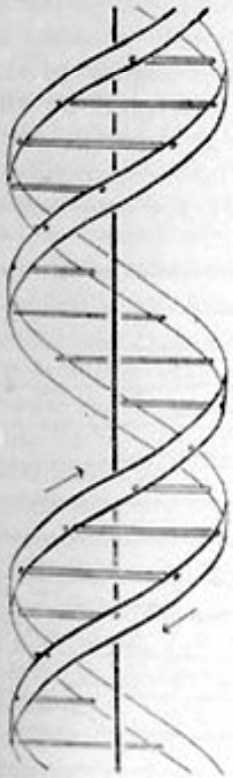
DNA replication & repair

Chapter 6: pp.195-215

British Understatement



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

on it.

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

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.

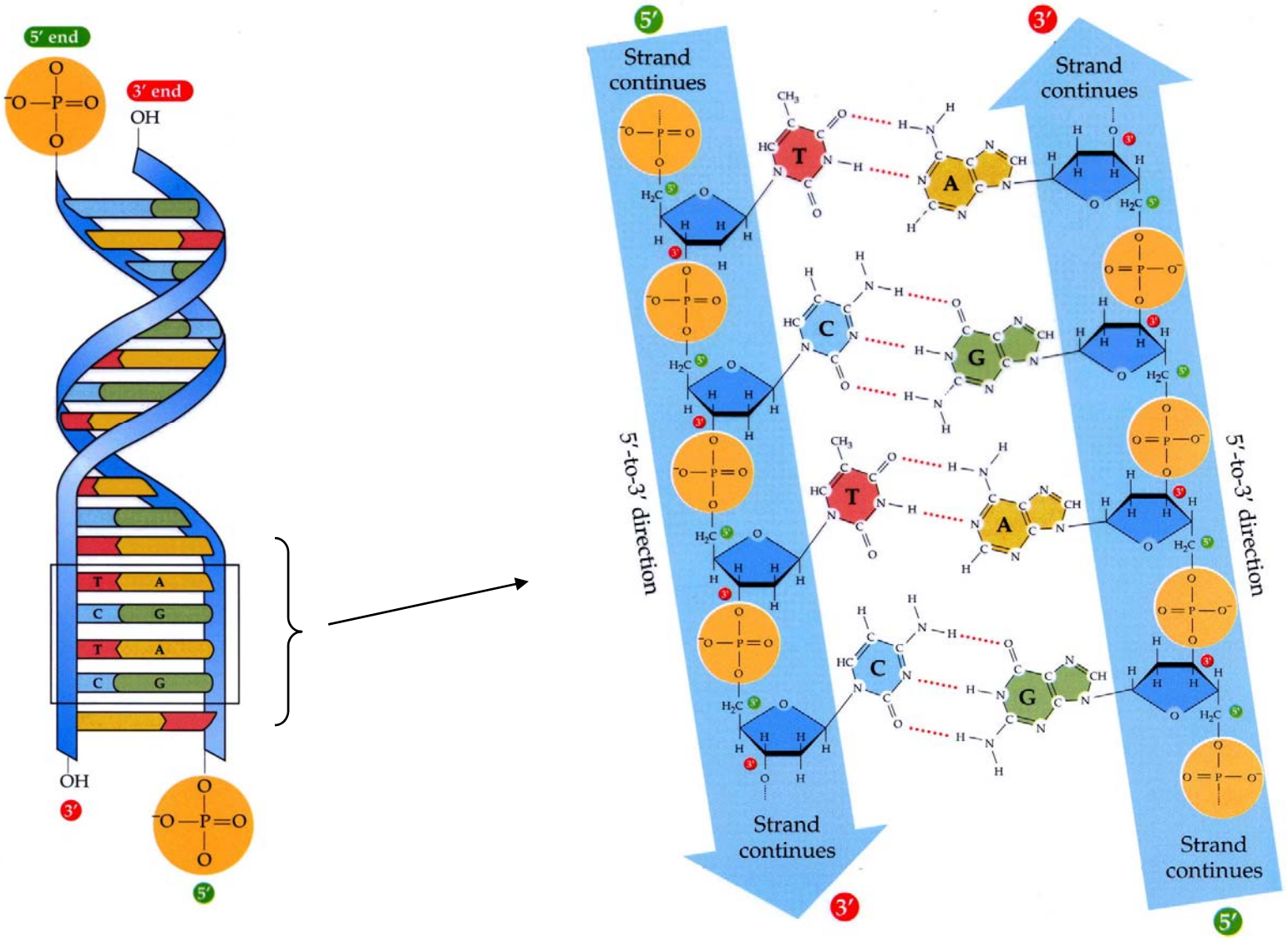
The previously published X-ray data[5,6] on deoxyribose nucleic acid are insufficient for a rigorous test of our structure. So far as we can tell, it is roughly compatible with the experimental data, but it must be regarded as unproved until it has been checked against more exact results. Some of these 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.

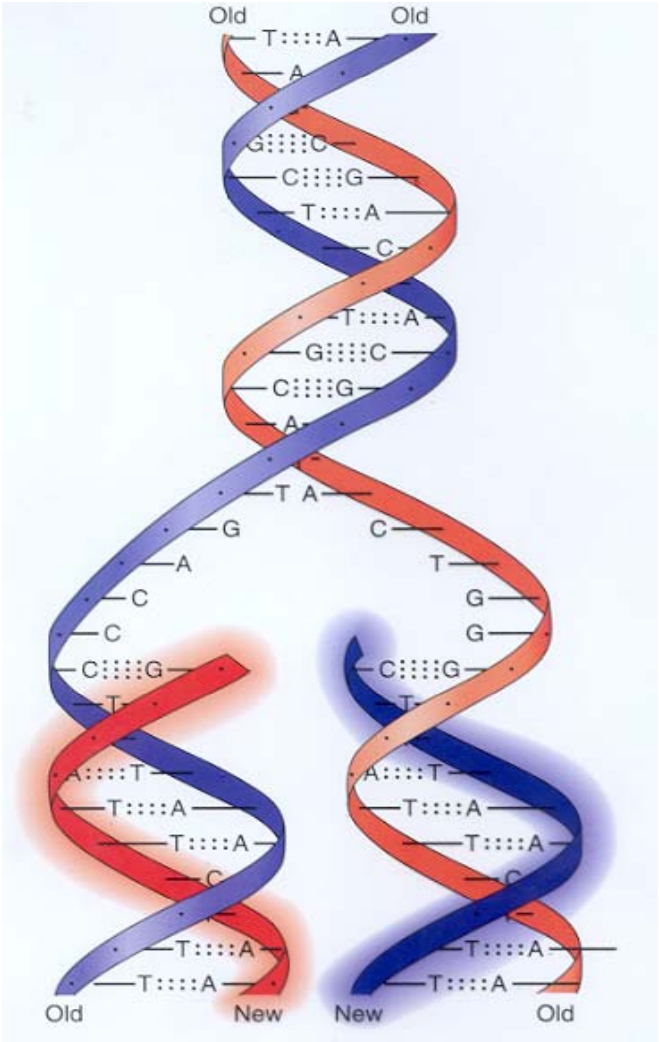
Full details of the structure, including the conditions assumed in building it, together with a set of co-ordinates for the atoms, will be published elsewhere.

