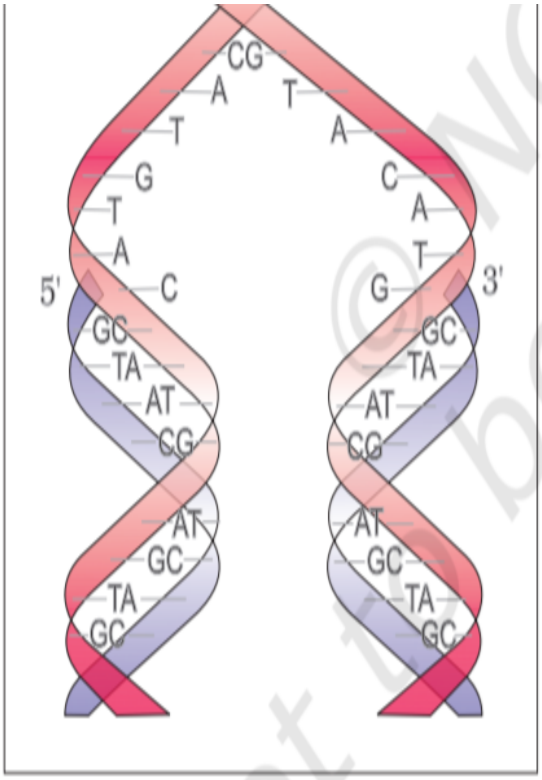


Figure 6.6 Watson-Crick model for semiconservative DNA replication

6.4 Replication

While proposing the double helical structure for DNA, Watson and Crick had immediately proposed a scheme for replication of DNA. To quote their original statement that is as follows:

"It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material" (Watson and Crick, 1953).

The scheme suggested that the two strands would separate and act as a template for the synthesis of new complementary strands. After the completion of replication, each DNA molecule would have one parental and one newly synthesised strand. This scheme was termed as **semiconservative** DNA replication (Figure 6.6).

6.4.1 The Experimental Proof

It is now proven that DNA replicates semiconservatively. It was shown first in *Escherichia coli* and subsequently in higher organisms, such as plants

and human cells. Matthew Meselson and Franklin Stahl performed the following experiment in 1958:

(i) They grew *E. coli* in a medium containing $^{15}NH_4Cl$ (^{15}N is the heavy isotope of nitrogen) as the only nitrogen source for many generations. The result was that ^{15}N was incorporated into newly synthesised DNA (as well as other nitrogen containing compounds). This heavy DNA molecule could be distinguished from the normal DNA by centrifugation in a cesium chloride (CsCl) density gradient (Please note that ^{15}N is not a radioactive isotope, and it can be separated from ^{14}N only based on densities).

(ii) Then they transferred the cells into a medium with normal $^{14}NH_4Cl$ and took samples at various definite time intervals as the cells multiplied, and extracted the DNA that remained as double-stranded helices. The various samples were separated independently on CsCl gradients to measure the densities of DNA (Figure 6.7).

Can you recall what centrifugal force is, and think why a molecule with higher mass/density would sediment faster?

The results are shown in Figure 6.7.

