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Mechanism of Cytoplasmic mRNA Translation

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Protein synthesis is a fundamental process in gene expression that depends upon the abundance and accessibility of the mRNA transcript as well as the activity of many protein and RNA-protein complexes. Here we focus on the intricate mechanics of mRNA translation in the cytoplasm of higher plants. This chapter includes an inventory of the plant translational apparatus and a detailed review of the translational processes of initiation, elongation, and termination. The majority of mechanistic studies of cytoplasmic translation have been carried out in yeast and mammalian systems. The factors and mechanisms of translation are for the most part conserved across eukaryotes; however, some distinctions are known to exist in plants. A comprehensive understanding of the complex translational apparatus and its regulation in plants is warranted, as the modulation of protein production is critical to development, environmental plasticity and biomass yield in diverse ecosystems and agricultural settings.

INTRODUCTION

Plant growth and function requires highly regulated spatial and temporal regulation of gene expression. The decoding of the mRNA into a polypeptide chain (protein) by the ribosome is a key step in the regulatory continuum from gene to protein to phenotype. The process of translation requires many RNAs, the messenger RNA (mRNA) transcript, transfer RNAs (tRNAs) and the ribosomal RNAs (rRNAs) of the ribosome as well as scores of soluble protein factors that function either as individual proteins or in multi-subunit complexes. The decoding of mRNA into a polymer of amino acids is a very ancient process, such that the machine for this process, the two-subunit ribosome, is conserved across all forms of life on Earth. The three basic phases of the process of translation – initiation, elongation and termination – are also generally conserved. Consequentially, many components of the complex apparatus involved are recognizable across phyla, especially across the plant, fungal and animal kingdoms. Despite this conservation of the basic chemistry and process of protein synthesis, nature has evolved many ways of starting the first phase, known as initiation. The second phase known as elongation, in which additional amino acids are covalently added to the polypeptide, and the third phase known as termination that completes the process are much more preserved across all kingdoms. Eubacteria have three proteins known as initiation factors to unite the mRNA, the initiator tRNA (usually $tRNA_i^{Met}$) and the small subunit of the ribosome and assemble them with the large subunit of the ribosome to commence the elongation process. *Archaea* and eukaryotes have expanded this machinery to include 10 or more proteins or protein complexes, although *Archea* lack the eIF3 and eIF4 families found in eukaryotes. In addition, a number of "flourishes" have been added to the nuclear-encoded eukaryotic mRNA such as a 5'-m^{7}GpppN cap structure at the 5' end and a stretch of adenine residues, the poly(A) tail at the 3' end. These features are added in the nucleus during transcription and are important in transcript stability during the journey from nucleus to cytoplasm and the lifetime of the mRNA. The translational apparatus has evolved to use these added mRNA features to facilitate the process of initiation in the cytoplasm. It is also thought that the role of the extended cohort of initiation factors in eukaryotes is to participate in exquisitely complicated schemes to regulate the process. What could be more important to a cell than the synthesis of the proteins that catalyze the chemistry of metabolism to make the energy for cell growth, division and function? It is therefore not surprising that the plant translational apparatus and its regulation varies from other eukaryotic organisms due to the specialized cellular biochemistry, developmental complexity and environmental plasticity that confers survival and reproduction centered around the capture of light energy and the conversion to chemical energy, i.e., photosynthesis. Another chapter of *The Arabidopsis Book* evaluates the regulation of translation of cytoplasmic mRNAs (Roy and von Arnim, 2013). Organellar mRNA translation (chloroplast and mitochondria) and its coordination with cytoplasmic translation is beyond the scope of this chapter; therefore, the reader is referred to recent reviews (Gonzalez and Giegé, 2014; Janska and Kwasniak, 2014; Tiller and Bock, 2014). Here, we detail the process of cytoplasmic translation, its machinery and regulation in plant cells as a drama that occurs in several acts.

DELIVERY OF THE SCRIPT: FROM PRE-mRNA TO QUALITY-CHECKED CYTOSOLIC mRNA

Following the selection of the transcript start site and polymerization of approximately the first 20 nucleotides of the pre-mRNA by RNA polymerase II, the 5'-end of the nascent transcript is modified by the addition of a 5'-m^{7}GpppN-cap structure. This event augments subsequent steps in pre-mRNA biogenesis including intron removal by the spliceosome (Izaurralde et al., 1994) and the cleavage event that marks the 3'-end and site of poly(A) addition (Cooke and Alwine, 1996; Hunt, 2011). Mechanisms of 5'-cap addition in plants are not well studied, but are thought to resemble those of other eukaryotes. The 5'-cap provides protection for the mRNA until it is removed by the decapping machinery and subsequent degradation occurs in a 5' to 3' manner (Jiao et al., 2008). As will be discussed, the cap structure also plays a definitive role in the selection of an mRNA for translation. At the 3' end of the pre-mRNAs, the process of cleavage and polyadenylation has both highly conserved eukaryotic and plant-specific features (Hunt, 2011). The 3'-poly(A) addition site of an individual gene transcript can vary, with ~25% of *Arabidopsis thaliana* genes displaying multiple 3' cleavage sites (Wu et al., 2011). This heterogeneity in the 3' untranslated region (3'UTR) is likely to impact mRNA stability as well as translation. Also pertinent to translation can be features of the 5'-leader sequence prior to the initiation codon of the protein-encoding open reading frame (ORF), referred to as the 5' untranslated region (5'UTR) or 5' leader. Sequences or secondary structures within the 5'UTR can predispose a transcript to distinct translational regulation (Arribere and Gilbert, 2013), as can the presence of short upstream ORFs (uORFs) (Roy and von Arnim, 2013). High-throughput mRNA sequencing (mRNA-seq) has further expanded appreciation for transcript isoform variants that arise due to selection of the site of transcript initiation and variation in intron selection that are regulated in environmental and developmental contexts (Yamamoto et al., 2009). Of these two, variation in intron removal appears to be more prevalent, but both lead to further diversity and potential regulation of protein expression (Filichkin et al., 2010; Li et al., 2010; Reddy et al., 2013).

The mechanism of constitutive intron splicing of plant pre-mRNAs is generally similar to the pathway detailed in yeast and mammals (Reddy, 2007; Koncz et al., 2012; Reddy et al., 2013). An aspect of this process is the recording of splicing events by binding of an exon junction complex (EJC) 20-30 nt upstream of the site of intron removal. There is modulation of intron removal through regulation of the selection of alternative splice sites and intron retention, affecting upwards of 60% of plant mRNAs during development or due to environmental influences (Filichkin et al., 2010; Wu et al., 2011; Filichkin and Mockler, 2012; Kalyna et al., 2012; Marquez et al., 2012; Syed et al., 2012; Leviatan et al., 2013; Staiger and Brown, 2013). Alternative splicing and intron retention events have numerous consequences, ranging from the generation of transcript isoforms that encode distinct proteins or are differentially regulated at the level of message stability, transport, localization or translation. When transcription or splicing produces a transcript containing a premature termination codon, typically upstream of an EJC, the mRNA is targeted for nonsense mediated decay (NMD) after the first round of translation (Reddy et al., 2013). Those transcripts that survive the pioneering round of translation are templates for protein synthesis until they are targeted for degradation or sequestered into translationally inactive complexes and removed from the "cast of actors" in the drama that is translation.

ACT 1: INITIATION OF TRANSLATION

The most well studied aspect of translation in eukaryotes is the initiation phase, which is by far considered currently to be the predominant level of regulation. Initiation of translation of a cytosolic mRNA utilizes both the 5'-m^{7}GpppN-cap and the 3'-poly(A) tail with initiation factors that specifically recognize these features to start the process of initiation of translation. Baker's yeast (*Saccharomyces cerevisiae*), has provided a genetic treasure trove for structural and functional insight of the highly interactive initiation machinery. Comparative studies have shown that the machinery and their functions are highly conserved, although there are some interesting differences across the spectrum of eukaryotes. In fact, there are remarkable tales of diversity in the machinery that are unique to various organisms and ecological niches (Hernández and Vazquez-Pianzola, 2005; Hernández et al., 2012). Several recent reviews on translation provide mechanistic and structural details of translation derived with *S. cerevisiae* and mammalian systems (Sonenberg and Hinnebusch, 2009; Jackson et al., 2010; Lorsch and Dever, 2010; Hinnebusch, 2011; Aitken and Lorsch, 2012; Dever and Green, 2012; Hernández et al., 2012; Hershey et al., 2012; Hinnebusch and Lorsch, 2012; Valasek, 2012; Voigts-Hoffmann et al., 2012; Lomakin and Steitz, 2013; Hinnebusch, 2014; Mead et al., 2014; Merrick and Harris, 2014). Translation in plants has been reviewed with different emphases in the past five years (Bailey-Serres et al., 2009; Muench et al., 2012; Muñoz and Castellano, 2012; Echevarría-Zomeño et al., 2013; Browning, 2014; Gallie, 2014), and several historical reviews provide the back story (Browning, 1996; Bailey-Serres, 1999; Kawaguchi and Bailey-Serres, 2002; Browning, 2004; Gallie, 2007). As will be described, the translational machinery of plants resembles that of *S. cerevisiae* and mammals. Because plants have unique biological activities, such as photosynthesis and the capacity to respond to stresses *in situ*, they have evolved translational control mechanisms relevant to their needs. This chapter will outline the process of initiation, elongation and termination as largely derived from detailed studies in *S. cerevisiae* and mammals, but will include specific aspects of the plant apparatus where known. Our knowledge of plant translation is based largely on the *Arabidopsis thaliana* accession Col-0 (referred to here as Arabidopsis) and the *in vitro* system derived from the germ (embryo) of hexaploid bread wheat (*Triticum aestivum*). Undoubtedly there will be myriad differences within the plant kingdom, not only in the translational apparatus but modulation of protein synthesis as is needed in particular ecological niches and environmental circumstances.

THE ACTORS: THE BASIC MACHINERY OF INITIATION

The current estimate of the number of "basic" initiation factors of eukaryotes is >16 and growing (see Table 1). The unified nomenclature for these proteins/complexes includes five categories for the different aspects of the initiation process (Safer, 1989). These are the eukaryotic initiation factors (eIF) 1 to 6. Table 1 includes several additional proteins that are now being considered part of the translational machinery, but have yet to be evaluated in plants, although in many cases a plant ortholog has been recognized. Initiation factor nomenclature is challenging, especially the names of the initiation factors which have evolved within eukaryotic phyla. eIFs include single or multi-subunit protein complexes that are distinguished by complex number (*i.e*., eIF2, 3, 4) and Roman letters or Greek letters (*i.e*., eIF2A or eIF2α), each of which is a different complex or protein. Some proteins were originally designated eIFs because of their ability to stimulate rabbit reticulocyte *in vitro* translation. For example, the eIF2C family turns out to correspond to the Argonautes that participate in RNA-mediated gene silencing (Chen, 2010). Why the addition of an AGO was found to stimulatory is unknown, but AGO1 copurifies with membrane-associated ribosomes during microRNA (miRNA)-mediated translational inhibition in Arabidopsis (Li et al., 2013b).

Table 1 presents the current nomenclature of proteins with known functional activities in mRNA translation in the model plant *Arabidopsis thaliana*. More than one functional gene encodes most of these factors or factor subunits. Therefore, there are multiple isoforms of each factor or factor subunit, which could accumulate in a distinct quantitative, spatial or temporal manner which may have functional consequence.

A schematic of the initiation process is shown in Figure 1 and emphasizes where plant translation is known to differ from that of yeast or mammals. In the next section we introduce and present details about the initiation factors and their interactions with other actors, mRNA, tRNAs and the ribosome, the *prima donna*. The order of the description of members of these acting troupes corresponds to the sequence of their appearance on the stage with the 40S ribosomal subunit. The 40S ribosome/associated factors and mRNA/associated factors are then joined by the 60S ribosome to form the functional 80S ribosome complex for elongation of the polypeptide. The 60S subunit possesses the peptidyl transferase activity to join together amino acids as directed by the codon sequence of the mRNA. After the introduction of the initiation factors, we consider the first act of protein synthesis: the sequence of events that culminates in initiation of polypeptide synthesis. The second and third acts, elongation and termination of protein synthesis will introduce several new performers (i.e., eEFs, eIF6, RACK1, ABCE1, and eRFs). There are also two *encore* events that have garnered attention in recent years that involve efficient recycling of ribosomes for maintained translation of an mRNA or in some cases re-initiation at a downstream open reading frame on an mRNA (von Arnim et al., 2014). There are also side shows of translation including mRNA turnover (see section on "Curtains for some mRNAs") and protein degradation (for recent reviews in this series see Callis, 2014; Choi et al., 2014).

eIF2 GROUP AND tRNA^{MET}

This group of factors functions in formation of the ternary complex comprised of Met-tRNA$_i^{Met}$ •eIF2•GTP and the exchange of GDP for GTP from eIF2•GDP by eIF2B (also called guanine nucleotide exchange factor or GEF). The eight known proteins of this group have challenging names (Table 1). eIF2A (not to be confused with eIF2α or eIF2Bα) and eIF2D (not to be confused with eIF2Bδ) are new members of the eIF2 group in animals (Komar et al., 2012). The genes appear to be conserved in plants, but their role in plant initiation is not currently known.

Also to be considered along with this group is the initiator Met-tRNA$_i^{Met}$, which has a very specific role in the selection of the correct initiation codon (AUG). This tRNA does not function in elongation and can be distinguished from Met-tRNA^{Met} used for addition of internal methionine residues. Initiator tRNAs have evolved several strategies for this role and avoiding interaction with elongation factor EF1A (reviewed in Kolitz and Lorsch, 2010). Plants and fungi appear to use a strategy of modification of a certain nucleotide in the T-loop with a large O-ribosylphosphate moiety to prevent interaction of initiator Met-tRNA$_i^{Met}$ with eEF1A.

eIF2 and Ternary Complex Formation

eIF2 is among the most studied of the translation initiation factors. The primary role of this heterotrimeric complex in both eukaryotes and *Archaea* is to bring the Met-tRNA$_i^{Met}$ and GTP to the ribosome, a task performed by the single polypeptide factor IF2 in eubacteria (reviewed in Schmitt et al., 2010). The eIF2 complex is composed of three subunits, designated eIF2α, eIF2β and eIF2γ. eIF2α and eIF2β interact with eIF2γ which forms the core of the complex and also contains the GTP nucleotide binding site. This factor has structural similarity to other GTP binding factors such as elongation factors EF-Tu (prokaryotic) or eEF1A (eukaryotic) (Schmitt et al., 2010). A zinc-binding domain is present in eIF2β that is similar to one found in eIF5 and is proposed to play a role during start codon recognition (Nanda et al., 2013). The major binding site of eukaryotic Met-tRNA$_i^{Met}$ appears to the eIF2γ subunit and there is less contribution to binding of Met-tRNA$_i^{Met}$ by eIF2α than eIF2β in eukaryotes; whereas in *Archaea* eIF2α and eIF2β comprise the major binding site of Met-tRNA$_i^{Met}$ (Schmitt et al., 2010). eIF2 and associated proteins have been purified from wheat germ and biochemically studied (Benne et al., 1980; Lax et al., 1982; Osterhout et al., 1983; Seal et al., 1983; Shaikhin et al., 1992; Benkowski et al., 1995a, b). Structural and functional similarity of plant eIF2 to yeast and mammalian eIF2 are expected, although there could be plant specific molecular interactions of the subunits or Met-tRNA$_i^{Met}$ given that plant eIF2β lacks the third poly-lysine region in the N-terminal domain found in other eukaryotic eIF2β subunits (Metz and Browning, 1997).

Table 1. Initiation Factors of Arabidopsis

Factor	Mr[a]	Function	Arabidopsis Gene[f]
eIF1	12,600	Formation of and scanning by pre-initiation complex; start site selection; controls GTP activating protein activity of eIF5	At4g27130, At5g54760, At5g54940, At1g54290
eIF1A (eIF4C[b])	17,600	Formation of and scanning by pre-initiation complex; start site selection	At5g35680, At2g04520
eIF2		Forms ternary complex with GTP and Met-$tRNA_i$; Binds Met-$tRNA_i$ to 40S subunit; GTPase activity in presence of eIF5	
α	42,000	Target for GCN2 kinase in plants	At2g40290, At5g05470
β	38,000		At5g20920, At5g01940, At3g07920
γ	50,000		At1g04170, At4g18330
eIF2A		Unknown in plants; in mammals participates in IRES mediated initiation	At1g73180
eIF2B[c]		Recycles eIF2•GDP to eIF2•GTP; unknown if similar function in plants	
α	42-65,000		At1g53880, At1g72340, At1g53900
β	43,800		At3g07300
γ	49,000		At5g19485
δ	37-73,000		At5g38640, At1g48970, At2g44070
ε	80,000		At3g02270, At2g34970, At4g18300
eIF3	13 subunits	Formation of and scanning by pre-initiation complex; start site selection; binding of mRNA to PIC; prevention of pre-mature 60S ribosome association	
a	114,000		At4g11420
b	85,000		At5g27640, At5g25780
c	103,000		At3g56150, At3g22860
d	67,000		At4g20980, At5g44320
e[d]	52,000		At3g57290
f	32,000		At2g39990
g	36,000		At3g11400, At5g06000
h	38,000	Required for efficient re-initiation of main open reading frame of mRNAs with upstream open reading frames	At1g10840
i[e]	36,000		At2g46280, At2g46290
j	25,000		At1g66070, At5g37475
k	26,000		At4g33250
l	60,200		At5g25754, At5g25757
m	50,000		At3g02200, At5g15610
eIF4A	47,000	ATP-dependent unwinding of mRNA Binds mRNA to 40S subunit	At3g13920, At1g54270
eIF4B	58,000	ATP-dependent unwinding of mRNA Binds mRNA to 40S subunit	At3g26400, At1g13020

Phosphorylation of eIF2

Mammals possess four eIF2 kinases: (HRI, heme-regulated inhibitor; PKR, double stranded RNA-dependent kinase; PERK, PKR-like ER kinase; GCN2, general control non-derepressible-2 kinase). All phosphorylate a conserved serine residue (Ser51) in mammalian eIF2α that inhibits initiation of translation in response to various stresses. eIF2B (see below) cannot easily dissociate from phosphorylated eIF2α and therefore guanine nucleotide exchange is inhibited depleting available eIF2 for ternary complex formation (reviewed in Donnelly et al., 2013). Despite early reports of a "PKR-like" activity in virus-infected plants (Hiddinga et al., 1988; Langland et al., 1995; Langland et al., 1996; Chang et al., 1999), no specific kinase could be purified and the sequence of a putative PKR ortholog is absent from plant genomes (Immanuel et al., 2012). GCN2 is therefore the only recognizable plant eIF2α kinase at this time and targets a similar serine residue in plant eIF2α (Halford et al., 2004; Byrne et al., 2012; Li et al., 2013a; Wang et al., 2014). Other eIF2α kinases may exist, but have yet to be described. GCN2 was identified in yeast in response to nutrient deprivation, particularly amino acid or purine starvation (Hinnebusch, 2005), but it is induced by other stresses (*e.g.,* UV, osmotic and oxidative stress) and functions similarly in mammals (Donnelly et al., 2013). The general amino acid control pathway in yeast is controlled by the transcription factor GCN4 which activates transcription of numerous genes in many biosynthetic pathways in response to nutrient deprivation (Hinnebusch, 2005). The translation of yeast *GCN4* mRNA, utilizes four short upstream open reading frames (uORFs) in the 5' leader sequence to control expression of the ORF encoding GCN4.

