

Chapter 2

Mechanism of Translation in Eukaryotes

Nancy Villa and Christopher S. Fraser

Contents

2.1	Introduction	8
2.2	Translation Initiation	11
2.2.1	Binding of eIF4F Complex Prepares the mRNA for Translation	15
2.2.2	Several Initiation Factors Prepare the 40S Ribosome for mRNA Recruitment and Form the 43S PIC	17
2.2.3	mRNA Recruitment to 43S PIC	19
2.2.4	5' to 3' Scanning	20
2.2.5	Initiation Codon Selection	21
2.2.6	60S Ribosome Binding and 80S Ribosome Formation	23
2.3	Translation Elongation and Termination	23
2.3.1	Translation Elongation	24
2.3.2	Translation Termination	25
2.4	Ribosome Recycling and Reinitiation	25
2.4.1	Ribosome Recycling	26
2.4.2	Reinitiation	26
2.5	Conclusions and Perspectives	27
	References	27

Abstract Recent years have seen a tremendous advance in our understanding of the mechanism of protein synthesis in eukaryotic cells. Furthermore, our understanding of the role of translation in cancer development and progression, as well as its significance in clinical medicine has also greatly increased. The process of messenger RNA (mRNA) translation is comprised of four main stages: initiation, elongation, termination and ribosome recycling. Each stage is promoted by many different protein factors that interact with mRNA, transfer RNA (tRNA) and the 40S and 60S ribosomes to ensure an mRNA is accurately translated into protein. Here, we will describe the fundamental mechanisms involved in selection, recruitment, and translation of an mRNA by the eukaryotic ribosome with an emphasis on aspects most relevant to the theme of translation and cancer.

C. S. Fraser (✉) · N. Villa

Section of Molecular and Cellular Biology, University of California, Davis, CA, USA

