Regulation of translational initiation in plants

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The abundance of cytosolic mRNA does not necessarily correspond to the quantity of polypeptide synthesized in plant cells. The initiation of mRNA translation is regulated at the global and message-specific levels. mRNAs compete for discriminatory initiation factors that couple the 5′-^{7m}GpppN-cap and the 3′-poly(A) tail of the RNA message. The resultant circularization of the mRNA promotes the association of the 43S pre-initiation complex that scans the 5′-leader for the initiation codon of the protein coding sequence. The physiological and developmental regulation of these events governs the level of polypeptide synthesis from endogenous and viral transcripts.

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Abbreviations

4EBP	eIF4E-binding protein
AMV	Alfalfa mosaic virus
CaMV	Cauliflower mosaic virus
eIF	eukaryotic initiation factor
FED1	*FERREDOXIN1*
GCN4	*GENERAL CONTROL NON-DEPRESSIBLE4*
HSP	heat shock protein
LOX2	LIPOXYGENASE2
nt	nucleotide
ORF	open reading frame
PABP	poly(A)-binding protein
RBCS	rubisco small subunit gene
RP	ribosomal protein
TAV	translational activator protein
TE	translational enhancer
TuMV	Turnip mosaic virus
uORF	upstream ORF
UTR	untranslated region
VPg	viral protein

Introduction

The abundance of a gene transcript is not a reliable indicator of the amount of polypeptide synthesized because of the regulation of mRNA translation. Examination of the association of mRNAs with ribosomes, by analysis of polyribosomes that are fractionated along a sucrose density gradient, can reveal distinctions in the translation of individual mRNAs (Figure 1a). For example, rubisco small subunit (*RBCS*) mRNA and ribosomal protein S6 (*RPS6*) mRNA differ in the extent to which they are associated with ribosomes in *Arabidopsis* leaves (Figure 1b). *RPS6* mRNA is poorly loaded with ribosomes compared to *RBCS* mRNA. Similar polyribosome-loading analyses have revealed alterations in mRNA translation following numerous environmental stimuli including light/dark transitions [1•,2•], heat stress [3,4], salt stress [4], water deficit ([5]; R Kawaguchi *et al.*, unpublished data), gravistimulation [6•], oxygen deprivation and pathogen infection [7]. Differential mRNA translation also occurs in response to the application of jasmonic acid [8], exogenous sucrose [9], and during pollen maturation [10,11] and germination [12•]. Recent studies reveal that differential translation of individual gene transcripts is determined by *cis*-acting mRNA sequences and translation factors. Sequence elements can be sufficient to maintain translation or inhibit translational initiation under certain physiological conditions or during certain developmental stages. The RNAs of plant viruses efficiently recruit ribosomes even when translation is globally repressed, thereby illuminating diverse mechanisms that facilitate efficient translation. Here, we consider the dynamic interactions between mRNAs and initiation factors that regulate the capture and maintenance of translating ribosomes.

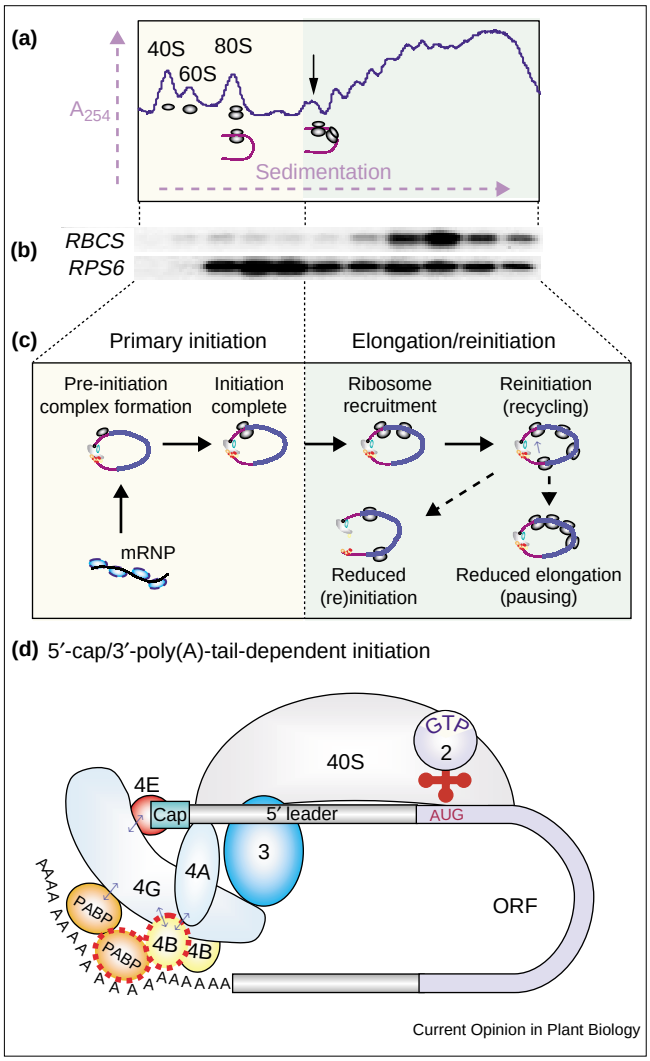
Initiation and reinitiation of mRNA translation

