Fundamentos de Biologia Molecular

Tradução

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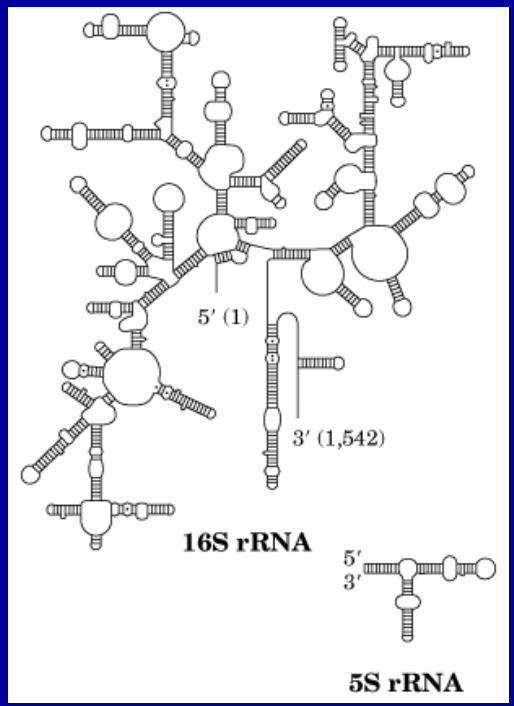
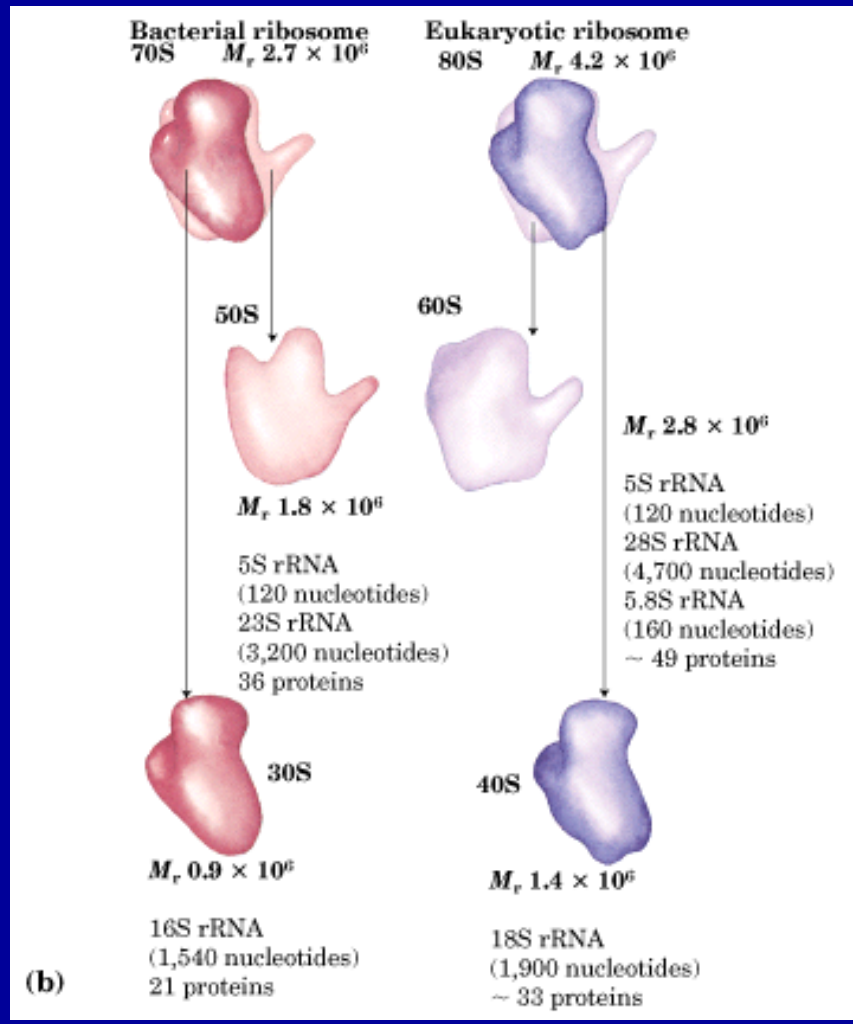
Ribossomos: estrutura e características

RNA and Protein Components of the *E. coli* Ribosome

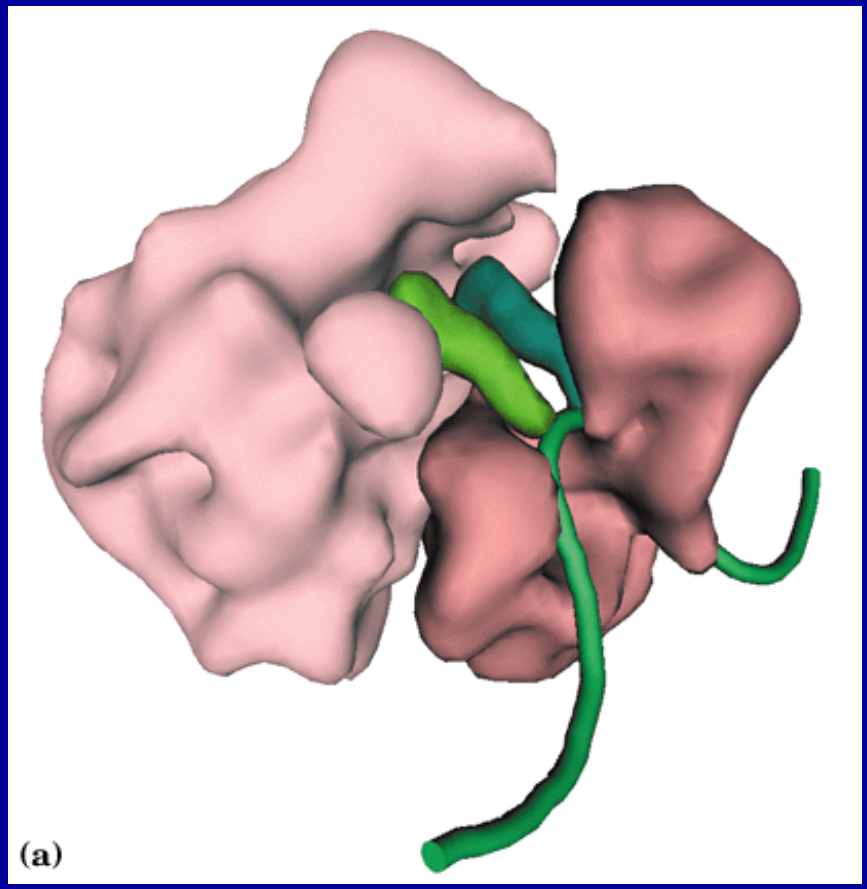
Subunit	Number of different proteins	Total number of proteins	Protein designations	Number and type of rRNAs
30S	21	21	S1–S21	1 (16S rRNA)
50S	33	36	L1–L36*	2 (5S and 23S rRNAs)

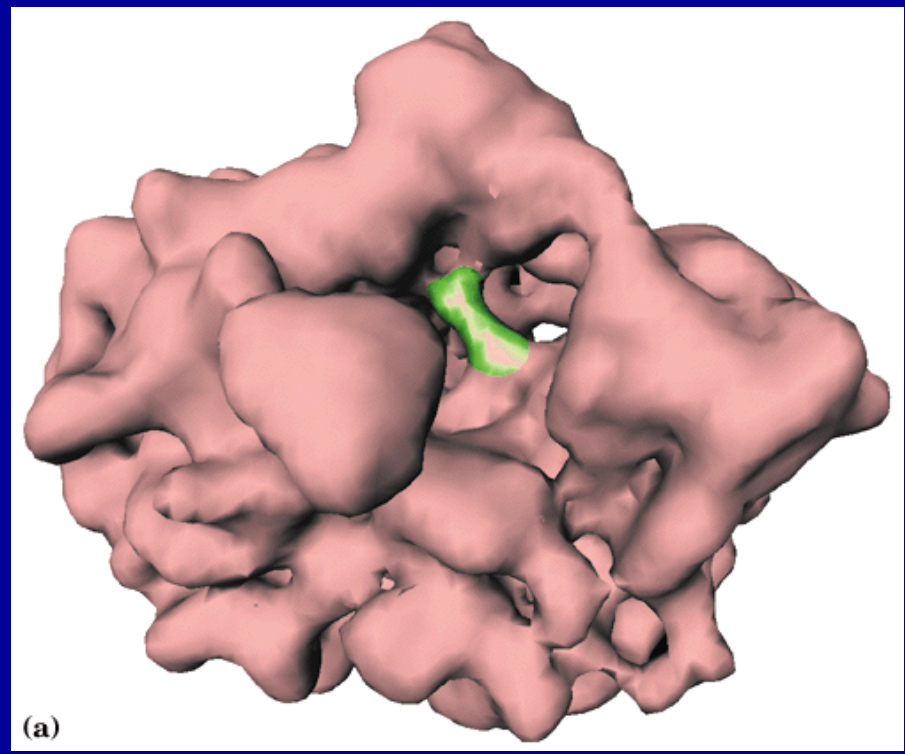
*The L1 to L36 protein designations do not correspond to 36 different proteins. The protein originally designated L7 is in fact a modified form of L12, and L8 is a complex of three other proteins. Also, L26 proved to be the same protein as S20 (and not part of the 50S subunit). This gives 33 different proteins in the large subunit. There are four copies of the L7/L12 protein, with the three extra copies bringing the total protein count to 36.



