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REVIEW ARTICLE

Translational control of eukaryotic gene expression

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Abstract

Translational control mechanisms are, besides transcriptional control and mRNA stability, the most determining for final protein levels. A large number of accessory factors that assist the ribosome during initiation, elongation, and termination of translation are required for protein synthesis. Cap-dependent translational control occurs mainly during the initiation step, involving eukaryotic initiation factors (eIFs) and accessory proteins. Initiation is affected by various stimuli that influence the phosphorylation status of both eIF4E and eIF2 and through binding of 4E-binding proteins to eIF4E, which finally inhibits cap-dependent translation. Under conditions where cap-dependent translation is hampered, translation of transcripts containing an internal ribosome entry site can still be supported in a cap-independent manner. An interesting example of translational control is the switch between cap-independent and cap-dependent translation during the eukaryotic cell cycle. At the G1-to-S transition, translation occurs predominantly in a cap-dependent manner, while during the G2-to-M transition, cap-dependent translation is inhibited and transcripts are predominantly translated through a cap-independent mechanism.

Keywords: *Initiation of translation; posttranscriptional regulation; IRES; cell cycle*

The gap between genomics and proteomics

Thanks to the development of a wide range of technologies to study the transcriptome, research has taken a huge step forward to discover pathways, leading to altered gene expression maps in response to a number of developmental or environmental stimuli. However, it has become increasingly clear that not all questions can be answered just by looking at the transcriptome. It is rather the proteome, i.e. the complete set of proteins encoded by the genome, that determines the cellular phenotype and the plasticity of cells in response to external signals. Protein levels do not only depend on the transcription rate of the corresponding genes, but also on additional control mechanisms, such as nuclear export and messenger RNA (mRNA) localization (Hieronymus and Silver, 2003), transcript stability (García-Martínez *et al.*, 2004), translational regulation (Beilharz and Preiss, 2004), and protein degradation (Beyer *et al.*, 2004). In addition, protein activity is affected by posttranslational modifications, such as glycosylation, nitrosylation, and phosphorylation or proteolytic cleavage (Mann and Jensen, 2003; Seo and Lee, 2004; Kwon *et al.*, 2006).

Several studies have confirmed that translational control is an important mechanism for protein abundance (e.g. Garcia-Sanz *et al.*, 1998; Mikulits *et al.*, 2000) and have shown that mRNA is sometimes a poor indicator of the corresponding protein levels (e.g. Tew *et al.*, 1996; Anderson and Seilhamer, 1997; Gygi *et al.*, 1999; Futcher *et al.*, 1999; Zong *et al.*, 1999; Ghaemmaghami *et al.*, 2003; Hedge *et al.*, 2003), because two transcripts, present in the cytoplasm in identical quantities might be translated at very different rates (Mathews *et al.*, 2000). The translational efficiency of an mRNA is determined by its ribosome loading profile. Translationally inactive mRNAs are often sequestered in messenger ribonucleoprotein particles (mRNPs) (Table 1) or associated with a single ribosome (monosome), while actively translated mRNAs are associated with multiple ribosomes (polysomes). Fractionation of mRNA populations into mRNPs and polysomes can be achieved by

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Table 1. List of acronyms.

Acronym	Full name	Function
4E-BP/Phas-I	eIF4E-binding protein	Regulation of initiation of translation
AMPK	AMP-activated protein kinase	Cellular energy status
ATF4	Activating transcription factor 4	Transcription factor; induced by eIF2 phosphorylation
CDK	Cyclin-dependent kinase	Regulation of cell cycle progression
CPEB	Cytoplasmatic polyadenylation element binding protein	Cytoplasmatic polyadenylation
EF	Elongation factor	Translational elongation
eIF	Eukaryotic initiation factor	Initiation of translation
Erk	Extracellular signal-regulated kinase	Signaling pathway for regulation of translation
FKBP12	FK506-binding protein 12	TOR signaling pathway
FOXO	Forkhead-box-binding protein	Transcription factor
GCN2	General control non-derepressible 2	Phosphorylation of eIF2
GCN4	General control non-derepressible 4	Transcriptional activator of amino acid biosynthetic genes
GSK3	Glycogen synthase kinase-3β	Signaling pathway for regulation of translation
hnRNP C1/C2	Heterogeneous nuclear nucleoproteins C1/C2	IRES-mediated translation
HRI	Haem-regulated inhibitor	Phosphorylation of eIF2
ITAF	IRES-trans-activating factor	IRES-mediated translation
IRES	Internal ribosome entry site	Cap-independent translation
LOX2	Lipoxygenase 2	Synthesis of plant regulatory molecules, such as jasmonic acid
mRNP	Messenger ribonucleoprotein particle	mRNA bound to ribosomes and other proteins
NAC	Nascent polypeptide-associated complex	Sorting of polypeptides containing a signal peptide
nCBP	Novel cap-binding protein	Binding to the cap of an mRNA
ODC	Ornithine decarboxylase	Biosynthesis of polyamines
PABP	Poly(A)-binding protein	Binding to the poly(A) tail
Paip	PABP-interacting protein	Binding to PABP
PDK1	3-Phosphatidylinositide-dependent protein kinase	Signaling pathway for regulation of translation
PERK	PKR-like endoplasmic reticulum kinase	Phosphorylation of eIF2
PI3K	Phosphatidylinositol 3-kinase	Signaling pathway for regulation of translation
PKB/Akt	Protein kinase B	Signaling pathway for regulation of translation
PKR	Double-stranded-RNA-dependent protein kinase	Phosphorylation of eIF2
PTB	Polypyrimidine tract-binding protein	IRES-mediated translation
PtdIns(3,4,5)P	Phosphatidylinositol-3,4,5-bisphosphate	Signaling pathway for regulation of translation
PtdIns(4,5)P	Phosphatidylinositol-4,5-bisphosphate	Signaling pathway for regulation of translation
Raptor	Regulatory associated protein of mTOR	TOR signaling pathway
Rheb	Ras-homolog enriched in the brain	TOR signaling pathway
Rictor	Rapamycin-insensitive companion of mTOR	TOR signaling pathway
S6K	S6 kinase	Phosphorylation of ribosomal protein S6
TSC	Tuberous sclerosis complex	TOR signaling pathway
TOR	Target of rapamycin	Ser/Thr kinase involved in translational control
TOS	Target of rapamycin signaling	TOR signaling pathway
Unr	Upstream of N-ras	IRES-mediated translation
uORF	Upstream open reading frame	

sucrose gradient centrifugation (de Jong *et al.*, 2006; Müllner and Garcia-Sanz, 1997). The migration of mRNAs in these gradients is directly proportional with their ribosome loading numbers, with free mRNPs found at the top of the gradient. As such, actively translated mRNAs associated with a particular cellular state can be isolated and the polysomal profile reflects the overall level of translational activity in a cell. On a genome-wide level, data from isolated polysomal RNA samples reflect the actual proteome more accurately (Branco-Price *et al.*, 2005).

Molecular mechanisms of translation

Translation of an mRNA into its cognate protein is accomplished in three successive steps controlled by a wide range of regulatory factors. During initiation of translation, Met-tRNA$_i$, together with the small ribosomal subunit (40S) and eukaryotic initiation factors (eIFs), form a complex onto the mRNA (Figure 1; Table 2). The reading frame for the protein is determined and the assembled ribosome can start elongation, which is regulated by elongation factors (EFs), adding amino acids to the growing

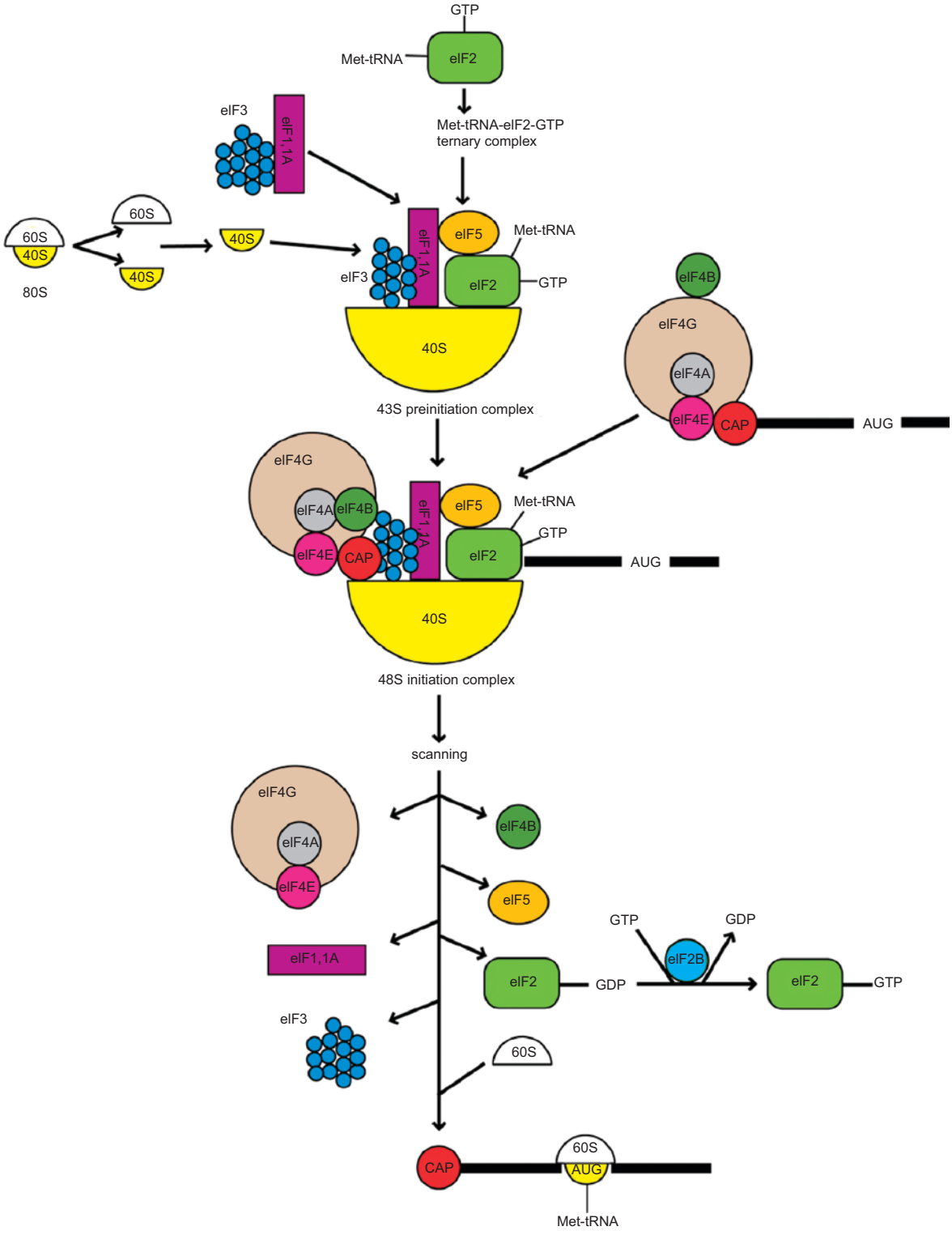

Figure 1. Schematic view of the scanning model for cap-dependent initiation of translation in eukaryotes. In the first step, a 43S preinitiation complex is formed, consisting of eIF1, eIF1A, eIF3, the ternary complex (eIF2-Met-tRNA-GTP), and the 40S subunit. eIF5 interacts with eIF2, after binding of the ternary complex to the 40S subunit. eIF4F is a heterotrimeric complex consisting of eIF4A, eIF4E, and eIF4G. eIF4A is responsible for the unwinding of the secondary structure in the leader of the mRNA, eIF4E binds to the 5′ cap, and eIF4G functions as a scaffold protein responsible for the coordinated attachment of the translation initiation machinery. The interaction of eIF3 and eIF4G recruits the 40S subunit to the 5′ cap of the mRNA and forms the 48S initiation complex. Scanning of the 5′ leader results in the location of the initiation codon. Subsequently, eIF5 triggers hydrolysis of GTP bound by the ternary complex, whereas eIF5B promotes 60S subunit joining with 80S ribosome formation and the start of translation as a result.

