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Review

The structure and function of initiation factors in eukaryotic protein synthesis

T. V. Pestova[a,b,*] and C. U. T. Hellen[a]

[a]Department of Microbiology and Immunology, State University of New York Health Science Center at Brooklyn, 450 Clarkson Avenue, Box 44, Brooklyn (New York 11203, USA), Fax + 1 718 270-2656, e-mail: tpestova@netmail.hscbklyn.edu
[b]A. N. Belozersky Institute of Physico-Chemical Biology, Moscow State University, 119899 Moscow (Russia)

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Abstract. Protein synthesis is one of the most complex cellular processes, involving numerous translation components that interact in multiple sequential steps. The most complex stage in protein synthesis is the initiation process. It involves initiation factor-mediated assembly of a 40S ribosomal subunit and initiator tRNA into a 48S initiation complex at the initiation codon of an mRNA and subsequent joining of a 60S ribosomal subunit to form a translationally active 80S ribosome. The basal set of factors required for translation initiation has been determined, and biochemical, genetic, and structural studies are now beginning to reveal details of their individual functions in this process. The mechanism of translation initiation has also been found to be influenced significantly by structural properties of the 5′ and 3′ termini of individual mRNAs. This review describes some of the major developments in elucidating molecular details of the mechanism of initiation that have occurred over the last decade.

Key words. Eukaryotic initiation factor; internal ribosomal entry site; mRNA; ribosome; translation.

Protein synthesis is one of the main energy-consuming steps in eukaryotic cells and it must therefore be integrated closely with other cellular metabolic pathways. It is also one of the most complex of cellular processes, involving the concerted action of over a hundred different RNA and protein molecules in multiple sequential steps. Protein synthesis can be divided into initiation, elongation, and termination phases. Initiation is by far the most complex of these phases and is the focus of the greatest number of regulatory processes. The last decade has seen many significant developments in elucidating molecular details of the mechanism of translation initiation, and this review will focus on them. The field cannot be covered completely, and the reader is therefore encouraged to consult a number of excellent reviews for background information on earlier data on the initiation process [1–4], as well as on recent developments in elucidating the mechanisms of translation elongation and termination [5–9].

* Corresponding author.

The ribosome

The ribosome provides the framework for protein synthesis in all organisms. Recent advances in cryoelectron microscopy and X-ray crystallography have revealed fine details of ribosomal structure [10–17]. The mammalian 80S ribosome consists of a 40S subunit [which comprises 18S ribosomal RNA (rRNA; 1869 bases) and ~ 33 different ribosomal proteins] and a 60S subunit

[which contains 5S rRNA (120 bases), 5.8S rRNA (159 bases), 28S rRNA (5025 bases), and ~49 different proteins] [18]. Comparative analysis of rRNAs and ribosomal proteins strongly suggests that all ribosomes have evolved from a common ancestral ribonucleoprotein (RNP) particle [18, 19]. Eukaryotic 80S ribosomes contain more proteins than their bacterial 70S counterparts, and eukaryotic 18S and 28S rRNAs contain several insertions relative to bacterial 16S and 23S rRNAs that map to peripheral regions of a common core [19]. Although 80S ribosomes are larger and more complex than 70S ribosomes, their structures show significant overall similarity in the architecture of the intersubunit cavity where the tRNA molecules are bound and in the location of the mRNA decoding and peptidyltransferase centers. The structural similarities in these functional centers of prokaryotic and eukaryotic ribosomes justify assumptions concerning the conservation of a universal mechanism of translation [20]. The path followed by mRNA in prokaryotic 30S subunits through two holes formed by non-covalent sub-domain interactions can be mapped onto the 40S subunit, suggesting that incoming mRNA is precisely clamped into position as it approaches the decoding area [14, 21]. Mapping of sites in 18S rRNA to which mRNA analogues bind after they have been cross-linked to 40S subunits suggests that the arrangement of rRNA with respect to the mRNA track is similar in eukaryotic and bacterial ribosomes [e.g., refs 22, 23].

Initiation factors

Protein synthesis can be divided into different phases, each of which can be described as a series of partial reactions that are promoted by soluble protein factors. Translation initiation involves at least ten eukaryotic initiation factors (eIFs) that together comprise over 26 different polypeptides. This degree of complexity reflects the multiple stages in the process of translation initiation and the elaborate mechanisms for its regulation. In the last year, sequencing of all known initiation factors has been completed, and the structures of several have been determined. We shall review these developments before describing current models for their role in translation initiation.

eIF1

eIF1 is the smallest initiation factor and has a similar length and conserved sequence in different eukaryotes [24, 25]. eIF1 exists in free form but also interacts weakly with the equivalent human p110 and yeast p93/Nip1 subunits of eIF3, and indeed is necessary for the activity of yeast eIF3 [26–29]. It is essential for viability in *Saccharomyces cerevisiae*, and human eIF1 can replace yeast eIF1 in vivo [30, 31]. The solution structure of human eIF1, a protein of 113 residues, has been determined by nuclear magnetic resonance (NMR) spectroscopy [29] (fig. 1). Residues 29–113 form a single tightly packed domain that has a novel fold comprising two α helices on one side of a mixed parallel and anti-parallel five-stranded β sheet. Residues that have been implicated in altering the accuracy of initiation site selection [30, 31] are clustered together, likely forming a binding site for another component of the translation apparatus [29]. There are several charged clusters of surface residues that are potential sites for electrostatic interaction with as yet unidentified components of the translation apparatus.

eIF1A

eIF1A is one of the most conserved factors in terms of structure and function: wheat and rabbit factors are interchangeable in vitro and human eIF1A can replace yeast eIF1A in vivo [32–34]. Moreover, there is significant sequence homology between eIF1A (143–151 amino acids in length) and the prokaryotic initiation factor IF1 (72–75 amino acids in length) [25, 35]. This region of homology corresponds to a β barrel oligomer-binding (OB) fold [36], and the structures of human eIF1A (residues 33–94) and *Escherichia coli* IF1 are very similar over this entire region [37, 38]. It is tempting to speculate that these structural similarities relate to common functions in the initiation process for the prokaryotic factor IF1 and eukaryotic eIF1A. eIF1A is highly charged and contains clusters of basic and acidic regions at its N and C termini, respectively [33, 34]. It is an RNA-binding protein [39] and NMR studies have identified a well-defined RNA-binding surface [38]. The nature of the RNA ligand for eIF1A is not yet known.

eIF2

eIF2 is a stable heterotrimeric protein consisting of essential α, β, and γ subunits. The principal function of eIF2 is formation of a ternary complex with GTP and aminoacylated initiator methionyl-tRNA (Met-tRNA$_i^{Met}$) which then binds to the 40S subunit, and in turn enables the resulting 43S complex to bind mRNA. After several intervening steps in the initiation process, eIF5 (see below) triggers hydrolysis of GTP bound to eIF2 and release of an eIF2-GDP complex. The GDP bound to eIF2 must be exchanged for GTP in a guanine nucleotide exchange reaction catalyzed by eIF2B before eIF2 can again form a ternary complex. Binding of Met-tRNA$_i^{Met}$ to the 40S subunit by eIF2 is a common step in translation of all eukaryotic mRNAs, and eIF2 is the focal point of several regulatory mechanisms that control overall protein synthesis rates [40].

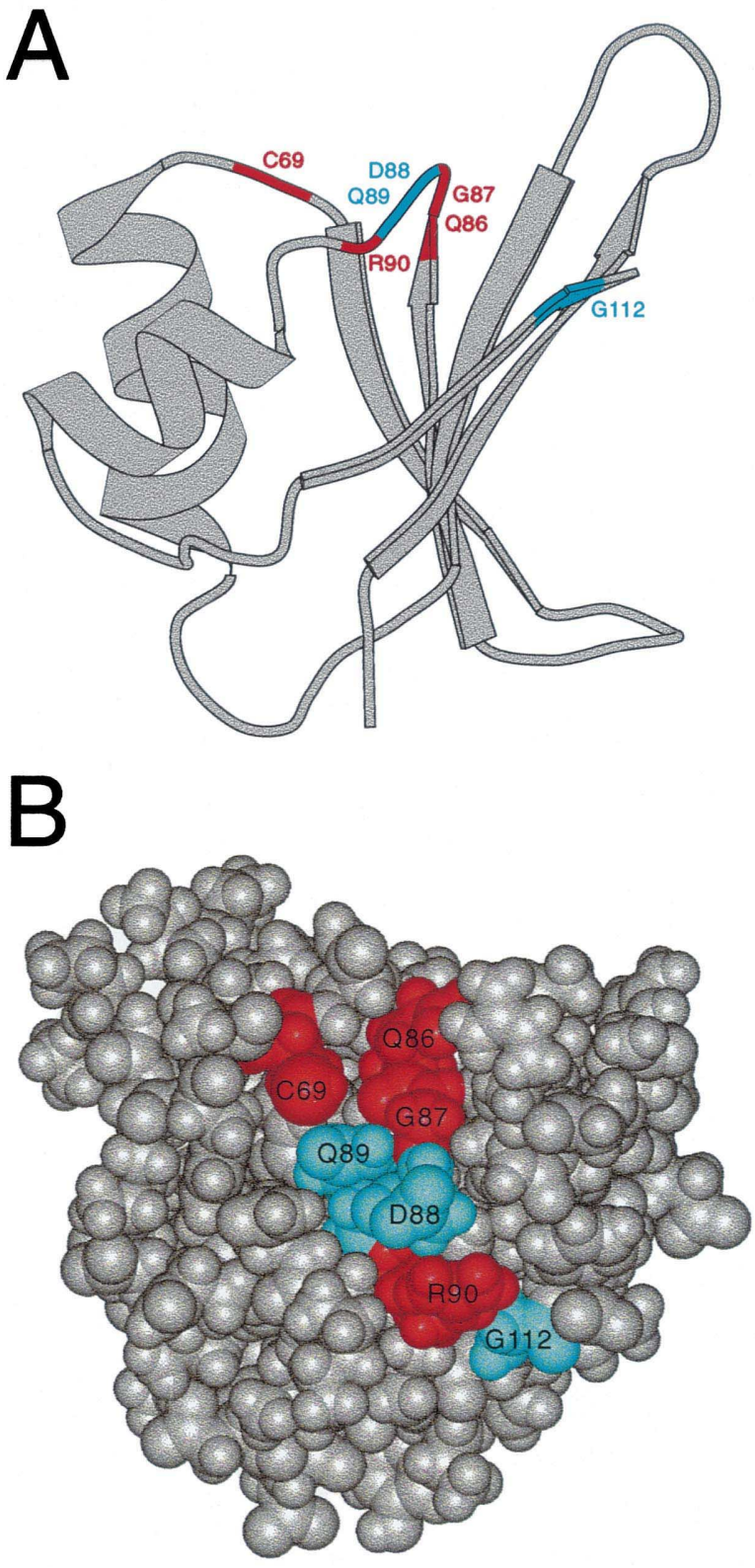

Figure 1. Structure of human eIF1. (*A*) Smoothed trace through the Cα positions of the folded region (residues 29–113) of human eIF1, showing residues (in blue) equivalent to mutations in *Saccharomyces cerevisae sui1* that allow initiation at non-AUG codons and adjacent conserved surface residues (in red). (*B*) Space-filling model of the same structure rotated 90° towards the viewer. Figure courtesy of G. Wagner and C. M. Fletcher [reprinted from ref. 29].

Significant progress has recently been made in characterizing the structure of eIF2 and interactions between it and various components of the translation apparatus. Yeast and human α subunits are most strongly conserved in the N-terminal third [41] which corresponds to an OB-like domain [42, 43]. Substitutions at the N terminus of this domain confer on yeast ribosomes the ability to initiate translation despite the absence of an AUG initiation codon [41]. This and other data (see below) implicate eIF2 in playing a role in ribosomal recognition of the initiation codon. The β subunit is the only subunit of eIF2 to bind mRNA strongly [44, 45]. Different eukaryotic eIF2β subunits range from 250 to 333 amino acid residues in length, but all contain two motifs that could interact with RNA. These are polylysine tracts in the poorly conserved N-terminal half of eIF2β and a C_2-C_2 zinc finger-like motif in its strongly conserved C-terminal half [46]. Neither binding of zinc to eIF2 nor a functional requirement for zinc by eIF2 have been observed. Cross-linking studies identified residues 205–333 of rabbit eIF2β which contain the C_2-C_2 motif as the primary site of mRNA binding [44], whereas in a different study, the N-terminal half of yeast eIF2β (which contains three polylysine tracts) bound mRNA more strongly than the C-terminal half [45]. These tracts are required for binding of mRNA but not tRNA to yeast eIF2 and are essential for eIF2 function in vivo [45, 47]. They also mediate binding of eIF2 to the eIF2Bε subunit and to eIF5 by interacting with bipartite motifs conserved in these two polypeptides [47–49]; other elements in the C-terminal third of eIF2β are also required for the interaction with eIF2B [50]. Interactions of eIF5 and eIF2B with eIF2β are mutually exclusive, consistent with these two factors promoting opposing reactions on the guanosine nucleotide bound to eIF2. The C_2-C_2 motif is required for the in vivo function of eIF2 [51] and has been implicated in start site selection during the scanning process: ten independent mutations in or adjacent to this motif allow initiation to occur at a UUG codon in the absence of an AUG initiation codon [51, 52]. These suppressor mutations do not alter the interaction of eIF2 with mRNA [45], but at least one conferred a high intrinsic rate of spontaneous GTP hydrolysis and a consequent high rate of initiator tRNA dissociation on eIF2 [53].

Initial data concerning the identity of the eIF2 subunit responsible for binding GTP and $tRNA_i^{Met}$ were contradictory: photoaffinity labelling of eIF2 with GTP analogues and cross-linking of eIF2 to Met-$tRNA_i^{Met}$ led to labelling of both β and γ subunits [54–56]. However, more recent biochemical and genetic evidence indicates that the eIF2 γ subunit is primarily responsible for binding these ligands [53, 57–59]. The eIF2γ subunits from various eukaryotes range in length from 446 to 527 residues but their sequences are remarkably conserved [$>72\%$ identity; refs 56, 60–62], particularly in the sequence blocks surrounding the consensus motifs that are found in a superfamily of GTP-binding proteins, including prokaryotic translation, initiation, and termination factors [63, 64] (fig. 2). Remarkably, eIF2γ shares strong homology with the prokaryotic elongation factor EF-Tu ($\sim 50\%$ similarity and 27% identity) and its sequence is compatible with the three-domain structure of EF-Tu [65]. The principal differences are that relative to EF-Tu, eIF2γ has a non-essential N-terminal extension and an essential insertion in domain 1 [60]. EF-Tu, the prototype for G protein translation factors, also binds aminoacylated (elongator) tRNAs in a GTP-dependent manner and might therefore serve as a useful model for GTP-dependent binding of initiator tRNA by eIF2γ. The GTP- and $tRNA_i^{Met}$-binding sites are in close proximity in EF-Tu [65]. Consensus motif G4 forms part of the guanine ring-binding loop in G proteins; mutations in this motif abrogated or strongly impaired the function of yeast eIF2γ in vivo and impaired binding of guanine nucleotides in vitro [57, 59]. Binding of initiator tRNA

		G1		G2		G3		G4		G5
		GxxxxGKT/S		**DxxxxxxxxxxT**		**DxxG**		**NKxD**		**SAK/L**
eIF2γ	43	NIGTI**G**HVAH**GKS**TVVKA	(6)	VRFKNELERNI**T**IKLGY	(45)	HVSFV**D**CP**G**HDILM	(42)	ILILQ**NKID**LVKES	(21)	PIIPI**SA**QLKYNI
eIF5B	628	ICVLM**G**HVDT**GKT**KILDK	(3)	THVQDGEAGGI**T**QQIGN	(26)	GMLII**D**TP**G**HESFS	(40)	FIVAL**NKID**RLYDW	(54)	SLVPT**SA**HTGDGM
IF2	388	VVTIM**G**HVDV**GKT**SLLDY	(3)	TKVASGEAGGI**T**QHIGA	(8)	MITFL**D**TP**G**HAAFT	(40)	VVVAV**NKID**KPEAD	(22)	QFVHV**SAK**AGTGI
EF-Tu	10	NVGTI**G**HVDH**GKT**TLTAA	(19)	**D**NAPEEKARGI**T**INTSH	(8)	HYAHV**D**CP**G**HADYV	(41)	IIVFL**NK**C**D**MVDDE	(24)	PIVRG**SAL**KALEG
EF-1	4	NIVVI**G**HVDS**GKS**TTTGH	(34)	**D**KLKAERERGI**T**IDISL	(8)	YVTII**DAPG**HRDFI	(48)	LIVGV**NKMD**STEPP	(38)	NMLEP**SA**NMPWFK

Figure 2. Sequence alignment of eukaryotic GTP-binding factors eIF2γ, eIF5B, and EF-1, and the prokaryotic translational GTP-binding factors IF2 and EF-Tu. Bold characters correspond to amino acid residues that are identical to those in the consensus sequences G1–G5 [63, 64, 185].

Table 1. Properties of mammalian and *Saccharomyces cerevisiae* eIF3 subunits.

