Chapter 7

INTRODUCTION TO DNA & PROTEIN SYNTHESIS

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This chapter covers a descriptive explanation for the replication of Deoxy-Ribose Nucleic Acid (DNA). The later half of this chapter covers transcription/translation of the genetic information embedded in DNA. Finally, the chapter concludes with a discussion of the *lac* operon system. This set of genes controls the expression of necessary proteins used to metabolize lactose sugars when glucose is not available.

Reference:

Chapter 13 & 14 of Starr & Tagart. 10th Ed.

1. http://www.pathology.washington.edu/Cytogallery
   This site de finds and describes a Genetic material

2. http://www .emc.maricopa.edu/faculty /farabee/BIOBK/BioBookDNAMOLGEN .html

3.. http://www.accessexcellence.com/AB/GG/protein synthesis.html
   This site provides narrative and pictures of protein synthesis process

4. http://web.indstate. edu/thcme/mwking/rna.html
   An Excellent site for explanations for and definitions of the protein synthesis process

5. http://photoscience.la.asu.edu/photosyn/ courses/BIO 343/lecture/DNA -RNAhtml
   Out standing drawing ofthe sugars of DNA and RNA (a comparison)

6. http://molbio.info.nih.gov/molbio/ gcode.html
   This last web site is the genetic code which you can also find in text book

Learning Objectives:

1. Describe the biochemical, structural, geometric aspects of DNA
   a. How does hydrogen bonding playa very big role in determining the pairing of the 4 bases composing DNA
2. Explain how DNA replicates
   a. Know why it is said DNA replication is semi-conservative in nature.
3. Explain "What are genes" in a biochemical sense. 4. Describe how copied genetic information from DNA, which never leaves the nucleus, is able to find its way into the cyptoplasm for the construction a polypeptide units.
5. Know the various forms of RNA in the transcription, translation, elongation & termination process of a polypeptide chain.
6. Be able to decode and demonstrate the use the "GENETIC CODE" for the synthesis of amino acids from given codon sequences.
7. Fully understand the differences between DNA replication and DNA transcription/translation

processes.

8. Understand the consequences of mutations, deletions, or frameshift reading errors that arise
9. Know the vocabulary associated with both chapters.

Chapter 7 DNA Synthesis

I. DNA Structure

A. Components of DNA

1. DNA is composed of 4 kinds of nucleotides, each of which consists of:

   a. A five-carbon sugar - deoxyribose

   b. A phosphate group

   c. One of four bases:

      Adenine (A)
      Guanine (G)
      Thymine (T)
      Cytosine (C)

2. There are 2 classes nucleotides that make DNA

   a. Pyrimidine:

      single-ring thymine and cytosine

   b. Purines:

      double ring adenine and guanine

3. DNA composed of 4 kinds of nucleotide bases pairing in an

   A ~ T and G ~ C fashion through the process of hydrogen bonding

   A ~ T forms two hydrogen bonds

   G ~ C forms three hydrogen bonds

   a. These base pairs differ in relative amounts from species to species

4. DNA exist as long, thin molecule of uniform diameter

   a. The structure is highly repetitive

   b. DNA is helical

B. Patterns of base Pairing

Watson and Crick model of DNA

1. Single ring 'T' hydrogen bonded with double ringed 'A' and single - ringed 'C' with double-ringed 'G'

   Examine the hydrogen bond linkage. This is a key factor here!

2. The backbone was made of chains of sugar-phosphate linkages

3. The molecule was double stranded and looked like a ladder with a twist to form a double helix

4. This base pairing is constant for all living species however the base pairing is different between species

II. DNA Replication and Repair

A. Making of Nucleotide Strands

1. Two strands of DNA unwind and expose their bases

2. Unattached nucleotides pair with exposed bases

3. Replication results in DNA molecules that consist of one "old" strand and one "new" strand; Note this is considered a 'semiconservative replication' method

B. Enzymes for Replication

1. One kind of enzyme unwinds the 2 nucleotide strands
2. DNA polymerase attach free nucleotides to the growing strand
3. DNA ligase seal new short stretches of nucleotides into one continuous strand

C. DNA polymerase, DNA ligase, and other enzymes engage in DNA repair

D. DNA polymerase "proof read" the new bases for mismatched pairs and will replace mismatches with correct base

DNA Structure and Function

DNA exists in the nucleus as a condensed, compact structure. To prepare DNA for replication, a series of proteins aid in the unwinding and separation of the double-stranded DNA molecule. These proteins are required because DNA must be single-stranded before replication can proceed.

