Overview of BCHM3081

- **Making/Breaking Proteins** (3 lectures)
    - Key concepts/revision
    - Protein Synthesis
    - Protein Degradation
- **Protein Targeting and Proteins in Cell Communication** (7 Lectures)
    - Secreted Proteins
    - Membranes and membrane proteins; Glycosylation
    - Disulfides and protein folding; Sorting to specific organelles
    - Vesicles and vesicular trafficking
    - Nuclear import and export; Lysosomes
    - Cytoskeleton
    - Cell-cell & cell-matrix adhesion
- **Protein Evolution, Engineering and Interactions** (6 Lectures)
    - Basics of Protein Evolution and Interactions
    - Evolutionary Advantages of Multidomain and Self-associating Proteins
    - Characterising Biomolecular Interactions
    - Finding Partners in the Cell and Networks of Protein-Protein Interactions
    - Intrinsic Disorder in Proteins
    - Engineering Binding Activity – DNA-binding, Artificial Transcription Factors and Nucleases
- **Protein Folding and Design** (7 Lectures)
    - Protein Folding and design 1 – An Introduction
    - Protein Folding and design 2 – Mechanisms of Folding & Serpins
    - Protein Folding and design 3 – Folding Assistance
    - Protein Folding and design 4 – Folding Diseases
    - Protein Folding and design 5 – Infectious Folding Diseases
    - Protein Folding and design 6 – Protein Design I
    - Protein Folding and design 7 – Protein Design II

Lecture 1&2 (Key Concepts + Protein Synthesis)

- You should be able to describe the key components for protein synthesis and their features, including:

- *The ribosome (subunits, proteins vs RNA, key sites)*
- *mRNA (rbs, start and stop sites, untranslated regions, and 5' methyl cap/polyA tail in eukaryotes)*
- *tRNA (anticodon loop featuring inosine and basepair wobbles, and acceptor stem)*
- *Amino acyl tRNA synthetases (amino acid activation and loading onto tRNA, proof reading esterase activity)*

- You should be able to describe the main steps of protein initiation, elongation and termination including:

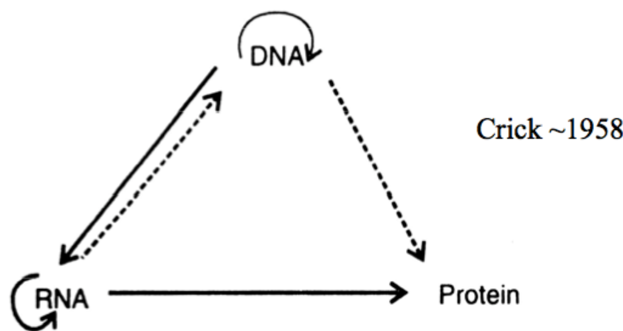
- *The importance of protein initiation elongation and termination factors*
- *The use of high-energy molecules (GTP and ATP)*
- *Differences between prokaryotic and eukaryotic initiation*
- *How eukaryotic mRNA is made circular*
- *IRES's (why do they exist and how do they work?)*
- *Polysomes and circular polysomes to amplify protein synthesis*
- *Mechanisms for rescuing stalling*

- Understand the role of shape complementarity in the different steps and regulation of protein synthesis

- Appreciate the role of the ribosome in directing protein trafficking and folding

- Understand the implications of balances between protein synthesis in the ER and the unfolded protein response, including the role of eIF2/EF-TU

Central dogma of MBLG



Crick ~1958

Prokaryotes	Eukaryotes
– RNA t/c & Protein t/l are linked (streamlined process) – 70S (50S + 30S) – ~2:1 (rRNA:Protein)	– RNA processing & t/c occurs in the NUCLEUS – t/l occurs in CYTOPLASM – **t/c and t/l are compartmentalised** – 80S (60S + 40S) – 1:1 (rRNA:Protein)

Universal genetic code

- 3 base codes → **codon**
- 64 codons for 20AA → give rise to **DEGENERACY**
- I.e. AUU, AUC, AUA all code for Ile (Thus anticodon can be IAU → See **WOBBLE** base pairs)
- There are deviants/variants in the genetic code → give rise to **uncommon naturally occurring AA**