Table 2. Functions of eIFs.

Subunit	Function	Interaction partners
eIF1	Inhibition eIF5-induced GTP hydrolysis	eIF5
	Selection start codon	
eIF1A	Catalysis of transfer of ternary complex to 40S subunit	eIF5
	Selection start codon	
eIF2	Binding of Met-tRNAi and GTP, GTPase	eIF5, 40S subunit
eIF2B	Catalysis of exchange of eIF2-GDP into eIF2-GTP	
eIF3	Stabilization of association of ternary complex to 40S subunit	eIF5, eIF4G, eIF4B, eIF1, 40S subunit
eIF3	Prevention of 80S ribosome formation	
eIF4A	Helicase necessary for unwinding of secondary structures in 5′ UTR	eIF4G
eIF4E	Binding to 5′ cap	eIF4G, eIF4E, eIF4A, eIF3
eIF4G	Bridge between cap and 40S subunit	
eIF4F	Complex of eIF4E, eIF4G, and eIF4A	
eIF4B	Stimulation of helicase activity of eIF4A	eIF3
eIF5	Activation of GTPase activity of eIF2	eIF2, eIF1, eIF1A
eIF5B	Subunit joining, GTPase	

peptide chain in accordance with the mRNA sequence (Browne and Proud, 2002; Frank, 2003; Thornton *et al.*, 2003). The termination phase begins after recognition of a stop codon. Subsequently, the 80S ribosome is released from the protein and dissociates into its subunits to be recycled in another round of peptide synthesis.

Cap-dependent initiation of translation initiation in eukaryotes

Formation of the 43S preinitiation complex

Initiation of translation requires the formation of a 43S preinitiation complex, consisting of the ternary complex eIF2-Met-tRNA-GTP, eIF1, eIF1A, eIF3, eIF5, and the 40S subunit (Figure 1). eIF2 consists of an α, β, and γ subunit. The γ subunit probably binds GTP, while the α and β subunits are required for the binding of Met-tRNA$_i$ (Flynn *et al.*, 1993; Dorris *et al.*, 1995; Erickson and Hannig, 1996). The β subunit of eIF2 interacts with eIF5 after the binding of the ternary complex to the 40S subunit (Chaudhuri *et al.*, 1994; Das *et al.*, 1997; Asano *et al.*, 1999). eIF5 is required for the activation of the GTPase activity of eIF2γ (Hershey and Merrick, 2000), while eIF1A catalyzes the transfer of the ternary complex to the 40S subunit (Chaudhuri *et al.*, 1994, 1999) and might be, together with eIF1, involved in the identification of the correct initiation codon by dissociating aberrant complexes from the mRNA (Pestova *et al.*, 1998). eIF3 is a large multi-subunit complex important for stabilizing the association of the ternary complex to the 40S subunit and preventing 80S ribosome formation (Chaudhuri *et al.*, 1999).

Binding of the 43S preinitiation complex to the mRNA

Nuclear processing of mRNAs includes the addition of a 5′ cap structure (m^{7}GpppN, where N represents any nucleotide) prior to its transport to the cytoplasm. This 5′ cap acts as a molecular tag that marks the recruitment position of the 40S subunit. This recruitment is mediated by eIF4F (Gingras *et al.*, 1999), consisting of eIF4A, eIF4G, and eIF4E (Figure 1). eIF4E, the small subunit of eIF4F, specifically recognizes the 5′ cap of the mRNA and is, therefore, necessary for the start of cap-dependent translation initiation. eIF4G, the large subunit of eIF4F, contains a binding site for eIF4E (Marcotrigiano *et al.*, 1997). Binding of eIF4E to eIF4G will induce a conformational change of eIF4G, suggesting an induced fit mechanism (Marcotrigiano *et al.*, 1999; Hershey *et al.*, 1999). eIF4G also forms a bridge between the 5′ cap (via eIF4E) and the 40S subunit (via eIF3) (Hentze, 1997; Gross *et al.*, 2003; Prévôt *et al.*, 2003), contains two binding sites for eIF4A, and is needed for the recruitment of eIF4A to the initiation complex. eIF4A is a member of the DEA(D/H)-box RNA helicase family that probably unwinds secondary structures in the 5′ untranslated region (UTR), thereby facilitating scanning of the 40S subunit for the initiation codon. eIF4A itself has weak ATPase activity and must to be stimulated by eIF4G and eIF4B (Rogers *et al.*, 2002). eIF4B increases the ATP binding affinity of eIF4A and the ATP-dependent helicase activity (Bi and Goss, 2000; Bi *et al.*, 2000). In addition to eIF4A, eIF4F (and eIFiso4F) also exhibits an RNA-dependent ATP hydrolysis activity and an ATP-dependent helicase activity, which is significantly stimulated by binding of eIF4A (Abramson *et al.*, 1988; Browning *et al.*, 1987; Lax *et al.*, 1985; 1986). Essentially, eIF4G functions as a scaffold necessary for the coordinated attachment of the translation initiation machinery and the ribosome to the mRNA and the organization of an ATP-dependent helicase complex at the 5′ cap to remove secondary structures in the 5′ leader during scanning.

Scanning and formation of the 48S initiation complex

The interaction between eIF3 and eIF4G results in the recruitment of the 43S preinitiation complex to the cap, and, subsequently, the formation of a 48S initiation complex. eIF3 is the largest initiation factor and contains 11 subunits in plants and mammals, of which 10 are core subunits, in addition to another species-specific subunit (Hershey and Merrick, 2000; Burks *et al.*, 2001). In *Saccharomyces cerevisiae*, eIF3 consists of five core subunits, orthologous to those of plants and animals, in addition to four yeast-specific subunits (Hershey and Merrick, 2000; Hinnebusch, 2000). A direct interaction between the mammalian eIF3a and eIF4B has been shown (Méthot *et al.*, 1996), while in yeast eIF4B directly interacts with eIF3g (TIF35) (Vornlocher *et al.*, 1999). As such, eIF4B also forms a bridge between eIF3 and eIF4F and might also participate in the formation of the 48S complex. The 48S initiation complex starts the ATP-dependent scanning of the 5′ leader to locate the initiation codon (Kozak, 1986b). Scanning will continue until an appropriate start codon is recognized (Kozak, 1980b, 2002). eIF5 will activate the GTPase activity of eIF2 only when the pause in scanning is long enough, for instance because of base pairing with the anti-codon present in the Met-tRNA$_i$ (Cigan *et al.*, 1988; Chakrabarti and Maitra, 1991; Huang *et al.*, 1997; Das and Maitra, 2001). eIF5 also interacts with eIF1 that acts as a negative regulator in preventing premature GTP hydrolysis and links codon–anticodon base pairing with eIF5-induced hydrolysis of eIF2-bound GTP (Valášek *et al.*, 2004). eIF1A is another player in the identification of the correct start codon. An interaction between eIF5 and eIF1A has been shown, which is strengthened after AUG recognition and results in an equilibrium shift between a scanning-competent open and a scanning-incompetent closed complex (Maag *et al.*, 2006; Passmore *et al.*, 2007). eIF1A will either stabilize the open conformation or destabilize the closed conformation. However, upon recognition of the start codon, eIF1 will dissociate from the 43S preinitiation complex or move onto eIF3 (Naranda *et al.*, 1996; Phan *et al.*, 2001; Unbehaun *et al.*, 2004). Not GTP hydrolysis per se, but rather the release of P$_i$ has been suggested to result in the AUG codon-dependent release of eIF1 (Maag *et al.*, 2005).

Dissociation of eIFs from the 48S initiation complex

Hydrolysis of GTP releases eIF2-GDP from the 40S subunit. eIF2 remains tightly associated with GDP and needs eIF2B, a guanine nucleotide exchange factor, to catalyze the exchange of GDP into GTP (Hershey and Merrick, 2000), forming a functional GTP-bound eIF2 ready for the next round of translation initiation. As a consequence, the release of eIF2-GDP will result in that of eIF3 from the 48S complex (Unbehaun *et al.*, 2004). Subsequently, eIF4B will mediate the joining of the 60S subunit and the assembly of a functional 80S ribosome with Met-tRNA$_i$ in the P site (Pestova *et al.*, 2000; Lee *et al.*, 2002).

Different isoforms of translation initiation factors

eIF4F is composed of eIF4G, eIF4E, and eIF4A and the existence of different isoforms is now widely accepted. Three eIF4E isoforms have been characterized in mammals (eIF4F-1, 4EHP, and eIF4E-3) (Sonenberg *et al.*, 1979; Rom *et al.*, 1998; Joshi *et al.*, 2004), three in plants (eIF4E, eIFiso4E, and nCBP) (Browning *et al.*, 1992; Ruud *et al.*, 1998), two in *S. pombe* (eIF4E-1 and eIF4E-2) (Ptushkina *et al.*, 1996, 2001, 2004), and only one in *S. cerevisiae* (Altmann *et al.*, 1987; Lejbkowicz *et al.*, 1992). In organisms possessing different eIF4E isoforms, one of them is responsible for default cap-dependent translation, while the others can have a specialized function during stress, at specific developmental stages, or in a tissue-specific manner. Some isoforms have been shown to function as translational repressors or to be involved in nuclear transport of specific mRNAs (for an overview, see Hernández and Vazquez-Pianzola, 2005).

Two eIF4G isoforms have been characterized in humans (eIF4G-I and eIF4G-II) (Bradley *et al.*, 2002; Byrd *et al.*, 2002; Gradi *et al.*, 1998), two from wheat (*Triticum aestivum*) (eIF4G and eIFiso4G) (Lax *et al.*, 1985; Allen *et al.*, 1992; Browning *et al.*, 1992), two from *S. cerevisiae* (TIF4631 and TIF4632) (Goyer *et al.*, 1993), and one from *S. pombe* (Hashemzadeh-Bonehi *et al.*, 2003). Although eIF4G isoforms have been suggested to exhibit largely similar functions (Gradi *et al.*, 1998; Goyer *et al.*, 1993), differences have been reported. For instance, in yeast, translation of uncapped, polyadenylated mRNAs is supported to a greater extent by eIF4G-II than by eIFG4-I (Tarun *et al.*, 1997). Wheat eIFiso4G binds preferably to linear structures devoid of secondary structures (Carberry and Goss, 1991; Gallie and Browning, 2001).

Three eIF4A isoforms have been discovered in humans (eIF4A-I, eIF4A-II and eIF4A-III) (Li *et al.*, 1999) and one in *S. cerevisiae* encoded by two genes (*TIF-1* and *TIF-2*) (Linder and Slonimski, 1989). Two eIF4A-encoding genes were found in *Arabidopsis thaliana* (Metz *et al.*, 1992), one in wheat (Metz and Browning, 1993), and more than 10 in tobacco (*Nicotiana tabacum*) (Brander *et al.*, 1995; Owttrim *et al.*, 1994). eIF4A isoforms seem to have a non-redundant function; and a role that depends on tissue, developmental stage, or growth status of the cell has been demonstrated.

