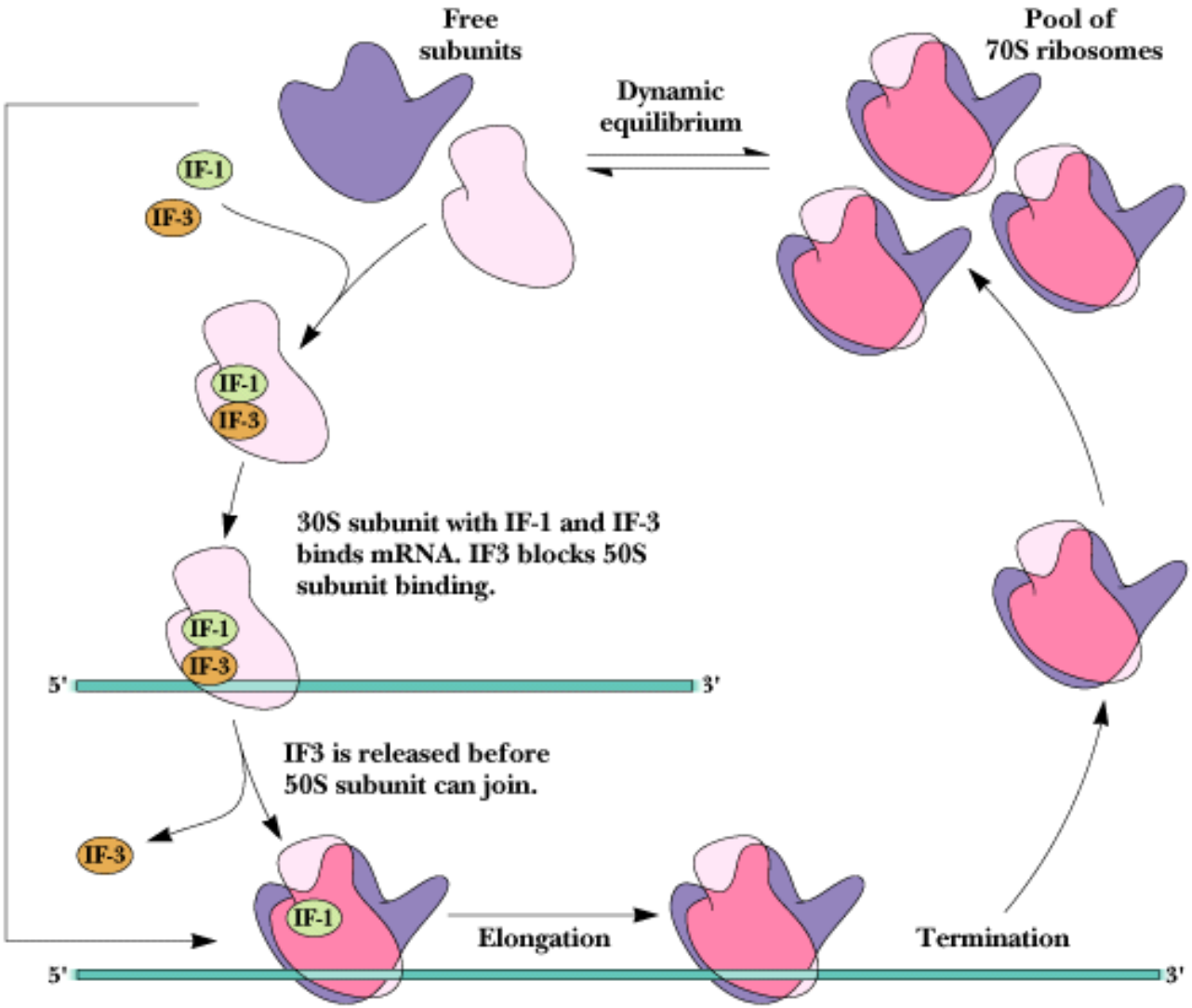
# Translational control

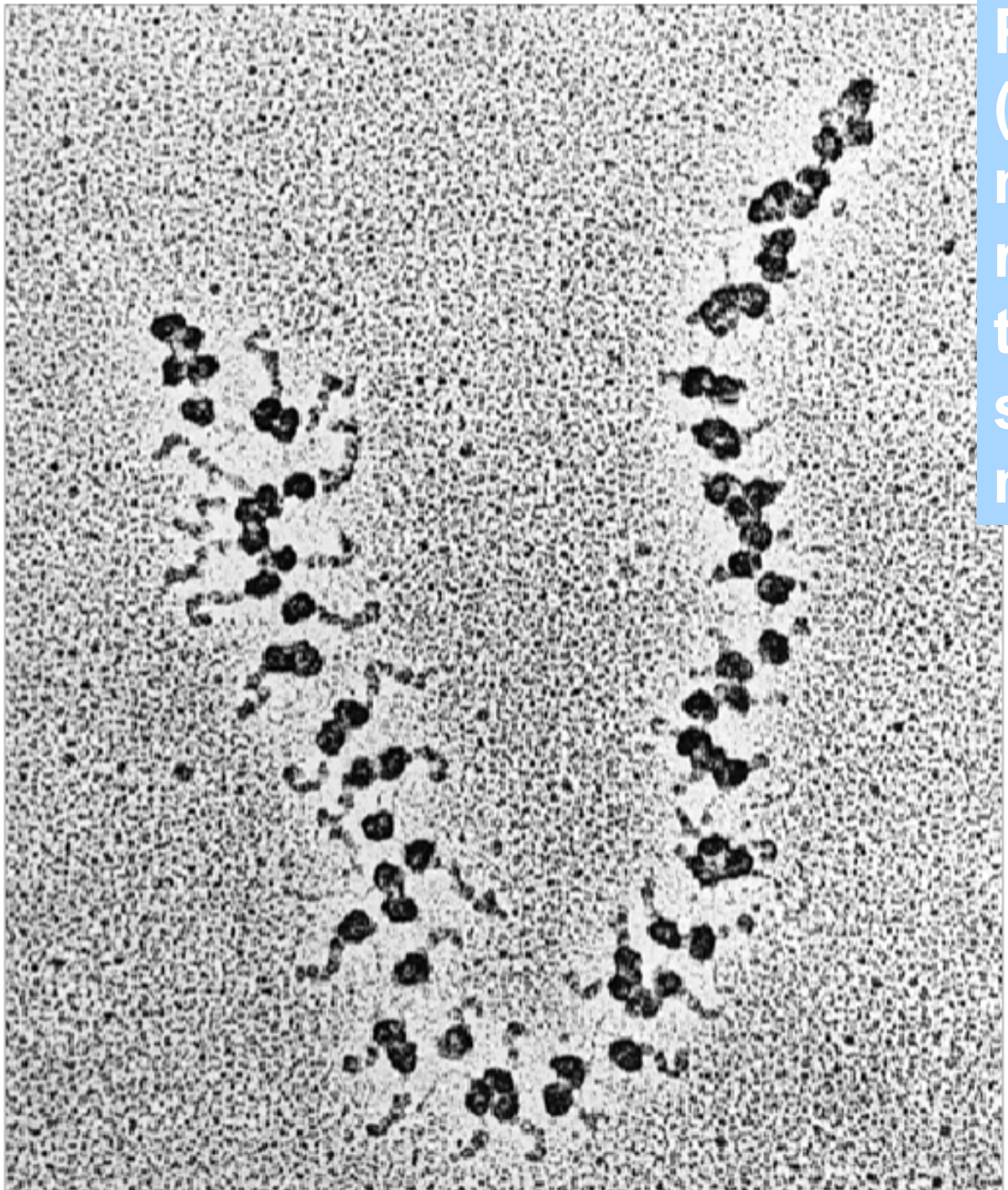
Gerardo Ferbeyre

BCM6026GF1

# Translation




Saunders College Publishing



**Polyribosomes (polysomes) - multiple ribosomes translating the same mRNA molecule**

# Translation in numbers

- M. genitalum, the smallest known cellular genome encodes 480 proteins, 101 function in translation. Together with tRNAs and rRNAs 45% of genes are devoted to translation in this organism.
- 44% of human body in dry weight is protein
- 75% of the energy budget goes for translation!
- rRNA and tRNA genes are often repeated, Number of repeats correlates with the size of the genome.
- Humans possess 2,000 copies of the 5S rDNA gene in a single cluster on chromosome 1. There are 280 copies of a repeat unit comprising the 28S, 5.8S and 18S rDNA genes grouped into 5 clusters of 50-70 repeats , one each on chromosomes 13, 14, 15, 21 and 22.

# Translation rate (FSR)

Isotopes (H3-leucine

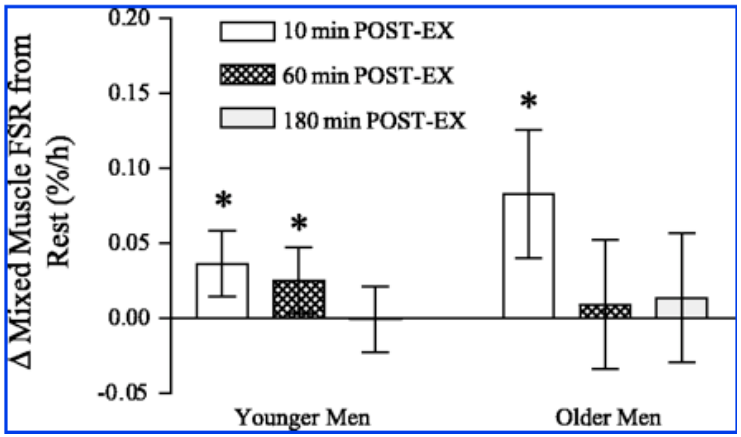
Amino acid pool
SA

TCA
precipitation

HCL hydrolysis

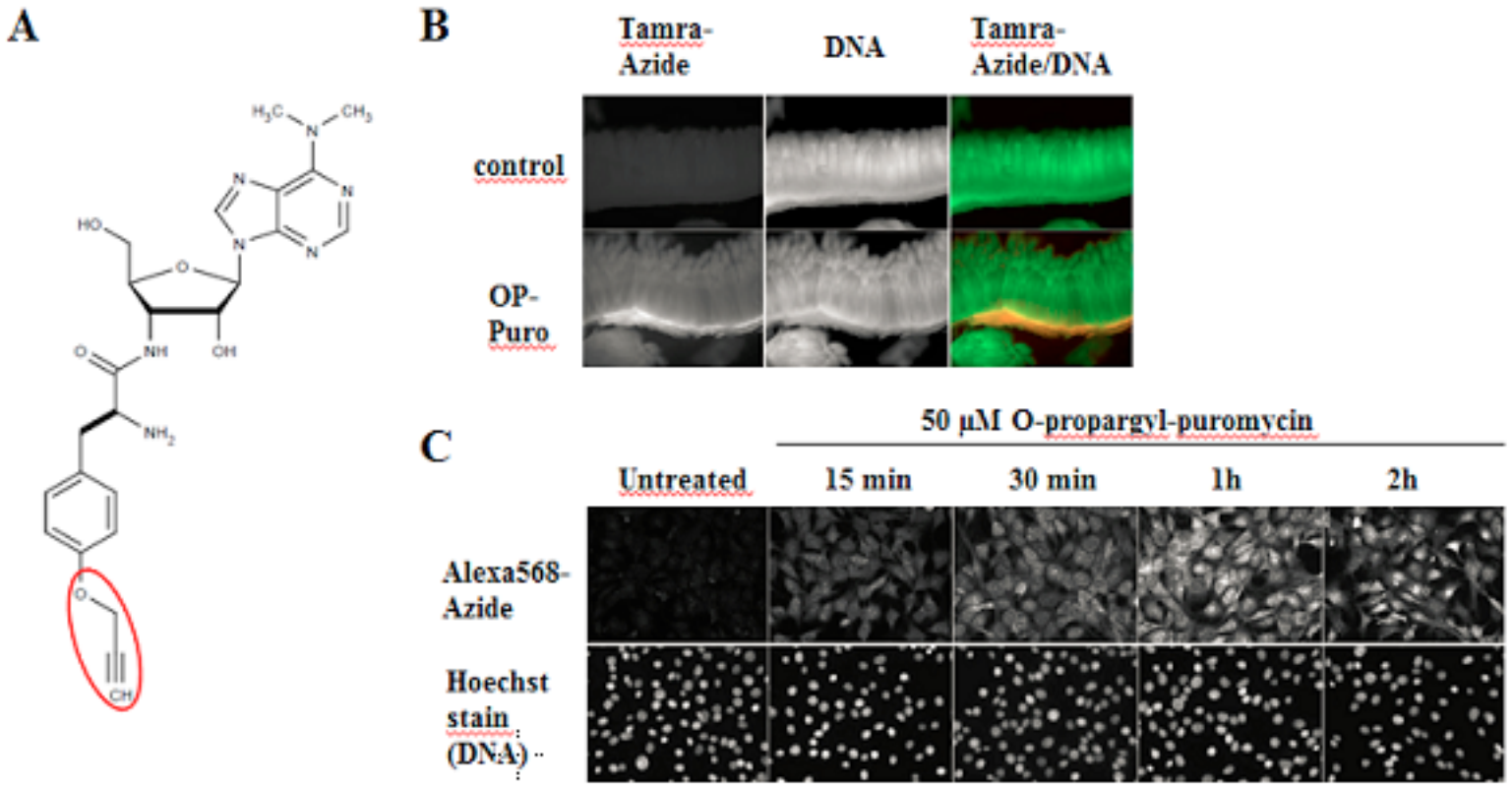
Protein
(aa incorporated)
SB

Fractional synthetic Rate (FSR)
FSR= SB/SA x t x 100 (%/day)




Am J Physiol Endocrinol Metab. 2004 Sep;287(3):E513-22.