Mammalian subunit	Number of amino acids	Yeast subunit	Yeast gene	Number of amino acids	Identity	Motifs/function	References
		p135	TIF31	1277			81
p170	1382	p110	Rpg1/TIF32	964	29%	PCI	82–84
p116	873	p90	Prt1	724	36%	RRM, PCI	85–87
p110	913	p93	Nip1	813	31%		81, 88
p66	548					binds RNA	89
p48	445					PCI	90
p47	320	p33	Tif35	274	32%	RRM	91–94
p40	352					Mov-34 family	89
p36	325	p39	Tif34	347	46%	WD40 repeats	79, 81
p35	258	p30	HCR1	265	26%		94, 97
(eIF1)	113	p16	Sui1	108	58%	binds p110/Nip1	29, 30

The subunits are named according to their electrophophoretic mobility, which often differs significantly from calculated molecular masses. eIF1 is not regarded as a subunit of mammalian eIF3 but interacts with it weakly, whereas yeast eIF1 exists in free form as well as associated with eIF3 [26–29]. A 135-kDa protein encoded by TIF31 is associated with eIF3 but has not definitively been characterized as a subunit of eIF3 [81]. The abbreviations used to designate sequence motifs in eIF3 subunits are: PCI, proteasome-COP9 complex-initiation, and RRM, RNA recognition motif.

to yeast eIF2 was impaired by N135K and Y142H mutations, which also resulted in increased initiation at non-AUG codons [53, 58, 59]. All three subunits of eIF2 are therefore implicated in start site selection during the scanning process.

eIF2B

The guanine nucleotide exchange factor eIF2B recycles eIF2 from its inactive GDP-bound state to its active GTP-bound state [66]. Phosphorylation of eIF2 inhibits recycling and leads to a general inhibition of translation. The mammalian α, β, γ, δ, and ε subunits are homologous to the yeast GCN3, GCD7, GCD1, GCD2, and GCD6 subunits, respectively; there is in turn significant homology between GCN3 (α), GCD7 (β), and GCD2 (δ) subunits on the one hand, and between GCD1 (γ) and GCD6 (ε) subunits on the other [67–70]; they form two separate sub-complexes that can bind eIF2 independently [71, 72]. GCD6 (ε) is the catalytic subunit and GCD1(γ) indirectly stabilizes its binding to eIF2 [72–74]. GCD6 (ε) contains a nucleotidyltransferase-related domain, suggesting that eIF2B catalyzes nucleotide exchange directly, rather than indirectly, for example by promoting dissociation of GDP from eIF2 [66]. The GCN3 (α) subunit is not required for eIF2B catalytic activity but plays a regulatory role, mediating the inhibitory effects of phosphorylated eIF2 as part of a GCN3-GCD7-GCD2 sub-complex that binds eIF2(P) through its GCD2 subunit [72–74]. This sub-complex has no nucleotide exchange activity but may act by preventing a productive interaction between eIF2-GDP and the catalytic sub-complex. The requirement for GCD7 (β) and GCD2 (δ) subunits for yeast viability suggests that they may play other roles in addition to regulating eIF2B function [70, 75].

eIF3

eIF3 plays a central role in initiation and is implicated in several consecutive steps in this process. It dissociates the 80S ribosome into 40S and 60S subunits and binds to 40S ribosomal subunits, thus acting as an anti-association factor [4]. It prevents dissociation of the initiator tRNA-eIF2-GTP ternary complex by mRNA, stabilizes binding of the ternary complex to the 40S subunit, and is required for binding of mRNA to the resulting 43S complex. Recent genetic evidence has suggested that eIF3 could also play a role in start site selection during scanning [76].

eIF3 is the largest and most complex initiation factor. Mammalian eIF3 has a molecular mass of $\sim$650 kDa, consists of ten polypeptides and can bind eIF1 (table 1). Yeast eIF3 appears to consist of between six and eight polypeptides including eIF1 [27, 28, 77, 78]. Two (p29 and p21) of the eight eIF3 subunits identified by Naranda et al. [77] are degradation products of other factors [79, J. Hershey, personal communication]. A 62-kDa protein (GCD10) associates with eIF3 but is not required for its function [80]. A 135-kDa protein associated with the p110 subunit of eIF3 has not definitively been characterized as a subunit of eIF3 [81]. The sequences of all mammalian and yeast eIF3 subunits have been determined and give some insights into the multiple functions of this factor.

The p116 and p44 subunits and their *S. cerevisiae* Prt1 and TIF35 homologues contain RNA recognition motif (RRM) domains present in many RNA-binding proteins [87, 92–94]. These and the p170 and p66 subunits of mammalian eIF3 bind rRNA, cellular mRNA, and viral mRNA [89, 94, 98]; the ability to bind rRNA may contribute to the interaction between eIF3 and 40S subunits [99]. The yeast TIF34 protein is essential for

the integrity of the eIF3 complex [79]. This protein and its mammalian p36 homologue contain seven WD repeat elements [79, 88] which are thought to interact with other proteins, promoting the assembly of macromolecular complexes [95]. Mutations in the most highly conserved WD repeats weaken the interaction of TIF34 with TIF35 and PRT1 [28]. Subunits of eIF3 contain additional homology domains also present in constituents of other macromolecular complexes that may mediate homo- or heterotypic subunit-subunit interactions. The N-terminal halves of mammalian p40 and p47 subunits and their lower eukaryotic homologues are similar to each other and all contain an $\sim$140-amino acid residue motif also found in members of the Mov-34 family of proteins [89]. Constituents of the same macromolecular complexes, including the p170, p116, and p48 subunits of eIF3 all contain a purely α-helical homology domain of $\sim$200 amino acid residues [96]. A number of interactions between subunits of mammalian and of yeast eIF3 have been identified [27, 28, 87, 93, 94]. Significantly, eIF3 also interacts with eIF5 and through its p110 (Nip1) subunit with eIF1 [27–29, 92]. eIF3 also binds eIF4F, probably to its eIF4G subunit [100] and to eIF4B [101].

eIF4A

eIF4A is an ATP-dependent RNA-binding protein, an RNA-dependent ATPase, and an RNA helicase [102–105]. It is thought to disrupt secondary and tertiary structure in the 5′-untranslated region of capped mRNAs in order to facilitate ribosomal attachment and to permit scanning to the initiation codon. eIF4A is the prototype of a large family of 'DEAD' box RNA helicases that contain specific consensus sequences in eight conserved motifs [106]. In helicases of known structure, these conserved motifs, including the ATP-binding site, line the cleft between two sub-domains [107]. The N-terminal ATPase domain of *S. cerevisiae* eIF4A (lacking the RNA-binding motifs) comprises a seven-stranded, twisted β sheet flanked by five α helices on one side and four on the other [108]. The positions of the conserved motifs in this eIF4A fragment are clustered together in an identical manner to those of the DNA helicase PcrA of *Bacillus stearothermophilus* [109]. This observation suggests that these enzymes utilize an identical mechanism for hydrolysis of ATP and possibly for transferring the energy of ATP hydrolysis to the (RNA or DNA) substrate. ATP is fixed at two sites in eIF4A: the adenine base fits into a conserved pocket and the phosphate is positioned by motif I. All eIF4A polypeptides are very similar in size ($\sim$410 amino acid residues) and their sequences are all closely related, particularly in the conserved motifs. The functions of some of these conserved motifs in *S. cerevisiae* and mouse eIF4A have been elucidated by mutational analyses [110–114]. Mutations in motif 1 (AxxxxGKT) render eIF4A incapable of binding ATP; motif 5 (DEAD) is critical for ATP hydrolysis and thus for RNA helicase activity, but is not essential for ATP binding. These observations are consistent with the location of these motifs in the crystal structure relative to the bound nucleotide [108]. Motif 6 (SAT) is dispensable for ATP binding and hydrolysis but is required for RNA helicase activity, and motif 8 (HRIGRxxR) is required for RNA binding and RNA helicase activity. RNA activates ATP binding, so mutations that impair RNA binding also impair ATP hydrolysis. Mutations in these domains yield proteins that are trans-dominant inhibitors of translation, probably because they are incorporated into eIF4F and subsequently inactivate this factor [114].

Duplex unwinding by eIF4A may result from a cycle of changes in protein conformation and RNA affinity caused by ATP binding and hydrolysis. Nevertheless, eIF4A alone is an inefficient helicase (maximally hydrolyzing ATP at a rate of $\sim$3 per minute) and is non-processive, because it dissociates from ssRNA faster than it hydrolyzes ATP [104, 105, 115]. The helicase activity of eIF4A is enhanced by eIF4B, eIF4H, and eIF4F [102, 104, 105, 112, 116]. It is not yet known whether and how these factors alter enzymatic and kinetic parameters of eIF4A interactions with its substrates and products, for example to increase its affinity for RNA and to confer processivity to its activity. In yeast, eIF4B suppresses a temperature-sensitive mutation in eIF4A in vivo and the same eIF4A mutant is synthetically lethal with eIF4B, eIF4E, and eIF4G [117–119]. In eukaryotes, eIF4A exists in free form and as part of eIF4F, binding to two sites in mammalian eIF4G and to a single site in yeast eIF4G [120–122]. It also binds to the translational regulators p97 and polyadenylate-binding protein (PABP)-interacting protein (PAIP) [123, 124].

eIF4B and eIF4H

Although eIF4B is thought to play a stimulatory role during translation initiation, no unique essential functions have been assigned to it in either mammals or in yeast. Mammalian eIF4B strongly stimulates the helicase activity of eIF4A and eIF4F [102, 125] and enhances (but is not essential for) ribosomal attachment to capped globin mRNA [126] and to the encephalomyocarditis virus (EMCV) internal ribosomal entry site (IRES) [127] (see below). In *S. cerevisiae*, genetic interactions between eIF4B and eIF4A, eIF4E, and eIF4G [117, 119] suggest a role for this factor in ribosomal binding to mRNA and in unwinding RNA secondary structure that is supported experimentally [118]. Nevertheless, *S. cerevisiae* eIF4B is not essential for growth

[117, 118]. Human eIF4B (611 amino acid residues long) has a modular structure consisting of an N-terminal RRM domain, a middle domain with a DRYG repeat motif [128] and a second, C-terminal RNA-binding region [129, 130]. Similar motifs are found in the smaller *S. cerevisiae* and *Saccharomyces pombe* factors (436 and 388 amino acid residues long, respectively). Human eIF4B may form a bridge between eIF3 and the 40S subunit, since the central domain mediates homodimerization and interaction with the p170 subunit of eIF3 [101] and the RRM may bind specifically to 18S rRNA [130]. The N- and C-terminal domains of yeast eIF4B also bind RNA, but the N-terminal RRM does not have a similar rRNA-binding specificity [131]. This protein does have an RNA-annealing activity [131, 132] which has been proposed to function reciprocally with the helicase activity of eIF4A to systematically open and close intractions between mRNA and rRNA, tRNA or mRNA during the scanning process. It has been noted that this process occurs too slowly and appears to require excessively high protein-to-RNA concentrations to be biologically relevant [133].

Recently, a new mammalian translation initiation factor termed eIF4H has been identified that shows significant homology with eIF4B, is five- to tenfold less abundant and has similar functions, enhancing the activities of eIF4A and eIF4F in translation and ATP hydrolysis assays [105, 134].

eIF4E

Cytoplasmic eukaryotic mRNAs have a 7-methyl-G(5′)ppp(5′)N 'cap' (where N is any nucleotide) at their 5′ termini that synergizes with the 3′ poly(A) tail in stimulating translation initiation [135, 136]. This 'cap' is specifically recognized by eIF4E, the cap-binding subunit of the heterotrimeric factor eIF4F. All eukaryotes appear to encode multiple isoforms of eIF4E that are strongly conserved, except for a non-essential N-terminal sequence of variable length [137, 138]. The structures of this conserved domain of murine eIF4E bound to the cap analogue 7-methyl-GDP and of *S. cerevisiae* eIF4E have been determined by X-ray crystallography and by NMR [138–141]. These proteins resemble a cupped hand consisting of a curved β sheet of eight anti-parallel strands (S1–S8) backed by three long helices that lie almost parallel to the top strand of the β sheet (fig. 3). The cap analogue binds in a narrow slot on the concave surface of eIF4E. Recognition is mediated by Π-Π stacking between the 7-methyl-guanine and the indole groups of two absolutely conserved tryptophan residues, by three Watson-Crick-like hydrogen bonds and by van der Waal's contact with a third conserved tryptophan [138]. Phosphorylation of eIF4E at Ser209 in murine eIF4E or its equivalent enhances binding of eIF4E to capped mRNA and stabilizes the interaction between eIF4E and eIF4G [142, 143]. Phosphorylation of this residue may enable it to form a salt bridge with Lys159 to form a retractable bridge over the slot, thereby stabilizing the binding of capped mRNA to eIF4E. These conclusions are consistent with mutational analyses [144, 145].

The eIF4G constituent of eIF4F uses an invariant Tyr-XXXX-Leu-Φ peptide segment (where X is variable and Φ is a hydrophobic residue) to recognize eIF4E (fig. 3); this recognition motif undergoes a disorder-to-order transition when it interacts with a phylogenetically invariant site on the convex surface of eIF4E [139]. Substitution of interacting residues in either eIF4E or eIF4G impairs or abolishes interactions between these factors [146–151]. A reduction in the affinity of eIF4E for eIF4G is likely to favor cap-independent translation over cap-mediated initiation. Remarkably, mammalian and yeast 4E-binding proteins that inhibit cap-dependent protein synthesis by competing with eIF4G for binding to eIF4E [152–154] are molecular mimics of eIF4G that undergo the same disorder-to-order transition on binding to the same site as eIF4GII on the convex surface of eIF4E (fig. 3) [139, 141].

eIF4G

The cap-binding complex eIF4F consists of eIF4A, eIF4E, and eIF4G subunits, although in lower eukaryotes eIF4A is bound less strongly [122, 155, 156]. A growing body of evidence indicates that eIF4G plays a central role in coordinating and enhancing the activities of various factors during the early stages of initiation [157, 158]. These include the cap-binding activity of eIF4E, the RNA helicase activity of eIF4A, the poly(A)-binding activity of PABP, and the mRNA and 40S subunit-binding activities of eIF3 [100, 121, 146, 153, 159–161]. In addition, eIF4G binds directly and in some instances specifically to mRNA [162–164] through a putatively RRM-like domain [165]. eIF4G binds each of these translational components directly (see below). Taken together, these observations suggest that eIF4G plays an essential bridging role as a ribosomal recruitment factor.

Mammalian, plant, and yeast cells all contain two eIF4G isoforms that differ from each other significantly in size, sequence, and activities. Mammalian eIF4GI is ten times more abundant than eIF4GII and shares only 46% amino acid identity with it [166]; yeast TIF3632 (914 amino acids in length and 53% identical to the TIF4631 protein) can be disrupted without effect in vivo, whereas disruption of TIF4631 (952 amino acids in length) confers a slow-growth phenotype [165]. The plant eIF4F complex contains a 220-kDa eIF4G polypeptide and a 26-kDa eIF4E component, whereas

the eIFiso4F complex contains eIFiso4G (86 kDa) and a 28-kDa eIF4E [167]. There are significant sequence homologies between various eIF4G polypeptides [156, 168] which have in some instances been shown to correspond to binding sites for other translation components. The binding site for PABP within yeast eIF4G has been localized [148, 159] to a 115-amino acid region N terminal to the binding site for eIF4E [153]. Mammalian eIF4G and wheat eIFiso4G proteins also contain a binding site for PABP [160, 168] N terminal to the eIF4E-binding site, which in mammals has been limited to 17 amino acid sequence [139, 146]. The central third of mammalian eIF4G binds eIF4A, eIF3, and RNA; the C-terminal third of eIF4G contains a second eIF4A-binding site [100, 121, 162]. This region is absent in yeast eIF4G and although present in wheat eIFiso4G appears not to be bound by eIF4A, which binds only to a central region of this factor [168].

