

Selective translation of cytoplasmic mRNAs in plants

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Translation of mRNA is emerging as an important mode of gene regulation in plants. It is frequently controlled at initiation and appears to be regulated by competition for limiting translational components, different requirements for specific factors and *cis*-acting mRNA elements. Recent studies indicate that interactions between the 5' and 3' ends of the message enhance translation, perhaps by facilitating recruitment of initiation factors or enhancing ribosome recycling. Normal development and environmental stimuli modulate the phosphorylation of components of the mRNA 5'-cap-binding complex, ribosomes and mRNA-binding proteins. These modifications might be responsible for changes in the hierarchy of mRNAs that are in competition for translation.

Translational efficiency is defined as the rate of polypeptide synthesis per mRNA per unit time. Levels of protein synthesis are therefore determined not only by message abundance but also by the ability of the transcript to be successfully translated. Translation is divided into three kinetic phases^{1,2}:

- Recruitment of mRNA to the ribosome (initiation),
- Polypeptide chain synthesis (elongation),
- Polypeptide chain release (termination).

The storage of mRNA on messenger ribonucleoproteins (mRNPs) and rates of reinitiation (ribosome recycling) also affect translation dynamics (Fig. 1).

Regulation of mRNA translation is evident in response to environmental stimuli and growth-regulatory substances and also during development. Such control most frequently occurs at initiation, but is also observed at elongation (Table 1). This review focuses on initiation because it is usually the rate-limiting step and very little is known about regulation of elongation and termination. Selective mRNA translation is most obvious under stress conditions that globally downregulate protein synthesis, such as heat shock and oxygen deprivation (anoxia), as translation is limited to a subset of cytoplasmic mRNAs. For example, in anoxic roots of maize, many normal cellular mRNAs are synthesized but their translation is extremely limited; by contrast, a few genes are induced by this stress at the level of transcription and their mRNAs are selectively translated^{3,4} (Fig. 2). The reduced association of normal cellular mRNAs with polysomes in anoxic roots suggests that these mRNAs are poorly recruited to the translational machinery. Also, selective translation of heat shock protein (Hsp) mRNAs and sequestration of many normal cellular protein mRNAs into perinuclear granules occurs in response to heat shock of tobacco suspension culture cells⁵. By contrast, jasmonic acid treatment only modestly affects total levels of protein synthesis but clearly promotes selective translation⁶. Perhaps even more striking is the dynamic modulation of translation of certain mRNAs in response to normal light-dark regimes⁷ (Fig. 2). Gene regulation at the level of mRNA recruitment to ribosomes allows for rapid responses to transient environmental stimuli and might also be important during development. Although our knowledge of the translational machinery and *cis*-acting elements involved in mRNA translation in plants is incomplete, it is evident that selective translation is an interesting and important mode of gene regulation.

Initiation of translation – the selection process

Recruitment of mRNA for translation is a complex process that requires interactions between the mRNA, the two ribosomal subunits and at least nine translation factors, as well as the hydrolysis of ATP and GTP (Refs 1,2). In cap-dependent translation, the eukaryotic initiation factor 4 (eIF4) components are responsible

for the selection of 5'-capped transcripts and the removal of the secondary structure to facilitate 5' to 3' migration of the 43S pre-initiation complex towards the initiator AUG (Table 2). The 43S pre-initiation complex includes the mRNA, eIF4 proteins, eIF2-tRNA^{met}-40S ribosomal subunit complex, eIF3, eIF1A and eIF5 (Refs 1,8). The pre-initiation complex scans for the first AUG triplet in the appropriate sequence context. Recognition of the initiation-codon is assisted by Watson-Crick base pairing, eIF1 and eIF1A. Initiation is completed by the binding of the 60S ribosomal subunit and hydrolysis of GTP (Refs 2,9). Specific *trans*-acting factors might be needed for efficient translation of certain messages.