Ribosome

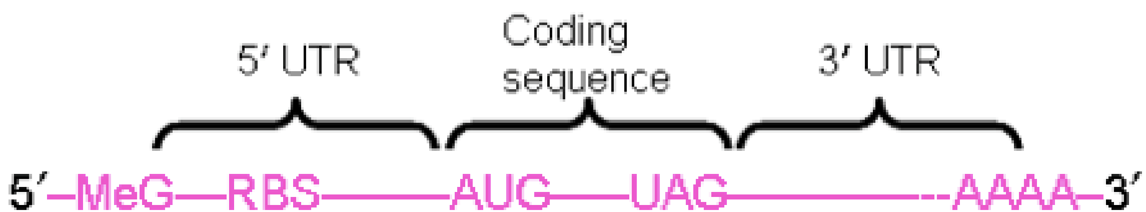
- Contain rRNA + proteins (ribozymes)
  - The **ratio of rRNA:Protein** differs for prokaryotes (~2:1) and eukaryotes (~1:1)
  - The human mitochondria has a ratio of ~1:2
- ~20nm in diameter
- Measured in **Svedberg (S unit)** → empirical measurement of how a particle sediments
  - S units are non-addictive
- The interface between the large/small subunits forms 3 local domains

A site = *aminoacylated tRNA* (site)
P site = *polypeptide* (growing site)
E site = *exit* (of tRNA)

mRNA

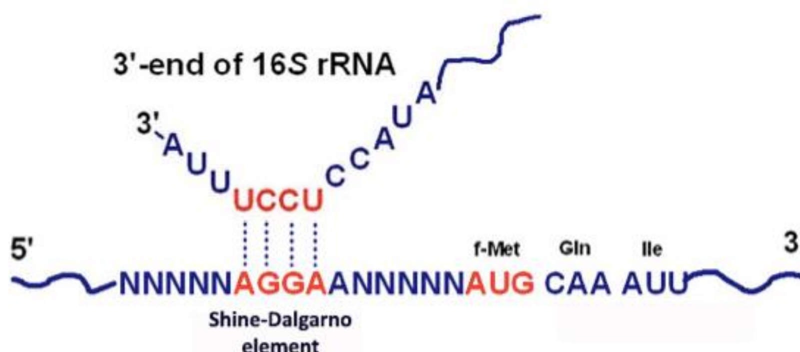
Diagram of a eukaryotic mRNA

- Note the 5' methylated CAP of **MeG** (Methylated guanine, don't confuse with methionine of tRNAi) & **polyA tail**



Shine-Dalgarno sequence

- Ribosomal binding site in bacterial and archaeal mRNA
- Located ~8 bases UPSTREAM of AUG
- Pairs with 3' end of 16S rRNA

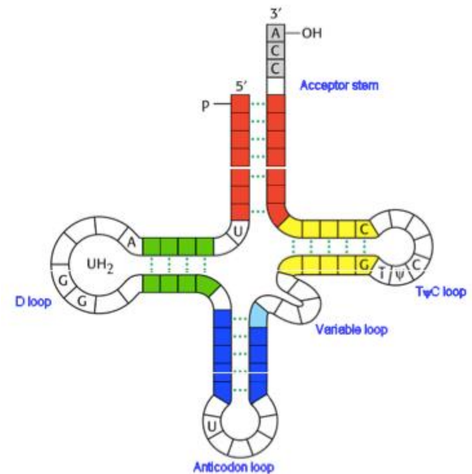


Kozak sequence

- Occurs in eukaryotic mRNA
- Major role in initiation of t/l

tRNA

- 70-80 nucleotides in length
- 4 stems held together by W-C bping + variable loop
- Acceptor stem (5’ + **3’ end containing CCA**)
- 3 short loops of 7-8 bases
    - **D-loop** with dihydrouridine (D)
    - **TψCG loop** containing ribothymidine (T) & pseudouridine (ψ)
    - **Anticodon loop** → (often) contain **inosine (I)**