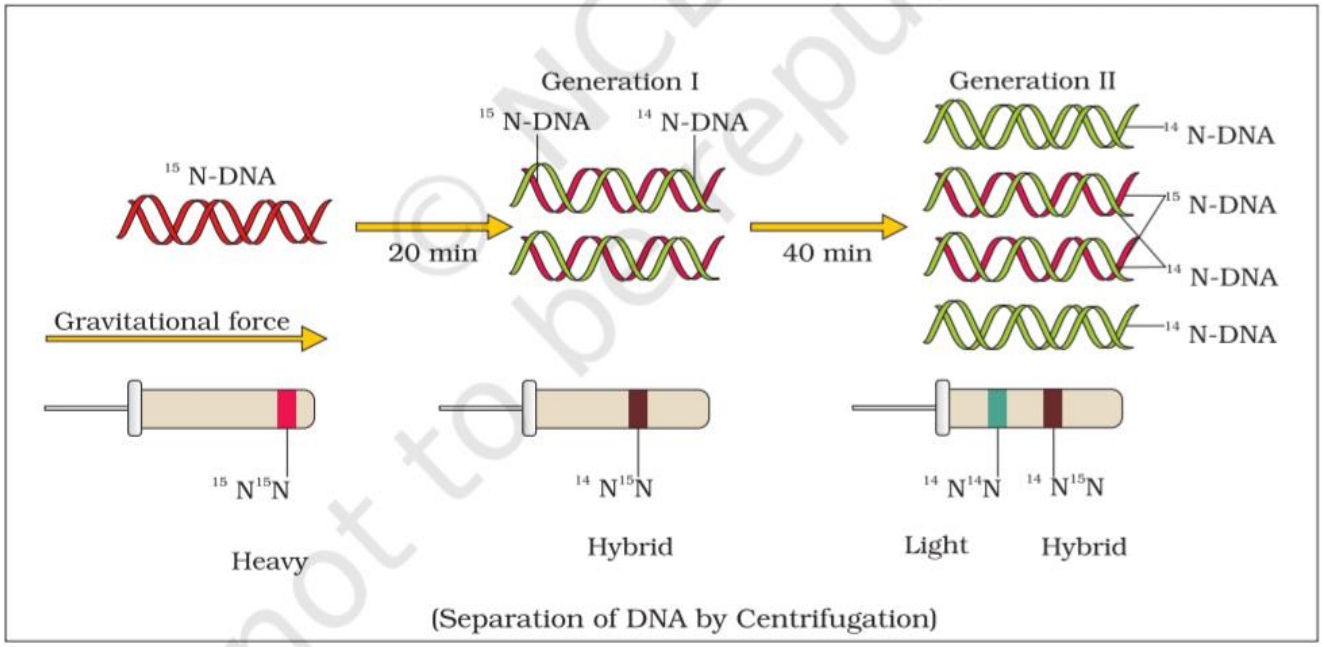


Figure 6.7 Meselson and Stahl's Experiment

(iii) Thus, the DNA that was extracted from the culture one generation after the transfer from ^{15}N to ^{14}N medium [that is after 20 minutes; *E. coli* divides in 20 minutes] had a hybrid or intermediate density. DNA extracted from the culture after another generation [that is after 40 minutes, II generation] was

composed of equal amounts of this hybrid DNA and of 'light' DNA.

If E. coli was allowed to grow for 80 minutes then what would be the proportions of light and hybrid densities DNA molecule?

Very similar experiments involving use of radioactive thymidine to detect distribution of newly synthesised DNA in the chromosomes was performed on *Vicia faba* (faba beans) by Taylor and colleagues in 1958. The experiments proved that the DNA in chromosomes also replicate semiconservatively.

6.4.2 The Machinery and the Enzymes

In living cells, such as *E. coli*, the process of replication requires a set of catalysts (enzymes). The main enzyme is referred to as DNA-dependent **DNA polymerase**, since it uses a DNA template to catalyse the polymerisation of deoxynucleotides. These enzymes are highly efficient enzymes as they have to catalyse polymerisation of a large number of nucleotides in a very short time. *E. coli* that has only 4.6×10^6 bp (compare it with human whose diploid content is 6.6×10^9 bp), completes the process of replication within 18 minutes; that means the average rate of polymerisation has to be approximately 2000 bp per second. Not only do these polymerases have to be fast, but they also have to catalyse the reaction with high degree of accuracy. Any mistake during replication would result into mutations. Furthermore, energetically replication is a very expensive process. Deoxyribonucleoside triphosphates serve dual purposes. In addition to acting as substrates, they provide energy for polymerisation reaction (the two terminal phosphates in a deoxynucleoside triphosphates are high-energy phosphates, same as in case of ATP).

In addition to DNA-dependent DNA polymerases, many additional enzymes are required to complete the process of replication with high degree of accuracy. For long DNA molecules, since the two strands of DNA cannot be separated in its entire length (due to very high energy requirement), the replication occur within a small opening of the DNA helix, referred to as **replication fork**. The DNA-dependent DNA polymerases catalyse polymerisation only in one direction, that is 5′→3′. This creates some additional complications at the replicating fork. Consequently, on one strand (the template with polarity 3′→5′), the replication is **continuous**, while on the other (the template with polarity 5′→3′), it is **discontinuous**. The discontinuously synthesised fragments are later joined by the enzyme **DNA ligase** (Figure 6.8).

