Replication

- DNA Synthesis in the cell cycle
- Functions required for replication
- Origins of replication
- Enzymes used in replication
- Chromosomal considerations
- Telomerase

The Cell Cycle

- S-phase is DNA Synthesis
- Before S phase, a time period is required (G1), presumably to “prepare” for synthesis
- After S phase, a time period is required (G2), presumably to complete segregation
- Mitosis cannot proceed until S phase and segregation are both complete (signal?)

Functions required for replication

- Melting of dsDNA
- Stabilization of ssDNA
- Priming with RNA
- Unwinding dsDNA
- Unlinking dsDNA
- Closing breaks in the phosphodiester backbone

Basic Rule of Molecular Biology

- Every regulatory event includes:
  - A sequence on DNA (or RNA)
  - A protein (or complex) that recognizes the regulatory sequence
  - Cellular regulation of binding or signaling
- A molecular event outcome
- Replication requires:
  - Origin sequence
  - Replication complex
  - Cell cycle signaling of DNA synthesis
  - DNA synthesis leading to mitosis.
DNA Polymerase Requirements

- Template
- Primer
  - basepaired to template
  - requires 3’ OH
- Deoxynucleotide triphosphate

Note - this is VERY IMPORTANT!!!!!

Rules of nucleic acid synthesis

- All nucleic acid synthesis is in the 5’ → 3’ direction. (How can both strands be copied?)
- Synthesis based on pre-existing template strands.
- DNA synthesis requires a primer. (How does synthesis begin?)
- DNA Replication requires a special growing fork. (How is DNA strand growth managed?)

Bidirectional Replication

- Replication machinery assembles on both strands of origin.
- RNA primer is synthesized on each strand.
- Melting/unwinding leads to synthesis on both forks.
- Human genome contains ~10,000-100,000 replicons; requires 8 hrs to replicate genome.

Bidirectional Synthesis

Discontinuous Synthesis

- Primer with 3’OH, base paired to template
- Base pairing requirement is element of error correction. Without base pairing, exo removes error.
- 3’OH requirement allows re-utilization of exo product (5’dNMP, leaving 3’OH on primer)
Mismatched base recognized by morphology of pol

Direction of Synthesis
- Since nucleotides charged with phosphates on their 5′ end, 5′dNTP’s are substrates for polymerases
- Since 3′-5′ exo leaves 3′OH product on primer, 5′dNTP may be added to proofread
- Therefore, the requirement for a 5′→3′ direction is necessary for proofreading.

Sites of Initiation
- Above is one yeast ARS.
- In general, contain multiple short repeated sequences, recognized by multimeric origin-binding proteins.
- Usually contain an AT-rich stretch (Why?).

Human Replication Origins
- Single chromosome ~150x10⁶ bp
- Rate of replication ~50 nt/sec
- Therefore, multiple origins per chromosome
- Origins are clustered: 20-80 origins/unit
- Units activated at different times in cycle
- Each unit’s origin spaced 30-300x10³ bp
- All DNA synthesis during one, regulated phase of cell cycle.

E. coli - DnaA Initiation

Primase - E. coli DnaG

Figure 3–12: Molecular Biology of the Cell, 4th Edition.
Primase activity

- Primer not required
- Product about 10 nt
- Next initiation site about 100-200 nt upstream

Strunk & White Moment

- None of these accessory enzymes provides DNA synthesis activity.
- None of these accessory enzymes provide DNA synthesis activity.

According to Strunk & White, which is correct?

“None” is singular

- Think of “no one” as equal “none.”
- “The number of the subject determines the number of the verb” (Strunk & White, p. 9).
- This seems like a tricky example, but watch for disagreement in number in your sentences.

DNA Ligase

- Forms phosphodiester linkage
- 3’OH + 5’ MP
- Uses ATP or NAD+ as source of energy

Helicase

- Hydrolyze ATP when bound to ssDNA
- Melt dsDNA when encountered
- 2 ATP hydrolyzed for each bp broken

Single-stranded binding proteins

- T4: Gene 32 protein lowers the $T_m$ of DNA by about 40°C; each molecule covers about 10 nt
- E. coli: single-strand-binding protein (Ssb)
- Eukaryotic cells: replication factor A (RFA)
E. coli DNA Pol III is Replicase

- Holoenzyme contains core polymerase activity and accessory proteins
- Holoenzyme is large (<600,000 daltons)
- Contains 10-20 polypeptides
- Asymmetric dimer

β-subunit clamp makes Pol III Processive

- Assembly of clamp requires ATP hydrolysis by a clamp loader enzyme complex.

Topological Considerations

- Unwinding DNA during melting leads to over-twisted strands
- Newly-synthesized double-stranded products are tangled around each other during replication

Clamp is Regulated

- Cuts one strand
- Allows swiveling of remaining strand to relieve torsion
- Energy for rotation provided by release of torsion
- Phosphodiester bond energy stored in phosphotyrosine linkage.
Topoisomerase II

- Also known as gyrase
- Uses ATP
- Cuts both strands of DNA, binds ends, allows another dsDNA to pass through cut
- Required for separation of circular DNAs
- Also required for separation of linear daughter chromatids

The Replication Complex

Chromatin Considerations

- Remember, eukaryotic DNA is not “free”—it is bound with nucleosomes to make chromatin.
- Histone proteins synthesized in S phase to match DNA synthesis.
- Histone mRNAs lack poly(A) tails. Become destabilized after S phase.
- So nucleosome synthesis and DNA synthesis are tightly coupled.

Replication and Chromatin

- The replication complex is able to pass through parental nucleosomes without displacing them.
- Both daughter strands inherit parental nucleosomes.
- New nucleosomes added (by assembly factors), later deacetylated.
Telomerase

- Ends of linear DNA will be shortened by replication
- Lagging strand cannot be primed beyond end of leading strand, but the leading strand is shortened due to priming.
- Therefore, chromosomal end must be repaired
- Telomerase is an RNA-directed DNA polymerase, containing RNA template.

Shortened ends without telomerase

Replication

- **Semi-conservative**—one parental strand becomes a strand of each daughter molecule.
- **Bidirectional**—long DNA molecules (chromosomes) have internal initiation sites and replication in both directions.
- **Sequence-directed**—specific sequences are required for initiation.