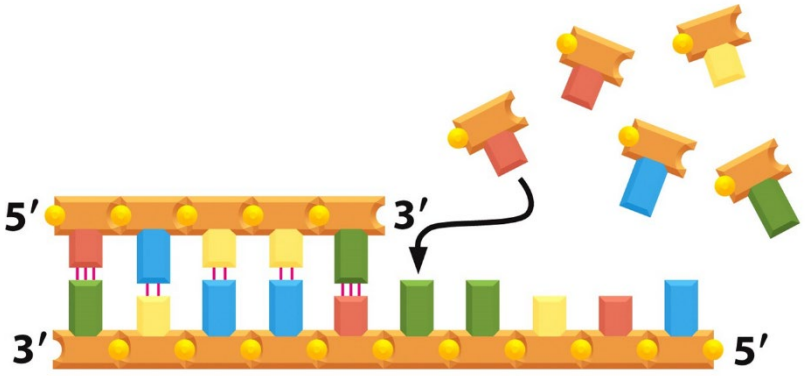
CCM121

REPLICAÇÃO DE DNA



GENETICAL IMPLICATIONS OF THE STRUCTURE OF DEOXYRIBONUCLEIC ACID

By J. D. WATSON and F. H. C. CRICK

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Nature, Vol. 171, No. 4361, May 30, 1953
pp. 964–967.

THE importance of deoxyribonucleic acid (DNA) within living cells is undisputed. It is found in l dividing cells, largely if not entirely in the nucleus, here it is an essential constituent of the chromo-omes. Many lines of evidence indicate that it is the arrier of a part of (if not all) the genetic specificity f the chromosomes and thus of the gene itself.

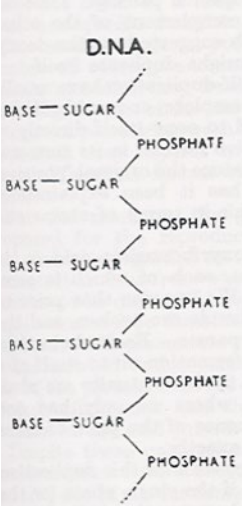


Fig. 1. Chemical formula of a single chain of deoxyribonucleic acid

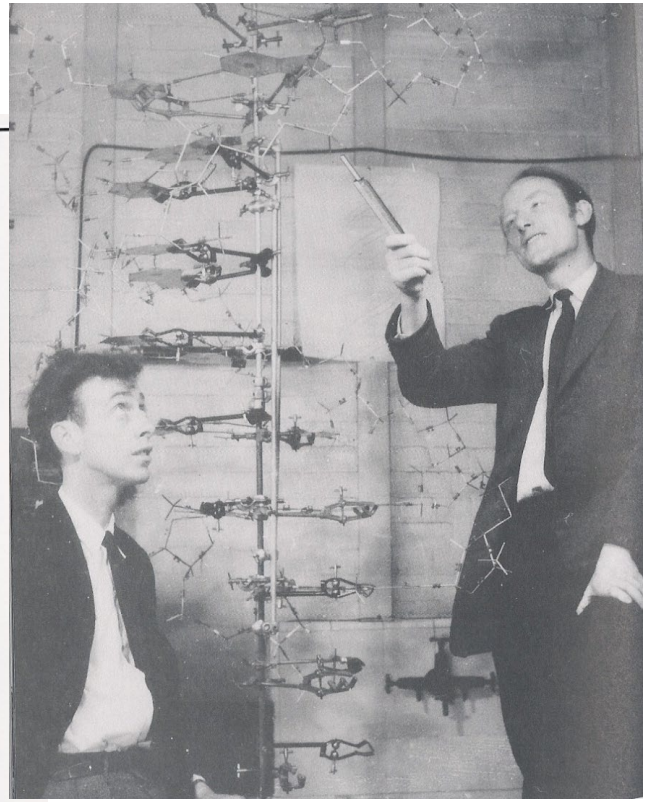
Fig. 2. This figure is purely diagrammatic. The two ribbons symbolize the two phosphate-sugar chains, and the horizontal rods the pairs of bases holding the chains together. The vertical line marks the fibre axis

Until now, however, no evidence has been presented to show how it might carry out the essential operation required of a genetic material, that of exact self-duplication.

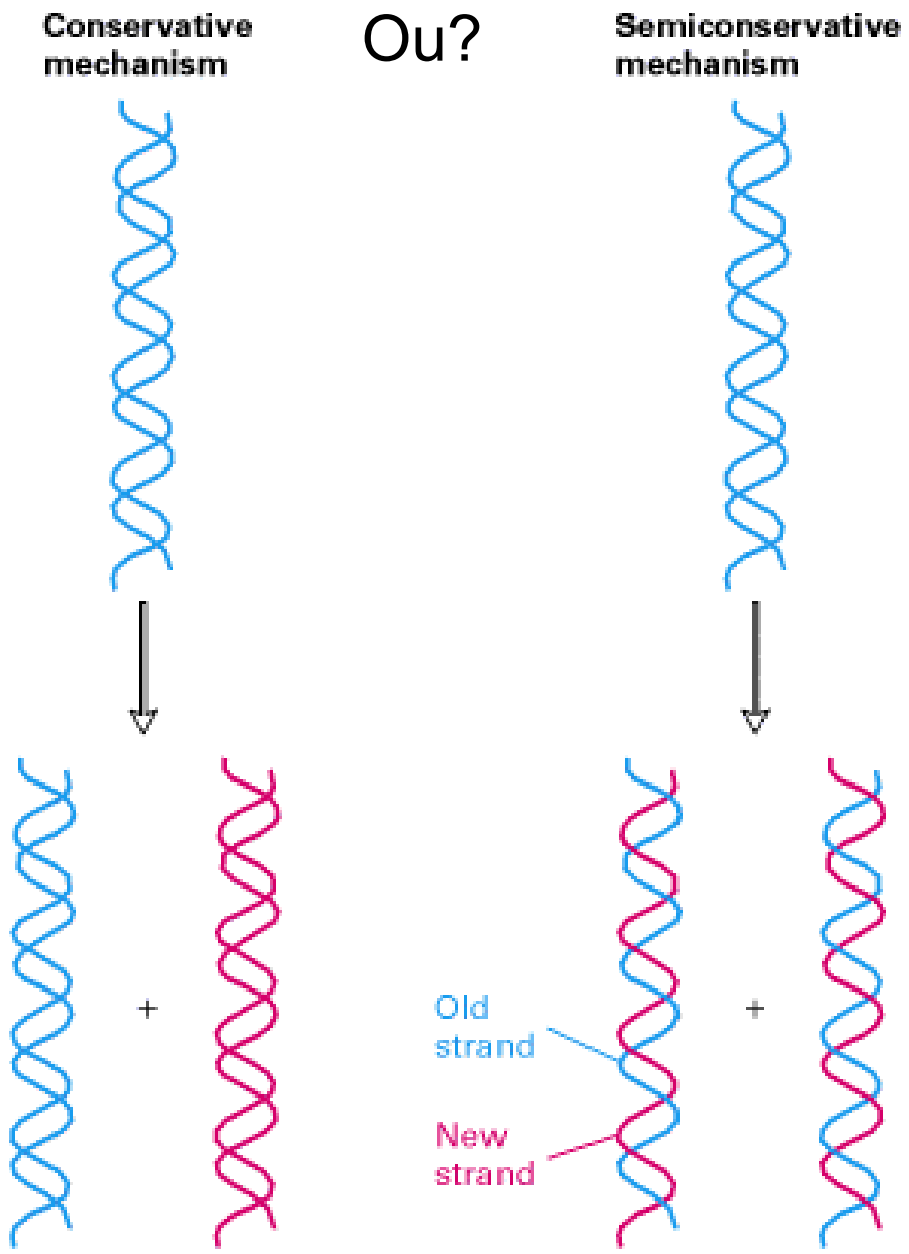
We have recently proposed a structure[1] for the salt of deoxyribonucleic acid which, if correct, immediately suggests a mechanism for its self-duplication. X-ray evidence obtained by the workers at King's College, London[2], and presented at the same time, gives qualitative support to our structure and is incompatible with all previously proposed structures[3]. Though the structure will not be completely proved until a more extensive comparison has been made with the X-ray data, we now feel sufficient confidence in its general correctness to discuss its genetical implications. In doing so we are assuming that fibres of the salt of deoxyribonucleic acid are not artefacts arising in the method of preparation, since it has been shown by Wilkins and his co-workers that similar X-ray patterns are obtained from both the isolated fibres and certain intact biological materials such as sperm head and bacteriophage particles[2,4].

The chemical formula of deoxyribonucleic acid is now well established. The molecule is a very long chain, the backbone of which consists of a regular alternation of s
in Fig. 1. To
base, which ca
considered 5-n
cytosine, since
structure.) Tw
guanine—are
and cytosine—
the sequence
The monomer
and base, is k

The first f
biological inte
but of two. T



"We have recently proposed a structure for DNA which, if correct, immediately suggests a mechanism for its self-duplication".

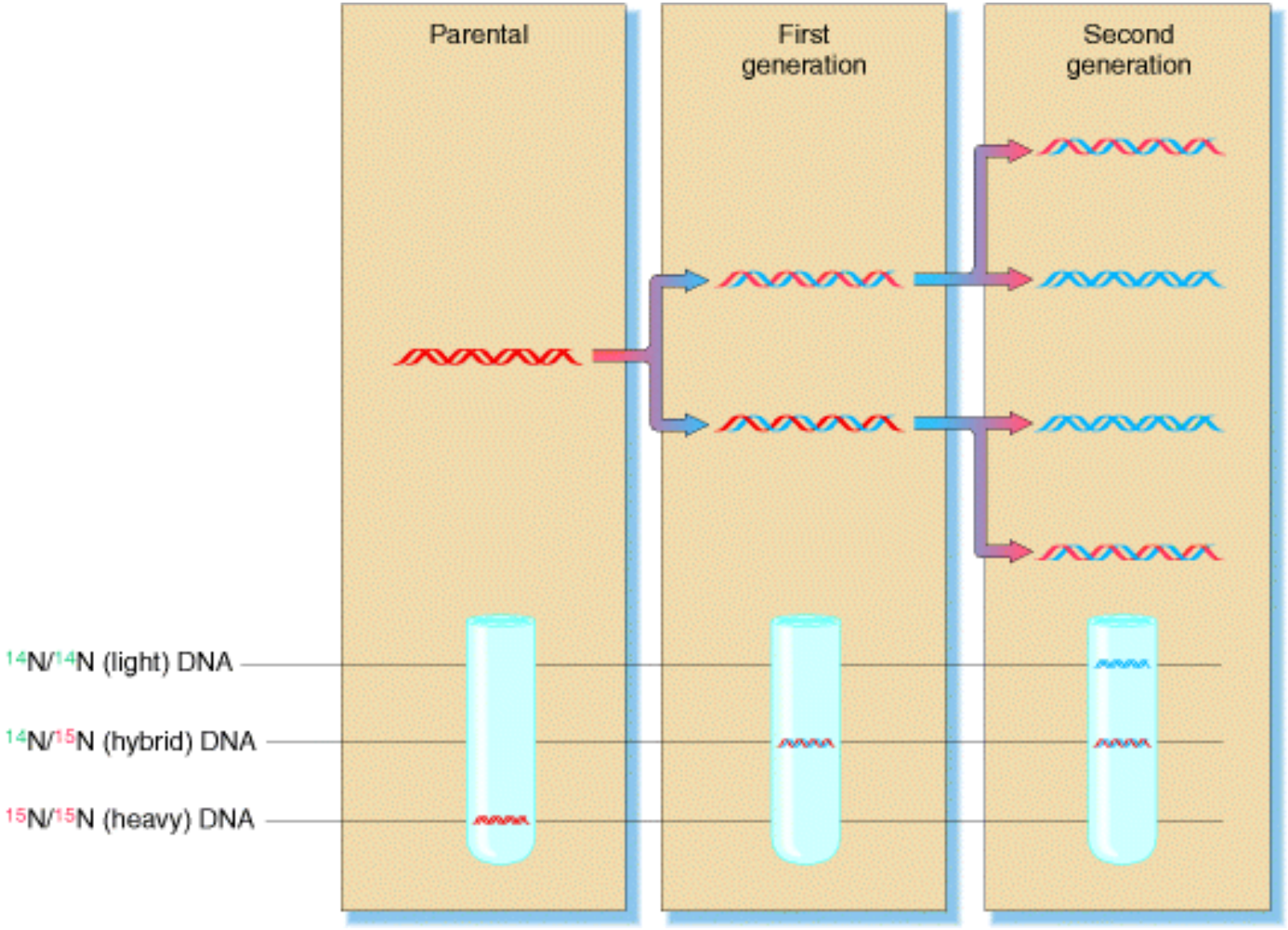

Conservative mechanism
Ou?
Semiconservative mechanism
+
Old strand
New strand
+

A replicação do DNA é semi-conservativa

O experimento que demonstrou que a replicação do DNA é semi-conservativa, em 1958, é considerado um dos mais elegantes da história: “Experimento de Meselson &Stahl”