e-mail: csfraser@ucdavis.edu

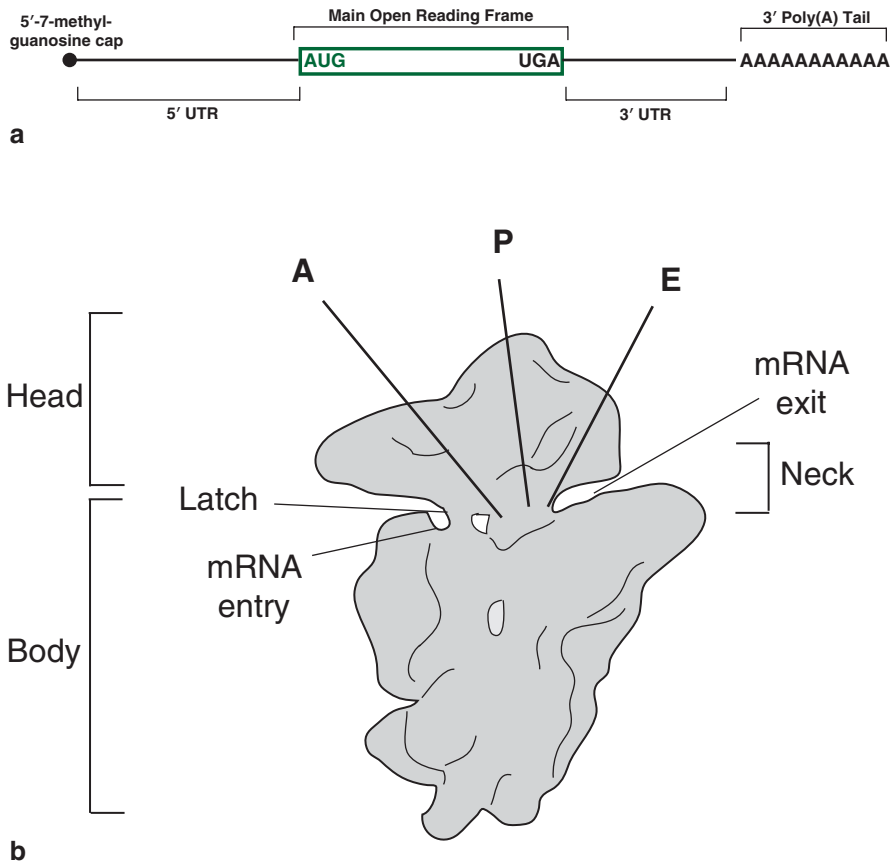


Fig. 2.1 Schematic diagrams of eukaryotic mRNA and 40S ribosome. **a** The mRNA 5' end is post-transcriptionally modified with a 7-methylguanosine cap and the 3' end with a poly(A) tail. The main open reading frame (ORF) is the region of the mRNA that encodes the protein, and usually begins with an AUG start codon and ends with one of three possible stop codons (UGA is used as an example here). Between the cap and the start codon is the 5' UTR. Between the stop codon and poly(A) tail is the 3' UTR. **b** The 40S subunit consists of three main regions: head, neck, and body. The neck is situated between the head and the body and delineates the mRNA-binding channel. The mRNA entry and exit sites are depicted together with the A-, P- and E-sites, which indicate respective tRNA-binding sites.

2.1 Introduction

In order for the information encoded in an mRNA to be translated into a protein, the mRNA must be recruited to a ribosome (Fig. 2.1). Protein synthesis occurs in four phases (initiation, elongation, termination, ribosome recycling), of which translation initiation is the most complex in as much as it requires the greatest number of protein factors. Initiation is also the stage most often implicated in cancer development and progression. During initiation, mRNA is recruited to the 40S ribosome, the start codon is located, and the 60S ribosome joins to form an elongation-competent 80S ribosome (Fig. 2.2). During elongation, the 80S ribosome migrates along the

