The Translation II

The Regulation of Translation

(Chapter 15)

LMR05.001 – 17
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Regulation of translation

- **Most gene expression regulation is at the level of transcription (mRNA production)**
- **Translational regulation**
  - **Fast response** (can skip may stages related to mRNA production on processing)
  - Functions mostly **at the level of initiation of translation**

Translation ft\Ŝ in a nutshell

- Initiation
- Elongation
- Termination

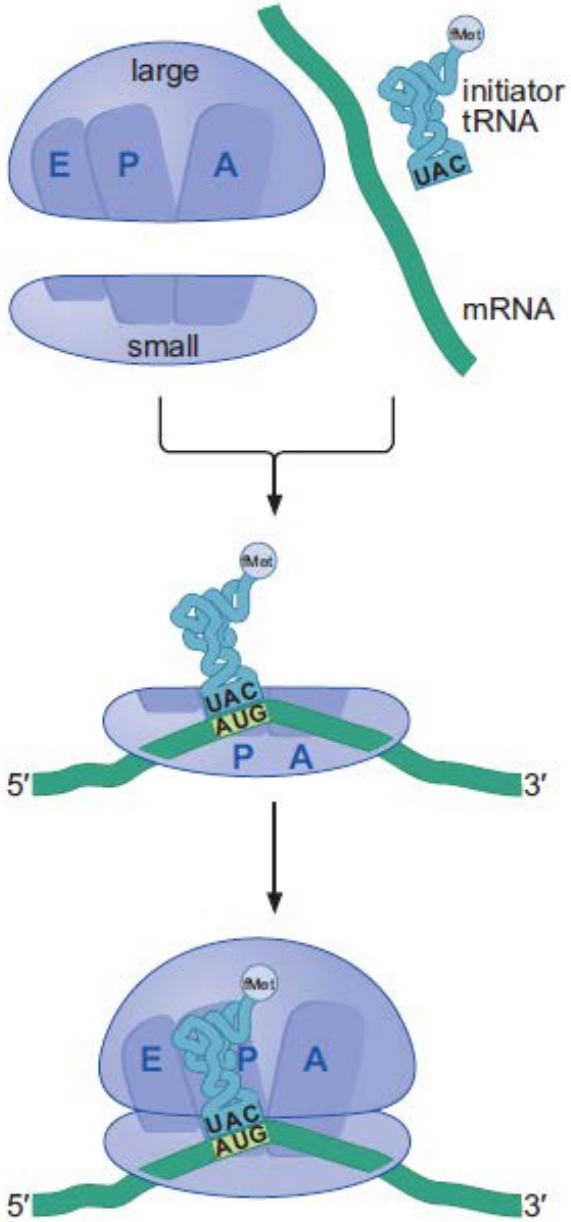


FIGURE 15-22 An overview of the events of translation initiation.

Step one in ptocaryotes (for remembering)

- **Find and recognize mRNA**

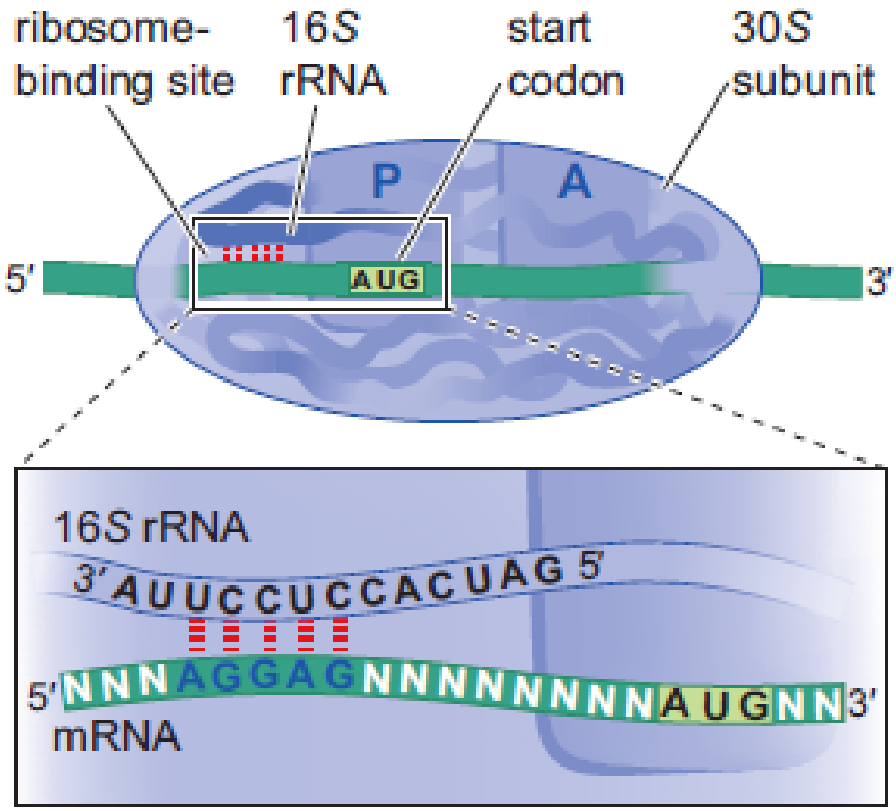


FIGURE 15-23 **The 16S rRNA interacts with the RBS to position the AUG in the P-site.** This illustration shows an mRNA with the ideal separation between the RBS and the initiating AUG. This spacing places the AUG in the region of the P-site. Many mRNA have non-ideal spacings leading to a reduced rate of translation initiation. Other mRNAs lack an RBS completely and recruit the ribosome by distinct mechanisms.

Eukaryotic initiation is more complicated

Initiation of translation in eukaryotes is similar to prokaryotic initiation in many ways.

- Both use start codon
- Both have dedicated initiator tRNA
- Both use initiation factors to form a complex with the small ribosomal subunit that assambles on the mRNA before addition of the large subunit.

Eucaryotes use at all the distinct method for recognition the mRNA and the start codon.

In eukaryotes, the small subunit is already associated with an initiator tRNA when it is recruited to the capped 5' end of the mRNA. It then "scans" along the mRNA in a 5' -> 3' direction until it reaches the first 5'-AUG-3'

Translation initiation is the only time a tRNA binds to the P-site without previously occupying the A-site. This event requires a special tRNA known as the initiator tRNA, which base-pairs with the start codon—usually AUG or GUG. AUG and GUG have a different meaning when they occur within an ORF, where they are read by tRNAs for methionine (tRNAMet) and valine (tRNAVal), respectively

Only the first AUG can be used as the start site of translation in eukaryotic cells!

NB!!! In this method of initiation is consistent with the fact that the vast majority of eukaryotic RNAs encode a single polypeptide (monocistronic); recognition of an internal start codon is generally neither required nor possible.

The Ribosome Has Three Binding Sites for tRNA

- A site
  - Aminoacyl-tRNA
- P site
  - Peptidyl-tRNA
- E site
  - Exit binding site for the tRNA that is released after the growing polypeptide chain has been transferred to the aminoacyl-tRNA

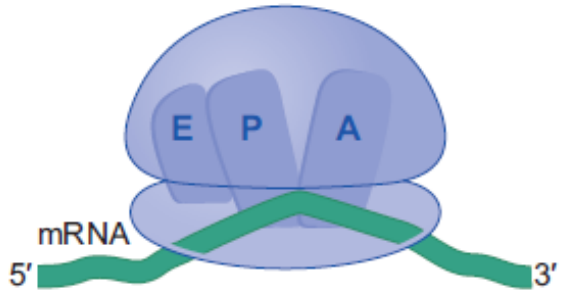


FIGURE 15-18 **The ribosome has three tRNA-binding sites.** The schematic illustration of the ribosome shows the three binding sites (E, P, and A) that each spans the two subunits.

Eukaryotic initiation is more complicated

MORE PROTEINS!!!

Assembly of the eukaryotic

small ribosomal subunit and initiator tRNA onto the mRNA!

- **Four steps:**
  - **Small subunit is associated with an initiator tRNA before being recruited to the capped 5' end of the mRNA**

  (see Kozak sequence)
  - **Set of factors to recognize mRNA**
  - **AUG scanning**
  - Recruitment of the **large subunit or binding it to small subunit.**

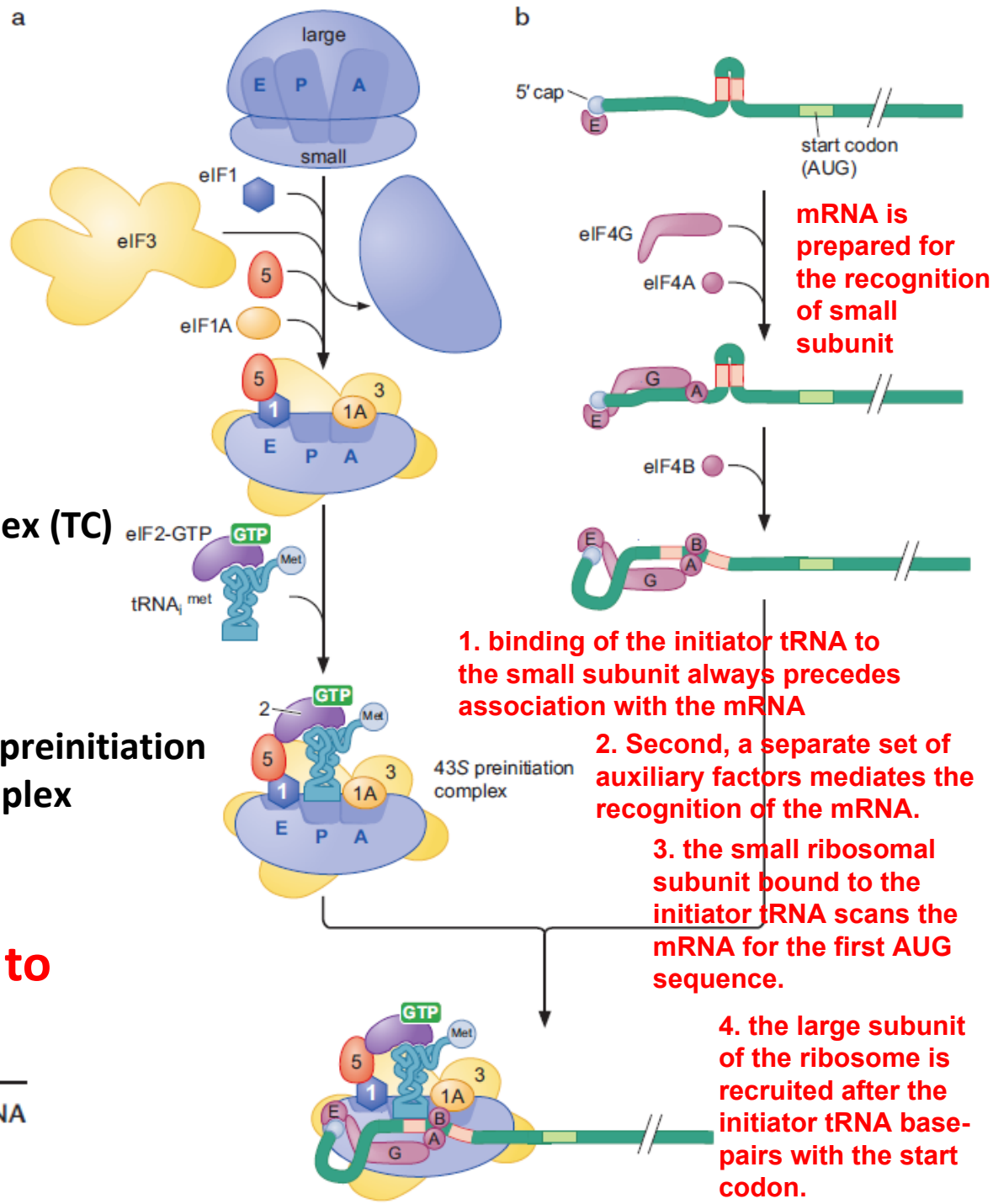


FIGURE 15-26 Assembly of the eukaryotic small ribosomal subunit and initiator tRNA onto the mRNA.

In some mRNAs, **of a purine three bases upstream of the start codon** and a guanine immediately downstream (5’-G/ANNAUGG-3’)
is the sequence was originally identified by Marilyn Kozak and is referred to as the **Kozak sequence**.

Many eukaryotic mRNAs lack these bases, but **their presence increases the efficiency of translation**.

In contrast to the situation in prokaryotes, these bases are thought to interact with the initiator tRNA, not with an RNA component of the ribosome.

Hide RBS from the ribosome

(using RNA-binding proteins)

- Primary target of regulators is to interfere with the RBS recognition
  - Associate with sequences near RBS and physically inhibit basepairing between the RBS and 16S rRNA
  - RNA-binding proteins that recognize RNA structures adjacent to the RBS
  - They do not bind to the RBS directly! (why?)

