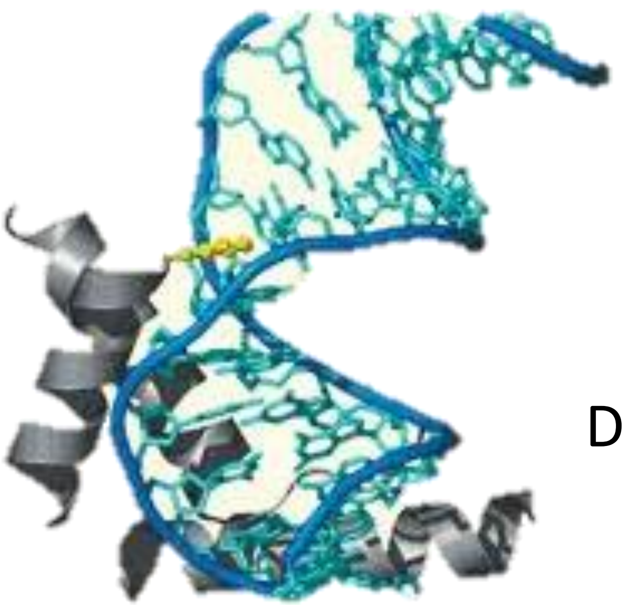
MECANISMOS BÁSICOS DA GENÉTICA MOLECULAR: DO GENE À PROTEÍNA (Parte 2)

Aula 3

LGN0232 – Genética Molecular



Maria Carolina Quecine
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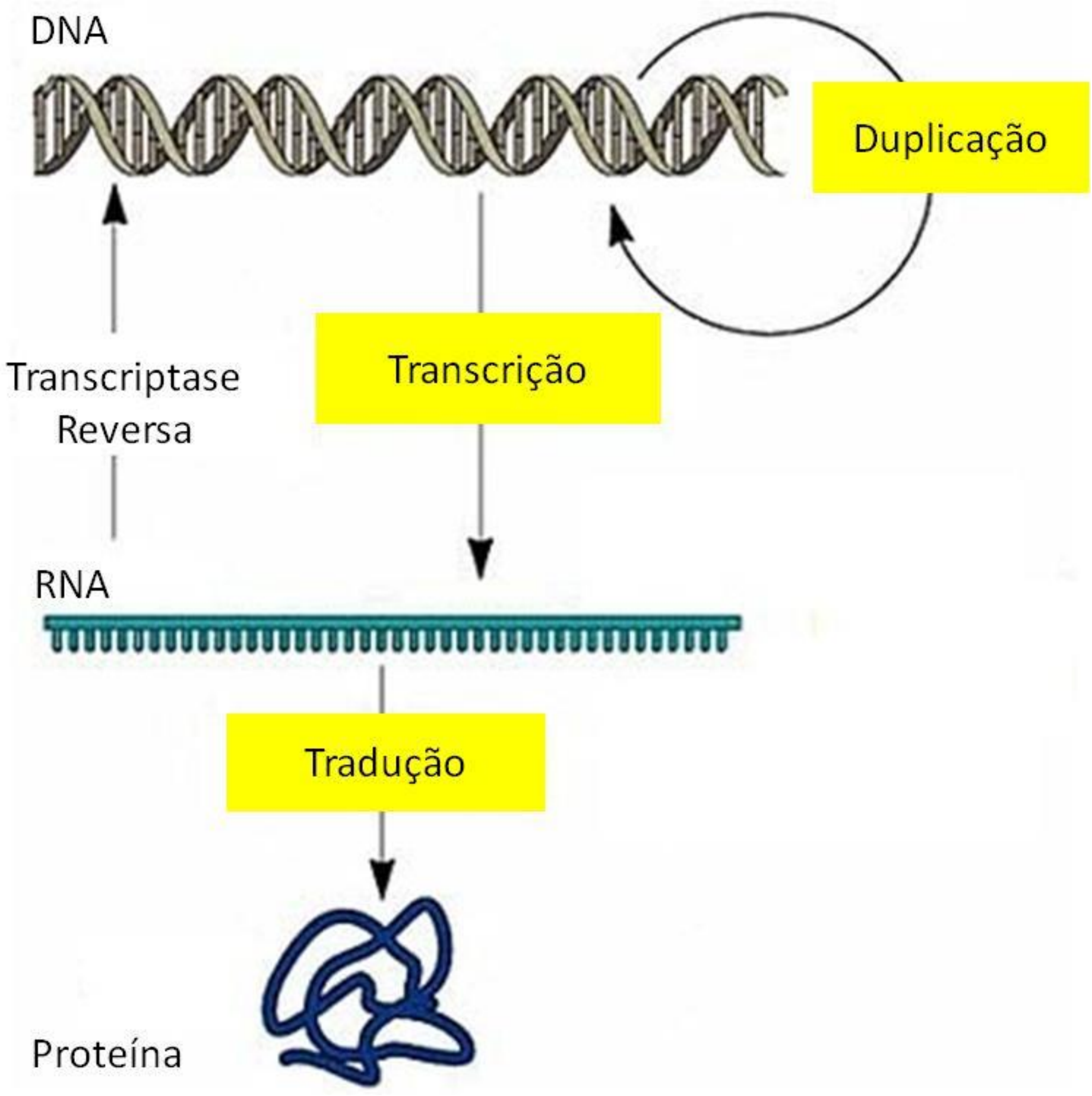
SUMÁRIO

- Fluxo da informação genética;
- Conceito de gene;
- O processo de tradução;
- Aplicação dos conhecimentos adquiridos
- Exercício mapa conceitual.

A MAQUINARIA GENÉTICA É A MESMA



MAS O QUE É UM GENE?

DEFINIÇÃO DE GENE

Wilhelm Johannsen

1909 → gene

- Um gene → unidade da informação genética que controla a síntese de polipeptídios ou uma molécula de RNA estrutural

mRNA → polipeptídeo

tRNA e rRNA → RNA estrutural

- Gene inclui as regiões 5´e 3´não codificantes, que estão envolvidas na regulação da transcrição e tradução, e todos os introns dentro do gene

GENE TÍPICO DE PROCARIOTOS

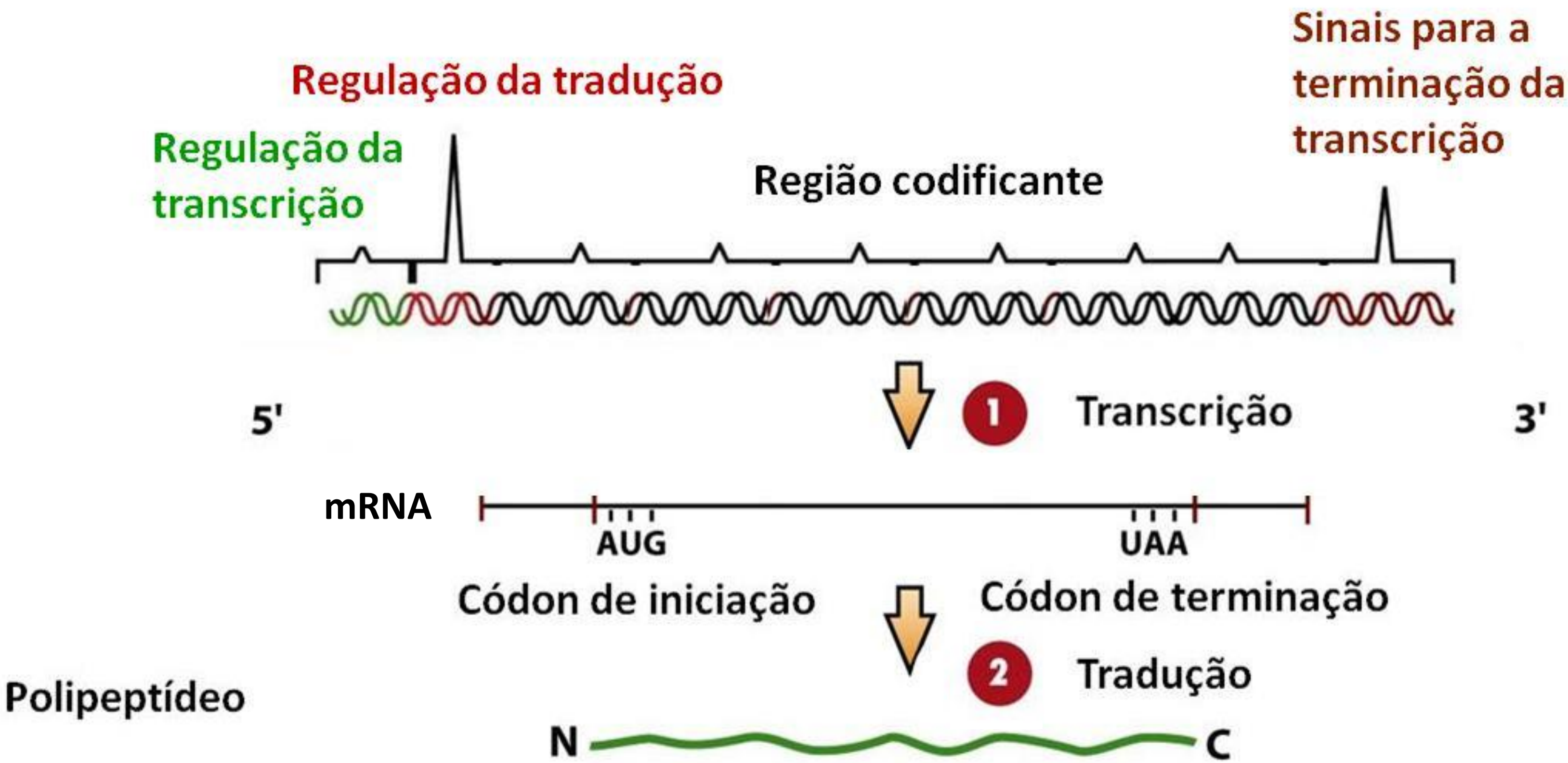


Figure 14-1b Principles of Genetics, 4/e

GENE TÍPICO DE EUCARIOTOS

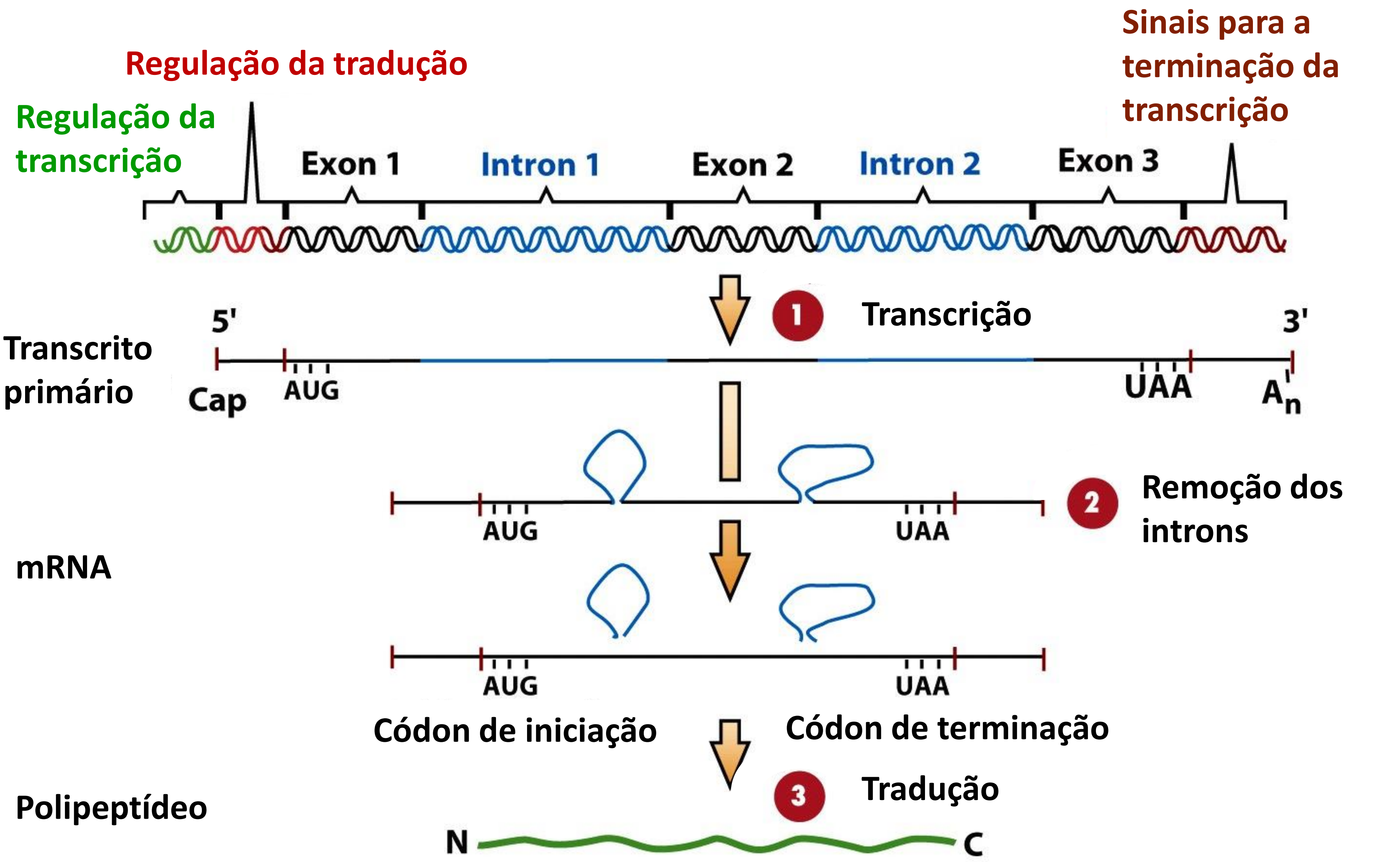
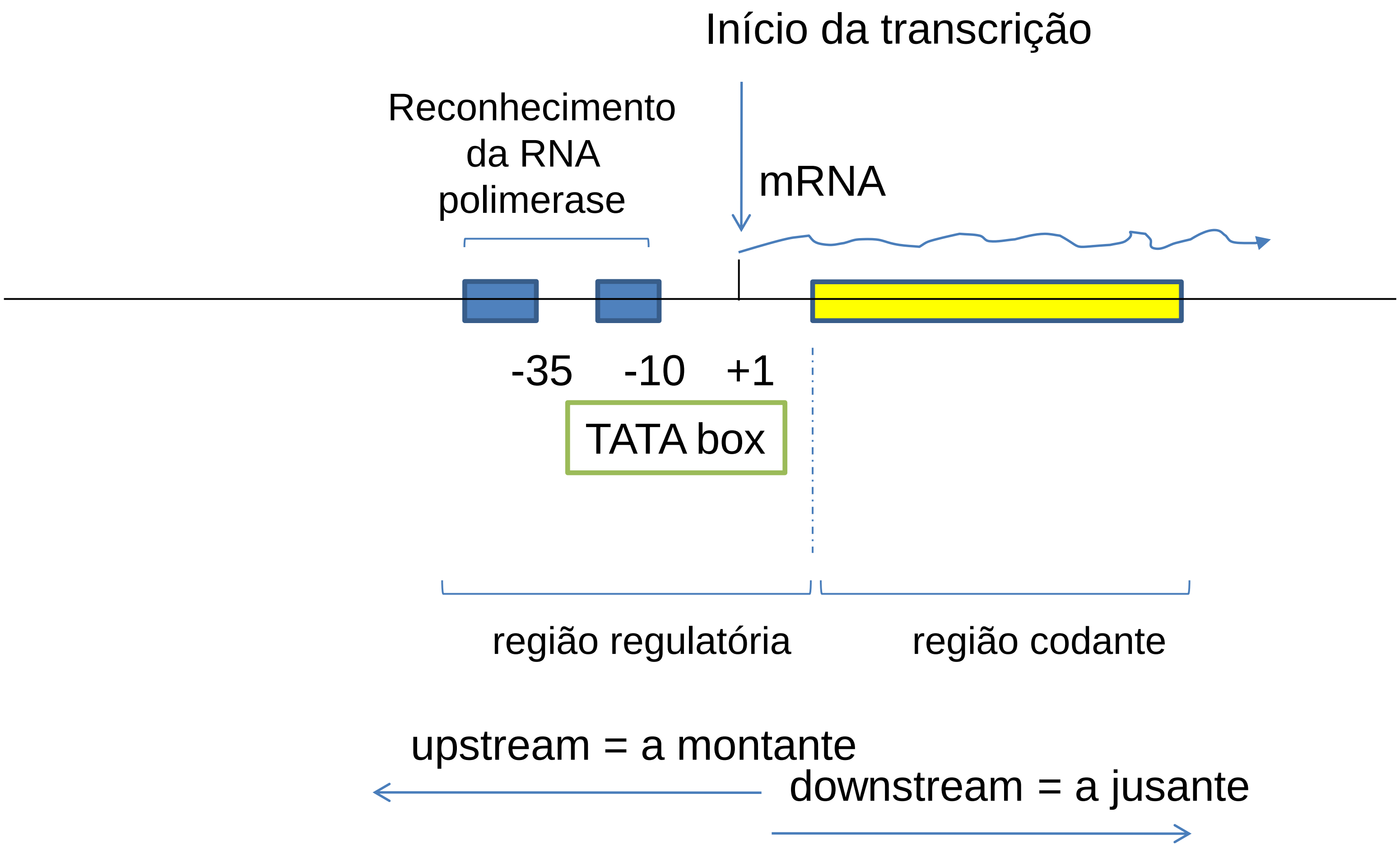
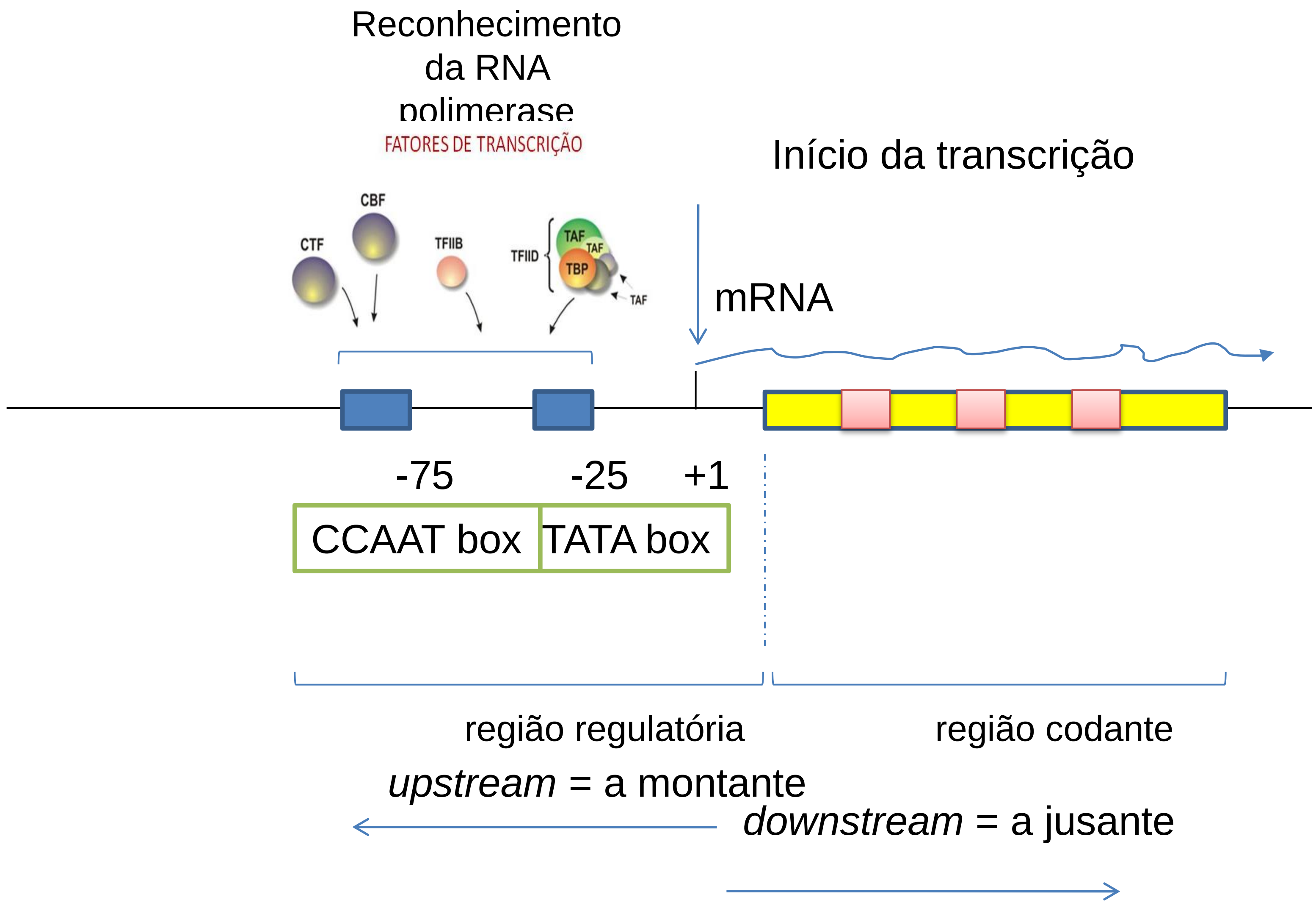