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

The DNA double helix is *anti-parallel*

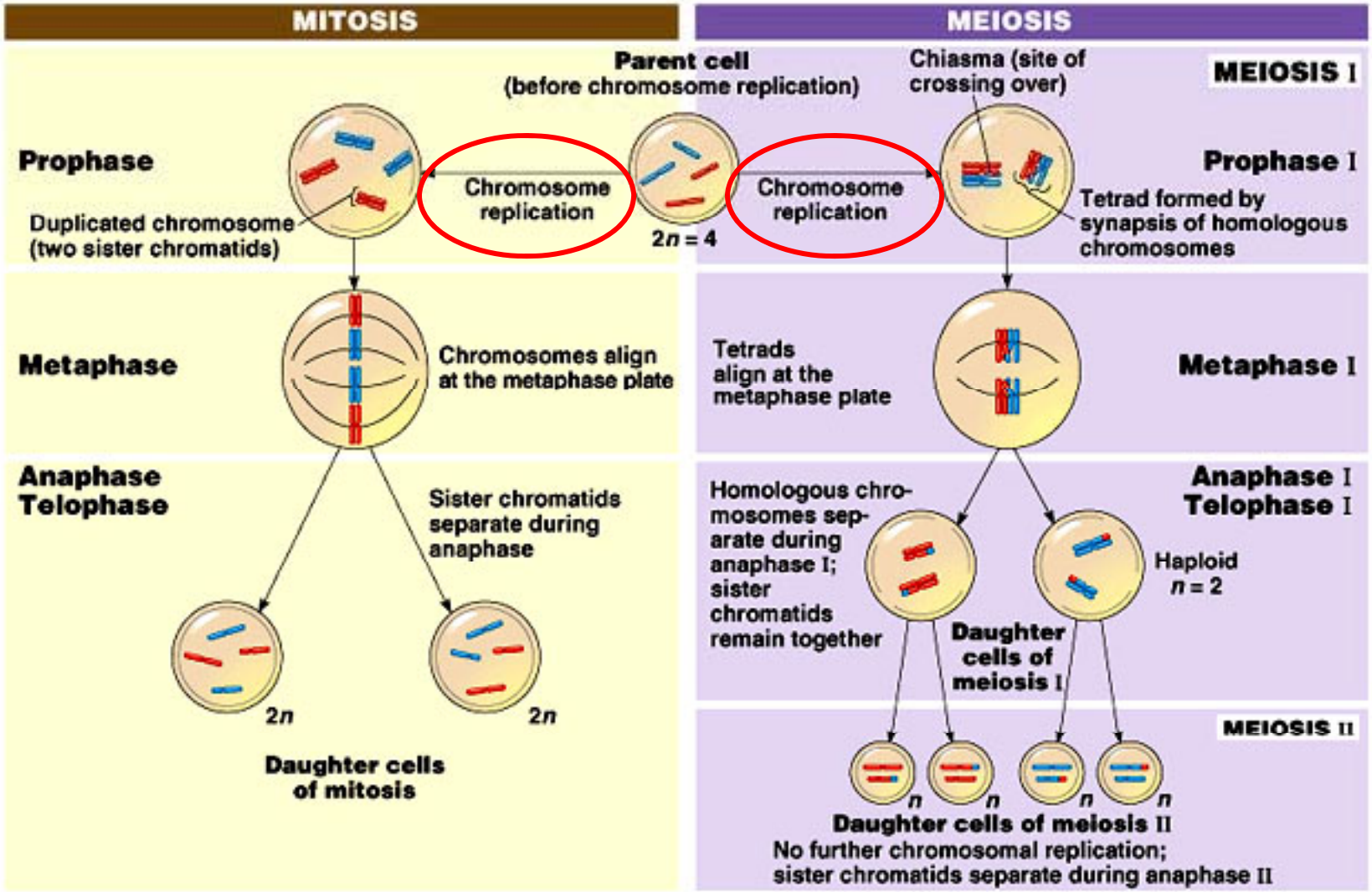


It can be opened up to create templates for copying

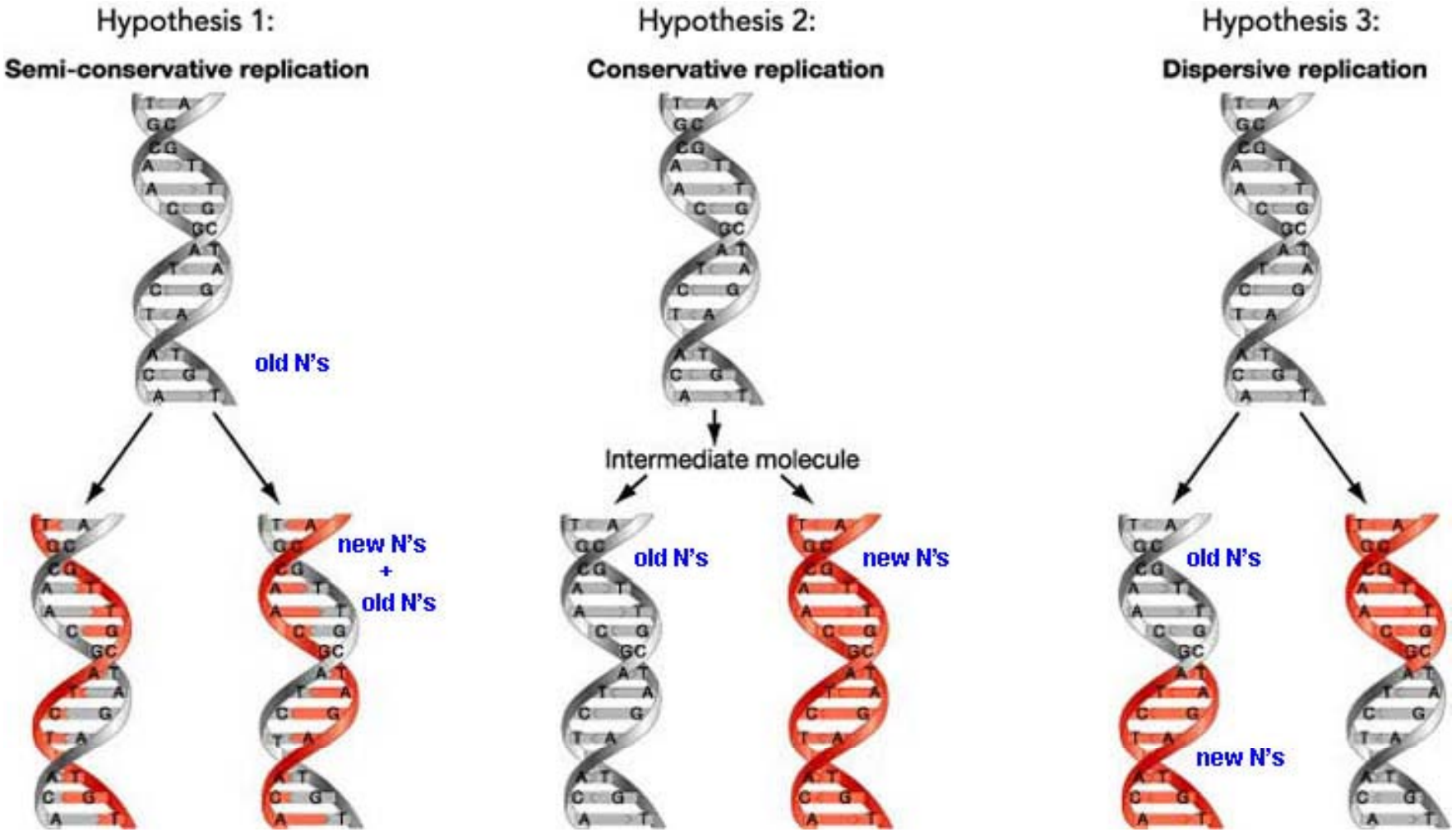


- Single stranded DNA can serve as a template for high-fidelity replication.
- Also as a template for making mRNA (transcription).

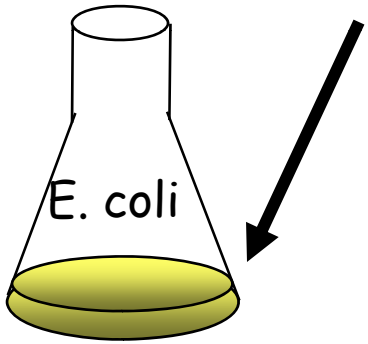
Cell division would go nowhere without DNA replication

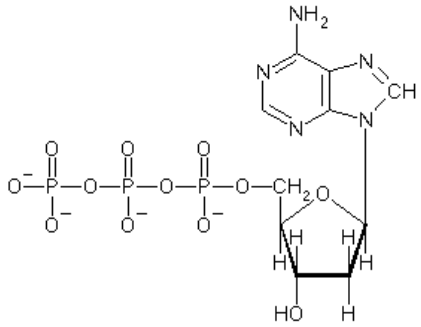


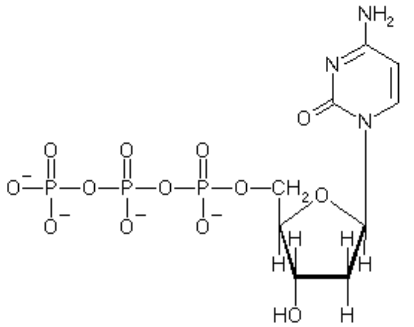
How does DNA replicate?

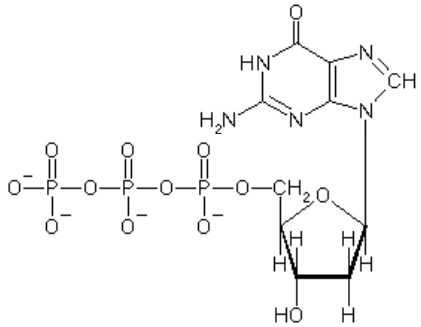


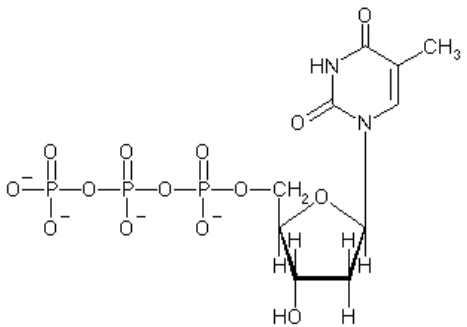
Meselson and Stahl, 1957




Deoxyadenosine 5'-triphosphate
(dATP)

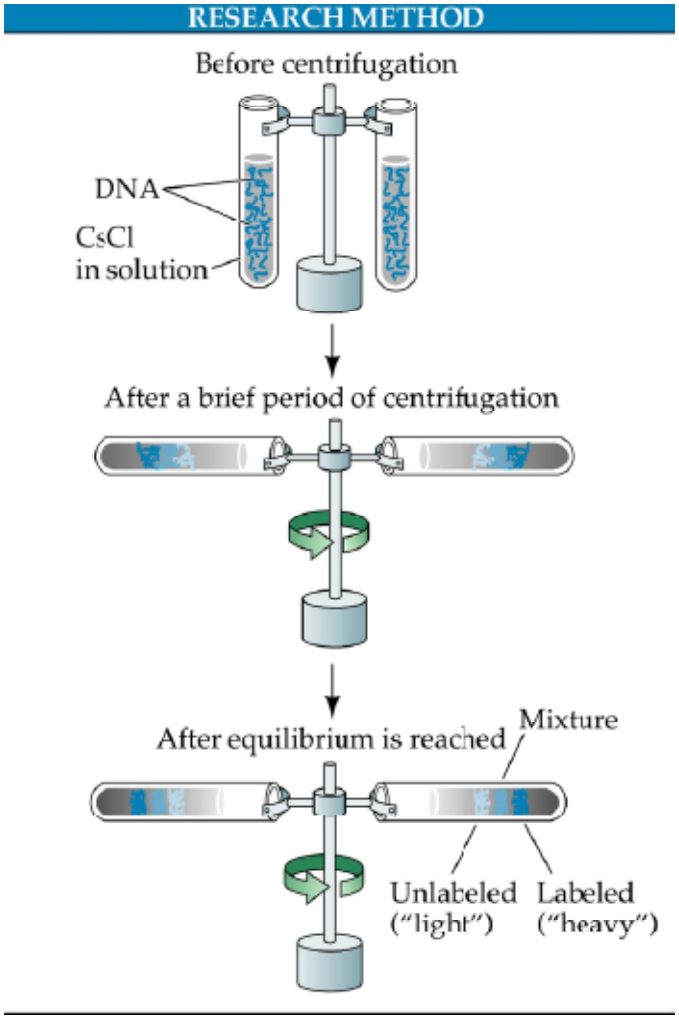

Deoxycytidine 5'-triphosphate
(dCTP)


Deoxyguanosine 5'-triphosphate
(dGTP)


Deoxythymidine 5'-triphosphate
(dTTP)

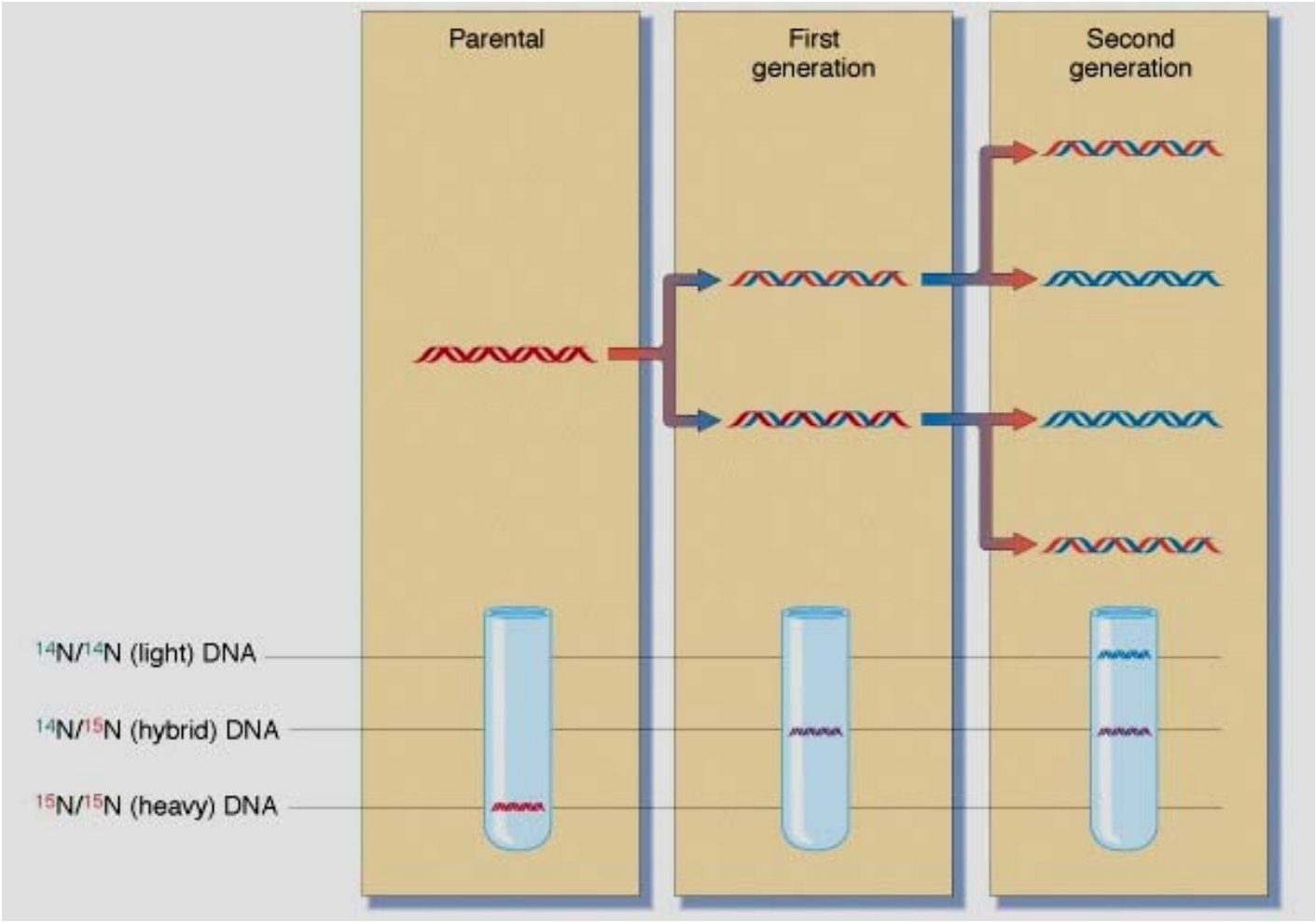
What kinds of molecules will ^{15}N end up in?

- -Lipids?
- -Carbohydrates/polysaccharides?
- -DNA?
- -Proteins?



In a cesium chloride density gradient, heavy stuff is centrifuged to the bottom and lighter stuff will be higher in tube.

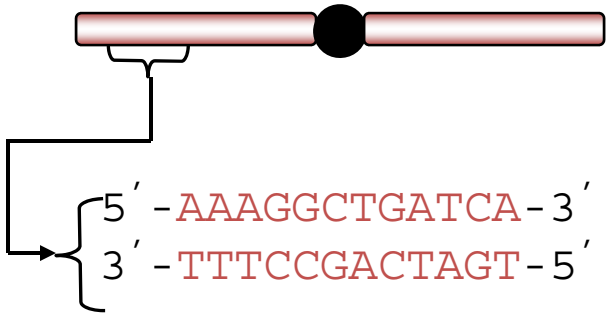
After one round of DNA replication, where will you see bands of DNA if the conservative model is correct? Semiconservative? Discontinuous?



