Lecture 08 – DNA Replication and Repair

Plasmids may carry genes that convey antibiotic resistance to the host bacterium, and may facilitate the transfer of genetic information from one bacterium to another.

1. Know the components and understand the steps in prokaryotic DNA synthesis including (i) initiation, (ii) elongation, and (iii) supercoiling requirements.

- **Initiation**: To initiate replication, you need some mechanism to unwind then double helix. You have to pull apart for them to some as templates. Helicases are enzymes that unwind or melt double DNA into single strand components and it requires energy.
  - ***origin of replication (OriC):*** a single 245-bp region that contains a tandem array of 13-mer "AT"-rich regions followed by 5 specific binding sites for DnaA protein. (the AT its AT because the H bonds is not so stable only 2 H bonds so its easier to pull apart)
  - ***origin recognition protein (DnaA):*** On bindingthe 5 recognition sequences, it forms a hexameric coiled DNA complex, which is required to recruitthe DNA helicase (DnaB) and helicase loader (DnaC).
  - ***helicase loader (DnaC):*** On binding DnaA, it recruits and loads DnaB onto the AT-rich regions.
  - ***DNA helicase (DnaB):*** On binding AT-rich regions, it forms a hexameric complex that begins unwinding dsDNA for trapping by SSB.
  - ***ssDNA binding proteins (SSB):*** bind to ssDNA regions to prevent re-annealing.

- **Elongation**: You cannot start new strand synthesis with deoxy-nucleotides but you can with ribo-nucleotides. The DNA polymerase is moving forward but it always checks to make sure that Base pairs a proper to the template strand before it moves on. If it doesn't Base pair correctly then you get a ball or bump and polymerase will back up and it uses water to hydrolyse out the mispair base pair.
  - ***Replication fork:*** the junction between dsDNA and strand separated ssDNA.
  - ***primase (DnaG):*** On binding, it converts the "*prepriming complex*" to a "*primosome*". Here it is a specific RNA polymerase that synthesizes short stretches of "*RNA primers*" (~10 nt) that are complementary and antiparallel to DNA template.
  - ***DNA helicases (DnaB):*** bind to replication fork and *continue* unwinding the DNA double helix.
  - ***ssDNA binding proteins (SSB):*** bind to the ssDNA regions to prevent re-annealing.
  - ***DNA polymerases:*** read DNA sequence of the parent or *template* strand in the 3'→5' direction and catalyze elongation of newly synthesized complementary ssDNA in the 5'→3' direction. *Note: Can only initiate at 3'-end of RNA primer of the RNA-DNA duplex.*6. ***leading strand:*** synthesized 5'→3' *continuously* in the direction of the advancing replication fork.
  - ***lagging strand:*** synthesized 5'→3' *discontinuously* in the direction away from the advancing fork.

- ***DNA polymerase III:*** polymerase used for synthesizing both the leading and lagging DNA strands.*5′→3′ polymerase*, strand elongation*3′→5′ exonuclease*, proofreading and excision of mispaired bases
- ***DNA polymerase I:*** polymerase used for excision of RNA primer and insertion of DNA.DNA strands.*5′→3′ exonuclease*, RNA primer excision, *5′→3′ polymerase*, gap filling with DNA,*3′→5′ exonuclease*, proofreading during gap filling. (The DNA is being removed one nucleotide at a time and the same time it is replacing the ribo-nucleotide with the deoxy-ribo-nucleotide)
- ***DNA ligase:*** catalyzes 3′→5′ phosphodiester bond formation between the original DNA strand (pol III) and gap filled DNA strand (pol I) during lagging strand synthesis.

