The role of the initiation surveillance complex in promoting efficient protein synthesis

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Abstract
Initiation is most often the rate-limiting step of translation. Translation initiation requires the involvement of numerous factors that assist binding of the 40 S ribosomal subunit to an mRNA and the assembly of the 80 S ribosome at the correct initiation codon. Recruitment of an initiation surveillance complex is required for translation and serves to identify mRNAs that are structurally and functionally competent for translation. For most cellular mRNAs, recruitment of the surveillance complex requires the 5′-cap and 3′-poly(A) tail. However, some cellular and viral mRNAs that naturally lack either of these have evolved alternatives that serve to recruit the complex. The initiation surveillance complex functions to stabilize eIF-4F (where eIF stands for eukaryotic initiation factor), the cap-binding complex, to the cap; promote eIF-4A helicase activity to remove secondary structure in the 5′-leader that might otherwise reduce 40 S ribosomal subunit scanning; promote eIF-4B binding to increase eIF-4A/eIF-4F function and stabilize binding of the poly(A)-binding protein to the poly(A) tail. The surveillance complex is regulated through changes in phosphorylation in response to environmental conditions or by developmental signals as a means to regulate globally protein synthesis. Thus the initiation surveillance complex ensures that only intact mRNAs are recruited for translation and serves to regulate protein synthesis.

Evidence for surveillance
The first evidence for translational surveillance was the observation that the poly(A) tail is as important as the 5′-cap in promoting protein synthesis and that both collaborate to recruit the 40 S ribosomal subunit [1,2]. These observations demonstrated that termini of an mRNA participate in the assembly of a competent translation-initiation complex that serves to exclude those RNAs not competent to produce an appropriate translational product. The requirement for both termini of an mRNA to promote initiation was observed even for mRNAs that naturally lack a 5′-cap or poly(A) tail. The cell-cycle-regulated histone mRNAs in metazoans and some algae are the only cellular mRNAs known to lack a poly(A) tail. Instead, these mRNAs terminate in a highly conserved stem-loop that binds the SLBP (stem-loop-binding protein) and serves as the functional equivalent to a cap in that it collaborates with the poly(A) tail to promote efficient translation [3,4].

Several viral mRNAs also lack a 5′-cap or poly(A) tail. The genomic mRNAs of TMV (tobacco mosaic virus), brome mosaic virus and turnip yellow mosaic virus terminate in a highly-structured 3′-UTR (3′-untranslated region), which, similar to the poly(A) tail, is essential for translation initiation [5,6]. The genomic mRNA of TEV (tobacco etch virus) lacks a 5′-cap but is polyadenylated. The TEV 5′-leader confers cap-independent translation and a functional analogue to a cap in that it collaborates with the poly(A) tail to promote efficient translation [5,7]. STNV (satellite tobacco necrosis virus) and barley yellow dwarf virus lack a 5′-cap and poly(A) tail but their 5′- and 3′-UTRs collaborate to promote translation [8,9]. Although considerable diversity exists in those RNA elements that promote translation, a common theme is the collaboration between functional elements at or near the termini of an mRNA to promote translation initiation.

Translatosome, the composition of the initiation surveillance complex
Assembly of a ribosome at an initiation codon requires the assistance of at least 11 conserved eIFs (eukaryotic initiation factors) that prepare an mRNA for binding to the 40 S ribosomal subunit, assist in initiation codon recognition and promote 60 S subunit joining [10]. Although their interaction with an mRNA is usually described as sequential, recent evidence indicates that several of these factors collaborate in an initiation surveillance complex that determines the functional competency of an mRNA. The composition of the complex as isolated by m′GTP-Sepharose or poly(A) agarose chromatography includes eIF-4E, eIF-4G, eIF-4A, eIF-4B, eIF-3 and the PABP (poly(A)-binding protein) [11]. eIF-4G is critical in organizing the complex in that it binds eIF-4E, the cap-binding subunit, and eIF-4A to form eIF-4F (Figure 1). eIF-4G also interacts with eIF-3, which recruits the 40 S subunit, and with PABP, which binds the poly(A) tail [12]. eIF-4A and eIF-4F exhibit RNA-dependent ATPase activity.

Key words: initiation factors, internal ribosomal entry site (IRES), phosphorylation, poly(A)-binding protein (PABP), protein–protein interactions, translation.