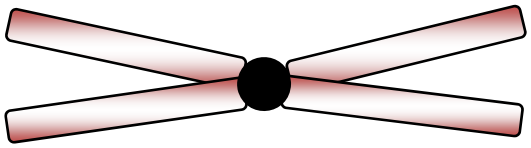
Cell division would go nowhere without DNA replication

One replicated chromosome (consisting of two sister chromatids)

One (unreplicated) chromosome



S phase



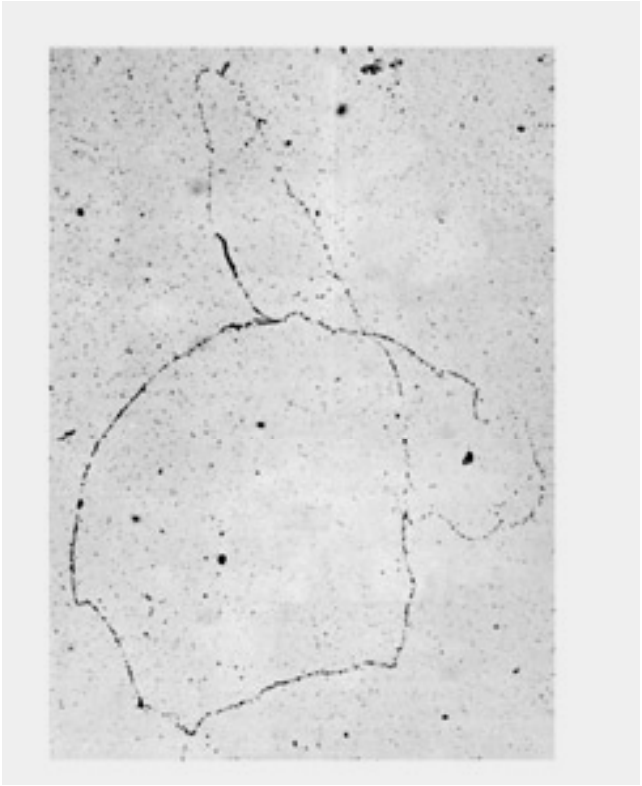
5′-AAAGGCTGATCA-3′
3′-TTTCCGACTAGT-5′

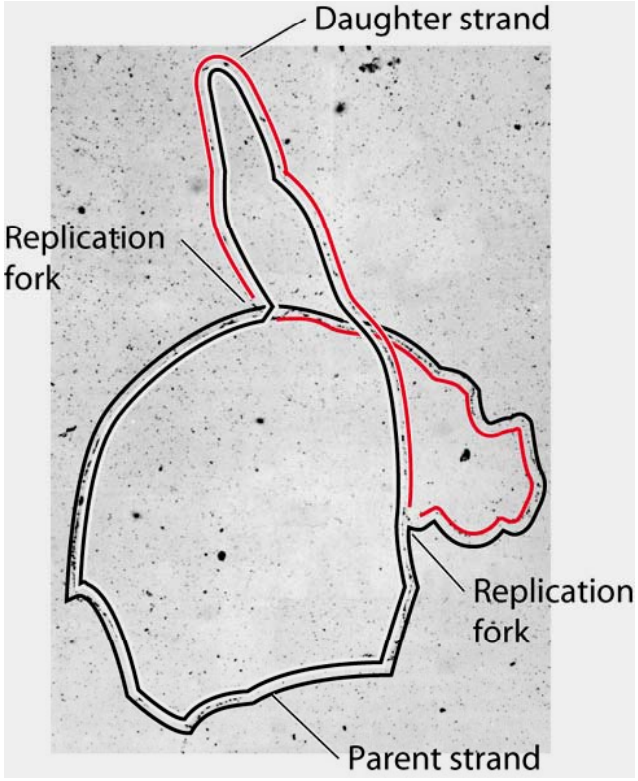
5′-AAAGGCTGATCA-3′
3′-TTTCCGACTAGT-5′

Mitosis, meiosis I

Prokaryotic origins of replication:

single replication origin

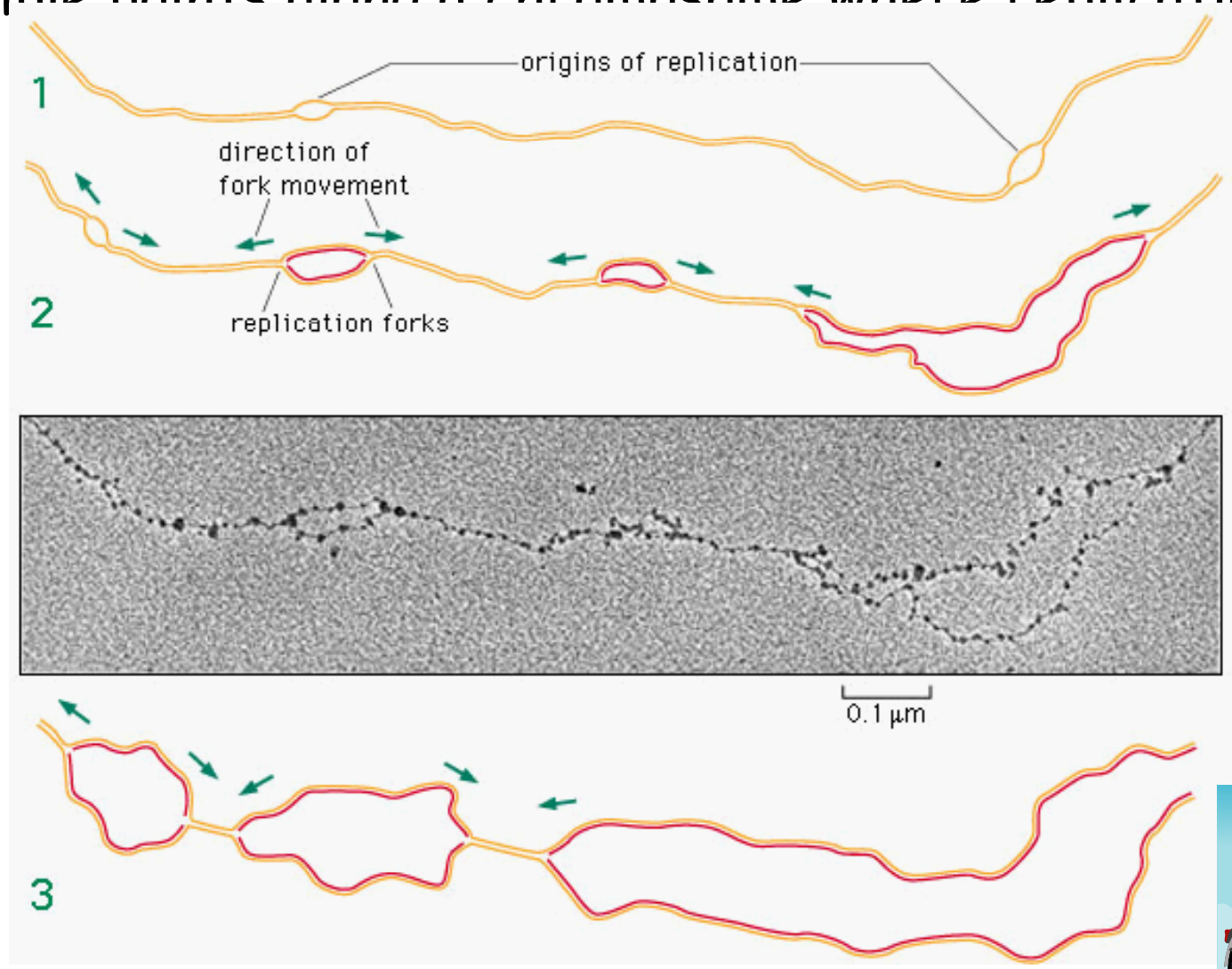




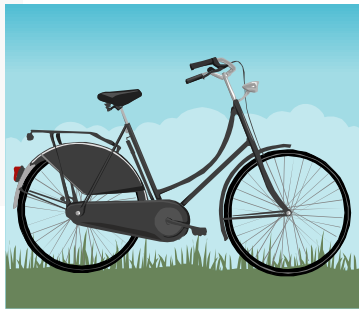
2000 nt/min

Eukaryotic origins of replication:

multiple points along a chromosome where replication begins

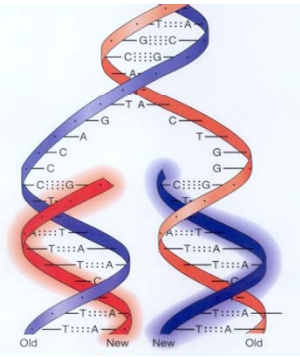


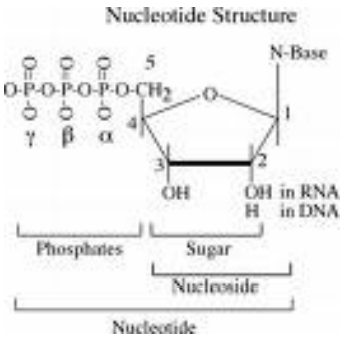
100 nt/min

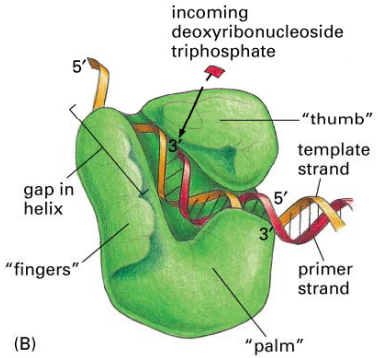


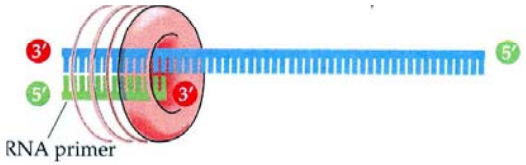
What do you need for DNA replication?

(Let's look at replication in *E.coli*)



