LGN0232 - Genética Molecular

Estrutura e Expressão de Genes

No. 4356 April 25, 1953 NATURE 737

equipment, and to Dr. G. E. R. Deacon and the captain and officers of R.R.S. *Discovery II* for their part in making the observations.

[1] Young, F. B., Gerrard, H., and Jevons, W., *Phil. Mag.*, **40**, 149 (1920).

[2] Longuet-Higgins, M. S., *Mon. Not. Roy. Astro. Soc., Geophys. Supp.*, **5**, 285 (1949).

[3] Von Arx, W. S., Woods Hole Papers in Phys. Oceanog. Meteor., **11** (3) (1950).

[4] Ekman, V. W., *Arkiv. Mat. Astron. Fysik. (Stockholm)*, **2** (11) (1905).

MOLECULAR STRUCTURE OF NUCLEIC ACIDS

A Structure for Deoxyribose Nucleic Acid

WE wish to suggest a structure for the salt of deoxyribose nucleic acid (D.N.A.). This structure has novel features which are of considerable biological interest.

A structure for nucleic acid has already been proposed by Pauling and Corey[1]. They kindly made their manuscript available to us in advance of publication. Their model consists of three inter-twined chains, with the phosphates near the fibre axis, and the bases on the outside. In our opinion, this structure is unsatisfactory for two reasons: (1) We believe that the material which gives the X-ray diagrams is the salt, not the free acid. Without the acidic hydrogen atoms it is not clear what forces would hold the structure together, especially as the negatively charged phosphates near the axis will repel each other. (2) Some of the van der Waals distances appear to be too small.

Another three-chain structure has also been suggested by Fraser (in the press). In his model the phosphates are on the outside and the bases on the inside, linked together by hydrogen bonds. This structure as described is rather ill-defined, and for this reason we shall not comment on it.

This figure is purely diagrammatic. The two ribbons symbolize the two phosphate—sugar chains, and the horizontal rods the pairs of bases holding the chains together. The vertical line marks the fibre axis

We wish to put forward a radically different structure for the salt of deoxyribose nucleic acid. This structure has two helical chains each coiled round the same axis (see diagram). We have made the usual chemical assumptions, namely, that each chain consists of phosphate di-ester groups joining β-D-deoxy-ribofuranose residues with 3′,5′ linkages. The two chains (but not their bases) are related by a dyad perpendicular to the fibre axis. Both chains follow right-handed helices, but owing to the dyad the sequences of the atoms in the two chains run in opposite directions. Each chain loosely resembles Furberg's[2] model No. 1; that is, the bases are on the inside of the helix and the phosphates on the outside. The configuration of the sugar and the atoms near it is close to Furberg's 'standard configuration', the sugar being roughly perpendicular to the attached base. There is a residue on each chain every 3·4 A. in the z-direction. We have assumed an angle of 36° between adjacent residues in the same chain, so that the structure repeats after 10 residues on each chain, that is, after 34 A. The distance of a phosphorus atom from the fibre axis is 10 A. As the phosphates are on the outside, cations have easy access to them.

The structure is an open one, and its water content is rather high. At lower water contents we would expect the bases to tilt so that the structure could become more compact.

The novel feature of the structure is the manner in which the two chains are held together by the purine and pyrimidine bases. The planes of the bases are perpendicular to the fibre axis. They are joined together in pairs, a single base from one chain being hydrogen-bonded to a single base from the other chain, so that the two lie side by side with identical z-co-ordinates. One of the pair must be a purine and the other a pyrimidine for bonding to occur. The hydrogen bonds are made as follows: purine position 1 to pyrimidine position 1; purine position 6 to pyrimidine position 6.

If it is assumed that the bases only occur in the structure in the most plausible tautomeric forms (that is, with the keto rather than the enol configurations) it is found that only specific pairs of bases can bond together. These pairs are: adenine (purine) with thymine (pyrimidine), and guanine (purine) with cytosine (pyrimidine).

In other words, if an adenine forms one member of a pair, on either chain, then on these assumptions the other member must be thymine; similarly for guanine and cytosine. The sequence of bases on a single chain does not appear to be restricted in any way. However, if only specific pairs of bases can be formed, it follows that if the sequence of bases on one chain is given, then the sequence on the other chain is automatically determined.

It has been found experimentally[3,4] that the ratio of the amounts of adenine to thymine, and the ratio of guanine to cytosine, are always very close to unity for deoxyribose nucleic acid.

It is probably impossible to build this structure with a ribose sugar in place of the deoxyribose, as the extra oxygen atom would make too close a van der Waals contact.

The previously published X-ray data[5,6] on deoxyribose nucleic acid are insufficient for a rigorous test of our structure. So far as we can tell, it is roughly compatible with the experimental data, but it must be regarded as unproved until it has been checked against more exact results. Some of these are given in the following communications. We were not aware of the details of the results presented there when we devised our structure, which rests mainly though not entirely on published experimental data and stereo-chemical arguments.

It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material.

Full details of the structure, including the conditions assumed in building it, together with a set of co-ordinates for the atoms, will be published elsewhere.

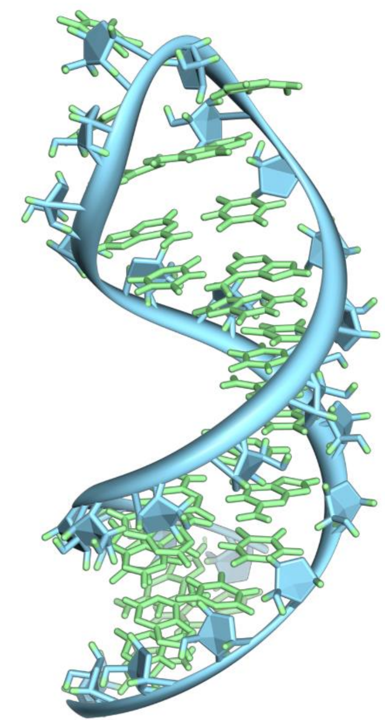
We are much indebted to Dr. Jerry Donohue for constant advice and criticism, especially on inter-atomic distances. We have also been stimulated by a knowledge of the general nature of the unpublished experimental results and ideas of Dr. M. H. F. Wilkins, Dr. R. E. Franklin and their co-workers at

©1953 Nature Publishing Group

Antonio Figueira

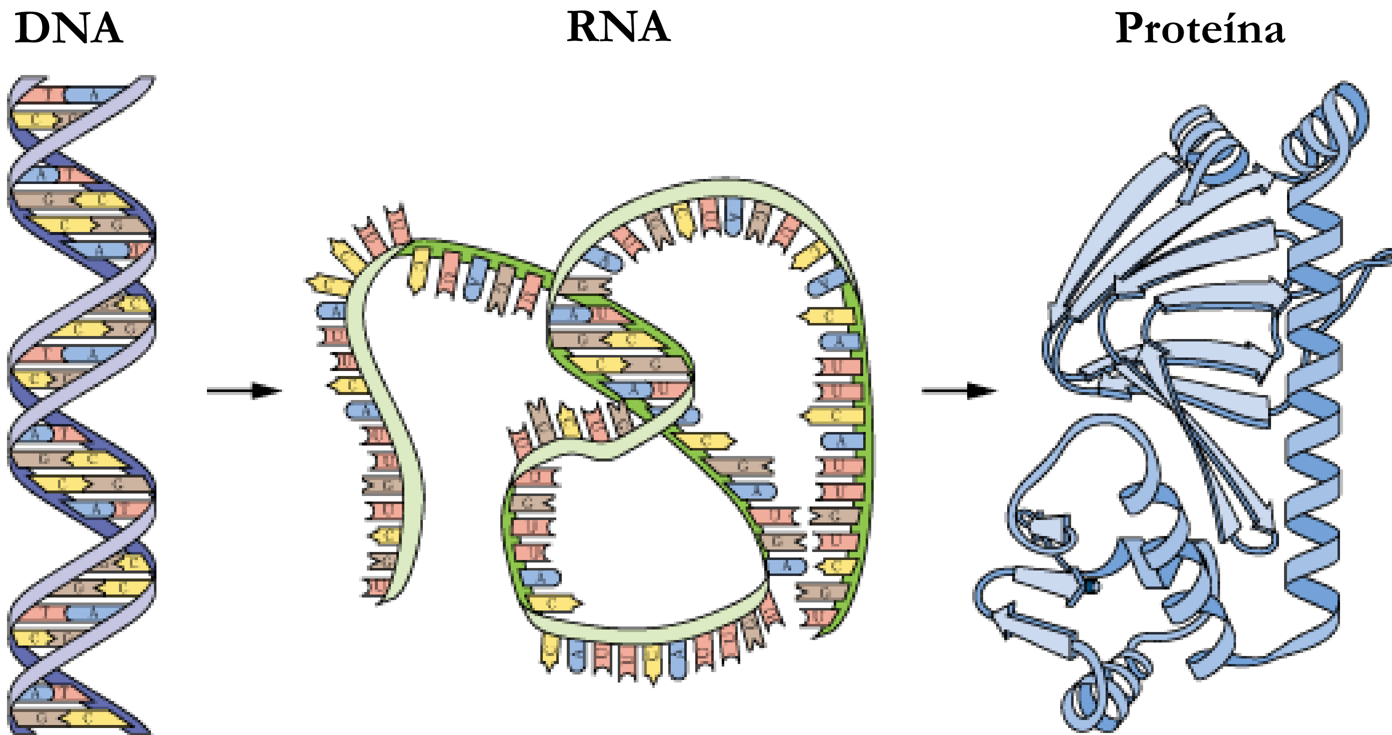
CENA

figueira@cena.usp.br

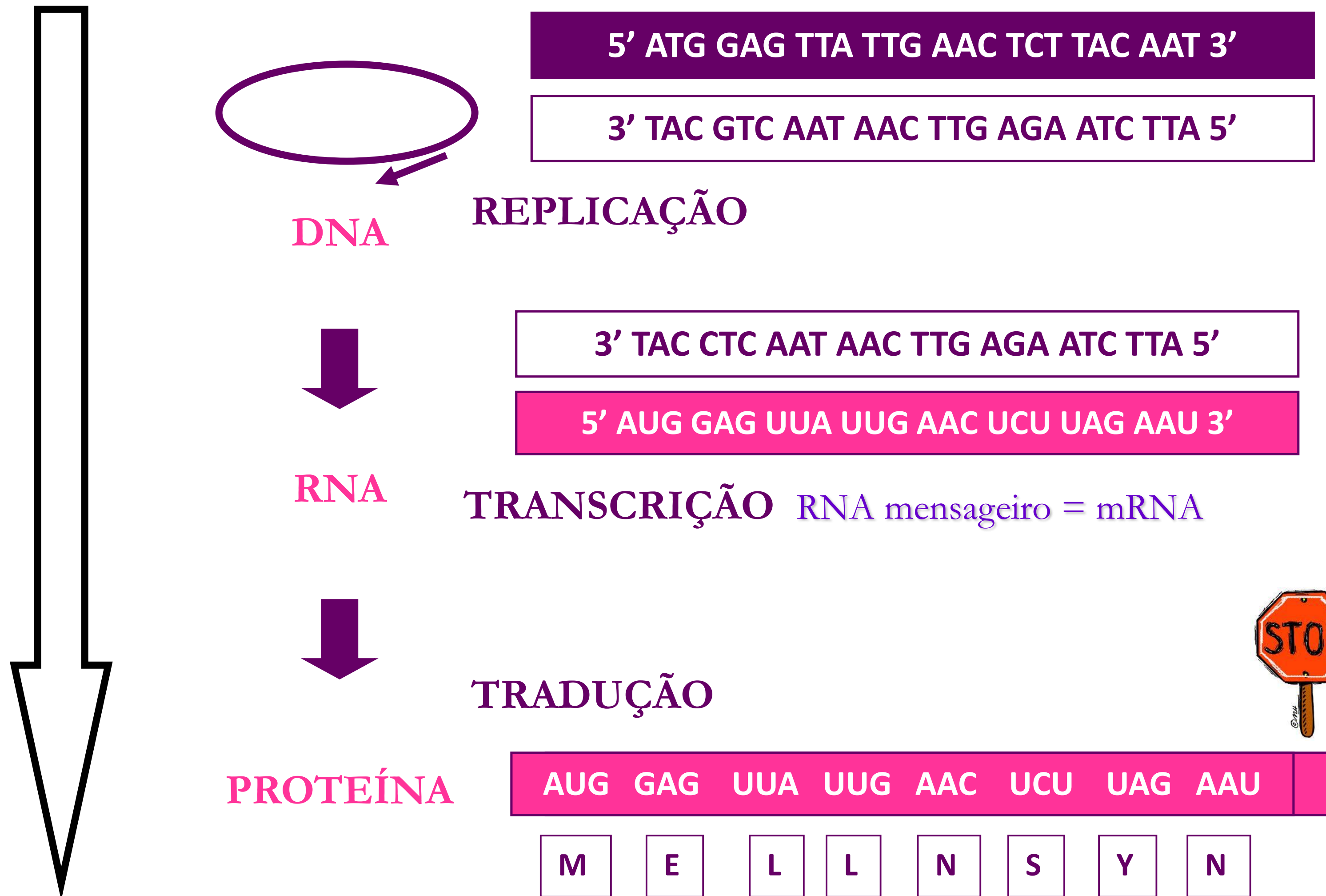


DOGMA CENTRAL DA BIOLOGIA

A informação genética, armazenada nos cromossomos, é transferida às células filhas através da **replicação do DNA**, sendo expressa por meio da **transcrição em mRNA** e **traduzida** subsequentemente em cadeias polipeptídicas (**proteínas**)



Fluxo da Informação Genética funciona do mesmo modo

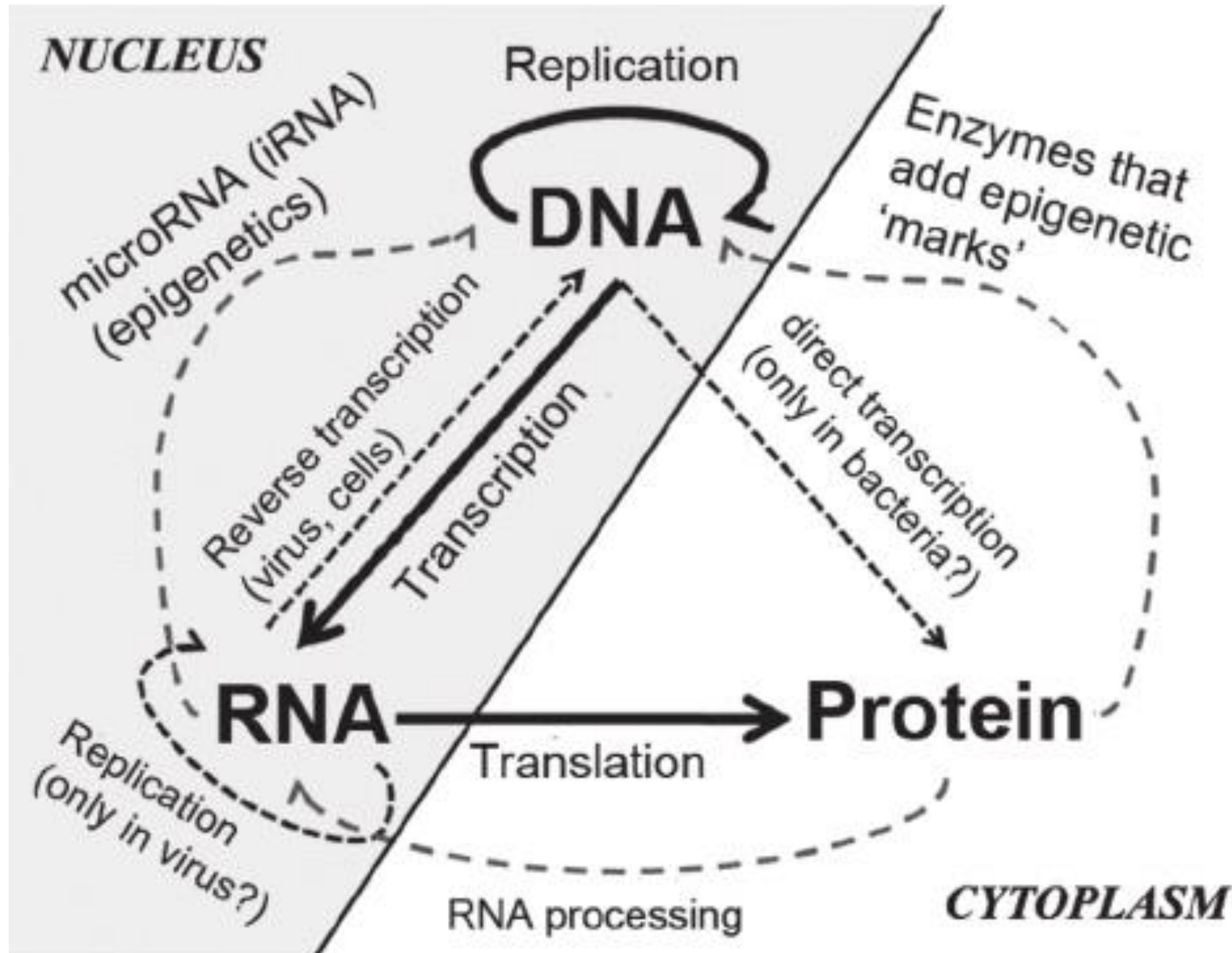


No. 4356 April 25, 1953 NATURE 737

MOLECULAR STRUCTURE OF NUCLEIC ACIDS

A Structure for Deoxyribose Nucleic Acid

Fluxo da Informação Genética



Dogma que não é Dogma!

Ácidos Nucleicos
DNA e RNA

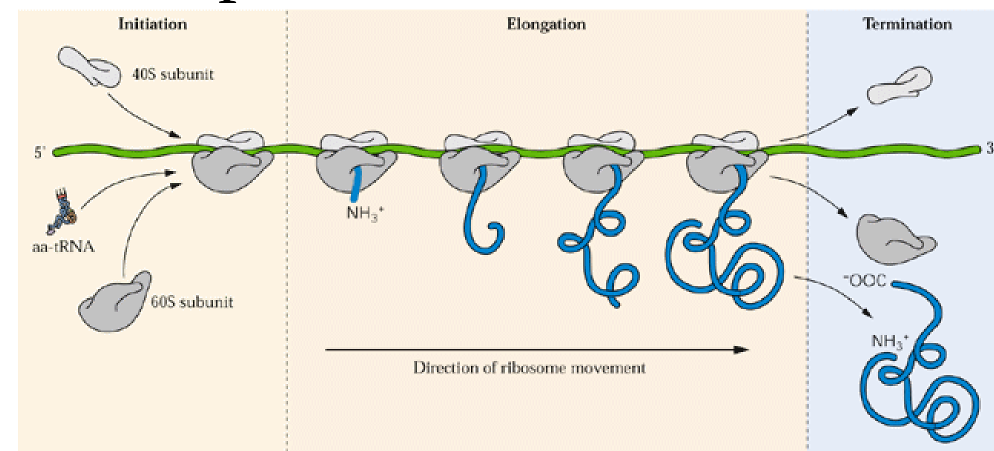
Ácidos Nucleicos

- **DNA**: Armazenamento da informação genética
  - **Estabilidade**
- **RNA**: síntese de macromoléculas - várias funções
  - **RNA ribossomal (rRNA)** - componentes estruturais de ribossomos
  - **RNA mensageiro (mRNA)** - contém a informação genética para a sequência de aminoácidos das proteínas
  - **RNA transferência (tRNA)** - identifica e transporta os aminoácidos até o ribossomo
  - snRNA, snoRNA, microRNA, ncRNA

snRNA = *small nuclear RNA* - spliceossomos

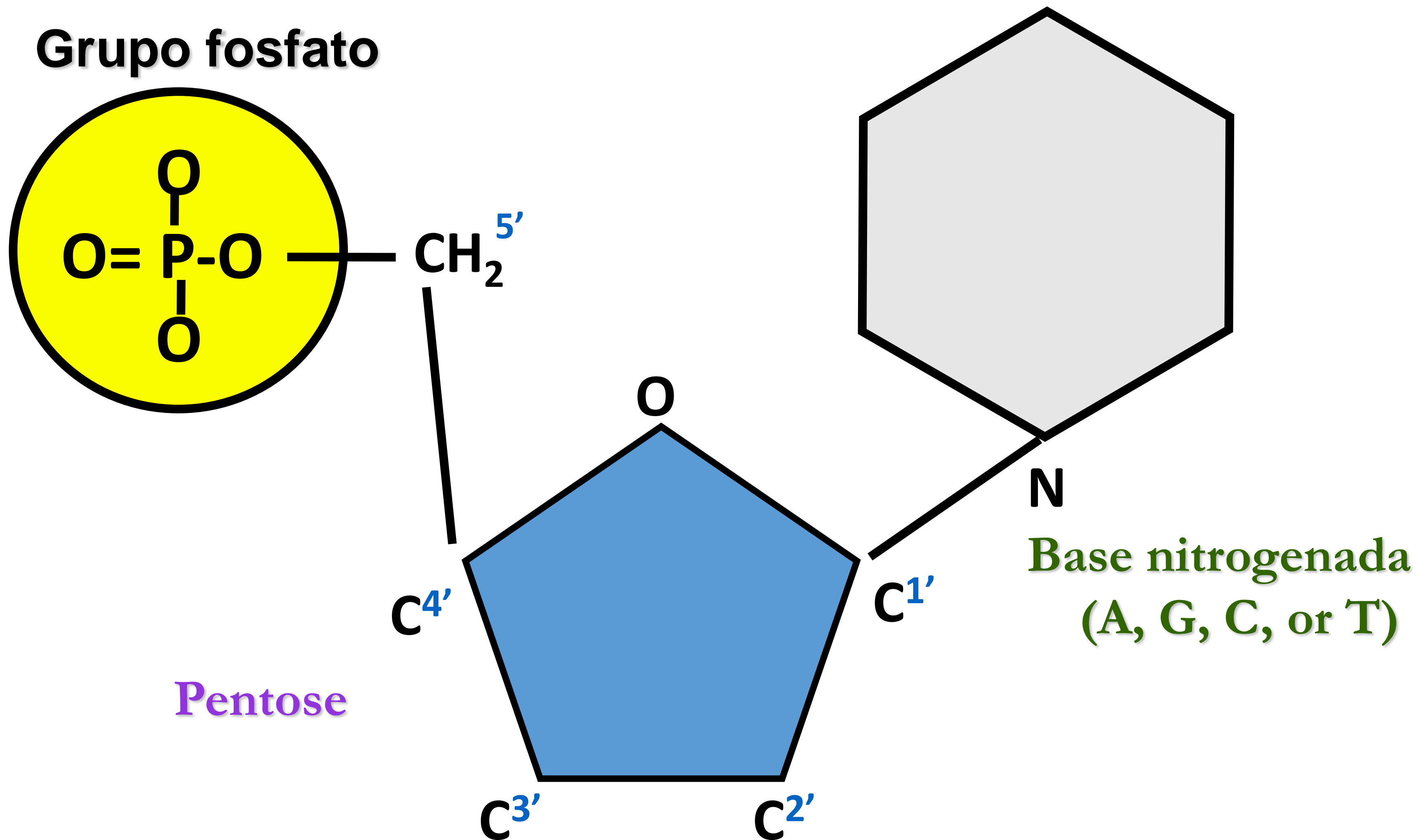
snoRNA = *small nucleolar RNA* – montagem ribossomo

ncRNA = *non-coding RNA* – RNA regulatórios



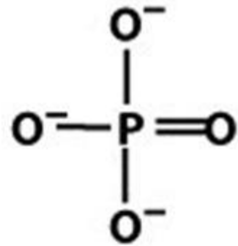
Ácidos Nucleicos

São polímeros de nucleotídeos



Componentes dos Nucleotídeos

(1)
Um grupamento fosfato:

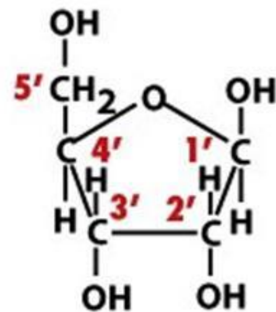


Carbono-5´

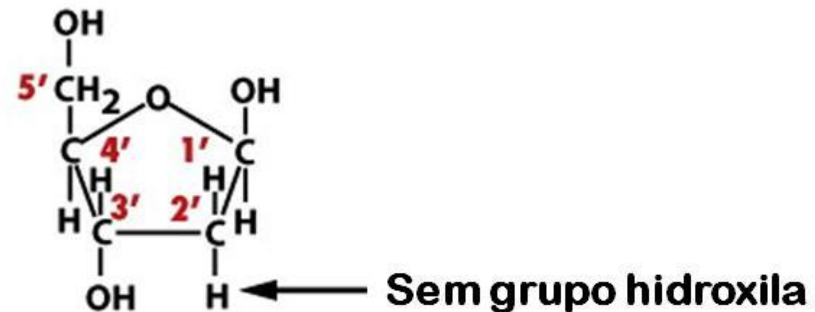
(2)

pentoses (açúcares de 5 carbonos)

(a) **RNA:**
Ribose



(b) **DNA:**
2-Desoxirribose



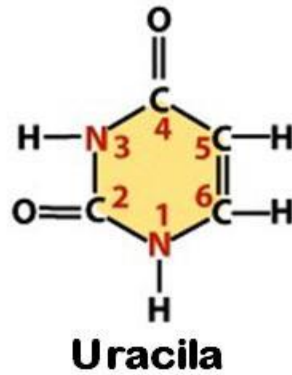
Carbono-2´

Componentes dos Nucleotídeos

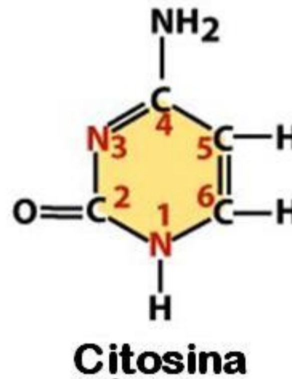
Purinas: A, G
Pirimidinas: U, T, C

(3)
Uma base cíclica contendo Nitrogênio

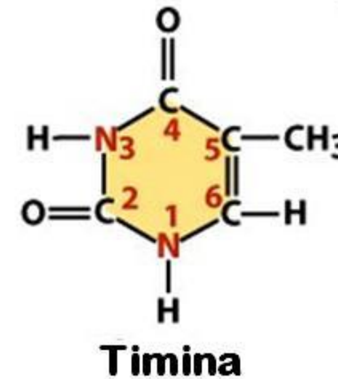
(a) RNA



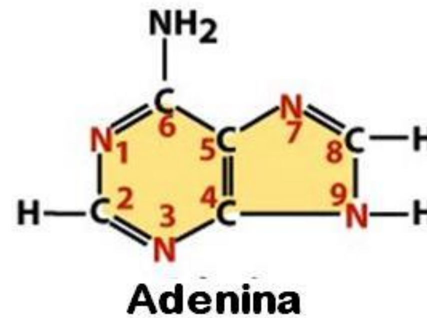
(b) DNA e RNA

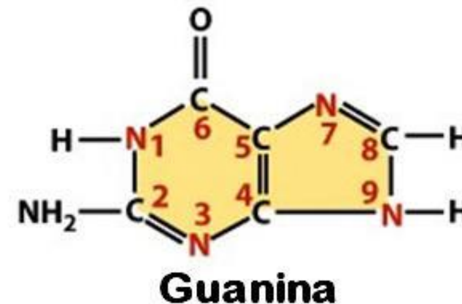


(c) DNA



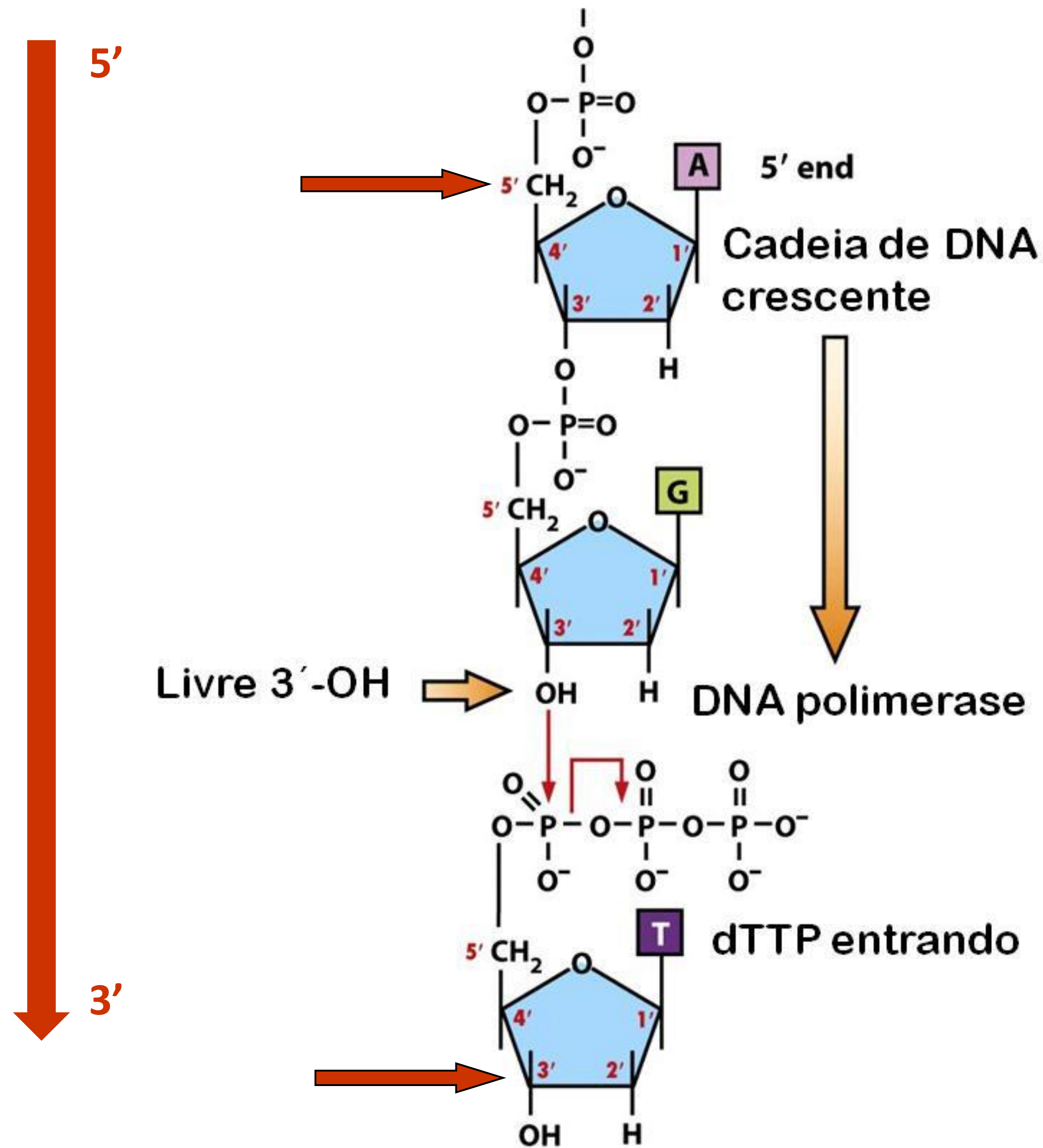
Pirimidinas

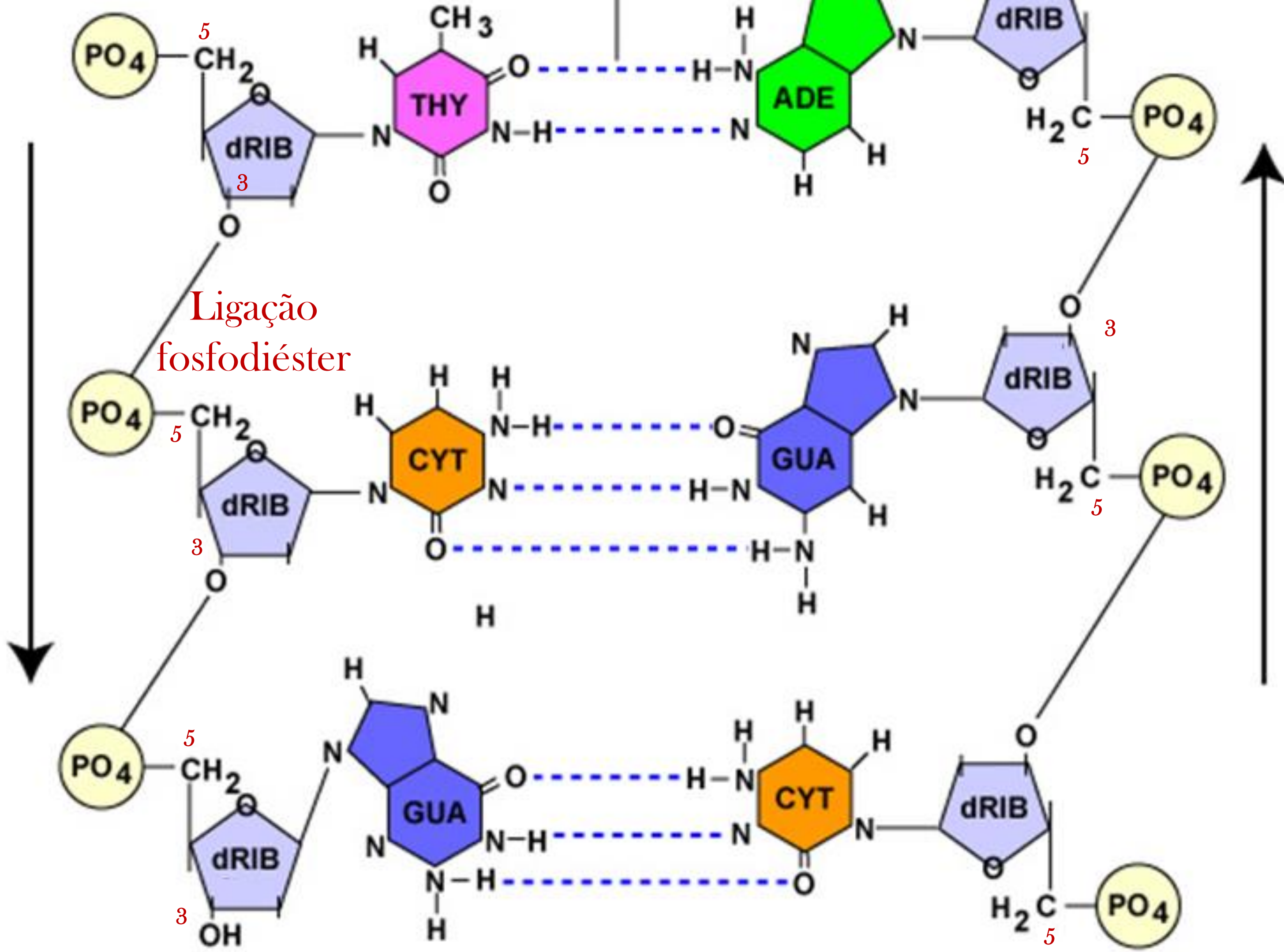




Purinas

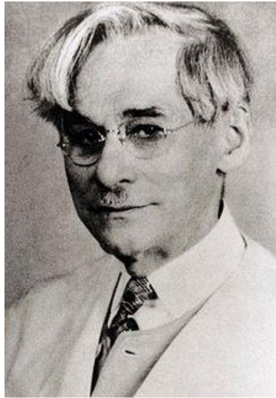
Ligações Fosfodiéster



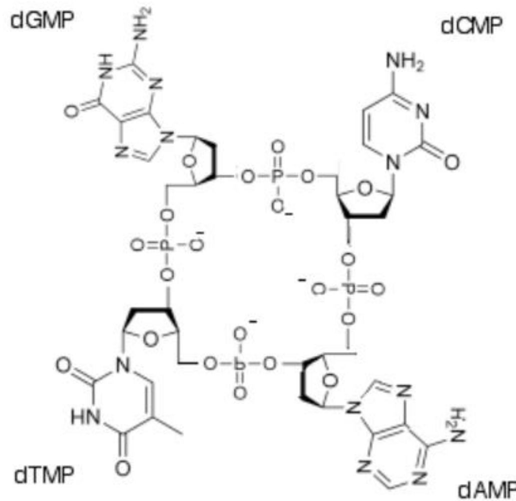


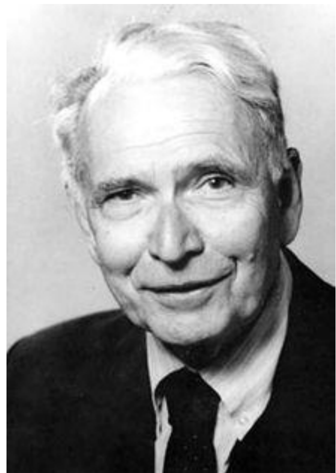
5'
3´
3'
5
Ponte de Hidrogênio
Ligação fosfodiéster
PO4
CH2
dRIB
THY
CH3
ADE
CYT
GUA
OH
H2C

Hipótese dos Tetranucleotídeos



Phoebus Levene





Erwin Chargaff

Regra de Chargaff

DNA SOURCE	ADENINE	THYMINE	GUANINE	CYTOSINE
Calf Thymus	1.7	1.6	1.2	1.0
Beef Spleen	1.6	1.5	1.3	1.0
Yeast	1.8	1.9	1.0	1.0
Tubercle Bacillus	1.1	1.0	2.6	2.4

equipment, and to Dr. G. E. R. Deacon and the captain and officers of R.R.S. *Discovery II* for their part in making the observations.

