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# Unveiling the functions of plastid ribosomal proteins in plant development and abiotic stress tolerance

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ABSTRACT

Translation of mRNAs into proteins is a universal process and ribosomes are the molecular machinery that carries it out. In eukaryotic cells, ribosomes can be found in the cytoplasm, mitochondria, and also in the chloroplasts of photosynthetic organisms. A number of genetic studies have been performed to determine the function of plastid ribosomal proteins (PRPs). Tobacco has been frequently used as a system to study the ribosomal proteins encoded by the chloroplast genome. In contrast, *Arabidopsis thaliana* and rice are preferentially used models to study the function of nuclear-encoded PRPs by using direct or reverse genetics approaches. The results of these works have provided a relatively comprehensive catalogue of the roles of PRPs in different plant biology aspects, which highlight that some PRPs are essential, while others are not. The latter ones are involved in chloroplast biogenesis, lateral root formation, leaf morphogenesis, plant growth, photosynthesis or chlorophyll synthesis. Furthermore, small gene families encode some PRPs. In the last few years, an increasing number of findings have revealed a close association between PRPs and tolerance to adverse environmental conditions. Sometimes, the same PRP can be involved in both developmental processes and the response to abiotic stress.

The aim of this review is to compile and update the findings hitherto published on the functional analysis of PRPs. The study of the phenotypic effects caused by the disruption of PRPs from different species reveals the involvement of PRPs in different biological processes and highlights the significant impact of plastid translation on plant biology.

## 1. Introduction

According to the central dogma of molecular biology, the last step in genetic information flow is translation (Crick, 1958, 1970), a process by which ribosomes translate the nucleotide sequence of an mRNA into the amino acid sequence of a peptide or protein with the participation of tRNA molecules. The ribosomes of all organisms are ribonucleoprotein structures, i.e. made up of ribosomal RNA molecules (rRNAs) and proteins (RPs), and they comprise two subunits: a larger one mainly with a catalytic function; a smaller one involved in mRNA decoding. Both eubacterial and archaeal ribosomes are smaller than eukaryotic ones, show a lower sedimentation coefficient (70S vs. 80S) and harbour fewer RPs (about 54 vs. 80) and rRNAs (3 vs. 4) (Tiller and Bock, 2014). Due to their endosymbiotic origin, chloroplasts and mitochondria also have their own ribosomes named chlororibosomes and mitoribosomes, respectively. Consequently, photosynthetic eukaryotes have three ribosome types that are located in the cytosol, chloroplasts and mitochondria. Throughout evolution, the number of genes in endosymbiotic genomes has drastically reduced following transfer of most of these genes to the nuclear genome. As a result, contemporary chloroplasts and mitochondria contain only a few dozen genes that are involved in organellar gene expression (OGE), photosynthesis and the electron transport chain. The transferred genes also include those encoding mitochondrial and chloroplast RPs, although the genomes of both organelles have retained some genes that encode RPs. In line with this, approximately two thirds of the genes encoding plastid ribosomal proteins (PRPs) are nuclear in the model plant *Arabidopsis thaliana* (thereafter Arabidopsis), while the remaining third reside in the plastid genome (plastome) (Allen, 2018). It is generally accepted that, during evolution, the preponderance of transcriptional regulation of gene expression in chloroplasts shifted towards the post-transcriptional control exerted at the RNA stability, processing and translation levels (Eberhard et al., 2002).

Plastid ribosomes are made of large (50S) and small (30S) subunits, which constitute a 70S ribosome that is similar in structure and size terms to bacterial ribosomes (Tiller and Bock, 2014). The 50S and 30S

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subunits are composed not only of rRNA molecules, which are all encoded by the organellar genome, but also of numerous PRPs encoded mostly by nuclear genes that must be imported to chloroplasts. This is an example of the participation of the nuclear genome in chloroplast genome expression. rRNAs form the core of the translational complex, while PRPs are primarily located on its surface, and mediate the interactions of the ribosome with other components, including regulatory proteins (Schippers and Mueller-Roeber, 2010). Plastid ribosomes contain a complete set of bacterial rRNAs (23S, 16S and 5S rRNAs) that, furthermore, serve as scaffolds for PRPs during ribosome assembly (Shajani et al., 2011; Maier et al., 2013). However unlike *Escherichia coli*, the genetic information encoding the 23S rRNA of plastid ribosomes is split between two distinct genes in the plastome: a large part corresponding to the 5′ end is encoded by the 23S rRNA gene; a small fragment at the 3′ end is encoded by the 4.5S rRNA gene (Whitfeld et al., 1978).

Other important changes to have occurred in plastid ribosomes during evolution have affected the protein component. The first complete catalogue of PRPs included six proteins, which were initially considered to lack bacterial homologues and were originally named plastid-specific RPs (PSRPs) PSRP1-PSRP6 (Yamaguchi and Subramanian, 2000; Yamaguchi et al., 2000). Today however, PSRP2 (cS22) and PSRP3 (cS23) of the 30S ribosomal subunit, and PSRP5 (cL37) and PSRP6 (cL38) of the 50S subunit, are considered to be the only RPs exclusively present in plastids (Bieri et al., 2017). Some evolutionarily conserved PRPs present N- or C-terminal extensions, and sometimes also internal expansions in relation to their orthologues in *E. coli* (Yamaguchi and Subramanian, 2000; Yamaguchi et al., 2000). Many of these terminal extensions and expansions mediate new interactions with rRNAs or with other PRPs that could structurally compensate, at least in part, the absence or modification of certain domains in plastid rRNAs (Ahmed et al., 2016; Graf et al., 2016; Bieri et al., 2017). Besides, some of these extensions differently configure the mRNA entry and exit sites to those in prokaryotic ribosomes (Bieri et al., 2017). It has also been proposed that the close association of PRP extensions with rRNAs could contribute to maintain the structural integrity of the plastid ribosome (Ahmed et al., 2016). It is noteworthy that PRPs bL25 and uL30 of the large 50S subunit were completely lost from the chlororibosome, and in some species, the plastid uL23c protein was replaced with that of the 80S cytosolic ribosome (Bubunenko et al. al., 1994; Yamaguchi and Subramanian, 2000). Furthermore, the protein content of plastid ribosomes is higher than in prokaryotic ribosomes. Accordingly, the protein:RNA ratio is 1:3 in bacteria, while it is 2:3 in plastids (Tiller and Bock, 2014). This difference is likely attributable to the larger size of PRPs due to their N- and C-terminal extensions (Yamaguchi et al., 2002; Yamaguchi and Subramanian, 2003; Manuell et al., 2007). Consistently with this, plastid ribosomes have a larger protein mass (approx. 170 kD) and a slightly lower RNA content (approx. 0.4 kD) than *E. coli* ribosomes (Zoschke and Bock, 2018).

The 30S subunit of the plastid ribosome includes the 16S rRNA and 24 PRPs, 21 of which present a homologous protein in *E. coli*. Twelve of these 24 PRPs are encoded by the plastome and the remaining 12 by the nuclear genome (Yamaguchi et al., 2000). The 50S subunit contains three rRNAs (23S, 5S and 4.5S) and 33 PRPs, 31 of which show homologous in *E. coli*, and 24 of the 33 PRPs are encoded by the nuclear genome and nine by the plastome (Yamaguchi and Subramanian, 2000).