GCN2 kinase, which is activated during nutrient deprivation by sensing tRNAs that are unchanged (i.e., low amino acid levels), phosphorylates eIF2α, preventing its interaction with eIF2B for guanine nucleotide recycling. The amount of available ternary complex falls and protein synthesis initiation is inhibited. When the levels of ternary complex are high, initiation occurs at the first AUG in the 5' leader and elongation and termination precede; subsequent reinitiation events are likely at uORFs 2-4 and therefore initiation at the

Table 1. (continued)

Factor	Mr[a]	Function	Arabidopsis Gene[f]
eIF4F		Complex of eIF4G and eIF4E; ATP-dependent unwinding of mRNA; Binds mRNA to 40S subunit	
eIF4G	188,000	Interaction with eIF4A, eIF4B, eIF5, eIF4E	At3g60240
eIF4E	26,000	Binds to eIF4G and m^7G cap on mRNA	At4g18040, At1g29590, At1g29550
eIFiso4F		Complex of eIFiso4G and eIFiso4E; Plant specific isoform of eIF4F; ATP-dependent unwinding of mRNA; Binds mRNA to 40S subunit	
eIFiso4G	84,000	Interaction with eIF4A, eIF4B, eIF5, eIF4E	At5g57870, At2g24050
eIFiso4E	22,500	Binds to eIFiso4G and m^7G cap on mRNA	At5g35620
eIF5	48,600	Joining of 60S subunit; GTPase activating protein	At1g77840, At1g36730
eIF5B	121-142,000	Positions Met-$tRNA_i$ at AUG with eIF1A	At1g76810, At1g21160
eIF5C	47,000	eIF5 "mimic protein" also called 5MP1 or BZW2; regulates eIF2 function by being both a mimic and competitor for eIF5; role unclear in plants	At5g36230, At1g65220
eIF6	26,000	Prevents association of 60S and 40S subunits	At3g55620, At2g39820
PABP	~60-74,000	Binds poly A on mRNA; interacts with eIF4G	At2g23350, At4g34110, At1g22760, At1g71770, At3g16380, At1g49760, At2g36660, At1g34140, At5g65250, At5g65250
4E2 (nCBP[b], 4EHP)	25,700	Unclear in plants	At5g18110

[a] Approximate molecular weight based on TAIR9 data.
[b] Prior nomenclature used in literature.
[c] Hypothetical genes based on similarity to mammalian eIF2B subunits; protein complex has not been isolated from plant source and shown to function as GDP exchange factor.
[d] Also known as INT6
[e] Also known as TRIP1 (TGF-beta receptor interacting protein)
[f] Links to various data bases using the Arabidopsis Gene Identifier can be found at http://browning.cm.utexas.edu/arabidopsis/fiat

AUG of the GCN4 coding region is limited. However, when ternary complex is low, reinitiation at uORFs 2-4 is less likely and the 40S ribosomes continue to scan, acquire ternary complex, reach the AUG of the GCN4 ORF and commence synthesis of GCN4 (Hinnebusch, 2005). The process also involves the transient maintenance of eIF3 association with the ribosome as it translates the first of the four uORFs under starvation conditions (Szamecz et al., 2008). This is an exquisitely complex regulatory system in yeast for sensing and response to nutrient status through translational control.

Arabidopsis GCN2 kinase complements yeast GCN2 kinase suggesting some aspects of the yeast general amino acid control (GAAC) mechanisms may be conserved in plants (Zhang et

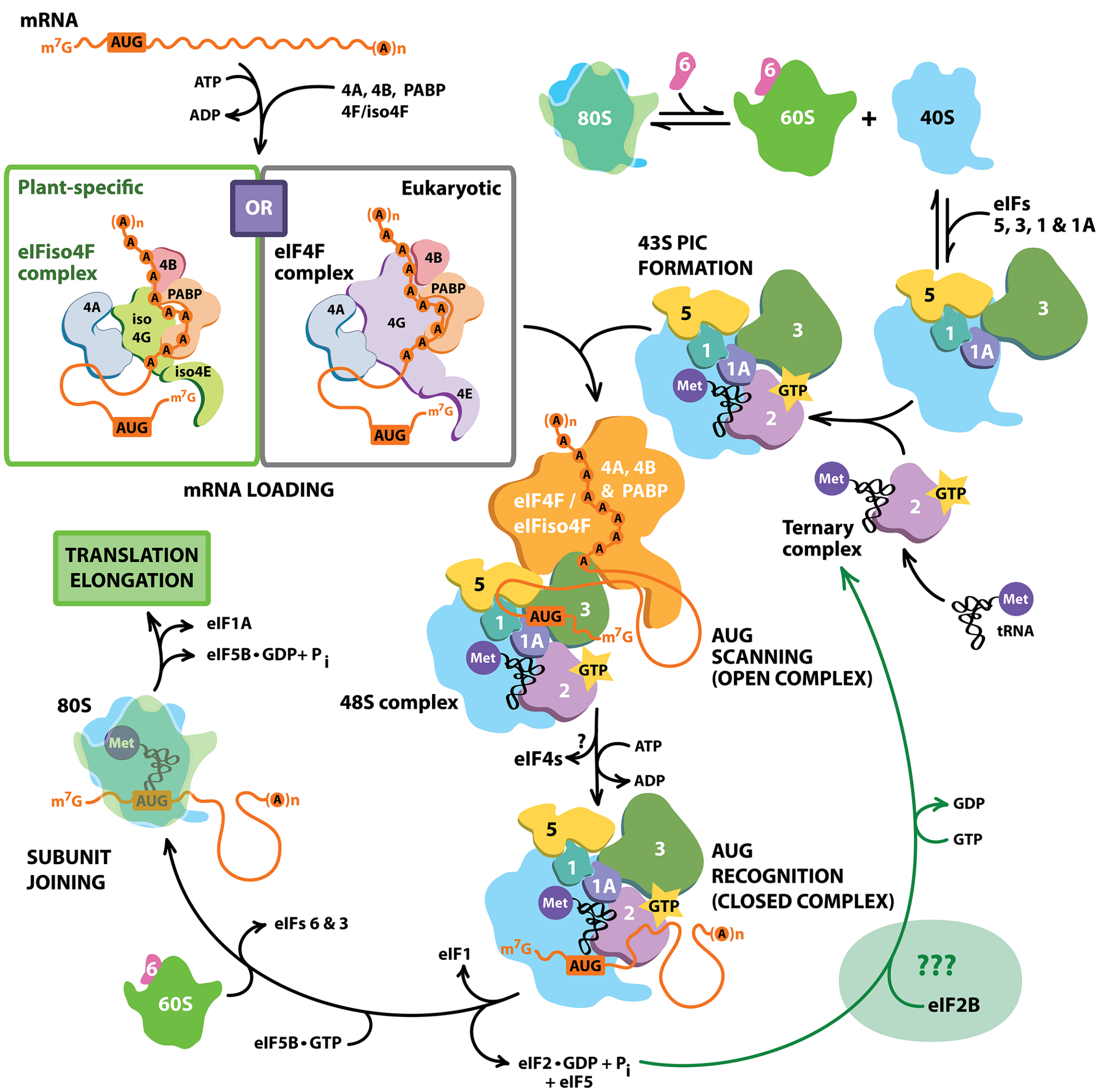

Figure 1. Overview of the steps of translation initiation in the cytoplasm of plants.

Once the mRNA has been exported from the nucleus into the cytoplasm it interacts with a cap-binding complex (eIF4F or plant-specific eIFiso4F) at the 5' end and PABP at the 3' end. Additional factors, eIF4A and eIF4B are recruited to the mRNA to promote ATP-dependent unwinding of secondary structure prior to interaction with the 43S PIC (pre-initiation complex). The 43S PIC is formed from the 40S ribosome and its associated factors eIF1, eIF1A, eIF3 and eIF5. eIF1, eIF1A, eIF3 and eIF5 form the multi-factor complex (MFC), although it is not clear if this assembles prior to interaction with the ribosome or on the ribosome. Addition of ternary complex (TC) of eIF2•Met-tRNA$_i$•GTP completes the preparation of the PIC. This then engages the mRNA and its associated factors (eIF4F/eIFiso4F, eIF4A, eIF4B, PABP) to form the 48S scanning complex (open conformation), which functions to scan the 5' untranslated region of the mRNA in the 5' to 3' direction in order to select the AUG (i.e., A/GXXA$_{+1}$UGG). Once the AUG is selected the 48S complex switches to the closed conformation securing the Met-tRNA$_i$ in the P-site and ejecting eIF1. eIF5B•GTP binds to the 48S ribosome complex and the process for joining with the 60S subunit commences, and eIFs 6, 5, 3, and 2 exit the 48S ribosome. Completion of joining of the 60S ribosome results in the hydrolysis of eIF5B•GTP and its release along with eIF1A. The now functional 80S ribosome may now start peptide elongation (see Figure 2). The role of eIF2B in guanine nucleotide recycling of eIF2 in plants is unclear at this time (shown with a green line). Note that the factors and ribosomal subunits are not to scale.

al., 2003). Herbicides (*i.e.,* chlorosulfuron, glyphosate) that inhibit amino acid synthesis and thus induce amino acid starvation result in induction of GCN2 and phosphorylation of eIF2α (Zhang et al., 2008b). GCN2 also functions in response to purine starvation, UV irradiation, wounding, hormones, cold shock (Lageix et al., 2008), cadmium stress (Sormani et al., 2011b), amino acid metabolism and sulfur signaling (Byrne et al., 2012), but evidently not virus infection (Zhang et al., 2008b). The phosphorylation of eIF2α in response to purine starvation was correlated with reduced large polysome complexes, suggesting that it can generally inhibit initiation (Lageix et al., 2008; Sormani et al., 2011b). To date, there is no direct evidence that GCN2 regulates ternary complex formation in plants or plays a role in translation of mRNAs with uORFs.

eIF2α and eIF2β subunits were reported to be targets of CK2 (formerly casein kinase II) *in vitro*, but the role of phosphorylation of these subunits *in vivo* is not known (Dennis and Browning, 2009; Dennis et al., 2009). Interestingly, none of the subunits of eIF2 were reported to be phosphorylated in a study of the effects of light/dark on the phosphoproteome of the translational apparatus (Boex-Fontvieille et al., 2013). There is still much to learn about the control of protein synthesis in plants in response to various types of stresses and to what level eIF2 subunit phosphorylation regulates the process.

In plants, "eIF2B or, not 2B, that is the question"

As described above, one of the major mechanisms used by yeast and particularly mammals for the regulation of initiation of translation is the phosphorylation of a single conserved serine residue on eIF2α. This phosphorylation event prevents eIF2B from dissociating from eIF2 during recycling of GDP for GTP, prohibiting the formation of a new ternary complex. This inability to recycle GDP/GTP effectively shuts down initiation in the absence of ternary complex formation (reviewed in Donnelly et al., 2013).

An eIF2B-like activity has not been purified from a plant source, although genes with similarity to mammalian eIF2B subunits are encoded by Arabidopsis (see Table 1) and phosphopeptides have been reported for the eIF2Bγ and eIF2Bδ subunits (Boex-Fontvieille et al., 2013), suggesting that the proteins are expressed and modified.

Evidence that eIF2B recycling may not be necessary, and thus phosphorylation of eIF2α may not have as strong an inhibitory effect on translation, is the report that binding of GDP to eIF2 is only about 10-fold higher than that of GTP in comparison to the ~100-fold difference for mammalian eIF2 (Shaikhin et al., 1992). Thus the requirement for eIF2B recycling may be less in plants allowing for continued translation even in the presence of eIF2α phosphorylation. There is a need for further studies to corroborate biochemically what is known about plant GCN2 kinase, its role in eIF2α phosphorylation and eIF2B (*i.e.* if it exists as a complex) activity in GDP recycling and global translational activity. It will also be intriguing to decipher the cross-talk that occurs between pathways that activate GCN2 and the Target of Rapamycin (TOR) pathway, which is likely involved in sensing sucrose and other nutrients in plants and regulating translation of certain mRNAs (Immanuel et al., 2012; Robaglia et al., 2012).

eIF3 Group

The sole performer of this group, eIF3, is a complex of six subunits (a, b, c, I, g, j) in yeast, but 13 subunits (a-m) in higher eukaryotes including plants (Browning et al., 2001). The principal role of eIF3 is to bind to the 40S subunit to participate in the formation of the 43S pre-initiation complex (PIC) comprised of the 40S subunit, and the ternary complex (eIF2•GTP•tRNA^{Met}), along with the factors eIF1, eIF5 and eIF1A. eIF3 acts as a bridge to facilitate binding of mRNA with its associated factors, eIF4F, eIF4A, eIF4B and Poly(A) binding protein (PABP), with the PIC, forming a 48S scanning complex. Recent cryo-EM and structural data for eIF3 suggest that it "hugs" the 40S ribosome with contacts that span the mRNA entry and exit sites (Hashem et al., 2013; Liu et al., 2014).

eIF3

The e**I**F3 complex shares "architectural" similarities to other large complexes, the 26S **p**roteasome lid and the **C**OP9/signalosome that are collectively known as **PCI** complexes (Pick et al., 2009). The eight proteins that form the octamer core of each of these complexes share motifs known as PCI and MPN domains. The characteristic composition of these PCI complexes are six subunits with PCI domains and two with MPN domains (Pick et al., 2009). Higher eukaryotic eIF3 subunits a, c, e, and l all have PCI domains, k and m have structurally related winged helix domains (Zhou et al., 2005), and f and h have MPN domains. The PCI/MPN containing subunits form the "octamer" core that is similar to that found in the proteasome lid (Querol-Audi et al., 2013). The remaining subunits, b, d and g have RNA Recognition Motif (RRM) domains (Cuchalova et al., 2010); subunits b and i have WD40 domains and eIF3g has a zinc-binding domain (Hinnebusch and Lorsch, 2012; Valasek, 2012; Voigts-Hoffmann et al., 2012; Hashem et al., 2013; Querol-Audi et al., 2013). Recent cryo-EM reconstructions of mammalian eIF3 and the 43S PIC indicate that eIF3 has five lobes and the PCI/MPN octamer forms the functional core of eIF3 (Siridechadilok et al., 2005; Khoshnevis et al., 2012; Hashem et al., 2013; Querol-Audi et al., 2013). The placement of the eIF3 subunits and their contacts with the ribosome and other initiation factors awaits further structural data, but a picture is beginning to emerge at the molecular level (Wilson and Doudna Cate, 2012; Hashem et al., 2013; Liu et al., 2014). Yeast eIF3 is also implicated in termination of protein synthesis, termination codon read-through and ribosome reinitiaton suggesting that we still have much to learn about this multi-functional factor and its roles during translation in all organisms (Pisarev et al., 2007; Beznosková et al., 2013).

Plant eIF3 (wheat and Arabidopsis) has been purified and its biochemical analysis suggests strong similarity both in number of subunits and sequence to mammalian eIF3 (Checkley et al., 1981; Lauer et al., 1985; Heufler et al., 1988; Burks et al., 2001). Subunits eIF3m and eIF3l were first described in plant eIF3 (Burks et al., 2001) and subsequently identified in mammalian eIF3. Biochemical and yeast-two hybrid analysis have implicated some of the Arabidopsis eIF3 subunits in association with the 26S proteasome and COP9 signalosome complexes or subunits (Karniol et al., 1998; Yahalom et al., 2001; Kim et al., 2004; Paz-Aviram et al., 2008). Both the 26S proteasome and COP9 signalosome play roles in protein turnover. These interactions with eIF3 or its subunits suggest that there may be additional unknown functions for some of the eIF3 subunits or some sort of communication between these large PCI complexes to coordinate various aspects of the cellular dramas of translation and protein degradation (Kim et al., 2001; von Arnim and Chamovitz, 2003).

eIF3 plays a pivotal role in initiation of translation via its interactions with numerous factors as well as the ribosome. It is therefore not surprising that only few Arabidopsis eIF3 subunit mutants have been reported. Five of the subunits for eIF3 (see Table 1) are encoded by a single gene in Arabidopsis (a, e, f, h, k) and eight are encoded by two genes (b, c, d, g, i, j, l, m). Mutations in the single genes for eIF3e or eIF3f cause male gametophytic lethality (Yahalom et al., 2008; Xia et al., 2010). Genotypes that only express *eIF3f* in pollen indicated that the absence of eIF3f is also embryo lethal (Xia et al., 2010). Partial loss of function alleles of the single *eIF3h* gene are viable but display multiple developmental defects, including reduced male gamete transmission. Interestingly, eIF3k appears to be non-essential under normal growth conditions (Tiruneh et al., 2013).

eIF3 and initiation/reinitiation

Several eIF3 subunits are implicated in translation of mRNAs with unusual 5' leader sequences. These include viral mRNAs and plant transcripts with that have one or more uORF upstream or overlapping with the main protein coding ORF (mORF) (See also, Reinitiation involving uORFs section).

Early insight into the nuanced roles *of* Arabidopsis eIF3 subunits came from the discovery of its function in the initiation of the 35S cauliflower mosaic virus (CaMV) mRNAs during infection. The 5' leader of this viral mRNA is long, highly structured and contains several short ORFs. A viral encoded protein called TAV (transactivation/viroplasmin) interacts with eIF3 through eIF3g to retain eIF3 on ribosomes and promote reinitiation at the initiation codon of the first long viral ORF (Park et al., 2001; Park et al., 2004). Another initiation factor, eIF4B is also involved in the TAV-mediated reinitiation process (see below). A host protein called RISP (reinitiation supporting protein) was described that supports reinitiation during CaMV infection and interacts with eIF3 through the eIF3a and eIF3c subunits (Thiebeauld et al., 2009). TAV binding to the TOR kinase was shown to be critical for the reinitiation event (Schepetilnikov et al., 2011). This suggests that eIF3 can be essential in reinitiation, in a manner exploited by CaMV and possibly other viruses and connects translation to the TOR signaling pathway in plants.

eIF3 is also important in translation of endogenous plant mRNAs that possess a uORF. Remarkably, over 30% of the protein coding mRNAs of Arabidopsis possess a 5' leader with one or more uORF. Of these, ~1% encode a peptide that is evolutionarily conserved among angiosperms (CPuORFs) (Jorgensen and Dorantes-Acosta, 2012). The presence of a uORF generally reduces the level of translation of the mORF, due to efficient initiation of translation of the uORF and limited initiation at the mORF. DNA microarray analysis of mRNA present on polysomes isolated from *eIF3h* mutants suggested that eIF3h is necessary for reinitiation on mRNAs with uORFs (Kim et al., 2004; Kim et al., 2007; Roy et al., 2010; Zhou et al., 2010a; Zhou et al., 2014a). Numerous studies document uORFs that regulate mORF translation in plants, including several that exert their regulation based on metabolite availability (Roy and von Arnim, 2013). uORF-containing mRNAs of Arabidopsis include those encoding the S class of bZIP transcription factors and Auxin Response Factors (ARFs) (Kim et al., 2004; Nishimura et al., 2005; Rahmani et al., 2009). Both eIF3h and the 60S ribosomal protein RPL24 are required for efficient reinitiation of translation of the *AtbZIP11 (ATB2*) and *ARF* mORF (Kim et al., 2004; Nishimura et al., 2005; Kim et al., 2007; Roy et al., 2010; Zhou et al., 2010a; Zhou et al., 2014a), although global analyses of polysomal RNA do not strongly support a role of RPL24 in this process (Tiruneh et al., 2013).

