Translational control of gene expression and disease

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In the past decade, translational control has been shown to be crucial in the regulation of gene expression. Research in this field has progressed rapidly, revealing new control mechanisms and adding constantly to the list of translationally regulated genes. There is accumulating evidence that translational control plays a primary role in cell-cycle progression and cell differentiation, as well as in the induction of specific cellular functions. Recently, the aetiologies of several human diseases have been linked with mutations in genes of the translational control machinery, highlighting the significance of this regulatory mechanism. In addition, deregulation of translation is associated with a wide range of cancers. Current research focuses on novel therapeutic strategies that target translational control, a promising concept in the treatment of human diseases.

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Translation of mRNA is the final step in gene expression, and regulation of this process allows the immediate and direct adaptation of protein levels, independent of nuclear pathways. Translational control is required for the fine-tuning of protein levels during cell proliferation and differentiation, and for spatial and temporal regulation of protein expression during embryogenesis. It also plays a role in the absence of transcription, for example, in the early embryo and in reticulocytes. There are two types of translational control mechanisms: 'global' and 'selective'. Global control acts on all mRNAs in a nonspecific manner, whereas selective translation regulation targets a specific subset of mRNAs. These specific mRNAs often have *cis*-regulatory sequences that sense subtle changes in the activity of the translation machinery. The activity of key components of the translation machinery can be altered in response to signalling cascades that sense external stimuli, such as fluctuations in nutrients, growth factors, mitogens and inducers of differentiation. Hence, it is no surprise that deregulation of translation is involved in the aetiology of human disease [1].

Translation initiation factors and signal transduction pathways

Two crucial events that take place during the initiation of translation are (1) recruitment of mRNA to the ribosomal complex, and (2) selection of the AUG initiation codon. Both are mediated by multiprotein complexes and are regulated by phosphorylation.

Assembly of the eukaryotic translation-initiation-factor complex, eIF4F, at the mRNA 5′ cap facilitates recruitment of the 40S ribosomal subunit, followed by scanning of the ribosomal complex to the first AUG initiation codon (Fig. 1a). Provision of the cap-binding protein, eIF4E, is a rate-limiting and regulated step in the assembly of the eIF4F complex [1,2]. eIF4E is held inactive by 4E-binding proteins (4E-BPs), and release of eIF4E only occurs after phosphorylation of the 4E-BPs, in response to mitogen- and growth-factor-signalling [3].

A second rate-limiting step in mRNA translation is the formation of a ternary complex between the G-protein, eIF2, the initiator Met–tRNA$_i^{Met}$ and GTP. This process facilitates AUG-codon recognition and the initiation of protein synthesis (Fig. 1b). After recognition of the initiation codon, bound GTP is hydrolyzed, and eIF2–GDP is released from the ribosomal complex. The activity of eIF2 is restored, ready for another round of initiation, by an associated guanine-nucleotide-exchange factor, known as eIF2B. Phosphorylation of the α subunit of eIF2 leads to strong inhibition of translation, through competitive binding to eIF2B. Four related eIF2α kinases [haem-regulated inhibitor kinase (HRI), RNA-dependent protein kinase (PKR), PKR-like endoplasmic-reticulum kinase (PERK), and GCN2] have been identified, which alter protein synthesis in a variety of stress conditions [1,2].

Mutations in components of the eIF2 cycle

Mutations affecting the regulation of eIF2 can cause severe inherited disorders. This discovery has firmly established the role of translational control in the aetiology of human disease.

Wolcott–Rallison Syndrome

The Wolcott–Rallison Syndrome (WRS) has been linked with mutations in the catalytic domain of the eIF2α kinase, PERK. The normal role of PERK is to phosphorylate and inactivate eIF2α, thus suppressing protein synthesis during the unfolded-protein response, a mechanism that protects the cell from irreversible damage caused by accumulation of unfolded proteins [4]. Mutations in PERK abolish its catalytic function [5], leading to the loss of pancreatic β-cells and, hence, to permanent diabetes. The disease then develops into multiple systemic disorders, including epiphyseal dysplasia, osteoporosis, and growth retardation. As with PERK mutations in humans, loss of PERK function in mice by gene targeting results in a deficiency of pancreatic β-cells and, therefore, in diabetes [6,7]. Destroying the