Wobble base pairs

1st position of anticodon				
C	A	G	U	I
can basepair with 3rd or wobble position in codon				
G	U	C U	A G	C A U

- **Inosine** @ 1st position of the anticodon (t/l to 3rd position on the codon)
- Another wobble bp is G-U

Aminoacyl-tRNA synthetase (aaRS)

- **Coupling enzyme** that links AA to the 3’ OH of adenosine @ the tRNA acceptor stem
- Require ATP to form a high energy bond (activated) → drives the formation of peptide bonds
- Mainly interact with the acceptor stem and anticodon loop of tRNA (to ensure correct AA binding)

Proofreading of aaRS

- Due to some AA having similar structures and tRNAs having similar overall shape

1) Kinetic proofreading

- Incorrect tRNA may bind → however, doesn’t induce a conformational change → NO ACTIVATION TRIGGER (unbinds)

2) Chemical proofreading (only occurs in select aaRS)

- Incorrect AA bound to tRNA can still get hydrolysed/”corrected”
- The **DOUBLE SIEVING MECHANISM** will not allow side chains that are too big to bind to the **SYNTHETIC ACTIVE SITE** → only hydrolyse residues that are appropriate sizes
- The mis-matching AA triggers conformational change in the tRNA → **EDITING DOMAIN** will then catalyse the removal of the incorrect AA

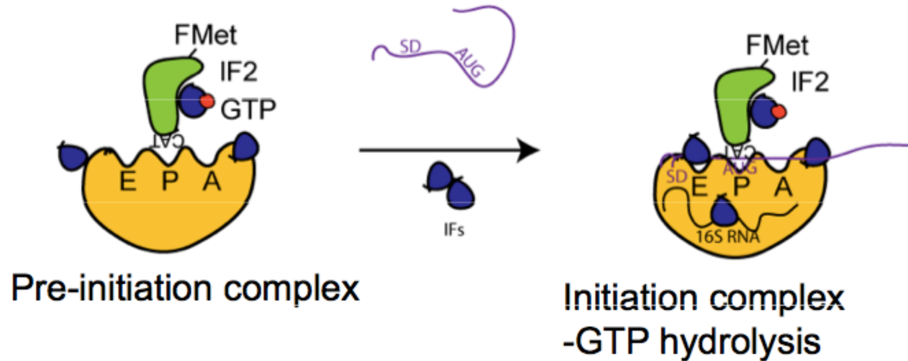
Mechanisms of protein synthesis (**TRANSLATION**)

1. Initiation
2. Elongation
3. Termination

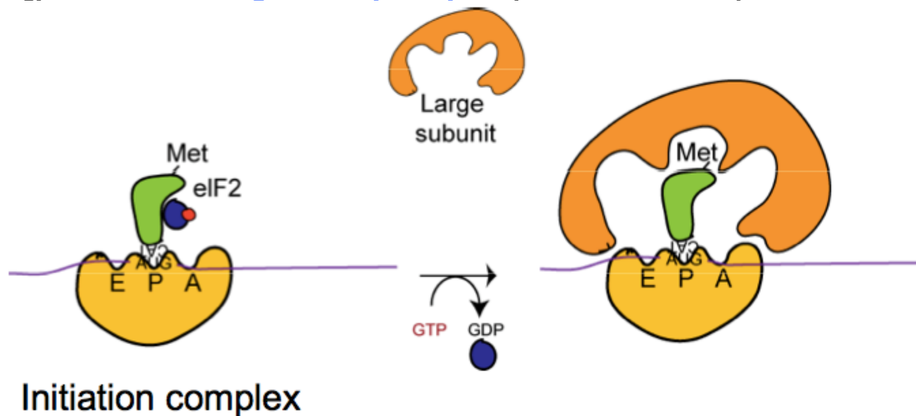
All steps require **protein cofactors** and often **GTP-binding**

1a) Prokaryotic INITIATION

- N-formyl methionine (**fMET-tRNA$_i^{Met}$**) → Brought into the **P-site** on the small subunit (30S) by IF2 → formation of the **pre-initiation complex**
- Pre-initiation complex binds mRNA (w/ help from IFs)