The atomic structure of the 70S chlororibosome from spinach has been recently resolved by cryo-EM (Bieri et al., 2017; Perez Boerema et al., 2018), which is helping to better understand its relation to that of ancestral bacterial ribosomes. Along these lines, and despite structural similarities with prokaryotic ribosomes, translation in plastid ribosomes is more complex, probably due to the presence of PSRPs at least in part. Another factor that contributes to the complexity of translation in plastids is the presence of small gene families that encode PRPs as a consequence of gene duplications. In plastid ribosomes, each PRP type is represented by a single polypeptide. However, several PRPs are encoded by two genes or more from the same family, which results from gene duplication (Table 1). Thus, in Arabidopsis, some PRPs and mitochondrial RPs (MRPs) are encoded by more than one gene (Table 1) (Sormani et al., 2011). Likewise, the 81 RP types of cytoplasmic ribosomes are encoded by 251 genes (Barakat et al., 2001). It has been proposed that translation in plants can be regulated by altering the composition of the proteins that form part of the ribosome. Indeed, the abundance and composition of polysomes vary during leaf growth and development in bean (Makrides and Goldthwaite, 1981). In *Brassica napus*, duplicated PRP genes have undergone remarkable functional divergence and given rise to paralogous proteins and highly specialised gene co-expression networks (Whittle and Krochko, 2009). The heterogeneity of plastid ribosomes may reflect functional diversity in at least some of their constituent proteins. This suggests that these proteins may be involved in various developmental processes and/or act at distinct times, and in different tissues or cell types. Because of this, the traditional view of the ribosome as cell maintenance machinery devoid of any selective function in protein synthesis has changed in recent years (Robles and Quesada, 2017; Scaltsoyiannes et al., 2022). Nonetheless, the specialised role of individual PRPs in plant biology remains largely unsolved.

Several proteomics studies on the dynamics and composition of ribosomes have been carried out to determine their function in plant biology (e.g., see Carroll, 2013; Hummel et al., 2015). Additionally, several genetic studies are contributing to unravel the function of each protein-coding gene of different plant ribosomes by characterising the effects caused by their loss-of-function mutant alleles (Carroll, 2013; Tiller and Bock, 2014; Robles and Quesada, 2017). Despite the relatively abundant scientific literature on the phenotypic effects of the perturbing PRP functions and the fact that exhaustive reviews have been written on the role of plastid translation in plant development (e.g., see Tiller and Bock, 2014), we are unaware of any review that specifically focuses on the role of PRPs in plant development and abiotic stress tolerance. Therefore, the main goal of this review is to provide a summary and update on the works hitherto published describing effects on the growth, development and environmental responses of mutations in PRPs from different plant species. In this way, we aim to gain insight into the relevant roles of these proteins in plant biology.

## 2. Effects on the plant growth and development of defective PRPs

Besides photosynthesis, other fundamental metabolic processes, such as biosynthesis of fatty acids, amino acids, vitamins and important steps in the biosynthesis of plant hormones like gibberellins (GA) or abscisic acid (ABA), also take place in plastids (Rolland et al., 2012), which makes these organelles indispensable for plant survival. Therefore, perturbed chloroplast translation due to mutations in PRPs is expected to severely impair chloroplast function and, consequently, plant growth and development.

Tobacco (*Nicotiana tabacum*) has been frequently used as a plant model system to study plastome-encoded PRPs because an efficient chloroplast transformation protocol is available for this species for quite long time. In contrast, Arabidopsis is often chosen as a model to study the function of the RPs encoded by nuclear genes using direct or reverse genetics approaches because an efficient chloroplast transformation protocol has only recently become available in this species (Ruf et al., 2019). Regardless of the genome that encodes them, only some PRPs appear to be essential (Table 1). In line with this, a mutational analysis by plastome transformation (transplastomics) in *N. tabacum* has revealed that several of the PRPs encoded by the plastome are indispensable for plant survival under autotrophic or heterotrophic growth conditions (Scharff and Bock, 2014). Remarkably, plant mutants with severe defects in chloroplast translation can be rescued under heterotrophic growth conditions, which suggests that chloroplast translation is perhaps essential only for autotrophic growth (Zubko and Day, 1998; Babiychuk et al., 2011). It is worth noticing that the classification of

**Table 1**
Essential and non-essential PRPs of the chlororibosome.

| Large ribosomal subunit (50S) | | | | Small ribosomal subunit (30S) | | | |
|---|---|---|---|---|---|---|---|
| Protein | [a]Genes in *A t.* | [b]Essentiality of PRPs | Reference | Protein | [a]Genes in *A.t.* | [b]Essentiality of PRPs | Reference |
| uL1c | 1 | Essential in *A.t.* (At3g63490) | Bryant et al. (2011); Romani et al. (2012) | bS1c | 3 | Non-essential in *A.t.* (At5g30510) | Romani et al. (2012) |
| uL2c | 2 | NA | | uS2c | 1 | Essential in *N.t.* (*rps2*) | Rogalski et al. (2008) |
| uL3c | 1 | NA | | uS3c | 1 | Essential in *N.t.* (*rps3*) | Fleischmann et al. (2011) |
| uL4c | 1 | Essential in *A.t.* (At1g07320) | Romani et al. (2012) | uS4c | 1 | Essential in *N.t.* (*rps4*) | Rogalski et al. (2008) |
| uL5c | 1 | Essential in *A.t.* (At4g01310) | Dupouy et al., 2022; Dupouy et al., 2022 | uS5c | 1 | Essential in *A.t.* (At2g33800) | Bryant et al. (2011); Lloyd and Meinke (2012) |
| uL6c | 1 | Essential in *A.t.* (At1g05190) | Lloyd and Meinke (2012) | bS6c | 1 | NA | |
| bL9c | 1 | NA | | uS7c | 2 | NA | |
| uL10c | 2 | Essential in *A.t.* (At5g13510) NC | Bryant et al. (2011) | uS8c | 2 | NA | |
| uL11c | 2 | Non-essential in *A.t.* (At1g32990) | Pesaresi et al. (2001) | uS9c | 1 | Essential in *Z.m.* (Zm00001eb058750) | Ma and Dooner (2004) |
| bL12c | 3 | Essential in *O.s.* (*LOC_Os01g47330*) | Zhao et al. (2016) | uS10c | 1 | NA | |
| uL13c | 1 | Essential in *A.t.* (At1g78630) NC | Bryant et al. (2011) | uS11c | 1 | NA | |
| uL14c | 1 | NA | | uS12c | 3 | NA | |
| uL15c | 1 | Essential in *A.t.* (At3g25920) | Bobik et al. (2019) | uS13c | 1 | Essential in *A.t.* (At5g14320) | Bryant et al. (2011) |
| uL16c | 1 | NA | | uS14c | 1 | Essential in *N.t.* (*rps14*) | Ahlert et al. (2003) |
| bL17c | 1 | NA | | uS15c | 1 | Non-essential in *N.t.* (*rps15*) | Fleischmann et al. (2011) |
| uL18c | 3 | Essential in *A.t.* (At1g48350) NC | Bryant et al. (2011) | bS16c | 3 | Essential in *N.t.* (*rps16*) | Fleischmann et al. (2011) |
| bL19c | 2 | NA | | uS17c | 1 | Non-essential in *A. t* (At1g79850)<br>Essential in *Z.m.* (Zm00001eb434460) | Romani et al. (2012)<br>Schultes et al. (2000) |
| bL20c | 1 | Essential in *N.t.* (*rpl20*) | Rogalski et al. (2008) | bS18c | 1 | Essential in *N.t.* (*rpl18*) | Rogalski et al. (2008) |
| bL21c | 1 | Essential in *A.t.* (At1g35680)<br>Essential in *O.s.* (*LOC_Os02 g15900*) | Yin et al. (2012)<br>Lin et al. (2015) | uS19c | 1 | NA | |
| uL22c | 1 | Essential in *N.t.* (*rpl22*) | Fleischmann et al. (2011) | bS20c | 1 | Essential in *A.t.* (At3g15190)<br>Essential in *O.s.* (*LOC-Os01g48690*) | Romani et al. (2012)<br>Gong et al. (2013) |
| uL23c | 2 | Essential in *N.t.* (*rpl23*) | Fleischmann et al. (2011) | bS21c | 2 | Non-essential in *A.t.* (At3g27160) | Morita-Yamamuro et al. (2004) |
| uL24c | 1 | Non-essential in *A t.* (At5g54600) | Tiller et al. (2012) | cS22 | 1 | Non-essential in *A.t.* (At3g52150) | Tiller et al. (2012) |
| bL27c | 1 | Essential in *A.t.* (At5g40950) | Romani et al. (2012) | cS23 | 2 | Non-essential in *A.t.* (At1g68590) | Tiller et al. (2012) |
| bL28c | 1 | Essential in *A.t.* (At2g33450) | Romani et al. (2012) | bTHXc | 1 | Non-essential in *A.t.* (At2g38140) | Tiller et al. (2012) |
| uL29c | 1 | NA | | | | | |
| bL31c | 1 | Essential in *A.t.* (At1g75350) NC | Bryant et al. (2011) | | | | |
| bL32c | 1 | Essential in *N.t.* (*rpl32*) | Fleischmann et al. (2011) | | | | |
| bL33c | 1 | Non-essential in *N.t.* (*rpl33*) | Rogalski et al. (2008) | | | | |
| bL34c | 1 | NA | | | | | |
| bL35c | 1 | Essential in *A.t.* (At2g24090)<br>Essential in *Z.m.* (Zm00001eb386680) | Romani et al. (2012)<br>Magnard et al. (2004) | | | | |
| bL36c | 1 | Non-essential in *N.t.* (*rpl36*) | Fleischmann et al. (2011) | | | | |
| cL37 | 1 | Non-essential in *A.t.* (At3g56910) | Tiller et al. (2012) | | | | |
| cL38 | 1 | Non-essential in *A.t.* (At5g17870) | Tiller et al. (2012) | | | | |

