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Eukaryotic translation initiation factors and regulators

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Significant progress has been made over the past several years on structural studies of the eukaryotic translation initiation factors that facilitate the assembly of a translation-competent ribosome at the initiation codon of an mRNA. These structural studies have revealed the repeated use of a set of common structural folds, highlighted the evolutionary conservation of the translation apparatus, and provided insight into the mechanism and regulation of cellular and viral protein synthesis.

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Current Opinion in Structural Biology 2003, **13**:56–63

This review comes from a themed issue on
Protein–nucleic acid interactions
Edited by Aneel K Aggarwal and Jennifer A Doudna

0959-440X/03/$ – see front matter
Published by Elsevier Science Ltd.

DOI 10.1016/S0959-440X(03)00009-5

Abbreviations

aa	amino acids
eIF	eukaryotic initiation factor
IF	initiation factor
OB	oligonucleotide/oligosaccharide-binding
PABP	poly-A-binding protein
rmsd	root mean square deviation
RRM	RNA recognition motif
TC	eIF2•GTP•Met–$tRNA_i^{Met}$ ternary complex
UTR	untranslated region

Introduction

Translation initiation can be subdivided into three steps: first, binding of the specific initiator Met–$tRNA_i^{Met}$ to the small ribosomal subunit; second, binding of the resulting complex to an mRNA and locating the initiation codon; and third, joining of the large ribosomal subunit to generate a translation-competent ribosome. All three steps are facilitated by soluble proteins termed translation initiation factors [1] and many of these factors have been characterized at the structural level (Table 1).

In this review, we survey the structural data that have been reported for the translation initiation factors and their regulators, and highlight the most recent findings. Of particular note is the reiterative use of common folds among the translation initiation factors. In addition, newly published results provide fresh insights into the regulation of protein synthesis by phosphorylation of the cap-binding protein eIF4E and by mRNA circularization mediated by proteins that bind to the 5′ and 3′ ends of the mRNA.

A core set of factors is conserved during evolution

The structures of the three prokaryotic initiation factors (IF1, IF2 and IF3) have been reviewed recently [2]. Like bacterial IF3, eukaryotic initiation factor 1 (eIF1) plays an important role in translation start site specificity. The NMR structure of eIF1 revealed an α/β fold with a five-stranded β sheet packed against two α helices [3], which resembles the structure of the C-terminal domain of IF3 (Figure 1a). The factors eIF1A and eIF5B are structural homologs of IF1 and IF2, respectively [4].

The core of IF1, a five-stranded β barrel of the oligonucleotide/oligosaccharide-binding (OB)-fold family, is embellished in eIF1A by the addition of an unstructured N-terminal tail and two C-terminal α helices followed by an unstructured tail [5•] (Figure 1b). The additional C-terminal appendage of eIF1A mediates a direct contact with a C-terminal α-helical extension of eIF5B, which is lacking in its prokaryotic homolog IF2 [6,7]. Mutations in residues contributing to the nonspecific RNA-binding activity of eIF1A impair assembly of initiation complexes at an AUG codon [5•]. This is consistent with the notion that eIF1A binds in the A site in the same manner as IF1 [8••].

The factor eIF5B promotes ribosomal subunit joining. The X-ray structure of eIF5B from archaea revealed a four-domain protein that resembles a chalice [9••] (Figure 1c). The GTP-binding domain I, the β-barrel domain II and the α/β/α-sandwich domain III form the cup of the chalice. These three domains are connected via a 40 Å α helix (the stem) to domain IV, a second β-barrel domain, which forms the base of the chalice. Consistent with the idea that the structure of eIF5B is conserved in IF2, domain IV of eIF5B and the C-terminal domain of bacterial IF2 are nearly identical, except for the addition of two C-terminal α helices in eIF5B that, as described above, bind to the C-terminal tail of eIF1A. Also, domains I and II of eIF5B are homologous to the corresponding domains of elongation factors EF-Tu and EF-G [9••], suggesting a common ribosomal interface for these three factors (Figure 1c). Comparison of the active eIF5B•GTP and inactive eIF5B•GDP structures revealed that modest

Table 1

Translation initiation factors.

