

Translation: elongation

Elongation factors

<i>Prokaryotes</i>	<i>Eukaryotes</i>	
EF-Tu	eEF1α	aa-tRNA transport
EF-Ts	eEF1$\beta\gamma$	recycling
EF-G	eEF2	translocation

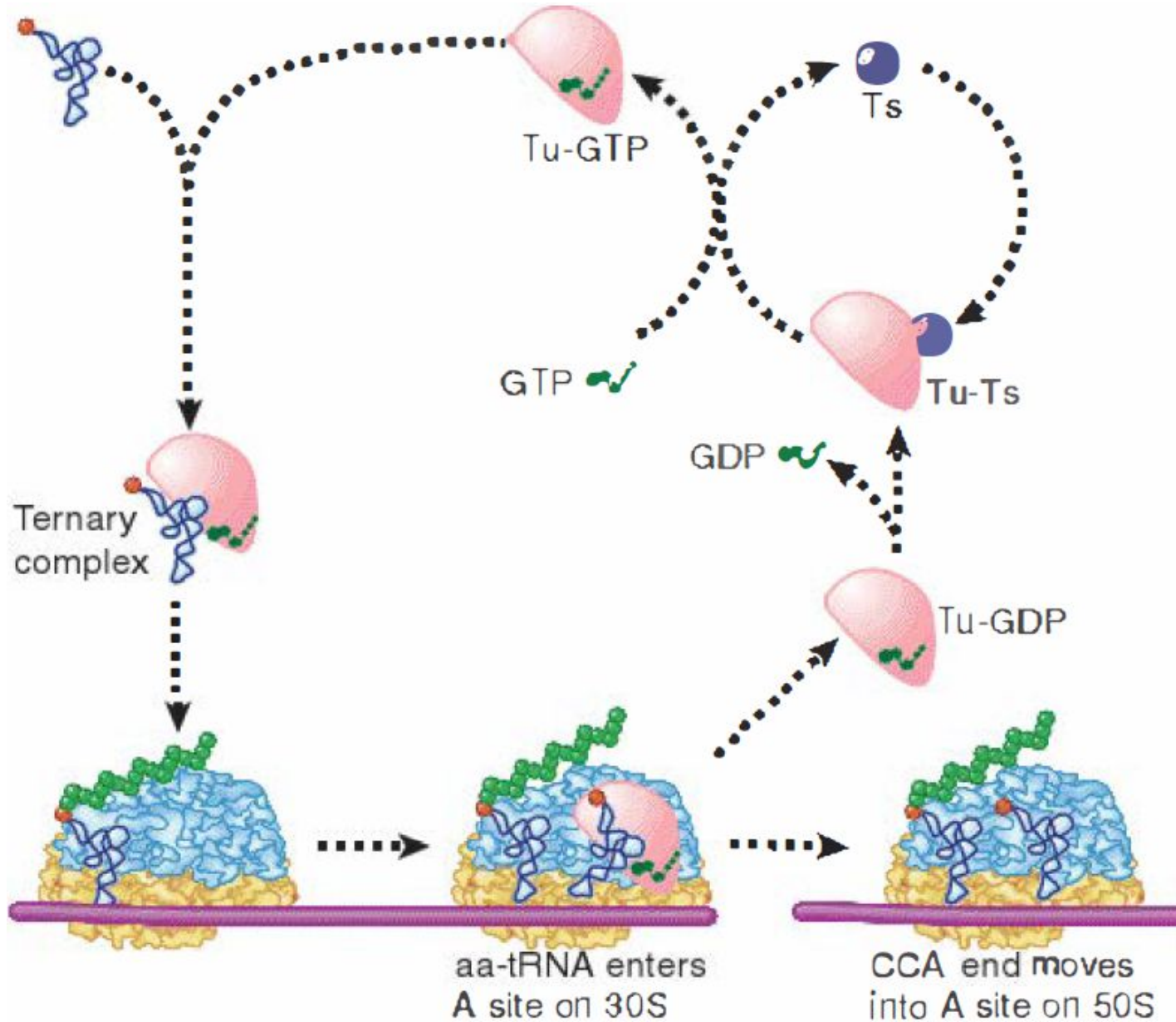


FIGURE 24.25 EF-Tu-GTP places aminoacyl-tRNA on the ribosome and then is released as EF-Tu-GDP. EF-Ts is required to mediate the replacement of GDP by GTP. The reaction consumes GTP and releases GDP. The only aminoacyl-tRNA that cannot be recognized by EF-Tu-GTP is fMet-tRNA_f, whose failure to bind prevents it from responding to internal AUG or GUG codons.

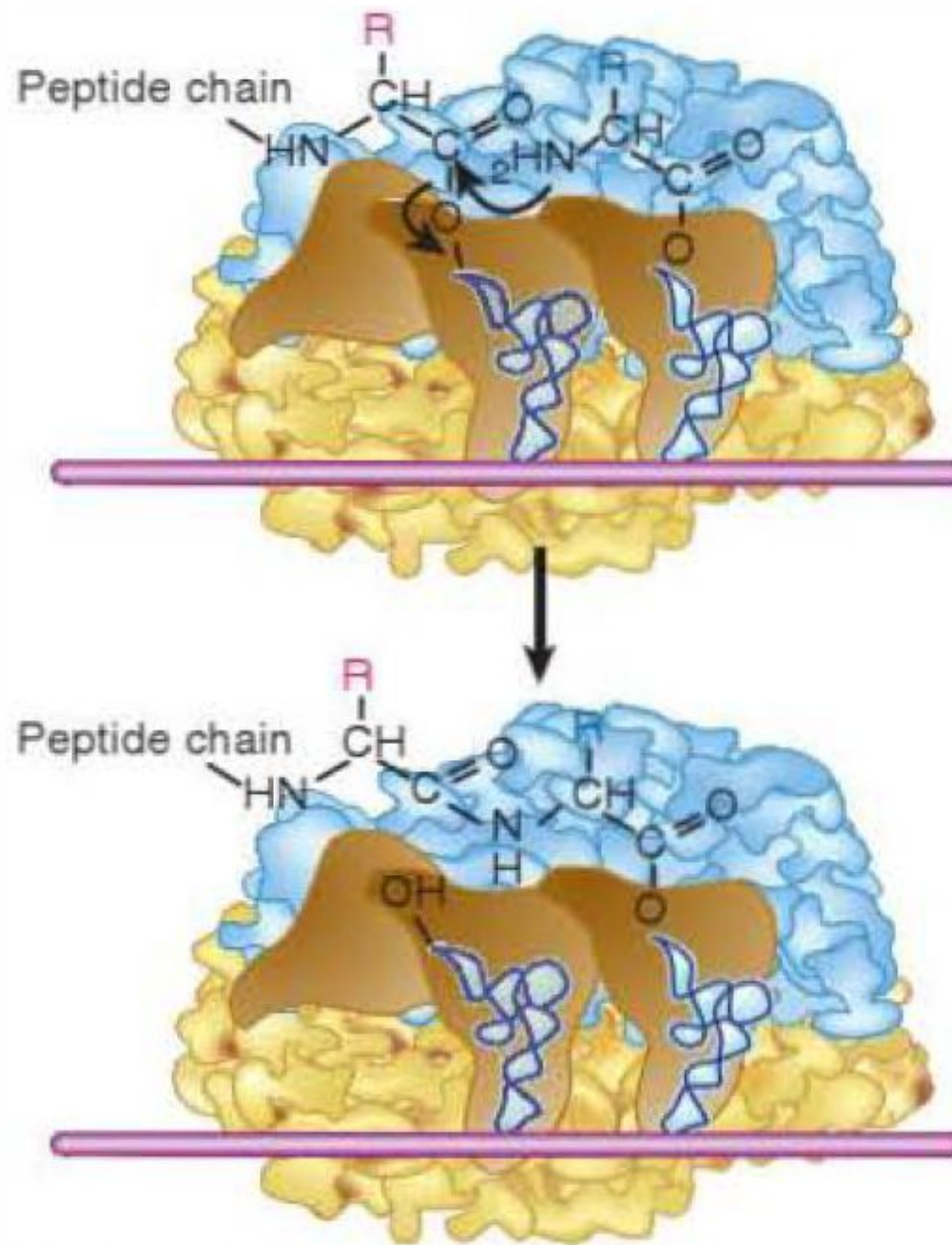
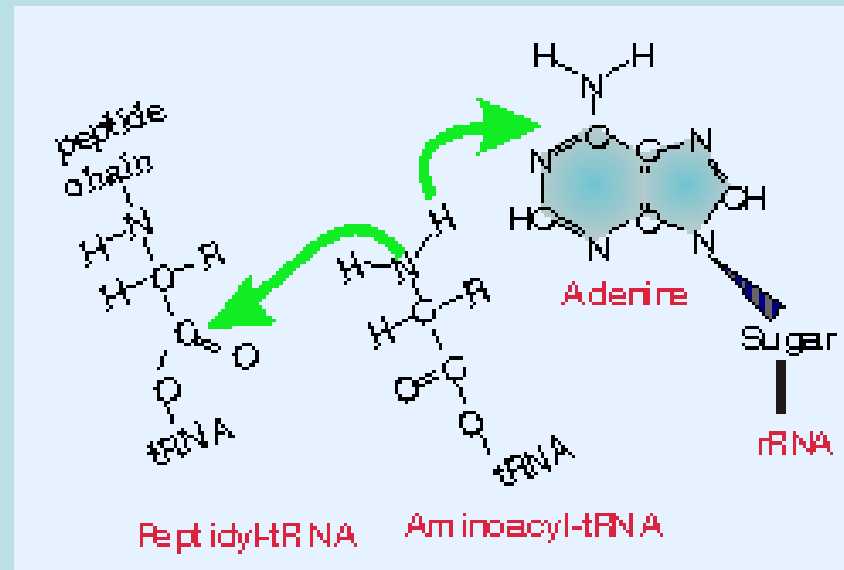
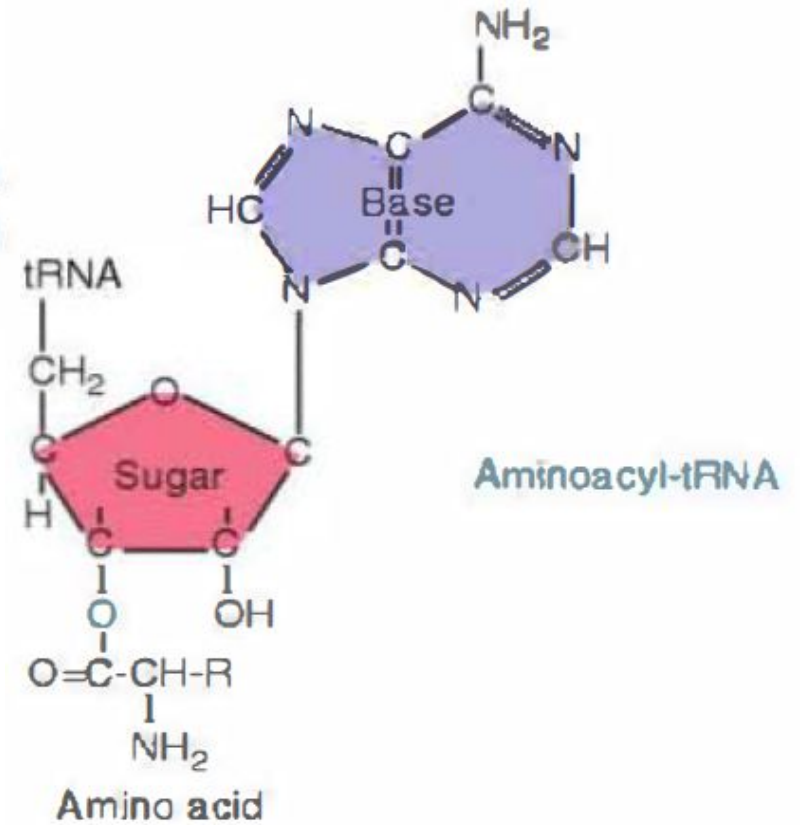
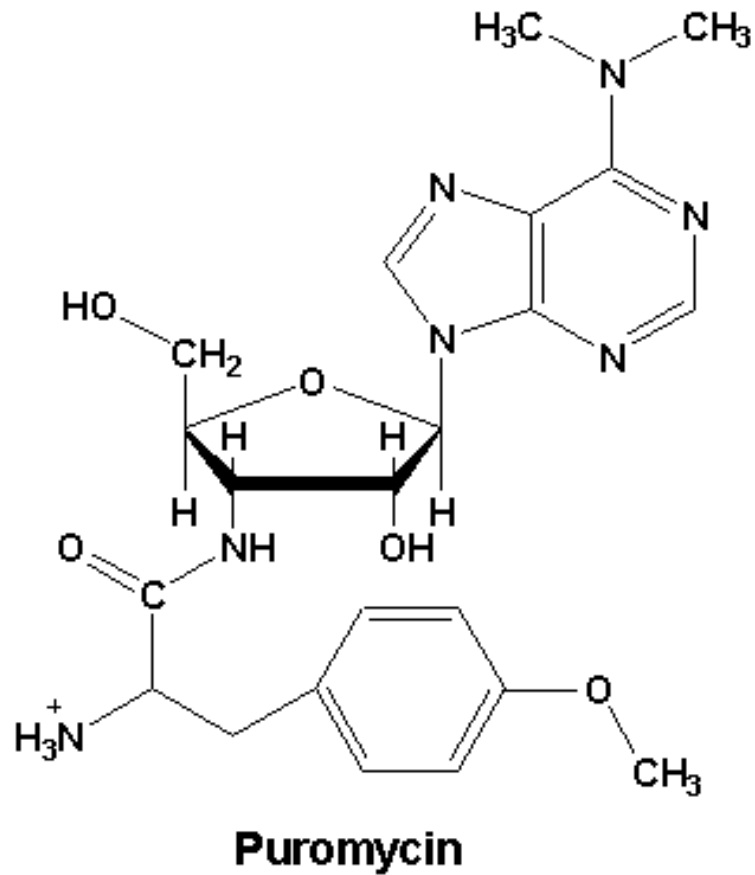


FIGURE 24.26 Peptide bond formation takes place by a reaction between the polypeptide of peptidyl-tRNA in the P site and the amino acid of aminoacyl-tRNA in the A site.

