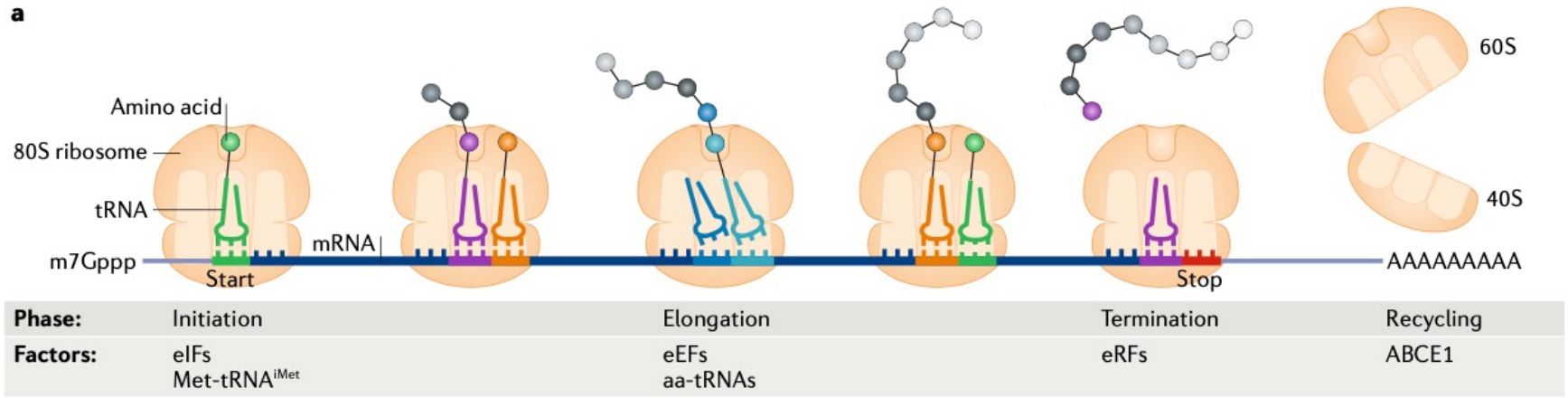
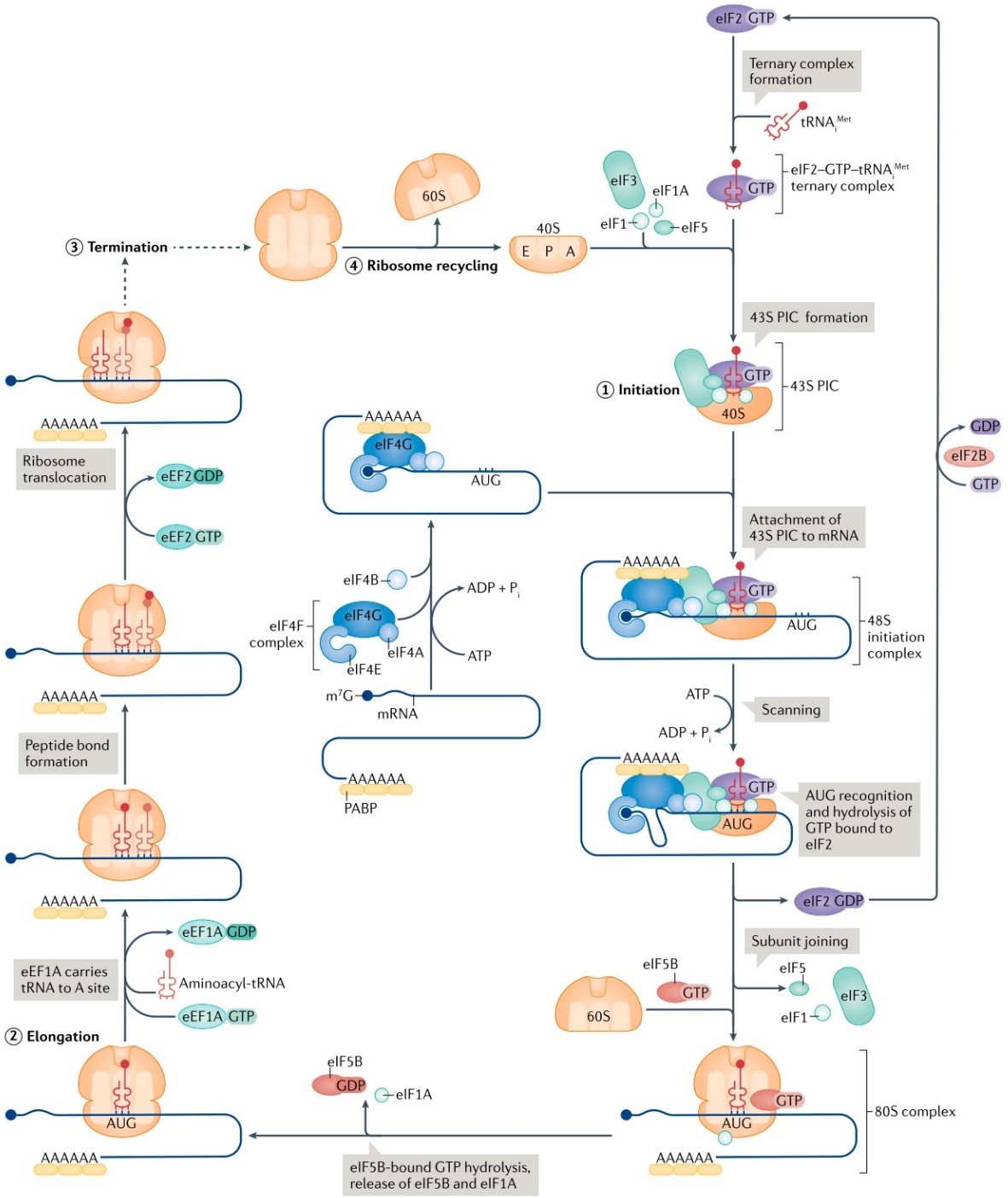
Translational control in eukaryotes

Overview of eukaryotic translation

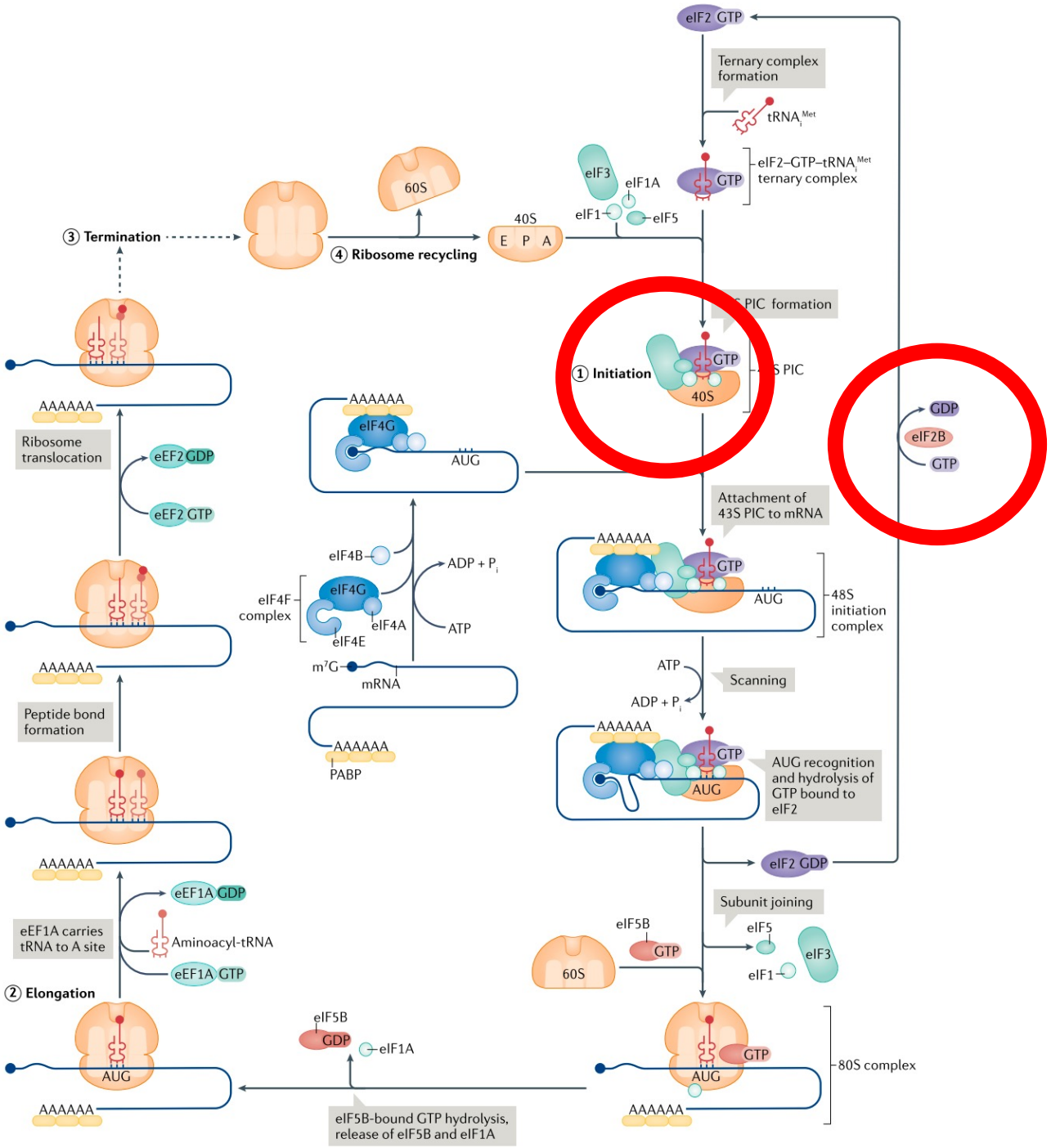
Translation begins with initiation, where the complex coordination of many eukaryotic translation initiation factors (eIFs), initiator methionyl-tRNA (Met-tRNA$_{iMet}$), the ribosomal subunits and the mRNA to be translated come together at the AUG start codon of the open reading frame. Next, elongation involves synthesis of the peptide chain through the coordinated actions of eukaryotic elongation factors (eEFs) and aminoacyl-tRNAs (aa-tRNAs) until the ribosome reaches a termination or stop codon. In this termination phase, the peptide is released through the actions of eukaryotic peptide chain release factors (eRFs). Finally, the ribosome subunits must be recycled by ATP-binding cassette subfamily E member 1 (ABCE1) for a subsequent round of translation.



Overview of eukaryotic translation



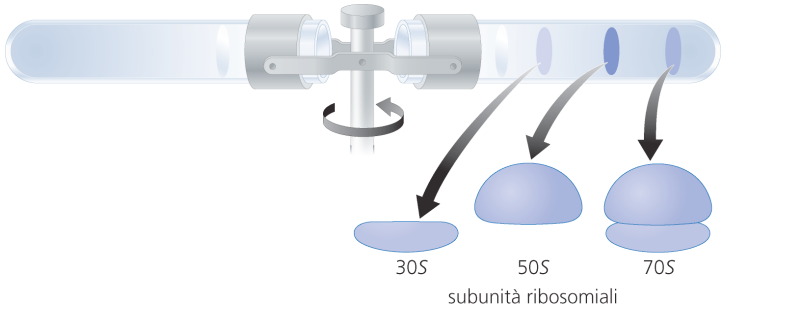
Overview of eukaryotic translation

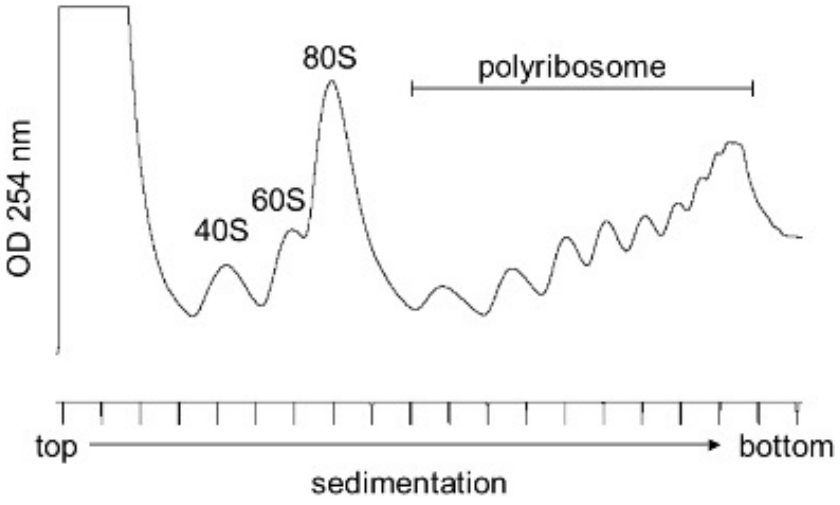


Methods Used to Quantify Translation

Polysome profiling

sucrose (10%-50%)

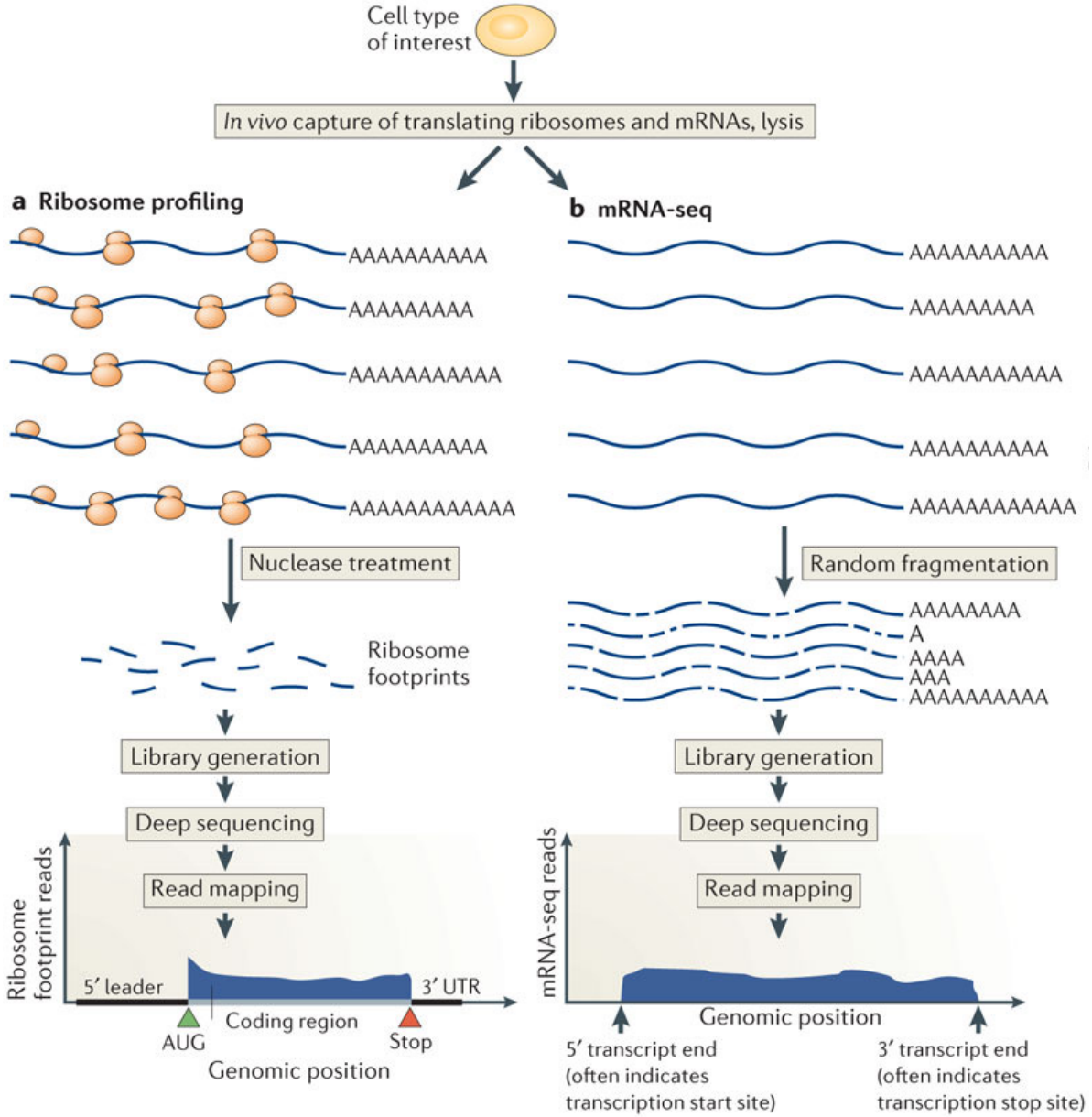




Northern blot , Real-time PCR, RNA-seq

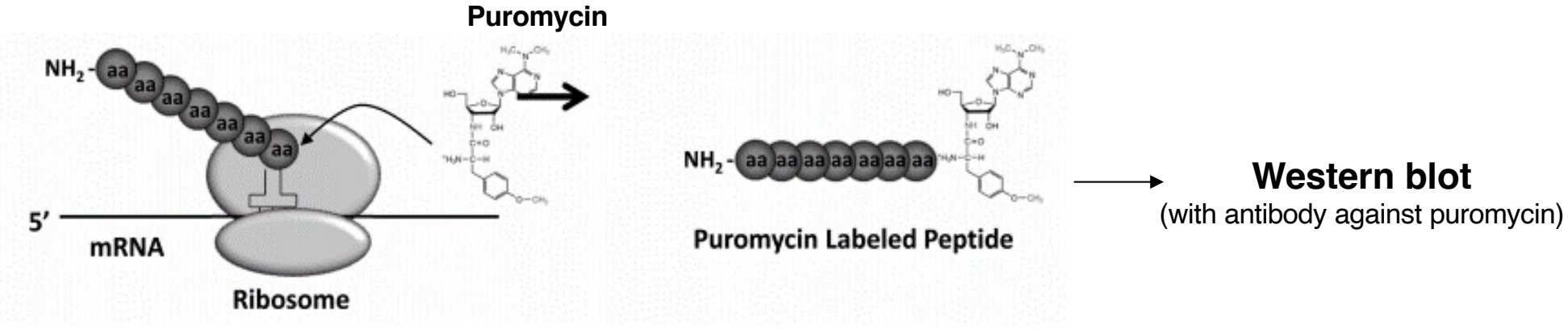
Methods Used to Quantify Translation

Ribosome footprinting (Ribo-seq)

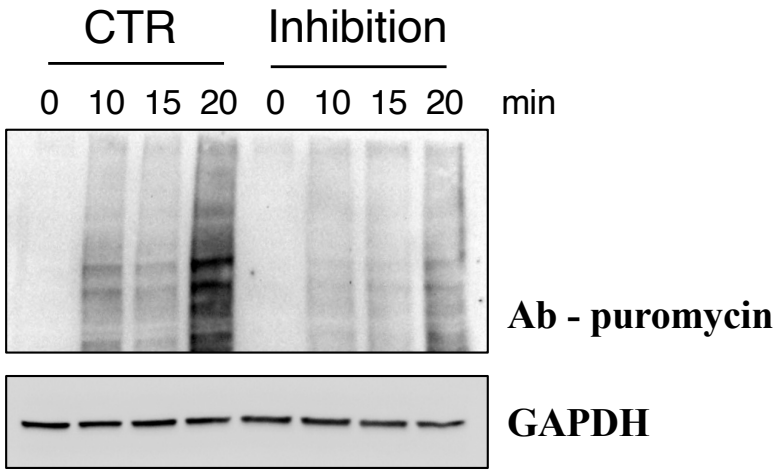


Methods Used to Quantify Translation

Surface sensing of translation (SUnSET)



If the level of ongoing translation is high, we expect to detect a large amount of newly generated proteins containing puromycin; if global translation is suppressed, we expect to observe a decrease in the amount of puromycin integrated into proteins.

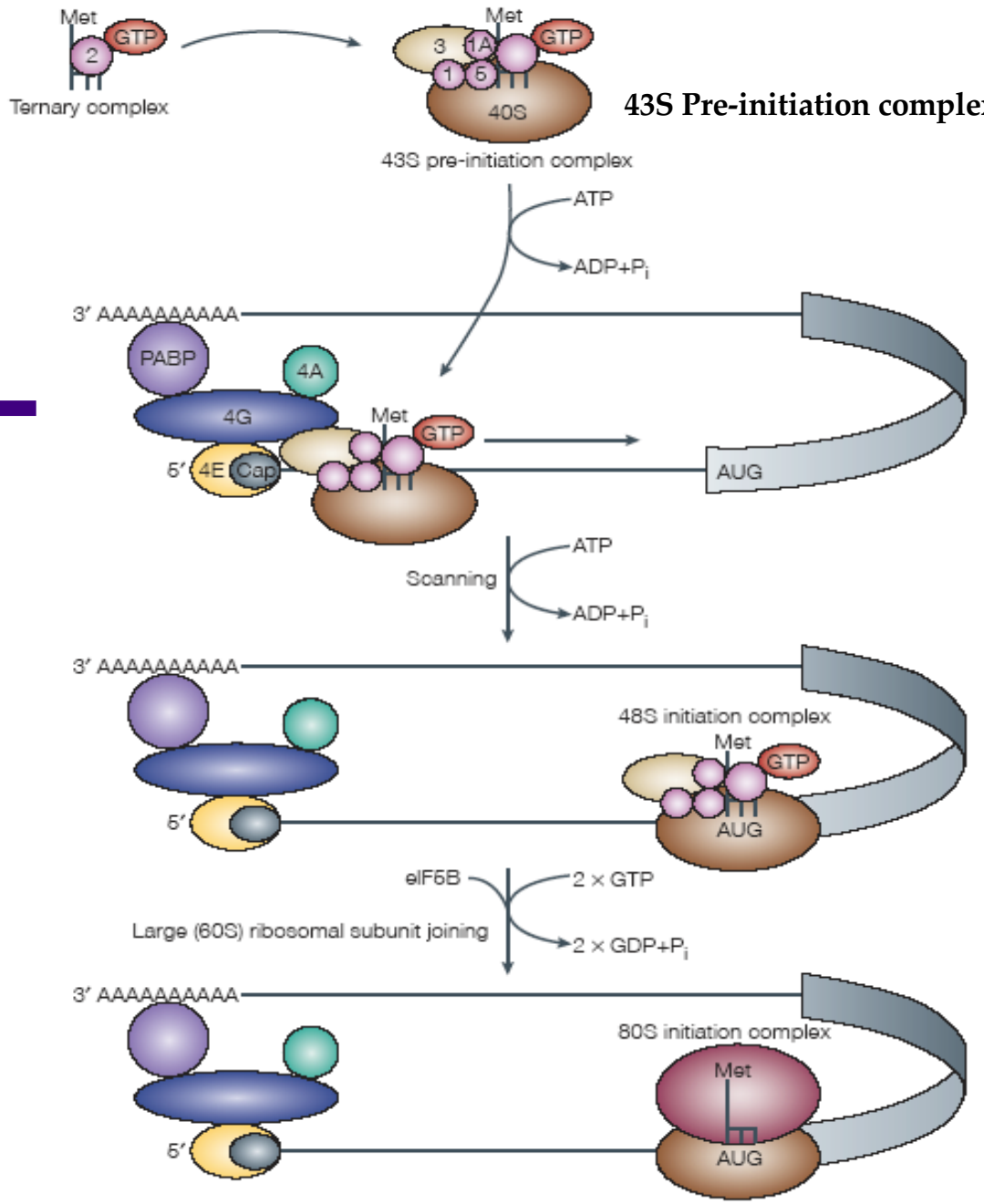


a) Global control of protein synthesis
(Initiation factors as target)

Initiation of translation in eukaryotes

a) Global control:
Initiation factor as target

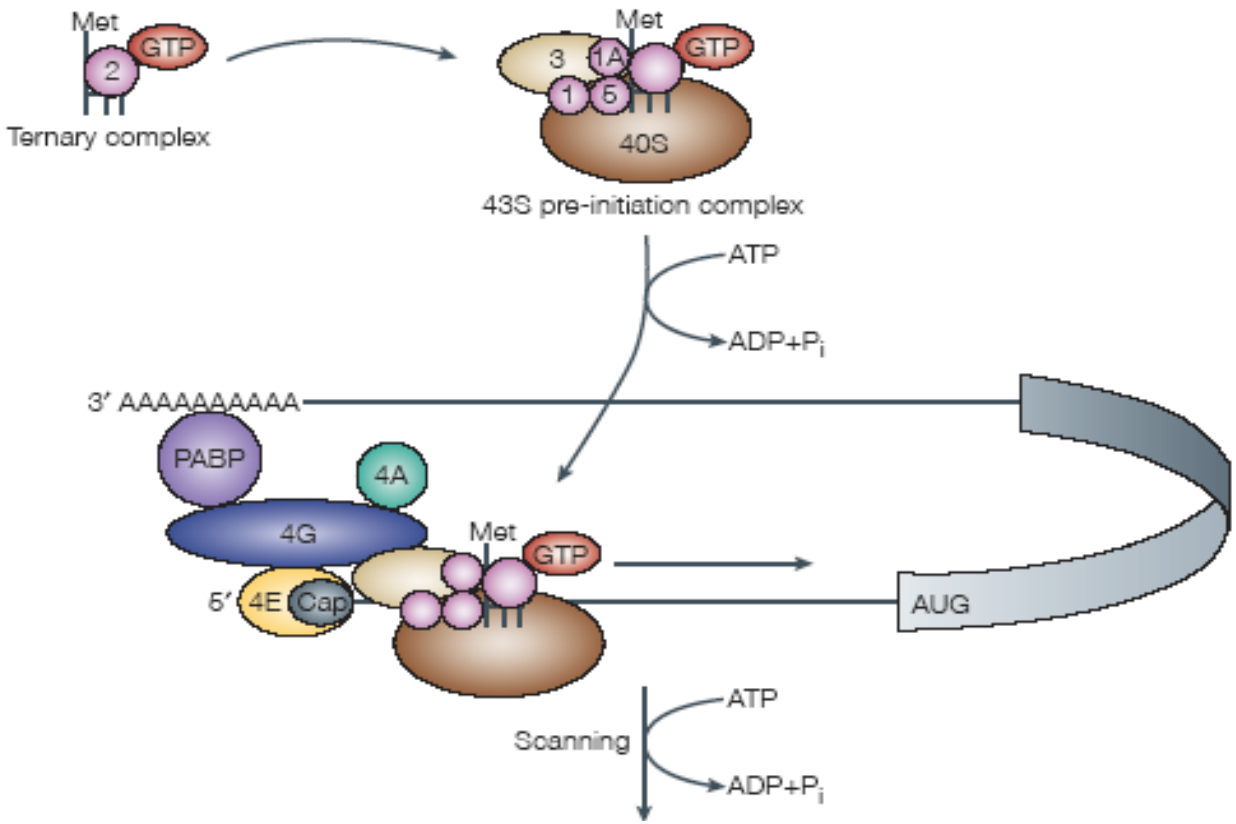
Formation of the Initiation complex (rate-limiting step)



