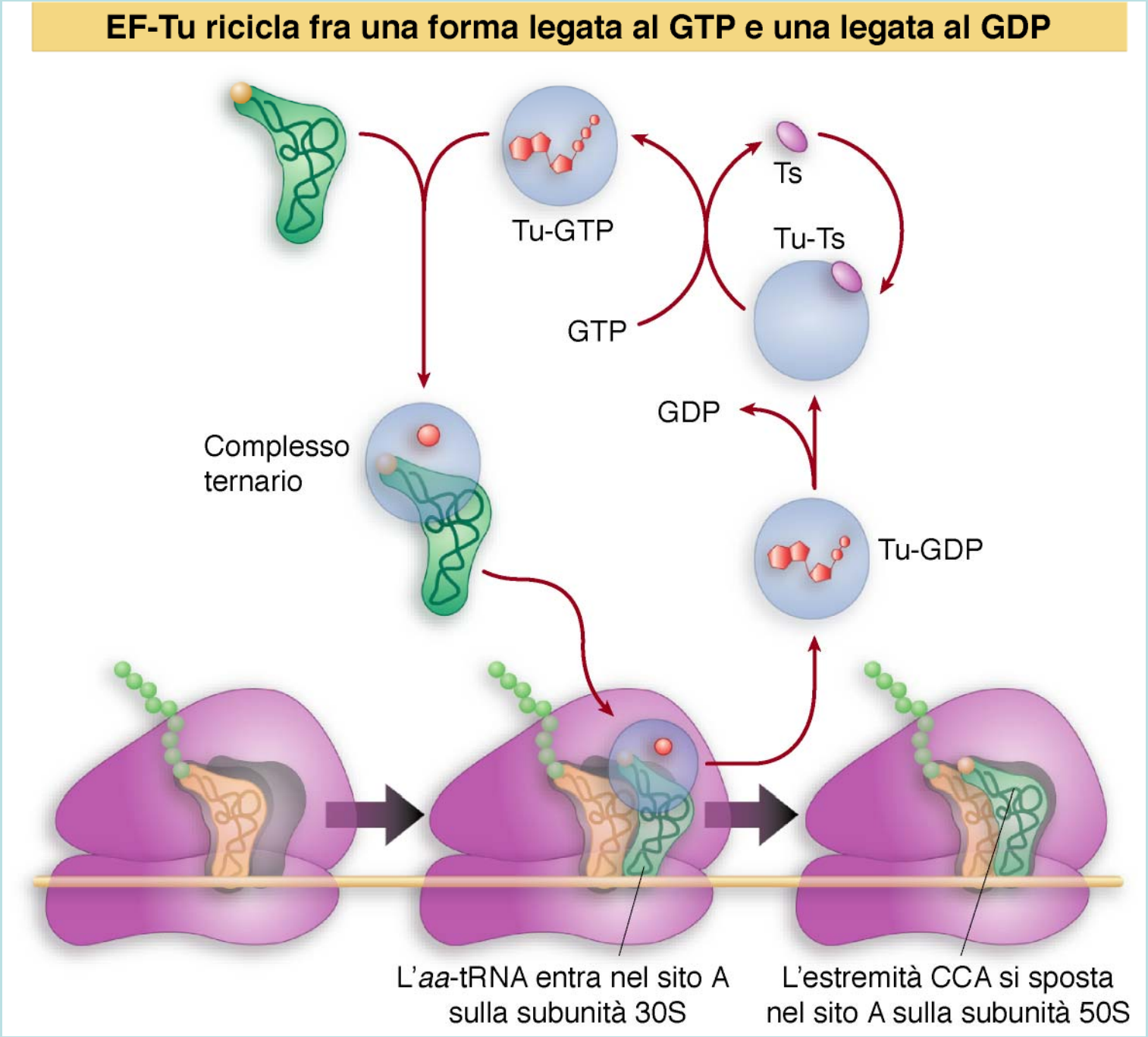
Traduzione: l'allungamento

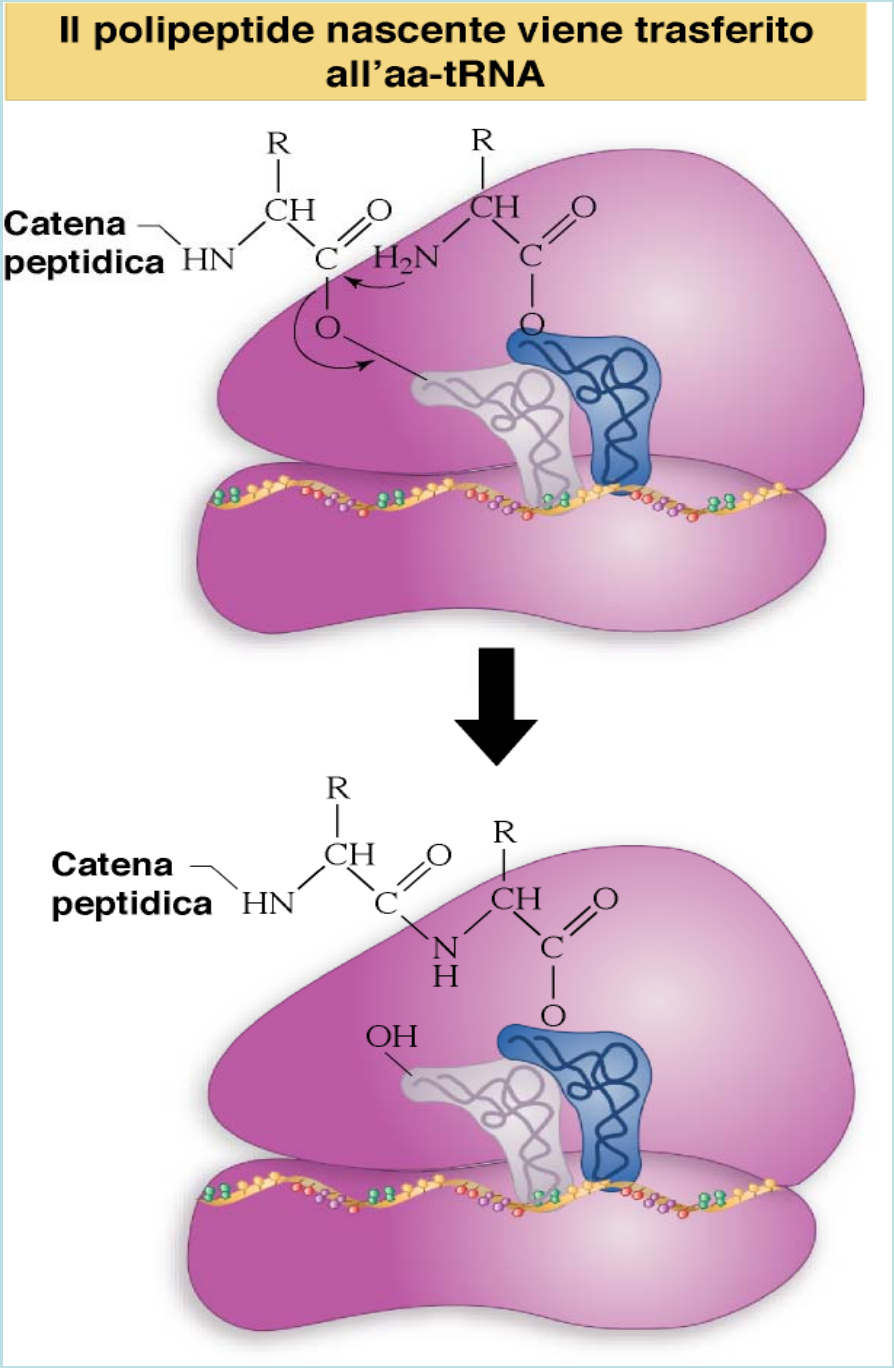
Fattori di allungamento

Procarioti	*Eucarioti*	
EF-Tu	**eEF1α**	**trasporto aa-tRNA**
EF-Ts	**eEF1βγ**	**riciclo**
EF-G	**eEF2**	**traslocazione**

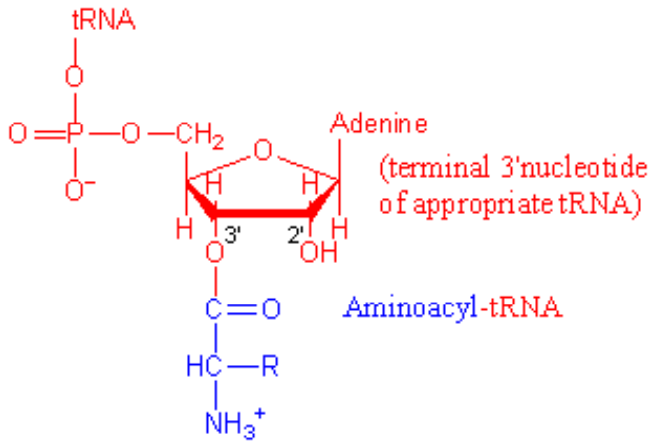
EF-Tu ricicla fra una forma legata al GTP e una legata al GDP

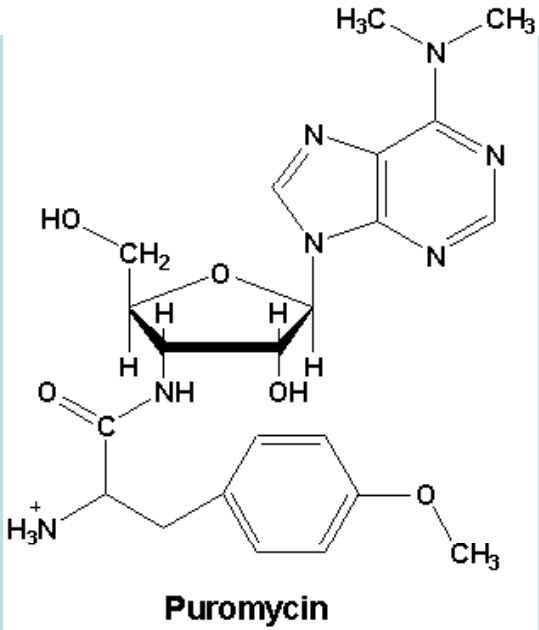


Il polipeptide nascente viene trasferito all'aa-tRNA



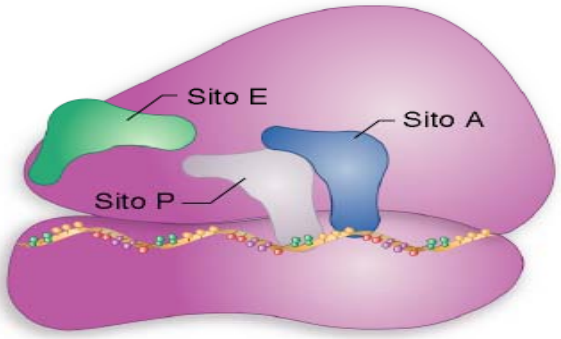
La puromicina è simile a un amminoacil-tRNA



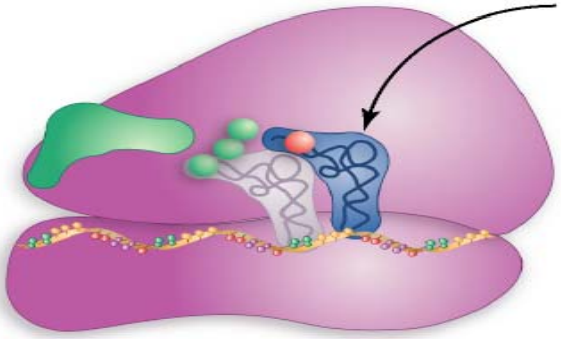


Puromycin is an aminonucleoside antibiotic, derived from the *Streptomyces alboniger* bacterium, that causes premature chain termination during translation taking place in the ribosome. It is <u>not selective</u> for either prokaryotes or eukaryotes.

