The Regulation of eIF4F During Cell Growth and Cell Death

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1 Introduction

Much effort has been focused on questions concerning the highly regulated processes of cell growth, proliferation and programmed cell death (apoptosis). Hormones, growth factors and ligands exert pleiotypic effects through activation of specific cell-surface receptors, and via transmembrane signalling and activation of common protein kinase/phosphatase cascades inside the cell. These, in turn, trigger an array of cellular responses, culminating in either cell growth and division, differentiation or cell death. One of the obligatory, early responses in all of these processes is a modulation of the rate of protein synthesis, mediated by regulating the phosphorylation of translation initiation factor polypeptides and their association into functional complexes. This chapter will review current knowledge about the regulation of these initiation factor proteins in response to cell growth and cell death.

2 Mechanism and Regulation of Initiation of Protein Synthesis

Protein synthesis involves a complex series of protein : protein and protein : RNA interactions culminating in the formation of peptide bonds between amino acids, as encoded by the mRNA being translated (reviewed in Merrick and Hershey 1996; Pain 1996; Gingras et al. 1999b). This process, summarised in Fig. 1, is described in more detail below. Briefly, the initiation phase of protein synthesis can be considered as four successive events: (1) the formation of a ternary complex between initiation factor eIF2, GTP and initiator methionyl tRNA (Met-tRNA$_f$); (2) binding of the ternary complex to the 40 S ribosomal subunit to form the 43 S initiation complex; (3) binding of mRNA to the 43 S initiation complex; (4) coupling of the 60 S ribosomal subunit to the mRNA/ribosome complex, release of bound initiation factors and formation of the 80 S initiation complex. Each stage involves the interaction of several

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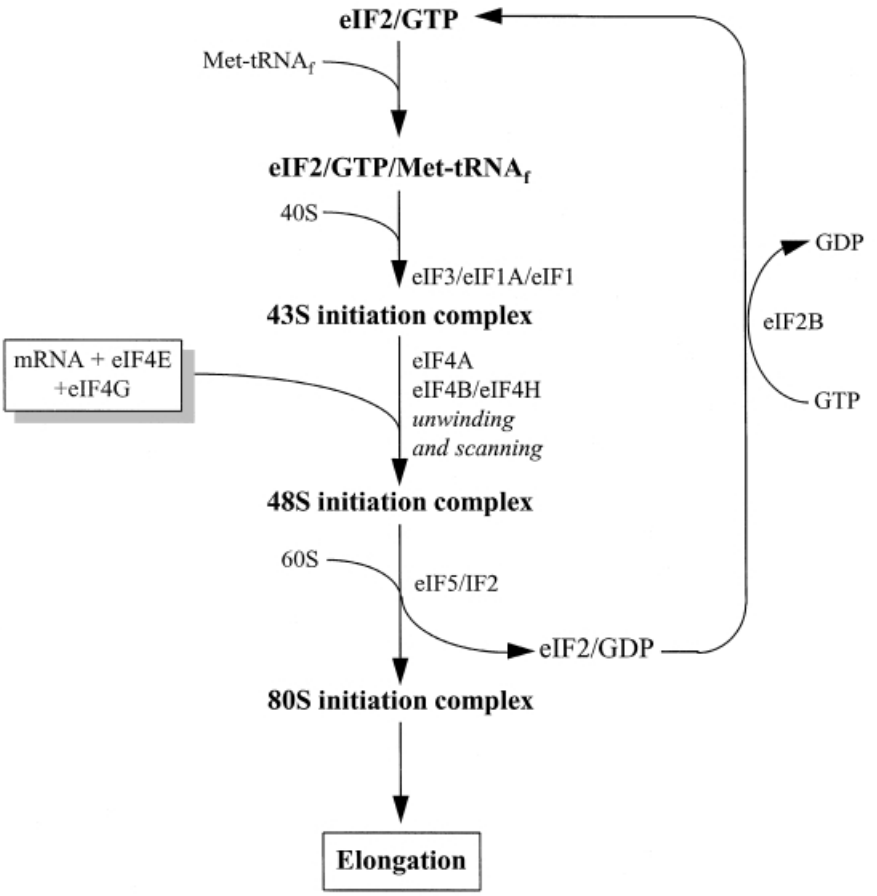