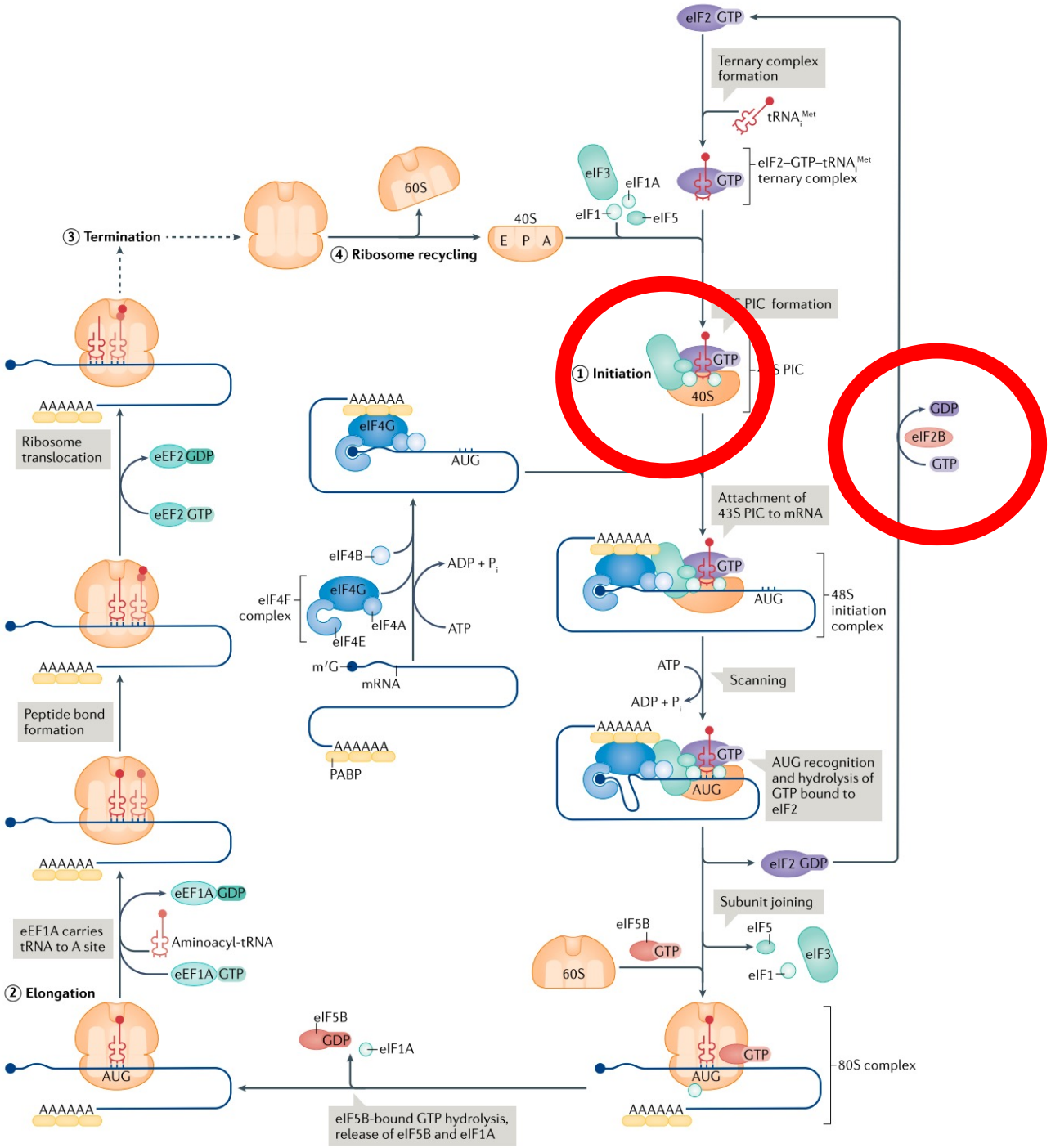
Global control of protein synthesis

1. Phosphorylation of factors involved in translation initiation

2. Degradation of translation factors

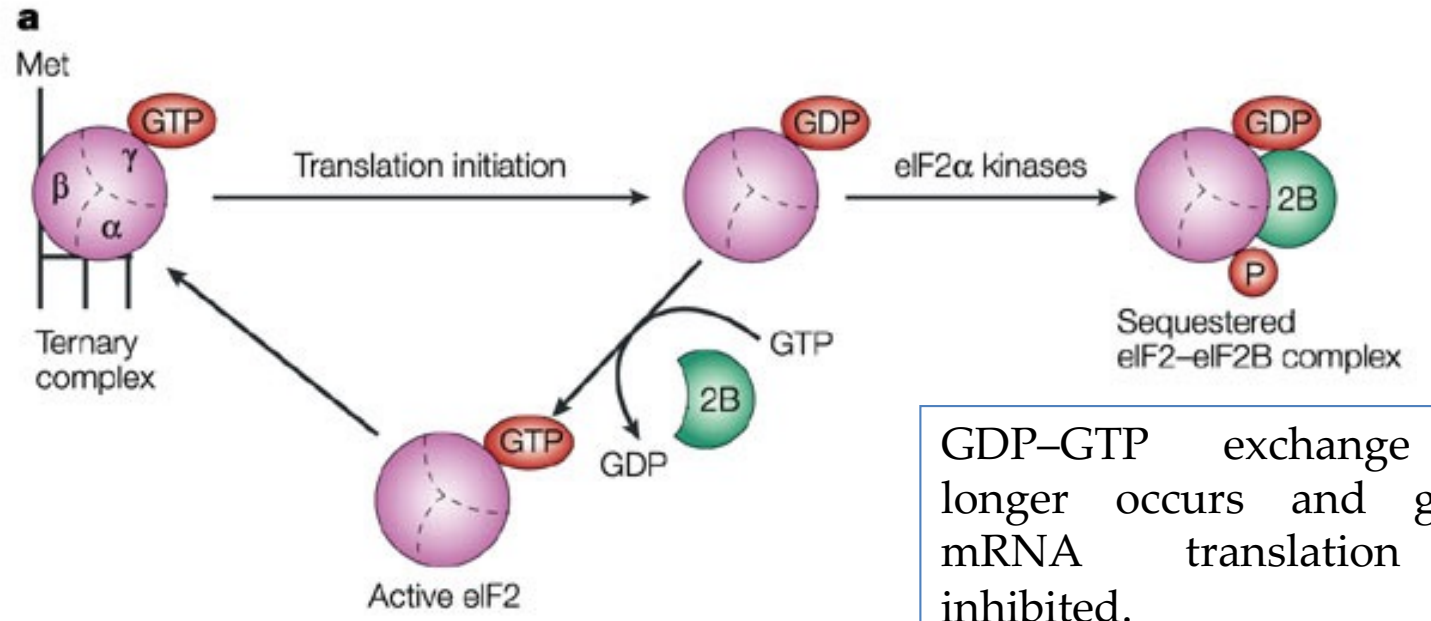


Overview of eukaryotic translation



1-Regulation of TC formation

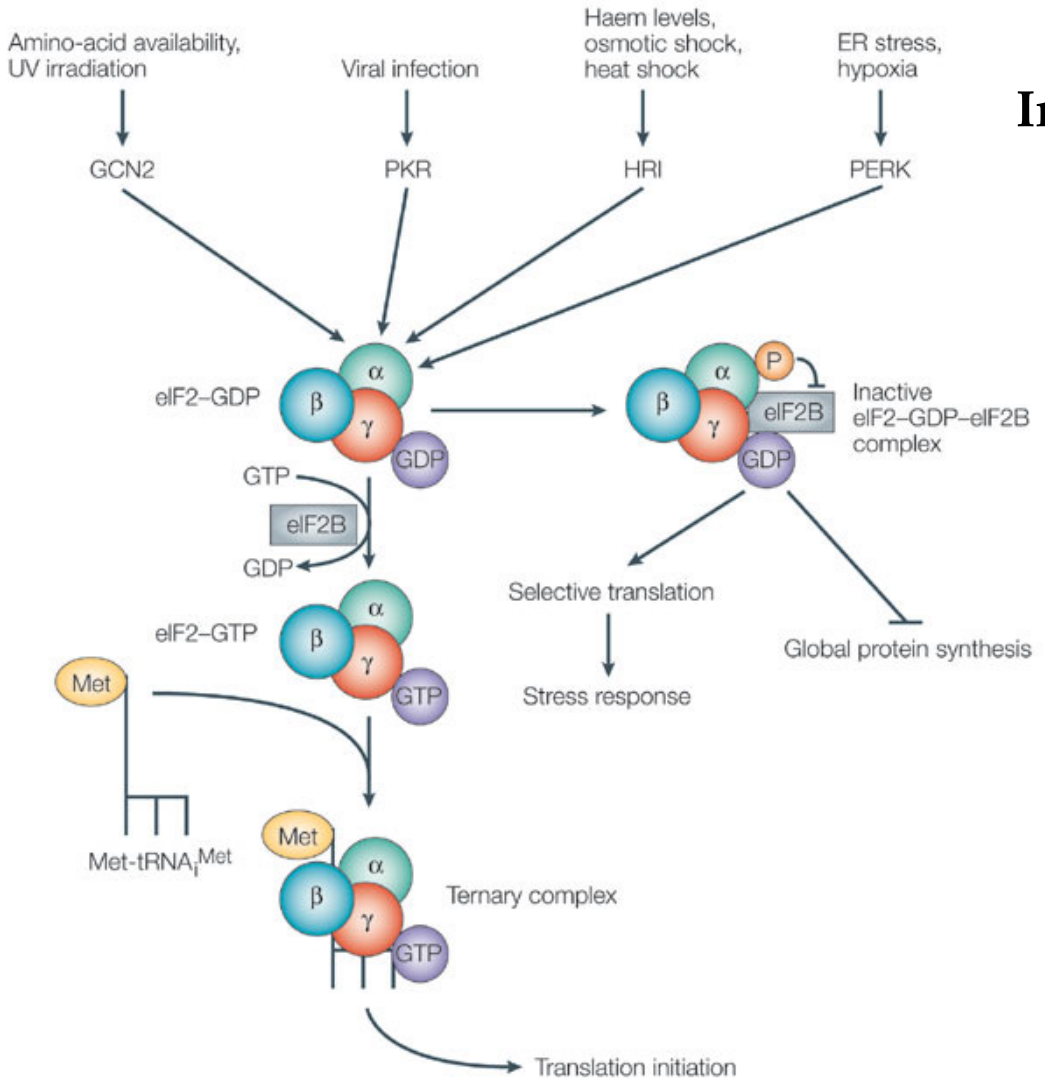
•phosphorylation of the **α subunit of eIF2** blocks the GTP-exchange reaction by reducing the dissociation rate of eIF2 from eIF2B, sequestering eIF2B in an inactive complex



GDP–GTP exchange no longer occurs and global mRNA translation is inhibited.

Phosphorylation of eukaryotic initiation factor-2a

•**A number of kinases that are activated under different conditions can phosphorylate eIF2a** (this modification can also result in the translational activation of specific mRNAs)



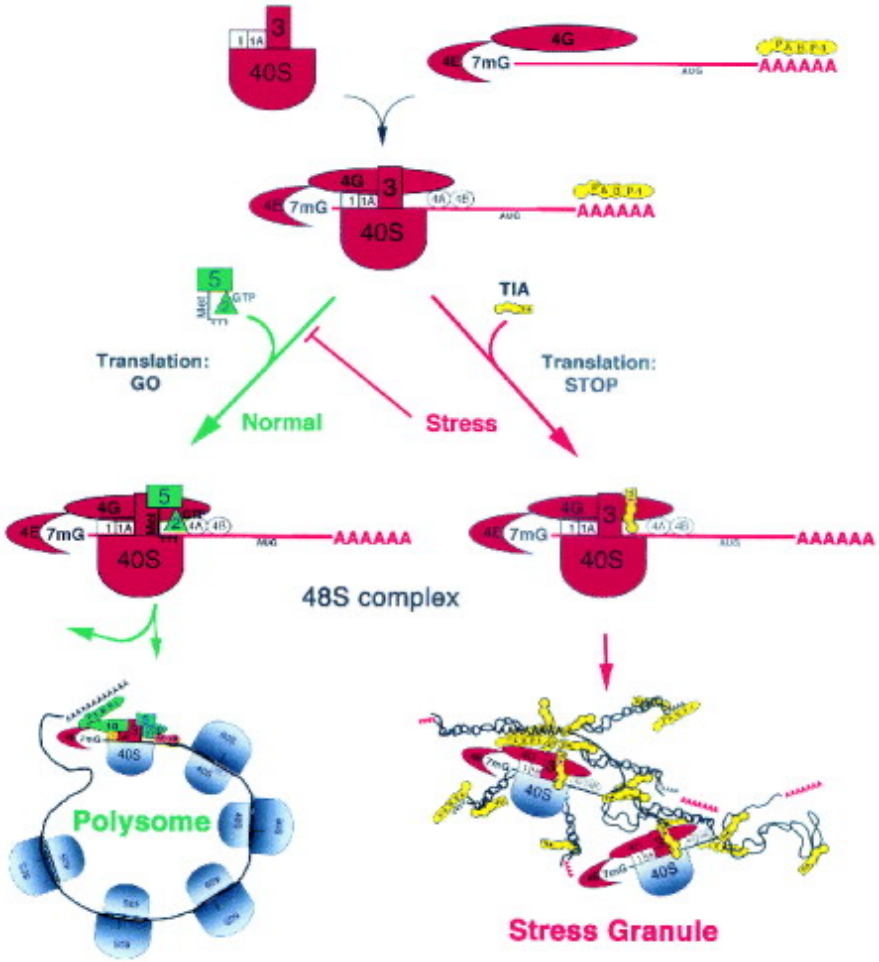
Integrated stress responce

Stress Granules (SGs)

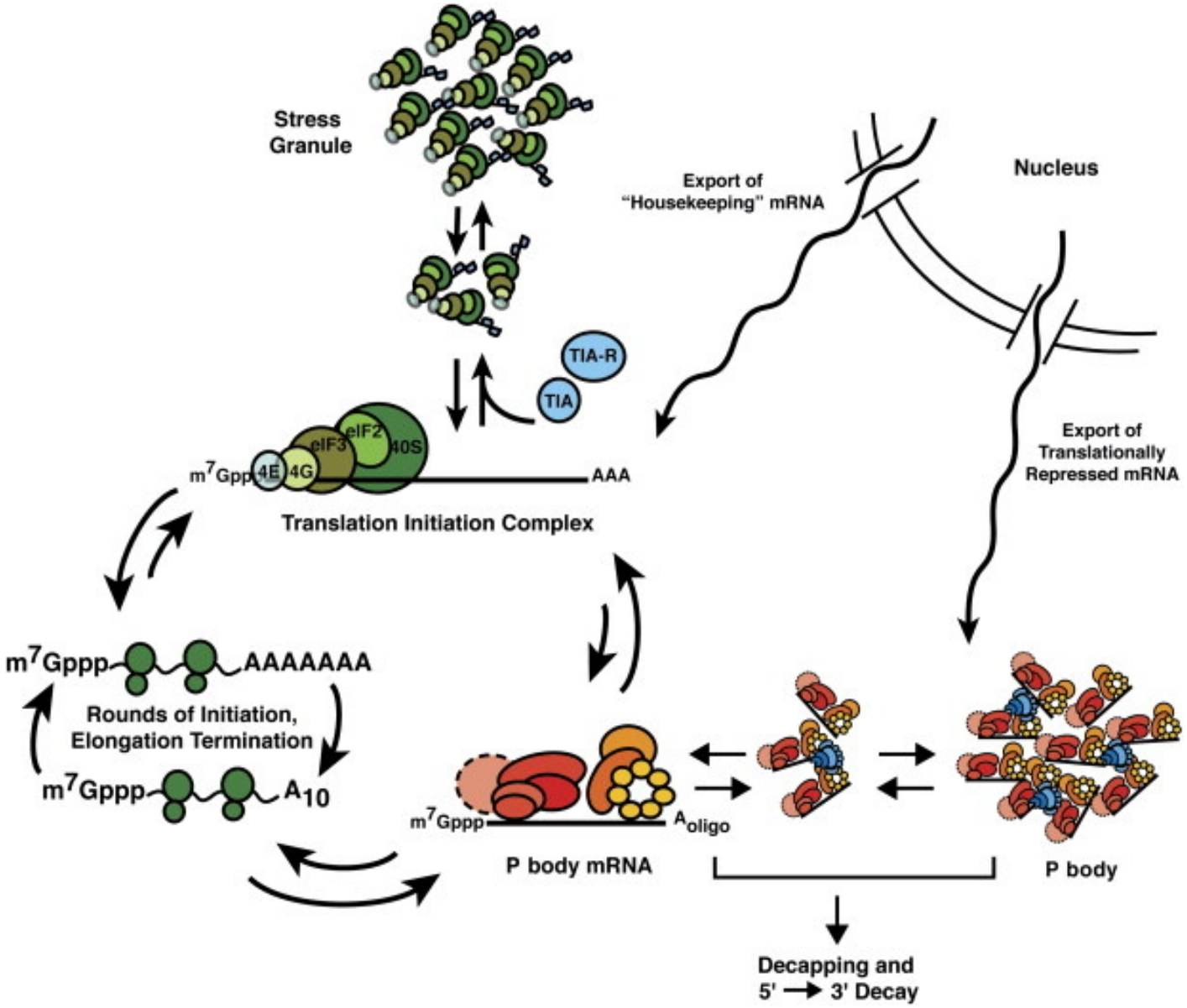
The phosphorylation of **eIF2** is critical for the assembly of SGs

•**SGs** appear in the cytoplasm of mammalian cells exposed to environmental stress (e.g., heat, oxidative conditions, UV irradiation, and hypoxia). mRNAs encoding stress-induced proteins such as HSP70 are excluded from SGs

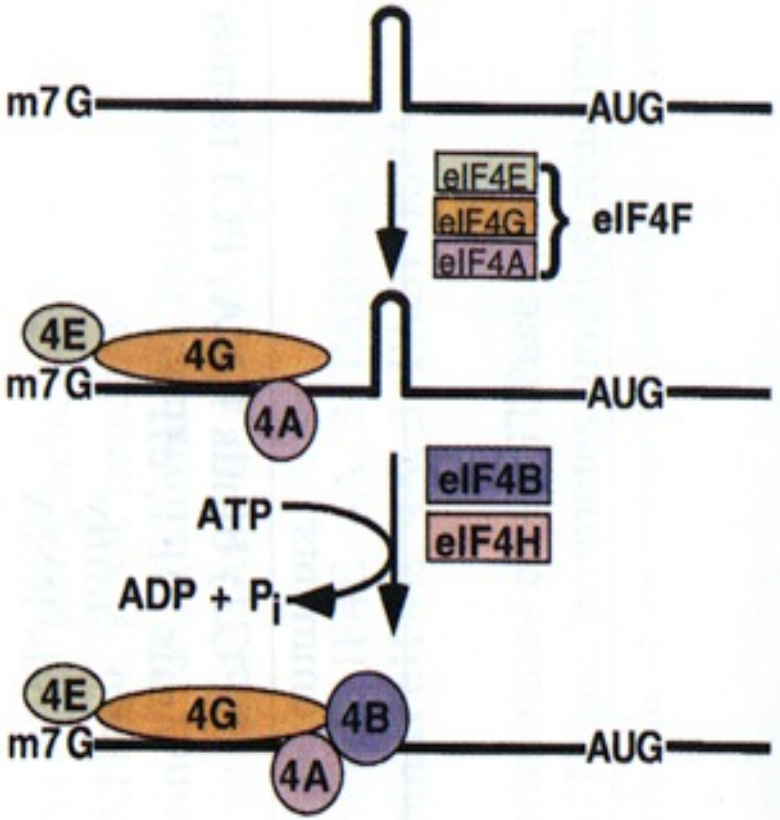
•Stalled **48S preinitiation complexes** are the core constituents of SGs, which include small but not large ribosomal subunits as well as the early translation initiation factors eIF2, eIF3, eIF4E, and eIF4G



The mRNA cycle

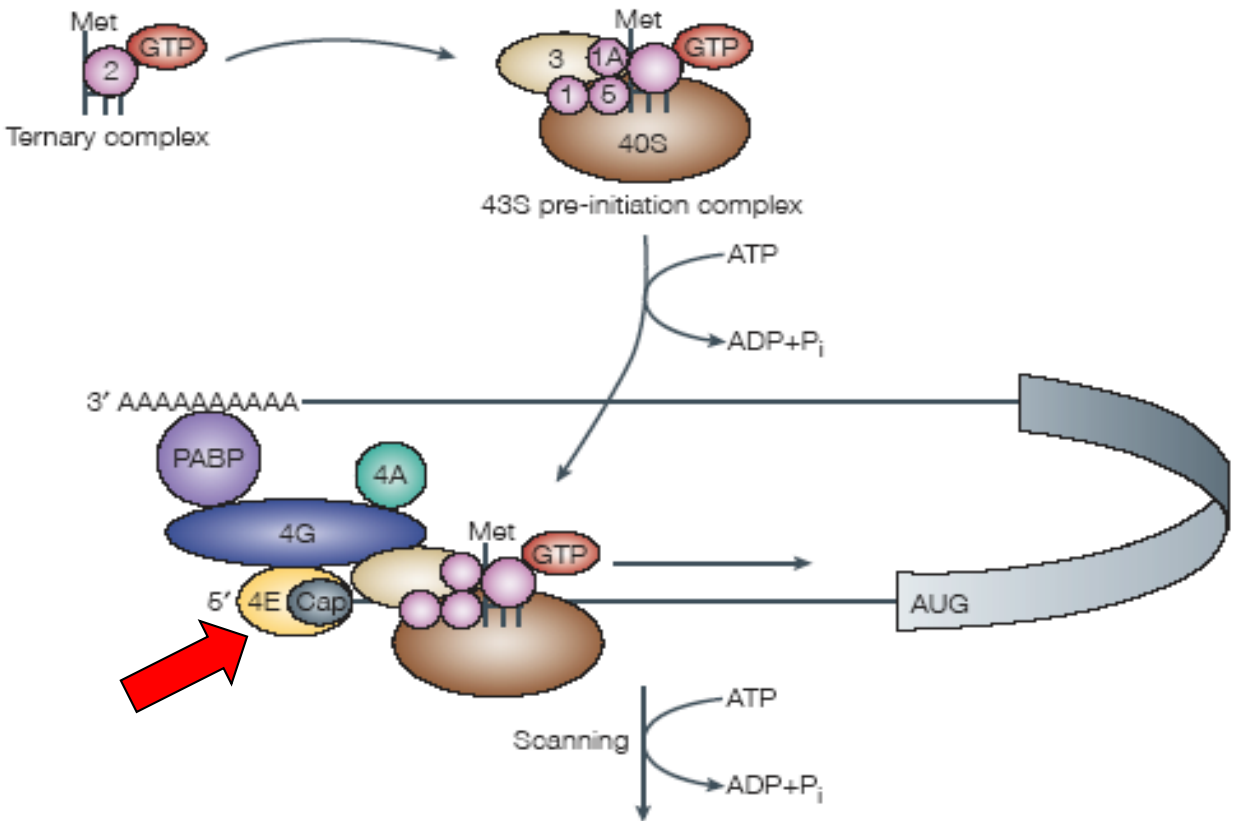


The Binding of eIF4F to the 5′ Cap of mRNA



2-Regulation of eIF4E

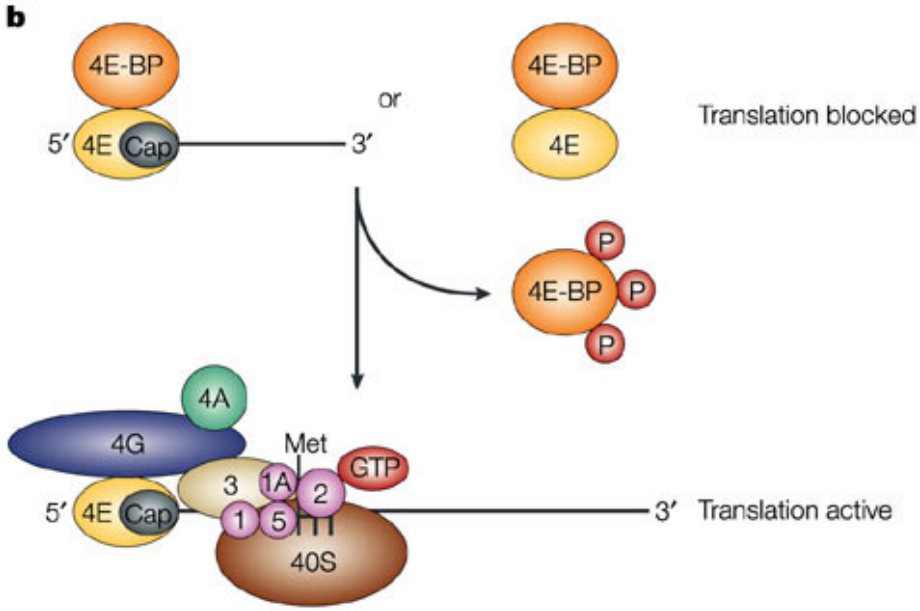
•**eIF4E** interacts with the scaffold protein **eIF4G** and is required for cap-mediated recruitment of the 43S ribosomal complex to the mRNA during translation initiation



4E Binding Proteins (4E-BPs)

•Association between **eIF4E** and **eIF4G** requires a small domain in eIF4G that is shared by a family of proteins known as **4E-BPs**

•**Hypophosphorylated 4E-BPs** bind to **eIF4E** and competitively displace **eIF4G**, which results in the inhibition of the association of the **43S** complex with the mRNA and, consequently, in translational repression. **4E-BP phosphorylation** liberates eIF4E from inhibition.



Regulation of eIF4E

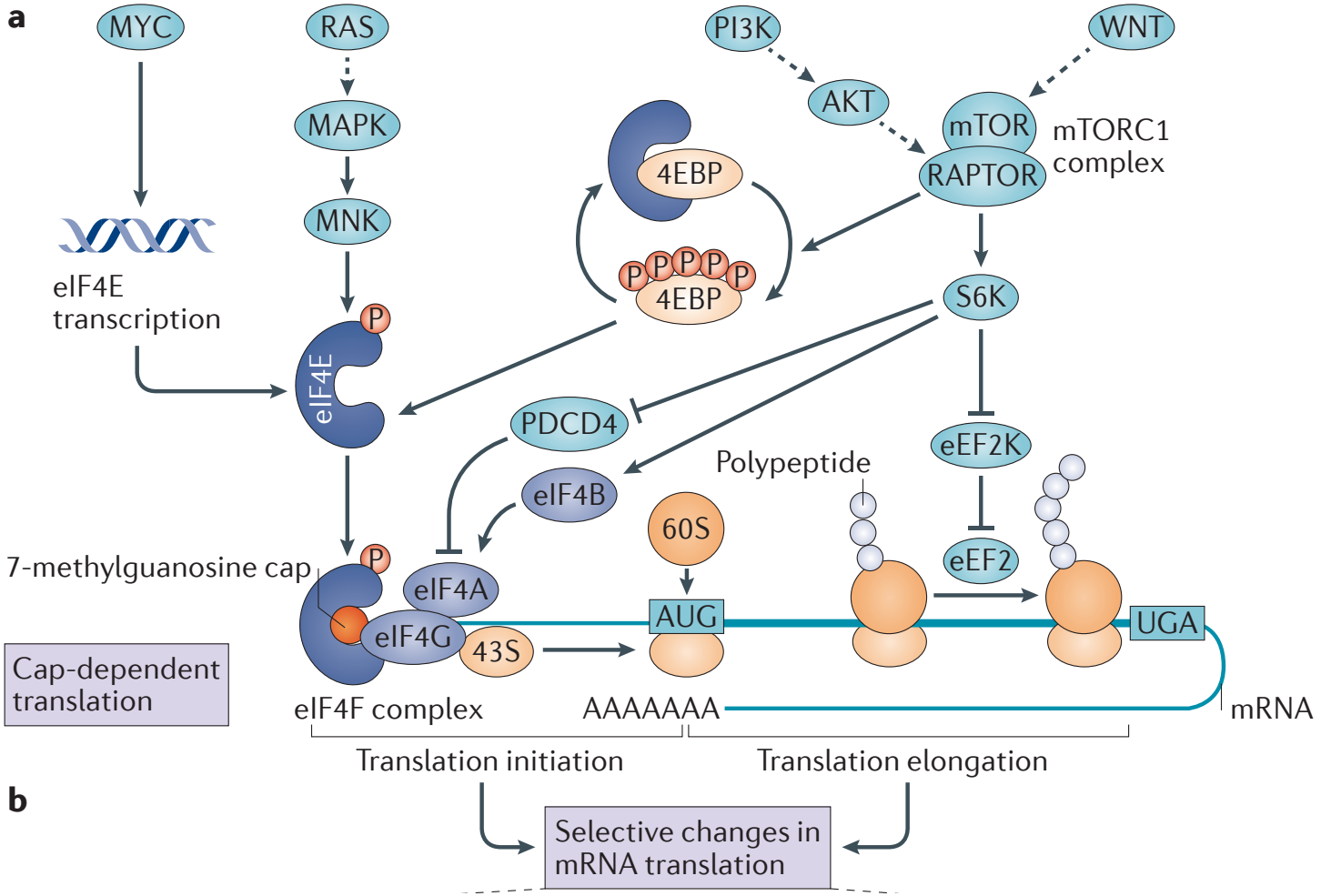
Historically, eIF4E was identified as the quantitatively limiting factor in the eIF4F complex. As such, even modest increasesor decreases in eIF4E expression were expected to profoundly influence mRNA translation and cellular function (overexpression of eIF4E is sufficient to drive transformation in cell lines and spontaneous tumorigenesis in mice).

The recent generation of an eIF4E-haplo insufficient mouse has created a paradigm shift in our understanding of the role of eIF4E levels in development and tumorigenesis. **Unexpectedly**, eIF4E-haploinsufficient mice were found to be physiologically normal, yet strikingly resistant to tumour formation.

eIF4E is present at levels that exceed those required for normal translational control and development and instead become limiting specifically for expression of the 'oncogenic translation programme'.

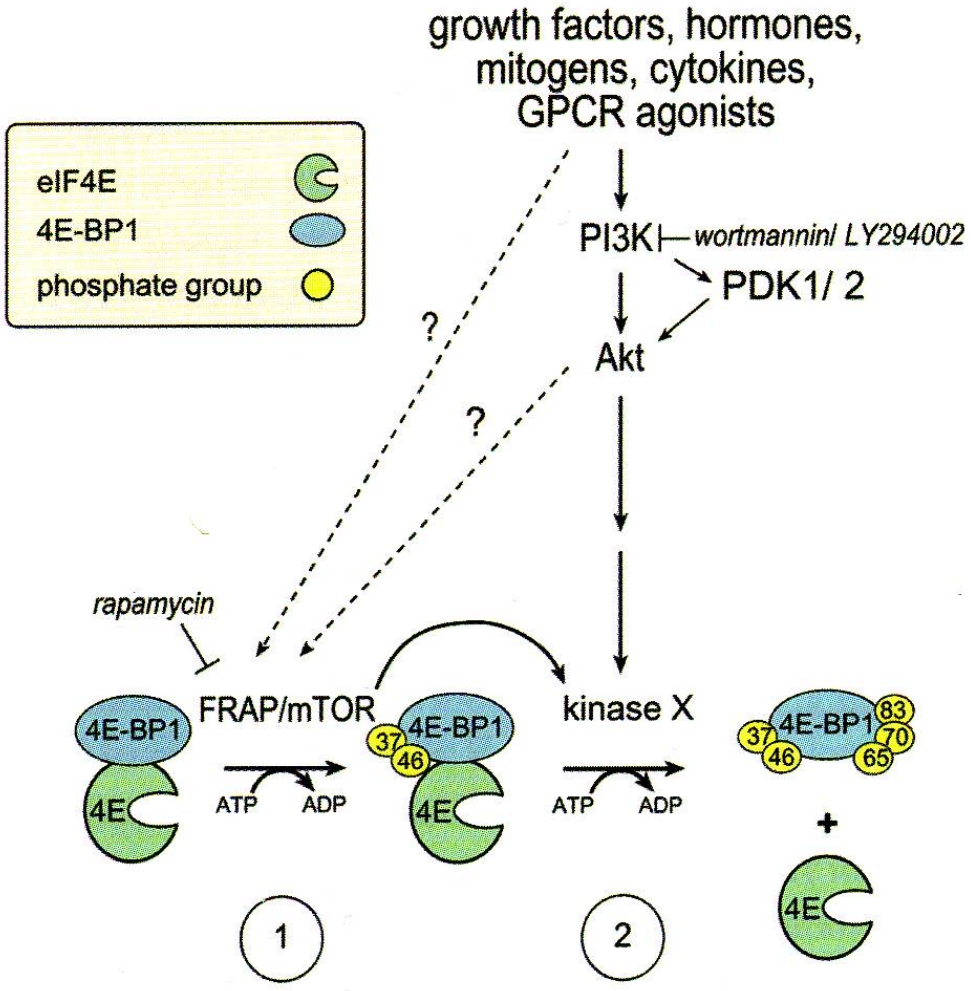
Regulation of eIF4E

Oncogenic signalling can hyperactivate eIF4E through enhanced transcription, through phosphorylation of eIF4E at serine 209 by the MAPK-interacting serine/threonine kinases (MNKs) and through mTOR complex 1 (mTORC1)-dependent phosphorylation and inactivation of the eIF4E inhibitors eIF4E-binding proteins (4EBPs).