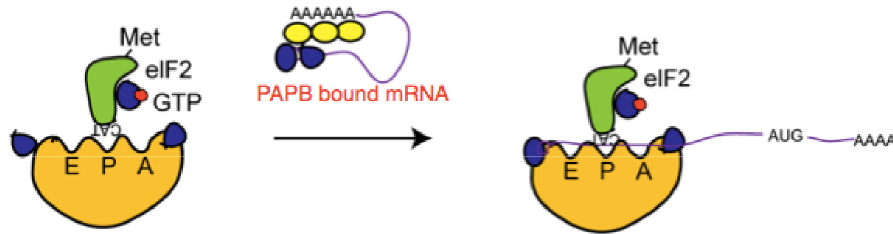
- When ribosome binding sites and AUG are found (recognition of Shine-Dalgarno sequence) → energy released **through GTP hydrolysis** (via one of the IFs)



- The large subunit (50S) is recruited

1b) Eukaryotic INITIATION (Note: eukaryotic protein cofactors has an *'e'* in front of them)

- Met is unmodified (unlike prokaryotes)
  - **tRNA$_i^{Met}$** = Only for initiation, binds P site (*note the i*)
  - **tRNA^{Met}** = Only for growing chain, binds A site

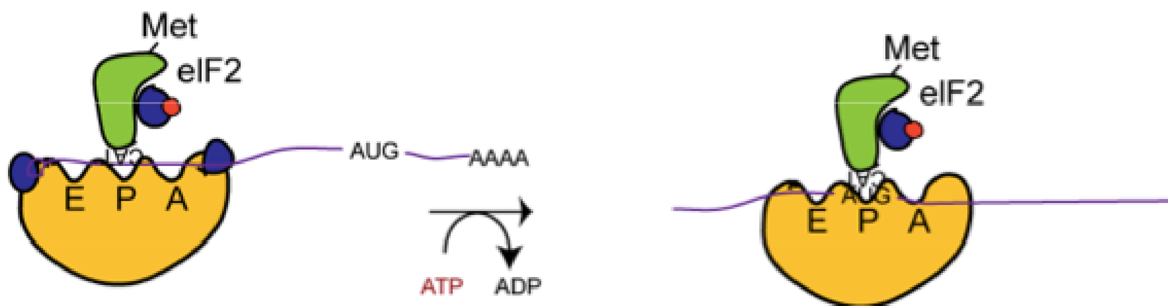


Pre-initiation complex

eIF4F complex – binds 5'cap and polyA-binding protein (PABP)

- Pre-initiation complex recognises the 5'CAP (via **eIF4F complex**)
  - **PolyA Binding Protein (PABP)** = Interact with the polyA tail of mRNA + eIFs (which is bound to 5'CAP) --> Formation of a circular mRNA (↑ Rate of protein synthesis)

- **eIF4F** contains an **ATP-dependent RNA-helicase** → scans mRNA (5' → 3') to find Kozak sequence



Initiation complex

- Similarly to prokaryotes → upon finding AUG → **GTP-hydrolysis** (via eIFs) → recruitment of the large ribosomal subunit (60S)

Non-cap mediated initiation → **Internal Ribosome Entry Sites (IRES)**

- Found originally on **mRNA of +RNA virus**, eventually, **cellular IRES** are found in mRNA or proteins that encode:
  - Apoptosis
  - Mitosis
  - t/c & t/l factors
- Located far downstream of 5'CAP
- ***Form the initiation complex on the P site w/o 5'CAP/fMet-tRNAi***
- ***Functionally mimic tRNA/mRNA complexes***

Viral IRES → shut down cell t/l by cutting key IFs for 5'CAP-PolyA binding

- Proceed to hijack the host's cellular protein machinery

Cellular IRES → During mitosis/apoptosis, protein translation is dampened by phosphorylation of IFs (which **inactivates** them)

- However, mRNA for apoptosis/mitosis specific proteins is unaffected due to IRES (Independent of IFs) → ↑ Translation of particular proteins

