DNA Replication

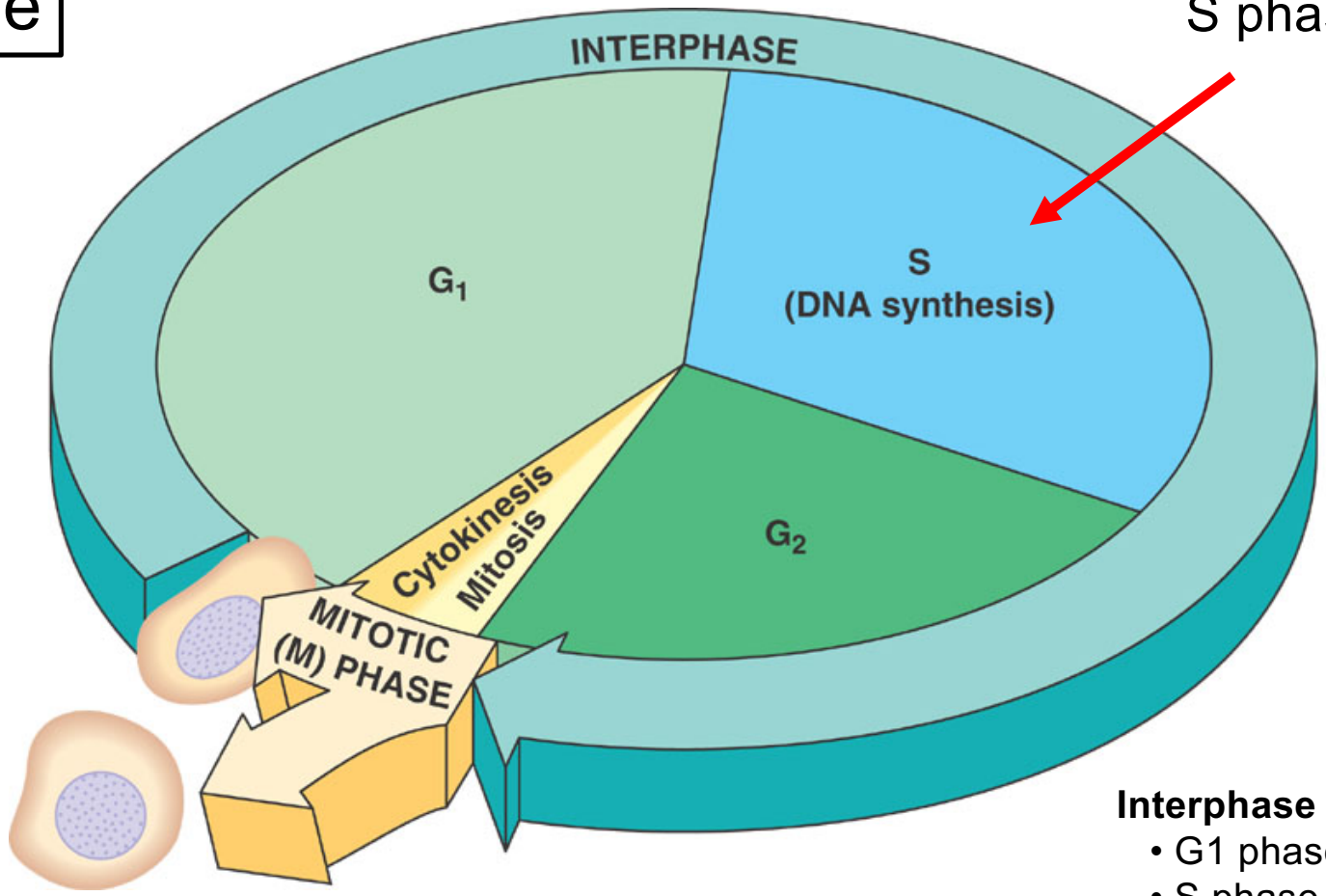
Occurs before division of cell into two daughters:
→ the genetic information (the chromosomes) must be duplicated
→ this copying is extremely fast: 1000 nucleotides/sec *
→ this process is called DNA Replication

Copying errors can occur during replication:
→ wrong nucleotide inserted into copied strand
→ machinery to remove and put correct nucleotide
→ this process is called DNA Repair mechanism

Replication speed : 1000 b/sec in E. coli
100 b/sec in Humans

Cell cycle

DNA replication occurs during S phase

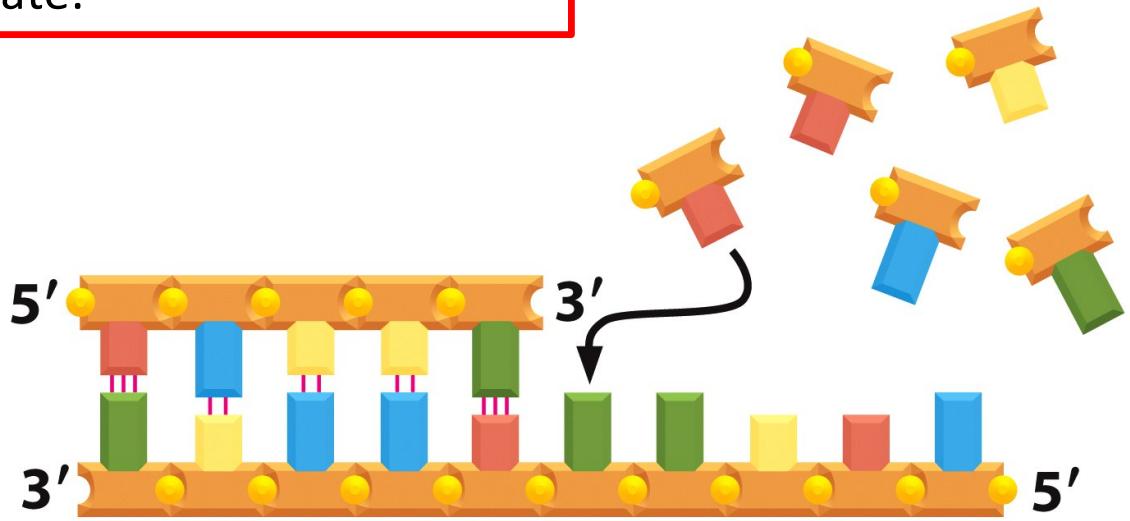


Interphase is subdivided into

- G1 phase
- S phase
- G2 phase

The “old” strand (parental strand):
-> serves as template

DNA polymerase can only elongate;
cannot initiate.



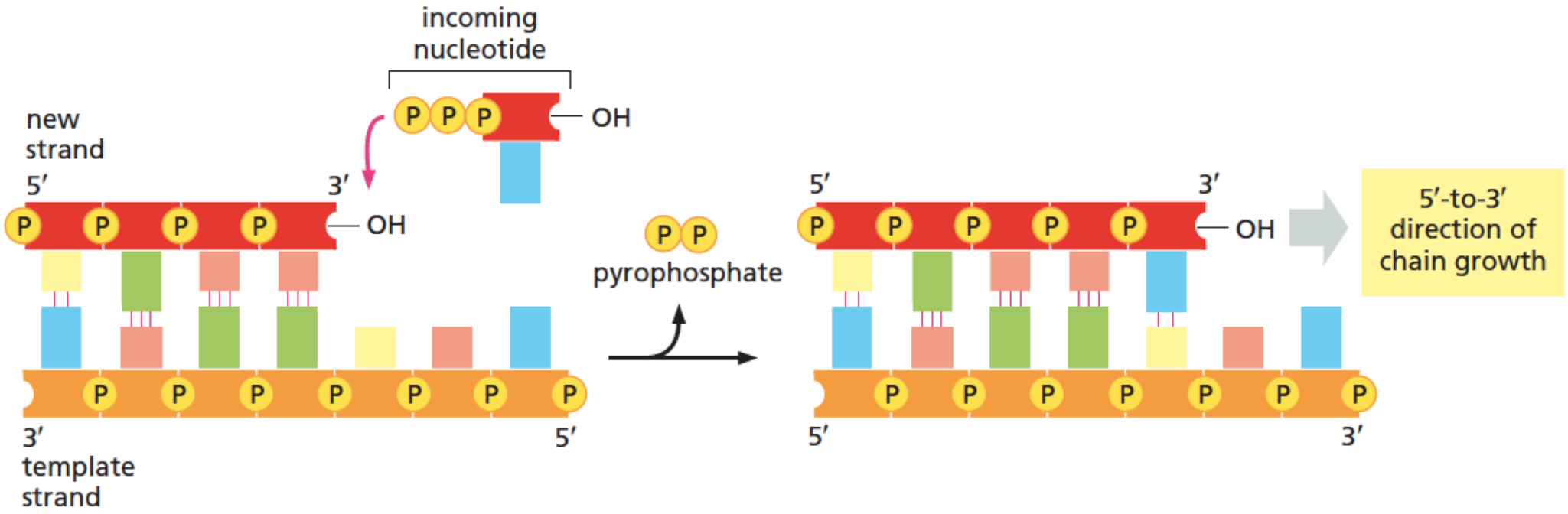
Building blocks :

- dATP
- dCTP
- dGTP
- dTTP

d = deoxy(ribose)