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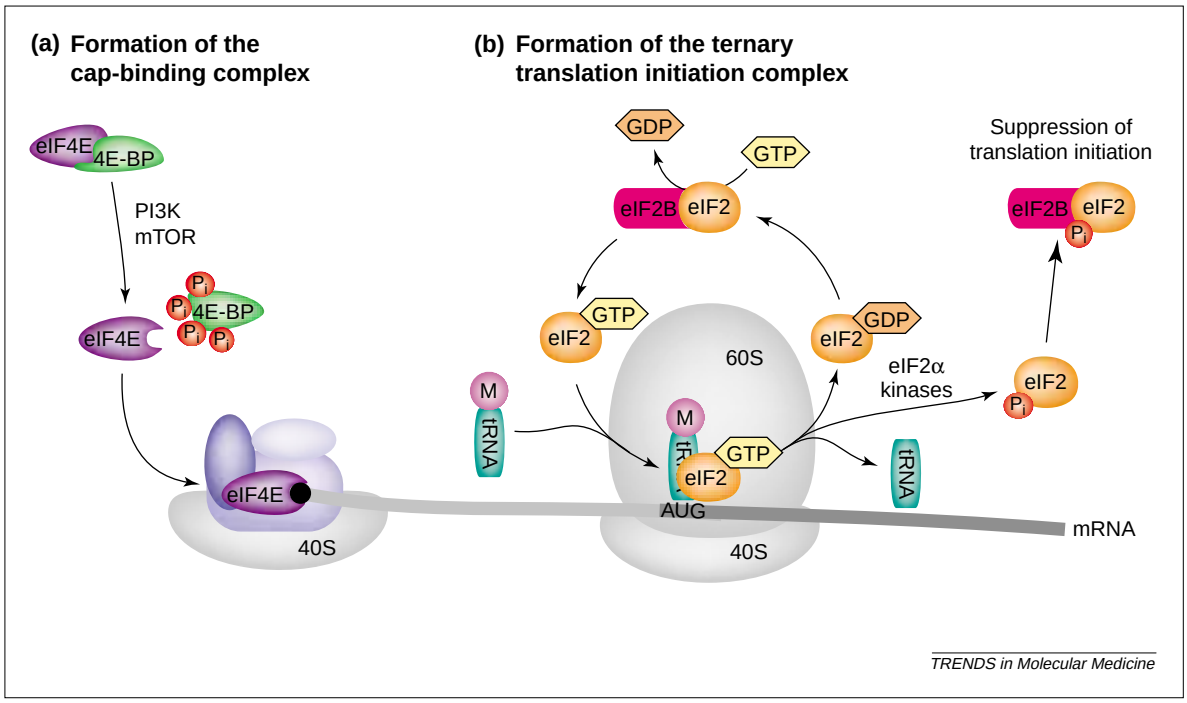

Fig. 1. Rate-limiting steps in the initiation of translation. (a) The eukaryotic translation initiation factor 4E (eIF4E) is retained in an inactive form by 4E-binding proteins (4E-BPs), but is released after phosphorylation of the 4E-BPs by phosphoinositol 3-kinase (PI3K) and mTOR (mammalian target of rapamycin). Free eIF4E then binds to the mRNA cap (black circle) as part of the eIF4F complex that recruits the small 40S ribosomal subunit. (b) A ternary complex, consisting of eIF2, Met–tRNA$_i^{Met}$ and GTP, facilitates AUG-codon recognition and initiation of protein synthesis. eIF2 is recycled through the exchange of GDP for GTP by the associated guanine-nucleotide-exchange factor, eIF2B. However, phosphorylation of the eIF2α subunit by an eIF2α kinase prevents dissociation of eIF2 from eIF2B, leading to inhibition of translation initiation. Abbreviations: M, methionine; P$_i$, phosphate.

PERK phosphorylation site on eIF2α by mutation has similar effects [8]. In summary, the PERK-regulated translational control system prevents overloading with misfolded proteins under stress conditions, which could otherwise lead to cell death.