Fig. 1. Summary of the initiation of protein synthesis. A complex of the initiation factor eIF2 with GTP binds the initiator Met-tRNA$_f$ to form a [Met-tRNA$_f$/eIF2/GTP] ternary complex. This then associates with a 40 S ribosomal subunit carrying eIF3 (and other factors such as eIF1/eIF1 A) to produce the 43 S preinitiation complex. mRNA binding to this complex involves the participation of the trimeric eIF4F complex, comprising the cap-binding protein eIF4E, the scaffold protein eIF4G and the RNA helicase eIF4A, together with the helicase-stimulatory factor eIF4B/eIF4H (see text for details). In the final common step, the anticodon of Met-tRNA$_f$ interacts with the AUG codon, facilitated by eIF2 and eIF4B, to generate the mature 48 S initiation complex. Following the location of the AUG initiation codon on the mRNA with the aid of the eIF1 group of proteins (Pestova et al. 1998, 2000), two additional events must occur. The GTP associated with the eIF2 is hydrolysed to GDP, in parallel with the dissociation of the previously bound initiation factors from the ribosome (Merrick and Hershey 1996; Dever 1999; Gingras et al. 1999). GTP hydrolysis requires the involvement of the eIF5 group of initiation factors and a homologue of bacterial IF2 (Pestova et al. 2000). The GDP that is generated remains associated with the eIF2 and must be exchanged for another molecule of GTP in a process catalysed by the guanine nucleotide exchange factor eIF2B (see C.G. Proud, Vol. I). The second event is the binding of the larger (60 S) ribosomal subunit to the initiation complex to form the complete 80 S initiation complex. (Modified from Merrick 1992; Pain 1996)

components, the majority of which have been purified to homogeneity and utilised to study the individual reactions in vitro. In the best characterised systems, control of protein synthesis is exerted at the level of initiation, although there is some evidence for regulation at the level of elongation (reviewed in Merrick and Hershey 1996; Pain 1996; Sonenberg 1996; Proud and Denton 1998; Kleijn et al. 1998; ; Dever 1999; Gingras et al. 1999b; Preiss and Hentze 1999; Rhoads 1999).

Two stages in the initiation phase have been found to be highly susceptible to regulation by physiological conditions, with the phosphorylation of the factors involved playing a key regulatory role. The first of these, the binding of the eIF2/GTP/Met-tRNA$_f$ (ternary) complex to the 43 S ribosomal subunit, is limited by the recycling of eIF2 and regulated through phosphorylation of eIF2α and the physiological control of initiation factor eIF2B (Fig. 1). This has been shown to be a key control point in the acute down regulation of protein synthesis in response to physiological stress or metabolic challenges and is discussed elsewhere in this volume. The second stage, which is the focus of this chapter, is the association of this pre-initiation complex with an mRNA molecule (Fig. 2). This event is mediated by initiation factors of the eIF4 family which act in concert to find the 5′ end of the mRNA, unwind any secondary structure therein, direct the binding of the 43 S initiation complex and, along with further unwinding of the 5′ untranslated region, allow migration of the complex to the initiation codon. As such, the eIF4 initiation factors are responsible for recruiting mRNA to the ribosome in a mechanism with strong analogies to transcription complex assembly at promoter sites on DNA (Morley 1996; Sachs and Buratowski 1997).