Abbreviations used: eIF, eukaryotic initiation factor; HSP, heat-shock protein; IRES, internal ribosomal entry site; PABP, poly(A)-binding protein; SLBP, stem-loop-binding protein; STNV, satellite tobacco necrosis virus; TEV, tobacco etch virus; TMV, tobacco mosaic virus; 3′-UTR, 3′-untranslated region
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Recruitment of the initiation surveillance complex

For typical cellular mRNAs, the 5′-cap and the poly(A) tail recruit the initiation surveillance complex through binding eIF4E and PABP respectively, both of which are required for 40 S subunit binding [18]. Collaboration of the 5′-cap and the poly(A) tail in the recruitment of the initiation surveillance complex suggests that the termini of an mRNA are held in close physical proximity during translation, a notion supported by electron micrographs of cells actively engaged in translation in which polysomes appear in circular or spiral forms [12] and in vitro when the surveillance complex components, eIF4E, eIF4G and PABP are bound to capped and polyadenylated mRNA [19]. The observation that translational efficiency increases with the length of the poly(A) tail can be understood through the co-operative binding of PABP to poly(A) [20–22], which increases its local concentration thus increasing the likelihood of recruitment of the initiation surveillance complex.

Those mRNAs that naturally lack a 5′-cap or poly(A) tail have often evolved alternative means to recruit the initiation surveillance complex. Cell-cycle-regulated histone mRNAs recruit the complex through the 5′-cap (thus tethering the complex through binding eIF4E) and the 3′-terminal-bound SLBP, which tethers the complex through its interaction with eIF4G and eIF3 [4]. Although PABP is not required for cell-cycle-regulated histone mRNA translation, the interaction of SLBP with the initiation surveillance complex does not affect the status of PABP in the complex [4]. In a similar manner, rotaviral mRNAs, which lack a poly(A) tail, recruit the initiation surveillance complex using their 5′-cap and a viral protein, NSP3, which binds the viral RNA 3′-terminus and eIF4G [23–25]. In contrast with SLBP, however, the interaction of NSP3 with eIF4G evicts PABP, but not eIF4A and eIF4E, from the surveillance complex.

The 68 nt 5′-leader (called Ω) of TMV binds HSP101 (heat-shock protein 101), which facilitates translation initiation independent of the 5′-cap through a functional interaction with eIF4G and eIF3 [26]. Ω exhibits partial functional redundancy with the 5′-cap or a poly(A) tail but not with the native TMV 3′-UTR [11], indicating that HSP101 enhances recruitment of the same initiation surveillance complex recruited by the 5′-cap and poly(A) tail. These results suggest that the HSP101–Ω complex is functionally similar to the eIF4E–5′-cap and PABP–poly(A) tail complexes and serves to enhance recruitment of the initiation surveillance complex. Of the two eIF4F proteins, i.e. eIF4F and eIFiso4F, present in plants, the former was specifically required for the function of Ω, suggesting that more than one type of initiation surveillance complex may exist (e.g. depending on which eIF4G isoform is present) and HSP101 may exhibit specificity in the recruitment. The 5′-leader of the cellular Ferredoxin 1 mRNA also binds HSP101 [12], demonstrating that some cellular mRNAs may also use HSP101 to enhance recruitment of the surveillance complex.

The requirement for a 5′-cap in recruiting the initiation surveillance complex can be circumvented if the mRNA contains an IRES (internal ribosomal entry site), which promotes 40 S subunit binding internally in an mRNA. The polyadenylated genomic mRNAs of picornaviruses such as encephalomyocarditis virus and poliovirus naturally lack a 5′-cap but contain an IRES within the 5′-leader to which eIF4G is recruited directly or indirectly and collaborates with the poly(A) tail to recruit the initiation surveillance complex [27,28]. The 5′-leader of the related virus, TEV, also contains an IRES that requires eIF4G, eIF4A, eIF4B and PABP for optimal function and exhibits a preference for the eIF4G-containing initiation surveillance complex over the eIFiso4G-containing complex [29]. The absence of eIF4E from the complex did not affect translation of TEV [29], suggesting that not all components of the surveillance complex may be necessary for the translation of all mRNAs, especially for those that naturally lack a 5′-cap or poly(A) tail.