Figure 14-1b Principles of Genetics, 4/e

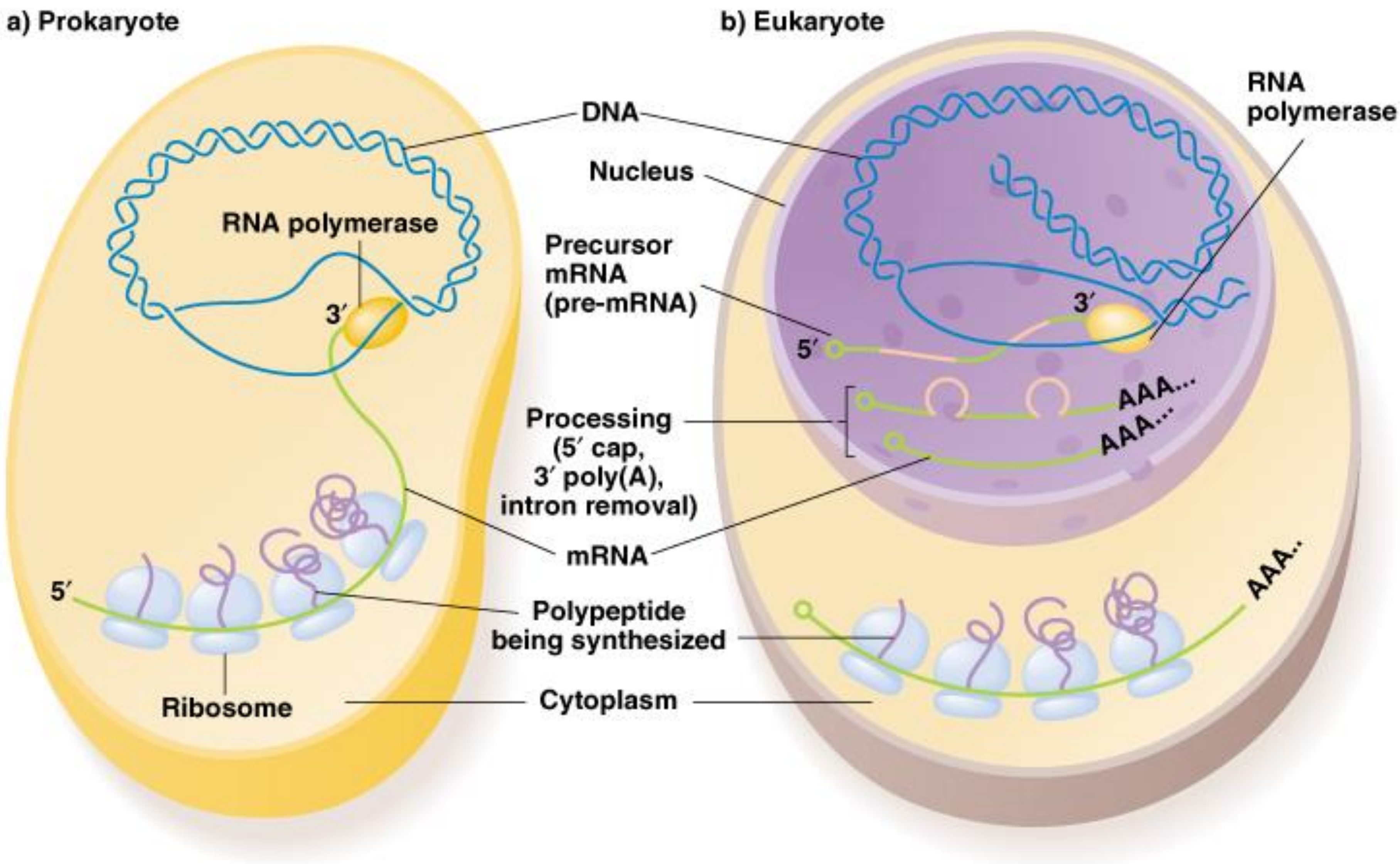
ESTRUTURA DO PROMOTOR EM PROCARIOTOS



ESTRUTURA DO PROMOTOR EM EUCARIOTOS



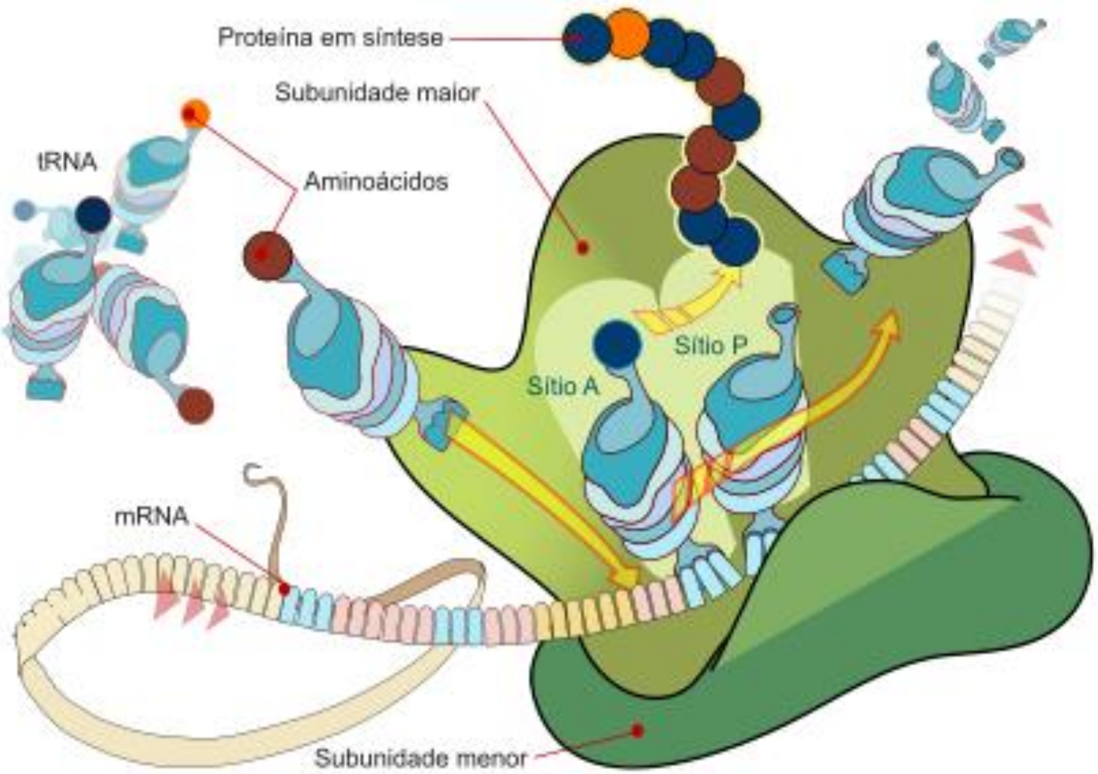
VISÃO GERAL



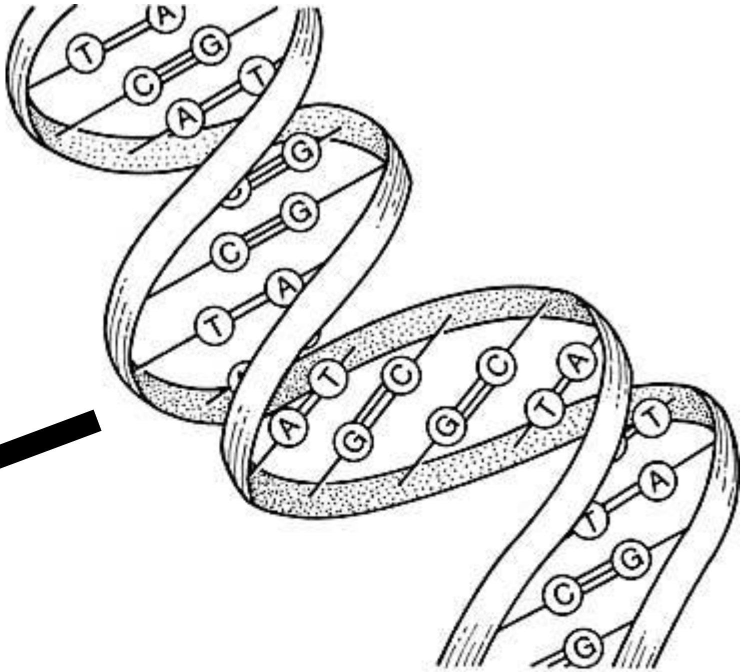
Animação: http://www.youtube.com/watch?v=983lhh20rGY&feature=related

TRADUÇÃO

Processo altamente conservado no qual as proteínas são sintetizadas a partir dos moldes de mRNA com auxílio de RNA estruturais.



TRADUÇÃO



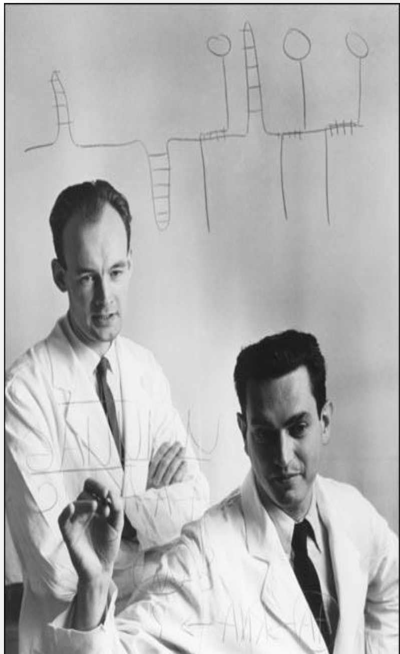
Laurel Cook Lhowe

Interpretação

AAGTCCTTTTAAATAAATAATTCTAGCTATATTTGCAAC
GTTGGAAAATTAGCTATTCTAATGTTATCGAAAGAAGAA
CACAGTTACTTAGTTTCTCGGCAAACTATATCAAAATGA
GAAGGTGAAAGAGTGGCATAATGATAAGCAAATCTGAAA
ATTTTTTGGTATAATAATCTTGATTGAAATTTGAATGGA
GTAGGCTTACCAAATGTTGGTAAATCAACCTTATTTAAC
ATTATCCTTTTGCGACTATTGATCCCAATGTTGGTATGG
GACAGAATTGATTACACCTAAAAAAACAGTTCCGACAAC
AAAGGTGCTTCTAGAGGGGAAGGTCTAGGAAATAAATTT
TTCATGTGGTACGTGCTTTTGATGATGAAAATGTCATGC
TCCTATAGCAGATATTGACACTATTAATCTTGAATTAAT
TATGCGCGTGTTGAAAAAATGGCACGAACTCAAAAAGAT
AAAAGATTAAACCTGTTTTGGAAGATGGGAAATCAGCTA
AGTTGTTAAAGGTCTCTTTTTATTAACAACTAAACCTGT
GTTGCTAATCTAGATGGTATTGATTATGTCAAACAAATT
TAGTTGTTATCTCAGCGCGTGCAGAAGAAGAAATTTCAG
GGAAGCTATCGGTCTTACTGAATCAGGCGTTGATAAATT
GGAACCTATTTTACAGCAGGTGAAAAAGAGGTTCGTGCT
AAGCTGCTGGTATTATCCATTCAGATTTTGAAAGAGGTT

Colocar em outra linguagem!!

Met -Lys –Trp...



The Nobel Prize in Physiology or Medicine 1968 was awarded jointly to Robert W. Holley, Har Gobind Khorana and Marshall W. Nirenberg "for their interpretation of the genetic code and its function in protein synthesis."

1961

Código Genético

https://history.nih.gov/exhibits/nirenberg/hs4_polyu.htm

1. Composto por trincas de bases nitrogenadas (portanto, existem 64 possibilidades - $4^3 = 64$);
2. São codificados, pelo código genético, 20 aminoácidos presentes nas proteínas;
3. Código genético é degenerado (e portanto, redundante), e linear;
4. Cada trinca (códon) codifica um único aminoácido, no entanto, um aminoácido pode ser codificado por mais do que uma trinca.