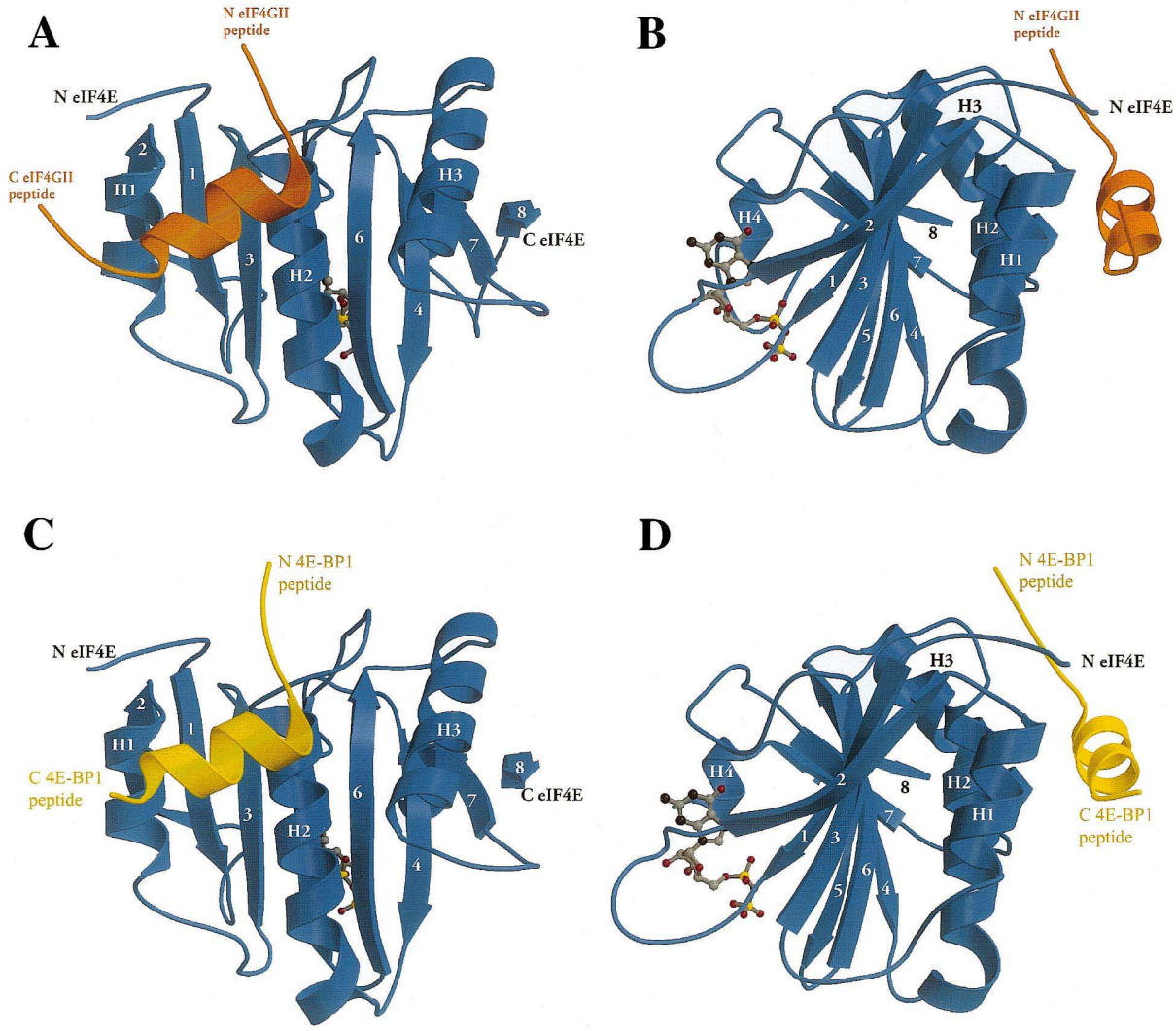

Figure 3. Structures of eIF4E bound to m^7GDP and either eIF4G peptides (*A*, *B*) or 4E-BP1 peptides (*C*, *D*). Ribbon diagrams of the ternary complex structures of murine eIF4E (blue) bound to m^7GDP (ball and stick representation) and eIF4GII or 4E-BP1 peptides, viewed perpendicular to the eIF4E β sheet (*A*, *C*) and in profile, rotated 90° about the vertical axis from the previous view (*B*, *D*). 7-methyl-GDP is in the cap-binding slot on the convex face of eIF4E. (*A*, *B*) The eIF4GII peptide (orange) forms an L-shaped structure that interacts with the N terminus of eIF4E and two of three long α helices (H1 and H2) on its convex dorsal surface. (*C*, *D*) The 4E-BP1 peptide (yellow) adopts a very similar conformation on binding to eIF4E. Figure courtesy of S. K. Burley and J. Marcotrigiano [reprinted from ref. 139].

eIF5

eIF5 is a GTPase-activating protein specific for eIF2 [169]. It interacts with the 48S complex to promote hydrolysis of eIF2-bound GTP which leads to the release of eIF2-GDP and Pi from the 40S subunit and eventually to its joining with a 60S subunit to form an active 80S ribosome [170]. *S. cerevisiae* eIF5 is an essential, single-copy gene that can be functionally replaced by rat eIF5 [171–173]. Sequence similarity between eIF5 moieties from different species is greatest over the N-terminal 160 amino acid residues, which contain a putative C_2-C_2 zinc finger that has been implicated in binding RNA [174], and a shorter C-terminal region that contains determinants of the eIF5 interaction with eIF2 [47–49]. Determinants of eIF5's interaction with the p110/Nip1 subunit of eIF3 are not known [27, 28, 92].

eIF5A

Formation of the first peptide bond, which marks the end of the initiation process and the beginning of elongation, is assayed using methionylpuromycin synthesis as a model reaction. This reaction is stimulated in vitro by eIF5A, which is therefore implicated late in the initiation process. Eukaryotic ($\sim$ 154 amino acids long) and archaebacterial eIF5A polypeptides have strongly conserved sequences and are all uniquely modified at a conserved lysine residue to form hypusine [25, 175]. Crystal structures of two archaebacterial eIF5A polypeptides show that they comprise an N-terminal β domain connected by a flexible linker to a C-terminal OB-like domain [176, 177]. The eIF5A protein and its hypusine modification are both essential in *S. cerevisiae* [178] but depletion of eIF5A does not affect the rate of protein synthesis significantly in vivo, casting some doubt on its requirement in translation [179, 180]. Alternative functions for eIF5A in mRNA turnover or cellular RNA export have been suggested [180]. However, other experiments in mammalian cells support a role for eIF5A in protein synthesis [181].

eIF5B

The term eIF5B has recently been suggested to designate a $\sim$175-kDa protein that is required with eIF5 for joining of the large (60S) ribosomal subunit to a 48S initiation complex assembled at the initiation codon of an mRNA [182]. This single large polypeptide is the eukaryotic homologue of IF2 and has itself previously been designated as eIF5 [1] and more recently as FUN12, yeast IF2 (yIF2), and human IF2 (hIF2) [183–186]. eIF5B polypeptides from different organisms range from $\sim$1000 to 1200 amino acid residues in length and have highly charged but otherwise relatively divergent N termini [183–186]. The extensive sequence homology in the C-terminal half of all family members is highest in the center of each protein in a region that contains all five sequence motifs characteristic of GTP-binding proteins (fig. 2). Human eIF5B can be UV cross-linked to GTP and has a GTPase activity that is absolutely dependent on the presence of ribosomal subunits [182]. Knockout experiments show that yeast eIF5B has an important function in translation [183, 184]. By analogy with prokaryotic IF2, this function could be recruitment of initiator tRNA to the small ribosomal subunit, adjustment of its position in the ribosomal P site, or subsequent joining of ribosomal subunits [187, 188]. The first of these seems unlikely since eIF5B does not bind initiator tRNA (S. A. Wilson, personal communication) and cannot complement lack of eIF2 in vivo [184]. Moreover, a defect in recruitment of initiator tRNA to the ribosome should enhance regulated reinitiation at the GCN4 initiation codon by permitting ribosomes to bypass the short upstream opening reading frames (ORFs) 2–4 but depletion of eIF5B in *S. cerevisiae* did not have this effect [184]. A role for eIF5B in adjusting the position of initiator tRNA in the ribosomal P site cannot be ruled out at present, but the principal step in the initiation process for which eIF5B is required is the joining of the large (60S) to the 48S initiation complex after it has assembled at the initiation codon of an mRNA (see below).

eIF6

Anti-association of dissociated ribsomal subunits is mediated by several factors, and had been thought to involve a 27-kDa protein termed eIF6 [189]. Although this protein binds strongly to 60S subunits, recent evidence suggests that it may be involved in ribosome biogenesis or assembly but plays no part in ribosomal anti-association [190–192].

PABP

The 3′ poly(A) tail of mRNAs stimulates their translation even in the absence of a 5′-terminal m^7G cap, and acts synergistically together with it to enhance translation [135, 136, 193, 194]. The effect of the poly(A) tail on translation is mediated by the cytoplasmic poly(A)-binding protein PABP [136, 195, 196]. The overall structure of PABP from divergent organisms is remarkably well conserved. PABP contains a proline-rich C-terminal domain and four highly conserved RNA-binding domains each consisting of an RRM of $\sim$80 amino acids. All four RRMs can bind RNA independently, but the highest affinity for poly(A) resides in the first two domains together [197, 198]. These two RRMs each contain four β strands and two α helices and are connected by a linker that contains a short α helix [199].

The two adjacent β sheets form the floor and an interstrand loop from each RRM and the RRM1-RRM2 and RRM2-RRM3 interdomain linkers form the sides of a continuous RNA-binding trough. It mediates specific, high-affinity binding to polyadenylate RNA, which adopts an extended conformation running through the entire length of the trough [199]. The less-conserved C-terminal domain does not contribute to RNA affinity or selectivity but enables formation of homodimers of PABP [198].

Experiments performed in vitro and in vivo have revealed that in the synergistic stimulation of translation of capped, polyadenylated mRNA, PABP participates in steps during initiation involving factors associated with the cap [135, 136, 194]. PABP has subsequently been found to bind to human and yeast eIF4G [159–161] and to wheat eIFiso4G and eIF4B [168]. PABP also binds to a human 480-amino acid PABP-interacting protein-1 (PAIP-1) which resembles the central portion of eIF4G [124]. Current models hold that PABP can stimulate poly(A)-dependent recruitment of 43S complexes to mRNA through its association with eIF4G, which in turn provides the link to this complex by interacting with its eIF3 constituent [200]. In addition, PABP can stimulate cap-dependent translation independently of its interaction with eIF4G and poly(A) by an unknown mechanism [200]. This stimulatory influence may be related to the role of PABP in the synergistic activation of translation of capped, polyadenyated mRNAs. These models for enhanced recruitment of 43S complexes do not rule out possible involvement of PABP in the subunit-joining reaction, and possibly in 'recycling' ribosomes from the 3′ end to the 5′ end of an mRNA after termination of translation [196].

The PABP-binding site in yeast eIF4G lies within a 115-amino acid region N terminal to the binding site for eIF4E [148, 159] and to an equivalent region in mammalian eIF4G [160, 161]. RRM2 of yeast PABP is required but not sufficient for its interaction with eIF4G [201]. The cocrystal structure of PABP RRMs $1+2$ with polyadenylate RNA [199] suggests phylogenetically conserved hydrophobic and acidic surface residues on the convex dorsal surface of PABP that may interact with eIF4G and PAIP-1. Synergistic activation of translation by the cap and the poly(A) tail can be destroyed in vitro by disruption of the interaction between eIF4E and eIF4G or between PABP and eIF4G, but additional stabilizing interactions possibly involving additional factors may occur in vivo, because mutations that weaken interactions in the eIF4E/eIF4G/PABP complex do not lead to cell lethality [147, 148, 159, 202]. Yeast PABP is an essential protein [202], so, alternatively, functions of PABP other than synergistic activation of translation are necessary for viability. The functional consequences of the interactions between PABP, eIF4G, and eIF4B are, at least in plants, to enhance the affinity of PABP for poly(A) and of eIF4F/eIFiso4F for the 5′ cap [168, 203], and in yeast to bring the termini of an mRNA into close physical proximity during initiation [204]. These RNA-protein interactions may synergize, contributing to enhanced recruitment of 43S complexes to the 5′ end of an mRNA.

The mechanism of translation initiation

Models for the mechanism of translation initiation in eukaryotes (fig. 4) are in large part based on the results of in vitro biochemical experiments, in the most part done using β-globin as a model capped mRNA. Although some steps in the proposed pathway have received independent support, most significantly from genetic studies in yeast, it is important to note that many steps in initiation are poorly understood and that all descriptions of them should be considered as working models. Specifically, (i) initiation of translation of a small but significant number of eukaryotic mRNAs apparently occurs by mechanisms such as internal ribosomal entry and shunting [205] that are incompatible with the canonical model and (ii) it is by no means certain that the full set of factors that mediate translation initiation on canonical mRNAs is known. Over the last 3 years, a significant number of novel factors have been implicated in translation initiation but have not yet been integrated into a coherent model of this process. These include the Ded1 protein [119, 206, 207], eIF4H [105, 134], p50 [208], the PABP-interacting protein PAIP-1 [124], and a family of eIF4E-related proteins [209, 210].

Dissociation of 80S ribosomes

Initiation of protein synthesis in all organisms begins with separated ribosomal subunits. In eukaryotes, ribosomes are released from mRNA after translation termination, but whether or not dissociation requires additional factors is not yet known. Physiological ionic concentrations favor the (premature) reassociation of subunits to form inactive 80S ribosomes, but this is prevented by the anti-association activities of eIF1A and eIF3, which bind to the 40S subunit to prevent it from binding to the 60S subunit [4]. The mechanism by which eIF3 prevents association is not known, but appears not to involve direct physical blockage of the subunit-subunit interface [211]. Until recently 'eIF6' was assumed to have had significant anti-association activity, resulting from its strong interaction with the 60S subunit, but it is now thought that this polypeptide plays no part in this process [187–189].

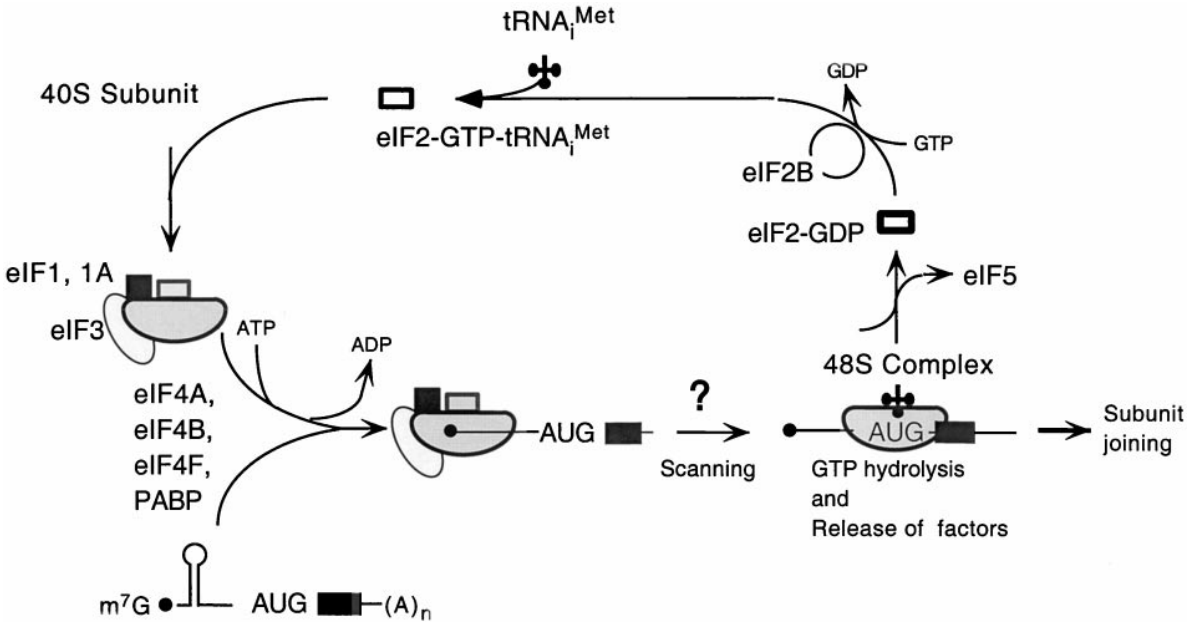

Figure 4. Schematic representation of the pathway of 48S complex formation on a capped eukaryotic mRNA. This model depicts the initial stage of action of some of the initiation factors that participate in initiation complex formation. A ternary complex comprising initiator tRNA, GTP, and eIF2 is bound to a 40S subunit associated with eIF1, eIF1A, and eIF3 to form a 43S preinitiation complex. At least four factors, eIF4A, eIF4B, eIF4F, and PABP cooperate in ATP-dependent mRNA binding to the 43S complex. The function of these factors is described in the text. The bound 43S complex then scans the 5′ non-translated region in a 5′–3′ direction until it recognizes the initiation codon. At this stage, hydrolysis of eIF2-bound GTP is triggered by eIF5, leading to release of eIF2-GDP and allowing the resulting 48S complex to join with a 60S ribosomal subunit to form a translationally competent 80S ribosome.

Recognition of initiator tRNA by eIF2

Aminoacylated initiator tRNA forms a ternary complex with eIF2 and GTP before it binds the 40S subunit. In the 80S subunit, initiator tRNA binds to the P site and is excluded from the ribosomal A site. Initiator tRNA has unique sequence and structural features that are not found in elongator tRNAs, some of which either determine its interaction with eIF2 or act as anti-determinants of interaction with elongation factors so that it is excluded from the ribosomal A site. The identity (methionine) and the presence of the aminoacyl residue are essential for ternary complex formation with eIF2 and GTP [212] and the A1-U72 base pair at the end of the acceptor stem determine the efficiency of its formation [213]. Mutation of this base pair to G1-C72 abolished the function of yeast initiator tRNA in vivo [214]. The amino acid residues in eIF2 that determine its recognition of Met-tRNA$_i^{Met}$ are not known. The factors eIF1A and eIF3 may associate with 40S subunits immediately after dissociation of 80S ribosomes (see above) and have been reported to stabilize binding of ternary complexes to 40S subunits [4, 215]. This activity may result from direct interactions between these factors and the ternary complex, or may be the result of induced changes caused by their binding to 40S subunits. A third factor, eIF4C, may also play a similar role, but although it has been cloned recently [216], little more is known about this protein.

Recruitment of 43S complexes to capped mRNA

The majority of eukaryotic mRNAs are capped and their binding to ribosomal 43S preinitiation complexes is thought to be mediated by the synergistic effects of the 5′-terminal cap and the 3′ poly(A) tail [135, 136, 193, 194] and an interacting network of initiation factors, including the eIF4E, eIF4A, and eIF4G subunits of eIF4F, eIF4B, and PABP. Physical and genetic interactions between several of these factors have been detected and shown to enhance binding of eIF4A and eIF4E to mRNA (see above). The cycling of eIF4A through eIF4F [120] and the inhibitory effect of 4E-binding proteins on eIF4E-eIF4G interaction [152–154] provide clear evidence for an equilibrium between eIF4F and its subunits. However, whether eIF4F assembles before or after binding to capped mRNA is not yet clear: conflicting evidence suggests either that eIF4E alone [217] or eIF4F [149, 218, 219] first binds to the

cap. The 43S complex then either binds eIF4G and is recruited to the eIF4E-bound capped mRNA via eIF4E-eIF4G association, or the 43S complex is recruited directly to the capped mRNA/eIF4F complex.

