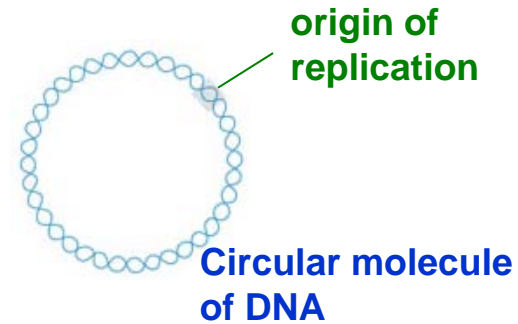
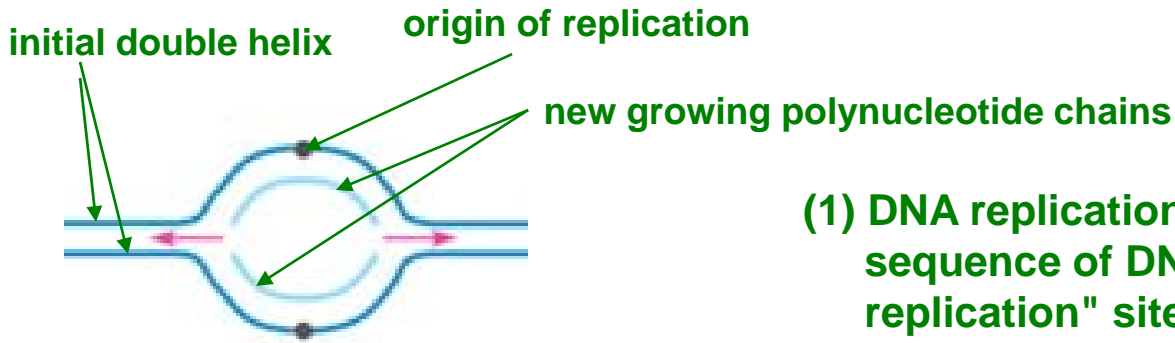


# BIO 311C

## Spring 2010

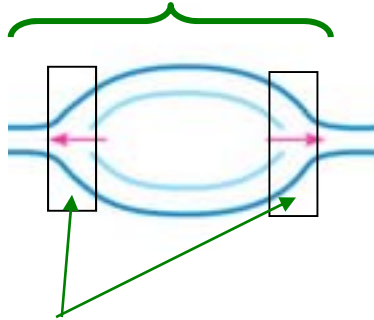
*Lecture 34 – Friday 23 Apr.*

# Summary of DNA Replication in Prokaryotes



(1) DNA replication starts at a specific sequence of DNA, called the "origin of replication" site. The polynucleotide chains of the initial double helix are separated at that point.

replication bubble



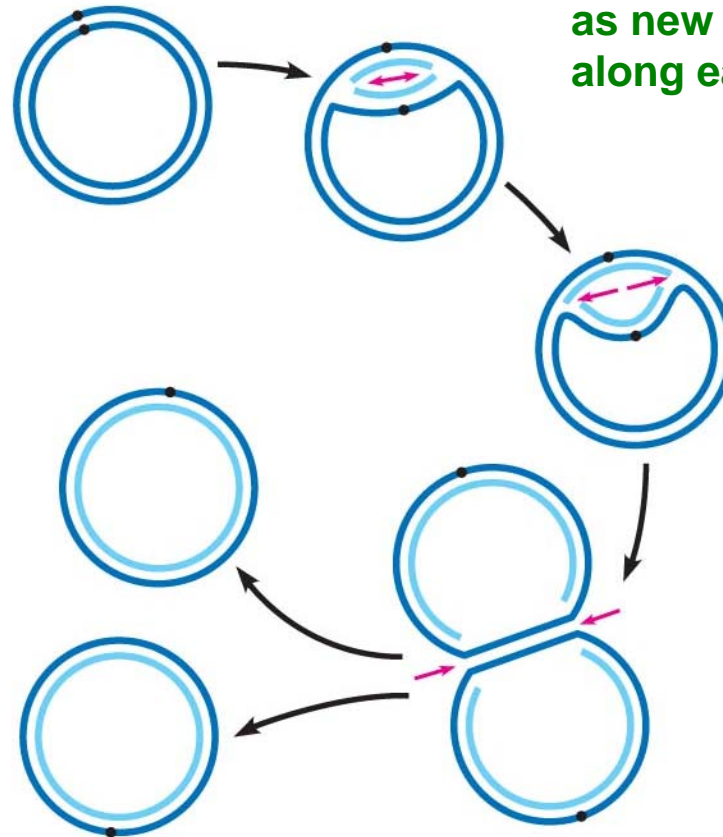
replication Y  
= replication fork

(2) DNA replication proceeds in both directions from the origin or replication, producing a replication bubble. The active site of synthesis in each direction is called the replication Y or replication fork. DNA polymerase and other proteins involved in replication function at the replication forks.



# Sequential Replication of DNA in a Prokaryote Cell with a Circular Molecule of DNA

Replication starts  
at a specific origin  
of replication site



A replication bubble is formed  
as new DNA is synthesized  
along each original strand.

The widening bubble  
develops into two  
replication forks. New DNA  
is synthesized at each of  
the two replication forks.

DNA synthesis is complete  
when the two replication forks  
meet, allowing the two DNA  
molecules to separate.