- **Supercoiling:** Unwinds the DNA to permit the replication to keep moving when you finish you end up with 2 circular plasmids that one linked so you have to cut the strand one last time and decatenation separates them.
  - ***superhelical tension:*** Positive supercoils are introduced as a consequence of helicases unwinding dsDNA during progression of the replication fork.
  - ***DNA gyrase***: introduces negative supercoils (or relaxes positive supercoils) into DNA. This activity is required for two processes:(i) relieve superhelical tension during replication (ii) separate chromosomes post replication
  - ***decatenation:*** When replication is complete, the two dsDNA chromosomes are interlocked. Chromosomal strands are separated by the DNA gyrase enzyme, due to its ability to catalyze passage of dsDNA through ds breaks.
  - ***topoisomerase II enzymes***: DNA gyrase is classified as a "*topoisomerase type II*" enzyme. Topo II mechanism:(i) dsDNA is looped. (ii) At the crossover point, a double stranded breakis introduced at one region of the double helix.(iii) The double helix is passed through the breakat the crossover before the double break is resealed. (Anticancer agents, such as *etoposide*, target human topoisomerase II. Bacterial DNA gyrase is a unique target of a group of antimicrobial agents called quinolones, for example, *ciprofloxacin*.)

2. Know the components and understand the steps in eukaryotic DNA synthesis including

Initiation

- **Origin of Replication**
  - ***origin of replication:*** In contrast to prokaryotic circular chromosomes that have one distinct origin, eukarytotic linear chromosomes have large numbers of origins, with much less well defined AT-rich regions.
  - ***origin of replication complex (ORC):*** six different proteins, analogous to DnaA, that likely form a similar hexameric coiled DNA complex, which is required to recruit the helicase (Mcm2-7) and its associated loaders (Cdc6/Cdt1).
- **DNA Strand Separation**
  - ***helicase loader (Cdc6/Cdt1):*** On binding ORC, it recruits and loads helicase onto the AT-rich regions.

- ***DNA helicase (Mcm2-7):*** On binding AT-rich regions, it forms a hexameric complex that begins unwinding dsDNA for trapping by **SSB.** (Instead of HOMOHEXAMERS like in prokaryotes, we have here HETEROHEXOMERS)
- ***replication protein A (SSB):*** bind to ssDNA regions to prevent re-annealing.

Elongation

- ***primase (pol α):*** This subunit of pol converts the "*prepriming complex*" to a "*primosome*". Here it isa specific RNA polymerase that synthesizes short stretches of "*RNA primers*" (~10 nt) that are complementary and antiparallel to DNA template.
- ***DNA helicases (Mcm2-7):*** bind to replication fork and *continue* unwinding the DNA double helix.
- ***ssDNA binding proteins (repA):*** bind to the ssDNA regions to prevent re-annealing.
- ***DNA polymerase (pol α):*** This subunit of pol initiates elongation of complementary ssDNA from the RNA primer, extending it only ~20 nt in the 5' → 3' direction before dissociating. *Note: the primase and polymerase subunits of pol α generate a hybrid RNA-DNA primer.*
- ***DNA polymerase (pol ε):*** replaces pol α and synthesizes leading strand 5' → 3' *continuously.*
- ***DNA polymerase (pol δ):*** replaces pol α and synthesizes lagging strands 5' → 3' *discontinuously.*
- ***flap endonuclease 1 (FEN1): ribonuclease H (RNase H):*** ribonucleases used for excision of RNA primer.
- ***DNA polymerases and :*** also used to fill in gaps left after RNA excision by FEN/RNaseH *5' → 3' polymerase*, strand elongation*3' → 5' exonuclease*, proofreading and excision of mispaired bases.
- ***DNA ligase:*** catalyzes 3' → 5' phosphodiester bond formation between the original DNA strand and gap filled DNA strand.

3. Know the characteristics of telomeres

Telomeres: Telomeres may be viewed as mitotic clocks in that their length in most cells is inversely related to the number of times the cells have divided. The study of telomeres is providing insight into the biology of aging and cancer.

- They are several thousand tandem repeats at the ends of linear eukaryotic chromosomes. The 5'-AGGGTT-3' repeat in the 3'-overhang of human DNA extends several hundred nt.
- ***duplex loop:*** The 3'-overhang is thought to fold back upon itself to form a loop that is stabilizedby *telomere binding proteins* (TBP's). Such a structure is thought to protect the ends of chromosomes from degradation by exonucleases.
- ***"end-replication" problem:*** Following excision of RNA primer from extreme 5'-end of lagging strand, there is no way to fill in remaining gap with DNA.
- ***"mitotic clock":*** In normal human somatic cells (T cells, red blood cells, muscle cells, pancreas cells and so on), linear chromosomes shorten with each cell division. Once shortened to critical length, cells cannot divide and enter *senescence* (means that it can no