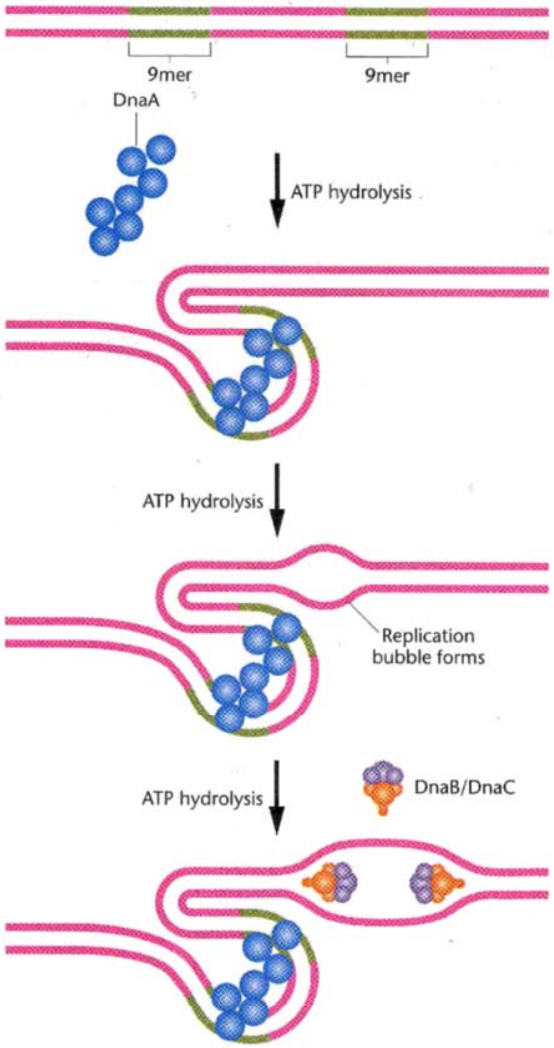






- Template
- Enzymes
- Primer
- dNTP's (d=dexyribose, N= base A,T,G,C)
- Energy

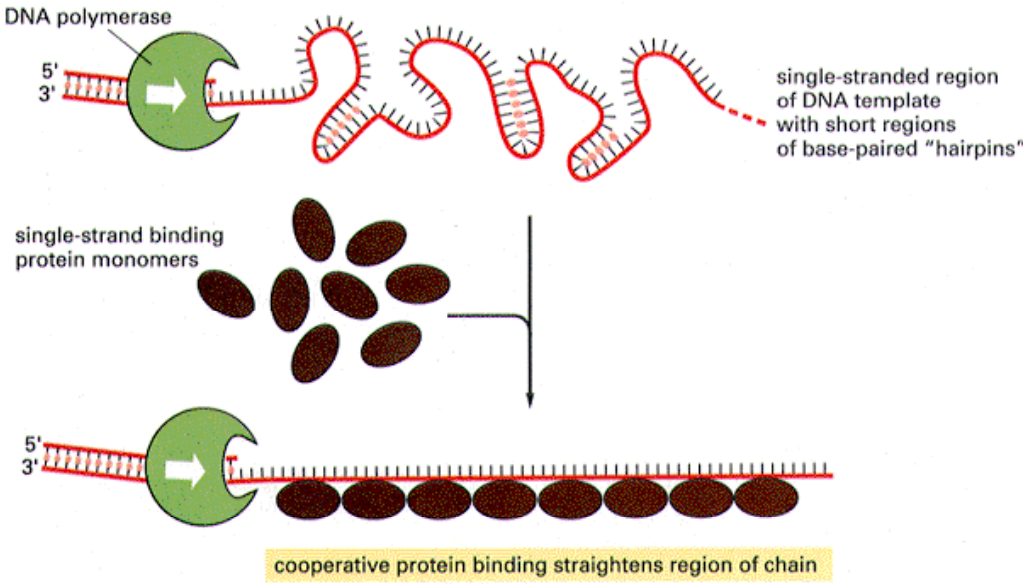
Enzymes for DNA replication: Helicases



Enzymes that unwind the DNA double helix for DNA replication proteins

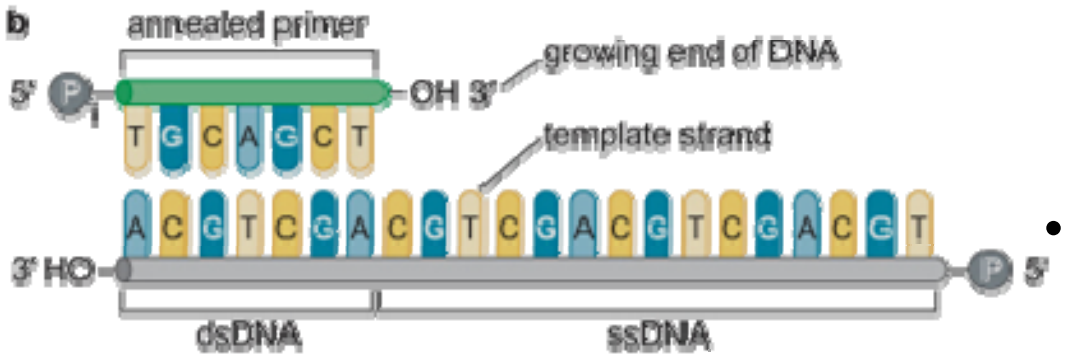
Bind to specific DNA sequences.

Proteins for DNA replication:
Single-stranded binding proteins



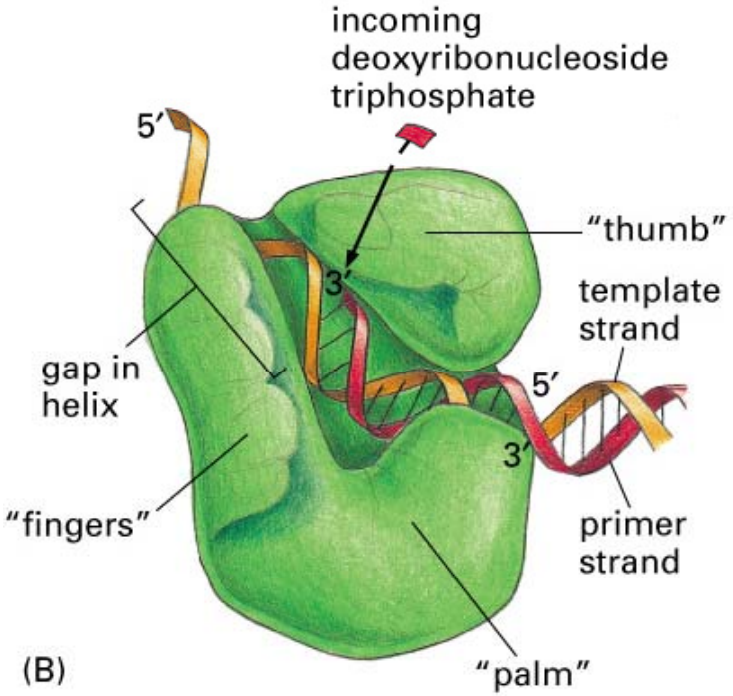
Proteins that stabilize the unwound single stranded DNA

Enzymes for DNA replication:
Primase



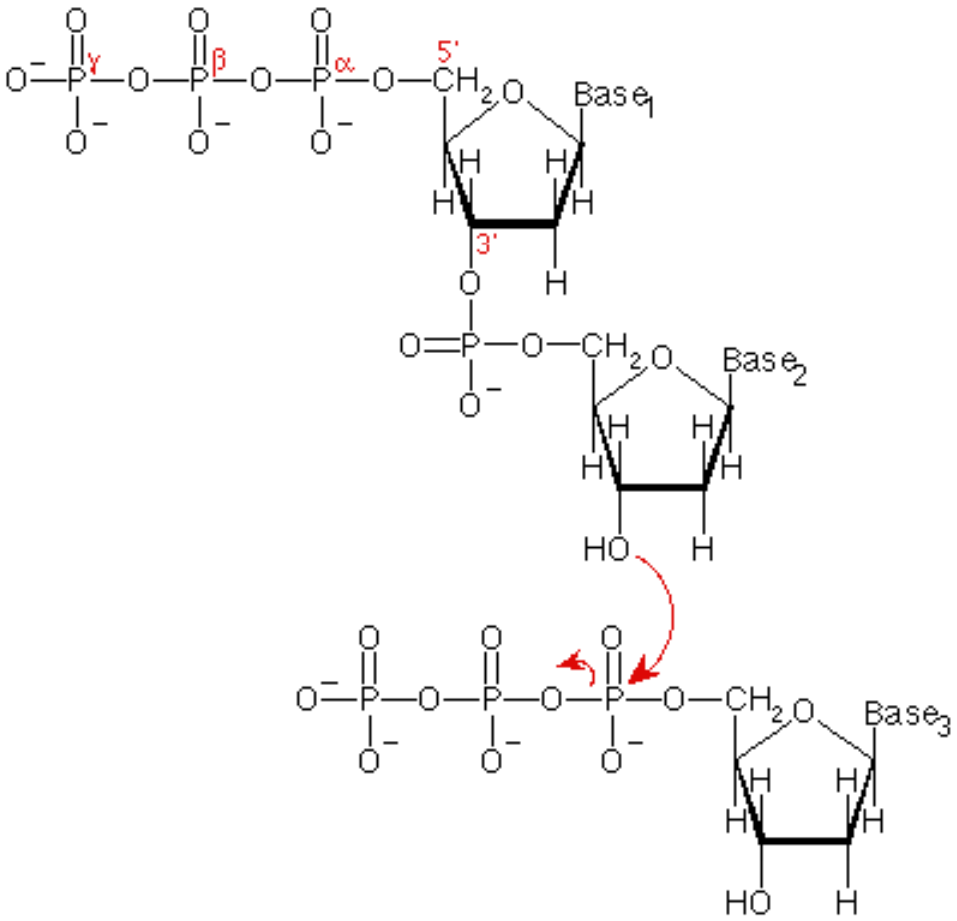
- Provides a short, complementary strand of RNA that is required for DNA synthesis from a naked DNA template.
- There is no known DNA polymerase that can initiate synthesis of a DNA strand – they can only add nucleotides to a pre-existing strand.

Enzymes for DNA replication:
DNA polymerase III

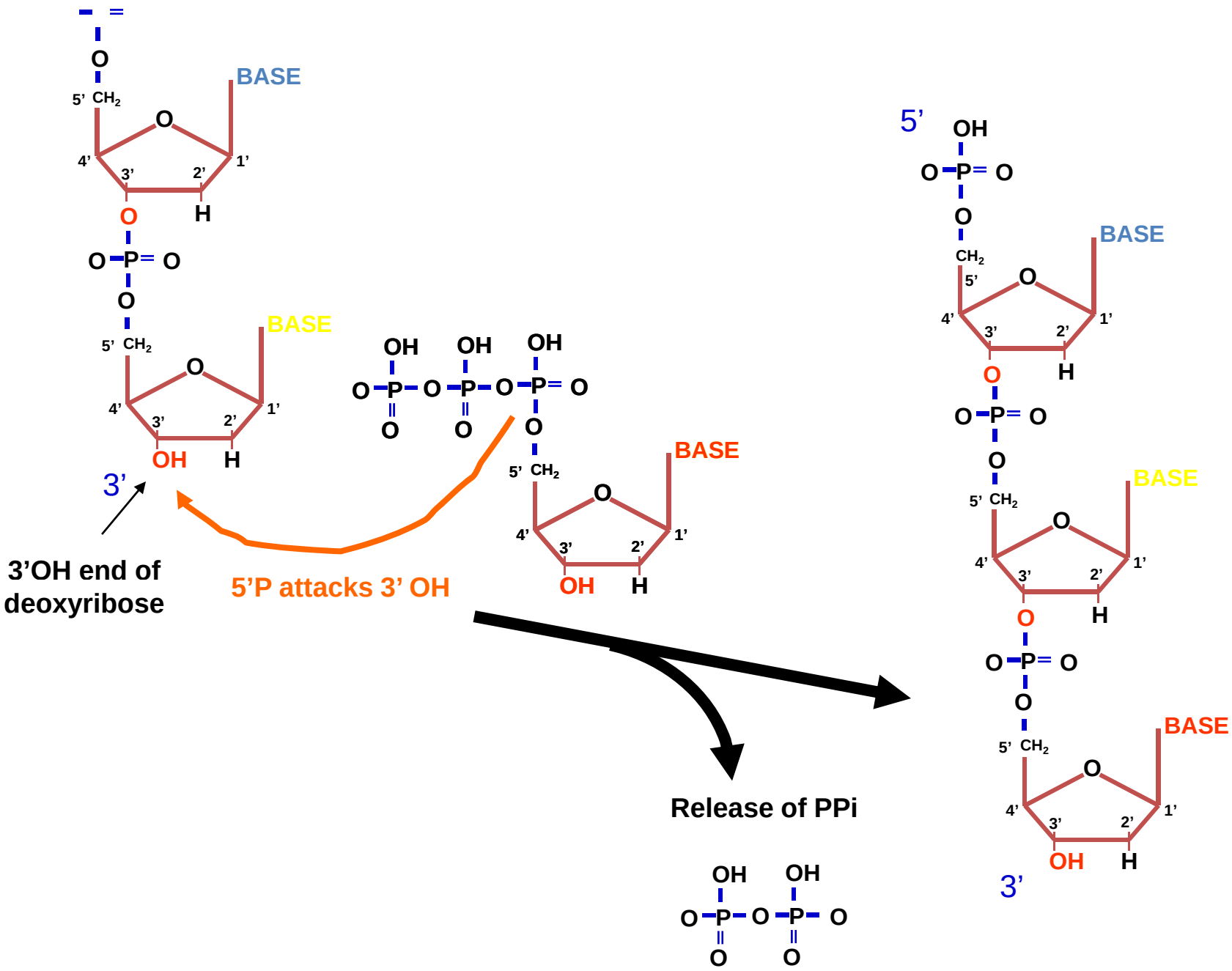


- DNA polymerase III
- The enzyme that adds complementary nucleotides to the backbone, based on the sequence of the single stranded template.
- *Can only work 5′ to 3′.*

