

Chapter for today: Chap. XIV**Major points for the day:**

1. More details about DNA replication
2. “The central dogma”: DNA RNA Protein
3. The process of transcription (synthesizing RNA from a DNA template)

Reprise:

Last time I discussed the nature of the genetic material, DNA. The evidence for the involvement of DNA in heredity began to accumulate starting in the 1940s and was generally accepted by the 1950s.

The experiment of Avery, MacLeod and McCarty provided the first clear evidence in favor of DNA’s hereditary role. Experiments in the 1950s made it clear that DNA was the genetic material and that it underwent a process of copying (replication) that involved the copying of a double stranded molecule into two double stranded daughters.

A question

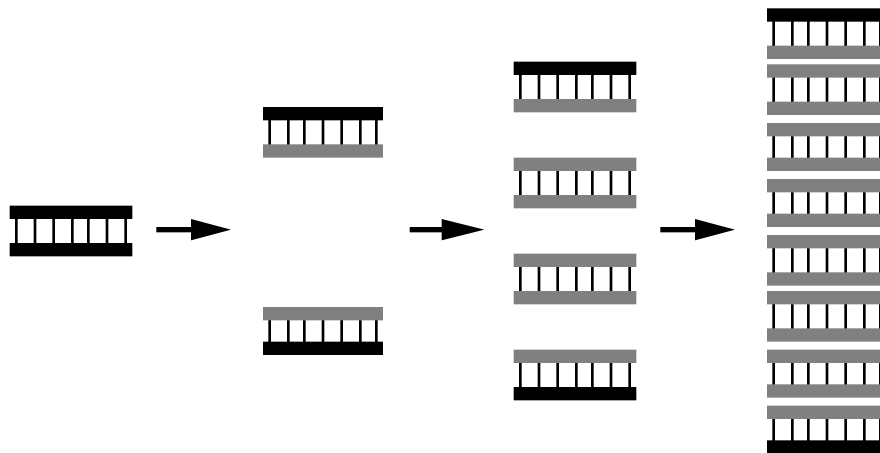
The Hershey–Chase experiment involved infecting cells with radioactive bacteriophage

- We know that only the DNA (^{32}P) enters the cell
- I told you that the virus makes multiple copies of itself after it enters the cell
- Those copies are identical in every way to the infecting phage

Question: If a ^{32}P -labeled bacteriophage infects a cell, replicates and bursts the cell open with the release of 100 new phage (a normal yield) how many of those phage will still be labeled?

To answer this question requires that we understand the mechanism of DNA replication

- It is semi-conservative, so that the product is 1/2 old DNA and 1/2 new DNA
- Think about the first three rounds of synthesis:



- Only two molecules of DNA will retain the label in the original molecule

That assumes something. What is it?

Today we are going to look at the events which are occurring during phage infection in addition to DNA replication—the conversion of genetic instructions into proteins

A closer look at replication

Last time I showed you the model for the DNA that Watson & Crick proposed in a now famous 1953 article. That article included the enigmatic comment that a method for the replication of DNA had not escaped their notice.

This method involves the unwinding of the double helix into two single strands. After unwound, new nucleotides can base pair with the old strands and become polymerized into new strands of DNA. The old DNA (“template”) provides the instruction for the assembly of the new strand because only A-T and G-C pairing is allowed in the structure.

The enzyme responsible for the construction of new strands is called DNA polymerase. During replication a small region of the DNA unwinds and two molecules of DNA polymerase assemble at the bubble. One molecule performs the synthesis of a continuous strand in the forward direction (“leading strand”) while the other makes a discontinuous set of shorter fragments running in the opposite direction (“lagging strand”). The short strands have to be linked together to form a second long strand of DNA, similar to the new leading strand. A second enzyme that joins these smaller units into the long strand of DNA is termed “DNA ligase”.

The two directions of the DNA correspond to opposite chemical directions in the molecule. Remember that nucleotides are asymmetric, meaning that a polymer of them would have directionality. Linkage in the molecule occurs between the deoxyribose part of the nucleotide using bridging phosphate molecules. Chemists call the linkage an ester, and because each phosphate makes two ester linkages to successive deoxyriboses, DNA is said to use phosphodiester linkage.

The ester linkage is between the phosphate and two positions on the deoxyribose, which chemists term the 5’ and 3’ positions. The directionality of DNA replication can be expressed in these terms. Each new nucleotide is added onto the 3’ end of the growing strand, making a new phosphodiester linkage. So, DNA replication occurs in a 5’ to 3’ direction.