Synthesis of new strand is catalyzed by an enzyme: **DNA polymerase**

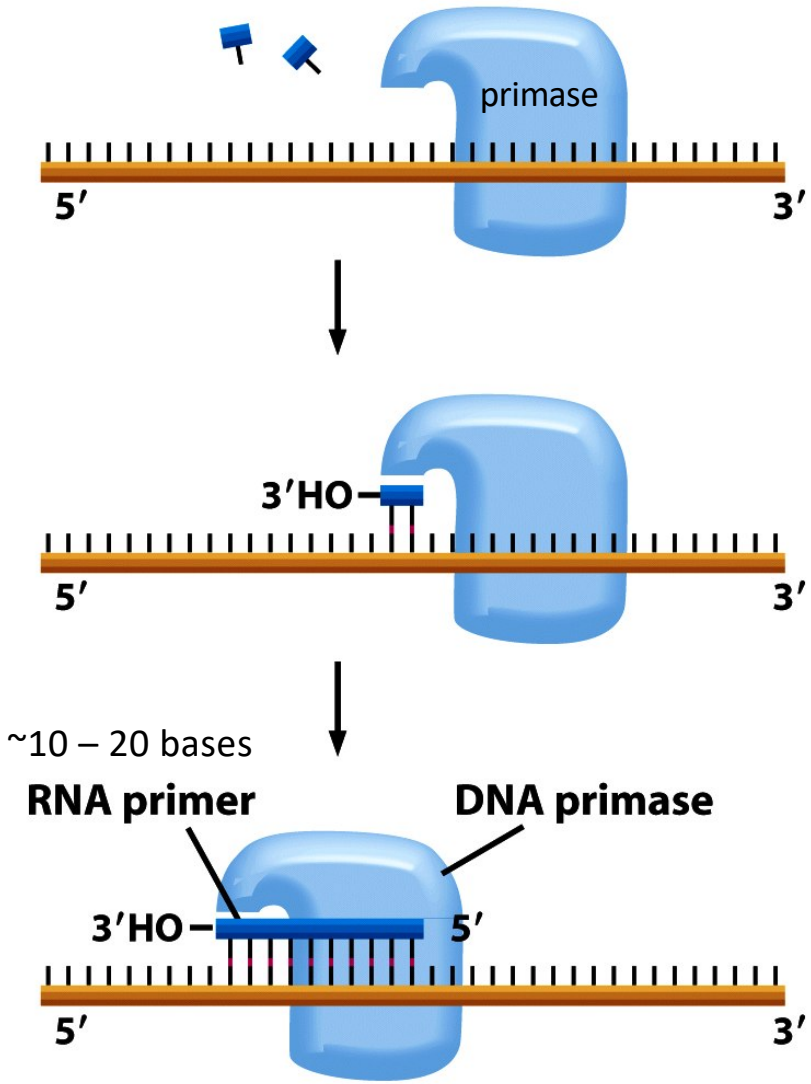
DNA polymerase assembles only nucleotides **tri**phosphate



Hydrolysis of high energy bonds drives the chemical reaction.

DNA polymerase can only elongate a sequence.

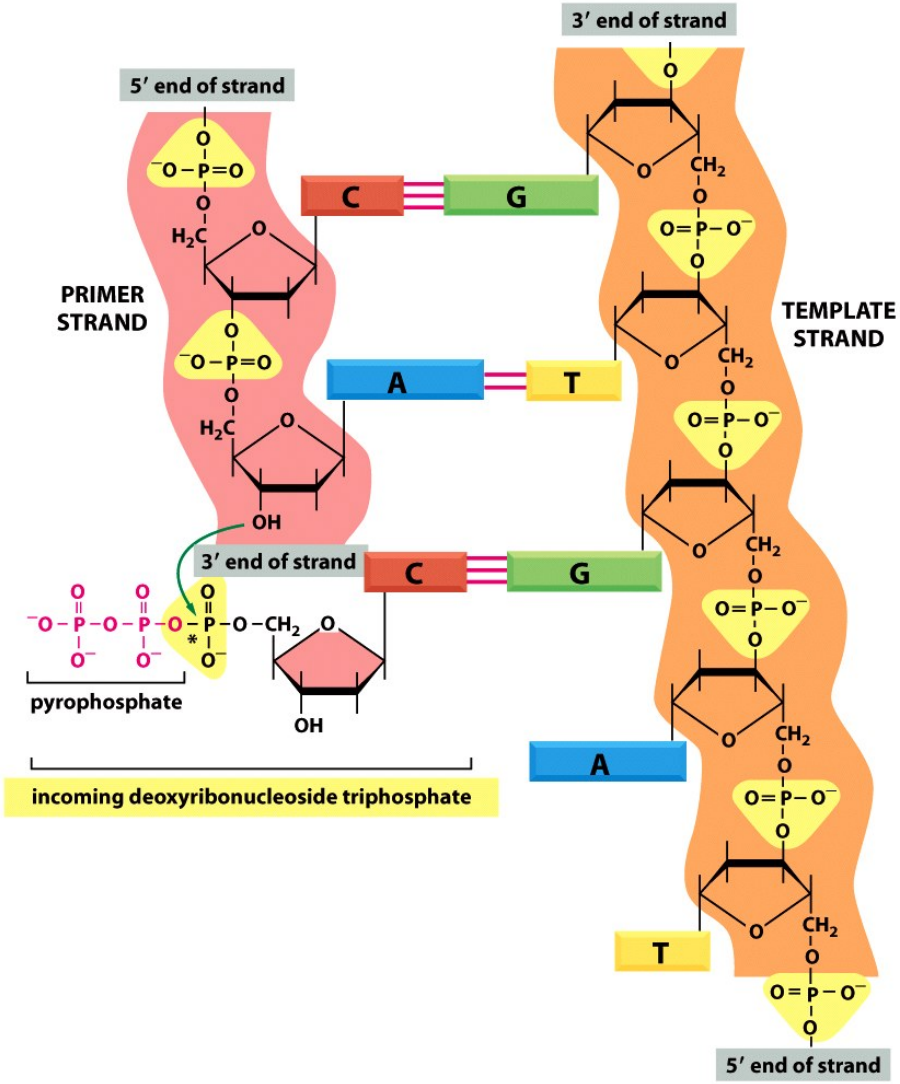
RNA polymerase can initiate the building of a strand.



Eventually, the RNA primer will be replaced by DNA.

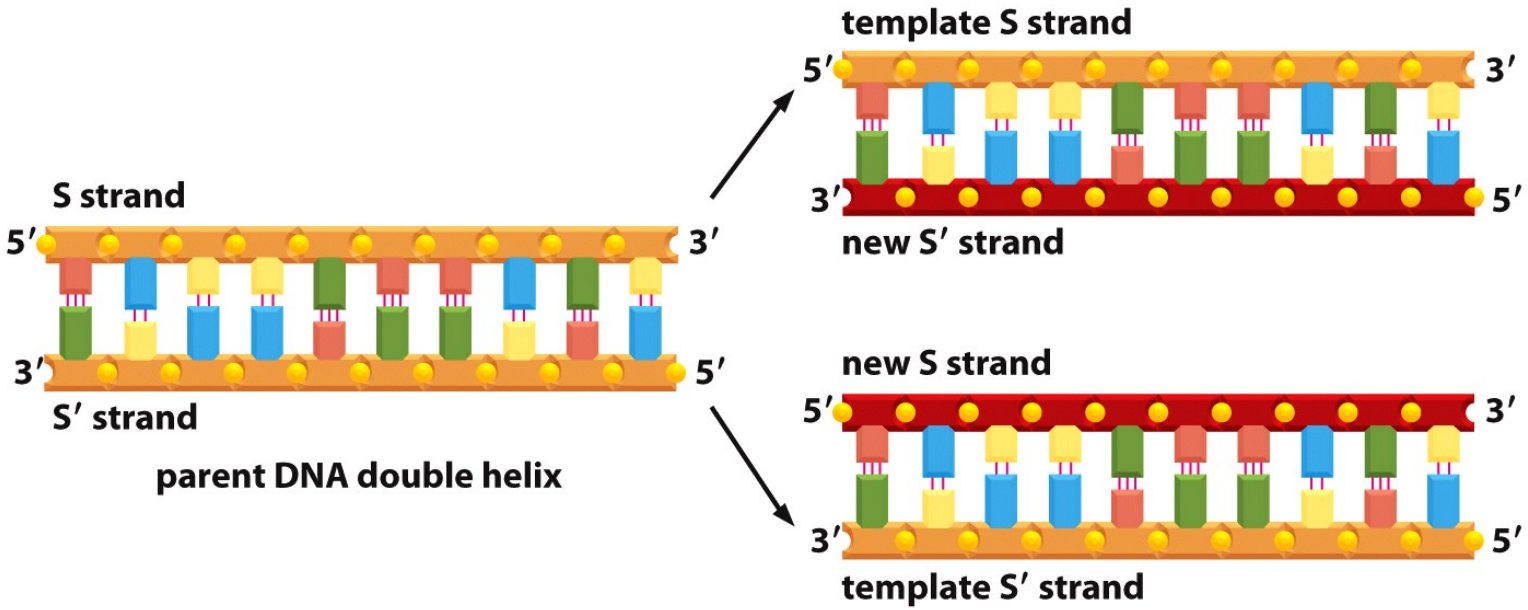
DNA replication works only in 5’ → 3’ direction

incoming nucleotide:
always added at 3’ OH-group of ribose
of last nucleotide by the DNA polymerase



Both parental strands are templates

1. strand S and strand S’ separate
2. S is template to polymerize a new S’
3. S’ is template to polymerize a new S

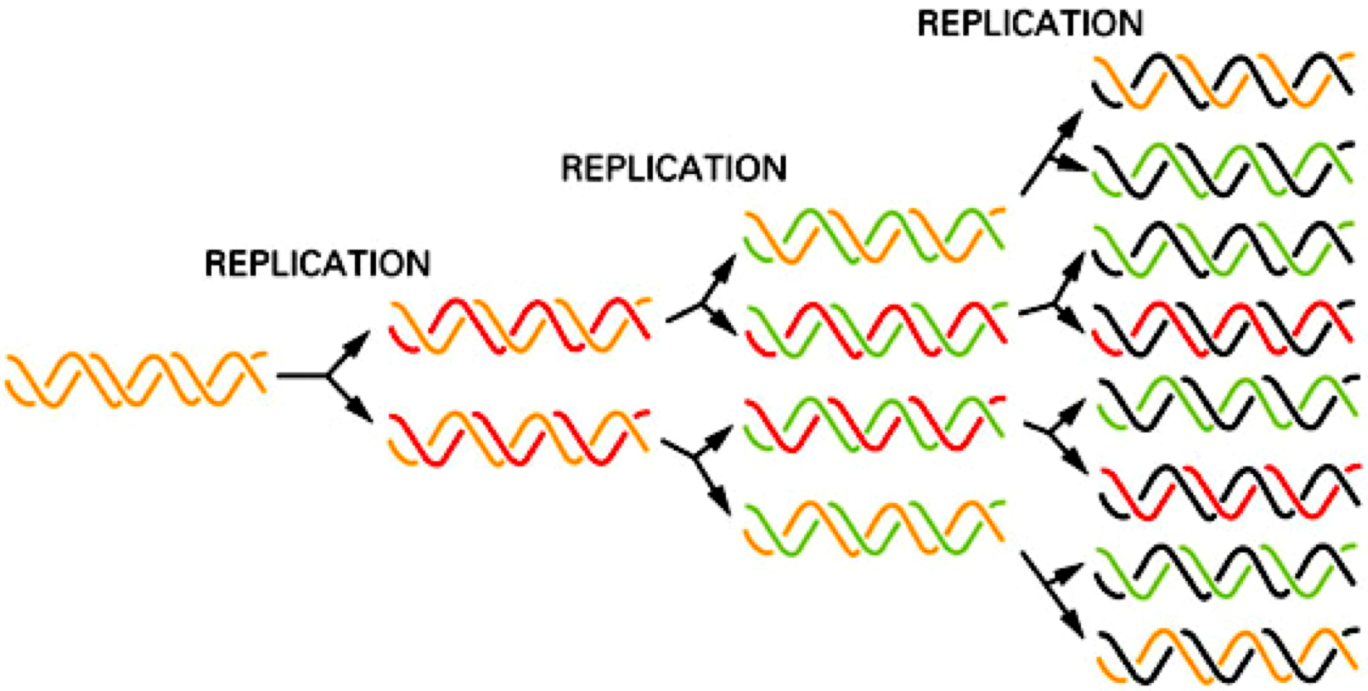


DNA replication is semiconservative

- chromosomes of each daughter cell are formed by:

→ one parental (old) strand

→ one new strand



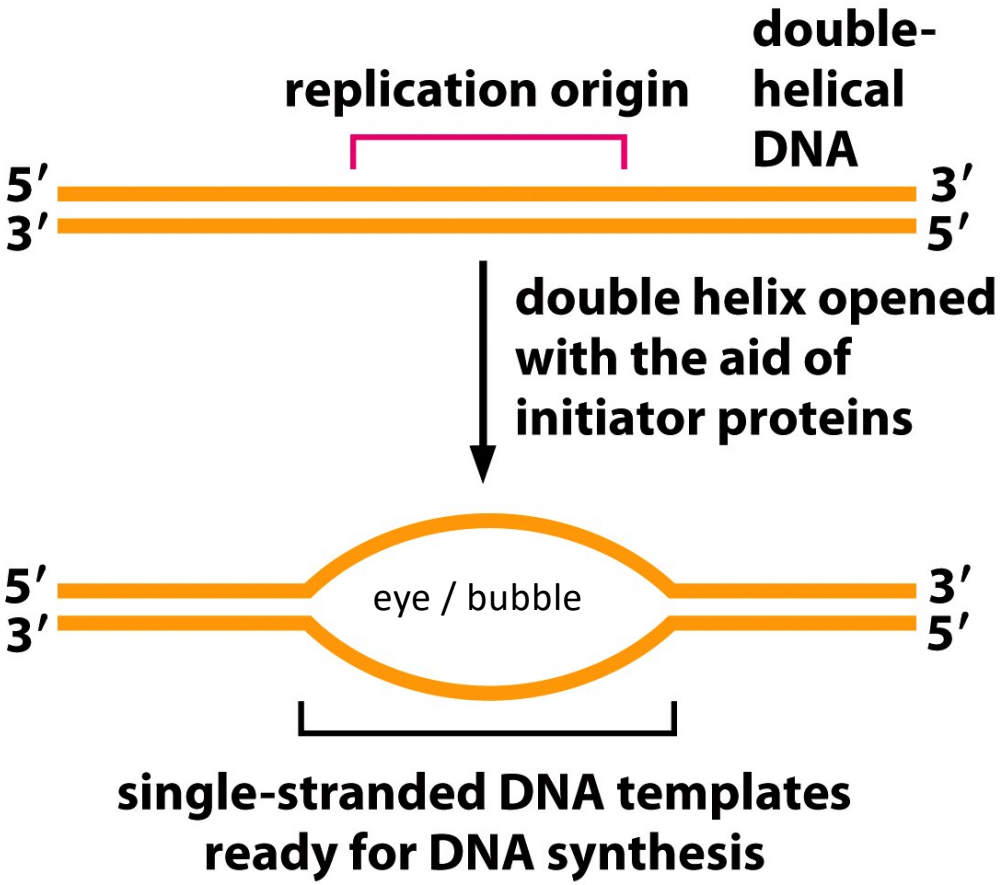
DNA synthesis begins at replication origins

- double-stranded DNA is "melted" at particular sequence stretch

- allows access for synthetizing enzymes

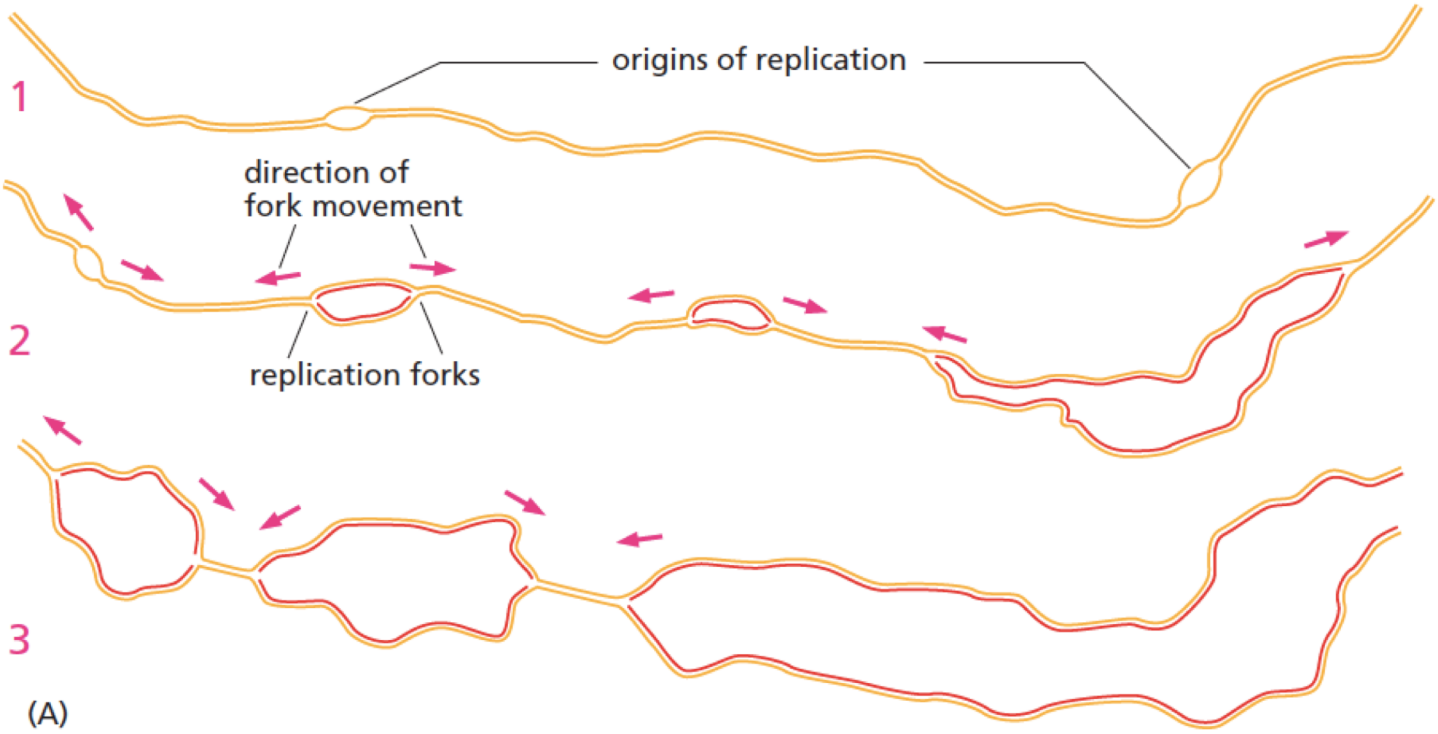
Origins number per genome :

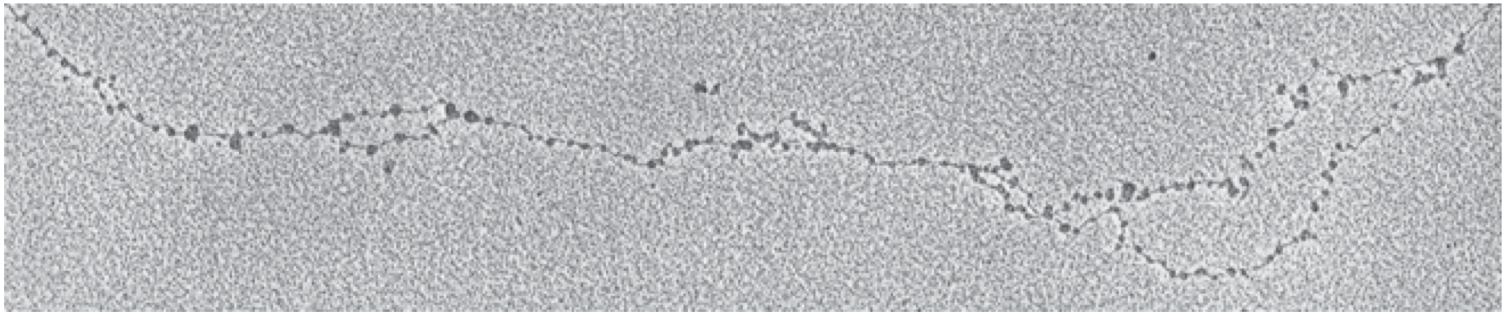
- only 1 origin for the E. coli genome
- thousand for the human genome



