



Protein synthesis

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▼ What are the key components of translation?

mRNA: carry the genetic information

tRNA: decipher the codon and carry the corresponding amino acids

rRNA: catalyse peptide bond formation

translation initiation factor

translation elongation factor

termination/release factor

ribosomal proteins: form the ribosomal subunits

▼ How tRNA deciphers the codon and carry the corresponding amino acids

▼ the structure of tRNA

- approx. 80 nt long
- RNA chain folds into 3D structure
- form 4 short double-helical segments, structure alike cloverleaf
- at 3'end: signature of 5'CCA3' - binding site of amino acid
- at 5' end: anticodon: complementary to the 3nt codon on the mRNA

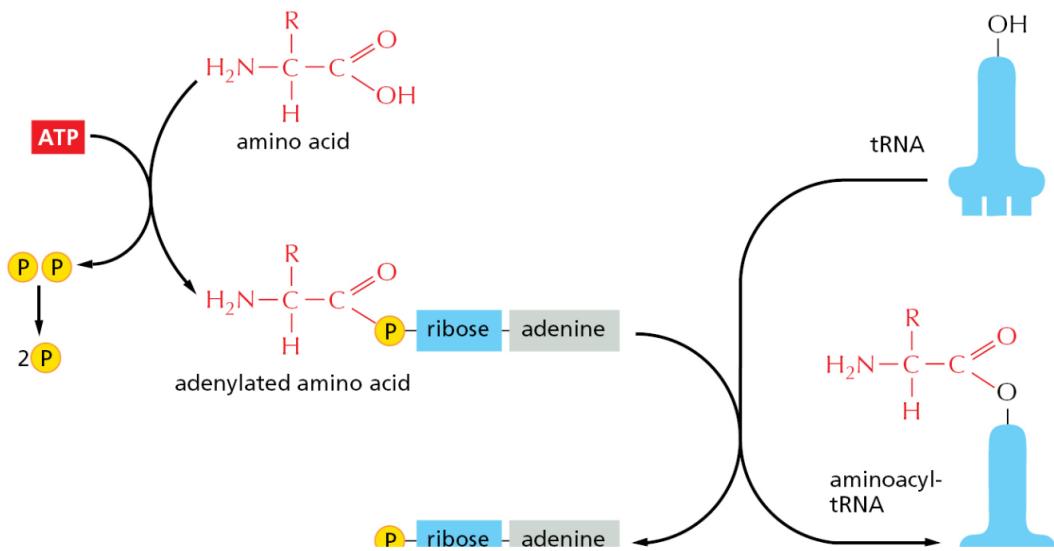
▼ Each amino acid has own set of tRNA

1. several different codons specify the identical amino acid: GGx codes for glycine — wobbling
2. This aligns with the finding that there are 100 different types of tRNA

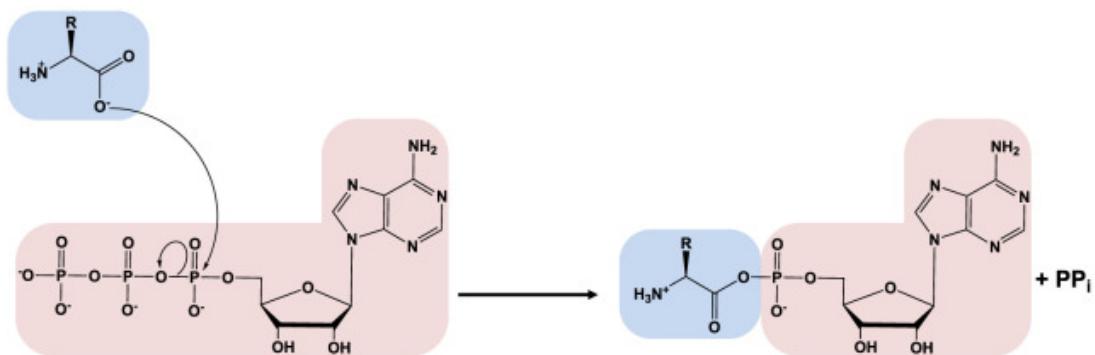
3. The number of types of tRNAs differ between species (prokaryotes have fewer than eukaryotes)

▼ How tRNA can correspond to 20 amino acids

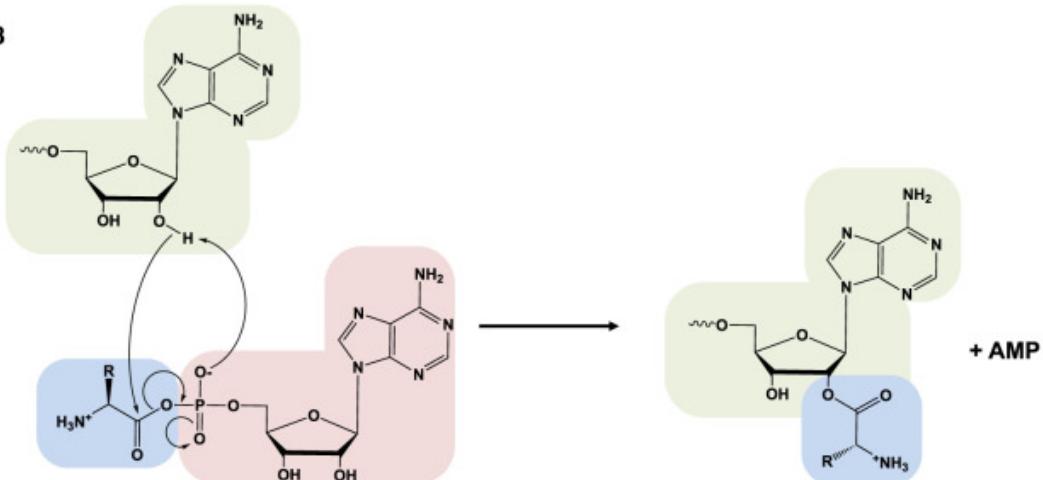
- aminoacyl-tRNA synthetases dictate the recognition and attachment of correct amino acids to the tRNAs
- therefore, there are only 20 types of aminoacyl-tRNA synthetases: one type recognises one specific amino acids
- one can only attach glycine to all types of tRNAs that recognise the codons for glycine
- the synthetases can attach the amino acid to the 3' end of tRNA, by 2 steps:
 - When the amino acid binds to the active site, the synthetase then facilitates high-energy bond formation between {{c1:the carboxyl}} group of the amino acid and the alpha phosphate group of ATP, and release the pyrophosphate — ATP is hydrolysed into **AMP (P-ribose-adenine)**, which then forms a high-energy bond with the carboxyl group of the amino acid using the released energy from hydrolysis. The adenylated amino acid is formed
 - adenylated amino acid is then transferred to 3'OH of the ribose of the tRNA nucleotide at 3' end; the amino acid is joint to the tRNA by forming an ester bond
- The high energy of this bond between the amino acid and tRNA is then used to covalently bind the amino acid residue to the growing polypeptide chain



A



B



- ▼ How can aminoacyl-tRNA synthetase make sure the correct amino acid is attached

- based on 2 consecutive steps
1. for amino acids of distinctive sizes: each type of the synthetase has the specific active-site pocket that has the greatest affinity to the correct amino acid, so the correct amino acid is favoured over the rest 19; this mechanism can effectively exclude the amino acids of larger size
 2. if the two amino acids are in similar sizes: when the amino acid is binding to tRNA, the synthetase forces the adenylated amino acid into the edit-site pocket. The precise orientation of the edit-site pocket will exclude the correct amino acid such that it does not bind to the site. The wrong amino acids will be removed from the edit-site pocket by hydrolysis

▼ Initiation of translation

▼ Key words

- rate limiting step
- two distinct mechanisms of the initiation complex
 1. Cap-dependent initiation: the initiation complex interact with the 5' cap structure and scan from 5' to 3' until the start codon
 2. IRES (internal ribosome entry site) - initiation complex binds between the 5' cap and start codon
- The culminating event is that the initiation complex (ribosome, Met-tRNA) attach to start codon

▼ Cap-dependent initiation

▼ Key steps

- mRNA circularisation
- recycling 40S subunit & formation of 43S complex
- Attachment of 43S complexes to mRNA
- Scan for the start codon

▼ mRNA circularisation

- 5' cap: consists of a methylated guanosine at 7 position that links the 5' end of mRNA — the 5' cap is also referred to as 7-methylguanylate (m7G) cap
- **elf4F complex:** cap-binding protein **elf4E**; RNA helicase **elf4A + elf4B** (regulatory factor of elf4A, enhances elf4A's helicase activity); **scaffolding (elf4G: binds elf4A, elf4E and PABP together)**; **poly(A)-binding protein (PABP)**

The initiation of translation



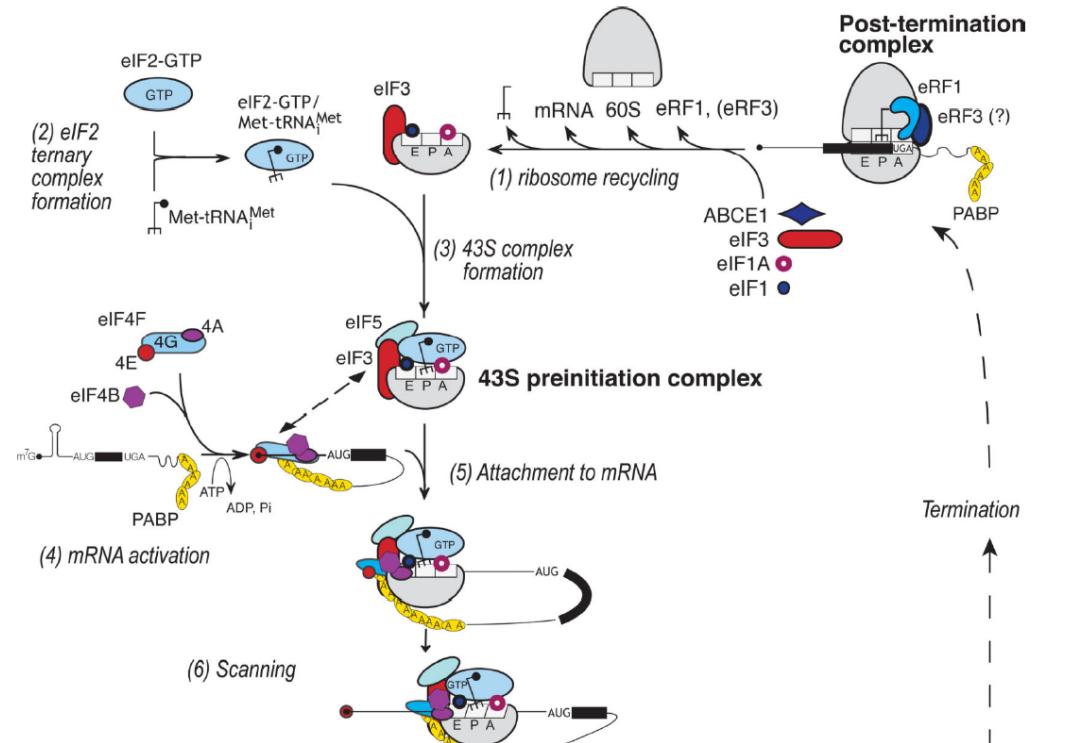
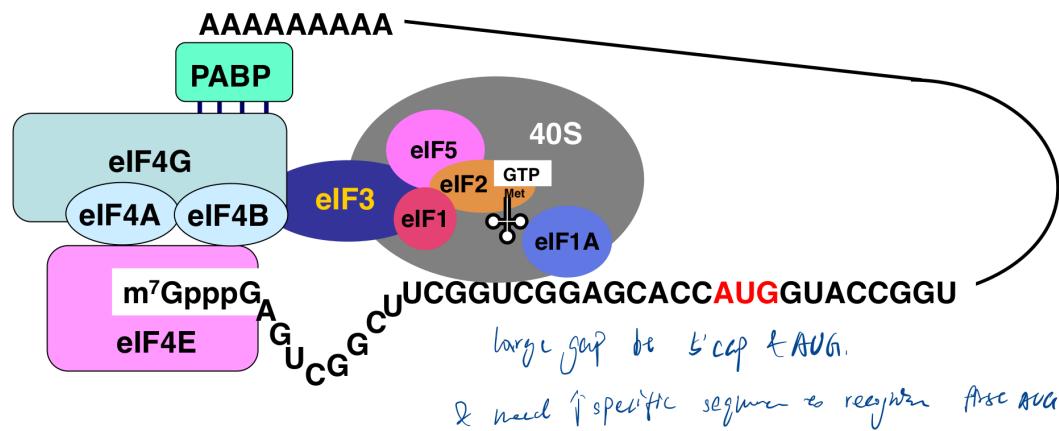
- Binding of elf4G to PABP circulates mRNA
- mRNA circularization is important because it increases the local concentration of important factors [ensure the mRNA is intact, not degraded]
- It also allows interaction between the events at the 5' Cap site and those of the 3' poly(A) tail
- This mechanism ensures the translation of only intact mRNA

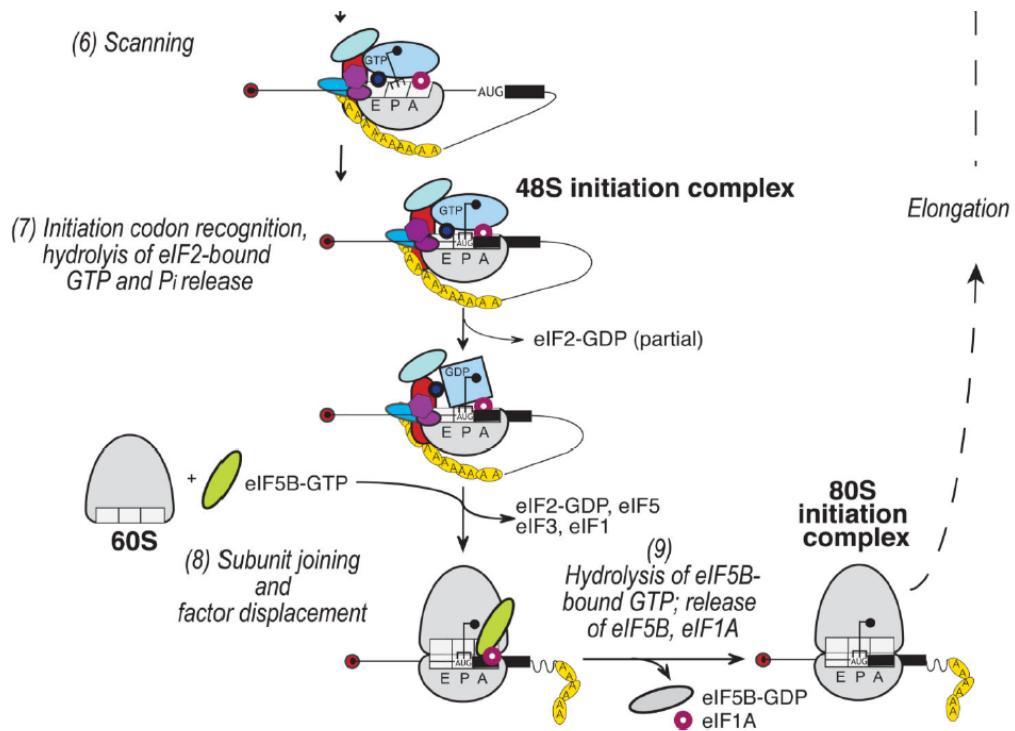
- Circularised mRNA can be seen by elf4E binding to the m7G cap and PABP binding to poly(A)-tail at 3' end
- mRNA circularisation is important for translation efficiency:
 1. circularisation brings the translation initiation factors in close proximity, thus facilitates the interaction of those factors to form translation initiation complex (elf4F)

- a. this can be equationalised by thinking that the local concentration of initiation factors increase: $r=k[IF]$, thus the rate of translation increases (initiation is the rate-limiting step)
- 2. Circularisation enables recycling ribosomal complex (40S) without them dissociating from mRNA — efficiently reinitiate translation: circularisation keeps ribosomes in close proximity to initiation factors, which then recycle the ribosomes for a new round of translation
- 3. Circularization can also protect the mRNA molecule from degradation by exonucleases. The closed-loop structure makes it more difficult for exonucleases to access and degrade the mRNA ends, thereby prolonging the lifespan of the mRNA molecule and allowing for more rounds of translation.