# O-Propargyl-puromycin: Non-radioactive monitoring of nascent protein synthesis

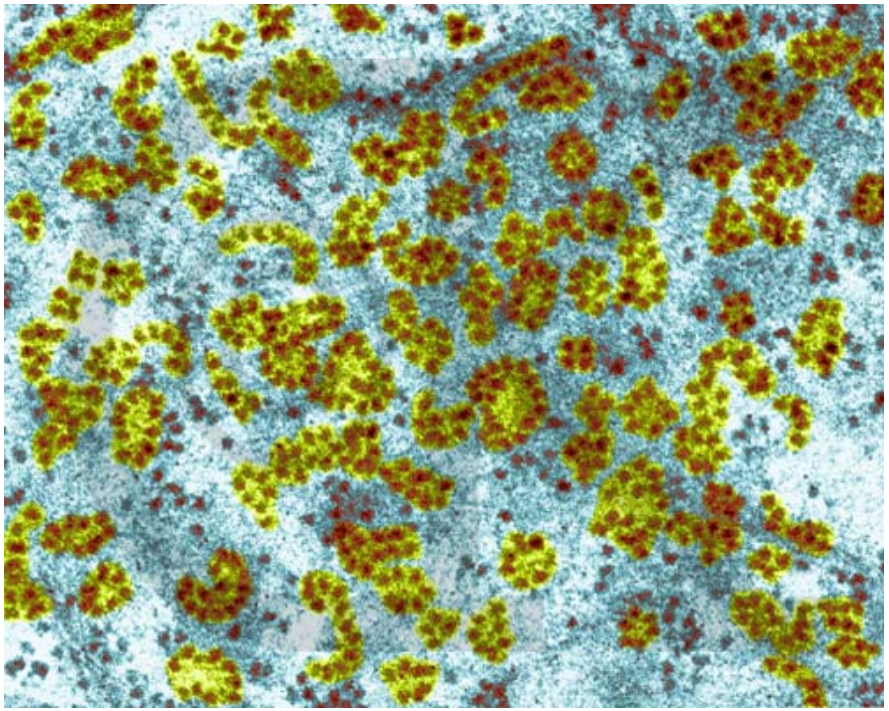



The resulting C-terminal alkyne labeled proteins can be detected via Cu(I)-catalyzed click chemistry that offers the choice to introduce a Biotin group (**Azides of Biotin**) for subsequent purification tasks or a fluorescent group (**Azides of fluorescent dyes**) for subsequent microscopic imaging

Liu *et al*. (2012) Imaging protein synthesis in cells and tissues with an alkyne analog of puromycin. *Proc. Natl. Acad. Sci. USA* **109(2)**:413.

# How many ribosomes are present in a single cell?

- A rapidly growing yeast cell contains 200,000 ribosomes, 40% of cyt vol. Yeast should produce 2000 ribosomes/min to grow.
- $10^7$ ribosomes in a single liver cell



- A process of such magnitude should be controlled, but where?
- Under steady state conditions #Init = # termination, therefore the rate of protein synthesis is determined by the rate of Initiation.

# What determines the number of initiation events?

- Amount of mRNA: total mRNA in the cytoplasm is in excess (30% of mRNA in cells is not bound to ribosomes)
- Abundance of ribosomes
- Activity and levels of any component of the translational apparatus: many translation factors are regulated by phosphorylation
- Most translational regulation occurs at the levels of initiation but some mRNAs are regulated at the level of elongation
- Individual mRNAs differ greatly in their translational efficiency: mRNA structure

# Why to control translation?

Logic will suggest to control transcription, which is the first step in gene expression. But…..

• Speed: in eukaryotes many other steps separate transcription from translation. Some genes are very large. Translation control is faster. Development requires rapid decisions

• Fine control: adjust protein levels to an optimun difficult to attain only by transcriptional regulation

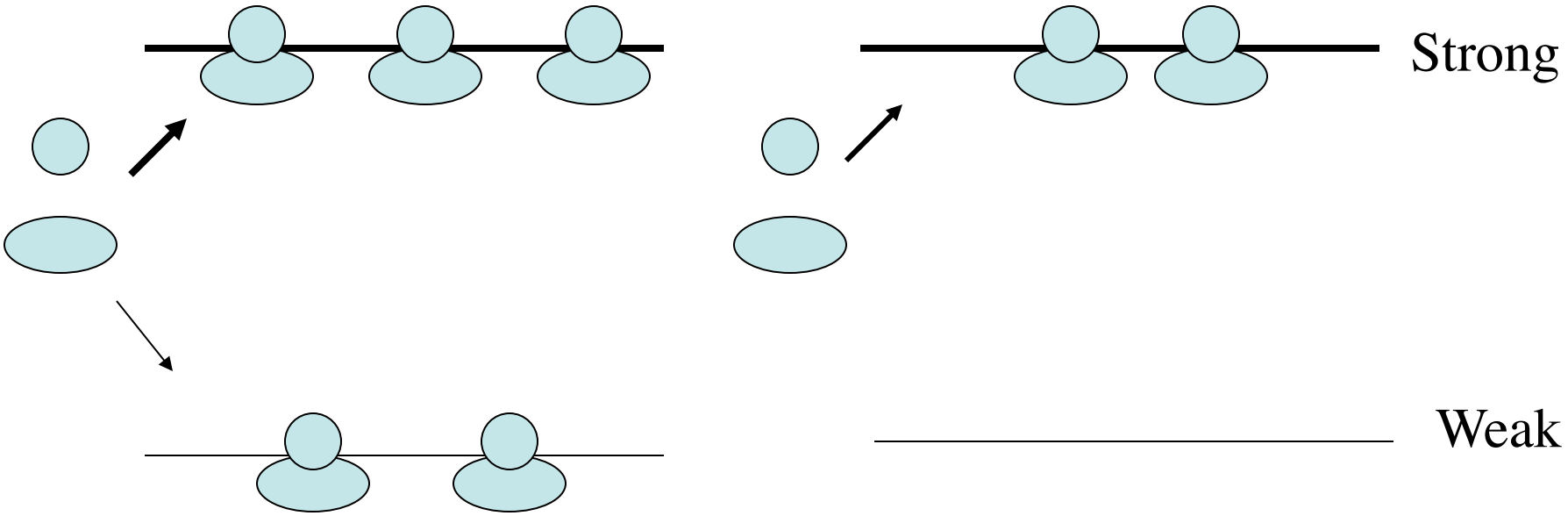
• Spatial control: local translation in synapsis (locasomes)

• In situations where there is no transcription (platelets, reticulocytes, early development)

# Global regulation vs. individual regulation

- Rapidly growing tissues have more ribosomes
- p53 and retinoblastoma (RB) repress Pol I and Pol III transcription. In cancer cells, which harbour inactivating mutations in these tumour suppressors, deregulation of Pol I and Pol III activity might contribute to tumorigenesis.
- Can global changes in translation efficiency have an impact on individual mRNAs translation?

