Lecture 14: Nucleic Acids and DNA Replication

I. Biological Background

A. Types of nucleic acids:
1. Deoxyribonucleic acid (DNA)
   a. Makes up genes that indirectly direct protein synthesis
   b. Contain information for its own replication
   c. Contains coded information that programs all cell activity
   d. Replicated and passed to next generation
   e. In eukarotic cells, it is found primarily in the nucleus
2. Ribonucleic acid (RNA)
   a. Functions in the synthesis of proteins coded for by DNA
   b. Messenger RNA (mRNA) carries encoded genetic message from the nucleus to the cytoplasm
   c. Information flow:
      DNA $\rightarrow$ RNA $\rightarrow$ Protein
   d. Sequence:
      (i) In the nucleus, genetic message is *transcribed* from DNA into RNA
      (ii) RNA moves into the cytoplasm
      (iii) Genetic message is *translated* into a protein

B. Nucleic acids are polymers
1. Nucleotides linked together by condensation reactions.

C. Nucleotide—building block of nucleic acids
1. Comprised of a five-carbon sugar covalently bonded to a phosphate group and a nitrogenous base.
2. Pentose—5-carbon sugar
   a. Two types:
      (i) Ribose—found in RNA
      (ii) Deoxyribose—found in DNA; lacks -OH group on carbon 2
3. Phosphate—attached to carbon 5 of the sugar
4. Nitrogenous base; two families:
   a. Pyrimidine—six member ring comprised of carbon and nitrogen
      (i) Cytosine (C)
      (ii) Thymine (T); only found in DNA
      (iii) Uracil (U); only found in RNA
   b. Purine—five member ring fused to a six member ring
      (i) Adenine (A)
      (ii) Guanine (G)
D. Functions of nucleotides:
1. Monomer for nucleic acids
2. Energy transfer (e.g., ATP)
3. Electron receptors in enzyme controlled redox reactions (e.g., NADPH)

E. Nucleic acids
1. Formed by phosphodiester linkages; bond between the phosphate of one nucleotide and the sugar of another
2. Backbone consists of repeating pattern of sugar-phosphate-sugar-phosphate
3. Varying nitrogenous bases are attached to the backbone
4. Genes are represented by linear sequence of nitrogenous bases which in turn is the unique code for linear sequence of amino acids in a protein.
Inheritance is based on the replication of the DNA double helix

1. DNA consists of two nucleotide chains wound in a double helix
2. Sugar-phosphate backbone is on the outside of the helix
3. The polynucleotide strands of DNA are held together by hydrogen bonding between paired nucleotide bases and by van der Wall attraction between stacked bases
4. Base pairing rules:
   a. A always with T
   b. G always with C
   c. In RNA, A always with U
5. The two strands are complementary and can serve as templates for new complementary strands
6. Most DNA molecules are long (often thousands or millions of bases)

II. Scientific process identifying DNA as the genetic material

By the 1940's, chromosomes were understood to carry heritable material. Chromosomes are comprised of protein and DNA. At this time little was known about DNA other than it was fairly uniform and apparently homogeneous. Proteins were recognized to be extremely heterogeneous and known to have a great deal of functional specificity. For these reasons, many scientists thought protein was likely to be the genetic material.

Experiments identifying DNA as the genetic material
1. Transformation Experiments (Frederick Griffith, 1928)
   Background: 
   *Streptococcus pneumoniae*, a bacterium that causes pneumonia, has two strains, "smooth" (S) and "rough" (R). These traits were inherited. Smooth have a polysaccharide coat, rough do not.

