

1 **Chapter 5**

DNA Replication, Repair, & Recombination

2 Maintenance of DNA Sequences
&
DNA Replication Mechanisms3

- Mutations
 - E. coli, C. elegans, & humans
 - 1 nucleotide change per 10^9 nucleotides per cell generation

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- Germ-line mutations
- Somatic cell mutations
- Central Dogma of Molecular Biology

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- 1000 nucleotides/sec
- DNA templating
 - Template strand
- 1957 DNA polymerase
 - Deoxyribonucleoside triphosphates

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- Semiconservative replication
- Replication fork
 - Multienzyme complex
 - DNA polymerase
 - Only adds in 5' to 3' direction
 - Okazaki fragments
 - Asymmetric
 - Leading strand
 - Lagging strand

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- Mismatching is possible (& common)
- Proofreading mechanisms remove mismatchings
- DNA polymerase
 - Needs preexisting 3' OH group
 - Conformational change checks base pair geometry
 - 5' to 3'
 - Exonuclease region/subunit

- 3' to 5'
 - RNA polymerase
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- 5' triphosphate provides energy
 - If on growing chain would be lost during proofreading
- 10

- Leading strand
 - Needs only 1 primer
 - Lagging strand
 - DNA primase
 - Adds RNA primer
 - Repair system
 - RNA to DNA at primer sites
 - DNA ligase
- 11

- DNA stability
 - DNA helicases
 - Separate DNA strands
 - Single strand DNA binding proteins
 - Cooperatively stabilize single strands
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- DNA polymerase tends to dissociate
 - Sliding clamp
 - Clamp loader complex
 - Primer template junction
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- Multienzyme replication complex
 - DNA helicase
 - 2 DNA polymerases
 - SSB proteins
 - DNA primase
 - Clamp loader
 - Clamps
 - DNA repair & ligase
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- Errors missed by DNA polymerase
- Strand-directed mismatch repair

- Distortions
- prokaryotes
 - Methylation of A's in GATC
- Eukaryotes
 - Single-strand breaks
 - Prior to DNA ligase?
- Cancer & proofreading proteins

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- Temporary covalent bond to phosphate in backbone
- Topoisomerase I
- Topoisomerase II

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- Eukaryotic
 - Conserved components
 - Replication fork
 - Additional components
 - Multi subunit proteins
 - Nucleosomes
 - 100-200bp vs. 1000-2000bp
 - speed

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Initiation and completion of DNA replication in chromosomes

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- Initiator proteins
 - Replication origins
 - A-T

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- Regulation
 - Nutrition
 - methylation
- Initiator proteins
- DNA helicase
 - & helix loader
- Primase
- Remaining proteins
- Dual replication forks

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- Slower than prokaryotes

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- S phase
 - 40min – 8hrs

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- Replication units
 - Spaced at 30,000-250,000bp
- Regional replication in a reproducible order
- microarrays

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- Active X chromosome early S phase
- Inactive X chromosome late S phase

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- Autonomously replicating sequence (ARS)
 - Origins of replication
- Excess origins to ensure chromosomes not lost

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- Minimal origin of replication
 - Binding site for ORC
 - Origin recognition complex
 - Rich A-T region
 - Binding site for proteins
 - to attract ORC
- Control
 - Protein kinases (Cdk)
 - G1- Form complexes
 - Cdk activity low
 - S - Activate & dissemble
 - Cdk activity high

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- Human
 - Same central roles
 - Less specific ORC binding sites

- 27 – Chromatin structure & DNA sequences

- Histones synthesized mainly in S phase
 - Feedback mechanism DNA –histone ratio
 -
 - Replication can pass through histones
 - Remodeling proteins

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- Conserved sequence
 - Protozoa, fungi, plants, mammals
 - GGGTTA repeating units
 - telomerase

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- Some cells
 - “immortal” maintain length
 - Stem cells (skin, bone marrow)
- Others
 - “age”
 - 100-200 nucleotides lost each division
 - Replicative senescence

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DNA Repair
295-304

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- Loss of adenine & guanine
 - 5000/cell/day
- Deamination
 - cytosine to uracil
 - 100 bases /cell/day
- Oxygen
- Environmental chemicals
- UV radiation
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- Complementary strand serves as template for repairs