The reinitiation downstream of the uORFs of *ARF3* mRNA is mediated by auxin as well as TOR kinase (Schepetilnikov et al., 2013). Auxin triggers TOR activation, followed by its association with polysomes, where it phosphorylates ribosomal protein S6 kinase (i.e., AtS6K1) rendering it active to phosphorylate eIF3h, evidently after dissociation from the ribosome. This discovery makes a direct link to auxin-mediated signaling through plant TOR/S6K1 and the translational apparatus needed to reinitiate translation of ARF mRNAs possessing uORFs and provides unequivocal evidence that TOR contributes to gene-specific translational control in plants (Schepetilnikov et al., 2013).

Functional characteristics of other eIF3 subunits are emerging. The overexpression of *eIF3g* in wheat appears to enhance tolerance to drought and other abiotic stresses (Singh et al., 2007; Singh et al., 2013). Interestingly, the only monoclonal antibody to wheat eIF3 subunits that showed any inhibitory activity *in vitro* was to eIF3g and it inhibited mRNA binding to 40S ribosomes *in vitro* (Lauer et al., 1985; Heufler et al., 1988). This observation suggests that eIF3g facilitates mRNA binding to 40S ribosomes. Further, eIF3g was shown to be involved in reinitiation events required to translate GCN4 mRNA in yeast (Cuchalova et al., 2010) and in the reinitiation of CaMV in plants (Park et al., 2001; Park et al., 2004). It can be speculated that eIF3g has a role(s) in reinitiation events through direct interaction with the mRNA and ribosome, but more work will be needed to further establish the function(s) of the eIF3g and other eIF3 subunits during initiation and reinitiation events.

Many observations suggest that there is "cross-talk" between the PCI/MPN complexes in the ribosome-mediated synthesis and proteasome-mediated degradation of proteins. Since these complexes share structural similarity in many of their subunits (Pick et al., 2009), it will be illuminating to figure out the structural and regulatory role of these subunits/complexes and their interactions, from their opening acts in synthesis to the "death scene" in degradation of proteins.

Regulation of eIF3 through Phosphorylation

Several subunits of eIF3 are reportedly phosphorylated by highly specific kinases. As mentioned, this includes TOR-regulated phosphorylation of eIF3h by AtS6K1 (Schepetilnikov et al., 2013). eIF3i was identified as a target of brassinosteroid insensitive receptor kinase (BRI1) and was shown to co-immunoprecipitate with BRI1 (Jiang and Clouse, 2001; Ehsan et al., 2005). This suggests a connection between brassinosteroid signaling and eIF3 function, although the effect of the phosphorylation of eIF3i on eIF3 function is not yet known. The brassinosteroid signaling pathway has been proposed to have similarities to TGF-β signaling in mammals. The TGF-β kinase targets the eIF3i subunit, suggesting that there may be conserved signaling pathways and regulation between plants and animals, albeit repurposed in the individual phyla.

Additional pleiotropic kinases such as CK2 (Mulekar and Huq, 2013) have been shown to be active against several plant initiation factors (eIF2α, eIF2β, eIF5) *in vitro*, including multiple phosphorylation sites in eIF3c (Dennis and Browning, 2009). An *in vivo* phosphoproteome study of the light/dark response identified multiple subunits of AteIF3 (b, c, d) as phosphorylation targets (Boex-Fontvieille et al., 2013), including eIF3c sites that are comparable to those identified as CK2 substrates in wheat (Dennis and Browning, 2009).

Clearly there is complicated regulation of translation through multiple eIF3 subunit phosphorylation events and it will be necessary to identify the various kinases and their roles in eIF3 phosphorylation regulation at the molecular level in plants and how it compares with regulation in other organisms.

eIF4 Group

This group of factors interacts with the mRNA and facilitates its binding to the 43S PIC (Jackson et al., 2010; Valasek, 2012; Hinnebusch, 2014). Within this group are the cap-binding complexes, including eIF4F (all eukaryotes) and eIFiso4F (plant-specific). The individual subunits of these complexes are designated as the cap-binding proteins, eIF4E or eIFiso4E, and the large scaffolding proteins, eIF4G or eIFiso4G. Other members of this group are eIF4A, a DEAD box RNA helicase and eIF4B, a RNA binding protein. Both eIF4A and eIF4B are single polypeptides and interact with the large subunits of eIF4F and eIFiso4F. Another member of this group is eIF4H in mammals; however, a comparable factor has not been identified in yeast or plants. Although PABP is not an official member of this group, it will be considered here as it binds to the poly(A) tail of mRNA and interacts with other eIF4 group members during binding of the mRNA to the ribosome. Additional RNA helicases have been identified as having roles in initiation such as yeast DED1 or mammalian DHX29. These have not yet been formally designated as “eIFs” but eventually may be added to the “cast of characters” (Jackson et al., 2010). Plants have comparable RNA helicases (Bush et al., 2009), but their role in plant translation has not been elucidated and they may also have other specific roles in post-transcriptional processes. For example, the nuclear-localized AteIF4AIII was shown to function in nuclear pre-mRNA/mRNA movement during hypoxia (Koroleva et al., 2009a; Koroleva et al., 2009b) and the DEAD box helicase AtRH57 appears to be involved in rRNA processing in response to glucose and abscisic acid (Hsu et al., 2013).

eIF4A

This was the first RNA helicase to be identified and has been called “the godfather of helicases” (Rogers et al., 2002). A number of reviews on eIF4A and the DEAD/DEAH family of helicases summarize its role in the initiation of translation in mammalian and yeast systems (Webster et al., 1991; Parsyan et al., 2011; Andreou and Klostermeier, 2013; Linder and Fuller-Pace, 2013; Marintchev, 2013; Putnam and Jankowsky, 2013). Despite being the founding member of the DEAD box helicases, so named for a conserved amino acid motif (DEAD), eIF4A is the “outlier” in the family. It possesses a minimal helicase core but lacks additional accessory domains found in other helicases (Andreou and Klostermeier, 2013, 2014). eIF4As are highly conserved proteins, and based on sequence similarity plant eIF4A is likely to share structural and mechanistic details with eIF4A from yeast or mammals.

eIF4A is a non-processive bi-directional RNA dependent ATPase that functions locally to unwind short duplexes and lacks any specificity for RNA sequence (Marintchev, 2013). It has two RecA domains that in the presence of RNA and ATP come together to form the “closed” catalytically active conformation in a dumbbell-like shape (Meng et al., 2014). eIF4G and eIF4B binding to eIF4A favors the closed conformation and likely stimulates P_i release and/or nucleotide exchange from eIF4A (see eIF4G below, reviewed in Marintchev, 2013). It is thought that eIF4A complexed with eIF4G and eIF4B interacts with the 5’ end of the mRNA to relax secondary structure in an ATP-dependent manner in preparation for the binding of the 43S PIC. eIF4A further functions to remove secondary structures and/or RNA binding proteins during the scanning of the 5’ leader by the PIC (Parsyan et al., 2011; Marintchev, 2013; Andreou and Klostermeier, 2014).

Biochemical studies of wheat eIF4A suggest that it is similar to mammalian and yeast eIF4A (Lax et al., 1986; Abramson et al., 1988; Balasta et al., 1993; Bi et al., 2000). Studies of Arabidopsis eIF4A during the cell cycle led to the suggestion that proliferating cells display high canonical eIF4A association with the cap binding complex whereas quiescent cells may have other types of RNA helicases associated with the cap-binding complexes (Bush et al., 2009). Overexpression of pea (*Pisum sativum*) *eIF4A* (PDH45) resulted in increased resistance to salt stress in rice (*Oryza sativa*) and tobacco (*Nicotiana tobaccum*), suggesting a role in stress responses (Tajrishi et al., 2011; Sahoo et al., 2012). A T-DNA insertion in one of the two *eIF4A* gene paralogs of *Brachypodium distachyon* resulted in a slow-growing, dwarf phenotype that could be partly reversed by heterologous expression of Arabidopsis *eIF4A-1* (Vain, et al, 2011). This phenotype is similar to a T-DNA insertion mutant in Arabidopsis *eIF4A-1* (Vain et al., 2011), one of the three genes encoding this protein (Table 1).

Phosphorylation of eIF4A

There is proteomic evidence that cytoplasmic eIF4A1/2 of Arabidopsis associates with the cyclin dependent kinase CDKA; however, an effect on eIF4A function by CDKA has not been demonstrated (Hutchins et al., 2004). eIF4A is rapidly phosphorylated in response to hypoxia in maize (*Zea mays*), but the relevant kinase or sites were not identified (Webster et al., 1991). Wheat eIF4A was also shown to be phosphorylated at an apparent single site in response to heat shock (Gallie et al., 1997). The recent phosphoproteomic analysis of the light/dark transition shows that *AteIF4A1*, *2* and *3* gene products are phosphorylated (Boex-Fontvieille et al., 2013).

eIF4B

This is the only initiation factor that lacks a high degree of sequence similarity between yeast, mammals and plants. It is largely accepted that eIF4B functions as a RNA binding protein and enhances the helicase activity of eIF4A, presumptively by augmenting both ATP and RNA binding (Hinnebusch and Lorsch, 2012). In yeast, eIF4B binds eIF4G and induces a conformational change that in turn promotes the binding of eIF4A and increases its RNA helicase activity (Park et al., 2012). Yeast eIF4B has also been shown to bind to the 40S ribosomal protein RPS20 near the mRNA entry site, which may facilitate interaction of eIF4A (Walker et al., 2012; Zhou et al., 2014b) and recent data suggest a mechanism in yeast for eIF4B to promote association of eIF4A with eIF4F (Park et al., 2012). eIF4B is not an essential gene in yeast, but its deletion produces a cold sensitive phenotype (Altmann et al., 1993).

Extensive biochemical and kinetic characterization of the interactions of wheat eIF4B, eIF4A, eIF4G/eIFiso4G and PABP confirm that the interactions are similar to other organisms (reviewed in Gallie, 2014; Le et al., 1997; Bi and Goss, 2000; Bi et al., 2000; Khan and Goss, 2005; Cheng and Gallie, 2006, 2007; Cheng et al., 2008; Khan et al., 2008; Khan et al., 2009; Mayberry et al., 2009; Cheng and Gallie, 2010; Yumak et al., 2010; Khan and Goss, 2012; Cheng and Gallie, 2013). Wheat eIF4B has two tandem domains for interaction with eIF4A and PABP separated by a RNA binding domain in addition to binding domains for eIF4G/iso4G and eIF3g. These tandem binding domains are the most highly conserved parts of plant eIF4B. This suggests that eIF4B may interact with more than one molecule of eIF4A or PABP at a time during initiation events. Further, the interactions with eIF4G/iso4G and eIF4A appear to be specific to plant eIF4B, suggesting divergent evolution of this factor from other eukaryotes (Gallie, 2014).

eIF4B also assists eIF3 in CaMV infection and the TAV-mediated reinitiation on the 35S transcript. Specifically, eIF4B interacts with eIF3g to form a stable 43S PIC in plant cells. Upon binding of the 60S ribosomal subunit at the final step of initiation, eIF4B and eIF3 are released and therefore not found in polysomes of cells not infected by CaMV. However, TAV keeps eIF3 associated with the ribosome, as eIF3 is found in polysomes of infected cells (Park et al., 2004). The overexpression of eIF4B prevents association of TAV with translating ribosomes by competing with TAV for binding to eIF3g (Park et al., 2004). These exploitations of the translation system by a virus suggest that the role of eIF4B may not be solely in stimulation of the helicase activity of eIF4A and eIF4F on mRNA.

Arabidopsis has two forms of eIF4B (eIF4B1 and eIF4B2), which similarly support *in vitro* translation (Mayberry et al., 2009). Interestingly, a heterozygous Arabidopsis T-DNA activation tagging line that showed necrotic lesions symptomatic of programmed cell death overexpresses *eIF4B2* and lines homozygous for a disruption of this gene were embryo lethal (Gaussand et al., 2011). Ectopic overexpression of *eIF4B2* recapitulated the necrotic phenotype, leading to the conclusion that too much eIF4B can cause disruptions in gene expression that trigger programmed cell death. These data suggest a fundamental role for eIF4B in the process of initiation in plants (Gaussand et al., 2011).

Phosphorylation of eIF4B

Plant eIF4B is a target of phosphorylation by CK2 and possibly additional kinases (Gallie et al., 1997; Dennis and Browning, 2009). Arabidopsis eIF4B isoforms show differential responses to the isoforms of CK2 (Dennis and Browning, 2009) suggesting that eIF4B activity could potentially be modulated by distinct CK2s. Support for eIF4B phosphorylation was found in the light to dark phosphoproteome (Boex-Fontvieille et al., 2013) and wheat eIF4B was shown to respond to heat shock by dephosphorylation at multiple sites (Gallie et al., 1997). The effect of phosphorylation of plant eIF4B on its various activities needs further study in light of the recent work showing that phosphorylation of mammalian eIF4B and eIF4G influence the formation of an eIF4F/eIF4A/eIF4B complex and stimulate interaction with eIF3 and the 43S PIC (Dobrikov et al., 2012).

The divergence in eIF4B protein sequences between kingdoms suggest that there may be wide latitude in evolutionary constraints and unique mechanisms for regulation for this factor by phosphorylation (Hernández et al., 2010) leaving much to explore for the role of this “nonconserved” factor.

eIF4F and eIFiso4F

The role of eIF4F is to bind to the “cap” on the 5’ end of the mRNA. This is a guanine residue methylated in the 7 position and attached to the first residue of the mRNA through a 5’ to 5’ linkage (m^7GpppX). This reverse linkage helps to protect the mRNA, as the decapping of mRNA is one of the first enzymatically-driven steps in a major mRNA degradation pathway (Li and Kiledjian, 2010; Milac et al., 2014) and likely plays roles in plant gene expression during stress and other developmental pathways (Zhang et al., 2013). The m^7G structure is recognized by the cap-binding protein eIF4E. eIF4E binds to the scaffold protein eIF4G to form the two-subunit complex called eIF4F, which is conserved in higher eukaryotes. eIF4G helps to assemble eIF4A, eIF4B and PABP on the mRNA to prepare it for binding to the 43S PIC (via interaction of eIF3 with eIF4G and eIF4B), in prepararion for scanning of the 5’ leader for an initiation codon (see below).

One distinction between the plant translational apparatus and that of other eukaryotes is the presence of a second cap-binding protein complex that differs from the canonical eIF4F. This two-protein complex, eIFiso4F, is comprised of eIFiso4G and eIFiso4E (Allen et al., 1992; van Heerden and Browning, 1994; Patrick and Browning, 2012). The cap binding proteins, eIF4E and eIFiso4E, have ~50% amino acid sequence similarity and form distinct and specific complexes with their respective binding partners, eIF4G and eIFiso4G (Mayberry et al., 2011). However, in the absence of the correct binding partner, mixed complexes will form that function in translation *in vitro* (Mayberry et al., 2011).

The larger scaffold subunits (eIF4G and eIFiso4G) share similarity in the C-terminal half with the eIF4E binding site and two HEAT (**H**untington, **e**longation factor 3, protein phosphatase 2**A**, and the yeast **T**OR1 kinase) domains; however, the N-terminal half of eIF4G is absent in eIFiso4G (Patrick and Browning, 2012). eIFiso4F appears to be necessary for proper growth and development of Arabidopsis, as deletion of both *eIFiso4G* genes results in slow and stunted growth, pale green rosettes and significant reproductive defects (Lellis et al., 2010). Based on phylogenetic analyses, eIFiso4G appeared in basal plant lineages before eIFiso4E and most likely formed a complex with eIF4E, whereas eIFiso4E emerged around the time flowering plants evolved (Patrick and Browning, 2012).

The role of the eIF4 factors is to bind the mRNA and prepare it for association with the 43S PIC. The current model for this process has the 5' cap binding to eIF4F through the eIF4E subunit. eIF4G (or eIFiso4G) serves as the scaffold for assembly of eIF4A, eIF4B and PABP (presumably binding the poly(A) tail and circularizing the mRNA at some time) as well as an interaction site for eIF3 (Jackson et al., 2010; Hinnebusch and Lorsch, 2012; Valasek, 2012). Although a structure for an eIF4F complex has not been determined, there are structures for portions of eIF4G in complex with eIF4E or eIF4A and considerable domain mapping for eIF4G from several organisms (Marintchev and Wagner, 2005; Marintchev et al., 2009; Dobrikov et al., 2012; Park et al., 2012), including plant eIF4G and eIFiso4G (Cheng and Gallie, 2010; Cheng and Gallie, 2013).

eIF4G and eIFiso4G

eIF4Gs have one to three HEAT domains depending upon the organism: yeast has one, plants have two, and mammals have three suggesting considerable evolution of the machinery (Hernández and Vazquez-Pianzola, 2005; Hernández et al., 2010; Hernández et al., 2012). HEAT domains are comprised of a series of alpha helices that form a coiled solenoid-like structure and provide surfaces for interaction with proteins and mRNA (Marintchev and Wagner, 2005; Valasek, 2012). Among these interactions are binding to eIF4A, eIF4B, eIF3 and PABP, which all function to bring the mRNA to the 40S subunit to initiate the scanning process (Hinnebusch and Lorsch, 2012).

The two eIF4A Rec A (N-terminal, C-terminal) domains interact with the middle section of the yeast HEAT domain or with the HEAT-1 and HEAT-2 domains of mammalian eIF4G (Marintchev et al., 2009; Hilbert et al., 2011). eIF4G promotes the transition from an "open" to a "closed" form of eIF4A based on crystal structures (Schütz et al., 2008; Marintchev et al., 2009). The model in yeast has been further refined by monitoring conformational changes in solution to include a "half-closed" intermediate for eIF4A that is stabilized upon binding of eIF4G (Hilbert et al., 2011). The closed state conformation of eIF4A is stimulated by ATP and RNA binding. It is also proposed that the closed form has a groove between the two interfaces of eIF4A and eIF4G that make the nucleotide binding site of eIF4A more accessible to ATP (Hilbert et al., 2011). The oscillations between the half-closed and closed forms may drive the release of ADP/P_i and rebinding of ATP to maintain mRNA binding affinity during scanning (Hilbert et al., 2011).