The recruitment of a ribosome to the initiating AUG codon of an mRNA is typically the rate-limiting step in polypeptide synthesis in eukaryotes, although polypeptide elongation can also be regulated [13]. Most mRNAs are translated via a mechanism that depends on the interaction of the 5′-^{7m}GpppN-cap with the 3′-poly(A) tail (Figure 1c,d). The initial step in translation is the assembly of a circular mRNA–protein complex. In plants, the 5′-^{7m}GpppN-cap of the transcript is recognized by eukaryotic initiation factor 4E (eIF4E) or eIFiso4E, which are bound to their respective partners, eIF4G and eIFiso4G [14]. eIF4G and eIFiso4G are scaffold proteins that also recruit: first, an RNA helicase (i.e. eIF4A, a monomer that facilitates the ATP-dependent unwinding of RNA [15]); second, an RNA-binding protein (i.e. eIF4B, a homodimer that binds poly(A), stabilizes the binding of ATP to eIF4A [16,17,18••], and promotes the RNA-dependent ATP-hydrolysis of eIF4E/eIF4G/eIF4A but not of eIFiso4E/eIFiso4G/eIF4A [19]); and third, poly(A)-binding protein (PABP; which binds poly[A] and enhances 5′-cap binding [16,18••,20,21•]). The assembly of these proteins circularizes the mRNA and stimulates RNA helicase activity, causing a synergistic enhancement of translation [13,20,21•,22•]. eIF4G and eIFiso4G also interact with the eIF3 complex (which includes 11 subunits, one of which is a plant-specific subunit [23•]) to position the 43S pre-initiation complex (i.e. the 40S subunit and the eIF2α–GTP–tRNA^{met} ternary complex) near the 5′ end of the mRNA. As secondary structure in the 5′-leader is relaxed, the 43S pre-initiation complex scans in a 5′ to 3′ direction until an AUG codon in the correct context is recognized and bound by tRNA^{met}, with the assistance of eIF1 and eIF1A [13,24•]. Completion of initiation

Figure 1 legend

Model of mRNA 5′-cap/3′-tail interactions that promote translational initiation. **(a)** Ultra-violet light absorbance profile of crude extracts from non-stressed *Arabidopsis* leaves (grown for 7 weeks under light) after centrifugation over a 20–60% sucrose gradient (R Kawaguchi *et al.*, unpublished data). The arrow indicates the position of disomes (i.e. two ribosomes on a mRNA). The non-polyribosomal region of the gradient (yellow side) includes the following complexes: mRNPs, ribosome subunits, the 43S pre-initiation complex, 80S monoribosomes and 80S monoribosomal mRNAs. The polyribosomal region of the gradient (green side) contains mRNAs that are associated with ribosomes. **(b)** Distribution of *RBCS* and ribosomal protein S6 (*RPS6*) mRNAs in the gradient fractions. The gradient shown was fractionated and RNA-blot analysis was performed with *RBCS* and *RPS6* cDNAs. **(c)** States of translation. 5′-cap/3′-poly(A)-tail interactions mediate the efficient initiation and re-initiation (i.e. recycling) of ribosomes. Dotted arrows propose alterations in translation status caused by either reduced primary initiation/reinitiation or elongation. Reduced 5′-cap/3′-tail interactions result in fewer ribosomes per mRNA. Impaired elongation, as a result of ribosome pausing, results in more ribosomes per mRNA without increased polypeptide synthesis. **(d)** Circularized initiation complex (see text for details). eIF4E (4E) or eIFiso4E binds to eIF4G (4G) or eIFiso4G, and the 5′-^{7m}GpppN-cap of the mRNA (Cap). Arrows indicate interactions between eIF4 subunits and PABP that result in the circularization of the mRNA. eIF4B may also interact with the 5′ UTR. The phosphorylation of PABP and eIF4B affects protein–protein and protein–poly(A) RNA interactions. Phosphoproteins are outlined with a dashed red line. eIF3 and 40S subunits are shown. Molecules are not drawn to scale. One PABP molecule protects approximately 25 nucleotides of poly(A) RNA. 2, eIF2; 3, eIF3; 4A, eIF4A; 4B, eIF4B.