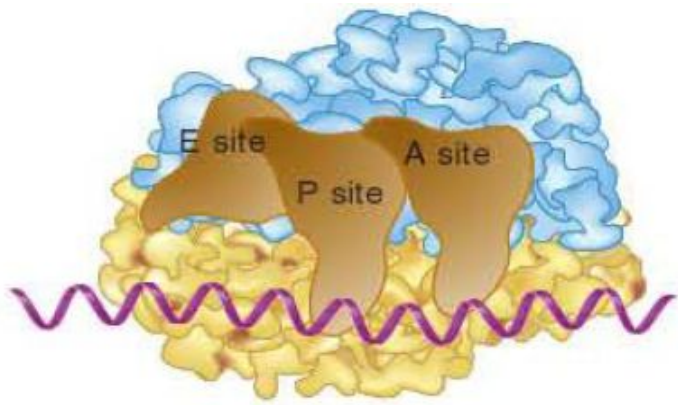


A basic adenine in 23S rRNA could accept a proton from the amino group of the aminoacyl-tRNA. This triggers an attack on the carboxyl group of the peptidyl-tRNA, leading to peptide bond formation.

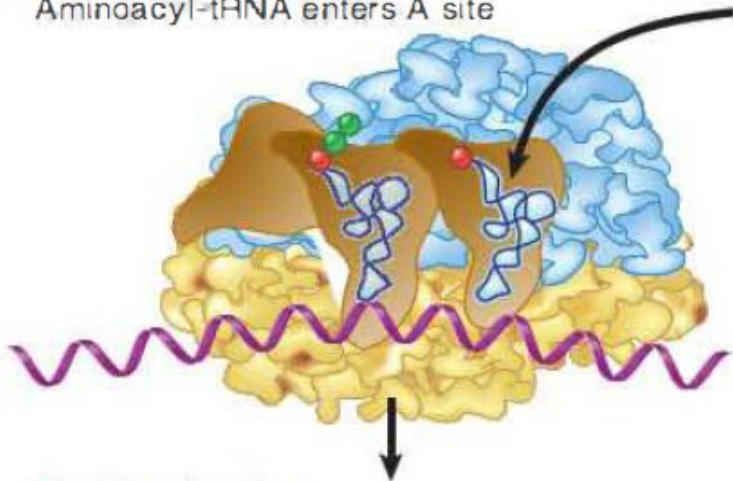
FIGURE 24.27 Puromycin mimics aminoacyl-tRNA because it resembles an aromatic amino acid linked to a sugar-base moiety.



Puromycin is an amino nucleoside antibiotic, derived from the *Streptomyces alboniger* bacterium, that causes premature chain termination during translation taking place in the ribosome. It is not selective for either prokaryotes or eukaryotes.



Pretranslocation:
 Peptidyl-tRNA is in P site;
 Aminoacyl-tRNA enters A site



Posttranslocation:
 Deacylated tRNA moves to E site;
 peptidyl-tRNA moves to P site

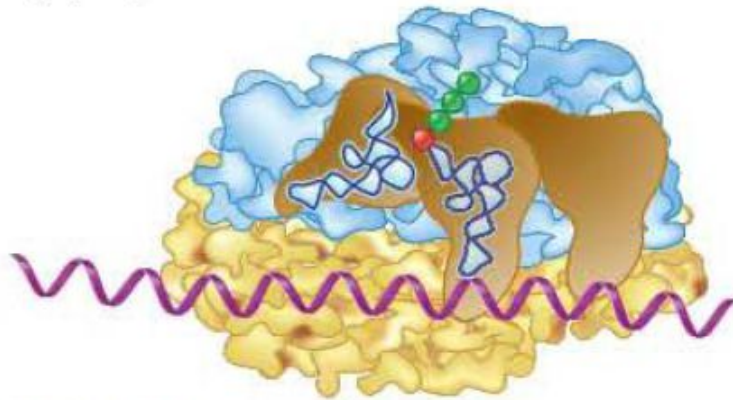


FIGURE 24.28 A bacterial ribosome has three tRNA-binding sites. Aminoacyl-tRNA enters the A site of a ribosome that has peptidyl-tRNA in the P site. Peptide bond synthesis deacylates the P site tRNA and generates peptidyl-tRNA in the A site. Translocation moves the deacylated tRNA into the E site and moves peptidyl-tRNA into the P site.

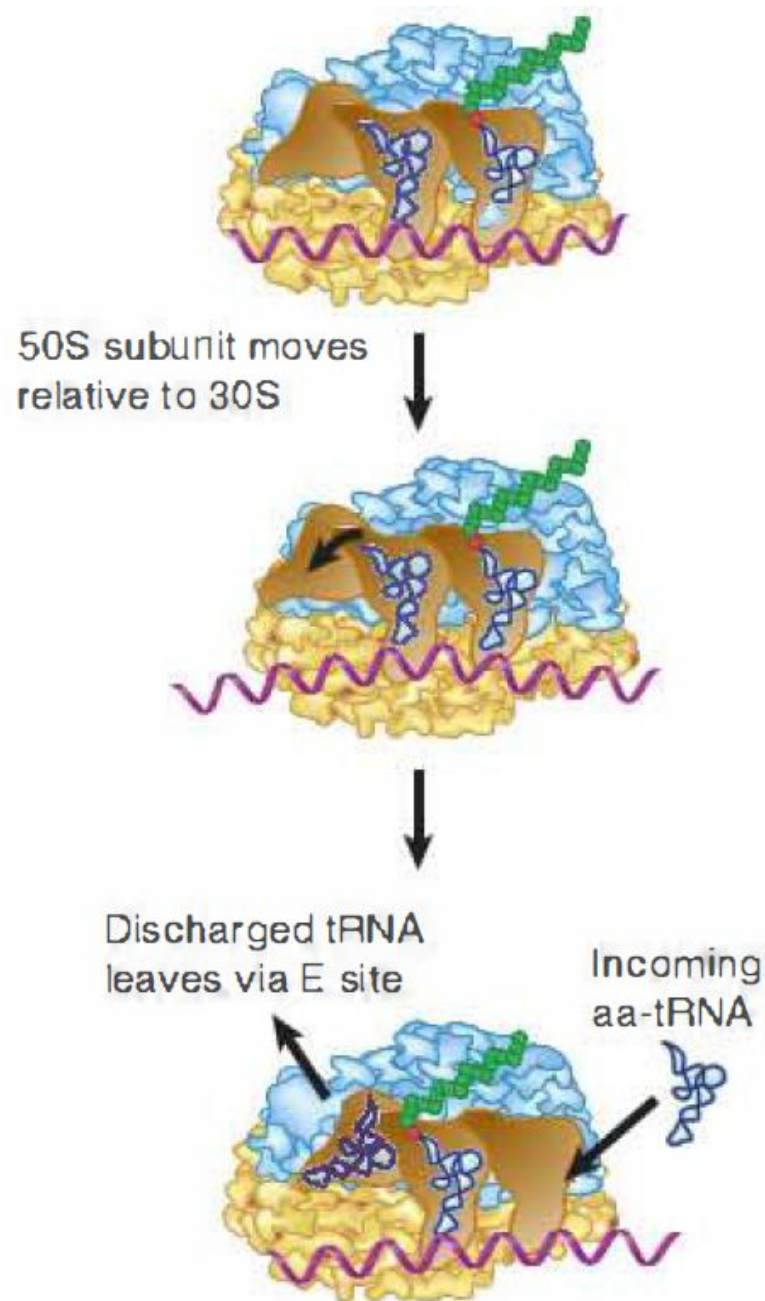
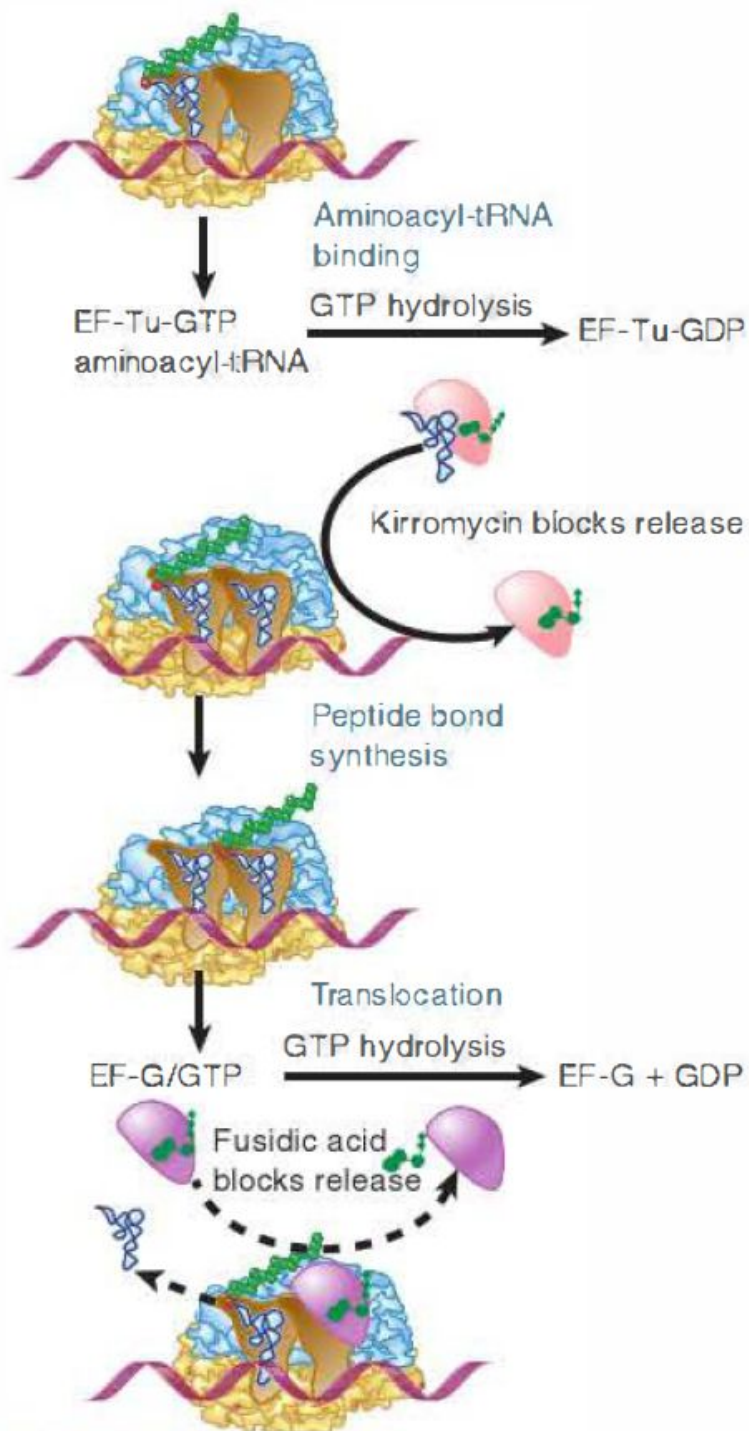


FIGURE 24.29 Models for translocation involve two stages. First, at peptide bond formation the aminoacyl end of the tRNA in the A site becomes relocated in the P site. Second, the anticodon end of the tRNA becomes relocated in the P site.



