CHAPTER 11: DNA and Its Role in Heredity

Introduction

- DNA is an information molecule.
- It typically has a double-helix structure, an icon often recognized even by those who are not scientists.
- It was originally thought that the genetic information-containing molecule was protein.
- There are 20 amino acids that make up proteins. This would provide ample characters to write a complex code.
- DNA consisted of just four different characters or molecular monomers.
- It seemed to be too few.
- Today, this would seem reasonable, though. Just as complex computer systems use binary, and exchange information in 0's and 1's, the genetic code is written with linear sequences of molecules designated A, C, G and T, a quaternary code.

I DNA: The Genetic Material

Circumstantial Evidence for DNA Being the Information Molecule:

- It was learned almost a century ago that DNA was in the nucleus as part of organized chromosomes.
- During cell division, what appeared as copies, were distributed evenly to daughter cells.
- Different species had different sizes and numbers of chromosomes, but within a species, the size and number of chromosomes are mostly consistent.
- It was found that sperm had half the amount of DNA as found in somatic (body's) cells.
- However, chromosomes are made of protein and DNA. Which was the genetic material? Was the DNA a structural component or an information molecule?

A. DNA from one type of bacterium genetically transforms another type

Direct Evidence for DNA Being the Genetic Information:

- 1920's - Griffith experimented with a pneumococcus.
- One strain of this bacteria, called S because of its shining appearance as small colonies on plates, is lethal to infected mice.
- The other is non-lethal, but does infect mice.
- The shininess of the S strain is caused by a polysaccharide coat, which serves to protect the bacteria from detection by the mouse’s immune system.
- R lacks this, and is defeated by a healthy mouse's immune system.
- Griffith discovered something astonishing and unexpected, simply as a result of his experimental design.

- He discovered that *killed* S-strain bacteria could "transform" live R type into the virulent S-type of bacteria.
- Something from a dead or killed organism could change the characteristics in a heritable manner of a living organism.

- See ***Figure 11.1*** for complete details.

B. The transforming principle is DNA

- Griffith failed to discover the agent responsible for the "transformation" event.
- Griffith cannot be faulted for this. It wasn't until 20 years later that this was determined.
- Eliminating possibilities one by one, lipids, carbohydrates, and proteins were selectively destroyed. Only with the elimination of DNA was the transformation process prevented.
- Also, pure DNA was used. DNA also transformed R to S.
- Work was published in 1944 by scientists: Avery, Macleod, and McCarty.
- This was again, a history-changing event.
  - Scientists of the day were too naive to fully appreciate these findings.
  - They weren't even sure that bacteria had heritable information or genes.

Additional Supporting Evidence:

C. Viral replication experiments confirm that DNA is the genetic material

- The Hershey and Chase experiment used virus.
- T2 bacteriophage - a bacterial virus was used.
- This virus is virtually a DNA molecule encased in a protein shell.
- Experiments were designed to see which entered the cell.
  - The possibilities were that the protein, the DNA or both would enter.
  - The interpretation of the results could be simple if either entered, but difficult if both did.
- Radioactive elements ^{35}S and ^{32}P were chosen because sulfur is present in proteins (cysteine and methionine) while phosphorus is found in DNA.
- By conducting separate experiments with T2, one for radioactively labeled protein (^{35}S) and one for radioactively labeled DNA (^{32}P), bacterial cells were infected, centrifuged to form a pellet and then their radioactivity was measured by sampling the pellet and the supernatant (solution, not the pellet). (Bacteria maked up the pellet found at the bottom of the tube.)
  - It was found that ^{32}P was mostly associated with the bacteria (pellet).
  - It was found that ^{35}S was mostly found in the supernatant, and not the pellet.
- This supported the hypothesis that DNA was the heritable material.
- See *Figure 11.3* and *Figure 11*.2.

II Structure of DNA

- How could a molecule composed of such few basic units contain information able to generate the variety and complexity of life forms?
- How could so many different shapes and forms be possible?
- The enquiry into the actual structure of the DNA molecule could be considered to be the defining moment in the history of humanity.

A. X ray crystallography provided clues to DNA structure

- X-ray defraction data provided clues to the structure and were generated by Rosalind Franklin.
- See Figure 11.4.

B. The chemical composition of DNA was known

- Erwin Chargaff had discovered that DNA molecules contained a balance in the amount of the base A with the base T and also a balance of G with C. He had no idea why, though.
- If 10% of the base A was found, then the amount of T would be 10%, C would be 40% and G would be 40%. This was called Chargaff's rule.