Where to begin?

When translation is not initiated at the start codon but in frame, an N-terminally extended and/or truncated

protein will be produced; but, when the initiation site is not in frame, the protein will be completely miscoded. Hence, it is pivotal that the correct initiation site is selected. The sequence context of the start codon plays an important role in this selection and initiating sites are designated as 'weak' or 'strong'. In mammals, the optimal context for translation initiation is GCCRCCaugG (with a purine for R) and is referred to as the 'Kozak sequence'. Generally, a strong context contains A at –3 and G at +4 (Kozak, 1987a). However, the 'Kozak sequence' has been evaluated with whole-genome expression data from animals, fungi, plants, and protists. Nucleotides –3A/G, –2A/C, and +5C were found to be common in mRNAs (Nakagawa *et al.*, 2008), with –3A/G crucial for the enhancement of translation initiation (Kozak, 1986b; Lukaszewicz *et al.*, 2000; Kochetov, 2005; Pesole *et al.*, 2000; Pisarev *et al.*, 2006). Furthermore, not nucleotides at certain positions, but rather repetitions of G or A are important in initiating translation. The signal *GCCGCC*AUG appears to be strong in monocotyledonous plants and vertebrates, and *AAAAAA*AUG in dicotyledonous plants and invertebrates. These sequence contexts are also correlated with the GC and AT contents of the respective genomes, with a preference for repetitions of A in AT-rich genomes, such as plants and yeast (Nakagawa *et al.*, 2008).

The importance of the poly(A)-binding protein

Interaction between the 5′ and the 3′ termini of mRNA

The poly(A) tail is added to mRNA molecules in the nucleus by poly(A) polymerase as part of mRNA processing (Preiss and Hentze, 1999). Binding of poly(A)-binding proteins (PABPs) will prevent deadenylation, which would result in mRNA decay (Caponigro and Parker, 1995). PABPs contain four continuous RNA recognition motifs (RRMs) in the N-terminal region, connected to a conserved C-terminal domain, referred to as the PABC (Mangus *et al.*, 2003). The RRM domains are required for the cooperative binding of the PABPs to the poly(A) tail, while the PABC domain is involved in protein–protein interactions and the recruitment of factors to the mRNA–RNP complex (Figure 2). The genome of *S. cerevisiae* encodes only one PABP, Pab1p, that is essential for viability (Sachs *et al.*, 1987), whereas the human genome contains three genes (*PAB1*, *PAB2*, *PAB3*) (Mangus *et al.*, 2003), and *Arabidopsis* a family of eight *PABP* genes (Belostotsky, 2003). Human PAB1 is important for translation and mRNA turnover, while PAB2 is involved in polyadenylation. The exact role of the different PABPs of *Arabidopsis* is unknown, except that at least PAB2 and PAB5 are essential (Bravo *et al.*, 2005). Both genes exhibit a different expression pattern, in which *PAB2* is ubiquitously expressed, while *PAB5* is specific for reproductive organs (Belostotsky and Maegher, 1996; Palanivelu *et al.*,

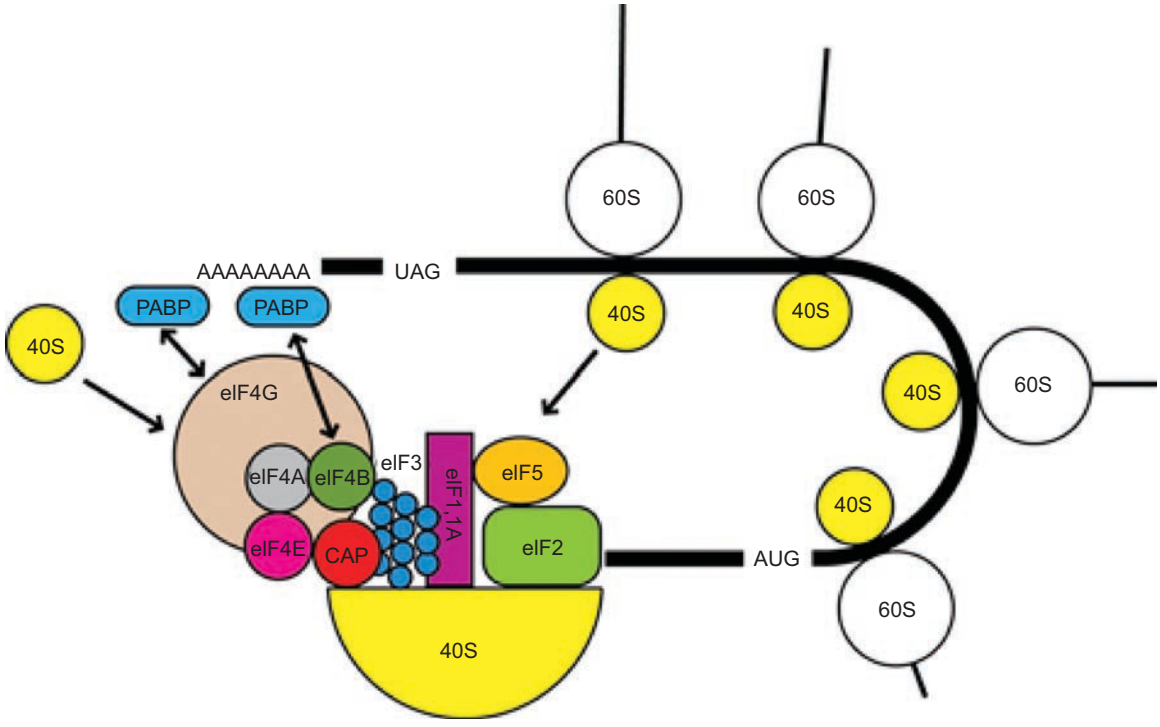

Figure 2. The interaction of PABP with the 48S initiation complex. The interaction between PABP, bound to the poly(A) tail, and eIF4G and eIF4B is indicated with arrows. The interaction results in the circularization of the mRNA.

2000). The eight PABPs from *Arabidopsis* are subdivided into four classes based on phylogenetic and expression analysis (Belostotsky, 2003). Class I contains the genes *PAB3* and *PAB5* encoding proteins that are mainly found in reproductive tissues and probably have a role as regulatory factors in gametogenesis and early development (Belostotsky and Maegher, 1996). Class II groups the highly and ubiquitously expressed genes *PAB2*, *PAB4*, and *PAB8* (Hilson *et al.*, 1993; Palanivelu *et al.*, 2000; Belostotsky, 2003). The class III genes, *PAB6* and *PAB7*, show a low and restricted expression, and class IV consists of only one gene, *PAB1*, which is expressed at low levels in roots (Belostotsky and Maegher, 1993; Belostotsky, 2003).

A functional, PABP-mediated interaction between the 5′ cap structure and the poly(A) tail has been observed (Gallie, 1991). This interaction promotes 40S subunit recruitment through a direct interaction with eIF4G (Tarun and Sachs, 1996; Imataka *et al.*, 1998), resulting in a circular conformation (Wells *et al.*, 1998) (Figure 2) that improves translation initiation efficiency by facilitating the utilization or recycling of 40S ribosomes (Jacobson, 1996; Kahvejian *et al.*, 2001). PABP also interacts with eIF4B in plants (Le *et al.*, 1997; Cheng and Gallie, 2007) and mammals (Bushell *et al.*, 2001), which probably increases the affinity of eIF4F (or eIFiso4F) for the 5′ cap (Wei *et al.*, 1998; Luo and Goss, 2001) and the ATPase and RNA helicase activities of the eIF4F-eIF4A-eIF4B complex (Bi and Goss, 2000). Subsequently, an increase in eIF4G recruitment will correspond with that of the 40S subunit and an efficient formation of the 48S initiation complex (Iizuka *et al.*, 1994; Tarun and Sachs, 1995). Furthermore, the relative translation efficiency of an mRNA with extensive secondary structure might be more sensitive to changes in PABP concentration than that of an unstructured mRNA (Gallie *et al.*, 2000; Gallie and Browning, 2001).

Other PABP-interacting proteins

In mammalian cells, the PABC domain of PABP binds to the PABP-interacting motif 2 (PAM2) of the PABP-interacting protein 1 (Paip1) and Paip2 (Craig *et al.*, 1998; Roy *et al.*, 2002). Another region involved in the interaction of Paip2 and Paip1 with PABP is the PAM1 domain that interacts with RRM2 and RRM3 (Khaleghpour *et al.*, 2001) and with RRM1 and RRM2 (Roy *et al.*, 2002), respectively. Paip1 has been shown to stimulate translation, to interact with eIF4A, and to be involved in mRNA turnover (Craig *et al.*, 1998; Grosset *et al.*, 2000). Paip2 competes with Paip1 for binding to PABPs, repressing translation of poly(A) mRNAs. On top of this, the binding sites of eIF4G and Paip2 in PABPs overlap, suggesting a competition between these proteins. Paip2 is also capable of displacing PABPs from the poly(A) tail (Khaleghpour *et al.*, 2001).

No real Paip orthologs have been reported for plants. However, the PABC domain was found to be present in six out of the eight *PABP* genes in *Arabidopsis*. The CTC-interacting domain 1 (CID1) and CID7 interact with the PABC domain of PAB2 from *Arabidopsis*. These proteins contain a domain highly similar to the PAM2 domain of the human Paip1 and Paip2 (Bravo *et al.*, 2005). CID1 corresponds to the previously identified early responsive to dehydration 15 (ERD15), of which the expression is induced after exposure to various abiotic stresses (Kiyosue *et al.*, 1994; Dunaeva and Adamska, 2001) and might be involved in the regulation of translation in response to dehydration and other stresses. Eleven additional PAM2 domain-containing proteins have been identified in *Arabidopsis* and rice (*Oryza sativa*). Six of these proteins, CID8 to CID13, are highly related RNA-binding proteins, containing two RRMs and a possible bipartite nuclear localization signal (NLS) (Bravo *et al.*, 2005). The mammalian cytoplasmatic polyadenylation element binding protein (CPEB), with two highly conserved RMM domains, is important for modulation of translation and mRNA localization (Hake *et al.*, 1998; Mendez and Richter, 2001) in several processes, such as early development and gametogenesis (Bally-Cuif *et al.*, 1998; Luitjens *et al.*, 2000), and contains two highly conserved RMM domains. In plants, no orthologs of CPEB have been found. The CIDs might modulate translation or location of specific mRNAs in a manner similar to the mammalian CPEBs (Bravo *et al.*, 2005).