Eukaryotes

Many replication origins on a DNA molecule



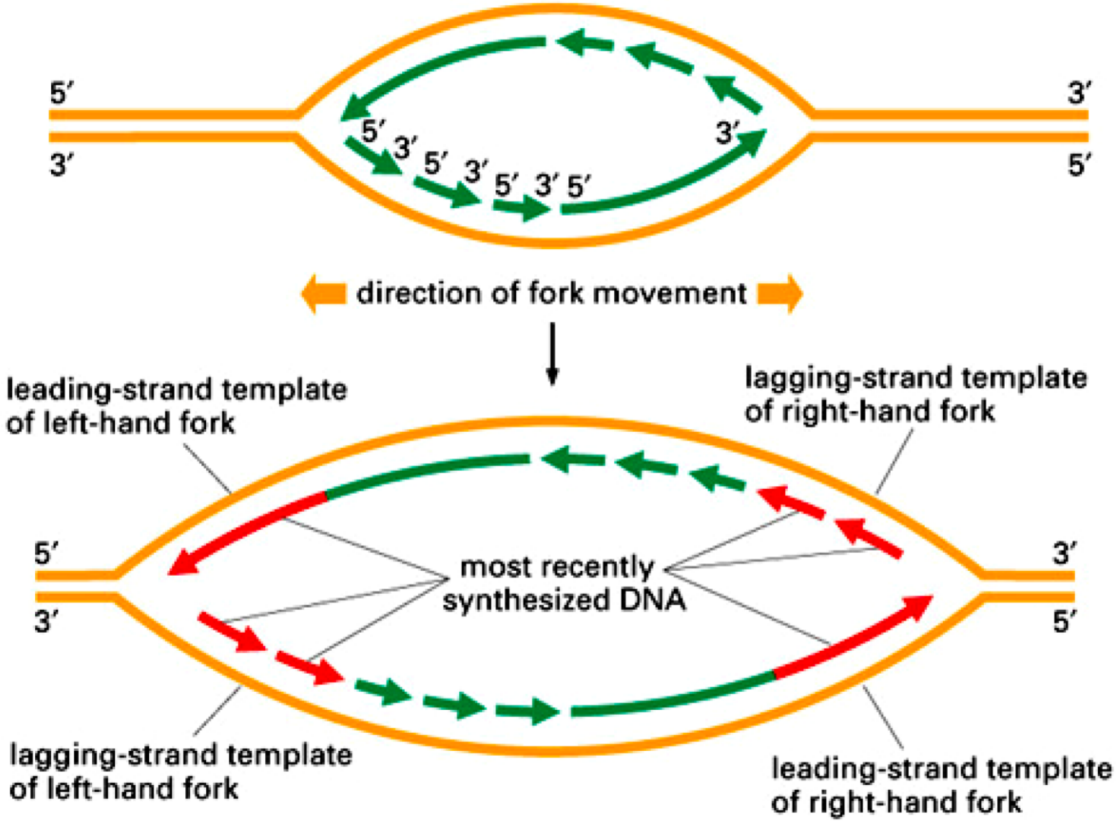


Consequence of 5’ → 3’ directionality:

→ *one strand is made discontinuously*

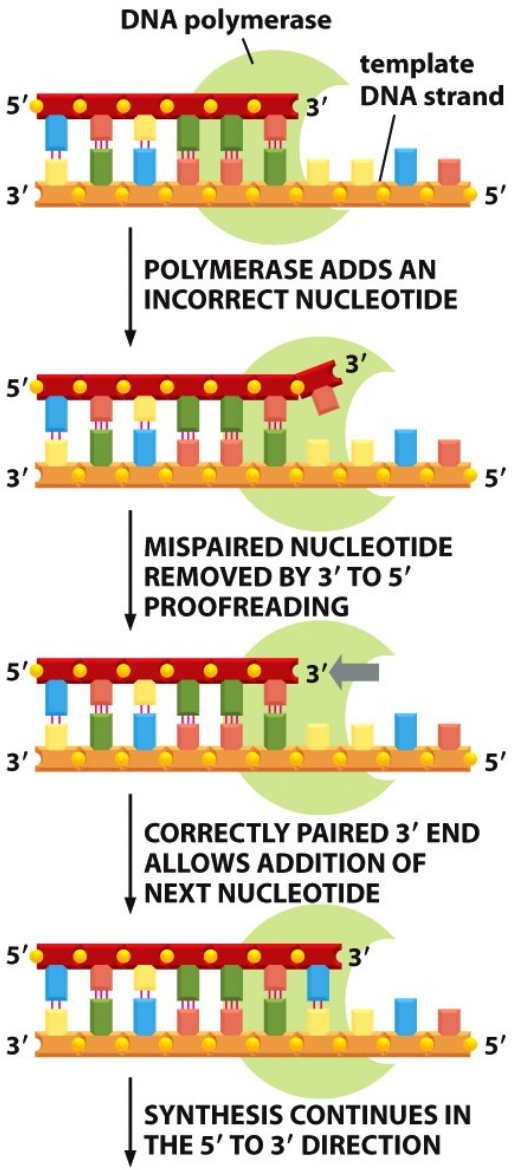
on one strand: continuous DNA synthesis

on other strand: synthesis on many short fragments
(Okazaki fragments),
which are later fused together

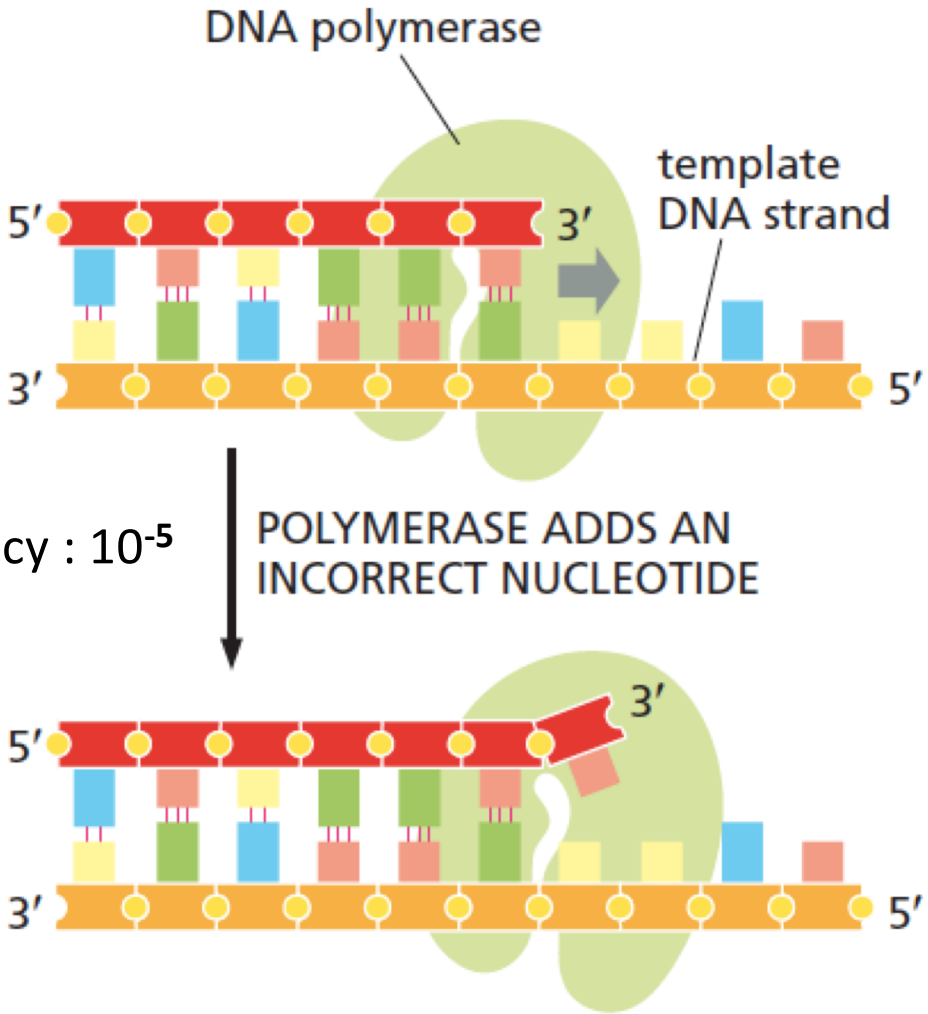


DNA polymerase is self-correcting

- DNA polymerase extremely accurate:
  → a wrong nucleotide: 1 per 10^5
  → but would still be too many mutations
- DNA polymerase possesses **proofreading**
  → checks previous base before adding next
- cuts out if wrong



Proofreading



Error frequency : 10^{-5}

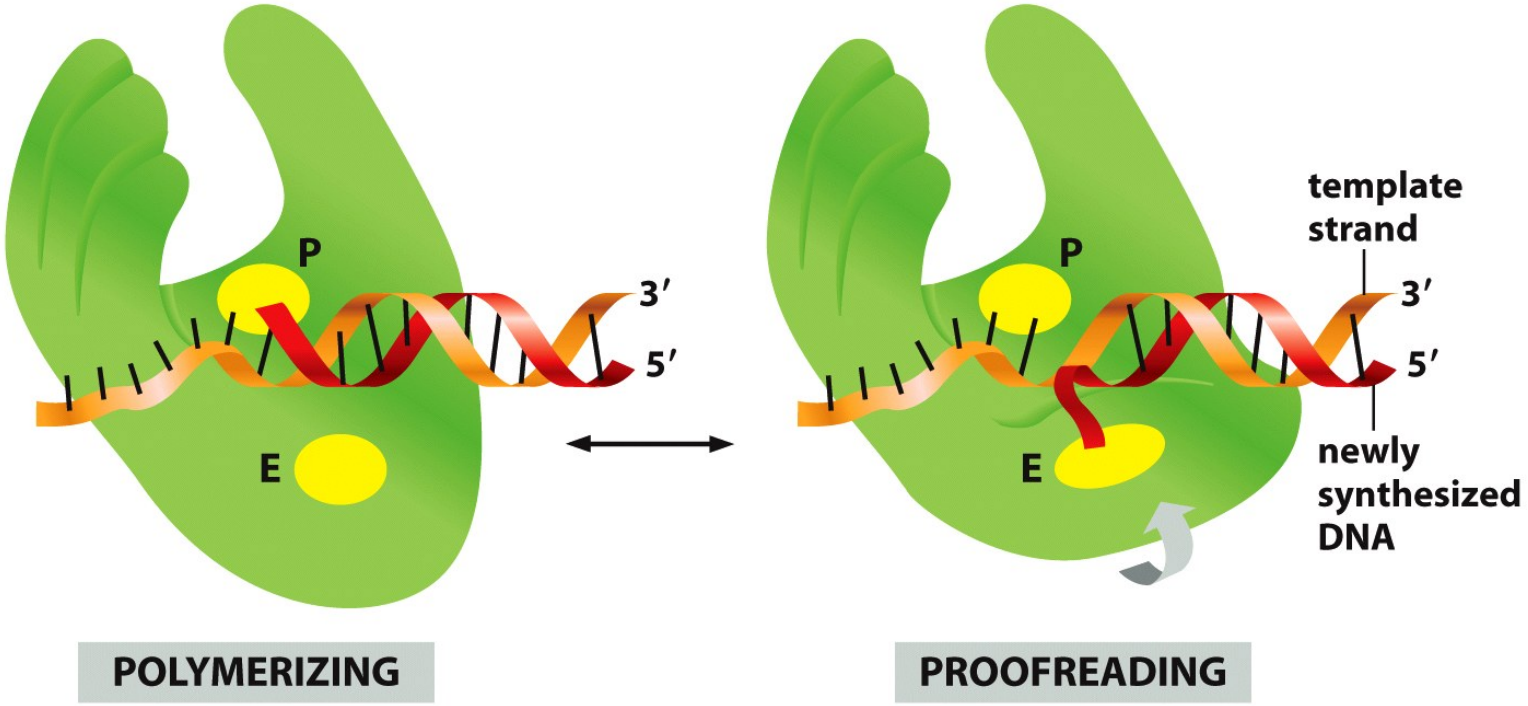
Because of the mispairing (mismatch)
the wrong nucleotide is not well positioned
to accept the next nucleotide.
Correction : 99%
Mutation : 1 % → 10^{-7}

There is still time to correct the mistake until the next replication.