From textbook Fig. 16.12, p. 313



# Replication of DNA in a Eukaryotic Cell with Linear Molecules of DNA

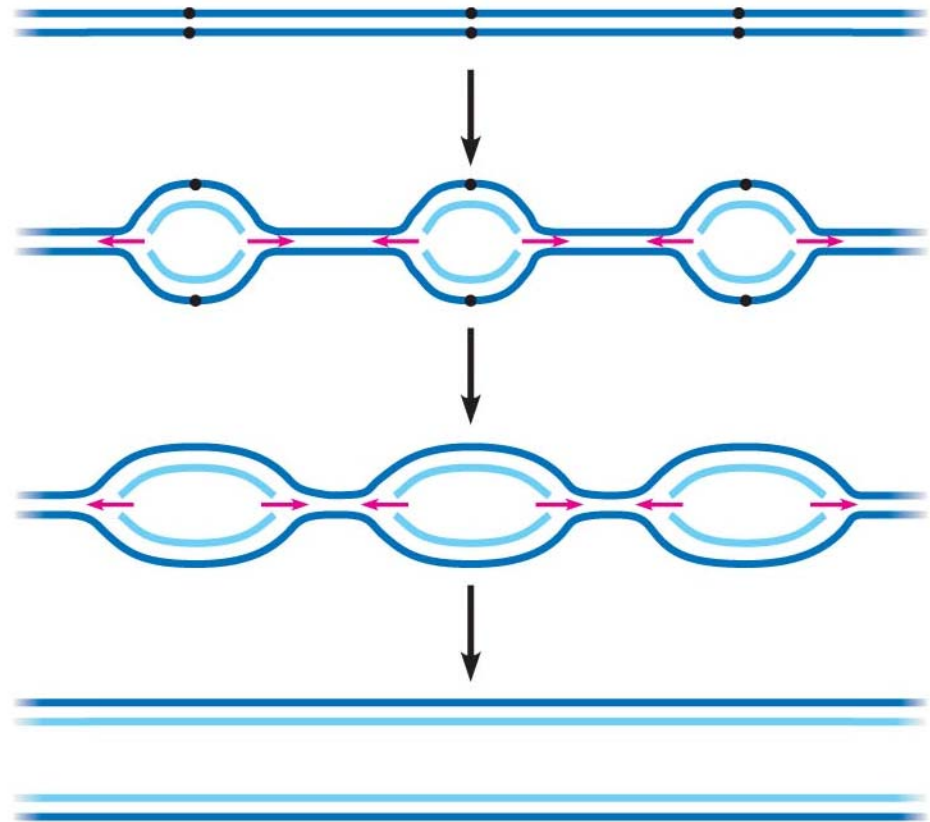
From textbook Fig. 16.12, p. 313

Replication starts at multiple “origin of replication” sites

A replication bubble is formed at each origin of replication site as new DNA is synthesized.

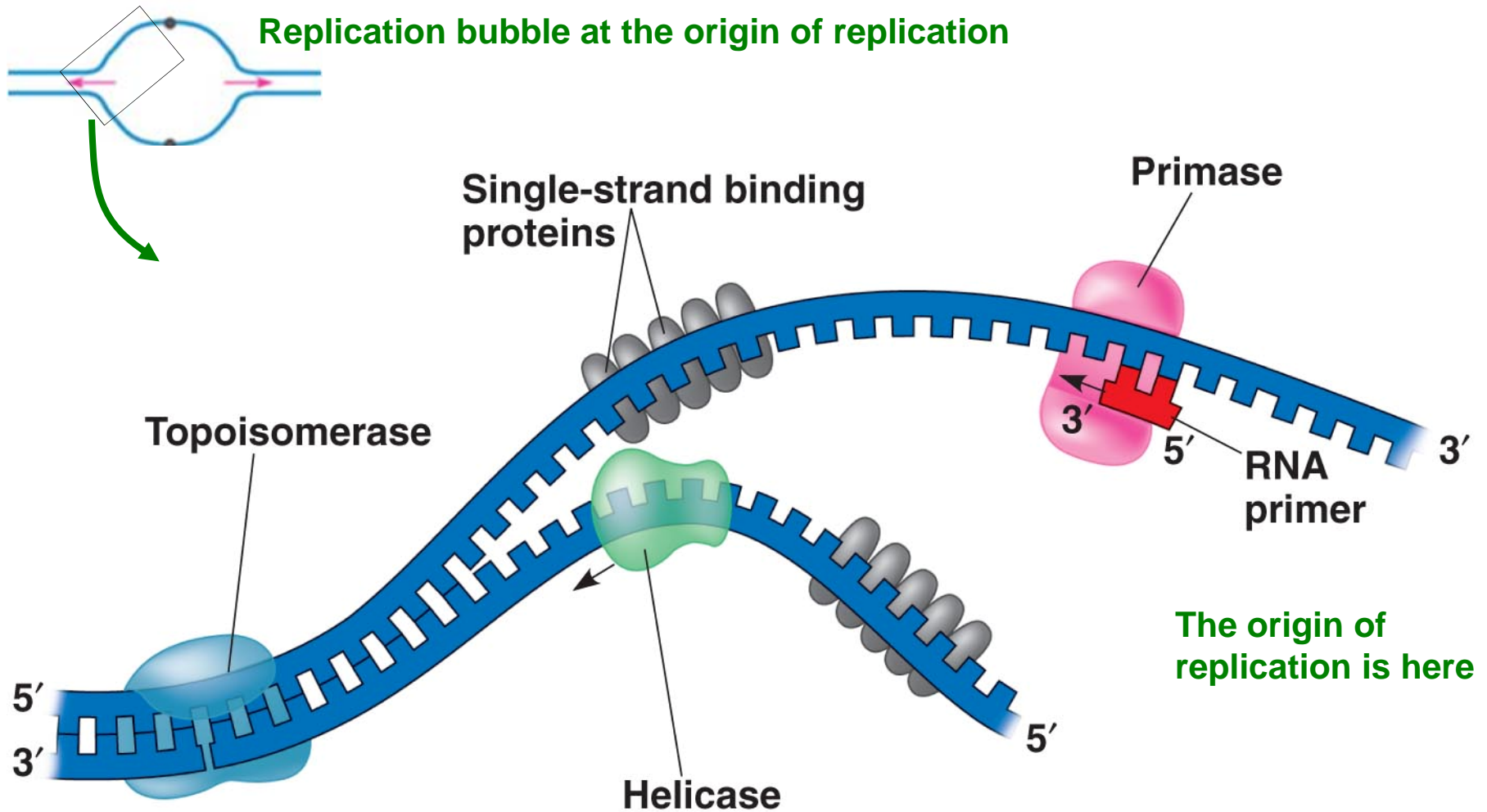
Each widening bubble develops into two replication forks. New DNA is synthesized at each of the two replication forks.

