DNA Replication III
& DNA Damage/Modification
Biochemistry 302

January 27, 2006
Bacterial replication organized into a membrane-bound replication “factory”

Two replisomes do not travel away from one another but rather are linked to each other.

This mechanism facilitates chromosome partitioning into daughter cells.

Lehninger Principles of Biochemistry, 4th ed., Ch 25
Features of DNA replication in eukaryotes

- Many origins > 5,000/genome which only initiate once per cell cycle
- **Ori**: less sequence conservation
- **Origin Recognition Complex (ORC)**
  - Six polypeptides, binds to yeast ARS in ATP-dependent manner & recruits MCM helicase complex
  - Role for phosphorylation, DNA methylation, and histone remodeling
- **Mitochondrial DNA**
  - Fixed origins but use two unidirectional forks (~1 h to complete replication)

![Fig. 28.18 from Lodish et al. Molecular Cell Biology, 3rd Edition](image1)

![Fig. 24.39](image2)
Licensing a site for initiation of DNA replication (E. coli vs budding yeast)

With the exception of fission yeast S. pombe, all eukaryotic ORCs characterized to date require ATP to bind DNA.

Eukaryotic Origins: no obvious sequence signature, noncoding regions, AT-richness, asymmetric strand composition

Kinetic features/enzymology of eukaryotic DNA replication

- Slower fork movement but larger number of origins (~$10^3$ per $10^8$ bp chromosome)
- Five different polymerases (distinguished by intracellular location, kinetic properties, and response to inhibitors):
  - Pol $\alpha$ - Okazaki fragment primer synthesis. (Primase activity but no 3$'$→5$'$ exonuclease activity and not very processive).
  - Pol $\delta$ - Major leading and lagging strand polymerase w/ 3$'$→5$'$ exonuclease activity. Highly processive w/ bound PCNA (analogous to E. coli Pol III).
  - Pol $\varepsilon$ - DNA repair and Okazaki fragment RNA primer removal (perhaps, not definitive).
  - Pol $\beta$ and Pol $\gamma$ - repair and mitochondrial DNA replication.
## Properties of eukaryotic DNA polymerases

<table>
<thead>
<tr>
<th></th>
<th>α</th>
<th>β</th>
<th>γ</th>
<th>δ</th>
<th>ε</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cell compartment</strong></td>
<td>nucleus</td>
<td>nucleus</td>
<td>mitochondrion</td>
<td>nucleus</td>
<td>nucleus</td>
</tr>
<tr>
<td><strong>Associated primase</strong></td>
<td>yes</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td><strong>Biological function</strong></td>
<td>lagging strand replication</td>
<td>DNA repair</td>
<td>mitochondrial DNA replication</td>
<td>leading strand replication</td>
<td>replication</td>
</tr>
<tr>
<td><strong>Number of subunits</strong></td>
<td>4</td>
<td>1</td>
<td>4 (identical)</td>
<td>2</td>
<td>?</td>
</tr>
<tr>
<td><strong>Mₚ, catalytic subunit, kilodaltons</strong></td>
<td>160–185</td>
<td>40</td>
<td>125</td>
<td>125</td>
<td>210–230 or 125–140</td>
</tr>
<tr>
<td><strong>Kₐ for dNTPs, μM</strong></td>
<td>2–5</td>
<td>10ᵃ</td>
<td>0.5</td>
<td>2–4</td>
<td>?</td>
</tr>
<tr>
<td><strong>Processivity (inherent)</strong></td>
<td>moderate</td>
<td>low</td>
<td>high</td>
<td>low</td>
<td>high</td>
</tr>
<tr>
<td><strong>Processivity (with PCNA)</strong></td>
<td>moderate</td>
<td>low</td>
<td>high</td>
<td>high</td>
<td>high</td>
</tr>
<tr>
<td><strong>3' exonuclease</strong></td>
<td>noᵇ</td>
<td>no</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td><strong>Sensitivity to 2',3'-dideoxy-NTPs</strong></td>
<td>low</td>
<td>high</td>
<td>high</td>
<td>low</td>
<td>moderate</td>
</tr>
<tr>
<td><strong>Sensitivity to arabinosyl-CTP</strong></td>
<td>high</td>
<td>low</td>
<td>high</td>
<td>low</td>
<td>high</td>
</tr>
<tr>
<td><strong>Sensitivity to aphidicolin</strong></td>
<td>high</td>
<td>low</td>
<td>low</td>
<td>low</td>
<td>high</td>
</tr>
</tbody>
</table>

ᵃKₐ values for repair synthesis in permeabilized cells are much lower.
ᵇA cryptic, or masked, 3' exonuclease activity is associated with polymerase α from *Drosophila*.