[1] Young, F. B., Gerrard, H., and Jevons, W., *Phil. Mag.*, **40**, 149 (1920).
[2] Longuet-Higgins, M. S., *Mon. Not. Roy. Astro. Soc., Geophys. Supp.*, **5**, 285 (1949).
[3] Von Arx, W. S., Woods Hole Papers in Phys. Oceanog. Meteor., **11** (3) (1950).
[4] Ekman, V. W., *Arkiv. Mat. Astron. Fysik. (Stockholm)*, **2** (11) (1905).

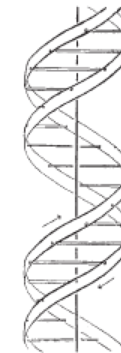
MOLECULAR STRUCTURE OF NUCLEIC ACIDS

A Structure for Deoxyribose Nucleic Acid

WE wish to suggest a structure for the salt of deoxyribose nucleic acid (D.N.A.). This structure has novel features which are of considerable biological interest.

A structure for nucleic acid has already been proposed by Pauling and Corey[1]. They kindly made their manuscript available to us in advance of publication. Their model consists of three intertwined chains, with the phosphates near the fibre axis, and the bases on the outside. In our opinion, this structure is unsatisfactory for two reasons: (1) We believe that the material which gives the X-ray diagrams is the salt, not the free acid. Without the acidic hydrogen atoms it is not clear what forces would hold the structure together, especially as the negatively charged phosphates near the axis will repel each other. (2) Some of the van der Waals distances appear to be too small.

Another three-chain structure has also been suggested by Fraser (in the press). In his model the phosphates are on the outside and the bases on the inside, linked together by hydrogen bonds. This structure as described is rather ill-defined, and for this reason we shall not comment on it.



This figure is purely diagrammatic. The two ribbons symbolize the two phosphate—sugar chains, and the horizontal rods the pairs of bases holding the chains together. The vertical line marks the fibre axis

We wish to put forward a radically different structure for the salt of deoxyribose nucleic acid. This structure has two helical chains each coiled round the same axis (see diagram). We have made the usual chemical assumptions, namely, that each chain consists of phosphate diester groups joining β-D-deoxyribofuranose residues with 3′,5′ linkages. The two chains (but not their bases) are related by a dyad perpendicular to the fibre axis. Both chains follow right-handed helices, but owing to the dyad the sequences of the atoms in the two chains run in opposite directions. Each chain loosely resembles Furberg's[2] model No. 1; that is, the bases are on the inside of the helix and the phosphates on the outside. The configuration of the sugar and the atoms near it is close to Furberg's 'standard configuration', the sugar being roughly perpendicular to the attached base. There is a residue on each chain every 3·4 A. in the z-direction. We have assumed an angle of 36° between adjacent residues in the same chain, so that the structure repeats after 10 residues on each chain, that is, after 34 A. The distance of a phosphorus atom from the fibre axis is 10 A. As the phosphates are on the outside, cations have easy access to them.

The structure is an open one, and its water content is rather high. At lower water contents we would expect the bases to tilt so that the structure could become more compact.

The novel feature of the structure is the manner in which the two chains are held together by the purine and pyrimidine bases. The planes of the bases are perpendicular to the fibre axis. They are joined together in pairs, a single base from one chain being hydrogen-bonded to a single base from the other chain, so that the two lie side by side with identical z-co-ordinates. One of the pair must be a purine and the other a pyrimidine for bonding to occur. The hydrogen bonds are made as follows: purine position 1 to pyrimidine position 1; purine position 6 to pyrimidine position 6.

If it is assumed that the bases only occur in the structure in the most plausible tautomeric forms (that is, with the keto rather than the enol configurations) it is found that only specific pairs of bases can bond together. These pairs are: adenine (purine) with thymine (pyrimidine), and guanine (purine) with cytosine (pyrimidine).

In other words, if an adenine forms one member of a pair, on either chain, then on these assumptions the other member must be thymine; similarly for guanine and cytosine. The sequence of bases on a single chain does not appear to be restricted in any way. However, if only specific pairs of bases can be formed, it follows that if the sequence of bases on one chain is given, then the sequence on the other chain is automatically determined.

It has been found experimentally[3,4] that the ratio of the amounts of adenine to thymine, and the ratio of guanine to cytosine, are always very close to unity for deoxyribose nucleic acid.

It is probably impossible to build this structure with a ribose sugar in place of the deoxyribose, as the extra oxygen atom would make too close a van der Waals contact.

The previously published X-ray data[5,6] on deoxyribose nucleic acid are insufficient for a rigorous test of our structure. So far as we can tell, it is roughly compatible with the experimental data, but it must be regarded as unproved until it has been checked against more exact results. Some of these are given in the following communications. We were not aware of the details of the results presented there when we devised our structure, which rests mainly though not entirely on published experimental data and stereochemical arguments.

It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material.

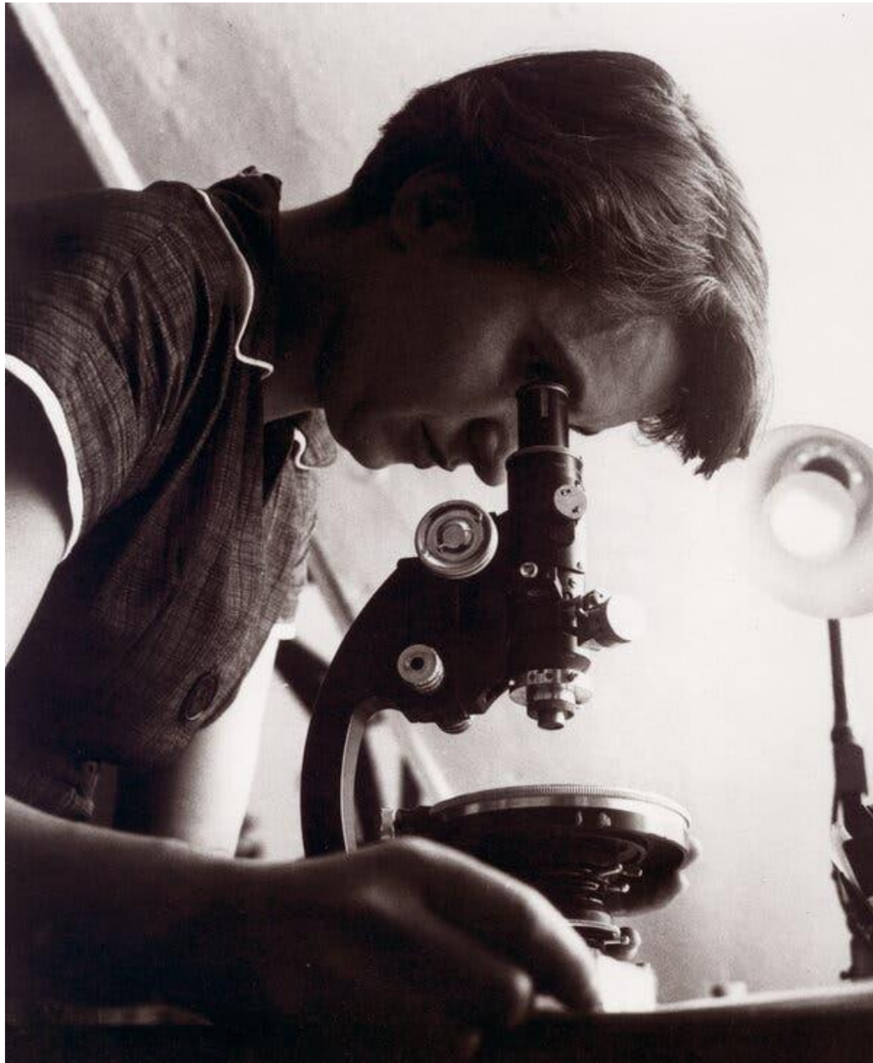
Full details of the structure, including the conditions assumed in building it, together with a set of co-ordinates for the atoms, will be published elsewhere.

We are much indebted to Dr. Jerry Donohue for constant advice and criticism, especially on interatomic distances. We have also been stimulated by a knowledge of the general nature of the unpublished experimental results and ideas of Dr. M. H. F. Wilkins, Dr. R. E. Franklin and their co-workers at

©1953 Nature Publishing Group

http://www.dnaftb.org/19/animation.html

DNA como material genético



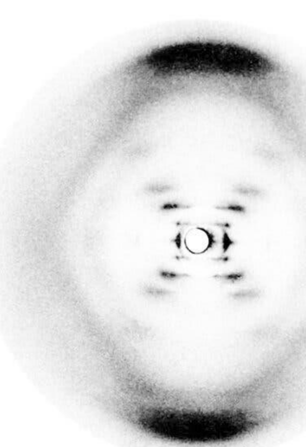


Foto 51

No. 4356 April 25, 1953 NATURE 737

equipment, and to Dr. G. E. R. Deacon and the captain and officers of R.R.S. *Discovery II* for their part in making the observations.

[1] Young, F. B., Gerrard, H., and Jevons, W., *Phil. Mag.*, **40**, 149 (1920).

[2] Longuet-Higgins, M. S., *Mon. Not. Roy. Astro. Soc., Geophys. Supp.*, **5**, 285 (1949).

[3] Von Arx, W. S., Woods Hole Papers in Phys. Oceanog. Meteor., **11** (3) (1950).

[4] Ekman, V. W., *Arkiv. Mat. Astron. Fysik. (Stockholm)*, **2** (11) (1905).

MOLECULAR STRUCTURE OF NUCLEIC ACIDS

A Structure for Deoxyribose Nucleic Acid

WE wish to suggest a structure for the salt of deoxyribose nucleic acid (D.N.A.). This structure has novel features which are of considerable biological interest.

A structure for nucleic acid has already been proposed by Pauling and Corey[1]. They kindly made their manuscript available to us in advance of publication. Their model consists of three intertwined chains, with the phosphates near the fibre axis, and the bases on the outside. In our opinion, this structure is unsatisfactory for two reasons: (1) We believe that the material which gives the X-ray diagrams is the salt, not the free acid. Without the acidic hydrogen atoms it is not clear what forces would hold the structure together, especially as the negatively charged phosphates near the axis will repel each other. (2) Some of the van der Waals distances appear to be too small.

Another three-chain structure has also been suggested by Fraser (in the press). In his model the phosphates are on the outside and the bases on the inside, linked together by hydrogen bonds. This structure as described is rather ill-defined, and for this reason we shall not comment on it.

This figure is purely diagrammatic. The two ribbons symbolize the two phosphate—sugar chains, and the horizontal rods the pairs of bases holding the chains together. The vertical line marks the fibre axis

We wish to put forward a radically different structure for the salt of deoxyribose nucleic acid. This structure has two helical chains each coiled round the same axis (see diagram). We have made the usual chemical assumptions, namely, that each chain consists of phosphate diester groups joining β-D-deoxyribofuranose residues with 3′,5′ linkages. The two chains (but not their bases) are related by a dyad perpendicular to the fibre axis. Both chains follow right-handed helices, but owing to the dyad the sequences of the atoms in the two chains run in opposite directions. Each chain loosely resembles Furberg's[2] model No. 1; that is, the bases are on the inside of the helix and the phosphates on the outside. The configuration of the sugar and the atoms near it is close to Furberg's 'standard configuration', the sugar being roughly perpendicular to the attached base. There is a residue on each chain every 3·4 A. in the z-direction. We have assumed an angle of 36° between adjacent residues in the same chain, so that the structure repeats after 10 residues on each chain, that is, after 34 A. The distance of a phosphorus atom from the fibre axis is 10 A. As the phosphates are on the outside, cations have easy access to them.

The structure is an open one, and its water content is rather high. At lower water contents we would expect the bases to tilt so that the structure could become more compact.

The novel feature of the structure is the manner in which the two chains are held together by the purine and pyrimidine bases. The planes of the bases are perpendicular to the fibre axis. They are joined together in pairs, a single base from one chain being hydrogen-bonded to a single base from the other chain, so that the two lie side by side with identical z-co-ordinates. One of the pair must be a purine and the other a pyrimidine for bonding to occur. The hydrogen bonds are made as follows: purine position 1 to pyrimidine position 1; purine position 6 to pyrimidine position 6.

If it is assumed that the bases only occur in the structure in the most plausible tautomeric forms (that is, with the keto rather than the enol configurations) it is found that only specific pairs of bases can bond together. These pairs are: adenine (purine) with thymine (pyrimidine), and guanine (purine) with cytosine (pyrimidine).

In other words, if an adenine forms one member of a pair, on either chain, then on these assumptions the other member must be thymine; similarly for guanine and cytosine. The sequence of bases on a single chain does not appear to be restricted in any way. However, if only specific pairs of bases can be formed, it follows that if the sequence of bases on one chain is given, then the sequence on the other chain is automatically determined.

It has been found experimentally[3,4] that the ratio of the amounts of adenine to thymine, and the ratio of guanine to cytosine, are always very close to unity for deoxyribose nucleic acid.

It is probably impossible to build this structure with a ribose sugar in place of the deoxyribose, as the extra oxygen atom would make too close a van der Waals contact.

The previously published X-ray data[5,6] on deoxyribose nucleic acid are insufficient for a rigorous test of our structure. So far as we can tell, it is roughly compatible with the experimental data, but it must be regarded as unproved until it has been checked against more exact results. Some of these are given in the following communications. We were not aware of the details of the results presented there when we devised our structure, which rests mainly though not entirely on published experimental data and stereochemical arguments.

It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material.

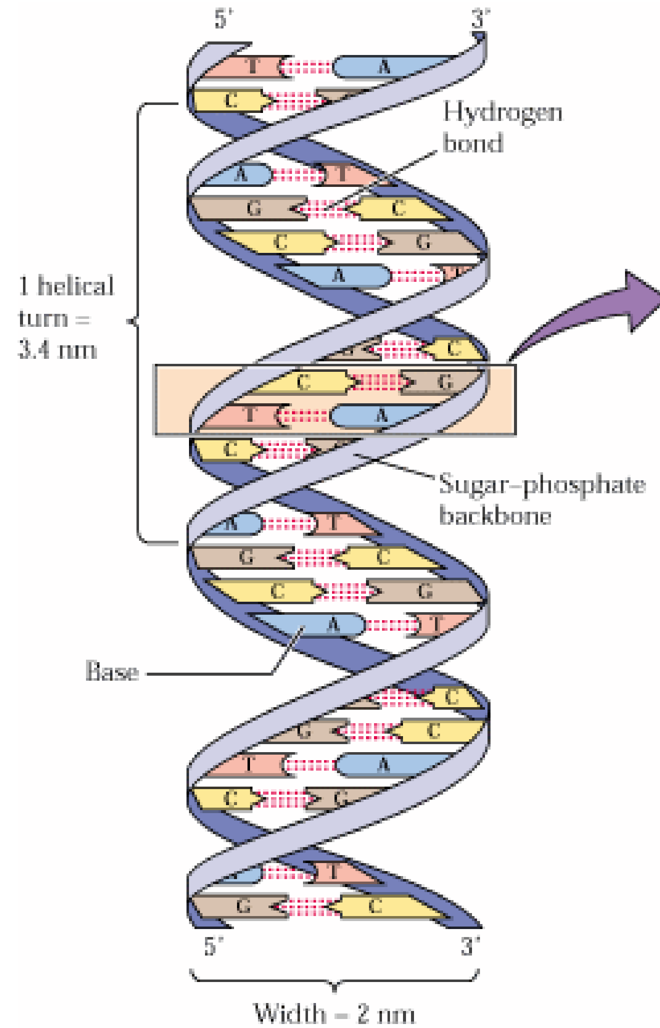
Full details of the structure, including the conditions assumed in building it, together with a set of co-ordinates for the atoms, will be published elsewhere.

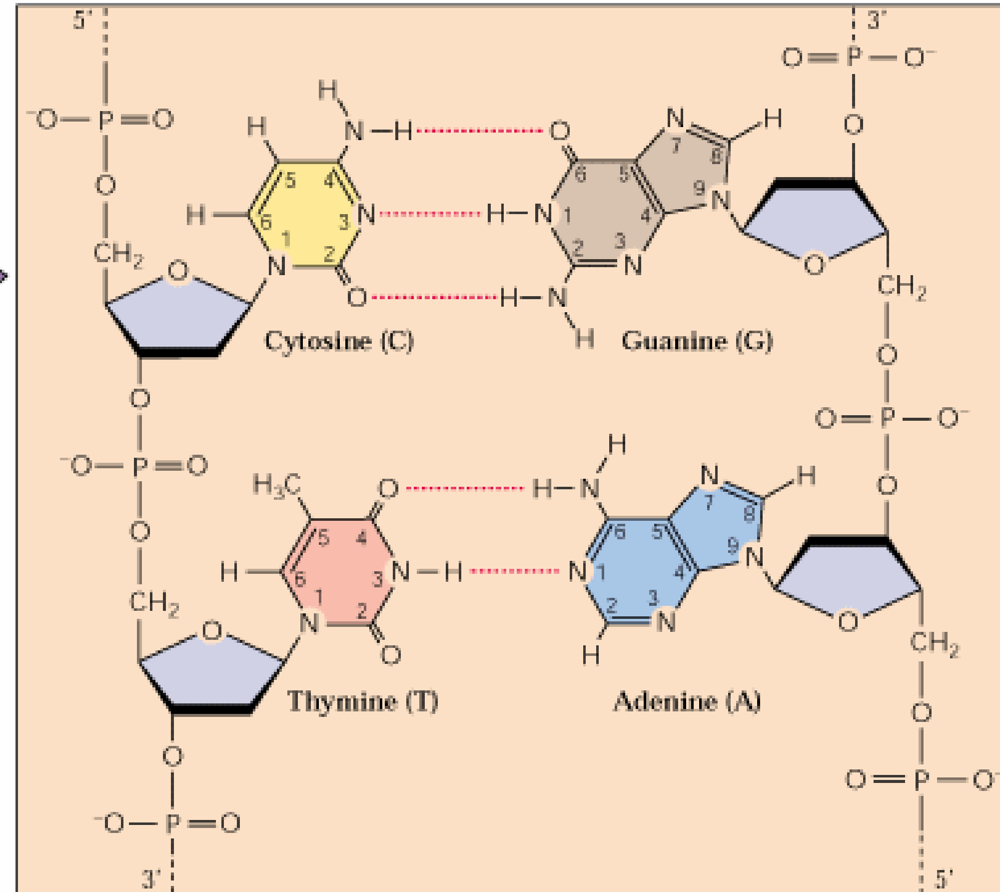
We are much indebted to Dr. Jerry Donohue for constant advice and criticism, especially on interatomic distances. We have also been stimulated by a knowledge of the general nature of the unpublished experimental results and ideas of Dr. M. H. F. Wilkins, Dr. R. E. Franklin and their co-workers at

©1953 Nature Publishing Group

Rosalind Franklin

DNA - Ácido Desoxirribonucleico





DNA – Fita Dupla!

- Duas cadeias independentes
- Dupla hélice, sentido direito
- Hélices antiparalelas
- Complementariedade das bases
    - Eixo externo hidrofílico - deoxiribose + fosfato
    - Bases hidrofóbicas (planas) no interior
    - Bases ligadas por pontes de H

Principais Tipos de RNA

RNAs ocorrem no núcleo e citoplasma

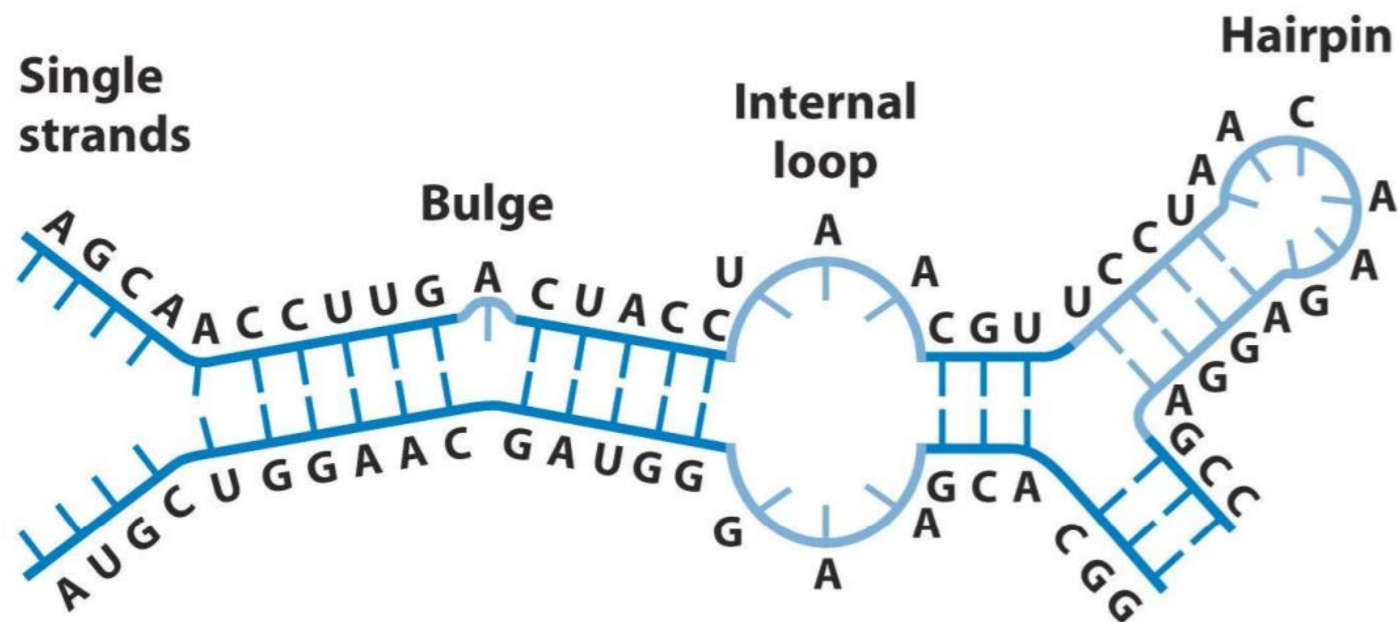
RNA mensageiro (mRNA): contém a informação genética para a sequência de aminoácidos das proteínas

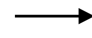
RNA transferência (tRNA): identifica e transporta os aminoácidos até o ribossomo

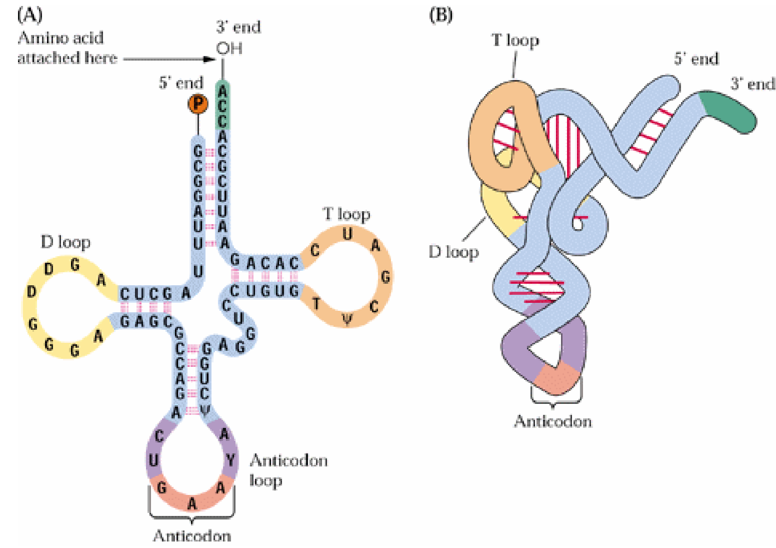
RNA ribossômico (rRNA): constituinte dos ribossomos

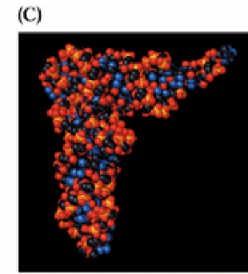
Estrutura de RNA

- RNA não possuem estrutura regular como DNA
- As estruturas de RNA são complexas e únicas
- Pareamento de bases similar a DNA forma estruturas

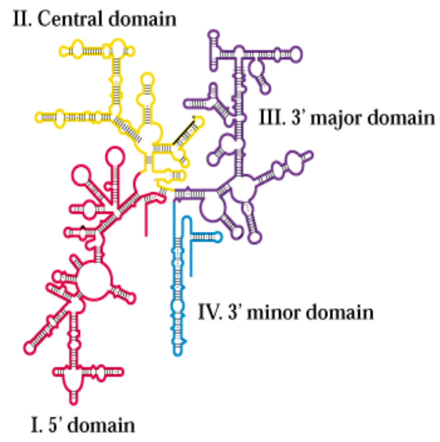


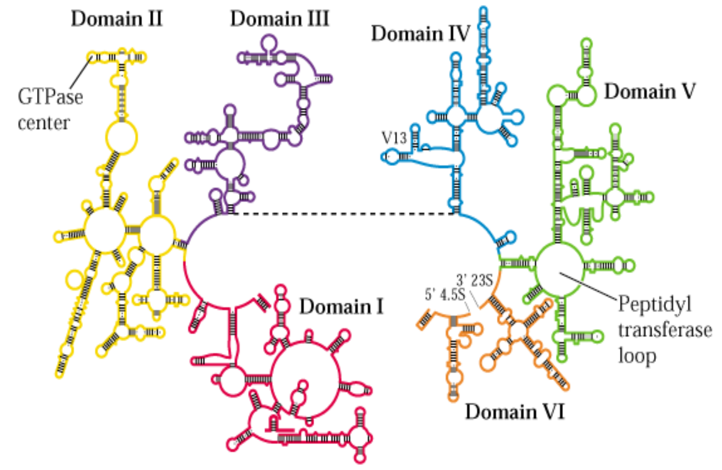
tRNA 

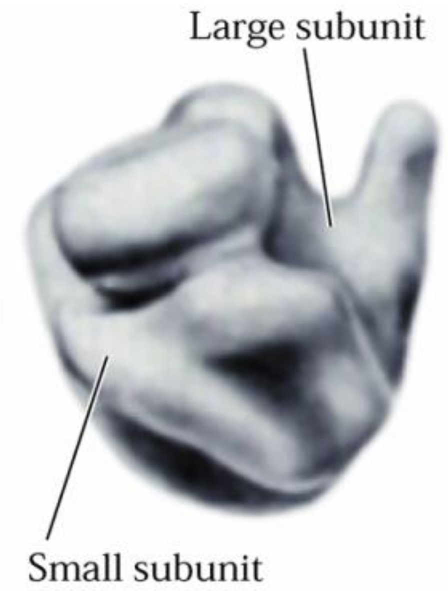




rRNA

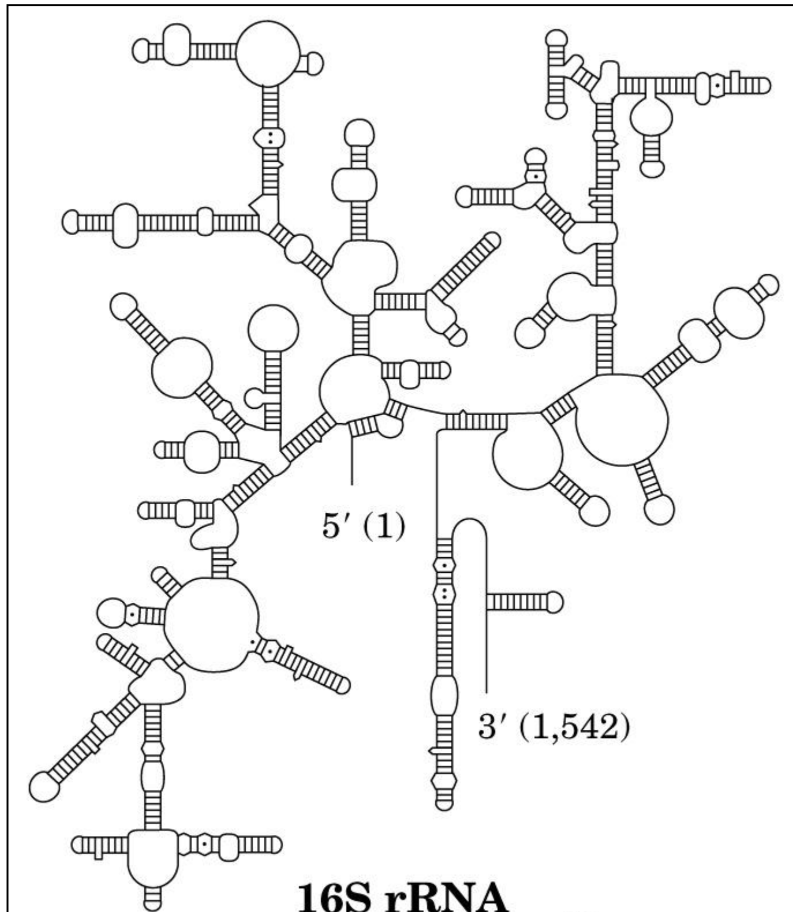






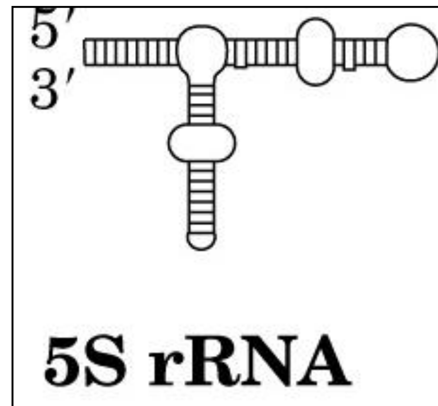
RNA ribossômico - rRNA

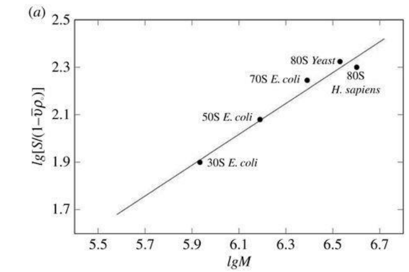
Possuem estrutura tridimensional específica visando promover a estabilidade e atividade catalítico nos ribossomos

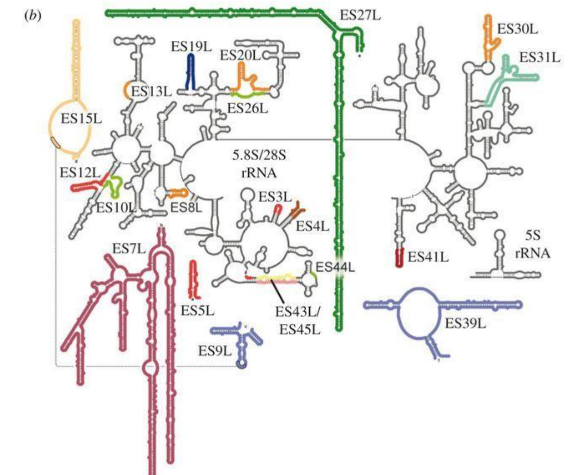


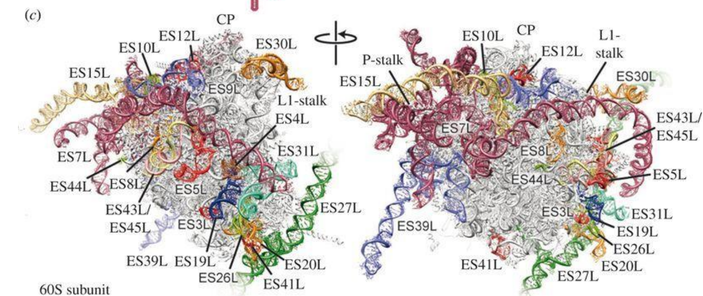
Exemplos de rRNAs:

- Estrutura secundária com grampos e alças









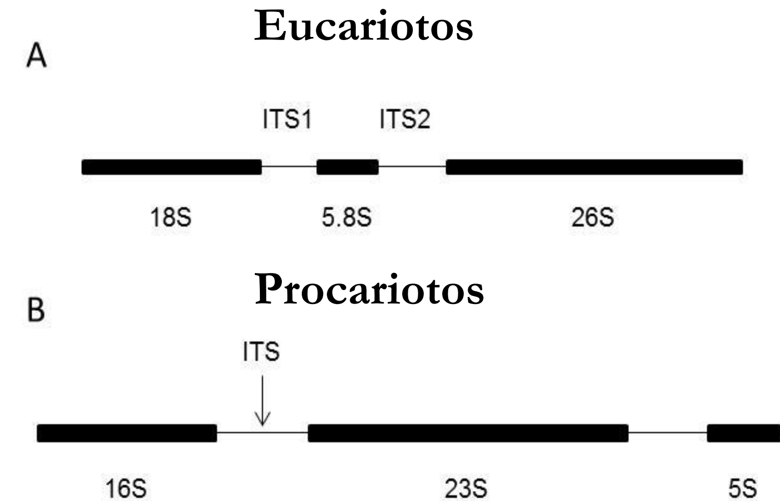
Yusupova & Yusupov 2017

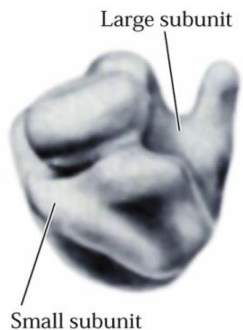
Ácidos Nucleicos - RNA

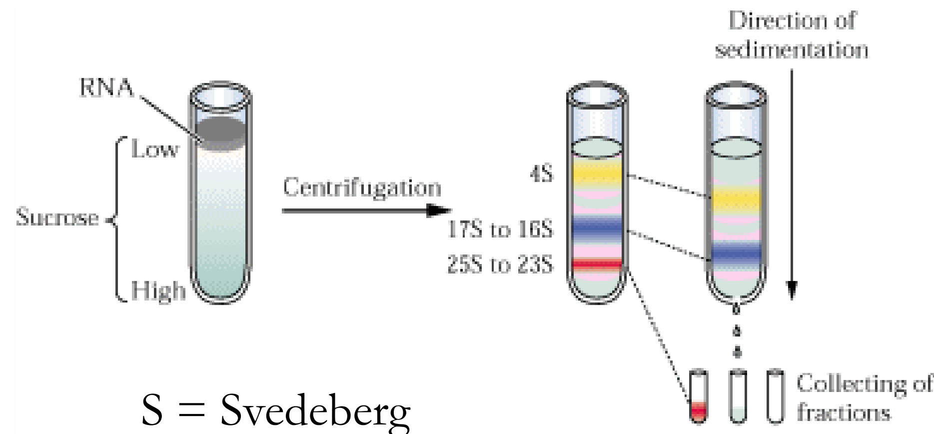
RNA ribossomal – rRNA

- mais abundante na célula (>80%)
- síntese nuclear
- combinado com proteínas - ribossomo
- procarioto 70S - (23S + 5S) (16S)
- eucarioto 80S - (28S+5.8S+5S) (18S)

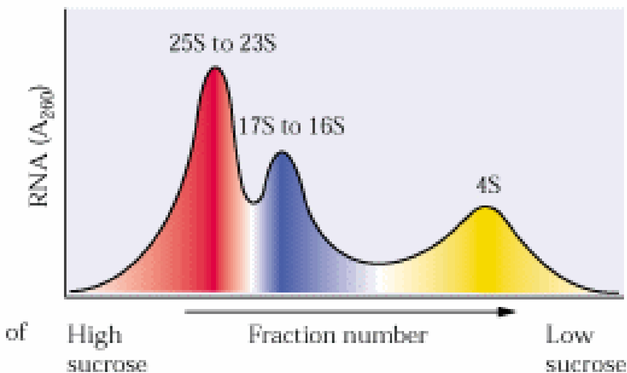
Cada um é um gene!!