Figure 1



requires the eIF5-assisted release of eIF2α–GDP and the eIF5B-assisted coupling of the 40S and 60S ribosomal subunits. It is thought that the 5′-cap/3′-tail connection may facilitate ribosome re-initiation (i.e. recycling) as well as the primary initiation event (Figure 1c).

Differential initiation of mRNA translation is mediated by eIF4F and eIFiso4F

The initiation of translation in plants is uniquely complicated by the existence of two heterodimeric cap-binding complexes, eIF4E/eIF4G (eIF4F) and eIFiso4E/eIFiso4G (eIFiso4F) [14]. eIF4E and eIFiso4E share 45–50% identity in amino-acid sequence, whereas eIF4G and eIFiso4G share only 35% identity [25,26••]. In a wheat germ cell-free translation system, both eIF4F and eIFiso4F facilitate 5′-cap–3′-poly(A) tail-dependent initiation in consort with eIF4A, eIF4B and PABP [14,26••]. The discrimination between mRNAs when levels of eIF4F and eIFiso4F are limiting is striking [26••,27•]. eIFiso4F is more abundant than eIF4F in wheat germ (i.e. embryos), maize root tips and cauliflower florets [14], suggesting that it is the general cap-binding complex of plant cells. Consistent with this, eIFiso4F preferentially translates 5′-capped–3′-poly(A) tailed mRNAs that lack a structured 5′ untranslated region (UTR). eIF4F is the more versatile factor. It efficiently translates 5′-capped–3′-poly(A)-tailed mRNAs as well as transcripts with a stem-loop structure near to the 5′-cap, mRNAs lacking a 5′-cap, and the downstream open reading frame (ORF) of uncapped dicistronic mRNAs [26••,27•].

Modulation of the subunit location, abundance or activity of eIF4F and eIFiso4F could differentially control mRNA translation. In mammals, protein synthesis can be regulated by phosphorylation of eIF4E and its interaction with eIF4E-binding protein (4EBP), a phosphoprotein that competes with eIF4G for binding [13]. Although eIF4E phosphorylation in mammals is frequently correlated with increased translation, phosphorylated eIF4E has a lower affinity for 5′-capped mRNA than unphosphorylated eIF4E [28]. This observation leads to the prediction that eIF4E phosphorylation occurs after the formation of the 43S pre-initiation complex, and that it promotes the release of the eIF4 complex and stimulates ribosome scanning. In plants, the abundance of eIF4E and eIFiso4E mRNAs and proteins is developmentally regulated [29,30]. Oxygen deprivation stimulates the rapid calcium-dependent hyperphosphorylation of eIF4E in maize root tips [25]. In contrast, the phosphorylation of eIF4G and eIFiso4G is partially reduced following heat shock [31] and modulated during development [18••]. The ramifications for translation

of changes in the abundance or phosphorylation of these proteins remain to be fully elucidated. An additional curiosity is that there is no plant ortholog of mammalian 4EBP. An *Arabidopsis* LIPOXYGENASE2 (LOX2) was shown to interact with eIFiso4E in the yeast two-hybrid system and *in vitro* [32•], but the significance of this interaction has not been investigated *in planta*.