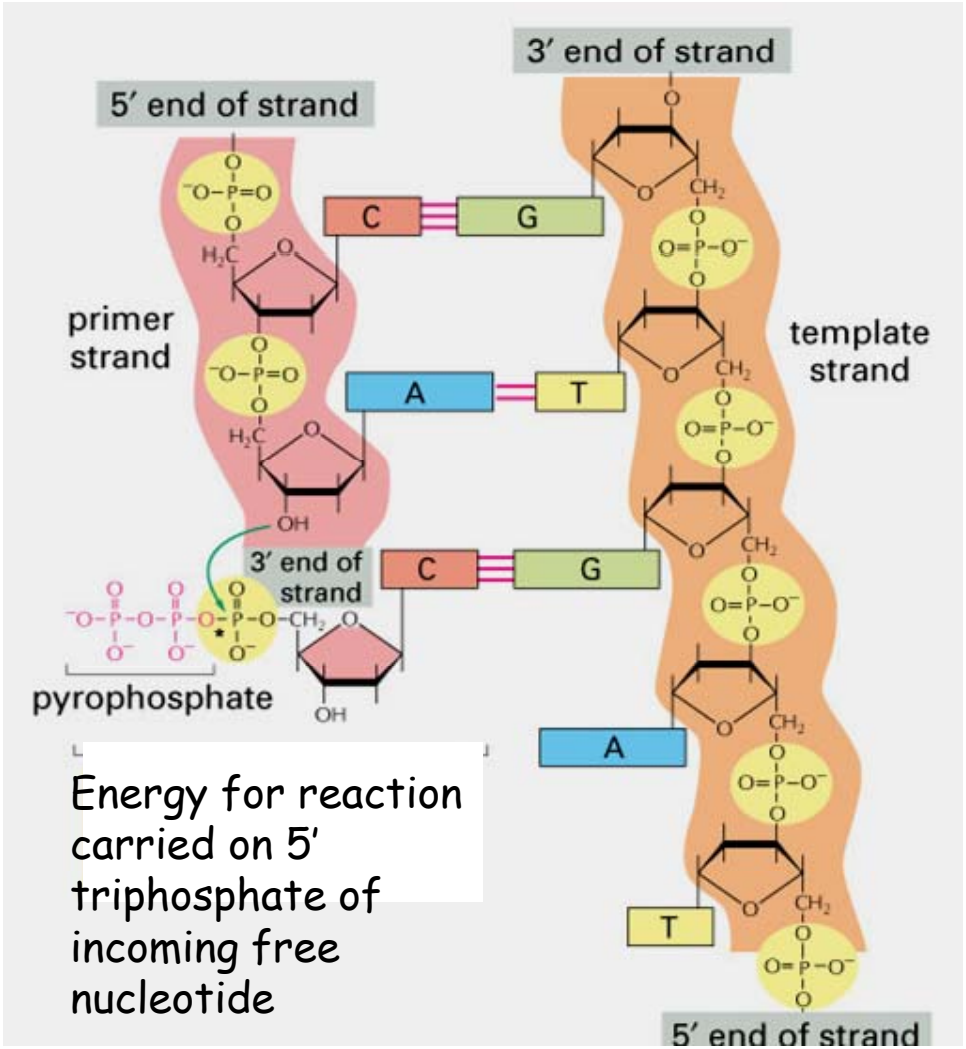
Enzymes for DNA replication:
DNA polymerase III



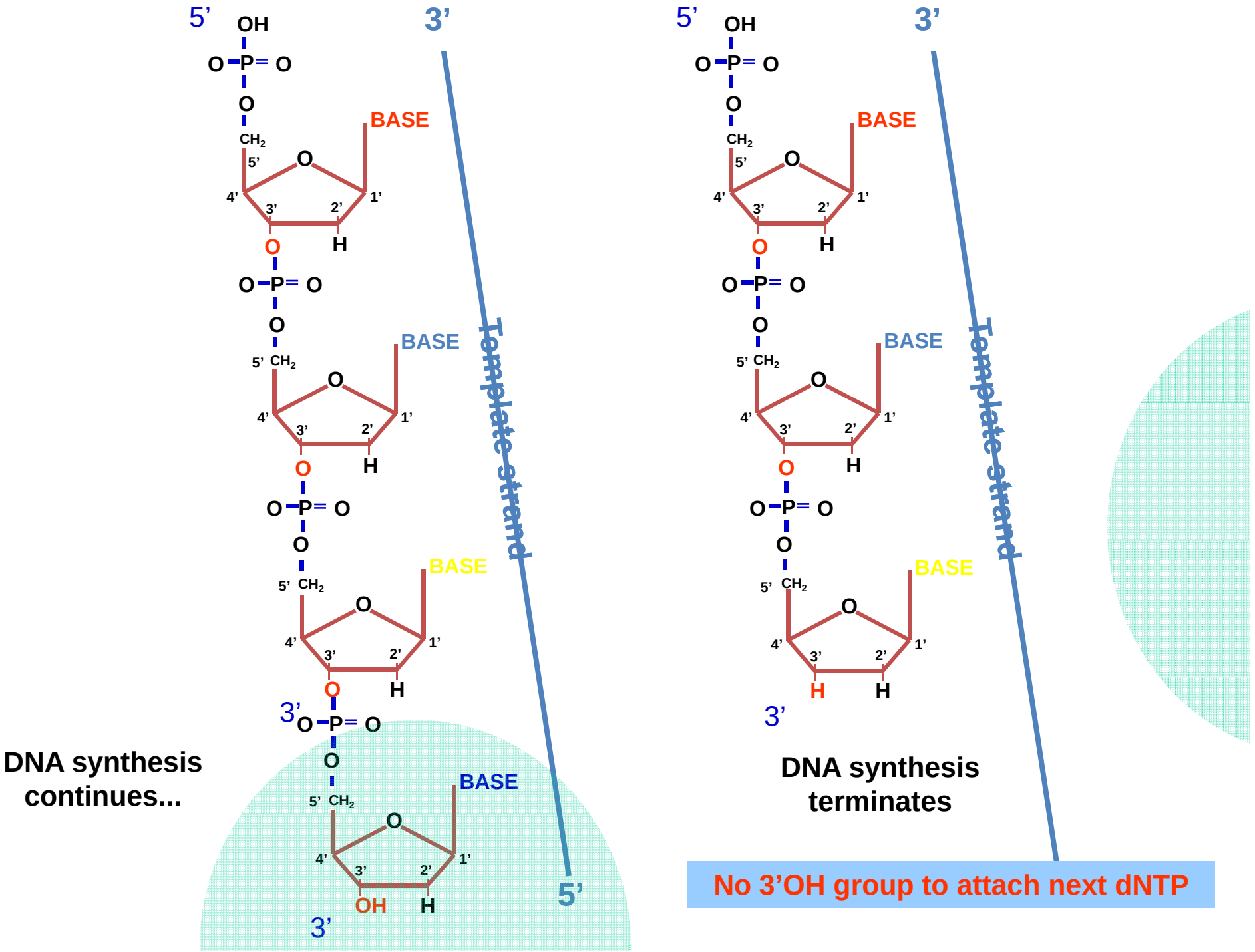
- Directionality of synthesis due to polarity of bond: incoming dNTP needs 3'OH to react with high energy phosphate bond.
- The nucleotides are added to the growing new strand **at the 3' end** – where the DNA strand has a free hydroxyl group.


BASE
5’ CH2
4’
3’
2’
1’
OH
H
3’
3’OH end of deoxyribose
5’P attacks 3’ OH
5’
Release of PPi

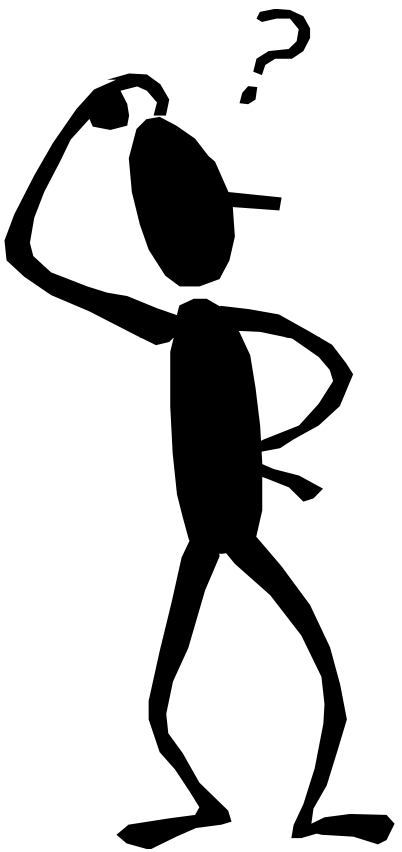
Enzymes for DNA replication: DNA polymerase III

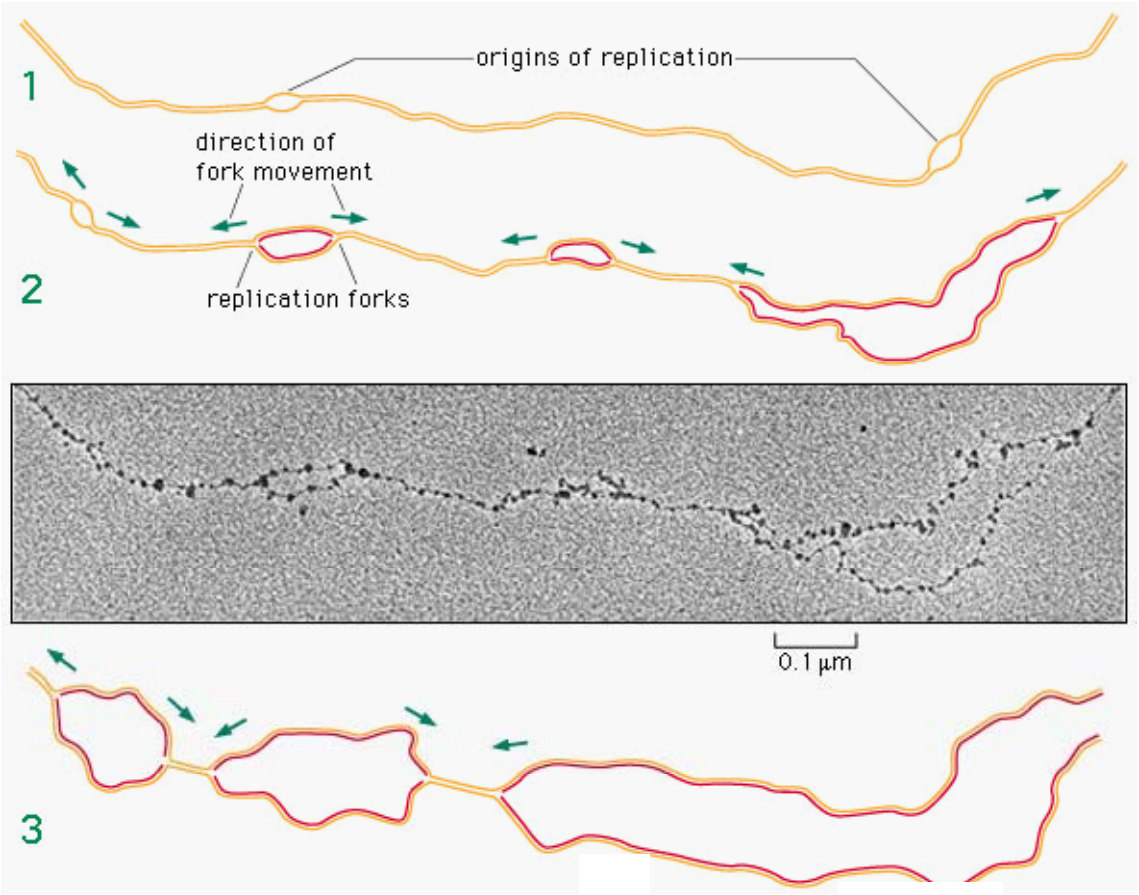


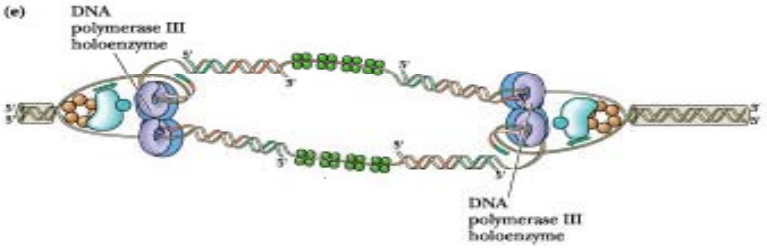
- Directionality of synthesis due to polarity of bond: incoming dNTP needs 3′OH to react with high energy phosphate bond.