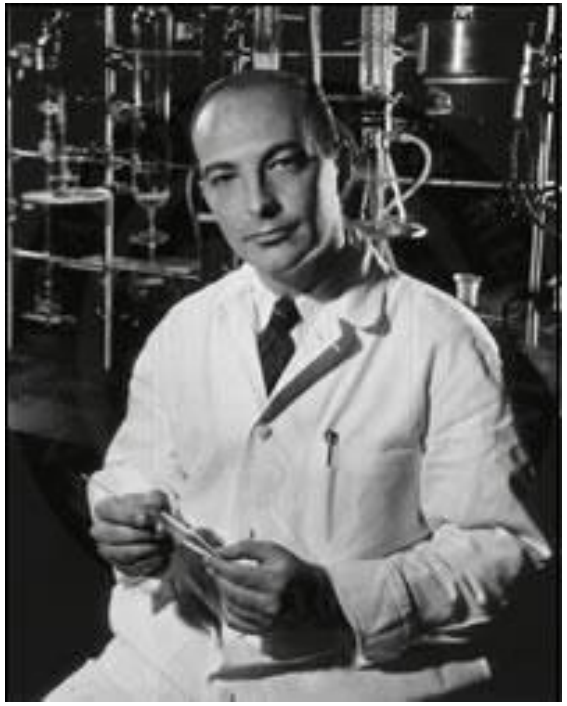
https://www.youtube.com/watch?v=frQbfdRtzBM

Experimento de
Meselson & Stahl



Centrifugação em
gradiente de densidade ($CsCl_2$)

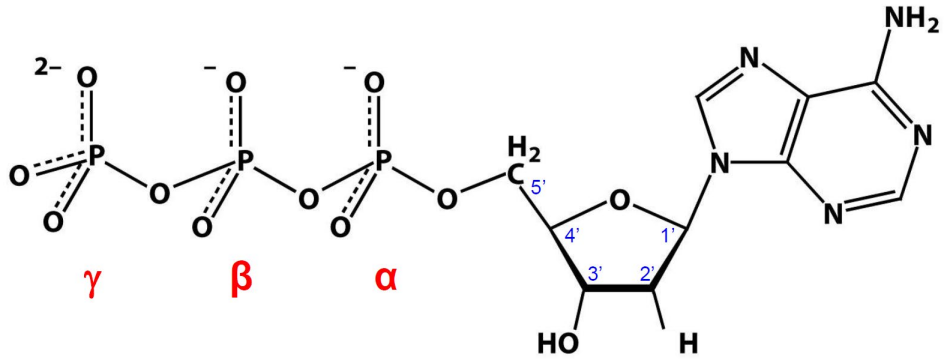
A química da replicação: enzima e substratos



Em **1958,** Arthur Kornberg revelou a descoberta da DNA polimerase de *E.coli*, enzima que sintetiza DNA novo usando uma das fitas como molde

As DNA polimerases:

-Sintetizam a fita no sentido 5’-3’;
-Requerem um iniciador (*primer*) com 3’OH livre
-Utilizam como substrato dNTPs;
-Requerem uma fita molde.
-Requerem Mg^{2+}como cofator.

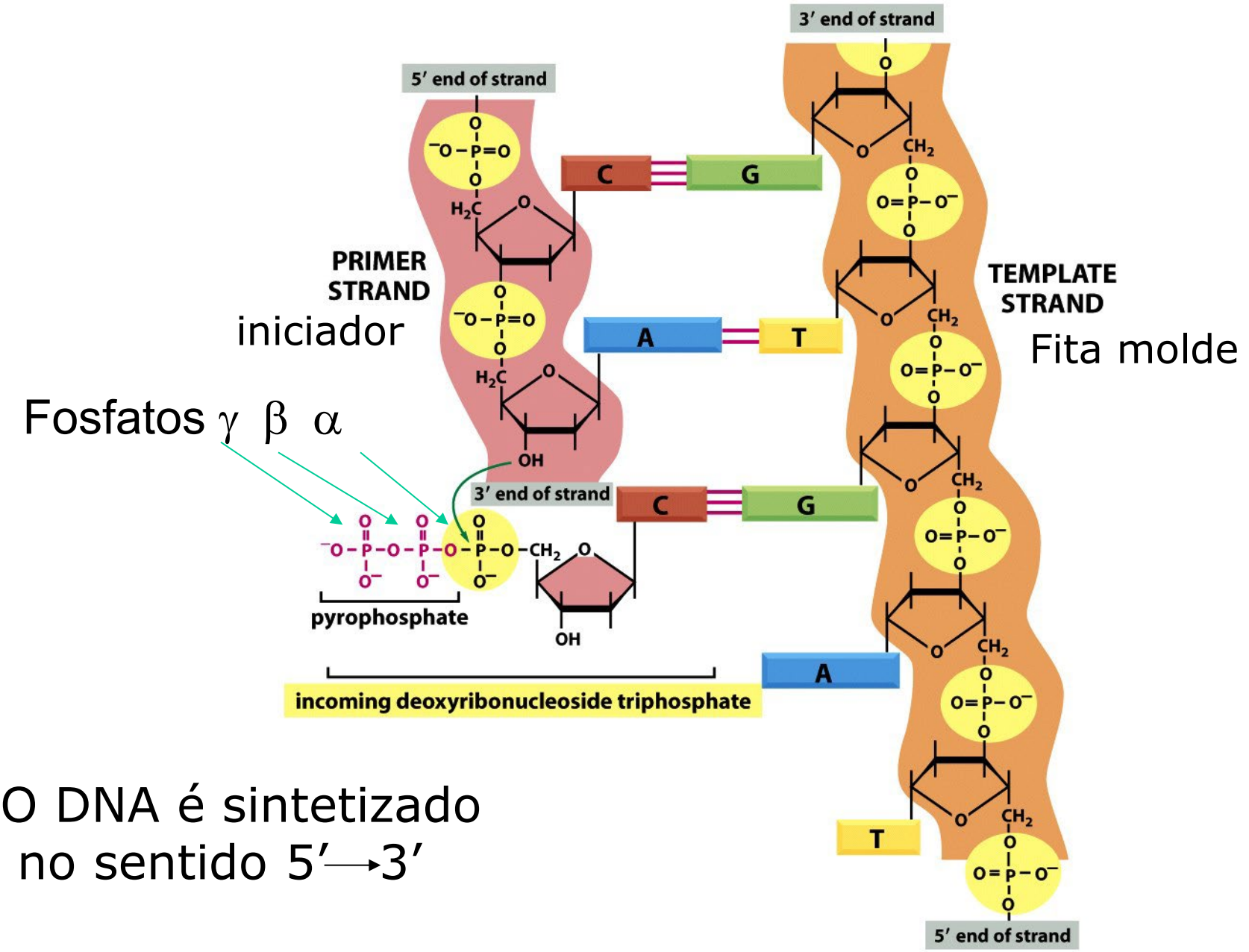


dATP

- dATP
- dGTP
- dCTP
- dTTP

dNTPs

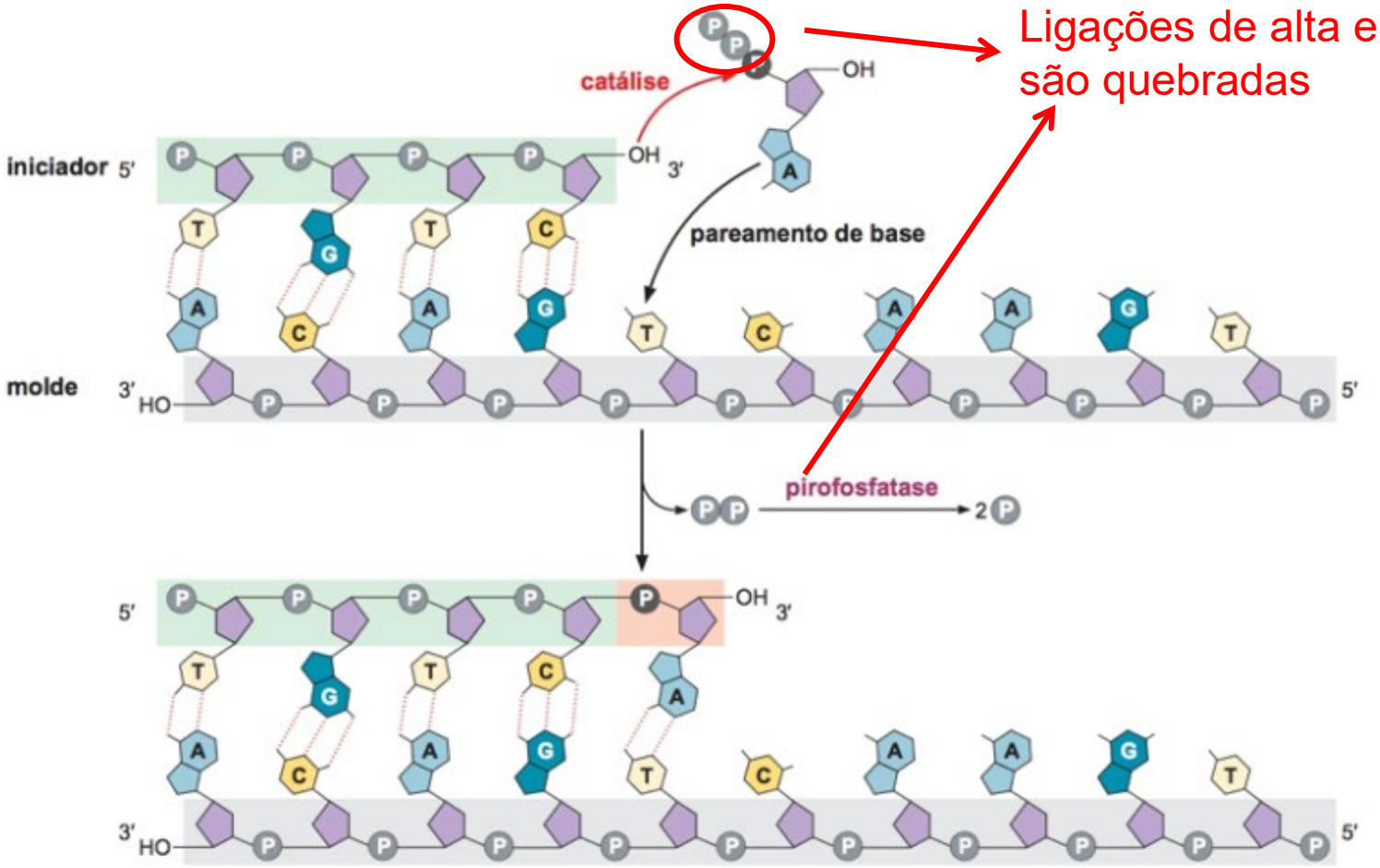
Síntese de uma cadeia de DNA pela DNA polimerase