Maintenance of interactions between the mRNA 5′-cap and the 3′-tail

The view that 5′-cap–3′-tail interaction facilitates the initiation of translation leads to the prediction that the stabilization or disruption of this interaction will impact polypeptide synthesis. The strategies evolved by viruses to achieve efficient translation of their RNAs illustrate the importance of 5′-cap–3′-tail interactions and efficient recruitment of initiation factors. A striking example is displayed by the polycistronic Barley yellow dwarf virus (BYDV) genomic RNA, which has no 5′-cap or 3′-poly(A) tail but is efficiently translated in plants [33••]. In this RNA, a 109 nucleotide (nt) translational enhancer (TE) that lies 5 kb downstream of the stop codon of the first ORF binds eIF4F and forms a stem-loop that interacts through base-pairing with a second stem-loop located in the 5′ UTR. This so-called kissing-loop results in an iconoclastic circularization of the transcript. Other initiation strategies are employed by Turnip mosaic virus (TuMV) and Alfalfa mosaic virus (AMV) genomic RNAs that also lack a 5′-cap and 3′-poly(A) tail. TuMV utilizes a viral protein (VPg) that is bound to its 5′-end to recruit eIFiso4E to the viral RNA [34•]. Sequestration of eIFiso4E by VPg likely results in a global impairment of translation, reminiscent of the role played by 4EBPs in mammals. Translation of AMV genomic RNA is stimulated by binding of several coat protein molecules to its 3′-end, apparently mimicking PABP function [35•]. On the other hand, *in vitro* translation of the non-capped/3′-poly(A)-tailed Tobacco etch virus (TEV) genomic RNA requires a 143-nt TE in the 5′ UTR that effectively recruits eIF4G [27•]. The TEV TE also promotes translation when positioned between the two ORFs of an uncapped dicistronic mRNA. In this case, initiation is not via the re-initiation of a ribosome after scanning the first ORF, but from initiation at a site between the two ORFs. PABP stimulates this eIF4G-mediated initiation event, possibly by stabilizing an interaction between eIF4G and the poly(A) tail. The animal encephalomyocarditis virus internal ribosome entry site (IRES) element was shown to promote translation of the downstream ORF of a dicistronic mRNA in tobacco [36•]. These final examples suggest that cap-independent internal ribosomal entry may regulate the translation of a subset of plant mRNAs.

The disruption of 5′-cap–3′-poly(A)-tail interactions may result in global repression of translation that only a subset of mRNAs can escape. Heat shock globally dampens protein synthesis because it reduces 5′-cap/3′-poly(A)-tail-dependent initiation [37]. Both the 5′ UTR of maize heat shock protein *HSP70* mRNA and the 5′ TE of Tobacco mosaic virus (TMV) genomic RNA (known as Ω) confer a translational advantage in heat shocked cells [38]. HSP101, a multifunctional protein with RNA-binding activity, binds the Ω sequence and functionally promotes the translation of mRNAs that have the TE in yeast cells [39]. HSP101 also binds the 5′ UTR of the pea *FERREDOXIN1* (*FED1*) mRNA *in vitro*. This leader, which integrates photosynthetic status with *FED1* mRNA stability and translation [1•,2•], reduces the thermal repression of translation in carrot cells [40•], plausibly as a result of its interaction with HSP101. Heat shock of wheat leaves causes rapid phosphorylation of eIF4A and partial dephosphorylation of eIF4B, eIF4G and eIFiso4G, but has no apparent effect on PABP [18••]. Comparison of the components of the cap-binding complex, by affinity purification with ^{7m}GTP or poly(A) RNA, reveals that heat shock reduces the frequency with which eIF4G and eIFiso4G are associated with PABP [18••]. The expected consequence is impaired translation of 5′-capped–3′-poly(A) mRNAs that require these interactions for initiation. One hypothesis to explain the efficient translation of *HSP* mRNAs under heat shock is that these mRNAs establish a thermostable complex that maintains initiation, perhaps by an alternative means of circularization. A likely candidate in this complex is HSP101. The translational advantage conferred by the 5′ and 3′ sequences of the maize *ALCOHOL DEHYDROGENASE1* (*ADH1*) mRNA in hypoxic cells also suggests that specialized interactions are also necessary to escape translational repression during oxygen deprivation [41]. It remains to be determined if 5′-cap–3′-tail interactions are involved in the pollen-stage-specific translation of tobacco *NTP303* (*Nicotiana tabacum* pollen303) and *LAT52* (*Lycopersicon esculentum* late pollen transcript52) mRNAs, which were shown to be mediated by 5′ UTR sequences [11,12•].