A ribosome binding site, or ribosomal binding site (**RBS**), is a **sequence of nucleotides upstream of the start codon** of an mRNA transcript that i**s responsible for the recruitment of a ribosome during the initiation of translation**

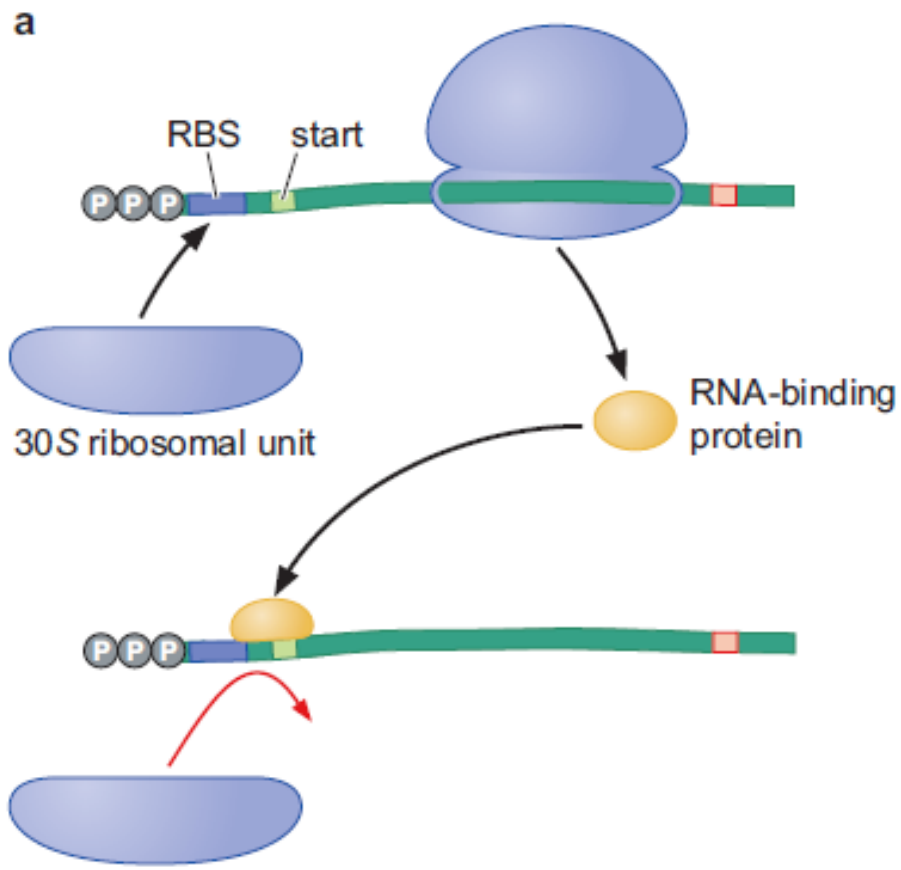


FIGURE 15-42 **Regulation of bacterial translation initiation by inhibiting 30S subunit binding.** (a) Protein binding to sites near the RBS prevents access of the 16S rRNA to the RBS. In this case, the protein encoded by the mRNA binds to its own RBS. (b) Intramolecular base pairing of the mRNA can interfere with base pairing by the 16S rRNA. In many cases, this inhibition is modulated by the translation of other genes in the same operon. If the region of the mRNA that is base pairing to the RBS proximal region is within an ORF, when that ORF is translated, the interfering base pairing is disrupted, allowing a second ribosome to recognize the previously blocked RBS.

The RBS in prokaryotes is a region upstream of the start codon. This region of the mRNA has the consensus 5'-AGGAGG-3', also called the Shine-Dalgarno (SD) sequence.

The complementary sequence (CCUCCU), called the anti-Shine-Dalgarno (ASD) is contained in the 3' end of the 16S region of the smaller (30S) ribosomal subunit. Upon encountering the Shine-Dalgarno sequence, the ASD of the ribosome base pairs with it, after which translation is initiated.

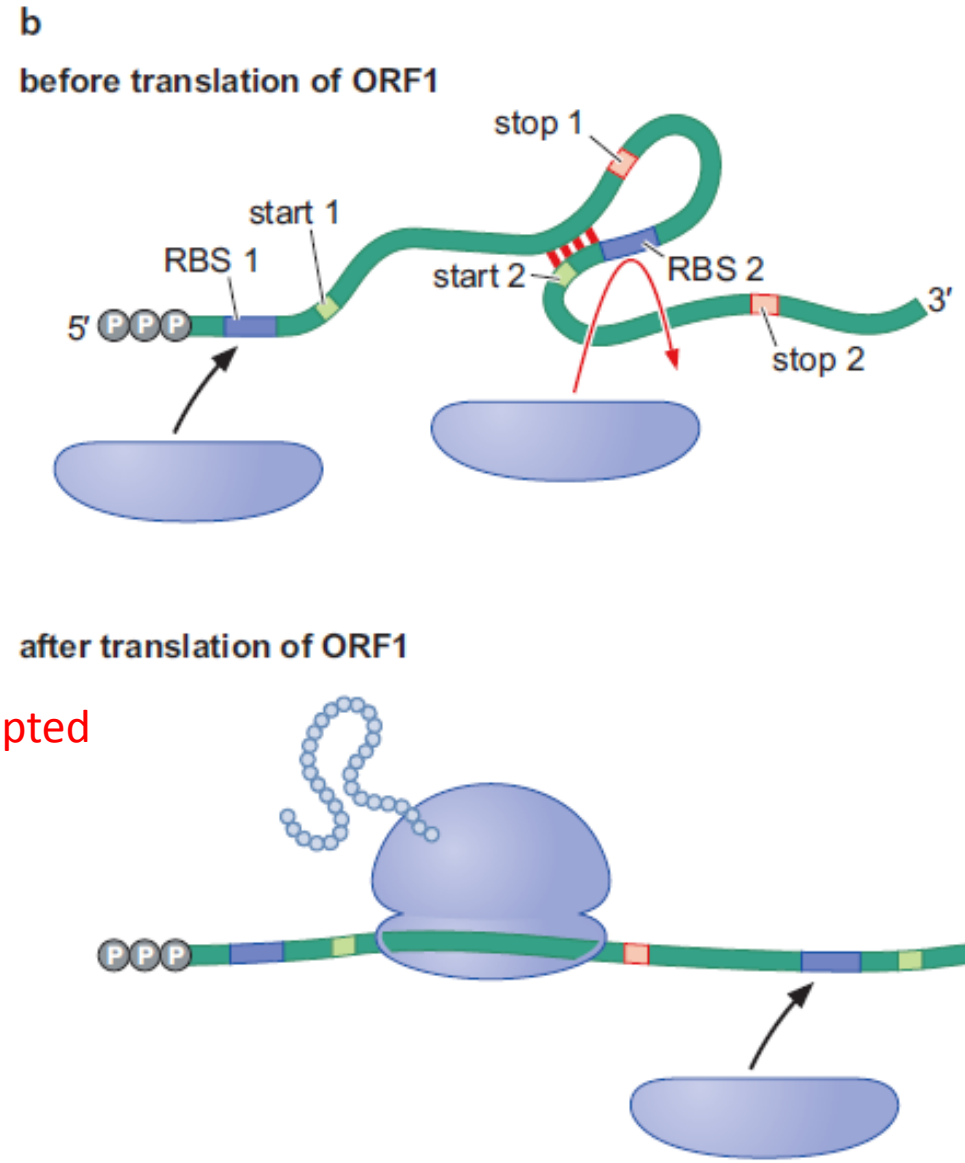
The Kozak consensus sequence (Kozak consensus or Kozak sequence) is a nucleic acid motif that functions as the protein translation initiation site in most eukaryotic mRNA transcripts.

Regarded as the optimum sequence for initiating translation in eukaryotes, the sequence is an integral aspect of protein regulation and overall cellular health as well as having implications in human disease. It ensures that a protein is correctly translated from the genetic message, mediating ribosome assembly and translation initiation. A wrong start site can result in non-functional proteins

The Kozak sequence is not to be confused with the ribosomal binding site (RBS), that being either the 5′ cap of a messenger RNA or an internal ribosome entry site (IRES).

Hide RBS from the ribosome

- RNA molecules can act as inhibitors
  - mRNA basepairs with itself
  - interaction needs to be disrupted for translation to proceed



- The masking of one or more RBSs
- The preventing of translation until the interaction is disrupted
- The another ribosoome can recognize the unmasked RBS

FIGURE 15-42 Regulation of bacterial translation initiation by inhibiting 30S subunit binding. (a) Protein binding to sites near the RBS prevents access of the 16S rRNA to the RBS. In this case, the protein encoded by the mRNA binds to its own RBS. (b) Intramolecular base pairing of the mRNA can interfere with base pairing by the 16S rRNA. In many cases, this inhibition is modulated by the translation of other genes in the same operon. If the region of the mRNA that is base pairing to the RBS proximal region is within an ORF, when that ORF is translated, the interfering base pairing is disrupted, allowing a second ribosome to recognize the previously blocked RBS.

What else could you regulate?

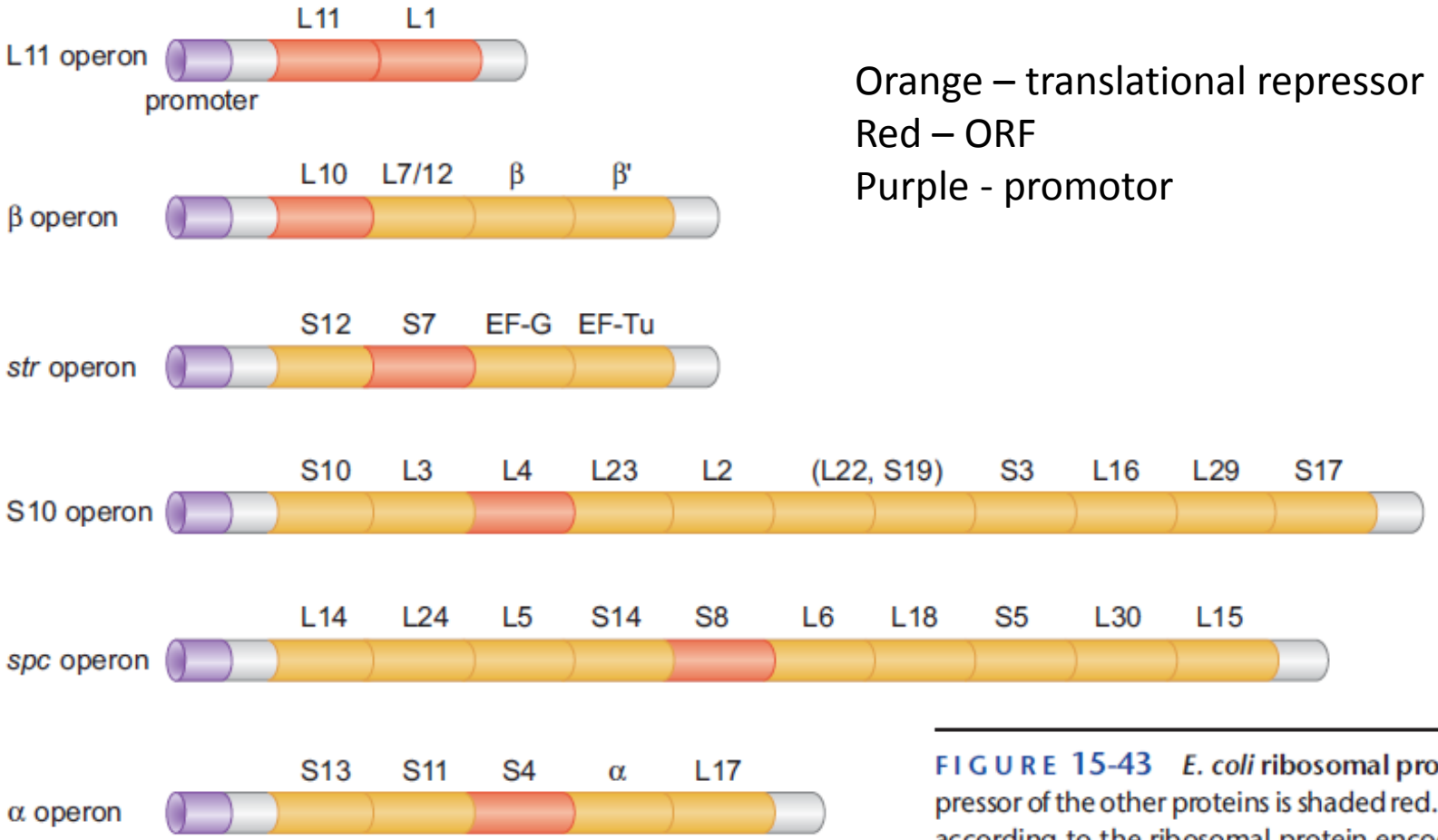
- tRNA synthesis?
- amino acid synthesis?
- ribosome synthesis?