O DNA é sintetizado no sentido 5′⟶3′

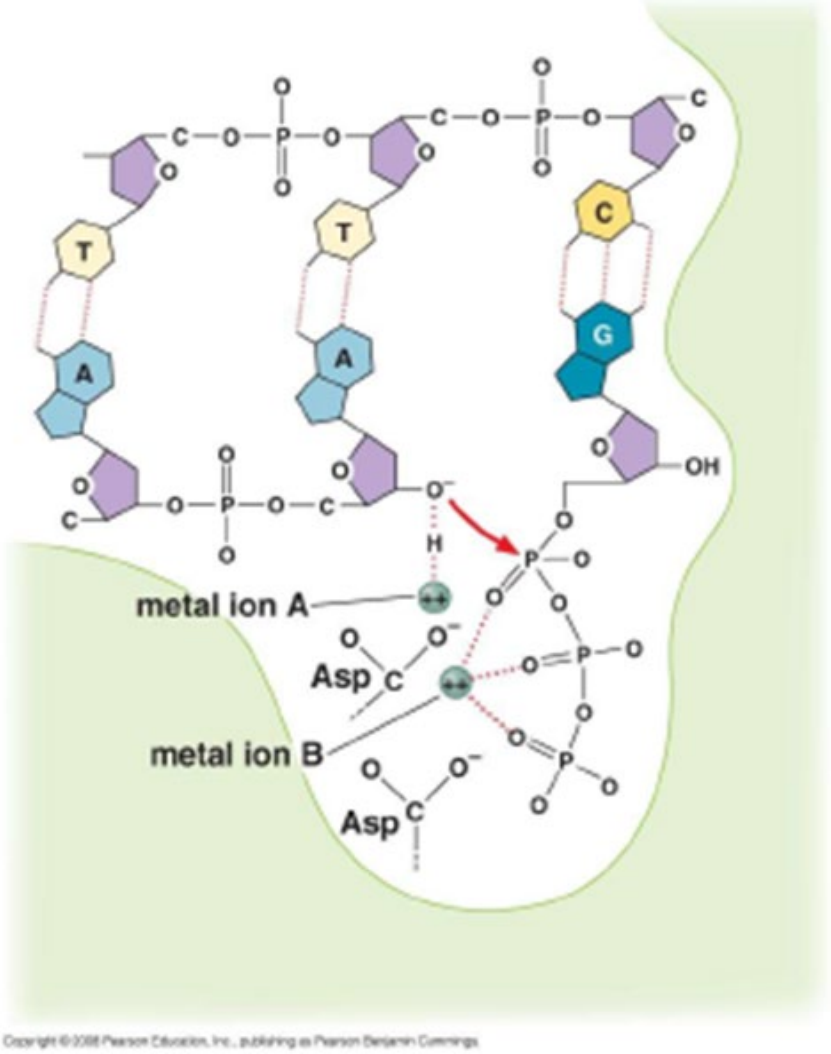
Figure 5-3 Molecular Biology of the Cell 5/e (© Garland Science 2008)

A química da replicação - a reação e sua energética



D., Watson, James, et al. *Biologia Molecular do Gene*. Grupo A, 2015. [USP Minha Biblioteca].

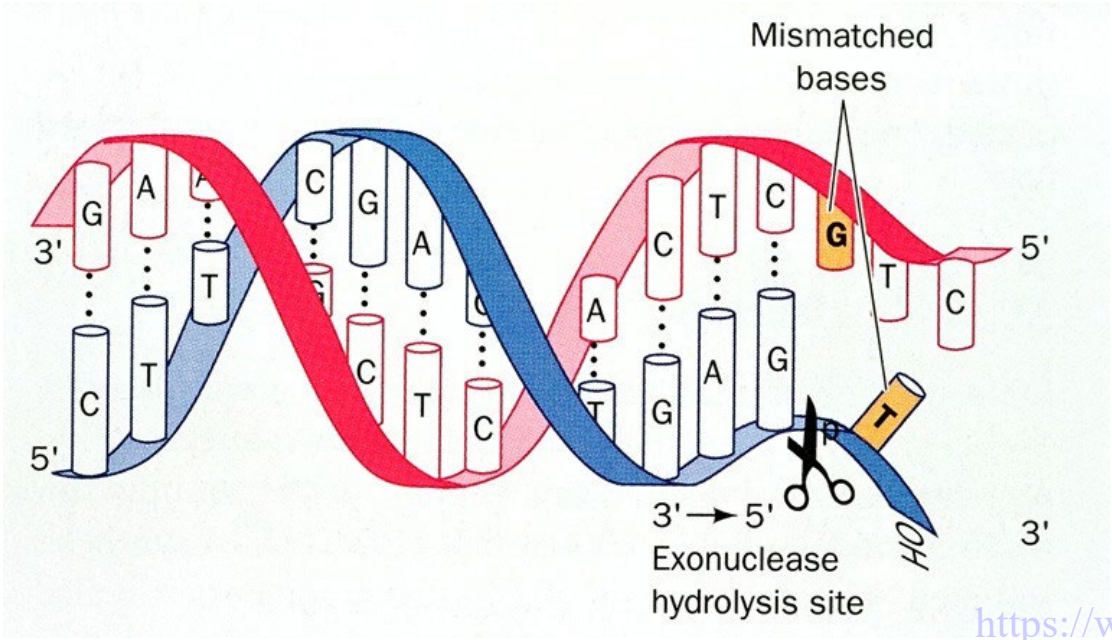
Magnésio é necessário como um cofator para a DNA polimerase



Reação no sítio ativo da DNA polimerase

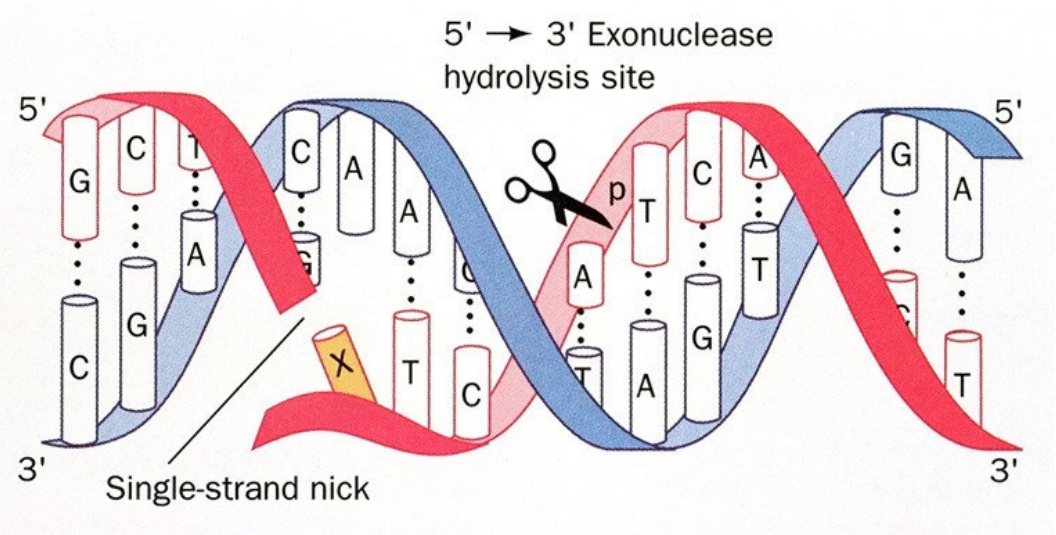
- Catálise depende da presença de dois átomos de metal no sítio ativo, em geral Mg2+
  - Taxa de erro = 10^{-5} - um nucleotídeo errado a cada 100.000 adicionados

Atividades exonucleolíticas da DNA polimerase



Atividade revisora da polimerase (*proofreading*) remove nucleotídeos mal pareados

https://www.youtube.com/watch?v=6O0qD6KCOVE



Presente apenas na DNApol I

Envolvidas na replicação do DNA

TABLE 25–1 Comparison of DNA Polymerases of *E. coli*

	DNA polymerase		
	I	II	III
Structural gene*	*polA*	*polB*	*polC* (*dnaE*)
Subunits (number of different types)	1	7	≥10
M_r	103,000	88,000†	791,500
3′⟶5′ Exonuclease (proofreading)	Yes	Yes	Yes
5′⟶3′ Exonuclease	Yes	No	No
Polymerization rate (nucleotides/s)	16–20	40	250–1,000
Processivity (nucleotides added before polymerase dissociates)	3–200	1,500	≥500,000

*For enzymes with more than one subunit, the gene listed here encodes the subunit with polymerization activity. Note that *dnaE* is an earlier designation for the gene now referred to as *polC*.

†Polymerization subunit only. DNA polymerase II shares several subunits with DNA polymerase III, including the β, γ, δ, δ', χ, and ψ subunits (see Table 25–2).

Como se inicia a replicação do DNA nas células?

Origem de replicação (Contém sequências ricas em AT)

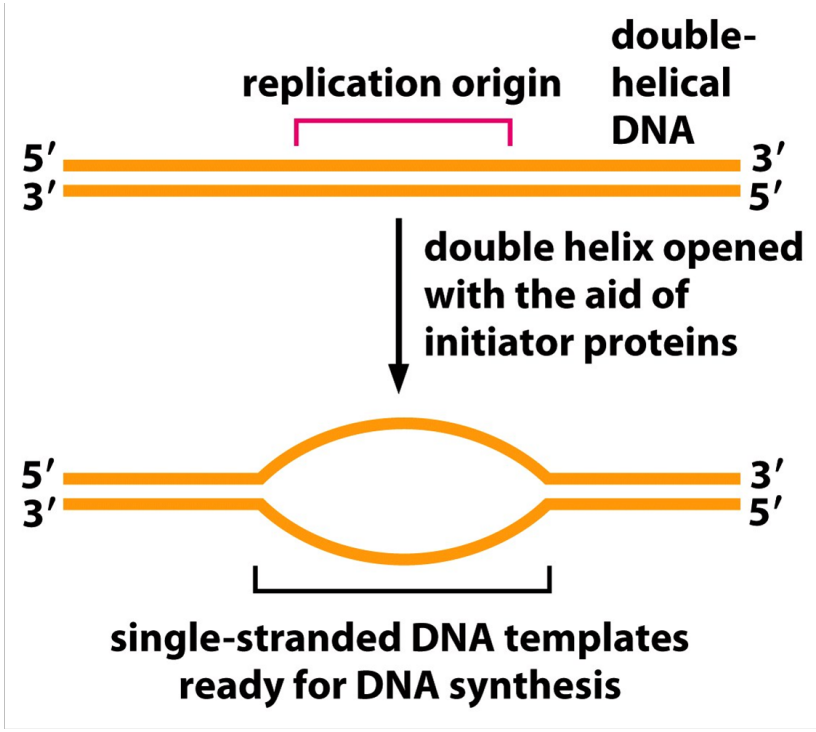
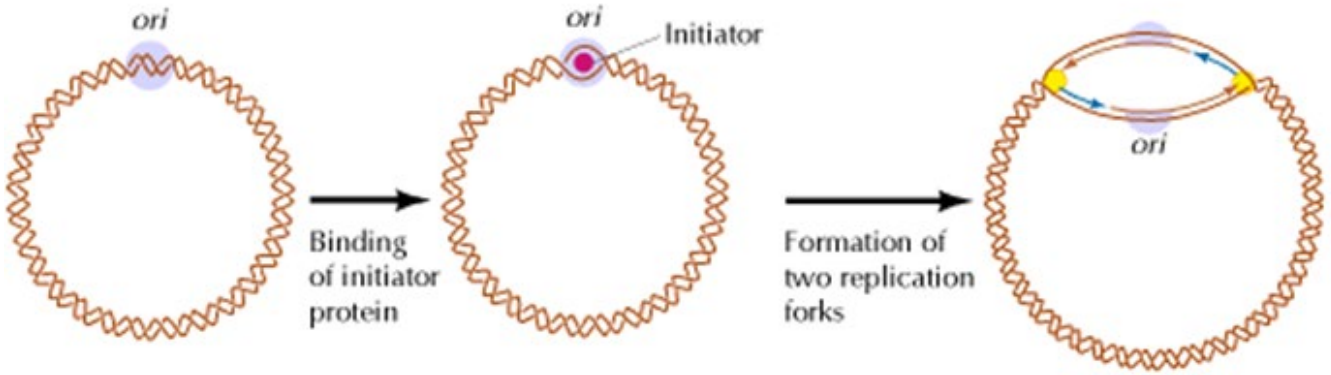


