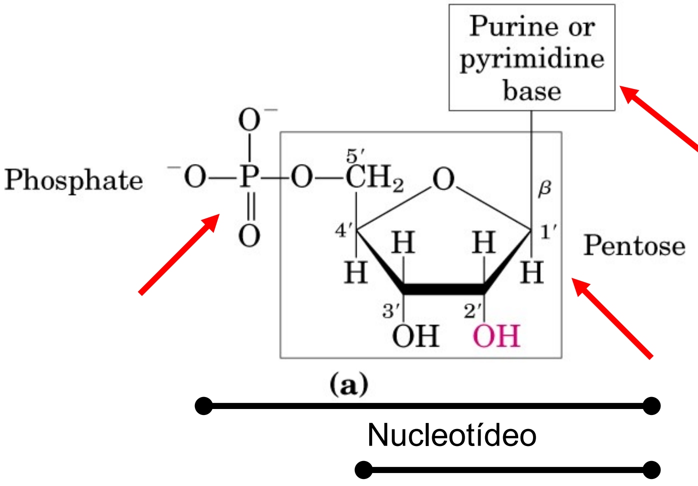
Recapitulando Biologia Molecular

Nucleotídeos e Ácidos Nucléicos

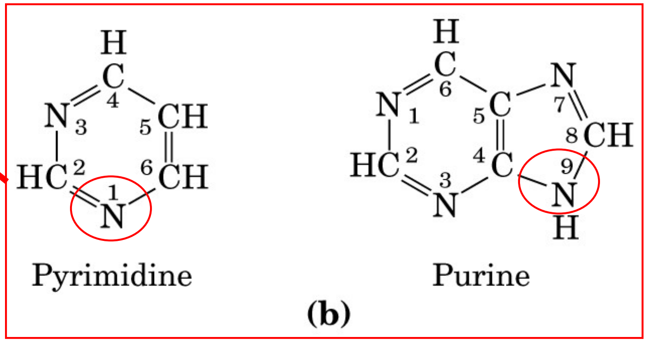
DNA - Acido Desoxirribonucléico
Armazenamento da informação genética da célula

RNA - Acido Ribonucléico
Funções variadas
RNA ribossomal - rRNA - Componente estrutural do ribossomo
RNA transportador - tRNA - Adaptadores da síntese de proteínas
RNA menssageiro - mRNA - Intermediários na síntese de proteínas
Unidade formadora: Nucleotídeos



Nucleotídeo

Nucleosídeo

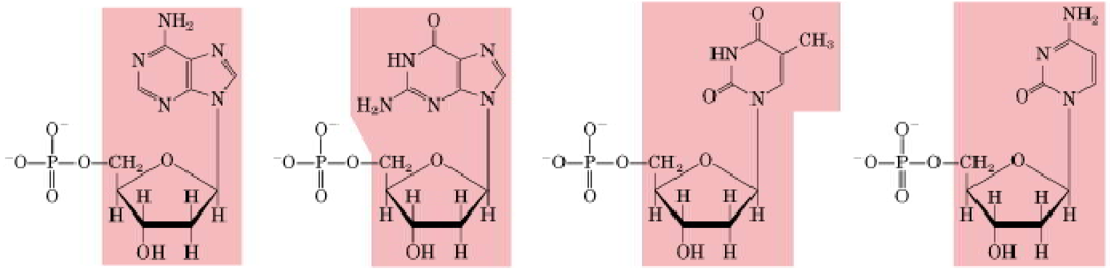


Numeração

Nucleotide and Nucleic Acid Nomenclature

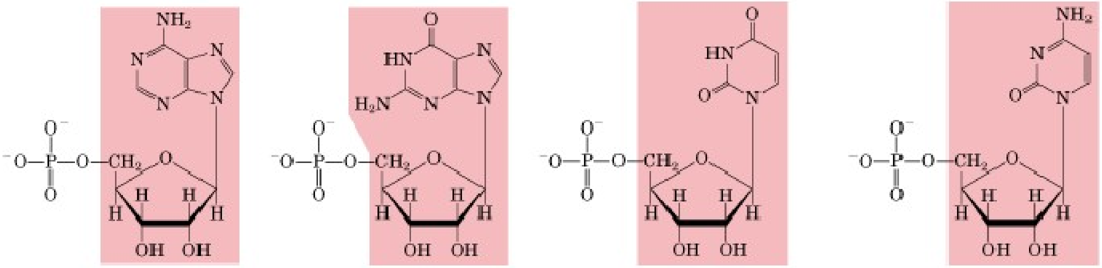
Base	Nucleoside*	Nucleotide*	Nucleic acid
Purines			
Adenine	Adenosine	Adenylate	RNA
	Deoxyadenosine	Deoxyadenylate	DNA
Guanine	Guanosine	Guanylate	RNA
	Deoxyguanosine	Deoxyguanylate	DNA
Pyrimidines			
Cytosine	Cytidine	Cytidylate	RNA
	Deoxycytidine	Deoxycytidylate	DNA
Thymine	Thymidine or deoxythymidine	Thymidylate or deoxythymidylate	DNA
Uracil	Uridine	Uridylate	RNA

Nucleotídeos



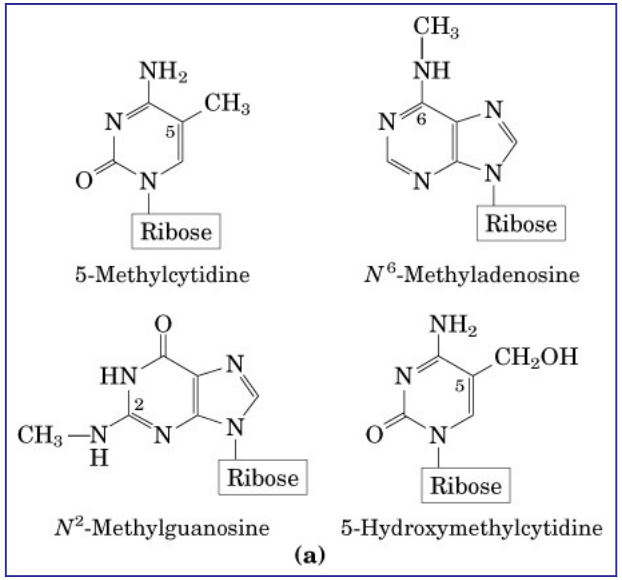
Nucleotide:	Deoxyadenylate (deoxyadenosine 5′-monophosphate)	Deoxyguanylate (deoxyguanosine 5′-monophosphate)	Deoxythymidylate (deoxythymidine 5′-monophosphate)	Deoxycytidylate (deoxycytidine 5′-monophosphate)
Symbols:	A, dA, dAMP	G, dG, dGMP	T, dT, dTMP	C, dC, dCMP
Nucleoside:	Deoxyadenosine	Deoxyguanosine	Deoxythymidine	Deoxycytidine

(a) Deoxyribonucleotides

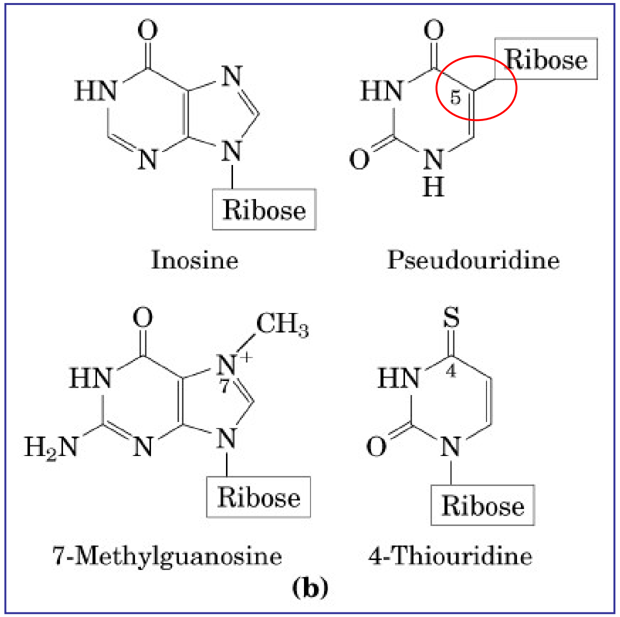


Nucleotide:	Adenylate (adenosine 5′-monophosphate)	Guanylate (guanosine 5′-monophosphate)	Uridylate (uridine 5′-monophosphate)	Cytidylate (cytidine 5′-monophosphate)
Symbols:	A, AMP	G, GMP	U, UMP	C, CMP
Nucleoside:	Adenosine	Guanosine	Uridine	Cytidine

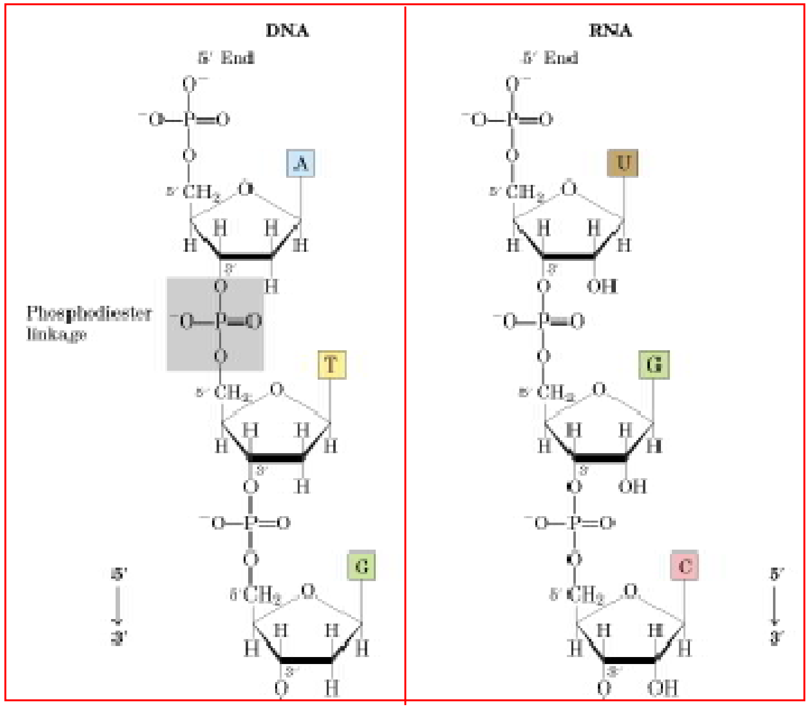
(b) Ribonucleotides

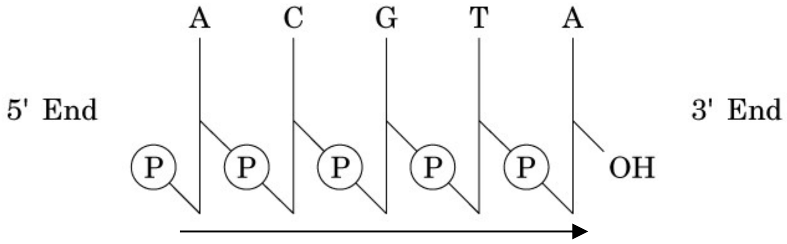


tRNA



Ligação fosfodiéster e hidrolise

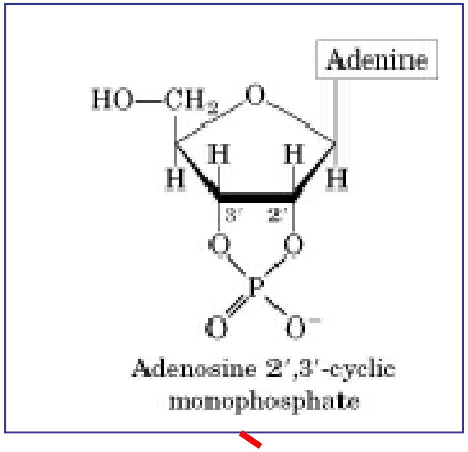




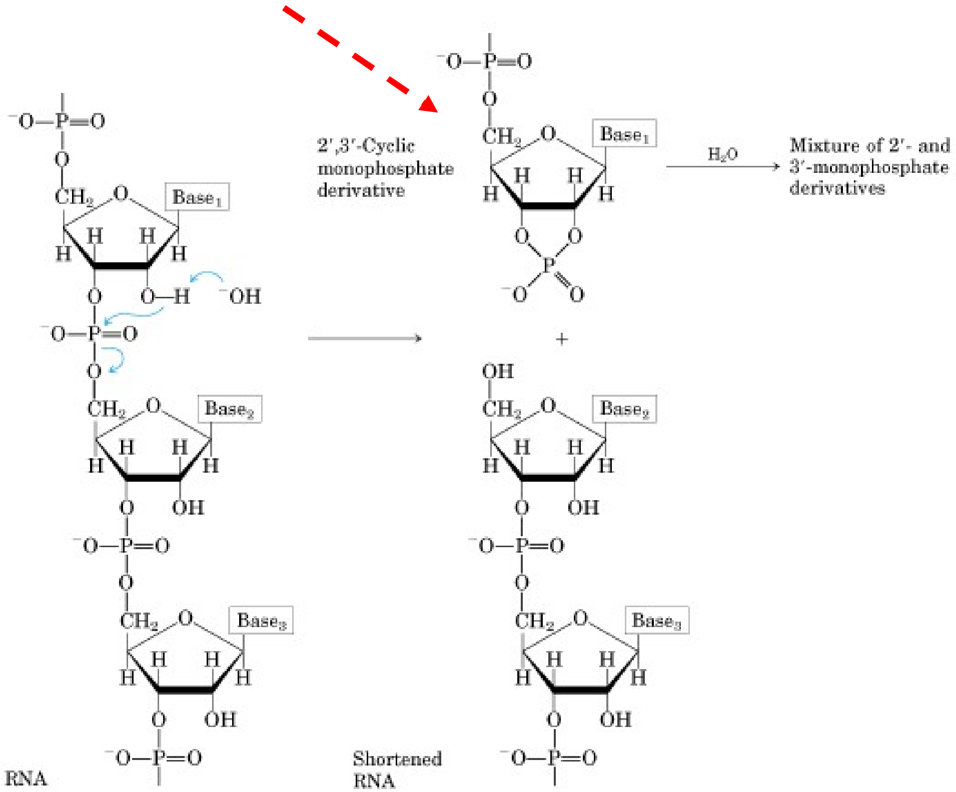
p-ACGTA

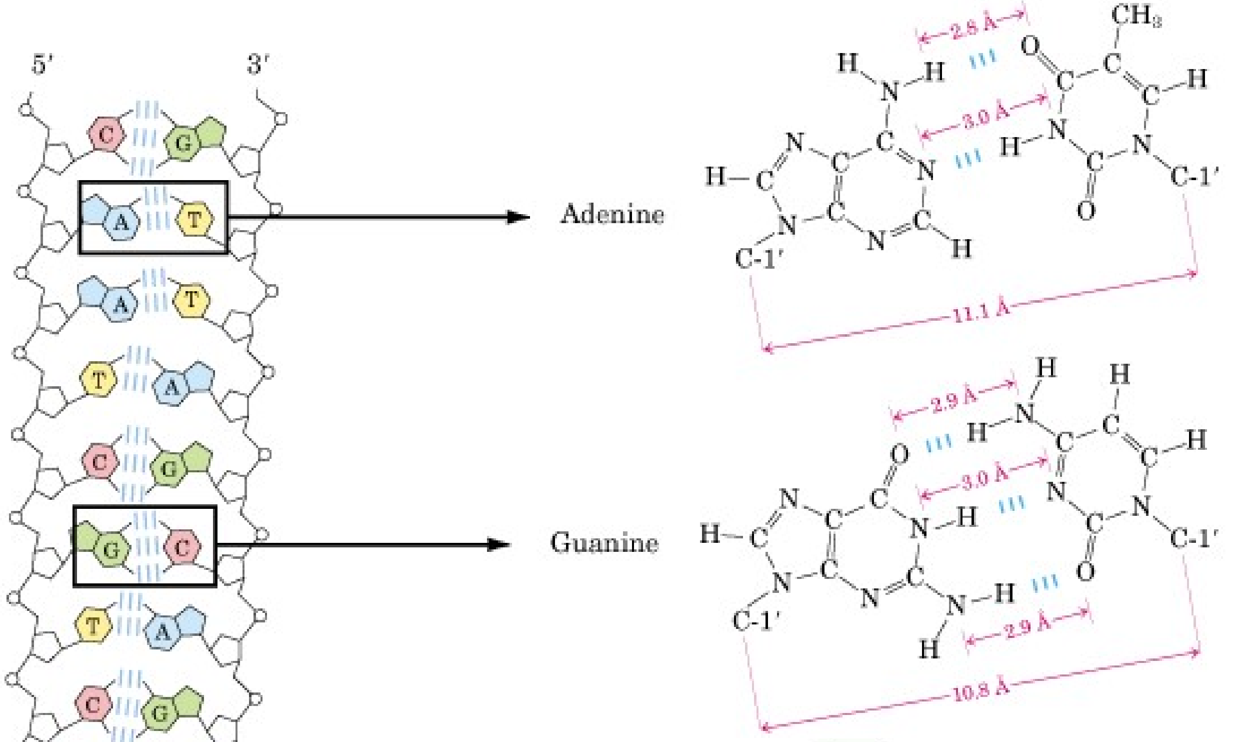
p-A-p-C-p-G-p-T-p-A

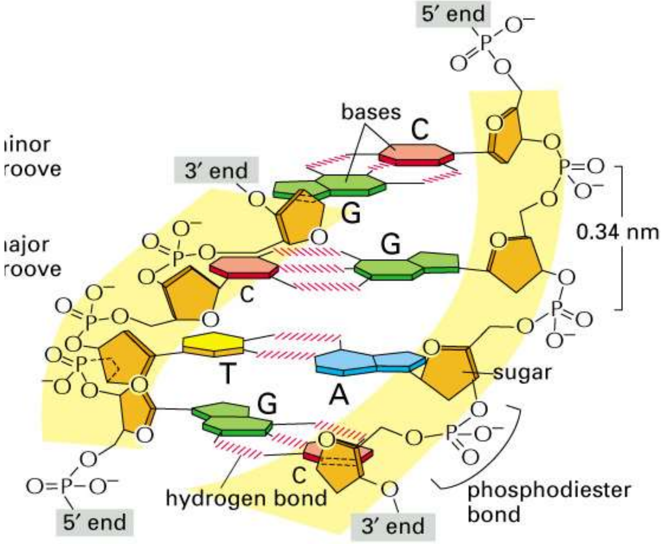
ACGTA

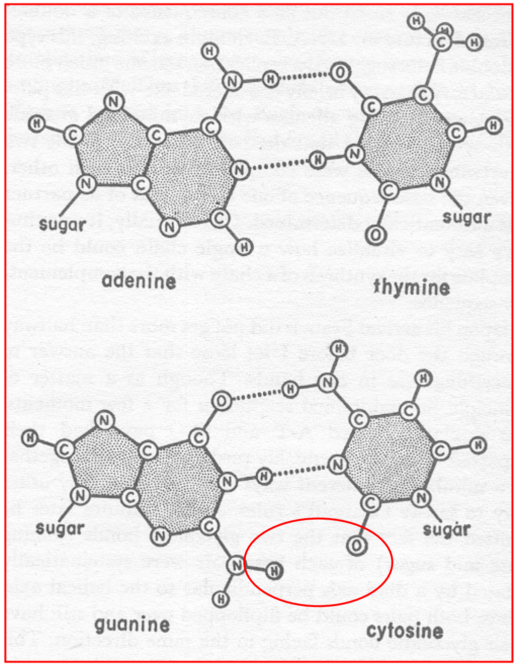


Adenosine 2′,3′-cyclic monophosphate



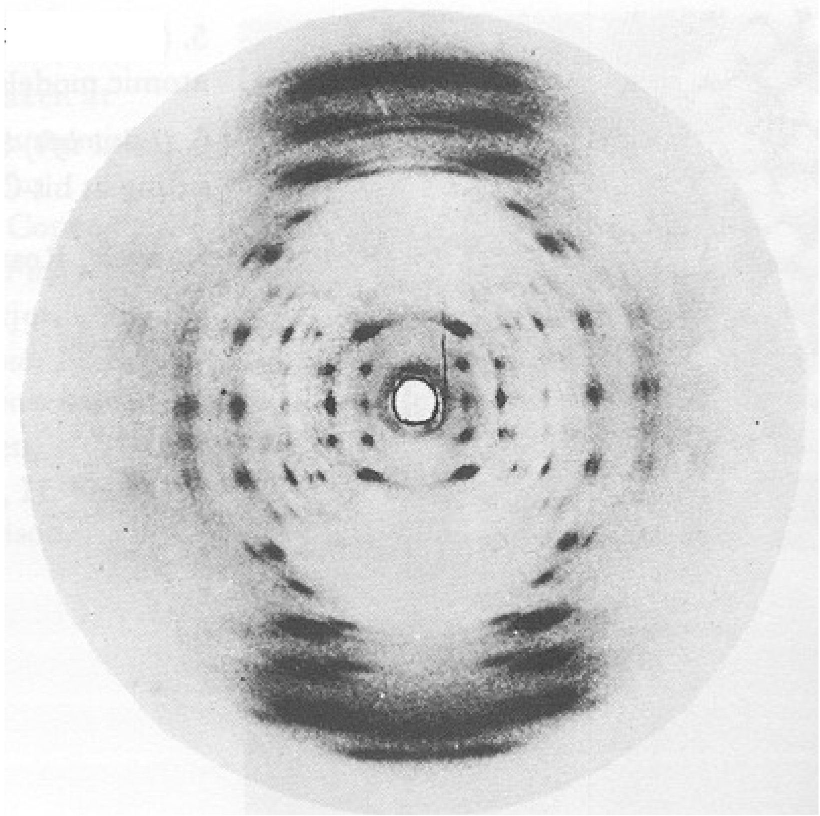

5′
3′
C
G
A
T
3′
5′
Adenine
Guanine
2.8 Å
3.0 Å
11.1 Å
CH3
C-1′
2.9 Å
3.0 Å
2.9 Å
10.8 Å
Thymine
Cytosine


5′ end
bases
C
3′ end
inor
roove
G
G
0.34 nm
ajor
roove
C
T
A
sugar
G
C
hydrogen bond
phosphodiester
bond
5′ end
3′ end

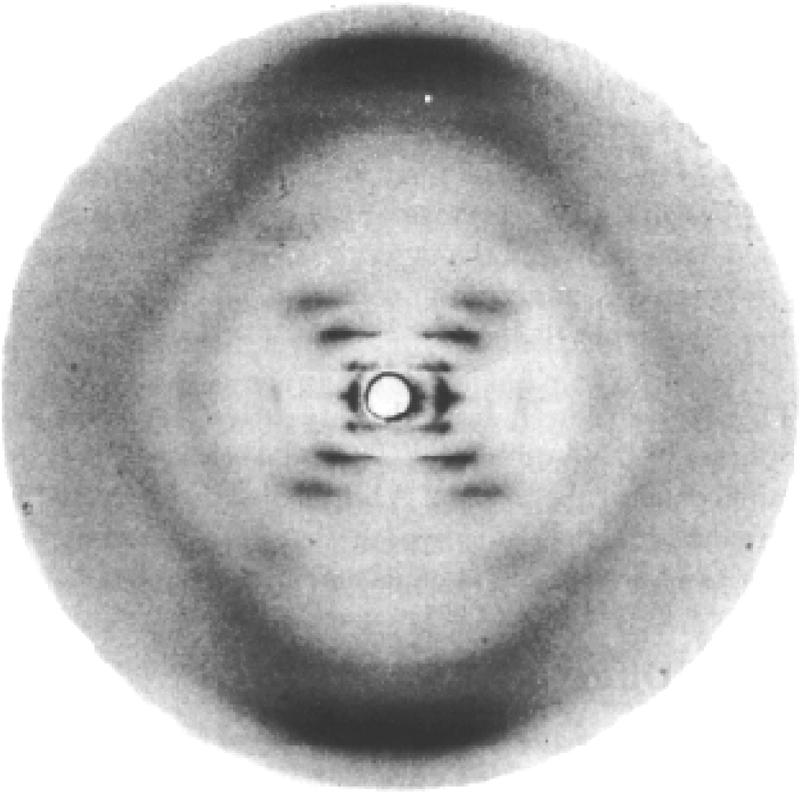

sugar
adenine
thymine
sugar
guanine
cytosine

Difração de Raios-X por Fibra de DNA

(fotos obtidas por Wilkins, Gosling e Stokes – King’s College)



DNA tipo “A”



DNA tipo “B”

Replicação do DNA

GENE

corresponde à seq. que codificam:

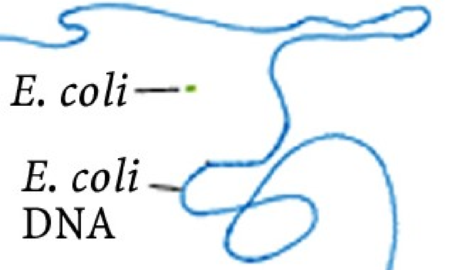
os polipeptídeos, rRNA, tRNA e outros, além de seq. não transcritas ou não traduzidas

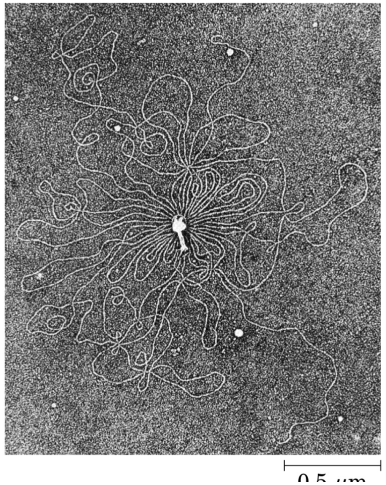
Em eucariotos: a maioria dos genes que codificam proteínas (genes estruturais) são transcritos individualmente.