DNA synthesis is complete when adjacent replication forks meet, allowing the two DNA molecules to separate.



\*

# Some Proteins Involved in the Initiation of DNA Synthesis



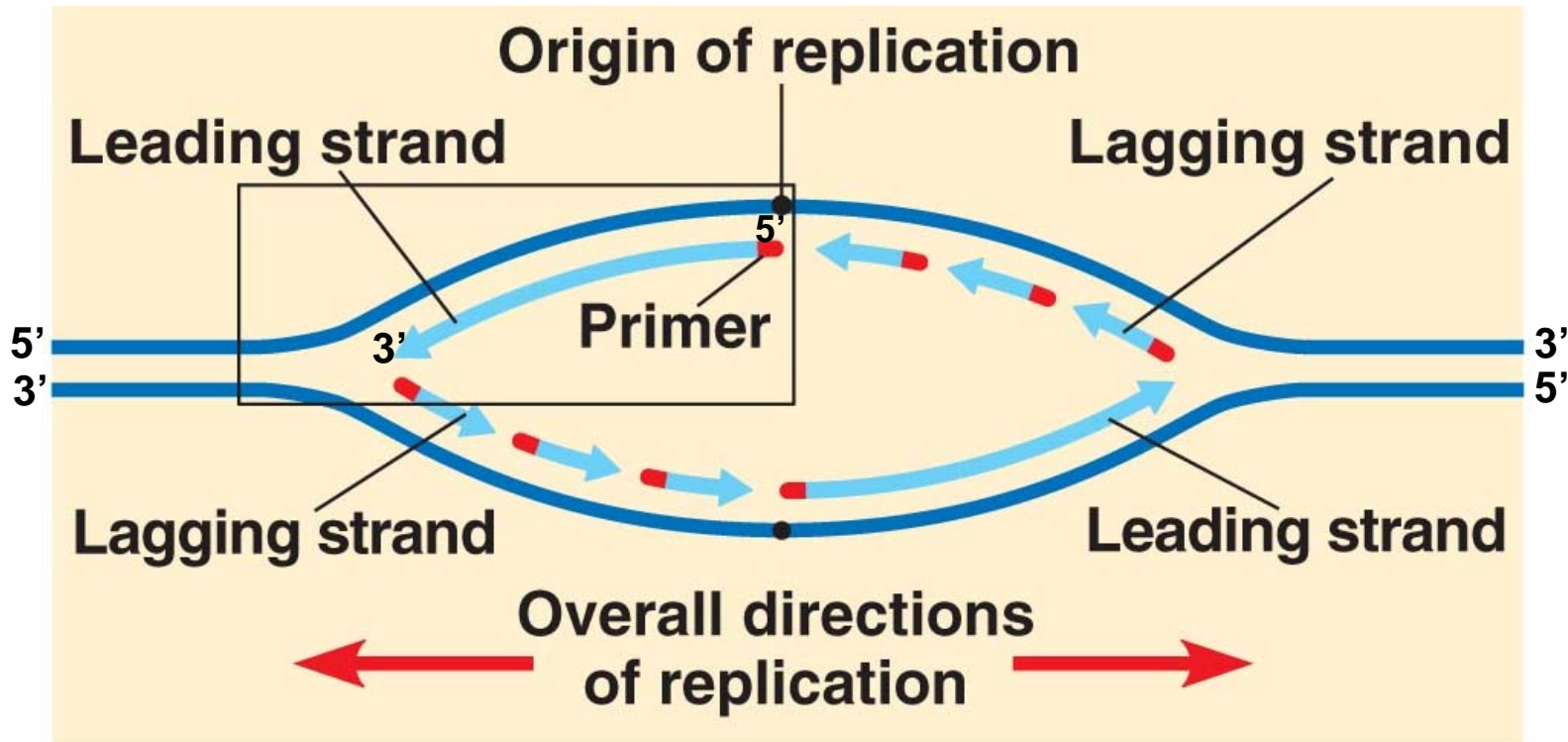
From textbook Fig. 16.13, p. 314



# Overview of the Sequence of Events in DNA Synthesis

From textbook Fig. 16.15, p. 315

Boxed area shows events in the synthesis of the leading strand.

