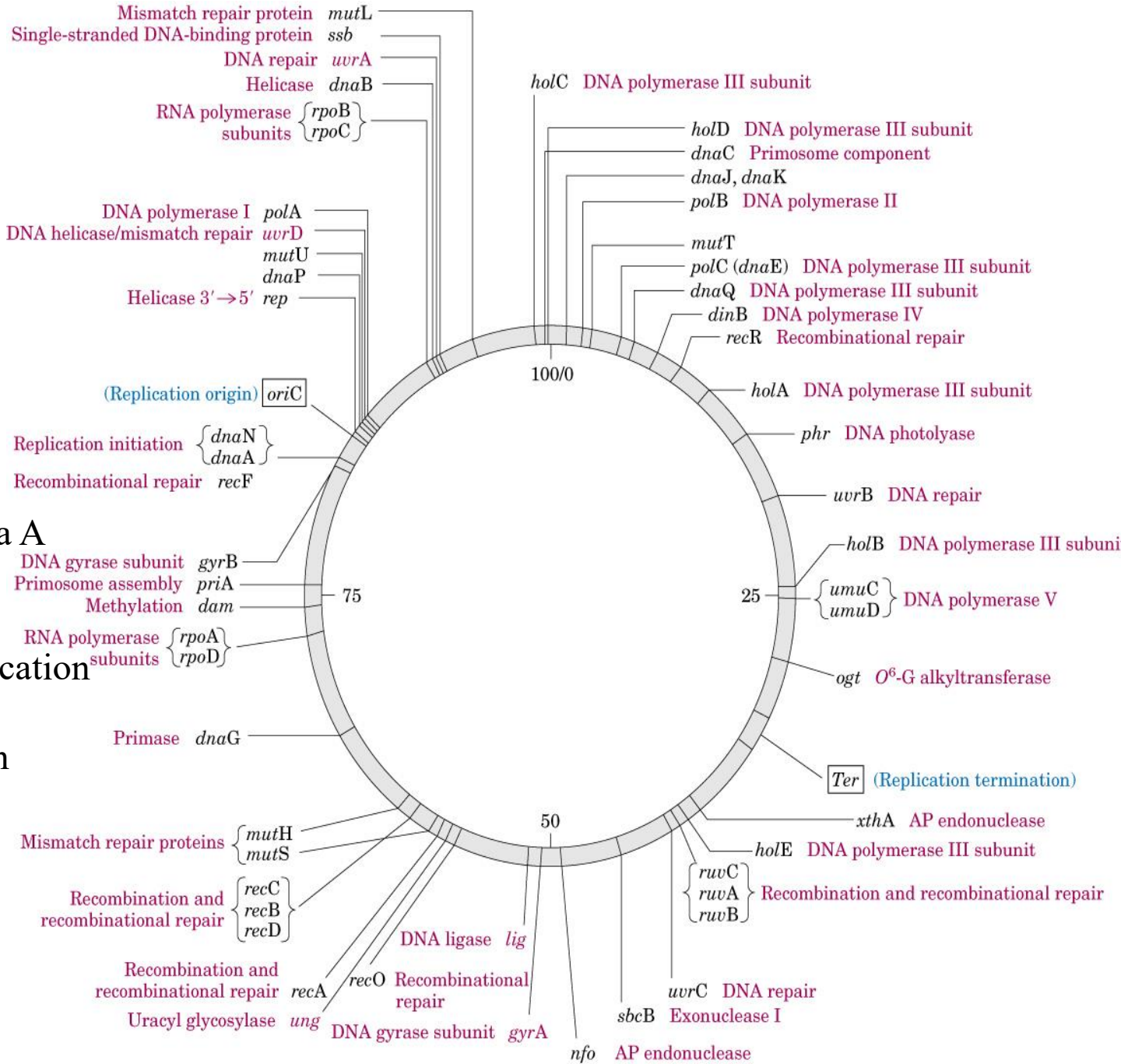
Map of E. coli chromosome

No. 0-100
Minutes (genetic measurement)
1 minute=40,000 bp.
Lower case, italics= Bacterial genes,
Name reflect function
A,B,C reflect order of discovery not order in rxn sequence
Upper case= protein product e.g. Dna A
dna= DNA replication
lig = DNA ligase
ter=termination of replication
pol=DNA polymerase
ori=origin of replication
rpo=RNA polymerase
uvr=UV resistance
rec=recombination
Dam=DNA adenine methylation



Watson – Crick model:

DNA structure revealed DNA strand serves as a template for replication.

Meselson- Stahl experiment:

Semiconservative replication:

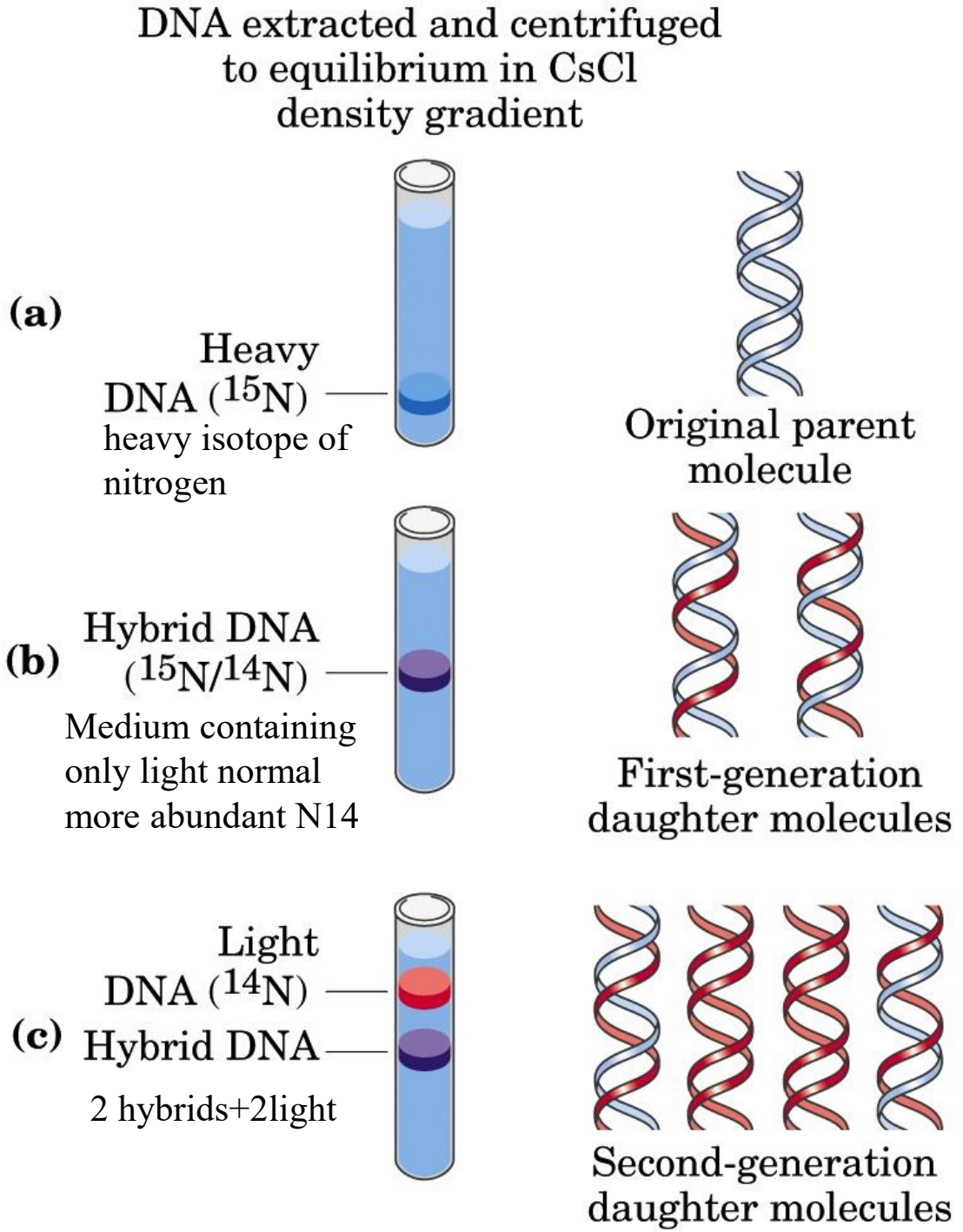
Grew E coli in medium containing only N15 as nitrogen source

Transferred to fresh medium containing only N14 till cell population allowed to double →

DNA isolated contained a single band showing hybrid

Transferred to fresh medium containing N14 DNA

isolated contained two bands.



Following the confirmation of semiconservative replication several questions:

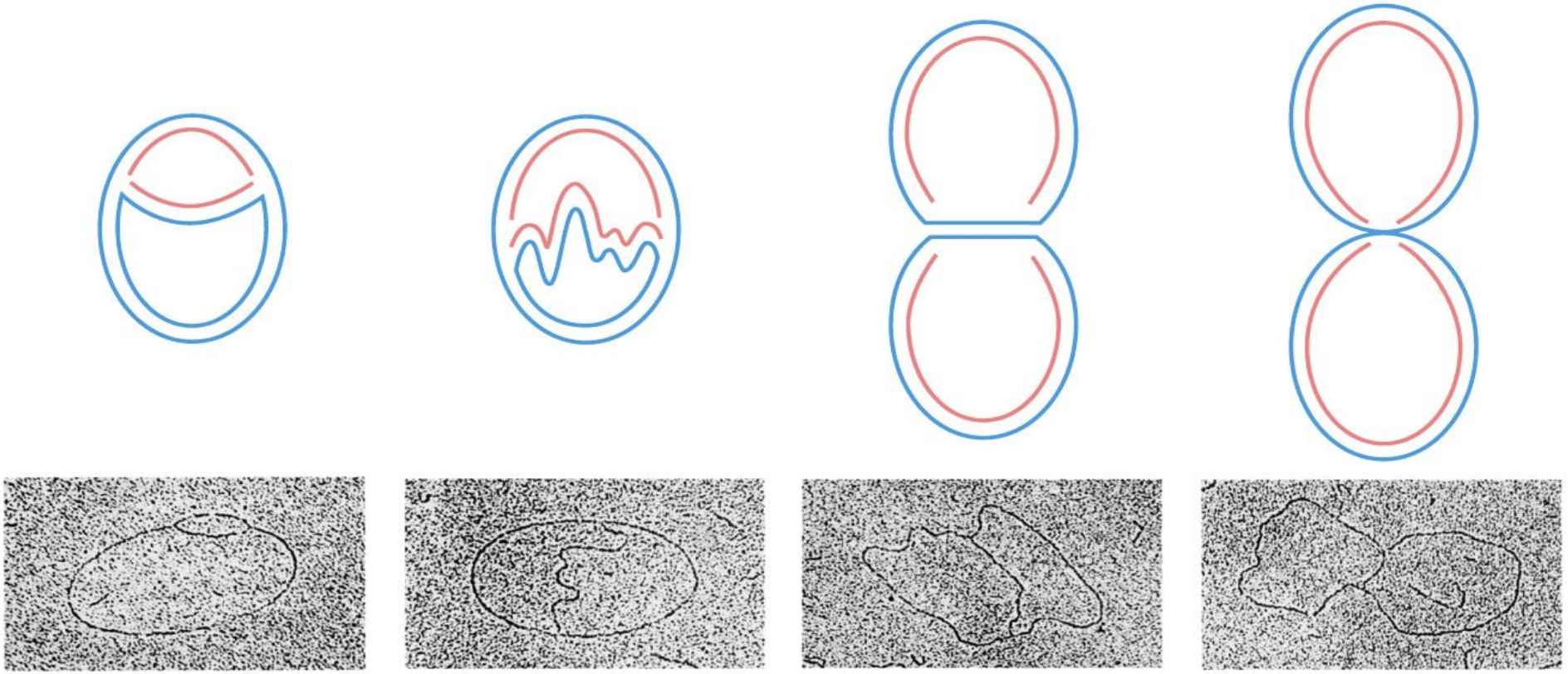
1- Are the parent DNA strands completely unwound before replication?

2- Does replication begin at random point or at a unique point ?

3- Does replication proceed in one direction or bidirectionally ?

Replication begins at an origin and proceeds bidirectionally:

Growing bacteria in medium containing thymidine labelled with tritum H3.
DNA isolated, spread, overlaid on autoradiograph. Radioactive thymidine residues generated "tracks" of silver grains producing an image of DNA.
Tracks revealed intact E. coli chromosome is single, huge DNA circle 1.7mm long.
Radioactive DNA isolated showed extra loop resulted from the formation of radioactive strands. One or both ends are dynamic ends called replication forks.



