

A new mechanism for translational control in plants

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The process of protein synthesis is well-conserved among eukaryotes [1]. However, regulation of translation differs between plants and animals [2]. One means of translational control involves eukaryotic initiation factor 4E (eIF4E), which binds the 5' cap structure of mRNA, and its partner eIF4G to recruit other initiation factors and ultimately the ribosome to the mRNA. Plants also contain an isoform, eukaryotic initiation factor iso4E (eIFiso4E), which pairs with eIFiso4G. The different biological roles of eIF4E/4G vs eIFiso4E/iso4G are unclear, but *Arabidopsis thaliana* (*At*) plants lacking eIFiso4E or eIFiso4G show developmental abnormalities [3]. In mammals, under low nutrient or various stress conditions, eIF4E-binding protein (4E-BP) inhibits translation initiation by preventing eIF4E from binding eIF4G and thus from recruiting the other factors to the mRNA (Fig. 1) [4]. Excessive translation initiation due to aberrant lack of inhibition of eIF4E by 4E-BP can lead to many types of cancer and metabolic disorders [5]. While in plants and animals eIF4E is structurally similar [6], plants lack an ortholog to the mammalian 4E-BP [7]. However, Patrick *et al.* [8] identified a different eIF4E binding protein (CBE1) in *Arabidopsis* that negatively regulates the accumulation of mRNAs that control the cell cycle and mitosis, via a mechanism that is currently still unknown.

In vertebrates, translation is often attenuated greatly during virus infection. This inhibits virus gene expression because viruses depend on the host's translation machinery. During virus infection, protein kinase R (PKR) becomes activated by binding to double-stranded

RNA, which is generated during RNA virus replication, and it then phosphorylates eIF2 α [9]. This prevents the exchange of GDP for GTP required for regeneration of the ternary complex eIF2•GTP•tRNA_iMet, necessary for the initiation of translation (Fig. 1). Plants lack a PKR homolog, and do not appear to use this mechanism as an antiviral response [10]. Given that translation attenuation is important for rapid response to changes in the environment or during virus infection, it is likely that such regulation takes place in plants by mechanisms that have yet to be discovered.

Indeed, a new mechanism of translation attenuation, apparently unique to plants, has been reported by the Bisaro lab in a recent issue of *The FEBS Journal* [11]. They provide evidence for translation attenuation mediated by phosphorylation of eIF4E and eIFiso4E by sucrose nonfermenting (SNF)-related kinase 1 (SnRK1). SnRK1 is an ortholog of yeast sucrose nonfermenting 1 (SNF1) and mammalian AMP-activated kinase (AMPK) that functions as a master regulator of energy homeostasis [12]. During biotic or abiotic stress, SNF1/AMPK1/SnRK1 activates energy-generating catabolic pathways and inhibits energy-consuming anabolic pathways, at the transcriptional level and post-translationally, by phosphorylation of key enzymes [13]. Here, Bruns *et al.* [11] identified two consensus SnRK1 target sites in *Arabidopsis* eIF4E and eIFiso4E that are absent in mammalian eIF4E. The purified SnRK1 kinase domain (SnRK1-KD), but not a catalytically inactive mutant (SnRK1-KDKR), phosphorylates the predicted target sites in *At* eIFiso4E. Consistent with this, SnRK1 interacts with *At* eIF4E and *At* eIFiso4E in a yeast

Abbreviations

4E-BP, eIF4E-binding protein; AMPK, AMP-activated kinase; eIF4E, eukaryotic initiation factor 4E; eIFiso4E, eukaryotic initiation factor iso4E; PKR, protein kinase R; SNF1, sucrose nonfermenting 1; SnRK1-KDKR, SnRK1 kinase domain inactive; SnRK1-KD, SnRK1 kinase domain; SnRK1, SNF1-related kinase 1.