2) ELONGATION (Steps cycle @ 3-5 AA per second)

- EF1a-GTP-aminoacyl-tRNA binds to A site via ***DIFFUSION***
- **GTP hydrolysis** proceeds when *correct A:AC recognition* occurs → conformational change in the ribosome → tight binding of aa-tRNA to the A site + release of EF1a-GDP

- **Peptidyl-transferase** activity mediated/catalysed by **rRNA (ribozyme)**
- **TRANSLOCATION** of ribosome along the mRNA (by one codon) driven by **GTP-hydrolysis of EF2-GTP**
    - Now $tRNA_i^{Met}$ (w/o Met attachment) @ E site
    - 2nd tRNA in P site w/ dipeptide
    - Conformation of the ribosome switches back to open the A site
    - Next aa-tRNA diffuses in → Process repeats until STOP codon
- Polypeptide chain (nascent protein) fed out through a channel

3) TERMINATION

- Hit STOP codon (no tRNA for STOP codon) → **Release Factors** (RF1 & 2 in prokaryotes & eRF1 in eukaryotes) mimics tRNA → **bind to STOP codon directly**

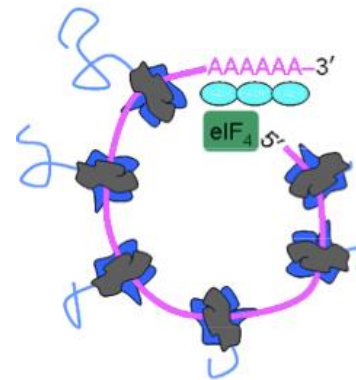
- OH joined to peptide chain via **peptidyl transferase** → RF3-GTP + other RFs break the tRNA-peptide bond → polypeptide chain released
- Ribosome dissociated by IFs → ready to be recycled

Antibiotic synthesis blockers → selectively INHIBIT prokaryotic protein synthesis

- I.e. Streptomycin (aminoglycosides) → inhibit initiation by mimicking and binding to the ribosomal site → causes misreading of prokaryotic mRNA
- I.e. Puromycin (very similar structure to tyrosyl-tRNA) → cause premature chain termination by acting as an analogue of tyrosyl-tRNA

Polyribosomes/Polysomes

- Multiple ribosomes/proteins bound to the SAME mRNA → makes multiple proteins (↑ Rate of protein synthesis)
- **Circular polysomes** are unique to eukaryotic mRNA due to PABP