Phosphorylation of 4EBP by mTOR and Upstream Pathway

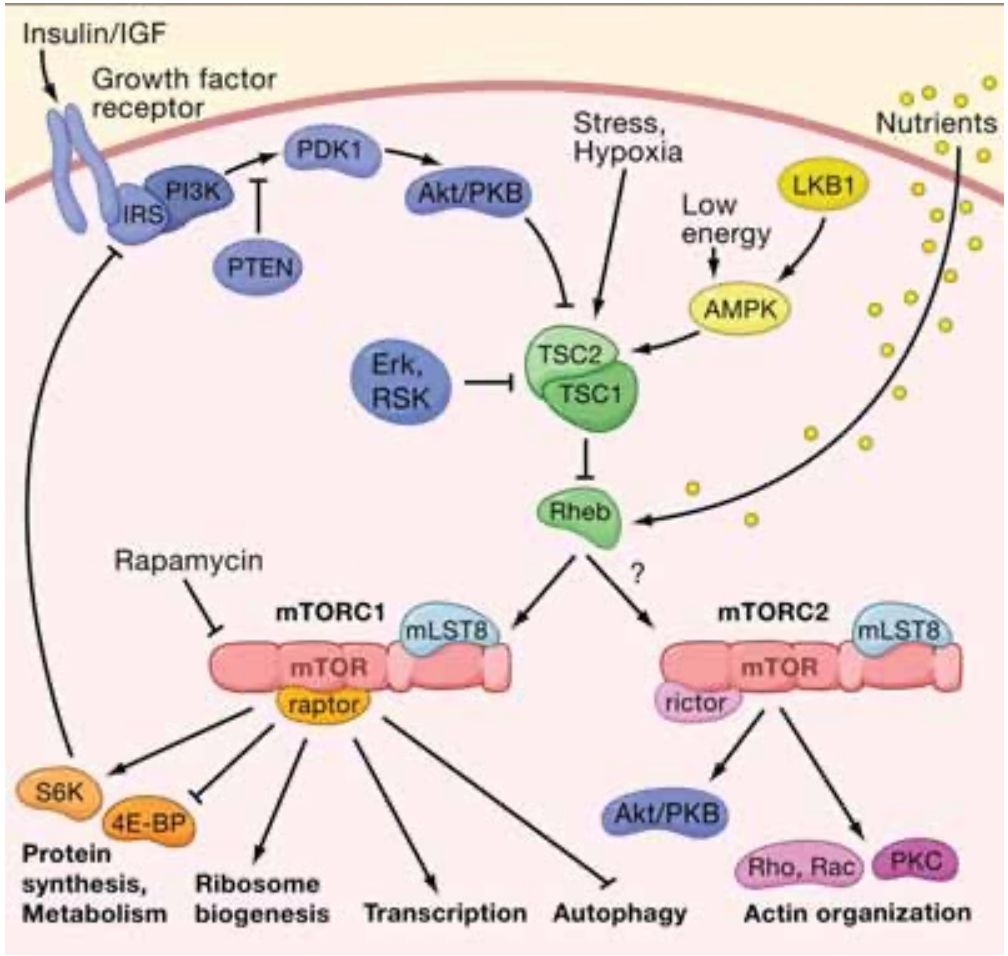
Extracellular signals (such as insulin) activate a signalling cascade that triggers 4E-BP phosphorylation.



eIF4E is overexpressed in numerous cancers and is implicated in mechanisms underlying oncogenesis and senescence. 4E-BPs inhibit eIF4E activity, and thereby act as suppressors of eIF4E-dependent pathways.

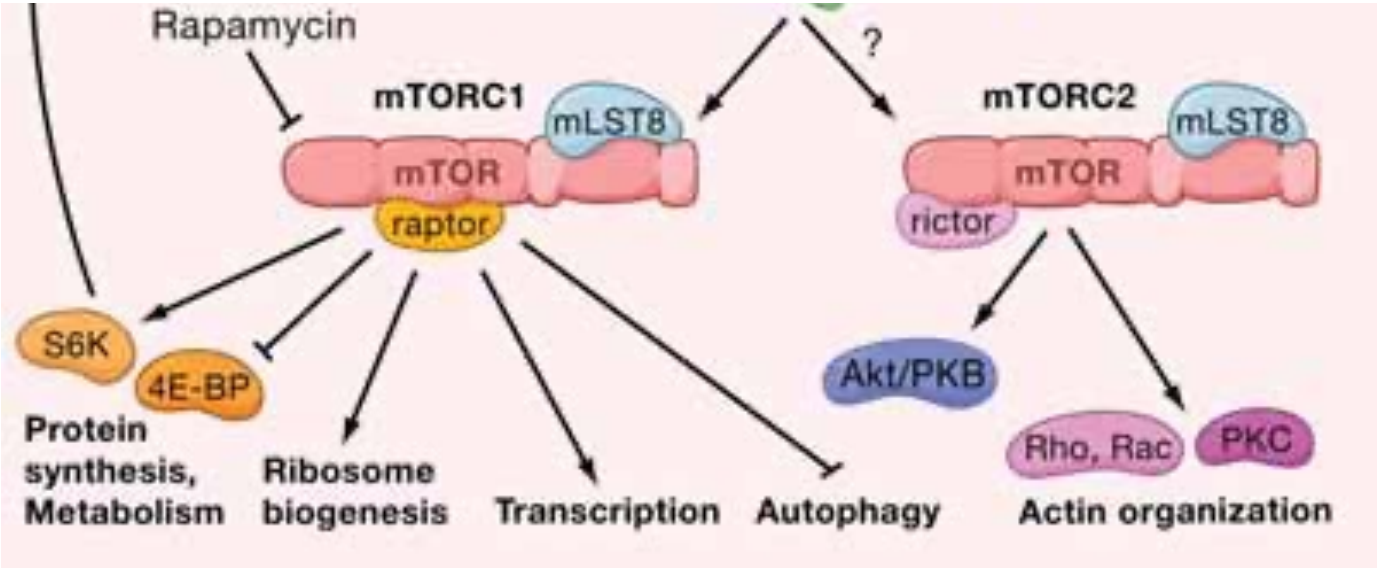
mTOR

The mammalian target of rapamycin (mTOR) is an serine/threonine kinase that is present in two distinct complexes. **mTORC1** integrates multiple signals reflecting the availability of growth factors, nutrients, or energy to promote either cellular growth when conditions are favourable or catabolic processes when conditions are unfavorable. Growth factors and hormones signal to mTORC1 via **Akt,** which inactivates **TSC2** to prevent inhibition of mTORC1. Alternatively, low ATP levels lead to the AMPK-dependent activation of TSC2 to reduce mTORC1 signaling. Amino acid availability is signaled to mTORC1 via a pathway involving the **Rag** proteins.



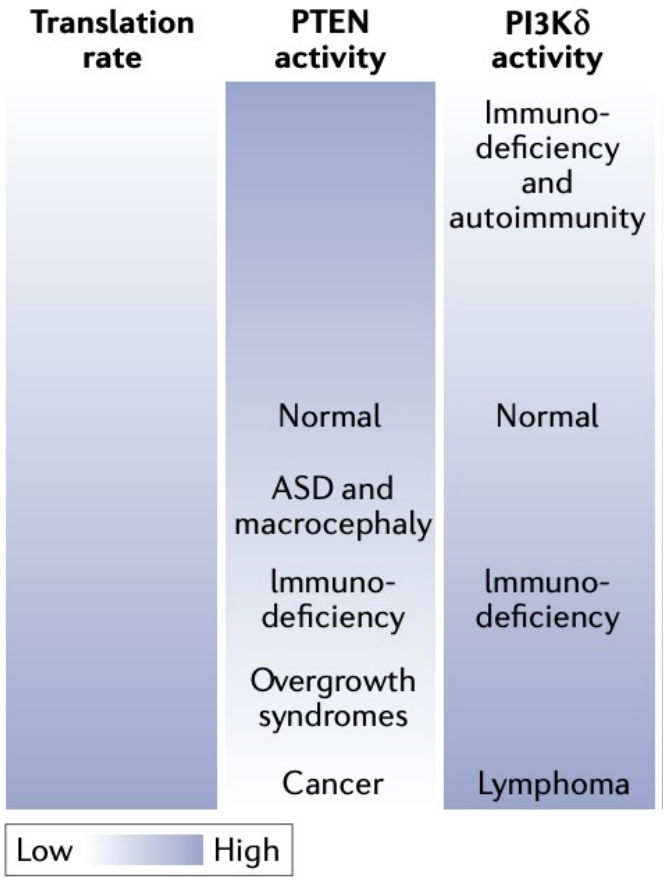
mTOR

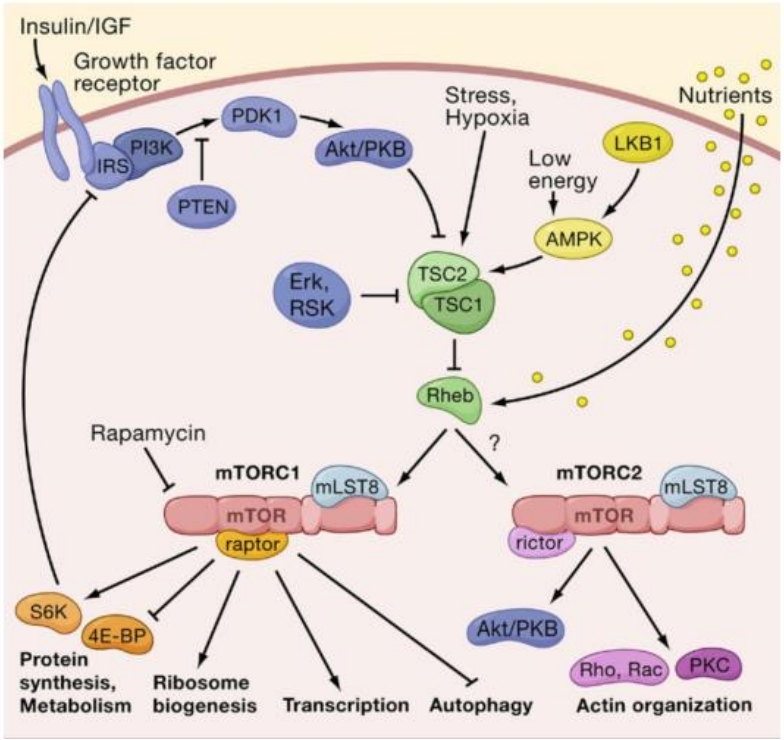
Active **mTORC1** has a number of downstream biological effects including translation of mRNA via the phosphorylation of downstream targets (4E-BP1 and p70 S6 Kinase), suppression of autophagy, ribosome biogenesis, and activation of transcription leading to mitochondrial metabolism or adipogenesis. **mTORC2** regulates cytoskeletal dynamics by activating PKCα and regulates ion transport and growth via SGK1 phosphorylation. Aberrant mTOR signaling is involved in many disease states including cancer, cardiovascular disease, and metabolic disorders.



Diseases caused by deregulation of protein synthesis

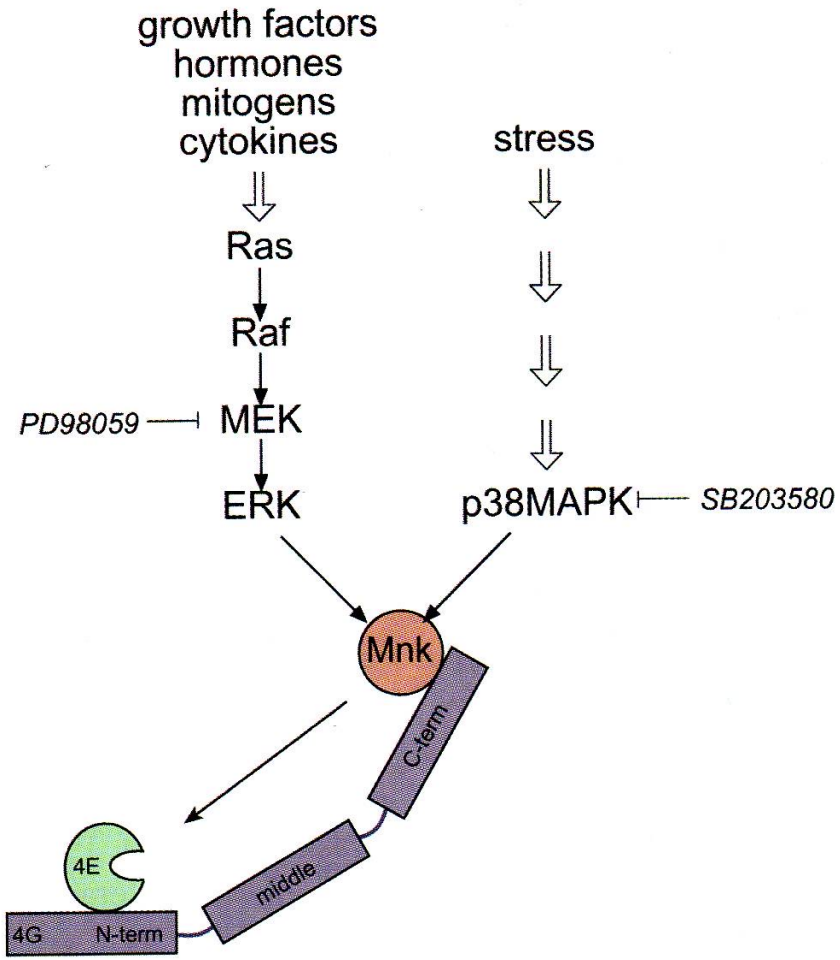
The dose-dependent effect of PTEN inactivation is well established in cancer and may also explain the tissue-specific phenotypes observed with various PTEN mutations. PTEN partial inactivation often correlates with autism spectrum disorder (ASD) and macrocephaly, whereas complete inactivation correlates with overgrowth syndromes or cancer. Both loss-of-function and gain-of-function mutations affecting PI3Kδ activity can cause an immunodeficiency phenotype, suggesting that precise regulation of PI3Kδ activity is required for immune cell functions.





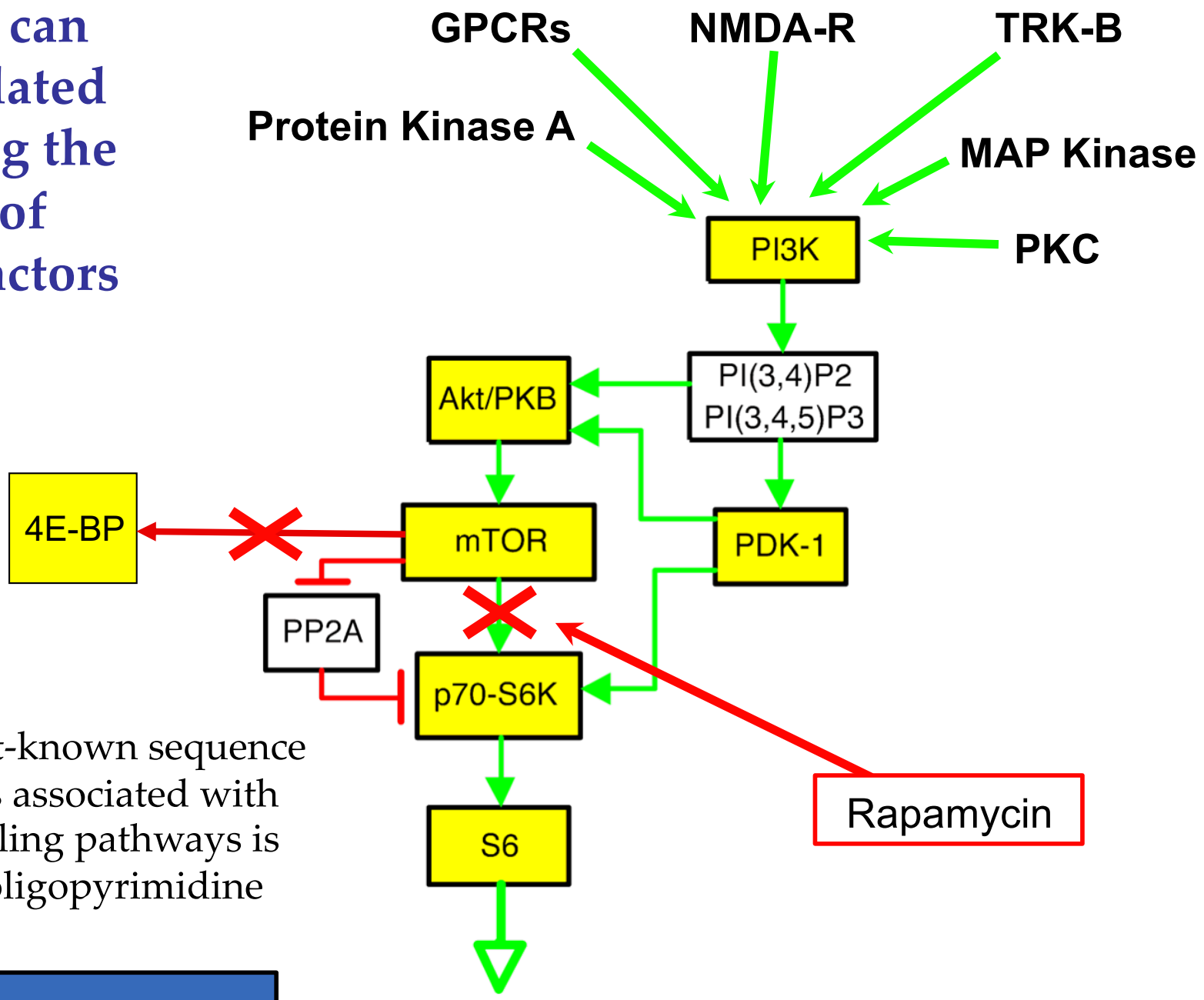
Phosphorylation of eIF4E and eIF4G

•Phosphorylation of **eIF4E** and **eiF4G** allows them to detach from the cap and recycle



MAPK-Dependent Phosphorylation of eIF4E is Mediated by the eIF4G Associated Kinase Mnk

Translation can also be regulated by controlling the synthesis of translation factors



One of the oldest-known sequence specific elements associated with oncogenic signalling pathways is the 5′ -terminal oligopyrimidine tract (5′TOP)

Proteins Involved in Translation

5′ terminal oligo-pyrimidine (5′TOP)

- **5′TOP** is an RNA sequence motif that typically starts with a cytosine residue followed by at least four uninterrupted pyrimidines (C or T) adjacent to the 5′cap moiety. The canonical 5′TOP motif is present in a relatively small subgroup of mRNAs (~ 100), but is particularly frequent in genes encoding ribosomal proteins s (e.g., it is present in 73 out of 80 human ribosomal genes) and translation-related proteins, such as eIF3A and eIF4B.

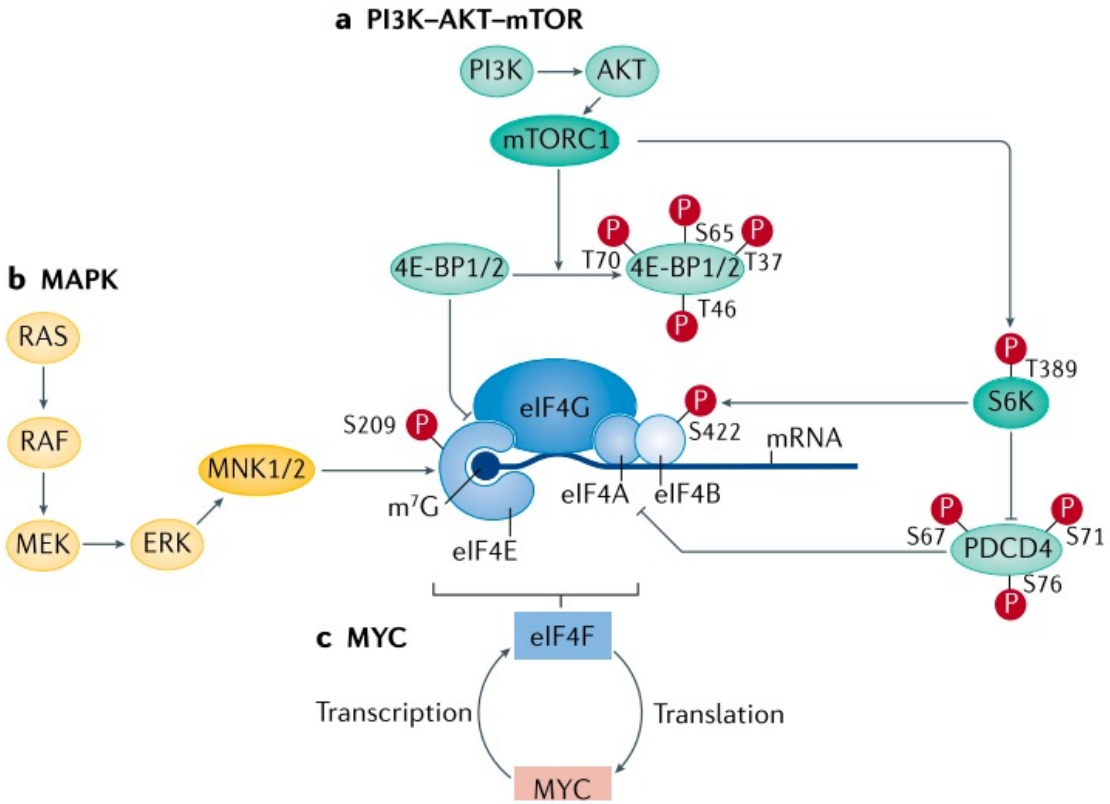
- While growth and proliferation stimulate the translation of 5′TOP-possessing mRNAs (TOP mRNAs), stress or metabolic shortages lead to its rapid inhibition.

- This condition-dependent translation is conferred by extra-sensitivity to the state of mTORC1, and inhibition of mTORC1 by different means leads to rapid translational arrest of multiple TOP mRNAs

- Translation of 5′TOP mRNAs depends on eIF4E and employs eIF4G to facilitate its interaction with the 5′cap. This type of translation initiation renders these mRNAs sensitive to the overall availability of the eIF4F complex, particularly to the depletion of eIF4G1 and the activity of 4E-BPs

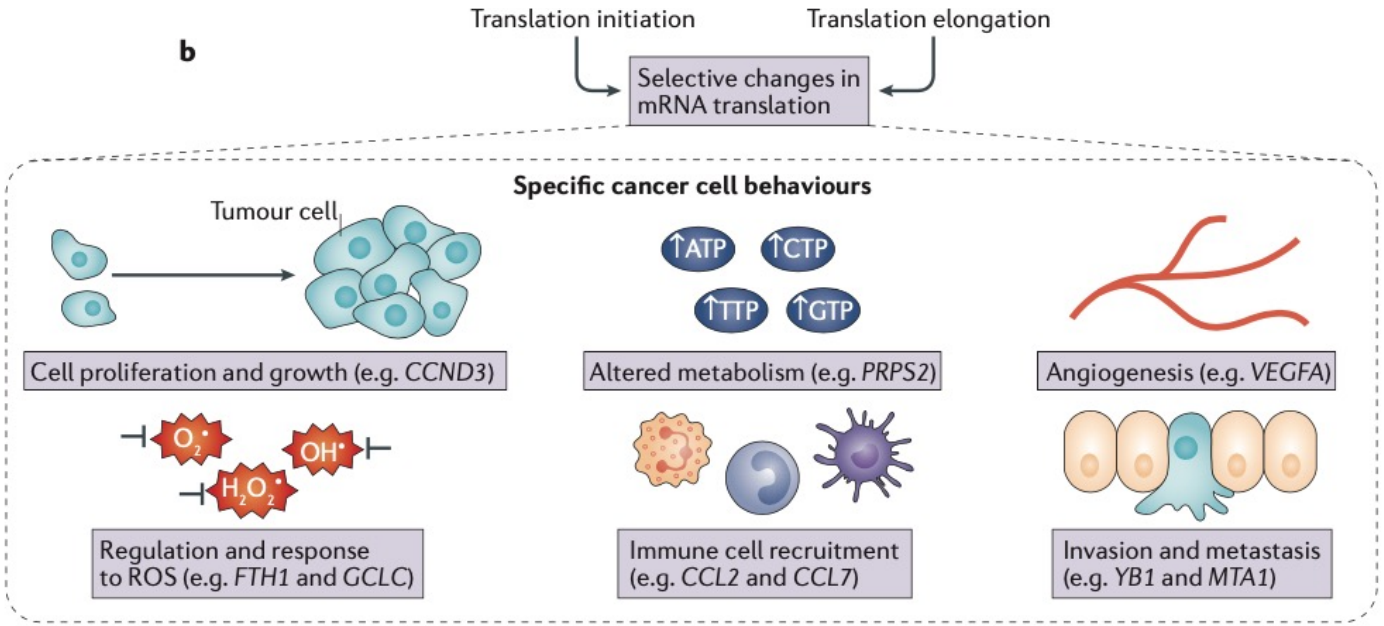
Oncogenic activation of mRNA translation

The PI3K–AKT–mTOR, MAPK and MYC oncogenic signalling pathways converge on the eIF4F complex to promote translation initiation. By binding to the m^7G, eIF4F recruits the 43S pre-initiation complex, thereby initiating cap- dependent translation. In cancer, the hyperactivation of oncogenic signalling reflects the hyperactivation of the eIF4F complex.



Oncogenic activation of mRNA translation

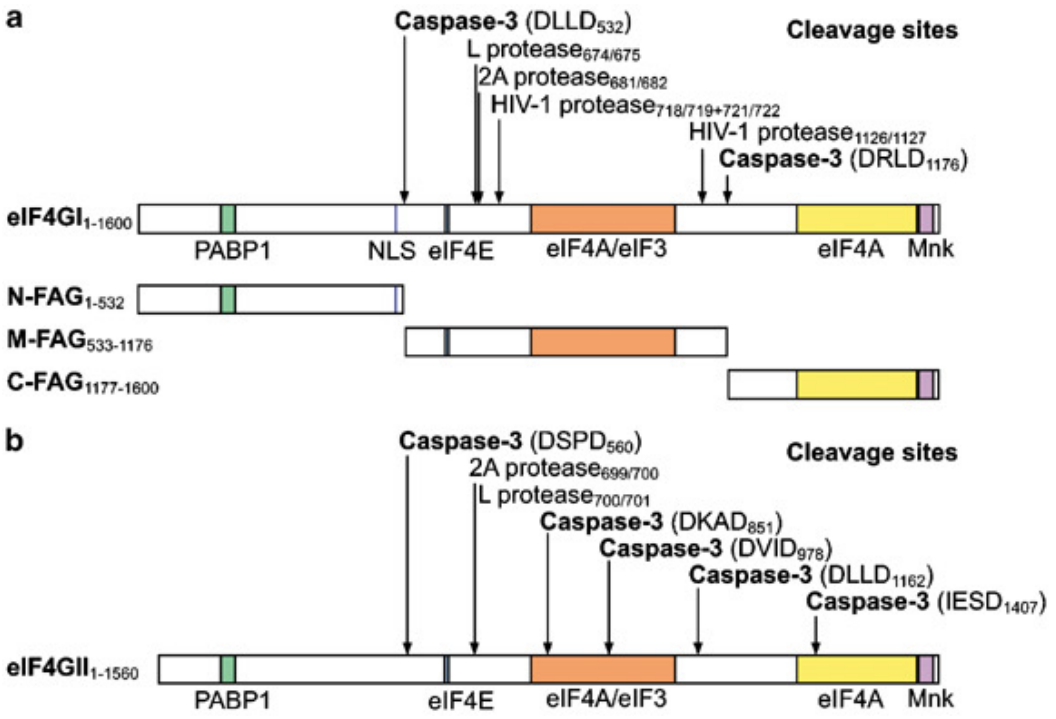
Oncogenic activation of translation initiation and elongation supports tumorigenesis in part by driving selective changes in the translation of specific mRNA transcripts independently of alterations in transcript levels or global increases in protein synthesis. This selective translational control of specific mRNA transcripts underlies the acquisition and execution of distinct cancer cell behaviours central to the transformed phenotype, such as increased cell growth and proliferation, altered metabolism, enhanced angiogenesis, proper reactive oxygen species (ROS) control, immune cell recruitment, and invasion and metastasis.