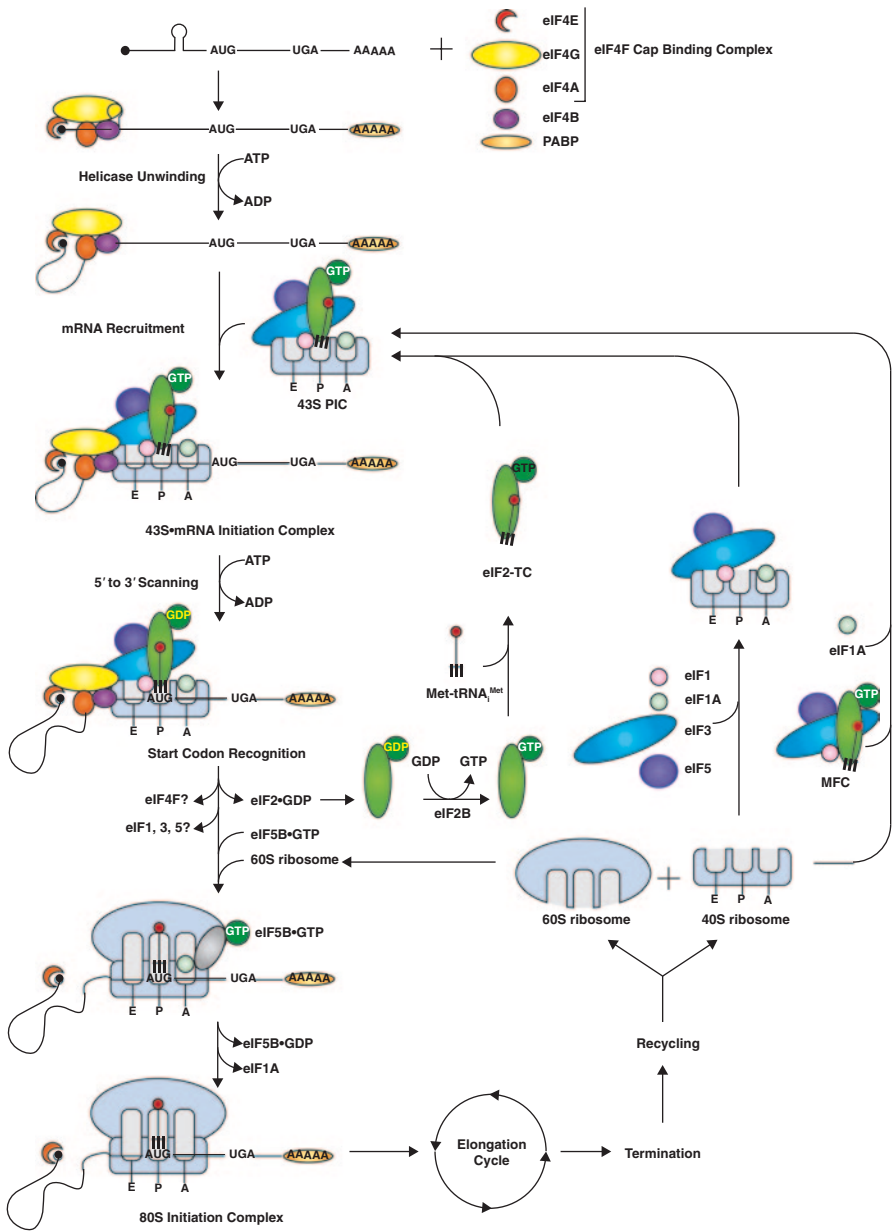


Fig. 2.2 Pathway of eukaryotic translation initiation. The target mRNA is recognized and bound by the eIF4F complex and PABP in preparation for recruitment to the 40S subunit. The eIF4F complex consists of three subunits (eIF4E, eIF4A, and eIF4G) and the auxiliary factor eIF4B, which together promote unwinding of secondary structure in the 5' UTR by eIF4A. The 40S subunit is prepared for mRNA recruitment by several factors, including eIF1, eIF1A, eIF2, eIF3 and eIF5. These factors may bind individually, or as a multifactor complex. eIF2 binds as a ternary complex, eIF2-TC, with GTP and the initiator tRNA. The message is directed to the 40S ribosome

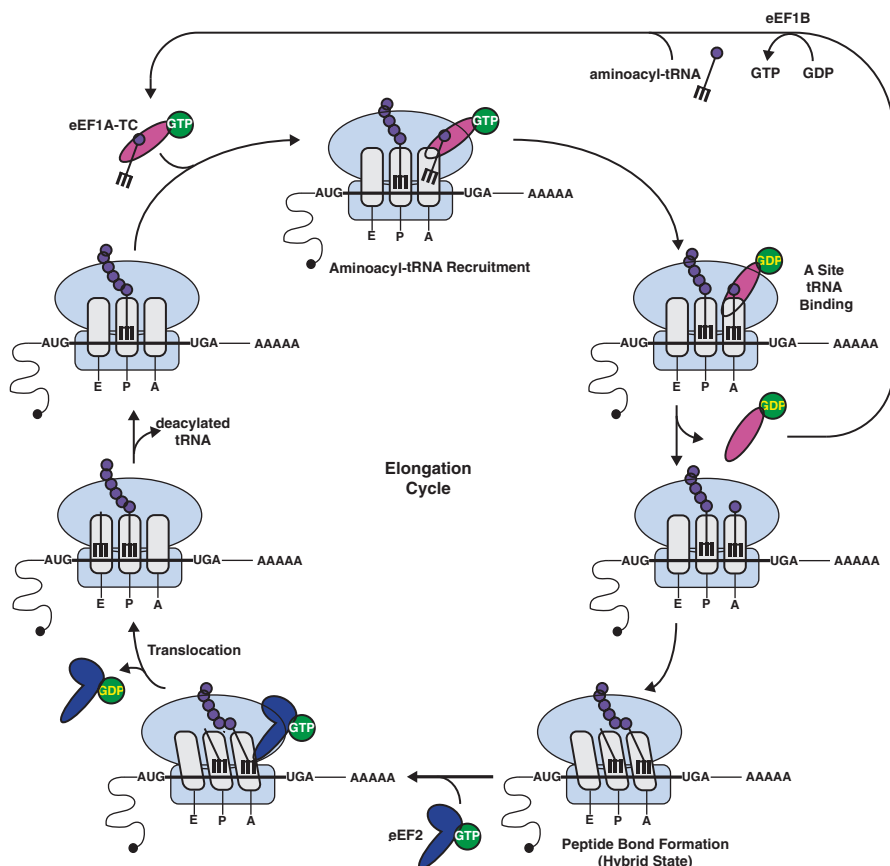


Fig. 2.3 The translation elongation cycle. During elongation, eEF1A-TC (eEF1A, GTP and aa-tRNA) recruits the aa-tRNA to the 80S ribosome. The aa-tRNA delivers the next amino acid to be incorporated into the growing polypeptide by base-pairing the tRNA anticodon with the mRNA codon in the A-site of the 80S ribosome. Peptide bond formation is catalyzed by the peptidyl transferase center in the 60S ribosomal subunit, resulting in the new amino acid being added to the polypeptide chain. This reaction results in spontaneous ratcheting of the ribosomal subunits forming a “hybrid state” in which tRNAs have partially translocated into the P/E- and A/P-sites. eEF2-GTP then binds the 80S ribosome and hydrolyzes its GTP. This promotes full translocation of the A-site tRNA to the P-site, and P-site tRNA to the E-site. The E-site tRNA and eEF2-GDP are then released from the ribosome leaving the next codon to be translated in the A-site.