   Experiment:
   a. Inject S strain into mice
      (i) Mice die of pneumonia
      (ii) Conclusion: Encapsulated strain is pathogenic
   b. Inject R strain into mice
      (i) Mice survive and are healthy
      (ii) Therefore, strain without polysaccharide is non-pathogenic
   c. Inject mice with heat-killed S strain
      (i) Mice survive and are healthy
      (ii) Conclusion: Polysaccharide coat did not cause pneumonia
   d. Mixture of heat-killed S strain and R strain injected into mice
      (i) Mice died from pneumonia
      (ii) Blood samples had live S strain
      (iii) Conclusion: R strain cells had acquired the ability to make polysaccharide coats from the dead S cells and this trait was inheritable
This phenomenon is known as *transformation*—change in phenotype due to the assimilation of externally acquired genetic material. From these experiments the nature of the inheritable material could not be determined.
2. DNA is the genetic material

Background:
A bacteriophage (phage) is a virus that infects bacteria. T2 is a particular type of phage that infects Escherichia coli (E. coli). Viruses are essentially DNA with a protein coat. When T2 infects a host, it reprograms the host cell's function, reproduces itself and then lyases the host.

Experiment (Hershey and Chase, 1952):
a. Label viral DNA and protein with radioactive labels
   (i) To label viral proteins, T2 and *E. coli* were grown in media with radioactive sulfur
       (^{35}\text{S}). T2 protein but not DNA was labeled.
   (ii) To label viral DNA, T2 and *E. coli* were grown in media with radioactive sulfur (^{32}\text{P}).
       T2 DNA but not protein was labeled.

b. Protein-labeled and DNA-labeled T2 phages were used to infect separate nonradioactive *E.
   coli*.

c. Cultures were agitated to dislodge phage particles that remained outside the bacterial cells.

d. Bacterial cells and viral particles were separated by centrifugation—the heavier bacterial cells
   pelleted and the lighter viral particles remained in the supernatant.

e. Radioactivity in the pellet and the supernatant was measured and compared

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<tr>
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<th>Protein-labeled T2</th>
<th>DNA-labeled T2</th>
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<tr>
<td>Supernatant</td>
<td>High</td>
<td>Low</td>
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<tr>
<td>Pellet</td>
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e. When bacteria containing DNA-labeled phages were returned to culture, phage progeny
   contained ^{32}\text{P} in their DNA

Conclusions:
1. Viral proteins remain outside the cells
2. Viral DNA is injected into the cells
3. Injected DNA causes cells to produce additional viruses
4. DNA rather than protein is the heritable material

Other evidence suggesting that DNA is the genetic material of cells:
1. Eukaryotic cells double their DNA content prior to mitosis
2. During mitosis, the doubled DNA is divided equally between the two daughter cells
3. An organism's diploid cells have twice as much DNA as its haploid cells

DNA composition as a function of species (Chargaff, 1947)
1. DNA composition is species specific
2. Amounts and ratios of nitrogenous bases varies from species to species
3. Regularity of base ratios:
   a. Number of adenosines is roughly equal to the number of thiamines
   b. Number of cytosines is roughly equal to the number of guanines
4. *Chargaff's rules*:
   a. A=T; G=C
III. Watson and Crick--the Double Helix

Rosalind Franklin used x-ray crystallography to image DNA. Watson and Crick used Franklin's x-ray data to deduce the following:

A. Characteristics
1. DNA is a helix with a uniform width of 2 nm. Each nucleic acid strand has a width of 1 nm, suggesting that DNA is double stranded.

2. Purine and pyrimidine bases are stacked 0.34 nm apart.

3. The helix makes one full turn each 3.4 nm along its length.

4. Each turn includes ten layers (10 x 0.34 = 3.4 nm) of nitrogenous bases.

5. The sugar-phosphate chains are on the outside and the hydrophobic nitrogenous bases are on the inside.

6. The molecule is ladder-like with the sugar-phosphate backbones serving as the uprights and the pairs of nitrogenous bases as rungs.

7. The two sugar-phosphate backbones of the helix are *antiparallel*—they run in opposite directions.

8. Nitrogenous bases are specifically paired:
   
   a. Structure dictates which bases can hydrogen bond.
   
   b. Width—DNA has a width of 2 nm.
   
   c. Base pairing rule:

   (b) Hydrogen bonding between base pairs adenine (A) and thymine (T) (top) and guanine (G) and cytosine (C) (bottom). The AT pair has two hydrogen bonds, the GC pair has four.
Purine | Pyrimidine | Base Pair | Hydrogen Bonds
---|---|---|---
Adenine (A) | Thymine (T) | A - T | 2
Guanine | Cytosine | G - C | 3