Leukoencephalopathy with vanishing white matter

Mutations in genes encoding subunits of the guanine-nucleotide-exchange factor, eIF2B, have been linked with the inherited brain disease, 'leukoencephalopathy with vanishing white matter' (VWM) [9]. Several mutations that cause VWM have been found in the enzymatic and in the regulatory subunits of eIF2B, suggesting that deregulation of eIF2 function is the most likely cause of this inherited disorder [9]. VWM patients who experience fever suffer major neurological deterioration, resulting in coma or death. These symptoms might be explained by a reduced ability to alter translational activity (which would normally be regulated by eIF2B) during mild stresses such as elevated body temperature (40–41°C).

Mutations in specific translational regulators

Several mRNA-binding proteins have been identified that specifically regulate translation of particular mRNAs. These proteins bind to *cis*-regulatory sequences in the untranslated regions (UTRs) and affect translation initiation by interaction with the translation machinery. Deregulated expression of such mRNA-binding proteins, or mutations in genes coding for these proteins, might contribute to the development of disease.

Fragile-X mental-retardation syndrome

Fragile-X mental-retardation syndrome is the most common form of inherited mental retardation, and is caused by a loss of fragile-X mental-retardation protein (FMRP) function that results from transcriptional silencing [10] or a single amino-acid change [11]. FMRP binds and regulates the translation of specific mRNAs, encoding proteins that are involved in synaptic maturation and function. FMRP is believed to shuttle these mRNAs from the nucleus to postsynaptic sites, where the mRNAs are held inactive until synaptic input alters FMRP activity and promotes translation [12]. It has been suggested that fragile-X mental-retardation syndrome is caused mainly by a lack of translation of FMRP-associated mRNAs [13,14].

Chronic myeloid leukaemia

In chronic myeloid leukaemia (CML; BCR-ABL) cells, expression of the RNA-binding protein, hnRNP (heterogeneous nuclear ribonucleoprotein) E2, is abnormally high. hnRNP E2 binds to the 5′ UTR of the mRNA encoding C/EBPα (CCAAT/enhancer-binding protein α), resulting in inhibition of its translation. Hence, C/EBPα expression is prevented in CML cells [15]. C/EBPα is a transcription factor that induces myeloid differentiation and has strong antiproliferative activity [16]. Suppression of C/EBPα also occurs in acute myeloid leukaemia