Fig. 2.2 (continued) through the direct interaction of eIF4G and eIF3. Following recruitment, the 40S subunit scans for the initiation codon by traveling along the message in the 5' to 3' direction, sampling codons on the mRNA with the initiator tRNA anticodon. When the initiation codon is recognized through base-pairing, eIF5 induces GTP hydrolysis in eIF2-TC, resulting in the release of the eIF2-GDP complex and other initiation factors from the 40S subunit. eIF2 is recharged with GTP by eIF2B and recruits another initiator tRNA to reform the eIF2-TC, and the factors are free to initiate a new round of translation on another mRNA. eIF1A remains on the 40S subunit until eIF5B-GTP binds and recruits the 60S subunit, after which both are released following GTP hydrolysis. The newly formed 80S ribosome then enters the elongation cycle, followed by termination and recycling steps, which result in free 40S and 60S subunits to be prepared for future rounds of translation.

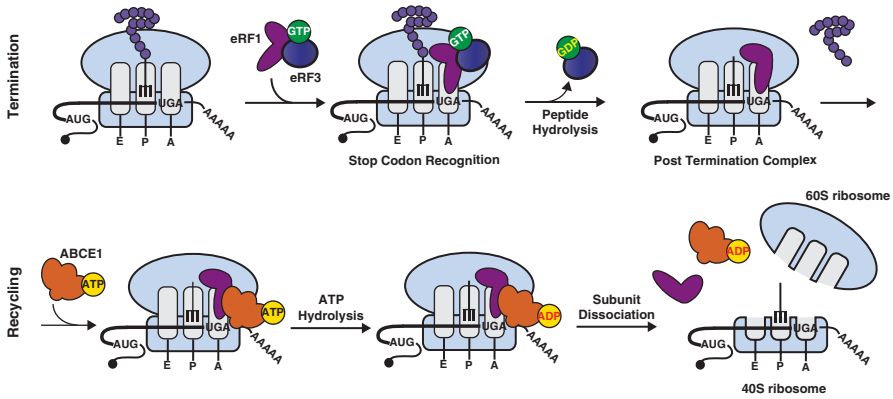


Fig. 2.4 Translation termination and ribosome recycling. **a** Translation termination occurs when a stop codon reaches the A-site of the 80S ribosome. Stop codons are not recognized by aa-tRNAs but by eRF1, which binds in a complex with eRF3 and GTP. This complex hydrolyzes GTP to promote peptide hydrolysis and release from the P-site tRNA and 80S ribosome. **b** Following peptide release, ABCE1 promotes ribosome dissociation and factor release in an ATP dependent reaction.

mRNA translating the information in each nucleotide triplet, or codon, to an amino acid, which is then incorporated into the growing polypeptide chain (Fig. 2.3). Stop codon recognition marks translation termination, where the newly formed protein is released (Fig. 2.4a). Lastly, ribosome recycling releases the mRNA and the 80S ribosome is separated into its component 40S and 60S subunits, which can then begin the translation cycle once again (Fig. 2.4b). The protein factors that function in each of these stages are summarized in Table 2.1, with known associations to the cancer etiology and pathogenesis (reviewed in Silvera et al. 2010; Spilka et al. 2013; Stumpf and Ruggero 2011). Each of these stages will be described in relevant detail in the following sections. Understanding the molecular mechanism of these processes is essential to understanding how they are regulated *in vivo*, and how they can become dysregulated in a transformed cell.