▼ recycling 40S subunit & form 43S complex

- The post-termination ribosomal complexes (post-TCs) are dissociated into 60S and tRNA- and mRNA-bound 40S subunit (by ABCE1)
- the 40S subunit is emptied by attachment of eIF3, eIF1, eIF1A
- eIF2-GTP is bound by the initiator tRNA carrying methionine (Met-tRNAMet) — forms the eIF2 ternary complex
- Then this ternary complex binds to eIF3, eIF1 & eIF1A complex; **eIF5**: an eIF2-specific GTPase-activating protein (GAP)
- eIF5, eIF-GTP Met-tRNAMet + eIF1A eIF1 eIF3 — 43S complex formation





- mRNA is activated by the translation initiation complex and circularise mRNA, in an ATP-dependent manner
- 43S complex then had the eIF3 binds to eIFAE that attaches at 5' cap
- The scanning initiates from 5'-3' direction

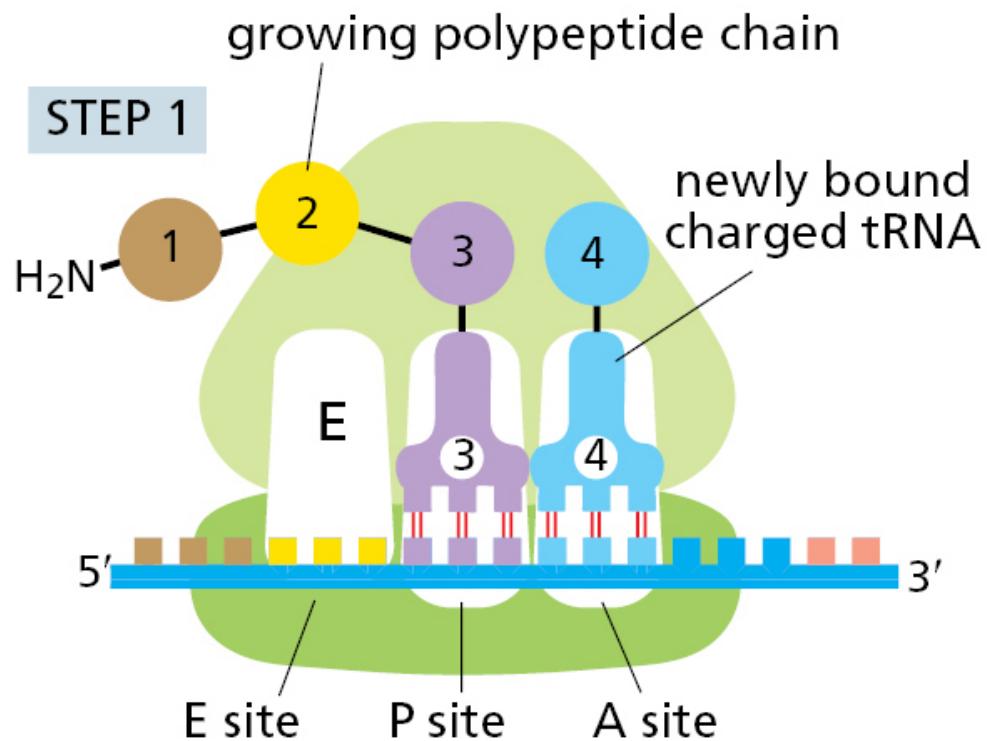
▼ scanning for the start codon and Ribosome recruitment

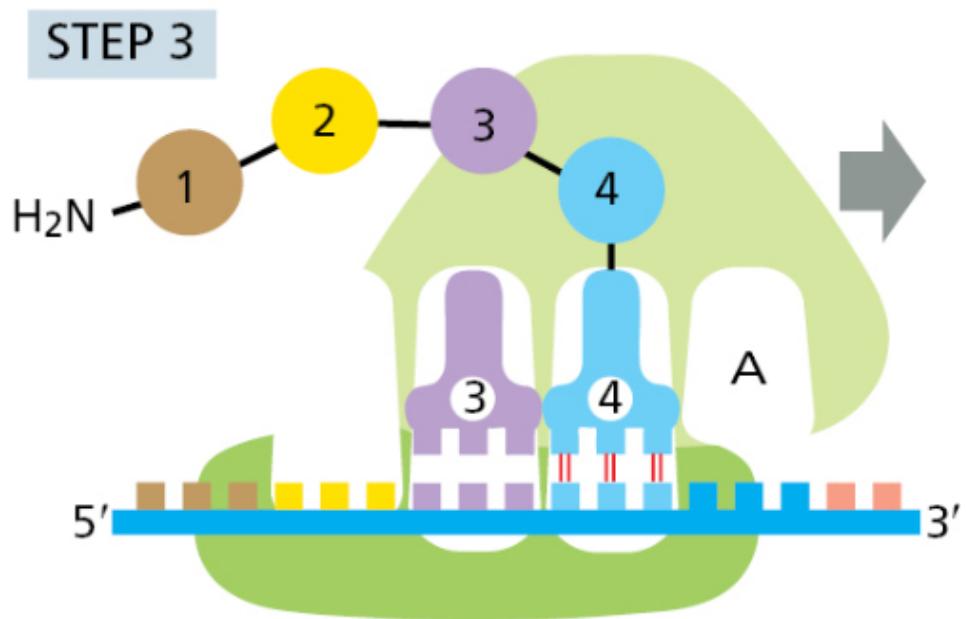
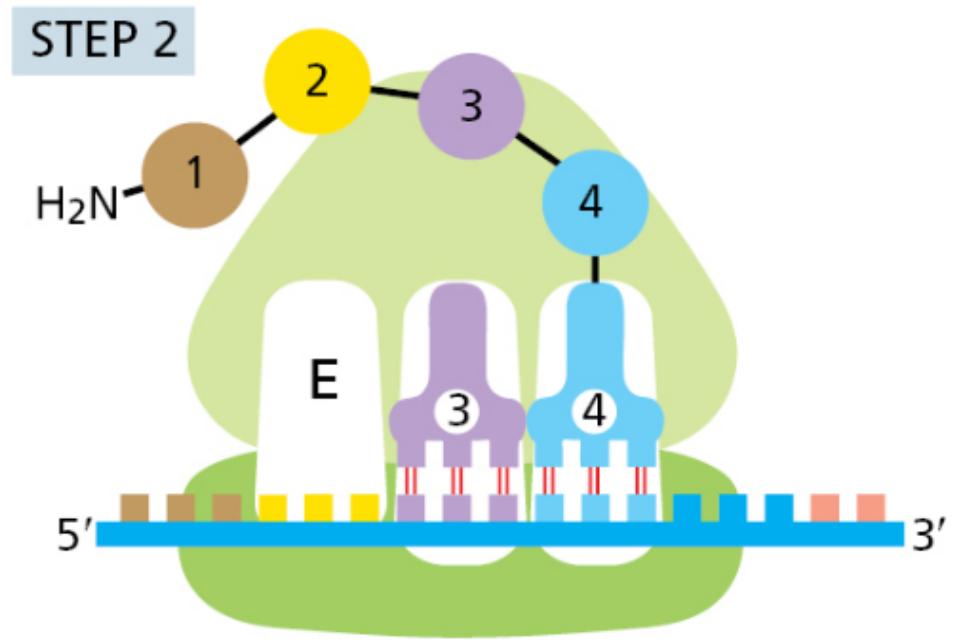
- when 43S complex recognises the Kozak sequence and the start codon (in eukaryotes) at 5' UTR: eIF1 is displaced by the start codon, followed by a change into a closed conformation, which is remarked as the 48S complex
- Formation of 48S complex leads to eIF5-mediated hydrolysis of eIF2-bound GTP
- induction of GTP hydrolysis by eIF5 serves as a final checkpoint for 80S assembly: **Joining of 60S subunits** and **dissociation** of other previous factors eIF1, eIF1A, eIF3 and residual eIF2-GDP are mediated by eIF5B (hydrolysis as a GTPase) —

- elongation-competent 80S ribosomes are formed

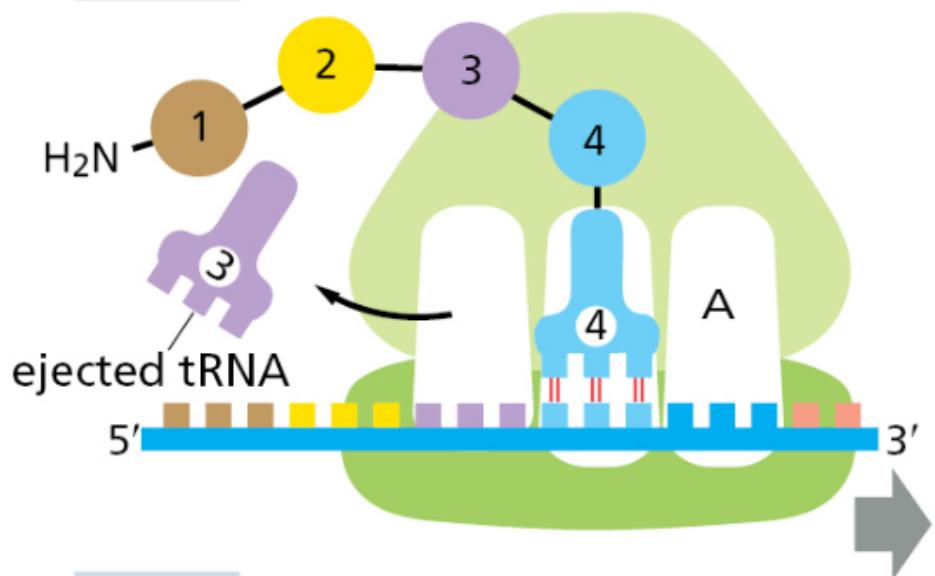
▼ Elongation Phase

- Each ribosome has one binding site for mRNA and three binding sites for tRNA:
 - A site: aminoacyl-tRNA
 - P site: peptidyl-tRNA
 - E site: exit
- Elongation basic steps:

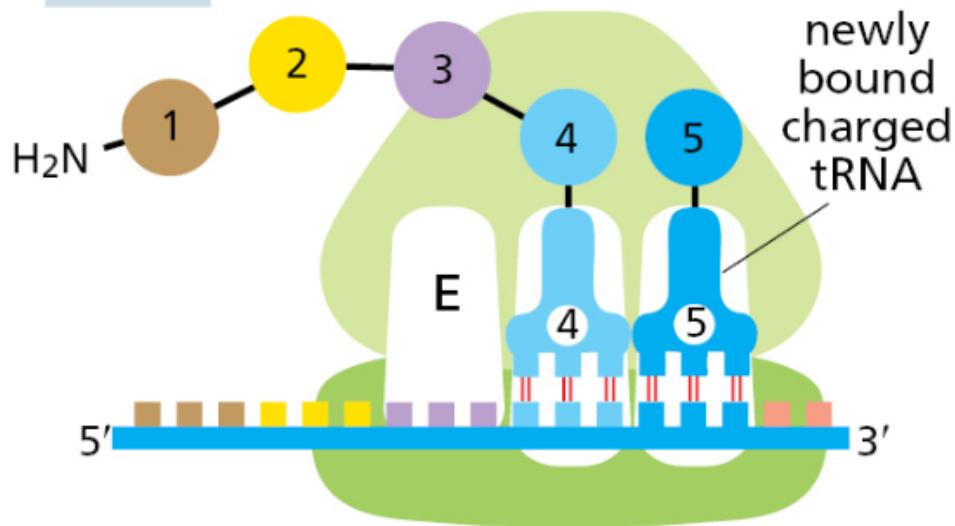




STEP 4



STEP 1



- aminoacyl-tRNA (the tRNA carrying the next amino acid) binds to mRNA by complementary base pairing
- the **carboxyl end of the polypeptide chain** is released from the tRNA P site — by breaking the **high-energy bond** between the tRNA and its amino acid & join the **free amino group** of the amino acid linked to the tRNA at the A site — new peptide bonds are formed

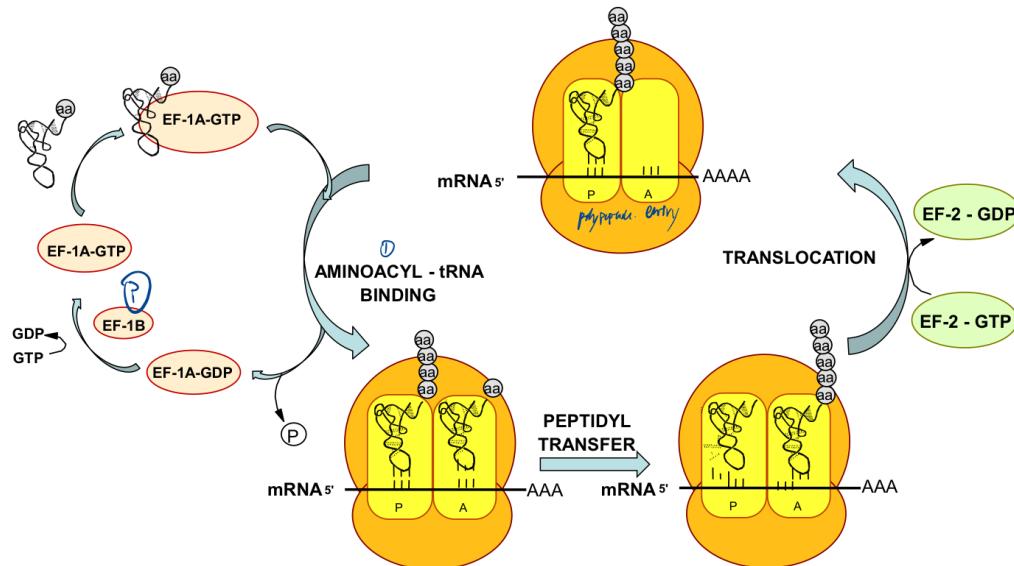
- Catalysed by a **peptidyl transferase** in the **large ribosomal subunit**

- Large subunit translocation (from 5' to 3')
- Small subunit translocation (from 5' to 3')
- This moves the empty tRNA at P site to E site
- Then Step 1 is repeated

▼ Elongation factors and mechanism

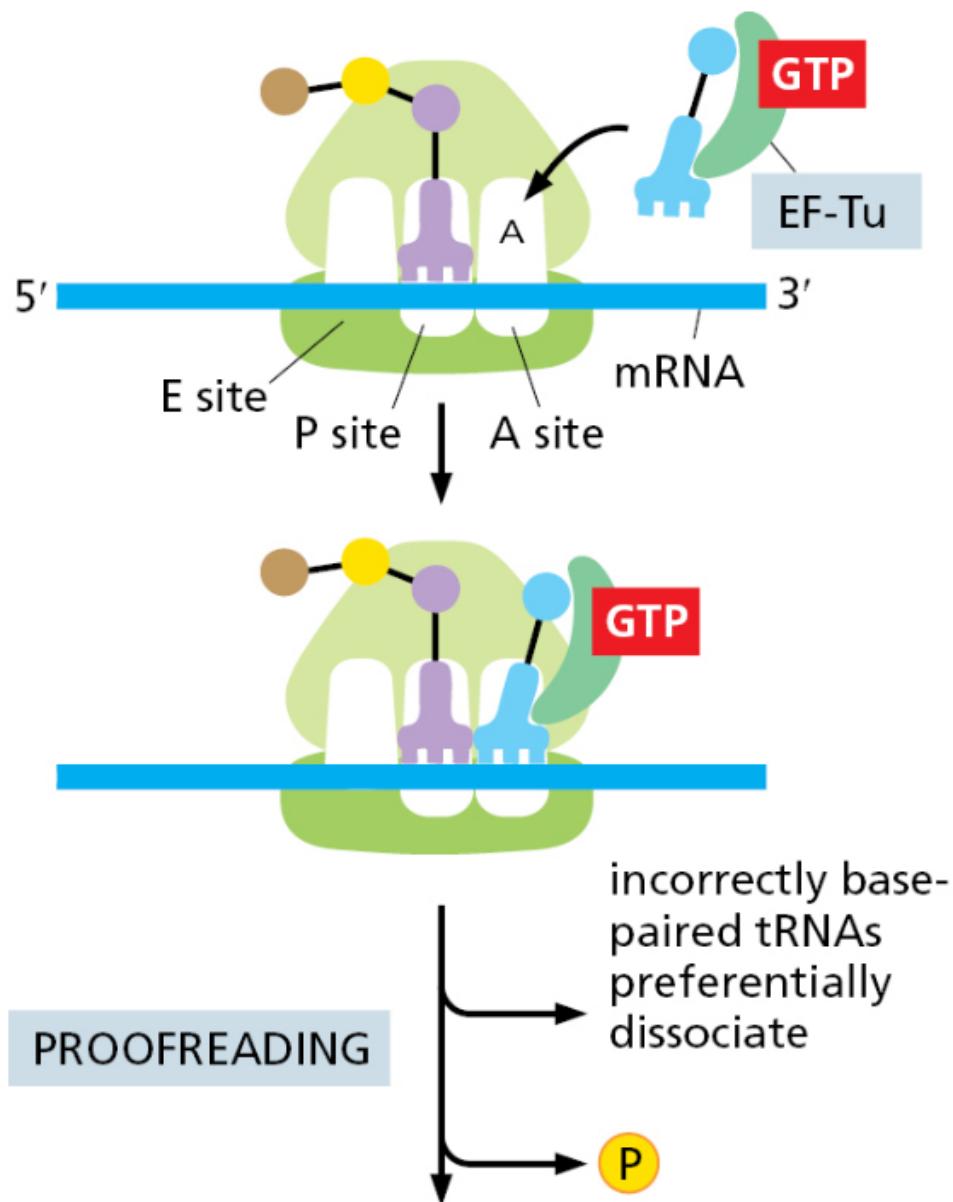
- EF-1 in eukaryotes (EF-Tu in prokaryotes) [EF-1A & EF-1B] & EF-2 (EF-G in prokaryotes)