Interfering with translation by Proteolytic cleavage of translation factors

•The apoptotic protein **caspase-3** cleaves **eIF4G** and **PABP** during apoptosis

•Cleavage of these factors by viral proteases serves as a common and successful mechanism to interfere with the translation of cellular mRNAs. As a consequence, some viral RNAs that do not require intact eIF4G and PABP are preferentially translated (as well as some cellular mRNAs that share such independence from intact eIF4G and PABP)



Specialised translation initiation

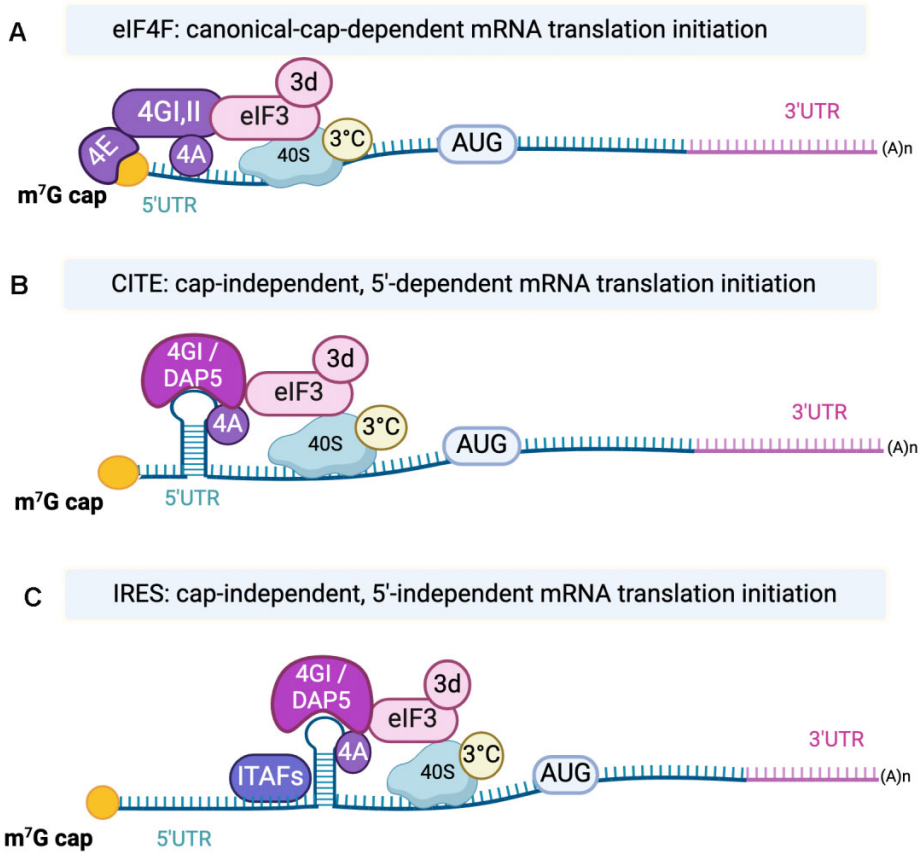
Translation of numerous mRNAs remains robust during cellular stress, early development, and cell cycle progression despite inactivation of eIF4E.

This relies on:

- **specific mRNA structures (IRES-internal ribosome entry sites)**
- **specific translation factors**
- **mRNA modifications (m^6A)**

Specific mRNA structures

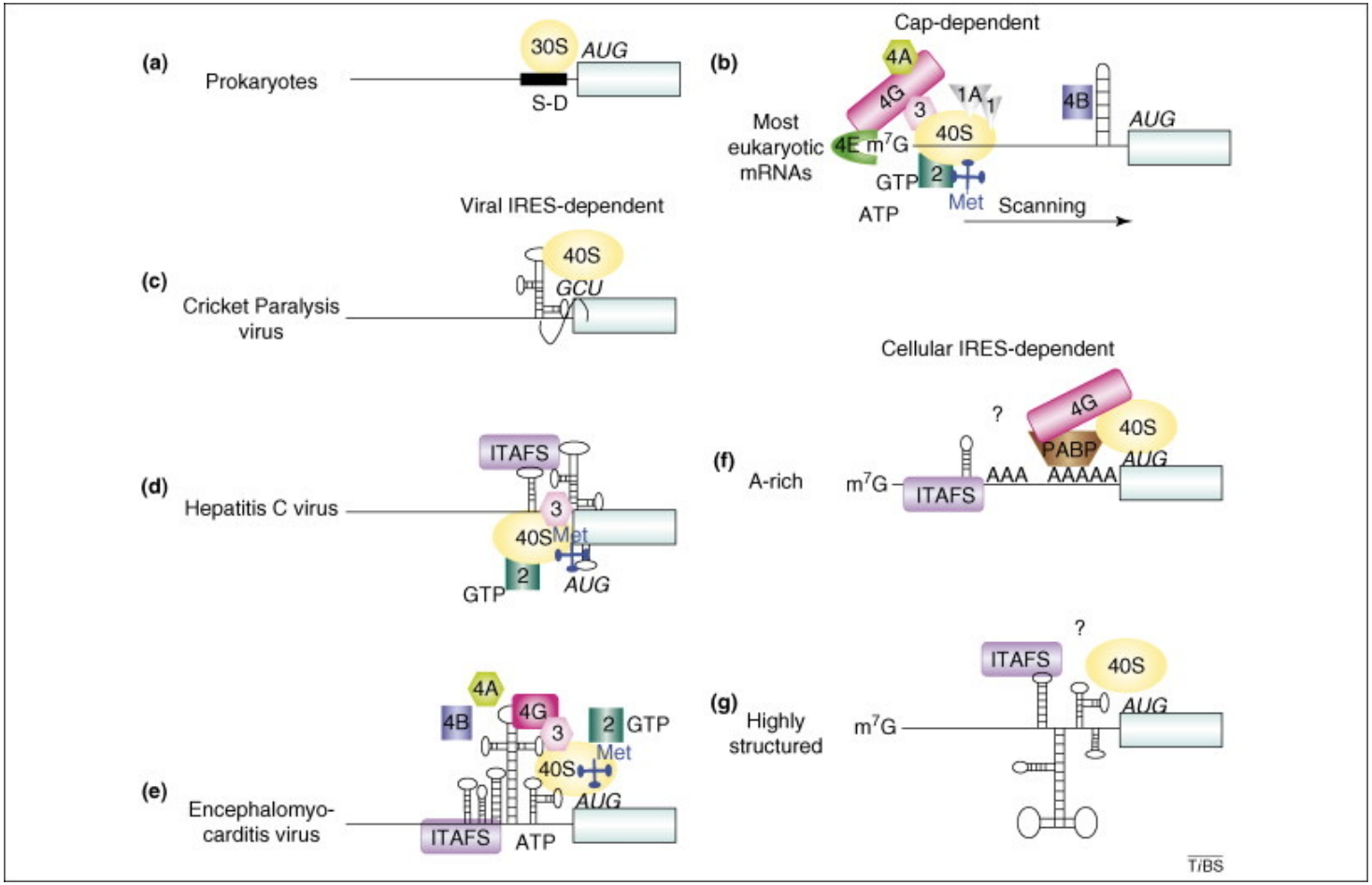
(A) Canonical cap/eIF4E-dependent translation initiation **(B)** CITE (Cap-Independent Translation Enhancer) cap/eIF4E-independent but 5′-UTR dependent translation involves structural elements or modifications in the 5′-UTR that are thought to bind directly to certain translation initiation factors that contain RNA binding motifs such as eIF4GI, II, or III, and / or eIF3, without the need for eIF4E and cap interaction, butin close proximity to the cap. **(C)** Cellular IRES-mediated mRNA translation initiation is thought to involve stable secondary hairpin structures, generally in the 5′-UTR, that can directly recruit the 40S small ribosomal subunit in the absence of cap and eIF4E interaction. IRES-mediated initiation typically requires interaction with IRES trans-acting factors (ITAFs), and either eIF4GI, eIF4GII or eIF4GIII (DAP5/eIF4G2), eIF3 and often eIF4A.



Internal Ribosome-Entry Sites (IRESs)

IRESs can functionally replace both the cap and also many (in some cases, all) of the proteins needed to recruit the ribosome to the start codon in a process that is RNA dependent.

IREs were discovered in viral RNAs but they <u>were also found in cellular mRNAs</u>



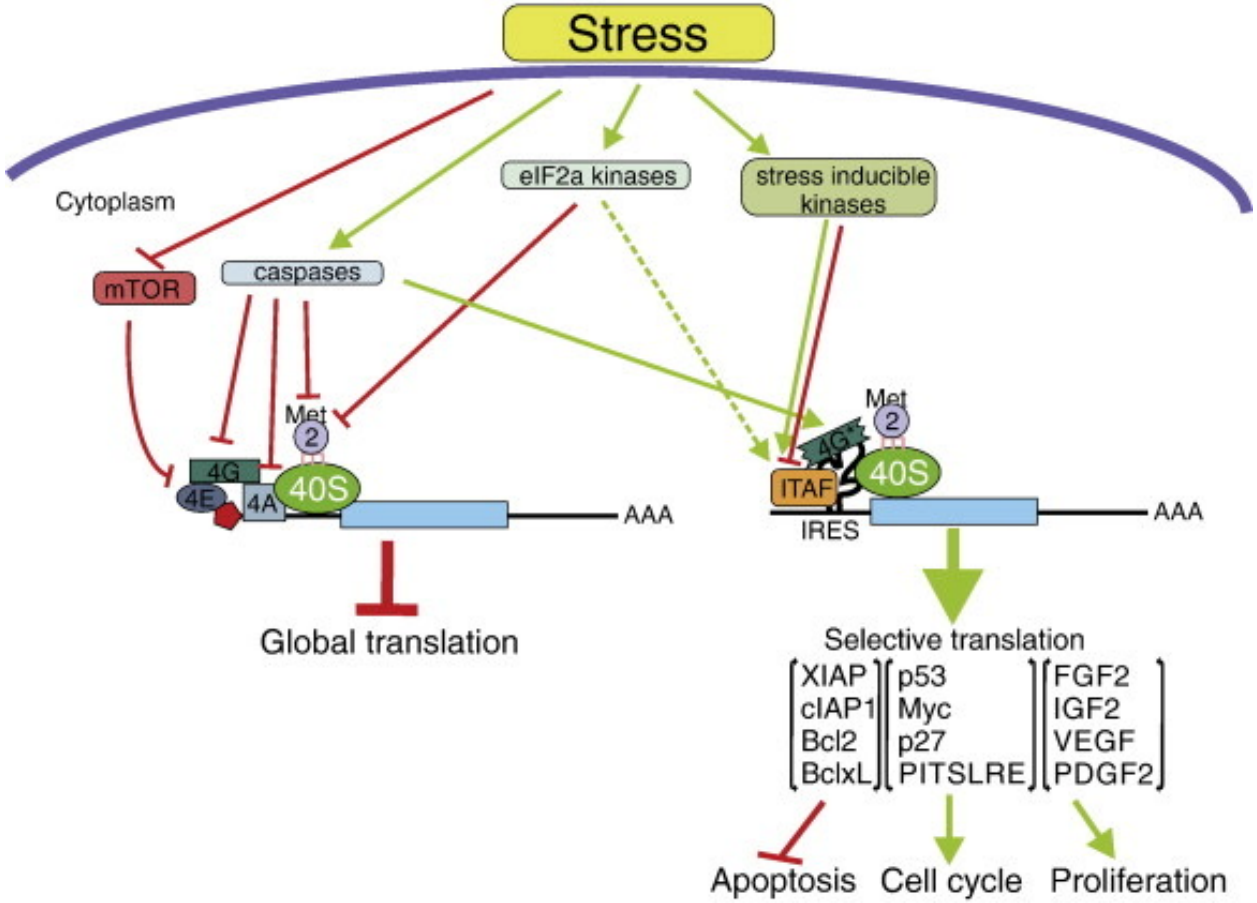
Cellular circumstances where cap-dependent translation is compromised but IRES-mediated translation maintains production of the proteins

It is estimated that about 3% of mRNAs in the cell could be translated by an IRES-dependent mechanism. Many proteins encoded by mRNAs with an IRES play important roles in cell survival (cIAP1, XIAP, Bcl-2, Bcl-xL, Apaf-1, Bag1), proliferation (Myc, FGF2, IGF2, PDGF2), cell cycle (p53, p27, PITSLRE) and angiogenesis (VEGF-A, HIF-1α), all processes that are important in cancer initiation and progression.

Cellular circumstance	***IRESs regulated***
Devlopment	Antennapedia, Ultrabithorax, c-*myc*, FGF-2
Apoptosis	c-*myc*, DAP5, XIAP, PKCδ
Genotoxic stress	c-*myc*
Cell cycle	Hairless, p58/P ITSLRE, PKCδ, ODC
Hypoxia	c-*myc*, HIF-1α, VEGFA
Differentiation	AML1/RUNX1, PDGF2/c-*sis*
Heat/cold shock	Bip, Bag-1, Rbm3
Amino-acid starvation	Cat-1

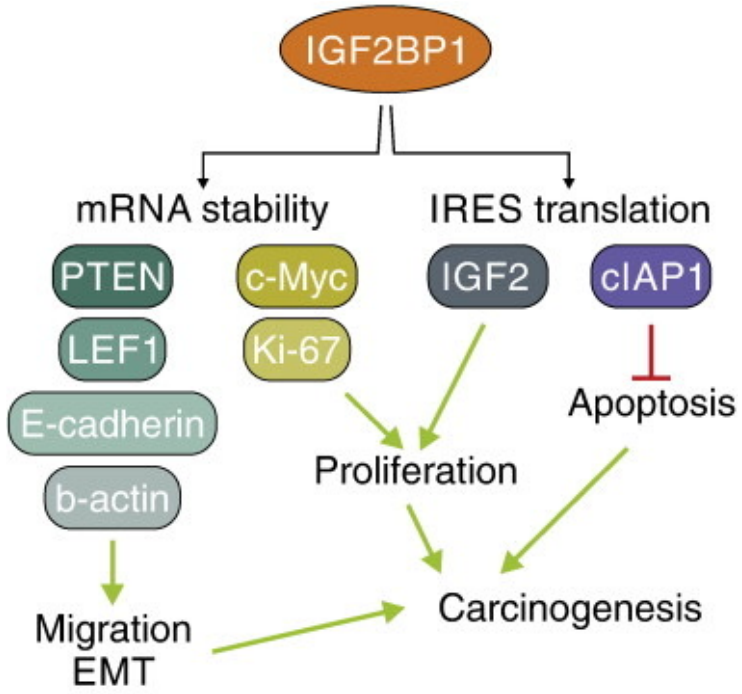
IRES trans-acting factors (ITAFs)

IRES trans-acting factors (**ITAFs**) are RNA-binding proteins that act to facilitate or block ribosome recruitment to the IRES, thus enhancing or inhibiting translation of these mRNAs. Interestingly, apart from their regulation of translation, many ITAFS are involved in other aspects of RNA metabolism that are important in carcinogenesis such as mRNA splicing, export and stability

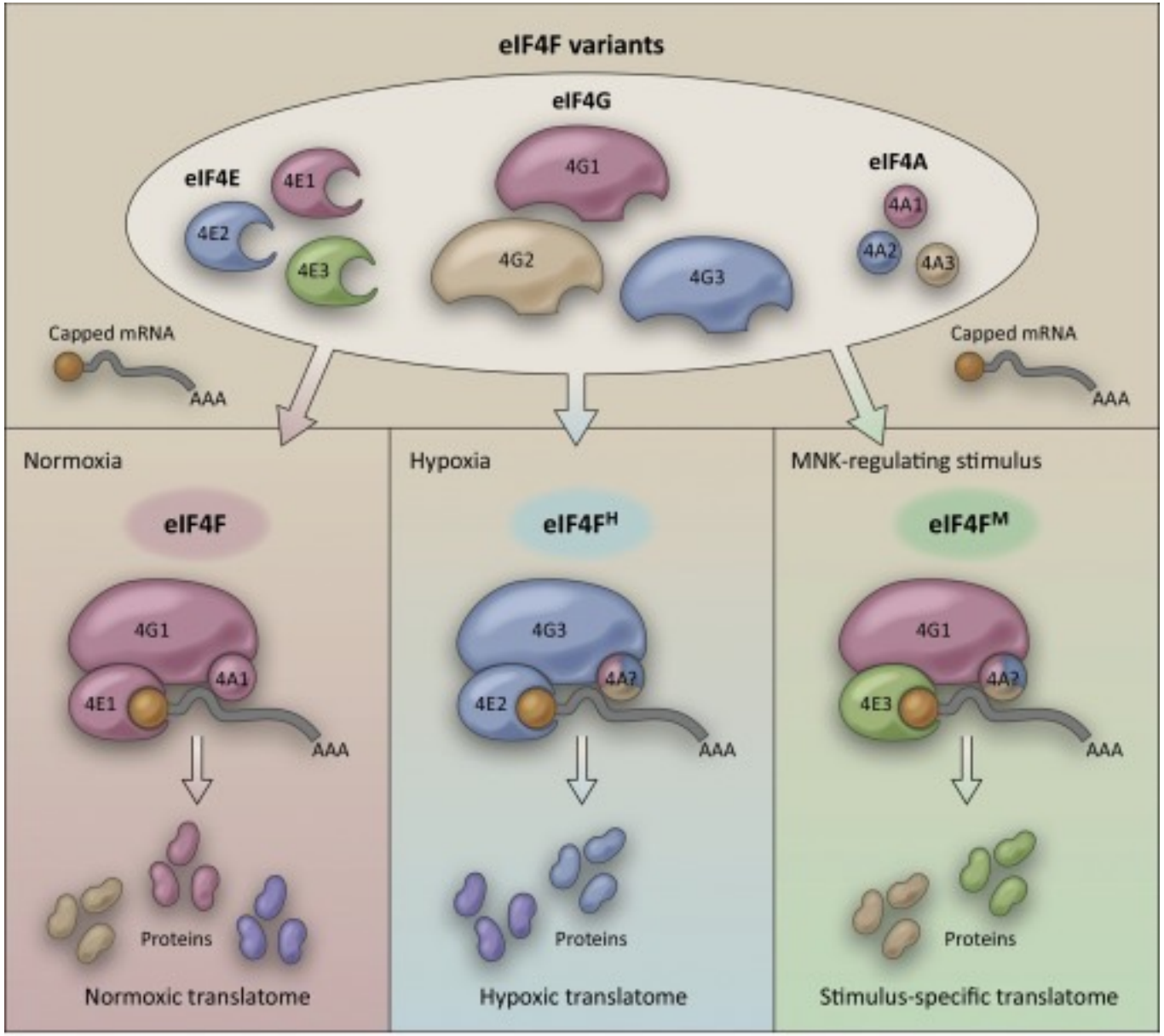


Insulin-like growth factor 2 mRNA binding protein 1 (IGF2BP1)

IGF2BP1 is an oncofetal protein that is expressed during embryogenesis and is silenced in adult tissues but becomes *de novo* expressed in many human cancers. Re-expression of IGF2BP1 enhances both the mRNA stability and IRES-mediated translation of key regulators of apoptosis, proliferation and epithelial-to-mesenchymal transition, ultimately driving carcinogenesis.



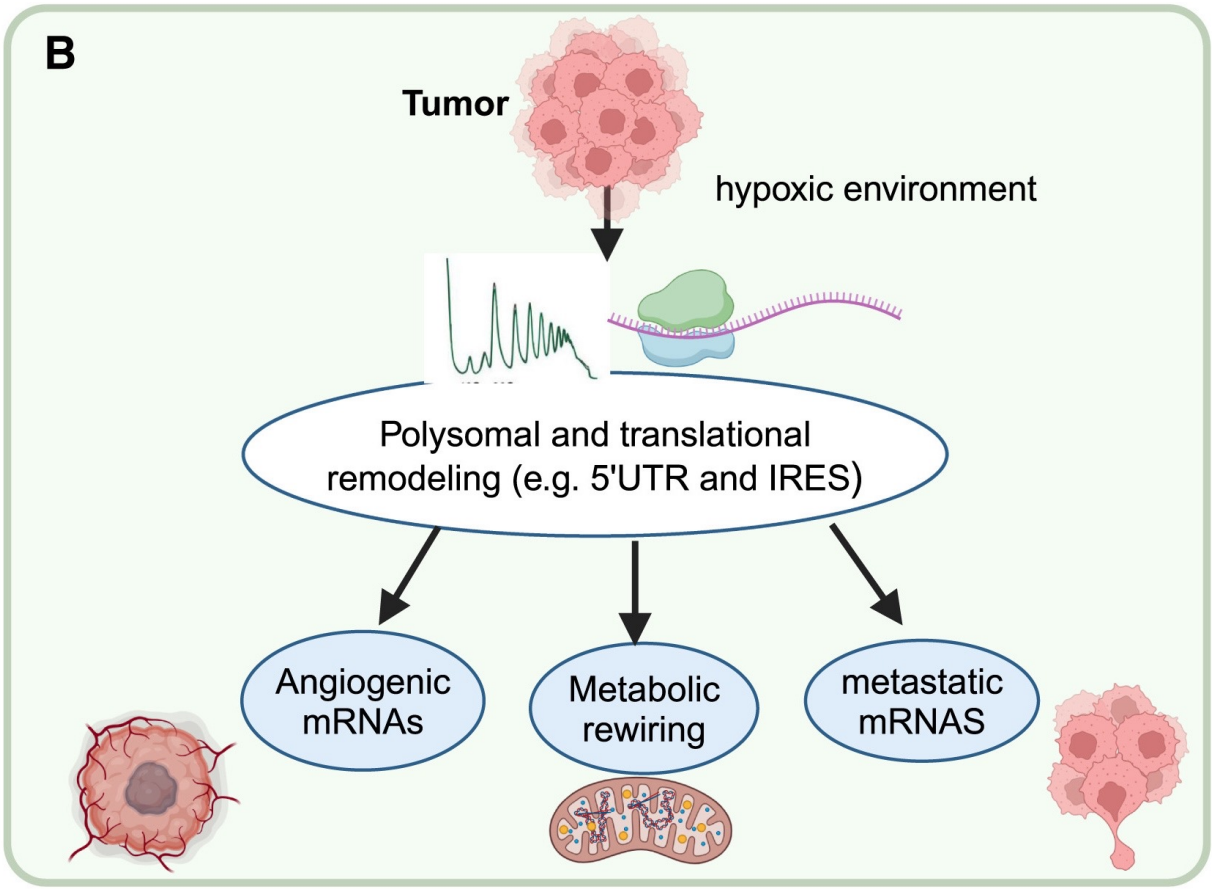
eIF4F Variants Reprogram the Cellular Translatome in Response to Distinct Stimuli



Trends in Biochemical Sciences

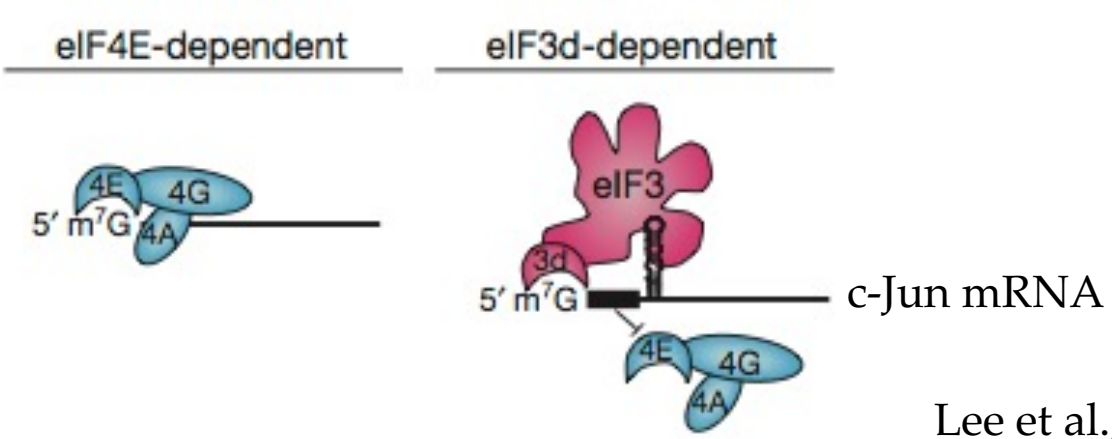
Non-canonical mRNA translation

In cancer cells, hypoxic environment leads to polysomal and translation machinery remodeling in a way that preferential translation of metabolic, angiogenic, and metastatic mRNAs occurs allowing tumor angiogenesis, metabolic rewiring, and tumor invasion.



eIF3 is required for specialized translation initiation

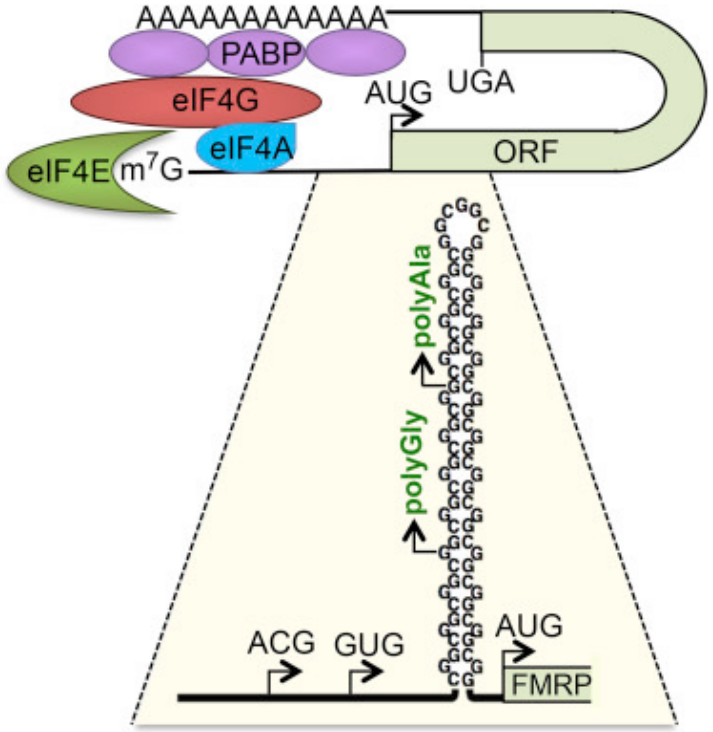
- eIF3 drives specialized translation on capped and non-capped.
- HCV IRES occurs through essential interactions between eIF3 and an IRES element in the viral genome.
- The eIF3 component **eIF3d** makes specific contacts with the cap and these interactions are essential for assembly of translation initiation complexes on eIF3-specialized mRNAs.
- An eIF4F-inhibitory RNA element ensures that mRNA translation occurs through an eIF3-specialized pathway.



Lee et al., 2016. Nature

Repeat-associated non-AUG (RAN) translation

Expansions within an open reading frame, such as the CAG repeats causing Huntington's disease or spinocerebellar ataxias, insert homopolymeric amino acids into the protein product, resulting in a loss and/or gain of function. In 2011, the Ranum lab discovered repeat-associated non-AUG (RAN) translation, in which microsatellite expansions promote translation of the repeats in multiple reading frames without a canonical AUG start codon.



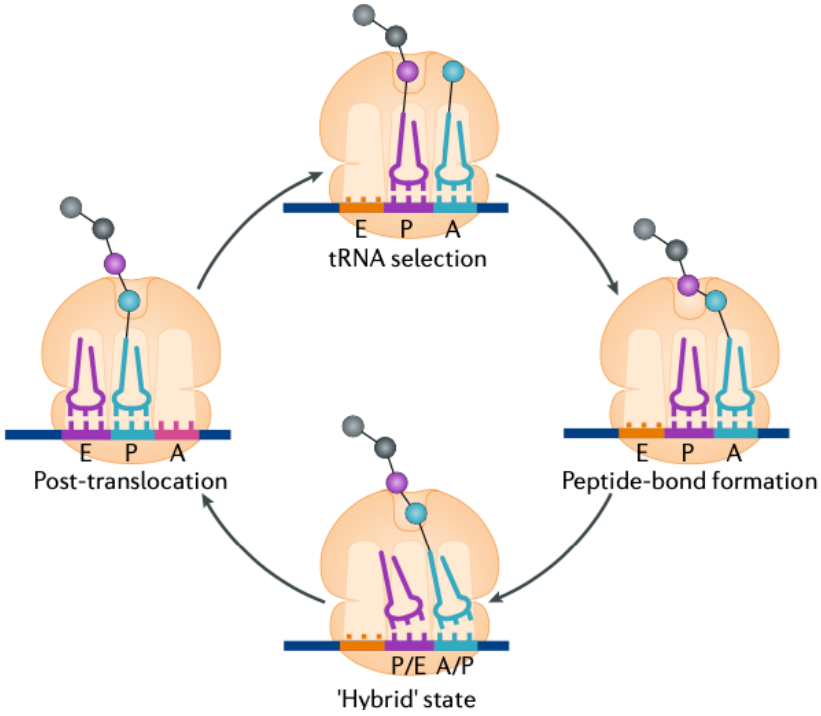
The efficiency of RAN translation is dependent on repeat length and the imperfect RNA hairpin structures formed by the expanded repeats.