Em procariotos: freqüentemente são transcritos unidos: policistrônicos

Unidades genéticas: OPERONS

Cromossomos de procariotos





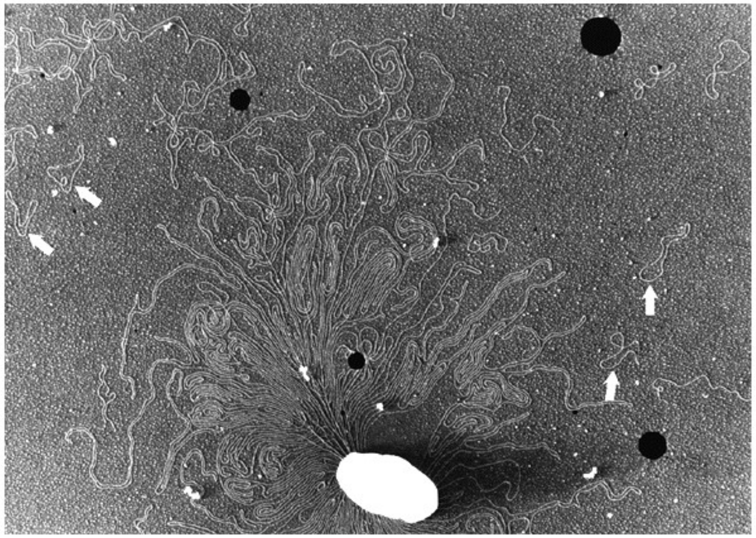


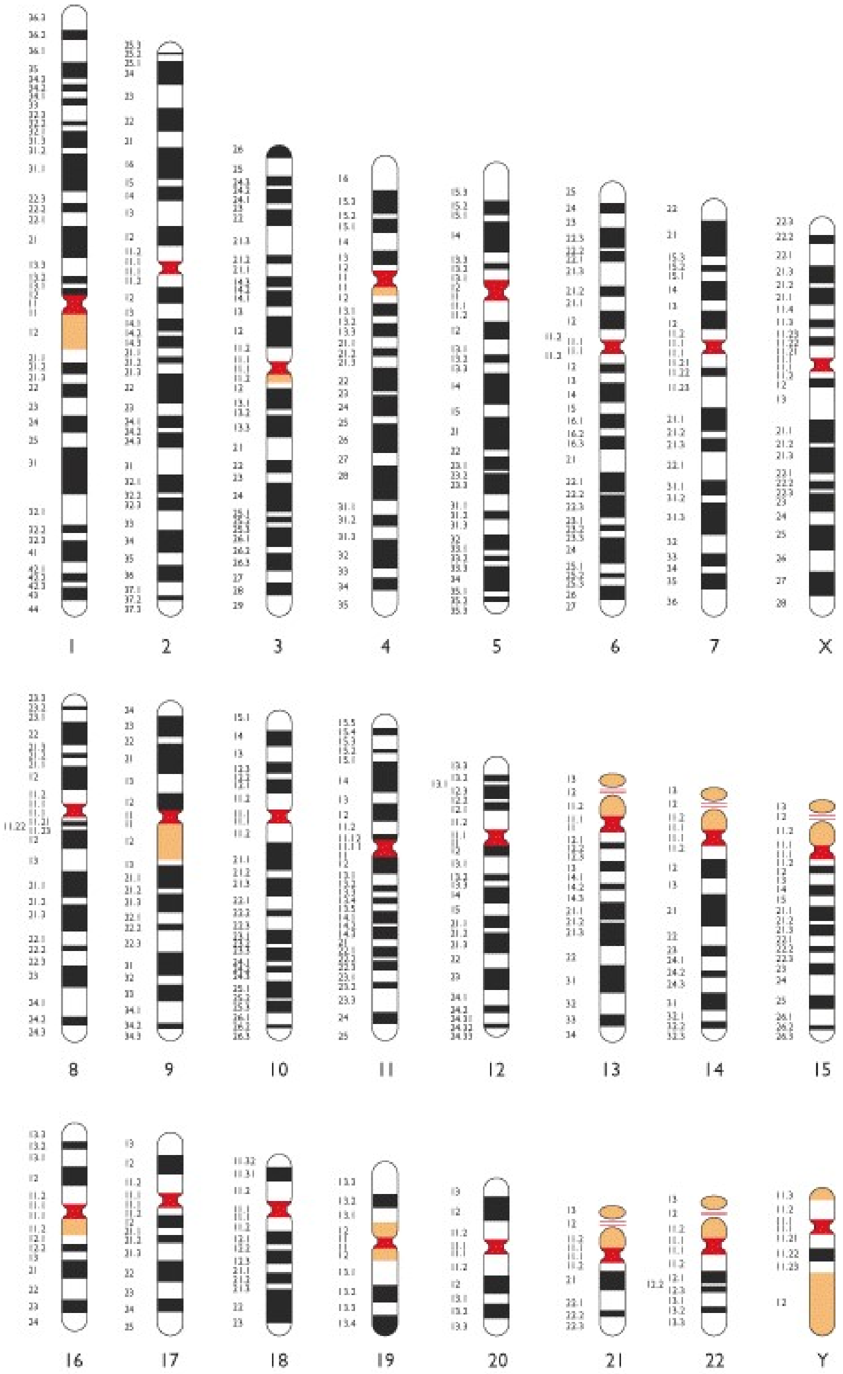
table 24–1

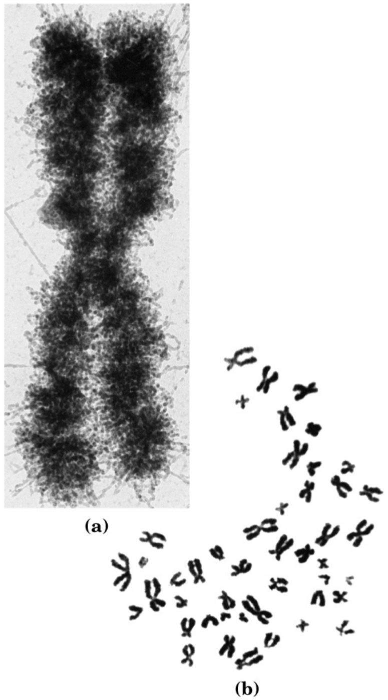
The Sizes of DNA and Viral Particles for Some Bacterial Viruses (Bacteriophages)

Virus	Number of base pairs in viral DNA*	Length of viral DNA (nm)	Long dimension of viral particle (nm)
ϕX174	5,386[†]	1,939[†]	25
T7	39,936	14,377	78
λ (lambda)	48,502	17,460	190
T4	168,889	60,800	210

* The complete base sequences of these bacteriophage genomes have been determined.

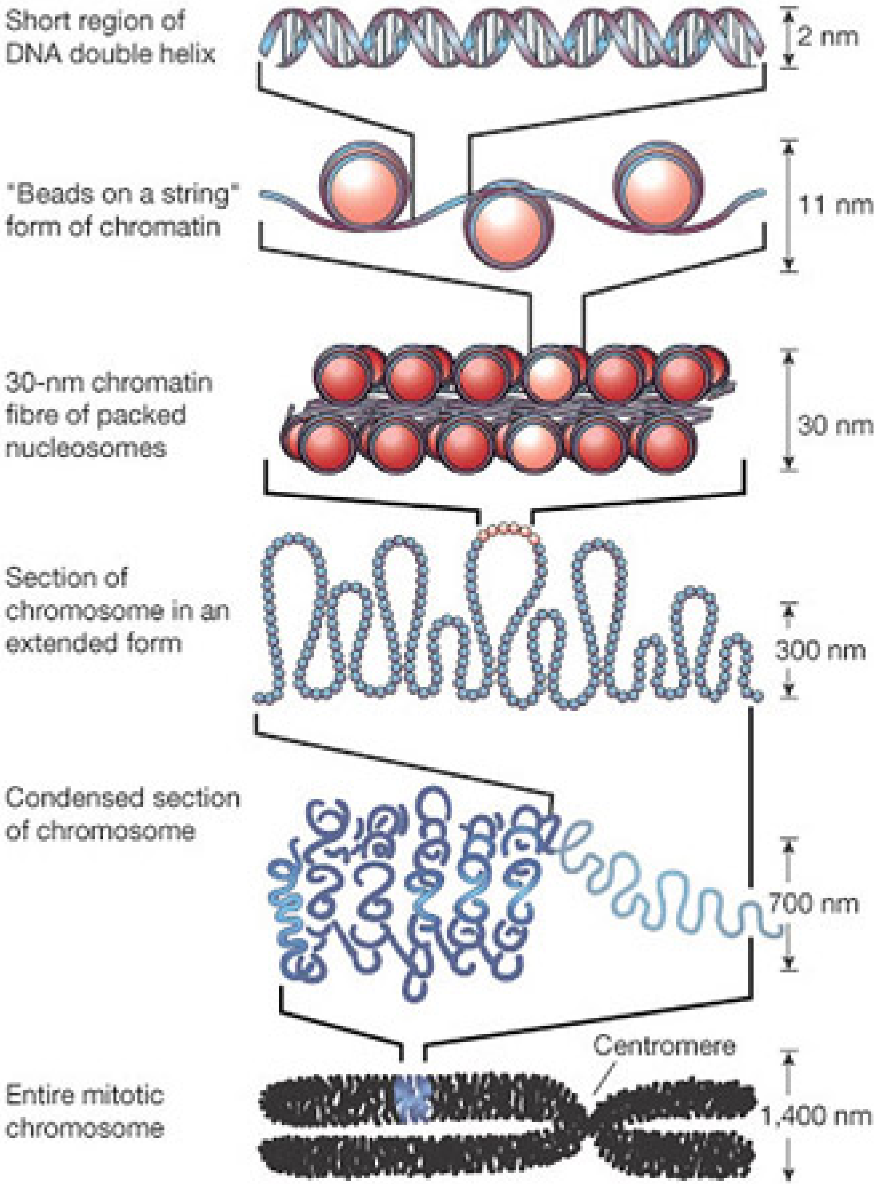
[†] Data are for the replicative form (double-stranded).

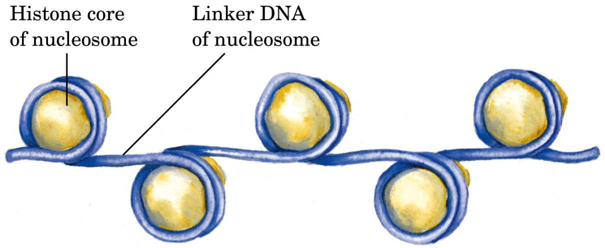


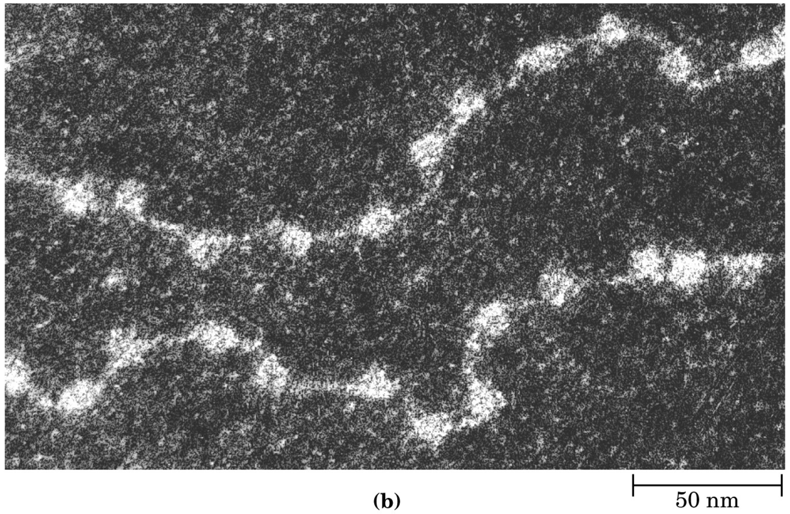


KEY
🟥 Centromere ═ rDNA 🟧 Constitutive heterochromatin

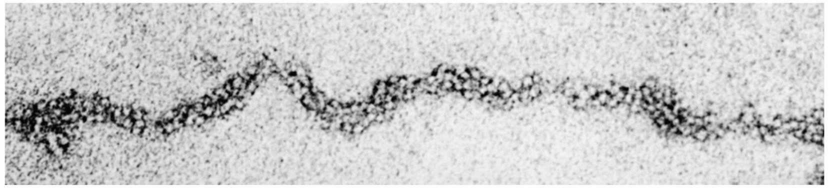
Cromatina

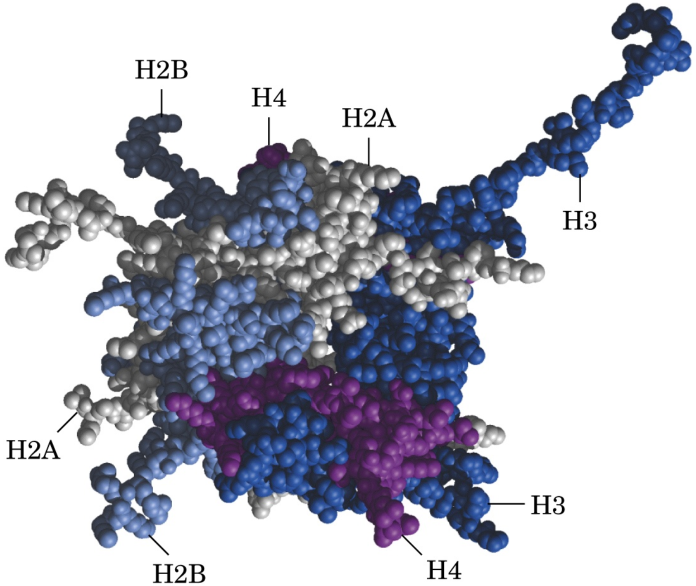

Short region of DNA double helix
2 nm
"Beads on a string" form of chromatin
11 nm
30-nm chromatin fibre of packed nucleosomes
30 nm
Section of chromosome in an extended form
300 nm
Condensed section of chromosome
700 nm
Centromere
Entire mitotic chromosome
1,400 nm


Histone core of nucleosome
Linker DNA of nucleosome


50 nm

(b)





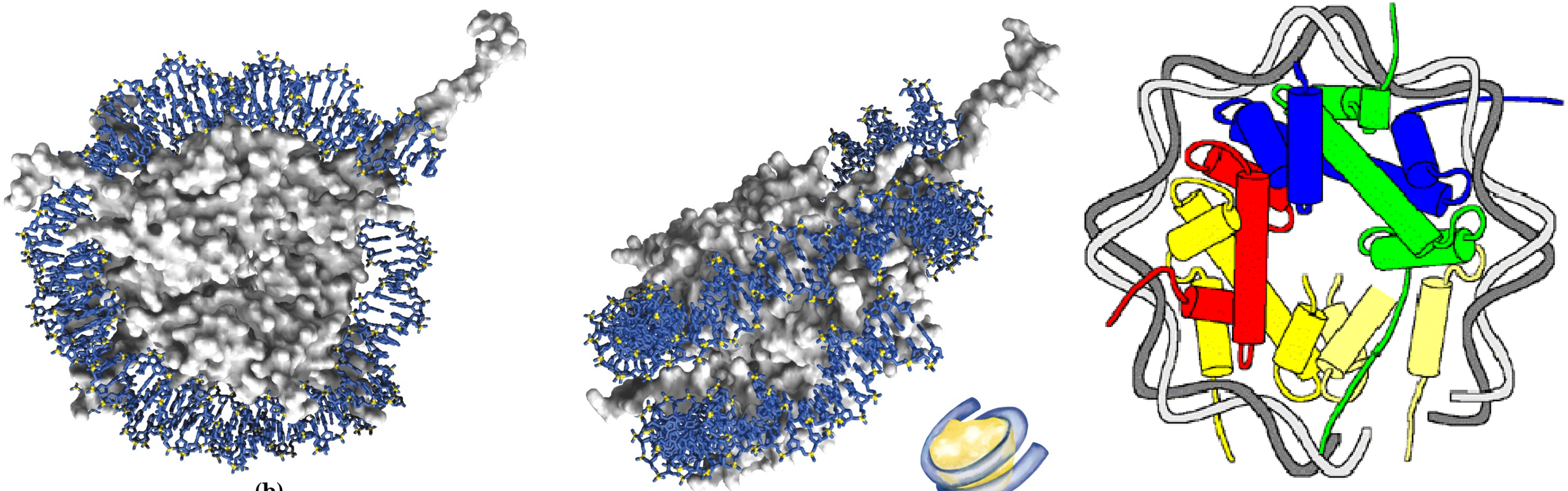
(a)

table 24–3

Types and Properties of Histones

Histone	Molecular weight	Number of amino acid residues	Content of basic amino acids (% of total)	
			Lys	Arg
H1*	21,130	223	29.5	1.3
H2A*	13,960	129	10.9	9.3
H2B*	13,774	125	16.0	6.4
H3	15,273	135	9.6	13.3
H4	11,236	102	10.8	13.7

*The sizes of these histones vary somewhat from species to species. The numbers given here are for bovine histones.



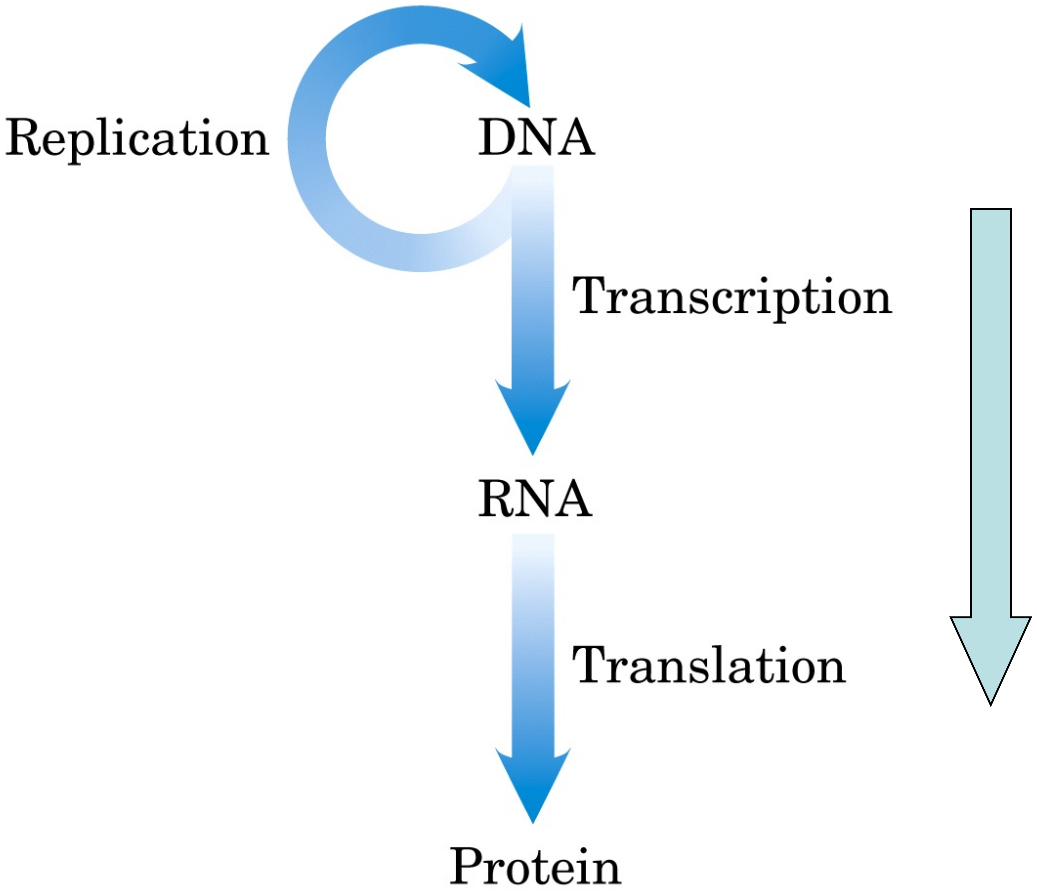
(b)

(c)