2.1 The mRNA-Binding Initiation Factors

2.1.1 Initiation Factor eIF4E

All cytoplasmic mRNAs have a unique cap structure at their 5′ terminus, with the general composition m^{7}G(5′)ppp(5′)N (where *N* is any nucleotide). The presence of this cap structure has a strong stimulatory effect on the translation of mRNA, with uncapped or nonmethylated mRNAs being less competent at 48 S and 80 S initiation complex formation (Sonenberg 1996). Several cytoplasmic proteins can specifically interact with the mRNA cap structure; as judged by cross-linking, four initiation factors, eIF4E, eIF4A, eIF4B and eIF4G have been defined. Of these, only the phosphoprotein eIF4E specifically interacts directly with the cap, either as an individual polypeptide or part of a protein complex, termed eIF4F (Fig. 2). Mutagenesis experiments on the eIF4E protein have suggested that highly conserved tryptophan residues are important for interaction with the cap structure (Marcotrigiano et al. 1997; Altmann et al. 1988). These findings have been confirmed and explained at the bio-

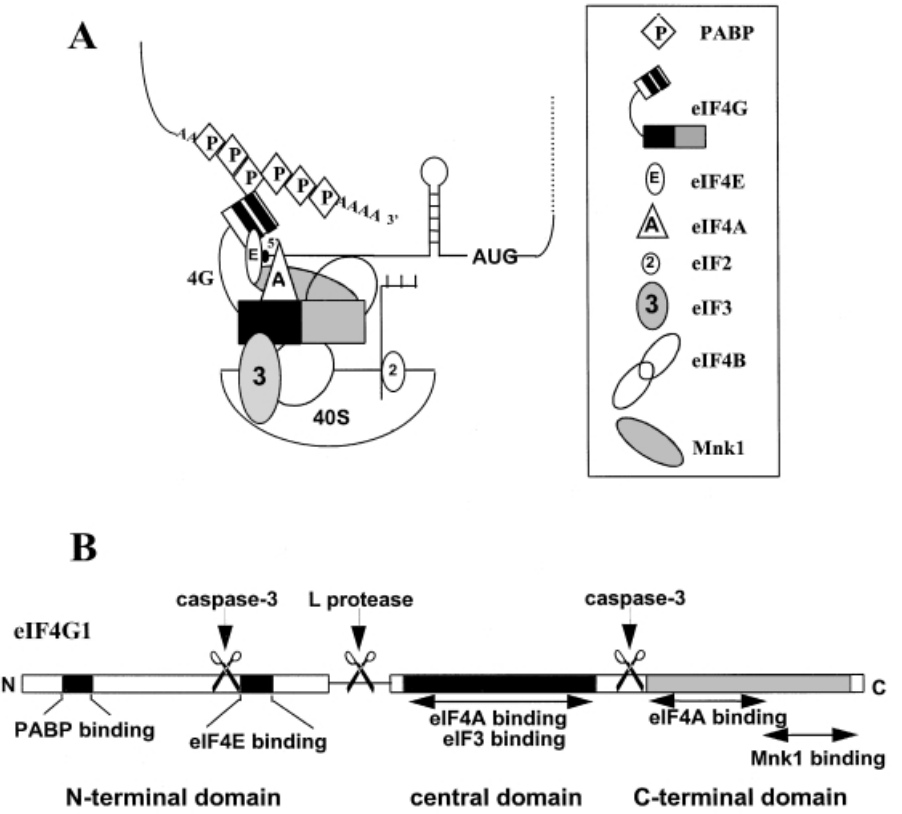

Fig. 2A,B. A current model for 48 S initiation complex formation in mammalian cells. **A** Interaction between individual initiation factors, the protein kinase, Mnk1, and the 5′ and 3′ ends of the mRNA (see text for details). **B** Diagrammatic representation of mammalian eIF4GI, showing known sites of interaction with PABP, eIF4E, eIF3, eIF4A and Mnk1 (Lamphear et al. 1995; Mader et al. 1995; Imataka et al. 1997, 1998; Morley et al. 1997; Piron et al. 1998; Gradi et al. 1998a; Gingras et al. 1999b; Pyronnet et al. 1999; Morino et al. 2000). Also indicated are the sites of cleavage by picornavirus L protease (Kirchweger et al. 1994) and caspase-3. (Bushell et al. 2000b)