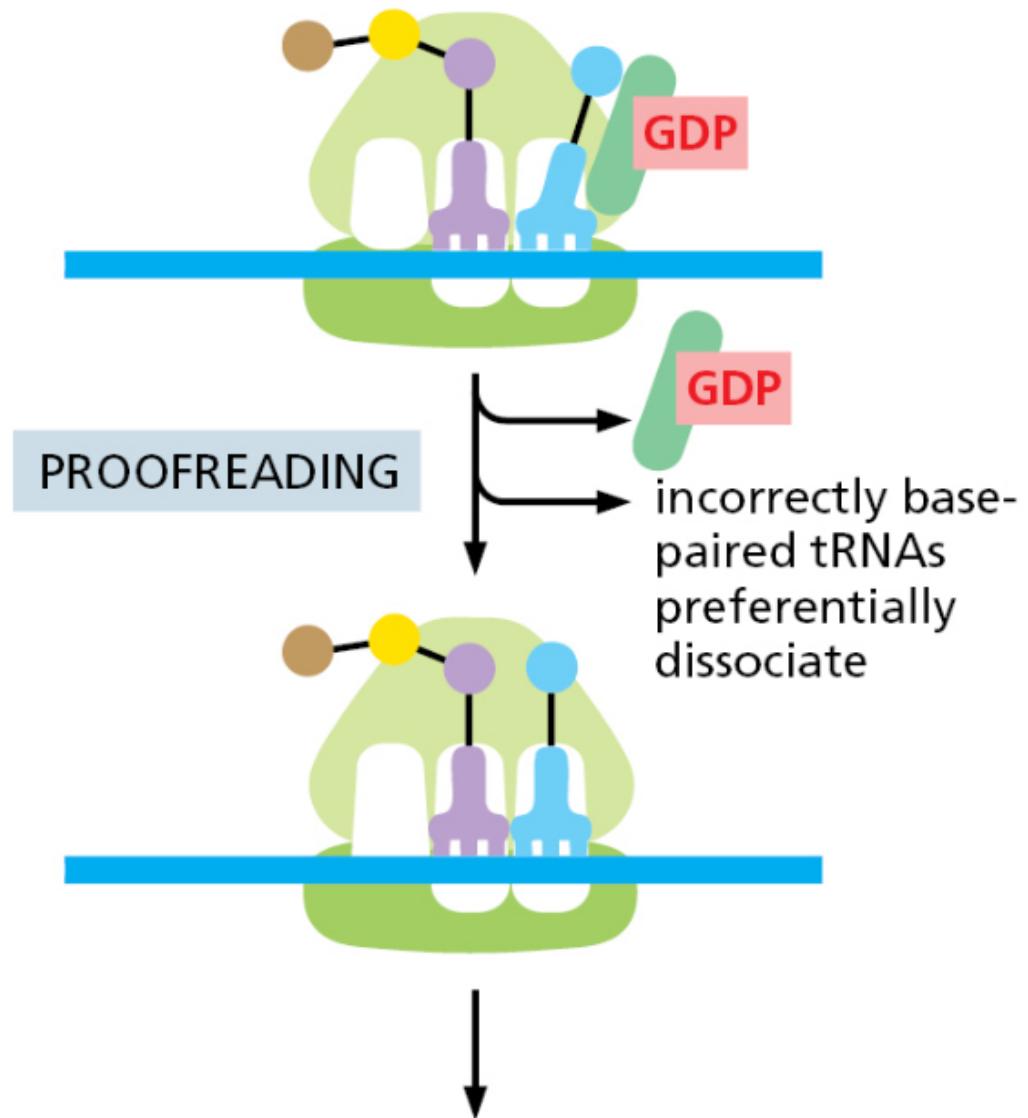
Elongation of protein synthesis

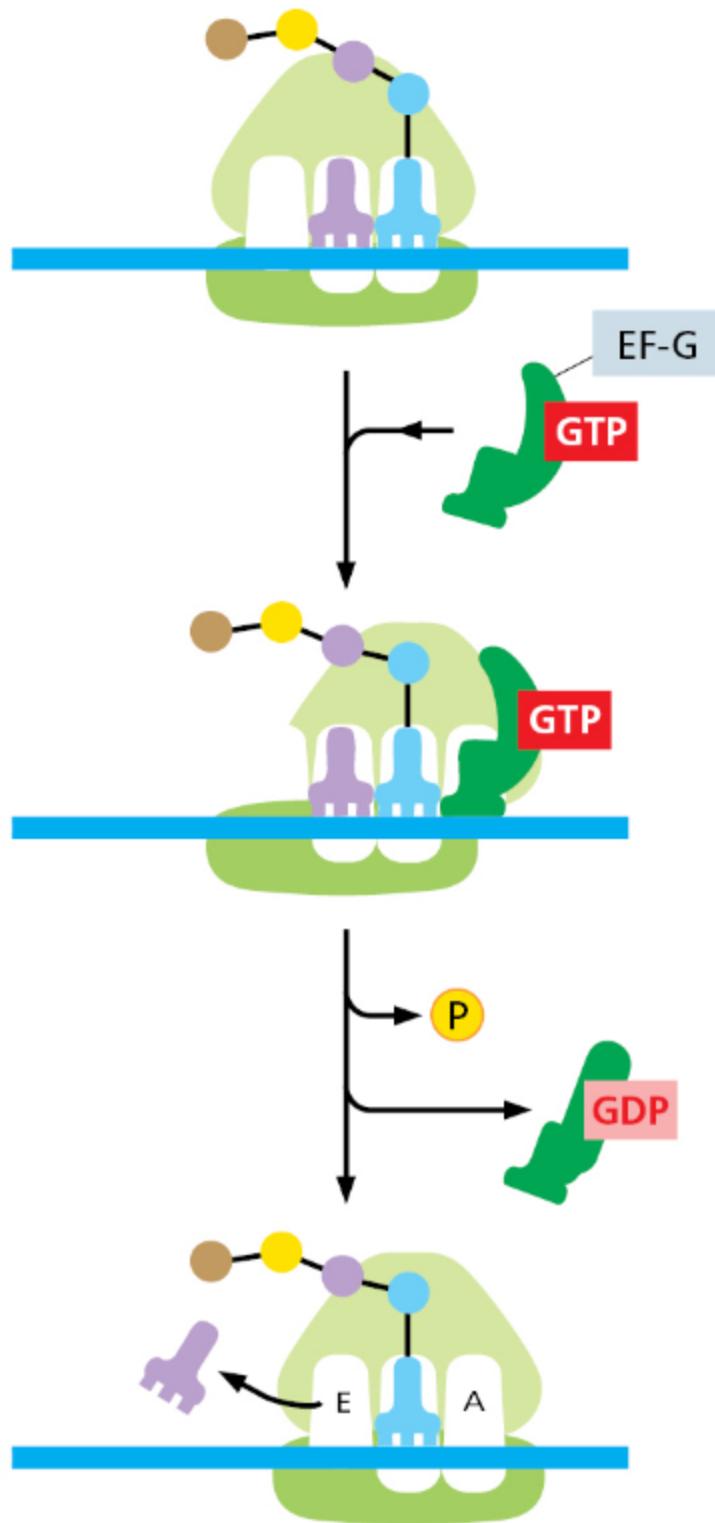


- EF-1A bound with GTP drives the translation in the forward reaction, by hydrolysing GTP to provide energy for new aminoacyl-tRNA binding to the P site
- EF-2-GTP facilitates the translocation of the deacylated tRNA to the E site

- ▼ Those two elongation factors provide two proofreading opportunity to ensure the accuracy of tRNA binding to the correct mRNA codon:







- Because the dissociation of incorrect ternary complexes (EF-Tu-GTP + aa-tRNA) occurs at a higher rate than the case where the base-pairing is correct; and the dissociation of the incorrect ternary

complexes takes place before GTP hydrolysis — ensure that the aa-tRNA does not remain bound to the ribosome

- balance between speed and accuracy

[lecturecast]

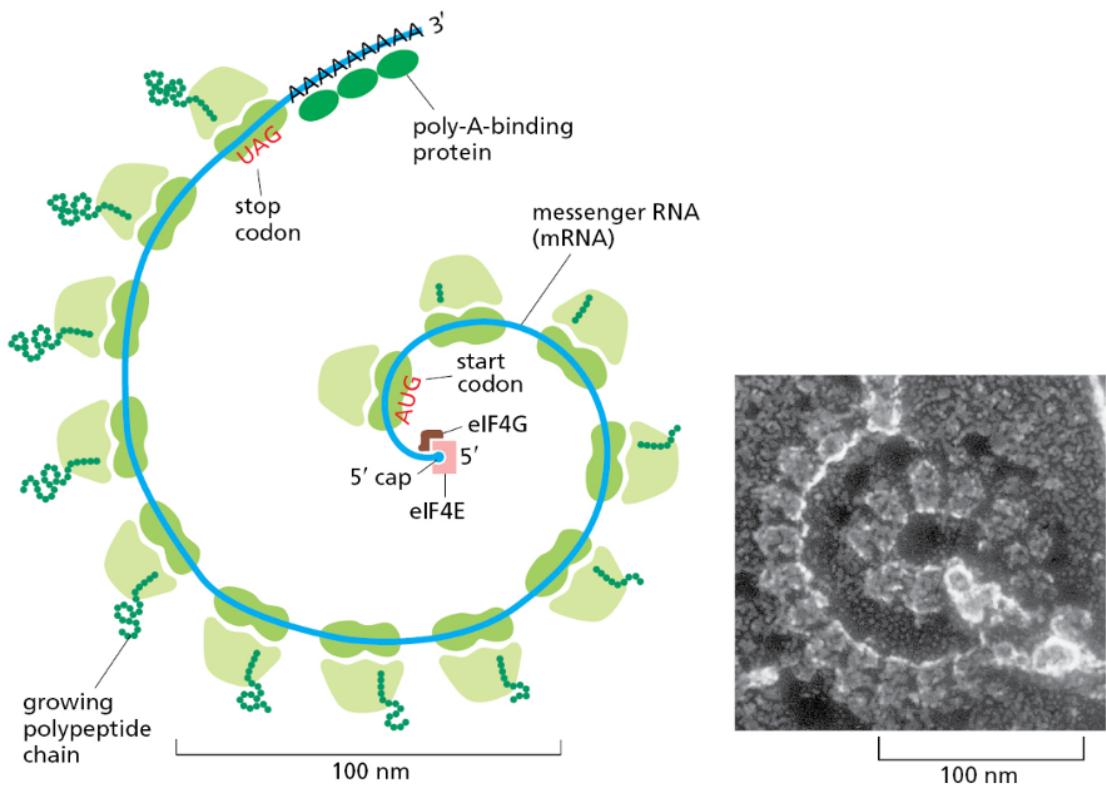
▼ Termination of translation

- eRF1 & eRF3-GTP - mimics the structure of tRNA-eEF1a-GTP
 - fits into the ribosomal A site, which is now located on the STOP codon
 - hydrolysis of GTP release energy, and it catalyses a nucleophilic attack on the ester bond between the peptide and the P-site tRNA (addition of water, instead of amino group)
 - Then the polypeptide chain is released from the ribosomes into the cytoplasm

▼ Contrast between eukaryotes & prokaryotes

Eukaryotes

- Leaky scanning: sometimes miss the first start codon and move to the second because the first recognition sequence is less identical to the consensus sequence (Kozak sequence) – produce two or more different proteins with different N-termini
- Proteins are made on polyribosomes: during short period, multiple initiations take place on one mRNA molecule – as soon as the preceding ribosome has translated long enough of the mRNA sequence, a new ribosome can be readily attached to the 5' end

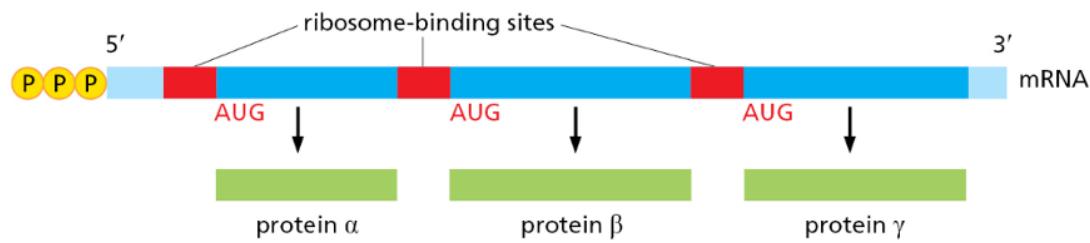


Prokaryotes

Bacterial mRNAs **have no 5' caps** to signal the ribosome where to begin searching for the start of translation. Instead, each bacterial mRNA contains a specific ribosome-binding site (called the **Shine–Dalgarno sequence**, named after its discoverers) that is located a few nucleotides upstream of the AUG at which translation is to begin. This nucleotide sequence, with the consensus 5'-AGGAGGU-3', **forms base pairs with the 16S rRNA of the small ribosomal subunit to position the initiating AUG codon in the ribosome**. A set of translation initiation factors orchestrates this interaction, as well as the subsequent assembly of the large ribosomal subunit to complete the ribosome.

A bacterial **ribosome can readily assemble directly on a start codon that lies in the interior of an mRNA molecule**, so long as a ribosome-binding site precedes it by several nucleotides. As a result, bacterial mRNAs are often **polycistronic**; that is, they encode several **entirely** different proteins, each of which is translated from the same mRNA molecule (Figure 6–75).

In contrast, a eukaryotic mRNA generally encodes only a single protein, or more accurately, **a single set of closely related proteins**. We will see in the next chapter that there are some exceptions to this generalization, where a eukaryotic mRNA can carry information for two or more distinct proteins (by alternative splicing – post-transcription).



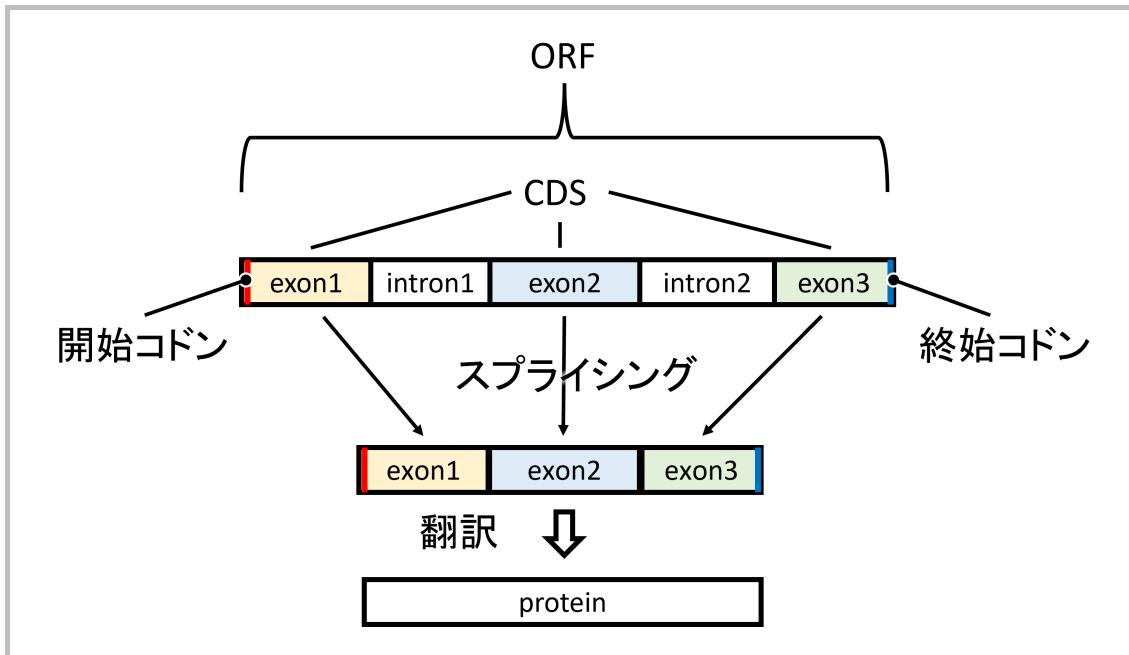
▼ Why recognition of 5' cap and 3' poly(A) tail is important for translation? — **recognition markers for complete & correct translation**

- some mRNA-processing is not complete in the nucleus before mRNA is transported to the cytosol
- some intact & correct mRNA can be damaged during the process of transporting
- Therefore, this is a backup mechanism to prevent the damaged or incomplete mRNA from being translated

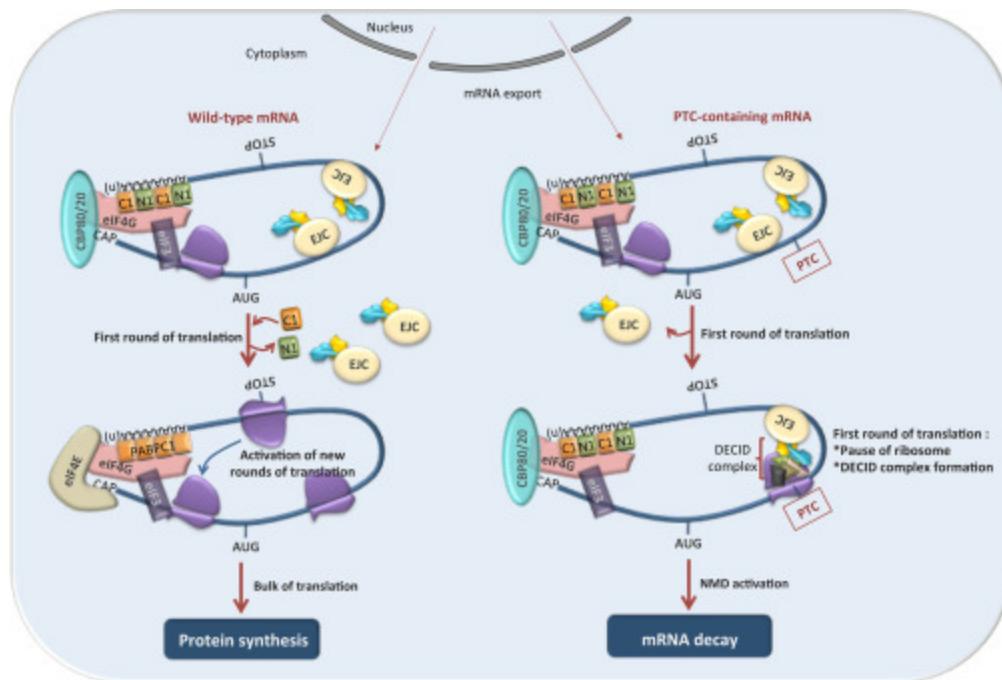
▼ **Nonsense-mediated mRNA decay** : resolution to premature stop codon