5’
3’
OH
O
P
O
O
CH2
5’
O
BASE
4’
3’
2’
1’
H
Template strand
3’
5’
DNA synthesis continues...
DNA synthesis terminates
No 3’OH group to attach next dNTP

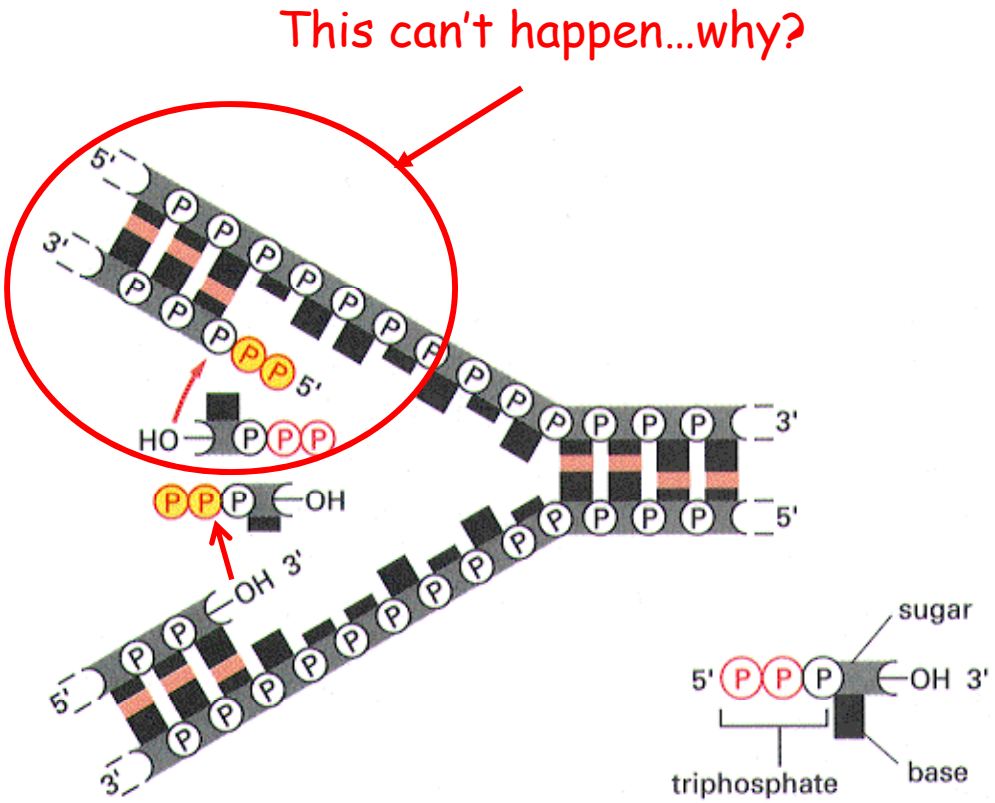
Houston, we have a problem…







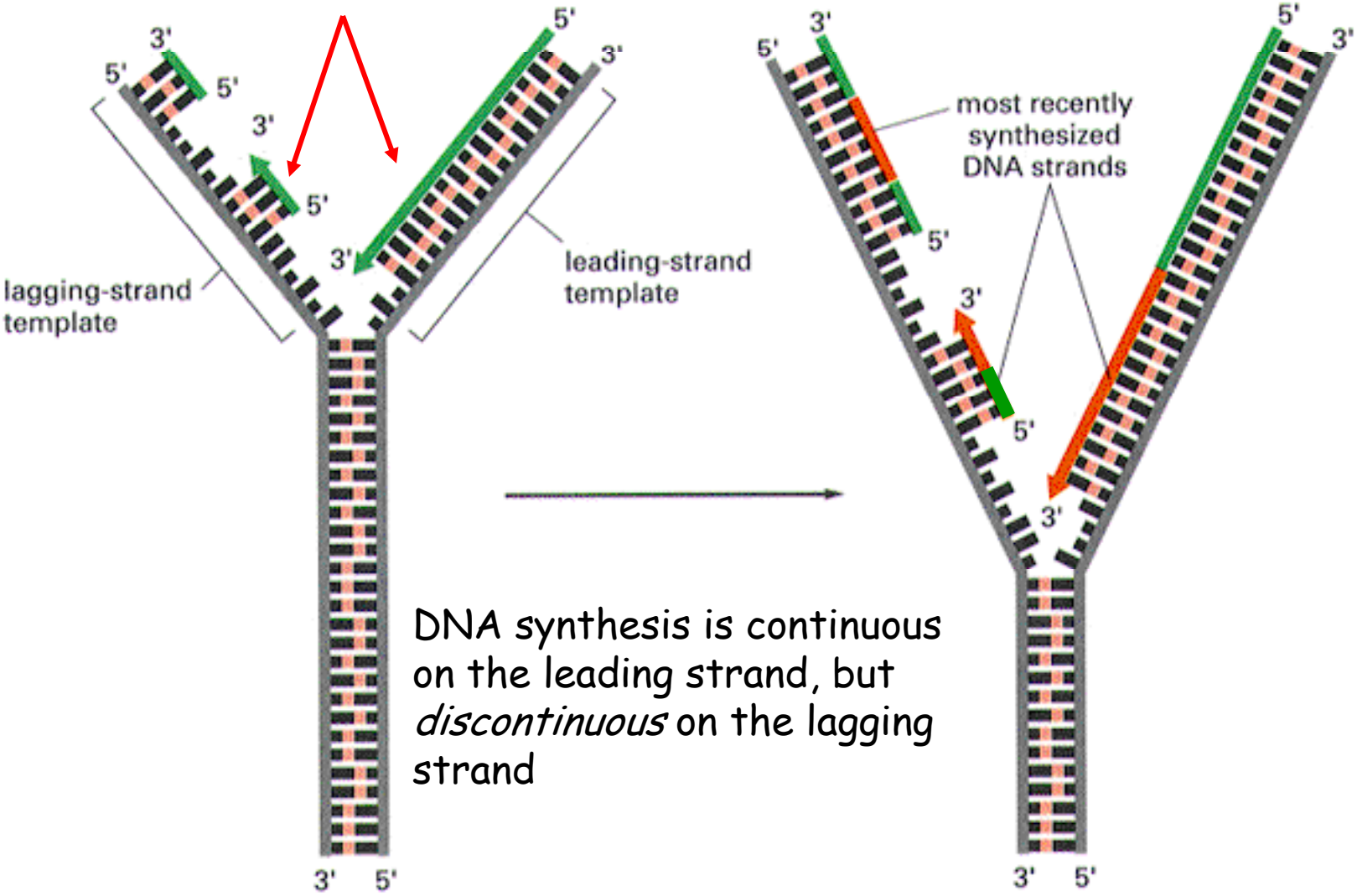
Houston, we have a problem…



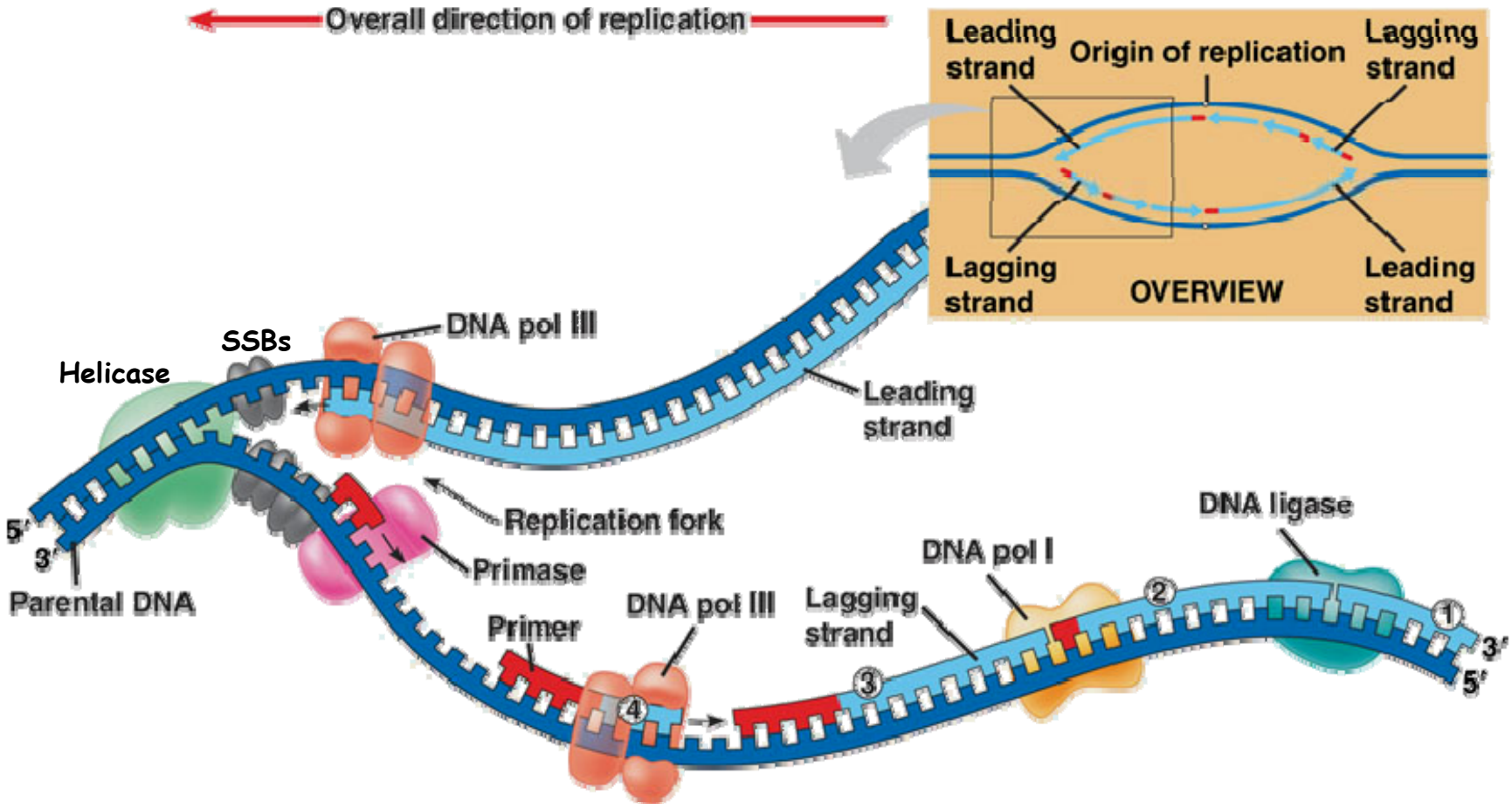
- How do we synthesize DNA on both strands simultaneously?
- Recall that the DNA double helix is *antiparallel.*
- One strand is OK – can go 5′-3′. But what about the other strand? Synthesis cannot be done in the 3′-5′ direction.

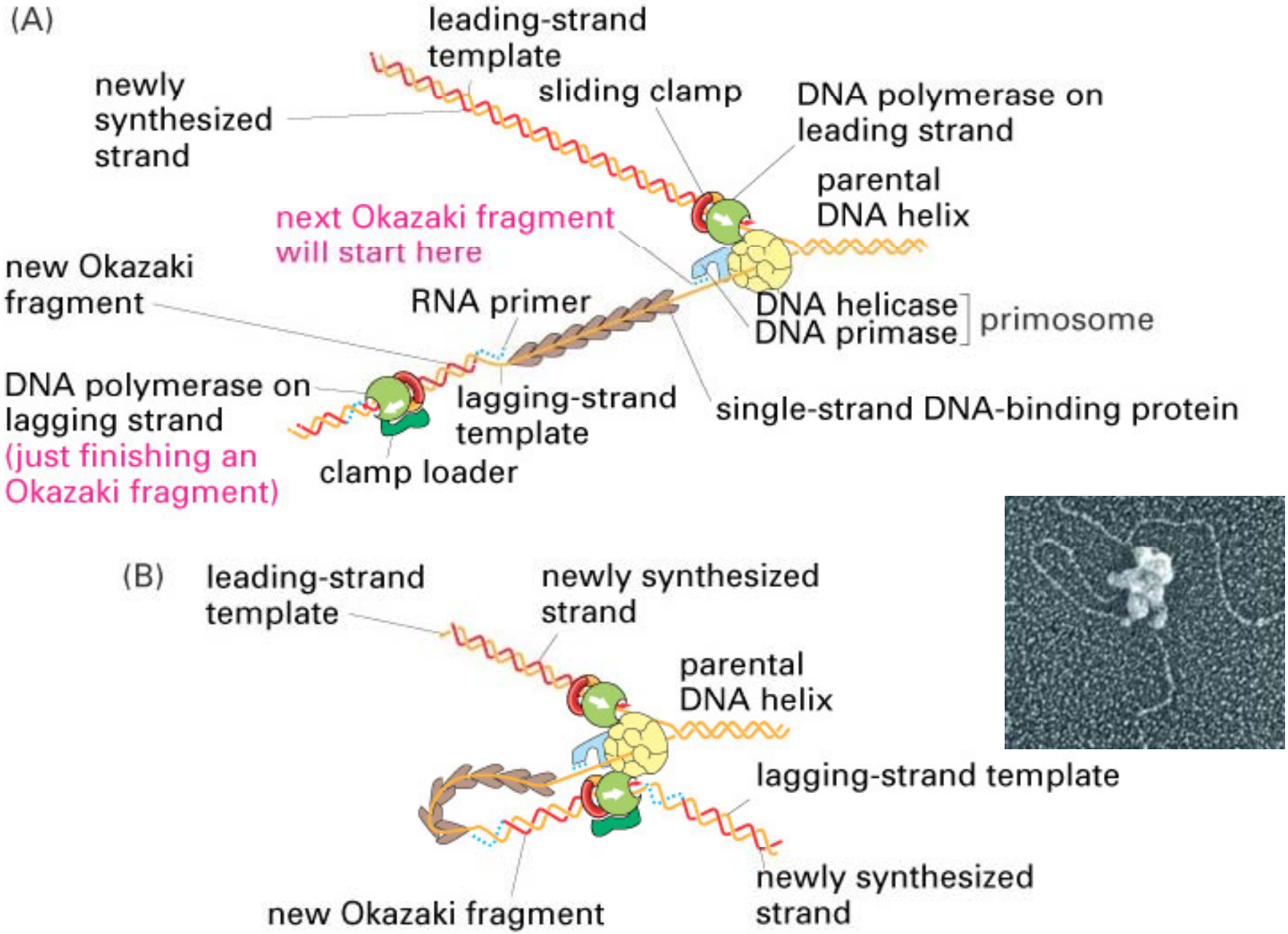
Solution: Okazaki fragments

Primase makes RNA primers for Okazaki fragments on lagging strand, and a single RNA primer on the leading strand.