*A.t.*: *Arabidopsis thaliana*; *N.t.*: *Nicotiana tabacum*; *O.s.*: *Oryza sativa*; *Z.m.*: *Zea mays*. NC: not confirmed by allelism tests or molecular complementation according to Bryant et al. (2011). NA: Not analysed.

[a] Number of Arabidopsis genes coding for the corresponding protein.

[b] Nuclear locus ID or plastome genes are indicated in brackets.

**Table 2**
PRPs characterised from the analysis of developmental mutants.

| Affected in | Mutant | PRP | Species | Mutant phenotype | Reference |
|---|---|---|---|---|---|
| Embryo development | *prps20-1* | bS20c[a] | *Arabidopsis thaliana* | Embryo lethal; alterations in cell division patterns and arrested embryo development before the heart stage | Romani et al. (2012) |
| | *prpl1-1* | uL1c[a] | | | |
| | *prpl4-1* | uL4c[a] | | | |
| | *prpl27-1* | bL27c[a] | | | |
| | *prpl35-1* | bL35c[a] | | | |
| | *asd* | bL21c[a] | *Arabidopsis thaliana* | Embryo lethal; arrest of embryos in the globular stage | Yin et al. (2012) |
| | *rpl15* | uL15c[a] | *Arabidopsis thaliana* | Embryo lethal; null mutations arrest embryo development in the globular stage and hypomorphic mutations increase intercellular trafficking | Bobik et al. (2019) |
| | *rpl5* | uL5c[a] | *Arabidopsis thaliana* | Embryo lethal; embryo failure to develop past the globular stage | Dupoy et al., 2022 |
| | *emb8516* | bL35c[a] | *Zea mays* | Embryo lethal; embryo morphogenesis is strongly aberrant, but endosperm develops normally | Magnard et al. (2004) |
| | *lem1* | uS9c[a] | *Zea mays* | Embryo lethal; embryo aborts before the transition stage, but endosperm develops almost normally | Ma and Dooner (2004) |
| Embryo-seedling transition | *prpl28-1* | bL28c[a] | *Arabidopsis thaliana* | Albino embryos germinate, but do not survive past the cotyledon stage | Romani et al. (2012) |
| Seedling growth and development | *psrp2-1* | cS22[a] | *Arabidopsis thaliana* | No detectable alteration in growth phenotype | Tiller et al. (2012) |
| | *psrp3/1-1* | cS23[a] | *Arabidopsis thaliana* | Light-green phenotype and retarded growth; severely disrupted mesophyll differentiation | |
| | *psrp4-R1* and *R2* | bTHXc[a] | *Arabidopsis thaliana* | Pale green leaves and severe growth retardation | |
| | *psrp5-1* | cL37[a] | *Arabidopsis thaliana* | Severely delayed plant growth and development. Plants do not often reach maturity. Altered leaf mesophyll differentiation | |
| | *psrp6-1* | cL38[a] | *Arabidopsis thaliana* | Phenotypically indistinguishable from wild-type plants | |
| | *ghs1* | bS21c[a] | *Arabidopsis thaliana* | Glucose-hypersensitive. Decreased leaf pigmentation, plant growth and photosynthetic activity | Morita-Yamamuro et al. (2004) |
| | *lpe2* | bS21c[a] | *Arabidopsis thaliana* | Disrupted thylakoid membrane composition. Suppressed response to the C/N balance at the physiological level | Dong et al. (2020) |
| | *prpl11* | uL11c[a] | *Arabidopsis thaliana* | Significantly decreased leaf pigmentation, plant growth and photosynthetic activity | Pesaresi et al. (2001) |
| | *prpl24* | uL24c[a] | *Arabidopsis thaliana* | Pale green leaves and severe growth retardation | Tiller et al. (2012) |
| | *rps17-1* | uS17c[a] | *Arabidopsis thaliana* | Pale green leaves and severe growth retardation | |
| | *ore4-1* | uS17c[a] | *Arabidopsis thaliana* | Yellow leaves and low leaf growth rate | Woo et al. (2002) |
| | *rfc3* | bS6c-like[a] | *Arabidopsis thaliana* | Pale leaves, reduced leaf photosynthetic activity and abnormal stem-cell patterning in lateral roots | Nakata et al. (2018) |
| | *asl1* | bS20c[a] | *Oryza sativa* | Albino seedling lethal phenotype | Gong et al. (2013) |
| | *asl2* | bL21c[a] | *Oryza sativa* | Albino seedling lethal phenotype | Lin et al. (2015) |
| | *asl4* | bS1c[a] | *Oryza sativa* | Albino seedling lethal phenotype | Zhou et al. (2021) |
| | *al1* | bL12c[a] | *Oryza sativa* | Albino seedlings do not survive past the three-leaf stage | Zhao et al. (2016) |
| | *wgl2* | uS9c[a] | *Oryza sativa* | Albino seedlings until the three-leaf stage and then gradually transitioned to green | Qiu et al. (2018) |
| | *hsf60-m1* | uS17c[a] | *Zea mays* | Unstable pale green seedling lethal phenotype | Schultes et al. (2000) |
| Leaf development | Δ*rps18* | bS18c[b] | *Nicotiana tabacum* | Misshapen leaves that lack leaf blade parts | Rogalski et al. (2006) |
| | Δ*rps2* | uS2c[b] | *Nicotiana tabacum* | Aberrant leaf morphologies; large leaf blade sectors missing | Rogalski et al. (2008) |
| | Δ*rps4* | uS4c[b] | *Nicotiana tabacum* | Aberrant leaf morphologies; large leaf blade sectors missing | |
| | Δ*rpl20* | bL20c[b] | *Nicotiana tabacum* | Aberrant leaf morphologies; large leaf blade sectors missing | |
| | Δ*rpl22* | uL22c[b] | *Nicotiana tabacum* | Misshapen leaves lacking part of the leaf blade | Fleischmann et al. (2011) |
| | Δ*rpl23* | uL23c[b] | *Nicotiana tabacum* | Misshapen leaves lacking part of the leaf blade | |
| | Δ*rpl32* | bL32c[b] | *Nicotiana tabacum* | Misshapen leaves lacking part of the leaf blade | |
| | Δ*rps3* | uS3c[b] | *Nicotiana tabacum* | Misshapen leaves lacking part of the leaf blade | |
| | Δ*rps16* | bS16c[b] | *Nicotiana tabacum* | Misshapen leaves lacking part of the leaf blade | |
| | Δ*rpl36* | bL36c[b] | *Nicotiana tabacum* | Very strong leaf phenotype; mutant leaf blades are extremely narrow | |
| | Δ*rps15* | uS15c[b] | *Nicotiana tabacum* | Mild growth phenotype under normal growth conditions, and much more apparent with chilling stress | |
| | *sca1* | uS5c[a] | *Arabidopsis thaliana* | Enhanced leaf polarity defects of the *asymmetric leaves2* mutant | Mateo-Bonmatí et al. (2015) |

*asd*: *aborted seed development*; *al1*: *albino-lethal 1; asl*: *albino seedling-lethal1; emb8516*: *embryo-specific8516*; *ghs1*: *glucose-hypersensitive1*; *hsf60-m1*: *high chlorophyll fluorescent 60-m1*; *lem1*: *lethal embryo1*; *lep2*: *low photosynthetic efficiency2*; *ore4-1*: *oresara4-1*; *rfc3*: *regulator of fatty acid composition3*; *sca1*: *scabra1*; *wgl2*: *white green leaf 2*; Δ: Knockout transplastomic lines.