- if mRNAs contain premature stop codons & with no stop codons, they are targeted for degradation
- The error is likely to arise from improper splicing of mRNA / nonsense mutation
- After the splicing the introns, exon junction complex is added to the site overlapping the two neighbouring exons
- mature mRNA is transported to cytosol through the nuclear pore
- during translation, ribosome removes the EJCs at CDS

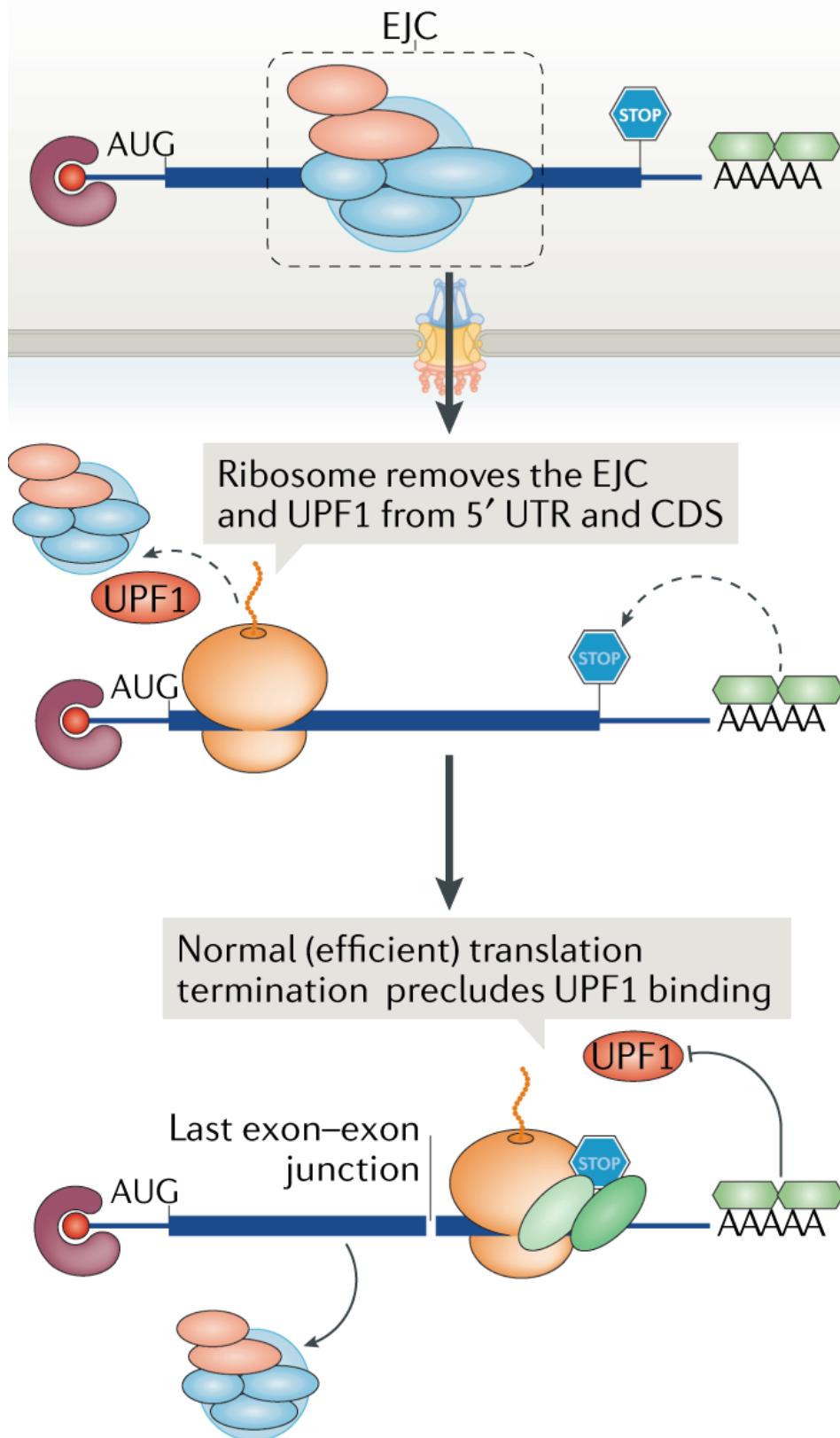
- thus, when the translation terminates at the normal stop codon, all the EJCs are removed



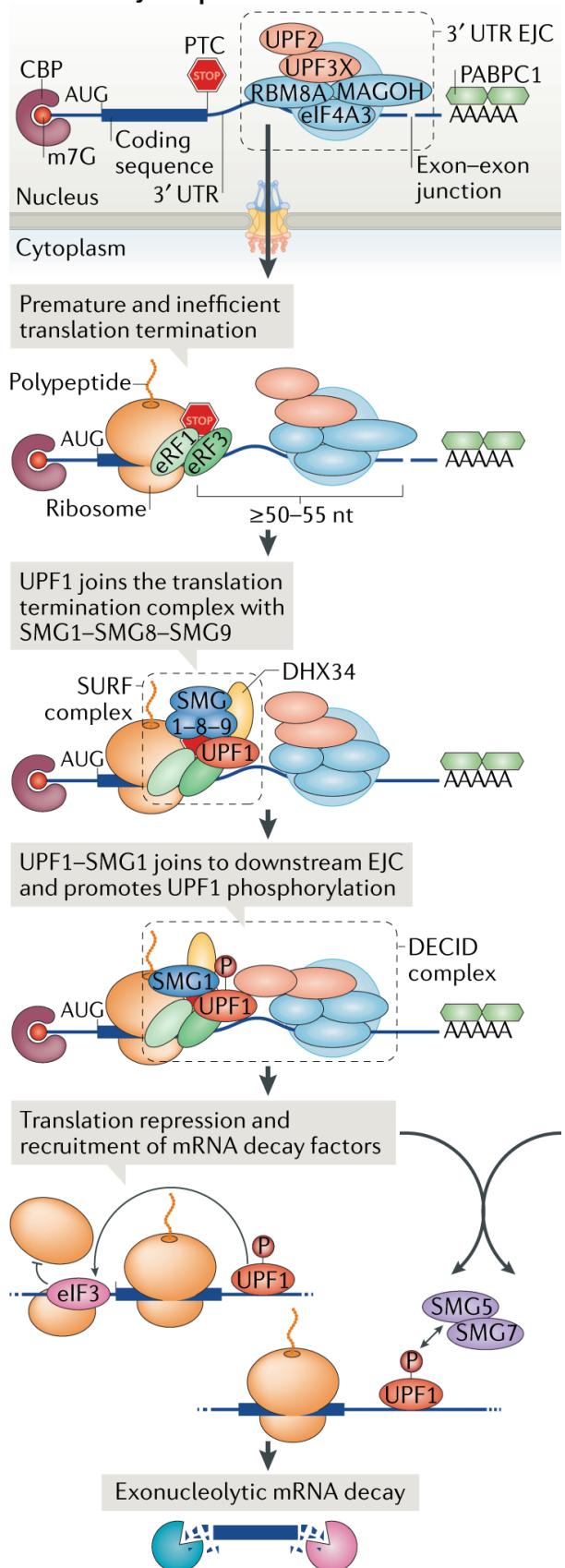
- However, when there are premature stop codons within the CDS:



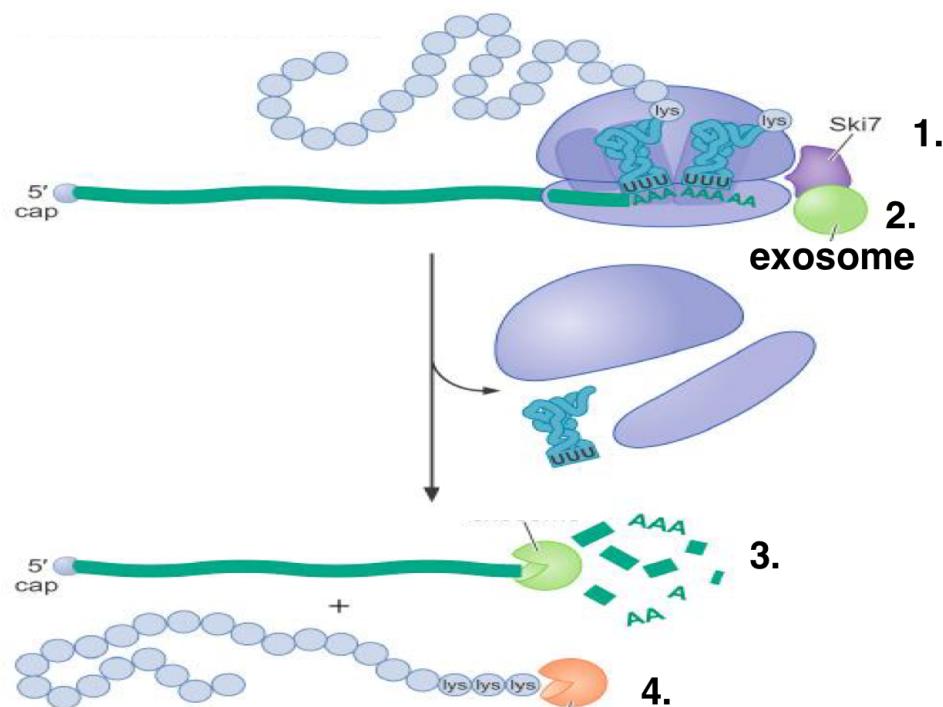
c No NMD



a 3' UTR EJC-dependent NMD



- The ribosome terminates before extra EJCs (normally within the EJC there are UPF proteins) — This recruits extra UPF protein binding to the ribosome
 - UPF is then phosphorylated
 - The phosphorylation of UPF then recruits mRNA decay factors & **decapping enzyme**— remove the ribosome & 5' cap & Upf proteins
 - recruits exonuclease to degrade the rest mRNA
- ▼ non-stop codon -mediated mRNA decay
- translate the poly(A) tail into poly-lysine chain
 - recruit **Ski7** → recruit exosome → ribosome tRNA dissociate & **exosome degrade the mRNA**
 - **Lysine specific protease** is recruit to remove the polypeptide chain



Regulation of Translation

- ▼ The significance of regulated translation — **translation costs great amount of energy!**

Therefore, the cell needs to turn on/off translation wisely:

- when the cell requires this protein for nutrient absorption / when cell "senses" the level of nutrients etc
- some proteins need to be expressed distantly and locally, so the translation is locally regulated —
 - e.g. mRNAs in neurons are transported to the axons which is quite distant from the cell bodies; and are translated locally when needed
- translation in response to **signalling**, e.g. insulin; IgG

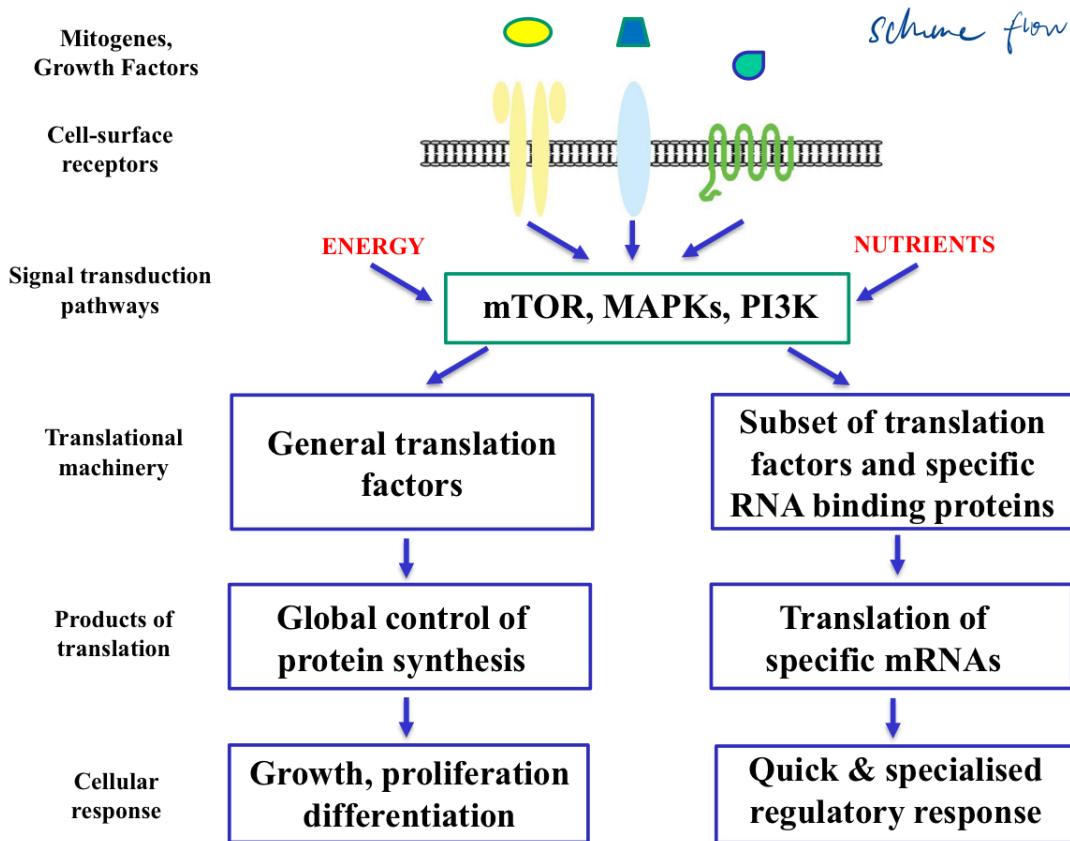
- ▼ Therefore, translation is mediated by diverse mechanisms

- Extracellular stimuli and stresses
- Nutrient availability
- Energy status
- Signal transduction pathways
- Cellular metabolites
- miRNA

- ▼ Protein homeostasis is mediated by:

- translation regulation
- post-translational modification
- folding
- degradation
- Dysregulation of this balance can lead to pathological changes; closely associated with diseases

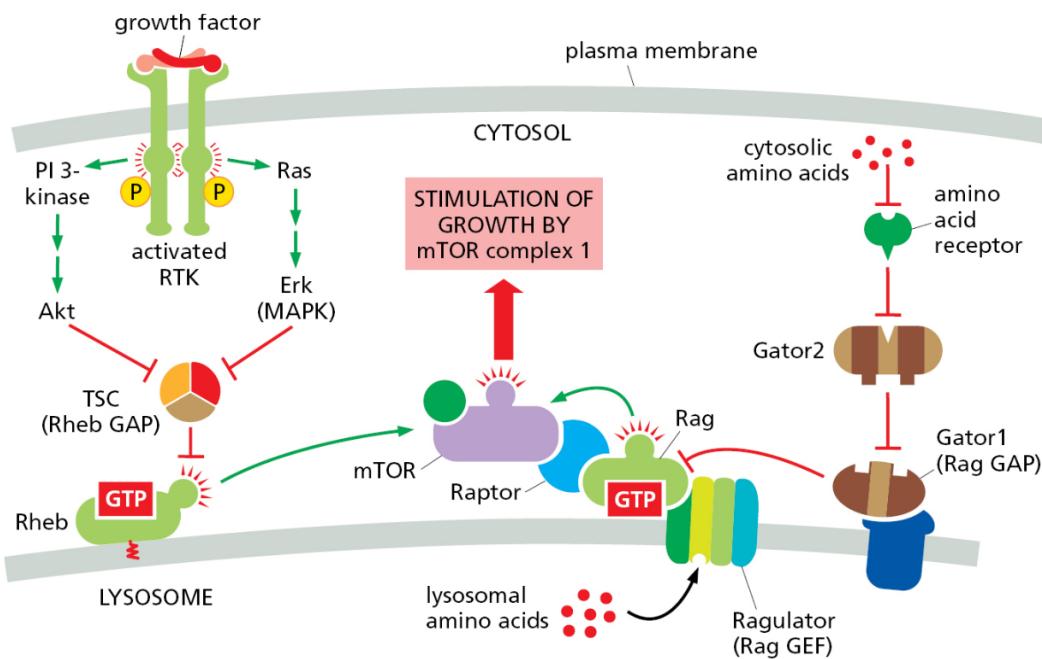
Regulation of translation by signalling pathways



▼ translation regulation via mTOR signalling

- mTOR: mammalian target of rapamycin — kinase
- two functionally distinct complexes:
 - mTORC1:
 - mTOR+ Raptor (determines the specificity of mTORC1) + G β L + Rheb
 - sensitive to rapamycin
 - two activators: growth factors (hormones) + nutrients (AMP: ATP)/ cytosolic amino acids

- Two upstream regulation pathway: PI3K-Akt pathway; Ras-Erk pathway
- downstream signalling pathway: 4E-BP1 & S6K
- regulation results: promote protein synthesis — cell growth and metabolism
 - cell growth / division: requires specific factors to regulate **cell cycle**



- mTORC2:

- mTOR + Rictor + G β L
- insensitive to rapamycin
- downstream signalling pathway: PKB (protein kinase B; aka **Akt**)
- activators: growth factors (? - lecturecast) / survival signals - PIP3 kinases pathway
- downstream signalling pathway: Akt signalling — cytoskeletal rearrangement & survival (inhibition of apoptosis)/ proliferation (?)