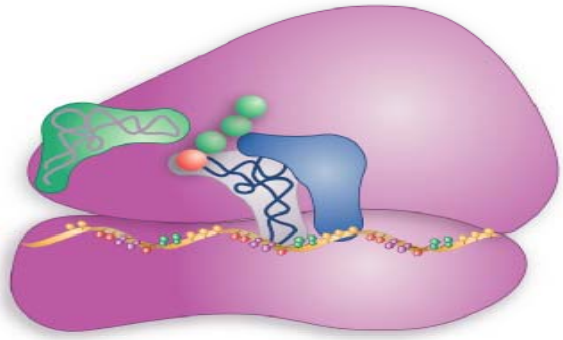
Il tRNA si sposta in 3 siti del ribosoma

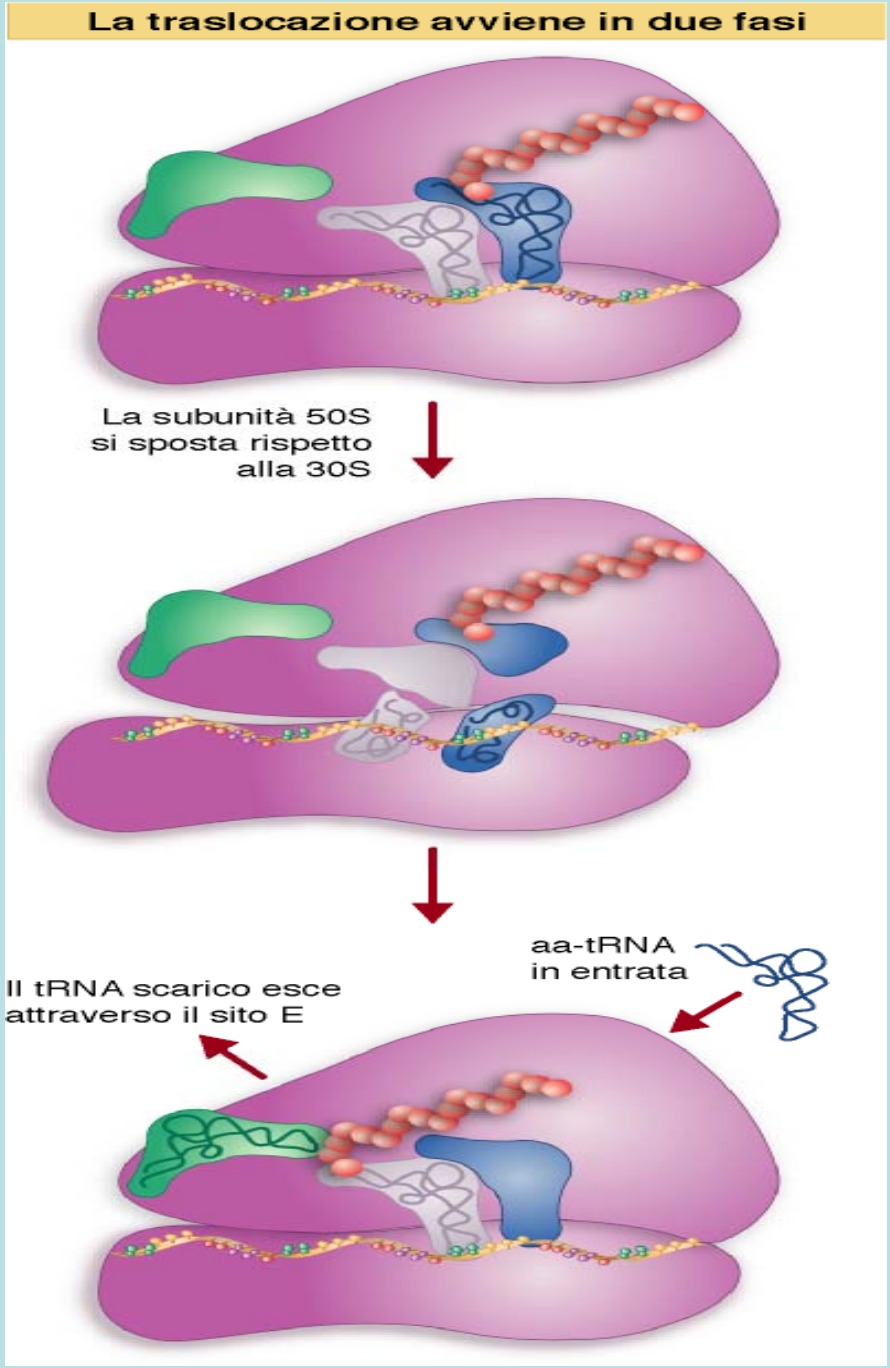


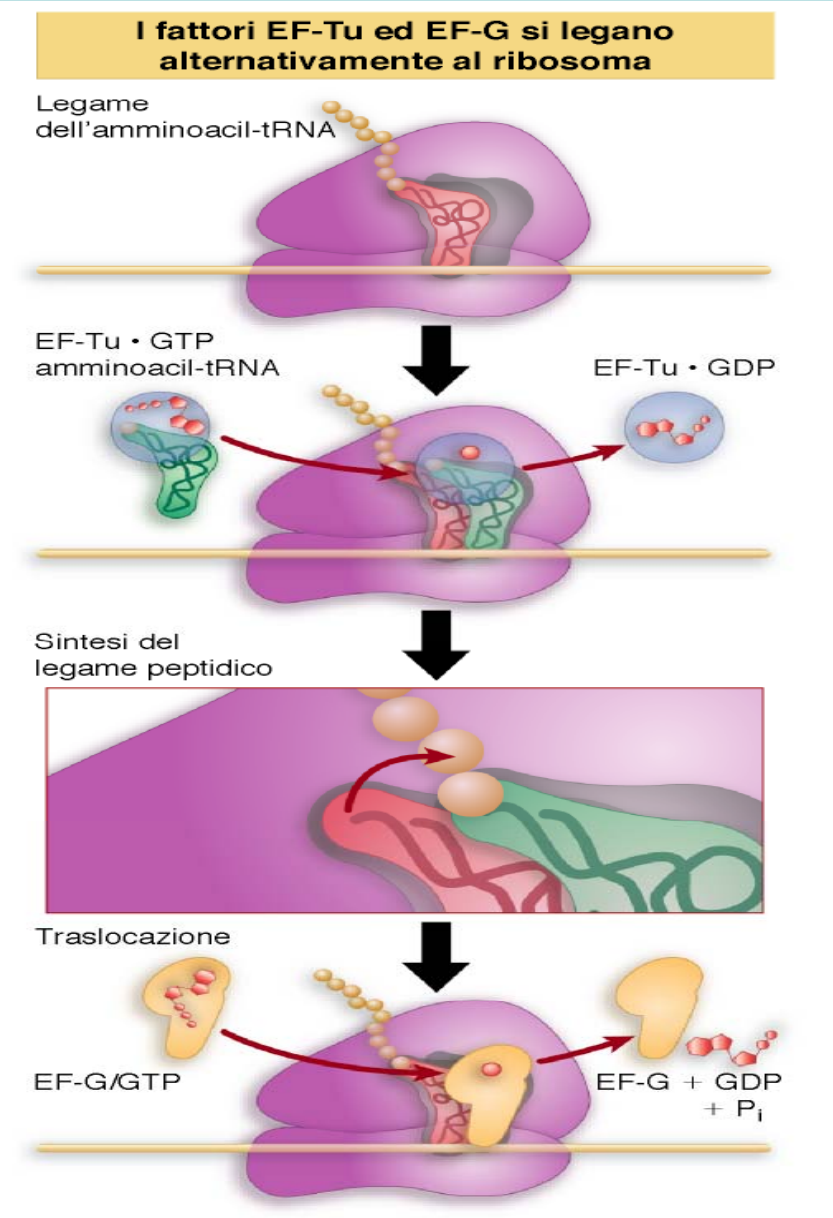
Pre-traslocazione:
il peptidil-tRNA è nel sito P;
l'amminoacil-tRNA entra nel sito A



Post-traslocazione:
il tRNA deacilato si sposta nel sito E;
il peptidil-tRNA si sposta nel sito P

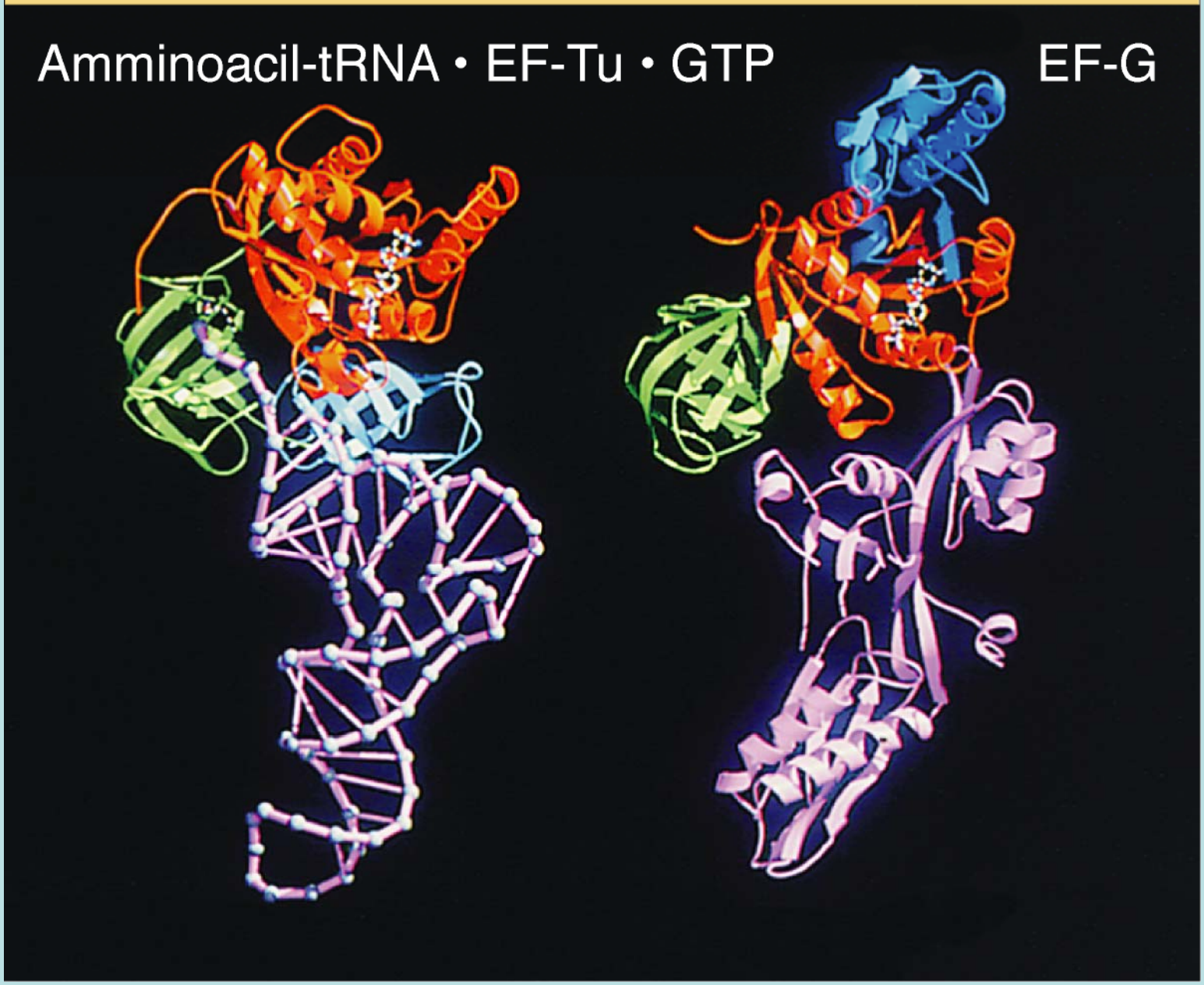



La traslocazione avviene in due fasi
La subunità 50S
si sposta rispetto
alla 30S
aa-tRNA
in entrata
Il tRNA scarico esce
attraverso il sito E