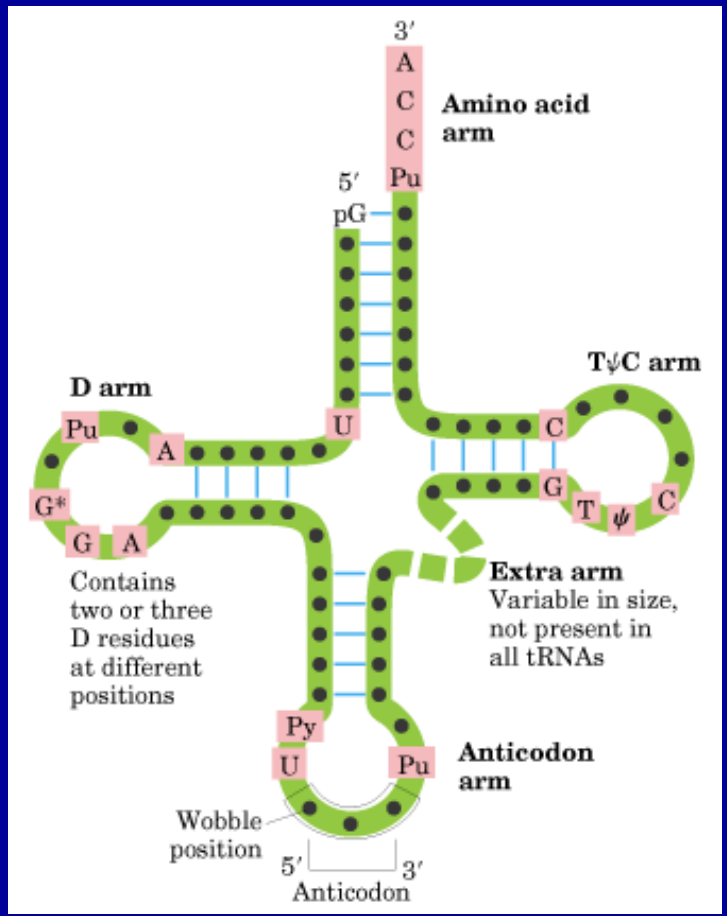


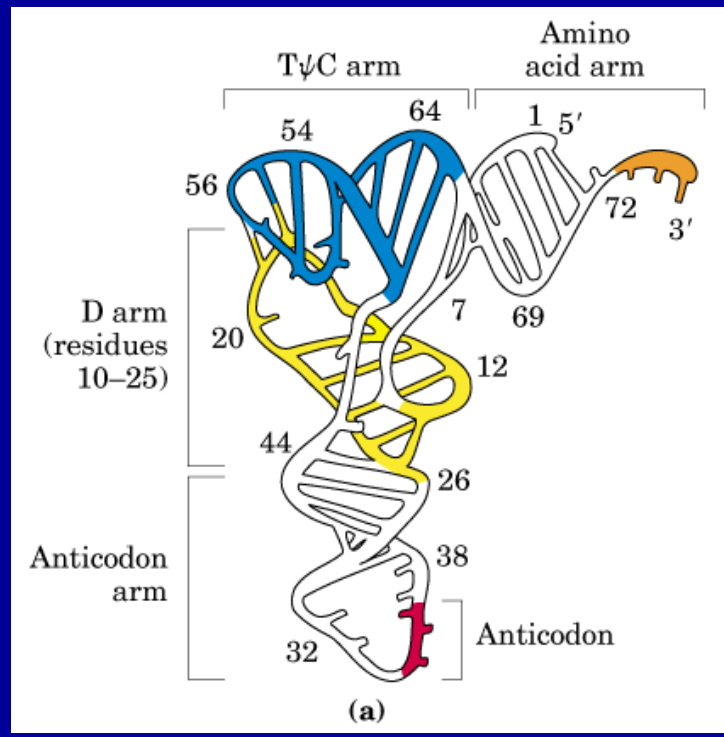
Ribossomos: estrutura

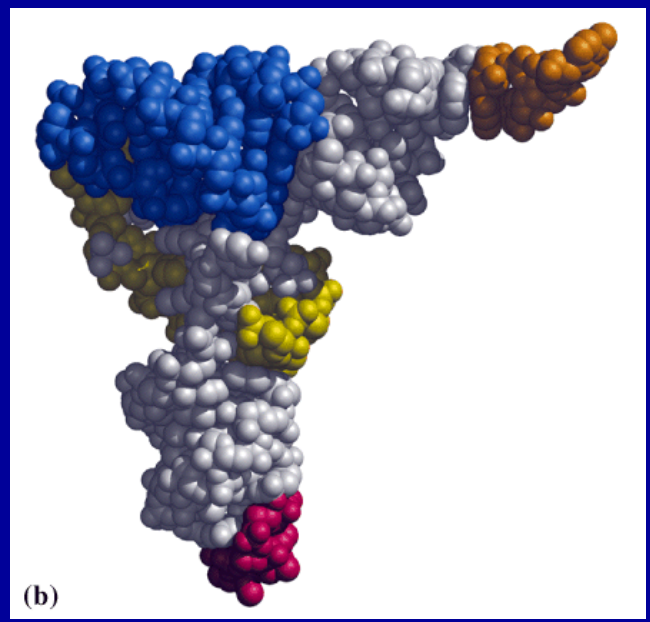




tRNA: estrutura





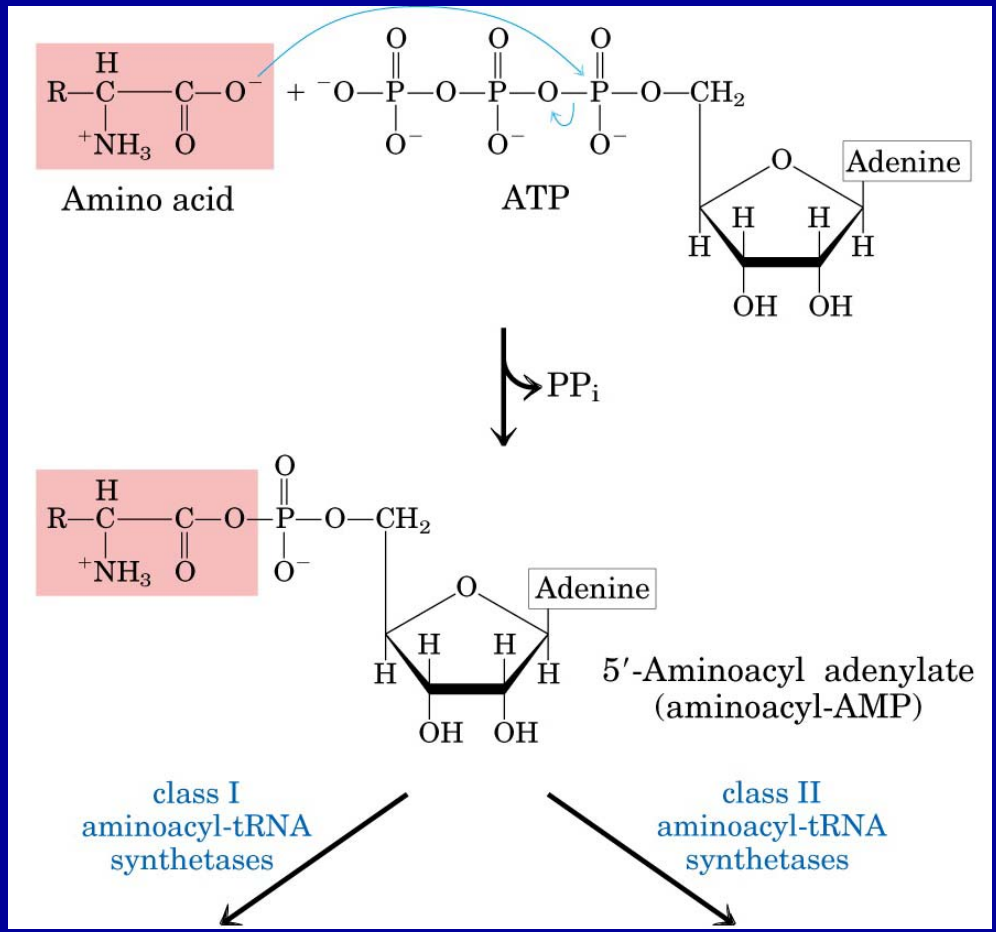


Etapas do processo traducional

Components Required for the Five Major Stages of Protein Synthesis in *E. coli*

Stage	Essential components
1. Activation of amino acids	20 amino acids 20 aminoacyl-tRNA synthetases 20 or more tRNAs ATP Mg^{2+}
2. Initiation	mRNA *N*-Formylmethionyl-tRNA Initiation codon in mRNA (AUG) 30S ribosomal subunit 50S ribosomal subunit Initiation factors (IF-1, IF-2, IF-3) GTP Mg^{2+}
3. Elongation	Functional 70S ribosome (initiation complex) Aminoacyl-tRNAs specified by codons Elongation factors (EF-Tu, EF-Ts, EF-G) GTP Mg^{2+}
4. Termination and release	Termination codon in mRNA Polypeptide release factors (RF_1, RF_2, RF_3) ATP
5. Folding and posttranslational processing	Specific enzymes, cofactors, and other components for removal of initiating residues and signal sequences, additional proteolytic processing, modification of terminal residues, and attachment of phosphate, methyl, carboxyl, carbohydrate, or prosthetic groups

1° estágio: Ativação dos aminoácidos - Síntese do aminoacil-tRNA



PPi ⟶ **2 Pi**

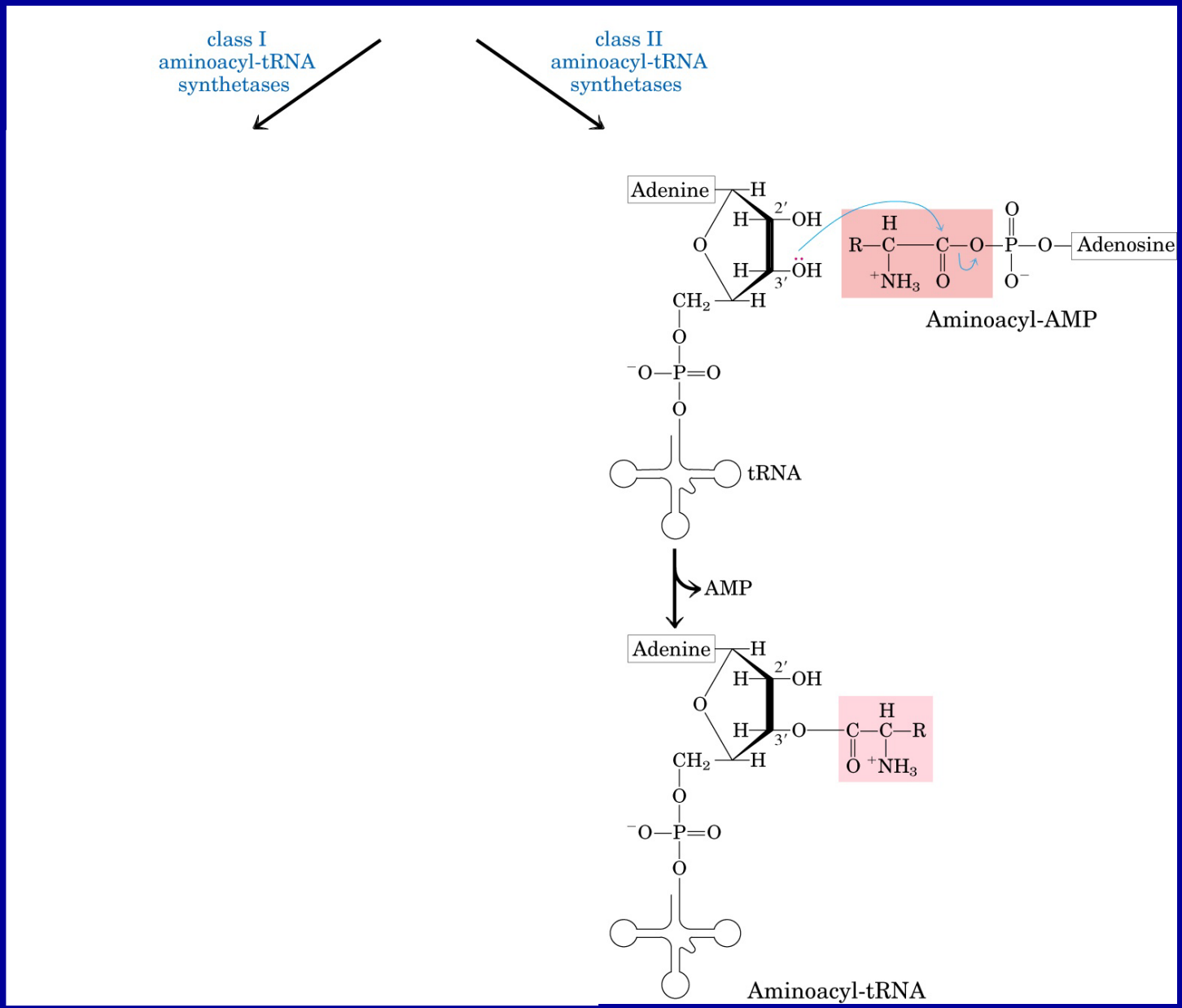
Pirofosfatase inorgânica

Two Classes of Aminoacyl-tRNA Synthetases*

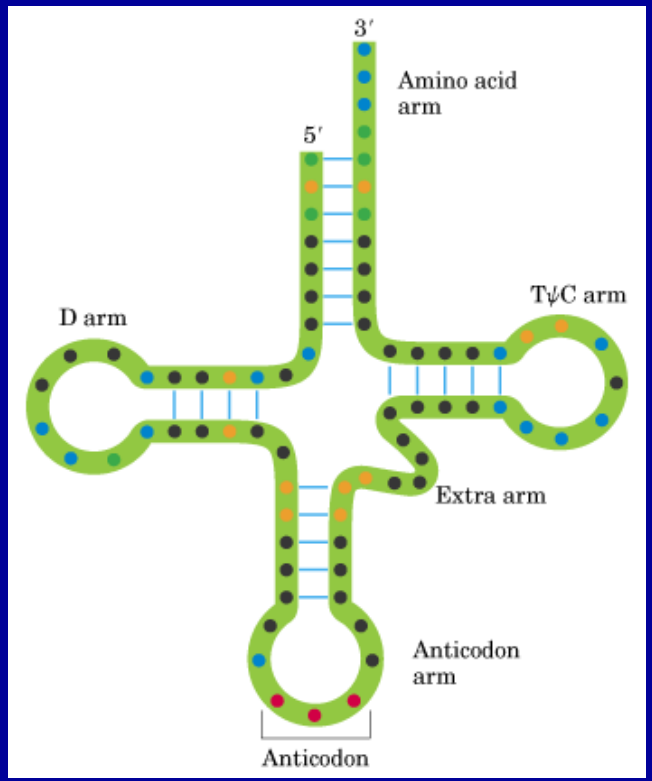
Class I	Class II
Arg	Ala
Cys	Asn
Gln	Asp
Glu	Gly
Ile	His
Leu	Lys
Met	Phe
Trp	Pro
Tyr	Ser
Val	Thr

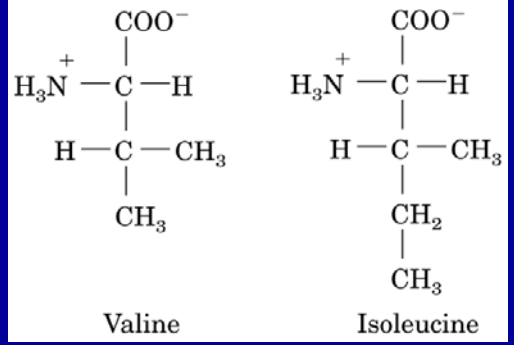
*Here, Arg represents arginyl-tRNA synthetase, and so forth. The classification applies to all organisms for which tRNA synthetases have been analyzed and is based on protein structural distinctions and on the mechanistic distinction outlined in Figure 27–16.