2.2 Translation Initiation

Translation initiation is almost always the rate-limiting step of protein synthesis, and as such, it is the most highly regulated (reviewed in Aitken and Lorsch 2012; Fraser 2009; Hinnebusch and Lorsch 2012; Jackson et al. 2010; Sonenberg and Hinnebusch 2009). Initiation rates can vary by many orders of magnitude. This variance can be due to differences in mRNA regulatory features, such as a highly structured 5' untranslated region (UTR), or regulation of initiation factors by key signaling cascades, such as the phosphoinositide 3-kinase (PI3K)/v-Akt murine thymoma viral oncogene homolog (AKT)/mechanistic target of rapamycin (mTOR) pathway and the mitogen-activated protein kinase (MAPK) pathway, which can affect factor availability and activity to alter the rate of translation. A recent study suggested that initiation

Table 2.1 Eukaryotic translation factors.

Initiation Factor	Subunits	Molecular Mass (kDa)	Function	Interacting Partners	Cancer Links
eIF1	1	12.7	Prepares 40S ribosome for mRNA loading and promotes scanning with eIF1A, fidelity of start site recognition	40S ribosome, eIF2, eIF3, eIF5	Chen et al. 2010; Lian et al. 1999
eIF1A	1	16.5	Prepares 40S ribosome for mRNA loading and promotes scanning with eIF1	40S ribosome, eIF5B	
eIF2	3	36.1, 38.4, 51.1	Binds and recruits initiator tRNA to the 40S ribosome	Initiator tRNA, 40S ribosome, eIF1, eIF2B, eIF3, eIF5	Rosenwald et al. 2001, 2003, 2008; Tejada et al. 2009; Wang et al. 1999
eIF2B	5	33.7, 39.0, 50.2, 59.7, 80.3	GTP exchange factor for eIF2, helps regenerate eIF2-TC	eIF2	
eIF3	13	~800 total	Scaffold that organizes the 43S PIC, increases eIF2-TC affinity for the 40S ribosome, prevents premature 60S ribosome binding, promotes mRNA recruitment with eIF4G	40S ribosome, eIF1, eIF1A, eIF4G, eIF5	Zhang et al. 2007
eIF4A	1	46.2	Member of eIF4F cap-binding complex; ATP dependent helicase, unwinds secondary structure in the 5' UTR of the mRNA	RNA, eIF4G, eIF4B	Eberle et al. 1997; Harris et al. 2004; Shuda et al. 2000
eIF4B	1	69.2	Stimulates helicase activity of eIF4A	RNA, eIF4A, eIF3	

Table 2.1 (continued)

Initiation Factor	Subunits	Molecular Mass (kDa)	Function	Interacting Partners	Cancer Links
eIF4E	1	25.1	Member of eIF4F cap-binding complex; Binds to the mRNA 5' 7-methylguanosine cap, stimulates eIF4A helicase activity with eIF4G	5' 7-methylguanosine cap, eIF4G	De Benedetti and Graff 2004; De Benedetti and Rhoads 1990; Flow-ers et al. 2009; Lazaris-Karatzas et al. 1990; Lazaris-Karatzas and Sonenberg 1992; Rosenwald et al. 2001, 2003, 2008; Ruggero et al. 2004; Tejada et al. 2009; Wang et al. 1999, 2001
eIF4G	1	175.5	Member of eIF4F cap-binding complex; acts as molecular scaffold, stimulates helicase and ATPase activity of eIF4A, promotes mRNA recruitment with eIF3	RNA, eIF4E, eIF4A, eIF3, PABP, MNK1	Fukuchi-Shimogori et al. 1997; Har-ris et al. 2004; Silvera et al. 2009
eIF4H	1	27.4	Homologous to the N-terminus of eIF4B, stimulates helicase activity of eIF4A	RNA, eIF4A	
eIF5	1	49.2	GTPase activating protein for eIF2 following start codon recognition	40S ribosome, eIF1, eIF2, eIF3	Harris et al. 2004
eIF5B	1	138.9	Ribosome dependent GTPase, pro-motes 80S ribosome formation	80S ribosome, eIF1A	
eIF6	1	26.6	60S ribosome biogenesis, binds 60S ribosomes and prevents premature association of 40S ribosomes	60S ribosome	Harris et al. 2004; Miluzio et al. 2011; Sanvito et al. 2000
DHX29	1	155.2	Binds the 40S ribosome and pro-motes scanning on highly struc-tured mRNAs	40S ribosome	Parsyan et al. 2009; Pisareva et al. 2008
PABP	1	70.7	Binds the mRNA poly(A) tail and eIF4G to promote translation initiation	eIF4G, poly(A) tail	Takashima et al. 2006