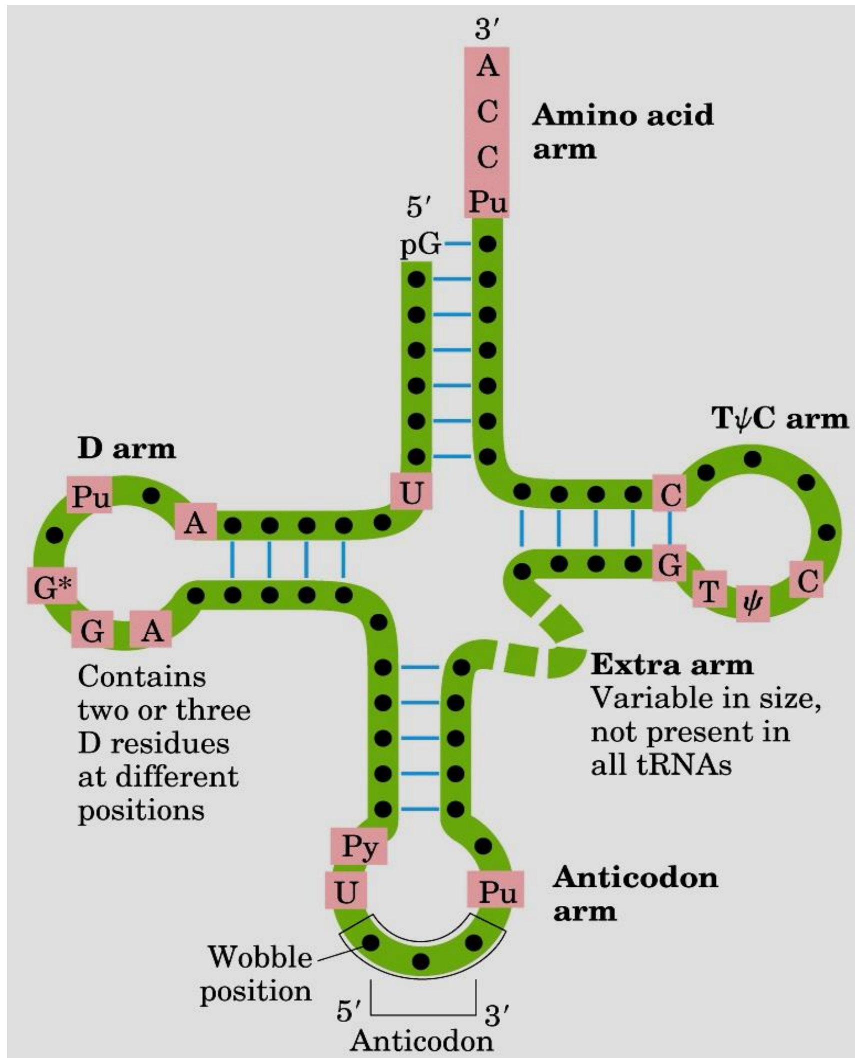


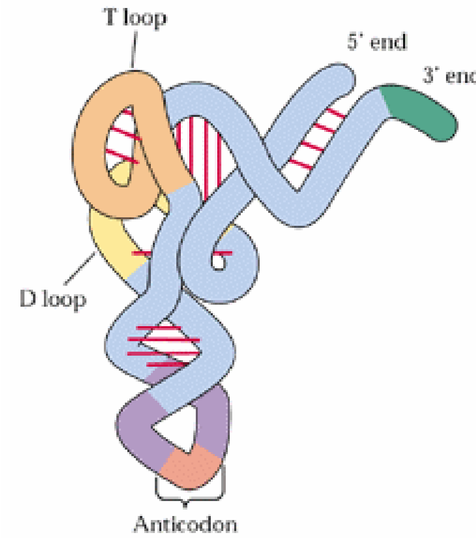


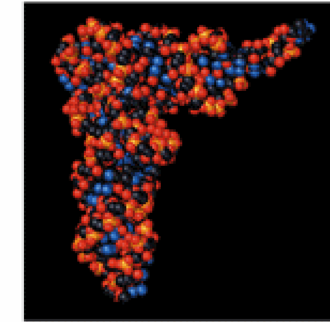
S = Svedeberg

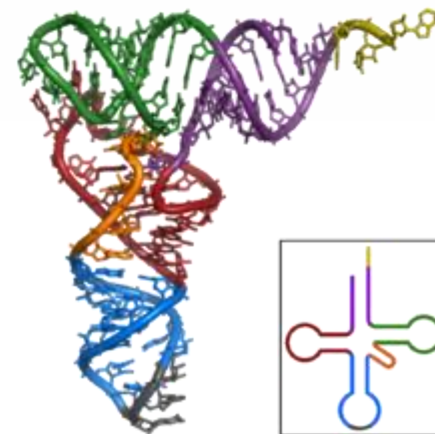


RNA transportador - tRNA







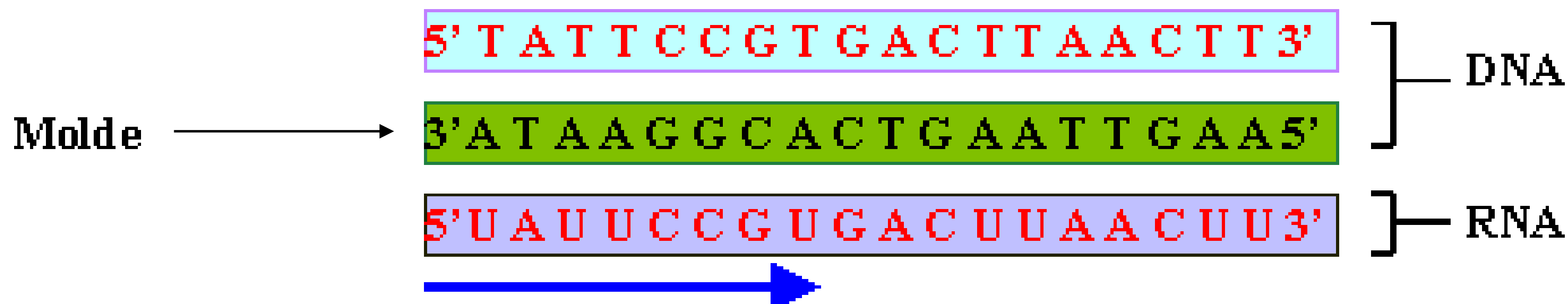


Cada tRNA = um gene

Reconhece códons em mRNA - anticódon

RNA mensageiro - mRNA

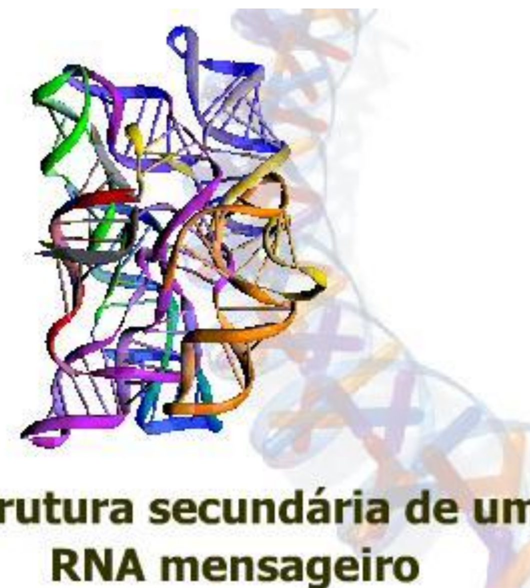
1 trinca de bases nitrogenadas = 1 códon



Sentido da transcrição

Segunda Base

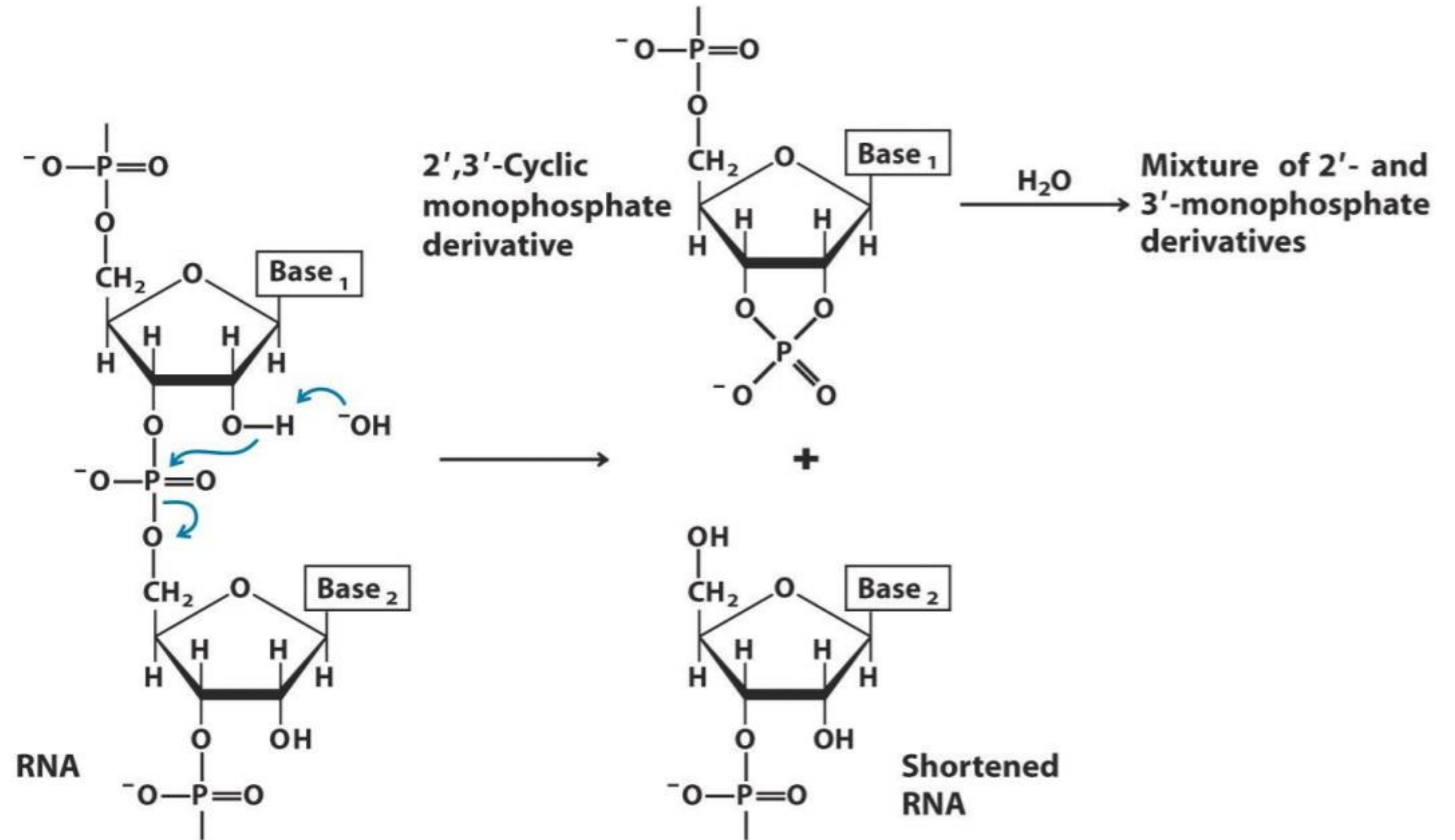
Primeira Base (5')	U	C	A	G	Terceira Base (3')
U	UUU, UUC – Fenil-alanina UUA, UUG – Leucina	UCU, UCC, UCA, UCG – Serina	UAU, UAC – Tirosina UAA – Stop codon UAG – Stop codon	UGU, UGC – Cysteine UGA – Stop codon UGG – Tryptophan	U C A G
C	CUU, CUC, CUA, CUG – Leucina	CCU, CCC, CCA, CCG – Prolina	CAU, CAC – Histidina CAA, CAG – Glutamina	CGU, CGC, CGA, CGG – Arginina	U C A G
A	AUU, AUC, AUA – Isoleucina AUG – Metionina start codon	ACU, ACC, ACA, ACG – Treonina	AAU, AAC – Asparagina AAA, AAG – Lisina	AGU, AGC – Serina AGA, AGG – Arginina	U C A G
G	GUU, GUC, GUA, GUG – Valina	GCU, GCC, GCA, GCG – Alanina	GAU, GAC – Ácido Aspártico GAA, GAG – Acido Glutâmico	GGU, GGC, GGA, GGG – Glicina	U C A G



Estrutura secundária de um RNA mensageiro

Porque o RNA é mais lábil?

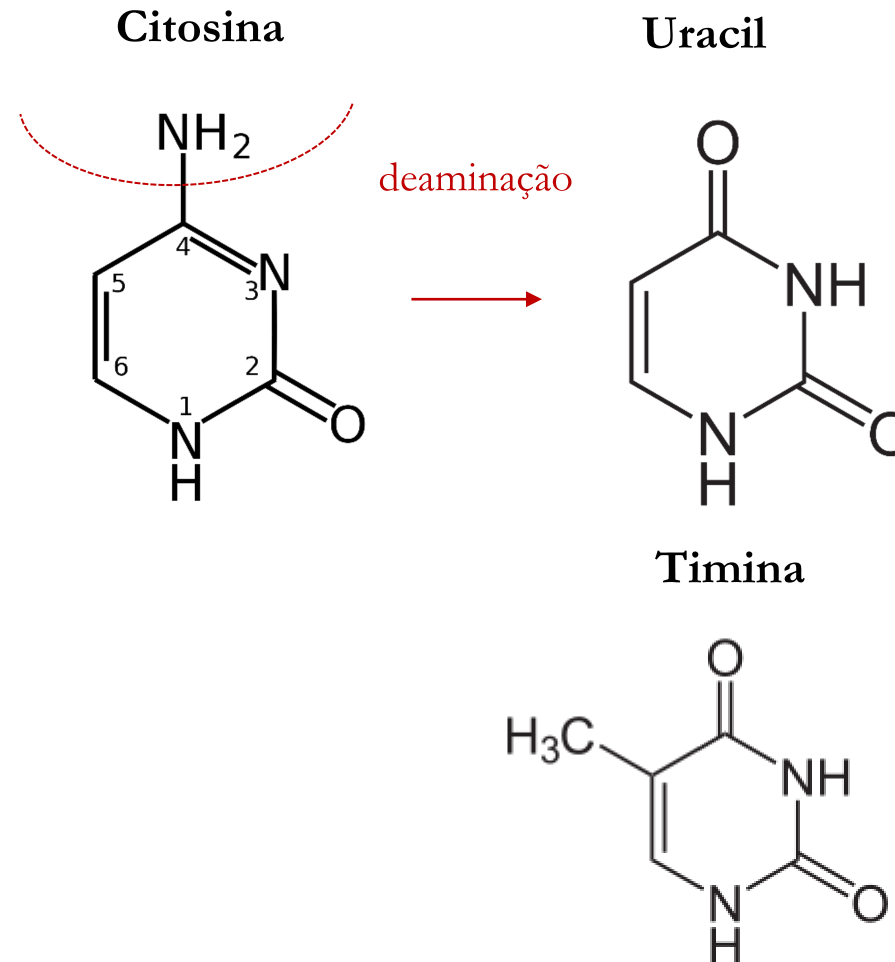
Efeito de pH alcalino



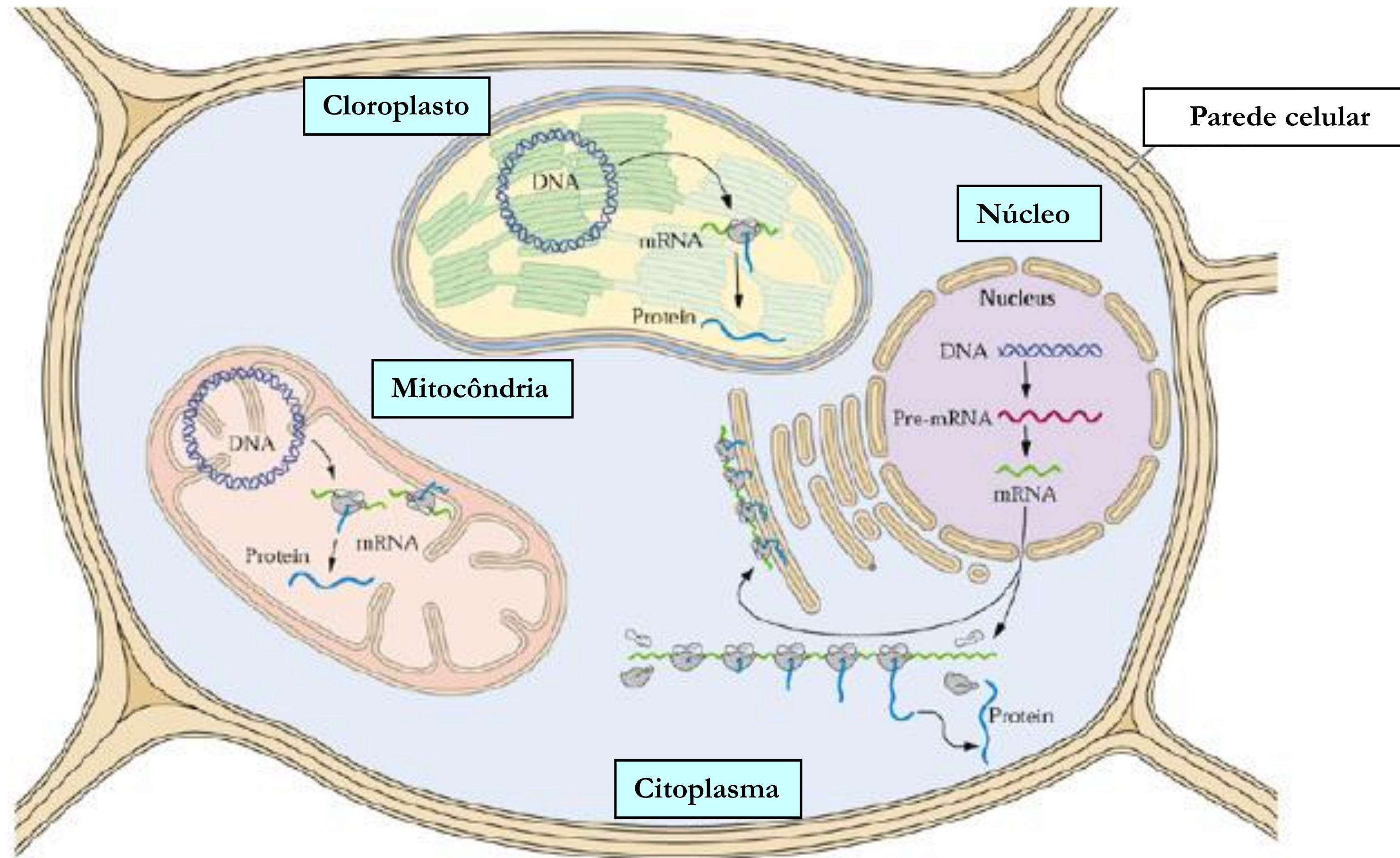
Porque o Timina (DNA) x Uracil (RNA)?

Deaminação de Citosina a Uracil
Ocorre naturalmente
Perda de amina em C vira U
Afeta o significado da sequência
Ao invés de C-G passa a ser U-G!!
Reconhecido em DNA

<u>Pareamento em D</u>NA
C – G passaria a ser U-G
Na replicação incorreria em erro!!

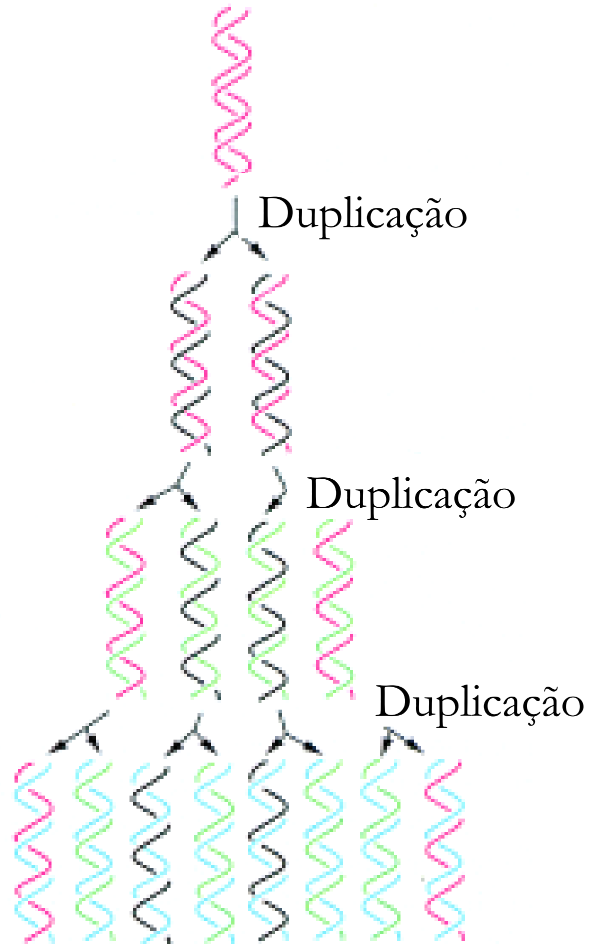


Três genomas em plantas: cromossomial, plastidial e mitocondrial



Replicação de DNA

Replicação de DNA



It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material.

Watson & Crick 1953

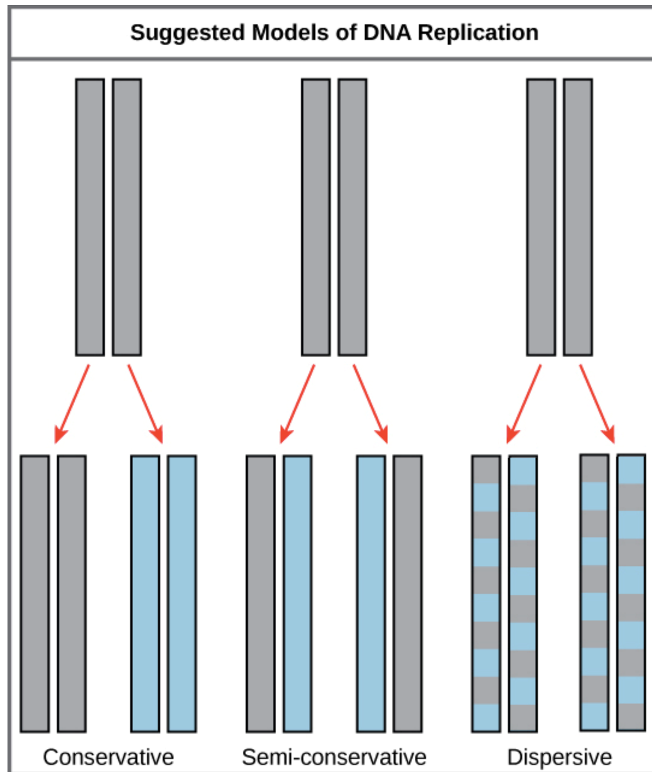
Meselson & Stalh 1958: replicação semiconservativa do DNA

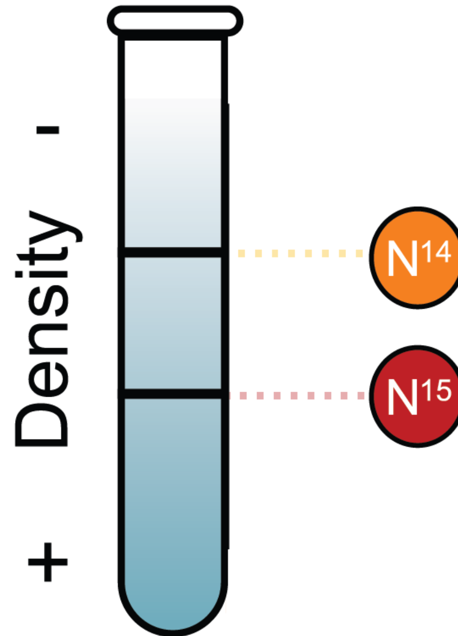
Kronenberg 1958: DNA polimerase

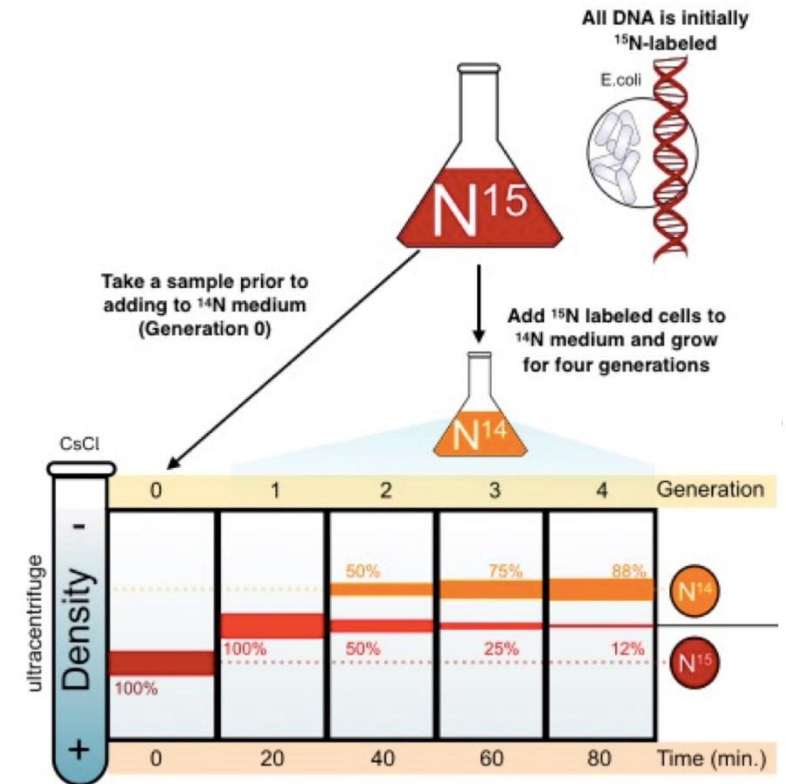
http://www.dnaftb.org/20/animation.html

Replicação de DNA

Conservativo, Semiconservativo ou Dispersivo??