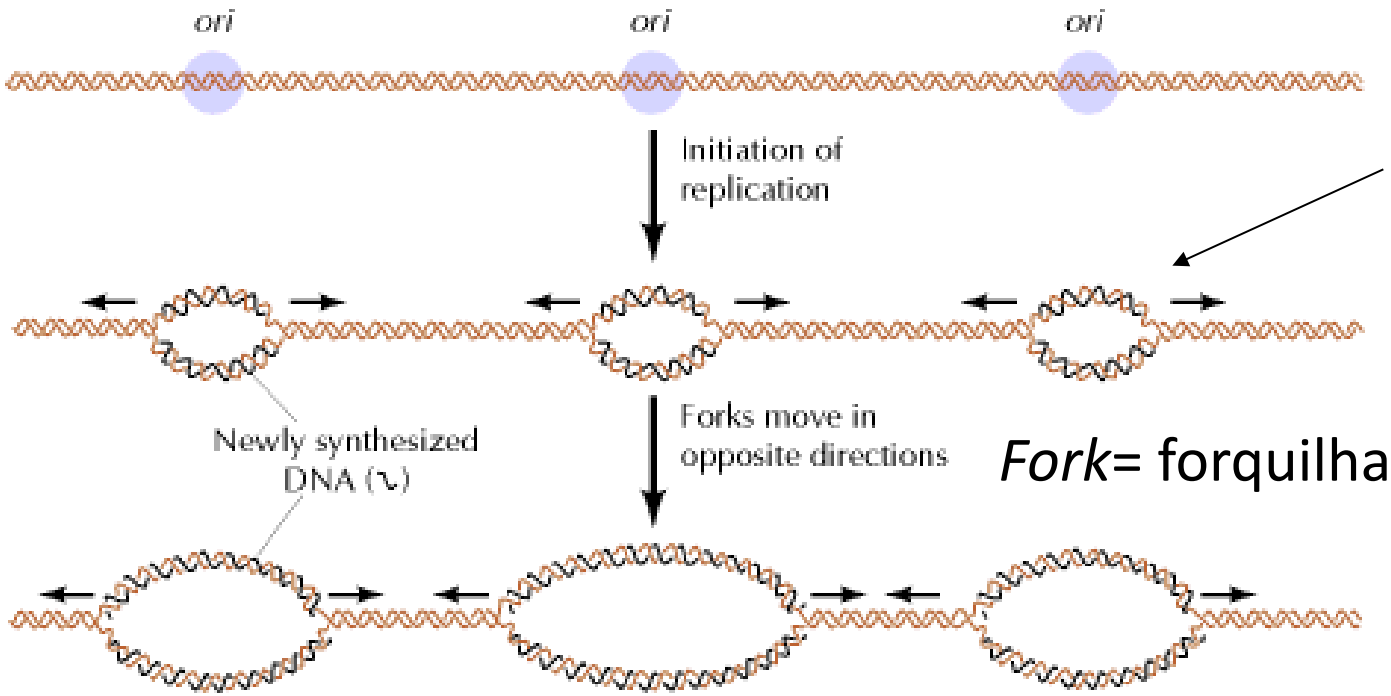
Figure 6-5 *Essential Cell Biology* (© Garland Science 2010)

Uma única origem de replicação em *E.coli*

Replicação do DNA *in vivo* é bidirecional



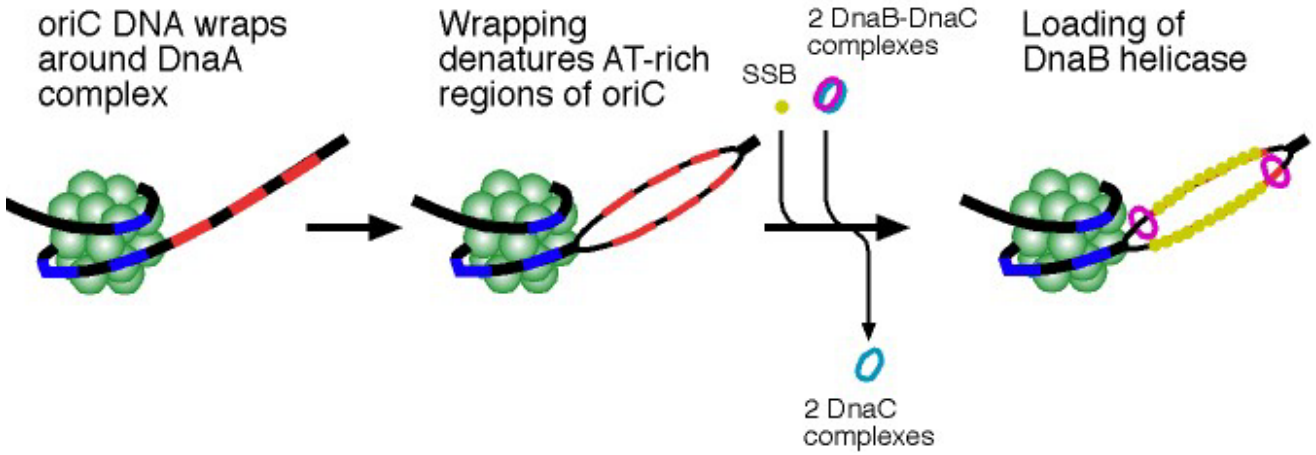
Várias origens de replicação em eucariotos

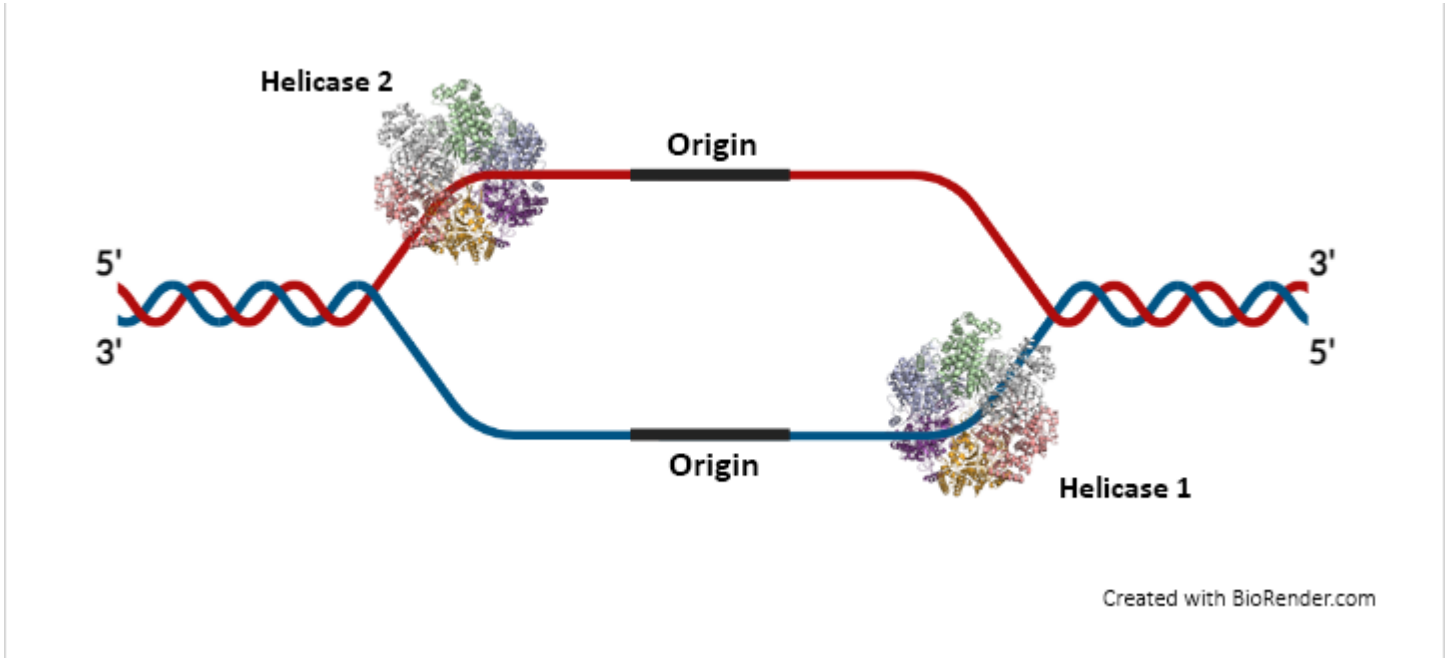


Bolhas de replicação

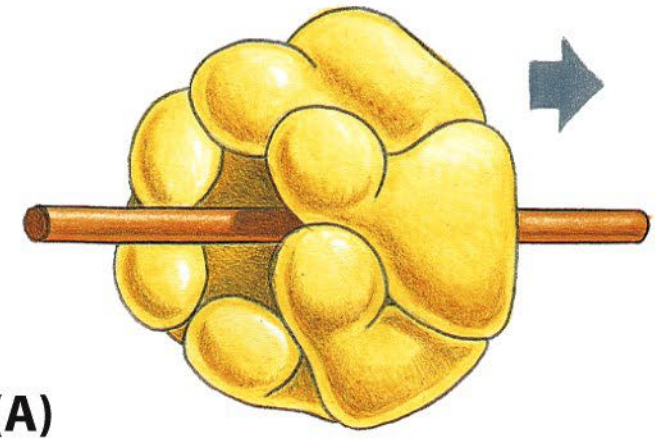
Fork= forquilha de replicação

ex. *E.coli*

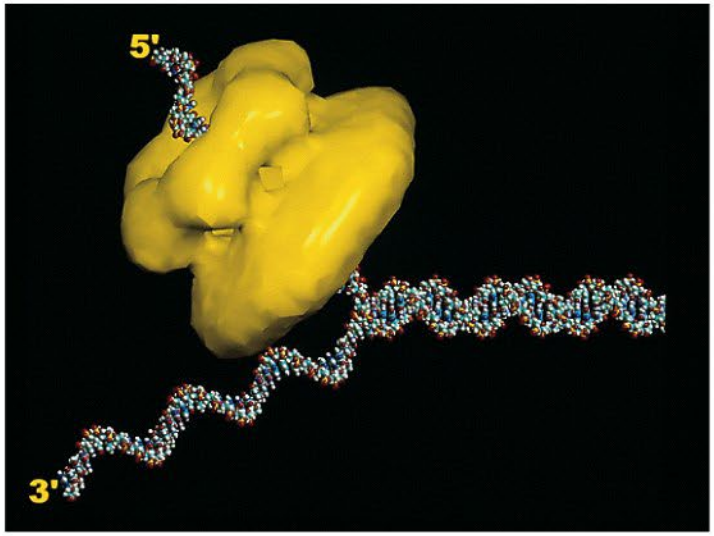

oriC DNA wraps around DnaA complex
Wrapping denatures AT-rich regions of oriC
SSB
2 DnaB-DnaC complexes
Loading of DnaB helicase
2 DnaC complexes