Fluxo da informação genética

Dogma Central da Biologia Molecular

Dogma – Grego Dogma – Decisão, Decreto



Replication

DNA

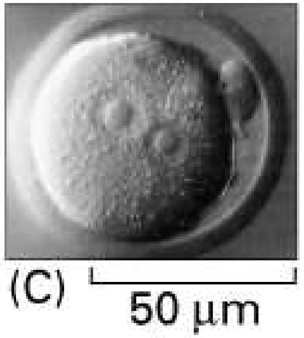
Transcription

RNA

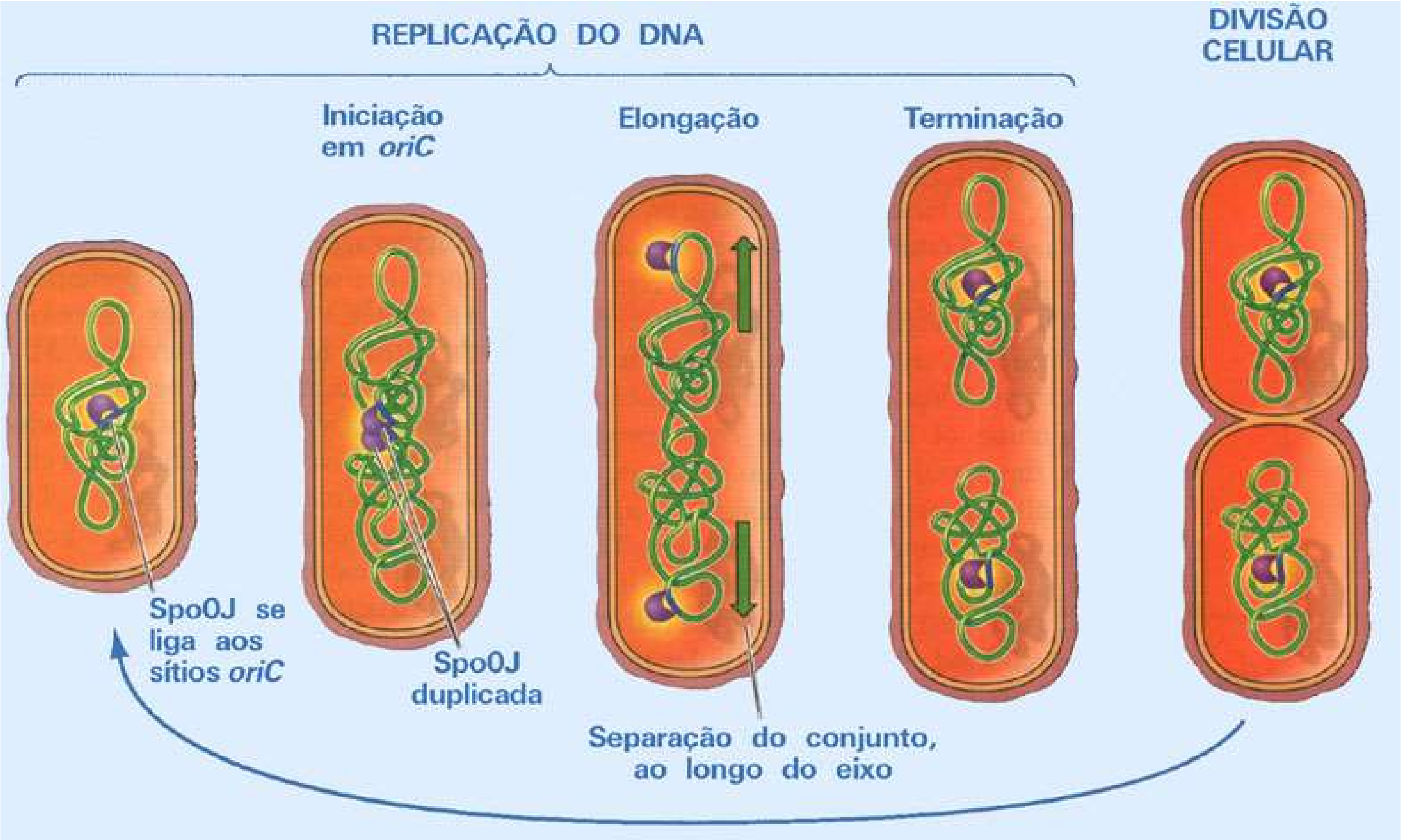
Translation

Protein

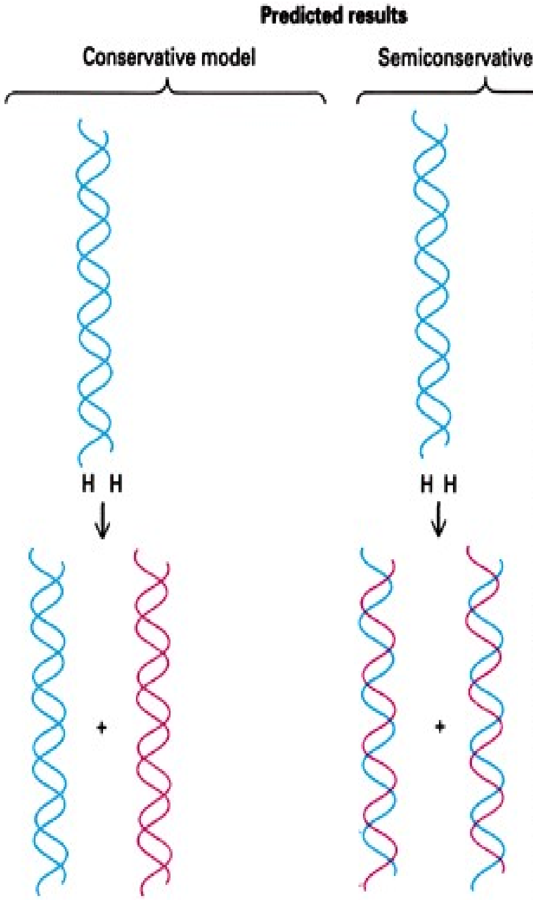
Replicação do DNA


(C)


(D)

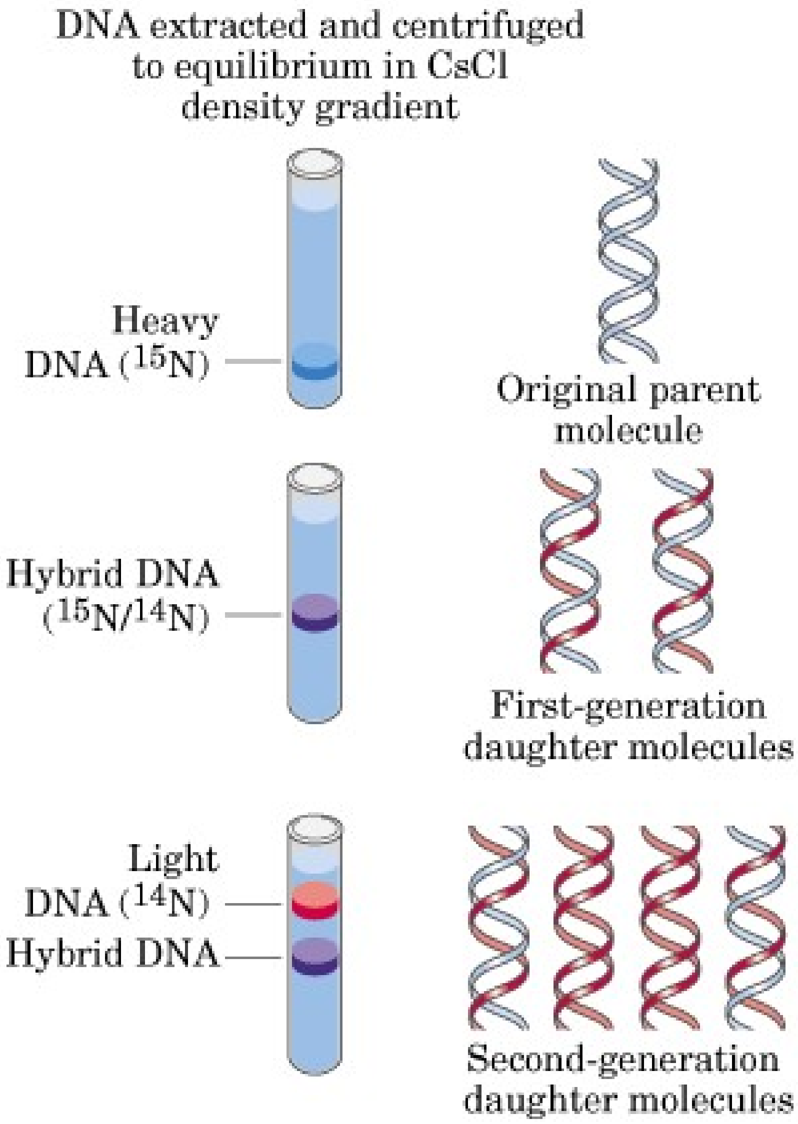

REPLICAÇÃO DO DNA
DIVISÃO CELULAR
Iniciação em *oriC*
Elongação
Terminação
Spo0J se liga aos sítios *oriC*
Spo0J duplicada
Separação do conjunto, ao longo do eixo

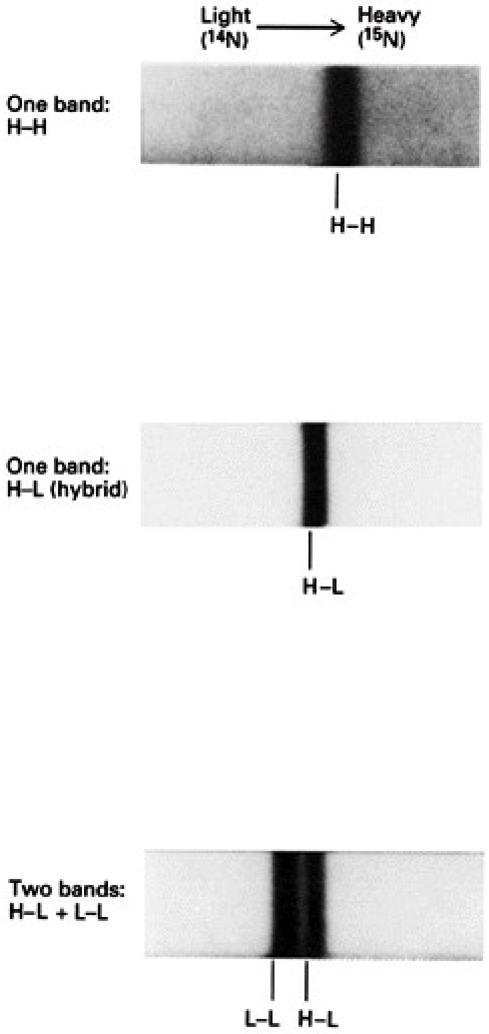
Replicação Conservativa ou semi-conservativa?



Meselson & Stahl

FIGURE 9-3. (Left) Matthew Meselson (b. 1930). (Right) Franklin W. Stahl (b. 1929). [Courtesy of M. Meselson.]


DNA extracted and centrifuged to equilibrium in CsCl density gradient
Heavy DNA (^{15}N)
Original parent molecule
Hybrid DNA (^{15}N/^{14}N)
First-generation daughter molecules
Light DNA (^{14}N)
Hybrid DNA
Second-generation daughter molecules

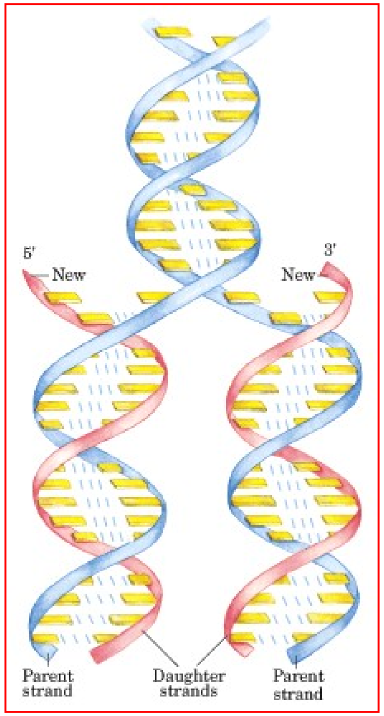

Light (^{14}N)
Heavy (^{15}N)
One band: H–H
H–H
One band: H–L (hybrid)
H–L
Two bands: H–L + L–L
L–L
H–L

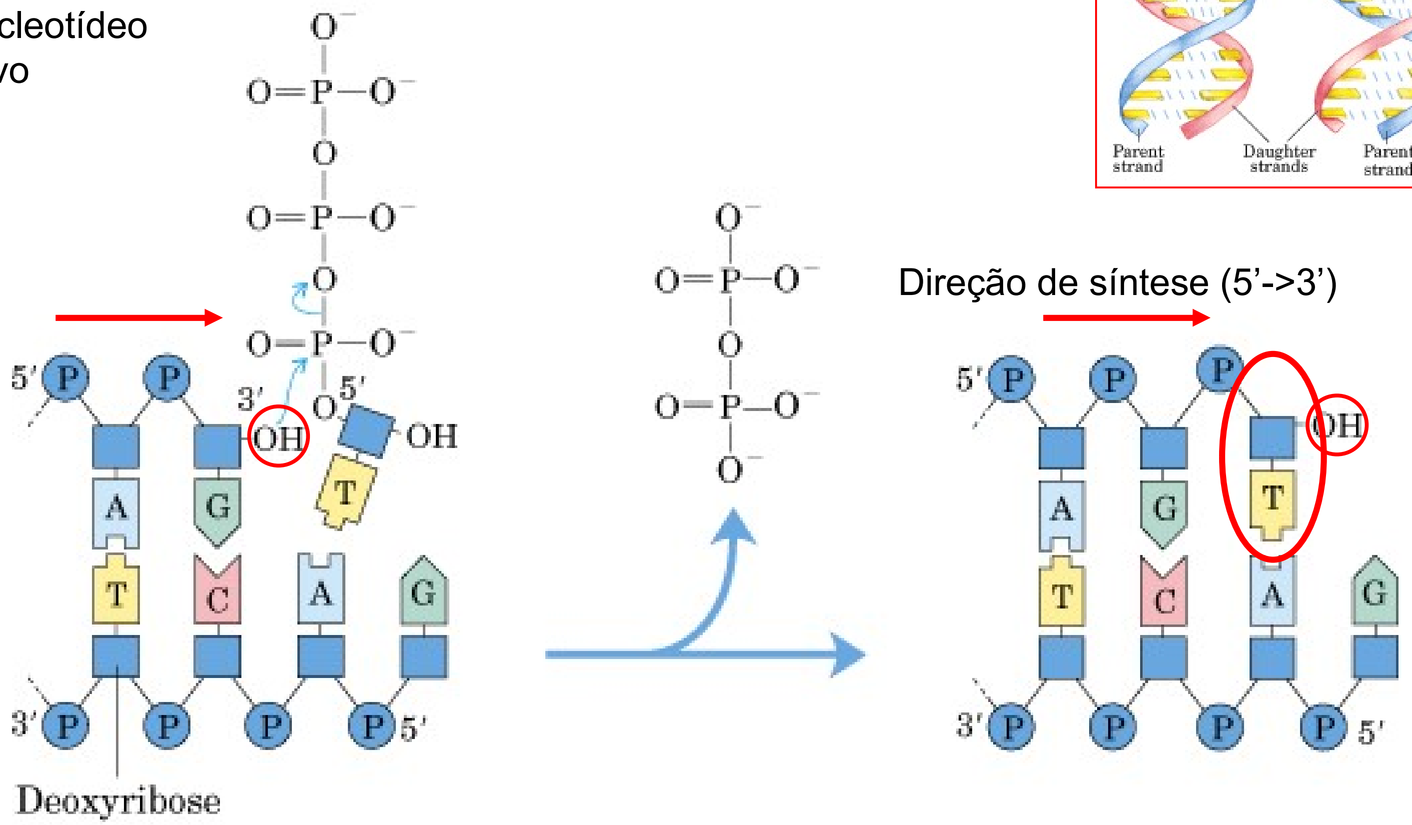
Nobel 1958

Processo de replicação do DNA.

Mediado por diversas enzimas

Principais enzima de síntese: DNA polimerases





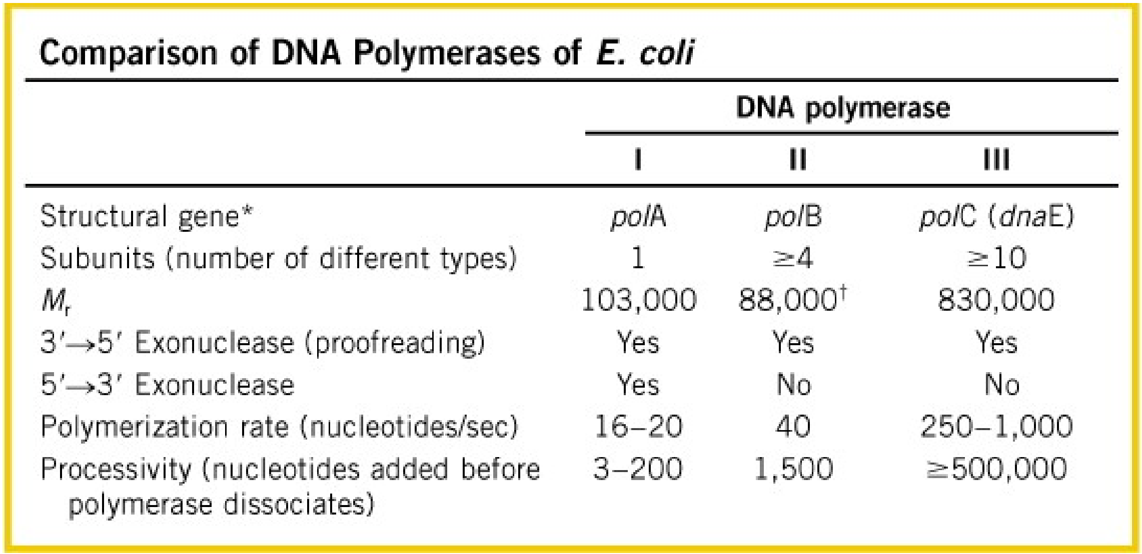
Nucleotídeo novo

Fita DNA sintetizada

Fita DNA molde

Direção de síntese (5’->3’)

Diversas DNA polimerases estão envolvidas na replicação do DNA.

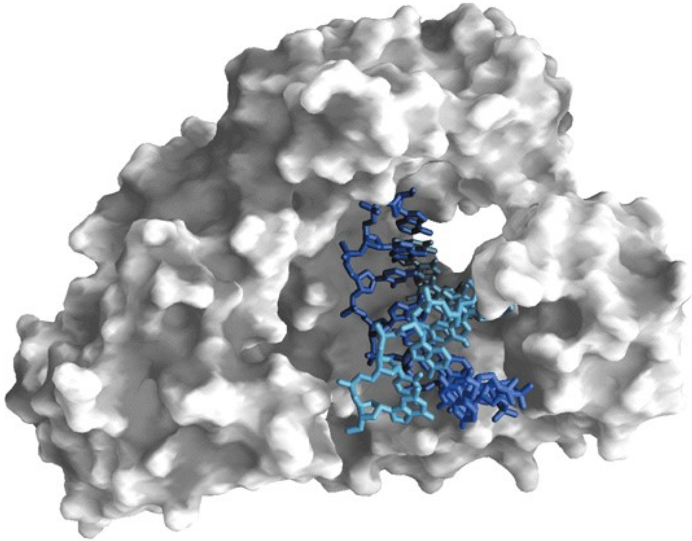


Comparison of DNA Polymerases of *E. coli*

	DNA polymerase		
	I	II	III
Structural gene*	*pol*A	*pol*B	*pol*C (*dna*E)
Subunits (number of different types)	1	≥4	≥10
M_r	103,000	$88{,}000^{\dagger}$	830,000
3′→5′ Exonuclease (proofreading)	Yes	Yes	Yes
5′→3′ Exonuclease	Yes	No	No
Polymerization rate (nucleotides/sec)	16–20	40	250–1,000
Processivity (nucleotides added before polymerase dissociates)	3–200	1,500	≥500,000

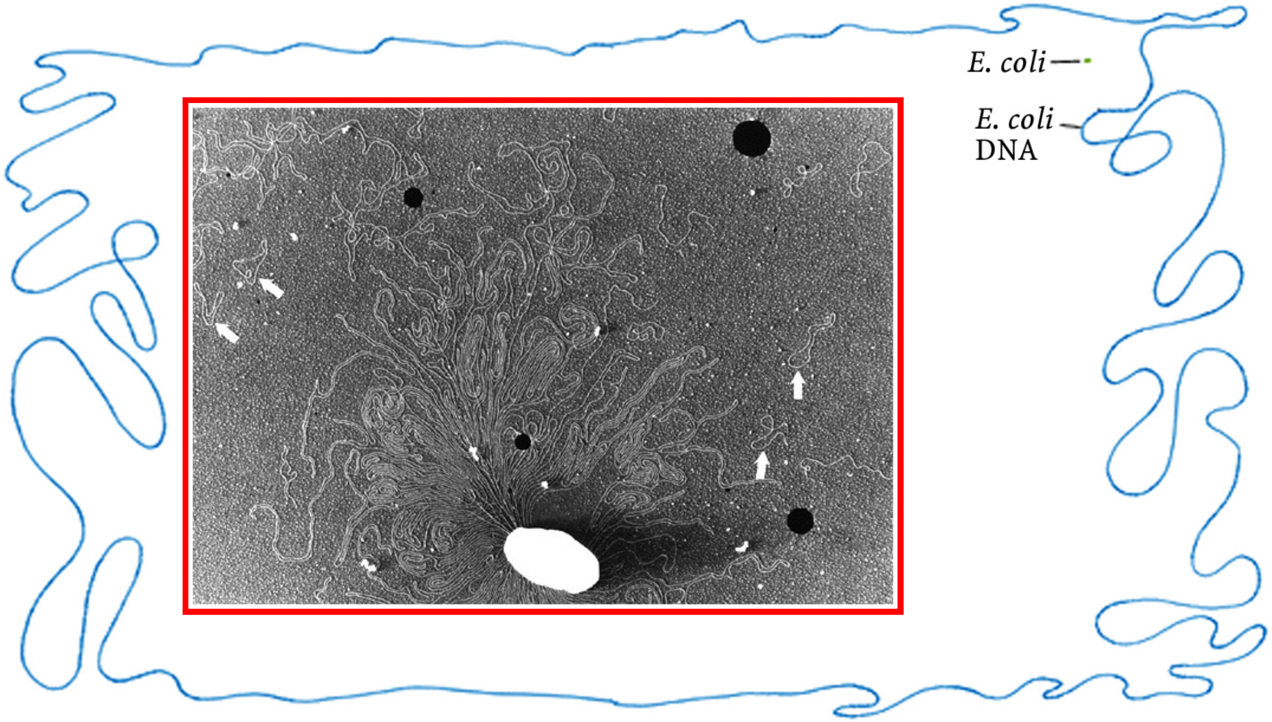
DNA polimerase I e II : reparo de DNA

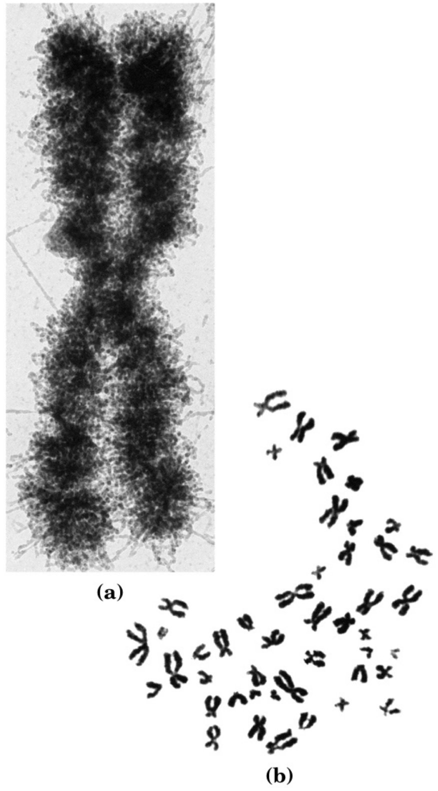
DNA polimerase III : replicação



DNA polimerase I de *Escherichia coli*
(Klenow fragment)