The factor **EF-G** catalyzes the translocation of the tRNA and mRNA down the ribosome at the end of each round of polypeptide elongation. Homologous to EF-Tu + tRNA, EF-G also binds to the ribosome in its GTP-bound state. When it associates with the A site, EF-G causes the tRNA previously occupying that site to occupy an **intermediate A/P position** (bound to the A site of the small ribosomal subunit and to the P site of the large subunit), and the tRNA in the P site is shifted to a **P/E hybrid** state. EF-G hydrolysis of GTP causes a conformation change that forces the A/P tRNA to fully occupy the P site, the P/E tRNA to fully occupy the E site (and exit the ribosome complex), and the mRNA to shift three nucleotides down relative to the ribosome due to its association with these tRNA molecules. The GDP-bound EF-G molecule then dissociates from the complex, leaving another free A-site where the elongation cycle can start again

FIGURE 24.30 Binding of factors EF-Tu and EF-G alternates as ribosomes accept new aminoacyl-tRNA, form peptide bonds, and translocate.

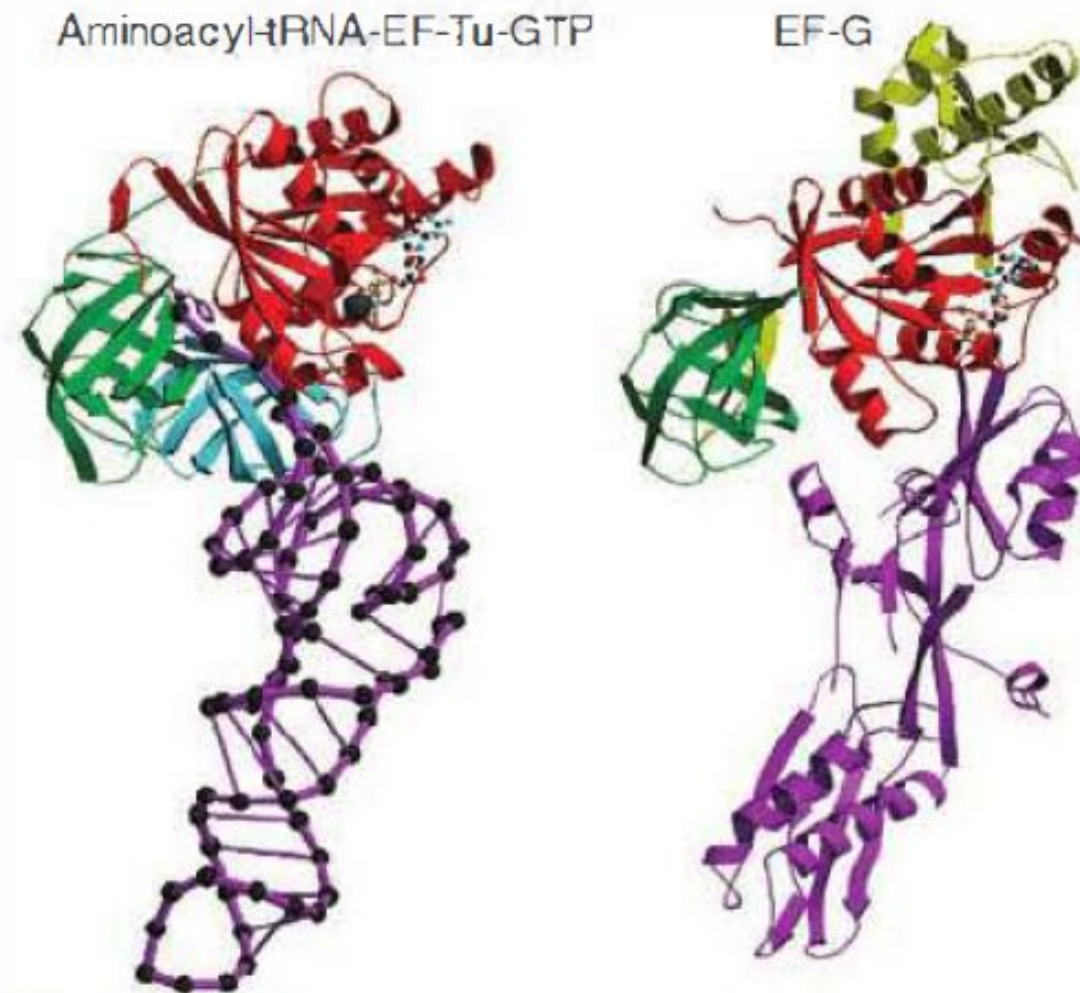


FIGURE 24.31 The structure of the ternary complex of aminoacyl-tRNA-EF-Tu-GTP (left) resembles the structure of EF-G (right). Structurally conserved domains of EF-Tu and EF-G are in red and green; the tRNA and the domain resembling it in EF-G are in purple. Photo courtesy of Poul Nissen, University of Aarhus, Denmark.

Decoding by the 70S ribosome

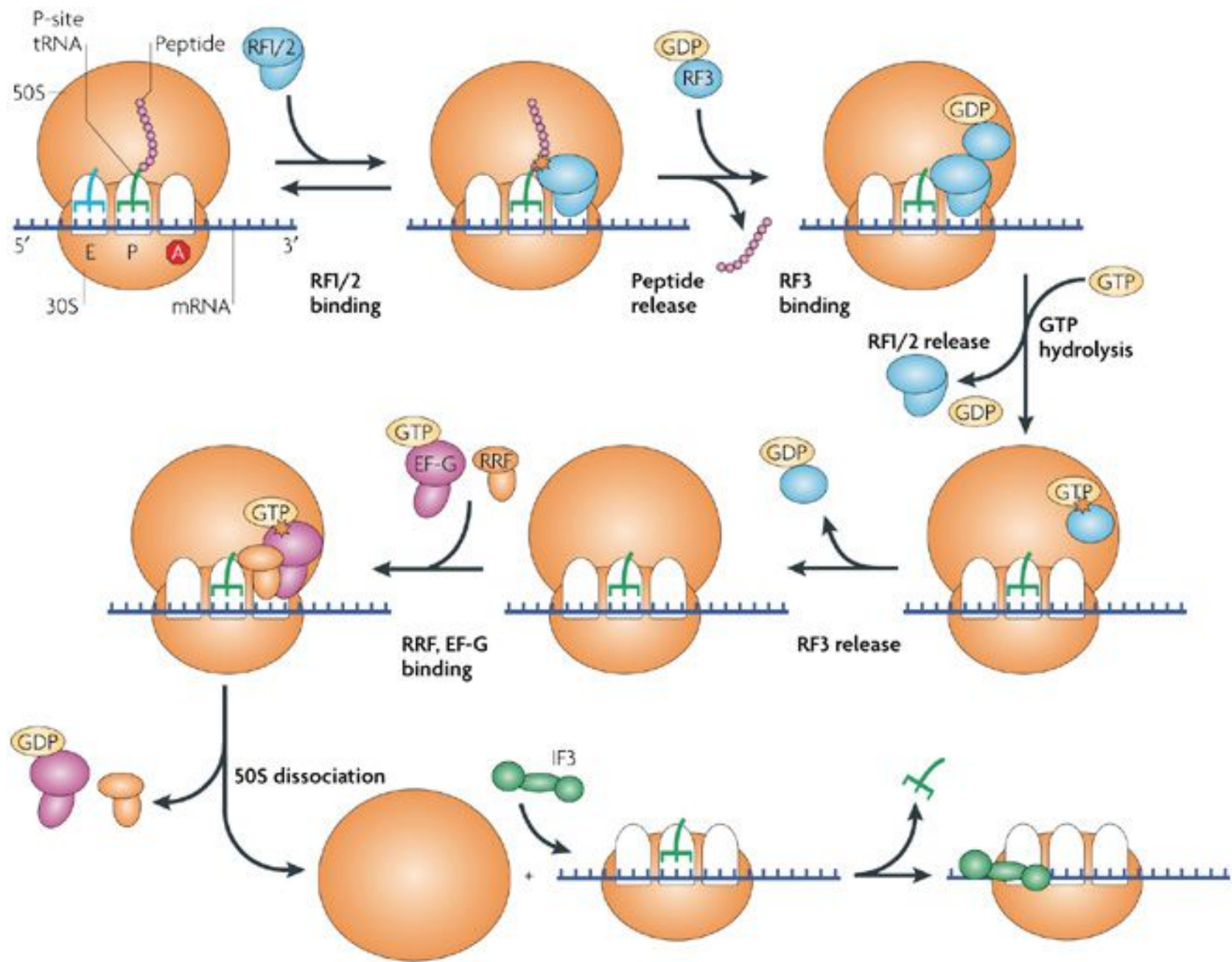
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Translation: termination

Termination factors

<i>Prokaryotes</i>	<i>Eukaryotes</i>	
RF1	eRF	identification UAA, UAG (ocher, amber)
RF2	“	identification UGA, UAA (opal)
RF3	eRF3	GTPase
RRF		release



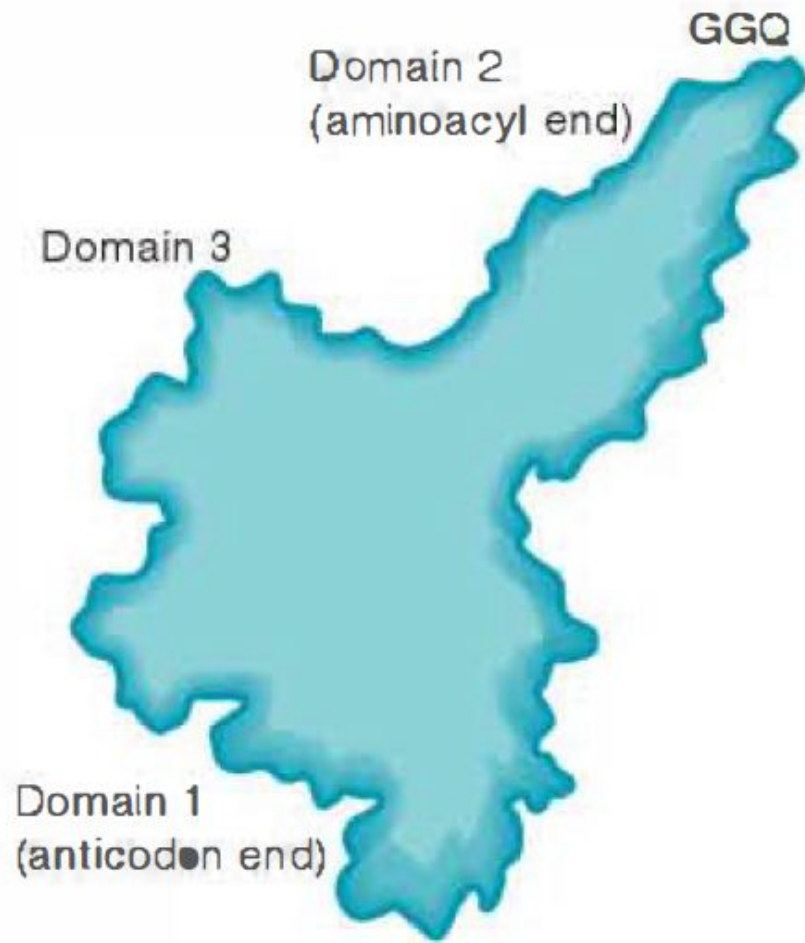


FIGURE 24.33 The eukaryotic termination factor eRF1 has a structure that mimics tRNA. The motif GGQ at the tip of domain 2 is essential for hydrolyzing the polypeptide chain from tRNA.