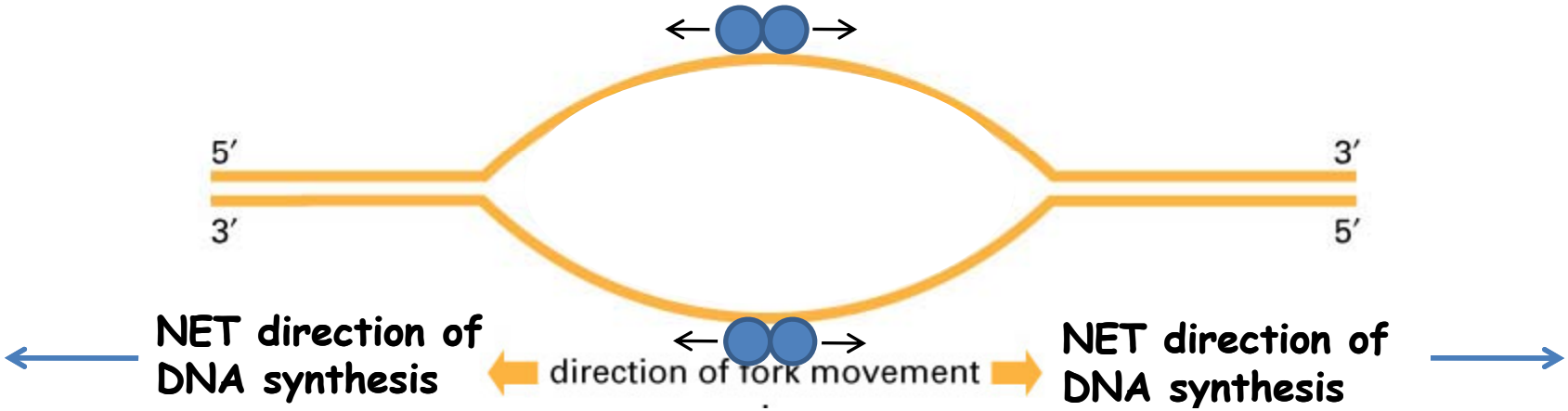


DNA synthesis is continuous on the leading strand, but *discontinuous* on the lagging strand

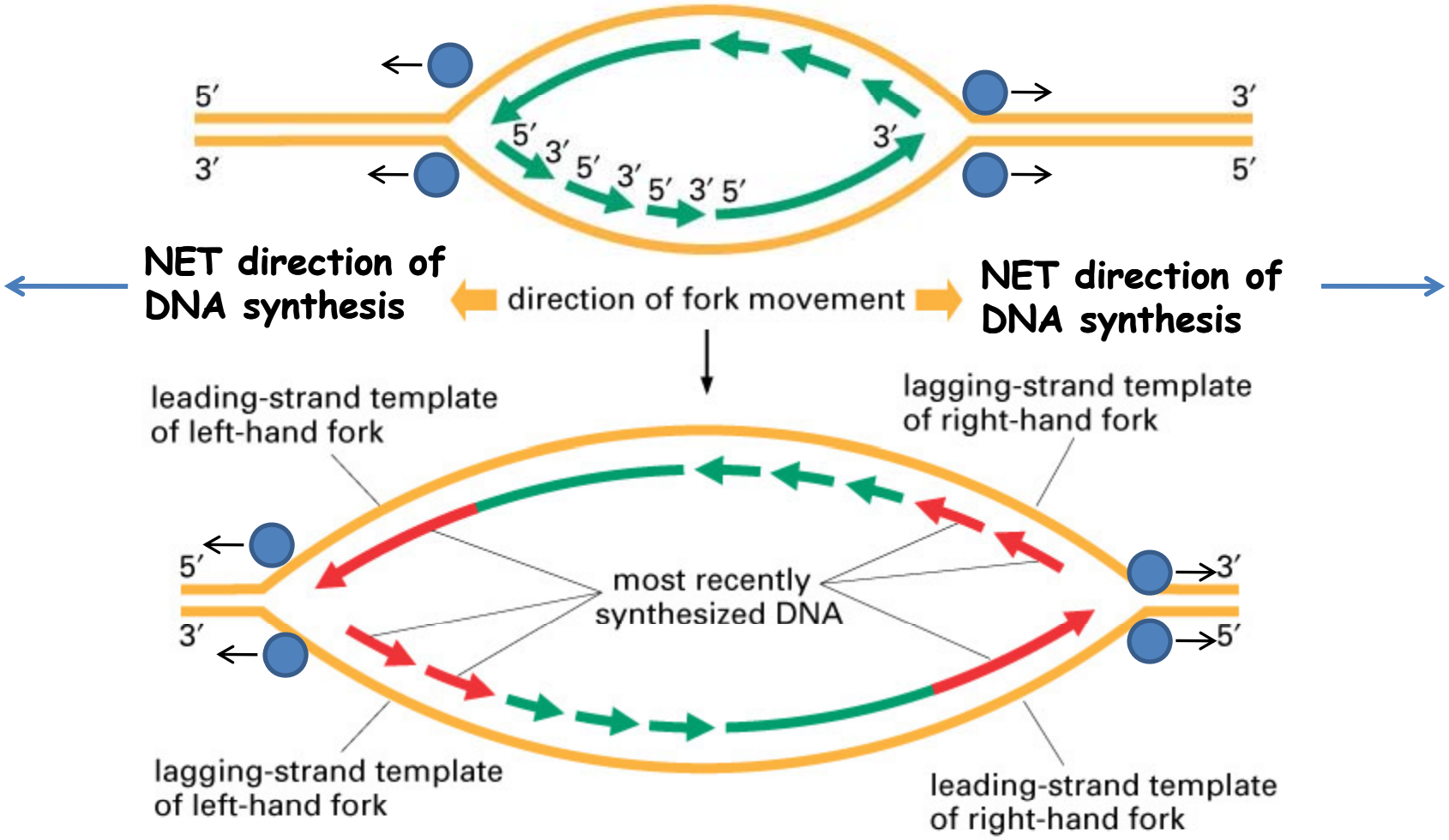

Overall direction of replication
Leading strand
Origin of replication
Lagging strand
Lagging strand
OVERVIEW
Leading strand
Helicase
SSBs
DNA pol III
Leading strand
Replication fork
5′
3′
Parental DNA
Primase
Primer
DNA pol III
Lagging strand
DNA pol I
DNA ligase
1
2
3
4
3′
5′


(A)
leading-strand template
newly synthesized strand
sliding clamp
DNA polymerase on leading strand
parental DNA helix
next Okazaki fragment will start here
new Okazaki fragment
RNA primer
DNA helicase
DNA primase
primosome
DNA polymerase on lagging strand (just finishing an Okazaki fragment)
lagging-strand template
single-strand DNA-binding protein
clamp loader
(B)
leading-strand template
newly synthesized strand
parental DNA helix
lagging-strand template
newly synthesized strand
new Okazaki fragment

The most important DNA replication slide!!!!!

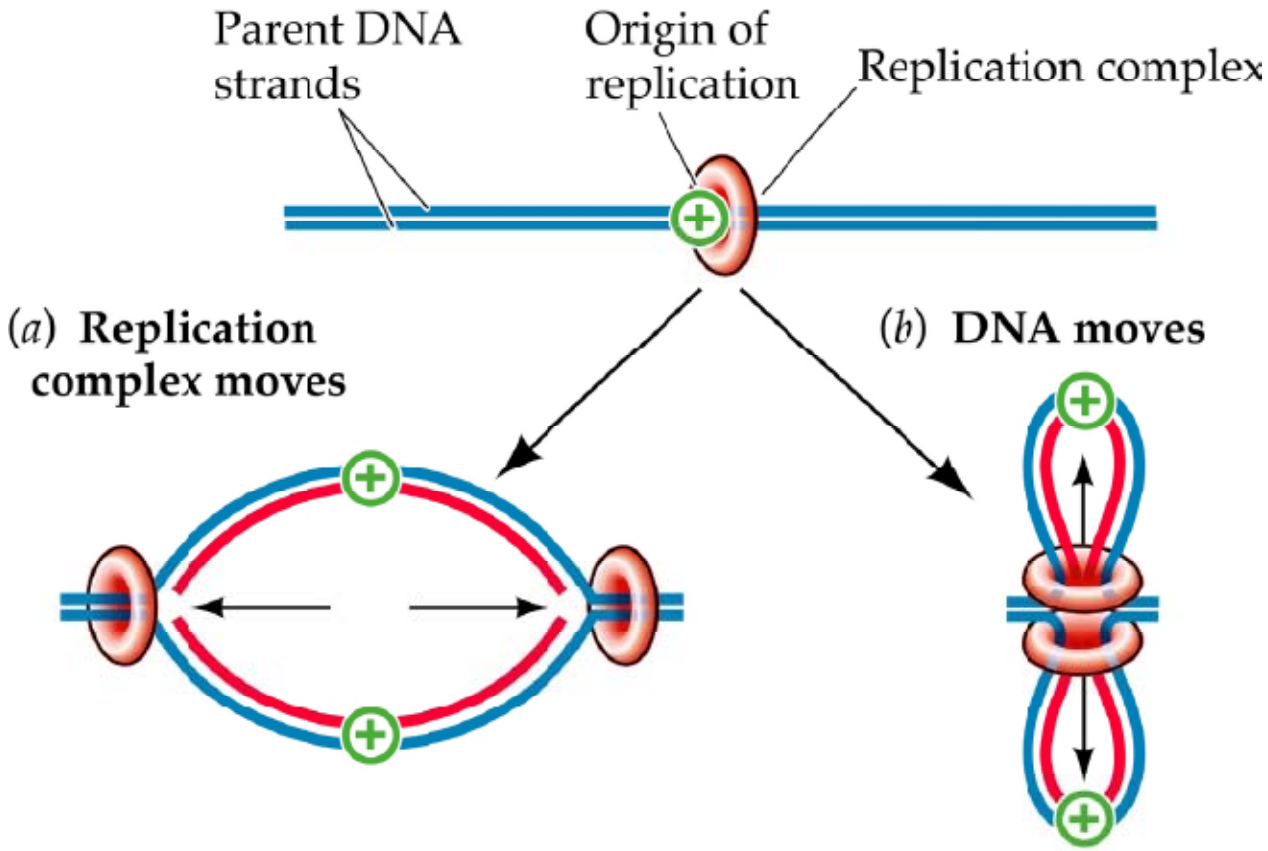


The most important DNA replication slide!!!!!



News Flash: The DNA replication complex is in a fixed location and DNA is threaded through it for replication.

- **Old idea** was via moving replication forks.



OK -- all this was worked out in bacteria. What about US???

- Basics conserved (denaturation of DNA, semi-conservative replication, 5′-3′ directionality, leading and lagging strands etc.)

- But eukaryotes have A LOT of DNA polymerases (named with Greek letters).

- And eukaryotic chromosomes are linear, so there is a problem at the **telomeres** (think about it… and see Fig. 6-18)

- Also, replication has to take place through the occluding effects of chromatin.

How fast is this?
How can it be accurate???

In bacteria, replication proceeds as fast as 2000 base pairs per minute. (Wow). How can this be accurate??? But it is...