chemical level by the solving of the three dimensional structure of mouse eIF4E. These structural studies showed that eIF4E resembles a cupped hand, with the m^7GTP cap occupying a narrow slot on the concave side and interacting proteins binding to separate, but overlapping, sites on the convex surface (Marcotrigiano et al. 1997, 1999; Gingras et al. 1999b; Ptushkina et al. 1999; Sonenberg, this Vol.). Forced overexpression of eIF4E results in transformation to a malignant phenotype (De Benedetti and Rhoads 1990; Lazaris-Karatzas et al. 1990). It has been suggested that this may reflect the enhanced translation of "inefficient" mRNAs (Koromilas et al. 1992), such as those encoding signalling molecules (Marth et al. 1988), ornithine decarboxylase (Rousseau et al. 1996), cyclin D1 (Rosenwald et al. 1995) and ribonucleotide reductase (Abid et

al. 1999). Moreover, raised levels of expression of eIF4E have been described in various human tumours (Li et al. 1997; Nathan et al. 1997b; Rosenwald et al. 1999), while down regulation of eIF4E strongly inhibits protein synthesis and cell growth (Rinker-Schaeffer et al. 1993).

2.1.2
eIF4E Phosphorylation

Although the site of phosphorylation of eIF4E was initially identified as Ser53 (Rychlik et al. 1987) and numerous studies were made with Ser53Ala mutant proteins (De Benedetti and Rhoads 1990; Joshi-Barve et al. 1990; Sonenberg 1994, 1996), it is now clear that phosphorylation of eIF4E occurs at Ser209 (Joshi et al. 1995; Flynn and Proud 1995; Whalen et al. 1996). To date, the effects of Ser209 mutations on cell growth have not been reported. As correlations exist between the enhanced phosphorylation of eIF4E and increased rates of protein synthesis (reviewed in Rhoads 1993; Morley 1996; Sonenberg 1996; Kleijn et al. 1998; Gingras et al. 1999b), important questions remain on the functional consequences of eIF4E phosphorylation and the signal transduction pathways which mediate it. While phosphorylation of eIF4E has been reported to increase its interaction with the cap structure (Minich et al. 1994), and the association of eIF4E with eIF4G enhances the binding of eIF4E to the mRNA cap (Haghighat and Sonenberg 1997), the precise mechanistic consequences of eIF4E phosphorylation are unclear. We (Morley 1997; Morley and McKendrick 1997; Fraser et al. 1999a, b), and others (Flynn and Proud 1996b; Wang et al. 1998; Gingras et al. 1999b) have shown that phosphorylation of eIF4E occurs via multiple signalling pathways. Indeed, recent studies have shown that the protein kinase Mnk1, which acts at the convergence point of mitogen-activated protein kinase (MAPK/ERK) and p38 MAP kinases (Waskiewicz et al. 1997; Wang et al. 1998), phosphorylates eIF4E at the physiological site in vitro and in vivo (Pyronnet et al. 1999; Waskiewicz et al. 1999). The related kinase, Mnk2, linked exclusively to the ERK pathway, phosphorylates eIF4E less efficiently (Waskiewicz et al. 1997). Both kinases interact directly with eIF4GI and eIF4GII (Pyronnet et al. 1999; Morino et al. 2000) bringing them in close proximity to eIF4E within the eIF4F complex (Fig. 2). However, although expression of a dominant-negative mutant of Mnk1 interfered with the phorbol ester-stimulated phosphorylation of co-transfected eIF4E (Waskiewicz et al. 1999), effects on global or specific translation were not reported.

2.2
Initiation Factor eIF4A

eIF4A shows the properties of RNA-dependent ATP hydrolysis and ATP-dependent RNA binding in vitro (Grifo et al. 1984; Ray et al. 1985; Pause and Sonenberg 1992; Pause et al. 1994b). In conjunction with eIF4B (see below),