CARACTERÍSTICAS GERAIS DA TRADUÇÃO

- ✓ Todos os RNAs mensageiros são lidos na direção 5’- 3’;
- ✓ As cadeias polipeptídicas são sintetizadas da extremidade amina (NH_3) para a carboxila terminal (COOH) – <u>ligação peptídica</u>;
- ✓ A tradução é realizada nos ribossomos, com os RNA transportadores como <u>adaptadores</u> entre o molde de mRNA e os aminoácidos;
- ✓ Cada aminoácido é especificado por três bases (códon) no mRNA – código genético universal.

CARACTERÍSTICAS GERAIS DA TRADUÇÃO

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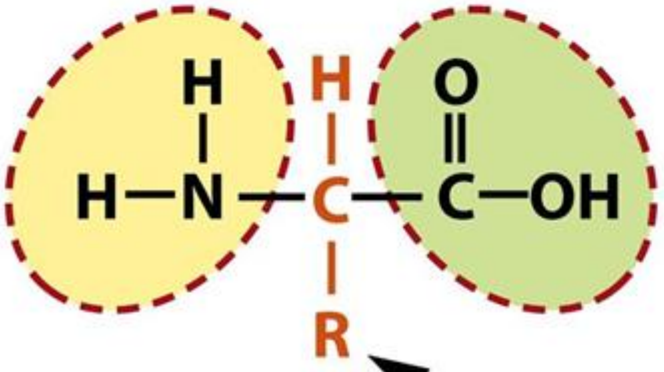
ESTRUTURA DA PROTEÍNA

Aminoácidos (20 tipo):

→ grupo amino;
→ grupo carboxila;
→ grupo lateral.

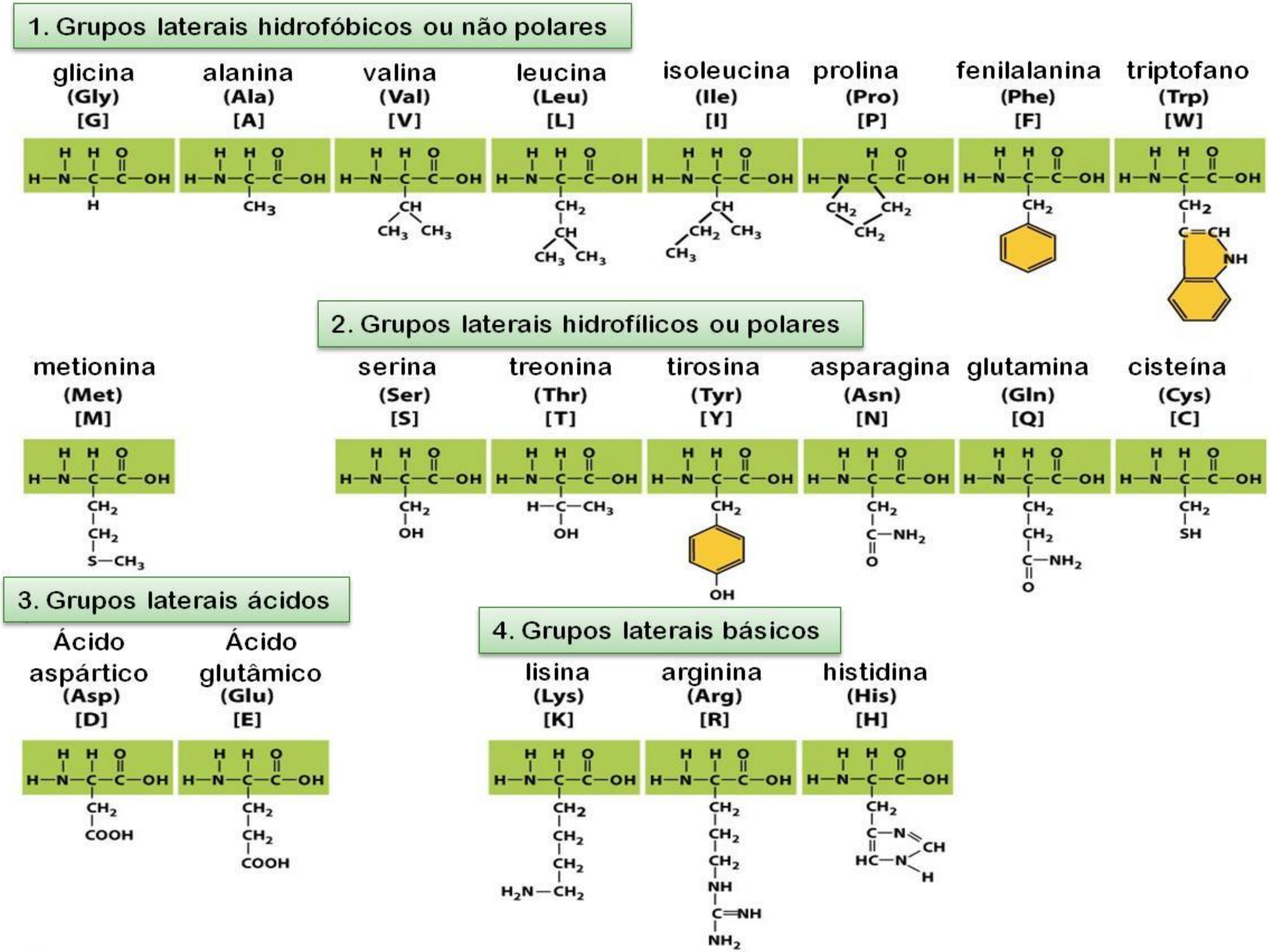
Grupo amino

Grupo carboxila

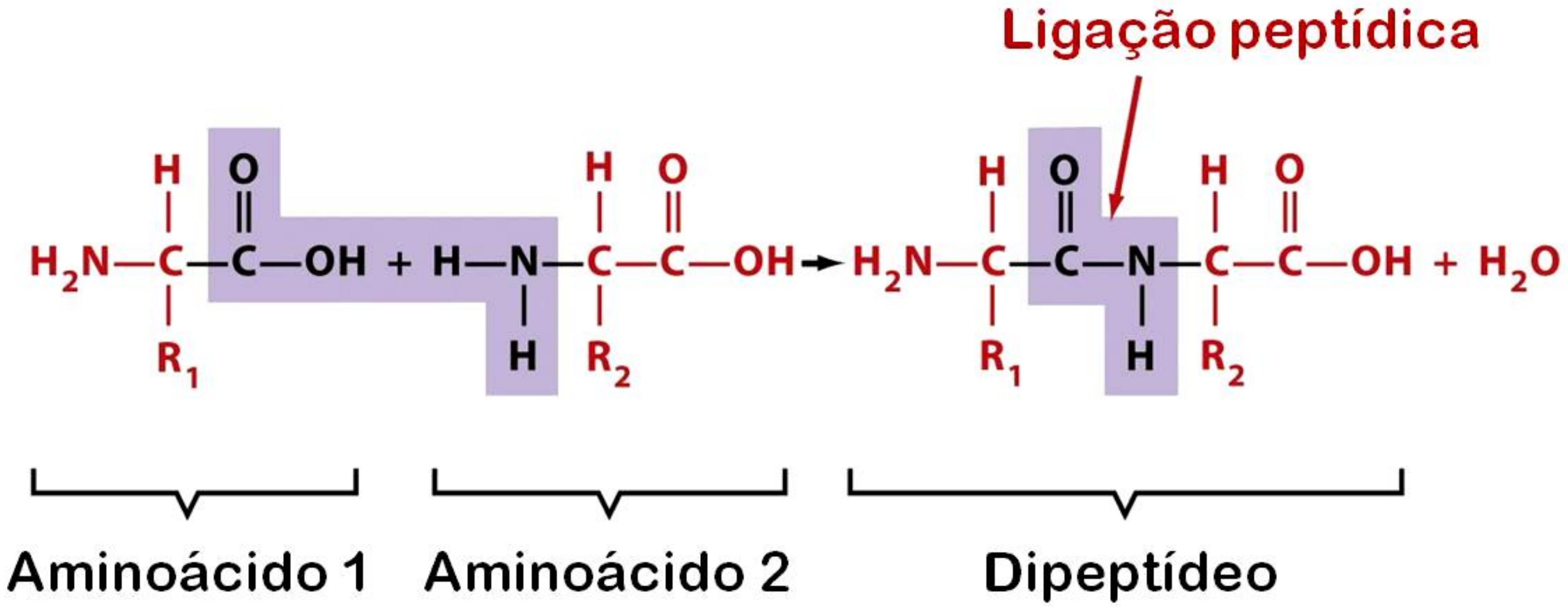


Grupo Lateral (R)

Unnumbered figure pg 312 Principles of Genetics, 4/e


1. Grupos laterais hidrofóbicos ou não polares
glicina (Gly) [G]
alanina (Ala) [A]
valina (Val) [V]
leucina (Leu) [L]
isoleucina (Ile) [I]
prolina (Pro) [P]
fenilalanina (Phe) [F]
triptofano (Trp) [W]
metionina (Met) [M]
2. Grupos laterais hidrofílicos ou polares
serina (Ser) [S]
treonina (Thr) [T]
tirosina (Tyr) [Y]
asparagina (Asn) [N]
glutamina (Gln) [Q]
cisteína (Cys) [C]
3. Grupos laterais ácidos
Ácido aspártico (Asp) [D]
Ácido glutâmico (Glu) [E]
4. Grupos laterais básicos
lisina (Lys) [K]
arginina (Arg) [R]
histidina (His) [H]

LIGAÇÃO PEPTÍDICA



Proteína - direção de sintese

GCA GCC GCG GCU	AGA AGG CGA CGC CGG CGU	GAC GAU	AAC AAU	UGC UGU	GAA GAG	CAA CAG	GGA GGC GGG GGU	CAC CAU	AUA AUC AUU	UUA UUG CUA CUC CUG CUU	AAA AAG	AUG	UUC UUU	CCA CCC CCG CCU	AGC AGU UCA UCC UCG UCU	ACA ACC ACG ACU	UGG	UAC UAU	GUA GUC GUG GUU	UAA UAG UGA
Ala	Arg	Asp	Asn	Cys	Glu	Gln	Gly	His	Ile	Leu	Lys	Met	Phe	Pro	Ser	Thr	Trp	Tyr	Val	stop
A	R	D	N	C	E	Q	G	H	I	L	K	M	F	P	S	T	W	Y	V	

Figure 7-24 Essential Cell Biology 3/e (© Garland Science 2010)

A leitura correta do código genético pelo ribossomo é vital!!!

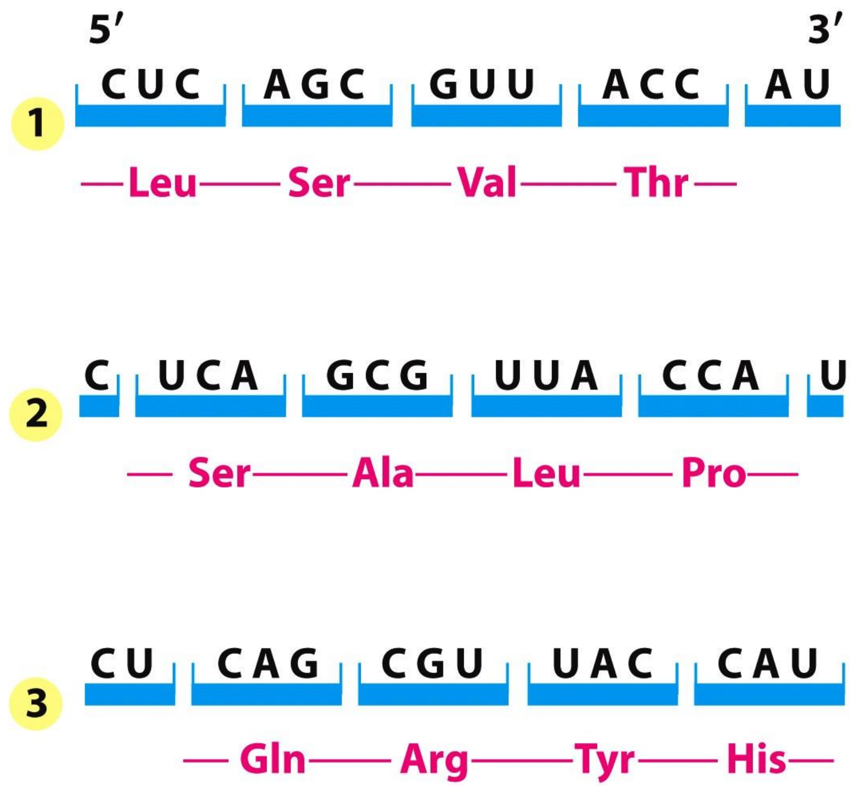


Figure 7-25 Essential Cell Biology 3/e (© Garland Science 2010)

START CODON E STOP CODON

Início: códon de iniciação da síntese protéica

– AUG -

↓

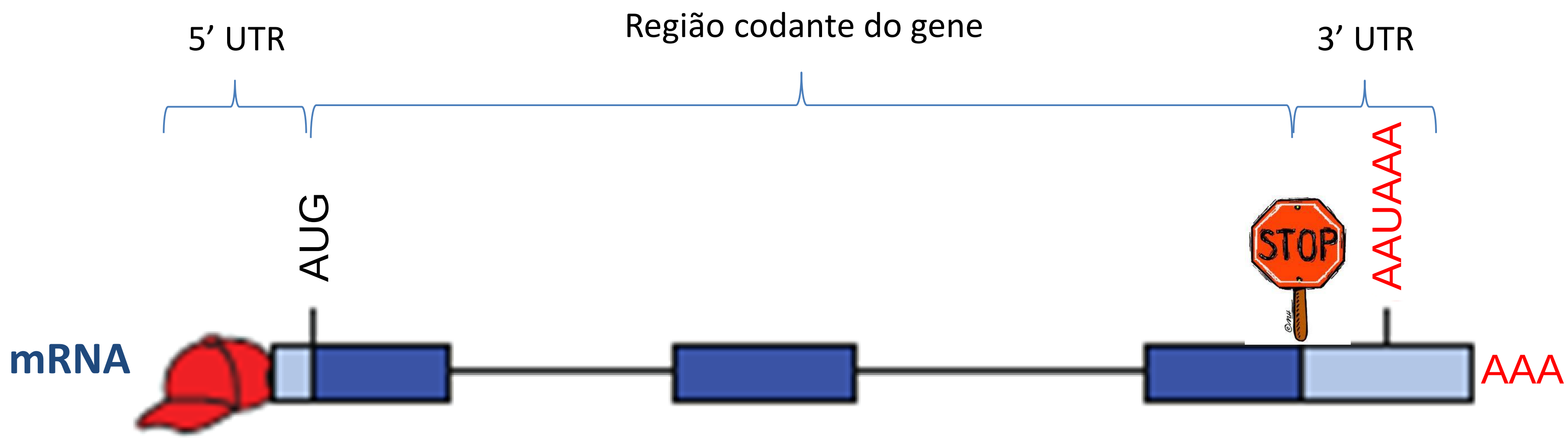
METIONINA

Terminação: três códons terminam a síntese protéica

– UAG – UAA – UGA -

START CODON E STOP CODON

- Delimitam a região codante (região que é transcrita e traduzida!)