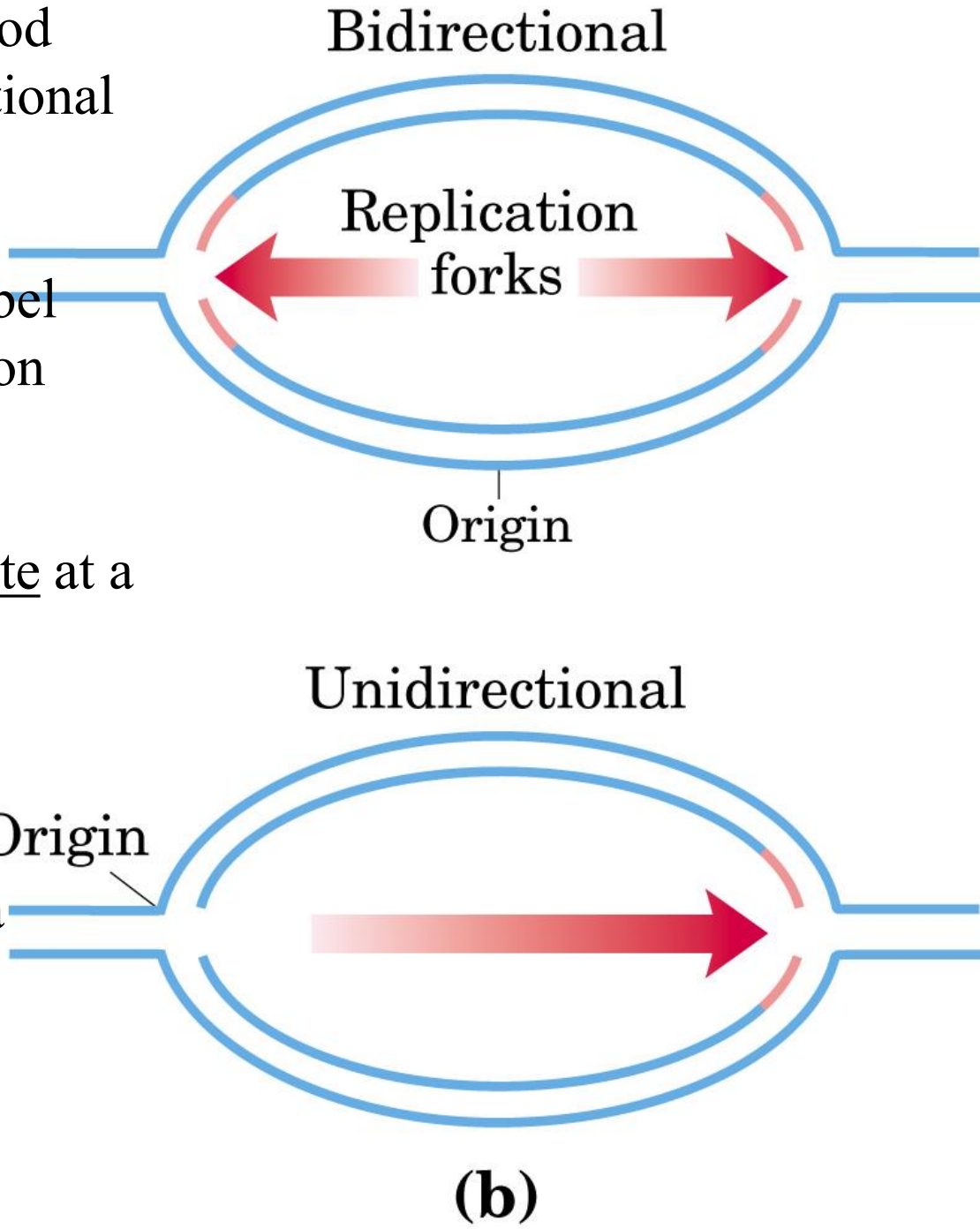
(a)

Addition of H3 for a short period Allows to distinct bw unidirectional and bidirectional replication.

By determining whether the label (red) is at one or both replication forks.

Replication loops always initiate at a unique point termed origin.

Two replication forks meet at a point on the side of the circle opposite to the origin.



DNA Synthesis

Requirements

1. Enzyme: DNA Polymerase
2. DNA Template
3. 3’ OH (primer of DNA or RNA)
4. Deoxynucleoside triphosphates: dATP, dGTP, dCTP, dTTP
5. Synthesis is 5’ to 3’

"problems" that must be overcome for DNA pol to copy DNA:

- The two strands in DNA duplex are opposite in chemical polarity, but all DNA pol catalyze nucleotide addition at the 3′-hydroxyl end of a growing chain, so strands can grow only in 5′ to 3′ direction.

  Solution: Okazaki fragment.

- All known DNA pol can only elongate a preexisting DNA or RNA strand (the primer) and are unable to initiate chains.

  Solution: RNA primers.

- DNA pol are unable to melt duplex DNA in order to separate the two strands that are to be copied.

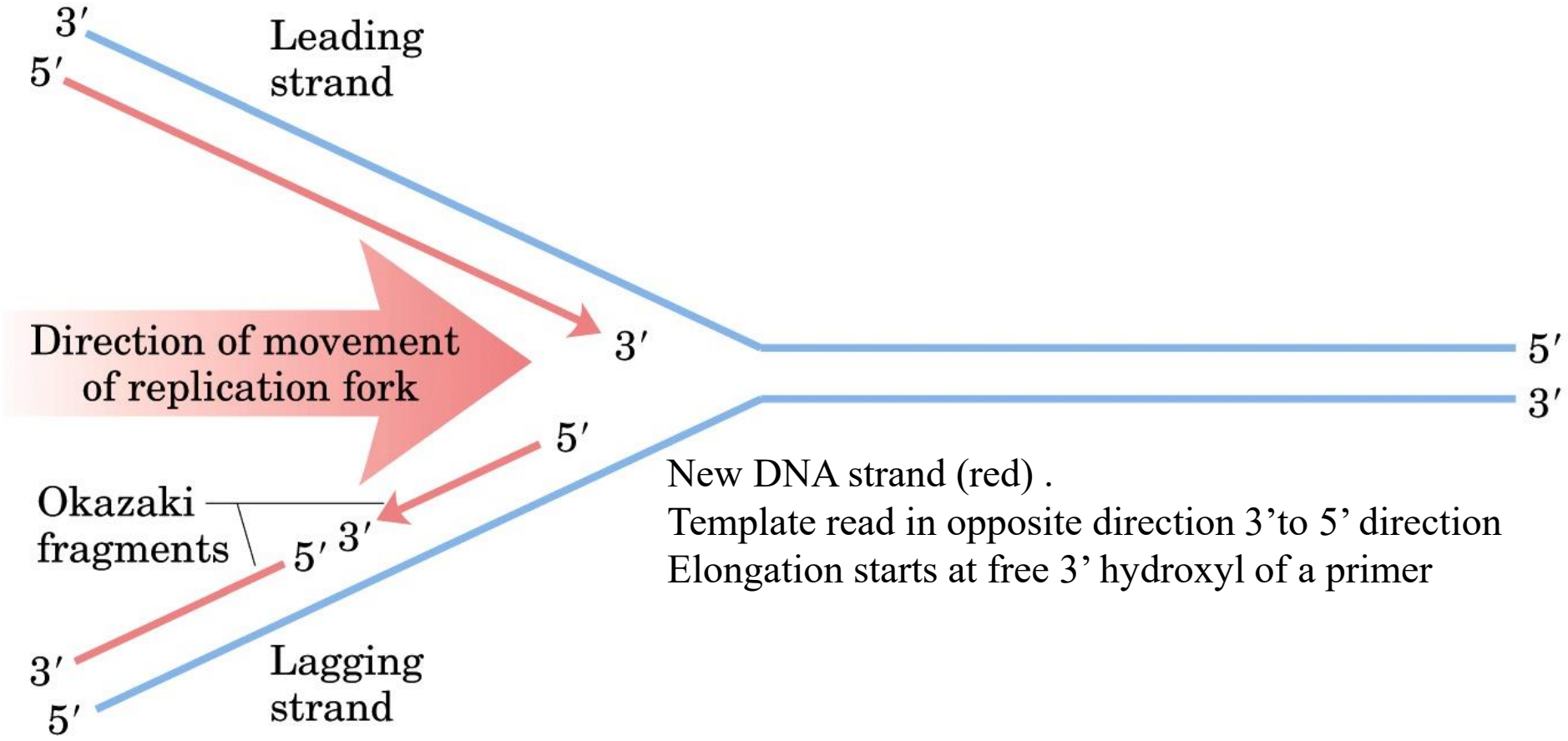
  Solution: helicase and SSB.

DNA synthesis proceeds 5'→3' direction and is Semicontinous.

If DNA synthesis proceeds 5'→3' direction how can both strands replicated continuously problem resolved by Okazaki.

One strand synthesized continously (leading strand), the other one (lagging strand) in short pieces (okazaki fragments).

Okazaki: bacteria 1000-2000 nucleotide ,eukaryotes 150-200 nucleotide long.



New DNA strand (red) .
Template read in opposite direction 3'to 5' direction
Elongation starts at free 3' hydroxyl of a primer

DNA degradation:

Nucleases degrade nucleic acids.

DNasess specific for DNA

RNases specific for RNA.

Exonuclease: degrade nucleic acids from one end of the molecule either from 3' or the 5' of dsDNA or ssDNA.

Endonuclease: degrade nucleic acids at internal sites.

Restriction endonucleases: cleaves only at specific internal sites important in Biotechnology.

DNA is synthesized by DNA pol.

Kornberg group purified and characterized DNA pol I from E. coli encoded by *polA* gene

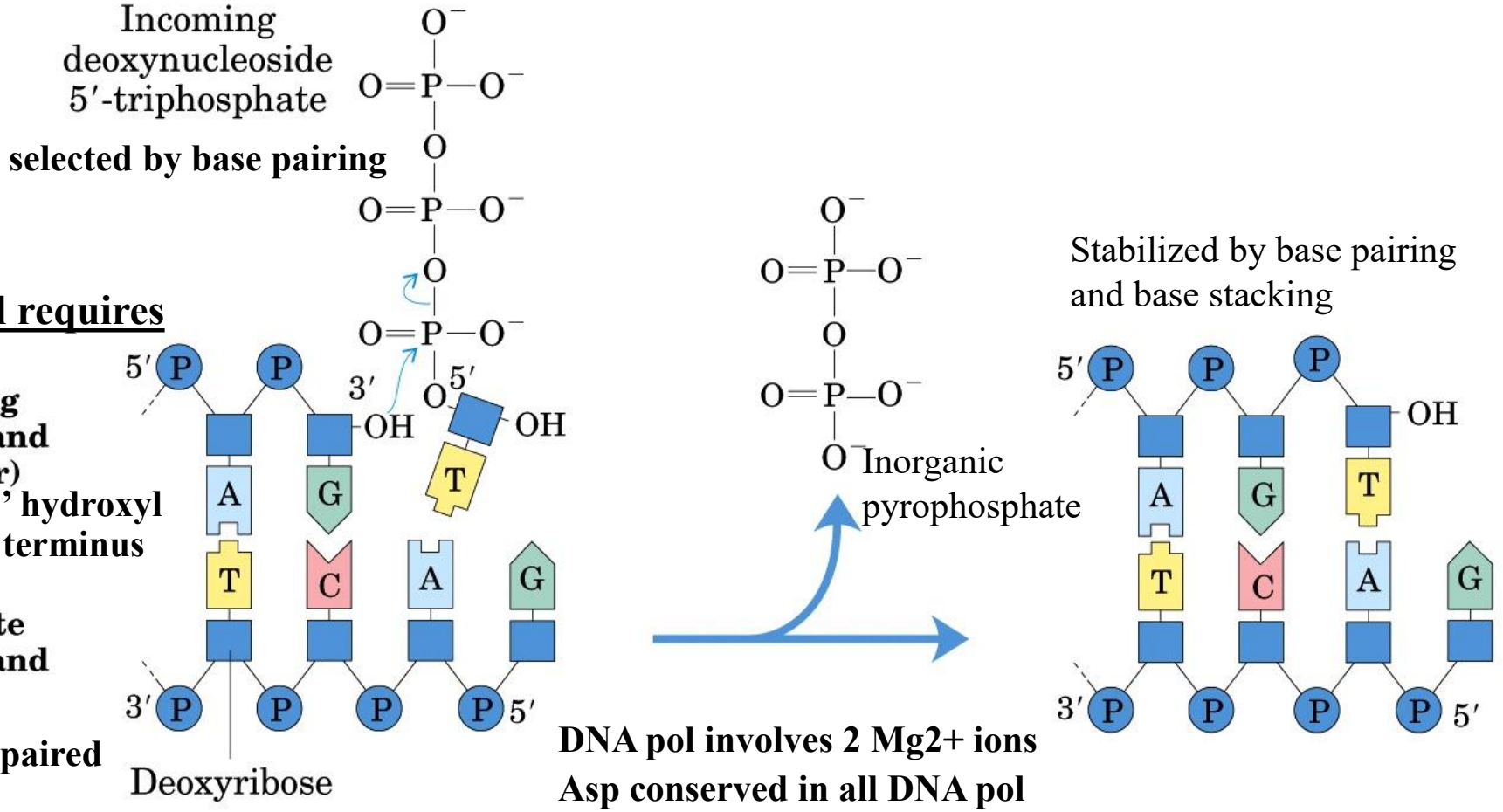
Later studies→E coli contains 4 other DNA pol.

Fundamental rxn is phosphoryl group transfer:

nucleophile = 3'hydroxyl group of the growing strand , nucleophilic attack at alpha phosphorus of deoxynucleoside 5'-triphosphate.

(dNMP)n + dNTP → (dNMP)n+1 + PPi

DNA **lengthened DNA**



Nucleotide addition guided by base pairing:

When A is present in template, a T nucleotide is added to the new strand.

Important discovery:

first example of a template leading the rxn.