RAN translation utilizes standard cap-dependent 40S ribosomal engagement with the mRNA 5′ end followed by scanning to find the translation start codon. A main difference appears to be the mechanism of start codon selection. Translation in the +1 reading frame was found to initiate predominantly from two non-AUG codons upstream of the CGG expansion while translation in the +2 reading frame initiates predominantly within the expansion. Furthermore, translation start sites varied depending on repeat length, indicating flexibility uncharacteristic of canonical translation.

Translation elongation

The process of elongation begins immediately after translation initiation has taken place and an 80S ribosome is positioned at an AUG start codon with a methionyl-tRNA^{iMet} in the P site. The elongation phase extends from the loading of the first aminoacyl-tRNA at the start of the ORF (after the initiation codon) until the ribosome reaches the termination codon at the end of the ORF and is thought to be mostly conserved relative to bacterial elongation. Translation elongation is composed of three basic steps that take place at the incorporation of each amino acid in the elongating peptide chain:

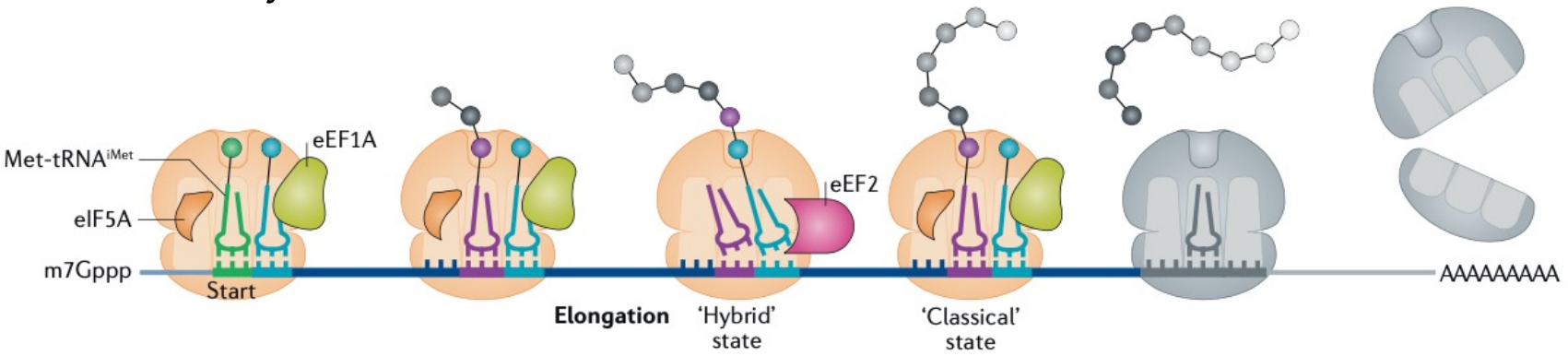
1. tRNA selection (or decoding),
2. peptide-bond formation,
3. translocation of the mRNA–tRNA complex.



Translation elongation

1. tRNA selection

tRNA selection is the process wherein the aminoacyl-tRNA with the proper anticodon to match the mRNA codon is loaded into the A site of the ribosome. Aminoacyl tRNAs are delivered to the ribosomal A site by the specialized GTPase **eEF1A** (elongation factor Tu (EFTu) in bacteria) in a ternary complex with GTP. Once cognate interactions between the codon and anticodon are sensed, eEF1A is activated and hydrolyses GTP to enable the tRNA to be fully accommodated into the A site.



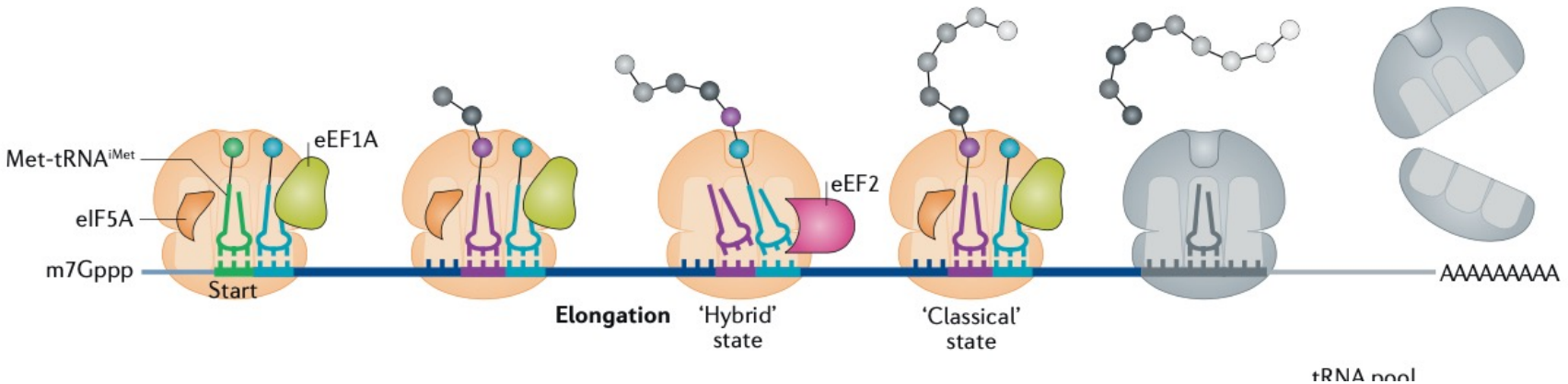
2. Peptide bond formation

The amino group of the incoming amino acid attacks the ester linkage on the peptidyl-tRNA in the ribosomal P site, and the growing peptide chain is transferred to the tRNA in the A site. As the peptide bond forms, the ribosomal subunits rotate with respect to one another, and the tRNAs adopt an altered conformation referred to as the 'hybrid' state, in which the anticodon end of the tRNAs remains positioned essentially in the P and A sites of the small ribosomal subunit, while the acceptor ends of the tRNA are positioned in the E site and P sites of the large subunit.

Translation elongation

3. translocation of the mRNA–tRNA complex

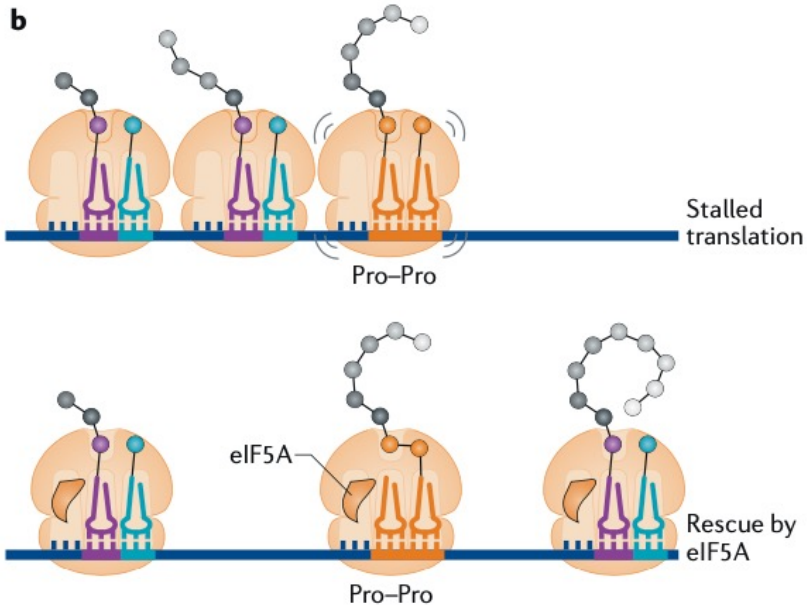
This rotated state of the ribosome is then the substrate for the action of another GTPase, **eEF2** (elongation factor G (EFG) in bacteria), which translocates the mRNA–tRNA complex relative to the ribosome and returns the tRNAs to their 'classical' states. This elongation cycle is repeated until each codon has been translated and a complete protein has been synthesized.



Translation elongation

As the ribosome elongates along an ORF, it can encounter a variety of problematic sequences that slow its progress.

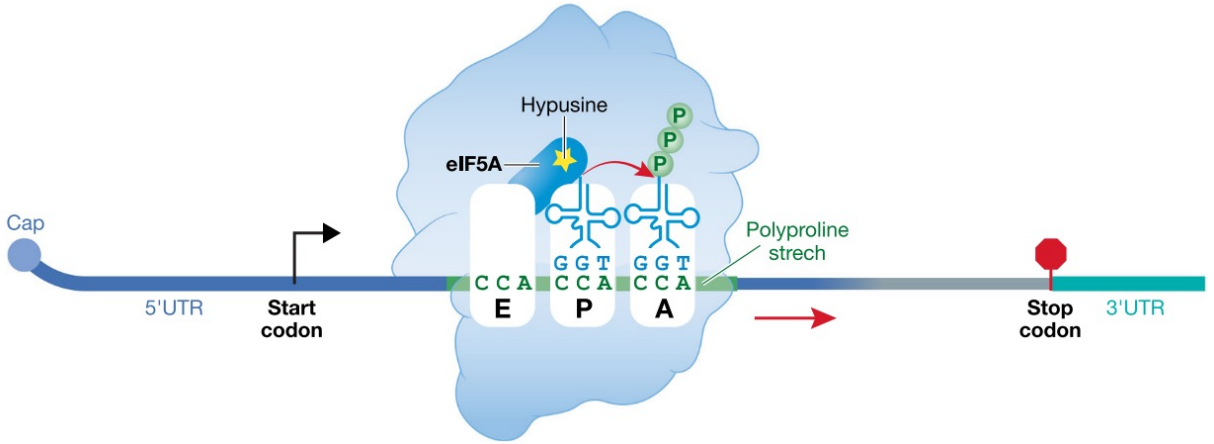
1. Certain **amino acid sequence combinations** can stall the ribosome, either because of poor reaction kinetics resulting from the nature of the amino acids themselves or from inhibitory conformations of the nascent peptide in the exit tunnel. Ribosome stalling due to slow peptidyl-transfer kinetics (such as during the formation of Pro–Pro) is rescued by eIF5A, which promotes peptide-bond formation.



This activity depends on its unique hypusine modification. eIF5A binds to the ribosomal E site free of tRNA and the eIF5A–80S complex has P-site tRNA present. Hypusine moiety of eIF5A interacts with the phosphate backbone of the A76 nucleotide at the tRNA 3′ (CCA) end in the peptidyl-transferase centre, suggesting that eIF5A promotes peptide-bond formation by stabilizing the conformation of the peptidyl-tRNA for nucleophilic attack by the aminoacyl-tRNA in the A site

Translation elongation

eIF5A was originally defined as an initiation factor; however, recent studies show its main role in translation elongation as a ribosomal pause relief factor. In humans, there are two eIF5A isoforms, eIF5A1 and eIF5A2, both of which contain the amino acid hypusine formed by a post-translational modification unique to a specific lysine residue. eIF5A regulates start codon selection of the MYC mRNA in cancer cells. In particular, loss of eIF5A promotes expression of an N-terminally extended c-Myc protein. Moreover, eIF5A may more generally regulate selective translation of oncogenes containing proline stretches or tripeptides (Met-Phe-Phe), which require eIF5A activity to prevent ribosome stalling. In this way, eIF5A may be a critical factor for maintenance of cancer cell fitness by releasing stalled ribosomes to support the increased metabolic burden of oncogenic transformation. This modified amino acid is essential for the activity of eIF5; therefore, it has attracted attention as a therapeutic target.



Translation elongation

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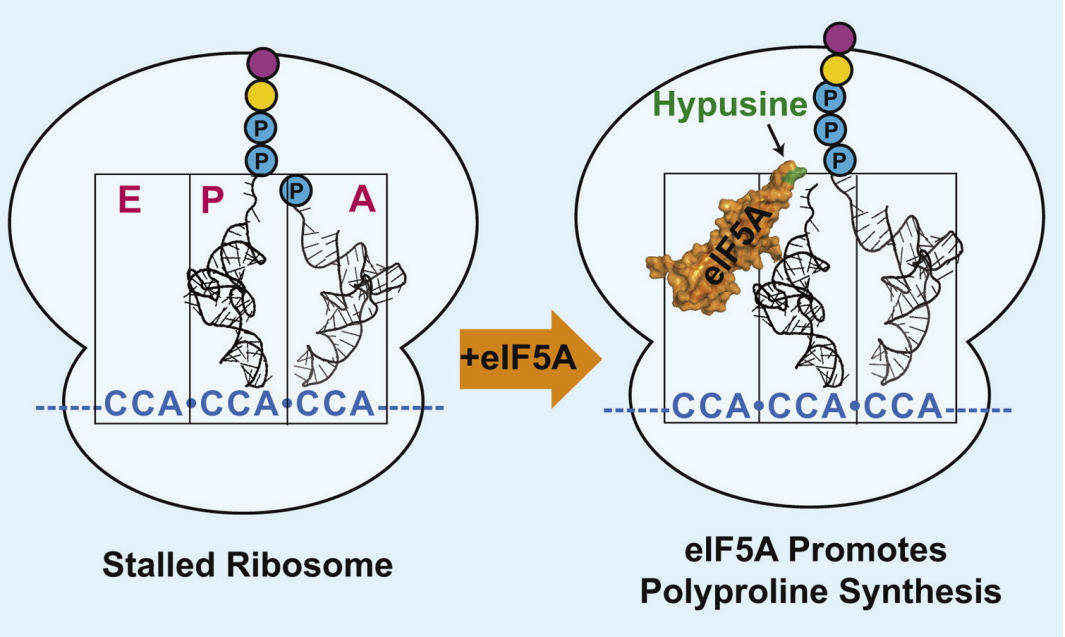
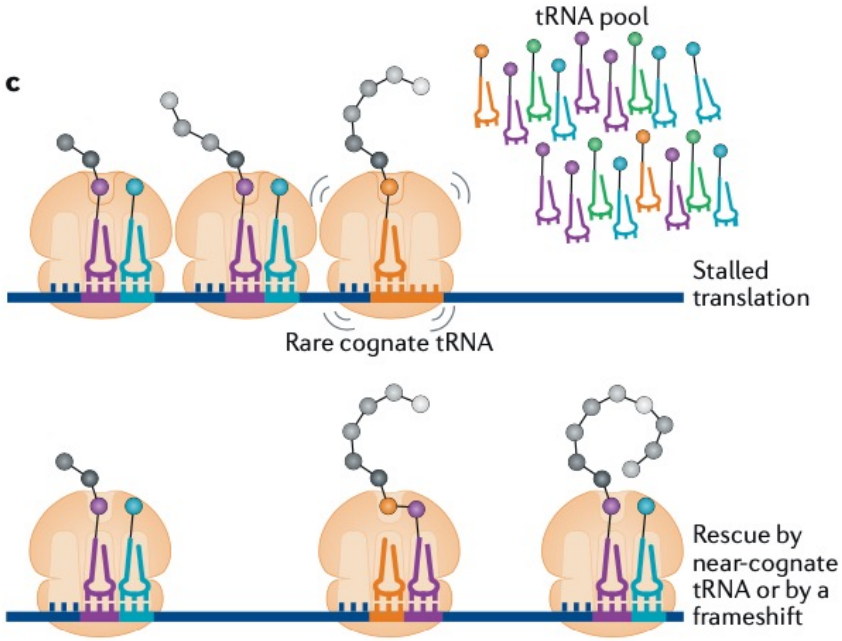


Translation elongation

As the ribosome elongates along an ORF, it can encounter a variety of problematic sequences that slow its progress.

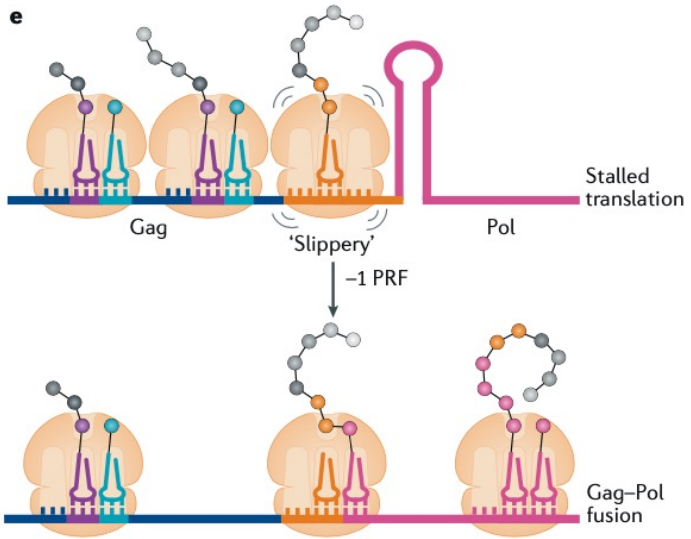
2. **mRNA sequences rich in codons of lowly expressed cognate tRNAs** also pause ribosomes. It can be rescued by misincorporation of near-cognate tRNAs or by frameshifting (represented as conversion of orange to purple). Additionally, the ribosome may encounter strong mRNA secondary structures, such as stem–loops or pseudoknots, that can arrest elongation



Translation elongation

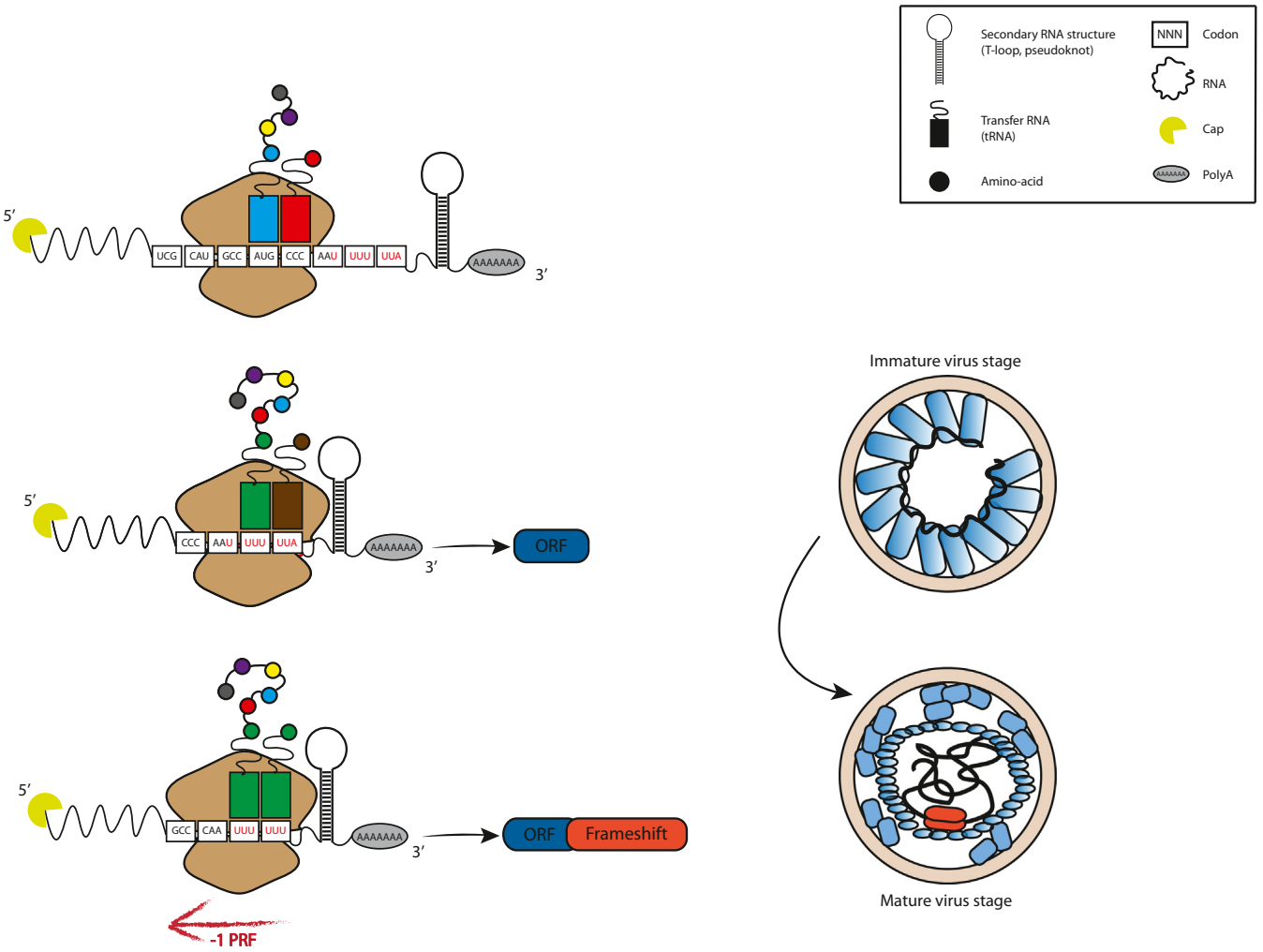
As the ribosome elongates along an ORF, it can encounter a variety of problematic sequences that slow its progress.

3. The ribosome may encounter **strong mRNA secondary structures**, such as stem–loops or pseudoknots, that can arrest elongation. Many of such sites are the product of natural selection and are referred to as **programmed ribosomal frameshifting** (**PRF**) sites. Viruses commonly use PRF sites to more efficiently encode genes in their limited genomes. Such as in HIV1, where a −1 PRF adjusts the Gag to Gag–Pol expression ratio needed for proper viral particle replication. The HIV1 PRF signal is comprised of two parts: a mRNA stem–loop structure and a U-rich slippery sequence. The structure leads to ribosome stalling directly over the slippery sequence, where slow reaction kinetics increase the likelihood of frameshifting that would permit translation of the downstream, out-of-frame pol gene.



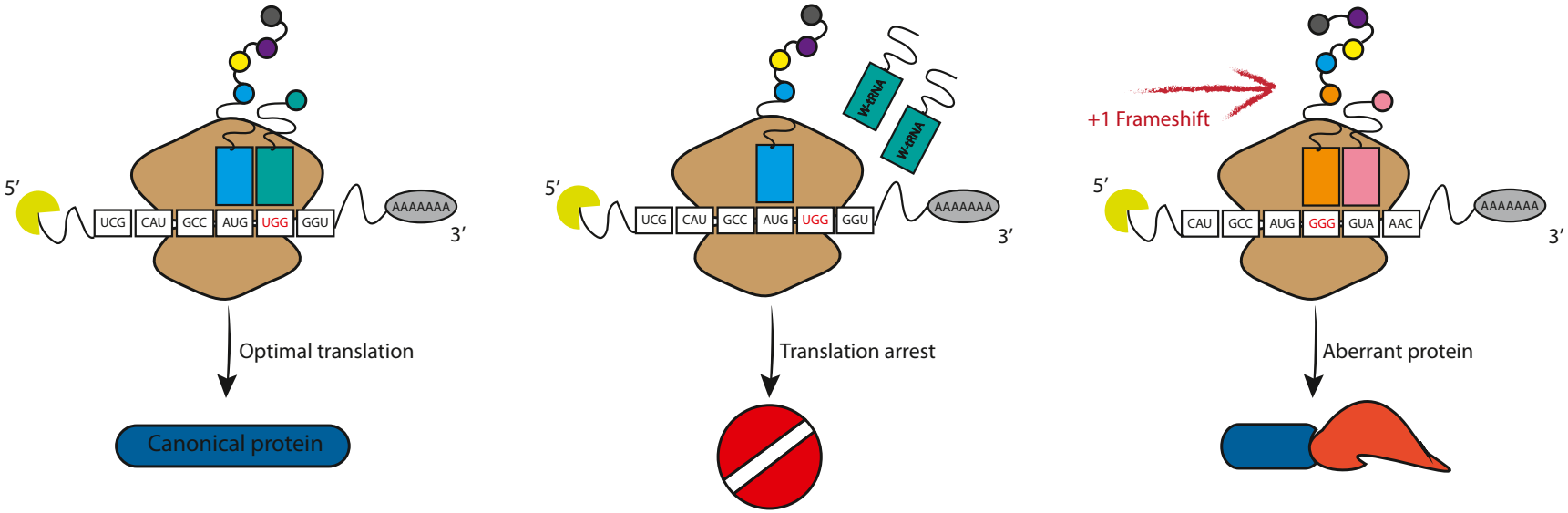
Programmed ribosomal frameshifting (PRF) in virus

The viral mRNA comprises a strong secondary RNA structure, such as a T-loop or a pseudoknot, preceded by a slippery sequence, depicted in red. The slippery sequence generally comprises a seven-nucleotide motif. When the ribosome encounters the secondary structure, the ribosome stalls and the slippery sequence allows ribosomal frameshifting (–1PRF), producing an alternative protein.



Ribosomal frameshifting (PRF) in cancer cells

Tryptophan shortage results in uncharged W-tRNA, inducing ribosome stalling at tryptophan codons (UGG, depicted in red). In sloppy cancer cells, ribosomal frameshifting is used to sustain protein synthesis, creating aberrant protein. Oncogenic pathways, such as mitogen-activated protein kinase (MAPK) or mammalian target of rapamycin (mTOR), stimulate ribosomal frameshifting by phosphorylating the ribosomal protein RPS6. Aberrant proteins resulting from ribosomal frameshifting are then processed, displayed at the cell surface, and can be recognized by dedicated T cells.

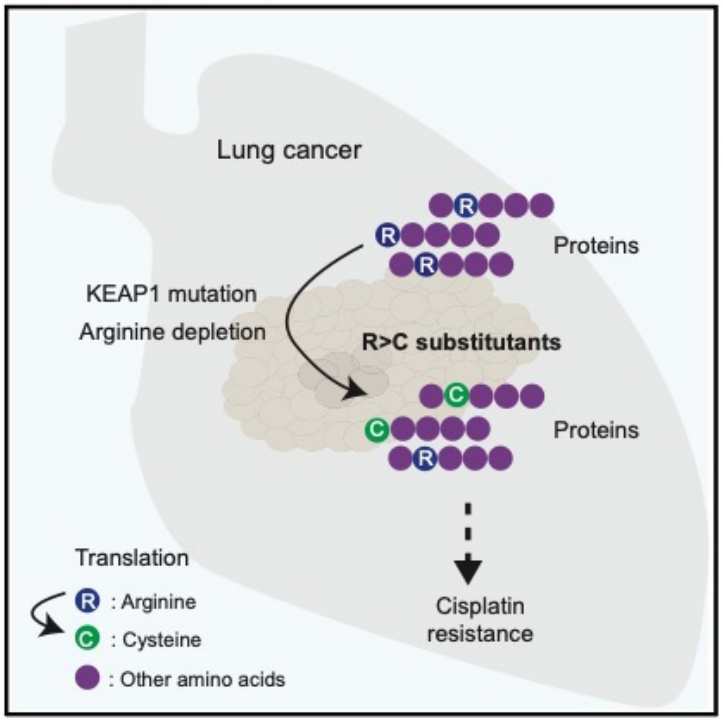


Molecular Cell

Article

Arginine deprivation enriches lung cancer proteomes with cysteine by inducing arginine-to-cysteine substitutants

Graphical abstract



Authors

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Correspondence

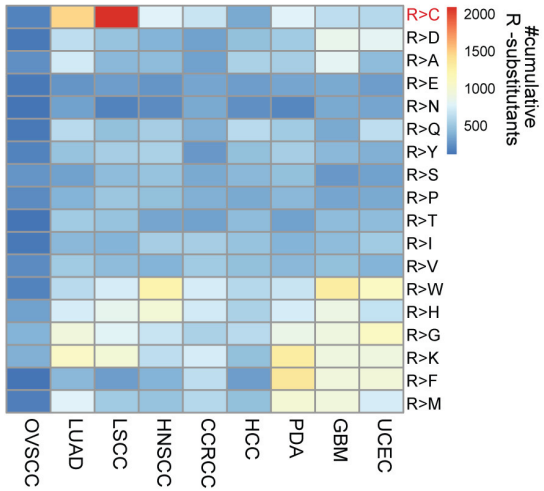
a.pataskar@nki.nl (A.P.), r.agami@nki.nl (R.A.)

In brief

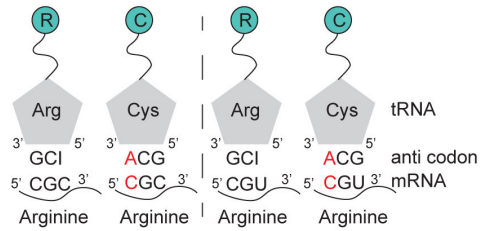
Yang, Pataskar, et al. investigated the impact of arginine shortage on aberrant mRNA translation in cancer. They report the enrichment of arginine-to-cysteine substitutants in human lung cancer, link it to a tRNA misalignment mechanism, and connect it to KEAP1 pathway mutations and platinum resistance.