Factor/regulator	Structure	Motif/domain	References
Prokaryotes			
IF1	NMR	OB-fold	[49]
	X-ray		[8••]
IF2 (C terminus)	NMR	β barrel	[50]
IF3	NMR		[51,52]
	X-ray		[53]
Eukaryotes			
eIF1	NMR		[3,54]
eIF1A	NMR	OB-fold	[5•,55]
eIF2α	X-ray	OB-fold	[14]
K3L	X-ray		[16••]
M156R	NMR		[17]
eIF2β	NMR	Zn-finger	[12•]
eIF2γ	X-ray	G domain, β barrel	[11•]
eIF2B (multiple subunits)	ND		
eIF3 (multiple subunits)	ND		
eIF4A	X-ray	DEAD-box helicase	[56]
eIF4B	ND	RRM	
eIF4E	X-ray		[20,22•]
	NMR		[21]
4E-BP	X-ray		[29]
eIF4G	X-ray	HEAT	[31••]
eIF4H	ND	RRM	
eIF5	ND		
eIF5A	X-ray	OB-fold	[57]
eIF5B	X-ray	G domain, β barrel	[9••]
	NMR		[7]
eIF6	X-ray		[58]
PABP	X-ray	RRMs	[43••,59]
	NMR		[42••]
NSP3	X-ray		[46••,47••]

ND, not determined.

conformational changes in the GTP-binding active site are amplified through a lever-type mechanism involving the long stem α helix and result in more significant motion of domain IV [9••]. Recent mutational analyses indicate that these switch-like conformational changes in eIF5B regulate the ribosome affinity of the initiation factor [10].

eIF2: delivery of initiator tRNA and regulation of translation

The eukaryotic-specific factor eIF2, consisting of three nonidentical subunits (α, β and γ), binds the initiator Met–tRNA$_i^{Met}$ to the P site of the small ribosomal subunit. GTP binding to eIF2 is necessary for formation of a stable eIF2•GTP•Met–tRNA$_i^{Met}$ ternary complex (TC). Following TC binding to the ribosome, base pairing between the anticodon of Met–tRNA$_i^{Met}$ and an AUG codon triggers GTP hydrolysis by eIF2 in a reaction also requiring eIF5. eIF2 is released from the ribosome in a stable binary complex with GDP and the five-subunit guanine-nucleotide exchange factor eIF2B is necessary to recycle eIF2•GDP to eIF2•GTP.