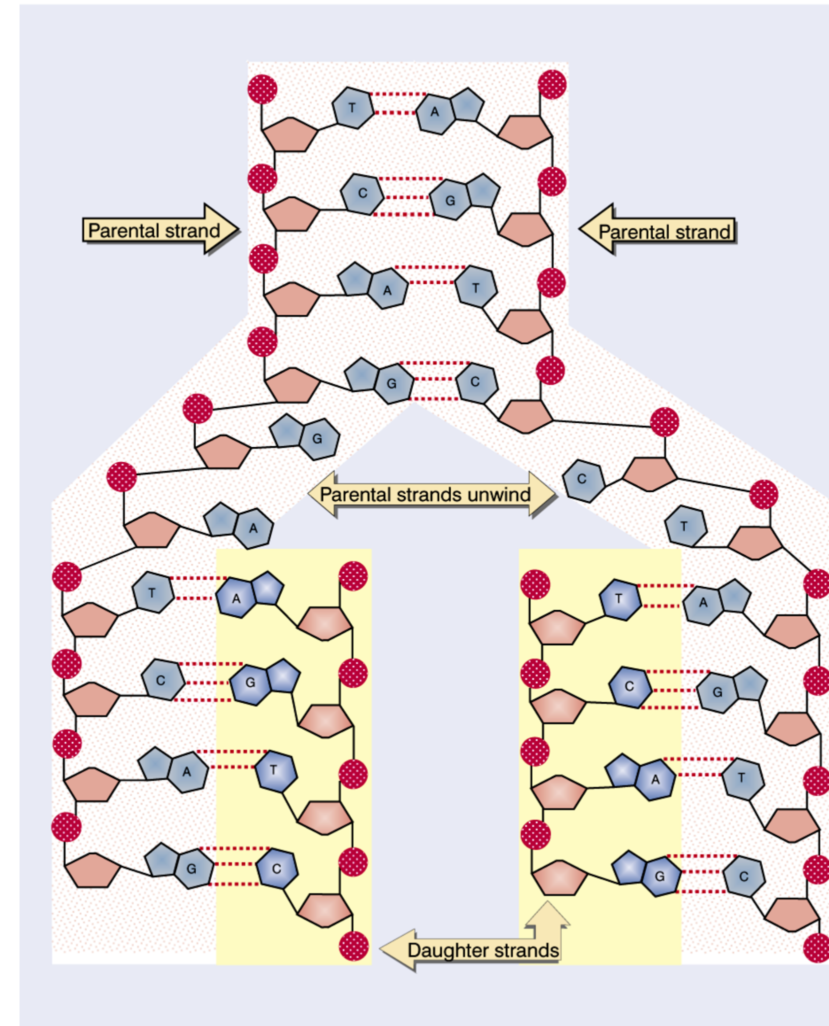
Meselson & Stalh 1958: replicação semiconservativa do DNA

Replicação de DNA

- A replicação do DNA é um mecanismo **semiconservativo**: a medida que as duas fitas complementares de uma dupla hélice parental se desenrolam e se separam, cada uma serve como um molde para a síntese de nova fita complementar

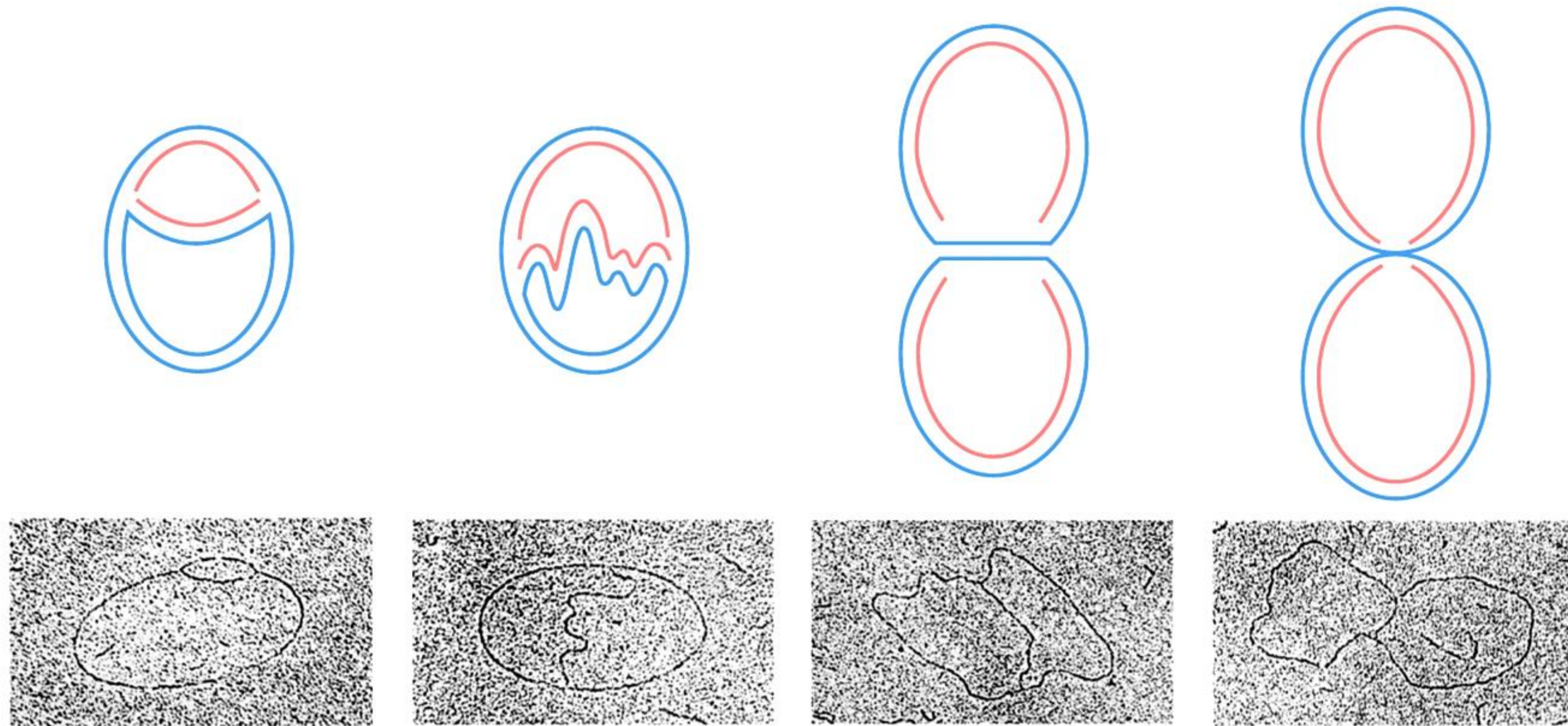
- Os potenciais de ligação das bases das fitas moldes especificam as sequências de bases complementares nas fitas de DNA nascentes

- A **replicação** é iniciada em **origens únicas** e em geral continua **bidirecionalmente** a partir de cada origem



http://www.dnaftb.org/20/animation.html

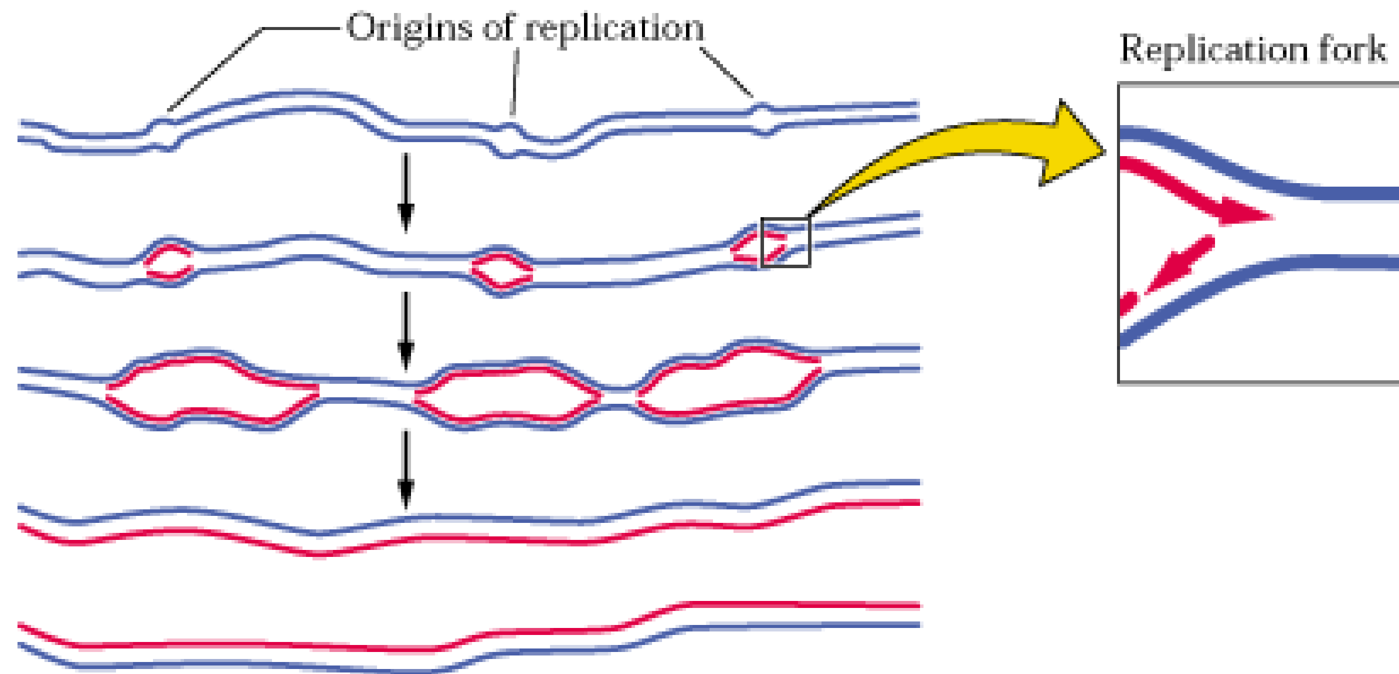
Replicação de Cromossomo Circular



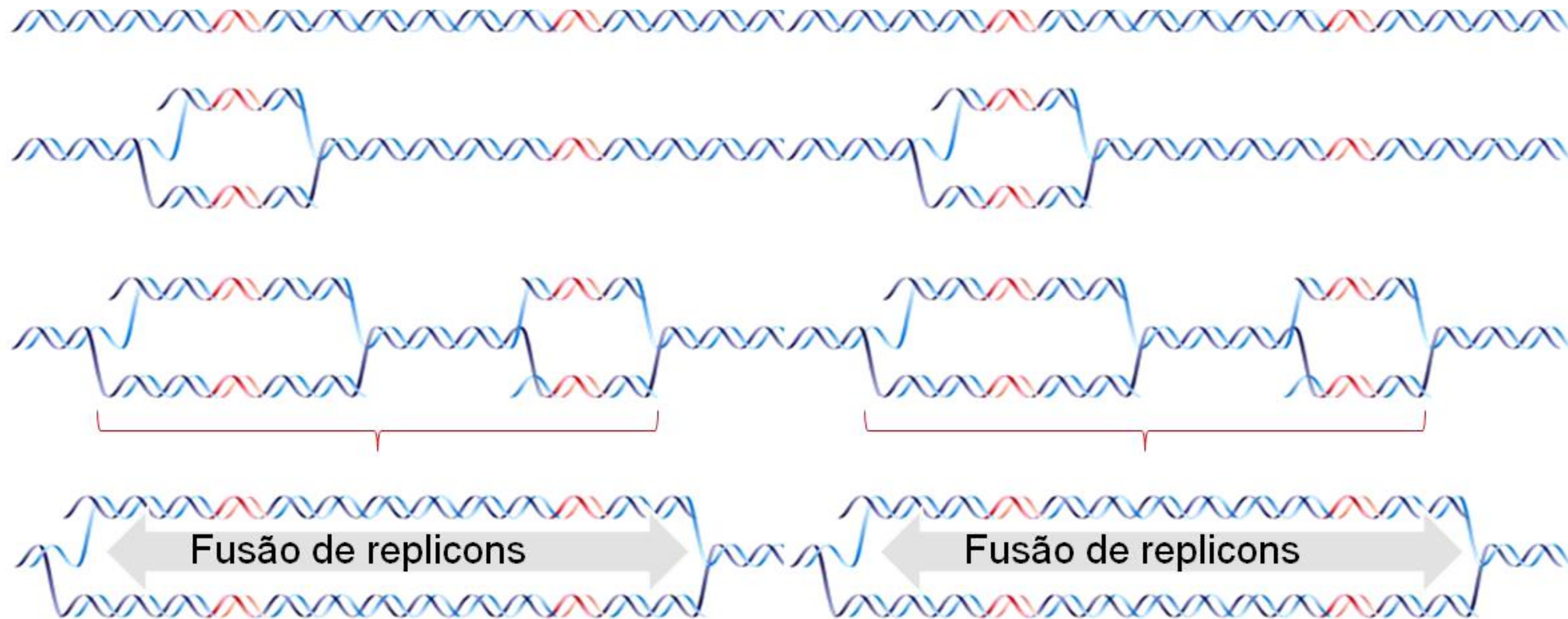
A replicação é bidirecional

- A velocidade da **forquilha** de replicação de **procarioto** é cerca de 30.000 pb/min
- 1 único replicon

Origem de replicação em Eucariotos



Replicação de cromossomo linear de eucarioto



- **A velocidade da forquillha de replicação de eucarioto é cerca de 100 pb/ segundo**
- Os **replicons** de eucariotos têm cerca de **40-100 kb** e são iniciados em tempos diferentes. (não conhecemos todos os fatores que determinam qual origem e em que momento ela fica ativa - O *timing* da replicação pode, por ex. ser determinado pela atividade do gene: genes mais transcritos são replicados primeiro)

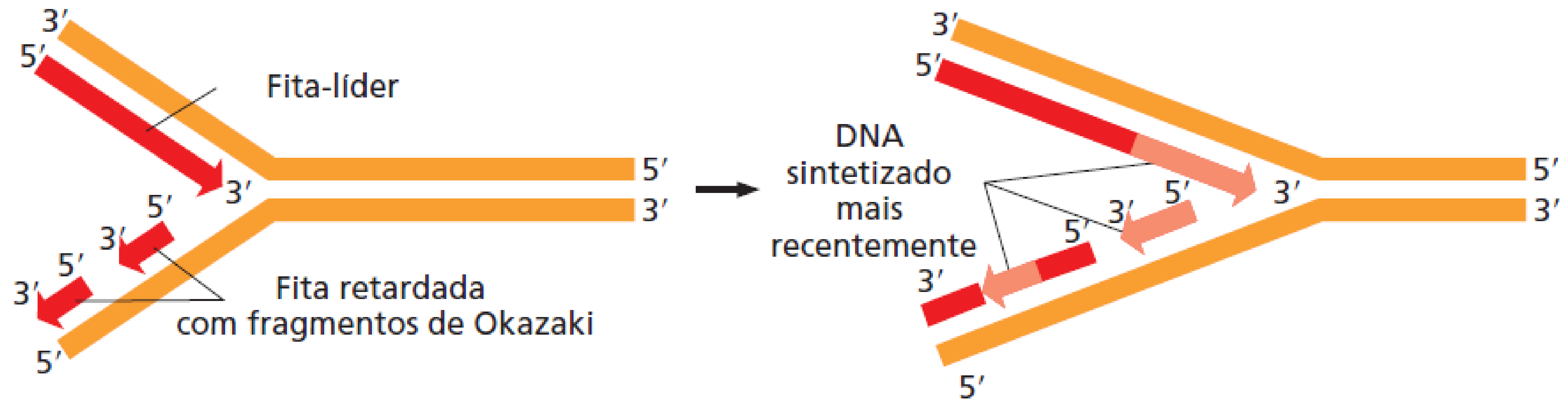
Principais Enzimas Envolvidas na Replicação

1. **DNA Polimerases** – Polimerização do DNA, retirada dos *primers* e reparo do DNA
2. **Helicases –** desenrolam a fita dupla
3. **Topoisomerases (girases) –** aliviam tensão de torsão devido a abertura da fita
4. **Primases –** síntese dos *primers*
5. **Ligases** - Une os fragmentos de Okasaki
6. ***Single strand binding* (SSB) -** Proteínas que se liga a fita simples de DNA
7. **Telomerases –** síntese das terminações

Replicação de DNA

- Se a replicação é semiconservativa e a polimerização deve ser sempre no sentido 5´→3´
- Mas o DNA é antiparalelo ou seja, uma fita ocorre no sentido 5’ → 3’ e a outra no sentido 3’ → 5’
- **Como ocorre, então, a replicação nos dois sentidos?**

Forquilha de duplicação de DNA



Alberts *et al.* (2012)

Pontos importantes sobre as DNA Polimerases

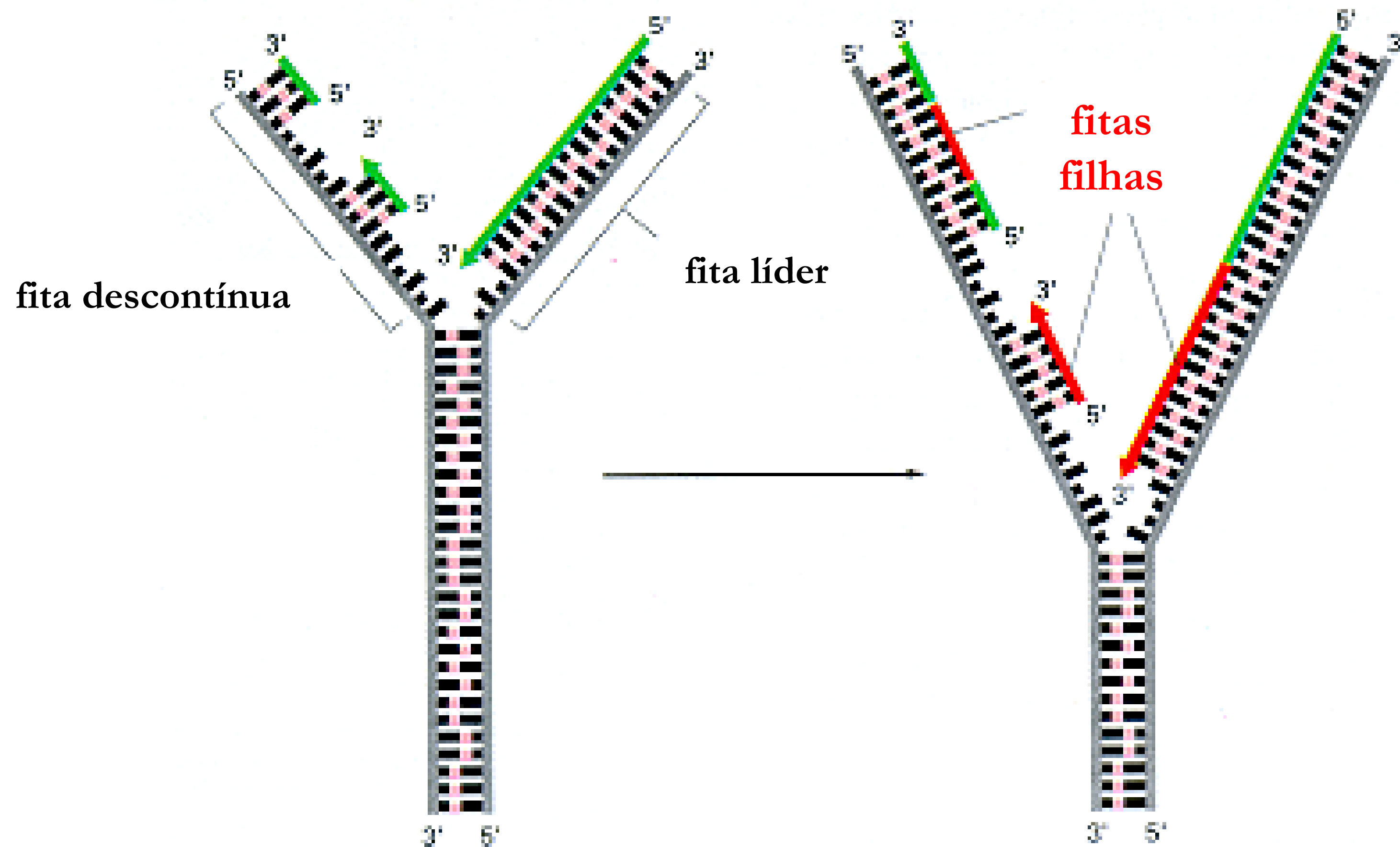
- A síntese de DNA é catalisada por enzimas chamadas **DNA polimerases**

- Todas as DNA polimerases precisam de um oligonucleotídeo (*primer*) para servir de ponto de origem de onde uma fita é sintetizada

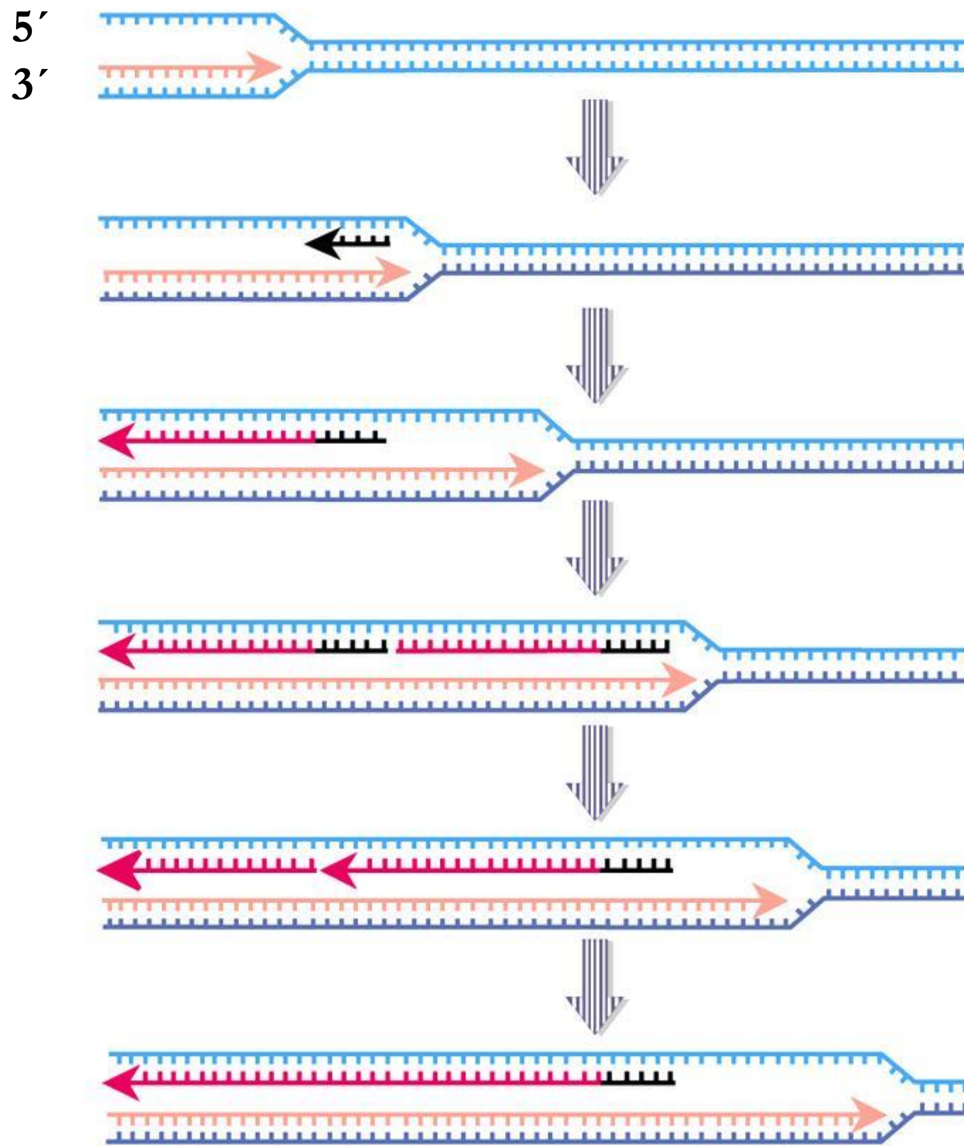
- Todas as DNA polimerases tem necessidade **absoluta** de uma 3'-OH livre do oligonucleotídeo (*primer*), e toda a síntese de DNA ocorre no sentido 5´→3'

- Atividades de **exonuclease** 3'→5' das DNA polimerases **revisam** as fitas nascentes à medida que eles são sintetizados, removendo quaisquer nucleotídeos mal pareados nas pontas 3' da fitas novas

Forquilha de duplicação de DNA



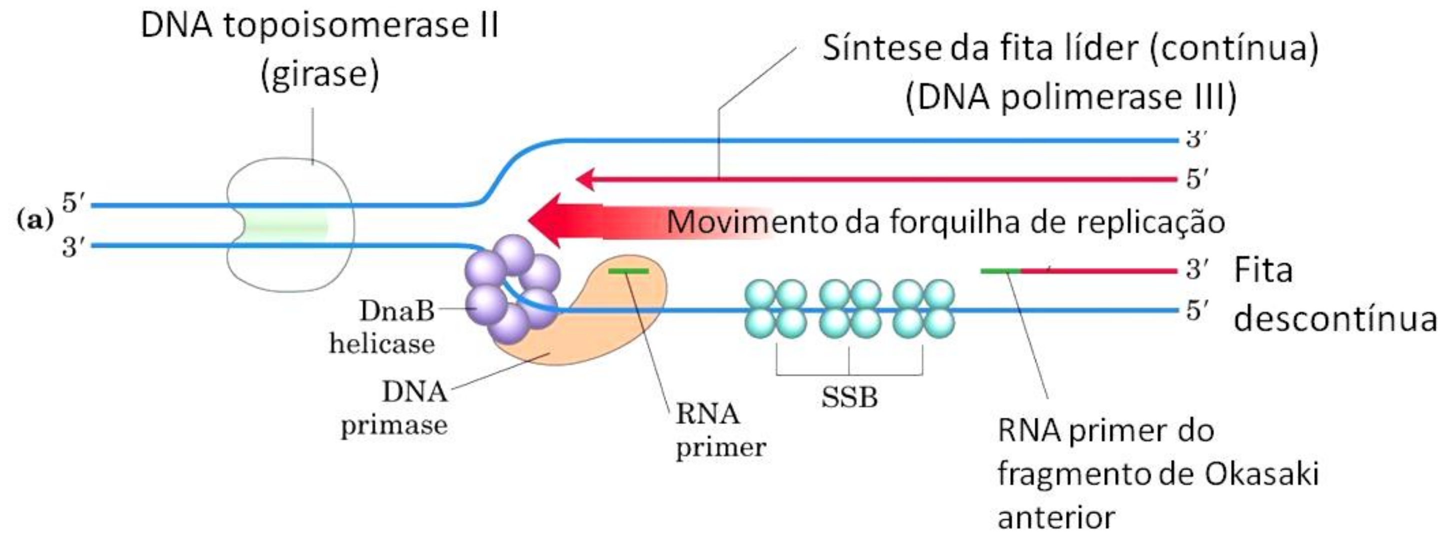
Alberts *et al.* (1997)

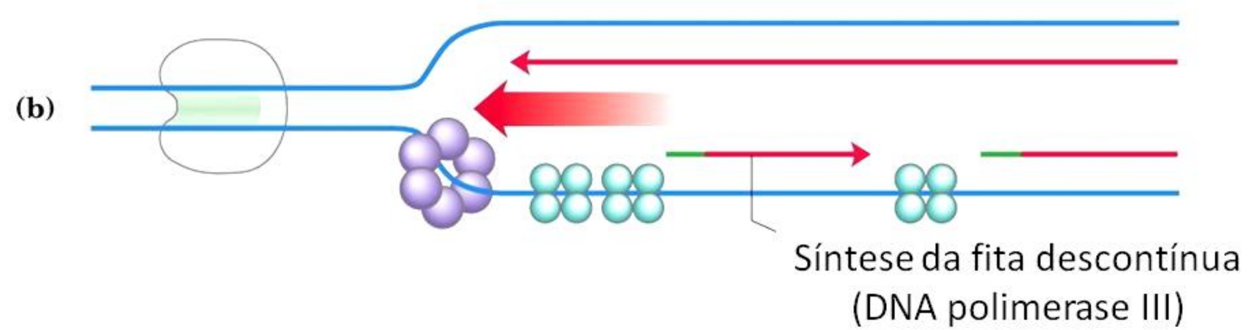


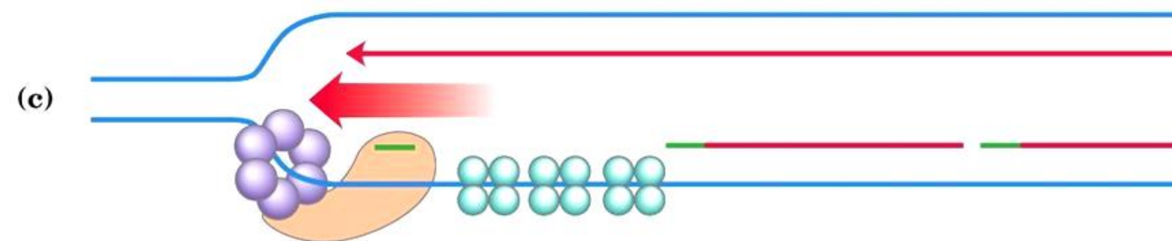
- Fragmentos de Okasaki ocorrem na fita descontínua
- A DNA polimerase III é responsável pela síntese da maior parte do DNA
- A DNA polimerase I remove o primer de RNA e preenche as lacunas
- A DNA ligase sela as quebras

Proteínas presentes na origem de replicação

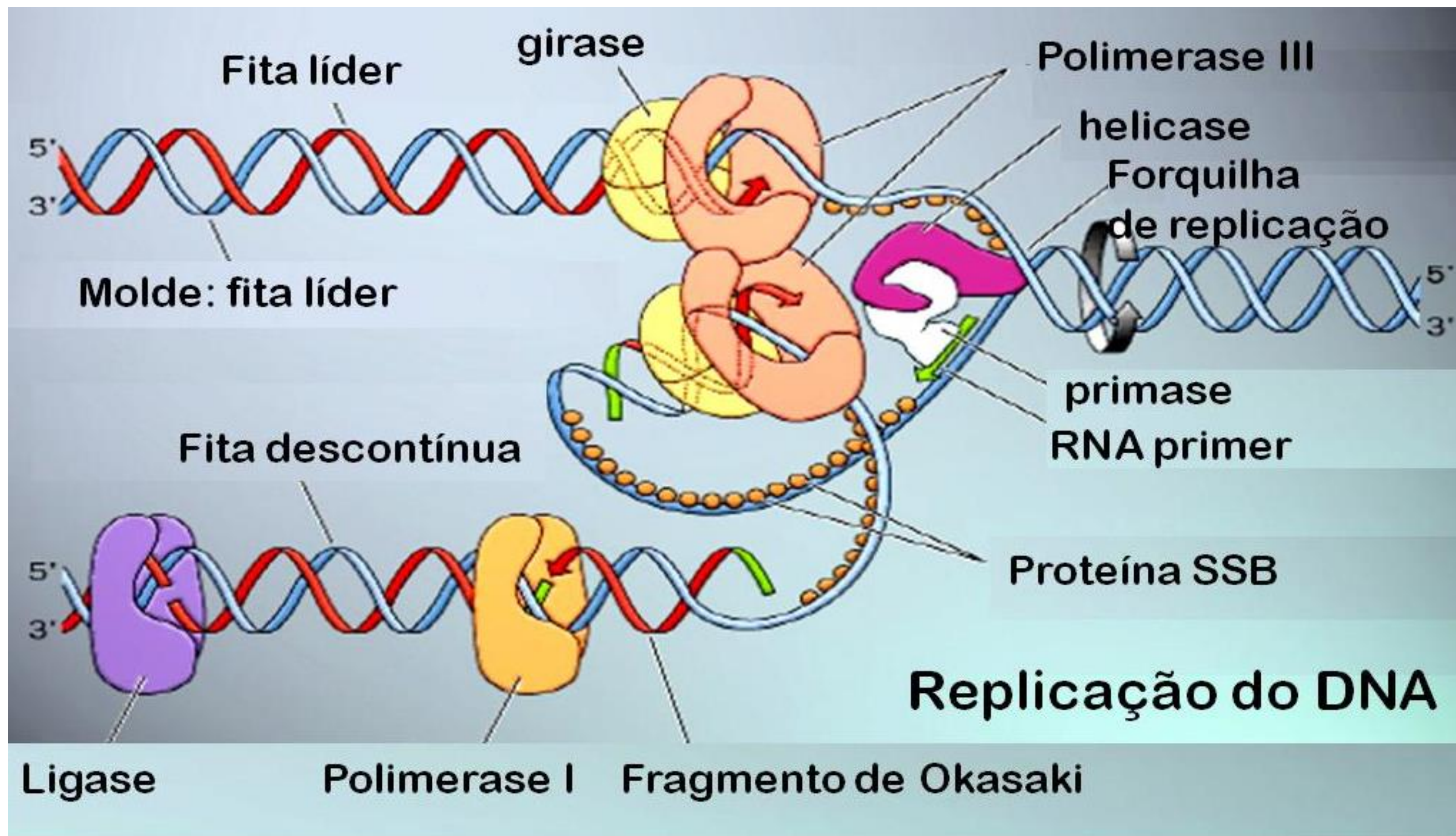
Helicase	Desenrola o DNA – forma a forquilha
DNA girase (topoisomerase)	Alivia a tensão de torção gerada pela abertura da dupla-fita
Primase	Sintetiza os *primers* de RNA
DNA polimerase III DNA Polimerase I	Polimerização do DNA e reparo do DNA Degradação dos *primers* de RNA
Single strand binding (SSB)	Liga a fita simples de DNA
DNA ligase	Une os fragmentos de Okasaki


DNA topoisomerase II
(girase)
Síntese da fita líder (contínua)
(DNA polimerase III)
(a)
5′
3′
3′
5′
Movimento da forquilha de replicação
3′
5′
Fita
descontínua
DnaB
helicase
DNA
primase
RNA
primer
SSB
RNA primer do
fragmento de Okasaki
anterior


(b)
Síntese da fita descontínua
(DNA polimerase III)


(c)

Síntese das fitas contínua e descontínua é independente

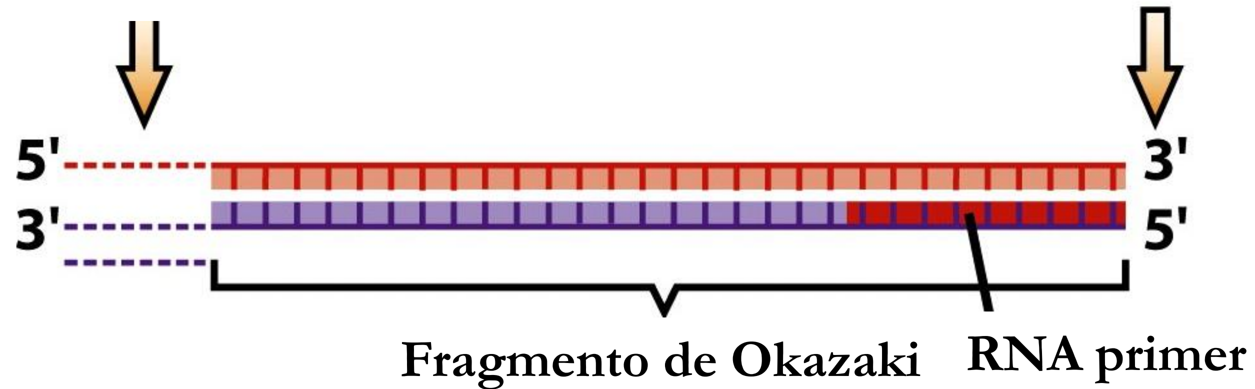


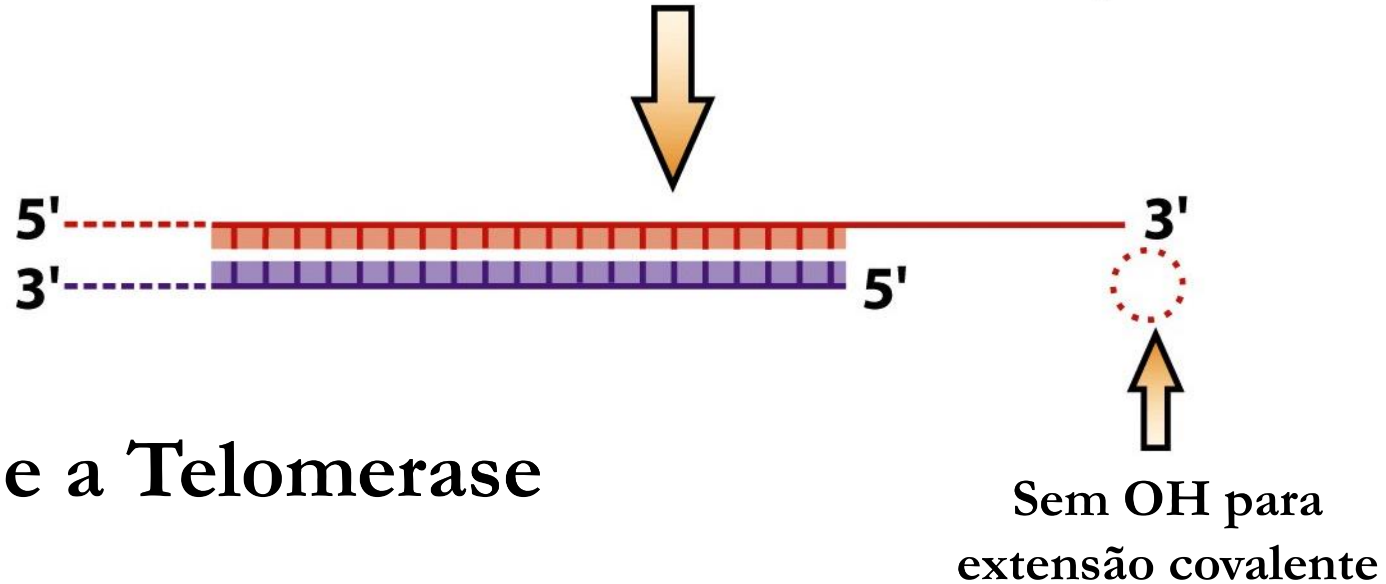
https://www.youtube.com/watch?v=TNKWgcFPHqw