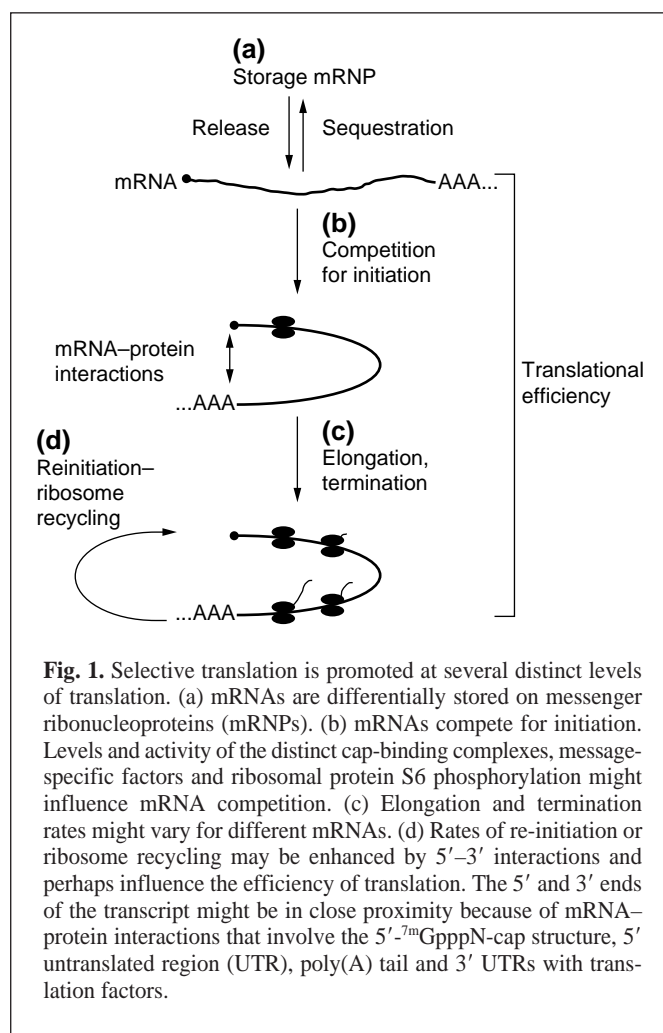


Fig. 1. Selective translation is promoted at several distinct levels of translation. (a) mRNAs are differentially stored on messenger ribonucleoproteins (mRNPs). (b) mRNAs compete for initiation. Levels and activity of the distinct cap-binding complexes, message-specific factors and ribosomal protein S6 phosphorylation might influence mRNA competition. (c) Elongation and termination rates might vary for different mRNAs. (d) Rates of re-initiation or ribosome recycling may be enhanced by 5'-3' interactions and perhaps influence the efficiency of translation. The 5' and 3' ends of the transcript might be in close proximity because of mRNA-protein interactions that involve the 5'-7mGpppN-cap structure, 5' untranslated region (UTR), poly(A) tail and 3' UTRs with translation factors.

Table 1. Examples of selective translation of plant gene transcripts

Stimulus	Plant	Translation dynamics	Proposed level of regulation ^a
Anoxia or hypoxia	Maize ^{3,4}	Selective synthesis of anaerobic polypeptides (ANPs) Impaired translation of normal cellular protein mRNAs	I I, E
	Potato ⁴⁴	Selective synthesis of ANPs Impaired translation of wound-response mRNAs	I E
Heat shock	Tomato suspension cultures ³	Selective synthesis of heat shock proteins (Hsps) Impaired translation of normal cellular protein mRNAs	I
	Carrot protoplasts ^{40,42}	Efficient translation of chimeric mRNAs with Hsp70 5' UTR Poor translation of capped-polyadenylated reporter transcripts	I
Light	Tobacco leaves ⁷	Translation of Lhcb and pea Fed-1 mRNA with photosynthesis	I
Auxin or cytokinin	<i>Arabidopsis</i> cell cultures ⁵	Required for rpS6 translation after stationary growth	I
Jasmonic acid	Barley leaves ⁶	Selective synthesis of plastid leucyl-tRNA synthetase Impaired translation of RbcS and Lhcb mRNA	I
Sucrose	<i>Arabidopsis</i> seedlings ⁴⁵	Translation of bZip protein ATB2 mRNA repressed by sucrose	I
Programmed cell death	Tobacco leaves ⁴⁶	Impaired translation of cytosolic ascorbate peroxidase mRNA	E

^aRegulation at initiation (I) or elongation (E); ^bF. Turck *et al.*, unpublished.

Abbreviations: UTR, untranslated region; Fed-1, pea ferredoxin-1; Lhcb, chlorophyll *a/b* binding protein; rpS6, ribosomal protein S6; RbcS, ribulose biphosphate carboxylase small subunit.

The mRNA selection machinery

The first phase of recruitment of mRNA for translation is mediated by a protein that binds to the 5'-7^mGpppN cap of the mRNA (Refs 1,2). In mammals, availability of the cap-binding protein eIF4E is regulated by its interactions with 4E-binding proteins (4EBPs)¹⁰. The eIF4E and 4EBP proteins form a complex that dissociates upon hyperphosphorylation of 4EBP, which allows for subsequent phosphorylation of eIF4E and interaction with eIF4G. The eIF4G protein has been described as the 'adaptor' molecule that forms a bridge from the 5' cap via its interaction with eIF4E to the eIF3-ribosome complex¹¹. This adaptor molecule also interacts with eIF4A – an RNA-dependant helicase – and eIF4B – an RNA-binding protein that is thought to interact with mRNA and 18S rRNA (Refs 1,2). In mammals, the formation of eIF4 complexes, which is regulated by the availability of eIF4E, governs the global rates of translation and mediates mRNA selection¹⁰.

Plants possess at least three distinct cap-binding proteins (Table 2). The plant eIF4E homolog interacts with the plant eIF4G homolog. There is also a second and distinct cap-binding protein, eIFiso4E, that is apparently unique to plants. This protein complexes with eIFiso4G, an 86 kDa protein unique to plants that also interacts with eIF4A, eIF4B and poly(A) binding protein (PABP)^{1,12}. Interestingly, eIFiso4G co-localizes with maize microtubules¹³, but the connection between this interaction and translation is not known. A third cap-binding protein, nCBP, was recently identified in *Arabidopsis*. It interacts with eIFiso4G in the yeast two-hybrid system and functionally replaces eIF4E or eIFiso4E in an *in vitro* translation system¹⁴. The function of nCBP is not known, although a higher binding affinity of nCBP to

7^mGpppG might suggest a role in mRNA sequestration¹⁴. An nCBP homolog also exists in mammals.