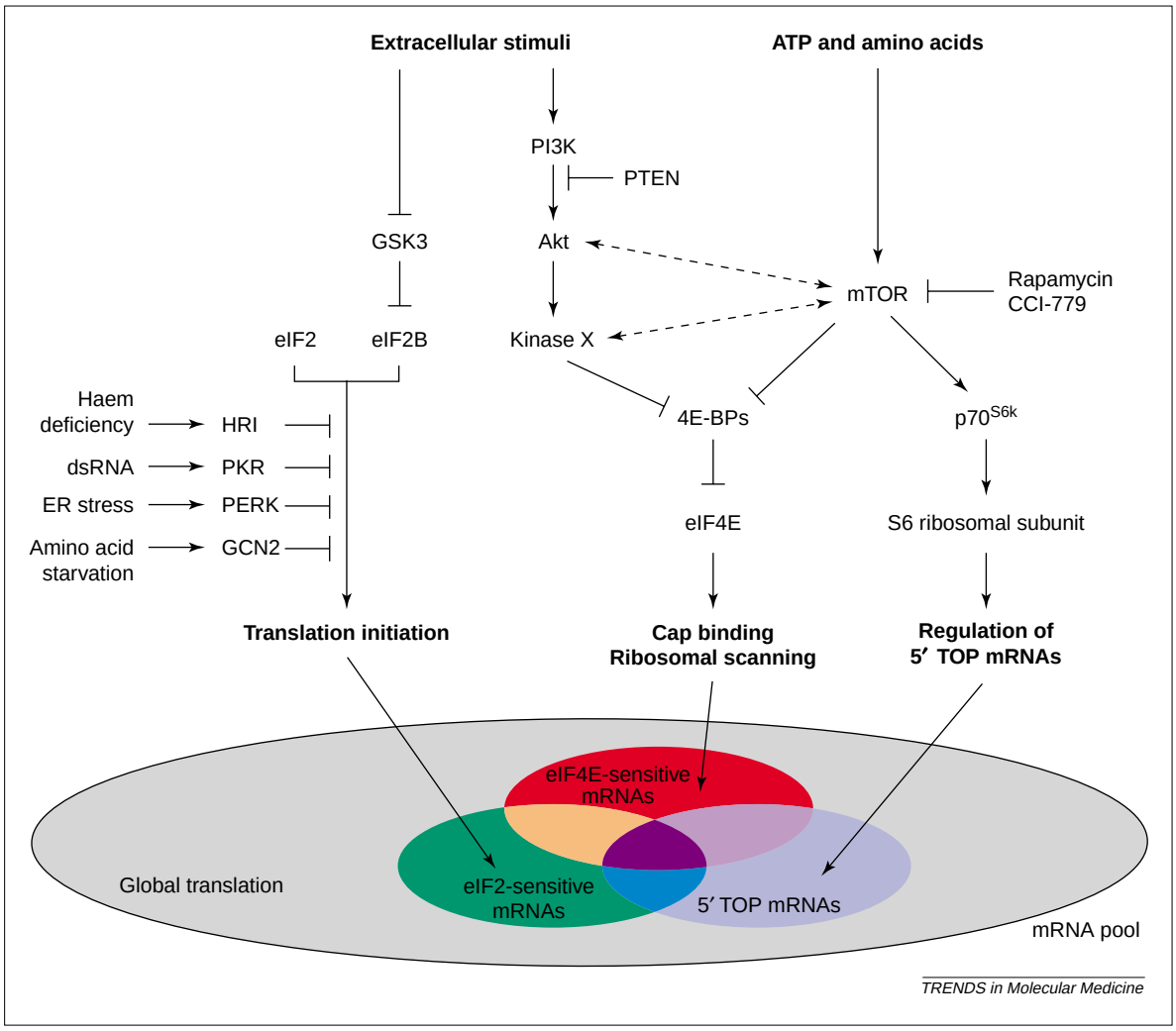

Fig. 2. Signal transduction pathways that regulate the function of general translation factors. Extracellular signals (growth factors, hormones, mitogens and cytokines) stimulate translation initiation via glycogen-synthase kinase 3 (GSK3) and phosphoinositol 3-kinase (PI3K). PI3K activates Akt through phosphorylation of phosphatidylinositol. This leads to activation of an as yet unidentified kinase(s) X and probably also of mTOR (mammalian target of rapamycin) kinase. Kinase(s) X and mTOR facilitate the release of the eukaryotic translation initiation factor 4E (eIF4E) by phosphorylating the 4E-binding proteins (4E-BPs). Released eIF4E can then join the complex eIF4F, leading to cap binding and ribosomal scanning. When sufficient amino acids and ATP are available, mTOR signals to $p70^{S6k}$ and possibly to kinase(s) X. $p70^{S6k}$ phosphorylates the S6 ribosomal subunit that is necessary to release inhibition of 5′ terminal oligopyrimidine tract (5′ TOP) mRNAs. These mainly encode components of the translational machinery. The tumour suppressor, PTEN, inhibits PI3K signalling through dephosphorylation of phosphatidylinositol. The drug, rapamycin, and its pharmacologically used derivative, CCI-779, inhibit mTOR. Active GSK3 phosphorylates eIF2B, thereby inhibiting the recycling of eIF2. When GSK3 is inhibited by extracellular signals, eIF2B is active and translation initiation is stimulated by the recycling of eIF2. The eIF2 cycle is also inhibited by phosphorylation of the eIF2α subunit. Conditions of stress lead to suppression of translation initiation by activation of the eIF2α kinases, haem-regulated inhibitor kinase (HRI), RNA-dependent protein kinase (PKR), PKR-like endoplasmic-reticulum kinase (PERK), and GCN2. Distinct translation initiation factors regulate the translation of specific mRNA pools, either alone or in combination with other factors. Abbreviations: dsRNA, double-stranded RNA; ER, endoplasmic reticulum.