DNApolymerase has 2 active sites :

P site : addition of one nucleotide (Polymerisation)

E site : Editing



At the E site
wrong bases are removed

Figure 6-14 *Essential Cell Biology* (© Garland Science 2010)

Mutations induced by DNA replication

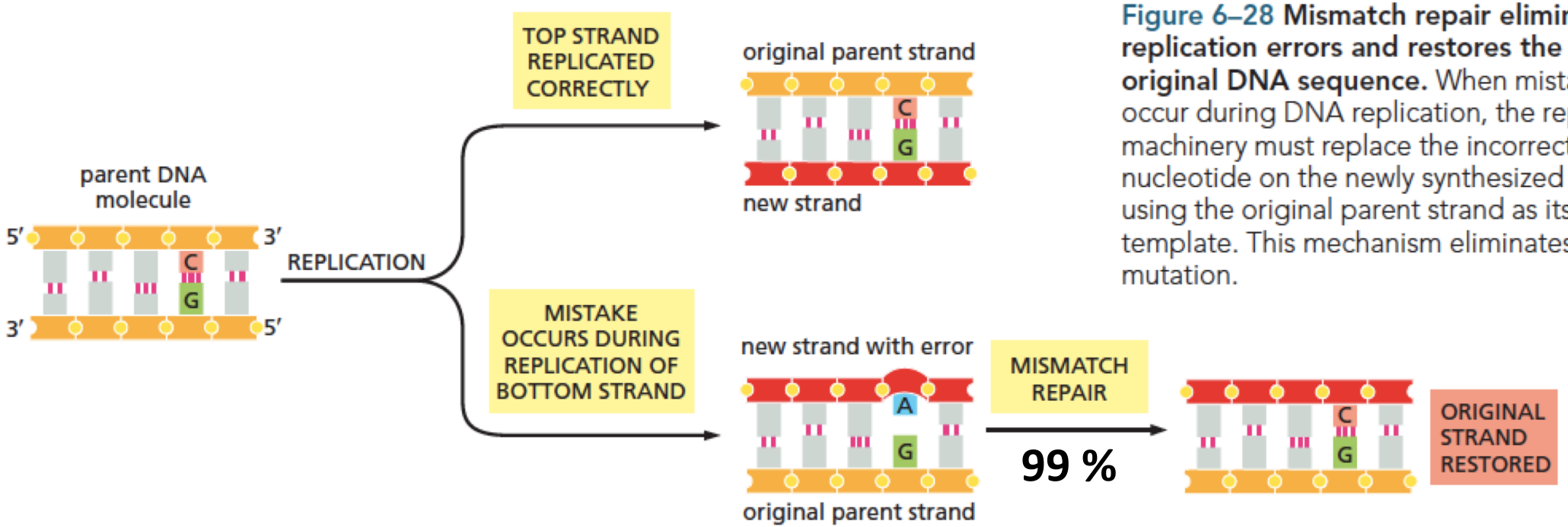


Figure 6–28 Mismatch repair eliminates replication errors and restores the original DNA sequence. When mistakes occur during DNA replication, the repair machinery must replace the incorrect nucleotide on the newly synthesized strand, using the original parent strand as its template. This mechanism eliminates the mutation.

Proofreading failed

10^{-7}

Mutations induced by DNA replication

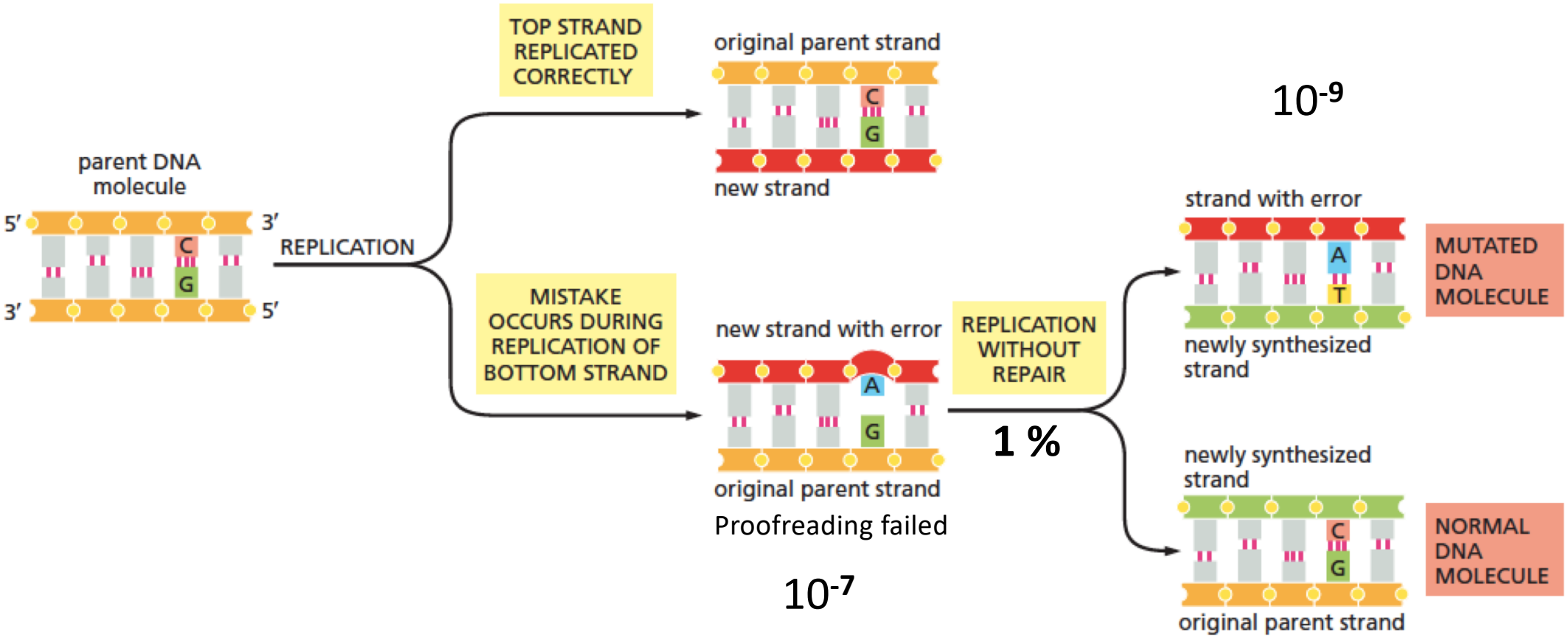


Table 5–1 The Three Steps That Give Rise to High-Fidelity DNA Synthesis

REPLICATION STEP	ERRORS PER NUCLEOTIDE
5′ → 3′ polymerization	1 in 10^5
3′ → 5′ exonucleolytic proofreading	1 in 10^2
Strand-directed mismatch repair	1 in 10^2
Combined	**1 in 10^9**

The third step, strand-directed mismatch repair, is described later in this chapter.