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

La struttura di EF-G imita l'amminoacil-tRNA



Decoding by the 70S ribosome

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Ramakrishnan Laboratory
MRC-Laboratory of Molecular Biology

Traduzione: terminazione

Fattori di terminazione

Procarioti	*Eucarioti*	
RF1	eRF	riconoscimento UAA, UAG (ocra, ambra)
RF2	"	riconoscimento UGA, UAA (opale)
RF3	eRF3	GTPase
RRF		rilascio

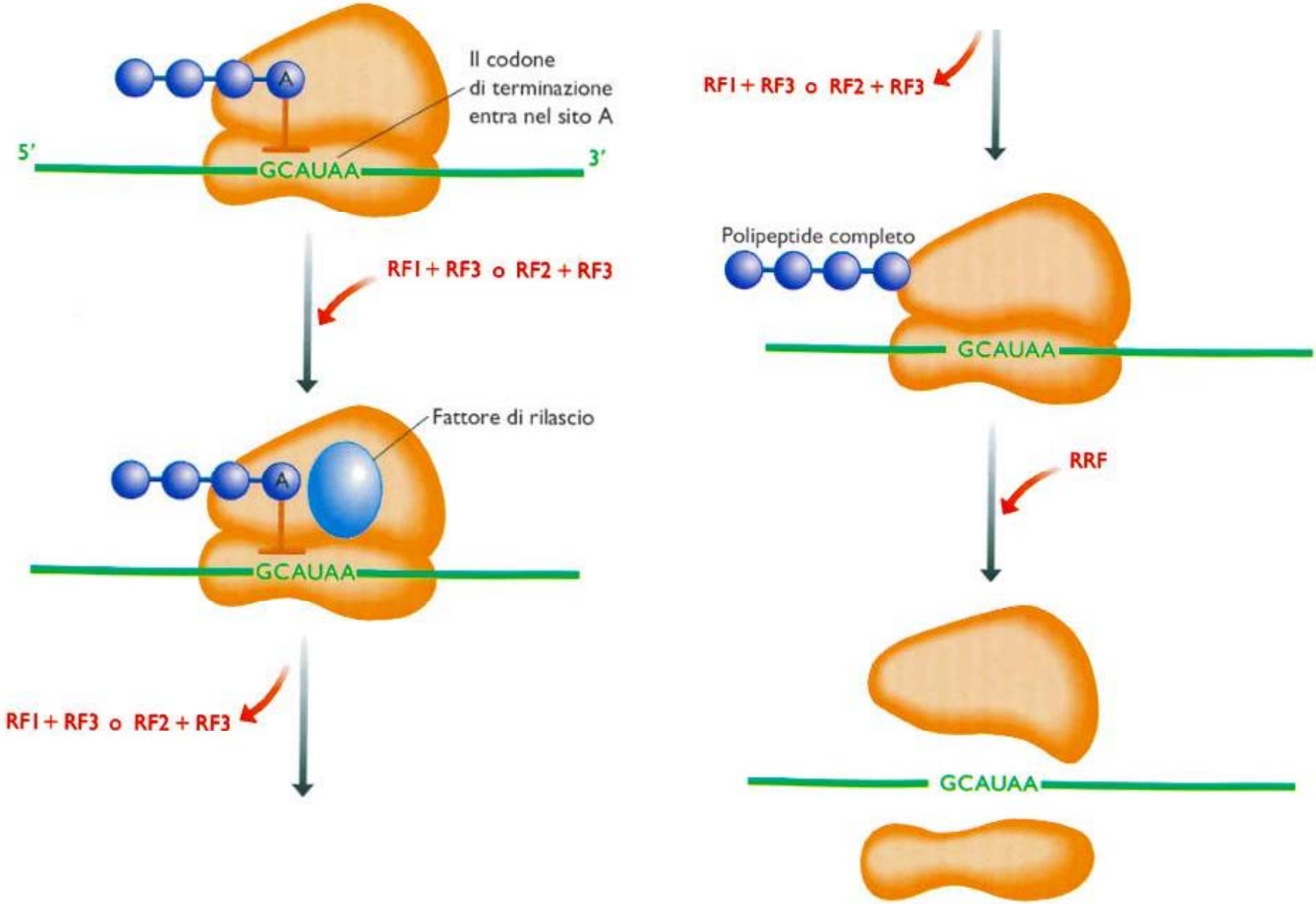
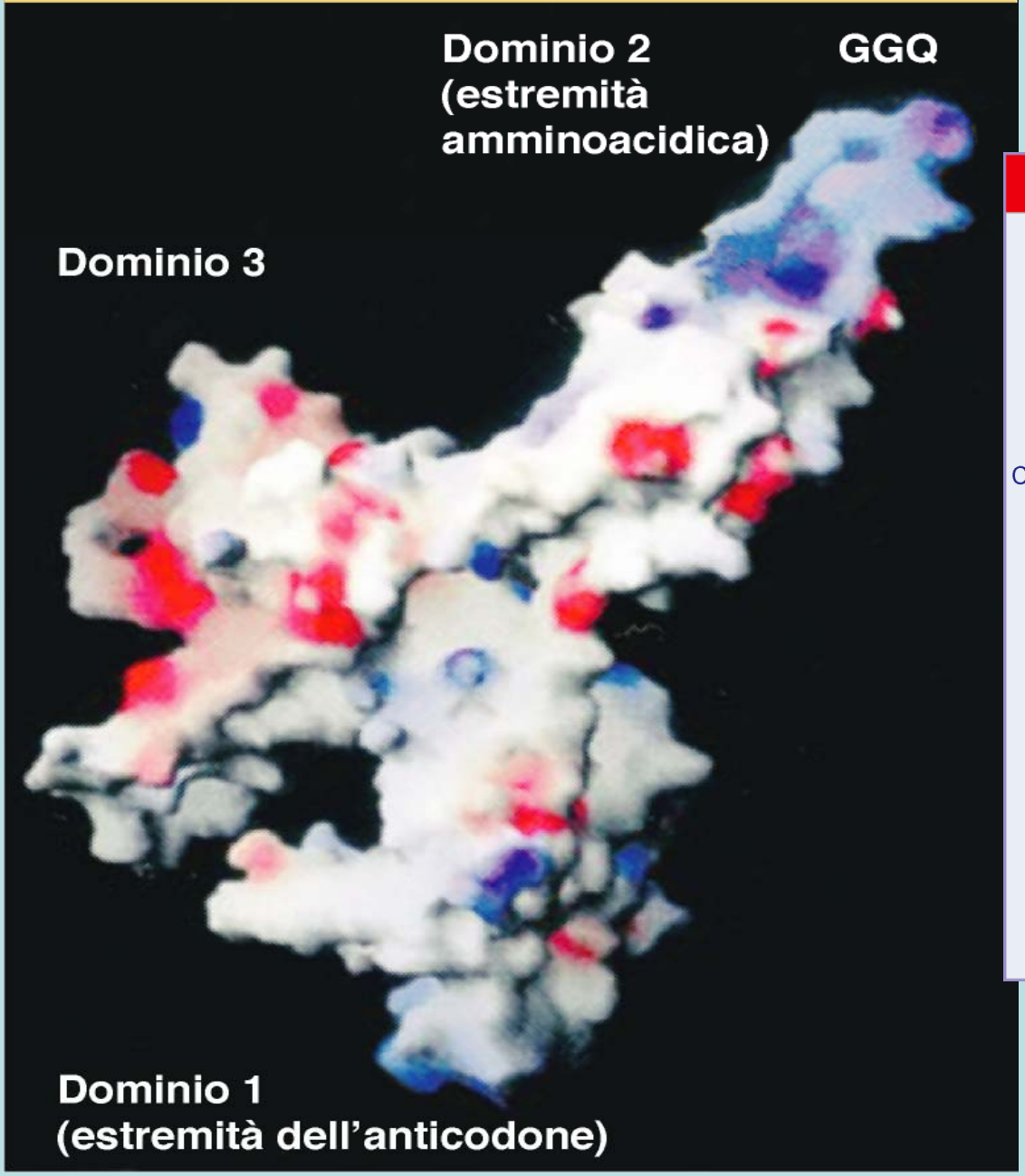
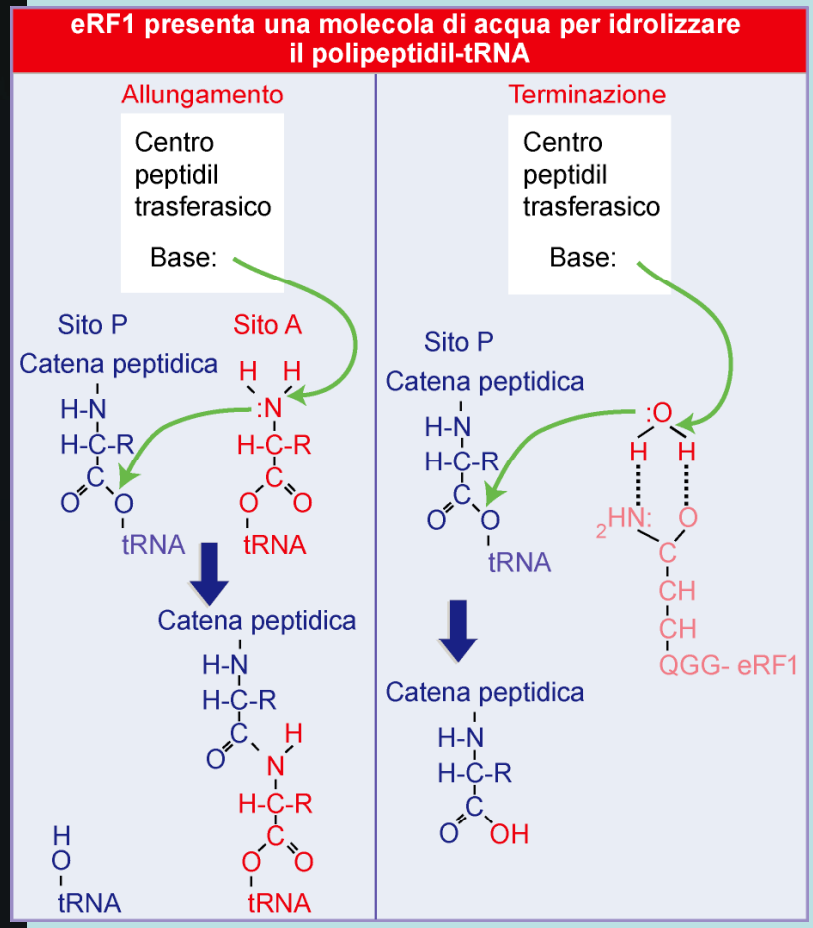


Figura 11.21 La terminazione della traduzione.