Unwinding protein: Helicases - These proteins bind to the double stranded DNA and stimulate the separation of the two strands.

Strand separating protein: *Single strand binding protein* - These proteins bind to the DNA as a tetramer and stabilize the single-stranded structure that is generated by the action of the helicases. Replication is 100 times faster when these proteins are attached to the single-stranded DNA.

Untangling proteins: Gyrase or topoisomerase -This enzyme catalyzes the formation of negative supercoils that is thought to aid with the unwinding process. In addition to these proteins, several other enzymes are involved in bacterial DNA replication.

Stress releasing protein

These topoisomerase proteins act by breaking DNA strands thereby allowing the molecule to rotate around a single strand

Primase: This enzyme is a short stretch of RNA (5 sequence long) which binds to complementary DNA base pair

1) RNA contains uracil in place of thymine
2) Also has ribose sugar instead of deoxyribose sugar

DNA polymerase: This enzyme catalyze the synthesis of a new DNA strand RNA polymerase, on the other hand, synthesizes mRNA **DNA Polymerase** - DNA Polymerase I (Pol I) was the first enzyme discoveredwith polymerase activity, and it is the best characterized enzyme. Although this was the first enzyme to be discovered that had the required polymerase activities, it is not the primary enzyme involved with bacterial DNA replication. That enzyme is DNA Polymerase III (Pol III). Three activities are associated with DNA polymerase I;

* 5' to 3' elongation (polymerase activity)

* 3' to 5' exonuclease (proof-reading activity

* 5' to 3' exonuclease (repair activity)

The second two activities of DNA Pol I are important for replication, but DNA Polymerase III (Pol III) is the enzyme that performs the 5'-3' polymerase function.

DNA polymerase III links nucleotides in a 5' to 3' direction

Energy to form the new covalent bonds comes from release of pyrophosphate

from ATP

-**DNA strands** are formed in an anti-parallel fashion

New DNA synthesis occurs in a 5' to 3' direction

One strand (leading strand) is made in continuous order on the 3' - 5' template while the other strand (lagging strand) is synthesized in short segments.

These segments are called OKAZAKI fragments which are usually 100 to 300 nucleotides long in Eukaryotes.

Each fragment is primed with RNA primes

DNA polyermase II removes the primer & replaces it with DNA

DNA Ligase - Nicks occur in the developing molecule because the RNA primer is removed and synthesis proceeds in a discontinuous manner on the lagging strand. The final replication product does not have any nicks because DNA ligase forms a covalent phosphodiester linkage between 3'-hydroxyl and 5'-phosphate groups.