[a] Nuclear gene.

[b] Plastome gene.

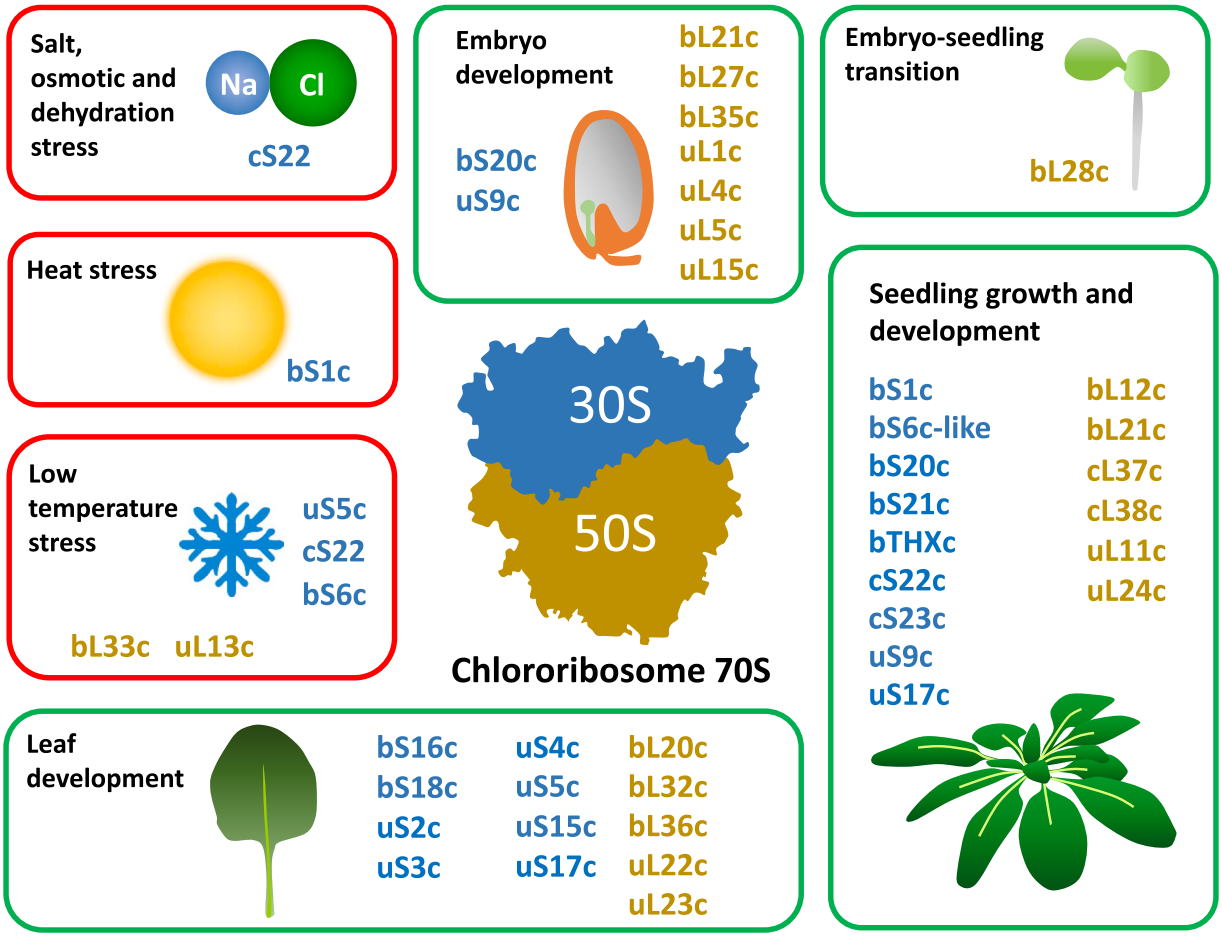


**Fig. 1. Summary of the PRPs described to be involved in different plant developmental processes, seedling growth and in the response to abiotic stresses.** The PRPs of the small (30S) and large (50S) chlororibosome subunits are depicted in blue and orange, respectively. Plant developmental processes and abiotic stress responses affected by mutations in genes encoding PRPs of the small (blue) or large (orange) plastid ribosome subunits are framed in green and red respectively. PRPs are named according to Ban et al. (2014).

PRPs as essential or non-essential is sometimes questionable and does not always coincide with that described for their corresponding orthologues in *E. coli*, despite the remarkable similarity in the composition of chloroplast and bacterial ribosomes. So the essentiality and non-essentiality of PRPs are incompletely conserved when comparing bacteria and plastid RPs (Tiller and Bock, 2014).

The mutant phenotypes hitherto characterised in different plant species, especially in Arabidopsis, rice and maize, demonstrate that PRPs are involved in many plant biology aspects (e.g. plastid biogenesis, embryogenesis, photosynthesis, photoautotrophic growth, lateral root formation, seedling development or leaf morphogenesis; Table 2 and Fig. 1). All this highlights the vital role played by chloroplasts, and by plastids by extension, in plants.

### 2.1. PRPs required for embryo development

By means of different reverse genetic screenings and analyses, more than 100 nuclear genes encoding plastid proteins have been reported to date to cause embryo lethality in Arabidopsis, where the genes that encode PRPs are well represented (Tzafrir et al., 2004; Meinke et al., 2008; Bryant et al., 2011; Romani et al., 2012; Yin et al., 2012; Bobik et al., 2019; Meinke, 2020). This reveals that plastid biology (particularly plastid translation) is clearly fundamental for embryo development.

From the characterisation of Arabidopsis mutants carrying T-DNA insertions in single-copy nuclear genes that encode nine PRPs of the small 30S (bS1c, uS17c and bS20c) and the large 50S (uL1c, uL4c, uL24c, bL27c, bL28c and bL35c) subunits, Romani et al. (2012) determined that six of them (bS20c, uL1c, uL4c, bL27c, bL28c, bL35c) are essential for normal embryo development (Table 1). Nonetheless, these proteins would be involved in different developmental stages. Accordingly, the embryos of the Arabidopsis mutants lacking bS20c, uL1c, uL4c, bL27c or bL35c cannot progress from the globular to the heart stage and beyond due to alterations in cell division patterns, whereas bL28c is essential later during the greening process (Table 2). Embryo lethality due to lack of uL1c and uL4c functions, as observed by Romani et al. (2012), corroborates the previous results reported by Bryant et al. (2011), although the latter did not study the phenotypes of the mutants defective in these genes in detail. Interestingly, Yin et al. (2012) reported for the Arabidopsis T-DNA insertional mutants lacking bL21c a different PRP, a similar embryo defect to those of the mutants affected in the aforementioned proteins bS20c, uL1c, uL4c, bL27c, bL28c and bL35c. Thus in the absence of bL21c, embryo development is arrested in the globular stage (Table 2). An interesting conclusion drawn by Romani et al. (2012) indicates that, despite the prokaryotic origin of plastids, the phenotypic severity due to the disruption of RP functions in bacteria (e.g. *E. coli*) does not allow to infer its effects on embryogenesis in Arabidopsis.