Table 2.1 (continued)

Elongation Factors	Subunits	Molecular Mass (kDa)	Function	Interacting Partners	Cancer Links
eEF1A	1	50.1	Binds and delivers aa-tRNA to the A-site as a ternary complex with GTP	80S ribosome, aa-tRNA	Harris et al. 2004
eEF1B	1	24.8	GTP exchange factor for eEF1A, helps regenerate eEF1A ternary complexes	eEF1A	
eEF2	1	95.3	Completes translocation of aa-tRNAs from the A- to P-site and the P- to E-site in the 80S ribosome	mRNA, tRNA, 80S ribosome	Nakamura et al. 2009
Termination Factors	Subunits	Molecular Mass (kDa)	Function	Interacting Partners	Cancer Links
eRF1	1	49.0	Binds to 80S ribosomes as a ternary complex with eRF3 and GTP, stop codon recognition, promotes peptide hydrolysis and release,	eRF3, 80S ribosome	
eRF3	1	55.8	Binds to 80S ribosomes as a ternary complex with eRF1 and GTP, hydrolyzes GTP to allow full accommodation of eRF1 into the A-site	eRF1, 80S ribosome	
Recycling Factors	Subunits	Molecular Mass (kDa)	Function	Interacting Partners	Cancer Links
ABCE1	1	67.3	Separates 80S ribosomes following termination	80S ribosome	

rates are likely to vary between 4s and 233s on different mRNAs in yeast (Shah et al. 2013). These findings emphasize the impact that translation initiation has on determining the overall rate of translation for any mRNA. Initiation of protein synthesis involves five basic steps, which will be described in further detail: (1) mRNA binding by the eIF4F cap-binding complex; (2) 43S preinitiation complex (PIC) formation; (3) mRNA recruitment to the ribosome; (4) localization of the initiation codon; and (5) 60S ribosome joining (Aitken and Lorsch 2012; Fraser 2009; Hinnebusch and Lorsch 2012; Jackson et al. 2010; Sonenberg and Hinnebusch 2009).

2.2.1 Binding of eIF4F Complex Prepares the mRNA for Translation

Eukaryotic mRNAs contain several key features involved in regulating translation (Fig. 2.1). Following transcription, a 7-methyl-guanosine cap is added to the 5' end while several adenosine residues are attached to the 3' end to form the polyadenosine (poly(A)) tail. Flanking the protein coding sequence of the mRNA are 5' and 3' UTRs, which may contain regulatory structures or sequences, which affect mRNA translation (see Chap. 3).

mRNAs are selected for translation by recognition of two of the aforementioned features: the 5' 7-methyl-guanosine cap (Carroll and Borden 2013; Topisirovic et al. 2011) and the 3' poly(A) tail (Fig. 2.1) (Mangus et al. 2003; Sachs et al. 1997). It has been known for many years that these features help to protect an mRNA from degradation and act synergistically to promote translation initiation (Gallie 1991; Searfoss et al. 2001). The eukaryotic translation initiation factor 4F (eIF4F) interacts directly or indirectly with these key features, and consists of three subunits: eIF4E (cap-binding), eIF4A (DEAD box helicase), and eIF4G (molecular scaffold) (Gingras et al. 1999; Grifo et al. 1983). The purpose of eIF4F is to locate the 5' end of the mRNA through cap recognition, then to ensure that the 40S ribosome will be able to bind to the 5' end of the mRNA by removing any inhibitory secondary structures in that region.

The cap-binding protein eIF4E specifically recognizes the 5' 7-methyl-guanosine cap by sandwiching it between two conserved tryptophans located in the cap-binding pocket of eIF4E (Marcotrigiano et al. 1997; Matsuo et al. 1997). Importantly, eIF4E is the least abundant initiation factor and therefore is generally regarded as the initiation factor that limits recruitment of mRNA to the ribosome (Duncan et al. 1987). Since cap recognition by eIF4E plays a critical role in mRNA recruitment the availability of this initiation factor is subject to strict regulation by eIF4E-binding proteins (4E-BPs), which can sequester eIF4E from eIF4G, thus inhibiting cap-dependent translation (see Chaps. 3 and 4). In a similar theme, programmed cell death 4 (PDCD4) can also inhibit cap-dependent translation by inhibiting the unwinding activity of eIF4A (Lankat-Buttgereit and Goke 2009) (see Chaps. 3, 5 and 6).