The DNA polymerases on their own cannot initiate the process of replication. Also the replication does not initiate randomly at any place in DNA. There is a definite region in *E. coli* DNA where the replication originates. Such regions are termed as **origin of replication**. It is

because of the requirement of the origin of replication that a piece of DNA if needed to be propagated during recombinant DNA procedures, requires a vector. The vectors provide the origin of replication.

Further, not every detail of replication is understood well. In eukaryotes, the replication of DNA takes place at S-phase of the cell-cycle. The replication of DNA and cell division cycle should be highly coordinated. A failure in cell division after DNA replication results into polyploidy(a chromosomal anomaly). You will learn the detailed nature of origin and the processes occurring at this site, in higher classes.

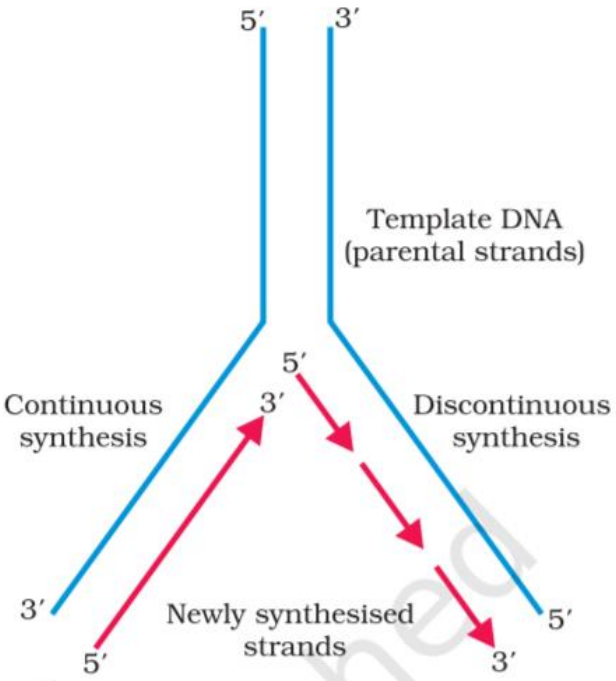


Figure 6.8 Replicating Fork

6.5 Transcription

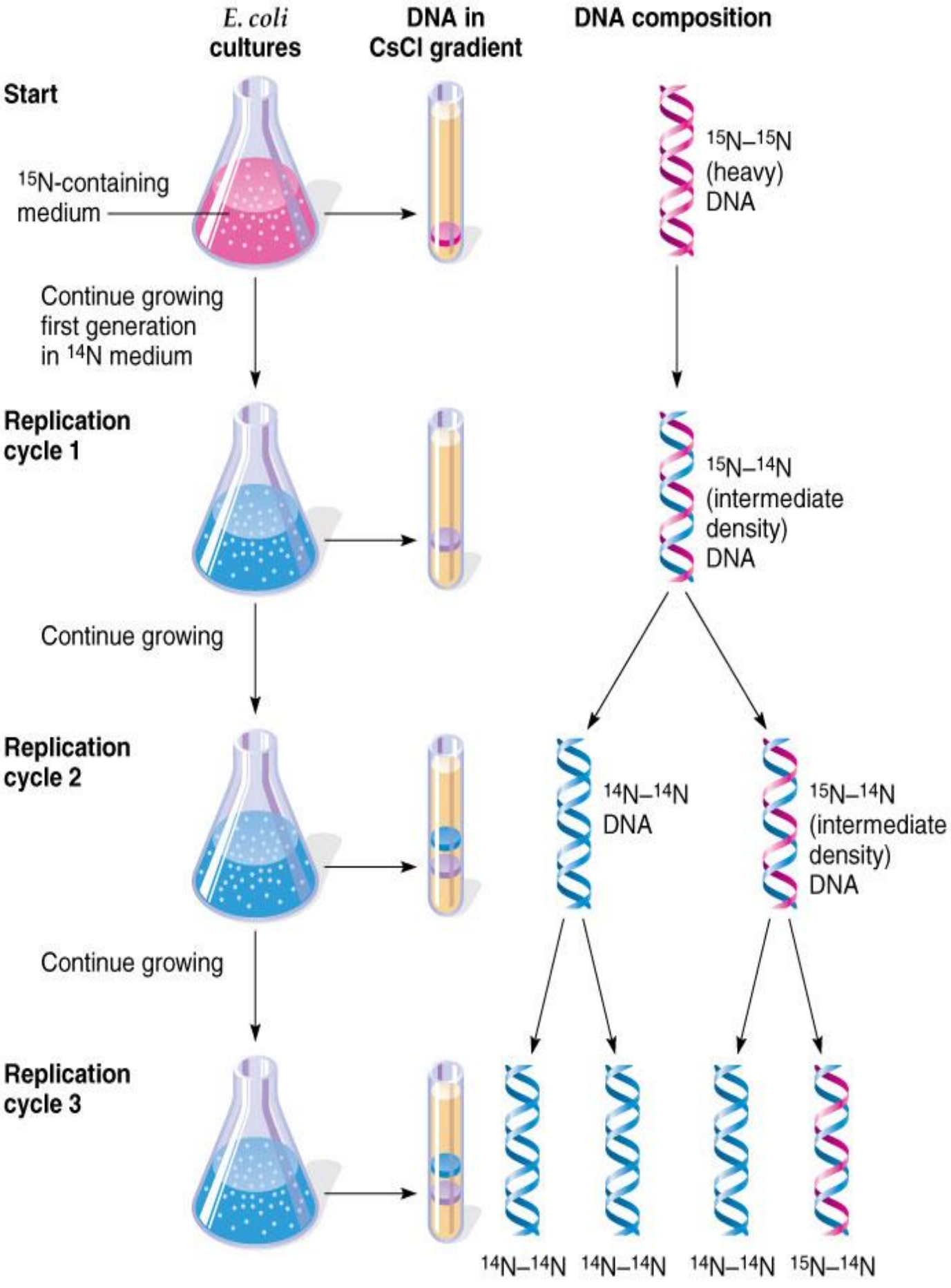
The process of copying genetic information from one strand of the DNA into RNA is termed as **transcription**. Here also, the principle of complementarity governs the process of transcription, except the adenosine complements now forms base pair with uracil instead of thymine. However, unlike in the process of replication, which once set in, the total DNA of an organism gets duplicated, in transcription only a segment of DNA and only one of the strands is copied into RNA. This necessitates defining the boundaries that would demarcate the region and the strand of DNA that would be transcribed.