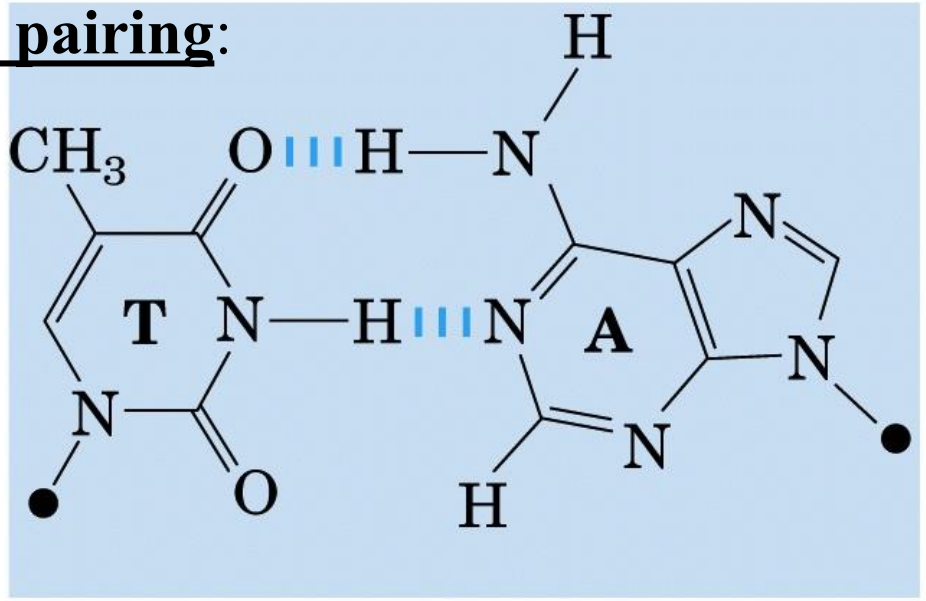
DNA pol requires a primer meaning part of the product /new strand is already present. All DNA pol require preexisting Primer synthesized by RNA pol when required.

After adding a nucleotide to a growing DNA strand , DNA either dissociates/ moves along the template to add more nucleotides. Dissociation and reassociation of pol affects /limits the polymerization rate.

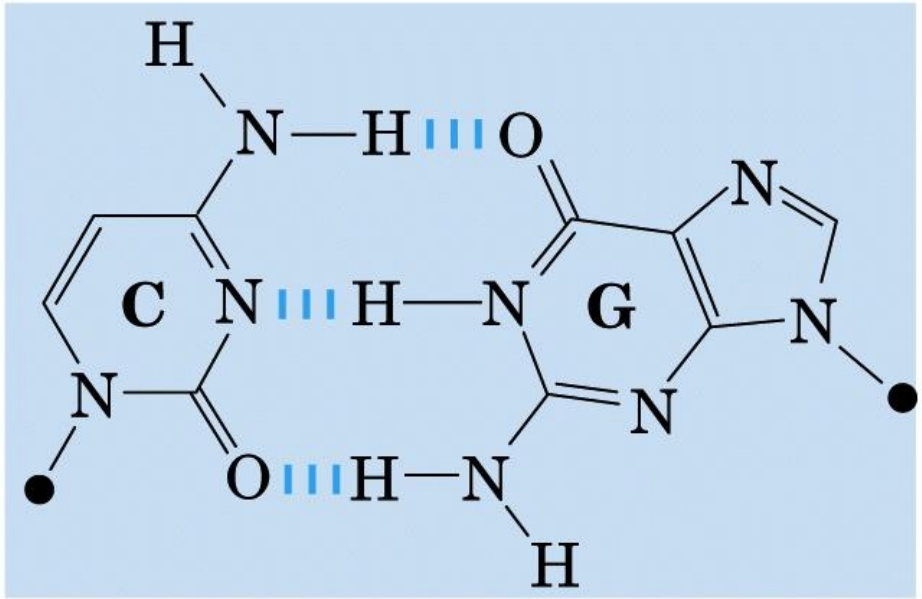
Processivity:

Av. number of nucleotides added before dissociating.

Some adds few nucleotides others thousands.



|| Similar Geometry || fit in active site



(a)

Replication is very accurate:

Replication proceeds with high fidelity.

In E coli a mistake occurs / 10^9-10^{10} nucleotides

Discrimination bw correct and incorrect nucleotide:

1- hydrogen bonding

2- common geometry of the standard A-T and G-C

(active site of DNA pol accommodate correct geometry

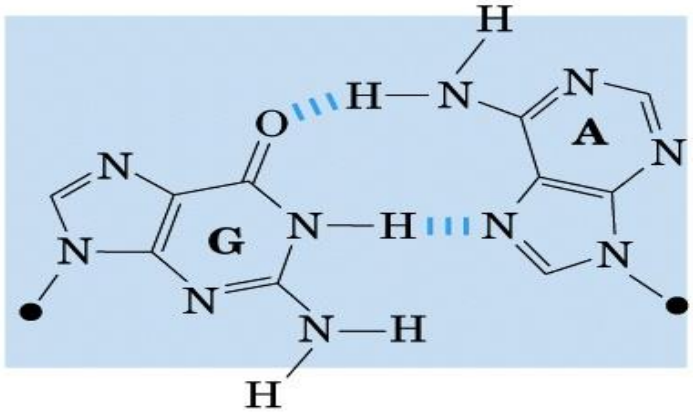
Incorrect bases rejected before phosphodiester bond formed.

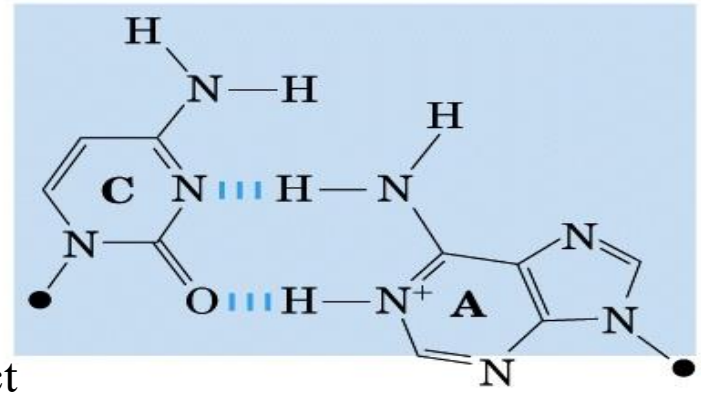
However, this doesn't account for high fidelity of pol.

in replication.

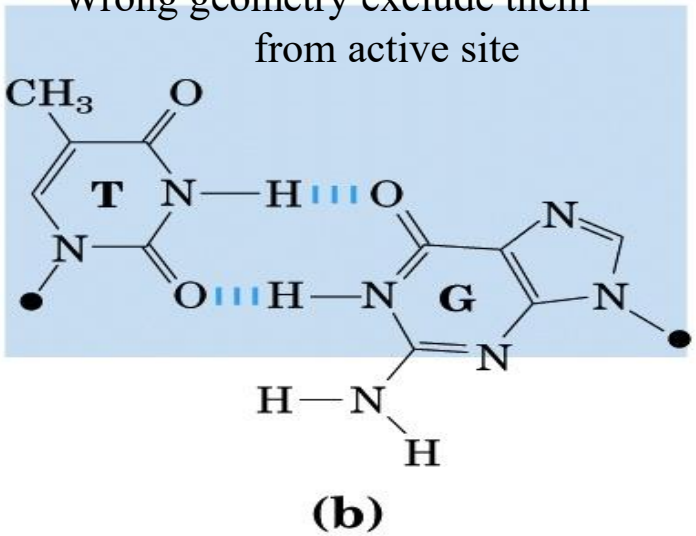
Studies showed DNA pol adds incorrect base / 10^4-10^5 correct one.

Another mechanism!!!





Wrong geometry exclude them from active site



Intrinsic property of all polymerases:

3'→5' exonuclease activity double checks each nucleotide after addition= **PROOFREADING**

If incorrect base added (mismatched base pairs) → Translocation of pol to next nucleotide inhibited = Kinetic pause = opportunity for correction.

3'→5' exonuclease activity removes mismatched

Then polymerase starts again.

Proofreading not reverse of polymerization (pyrophosphate is not involved).

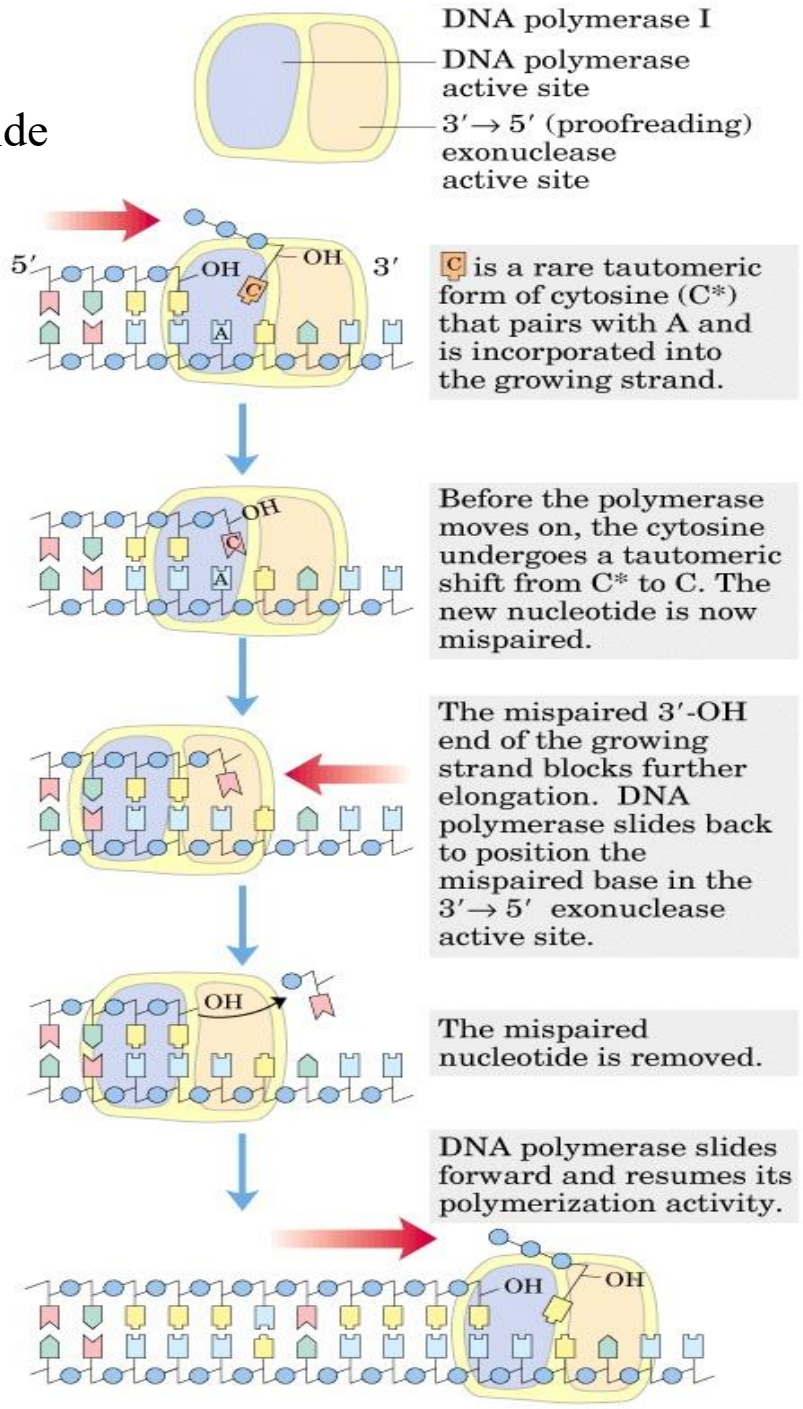
Proofreading improves polymerization accuracy

One error/ 10^6-10^8 bases added.

Still measured accuracy is much higher.!!!!

Other mechanism!!!

Repair System



➢DNA pol I

1- The rate it adds nucleotides 600 nucleotide/min too slow (< factor of 100) than the rate of replication fork in E. coli.

2- DNA pol I has slow processivity.

3- genetic studies → many genes therefore proteins are involved (pol I not acting alone)

4- An isolated bacterial strain with mutated DNA pol I (sensitive to DNA damage) but viable !!

table 25–1

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†	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

*For enzymes with more than one subunit, the gene listed here encodes the subunit with polymerization activity. Note that *dna*E is an earlier designation of the gene now referred to as *pol*C.

†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).

