Lecture #6
The Cell as a Machine

DNA Replication

Adapted from Alberts et al., IVth Edition, Chapter 5

Summary: When the right point is reached in the cell cycle, the cell will initiate the process of DNA replication (S phase or DNA synthesis phase). Commitment to synthesis is critical in that it will disrupt RNA synthesis and processing and the cell should have sufficient metabolic reserve to complete the cell cycle.
Problems: 1. The basic goal is to make 1 and only 1 copy of each chromosome with high fidelity (on average 1 error in 10^9 base pairs).
2. Unwind before and rewind during synthesis
3. Synthesize both strands simultaneously
4. Because of time constraints, synthesis must be going at multiple regions simultaneously.

1. Direction of Polymerization (5' to 3')
2. Need for Okazaki fragments (1000-2000 bp in bacteria and 100-200 in eucaryotes)
3. Initiation site for polymerization (rate for bacterial polym 500/s but eukaryotic 50/s which would mean for an average human chromosome of 150 million bp 3 million sec. or 35 days. Clusters are activated at the same time.
4. Helicase is critical for rep. and SV40 T antigen is specific for viral DNA
5. Other elements of complex for Rep.
6. nucleosome, etc
7. telomerase
8. Proof-reading (DNA repair)

How is DNA Replicated?

From the viewpoint of the engineer, the copying of DNA in the confines of the nucleus is a major task. Basically, the job is to produce one complete copy of the complete DNA sequence with high fidelity. The task is broken into two tasks, replication and proofreading. The task of replication can be broken into several different steps; activation of replication, origination of replication, replication of the forward strand, replication of the rearward strand (which has several steps itself), and assembly of chromatin. In turn, each step involve s multiple protein complexes that must communicate for the whole operation to be completed in an orderly fashion during the allotted period of about 2 hours.

The cell moves through the first growth phase (G1) after mitosis growing in size until a signal is developed that tells the cell that it can devote resources to making another copy of DNA. How that decision is made by the cell is not completely understood but the synthesis of cyclins is an important factor. Cyclins are proteolyzed at mitosis and levels gradually rise until they reach a critical point that catalyzes the phosphorylation of components that activate the pre-initiation complex, which is already bound to the

initiation sites. Fusion studies provide important insights into the activation process. When an S phase cell is fused to a cell in G1, the nucleus of the G1 cell begins DNA synthesis. Thus, the pre-initiation complex that is bound to the DNA in G1 is competent to polymerize and only needs the active kinases from the S cell to become active.

An origin recognition complex (ORC) is initially formed; and after binding of Cdc6, there is the assembly of Mcm (individual subunits of the AAA ATPase, helicase) to form the pre-replicative complex. S-Cdks trigger S phase by phosphorylating Cdc6, which then releases and is degraded. In the mean time, all free Cdc6 and Mcm has been degraded as a result of phosphorylation and ubiquitylation. This prevents the initiation of a second round of replication and assures that more than one copy of DNA is not made.

The actual DNA polymerase activity is accomplished by two different polymerase complexes, because polymerization only occurs from 5' to 3' direction. A logical reason for only 5' to 3' polymerization is that the triphosphate moiety of the last base is cleaved, whereas in 3' to 5' polymerization, the triphosphate moiety is still present on the last base. If the last base is improperly base paired, then it is often removed by enzymatic cleavage (endonuclease). In the case of 3' ends (normal direction of polymerization), a new nucleotide can be added because the energy from triphosphate cleavage will drive the reaction. However, cleavage of a 5' end removes the nucleotide triphosphate, leaving a monophosphate on the end, which cannot provide the energy to add the next nucleotide.

Molecular Nature of DNA Polymerases

Current studies of the DNA polymerases are focusing on the processes at the level of single molecules. A controversy now concerns the interpretation of the kinetics of the polymerase under high force loads (Goel et al., 2002; Maier et al., 2000). These molecular details are critical to understand the process of polymerization in vivo.

Template problems

There are a variety of situations where DNA damage results in the blockade of the normal polymerase complex. In those cases, a second DNA polymerase is brought in and used to bridge the gap in normal bases (Goodman, 2000).

How are the Ends of the Chromosomes modified by Telomerases?

It has been demonstrated that a telomeric G-rich single-strand overhang, presumed to be a necessary substrate for telomerase action, is either generated or extended during late
S phase in *S. cerevisiae*, suggesting that telomerase addition occurs at or near the time when the conventional replication machinery copies the chromosome ends (Chan and Blackburn, 2002; Shore, 2001). Using a site-specific recombination system to rapidly shorten a specific
TG_{1-3} tract, Marcand *et al.* [8••] have provided the first direct evidence that telomerase elongation and normal DNA replication are in fact coincident. This conclusion is consistent with results of Diede and Gottschling [9••], who showed that telomerase addition at an HO endonuclease generated double-strand break (DSB) occurs only if cells are allowed to pass through S phase. Cells held in G_1, though they contain an active telomerase enzyme, are unable to extend the break. The temporal coincidence of

telomeric DNA replication and telomerase action suggests that these two processes might be mechanistically coupled. In strong support for this idea, telomerase addition at a DSB requires S phase

Questions:

1. To complete the replication of DNA in the normal S period of the cell cycle requires that multiple sites of DNA replication be initiated. For our average chromosome of 150 million bps, how many polymerases are needed to complete the replication in 8 hours with polymerases that move at 50 bp/s?

2. Estimates of the number of genes in the human genome are in the range of 50,000. If we assume that the coding region of each gene is 3,000 bp (corresponding to a molecular weight of 120,000 for an average protein—this is a little high), then how many replication events (cell divisions) are needed before a genetic mutation is generated on average in every gene (remember that there are 6,000,000,000 bp in the human genome) for the normal error frequency of 1 bp in 1,000,000,000 bp? Assume that the average volume of your cells is 3,000 microns cubed, then a 75 kg person will have approximately 2.5 $x10^{13}$ cells that arose by binary division. How many mutant genes will the person have in at least one cell?

Reference:

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