(i) Explains Chargaff's rules
(ii) Does not limit linear sequence along the length of a DNA strand
(iii) Suggests a general mechanism for DNA replication--bases form specific pairs, therefore the information in one strand compliments the other

IV. DNA Replication and Repair

A. Watson and Crick proposed during replication:
1. The two DNA strands separate
2. Each strand is template for assembling a complementary strand
3. Nucleotides line up singly along the strand in accordance with base pairing (A - T, G - C)
4. Enzymes link the nucleotides together at their sugar-phosphate groups

B. This model is a semiconservation model for replication.
1. When the helix is replicated, each daughter molecule will have one conserved strand (i.e., strand from the original molecule) and one newly created strand

Experimental evidence:
Background:
There are three alternate hypotheses for the pattern of DNA replication:
A. DNA replication is *conservative*—parent molecule remains intact and the second DNA molecule is constructed on entirely new DNA

B. DNA replication is *semiconservative*—each of the two resulting DNA molecules is comprised of one parental molecule and one newly created molecule

C. DNA replication is *dispersive*—each of the two resulting DNA molecules is comprised of a mixture of old and new DNA

**Experiment:**

- Bacteria are grown in $^{15}$N (heavy) medium. All DNA is heavy.
- Some cells are transferred to $^{14}$N (light) medium.
- Some cells continue to grow in $^{14}$N medium.

First generation

Second generation

- DNA is mixed with CsCl solution, placed in an ultracentrifuge, and centrifuged at very high speed for about 48 hours.

The greater concentration of CsCl at the bottom of the tube is due to sedimentation under centrifugal force.

- $^{14}$N (light) DNA
- $^{14}$N–$^{15}$N hybrid DNA
- $^{15}$N (heavy) DNA

DNA molecules move to positions where their density equals that of the CsCl solution.
1. Label DNA strands with $^{15}$N (heavy) by growing *E. coli* for several generations in medium containing $^{15}$N.

2. Transfer *E. coli* to a medium with $^{14}$N (light).

3. Separate using CsCl ultracentrifugation
   a. Centrifugal force creates a concentration gradient in the centrifugation tube (i.e., higher concentration towards the bottom).
   b. During centrifugation, DNA molecules move to a position where their density is equal to that of CsCl.
   c. Results:
      (i) DNA from *E. coli* grown in $^{15}$N was heavier than DNA grown in $^{14}$N.
      (ii) First generation DNA was of all of an intermediate density.
      (iii) Second generation DNA contained intermediate and light density.

Conclusions:
1. First generation DNA was a hybrid containing one parental strand and a newly synthesized light one.
2. This finding eliminated the possibility that replication was conservation but left the possibility of dispersive replication.
3. If replication was dispersive, second generation should contain only intermediate density DNA; since it also contains light DNA, semiconservation replication is favored.
V. Enzymes and proteins involved in replication

A. DNA replication
1. Complex--helical molecule must be untwisted while it copies two antiparallel strands simultaneously
   a. Requires over a dozen enzymes and proteins
2. Rapid--500 bases/sec; a few hours to replicate the 6 billion bases of a single human cell
3. Accuracy--only one in a billion bases is mis-paired

B. Overview
1. Initiation of replication
2. Elongating the new strand
3. Continuous and discontinuous synthesis of anti-parallel strands
4. Priming DNA synthesis
5. Separation of parental strands
6. Proofreading
7. Replication of terminal regions
C. Initiation of replication

![DNA polymerase diagram]

1. Terminology:
   a. Origin of replication
      i. Replication begins at particular sites that have a specific sequence of nucleotides
      ii. Mediated by specific proteins that bind to each origin
      iii. Bacterial or viral DNA have only a single replication origin; eukaryotes may have hundreds or thousands
   b. Replication fork
      i. Opening formed in the DNA double helix at the origin
      ii. Y-shaped site where new strands are growing
   c. Replication bubble
      i. Opening spreads away from the central initiation point (two forks, each going away from the origin)