The presence of multiple cap-binding proteins raises the engaging question of whether these factors regulate selective mRNA translation through their abundance, availability or activity. Levels of eIF4E and eIFiso4E are modulated during seed development in wheat¹⁵, and eIFiso4F (the complex of eIFiso4E and eIFiso4G) is more abundant than eIF4F (the complex of eIF4E and eIF4G) in wheat germ and maize root tips¹. In addition, mRNAs that encode eIF4E and eIFiso4E are differentially expressed during *Arabidopsis* development¹⁶.

Biochemical analyses reveal that wheat eIF4F and eIFiso4F are functionally distinct in their interaction with the mRNA 5' cap structure¹. It has been shown that eIFiso4F has a lower affinity for monomethylated cap analogs than for dimethylated cap analogs, whereas the binding preference for eIF4F is the opposite. Given this, it would be interesting to determine if there is differential cap methylation in plants or unusual forms of caps, as observed in *Caenorhabditis elegans*. Furthermore, wheat eIFiso4F binds more efficiently to oligoribonucleotides with a more exposed cap than does eIF4F (Ref. 1).

As cytosolic acidification frequently occurs in response to environmental stimuli, it might be significant that eIFiso4F has a narrow pH optimum at pH 7.6 for binding of 7^mGpppG, whereas binding by eIF4F is not markedly different within the physiological pH range of pH 6.8 to 7.6. Interestingly, eIF4E is phosphorylated in response to hypoxia in maize root tips, whereas no change in eIFiso4E electrophoretic mobility can be detected (S. Manjunath and J. Bailey-Serres, unpublished). This phosphorylation can be

stimulated by treatment of roots with caffeine, which increases the level of cytosolic calcium in maize protoplasts¹⁷. These observations might be of significance if the eIF4E isoforms have different affinities for the 5'-mRNA cap structure or another protein.