Arabidopsis INCREASED SIZE EXCLUSION LIMIT2 (ISE2) is a nucleus-encoded chloroplast RNA helicase required for chloroplast rRNA processing and chlororibosome assembly (Bobik et al., 2017). The *ise2* mutant is defective in intercellular trafficking via plasmodesmata

(Kim et al., 2002). To look closely at the ISE2 function in chloroplast translation, Bobik et al. (2019) performed yeast two-hybrid screen and pull-down assays, and identified that PRP uL15c interacts with ISE2. These authors found that *RPL15* is an essential gene for autotrophic Arabidopsis growth because *rpl15* null mutants are embryo-lethal. Noteworthy, low uL15c levels cause aberrant chloroplast rRNA processing, defective chloroplast translation and diminished chloroplast-encoded proteins accumulation. Moreover, knockdown of *RPL15* increases intercellular trafficking, a phenotype previously reported in plants with low ISE2 levels (Kim et al., 2002). Therefore, these findings reveal a role for chloroplast function in intercellular trafficking via plasmodesmata. However, the mechanism by which organelles can influence cell-to-cell communication in plants is currently unknown.

Very recently, another essential PRP was identified in Arabidopsis, uL5c. *prpl5* loss-of-function mutants show an embryo abortion phenotype associated with aberrant plastid biogenesis, in which embryo development is arrested past the post-globular stage (Dupouy et al., 2022, Table 2). This indicates that uL5c is required for normal embryo post-globular development in Arabidopsis. Dupouy and collaborators investigated the role of the uL5c evolutionary conserved N-terminal and, surprisingly, C-terminal localisation signals. Unexpectedly, these authors found a functional nuclear localisation signal on the C-terminal end of uL5c, which suggests a function for this protein in the nucleus. In line with this, other authors have identified Arabidopsis PRPs in the proteome extracted from cultured cells nuclei (Goto et al., 2019) or protoplast nuclei (Sakamoto and Takagi, 2013). Dupouy et al. (2022) have hypothesised that uL5c might relocate in the nucleus in response to stressful conditions to perform a different function than under normal growth conditions. This might explain the effect on plant development of some defective PRPs only under stress, and would link plastid translation with plant development and abiotic stress tolerance (see section below). Nevertheless, the function/s in the nucleus of uL5c, and the other PRPs detected in this cellular compartment, require/s extensive further research.

Apart from Arabidopsis, some mutants showing alterations in embryogenesis have also been identified and characterised in maize (*Zea mays*). Remarkably, defective plastid translation in maize may or may not result in embryo lethality depending on the genetic background (Parker et al., 2014). One of these mutants, *embryo-specific8516* (*emb8516*), is affected in one of the two genes (*ZmPRPL35-1*) that encodes a functional bL35c protein (Magnard et al., 2004). Similarly to Arabidopsis (see above), loss of function of the *ZmPRPL35-1* gene results in aberrant embryogenesis and leads to non-viable seeds, although the endosperm develops normally (Table 2). Another maize mutant, *lethal embryo 1* (*lem1*) affected in PRP uS9c, also shows perturbed embryo development and, as in *emb8516*, the endosperm develops almost normally (Ma and Dooner, 2004). *lem1* embryos abort very early and seeds are not viable (Table 2). Ma and Dooner propose that the *lem1* mutant phenotype might be explained by the fact that functional plastids are essential for normal embryo development and/or because uS9c performs an extraribosomal function that is required for embryogenesis. On the explanation for the differential effect of PRP mutations on embryo and endosperm in maize, Magnard et al. (2004) point out completely different developmental fates of embryo and endosperm and, consequently, their likely different requirements for the molecules synthesised in plastids.

It is not well-known whether the need for the above-mentioned Arabidopsis and maize PRP-encoding genes in embryo development is related to the insufficient synthesis of proteins required for embryogenesis. However, it does not seem related to the participation of plastid ribosomes in photosynthesis because embryos are normal in some of the mutants that lack the components involved in electron transport in thylakoids (Romani et al., 2012). Furthermore, some PRPs are required very early in embryo development, even before any photosynthetic activity (Dupouy et al., 2022). Along these lines, one first proposal states that the function of a single gene in Arabidopsis, the plastome gene *accD* that encodes the β-subunit of the heteromeric acetyl-CoA carboxylase (ACCase) enzyme involved in fatty acid biosynthesis in chloroplasts, might explain why plastid translation failure results in non-viable embryos in Arabidopsis (Bryant et al., 2011). Lipids are the building blocks of membranes and can also be either involved in signalling processes or required in enzymatic activities (Boutté and von Jaillais, 2020). Thus alterations in lipid biosynthesis can have serious consequences during embryogenesis. In line with this, a null mutation in a nuclear gene that encodes another subunit of heteromeric plastid ACCase also causes embryo lethality (Li et al., 2011). Notwithstanding, direct genetic evidence from *accD* knockout mutants is still missing, and is required to demonstrate that lack of *accD* results in embryo lethality (Li et al., 2021).

In contrast, the situation is different in maize and other monocot grasses that lack plastid *accD*. This is due to the presence of duplicated nuclear genes that can compensate loss of the plastid *accD* function by producing malonyl-CoA, the product of ACCase activity, in the absence of a functional plastid *accD* gene (Bryant et al., 2011; Romani et al., 2012). To explain the specific embryo lethality associated with defective plastid translation in maize, loss of a retrograde signalling produced in functional plastids that activates the expression of nuclear genes (including those required for embryonic, but not for endosperm development) has been suggested (Parker et al., 2014; Meinke, 2020). This would account for the normal formation of the endosperm tissues in the embryo-lethal mutants of maize.

### 2.2. Influence of PRPs on seedling growth and development

As most of the proteins that are encoded by the plastome participate in either OGE or photosynthesis (see the Introduction), defective plastid translation due to perturbed PRP functions is expected to alter photosynthesis and plant growth. This might eventually result in early lethality after germination.

Similarly, Tiller et al. (2012) investigated the functions of Arabidopsis PSRPs cS22, cS23, cL37, cL38 and bTHXc (formerly PSRP4) (see the Introduction) by analysing T-DNA insertion mutants and RNAi lines. Knockdown of proteins cS23, bTHXc and, principally cL37, resulted in delayed growth and altered development, although none led to embryo or seedling lethality (Tiller et al., 2012). Based on their phenotypic and molecular analyses, Tiller et al. (2012) classified these PSRPs into two groups: one comprised cS23, bTHXc and cL37, which could be considered *bona fide* RPs; the other included cS22 and cL38, which would not be required for stable chlororibosome accumulation and hence, chloroplast translation.

The first mutation in a gene that encodes a PRP and caused lethality early after germination was identified and characterised in maize. Thus maize *high chlorophyll fluorescent 60 m1* (*hsf60-m1*) mutation affects PRP uS17c, severely compromises photosynthesis and reduces plastid translation, which lead to an unstable pale green seedling lethal phenotype because it depends on temperature and light growth conditions (Schultes et al., 2000). However, the absence of its orthologous protein in Arabidopsis is not lethal (Woo et al., 2002; see onwards).

In the last few years, several rice (*Oryza sativa*) mutants dubbed *albino seedling lethal1, 2* and *4* (*asl1, 2* and *4*) have been identified and characterised. All of them, as their name suggests, exhibit an albino lethal phenotype in the seedling stage associated with low chlorophyll content and abnormal chloroplast development (Gong et al., 2013; Lin et al., 2015; Zhou et al., 2021). Cloning the *ASL1*, *2* and *4* genes revealed that they respectively encode PRPs bS20c, bL21c and bS1c (Gong et al., 2013; Lin et al., 2015; Zhou et al., 2021) (Table 2). *ASL1* and *ASL2* expression, and very likely that of *ASL4,* is regulated by light, and *asl1, 2* and *4* mutations perturb the expression of nuclear and plastid genes that encode proteins involved in chlorophyll biosynthesis, chloroplast development and photosynthesis (Gong et al., 2013; Lin et al., 2015; Zhou et al., 2021). Another rice mutant, *albino lethal 1* (*al1*), mutated in the nuclear gene that encodes bL12c, also exhibits an albino seedling phenotype, and *al1* individuals are unable to survive beyond the

three-leaf stage (Zhao et al., 2016) (Table 2). As with the *asl* mutants, lack of pigmentation of *al1* seedlings is associated with low chlorophyll levels and perturbed chloroplast morphology, likely attributable to an altered expression of nuclear and plastid genes involved in chloroplast biogenesis and photosynthesis (Zhao et al., 2016). The effect on the expression of nuclear genes in the *asl* and *al1* mutants is probably indirect, due to retrograde signalling from the chloroplasts to the nucleus. Interestingly, *al1* is a spontaneous single-nucleotide mutation that causes an amino acid substitution from leucine in AL1 wild-type protein to phenylalanine in the al1 mutant protein, which abolishes the interaction between proteins uL10c and bL12c and renders the mutated protein unstable.