Features of DNA Polymerases

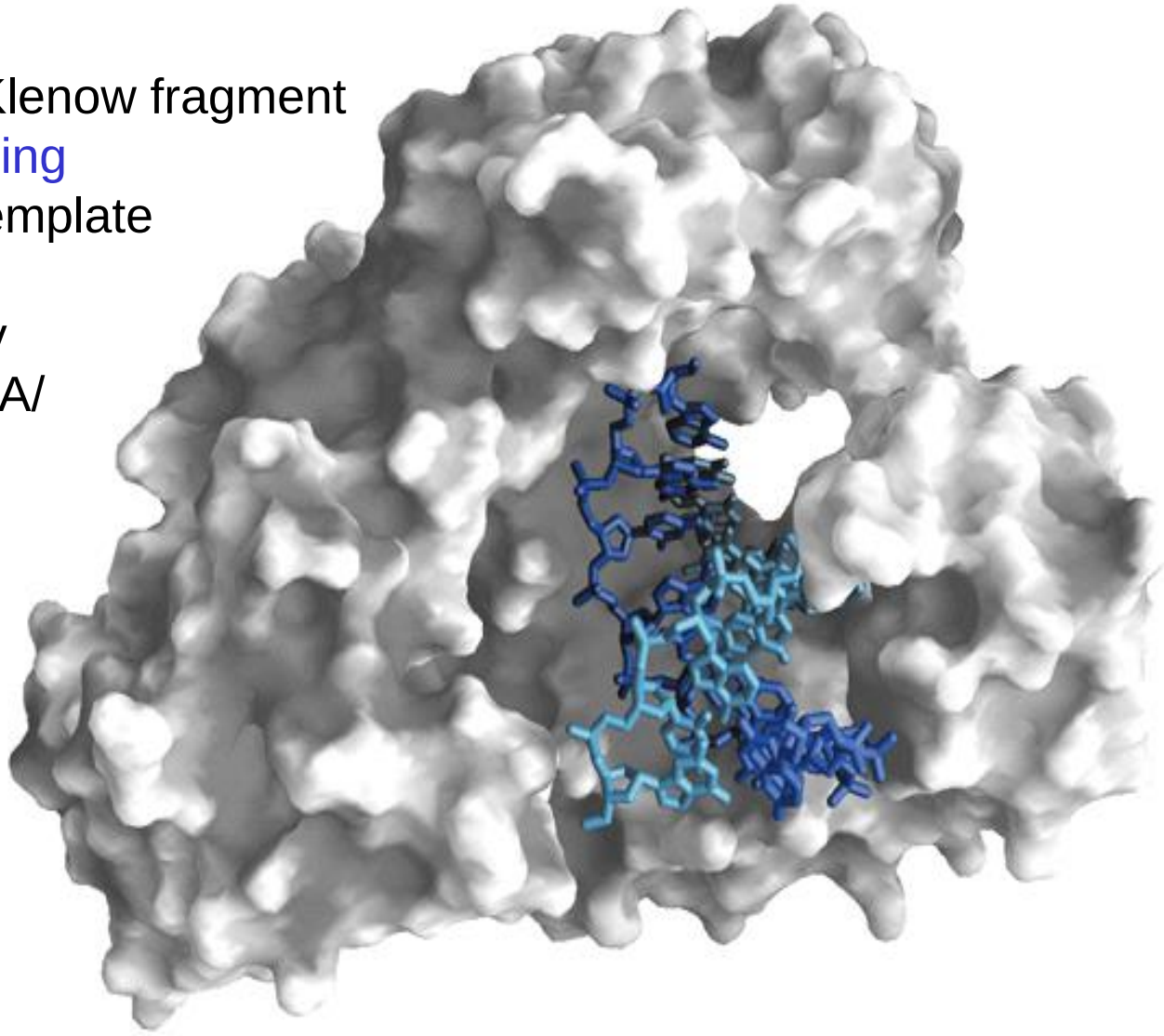
	activity	function
5’ → 3’	polymerase	synthesis
3’ → 5’	exonuclease (to remove non H-bonded base)	editing “proof-reading”
5’ → 3’	exonuclease removes only H-bonded base)	primer removal

DNA pol I:

not the primary enzyme of replication its special in 5'→3' exonuclease activity this activity in a structural domain separated from the enzyme by protease treatment to give **large fragment = Klenow**

Active site is deep in the Klenow fragment
polymerization+proofreading
Dark blue= DNA strand=template

5'→3' exonuclease activity replaces a segment of RNA/ DNA bound to template in a process = nick translation.



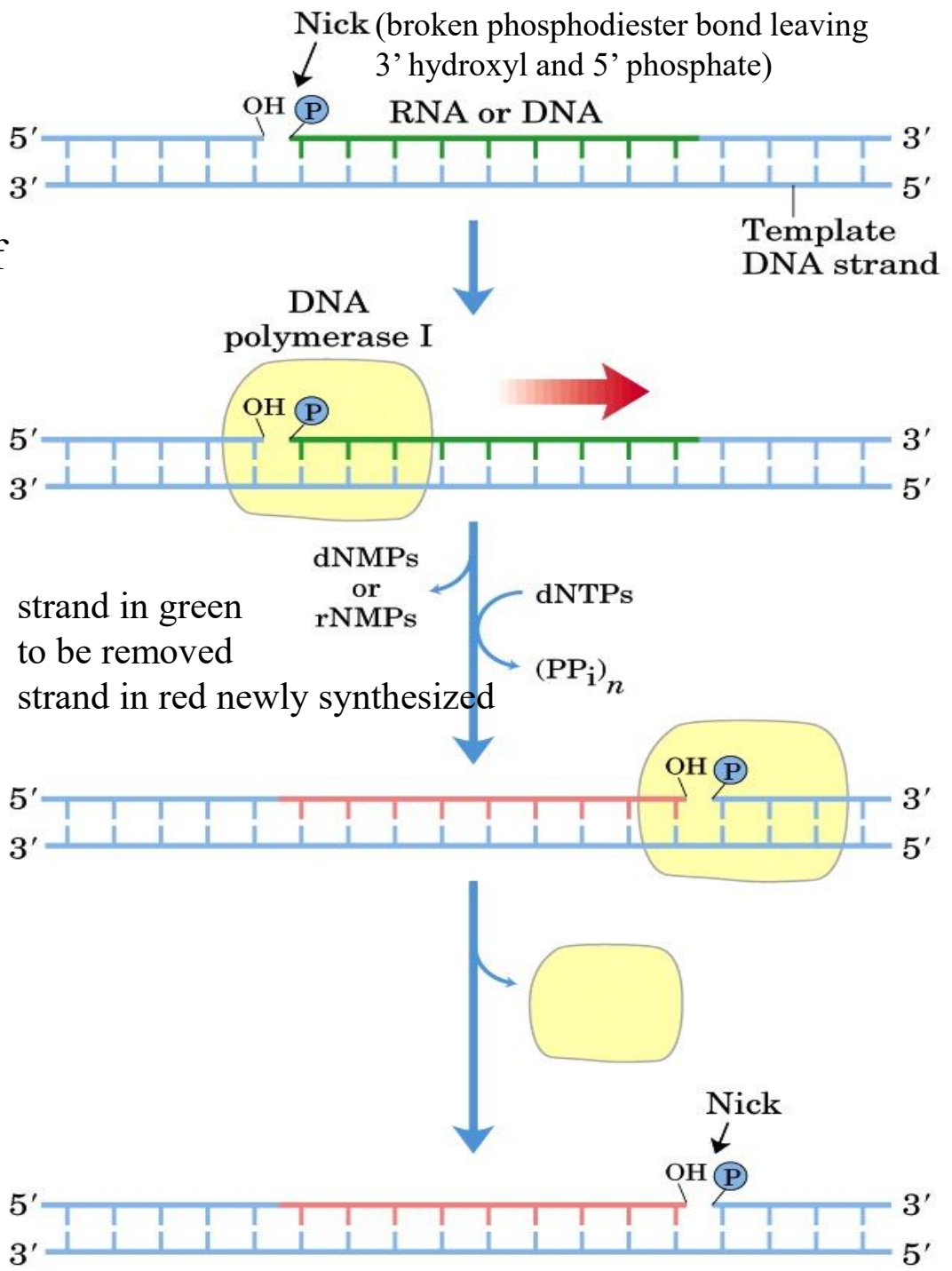
Nick translation:

DNA/RNA strand paired to DNA template degraded by 5'→3' exonuclease activity of DNA pol I and replaced by the same enzyme .

These activities have a role in DNA repair and primer removal during replication.

DNA synthesis begins at a nick , pol I extends the non template DNA strand and moves the nick along the DNA

pol I dissociates, nick sealed by ligase.



DNA fidelity maintained by:

1- base selection by DNA polymerase.

2- proofreading exonuclease 3’→5’ activity.

3- mismatch repair mechanism by 5’→3’ exonuclease activity.

DNA pol III more complex than DNA pol I (ten subunits)

B subunits associates in pairs to form donut-shaped structures that encircle DNA and act like clamps.

table 25–2

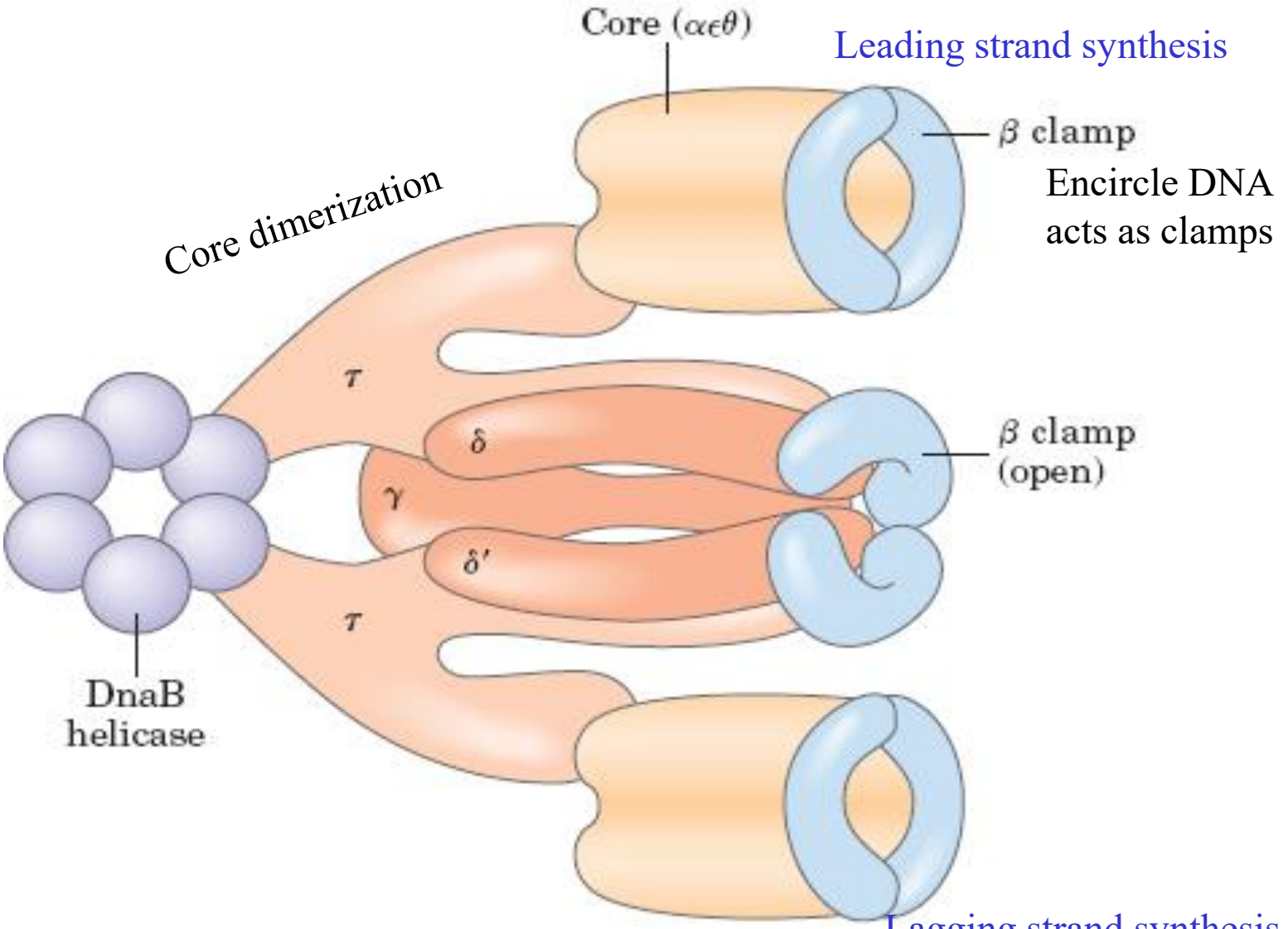
Subunits of DNA Polymerase III of *E. coli*

Subunit	Number of subunits per holoenzyme	M_r of subunit	Gene	Function of subunit	
α	2	132,000	*pol*C (*dna*E)	Polymerization activity	Core polymerase
ϵ epsilon	2	27,000	*dna*Q (*mut*D)	3′→5′ Proofreading exonuclease	
θ	2	10,000	*hol*E		Polymerize DNA with minimum processivity
τ tau	2	71,000	*dna*X	Stable template binding; core enzyme dimerization	
γ	2	52,000	*dna*X*	Clamp-loading complex that loads β subunits on lagging strand at each Okazaki fragment	
δ	1	35,000	*hol*A		
δ'	1	33,000	*hol*B		
χ chi	1	15,000	*hol*C		
ψ psi	1	12,000	*hol*D		
β	4	37,000	*dna*N	DNA clamp required for optimal processivity	Increase processivity to >500,000

*The γ subunit is encoded by a portion of the gene for the τ subunit, such that the amino-terminal 80% of the τ subunit has the same amino acid sequence as the γ subunit. The γ subunit is generated by a translational frameshifting mechanism (see Box 28–1) that leads to premature translational termination.