Translational regulation of mRNAs with upstream ORFs

One or more small upstream ORFs (uORFs) with at least two codons are found in the 5′-leader of a subset of plant mRNAs. uORFs are evolutionarily conserved in mRNAs encoding *S*-adenosylmethionine decarboxylase (AdoMetDC) [42,43], ornithine decarboxylase [44], H^+-ATPase [45], and basic leucine zipper (bZIP) and myb transcription factors [46,47]. mRNAs of plant pararetroviruses, which include Cauliflower mosaic virus (CaMV), also possess uORFs [48]. The relevance of uORFs to translation depends upon one or more of the following: the length of the uORF, the peptide encoded by the uORF, the spacing and/or sequences in the intercistronic region, and the physiological status of the cell [43].

The presence of uORFs usually impedes initiation at the downstream coding ORF. A classical example of this is the translation of yeast *GENERAL CONTROL NON-DEPRESSIBLE4* (*GCN4*) mRNA, which has four uORFs and is translated only under amino-acid starvation when protein synthesis is globally repressed. The inhibitory effect of *GCN4* uORF4 is released when

eIF2α phosphorylation reduces the availability of the eIF2α–GTP–tRNA^{met} ternary complex, resulting in the prolonged scanning of the 5′-leader by the 40S subunit [13,43]. Recently, the phosphorylation-mediated regulation of *GCN4* mRNA translation was functionally complemented in yeast with wheat eIF2α [49••], suggesting that the regulation of eIF2α phosphorylation and its role in uORF translation may be conserved in plants. The maize *R-Lc* mRNA, encoding a transcriptional activator, is poorly translated due to a 5′ proximal stem-loop structure that inhibits primary initiation of translation, an uORF that impairs reinitiation, and an AU-rich intercistronic sequence that inhibits reinitiation at the coding ORF [50,51]. Similarly, repression of translation of the rice *MYB7* mRNA depends on an uORF and the intercistronic region [47]. In contrast, four uORFs are necessary for the sucrose-mediated repression of *ATB2* mRNA, encoding a bZIP protein, in *Arabidopsis* [9]. The inhibitory nature of an uORF can be due to the synthesis of an uORF-encoded polypeptide that locally blocks reinitiation under certain conditions, as observed for mammalian AdoMetDC [43]. Chang *et al.* [52] provide evidence that a uORF-encoded peptide of an arginine decarboxylase mRNA of tomato inhibits translation *in vitro*, suggesting that translational regulation is important in polyamine biosynthesis.

The presence of an uORF can also result in a translational advantage, as elegantly demonstrated for the polycistronic CaMV 35S mRNA. Cap-dependent translation of the 35S transcript involves an uORF that is positioned just 5′ of a stable 478-nt hairpin in the 600-nt 5′-leader. Translation of a downstream ORF requires the correct termination of translation of the uORF, which allows the ribosome to 'shunt' past the hairpin and reinitiate translation [53•]. The translational activator protein (TAV), an RNA-binding protein encoded by the virus, facilitates ribosome shunting by relocating eIF3 from the 40S subunit and the 60S subunit after translation of the uORF. After termination and peptide release, eIF3 is relocated to the 40S subunit via TAV and the 40S-subunit–TAV–eIF3 complex efficiently recruits the eIF2α–GTP–tRNA^{met} ternary complex allowing the shunted ribosome to scan and re-initiate at a downstream ORF [54••]. It is plausible that ribosome shunting may occur on endogenous plant mRNAs.

Conclusions

Regulation of the initiation of translation can contribute significantly to the level of expression of a gene. Efficiently translated mRNAs are adept at recruiting initiation factors, maintaining interactions between the 5′- and 3′-ends of the mRNA, ribosome scanning and/or recognizing the initiation codon. However, the translation of individual transcripts can be affected by global repression of protein synthesis, developmental program or changes in cell physiology. It is anticipated that future genomic-level examination of translational regulation will define mRNA sequences and translation factor modifications that interpret complex and dynamic changes in the cellular milieu to modulate protein synthesis.

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