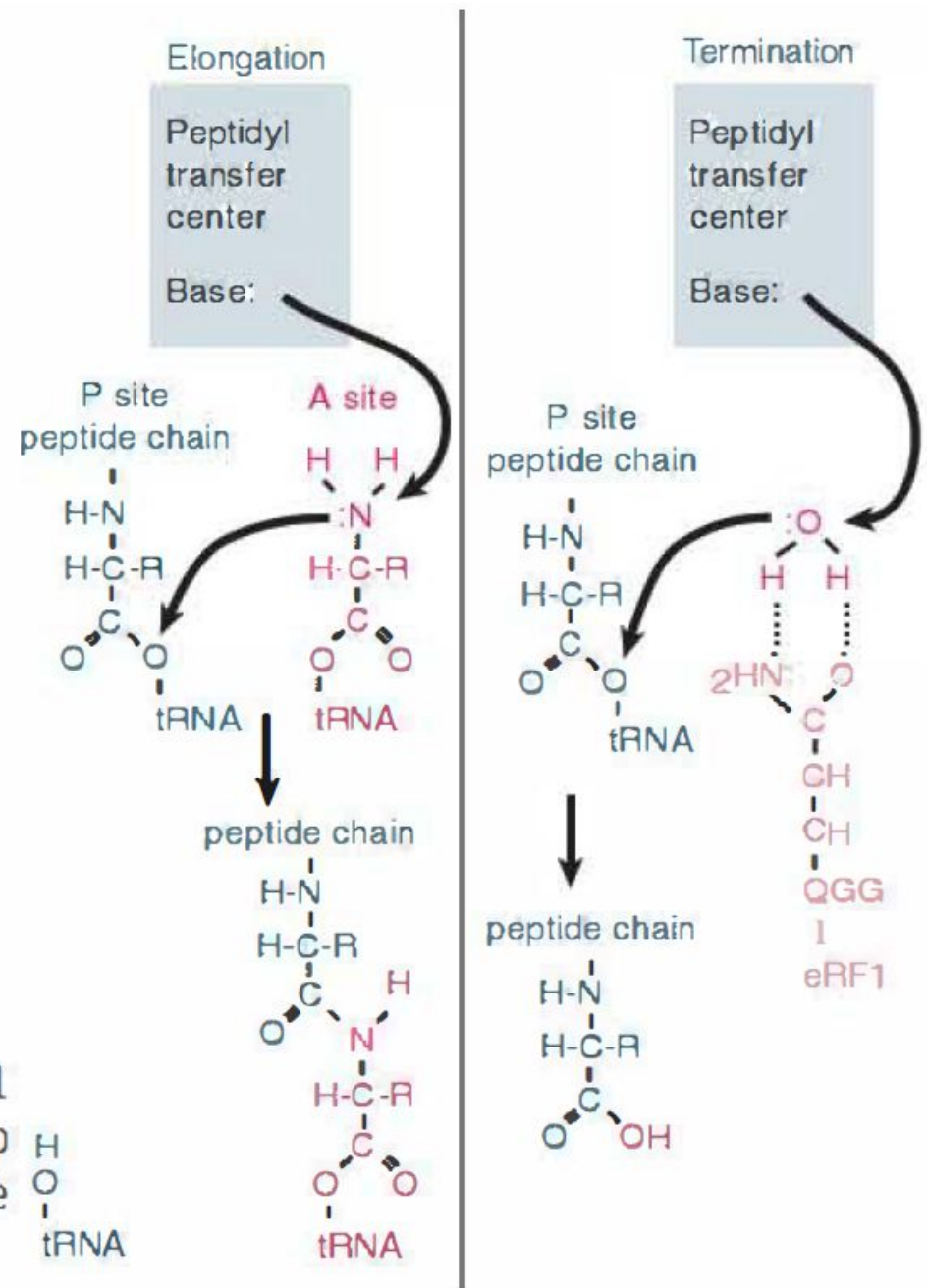


FIGURE 24.34 Peptide transfer and termination are similar reactions in which a base in the peptidyl transfer center triggers a transesterification reaction by attacking an N-H or O-H bond, releasing the N or O to attack the link to tRNA.

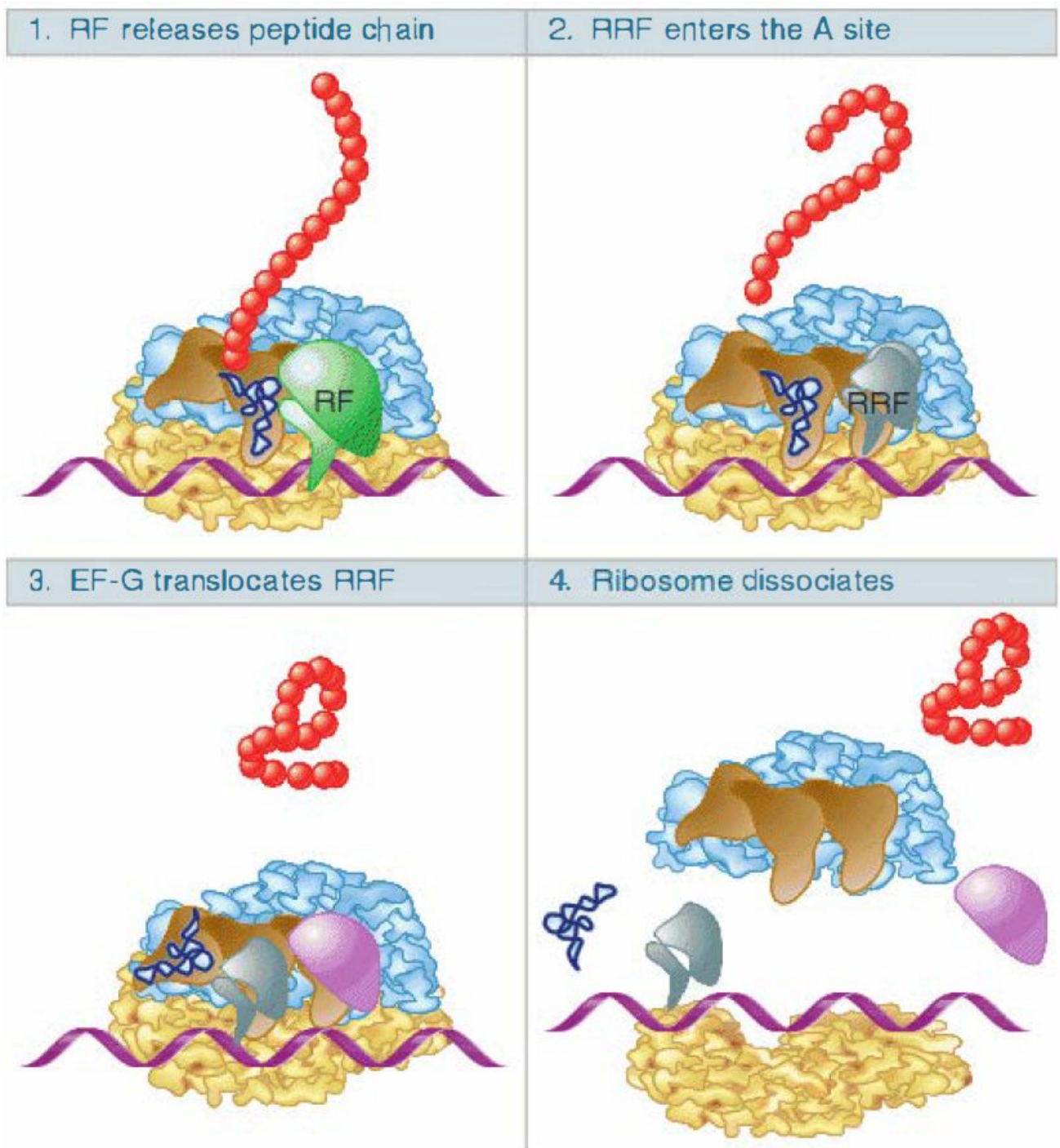


FIGURE 24.35 The RF (release factor) terminates translation by releasing the polypeptide chain. The RRF (ribosome recycling factor) releases the last tRNA, and EF-G releases RRF, causing the ribosome to dissociate.

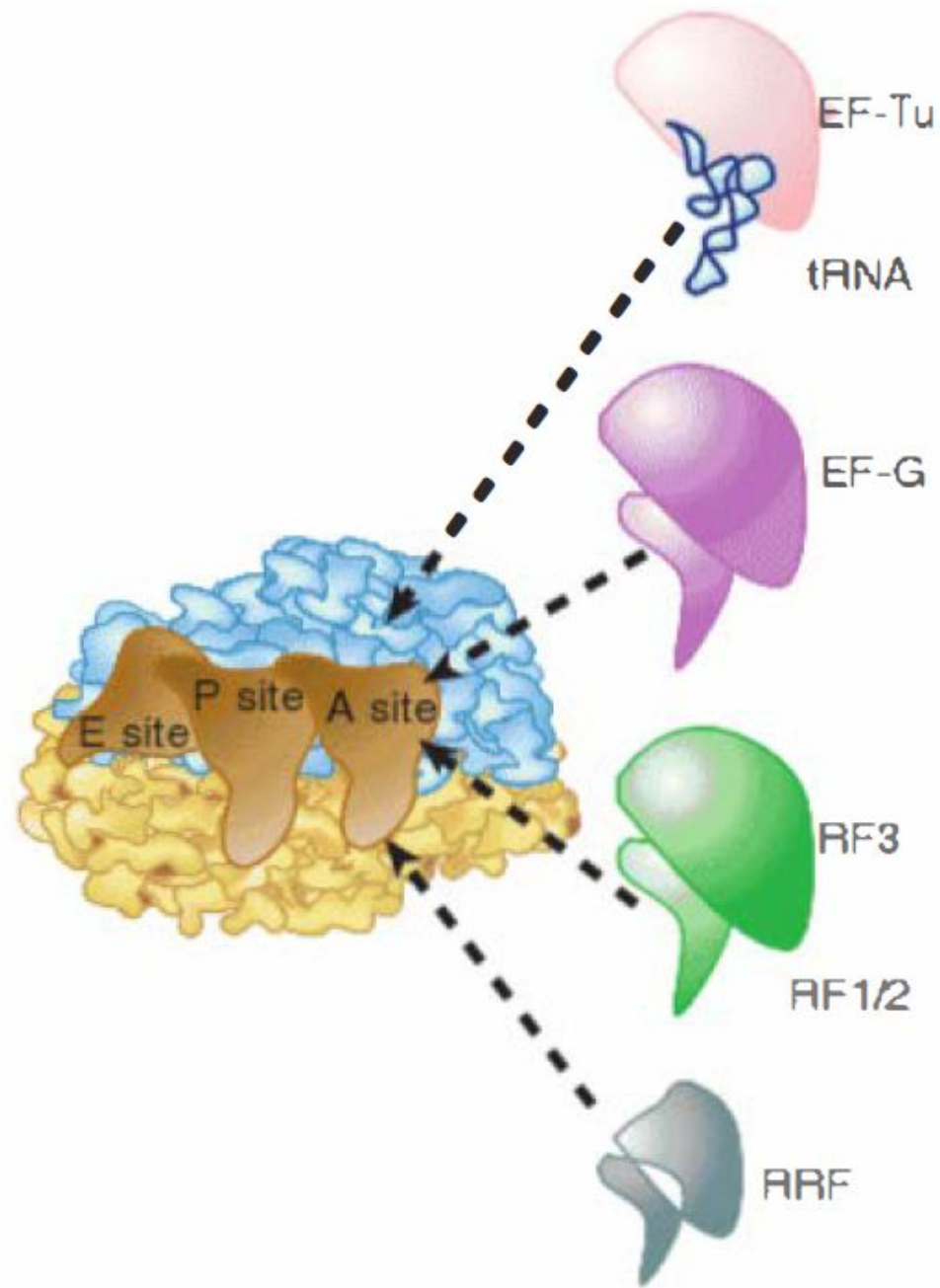







FIGURE 24.3 2 Molecular mimicry enables the elongation factor Tu-tRNA complex, the translocation factor EF-G, and the release factors RF1/2-RF3 to bind to the same ribosomal site. RRF is the ribosome recycling factor.

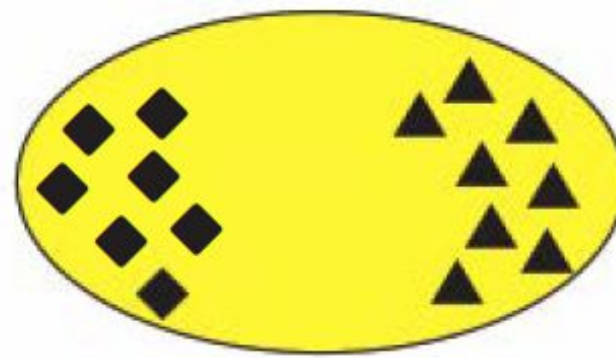
Inhibitor	Effect
Chloramphenicol	inhibits prokaryotic peptidyl transferase.
<u>Streptomycin</u> (Neomycin)	inhibits prokaryotic initiation, also induces mRNA misreading.
Tetracycline	inhibits prok. aminoacyl-tRNA binding to the ribosome small subunit.
Erythromycin	inhibits prokaryotic translocation through the ribosome large subunit.
<u>Fusidic acid</u>	similar to erythromycin only by preventing EF-G from dissociating from the large subunit.
<u>Puromycin</u>	resembles aa-tRNA, interferes with peptide transfer resulting in premature termination in prok. and euk.
Diphtheria toxin	catalyzes ADP-ribosylation of and inactivation of eEF-2.
Ricin	found in castor beans, catalyzes cleavage of the euk. 28S rRNA
Cycloheximide	inhibits eukaryotic peptidyltransferase.