In the previous section, we point out that a defective uS9c protein results in embryo lethality in maize (Ma and Dooner, 2004). Nevertheless, mutations in the *WHITE GREEN LEAF 2* (*WGL2*) gene that encodes the rice uS9c protein are not lethal, although mutant seedlings display an albino phenotype from germination through to the three-leaf stage. However, these albino seedlings gradually accumulate pigmentation and turn green in later developmental stages (Qiu et al., 2018) (Table 2). This phenotypic trait differentiates *wgl2* mutants from the *asl1*, *2*, *4* and *al1* rice seedling albino mutants discussed above, which were all lethal. Nonetheless, *wgl2* individuals like *asl1*, *2*, *4* and *al1* seedlings also show abnormal transcript levels of genes involved in chloroplast development, photosynthesis, chlorophyll biosynthesis and plastid ribosome assembly, which are the likely cause of the phenotype of the *wgl2* mutants (Qiu et al., 2018).

Regarding Arabidopsis, knockout mutants *ghs1* (*glucose hypersensitive 1*), *prpl11* and *prpl24* that respectively lack bS21c, uL11c and uL24c, as well as mutant *oresara4-1* (*oresara* means "long living" in Korean) defective in the uS17c protein and displaying extended leaf longevity, are all fully viable and can complete their entire life cycle (Pesaresi et al., 2001; Woo et al., 2002; Morita-Yamamuro et al., 2004; Tiller et al., 2012). Nonetheless, all these mutants exhibit significantly decreased leaf pigmentation, retarded growth, little photosynthetic activity, as well as diminished plastid protein synthesis (Table 2). Interestingly, the *gsh1* mutant, as its names indicates, also shows a glucose hypersensitive phenotype, which reveals a relation between sugar response and chlororibosomes (Morita-Yamamuro et al., 2004). This has already been supported by the phenotype of another mutant, *rfc3* (*regulator of fatty acid composition 3*), which is affected in the bS6c-like protein and displays a sucrose-conditional defect in the patterning of distal elements in lateral root (LR) meristems (Horiguchi et al., 2003). In a more recent work, the involvement of the plastid-located bRPS6 family member RFC3 protein in stem cell patterning in LR primordia was further investigated (Nakata et al., 2018). *rfc3* mutations affect roots and the aerial part of seedlings. *rfc3* mutants display pale leaves and scarce leaf photosynthetic activity, in addition to abnormal stem-cell patterning in LR, and are likely caused by impaired gene expression and decreased accumulation of rRNAs in plastid roots (Nakata et al., 2018) (Table 2). Interestingly, wild-type and plastid-translation defective mutant *prps17* plants treated with the plastid translation inhibitor spectinomycin, phenocopy the LR phenotype of *rfc3*. This finding demonstrates that defective plastid translation is likely the cause of defective stem cell patterning in *rfc3* LR, which connects translation in non-green plastids with root development (Nakata et al., 2018).

More recently, Dong et al. (2020) identified T-DNA viable insertional mutants that exhibited low photosynthetic efficiency by fluorescence imaging. Accordingly, they were named *low photosynthetic efficiency2* (*lpe2*). As with *gsh1* mutants, *lpe2* plants are also affected in the nuclear gene that encodes PRP bS21c. The characterisation of loss-of-function *lpe2* mutants reveals that impaired LPE2 activity disrupts thylakoid membrane composition and alters plastid protein levels. Remarkably, the transcriptome analysis showed that *lpe2* mutations perturb the expression of nuclear genes involved in the response to the C/N balance. Accordingly, physiological experiments revealed that *LPE2* deficiency suppresses the response to the C/N balance (Dong et al., 2020) (Table 2).

Taken together, the characterisation of mutants *gsh1, rfc3* and *lpe2* highlights that disrupted plastid translation has repercussions beyond photosynthesis and may also affect other plant metabolic or developmental processes.

### 2.3. Mutations in PRPs impact leaf development

Chloroplast function and leaf development are closely linked. Indeed genetic screenings for mutants displaying altered leaf morphology have identified a number of nuclear genes involved in plastid gene expression at the transcriptional or post-transcriptional levels, which usually show defects in palisade cell development (Hricová et al., 2006; Wang et al., 2010; Moschopoulos et al., 2012; Mateo-Bonmatí et al., 2015; Robles et al., 2015, 2018). Perturbed leaf architecture is usually attributed to retrograde signalling from plastids to the nucleus because none of the functions of plastome-encoded proteins seems to be directly involved in leaf development (Tiller and Bock, 2014). Retrograde signalling provides an effective means of communicating the developmental (biogenic) and functional (operational) state of the plastid to the nucleus (Pogson et al., 2008) by adjusting the expression of nuclear genes (de Souza et al., 2017). Several leaf shape mutants bear mutations in PRPs, which hints at a role of plastid translation in leaf development. Additionally, plastid translational activity is required for the generation of the retrograde signal/s that modulate/s leaf shape (Fleischmann et al., 2011).

Genetics studies in evening primroses (*Oenothera odorata* and *O. berteriana*) in the 1940s provided early evidence for the influence of plastid genotypes on leaf shape, particularly on leaf blade width and the degree of serration of the leaf margin (Schwemmle, 1941, 1943). In the present century, work has been carried out about *N. tabacum* on the inactivation of plastid genes that encode PRPs, and has highlighted the importance of these proteins and, therefore, of plastid translation, in leaf morphology. The phenotypic analysis of transplastomic lines in which the plastid gene that encodes the PRP bS18c was inactivated, revealed that *rps18* knockout plants (Δ*rps18*) exhibit a wide range of leaf shape abnormalities (Rogalski et al., 2006). Thus misshapen leaves that lack leaf blade parts are normally observed and, in the severest cases, leaves lacking the entire blade appear to resemble needle-like structures (Rogalski et al., 2006) (Table 2). Besides, irregular leaf branching was also observed. Interestingly, the intensity of developmental defects depends on the frequency of somatic segregation into homoplasmy for the *rps18* knockout allele. By the transplastomic approach, Rogalski et al. (2008) later studied the function of four additional PRPs encoded by the tobacco plastome: uS2c, uS4c, bL20c and bL33c. The knockout lines for proteins uS2c, uS4c or bL20c (Δ*rps2,* Δ*rps4,* and Δ*rpl20*, respectively) frequently displayed similar aberrant leaf morphologies to those previously reported for the Δ*rps18* lines (see above). Accordingly, large leaf blade sectors were missing, which resulted from the lack of entire cell lineages during leaf development caused by homoplasmic mutant cells, which did not survive (Table 2). In contrast to lines Δ*rps2,* Δ*rps4* and Δ*rpl20*, lack of the *rpl33* gene did not affect plant viability and growth under standard conditions. Together, these results show that uS2c, uS4c, bL20c are essential for cell survival under normal growth conditions, while bL33c is not. Rogalski et al., also found that bL33c is required for recovery from chilling stress, and this reveals a role for plastid translation in chilling stress tolerance (see the next section).