(AML), through mutation of the *C/EBPα* gene [17]. Hence, downregulation of C/EBPα expression appears to be a crucial step in myeloid leukaemogenesis.

Hereditary hyperferritinaemia-cataract syndrome

Translational regulation by iron-response proteins (IRPs) allows rapid and coordinated expression of the proteins that are required for cellular iron homeostasis, such as ferritins, transferrin and the transferrin receptor. Under conditions of iron depletion, IRPs inhibit the translation of target mRNAs by binding to iron-response elements (IREs) in the UTRs [18]. Hereditary hyperferritinaemia-cataract syndrome (HHCS) is an autosomal dominant disorder caused by mutations in the IRE of L-ferritin. This results in reduced IRP binding affinity [19], leading to increased translation of ferritin mRNA and, hence, elevated serum levels of ferritin. This causes an early onset of nuclear cataract, an eye disease that eventually progresses to total blindness [18].

Translational control and cancer development

The nucleolus is at the centre of ribosome biogenesis, a process that is significantly upregulated in malignant cells. Hence, the size of the nucleolus is a prominent marker for cancer diagnosis. The significance of translational control in the oncogenic transformation of cells is also evident from analysis

of the effects on translation of known oncogenic signalling pathways.

Deregulation of eIF4E in cancer

The phosphoinositol 3-kinase (PI3K) pathway bifurcates into those effector pathways that stimulate cell-cycle progression and those that inhibit apoptosis [20]. Constitutive activation of signalling via PI3K or its downstream target, Akt (also known as protein kinase B), promotes oncogenic conversion. This can occur through (1) amplification of the PI3K catalytic subunit (p110α) [21], (2) amplification of Akt [22], or (3) loss of the phosphatase, PTEN, which inhibits PI3K [22]. Upon activation of PI3K signalling, 4E-BPs are phosphorylated and eIF4E is released, resulting in the stimulation of translation initiation (Fig. 1a) [3,23]. For effective release of eIF4E, additional phosphorylation of 4E-BPs, through the mTOR [mammalian target of rapamycin; also known as FKBP12-rapamycin-associated protein (FRAP)] kinase signalling cascade, is required (Fig. 2) [24]. mTOR is activated in response to high levels of ATP and amino acids, and is believed to link metabolic and nutritional status with mitogenic signals [25,26]. In addition to activating eIF4E function, stimulates the translation of mRNAs that code for general components of the translation machinery. The mRNAs of these components have *cis*-regulatory elements, known as 5′ TOP (5′ terminal oligopyrimidine tract) sequences, in their 5′ UTRs. 5′ TOP inhibits translation in the absence of activation by $p70^{s6k}$ kinase, a downstream effector of mTOR signalling [27].

Although no components of the mTOR signalling pathway have been identified as oncogenes, loss of their translational control function seems to be required for the transformation potential of the Akt pathway. Indeed, downregulation of translation by inhibition of mTOR with the drug, rapamycin, effectively quenches transformation by the oncoproteins, PI3K and Akt [28]. Although the exact relationship between the PI3K–Akt and mTOR signalling pathways has not been clarified, it is thought that they regulate translation via separate, but interdependent, mechanisms (Fig. 2). In addition to modulating translation, activation of PI3K signalling affects transcription and post-translational protein modification, thereby inhibiting apoptosis and stimulating cell-cycle progression [20].

The tumour suppressor, PTEN, inhibits PI3K–Akt signalling by dephosphorylating inositol trisphosphate (IP_3), a product of PI3K-mediated phosphorylation of phosphatidylinositol that activates Akt (Fig. 2). Mutation of PTEN has been noted in a wide variety of sporadic cancers, as well as in the autosomal dominant cancer-prone syndromes, Cowden disease, Chermitte–Dudos Syndrome and Bannayan–Zonana Syndrome [29]. PTEN deficiency in mice results in enhanced $p70^{S6k}$ activation and 4E-BP phosphorylation, and correlates with increased cell size, cell proliferation and tumour formation [30].