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**PCNA ~ *E. coli* β sliding clamp of Pol III holoenzyme**

**RFC ~ *E. coli* clamp loader γ complex of Pol III holoenzyme**

**RFA ~ *E. coli* SSB; RNA primer removal by FEN-1/RNaseH1 or Pol ε**
Nuclear genomes & linear chromosomes: nucleosome replication & gaps on 5′ ends
Replicating and protecting the ends of linear chromosomes

T-loop formation: telomere repeat binding factors

EM mouse hepatocyte chromosome T-loop

Lehninger Principles of Biochemistry, 4th ed., Ch 26
Mechanisms for ensuring fidelity of DNA replication

- **Metabolic:** balanced levels of dNTPs
- **Structural 1:** complementary base pairing between dNTP and template (Error ~1 in $10^3$ to $10^4$ bp/round of replication)
- **Structural 2:** induced fit between Pol and DNA (Cumulative Error ~1 in $10^5$ to $10^6$)
- **Enzymatic 1:** proofreading by 3’ to 5’ exonuclease (Cumulative Error ~1 in $10^7$ to $10^8$)
- **Enzymatic 2:** mismatch and other repair systems (1 in $10^{10}$ bp/generation)
Overview of DNA Restructuring

Restriction and Modification (protective mechanisms in prokaryotes, useful in recombinant DNA techniques)

Repair (in response to DNA damage)

Recombination (redistribution of genomic contents, reproduction, repair)

Transposition and Amplification (developmental processes and/or responses to external stress)

Fig. 25.1
Consequences of DNA damage.....

DNA Damage

DNA Repair → X

DNA Replication

Mutations
Replication Errors
Persistent Damage
Genomic Instability

Cancer
Aging
Mechanisms ensuring that information content is transmitted w/o error

- High accuracy replication and editing
  - 3’ exonuclease-mediated proofreading
  - Uracil DNA glycosylase (1 in *E. coli*, 4 types in humans)
    - Mis-incorporation of dUMP (rare)
    - Cytosine deamination (common occurrence)
    - Associated w/ the replisome (human UNG)

- Repairing damaged DNA arising from….
  - Replicative errors not corrected by proofreading activity of DNA polymerases
  - Spontaneous alterations in covalent structure of nucleotide (very slow but physiologically significant, permanent change = mutation)
  - Environmental damage (chemical or photochemical)
Spontaneous non-enzymatic reactions of nucleotides: deamination

- Loss of exocyclic amino group
- C → U (1 out of every $10^7$ cytidine residues/24 h) so ~100 events/day in a mammalian cell. Rate higher in ssDNA.
- Oxidative deamination of adenine and guanine occurs at ~1/100 the rate of cytosine deamination.
- What is the potential genetic consequence of such an event?
DNA Damage: Chemical structure of bases that are mutagenic (i.e. produce altered, non-Watson-Crick base-pairs)

Deamination

Cytosine

Uracil

5-Bromouracil

5-Azacytosine

bp w/T & G

bp w/T & C

Adenine

Hypoxanthine

2-Aminopurine

6-thioguanine

Base analogs: used experimentally as mutagens
Spontaneous non-enzymatic reactions of nucleotides: depurination

- Hydrolysis of the $N$-$\beta$-glycosyl bond
- Higher rate for purines than pyrimidines (1 out of every $10^5$ guanosine residues or $10^4$ events/24 h in mammalian cell)
- Slower in ribonucleotides and RNA (probably not physiologically significant)
- Accelerated by low pH $\sim$3 resulting in apurinic acid $\rightarrow$ ring opening, linear aldehyde configuration
Environmental DNA damage: Radiation and pyrimidine dimers

- One of the first forms of DNA damage discovered
- Irradiation of bacteria with 260 nm light → condensation of adjacent ethylene groups
- Human skin cells particularly susceptible
- Ionizing radiation (x-rays and gamma rays)
  - Ring opening and base fragmentation
  - Breaks in DNA backbone
- UV + ionizing radiation exposure accounts ~10% of all DNA damage caused by environmental agents

Lehninger Principles of Biochemistry, 4th ed., Ch 8
Environmental DNA damage: Reactive chemical agents

- Direct action vs damage cause by metabolic by-products
- Precursors of nitrous acid (HNO₂) e.g. NaNO₂, NaNO₃, and nitrosamine
  - Accelerate deamination
  - Food preservatives
- Alkylating agents
  - Replace H atom
  - Methylation of purines results in altered base-pairing (O⁶-methylguanine cannot pair with cytosine)
  - Used experimentally as DNA modifying agents (mutagens)
Summary of types of DNA damage