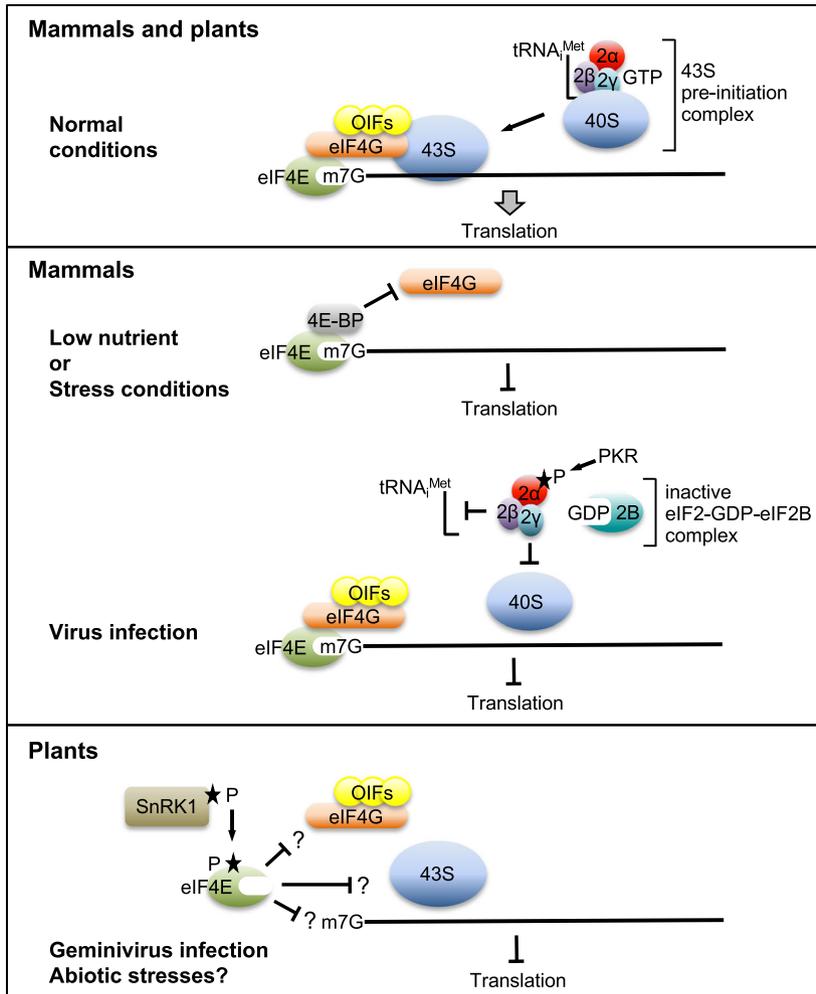


Fig. 1. Negative regulation of translation in mammals and plants. Top: In normal, unstressed conditions, cap-binding protein eIF4E simultaneously binds the m7GpppN cap structure on the mRNA and scaffolding protein eIF4G, which binds other initiation factors (OIFs) to recruit the 43S complex consisting of the 40S ribosomal subunit bound to the ternary complex of eIF2•GTP•tRNA^{Met}. Middle: In mammals, under low nutrient or stress conditions, eIF4E binding protein (4E-BP) binds eIF4E to prevent eIF4G binding, thus blocking translation initiation. In response to many virus infections, PKR phosphorylates eIF2 α , preventing exchange of GTP with GDP in eIF2B, which, in turn, prevents formation of the ternary complex. Bottom: In plants, Bruns *et al.* provide evidence that phosphorylation of eIF4E by SnRK1 attenuates translation initiation, but the interactions disrupted by this modification are unknown (question marks).

two-hybrid assay and in bimolecular fluorescent complementation assays in plant cells. Furthermore, SnRK1 phosphorylates *At* eIFiso4E when both are transiently co-expressed in plant cells.

To determine the effect of SnRK1-mediated phosphorylation on *At* eIF4E/iso4E activity, the authors expressed SnRK1 under a copper inducible promoter in a yeast strain that lacks endogenous eIF4E (*cdc33Δ*) but expresses *At* eIF4E, *At* eIFiso4E, or human eIF4E (*Hs* eIF4E). Interestingly, induction of SnRK1 increased doubling time of yeast that depended on *At* eIF4E or *At* eIFiso4E but not *Hs* eIF4E. This reduced growth of yeast is likely due to phosphorylation of *At* eIF4E/iso4E by SnRK1 because *Hs* eIF4E lacks the SnRK1 phosphorylation site present on plant eIF4E/iso4E. In the SnRK1-growth-inhibited yeast, there was a substantial reduction in translation as measured by a 50% reduction in the ratio of polysomes (ribosome-studded, actively translating mRNAs) to nonpolysomes (non-translating ribosomes). However, as predicted, no such

SnRK1-mediated reduction in translation was observed in yeast complemented with *Hs* eIF4E or nonphosphorylatable mutant *At* eIF4E-T67V/T91A (called *At* eIF4E-VA). A similar reduction in polysome/nonpolysome ratio was also observed in *Nicotiana benthamiana* plants engineered to overexpress SnRK1. Finally, transient overexpression of *At* eIF4E or *At* eIF4E-VA in SnRK1-overexpressing plants restored the polysome/nonpolysome ratio to that found in wild-type plants, presumably because the amount of overexpressed eIF4E was sufficient to allow full translation. As expected, overexpression of the phosphomimic mutant, *At* eIF4E-T67D, had no effect on the reduced polysome/nonpolysome ratio, because it behaves as a phosphorylated (inactivated) eIF4E under all conditions.

The results described in Bruns *et al.* [11] provide compelling evidence for translation attenuation due to SnRK1-mediated phosphorylation of eIF4E and eIFiso4E. However, many questions remain. How does phosphorylation inhibit eIF4E/iso4E activity (Fig. 1)?

Does it affect binding to eIF4G or to the 5' cap on the mRNA or other components of the translation machinery? Does SnRK1-mediated phosphorylation of eIF4E functionally replace the role that 4E-BP plays in mammals? If it does, then the phosphorylation state of eIF4E should increase under nutrient stress/low energy conditions in plants. Does SnRK1-mediated translation inhibition play an antiviral role? Previously, Bisaro's group showed that SnRK1 overexpression increases resistance to a geminivirus, while SnRK1 knockdown makes plants more susceptible to the virus [14]. Therefore, translation attenuation would inhibit geminivirus gene expression because its mRNAs, generated by host transcription machinery from the viral DNA, are translated by normal cellular mechanisms, unlike many RNA viruses that are translated cap-independently [15] and thus may not be subject to eIF4E regulation. How is translation of individual mRNAs affected by eIF4E phosphorylation? It is unlikely that all are affected equally. One would predict that mRNAs needed during low energy conditions would translate more efficiently than those that are not. Ribosome profiling [16] could be one approach to answer this question.

In summary, Bruns *et al.* have provided one answer to the complex question of how plants negatively control translation, identifying a novel regulatory mechanism that is unknown in mammals. How SnRK1-mediated eIF4E phosphorylation is regulated, and the biological pathways controlled by this mechanism remain to be determined.

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Conflict of interest

The authors declare no conflict of interest.

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