In a later work also performed with tobacco, Fleischmann et al. (2011) thoroughly analysed the essentiality of plastome-encoded RPs. To this end, as a candidate for non-essential PRPs, they considered those proteins identified as non-essential in bacteria and those whose genes are lost from the extremely reduced plastid genomes of non-photosynthetic plastid-bearing species. Following these criteria, seven plastid potentially non-essential genes (*rpl22*, *rpl23*, *rpl32*, *rpl36*, *rps3*, *rps15*, *rps16*) were identified and the knockout alleles of them all were generated in tobacco plastids. When genes *rpl22, rpl23, rpl32, rps3* or *rps16*, which respectively encode PRPs uL22c, uL23c, bL32c, uS3c

and bS16c, were inactivated, plants typically exhibited misshapen leaves that lacked part of the leaf blade. This finding suggests that these genes are indispensable for cellular viability despite their absence from the plastomes of some non-photosynthetic plastid-bearing species. Knockout of genes *rpl36* and *rps15*, encoding proteins bL36c and uS15c respectively, resulted in homoplasmic transplastomic mutants, which indicates that these were non-essential PRPs (Fleischmann et al., 2011) (Table 1). Notwithstanding, Δ*rpl36* lines showed a severe leaf phenotype because mutant leaf blades were extremely narrow compared to the wild-type leaves. On the contrary, Δ*rps15* lines displayed only a mild growth phenotype under normal growth conditions (despite the considerable reduction in photosynthetic complex accumulation), which became much more apparent with chilling stress (Table 2). The enhanced phenotype of Δ*rps15* plants under cold stress correlated with reduced plastid translation efficiency compared to the wild type (Fleischmann et al., 2011). These findings reveal that the maintenance of plastid translational capacity at low temperatures is essential for cold stress adaptation. The association between plastid translation and abiotic stress responses is discussed in more detail in the next section of this review.

The above-mentioned results highlight the effects of disrupting the function of plastid genes that encode RPs on tobacco leaf shape. Mutations in PRPs that alter leaf morphogenesis have also been described in Arabidopsis. In this case, as only recently an efficient plastid transformation protocol in this species has become possible (Ruf et al., 2019), mutations that perturb PRPs have been hitherto reported in nuclear genes but not in plastid genes. Thus *scabra1* (*sca1*) mutants affected in the nuclear gene that encode PRP uS5c show defective chloroplast function, paleness and severely perturbed mesophyll development (Mateo-Bonmatí et al., 2015). Furthermore, *sca1* loss-of-function mutations enhance the leaf polarity defects of the *asymmetric leaves 2* (*as2*) mutant (Table 2). This was an unexpected result because the enhancement of the leaf phenotype of mutants *as1* and *as2* has been previously reported for cytosolic RPs, but not for PRPs (Pinon et al., 2008; Casanova-Sáez et al., 2014). These findings unveil a new role for plastid translation in leaf morphogenesis: the establishment of adaxial-adaxial leaf patterning, which might be a consequence of the evolution in land plants of the adaxial-abaxial polarity to optimise photosynthesis (Mateo-Bonmatí et al., 2015). In addition to leaf patterning, Arabidopsis uS5c has been reported as being necessary for cold tolerance (Zhang et al., 2016; see the next section).

The *suppressor of variegation 8* (*svr8*) mutant of Arabidopsis is another example of the link between plastid translation and leaf development. The *SVR8* gene encodes PRP uL24c (mentioned in the previous section) and *svr8* mutation was found in a genetic suppressor screen to identify those mutants able to reverse the leaf variegation phenotype of the *yellow variegated 2* (*var2*) mutant (Liu et al., 2013). *var* mutants have been widely used for investigating the genetic regulation of chloroplast biogenesis (Yu et al., 2007). Mutations in *SVR8* suppress the *var2* leaf phenotype under normal growth conditions and partially suppress cold stress-induced *var2* chlorosis. Interestingly, Liu et al., found that the loss-of-function mutations in another PRP of the small subunit, bS21c, do not suppress *var2* leaf variegation despite loss of bS21c and, like that of uL24c, they also lead to aberrant chloroplast rRNA processing. These results prompted Liu et al. (2013) to hypothesise that particular chloroplast translation aspects other than a general translation defect in this organelle lead to *var2* phenotype suppression. Besides uL24c, several other chloroplast translation factors have been identified with *var2* suppressor screens (Liu et al., 2010a), which emphasises the influence of chloroplast translation on leaf morphogenesis.

## 3. Effects on the abiotic stress tolerance of mutations in PRPs

As indicated in the previous sections, the effects of loss-of-function mutations on PRPs have been generally related to perturbations in growth and developmental processes. In addition, it is well-known that the translational machinery of plastids is sensitive to abiotic stress, particularly to temperature stress. The results of Grennan and Ort (2007) suggest that chilling stress can interfere with the synthesis of proteins in plastids by leading to ribosome pausing and, as a consequence, to delayed translation elongation. In line with this, Arabidopsis mutants *svr3* (*suppressor of variegation 3*), which are affected in a nuclear gene that encodes a chloroplast translation elongation factor highly similar to the *E. coli* TypA translation factor, display a chilling-sensitive phenotype when grown at 8 °C (Liu et al., 2010b). This indicates that SVR3 is required for normal chloroplast function at low temperatures. Similarly, substantial changes in the expression of genes that encode plant ribosomal proteins in response to different abiotic stress conditions have been reported and proposed to be important for efficient translation apparatus rebuilding under adverse environmental conditions. Remarkably, some identified ribosomal proteins are located in plastids (Omidbakhshfard et al., 2012). Furthermore, the characterisation in recent years of mutants affected in PRPs and exhibiting an altered response to abiotic stress has revealed an increasing association between these proteins and different environmental stresses, mainly with temperature and salinity (Table 3 and Fig. 1).

It is worth noting that a mutation in a PRP can sometimes lead to developmental and stress phenotypes. Along these lines, the Arabidopsis *rps5* mutant affected in PRP uS5c shows abnormal chloroplast development, severe impairment of plastid 16S rRNA processing and accumulation, enhanced cold sensitivity and a low level of proteins related to the cold stress response (Zhang et al., 2016). As previously mentioned (see the previous section), the mutations in the Arabidopsis gene that encodes the uS5c protein also alter adaxial-adaxial leaf polarity in the *as2* mutant (Mateo-Bonmatí et al., 2015). Interestingly, the overexpression of the gene that encodes uS5c results in enhanced tolerance to low temperatures, which suggests a possible active role for this protein in cold stress adaptation (Zhang et al., 2016, Table 3).

Mutations in the Arabidopsis nuclear gene that encodes PRP bS1c inhibit HsfA2 (*Heat shock transcription factor A2*)-dependent heat stress responses in chloroplasts. This implies a connection between the heat

**Table 3**
Mutants affected in PRPs showing an abiotic stress phenotype.

| Mutant | PRP | Species | Mutant Stress Phenotype | Reference |
|---|---|---|---|---|
| *rps1* | bS1c[a] | *Arabidopsis thaliana* | Heat tolerance loss | Yu et al. (2012) |
| *rps5* | uS5c[a] | *Arabidopsis thaliana* | Increased cold sensitivity and low level of proteins related to the cold stress response. *RPS5* overexpression results in enhanced cold tolerance | Zhang et al. (2016) |
| *psrp2* | cS22[a] | *Arabidopsis thaliana* | Enhanced tolerance to salt, dehydration and cold stress. *PSRP2* overexpression causes reduced tolerance to salt, dehydration and cold stress | Xu et al. (2013) |
| *wlp1* | uL13c[a] | *Oryza sativa* | Albino-mutant leaf phenotype enhanced in plants exposed to low temperature | Song et al. (2014) |
| *tcd11* | bS6c[a] | *Oryza sativa* | A thermo-sensitive phenotype, albino- and seedling-lethal when grown at 20 °C, but the same phenotype as the wild type when both are grown at 32 °C | Wang et al. (2017) |
| *Δrpl33* | bL33c[b] | *Nicotiana tabacum* | Chilling stress-sensitive; marked delay in recovering from prolonged chilling stress at 4 °C | Rogalski et al. (2008) |

*tcd11*: *thermo-sensitive chlorophyll-deficient mutant 11*; *wlp1*: *white leaf and panicles 1*; Δ: Knockout transplastomic lines.
[a] Nuclear gene.
[b] Plastome gene.

stress response and plastid translation (Yu et al., 2012). As a result, *rps1* mutant plants display significant heat tolerance loss (Table 3). Interestingly, the phosphorylation of bS1c is regulated by the activation of the MKK9-MPK6 cascade, which mediates the salt stress response in Arabidopsis. Accordingly, bS1c phosphorylation was upregulated by salt treatment in wild-type seedlings but the level of phosphorylation of this protein was reduced in the *mkk9* and *mpk6* null mutants, which indicates that bS1c is downstream of the MKK9-MPK6 cascade in the salt stress response pathway (Liu et al., 2015). Because a number of the proteins with salt-induced phosphorylation identified by Liu and collaborators were localized in the chloroplast, it would be worth to further investigate if these proteins are phosphorylated by the MKK9-MPK6 cascade inside the organelle or in the cytosol and later imported into the chloroplast. In any case, these results extends the involvement of bS1c in abiotic stress to plant salt tolerance.