The origin of the “central dogma” of molecular biology

In the first few years following Watson & Crick’s proposal of a structure for DNA the focus of research interest rapidly moved to the question of how the genetic instructions of DNA could be used by the cell

It had long been known that the major informational content of the cell was in the primary sequence of its proteins

- The belief in proteins as the genetic material centered on the idea that proteins must construct other proteins
- How this would be accomplished was unknown, and no good hypotheses were forthcoming

DNA as the genetic material was in some ways a larger problem

- Proteins seemed to be the intended products of genetic instructions
 - Proteins and nucleic acids had no chemical relationship to each other
- How could nucleic acids program the construction of proteins?

The answer is that there is a process in which the DNA instructions are “translated” from the language of nucleic acid into the language of protein, i.e. from nucleotides to amino acids

- The two “languages” are distinct with their own words
- However, there is a one-to-one correspondence between the words in the two languages

- The cell includes a “translation machine” which converts the nucleotide “words” into amino acid “words”

It is fortunate that biologists had the presence of mind to call this process **translation**

However, proteins are not translated directly from DNA. Rather, a messenger form of nucleic acid is synthesized which moves to the cytoplasm, and is then translated into its protein product.

- Copying DNA into RNA does not involve “translating” into a new chemical language
- Rather the RNA is a faithful copy of a region of the DNA molecule
- The metaphor best suited to this event is the copying of a manuscript by a professional copier, a scribe, an event called “transcribing”
- Fortunately again, biologists call this process **transcription**

So the process of expressing genetic information involves movement of information from DNA to RNA and finally to protein

This is the central dogma of molecular biology: DNA RNA Protein

The process of transcription

Ribonucleic acid (RNA) resembles DNA in being composed of nucleotides, however the nucleotides are slightly different

- DNA nucleotides are composed of a base, deoxyribose and a phosphate
- RNA nucleotides are composed of a base, ribose and a phosphate
- Ribose differs from deoxyribose by the substitution of a hydroxyl (OH) for a hydrogen (H) at the 2' position of the sugar ring
 - This difference makes RNA more unstable since this hydroxyl makes the backbone of the molecule more susceptible to degradation
- In addition, RNA has no thymidine which is replaced by a similar molecule called uracil (thymidine is simply uracil with an added methyl group)
 - Uracil base pairs with adenine (U•A)

The DNA molecule acts as the template for synthesis of an RNA copy

- As in replication, RNA nucleotides can be assembled into a chain using the existing chain of DNA as a guide to indicate the order necessary
- This process is catalyzed by a special enzyme called RNA polymerase
- Only a single strand of each transcribed region is copied into RNA. This strand encodes a protein (the “sense” strand) the opposite strand does not (the “antisense” strand)

RNA polymerase begins synthesis of the RNA chain at a region called a “promoter”. The role of the promoter is to position the polymerase correctly so that the right region will be transcribed.

- RNA is synthesized by base pairing between DNA nucleotides and new RNA nucleotides
- Since the normal double-helical structure of the DNA would not allow this to occur, the helix must be opened up, breaking the DNA base pairs
- RNA polymerase catalyzes this opening of the helix, and directs the synthesis of a faithful copy of the DNA into RNA
- The enzyme proceeds down the DNA molecule unwinding base pairs ahead of it while those behind reform, and copying the DNA into RNA

- At a special signal in the DNA called a “terminator” the polymerase stops synthesis of the RNA, releases it, and dissociates from the DNA

I noted the fact that nucleic acids have a polarity (the two strands of DNA run in opposite directions)

- This polarity is expressed in terms of the structure of the sugar moiety
- The phosphate group bridges between the 5' and 3' positions of adjacent nucleotide sugars
- The DNA polymerase synthesizes DNA from the 5' to the 3' direction (the free end at the beginning is a 5', and the free end at the end is a 3')
- RNA polymerase has the same polarity—from 5' to 3'

In prokaryotes, the RNA product of transcription is directly used in the synthesis of proteins

In eukaryotes the RNA is modified after transcription

- Special modifications are made at the 5' and 3' ends.
- Internal regions of the RNA can be excised, with rejoining of the adjacent segments of RNA
 - By analogy to editing of film or tape, this process is called “splicing”

Next time

We will continue with a discussion of how the information contained in DNA is transmitted to the cell to do useful work in the second stage of the process, translation.