Non-classical cap-dependent initiation mechanisms

Leaky scanning

In most mRNAs, initiation occurs at the first AUG codon encountered proximal to the 5′ end of the mRNA. However, in some cases, this AUG codon can be bypassed in a process called leaky scanning (Figure 3). First, when the first start codon is in a poor context and the next, or rarely the third, is in a good context, the 40S subunits can skip the first AUG and initiate translation at the second or third AUG. Secondly, when the first AUG is too close to the 5′ end to be recognized efficiently (Kozak, 1991; Slusher *et al.*, 1991; Kaneda *et al.*, 2000) or when ribosomes initiate at an upstream non-AUG codon in addition to the first AUG (Kozak, 1991), initiation from two different AUG codons will lead to two different proteins. When the codons are in frame, the produced proteins differ only in length and might be targeted to different compartments of the cell. For example, alternative translation initiation of the insulin-degrading enzyme (IDE) mRNA results in an N-terminally extended protein that is targeted to mitochondria (Leissring *et al.*, 2004). However, when the first and second initiation codons are in different

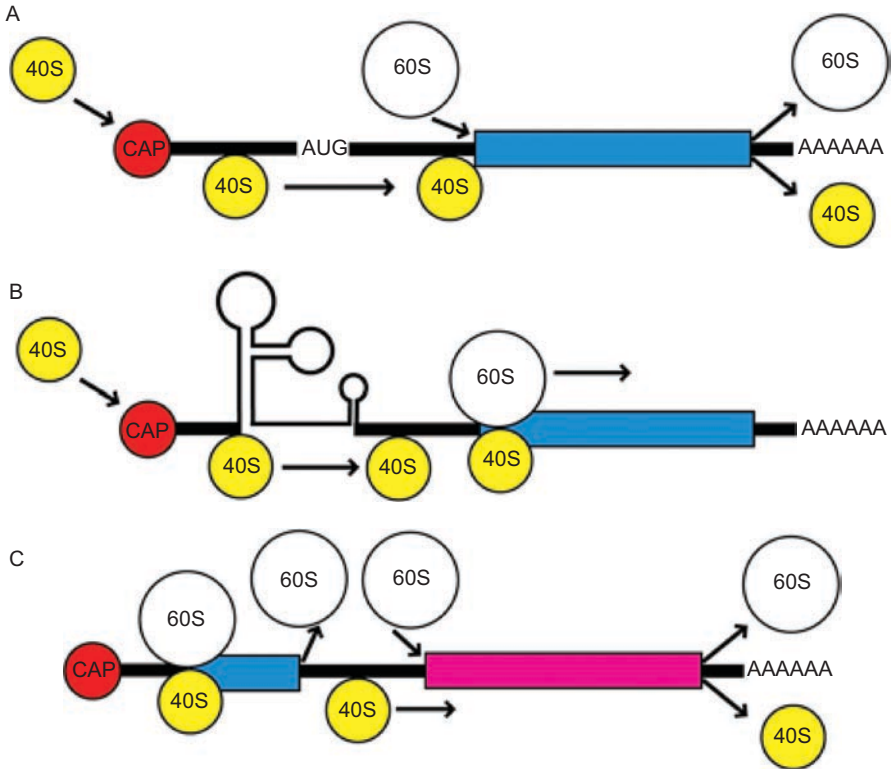

Figure 3. Nonclassical cap-dependent initiation mechanisms. (A) Leaky scanning. The first AUG start codon can be bypassed and translation can start at the next AUG codon. (B) Ribosome shunting. The ribosome shunts over a large segment with extensive secondary structure, possibly containing start codons. (C) Reinitiation. After initiation at the first start codon, the 40S subunit remains bound to the mRNA and resumes scanning, leading to initiation from the downstream AUG codon.

reading frames, one mRNA can produce two different proteins. The bifunctional mRNA from HIV-1 viruses encodes an accessory protein VPU in a different reading frame upstream from the envelope protein ENV. To allow translation from the downstream *Env* reading frame, the initiation context of *Vpu* is weak (Schwartz *et al.*, 1992). Leaky scanning has been suggested to be modulated by growth phase or temperature (Liu *et al.*, 1997; Spotts *et al.*, 1997).

Ribosome shunting

Normally, the scanning ribosome will unwind hairpin loops present in the 5′ UTR (Kozak, 1980a, 1986a; Pelletier and Sonenberg, 1987), but in some cases, the ribosome can shunt over a large segment, thereby bypassing possible AUG codons and secondary structures that normally would block the scanning process (Figure 3). For instance, ribosome shunting has been demonstrated for the 35S RNA of the cauliflower mosaic virus (CaMV) (Fütterer *et al.*, 1993), and adeno late virus mRNAs (Yueh and Schneider, 1996).

Reinitiation

When the 5′ proximal AUG codon is followed by a short upstream open reading frame (uORF), ribosomes can start at the first AUG codon to produce the small peptide encoded by the uORF and subsequently from a second, downstream AUG without becoming disengaged from the mRNA. Although the mechanism is not completely clear, the 60S subunit presumably dissociates from the mRNA when the stop codon of the uORF is reached, while the 40S subunit remains bound and resumes scanning (Figure 3). The reinitiation frequency increases when the distance augments between the stop codon of the uORF and the reinitiation site (Kozak, 1987b; Abastado *et al.*, 1991), probably because the 40S subunit needs to recruit the ternary complex (eIF2-Met-tRNA$_i$-GTP) before reinitiation can occur. As such, enough time or length has to be available before arriving at the next AUG (Hinnebusch, 1997). Although reinitiation is never efficient, it might serve as a mean to regulate gene expression. First, reinitiation can regulate translation of mRNAs with a toxic or harmful effect when translated in high amounts (Pecqueur *et al.*, 2001; Kriaucionis and Bird, 2004). Secondly, when the levels of eIF2-GTP are low, the closest downstream AUG codon can be bypassed and reinitiation can occur from a more downstream-located AUG. The eIF2-GTP levels can be modulated by kinases depending on growth or other conditions (Clemens, 2001), determining the site where reinitiation occurs. A well-known example of this kind of regulation is translation of the yeast *GCN4*

gene. GCN4 regulates transcription of genes involved in amino acid biosynthesis. The GCN4 mRNA contains four uORFs. After translation of uORF1, reinitiation can start either from uORF4 or from the GCN4 ORF. When uORF4 is translated, translation of the GCN4 ORF is precluded. GCN2 is a kinase responsible for phosphorylation of eIF2 that impairs the exchange of GDP to GTP and that is activated when uncharged tRNAs accumulate, resulting in a slow exchange of GDP into GTP. As such, a part of the 40S subunits will bypass uORF4, leading to translation of the GCN4 ORF (Gaba *et al.*, 2001; Hinnebusch, 1997). Thirdly, the peptide encoded by the uORF might have a regulatory effect (Alderete *et al.*, 2001; Fang *et al.*, 2004; Law *et al.*, 2001).

Regulation of translation

Cell and tissue growth depends on protein synthesis. For cell viability, it is very important that translation is regulated in a controlled fashion, because deregulation of components of the translational machinery can provoke cell transformation and several cancers. Furthermore, modulation of translational initiation allows a faster response than transcriptional induction in response to hormones, growth factors, or nutrients. Conversely, the rate of protein synthesis can decrease within several minutes in response to stressful conditions, such as oxidative or osmotic stress, DNA damage, or nutrient withdrawal. The need for translational control is also emphasized by the fact that protein synthesis needs amino acids and metabolic energy in the form of ATP and GTP, implying a balance between availability of both amino acids and metabolic energy.

eIF2 and eIF4E are initiation factors involved in translational control, which occurs primarily at the initiation level, although control during the elongation phase is possible to some extent.

The interaction of eIF4E with 4E-BPs

eIF4E can interact with 4E-binding proteins (4E-BPs) (also known as Phas-I) via a site that is also bound by eIF4G (Figure 4). Three 4E-BPs have been described (4E-BP1, 4E-BP2, and 4E-BP3) in mammals (Haghighat *et al.*, 1995; Mader *et al.*, 1995; Marcotrigiano *et al.*, 1999), but only one ortholog in invertebrates (Bernal and Kimbrell, 2000; Cormier *et al.*, 2001; Miron *et al.*, 2001) and none in plants. When eIF4E is bound to the 4E-BPs, the formation of an active initiation complex with eIF4G will be inhibited. As such, 4E-BPs can function as inhibitors of cap-dependent translation (Lawrence and Abraham, 1997; Gingras *et al.*, 1999). The interaction strength depends on the phosphorylation status of the 4E-BPs and is strong when the 4E-BPs are in a hypophosphorylated state. On the other hand, 4E-BPs will dissociate from eIF4E when they are in a hyperphosphorylated state, forming an active eIF4F complex. The 4E-BPs are phosphorylated in response to amino acids, insulin, and growth factors among numerous stimuli (Gingras *et al.*, 1999; Haghigat and Sonenberg, 1997; Kimball, 2001). 4E-BP1 contains at least seven sites of phosphorylation, of which four are known to be regulated via signaling pathways (Heesom *et al.*, 2001). 4E-BP1 is a downstream target of the Akt/PKB and mammalian target of rapamycin (mTOR) signaling pathways (Sonenberg and Gingras, 1998; Gingras *et al.*, 2001; Wang *et al.*, 2005). Hypoxia will cause a reduction in 4E-BP1 phosphorylation and an increase of eIF4E associated with 4E-BP1 (Shenberger *et al.*, 2005).

In addition to the 4E-BPs, other eIF4E-binding proteins have already been identified. These proteins seem to be involved in translational control of specific mRNAs. For instance, Maskin is able to disrupt the eIF4E–eIF4G interaction, resulting in translational inhibition of cytoplasmatic polyadenylation element (CPE)-containing mRNAs during oocyte development (Stebbins-Boaz

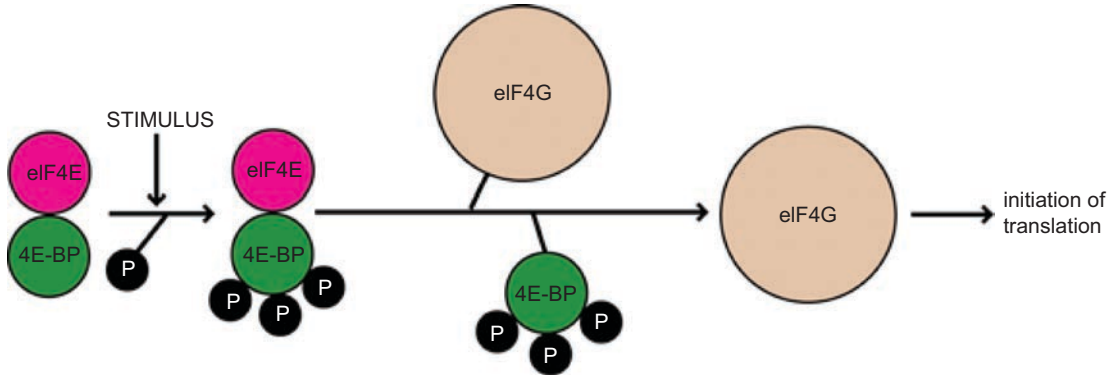

Figure 4. Interaction of eIF4E with 4E-BP. The interaction between eIF4E and 4E-BP depends on the phosphorylation status of the 4E-BPs. In response to the appropriate stimulus, 4E-BP is phosphorylated and dissociates from eIF4E.

et al., 1999). For an overview of other eIF4E-interacting proteins, see Richter and Sonenberg (2005).

The yeast p20 protein, encoded by a non-essential gene, binds to eIF4E and blocks binding of eIF4G. No plant 4E-BP orthologs have been identified, suggesting either a different manner of regulation or the existence of a structurally divergent protein with a function comparable to that of the 4E-BPs. However, an *in vitro* interaction between eIF4E and the nucleus-encoded chloroplast lipoxygenase 2 (AtLOX2) of *Arabidopsis* has been reported that was disrupted by eIFiso4G (Freire *et al.*, 2000). The *Arabidopsis* AtBTF3 is another eIF4E-interacting protein that can disrupt the interaction between eIF4G and eIF4E (Freire, 2005). AtBTF3 is the β subunit of the nascent polypeptide-associated complex (NAC) (Wiedmann *et al.*, 1994) that consists of an α and a β subunit. This complex binds to polypeptides emerging from the ribosome and appears to be important for sorting polypeptides containing a signal peptide (Wiedmann *et al.*, 1994; Lauring *et al.*, 1995; Powers and Walter, 1996). Deletion of the signal peptide of AtLOX2 diminishes the interaction between eIFiso4E and AtLOX2, suggesting that signal peptides might contain interaction sites with eIFiso4E and a link between translation (via eIFiso4E) and sorting of nascent polypeptides (via AtBTF3 and signal peptides) (Freire *et al.*, 2000; Freire, 2005).