Plant eIFiso4G and eIF4G domains have been mapped and shown to have similar types of interactions with eIF4A, eIF4B and PAPB, but have some differences from the mammalian or yeast interactions (Gallie, 2014). Further structural details will be required to understand how similar or different these plant factor interactions are to other eukaryotes.

An alternative to the current "script" of mRNA binding and unwinding by associated factors prior to binding to the 43S PIC, is that the eIF4 factors assemble on the 43S PIC and then recruit and unwind the mRNA, feeding it directly into the scanning complex (Hinnebusch and Lorsch, 2012). This model has many elements that explain some of the biochemical data but will require further testing not only in yeast but also in mammals and plants to determine if this is an accurate depiction of the scene within the cell.

"4F or, not 4F, that is the question"

The presence of two eIF4F complexes in plants raises the question of whether they have distinct biological activities. eIF4F promotes translation of reporter mRNAs with more secondary structure better than eIFiso4F in a cell-free translation system derived from wheat germ (Gallie and Browning, 2001). In addition, cellular mRNAs were shown to have varying levels of dependence upon eIF4F or eIFiso4F, as well as eIF4B, for optimal translation (Mayberry et al., 2009). These results suggest that plant eIFiso4F and eIF4F may have evolved selective abilities to promote or otherwise regulate translation of specific mRNA populations. What advantage distinct eIF4Fs provide to plants is not yet clear, but they may have pleiotropic roles in the synthesis of proteins involved in plant-specific functions, such as photosynthesis, cellulose biosynthesis, *etc.*. eIFiso4F was implicated in the specific regulation of translation of an enzyme involved in the synthesis of chlorophyll (Chen et al., 2014) which fits with the observed pale green phenotype of Arabidopsis plants lacking eIFiso4G (Lellis et al., 2010).

Ultimately, structures of plant eIF4F or eIFiso4F in association with the 43S PIC will be needed to determine if there are functional differences in assembly of the initiation complexes in plants as compared to yeast and mammalian systems.

The cast of cap-binding proteins: eIF4E, eIFiso4E and 4EHP

All higher plants have three forms of the cap-binding proteins, eIF4E, eIFiso4E (plant-specific) and 4EHP (4E homologous protein) which all bind to m^7GTP-Sepharose (Ruud et al., 1998; Patrick et al., 2014; Kropiwnicka et al., 2015). eIF4E and eIFiso4E are both class 1 cap-binding proteins (Joshi et al., 2005) and form the eIF4F and eIFiso4F complexes with their respective subunits, eIF4G and eIFiso4G (Mayberry et al., 2011; Patrick and Browning, 2012). 4EHP is termed a class 2 cap-binding protein (Joshi et al., 2005) and does not appear to function in canonical translation but has been implicated in regulatory roles for mRNAs during animal development in association with proteins that are not considered part of the canonical translational apparatus (Rom et al., 1998; Rhoads, 2009; Morita et al., 2012). The role of plant 4EHP (previously termed nCBP in plants for "novel" cap-binding protein) is unknown, although it has shown modest ability to stimulate translation with wheat eIFiso4G and appears to bind m^7GTP more tightly than other cap-binding proteins (Ruud et al., 1998). There is more to learn about this unusual cap-binding protein and its role(s) in various cellular processes as capped non-coding and small RNAs are discovered and their functions elucidated.

It is also worth mentioning that there is a nuclear cap-binding protein complex (CBP20/CBP80) that shares structural and ancestral similarity to the eIF4E/eIFiso4E cap-binding proteins (CBP20) and eIF4G/eIFiso4G large subunits (CBP80). This complex does not function directly in translation but is important in pre-mRNA splicing, miRNA processing and export of mRNA in plants (Hugouvieux et al., 2001; Papp et al., 2004; Marintchev and Wagner, 2005; Topisirovic et al., 2011; Rogers and Chen, 2013; Gonatopoulos-Pournatzis and Cowling, 2014). There are many unanswered questions about the timing and location of exchange of CPB20 and eIF4E/eIFiso4E from the cap of mRNAs as it transitions from the nucleus to the cytoplasm.

The canonical type 1 plant cap-binding proteins, eIF4E and eIFiso4E, form tight complexes with their respective eIF4G subunits at the nanomolar to sub-nanomolar level (Mayberry et al., 2011). It is unlikely that these complexes readily dissociate given the tight binding affinity. They also do not appear to be regulated by any of the pathways associated with mammalian eIF4E that require dissociation/reassociation (see 4E Binding Proteins below). The crystal structure of wheat eIF4E shows similarities to both mammalian and yeast eIF4E, but revealed an intermolecular disulfide bridge between two plant-specific cysteine residues (Cys-113 and Cys151) that form under oxidizing conditions (Monzingo et al., 2007). This leads to the hypothesis that eIF4E could function as a redox sensor at the level of translational initiation. A constitutively reduced mutant (C113S) and oxidized forms of eIF4E bound m^{7}GTP with a modest 1.5x difference in k_{off} values in NMR (nuclear magnetic resonance) solution studies (Monzingo et al., 2007). Further studies using mass spectrometry and a lysine-specific chemical probe indicated structural changes occurred upon altering the redox state and support the hypothesis of a redox sensor or switch for eIF4E (O'Brien et al., 2013). It remains a tantalizing possibility that the oxidation state of eIF4E (and/or eIFiso4E) may modulate cap binding in a redox-sensing manner in plants during retrograde signaling from the chloroplast to nucleus or other processes that could regulate the level of translation in response to the redox state of the cell.

Arabidopsis thaliana has three genes for the class 1 eIF4E (*eIF4E, eIF4E1b, eIF4E1c*) and one gene for eIFiso4E (Patrick and Browning, 2012; Patrick et al., 2014). *eIF4E* is the most highly expressed; the other two eIF4E genes (*eIF4E1b, eIF4E1c*) correspond to a tandem duplication event within the *Brassicaceae* with evidence that only *eIF4E1b* generates a transcript (Patrick and Browning, 2012) (see Table 1 for accession numbers). A number of forms of eIF4E have evolved in other organisms with special functions such as recognition of tri-methylated caps or tissue-specific regulation; however, some of these proteins have lost either their ability to bind m^{7}G or eIF4G poising them as potential inhibitors or repressors of initiation (Joshi et al., 2005; Rhoads, 2009). eIF4E1b and eIF4E1c from Arabidopsis were found to bind eIF4G and to m^{7}G-affinity resin and thus function biochemically *in vitro* as canonical cap-binding proteins (Patrick et al., 2014; Kropiwnicka et al., 2015). However, the double mutant *eIF4E eIFiso4E* is lethal suggesting that eIF4E1b and eIF4E1c are not able to replace eIF4E *in vivo* (Callot and Gallois, 2014; Patrick et al., 2014).

Several laboratories made the simultaneous discovery that mutations in the genes encoding eIFiso4E or eIF4E confer naturally occurring virus resistance and prevent viral replication (Jiang and Laliberte, 2011; Wang and Krishnaswamy, 2012). Plants and many of their RNA viruses have co-evolved and a significant portion of this evolution appears to center on the use of eIF4F and eIFiso4F subunits by viruses for replication. Interestingly, neither Arabidopsis *eIF4E1b* nor *eIF4E1c* are recognized as virus resistance genes in contrast to dozens of examples for *eIF4E* and *eIFiso4E* (Robaglia and Caranta, 2006; Charron et al., 2008; Moury et al., 2013). Likely, other plants have multiple *eIF4E*/*eIFiso4E* genes, some of which may have evolutionary advantages for specific functions during stress or development.

The viral 5' linked protein (VPg) of potyviruses was found to interact directly with eIF4E or eIFiso4E, as well as other translation factors (eIF4G, eIFiso4G, PABP, eIF4A, eEF1A) (Jiang and Laliberte, 2011; Wang and Krishnaswamy, 2012). A number of mutations in subunits of cap-binding complexes interfere with virus reproduction, yet these mutations do not appear to compromise host protein synthesis. This suggests that it is not the protein synthesis activity *per se* that confers virus resistance, but some other aspect that has yet to be discovered. There is interest in using these genes to engineer better virus resistance for economically important crops (Wang and Krishnaswamy, 2012; Moury et al., 2013; Kim et al., 2014a) and it is likely that virus/host co-evolution has shaped the roles of these initiation factors in plants (Moury et al., 2013).

Positive strand plant viral RNAs recruit eIF4F and/or eIFiso4F using structural elements in the 3' UTRs termed 3' cap-independent translation enhancers (3'CITES) (reviewed in Simon and Miller, 2013). These varied structural RNA elements appear to function as "cap substitutes" through direct interaction with eIF4F or eIFiso4F (or both). Plant viral 3'CITES are unlike the internal ribosome entry site (IRES) elements associated with animal and insect viruses that use internal initiation as their hallmark and vary in their initiation factor requirement (Komar et al., 2012; Jackson, 2013). Plant viral 3'CITES typically make use of RNA-RNA interactions that base pair a portion of the 3'CITE with a 5' UTR loop ("kissing loop") in a manner reminiscent of the 5' to 3' interactions of the canonical initiation process involving the 5' cap and 3' poly(A) tail (Simon and Miller, 2013). Additionally, some plant viral RNA 3' UTRs use molecular mimicry by folding into structures that resemble tRNAs recruiting ribosomes directly (Simon and Miller, 2013). There is much to be learned from these interesting structures and the co-evolution of viruses and eIF4F/eIFiso4E host proteins. Since viruses are adept at co-opting host systems and using them to their advantage, it is likely that at least some host mRNAs may have elements similar to a 3'CITE.

eIF4E-Binding Proteins: Are they actors on the plant stage?

A major form of regulation of mammalian translation is through 4E binding proteins (4EBPs) that are regulated via phosphorylation by mammalian TOR (mTOR) in the PI3K-Akt signaling pathway that responds to many types of stress and environmental cues (Carrera, 2004; Richter and Sonenberg, 2005; Hernández et al., 2010). Phosphorylated mammalian 4EBPs dissociate from eIF4E, allowing it to interact with eIF4G to form a functional complex, whereas unphosphorylated 4EBPs bind to eIF4E and sequester it from interaction with eIF4G, thereby limiting eIF4F/

cap-dependent initiation (Carrera, 2004; Richter and Sonenberg, 2005). Plants have a functional TOR system that senses metabolic states (Ren et al., 2012; Robaglia et al., 2012; Xiong and Sheen, 2012; Caldana et al., 2013; Dobrenel et al., 2013; Xiong et al., 2013; Xiong and Sheen, 2013, 2014), but appear to lack 4EBPs that regulate eIF4F complex formation (Verma and Chatterjee, 2009). Given the sub/nanomolar binding of the plant eIF4F and eIFiso4F subunits to each other, it seems unlikely that these complexes will dissociate once formed (Mayberry et al., 2011). Plants have proteins with canonical eIF4E binding sites that have been shown to bind to eIF4E or eIFiso4E, such as lipoxygenase 2 and BTF3 (beta subunit of the nascent polypeptide-associated complex (Freire et al., 2000; Freire, 2005); however, the role(s) of these protein interactions with plant cap-binding proteins is still unclear.

Phosphorylation of eIF4G/iso4G and eIF4E/iso4E

Although there are reports of multiple isoelectric states of wheat eIF4F/iso4F subunits, the effect on activity and the kinases involved have not been identified (Gallie et al., 1997). Neither eIF4F nor eIFiso4F subunits were targets of CK2, unlike eIF4B (Dennis and Browning, 2009). Only eIF4G was found to be phosphorylated in the phosphoproteome of the light to dark transition (Boex-Fontvieille et al., 2013). Given the importance of phosphorylation of eIF4E, 4E-BP and eIF4G in mammalian systems, it remains to be discovered if plants have evolved a different system for regulation of these subunits through phosphorylation, redox-sensitive structure regulation or other means.

Poly(A) Binding Protein

Although not an "official" initiation factor, PABP binds to the 3' poly(A) tail of the mRNA and interacts with eIF4G and eIF4B, suggesting that the mRNA may be circularized at least transiently during initiation (Park et al., 2011). In mammals, PABPs have extensive roles in the nucleus and cytoplasm in mRNA processing, translation and degradation, as well as a role in miRNA-mediated processes (reviewed in Goss and Kleiman, 2013). Higher eukaryotes have multiple genes for PABP. In the case of *X. laevis, PABP* gene products have been shown to function similarly in translation, but are distinctly required for development indicating there may be mRNAs whose processing, expression or degradation requires a specific PABP (Gorgoni et al., 2011).

Plants have an extensive family of genes encoding PABP with considerable protein sequence diversity. The eight *PAB* genes in *A. thaliana* fall into four phylogenetic sub-groups with varying tissue specific expression (Le and Gallie, 2000; Belostotsky, 2003). In general, Arabidopsis and other plant PABPs have four RRM domains that consist of two α-helices and four anti-parallel β-sheets. A separately folded C-terminal domain called PABC is composed of 4 to 5 α-helices and contains a **PA**BC interaction **m**otif (PAM2) for protein-protein interaction. The solution structure of wheat PABC has a similar fold to the mammalian PABC domain and also contains the PABC-Interacting Motif (PAM-2) protein interaction domain (Siddiqui et al., 2007). *A. thaliana* PABC/PAM2 was shown to have multiple binding partners, several of which interfere with *in vitro* translation or are implicated in RNA metabolism (Wang and Grumet, 2004; Bravo et al., 2005; Siddiqui et al., 2007). Some plant PABPs have been reported to have two instead of four RRMs, suggesting specifically evolved functions for these proteins (Belostotsky, 2003). It is also well established that there can be multiple PABPs bound to the poly(A) tail of the mRNA at any given time, thus leading to a diversity of possible PABP molecules on one transcript, each perhaps recruiting different binding partners via the PAM2 interface. Adding further complexity is post-translational modification by phosphorylation, which also affects PABP's interactions with binding partners (Le et al., 2000).

Extensive biochemical analysis of wheat PABP has shown that the presence of eIF4G or eIF4B enhances its RNA binding activity and in turn the presence of PABP increases the affinity of the eIF4F complex for the cap and stimulates the ATPase and RNA helicase activities of eIF4A, eIF4F/eIFiso4F and eIF4B (reviewed in Gallie, 2014; Le et al., 1997; Wei et al., 1998; Bi and Goss, 2000; Luo and Goss, 2001; Khan and Goss, 2005; Cheng and Gallie, 2010). It has been further shown that plant eIF4G has an additional PABP binding domain that binds eIF4B in a competitive and mutually exclusive manner (Cheng and Gallie, 2010; Cheng and Gallie, 2013). This second domain is absent in mammals and yeast PABP. PABP is also implicated in viral replication (Smith and Gray, 2010) and plant PABP was shown to interact with the reverse transcriptase of turnip mosaic virus (TuMV) (Dufresne et al., 2008) and with the 3'UTR of tobacco etch virus (TEV) to promote internal initiation (Khan et al., 2008; Khan et al., 2009; Yumak et al., 2010; Iwakawa et al., 2012).

There are still many questions about the role of PABP in initiation, its interactions with various initiation factors and other proteins, such as the PAM2-domain containing Early Responsive to Dehydration 15 (ERD15) family members (Aalto et al., 2012). PABP function likely extends beyond initiation. For example, an Arabidopsis *pab2 pab8* loss-of-function mutant maintained translation of late-embryogenesis mRNAs in young seedlings, leading to the suggestion that PABP contributes to turnover of abundant seed transcripts during early seedling development (Tiruneh et al., 2013).

eIF1 Group

This group is involved in the stimulation and assembly of the 43S PIC and includes eIF1 (called SUI1 in yeast) and eIF1A (formerly known as eIF4C). Both of these small factors (~12-17 kDa) are single polypeptides and conserved across all eukaryotes. eIF1 has structural similarity to the initiation factor (IF)3 C-terminal domain in prokaryotes and eIF1A is the functional equivalent to prokaryotic IF1 (Valasek, 2012).

eIF1 and eIF1A

eIF1 binds near the peptidyl (P)-site of the 40S subunit and precludes Met-tRNA$_i^{Met}$ bound to eIF2•GTP (the ternary complex) from being fully engaged in the peptidyl (P)-site of the 43S PIC until the initiation codon is accurately identified (Nanda et al., 2013; Martin-Marcos et al., 2014). eIF1A has an interesting structure with a folded central region that binds in the acceptor (A)-site of the 40S subunit; however, its N- and C terminal tails are unstructured and extend into the P-site. Similar to eIF1, eIF1A participates in preventing full P-site engagement of the Met-tRNA$_i^{Met}$ until the correct initiation codon is identified (Nanda et al., 2013).