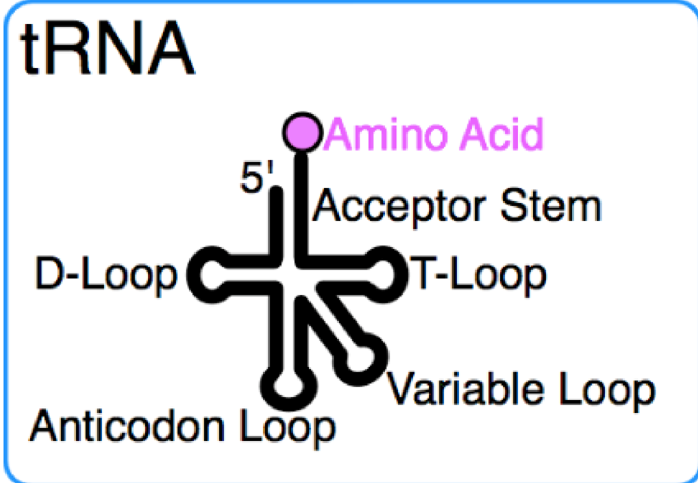
STALLED ribosomes

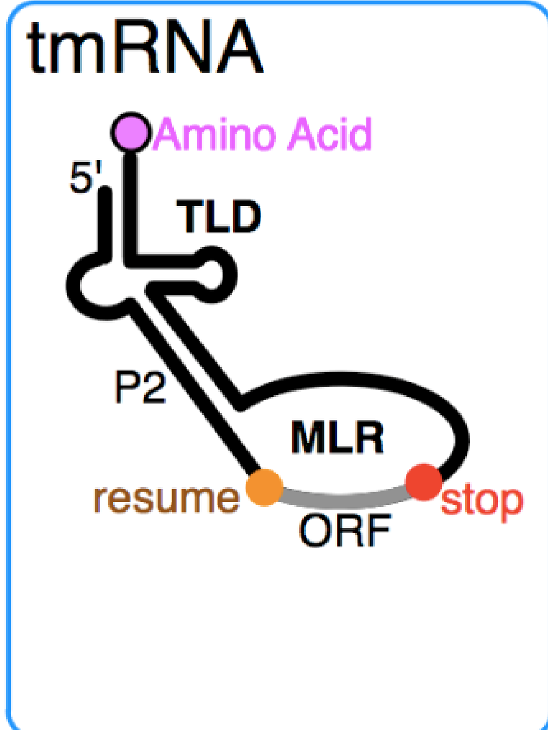
- Ribosome **stalls** when mRNA is truncated OR when there is no aa-tRNA to occupy the A site
  - It is not terminated (since termination requires STOP codon + RFs)
- Difference between prokaryotes and eukaryotes

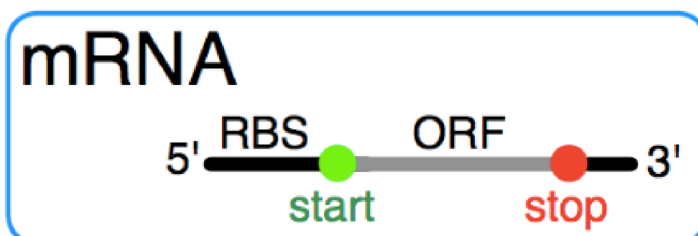
Rescuing stalled ribosomes – Prokaryotes: **tmRNA**

tmRNA (present in bacteria) → function as both tRNA and mRNA

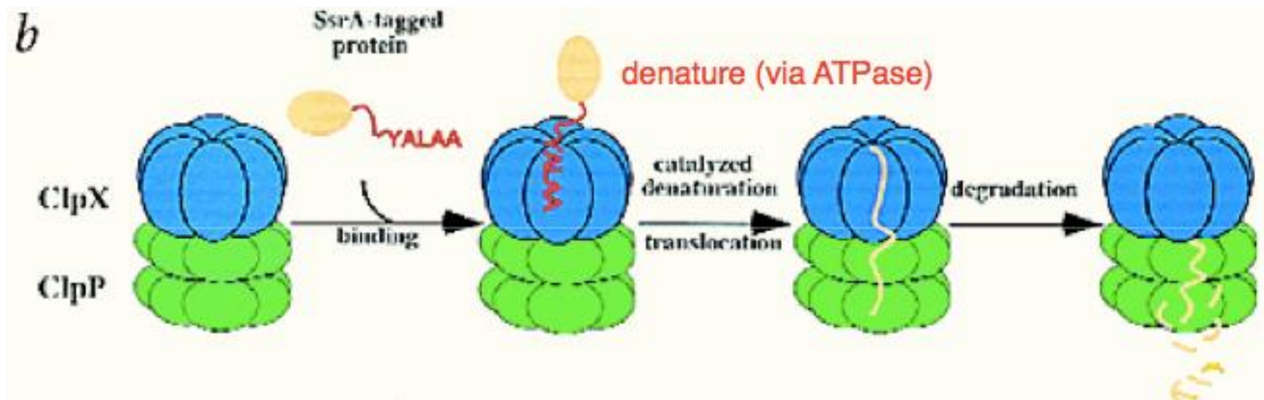
- Recycles the stalled ribosome
- Adds a **proteolysis-inducing tag** to the unfinished polypeptide
- Facilitates the **degradation of the aberrant mRNA**







- tmRNA-EF-Tu-SmpB complex recognise stalled ribosome
- tmRNA carries out its tRNA-like role by binding in the A site via **TLD (alanyl-tRNA Like Domain)**
- **Template switch** → original template mRNA **SWITCHED** from to the tmRNA ORF
  - tmRNA ORF encodes a **peptidyl degradation tag**
- **Trans-translation** = Translation occurs in this tmRNA ORF → when t/l is complete → ribosome is freed/rescued
- The partly expressed protein w/ degradation tag → sent to **special sets of proteases**
  - Broken down & AA recycled