https://www.dnalc.org/resources/3d/04-mechanism-of-replication-advanced.html

Próximo ao centrômero

Fim do cromossomo

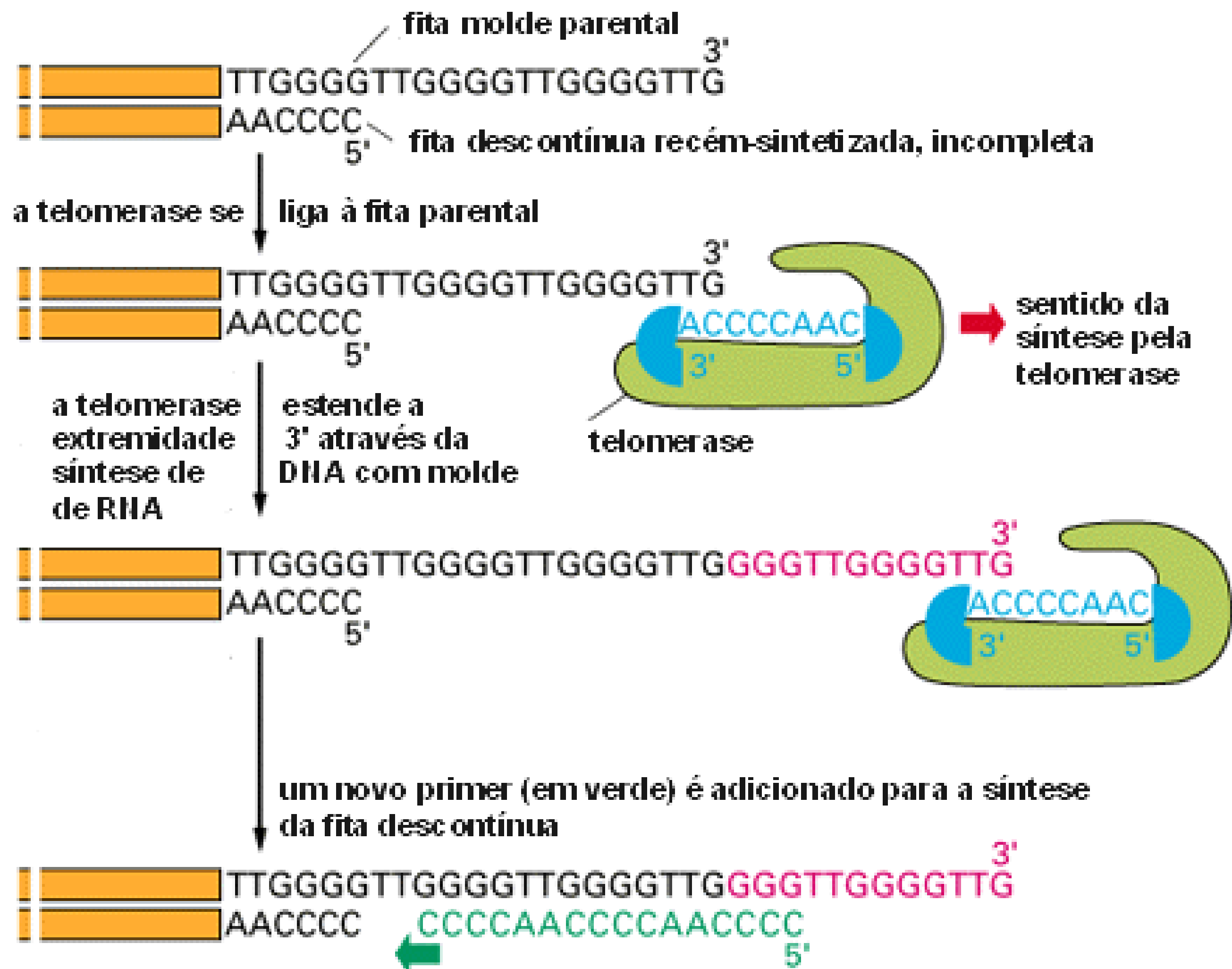


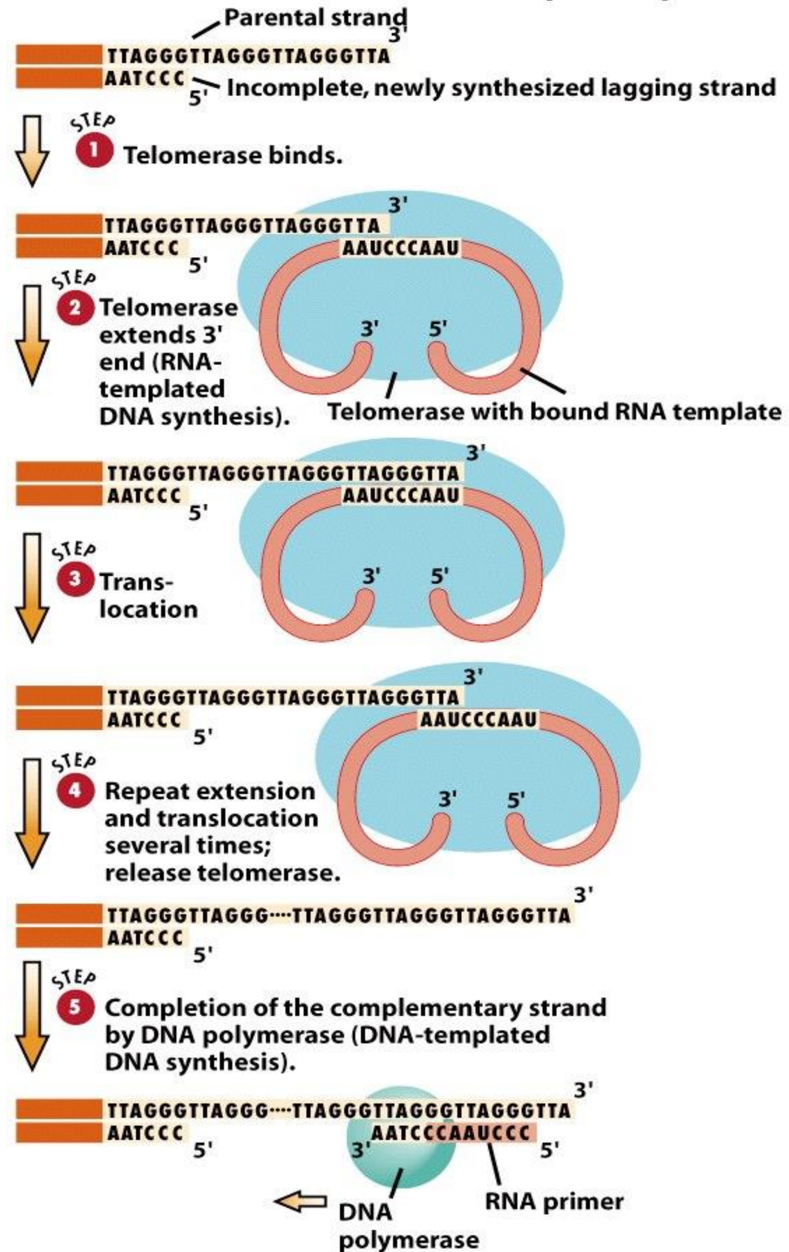


Telomeros e a Telomerase

Figure 10-33a Principles of Genetics, 4/e

© 2006 John Wiley & Sons


fita molde parental
3'
TTGGGGTTGGGGTTGGGGTTG
AACCCC
5'
fita descontínua recém-sintetizada, incompleta
a telomerase se liga à fita parental
3'
TTGGGGTTGGGGTTGGGGTTG
AACCCC
5'
ACCCCAAC
3'
5'
sentido da síntese pela telomerase
telomerase
a telomerase estende a extremidade 3' através da síntese de DNA com molde de RNA
3'
TTGGGGTTGGGGTTGGGGTTGGGGTTGGGGTTG
AACCCC
5'
ACCCCAAC
3'
5'
um novo primer (em verde) é adicionado para a síntese da fita descontínua
3'
TTGGGGTTGGGGTTGGGGTTGGGGTTGGGGTTG
AACCCC
CCCCAACCCCAACCCC
5'



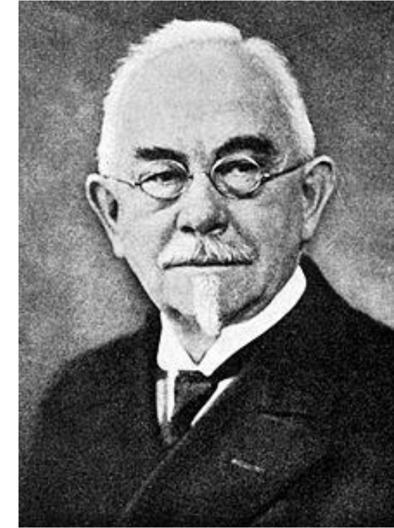
Elizabeth Blackburn
Nobel 2009
Carol Greider

Mas o que é um gene?

Definição de Gene

Wilhelm Johannsen

1909 → gene



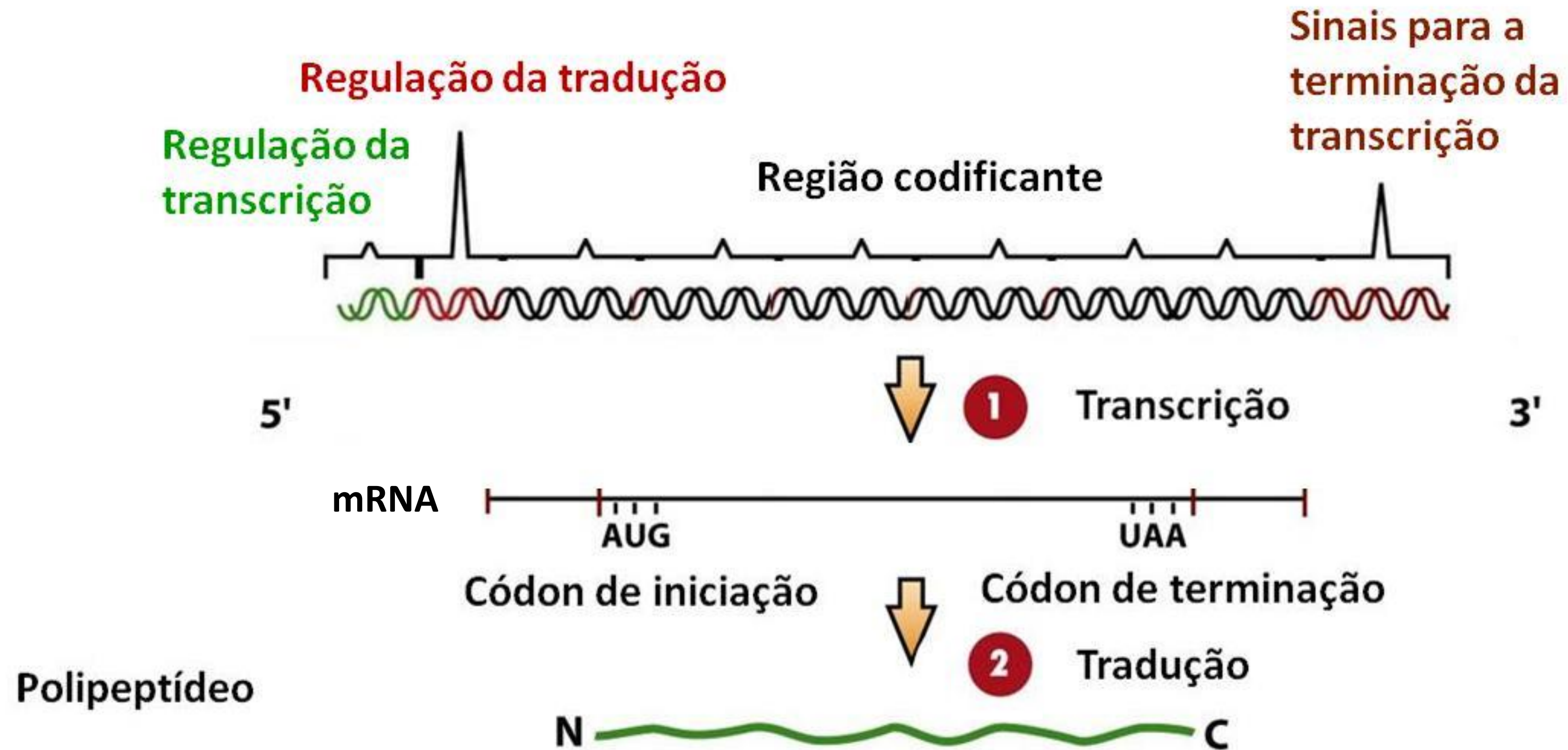
Gene → unidade da informação genética (hereditária) que codifica a síntese de polipeptídeo ou uma molécula de RNA estrutural

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



Gene Típico de Eucariotos

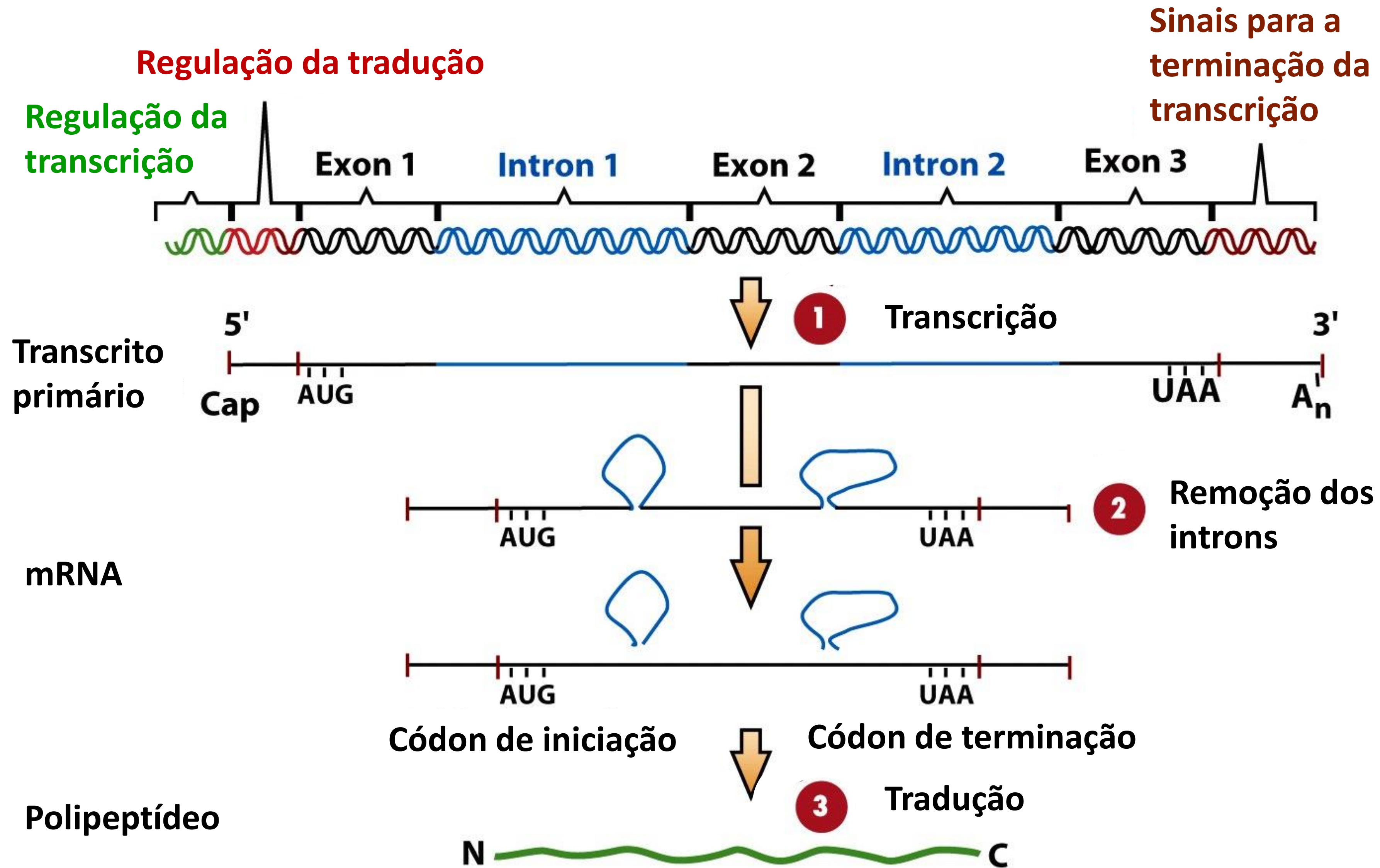
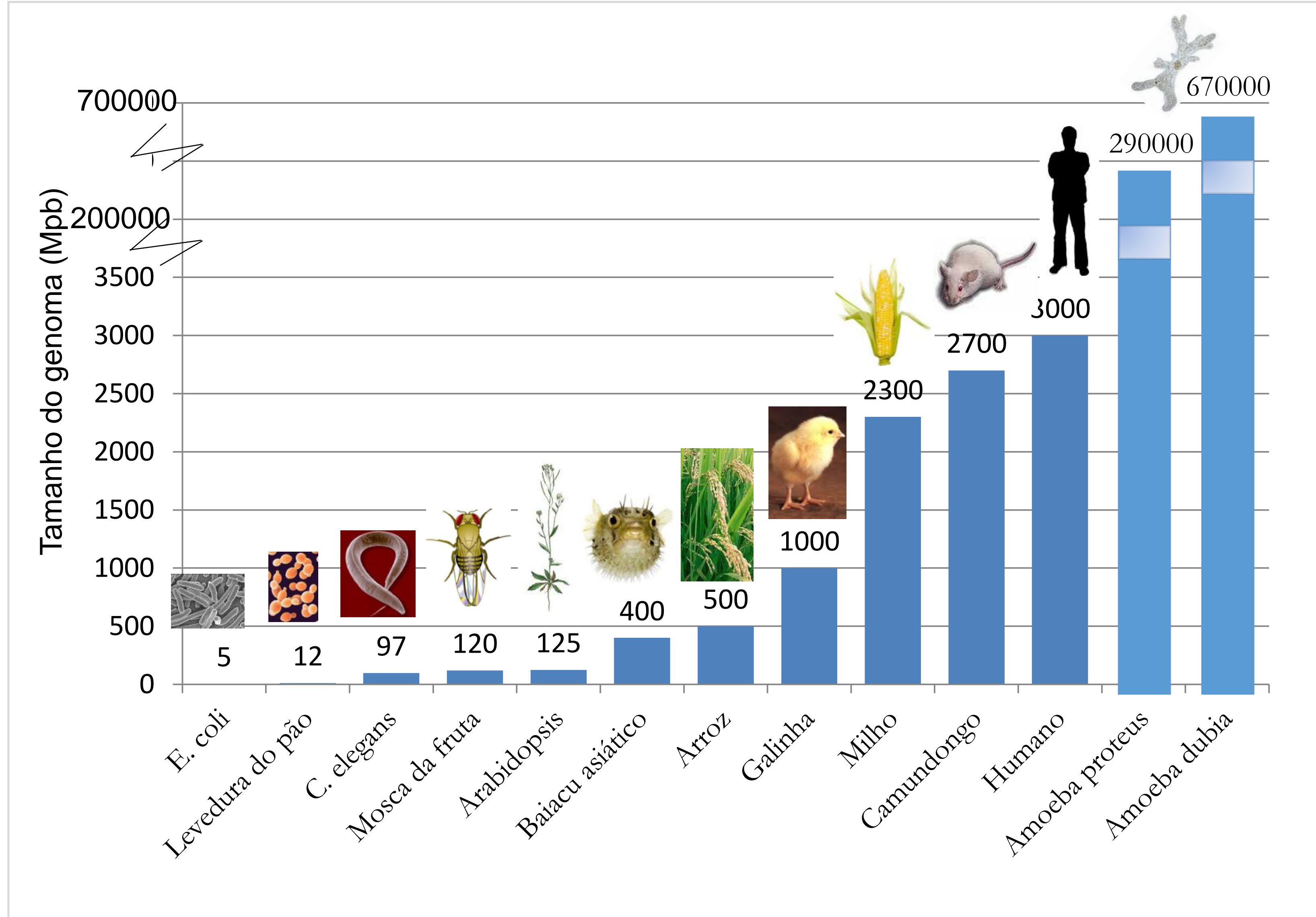


Figure 14-1b Principles of Genetics, 4/e
© 2006 John Wiley & Sons

Número de genes em Eucariotos

Espécies	Genoma (Mb)	Genes
D. melanogaster	165	~12.000
S. cerevisiae	13	~6.000
C. elegans	97	~20.000
H. sapiens	3.300	~30.000

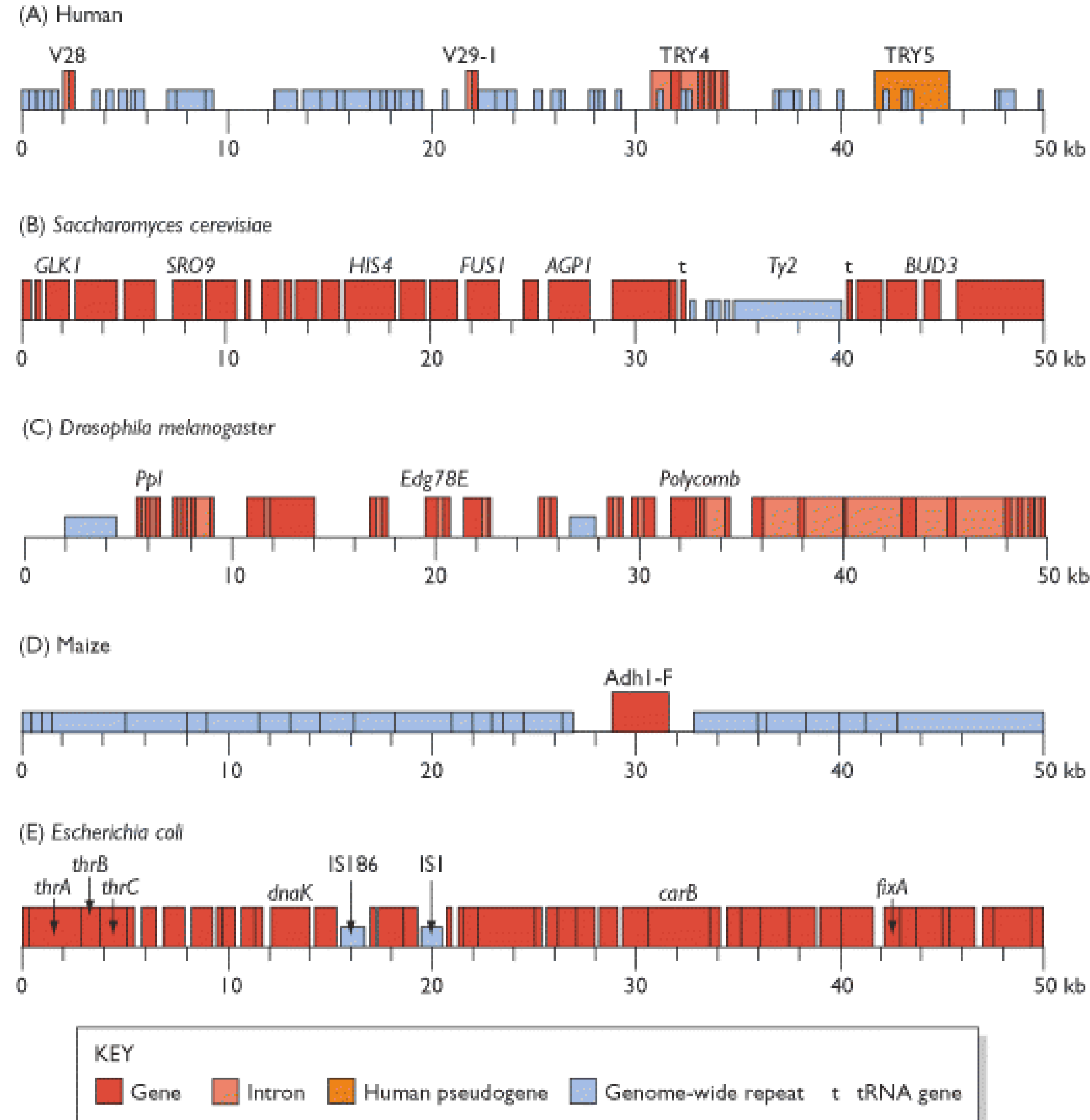
Comparação do Tamanho de Genomas



Paradoxo-C
O que seria?

A complexidade de um organismo não é diretamente proporcional ao tamanho do genoma

Arquitetura de Genomas



50 kpb

T. A. Brown - Genome

Transcrição

DNA ➡ RNA

Do DNA ao RNA - Transcrição

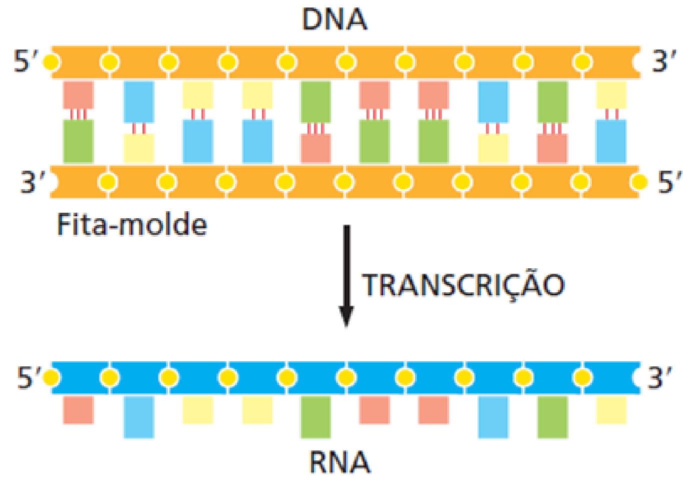


Figura 6-8 A transcrição do DNA produz uma molécula de RNA de fita simples que é complementar a uma das fitas da dupla--hélice de DNA. Observe que a sequência de bases na molécula de RNA produzida é a mesma que a sequência de bases da cadeia de DNA não molde, exceto que uma base U substitui cada base T do DNA.

Controle da Expressão Gênica

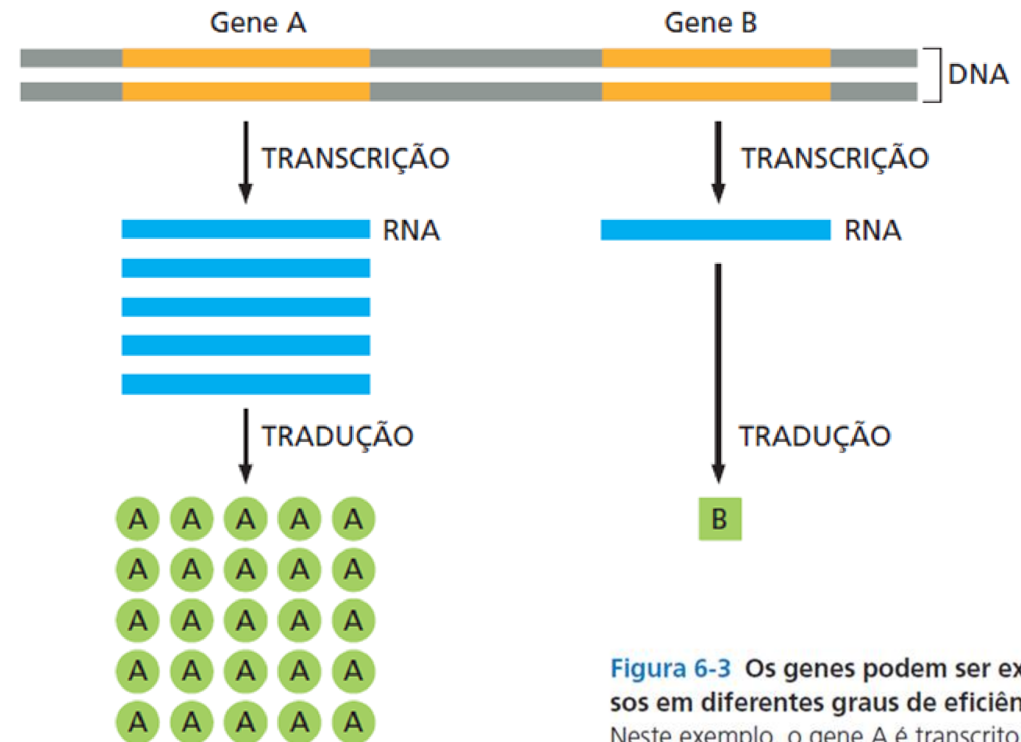


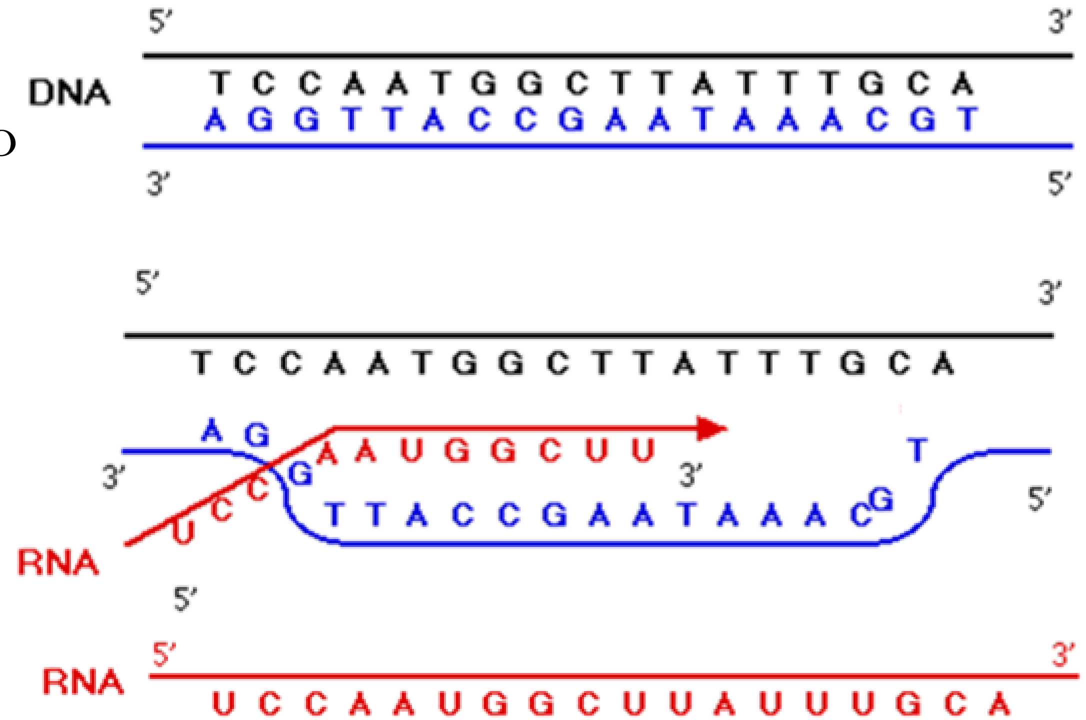
Figura 6-3 Os genes podem ser expressos em diferentes graus de eficiência. Neste exemplo, o gene A é transcrito de maneira mais eficiente do que o gene B e cada molécula de RNA que ele produz também é traduzida mais frequentemente. Isso torna a quantidade da proteína A na célula muito maior do que a quantidade da proteína B.

Alberts et al. 2010 3ª ed.