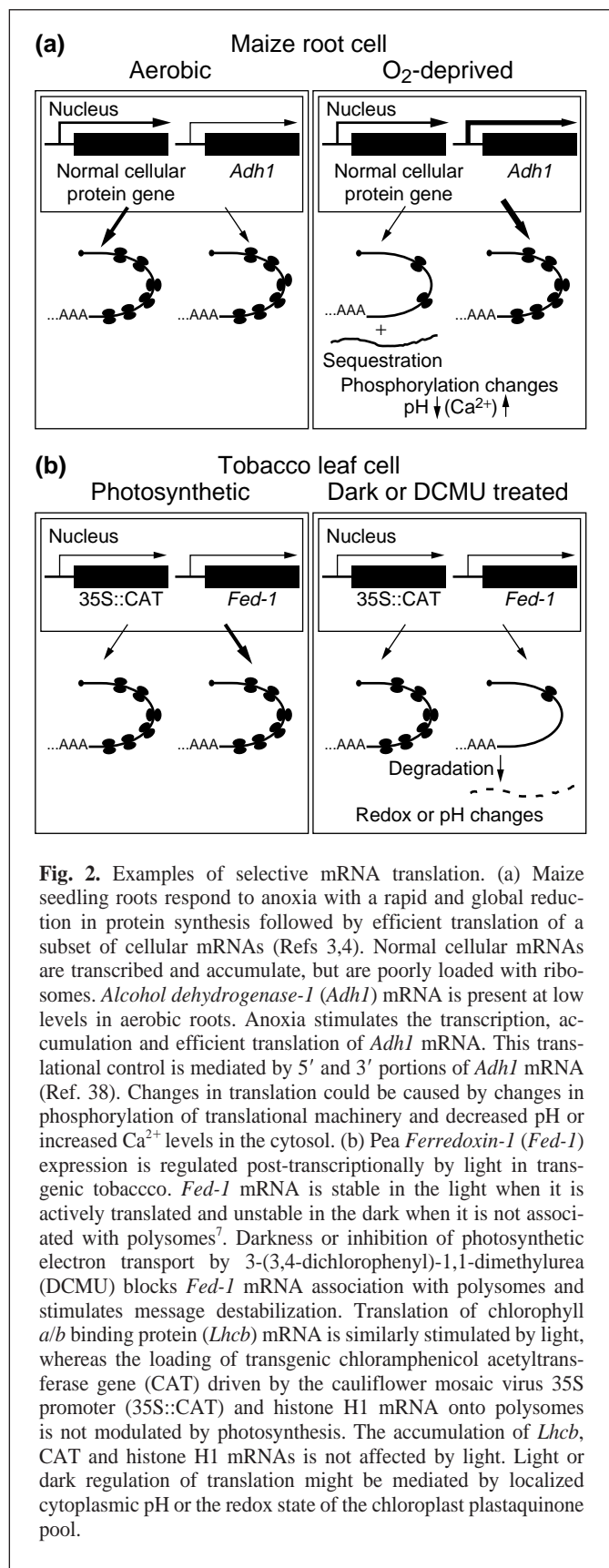


Fig. 2. Examples of selective mRNA translation. (a) Maize seedling roots respond to anoxia with a rapid and global reduction in protein synthesis followed by efficient translation of a subset of cellular mRNAs (Refs 3,4). Normal cellular mRNAs are transcribed and accumulate, but are poorly loaded with ribosomes. *Alcohol dehydrogenase-1 (Adh1)* mRNA is present at low levels in aerobic roots. Anoxia stimulates the transcription, accumulation and efficient translation of *Adh1* mRNA. This translational control is mediated by 5' and 3' portions of *Adh1* mRNA (Ref. 38). Changes in translation could be caused by changes in phosphorylation of translational machinery and decreased pH or increased Ca²⁺ levels in the cytosol. (b) Pea *Ferredoxin-1 (Fed-1)* expression is regulated post-transcriptionally by light in transgenic tobacco. *Fed-1* mRNA is stable in the light when it is actively translated and unstable in the dark when it is not associated with polysomes⁷. Darkness or inhibition of photosynthetic electron transport by 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) blocks *Fed-1* mRNA association with polysomes and stimulates message destabilization. Translation of chlorophyll *a/b* binding protein (*Lhcb*) mRNA is similarly stimulated by light, whereas the loading of transgenic chloramphenicol acetyltransferase gene (CAT) driven by the cauliflower mosaic virus 35S promoter (35S::CAT) and histone H1 mRNA onto polysomes is not modulated by photosynthesis. The accumulation of *Lhcb*, CAT and histone H1 mRNAs is not affected by light. Light or dark regulation of translation might be mediated by localized cytoplasmic pH or the redox state of the chloroplast plastoquinone pool.

Both eIF4A and eIF4B are also post-translationally modified in plants (Table 2). Moreover, eIF4A is present in phosphorylated and non-phosphorylated forms. The level of phosphorylated eIF4A increases to ~50% in response to oxygen deprivation¹⁸ and heat shock¹⁹. Increased eIF4A phosphorylation is also observed during pollen tube germination in tobacco²⁰ and seed maturation in wheat, when the maternal nucellus undergoes apoptosis²¹. Hence, eIF4A phosphorylation could be involved in selective translation. Multiple phosphorylation of the RNA binding-protein eIF4B is observed when levels of protein synthesis are high, such as during seed germination, but eIF4B phosphorylation decreases during late stages of seed maturation²¹ and in response to heat shock¹⁹, when levels of protein synthesis are reduced. The phosphorylation status of eIF4A and eIF4B might influence protein-protein or protein-mRNA interactions that are important for mRNA selection or global levels of translation.

Ribosomal protein S6 phosphorylation

The phosphorylated ribosomal protein S6 plays an important role in the selective translation of mRNAs that possess a 5' terminal oligopyrimidine tract (5' TOP) in mammals²². The 5' TOP mRNAs encode many components of the translational machinery, such as ribosomal proteins. S6 is dephosphorylated in serum-starved cells and 5' TOP mRNAs are not associated with polysomes. Stimulation of mitogenesis promotes S6 phosphorylation, concomitant with recruitment of 5' TOP mRNAs onto polysomes. This selective translation is probably mediated via interaction of a *trans*-acting factor with a structural element of the mRNA.

In plants the modulation of S6 phosphorylation is observed in several instances (Table 2). In *Arabidopsis*, heat shock results in S6 dephosphorylation by heat inactivation of the S6 kinase²³. The mRNA that encodes ribosomal protein S6 is not associated with polysomes and S6 is dephosphorylated in cultured *Arabidopsis* cells before subculture into fresh medium; whereas this message shifts to large polysomes and S6 is phosphorylated in cells transferred to fresh medium containing auxin and cytokinin (F. Turck *et al.*, unpublished). Interestingly, polypyrimidine-rich tracts are located within the 5' untranslated regions (UTRs) of many plant gene transcripts but not specifically at the 5' termini of ribosomal protein mRNAs (A.J. Williams and J. Bailey-Serres, unpublished). It will be important to determine if S6 phosphorylation alone, or in concert with other changes in the translational machinery, impacts the selection of mRNAs for translation.

Role of the two ends of the message in selection

Plant cytoplasmic mRNAs are typically monocistronic and possess a terminal ^mG(5')ppp(5')N-cap structure (where N is the first nucleotide of the mRNA), a 5' UTR, a single open reading frame (ORF), a 3' UTR and a 3' poly(A) tail. Initiating translation of most cytoplasmic mRNAs involves a 5' cap-dependent scanning procedure, but cap-independent initiation and exceptional modes of cap-dependent initiation also occur (Fig. 3). Several unusual modes of initiation have been observed for viral mRNAs and a few plant transcripts (for reviews see Refs 24,25). Modes of initiation used by viruses are important to consider because similar schemes might regulate the expression of some plant-encoded genes.