The mechanism of 43S preinitiation complex binding to capped mRNA has not been characterized in detail, but several individual steps in this process have been suggested. First, binding of the eIF4A subunit of eIF4F and associated eIF4B to the cap-proximal region of an mRNA results in localized unwinding of the 5′ nontranslated region (5′NTR) in an ATP-driven reaction, yielding single-stranded mRNA that is potentially a ribosome-binding site [102, 103]. A reaction of this type would explain the ATP requirement for ribosomal recruitment to mRNA [220, 221]. Subsequent ribosomal recruitment to a 'prepared' mRNA could then be mediated by bridging interactions between eIF3 and eIF4G [100], eIF4B [101], mRNA [77, 89, 91, 94], and the 40S subunit [2, 99], as well as by direct binding of eIF4A, eIF4B, eIF4G, and the 40S subunit to mRNA. Disruption of the yeast eIF4B gene led to preferential inhibition of translation of mRNAs with stable secondary structure in the 5′NTR [118], and over-expression of eIF4E in mammalian cells facilitated translation of such mRNAs [224]. Nevertheless, some of these interactions may be redundant: eIF4B enhances initiation but is non-essential in vivo in *S. cerevisiae* [117, 118] and in vitro in reconstituted mammalian initiation [126], and initiation on uncapped mRNAs occurs as a result of 5′ end-dependent scanning [222] even though it does not require eIF4E or the N-terminal third of eIF4G [147, 223]. The component of the translation apparatus that promotes attachment of 43S complexes to the 5′ termini of uncapped mRNAs is not known, but the properties of eIF3 and its presence at the entry site of the mRNA-binding cleft of eukaryotic 40S subunits suggest that it is a candidate. Recent genetic experiments have implicated additional factors in ribosomal recruitment: the essential DEAD box helicase Ded1p and its non-essential Dbp1 homologue (72% identity) both suppressed a temperature-sensitive defect in eIF4E, and mutants of the former protein were synthetically lethal with eIF4A and eIF4E mutants and synthetically lethal in cells lacking eIF4B or one of the two eIF4G genes [119, 206, 207]. The function of Ded1p in translation appears to be evolutionarily conserved because the yeast DED1 gene could be functionally replaced by a mouse homologue [206].

Recruitment of 43S complexes by internal ribosomal entry

Initiation of translation of several viral and cellular mRNAs is 5′ end independent and occurs instead by ribosomal attachment to an IRES [225–228]. All IRESs are long RNAs with extensive secondary structure and are functionally defined by their ability to promote translation of the downstream cistron in a dicistronic mRNA independently of translation of the upstream cistron (fig. 5). Related viruses contain similar IRESs, but otherwise most IRESs differ significantly from one another in terms of length, sequence and structure, and thus very likely in details of the mechanism by which they bind ribosomes. The number of mRNAs known to contain an IRES is increasing continually, but only three groups of IRESs have been characterized in significant detail.

Hepatitis C virus and related viruses contain a compact ~330 nucleotide-long IRES that extends ~30 nucleotides into the coding region. Initiation occurs at the site of ribosomal attachment and does not involve scanning [229, 230]. This mode of direct ribosomal attachment may explain why the initiation codon context has little influence on the efficiency of initiation and why initiation can occur efficiently if the initiation codon is replaced by non-AUG triplets. Remarkably, 40S subunits form stable binary complexes with this IRES by binding directly at the initiation codon without any requirement for initiation factors [231]. This type of interaction is reminiscent of the interaction of prokaryotic 30S subunits with Shine-Dalgarno sequences but not exactly analogous, because ribosomal-binding determinants in this IRES do not comprise a single linear sequence. A model for ribosomal binding to the hepatitis C virus (HCV) IRES suggests that interaction with at least one 'recruitment' site within the IRES is followed by accommodation of the initiation codon region within the mRNA-binding cleft of the 40S subunit [231]. The HCV IRES also contains a binding site for eIF3 [98, 231] and its interactions with these two components of a 43S preinitiation complex are sufficient to promote both ribosomal recruitment and accurate placement of initiator tRNA in the ribosomal 'P' site (fig. 6).

EMCV exemplifies a group of viral IRESs that also includes foot-and-mouth disease virus (FMDV) and Theiler's murine encephalomyelitis virus (TMEV) [232–234]. The EMCV IRES is more than 100 nucleotides longer than the HCV IRES and has an unrelated sequence and structure, but the initiation codon (AUG_{834}) is nevertheless also at or near the initial site of ribosomal attachment [235]. TMEV and FMDV IRESs promote initiation at two sites, one at and one downstream of a position equivalent to EMCV AUG_{834}. Whether ribosomes reach the second start site by scanning from the vicinity of the first or by some other mechanism has not yet been established [e.g., refs 236, 237]. Initiation on the EMCV IRES to the stage of 48S complex formation requires a basal set of factors (eIFs 2, 3, 4A, and 4G), is enhanced by eIF4B and a cellular RNA-

binding protein termed the pyrimidine tract-binding protein (PTB), and does not involve eIF4E [127, 162]. Direct and specific binding of eIF4G to a domain upstream of the initiation codon promotes binding of eIF4A and eIF4B to the EMCV IRES, and is required (but is not sufficient) for ribosomal recruitment to this and all related IRESs [e.g., refs 127, 162, 163] (fig. 6). Interaction of eIF4G with these IRES-containing mRNAs may therefore play an analogous role in their translation to that of eIF4E with the cap in cap-mediated translation. The requirement for PTB in this initiation mechanism is not absolute and it seems likely that when PTB is required, its binding to the IRES helps to maintain its higher-order structure in an appropriate active conformation [238].

A third group of IRESs includes poliovirus and rhinovirus. These IRESs are similar in size to the EMCV IRES but differ from it significantly in terms of sequence and secondary structure and do not overlap the initiation codon but instead promote initiation at start sites that are between 31 and 154 nucleotides from their 3′ border [205, 234]. The mechanism by which ribosomes reach the start site after initial recruitment to the IRES is obscure; arguments have been advanced both for scanning from the 3′ border of the IRES and for IRES-mediated binding at sites downstream of its 3′ border [234–241]. The latter mechanism is analogous to the proposed two-step binding of 40S subunits to the HCV IRES, binding of residues flanking the initiation codon being dependent on an initial interaction between the body of the IRES and a site in the 43S complex [231]. The full set of factors required for ribosomal recruitment to poliovirus and rhinovirus IRESs is not yet known. Poliovirus translation is inhibited by a trans-dominant eIF4A mutant, so eIF4A is required [114]. These viruses both encode a protease that cleaves eIF4G, separating its eIF3/eIF4A-binding domain from the N-terminal PABP/eIF4E binding domain [100]. Neither it nor eIF4E is required for IRES function [223]. Poliovirus and rhinovirus translation also requires additional (non-canonical) factors not involved in cap-mediated initiation; the activity of these IRESs is severely restricted in some tissue and cell types that support efficient translation of capped mRNAs. Several candidate trans-acting factors have been identified [242–245]. Poliovirus translation is enhanced in rabbit reticulocyte lysate by a combination of PTB and the poly(C)-binding protein-2 (PCBP-2), whereas rhinovirus translation is most responsive to a combination of PTB and *unr*, a protein that like PTB and PCBP-2 contains multiple RNA-binding domains [245]. These three proteins may contact the IRES at several different points, thereby

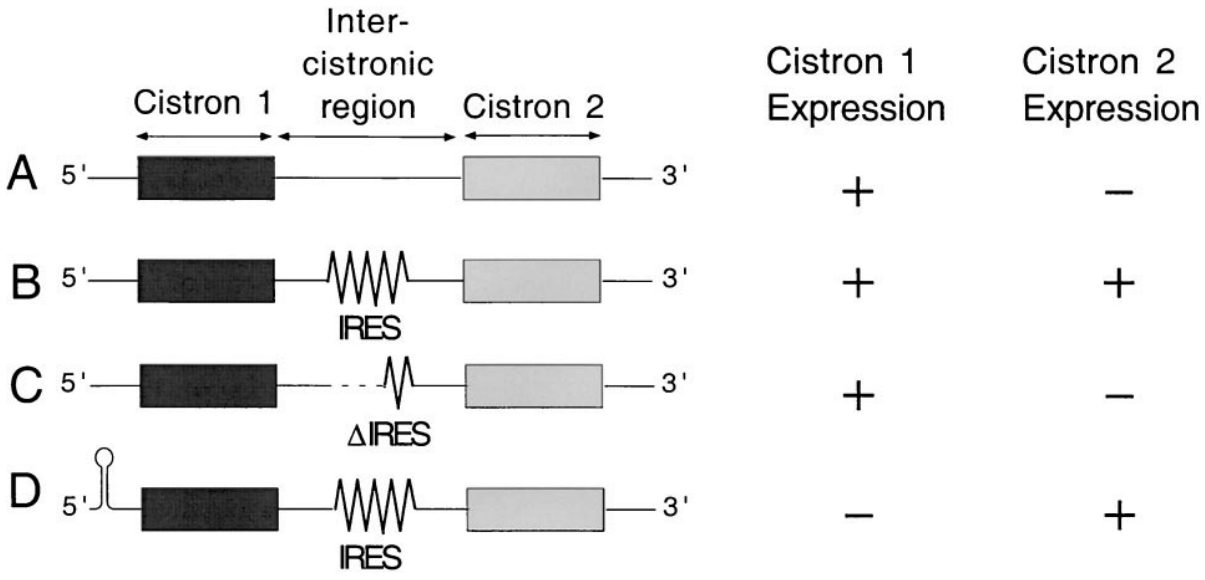

Figure 5. Schematic structure of dicistronic mRNAs used to prove the existence of an IRES and the resulting pattern of expression of upstream and downstream cistrons. Coding regions are boxed and non-coding regions are shown as thin lines, except segments of the putative IRES in the intercistronic area which are drawn as thick zig-zag lines. The putative IRES is partially deleted in construct C and cistron 1 is preceded by a stable hairpin in construct D. Expression of cistrons 1 and 2 is as indicated. This strategy has been used to identify and characterize IRESs in the 5′ non-translated regions of the RNA genomes of encephalomyocarditis virus, poliovirus, and hepatitis C virus, amongst others [e.g., 225–228].

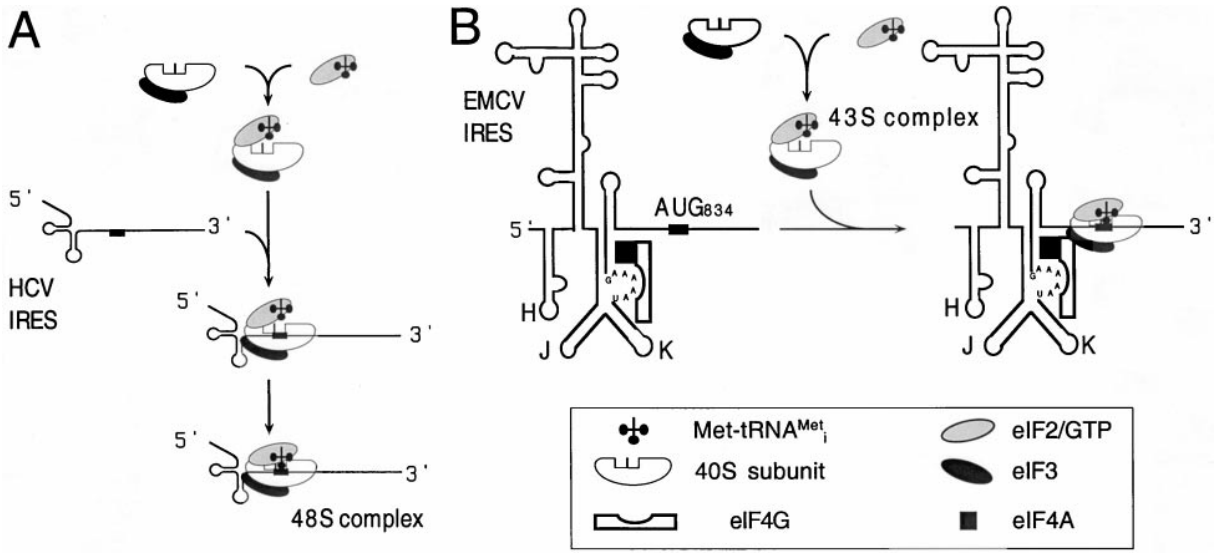

Figure 6. Models for internal ribosomal entry on the IRESs of hepatitis C virus (HCV) (*A*) and encephalomyocarditis virus (EMCV) (*B*), leading to 48S complex formation. (*A*) A 43S preinitiation complex is recruited to the HCV IRES as a result of specific interactions between the IRES and both eIF3 and the 40S subunit. The resulting complex then undergoes structural rearrangements that place initiator tRNA in the ribosomal P site so that its anticodon can base pair with the HCV initiation codon. (*B*) eIF4G binds to a Y-shaped J-K domain upstream of the EMCV initiation codon, and then recruits eIF4A and the 43S preinitiation complex to the IRES, leading to formation of a 48S complex. The IRESs of some EMCV strains require binding of pyrimidine tract-binding protein to domains H and K to stabilize the IRES in an active conformation.

stabilizing the IRES in a tertiary conformation required for internal initiation.

Initiation of HCV and EMCV translation occurs by very different mechanisms and it would clearly be premature to assume that other IRESs will not utilize yet other mechanisms to promote internal ribosomal entry. Nevertheless, some common aspects of initiation mediated by these different IRESs are apparent. First, initiation by internal ribosomal entry requires a smaller set of canonical factors than cap-mediated initiation, which may enable IRES-mediated initiation to continue under conditions (such as reduced activity of eIF4E or dissociation of eIF4E from the eIF4F complex) that inhibit cap-mediated initiation [246]. This ability would enable a subset of cellular mRNAs to be translated during conditions such as mitosis, heat shock, or hypoxia. Second, the activity of some IRESs is strongly dependent on non-canonical factors, often RNA-binding proteins, that are presumed to stabilize the IRES in a tertiary conformation required for internal initiation. Tissue-specific or developmental expression or regulation of such factors would in turn regulate IRES function without affecting cap-mediated initiation. Third, IRESs play a direct role in loading a 43S complex on an mRNA and in directing its binding to a specific location on it. This ability is probably determined by the correct spatial dispositon of multiple binding sites on an IRES and possibly by its ability to undergo defined structural transitions. This model explains the large size and structural complexity of IRESs and the functional requirement for structural integrity. It is consistent with observations that the sequences of related IRESs are less strongly conserved than their secondary structures, and that conserved residues tend to occur in unpaired loops and bulges that could participate in RNA-RNA or RNA-protein interactions [205, 233, 234].

Scanning

Ribosomes locate the initiation codon on conventional capped mRNAs by 'scanning' 50–100 nucleotides downstream from the initial binding site at the 5′ terminus of an mRNA. Scanning is defined in terms of the effect of mRNA structure on initiation [247] but the molecular mechanism of this process remains very poorly characterized. Initiation is with few exceptions limited to AUG codons, which are recognized specifically by the anticodon of initiator tRNA [248]. Initiation efficiency is reduced if the sequence 'context' surrounding the initiation codon deviates from a preferred optimum. However, the component of the translation apparatus that detects context residues is not

known. Initiation on eukaryotic mRNAs is usually limited to the first AUG triplet from the 5′ end of an mRNA, which is best explained by an overall 5′–3′ movement by scanning ribosomes so that the priority rule is imposed by initial attachment at or near the 5′ end. Specificity of ribosomal attachment has been argued as being mediated by binding of the eIF4F/PABP complex to the cap and to the poly(A) tail [194] but, notably even uncapped and unpolyadenylated mRNAs are translated by a 5′ end-dependent scanning mechanism [222]. A further argument in favor of the scanning model is that hairpin structures in the 5′NTR reduce initiation efficiency, in proportion to their stability and increasing with increasing proximity to the 5′ cap, presumably by impairing forward movement of the scanning ribosomal complex to the initiation codon [247]. The observation that 43S complexes appear to be arrested upstream of a stable iron-responsive element/iron-regulatory protein complex placed in a cap-distal position is consistent with this model [249]. Arrest of scanning ribosomes at the initiation codon by a downstream hairpin enhances start site recognition [250].

The minimal set of factors (eIFs 1, 1A, 2, 3, and 4F) required for 48S complex assembly at the initiation codon of β-globin mRNA (a model capped cellular mRNA) have been defined biochemically [126]. A requirement for eIF4A distinct from eIF4F was not investigated: eIF4B enhanced but was not essential for initiation complex formation. A consideration of the role of individual factors in scanning may be facilitated by considering it as consisting of several linked processes. They are (i) dissociation of the ribosomal complex from the 5′ cap to enter the processive scanning mode, (ii) unwinding of structured RNA in the 5′NTR, (iii) ribosomal movement, (iv) inspection of the 5′NTR for the initiation codon, (v) rejection of mismatched interactions between triplets in the 5′NTR and the anticodon of initiator tRNA and (vi) dissociation of arrested complexes from the 5′NTR.

A 43S complex that is recruited to the 5′-proximal end of an mRNA by the concerted actions of eIF4F and PABP must at some subsequent stage dissociate from these factors. Rearrangement of the preinitiation complex sufficient to cause factor dissociation is most likely to occur either when this complex enters a processive scanning mode after initial attachment or during the subunit joining stage. The evidence on which these alternatives can be assessed is sparse. Recycling of eIF4E has been attributed to eIF4B [251] and more recently to disruption of the eIF4G-eIF4E interaction and a consequent reduction of eIF4E cap-binding affinity [149]. Assays of the association of eIF4E with fractionated ribosomal complexes have given contradictory results, not least because of the tendency of sucrose density gradient centrifugation to strip factors from ribosomal complexes [217, 252]. Factors such as eIF4F or its eIF4G subunit that mediate recruitment of a ribosomal complex to capped mRNA could participate in subsequent processes if they are not released from the complex as it begins scanning. If they remain tethered to the 5′ end of an mRNA, they could enable ribosomal complexes that dissociate prematurely during scanning to rapidly rebind to cap-proximal regions of the same mRNA [253]. If they dissociate from the 5′ end of an mRNA, they might nevertheless play an active role in the scanning process itself (such as increasing the processivity of the helicase eIF4A or coupling its activity directly to ribosomal movement).