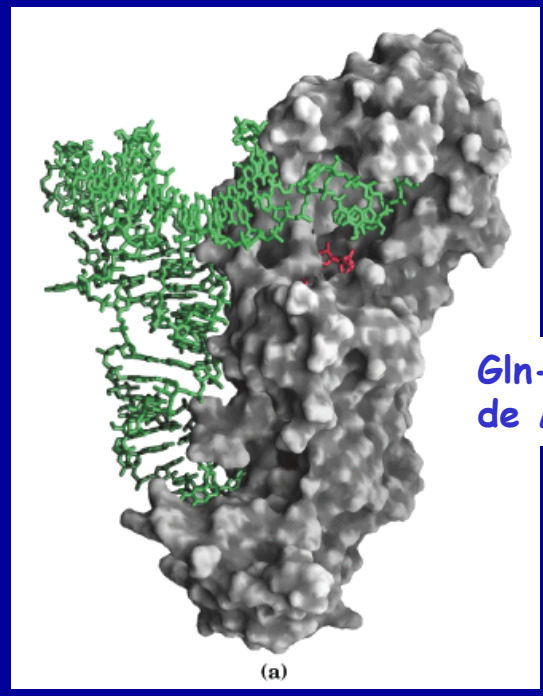
Síntese do aminoacil-tRNA:



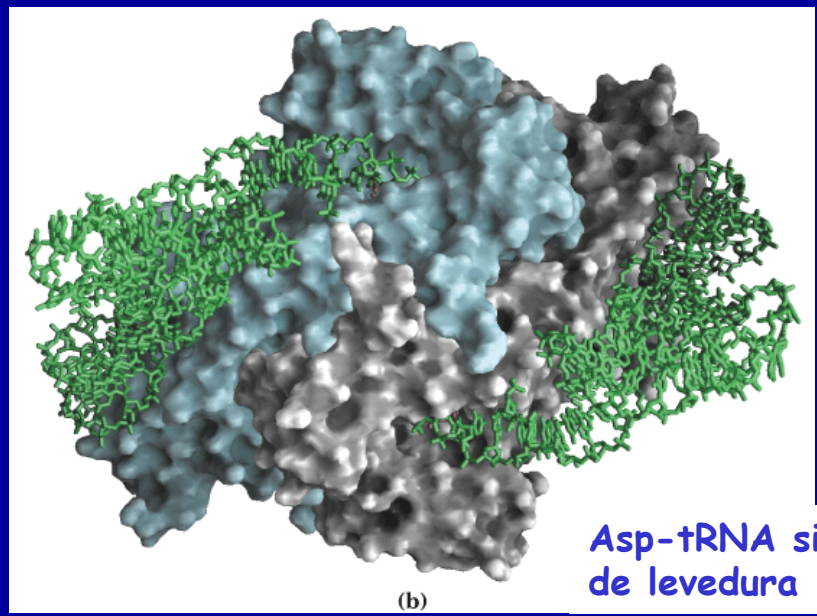
Interação entre as aminoacil-tRNA sintetases e os tRNA: O "Segundo Código Genético"







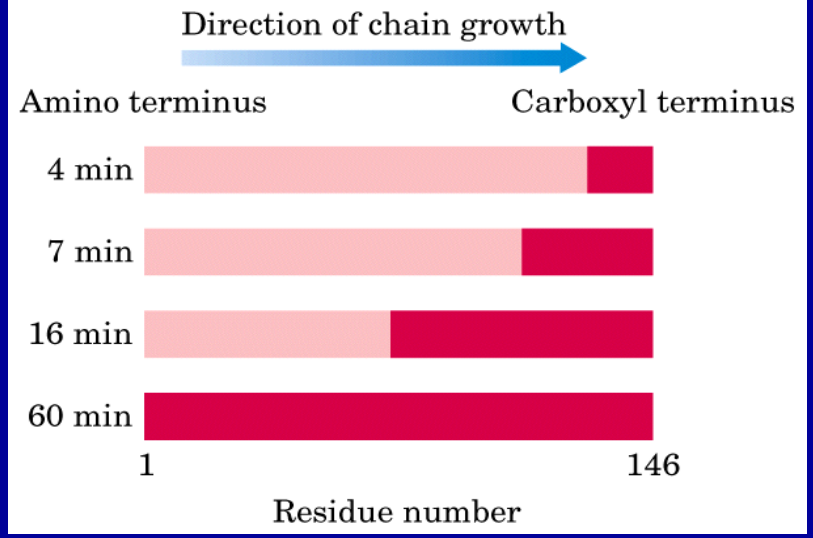
Gln-tRNA sintetase de *E. coli*

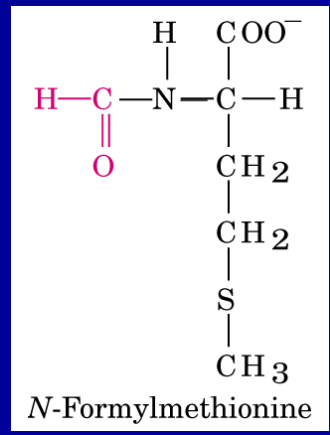


Asp-tRNA sintetase de levedura

2° estágio: Iniciação da tradução – Especificidade por metionina/*N*-formilmetionina

Experimento de H. Dintzis (1961):

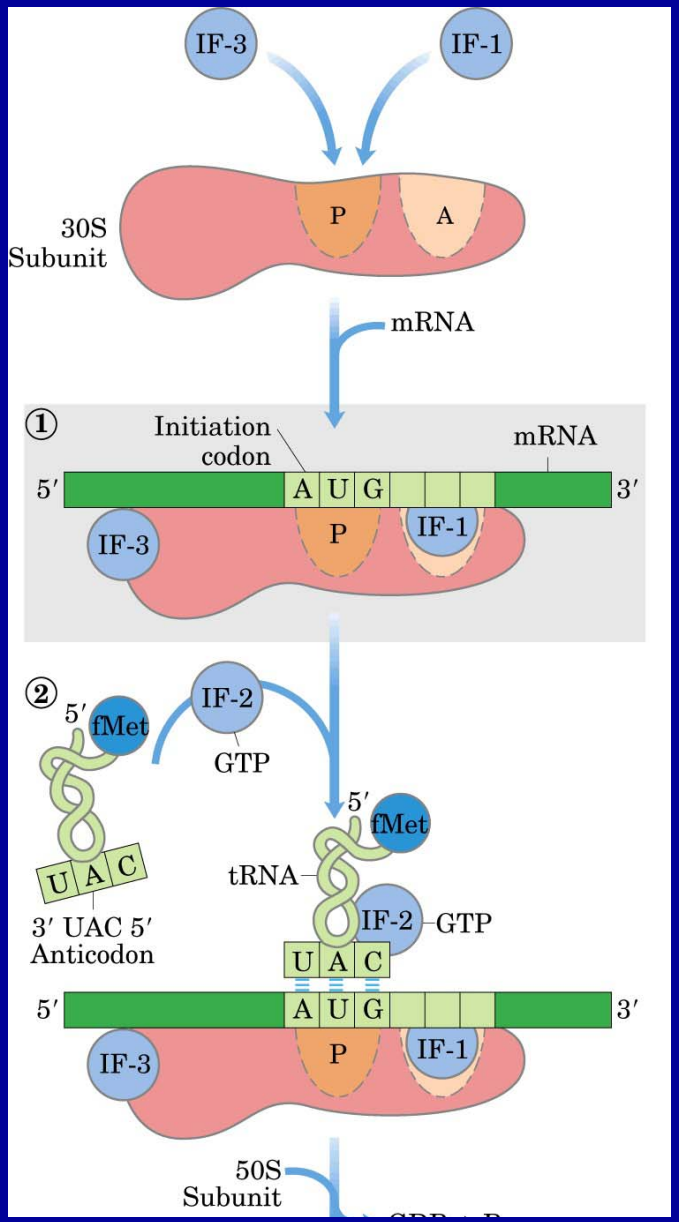


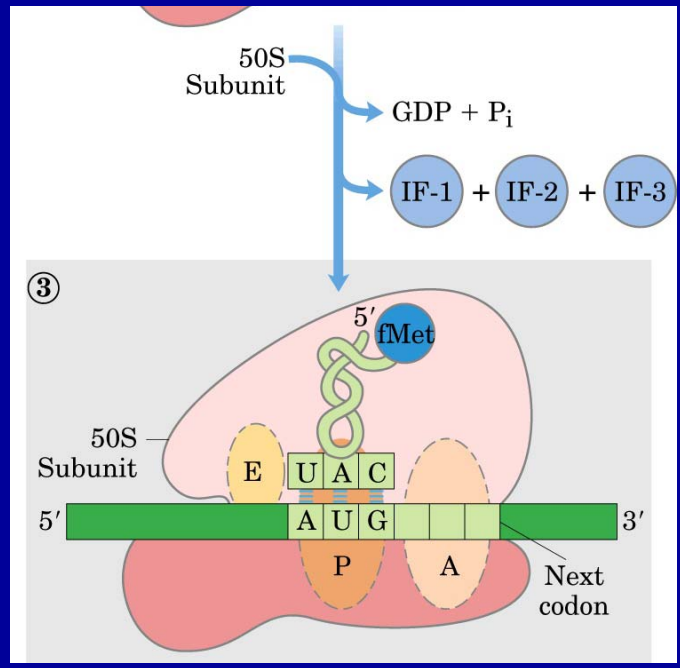


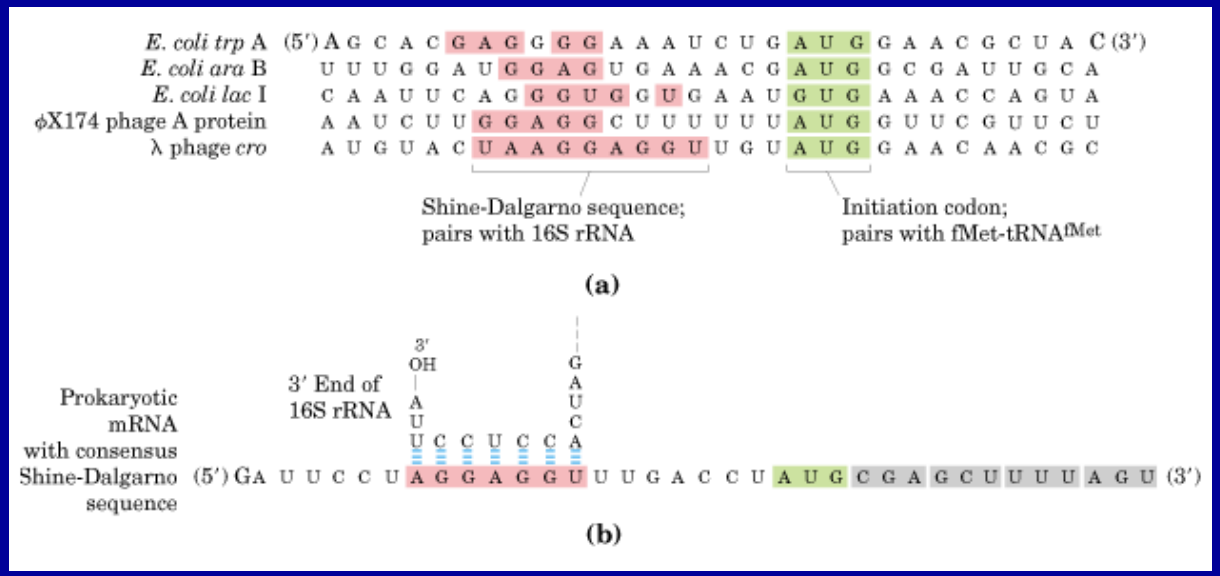
N-Formylmethionine

N^{10}-Formiltetrahidrofolato + Met-$tRNA^{fMet}$ ⟶ (transformilase) tetrahidrofolato + fMet-$tRNA^{fMet}$

Iniciação da tradução: procariontes







Iniciação da tradução: procariontes x eucariontes

Protein Factors Required for Initiation of Translation in Bacterial and Eukaryotic Cells