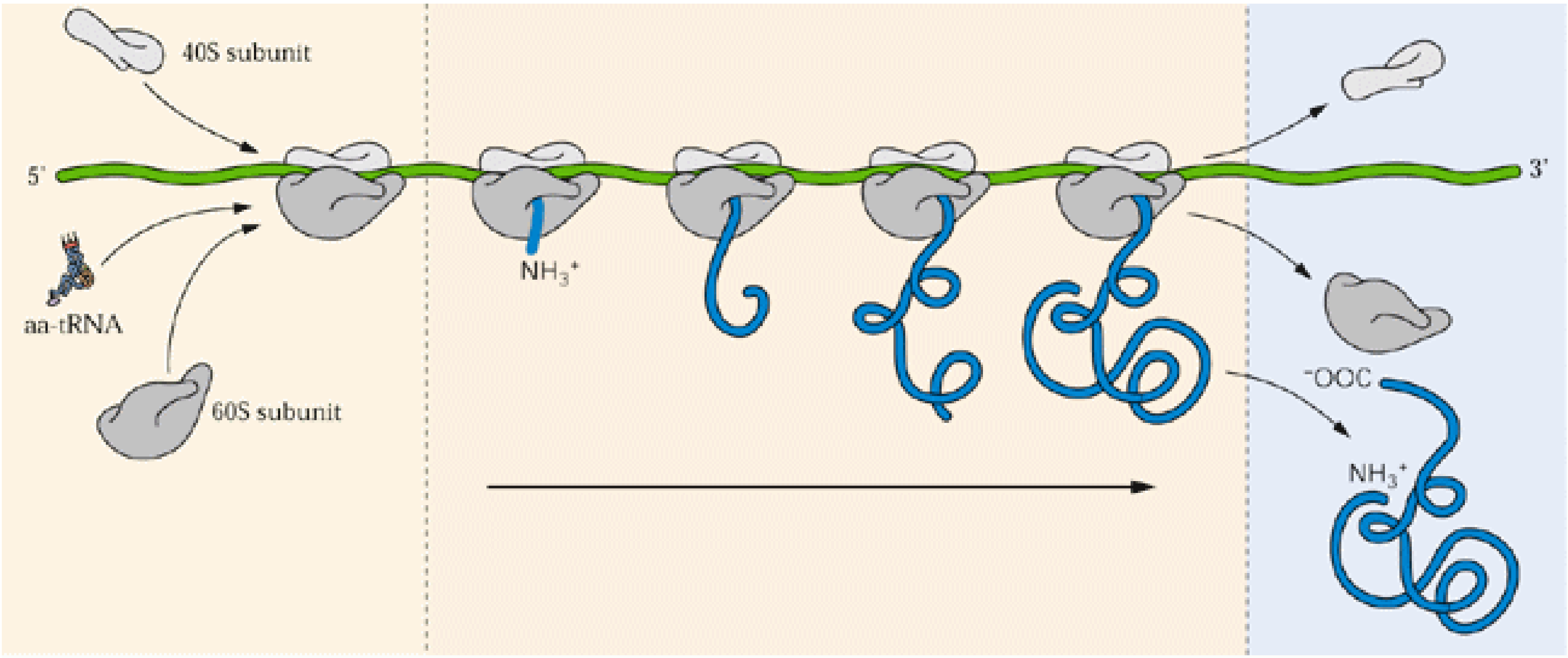


TRADUÇÃO: INÍCIO E FIM

TTCATACTTGGTTAAGACCTTTACAAGCCGACCAACGTGGTGAC
AGTGTCGTCCTTTACGCACCGAATCCCTTTATCATTGAATTAGT
AGAAGAGCGATACTTAGGACGTCTTCGG**ATG**GAATCTTGGTCCC
GTTGCCTGGAACGTCTTGAAACTGAATTCCCGCCAGAAGATGTT
CATACTTGGTTAAGACCTTTACAAGCCGACCAACGTGGTGACAG
TGTCGTCCTTTACGCACCGAATCCCTTTATCATATTGAATTAGT
AGAAGAGCGATACTTAGGACGTCTTCGGGAATTGTTATCCTATT
TCTCAGGAATACGTGAAGTAGTCCTTGCAATTGGCTCACGACCT
AAAACAACAGAACTACCCGTACCAGTAGACACTACAGGACGTTT
GTCTTCAACAGTCCCATTTAACGGAAATCTCGACACACACTATA
ACTT**TGA**TAATTTTGTTGAGGGACGAAGCAATCAACTCGCTCGT
GCTGCAGCTTGGCAAGCGGCACAGAAACCGGGAGACCGTACTCA
CAACCCTCTATTGCTCTATGGTGGGACTGGTTTGGGTAAAACCC
ATTTAATGTTTGCTGCAGGTAACGTAATGCGGCAAGTAAACCCA
ACTTATAAAGTAATGTATCTTCGTTCGGAACAGTTTTTCAGCGC
CATGATAAGAGCGTACAAGATAAAAGTATGGATCATAAGGTAA

TRADUÇÃO

INICIAÇÃO ALONGAMENTO TÉRMINO



DOBRAMENTO DE PROTEÍNAS

5’ → 3’

DNA

PROMOTOR

-35 -10 +1

START CODON

STOP CODON

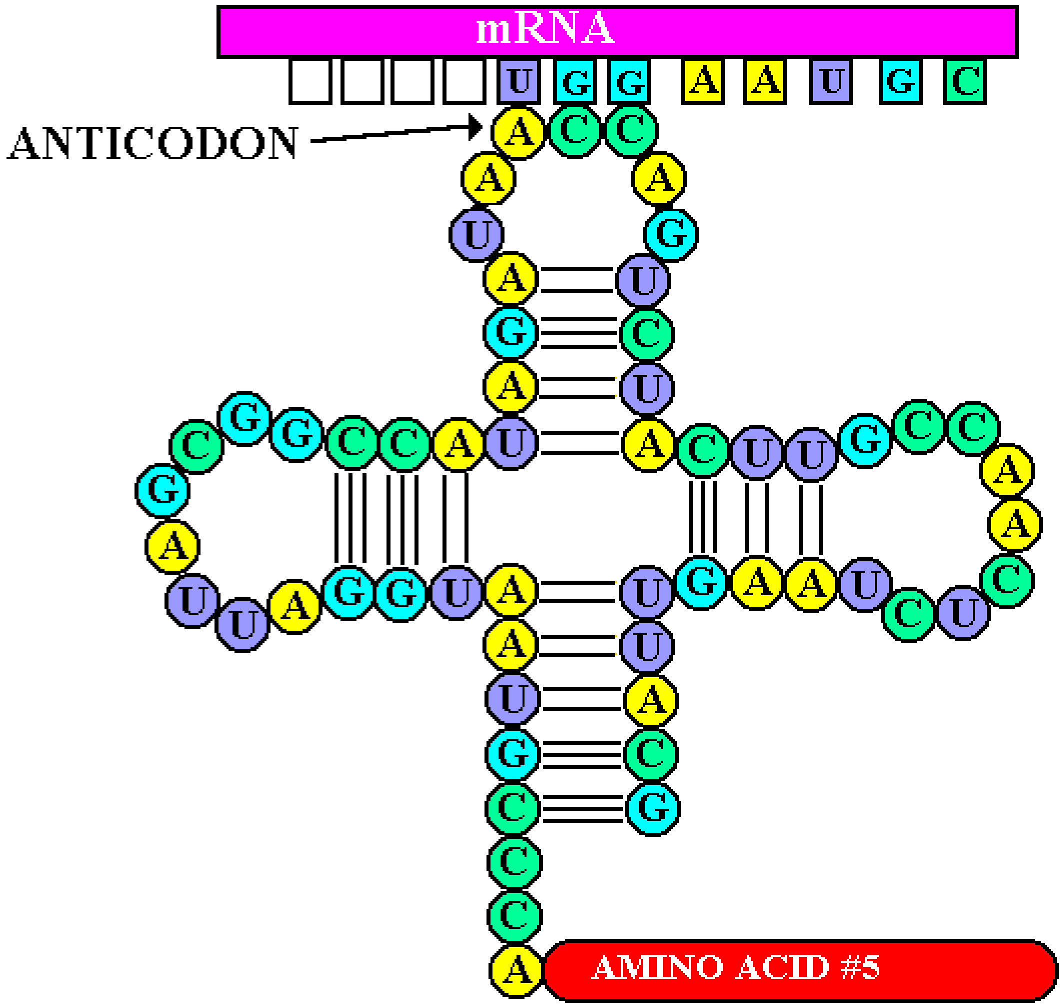
TTGACA-14N-TATAATAA-9N-CACAGGAGGATTATTTATGAAATTCCCTTCTTGGTGA

mRNA CACAGGAGGAUUAUCCAUGAAAUUCCCUUCUUGGUGA

PROTEINA MET LYS PHE PRO SER TRY

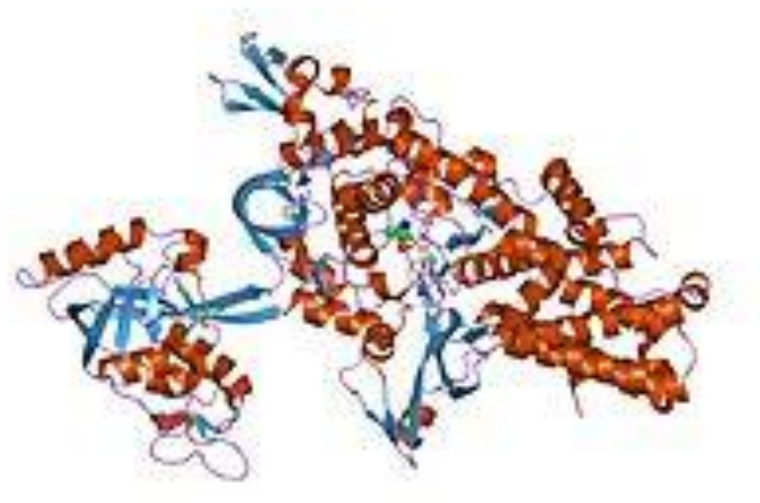
TRADUÇÃO EM PROCARIOTOS

CÓDON E ANTICÓDON

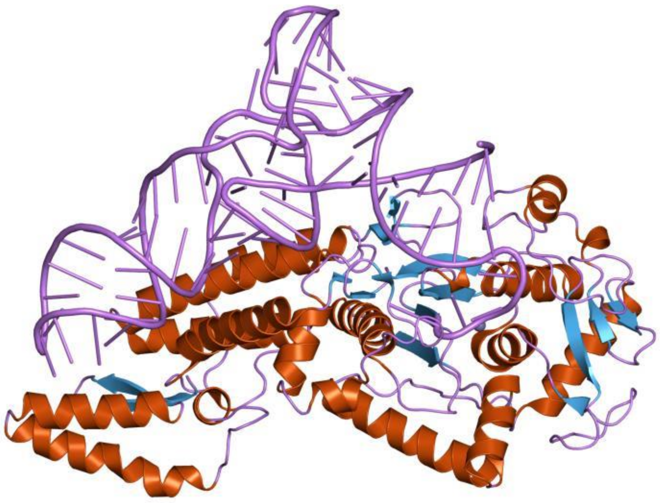


As aminoacil - tRNA sintetases:

- Para que uma molécula de tRNA se ligue ao aminoácido correto são necessárias 20 enzimas diferentes que reconheçam, especificamente, aminoácidos e seus tRNAs compatíveis.
- Cada enzima liga um aminoácido específico a seu tRNA correspondente e é capaz de reconhecer diferentes tRNAs para o mesmo aminoácido. Estas enzimas ligam o aminoácido à hidroxila 3' livre da adenosina terminal do tRNA.



LeuRS



ProRS

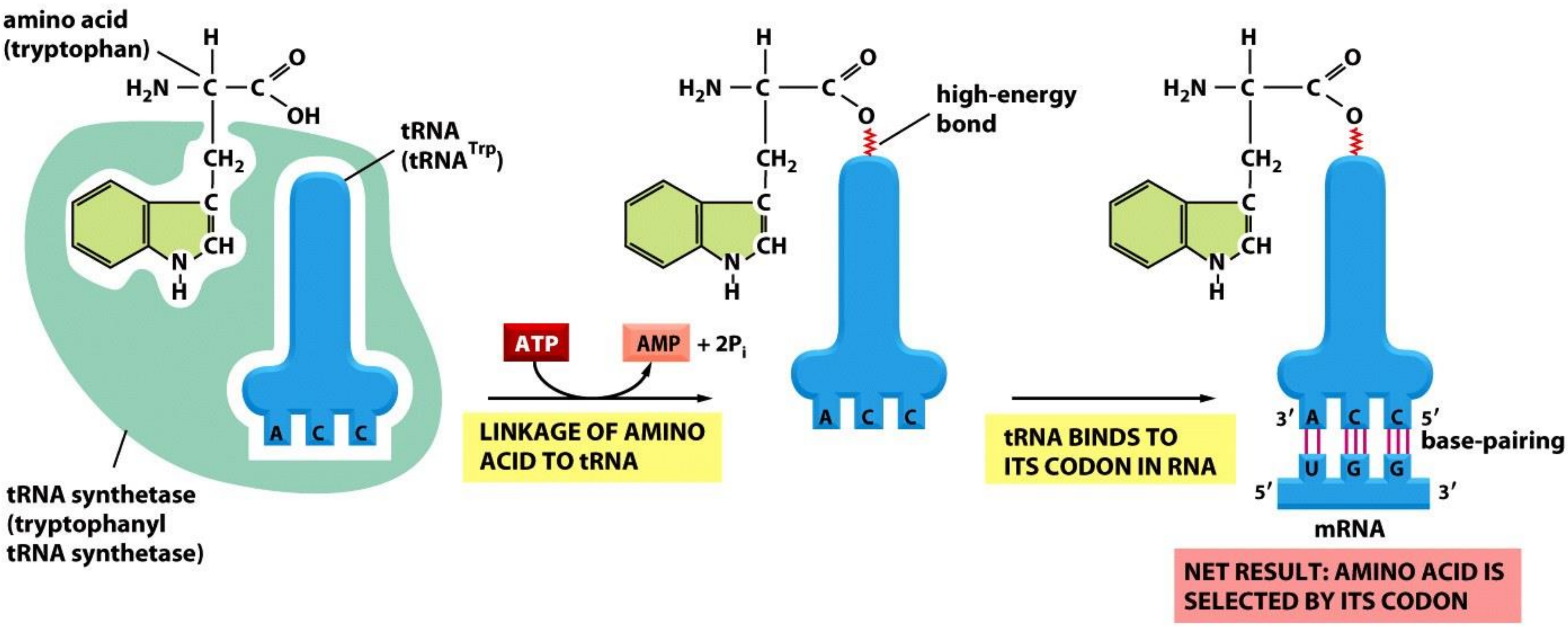


Figure 7-29 Essential Cell Biology 3/e (© Garland Science 2010)

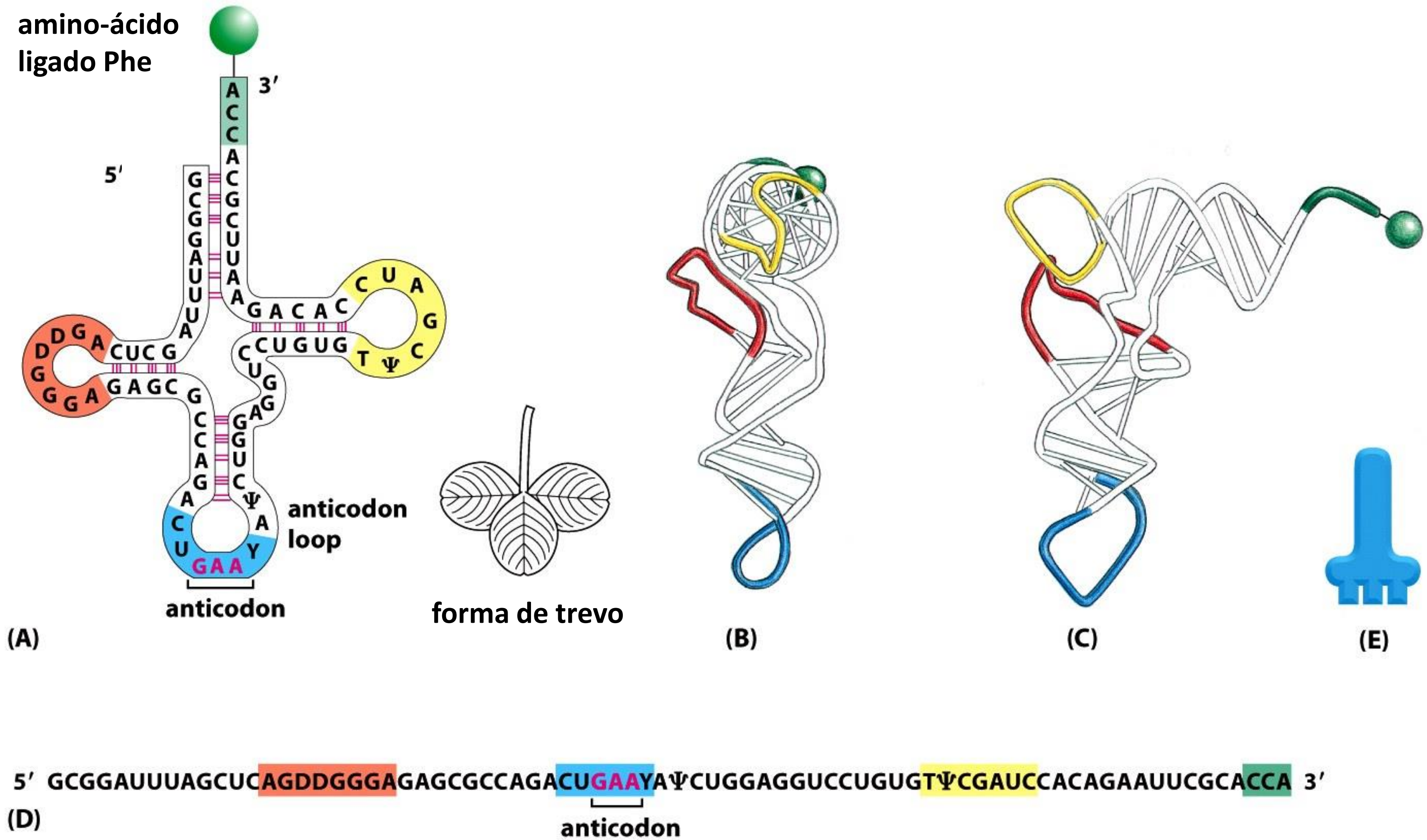


Figure 7-28 Essential Cell Biology 3/e (© Garland Science 2010)