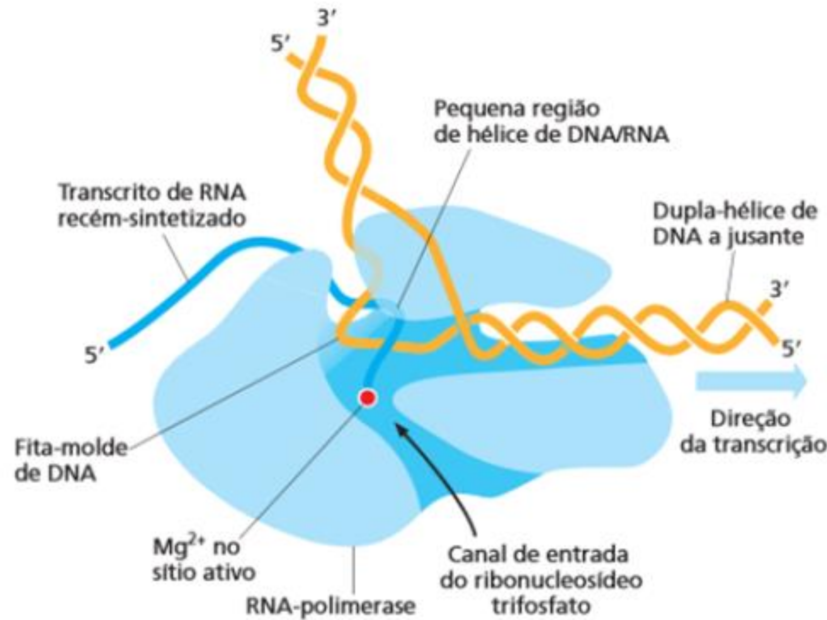
Transcrição

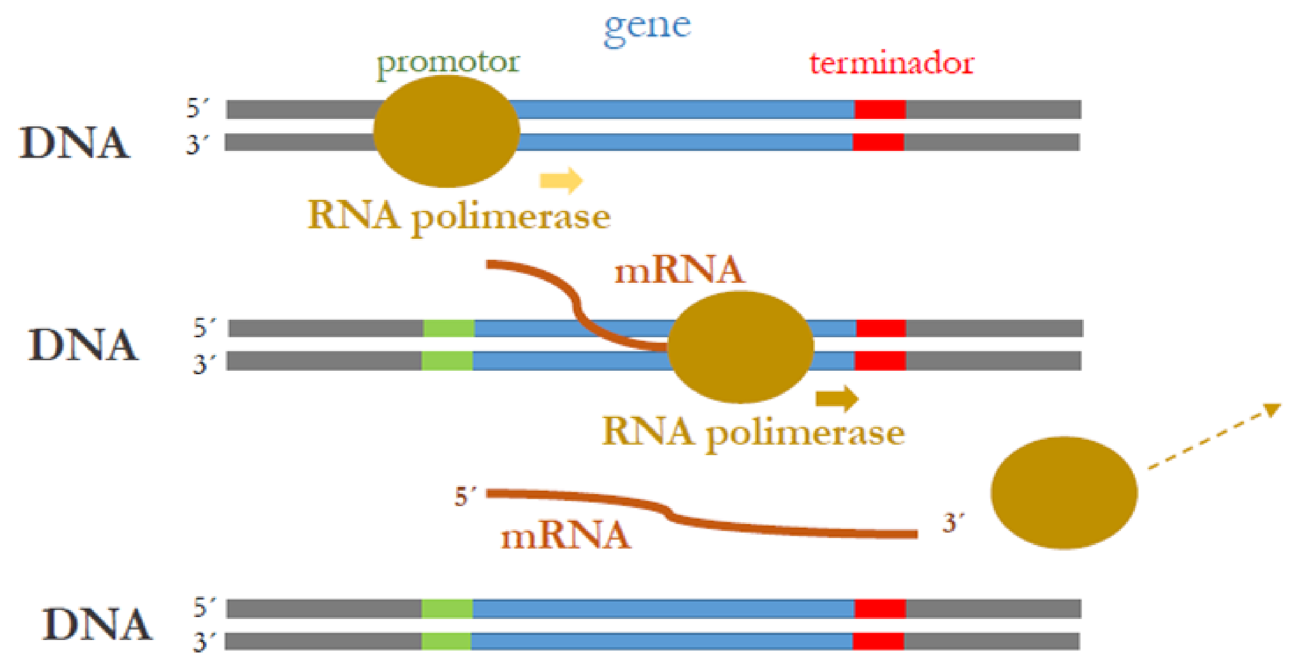
- A informação genética contida num segmento do DNA é reescrita em uma fita simples de RNA

- Uma fita apresenta uma sequência de ribonucleotídeos complementar a uma das fitas da dupla hélice de DNA (**molde**) e idêntica à sequência da outra fita (**codificadora**), com substituição de T por U

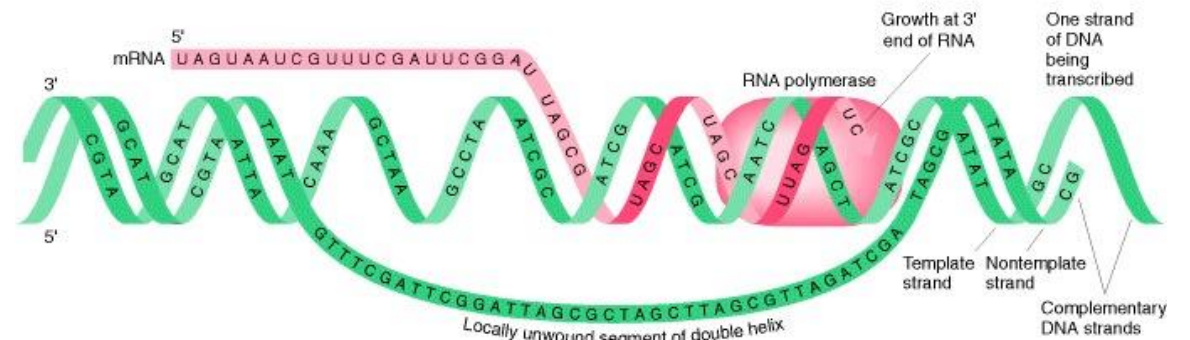


Transcrição





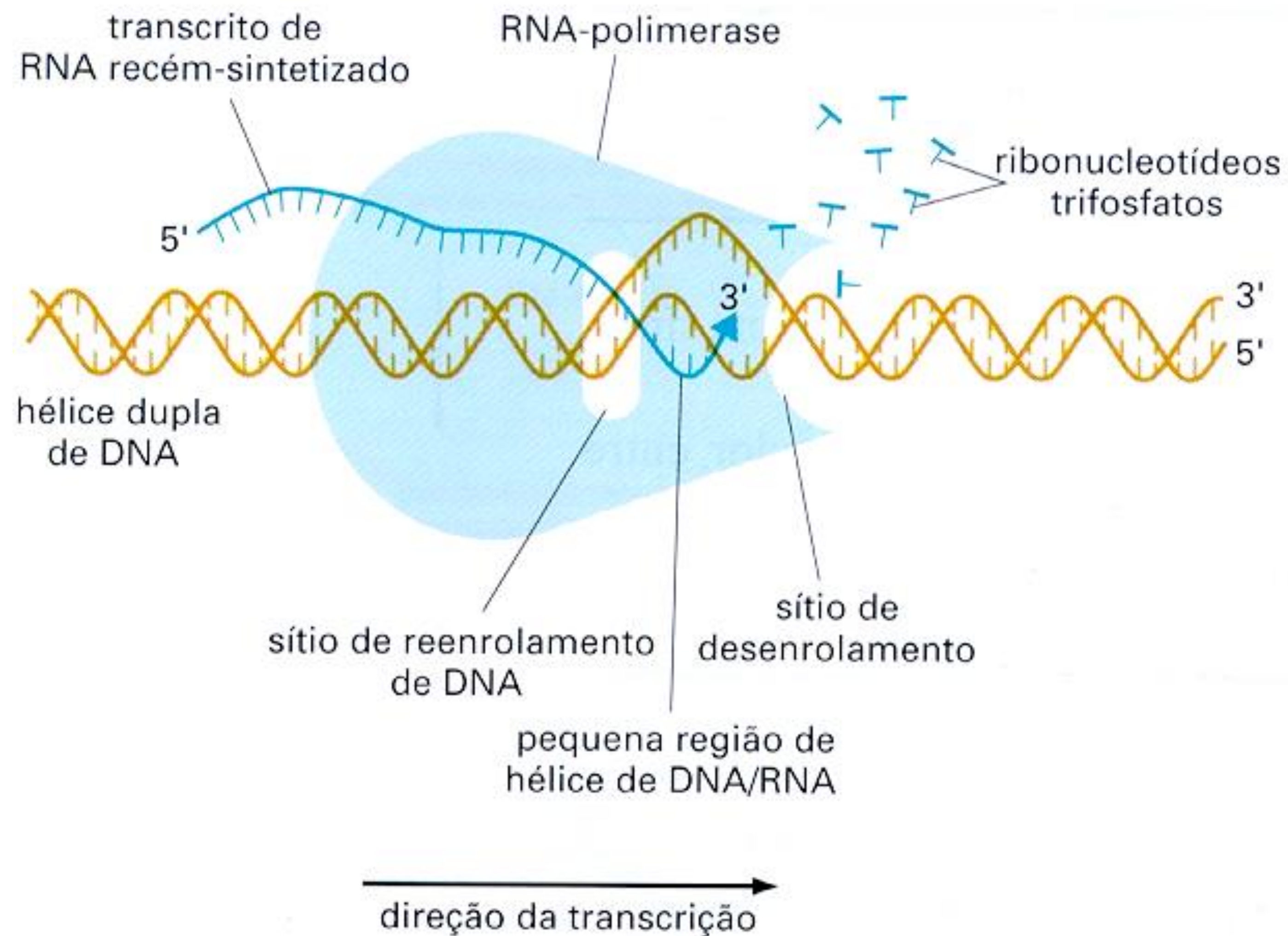
RNA Polimerase



Animação sobre Transcrição

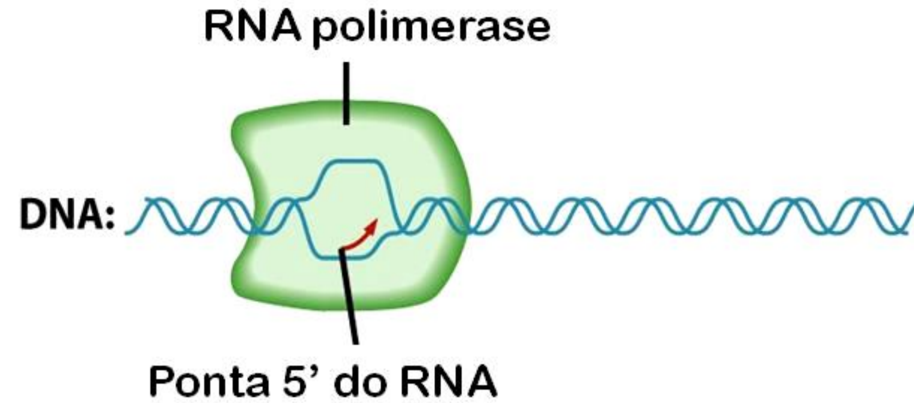
Alberts et al. 2010 3ª ed.

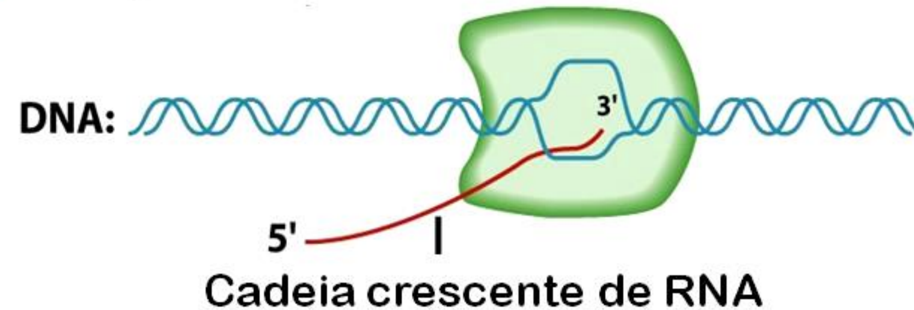
Enzima RNA Polimerase

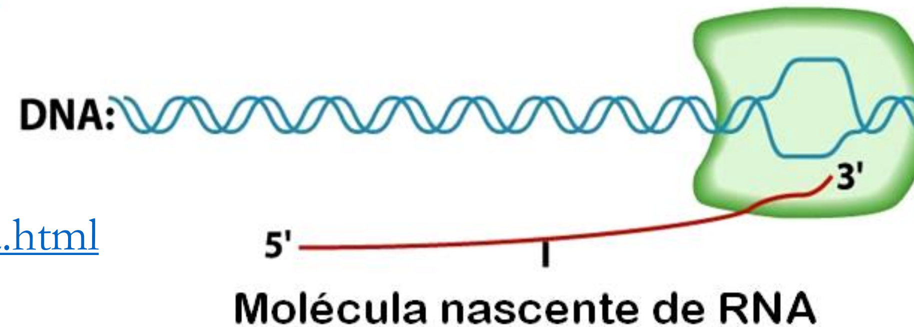


Alberts et al. (1999)

Etapa da Transcrição

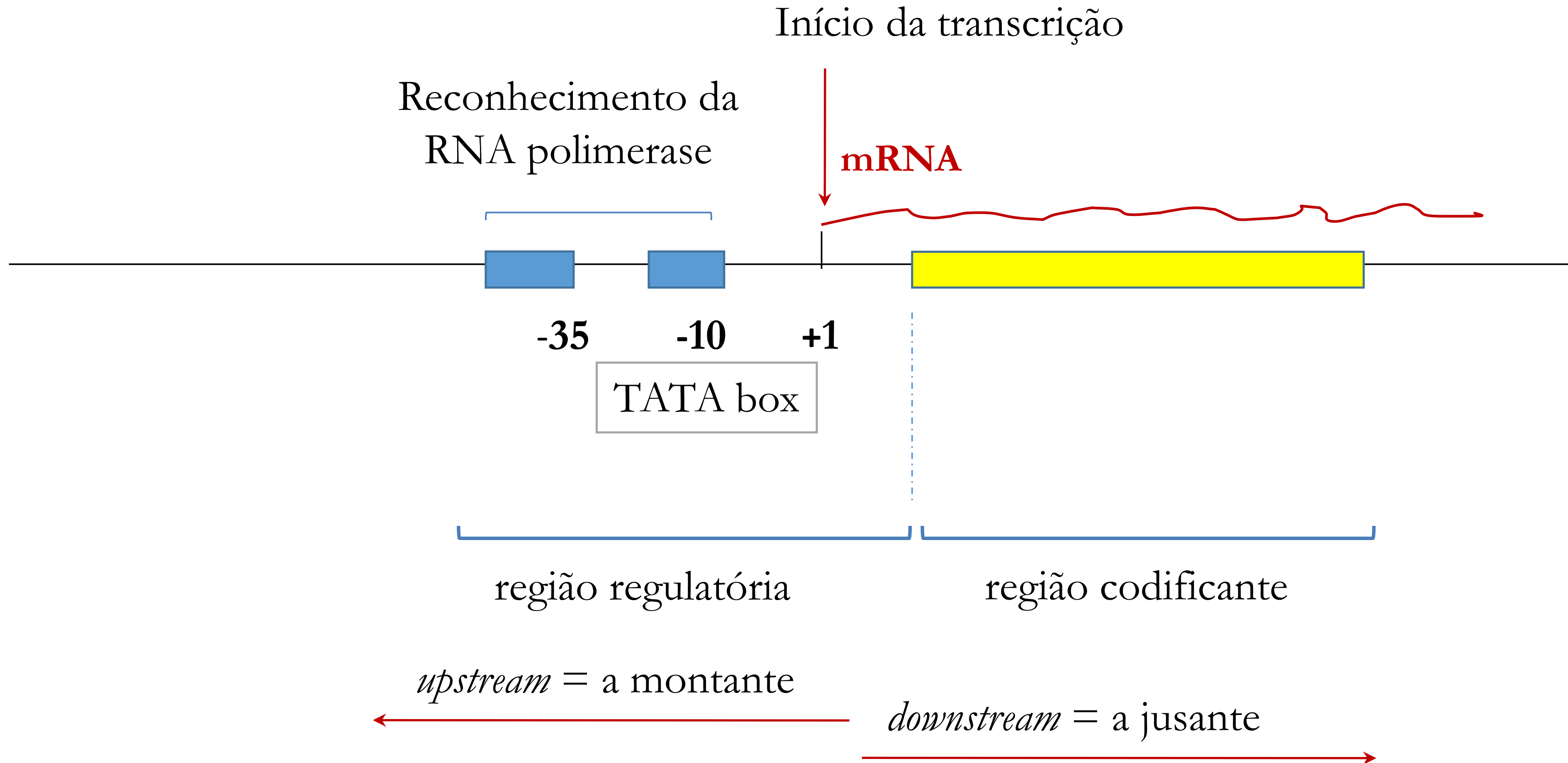




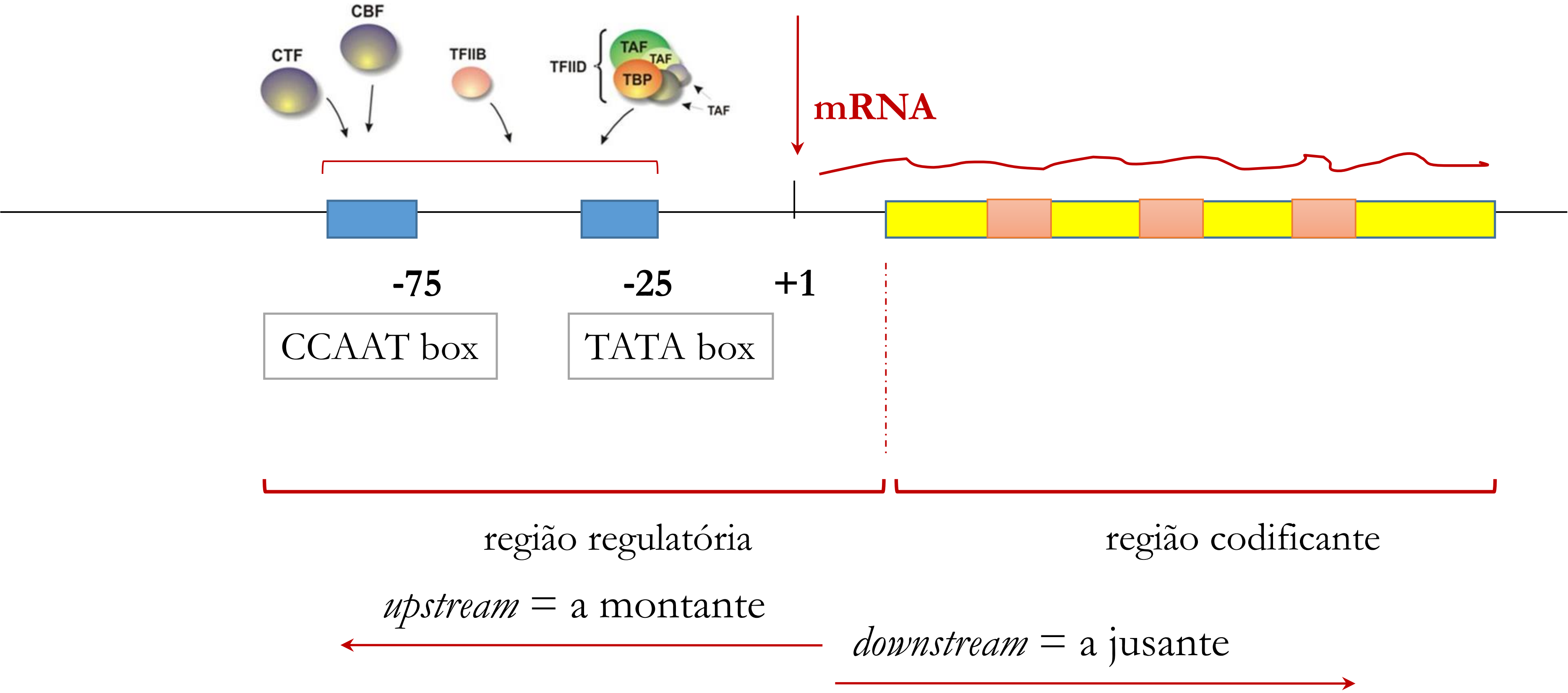


https://www.dnalc.org/resources/3d/central-dogma.html

Região Promotora de Gene Procarioto



Região Promotora de Gene Eucarioto



Características Gerais da Síntese de RNA

1. Os precursores são ribonucleotídeos
2. Apenas 1 fita de DNA é utilizada como molde para a síntese de RNA complementar
3. As cadeias de RNA são sintetizadas **sem** a necessidade de um filamento *primer* preexistente (atuação da RNA polimerase)
4. Síntese é complementar ao DNA, mas A → U
5. Polimerização sentido 5' → 3'
6. **RNA polimerase inicia a transcrição em sequências específicas de nucleotídeos → promotores (regiões regulatórias)**
7. **RNA polimerase termina a transcrição em sequências específicas de nucleotídeos → terminadores (finalizadores)**

Término da Transcrição

✓ o término das cadeias de RNA ocorre quanto a RNA polimerase encontra um sinal de término, e quando isso ocorre o complexo é liberado

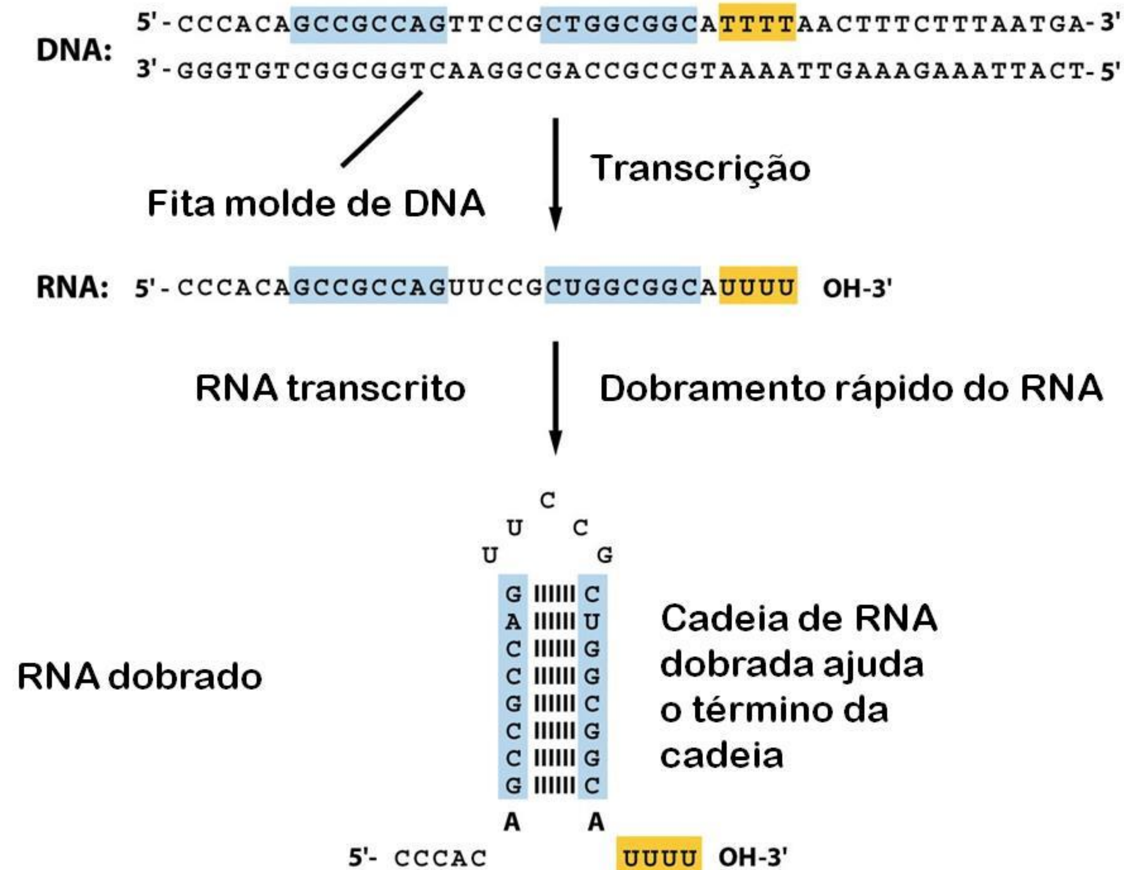
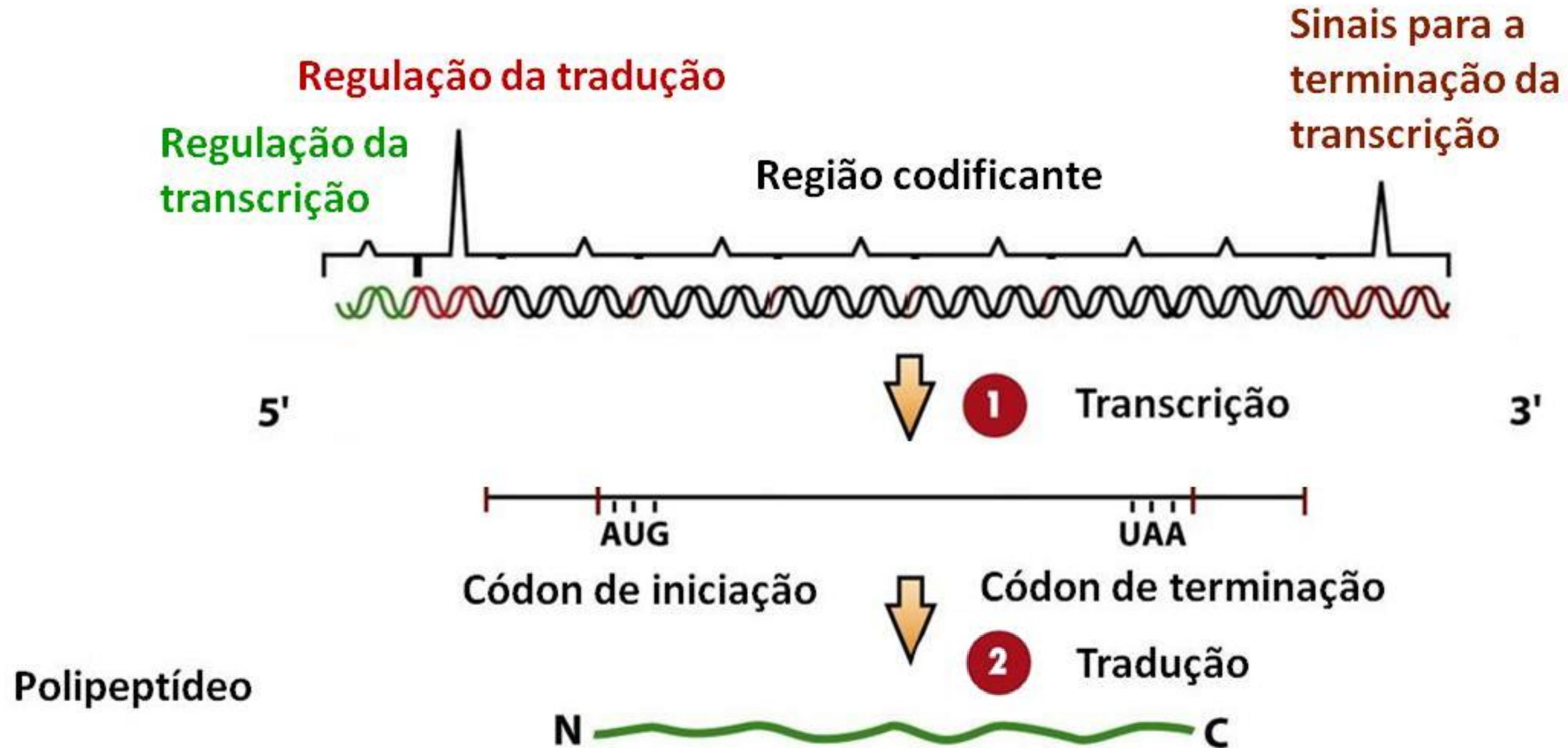
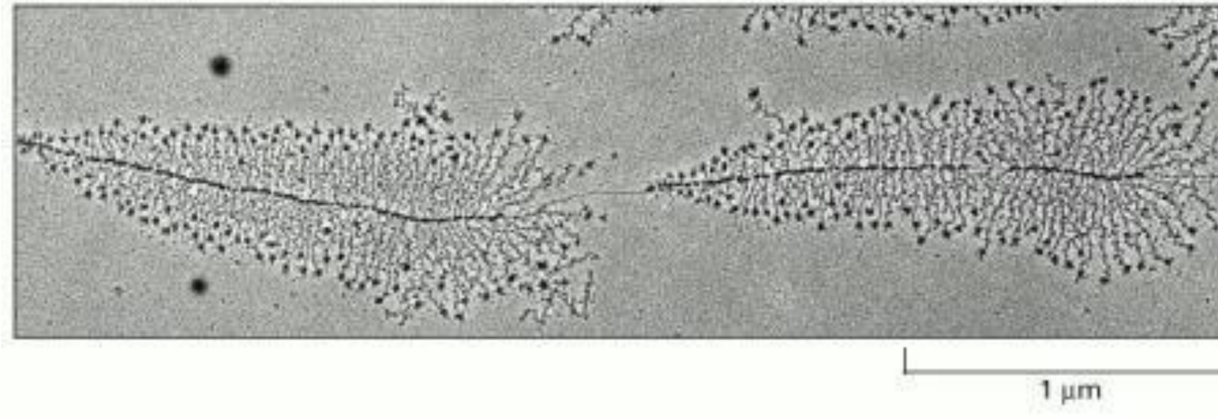


Figure 11-13 Principles of Genetics, 4/e
© 2006 John Wiley & Sons

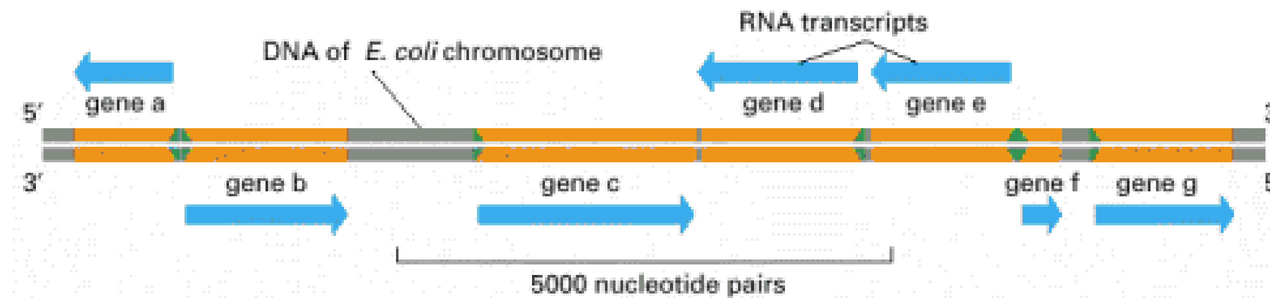
Gene de Procarioto



Transcrição simultânea múltipla - Procariotos

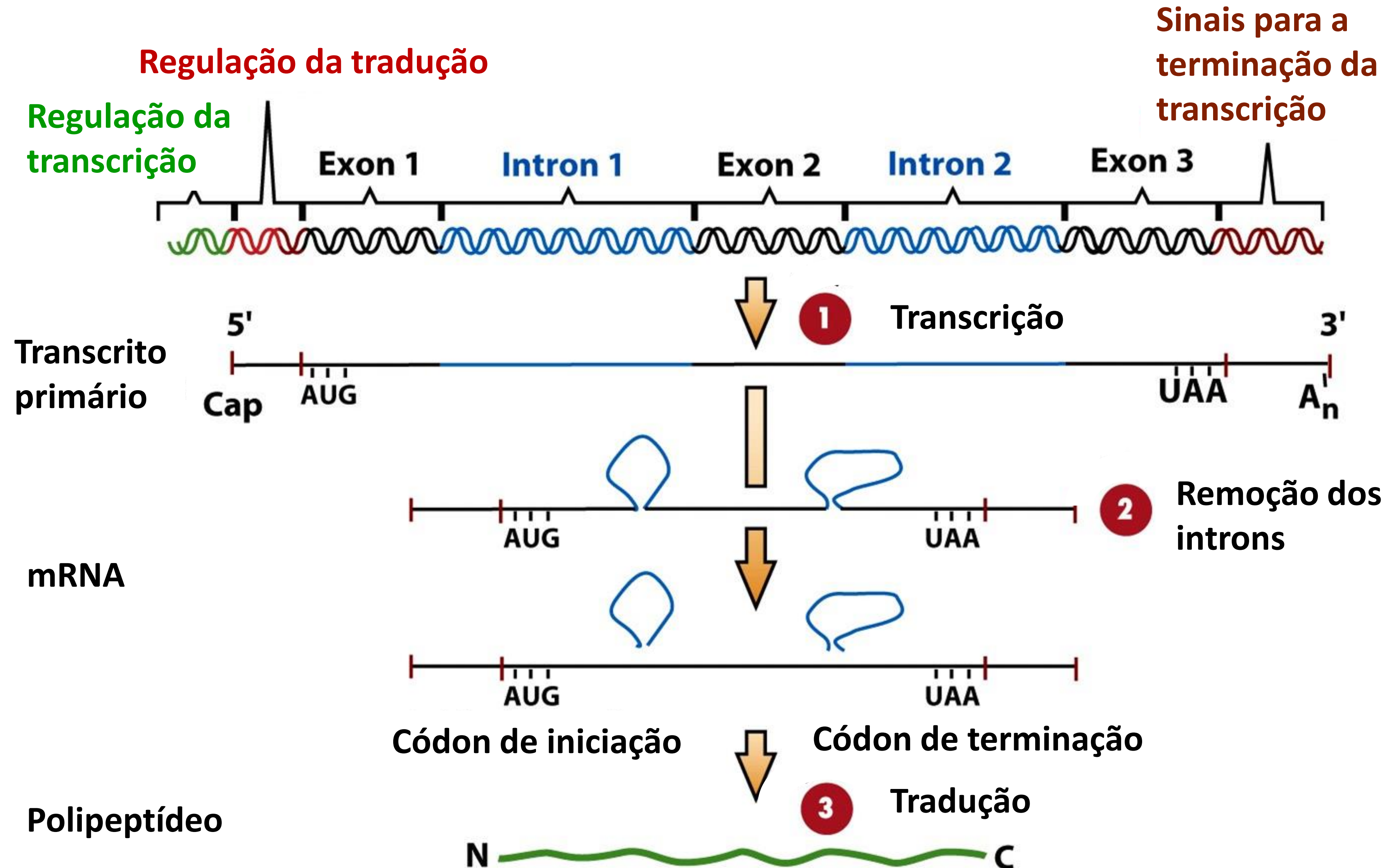


Direção de transcritos em Procariotos

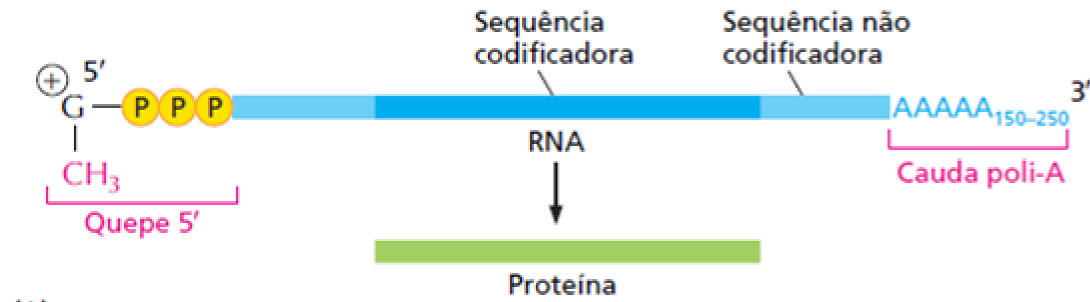


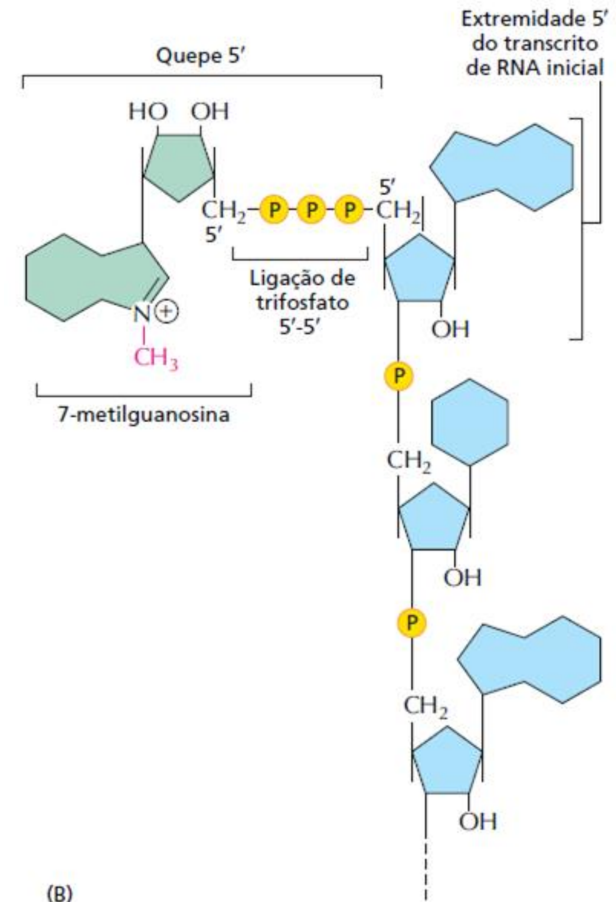
Alberts et al. 2010 3ª ed.