Recombinant wheat eIF1 was shown to function in formation of a multifactor complex (MFC) *in vitro* similar to that found in yeast and mammals (Asano et al., 2000; Dennis et al., 2009; Hinnebusch and Lorsch, 2012; Sokabe et al., 2012; Hinnebusch, 2014). The MFC, consisting of eIF1, eIF2, eIF3 and eIF5, presumably helps to organize these factors prior to binding to the 43S PIC. It also appears to stabilize binding of the ternary complex to the 40S subunit. Plant eIF1 interacts directly with eIF5 and the N-terminal domain of eIF3c (Dennis et al., 2009). On the other hand, eIF1A binds to the 40S subunit independently of the MFC. eIF1A purified from wheat germ substitutes biochemically for rabbit reticulocyte eIF1A suggesting a highly conserved function (Timmer et al., 1993). Overexpression of *eIF1* and *eIF1A* have been reported to improve salt tolerance in plants (Rausell et al., 2003; Latha et al., 2004; Diedhiou et al., 2008; Sun and Hong, 2013), suggesting a role in stress acclimation.

eIF5 Group

Two members (eIF5, eIF5B) of this group function in the selection of the start site and engagement of codon-anticodon base pairing, whereas the third (eIF5A), functions in elongation. All three group members, eIF5, eIF5A and eIF5B are found in plants and other eukaryotes, suggesting ancient origin and conserved functions. eIF5 has GTPase activating protein function. eIF5B resembles prokaryotic IF2, but does not bind Met-tRNA$_i^{Met}$ and functions to promote binding of the 60S subunit (subunit joining) which in turn promotes GTP hydrolysis and release of eIF5B as the last step of the initiation process. eIF5A (nee eIF4D), although initially designated an initiation factor, is the “imposter” in the group and promotes the peptidyl transferase reactions of poly-prolyl residues during elongation (Gutierrez et al., 2013) Given the role for eIF5A in elongation, it has been proposed to rename this factor eEF5 (Dever et al., 2014) (See section on Elongation Factors and Table 2.

eIF5

This protein promotes hydrolysis of GTP bound to the ternary complex during start site recognition (Jennings and Pavitt, 2010; Aitken and Lorsch, 2012; Hinnebusch and Lorsch, 2012; Valasek, 2012; Nanda et al., 2013; Hinnebusch, 2014). eIF5 has two functional domains, N-terminal (NTD) and C-terminal (CTD) with a linker connection (Conte et al., 2006; Wei et al., 2006). An “arginine finger” (Arg-15) required for GTPase activity is positioned near the N-terminus in an unstructured region. This unstructured region is free to interact with the GTP-binding region of eIF2γ to promote GTP hydrolysis. In addition, the NTD has structural similarity to eIF1, which may play a role in events during initiation codon selection (Nanda et al., 2013). The eIF5 CTD contains a HEAT domain that interacts with eIF1, the NTD of eIF3c, and N-terminal tail of eIF2β to stabilize the MFC mentioned earlier that is formed by these factors (Nanda et al., 2013). In addition, the CTD of yeast eIF5 interacts with an unstructured region of eIF4G that is proposed to promote binding of the mRNA to the 43S PIC and assist in scanning and subsequent release of eIF1 (Singh et al., 2012). Whether this occurs with mammalian or plant eIF4G is not known. eIF5 has been shown to be released from mammalian PIC with eIF2•GDP and appears to have a role as GDP dissociation inhibitor during recycling of eIF2•GDP by eIF2B (Jennings et al., 2013). eIF5 from plants has received little attention, except studies that show that the wheat factor functions in the formation of a MFC in a manner enhanced by phosphorylation of members of the MFC by CK2 (Dennis et al., 2009). One of the *in vitro* CK2 sites of eIF5 was confirmed in the light/dark phosphoproteome (Boex-Fontvieille et al., 2013).

eIF5B

As the structural homolog of eubacterial IF2, eIF5B carries out a similar function in eukaryotes (Allen and Frank, 2007). Upon recognition of the initiation codon, a series of events including the release of eIF1 from its position near the P-site and hydrolysis of the GTP bound to ternary complex, leads to conformation changes in eIF2 (Allen and Frank, 2007). At this point eIF5B•GTP binds to the complex via contacts with the C-terminal tail of eIF1A and may stabilize the binding of the Met-tRNA$_i^{Met}$ in the P-site (Pisareva and Pisarev, 2014). The binding of eIF5B•GTP likely displaces many of the associated factors (eIF2, eIF3 and eIF5) to open a surface for 60S subunit attachment. Hydrolysis of eIF5B•GTP is promoted by the GTPase activating activity of the 60S subunit, triggering release of eIF5B•GDP and eIF1A as the newly formed 80S complex is established (Allen and Frank, 2007). Mutations in mammalian eIF5B show this factor may play multiple roles during initiation *in vitro* (Pisareva and Pisarev, 2014). Pea eIF5B has been biochemically characterized as a heat stable protein with potential properties of a chaperone and binds to GTP as expected (Rasheedi et al., 2010; Suragani et al., 2011).

eIF6 Group

Designated as an initiation factor and the sole performer of this group, eIF6 interacts with the 60S subunit and functions in the prevention of premature association of the 60S ribosomal subunit with the 43S PIC. It also has a role in the assembly of the 40S and 60S ribosomal subunits (Miluzio et al., 2009; Brina et al., 2011). eIF6 was first discovered in wheat germ as a ribosome disassociation factor that bound 60S ribosomes (Russell and Spremulli, 1978, 1979, 1980) and subsequently identified in yeast (Si et al., 1997) and mammals (Valenzuela et al., 1982; Raychaudhuri et al., 1984).

In sequenced plant genomes, eIF6 is typically encoded by multiple genes; in the case of Arabidopsis a single eIF6 gene (*AteIF6A*) is broadly expressed and a second displays more regional and regulated transcript accumulation (*AteIF6B*) (Guo et al., 2011a). The role of eIF6 in subunit joining involves its interaction with the conserved ribosome-associated protein receptor of activated C kinase 1 (RACK1) (see section below on RACK1).

Table 2. Elongation and Termination Factors of *Arabidopsis*

Factor	M_r[a]	Function	Arabidopsis Gene[h]
eEF1A (EF-1α[b])	52,000	Bind aminoacyl-tRNA and GTP	At1g07920, At1g07930, At1g07930, At1g07940, At5g60390
eEF1B		Recycle eEF1A·GDP	
α[c]	24,000		At5g12110, At5g19510
β[d]	28,000		At1g30230, At2g18110
γ	46,000		At1g09640, At1g57720
eEF2[e]	92,000	Translocation	At1g56070, At3g12915
eEF5[f] (eIF4D, eIF5A)[b]	17,200	Elongation of poly-proline/glycine regions	AT1g26630, AT1g69410, AT1g13950
eRF1	49,000	Termination/peptide release	At5g47880, At1g12920, At3g26618
eRF3	60,500	Termination/peptide release	At1g18070
ABCE1/RLI1[g]	68,000	Ribosome recycling	At3G13640, At4g19210[g]

[a] Approximate molecular weight based on TAIR9 data.
[b] Prior nomenclature used in literature.
[c] The old *Artemia* and mammalian designation was EF-1δ The old plant designation was EF-1β.
[d] The old *Artemia* and mammalian designation was EF-1β. The old plant designation was EF-1β'.
[e] A conserved histidine residue is post-translationally modified to a diphthamide.
[f] Originally designated eIF4D and later eIF5A; now known to participate in elongation and renamed eEF5; contains the unique amino acid hypusine.
[g] We recommend At3G13640 be called *ABCE1A* and At4g19210 be called *ABCE1B* rather than *ABCE1* and *ABCE2*, respectively.
[h] Links to various data bases using the Arabidopsis Gene Identifier can be found at http://browning.cm.utexas.edu/arabidopsis/fiat

eIF6 also functions in ribosome biogenesis and the transport of ribosomal subunits from the nucleolus to the cytoplasm in a process that requires phosphorylation of a CK1 site that is conserved in *AteIF6A*, but lost in *AteIF6B* mutants (Guo et al., 2011a).

The Ribosome: the *Prima Donna*

The peptidyl transferase reaction is catalyzed by the ribosome, a highly evolutionarily conserved macromolecular complex of two subunits that is comprised of RNA and proteins (Yusupova and Yusupov, 2014). Without exception, the role of the small subunit of the ribosome is to decode the mRNA whereas the large subunit catalyzes the peptidyl transferase reaction (peptide bond formation). Decoding involves the A-, P- and exit (E)-site positions transiently occupied by tRNAs as they bring amino acids to transfer to the growing polypeptide chain and exit empty to be recharged.

Subunit composition

Ribosomes, their subunits, and rRNAs are measured in Svedberg (S) units corresponding to their sedimentation coefficient measured by ultracentrifugation. When joined together, cytosolic ribosomes of higher eukaryotes (including plant 40S and 60S subunits) sediment at 80S. The 40S subunit is formed with 18S rRNA and small ribosomal proteins (RPSs) and the 60S by the 5S, 5.8S, and 25-28S rRNAs and large ribosomal proteins (RPLs). The subunits of lower eukaryotes have the same four rRNA molecules and are therefore slightly smaller. The ribosomes of bacteria, mitochondria and plastids are significantly smaller, typically consisting of a 30S small subunit with a 16S rRNA and a 50S large subunit with 23S and 5S rRNAs and no 5.8S rRNA. The rRNAs of eukaryotes possess several expanded segments and variable regions relative to their bacterial counterparts. It was postulated that these expansion regions are associated with the more complex control of translation. Accompanying the rRNA distinctions are eukaryote-specific RPs, all of which are encoded in higher plants (Barakat et al., 2001). The remaining RPs fall into two groups of either eubacterial (found across kingdoms) or archaea/eukaryote-specific origin (Armache et al., 2010b, a). A total of 54 RPs are recognized in eubacteria, 79 in yeast, and 79- 80 RPs in higher eukaryotes. With the exception of the RPs that form a flexible lateral stalk of the large subunit, all are present in a single copy per ribosome (Yusupova and Yusupov, 2014).

Ribosome architecture

High-resolution crystal structure analyses confirm pronounced conservation of the three-dimensional structure of ribosomes between eubacterial, archaebacterial and eukaryotic kingdoms

(Klinge et al., 2012; Voigts-Hoffmann et al., 2012; Yusupova and Yusupov, 2014). The larger mass of eukaryotic ribosomes has presented a greater challenge for obtaining crystals with high-quality diffraction characteristics. Consequentially, insights into eukaryote-specific structural features of ribosomes have been gleaned from cryo-EM structural analyses, including models at 38 Å (Verschoor et al., 1996) and at <10 Å resolution for translating wheat, yeast, insect, and mammalian ribosomes (Armache et al., 2010b, a). These models provide a wealth of insight into the position and structure of rRNAs and RPs.

Eukaryotic-specific RPs are located at several key positions within animal, yeast and wheat ribosomes, including the sites associated with decoding and tRNA binding, and mRNA exit on the 40S subunit. RPs specific to both subunits interact with the eukaryote-specific factor eIF3. The cryo-EM studies of insect and mammalian ribosomes emphasize the presence of rRNA expansion regions on the outer periphery that dynamically form RP-rRNA and rRNA-rRNA interactions (Anger et al., 2013). The wheat ribosome is more like that of yeast with a less extensive outer rRNA-protein layer. Further studies of plant ribosome architecture are needed to better appreciate kingdom-specific features as well as structural variations that might be associated with specific RP isoforms.

Interest in plant ribosomes, as well as other organisms, centers around several questions: Do ribosomes play specific roles in global translational activity or the translation of individual mRNAs? Is there ribosome heterogeneity due to differences in protein isoform composition or modification and if so, what is its function(s)? Are ribosome biogenesis and protein synthesis tightly regulated as a means of energy conservation and does management of these investments pace growth during the diurnal cycle or under abiotic environment? Is a threshold level of ribosomes necessary for cellular and organismal homeostasis?

Cytosolic ribosomal proteins

Eighty RP gene families of two to five paralogous members were identified in the Arabidopsis genome by comparison to the amino acid sequences of animal, yeast and *Archaea* RPs (Barakat et al., 2001) (see Table 3). The vast majority of RPs are basic in charge (pI > 8.0) and ≤ 45 kDa in mass. There are, however, a handful of conserved acidic RPs (pI < 5). Eukaryotes have two RP gene families that encode small acidic phosphoproteins that dimerize and bind the larger RPP0 to form a flexible lateral stalk structure on the large ribosomal subunit. Higher plants have a RPP1 and RPP2 family, as well as a third acidic RP called RPP3 (Szick et al., 1998; Chang et al., 2005). This stalk structure promotes eEF2 binding and GTP hydrolysis in yeast (Gonzalo and Reboud, 2003) and is important in the binding of ribosome inactivating proteins, such as the ricin toxin (Li et al., 2013c).

Several mass spectrophotometric studies have explored the proteome of Arabidopsis ribosomes. Ribosomes purified by differential centrifugation yielded 31-33 of the putative 40S RPs and 48 of the putative 60S RPs, respectively (Chang et al., 2005; Giavalisco et al., 2005; Carroll et al., 2008; Piques et al., 2009; Turkina et al., 2011; Carroll, 2013). An analysis performed on ribosomes captured by immunopurification, thereby limiting contamination by organellar ribosomes and other co-sedimenting complexes, confirmed products from 204 of the estimated 232 functional RP genes of *A. thaliana* based on two or more prototypic peptides (Hummel et al., 2012). This corresponded to 64 of the 80 putative RP families and included RACK1. Of these, 74 Arabidopsis RPs were positioned in a high-resolution 80S ribosome structure map relative to the rRNA structure (Armache et al., 2010b).

Ribosome heterogeneity

The biogenesis of a ribosome requires coordinated synthesis of rRNAs and RPs. In Arabidopsis, the 18S, 5.8S and 25S rRNA are encoded by the 45S rDNA genic repeat that is tandemly duplicated over 500 times on the short arms of chromosome 2 and 4, whereas the pre-5S rRNA is encoded by repetitive pericentromeric regions on chromosomes 3, 4 and 5 (Layat et al., 2012). The 18S, 5.8S and 25S rRNA precursor of Arabidopsis is sometimes referred to as the 35S pre-rRNA. The transcription of the Arabidopsis 45S rDNA units by RNA polymerase I is regulated by direct binding of TOR to the promoter region located in the intergenic region (spacer) between the 25S and 18S genes (Ren et al., 2011). The subsequent pre-rRNA processing pathway is a complicated pathway involving cleavage and nucleotide modification events. Synthesis of 5S rRNA by RNA polymerase III is regulated by mTOR in animals (Kantidakis et al., 2010); however, the regulation of plant 5S rRNA synthesis is not well characterized.

Arabidopsis *RP* genes are significantly co-regulated at transcriptional and post-transcriptional levels during development, in response to various stimuli and in some translational apparatus mutants. The coordinate transcriptional and posttranscriptional regulation of many *RP* genes reflects the presence of common *cis*-regulatory elements in gene promoters and features of mRNA 5'UTRs (Kawaguchi et al., 2004; Branco-Price et al., 2005; McIntosh and Bonham-Smith, 2006; Nicolaï et al., 2006; Tiruneh et al., 2013; Wang et al., 2013). Close inspection of Arabidopsis RP transcript accumulation data indicate that there is more than one *RP* transcription network regulated in response to carbon and nitrogen availability, as well as other environmental inputs (Sormani et al., 2011a; Wang et al., 2013). *RP* gene co-regulation was also noted when monitoring polysome-associated transcript levels. A coordinate shift of Arabidopsis RP mRNAs out of polysome complexes was observed in response to a number of environmental stresses (Kawaguchi et al., 2004; Branco-Price et al., 2005; McIntosh and Bonham-Smith, 2006; Nicolaï et al., 2006; Branco-Price et al., 2008; Pyl et al., 2012; Tiruneh et al., 2013; Wang et al., 2013). Conversely, coordinated up-regulation of the translation of a large majority of Arabidopsis *RP* mRNAs was recorded in two mutants of the translational apparatus (*eif3h and rpl24b*) (Tiruneh et al., 2013). Co-regulation of *RP* gene transcript accumulation and translation has also been noted in *Chlamydomonas* (Schmollinger et al., 2014).

The stereotype of a ribosome is that all are the same, but there is some evidence of specialized differences in cytosolic ribosomes of Arabidopsis and other plants that may contribute to the regulation of translation (Horiguchi et al., 2012; Hummel et al., 2012). Ribosome heterogeneity is predicted to be the con-

Table 3. Cytosolic Ribosomal Protein Genes of *Arabidopsis*[a]

Small Subunit Genes

Protein Family Name	Gene Names	Arabidopsis Gene Identifier of Gene Family Members
Sa	*RPSaA*	At1g72370
	RPSaB	At3g04770
S2	*RPS2A*	At1g58380
	RPS2B	At1g59359
	RPS2C	At2g41840
	RPS2D	At3g57490
	RPS2E	At1g58684
	RPS2F	At1g58983
S3	*RPS3A*	At2g31610
	RPS3B	At3g53870
	RPS3C	At5g35530
S3a	*RPS3aA*	At3g04840
	RPS3aB	At4g34670
S4	*RPS4A*	At2g17360
	RPS4B	At5g07090
	RPS4D	At5g58420
S5	*RPS5A*	At2g37270
	RPS5B	At3g11940
S6	*RPS6A*	At4g31700
	RPS6B	At5g10360
S7	*RPS7A*	At1g48830
	RPS7B	At3g02560
	RPS7C	At5g16130
S8	*RPS8A*	At5g20290
	RPS8B	At5g59240
S9	*RPS9B*	At5g15200
	RPS9C	At5g39850
S10	*RPS10A*	At4g25740
	RPS10B	At5g41520
	RPS10C	At5g52650
S11	*RPS11A*	At3g48930
	RPS11B	At4g30800
	RPS11C	At5g23740
S12	*RPS12A*	At1g15930
	RPS12C	At2g32060
S13	*RPS13A*	At3g60770
	RPS13B	At4g00100
S14	*RPS14A*	At2g36160
	RPS14B	At3g11510
	RPS14C	At3g52580
S15	*RPS15A*	At1g04270
	RPS15B	At5g09490

Large Subunit Genes

Protein Family Name	Gene Names	Arabidopsis Gene Identifier of Gene Family Members
P0	*RPP0A*	At2g40010
	RPP0B	At3g09200
	RPP0C	At3g11250
P1	*RPP1A*	At1g01100
	RPP1B	At4g00810
	RPP1C	At5g47700
	RPP1D	At5g24510
P2	*RPP2A*	At2g27720
	RPP2B	At2g27710
	RPP2C	At3g28500
	RPP2D	At3g44590
	RPP2E	At5g40040
P3	*RPP3A*	At4g25890
	RPP3B	At5g57290
L3	*RPL3A*	At1g43170
	RPL3B	At1g61580
L4	*RPL4A*	At3g09630
	RPL4D	At5g02870
L5	*RPL5A*	At3g25520
	RPL5B	At5g39740
L6	*RPL6A*	At1g18540
	RPL6B	At1g74060
	RPL6C	At1g74050
L7	*RPL7A*	At1g80750
	RPL7B	At2g01250
	RPL7C	At2g44120
	RPL7D	At3g13580
L7a	*RPL7aA*	At2g47610
	RPL7aB	At3g62870
L8	*RPL8A*	At2g18020
	RPL8B	At3g51190
	RPL8C	At4g36130
L9	*RPL9B*	At1g33120
	RPL9C	At1g33140
	RPL9D	At4g10450
L10	*RPL10A*	At1g14320
	RPL10B	At1g26910
	RPL10C	At1g66580
L10a	*RPL10aA*	At1g08360
	RPL10aB	At2g27530
	RPL10aC	At5g22440
L11	*RPL11A*	At2g42740
	RPL11B	At3g58700
	RPL11C	At4g18730
	RPL11D	At5g45775
L12	*RPL12A*	At2g37190
	RPL12B	At3g53430
	RPL12C	At5g60670

continued

Table 3. (continued)

Small Subunit Genes

Protein Family Name	Gene Names	Arabidopsis Gene Identifier of Gene Family Members
	RPS15C	At5g09500
	RPS15D	At5g09510
	RPS15E	At5g43640
	RPS15F	At5g63070
S15a	*RPS15aA*	At1g07770
	RPS15aB b	At2g19720
	RPS15aC	At2g39590
	RPS15aD	At3g46040
	RPS15aE b	At4g29430
	RPS15aF	At5g59850
S16	*RPS16A*	At2g09990
	RPS16B	At3g04230
	RPS16C	At5g18380
S17	*RPS17A*	At2g04390
	RPS17B	At2g05220
	RPS17C	At3g10610
	RPS17D	At5g04800
S18	*RPS18A*	At1g22780
	RPS18B	At1g34030
	RPS18C	At4g09800
S19	*RPS19A*	At3g02080
	RPS19B	At5g15520
	RPS19C	At5g61170
S20	*RPS20A*	At3g45030
	RPS20B	At3g47370
	RPS20C	At5g62300
S21	*RPS21B*	At3g53890
	RPS21C	At5g27700
S23	*RPS23A*	At3g09680
	RPS23B	At5g02960
S24	*RPS24A*	At3g04920
	RPS24B	At5g28060
S25	*RPS25A*	At2g16360
	RPS25B	At2g21580
	RPS25D	At4g34555
	RPS25E	At4g39200
S26	*RPS26A*	At2g40510
	RPS26B	At2g40590
	RPS26C	At3g56340
S27	*RPS27A*	At2g45710
	RPS27B	At3g61110
	RPS27D	At5g47930