Ribosome synthesis

- Each component needs to be synthesized stoichiometrically
  - rRNA (3-4 species)
  - r-proteins (54+ proteins)
- Rate of ribosome production must follow cell's growth rate
  - Changes in growth conditions quickly lead to an appropriate increase or decrease in the rate of synthesis of all ribosomal components
- Operons

Operons

- Up to 11 r-protein genes in an operon, regulated at the level of RNA synthesis



Orange – translational repressor
Red – ORF
Purple - promotor

To coordinate regulation of ribosomal protein genes is simplified by their organization into several operons, each containing genes for up to 11 ribosomal proteins (Fig. 15- 43).

As with other operons, these gene clusters are regulated at the level of RNA synthesis; however, **the most important control of ribosomal protein synthesis is at the level of translation of the mRNA.**

FIGURE 15-43 *E. coli* **ribosomal protein operons.** The protein that acts as a translational repressor of the other proteins is shaded red. The promoter is shown in purple, and each ORF is labeled according to the ribosomal protein encoded (e.g., L14 is large ribosomal protein 14). (Adapted, with permission, from Nomura M. et al. 1984. *Annu. Rev. Biochem.* 53: 75–117. © Annual Reviews.)

- Self-regulation, free protein regulates its own synthesis
  - Additional operons increase mRNA amounts but not r-protein amounts

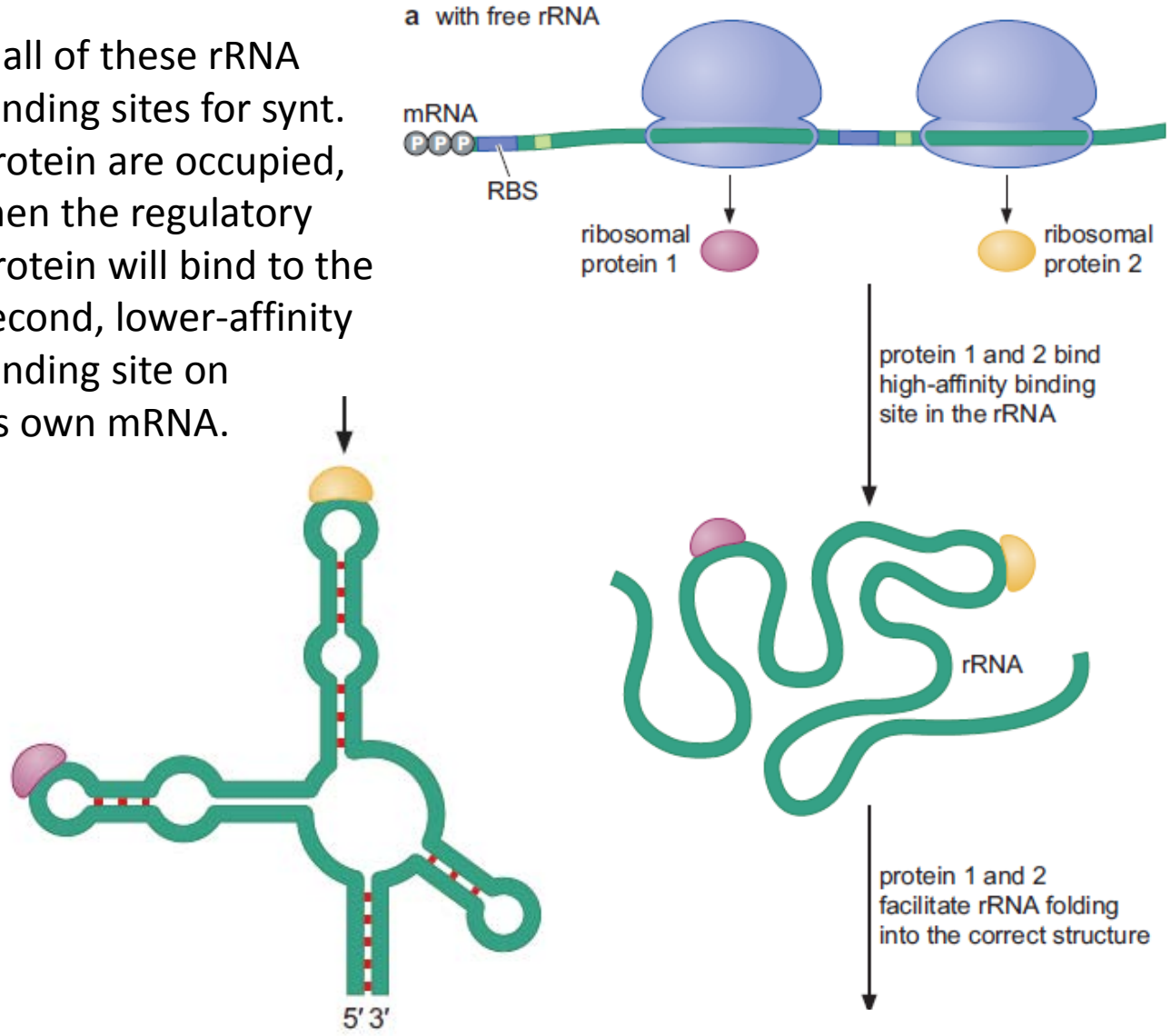
- Autorepression
  - Protein (or two) binds to its own mRNA, inhibiting synthesis

- mRNA or rRNA – which one to choose?

The ribosomal protein operon mRNAs (rRNA) frequently are folded into structures that only allow recognition of internal RBSs if earlier genes in the mRNA are being translated.

Self-control

If all of these rRNA binding sites for synt. protein are occupied, then the regulatory protein will bind to the second, lower-affinity binding site on its own mRNA.



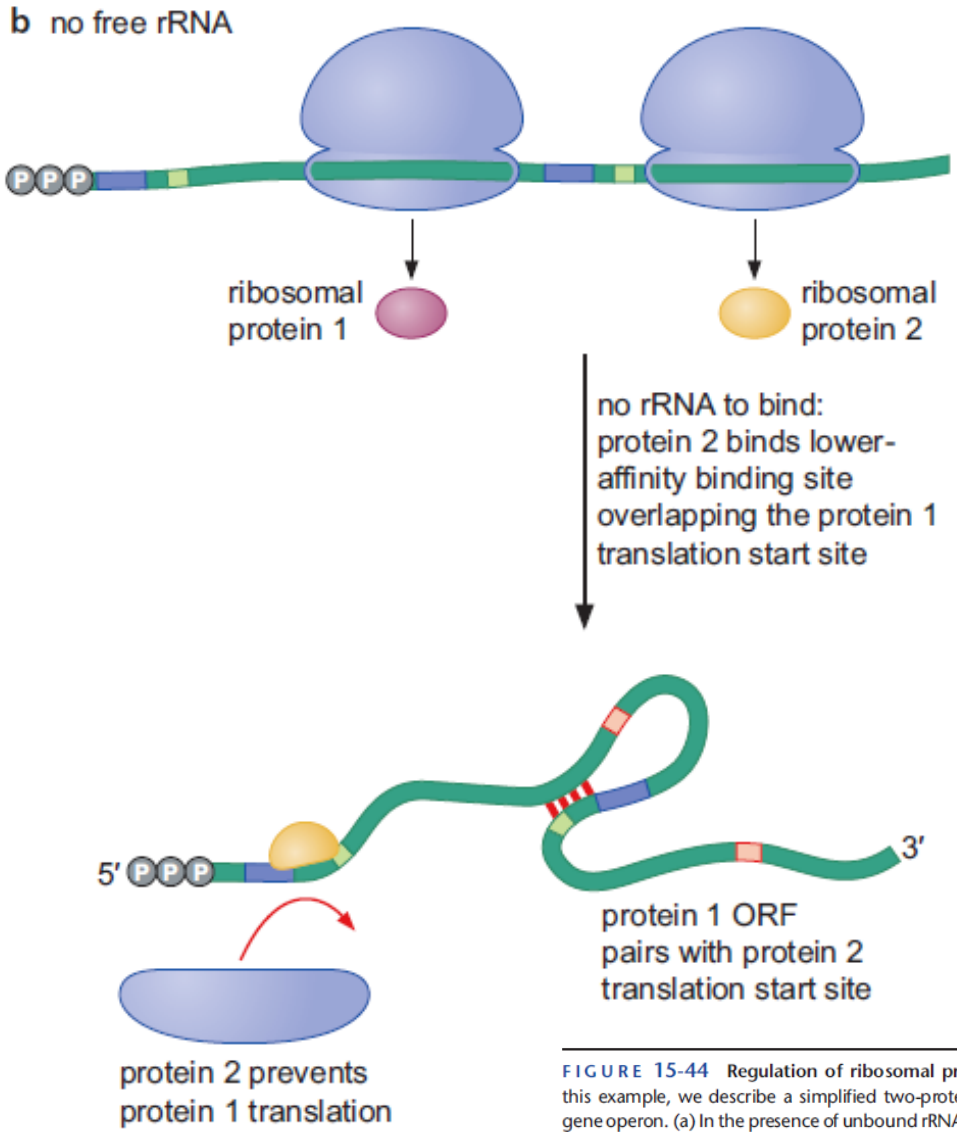
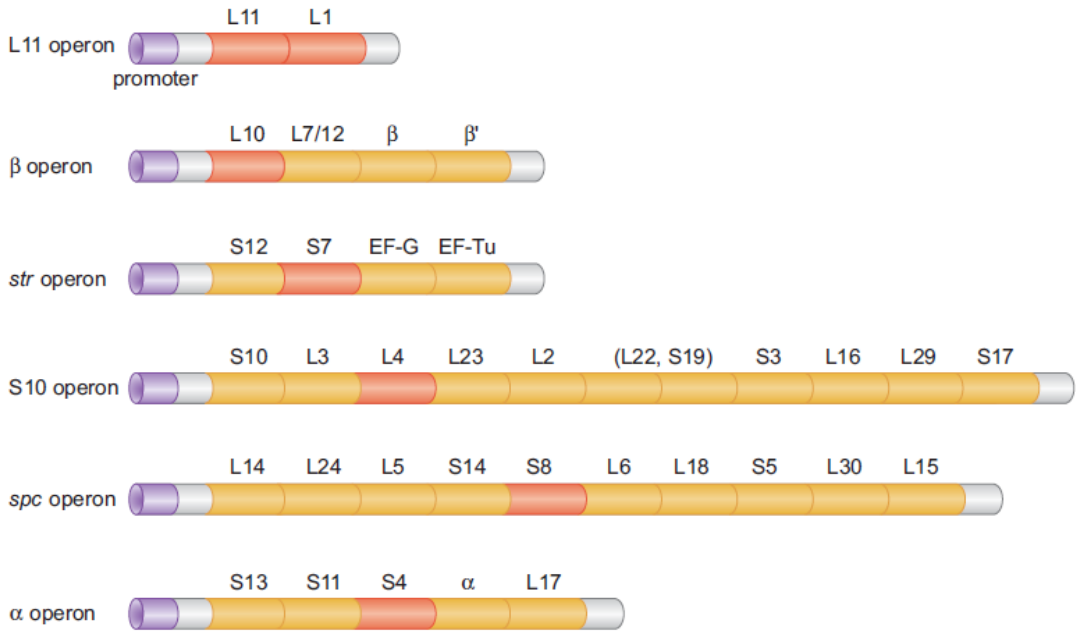
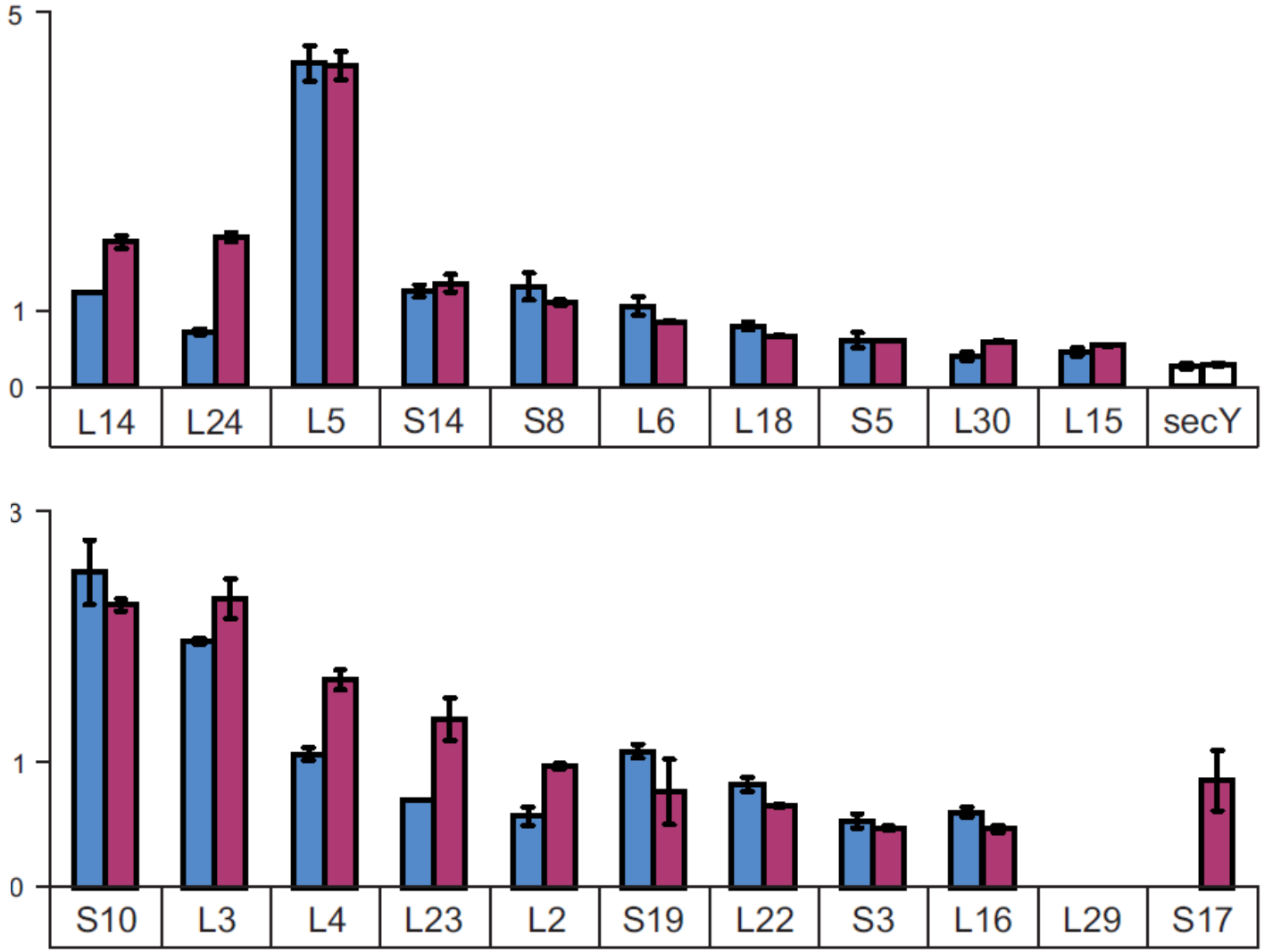


