BICH/GENE 431 KNOWLEDGE OBJECTIVES

Chapter 8 – DNA Replication

origin of replication
replicons
unidirectional replication vs. bidirectional replication
leading strand vs. lagging strand / continuous vs. discontinuous replication
Overall, semidiscontinuous replication
Okazaki fragments and RNA primers
dNTPs; α, β, γ phosphates; incorporation of α phosphate into DNA chain
chemical reaction during DNA synthesis – pyrophosphate released and then hydrolyzed to inorganic phosphates by pyrophosphatase
incorporation assay to monitor DNA synthesis

- either α-32P-dNTP or fluorescently-labeled T base in TTP as substrate
- separate synthesized DNA (bigger) from unincorporated dNTPs (smaller) using filter binding or gel electrophoresis
- measure radioactivity or fluorescence incorporated into DNA

3D structures of several DNA polymerases known – model structural features after right hand (palm, fingers, thumb)
processivity of DNA polymerases
fidelity of DNA polymerases

- approximate error rates in the cell, and by enzyme with/without proofreading
- kinetic control of fidelity
- proofreading activity: 3' to 5' exonuclease

many different DNA polymerase enzymes in E. coli and humans
Two major DNA pols in E. coli + 3 others

- DNA pol I: discovered first by Arthur Kornberg, most abundant, single subunit, not very processive; 3 activities: 5' to 3' polymerase, 3' to 5' exonuclease (proofreading), 5' to 3' exonuclease for endfilling after removal of Okazaki fragments
- DNA pol III: major replicative enzyme, highly processive, multiple subunits to be discussed later

Three major DNA pols in eukaryotes + many others

- Polα used for primer synthesis
- Polδ used for lagging strand synthesis
- Polε used for leading strand synthesis

Many other proteins, enzymes at the replication fork in addition to DNA polymerase
Primase catalyzes addition of RNA primers
DNA helicases unwind DNA
Single-stranded binding proteins (SSBs) keep DNA single-stranded after unwinding
Topoisomerases relax supercoils that are introduced into DNA because of unwinding
Know names of these proteins, enzymes in E. coli, humans (Table 8-1)
Process used to remove RNA primer and seal phosphodiester backbone after completing new synthesis in E. coli:

- RNase H removes most of RNA; 5' to 3' exonuclease activity of DNA pol I removes rest of RNA primer; then DNA pol I fills in the gap with new DNA; DNA ligase (+ATP) seals the nick

DNA polymerase switching in eukaryotes

- DNA polα is associated with primase: synthesizes RNA, then a little DNA to make primer (50-100 nt long)
- DNA polδ replaces polα on lagging strand; DNA polε replaces on leading strand

sliding clamp – multisubunit protein associated with DNA polymerase

- encloses DNA like a donut
- E. coli: β subunit of DNA pol III holoenzyme
- eukaryotes: PCNA (proliferating cell nuclear antigen)
- open up to come on/off DNA

sliding clamp loader catalyzes assembly or removal of sliding clamp from DNA

- requires ATP binding and hydrolysis in this process
- a member of the AAA+ protein family (ATPases Associated with various Activities)
- in E. coli, it is the γ complex that is part of DNA pol III holoenzyme

replisome complex coordinates leading and lagging strand synthesis

understand trombone model of replisome action in E. coli and sequence of events (Fig. 8-22)

understand distinction between replicator vs origin of replication

general structures of replicators: initiator protein binding site, easily unwound sequences (A/T-rich)

initiator proteins: DnaA in E. coli, ORC (origin recognition complex) in eukaryotes; bind specific sequences in replicator and recruit other proteins

know details about initiation of replication initiation in E. coli (Fig. 8-27)

- DnaA (+ATP) binds 9-mer sites and induces unwinding at 13-mer sites
- then recruits DnaB (helicase) that requires action of a loader protein (DnaC)
- bidirectional (two forks), so two molecules of DnaB loaded
- recruits primase (DnaG) to make RNA primers
- then recruit DNA polymerase III holoenzyme and away they go….

know how E. coli replication initiation is regulated by methylation of DNA, SeqA binding, and DnaA binding

pre-RC complex formation in eukaryotes (ORC, Cdc6, Cdt1, MCM helicase)

understand cell cycle control of replication initiation in eukaryotes

- regulation of pre-RC activation by Cdk in S phase
- inhibition of pre-RC formation by Cdk in S, G2 and M phases
- pre-RC assembles in G1 phase
- ORC binding to replicator not regulated

understand end replication problem with linear DNAs

- one solution is protein priming (linear bacterial chromosomes; linear viral genomes)
- usual solution in eukaryotes is to extend telomeric DNA with telomerase followed by normal lagging strand replication

telomerase enzyme – TER RNA subunit + TERT (reverse transcriptase) + other proteins
telomerase discovered and characterized by Elizabeth Blackburn, Jack Szostak, Carol Greider – Nobel Prize in 2009
many telomere binding proteins help to regulate telomerase and length of telomeric DNA
Hayflick limit for cell division
telomerase inactivity in somatic cells vs. activity in cancer cells and stem cells
telomerase is a potential target for anticancer treatment