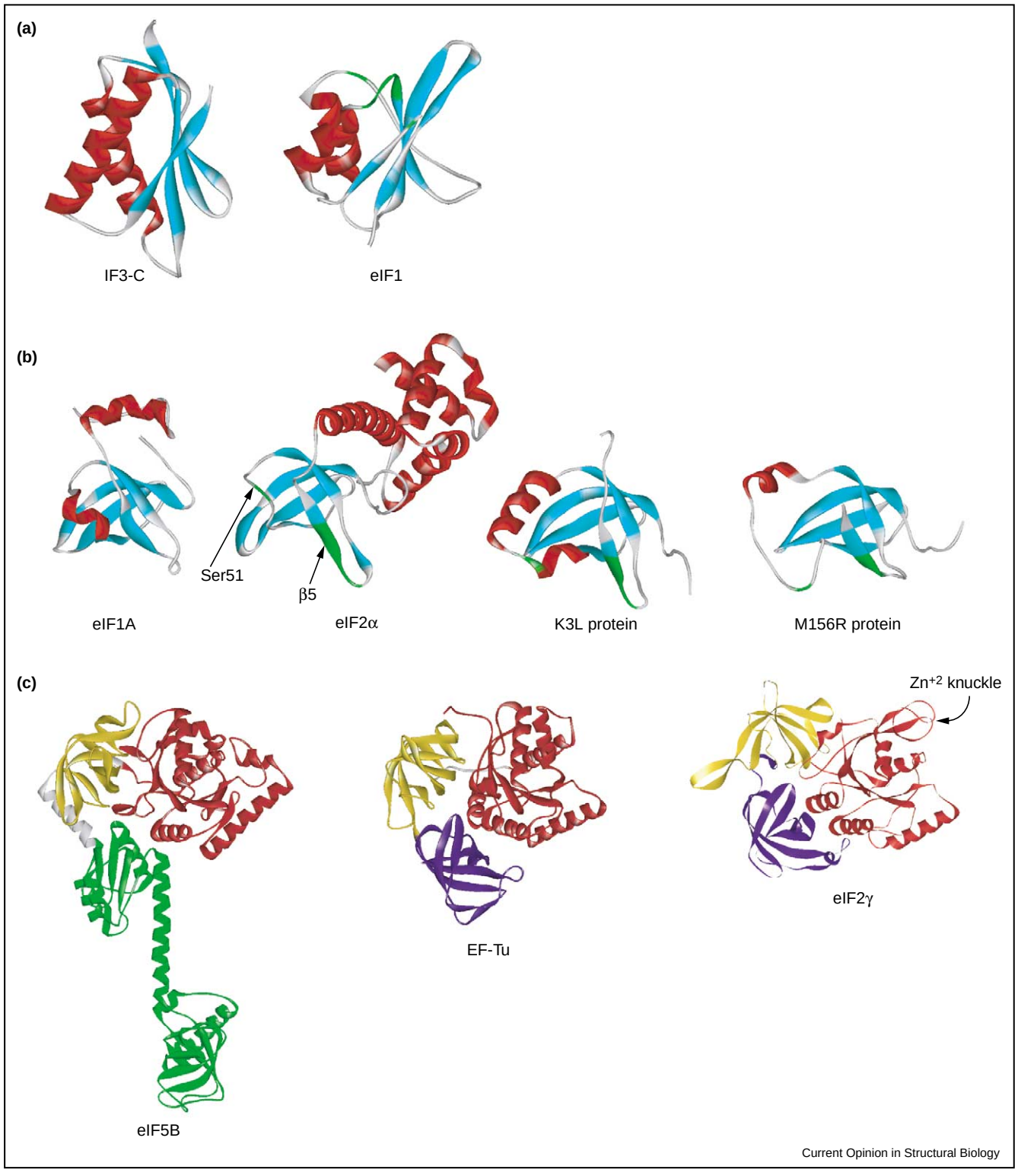
eIF2γ resembles the prokaryotic translation elongation factor EF-Tu [11•] (Figure 1c). Domain I is the GTP-binding domain, and domains II and III are β-barrel folds. A unique feature of eIF2γ is the presence of a Zn-binding knuckle of unknown function located between the conserved switch I and switch II elements in the GTP-binding domain. Relatively small structural changes were observed when comparing the inactive GDP and active GTP forms of eIF2γ. In addition, the structure of the inactive eIF2γ•GDP complex resembles the structure of the active EF-Tu•GTP complex [11•]. Thus, the structure of eIF2γ has not provided an explanation for the GTP dependence of Met–tRNA$_i^{Met}$ binding by eIF2. It is possible that the structural transitions regulating Met–tRNA$_i^{Met}$ binding will only be apparent in the eIF2 complex. Alternatively, as the GTP structure was obtained for a domain II (G235D) mutant form of *Pyrococcus abyssi* eIF2γ, it is possible that the mutation affected structural transitions in the factor.