Helicase 2
Origin
5'
3'
3'
5'
Origin
Helicase 1
Created with BioRender.com

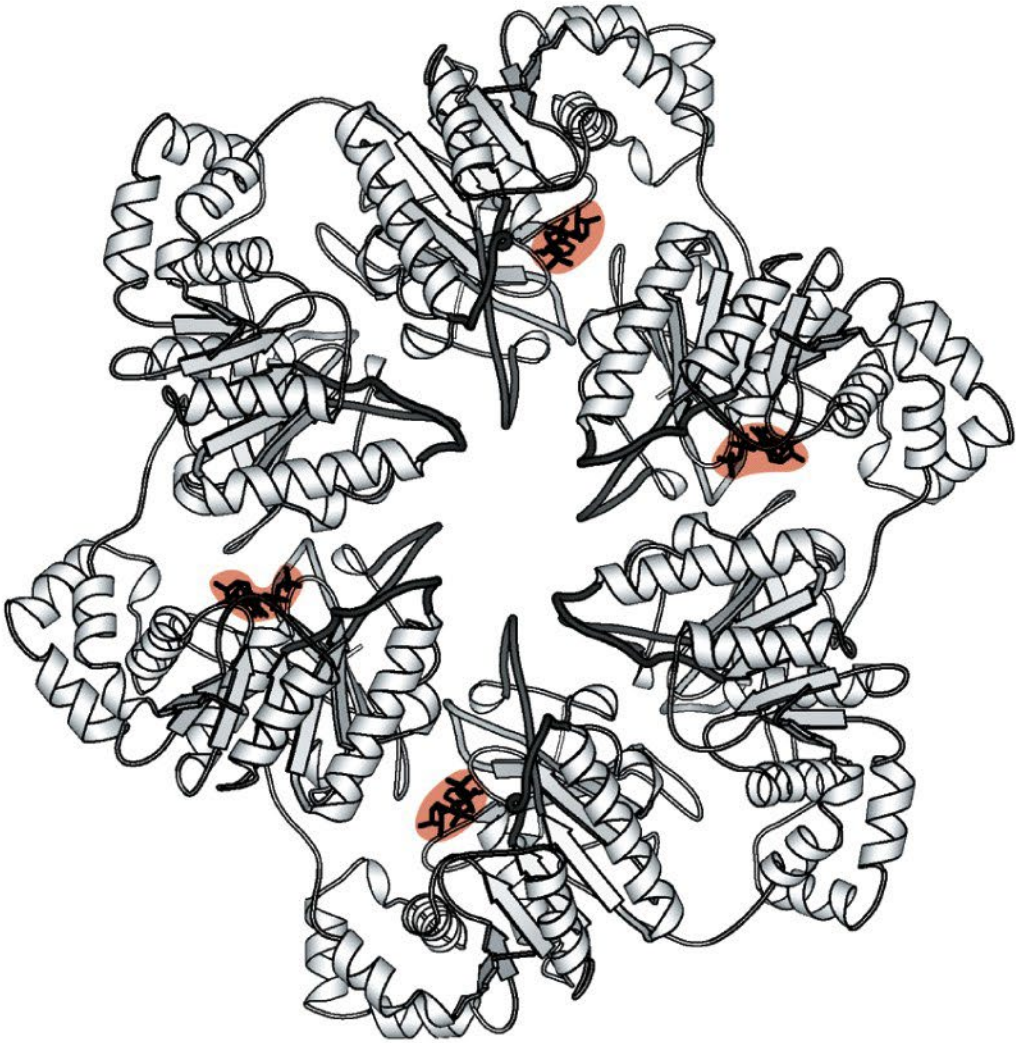
A estrutura da helicase



(A)



(B)



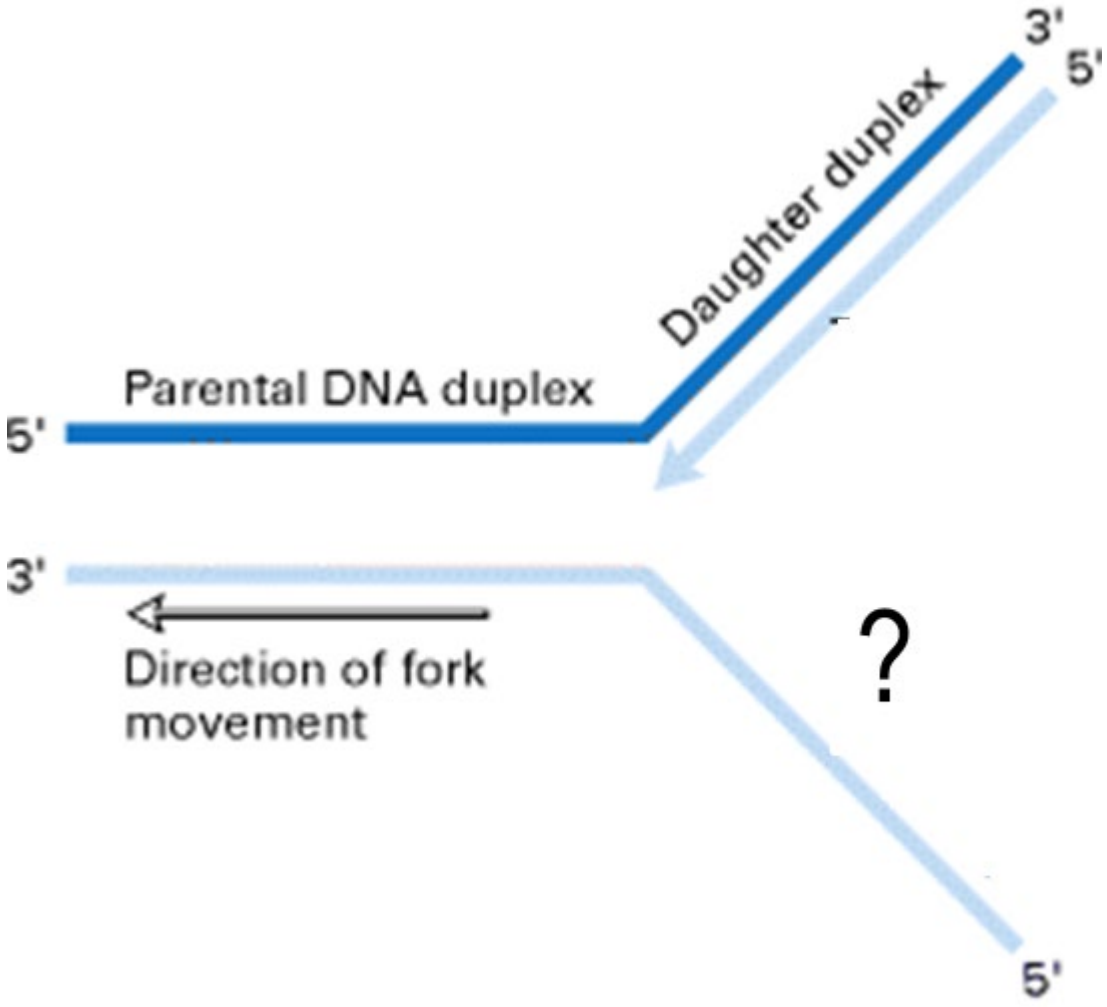
(C)

https://www.youtube.com/watch?v=n6lAvjqby_4

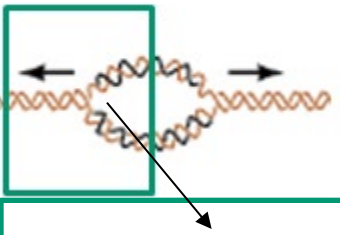
Figure 5-15 Molecular Biology of the Cell 5/e (© Garland Science 2008)

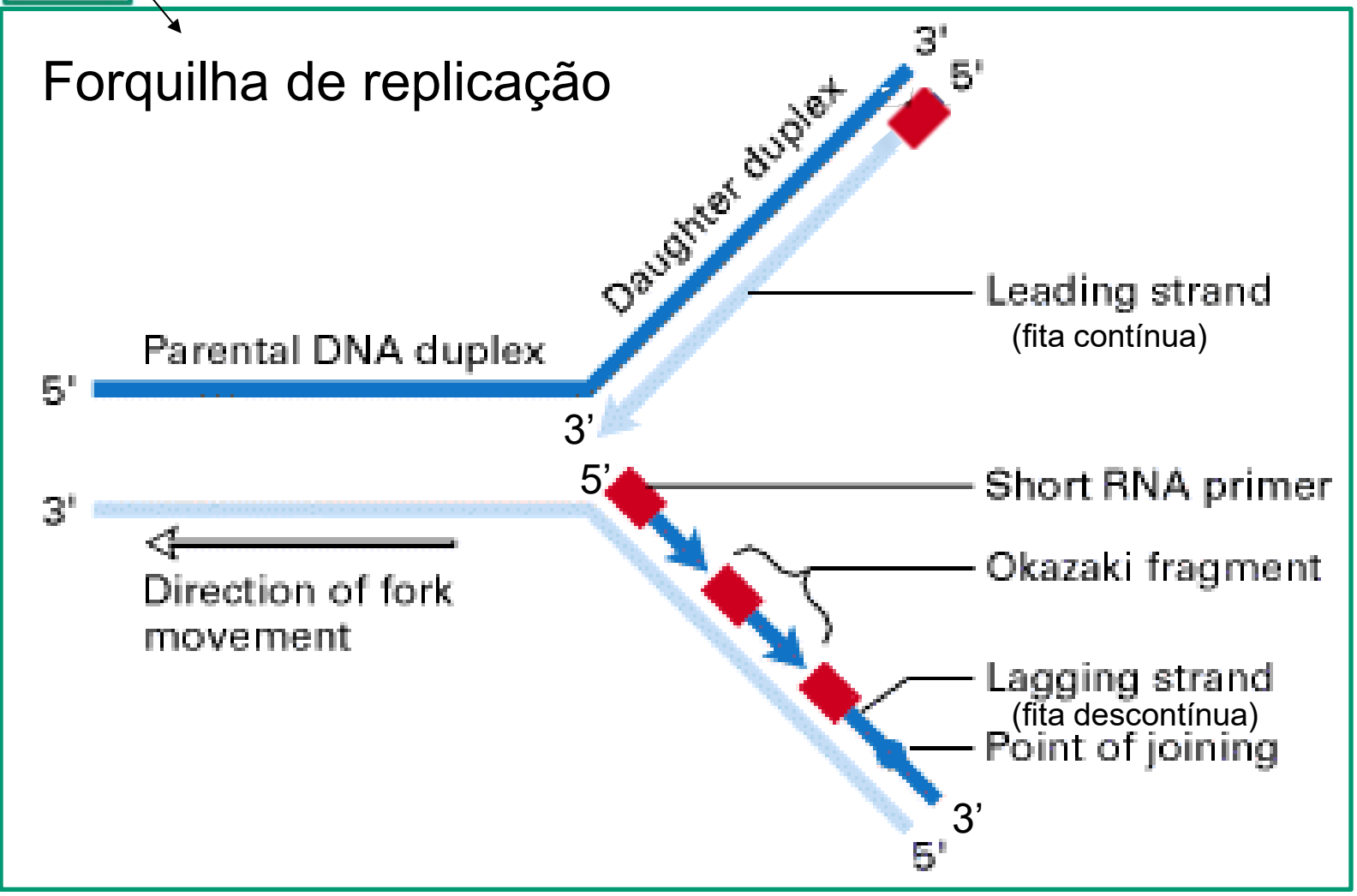
A polaridade da síntese de DNA cria um problema para a replicação

Como replicar as duas fitas



O processo de replicação é semi-descontínuo

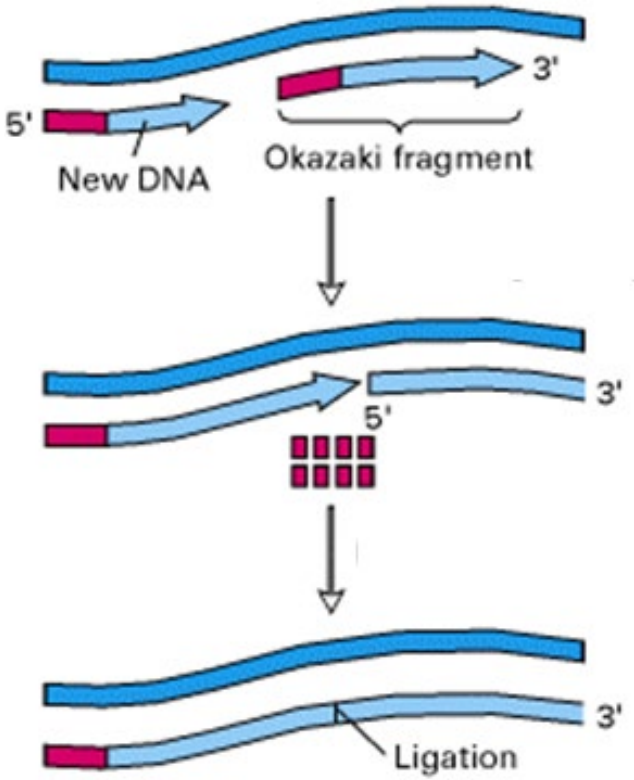




Primase sintetiza primers de RNA

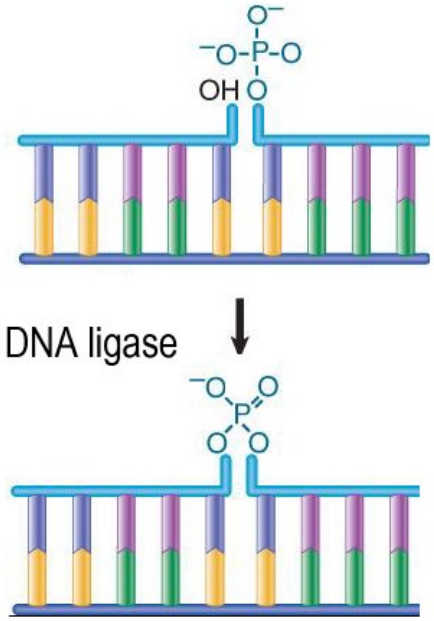
DNA pol III extende os primers sintetizando a nova fita de DNA

