*. The future research in model plants and similar analyses in some other plant species may show some new interesting mechanisms of specific translation regulation and could develop transgenic plants with enhanced resistance to environmental stress that could be truly useful in the coming decades.

7. References

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