C. Watson and Crick described the double helix

- Watson and Crick combined data from Chargaff, Franklin and others.
- One crucial bit of information came from a physical biochemist, who explained that the structures of the DNA monomers that scientists of the day were attempting to use, were in fact wrong.
- After several attempts, a successful model was made out of *cardboard* in 1953.
- The same molecular model stands today.
- See *Figure 11.6*.

D. Four key features define DNA structure

1. DNA is usually double stranded.
2. Helical
3. The molecules are twisted to the right. When holding two pieces of string parallel to each other, vertically, and then twisting the top of the strings to the right (counter clockwise), while holding the bottom still, the a right-handed double helix model can be made.
4. The strands are antiparallel.

- DNA monomers are called nucleotides.
  - The sugar part of the molecule, the pentagonal shaped central portion, is called a deoxyribose, because it is a ribose minus one oxygen.
  - The oxygen atom is missing from the 2' carbon of the sugar.
  - RNA monomers have oxygen in the 2' position; in fact, it is having or not having the 2' oxygen that distinguishes RNA from DNA.

- Nucleotides contain at least one phosphate group, a deoxyribose sugar and a nitrogenous group.
  - Assuming that the number of phosphates on each molecule is the same, what distinguishes one type of deoxyribonucleotide from another is the nitrogenous group (or base).
  - There are four different possibilities for these.
  - Nucleotides are not symmetrical.
  - There's a 5' carbon, which is bonded to a phosphate group, and a 3' carbon, where an OH is covalently attached.

Antiparallel:

- If you think of the 5' as the head and the 3' as feet, then you can easily visualize the polarity issues about to be discussed.
  - If you place a row of people on the floor, the first with his/her head pointing north, the next person's head would be aligned with the first person's feet.
  - Continue this and you would have one strand.
  - The anti-parallel complementary strand would align with the first strand except that the heads and feet would be pointing in the opposite directions:
    :)><   :)><   :)><   :)><   :)><   :)><   :)><
    ><(:   ><(:   ><(:   ><(:   ><(:   ><(:   ><(:
  - *See Figure 11.7.*
- The two strands are complementary such that at each point where an adenine is on one strand, the other strand will have a thymine, also vise versa.
- The force that maintains the interaction of the two separate but complementary strands is hydrogen bonding.
- Hydrogen bonding is caused by charge attractions. The bonding strength is weak but the bonds along the molecule are somewhat additive.
- The hydrogen bonds form between A and T, but not usually between A and C or G.
- Two form between A and T; three form between C and G. It is the fact that an A is across from a T or that a G is across from a C that establishes the hydrogen bonds.
- Hydrogen bondings' weakness means that just heating the DNA is sufficient to separate the strands.
- It is the fact that the strands can be easily separated that makes the molecule useful as an information molecule.
- When DNA is heated to separate DNA molecules, it is called denaturing or melting the DNA.
  - If the concentration of complementary molecules is high enough, some will come back together again upon cooling.
  - This is called annealing, or re-annealing.

E. The double helical structure of DNA is essential to its function

The following functions must be provided by the DNA:

- Store an organisms genetic information
- Precisely replicate
- Change slowly over time
- Be expressible as to generate a phenotype

III DNA Replication

- Watson and Crick (W/C) postulated that one strand of DNA molecule could act as a template for the creation of a new strand.
- It would be expected then, that for each new double-strand DNA molecule replicated, one of the strands would be the template or "old strand", whilst the other would be a newly synthesized strand.
- This hypothesis was proposed along with the structure in Watson and Cricks original paper.

A. Three modes of DNA replication appeared possible

- Often when an experimenter designs an experiment to prove or disprove a hypothesis, one outcome explains, and others fail to.
- When an experiment is designed that has only a few possible outcomes and any would prove one of the possible alternative hypotheses, it is called a critical experiment.
- In some respects the experiment by Meselson and Stahl is a paradigm of how geneticists approach questions.
- How many possible ways are there for how DNA might replicate?
    1. Dispersive: Fragments of the original molecule serve as templates and then assembly yields a mixture of old and new parts. This is a difficult to imagine alternative, but is one of the possibilities.
    2. Conservative: Each double helix serves as a template for a whole new double helix - both strands of the one would be old - both of the newly synthesized would be new. This isn't what Watson and Crick expected but is another possibility.
    3. Semi conservative – W/C’s model: Each molecule consists of an old and new strand

B. Meselsen and Stahl demonstrated that DNA replication is semiconservative

- The experimental results could distinguish between the three possible outcomes.
- They actually invented the technologies needed to answer the questions.
- First, how could old from new strands he distinguished?