Síntese da fita descontínua (*continuação*)



Remoção dos 5’ RNAs (primers) pela DNA pol I (com sua atividade exonucleolítica 5’-3’) e preenchimento da lacuna

Ligação covalente dos fragmentos adjacentes pela DNA ligase



DNA ligase forma uma ligação fosfodiéster entre as duas fitas de DNA

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

Maquinaria de replicação: proteínas envolvidas na replicação

Principais componentes:

- Helicase (abertura da dupla fita)
- Proteínas ligantes de DNA fita simples (*single stranded DNA binding protein*, SSB)
- DNA polimerase replicativa (Pol III)
- DNA polimerase I (Pol I)
- Primase (síntese do primer de RNA)
- *Sliding clamp* (grampo deslizante, subunidades β)
- Topoisomerase (que desfaz a tensão do superenrolamento)

O grampo deslizante (subunidades beta) tem como função conferir processividade à DNA polimerase

DNA polimerase III apresenta maior processividade (capacidade de ficar ligada ao DNA)

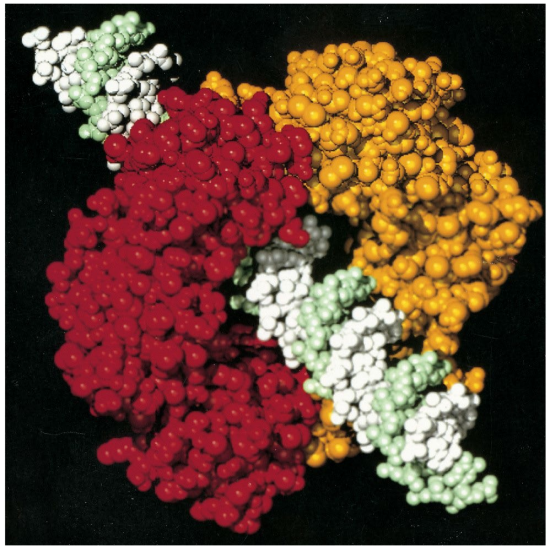
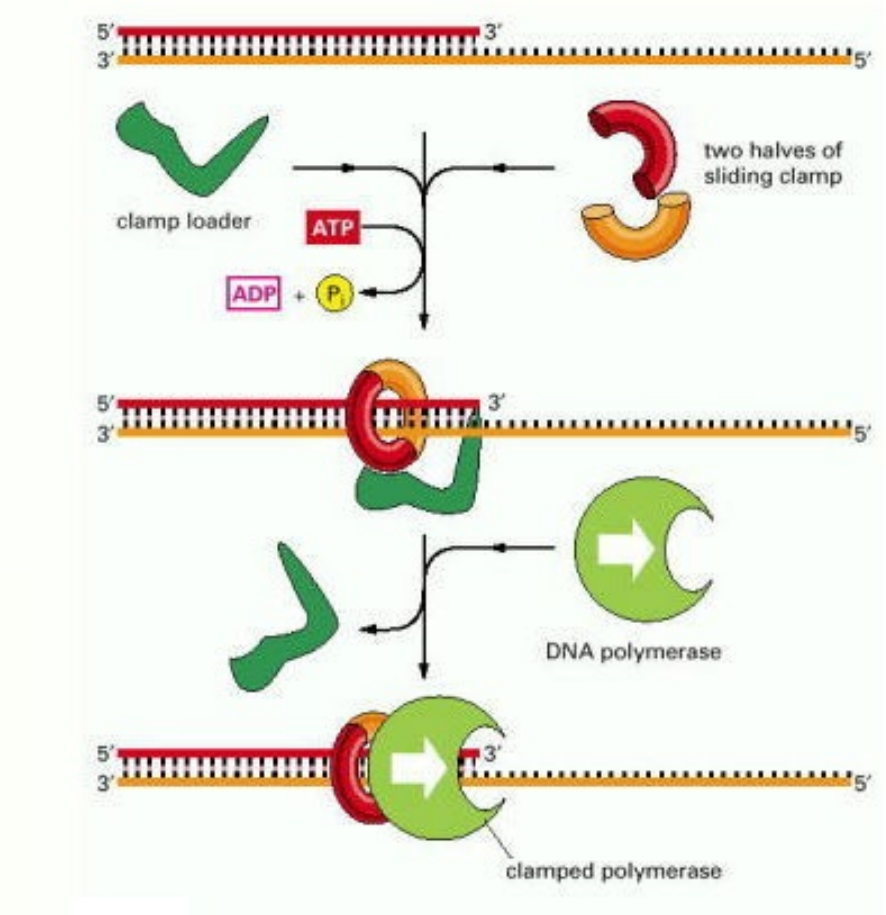


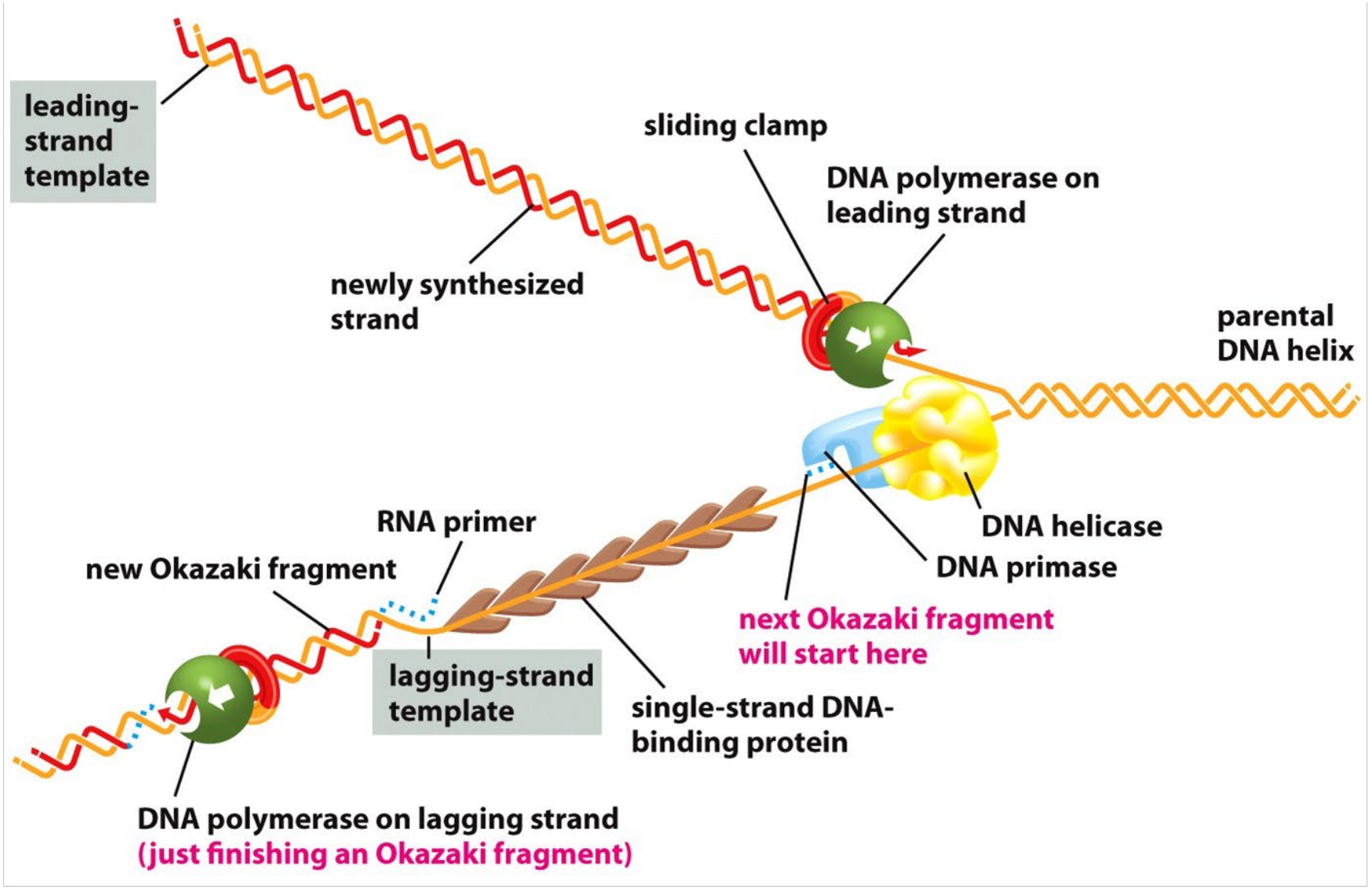
Figure 5-18a Molecular Biology of the Cell 5/e (© Garland Science 2008)

subunidades β
da DNA pol III



Processividade: quanto tempo a enzima fica associada ao seu molde antes que se solte espontaneamente
~500.000 nt por “corrida” para a Pol III!