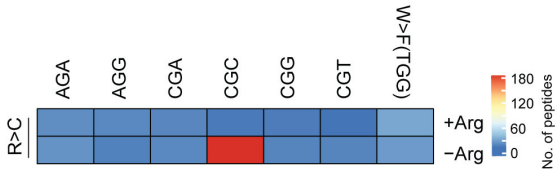
Highlights

- Arginine-to-cysteine (R>C) substitutants are enriched in lung cancers with KEAP1 pathway mutations
- Arginine deprivation induces R>C substitutants
- tRNA misalignment is a proposed mechanism for R>C substitutants in lung cancer
- R>C substitutants may enhance resistance to cisplatin treatment

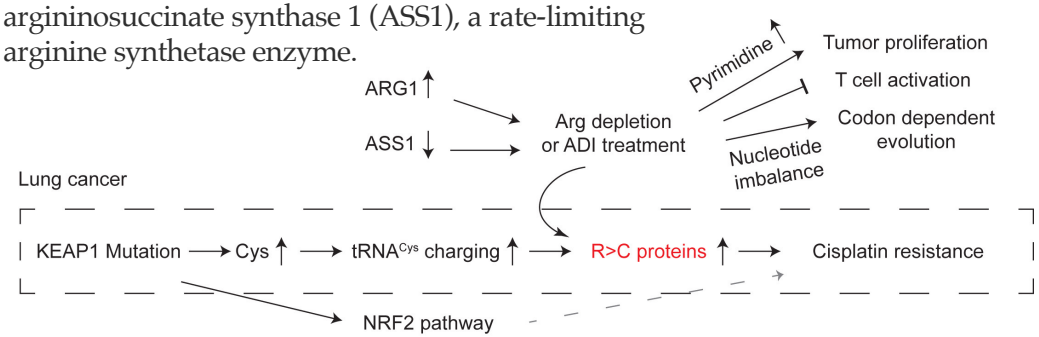


lung squamous cell carcinoma (LSCC)
lung adenocarcinoma (LUAD)

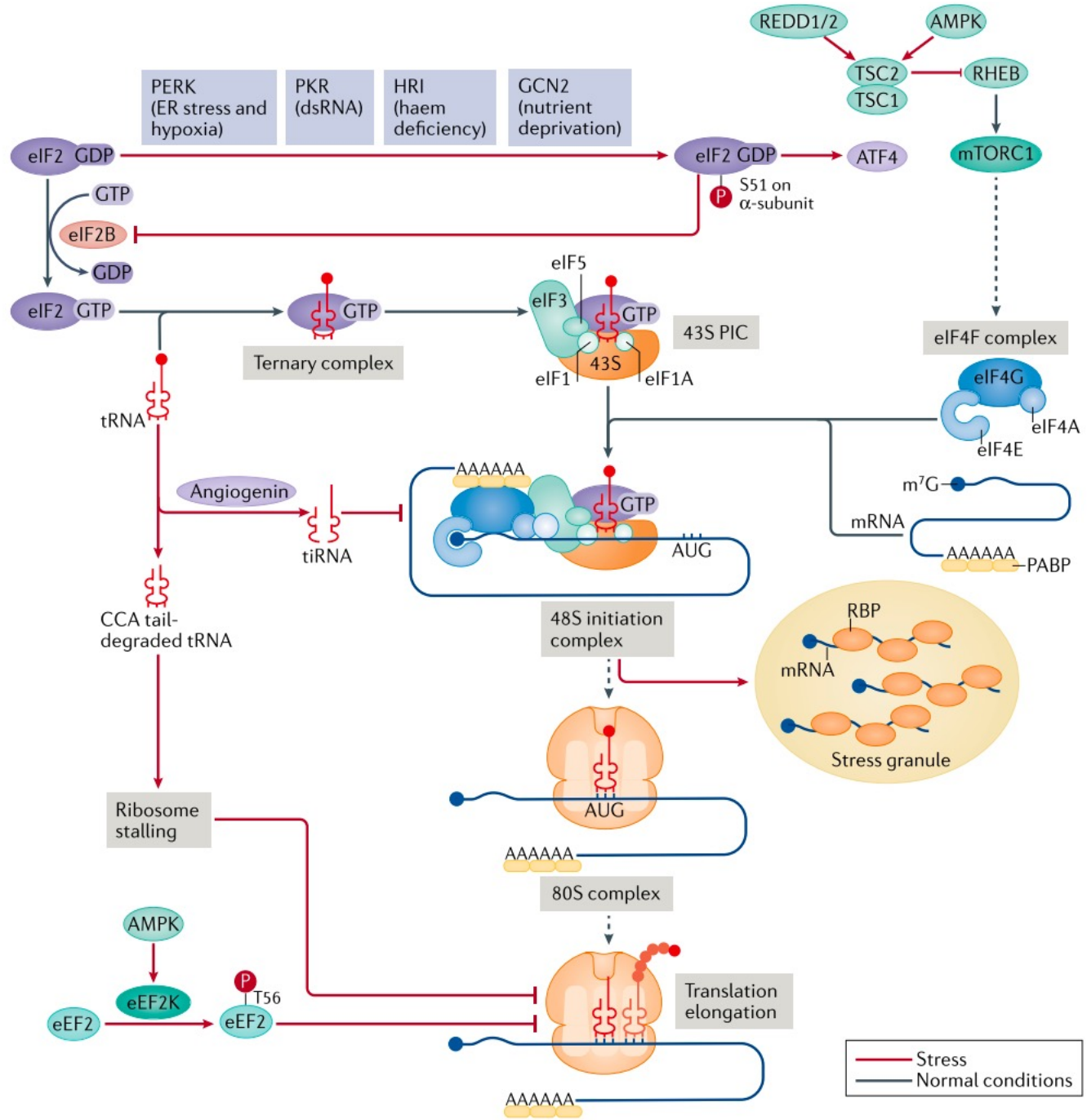




argininosuccinate synthase 1 (ASS1), a rate-limiting arginine synthetase enzyme.

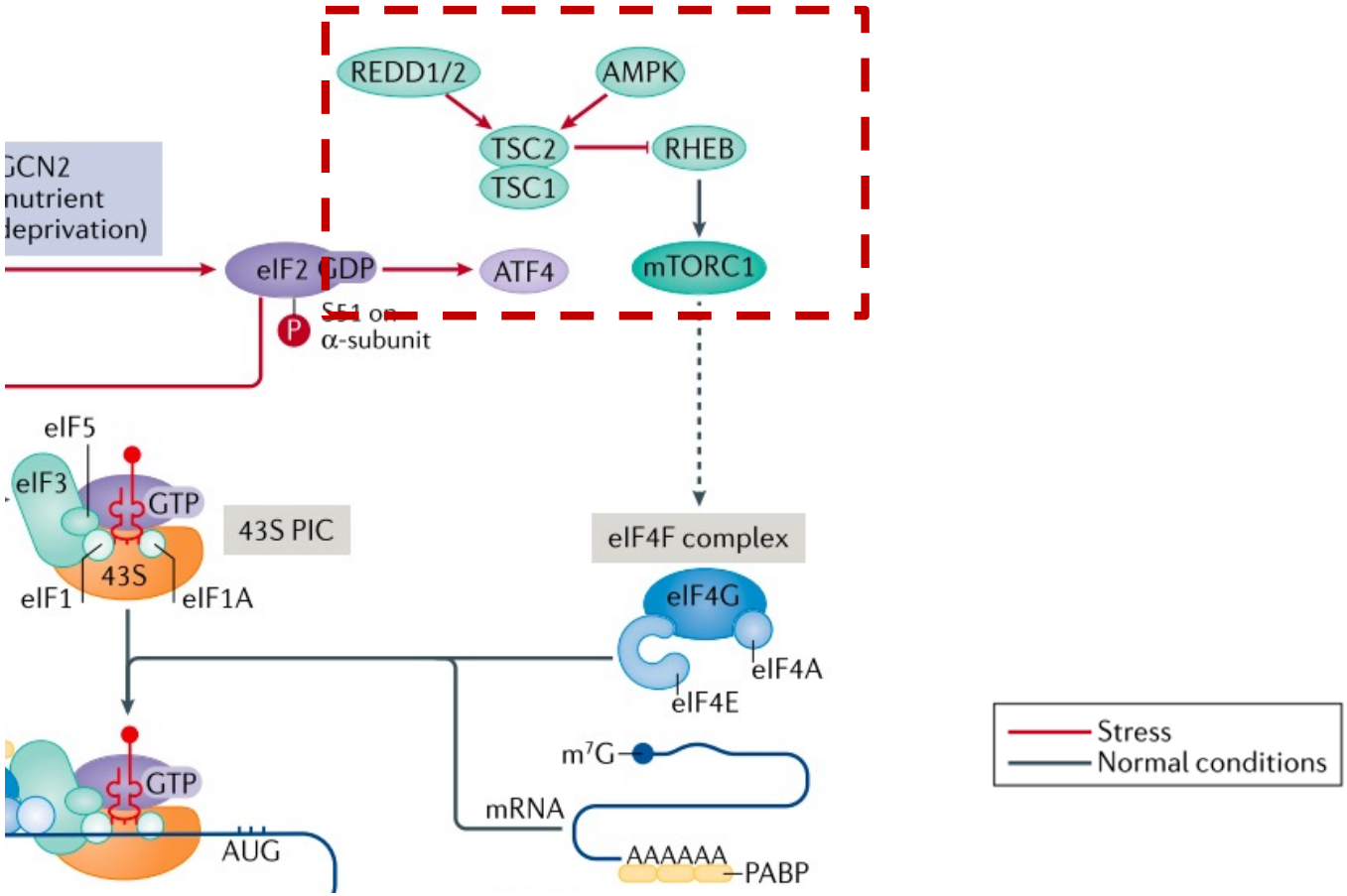


Stress-induced shutdown of translation



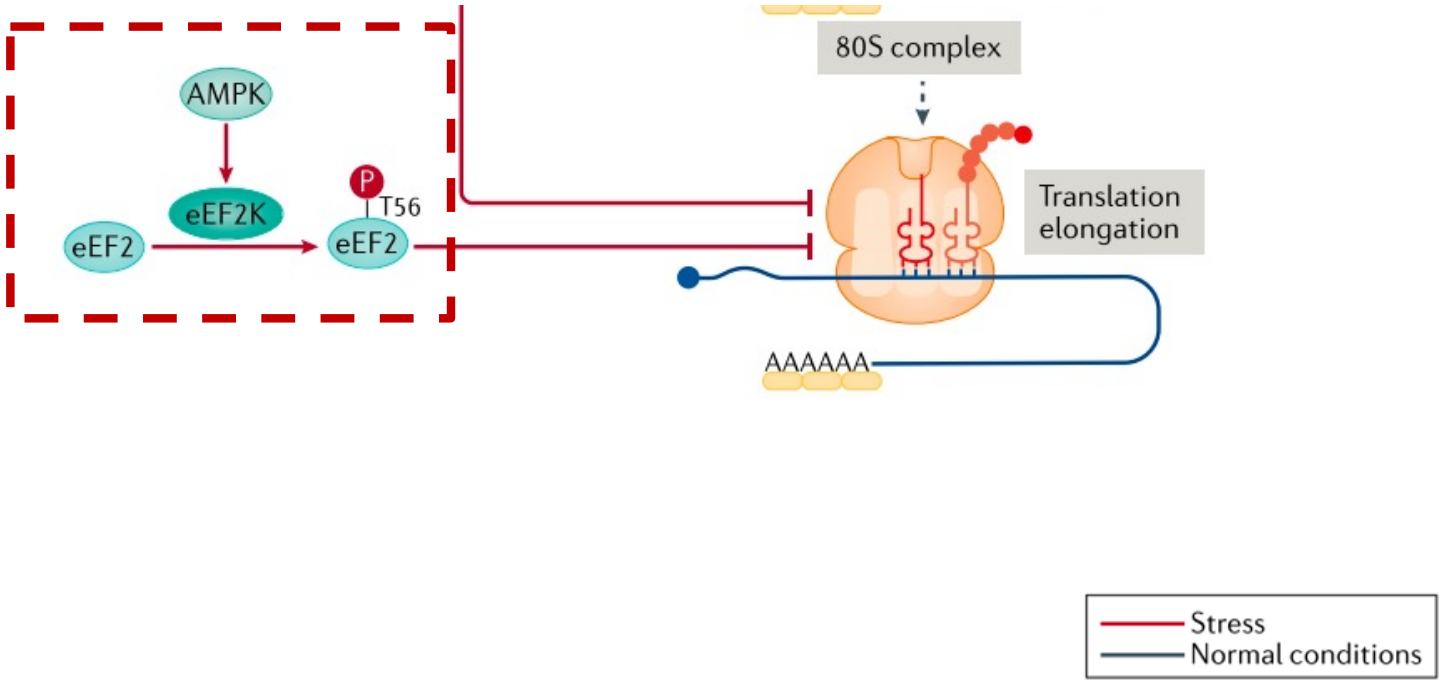
Stress-induced shutdown of translation

Hypoxia and nutrient deprivation inactivate mTORC1 primarily by activating protein regulated in development and DNA damage response 1 (REDD1) and REDD2, and the energy sensor AMPK, which activate the mTORC1 inhibitors tuberous sclerosis 1 protein (TSC1) and TSC2. TSC1 and TSC2 inactivate the GTPase RHEB by functioning as its GTPase-activating protein, thereby inhibiting mTORC1 kinase activity.



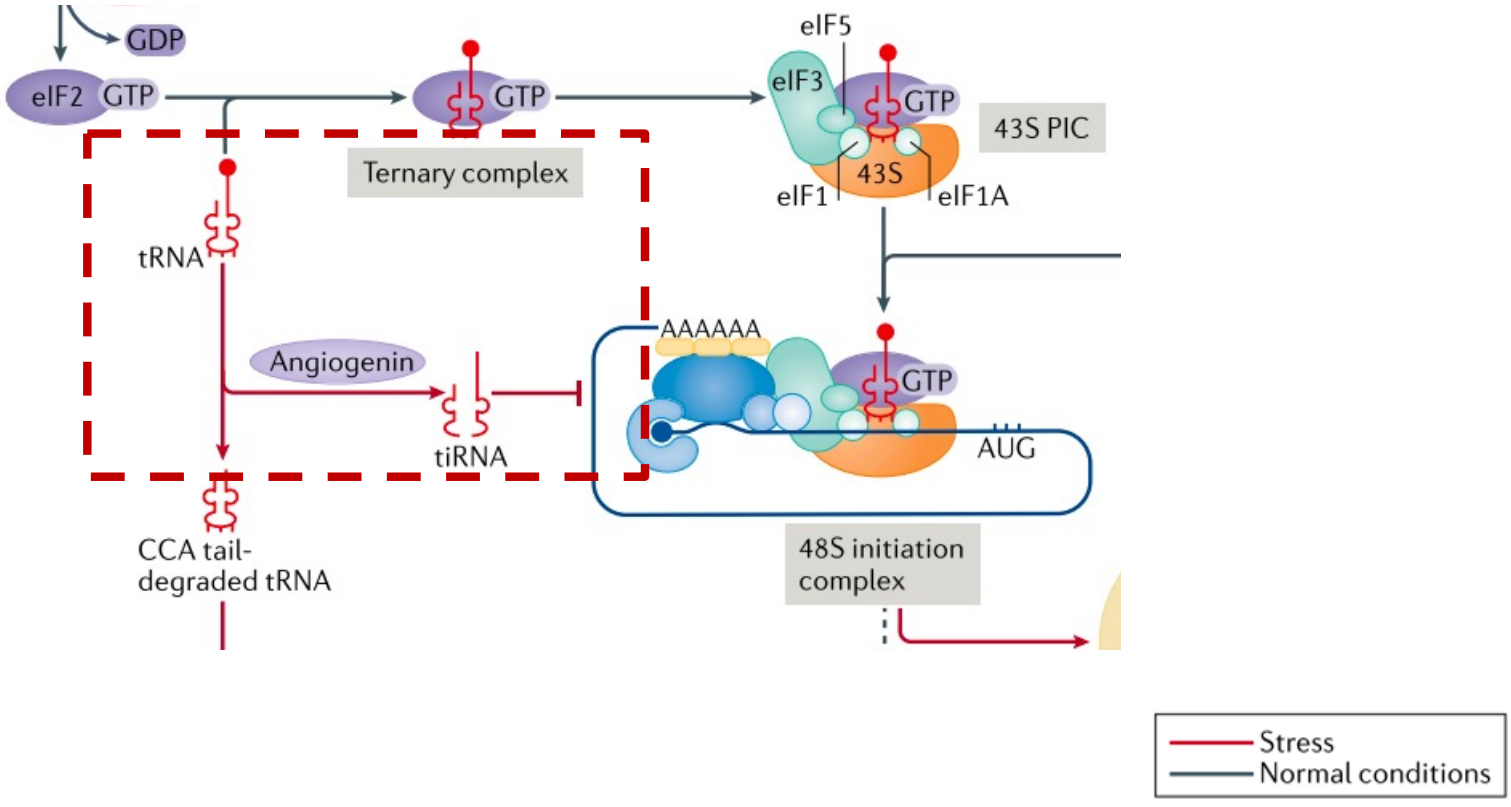
Stress-induced shutdown of translation

Protein synthesis during stress is also inhibited during elongation. AMPK eukaryotic elongation factor 2 kinase (eEF2K), which controls elongation by inhibiting eEF2.

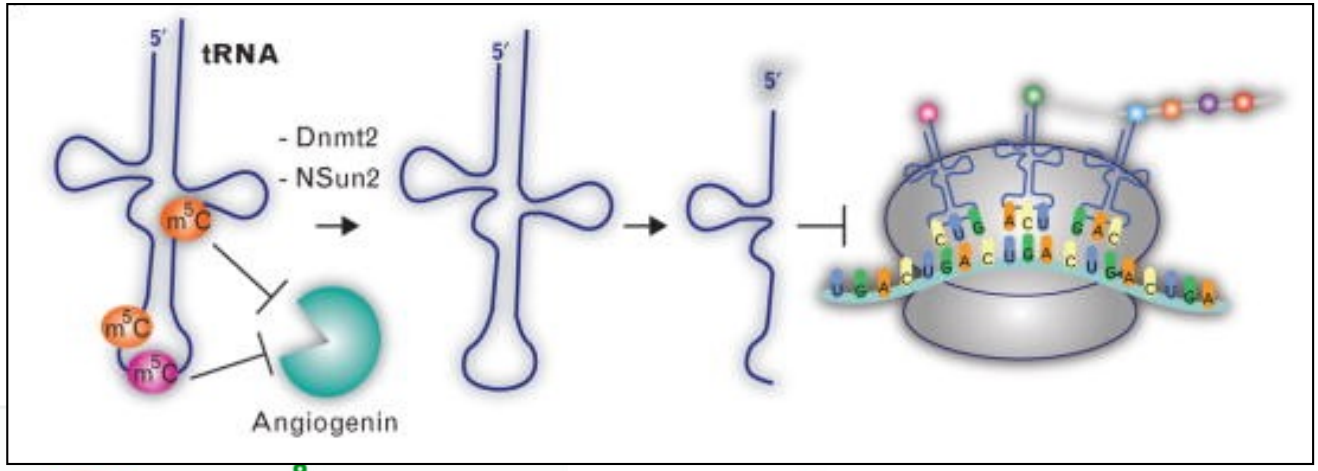


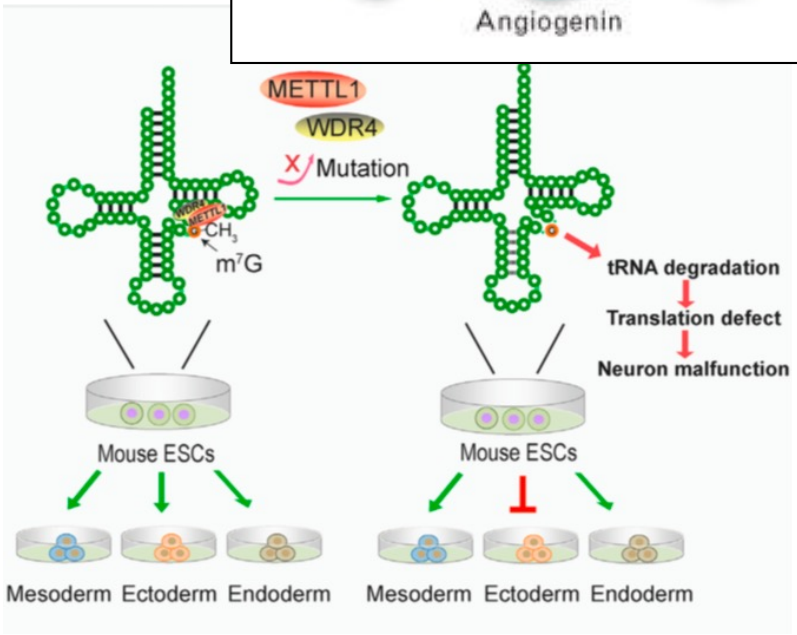
Stress-induced shutdown of translation

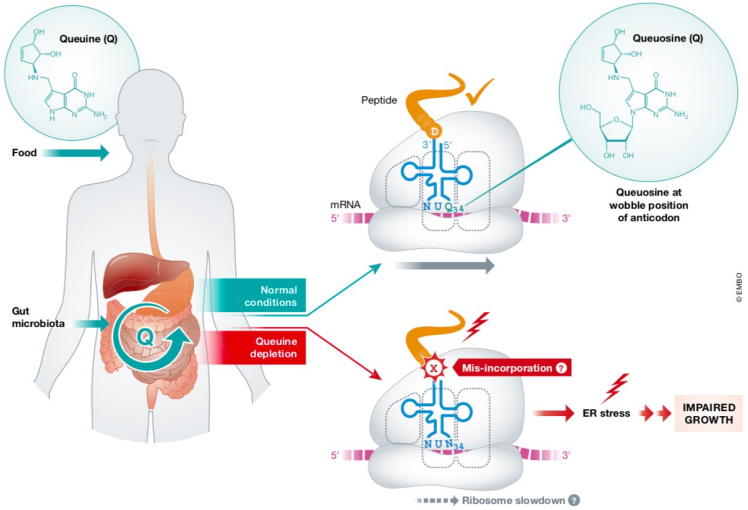
Translation initiation can also be inhibited via the angiogenin- induced cleavage of tRNA within the anticodon loops to produce tRNA-derived stress-induced fragments (tiRNAs), which displace eIF4F from the cap.



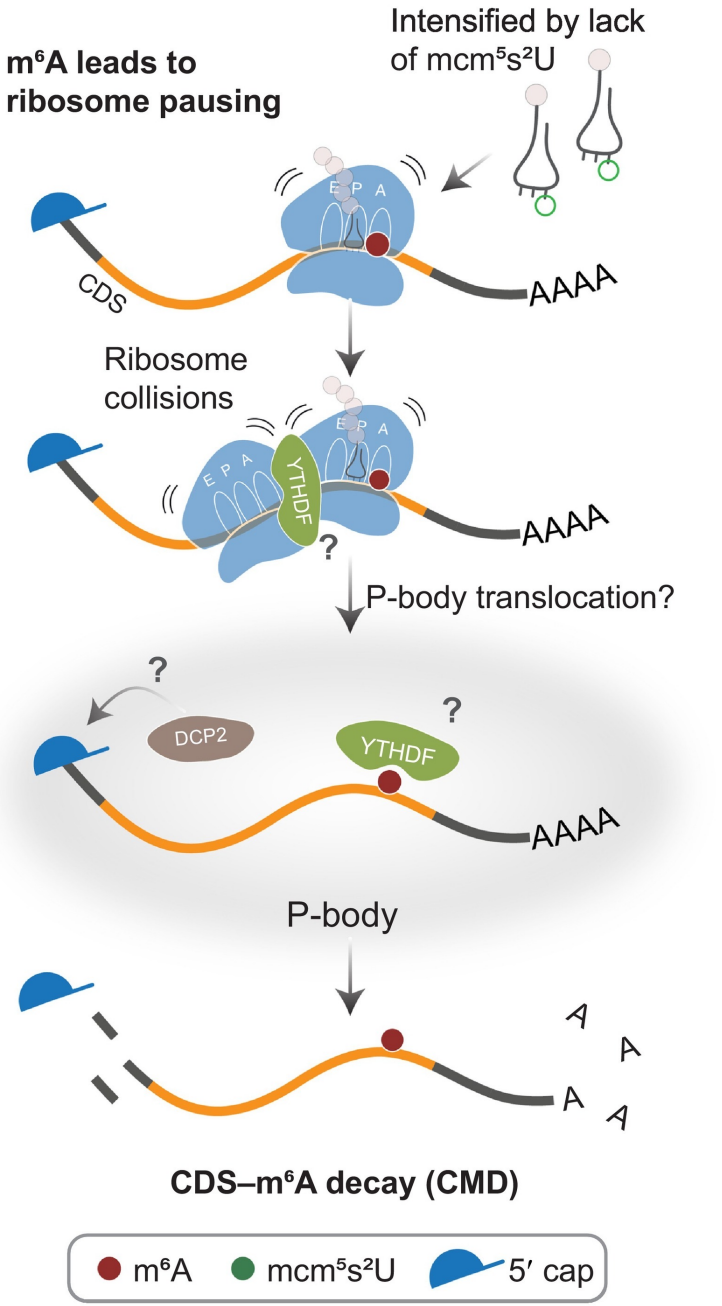
tRNA modifications and tRNA fragments can regulate global translation



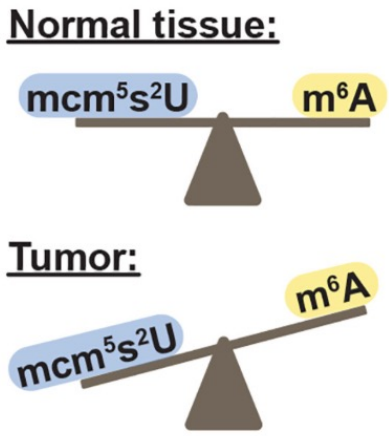




Impact of the m^{6}A modification on translation



m6A modification within A-site codons slows down ribosome decoding, which may result in ribosome pausing and degradation of mRNA. Ribosome collisions, potentially intensified by loss of tRNA modifications such as 5-methoxycarbonylmethyl-2-thiouridine (mcm5s2U) – amplify decay signals. It remains unclear whether degradation occurs at the site of pausing, within P-bodies, or both, and whether decapping enzymes such as DCP2 play a role in this process.



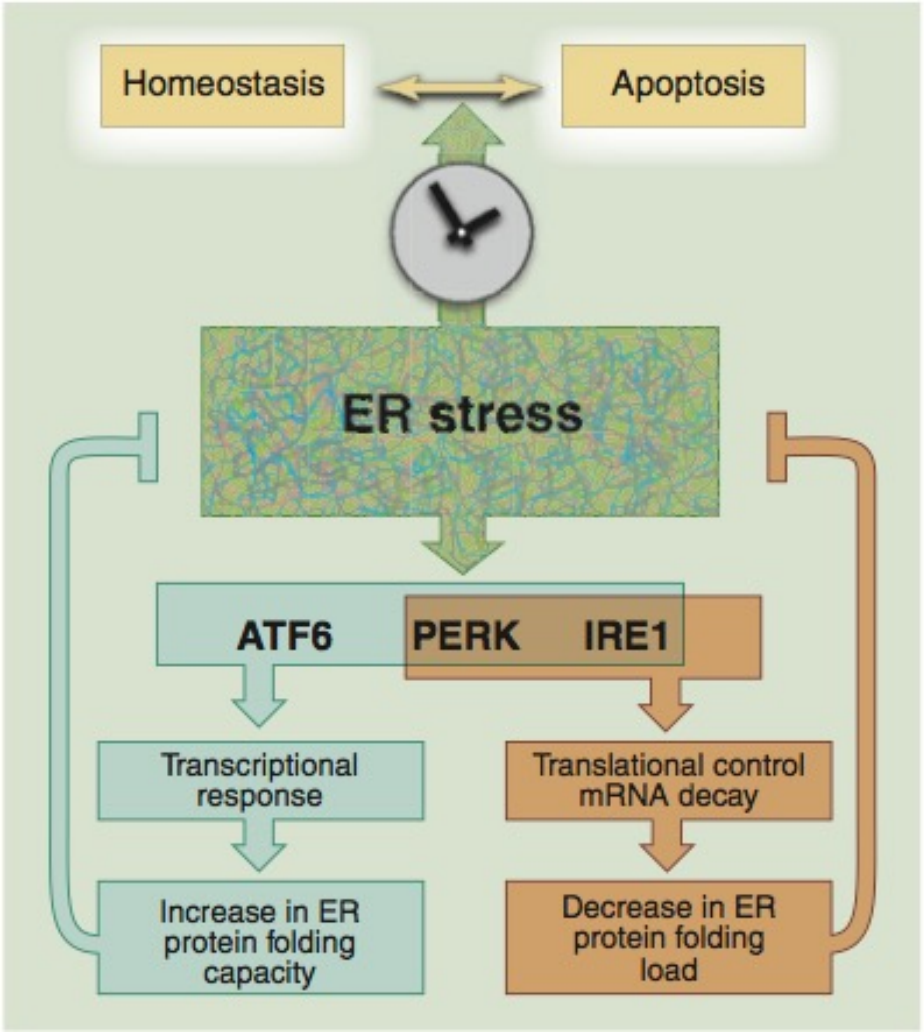
The Unfolded Protein Response (UPR)

The vast majority of proteins that a cell secretes or displays on its surface first enter the **endoplasmic reticulum (ER)**, where they fold and assemble. Only properly assembled proteins advance from the ER to the cell surface.

To ascertain fidelity in protein folding, cells regulate the protein-folding capacity in the ER according to need. The ER responds to the burden of unfolded proteins in its lumen (ER stress) by activating intracellular signal transduction pathways, collectively termed **the unfolded protein response (UPR)**.