Large Subunit Genes

Protein Family Name	Gene Names	Arabidopsis Gene Identifier of Gene Family Members
L13	*RPL13B*	At3g49010
	RPL13C	At3g48960
	RPL13D	At5g23900
L13a	*RPL13aA*	At3g07110
	RPL13aB	At3g24830
	RPL13aC	At4g13170
	RPL13aD	At5g48760
L14	*RPL14A*	At2g20450
	RPL14B	At4g27090
L15	*RPL15A*	At4g16720
	RPL15B	At4g17390
L17	*RPL17A*	At1g27400
	RPL17B	At1g67430
L18	*RPL18A*	At2g47570
	RPL18B	At3g05590
	RPL18C	At5g27850
L18a	*RPL18aB*	At2g34480
	RPL18aC	At3g14600
L19	*RPL19A*	At1g02780
	RPL19B	At3g16780
	RPL19C	At4g02230
L21	*RPL21A*	At1g09590
	RPL21C	At1g09690
	RPL21E	At1g57660
	RPL21G	At1g57860
L22	*RPL22A*	At1g02830
	RPL22B	At3g05560
	RPL22C	At5g27770
L23	*RPL23A*	At1g04480
	RPL23B	At2g33370
	RPL23C	At3g04400
L23a	*RPL23aA*	At2g39460
	RPL23aB	At3g55280
L24	*RPL24A*	At2g36620
	RPL24B	At3g53020
	RPL24C	At2g44860
L26	*RPL26A*	At3g49910
	RPL26B	At5g67510
L27	*RPL27A*	At2g32220
	RPL27B	At3g22230
	RPL27C	At4g15000
L27a	*RPL27aA*	At1g12960
	RPL27aB	At1g23290
	RPL27aC	At1g70600
L28	*RPL28A*	At2g19730
	RPL28C	At4g29410
L29	*RPL29A*	At3g06700

Table 3. (continued)

Small Subunit Genes

Protein Family Name	Gene Names	Arabidopsis Gene Identifier of Gene Family Members
S27a	*RPS27aA*	At1g23410
	RPS27aB	At2g47110
	RPS27aC	At3g62250
S28	*RPS28A*	At3g10090
	RPS28B	At5g03850
	RPS28C	At5g64140
S29	*RPS29A*	At3g43980
	RPS29B	At3g44010
	RPS29C	At4g33865
	RPS30A	At2g19750
S30	*RPS30B*	At4g29390
	RPS30C	At5g56670
RACK1 [c]	*RACK1A*	At1g18080
	RACK1B	At1g48630
	RACK1C	At3g18130

Large Subunit Genes

Protein Family Name	Gene Names	Arabidopsis Gene Identifier of Gene Family Members
	RPL29B	At3g06680
L30	*RPL30A*	At1g36240
	RPL30B	At1g77940
	RPL30C	At3g18740
L31	*RPL31A*	At2g19740
	RPL31B	At4g26230
	RPL31C	At5g56710
L32	*RPL32A*	At4g18100
	RPL32B	At5g46430
L34	*RPL34A*	At1g26880
	RPL34B	At1g69620
	RPL34C	At3g28900
L35	*RPL35A*	At3g09500
	RPL35B	At2g39390
	RPL35C	At3g55170
	RPL35D	At5g02610
L35a	*RPL35aA*	At1g07070
	RPL35aB	At1g41880
	RPL35aC	At1g74270
	RPL35aD	At3g55750
L36	*RPL36A*	At2g37600
	RPL36B	At3g53740
	RPL36C	At5g02450
L36a	*RPL36aA*	At3g23390
	RPL36aB	At4g14320
L37	*RPL37A*	At1g15250
	RPL37B	At1g52300
	RPL37C	At3g16080
L37a	*RPL37aB*	At3g10950
	RPL37aC	At3g60245
L38	*RPL38A*	At2g43460
	RPL38B	At3g59540
L39	*RPL39A*	At2g25210
	RPL39B	At3g02190
	RPL39C	At4g31985
L40	*RPL40A*	At2g36170
	RPL40B	At3g52590
L41	*RPL41C*	At2g40205
	RPL41D	At3g08520
	RPL41E	At3g11120
	RPL41G	At3g56020

[a] RP genes that have not been identified as pseudogenes (Barakat et al., 2001; Hummel et al., 2012; The Arabidopsis Information Resource, October 2014). Proteomic studies using 1D and 2D mass spectrometry have obtained evidence for the product of a least one paralog of each RP family in purified ribosomes, with the exception of the small (~3.5 kDa) and highly basic RPL41 (reviewed by Carroll, 2013).
[b] Two *RPS15a* paralogs encode RPs that assemble into mitochondrial and not cytosolic ribosomes (Carroll et al., 2008).
[c] Three *RACK1* paralogs encode a protein stably associated with the ribosome; RACK1s also function in signaling outside of the ribosome.

sequence of ancestral genome duplication and subsequent neo-functionalization of members of some RP gene families as well as regulated post-translational modification of some RPs. For example, transcripts of Arabidopsis *RP* paralogs are regulated by environmental inputs including carbon, phosphate and metals (Hummel et al., 2012; Wang et al., 2013). *RP* transcript levels are also differentially regulated between cell types (*e.g.*, (Mustroph et al., 2009). Other sources of ribosome heterogeneity include N-terminal methionine removal, N-terminal acetylation, methylation of lysine and proline residues and phosphorylation of serine and threonine residues of RPs (Bailey-Serres and Freeling, 1990; Bailey-Serres et al., 1997; Szick-Miranda and Bailey-Serres, 2001; Williams et al., 2003; Chang et al., 2005; Carroll et al., 2008; Turkina et al., 2011; Hummel et al., 2012; Boex-Fontvieille et al., 2013; Carroll, 2013). The functional consequence of ribosome heterogeneity is largely unknown, but may provide for a complex regulatory network that impacts translation at the global or mRNA specific level.

Ribosomal protein phosphorylation

The most well studied phosphorylated RP is the 40S subunit protein RPS6, which is modified at multiple serine residues at its C-terminus. This region of the protein extends into the mRNA exit channel of the ribosome (Anger et al., 2013). In mammals, RPS6 is phosphorylated by p70S6k, whose activity is mediated by the mTOR kinase which also phosphorylates other proteins that regulate translation (Zoncu et al., 2011). The direct impact of RPS6 phosphorylation on mammalian translation may be negligible, but provides an effective readout for p70S6k activity in actively dividing cells, which promotes efficient translation of mRNAs with a polypyrimidine track at their 5' end (5'TOP). This feature is a characteristic of mRNAs encoding RPs and a number of core translation factors in mammals, but there is only limited evidence of functional 5'TOPs in plants (Jiménez-López et al., 2011). In Arabidopsis, RPS6 phosphorylation is promoted during the day (Turkina et al., 2011; Boex-Fontvieille et al., 2013) and in response to auxin and cytokinin (Turck et al., 2004), but is repressed by hypoxia (Chang et al., 2005). In monocots, RPS6 is phosphorylated in embryos during germination (Beltrán-Peña et al., 2002) and its phosphorylation rapidly declines following hypoxia, heat shock and singlet oxygen treatment (Williams et al., 2003; Khandal et al., 2009). The RPS6 kinase of Arabidopsis, AtS6K1, is important in regulating cell division and growth (Henriques et al., 2010; Shin et al., 2012). New data suggest a non-ribosomal function of RPS6 in epigenetic regulation of rDNA transcription in Arabidopsis (Kim et al., 2014c). Further clarification will require mutational analyses to determine if RPS6 phosphorylation is biologically significant or simply a hallmark of S6K activity in plants. Clearly there are many layers of S6K regulation yet to be explored and explained at the molecular level.

Most RPs assemble into pre-ribosomes in the nucleolus at a stoichiometry of one copy per ribosome. By contrast, the acidic proteins RPP1 and RPP2 complex in the cytoplasm with one another and then assemble onto the ribosome (Gonzalo and Reboud, 2003). The acidic proteins can be absent or present in multiple copies on the ribosome. Plant ribosomes possess a related third plant-specific protein called RPP3 (Szick et al., 1998). A source of heterogeneity of plant ribosomes is developmentally and environmentally regulated by modulation of RPP1, 2 and 3 levels and phosphorylation status (Bailey-Serres et al., 1997; Szick-Miranda and Bailey-Serres, 2001; Turkina et al., 2011; Boex-Fontvieille et al., 2013). The biological relevance of P-protein phosphorylation also deserves additional investigation.

Phosphoproteomic studies have also provided insight into the modulation of RP phosphorylation. In a non-targeted phosphoproteomics study, one or more isoforms of Arabidopsis RPs displayed distinct patterns of phosphorylation according to availability of CO_2 and light (Boex-Fontvieille et al., 2013). This included significant quantitative differences in phosphorylation state of RPS6A, RPS6B, RPL13D and RPL14A. Clearly further work on the functional consequences of RP phosphorylation is needed to better understand the complex interplay of signaling with protein modification and translational control in plants.

RACK1, A Ribosome Interacting Player

RACK1 is an interesting protein that is soluble, plasma-membrane-associated or ribosome-bound via interactions with the 18S rRNA and several 40S RPs (Valasek, 2012). RACK1 is stably associated with the 40S ribosomal subunit of Arabidopsis (Chang et al., 2005; Giavalisco et al., 2005; Carroll et al., 2008; Piques et al., 2009; Turkina et al., 2011; Hummel et al., 2012; Carroll, 2013), as in other eukaryotes. This protein has a seven bladed β-propeller domain that allows it to act as a scaffold, in a manner homologous to the heterotrimeric G protein Gβ subunit. RACK1 is involved in diverse signaling events as well as in translation (Gandin et al., 2013a). Its roles in mammals and yeast include recruitment of the protein kinase C that phosphorylates eIF6 to promote its release from the 60S. This event must take place before the joining of the 40S and 60S subunits can occur at the end of the initiation phase. Interestingly, Arabidopsis eIF6A/B appear to have both lost this protein kinase C phosphorylation site and only eIF6A retains a CK1 phosphorylation site (Guo et al., 2011a). RACK1 may have additional functions associated with protein synthesis, including recruitment of eIF3, regulation of RP synthesis, and promotion of the turnover of improperly folded nascent proteins (Gandin et al., 2013b). These distinct roles may involve different kinases recruited to the RACK1 scaffold. RACK1 was connected to the activity of miRNA in humans, *C. elegans* and Arabidopsis (Speth et al., 2013).

There are three *RACK1* paralogs in Arabidopsis. All three Arabidopsis RACK1s are detected in ribosomes (Chang et al., 2005; Giavalisco et al., 2005; Carroll et al., 2008; Piques et al., 2009; Turkina et al., 2011; Hummel et al., 2012; Carroll, 2013), functionally complement a CPC2/RACK mutant of yeast, and interact with eIF6 (Guo et al., 2011a). Interestingly, single and multiple *RACK1* mutants cause a variety of developmental abnormalities and enhance responsiveness to abscisic acid (ABA) (Guo et al., 2009; Guo et al., 2011b). Double mutants of *rack1a rack1b* are hypersensitive to anisomycin, an inhibitor of peptide elongation and display slightly reduced levels of 80S ribosomes under normal growth conditions and following ABA treatment (Guo and Chen, 2008). The multiple locations and functions of RACK1 makes interpretation of the double mutant phenotypes difficult. For example, RACK1s participates in pre-miRNA processing via interaction with SERRATE, a partner of DICER-LIKE 1 and the nuclear-cap binding complex (CBP20/80) in Arabidopsis (Speth et al., 2013; Raczynska et al., 2014). This apparent nuclear role of RACK1 contrasts to its invovlement with miRNAs in *C. elegans,* which is at the level of recruitment of the Ago2-miRNA silencing complex to polysomes for translational inhibition (Jannot et al., 2011). An unresolved question is whether ribosome-associated RACK1 functions in ALTERED MERISTEM PROGRAM 1 (AMP1)/AGO1/miRNA-mediated translation repression in Arabidopsis (Li et al., 2013b). In summary, plant RACK1 is ribosome-associated and functions in a conserved manner but also has extra-ribosomal functions that may be plant-specific.

Ribosomal protein mutants

Over 20 *RP* gene mutants of Arabidopsis have been characterized (reviewed by Byrne, 2009; Horiguchi et al., 2012; Roy and von Arnim, 2013). Often, single or multiple loss-of-function mutations for individual RPs result in embryo-lethality or pleiotropic developmental phenotypes affecting organ size or shape. These include asymmetric or pointed first leaves and reduced rosette size. In many cases *RP* mutants display phenotypes related to defects in auxin-mediated processes. At the mechanistic level, there are at least four possible causes of *RP* mutant phenotypes: (1) insufficient ribosomes affecting mRNAs equally or specifically (2) non-functional ribosomes, (3) a requirement for a distinct ribosome form for translation of specific mRNAs, or (4) an extra-ribosomal function of the protein (reviewed by Horiguchi et al., 2012).

Ribosome insufficiency, for example, could arise when reduced levels of a specific RP limits the biogenesis of a ribosomal subunit (Horiguchi et al., 2012). Phenotypes associated with *RP* mutants, such as smaller plant rosette size could be the consequence of reduced ribosome biogenesis. The synthesis of both subunits is tightly coordinated within the nucleolus, culminating in export of individual subunits to the cytoplasm. The co-expression of multiple *RP* gene paralogs in the same cell-type may limit ribosome insufficiency. However, reduction in a core ribosome component could limit overall ribosome numbers, rather than just the stoichiometry of an individual subunit. This could be important, since ribosome levels may be tightly regulated to meter the amount of energy consumed in translation at specific developmental states (*i.e*., rapidly dividing versus differentiated cells) or under non-favorable environmental conditions. Indeed, the defects in cellular expansion of Arabidopsis leaves, a prominent phenotype of *RP* and ribosome biogenesis mutants, might be attributed to global reduction of ribosomes (Roy and von Arnim, 2013).

In the second scenario, *RP* mutants may not disrupt ribosome biogenesis but the subunits or complexes that form might be defective in overall activity or a specific function. This could occur if individual RPs have a specific role in translation. For example, studies of RPL24 indicated its importance in translation of mRNAs with small uORFs. RPL24 was also shown to be important in the intricate regulation of initiation of translation on CaMV 35S mRNA (reviewed by Roy and von Arnim, 2013). Levels of RPL4 and RPL5 were recognized as critical for translation of uORF-containing mRNAs encoding proteins important for auxin responses (Rosado and Raikhel, 2010; Rosado et al., 2012).

The third possibility is that ribosome heterogeneity, due to the product of a specific *RP* gene paralog, is necessary for translation of a sub-set of transcripts. This last concept was detailed by Horiguchi et al. (2012), but definitive examples of *RP* gene paralogs of distinct function remain limited. A possible example is provided by the Arabidopsis *RPL10* paralogs, which appear to have non-redundant functions in male gametophyte development (Falcone Ferreyra et al., 2010; Falcone Ferreyra et al., 2013). However, it is necessary to rule out the possibility that RPL10 may have an extra-ribosomal function, as shown for a number of RPs in diverse eukaryotes (Warner and McIntosh, 2009; Xue and Barna, 2012).

Extra-ribosomal function has been suggested for several Arabidopsis RPs as well as RACK1. An example of an extra-ribosomal function is the proposed role played by RPS6 in the regulation of transcription of rDNA and some *RP* gene transcripts in Arabidopsis (Kim et al., 2014c). This function involves direct interaction of non-phosphorylated RPS6 with Histone Deacetylase 2B (HD2B), which suppresses rDNA transcription. It was hypothesized that phosphorylation of free RPS6 could reduce HD2B inhibition, thereby promoting rDNA transcription or processing. If regulated by TOR as proposed, this could place ribosome biogenesis and translational regulation under unified control (Kim et al., 2014c).

To move forward in our understanding of plant ribosomes there needs to be further consideration of whether limitation, excess, or modification of individual RPs modulates ribosome biogenesis or impacts translation of specific mRNAs. The use of gene silencing constructs equipped with inducible promoters or targeted gene editing as well as examinations limited to specific cells may aid in this challenge. Significant advancements would be generated by further structural analyses of 80S ribosomes or subunit-initiation/elongation factor complexes of plant ribosomes.

Ribosomes and energy for their synthesis

The biogenesis and activity of ribosomes requires a considerable investment in energy to power the synthesis of RPs and rRNAs. In rapidly dividing cells, the synthesis of rRNAs and RPs necessary for ribosome biogenesis may utilize more than half of all cellular energy, as each amino acid addition to a nascent peptide consumes at least four NTP molecules: (2 ATP for generating each amino-acyl tRNA and 2 GTP for each elongation event) (Figure 2). For ribosome biogenesis there is the additional energy outlay for rRNA synthesis. It therefore is not surprising that both ribosome biogenesis and global levels of translation are central to energy management (Piques et al., 2009; Pyl et al., 2012; Pal et al., 2013). Situations that limit ATP and GTP availability such as hypoxia, unanticipated darkness and extended nighttime limit *RP* mRNA translation in Arabidopsis (Branco-Price et al., 2008; Piques et al., 2009; Pal et al., 2013). In seedlings, cytosolic *RP* mRNAs account for ~10% of total cellular mRNA (Branco-Price et al., 2008). These transcripts are stable but rapidly translationally repressed during hypoxia due to sequestration in aggregates of oligouridylate binding protein 1C

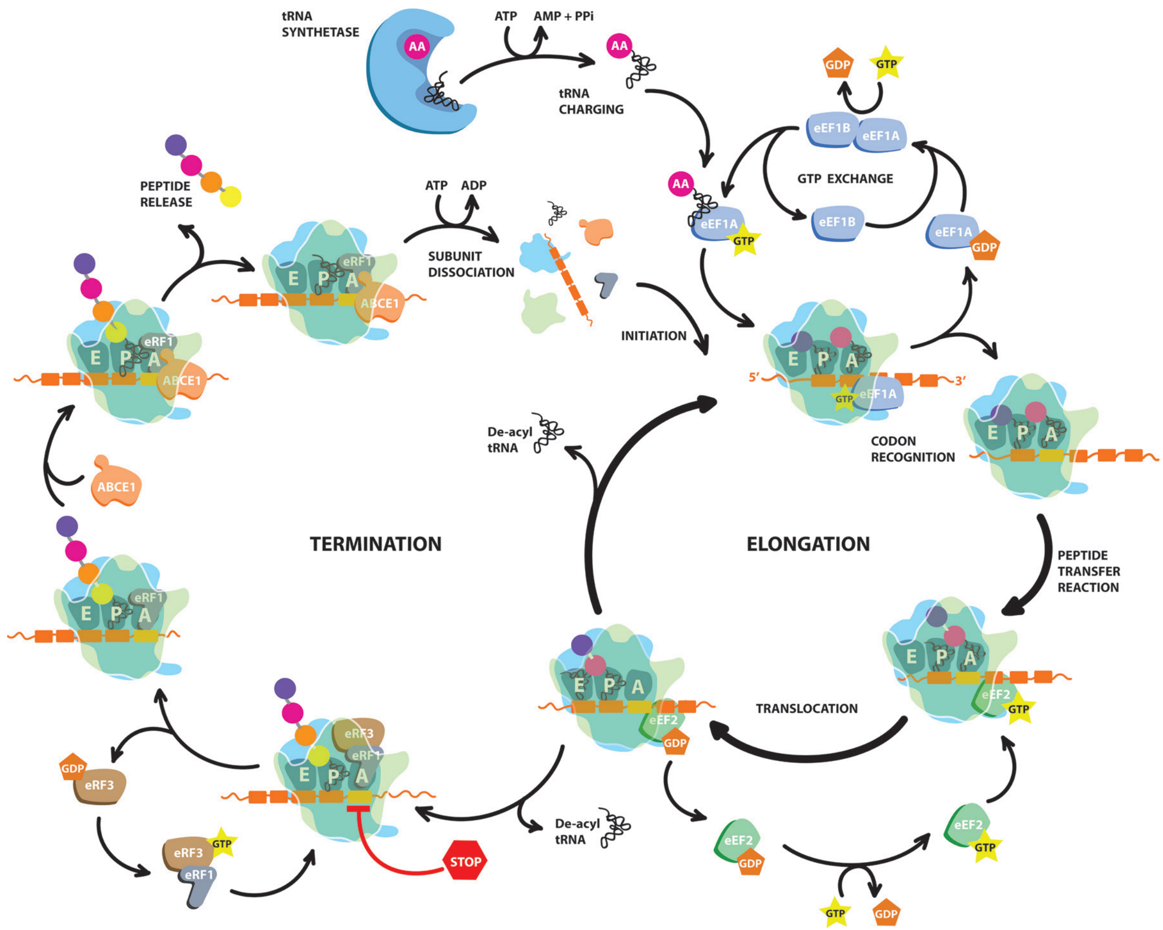

Figure 2. Overview of the steps of plant cytoplasmic translation elongation and termination cycles.