5' UTRs

Features of the 5' UTR that affect the translational efficiency include the 5' cap structure, the leader length and sequence, the presence of secondary structure(s), the context of the AUG codon and the presence of upstream open reading frames (uORFs). The 5'-cap facilitates cap-dependent initiation through its interaction

Table 2. Proteins involved in recruitment of mRNA for translation

Factors	Description	Effect of various stimuli on phosphorylation
mRNA binding to 40S subunit pre-initiation complex		
eIF4G	~200 kDa phosphoprotein ¹	None reported
eIF4E	26 kDa cap-binding protein; interacts with eIF4G ¹	Anoxia (maize) – phosphorylation ^a Heat shock (wheat) – no effect ¹⁹
eFiso4G	86 kDa phosphoprotein; microtubule-associated protein ¹	None reported
eFiso4E	28 kDa cap-binding protein; interacts with eFiso4G ¹	Anoxia (maize) – no effect ^a Heat shock (wheat) – no effect ¹⁹
nCBP	24 kDa novel cap-binding protein; might interact with eFiso4G; might sequester capped transcripts ¹⁴	None reported
mRNA unwinding in association with the cap-binding complex		
eIF4A	46 kDa ATPase, RNA helicase; interacts with eIF4B ¹	Anoxia (maize) – phosphorylation ¹⁸ Heat shock (wheat) – phosphorylation ¹⁹ Seed development (wheat) – phosphorylation ²¹ Germination (wheat) – dephosphorylation ²¹ Pollen tube germination (tobacco) – phosphorylation ²⁰
eIF4B	59 kDa RNA-binding protein; interacts with eIF4A ¹ , 18S rRNA and poly(A)-binding protein ¹²	Heat shock (wheat) – dephosphorylation ¹⁹ Seed desiccation (wheat) – dephosphorylation ²¹ Germination (wheat) – phosphorylation ²¹
mRNA–40S-subunit interaction^b		
Ribosomal protein S6	30 kDa, 40S subunit protein ¹	Anoxia (maize) – dephosphorylation ⁴⁷ Heat shock (tobacco) – dephosphorylation ⁵ Heat shock (<i>Arabidopsis</i>) – dephosphorylation ^c Germination (maize) – phosphorylation ⁴⁸ Cell subculture (<i>Arabidopsis</i>) – phosphorylation ²³
5' and 3' UTR interactions		
p102	Hsp101 (Ref. 32); RNA-binding protein interacts with 5' and 3' UTRs of TMV	None reported
Poly(A) binding protein	68 kDa, binds poly(A) tail; interacts with eFiso4G and eIF4B ¹²	Heat shock (wheat) – no effect ¹⁹
Initiation on CaMV 35S mRNA and dicistronic mRNAs		
TAV	60 kDa transactivator ²⁴ RNA-binding protein of CaMV; stimulates translation of downstream ORF	None reported

^aS. Manjunath and J. Bailey-Serres, unpublished.

^bThe interaction may be mediated through a general or specific translation factor or mRNA-binding protein.

^cF. Turck *et al.*, unpublished.

Abbreviations: eIF, eukaryotic initiation factor; UTR, untranslated region; Hsp, heat shock protein; TMV, tobacco mosaic virus; CaMV, cauliflower mosaic virus; TAV, CaMV-encoded RNA-binding protein.

with a cap-binding protein. The only known uncapped transcripts in plant cells are of viral origin. The 5' UTRs of plant mRNAs are usually <100 nucleotides (nt) and generally lack GC or AU tracts that might form stable hairpin structures that could impede the scanning of the 43S pre-initiation complex²⁶. Extremely long leaders (>200 nt) might employ unusual modes of initiation. The nucleotide sequence context of the AUG codon also plays a role in the efficiency of translation initiation²⁴. The statistically-determined consensus sequence of monocot initiation codons is (A/G)(A/C)cAUGGC and that of dicots is A(A/C)aAUGGC (Ref. 27). Optimization of the AUG context improves expression of heterologous genes, although initiation at poor context AUGs and at non-AUG codons occurs on certain viral mRNAs, and at reduced efficiency in plant cell protoplasts²⁴.

3' UTRs

The 3' UTRs of plant transcripts are two to three times longer (average length of 240 nt), significantly more U-rich than 5' UTRs and terminate with a 3' poly(A) tail of 50–150 nt (Ref. 22). The poly(A) tail is involved in mechanisms that regulate stability as well as translation²⁸. Frequently there is heterogeneity in the site selected for pre-mRNA cleavage and polyadenylation, resulting in variation in 3' UTR length. Modulation of poly(A) tail length has also been observed. Although no ramifications on translation by poly(A) site selection or natural variation in poly(A) tail length have been reported in plants, either could engender translational regulation.

5'-cap and 3'-poly(A) tail interactions

Presence of a 5' cap structure and a 3' poly(A) tail promotes a synergistic enhancement of translation of mRNAs in plant and