Rescuing stalled ribosome – Eukaryotes: **Ski7p**

Exosome associated protein Ski7p rescues stalled ribosome in eukaryotes (thought to resemble eEFs/eRFs)

- Bind in A site and bring the **exosome** to the stalled mRNA

Emerging nascent proteins

- Ribosome also acts as a platform for **folding**, **targeting** and **processing** of newly synthesised proteins.
- In prokaryotes:
  - **Peptide deformylase** binds and removes the formyl group
  - Then Met is removed by **methionine aminopeptidase**
  - Then **targeting proteins** (i.e. Signal Recognition Particles, SRP) binds
  - Longer chains often need more protection from aggregation → **trigger factor (bacterial folding chaperone)** binds near the exit of the protein channel

Ribosome can be BOTH:

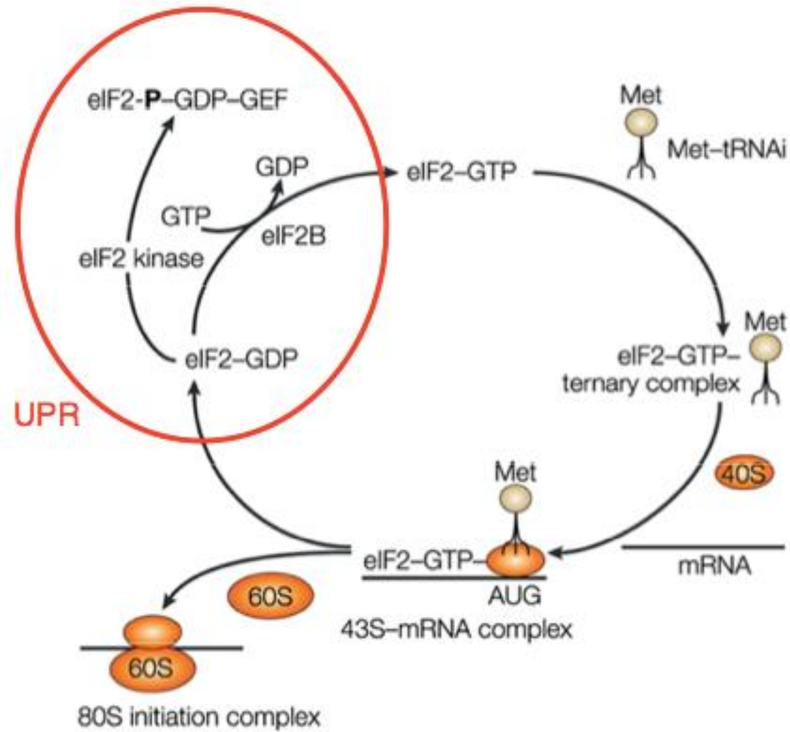
- **Free in the cytoplasm**
- **Membrane-bound** → feed nascent protein directly into ER for tp to other cellular/extracellular destinations

Coupling between folding in the ER & mRNA translation initiation → **Unfolded Protein Response**

- Rates of protein synthesis, folding and degradation are **LINKED** within the cell
- Proteins are **TRANSLOCATED** into the ER in an **UNFOLDED** state

Unfolded Protein Response (UPR)

- Natural response in cells to maintain balance
- Activated when abundance of unfolded proteins accumulate in the ER → triggered UPR → ↓ **Rate of protein synthesis by phosphorylation of eIF2a**
  - **eIF2a** delivers the initiation met-tRNA$_i^{Met}$ complex to the small ribosomal subunit
  - **P-eIF2a** CANNOT recycle GDP → effectively rendered useless (translation attenuation)



Viral mRNA elicit UPR to downregulate host protein synthesis → allow virus to hijack the host's cellular machinery

- This means that the viral mRNA would require non-cap mediated initiation for t/l (i.e. IRES)