Consistent with its being the limiting factor, modest overexpression of eIF4E by 2.5-fold is able to transform immortalized cells and form tumors in mice (De

Benedetti and Rhoads 1990; Lazaris-Karatzas et al. 1990; Ruggero et al. 2004). In addition to binding the cap, eIF4E forms a high affinity interaction with eIF4G using a number of conserved residues located on the convex dorsal surface of eIF4E on the opposite side of the cap-binding pocket (Marcotrigiano et al. 1999). This interaction is necessary to position eIF4G near the 5' end of the mRNA so that it can prepare the mRNA for recruitment to the 40S subunit. It is noteworthy to mention that in addition to its role in mRNA recruitment to the 40S subunit, eIF4E is also involved in promoting the export of specific mRNAs from the nucleus (Culjkovic et al. 2005; Rousseau et al. 1996), many of which are linked to cell cycle progression and survival (Culjkovic et al. 2006).

The largest component of the eIF4F complex is the 175 kDa protein named eIF4G (Gingras et al. 1999; Hentze 1997; Imataka et al. 1998; Keiper et al. 1999; Prevot et al. 2003; Yan et al. 1992) (see Chap. 7). This protein contains binding domains for RNA (Berset et al. 2003; Goyer et al. 1993; Park et al. 2011), eIF4E (Lamphear et al. 1995; Mader et al. 1995), eIF4A (Imataka and Sonenberg 1997; Korneeva et al. 2001), eIF3 (Korneeva et al. 2001; Lamphear et al. 1995), poly (A)-binding protein (PABP) (Imataka et al. 1998; Tarun and Sachs 1996), and the MAPK-interacting kinases (MNKs) (Pyronnet et al. 1999). The protein can therefore be thought of as a molecular scaffold that recruits and coordinates the activities of these other initiation components. One function of eIF4G may be to stabilize the interaction of eIF4E with the cap structure and PABP with the poly(A) tail. Data to support this concept comes from a study which used crosslinking assays to show that eIF4E crosslinking to the cap structure is enhanced in the presence of eIF4G (Yanagiya et al. 2009). However, quantitative binding assays have provided inconsistent results as to whether eIF4G stimulates the interaction between eIF4E and the cap structure (Niedzwiecka et al. 2002; Slepnev et al. 2008; Yanagiya et al. 2009). Quantitative binding assays show that eIF4G does indeed increase the affinity of PABP to the poly(A) tail, which may help to circularize the mRNA and stimulate translation by facilitating reinitiation of a terminating ribosome on the same mRNA (Le et al. 1997; Wells et al. 1998). It is worth noting, however, that whether an mRNA can actually form closed circles as a result of this interaction *in vivo* is not clear (Amrani et al. 2008; Rajagopal et al. 2012). In addition to stabilizing these interactions, mammalian eIF4G has two additional pivotal roles in mRNA recruitment to the ribosome. Through its direct interaction with eIF3, which will be discussed in the next section, eIF4G helps bridge the eIF4F-mRNA complex and the 43S PIC (Hinton et al. 2007; Lamphear et al. 1995; Villa et al. 2013). Moreover, its interaction with DEAD box helicase eIF4A is required to recruit this helicase to the mRNA, which ultimately functions to unwind secondary structures located in the 5' UTR in order to facilitate ribosome recruitment and scanning.

It has been known for some time that the presence of secondary structure in the 5' UTR of eukaryotic mRNAs plays a key role in controlling recruitment to the ribosome (reviewed in Mauger et al. 2013; Parsyan et al. 2011). Secondary structures typically form when complementary regions of the mRNA base-pair to form stable hairpin structures. These structures can be particularly inhibitory when located near the cap since they can sterically hinder the recruitment of the mRNA to the 40S

Translation and Its Regulation in Cancer Biology and
Medicine

Parsyan, A. (Ed.)

2014, XXXIV, 697 p. 52 illus., 44 illus. in color.,

Hardcover

ISBN: 978-94-017-9077-2