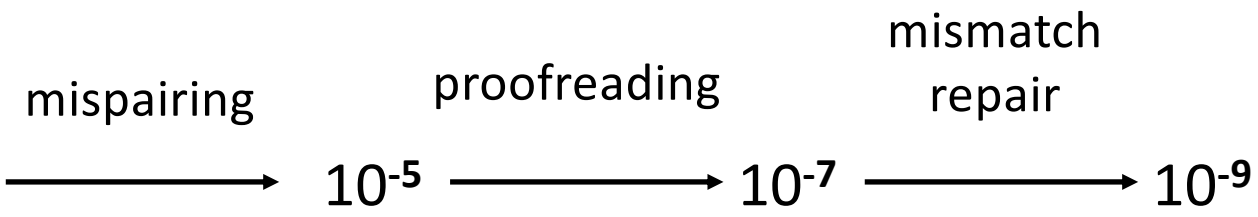
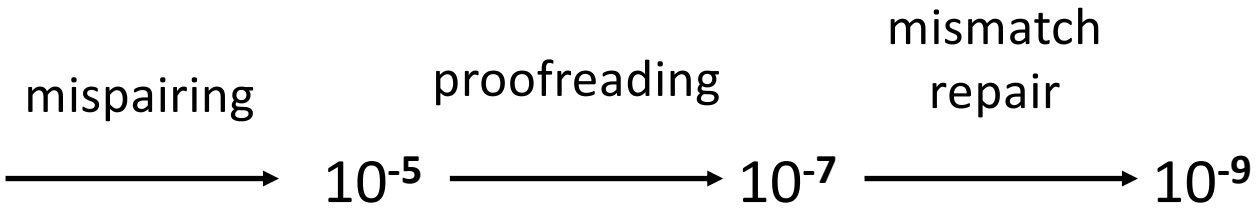
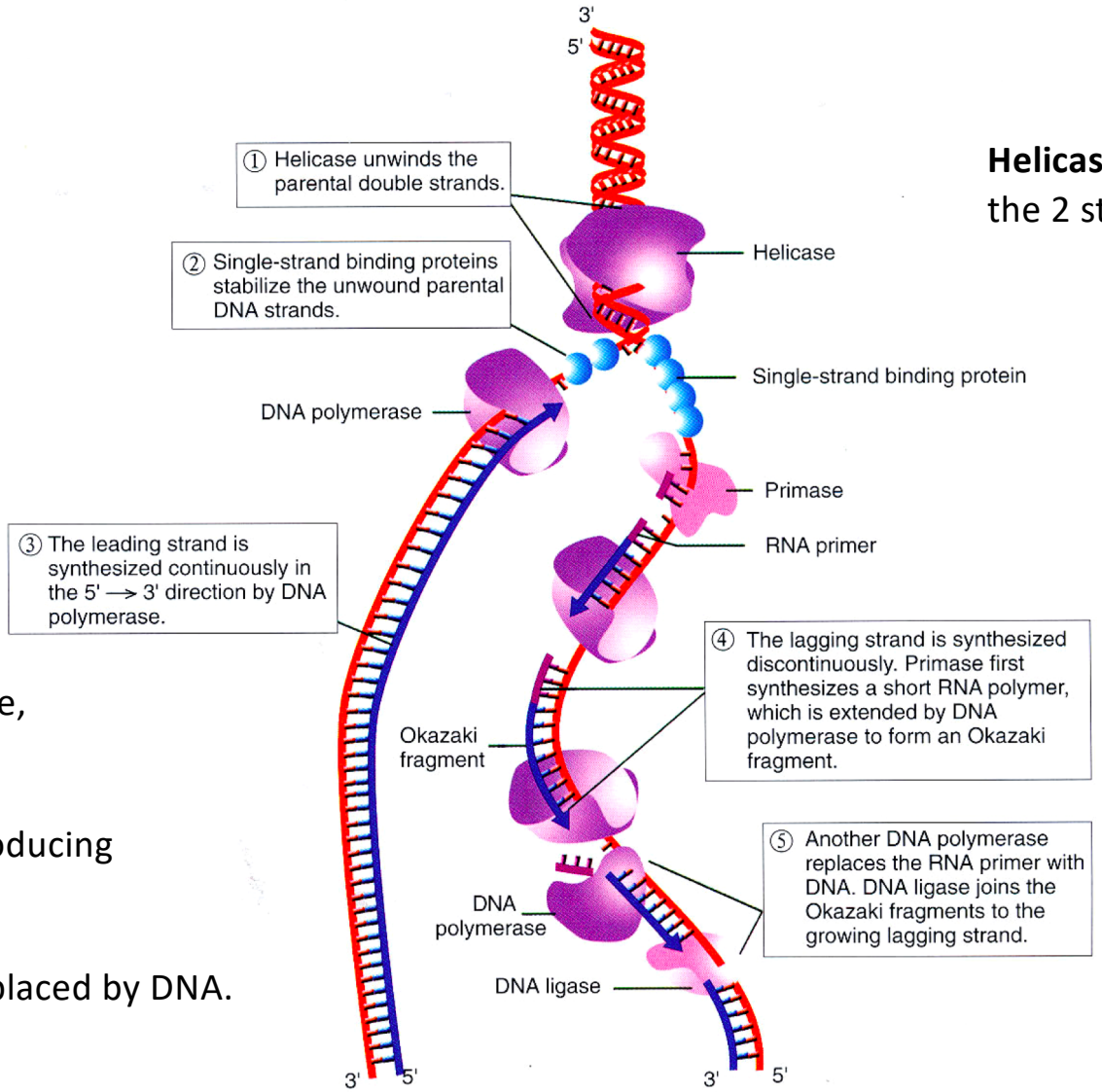


TABLE 6–1 ERROR RATES	
US Postal Service on-time delivery of local first-class mail	13 late deliveries per 100 parcels
Airline luggage system	1 lost bag per 200
A professional typist typing at 120 words per minute	1 mistake per 250 characters
Driving a car in the United States	1 death per 10^4 people per year
DNA replication (without mismatch repair)	1 mistake per 10^7 nucleotides copied
DNA replication (including mismatch repair)	1 mistake per 10^9 nucleotides copied



A complicated machinery of different proteins works at the replication fork



Helicase separates the 2 strands

DNA polymerase cannot initiate, it can only elongate **a primer**.

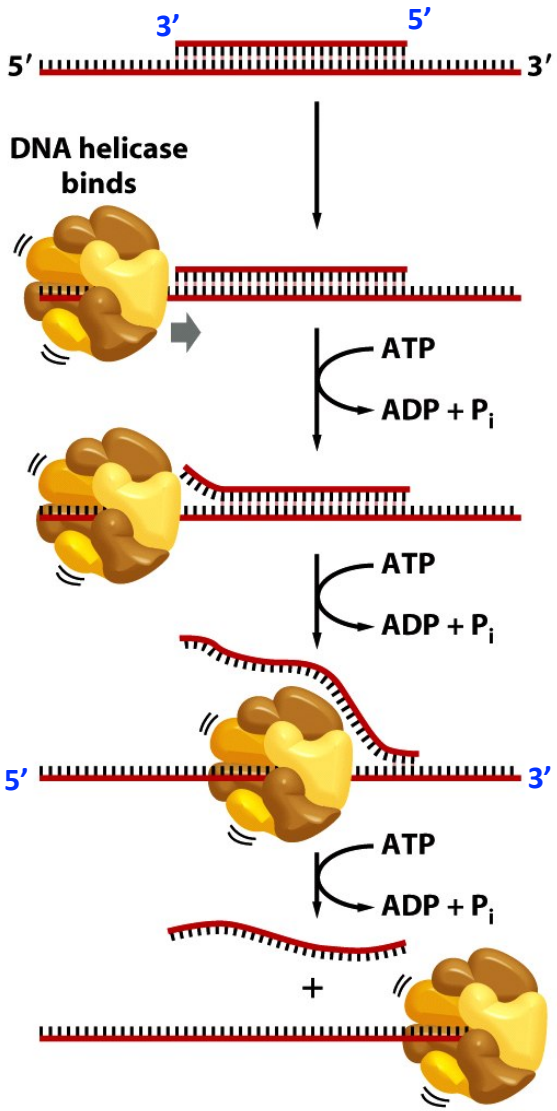
Primase = RNA polymerase producing primers.

Eventually RNA primers are replaced by DNA.

Helicase

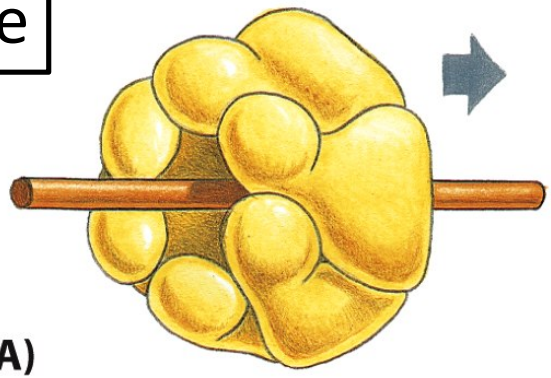
6 subunits

is a motor

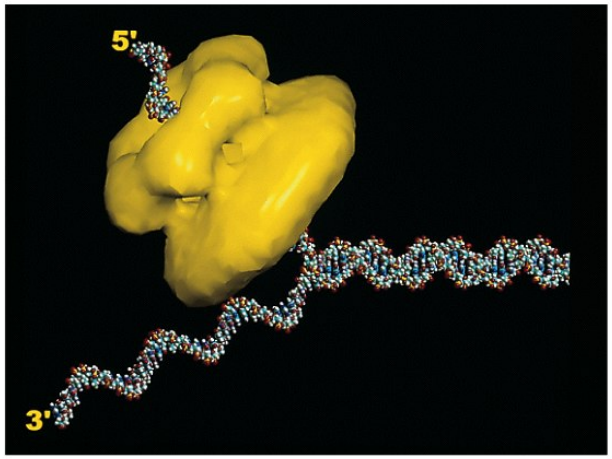


ATP provides energy

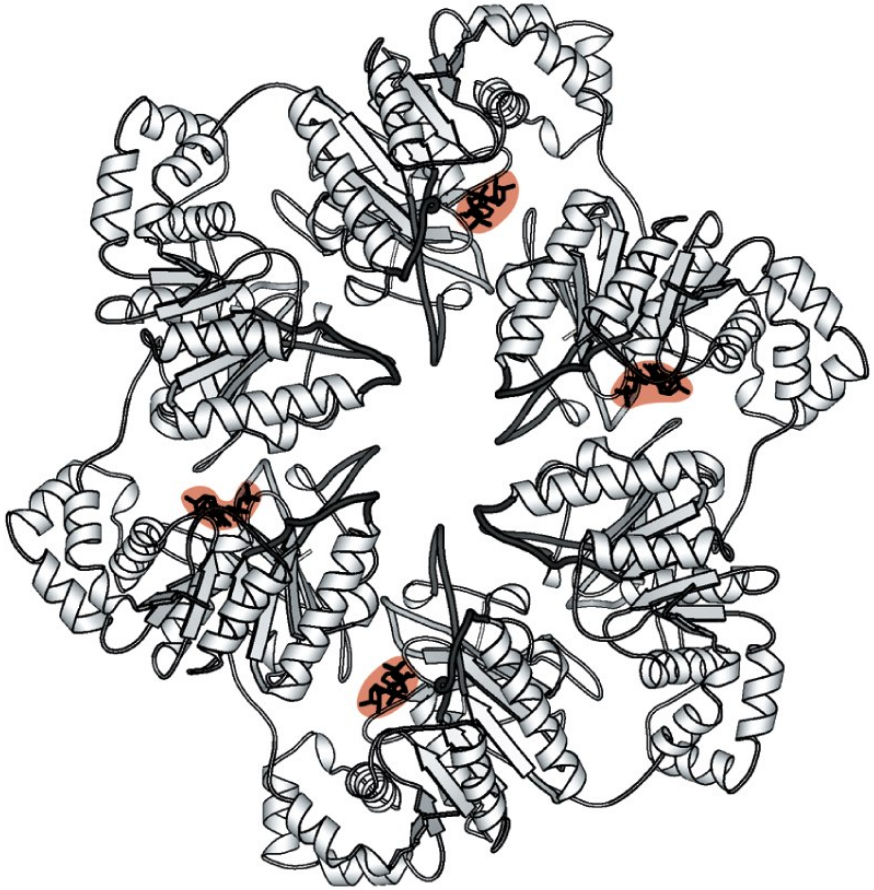
Helicase



(A)



(B)



(C)