STNV, which lacks a 5′-cap and poly(A) tail, recruits eIF4E, and presumably the entire surveillance complex, to its 3′-UTR, which may then interact with the 5′-leader to promote ribosome binding [8], demonstrating that even when an mRNA naturally lacks a 5′-cap, the requirement for eIF4E in the surveillance complex can still be important.
Function of the initiation surveillance complex

The association of the components of the initiation surveillance complex has several functional consequences: eIF4G and eIF4B individually and together synergistically stabilize PABP binding to poly(A) RNA [16], whereas the presence of PABP in the complex stabilizes eIF4F binding to the 5′-cap [30], suggesting that interaction between PABP and eIF4G increases eIF4E cap-binding function. This co-operativity allows binding of the complex to serve as a means to test for the suitability and integrity of RNA for translation thus preventing the recruitment of any inappropriate RNA (e.g. tRNAs, rRNAs, snRNAs etc.) or RNA whose integrity had been compromised (e.g. mRNA degradation intermediates).

The presence of PABP in the complex also increases the ATPase and RNA helicase activities of the eIF4F–eIF4A–eIF4B complex [31,32], suggesting that translation of mRNAs with a structured 5′-leader might benefit most from recruitment of the initiation surveillance complex. This prediction was supported in Xenopus oocytes in which the surveillance complex is deficient in PABP due to low expression of the latter before embryo development [33]. Increasing the amount of PABP assembled into the initiation surveillance complex by increasing PABP expression preferentially improved translation of those mRNAs capable of recruiting the complex (i.e. capped and polyadenylated mRNAs) and particularly so for those containing a structured 5′-leader [33]. The initiation surveillance complex thus serves to stabilize binding components of the complex to an mRNA and prepare the mRNA for 40 S subunit binding and scanning. Binding of the initiation surveillance complex also serves to inhibit entry of an mRNA into a decay pathway [34], to facilitate translation reinitiation through the physical proximity of the termini, and to ensure that 40 S subunits bind upstream of the initiation codon to prevent synthesis of N-terminally truncated proteins [35]. Redirecting binding of the complex to sites, e.g. by IRES elements, distal to the 5′-cap results in internal initiation [36].

Regulation of surveillance complex

The poly(A) tail is typically long enough to accommodate multiple molecules of PABP. Co-operative binding in plants is controlled by phosphorylation whereby phosphorylated PABP co-operatively binds poly(A) RNA, whereas hypophosphorylated PABP binds as a monomer [21]. The greatest degree of co-operative binding occurs between PABP of differing phosphorylation states suggesting that PABP molecules assembled on a poly(A) tail probably differ in the phosphorylation state. eIF4G promotes co-operative binding of hypophosphorylated PABP, whereas eIF4B increases the poly(A)-binding affinity of phosphorylated PABP [21]. Phosphorylation of eIF4G and eIF4B by developmental signals or by serum stimulated their interaction with PABP [12], suggesting that phosphorylation regulates subunit interaction within the initiation surveillance complex. That phosphorylation of eIF4B correlates with active translation, whereas its dephosphorylation occurs following stress or during developmentally quiescent stages, supports this conclusion [12,37]. Subunit interaction within the initiation surveillance complex was disrupted after heat-stress such that the association of eIF4G, eIF4B and PABP with the complex was reduced as was the affinity of PABP for poly(A) [21], observations correlating with the heat-induced dephosphorylation of eIF4G and eIF4B and loss of cap and poly(A) tail-dependent translation [21,37,38].

Conclusions

Recruitment of the initiation surveillance complex is an essential early step for the assembly of a translationally competent 80 S ribosome at the initiation codon. The initiation surveillance complex requires collaboration between the termini of an mRNA for its recruitment, and as such, serves as a means to test for the suitability and integrity of an RNA for translation. In most cases examined to date, those mRNAs that naturally lack a 5′-cap and/or a poly(A) tail have evolved functional analogues that recruit the initiation surveillance complex. Phosphorylation of components of the initiation surveillance complex regulates the stability and specificity of subunit interactions and regulates the binding affinity of the complex to an mRNA. Thus global changes in cellular protein synthesis following exposure to abiotic stress or during development may be controlled by the regulation of surveillance complex stability and activity.

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References


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