FIGURE 15-44 **Regulation of ribosomal protein expression.** In this example, we describe a simplified two-protein ribosomal protein gene operon. (a) In the presence of unbound rRNA, both ribosomal proteins expressed from the operon bind to the rRNA and assist in the proper assembly of the ribosome. Under this circumstance, ribosomal protein 2 does not bind to the lower-affinity binding site adjacent to the ribosomal protein 1 RBS. (b) When there is no available rRNA for the ribosomal proteins to associate with, ribosomal protein 2 binds adjacent to the ribosomal protein 1 RBS, inhibiting translation of this ORF. In the absence of translation of ribosomal protein 1, sequences that are complementary to sequences adjacent to the ribosomal protein 2 RBS base-pair and prevent binding of the 30*S* subunit to this site. As discussed in the text, translational coupling could also act to inhibit translation of downstream ORFs.

Sidenote - antibiotics

- Chloramphenicol
  - Inhibits peptidyl transferase
- Erythromycin
  - Inhibits translocation





Inhibit translation initiation globally

- eIF2 (initiator tRNA binding to the 40S subunit)
- eIF4E (recognition of the mRNA)

In addition to globally regulating translation, binding to eIF4E is also used to regulate the translation of specific mRNAs.

Eukaryotic initiation - ribosome small subunit

- Ribosome subunits are dissociated
- eIF1, eIF1A, eIF3, and eIF5 bind to the small subunit
  - They act similar to IF1 and IF3 in bacteria
- G-protein eIF2 brings in initiator tRNA
  - Only in GTP-bound state!
  - Called ternary complex (TC)
- eIF2 positions Met-tRNA$^{Met}_{i}$ in the P-site, forms 43S PIC

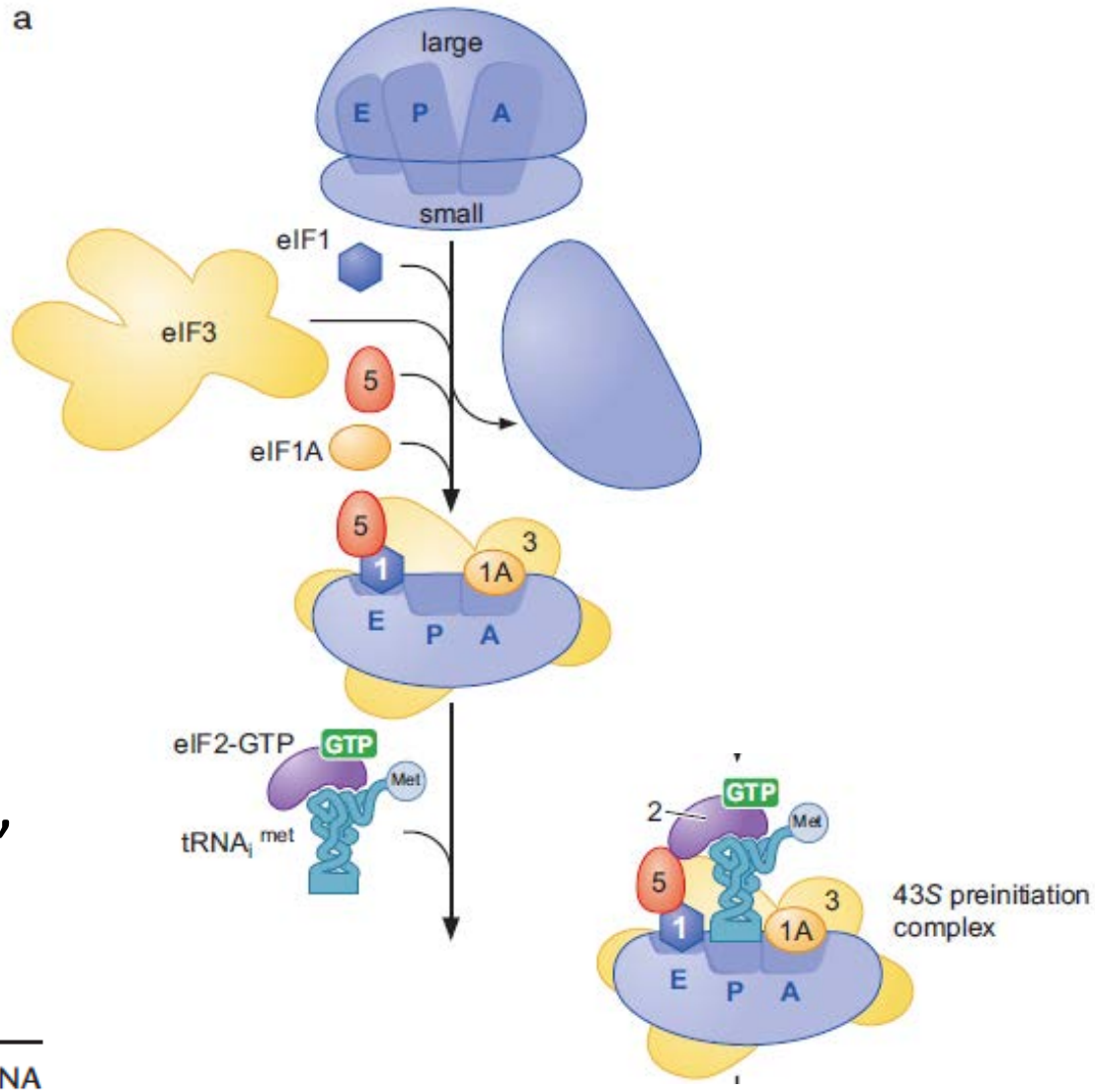


FIGURE 15-26 Assembly of the eukaryotic small ribosomal subunit and initiator tRNA onto the mRNA.

Phosphorylation is crucial!

- eIF2 phosphorylation inhibits eIF2:GTP formation (needed for the delivery of initiator-tRNA to the P-site)

Eukaryotic initiation - mRNA

- 5’ cap is recognized by the cap-binding protein eIF4E
- eIF4G binds to the eIF4E and mRNA
- eIF4A binds to the eIF4G and mRNA
  - the overall level of translation is controlled at this step by eIF4E-binding proteins that compete with eIF4G
- **eIF4B activates helicase activity of eIF4A**

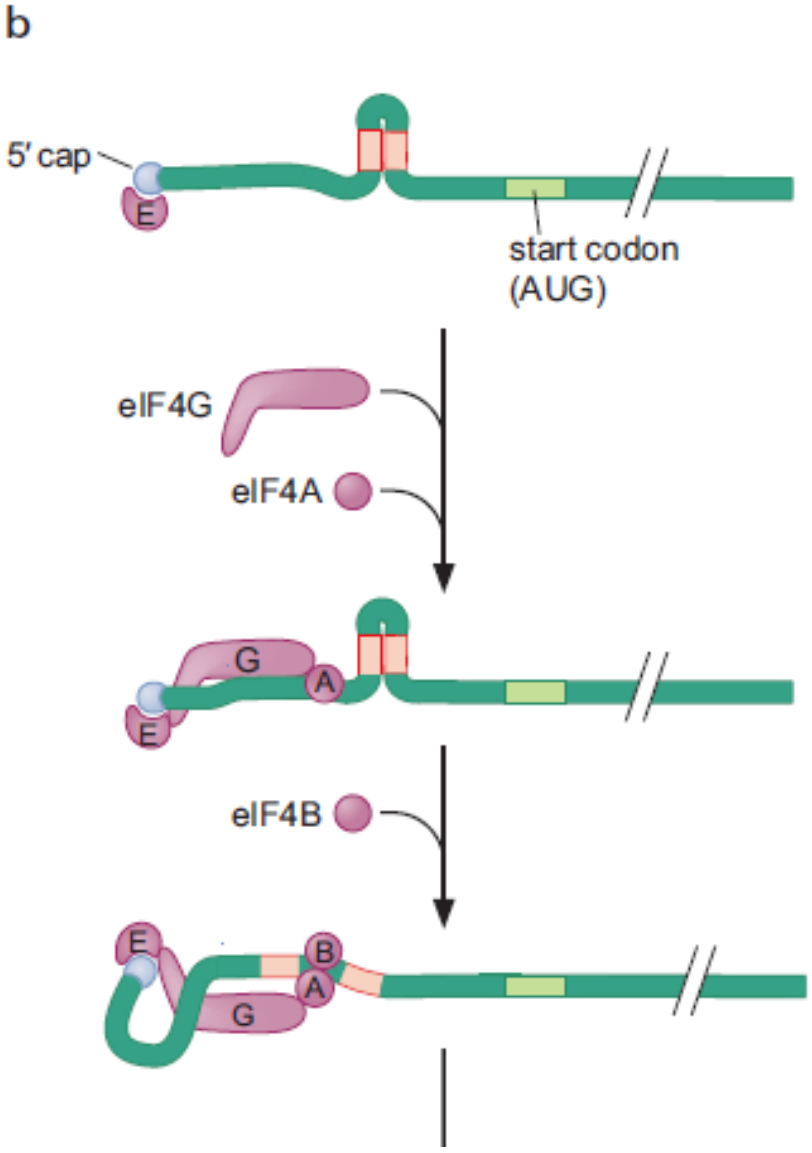


FIGURE 15-26 Assembly of the eukaryotic small ribosomal subunit and initiator tRNA onto the mRNA.

- Phosphorylation is crucial!
  - Unphosphorylated 4E-BPs (eIF4E binding proteins) inhibit translation

4E-BPs

(eIF4E-binding protein)

- Phosphorylation controlled by mTor kinase

(phosphorylates the eLF4E)

- Cell division stimulators activate mTor and increase translation

Indeed, overexpression of eIF4E can result in cancerous transformation of cells, and inhibitors of mTor (e.g., rapamycin) are effective chemotherapy agents.