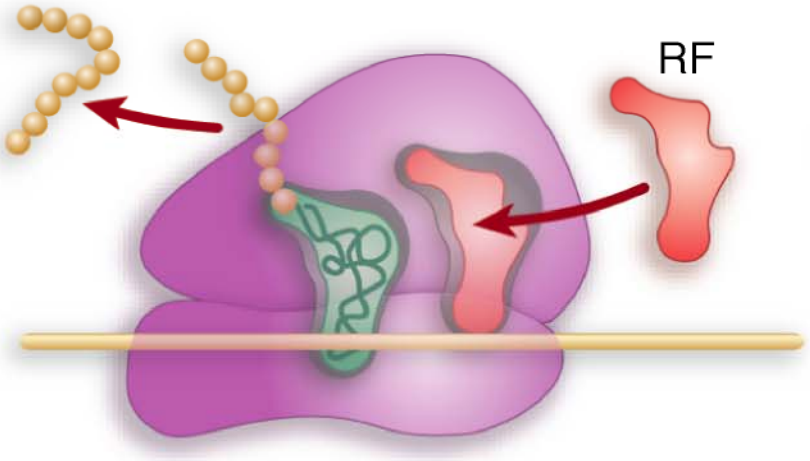
eRF1 imita il tRNA



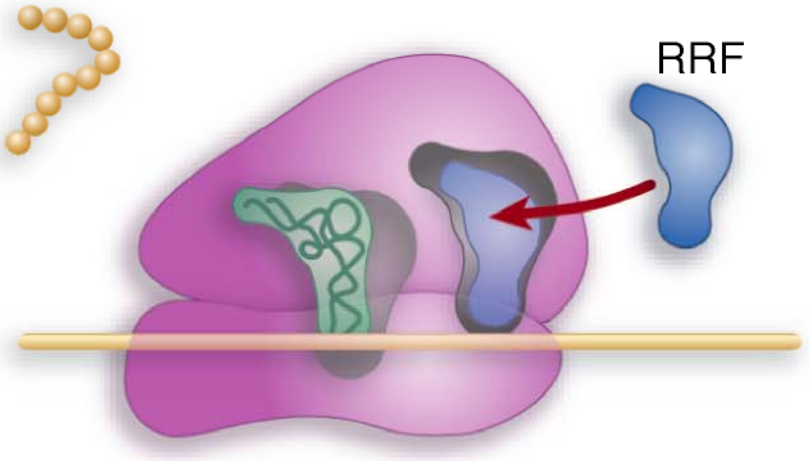


La terminazione richiede molteplici fattori proteici

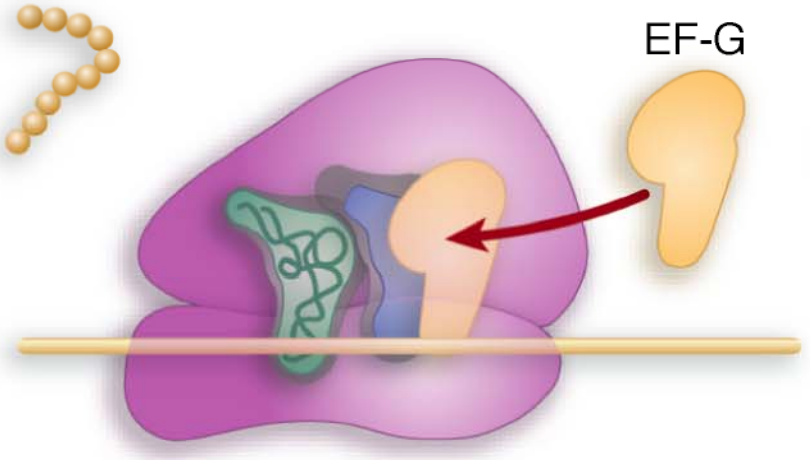
1. RF rilascia la catena proteica



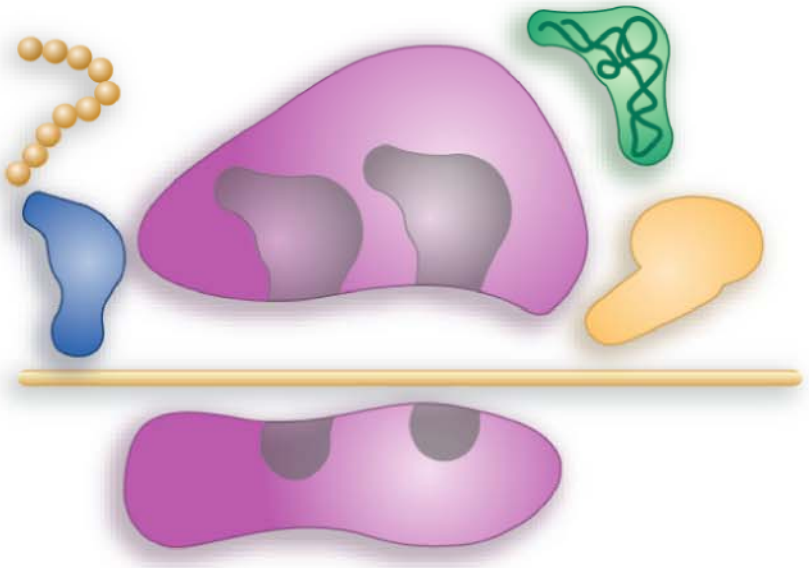
2. RRF entra nel sito A



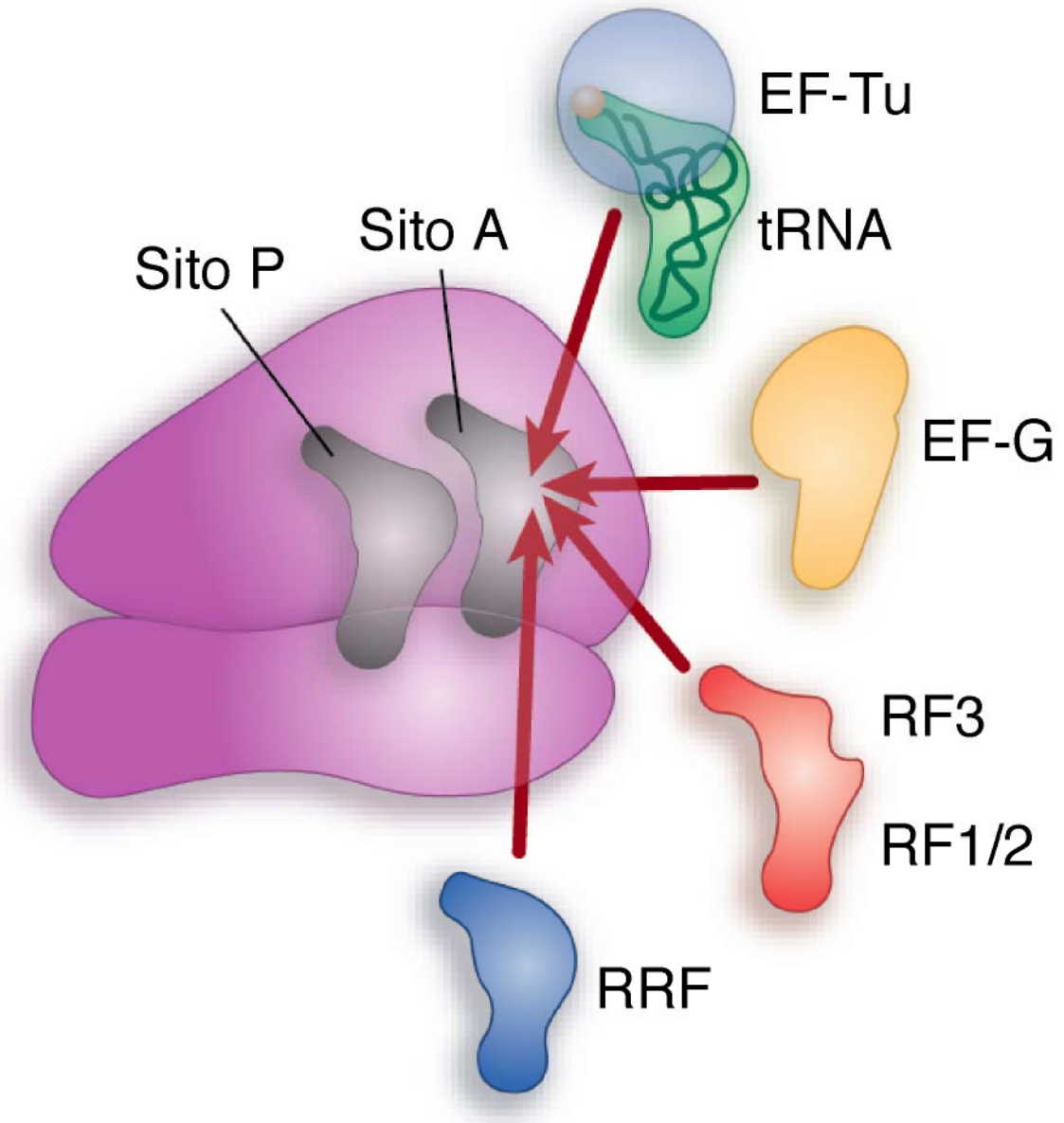
3. EF-G trasloca RRF



4. Il ribosoma si dissocia



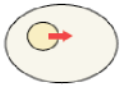
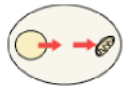
Vari fattori sono simili nella forma