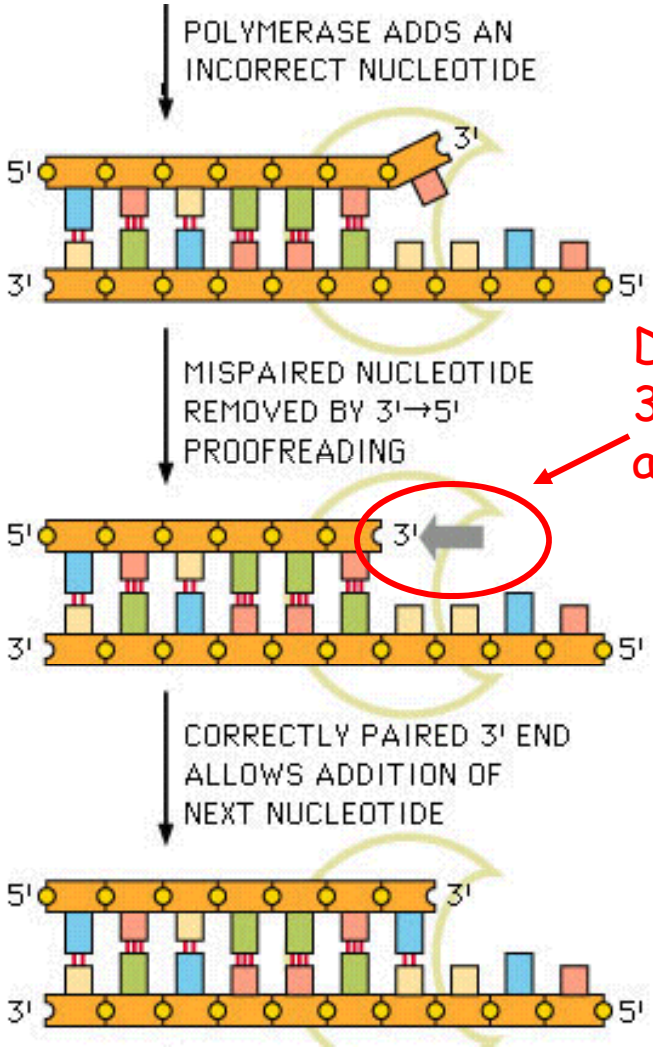
There is about about one error in 10^6 nucleotides bases added in DNA replication, repaired by: proofreading, mismatch repair, and excision repair.

DNA repair mechanisms lower the error rate to about one base in 10^9.

Proof-reading by the DNA polymerase *during synthesis*

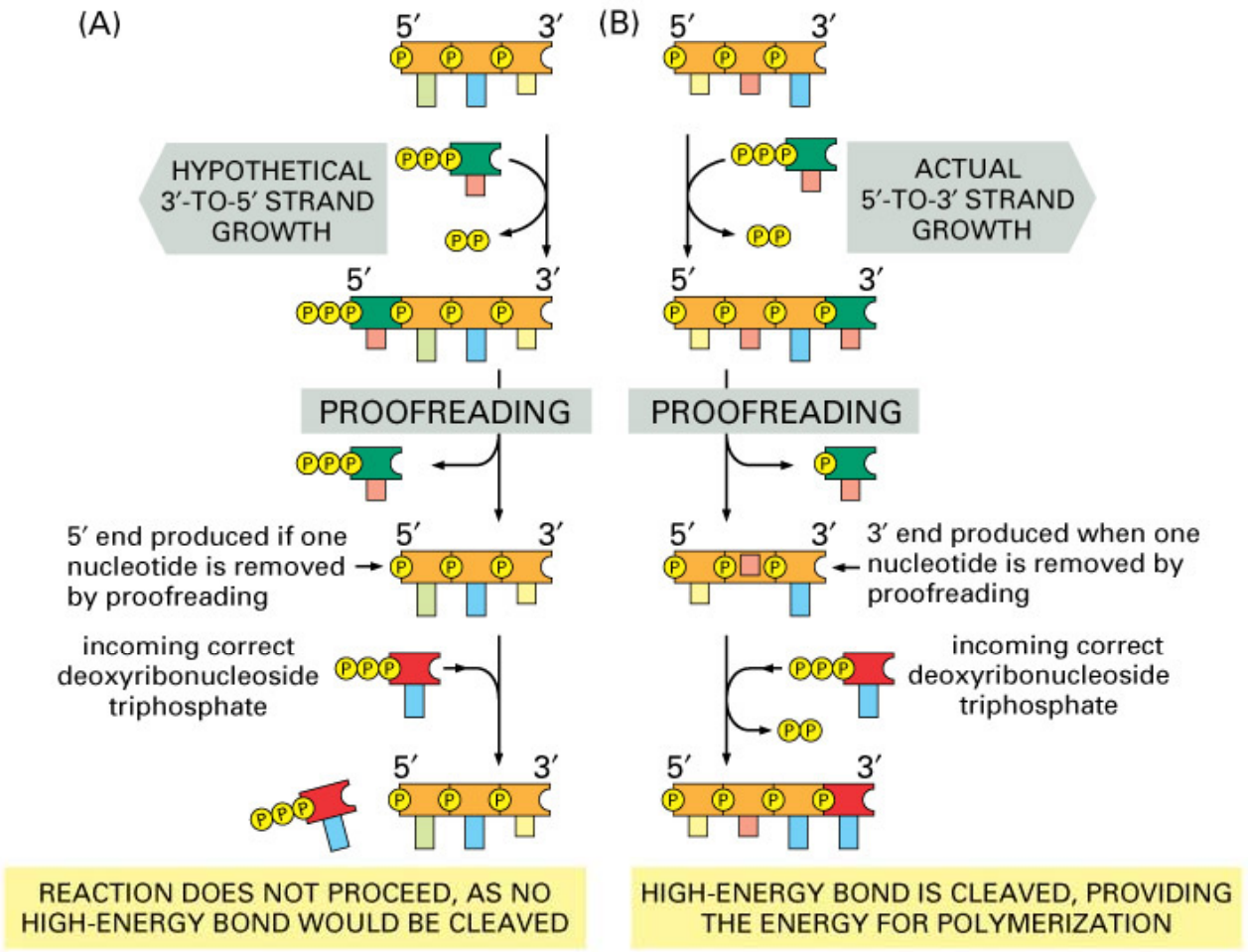
- The polymerase edits the DNA sequence by removing mispaired nucleotides that have been incorrectly inserted during the polymerization process.
- DNA polymerase has a 3'-5' exonuclease function that acts as a proof-reader!

Proof-reading by the DNA polymerase



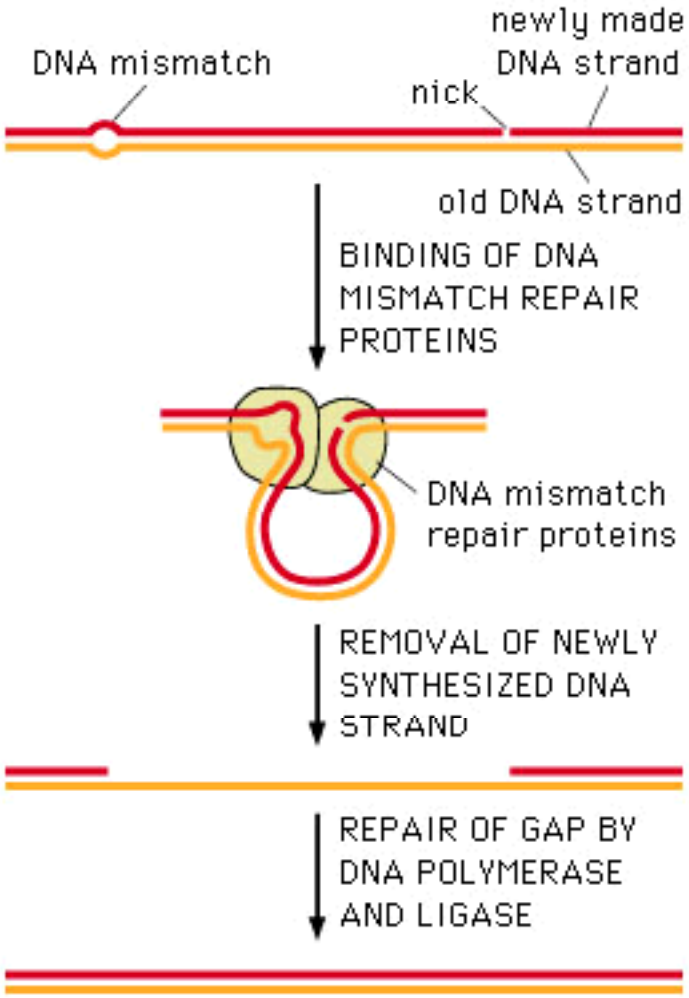
DNA polymerase has a 3'-5' exonuclease activity

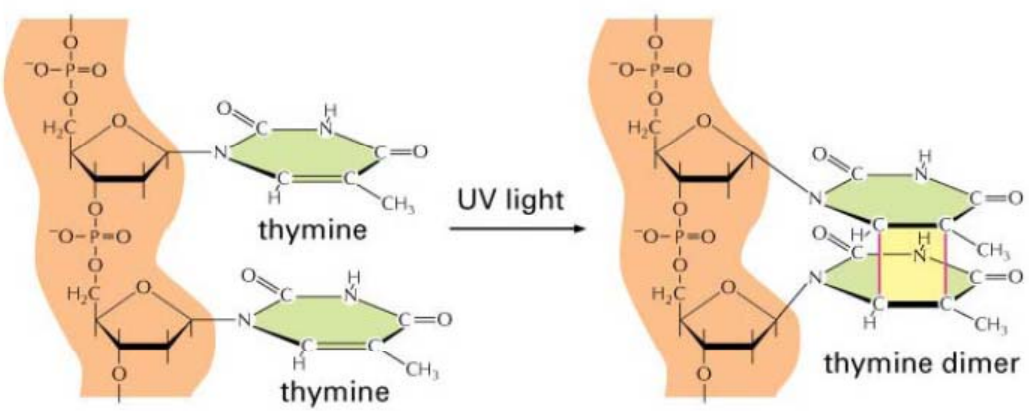
Proof-reading by the DNA polymerase

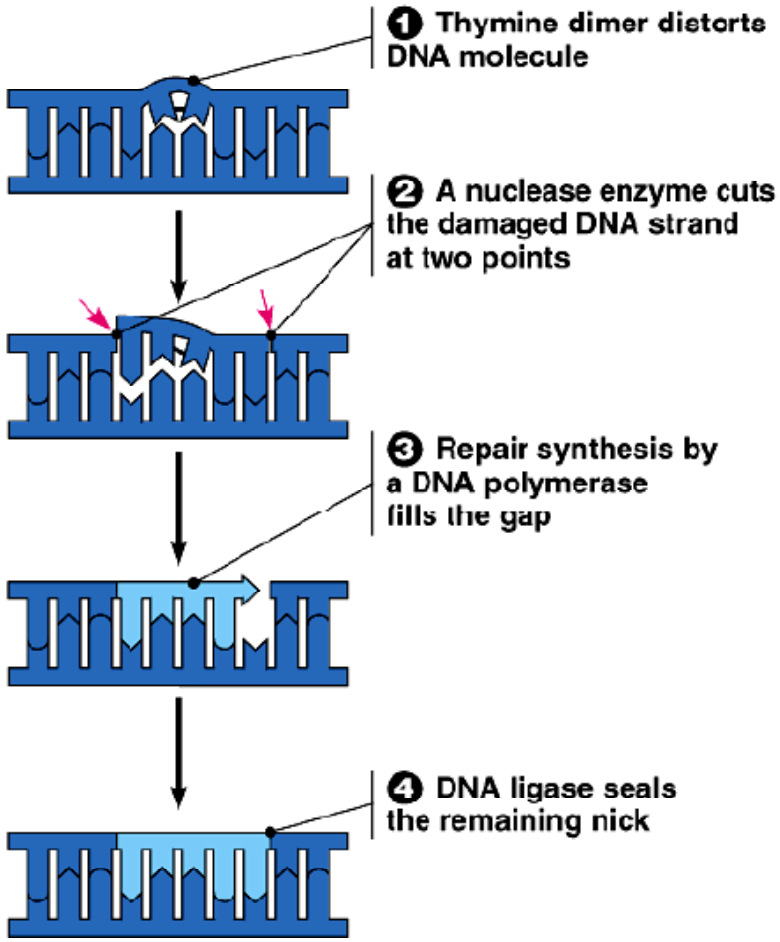


Post-synthesis repair mechanisms

- Correction of errors that remain after proofreading.
- A variety of mechanisms.
- Detection of a mismatched base pair, removal of the mismatched nucleotide, and replacement with the correct nucleotide.
- Interesting question: how does the enzymatic machinery that does this know which of the two mismatched bases to repair?




thymine
thymine
UV light
thymine dimer


1 Thymine dimer distorts DNA molecule
2 A nuclease enzyme cuts the damaged DNA strand at two points
3 Repair synthesis by a DNA polymerase fills the gap
4 DNA ligase seals the remaining nick

Final error rate?

- Net (final) error rate after post-synthesis correction is estimated to be about 1 in 10^9 to 1 in 10^{10}. (One mistake per one billion to ten billion nucleotides replicated).
- It appears that the error rate in *E.coli* is about the same as in humans (despite the VERY large difference in genome size).
- Maybe this is as good as it gets, biochemically.
- NOTE: error rates in replication of viral genomes is much higher (missing error correction capabilities).

Consequences of errors in replication