Molecular Cell Biology A

“DNA Replication and Repair”

BIOX24ZL
Tuesdays 9-10:30
Ray LC

DNA replication is semiconservative: an elegant experiment.

- Complementary base pair allows replication with either strand by cluster of proteins
- Watson: DNA replication is semiconservative
- Delbruck: breaks and unions, patchwork of old and new nucleotides but no unwinding
- Others: conservative, daughter 2 new strands
- Meselson & Stahl: grow e. coli in N15 and N14 nitrogen media (heavy & light DNA), centrifuge in cesium chloride allows separation, grow N15 e.coli in N14 for one gen, heat up after to separate strands to differentiate with Delbruck
Heat up DNA to find separate bands after 2\textsuperscript{nd} generation: not dispersive.
DNA replication is bidirectional at each replication origin (slow in eukaryotes).

- Initiator proteins bind to replication origins, opening H bonds weak at ordinary temp
- Rep origins has AT (fewer H bonds than GC)
- One origin in bacteria, 10000 in humans
- Two replication forks one in each direction, 10x slower in humans due to chromatin
- DNA Polymerase adds nucleotides at 3’ hydroxyl end of DNA chain to 5’ phosphate of nucleotide using energy of deoxyribonucleoside triphosphate itself releasing pyrophosphate -> inorganic phosphate, irreversible reaction
DNA polymerase can correct its own errors before adding next nucleotide.

- Replication fork is asymmetric 5’->3’, 3’->5’
- So 3’->5’ is made discontinuously and joined as Okazaki fragments (lagging strand)
- GT and AC less stable pairs 1/10^7 error
- DNA polymerase monitors nucleotide addition
- 3’->5’ exonuclease proofreading: if incorrect, clips phosphodiester backbone using nuclease on diff catalytic domain (separate from post repair sys)
- Backstitching on works if polymer 5’->3’ only
Primase make ~10 nucleotide long RNA primers for DNA replication.

- Use U instead of T to make RNA from DNA
- Only one primer on leading, many primers on lagging, lets DNA poly begin new Okazaki frag, primers can have greater mutation rates
- Nuclease degrades RNA primer -> repair polymerase replaces RNA with DNA using Okazaki as primer -> DNA ligase joins DNA frag
- Primase does not proofread but DNA polymerases proofreads as RNA is replaced
Complex of replication machinery cooperate to synthesize DNA.

- DNA helicase uses ATP hydrolysis to pry apart double helix; Single-strand DNA-binding protein prevent base repairing holds template elongated.
- DNA topoisomerase relieves tension of excess twisting in front of replication fork using temp nicks in backbone, and untangle intertwined DNA.
- Sliding clamp forms ring on new helix, keeps DNA polymerase on template w/o falling off.
- Clamp loader locks in clamp using ATP, loads each time a new Okazaki fragment is begun.
Figure 5-12: Molecular Biology of the Cell (Garland Science 2015)
Telomeres are noncoding long repeats at the ends of chromosomes.

- DNA replication only 5’-3’ leaves lagging strand short of completion
- Bacteria solves problem using circular DNA, eukaryotes have repeat sequences at ends
- Telomerase adds copies of DNA repeats to template strand using RNA as template
- Telomeres allow recognition of natural ends of chromosomes distinct from accidental breaks
Figure 5-13: Molecular Biology of the Cell (S. Garland, Science 2015)

Figure 5-14: Molecular Biology of the Cell (S. Garland, Science 2015)
Team work

During DNA replication in the cell, DNA primase makes short primers that are then extended by the replicative DNA polymerases. These primers ...

• A. provide a 3′-phosphate group for the DNA polymerases to extend.
• B. are made up of DNA.
• C. are made more frequently in the leading strand than the lagging strand.
• D. are joined in unmodified form to the neighboring DNA.
• E. generally have a higher number of mutations compared to their neighboring DNA.

The telomerase enzyme in human cells ...

• A. creates the “end-replication” problem.
• B. polymerizes the telomeric DNA sequences without using any template.
• C. removes telomeric DNA from the ends of the chromosomes.
• D. has an RNA component.
• E. extends the telomeres by its RNA polymerase activity.