mRNA transport and localization

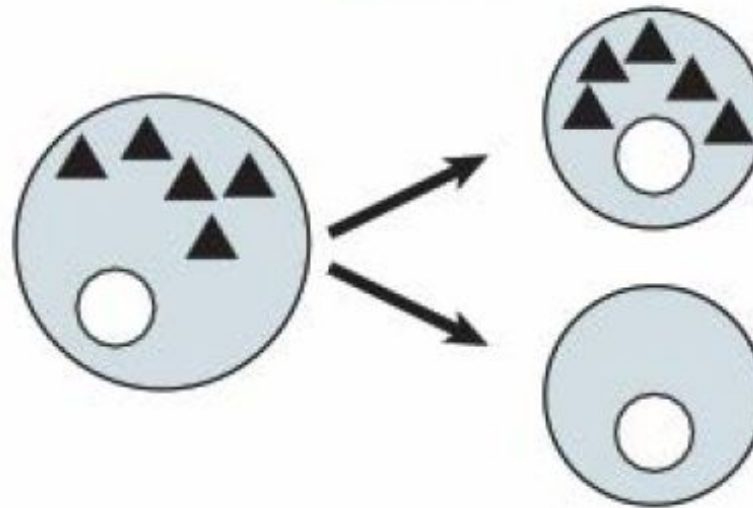
Eukaryotic RNA is translocated and can be localized

RNA can be transported between cell compartments			
RNA	Transport	Location	
All RNA	Nucleus→cytoplasm	All cells	
tRNA	Nucleus→mitochondrion	Many cells	
mRNA	Nurse cell→oocyte	Fly embryogenesis	
mRNA	Anterior→posterior oocyte	ditto	
mRNA	Cell→cell	Plant phloem	

Pattern formation and fate specification in oocytes and embryos



Generation of different daughter cells in asymmetric cell division



Compartmentalization of a cell into specialized regions

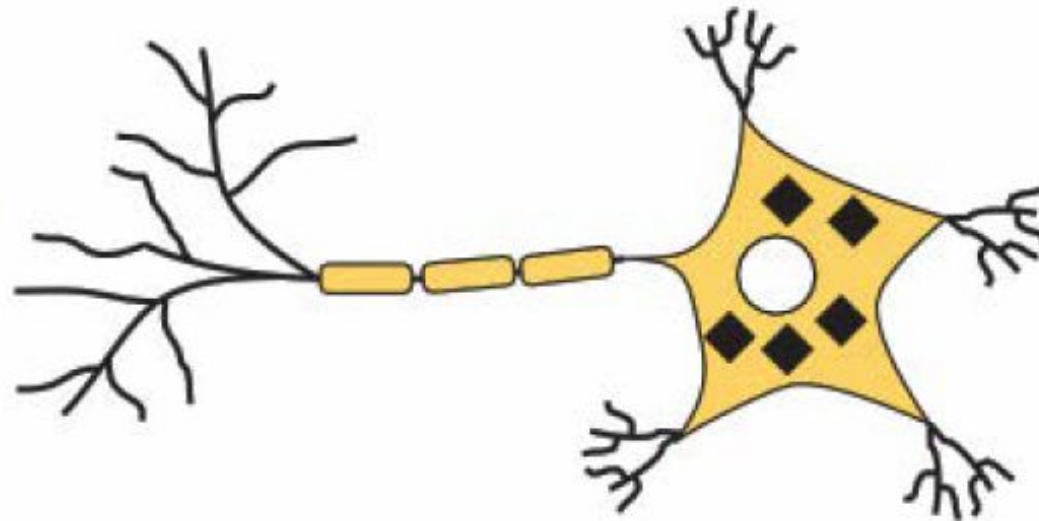
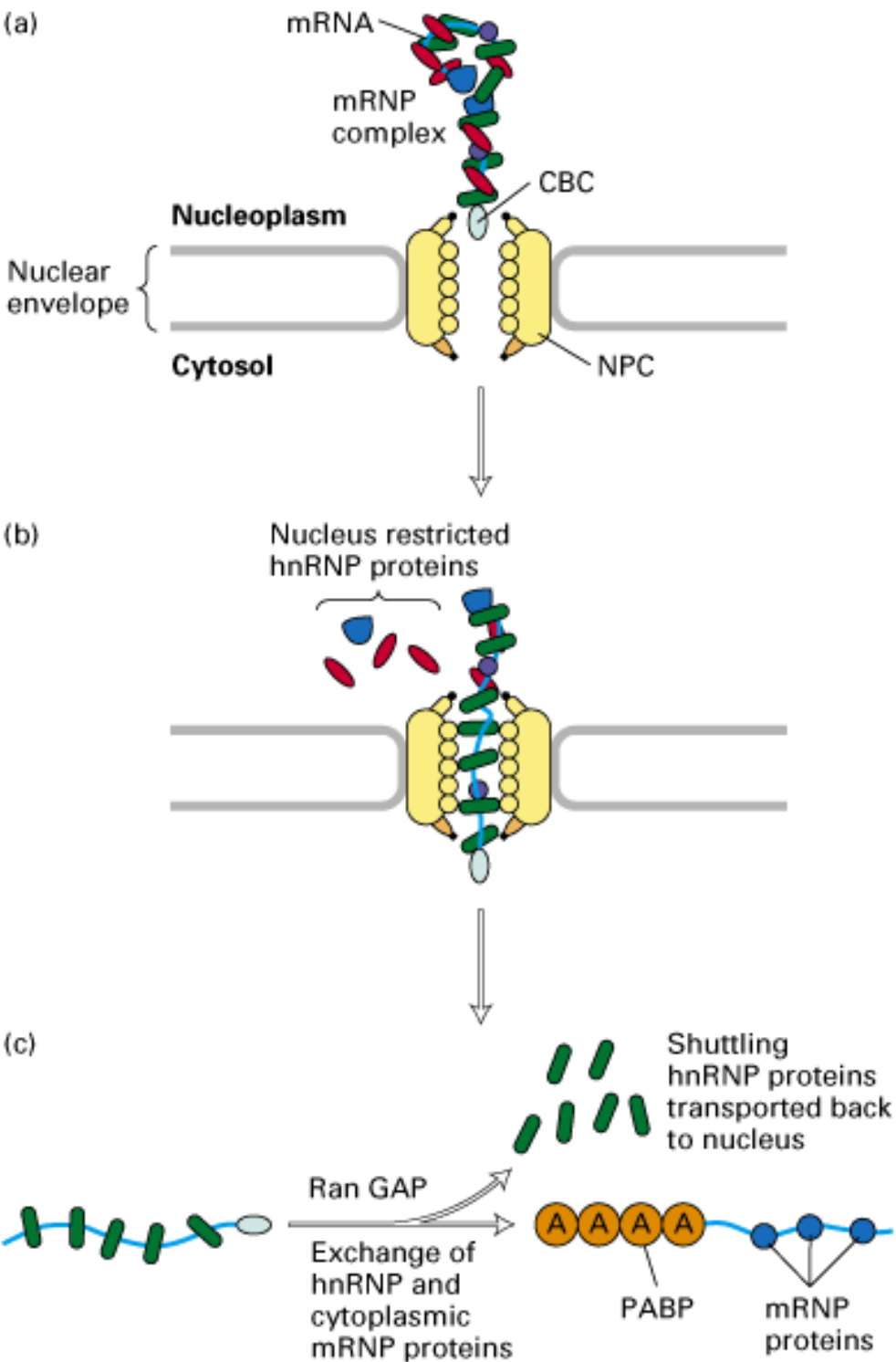
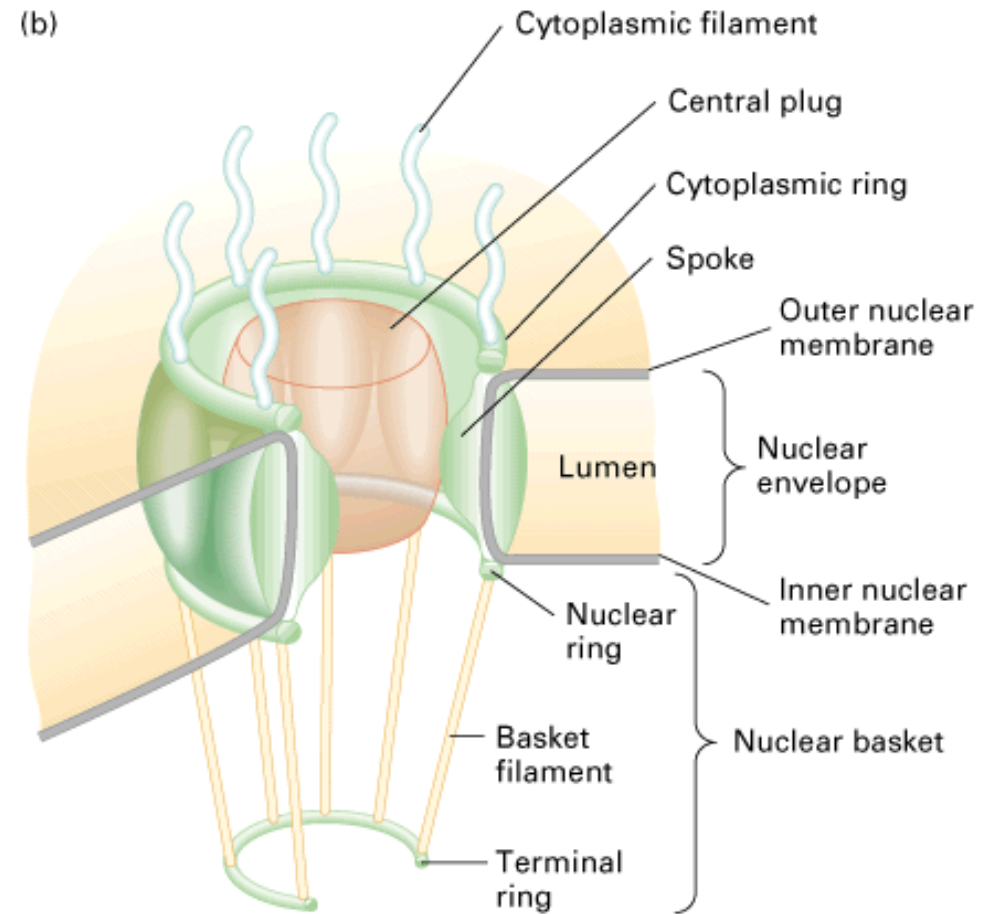


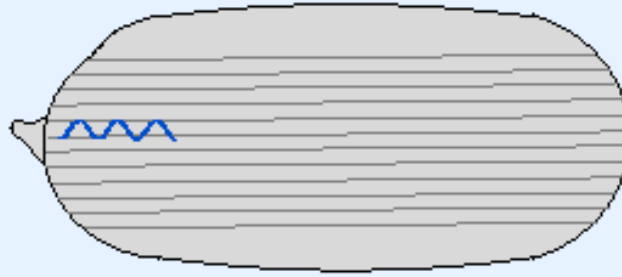
FIGURE 22.16 Three main functions of mRNA localization.



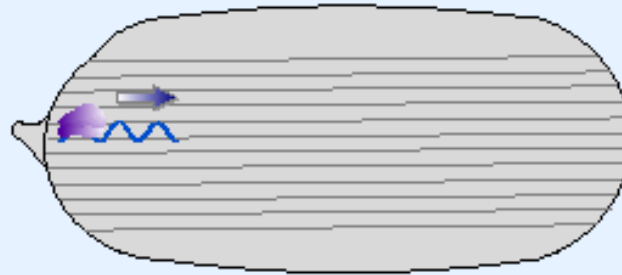
Nuclear Pore Complex



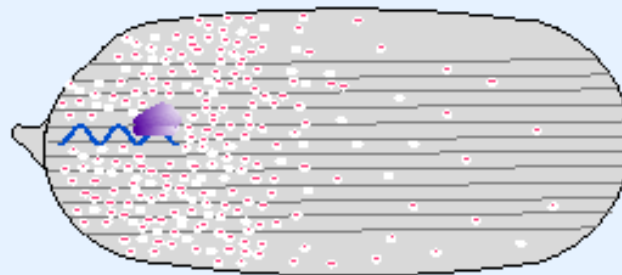
mRNA is localized at anterior end



mRNA is translated



Protein diffuses from site of synthesis

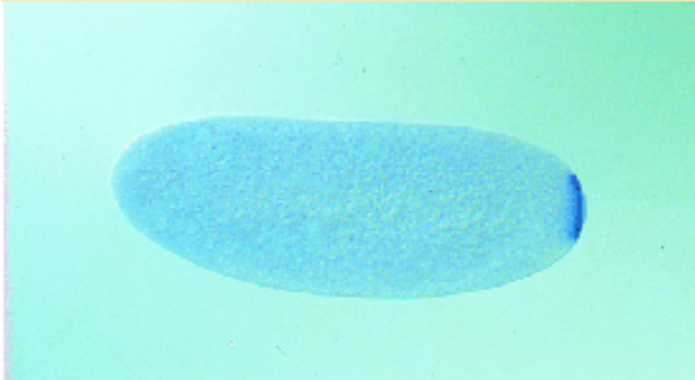


Translation of a localized mRNA generates a gradient of protein as the products diffuse away from the site of synthesis.