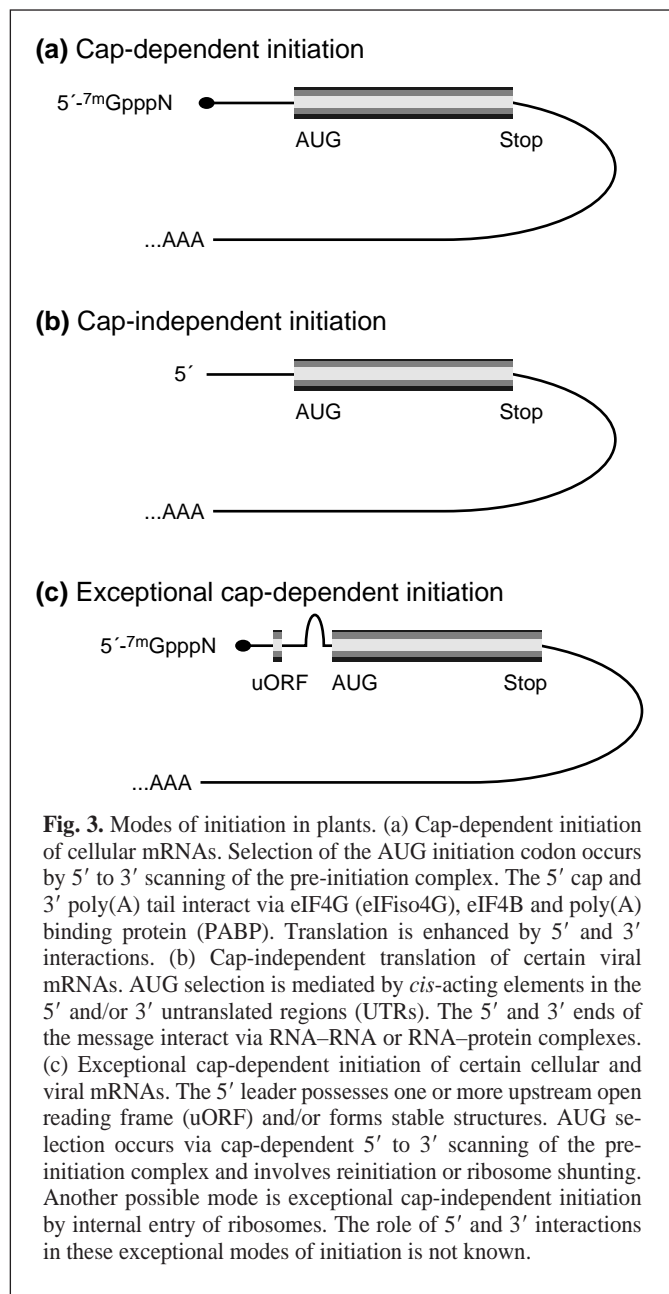


Fig. 3. Modes of initiation in plants. (a) Cap-dependent initiation of cellular mRNAs. Selection of the AUG initiation codon occurs by 5' to 3' scanning of the pre-initiation complex. The 5' cap and 3' poly(A) tail interact via eIF4G (eIFiso4G), eIF4B and poly(A) binding protein (PABP). Translation is enhanced by 5' and 3' interactions. (b) Cap-independent translation of certain viral mRNAs. AUG selection is mediated by *cis*-acting elements in the 5' and/or 3' untranslated regions (UTRs). The 5' and 3' ends of the message interact via RNA–RNA or RNA–protein complexes. (c) Exceptional cap-dependent initiation of certain cellular and viral mRNAs. The 5' leader possesses one or more upstream open reading frame (uORF) and/or forms stable structures. AUG selection occurs via cap-dependent 5' to 3' scanning of the pre-initiation complex and involves reinitiation or ribosome shunting. Another possible mode is exceptional cap-independent initiation by internal entry of ribosomes. The role of 5' and 3' interactions in these exceptional modes of initiation is not known.

yeast cell protoplasts and in a yeast *in vitro* translation system²⁸. These observations led to the prediction that interaction between the 5' and 3' transcript termini causes circularization of actively translated mRNAs. Biochemical studies indicate that the interaction of 5' and 3' termini involves PABP and components of the cap-binding complex, eIF4G (or eIFiso4G) and eIF4E (or eIFiso4E)^{12,29}. It was also shown that PABP increases the binding of the cap-binding complex to cap analogs *in vitro*³⁰. The circular translation complex model is further supported by atomic force microscopy visualization of a circular complex formed following incubation of 5'-capped mRNA with yeast eIF4E, eIF4G and PABP (Ref. 31). In plants, 5'–3' interactions via PABP and the cap-binding complex might be further promoted by eIF4B, because this factor stimulates the binding of PABP to poly(A)^{12,28}. The 5'–3' interactions might promote the selection of mRNA for initiation, ribosome recycling, and/or ensure that scanning proceeds from the 5' end of correctly capped and polyadenylated transcripts.

Role of specific mRNA elements in selection

There is limited information on *cis*-acting mRNA elements that regulate initiation in plants. Sequences that enhance translation might serve to improve the recruitment of the translational machinery to the message. Intriguingly, *cis*-acting elements have been found at both the 5' and 3' ends of transcripts. Hence, certain *cis*-acting elements might promote 5'–3' interactions that improve the effective use of limiting translational components. Such mRNA elements or regions known to affect the recruitment of the translational apparatus fall into three categories:

- 5' and/or 3' UTRs of viral mRNAs that enhance translation in host cells,
- 5' and 3' regions of plant mRNAs that enhance translation under specific environmental conditions or in certain cell types,
- Complex 5' leaders that promote unusual modes of initiation.

Viral 5' and 3' UTR sequences that enhance translation

Several viral mRNAs that lack a 5' cap and/or a 3' poly(A) tail are competitively translated because of the presence of *cis*-acting elements in their 5' and/or 3' UTRs (Fig. 4). Tobacco mosaic virus (TMV) mRNA, for example, is a 5'-capped and non-polyadenylated transcript that is efficiently translated. The 68 nt 5' translational enhancer sequence (Ω) of TMV interacts with a 3' UTR pseudo-knot structure via a 102-kDa protein that was identified as Hsp101 by complementation in yeast³². Genetic analyses demonstrate that the interaction between Ω and Hsp101 requires eIF3 and, specifically, one of the two eIF4Gs of yeast³². It will be interesting to study the role of Hsp101 in plants because its stimulation of Ω -*luc* mRNA translation is reduced in yeast by amino acid starvation and in late-exponential growth.