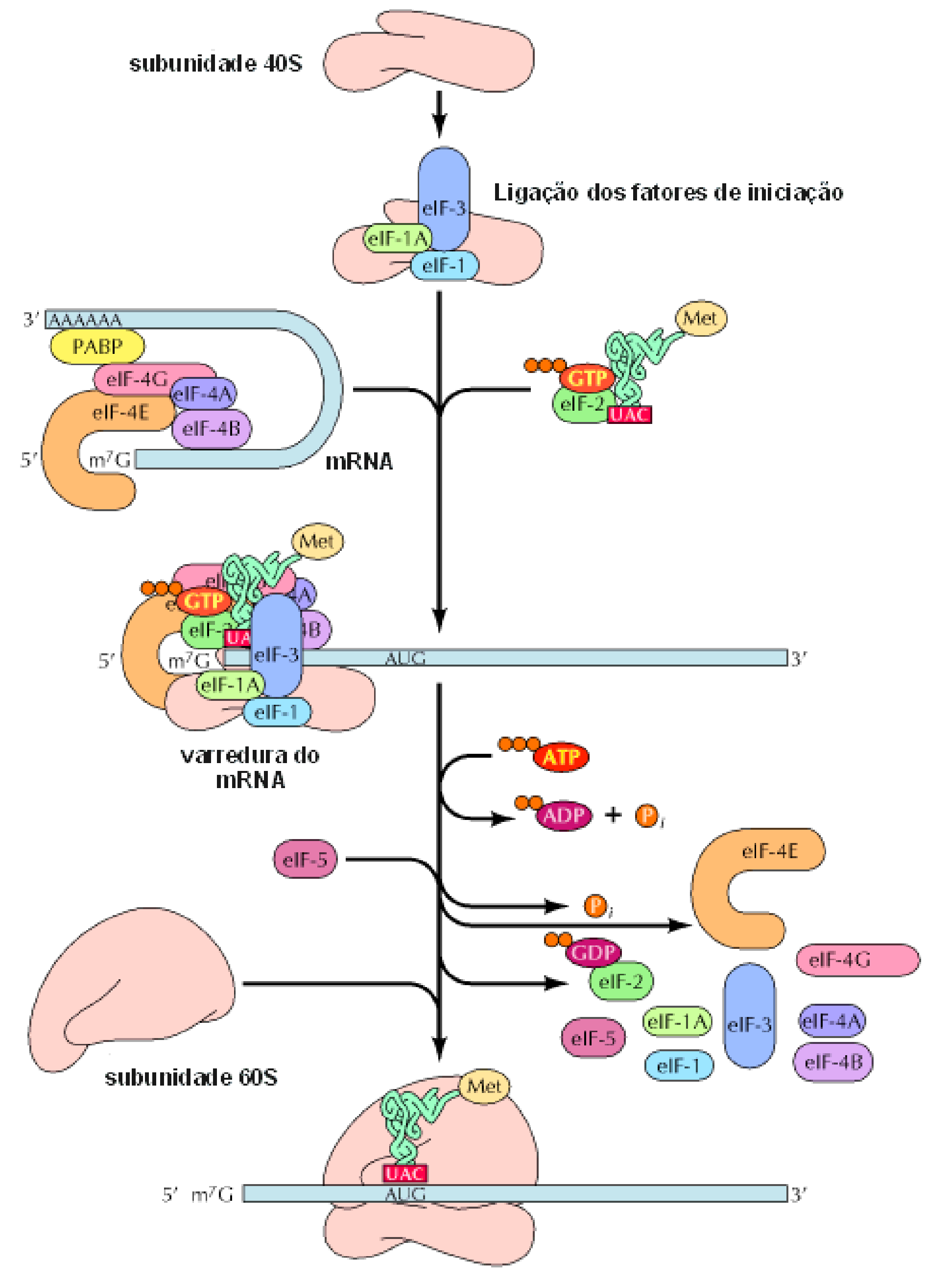
O CÓDIGO GENÉTICO É REDUNDANTE!

Amino acid	3-Letter code	1-Letter code	Codons
Alanine	Ala	A	GCC, GCU, GCG, GCA
Arginine	Arg	R	CGC, CGG, CGU, CGA, AGA, AGG
Asparagine	Asn	N	AAU, AAC
Aspartic acid	Asp	D	GAU, GAC
Cysteine	Cys	C	UGU, UGC
Glutamic acid	Glu	E	GAA, GAG
Glutamine	Gln	Q	CAA, CAG
Glycine	Gly	G	GGU, GGC, GGA, GGG
Histidine	His	H	CAU, CAC
Isoleucine	Ile	I	AUU, AUC, AUA
Leucine	Leu	L	UUA, UUG, CUA, CUG, CUU, CUC
Lysine	Lys	K	AAA, AAG
Methionine	Met	M	AUG
Phenylalanine	Phe	F	UUC, UUU
Proline	Pro	P	CCU, CCC, CCA, CCG
Serine	Ser	S	UCU, UCC, UCA, UCG, AGU, AGC
Threonine	Thr	T	ACU, ACC, ACA, ACG
Tyrosine	Tyr	Y	UAU, UAC
Tryptophan	Trp	W	UGG
Valine	Val	V	GUU, GUC, GUA, GUG
"Stop"	—	—	UAA, UAG, UGA

start codon -iniciador

stop codon -terminador

Vários polipeptideos auxiliam o início da tradução!



TRADUÇÃO

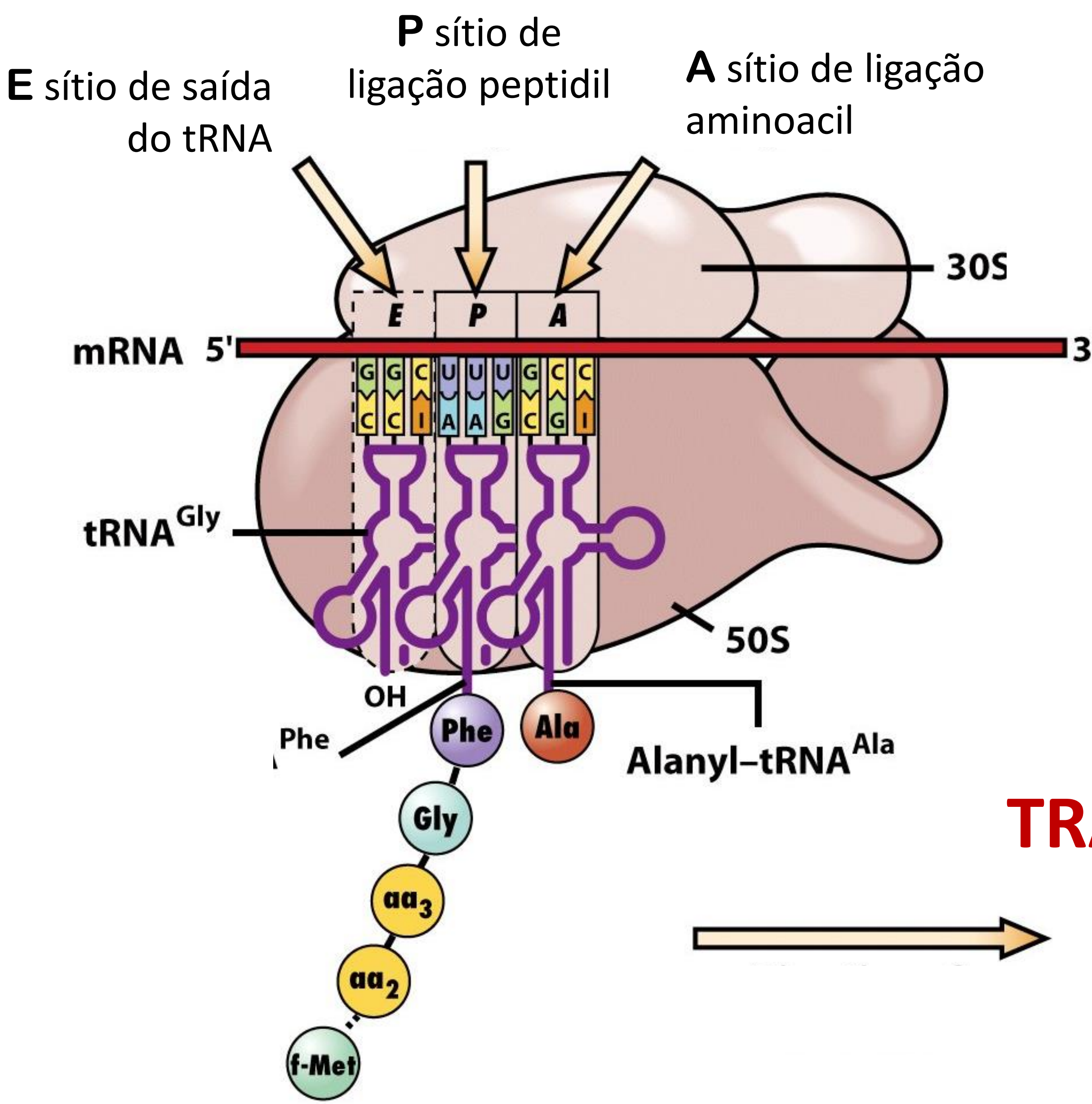


Figure 12-14a Principles of Genetics, 4/e

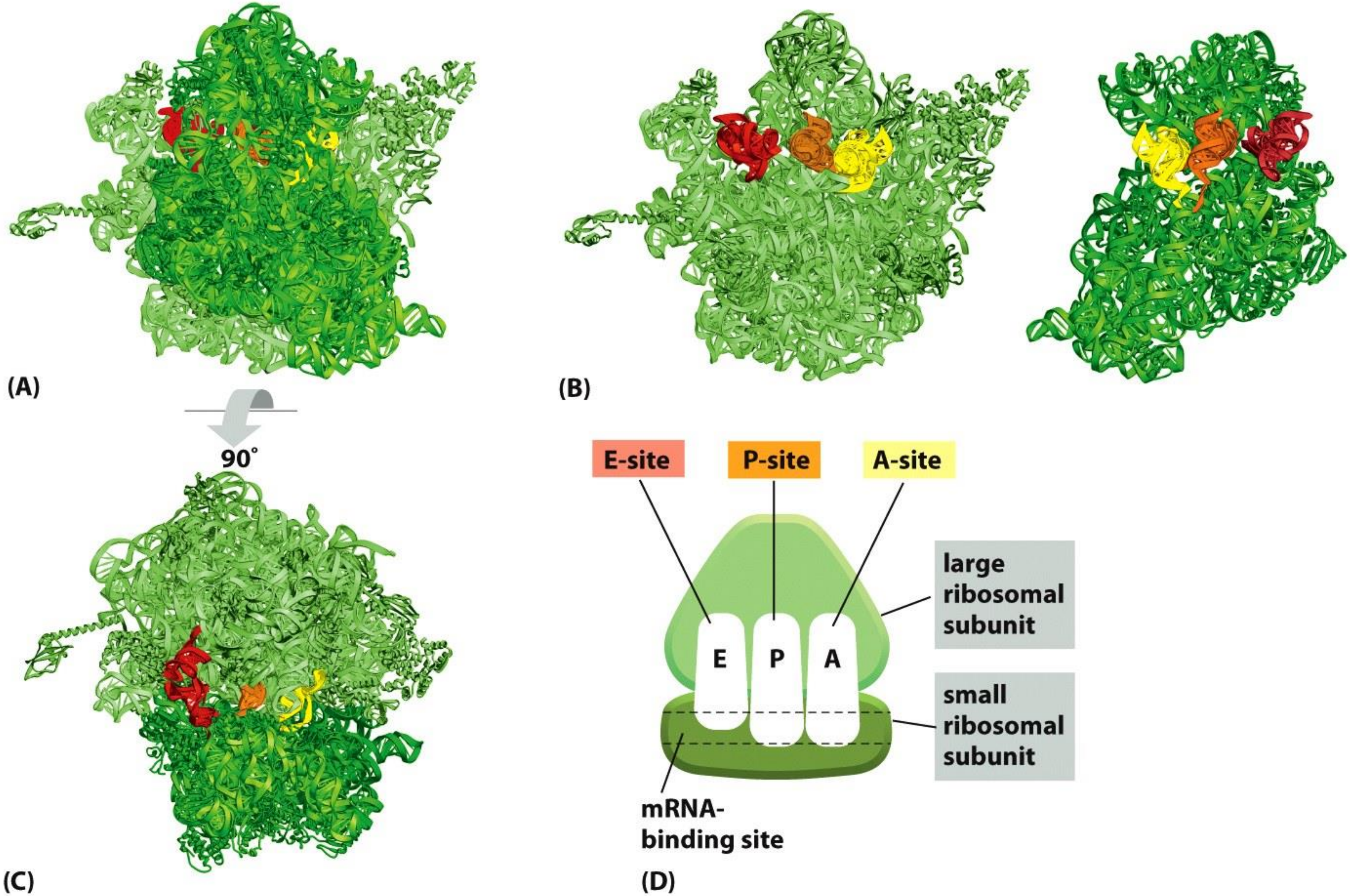


Figure 7-32 Essential Cell Biology 3/e (© Garland Science 2010)