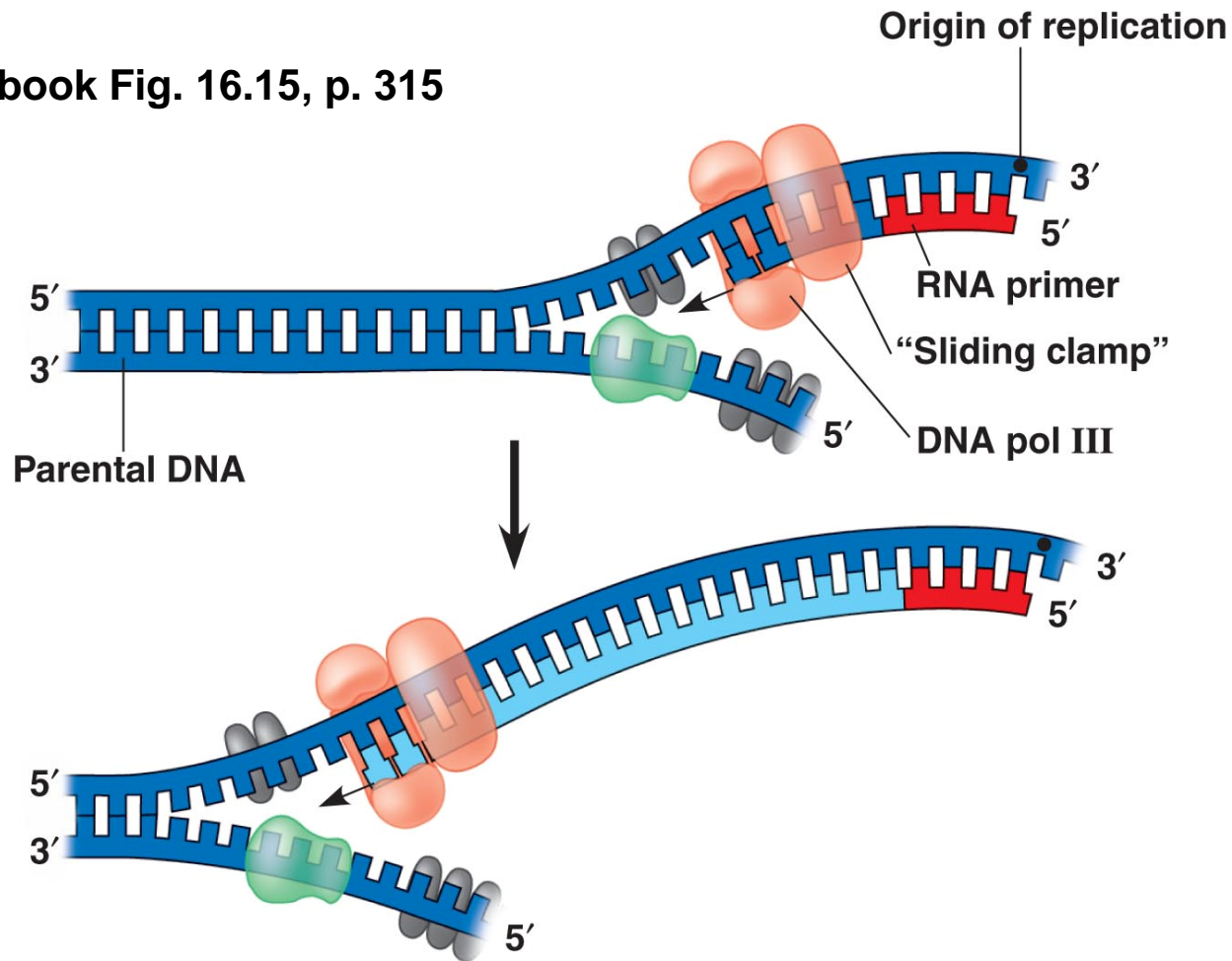


Polynucleotide chains in living cells are always extended in the 5'-to-3' direction.



# Synthesis of the Leading Strand During DNA Replication

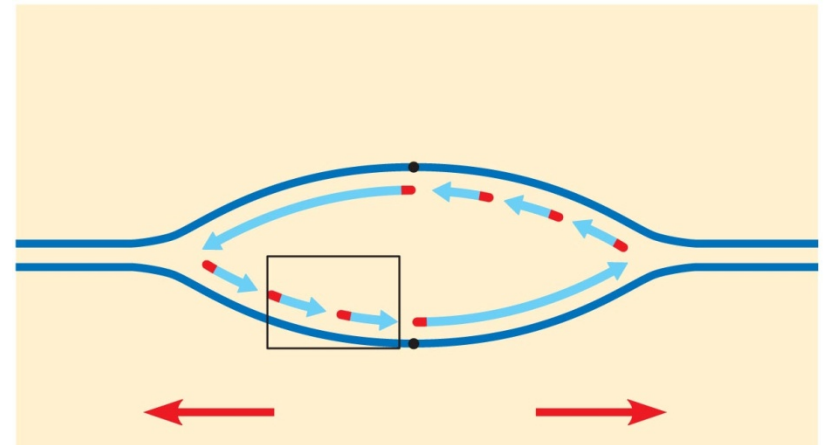
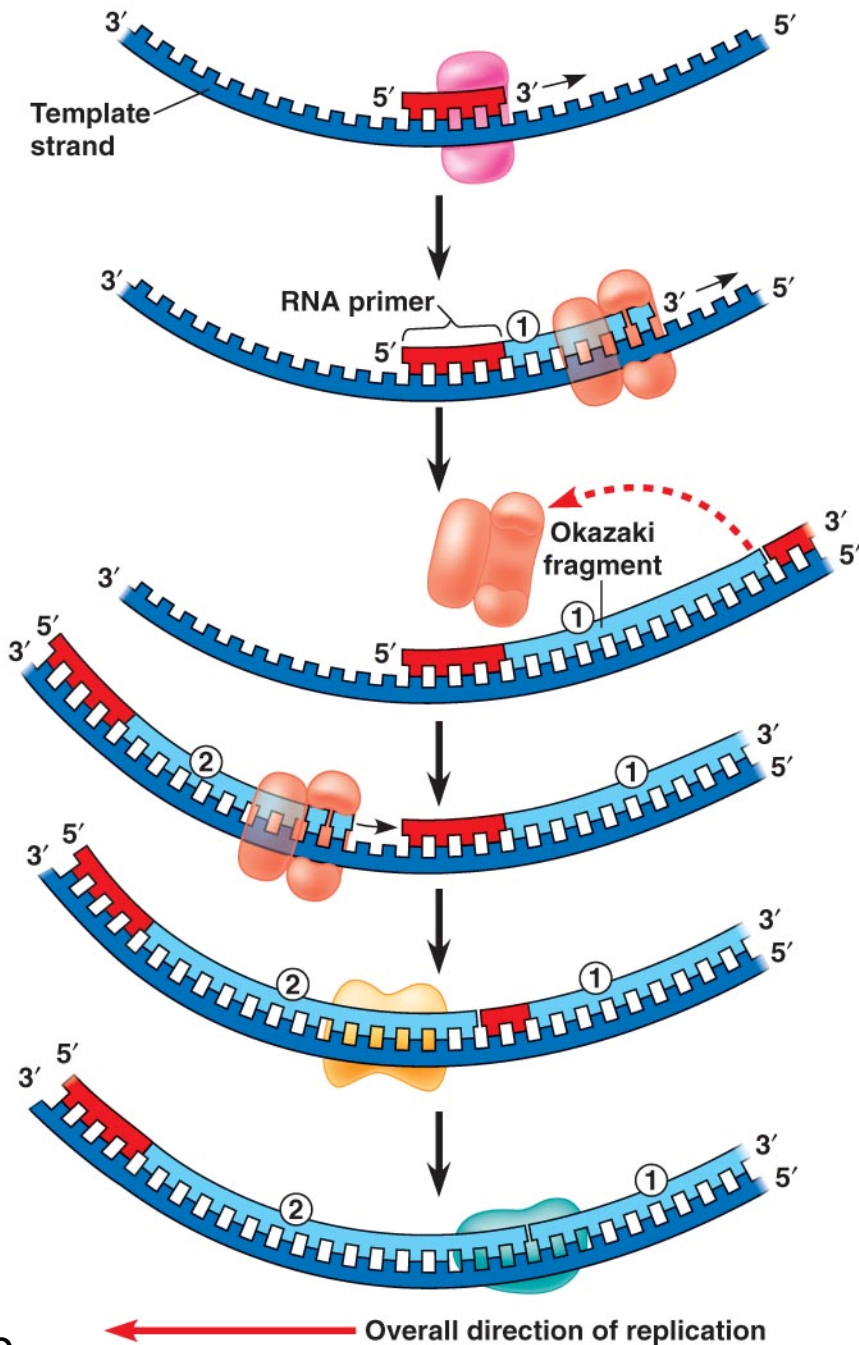
From textbook Fig. 16.15, p. 315