A replicação bidirecional do DNA permite completar a tarefa em menor tempo



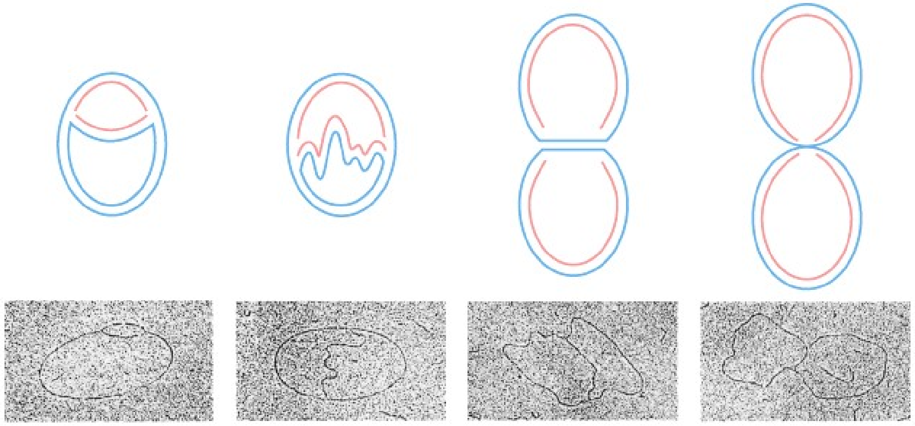


Somado = 2m

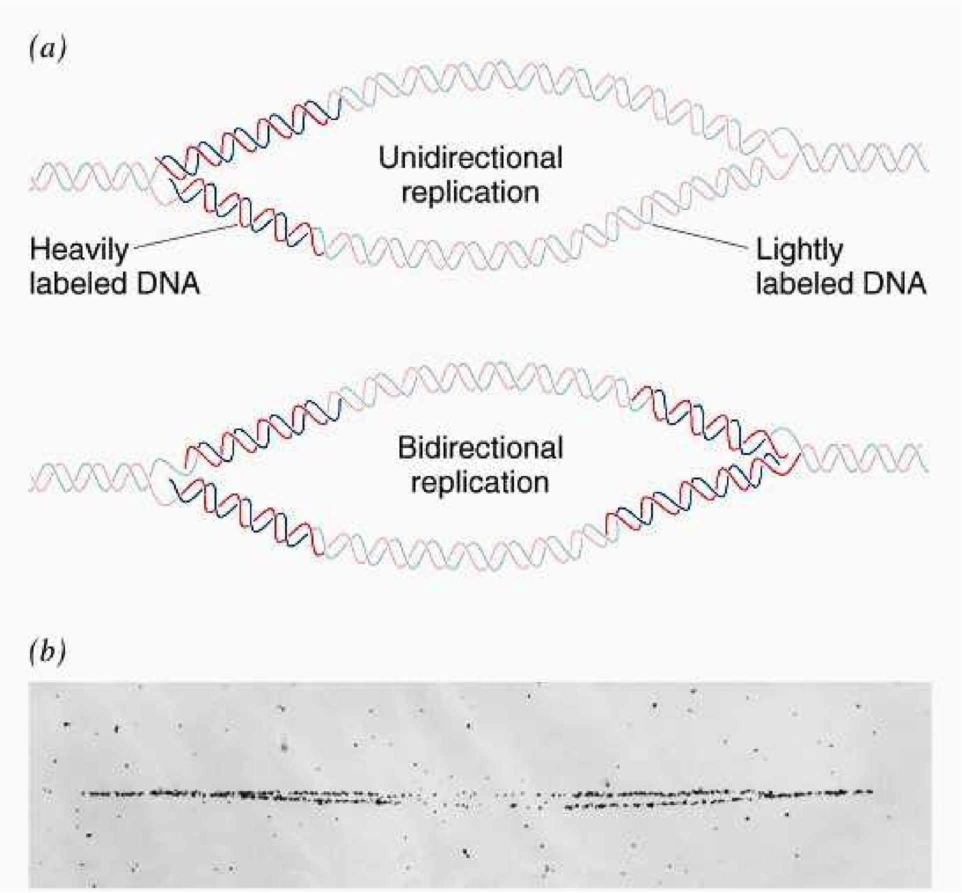
Problema de tamanho: Genoma bacteriano +/- 10.000.000 pbs (pares de bases)
DNA pol III sintetiza entre 250 e 1000 bases por segundo
Levaria entre 2:48 horas e 11 horas para completar o genoma
E. coli se divide a cada 30 minutos (condições ideais)

Genoma Humano: +/- 3.000.000.000 pbs
Tempo de replicação: 34 a 139 dias

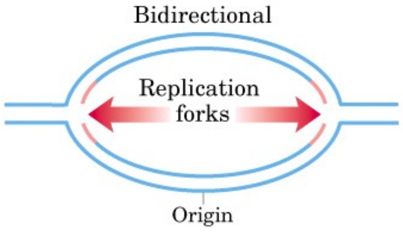
Visualização da replicação bidirecional do DNA

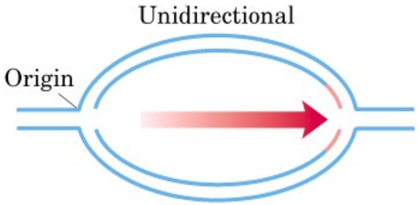


(a)

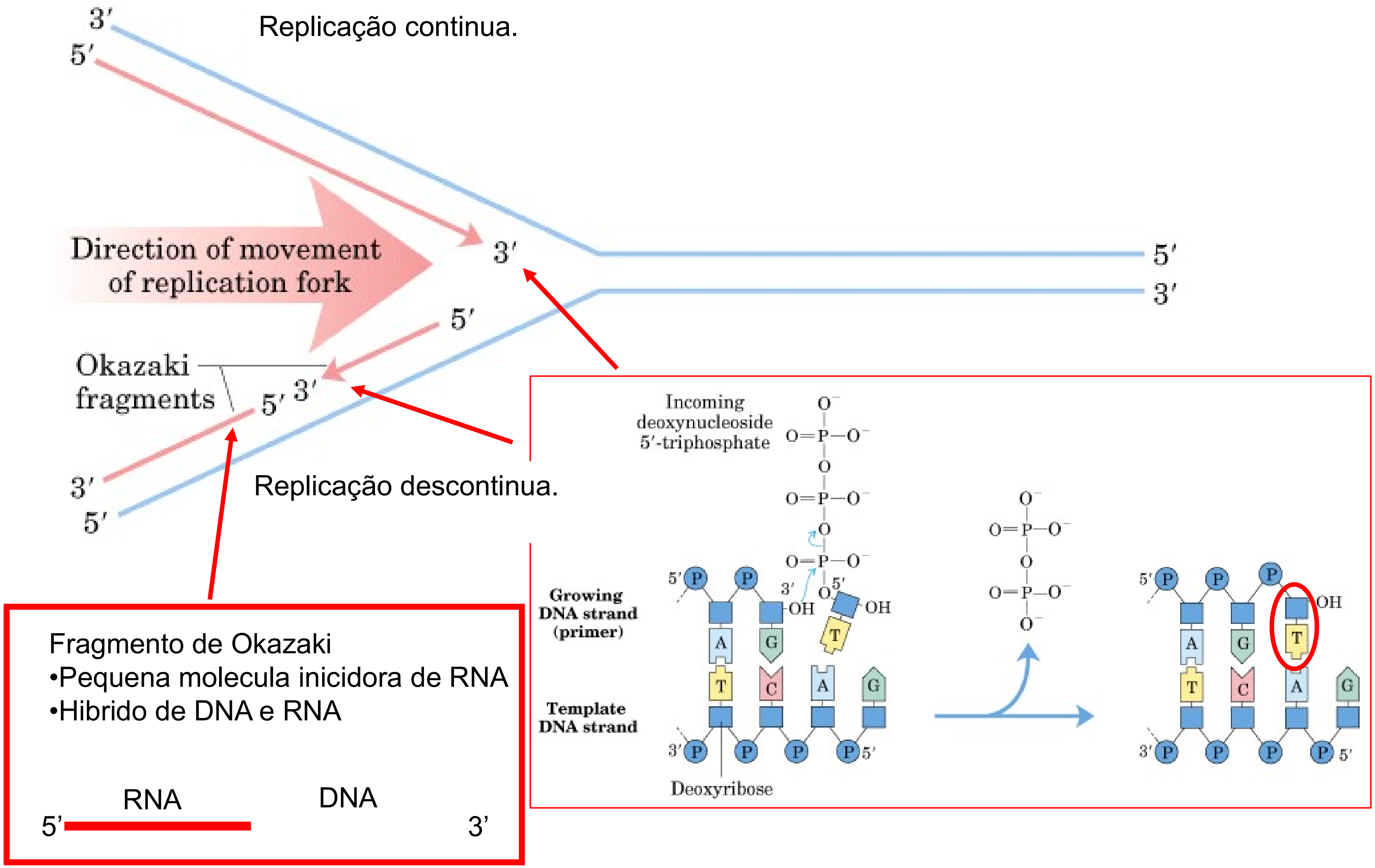


A replicação progride do 5’ para o 3’ do DNA Inicia-se em um ponto da molécula e se estende em ambas as direções.





Replicação do DNA: continua e descontinua



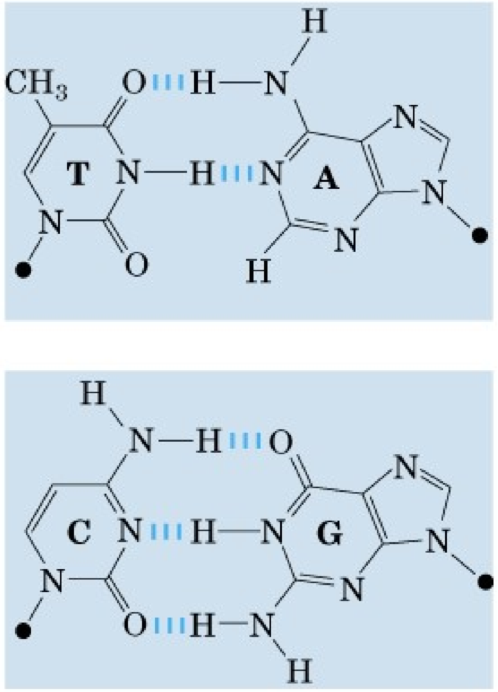
Replicação continua.

Replicação descontinua.

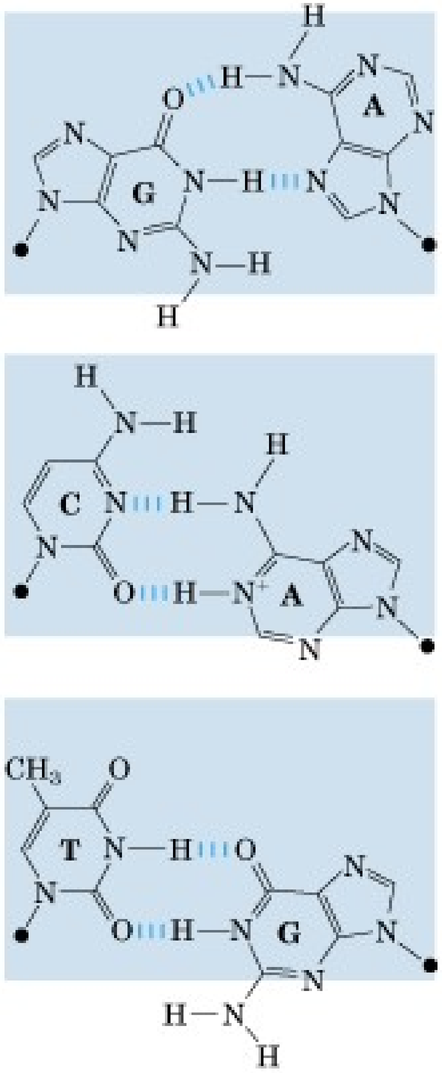
Fragmento de Okazaki
- Pequena molecula inicidora de RNA
- Hibrido de DNA e RNA

RNA DNA
5’ 3’

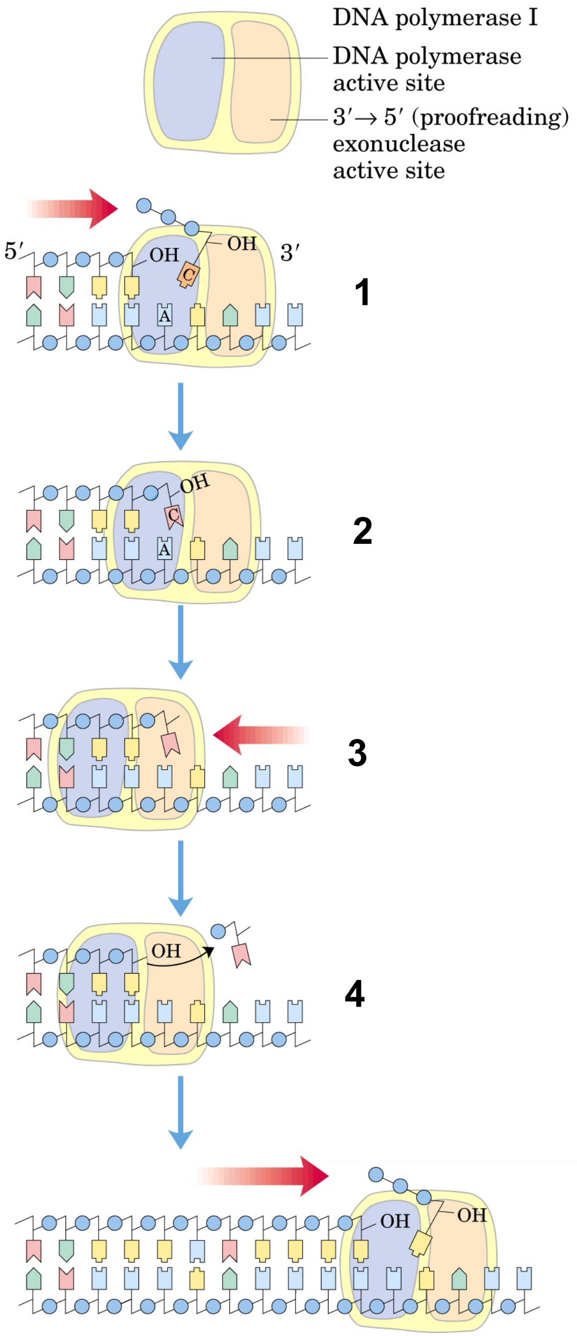
A geometria do pareamento de bases contribui para a fidelidade da replicação



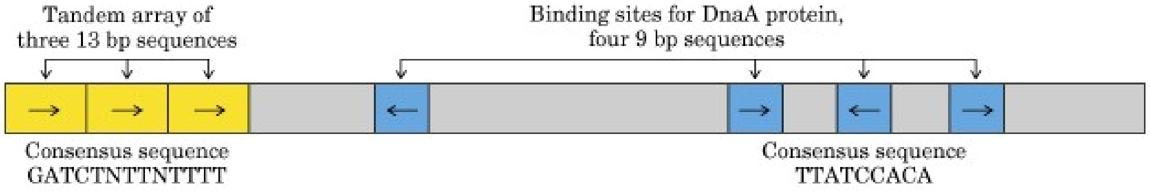
Pareamento correto

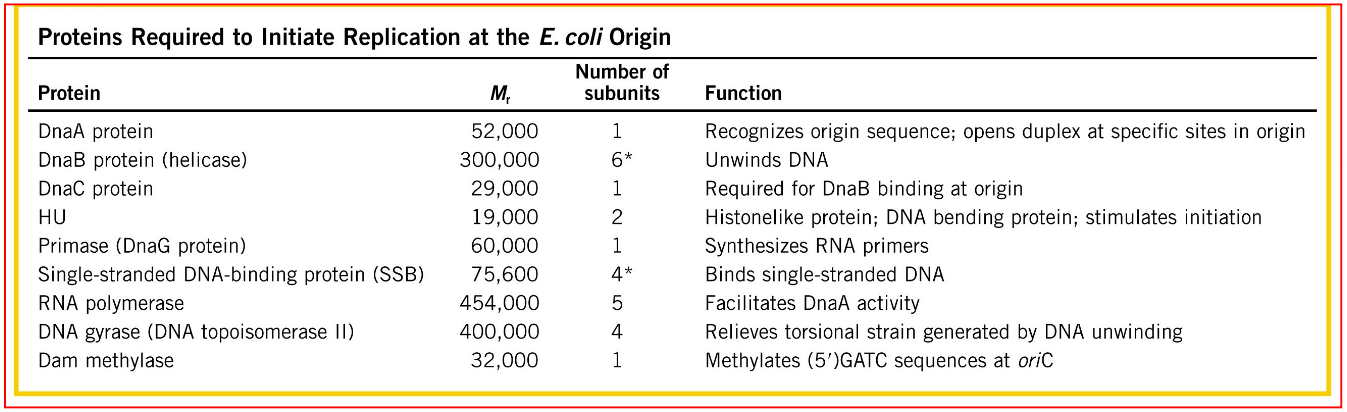


Pareamento incorreto



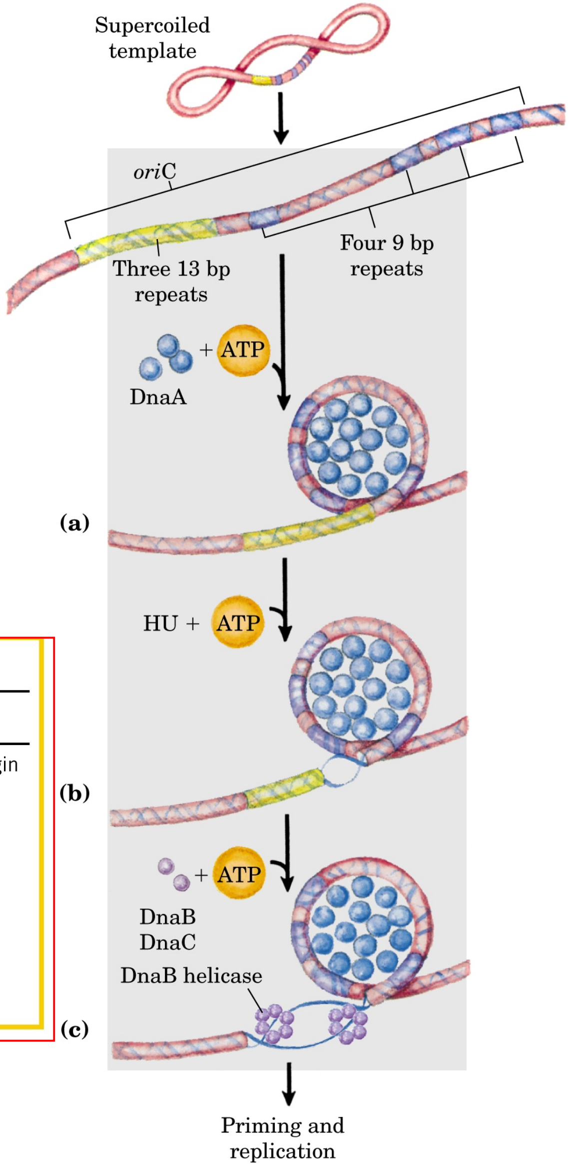
Inicio da replicacao



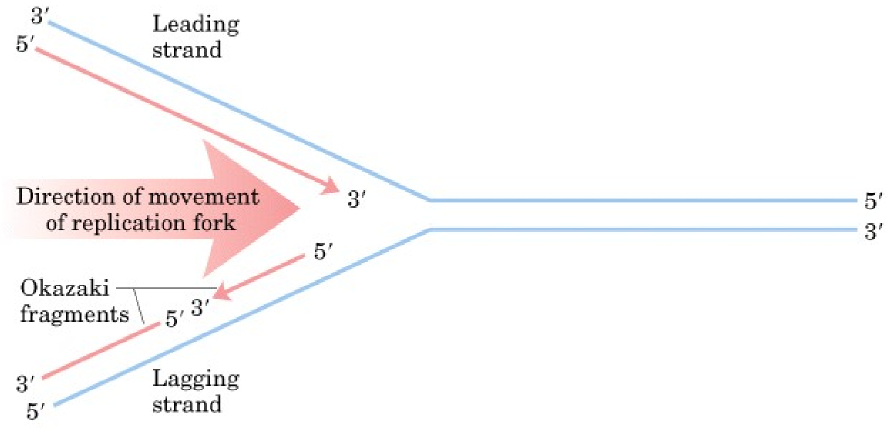


Proteins Required to Initiate Replication at the *E. coli* Origin

Protein	M_r	Number of subunits	Function
DnaA protein	52,000	1	Recognizes origin sequence; opens duplex at specific sites in origin
DnaB protein (helicase)	300,000	6*	Unwinds DNA
DnaC protein	29,000	1	Required for DnaB binding at origin
HU	19,000	2	Histonelike protein; DNA bending protein; stimulates initiation
Primase (DnaG protein)	60,000	1	Synthesizes RNA primers
Single-stranded DNA-binding protein (SSB)	75,600	4*	Binds single-stranded DNA
RNA polymerase	454,000	5	Facilitates DnaA activity
DNA gyrase (DNA topoisomerase II)	400,000	4	Relieves torsional strain generated by DNA unwinding
Dam methylase	32,000	1	Methylates (5′)GATC sequences at *ori*C



Replication fork



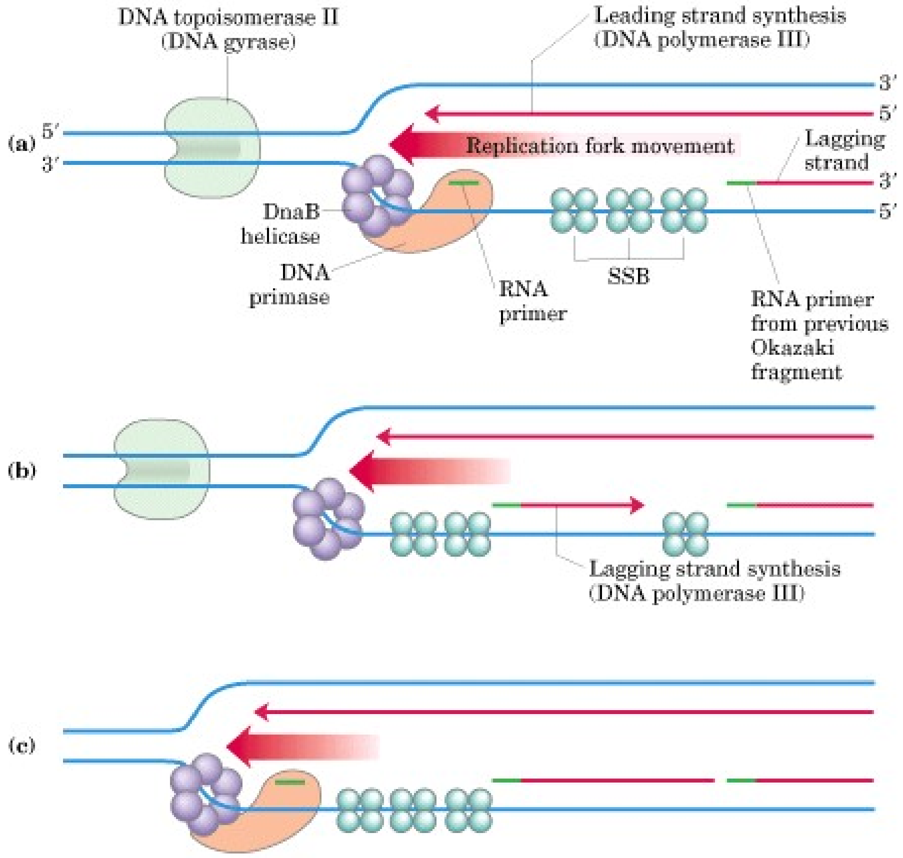
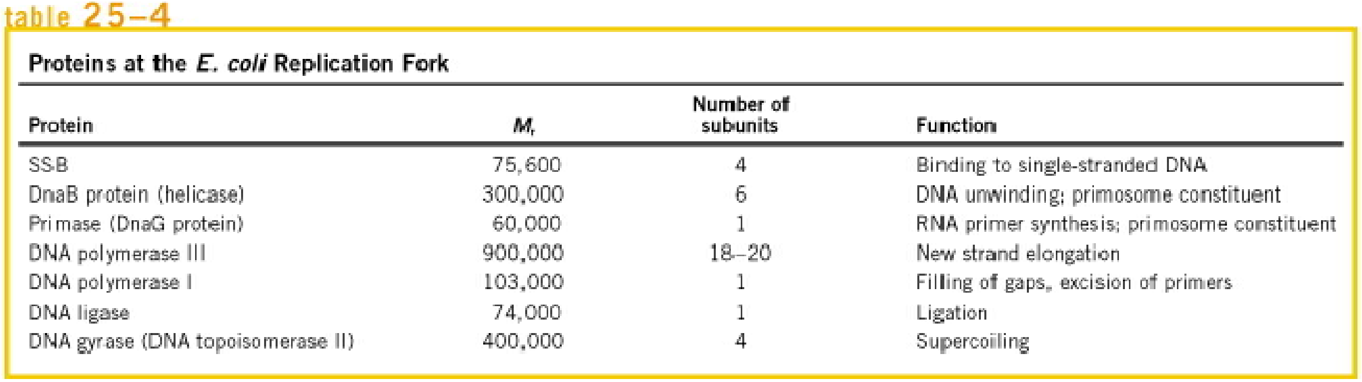


table 25–4



Proteins at the *E. coli* Replication Fork

Protein	M_r	Number of subunits	Function
SSB	75,600	4	Binding to single-stranded DNA
DnaB protein (helicase)	300,000	6	DNA unwinding; primosome constituent
Primase (DnaG protein)	60,000	1	RNA primer synthesis; primosome constituent
DNA polymerase III	900,000	18–20	New strand elongation
DNA polymerase I	103,000	1	Filling of gaps, excision of primers
DNA ligase	74,000	1	Ligation
DNA gyrase (DNA topoisomerase II)	400,000	4	Supercoiling

Modified from Kornberg, A. (1982) *Supplement to DNA Replication*, Table S11-2, W.H. Freeman and Company, New York.