Gene de Eucarioto

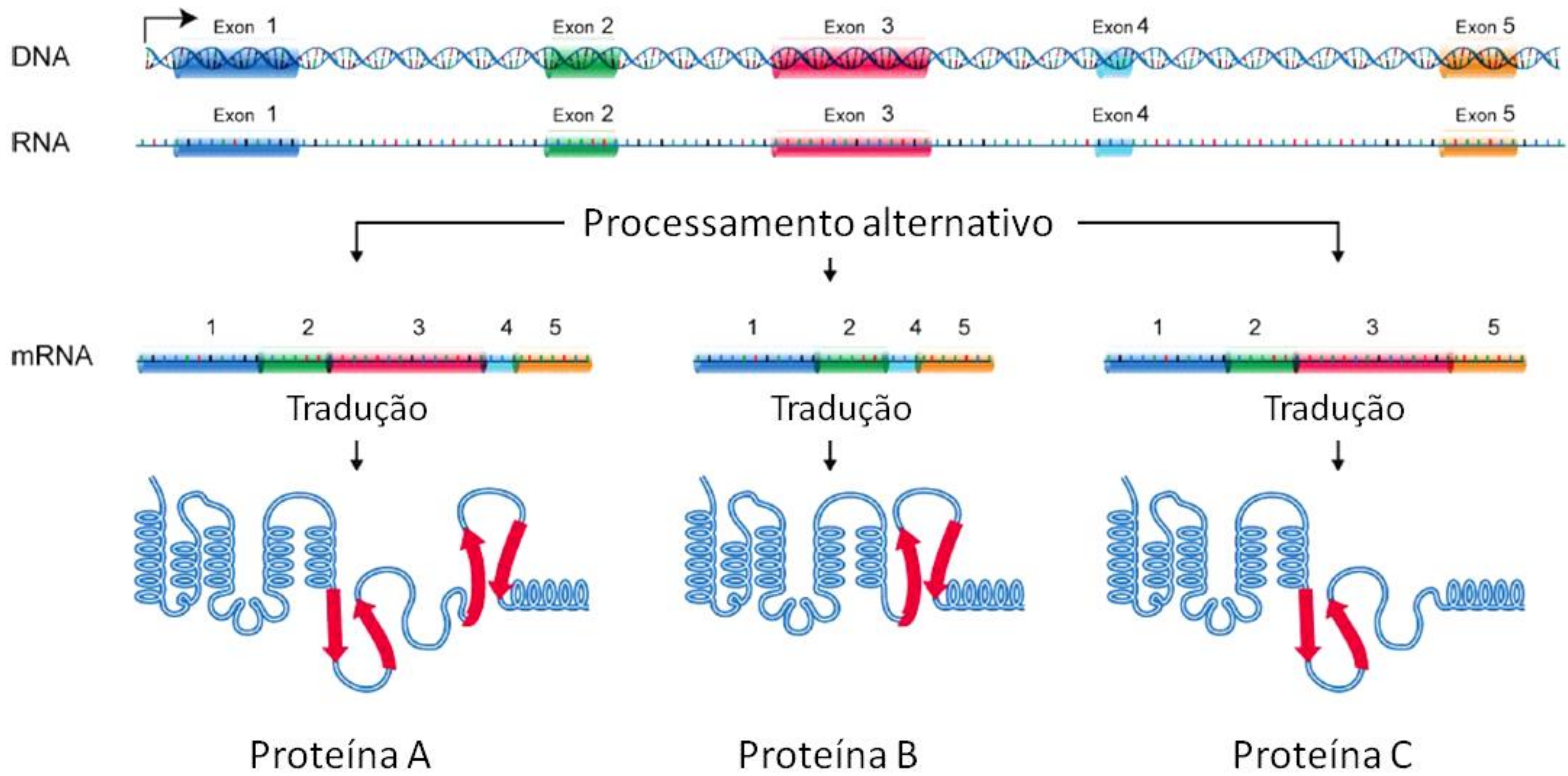


Gene de Eucarioto



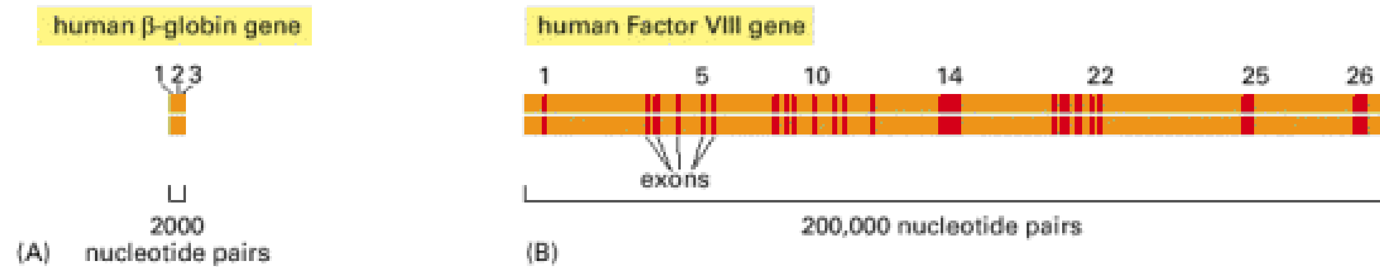


Splicing Alternativo gerando diversas Proteínas

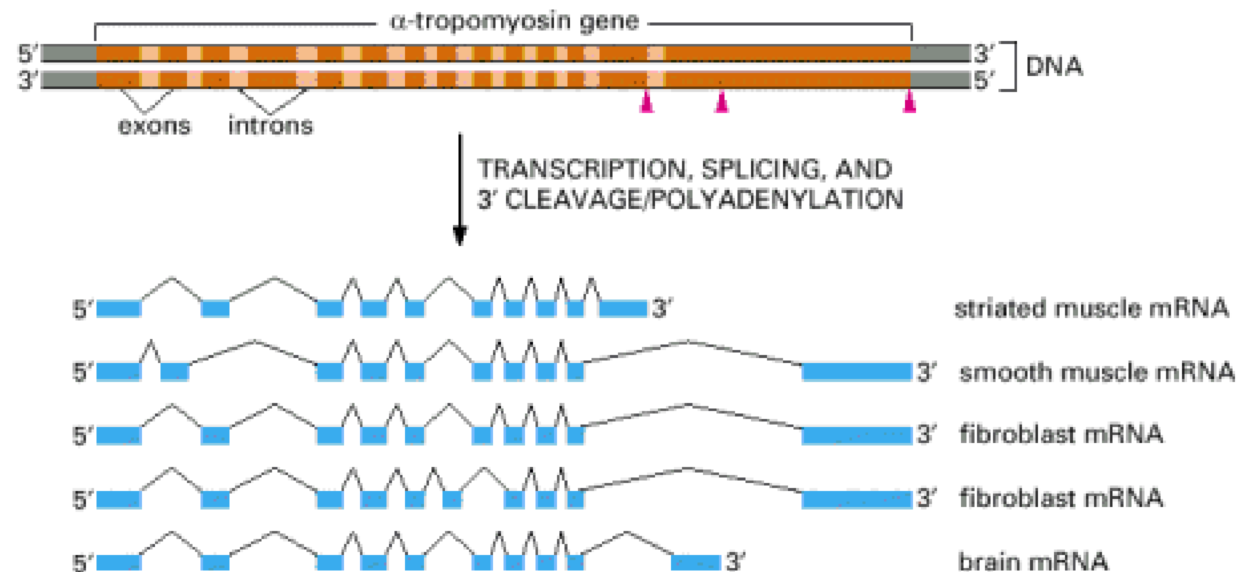


Eucariotos

Estrutura de genes humanos com arranjos de exons e introns

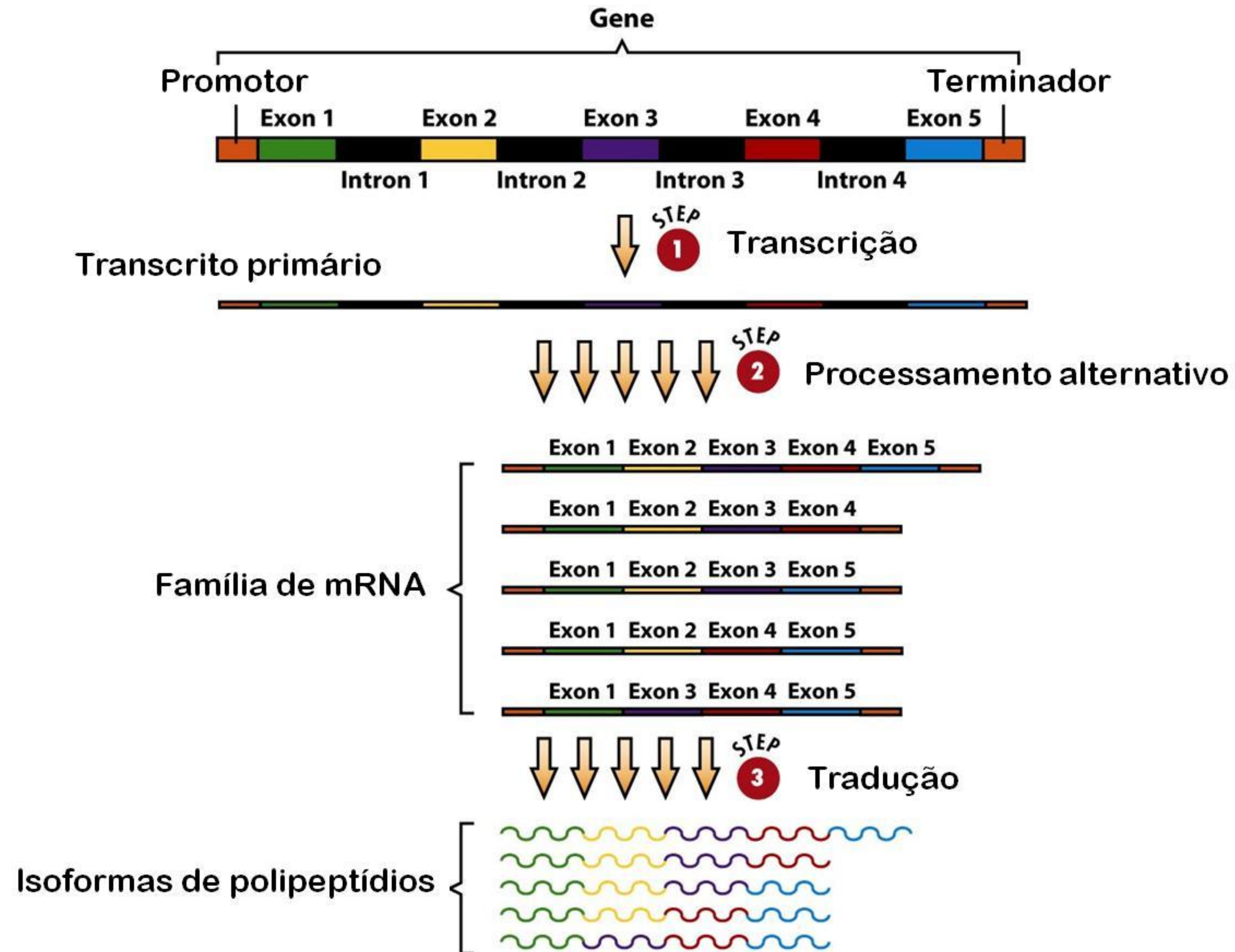


Splicing alternativo do gene de α-tropomiosina de rato

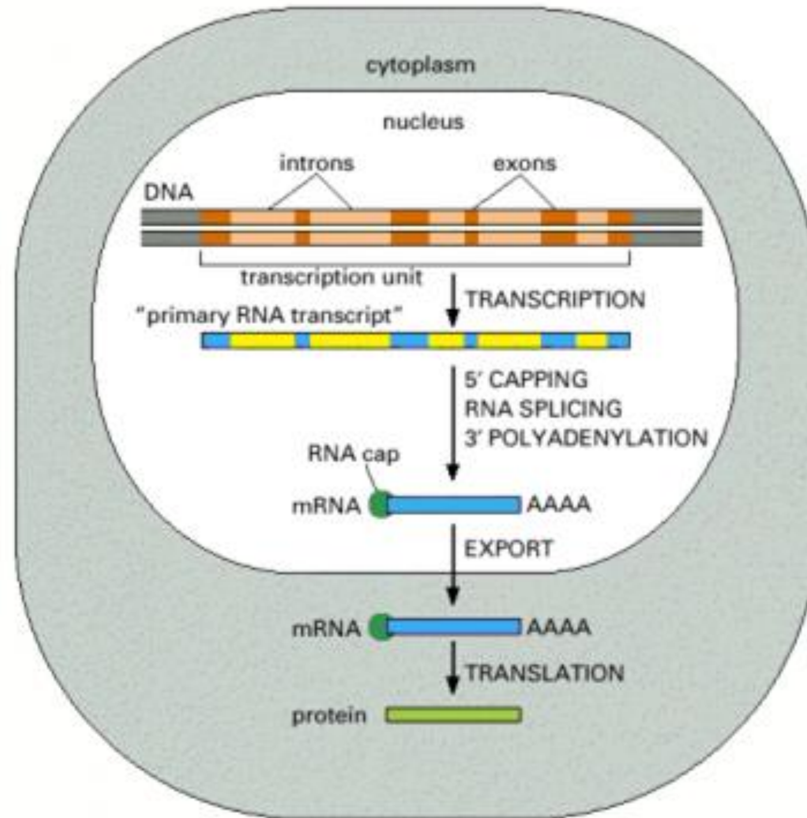


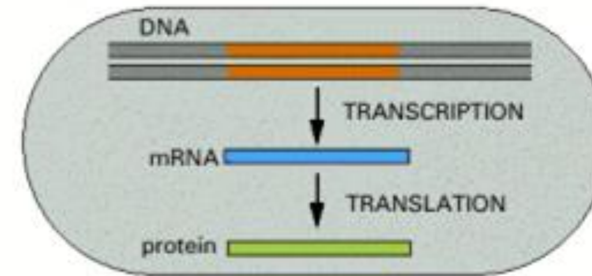
Alberts et al. 2010 3ª ed.

Isoformas de Proteínas



Etapas características da transcrição





Procariotos

Animação Splicing

Eucariotos

Alberts et al. 2010 3ª ed.

Tradução
RNA ➡ Proteína

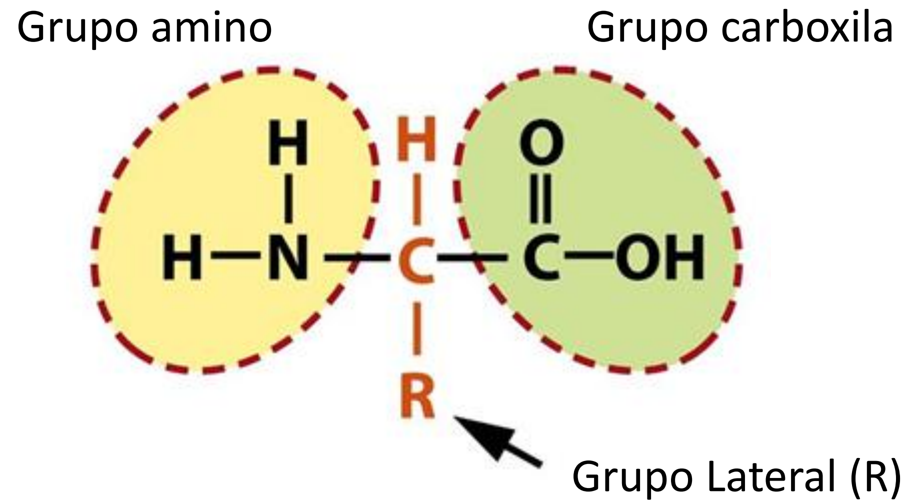
Características Gerais da Tradução

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

Estrutura da Proteína

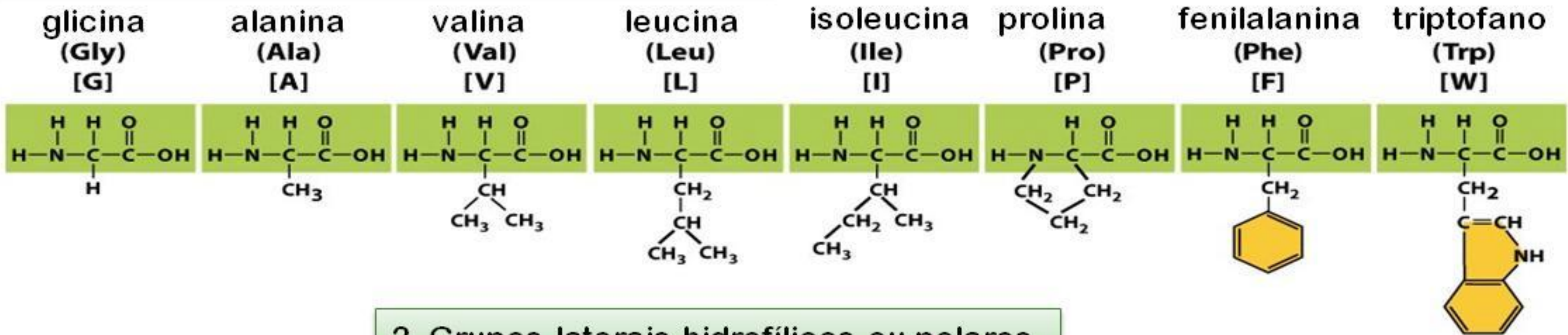
Aminoácidos (20 tipos):

→ grupo amino
→ grupo carboxila
→ grupo lateral

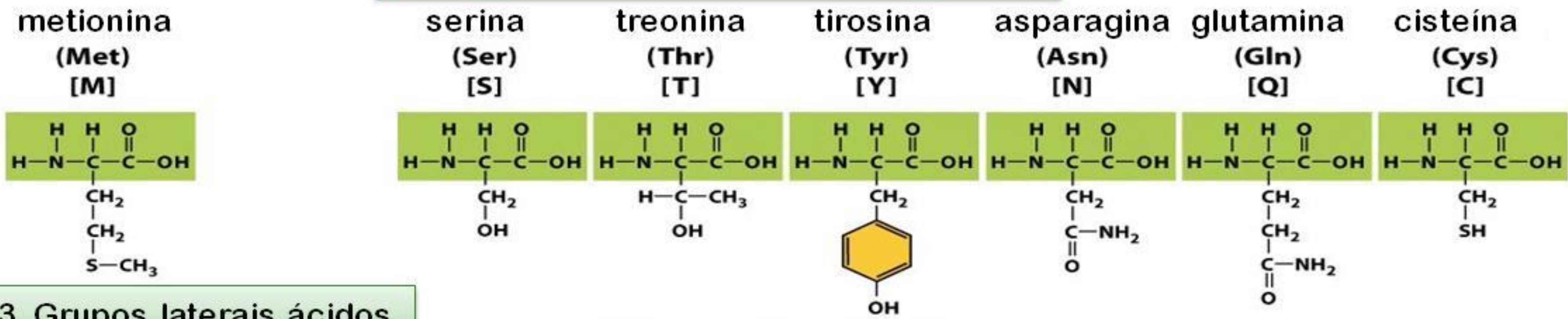


Unnumbered figure pg 312 Principles of Genetics, 4/e
© 2006 John Wiley & Sons

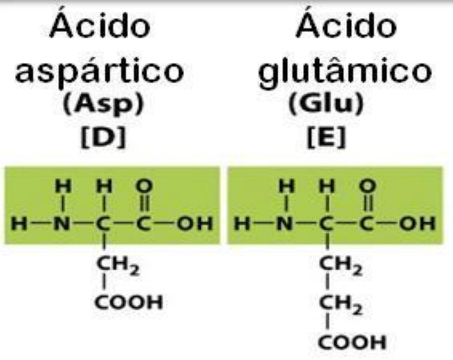
1. Grupos laterais hidrofóbicos ou não polares



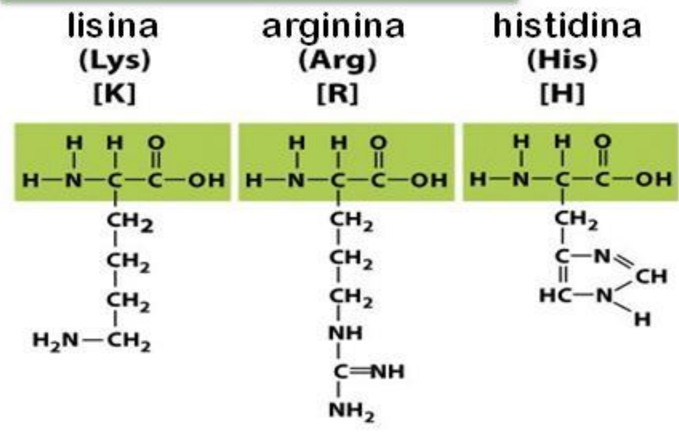
2. Grupos laterais hidrofílicos ou polares



3. Grupos laterais ácidos



4. Grupos laterais básicos



Ligação Peptídica

Ligação peptídica

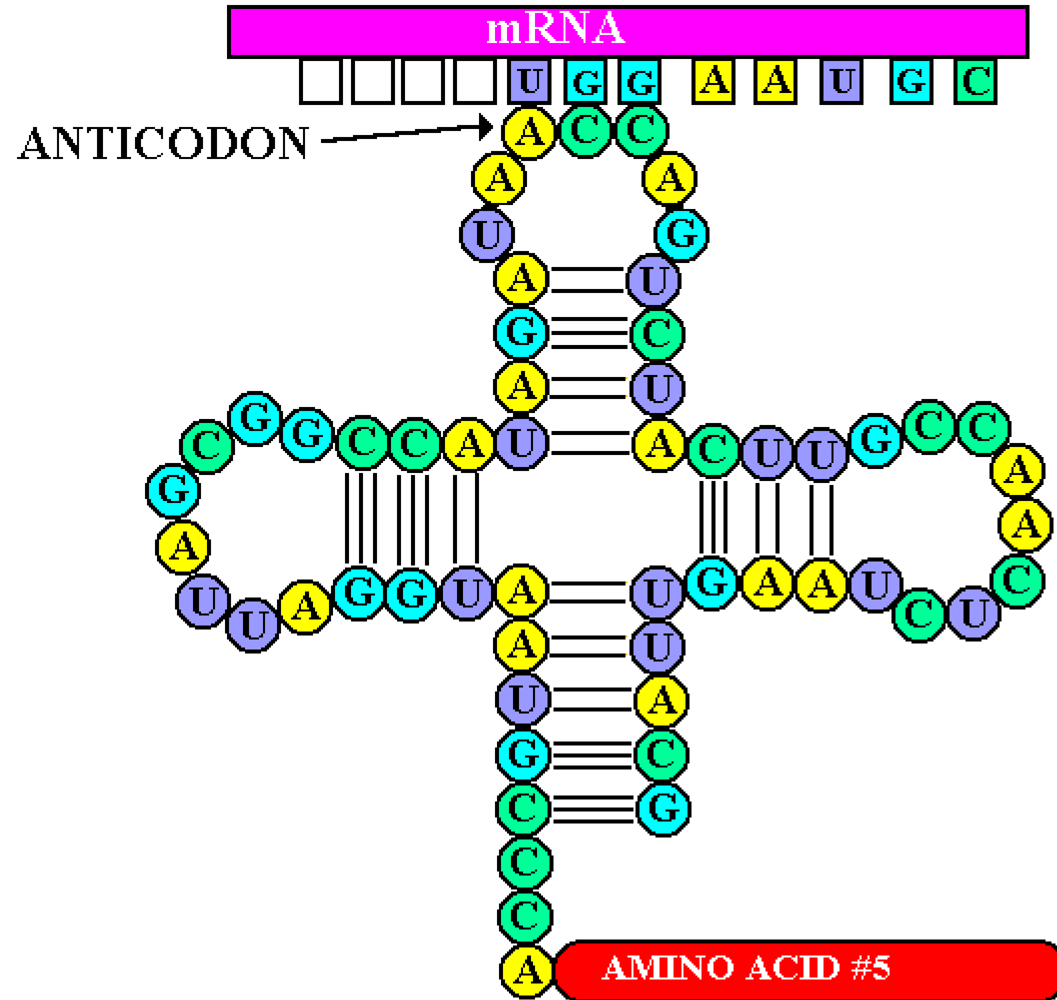
$$H_2N{-}C(H)(R_1){-}C(=O){-}OH + H{-}N(H){-}C(H)(R_2){-}C(=O){-}OH \rightarrow H_2N{-}C(H)(R_1){-}C(=O){-}N(H){-}C(H)(R_2){-}C(=O){-}OH + H_2O$$

Aminoácido 1 | Aminoácido 2 | Dipeptídeo

N — C

Proteína - direção de síntese

Codon e Anticodon



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	
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

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

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

5′ 3′

1 CUC AGC GUU ACC AU

—Leu—Ser—Val—Thr—

2 C UCA GCG UUA CCA U

— Ser—Ala—Leu—Pro—

3 CU CAG CGU UAC CAU

— Gln—Arg—Tyr—His—

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

Frameshift!

QUADRO DE LEITURA

Start codon e Stop codon

Início: códon de iniciação da síntese protéica

– AUG -

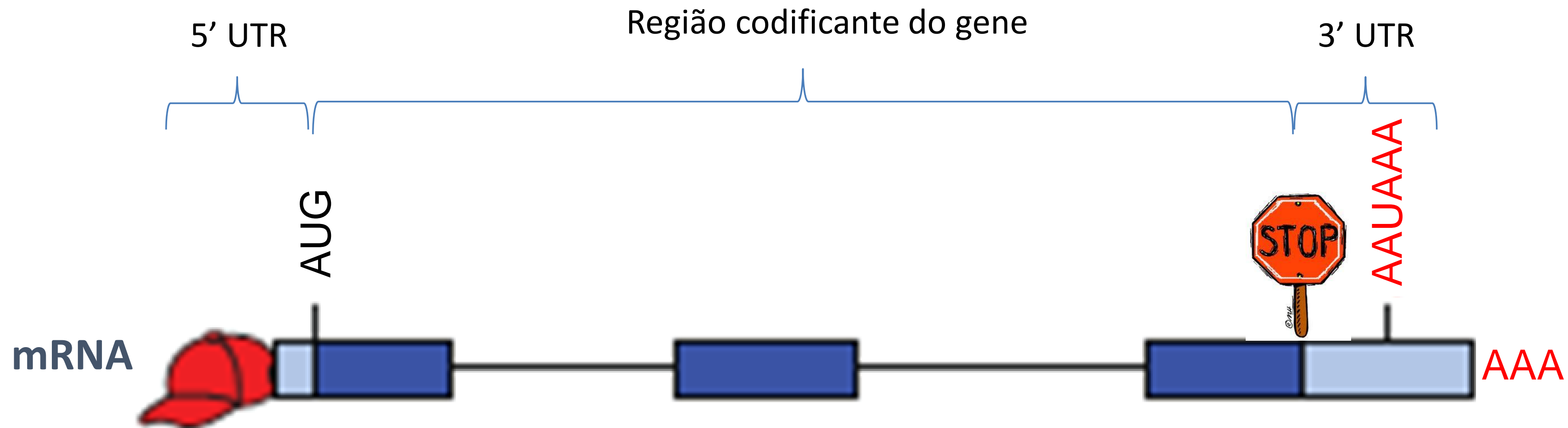
METIONINA

Terminação: três códons terminam a síntese protéica

– UAG – UAA – UGA -

Start codon e Stop codon

❖ Delimitam a região codificante (região que é transcrita e traduzida!)

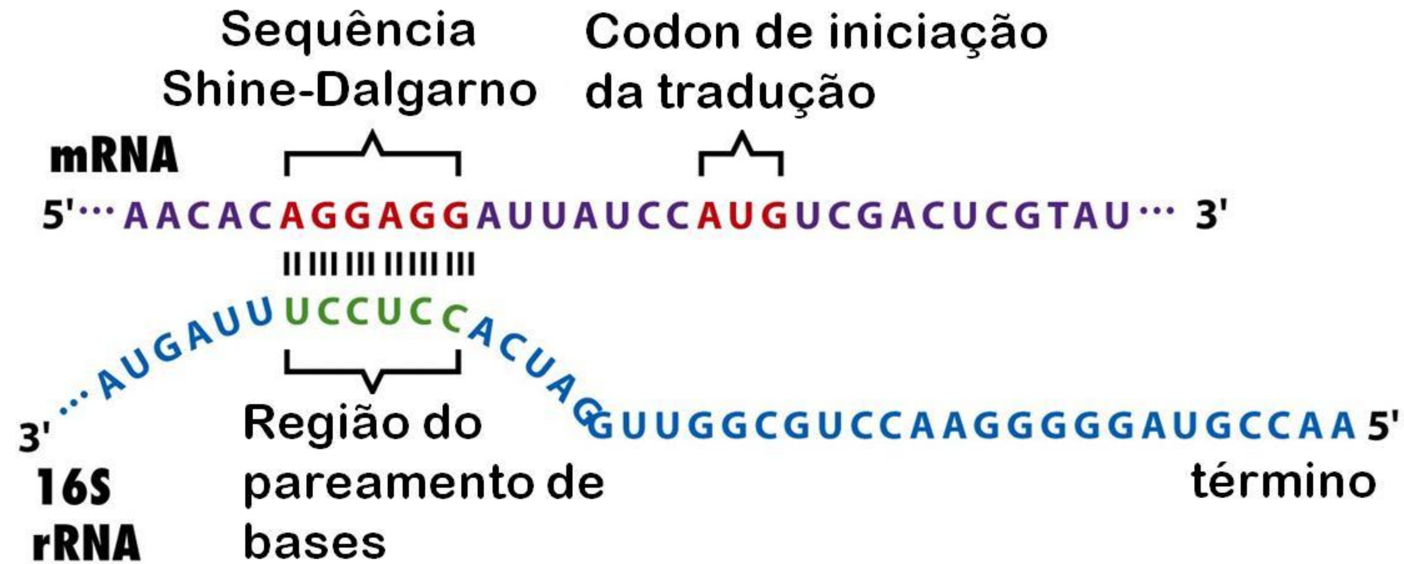


Tradução: Início e Fim

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

Sinais para o início da Tradução

PROCARIOTO



EUCARIOTO

(Sequência de Kozak)

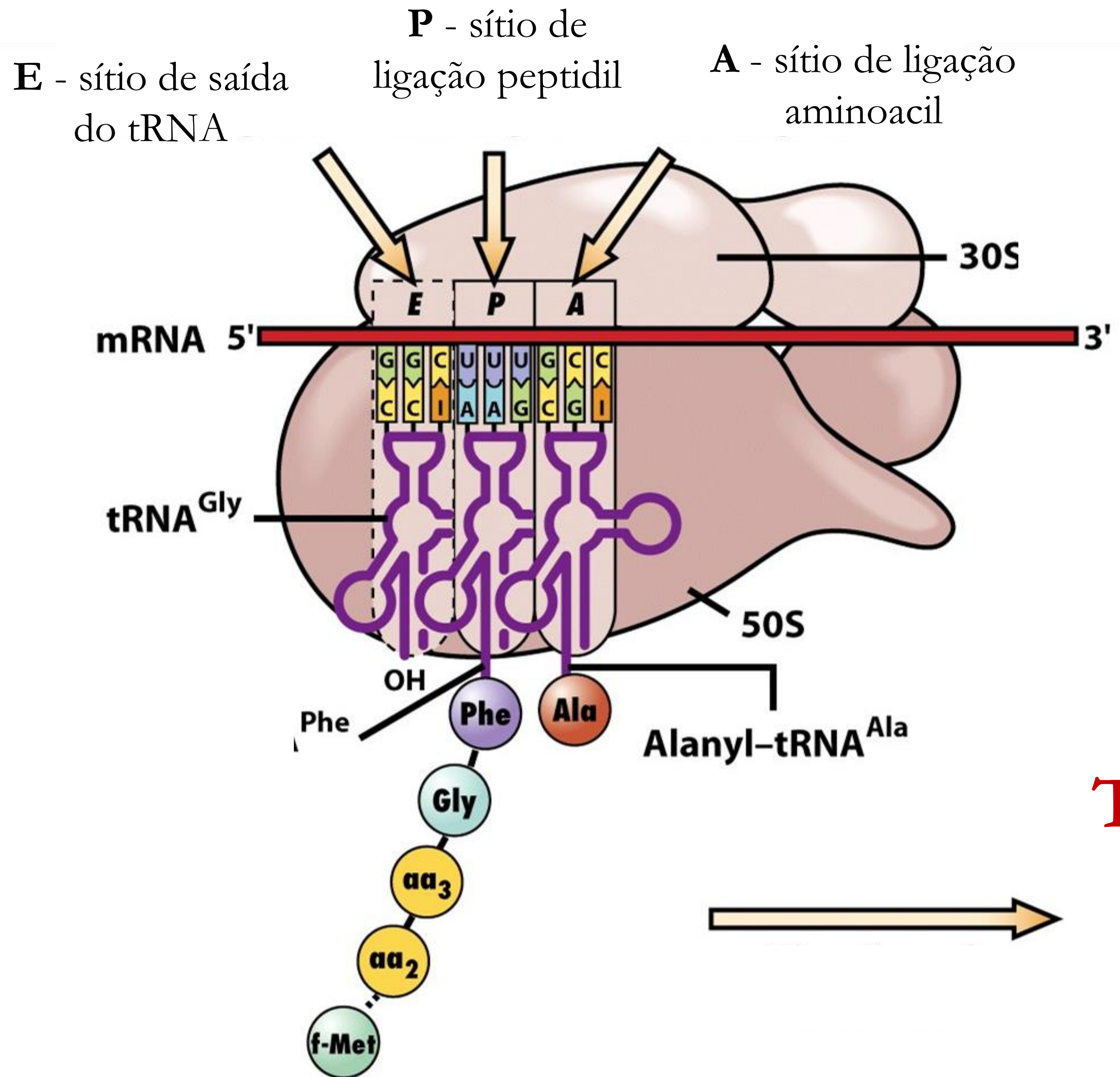
5´ - GCC (A ou G) CC **AUG**G - 3`

Sinal para o início da tradução em eucariotos

mRNA

CAP5′
Região definitivamente traduzida
Códon de Término
3'ʚRNNNNNNGCCRCCAUGGNNNNNNNNNNNNNNNNNNNNNNNUGANNNNNNNNNAAAAAAAAAAA3'
Seqüência de Kozak
Códon de Iniciação
Cauda Poli-A
5′UTR
3′UTR

Regiões UTR = regiões não-traduzidas (***UnTranslated Region***)
Regiões anteriores e posteriores a codificadora
5´ UTR
3´UTR