# Synthesis of the Lagging Strand During DNA Replication

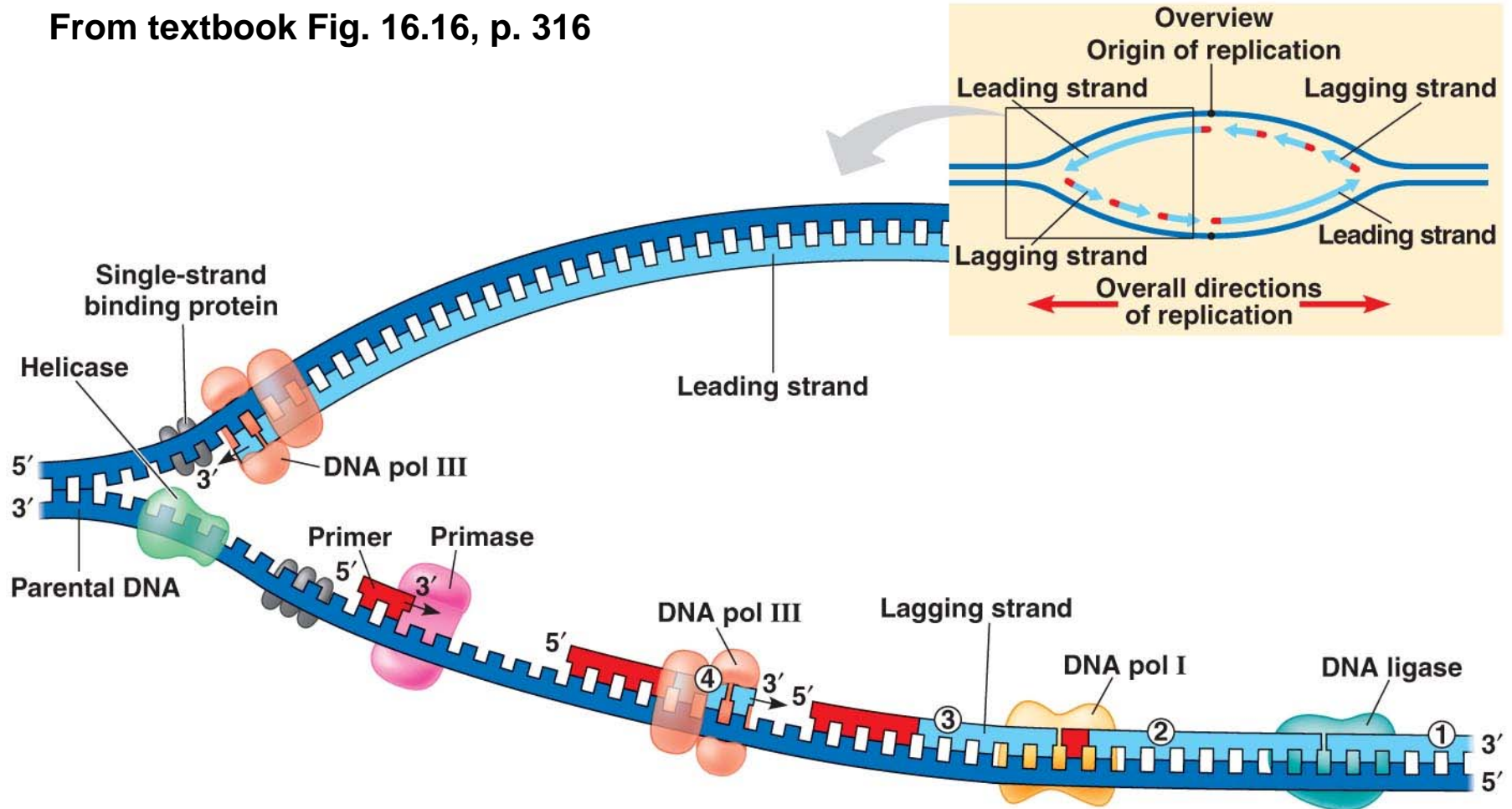
From textbook Fig. 16.16, p. 316





# A Summary of DNA Replication in Prokaryotes

From textbook Fig. 16.16, p. 316



DNA replication in Eukaryotic cells is very similar, but somewhat more complex.