Interestingly, eIF4E itself appears to have oncogenic potential, because overexpression of eIF4E in 3T3 mouse fibroblasts induces malignant transformation [31]. Furthermore, eIF4E can transform primary rat-embryo fibroblasts, in cooperation with either v-myc or the adenovirus E1A oncoprotein [32]. Increased levels of eIF4E have been found in several cancers [33], including colon adenomas and carcinomas [34], breast carcinomas [35,36], non-Hodgkin's lymphomas [37] and primary bladder cancers [38]. The product of the *Myc* oncogene enhances eIF2 and eIF4E expression [39].

In summary, deregulated translation as a result of disturbed PI3K–Akt signalling contributes to oncogenesis. This might prove to be as important as the well-known transcriptional- and post-translational-effects of PI3K–Akt signalling.

Deregulation of eIF2 in cancer

Activation of eIF2α kinases and phosphorylation of eIF2α results in downregulation of translation initiation. Constitutive activation of eIF2, either using a dominant negative form of the eIF2α kinase, PKR, or by overexpression of an eIF2α protein that carries a mutated eIF2α-kinase phosphorylation site, results in malignant transformation of cells in culture [40]. However, although deregulation of eIF2 is frequently observed in cancer cells [37,41], there is no direct evidence that this defect contributes to the development of cancer. Nevertheless, three compounds, eicosapentaenoic acid, thiazolidinedione and clotrimazole, that indirectly enhance eIF2α phosphorylation via partial depletion of intracellular Ca^{2+} stores, are potent inhibitors of tumour growth *in vitro* and in animal models [42–44].

***cis*-regulatory mRNA elements**

Global changes in protein synthesis following hormone- or growth-factor-stimulation are relatively small (up to twofold), but a subgroup of mRNAs exhibits a dramatic change in translation rates. In this way, general stimuli can selectively induce or suppress the translation of this set of mRNAs. Most of these mRNAs possess specialized *cis*-regulatory elements that make them receptive to translational control, such as upstream initiation codons, upstream open reading frames (uORF) and internal ribosomal entry sites (IRES) [1,2].

Only ~10% of vertebrate mRNAs can be regulated in this manner, with approximately two-thirds of these transcripts encoding key regulatory proteins, such as growth factors, cytokines, proto-oncogenes and components of the cell cycle [45]. Examples of proteins that can be regulated at the translational level include the cell-cycle regulators p27 [46] and cyclin D1 [47], the growth factor thrombopoietin [48], the apoptosis regulator BCL-2 [49], and the transcription factors CCAAT/enhancer-binding proteins (C/EBPs) -α and -β [50], and activating transcription factor (ATF)-4 [51]. This list reflects