• UV photoproducts
• Depurination
• Deamination
• Alkylation
• Oxidation (maybe most important)
  – ROS, reactive oxygen species (H₂O₂, hydroxyl radicals, and superoxide radicals)
  – ROS generated during irradiation or as byproducts of aerobic metabolism
  – Defense systems (e.g. catalase and superoxide dismutase)
  – Oxidants escaping cellular defense can promote...
    • Deoxyribose oxidation
    • Base oxidation
    • Strand breaks
Epigenetic site-specific DNA methylation

• **Bacteria**
  – Restriction & modification
  – Mismatch error correction

• **Eukaryotes**
  – Tissue-specific inactivation of genes during development
  – Suppression of transposon migration
  – Formation of Z-DNA

• **Occurrence**
  – *E. coli*: \(N^6\) of adenine (major), \(N^4\) of cytosine (minor), GATC restriction sites
  – Eukaryotes: 3-5% of cytosines (C5), mainly in CpG islands (animal cells and higher plants, absent in insects)
Type II restriction endonucleases (work horses of recombinant DNA technology)

- Recognize simple symmetrical sequences
- Cut un-methylated DNA generating 3′OH & 5′-phosphate termini with either blunt or overhanging ends
- Most are homodimers with subunits of 30-40 kDa and require a divalent cation for catalysis
- Counterpart methylase: recognizes un- and hemi-methylated DNA, AdoMet

**Table 25-2**

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Bacterial Source</th>
<th>Restriction and Modification Site*</th>
</tr>
</thead>
<tbody>
<tr>
<td>BamHI</td>
<td><em>Bacillus amyloliquefaciens</em> H</td>
<td>G↓GATCC</td>
</tr>
<tr>
<td>EglII</td>
<td><em>B. globigii</em></td>
<td>A↓GATCT</td>
</tr>
<tr>
<td>EcoRI</td>
<td><em>Escherichia coli</em> RY13</td>
<td>G↓AATTC</td>
</tr>
<tr>
<td>EcoRII</td>
<td><em>E. coli</em> R245</td>
<td>CC↓GG</td>
</tr>
<tr>
<td>HaeIII</td>
<td><em>Haemophilus aegyptius</em></td>
<td>GG↓CC</td>
</tr>
<tr>
<td>HpaI</td>
<td><em>H. gallinarum</em></td>
<td>GACGGNNNNNN↓CTG</td>
</tr>
<tr>
<td>HpaII</td>
<td><em>H. haemolyticus</em></td>
<td>G↓GG↓C</td>
</tr>
<tr>
<td>HinIII</td>
<td><em>H. influenzae</em> Rd</td>
<td>G↓AGCTT</td>
</tr>
<tr>
<td>HincI</td>
<td><em>H. influenzae</em> Ri</td>
<td>G↓ANTC</td>
</tr>
<tr>
<td>HpaI</td>
<td><em>H. parainfluenzae</em></td>
<td>GT↓AAC</td>
</tr>
<tr>
<td>HpaII</td>
<td><em>H. parainfluenzae</em></td>
<td>C↓GGG</td>
</tr>
<tr>
<td>MspI</td>
<td><em>Mesorina sp.</em></td>
<td>C↓GGG</td>
</tr>
<tr>
<td>NcoI</td>
<td><em>Nocardia rubra</em></td>
<td>GC↓GCGCG</td>
</tr>
<tr>
<td>PstI</td>
<td><em>Pseudomonas lemoignei</em></td>
<td>GAGTCNNNN↓CTCAGNNNNN↓</td>
</tr>
<tr>
<td>EcoI</td>
<td><em>Providencia stuartii</em></td>
<td>CTGCA↓G</td>
</tr>
<tr>
<td>SalI</td>
<td><em>Streptomyces albus</em> G</td>
<td>G↓TCGAC</td>
</tr>
<tr>
<td>SmaI</td>
<td><em>Serratia marcescens</em> Sb</td>
<td>CC↓GGG</td>
</tr>
<tr>
<td>XbaI</td>
<td><em>Xanthomonas badini</em></td>
<td>T↓CTAGA</td>
</tr>
</tbody>
</table>

*The methylated base in each site, where known, is identified with the letter m. All sequences read 5′ to 3′, left to right. The cleavage on the opposite strand in each case can be inferred from the symmetry of the site (except for HpaI and PstI, each of which has an asymmetric site). Pu = purine, Py = pyrimidine, N = any base.