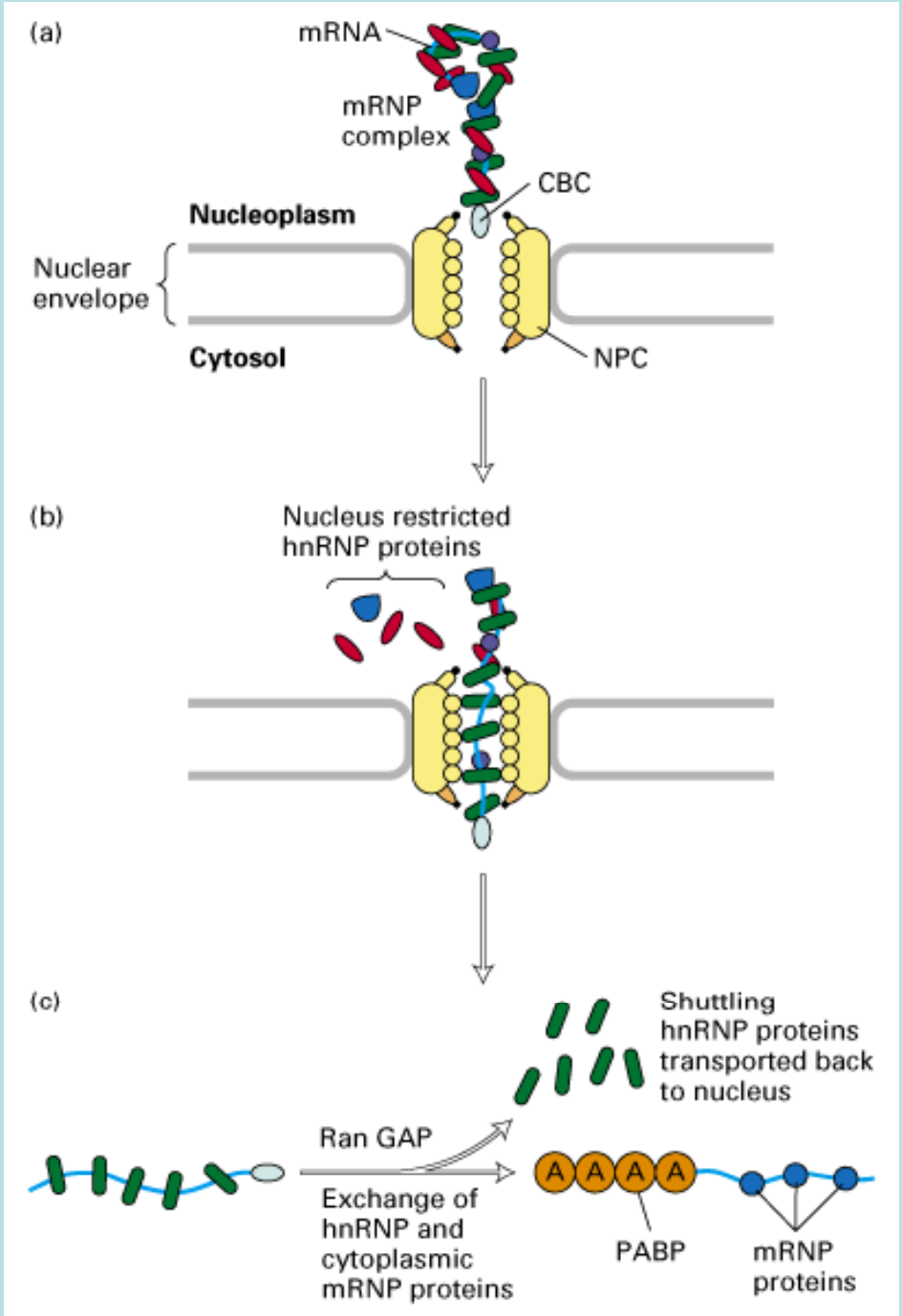


Inhibitor	Effect
Chloramphenicol	inhibits prokaryotic peptidyl transferase.
Streptomycin (Neomycin)	inhibits prokaryotic initiation, also induces mRNA misreading.
Tetracycline	inhibits prok. aminoacyl-tRNA binding to the ribosome small subunit.
Erythromycin	inhibits prokaryotic translocation through the ribosome large subunit.
Fusidic acid	similar to erythromycin only by preventing EF-G from dissociating from the large subunit.
Puromycin	resembles aa-tRNA, interferes with peptide transfer resulting in premature termination in prok. and euk.
Diptheria 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.

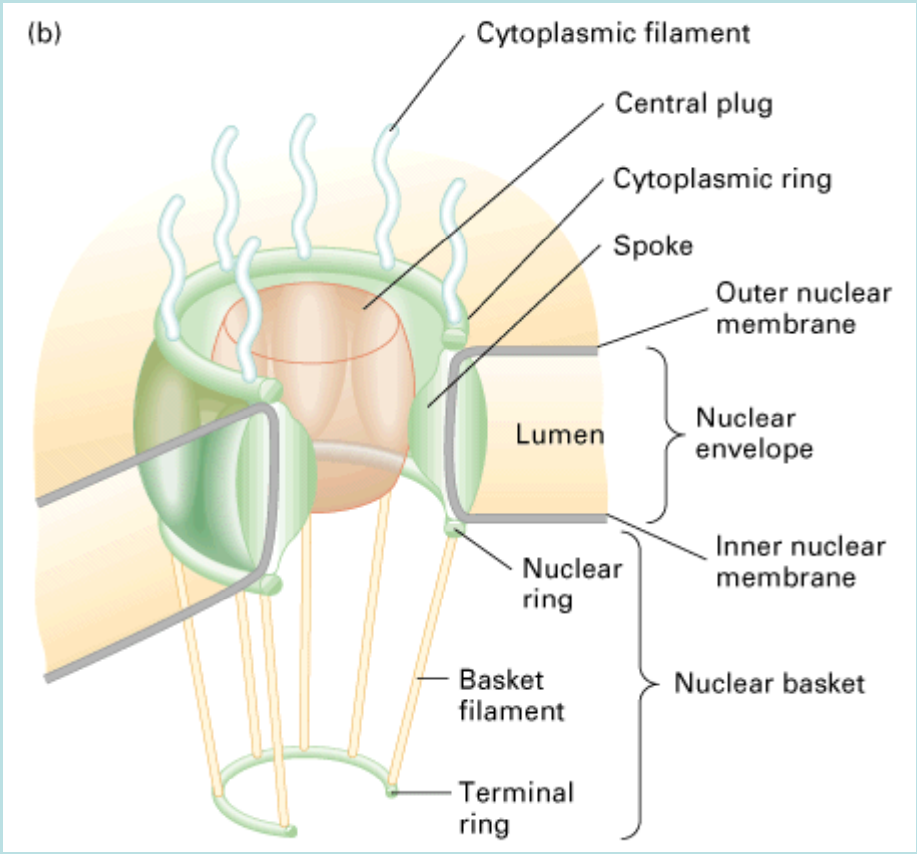
Trasporto e localizzazione degli mRNA

Negli eucarioti l'RNA viene traslocato e può essere localizzato

L'RNA può essere trasportato fra comparti cellulari			
RNA	Trasporto	Localizzazione	
Tutti gli RNA	Nucleo → citoplasma	Tutte le cellule	
tRNA	Nucleo → mitocondrio	Molte cellule	
mRNA	Cellula nutrice → oocita	Embriogenesi di *Drosophila*	
mRNA	Polo anteriore → polo posteriore dell'oocita	Embriogenesi di *Drosophila*	
mRNA	Cellula → cellula	Floema delle piante	

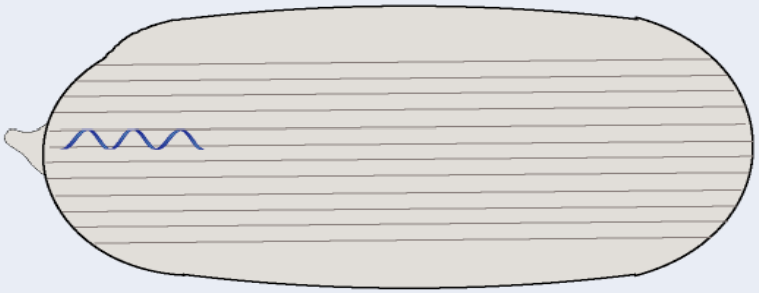


Nuclear Pore Complex

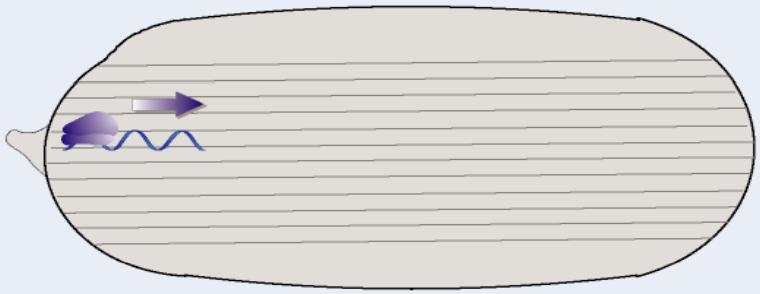


La localizzazione dell'mRNA genera un gradiente di proteina

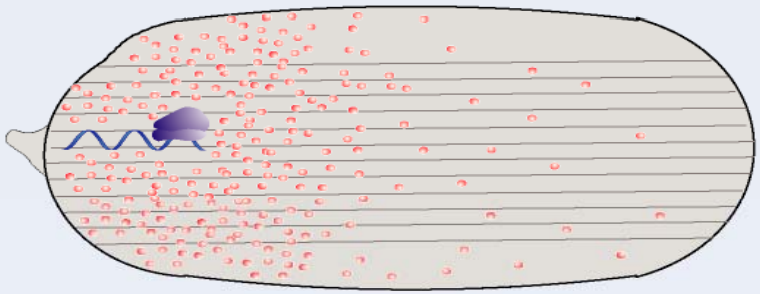
L'mRNA è localizzato all'estremità anteriore



L'mRNA è tradotto



La proteina diffonde dal sito di sintesi



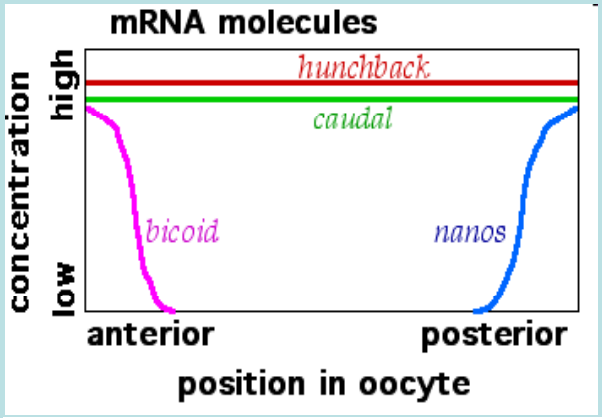
L'RNA e la proteina nanos sono posteriori

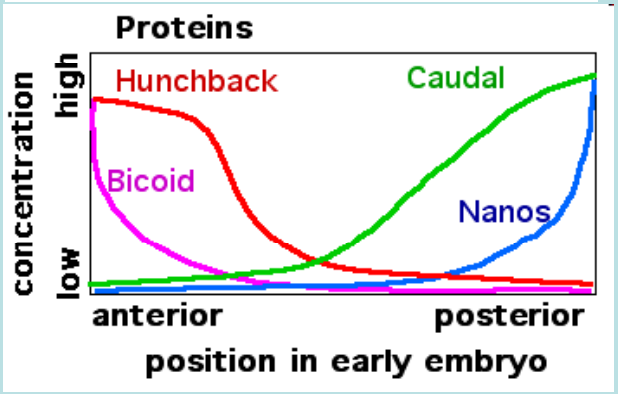
L'RNA *nanos* è localizzato all'estremità posteriore alla terza divisione

La proteina nanos diffonde dall'estremità posteriore all'ottava divisione

<u>The developing egg (oocyte) is polarized by differentially localized mRNA molecules.</u>

The genes that code for these mRNAs, called maternal effect genes, encode for proteins that get translated upon fertilization to establish <u>concentration gradients</u> 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.