The structure of archaeal eIF2β, which lacks an N-terminal interaction domain found in eukaryotic eIF2β, revealed two domains. The N-terminal α/β-fold domain consists of a four-stranded β sheet with two α helices packed on one side. The C-terminal Zn-ribbon domain is similar in structure to ribosomal protein L36, consisting of three antiparallel β sheets with the residues coordinating the Zn located in the turns or loops at one end of the sheet [12•]. The NMR data on archaeal eIF2β indicated that the N- and C-terminal domains are connected by a 15-residue linker that is likely to be α-helical in structure. Finally, conserved residues in the central region of yeast eIF2β (corresponding to the N-terminal region of archaeal eIF2β) mediate the interaction with eIF2γ [13].

eIF2α is a key target in translational regulation. Phosphorylation of eIF2α on Ser51 converts eIF2 from a substrate to a competitive inhibitor of its exchange factor, eIF2B. The X-ray structure of the N-terminal two-thirds of human eIF2α revealed two domains: an N-terminal OB-fold domain and a compact α-helical domain [14] (Figure 1b). The Ser51 phosphorylation site is located adjacent to a 3_{10} helix in an extended loop connecting two β strands in the OB-fold domain. This loop was not resolved in the X-ray structure, indicating that it is highly mobile. Mutations in eIF2α that impair binding to the eIF2Bαβδ regulatory subcomplex map to the residues flanking Ser51 and to strand β5, suggesting that eIF2B contacts eIF2α across one face of the OB-fold domain [15].

The X-ray structure of the vaccinia virus K3L protein [16••], a pseudosubstrate inhibitor of the eIF2α kinases, and the NMR structure of the myxoma virus M156R protein [17] revealed five-stranded β barrels of the OB-fold family, homologous to the N-terminal domain of eIF2α (Figure 1b) and the RNA-binding domain of the

Figure 1



Conservation of structural domains among the translation initiation factors. **(a)** α/β folds from the C-terminal domain of IF3 (left) and from eIF1 (right). Conserved surface residues and residues mutated in yeast eIF1 (*sui1*) that affect AUG start site selection are colored green. **(b)** OB-fold domains from eIF1A, eIF2α, K3L protein and M156R protein. The Ser51 phosphorylation site and conserved residues near strand β5 in eIF2α, and the related elements in the K3L and M156R proteins are highlighted in green. **(c)** Conservation of the GTP-binding domain (red) and β-barrel domain II (yellow) among eIF5B (GDPNP bound), EF-Tu (GDPNP bound, *T. aquaticus*) and eIF2γ (apo form). Domain III (purple), another β barrel, is conserved in EF-Tu and eIF2γ, but not in eIF5B.

ribosomal protein S1 (S1 domain). The K3L protein lacks a phosphorylatable residue corresponding to Ser51 in eIF2α, whereas a tyrosine residue in the corresponding position in the highly mobile loop of the M156R protein is readily phosphorylated by the eIF2α kinase PKR [17]. By analyzing mutants of the K3L protein that impair inhibition of PKR, the PKR-binding surface was mapped to a surface-exposed patch near β5, which was previously implicated in eIF2B interactions [15,16••]. Thus, the eIF2α kinases and eIF2B may interact with a common face on eIF2α.

Ribosome recruitment to eukaryotic mRNAs

Unlike the initiation factors described above, those that effect the recruitment of ribosomes to the eukaryotic mRNA do not have counterparts in prokaryotes. These include eIF4A, eIF4B eIF4E, eIF4G and eIF4H [1,18]. eIF4A, eIF4E and eIF4G form a stable ternary complex in mammals termed eIF4F, which binds directly to the mRNA 5′ cap structure (m^7GpppN, where N is any nucleotide) via the eIF4E subunit. eIF4A is a DEAD family RNA helicase, which is thought to unwind secondary structure in the mRNA 5′ untranslated region (UTR) to facilitate ribosome binding to the mRNA and its subsequent scanning to reach the initiation codon. eIF4G is a large modular scaffolding protein that interacts with the other eIF4 subunits and the multisubunit initiation factor eIF3, which binds directly to the ribosome. Importantly, eIF4G also interacts with the poly-A-binding protein (PABP), which binds with high affinity to the poly-A tail [19]. Thus, the PABP–eIF4G interaction brings about the circularization of the mRNA.