D. Elongation

1. Synthesis of a new DNA strands is catalyzed by enzymes called DNA polymerases.
2. Events:
   a. According to base-pairing rules, new nucleotides align themselves along the templates of the parent DNA strands
   b. Nucleotides are added to the 3′ end of the growing strand
   c. DNA polymerase links the nucleotides in the 5′→3′ direction
   d. Energy for this reaction is provided by the hydrolysis of nucleoside triphosphates (i.e., nucleotide with a triphosphate covalently linked to the 5′ carbon of the pentose)
   e. Nucleoside triphosphates are the building blocks for DNA
   f. Hydrolysis of nucleoside triphosphate:
      i. Pyrophosphate (i.e., two phosphates) is lost when the nucleoside is covalently bonded to the growing chain
   g. Energy released (i.e., exergonic) drives the synthesis (i.e., endergonic) of DNA (energy is required to form the new covalent bond)

E. Continuous and discontinuous synthesis

1. Continuous synthesis at the replication fork is not possible.
2. The two strands are anti-parallel--the two complementary strands run in opposite directions
3. The strands have polarity--at the 3′ end, a OH is attached to the 3′ carbon of the terminal deoxyribose; at the 5′ end, a phosphate is attached to the 5′ carbon of the terminal deoxyribose
4. DNA polymerase can only elongate from the 5′ end to the 3′ end
5. To overcome these limitations:
   a. Synthesis of the leading strand is continuous
   b. Synthesis of the lagging strand is discontinuous
F. Terms
1. Leading strand
   a. DNA strand which is synthesized as a single polymer in the 5’→3’ direction towards the replication fork
2. Lagging strand
   a. Strand that is discontinuously synthesized against the overall direction of replication
3. Okazaki fragments
   a. Short 5’→3’ segments produced during replication of the lagging strand
   b. Fragments are 100-200 bases long in eukaryotes and up to 2000 bases in prokaryotes

G. Replication of the lagging strand
1. Okazaki fragments are produced by DNA polymerase
2. Intervening spaces are ligated together by covalently bonding the 3’ end of each Okazaki fragment to the 5’ end of the growing chain
   a. Enzymatic reaction catalyzed by DNA ligase
VI. Priming

A. Replication requires that the parental DNA must be primed.
   1. Terms
      a. **Primer**—short RNA sequence that is complementary to a specific sequence of DNA necessary to initiate DNA replication
         i. About 10 nucleotides in eukaryotes
      b. **Primase**—enzyme that polymerizes the short RNA primer sequence

B. Sequence of events:
   1. Primer is polymerized from nucleotides by primase
      a. Priming occurs at a specific site on the DNA
      b. Priming precedes replication
      c. Establishes correct base pairing for addition of nucleotides by DNA polymerase
      d. One primer is required for the leading strand
      e. Many primers are required for the lagging strand
         i. Each Okasaki fragment needs a primer
         ii. DNA polymerase removes the primer on the lagging strand and replaces it with DNA
         iii. DNA ligase catalyzes the linkage between the 3’ end of the Okasaki fragment and the 5’ end of the growing chain

VII. Parental strand separation

A. Replication requires that the helical double helix be separated
   1. Two types of proteins are required:
      a. **Helicases**—enzymes that unwind the parental double helix to expose the template
      b. **Single-strand binding proteins**—proteins which keep the separated DNA single strand apart and stable until the complementary strand is synthesized

VIII. DNA proofreading

A. Accuracy of DNA replication is not solely dependent on the precision base-pairing.
B. Mechanisms for repair:
   1. **Mismatch repair**—corrects errors when DNA is synthesized
      a. DNA polymerase proofreads each new nucleotide against its template
      b. If an error is detected, DNA polymerase removes the incorrectly paired nucleotide and replaces it before preceding
   2. **Excision repair**—corrects accidental changes that occurs in existing DNA
During an organism’s lifetime, existing DNA may be damaged by chemicals, UV radiation, radioactivity, etc.

a. There are more than 50 different types of repair enzymes

b. In excision repair:
   (i) Damage is removed by one repair enzyme
   (ii) DNA polymerase and DNA ligase fill in the excised segment