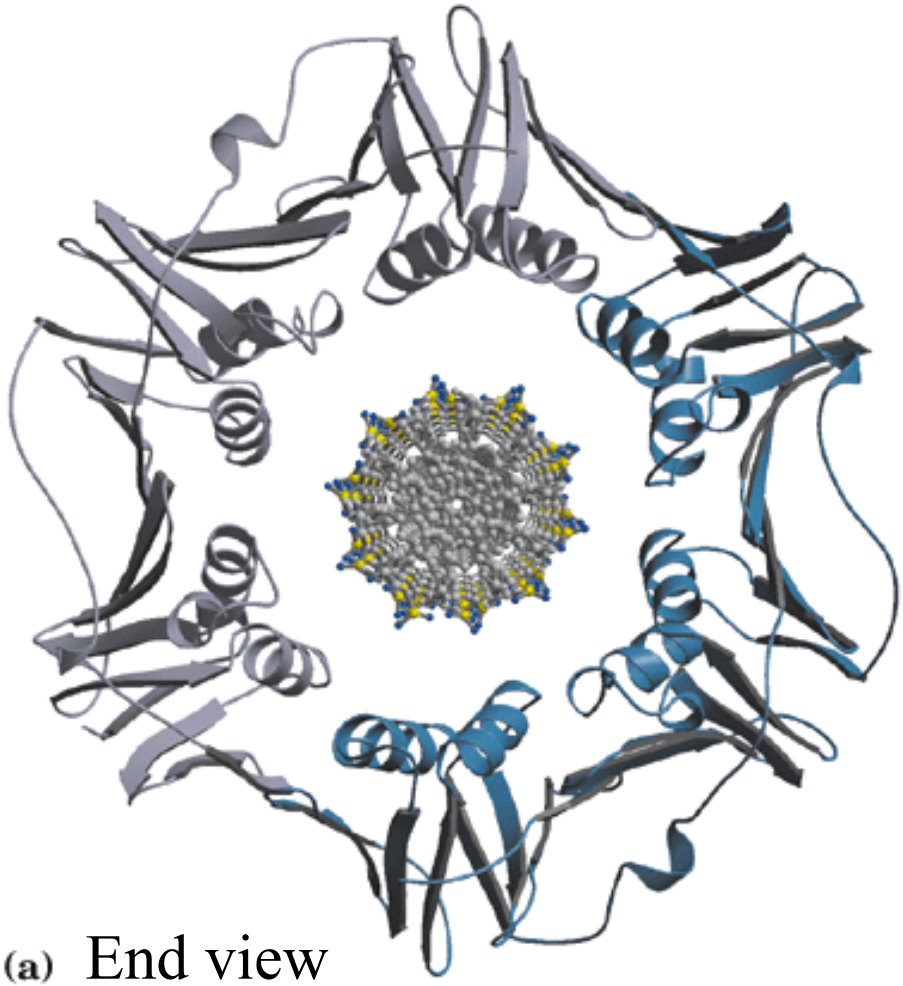
Bacterial DNA pol III :

2 core domains, 2 clamps each a dimer of B subunit, core complex interacts with helicase through tau.

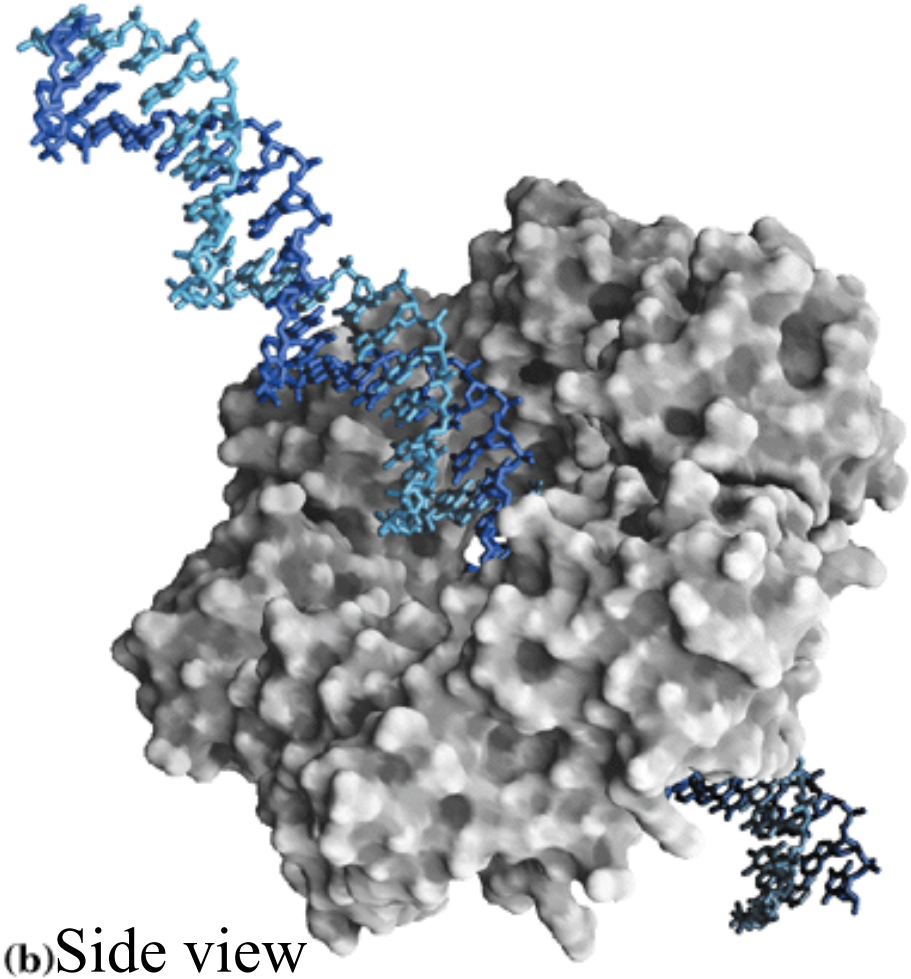


Two β-subunits of E. coli pol III:

Form circular clamp surrounding DNA. The clamp slides along DNA increasing processivity to > 500,000 by preventing its dissociation from DNA.



(a) End view



(b) Side view

DNA replication requires not only a single DNA pol. but >20 different enzymes and protein.
The entire complex = **DNA replicase system = replisome**.

Access to DNA strands requires
1) **helicases**=enzymes move along DNA and separate dsDNA using ATP
2) **topoisomerase** to relieve stress created from strand separation/ unwinding of the helical DNA.
3) **DNA binding proteins** to stabilize separated strands.
4) **Primases** to synthesize primers (short segments of RNA removed and replaced by DNA pol I.
5) **DNA ligase** to seal the nick /gap (broken phosphodiester bond) after primer removal.

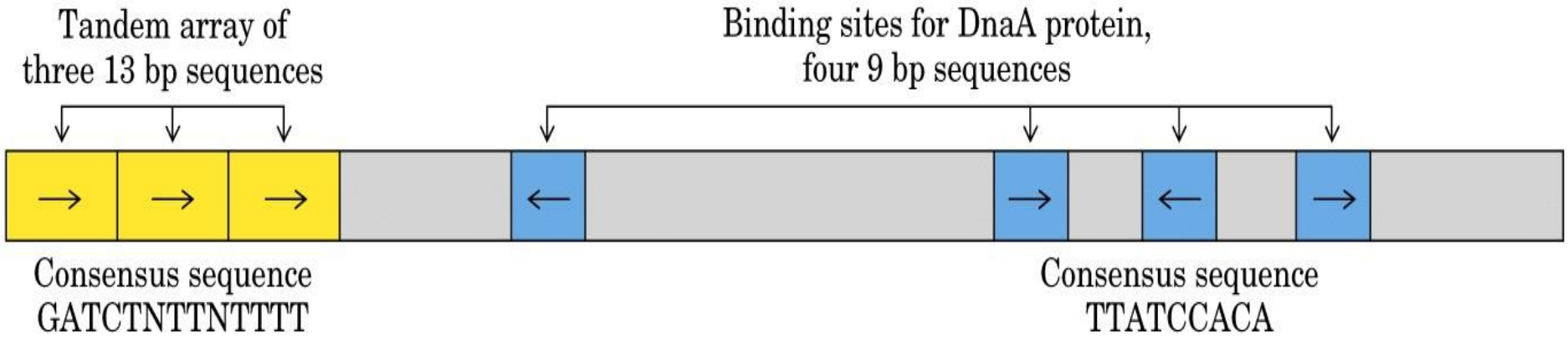
Synthesis of DNA divided into 3 stages:

Initiation, Elongation, Termination

1) Initiation:

Ori C (245bp) contain 2 sequences of short repeats, three repeats of a 13bp sequence and four repeats of a 9bp sequence.

Consensus sequence: contains highly conserved sequence.



N represents any of the four nucleotides.

At least 9 enzymes are involved in the initiation phase of replication.

They open the DNA helix at the origin.

DnaA = the crucial component for initiation.

table 25–3

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

*Subunits in these cases are identical.

DnaA crucial for initiation:

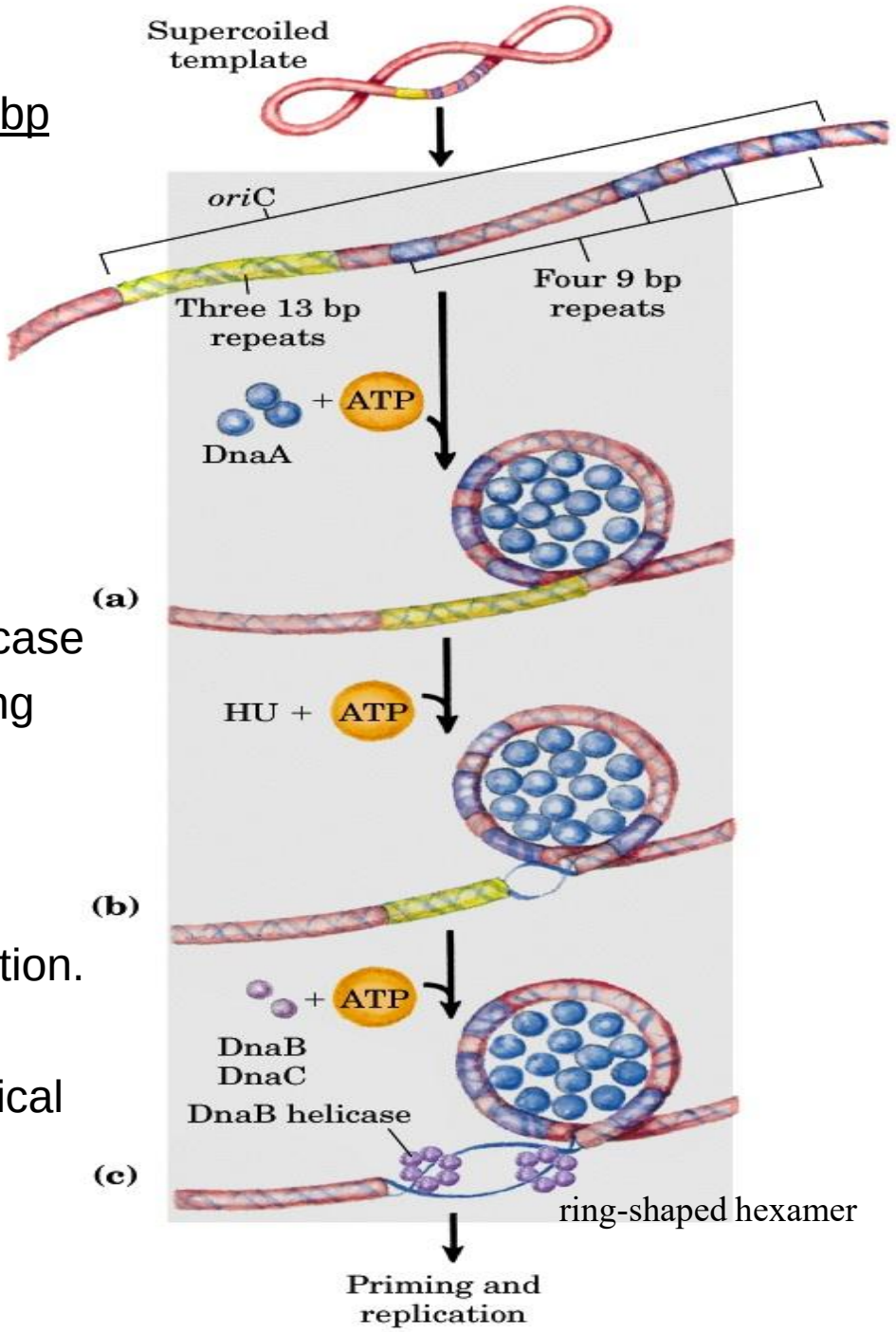
a) DnaA protein molecules bind to the four 9bp repeats in the origin.

b) recognizes→denatures DNA in the region of 13bp repeats (A=T rich) using ATP+ HU.

c) DnaC protein loads DnaB onto unwound region. 2 ring shaped DnaB hexamers, one loaded onto each strand of DNA, act as helicase Helicases unwind DNA bidirectionally creating 2 replication forks.

SSB=single stranded DNA binding proteins, stabilize separated strands prevent renaturation.

Topoisomerase II (gyrase)= relieves topological stress produced by helicase.



Initiation: the only phase in DNA replication that is regulated:

1) Methylation

- oriC DNA methylated by Dam methylase (DNA adenine methylation)

  methylates at N6 position of adenine in 5'-GATC-3' which is highly abundant in oriC 11/245bp. (in E.coli whole chromosome 1/245bp).

- After replication parent DNA oriC is methylated but newly synthesized strands aren't →hemimethylated oriC sequestered by interaction with plasma mb.

- After a time oriC released from plasma mb →→fully methylated by Dam methylase →→ binds DnaA.