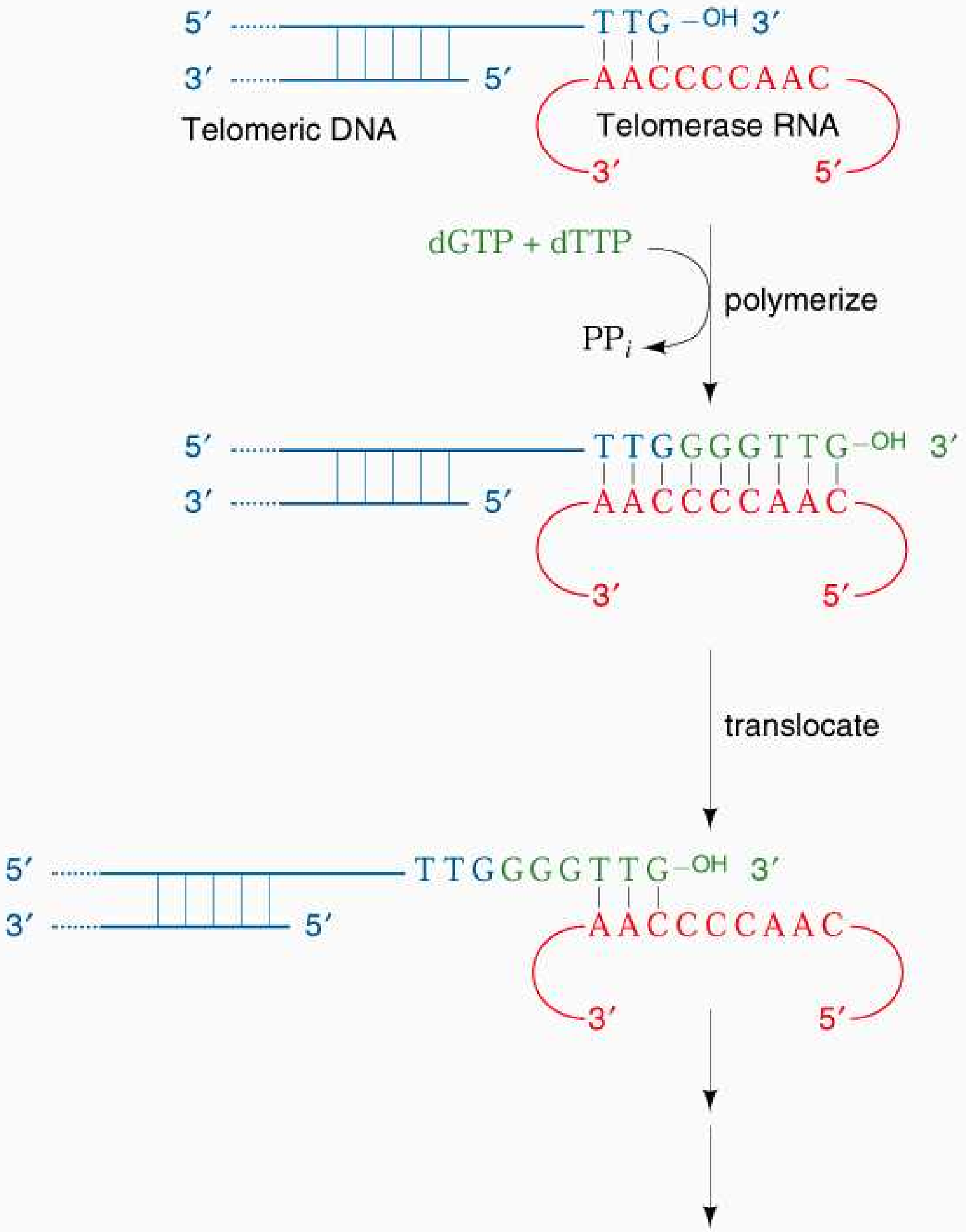
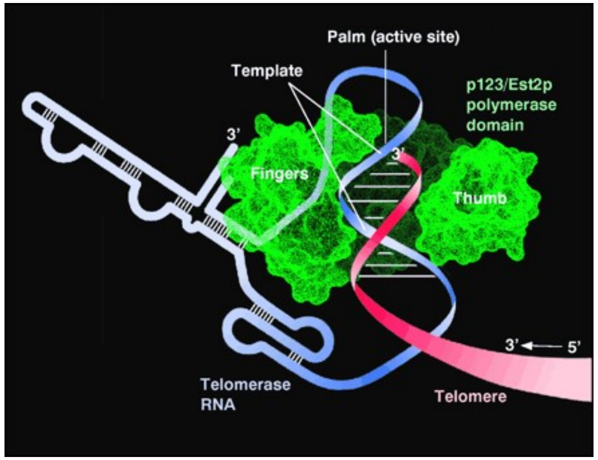


Figure 24-21. The proposed mechanism for the synthesis of telomeric DNA by *Tetrahymena* telomerase. [After Greider, C.W. and Blackburn, E.H., *Nature* 337, 336 (1989).]

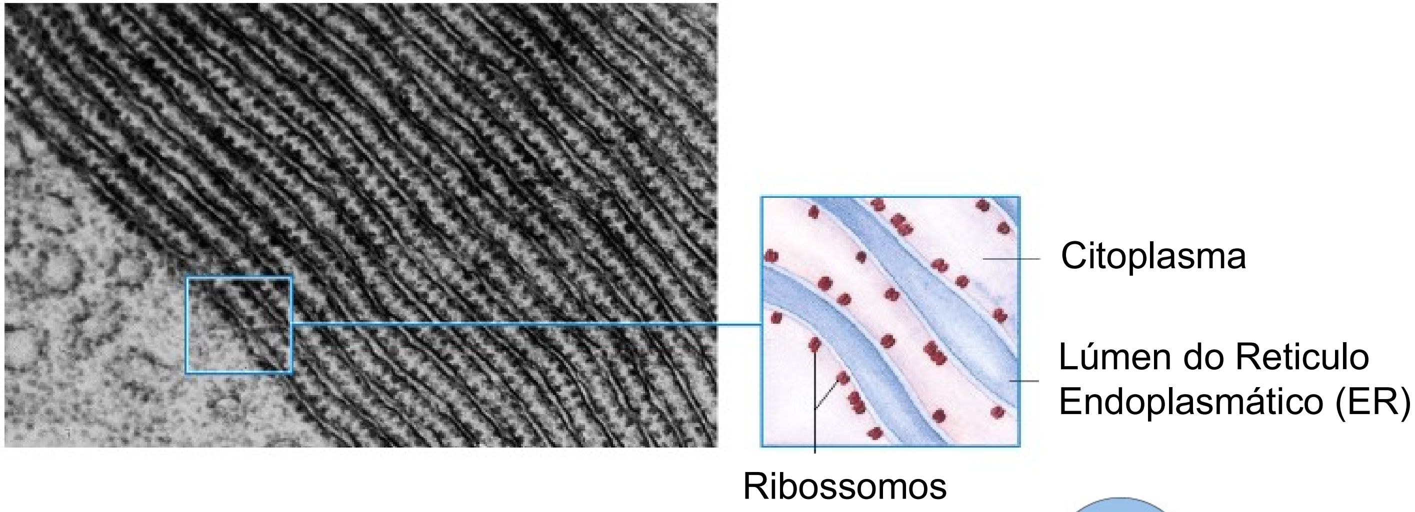
Copyright 1999 John Wiley and Sons, Inc. All rights reserved.



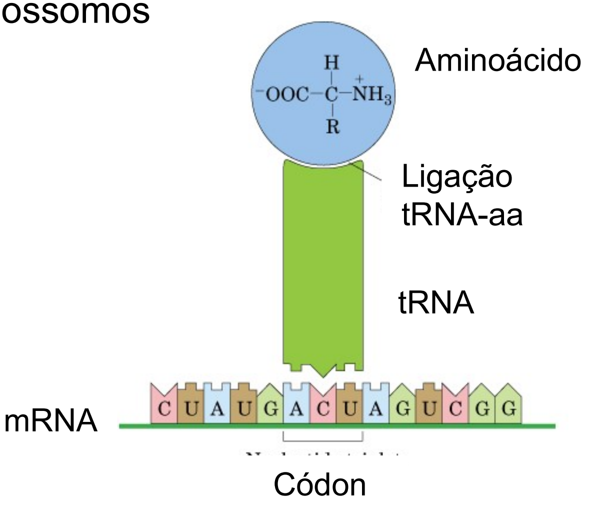
Código Genético. Ativação de aminoácidos e Síntese de Proteínas

Código genético e Síntese protéica

Início da década de 1950: Paul Zamecnik et. al
Identificaram a síntese de proteínas ocorrendo em partículas – Ribossomos



Identificaram a existência de um intermediário RNA (tRNA) e a proteína de transferência (aminoacil-tRNA-sintetase).



Código Genético

- O mRNA não reconhece diretamente um aminoácido (aa);
- mRNA liga-se a moléculas de tRNA que carregam o aa correspondente;
- cada tRNA tem um anticódon;
- o código genético “padrão” não é universal;
- a tradução correta requer 2 etapas de reconhecimento:
- Ligação do aa correto ao tRNA (por meio de uma aminoacil tRNA sintetase);
- O aminoacil-tRNA (anticódon) deve parear com um códon do mRNA no ribossomo;
- Mais de um tRNA pode ser carregado com um determinado

Tradução.

Qual a correspondência entre ácidos nucléicos e seqüência de proteínas?

O código genético:

seqüência de nucleotídeos → seqüência de aminoácidos

4 tipos de resíduos → 20 tipos de resíduos

(A, C, G, T ou U)

$4^1 = 4$ possibilidades teóricas

$4^2 = 16$ possibilidades teóricas

$4^3 = 64$ possibilidades teóricas

certos aa são determinados por mais de um códon

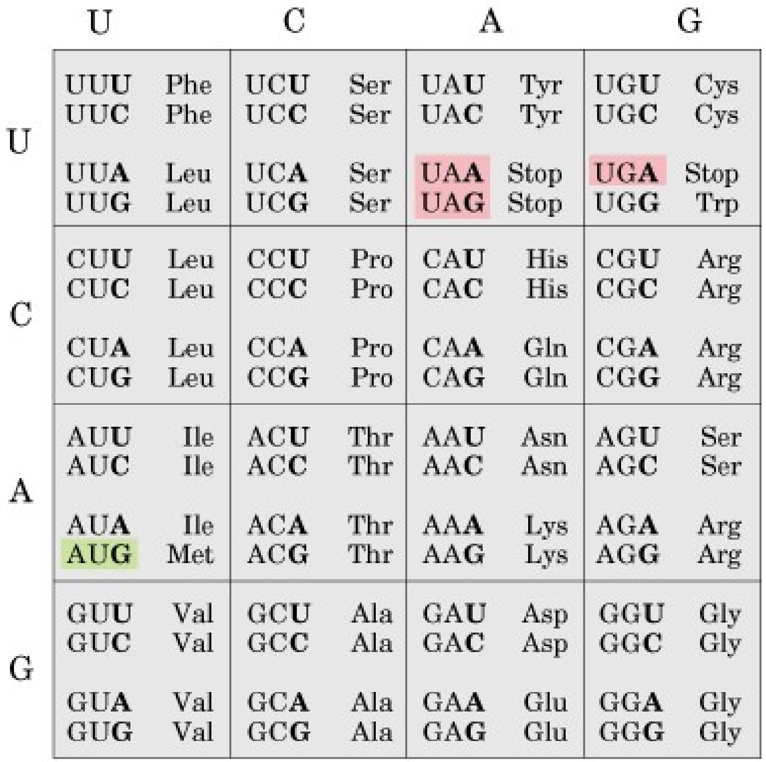
código degenerado

códons: são trincas lidas seqüencialmente

ALI VEM MEU PAI COM SEU TIO
..A LIV EMM EUP AIC OMS EUT IO.
.AL IVE MME UPA ICO MSE UTI O..

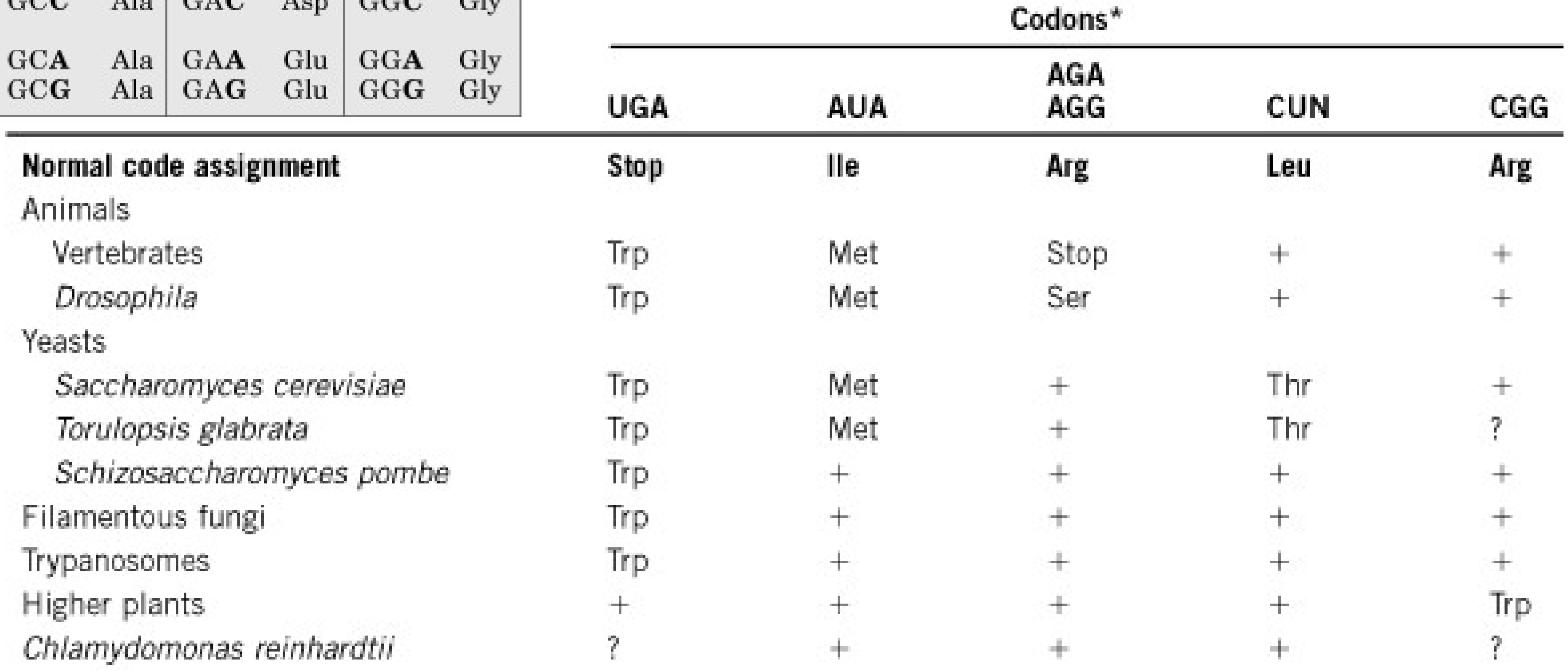
Fases de leitura

Variações naturais da interação códon-anticódon



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

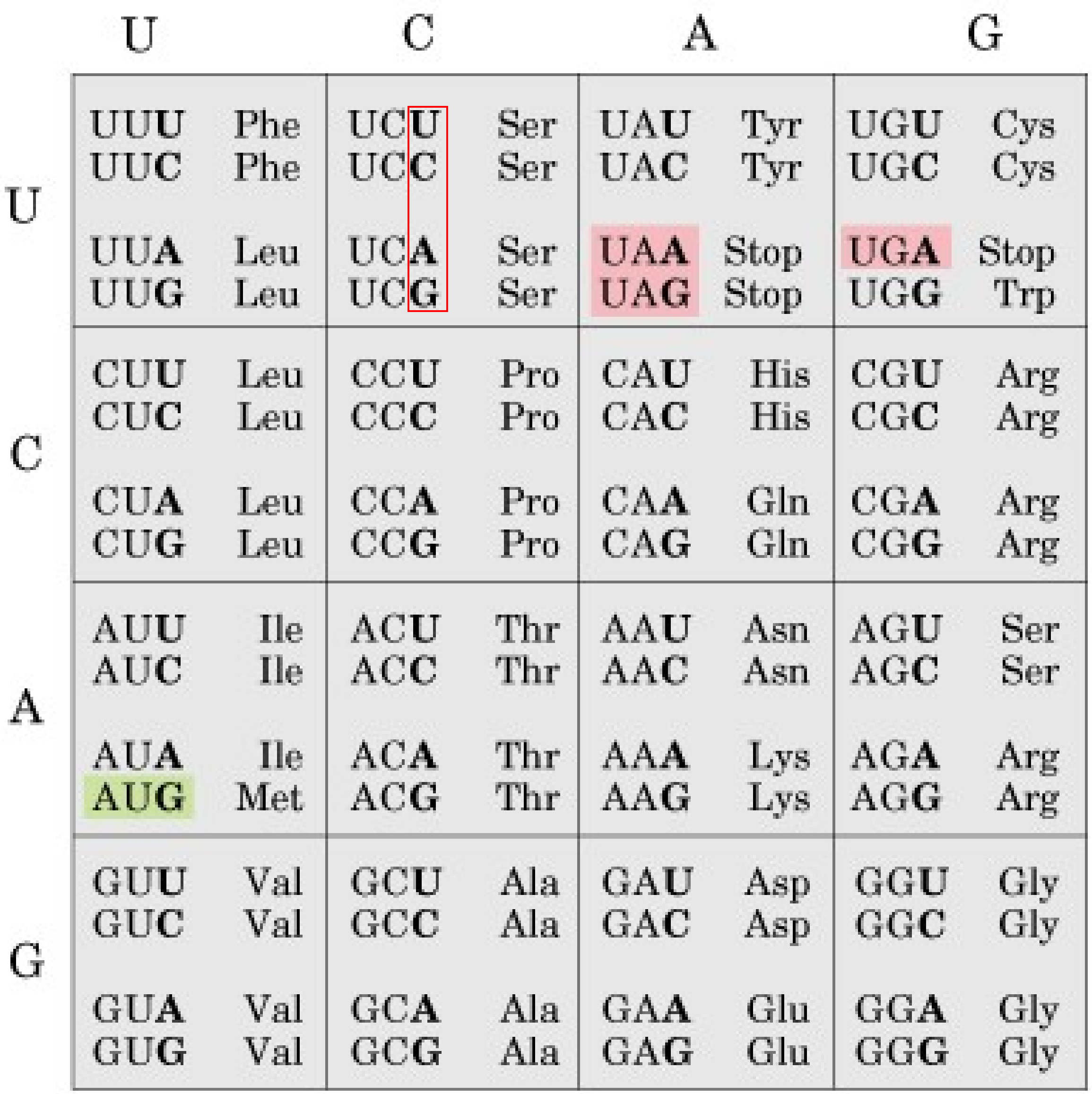
Casos observados nas mitocôndrias



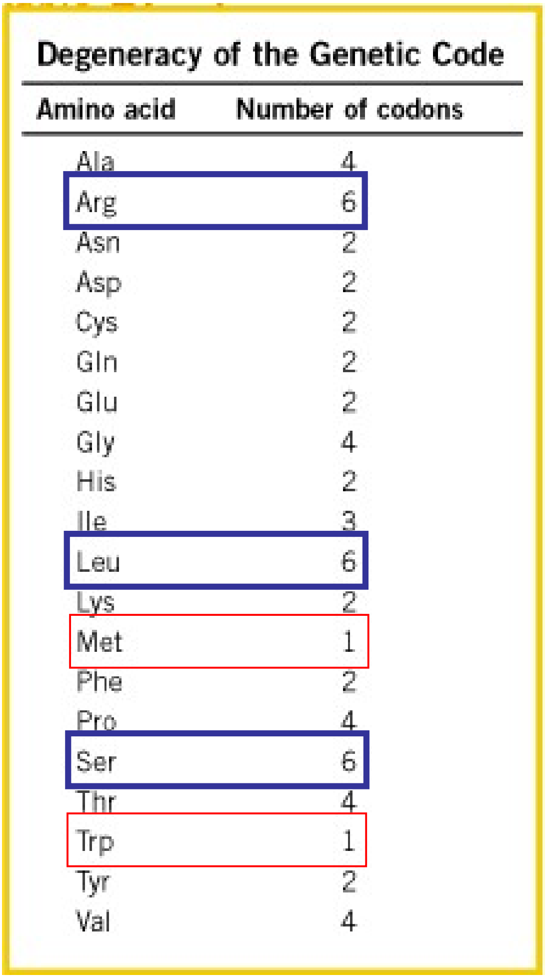
	Codons*				
	UGA	AUA	AGA AGG	CUN	CGG
Normal code assignment	**Stop**	**Ile**	**Arg**	**Leu**	**Arg**
Animals					
Vertebrates	Trp	Met	Stop	+	+
Drosophila	Trp	Met	Ser	+	+
Yeasts					
Saccharomyces cerevisiae	Trp	Met	+	Thr	+
Torulopsis glabrata	Trp	Met	+	Thr	?
Schizosaccharomyces pombe	Trp	+	+	+	+
Filamentous fungi	Trp	+	+	+	+
Trypanosomes	Trp	+	+	+	+
Higher plants	+	+	+	+	Trp
Chlamydomonas reinhardtii	?	+	+	+	?