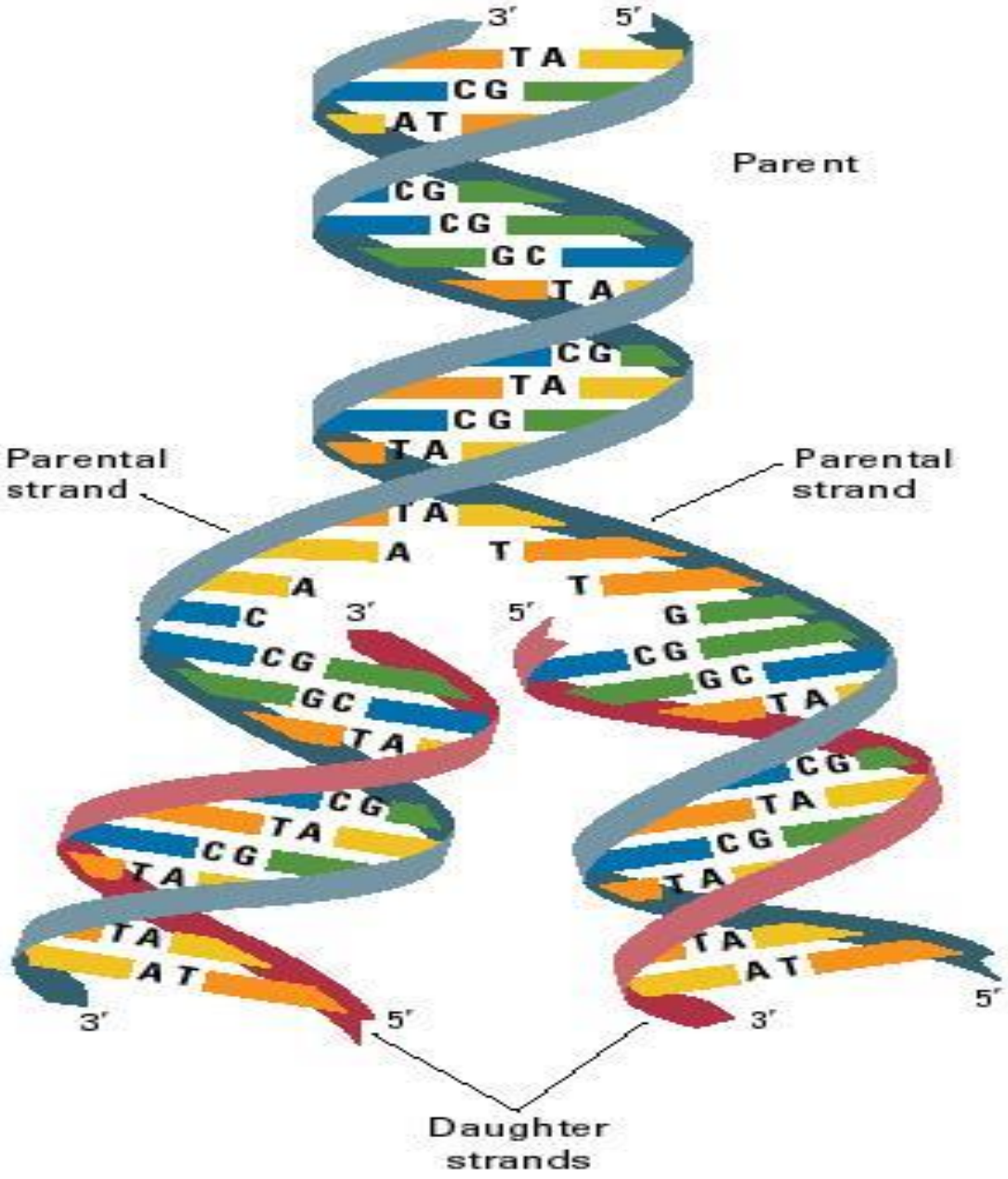
Why both the strands are not copied during transcription has the simple answer. First, if both strands act as a template, they would code for RNA molecule with different sequences (Remember complementarity does not mean identical), and in turn, if they code for proteins, the sequence of amino acids in the proteins would be different. Hence, one segment of the DNA would be coding for two different proteins, and this would complicate the genetic information transfer machinery. Second, the two RNA molecules if produced simultaneously would be complementary to each other, hence would form a double stranded RNA. This would prevent RNA from being translated into protein and the exercise of transcription would become a futile one.