The characterisation of the rice *wlp1* (*white leaf and panicles 1*) mutant, which exhibits abnormal chloroplast development, particularly at low temperatures (Table 3), revealed that it is affected in PRP uL13c (Song et al., 2014). The mutant phenotype of *wlp1* is attributed by the authors to low levels of the synthesis of plastid RNA polymerase PEP (Plastid-Encoded Polymerase) encoded by the *rpoA*, *rpoB*, *rpoC1* and *rpoC2* genes of the plastome due to impaired plastid translation (Song et al., 2014). Another rice mutant, *tcd11* (*thermo-sensitive chlorophyll-deficient mutant 11*), which is affected in the nuclear gene that encodes PRP bS6c, is thermo-sensitive, chlorophyll-deficient and displays altered chloroplast development (Wang et al., 2017). While the *tcd11* mutant shows an albino phenotype and dies when grown at 20 °C, it exhibits the same phenotype as the wild type when cultivated at higher temperature (32 °C) (Table 3). Interestingly, the impaired *TCD11* function at 20 °C leads to a sharp drop in the transcript levels of several genes related to chloroplast development, which may affect the assembly of chloroplast ribosomes at low temperature. In contrast, when *tcd11* and wild-type plants are grown at 32 °C, the expression of the examined genes is not affected in the mutant (Wang et al., 2017). These findings suggest that the bS6c function is vital for correct chloroplast development and, consequently, for plant survival at lower than optimal temperatures (Wang et al., 2017).

As mentioned in the previous section, Rogalski et al. (2008) found that tobacco PRP bL33c is involved in plant tolerance to low-temperature stress. Thus the *rpl33* loss-of-function mutant plants did not recover like the wild-type plants did after exposure for 5 weeks at 4 °C and returned to standard growth conditions. This indicates that functional bL33c is required to maintain chloroplast translation in response to chilling conditions. Furthermore, the simultaneous deletion of the tobacco plastid genes that encode bL33c and uS15c (the latter is another non-essential PRP) results in lethality under autotrophic growth (Ehrnthaler et al., 2014). Nonetheless, the synthetic lethality of the double mutant is rescued when the plants lacking bL33c and uS15c are continuously grown at a higher temperature (35 °C). These plants exhibit a wild-type phenotype caused by improved efficiency in the biogenesis of chlororibosomes (Ehrnthaler et al., 2014).

The involvement of the plastid translational apparatus in plant adaptation to salinity has also been investigated. Salt stress negatively affects plant growth and development by, among other things, significantly altering the function of chloroplasts and mitochondria (Leister et al., 2017; Robles and Quesada, 2019). By qRT-PCR, Omidbakhshfard et al. (2012) studied in Arabidopsis seedlings the changes in expression of 170 nuclear genes related to protein synthesis after exposing wild-type plants to 150 mM NaCl at different time points. Some of the up-regulated genes encode chloroplast translation-related proteins, such as uL11c, ATAB2 (PROTEIN TAB2 HOMOLOG, an RNA-binding protein in A/U-rich regions which probably acts as a translation activator) and PDF1B (PEPTIDE DEFORMYLASE 1B required to remove the N-formyl group from nascent peptides). These genes are considered important for chloroplast development and have been proposed as potential biotechnological targets for optimising salinity tolerance in plants (Omidbakhshfard et al., 2012). For uL11c, Omidbakhshfard et al. (2012) proposed that the salt-stress up-regulation of the gene that encodes this PRP might contribute to chloroplast functionality withstanding the initial salt stress phase.

To our knowledge, only one mutant defective in a PRP that displays an altered response to salinity has been described: *psrp2*, which is affected in Arabidopsis chloroplast-specific protein cS22. Specifically, the *psrp2* mutant grows more under salt stress conditions than the wild type (Table 3). In contrast, the transgenic plants that overexpress cS22 exhibit delayed germination and reduced seedling growth in response to salt stress (Xu et al., 2013, Table 3). These results suggest that cS22 functions as a negative regulator of the salinity response. cS22 contains two RNA recognition motifs, is able to bind single-stranded RNA and DNA, and also possesses RNA chaperone activity. This led Xu et al. (2013) to propose a relation between the translation and regulation of RNA metabolism in chloroplasts.

Taken together, the aforementioned findings provide solid evidence for the close link between PRPs, which are key components of chloroplast translational machinery, and of the response and adaptation of higher plants to abiotic stress. This is not altogether unexpected because plastid gene expression must appropriately respond to developmental signals and environmental stress conditions so that plants can adapt to internal and/or exogenous changes. This is achieved mainly by the coordination and integration of the expression of nuclear and plastome genes by retrograde and anterograde signalling (Leister et al., 2017; Robles and Quesada, 2019).

## 4. Conclusions and future perspectives

The results presented in this review highlight that the impaired function of some PRPs cause lethality in embryo or seedling stages by revealing the essentiality of these PRPs. In contrast, other PRPs are not essential, but their loss leads to phenotypic alterations, such as growth retardation, decreased pigmentation and photosynthesis, defective chloroplast biogenesis or perturbed development (e.g. altered lateral root formation or leaf morphogenesis). All these phenotypes can ultimately be attributed to defective plastid translation and/or ribosome assembly. Remarkably, defective PRPs can also result in altered tolerance to abiotic stress, mainly to low temperatures. All this reveals the involvement of PRPs in different processes of plant growth, development and response to adverse environmental cues. This is noteworthy because the mutants affected in plastid ribosomes should share the same type of perturbation at the molecular level, and they would be expected to exhibit similar phenotypes. However, this is not the case because not all PRP-defective mutants display the same phenotype. This could be attributed to differences in the specificities of PRP functions, and perhaps even to their locations other than chloroplasts and/or their involvement in extraribosomal functions. The presence of small families of PRP-encoding genes, which results from gene duplications, in different plant species might contribute to the functional diversity of PRPs and to the heterogeneity of plastid ribosomes. In line with this, specific PRPs could selectively influence distinct developmental stages, tissues and/or cell types, which might support the existence of specialised ribosomes with differential translational activity. This would explain why some gene families show little or no gene redundancy, because the loss of function of one of their members results in lethality or an alteration in growth and/or development that is not compensated for by the existence of another functional member. It is also likely that some PRPs are more important than others for plastid ribosome activity depending on their position and functional role within the ribosome structure.

Despite much progress having been made in the recent years, we are still far from knowing in depth the molecular functions of each PRP and the specific biological processes in which they would be involved. The identification and characterisation of new PRP-defective single mutants (or many mutants in the case of PRP families) for which the effects of the

perturbation of PRP functions are still unknown, will help to extend our current knowledge on the impact that plastid translational activity has on plant biology. And last but not least, it would also be valuable to obtain viable mutant alleles of essential PRPs to study the roles of these PRPs in developmental stages other than those at which they are indispensable for plants.

## Authors' contributions

VQ conceived the study; VQ and PR collected and analyzed the information: VQ wrote the draft paper. PR and VQ reviewed and edited the manuscript; PR designed and made the Figure; both authors have read and approved the manuscript final version.

## Author agreement

The authors have seen and approved the final version of the manuscript. The authors confirm that manuscript is the authors' original work, has not received prior publication and is not under consideration for publication elsewhere.

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The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

No data was used for the research described in the article.

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