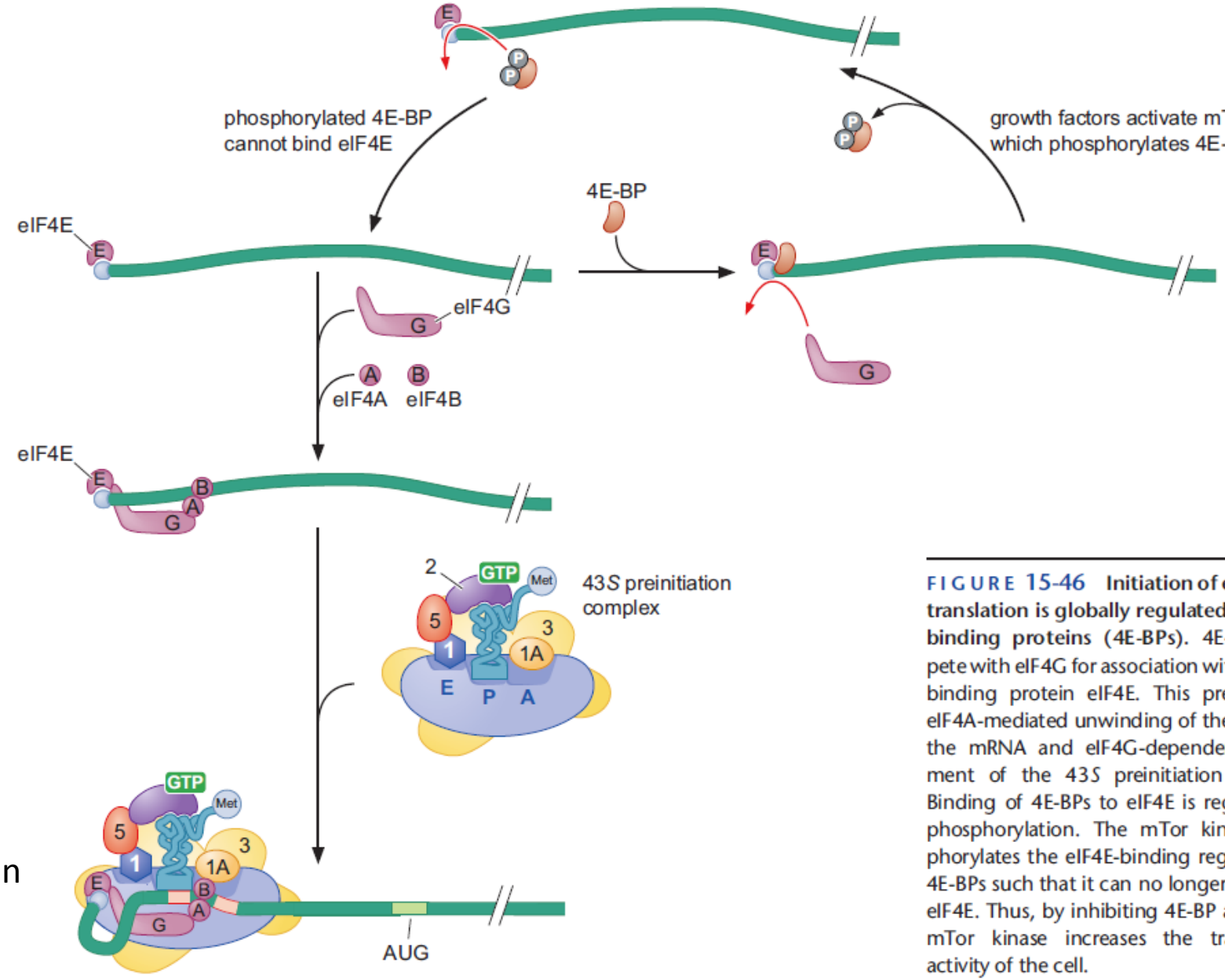
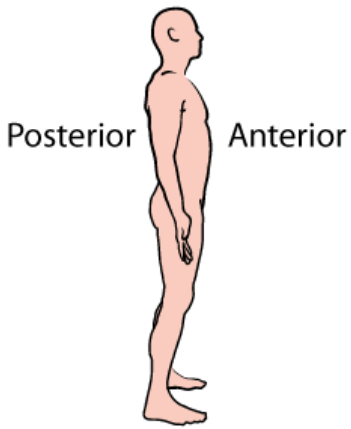


FIGURE 15-46 **Initiation of eukaryotic translation is globally regulated by eIF4E-binding proteins (4E-BPs).** 4E-BPs compete with eIF4G for association with the cap-binding protein eIF4E. This prevents the eIF4A-mediated unwinding of the 5′ end of the mRNA and eIF4G-dependent recruitment of the 43*S* preinitiation complex. Binding of 4E-BPs to eIF4E is regulated by phosphorylation. The mTor kinase phosphorylates the eIF4E-binding region of the 4E-BPs such that it can no longer recognize eIF4E. Thus, by inhibiting 4E-BP action, the mTor kinase increases the translational activity of the cell.

Does it matter in which corner I sit?

- 4E-BP = Cup
- Oskar protein – posterior
- *Oskar* mRNA – synthesized *anterior*, transported to *posterior*
  - How to make sure translation only happens in posterior?



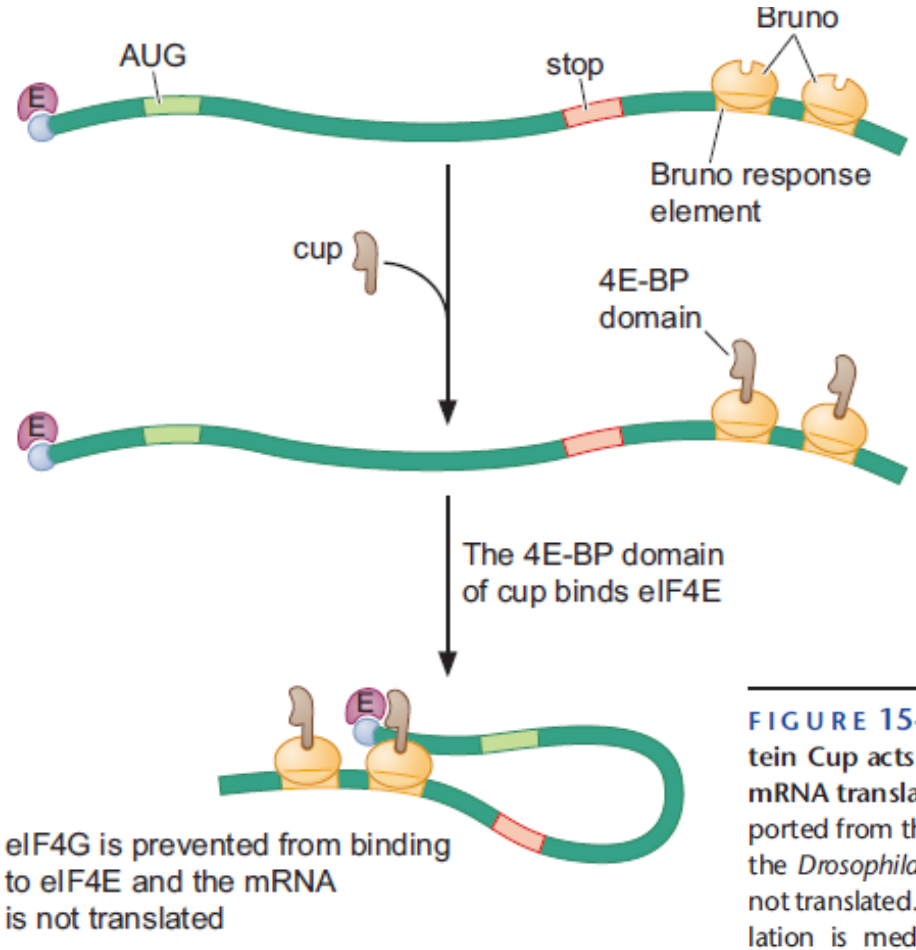


FIGURE 15-47 The eIF4E-binding protein Cup acts to specifically inhibit *Oskar* mRNA translation. As *Oskar* mRNA is transported from the anterior to the posterior of the *Drosophila* oocyte, it is critical that it is not translated. The inhibition of Oskar translation is mediated by two proteins. The RNA-binding protein Bruno binds to multiple sequences in the 3′-UTR of *Oskar* mRNA called Bruno response elements (BREs). Bruno then recruits the 4E-BP Cup to the mRNA. When localized to the mRNA, Cup outcompetes eIF4G for binding to eIF4E, inhibiting translation of this mRNA.

As Oskar mRNA is transporter from the anterior to the posterior of the Drosophila oocyte, it is critical that it is not translated.

Can you taste the blood?

- An Iron-Regulated, RNA-Binding Protein Controls Translation of Ferritin
- Ferritin Iron-binding protein
  - Stores and releases iron in a controlled manner

- Iron-regulatory proteins (IRPs)
  - RNA-binding proteins, recognize a hairpin at the 5’ end of ferritin mRNA
  - Low free iron – IRPs bind to IRE (iron regulatory element), inhibit eIF4A/B to unwind the IRE hairpin (no protein)
  - High free iron – IRPs bind iron and cannot bind to IRE (protein produced)

Can you taste the blood?

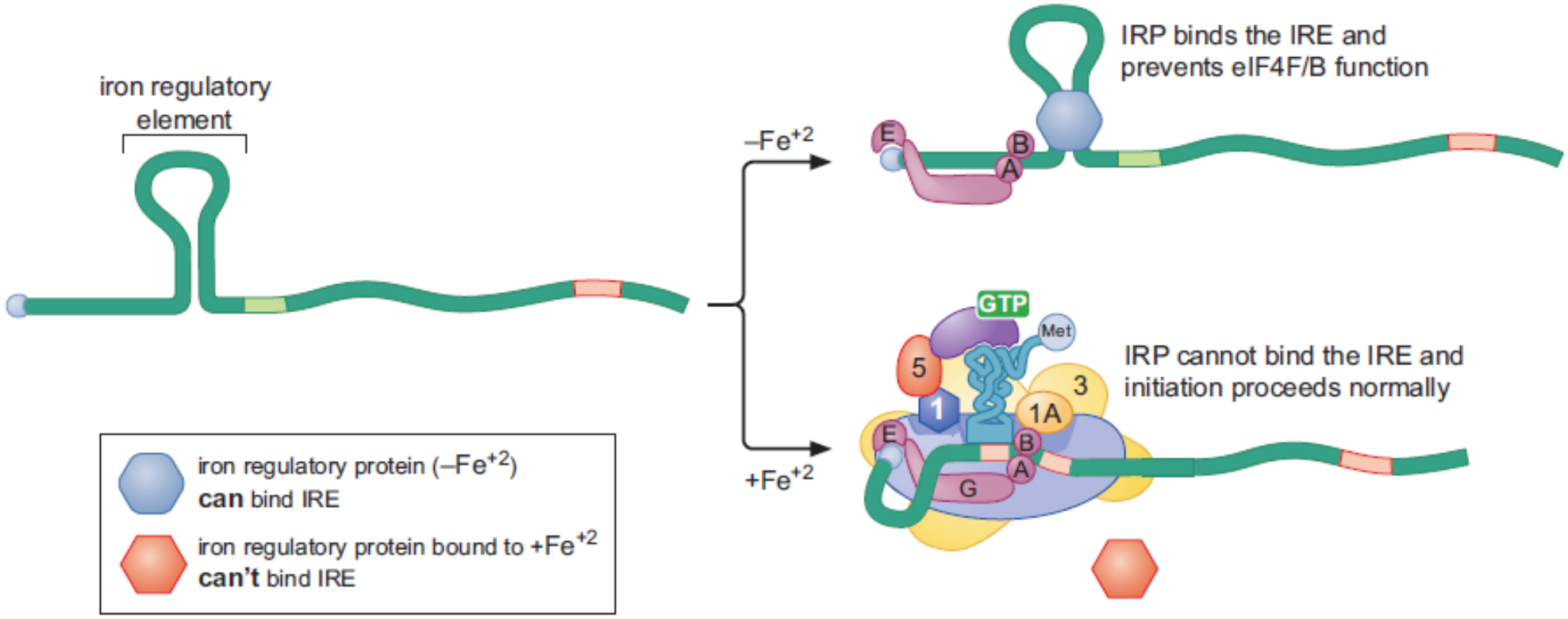


FIGURE 15-48 Regulation of Ferritin translation by iron. The 5′-UTR of the *ferritin* genes includes a stem–loop structure called the iron regulatory element (IRE). The iron regulatory protein (IRP) binds tightly to this site when it is not bound to Fe^{2+}. By stabilizing the stem–loop structure of the IRE, IRP prevents eIF4A from removing this structure from the end of the *ferritin* mRNA. Under these conditions, association of the 43*S* preinitiation complex with the mRNA cannot occur and the *ferritin* genes are not translated. When iron levels are elevated and Ferritin protein is needed, IRP binds to Fe^{2+}, which inhibits its ability to bind to the IRE and, therefore, allows translation of the Ferritin protein.

Gcn4 and the order of the amino acids

Gcn4 is a yeast transcriptional activator that regulates the expression of genes encoding enzymes that direct amino acid biosynthesis.

