C. DNA Replication

Conservative vs. Semi-Conservative Method

- Meselson and Stahl supported Watson and Crick's discovery in 1958 by developing the concept of **semiconservative** DNA replication
- Figure 1, p. 217 illustrates both the **conservative** method and the semiconservative method
- in the conservative method, one daughter duplex consists of two newly synthesized chains, and the other consists of the original parent duplex – thus all the DNA is conserved
- in the semiconservative method, each daughter duplex receives one strand from the parent molecule plus one newly synthesized strand – thus ½ of the DNA is conserved
- to determine which kind of replication occurred, Meselsen and Stahl grew two types of *E. coli* cultures – one type in a medium of ^{15}N (heavy nitrogen) isotope and another type in a medium of ^{14}N (normal nitrogen)
- once the bacteria was able to replicate, its DNA was removed using density gradient centrifugation
- heavy (^{15}N) DNA would accumulate at the bottom (see Figure 2a, p. 218) of the tube, and light (N^{14}) DNA would accumulate on top
- when bacteria with heavy (labeled) DNA was cultured in an ^{14}N medium, the newly synthesized DNA, after one generation, possessed an intermediate density – which is consistent with the semiconservative method of DNA replication (see Figure 2b, p. 218)
- subsequent generations of DNA replication resulted in both intermediate DNA and light DNA bands (see Figure 2c, p. 218), which further supports the semiconservative method

The Process of DNA Replication

- Figure 3, p. 219 shows the **replication fork** of the double-stranded DNA that is unwound and separated by the enzyme **helicase**
- helicase uses ATP energy to break the H-bonds beginning at the **origins of replication** running along the entire DNA molecule
- the **gyrase** or **topoisomerase** enzyme helps prevent the unwound duplex from tangling, twisting, and knotting in certain "stress" regions, and it also prevents the separated complimentary base pairs of the parent duplex from re-annealing by making transient breaks to relieve the stress – they later rejoin these template breaks after the daughter strands are replicated
- once the H-bonds are broken between complimentary base pairs in the parent DNA molecule, the bases are exposed to the nucleoplasm, which makes the molecule susceptible to degradation
- as well, the exposed bases have a natural propensity to anneal with their complimentary base
- to prevent degradation and base pair re-annealment, **single-stranded DNA binding proteins** or **SSBs**, bind to the exposed bases in the replication bubbles – they sort of prevent the two template parent DNA strands from re-establishing H-bonds

- there is strong evidence to suggest that many of the essential proteins and enzymes that contribute to the efficient coordination and replication of DNA, are associated together in a large complex called a **replisome**, which consists of helicase, gyrase, SSBs, DNA polymerases, and other functional molecules
- DNA replication proceeds in the direction of the replication fork on one strand called the **leading strand**, and away from the fork on the other strand, referred to as the **lagging strand**
- when two replication forks are relatively near each other a **replication bubble** forms (see Figure 4, p. 220)
- the replication bubbles originate at the origins of replication, and once the daughter strands are created within the bubble, the DNA rewinds back into the helical form, and new bubbles are made in non-replicated regions
- this action continues until the two semiconservative daughter DNA molecules are made
- in prokaryotes, **DNA polymerase I, II, and III** are the three enzymes known to function in replication and repair
- in eukaryotes, there are five different types of DNA polymerases that are at work
- the enzyme responsible for "stringing" nucleotides together to make the newly synthesized DNA for prokaryotes is called **DNA polymerase III** – it catalyzes the formation of S-P bonds between adjacent **deoxyribonucleoside triphosphates**
- to view how deoxyribonucleosied triphosphates are added onto one another to create a deoxyribonucleic acid chain, click on http://bioweb.uwlax.edu/GenWeb/Molecular/Theory/DNA_sequencing/dnaseq.mov
- DNA polymerase III functions only under certain conditions
- DNA polymerase III will work if the following are present: a pre-existing DNA **template** – the master copy , all four types of nucleotides, and a short region of an RNA duplex called a **primer strand** – 10 to 60 base pairs long
- the primer strand helps the enzyme DNA polymerase III start a new chain of connected nucleotides – this is because DNA polymerase III can only start adding nucleotides onto a free 3' – OH end of one existing primer strand, while it uses the other as a template
- since the start of the template strand begins at 3', then its other end must be 5', thus the direction of the new chain can be in the 5'-3' direction only
- Figure 5, p. 220 illustrates how DNA polymerase III adds complimentary nucleotides in the 5' to 3' direction using RNA primers as starting points
- once the DNA polymerase III is in place, the elongation of the daughter strand occurs where each deoxyribonucleoside triphosphate is added to the previous one
- free bases in the nucleoplasm are used to generate the deoxyribonucleoside triphosphates that will be linked together to form the complimentary strand by DNA polymerase III (see Figure 6, p. 221)
- as the two tail end phosphates break off the deoxyribonucleoside triphosphate, it releases free energy, which in turn, is used to create the phosphodiester bond between two adjacent nucleotides
- the energy needed for DNA synthesis comes from the breaking off of two tail end phosphates by the work of nucleoside triphosphosphase enzymes
- to view an animation of how the replication fork works, click on http://bioweb.uwlax.edu/GenWeb/Molecular/Theory/Replication/replicat.mov
- the leading strand is built toward the replication fork and is strung together in a continuous manner
- the lagging strand is synthesized discontinuously in short fragments in the opposite direction (away from the fork)
- this means the lagging strand consists of periodic primers and DNA polymerase III-built fragments called **Okasaki fragments** – named after the person who discovered them
- the lagging strand fragments are about 100 to 200 nucleotides long in eukaryotes and 1000 to 2000 nucleotides long in prokaryotes
- Figure 7, p. 221 illustrates the processes that take place in the lagging strand

- **DNA polymerase I** then removes the RNA primers form the leading strand and from the Okasaki fragments, and replaces them with the appropriate deoxyribonucleotides
- the enzyme **DNA ligase** then joins the Okasaki fragments into one strand by creating phosphodiester bonds between each fragment
- once the newly synthesized daughter strand is created, it automatically twists into a helix together with the original parent strand, forming the new duplex DNA molecule
- Figure 8, p. 222 effectively summarizes the events and species involved in the replication of DNA
- once DNA is synthesized, DNA polymerase III and DNA polymerase I act as quality control checkers by proofreading the newly synthesized daughter strand – either of the two enzymes function as an **exonucleases** – these are enzymes that remove incorrectly-placed nucleotide sequences and replace them with the correct ones
- if this is not done immediately, a mistake can be subsequently copied or a mistake may be transcribed immediately resulting in abnormal protein synthesis
- to view a DNA replication animation click on http://www.johnkyrk.com/DNAreplication.html, and for a complete interactive tutorial of DNA replication click on http://www.nobel.se/medicine/educational/dna/intro.html

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