Phosphorylation of eIF4E

In mammals, eIF4E is phosphorylated *in vivo* by the MAP kinases Mnk1 and Mnk2 at Ser209 (Flynn and Proud, 1995; Joshi *et al.*, 1995). All human Mnk proteins can interact with eIF4F complexes *in vivo* (Waskiewicz *et al.*, 1999; Pyronnet *et al.*, 1999; Scheper *et al.*, 2001) by binding to the C-terminal part of eIF4G and mediate phosphorylation of eIF4E *in vivo* (Pyronnet *et al.*, 1999; Ueda *et al.*, 2004). Both Mnk1a and Mnk2a are activated through phosphorylation by an extracellular signal-regulated kinase (Erk) and by p38 MAP kinase *in vitro* (Fukunaga and Hunter, 1997; Waskiewicz *et al.*, 1997; Scheper *et al.*, 2001). However, *in vivo*, Mnk1a has a low basal activity that can be enhanced by agents that activate either the Erk or the p38 MAK pathway (Wang *et al.*, 1998; Fukunaga and Hunter, 1997; Waskiewicz *et al.*, 1997). In contrast, Mnk2a has a high basal activity and is not further enhanced by agents that activate these pathways (Scheper *et al.*, 2001). Growth factors, phorbol esters, and, in some cell types, insulin will activate Mnk kinases through the MEK/Erk pathway (Flynn and Proud, 1996; Wang *et al.*, 1998; Waskiewicz *et al.*, 1999; Tschopp *et al.*, 2000), while certain cytokines and stressful conditions activate the p38 MAP kinase pathway (Wang *et al.*, 1998; Morley and McKendrick, 1997).

Yeast and plant eIF4G, however, lack the Mnk1 interaction domain present in the mammalian eIF4G. It is not known whether plants and yeast miss a Mnk1-like kinase activity or whether the interaction with eIF4G involves another region.

Phosphorylation of eIF2

After translation initiation, eIF2 leaves the 40S subunit as an inactive eIF2-GDP. Regeneration of the active eIF2-GTP requires the heteropentameric guanine-nucleotide exchange factor, eIF2B. Phosphorylation of serine 51 of eIF2α increases the binding affinity of eIF2B to eIF2 and the formation of an inactive eIF2–eIF2B complex (Hinnebusch, 2000), in which eIF2B is present in limited amounts compared to eIF2. Hence, a small increase in phosphorylation of eIF2α can have a high impact on protein synthesis (Hershey, 1991).

Several kinases responsible for eIF2 phosphorylation have been identified in yeast and mammals and include the double-stranded-RNA-dependent protein kinase (PKR), the heme-regulated inhibitor (HRI), general control non-derepressible-2 (GCN2) and the PKR-like endoplasmic reticulum kinase (PERK) (Kaufman, 2000; Chen, 2000; Hinnebusch, 2000; Ron and Harding, 2000). These kinases have a similar catalytic and a different regulatory domain, allowing control in different circumstances (Dever *et al.*, 2007). eIF2α is phosphorylated in response to heat shock, viral infection, heme deprivation, ER or nutrient stress, with reduced protein synthesis as a consequence (Duncan and Hershey, 1989; Pain, 1994; Wek, 1994). In contrast, eIF2α is dephosphorylated when mitogens are added to non-growing cells (Montine and Henshaw, 1989). In the case of amino acid starvation, free tRNAs accumulate, activating the kinase GCN2 in yeast and mammals. Subsequently, eIF2α is phosphorylated and inhibits translation initiation (Hinnebusch, 1997; Berlanga *et al.*, 1999; Sood *et al.*, 2000). However, limited phosphorylation of eIF2α can promote the translation of a subset of mRNAs, such as that of the mammalian activating transcription factor 4 (ATF4) mRNA (Vattem and Wek, 2004) or the yeast GCN4 mRNA (Hinnebusch, 1997). In plants, the situation is seemingly different. Specific phosphorylation of Ser51 of eIF2α has been demonstrated in wheat and barley (*Hordeum vulgare*) in which a plant PKR (pPKR) kinase activity has been observed (Langland *et al.*, 1995, 1996). However, no pPKR and no AtGCN4 orthologs have been found after sequencing of the *Arabidopsis* genome. AtGCN2 appears to be the only eIF2α kinase present, which is phosphorylated in response to herbicides affecting amino acid biosynthesis. The involvement of AtGCN2 in the regulation of amino acid biosynthesis is still unclear (Zhang *et al.*, 2008). Other phosphorylation sites have been identified and the phosphorylation status of eIF2α seems to be developmentally controlled. The question of how these

phosphorylation events influence the interaction with eIF2B is still open. eIF2α has been identified as intermediately phosphorylated and hyperphosphorylated during developmental periods that coincide with a high and low rate of protein synthesis, respectively. Thus, phosphorylation on certain sites might promote protein synthesis, while on other sites it might inhibit eIF2 activity and subsequent protein synthesis (Gallie *et al.*, 1997; Le *et al.*, 1998).

Upstream signaling to the translational machinery

In animal systems, response to insulin and insulin-like growth factors results in receptor–ligand binding and activation of the lipid kinase phosphatidylinositol 3-kinase (PI3K) (Figure 5). This activation in turn converts phosphatidylinositol-4,5-bisphosphate (PtdIns(4,5)P) into phosphatidylinositol-3,4,5-trisphosphate (PtdIns(3,4,5)P), which will bind to the 3-phosphatidylinositide-dependent protein kinase (PDK1) that activates effectors, such as protein kinase B (PKB; also Akt), $p70^{S6K}$ (S6Ks), and $p90^{RSK}$ (RSKs) (Alessi *et al.*, 2001; Brazil *et al.*, 2004). PKB/Akt phosphorylates and inhibits the activity of the α and β isoforms of GSK3 against their substrates, including eIF2Bε (Welsh and Proud, 1993; Cohen and Frame, 2001). Thus, insulin and other factors lead to the activation of PKB/Akt the inactivation of GSK3, the subsequent dephosphorylation of the inhibitory GSK3 phosphorylation site of eIF2Bε, and finally, the activation of eIF2Bε.

No PtdIns(3,4,5)P is found in plant cells, while phosphatidylinositol 3-phosphate (PtdIns(3)P) is present at very low concentrations. PI3K, encoded by the *VPS34* gene, is responsible for the formation of PtdIns(3)P and is essential for normal plant growth (Welters *et al.*,1994). A role for PtdIns(3)P has been demonstrated in root hair growth, among others (Lee *et al.*, 2008), but a function similar to that in animal systems is not discovered yet.

The classical MAPK (MEK/Erk) pathway and the p38 MAPK α/β pathways are MAPK signaling modules that are involved in the control of the translational machinery, activating Mnk1/2. Besides Mnk1/2, Erk activates members of the $p90^{RSK}$ group of kinases (RSK1-4) that, in turn, phosphorylate eIF4B, a factor that enhances the helicase activity of eIF4A and its association with eIF3 (Shahbazian *et al.*, 2006). In mammals and plants, six to

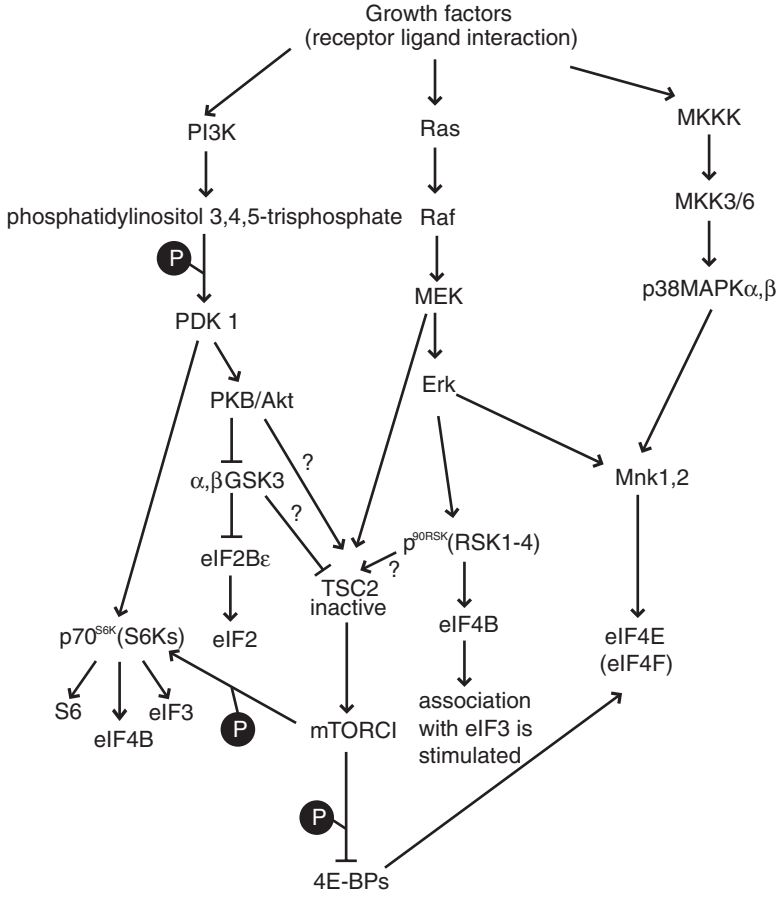

Figure 5. Upstream signaling to the translational machinery. The lines with a question mark indicate signaling connections that are still unclear.

eight eIF4B phosphorylated isoforms have been identified, depending on developmental cues or external signals (Duncan and Hershey, 1989; Gallie *et al.*, 1997; Le *et al.*, 1998). The presence of hyperphosphorylated and dephosphorylated eIF4B correlates with active translation and the loss of protein synthesis, respectively. For instance, in wheat, heat shock is followed by a rapid decrease in eIF4B phosphorylation (Gallie *et al.*, 1997), while in mammalian cells eIF4B undergoes dephosphorylation after heat shock and serum depletion (Duncan and Hershey, 1985; 1989). Moreover, RSKs might be responsible for the phosphorylation of tuberous sclerosis complex 2 (TSC2), leading to the activation of mTORC1 signaling (Roux *et al.*, 2004; Rolfe *et al.*, 2005). Phosphorylation of GSK3, a downstream member of the PI3K/PKB pathway, results in phosphorylation of TSC2 on two sites and provides another link to the mTORC1 pathway. Hence, both the PI3K/PKB and MEK/Erk pathways gather a number of components of the translational machinery to promote assembly of initiation complexes and translation. Although plants possess a large number of MAPKs, their possible role in posttranscriptional regulation has not been established yet.