- Translation of the Yeast Transcriptional Activator Gcn4 Is Controlled by Short Upstream ORFs and Ternary Complex Abundance
- Low levels of amino acids – *Gcn4* mRNA is translated -> biosynthetic enzymes are expressed
- High levels of amino acids – no Gcn4 mRNA translation
- Regulated over four uORFs

Gcn4 and the order of the amino acids

NORMAL CONDITION - no starvation

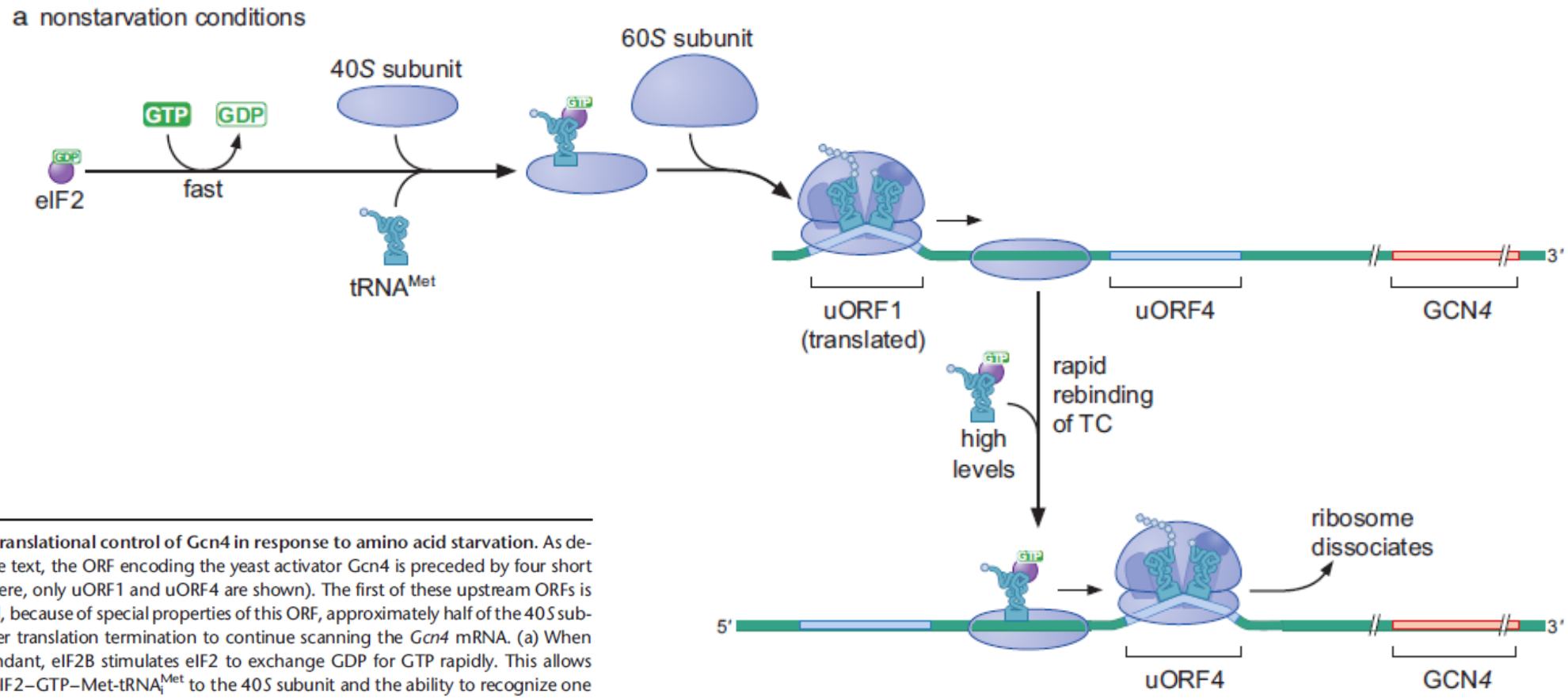


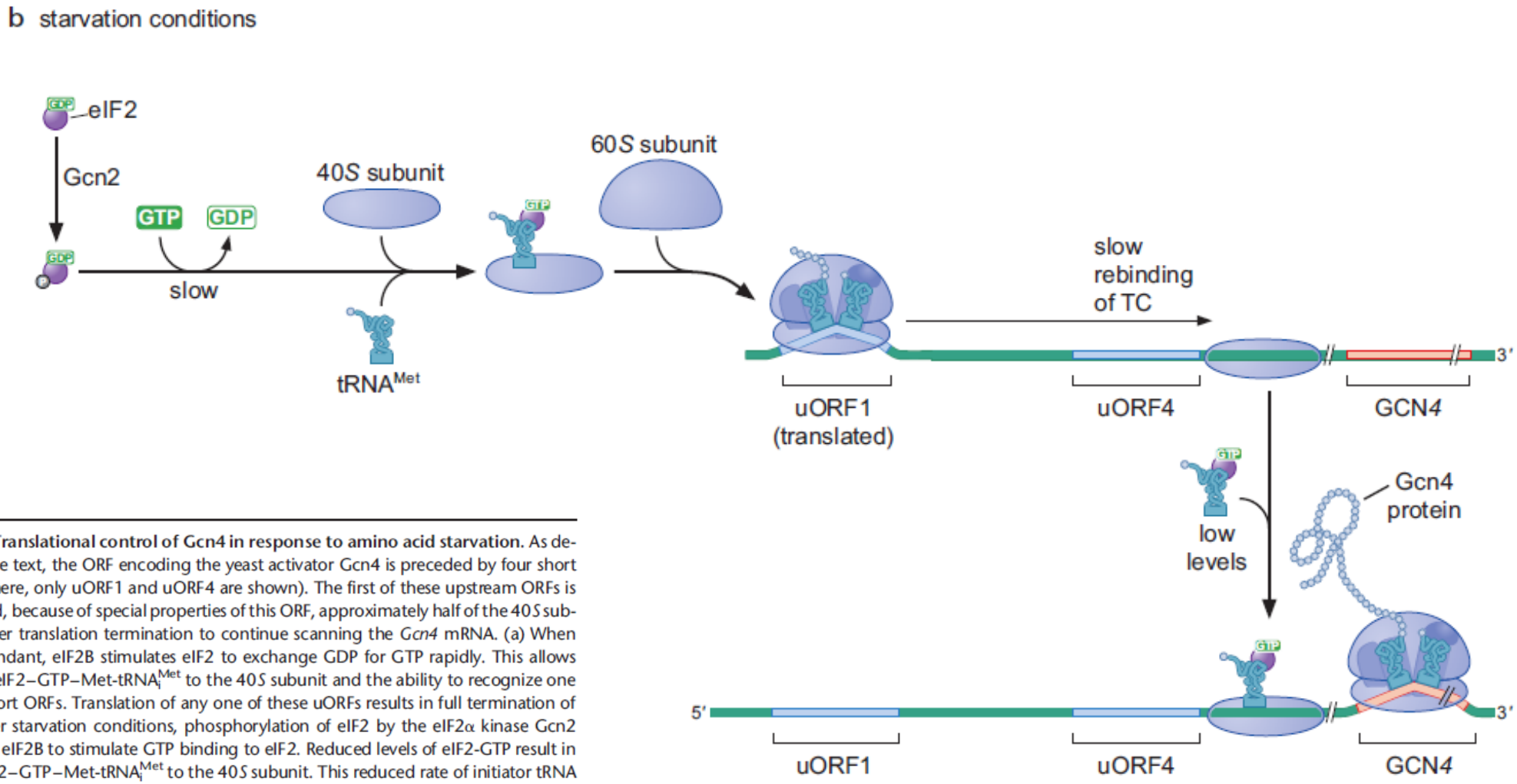
FIGURE 15-49 **Translational control of Gcn4 in response to amino acid starvation.** As described in detail in the text, the ORF encoding the yeast activator Gcn4 is preceded by four short ORFs called uORFs (here, only uORF1 and uORF4 are shown). The first of these upstream ORFs is translated initially and, because of special properties of this ORF, approximately half of the 40*S* subunits are retained after translation termination to continue scanning the *Gcn4* mRNA. (a) When amino acids are abundant, eIF2B stimulates eIF2 to exchange GDP for GTP rapidly. This allows for rapid binding of eIF2–GTP–Met-tRNA$_i^{Met}$ to the 40*S* subunit and the ability to recognize one of the three other short ORFs. Translation of any one of these uORFs results in full termination of translation. (b) Under starvation conditions, phosphorylation of eIF2 by the eIF2α kinase Gcn2 reduces the ability of eIF2B to stimulate GTP binding to eIF2. Reduced levels of eIF2-GTP result in slower binding of eIF2–GTP–Met-tRNA$_i^{Met}$ to the 40*S* subunit. This reduced rate of initiator tRNA binding increases the chance that the scanning ribosome will pass uORF4 before being able to recognize an AUG and therefore favors the translation of Gcn4. (Modified, with permission, from Hinnebusch A.G. 1997. *J. Biol. Cell* **272**: 21661–21664, Fig. 1. © American Society for Biochemistry & Molecular Biology.)

Gcn4 and the order of the amino acids

- High levels of amino acids – no *Gcn4* mRNA translation
  - uORF1 is translated, new TC (ternary complex) is recruited quickly and one of the remaining three uORFs is translated
  - Ribosome does not reach Gcn4 ORF!

Gcn4 and the order of the amino acids

STARVATION CONDITIONS)



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Gcn4 and the order of the amino acids

- Low levels of amino acids – *Gcn4* mRNA is translated -> biosynthetic enzymes are expressed
  - TC binding rate is reduced, no tRNA in the P-site
  - Ribosome can scan for longer and will skip remaining uORFs (AUG codons for these are closer to uORF1, Gcn4 AUG codon is further away)

Broken mRNA = broken protein?

- At some frequency, mRNAs will get damaged/broken
- Such mRNAs will block translation by stalling ribosomes on them

- Any way out?

Broken mRNA ≠ broken protein

The SsrA RNA rescues ribosomes - it translates broken mRNAs

- Bring in SsrA – part tRNA, part mRNA (**tmRNA**)
  - 457 nucleotides
  - 5’ end mimics $tRNA^{Ala}$
  - 3’ end encodes 10 amino acids (Ala-Asn-Asp-Glu-Asn-Tyr-Ala-Leu-Ala-Ala)
  - ~4 times larger than normal tRNA

Broken mRNA ≠ broken protein

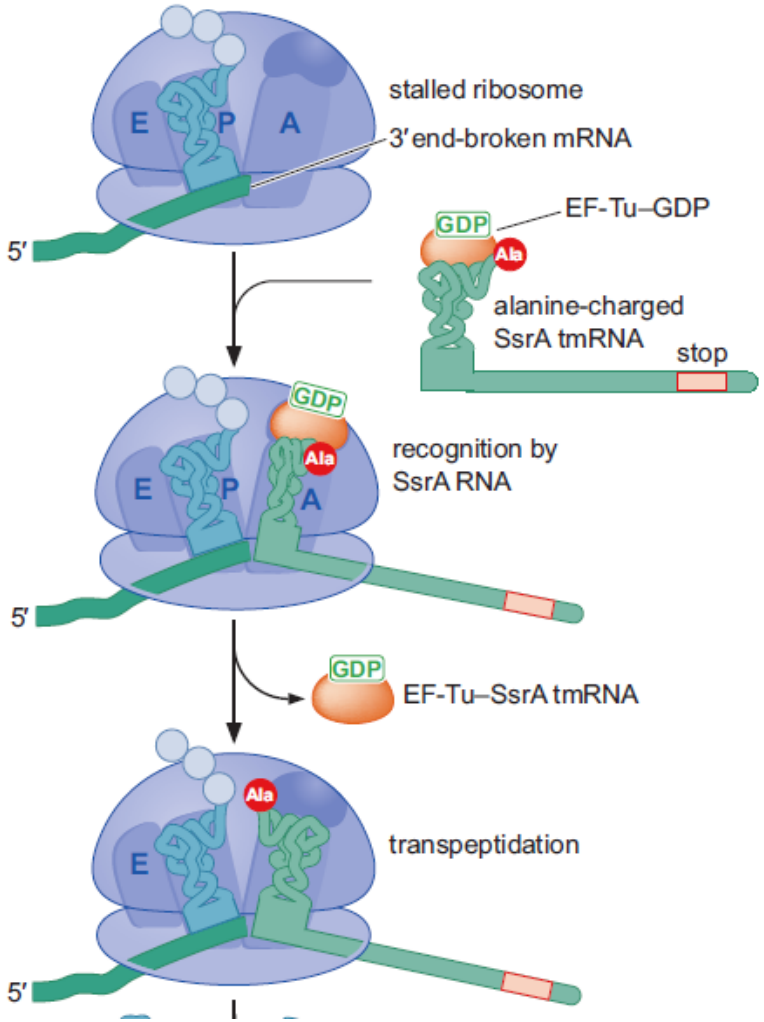
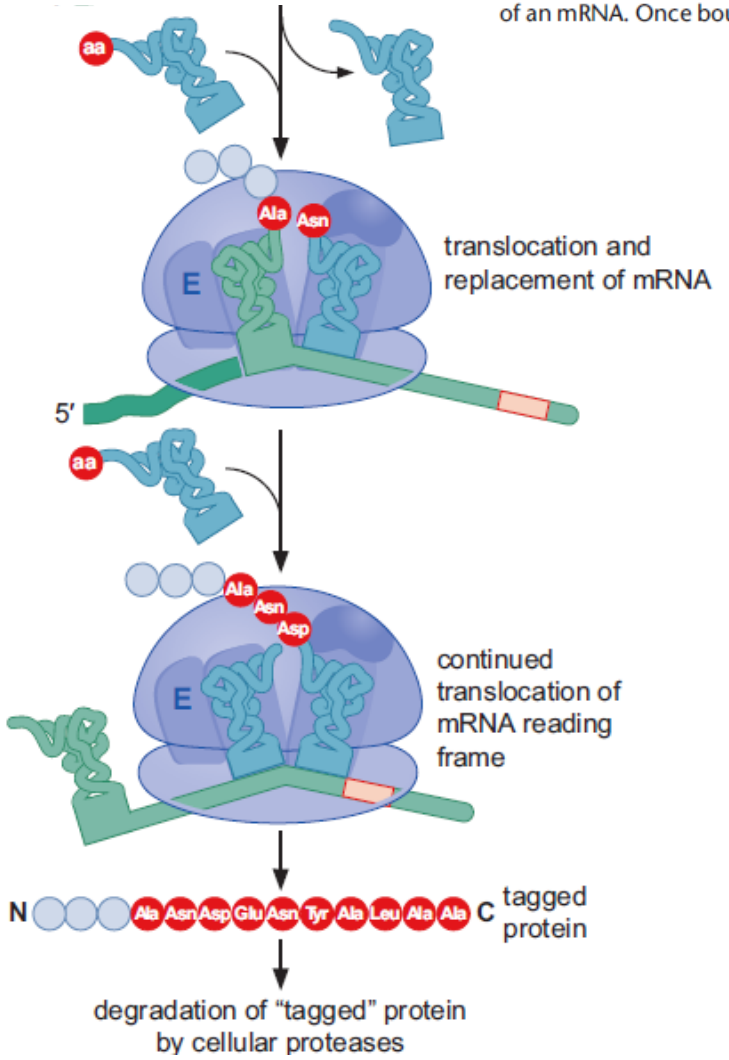