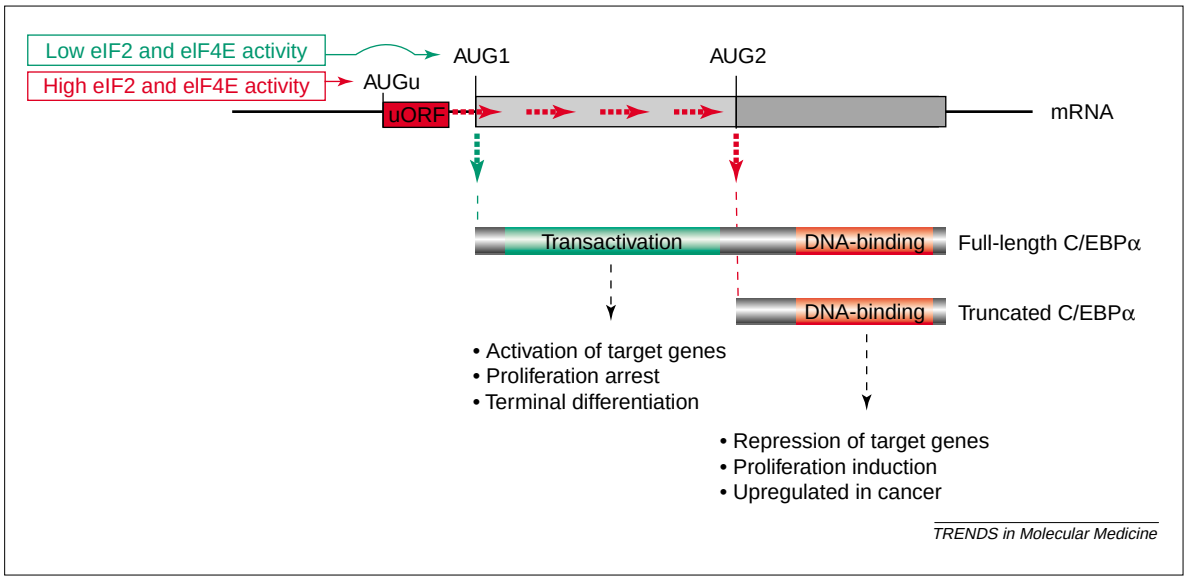

Fig. 3. Translational control of CCAAT/enhancer-binding protein α (C/EBPα) isoform expression. A small upstream open reading frame (uORF) serves as a *cis*-regulatory element that controls the site of translation initiation by monitoring the activity of the translation initiation factors, eIF2 and eIF4E. At high eIF activity, the uORF is translated with subsequent scanning to, and efficient reinitiation at, AUG2, resulting in the expression of a truncated C/EBPα isoform. At low eIF activity, most ribosomes miss out the uORF and initiate at AUG1, leading to expression of the full-length isoform. Truncated C/EBPα suppresses target genes and allows or induces proliferation, whereas full-length C/EBPα activates the transcription of target genes and is antiproliferative.

the importance of translational control in cell growth, proliferation and differentiation. In many cases, signal-transduction pathways and the mechanisms controlling the translation of these mRNAs, as well as the biological consequences of their translational deregulation, have yet to be clarified. However, it is likely that several of these mRNAs are deregulated in diseases in which altered eIF functions are evident.

Translational control of C/EBPα expression

The deregulated expression of the transcription factors, C/EBPα and C/EBPβ, that has been observed in some cancers might be caused by disturbed translational control. The expression of full-length versus N-terminally truncated isoforms of C/EBP is controlled by the levels of eIF4E and eIF2 activity (Fig. 3) [50]. C/EBP isoforms have different and partially opposing functions in gene regulation and proliferation control; full-length isoforms are transcriptional activators and induce differentiation and cell-cycle arrest, whereas truncated isoforms have only restricted transcriptional activity and support proliferation [52–55]. Control of C/EBP-isoform expression is mediated via a conserved *cis*-regulatory uORF in the 5′ UTR that senses the activities of both eIF4E and eIF2 (Fig. 3) [50]. Interestingly, enhanced eIF2 or eIF4E activity, and concomitant upregulation of truncated C/EBP isoforms, are seen in mammary-epithelial and intestinal-epithelial cancer cells [34,56,57]. Furthermore, experimentally induced expression of truncated C/EBP isoforms in cultured adipoblasts promotes cellular transformation [50]. This suggests that deregulation of C/EBP-isoform expression through enhanced eIF activities might contribute to the development of some cancers.

Hereditary thrombocythaemia

Genetic analysis of hereditary thrombocythaemia has revealed that this disease might be caused by mutations in uORFs [18]. The disease is characterized by sustained proliferation of megakaryocytes in the bone marrow, resulting in a marked increase in the number of blood platelets. Symptoms are mostly mild and include aberrant blood clotting and bleeding. The cytokine, thrombopoietin (TPO), is the primary regulator of megakaryopoiesis and platelet production. The normal serum concentration of TPO is low because the translation of TPO mRNA is almost completely inhibited by the presence of uORFs, especially an uORF that extends over the TPO translation initiation site. Removal of upstream initiation codons, by point mutation or by deletion of the entire 5′ UTR, greatly improves the efficiency of TPO translation [48]. The mutations identified in patients with hereditary thrombocythaemia are characterized by loss of uORF function, leading to increased TPO expression [18].