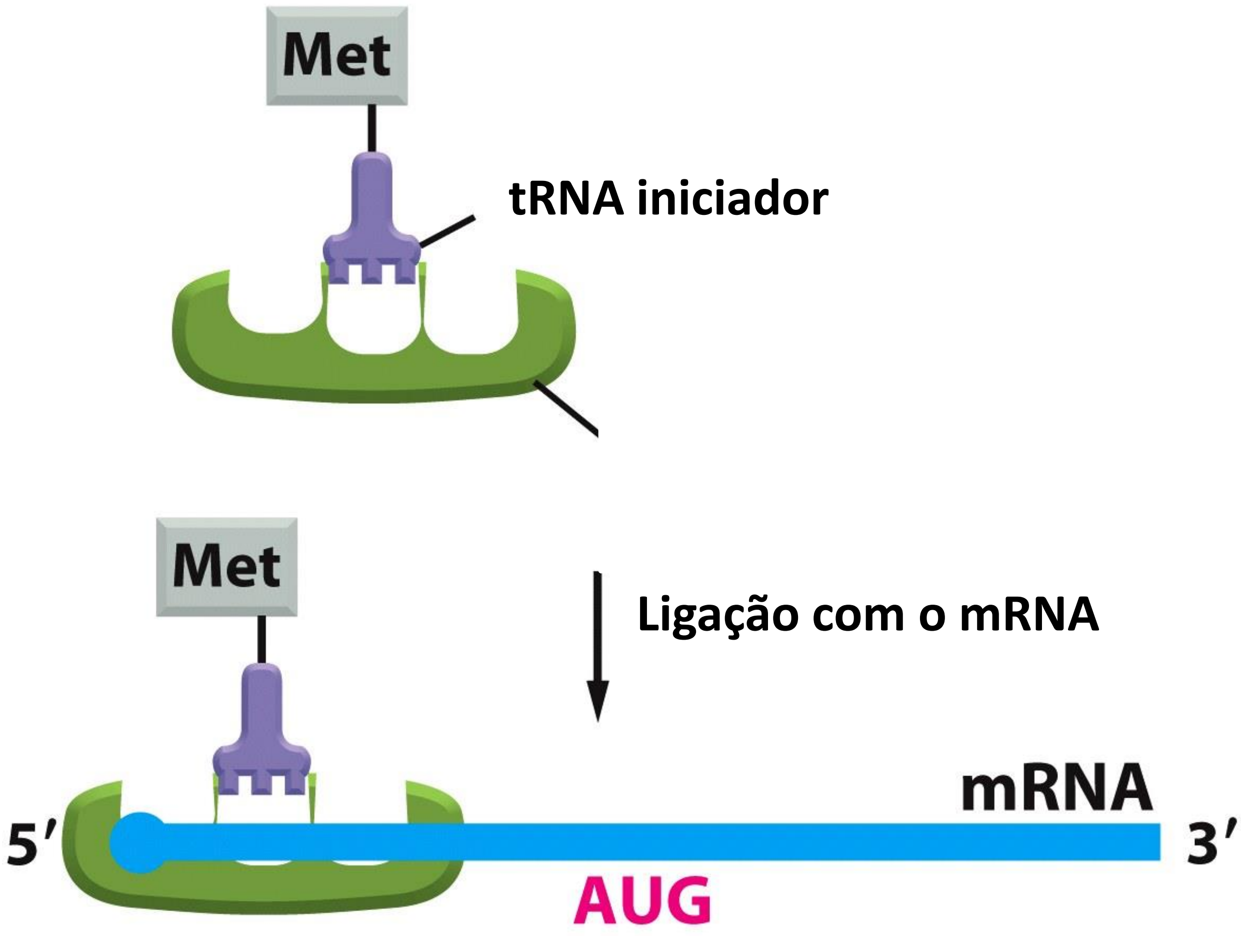
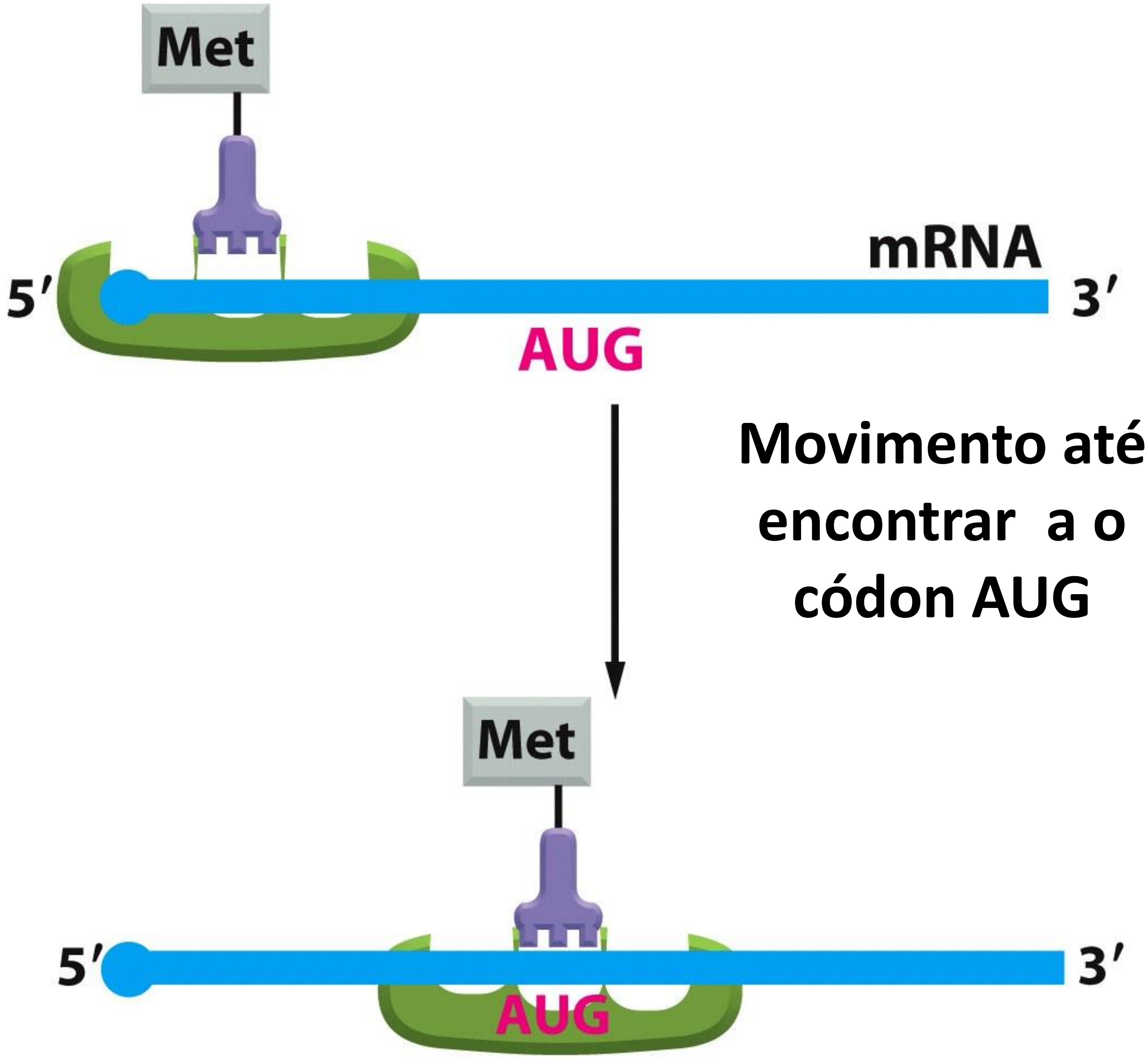


Figure 7-35 part 1 of 5 Essential Cell Biology 3/e (© Garland Science 2010)



Movimento até encontrar a o códon AUG

Figure 7-35 part 2 of 5 Essential Cell Biology 3/e (© Garland Science 2010)

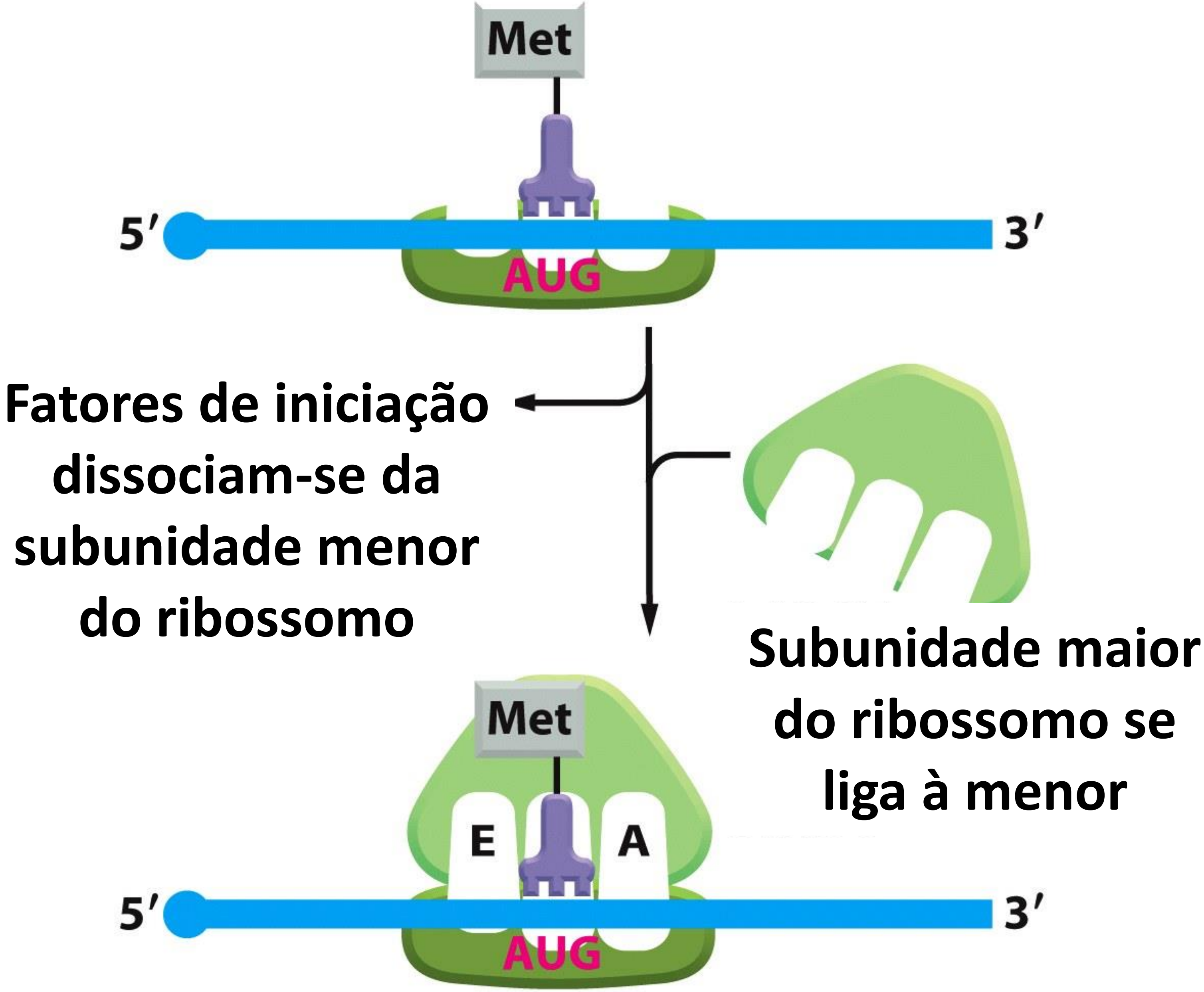
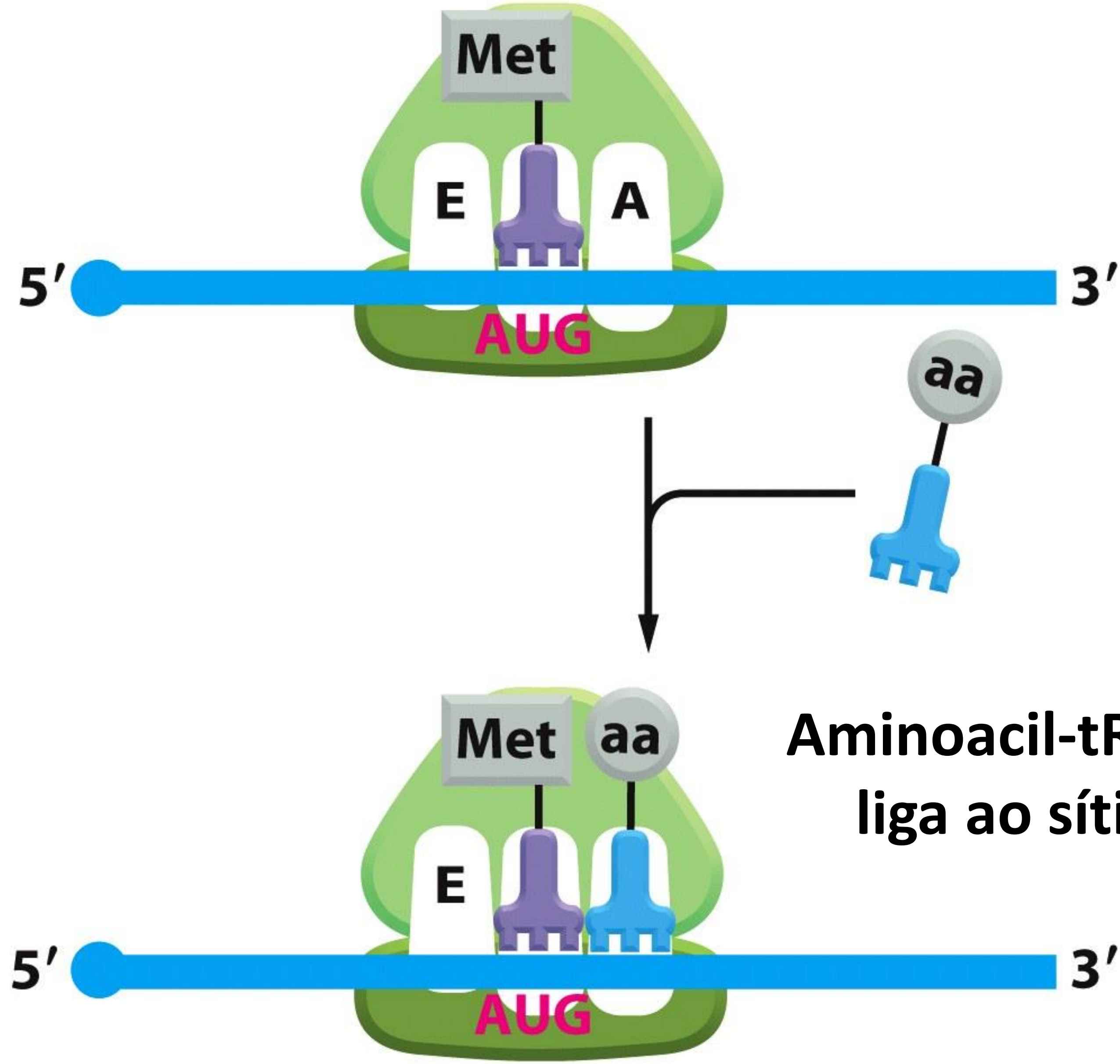
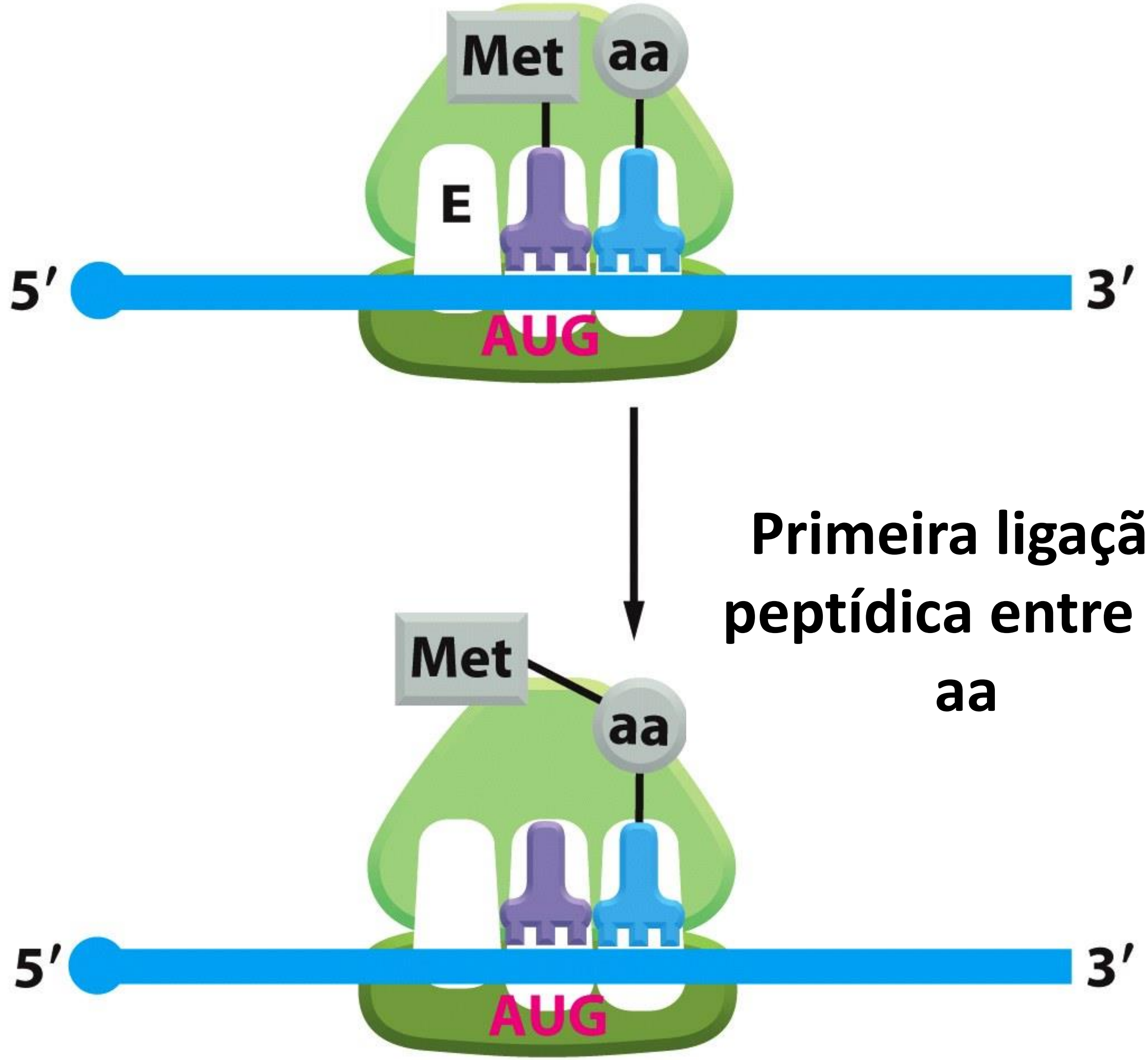


Figure 7-35 part 3 of 5 Essential Cell Biology 3/e (© Garland Science 2010)



Aminoacil-tRNa se liga ao sítio A

Figure 7-35 part 4 of 5 Essential Cell Biology 3/e (© Garland Science 2010)



Primeira ligação peptídica entre os aa

Figure 7-35 part 5 of 5 Essential Cell Biology 3/e (© Garland Science 2010)

E continuadamente....