1. This was done by labeling the DNA with an isotope of nitrogen, ^{15}N. DNA containing this isotope is denser than that which contains the common isotope ^{14}N.
2. A method to determine the density of the strands of DNA had to be developed.
   - For this they invented cesium chloride density centrifugation.
   - When a solution containing cesium chloride is spun at remarkable speeds, enough to create 100,000 times earth's gravity, the solution becomes far more concentrated at the bottom of the tube than the top.
   - This is a density gradient. DNA spun in such a solution will end up in a region of the tube that has the same density as its own.

- With these systems in place, bacteria were grown for 15 generations in medium in which the nitrogen source was ^{15}N.
- Just to test the separation system, DNA from the 15 generations' tube was spun with DNA from ^{14}N standard cultures. One low band and one high band were observed. The assay was working. To visualize the DNA, a DNA binding stain that fluoresces upon illumination with ultraviolet light was used. The bands glow.
- Then ^{15}N grown bacteria were placed into ^{14}N solution and grown. Some were removed after a single generation (or replication), and some after two.
- The *ifs* and *thens*:
  1. If after one generation, there were two bands, one that corresponds to the control ^{15}N grown and one that corresponds to the control ^{14}N grown, then this would be consistent with conservative replication.
  2. If after one generation there was one band found intermediate in position to the control ^{15}N and ^{14}N bands, this might mean semiconservative replication - W/C's model, or possibly the dispersive model.
  3. After two generations, if one low and one high (more fluorescence this time in the high band) were observed, then conservative replication would be supported.
  4. After two generations, if one band between the midpoint and high band (or smear) were observed, then dispersive replication would be supported.
  5. After two generations, if two bands, a high and an intermediate were observed, then semiconservative would be supported.
  6. This is what was observed, as described in 5.

See *Figure 11-10*.

IV Replication Mechanism.

- DNA is routinely replicated cell-free in labs all over the world.
- A template, a double stranded DNA molecule is required.
- Needed are the four deoxyribonucleoside triphosphates. These are the basic building blocks for DNA, and also provides the energy needed for synthesis.
- Needed is an enzyme, a *DNA polymerase*.

- Bases are added to an existing DNA strand. Each nucleotide's 5' end is added to the existing chain's 3' end. This would be like adding one more person in the row by placing him/her next to the feet of the last one already in the row. All DNA synthesis and even RNA synthesis occurs in this 5' to 3' direction.
- As each base is added, 2 phosphate groups come off as a single pyrophosphate molecule leaving one phosphate incorporated into the DNA molecule (See *Figure 11.11*)
- The process of replication involves the use of a 3' to 5' template to generate a 5' to 3' molecule.

A. DNA is threaded through a replication complex

- Currently, it has been suggested that DNA synthesis occurs at replication complexes. These stay still relatively as DNA is threaded through and is replicated.
- However, it is easiest to first understand the steps and process that must occur.
  1. DNA must be unwound: DNA helicase unwinds the DNA.
  2. RNA primase, an RNA polymerase that is used specifically for DNA synthesis, must make a stretch of RNA complementary to the DNA; since DNA cannot start at a single base, but must add on to an existing chain.
  3. DNA polymerase adds nucleotides in the order directed by the template strand.
  4. RNA is removed and replaced with DNA by another DNA polymerase.
  5. An enzyme DNA ligase seals gaps left between segments.
- Review *Figure 11.13* for details on the general movement of replication forks.

B. Small, circular DNA's replicate from a single origin, while large linear DNA's have many origins

- DNA replication begins at one spot in bacterial chromosomes, a site called the origin of replication, and it then progresses with replication forks moving in opposite directions (see *Figure 11.12*).
- There are multiple copies of origins of replication in eukaryotes.

C. Most DNA polymerases need a primer

- DNA must add to an existing chain.
- RNA can add a single base and then elongate.
- The complex that makes the RNA primer is called a primosome.
- DNA must be synthesized in the 5' to 3' direction. So for the 3' to 5' template strand, a single RNA primer gets the process going. This strand is called the leading or continue strand.
- However, since the replication fork must generate both strands of DNA while moving along the original double-stranded molecule, how do you create the strand of DNA that is made using the 5' to 3' template? It must be made in the

5' to 3' direction, reading the that DNA template in the 3' to 5' direction, but how?