See the animation on Moodle

Telomerase maintains the ends of chromosomes

- replication at discontinuous strand would not go up to chromosome end
- telomerase adds telomere sequences

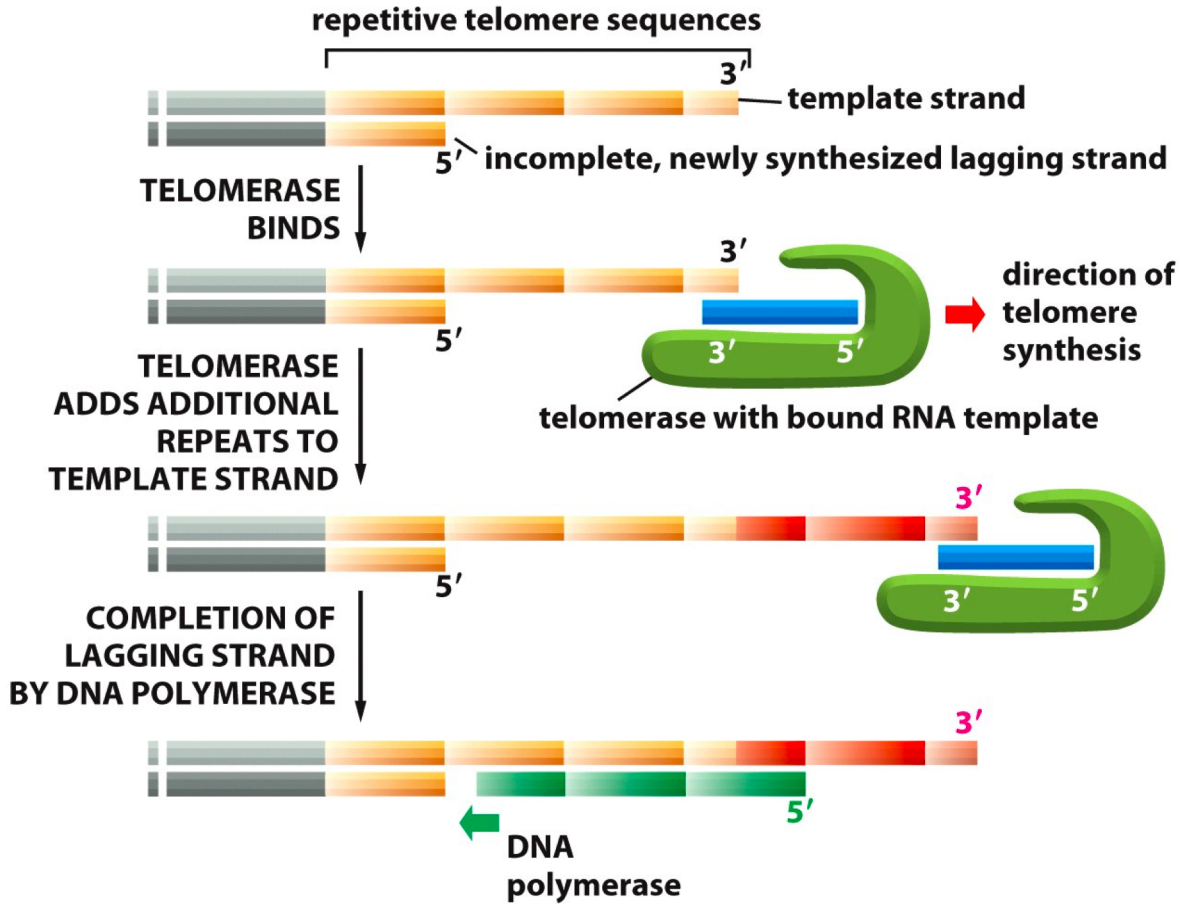


Figure 6-18 Essential Cell Biology 3/e (© Garland Science 2010)

Replication of linear molecule :

the first primer cannot be replaced by DNA.

→ copy shorter than template

Telomerase :
proteins + RNA

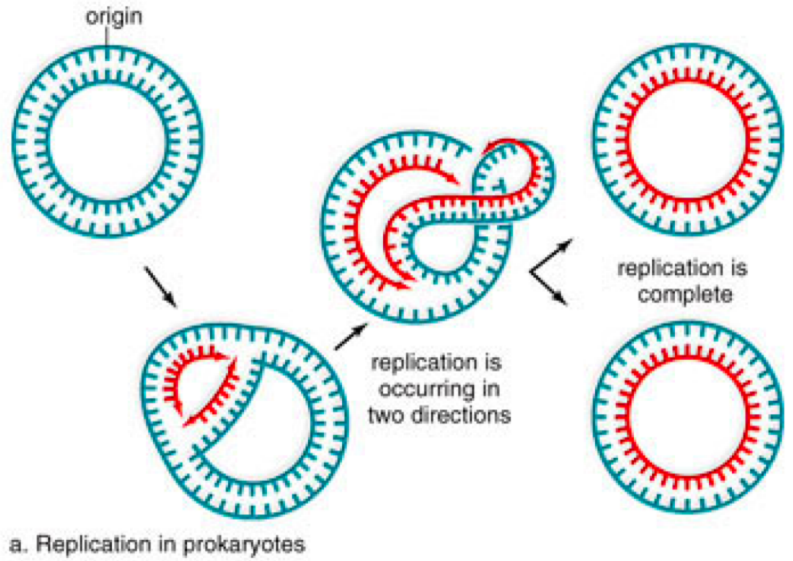
DNA replication

Bacterial genomes are circular

When the genome is circular there is no ends problem.

No need for telomers

No need for telomerase



DNA Repair

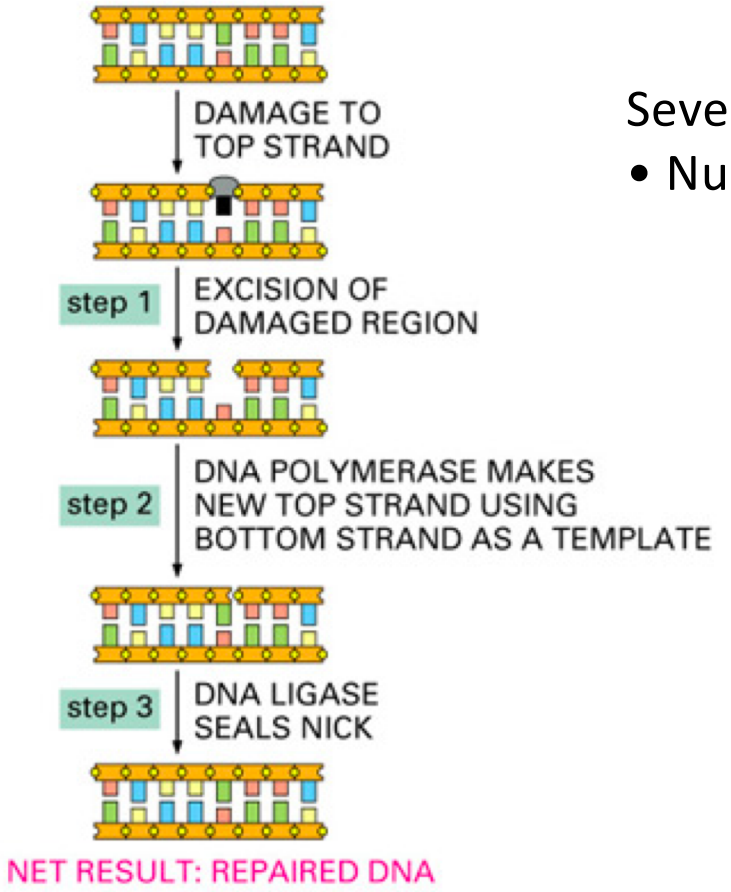
- a permanent change in DNA: mutation
- source of "improvement" during evolution
- but: very dangerous for individual organism
- mutations can arise from spontaneous, or UV- or chemical induced modifications of bases: mismatches between bases
- damaged sequence must be removed and re-synthetized

The steps in DNA repair

Several types of damage :