Proteínas na forquilha de replicação de *E.coli*



A forquilha de replicação em movimento

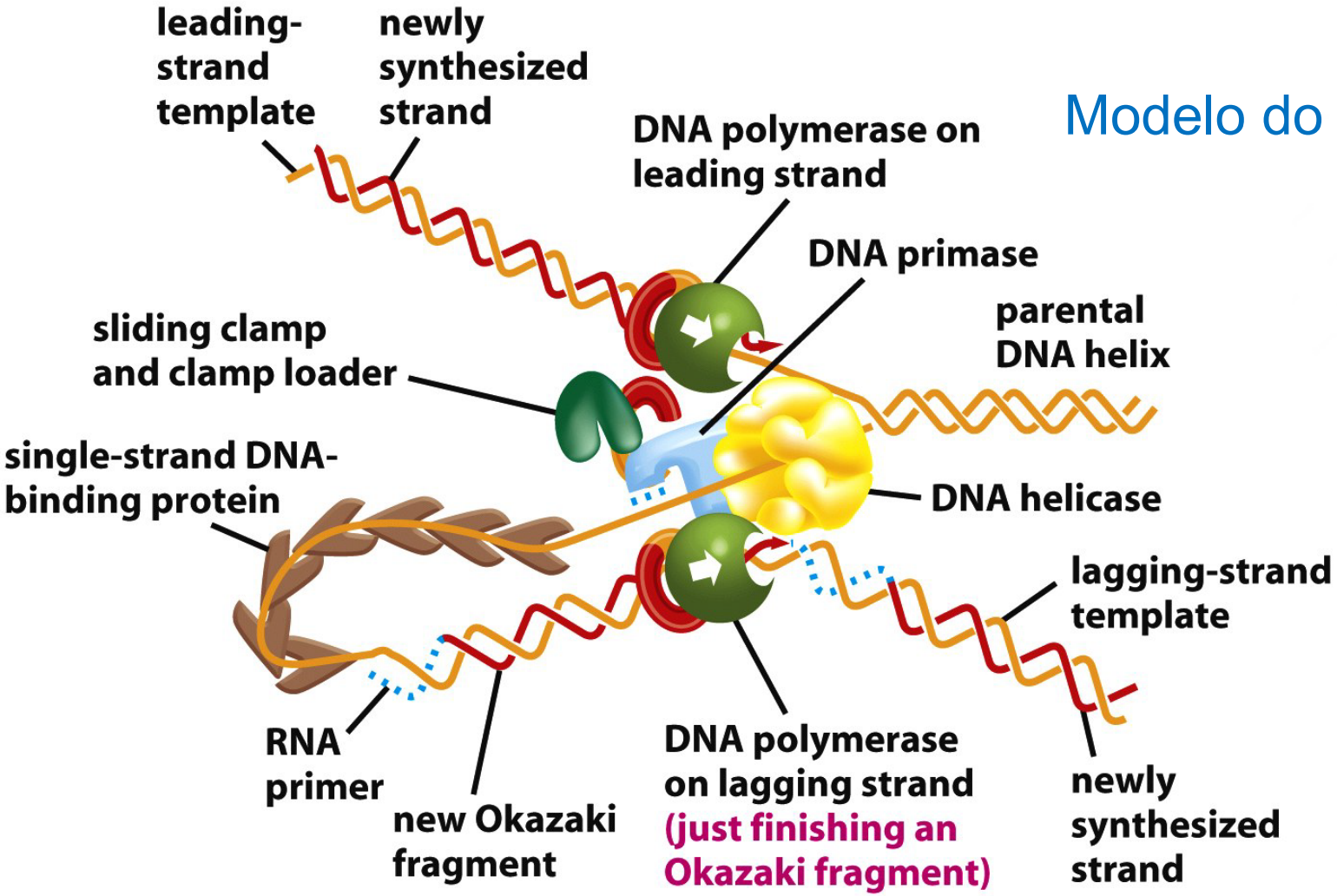
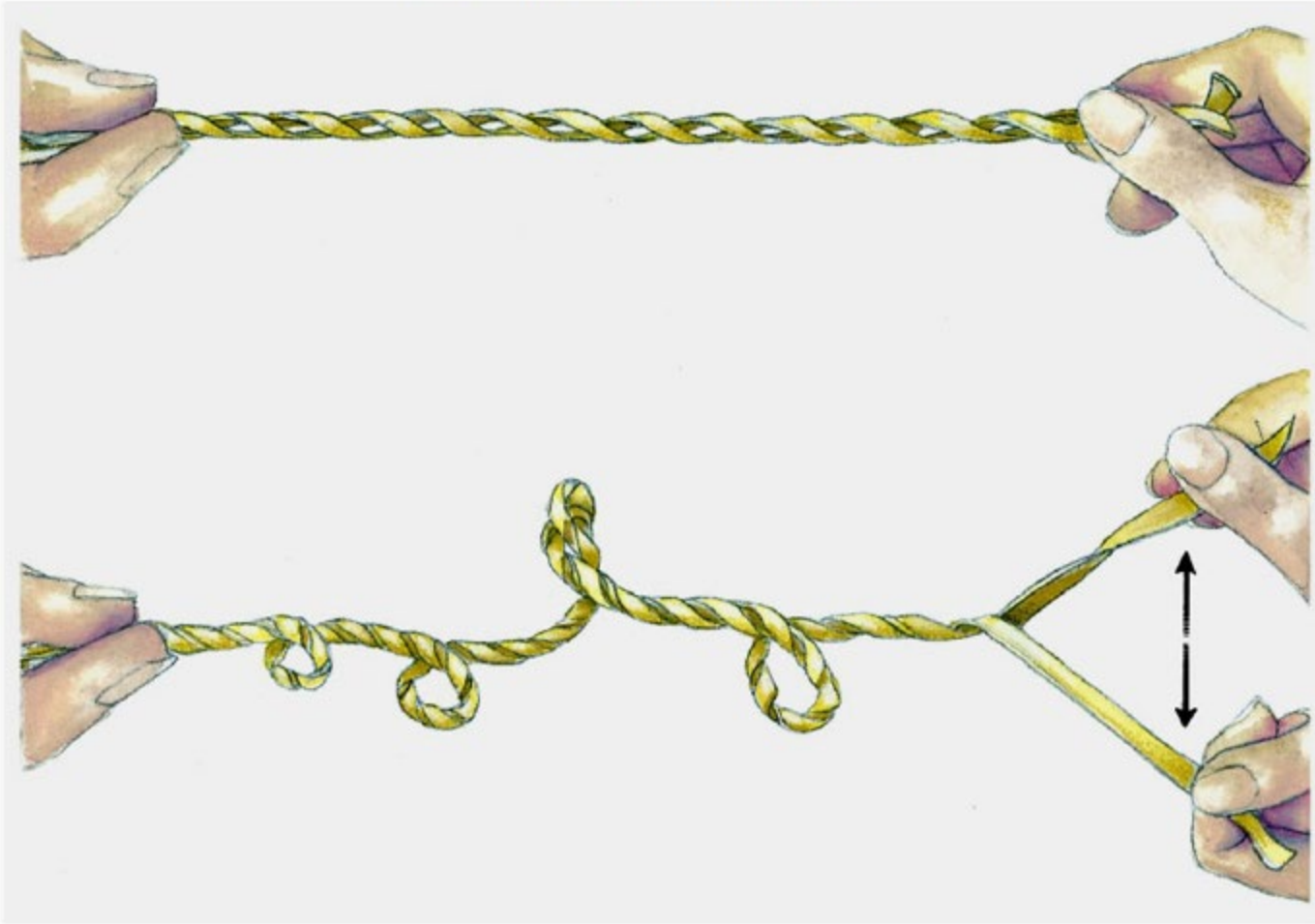


Figure 5-19a Molecular Biology of the Cell 5/e (© Garland Science 2008)

Modelo do trombone

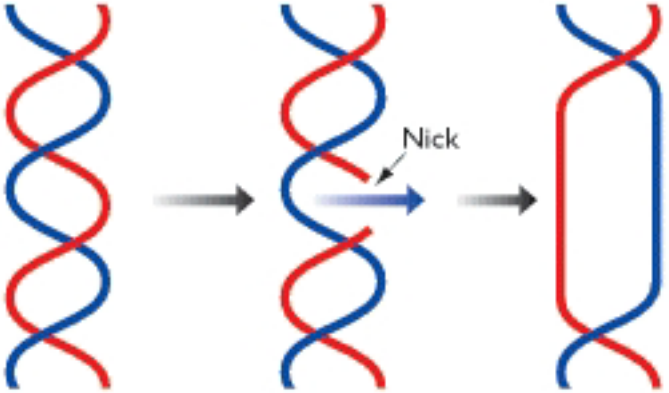
https://app.jove.com/es/science-education/v/11552/the-replisome

Superenrolamento para anular a tensão criada pela abertura da hélice

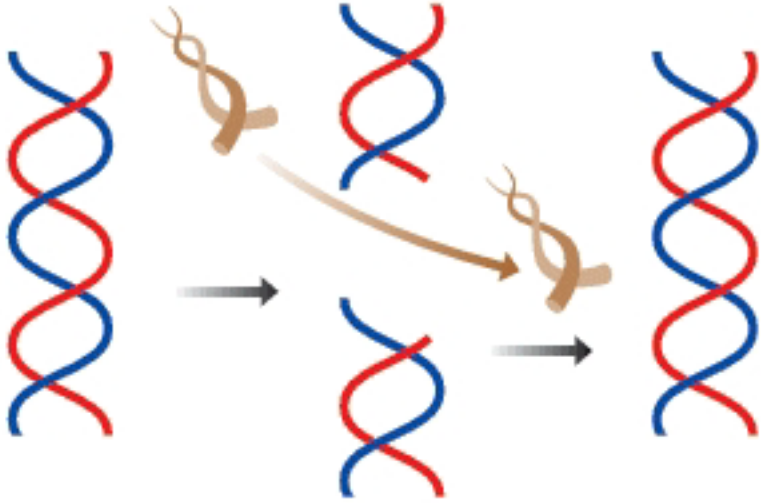


Topoisomerases

Tipo I



Tipo II

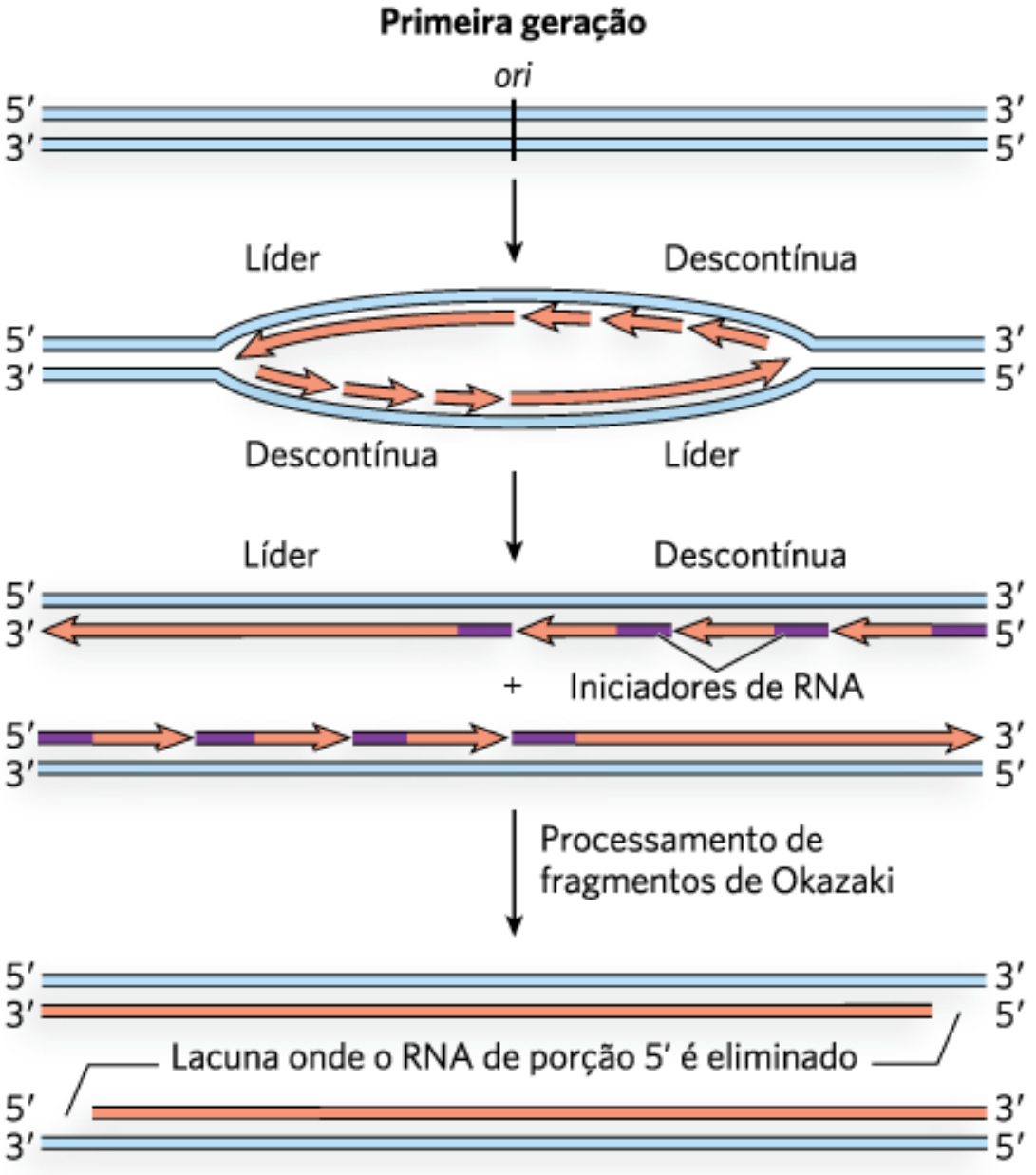


Podem relaxar super espiralamentos no DNA, remover emaranhados de DNA

https://app.jove.com/embed/player?id=11554&t=1&s=1&fpv=1

A cada ciclo de replicação ocorre encurtamento do cromossomo

'End-replication problem'



A remoção dos *primers* causa encurtamento das fitas filhas!!!