# Weak vs Strong genes

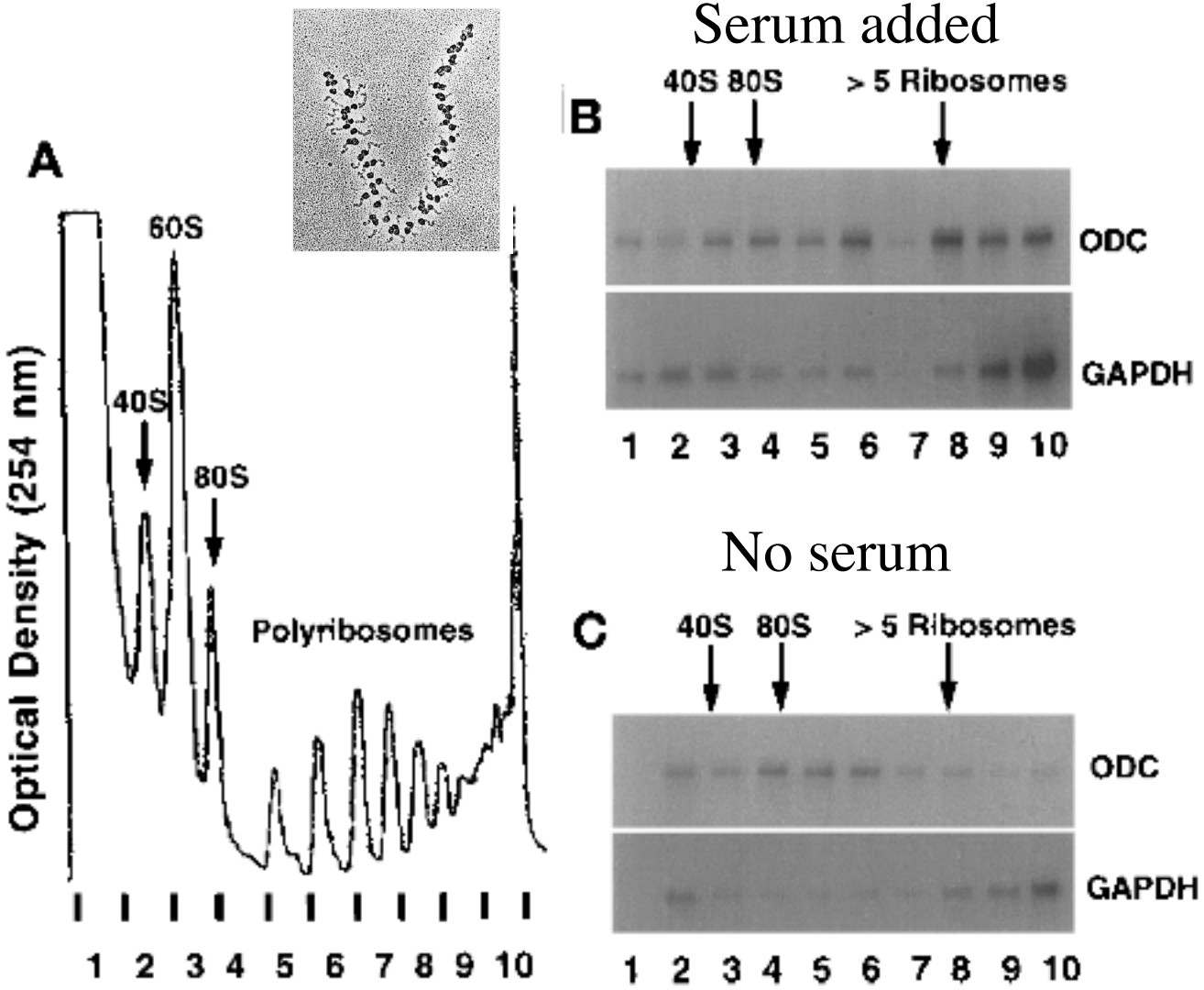



High efficiency

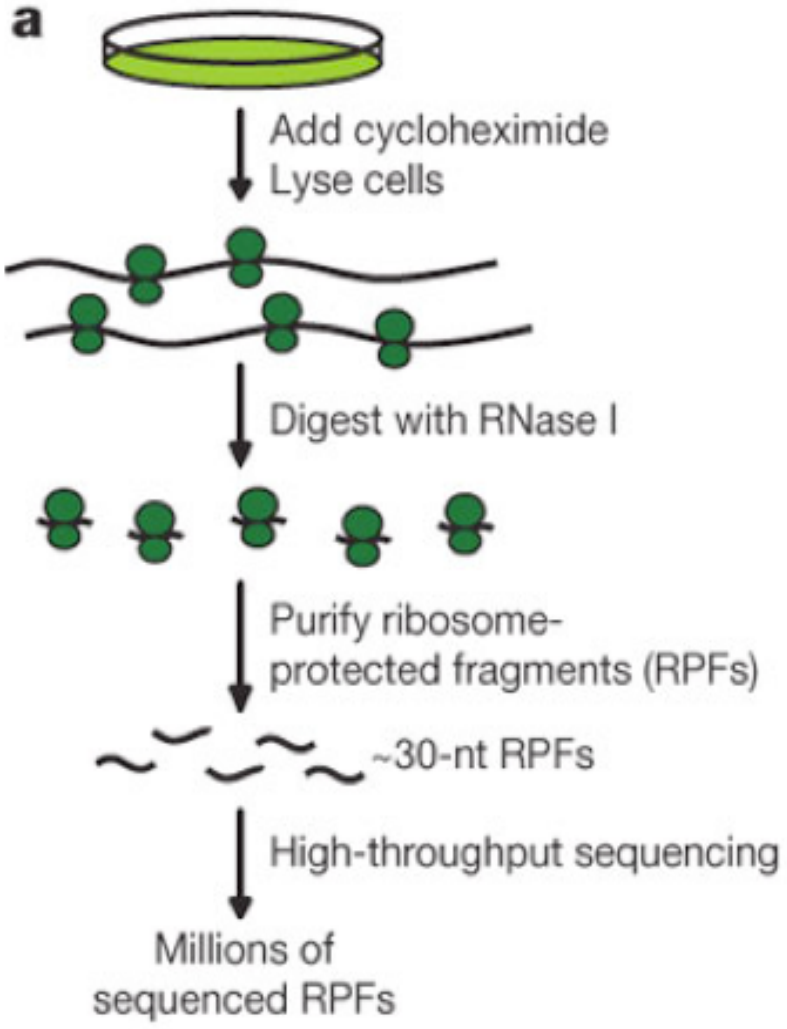
Low efficiency

The question is how the cell regulates translational efficiency?

# Polysome distribution of ODC mRNA



# Ribosome profiling

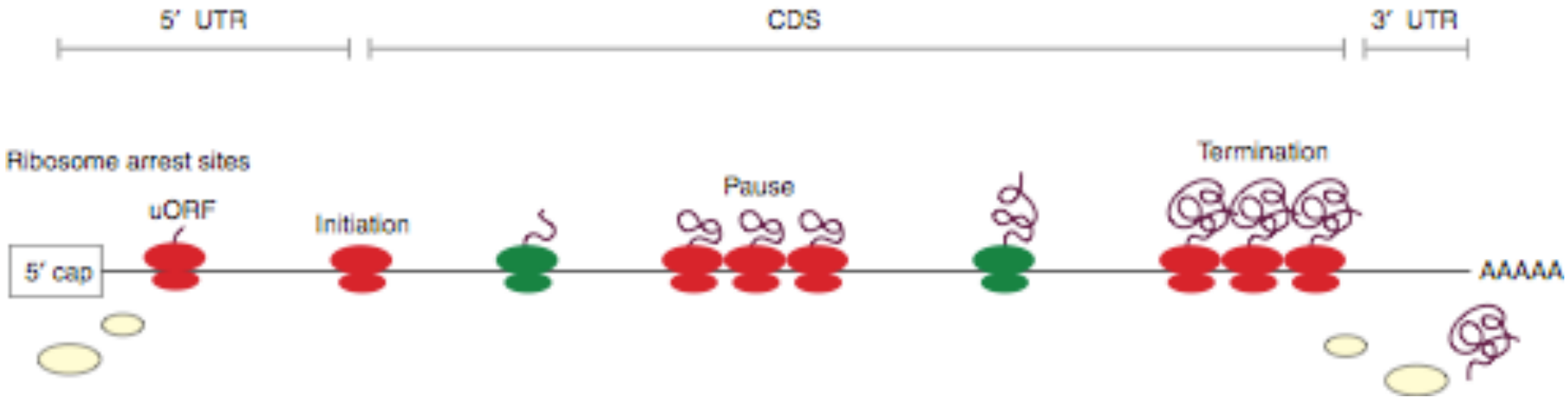



-Eukaryotic ribosomes carrying out translation protected around 30 nucleotides of mRNA sequence from digestion by Rnase.

- Ingolia and colleagues implemented an intramolecular ligation strategy to generate directional, unbiased cDNA libraries for sequencing ribosome-protected RNA fragments.

- Guo et al (picture) used RNA seq

# RPF to study translational control




-RPF density was highest at the start and stop codons, reflecting known pauses at these positions

Ingolia et al found apparent abundance of uORFs with non-AUG starts throughout the yeast transcriptome.

Translation divided in 3 steps

Cells spend many resources for translation

Initiation as the most important control step

Logic behind translational control

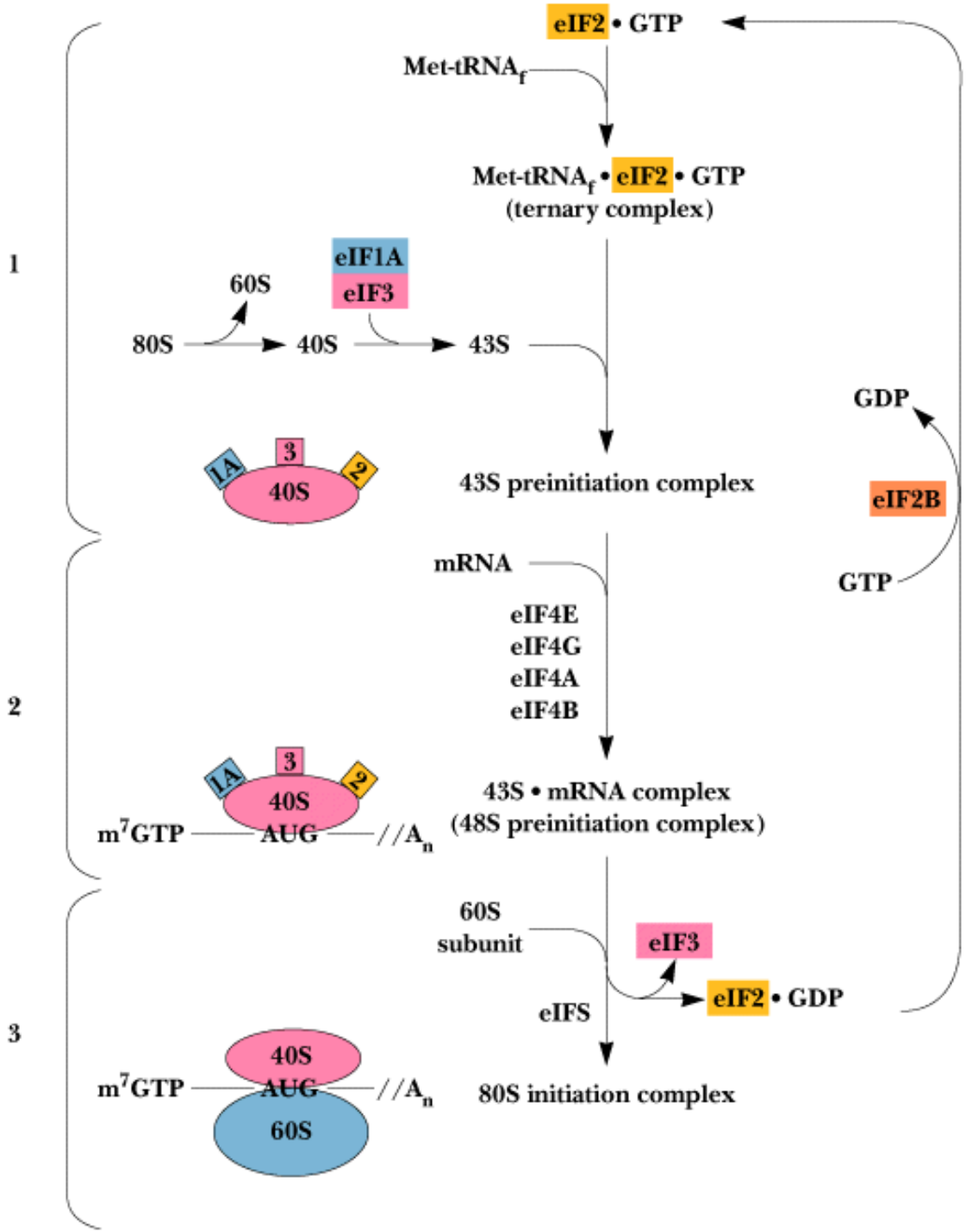
Globalization affects individuals

Weak and Strong RNAs

Polysome gradient

Ribosome profiling

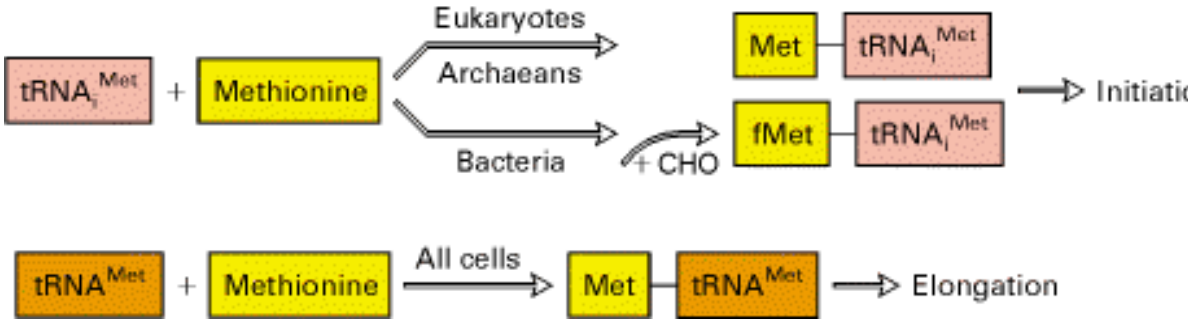
# Initiation of translation in eukaryotes

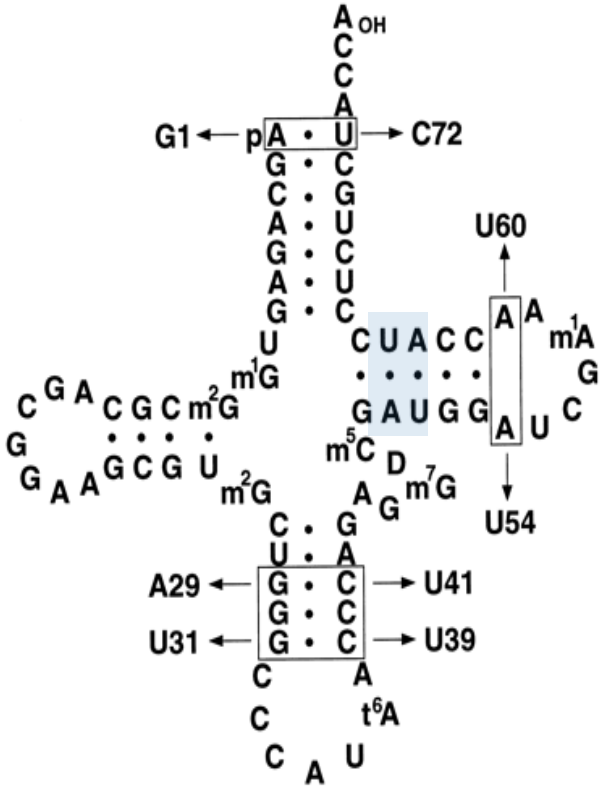


# Eukaryotic Initiation

- Begins with formation of ternary complex of eIF-2, GTP and Met-$tRNA_i^{Met}$
- This binds to 40S ribosomal subunit: eIF-3:eIF1A complex to form the 43S preinitiation complex
- No mRNA yet, so no codon association with Met-$tRNA_i^{Met}$
- mRNA then adds with several other factors, forming the 48S pre-initiation complex
- ATP is required!
- Based mostly on protein-protein and protein RNA interactions