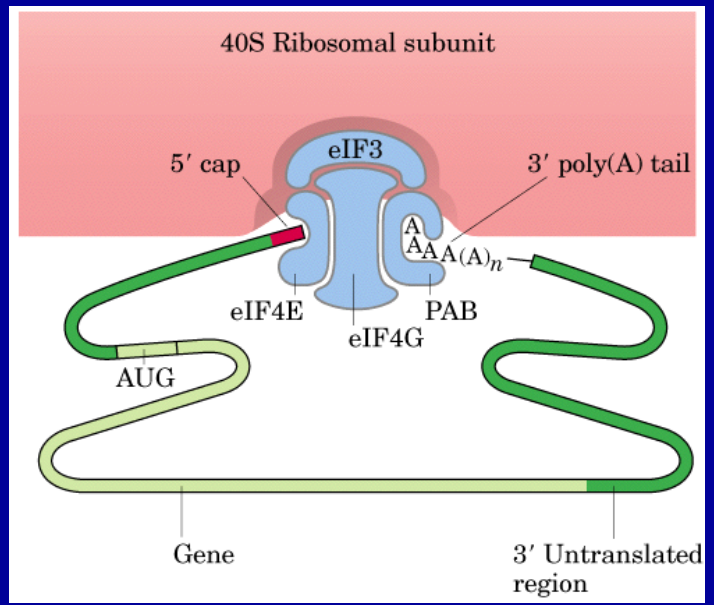
Bacterial

Factor	Function
IF-1	Prevents premature binding of tRNAs to A site
IF-2	Facilitates binding of fMet-tRNA^{fMet} to 30S ribosomal subunit
IF-3	Binds to 30S subunit; prevents premature association of 50S subunit; enhances specificity of P site for fMet-tRNA^{fMet}

Eukaryotic

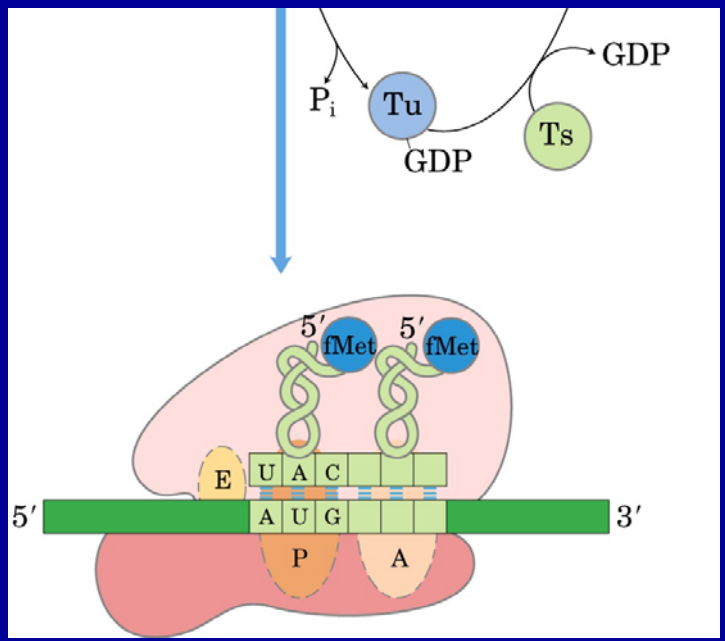
Factor*	Function
eIF2	Facilitates binding of initiating Met-tRNA^{Met} to 40S ribosomal subunit
eIF2B, eIF3	First factors to bind 40S subunit; facilitate subsequent steps
eIF4A	RNA helicase activity removes secondary structure in the mRNA to permit binding to 40S subunit; part of the eIF4F complex
eIF4B	Binds to mRNA; facilitates scanning of mRNA to locate the first AUG
eIF4E	Binds to the 5′ cap of mRNA; part of the eIF4F complex
eIF4G	Binds to eIF4E and to poly(A) binding protein (PAB); part of the eIF4F complex
eIF5	Promotes dissociation of several other initiation factors from 40S subunit as a prelude to association of 60S subunit to form 80S initiation complex
eIF6	Facilitates dissociation of inactive 80S ribosome into 40S and 60S subunits

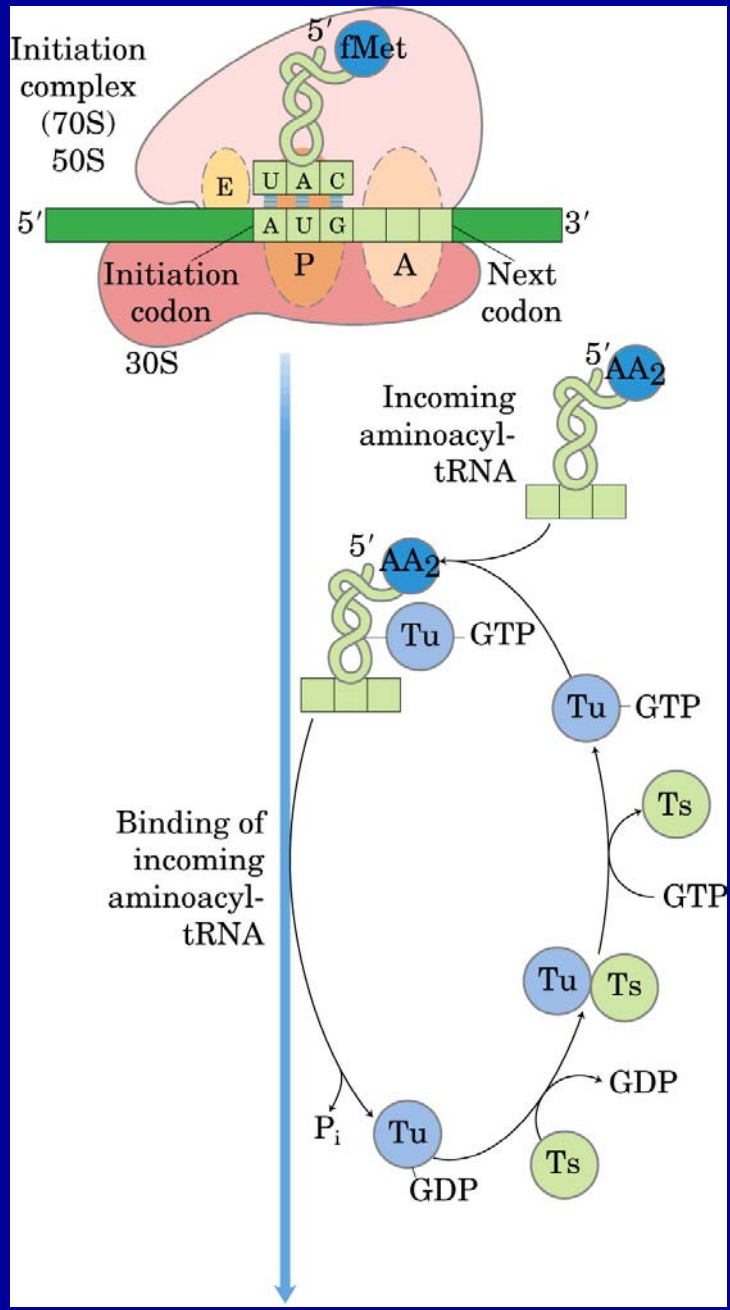
*The prefix "e" identifies these as eukaryotic factors.