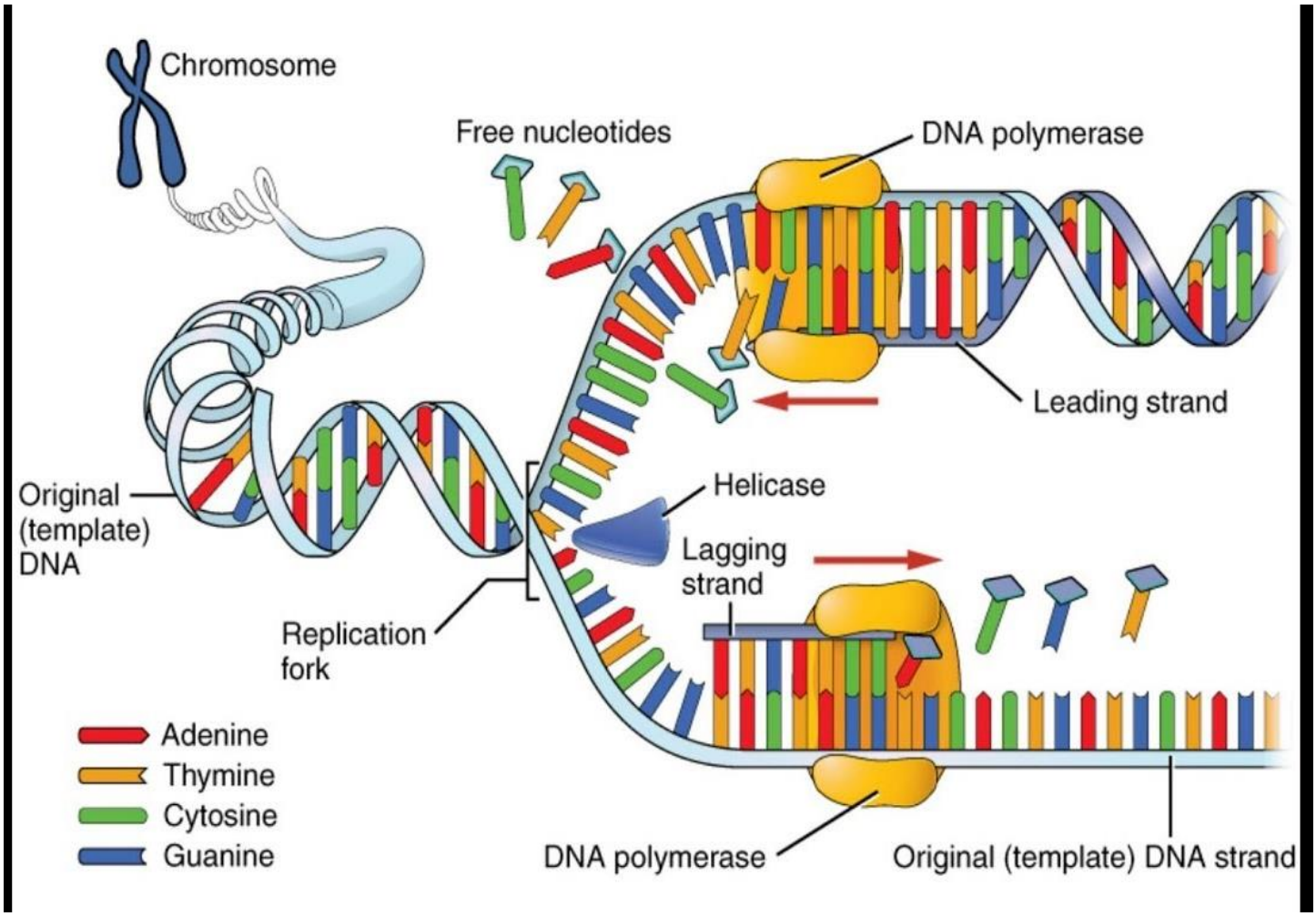
6.5.1 Transcription Unit

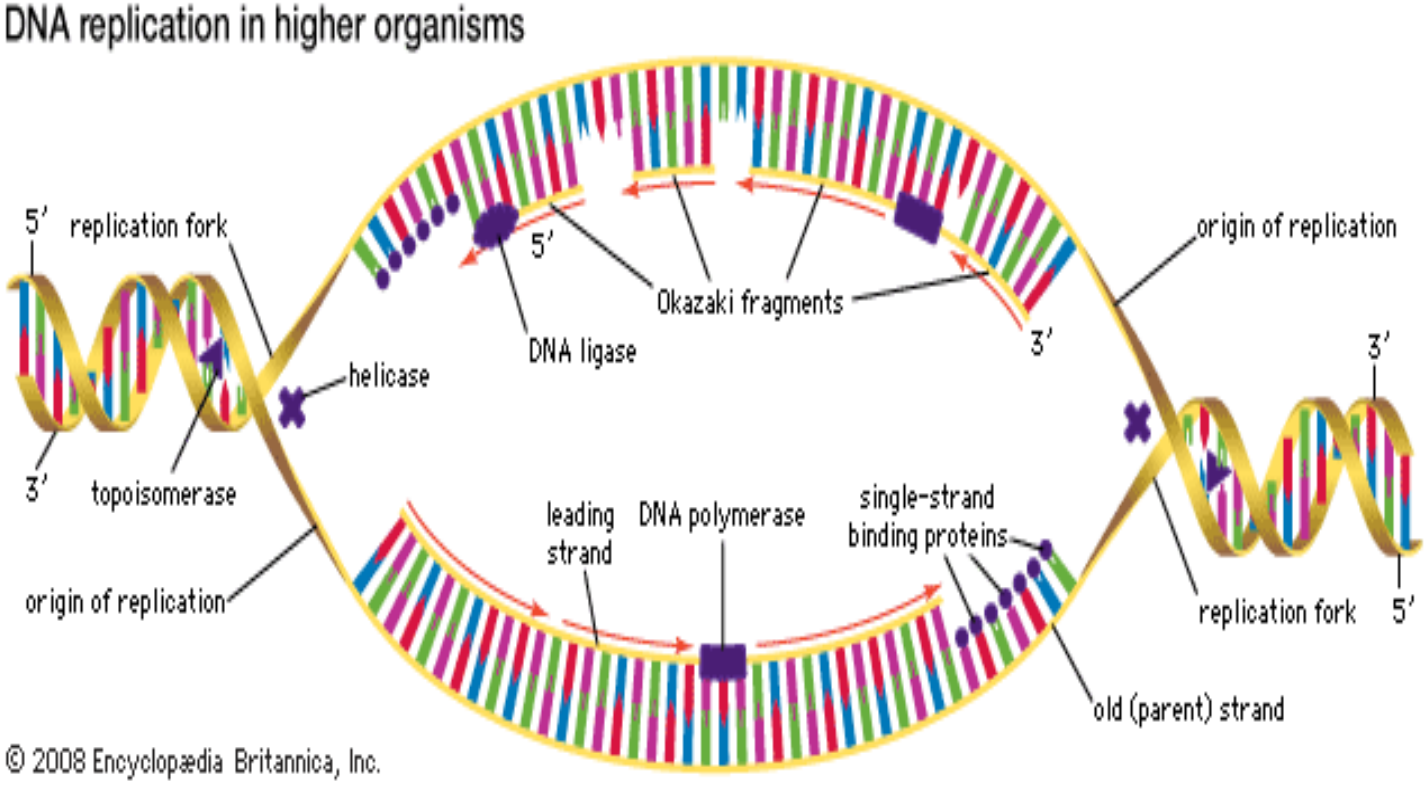
A transcription unit in DNA is defined primarily by the three regions in the DNA:

(i) A Promoter
(ii) The Structural gene
(iii) A Terminator


E. coli cultures
DNA in CsCl gradient
DNA composition
Start
15N-containing medium
15N–15N (heavy) DNA
Continue growing first generation in 14N medium
Replication cycle 1
15N–14N (intermediate density) DNA
Continue growing
Replication cycle 2
14N–14N DNA
15N–14N (intermediate density) DNA
Continue growing
Replication cycle 3
14N–14N
14N–14N
14N–14N
15N–14N


3′
5′
Parent
Parental strand
Parental strand
3′
5′
3′
5′
3′
5′
Daughter strands


Chromosome
Free nucleotides
DNA polymerase
Leading strand
Original (template) DNA
Helicase
Lagging strand
Replication fork
Adenine
Thymine
Cytosine
Guanine
DNA polymerase
Original (template) DNA strand


DNA replication in higher organisms
5′
replication fork
3′
topoisomerase
helicase
origin of replication
5′
DNA ligase
Okazaki fragments
3′
origin of replication
leading strand
DNA polymerase
single-strand binding proteins
old (parent) strand
3′
5′
replication fork
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ASSIGNMENT 4 (MOLECULAR BASIS OF INHERITANCE)

1. WITH A WELL LABELLED DIAGRAM EXPLAIN THE EXPERIMENT OF MESELSON AND STAHL. WHAT HAS BEEN CONCLUDED FROM THIS EXPERIMENT?
2. DESCRIBE THE STUCTURE OF REPLICATION FORK. STATE THE DIFFERENCES BETWEEN LEADING STRAND AND LAGGING STRAND.
3. STATE THE FUNCTION OF THE FOLLOWING ENZYME:
   A. DNA POLYMERASE
   B. DNA LIGASE
   C. HELICASES
4. WHAT DO YOU MEAN BY ORIGIN OF REPLICATION?
5. WHAT IS THE RESULT OF FAILURE OF IN CELL DIVISION AFTER DNA REPLICATION?