2

1



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



Degeneracy of the Genetic Code

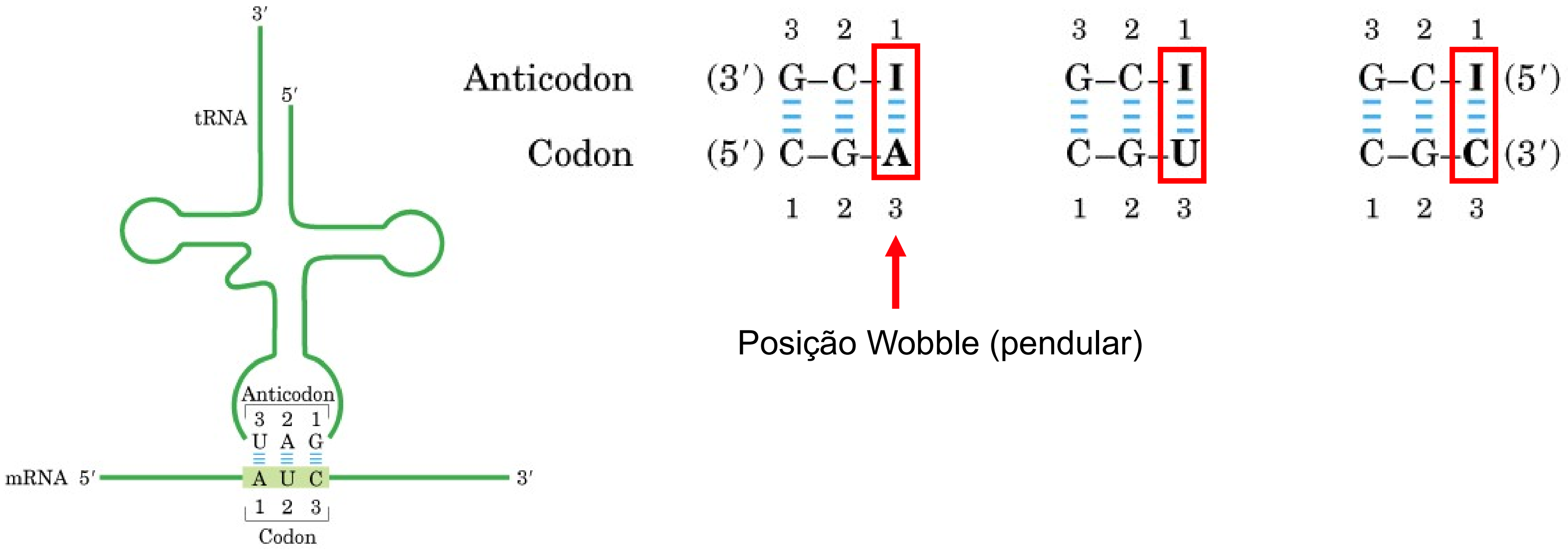
Amino acid	Number of codons
Ala	4
Arg	6
Asn	2
Asp	2
Cys	2
Gln	2
Glu	2
Gly	4
His	2
Ile	3
Leu	6
Lys	2
Met	1
Phe	2
Pro	4
Ser	6
Thr	4
Trp	1
Tyr	2
Val	4

5’ – CCA – 3’ codon
3’ – GGU – 5’ anticodon

Interações códon-anticódon

- O tRNA apropriado é selecionado pela interação códon-anticódon;
- o pareamento é antiparalelo;
- muitos tRNAs ligam-se a 2 ou 3 códons que especificam seu aa correspondente. Por ex. IGC (I = inosina)

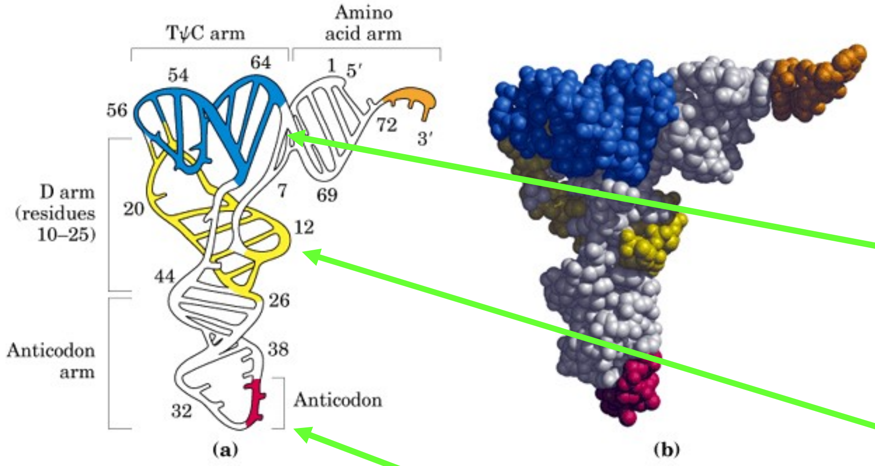
```
CGI          CGI          CGI
 |||         |||           |||
GCU          GCC          GCA
```

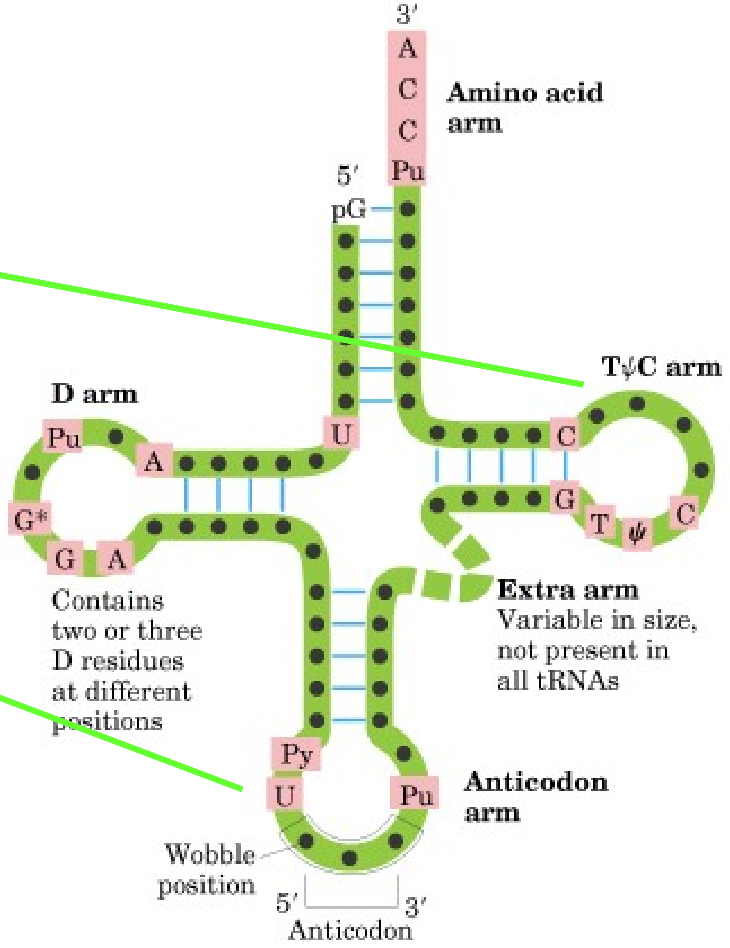


Posição Wobble (pendular)

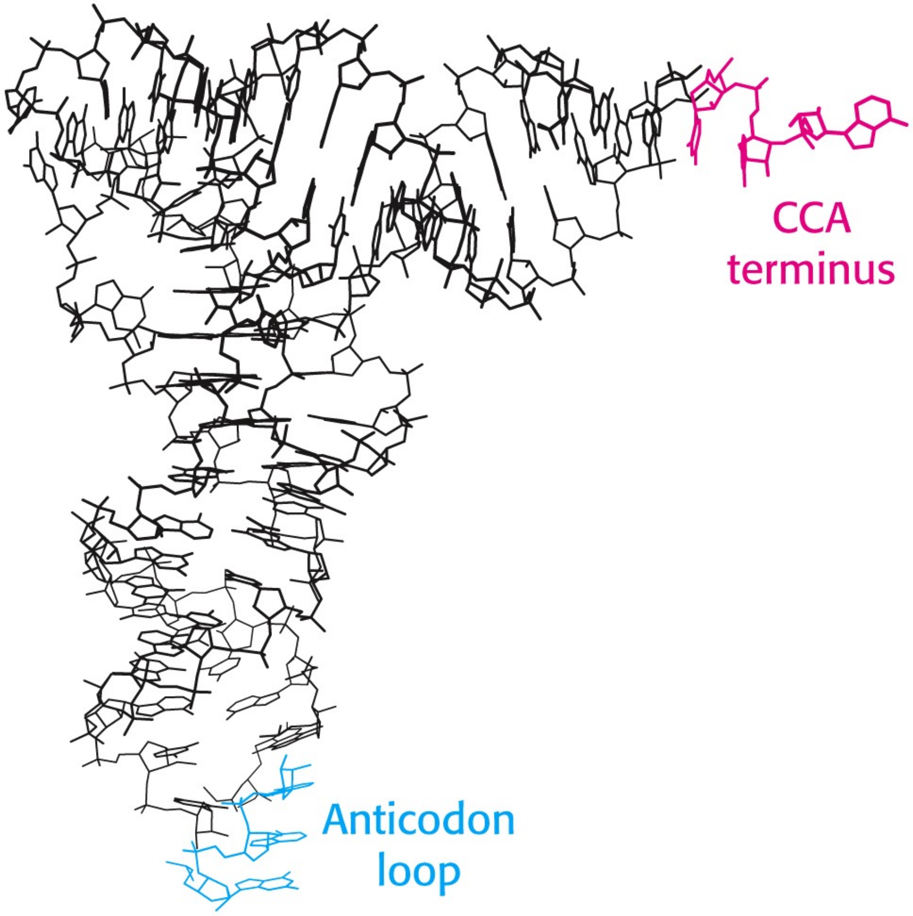
RNA transportador (tRNA)

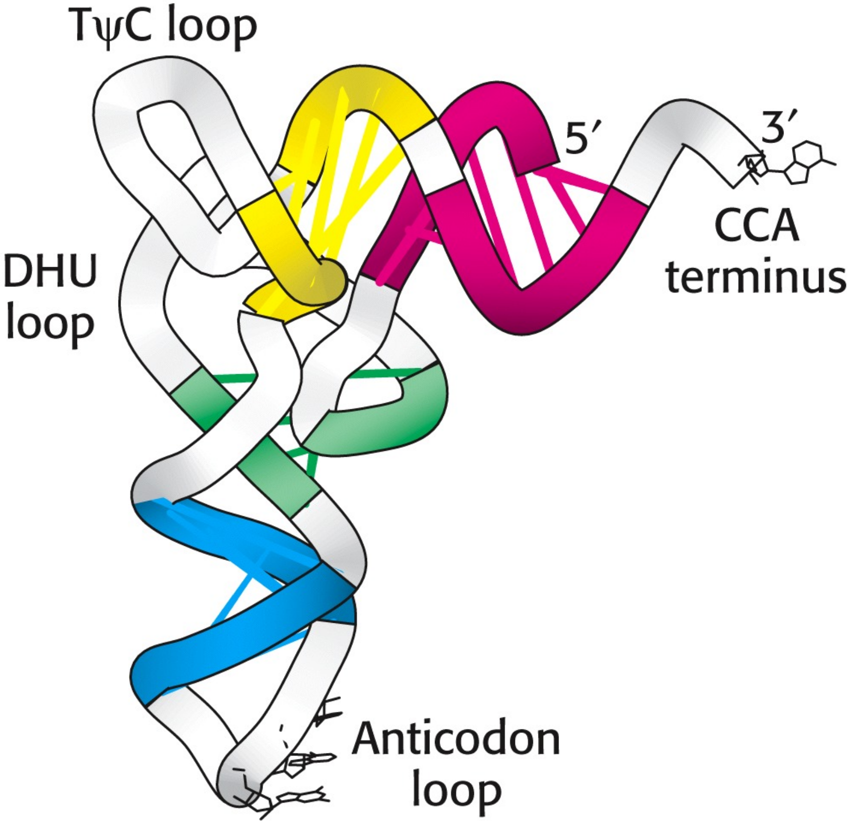
Estrutura e características
gerais dos tRNAs





Os tRNA são quimicamente modificados

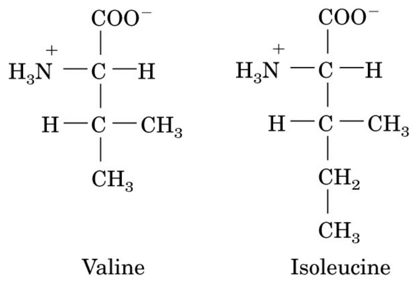

CCA
terminus
Anticodon
loop

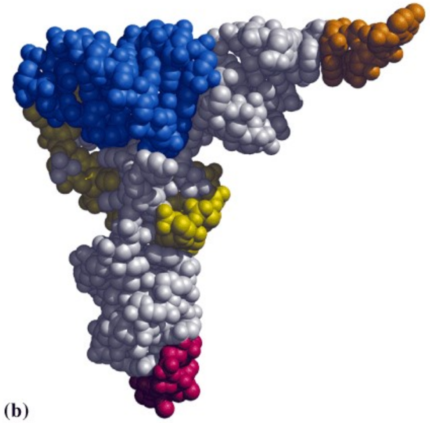

TψC loop
5′
3′
DHU
loop
CCA
terminus
Anticodon
loop

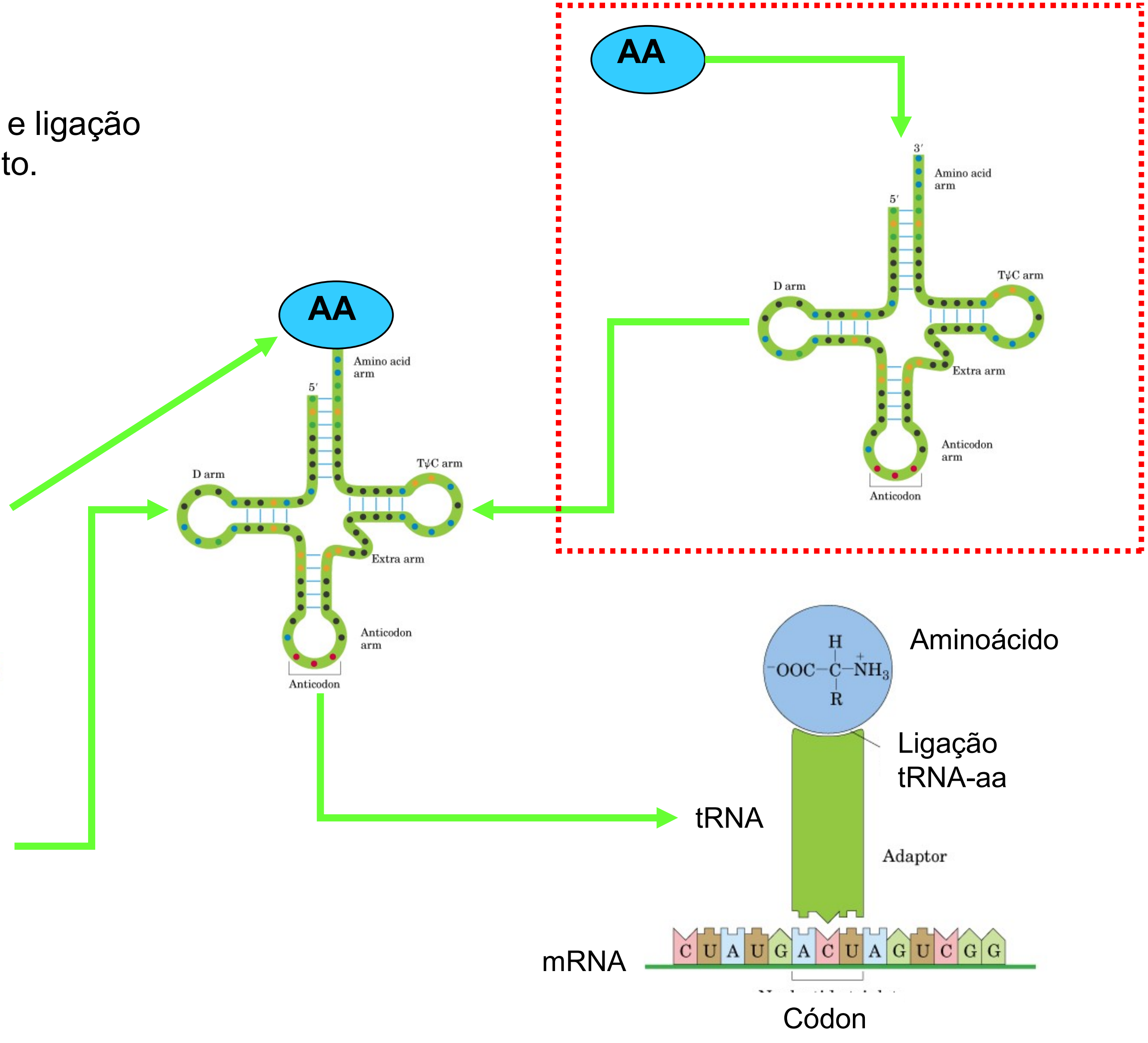
Ativação de aminoácidos

Processo de reconhecimento e ligação do aminoácido ao tRNA correto.

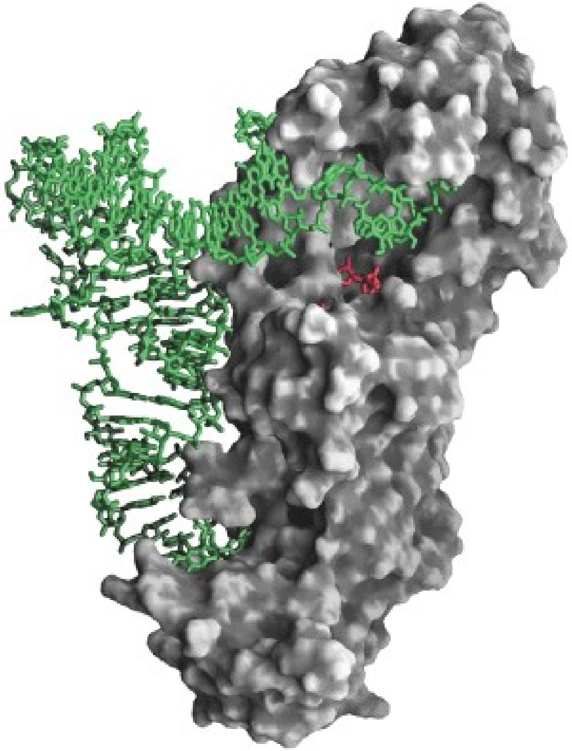
Enzimas envolvidas:
Aminoacil-tRNA-sintetases





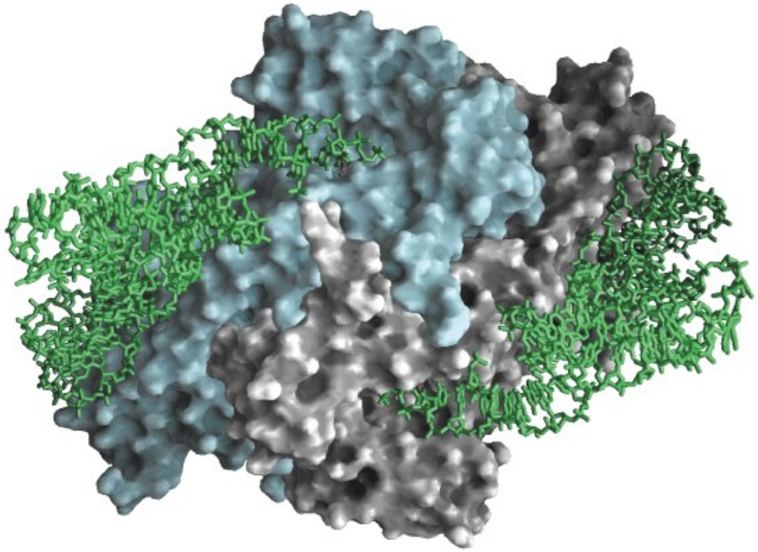


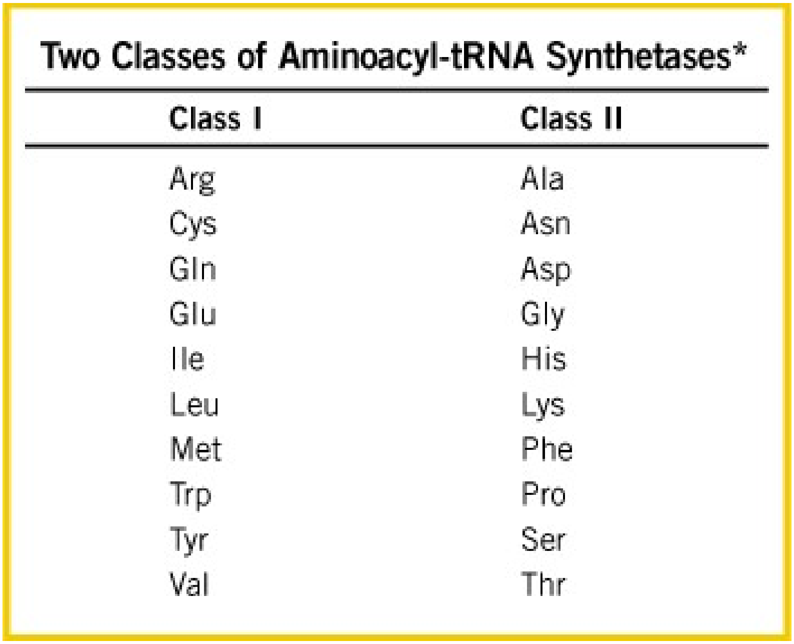
Aminoacil-tRNA-sintetases (aaRS)



Classe I

Classe II



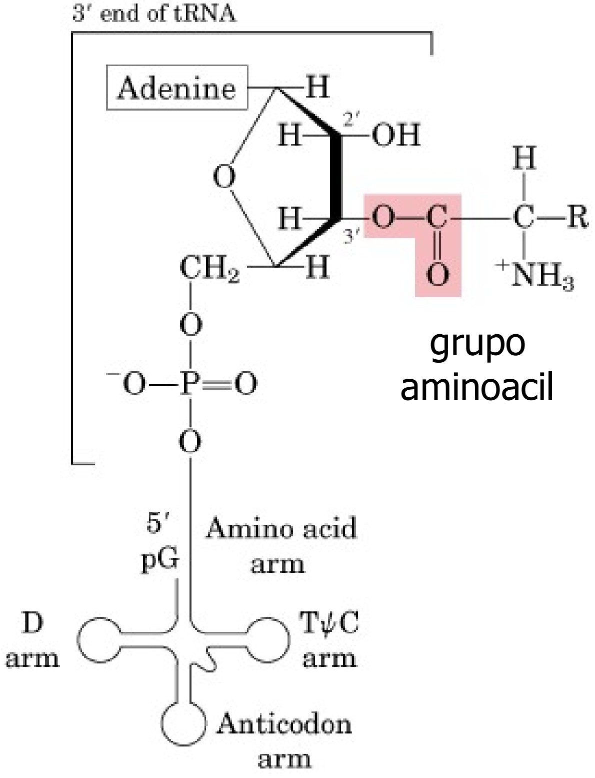


Two Classes of Aminoacyl-tRNA Synthetases*

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

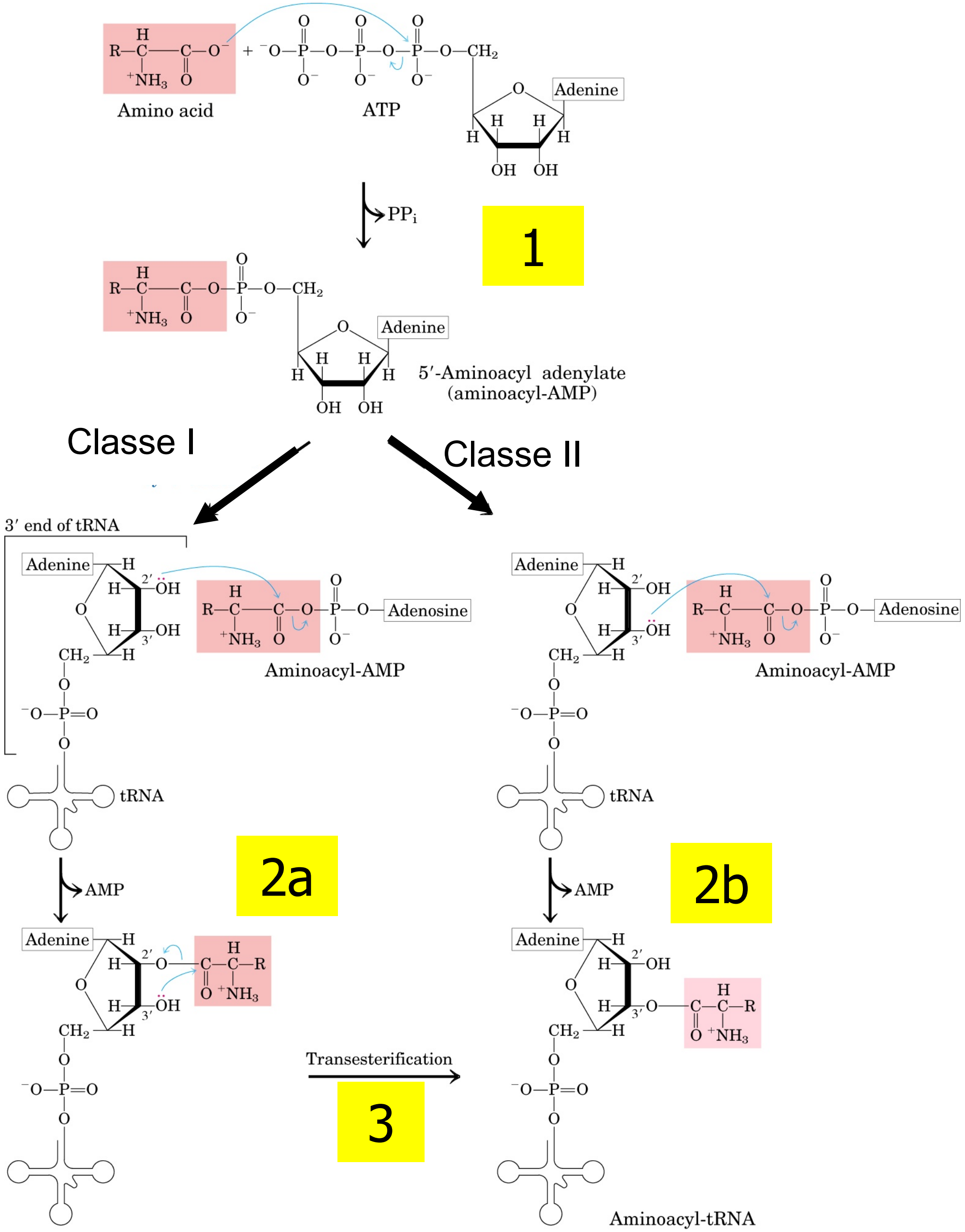
Aminoacil-tRNA-sintetases (aaRS)