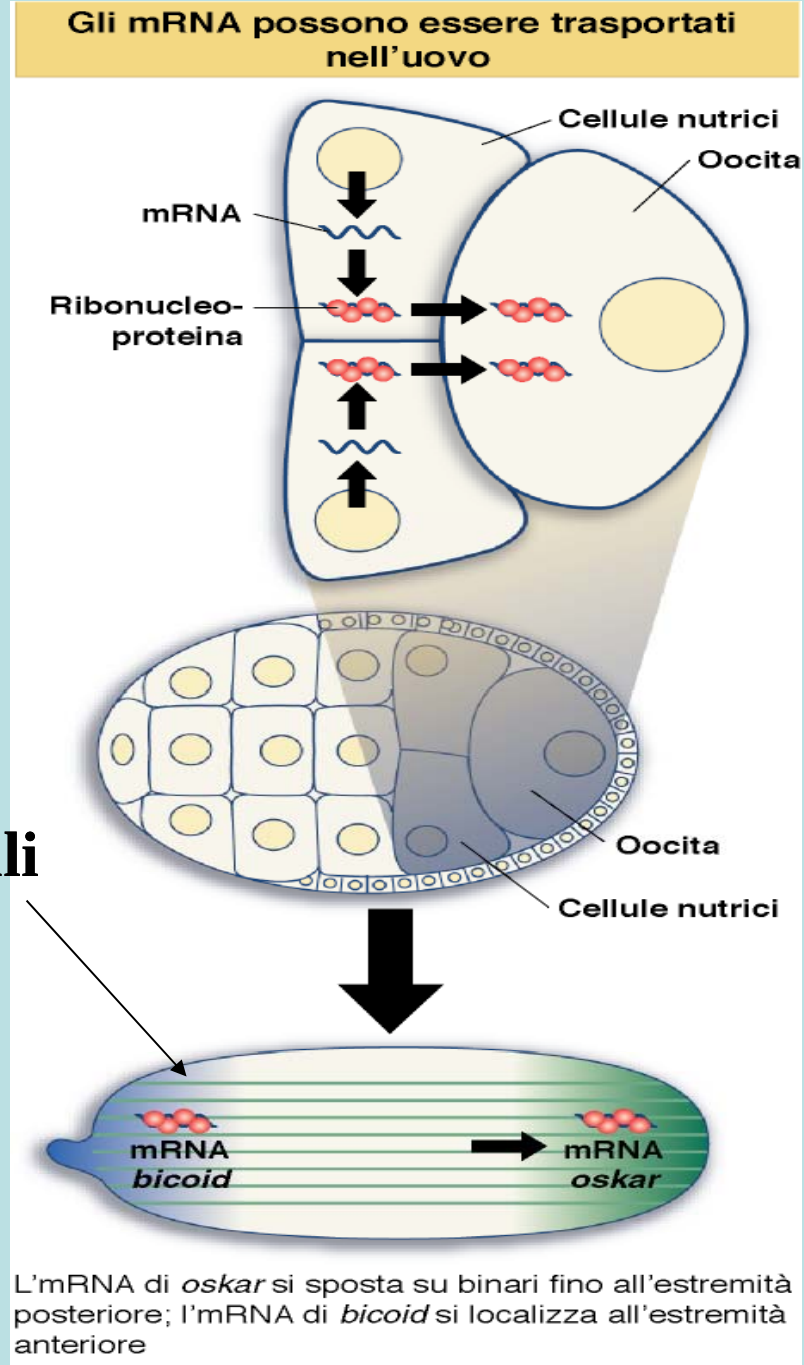




Meccanismi di localizzazione

- Trasporto specifico
- Degradazione selettiva
- Ancoraggio a siti specifici

Microtubuli

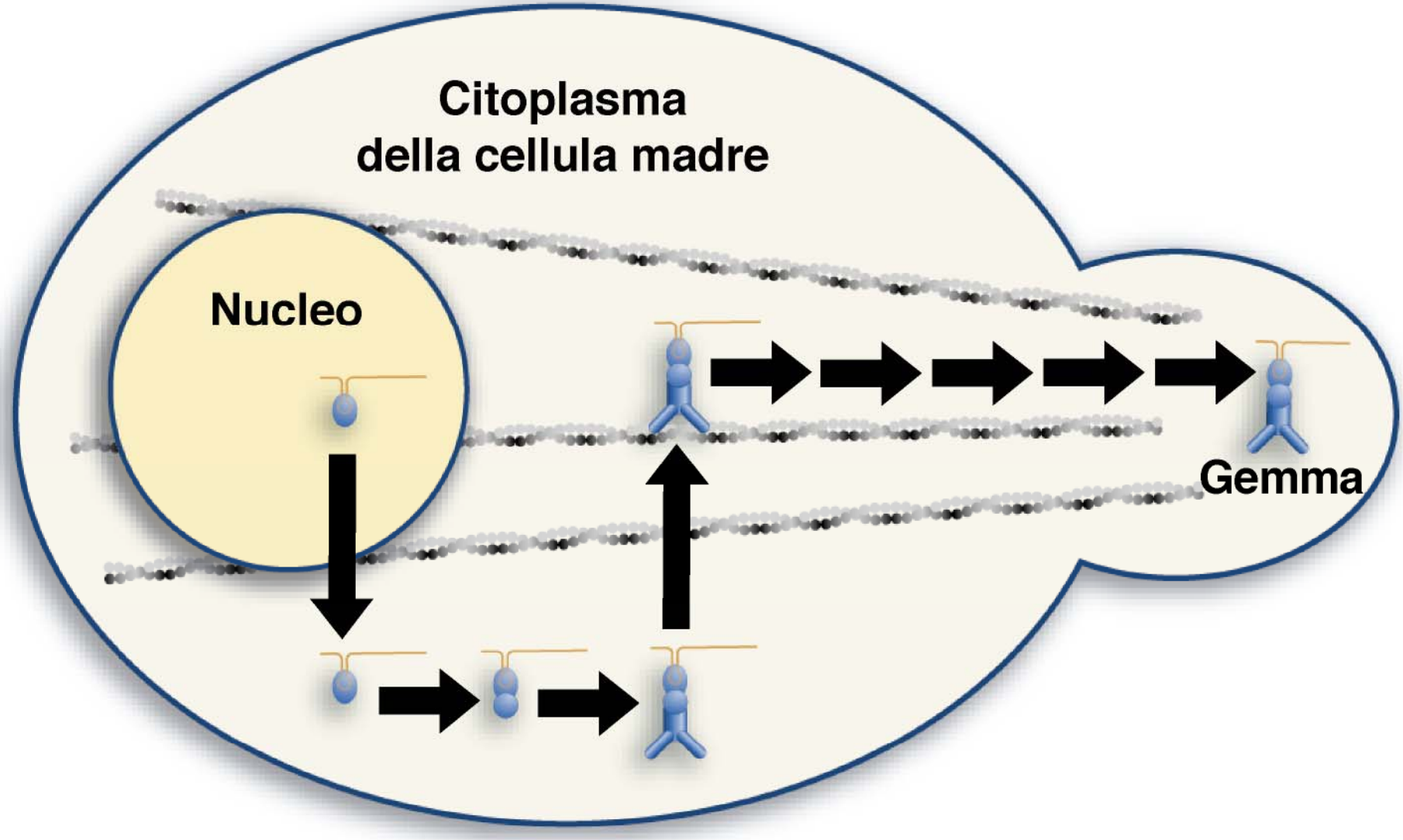


La localizzazione anteriore è determinata da particolari sequenze all'estremità 3' non tradotta (UTR) dell'mRNA

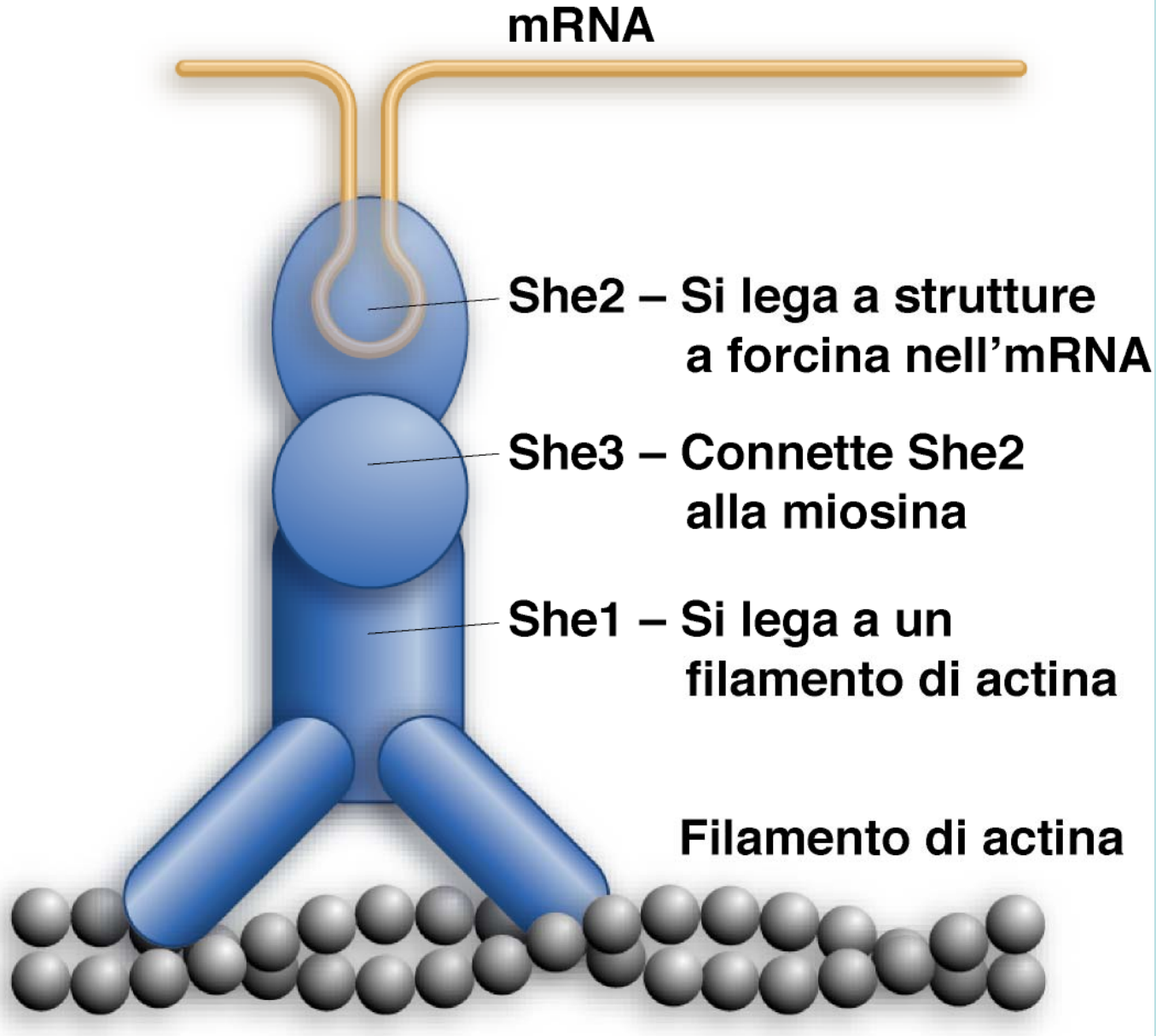
All'interno dell'oocita ci saranno concentrazioni diverse delle 2 proteine ai 2 poli della cellula → influenza sullo sviluppo dell'embrione

Nei lieviti alcuni mRNA si spostano dalla cellula madre alla gemma.