Ribosomal recruitment and scanning both require ATP [220]. ATP is hydrolyzed by eIF4A and associated factors that can melt structured RNA in the 5′NTR that would otherwise impair ribosomal recruitment to and subsequent movement from the 5′-proximal region. This raises the question of whether ATP is required for ribosomal movement per se (distinct from unwinding of structured RNA), and if it is, whether ATP is hydrolyzed by 40S subunits themselves or by associated eIF4A. The minimal stimulatory effect of ATP on the ability of 40S subunits to reach the initiation codon of unstructured mRNAs in depleted cell-free extracts [254] and the lack of requirement for ATP for 48S complex formation on such mRNAs in in vitro reconstituted assembly reactions [T. Pestova, unpublished data] suggests strongly that ribosomal movement per se does not consume chemical energy. The stimulatory effect on initiation of a hairpin downstream of the initiation codon [250] argues against uncoupled helicase-catalyzed unwinding of structured mRNAs by eIF4A [103]. Coupling of RNA unwinding to ribosomal movement might be mediated by eIF4B or eIF4G (which both interact with both eIF4A and eIF3), would serve to minimize futile energy expenditure, and might enhance the processivity of scanning during the scanning process.

The function of ribosomal scanning is for the 40S subunit to locate the initiation codon rapidly and accurately. Many nucleic acid-binding proteins that mediate or regulate biological processes must similarly locate their target sites rapidly and accurately and often do so by being able to diffuse in the reduced dimensions of polymeric RNA or DNA [255, 256]. Electrostatic non-sequence-specific binding to nucleic acids allows for either linear diffusion (sliding) or transient dissociation-reassociation (short hops or interstrand transfer). These processes can be considered as a ‘random walk’ driven by thermal fluctuations in the medium in contrast to chemical energy-dependent translocation of helicases. Sliding is a widespread phenomenon, used to locate target sequences by many RNA- and DNA-binding proteins, including repressors, polymerases, methyltransferases, and nucleases [257–259]. We have sug-

gested that facilitated diffusion might be an instructive model for the ribosomal scanning process [253]. Linear diffusion is theoretically not exclusively unidirectional, and both prokaryotic and eukaryotic ribosomes are indeed capable of bidirectional movement on mRNA during reinitiation [222, 260, 261]. The failure of eukaryotic ribosomes to discriminate in favor of the upstream codon of the two AUG codons in the sequence -AAA**AUG**ACA**AUG**CUA- in the NA/NB mRNA of influenza type B virus [262] has been suggested by Jackson [205] as evidence for the possibility that cap-mediated initiation is a random diffusion process.

DNA-bound *Eco*R1 endonuclease is thought to be in continuous transition between a tight, specific and immobile DNA-binding mode (which leads to DNA cleavage) and a loose, non-specific binding mode (which allows for linear diffusion) that depends on the similarity between the recognition sequence and the DNA sequence being encountered by the enzyme [263]. A similar transition may occur in the scanning ribosomal preinitiation complex as it inspects an mRNA for the initiation codon. The critical interaction that leads to ribosomal recognition of a start site is the base pair interaction between the anticodon of $tRNA_i^{Met}$ and the AUG codon; translation initiation can occur at an AGG triplet as long as the anticodon of $tRNA_i^{Met}$ is altered from UAC to UCC [248]. Genetic suppressor analysis has identified additional components of the yeast translation apparatus that are important determinants of ribosomal start site recognition. Factors implicated in this process include eIF1 [30], all three subunits of eIF2 [41, 51–53], and eIF5 [53]. The Nip1 subunit of eIF3, which binds to both eIF1 and eIF5, may also be part of a complex involved in start site selection [76]. These studies have led to a model in which this complex of wild-type factors in association with initiator tRNA and the scanning 40S subunit triggers hydrolysis of eIF2-bound GTP only when a 3-bp codon/anticodon interaction is realized at an AUG initiation codon [53]. GTP hydrolysis leads to the release of some or all initiation factors, leaving initiator tRNA at the initiation codon so that 60S subunit joining can occur. The scanning complex may encounter and pause at a UUG triplet in the 5′NTR but in the absence of a 3-bp codon/anticodon interaction, GTP hydrolysis is not induced and the ribosome continues to scan the 5′NTR. Mutations in eIF1, eIF2, or eIF5 impair the ability of the scanning complex to reject mismatched interactions between triplets in the mRNA and the anticodon of initiator tRNA, by promoting premature hydrolysis of GTP, thereby allowing initiation factors to dissociate and leaving the initiator tRNA in the ribosomal P site with partially mismatched base pairing between $tRNA_i^{Met}$ and the non-cognate codon.

In the absence of eIFs 1 and 1A, 43S complexes can bind a model capped mRNA (β-globin) but dissociate again rapidly rather than scanning to the initiation codon [126]. These complexes become competent to scan to the initiation codon only in the presence of eIFs 1 and 1A, suggesting that they directly or indirectly stabilize ribosomal binding and prevent premature dissociation during scanning. A direct effect might involve formation of an initiation factor 'clamp' that regulates association of the 5′NTR with the 40S subunit mRNA-binding cleft [126, 205]. Formation of a transient 'clamp' between one or more factors and the 40S subunit [14, 21] could be reversed before a scanning preinitiation complex reaches an initiation codon, either in a programmed event such as non-linear scanning or shunting (during which scanning ribosomes seem to skip over large segments of a 5′NTR [264–266]) or randomly, leading to dissociation of scanning complexes before they reach the initiation codon [205].

Subunit joining

The final stage in the initiation process is joining of a 60S ribosomal subunit to the 48S complex assembled at the initiation codon of an mRNA. This subunit joining step leads to release of some or all initiation factors from the 40S subunit and formation of an 80S ribosome that is competent to begin translation. Investigation of the subunit joining process has led to apparently contradictory results; initial reports indicated that a $\sim$125- to 160-kDa factor was sufficient to mediate subunit joining [1, 4] but was subsequently discounted as an irrelevant contaminant of a smaller factor that is now termed eIF5 [169, 174]. This confused picture most likely arose because experiments were done with over-simplified assay systems: some factors essential for 48S complex formation on an mRNA can be omitted when this process is recapitulated on an AUG triplet. These earlier reports are reconciled by a recent experiment using a full complement of initiation factors (eIFs 1, 1A, 2, and 3) rather than a minimal set to assemble 48S complexes on an AUG triplet that identifies eIF5 and the high molecular-weight factor eIF5B as both being required for subunit joining [182].

The scanning ribosomal complex contains eIF2 and eIF3 and possibly eIF5, which binds both of these factors [27, 28, 47–49, 92]. This ribosomal complex is arrested at the initiation codon following its base pairing with the anticodon of initiator tRNA. This interaction induces eIF5 to activate the intrinsic GTPase activity of the eIF2 γ subunit, promoting quantitative hydrolysis of eIF2-bound GTP and in turn leading to release of eIF2-GDP and Pi from the 40S subunit, leaving initiator tRNA in the ribosomal 'P' site [53, 169, 170]. Molecular details of the mechanism by which

correct codon/anticodon base pairing is recognized and leads to this chain of events are not known but, as described above, genetic analysis has implicated eIF1, eIF2, and eIF5 in this process [30, 41, 51–53]. The eIF5-induced hydrolysis of bound GTP on a 48S complex is necessary but not sufficient for subunit joining, which additionally requires eIF5B, which has a GTPase activity that is strictly dependent on the presence of ribosomal subunits [182]. The requirement for both eIF5 and eIF5B is only apparent when assembly reactions contain eIF1 and eIF3, which are both necessary for 48S complex formation on capped cellular mRNAs but not for assembly on an AUG triplet. In their presence, eIF5B is unable to promote hydrolysis of eIF2-bound GTP and eIF5 may not be sufficient to displace all factors from the 40S subunit to prepare it for joining to the 60S subunit. The stage at which these factors are displaced from the 48S complex and the mechanism by which this occurs have not been determined. eIF5B must bind GTP to assume an active conformation that enables it to promote subunit joining, but this process does not require hydrolysis of this GTP molecule; however, hydrolysis is required subsequently so that eIF5B may dissociate from the 80S ribosome after its assembly [182].

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1 Moldave K. (1985) Eukaryotic protein synthesis. Annu. Rev. Biochem. **54:** 1109–1149
2 Nygard O. and Nilsson L. (1990) Translational dynamics: interactions between the translational factors, tRNA and ribosomes during eukaryotic protein synthesis. Eur. J. Biochem. **191:** 1–17
3 Trachsel H. (1991) Translation in Eukaryotes, CRC, Boca Raton, Fla.
4 Merrick W. C. (1992) Mechanism and regulation of eukaryotic protein synthesis. Microbiol. Rev. **56:** 291–315
5 Riis B., Rattan S. I., Clark B. F. and Merrick W. C. (1990) Eukaryotic protein elongation factors. Trends Biochem. Sci. **15:** 420–424
6 Negrutskii B. S. and El'skaya A. V. (1998) Eukaryotic translation elongation factor 1α: structure, expression, functions, and possible role in aminoacyl-tRNA channeling. Prog. Nucleic Acids Res.Mol. Biol. **60:** 47–78
7 Czworkowski J. and Moore P. B. (1996) The elongation phase of protein synthesis. Prog. Nucleic Acids Res. Mol. Biol. **54:** 293–332
8 Nakamura Y. and Ito K. (1998) How protein reads the stop codons and terminates translation. Genes Cells **3:** 265–278
9 Kisselev L. L. and Frolova L. Y. (1999) Termination of translation in eukaryotes: new results and new hypotheses. Biochemistry (Moscow) **64:** 8–16
10 Verschoor A., Srivastava S., Grassucci R. and Frank J. (1996) Native 3D structure of eukaryotic 80S ribosome: morphological homology with the *E. coli* 70S ribosome. J. Cell Biol. **133:** 495–505
11 Stark H., Orlova E. V., Rinke-Appel J., Jünke N., Brimacombe R. and Heel M. van (1997) Arrangement of tRNAs in pre- and posttranslocational ribosomes revealed by electron cryomicroscopy. Cell **88:** 19–28
12 Ban N., Nissen P., Nasen J., Capel M., Moore P. B. and Steitz T. A. (1999) Placement of protein and RNA structures into a 5Å resolution map of the 50S ribosomal subunit. Nature **400:** 841–847
13 Dube P., Wieske M., Stark H., Schatz M., Stahl J., Zemlin F. et al. (1998) The 80S rat liver ribosome at 25 Å resolution by electron cryomicroscopy and angular reconstitution. Structure **6:** 389–399
14 Dube P., Bacher G., Stark H., Mueller F., Zemlin F., Heel M. van et al. (1998) Correlation of the expansion segments in mammalian rRNA with the fine structure of the 80S ribosome: a cryoelectron microscopic reconstruction of the rabbit reticulocyte ribosome at 21Å resolution. J. Mol. Biol. **279:** 403–421
15 Cate J. H., Yusupov M. N., Yusupova G. Z., Earnest T. N. and Noller H. F. (1999) X-ray crystal structures of 70S ribosome functional complexes. Science **285:** 2095–2104
16 Verschoor A., Warner J. R., Srivastava S., Grassucci R. and Frank J. (1998) Three-dimensional structure of the yeast ribosome. Nucleic Acids Res. **26:** 655–661
17 Clemons W. M., May J. L., Wimberly B. T., McCutcheon J. P., Capel M. S. and Ramakrishnan V. (1999) Structure of a bacterial 30S ribosomal subunit at 5.5 Å resolution. Nature **400:** 833–840
18 Wool I., Chan Y.-L. and Glück A. (1996) Mammalian ribosomes: the structure and the evolution of proteins. In: Translational Control, pp. 685–732, Hershey J. W. B., Mathews M. B. and Sonenberg N. (eds), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
19 Gerbi S. A. (1996) Expansion segments: regions of variable size that interrupt the universal core secondary structure of ribosomal RNA. In: Ribosomal RNA: Structure, Evolution, Processing, and Function in Protein Biosynthesis, pp. 71–87, Zimmerman R. A. and Dahlberg A. E. (eds), CRC, New York
20 Wilson K. S. and Noller H. F. (1998) Molecular movement inside the translational engine. Cell **92:** 337–349
21 Mueller F., Stark H., Heel M. van, Rinke-Appel J. and Brimacombe R. (1997) A new model for the three-dimensional folding of *Escherichia coli* 16S ribosomal RNA. III. The topography of the functional centre. J. Mol. Biol. **271:** 566–587
22 Graifer D.M., Juzumiene D. I., Wollenzien P. and Karpova G. G. (1994) Cross-linking of mRNA analogues containing 4-thiouridine residues on the 3′- or 5′-side of the coding triplet to the mRNA binding center of the human ribosome. Biochemistry **33:** 3878–3884
23 Bulygin K. N., Graifer D. M., Repkova M. N., Smolenskaya I. A., Veniyaminova A. G. and Karpova G. G. (1997) Nucleotide G-1207 of 18S rRNA is an essential component of the human 80S ribosomal decoding center. RNA **3:** 1480–1485
24 Fields C. and Adams M. D. (1994) Expressed sequence tags identify a human isolog of the *sui1* translation initiation factor. Biochem. Biophys. Res. Commun. **198:** 288–291
25 Kyrpides N. C. and Woese C. R. (1998) Universally conserved translation initiation factors. Proc. Natl. Acad. Sci. USA **95:** 224–228
26 Naranda T., Macmillan S. E., Donahue T. F. and Hershey J. W. B. (1996) SUI1/p16 is required for the activity of eukaryotic translation initiation factor 3 in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **16:** 2307–2313
27 Asano K., Phan L., Anderson J. and Hinnebusch A. (1998) Complex formation by all five homologues of mammalian translation initiation factor 3 subunits from yeast *Saccharomyces cerevisiae*. J. Biol. Chem. **273:** 18573–18585
28 Phan L., Zhang X., Asano K., Anderson J., Vornlocher H.-P., Greenberg J. et al. (1998) Identification of a translation initiation factor 3 (eIF3) core complex, conserved in yeast and mammals, that interacts with eIF5. Mol. Cell. Biol. **18:** 4935–4946