The TOR signaling pathway

The TOR signaling pathway has been studied mostly in animal systems, where it plays a role in the phosphorylation of a number of components of the translation machinery. Hyperactivation of the TOR signaling pathway has been shown to affect cell and tissue growth (Sarbassov *et al.*, 2005). Furthermore, TOR signaling appears to be important for both short-term (minutes) and long-term (hours) induction of translation by increasing the levels of ribosomes and translational factors. The TOR protein is related to lipid kinases and displays Ser/Thr kinase activity *in vitro* (Dennis *et al.*, 2001). TOR contains a region that binds the immunophilin FK506-binding protein 12 (FKBP12), when linked to rapamycin (Wullschleger *et al.*, 2006), which inhibits some, but not all, functions of TOR.

In mammals, TOR can form at least two different complexes, designated mTORC1 and mTORC2, in which the former mediates rapamycin-sensitive and the latter rapamycin-insensitive effects (Sarbassov *et al.*, 2005). mTORC1 consists of TOR, regulatory associated protein of mTOR (Raptor) and KOG1 in yeast, and the protein GβL (or mLst8). mTORC2 is composed of TOR, GβL and rapamycin-insensitive companion of mTOR or mAVO3 (Rictor). Raptor interacts with targets of rapamycin signaling (TOS) motifs present in targets of TOR, enhancing the ability of TOR to phosphorylate 4E-BP1 *in vitro* (Nojima *et al.*, 2003; Schalm *et al.*, 2003).

Hormones, growth factors, amino acids, and cellular energy status can regulate the TOR signaling pathway. First, insulin can activate PKB/Akt, resulting in the phosphorylation of TSC2 (Figure 6), which is part of a dimeric complex, also containing TSC1. TSC2 is a GTPase-activating protein (GAP) for Ras-homolog enriched in brain (Rheb), a small G protein that interacts with TOR irrespective of its guanine-nucleotide-binding status (Long *et al.*, 2005; Smith *et al.*, 2005) but that stimulates the *in vitro* kinase activity of TOR only in its GTP-bound form (Long *et al.*, 2005). Phosphorylation of TSC2 probably inhibits its GAP activity toward Rheb and leads to the accumulation of active, GTP-bound Rheb and activation of mTORC1 (Inoki *et al.*, 2002; Potter *et al.*, 2002). Another possibility is that phosphorylation of TSC2 alters its stability, its association with TSC1, or its subcellular localization (Avruch *et al.*, 2006; Kwiatkowski and Manning, 2005). mTORC1 signaling also depends on the cellular energy status. The AMP-activated protein kinase (AMPK) is triggered by increasing AMP levels (Hardie *et al.*, 2003) and phosphorylates TSC2 at a different site from that of PKB, which turns off the mTORC1 signaling, probably by stimulating its GAP activity toward Rheb (Inoki *et al.*, 2003). Furthermore, the RSKs (or $p90^{RSK}$ proteins), which are activated by the MEK/Erk MAPK pathway, most probably activate mTORC1 as well. TSC2 can be phosphorylated by RSKs, which might, as is the case for PKB, impair the GAP activity of Rheb by keeping it in an active GTP-bound form. Growth factors and mitogens can also turn on mTORC1 signaling (Roux *et al.*, 2004; Rolfe *et al.*, 2005).

Targets of mTORC1 are the 4E-BPs and the S6Ks that all contain a TOS motif for binding to Raptor. Rapamycin probably inhibits TOR activity by disrupting the TOR-Raptor interaction (Oshiro *et al.*, 2004). S6Ks are activated by phosphorylation, while 4E-BPs are deactivated. S6Ks are responsible for the phosphorylation of S6, a component of the 40S ribosomal subunit, eIF4B, and eIF3, triggering translation by recruitment of eIF4B into complexes with eIF3 (Holz *et al.*, 2005; Raught *et al.*, 2004; Shahbazian *et al.* 2006). The physiological role of S6K phosphorylation is still not clear, but it probably plays a role in cell size control. Mice producing S6 proteins, in which all five phosphorylation sites are mutated, display a decreased cell phenotype, which is not due to faster cell divisions, but to a growth defect (Ruvinsky *et al.*, 2005). Phosphorylation of S6 is strongly inhibited by rapamycin.

TOR signaling is essential for regulation of the cellular capacity for protein synthesis. Ribosomes consist of rRNA, synthesized in the nucleolar compartment by RNA polymerase I, and ribosomal proteins. Translation of mRNAs encoding ribosomal proteins is suppressed under basal conditions (Meyuhas and Hornstein, 2000). However, in response to stimulation of serum-starved cells by adding fresh medium, these mRNAs are translated. This effect can be partially inhibited by rapamycin

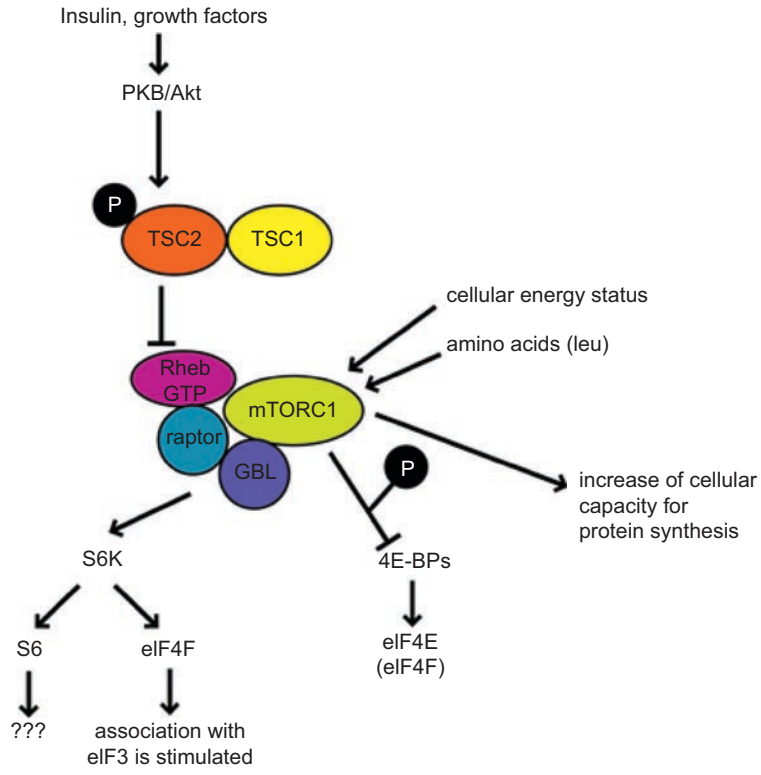

Figure 6. Schematic view of the TOR signaling pathway. Activation of the TOR signaling pathway results in both a short-term and a long-term activation of translation. A variety of growth factors can trigger the TOR signaling pathway through activation of the PKB/Akt pathway, phosphorylating TSC2. TSC2 forms a complex with TSC1 and functions as a GAP for Rheb, a small G-protein that stimulates the kinase activity of TOR only in its GTP-bound form. The kinase activity of mTORC1 can also be stimulated by amino acids (with Leu being the most effective) and depends on the cellular energy status. Targets of mTORC1 are the 4E-BPs and the S6 kinases.

(Jefferies *et al.*, 1994). The exact mechanism by which TOR controls the translation of these mRNAs is unclear. TOR signaling is required for the activation of rRNA transcription (Hannan *et al.*, 2003) and the positive regulation of phosphorylation of upstream binding factor (UBF), an rRNA transcription factor. TOR controls polI transcription through the activity of TIF-1A, a regulatory factor that senses the availability of nutrients and growth factors (Mayer *et al.*, 2004).

The involvement of TOR signaling in other processes besides initiation of translation has been revealed by a number of studies performed in yeast and plants. First, TOR has been demonstrated to influence mitochondrial gene expression. In yeast, inhibition of TOR signaling results in an increased life span, probably the consequence of increased respiration due to enhanced mitochondrial gene expression (Bonawitz *et al.*, 2007). *Arabidopsis* contains only one TOR-encoding gene and the protein is insensitive to rapamycin (Menand *et al.*, 2002), probably because of a mutation in the FKBP12 protein that makes it unable to bind to AtTOR when bound to rapamycin (Mahfouz *et al.*, 2006). In contrast, maize (*Zea mays*) TOR has been shown to be inhibited by rapamycin (Agredano-Moreno *et al.*, 2007). Although AtTOR mRNA is present in all plant tissues in nearly equal amounts, the protein is produced only in developing endosperm, embryo, and primary root or shoot meristems. Embryos arrested at the globular stage are obtained by homozygous AtTOR knockout mutations, although some cells still divide, suggesting a function of AtTOR in developmental stages, where cell division is coupled to an increase in cell mass and cytoplasm, as is the case in primary meristems. As a consequence, AtTOR might be involved in premitotic cytoplasmatic growth in plants (Menand *et al.*, 2002; Robaglia *et al.*, 2004). Induced silencing of AtTOR results in leaf senescence, arrest in organ growth, and reduced translation. The expression levels of *AtTOR* and *AtEBP1*, a regulator of ribosome assembly and translation (Horváth *et al.*, 2006; Deprost *et al.*, 2007) are seemingly correlated, or in other words, AtEBP1 might be a downstream target of AtTOR (Deprost *et al.*, 2007). The discrepancy between AtTOR mRNA and protein levels implies a posttranscriptional regulation mechanism. A short micro ORF was found in the 5′ leader of the *Arabidopsis* and rice TOR mRNAs, which might result in this posttranscriptional regulation (Robaglia *et al.*, 2004; Bonnet *et al.*, 2004). Overexpression of *AtTOR* enhances growth of root and

shoots, increased seed production due to increased growth of the inflorescence, and insensitivity of primary root growth to salt (Deprost *et al.*, 2007). Two Raptor homologs are found in *Arabidopsis*, AtRaptor 1 and AtRaptor 2 (AtRaptor1B and AtRaptor1A, respectively), of which the N-terminal domain is homologous to yeast and plant metacaspases (Ginalski *et al.*, 2004). AtRaptor 1 shows a higher expression than AtRaptor 2, with similar levels in roots, leaves, and inflorescences (Deprost *et al.*, 2005). A knockout mutation of AtRaptor 2 had no visible phenotype, in contrast to homozygous AtRaptor 1 –/– plants. Whereas Deprost *et al.* (2005) were not able to recover homozygous AtRaptor 2 –/– plants, Anderson *et al.* (2005) could obtain plants with a defect in primary shoot apical meristem (SAM) maintenance, combined with a failure to repress axillary meristem activity. These results suggest that AtRaptor, together with AtTOR, functions in postembroyonic meristem-driven growth, while during embryonic development, AtTOR probably functions without AtRaptor (Anderson *et al.*, 2005). In yeast, Mei2 is a putative target of TOR signaling and is bound by the yeast Raptor-homologous Mip1. Under high nutrient conditions, Mei2 is an inactive phosphoprotein. In diploid plants, it accumulates in an unphosphorylated state when nutrients are scarce and translocates to the nucleus, where it binds to noncoding, mRNA-like meiRNA while being transcribed, which precedes meiosis (Watanabe *et al.*, 1988; 1997; Watanabe and Yamamoto, 1994; Li and Mcleod, 1996; Yamashita *et al.*, 1998; Shinozaki-Yabana *et al.*, 2000; Shimada *et al.*, 2003). An interaction between *Arabidopsis* Mei2-like 1 (AML1) and AtRaptor 1 has been demonstrated, suggesting that AML1 might also be a downstream target of AtTOR-AtRaptor signaling. Homozygous AML1 insertion mutants show an early flowering phenotype, again hinting at a role for AtTOR-AtRaptor complexes in postembryonic growth (Anderson and Hanson, 2005). Furthermore, an *in vivo* interaction has been observed between AtRaptor 1 and S6K1, which is the *Arabidopsis* $p70^{S6K}$ homolog, leading to phosphorylation and activation of S6K1. Under osmotic stress, the activity of S6K1 is attenuated, decreasing the rate of protein and ribosome synthesis (Mahfouz *et al.*, 2006). In animal systems, S6K is activated through consecutive phosphorylation by TOR, MAPK, and PDK1 (Pullen and Thomas, 1997); in plants, activation of S6K1 seemingly requires phosphorylation by AtTOR and the plant PDK1 as well (Mahfouz *et al.*, 2006).