The alfalfa mosaic virus (AMV) coat protein mRNA is also capped and non-polyadenylated and possesses elements that enhance translation in its 5' and 3' UTRs (Ref. 33). Competitive translation of the 5' capless but polyadenylated tobacco etch virus (TEV) mRNAs involves a 5' UTR enhancer and requires the poly(A) tail^{24,28}. Interestingly, mRNAs that lack both a 5' cap and a 3' poly(A) tail, such as the barley yellow dwarf (BYDV) and satellite tobacco necrosis (STNV) virus mRNAs can outcompete plant-encoded 5' capped mRNAs for translation because of lower requirements for cap-binding factors^{34,35}. The BYDV enhancer is situated 5 kb downstream of the stop codon and functions as a surrogate cap in recruitment of eIF4F (Ref. 35). The 120 nt STNV enhancer domain is immediately downstream of the stop codon, functions as a cap, and even further stimulates translation of capped transcripts^{36,37}. It was recently shown that both the BYDV and STNV 3' enhancers function when placed in the 5' portion of a message^{35,37}, raising the possibility that 5'–3' interactions are not an absolute requirement for efficient translation.

Plant 5' and 3' sequences regulate translation

Sequences in both the 5' and 3' UTRs of plant mRNAs modulate developmentally and environmentally regulated expression (Fig. 4). For example, electroporation of capped and polyadenylated mRNA constructs into protoplasts demonstrates that the 5' UTR, a portion of the coding sequence and the 3' UTR of the maize *Adh1* mRNA is required for efficient expression in hypoxic protoplasts³⁸. In this case the sequence encoding the first 18 amino acids is required for efficient translation. Electroporation studies also demonstrate that both the 5' and 3' UTRs of the barley α -amylase gene are required for efficient expression in protoplasts derived from the aleurone layer of maize kernels³⁹. This translational enhancement is observed only in cells that normally express α -amylase. By contrast, mRNA electroporation studies show that the 5' UTR of Hsp70 mRNA is sufficient for translational enhancement in heat-shocked