- This is called the lagging strand.

D. The lagging strand is synthesized from Okazaki fragments

- It is made in short segments.
- Each segment begins with an RNA primer.
- In eukaryotes the segments are about 100-200 base pairs.
- In prokaryotes: 100 to 2000 base pairs.
- Discontinuous stretches are synthesized at the same time as the leading strand is synthesized for a given region of the chromosome.
- The discontinuous stretches are called Okazaki fragments in honor of Dr. Okazaki who discovered them.
- After the replication fork moves forward, the Okazaki fragments are removed by DNA polymerase, in a pacman-like manner. DNA polymerase can digest the RNA and replace it with DNA.
- DNA polymerase I does this because it can recognize a gap in linkage between the start of one fragment and the end of another.
- DNA Polymerase I replaces bases whenever it locates a gap. It adds a characteristic number of bases before dissociating from the DNA strand.
- Once DNA polymerase I replaces the stretch of RNA, an enzyme called ligase seals the gap.
- Ligase can only seal gaps between adjacent DNA-molecules. This is why the gaps remain as long as RNA exists in the strand.

V DNA Proofreading and Repair

- Proofreading: After DNA incorporates a base, it verifies it. If it is wrong, endogenous exonuclease activity removes it.
- A new base is then put in again.

A. Proofreading and repair mechanisms ensure that DNA replication is accurate

- Mismatch repair recognizes A=C for example, as a mistaken pairing and must decide which base is wrong.
- If the DNA is newly synthesized, the old strand, which can be identified as old by the presence of some $-CH_3$ groups on some cytosines, is assumed to be the correct reference.
- Other times, the system relies on chance to repair correctly.
- Some types of mistakes occur more frequently, and the system might then assume one type of mistake has occurred, the more frequent type, improving the odds that the decision made is correct.
- Excision repair involves enzymes that can act on defective strands of DNA by cutting out regions around the mistakes.

- DNA polymerase I recognizes the gap and replaces the stretch and ligase seals the gap.
- DNA repair is essential for repair of damage caused by the ultraviolet light found in sunlight, compounds found in our environment, and even substances produced by our own bodies that can alter our DNA.

B. DNA synthesis and repair requires energy

- The slightly endergonic reaction gets energy from hydrolysis of the phosphate bonds.
- The reaction is kept from reversing by the hydrolysis of the by-product inorganic pyrophosphate.
- Repair of DNA damage requires investments in the making of the relevant enzymes as well as use of energy during the repair process.

VI Practical Applications of DNA Replication

A. Polymerase chain reaction makes multiple copies of DNA

- Polymerase Chain Reaction is a technique that replicates DNA in vitro.
- Ingredients include NTPs, DNA polymerase (thermal stable polymerase I from a thermophilic organism), a template, DNA primers and a buffer for control of conditions and to provide magnesium, which is a cofactor for DNA polymerase.
- The reaction tube is heated and cooled repeatedly.
- When heated, the DNA strands denature, and become single-stranded.
- Cooling causes the primers to anneal.
- The temperature can be raised to accelerate the polymerization reaction rate. Typically around 70 base pairs per second are incorporated.
- Each time the temperature cycles, the amount of DNA doubles (in theory).
- The less than perfect efficiency reduces this multiplying effect somewhat.

B. The nucleotide sequence of DNA can be determined

- The sequence of fragments of DNA up to 500 base pairs can be determined.
- A reaction similar to PCR is run.
- Included in the tube along with those components used for PCR are special nucleotides called di-deoxyribonucleoside triphosphates.
    - There are four types of ddNTPs included and each type is tagged with its own fluorescent label.
    - Dideoxyribonucleotides lack an OH at the 3' position, so they terminate the synthesis when incorporated into a DNA molecule.
- Random termination of replication during the reaction causes fragments of different lengths to be generated.

    - If the shortest sequence of the mixture fluoresces the color of A, then A must have been the first base incorporated.
    - If the second longest sequence fluoresces the color of C, then C was incorporated next.
- A special instrument separate the molecules in the reaction by size.
    - The smallest goes through first.
    - A laser and detector determines the base color.
    - Using this system the order of the bases is determined.