Distinção das classes de aaRS:
pela estrutura primária e mecanismo
de reação de aminoacilação



grupo aminoacil

Estrutura geral dos aminoacil-tRNAs



1

Classe I

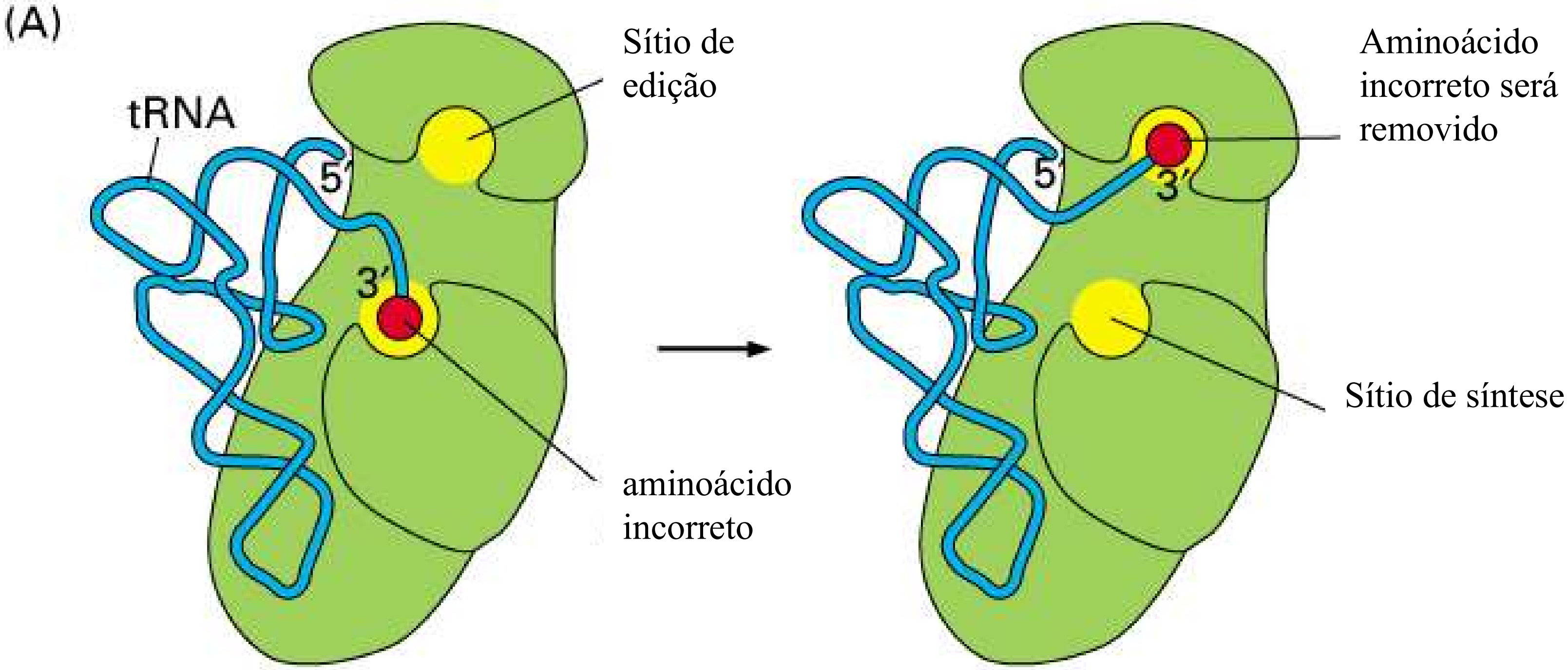
Classe II

2a

2b

3

Um mecanismo para manter a alta fidelidade da síntese de proteínas é a alta fidelidade das aa-tRNA sintetases



Sítio de edição

aminoácido incorreto

Aminoácido incorreto será removido

Sítio de síntese

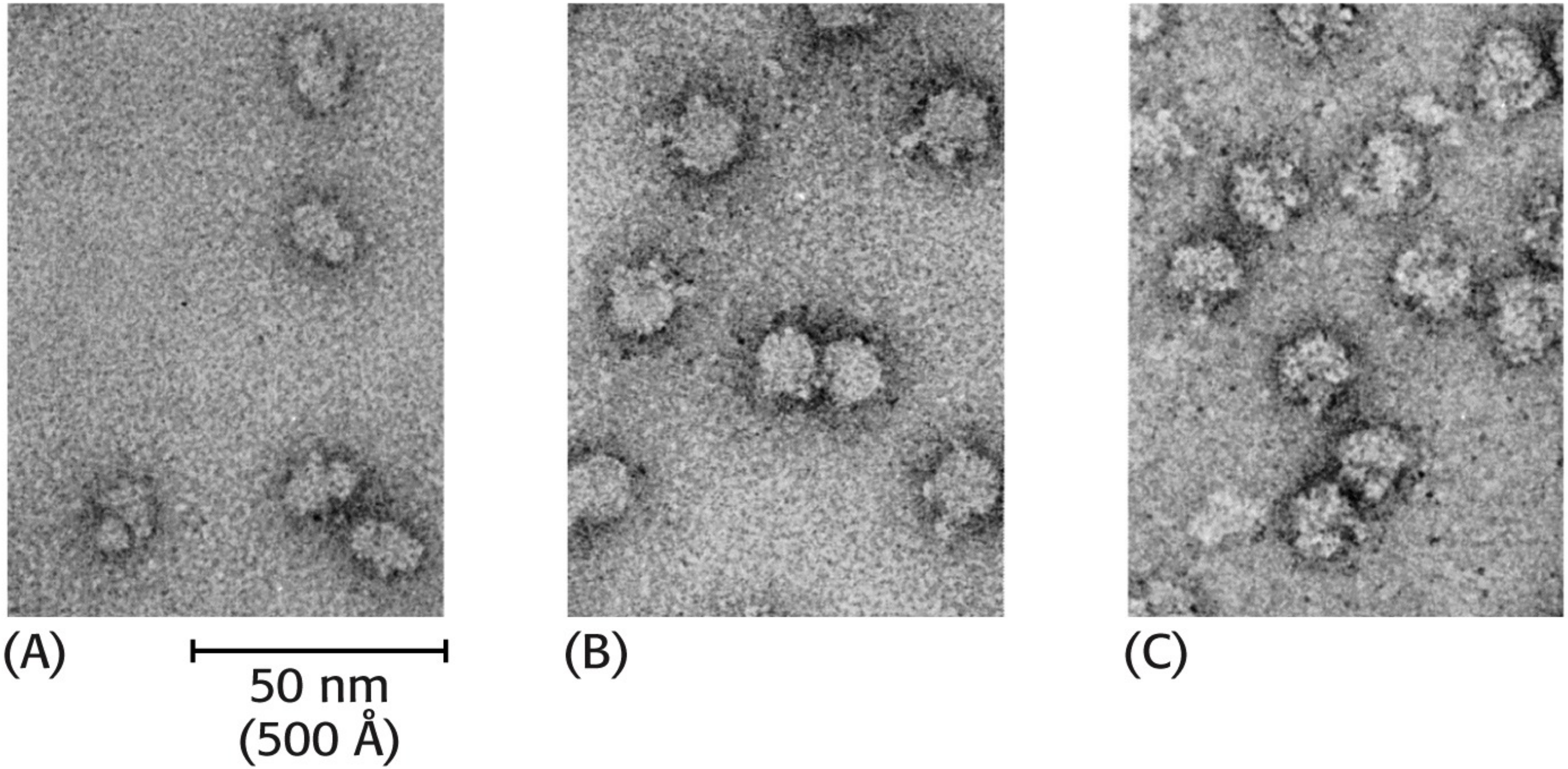
sintetizando

editando

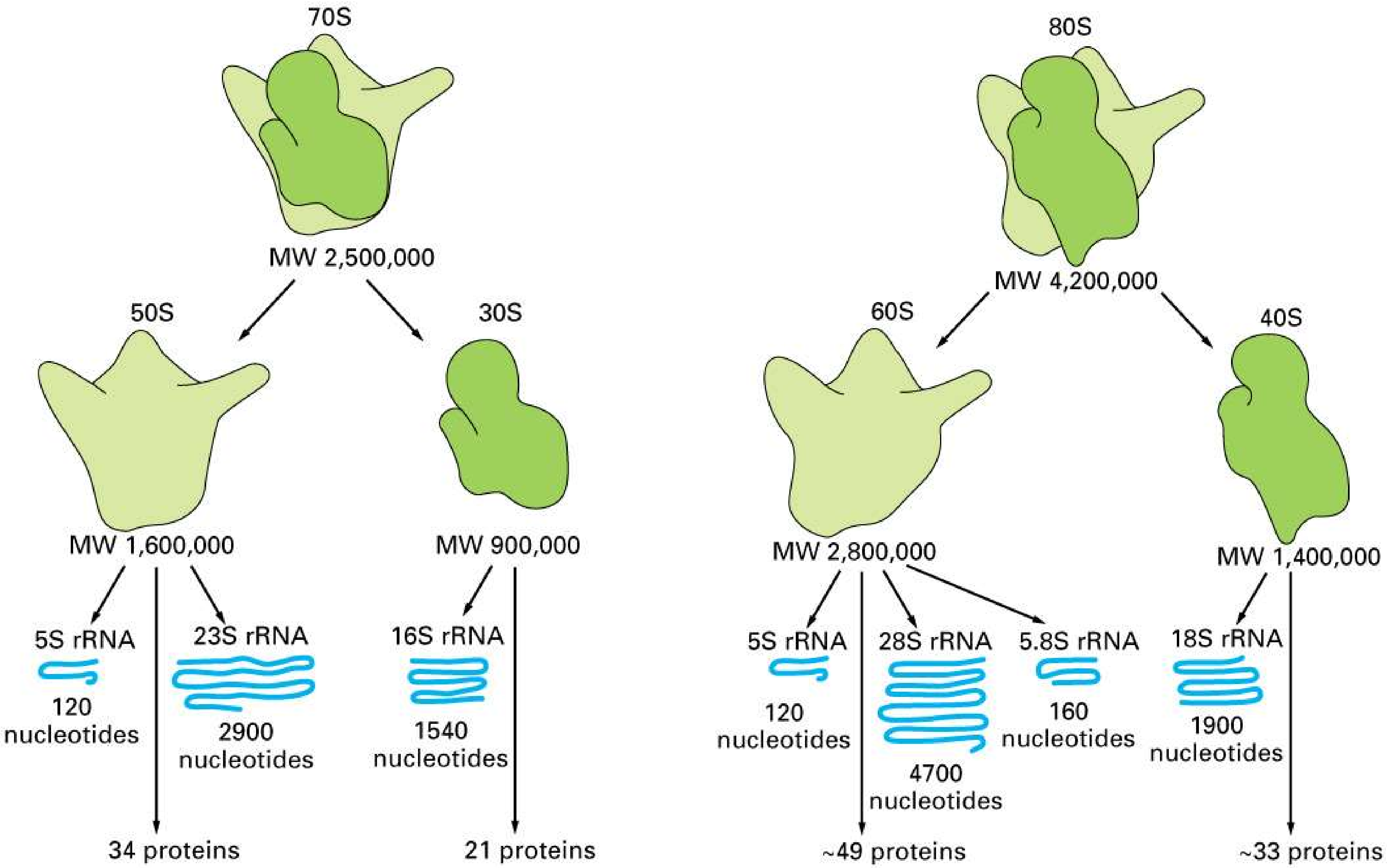
A tRNA sintetase remove seus próprios erros de pareamento por meio da edição hidrolítica de aminoácidos incorporados incorretamente. O aa correto é rejeitado pelo sítio de edição.

Síntese de proteínas ocorre nos ribossomos

A síntese de proteínas ocorre nos ribossomos



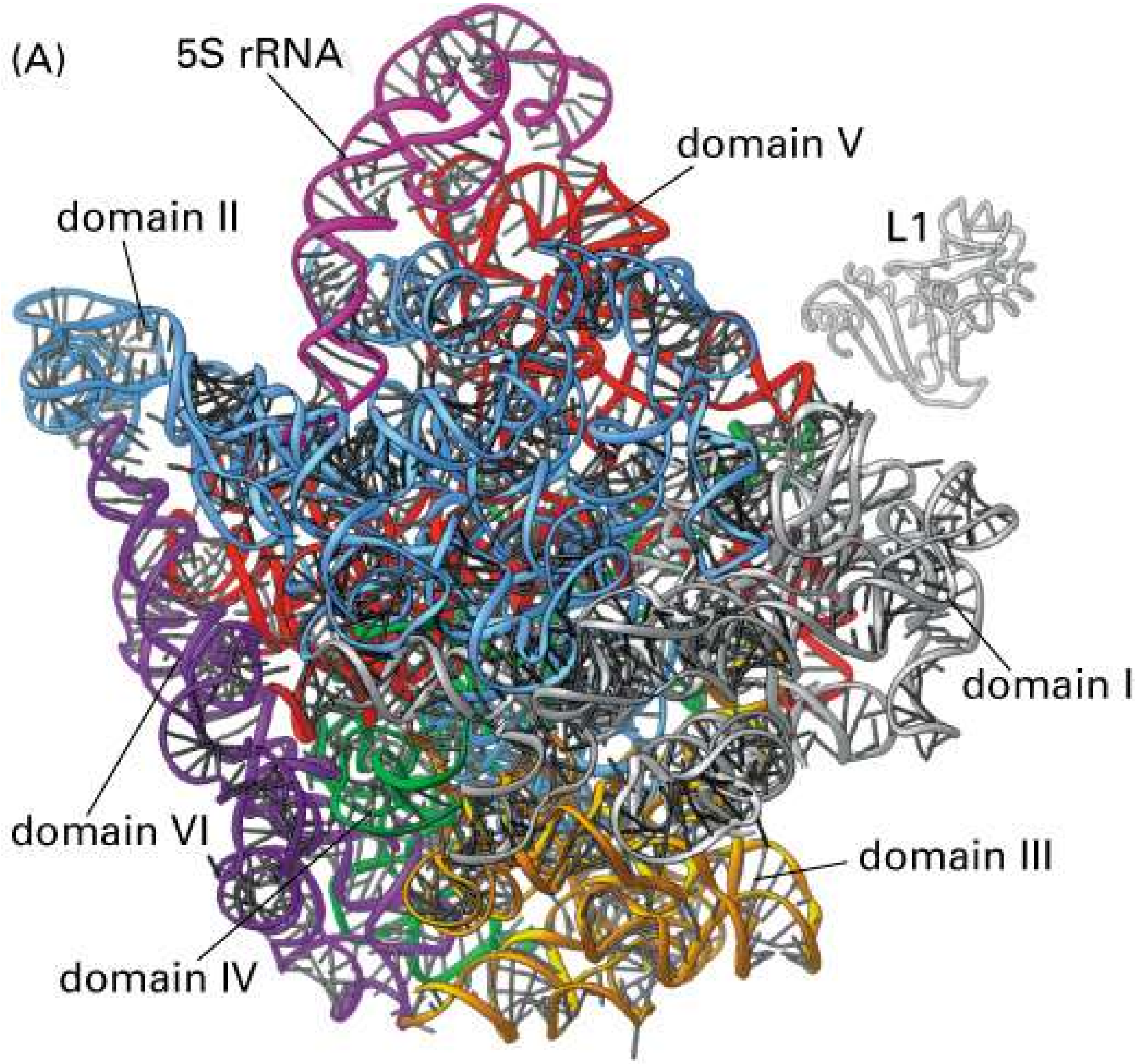
Comparação das estruturas de ribossomos procarióticos e eucarióticos



Ribossomo procariótico, mitocôndria e cloroplasto

Ribossomo eucariótico

Estrutura dos rRNAs na subunidade maior de um ribossomo bacteriano. Estruturas 3D dos rRNAs 5S e 23S



The Nobel Prize in Chemistry 2009

"for studies of the structure and function of the ribosome"

Venkatraman Ramakrishnan
MRC Laboratory of Molecular
Biology
Cambridge, United Kingdom

Thomas A. Steitz
Yale University
New Haven, CT, USA;

Ada E. Yonath
Weizmann Institute of Science
Rehovot, Israel

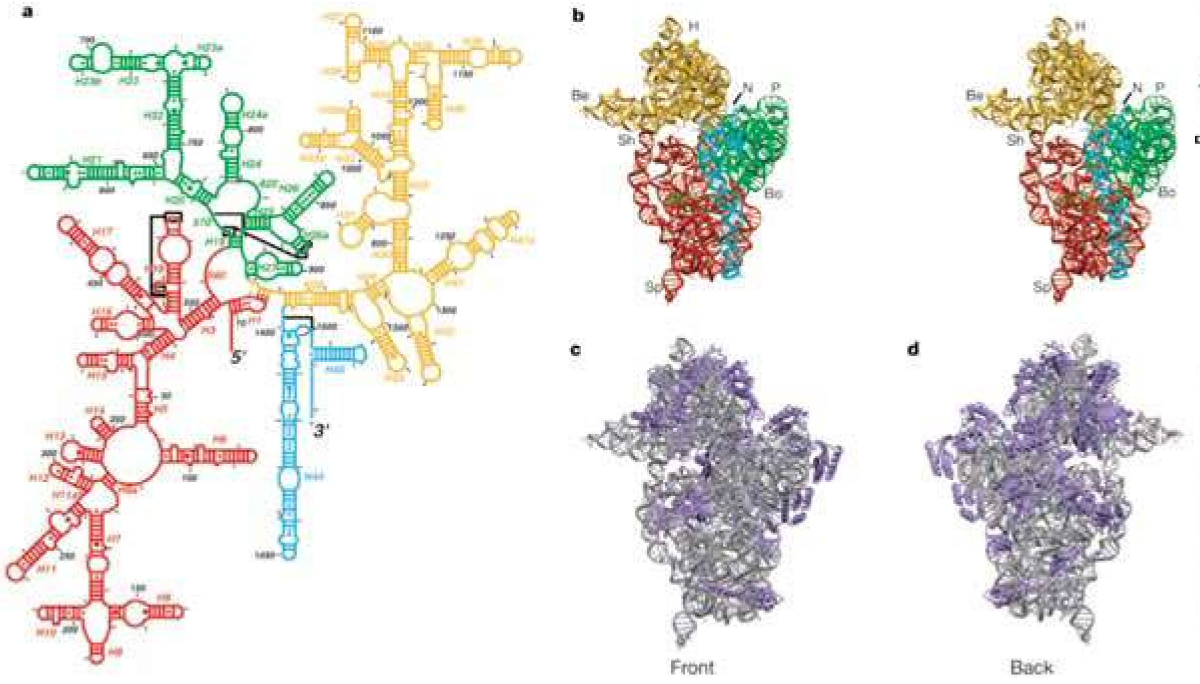
The Complete Atomic Structure of the Large Ribosomal Subunit at 2.4 Å Resolution

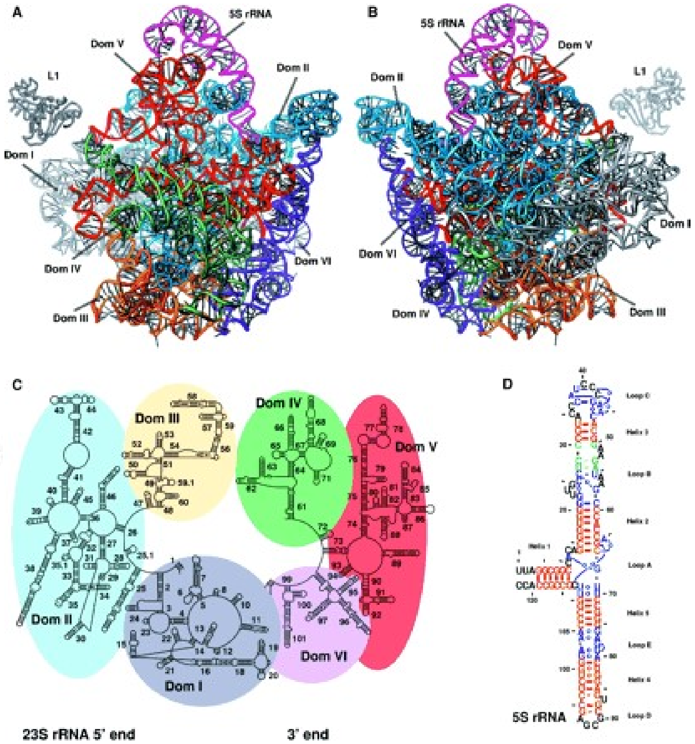
Nenad Ban,[1]* Poul Nissen,[1]* Jeffrey Hansen,[1] Peter B. Moore,[1,2]
Thomas A. Steitz[1,2,3]†

The Structural Basis of Ribosome Activity in Peptide Bond Synthesis

Poul Nissen,[1]* Jeffrey Hansen,[1]* Nenad Ban,[1]* Peter B. Moore,[1,2]
Thomas A. Steitz[1,2,3]

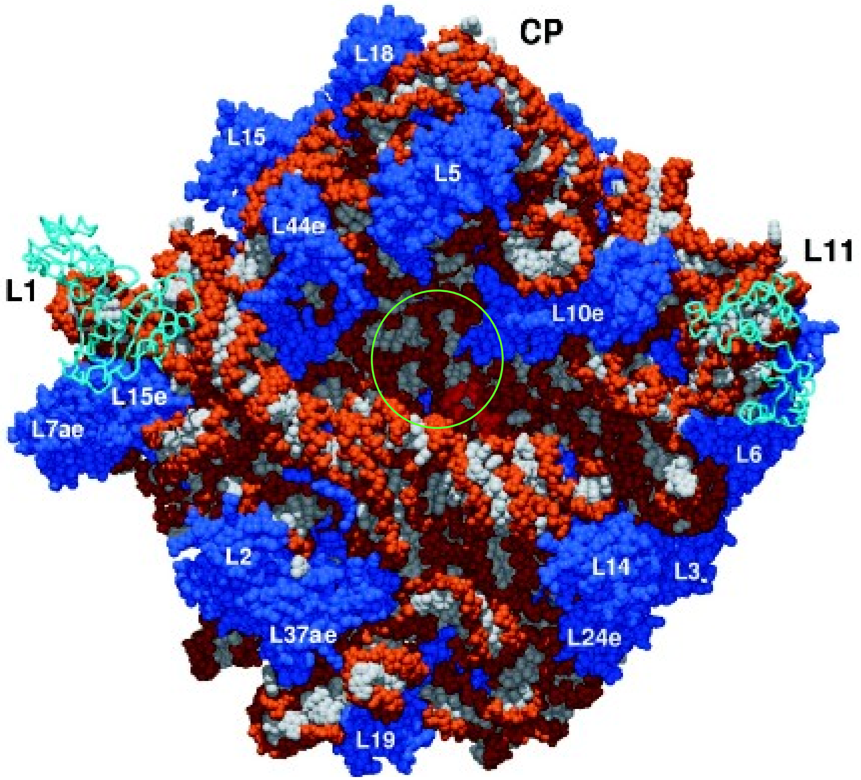
SCIENCE VOL 289 11 AUGUST 2000

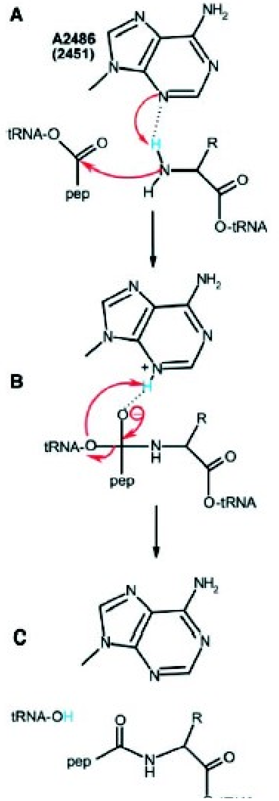


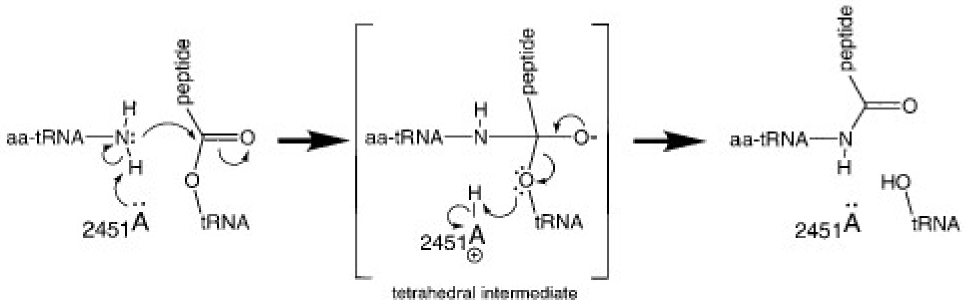


As proteínas ribossomais são importantes para manutenção da estabilidade e integridade do ribossomo, mas NÃO para a catálise

O RNA ribossomal é uma ribozima!