Tradução

Figure 12-14a Principles of Genetics, 4/e
© 2006 John Wiley & Sons

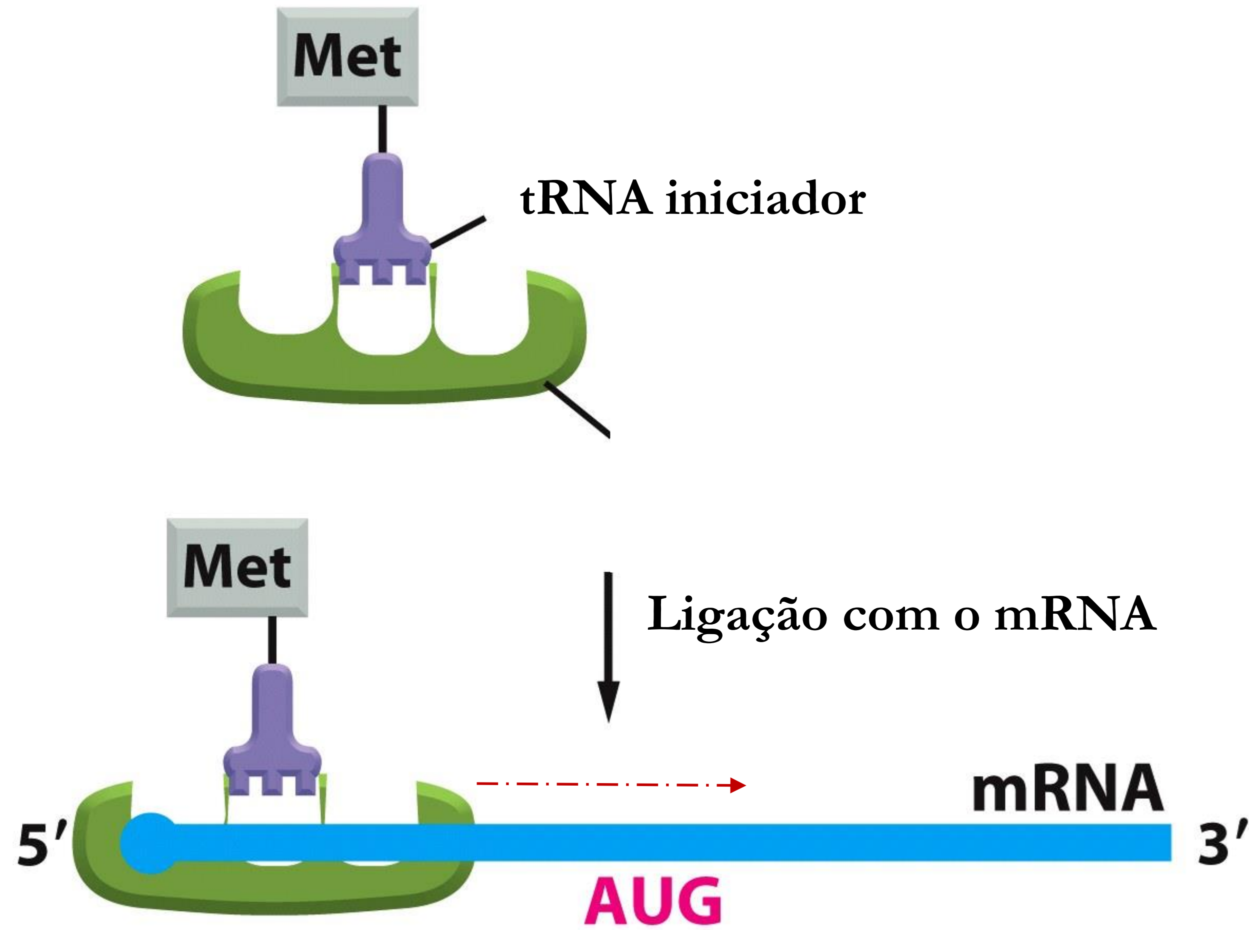


Figure 7-35 part 1 of 5 Essential Cell Biology 3/e (© Garland Science 2010)

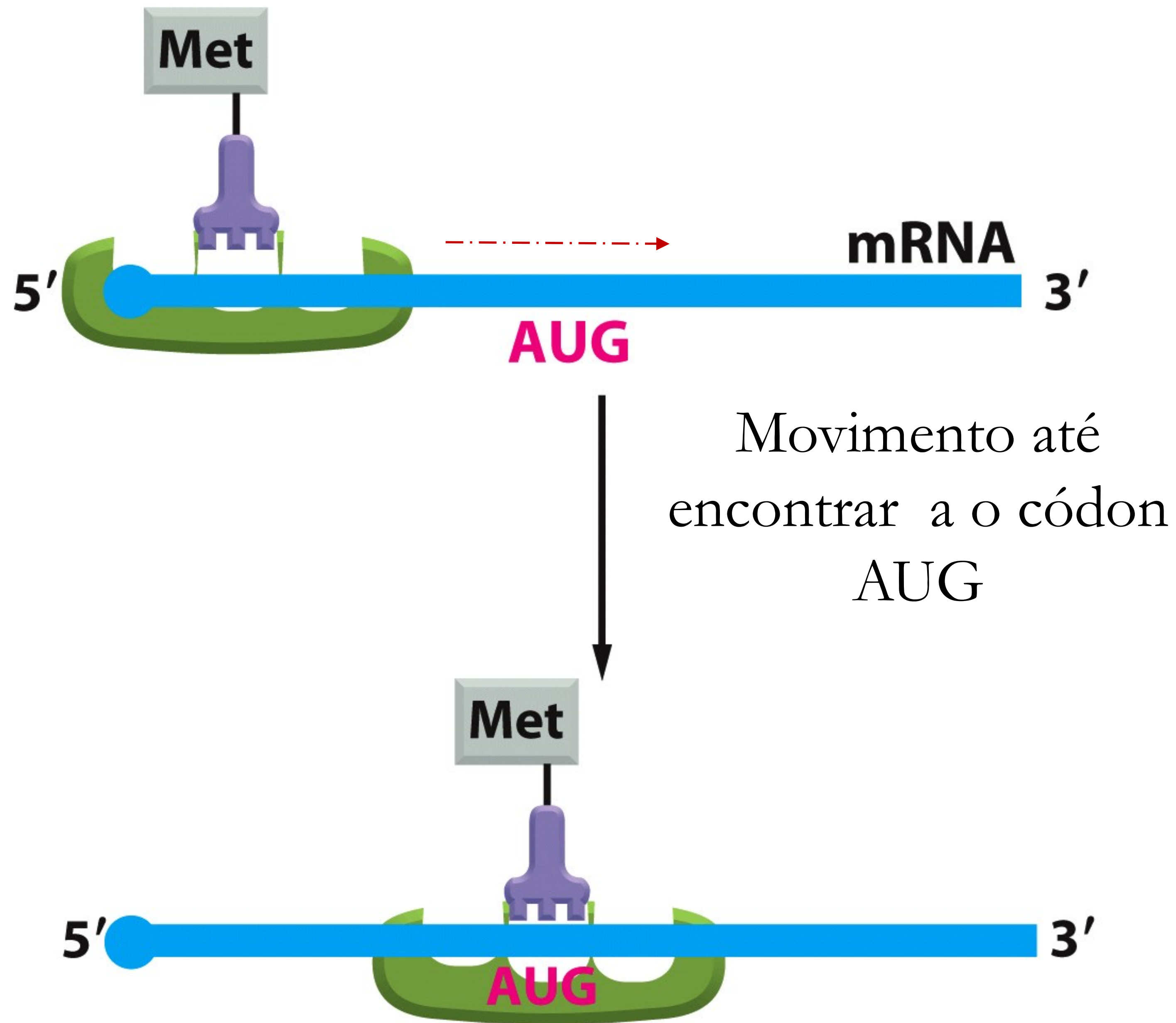


Figure 7-35 part 2 of 5 Essential Cell Biology 3/e (© Garland Science 2010)

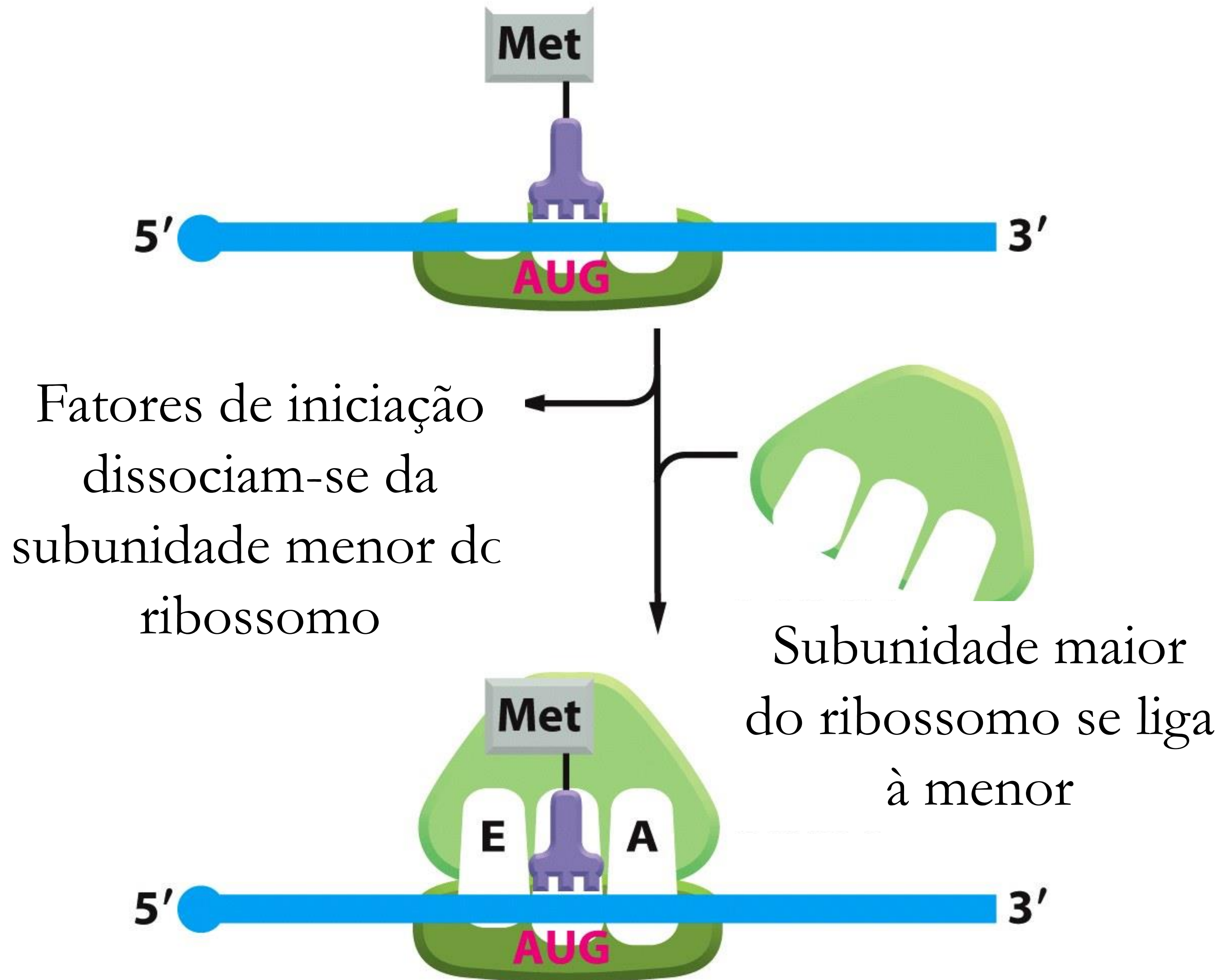
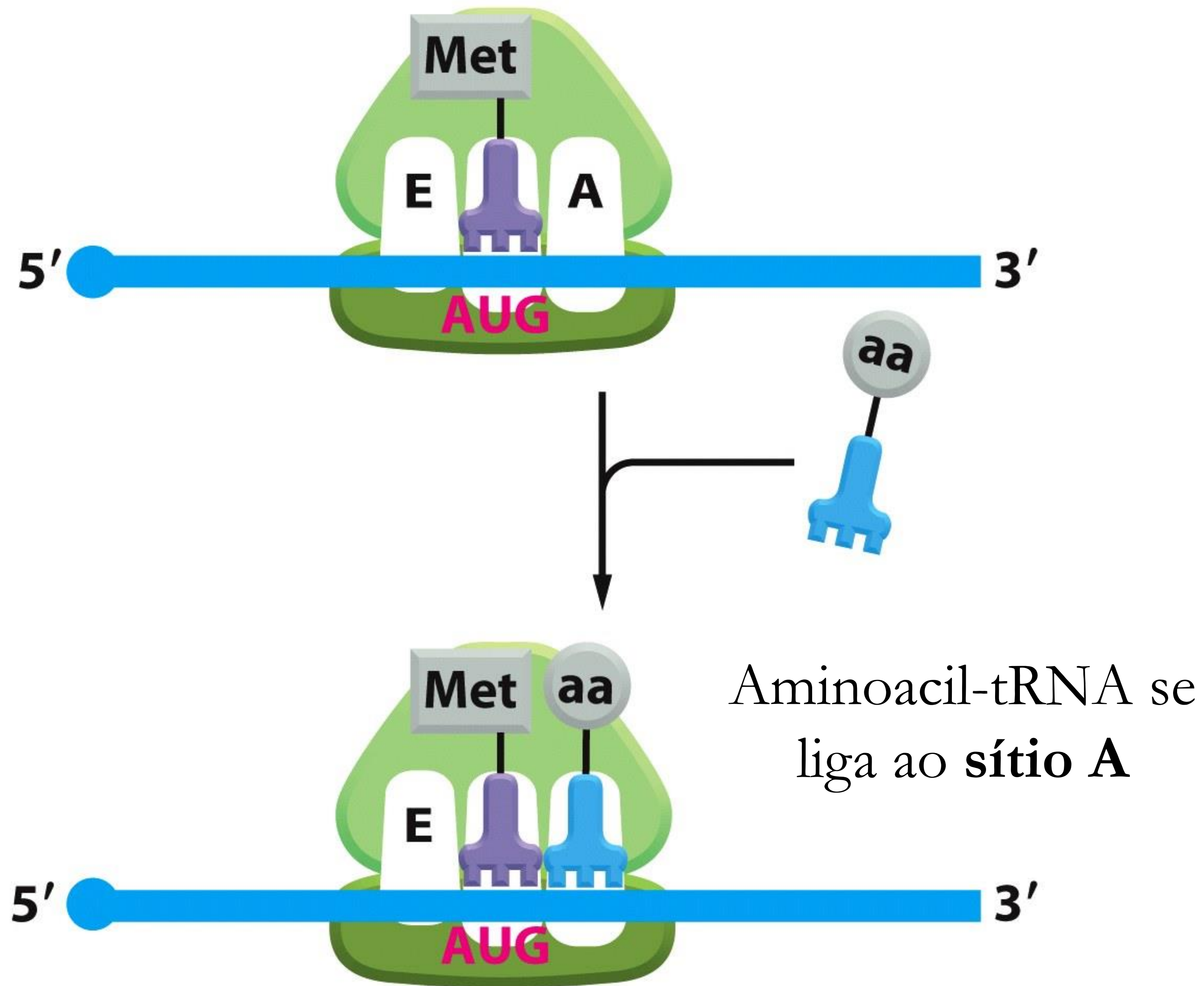
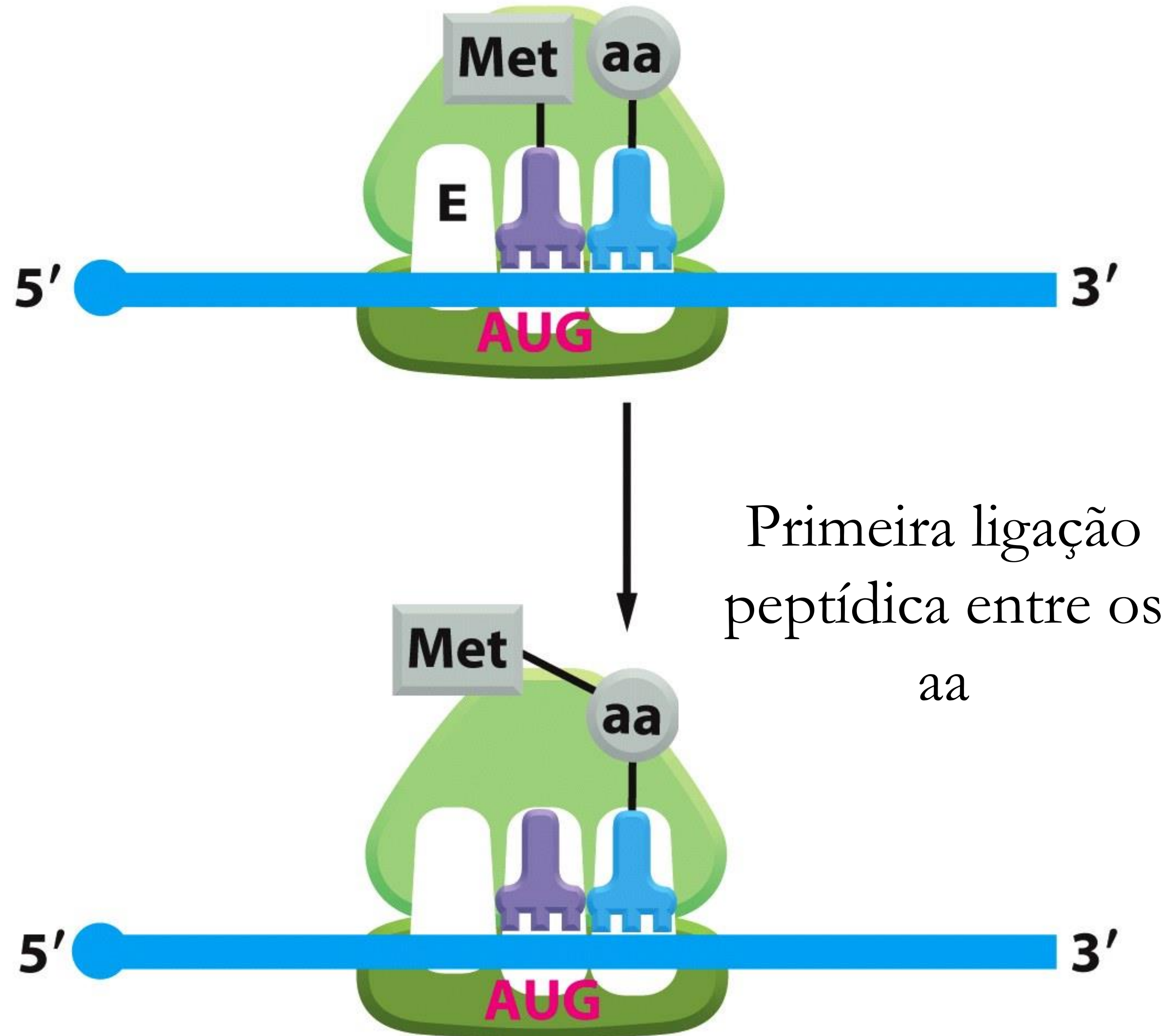


Figure 7-35 part 3 of 5 Essential Cell Biology 3/e (© Garland Science 2010)



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

E continuadamente….

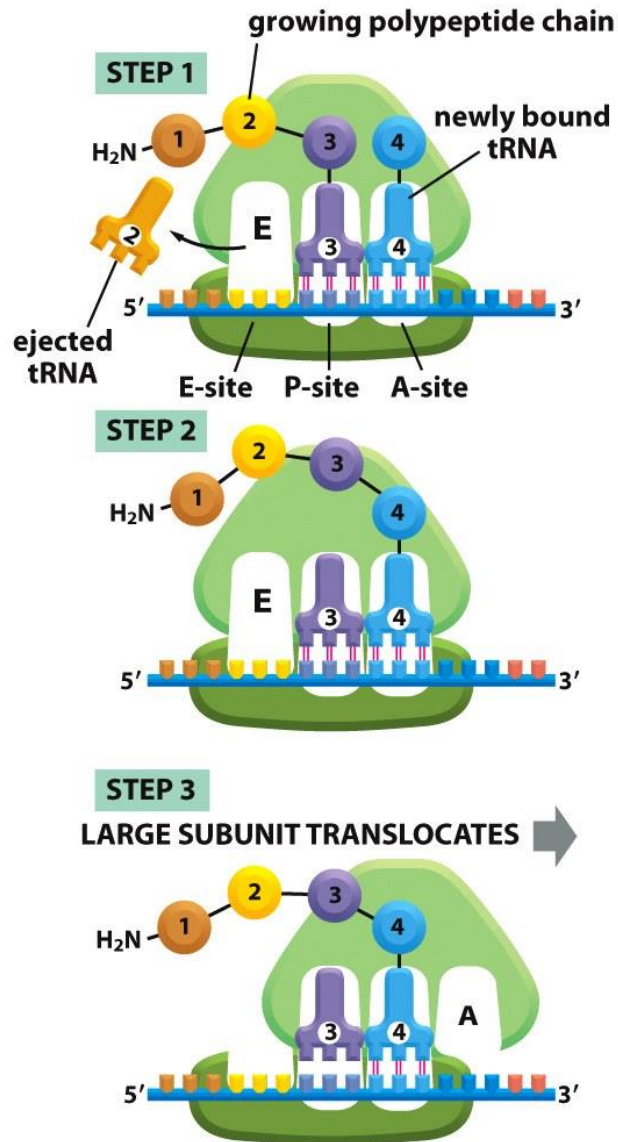
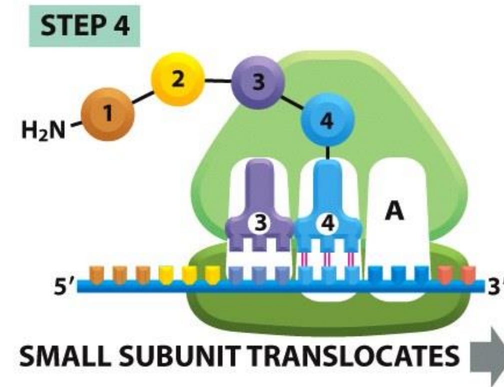
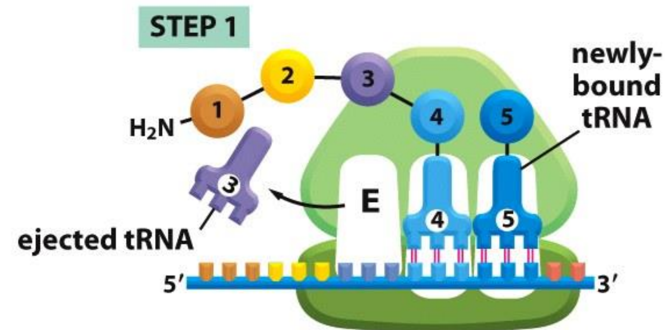


Figure 7-33 Essential Cell Biology 3/e (© Garland Science 2010)





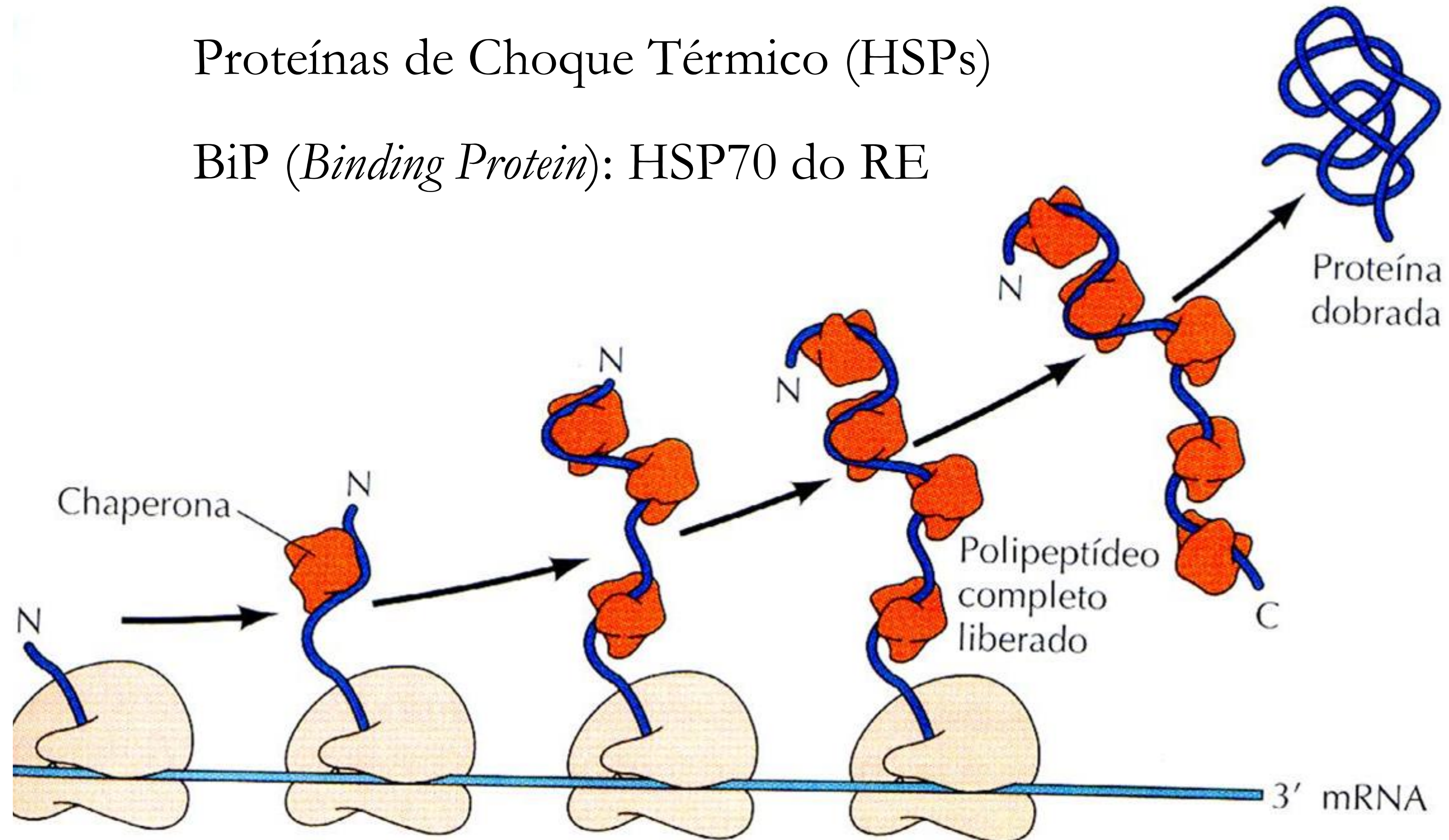
https://www.dnalc.org/resources/3d/16-translation-advanced.html

Chaperonas e Tradução

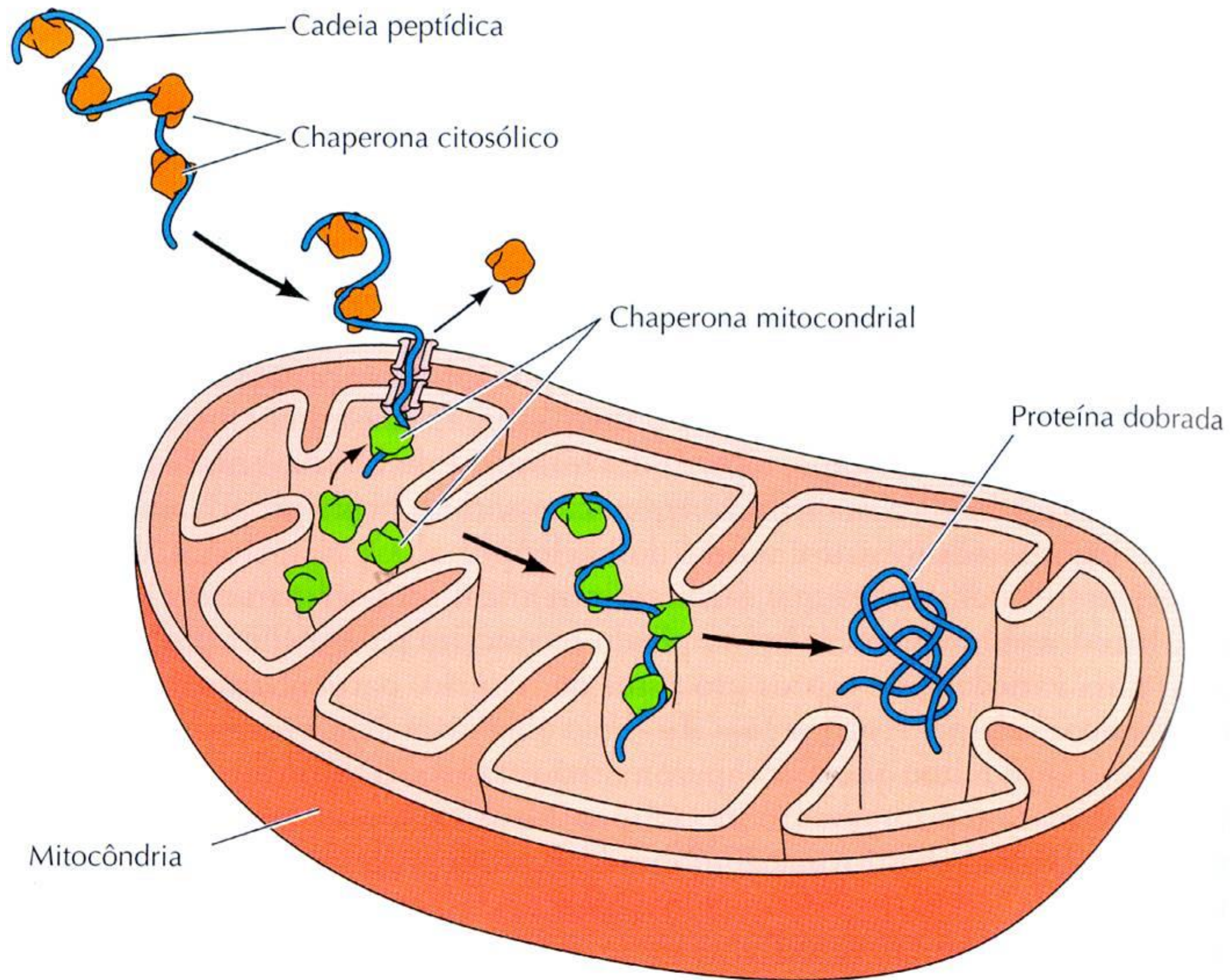
Dobramento de proteínas

Proteínas de Choque Térmico (HSPs)

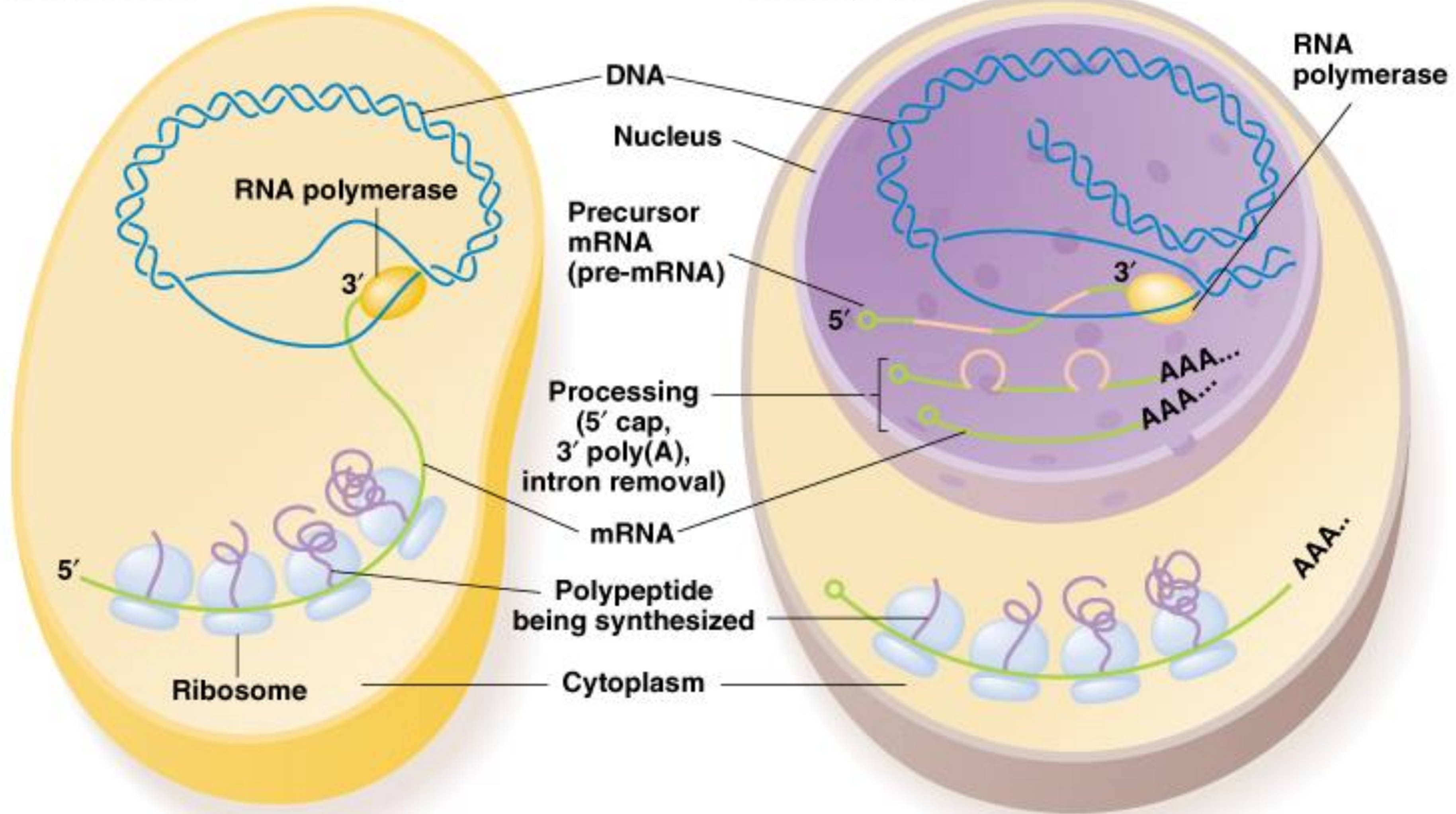
BiP (*Binding Protein*): HSP70 do RE



Chaperonas e Transporte de Proteínas

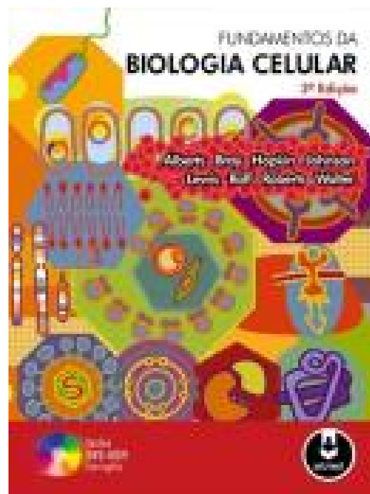


Visão Geral



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

Leitura Recomendada



FUNDAMENTOS DA BIOLOGIA CELULAR

Formato: Livro
Autor: ALBERTS, BRUCE
Idioma: PORTUGUES
Editora: ARTMED -
Assunto: CIÊNCIAS BIOLÓGICAS - BIOLOGIA

Capítulos: 5, 6 e 7 – Estarão no no Stoa!

Estudo Dirigido

1. Diferenças fundamentais entre DNA e RNA;
2. Estrutura e função do DNA;
3. Principais características da dupla hélice do DNA;
4. Principais tipos e funções dos RNAs;
5. Definição de gene;
6. Diferença na estrutura dos genes de eucariotos e procariotos;
7. Região promotora e sua importância para a transcrição em eucariotos e procariotos;
8. Região codificante: start codon e stop codon.
9. Processo de tradução