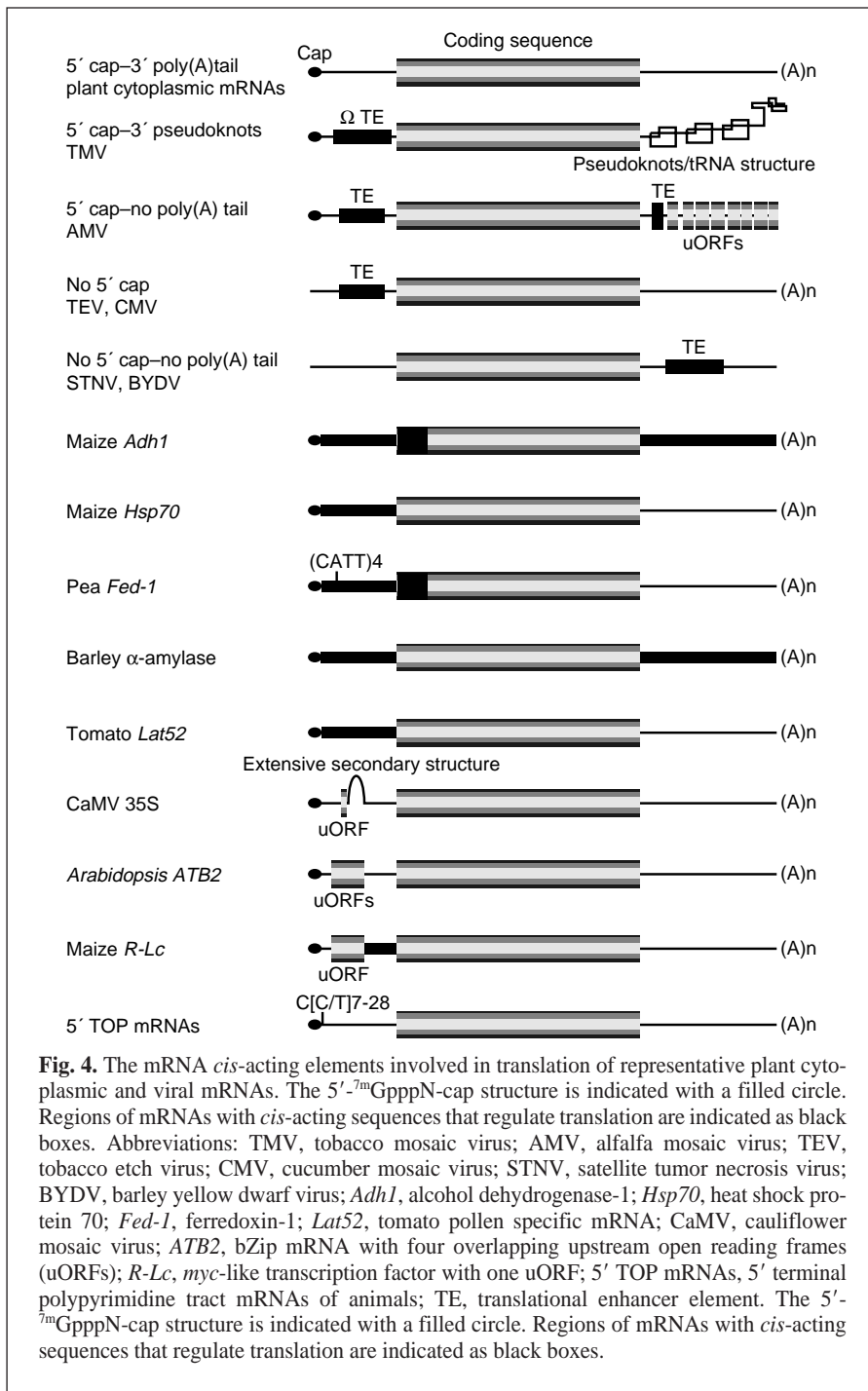


Fig. 4. The mRNA *cis*-acting elements involved in translation of representative plant cytoplasmic and viral mRNAs. The 5'-7^mGpppN-cap structure is indicated with a filled circle. Regions of mRNAs with *cis*-acting sequences that regulate translation are indicated as black boxes. Abbreviations: TMV, tobacco mosaic virus; AMV, alfalfa mosaic virus; TEV, tobacco etch virus; CMV, cucumber mosaic virus; STNV, satellite tumor necrosis virus; BYDV, barley yellow dwarf virus; *Adh1*, alcohol dehydrogenase-1; *Hsp70*, heat shock protein 70; *Fed-1*, ferredoxin-1; *Lat52*, tomato pollen specific mRNA; CaMV, cauliflower mosaic virus; *ATB2*, bZip mRNA with four overlapping upstream open reading frames (uORFs); *R-Lc*, *myc*-like transcription factor with one uORF; 5' TOP mRNAs, 5' terminal polypyrimidine tract mRNAs of animals; TE, translational enhancer element. The 5'-7^mGpppN-cap structure is indicated with a filled circle. Regions of mRNAs with *cis*-acting sequences that regulate translation are indicated as black boxes.

protoplasts⁴⁰, and that the 5' UTR of pollen-specific mRNA mediates efficient translation during late pollen development⁴¹. Neither of these analyses studied the effect of 3' UTR sequences on expression. It would be interesting to know if *Hsp70* mRNA translation occurs independently of 5' and 3' interactions, because heat shock disrupts the synergism between the cap and poly(A) tail of non-*Hsp* mRNAs in protoplasts⁴². There is clearly much more to be learned about specific nucleotide sequences and their roles in the regulation of plant mRNA translation.

Complex 5' UTRs that regulate translation

The most well-documented exceptional mode of initiation exists for the polycistronic 35S mRNA of cauliflower mosaic virus (CaMV) and other pararetroviral mRNAs (Fig. 4)^{24,25}. Cap-dependent

initiation from the 600 nt leader of 35S mRNA requires the presence of a short uORF directly preceding a stable hairpin structure. Ribosomes are thought to bypass the stable stem structure and re-initiate at the downstream functional ORF. The CaMV-encoded RNA-binding protein TAV stimulates this re-initiation on the downstream coding sequence of the 35S mRNA. TAV also facilitates translation of the second cistron of synthetic dicistronic mRNAs in protoplasts. Perhaps the role of TAV is to promote efficient translation of CaMV mRNA at the boundary of the cytoplasmic viral inclusion body.

Finally, a small proportion of plant genes possess one or more short uORFs upstream of the functional coding region²⁴. Translation of these mRNAs is generally repressed owing to poor re-initiation of ribosomes that have translated the uORF, but the exact mechanisms of repression differ between genes. In the case of the maize transcription factor *R-Lc*, translational repression results from the presence of a short uORF and the sequence that follows the ORF (Ref. 43). Remarkably, the mRNA that encodes an *Arabidopsis* bZip protein, *ATB2*, has four partially overlapping uORFs and is translationally regulated by levels of sucrose in vascular tissue⁴⁵. Repression of *ATB2* mRNA translation by sucrose requires the uORF region of the leader (Fig. 4). Although the regulation of translation by metabolites is well documented in yeast, this is the first indication of metabolite-mediated translational control in plants.

Future considerations

Selective mRNA translation provides a rapid mechanism for the modulation of protein synthesis in response to changes in cellular homeostasis and during development in plants. New tools, such as gene microarray technology, could be used to more rigorously demonstrate the modulation of mRNA recruitment to polysomes. Evidence to date indicates that selective translation is mediated by differential initiation factor requirements and/or changes in abundance and

activity of the translational apparatus. The subcellular localization of mRNA transcripts might also be important. The confirmation of interactions between the 5' and 3' ends of the message raises the question of whether these interactions promote the primary initiation event or ribosome recycling. Further biochemical and genetic analyses should reveal the identity and role of *cis*-acting elements and specific translation factors in translational dynamics, and might reveal the role of localized changes in pH, calcium and nucleotide triphosphates on mRNA selection.

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