# Steps in the Replication of a Molecule of DNA (1)

1. The two polynucleotide strands of the DNA molecule become separated at the origin of replication site by a specific protein complex. Eukaryotic nuclear DNA molecules contain multiple origin of replication sites on each molecule of chromatin (chromosome), while prokaryotic cells have a single origin of replication site on their chromosome.
2. As the protein complex continues to pry apart the two strands at each origin of replication, two replication forks (replication Y) are generated and stabilized. Helicase, single-strand-binding proteins and topoisomerase each have important roles in widening and stabilizing the replication fork.
3. A separate molecule of the enzyme called primase binds to each of the two strands of separated DNA. Primase catalyzes the synthesis of a molecule of RNA with bases complementary to a short segment of each separated strands of DNA. The base pairing rules apply, with RNA nucleoside U inserted adjacent to, and hydrogen bonded to) dA on the DNA. This segment of RNA (less than 200 nucleotides long) forms a hybrid double helix with the complementary segment of DNA. The primer is always synthesized in the 5'-to-3' direction, so it adds nucleotides in the direction toward the replication fork on one strand of the DNA (called the leading strand) and adds nucleotides in the direction away from the replication fork on the other strand of DNA (called the lagging strand).



# Steps in the Replication of a Molecule of DNA (2)

4. A separate DNA Polymerase 3 (pol III) binds to the short segment of DNA-RNA hybrid double helix of each strand. Each pol III, then begins to prepare a new polydeoxyribonucleotide chain (a new strand of DNA) in the 5'to-3' direction, starting at the 3' end of the RNA primer. The pol III bound to the leading strand adds deoxyribonucleotides in the direction toward the continuously-opening replication fork, while the pol III bound to the lagging strand adds deoxyribonucleotides in the direction away from the replication fork.
5. The pol III bound to the leading strand can continue to add deoxyribonucleotides to the growing polydeoxynucleotide chain continuously (all the way around a prokaryotic chromosome), or until it runs into another replication fork in a eukaryote molecule of DNA) as the replication fork continues to open. The pol III bound to the lagging strand only adds nucleotides until it runs into the origin of replication or a segment of DNA that has already been replicated.
6. As a new segment of DNA opens up at the constantly opening replication fork, primase synthesizes a new RNA primer on the lagging strand. Pol III then binds to the new hybrid double helix to synthesize a new strand of DNA that elongates in the direction away from the replication fork, as described in step 4.



# Steps in the Replication of a Molecule of DNA (3)

7. The new lagging strand of DNA elongates until it runs into the primer strand of RNA that was made previously. Pol III then is released. The resulting short piece of DNA, with a segment of RNA attached to its 3' end and another segment of RNA adjacent (but not attached) to its 5' end, is called an Okazaki fragment.
8. The enzyme “DNA polymerase I (pol I) then sits where the pol III was released on the lagging strand, and begins to slide over the DNA-RNA hybrid. It replaces ribonucleotides one at a time with deoxyribonucleotides, thereby continuing to extend the length of the new strand of DNA in the 5' to 3' direction.
9. Pol I is released from the lagging strand after it replaces the last ribonucleotide of RNA with a deoxyribonucleotide of DNA. The new strand of DNA is then adjacent to (but still not covalently attached to) the previously synthesized segment of DNA (see Step 5).
10. An enzyme called ligase binds to the site where the two segments of lagging strand DNA are adjacent to each other. It catalyzes the formation of a phosphodiester bond between the two segments to form a single continuous polynucleotide chain.