Replication Timing depends on methylation and plasma mb interaction

2) Phosphorylation

Involves slow hydrolysis of ATP

DnaA (bound to ATP) →→ DnaA (bound to ADP)

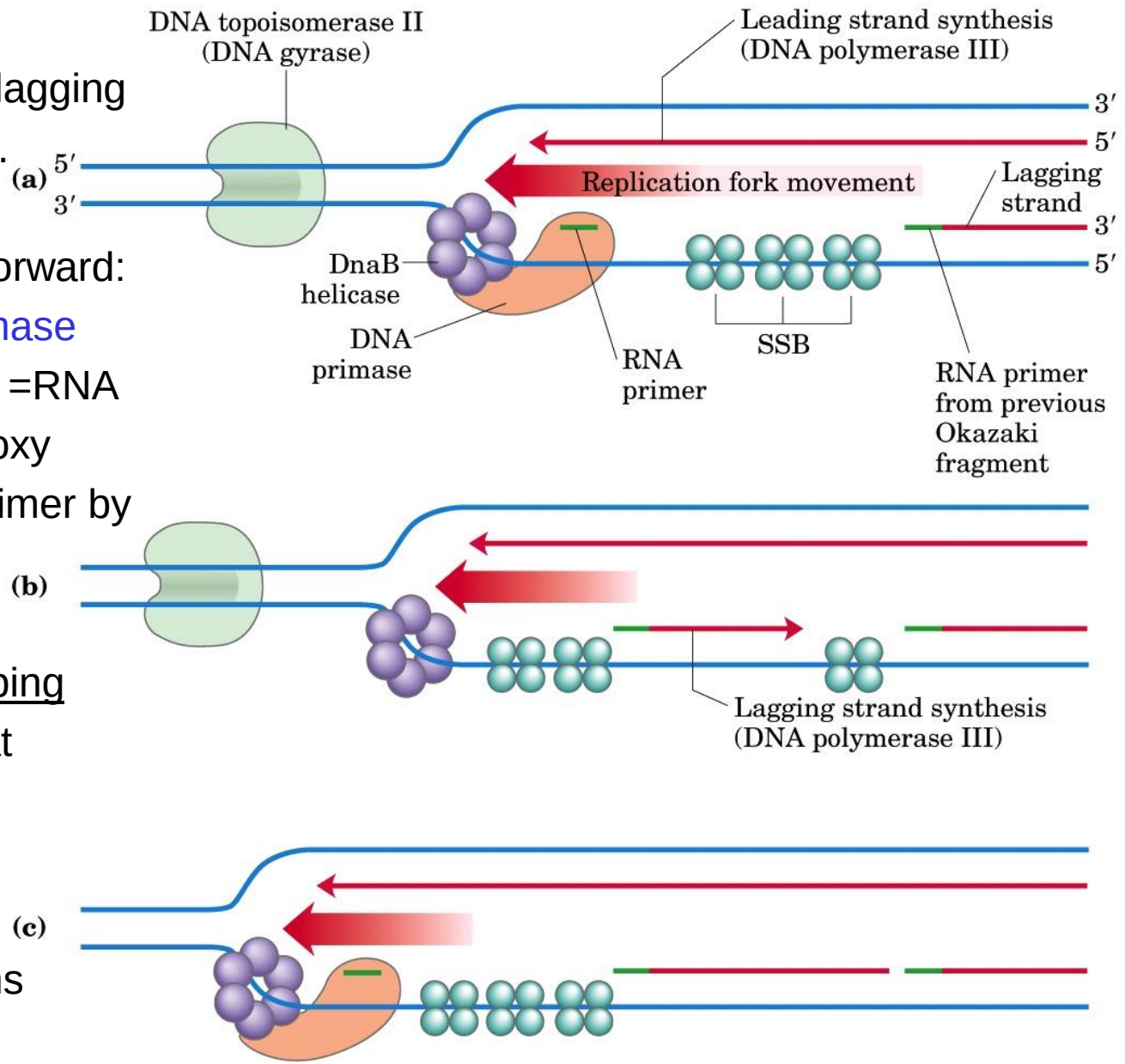
Active **inactive**

2) <u>Elongation</u>: involves 2 stage leading and lagging strand synthesis
-DNA helicases unwound DNA , topo II relive stress, each separated strand stabilized by SSB.

from this point leading and lagging strand synthesis is different.

1) <u>Leading strand</u> straight forward: Begin with synthesis of primase (DnaG) to 10-60 nucleotide =RNA primer at replication ori, deoxy ribonucleotides added to primer by DNA polyIII .
Leading strand synthesis proceeds continuously <u>keeping pace</u> with DNA unwinding at replication fork.

2) <u>lagging</u>: same as above, but for each fragment seems continuous.



Proteins acting in the entire complex at the replication fork (Replisome):

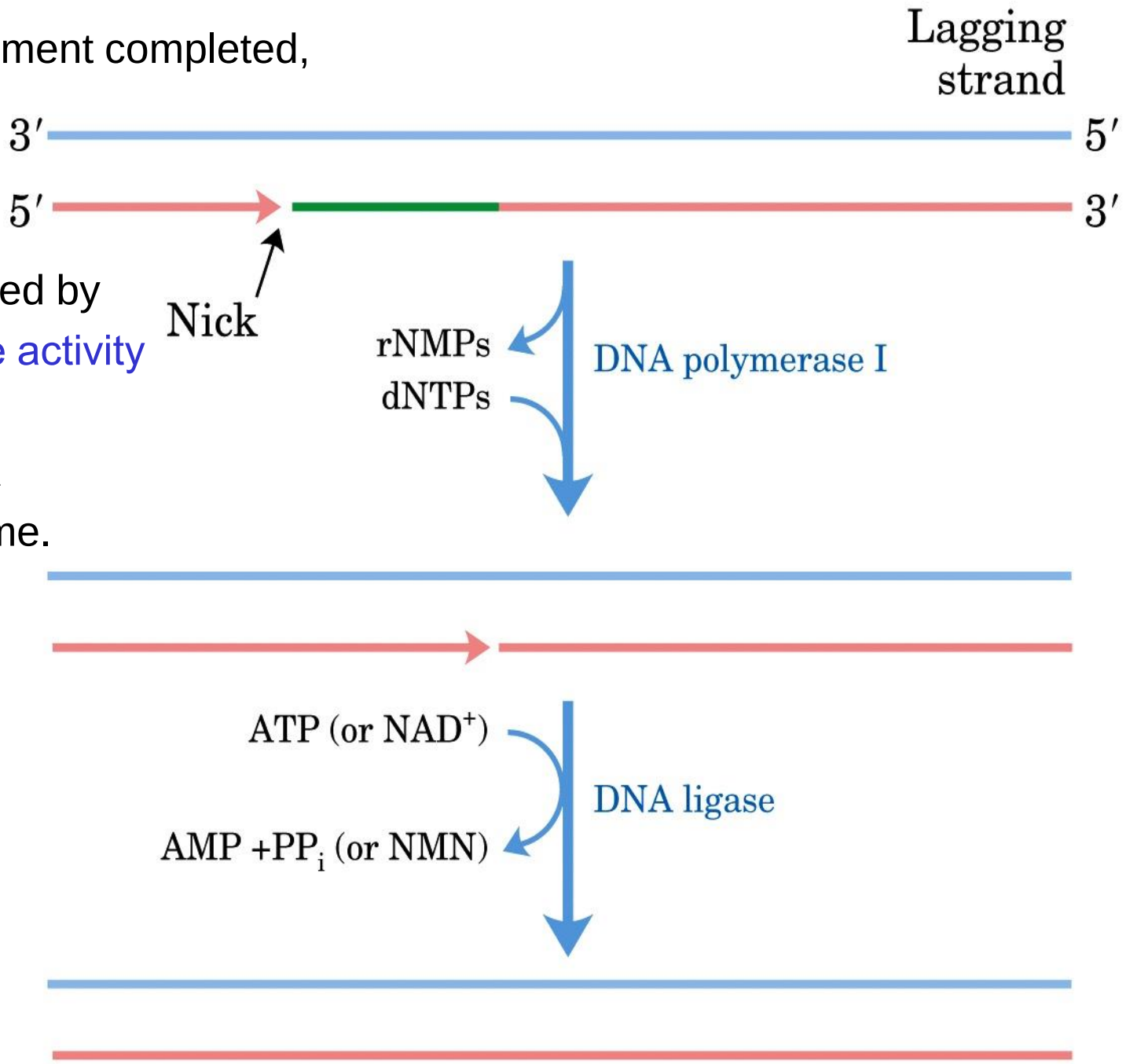
table 25–4

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.

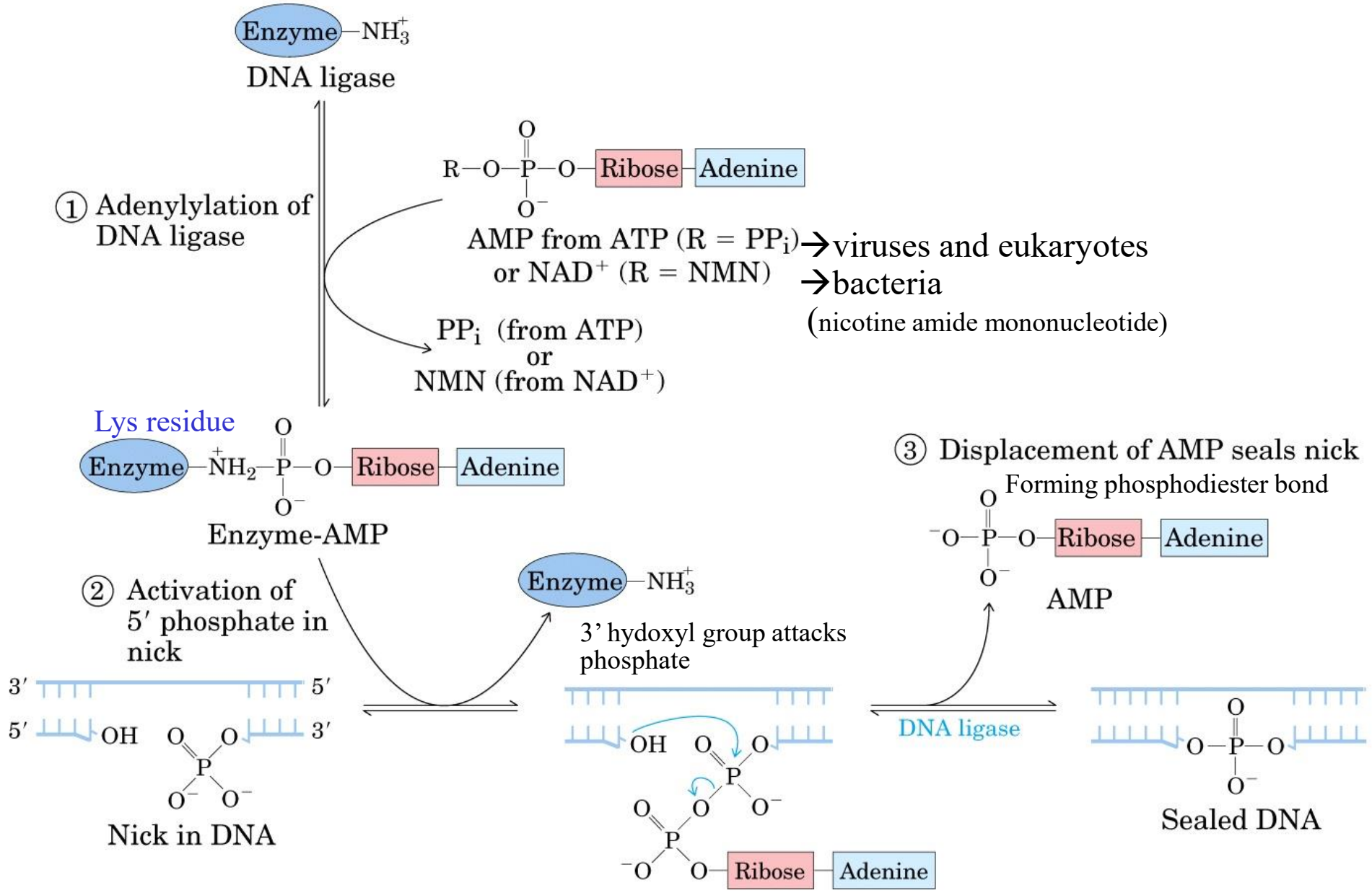
Once Okazaki fragment completed,



RNA primer removed by
5'→3' exonuclease activity
of DNA pol I ,
replaced with DNA
by the same enzyme.

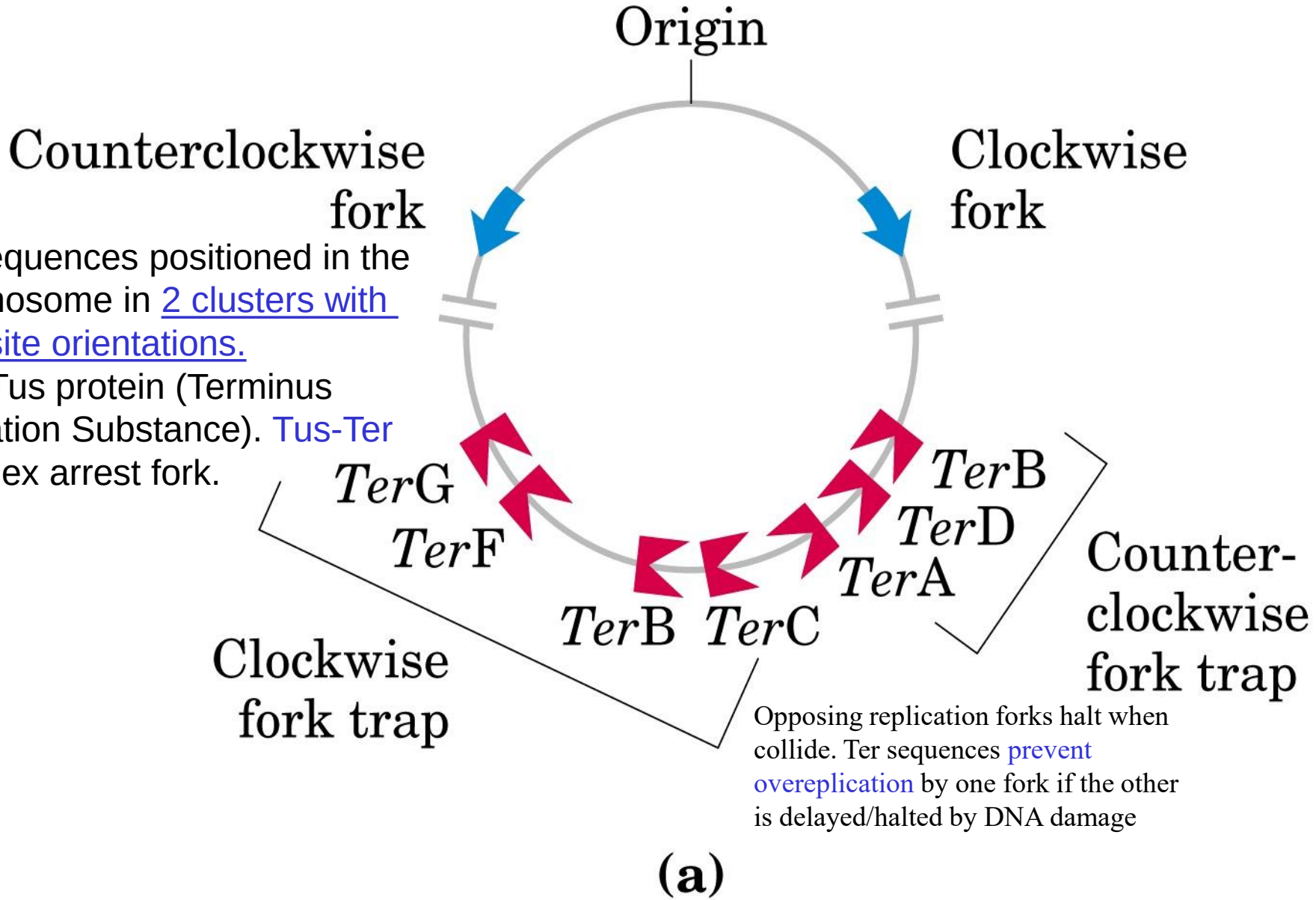
nick sealed by
DNA ligase.