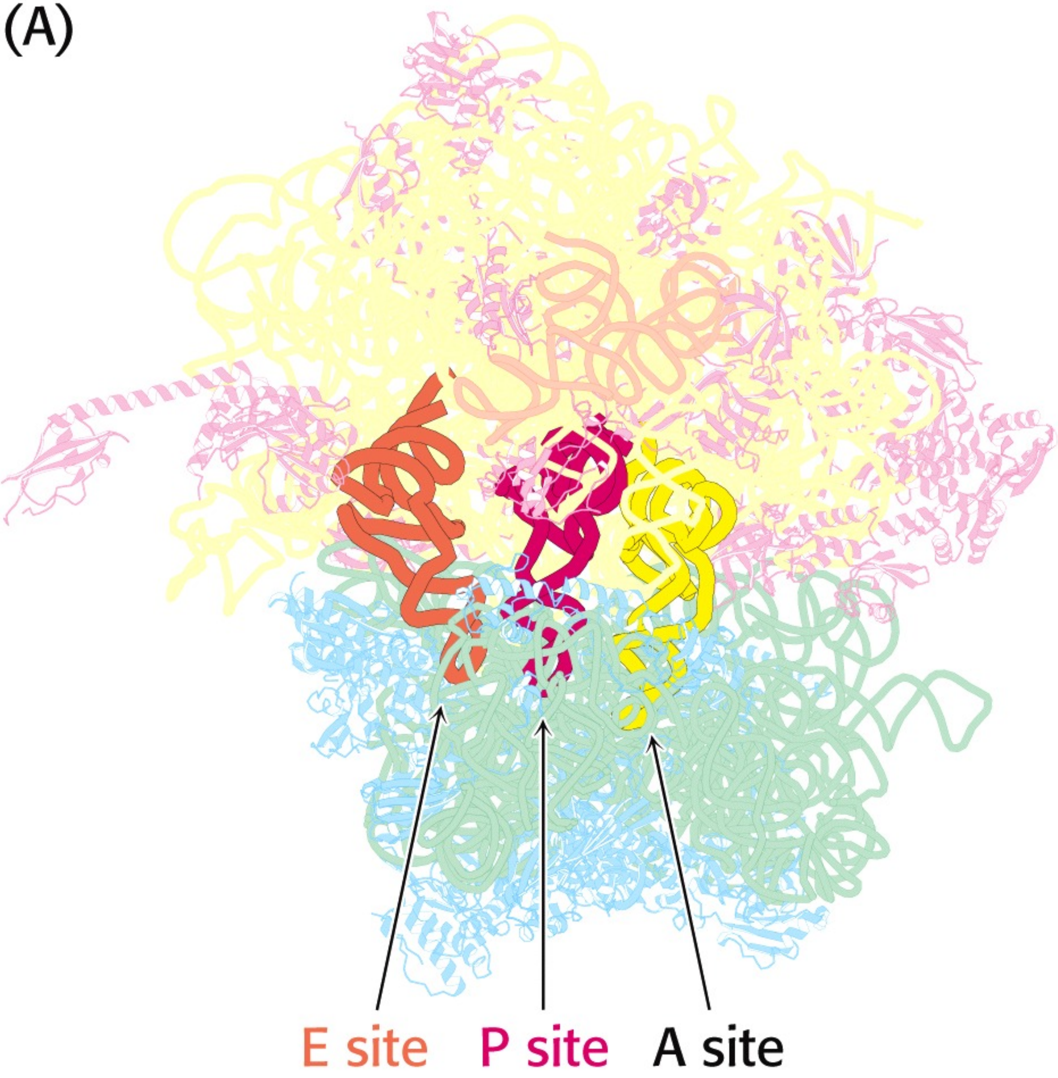


A Single Adenosine with a Neutral pK_a in the Ribosomal Peptidyl Transferase Center

Gregory W. Muth, Lori Ortoleva-Donnelly, Scott A. Strobel*

SCIENCE VOL 289 11 AUGUST 2000

A associação das subunidades grande e pequena cria características estruturais no ribossomo que são essenciais para a síntese protéica

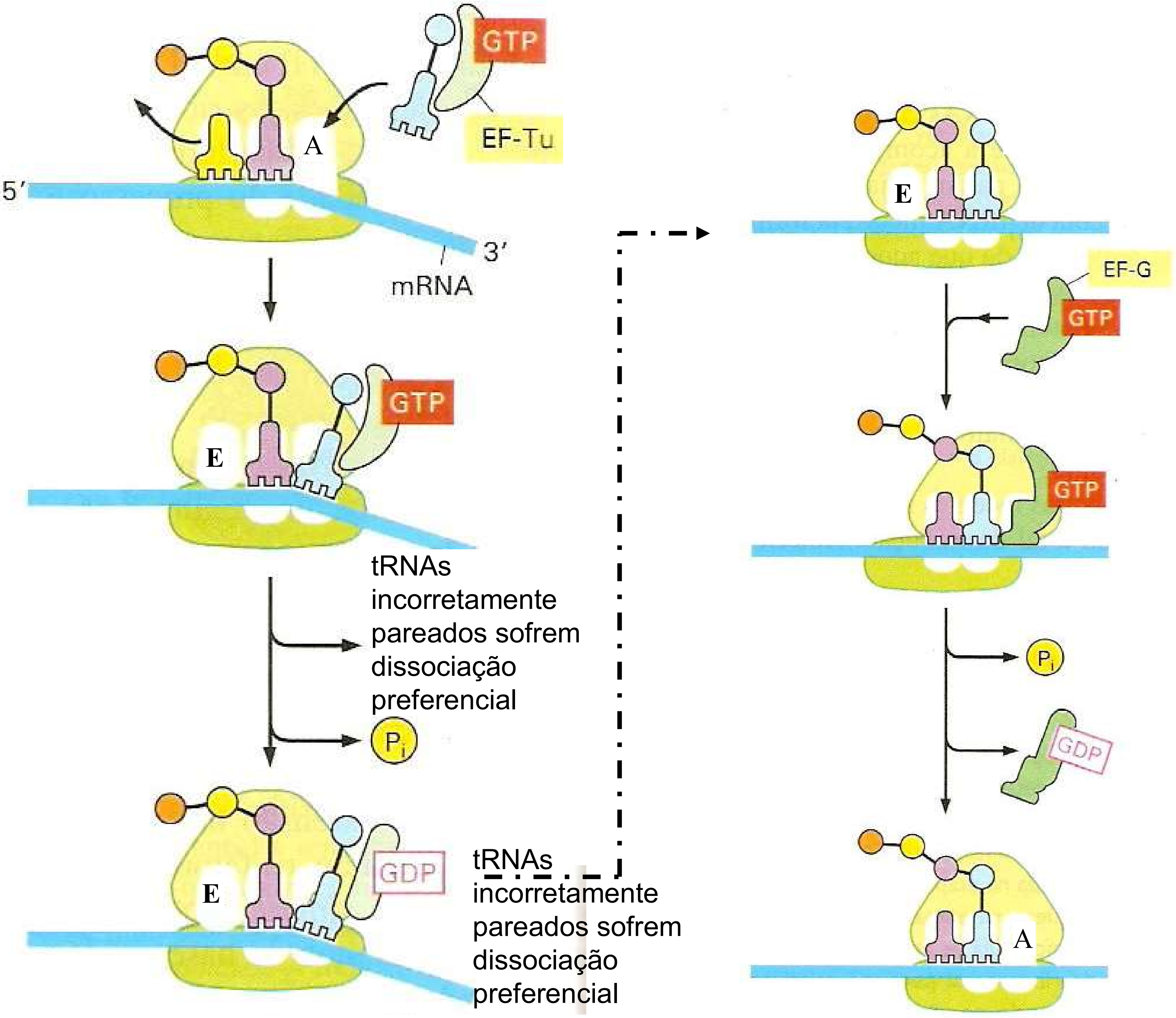


Há três sítios de ligação no tRNA :

Sítio A = sítio de ligação do aminoacil tRNA

Sítio P = sítio de ligação do peptidil-tRNA

Sítio E = sítio de saída

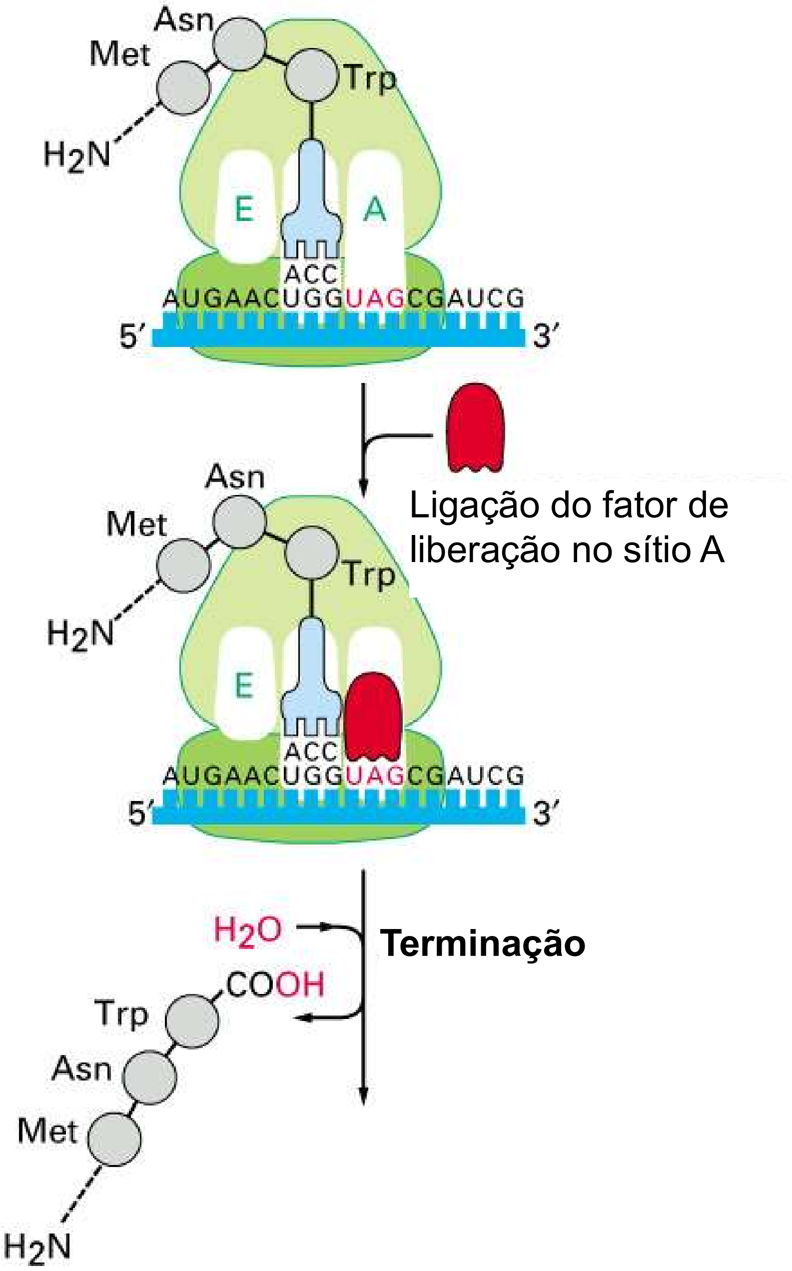


tRNAs incorretamente pareados sofrem dissociação preferencial

tRNAs incorretamente pareados sofrem dissociação preferencial

Terminação da tradução é disparada pelo *stop codon*

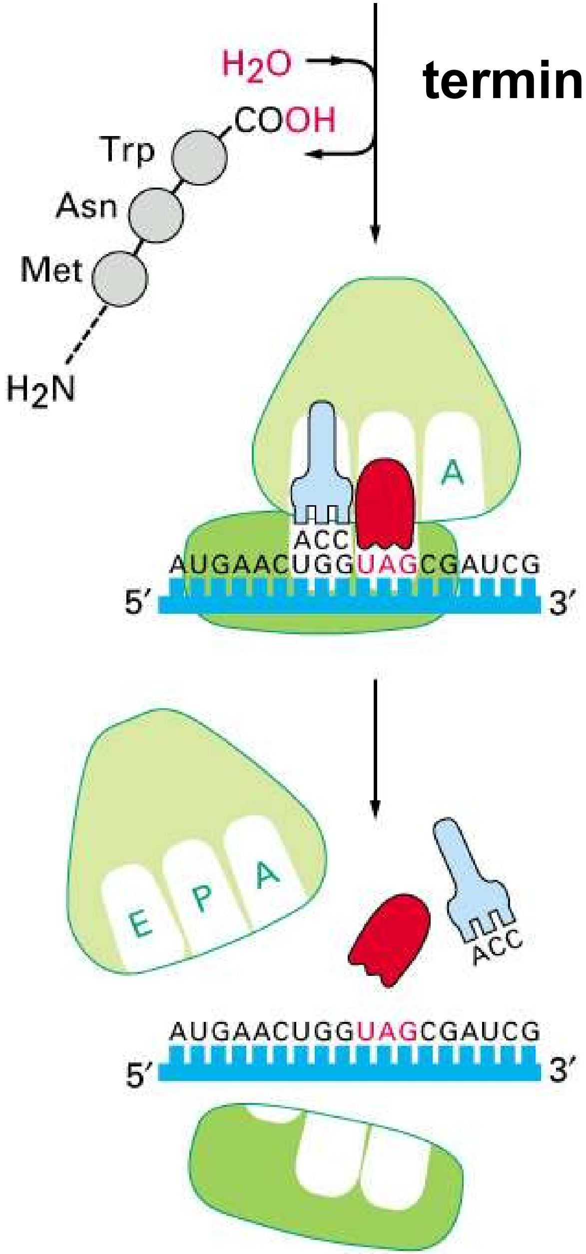
O ***Fator de liberação*** entra no sítio A e dispara a hidrólise da ligação peptidil-tRNA, levando à liberação da proteína.



Ligação do fator de liberação no sítio A

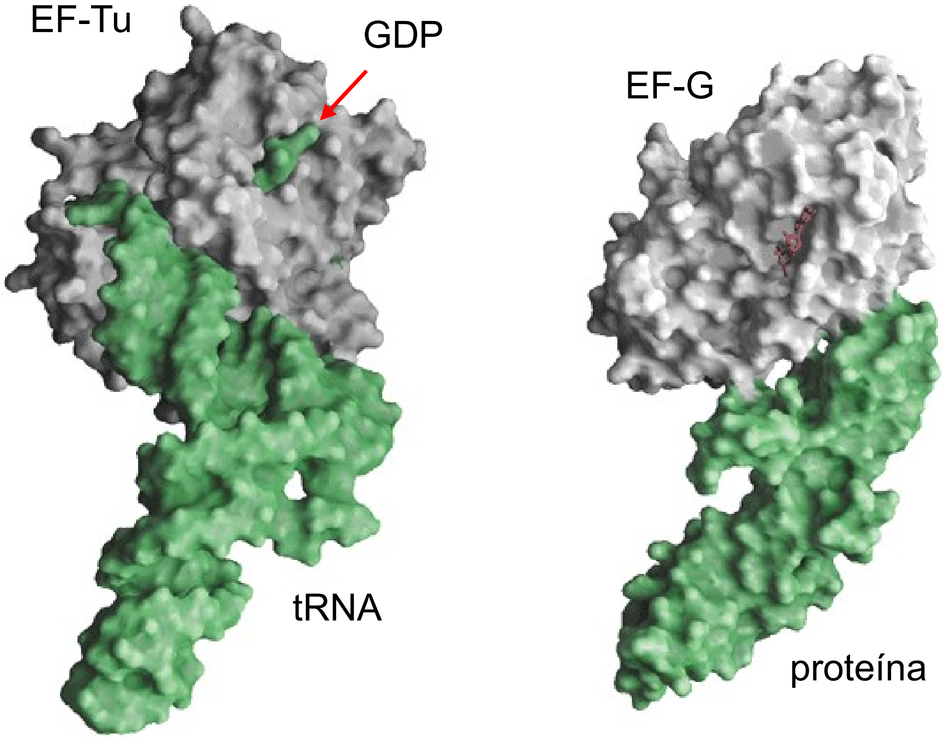
Terminação

terminação



A liberação da proteína causa
a desassociação das subunidades
constituintes do ribossomo

Terminação da cadeia é mediada por proteínas



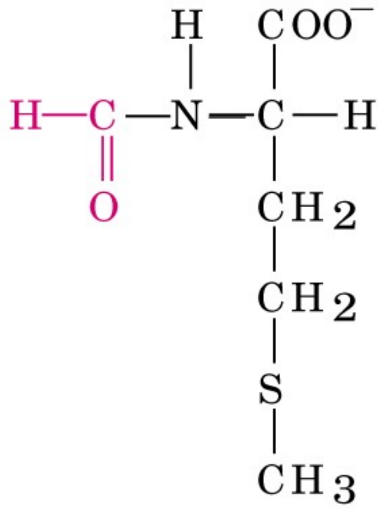
http://pubs.acs.org/cen/multimedia/85/ribosome/translation_bacterial.html

http://www.yale.edu/steitz/movies.html

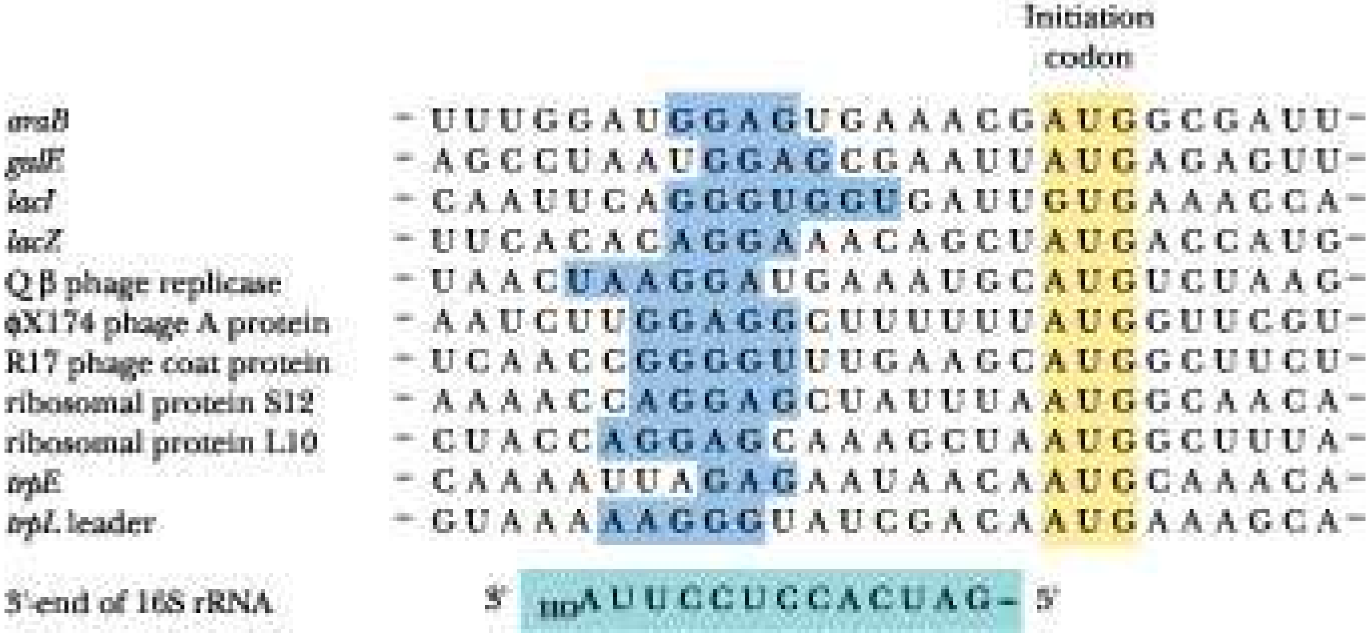
INICIAÇÃO da cadeia

início com Met ou fMet.

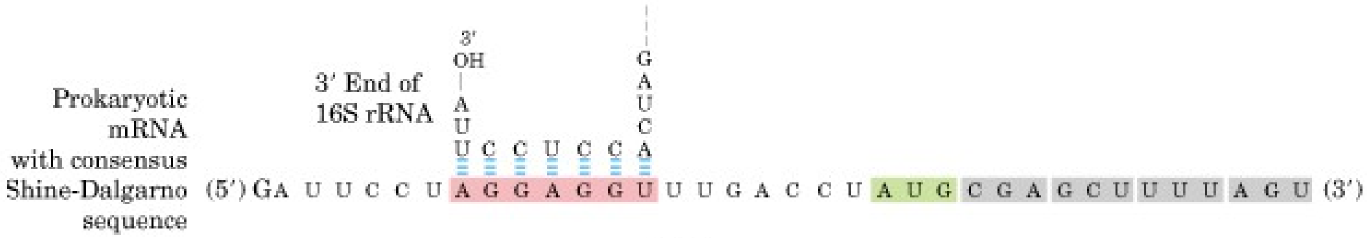
- Deve haver mais especificações que apenas o códon de iniciação
- Em *E. coli*: Shine-Dalgarno (3 a 10 nt ricos em purinas antes do AUG)



N-Formylmethionine



(a)



(b)

A tradução pode ocorrer simultaneamente a transcrição em procariotos, formando polissomos