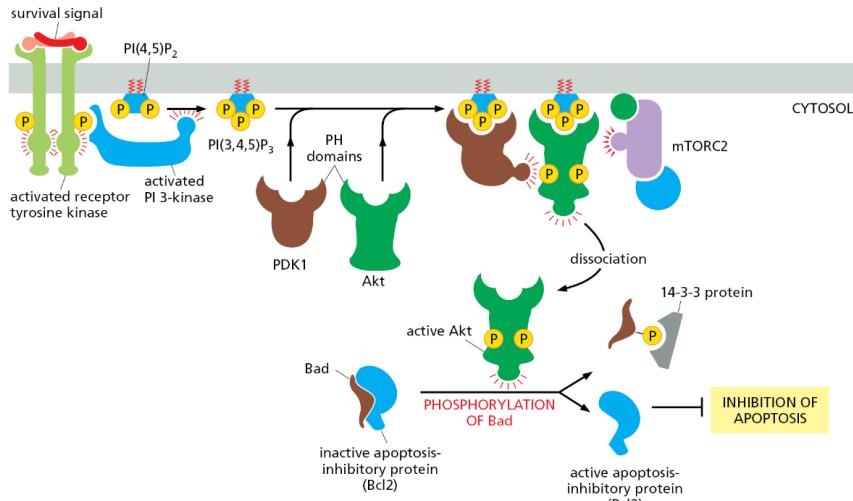


Figure 15–54 One way in which signaling through PI 3-kinase can promote cell survival. An extracellular survival signal activates an RTK, which recruits and activates PI 3-kinase. The PI 3-kinase produces PI(3,4,5)P₃, which can act as a docking site for two serine/threonine kinases with PH domains—Akt and the phosphoinositide-dependent kinase PDK1—and bring them into proximity at the plasma membrane. The Akt is phosphorylated on a serine by a third membrane-associated kinase mTORC2, or mTORC1, which alters the conformation of the Akt so that it can be phosphorylated on a threonine by PDK1, which activates the Akt. The activated Akt may dissociate from the plasma membrane and phosphorylates various target proteins, including the Bad protein. When unphosphorylated, Bad holds the apoptosis-inhibitory Bcl2 in an inactive state (Bad and Bcl2 are both members of a family of proteins that regulates apoptosis, as discussed in Chapter 13). Once phosphorylated, Bad releases Bcl2, which now can block apoptosis and thereby promote cell survival. The phosphorylated Bad binds to a ubiquitous cytosolic protein called 14-3-3, which keeps the protein out of action, as shown.

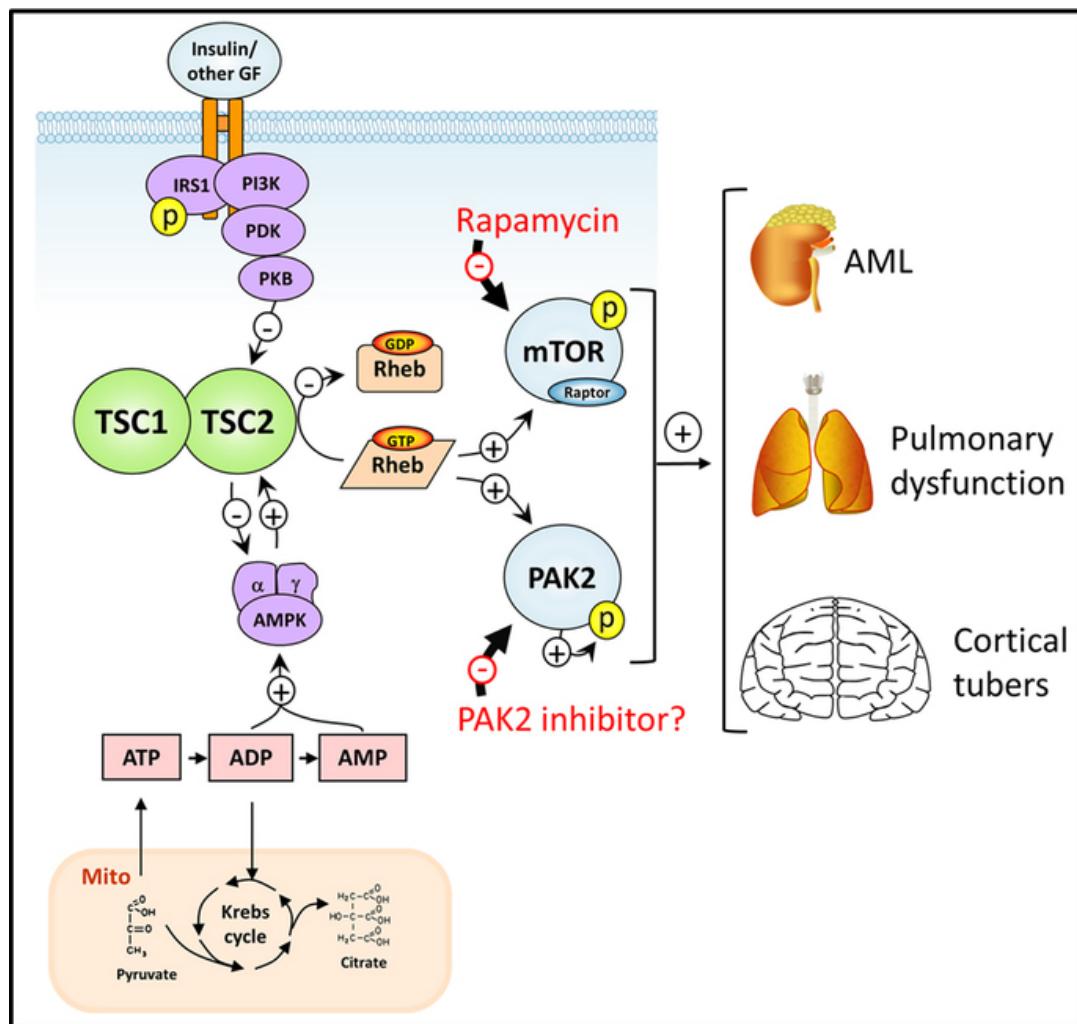
▼ Integrated knowledge with CELL0010

- GEF: guanine nucleotide exchanging factor: GDP → GTP: initiate signalling
- GAP: GTP-activating proteins: hydrolysis of GTP → GDP: terminate signalling
- Ras: one family of G-protein (aka GTPase: GTP-binding proteins); others: Rho, Rab, Arf and Ran
- Sos: one classic GEF for Ras

▼ mTORC downstream response to growth factor stimulation

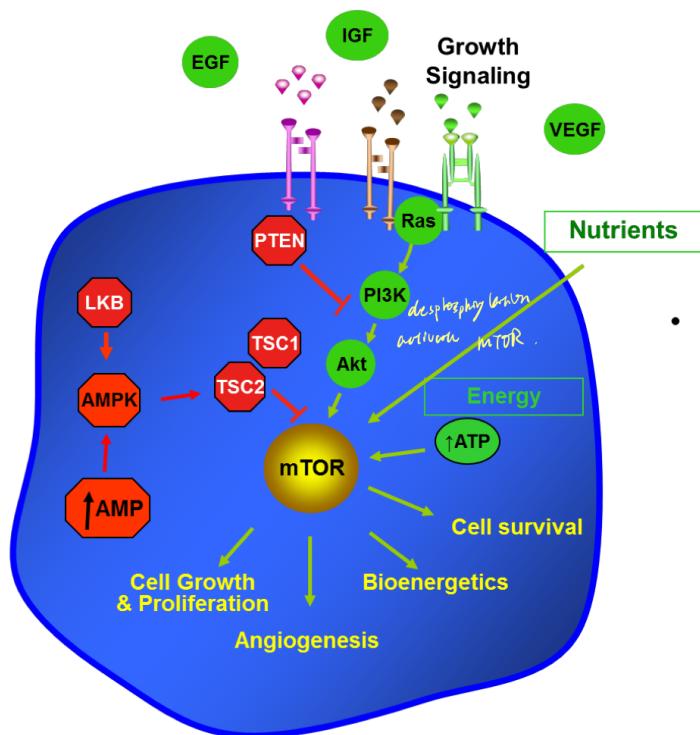
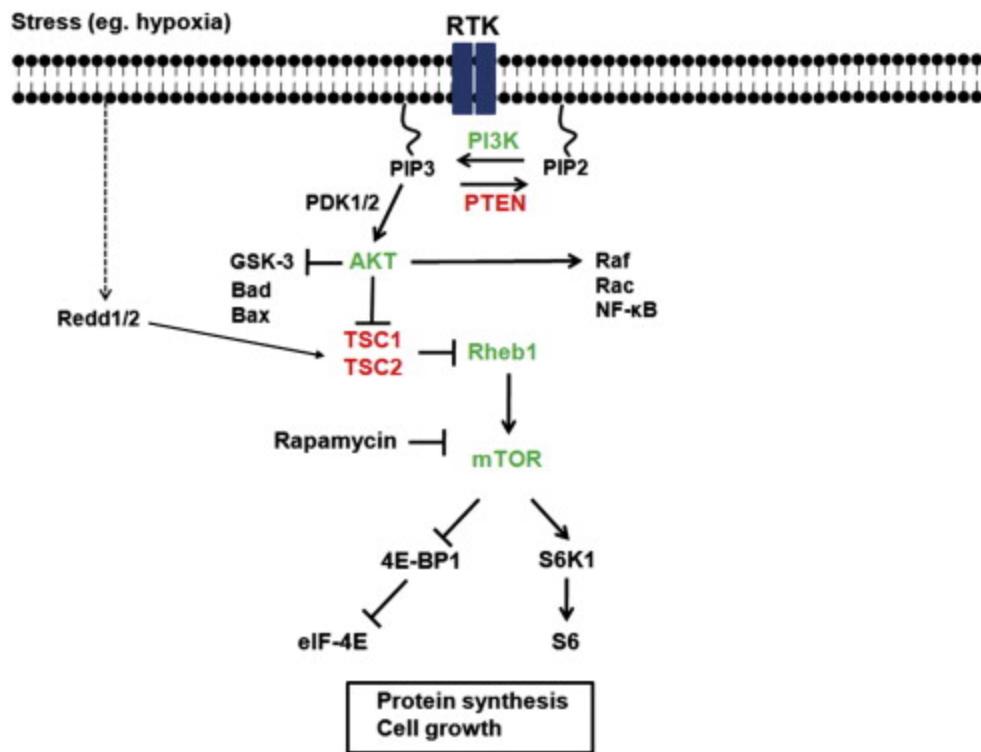
- 4E-BP1: binds to eIF4E then **inhibits** eIF4G binding to eIF4E (→form eIF4F complex at 5' cap to initiate translation)
 - phosphorylation of 4E-BP1 leads to its dissociation from eIF4E — allow eIF4E binding to eIF4G and eIF4A
 - why 4P - 4E-BP1 has at least 7 phosphorylation sites, four of which is mediated by mTOR
 - hypophosphorylated 4E-BP can still tightly bind to eIF4E
- S6K: 40S ribosomal protein - S6 kinase

- recruits eIF4B to eIF4A — enhances eIF4A helicase activity - upregulate translation efficiency
- mTORC1 phosphotransferase activity
 - stimulated by Rheb-GTP
 - Rheb activity is regulated by TSC1 and TSC2; **TSC2** exhibits **GAP** activity towards Rheb → **inhibit** Rheb activity by hydrolysing GTP to GDP



▼ Inactivation of mTOR signalling

- when lack of nutrients (\uparrow AMP:ATP)

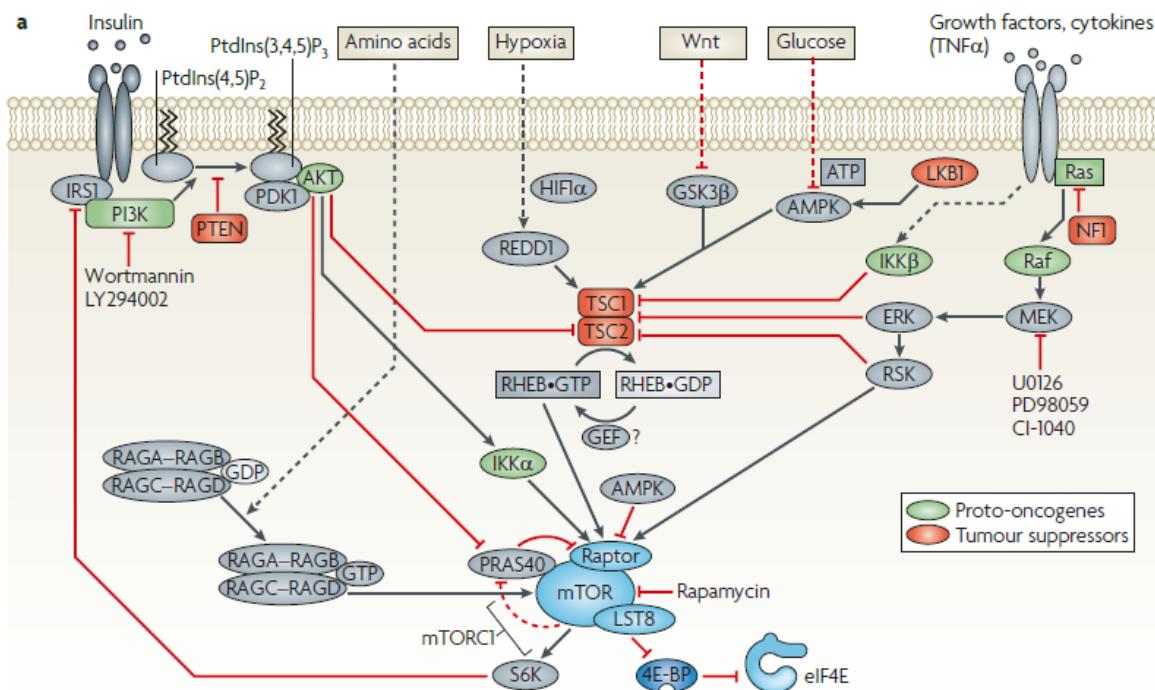


- The upregulated [AMP] (marking the low energy status; low ATP condition) can be sensed by LKB1, which then phosphorylates AMPK

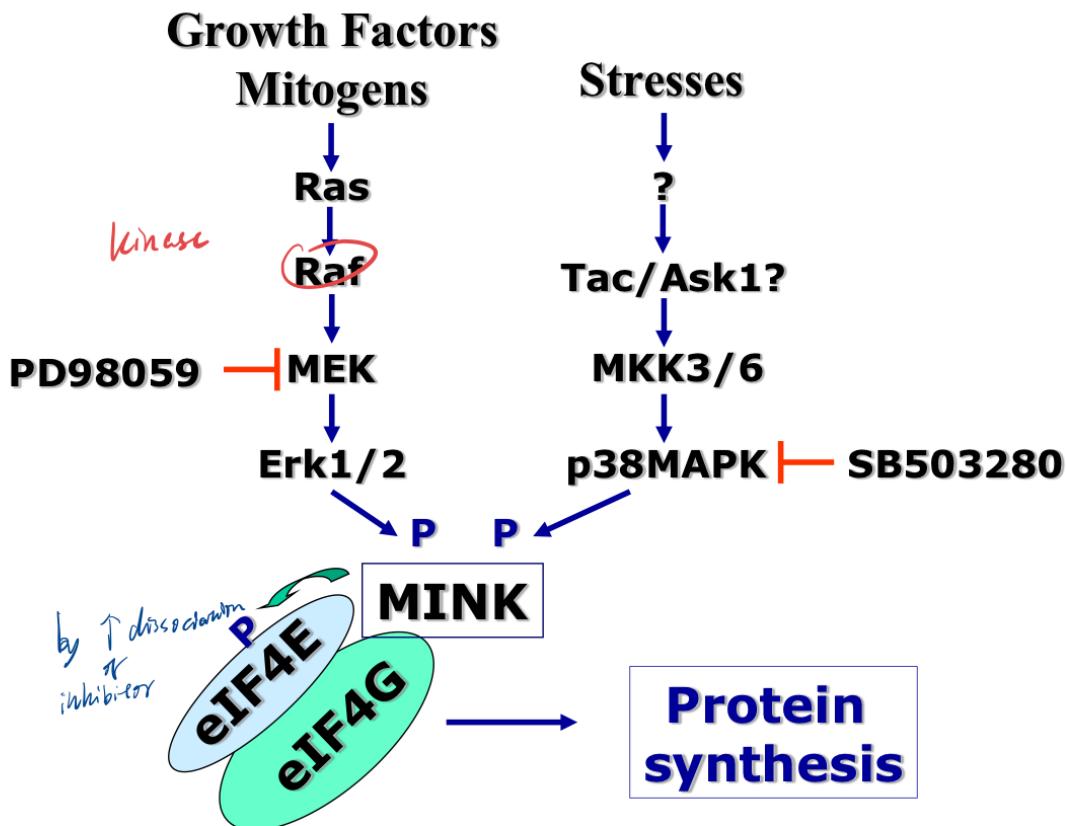
to upregulate its activity

- AMPK is a metabolic master regulator that is activated in response to reduced energy availability [high cellular AMP: ATP ratios] or hypoxic stress
- Then AMPK can phosphorylate TSC2 to inhibit the activity of mTORC1
- ▼ PTEN regulation