Therapeutic strategies

The examples discussed in this review show that defects in translational control at any level (signal transduction pathways, general or specific translation factors, or *cis*-regulatory sequences in the mRNA) can contribute to the development of disease. We have only recently begun to appreciate the relevance of deregulation of translation in the development of disease. Hence, intervention at the level of translational control has scarcely been adopted as a therapeutic concept. Most genes that are under translational control encode key regulatory proteins

involved in cell-cycle progression, cell differentiation and metabolic pathways. Deregulation of these genes at the level of translation has been associated with numerous diseases, suggesting a potentially significant role for therapeutic intervention at this level. Intriguingly, several agents with known anti-cancer effects, such as rapamycin, eicosapentaenoic acid, thiazolidinedione and clotrimazole, target signalling pathways that regulate either eIF4E-binding-protein function or the eIF2 cycle [42–44]. Rapamycin has merited particular attention because it is highly active against a broad range of tumour cells, in particular those that lack PTEN activity [58,59]. Furthermore, rapamycin inhibits cellular transformation induced by oncogenic PI3K–Akt signalling or by the *Gli* oncogene, a transcription factor that operates in the hedgehog-patched signalling pathway [60]. Moreover, rapamycin suppresses tumour growth by inhibition of neo-angiogenesis, suggesting that vascularization of tumours is also translationally regulated [36,61]. The compound, CCI-779, a water-soluble ester analogue of rapamycin with improved pharmaceutical properties, is currently undergoing clinical investigation [58]. In light of their potentially broad therapeutic application, we hope that more such drugs that specifically interfere with translational regulation mechanisms and signalling pathways will be identified.

It is now widely accepted that translation control is a key regulatory principle, modulating the expression of many proteins that are crucial in regulating cell physiology. The numerous studies addressing translational control in the aetiology of human diseases will hopefully contribute to the development of novel therapeutic strategies.

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Owing to the concise nature of this review, it was not possible to address all aspects of translational control in disease. Notably, translational control plays a major role in the pathology of viruses and host defense mechanisms against viral infection. This subject has been extensively reviewed elsewhere (e.g. Ref. [1]).

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Urea-cycle disorders as a paradigm for inborn errors of hepatocyte metabolism

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Urea-cycle disorders (UCDs) are a group of inborn errors of hepatocyte metabolism that are caused by the loss of enzymes involved in the process of transferring nitrogen from ammonia to urea, via the urea cycle (UC). Recent genetic analyses of inherited disorders that present with hyperammonemia demonstrate the function of cellular transporters that regulate the availability of UC intermediates. The regulation of UC intermediates, such as arginine, could have far reaching implications on nitric-oxide synthesis and vascular tone. Hence, each UCD and UC-related disorder constitutes a unique gene-nutrient interaction that is crucial for postnatal homeostasis. Recent advances in the diagnosis and management of UCDs include the application of *in vivo* metabolic-flux measurements. Cumulative morbidity is still high despite dietary and pharmacological therapies and, hence, both cell and gene therapies are being pursued as possible long-term corrective treatments. Although gene-replacement therapy has suffered recent clinical setbacks, new vector developments offer hope for the treatment of cell-autonomous defects of hepatocyte metabolism.

Urea-cycle disorders (UCDs) are seen as models of classic inborn errors of hepatocyte metabolism. Hence, new developments in their diagnosis and treatment are relevant to a host of other disorders, including (but not limited to) organic acidemias, fatty-acid-oxidation disorders and amino acidopathies. UCDs are cell-autonomous disease processes and, therefore, a cure requires the correction of significant numbers of hepatocytes. These disorders also represent a unique genetic model for understanding how gene–environment interactions affect nitrogen homeostasis, which is affected by the intake of both energy molecules and protein. The recent discoveries of several cellular transporters show how the availability of urea cycle (UC) intermediates further impacts these processes.

Urea cycle: pathway to phenotype

The UC is the only metabolic pathway capable of disposing of excess nitrogen. It converts nitrogen, derived from dietary protein intake (i.e. enteral sources) and the breakdown of endogenous protein (i.e. peripheral sources), into urea, which is water-soluble and easily excreted from the body (Fig. 1).

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