# Two types of methionine tRNA are found in all cells.

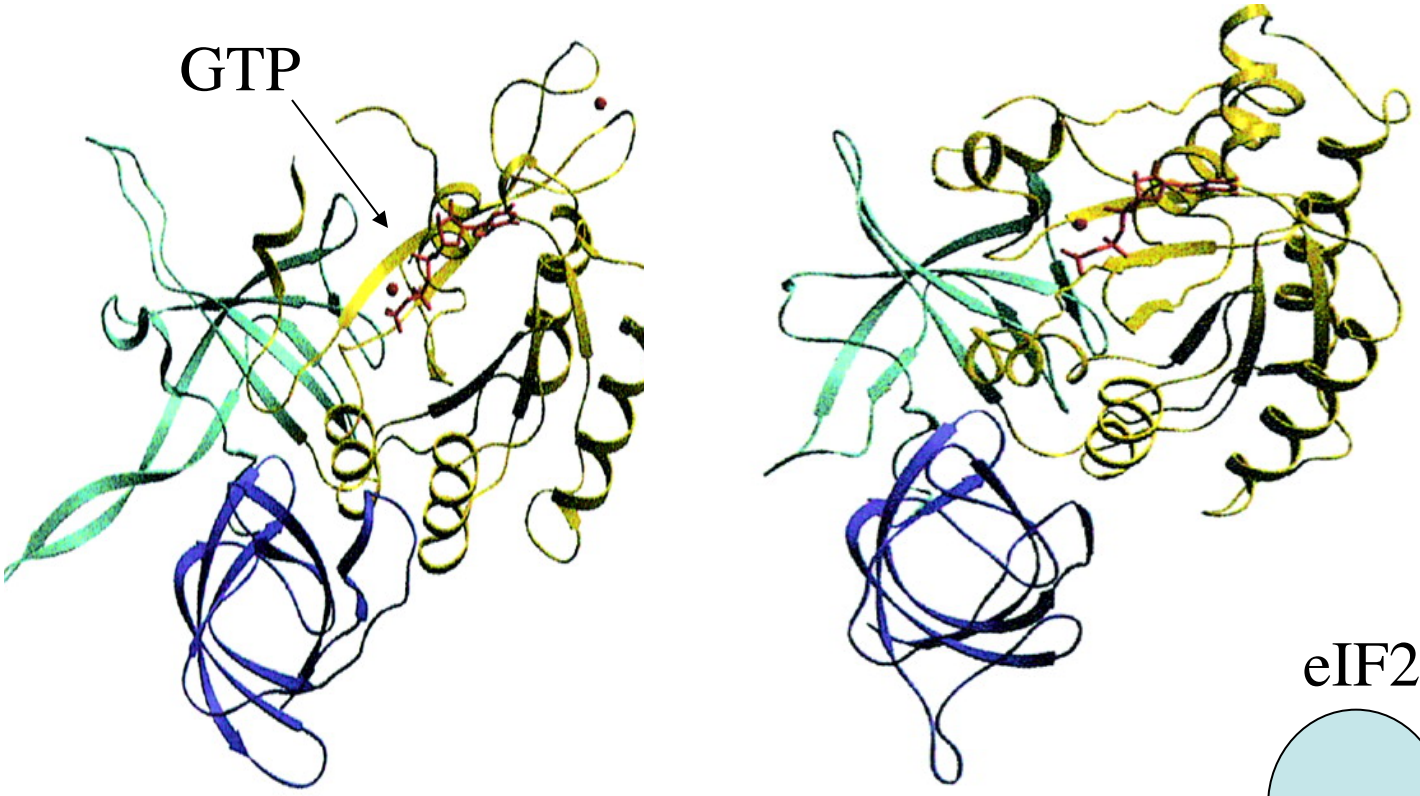



tRNAiMet, is used exclusively to start protein synthesis

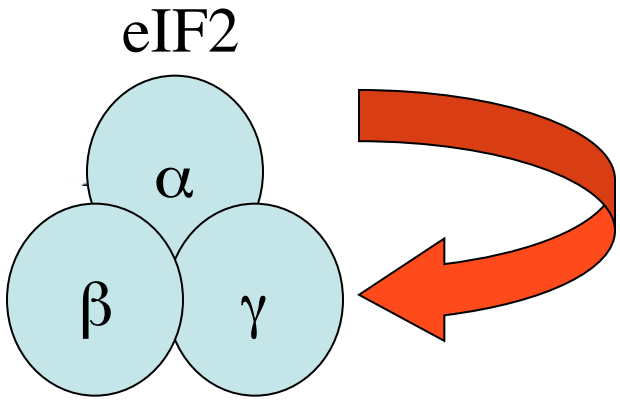
tRNAMet, delivers methionine to internal sites in a growing protein chain.

# eIF2 is a GTP binding protein

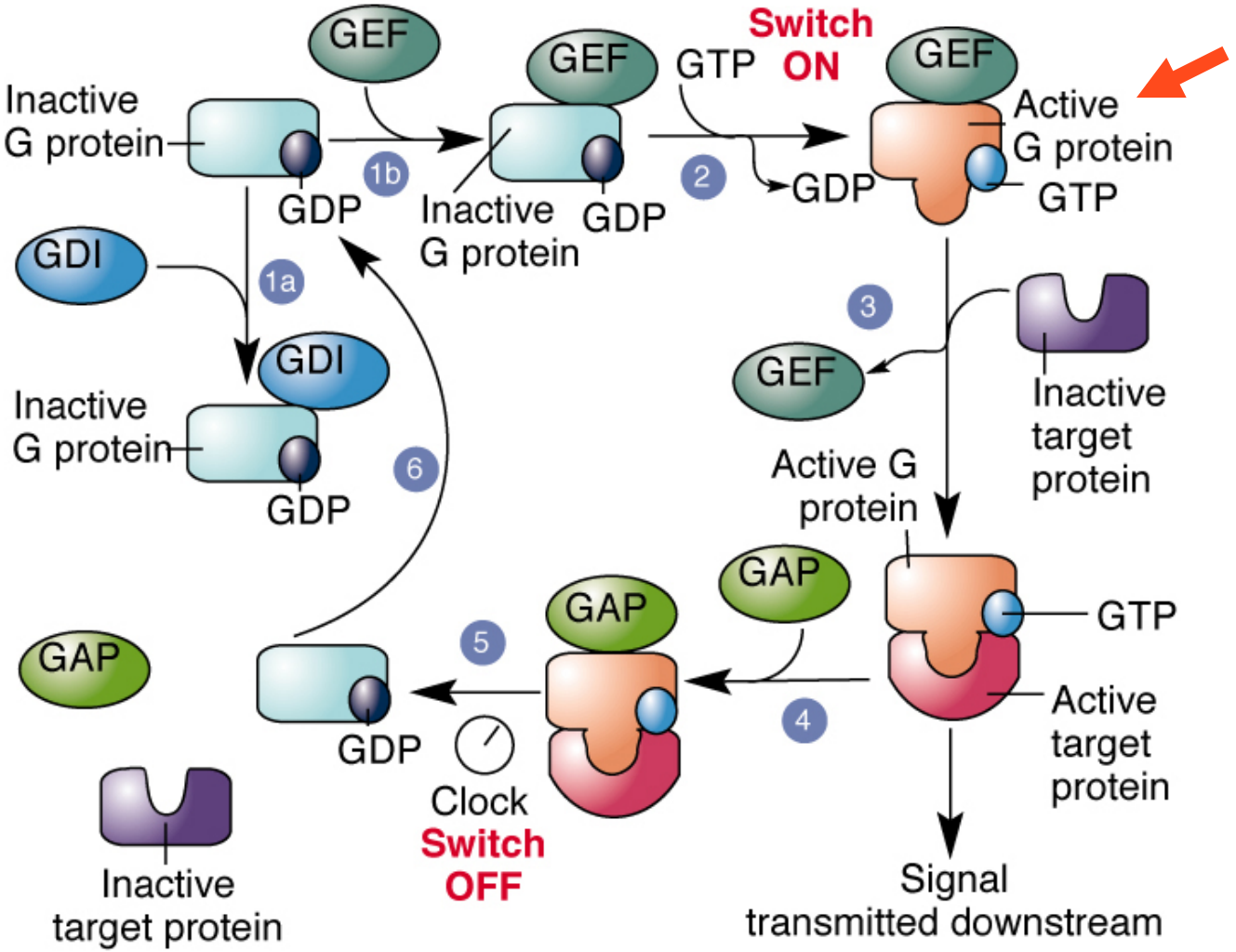



eIF2-Gamma is a GTP binding protein like EFTu

it binds to the initiator tRNA.



# The G Protein Cycle



- What is the favored bound nucleotide in the resting cell? G-GTP or G-GDP?

  In the basal state, G proteins release GDP at a slow rate compared to its rate of GTP hydrolysis. This kinetic balance ensures a very low population of activated G protein molecules, and maintains the cell in a resting state.

# Why do we need GEFs?

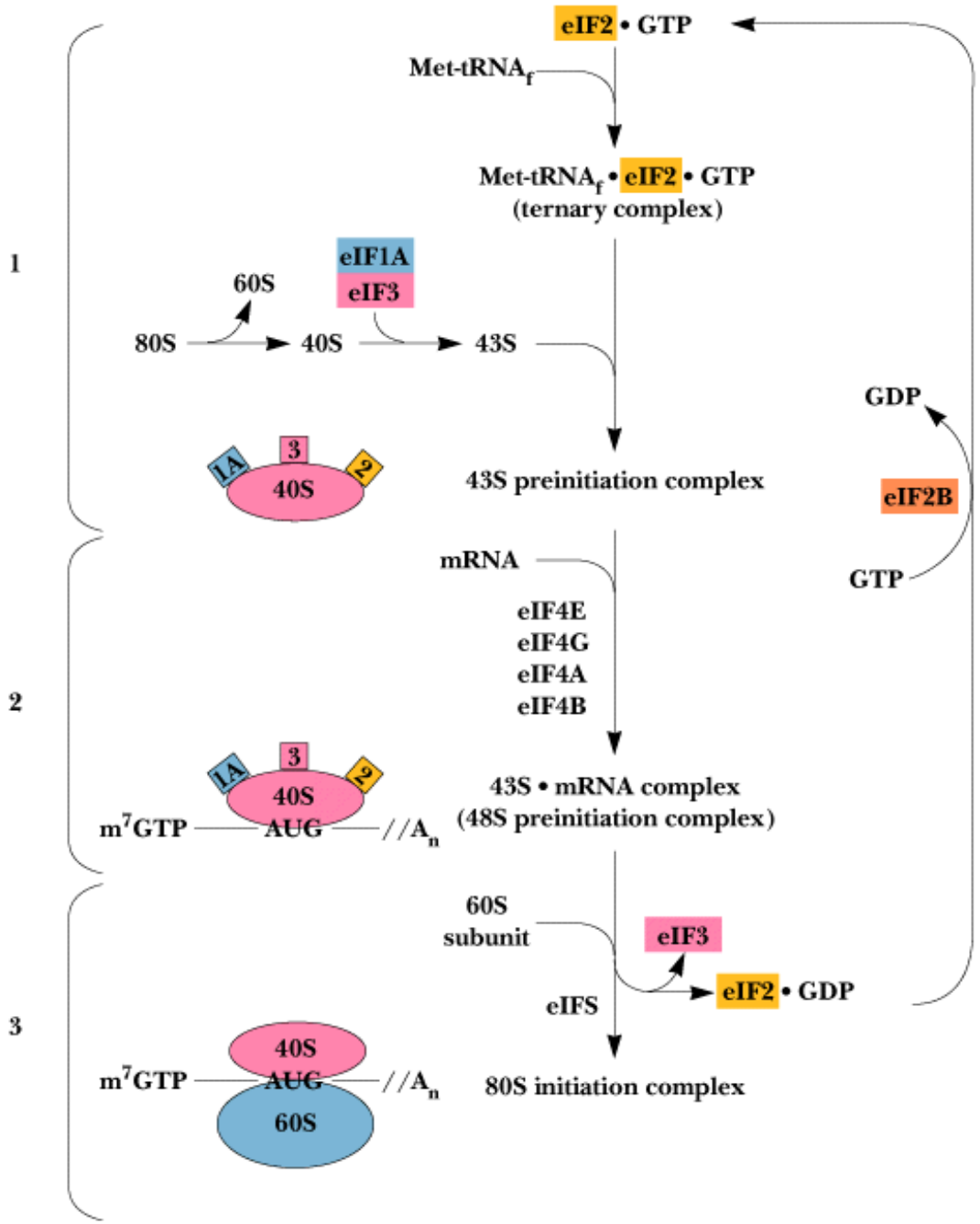
The kinetic barrier to product (GDP) release is high, even though GTP is in 10-fold molar excess to GDP in the cytosol. Replacement of GDP by GTP in the active site of a G protein is the turn-on signal that almost invariably requires the assistance of a guanine nucleotide exchange factor, or GEF.