The elongating ribosome binds the incoming eEF1A•GTP•aa-tRNA in the A-site. If there is a match between the codon and anticodon of the tRNA, GTP hydrolysis occurs and eEF1A•GDP exits. Peptide bond formation occurs at the peptidyl transferase site; this reaction is mediated by the ribosome. eEF2•GTP binds and hydrolysis of GTP promotes translocation of the mRNA by three nucleotides, moving the now empty tRNA into the E-site, the newly elongated peptide•tRNA into the P-site, generating an empty A-site ready to accept another eEF1A•GTP•aa-tRNA. eEF1A•GDP requires the action of eEF1B to exchange GDP for GTP. eEF2 does not require a guanine exchange factor to acquire another GTP molecule. It is clear that each step of elongation is expensive in energy. Two GTP are required in the ribosome during elongation and each incoming eEF1A•GTP•aa-tRNA requires the functional equivalent of two ATP molecules to activate the amino acid and add it to the tRNA acceptor arm site. The AMP formed in this process requires two ATP to regenerate back to ATP; thus even though one ATP is consumed in the aminoacylation reaction, two ATP are ultimately consumed. The arrival of the termination codon in the A-site triggers the binding of the eRF1•eRF3•GTP complex into the A-site. Upon GTP hydrolysis, the eRF3•GDP is released. ABCE1 binds at the A-site to the remaining eRF1, promoting release of the polypeptide. Subsequent ATP hydrolysis by ABCE1 dissociates the ribosomal subunits, releasing ABCE1, eRF3, the deacetylated tRNA in the P-site and the mRNA. Note that the factors and ribosomal subunits are not to scale.

(UBP1C) (Sorenson and Bailey-Serres, 2014). This sequestration is quickly reversed upon reoxygenation, facilitating energy management during stress events.

RP mRNA translation in animals is largely mediated by mTOR activity, the presence of a 5'TOP, and the RNA binding protein La-related protein 1 (Thoreen et al., 2012; Tcherkezian et al., 2014). A characteristic of many other mammalian mRNAs that require mTOR activity is extensive secondary structure within their 5' leader sequences. In the case of higher plants, it is not clear yet if coordinate regulation of *RP* mRNA translation is TOR or 5'TOP regulated. Arabidopsis *RP* mRNAs typically have GC-rich untranslated leaders (Kawaguchi et al., 2004; Branco-Price et al., 2005) and some have termini reminiscent of 5'TOPs. Whether or not a specific mRNA sequence or feature (i.e. structure) is involved, the manipulation of TOR levels in Arabidopsis influences overall levels of polysomes (Deprost et al., 2007). RNA binding proteins may also be a factor, as translation of a large number of *RP* transcripts was enhanced at subfreezing temperatures (4°C) by the RNA chaperone Cold Shock Protein 1, which has double-stranded RNA helicase activity (Juntawong et al., 2013). In sum, ribosome biogenesis is highly regulated due to the energy investment in both the transcription of rRNA and translation of *RP* mRNAs. Further study is needed to clarify the connections of these processes to TOR and S6K regulated energy sensing.

THE DRAMA OF INITIATION

The actors introduced in the previous section, initiation factors eIF1, eIF1A, eIF2, eIF3, and eIF5, come together on the 40S subunit to form the 43S PIC. This complex interacts with the mRNA and its associated factors (eIF4s) to form a 48S complex that is competent to search in the 5’ to 3’ direction for the initiation codon using the ATP-dependent scanning model proposed by Kozak (Kozak, 1978, 1980). The selection of the initiation codon depends upon its nucleotide sequence context and possibly other features in the mRNA. Upon selection of the initiation codon, a series of molecular events occurs that transforms the open scanning form of the 48S scanning complex to the closed form that is ready to engage the 60S subunit (Asano, 2014). This completes the initiation phase with an elongation competent 80S ribosome at the start of the desired ORF.

Formation of the 43S Pre-Initiation Complex

The 40S subunit, ternary complex (eIF2•GTP•Met-tRNA$_i^{Met}$), eIF1, eIF1A and eIF3 interact to form the 43S PIC. The ternary complex, eIF1, eIF1A, eIF3 and eIF5 can also form the MFC prior to interaction with the small ribosomal subunit. MFC formation in yeast promotes assembly and stability of the 43S PIC (Hinnebusch et al., 2007). The MFC has been shown *in vitro* to form in yeast (Asano et al., 2000), mammals (Sokabe et al., 2012) and plants (Dennis et al., 2009), suggesting that these protein interactions are part of a conserved mechanism.

Models for mRNA Binding of the eIF4s

The canonical model begins with mRNA interacting with the cap-binding complex through the eIF4E subunit, which is complexed with eIF4G. eIF4G then serves as the scaffold for assembly of eIF4A, PABP and eIF4B. This mRNA-factor complex is thought to then unwind the mRNA in an ATP-dependent manner for interaction with the 43S PIC. Since eIF4A is not a processive helicase, it is not clear exactly how an unwound region is initiated and maintained to enable ribosome binding. A more recent model of initiation (Aitken and Lorsch, 2012), places the eIF4s directly on the 43S PIC. In this scenario, the mRNA is subsequently recruited and unwound on the 40S subunit directly channeling the transcript. eIF4F/eIF4A could be bound to the mRNA (via the 5’ cap or other RNA binding regions on eIF4G) as a “chaperone” which facilitates its interaction with 43S PIC associated factors such as eIF4B, eIF3 and/or eIF5. This model would explain a number of protein-protein interactions that are known to occur in yeast (*e.g.* eIF4G-eIF3, eIF4G-eIF5, eIF4B-40S subunit). However, some of these interactions have yet to be shown to occur in other eukaryotes (*e.g.* eIF4G-eIF5) and further biochemical analysis is needed to confirm the myriad of protein-protein interactions in the 43S PIC•mRNA/eIF4s complex and when/where they occur during the initiation process. Whichever model(s) proves to be true, the key aspect of the process is the relaxation of secondary structure in the 5’ region of the transcript to facilitate ribosome binding, scanning, and eventually initiation codon recognition.

Start Site Recognition

Once the 43S PIC associates at the 5’ end of the mRNA, scanning proceeds from 5’ to 3’ until a start codon is selected (Kozak, 1986; Asano, 2014). This is facilitated by binding of eIF1 to the “open” or scanning form of the 43S PIC that is stabilized by contacts with the N-terminal tail of eIF2β (Nanda et al., 2013). The zinc-binding domain in the C-terminus of eIF5 lies in close proximity to eIF1 and displaces the zinc-binding domain of eIF2β. This close proximity allows the N-terminal tail of eIF5 with its arginine finger required to interact with eIF2 and stimulate the GTPase activity of eIF2γ. The N-terminal tail of eIF5 prevents P_i release from the eIF2 ternary complex from this “open” conformation during the scanning process. When the scanning complex encounters the initiation codon in the suitable context in the P-site of the 40S subunit, the formation of the codon/anticodon base pair promotes full engagement of the Met-tRNA$_i^{Met}$. This disrupts eIF1 interaction with N-terminal tail of eIF2β and results in eIF1 release. The eIF2β N-terminal tail then interacts with the C-terminal domain of eIF5. The N-terminal domain of eIF5 is then able to interact with the C-terminal tail of eIF1A, which promotes the release of P_i from the ternary complex resulting in scanning arrest at a suitable initiation codon and conversion to a “closed” PIC (Asano, 2014; Hinnebusch, 2014; Saini et al., 2014).

A suitable initiation codon context is an A residue and to a lesser extent a guanine (G) residue at position -3 and a G residue at position +4 relative to the A$_{+1}$UG codon of the mRNA. It is thought that nucleotides surrounding the start codon help to engage the closed PIC conformation as the AUG codon is recognized (Hinnebusch and Lorsch, 2012; Asano, 2014; Hinnebusch, 2014). The A at position -3 corresponds to the first nucleotide of the E-site of the 40S subunit and is occupied by eIF2 as the codon-anticodon interaction is established in the P-site. In Arabidopsis plants exposed to dehydration stress, transcripts that were better associated with polysomes during the stress were enriched in A nucleotides just upstream of the start codon (Kawaguchi and Bailey-Serres, 2005). A recent study of Arabidopsis 5’UTRs further showed that the positions of A residues in the -1 to -5 region from the AUG were highly correlated with translational efficiency and uracil (U) residues in the same region were negatively correlated (Kim et al., 2014b). These results suggest that the region 5’ to the AUG in Arabidopsis strongly influences translational efficiency in plants (Kim et al., 2014b). mRNAs with a 5’UTR that was shorter than average (125 nt) and had low potential for secondary structure formation had higher levels of ribosome occupancy. Consistently, the G+C nucleotide content of the 5’UTR was inversely correlated with translational activity during a variety of environmental stresses including dehydration, hypoxia and darkness (Branco-Price et al., 2005; Kawaguchi and Bailey-Serres, 2005; Juntawong and Bailey-Serres, 2012).

Non-AUG codons

There are also rare examples of initiation at non-AUG codons on Arabidopsis mRNAs, including *AGAMOUS*, *FCA* and *POLyG*. The latter encodes a RNA polymerase targeted to the plastid or mitochondrion based on the AUG selected (Riechmann et al., 1999; Wamboldt et al., 2009; Simpson et al., 2010). Mutational studies that evaluated the ramifications of initiation codon context, secondary structure, 5'UTR length and presence of uORFs on the rate of initiation on the protein coding ORFs of plants have provided insight into use of an CUG triplet as a functional initiation codon for the *FCA* transcript (Simpson et al., 2010). Advances in nucleotide-level resolution of ribosome position (Liu et al., 2013; Juntawong et al., 2014) and *in vivo* secondary structure (Ding et al., 2014) are likely to yield additional examples of non-AUG initiation and information on the surrounding mRNA region that will provide new insight into flexibility in start site selection in plants.

Reinitiation involving uORFs

The presence of one or more short uORFs that precedes a mORF presents a special situation to the scanning ribosome. Based on the characterization of *GCN4* mRNA translation in yeast, the length of the uORFs, the spacing between the uORFs and the mORF, and specific mRNA sequence features contribute to the subtle regulation of subsequent reinitiation events that determine amount of GCN4 synthesized (Valasek, 2012). In plants, the amino acid sequence of the uORF peptide can also contribute to the translational regulation (Rahmani et al., 2009; Jorgensen and Dorantes-Acosta, 2012; Roy and von Arnim, 2013; von Arnim et al., 2014). In the case of mRNAs with multiple ORFs (polycistronic), ribosomes will initiate in the normal manner at the first AUG in a suitable context and elongation will proceed. When the termination codon is encountered, the termination process that dissociates the ribosome subunits is likely to occur. If the uORF is short there may be lingering association of eIF3 with the 40S subunit and the reassembly of the MFCmay occur (Asano, 2014). In plants, this process is enhanced when the eIF3h subunit is present and phosphorylated by S6K in a TOR kinase-dependent manner (Schepetilnikov et al., 2013).

Assembly of the 80S ribosome, the final scene of initiation

Upon formation of the "closed" PIC, mammalian eIF5 is released in complex with eIF2•GDP. eIF5's role at this point is as a GDP dissociation inhibitor for eIF2•GDP until eIF2B is able to stimulate the replacement of GDP with GTP. The function of mammalian eIF2B is crucial as it allows eIF2•GDP to exchange for GTP and acquire a new Met-tRNA$_i^{Met}$ for participation in another round of initiation (Jennings and Pavitt, 2010; Jennings et al., 2013); however, as described above it is not clear what the role and importance of plant eIF2B are at this time. The release of eIF5/eIF2•GDP opens the surface of the 40S for binding of the 60S subunit, whereas eIF5B•GTP facilitates the 60S ribosome joining through interactions with the C-terminal tail of eIF1A, and then eIF5B•GDP readily dissociates from the complex.

eIF1A plays a central role in the entire process of initiation. First, through its interactions with the eIF1 C-terminal tail to stabilize the "open" PIC by preventing Met-tRNA$_i^{Met}$ to fully engage in the P-site. Second, upon arrival at the correct AUG, the C-terminal tail of eIF1A is displaced and interacts with eIF5 to promote the release of P$_i$ generated by hydrolysis of eIF2•GTP by eIF5. Lastly, eIF1A facilitates the formation of the 80S ribosome and is the last initiation factor to exit after eIF5B•GTP. Thus, the 80S ribosome positioned at the correct initiation codon is now ready to move on to the next act, elongation.

ACT 2: ELONGATION

Translational elongation is an evolutionarily conserved progression of ribosome catalyzed polypeptide formation through mRNA decoding (see Figure 2 and Table 2). Once the subunit joining is complete with the Met-tRNA$_i^{Met}$ in the P-site of the ribosome, the second codon in the A-site awaits interaction with the anticodon of an aminoacyl (aa)-tRNA coupled to eEF1A•GTP (Dever and Green, 2012). Appropriate codon-anticodon interactions at the A-site will stimulate the peptidyl transferase reaction that generates a peptide bond between the Met-RNA$_i^{Met}$ and the aa-tRNA, leaving a deacylated tRNA$_i^{Met}$ in the P-site. The subsequent translocation of the mRNA by one codon shifts the peptidyl-tRNA to the P-site and the deacylated tRNA to the E-site, freeing the A-site for the next appropriate aa-tRNA and continuation of the cycle (Dever and Green, 2012; Doerfel et al., 2013). Translocation is facilitated by eEF2•GTP binding and GTP hydrolysis.

The principal cast for this process includes the aa-tRNAs, eukaryotic elongation factor (eEF)1A (homolog of bacterial EF-Tu), eEF1B (homolog of bacterial EF-Ts), eEF2 (homolog of bacterial EF-G) and the ribosome. The role of eEF5 (nee eIF5A) in the elongation process is emerging, and like its prokaryotic homolog (EF-P) appears to involve elongation of amino acid sequences enriched in runs of proline and/or glycine (Doerfel et al., 2013; Gutierrez et al., 2013; Ude et al., 2013). Aminoacyl tRNA synthetases (aa-synthetases) participate backstage. These enzymes are encoded by a large family of nuclear genes in Arabidopsis that couple cognate tRNAs to their amino acids to form acetylated tRNA (aa-tRNA) in an ATP dependent reaction.

THE ACTORS IN ELONGATION

eEF1A

eEF1A is the ortholog of bacterial elongation factor-Tu (EF-Tu). This factor forms a ternary complex with GTP and an aa-tRNA, which it delivers to the peptidyl transferase center when the corresponding codon is present in the A-site. Initial loose binding is followed by a recognition event that involves the hydrolysis of GTP and structural rearrangements of the tRNA, eEF1A and the ribosome. eEF1A•GDP is then released for recycling by eEF1B, a complex with GEF activity that recovers eEF1A•GTP (described below).

eEF1A is a highly abundant protein that may constitute up to 1% of the total protein in a cell. The protein is encoded by four paralogs in Arabidopsis. Seed endosperm of the maize *opaque 2* mutant has increased levels of eEF1A from multiple genes and is associated with improved lysine content (Lopez-Valenzuela et al., 2003; Lopez-Valenzuela et al., 2004). Interestingly, eEF1A has functions and interactions outside of its role in translation including association with cytoskeleton (which may reflect an association of the translation process with cytoskeleton anchors), nuclear export, proteolysis, apoptosis and viral propagation (Browning, 1996; Sasikumar et al., 2012). This factor is also known to participate in processes including export of tRNAs from the nucleus and the targeting of damaged and misfolded proteins to the proteasome (Sasikumar et al., 2012). EF1A is also reported to have interactions with the tombusvirus replication complex and the 3' tRNA-like structure of turnip yellow mosaic virus (Matsuda et al., 2004; Li et al., 2009).

eEF1B

In prokaryotes, EF-Tu•GDP cannot recycle the GDP for GTP without assistance from EF-Ts. Similarly, eEF1A requires an exchange factor, eEF1B. In contrast to the single polypeptide EF-Ts, eEF1B is a complex of proteins that varies in complexity from eukaryote to eukaryote. eEF1B has three components in plants, eEF1Bα, eEF1Bβ and eEF1Bγ (Table 2), two in yeast and three in mammals (Sasikumar et al., 2012). In some organisms eEF1B also includes a valyl tRNA synthetase. Very little is known specifically about eEF1B from plants other than it has been purified from wheat germ (Lauer et al., 1984) and was shown to play a role in viral replication (Sasvari et al., 2011; Hwang et al., 2013), as have eEF1A (Matsuda et al., 2004; Li and Nagy, 2011) and cap-binding complex subunits (Wang and Krishnaswamy, 2012).