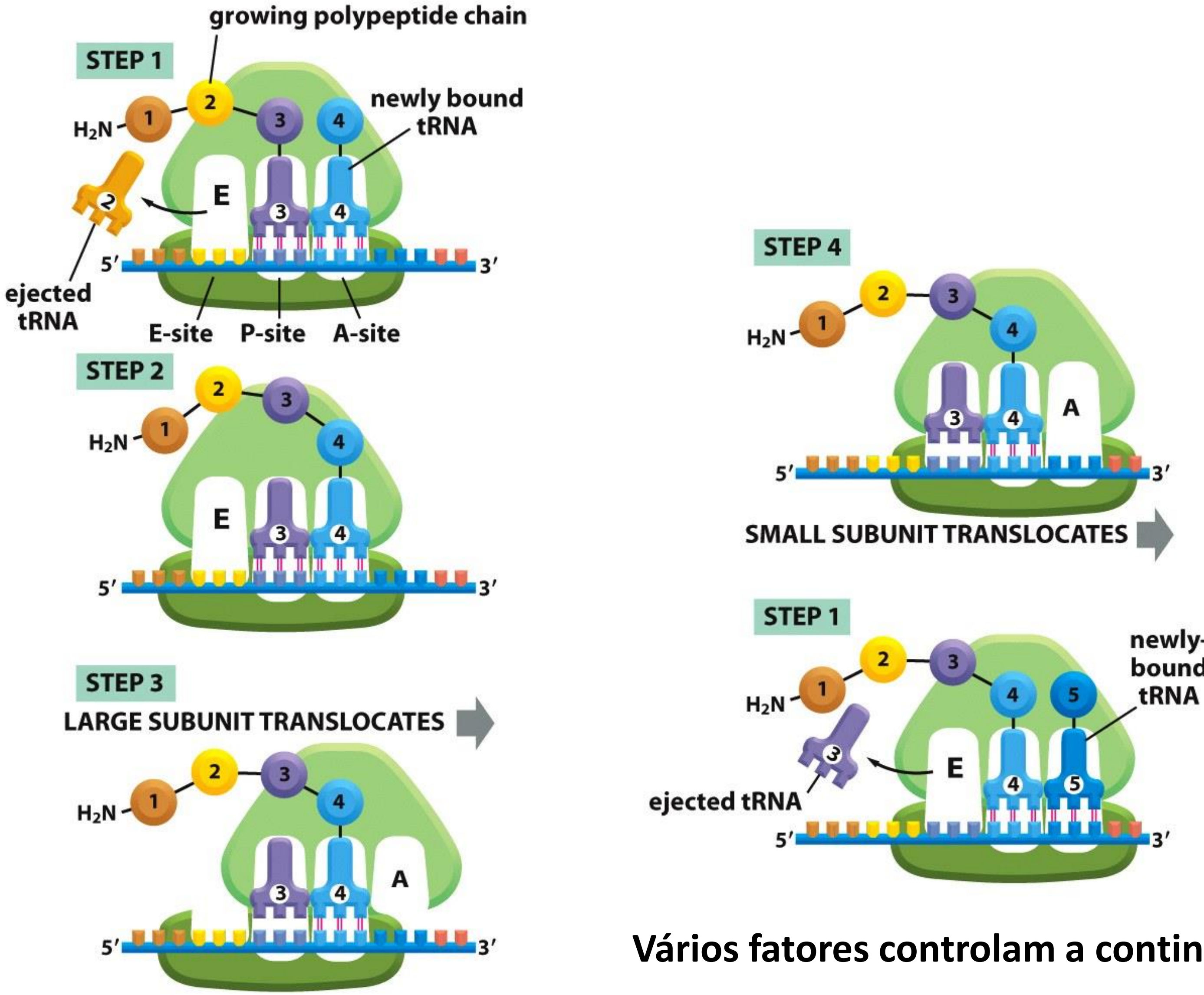
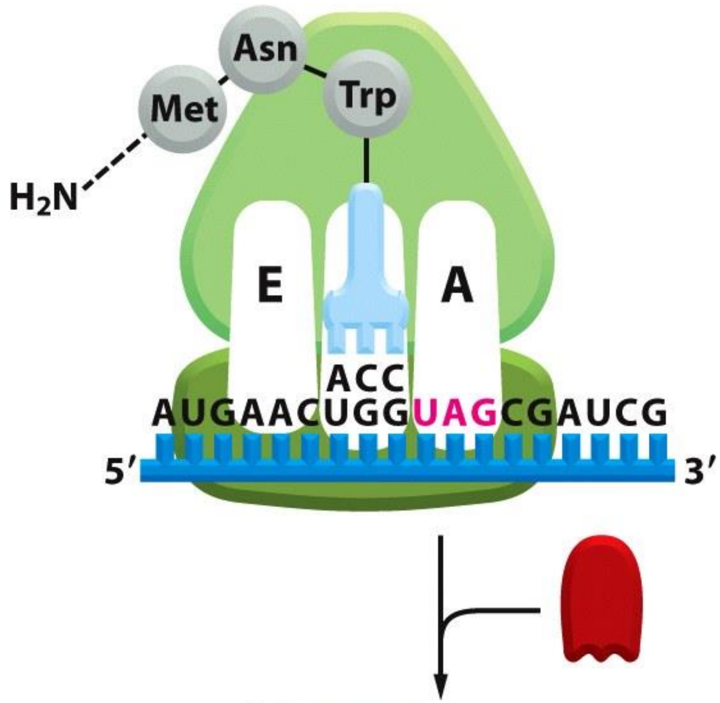


Figure 7-33 Essential Cell Biology 3/e (© Garland Science 2010)

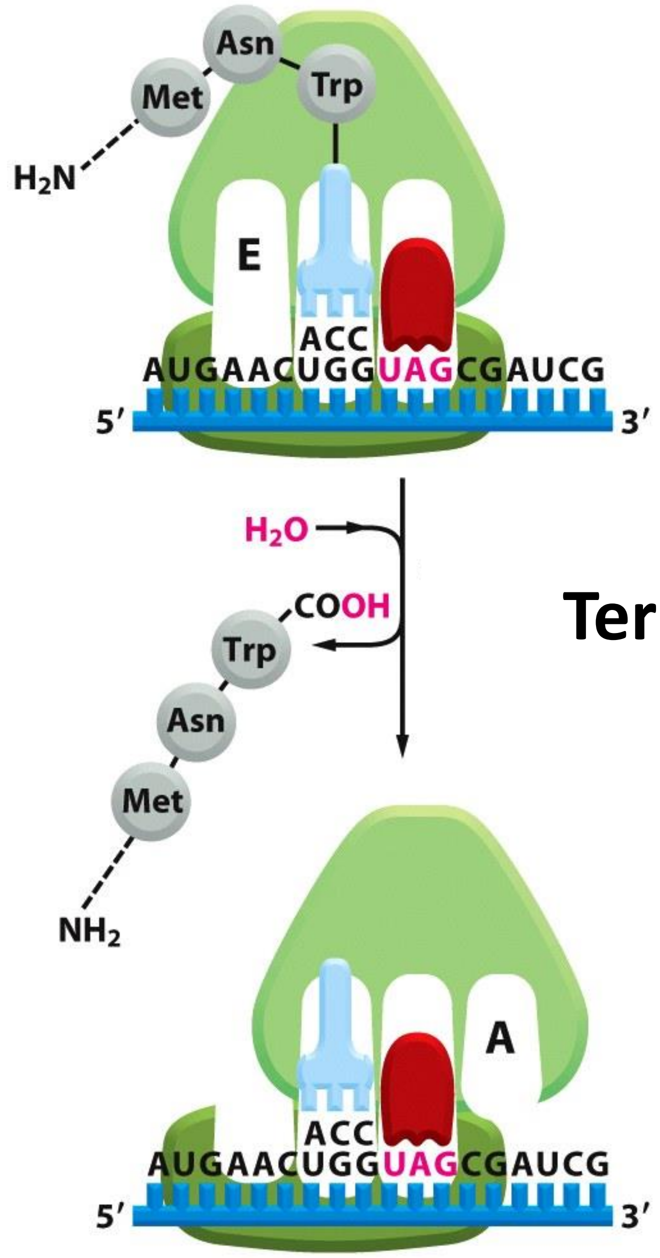
Vários fatores controlam a continuação da tradução!



Ligação do fator de liberação no síto A

Figure 7-37 part 1 of 3 Essential Cell Biology 3/e (© Garland Science 2010)

Terminação



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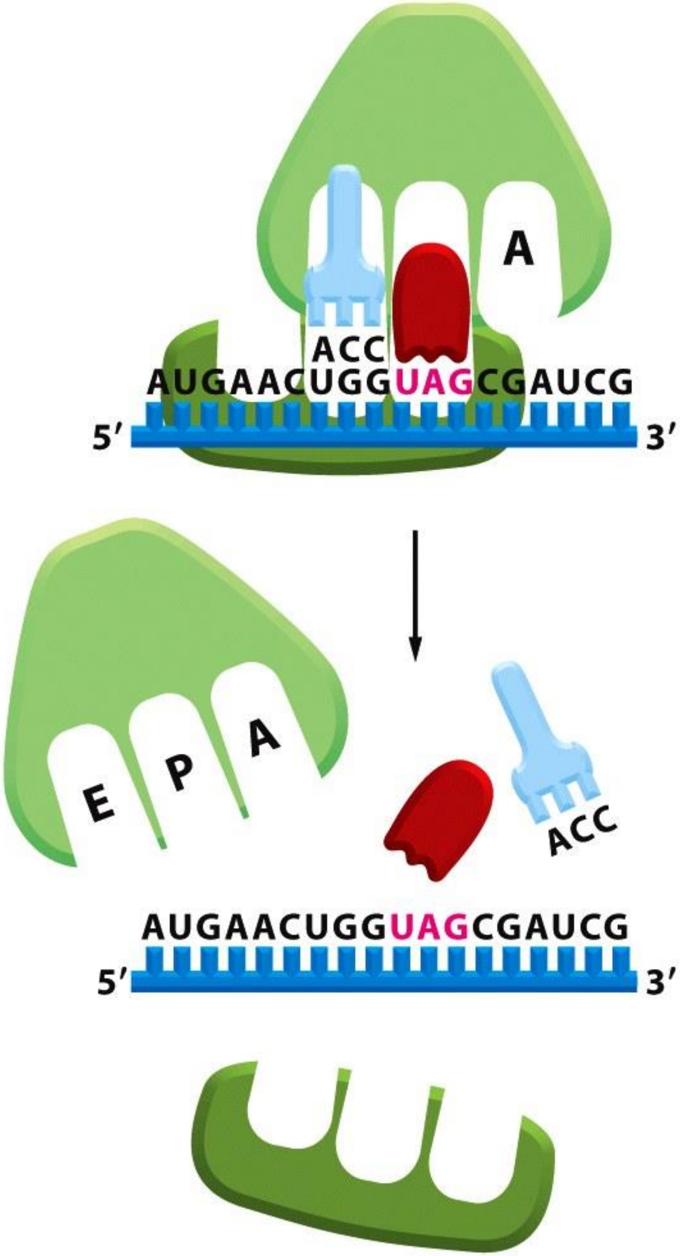
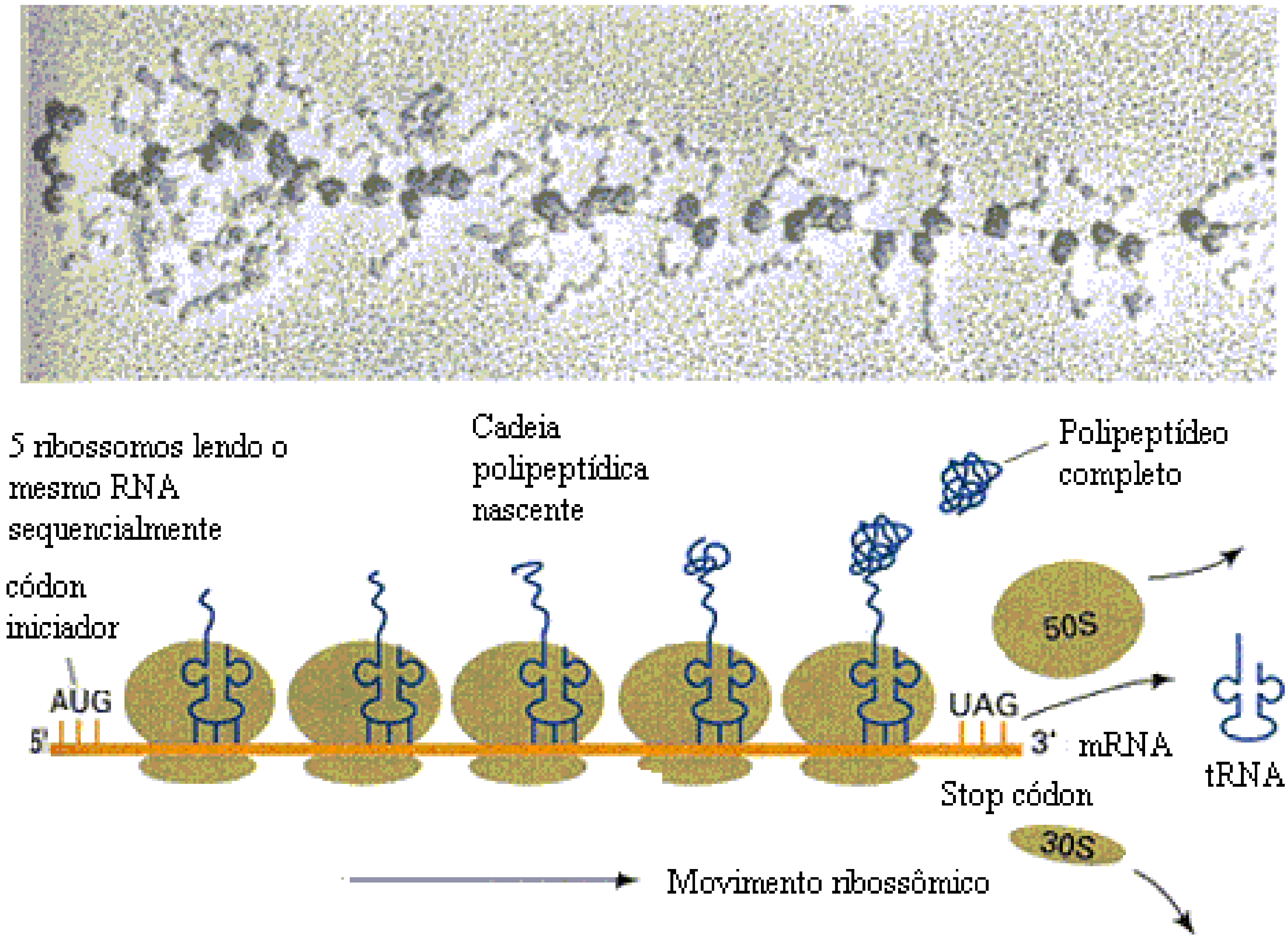
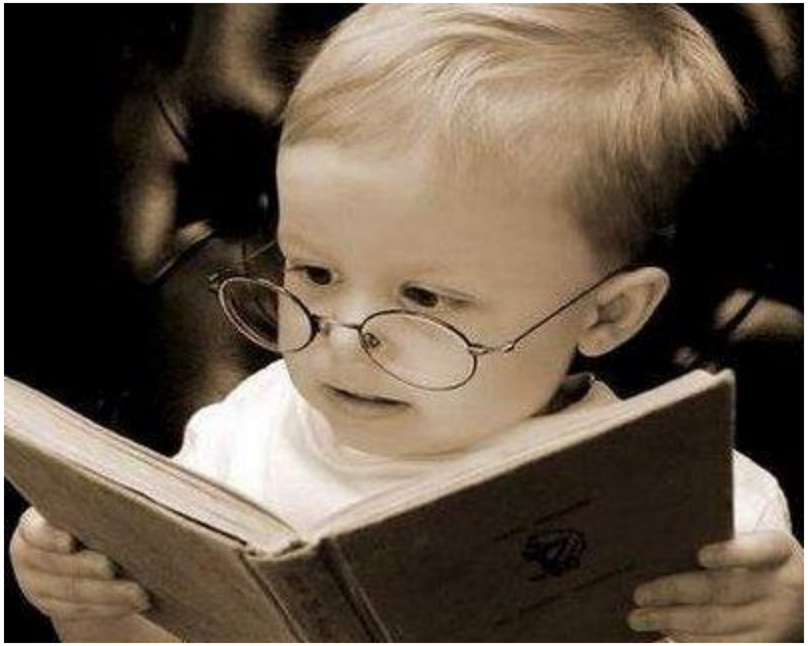