# Why do we need GAPs?

# Answer

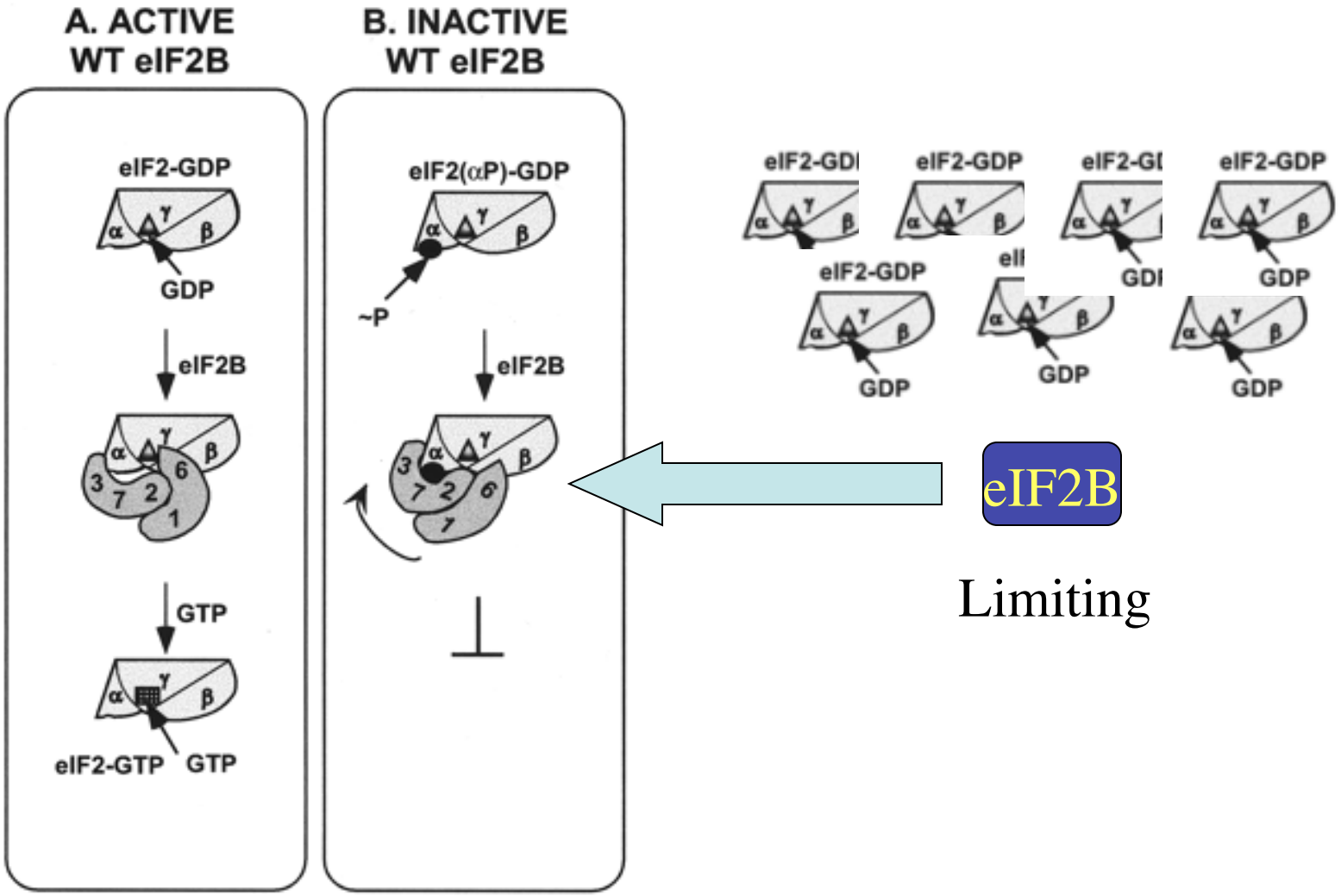
- The kinetic barrier to GTP hydrolysis is substantial, allowing G proteins to maintain the active signaling state for seconds, potentially hours. Hence, GTPase-activating proteins, or GAPs, are required to assist G proteins in hydrolyzing GTP.

# Initiation of translation in eukaryotes

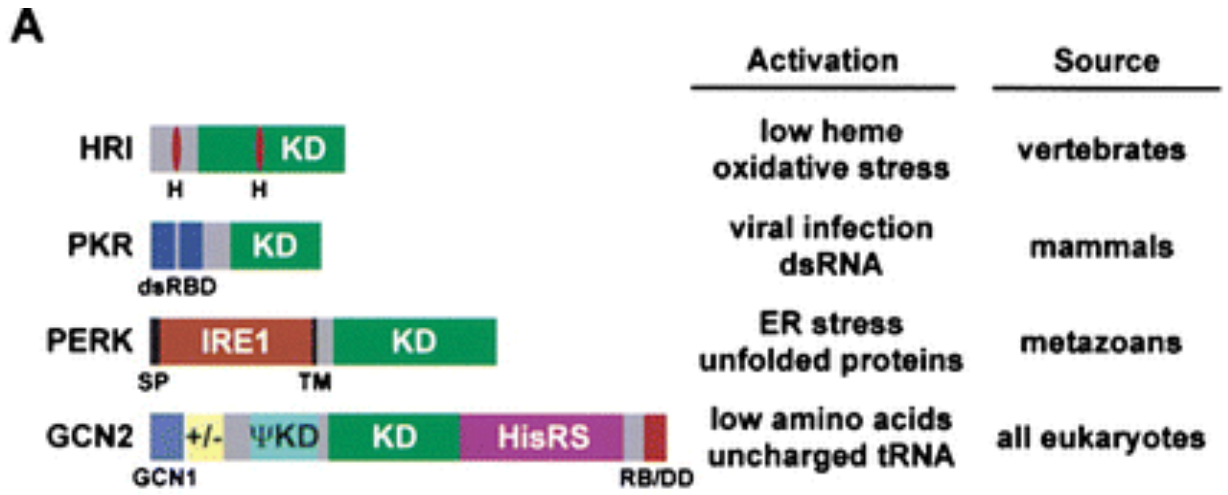



Regulation

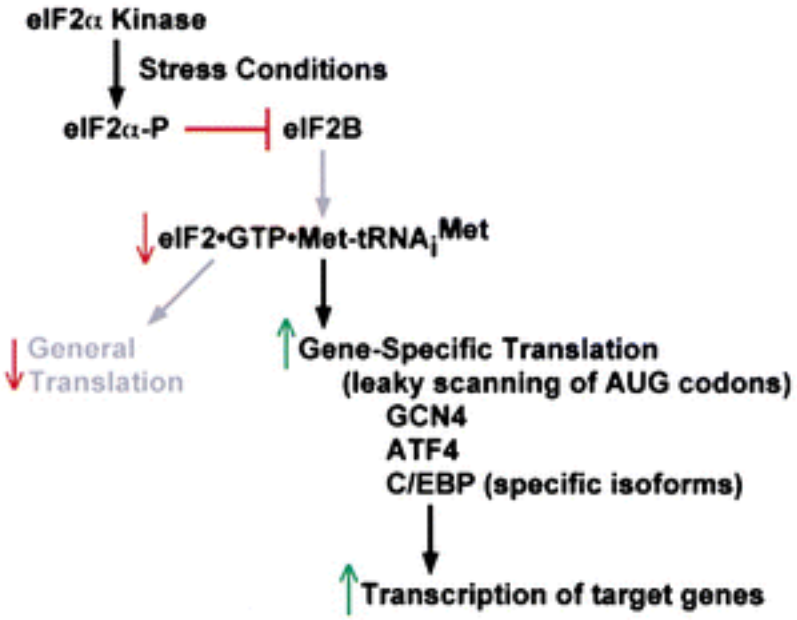
# Model for negative regulation of the guanine nucleotide exchange activity of eIF2B by eIF2(P).



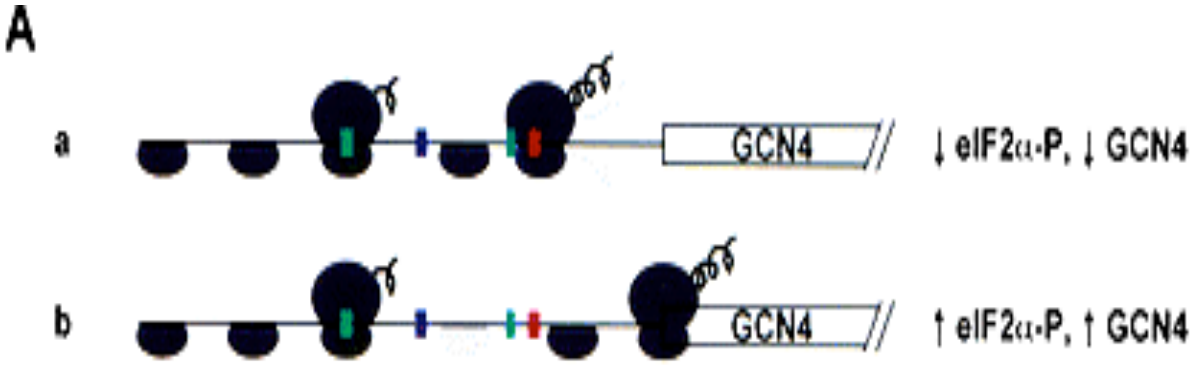
# eIF2alpha kinases



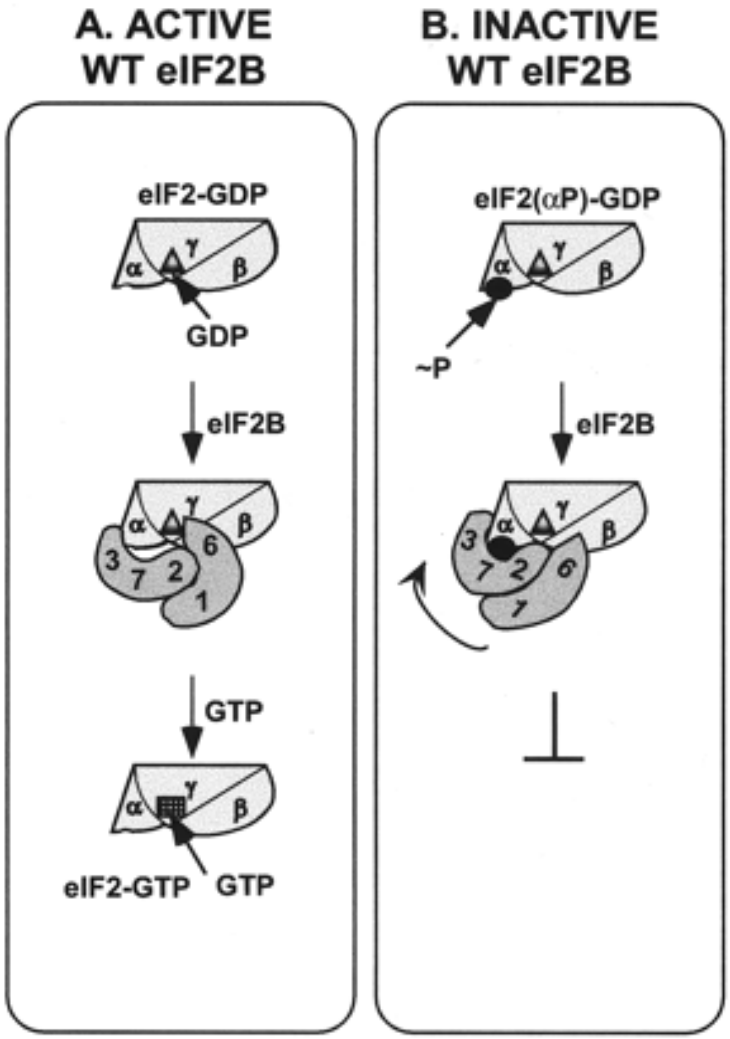
# Stress-Responsive eIF2alpha Kinases Inhibit General Translation yet Stimulate Expression of a Special Class of Genes