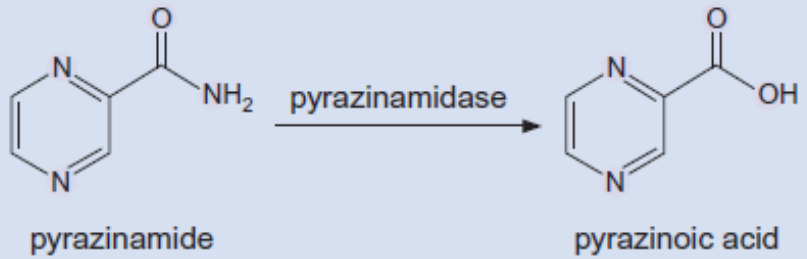
FIGURE 15-50 The tmRNA SsrA rescues ribosomes stalled on prematurely terminated mRNAs. The SsrA RNA mimics a tRNA but can only bind a ribosome that is stalled at the 3′ end of an mRNA. Once bound, the SsrA RNA substitutes part of its sequence to act as a new "mRNA."



Mimicri!!!
The SsrA RNA mimics a tRNA but can only bind a ribosome that is stalled at the 3' end of an mRNA. Once bound, the SsrA RNA substitutes part of its sequence to act as a new "mRNA."

Can we use it for our advantage?

- Tuberculosis
- A Frontline Drug in Tuberculosis Therapy Targets SsrA Tagging
  - Rifampicin (RNA polymerase inhibitor)
  - Isoniazid (cell envelope synthesis inhibitor)
  - Ethambutol (celle nvelope synthesis inhibitor)
  - Pyrazinamide (blocks the binding of SsrA RNA)



BOX 15-7 FIGURE 1 Conversion of pyrazinamide to pyrazinoic acid.

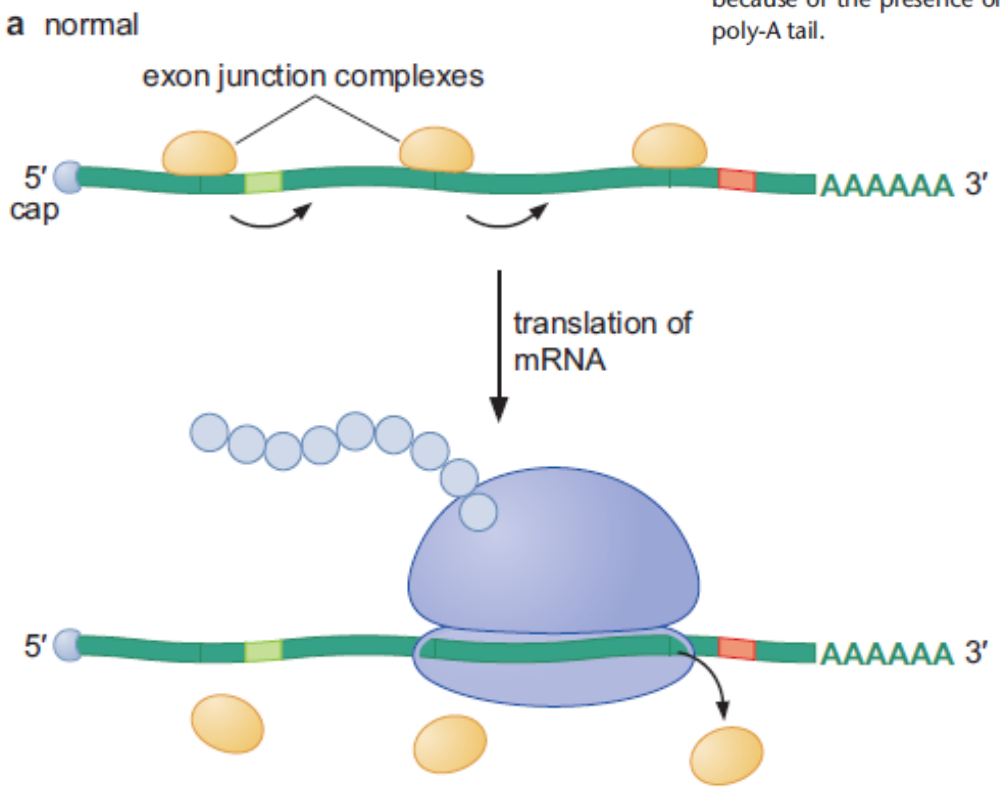
- Translation and mRNA decay or destoring are linked

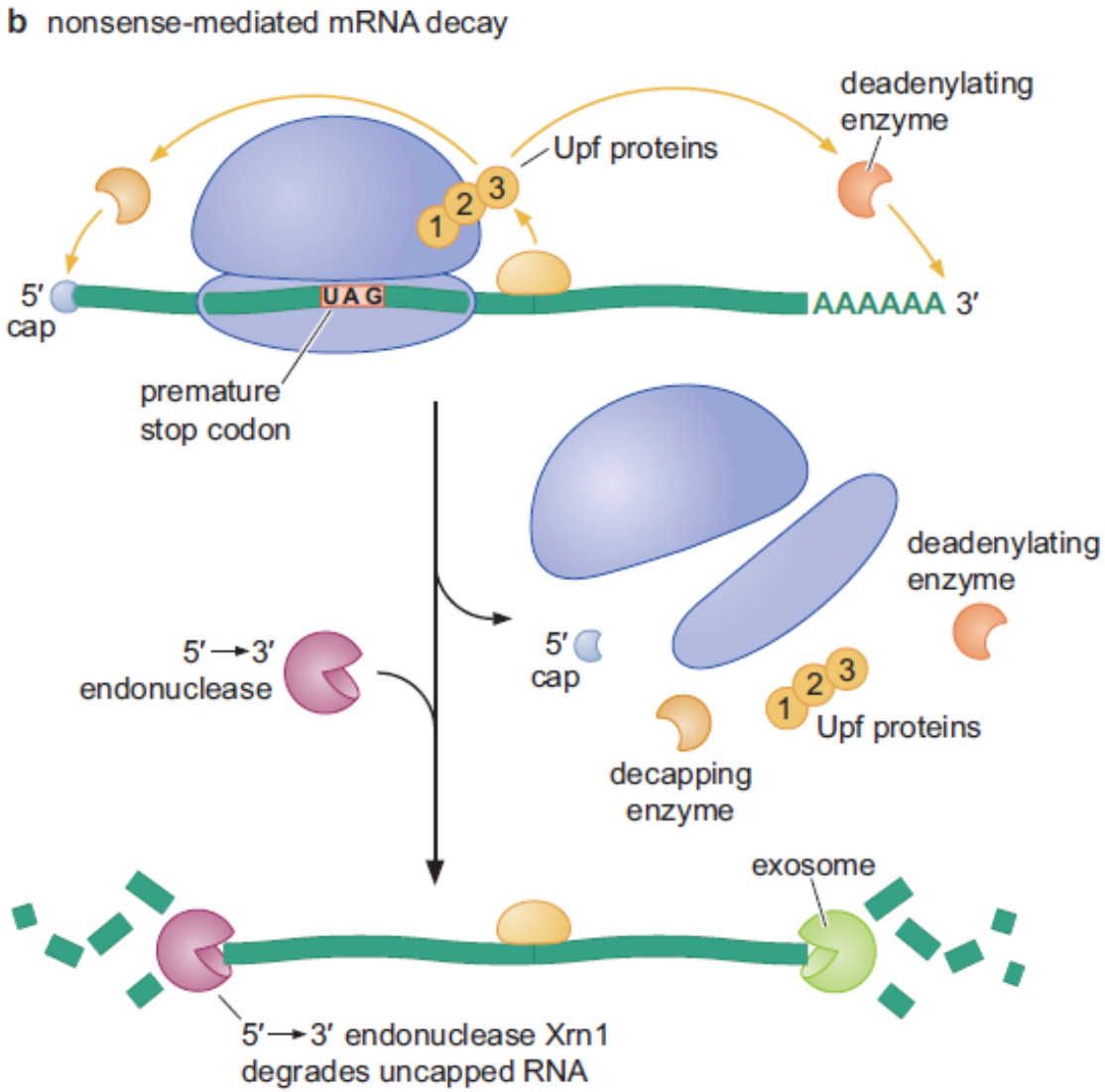
- What if an mRNA contains premature stop codon?
  - Nonsense-mediated mRNA decay (NMD)

NMD

- **Nonsense-mediated mRNA decay**

FIGURE 15-51 **Eukaryotic mRNAs with premature stop codons are targeted for degradation.** (a) Translation of a normal mRNA displaces all of the exon–junction complexes. (b) Nonsense-mediated decay. Translation of an mRNA with a premature stop codon does not displace one or more of the exon–junction complexes. This results in the recruitment of the Upf1, Upf2, and Upf3 proteins to the ribosome. Once bound to the ribosome, these proteins activate a decapping enzyme that removes the 5′ cap and a deadenylating enzyme that removes the poly-A tail of the mRNA. The uncapped and deadenylated mRNA is then rapidly degraded by 5′-to-3′ (Xrn1) and 3′–5′ (exosome) exonucleases that are normally unable to degrade the mRNA because of the presence of the 5′ cap and poly-A tail.





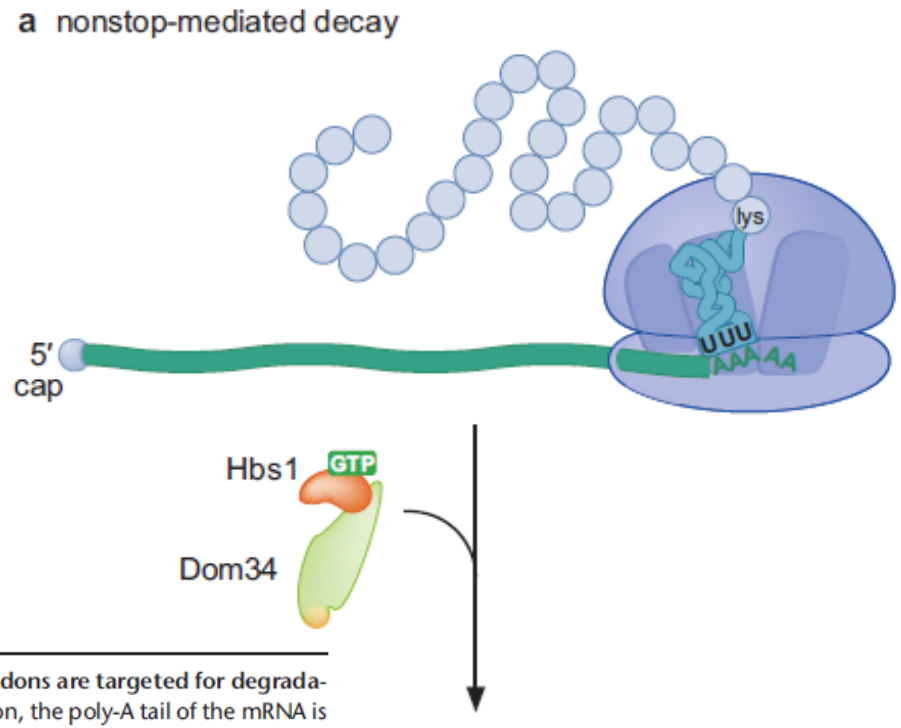
Sir, this is nonstop nonsense!

- What if an mRNA does not contain a stop codon?
  - Nonstop-mediated decay
- What if an mRNA stalls ribosomes?
  - No-go-mediated decay

Eukaryotic mRNAs with premature stop codons are targeted for degradation.

Sir, this is nonstop nonsense!