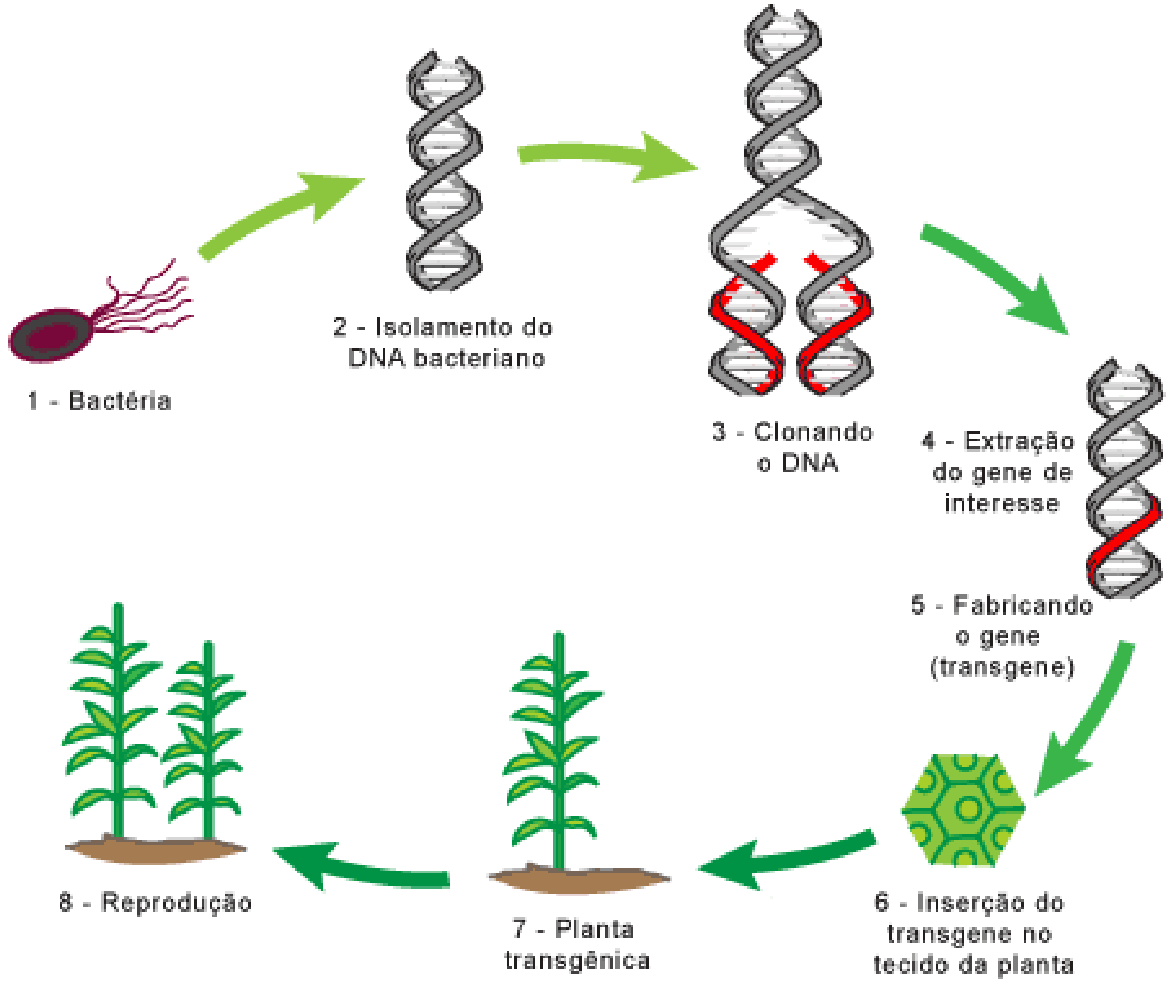
Figure 7-37 part 3 of 3 Essential Cell Biology 3/e (© Garland Science 2010)


5 ribossomos lendo o mesmo RNA sequencialmente
Cadeia polipeptídica nascente
Polipeptídeo completo
códon iniciador
AUG
5'
50S
UAG
3'
mRNA
tRNA
Stop códon
30S
Movimento ribossômico

APLICANDO O CONHECIMENTO…



OBTENÇÃO DE ORGANISMOS GENETICAMENTE MODIFICADOS



Pesq. agropec. bras. vol.42 no.11 Brasília Nov. 2007

CONSTRUÇÃO PRESENTE NA SOJA RR®

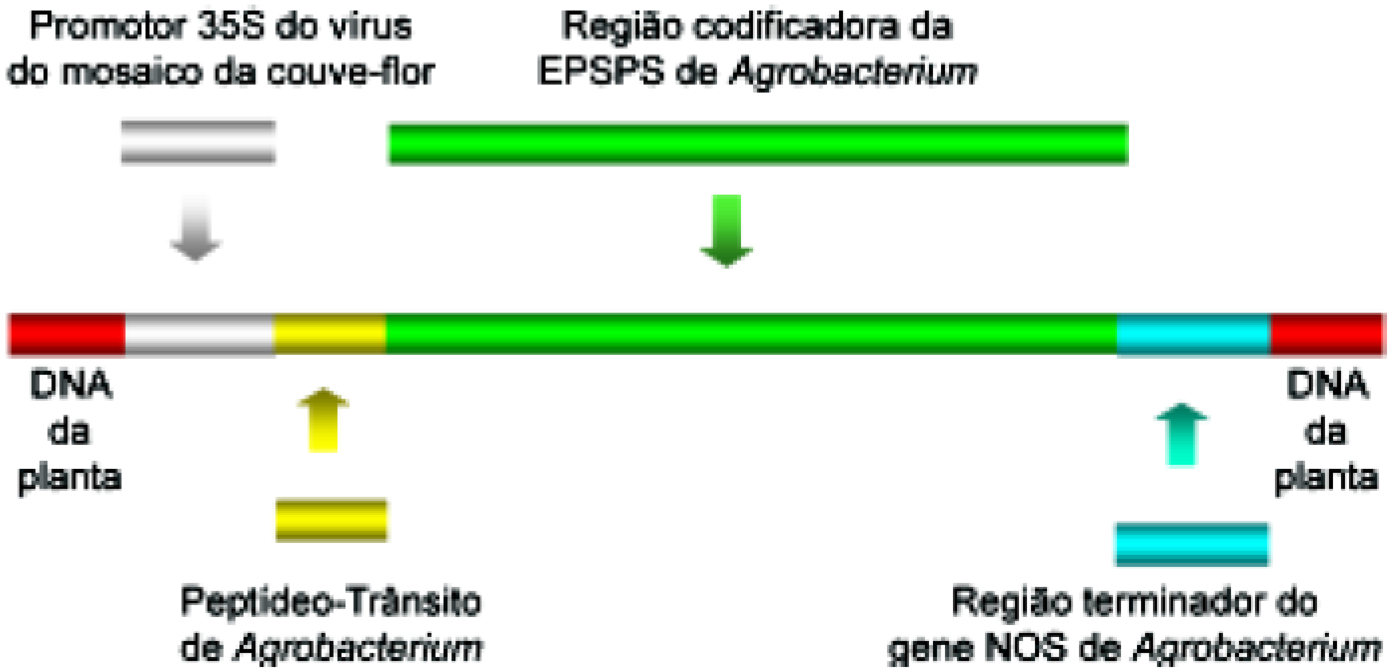


Figura 1 - Representação da construção presente na soja RR® (*Roundup Ready*). Região promotora 35S do vírus do mosaico da couve flor, peptídeo de trânsito de Petúnia, gene que codifica a proteína EPSPS, que confere a resistência ao herbicida, e o terminador do gene da nopalina sintase (NOS).

APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Apr. 2009, p. 2099–2110 Vol. 75, No. 7
0099-2240/09/$08.00+0 doi:10.1128/AEM.02066-08

Codon-Optimized Fluorescent Proteins Designed for Expression in Low-GC Gram-Positive Bacteria▽

Inka Sastalla, Kannie Chim, Gordon Y. C. Cheung, Andrei P. Pomerantsev, and Stephen H. Leppla*

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Fluorescent proteins have wide applications in biology. However, not all of these proteins are properly expressed in bacteria, especially if the codon usage and genomic GC content of the host organism are not ideal for high expression. In this study, we analyzed the DNA sequences of multiple fluorescent protein genes with respect to codons and GC content and compared them to a low-GC gram-positive bacterium, *Bacillus anthracis*. We found high discrepancies for cyan fluorescent protein (CFP), yellow fluorescent protein (YFP), and the photoactivatable green fluorescent protein (PAGFP), but not GFP, with regard to GC content and codon usage. Concomitantly, when the proteins were expressed in *B. anthracis*, CFP- and YFP-derived fluorescence was undetectable microscopically, a phenomenon caused not by lack of gene transcription or degradation of the proteins but by lack of protein expression. To improve expression in bacteria with low genomic GC contents, we synthesized a codon-optimized *gfp* and constructed optimized photoactivatable *pagfp*, *cfp*, and *yfp*, which were in contrast to nonoptimized genes highly expressed in *B. anthracis* and in another low-GC gram-positive bacterium, *Staphylococcus aureus*. Using optimized GFP as a reporter, we were able to monitor the activity of the protective antigen promoter of *B. anthracis* and confirm its dependence on bicarbonate and regulators present on virulence plasmid pXO1.

Molecular Cell
Review

CellPress

Codon Bias as a Means to Fine-Tune Gene Expression

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The redundancy of the genetic code implies that most amino acids are encoded by multiple synonymous codons. In all domains of life, a biased frequency of synonymous codons is observed at the genome level, in functionally related genes (e.g., in operons), and within single genes. Other codon bias variants include biased codon pairs and codon co-occurrence. Although translation initiation is the key step in protein synthesis, it is generally accepted that codon bias contributes to translation efficiency by tuning the elongation rate of the process. Moreover, codon bias plays an important role in controlling a multitude of cellular processes, ranging from differential protein production to protein folding. Here we review currently known types of codon bias and how they may influence translation. We discuss how understanding the principles of codon bias and translation can contribute to improved protein production and developments in synthetic biology.

Review

Zinc-finger transcription factors in plants*

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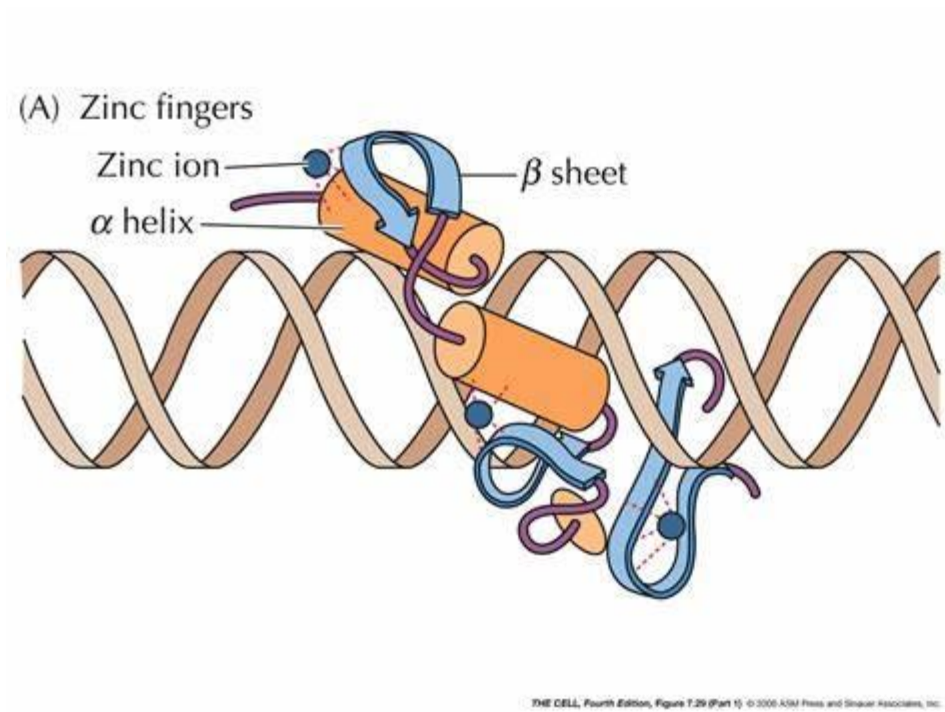

(wileyonlinelibrary.com) DOI 10.1002/ps.4099

Detection of the F129L mutation in the cytochrome *b* gene in *Phakopsora pachyrhizi*

Ana C Klosowski,[a*] Louise L May De Mio,[a] Simone Miessner,[b] Ronaldo Rodrigues[c] and Gerd Stammler[b]

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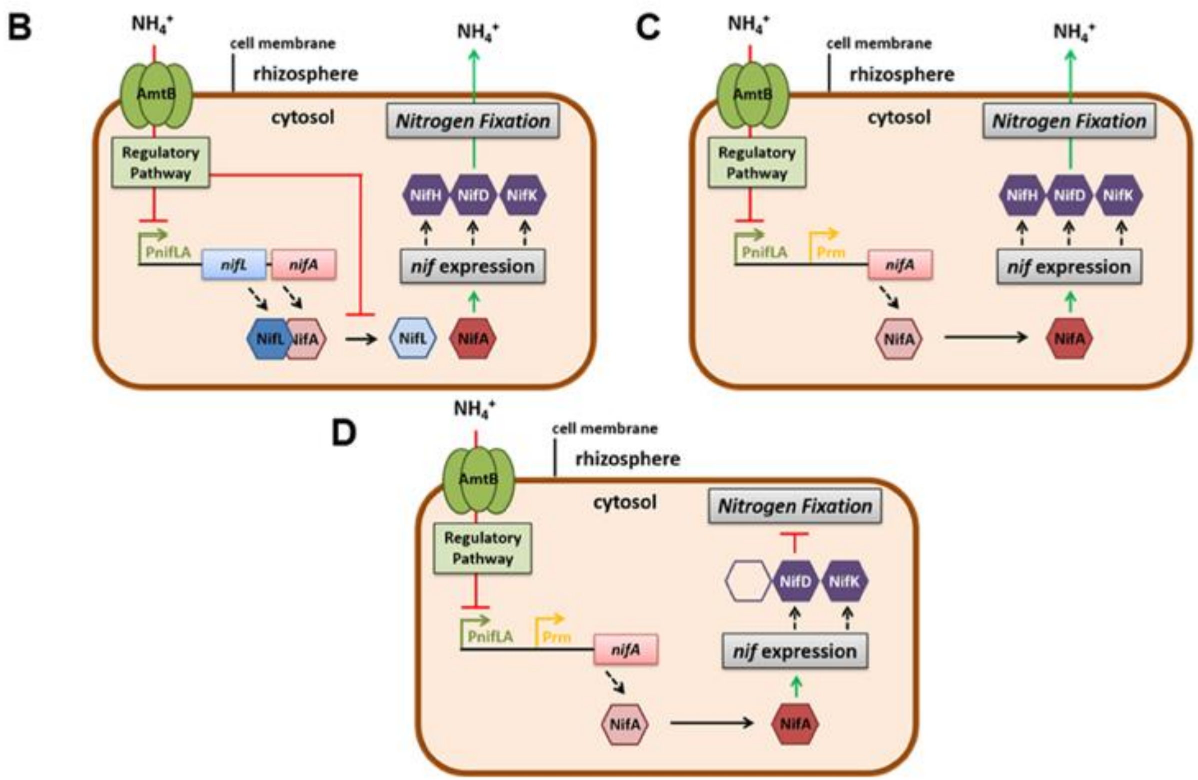
Enabling Biological Nitrogen Fixation for Cereal Crops in Fertilized Fields



VISUALIZANDO O PROCESSO...

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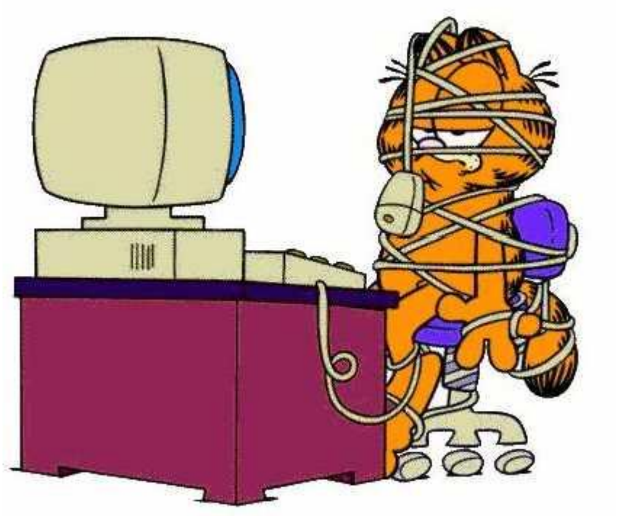

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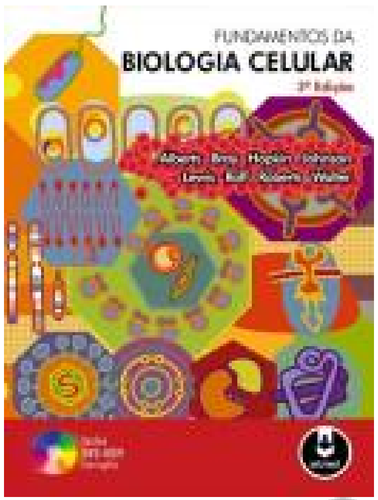

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ESTUDO DIRIGIDO

1.Definição de gene;
2. Diferença na estrutura dos genes de eucariotos e procariotos;
3. Região codante: start codon e stop codon.
4.Processo de tradução

Dogma Central da Biologia Celular - Mapa conceitual

Entrega dia 13 de Setembro!