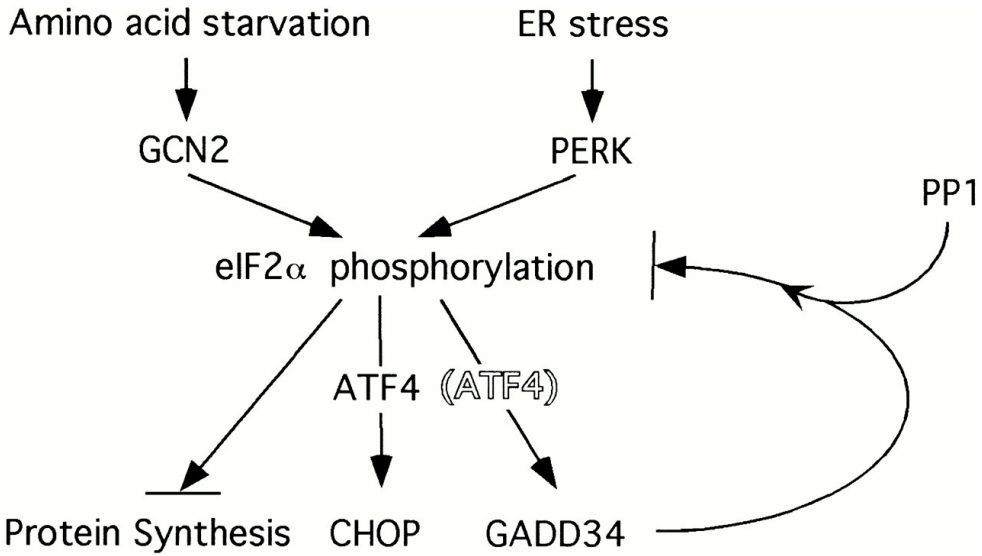
# Reinitiation mode and Leaky Scanning of AUG Codons Form the Basis of Translational Control of the *GCN4*, *ATF4*, and C/EBP mRNAs



# After the stress the system should go back to normal: but how?

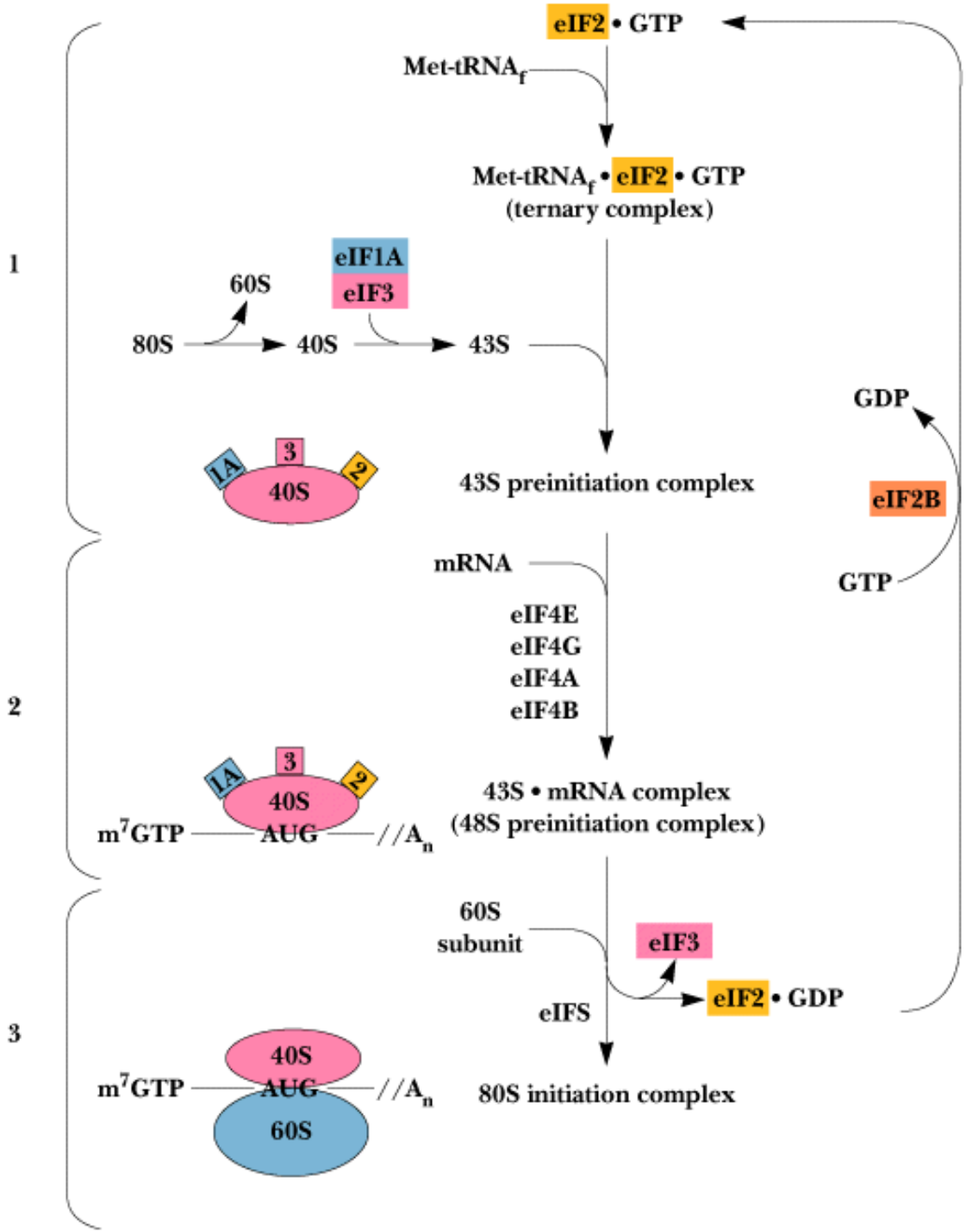



Dephosphorylation of Ser51
Dissociation of trapped eIF2B
Increase expression of eIF2B



- G proteins GEFs and GAPS control translation
- Global control of translation by blocking formation of the ternary complex Met-tRNA-eIF2-GTP
- Rebels achieve translation by leaky scanning
- Cellular Stresses activate Ser51 kinases

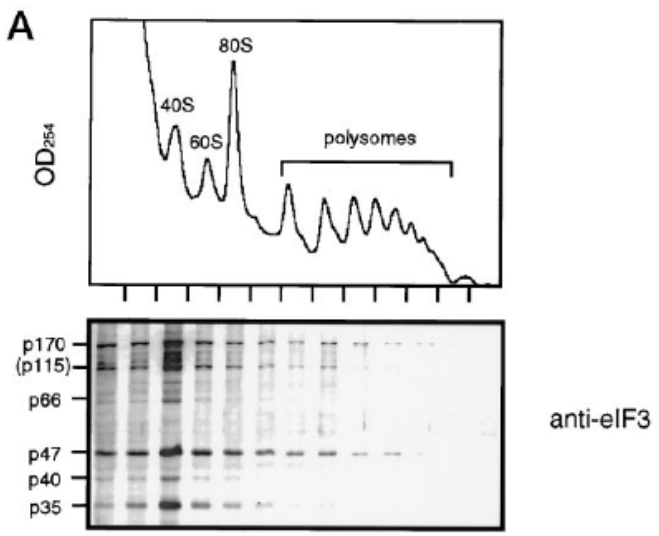
# Initiation of translation in eukaryotes

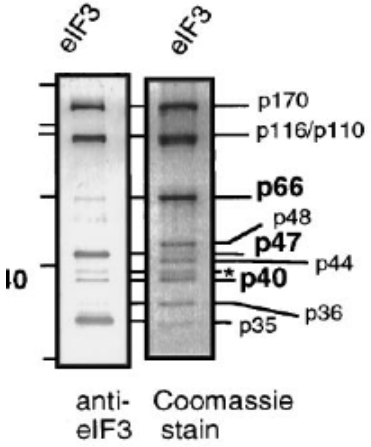


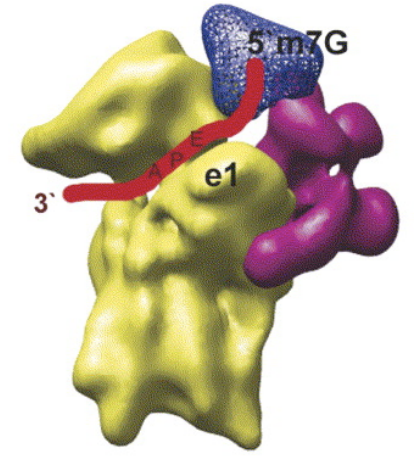
# eIF3 acts as a large scaffold anchored to the back of the 40S subunit

Summary of eIF3 subunits in selected eukaryotes

| Unified nomenclature | *H. sapiens* | *A. thaliana* | *S. cerevisiae* |
|---|---|---|---|
| eIF3a[a] | p170 | p114 | TIF32 |
| eIF3b[a] | p116 | p82 | PRT1 |
| eIF3c[a] | p110 | p105 | NIP1 |
| eIF3d | p66 | p66 | – |
| eIF3e | p48 | p51 | – |
| eIF3f | p47 | p32 | – |
| eIF3g[a] | p44 | p33 | TIF35 |
| eIF3h | p40 | p38 | – |
| eIF3i[a] | p36 | p36 | TIF34 |
| eIF3j | p35 | – | HCR1 |
| eIF3k | p28 | p25 | – |
| eIF3l | p67 | p60 | – |
| eIF3m | GA17 | – | – |



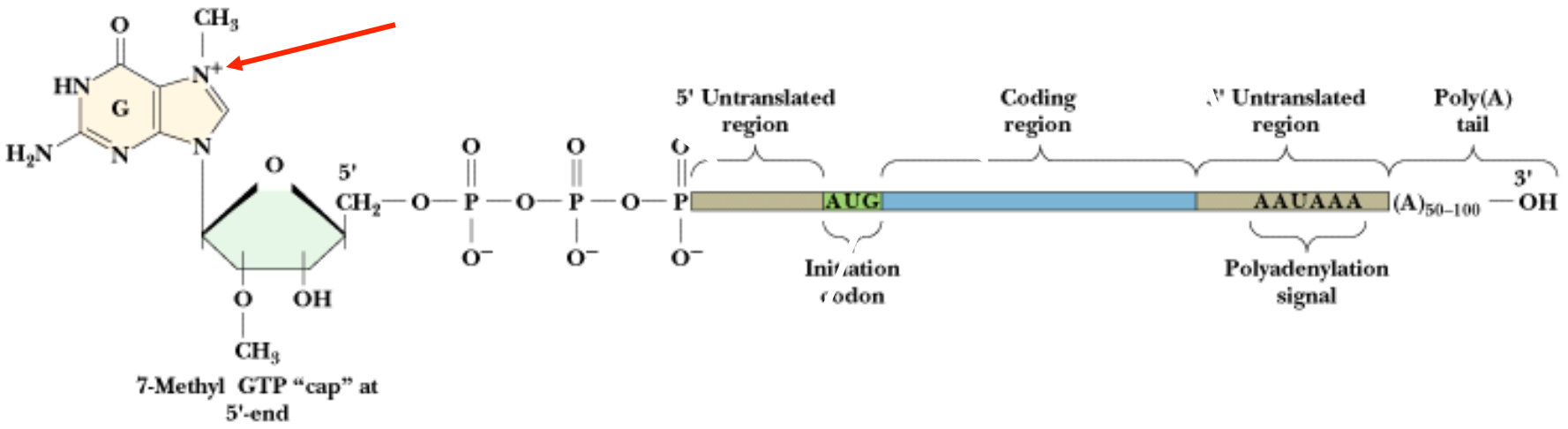




The Journal Of Biological Chemistry
Volume 280, Issue 31 , August 5, 2005, Pages 28251-28264

# Structure of eukaryotic mRNA

Cap | 5’-UTR | Coding region | 3’-UTR | Poly-A




Initiation (AUG)

Termination (AUG, UGA, UAA)

# eIF4F

Originally isolated based on its ability to bind the Cap-nucleotide $^{7Me}$GTP.