DNA ligase catalyzes the formation of a phosphodiester bond bw a 3' hydroxyl at one end + 5'phosphate at the other.



3) **Termination:**

2 replication forks meet eventually at multiple copies of 20bp sequence called Ter (terminus). Organized like a trap so that the replication fork can enter but cant leave.



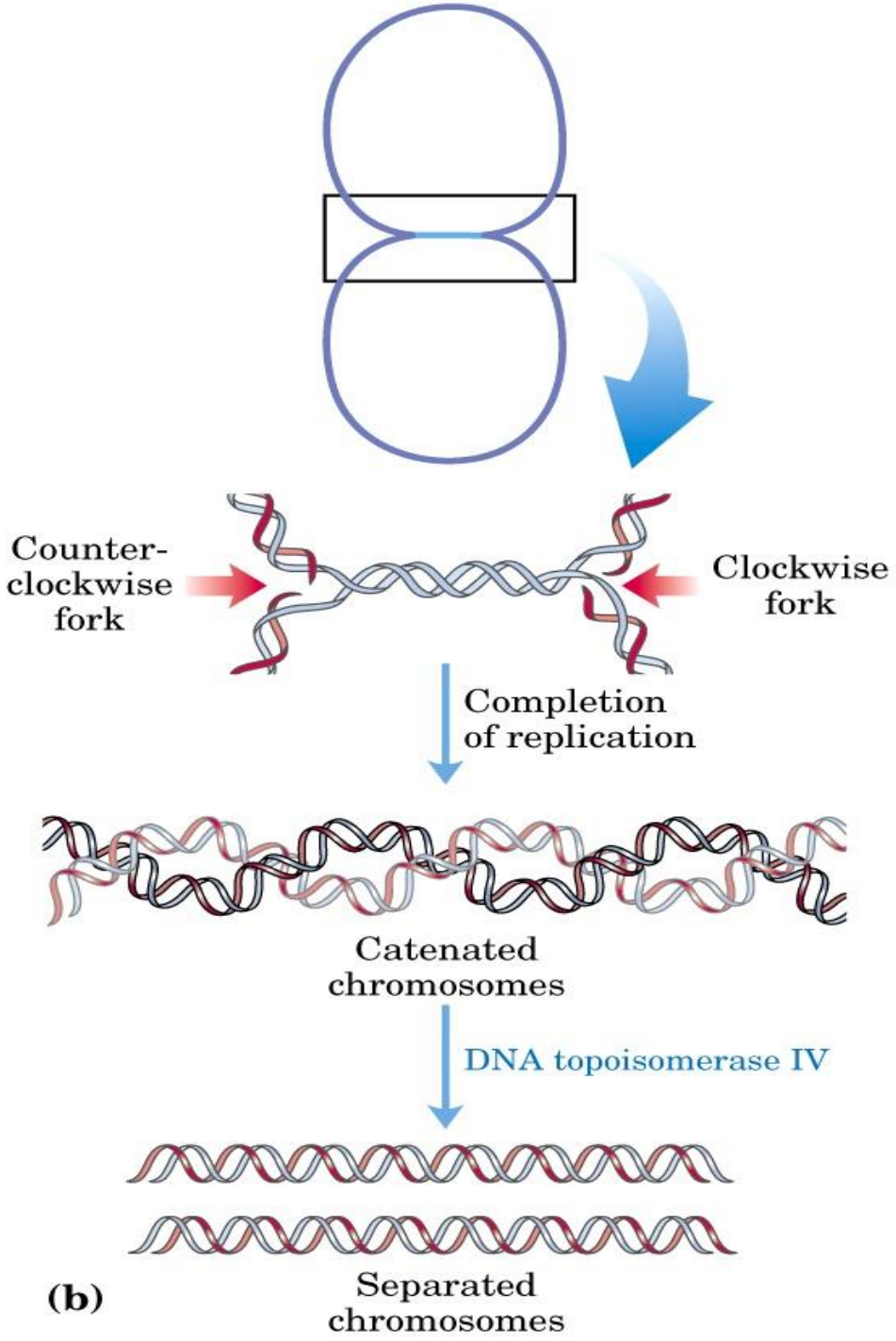
Ter sequences positioned in the chromosome in 2 clusters with opposite orientations.

Bind Tus protein (Terminus Utilization Substance). Tus-Ter complex arrest fork.

Opposing replication forks halt when collide. Ter sequences prevent overeplication by one fork if the other is delayed/halted by DNA damage

Termination of chromosome replication:

Replication of DNA separating the opposing replication forks. leaves the completed chromosome joined as **catenanes**= interlinked circles.
Circles aren't covalently linked but interwound and closed they cant be separated. Need for topoiomerase II type known as IV (for separation of catenated chromosomes) :
Breaks both DNA strands of one chromosome allowing the other chromosome to pass through the break.



Replication in eukaryotes is complex: Variations:

Essential features the same as well as the proteins functionally + structurally conserved.

DNA in eukaryotes >> bacteria+ organized into nucleoprotein structures (chromatin).

1- Replication fork rate in eukaryotes **50 nucleotide/s** = 1/20 of E. coli.

Solution = **multiple origins**, spaced 30,000-300,000 bp apart.

2- Origins called **Autonomously Replicating Sequences (ARS) / replicators**.

in yeast around 400 replicators / 16 chromosome. Each around 150bp+conserved seq.

3- Initiation starts binding of a protein (**Origin Recognition complex=ORC**) binds ARS. This regulated by several proteins involved in cell cycle regulation. (similar to DnaA)

4- CDC6 (**C**ell **D**ivision **C**ycle) , CDT1 (**C**DC10-**D**ependent **T**ranscript 1) both bind ORC mediate loading of MCM2 to MCM7 (**M**ini**C**hromosome **M**aintenance proteins)= heterohexamers forming ring shaped helicase analogous to bacterial DnaB helicase. Role of CDC6 and CDT1 similar to bacterial DnaC loading MCM helicases onto replication origin.

Several polymerases

DNA polymerase α: multisubuint enzyme similar in all eukaryotes.

- primase activity , polymerization.
- No proofreading 3'→5' exonuclease activity. (unsuitable for high fidelity DNA replication)
- function: primer synthesis (RNA/DNA) for okazaki on lagging.

DNA polymerase δ:

-function: extends the primers + 3'→5' exonuclease activity.

- stimulated by PCNA (abundant in nuclei of proliferating cells).

-PCNA similar to β-subunit in E. coli forming a circular clamp increasing processivity.

-Pol δ carry out leading and lagging strand synthesis similar to bacterial DNA pol III.

-RFC: (replication factor C)= clamp loader of PCNA(similar to bacterial clamp loading γ complex.

DNA polymerase ε:

- Function: DNA repair + removing primers at replication fork

Additional differences:

- RPA: (replication protein A) = eukaryotic ssDNA binding protein (similar to SSB).

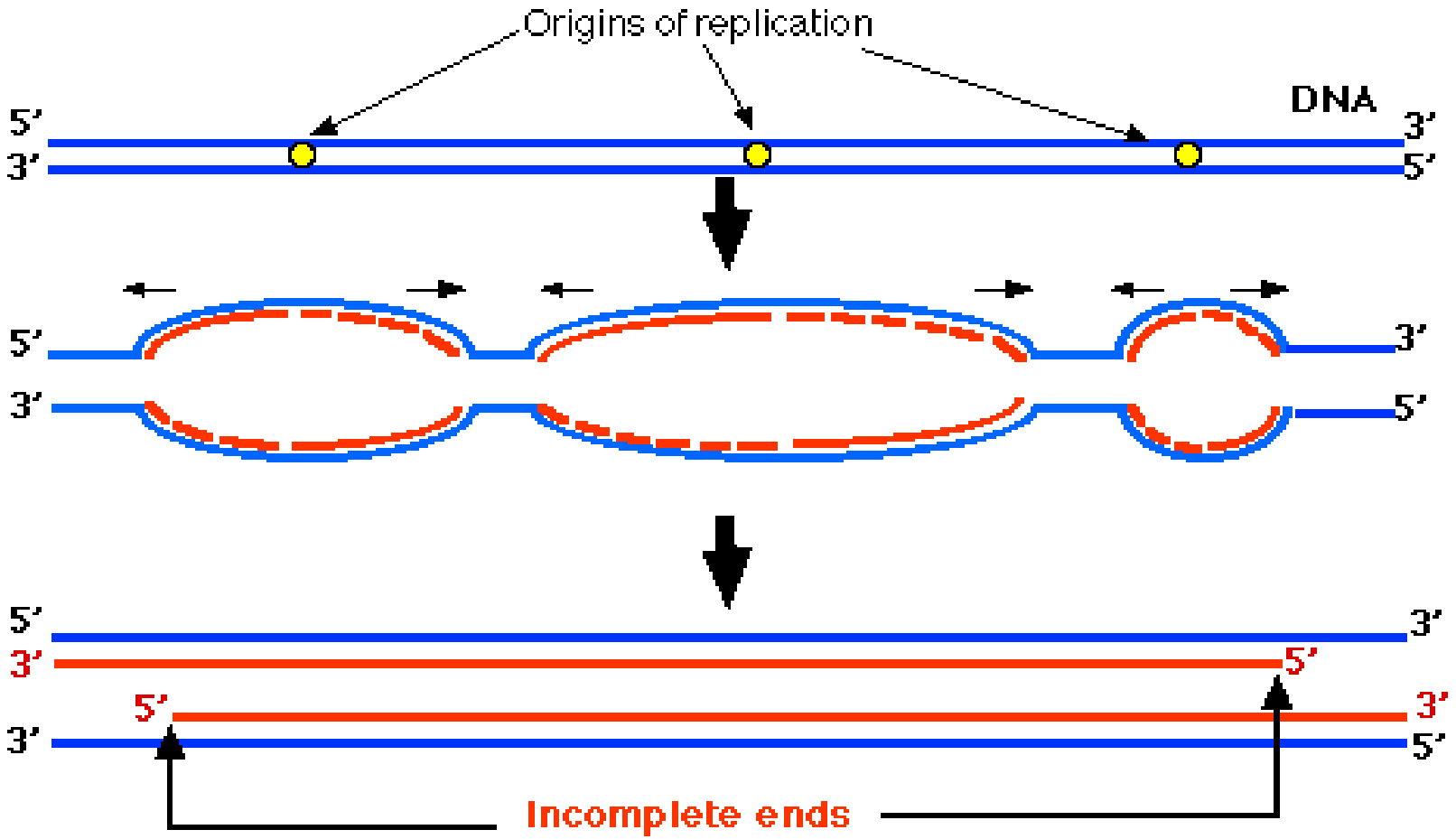
- Termination involves synthesis of telomeres.

