LECTURE 21

DNA Replication

A. Watson & Crick (1952)

1. Proposed a model where hydrogen bonds break, the two strands separate, and DNA synthesis occurs semi-conservatively in the same net direction. While a straightforward and simple model, subsequent studies revealed that the polarity requirements of DNA polymerizing enzymes would not permit replication to occur in the same direction on both strands.

B. Meselsohn-Stahl (1958)

1. Demonstrated in a classic CsCl density-gradient experiment involving light (N^{14}) and heavy (N^{15}) nitrogen that DNA replication in *E. coli* was semi-conservative.

   (a) Grew cells for several generations on N^{15} so that both DNA strands would be N^{15}/N^{15}.
   (b) Transferred the cells to N^{14} medium. This is Generation 0.
   (c) Extracted DNA in every generation and centrifuge in CsCl density gradient.
   (d) The results were consistent with semi-conservative, not conservative replication.
   (e) Semi-conservative replication in eukaryotes was demonstrated some time later.

C. Cairns (1963) autoradiographic experiment [OVERHEAD]

1) Demonstrated that replication in *E. coli* was semi-conservative from a single origin of replication, and that DNA synthesis occurred in the same *net* direction. This again raises the "polarity" problem.

2) Note: Cairns was correct that replication was initiated from a single *origin*, but incorrect initially in that he proposed that replication proceeded unidirectionally. DNA replication in *E. coli* (and elsewhere as well) is *bidirectional* and proceeds is either direction (on both strands) from the origin of replication. This, too, raises the "polarity" problem.

3) The origin of replication in *E. coli* is called **OriC** and is well characterized molecularly.

   a) OriC is 245 bp in length, containing two different repeat sequences.

   b) One repeat (13 bp in length) is repeated three times in tandem. It is enriched in AT base pairs and serves as region where double strands separate, facilitating a structure called the *replication bubble*.

   c) The other repeat (9 bp in length) is interspersed (not tandem) in OriC and serves as binding sites for a protein involved in formation of the replication bubble.

D. DNA polymerases [DNA dependent DNA polymerases]

1) A DNA polymerizing enzyme was first discovered in *E. coli* by Arthur Kornberg in1957. The enzyme historically has been called the "Kornberg enzyme" but also goes by the name DNAP-I.

   a) Kornberg demonstrated that the enzyme could synthesize DNA *in vitro* (i.e., in a test tube). All that was needed were the four deoxyribonucleotide triphosphates (ATP, GTP, CTP, TTP), magnesium (Mg^{++}), the Kornberg enzyme, and a pre-existing DNA that provided both a priming site and a template.

   b) The priming site was a free OH (hydroxyl) group at the 3' end to which nucleotides were added. DNAP-I (in fact all polymerases) cannot initiate synthesis of DNA from "nothing" and require a pre-existing 3'-OH group. Enzymatically, DNAP-I catalyzes the formation of the phosphodiester link between the 3'-OH of the primer and the 5'phosphate of the incoming nucleotide tri-phosphate. This requirement for a pre-existing 3'-OH group is the "polarity" problem and the reason why DNA polymerases "read" a 3'→5' template and synthesize "new" DNA in a 5'→3' direction.

   c) The template "function" of the pre-existing DNA provides the nucleotide sequence for the addition of complementary bases (A w/T, G w/C, etc.).

2) DNAP-I is a multifunctional enzyme that has <u>both</u> 5'→3' and 3'→5' exonuclease activity as well as polymerizing activity, i.e., can degrade DNA as well as synthesize (polymerize) DNA. DNAP-I, however, is *not* the replicator in *E. coli*.

   a) Cairns' isolated a mutant (termed the *pol*A mutant) in *E. coli* that had no Kornberg enzyme (DNAP-I) but replicated just fine, albeit a bit more slowly.

   b) *pol*A mutants were ultrasensitive to UV light, suggesting that DNAP-I might be involved in DNA repair. As it turned out, DNAP-I is involved in <u>both</u> DNA repair and DNA replication. Its role in DNA replication presumably is filled by other DNAPs in the *pol*A mutant.