- Growth factors, such as insulin, act through receptor tyrosine kinases to activate PI3K, which catalyzes the conversion of phosphatidylinositol(4,5)-bisphosphate (PIP2) to phosphatidylinositol(3,4,5)-trisphosphate (PIP3). PIP3 stimulates Akt kinase activity, which in turn stimulates mTOR by phosphorylating and inactivating TSC2
- PTEN is a negative regulator, acts as a lipid phosphatase, which converts PIP3 back to PIP2, such that the Akt activity is downregulated



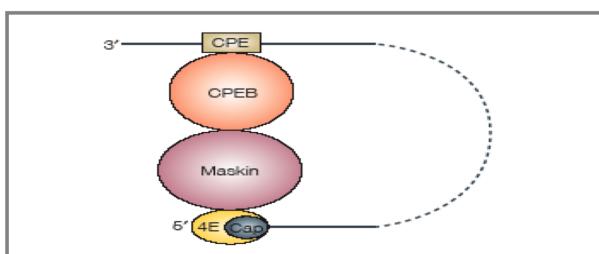
Regulation of protein synthesis via MAPK and stress-induced pathways



Ras-Raf-MEK (MEK: MAPK/ERK kinase)- MAPK pathway

- ▼ Regulation of Cyclin B1 translation (Cell cycle) by eIF4E-Maskin dissociation

Sequestration of eIF-4E



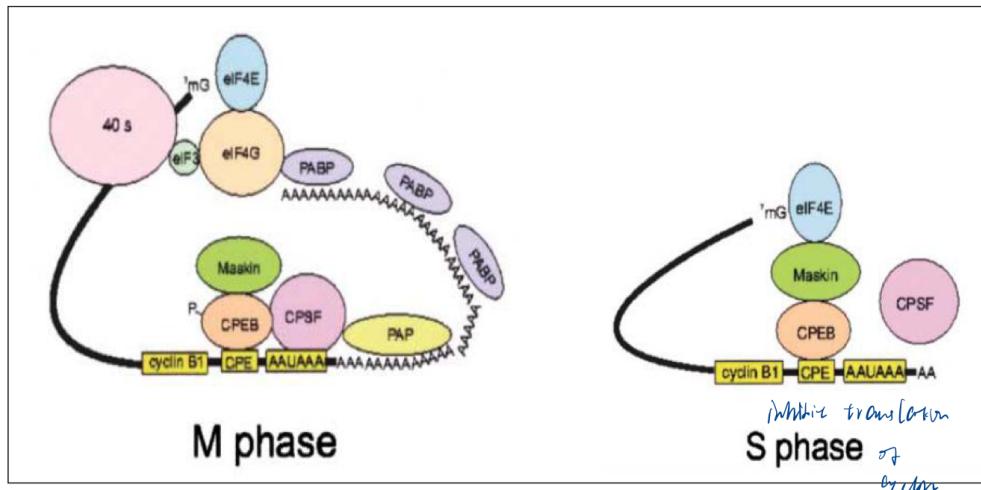
Maskin binds to and prevents eIF-4E function
Early development in Xenopus

CPEB: cytoplasmic polyadenylation element binding protein

CPE: 3' UTR site

Maskin: binds to eIF4E to prevent eIF4G from binding to form eIF4F initiation complex

Regulation of Cyclin B1 translation during the cell cycle



Translation of Cyclin B1 is induced during M phase by PABP-mediated dislodgment of Maskin from eIF4E

CPEB - cytoplasmic polyadenylation element binding protein

Src

▼ What is Src: tyrosine kinase family

- N-terminus — Src homologous 3 (SH3) domain — SH2 domain — kinase domain (small lobe + large lobe) — C-terminal tyrosine phosphorylation site — C terminal

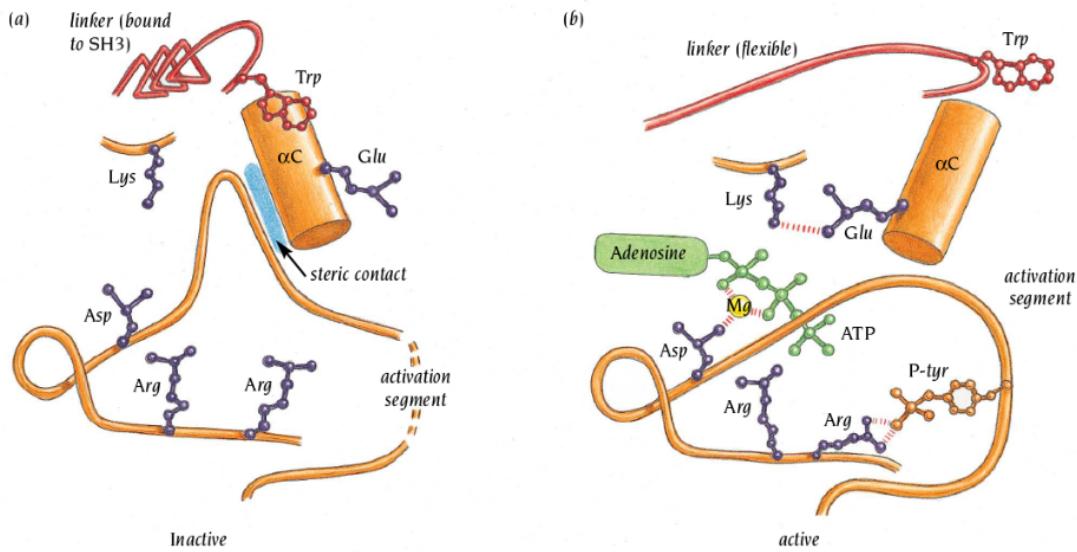
▼ Autoinhibition:

1. The C-terminus contains a tyrosine phosphorylation site for another tyrosine kinase called Csk (C-terminal Src kinase) — the site is Tyr-527 is phosphorylated

2. Intramolecular interaction between SH2 and Src's C-terminal
 - a. The sequence following Y527 is not the optimal consensus sequence pY-E-E-I
→ SH2 binds to the C tail in a low affinity → high koff
 - b. SH2 domain will keep "seek" for the more optimal sequence
 - c. KD for SH2 binding to the C-terminal tyrosine is small (koff/kon is small) → a fast association rate has to compensate for the fast dissociation rate [this makes sense because SH2 and the C-terminal are in close proximity]
3. SH3 domain binds to the back of the kinase domain (linker helix)
 - a. The linker resembles a "type II polyproline helix" that runs at the back of the two lobes of the kinase domain which SH3 would bind to
 - b. The binding of SH3 domain causes the linker to make steric clashes with the elements of the catalytic site — kinase domain is not competent to function
 - c. again, this is a low-affinity binding — SH2 and SH3 readily dissociate and randomly orient into a active state conformation

▼ During activation:

1. tyrosine phosphatase removes the inhibitory phosphate on the C-terminus tail
2. SH2 recognises and is now able to bind to a optimal consensus sequence
3. Also the activation requires **SH3 to dissociate from the linker**
4. The **activation loop** Tyr is phosphorylated, usually by other active Src molecules (**trans auto-phosphorylation**)
5. → H interacts with the -ve Phosphate → the C-helix equivalent (PSTAIRE helix in CDK) can now rotate and orient correctly: The **E** in C-helix can interact with **K** → **co-ordinates the α- and β-ATP phosphate**

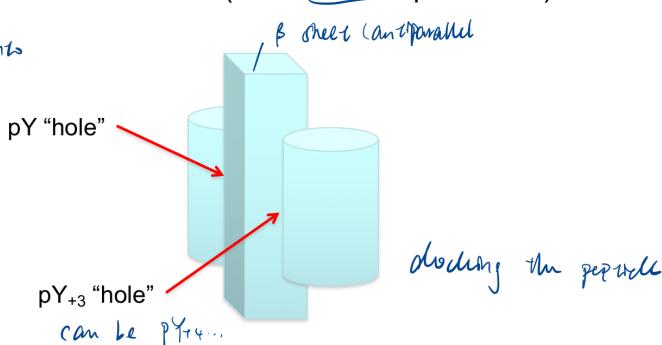


6. In the activation loop: upstream Arg binds to the pTyr + another Arg binds interact with γ -ATP phosphate + Asp binds to Mg²⁺ at the active site

▼ Structure of SH2 domain

- A central anti-parallel beta sheet with single helices at either side
- pTyr binding pocket between one helix and the sheet
- pY+3 binding pocket between the other helix and the sheet
- Mixed alpha-helices and beta-strands.
- In essence a **central anti-parallel beta sheet** with single helices packed on either side.
- A phospho-tyrosine binding **pocket** between one helix and the sheet
- A pY₊₃ binding pocket (although some SH2 domains accommodate more residues) between the other helix and the sheet (with some loops as well)

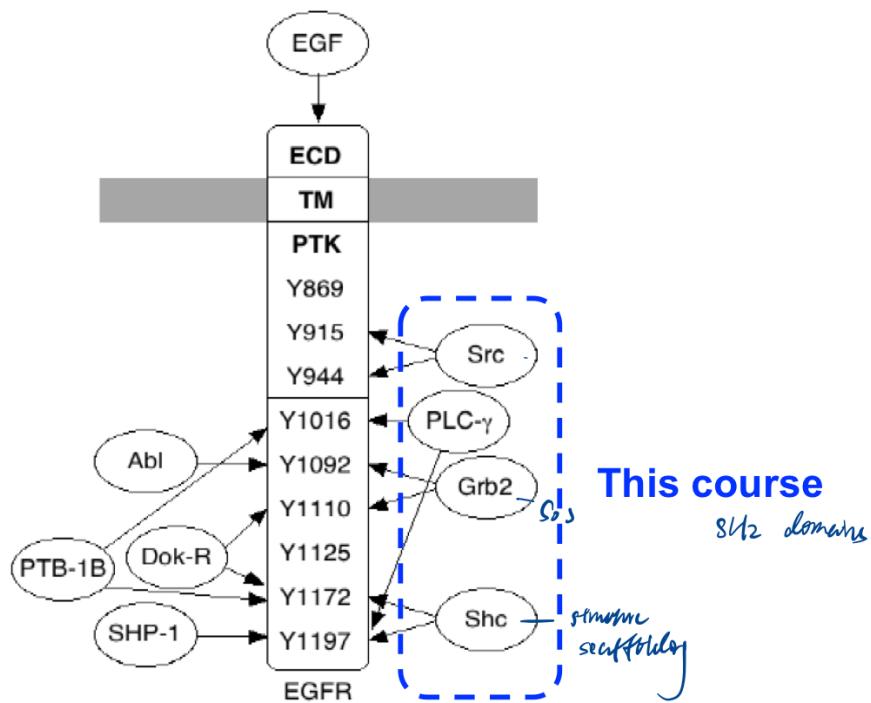
for P tyrosine to plug in



Tyrosine Kinase Receptor activation & signalling cascade

- ▼ pTyr signalling cascade EGFR as an example

Autophosphorylation sites and binding partners



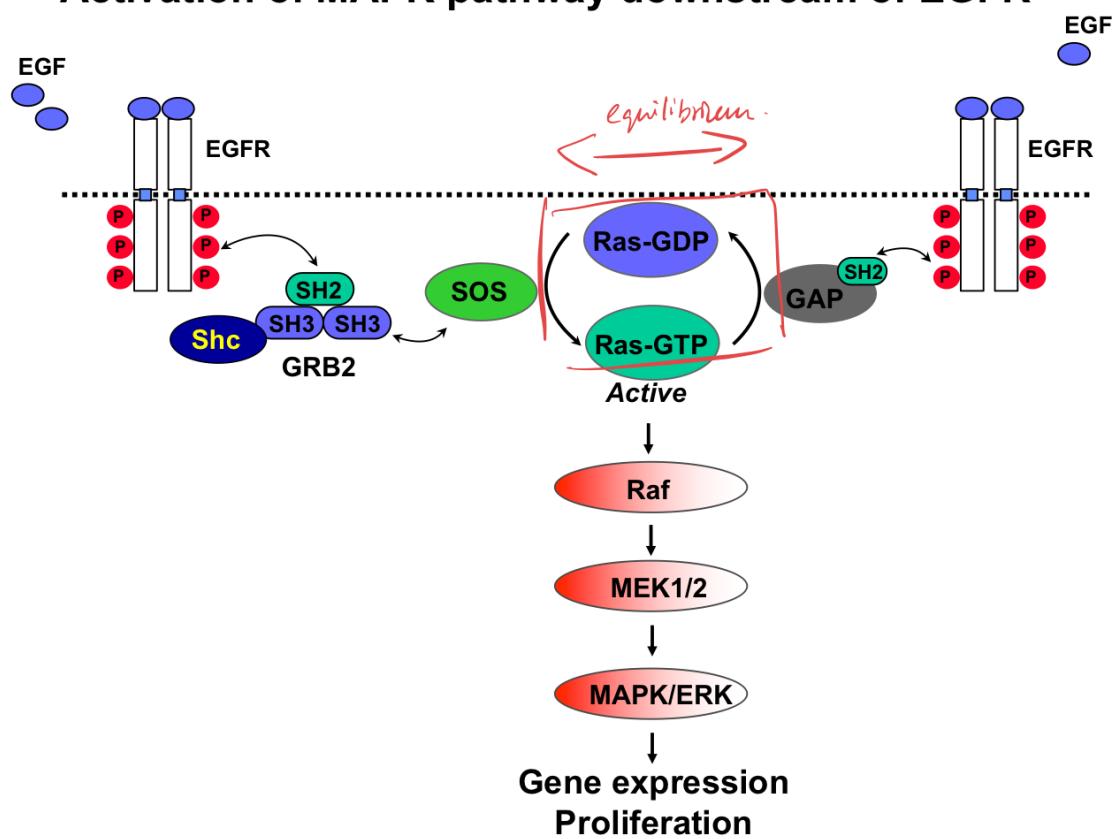
Cellular proteins interact with activated EGFR via:

- SH2 domains (recognise specifically pTyr)
- SH3 domain (recognise specifically Pro-rich motifs)
- Indirect association via adapter proteins (Shc, Grb2, Crk etc) that contain SH2 domains
- Clathrin binding via AP-2 (yes... the same as GPCRs)
endocytosis / internalisation