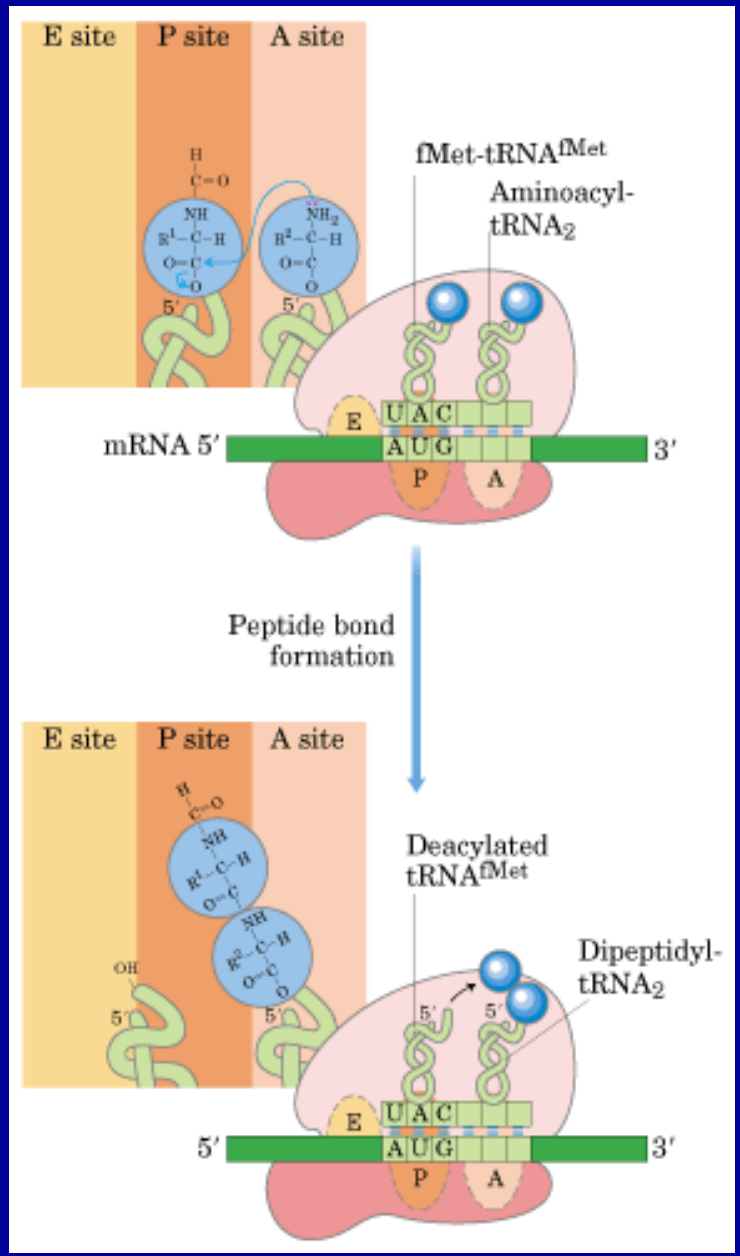
3° estágio: Elongamento

a) Ligação do aminoacil-tRNA

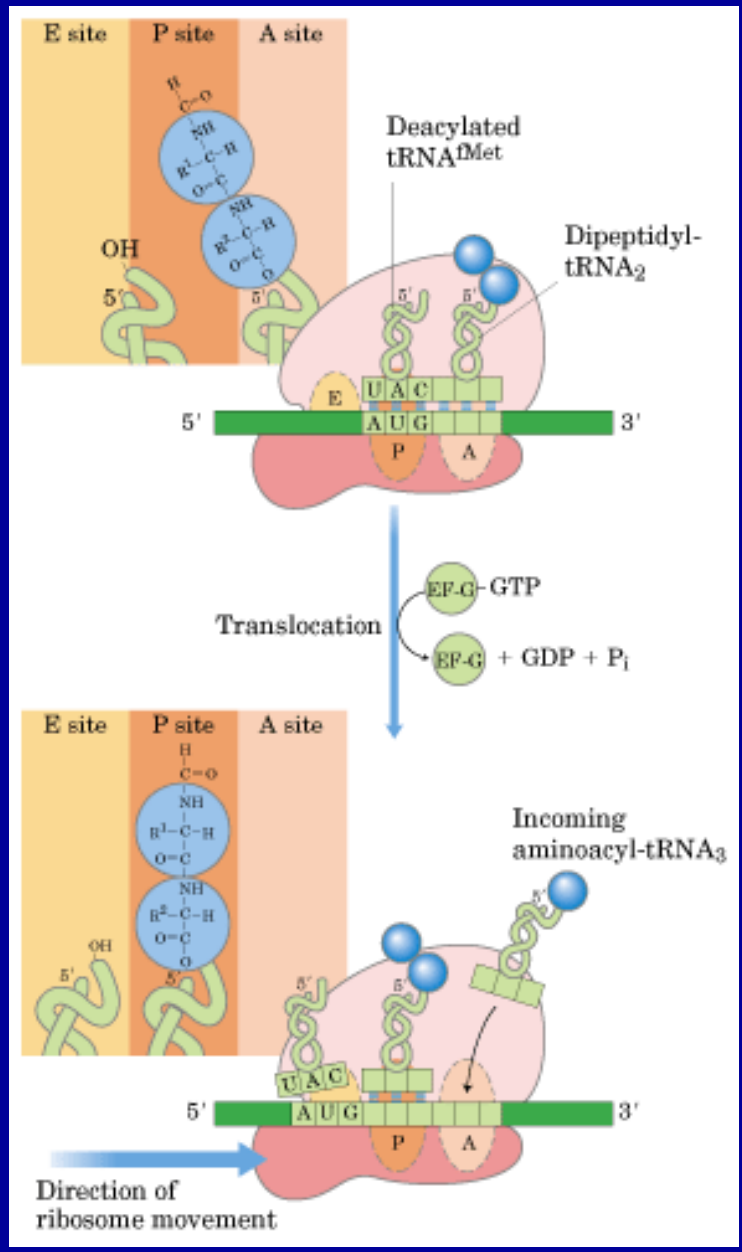




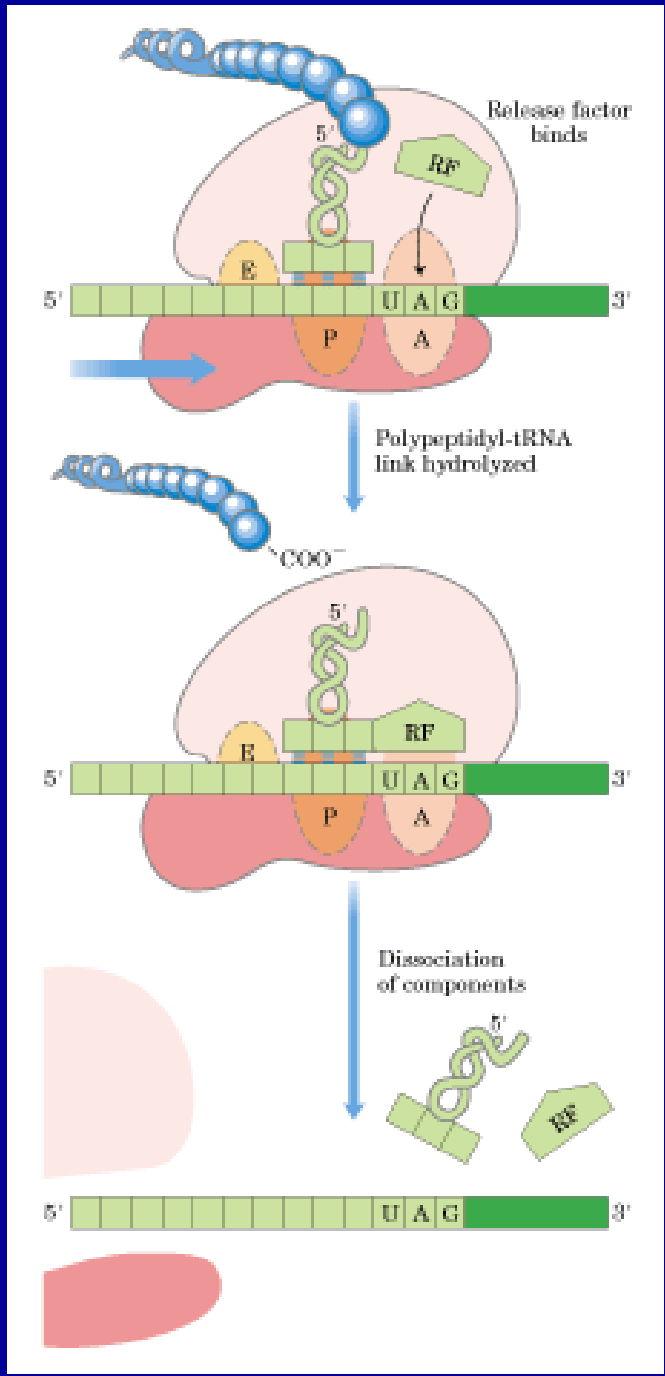
b) Formação da ligação peptídica

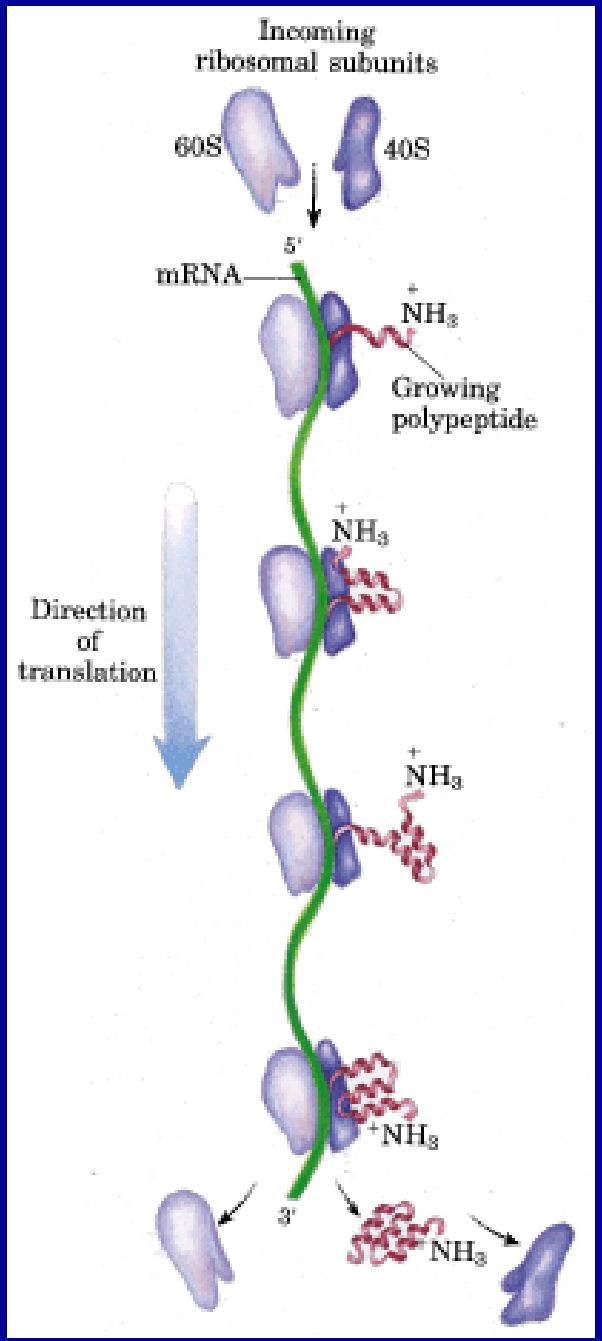


c) Translocação

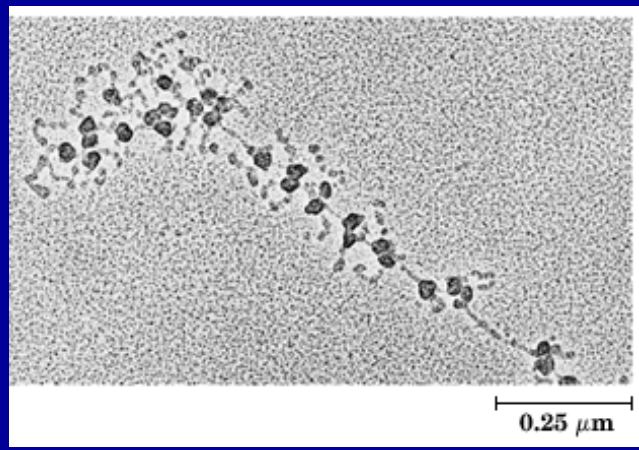


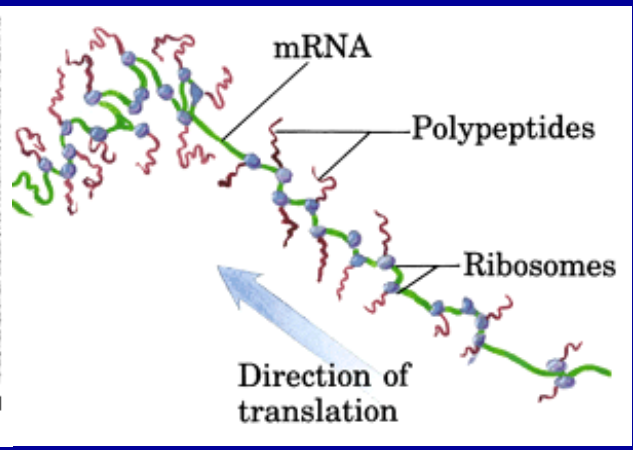
4° estágio: Terminação



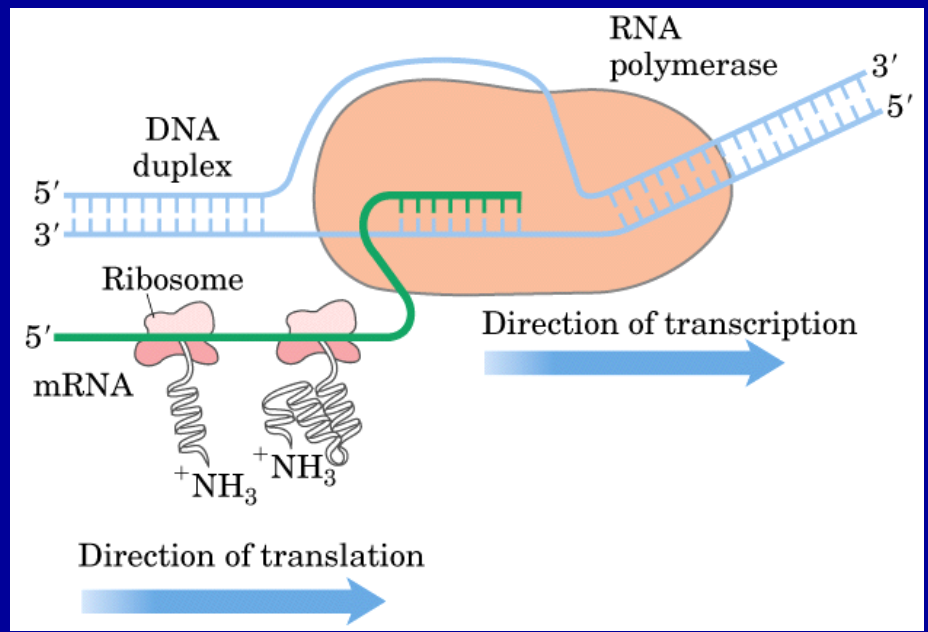


Polissomos:



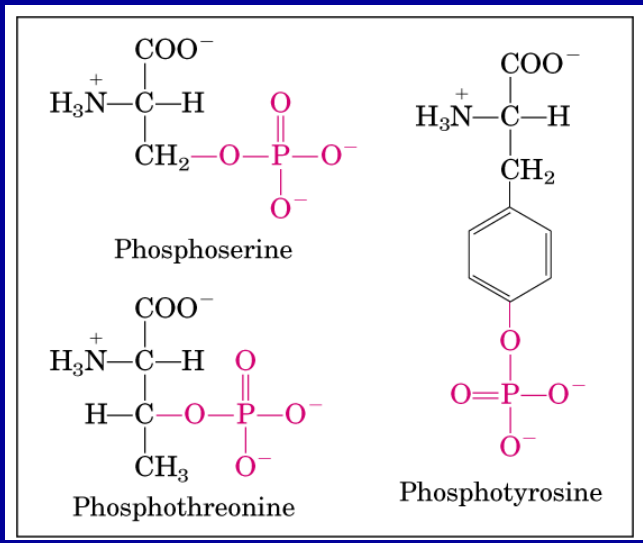


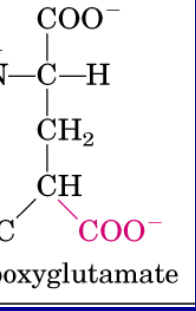
Acoplamento transcrição-tradução em bactérias:

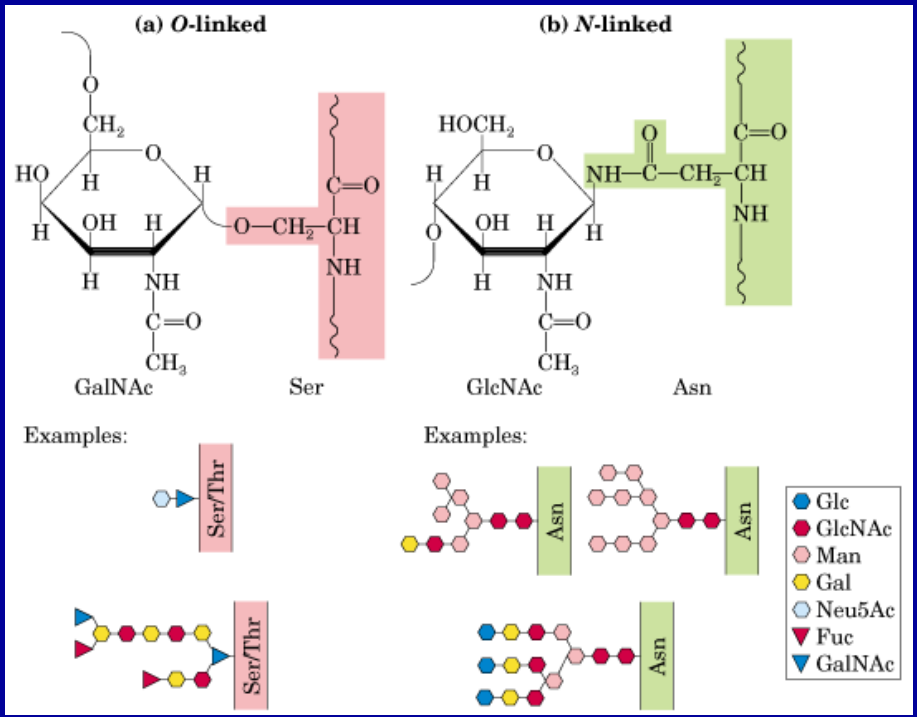


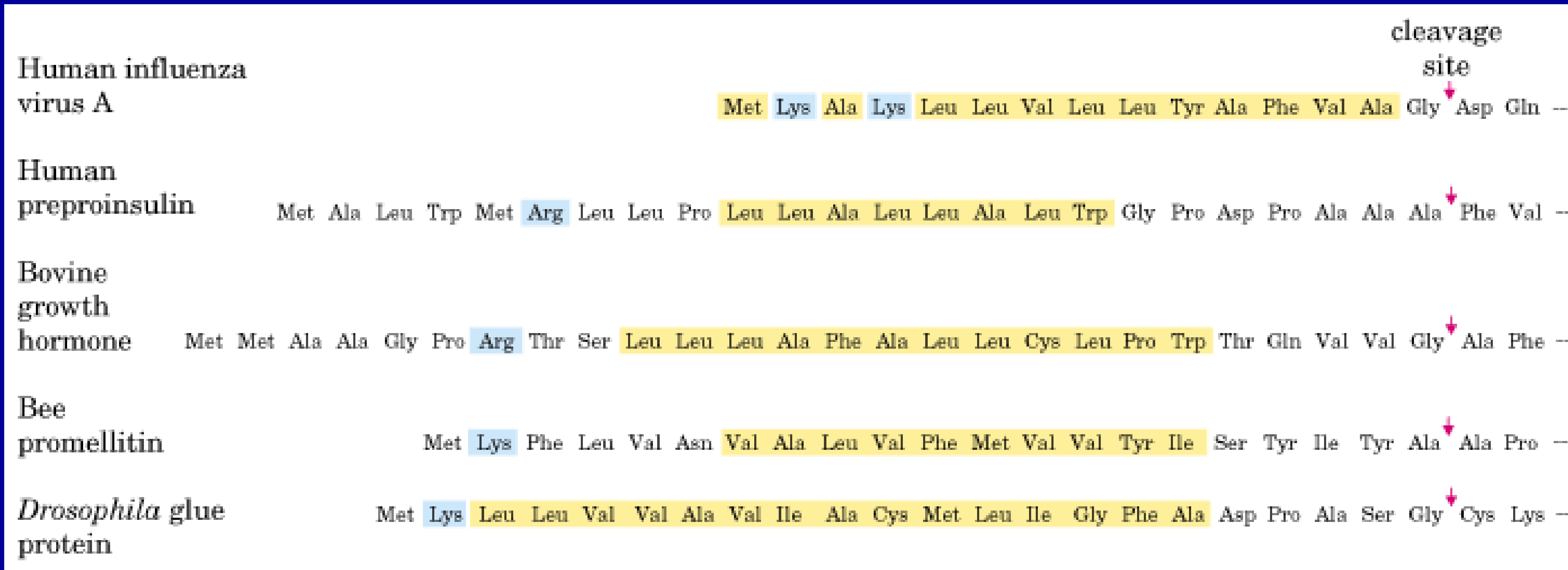
5° estágio: Folding e modificações pós traducionais.

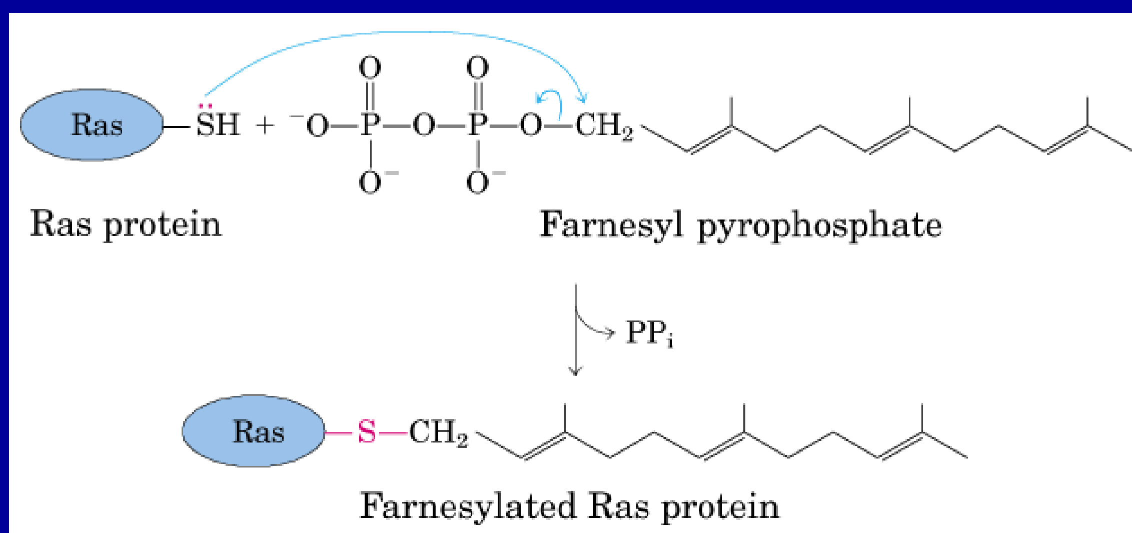
- Modificações no N-terminal e no C-terminal;
- Formação de ligações dissulfeto;
- Modificações individuais em aminoácidos;
- Ligações com carboidratos;
- Adição de grupos prostéticos;
- Processamento proteolítico;
- Perda de sequência(s) sinal;
- Adição de grupos isoprênicos.





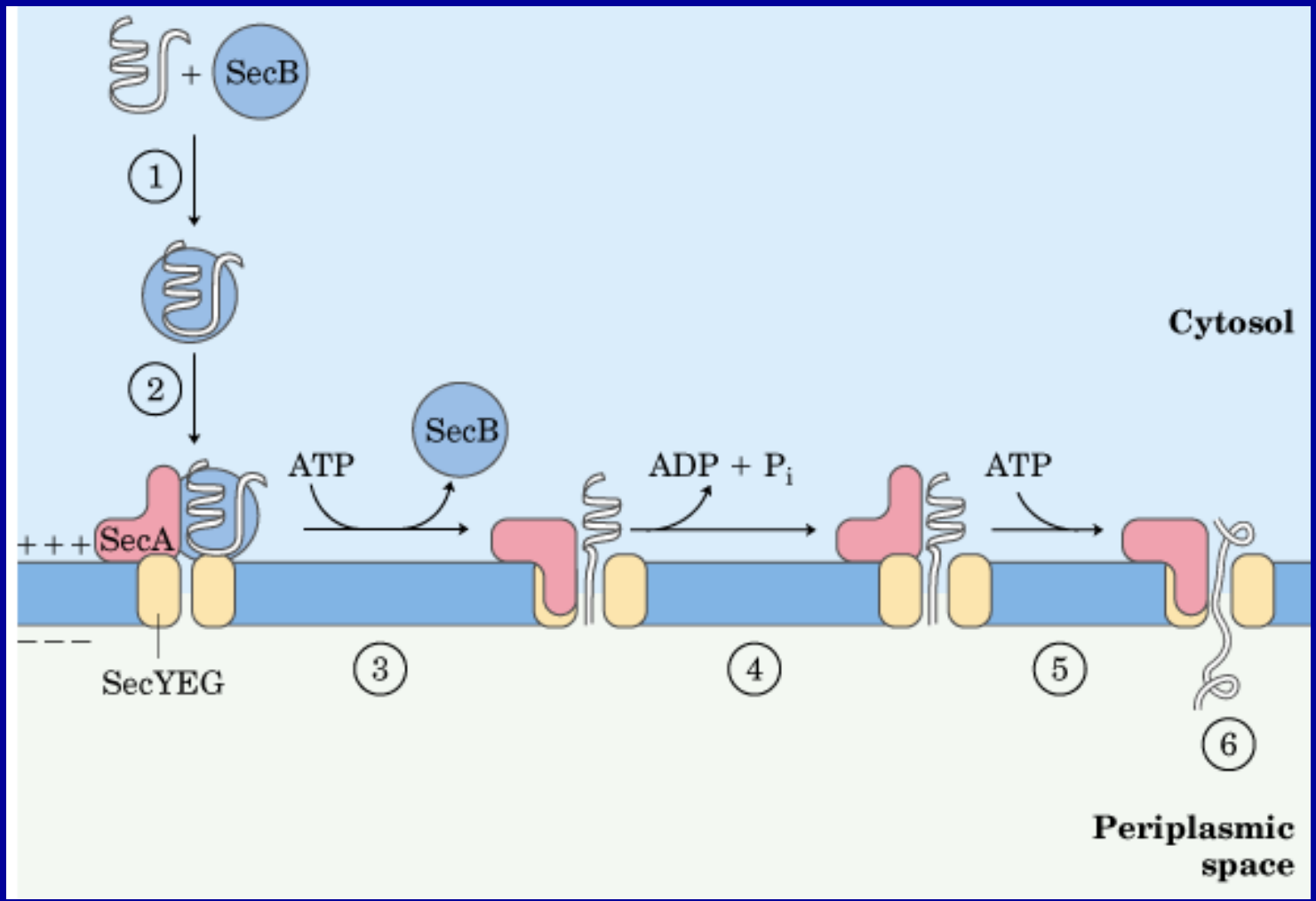



cleavage site
Human influenza virus A
Met Lys Ala Lys Leu Leu Val Leu Leu Tyr Ala Phe Val Ala Gly Asp Gln --
Human preproinsulin
Met Ala Leu Trp Met Arg Leu Leu Pro Leu Leu Ala Leu Leu Ala Leu Trp Gly Pro Asp Pro Ala Ala Ala Phe Val --
Bovine growth hormone
Met Met Ala Ala Gly Pro Arg Thr Ser Leu Leu Leu Ala Phe Ala Leu Leu Cys Leu Pro Trp Thr Gln Val Val Gly Ala Phe --
Bee promellitin
Met Lys Phe Leu Val Asn Val Ala Leu Val Phe Met Val Val Tyr Ile Ser Tyr Ile Tyr Ala Ala Pro --
Drosophila glue protein
Met Lys Leu Leu Val Val Ala Val Ile Ala Cys Met Leu Ile Gly Phe Ala Asp Pro Ala Ser Gly Cys Lys --