29 Fletcher C. M., Pestova T. V., Hellen C. U. T. and Wagner G. (1999) Structure and interactions of translation initiation factor eIF1. EMBO J. **18:** 2631–2637
30 Yoon H. and Donahue T. F. (1992) The *sui1* suppressor locus in *Saccharomyces cerevisiae* encodes a translation factor that functions during $tRNA_i^{Met}$ recognition of the start codon. Mol. Cell. Biol. **12:** 248–260
31 Cui Y., Dinman J. D., Kinzy T. G. and Peltz S. W. (1998) The *mof2/Sui1* protein is a general monitor of translational accuracy. Mol. Cell. Biol. **18:** 1506–1516
32 Timmer R. T., Lax S. R., Hughes D. L., Merrick W. C., Ravel J. M. and Browning K. S. (1993) Characterization of wheat germ protein synthesis initiation factor eIF-4C and comparison of eIF-4C from wheat germ and rabbit reticulocytes. J. Biol. Chem. **268:** 24863–24867
33 Dever T. E., Wei C.-L., Benkowski L. A., Browning K., Merrick W. C. and Hershey J. W. B. (1994) Determination of the amino acid sequence of rabbit, human, and wheat germ protein synthesis factor eIF-4C by cloning and chemical sequencing. J. Biol. Chem. **269:** 3212–3218
34 Wei C.-L., Kainuma M. and Hershey J. W. B. (1995) Characterization of yeast translation initiation factor 1A and cloning of its essential gene. J. Biol. Chem. **270:** 22788–22794
35 Keeling P. J. and Doolittle W. F. (1995) An archaebacterial eIF-1A: new grist for the mill. Mol. Microbiol. **17:** 399–400
36 Murzin A. G. (1993) OB (oligonucleotide/oligosaccharide binding) fold – common structural and functional solution for nonhomologous sequences. EMBO J. **12:** 861–867
37 Sette M., Tilborg P. van, Spurio R., Kaptein R., Paci M., Gualerzi C. O. et al. (1997) The structure of the translational initiation factor IF1 from *E. coli* contains an oligomer-binding motif. EMBO J. **16:** 1436–1443
38 Battiste J., Pestova T. V., Hellen C. U. T. and Wagner G. (2000) The eIF1A solution structure reveals a large RNA-binding surface important for scanning function. Mol. Cell **5:** 109–119
39 Wei C.-L., MacMillan S. E. and Hershey J. W. B. (1995) Protein synthesis initiation factor eIF-1A is a moderately abundant RNA-binding protein. J. Biol. Chem. **270:** 5764–5771
40 Clemens M. J. (1996) Protein kinases that phosphorylate eIF2 and eIF2B, and their role in eukaryotic cell translational control. In: Translational Control, pp. 139–172, Hershey J. W. B., Mathews M. B. and Sonenberg N. (eds), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
41 Cigan A. M., Pabich E. K., Feng L. and Donahue T. F. (1989) Yeast translation initiation suppressor *sui2* encodes the α subunit of eukaryotic initiation factor 2 and shares sequence identity with the human α subunit. Proc. Natl. Acad. Sci. USA **86:** 2784–2788
42 Gribskov M. (1992) Translation initiation factor IF1 and factor eIF2α share an RNA-binding motif with prokaryotic ribosomal S1 and polynucleotide phosphorylase. Gene **119:** 107–111
43 Bycroft M., Hubbard T. J. P., Proctor M., Freund S. M. V. and Murzin A. G. (1997) The solution structure of the S1 RNA-binding domain: a member of an ancient nucleic-acid-binding fold. Cell **88:** 235–242
44 Flynn A., Shatsky I. N., Proud C. G. and Kaminski A. (1994) The RNA-binding properties of protein synthesis initiation factor eIF-2. Biochim. Biophys. Acta **1219:** 293–301
45 Laurino J. P., Thompson G. M., Pacheco E. and Castilho B. A. (1999) The β subunit of eukaryotic translation initiation factor 2 binds mRNA through the lysine repeats and a region comprising the C_2-C_2 motif. Mol. Cell. Biol. **19:** 173–181
46 Metz A. M. and Browning K. S. (1997) Assignment of the β-subunit of wheat eIF2 by protein and DNA sequence analysis and immunoanalysis. Arch. Biochem. Biophys. **342:** 187–189
47 Asano K., Krishnamoorthy T., Phan L. and Hinnebusch A. G. (1999) Conserved bipartite motifs in yeast eIF5 and eIF2Bε, GTPase-activating and GDP-GTP exchange factors in translation initiation, mediate binding to their common substrate eIF2. EMBO J. **18:** 1673–1688
48 Koonin E. V. (1995) Multidomain organization of eukaryotic guanine nucleotide exchange translation initiation factor eIF-2B subunits revealed by analysis of conserved sequence motifs. Protein Sci. **4:** 1608–1617
49 Das S., Maiti T., Das K. and Maitra U. (1997) Specific interaction of eukaryotic translation initiation factor 5 (eIF5) with the β-subunit of eIF2. J. Biol. Chem. **272:** 31712–31718
50 Kimball S. R., Heinzinger N. K., Horetsky R. L. and Jefferson L. S. (1998) Identfication of interprotein interactions between the subunits of eukaryotic initiation factors eIF2 and eIF2B. J. Biol. Chem. **273:** 3039–3044
51 Castilho-Valavicius B., Thompson G. M. and Donahue T. F. (1992) Mutation analysis of the Cys-X_2-Cys-X_{19}-Cys-X_2-Cys motif in the β subunit of eukaryotic translation initiation factor 2. Gene Expression **2:** 297–309
52 Donahue T. F., Cigan A. M., Pabich E. K. and Castilho-Valavicius B. (1988) Mutations at a Zn(II) finger motif in the yeast eIF-2β gene alter ribosomal start-site selection during the scanning process. Cell **54:** 621–632
53 Huang H.-K., Yoon H., Hannig E. M. and Donahue T. F. (1997) GTP hydrolysis controls stringent selection of the AUG start codon during translation initiation in *Saccharomyces cerevisiae*. Genes Dev. **11:** 2396–2413
54 Bommer U. A., Kraft R., Kurzchalia T. V., Price N. T. and Proud C. G. (1991) Amino acid sequence analysis of the beta- and gamma-subunits of eukaryotic initiation factor eIF-2: identification of regions interacting with GTP. Biochim. Biophys. Acta **1079:** 308–315
55 Anthony D. D., Kinzy T. G. and Merrick W. C. (1990) Affinity labeling of eukaryotic initiation factor 2 and elongation factor 1 alpha beta gamma with GTP analogs. Arch. Biochem. Biophys. **281:** 157–162
56 Gaspar N. J., Kinzy T. G., Scherer B. J., Hümbelin M., Hershey J. W. B. and Merrick W. C. (1994) Translation initiation factor eIF-2: cloning and expression of the human cDNA encoding the γ-subunit. J. Biol. Chem. **269:** 3415–3422
57 Naranda T., Sirangelo I., Fabbri B. J. and Hershey J. W. B. (1995) Mutations in the NKXD consensus element indicate that GTP binds to the γ-subunit of translation initiation factor eIF2. FEBS Lett. **372:** 249–252
58 Dorris D. R., Erickson F. L. and Hannig E. M. (1995) Mutations in *GCD11*, the structural gene for eIF-2γ in yeast, alter translational regulation of *GCN4* and the selection of the start site for protein synthesis. EMBO J. **14:** 2239–2249
59 Erickson F. L. and Hannig E. M. (1996) Ligand interactions with eukaryotic translation initiation factor 2: role of the gamma-subunit. EMBO J. **15:** 6311–6320
60 Erickson F. L., Harding L. D., Dorris D. R. and Hannig E. M. (1997) Functional analysis of homologs of translation initiation factor 2γ in yeast. Mol. Gen. Genet. **253:** 711–719
61 Hannig E. M., Cigan A. M., Freeman B. A. and Kinzy T. G. (1994) *GCD11*, a negative regulator of *GCN4* expression, encodes the γ subunit of eIF2 in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **13:** 506–520
62 Ehrmann I. E., Ellis P. S., Mazeyrat S., Duthie S., Brockdorff N., Mattei M. G. et al. (1998) Characterization of genes encoding translation initiation factor eIF-2γ in mouse and human: sex chromosome localization, escape from X-inactivation and evolution. Hum. Mol. Genet. **7:** 1725–1737
63 Bourne H. R., Saunders D. A. and McCormick F. (1991) The GTPase superfamily: conserved structure and molecular mechanism. Nature **349:** 117–127
64 Laalami S., Grentzmann G., Bremaud L. and Cenatiempo Y. (1996) Messenger RNA translation in prokaryotes: GTPase centers associated with translational factors. Biochimie **78:** 577–589

65 Kjeldgaard M. and Nyborg J. (1992) The refined structure of elongation factor Tu from *Escherichia coli*. J. Mol. Biol. **223:** 721–742

66 Trachsel H. (1996) Binding of initiator methionyl-tRNA to ribosomes. In: Translational Control, pp. 113–138, Hershey J. W. B., Mathews M. B. and Sonenberg N. (eds), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

67 Price N. T., Francia G., Hall L. and Proud C. G. (1995) Guanine nucleotide exchange factor for eukaryotic initiation factor-2: cloning of cDNA for the δ-subunit of rabbit translation initiation factor-2B. Biochim. Biophys. Acta **1217:** 207–210

68 Price N. T., Kimball S. R., Jefferson L. S. and Proud C. G. (1996) Cloning of cDNA for the γ-subunit of mammalian translation initiation factor 2B, the guanine nucleotide-exchange factor for eukaryotic initiation factor 2. Biochem. J. **318:** 631–636

69 Price N. T., Mellor H., Craddock B. L., Flowers K. M., Kimball S. R., Wilmer T. et al. (1996) eIF2B, the guanine nucleotide-exchange factor for eukaryotic initiation factor 2: sequence correspondence between the α, β and δ subunits of eIF2B from mammals and plants. Biochem. J. **318:** 637–643

70 Bushman J. L., Asuru A. I., Matts R. L. and Hinnebusch A. G. (1993) Evidence that GCD6 and GCD7, translational regulators of GCN4, are subunits of the guanine nucleotide exchange factor for eIF-2 in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **13:** 1920–1932

71 Yang W. and Hinnebusch A. G. (1996) Identification of a regulatory subcomplex in the guanine nucleotide exchange factor eIF2B that mediates inhibition by phosphorylated eIF2. Mol. Cell. Biol. **16:** 6603–6616

72 Pavitt G. D., Ramaiah K. V. A., Kimball S. R. and Hinnebusch A. G. (1998) eIF2 independently binds two distinct eIF2B subcomplexes that catalyze and regulate guanine-nucleotide exchange. Genes Dev. **12:** 514–526

73 Fabian J. R., Kimball S. R., Heinzinger N. K. and Jefferson L. S. (1997) Subunit assembly and guanine nucleotide exchange activity of eukaryotic initiation factor-2B expressed in Sf9 cells. J. Biol. Chem. **272:** 12359–12365

74 Kimball S. R., Fabian J. R., Pavitt G. D., Hinnebusch A. G. and Jefferson L. S. (1998) Regulation of guanine nucleotide exchange through phosphorylation of eukaryotic initiation factor eIF2alpha: role of the alpha- and delta-subunits of eIF2B. J. Biol. Chem. **273:** 12841–12845

75 Foiani M., Cigan A. M., Paddon C. J., Harashima S. and Hinnebusch A. G. (1991) GCD2, a translational repressor of the GCN4 gene, has a general function in the initiation of protein synthesis in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **11:** 3203–3216

76 Greenberg J. R., Phan L., Gu Z., deSilva A., Apolito C., Sherman F. et al. (1998) Nip1p associates with 40S ribosomes and the Prt1p subunit of eukaryotic initiation factor 3 and is required for efficient translation initiation. J. Biol. Chem. **273:** 23485–23494

77 Naranda T., MacMillan S. E. and Hershey J. W. B. (1994) Purified yeast translational initiation factor eIF-3 is an RNA-binding protein complex that contains the PRT1 protein. J. Biol. Chem. **269:** 32286–32292

78 Danaie P., Wittmer B., Altmann M. and Trachsel H. (1995) Isolation of a protein complex containing translation initiation factor Prt1 from *Saccharomyces cerevisiae*. J. Biol. Chem. **270:** 4288–4292

79 Naranda T., Kainuma M., MacMillan S. E. and Hershey J. W. B. (1997) The 39-kilodalton subunit of eukaryotic translation initiation factor 3 is essential for the complex's integrity and for cell viability in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **17:** 145–153

80 Anderson J., Phan L., Cuesta R., Carlson B. A., Pak M., Asano K. et al. (1998) The essential Gcd10p-Gcd14p nuclear complex is required for 1-methyladenosine modification and maturation of initiator methionyl-tRNA. Genes Dev. **12:** 3650–3662

81 Vornlocher H.-P., Hanachi P., Ribeiro S. and Hershey J. W. B. (1999) A 110-kilodalton subunit of translation initiation factor eIF3 and an associated 135-kilodalton protein are encoded by the *Saccharomyces cerevisiae* TIF32 and TIF31 genes. J. Biol. Chem. **274:** 16802–16812

82 Johnson K. R., Merrick W. C., Zoll W. L. and Zhu Y. (1997) Identification of cDNA clones for the large subunit of eukaryotic translation initiation factor 3: comparison of homologues from human, *Nicotiana tabacum*, *Caenorhabditis elegans* and *Saccharomyces cerevisiae*. J. Biol. Chem. **272:** 7106–7113

83 Kovarik P., Hasek J., Valasek L. and Ruis H. (1998) RPG1: an essential gene of *Saccharomyces cerevisiae* encoding a 110-kDa protein required for passage through the G1 phase. Curr. Genet. **33:** 100–109

84 Valasek L., Trachsel H., Hasek J. and Ruis. H. (1998) Rpg1, the *Saccharomyces cerevisiae* homologue of the largest subunit of mammalian translation initiation factor 3, is required for translational activity. J. Biol. Chem. **273:** 21253–21260

85 Evans D. R. H., Rasmussen C., Hanic-Joyce P. J., Johnston G. C., Singer R. A. and Barnes C. A. (1995) Mutational analysis of the Prt1 protein subunit of yeast translation initiation factor 3. Mol. Cell. Biol. **15:** 4525–4535

86 Hanic-Joyce P. J., Singer R. A. and Johnston G. C. (1997) Molecular characterization of the yeast PRT1 gene in which mutations affect translation initiation and regulation of cell proliferation. J. Biol. Chem. **262:** 2845–2851

87 Méthot N., Rom E., Olsen H. and Sonenberg N. (1997) The human homologue of the yeast Prt1 protein is an integral part of the eukaryotic initiation factor 3 complex and interacts with p170. J. Biol. Chem. **272:** 1110–1116

88 Asano K., Kinzy T. G., Merrick W. C. and Hershey J. W. B. (1997) Conservation and diversity of eukaryotic translation initiation factor eIF3. J. Biol. Chem. **272:** 1101–1109

89 Asano K., Vornlocher H.-P., Richter-Cook N. J., Merrick W. C., Hinnebusch A. G. and Hershey J. W. B. (1997) Structure of cDNAs encoding human eukaryotic initiation factor 3 subunits: possible roles in RNA binding and macromolecular assembly. J. Biol. Chem. **272:** 27042–27052

90 Asano K., Merrick W. C. and Hershey J. W. B. (1997) The translation initiation factor eIF3-p48 subunit is encoded by *int*-6, a site of frequent integration by the mouse mammary tumor virus genome. J. Biol. Chem. **272:** 23477–23480

91 Hanachi P., Hershey J. W. B. and Vornlocher H.-P. (1999) Characterization of the p33 subunit of eukaryotic translation initiation factor-3 from *Saccharomyces cerevisiae*. J. Biol. Chem. **274:** 8546–8553

92 Bandyopadhyay A. and Maitra U. (1999) Cloning and characterization of the p42 subunit of mammalian translation initiation factor 3 (eIF3): demonstration that eIF3 interacts with eIF5 in mammalian cells. Nucleic Acids Res. **27:** 1331–1337

93 Verlhac M.-H., Chen R.-W., Hanachi P., Hershey J. W. B. and Derynck R. (1997) Identification of partners of TIF34, a component of the yeast eIF3 complex, required for cell proliferation and translation initiation. EMBO J. **16:** 6812–6822

94 Block K. L., Vornlocher H.-P. and Hershey J. W. B. (1998) Characterization of cDNAs encoding the p44 and p35 subunits of human translation initiation factor eIF3. J. Biol. Chem. **273:** 31901–31908

95 Smith T. F., Gaitatzes C., Saxena K. and Neer E. J. (1999) The WD repeat: a common architecture for diverse functions. Trends Biochem. Sci. **24:** 181–185

96 Hofmann K. and Bucher P. (1998) The PCI domain: a common theme in multiprotein complexes. Trends Biochem. Sci. **23:** 204–205

97 Valasek L., Hasek J., Trachsel H., Imre E. M. and Ruis H. (1999) The *Saccharomyces cerevisiae* HCR1 gene encoding a homologue of the p35 subunit of human translation initiation factor 3 (eIF3) is a high copy suppressor of a temperature-sensitive mutation in the Rpg1 subunit of yeast eIF3. J. Biol. Chem. **274:** 27567–27572