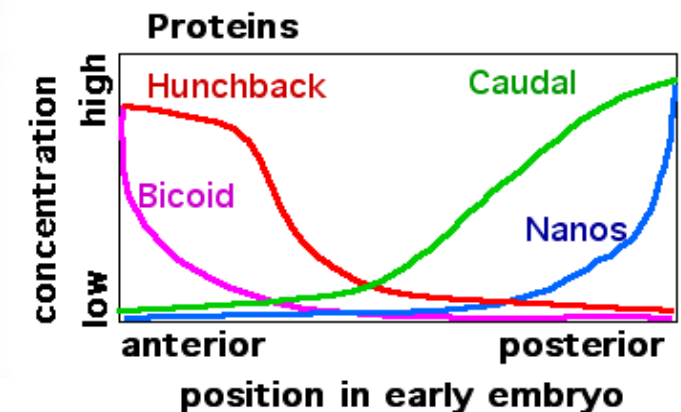
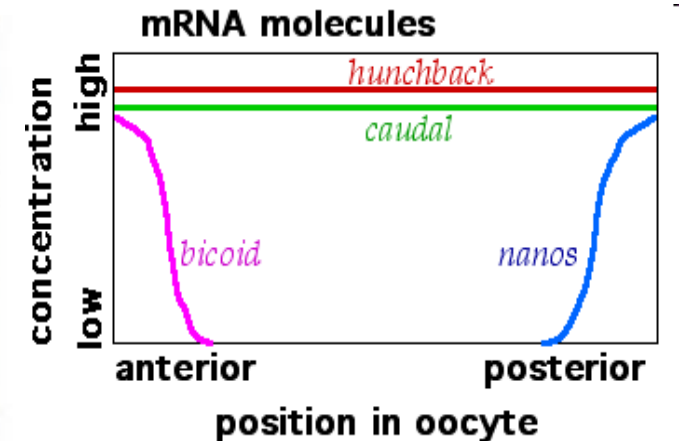
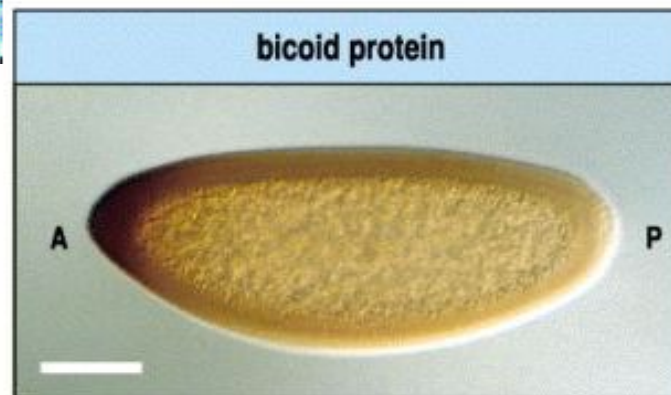
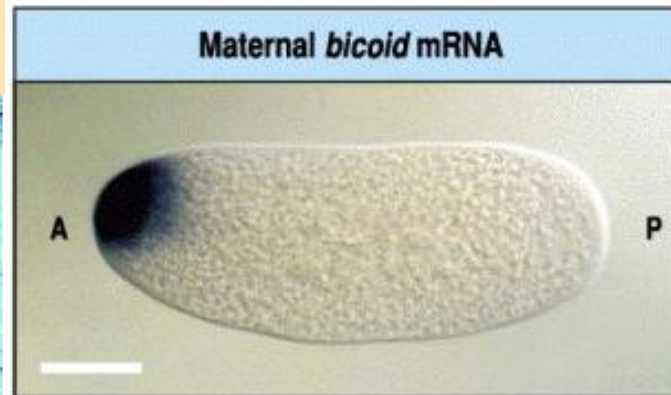
The developing egg (oocyte) is polarized by differentially localized mRNA molecules.

The genes that code for these mRNAs, called **maternal effect genes**, encode for proteins that get translated upon fertilization to establish concentration gradients that span the egg. *Bicoid* and *hunchback* are the maternal effect genes that are most important for patterning of anterior parts (head and thorax) of the *Drosophila* embryo. *Nanos* and *Caudal* are maternal effect genes that are important in the formation of more posterior abdominal segments of the *Drosophila* embryo.

nanos RNA is localized at the posterior end at the 3rd division

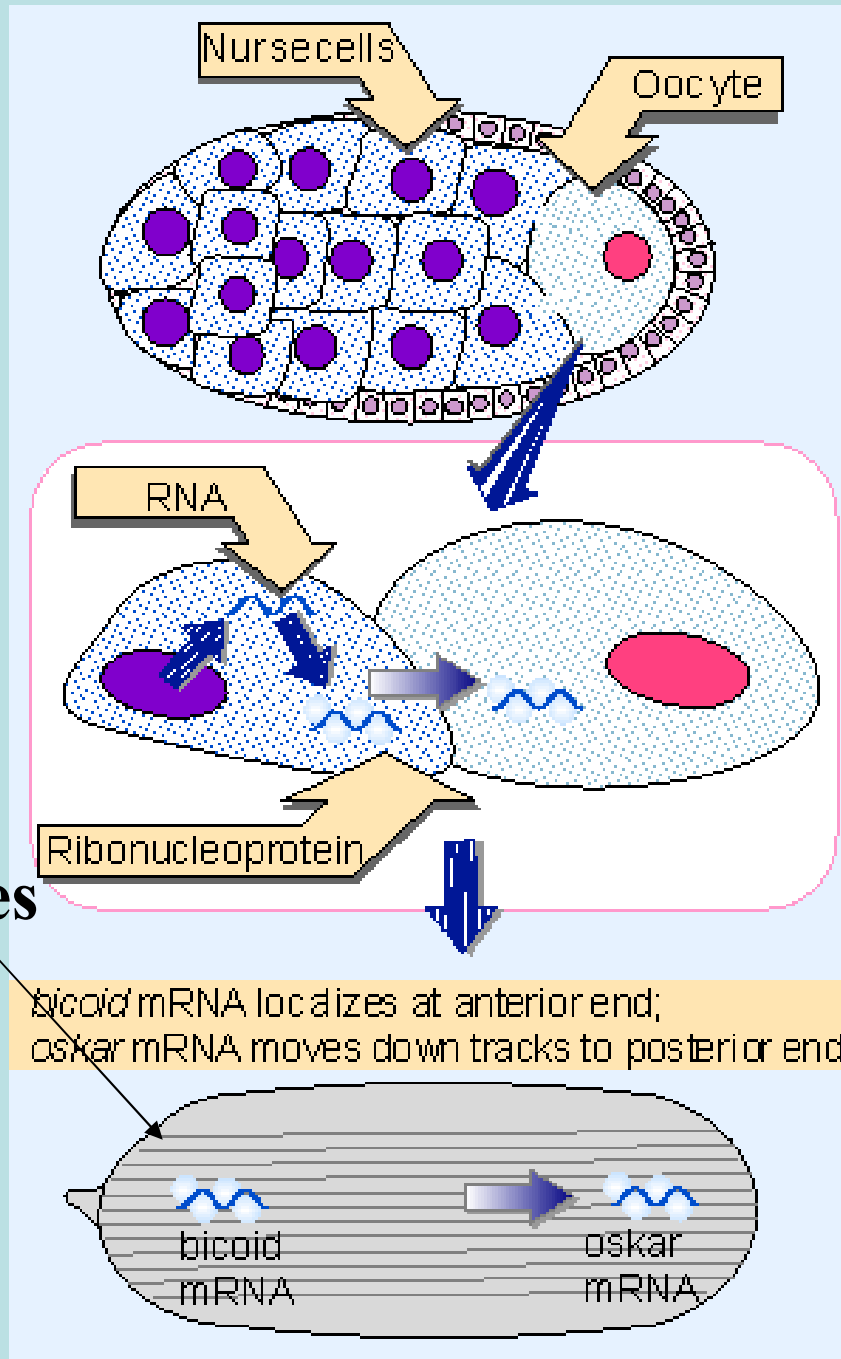


nanos protein spreads from the posterior end at the 8th division



Localization mechanisms

- *Specific transport*
- *Selective degradation*
- *Site specific anchoring*



Microtubules

bicoid mRNA localizes at anterior end;
oskar mRNA moves down tracks to posterior end

The anterior localization is determined by particular sequences at the 3' non translated end (UTR) of mRNA

Inside the oocyte there are different concentrations of the 2 proteins at the 2 antipodes of the cell → this can influence the embryo development

In yeast some mRNAs move from the mother cell to the bud.

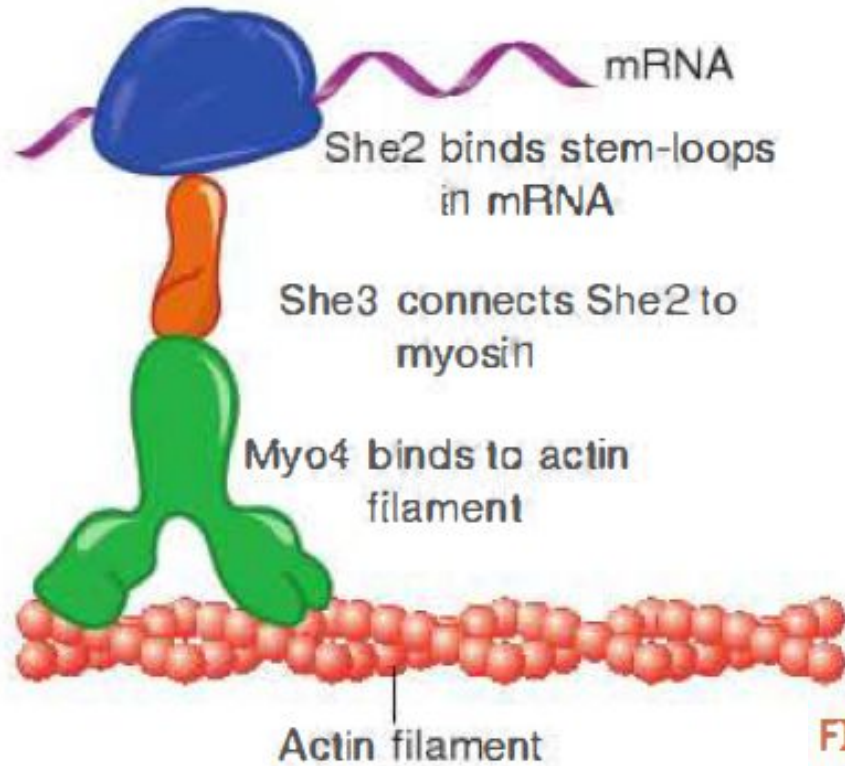
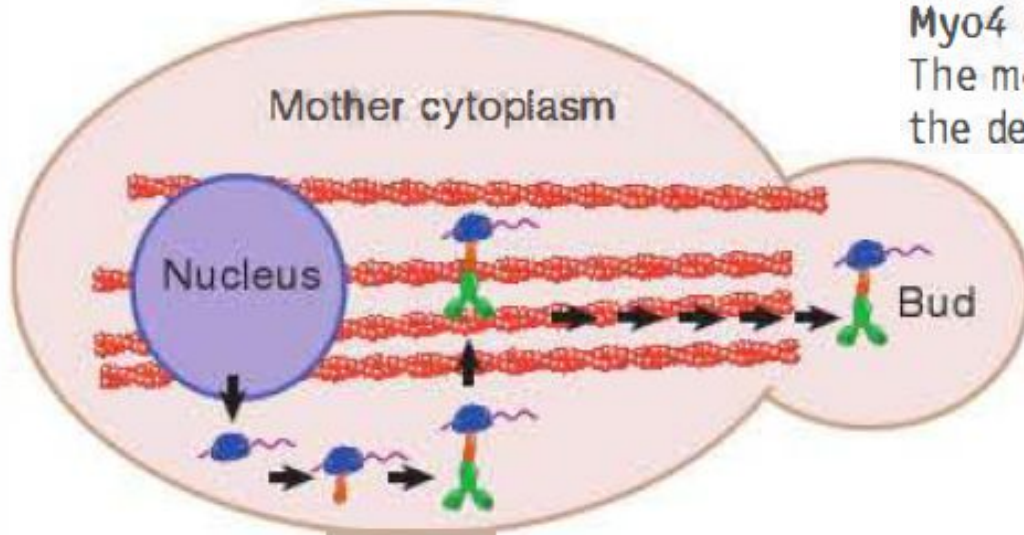
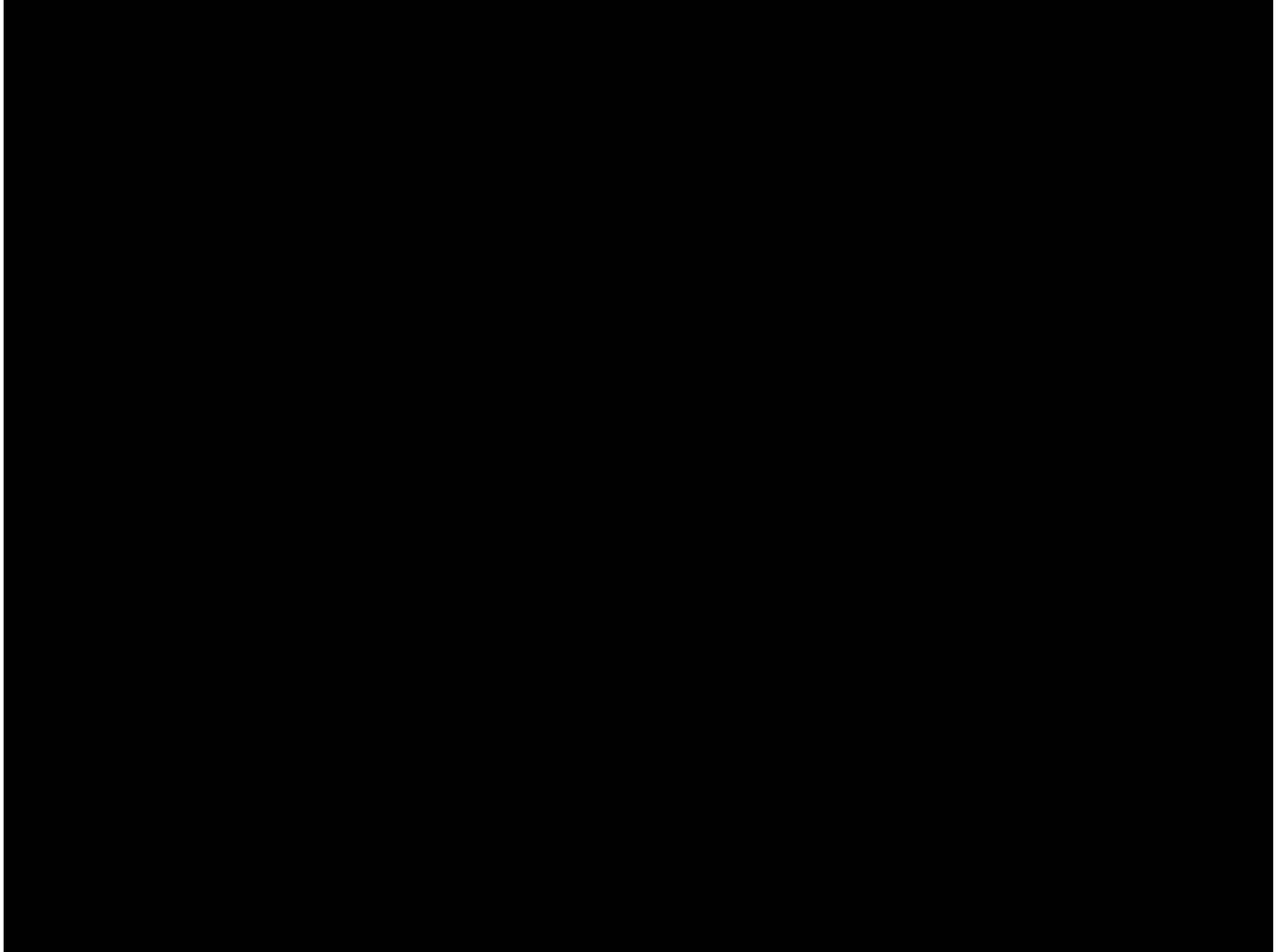