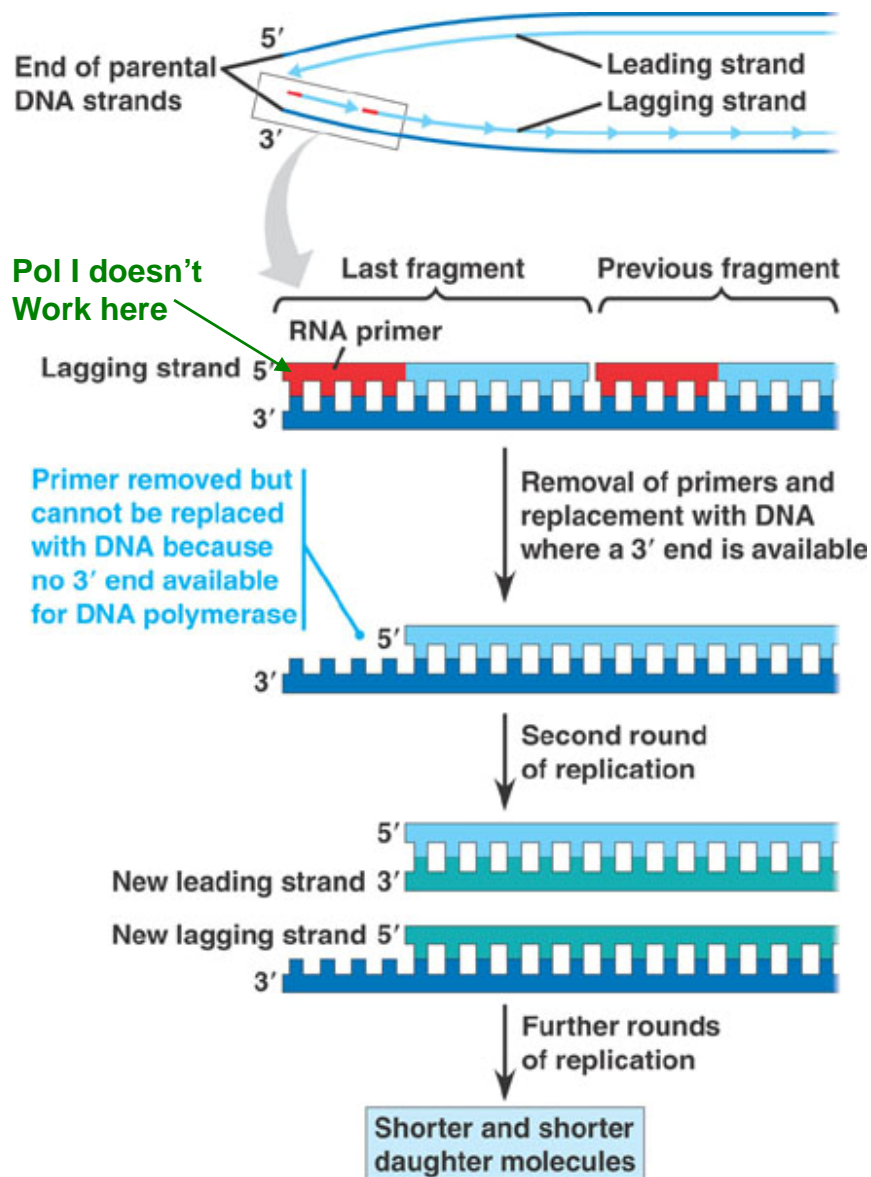


# Steps in the Replication of a Molecule of DNA (4)

11. Steps 5 – 10 are repeated over and over as the replication fork continues to open. Thus, new lagging-strand DNA is synthesized one segment at a time, and the segments are ligated together to produce one continuous polydeoxyribonucleotide chain. This process of synthesizing lagging-strand DNA in individual segments that are individually attached together is sometimes called “backstitching”.
12. The leading strand, whose synthesis is described in Steps 3 – 5, retains a RNA primer attached to the 3’end of the molecule as it continues to elongate at the replication fork. Pol I and ligase replace this piece of RNA with DNA as was described for the lagging strand in steps 8 – 10.



# The length of eukaryotic DNA decreases with each round of replication.



A sequence of nucleotides at each end of each molecule of eukaryotic nuclear DNA is called a telomere.

The telomere DNA does not code for useful genetic information.

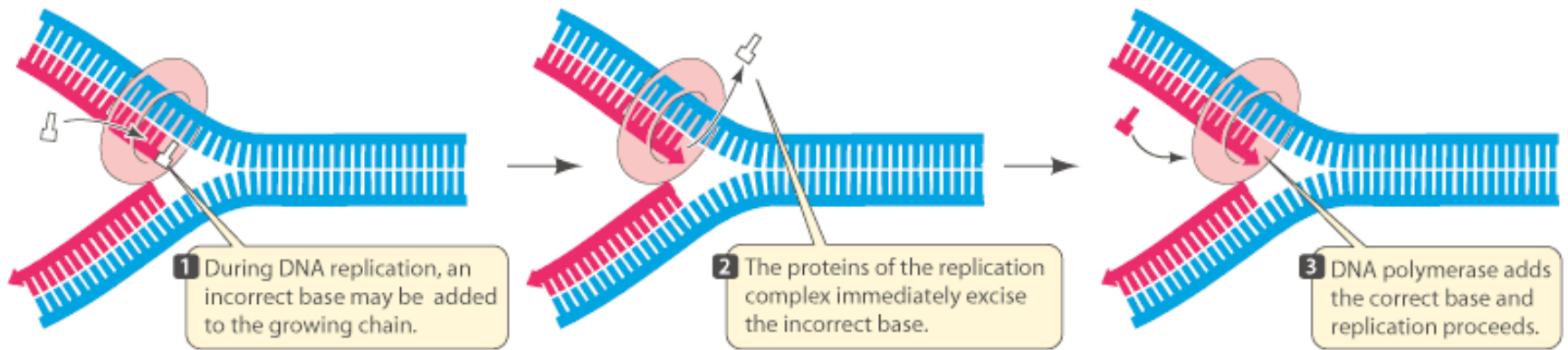
Each time the DNA replicates, the new polynucleotide chains get somewhat shorter at each end, thus decreasing the length of the telomere.

After replicating many times the telomere is completely lost. Subsequent replications then begin to lose useful genetic information at the ends of the remaining DNA, eventually causing the cell to die.

Many kinds of eukaryotic cells can only replicate a certain number of times before they die, perhaps due to the loss of telomeres.



# DNA Proofreading During Replication



The figure illustrates a replication fork, with red lines representing nucleotides of the new growing polynucleotide chains.

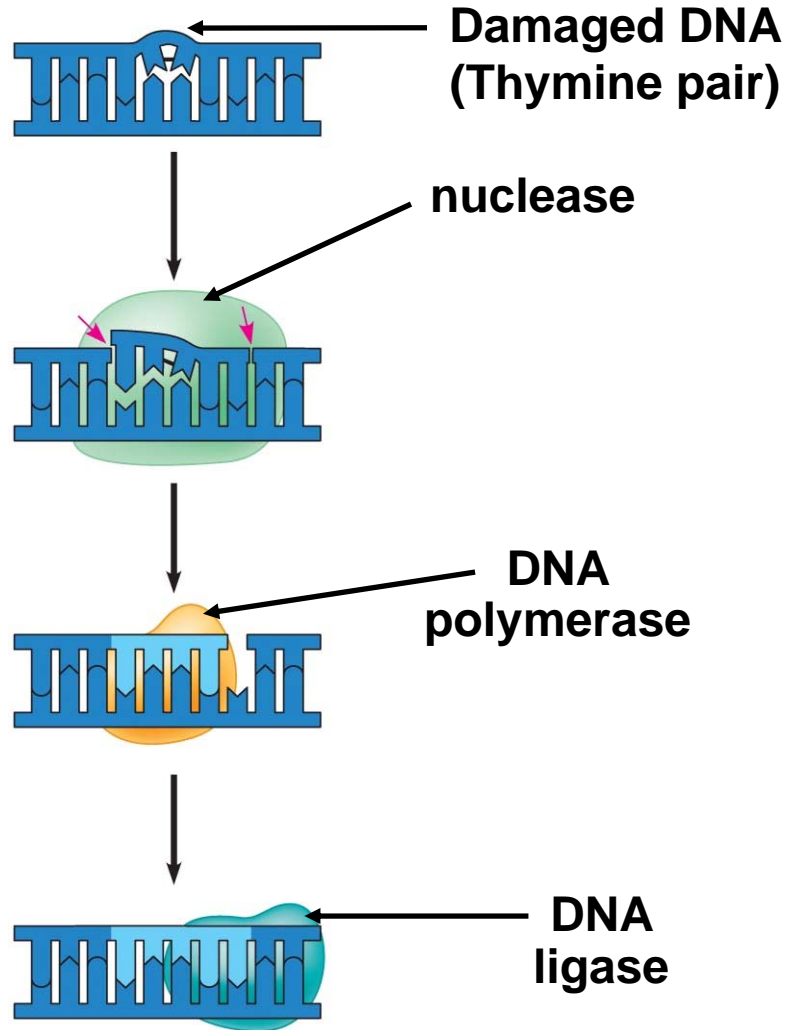
DNA polymerase is almost error-free in inserting new nucleotides into the growing polynucleotide chain, but occasionally a mistake does occur (approximately 1 in  $10^5$  nucleotides inserted is incorrect).