98 Sizova D. V., Kolupaeva V. G., Pestova T. V., Shatsky I. N. and Hellen C. U. T. (1998) Specific interaction of eukaryotic translation initiation factor 3 with the 5′ nontranslated regions of hepatitis C virus and classical swine fever virus RNAs. J. Virol. **72:** 4775–4782
99 Melander Y., Holmberg L. and Nygard O. (1997) The structure of 18S ribosomal RNA in native 40S ribosomal subunits. J. Biol. Chem. **272:** 3254–3258
100 Lamphear B. J., Kirchweger R., Skern T. and Rhoads R. E. (1995) Mapping of functional domains in eukaryotic protein synthesis initiation factor 4G (eIF4G) with picornaviral proteases: implications for cap-dependent and cap-independent translational initiation. J. Biol. Chem. **270:** 21975–21983
101 Méthot N., Song M. S. and Sonenberg N. (1996) A region rich in aspartic acid, arginine, tyrosine and glycine (DRYG) mediates eukaryotic initiation factor 4B (eIF4B) self-association and interaction with eIF3. Mol. Cell. Biol. **16:** 5328–5334
102 Rozen F., Edery I., Meerovitch K., Dever T. E., Merrick W. C. and Sonenberg N. (1990) Bidirectional RNA helicase activity of eucaryotic translation initiation factors 4A and 4F. Mol. Cell. Biol. **10:** 1134–1144
103 Jaramillo M., Dever T. E., Merrick W. C. and Sonenberg N. (1991) RNA unwinding in translation: assembly of helicase complex intermediates comprising eukaryotic initiation factors eIF-4F and eIF4-B. Mol. Cell. Biol. **11:** 5992–5997
104 Lorsch J. R. and Herschlag D. (1998) The DEAD box protein eIF4A. 1. A minimal kinetic and thermodynamic framework reveals coupled binding of RNA and nucleotide. Biochemistry **37:** 2180–2193
105 Rogers G. W., Richter N. J. and Merrick W. C. (1999) Biochemical and kinetic characterization of the RNA helicase activity of eukaryotic initiation factor 4A. J. Biol. Chem. **274:** 12236–12244
106 Gorbalenya A. E. and Koonin E. V. (1993) Helicases: amino acid sequence comparisons and structure-function relationships. Curr. Opin. Struct. Biol. **3:** 419–429
107 Bird L. E., Subramanya H. S. and Wigley D. B. (1998) Helicases: a unifying structural theme? Curr. Opin. Struct. Biol. **8:** 14–18
108 Benz J., Trachsel H. and Baumann U. (1999) Crystal structure of the ATPase domain of translation initiation factor 4A from *Saccharomyces cerevisiae* – the prototype of the DEAD box protein family. Structure **7:** 671–679
109 Velankar S. S., Soultanas P., Dillingham M. S., Subramanya H. S. and Wigley D. B. (1999) Crystal structures of complexes of pcrA DNA helicase with a DNA substrate indicate an inchworm mechanism. Cell **97:** 75–84
110 Schmid S. R. and Linder P. (1991) Translation initiation factor 4A from *Saccharomyces cerevisiae*: analysis of residues conserved in the D-E-A-D family of RNA helicases. Mol. Cell. Biol. **11:** 3463–3471
111 Blum S., Schmid S. R., Pause A., Buser P., Linder P., Sonenberg N. et al. (1992) ATP hydrolysis by initiation factor 4A is required for translation initiation in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **89:** 7664–7668
112 Pause A. and Sonenberg N. (1992) Mutational analysis of a DEAD box RNA helicase: the mammalian translation initiation factor eIF-4A. EMBO J. **11:** 2643–2654
113 Pause A., Methot N. and Sonenberg N. (1993) The HRIGRXXR region of the DEAD box RNA helicase eukaryotic translation initiation factor 4A is required for RNA binding and ATP hydrolysis. Mol. Cell. Biol. **13:** 6789–6798
114 Pause A., Methot N., Svitkin Y., Merrick W. C. and Sonenberg N. (1994) Dominant negative mutants of mammalian translation initiation factor eIF-4A define a critical role for eIF-4F in cap-dependent and cap-independent initiation of translation. EMBO J. **13:** 1205–1215
115 Lorsch J. R. and Herschlag D. (1998) The DEAD box protein eIF4A. 2. A cycle of nucleotide and RNA-dependent conformational changes. Biochemistry **37:** 2194–2206
116 Methot N., Pause A., Hershey J. W. B. and Sonenberg N. (1994) The translation initiation factor eIF-4B contains an RNA-binding region that is distinct and independent from its ribonucleoprotein consensus sequence. Mol. Cell. Biol. **14:** 2307–2316
117 Coppolecchia R., Buser P., Stotz A. and Linder P. (1993) A new yeast translation initiation factor suppresses a mutation in the eIF-4A RNA helicase. EMBO J. **12:** 4005–4011
118 Altmann M., Muller P. P., Wittmer B., Ruchti F., Lanker S. and Trachsel H. (1993) A *Saccharomyces cerevisiae* homologue of mammalian translation initiation factor 4B contributes to RNA helicase activity. EMBO J. **12:** 3997–4003
119 Cruz J. de la, Iost I., Kressler D. and Linder P. (1997) The p20 and Ded1 proteins have antagonistic roles in eIF4E-dependent translation in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **94:** 5201–5206
120 Yoder-Hill J., Pause A., Sonenberg N. and Merrick W. C. (1993) The p46 subunit of eukaryotic initiation factor (eIF)-4F exchanges with eIF-4A. J. Biol. Chem. **268:** 5566–5573
121 Imataka H. and Sonenberg N. (1997) Human eukaryotic translation initiation factor 4G (eIF4G) possesses two separate and independent binding sites for eIF4A. Mol. Cell. Biol. **17:** 69406947
122 Neff C. L. and Sachs A. B. (1999) Eukaryotic translation initiation factors 4G and 4A from *Saccharomyces cerevisiae* interact physically and functionally. Mol. Cell. Biol. **19:** 5557–5564
123 Imataka H., Olsen H. S. and Sonenberg N. (1997) A new translational regulator with homology to eukaryotic translation initiation factor 4G. EMBO J. **16:** 817–825
124 Craig A. W., Haghighat A., Yu A. T. and Sonenberg N. (1998) Interaction of polyadenylate-binding protein with the eIF4G homologue PAIP enhances translation. Nature **392:** 520–523
125 Lawson T. G., Lee K. A., Maimone M. M., Abramson R. D., Dever T. E., Merrick W. C. et al. (1989) Dissociation of double-stranded polynucleotide helical structures by eukaryotic initiation factors, as revealed by a novel assay. Biochemistry **28:** 4729–4734
126 Pestova T. V., Borukhov S. I. and Hellen C. U. T. (1998) Eukaryotic ribosomes require initiation factors 1 and 1A to locate initiation codons. Nature **394:** 854–859
127 Pestova T. V., Hellen C. U. T. and Shatsky I. N. (1996) Canonical eukaryotic initiation factors determine initiation of translation by internal ribosomal entry. Mol. Cell. Biol. **16:** 6859–6869
128 Milburn S. C., Hershey J. W., Davies M. V., Kelleher K. and Kaufman R. J. (1990) Cloning and expression of eukaryotic initiation factor 4B cDNA: sequence determination identifies a common RNA recognition motif. EMBO J. **9:** 2783–2790
129 Naranda T., Strong W. B., Menaya J., Fabbri B. J. and Hershey J. W. B. (1994) Two structural domains of initiation factor eIF-4B are involved in binding to RNA. J. Biol. Chem. **269:** 14465–14472
130 Méthot N., Pickett G., Keene J. D. and Sonenberg N. (1996) In vitro RNA selection identifies RNA ligands that specifically bind to eukaryotic translation initiation factor 4B: the role of the RNA recognition motif. RNA **2:** 38–50
131 Niederberger N., Trachsel H. and Altmann M. (1998) The RNA recognition motif of yeast translation initiation factor Tif3/eIF4B is required but not sufficient for RNA strand-exchange and translational activity. RNA **4:** 1259–1267
132 Altmann M., Wittmer B., Methot N., Sonenberg N. and Trachsel H. (1995) The *Saccharomyces cerevisiae* translation initiation factor Tif3 and its mammalian homologue, eIF-4B, have RNA annealing activity. EMBO J. **14:** 3820–3827
133 McCarthy J. E. G. (1998) Posttranscriptional control of gene expression in yeast. Microbiol. Mol. Biol. Rev. **62:** 1492–1553
134 Richter-Cook N. J., Dever T. E., Hensold J. O. and Merrick W. C. (1998) Purification and characterization of a new eukaryotic protein translation factor: eukaryotic initiation factor 4H. J. Biol. Chem. **273:** 7579–7587

135 Gallie D. R. (1991) The cap and poly(A) tail function synergistically to regulate mRNA translational efficiency. Genes Dev. **5:** 2108–2116

136 Tarun S. and Sachs A. B. (1995) A common function for mRNA 5′ and 3′ ends in translation initiation in yeast. Genes Dev. **9:** 2997–3007

137 Vasilescu S., Ptushkina M., Linz B., Müller P. P. and McCarthy J. E. G. (1996) Mutants of eukaryotic initiation factor eIF4E with altered cap binding specificity reprogramme mRNA selection by ribosomes in *Saccharomyces cerevisiae*. J. Biol. Chem. **271:** 7030–7037

138 Marcotrigiano J., Gingras A.-C., Sonenberg N. and Burley S. K. (1997) Cocrystal structure of the messenger RNA 5′ cap-binding protein (eIF4E) bound to 7-methyl-GDP. Cell **89:** 951–961

139 Marcotrigiano J., Gingras A.-C., Sonenberg N. and Burley S. K. (1999) Cap-dependent translation initiation in eukaryotes is regulated by a molecular mimic of eIF4G. Mol. Cell **3:** 707–716

140 Matsuo H., Li H., McGuire A. M., Fletcher C. M., Gingras A.-C., Sonenberg N. et al. (1997) Structure of translation factor eIF4E bound to m7GDP and interaction with 4E-binding protein. Nat. Struct. Biol. **4:** 717–724

141 Fletcher C. M., McGuire A. M., Gingras A.-C., Li H., Sonenberg N. and Wagner G. (1998) 4E binding proteins inhibit the translation factor eIF4E without folded structure. Biochemistry **37:** 9–15

142 Bu X., Haas D. and Hagedorn C. H. (1993) Novel phosphorylation sites of eukaryotic initiation factor 4F and evidence that phosphorylation stabilizes interactions of the p25 and p220 subunits. J. Biol. Chem. **268:** 4975–4978

143 Minich W. B., Balasta M. L., Goss D. J. and Rhoads R. E. (1994) Chromatographic resolution of in vivo phosphorylated and nonphosphorylated eukaryotic translation factor eIF-4E: increased cap affinity of the phosphorylated form. Proc. Natl. Acad. Sci. USA **91:** 7668–7672

144 Morino S., Hazama H., Ozaki M., Teraoka Y., Shibata S., Doi M. et al. (1996) Analysis of the mRNA cap-binding ability of human eukaryotic initiation factor-4E by use of recombinant wild-type and mutant forms. Eur. J. Biochem. **239:** 597–601

145 Shibata S., Morino S., Tomoo K., In Y. and Ishida I. (1998) Effect of mRNA cap structure on eIF-4E phosphorylation and cap binding analyses using Ser209-mutated eIF-4Es. Biochim. Biophys. Res. Commun. **247:** 213–216

146 Mader S., Lee H., Pause A. and Sonenberg N. (1995) The translation initiation factor eIF4E binds to a common motif shared by the translation factor eIF-4G and the translational repressors 4E-binding proteins. Mol. Cell. Biol. **15:** 4990–4997

147 Tarun S. Z. and Sachs A. (1997) Binding of eukaryotic translation initiation factor 4E (eIF4E) to eIF4G represses translation of uncapped mRNA. Mol. Cell. Biol. **17:** 6876–6886

148 Tarun S. Z., Wells S. E., Deardorff J. A. and Sachs A. (1997) Translation initiation factor eIF4G mediates in vitro poly(A) tail-dependent translation. Proc. Natl. Acad. Sci. USA **94:** 9046–9051

149 Ptushkina M., Haar T. van der, Vasilescu S., Birkenhage R. and McCarthy J. E. (1998) Cooperative modulation by eIF4G of eIF4E-binding to the mRNA 5′ cap in yeast involves a site partially shared by p20. EMBO J. **17:** 4798–4808

150 Ptushkina M., Haar T. van der, Karim M. M., Hughes J. M. X. and McCarthy J. E. G. (1999) Repressor binding to a dorsal regulatory site traps human eIF4E in a high cap-affinity state. EMBO J. **18:** 4068–4075

151 Pyronnet S., Imataka H., Gingras A.-C., Fukunaga R., Hunter T. and Sonenberg N. (1999) Human eukaryotic translation initiation factor 4G (eIF4G) recruits Mnk1 to phosphorylate eIF4E. EMBO J. **18:** 270–279

152 Pause A., Belsham G. J., Gingras A.-C., Donzé O., Lin T.-A., Lawrence J. C. et al. (1994) Insulin-dependent stimulation of protein synthesis by phosphorylation of a regulator of 5′-cap function. Nature **371:** 762–767

153 Altmann M., Schmitz N., Berset C. and Trachsel H. (1997) A novel inhibitor of cap-dependent translation initiation in yeast – p20 competes with eIF4G for binding to eIF4E. EMBO J. **16:** 1114–1121

154 Haghighat A., Mader S., Pause A. and Sonenberg N. (1995) Repression of cap-dependent translation by 4E-binding protein 1: competition with p220 for binding to eukaryotic initiation factor-4E. EMBO J. **14:** 5701–5709

155 Dominguez D., Altmann A., Benz J., Baumann U. and Trachsel H. (1999) Interaction of translation initiation factor eIF4G with eIF4A in the yeast *Saccharomyces cerevisiae*. J. Biol. Chem. **274:** 26720–26726

156 Hernandez G., Mar Castellano M. del, Agudo M. and Sierra J. M. (1998) Isolation and characterization of the cDNA and the gene for eukaryotic translation initiation factor 4G from *Drosophila melanogaster*. Eur. J. Biochem. **253:** 27–35

157 Hentze M. (1997) eIF4G: a multipurpose ribosome adaptor? Science **275:** 500–501

158 Morley S. J., Curtis P. S. and Pain V. M. (1997) eIF4G: translation's mystery factor begins to yield its secrets. RNA **3:** 1085–1104

159 Tarun S. Z. and Sachs A. B. (1996) Association of the yeast poly(A) tail binding protein with translation initiation factor eIF-4G. EMBO J. **15:** 7168–7177

160 Imataka H., Gradi A. and Sonenberg N. (1998) A newly identified N-terminal amino acid sequence of human eIF4G binds poly(A)-binding protein and functions in poly(A)-dependent translation. EMBO J. **17:** 7480–7489

161 Piron M., Vende P., Cohen J. and Poncet D. (1998) Rotavirus RNA-binding protein NSP3 interacts with eIF4GI and evicts the poly(A) binding protein from eIF4F. EMBO J. **17:** 5811–5821

162 Pestova T. V., Shatsky I. N. and Hellen C. U. T. (1996) Functional dissection of eukaryotic initiation factor 4F: the 4A subunit and the central domain of the 4G subunit are sufficient to mediate internal entry of 43S preinitiation complexes. Mol. Cell. Biol. **16:** 6870–6878

163 Kolupaeva V. G., Pestova T. V., Hellen C. U. T. and Shatsky I. N. (1998) Translation eukaryotic initiation factor 4G recognizes a specific structural element within the internal ribosome entry site of encephalomyocarditis virus RNA. J. Biol. Chem. **273:** 18599–18604

164 Kim C. Y., Takahashi K., Nguyen T. B., Roberts J. K. and Webster C. (1999) Identification of a nucleic acid binding domain in eukaryotic initiation factor eIFiso4G from wheat. J. Biol. Chem. **274:** 10603–10608

165 Goyer C., Altmann M., Lee H. S., Blanc A., Deshmukh M., Woolford J. L. Jr et al. (1993) TIF4631 and TIF4632: two yeast genes encoding the high-molecular-weight subunits of the cap-binding protein complex (eukaryotic initiation factor 4F) contain an RNA recognition motif-like sequence and carry out an essential function. Mol. Cell. Biol. **13:** 4860–4874

166 Gradi A., Imataka H., Svitkin Y. V., Rom E., Raught B., Morino S. et al. (1998) A novel functional human eukaryotic translation initiation factor 4G. Mol. Cell. Biol. **18:** 334–342

167 Metz A. M. and Browning K. S. (1996) Mutational analysis of the functional domains of the large subunit of the isozyme form of wheat initiation factor eIF4F. J. Biol. Chem. **271:** 31033–31036

168 Le H., Tanguay R. L., Balesta M. L., Wei C.-C., Browning K. S., Metz A. M. et al. (1997) The translation initiation factors eIFiso4G and eIF4B interact with the poly(A) binding protein to increase its RNA binding affinity. J. Biol. Chem. **272:** 16247–16255

169 Raychaudhuri P., Chaudhuri A. and Maitra U. (1985) Eukaryotic initiation factor 5 from calf liver is a single polypeptide chain of Mr = 62,000. J. Biol. Chem. **260:** 2132–2139

170 Chakrabarti A. and Maitra U. (1991) Function of eukaryotic initiation factor 5 in the formation of an 80S ribosomal polypeptide chain initiation complex. J. Biol. Chem. **266:** 14038–14045