The End Replication Problem:

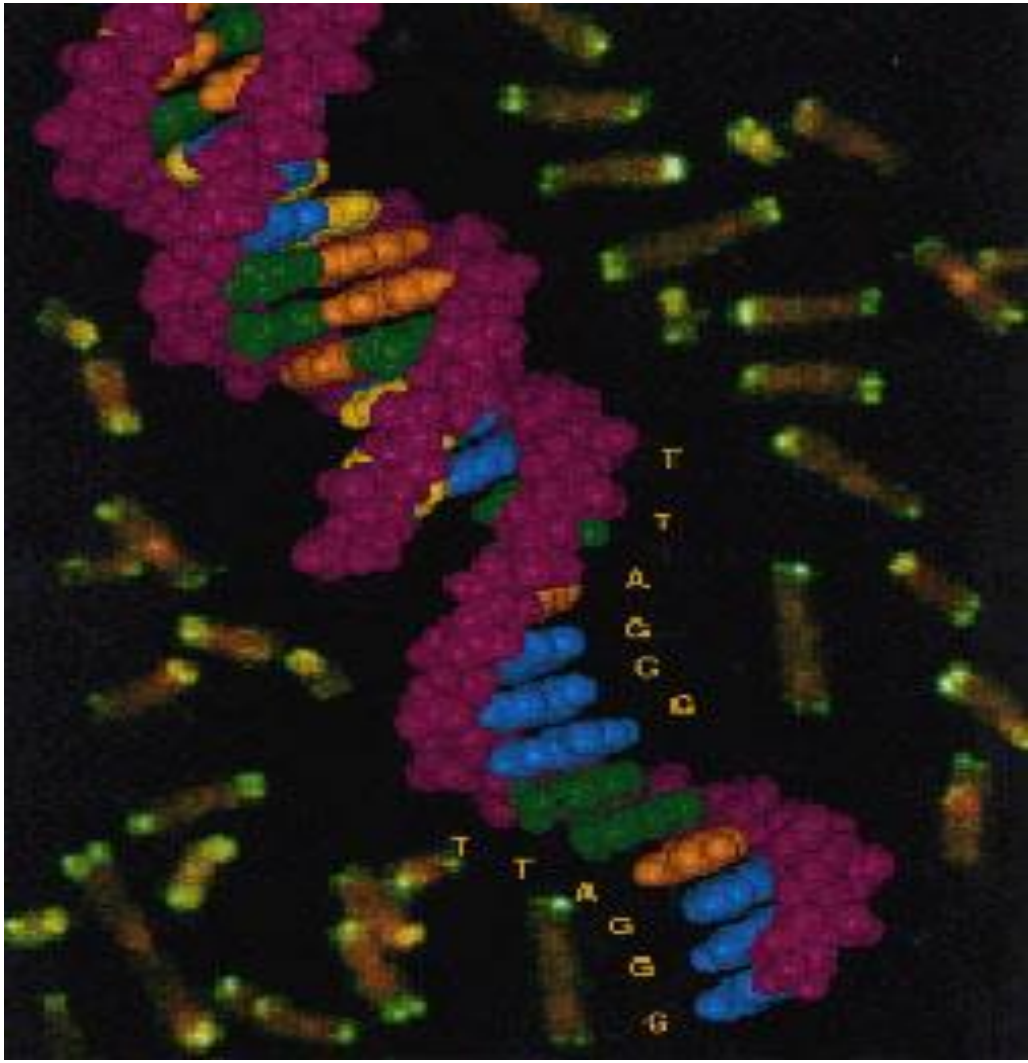
Telomeres shorten with each S phase

DNA replication is bidirectional, 5'→3' Requires a labile primer

Each round of DNA replication leaves ~100bp DNA unreplicated at the 3' end



Telomeres 'cap' chromosome ends



Solution for End Replication Problem :

Telomerase:

Telos=end,

meros=component

Telomerae specific DNA polymerase.

a ribonucleoprotein enzyme RNA template +catlytic unit (reverse transcriptase) has been referred to as a cellular immortalizing enzyme.

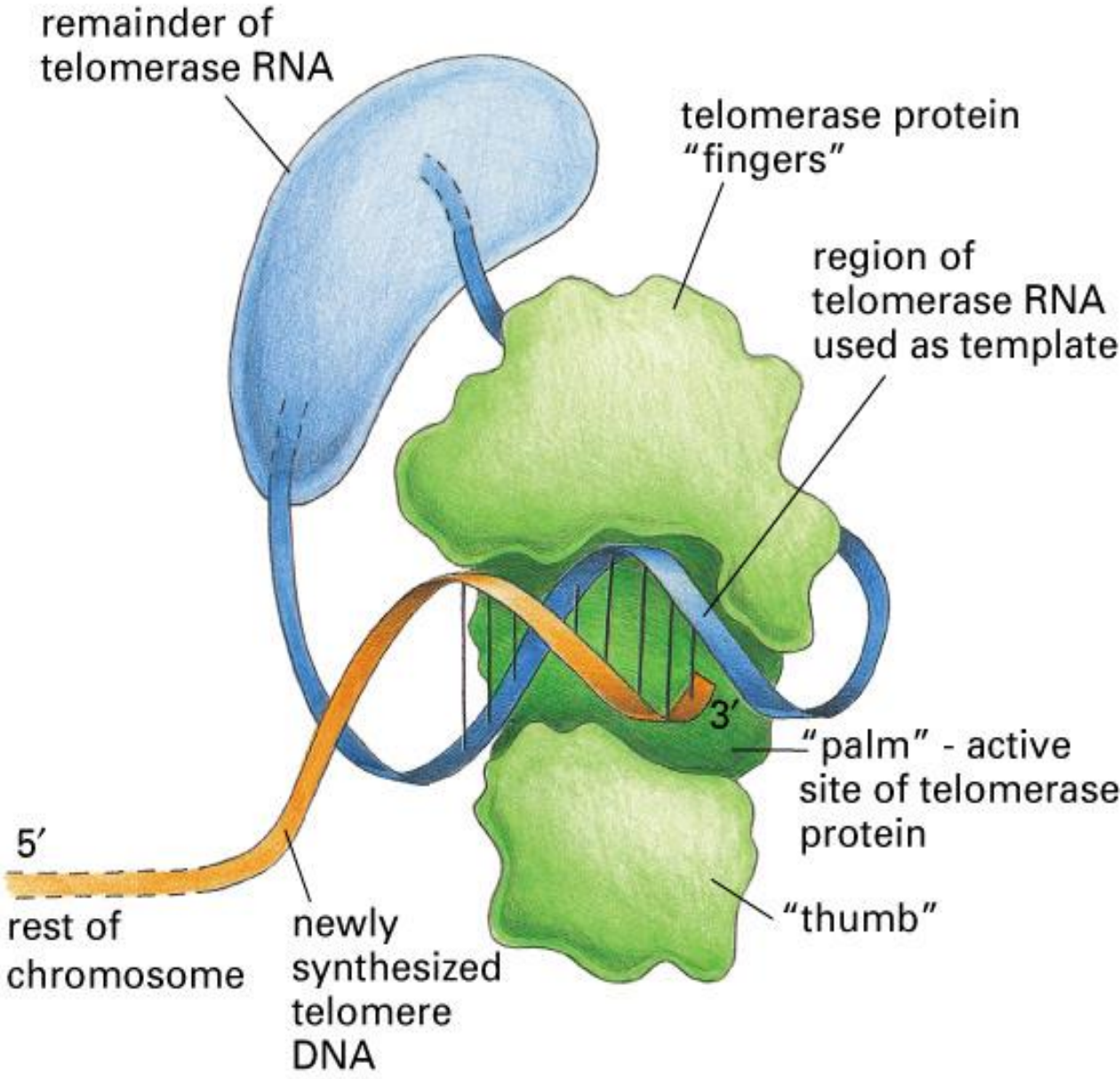


Figure 5–42. Molecular Biology of the Cell, 4th Edition.

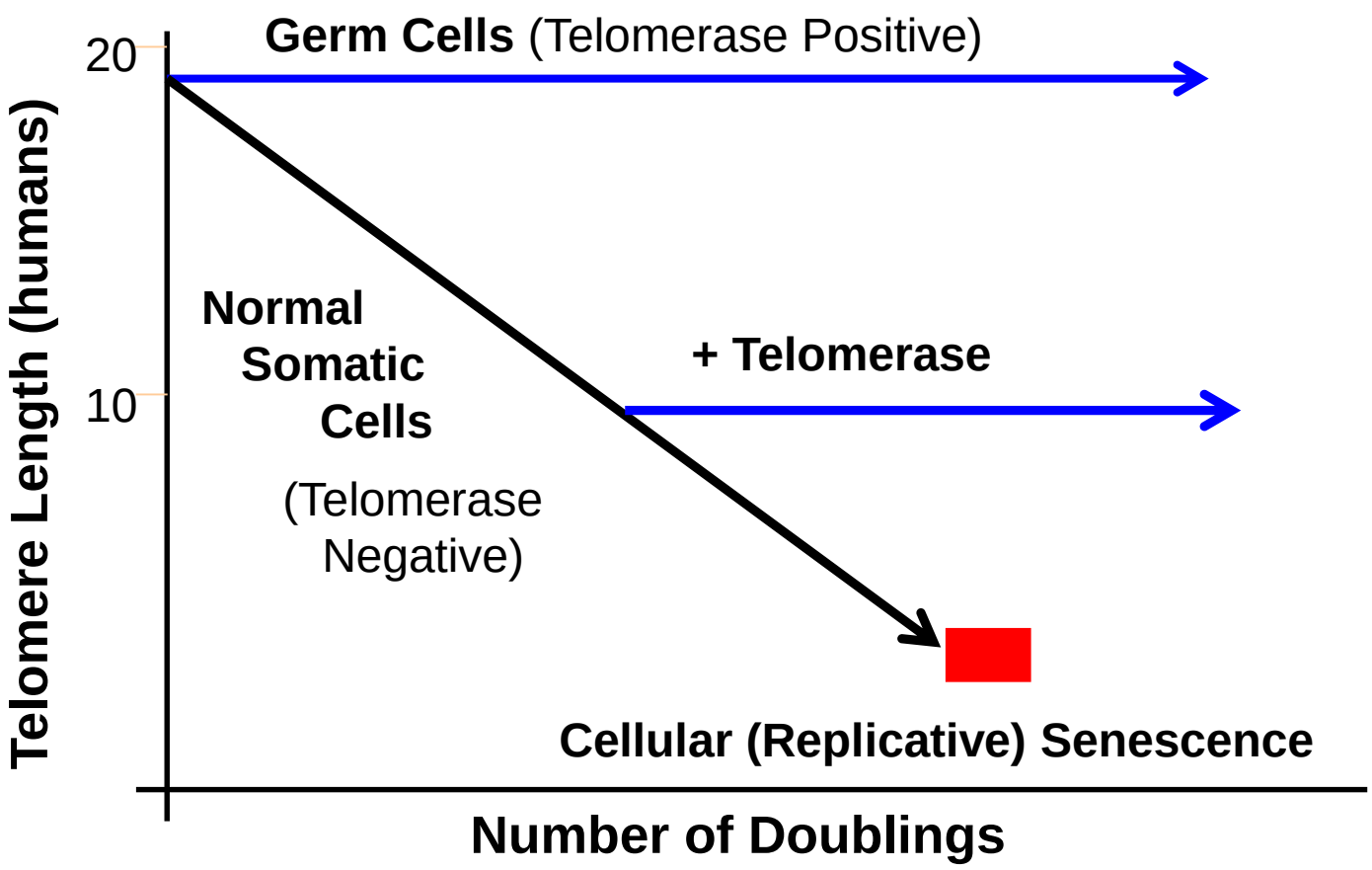
- **In the laboratory:**

  cells in tissue culture with introduced telomerase

  → extended their telomere length.

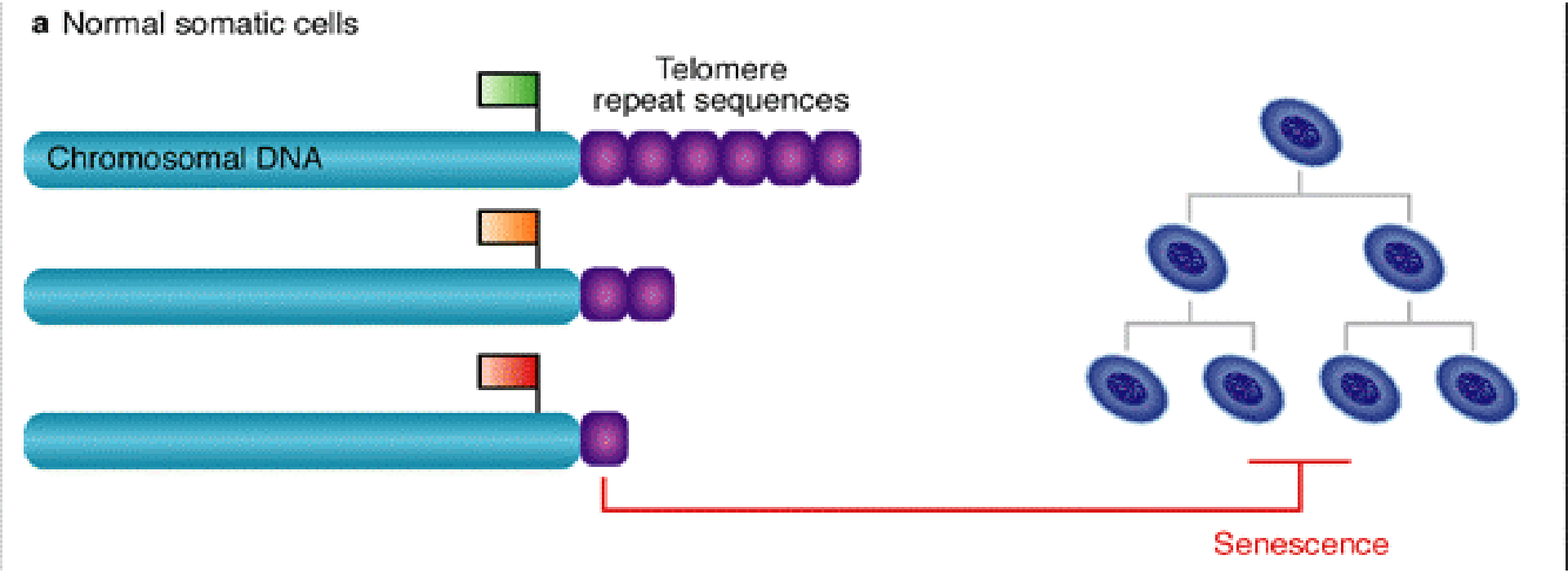
  → divided for 250 generations past the time they normally would stop dividing, and are continuing to divide normally, giving rise to normal cells with the normal number of chromosomes.

Telomere Length and Cell Division Potential



Telomerase and Senescence

In most somatic tissues, telomerase is expressed at very low levels or not at all -- as cells divide, telomeres shorten



Short telomeres may be a signal for cells to senesce (stop dividing)

Telomerase and Cancer

The presence of telomerase in cancer cells allows them to maintain telomere length while they proliferate.