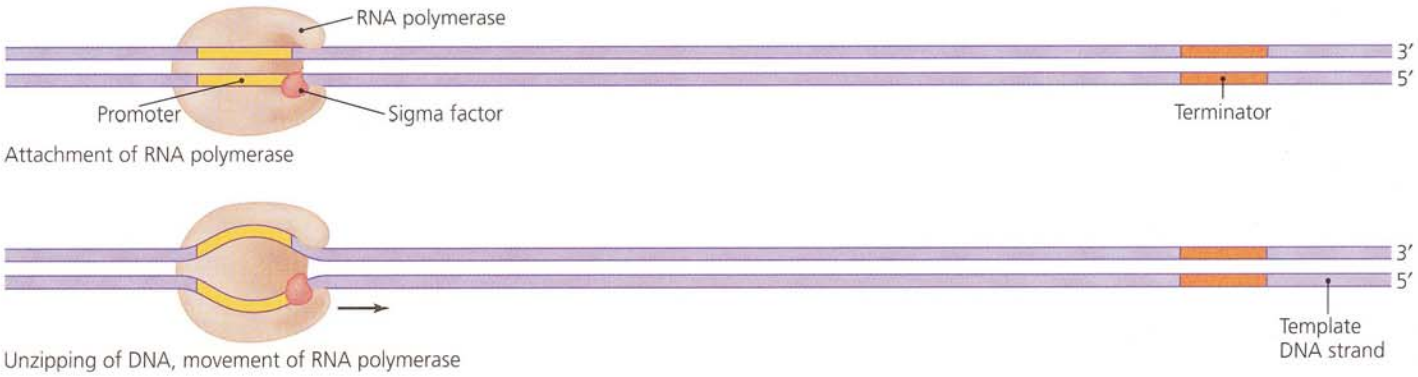
Proof reading the DNA : There is a high degree of accuracy in DNA replication

In bacteria there can be an error in the order of 1:10,000 base pairs

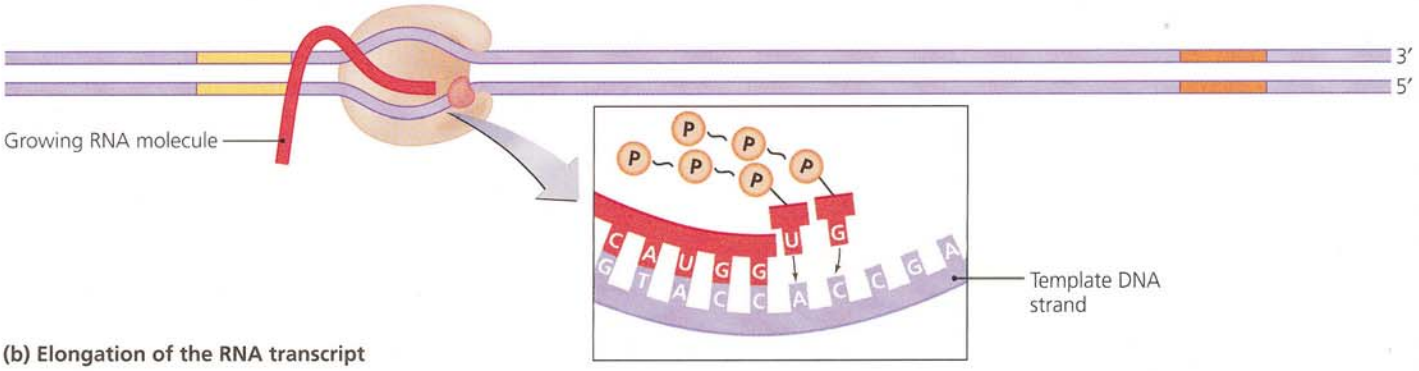
DNA polymerase I backs up and removes the corrupted nucleotide sequence in a 5' - 3' direction

The analysis of temperature-sensitive mutants of E. coli has defined a series of genes and their role in DNA synthesis. The following table list some of the genes and their role in E. coli DNA replication.