The role of microRNAs in translational control

microRNAs (miRNAs) are 20–22 nucleotides in length and form a group of important regulatory, nonprotein-coding molecules involved in posttranscriptional regulation of gene expression. Approximately 30% of the protein-coding genes in animals are estimated to be regulated by miRNAs (Krek *et al.*, 2005; Lewis *et al.*, 2005). A number of differences exist between plant and animal miRNAs. Firstly, in contrast to animal miRNAs, plant miRNAs show a high degree of complementarity to target mRNAs (Ambros, 2004; Bartel, 2004). As a consequence, animal miRNAs mostly target a large number of transcripts (Ambros, 2004), while plant miRNAs are restricted to a limited number of mRNAs (Rhoades *et al.*, 2002). In addition, animal mRNAs contain various numbers of miRNA target sites improving the degree of repression (Sætrom *et al.*, 2007; Tay *et al.*, 2008). In animals, the target site of miRNAs is located in the 3′ UTR (Lee *et al.*, 1993; Reinhart *et al.*, 2000), while in plants, they are found in the coding region and sometimes in the UTRs. The mechanism by which miRNAs regulate gene expression has often been stated to depend on the degree of complementarity between the miRNA and its target (Hutvágner and Zamore, 2002). When the miRNA pairs perfectly or near-perfectly to the target mRNA, site-specific endonucleolytic mRNA cleavage (slicing) occurs (Rhoades *et al.*, 2002; Bartel, 2004), while translational inhibition or mRNA decay happens when it pairs imperfectly to the target (Tang, 2005). Recently, it has become clear that these mechanisms might be combined or superimposed to each other (Wu *et al.*, 2006). For instance, in *Arabidopsis*, slicing is often combined with translational inhibition, irrespective of the degree of complementarity (Brodersen *et al.*, 2008).

Translation can be inhibited at the initiation or post-initiation levels. Repression at the initiation level has been demonstrated in experiments, in which the target mRNA shifts from the polysomal fraction to the mRNP fraction after miRNA-mediated repression (Pillai *et al.*, 2005; Bhattacharyya *et al.*, 2006). miRNAs might interfere with the 'closed loop', in which PABP, bound to the poly(A) tail, interacts with eIF4G, bound to the 5′ end of the mRNA (Humphreys *et al.*, 2005; Kiriakidou *et al.*, 2007). In other experiments, the polysomal distribution of the target mRNA remains unchanged after introduction of the miRNA, reflecting that the initiation step is not affected (Olsen and Ambros, 1999; Seggerson *et al.*, 2002; Petersen *et al.*, 2006). Furthermore, a strong reduction in protein production has been suggested to be the consequence of a specific cotranslational degradation mechanism of the nascent protein chain (Olsen and Ambros, 1999). Likewise, no protein product could be detected after pulse-labeling, consistent with the cotranslational degradation hypothesis (Petersen *et al.*, 2006).

Promotion of mRNA decay is another mechanism of gene regulation by miRNAs (Lim *et al.*, 2005). Target mRNA is degraded by deadenylation, followed by decapping and subsequently by a 5′→3′ exonuclease activity, but not by an siRNA-like mechanism of endonucleolytic cleavage (Schmitter *et al.*, 2006; Wu *et al.*, 2006).

In conclusion, miRNA-mediated repression of translation probably results from translational inhibition and mRNA decay. The contribution of each mechanism can vary according to cell type, stability of the particular miRNA–mRNA pair, and physiological conditions, and could be influenced by additional proteins associated with the mRNA, which might confer different accessibility to nucleases (Behm-Ansmant *et al.*, 2006; Schmitter *et al.*, 2006; Wu *et al.*, 2006).

Cap-independent translation initiation

The discovery of IRES elements

More than 20 years ago, only prokaryotic ribosomes had been reported to be able to bind to circular RNA molecules, suggesting that eukaryotic ribosomes can enter mRNAs exclusively via the free 5′ end (Kozak 1979; Konarska *et al.*, 1981). In 1988, however, picornaviral mRNAs were found to be translated in a manner distinct from ribosomal shunting, enabling ribosomes to initiate effectively on highly structured regions within the 5′ UTR (Jang *et al.*, 1988; Pelletier and Sonenberg, 1988). These regions are designated internal ribosome entry site or IRES elements. The first proof supporting the hypothesis that picornaviral mRNAs are translated via a cap-independent mechanism came from experiments in which the 5′ UTRs of the encephalomyocarditis virus (EMCV) and poliovirus were placed in a dicistronic construct between two cistrons. Translation of the second cistron occurred even when translation of the first cistron was inhibited (Jackson *et al.*, 1995). Furthermore, small deletions or insertions in the IRES element severely reduced its activity (Svitkin *et al.*, 1985; Kuge and Nomoto, 1987; Trono *et al.*, 1988).

Since then, other RNA and DNA viruses have been found to contain IRES elements that initiate internal translation. In addition, not only viral, but also cellular mRNAs have been found that harbor IRES elements. The first cellular, IRES-containing mRNA that had been discovered was the mRNA encoding the immunoglobulin heavy chain binding protein BiP, thanks to the continued translation of this mRNA in poliovirus-infected cells, in which cap-dependent translation was inhibited (Sarnow, 1989; Macejak and Sarnow, 1991). Cellular mRNAs containing an IRES element in mammalian systems include mRNAs encoding translation initiation factors, transcription factors, oncogenes, growth factors, homeotic genes, survival proteins, and proteins involved in cell cycle progression and stress response. For instance, in *Drosophila melanogaster*, insulin and nutrients activate PKB/Akt, which subsequently phosphorylates and inactivates 4E-BP and the Forkhead-box-binding protein (FOXO). FOXO is a transcription factor that triggers transcription of 4E-BP and the insulin-like receptor (INR). Phosphorylation of FOXO results in its sequestration into the cytoplasm and, hence, transcriptional inhibition of its target genes, whereas inactivation of 4E-BP leads to an efficient translation. However, when nutrients are scarce, 4E-BPs are not phosphorylated and inhibit cap-dependent translation. In addition, FOXO translocates to the nucleus where it transcribes the target genes 4E-BP and INR (Puig *et al.*, 2003; Puig and Tjian, 2005). Although general translation is inhibited, INR, which harbors an IRES element in the 5′ UTR, can be translated efficiently under these conditions, resulting in an increased amount of proteins and an improved cell capacity for nutrient sensing (Marr *et al.*, 2007). Another example of the importance of IRES elements during stress response is the translation of various yeast genes involved in invasive growth, due to morphological changes caused by glucose deprivation (Cullen and Sprague 2000; Ashe *et al.*, 2000). This starvation-induced differentiation is accompanied by inhibition of cap-dependent translation. However, IRES-mediated translation was demonstrated for a number of transcripts under these conditions, as well as for eIF4G2, of which the synthesis allows translation of the invasive growth genes (Gilbert *et al.*, 2007).

Only a few IRES-containing mRNAs have been described in plants. For instance, the 5′ UTR of the maize Hsp101 can mediate translation during heat stress (Dinkova *et al.*, 2005). The leader of the ribosomal S18C mRNA can mediate internal initiation of translation (Vanderhaeghen *et al.*, 2006) and the alcohol dehydrogenase-1 (ADH1) mRNA is translated efficiently under oxygen deprivation in maize. The ADH1 leader contains an IRES, but its activity appears to be too weak to fully explain translation of the messenger under stress conditions (Mardanova *et al.*, 2007, 2008).

The search for IRES sequences

In general, IRES-containing mRNAs are not subjected to translational regulatory mechanisms. IRES-mediated translation allows an enhanced or continued production of proteins under conditions in which cap-dependent translation is inhibited or compromised, such as irradiation (Holcik *et al.*, 2000), hypoxia (Lang *et al.*, 2002; Stein *et al.*, 1998), angiogenesis (Akiri *et al.*, 1998), apoptosis (Stoneley *et al.*, 2000), and amino acid starvation (Álvarez *et al.*, 2003). This kind of regulation probably serves as a mechanism to help cells to cope with transient stress situations. Furthermore, IRES-mediated translation has been demonstrated during the G2-to-M transition, when cap-dependent translation is also inhibited. Hence, IRES elements may be important for the maintenance of protein synthesis in normal physiological processes, such as the cell cycle (Cormier *et al.*, 2003; Pyronnet and Sonenberg, 2001; Sachs, 2000). In many cases, the activity of the IRES element is restricted to a specific cell type,

possibly because IRES-mediated translation depends on noncanonical translation initiation factors (ITAFs), not present in all cell types. Databases of published IRES elements are available at http://ifr31w3.toulouse.inserm.fr/IRESdatabase (Bonnal *et al.*, 2003) and http://www.iresite.org/IRESite_web.php.

Translational control during the cell cycle

As early as 1966, protein synthesis has been discovered to be regulated during the cell cycle (Scharff and Robbins, 1966). In mammalian cells, a reduction of 50% in protein synthesis prevents DNA replication in response to mitogenic stimuli (Zetterberg and Larsson, 1985; Zetterberg *et al.*, 1995). Hence, protein synthesis is a critical feature in DNA replication and progression through the cell cycle (O'Farrell, 2001). Translation of G1 cyclins seems to be a regulatory step for G1-to-S transition, while the translation of cyclin B is important for G2-to-M transition. During the G1-to-S transition, proteins are translated via a cap-dependent mechanism, while cap-dependent translation is inhibited in the G2-to-M phase (Pyronnet and Sonenberg, 2001).

G1-to-S transition

In budding yeast, two waves of cyclin expression are observed during the G1-to-S transition. In contrast to the other cyclins, the *CLN3* transcript is present throughout the cell cycle and is only translated early in G1 (Nasmyth and Dirick, 1991; Tyers *et al.*, 1993). Its translation depends on eIF3 and eIF4E (Danaie *et al.*, 1999; Polymenis and Schmidt, 1997). The 5′ UTR of the CLN3 mRNA is long and structured and contains an uORF (Danaie *et al.*, 1999). Loss of TOR function in *S. cerevisiae* inhibits translation, probably causing a cell cycle arrest in early G1 (Barbet *et al.*, 1996). Furthermore, rapamycin was initially characterized as an inhibitor of G1 progression and affects translation similarly as the inactivation of eIF4E (Thomas and Hall, 1997). Thus, a role for the mTOR signaling pathway in G1-to-S transition has been suggested. Targets of mTOR include S6Ks and the 4E-BPs (Gingras *et al.*, 1999; Burnett *et al.*, 1998). This phosphorylation results in an increased formation of eIF4F complexes and, hence, of cap-dependent translation, necessary for the G1-to-S transition (Burnett *et al.*, 1998; Fumagalli and Thomas, 2000).