FIGURE 22.17 Localization of *ASH1* mRNA. Newly exported *ASH1* mRNA is attached to the myosin motor Myo4 via a complex with the She2 and She3 proteins. The motor transports the mRNA along actin filaments to the developing bud.

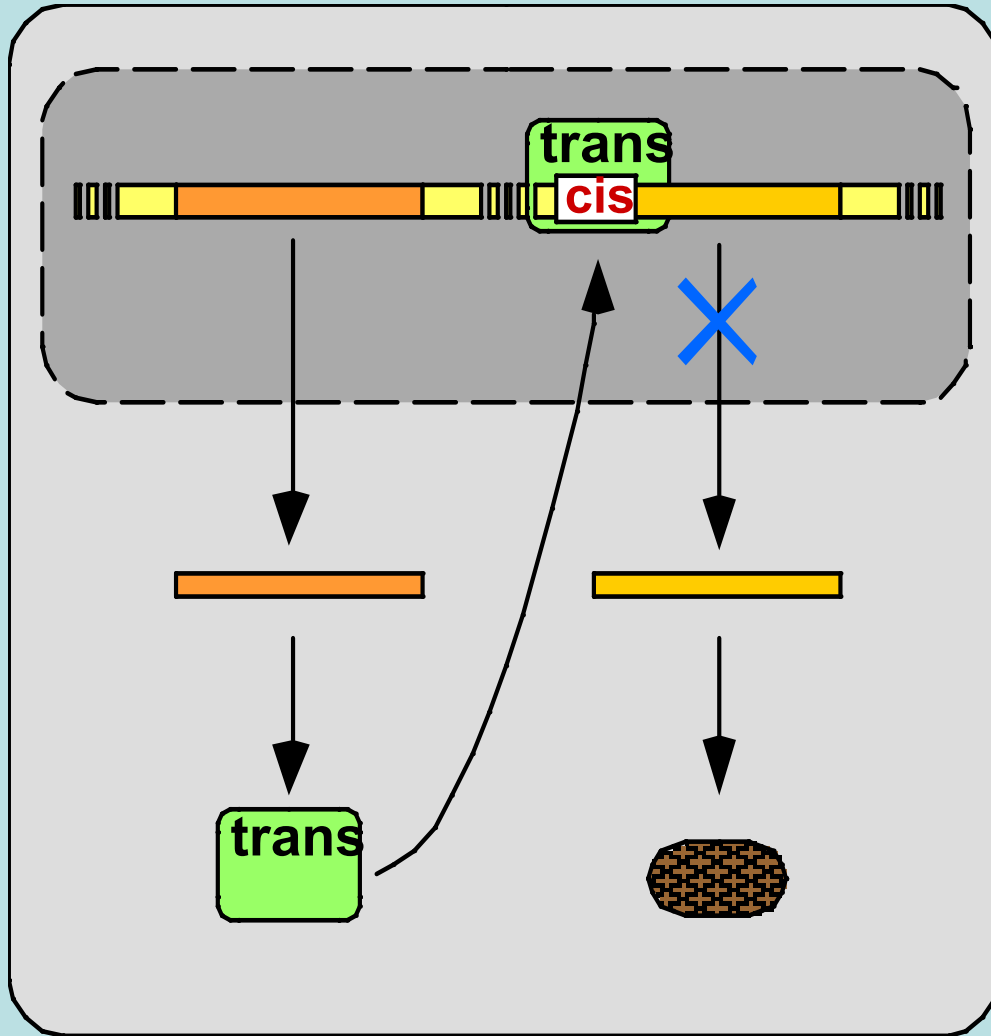




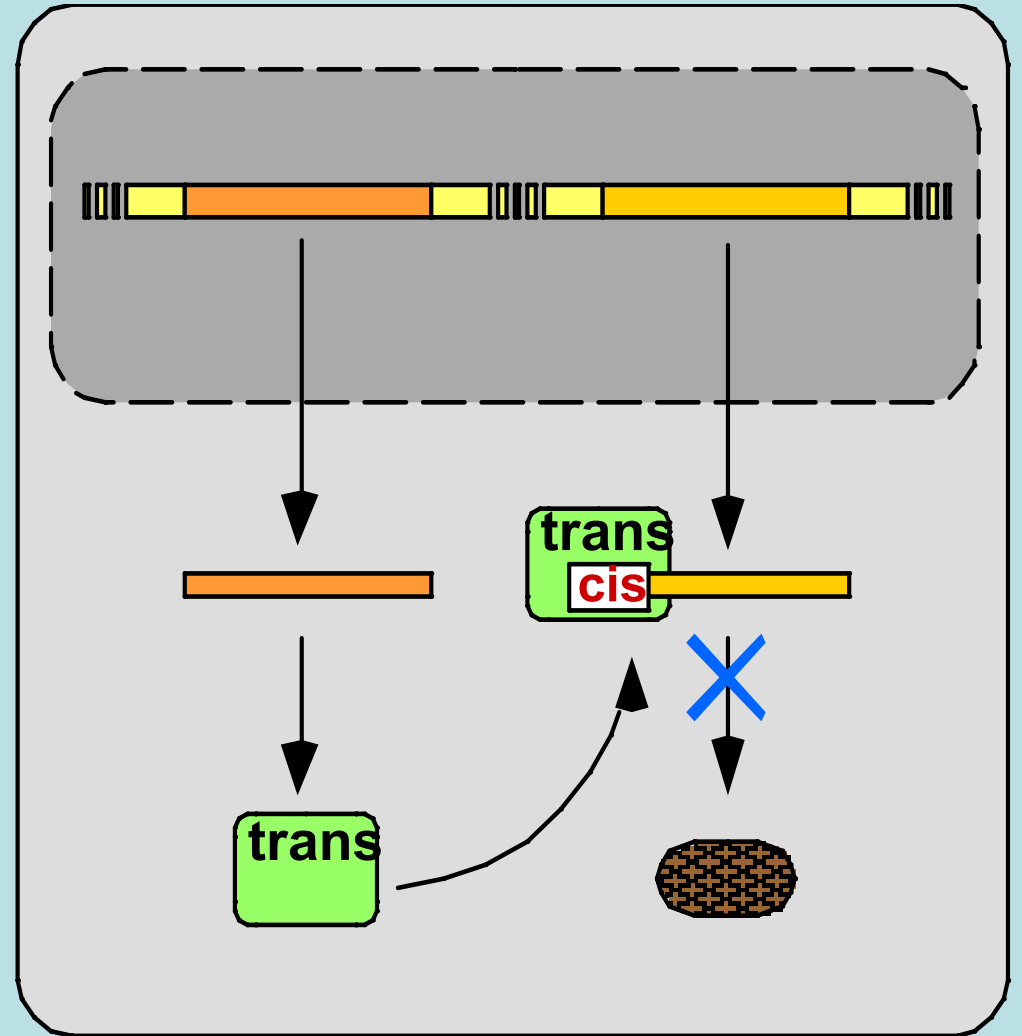
Regulation of translation

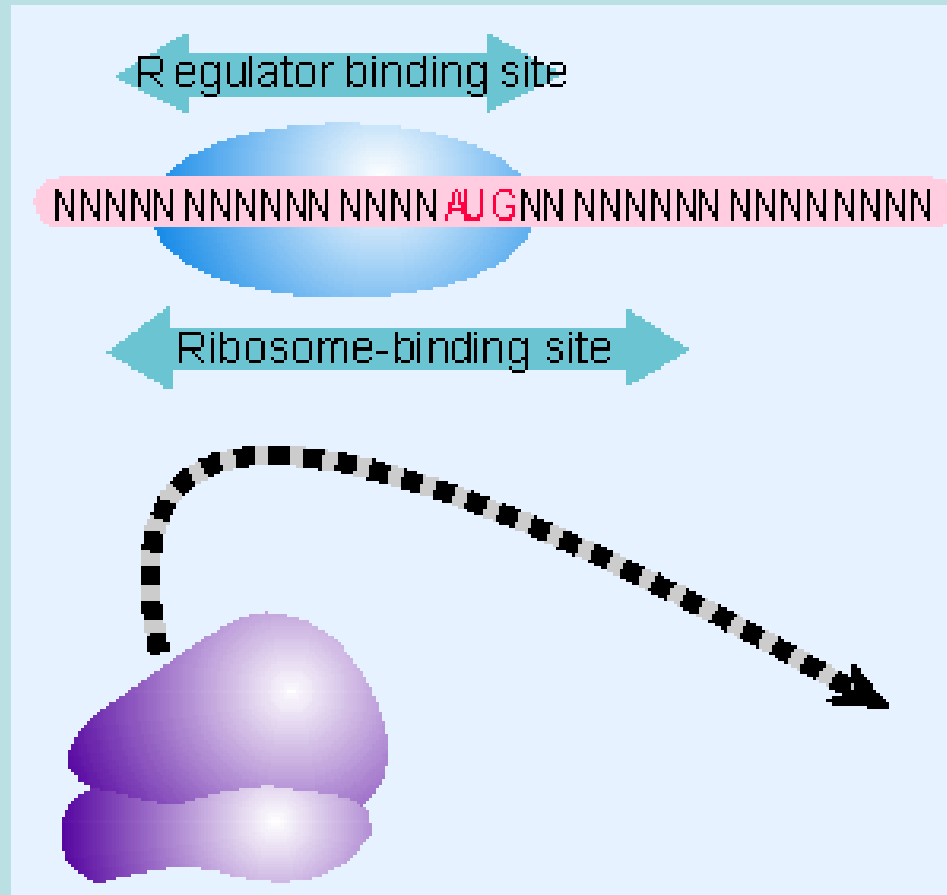
- **General**
- **Specific**

Transcriptional regulation



Translational regulation





A regulator protein may block translation by binding to a site on mRNA that overlaps the ribosome-binding site at the initiation codon.