171 Chakravarti D. and Maitra U. (1993) Eukaryotic translation initiation factor 5 from *Saccharomyces cerevisiae*: cloning, characterization, and expression of the gene encoding the 45,346-Da protein. J. Biol. Chem. **268:** 10524–10533
172 Das K., Chevesich J. and Maitra U. (1993) Molecular cloning and expression of cDNA for mammalian translation initation factor 5. Proc. Natl. Acad. Sci. USA **90:** 3058–3062
173 Maiti T. and Maitra U. (1997) Characterization of translation initiation factor 5 (eIF5) from *Saccharomyces cerevisiae*: functional homology with mammalian eIF5 and the effect of depletion of eIF5 on protein synthesis in vivo and in vitro. J. Biol. Chem. **272:** 18333–18340
174 Ribera I. L., Ruiz-Avila L. and Puigdomenech P. (1997) The eukaryotic translation initiation factor 5, eIF-5, a protein from *Zea mays*, containing a zinc-finger structure, binds nucleic acid in a zinc-dependent manner. Biochem. Biophys. Res. Commun. **236:** 510–516
175 Bartig D., Lemkemeier K., Frank J., Lottspeich F. and Klink F. (1992) The archaebacterial hypusine-containing protein: structural features suggest common ancestry with eukaryotic translation initiation factor 5A. Eur. J. Biochem. **204:** 751–758
176 Kim K. K., Hung L.-W., Yokota H., Kim R. and Kim S.-H. (1998) Crystal structures of eukaryotic translation initiation factor 5A from *Methanococcus jannaschii* at 1.8 Å resolution. Proc. Natl. Acad. Sci. USA **95:** 10419–10424
177 Peat T. S., Newman J., Waldo G. S., Berendzen J. and Terwilliger T. C. (1998) Structure of translation initiation factor 5A from *Pyrobaculum aerophilum* at 1.75 Å resolution. Structure **6:** 1207–1214
178 Schnier J., Schwelberger H. G., Smit-McBride Z., Kang H. A. and Hershey J. W. B. (1991) Translation initiation factor 5A and its hypusine modification are essential for cell viability in the yeast *Saccharomyces cerevisiae*. Mol. Cell. Biol. **11:** 3105–3114
179 Kang H. A. and Hershey J. W. B. (1994) Effect of initiation factor eIF-5A depletion on protein synthesis and proliferation of *Saccharomyces cerevisiae*. J. Biol. Chem. **269:** 3934–3940
180 Zuk D. and Jacobson A. (1998) A single amino acid substitution in yeast eIF-5A results in mRNA stabilization. EMBO J. **17:** 2914–2925
181 Shi X.-P., Yin K.-H. and Waxman L. (1997) Effects of inhibitors of RNA and protein synthesis on the subcellular distribution of the eukaryotic translation initiation factor, eIF-5A, and the HIV-1 Rev protein. Biol. Signals **6:** 143–149
182 Pestova T. V., Lomakin I., Lee J. H., Choi S. K., Dever T. E. and Hellen. C. U. T. (2000) The joining of ribosomal subunits in eurkaryotes requires eIF5B. Nature **403:** 332–335
183 Sutrave P., Shafer B. K., Strathern J. N. and andHughes S. H. (1994) Isolation, identification and characterization of the FUN12 gene of *Saccharomyces cerevisiae*. Gene **146:** 209–213
184 Choi S. K., Lee J. H., Zoll W. L., Merrick W. C. and Dever T. E. (1998) Promotion of Met-tRNA$_i^{Met}$ binding to ribosomes by yIF2, a bacterial IF2 homolog in yeast. Science **280:** 1757–1760
185 Lee J. H., Choi S. K., Roll-Mecak A., Burley S. K. and Dever T. E. (1999) Universal conservation in translation initiation revealed by human and archaeal homologs of bacterial translation initiation factor IF2. Proc. Natl. Acad. Sci. USA **96:** 4342–4347
186 Wilson S. A., Sieiro-Vasquez C., Edwards N. J., Iourin O., Byles E. D., Kotsopoulou E. et al. (1999) Cloning and characterization of hIF2, a human homologue of bacterial translation initiation factor two and its interaction with HIV-1 matrix. Biochem. J. **342:** 97–103
187 Luchin S., Putzer H., Hershey J. W., Cenatiempo Y., Grunberg-Manago M. and Laalami S. (1999) In vitro study of two dominant inhibitory GTPase mutants of *Escherichia coli* translation initiation factor IF2: direct evidence that GTP hydrolysis is necessary for factor recycling. J. Biol. Chem. **274:** 6074–6079
188 La Teana A., Pon C. L. and Gualerzi C. O. (1996) Late events in translation initiation: adjustment of fMet-tRNA in the ribosomal P-site. J. Mol. Biol. **256:** 667–675
189 Si K., Chaudhuri J., Chevesich J. and Maitra U. (1997) Molecular cloning and functional expression of a human cDNA encoding translation initiation factor 6. Proc. Natl. Acad. Sci. USA **94:** 14285–14290
190 Sanvito F., Piatti S., Villa A., Bossi M., Lucchini G., Marchisio P. C. et al. (1999) The beta4 integrin interactor p27(BBP/eIF6) is an essential nuclear matrix protein involved in 60S ribosomal subunit assembly. J. Cell Biol. **144:** 823–838
191 Si K. and Maitra U. (1999) The *Saccharomyces cerevisiae* homologue of mammalian translation initiation factor 6 does not function as a translation initiation factor. Mol. Cell. Biol. **19:** 1416–1426
192 Wood L. C., Ashby M. N., Grunfeld C. and Feingold K. R. (1999) Cloning of murine translation initiation factor 6 and functional analysis of the homologous sequence YPR016c in *Saccharomyces cerevisiae*. J. Biol. Chem. **274:** 11653–11659
193 Iizuka N., Najita L., Franzusoff A. and Sarnow P. (1994) Cap-dependent and cap-independent translation by internal initiation of translation of mRNAs in cell extracts prepared from *Saccharomyces cerevisiae*. Mol. Cell. Biol. **14:** 7322–7330
194 Preiss T. and Hentze M. W. (1998) Dual function of the messenger RNA cap structure in poly(A)-tail-promoted translation in yeast. Nature **392:** 516–520
195 Grossi de Sa M.-F., Standart N., Martins C., Akhayat O., Huesco M. and Scherrer K. (1988) The poly(A)-binding protein facilitates in vitro translation of poly(A)-rich mRNA. Eur. J. Biochem. **176:** 521–526
196 Jacobson A. (1996) Poly(A) metabolism and translation: the closed-loop model. In: Translational Control, pp. 451–480, Hershey J. W. B., Mathews M. B. and Sonenberg N. (eds), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
197 Burd C. G., Matunis E. L. and Dreyfuss G. (1991) The multiple RNA-binding domains of the mRNA poly(A)-binding protein have different RNA-binding affinities. Mol. Cell. Biol. **11:** 3419–3424
198 Kühn U. and Pieler T. (1996) *Xenopus* poly(A) binding protein: functional domains in RNA binding and protein-protein interaction. J. Mol. Biol. **256:** 20–30
199 Deo R. C., Bonanno J. B., Sonenberg N. and Burley S. K. (1999) Recognition of polyadenylate RNA by the poly(A)-binding protein. Cell **98:** 835–845
200 Otero L. J., Ashe M. P. and Sachs A. B. (1999) The yeast poly(A)-binding protein Pab1p stimulates in vitro poly(A)-dependent and cap-dependent translation by distinct mechanisms. EMBO J. **18:** 3153–3163
201 Kessler S. H. and Sachs A. B. (1998) RNA recognition motif 2 of yeast Pab1p is required for its functional interaction with eukaryotic translation initiation factor 4G. Mol. Cell. Biol. **18:** 51–57
202 Deardorff J. A. and Sachs A. B. (1997) Differential effects of aromatic and charged residue substitutions in the RNA binding domains of the yeast poly(A)-binding protein. J. Mol. Biol. **269:** 67–81
203 Wei C. C., Balesta M. L., Ren J. and Goss D. J. (1998) Wheat germ poly (A) binding protein enhances the binding affinity of eukaryotic initiation factor 4F and iso(4F) for cap analogues. Biochemistry **37:** 1910–1916
204 Wells S. E., Hillner P. E., Vale R. D. and Sachs A. B. (1998) Circularization of mRNA by eukaryotic translation initiation factors. Mol. Cell **2:** 135–140
205 Jackson R. J. (1996) A comparative view of initiation site selection mechanisms. In: Translational Control, pp. 71–112, Hershey J. W. B., Mathews M. B. and Sonenberg N. (eds), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

206 Chuang R.-Y., Weaver P. L., Liu Z. and Chang T.-H. (1997) Requirement of the DEAD-box protein Ded1p for messenger RNA translation. Science **275:** 1468–1471
207 Iost I., Dreyfus M. and Linder P. (1999) Ded1p, a DEAD-box protein required for translation initiation in *Saccharomyces cerevisiae*, is an RNA helicase. J. Biol. Chem. **274:** 17677–17683
208 Evdokimova V. M., Kovrigina E. A., Nashchekin D. V., Davydova E. K., Hershey J. W. B. and Ovchinnikov L. P. (1998) The major core protein of messenger ribonucleoprotein particles (p50) promotes initiation of protein biosynthesis in vitro. J. Biol. Chem. **273:** 3574–3581
209 Ruud K. A., Kuhlow C., Goss D. J. and Browning K. S. (1998) Identification and characterization of a novel cap binding protein from *Arabidopsis thaliana*. J. Biol. Chem. **273:** 10325–10330
210 Rom E., Kim C., Gingras A.-C., Marcotrigiano J., Favre D., Olsen H. et al. (1998) Cloning and characterization of 4EHP, a novel mammalian eIF4E-related cap-binding protein. J. Biol. Chem. **273:** 13104–13109
211 Srivastava S., Verschoor A. and Frank J. (1992) Eukaryotic initiation factor 3 does not prevent association through physical blockage of the ribosomal subunit-subunit interface. J. Mol. Biol. **226:** 301–304
212 Wagner T., Gross M. and Sigler P. B. (1984) Isoleucyl initiator tRNA does not initiate eucaryotic protein synthesis. J. Biol. Chem. **259:** 4706–4709
213 Farrugio D., Chaudhuri J., Maitra U. and RajBhandary U. L. (1996) The A1-U72 base pair conserved in eukaryotic initiator tRNAs is important specifically for binding to the eukaryotic translation initiation factor eIF2. Mol. Cell. Biol. **16:** 4248–4256
214 Pawel-Rammingen U. van, Bystrom S. and Bystrom A. S. (1992) Mutational analysis of conserved positions potentially important for initiator tRNA function in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **12:** 1432–1442
215 Chaudhuri J., Si K. and Maitra U. (1997) Function of eukaryotic translation initiation factor 1A (eIF1A) (formerly called eIF-4C) in initiation of protein synthesis. J. Biol. Chem. **272:** 7883–7891
216 Zou C., Zhang Z., Wu S. and Osterman J. C. (1998) Molecular cloning and characterization of a rabbit eIF2C protein. Gene **211:** 187–194
217 Joshi B., Yan R. and Rhoads R. E. (1994) In vitro synthesis of human protein synthesis initiation factor 4 γ and its localization on 43S and 48S initiation complexes. J. Biol. Chem. **269:** 2048–2055
218 Haghighat A. and Sonenberg N. (1997) eIF4G dramatically enhances the binding of eIF4E to the mRNA 5′-cap structure. J. Biol. Chem. 1997 **272:** 21677–21680
219 Muckenthaler M., Gray N. K. and Hentze M. W. (1998) IRP-1 binding to ferritin mRNA prevents the recruitment of the small ribosomal subunit by the cap-binding complex eIF4F. Mol. Cell **2:** 383–388
220 Kozak M. (1980) Role of ATP in binding and migration of 40S ribosomal subunits. Cell **15:** 1109–1123
221 Jackson R. J. (1991) The ATP requirement for initiation of eukaryotic translation varies according to the mRNA species. Eur. J. Biochem. **200:** 285–294
222 Gunnery S., Maivali U. and Mathews M. B. (1997) Translation of an uncapped mRNA involves scanning. J. Biol. Chem. **272:** 21642–21646
223 Ohlmann T., Rau M., Pain V. M. and Morley S. J. (1996) The C-terminal domain of eukaryotic protein synthesis initiation factor (eIF) 4G is sufficient to support cap-independent translation in the absence of eIF4E. EMBO J. **15:** 1371–1382
224 Koromilas A. E., Lazaris-Karatzas A. and Sonenberg N. (1992) mRNAs containing extensive secondary structure in their 5′ non-coding region translate efficiently in cells overexpressing initiation factor eIF-4E. EMBO J. **11:** 4153–4158
225 Jang S. K., Krausslich H. G., Nicklin M. J., Duke G. M., Palmenberg A. C. and Wimmer E. (1988) A segment of the 5′ nontranslated region of encephalomyocarditis virus RNA directs internal entry of ribosomes during in vitro translation. J. Virol. **62:** 2636–2643
226 Pelletier J. and Sonenberg N. (1988) Internal initiation of translation of eukaryotic mRNA directed by a sequence derived from poliovirus RNA. Nature **334:** 320–325
227 Macejak D. G. and Sarnow P. (1991) Internal initiation of translation mediated by the 5′ leader of a cellular mRNA. Nature **353:** 90–94
228 Tsukiyama-Kohara K., Iizuka N., Kohara M. and Nomoto A. (1992) Internal ribosome entry site within hepatitis C virus RNA. J. Virol. **66:** 1476–1483
229 Reynolds J. E., Kaminski A., Kettinen H. J., Grace K., Clarke B. E., Carroll A. R. et al. (1995) Unique features of internal initiation of hepatitis C virus RNA translation. EMBO J. **14:** 6010–6020
230 Reynolds J. E., Kaminski A., Carroll A. R., Clarke B. E., Rowlands D. J. and Jackson R. J. (1996) Internal initiation of translation of hepatitis C virus RNA: the ribosome entry site is at the authentic initiation codon. RNA **2:** 867–878
231 Pestova T. V., Shatsky I. N., Fletcher S. P., Jackson R. J. and Hellen C. U. T. (1998) A prokaryotic-like mode of cytoplasmic eukaryotic ribosome binding to the initiation codon during internal translation initiation of hepatitis C and classical swine fever virus RNAs. Genes Dev. **12:** 67–83
232 Agol V. I. (1991) The 5′-untranslated region of picornaviral genomes. Adv. Virus Res. **40:** 103–180
233 Hellen C. U. T. and Wimmer E. (1995) Translation of encephalomyocarditis virus RNA by internal ribosomal entry. Curr. Top. Microbiol. Immunol. **203:** 31–63
234 Jackson R. J. and Kaminski A. (1995) Internal initiation of translation in eukaryotes: the picornavirus paradigm and beyond. RNA **1:** 985–1000
235 Kaminski A., Howell M. T. and Jackson R. J. (1990) Initiation of encephalomyocarditis virus RNA translation: the authentic initiation site is not selected by a scanning mechanism. EMBO J. **9:** 3753–3759
236 Pilipenko E. V., Gmyl A. P., Maslova S. V., Belov G. A., Sinyakov A. N., Huang M. et al. (1994) Starting window, a distinct element in the cap-independent internal initiation of translation on picornaviral RNA. J. Mol. Biol. **241:** 398–414
237 Lopez de Quinto S. and Martinez-Salas E. (1999) Involvement of the aphthovirus RNA region located between the two functional AUGs in start codon selection. Virology **255:** 324–336
238 Kaminski A. and Jackson R. J. (1998) The polypyrimidine tract binding protein (PTB) requirement for internal initiation of translation of cardiovirus RNAs is conditional rather than absolute. RNA **4:** 626–638
239 Pilipenko E. V., Gmyl A. P., Maslova S. V., Svitkin Y. V., Sinyakov A. N. and Agol V. I. (1992) Prokaryotic-like cis elements in the cap-independent internal initiation of translation on picornavirus RNA. Cell **68:** 119–131
240 Pestova T. V., Hellen C. U. T. and Wimmer E. (1994) A conserved AUG triplet in the 5′ nontranslated region of poliovirus can function as an initiation codon in vitro and in vivo. Virology **204:** 729–737
241 Hellen C. U. T., Pestova T. V. and Wimmer E. (1994) Effect of mutations downstream of the internal ribosome entry site on initiation of poliovirus protein synthesis. J. Virol. **68:** 6312–6322
242 Borman A., Howell M. T., Patton J. and Jackson R. J. (1993) The involvement of a spliceosome component in internal initiation of human rhinovirus RNA translation. J. Gen. Virol. **74:** 1775–1788
243 Hellen C. U. T., Witherell G. W., Schmid M., Shin S. H., Pestova T. V., Gil A. et al. (1993) A cytoplasmic 57-kDa protein that is required for translation of picornavirus RNA by internal ribosomal entry is identical to the nuclear pyrimidine tract-binding protein. Proc. Natl. Acad. Sci. USA **90:** 7642–7646
244 Blyn L. B., Towner J. S., Semler B. L. and Ehrenfeld E. (1997) Requirement of poly(rC) binding protein 2 for translation of poliovirus RNA. J. Virol. **71:** 6243–6246

245 Hunt S. L., Hsuan J. J., Totty N. and Jackson R. J. (1999) unr, a cellular cytoplasmic RNA-binding protein with five cold-shock domains, is required for internal initiation of translation of human rhinovirus RNA. Genes Dev. **13:** 437–448
246 Sonenberg, N. (1996) mRNA 5′ cap-binding protein eIF4E and control of cell growth. In: Translation Control, pp. 271–294, Hershey J. W. B., Mathews M. B. and Sonenberg N. (eds), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
247 Kozak M. (1991) Structural features in eukaryotic mRNAs that modulate the initiation of translation. J. Biol. Chem. **266:** 19867–19870
248 Cigan A. M., Feng L. and Donahue T. F. (1988) $tRNA_i^{Met}$ functions in directing the scanning ribosome to the start site of translation. Science **242:** 93–97
249 Paraskeva E., Gray N. K., Schlager B., Wehr K. and Hentze M. W. (1999) Ribosomal pausing and scanning arrest as mechanisms of translational regulation from cap-distal iron-responsive elements. Mol. Cell. Biol. **19:** 807–816
250 Kozak M. (1990) Downstream secondary structure facilitates recognition of initiator codons by eukaryotic ribosomes. Proc. Natl. Acad. Sci. USA **87:** 8301–8305
251 Ray B. K., Lawson T. G., Abramson R. D., Merrick W. C. and Thach R. E. (1986) Recycling of messenger RNA cap-binding proteins mediated by eukaryotic initiation factor 4B. J. Biol. Chem. **261:** 11466–11470
252 Hiremath L. S., Hiremath S. T., Rychlik W., Joshi S., Domier L. L. and Rhoads R. E. (1989) In vitro synthesis, phosphorylation, and localization on 48S initiation complexes of human protein synthesis initiation factor 4E. J. Biol. Chem. **264:** 1132–1138
253 Pestova T. V. and Hellen C. U. T. (1999) Ribosome recruitment and scanning: what's new? Trends Biochem. Sci. **24:** 85–87
254 Jackson R. J. (1991) The ATP requirement for initiation of eukaryotic translation varies according to the mRNA species. Eur. J. Biochem. **200:** 285–294
255 Hippel P. H. von and Berg O. G. (1989) Facilitated target location in biological systems. J. Biol. Chem. **264:** 675–678
256 Shimamoto N. (1999) One-dimensional diffusion of proteins along DNA: its biological and chemical significance revealed by single-molecule measurements. J. Biol. Chem. **274:** 15293–15296
257 Surby M. A. and Reich N. O. (1996) Facilitated diffusion of the EcoRI DNA methyltransferase is described by a novel mechanism. Biochemistry **35:** 2209–2217
258 Jeltsch A., Wenz C., Stahl F. and Pingoud A. (1996) Linear diffusion of the restriction endonuclease EcoRV on DNA is essential for the in vivo function of the enzyme. EMBO J. **15:** 5104–5111
259 Kelemen B. R. and Raines R. T. (1999) Extending the limits to enzymatic catalysis: diffusion of ribonuclease A in one dimension. Biochemistry **38:** 5302–5307
260 Peabody D. S. and Berg P. (1986) Termination-reinitiation occurs in the translation of mammalian cell mRNAs. Mol. Cell. Biol. **6:** 2695–2703
261 Adhin M. R. and Duin J. van (1990) Scanning model for translational reinitiation in eubacteria. J. Mol. Biol. **213:** 811–818
262 Williams M. A. and Lamb R. A. (1989) Effect of mutations and deletions in a bicistronic mRNA on the synthesis of influenza B virus NB and NA glycoproteins. J. Virol. **63:** 28–35
263 Jeltsch A. and Pingoud A. (1998) Kinetic characterization of linear diffusion of the restriction endonuclease EcoRV on DNA. Biochemistry **37:** 2160–2169
264 Futterer J., Kiss-Laszlo Z. and Hohn T. (1993) Nonlinear ribosome migration on cauliflower mosaic virus 35S RNA. Cell **73:** 789–802
265 Yueh A. and Schneider R. J. (1996) Selective translation initiation by ribosome jumping in adenovirus-infected and heat-shocked cells. Genes Dev. **10:** 1557–1567
266 Latorre P., Kolakofsky D. and Curran J. (1998) Sendai virus Y proteins are initiated by a ribosomal shunt. Mol. Cell. Biol. **18:** 5021–5031