Telomerase: adiciona os telômeros

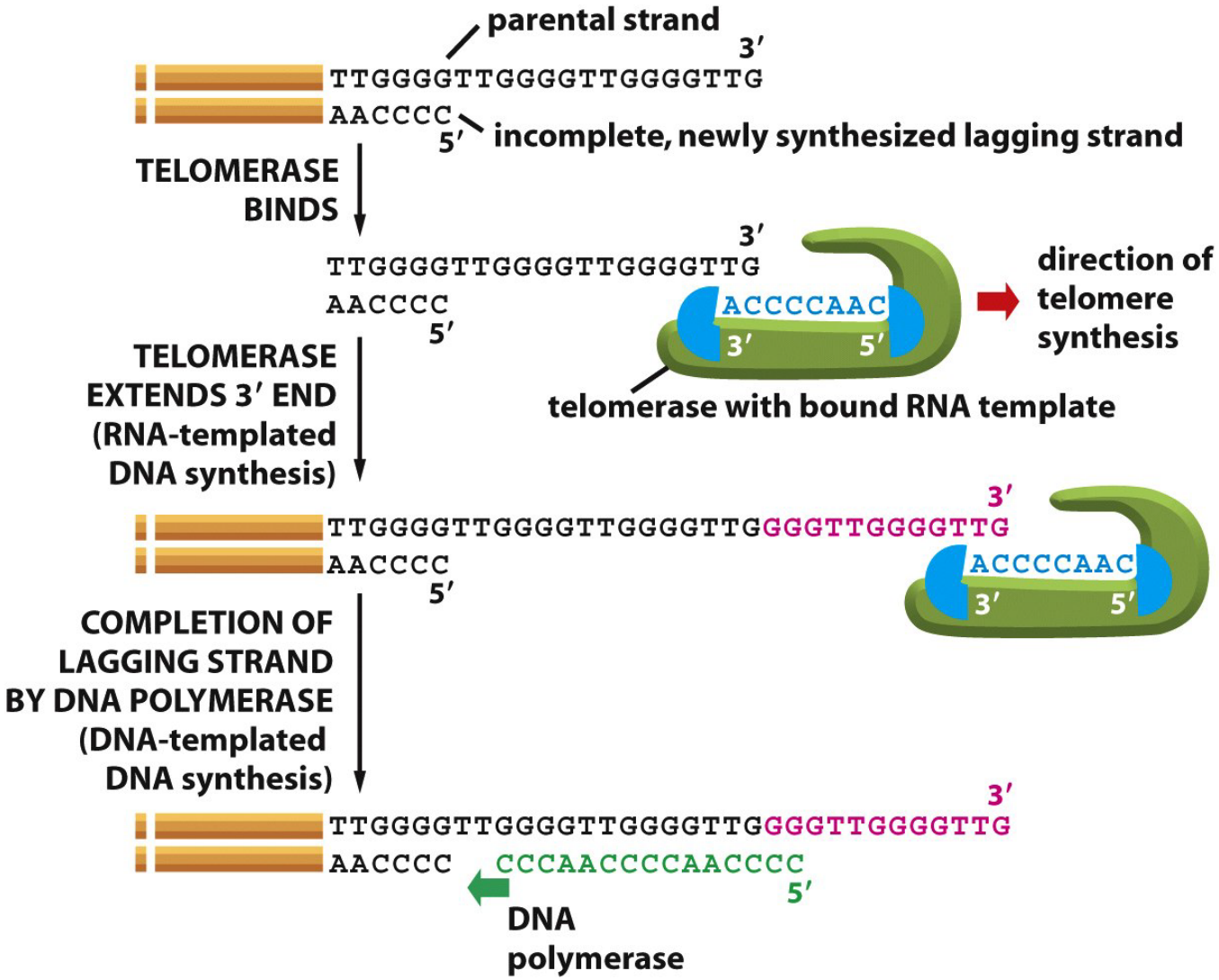
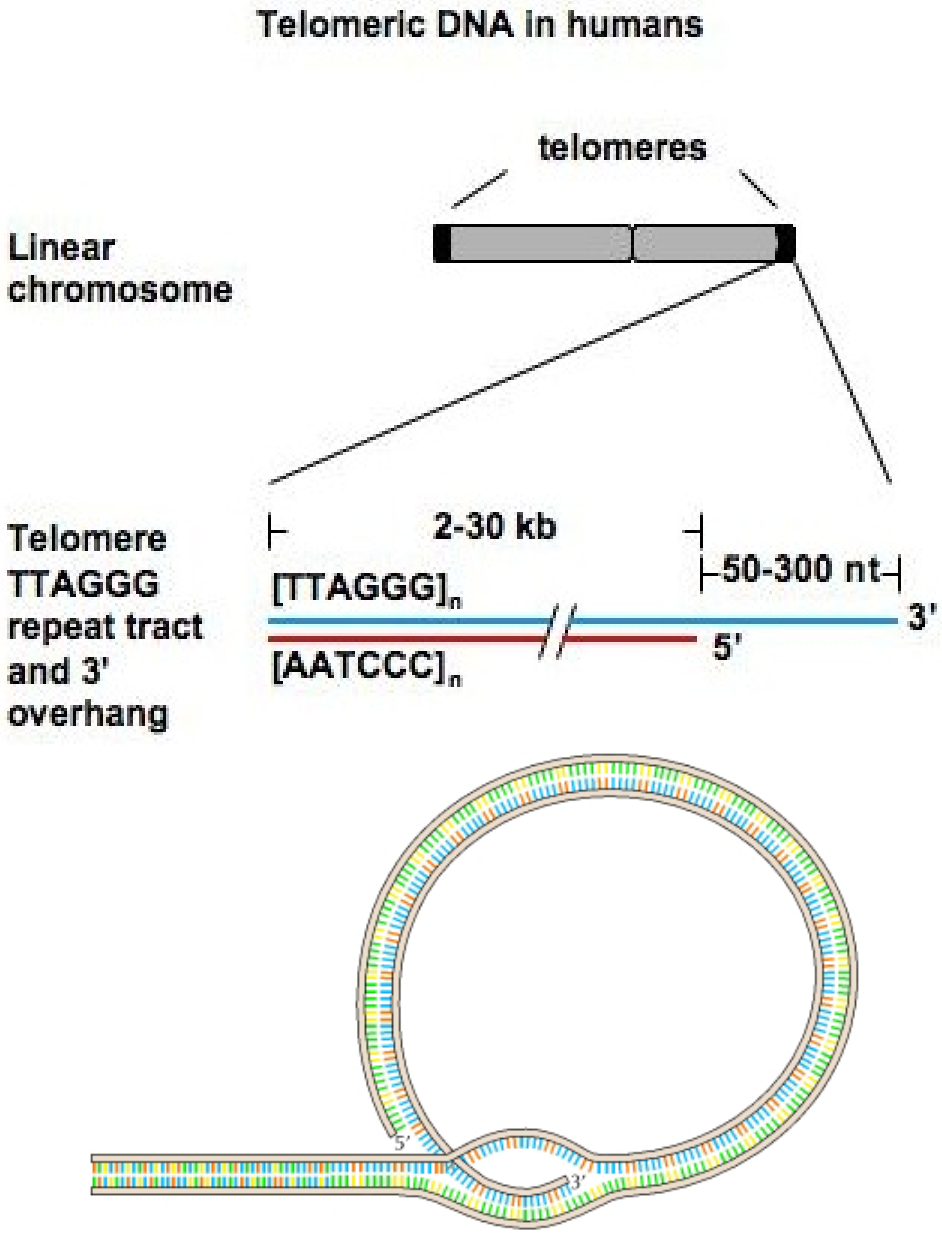


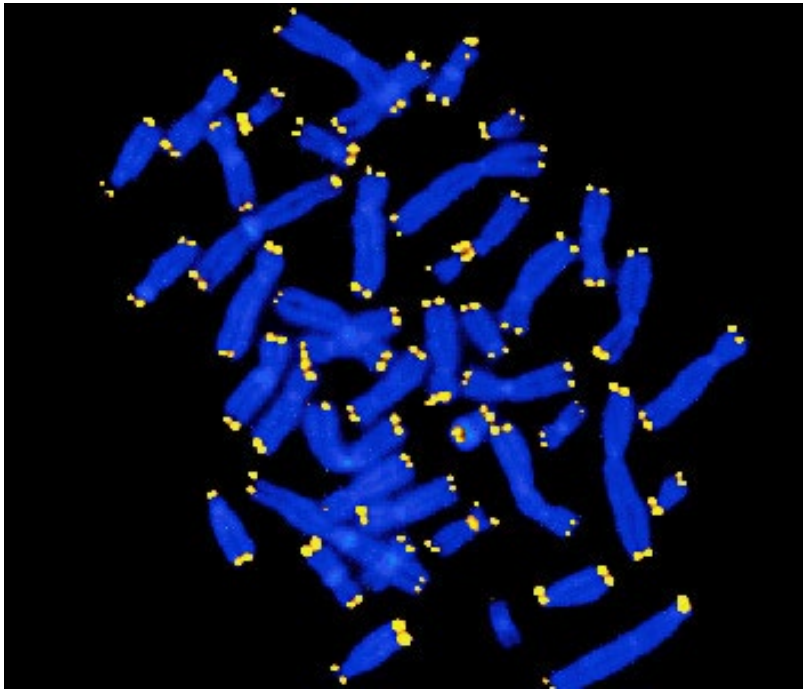
Figure 5-41 Molecular Biology of the Cell 5/e (© Garland Science 2008)

https://www.youtube.com/watch?v=2NS0jBPurWQ

Telômeros

Sequências repetidas nas extremidades dos cromossomos eucarióticos

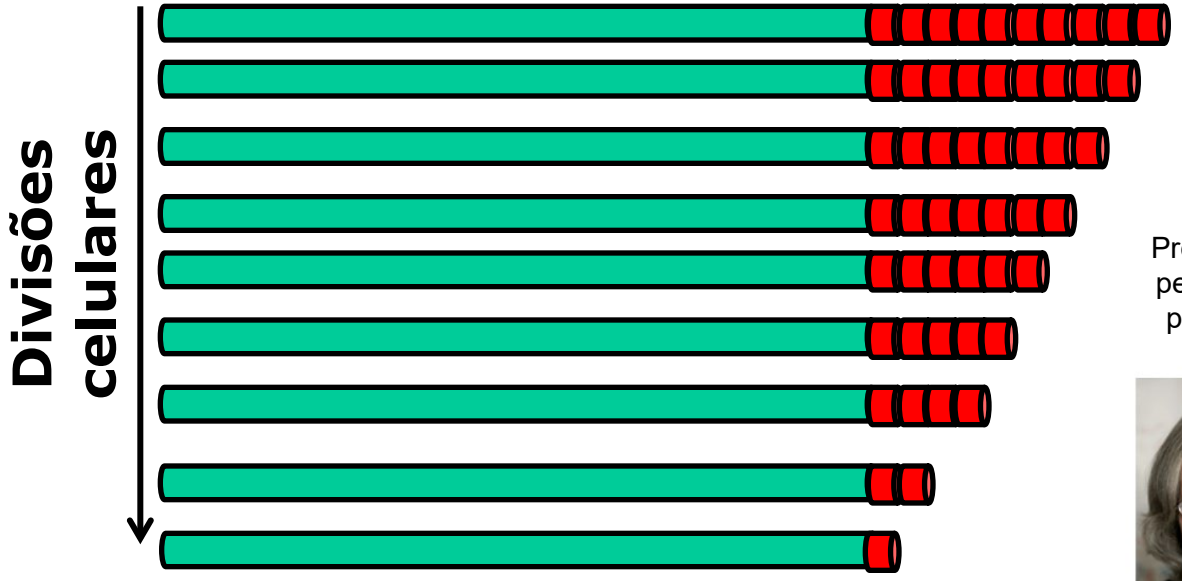




Marcação fluorescente de Telômeros

azul: DNA
amarelo: TTAGGG (repetição telomérica)

Sucessivos eventos de replicação → telômeros diminuem seu tamanho → células entram em **senescência celular replicativa**



Prêmio Nobel em Fisiologia ou Medicina, 2009, pela descoberta de como os cromossomos são protegidos pelos telômeros e pela telomerase


Elizabeth H. Blackburn


Carol W. Greider


Jack W. Szostak

Limite de Hayflick

Limite no número de vezes que as células mitóticas podem se dividir, que se correlaciona com o tamanho dos telômeros.