The 3D structures of the whole or portions of the eIF4F subunits have been solved. These include the almost complete structures of eIF4E and eIF4A, and several protein-binding domains of eIF4G. Recent advances concerning the eIF4E and eIF4G structures are described below.

eIF4E

The 3D structure of eIF4E is phylogenetically conserved from yeast to human [20,21,22•]. The X-ray and NMR co-structures of slightly truncated mouse and yeast eIF4E (aa 27–217), respectively, with the cap analog m^7GDP revealed a novel α/β fold. It resembles a cupped hand or a baseball glove, and consists of eight antiparallel β strands, which form a curved β sheet; a narrow hydrophobic cap-binding slot resides on the concave surface [20,21].

In the original structure [20], the C-terminal portion of the protein, including the Ser209 phosphorylation site (see below), was partly disordered. The reason for this became evident when the crystal structure of full-length human eIF4E complexed with m^7GpppA was solved [22•]. Although the core structures of human and mouse eIF4E are highly conserved, the C-terminal portions (aa 200–217) of the structures are significantly different (rmsd 0.9 Å). This difference is probably due to the fact that human eIF4E was co-crystallized with the full cap structure, m^7GpppA, and not just m^7GDP. The adenosine in m^7GpppA interacts with several residues in the flexible C-terminal region (Thr205 and Thr211 through hydrogen bonds, and Ser207 through van der Waals interactions), which stabilizes the eIF4E structure. The co-crystal structure of a mouse eIF4E–m^7GpppG complex was also determined, but no electron density for the penultimate G residue was detected [23]. Because the m^7GpppG cap exhibits a twofold higher affinity for eIF4E than m^7GpppA [24•], the reason for this discrepancy is not immediately clear.

Ser209 phosphorylation

The 3D structure of eIF4E also provided new insight into the possible function of Ser209 phosphorylation in regulating translation. Generally, but not always, phosphorylation of Ser209 positively correlates with increased translational activity [25]. *Drosophila* expressing a Ser209-Ala mutant exhibit slow development and are smaller than wild-type flies [26]. On the basis of the original murine eIF4E–m^7GDP co-crystal structure [20], it was suggested that phosphorylated Ser209 forms a salt bridge with Lys159, which lies just across (~7 Å) the groove that forms the trajectory of the mRNA [20]. The salt bridge was proposed to act as a clamp to stabilize mRNA–eIF4E interaction. This is consistent with earlier data showing that phosphorylated eIF4E binds with higher affinity than unphosphorylated eIF4E to the cap structure [27]. However, using different methods, two recent papers [24•,28•] demonstrated that Ser209 phosphorylated eIF4E exhibits reduced affinity (2–4-fold) for cap analogs. Could the structural data explain the negative effects of Ser209 phosphorylation on cap recognition?

The new structure of eIF4E–m^7GpppA reveals that the distance between the Cα positions of Ser209 and Lys159 is ~19 Å, which is too far to permit salt bridge formation, thus arguing against a clamping mechanism. It was suggested that electrostatic repulsion between the phosphate of the penultimate adenosine of the cap structure and phosphorylated Ser209 might reduce the eIF4E binding affinity [28•]; however, the distance might be too far (7 Å) for such a repulsion to function. Thus, the mechanism by which phosphorylation of Ser209 lowers cap affinity is not clear; regardless, the decreased affinity is proposed to stimulate release of eIF4E from the cap structure, so that the initiation complex can scan towards the initiation codon [28•]. This mechanism resembles the paradigm for transcription promoter clearance following phosphorylation of transcription initiation complexes [28•].

eIF4G

The 3D structures of two protein-interacting domains of eIF4G have been solved by crystallography and NMR

spectroscopy. The first is an unstructured region that becomes structured by an induced-fit mechanism when it binds to eIF4E. This region of mammalian eIF4G assumes the structure of an α helix with two turns when bound to eIF4E [29]. In yeast, a larger unstructured region of eIF4G (98 aa) undergoes an induced fit when bound to eIF4E [30].