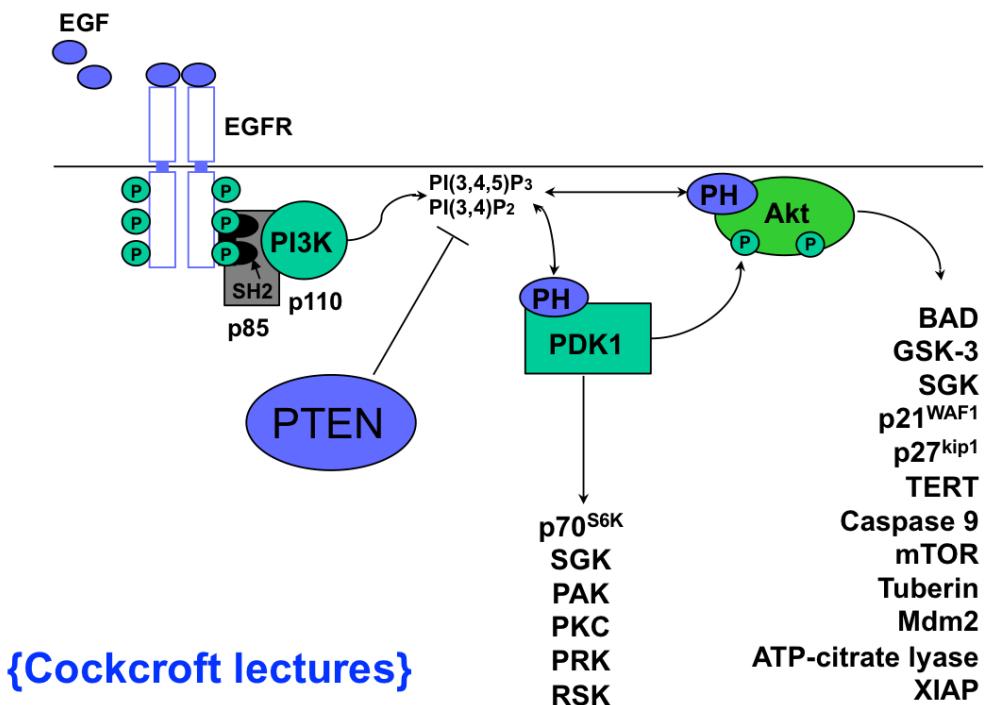
Key signalling substrates for EGFR

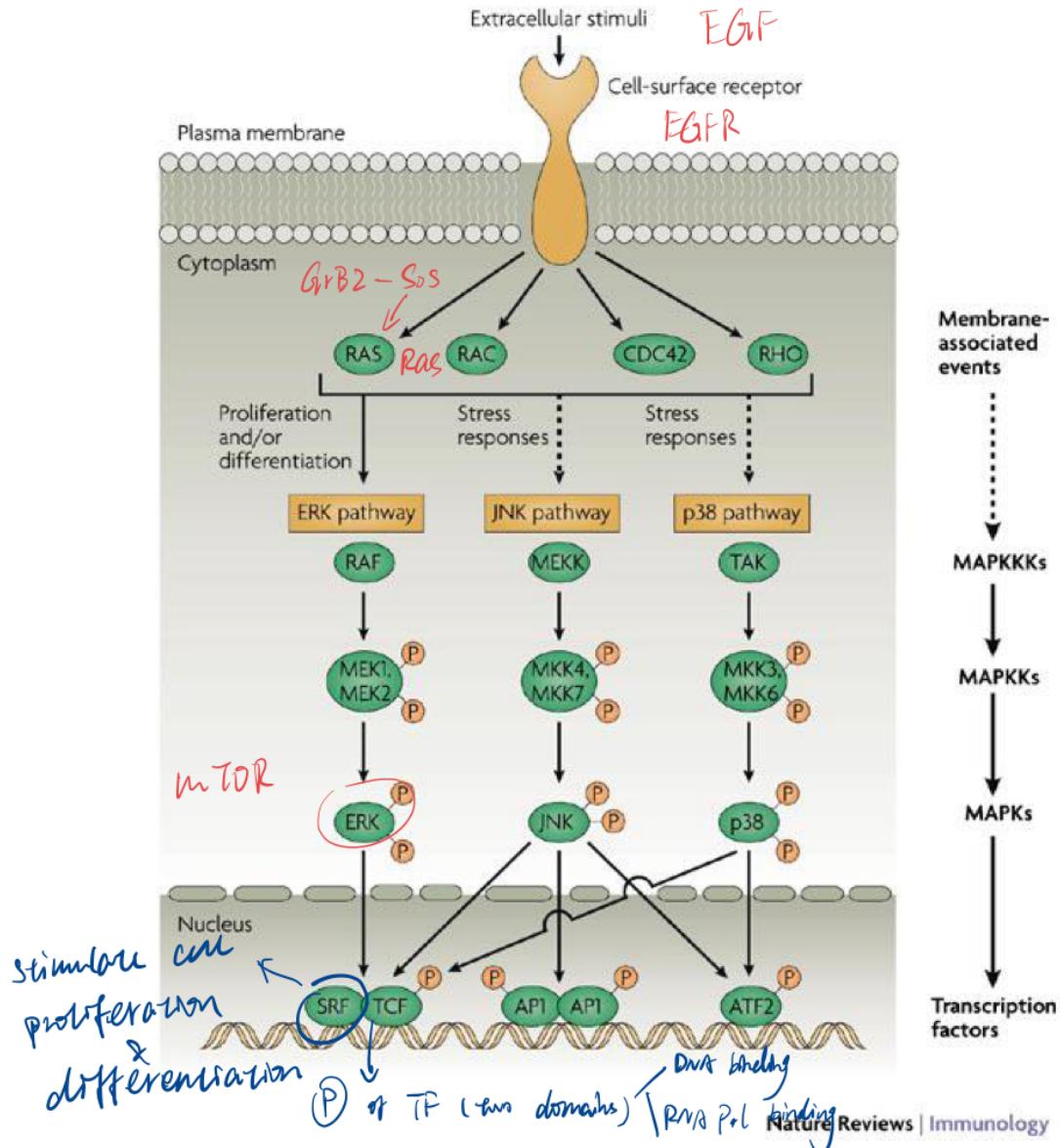
- EGFR intracellular domain
- Grb2/Sos {Thomas L5 & L6}
- Ras-GAP {Thomas L5 & L6}
- Shc (adaptor protein) {Thomas L6}
- Phospholipase C γ {Cockcroft lectures}
- Phosphoinositol-3-Kinase {Cockcroft lectures}
- p91 transcription factor
- Cbl

Activation of MAPK pathway downstream of EGFR



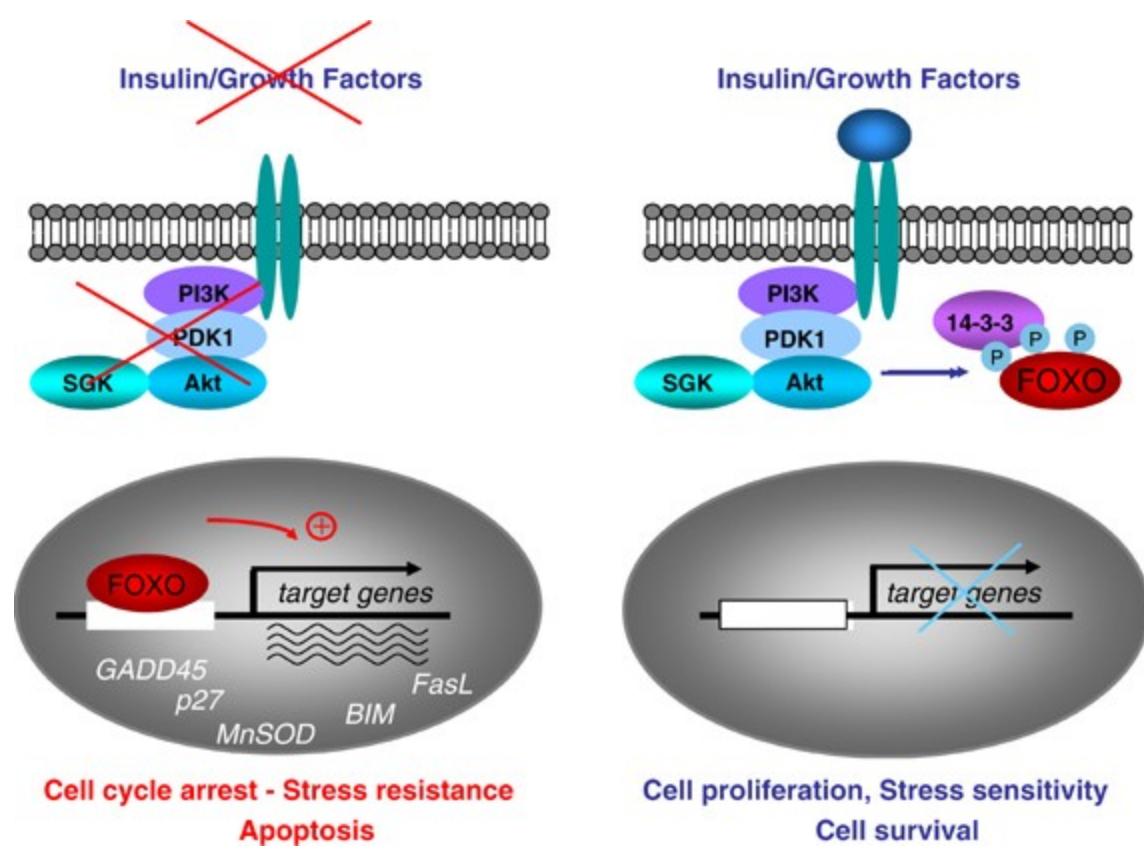
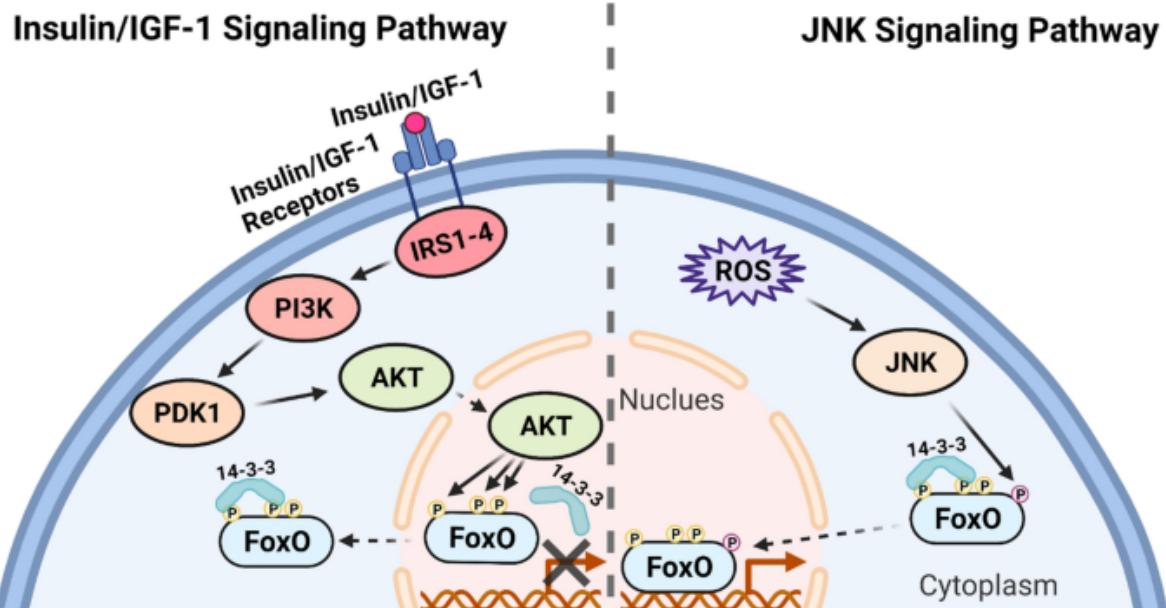
Activation of PI3K pathway and cell survival downstream of EGFR





▼ FOXO signalling pathway

- FOXO: transcription factor: **metabolism, cellular proliferation, stress resistance, and apoptosis**
- regulated by phosphorylation, acetylation, ubiquitylation



▼ Akt-dependent signalling

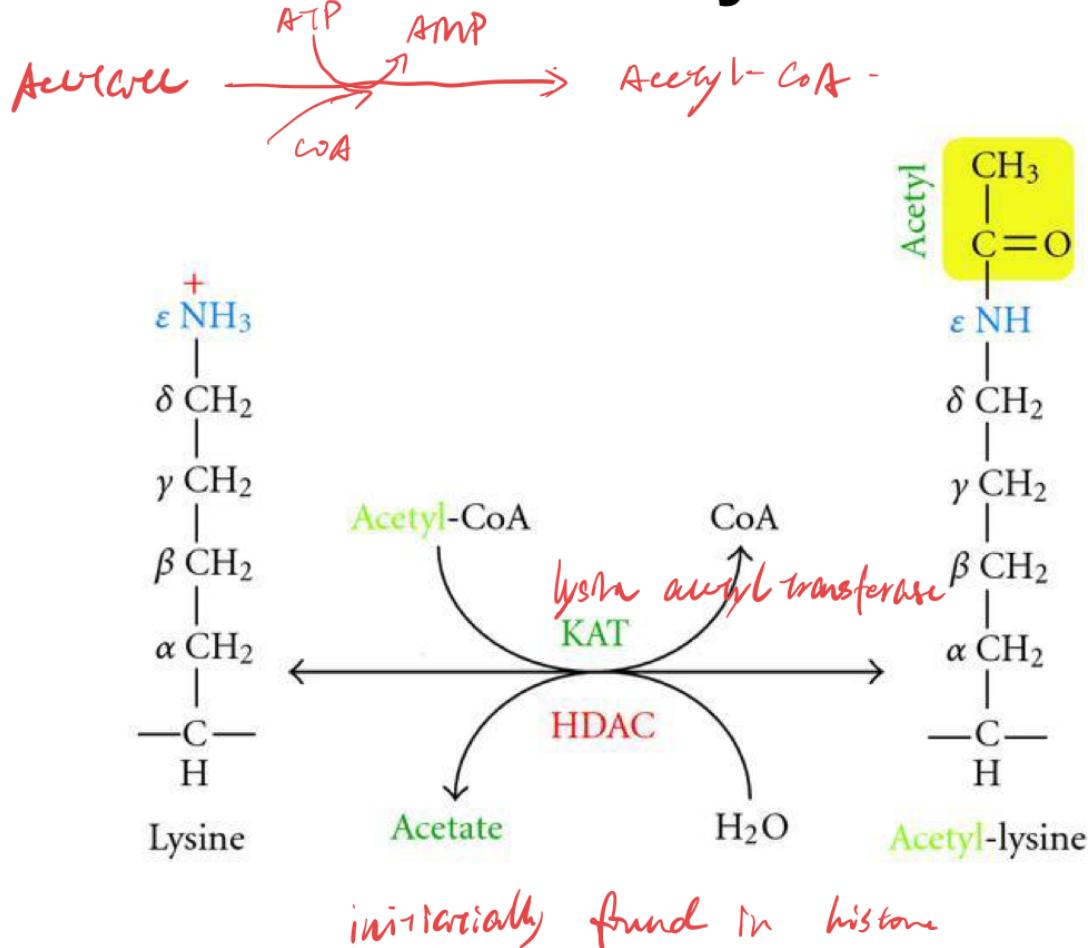
1. Insulin / Growth factor tyrosine kinase signalling
2. Activate PI3K to phosphorylate PIP2 to PIP3

3. PIP3 then activates Akt
4. Akt enters the nucleus and then phosphorylates foxO
5. the phosphorylated foxo then binds to 14-3-3, which then shutters foxO out of the nucleus
6. This inhibits the transcription of the target genes, leading to inhibition of apoptosis → cell survival

▼ Acetylation

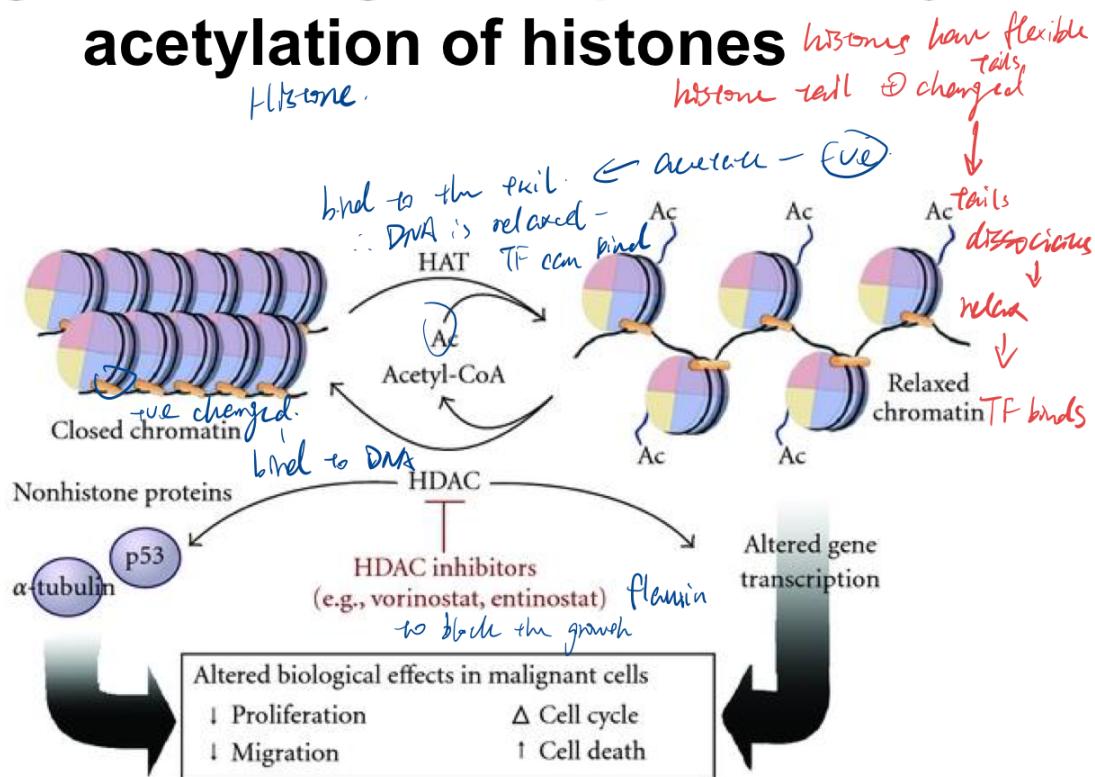
- take place in **lysine** (straight chain + amino group at terminus)
- originally found in histones

Protein acetylation



- Tails of histone is **+ve charged & compacted with chromatin** → acetate is -ve charged → When histone tail is acetylated → tails become more flexible → histone complexes are relaxed → TFs can bind to chromatin
- such as **bromoprotein** can bind

Regulation of gene expression by acetylation of histones

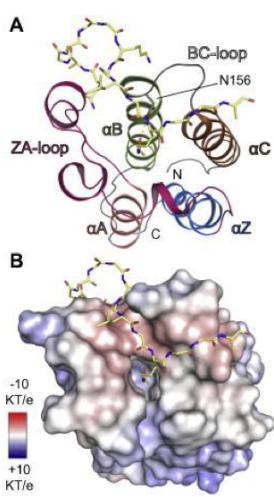


▼ Methylation

- methyl groups are neutral → the binding cannot relax the histone tail
- but the methyl groups can recruit chromoproteins → inhibit the transcription