- **Nonstop-mediated mRNA decay**



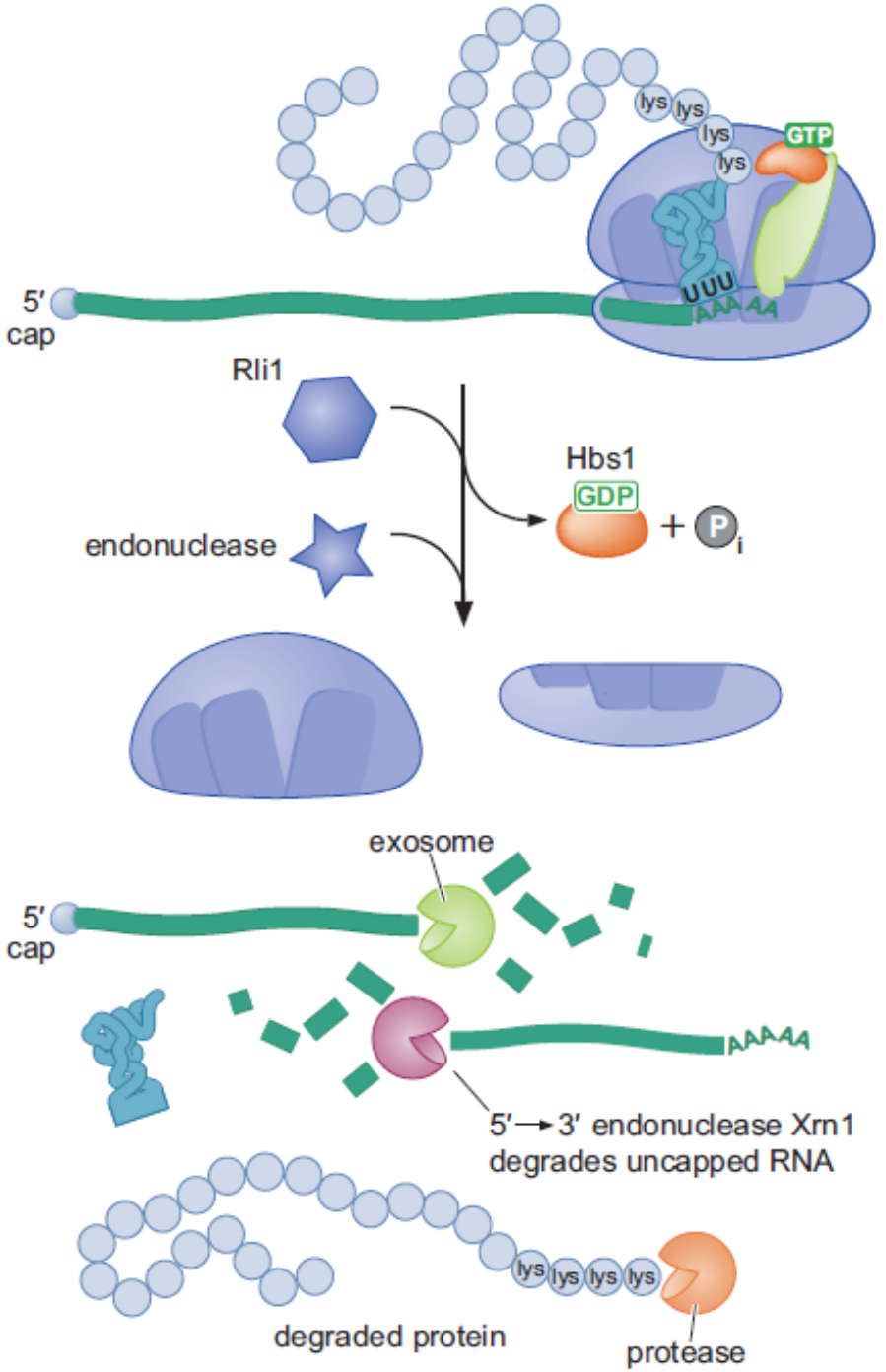
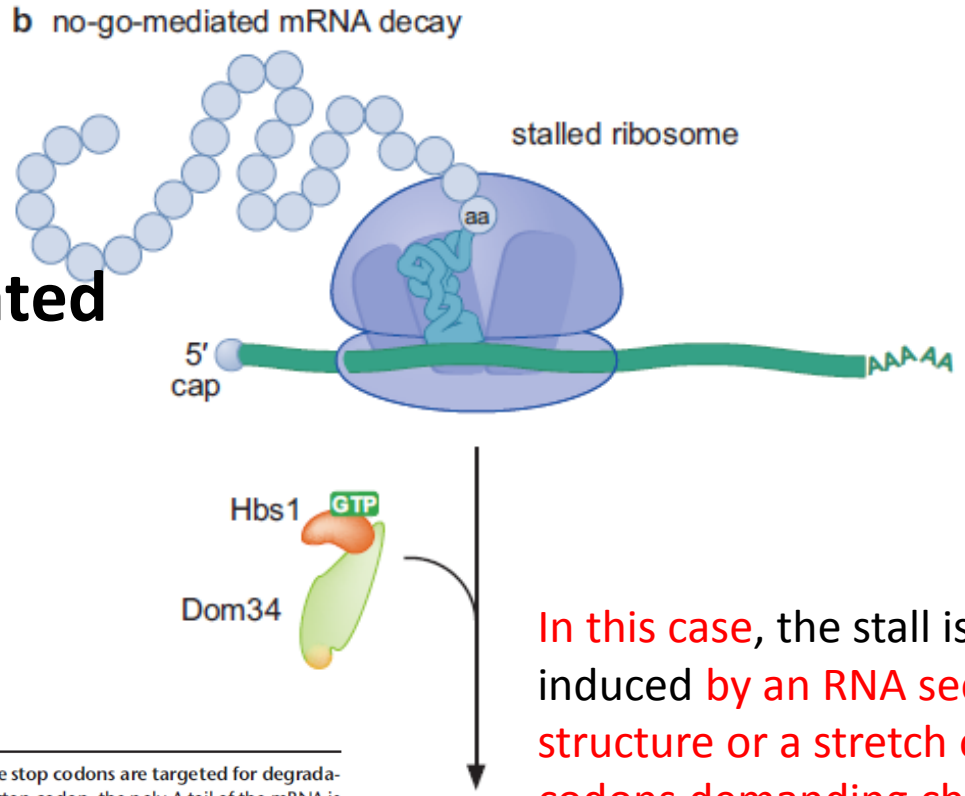


FIGURE 15-52 **Eukaryotic mRNAs with premature stop codons are targeted for degradation.** (a) Nonstop-mediated decay. In the absence of a stop codon, the poly-A tail of the mRNA is translated, leading to the addition of polylysine (AAA encodes Lys) to the end of the protein. Upon reaching the 3′ end of the template, the stalled ribosome is recognized by a complex of Dom34 and Hbs1. After delivering Dom34 to the ribosome, Hbs1 hydrolyzes GTP and is released. In combination with the Rli1 ATPase, Dom34 acts to disassemble the ribosome into its two subunits and recruit an endonuclease that cuts the mRNA upstream of the ribosome. The resulting mRNA fragments are degraded by 5′→3′ and 3′→5′ exonucleases. The protein with the polylysine at the end is also subject to proteolysis. (b) No-go-mediated decay. As with nonstop-mediated decay, no-go-mediated decay is initiated when the ribosome stalls. In this case, the stall is induced by an RNA secondary structure or a stretch of codons demanding charged tRNAs that are present in low abundance (often referred to as rare codons). The stalled ribosome is recognized by Dom34 and Hbs1, and the ribosome is released and the mRNA degraded in a similar manner to non-stop-mediated decay.

No, you can’t go further!

- **No-go-mediated mRNA decay**
- Stopping



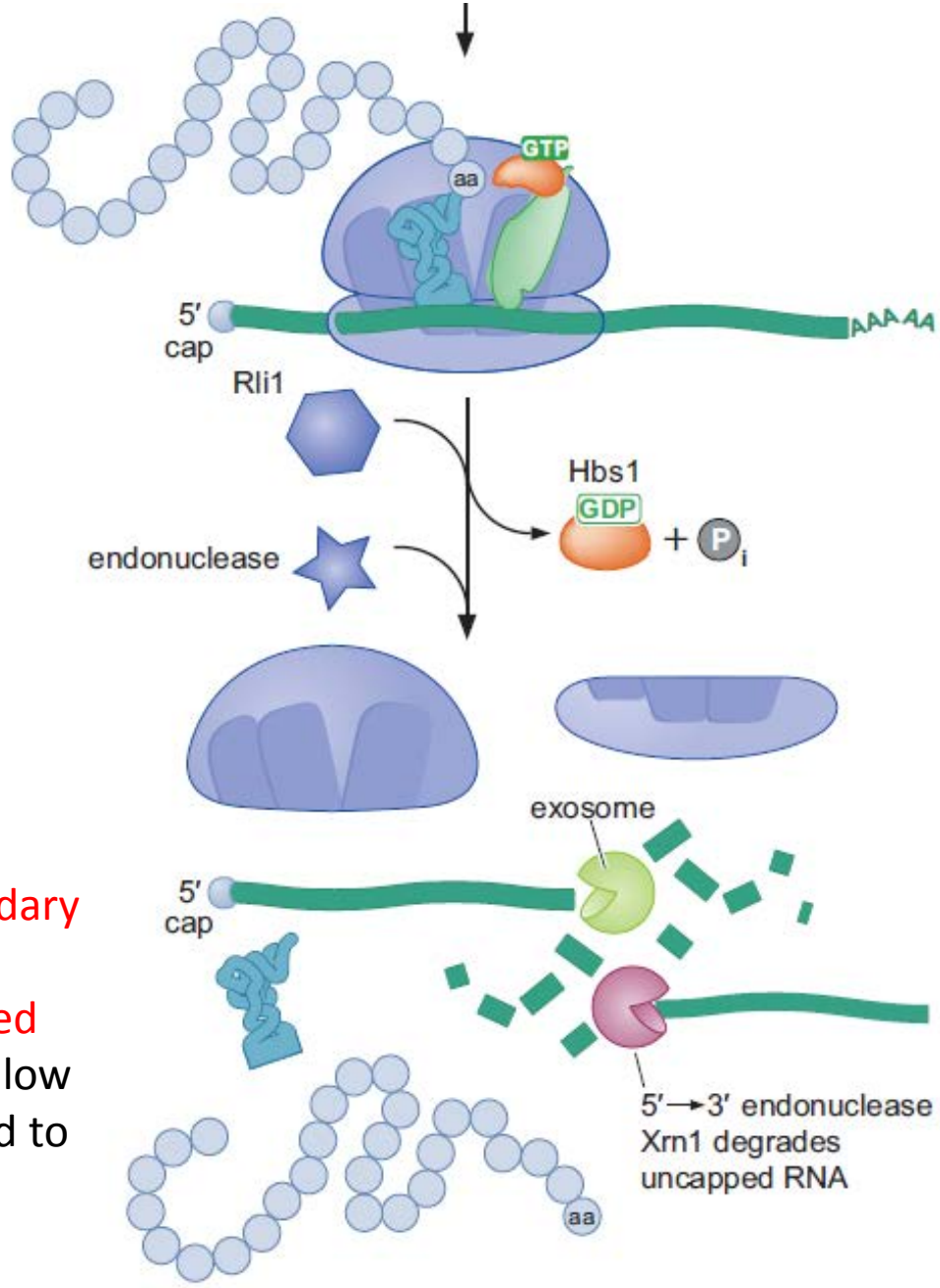


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FIGURE 15-52 **Eukaryotic mRNAs with premature stop codons are targeted for degradation.** (a) Nonstop-mediated decay. In the absence of a stop codon, the poly-A tail of the mRNA is translated, leading to the addition of polylysine (AAA encodes Lys) to the end of the protein. Upon reaching the 3′ end of the template, the stalled ribosome is recognized by a complex of Dom34 and Hbs1. After delivering Dom34 to the ribosome, Hbs1 hydrolyzes GTP and is released. In combination with the Rli1 ATPase, Dom34 acts to disassemble the ribosome into its two subunits and recruit an endonuclease that cuts the mRNA upstream of the ribosome. The resulting mRNA fragments are degraded by 5′→3′ and 3′→5′ exonucleases. The protein with the polylysine at the end is also subject to proteolysis. (b) No-go-mediated decay. As with nonstop-mediated decay, no-go-mediated decay is initiated when the ribosome stalls. In this case, the stall is induced by an RNA secondary structure or a stretch of codons demanding charged tRNAs that are present in low abundance (often referred to as rare codons). The stalled ribosome is recognized by Dom34 and Hbs1, and the ribosome is released and the mRNA degraded in a similar manner to non-stop-mediated decay.

Translation helps to keep mRNA quality!

- A fascinating feature of nonsense-, non-stop- and no-go-mediated mRNA decay is that each of these processes requires translation of the damaged mRNA **to detect the defect and degrade the mRNA**.
- In the absence of translation, the damaged mRNAs are not rapidly degraded and have normal stability.
- Thus, although indirect, **eukaryotic cells rely on translation as a mechanism to proofread their mRNAs**.