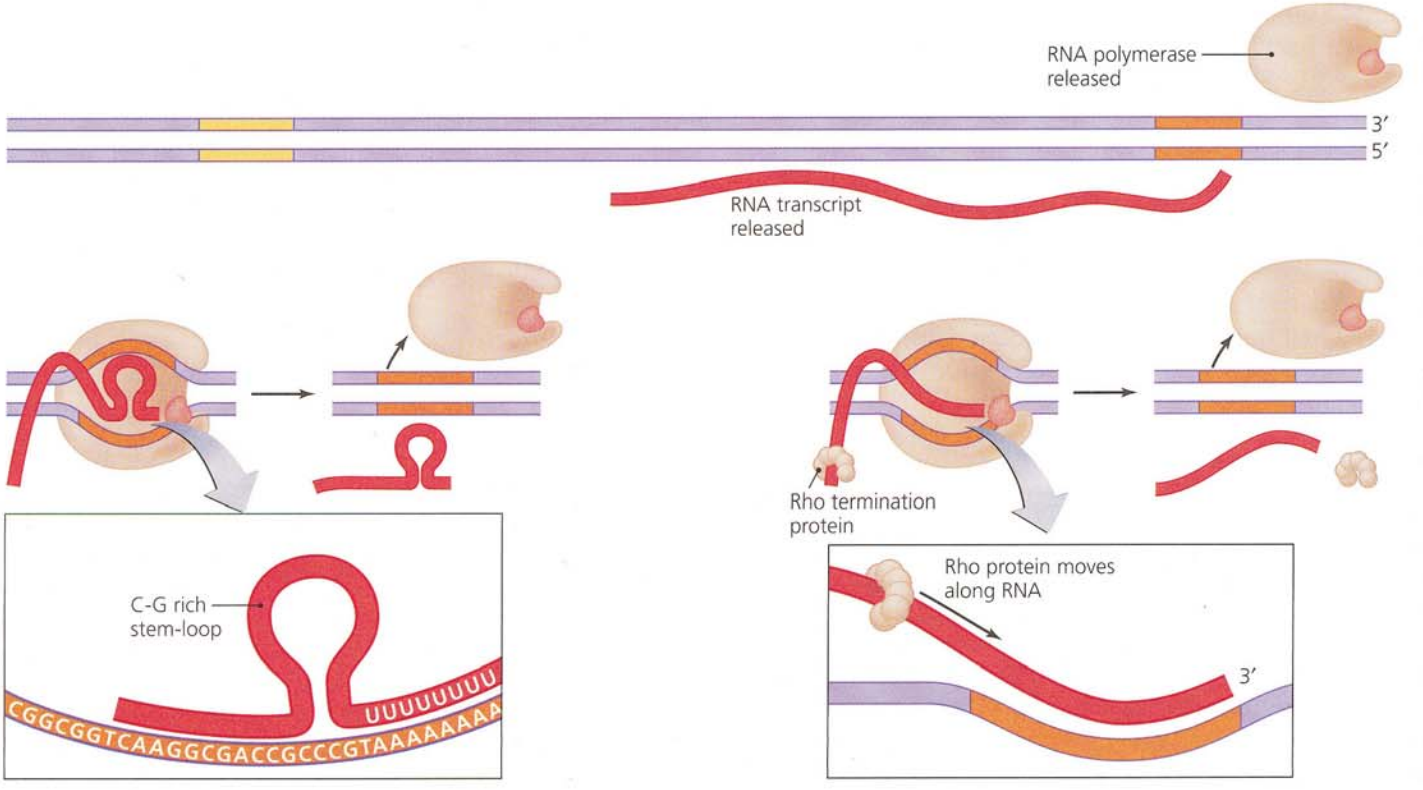
Gene	Function
dnaA,I,P	Initiation
dnaB,C	Helicase at oriC
dnaE,N,Q,X,Z	Subunits of DNA polymerase III
dnaG	Primase
gyrA,B	Subunits of gyrase
lig	Ligase
oriC	Origin of Replication
polA	DNA polymerase I
polB	DNA polymerase II
rep	Helicase
ssb	Single-stranded DNA binding proteins



(a) Initiation of transcription



(b) Elongation of the RNA transcript



(c) Termination of transcription

The function of important enzymes involved in DNA replication.

TABLE 9.1 Some Enzymes Involved in DNA Replication and Their Functions

Enzyme	Function
Helicase	Unzipping the DNA helix
Primase	Synthesizing an RNA primer
DNA polymerase III	Adding bases to the new DNA chain; proofreading the chain for mistakes
DNA polymerase I	Removing primer, closing gaps, repairing mismatches
Ligase	Final binding of nicks in DNA during synthesis and repair
Gyrase	Supercoiling

Table 9.1 Some enzymes involved in DNA replication