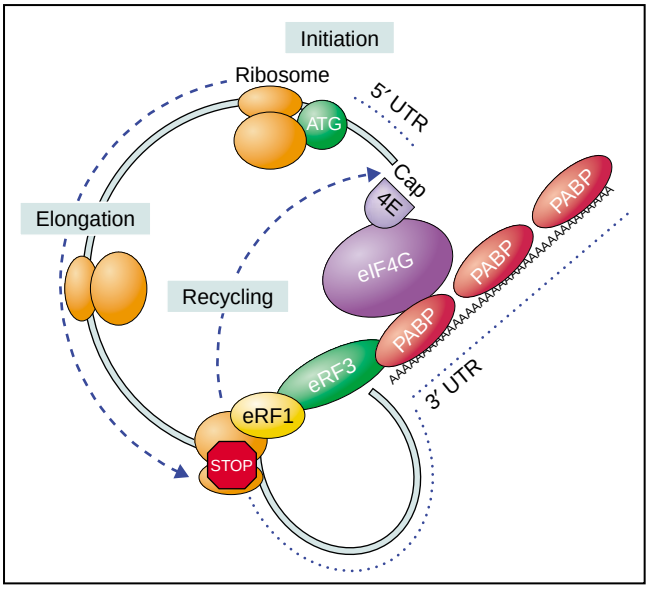
The X-ray structure of a second functional domain in eIF4G has been resolved. This middle domain binds several molecules, including eIF4A, eIF3 and RNA. The domain is crescent shaped and consists of ten α helices, which comprise five HEAT repeats [31••]. Mutational analysis based on the structure identified two separate but adjacent amino acid patches that bind eIF4A and the encephalomyocarditis virus (EMCV) RNA.

Poly-A-binding protein

PABP is a phylogenetically conserved protein that contains at its N terminus four highly conserved RNA-binding domains (RNA recognition motifs; RRMs), whereas its C terminus is less conserved, except for a highly conserved 74 aa segment. PABP interacts with the poly-A tail of the eukaryotic mRNA and with several proteins, including eIF4G [19], eIF4B [32], the termination factor eRF3 (GSPT2) [33] and two regulatory proteins, PABP-interacting proteins 1 and 2 (Paip1 and Paip2) [34,35••].

The PABP–eIF4G interaction brings about the circularization of the mRNA [36]. Such circularization explains the synergism between the cap structure and the poly-A tail in stimulating translation initiation [37]. It is thought that circularization of the mRNA stimulates translation either by shunting terminating ribosomes to the 5′ end of the mRNA and/or by stimulating the interaction of eIF4E with the cap structure [38,39]. Paip1 and Paip2 modulate translation, most probably, by affecting circularization. Paip1 and Paip2 both contain two distinct binding sites for PABP [40•,41•]. Interestingly, the amino acid sequence motif of one of these sites that binds to the highly conserved 74 aa C terminus of PABP is present in several unrelated proteins, including eRF3, termination factor 3 [42••,43••]. The interaction of eRF3 with the C terminus of PABP is of special interest because it provides further support for the idea that mRNA circularization plays an important role in translation. The interaction between eRF3 and PABP is expected to result in looping out of the 3′ UTR, which would ensure that, for mRNAs that possess long 3′ UTRs, the terminating ribosome is efficiently delivered to the mRNA 5′ end (see Figure 2 for model). Indeed, the interaction of eRF3 with PABP was shown to enhance translation [44••]. These findings are also consistent with the notion that, upon termination of translation, ribosomes might not continue to scan the 3′ UTR, but might dissociate and be recruited for the next cycle of translation initiation.

Figure 2



mRNA circularization. A model for the role of termination factor GSPT/eRF3 in the recycling of ribosomes on the same mRNA. GSPT/eRF3 is associated with eIF4G through PABP and eIF4G interacts with the mRNA 5′ cap structure through eIF4E, resulting in the circularization of the mRNA. This circularization explains the synergistic effects of the cap and PABP on translation. Modified from [44••].