ASH1 si sposta lungo filamenti di actina fino alla gemma



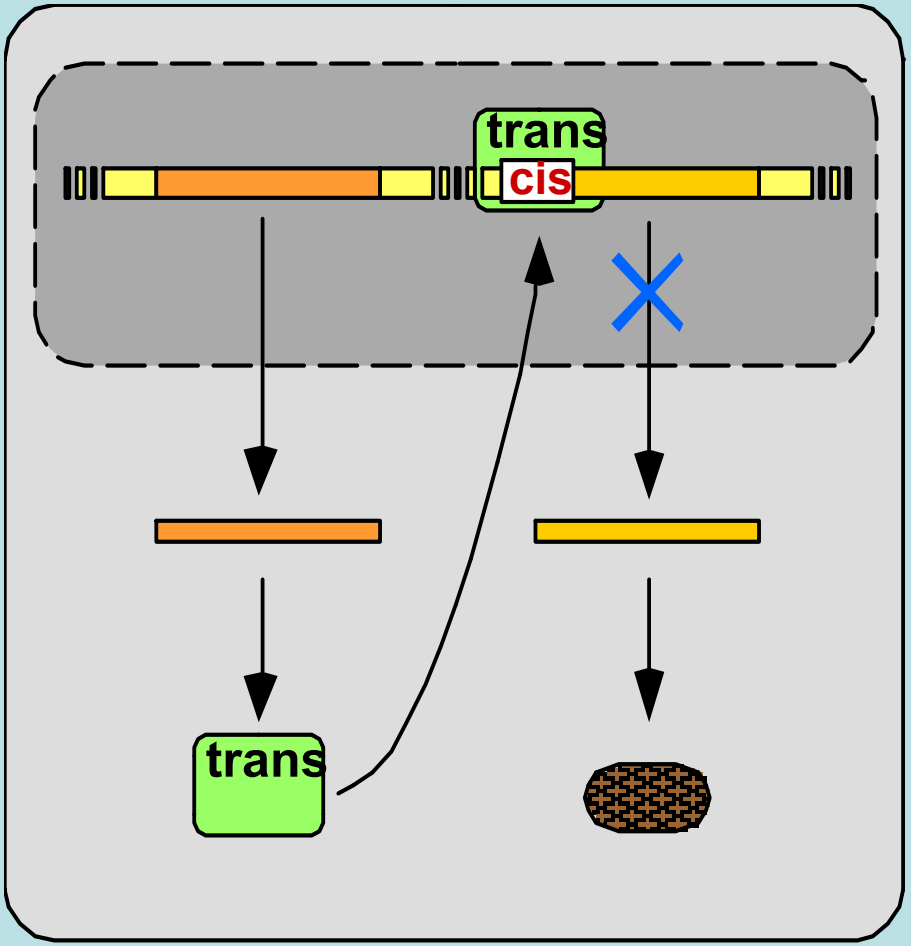
L'mRNA di ASH1 è legato a un motore



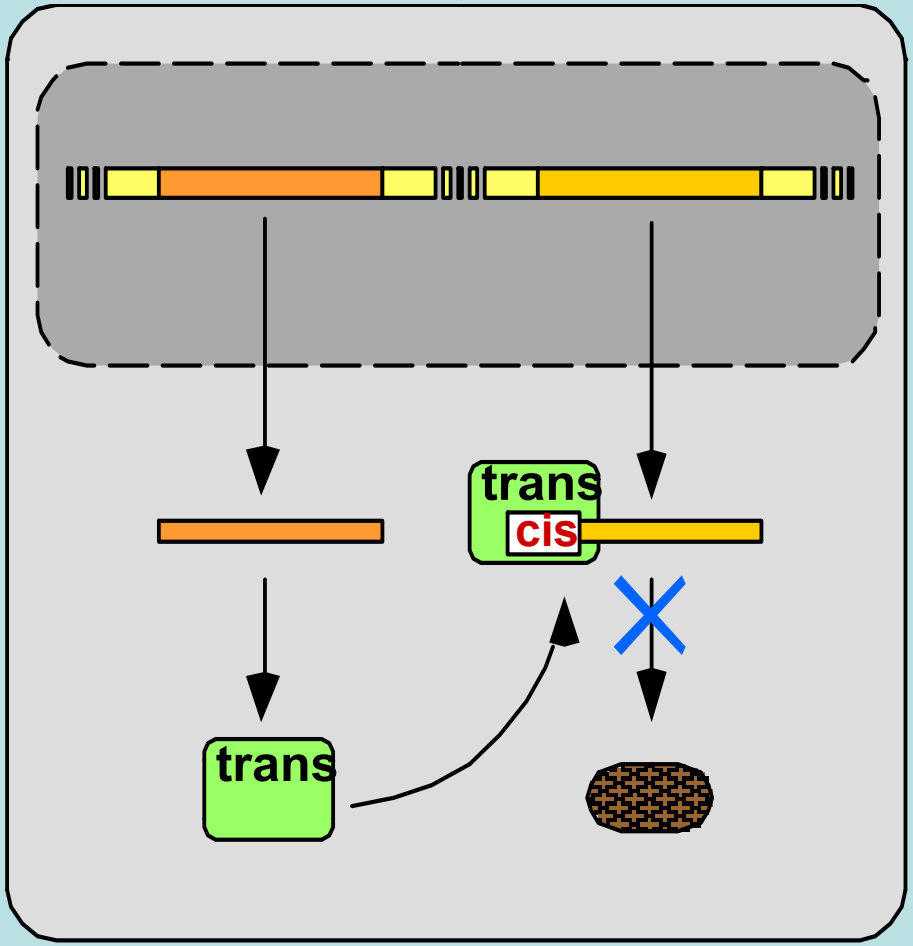
Regolazione della traduzione

- Generale
- Specifica

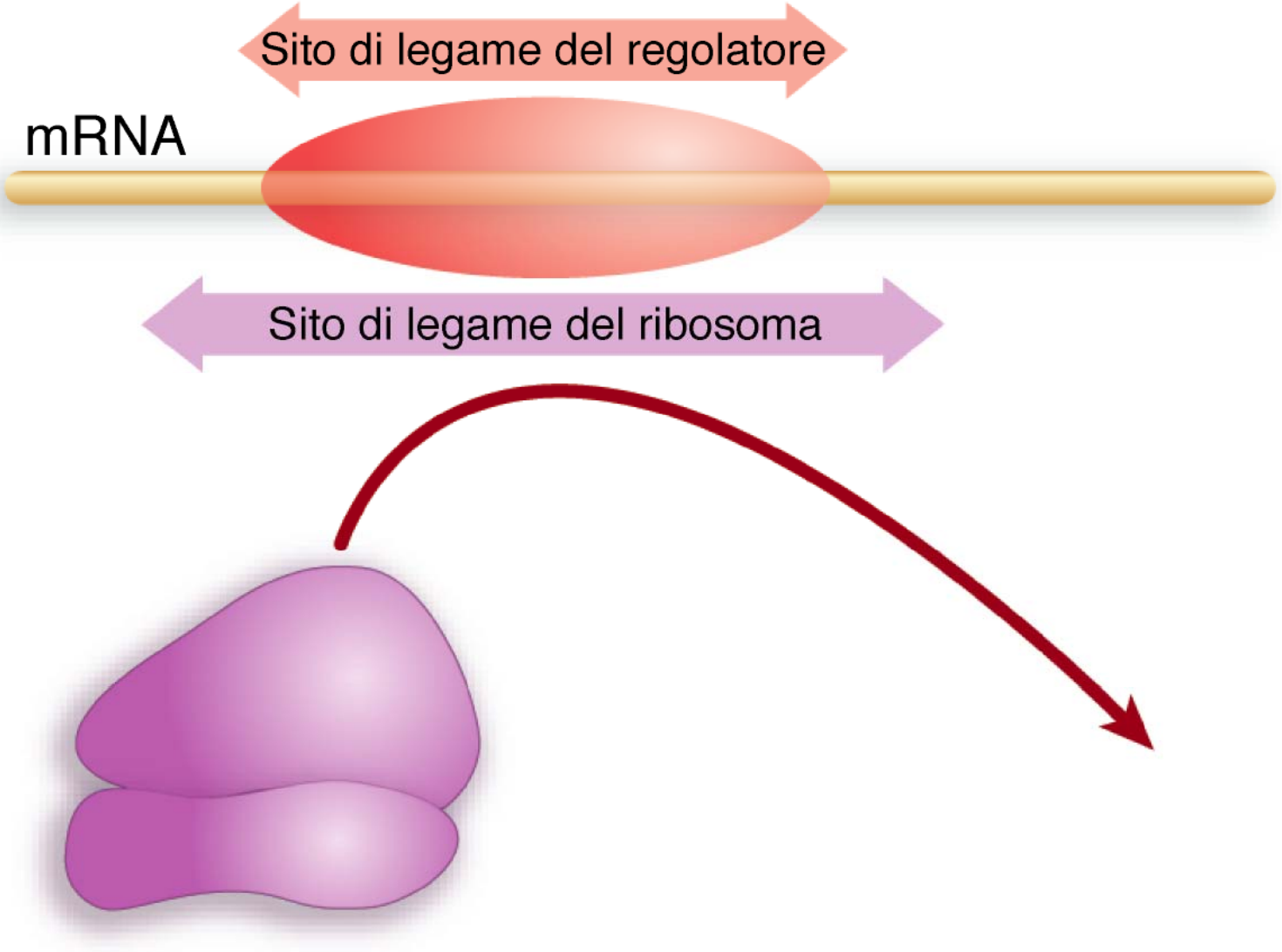
Regolazione trascrizionale

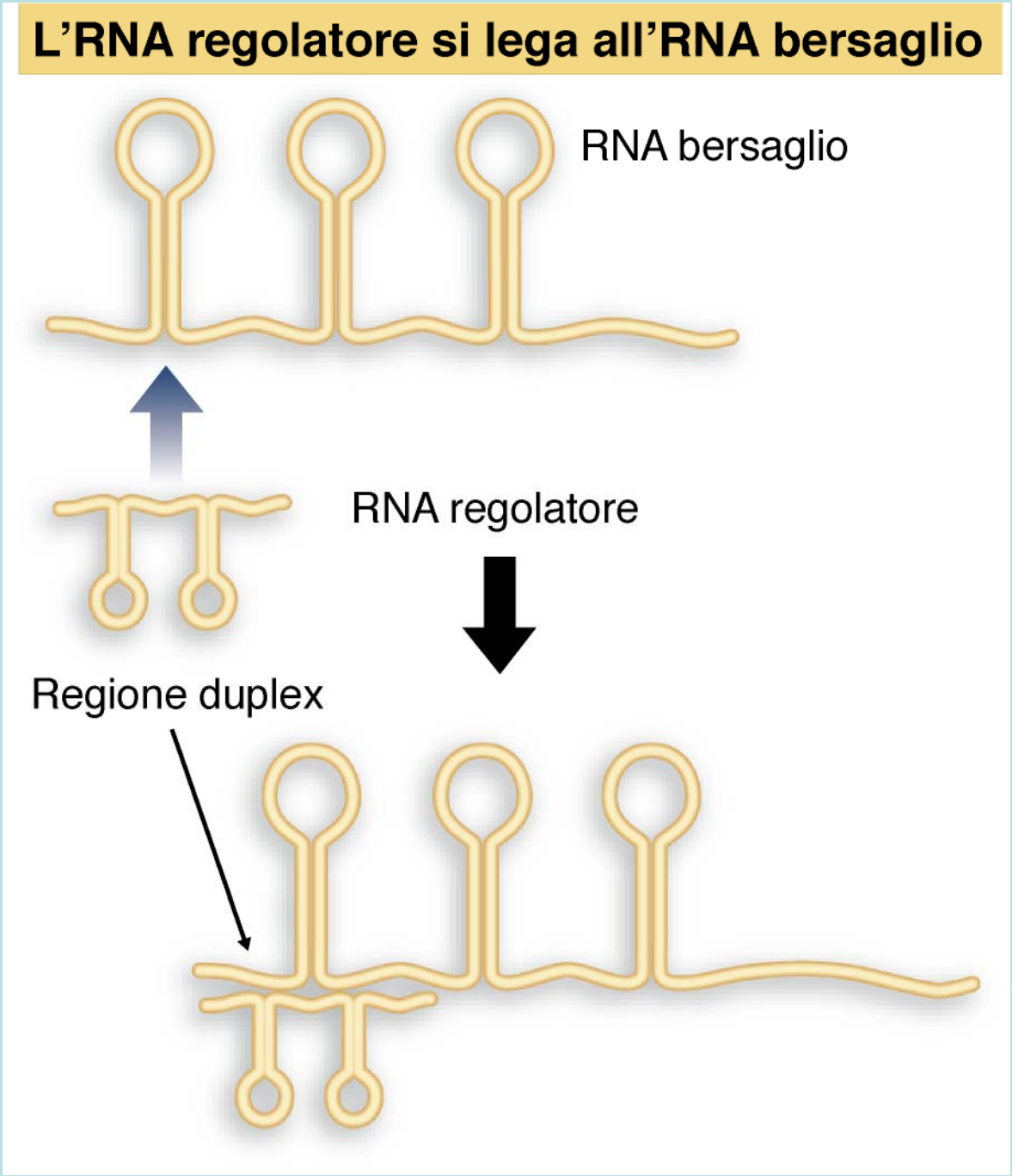


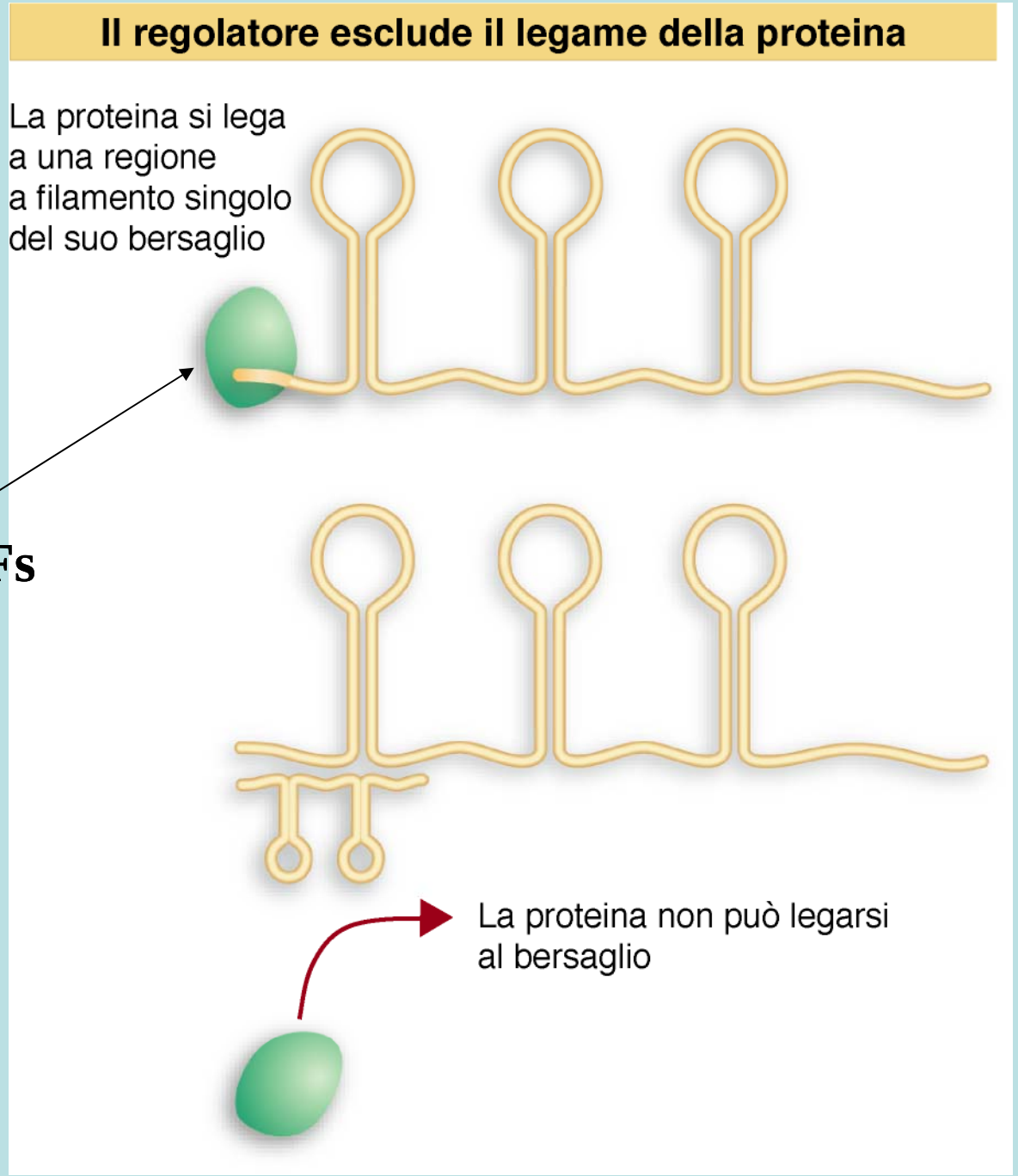
Regolazione traduzionale



Un regolatore può bloccare il legame del ribosoma




L’RNA regolatore si lega all’RNA bersaglio
RNA bersaglio
RNA regolatore
Regione duplex


Il regolatore esclude il legame della proteina
La proteina si lega a una regione a filamento singolo del suo bersaglio
La proteina non può legarsi al bersaglio

Es. **eIFs**

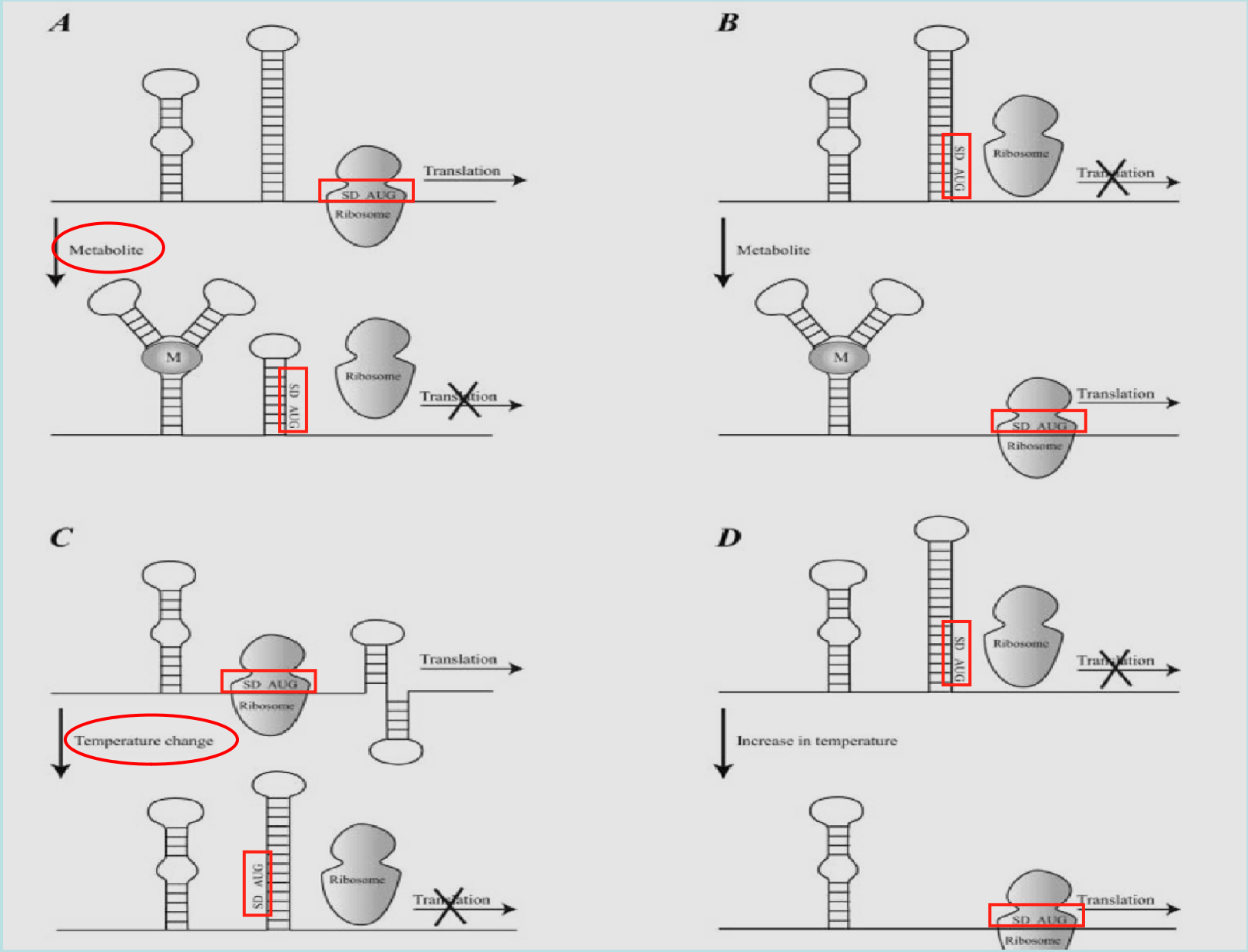


FIG. 13. Examples of translational regulation mechanisms. (*A*) Repression of translation by binding of a metabolite that stabilizes an alternative mRNA secondary structure and leaves the SD sequence and initiation codon (AUG) in a base-paired region. (*B*) Activation of translation by binding of a metabolite 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.

I repressori traduzionali si legano all'mRNA

Repressore	Gene bersaglio	Sito di azione
Proteina del rivestimento di R17	Replicasi di R17	Forcina che comprende il sito di legame del ribosoma
RegA di T4	mRNA precoci di T4	Varie sequenze che comprendono il codone di inizio
DNA polimerasi di T4	DNA polimerasi di T4	Sequenza Shine-Dalgarno
p32 di T4	Gene 32	Leader 5′ a filamento singolo