3) DNA polymerases in *E. coli* [at least three]

DNAP-I	The Kornberg enzyme; DNA repair and DNA replication
DNAP-II	DNA repair
DNAP-III	DNA synthesis (replication)

   Note: DNAP-II & DNAP-III have 5'→3' polymerase activity and 3'→5' exonuclease activity, but <u>not</u> 5'→3' exonuclease activity. The last is limited to DNAP-I.

4) DNA polymerases in eukaryotes [at least five]

DNAPα (I)	replication of nuclear DNA	[discontinuous strand]
DNAPβ	DNA repair	
DNAPγ	replication of mitochondrial DNA	
DNAPδ (III)	replication of nuclear DNA	[continuous strand]
DNAPε (II)	DNA repair	

a) All five polymerize by adding nucleotides to a 3'-OH group, i.e., the DNA polymerases "read" a 3'→5' template and synthesize "new" DNA in a 5'→3' direction.

b) Only DNAPγ, DNAPδ, and DNAPε have 3'→5' exonuclease activity. DNAPα and DNAPβ do not have nuclease activity at all.

c) None of the eukaryotic DNAPs have 5'→3' exonuclease activity.

5) 3'→5' exonuclease activities of DNA polymerases are involved in "proofreading" the growing DNA chain. When an improper base is added at the 3' end, the exonuclease activity of the enzyme "clips" off the unpaired base, and the 5'→3' polymerase activity fills in the correct base.

a) All known DNA polymerases except DNAPα and DNAPβ (in eukaryotes) have this proofreading capability.

E. Semi-Discontinuous Model of DNA Synthesis (in *E. coli*)

1. In *E. coli*, DNA replication begins at *Ori*C with the formation of a localized region of strand separation – the replication bubble – formed by the interaction of "prepriming proteins" with *Ori*C.

a) These prepriming proteins are DnaA (largely responsible for the "bubble"), DnaB (DNA helicase), DnaC, DNA gyrase, and SSB (single-strand binding) proteins. The overall net result is formation of the "replication fork."

b) DNA helicases catalyze the "unwinding" of the DNA helix (at approximately 3,000 rpm). Once the DNA is unwound, it must remain in an extended single-stranded form. A coating of SSBs maintains the single-stranded state and prevents "snap-back" into a double-stranded molecule.

c) DNA topoisomerases (DNA gyrase) promote and maintain the DNA in a negative supercoil.

2. DNA synthesis along one strand is continuous and requires only a single RNA primer. This strand is called the "leading" strand. DNA synthesis on the other strand (the "lagging" strand) is discontinuous (and "backward") and requires numerous DNA primers. In eukaryotes, it is thought that DNA polymerization on the continuously synthesized (leading) strand is catalyzed by DNAPδ, whereas DNA polymerization on the discontinuously synthesized (lagging) strand is catalyzed by DNAPα.

   a) DNA primase (*dna*G gene) makes an RNA primer (RNA:DNA heteroduplex) at the origin (*Ori*C) for the continuously synthesized (leading) strand. The RNA primer(s) provide the necessary 3'-OH group required for covalent extension by DNAP enzymes. Synthesis along this strand is more-or-less continuous from the origin and proceeds 5'→3' (reading a 3'→5' template). RNA primers in prokaryotes are up to 50-60bp in length; whereas RNA primers in eukaryotes are only 10 or so bp in length.

   b) For the discontinuously synthesized (lagging) strand, DNA synthesis is in short bursts "backward" but in the same net direction. Here, there are several RNA primers (RNA:DNA duplexes made by DNA primase), and synthesis at each is primed by a 3'-OH group. The small fragments produced are called "Okazaki" fragments, and DNAP-III terminates an Okzaki fragment when during synthesis it "runs into" the RNA primer of the preceding Okazaki fragment.

3. The DNA primers (RNA bases) are removed by the 5'→3' exonuclease activity of DNAP-I and the "gap" is filled with appropriate DNA bases by the 5'→3' polymerase activity of DNAP-I. DNAP-I uses the 3'-OH group provided by the terminal base of the Okazaki fragment.

4. Okazaki fragments are linked together by DNA (polynucleotide) ligase.

F. Replication in eukaryotes

1. Essentially the same but with differences noted above. There are several, independent origins of replication in eukaryotes. Each replicating unit is known as a **replicon**.