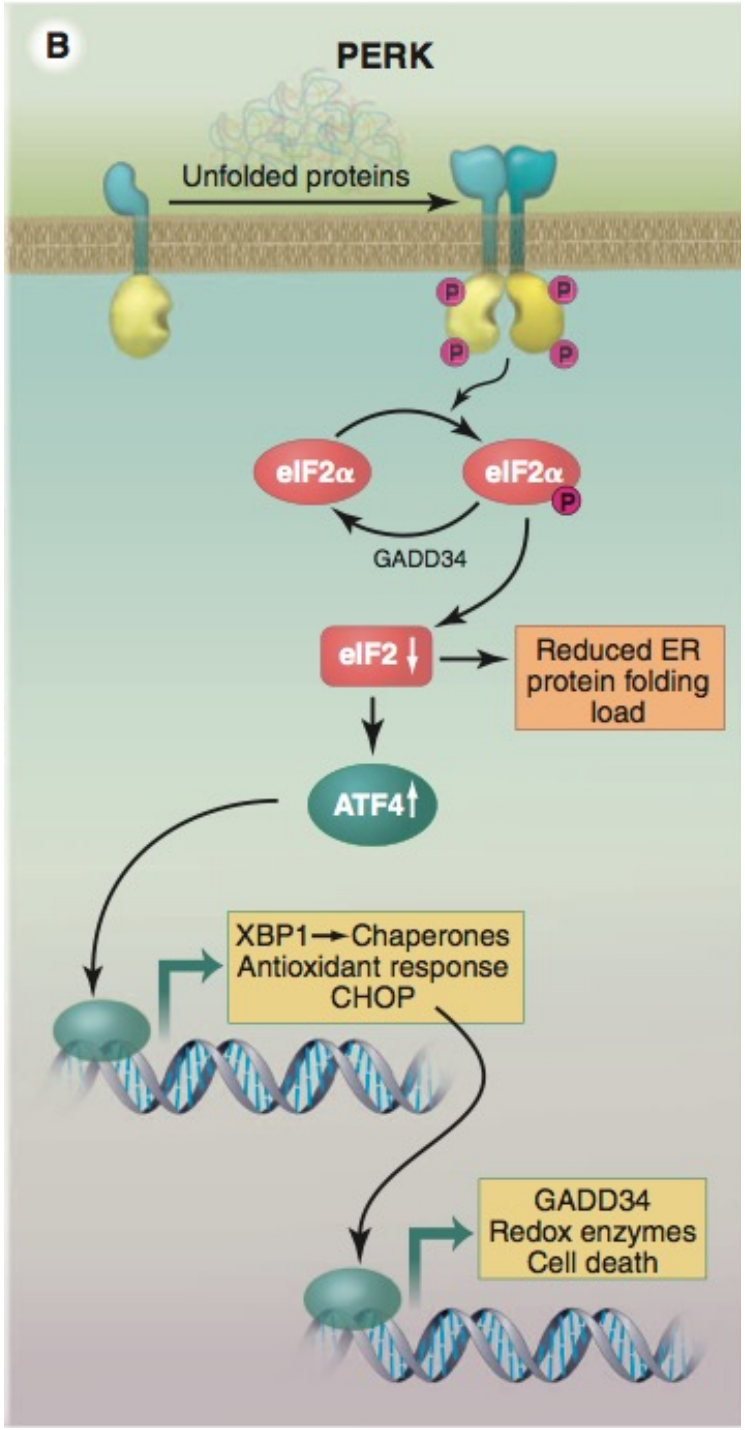
The Unfolded Protein Response (UPR)

Together, three mechanistically distinct branches of the UPR regulate the expression of numerous genes that maintain homeostasis in the ER or induce apoptosis if ER stress remains unmitigated. The branches operate in parallel and use unique mechanisms of signal transduction. Each branch is defined by a class of **transmembrane ER-resident signaling components**: **PERK** [double-stranded RNA-activated protein kinase (PKR)–like ER kinase], **ATF6** (activating transcription factor 6) and **IRE1** (inositol requiring enzyme 1). The IRE1 branch is the most conserved and sole branch of the UPR in lower eukaryotes. Evolution later added the PERK and ATF6 branches to metazoan cells.



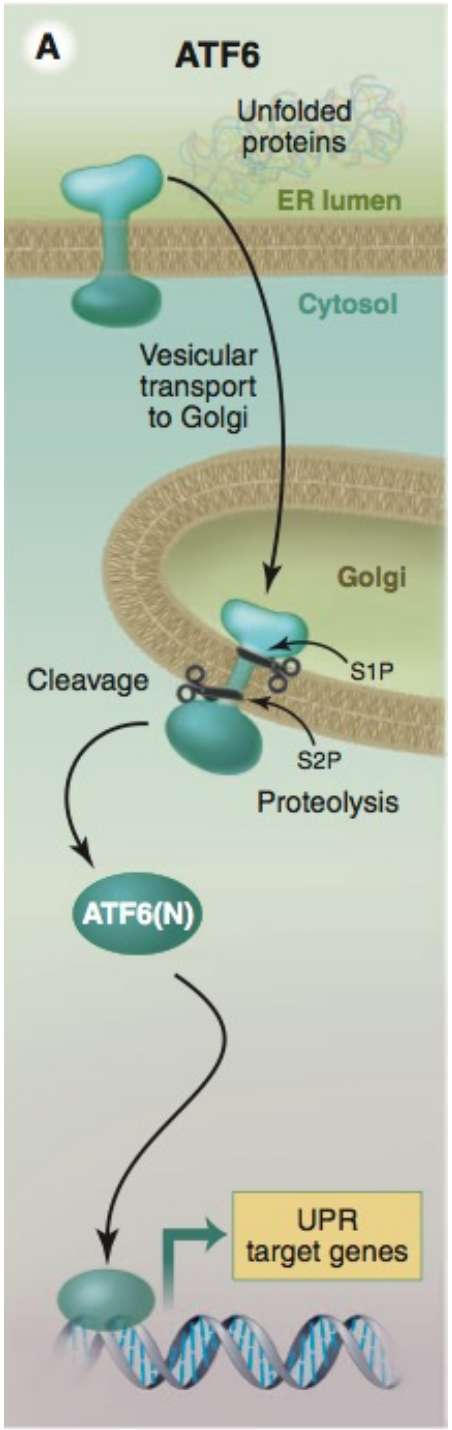
The PERK branch:

When activated upon sensing ER stress, **PERK** oligomerizes and phosphorylates itself and the translation initiation factor eIF2a, inhibiting mRNA translation. In this way, PERK helps reduce the flux of protein entering the ER to alleviate ER stress. However, some mRNAs containing short open reading frames in their 5′-UTR are preferentially translated. when eIF2 is limiting. One of these encodes the transcription factor **ATF4**, which induces **CHOP** and **GADD34**. CHOP is a transcription factor that controls genes encoding components involved in apoptosis. GADD34 encodes a PERK-inducible regulatory subunit of the protein phosphatase PP1C that counteracts PERK by dephosphorylating eIF2a.



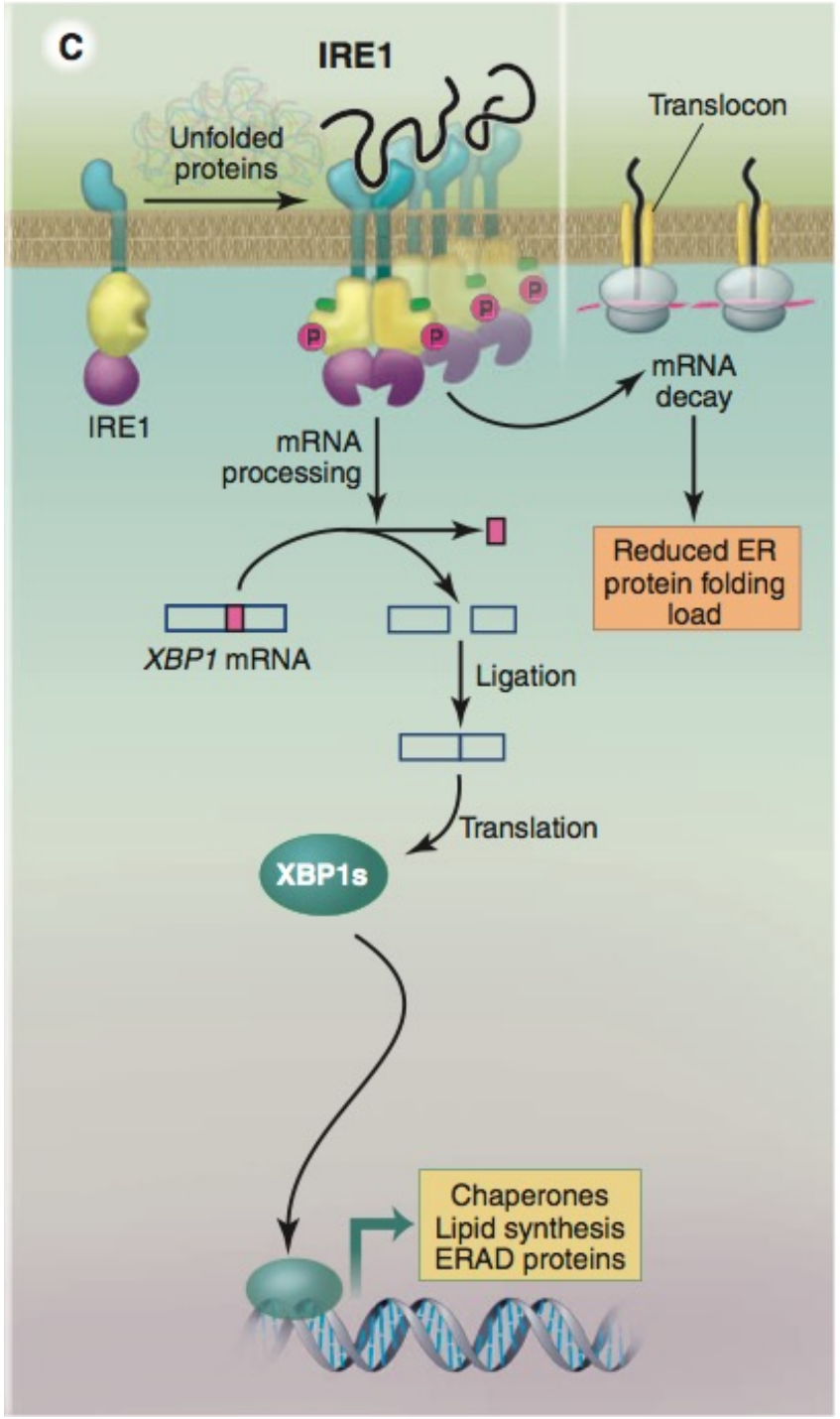
The ATF6 branch:

ATF6 is a transcription factor that is initially synthesized as an ER-resident transmembrane protein bearing a large ER-luminal domain. Upon accumulation of unfolded proteins, it is packaged into transport vesicles that pinch off the ER and deliver it to the Golgi apparatus. There, it encounters two proteases, **S1P** and **S2P** (site-1 and site-2 protease), that sequentially remove the luminal domain and the transmembrane anchor, respectively. The liberated N-terminal cytosolic fragment, ATF6(N), then moves into the nucleus to activate UPR target genes. Among ATF6's targets are prominent ER-resident proteins involved in protein folding.



The IRE1 branch:

IRE1 is a bifunctional transmembrane kinase/endoribonuclease that uses a unique mechanism of nonconventional mRNA splicing to transmit the UPR signal. Its RNase function is activated by conformational changes following lateral IRE1 oligomerization in the ER membrane. When activated, IRE1 cleaves the mRNA encoding a UPR-specific transcription factor, called **XBP1** in metazoans, in two specific positions, excising an intron. The exons are then ligated (by tRNA ligase) giving rise to a spliced mRNA that is translated to the active forms of the transcription factor **XBP1^{s}**. XBP1^{s} regulates lipid biosynthetic enzymes and ER-associated degradation components.



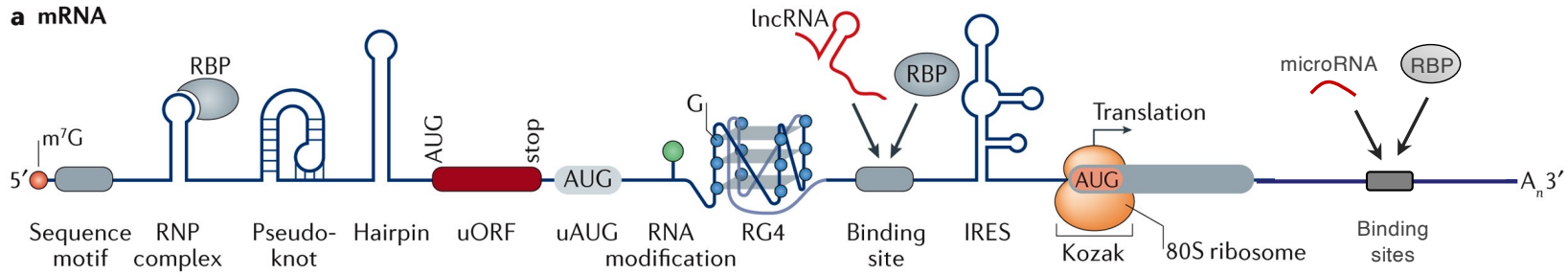
Translational control in eukaryotes

Part II

b) Specif control of protein synthesis

(mRNAs as target)

Elements that influence translation of mRNA



- The 7-methylguanosine (m^7G) 5′ cap structure (circle) at the 5′ end of the mRNA and the poly(A) tail (A_n) at the 3′ end stabilize the mRNA and stimulate translation.

- The **5′-UTR** contains secondary and tertiary structures and other sequence elements. RNA structures such as **pseudoknots**, **hairpins** and RNA **G-quadruplexes (RG4s)**, as well as **upstream open reading frames (uORFs)** and **upstream start codons (uAUGs)**, mainly inhibit translation.

- **Internal ribosomal entry sites (IRESs)** mediate translation initiation independently of the cap.

- **RNA modifications**, or **RNA-binding proteins (RBPs)** and **lncRNAs** that interact with RNA binding sites or form ribonucleoprotein (RNP) complexes, as well as the **Kozak sequence** around the start codon, can regulate translation initiation.

- The **3′-UTR** contains binding site for **RBPs** and **microRNAs** that can additionally regulate translation.

Methods Used to study mRNA translational regulation

- Ratio between mRNA and protein levels
- Association with ribosomes (analysis of specific mRNA in polysome profiling or IP with ribosomal proein)
- Reporter genes fused to mRNA regulatory regions

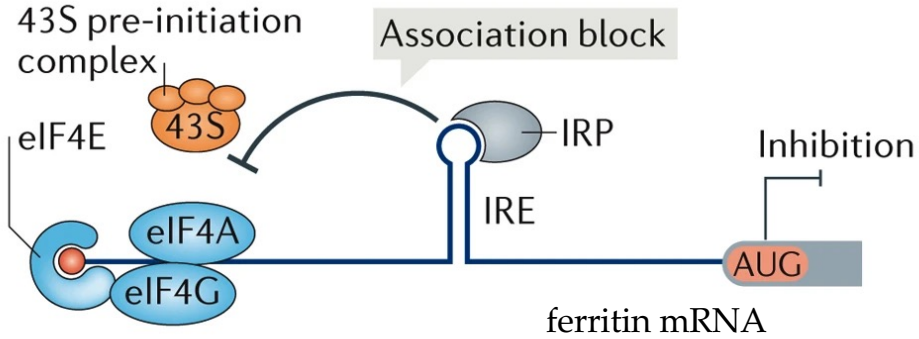
mRNP-specific regulation of the initiation complex assembly

1. Steric blockage
2. Scanning inhibition
3. Interfering with eIF4F complex
4. Cap-independent inhibition

Steric blockage

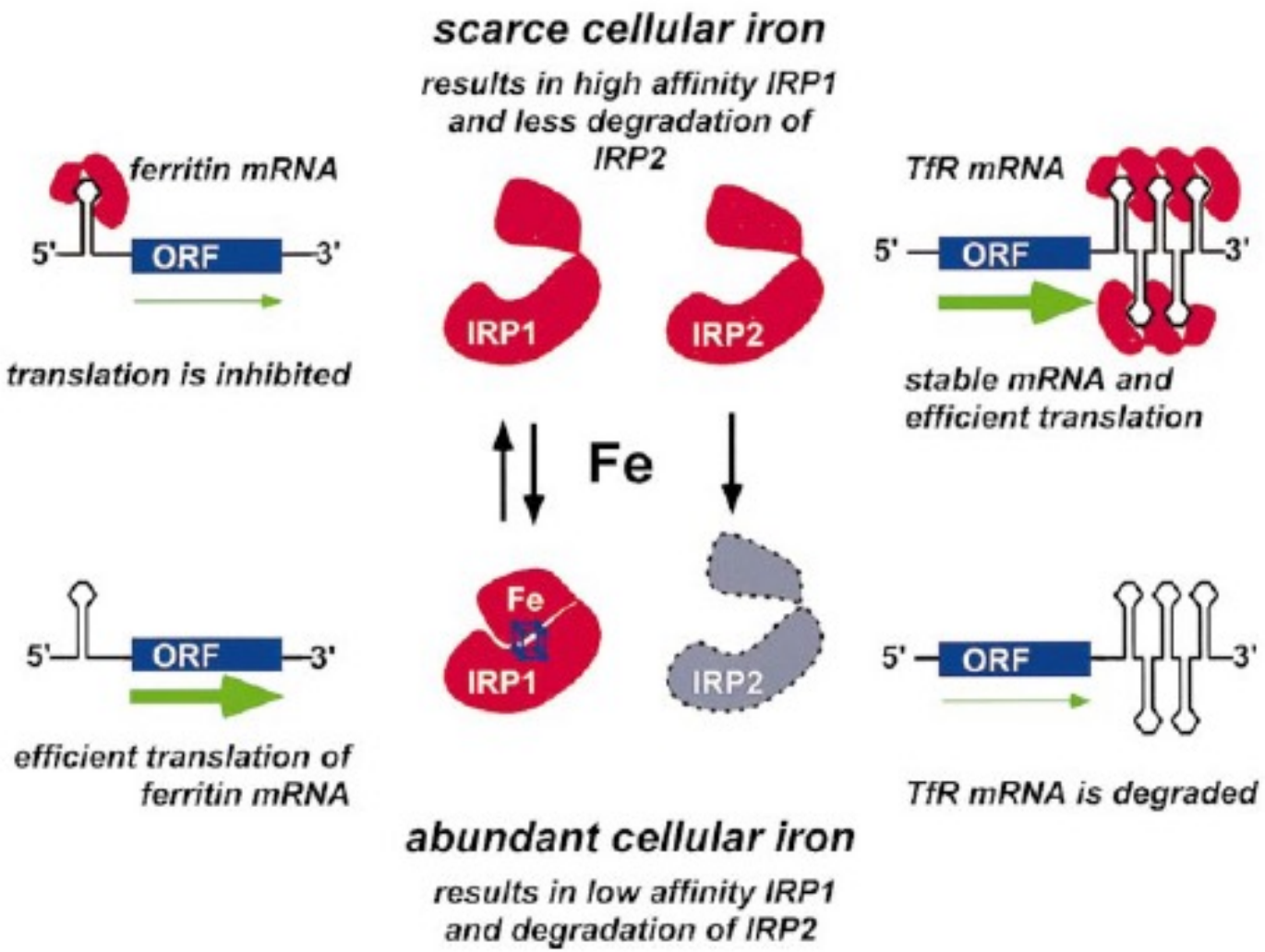
•**Iron Responsive Protein (IRP)** controls iron homeostasis by regulating the translation of the **ferritin**, which is required for cellular iron storage.

•In iron-deficient cells, IRP binds to an **iron-responsive element (IRE)**, in the 5′-UTR of the **ferritin** mRNAs. The IRE is located within 40 nt of the cap structure, and IRP binding blocks the recruitment of the 43S complex to ferritin mRNA that is engaged with the eIF4F complex



Replacing the IRE–IRP interaction by an RNA-binding interaction that involves other proteins with no physiological function in eukaryotic translation can fully recapitulate translational repression

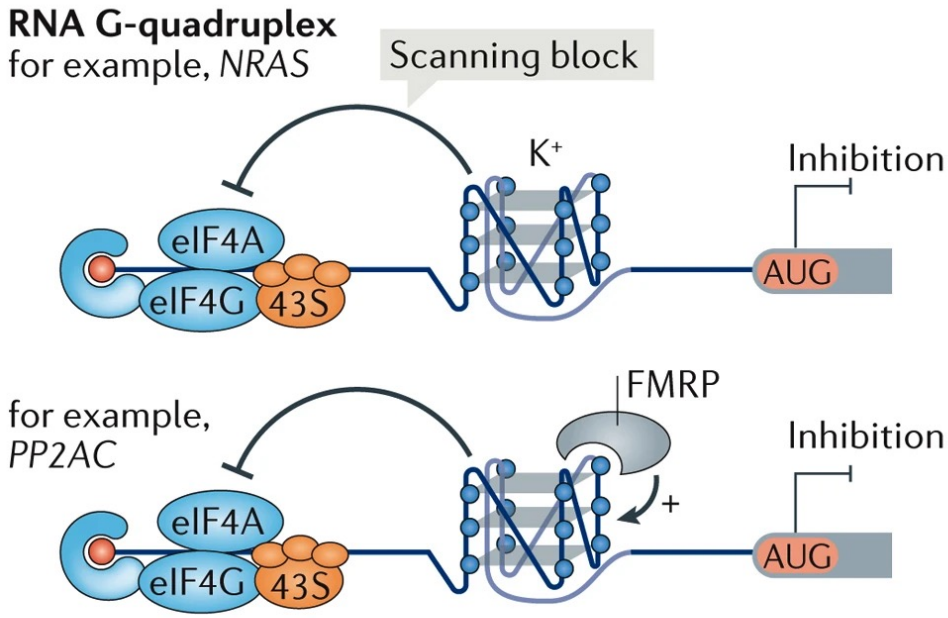
Cellular control of iron



Transferrin receptor (**TfR**) binds **Transferrin**, the carrier of Iron in the plasma

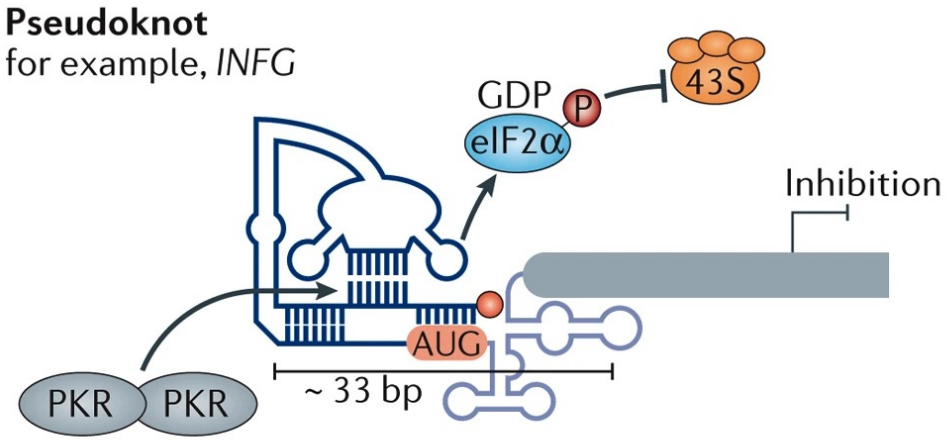
Scanning inhibition

RNA G-quadruplex structures (RG4s) are stable *in vitro*, with melting temperatures that are higher than physiological temperature, especially in the presence of potassium ions (K^+), which are specifically chelated inside G-quartets. As the cytoplasm contains high concentrations of K^+, it has been assumed that RG4s also fold *in vivo*. The formation of RG4 structures — if validated inside cells — would represent the most stable RNA structure that could block ribosome scanning. However, the inhibitory effect of 5′-UTR RG4s on scanning is still speculative. Scanning inhibition is thought to be further increased by recruitment of RG4-stabilizing proteins such as fragile X mental retardation protein (**FMRP)**, which binds to many RG4-harbouring mRNAs.



Scanning inhibition

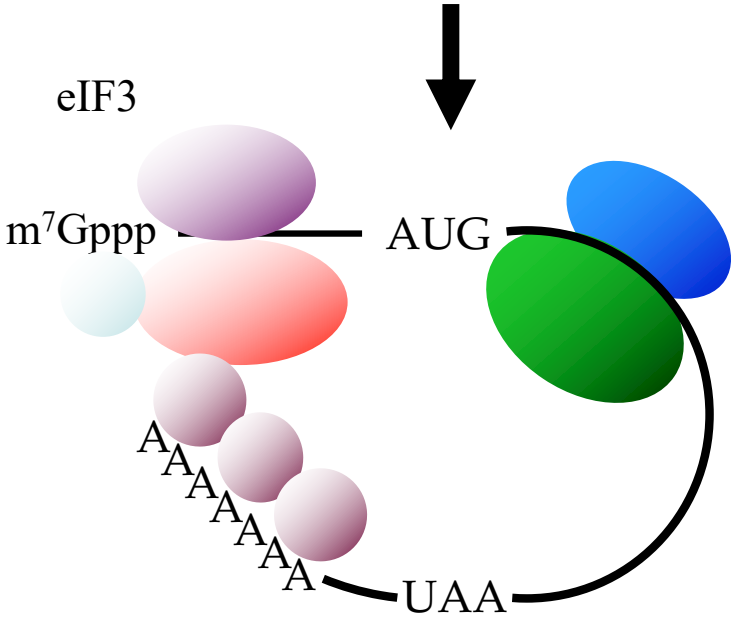
RNA secondary structures can form higher-order interactions to assemble tertiary structures or intermolecular RNA complexes. For example, **pseudoknots** are complex intramolecular RNA structures consisting of at least two intercalated stem–loop structures that form a knot-like three-dimensional shape. A pseudoknot structure conserved across mammals, along with contiguous helices, has been proposed to reside in the 5′-UTR of **human interferon gamma (*IFNG*) mRNA**. This pseudoknot signals to another member of the innate immunity pathway, (PKR), which is induced by interferon. PKR is typically activated by double-stranded RNAs of >33 bp in length, which do not appear naturally in the cell but are commonly generated by viruses during infection. Normally, initiating ribosomes unfold the pseudoknot in the IFNG 5′-UTR. The pseudoknot structure refolds as part of a larger, base-paired RNA structure of sufficient length to attract a PKR dimer. The interaction of PKR with the IFNG 5′-UTR is thought to locally activate the kinase, which phosphorylates eIF2α and results in repression of IFNG translation . Thus, as part of a feedback loop, this RNA structure adjusts translation of its mRNA to PKR activity levels to prevent excess interferon synthesis

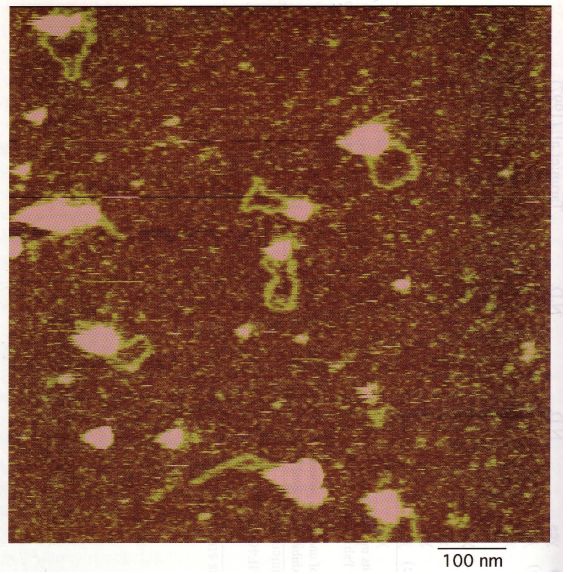


Interfering with eIF4F complex

• PABP binding circularizes the mRNA in translation and allows ribosomal subunits to start new ribosomes.