DNA polymerase is actually part of a larger aggregation of proteins called a replication complex. A site on this complex is able to detect that an incorrect nucleotide has been inserted, and remove the incorrectly inserted nucleotide. The replication complex then inserts the correct nucleotide. After this correction the error rate approximately 1 in  $10^{10}$ .





## A mechanism for repair of damaged DNA



Although DNA is replicated almost perfectly, on rare occasion an incorrect base is inserted. Also, DNA is occasionally damaged after it is synthesized.

Cells have elaborate repair mechanisms for correcting various kinds of errors. Damage-detecting proteins scan the DNA for errors, then recruit repair enzymes to sites of damage.

The example shown here, nucleoside excision repair of damage due to the fusion of two adjacent thymine bases, is only one of many kinds of repair mechanisms.

From Textbook Fig. 16.18,  
P. 318



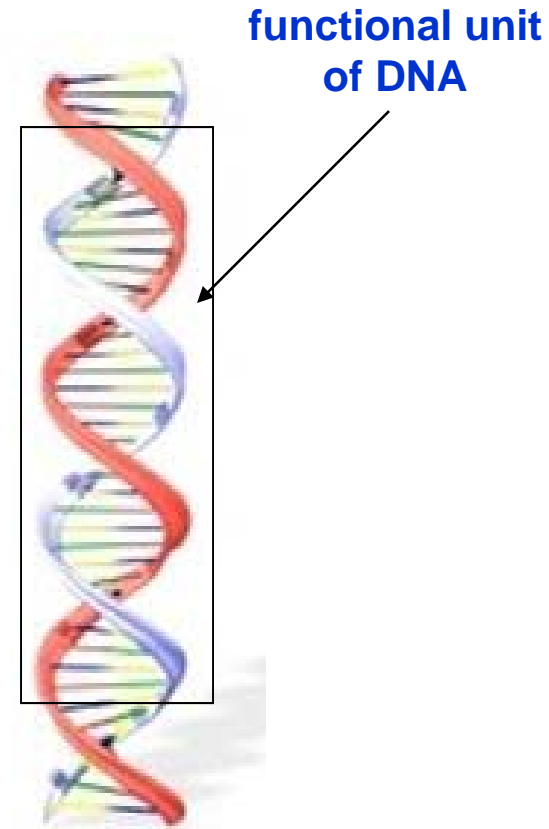
# DNA as a Series of Functional Units

A molecule of DNA can be thought of as a linear sequence of thousands of specific functional units, interspersed with stretches of DNA with no known function. Individual functional units vary in length from just a few nucleotide-pairs to many thousands of nucleotide-pairs. The character of each functional unit is determined by the sequence of its nucleotides. These units of function are called genes when their function is known.

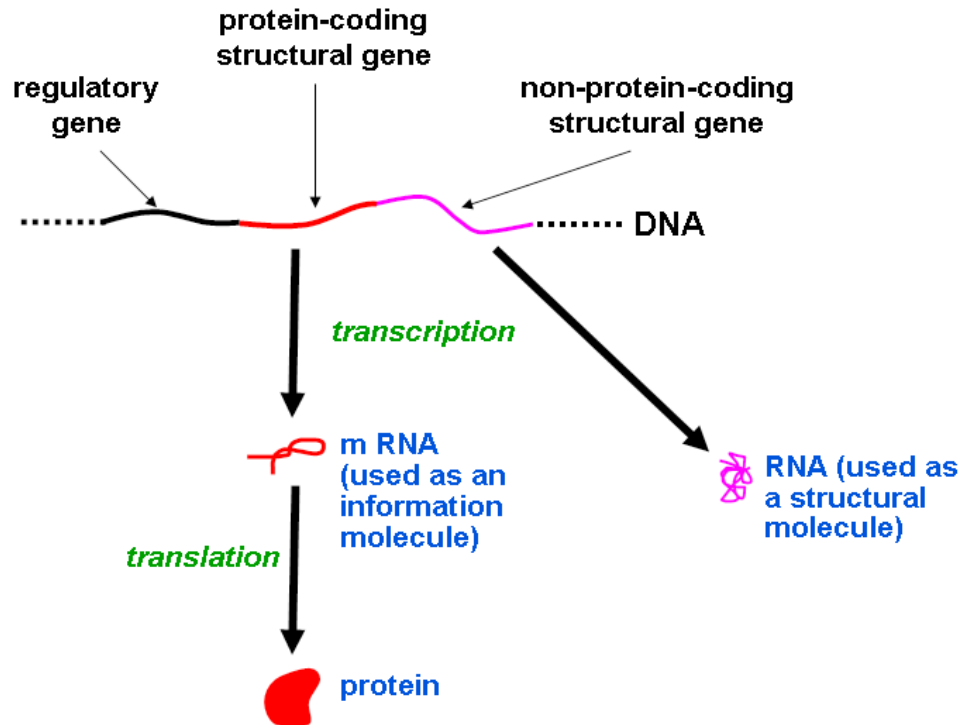
Genes along a molecule of DNA can be divided into three main categories:

- a. structural genes, whose information content is used to synthesize RNA during transcription,
- b. regulatory genes, whose function is to control the rate of transcription,
- c. genes that are not directly involved in transcription.

Some stretches of DNA have no known function and in the past have been called junk DNA. That is almost certainly a misleading term.



# Structural and Regulatory Genes



A structural gene is a gene whose information content is used to synthesize a complementary molecule of RNA during transcription. (i.e. It generates a product).

The term "structural molecule" means something different than does "structural gene". A structural molecule in a cell is a molecule that is a component of some structure within the cell.