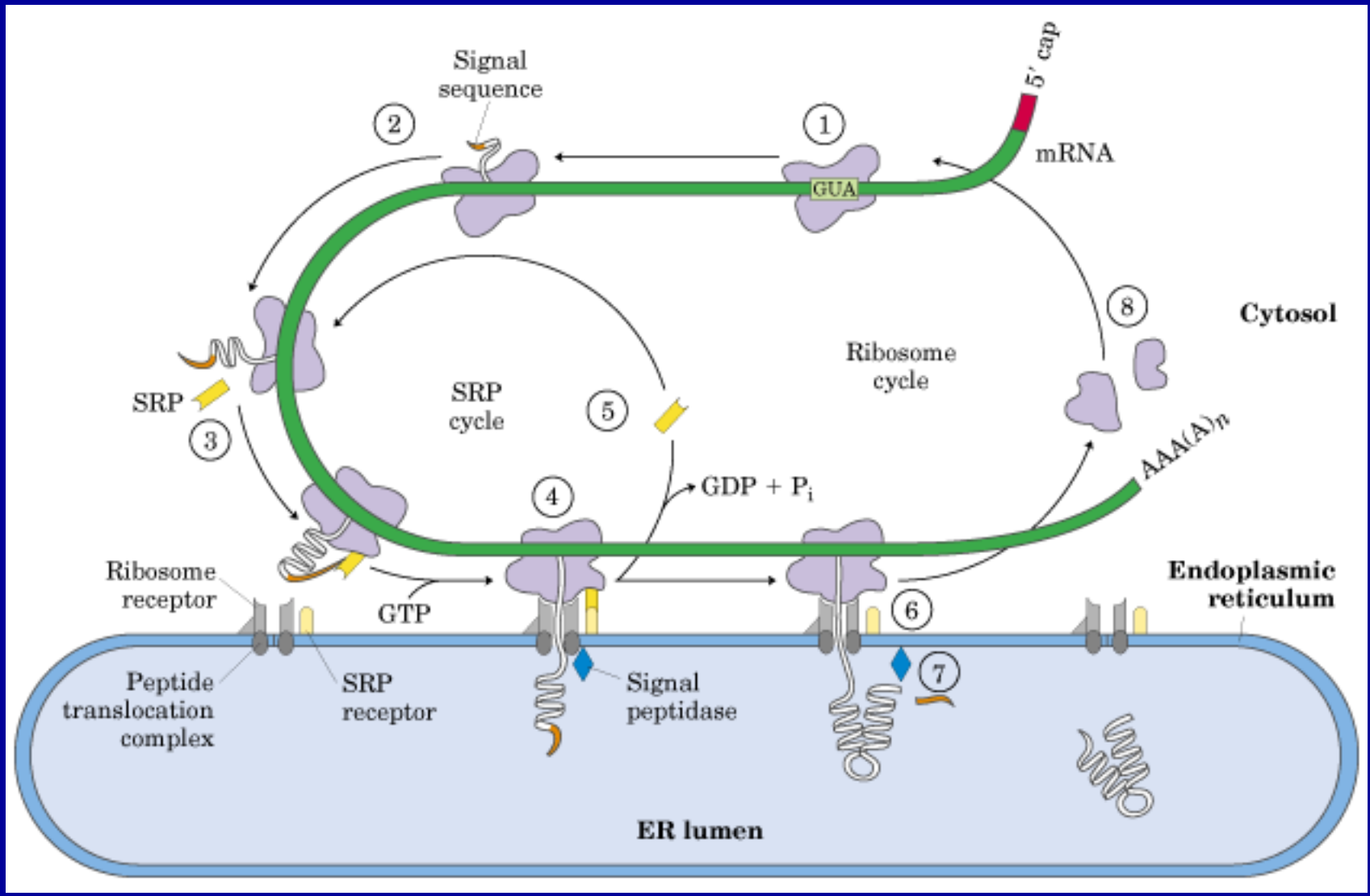

Ras
SH
O
P
O
P
O
CH2
O
O
O
O
Ras protein
Farnesyl pyrophosphate
PPi
Ras
S
CH2
Farnesylated Ras protein

Direcionamento de proteínas em procariotos:

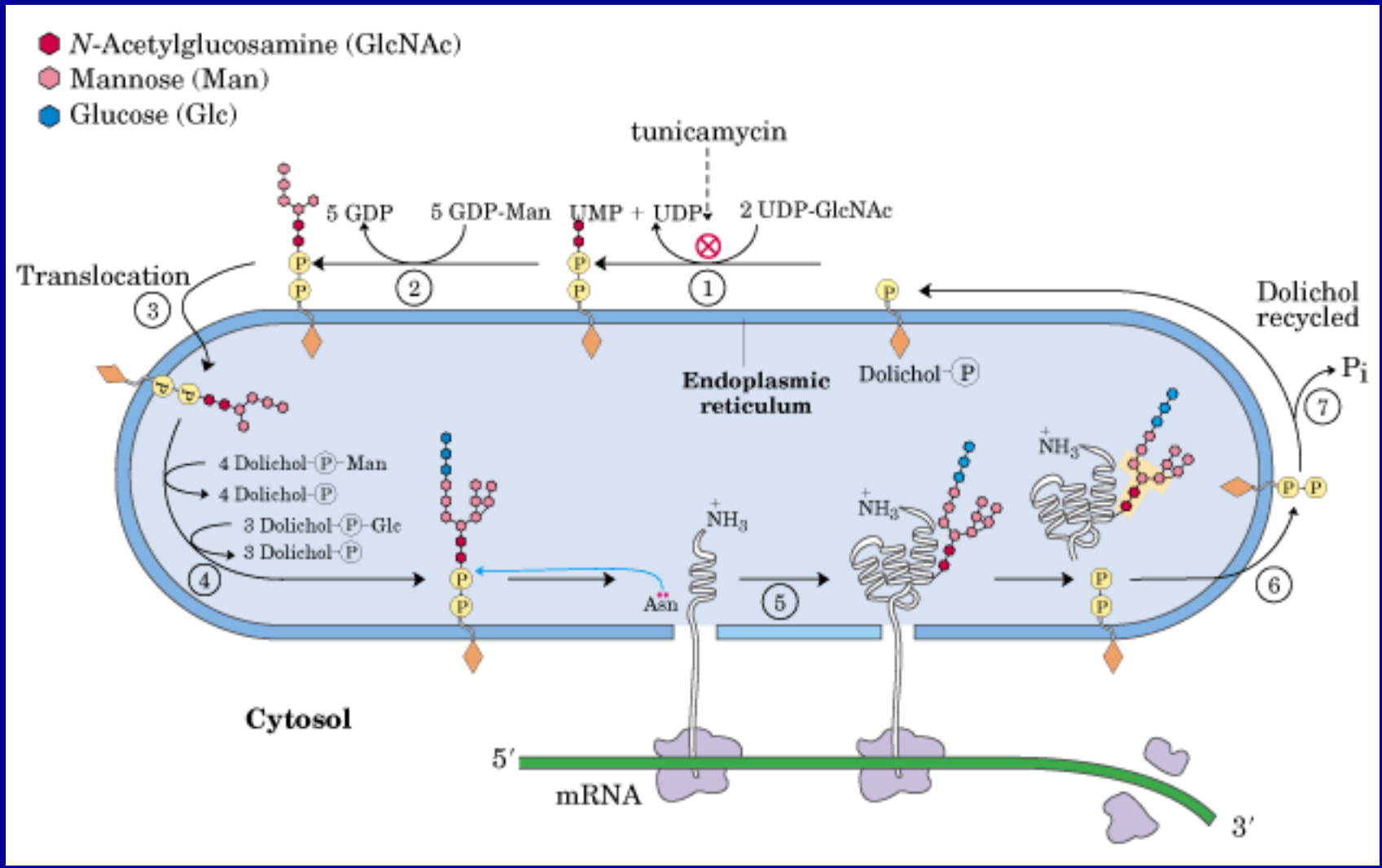
Direcionamento de proteínas em procariotos:



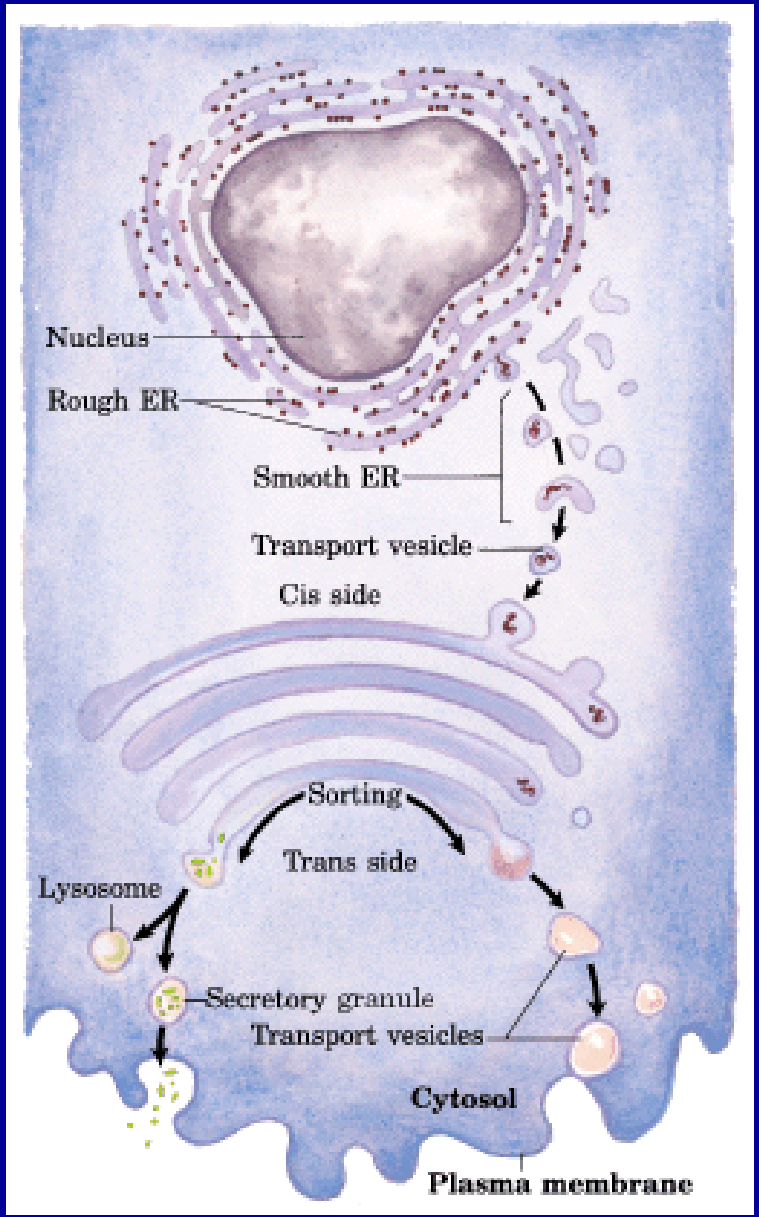
Direcionamento de proteínas em eucariotos: retículo endoplasmático

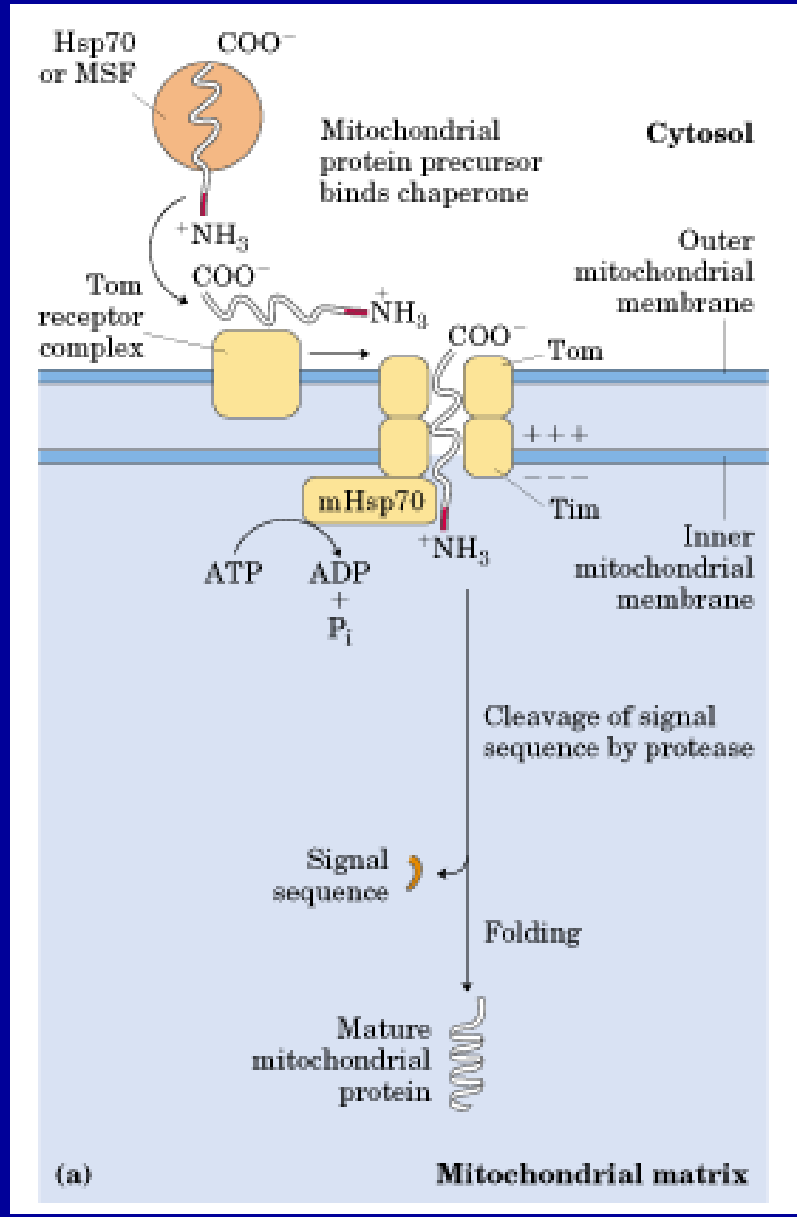


Síntese da porção oligossacarídica para as glicoproteínas:

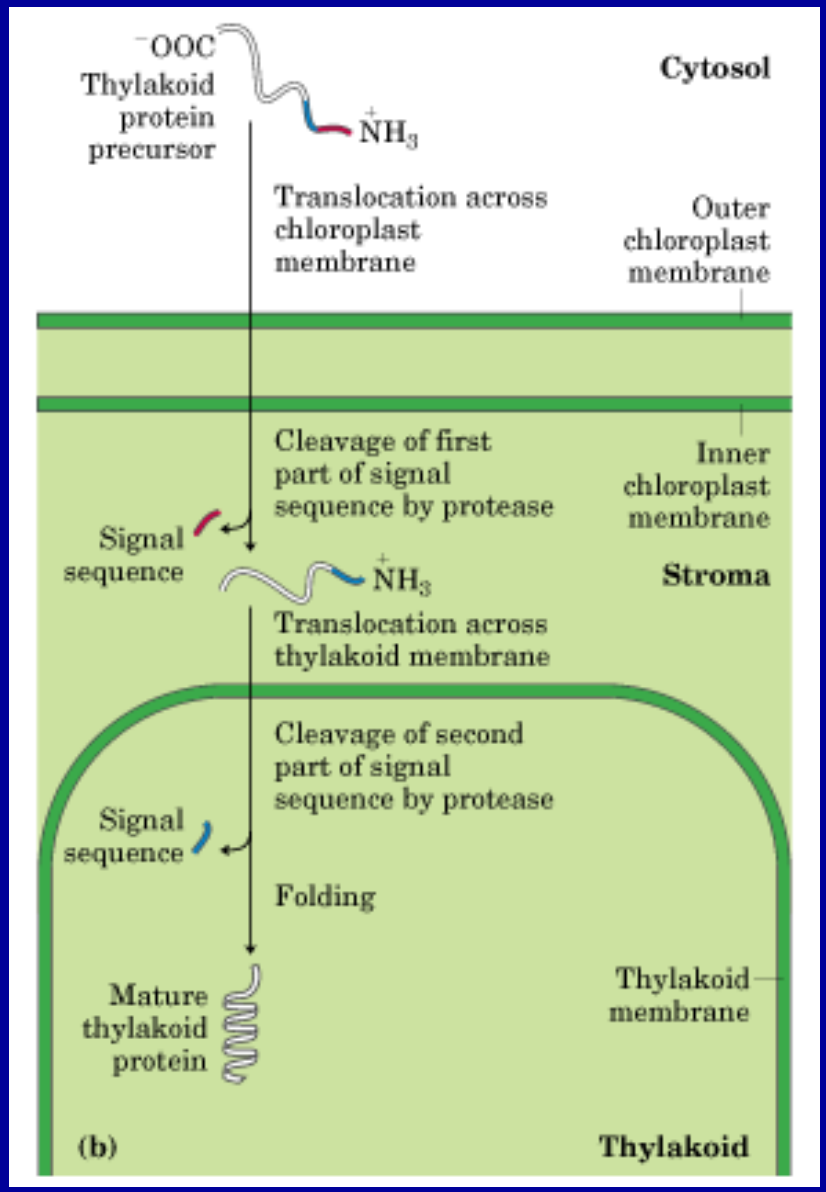


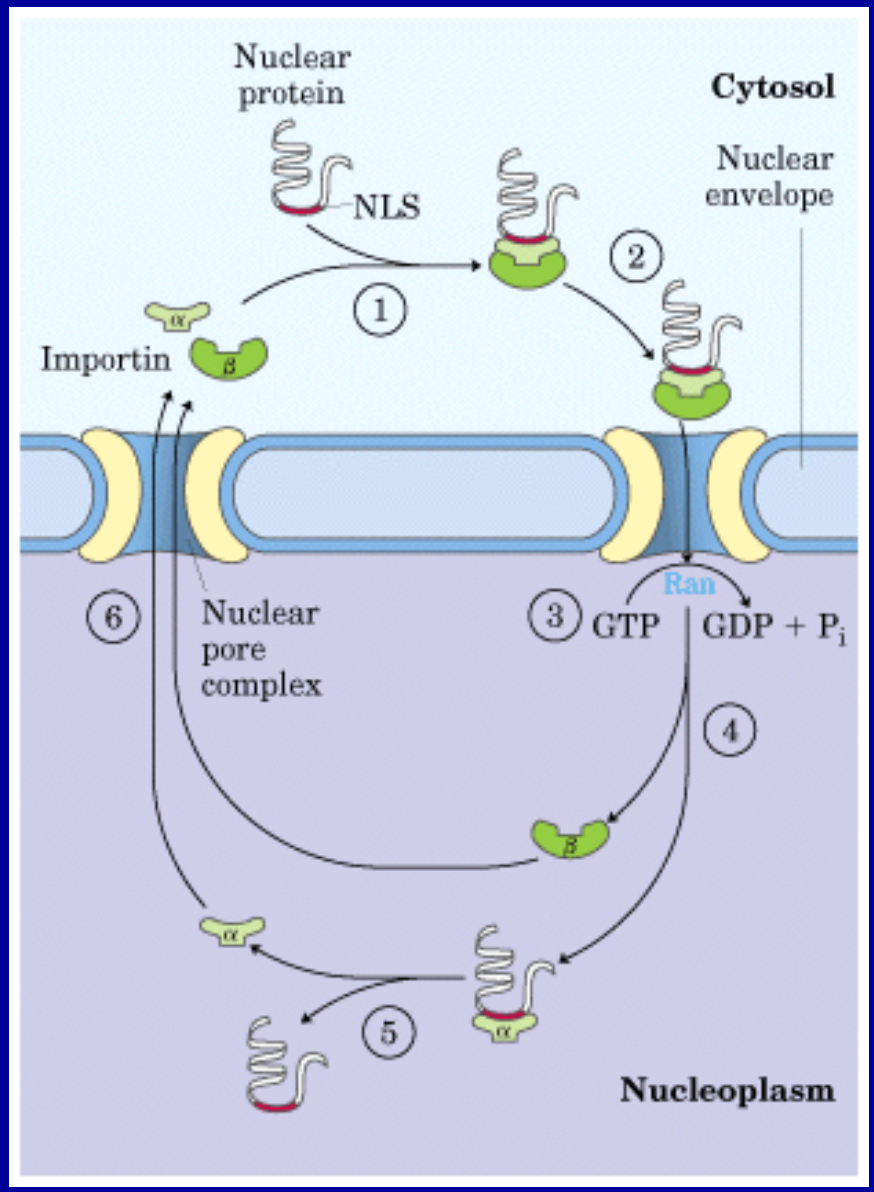
Direcionamento de proteínas em eucariotos:





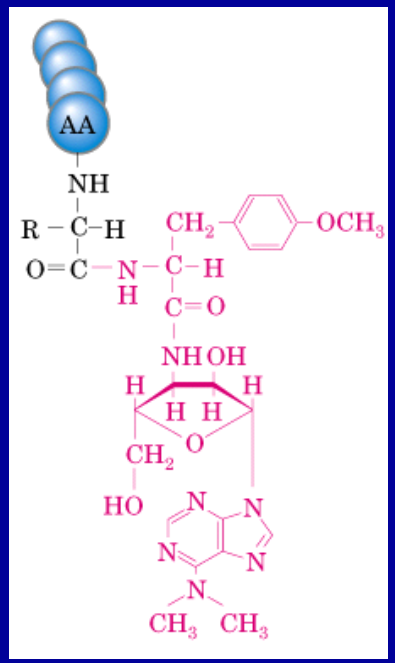
Direcionamento de proteínas em eucariotos:

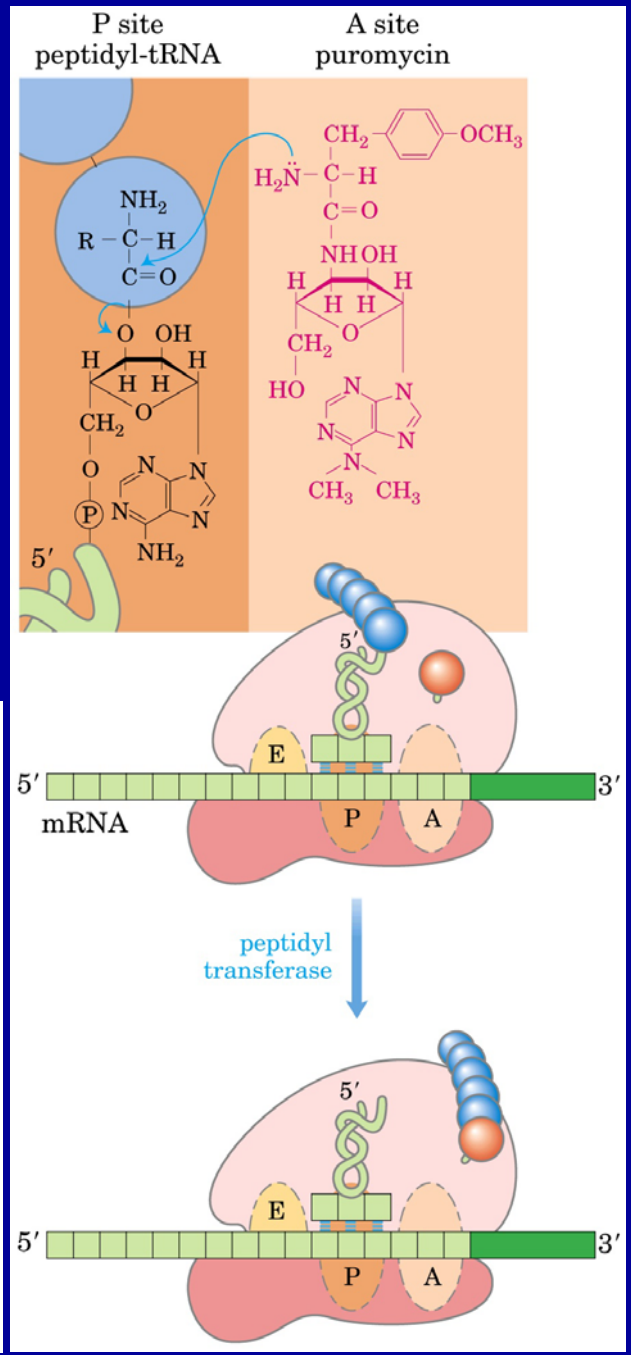




Inibição da síntese de proteínas por antibióticos:

- Puromicina: semelhante à região 3' do aminoacil-tRNA;
- Tetraciclinas: bloqueadores do sítio A;
- Clorafenicol: bloqueia a tranferência de peptídeos em procariontes (*);
- Cicloheximida: bloqueia a tranferência de peptídeos em eucariontes (80S);
- Estreptomicina: inibe a leitura do código genético e a etapa de iniciação;





Degradação protéica em eucariotos: proteassomo

Relationship between Protein Half-Life and Amino-Terminal Amino Acid Residue

Amino-terminal residue	Half-life*
Stabilizing	
Met, Gly, Ala, Ser, Thr, Val	>20 h
Destabilizing	
Ile, Gln	~30 min
Tyr, Glu	~10 min
Pro	~7 min
Leu, Phe, Asp, Lys	~3 min
Arg	~2 min

Source: Modified from Bachmair, A., Finley, D., & Varshavsky, A. (1986) In vivo half-life of a protein is a function of its amino-terminal residue. *Science* **234,** 179–186.

*Half-lives were measured in yeast for a single protein modified so that in each experiment it had a different amino-terminal residue. (See Chapter 29 for a discussion of techniques used to engineer proteins with altered amino acid sequences.) Half-lives may vary for different proteins and in different organisms, but this general pattern appears to hold for all organisms: amino acids listed here as stabilizing when present at the amino terminus have a stabilizing effect on proteins in all cells.