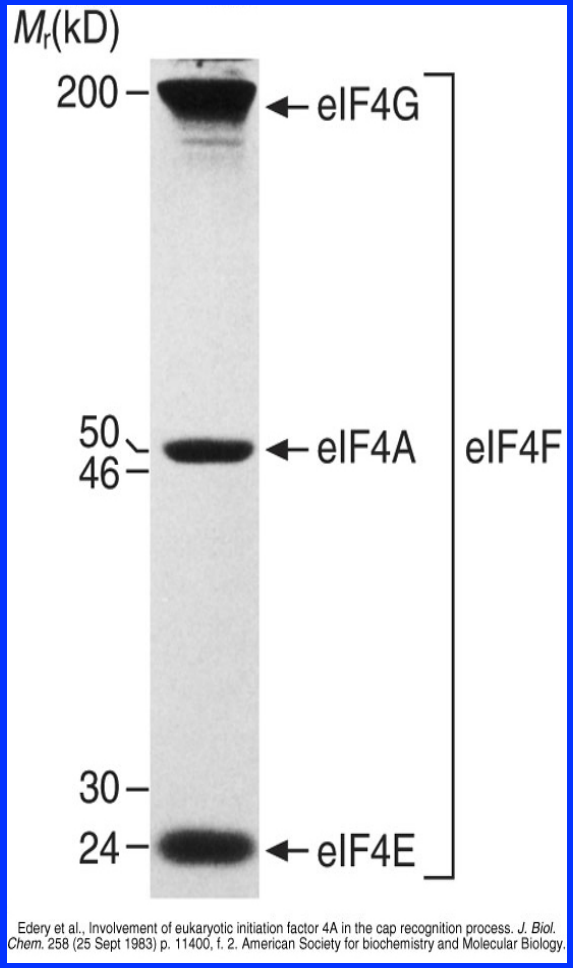
It was found to be composed of 3 subunits, a 24 kDa protein that binds the Cap, and 2 others that stabilized the complex.

These proteins now known as:

1. eIF4E - binds the Cap
2. eIF4A - RNA helicase
3. eIF4G - versatile adaptor

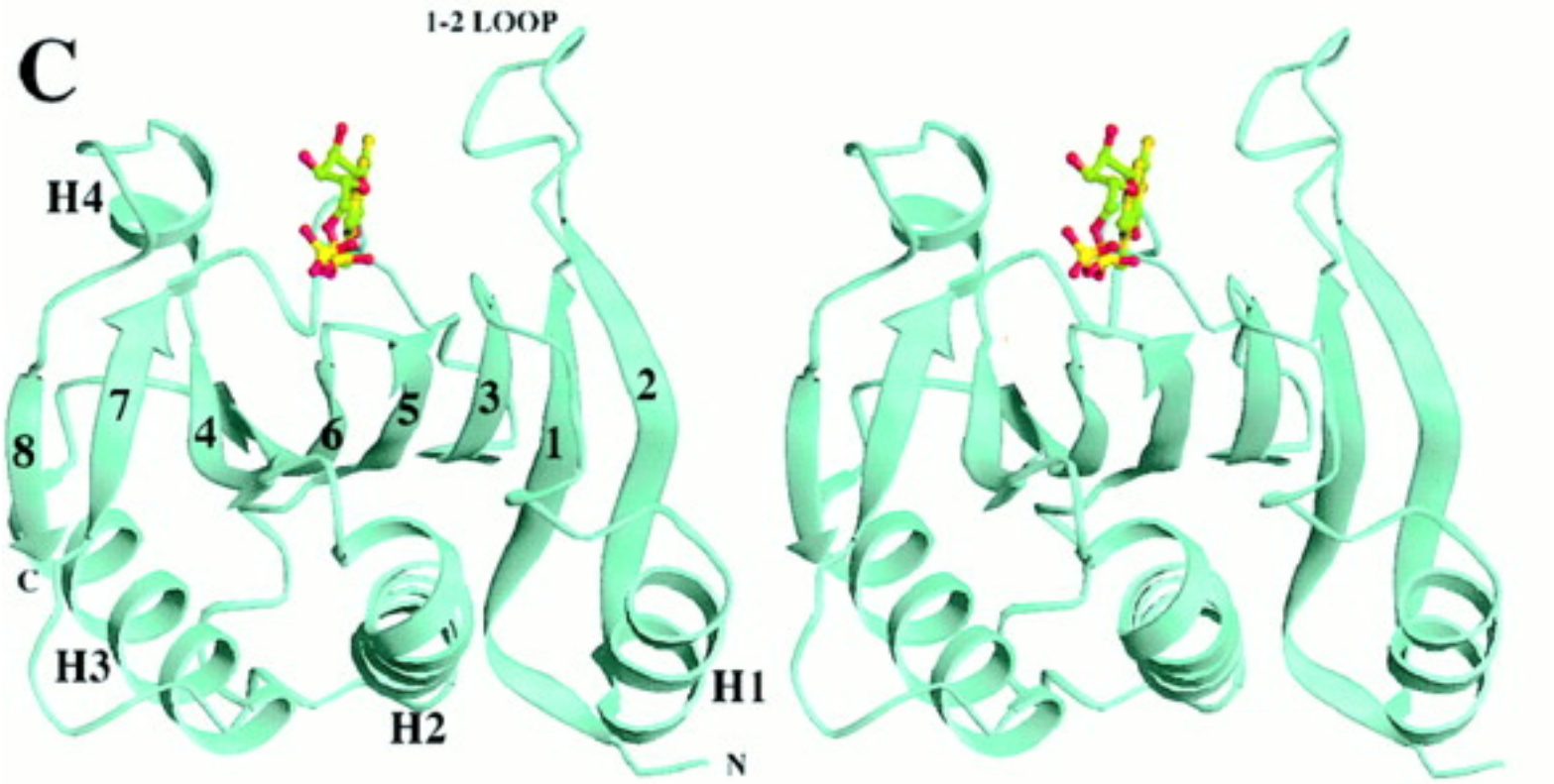
Other helicases:

DHX29



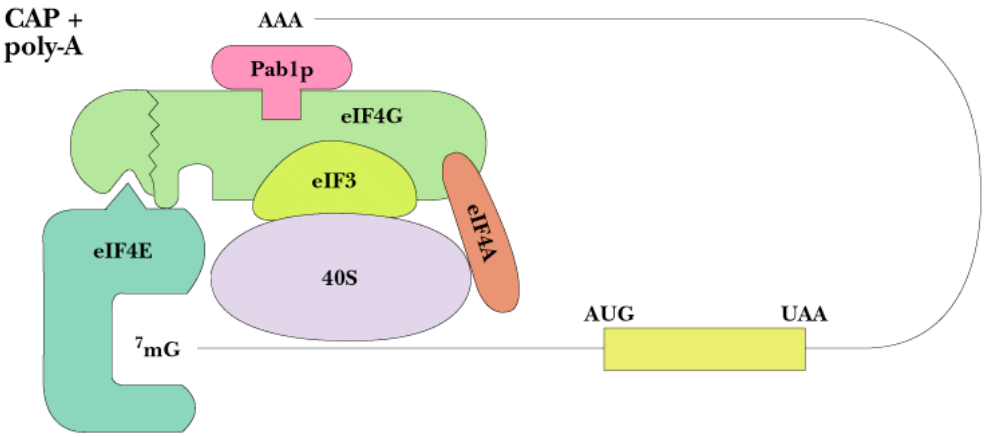
Edery et al., Involvement of eukaryotic initiation factor 4A in the cap recognition process. *J. Biol. Chem.* 258 (25 Sept 1983) p. 11400, f. 2. American Society for biochemistry and Molecular Biology.

# Structure of the Murine eIF4E-7-methyl-GDP Complex

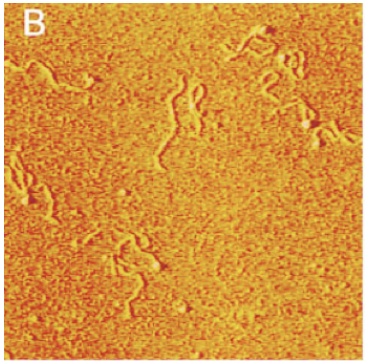


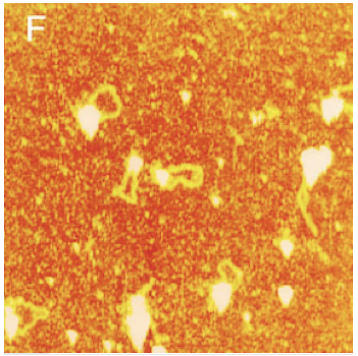
# Initiation of translation in eukaryotes - role of factor eIF4G as a multipurpose adapter to organize 40S, cap, polyA and other factors

Garrett & Grisham: Biochemistry, 2/e
Figure 33.24




Saunders College Publishing



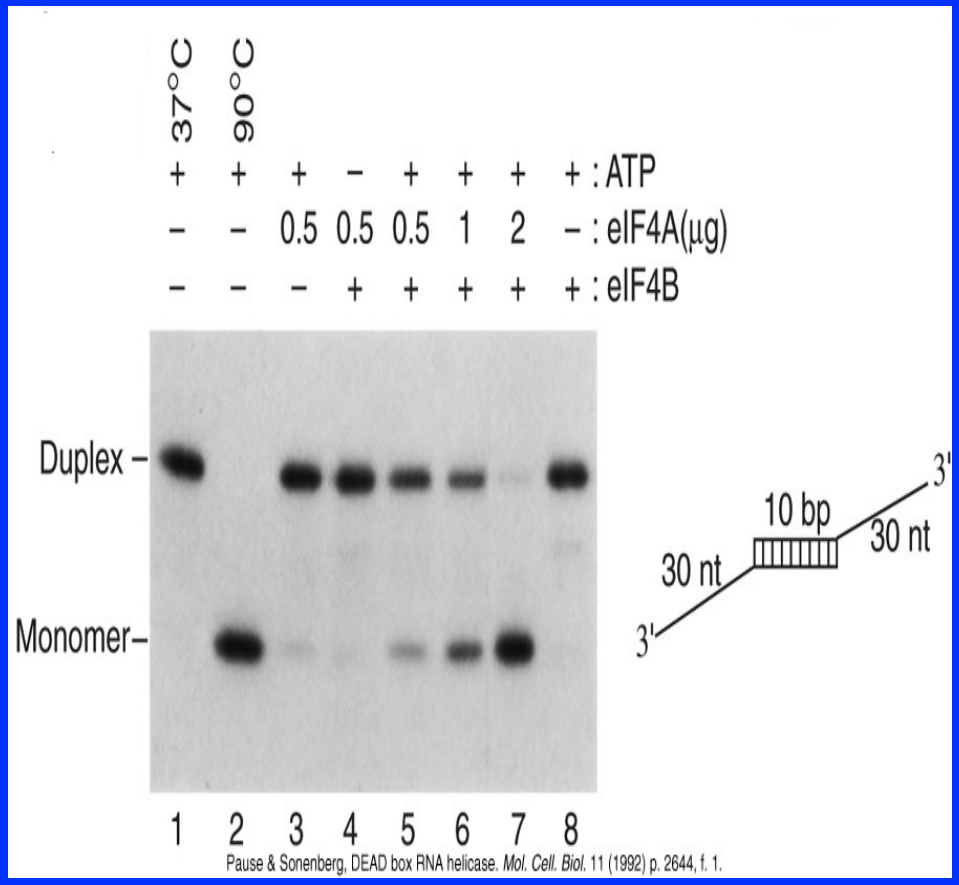




Sachs and colleagues Mol Cell 1998

# eIF4A and eIF4B

## eIF4A

- also exists outside of the eIF4F complex
- contains a DEAD motif (aspartate-glutamate-alanine-aspartate) characteristic of RNA helicases
- RNA helicase activity was demonstrated (right panel) and found to require ATP and to be stimulated by another protein, eIF4B



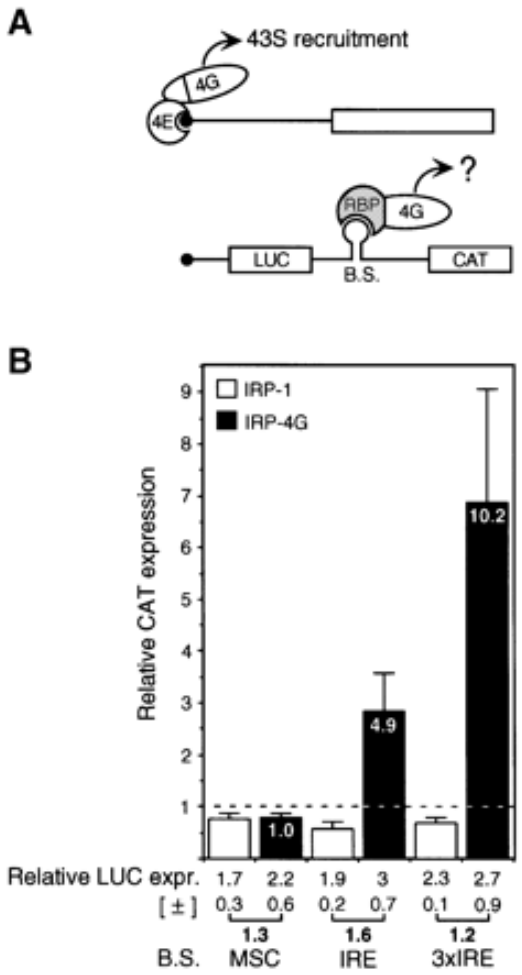
Pause & Sonenberg, DEAD box RNA helicase. *Mol. Cell. Biol.* 11 (1992) p. 2644, f. 1.