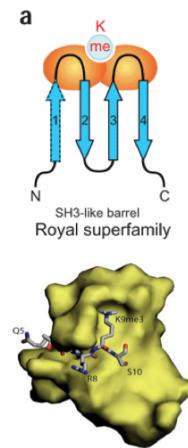
Function of bromo and chromodomains

Bromodomain



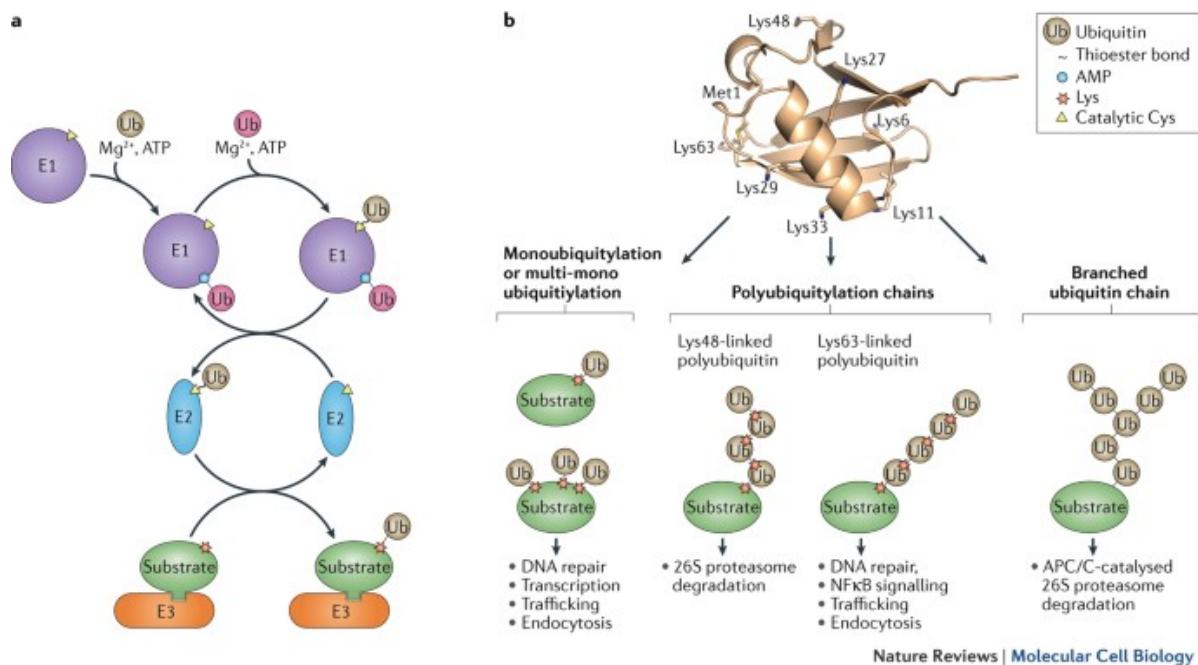
- Bromodomains are ~110aa in length
- Recognize acetylated lysine residues

Chromodomain



- Chromodomains are ~~40-50aa~~ in length
- Recognize methylated proteins
- HP1, a chromodomain protein that binds to histone H3 methylated at Lys 9

▼ Ubiquitination



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- Ubiquitin is a highly stable protein
- Proteins have **multiple lysine residues** that can act as the ubiquitin sites
- There are **mono-ubiquitination** and **poly-ubiquitination** → which can have different effects
- monoubiquitylation → endocytosis & protein sorting
- polyubiquitylation →
 - Lys48 linked : proteosomal degradation;
 - Lys63 linked: DNA repair; endocytosis

▼ Involved enzymes:

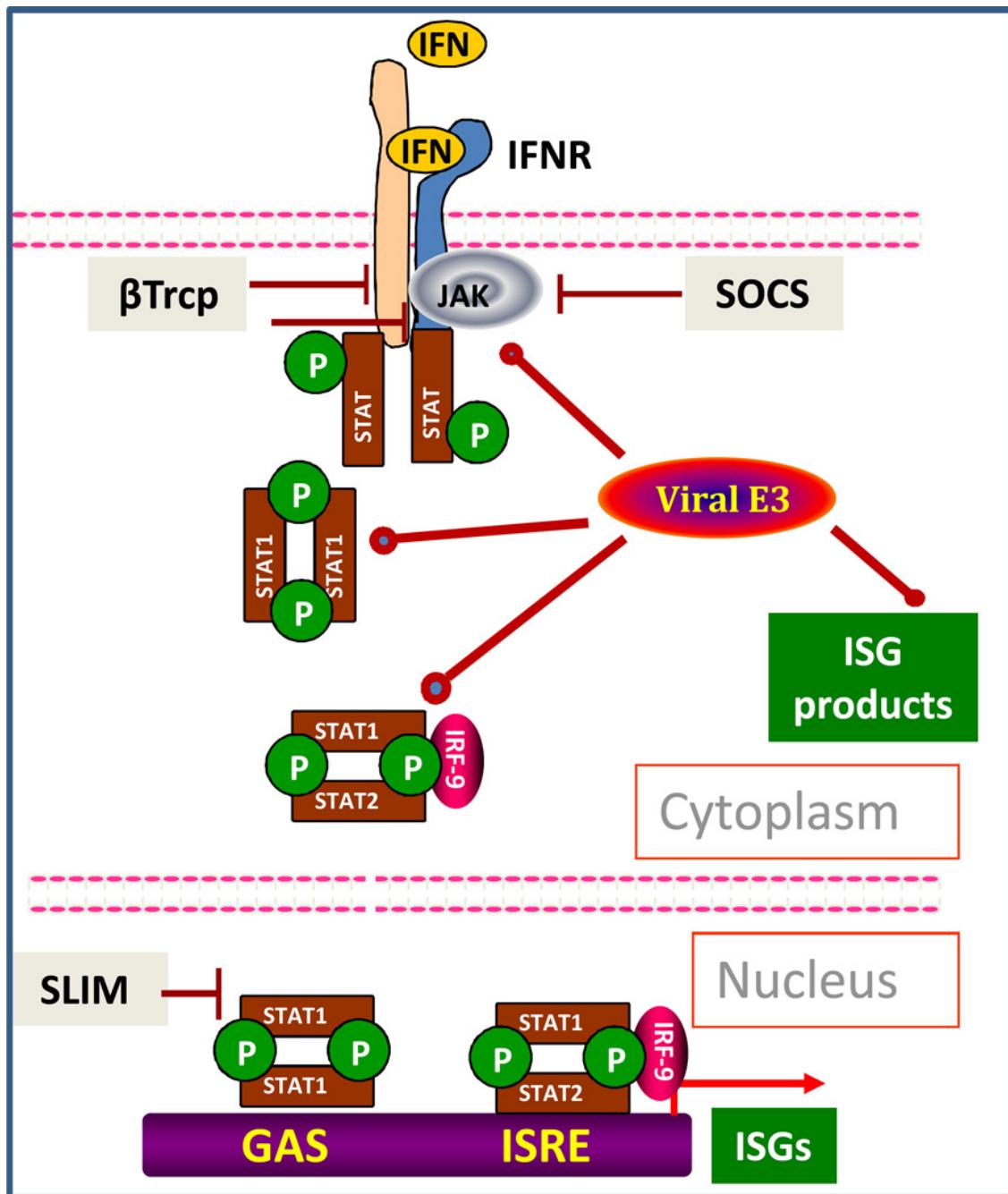
E1: Ubiquitin-**activating enzyme** (binding to ubiquitin via cisteine bridge)

E2: Ubiquitin-**conjugating enzyme**

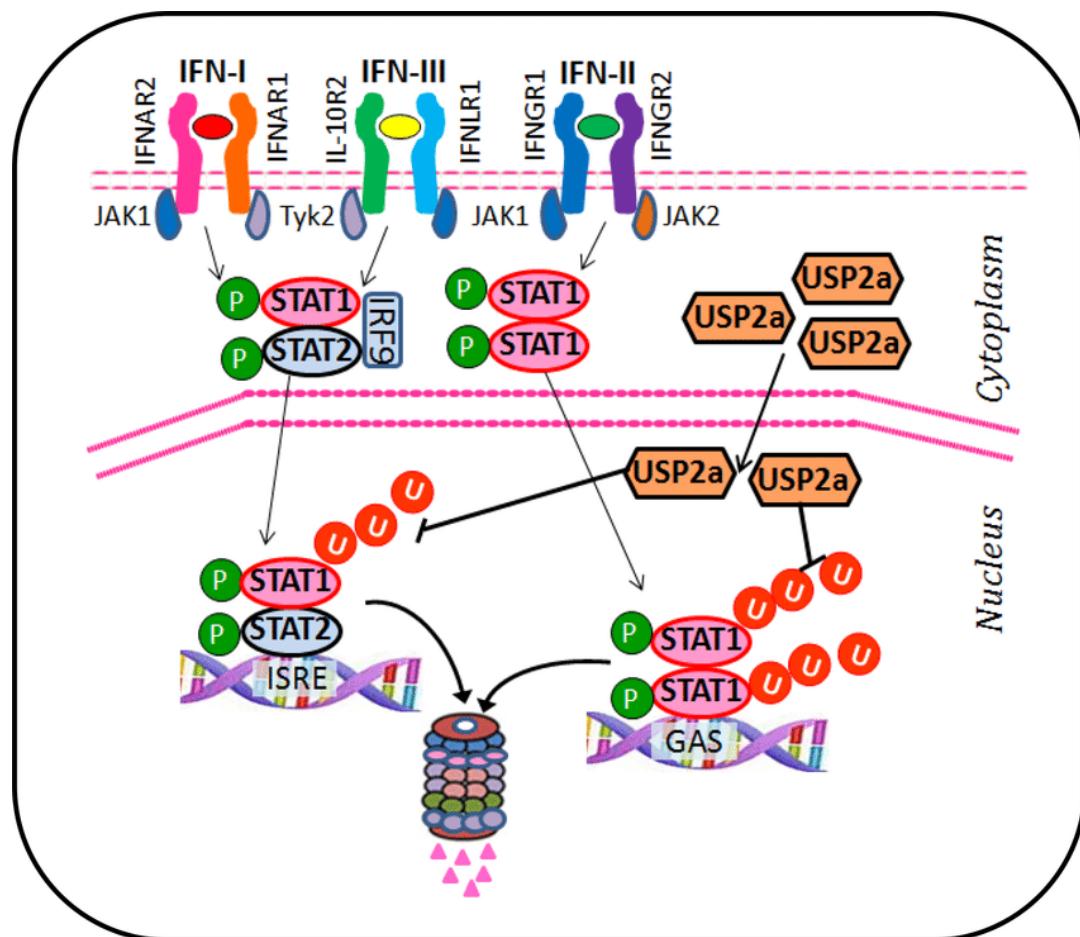
E3: Ubiquitin **ligase**: transfer ubiquitin from E2 to substrate

a single ubiquitin-protein ligase molecule bound to the target protein can catalyse the successive transfer of several ubiquitin molecules, **resulting in ubiquitination of multiple lysines**

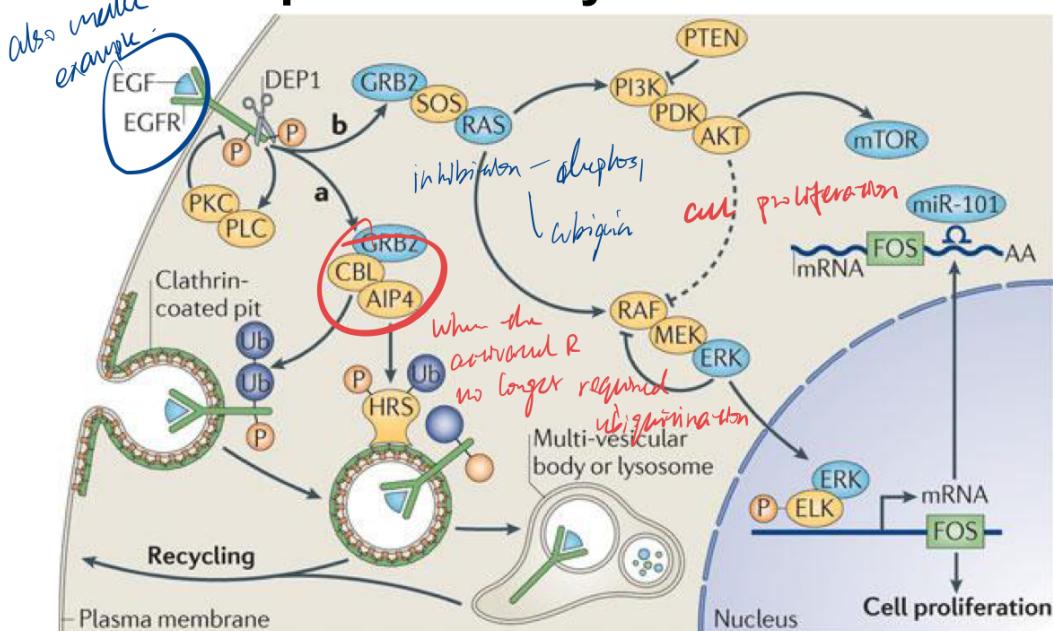
- Proteasome-mediated degradation: STAT as example
 - in response to IFN signalling
 - translocate to the nucleus and induce the expression of a diverse family of interferon-stimulated genes (ISGs, Figure 1). Protein products of these genes act in concert to mediate the anti-viral, anti-tumorigenic and immunomodulatory effects of IFNs



- However, when STAT is phosphorylated, the ubiquitylation site is also open → when it is not needed
- ubiquitination & poly-ubiquitylated STAT complex will be degraded in nucleus by proteasome
- **IFNs promote the nuclear import of the deubiquitinase USP2a, which binds to pY701-STAT1 and cleaves K48-linked ubiquitin chains of pY701-STAT1, and therefore sustains pY701-STAT1 levels in the nucleus. The positive regulation of pY701-STAT1 by USP2a enhances all three types of IFNs-induced signaling and antiviral function.**



Ubiquitination: degradation of plasma proteins in lysosomes



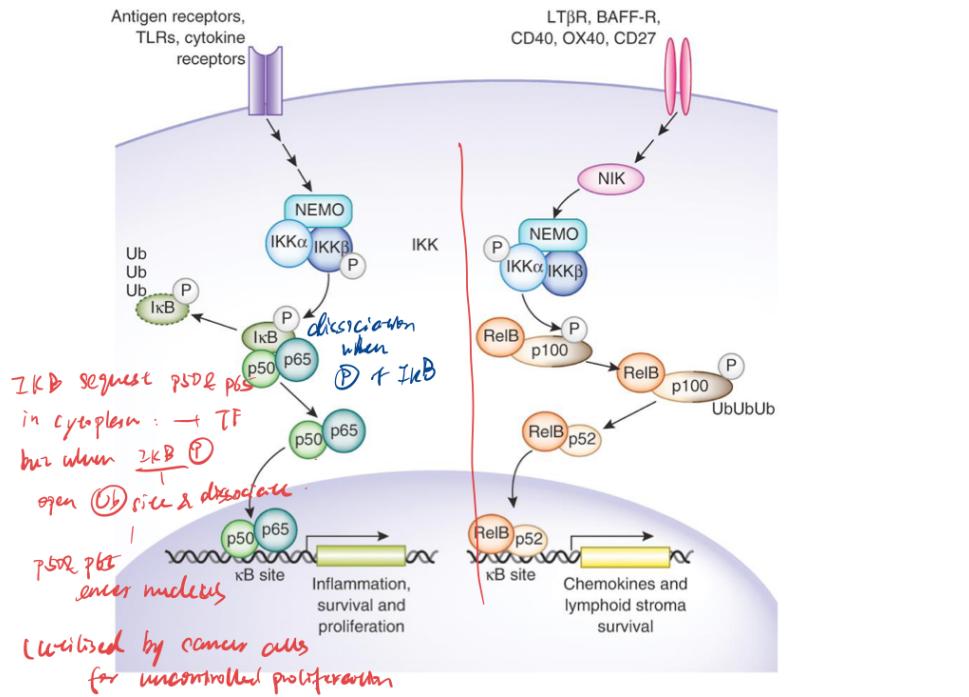
Mediated by monoubiquitination or by Lys63-linked polyubiquitination

Example: downregulation of EGF-EGFR signalling by internalization and Cbl-mediated ubiquitination and lysosomal degradation

- Ubiquitin-proteosomal degradation example two: IkBa - p50&p65
 - inactive IkBa sequest p50 & p65 (transcription factor)
 - NF-kappaB p50/p65 heterodimer is **the classical member of the Rel family of transcription factors which regulate diverse cellular functions such as immune response, cell growth, and development**
 - So p50 & p65 is located at cytoplasm when rest state → low transcription level
 - activation of antigen receptors/ cytokine receptors → **phosphorylation of IkBa**
 - active IkBa open its ubiquitination site → dissociation

- p50 & p65 enter nucleus and bind to kappa B site to switch on transcription, for **proliferation**

Blocking the ubiquitin-proteosomal pathway as anti-cancer strategy



Example: degradation of I κ B α

Bortezomib (Velcade) blocks the degradation of I κ B α and is used in the clinic for treating multiple myeloma

- Distinct function of ubiquitination — PTEN as example
 - mono-ubiquitination → translocation from cytoplasm to nucleus → drive nuclear apoptosis by inhibiting Akt
 - poly-ubiquitination → proteasomal degradation of PTEN

Regulation of protein localization: monoubiquitination drives nuclear import of PTEN