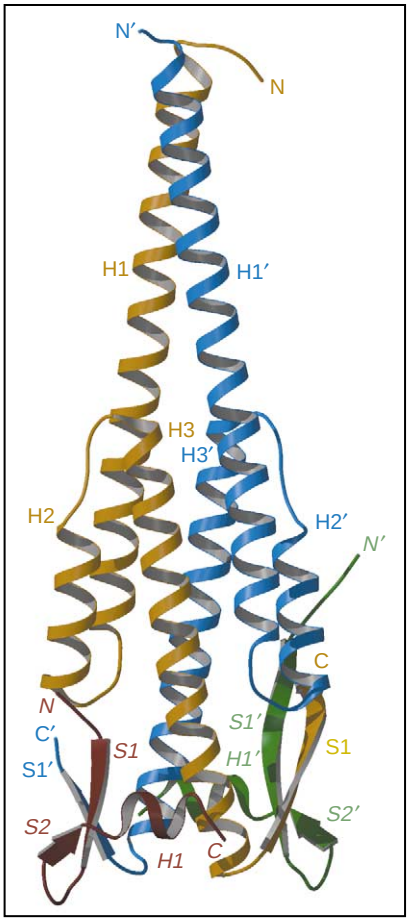
The solution structure of the highly conserved 74 aa C terminus of PABP has been determined by NMR spectroscopy. It consists of five α helices that form a right-handed supercoil arranged as an arrowhead [42••]. The contacts with Paip2 were inferred from chemical shift changes and involve an amino acid sequence that is conserved amongst the PABP-interacting proteins. A very similar structure was determined by X-ray crystallography for an ortholog of the C-terminal domain of PABP, the human hyperplastic discs (HYD) protein [43••].

PABP interaction with eIF4G

The reciprocal binding sites on eIF4G and PABP have been determined in yeast and mammals. eIF4G binds to RRM2 of PABP through an N-terminal segment that was mapped to a 48 aa sequence in human eIF4G [45]. The 3D structure of the eIF4G–PABP complex has not been determined.

Nevertheless, important progress in the understanding of eIF4G–PABP molecular interactions has been made by solving the co-crystal structure of the C-terminal domain of NSP3 in complex with a fragment of eIF4G (Figure 3) [46••]. NSP3 is a rotavirus nonstructural protein that binds via its N-terminal domain to a conserved tetranucleotide sequence at the 3′ end of the viral RNA [47••]. NSP3 also binds eIF4G through a C-terminal domain [48]. These dual interactions bring about the circulariza-

Figure 3



Structure of the heterotetrameric complex of the C-terminal domain of NSP3 (NSP3-C) with eIF4G. The NSP3-C homodimer forms a six-helix bundle (monomers are colored gold and blue). Two eIF4G fragments (red and green) bind to hydrophobic pockets in each of the NSP3-C monomers. Reproduced from [46••].

tion of the mRNA in analogy to the PABP–eIF4G interaction. NSP3 and PABP compete for a shared eIF4G binding site. Groft and Burley [46••] showed by using mutagenesis that hydrophobic residues in eIF4G, which mediate van der Waals interactions with NSP3, are also critical for the interaction with PABP. Indeed, the dorsal surface of the NSP3 C-terminal domain exhibits a patch of hydrophobic amino acids that resembles that on RRM2 of PABP. In addition, some solvent-exposed basic and acidic residues in NSP3 might have counterparts in PABP.

Perspectives

With the solution of structures for many of the single polypeptide factors, an important target for future studies will be the structures of the eIF2 and eIF4F complexes, as well as of components of the multisubunit factors eIF2B and eIF3. In addition, co-crystal structures or cryo-electron microscopy studies of factors bound to the ribosome offer the opportunity to relate the structure of the factors to their function in promoting the assembly and function of a translating ribosome.

Acknowledgements

Research support is acknowledged from the Canadian Institute of Health Research, National Cancer Institute of Canada and the Howard Hughes Medical Institute International Scholar Program. We thank Alan Hinnebusch and Gerhard Wagner for communicating results before publication. We also thank Stephen Burley, Antonina Roll-Mecak, Kalle Gehring and Avak Kahvejian for helpful discussions, and Caroline Groft for kindly providing Figure 3.

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