## eIF4B

- binds RNA, stimulates eIF-4A

**Function in translation**: Unwind hairpins in the 5' UTRs

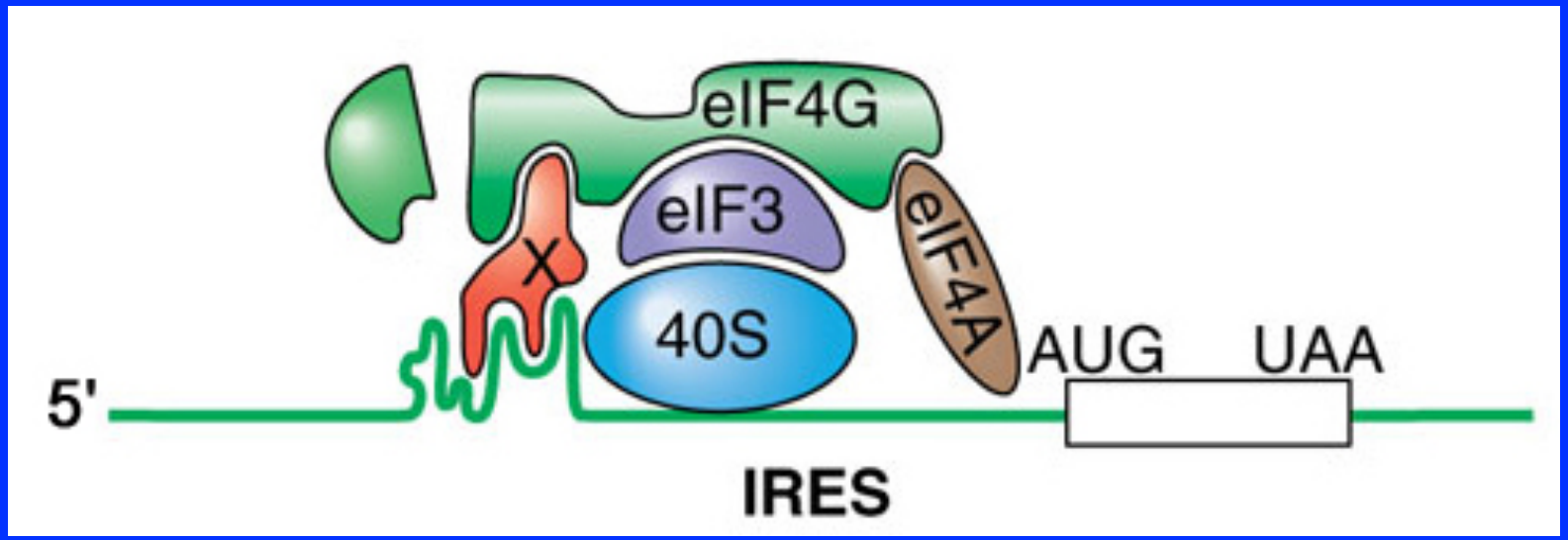
# Ribosome recruitment



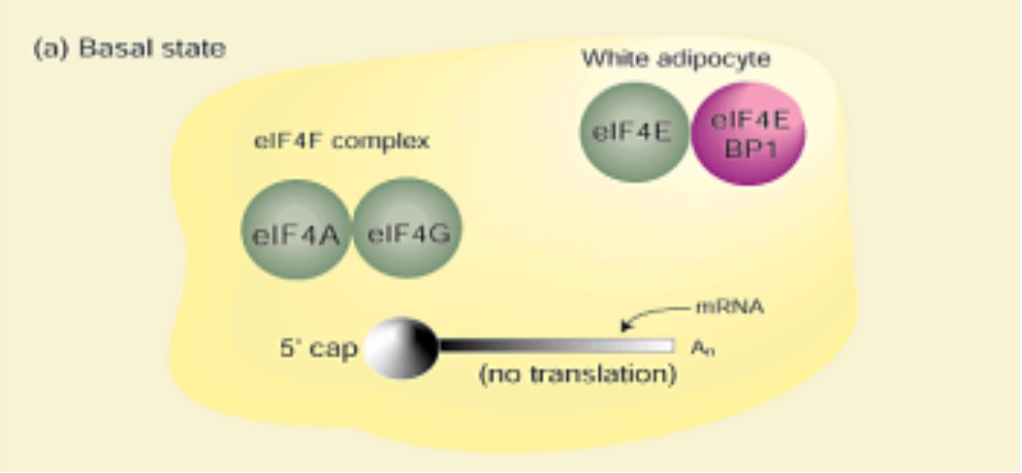

- Identify the central region of eIF4GI as an active `ribosome recruitment core' which requires no more than a means to bind upstream of an open reading frame to recruit all additional factors necessary for at least basal translation *in vivo*.
- The role of the `ribosome recruitment core' in translation is reminiscent of the function of transcription activation domains, which when fused to DNA binding proteins can drive transcription from promoters bearing (multiple) suitable binding sites.

**Observation**: Some **viral** mRNAs (such as Polio virus) are not capped, yet are preferentially translated. Some are also translated via internal ribosome entry sites (IRES) (apparently without scanning to them).

**Mechanism**: Viral protease clips off N-terminus of eIF4G, so it can't bind eIF4E. eIF4G binds a viral protein (X), that binds to the IRES, promoting translation of the uncapped viral mRNAs.



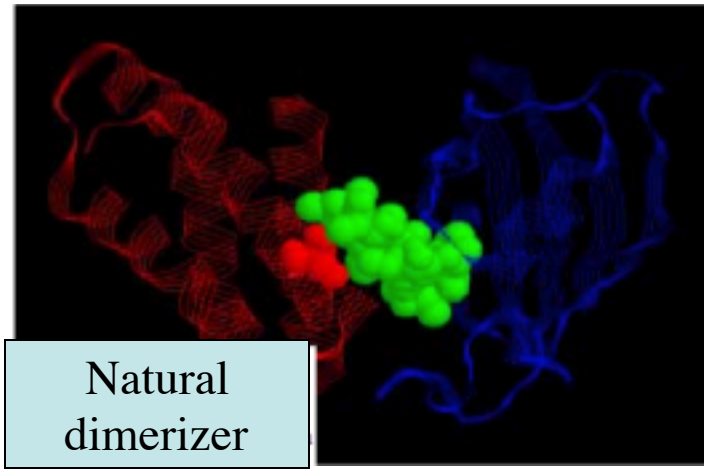
# Translational repressors: 4E-BPs




- Small 12kD eIF4E binding proteins (Far western).
- Molecular mimics of the eIF4G binding site for eIF4E
- Inhibits cap-dependent translation
- Phosphorylation of 4E-BP blocks interaction with eIF4E
- Hormones, GFs, cytokines, GPCR ligands increase translation via phosphorylation of 4E-BPs

# Target of rapamycin

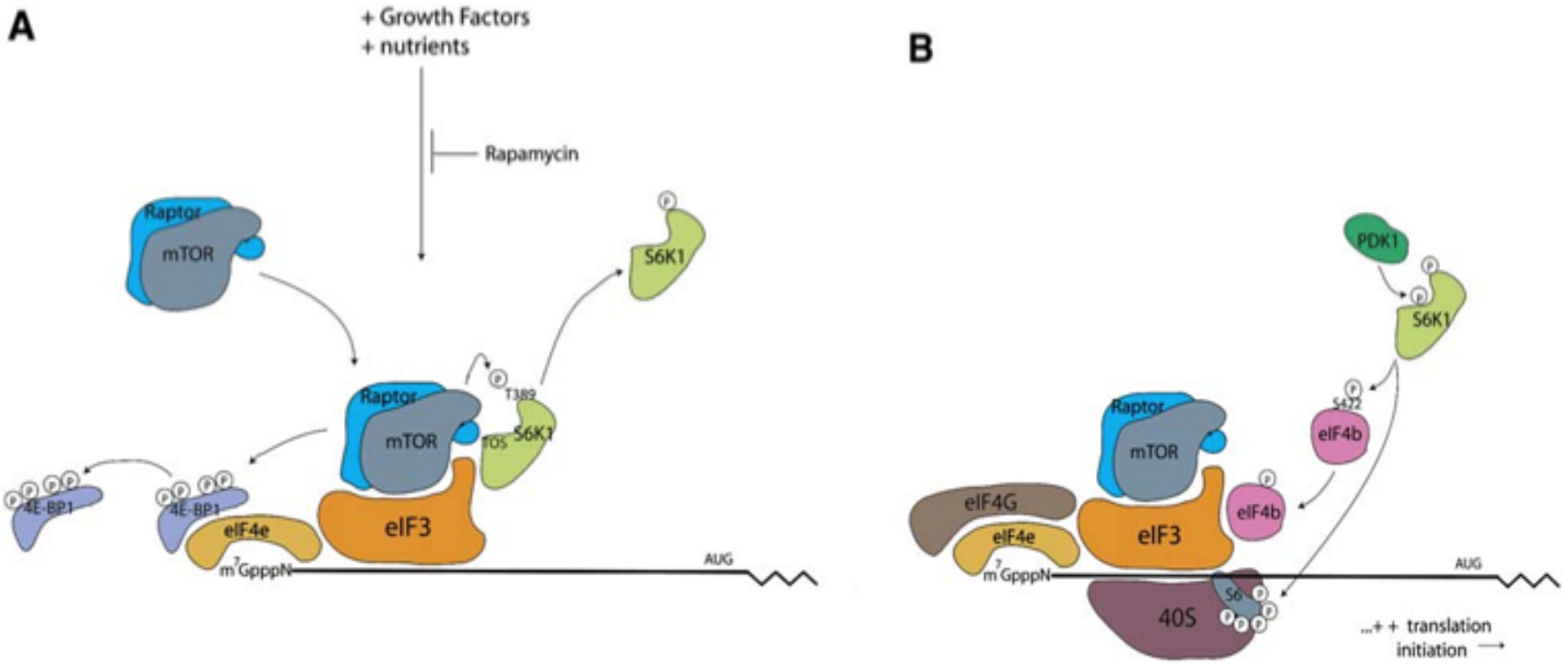
- A macrolyde isolated from Streptomyces
- G1 arrest in different cells (arrest of T cells cause immunosuppression)
- blocks protein synthesis
- FKBP12 a 12kD protein is the cellular receptor for Rapamycin
- The complex of FKBP12 and rapamycin interacts with TOR inhibiting their function
- TOR is a PI3K like protein that functions as a protein kinase (ATM, ATR)




Clardy Lab crystal structure.

Target Of Rapamycin

# Scaffolding initiation by eIF3



- eIF4F is a Chapter of biochemistry Made in Montreal
- eIF4G is a big adaptor protein and a ribosome recruitment factor
- eIF4G mediates the stimulation of translation by the poly A tail
- IRES allows cap-independent translation without scanning
- eIF4E is a key regulator of protein synthesis and a limiting factor for initiation.
- eIF4A and B cooperate to disrupt strong secondary structures during initiation
- 4EBPs are translational repressors
- TOR phosphorylates 4EBP and stimulate cap-dependent translation

**I- Protein synthesis rate**

Cell. 2014 Apr 24;157(3):624-35. doi: 10.1016/j.cell.2014.02.033. Li GW, Burkhardt D, Gross C, Weissman JS. Quantifying absolute protein synthesis rates reveals principles underlying allocation of cellular resources.

Jose, Natasha, Jeniffer

**II Cap-independent translation**

K.D. Meyer, D.P. Patil, J. Zhou, A. Zinoviev, M.A. Skabkin, O. Elemento, T.V. Pestova, S.-B. Qian, S.R. Jaffrey. 5'UTR $m^6A$ Promotes Cap-Independent Translation. Cell, 163 (2015), pp. 999–1010

David, Melanie

**III Ribosome profiling**

Fields AP[1], Rodriguez EH[1], Jovanovic M[2], Stern-Ginossar N[3], Haas BJ[2], Mertins P[2], Raychowdhury R[2], Hacohen N[4], Carr SA[2], Ingolia NT[5], Regev A[6], Weissman JS[7]. Mol Cell. 2015 Dec 3;60(5):816-27. Regression-Based Analysis of Ribosome-Profiling Data Reveals a Conserved Complexity to Mammalian Translation.

MC, Ana, Christina