- UV light → thymine dimer
- x ray → strand breaks
- radioactivity

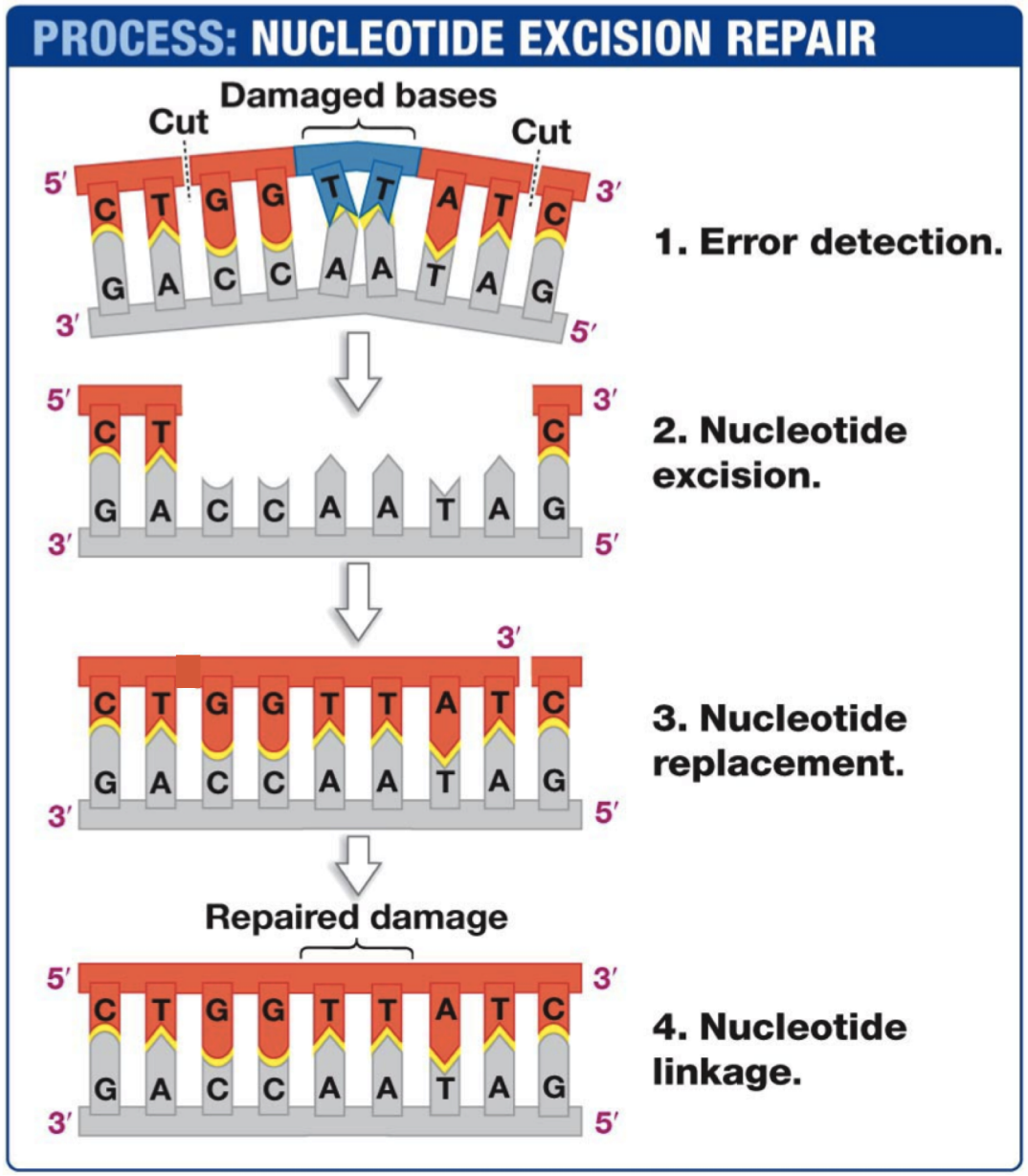
- chemical



Several types of repairs :

- Nucleotides Excision Repair (NER)

Repair of
UV-induced damage



NER deficient

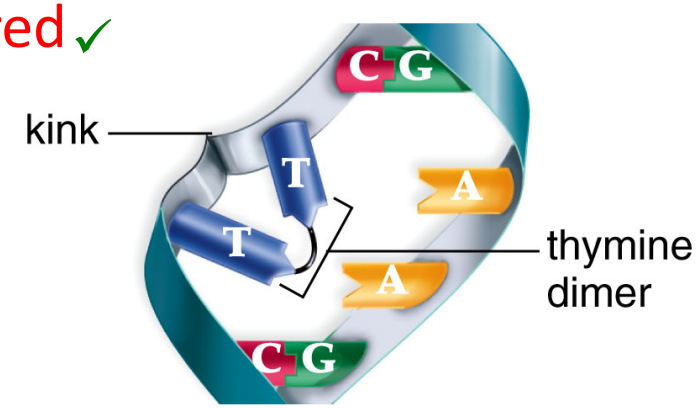
UV –induced DNA damage:

Mutations → **cancer**

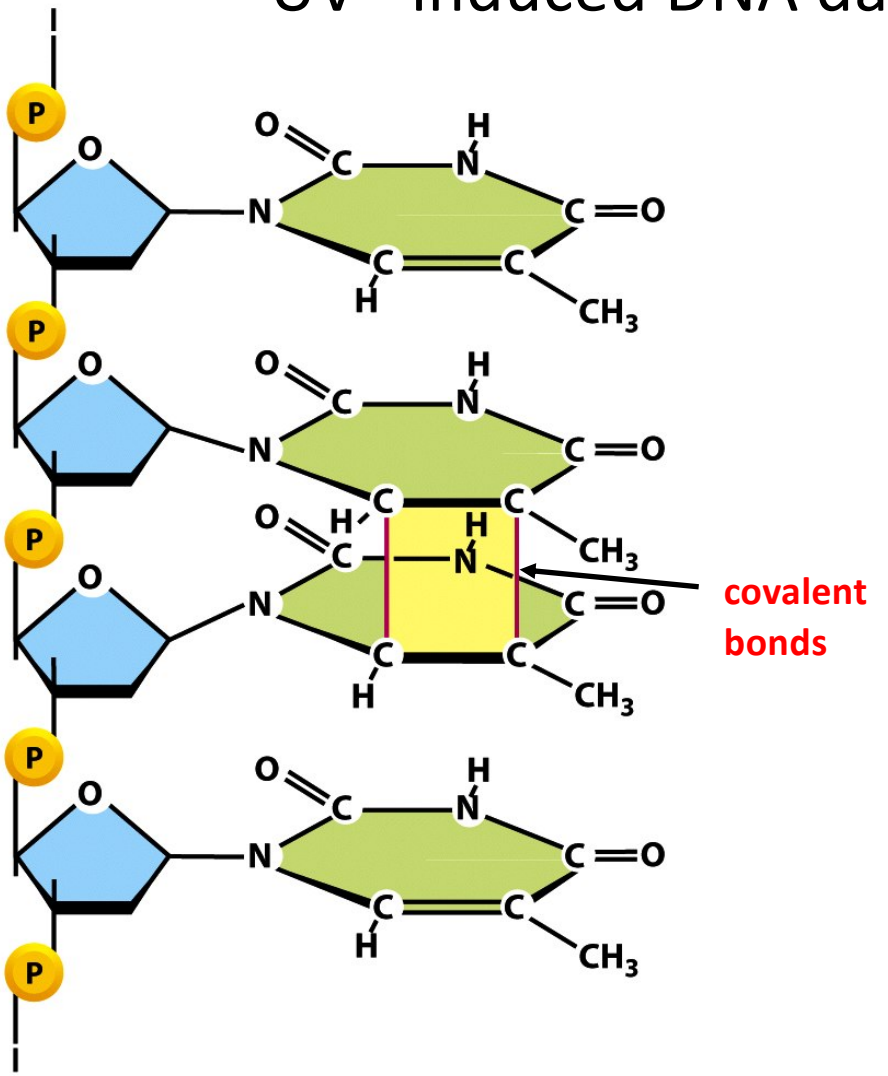
Figure 7.22 The inability to repair UV-caused dimers. **(a)** Sunbathers acquire dimers caused by UV radiation, which can cause skin cancer if they are not repaired. *(Steven Frame/Stock Boston)* **(b)** Xeroderma pigmentosum is a genetic disease in which the enzymes that normally repair UV damage to DNA are defective, and exposure to sunlight results in multiple skin cancers. *(Dr. Ken Greer/Visuals Unlimited)*

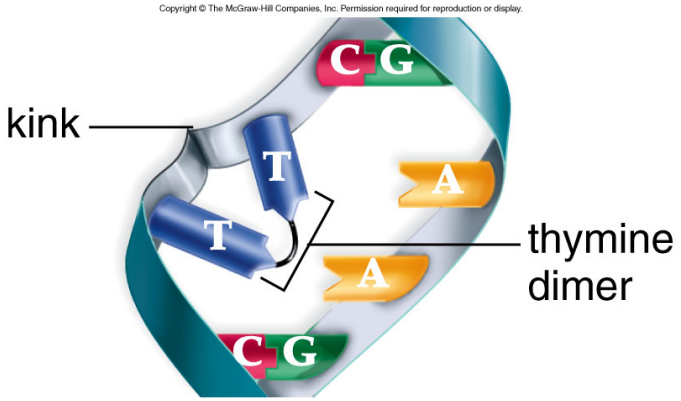
Region with low exposure

repaired ✓



UV –induced DNA damage:





UV –induced DNA damage:

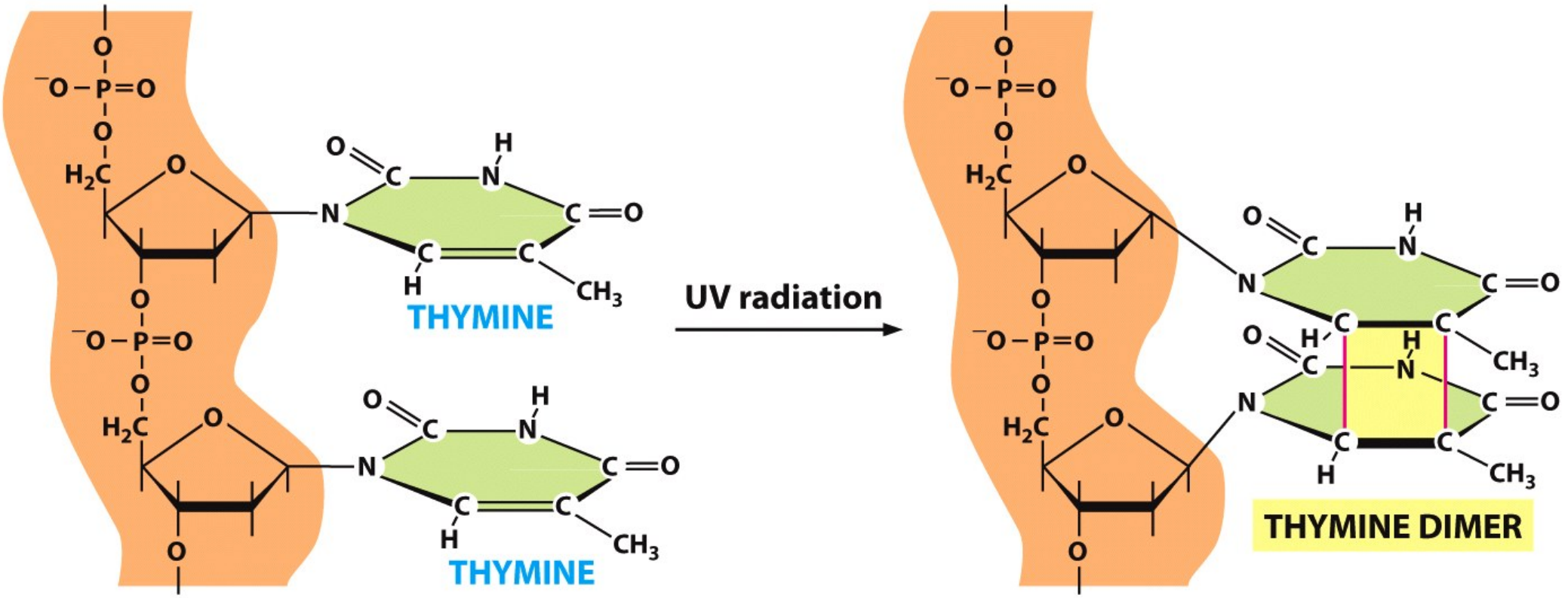


Figure 6-24 *Essential Cell Biology* (© Garland Science 2010)