Both eEF1A and eEF1B are post-translationally modified by phosphorylation involving several kinases and by methylation, which may influence various activities such as interaction with actin (Lopez-Valenzuela et al., 2003). Presumably these modifications reflect highly complex mechanisms of regulation in eukaryotes (Le Sourd et al., 2006; Sasikumar et al., 2012). Phosphorylation of elongation factors under photosynthetic control was not reported for Arabidopsis (Boex-Fontvieille et al., 2013).

eEF2

eEF2 is the functional equivalent to EF-G of prokaryotes. Peptide bond formation occurs rapidly following acceptance of the aa-tRNA into the A-site of the peptidyl transferase center within the large ribosomal subunit. This region is largely comprised of rRNA and is highly conserved between prokaryotic and eukaryotic ribosomes, indicating that the process of peptide bond formation is quite ancient (Dever and Green, 2012). After the peptide bond is formed in the peptidyl transferase reaction catalyzed by the ribosome, it is necessary to move the now uncharged tRNA from the P-site into the E-site, freeing the A-site for the next incoming aa-tRNA•eEF1A•GTP. A GTP•eEF2 complex binds to the ribosome and its GTP hydrolysis promotes the movement of the mRNA•tRNA•tRNA hybrid forward by three nucleotides, coinciding with the movement of the P-site deacylated tRNA to the E-site and ejection of the deacylated-tRNA from the E-site.

eEF2 is post-translationally modified at a conserved histidine residue to dipthamide. This modification makes eEF2 the target for ADP-ribosylation by diphtheria-like toxins (Ortiz et al., 2006; Zhang et al., 2008a). The biological significance of this unusual modification is unknown despite its conservation across all eukaryotes and *Archaea.* Wheat eEF2 was shown to have this modification as evidenced by ADP-ribosylation by diphtheria toxin (Lauer et al., 1984). eEF2 is also a substrate for phosphorylation by the Ca^{2+}/calmodulin-dependent eEF2 kinase (eEF2K), which reduces eEF2 association with the ribosome. The phosphorylation site in mammals is a conserved threonine residue (T56). The mammalian AMP kinase and mTOR-signaling pathways converge to inhibit Ca^{2+}-dependent eEF2K activity, thereby limiting translational elongation under nutrient limiting conditions (Leprivier et al., 2013). Conversely, hypoxia in mammals promotes eEF2K phosphorylation and accumulation. Because eEF2 phosphorylation is Ca^{2+}-regulated, it is thought to regionally fine-tune protein synthesis, such as in dendrites of activated neurons (Heise et al., 2014). When purified wheat germ eEF2 was phosphorylated with a mammalian Ca^{2+}/calmodulin-dependent kinase, its activity in the *in vitro* translation system was reduced (Smailov et al., 1993). Although a plant eEF2 kinase has not been recognized, phosphoproteomic analyses focused on translation factors detected P-Ser558 of Arabidopsis eEF2 (Guillaume Tcherkez, personal communication). This site is conserved relative to Ser595 of mammals. Interestingly, phosphorylation of Ser595 by cyclin A in mammals promotes phosphorylation of Thr56 by eEF2K (Hizli et al., 2013). The finding that the *AteEF2* (*LOS1*) is important for protein synthesis at low temperatures (Guo et al., 2002) hints that regulation of eEF2 activity is relevant to cold acclimation and likely other stress conditions.

eEF5 (nee eIF5A/eIF4D)

eEF5 was formerly known as eIF5A/eIF4D due to its initial report as a stimulator of Met-puromycin synthesis *in vitro,* a model assay for initiation. eEF5 was later recognized as a facilitator of elongation (Nanda et al., 2009) and confirmed as the functional and structural equivalent of elongation factor P (EF-P) of eubacteria. Both EF-P and eEF5 are involved in the efficient elongation of proteins with runs of proline or glycine residues (Doerfel et al., 2013; Gutierrez et al., 2013; Ude et al., 2013). Why certain combinations of amino acids pose difficulties during elongation is not fully understood, but at least for the imino acid proline (lacking the hydrogen at the amino group) it may be due to structural constraints introduced in the peptide backbone by its presence.

eEF5 has features that distinguish it from EF-P and is truncated on its C-terminus relative to EF-P, which limits its contacts with the 60S subunit (Gutierrez et al., 2013). eEF5 is the only eukaryotic protein known to be post-translationally modified with hypusine, a modification derived from spermidine, which is required for its activity. Similarly, prokaryotic EF-P is modified by β-lysylation, also a spermidine derivative (Allen and Frank, 2007; Bullwinkle et al., 2013). The hypusine/β-lysine modification is postulated to help eEF5 to engage the ribosome and bring proline residues into closer proximity in the peptidyl transferase center for peptide bond formation (Gutierrez et al., 2013). Given this proposed function, it may be informative to evaluate the density of ribosomes in regions of mRNAs enriched in proline codons in genotypes that vary in eEF5 abundance and hypusination. eEF5 also appears to be modified by phosphorylation in the light/dark transition (Boex-Fontvieille et al., 2013).

A number of studies of plant eEF5 have indicated a role in stress responses (abiotic, pathogen, iron deficiency), growth and development (Wang et al., 2003; Chou et al., 2004; Hopkins et al., 2008; Ma et al., 2010; Lan and Schmidt, 2011; Wang et al., 2012). eEF5 was found to be associated with eEF2 in pumpkin phloem (Ma et al., 2010). In Arabidopsis, an *eEF5* paralog (reported as *eIF5A-2*) is necessary for cytokinin-mediated promotion of protoxylem development in seedling roots through genetic interaction with Cytokinin Response 1 (CRE1), a histidine kinase that binds cytokinin and the phophotransferase AHP6, that negatively regulates signaling by cytokinin (Ren et al., 2013). Given that these processes appear unrelated to translation, eEF5 may have additional biological function(s) in plants; alternatively, these may represent downstream outcomes due to translation defects involving proteins with poly-prolyl or glycyl residues whose translation may depend upon this factor.

Compared to initiation in plant translation, there has been less work on the elongation process and its factors. Whether there will be aspects of elongation or its control that are specific to plants await further discovery.

ACT 3, THE *FINALE:* FACTORS AND EVENTS OF TERMINATION

Elongation ends when translocation places one of the three stop codons (UAA, UGA, or UAG) into the A-site of the ribosome. This initiates the termination phase, which ends with disengagement of the peptide from the ribosome (Dever and Green, 2012; Jackson et al., 2012). There are two eukaryotic release factors (eRF1 and eRF3, see Table 2 and Fig. 2) in plants, the same as in mammals. The fate of the translation complex upon termination is still lacking in molecular details. Termination is usually followed by ribosome release, but may be followed by reinitiation of translation after a short coding sequence (i.e., a uORF) (Roy and von Arnim, 2013). In the special case of premature termination at a nonsense codon (a termination codon 5' of an EJC), degradation of the transcript occurs via the NMD pathway (see below).

Prior to the final "act", a complex of release factors (RF) and GTP must form in the cytosol in preparation for interaction with the ribosome (see Table 2). eRF3•GTP binds to eRF1 which acts as a GTP dissociation inhibitor. When the elongating ribosome arrives at a stop codon on the mRNA, the eRF3•GTP•eRF1 complex is recruited to the A-site, preventing further entry of eEF1A•aa-tRNA complexes. Unlike prokaryotic termination factors that have specificity for one or more termination codons, the eukaryotic ternary complex of eRF3•GTP•eRF1 recognizes all three stop codons (UAA, UGA, UAG) by a little known mechanism (reviewed in Dever and Green, 2012; Jackson et al., 2012).

THE ACTORS IN TERMINATION

eRF1 and eRF3

eRF1 is evolutionarily related to bacterial RF1 and RF2, class 1 RFs. The structure of these proteins resembles a tRNA, allowing the N-terminal domain of the RF to dock in the A-site and directly interact with the stop codon (Dever and Green, 2012; Jackson et al., 2012). The high fidelity of this binding coupled with the GTPase activity of eRF3 promotes the peptidyl tRNA hydrolysis necessary to release the polypeptide from the P-site and from the ribosome (Dever and Green, 2012; Jackson et al., 2012). At the structural level, the N-terminal region of eRF1 binds the stop codon, the middle domain enters the peptidyl transferase center where it promotes the hydrolytic release of the polypeptide, whereas the C-terminal region interacts with eRF3. eRF3's GTPase activity is necessary both to increase the rate of peptide hydrolysis by eRF1 and the efficiency of termination (Dever and Green, 2012; Jackson et al., 2012). The structure of the eRF3•GTP•eRF1 ternary complex on the ribosome was determined, revealing a number of features that suggest it has a very similar GTPase activation mechanism to the prokaryotic aa-tRNA•EF-Tu•GTP complex (des Georges et al., 2014). eRF1 is retained after termination and is important for recycling of the ribosome by recruiting the ABCE1/RIL1 protein (see below and Fig. 2) which functions with eIF6 in the dissociation of the ribosome into subunits for recycling (Pisarev et al., 2010). Yeast eRF1 has been shown to have additional functions that affect the cytoskeleton and cell cycle (Valouev et al., 2002) and appears to "moonlight" as observed for the eEFs (Le Sourd et al., 2006; Sasikumar et al., 2012)

Arabidopsis has three *AteRF1* genes that encode functional RFs (Chapman and Brown, 2004). The overexpression of *AteRF1-1* resulted in the silencing of *AteRF1-1* and to some extent *AteRF1-2* and *AteRF1-3* causing a phenotype known as *broomhead* (altered spacing between inflorescence stems cause a broom-like appearance) and is associated with perturbations in cell division and cell elongation (Petsch et al., 2005). *AteRF1-2* mRNA levels are induced by high glucose levels and *AteRF1-2* overexpression lines display increased glucose-mediated repression of germination (Zhou et al., 2010b). These genotypes are also hypersensitive to paclobutrazol, an inhibitor of gibberellin biosynthesis, as well as abscisic acid. Consistently, T-DNA insertion mutants of *AteRF1-2* showed resistance to gibberellin synthesis inhibitors during germination. It is not yet clear if the role *AteRF1-2* plays in glucose sensing or phytohormone responses reflects its role in termination or some other cellular role. It is important to determine if the *broomhead* and other phenotypes associated with eRF1 mutants in Arabidopsis are related to translation or other processes. Interestingly, a mutant (*Or*) that produces an orange color in cauliflower heads (infloresence meristems) due to an increase in β-carotene and encodes, a protein shown to interact with eRF1-2. The *Or* mutant displays altered petiole elongation and other developmental alterations suggesting a role for termination in developmental programs (Zhou et al., 2010c). Much more needs to be learned about plant termination and its actors.

NONSENSE MEDIATED mRNA DECAY: CURTAINS FOR SOME mRNAs

In special cases, termination can trigger mRNA decay (Belostotsky and Sieburth, 2009). This mechanism, termed nonsense mediated decay (NMD), provides quality control of mRNAs as they transit from the nucleus to active translation complexes. The process provides continuity between the nuclear process of intron splicing and cytoplasmic translation. A key feature in the process is the EJC, which is deposited just 5' of exon-exon junctions following splicing, and serves as a talisman in the pioneering (first) round of translation of an mRNA. Following translational initiation, the elongating ribosome is thought to displace many of the RNA binding proteins bound to the mRNA as it translocates from codon to codon. If an EJC lies 3' of a stop codon or the transcript has an unusually long 3' UTR (>300 bp), then eRF3, responsible for termination and release of the nascent polypeptide, associates with UPF1 (helicase up-frameshift 1), a protein needed to initiate NMD. Two other proteins required for this process, UPF2 and UPF3, bind to the EJC after splicing. mRNAs with an EJC 3' of the termination codon properly position UPF1-3 such that the destruction of the mRNA is triggered (Chang et al., 2007). NMD functions similarly in plants based on the presence of orthologs of NMD components and evidence of NMD coupled to the turnover of mRNAs with premature termination codons (Kerényi et al., 2008; Reddy et al., 2013). The targeting of alternatively spliced transcripts with premature termination codons for NMD provides an example of a mechanism coupled to translation that modulates mRNA abundance in response to environmental cues (Kalyna et al., 2012). Thus the half-life of an mRNA can be intimately entwined with its translation.

The many factors, complexes and processes involved in the temporal and spatial regulation of mRNA decay in plants have received limited attention until quite recently (Maldonado-Bonilla, 2014). It is important to understand the connection between translation and decay processes, including miRNA-mediated translational inhibition and mRNA turnover (Li et al., 2013b; Rogers and Chen, 2013).

RECYCLING: IS THERE AN *ENCORE*?

The emerging view is that termination is followed by "recycling", efficient reuse of the ribosome. At this point in the translational process the 80S ribosome, mRNA and tRNA-polypeptide chain are still coupled. This requires that the two subunits of the ribosome dissociate and eRF1 as well as the deacylated tRNA be released. In prokaryotes ribosome recycling is promoted by EF-G and a dedicated ribosome recycling factor (RRF), present only in prokaryotes. Currently, it is thought that an essential protein, ATP-binding cassette E (ABCE1)/RNASE L INHIBITOR 1 (RLI1), conserved in eukaryotes and *Archaea*, promotes polypeptide release and ribosome recycling (Pisarev et al., 2010) in a process that requires ATP hydrolysis (Dever and Green, 2012; Jackson et al., 2012). Recent structural studies show that following eRF3•GDP release, ABCE1 binds to eRF1 and within the ribosome (Preis et al., 2014). This binding is associated with a dramatic conformational change in eRF3 that positions its central domain in the peptidyl transferase center, where it catalyzes the release of the polypeptide.

Other factors may be important in recycling of ribosomes on cytosolic mRNAs of eukaryotes. First, the proximity between the 3' and 5' ends of the message, fostered by the presumed interaction between PABP and eIF4s (Jackson et al., 2010; Valasek, 2012), may enable loosely associated 40S subunits to reform a PIC and recommence the initiation phase. There is, however, some debate about the importance of PABP/eIF4G interactions in the closed-loop mRNA model (Afonina et al., 2014). Studies with yeast and mammals (Dever and Green, 2012) point to a role of ABCE1/RLI1 in recruiting the MFC to the 40S subunit once the ribosome is dissociated. Interestingly, there is evidence from mammals that if eIF3, eIF1, eIF1A, and eIF2•$tRNA_i^{Met}$ remain associated with the 40S subunit after termination then bidirectional scanning by the 40S or 80S complex occurs. Such a scenario would enable initiation at AUGs of downstream or upstream open reading frames (i.e., uORFs) that precede the ORF encoding the functional protein (Skabkin et al., 2013). The clever use of mimicry of tRNA shapes in some plant viral 3' UTRs serves to recruit or recycle ribosomes, suggesting that recycling may be a common cellular event.

THE ACTORS IN RECYCLING

In addition to eRF1, the ABC-type ATPase ABCE1 is a key player in ribosome recycling. The Arabidopsis genome encodes two *ABCE1* genes, which are characterized by an N-terminal Fe-S cluster and two nucleotide binding domains. A point mutation in a ABCE1/RLI1 ortholog in *Cardamine hirsuta,* a relative of Arabidopsis*,* converts the highly lobed leaf into a simple leaf and causes other downstream phenotypes (Kougioumoutzi et al., 2013). These findings suggest that ABCE1 plays an important role in numerous cellular developmental processes. Developmental dysfunctions including alterations in auxin homeostasis are quite frequent for mutants affecting ribosome biogenesis as described above. But caution is needed in interpreting these results, as it remains to be shown if ABCE1 has other roles or the efficiency of ribosome recycling is critical for development. Other proteins that act in the recycling of the translational apparatus in segue from termination to a new initiation event are unknown, with the exception of eIF6 which promotes subunit dissociation.

FUTURE PROSPECTS

The ease of isolation of mRNA and methods for global analyses of mRNA abundance has resulted in intense research on gene regulation in plants and other eukaryotes. Although, transcriptional regulation is frequently presumed to be the default mechanism that modulates steady-state transcript abundance, regulation that occurs at post-transcriptional levels including the processes that determine mRNA maturation, transport, stabilization, turnover and, in particular, translation have become increasingly apparent. In plants, these processes all contribute to dynamics in quantity, location and function of the gene product and are not readily discerned from steady-state transcript data. Technologies that enable the isolation of mRNAs associated with polysomes, such as translating ribosome affinity purification (TRAP) have helped to illuminate translational regulation of individual transcripts, particularly in Arabidopsis (Zanetti et al., 2005). Resolution of dynamics in mRNA translation will be enhanced by the ability to identify the position and frequency of ribosomes as they transit gene transcripts. This "ribosome profiling" strategy has been applied to examine changes in ribosome distribution along Arabidopsis mRNAs in seedlings upon illumination-triggering photomorphogenesis and during hypoxia (Liu et al., 2013; Juntawong et al., 2014). Translational dynamics occur in response to environmental stress, metabolites, and over the course of development (reviewed by Roy and von Arnim, 2013) and as a means for overall regulation of cellular energy over the diurnal cycle (Pal et al., 2013; Sulpice et al., 2014). Translational regulation may also be important in the tolerance of polyploidy in plants, as a comparison of total and polysomal mRNAs in the allopolyploid *Glycine dolichocarpa* indicated that selective translation contributes to dominance of expression of specific homoeologous genes as well as physically linked genes (Coate et al., 2014).

Further studies of the translational apparatus is needed, including the soluble factors, ribosomes and the cadre of RNA binding proteins that act as stagehands to fine-tune translational regulation. The use of genetic approaches to dissect the roles of the apparatus will most likely benefit from inducible constructs that reduce endogenous transcript levels or produce isoforms with specific features at controlled levels. In addition to a focus on endogenous mRNAs, the study of the performances of plant viruses in translation can be helpful. In the end, the data generated over the next decade will provide key insights, but is likely to also raise more enigmas. There are currently many questions about plant translation that are unanswered:

- What is the role of eIF2 phosphorylation by GCN2 in regulating translation and are there other eIF2 kinases that might regulate global levels of translation?
- Is there a plant version of eIF2B and what is its function?
- What is the role of the plant-specific eIFiso4F and why did it evolve?
- Are there other specific differences in plant initiation complexes compared to other eukaryotes?
- What are the molecular interactions of the initiation factors with the ribosomes? Do they differ from other eukaryotes?
- What factors besides eIF3h and the ribosome are important in uORF translation?
- Is ribosome heterogeneity of biological significance?
- Do specific ribosomal proteins regulate translation of individual gene transcripts or cohorts of mRNAs during development or in response to environmental cues?
- What is the role of nutrient availability and TOR in ribosome biogenesis (including rRNA synthesis, *RP* mRNA transcription and translation), and other processes of translation?
- What are the signals from chloroplast to nucleus that regulate coordinated synthesis of nuclear encoded photosynthetic proteins?
- What RNA sequences or structures and RNA binding proteins contribute to differential translation, targeting, stability and trafficking of mRNAs?
- What mechanisms sequester mRNAs into untranslatable pools, and how do they regain their ribosome loading?
- What are the levels of interaction between chromatin, transcription, nuclear processing, translation, and mRNA turnover involving NMD, miRNA or general decay mechanisms?

As these questions are answered we will acquire a greater appreciation of the multi-dimensional and integrated performance within the cell nucleus and cytoplasm that culminate in the highly regulated “action drama” of protein synthesis in plants.

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