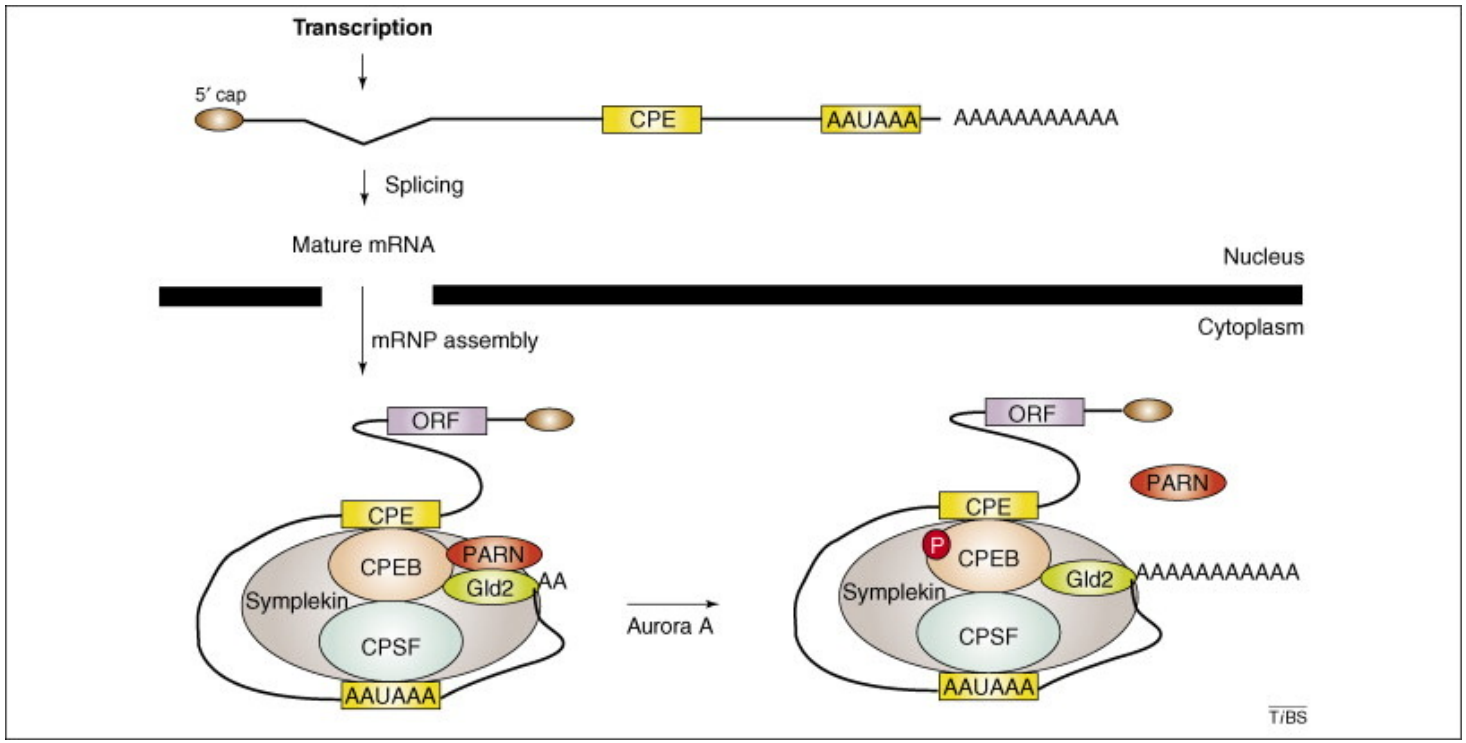
• Some translational regulators that function during embryonic development target the formation of the eIF4F complex by **blocking eIF4F-PABP interaction**





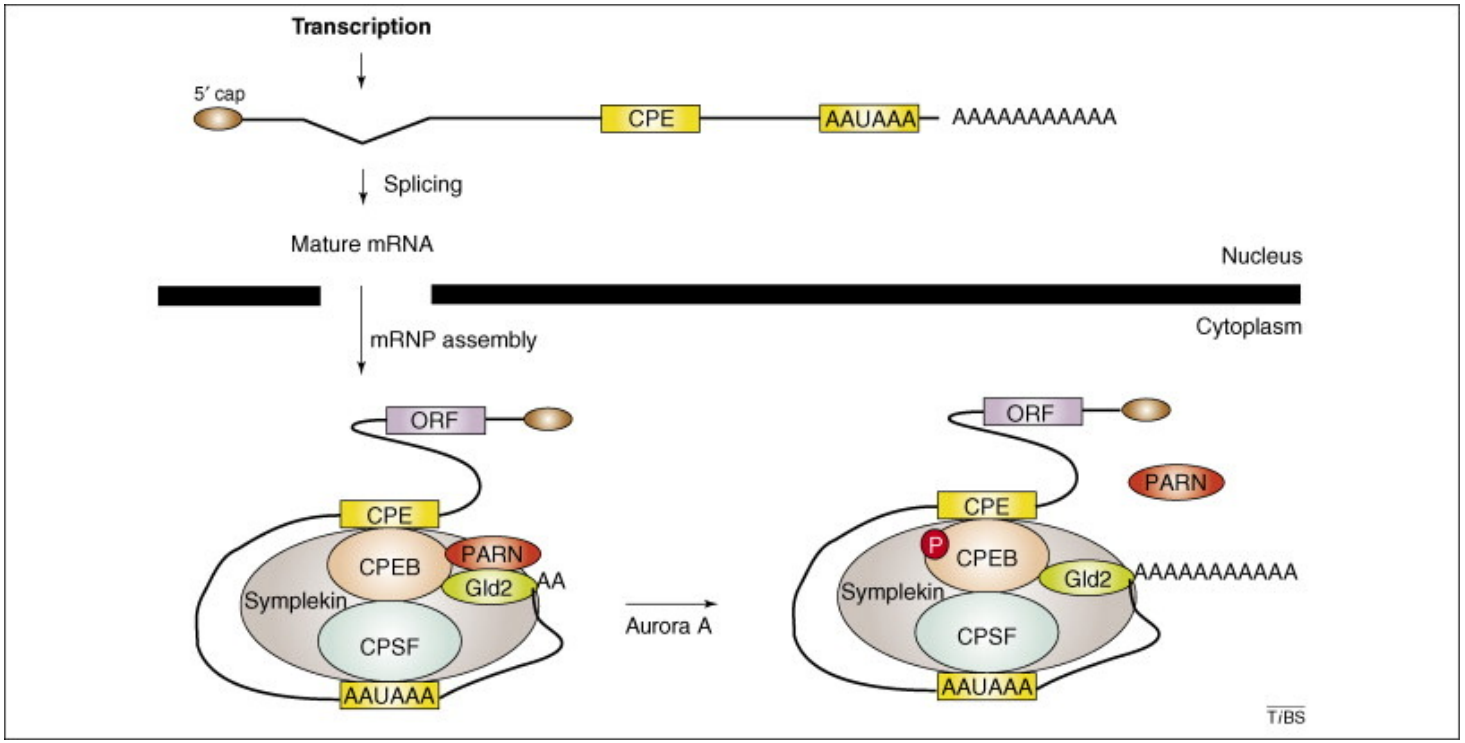
Interfering with the eIF4F complex

The **cytoplasmic-polyadenylation-element-binding protein (CPEB)** regulates the translation of maternal mRNA during vertebrate oocyte maturation and early development. This protein binds to a uridine-rich sequence — the **cytoplasmic polyadenylation element (CPE)** — that is located in the 3' UTR of target mRNAs and promotes both silencing of the mRNA before oocyte maturation as well as subsequent cytoplasmic polyadenylation and translational activation



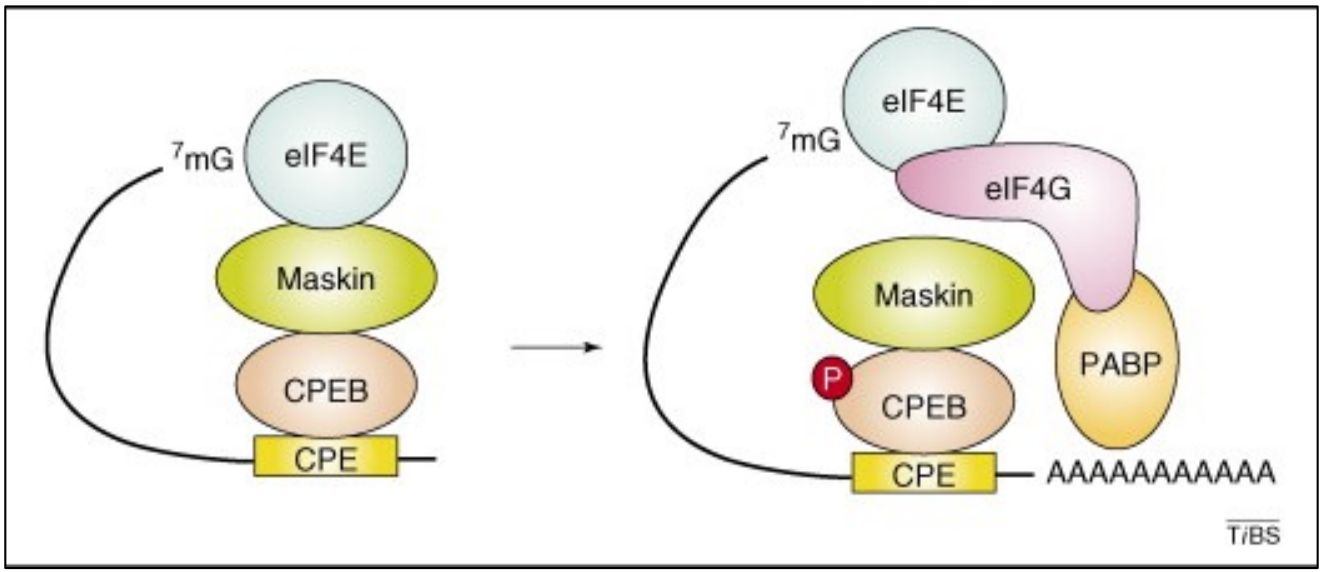
Interfering with the eIF4F complex

Following splicing and RNA export, the CPEB-containing RNAs assemble into a ribonucleoprotein (RNP) complex that is nucleated by CPEB. The other factors in this complex include: **CPSF**, which recognizes the AAUAAA polyadenylation hexanucleotide; **PARN**, a deadenylating enzyme; **Gld2**, a poly(A) polymerase; and **symplekin**, a scaffold protein. Although PARN and Gld2 are both active, PARN activity is more robust; it thus removes the poly(A) tail and keeps it short although Gld2 continues to catalyze polyadenylation. Upon the induction of oocyte maturation, the **kinase Aurora A** phosphorylates CPEB Ser174, which causes the expulsion of PARN from the RNP complex. Thus, by default, Gld2 elongates.

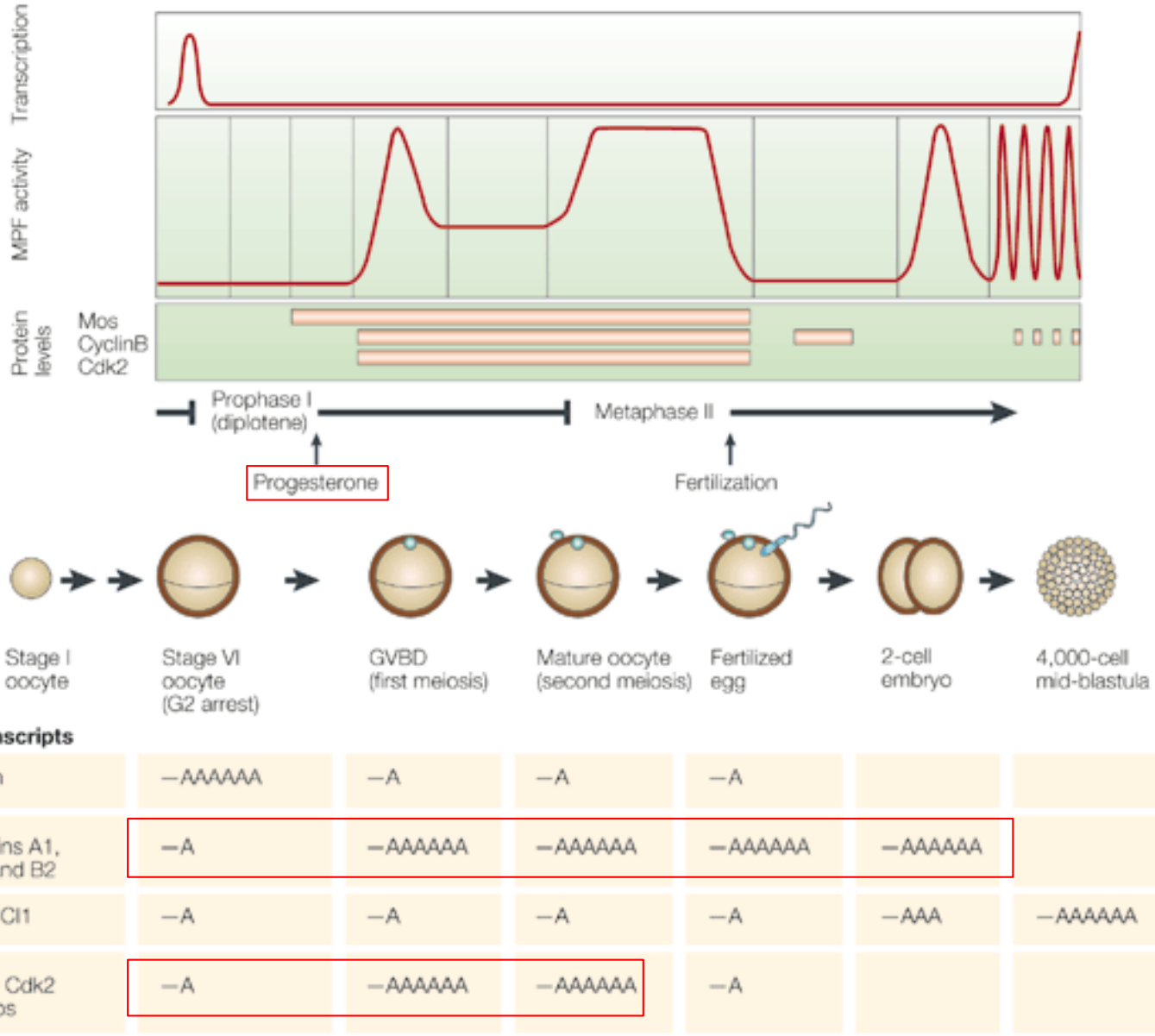


Interfering with the eIF4F complex

CPEB associates with both CPE-containing mRNAs and **Maskin**. Maskin, in turn, interacts with the cap (m^7G)-binding factor eIF4E. In this configuration, Maskin binding to eIF4E precludes eIF4G from binding eIF4E, thus, inhibiting translation. Following CPEB phosphorylation and polyadenylation, PABP binds the newly elongated poly(A) tail; PABP also binds eIF4G and helps it displace Maskin from eIF4E. Because eIF4G is indirectly associated with the 40S ribosomal subunit, translation initiation proceeds.



Oocytes synthesize and store a complex population of mRNAs. Maturation is accompanied by a cessation of transcription and a complex network of translational activation and repression of stored maternal mRNAs

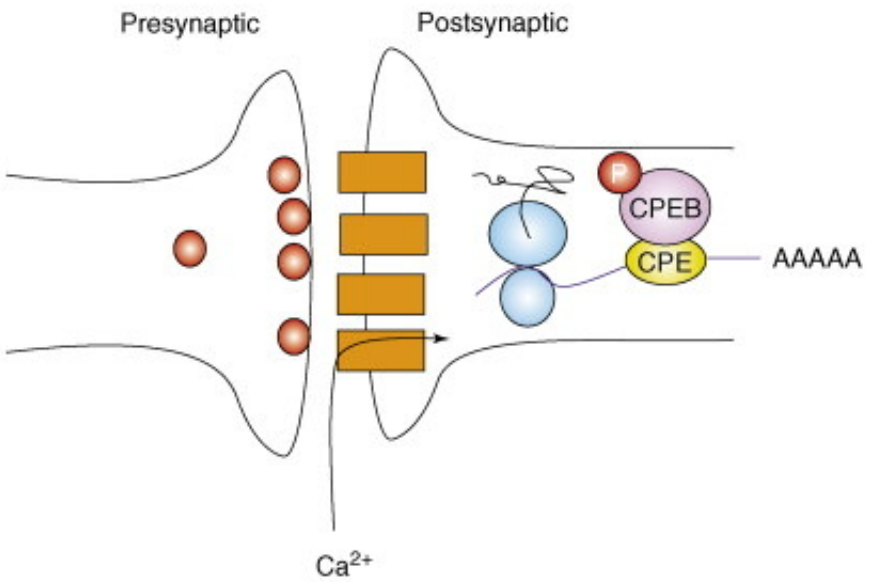


regulated by CPEB

Interfering with the eIF4F complex

CPEB control of synaptic plasticity and learning and memory.

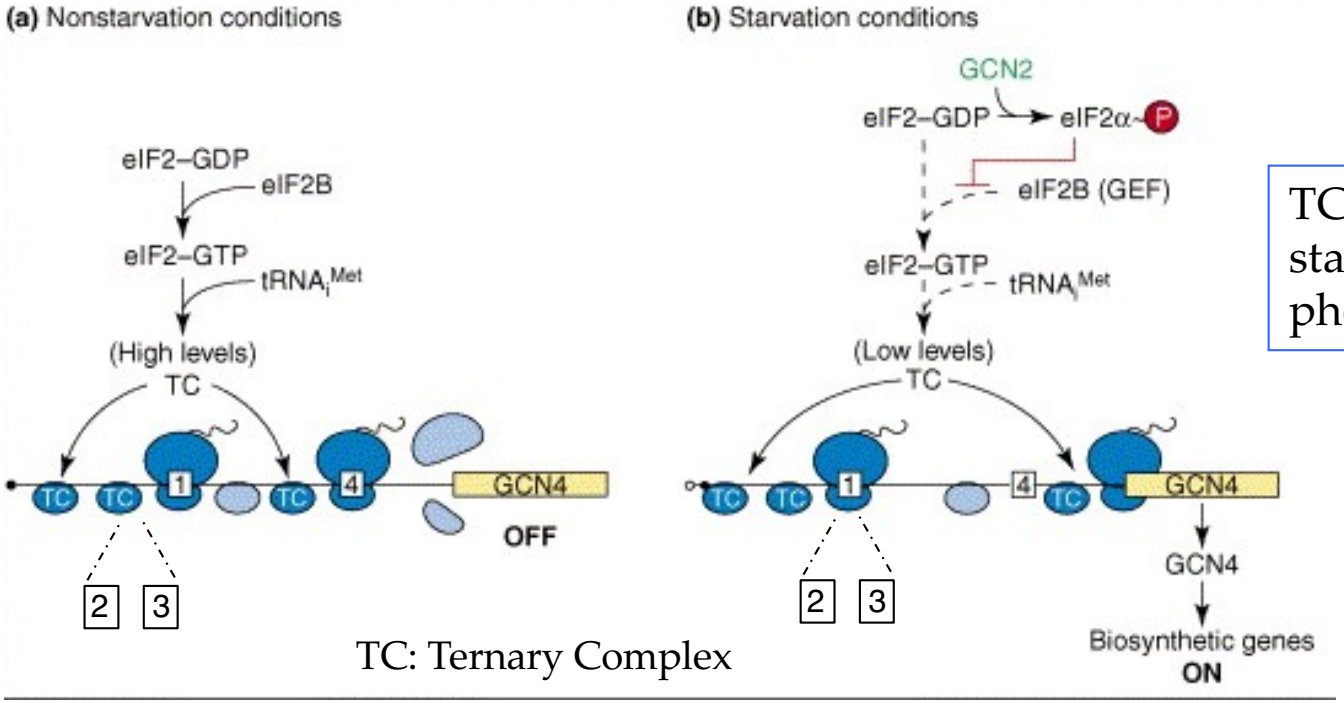
Presynaptic vescicles release N-methyl-D-aspartate (NMDA), which interacts with NMDA receptors on the postsynaptic membrane (orange blocks). The NMDA receptors enable calcium entry into the synapse, which leads to CPEB phosphorylation, polyadenylation and translation of CPE-containing RNAs. The protein products of these mRNAs might then serve as synaptic tags to distinguish naïve from experienced synapses.



Short ORFs regulate translation of the main ORF

GCN4 (transcriptional activator of amino acid biosynthetic genes)

After translation of uORF1 (only 3 codons), a large fraction of the 40S subunits remain attached to the mRNA and resume scanning. (a) **Under normal conditions**, the 40S subunit quickly rebinds TC and reinitiates translation at uORF2, uORF3 or uORF4.The 80S ribosome dissociates from the mRNA after terminating at uORF4, leaving the GCN4 ORF untranslated. (b) **Under conditions of amino acid starvation**, many 40S ribosomes fail to rebind TC until they have scanned past uORF4, because the TC concentration is low and the rate of TC loading is diminished; thus, translation reinitiation occurs at GCN4 instead.

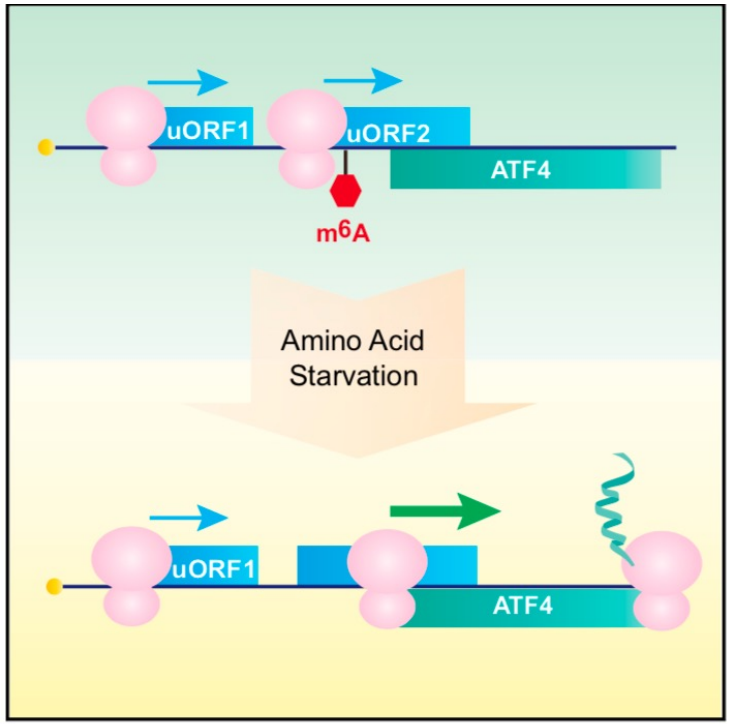


TC: Ternary Complex

TC levels are reduced in starved cells by phosphorylation of eIF2α

Short ORFs regulate translation of the main ORF

ATF4 (activating transcription factor 4)
ATF4 is induced during the **integrated stress response (ISR)** that is activated following various cellular stresses.
In response to amino acid starvation, the reinitiation of ATF4 is not only governed by the eIF2 signaling pathway, but is also subjected to regulation by m^6A. The translation of uORF2 depends on its m^6A methylatin. While depleting m^6A demethylases (high uORF2 m^6A) represses ATF4 reinitiation, knocking down m^6A methyltransferases low uORF2 m^6A) promotes ATF4 translation.



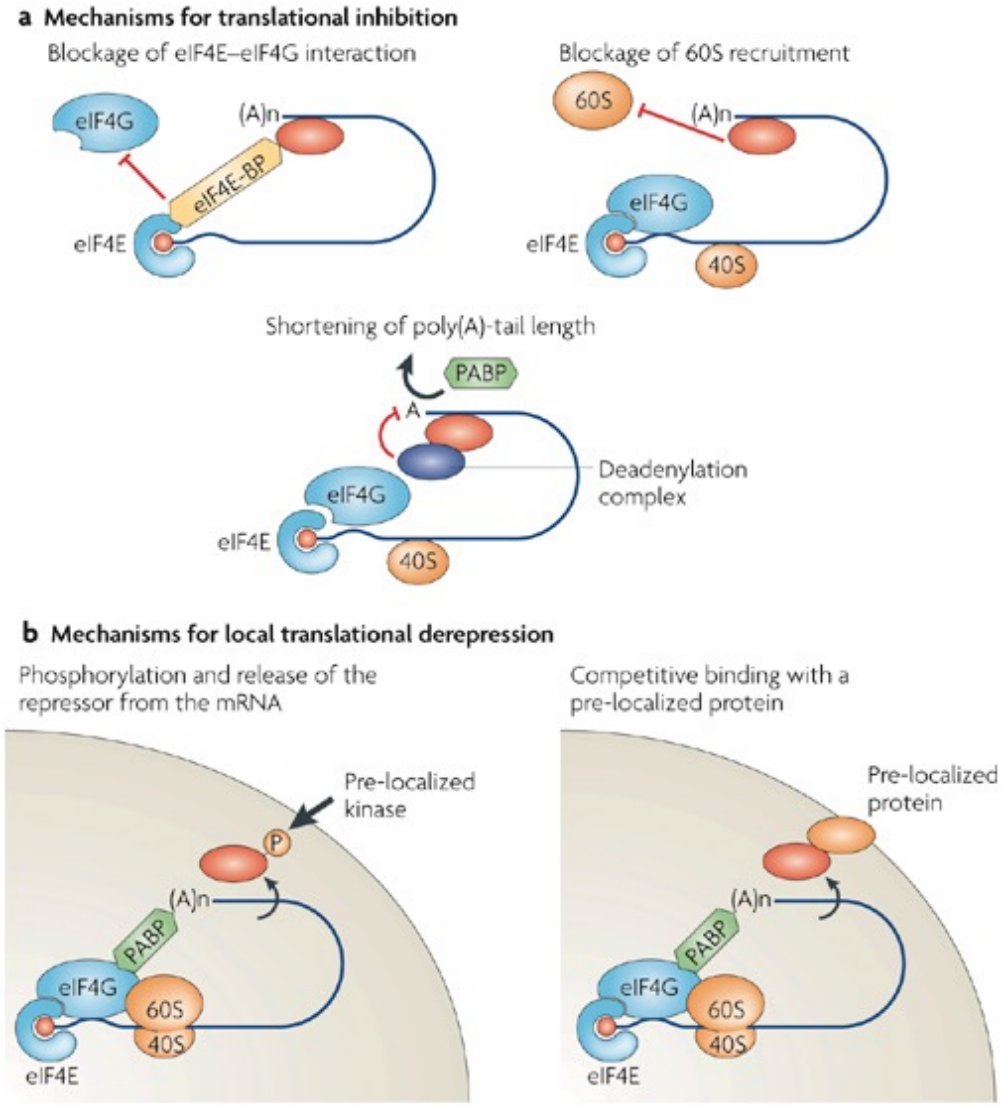
Zhou et al., 2018 Mol Cell

Translational control of localized mRNAs

•Translation of localizing mRNAs is silenced during their transport and is activated when they reach their final destination

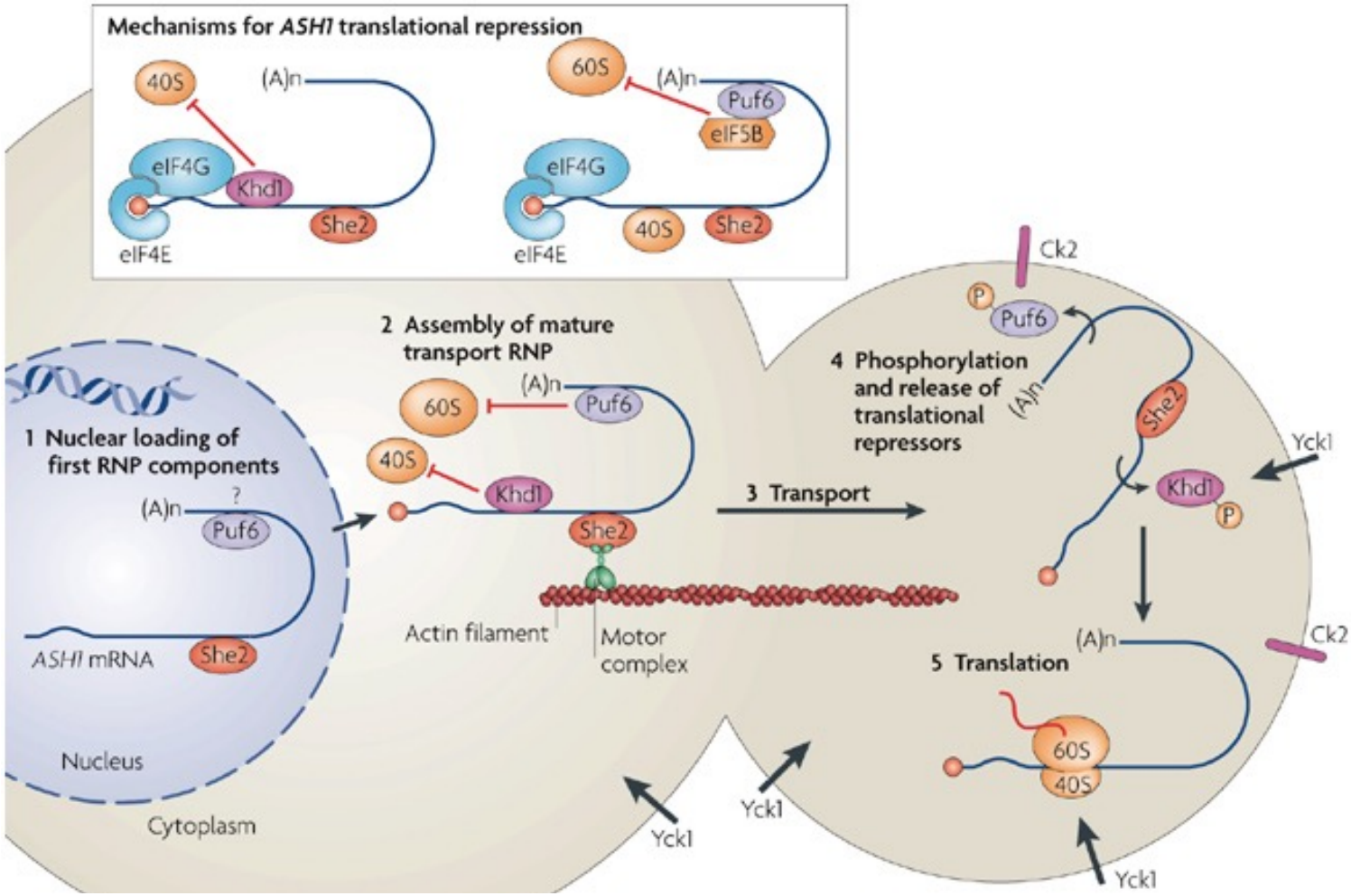
•Translation of these mRNA is controlled by repressors, which are specifically recruited to transport ribonucleoprotein particles and block translation at different steps

•Inactivation of these repressors, either by pre-localized proteins or in response to conserved signalling pathways, triggers local protein synthesis



ASH1 mRNA localization to the tip of the daughter cell in yeast

•Localization-dependent translational activation of ASH1 mRNA, which encodes a repressor of mating-type switching, ensures its restriction to the daughter cell and thus the generation of two cells of distinct types, a prerequisite for mating



Signal-induced translational activation

a) Regulation of general translation factors: the cap-dependent **eIF4E** and **eIF4E-BP** are phosphorylated by the **ERK** and **mTOR** signalling pathways, leading both to changes in global translation rate and mRNA-specific translational activation. In addition, phosphorylation of eukaryotic elongation factor-2 (eEF2) is controlled by various stimuli and modulates these two processes.

b) Regulation of mRNA-specific repressors: the translational repressors and transport factors is differentially regulated in response to selective stimuli.