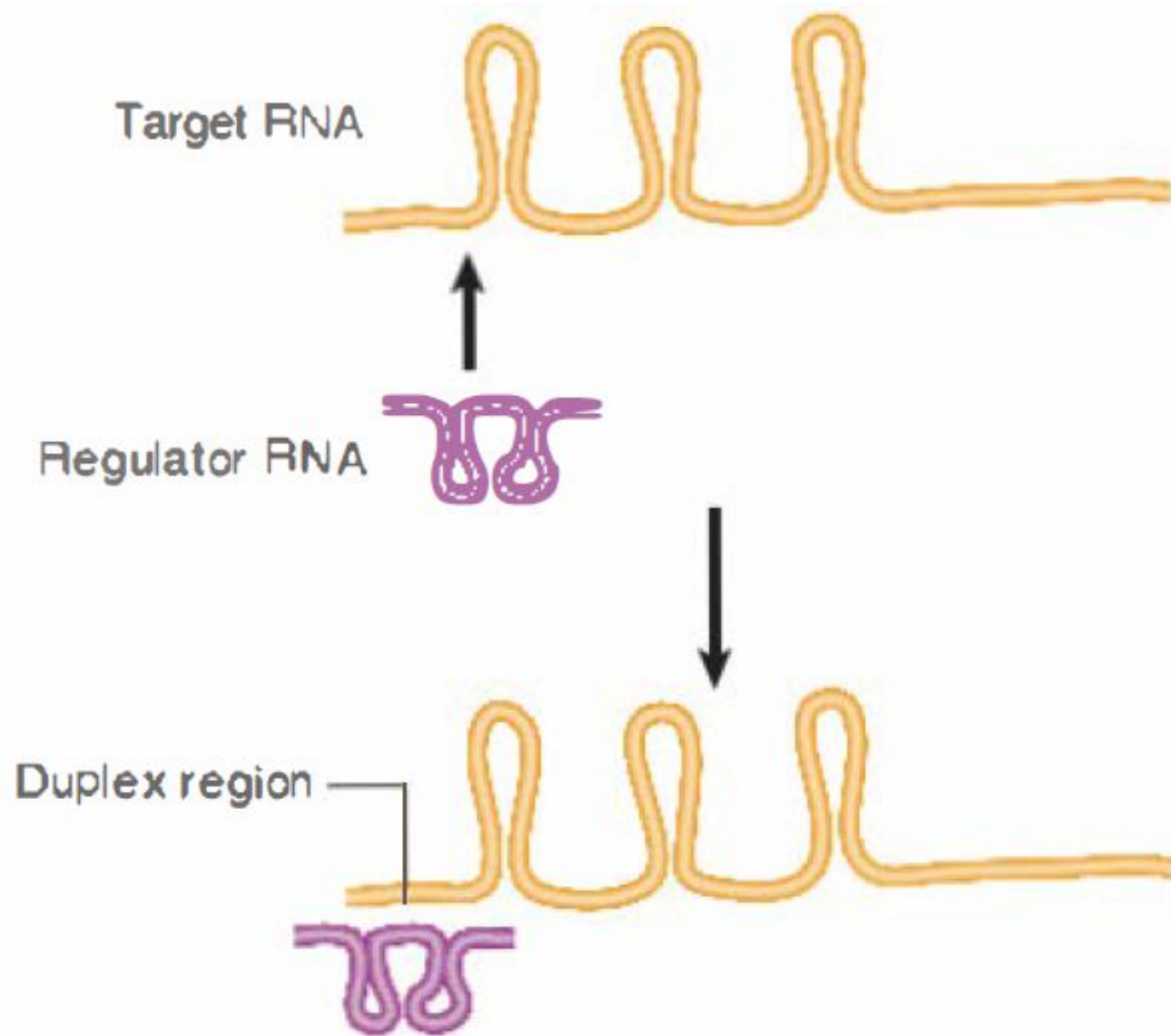


FIGURE 30.1 A regulator RNA is a small RNA with a single-stranded region that can pair with a single-stranded region in a target RNA.

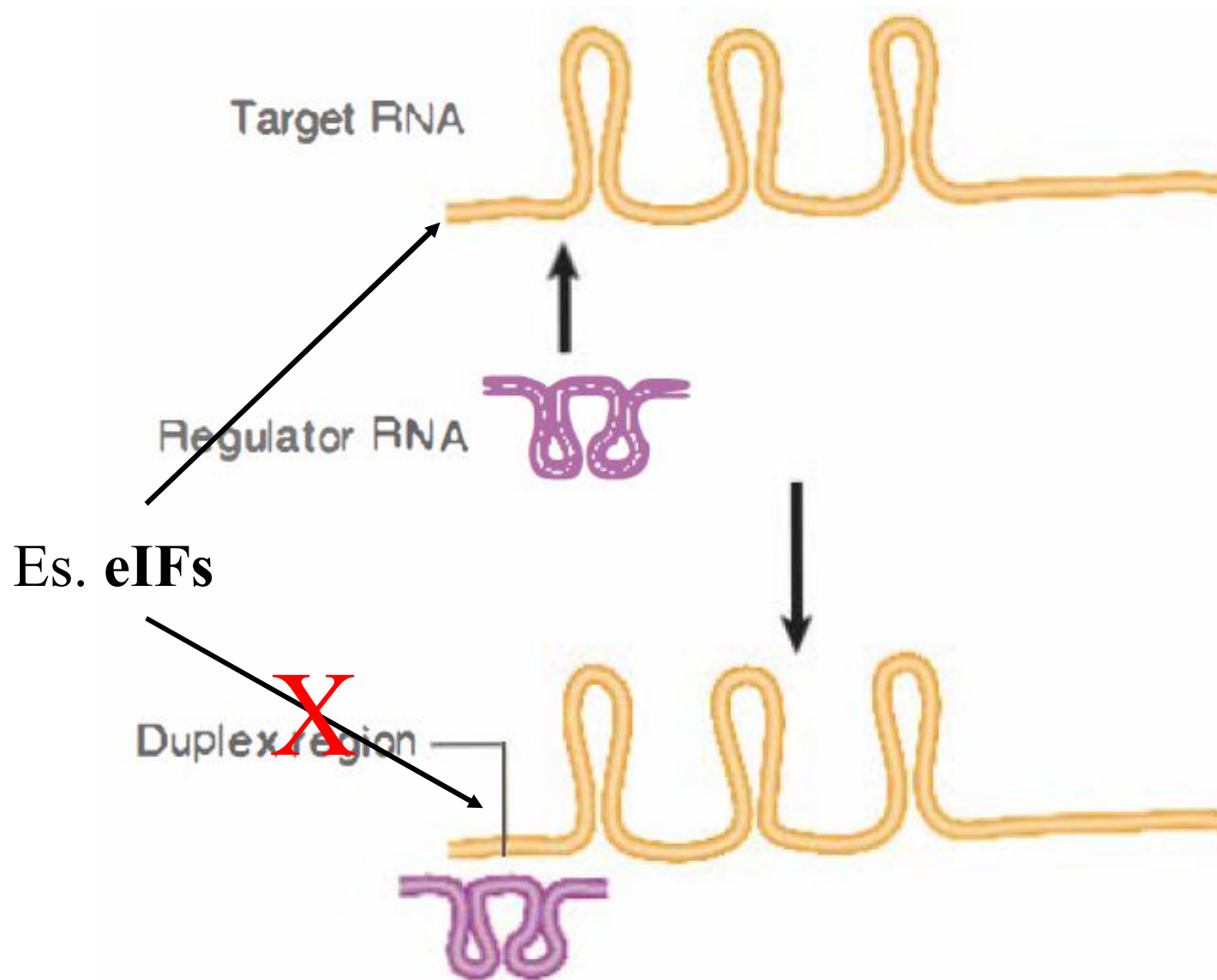


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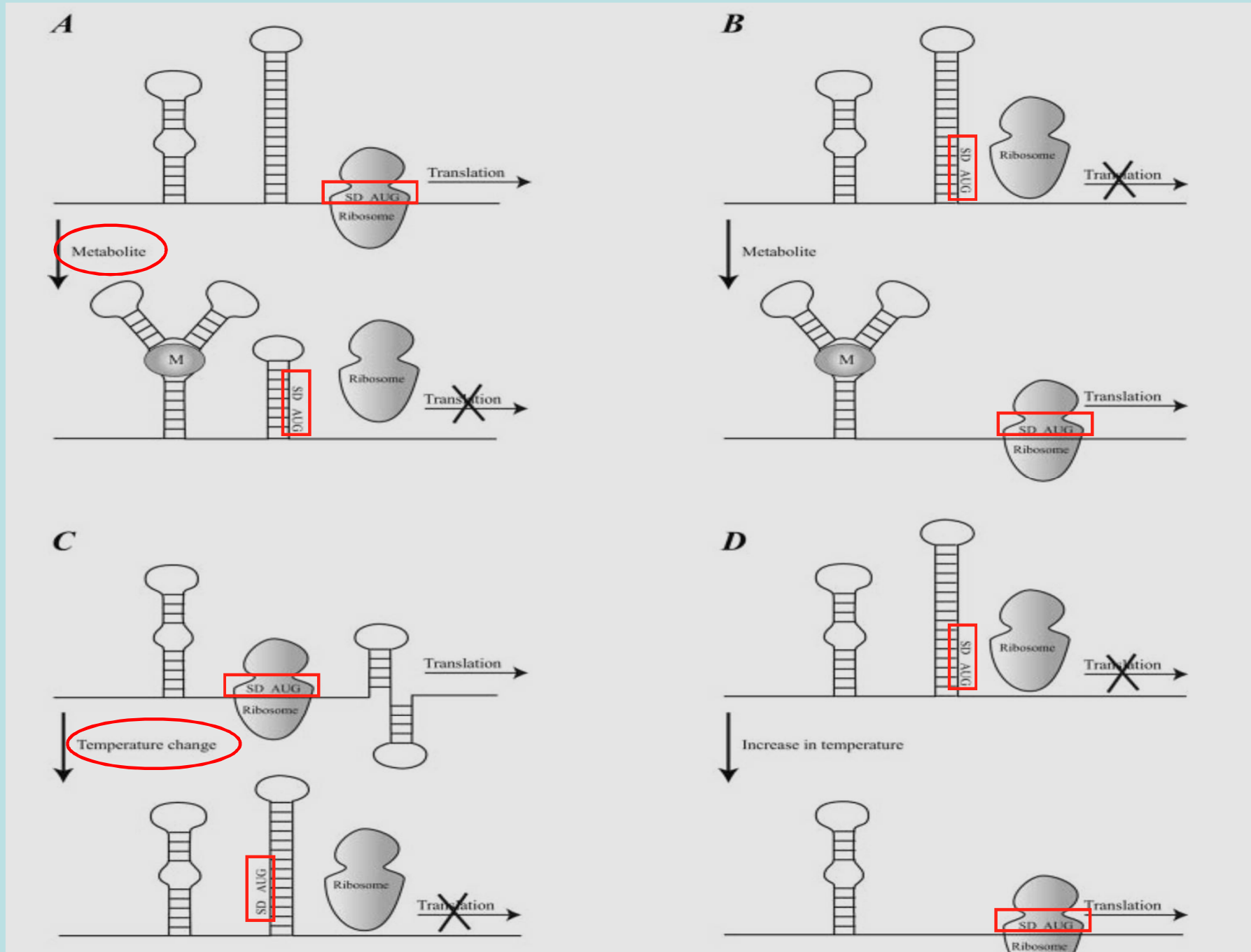


FIG. 13. Examples of translational regulation mechanisms. (A) Repression of translation by binding of a metabolite that stabilizes an alternative mRNA secondary structure and leaves the SD sequence and initiation codon (AUG) in a base-paired region. (B) Activation of translation by binding of a metabolite that stabilizes an alternative mRNA secondary structure and leaves the SD sequence and initiation codon (AUG) in an unpaired region, thus providing ribosomal access. (C) Repression of translation by the formation of an alternative mRNA secondary structure as a result of a change in temperature. (D) Activation of translation by an increase in temperature, causing a local melting of the mRNA secondary structure covering the SD and AUG region.

Repressor	Target Gene	Site of Action
R17 coat protein	R17 replicase	Hairpin that includes ribosome-binding site
T4 RegA	Early T4 mRNAs	Various sequences including initiation codon
T4 DNA polymerase	T4 DNA polymerase	Shine-Dalgarno sequence
T4 p32	Gene 32	Single-stranded 5' leader

FIGURE 24.51 Proteins that bind to sequences within the initiation regions of mRNAs may function as translational repressors.