However, other signaling pathways seem to be involved in the G1-to-S progression. MAPKs are responsible for phosphorylation of Mnk1, which in turn phosphorylates eIF4E only when eIF4E is in complex with eIF4G (Fukunaga and Hunter, 1997; Waskiewicz *et al.*, 1997, 1999; Pyronnet *et al.*, 1999). Moreover, PKR activities that phosphorylate eIF2 vary during the cell cycle: highest peak in the early G1 phase and a second peak at the G1-to-S boundary. The fact that cells producing a catalytically inactive form of PKR or lacking it show a prolonged G1 phase with fewer cells engaged in S phase is also in favor of a role for PKR in cell cycle progression (Petryshyn *et al.*, 1994; Zamanian-Daryoush *et al.*, 1999). PKR has been identified as an E2F1-induced gene product with a role in E2F1-mediated apoptosis (Vorburger *et al.*, 2002).

G2-to-M transition

Several studies in mammalian cells have shown that the rate of protein synthesis at mitosis drops to 25–30% of that of interphase cells (Fan and Penman, 1970; Tarnowka and Baglioni, 1979; Qin and Sarnow, 2004). Cap-dependent translation is inhibited during mitosis due to several factors that, ultimately, all contribute to this drop in protein synthesis. First, the inhibition of cap-dependent translation coincides seemingly with eIF4E dephosphorylation (Scharff and Robbins, 1966; Bonneau and Sonenberg, 1987), which is in contrast with the activation of MAPKK, MAPK, and its direct substrate Mnk1, during mitosis and their requirement for G2-to-M transition (Tamemoto *et al.*, 1992; Wright *et al.*, 1999). When cells enter mitosis, 4E-BP1 is in a hypophosphorylated form (Pyronnet *et al.*, 2001; Heesom *et al.*, 2001) and associates with eIF4E, thereby disrupting the eIF4F complex and separating eIF4E and eIF4G. Hence, the eIF4G-associated Mnk1 kinase can no longer phosphorylate eIF4E (Pyronnet *et al.*, 2001). In addition, phosphorylation of 4E-BP1 increases again during mitosis and a complex containing the cyclin-dependent kinase 1 (CDK1) is responsible for the phosphorylation of at least two of the seven identified phosphorylation sites of 4E-BP1. Protein synthesis resumes quickly when cells enter the G1 phase and these phosphorylation events that dissociate the 4E-BP1-eIF4E complex might be a means to resume cap-dependent translation (Heesom *et al.*, 2001). Secondly, phosphorylation of eIF2 inhibits translation (Hinnebusch, 2000). The p67 glycoprotein, associated with eIF2, protects eIF2α from inhibitory phosphorylation only when it is glycosylated. In mammalian systems, the G2-to-M transition is correlated with a reduced level of p67 glycosylation and, hence, an increased level of eIF2α phosphorylation (Datta *et al.*, 1999). Finally, S6Ks have been shown to be phosphorylated and inactivated by CDK1/cyclin B during the M phase. Thus, the availability of ribosomes and initiation factors decreases at the M phase (Papst *et al.*, 1998; Shah *et al.*, 2003). However, translation of proteins is critical for progression through G2-to-M and mitosis. Since polioviral IRES-containing mRNA is still efficiently translated during mitosis (Bonneau and Sonenberg, 1987), IRES-directed translation might

proceed when cap-dependent translation is inhibited. Indeed microarray analysis revealed that approximately 3% of the cellular mRNAs remain associated with polysomes during the G2-to-M transition and all, but one, of the tested mRNAs score positively for IRES activity (Qin and Sarnow, 2004). Cellular IRES-containing mRNAs important for the G2-to-M transition include mRNAs encoding ornithine decarboxylase (ODC) (Pyronnet *et al.*, 2000), the protein kinase p58^{PITSLRE} (Cornelis *et al.*, 2000), upstream of N-ras (Unr) (Cornelis *et al.*, 2005; Schepens *et al.*, 2007), Sjogren syndrome autoantigen B (La) (Qin and Sarnow, 2004), and c-Myc (Nanbru *et al.*, 1997; Stoneley *et al.*, 1998; Pyronnet *et al.*, 2000; Kim *et al.*, 2003; Kobayashi *et al.*, 2003).

ODC is the rate-limiting enzyme in the biosynthesis of polyamines (putrescine, spermidine, and spermine) and catalyzes the transformation of ornithine into putrescine. Polyamines have multiple functions, such as DNA replication during S phase and chromosome condensation and mitotic spindle organization during mitosis (Pollard *et al.*, 1999; Childs *et al.*, 2003). Depletion of polyamines causes chromosomal aberrations in mammalian cells (Pohjanpelto and Knuutila, 1982), disruption of the higher order organization of chromatin in yeast (Pollard *et al.*, 1999), and inhibition of cytokinesis with multinucleated cells in various cell types as a consequence (Sunkara *et al.*, 1979; Pohjanpelto *et al.*, 1981; Heiskala *et al.*, 1999). ODC is a very short-lived enzyme (Tabor and Tabor, 1984) and its protein synthesis is highly controlled. The 5′ UTR of ODC is long and structured and consists of two segments: the cap-proximal segment, a stable hairpin structure followed by a uORF that represses cap-dependent translation, and the downstream segment, located just upstream from the initiation codon and containing an IRES element, responsible for translational control in a cell cycle-dependent manner (Pyronnet *et al.*, 2000). This control is negatively exerted at the G1-to-S phase, but functions positively in G2-to-M (Pyronnet *et al.*, 2000; Tinton *et al.*, 2005).

The IRES of p58^{PITSLRE} is located in the coding region of the PITSLRE mRNA. PITSLRE kinases (also named CDK11) are protein kinases important for normal cell cycle progression and are encoded by two duplicated genes *Cdc2L1* and *CdcL2* in mammals. These genes give rise to two isoforms of the PITSLRE kinase, p110^{PITSLRE} and p58^{PITSLRE}, the latter encoded by a C-terminally located ORF in the coding region of the former (Lahti *et al.*, 1995). While p110^{PITSLRE} is produced throughout the cell cycle, p58^{PITSLRE} is predominantly active at the G2-to-M transition (Cornelis *et al.*, 2000). p110^{PITSLRE} is probably involved in pre-mRNA splicing and the regulation of RNA transcription (Trembley *et al.*, 2002), whereas p58^{PITSLRE} is associated with centrosomes during mitosis and has an important role in the maturation of centrosomes and bipolar spindle formation (Petretti *et al.*, 2006). The Unr protein acts as a stimulatory ITAF for the p58^{PITSLRE} IRES *in vitro* and is produced in a cell cycle-dependent manner with a peak at G2-to-M, coinciding with the activation of the IRES activity of PITSLRE (Ferrer *et al.*, 1999; Tinton *et al.*, 2005). However, deletion of the Unr consensus-binding site in the IRES does not completely destroy the IRES activity, but results in a strong inhibition. Hence, other factors might be needed for IRES activity and Unr might function as a chaperone, inducing a conformational change of the IRES to bind other factors (Tinton *et al.*, 2005). A 5′-located IRES element has been shown to be present in the Unr mRNA responsible for its cap-independent translation. The Unr IRES activity is significantly higher during G2-to-M than during the S phase or in asynchronously growing cells. PTB binds to the IRES of Unr and acts as a negative regulator of its IRES-dependent translation. Additional ITAFs for the Unr IRES include the heterogeneous nuclear nucleoproteins C1/C2 (hnRNP C1/C2) and Unr itself. hnRNP C1/C2 function as stimulating factors, while Unr acts as an inhibitory factor (Cornelis *et al.*, 2005; Schepens *et al.*, 2007). hnRNP C1/C2 reside in the nucleus during the interphase, but migrate to the cytoplasm during mitosis by an unknown mechanism, probably before the nuclear envelope breaks down. Once located in the cytoplasm, these proteins can bind to the Unr IRES or other IRES elements. Binding of hnRNP C1/C2 coincides with a decreased binding of PTB and Unr to the IRES of Unr. Since the binding site for PTB and hnRNP C1/C2 partially overlap, the diminished binding of PTB to the Unr IRES might probably be a consequence of direct competition between these proteins. However, the Unr binding site is located farther away from the hnRNP C1/C2 site. Hence, reduced IRES binding of Unr probably results from a conformational change of the Unr IRES induced by hnRNP C1/C2. Binding of hnRNP C1/C2 is required for Unr IRES stimulation during mitosis, as revealed by the fact that depletion of hnRNP C1/C2 causes cell cycle arrest in the G2/M phase (Schepens *et al.*, 2007). The polysomal association of c-Myc and ODC could not be confirmed (Qin and Sarnow, 2004). The reason for this discrepancy may be that different synchronization protocols and cell lines had been used or that the up-regulation of ODC mRNA and protein occurred in the G2 phase, a cell cycle phase that is missing in the study of Qin and Sarnow (2004).

Mice overexpressing Myc in the B-cell compartment (*minute* mutants) display an increase in protein synthesis, cell size, and accelerated cell cycle progression, which is independent from the transcriptional activation of genes downstream of Myc. Furthermore, cap-dependent translation during mitosis is not impaired and the switch to IRES-mediated translation necessary for mitosis does not occur. As a result, p58^{PITSLRE} is not translated, leading to cytokinetic defects and genome instability. However, the phenotype is rescued when these mice are

intercrossed with *L24*$^{+/-}$ mice (ribosomal protein L24), probably because the translational machinery is again available for the translation of IRES-containing mRNAs. These results indicate that the switch from cap-dependent to cap-independent translation is crucial for mitotic progression (Barna *et al.*, 2008).

The switch to IRES-mediated translation of a specific subset of mRNAs is probably influenced by the increasing availability of ITAFs during M phase. Most of the known ITAFs play a role in nuclear RNA metabolism and these factors can diffuse into the cytoplasm because of the breakdown of the nuclear envelope in mitosis. Hence, this cytoplasmatic enrichment of specific ITAFs might be the trigger for the increased activity of specific IRES elements. Consistently, during mitosis, the cytoplasm is enriched with hnRNP C (Kim *et al.*, 2003) and Unr (Tinton *et al.*, 2005), stimulating the activity of the c-Myc IRES (Kim *et al.*, 2003). Furthermore, other known ITAFs, such as PCB2, PTB, hnRNP L, eIF3, and La protein were shown to be induced during S and G2-to-M phase (Honda *et al.*, 2005).

Another protein involved in translational control during the G2-to-M transition is 14-3-3σ. Members of the 14-3-3 family had already been demonstrated to function in the G1-to-S and the G2-to-M transitions. 14-3-3σ binds directly to eIF4B and eIF2α and this interaction depends on a phosphorylation event. Furthermore, knockout of this protein results in multinucleate cells with a defect in cytokinesis. In these cells, cap-dependent translation is not inhibited during mitosis, because no 14-3-3σ protein binds to eIF4B. Cap-independent translation is also inhibited, because the IRES-containing p58^{PITSLRE} is not translated. In conclusion, 14-3-3σ is another protein seemingly important for the switch between cap-dependent and cap-independent translation during mitosis (Wilker *et al.*, 2007).

Not much is known about the extent of translational control during the plant cell cycle. Activity of CDK-cyclin complexes is crucial for proper cell cycle progression. The CDK subunit is responsible for phosphorylation of target proteins, while the activity is regulated by the associated cyclin partner (for a review, see Inzé and De Velder, 2006). In proliferating cells, an *in vivo* interaction between eIF4A and CDKA has been demonstrated. This eIF4A-CDKA complex is active and furthermore, eIF4A contains a CDKA phosphorylation site. These results might suggest a link between cell cycle progression and translational control in plants. However, the cyclin partner or other interaction partners still have to be identified (Hutchins *et al.*, 2004).

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