CHROMOSOMAL DNA REPLICATION

Date:

KEY CONCEPTS AND LEARNING OBJECTIVES

1. Nucleic acid synthesis is governed by four important characteristics:
   - A pre-existing nucleic acid strand is copied by rules of Watson-Crick base pairing;
   - Nucleic acid strands grow in only one direction, 5'→3';
   - Polymerases synthesize nucleic acids;
   - Duplex DNA synthesis requires a special growing fork because the strands are antiparallel.
   
   a. Identify 3' and 5' ends of DNA chains.
   b. Identify and name complementary base pairs.
   c. Assess the unique properties of DNA polymerases.
   d. Describe Okazaki fragments and their function.

2. DNA replication utilizes six specialized mechanisms: initiation, unwinding, priming, unidirectional fork movement, untangling, and termination.

   a. Describe important features of a replication origin and the mechanism for the fork initiation reaction.
   b. Compare DNA replication on leading and lagging strands.
   c. Describe 5' to 3' polymerization in DNA replication.
   d. Understand that DNA polymerase has editing (3' to 5' exonuclease activity) as well as polymerizing function, why this is important for high fidelity replication, and potential impact of mutations in the proofreading domain of DNA polymerase.
   e. Explain the role of primers and why they are RNA and not DNA; compare the synthesis and removal of primers.
   f. Compare the function and mechanisms of helicase and topoisomerases.
   g. Compare the role of SSB proteins in denaturing and stabilizing DNA.
   h. Describe the functions of the subunits of the replication machine and explain how leading and lagging strand synthesis occurs simultaneously.
   i. Describe the reaction catalyzed by ligase.
   j. Compare the function and mechanism of topoisomerase I and II.
   k. Understand the use of DNA topoisomerase inhibitors in cancer treatment
   l. Predict the experimental results of density labeling newly synthesized DNA and inhibiting protein synthesis during replication.
3. Eukaryotic chromosomal DNA replication differs from prokaryotic DNA replication in three ways:
   - It is compartmentalized within the nucleus, partitioning it from the site of synthesis of replication proteins and precursors and from extracellular stimuli that may trigger initiation of synthesis;
   - It begins at multiple origins, activated throughout S-phase in a precise and temporally regulated manner;
   - The nucleosomal proteins must be duplicated along with the DNA to maintain proper chromosomal organization.

   a. Identify important chromosomal DNA sequences and their functions.
   b. Identify a chromatin property that would contribute to early vs. late replicating DNA.
   c. Describe the fate of nucleosomes during DNA replication.

4. Telomeres function to protect single-stranded chromosome ends - left by removal of primer RNA at the ends of replicated linear chromosomes - from recombination, fusion, and from being recognized as damaged DNA. Telomere shortening can trigger replicative senescence in human cells.

   a. Explain the “end-replication” problem.
   b. Describe the mechanism for the addition of telomere repeat sequences.
   c. Analyze the relationship of telomere length with cellular life span.
   d. Explore the potential of a telomerase template antagonist as an anticancer agent.

5. Cell controls assure that each region of eukaryotic DNA is usually replicated only once and that all DNA is replicated before the onset of mitosis.

   a. Describe factors that contribute to gene replication timing.
   b. Describe and assess cell controls that assure that each region of eukaryotic DNA is replicated only once per cell cycle and that all DNA is replicated before onset of mitosis.

6. Slipped mispairing during DNA replication is a likely mechanism for triplet repeat expansion. The wild-type genes associated with triplet repeat disorders contain variable but relatively low numbers of tandem (adjacent) trinucleotide units. Individuals with genes carrying repeats above a threshold number are affected.

   a. Identify common DNA-repeat expansion diseases.
   b. Describe significant features of DNA-repeat expansion diseases.
   c. Relate location of the DNA expansion with effect on transcription and translation.
   d. Relate genetic features with clinical features of a DNA repeat expansion disease.
1. Nucleic acid synthesis is governed by four important characteristics:
- A pre-existing nucleic acid strand is copied by rules of Watson-Crick base pairing;
- Nucleic acid strands grow in only one direction, $5' \rightarrow 3'$;
- Polymerases synthesize nucleic acids;
- Duplex DNA synthesis requires a special growing fork because the strands are antiparallel.

DNA replication is bi-directional. Two forks move in opposite directions from one origin.

Direction of synthesis is $5' \rightarrow 3'$
One strand synthesized continuously (leading strand) and other discontinuously (lagging strand).
2. DNA replication utilizes six specialized mechanisms: initiation, unwinding, priming, unidirectional fork movement, untangling, and termination.

Helicase is an allosteric motor protein.

SSB proteins prevent reannealing without preventing base pairing.
DNA synthesis requires an RNA primer.

A regulated sliding clamp permits processive DNA synthesis on the leading strand and rapid reassembly of the replication complex on the lagging strand.

Schematic illustration showing how the clamp (with red and yellow subunits) is loaded onto DNA to serve as a tether for a moving DNA polymerase molecule. The structure of the clamp loader (dark green) resembles a screw nut, with its threads matching the grooves of double-stranded DNA. The loader binds to a free clamp molecule, forcing a gap in its ring of subunits so that this ring is able to slip around DNA. The clamp loader, thanks to its screw-nut structure, recognizes the region of DNA that is double-stranded and latches onto it, tightening around the complex of a template strand with a freshly synthesized elongating (primer) strand. It carries the clamp along this double-stranded region until it encounters the 3' end of the primer, at which point the loader hydrolyzes ATP and releases the clamp, allowing it to close around the DNA and bind to DNA polymerase.

The RNA primer is removed and replaced by DNA.

Ligase synthesizes the phosphodiester bond that links Okazaki fragments.
DNA polymerase “proofreads” as it synthesizes DNA.

The replication proteins act together as one large multienzyme complex requiring the lagging strand to fold back.

Germline mutations affecting DNA polymerases (E and D) proofreading domains predispose to colorectal adenomas and carcinomas.
Topoisomerases relieve supercoils and untangle DNA during replication.

**Topo I**: relieve supercoiling ahead of replication fork

**Topo II**: untangle DNA after it is replicated; is target of chemotherapeutic drugs

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**Topoisomerase I**

- cannot rotate relative to the other end
- type I DNA topoisomerase with tyrosine at the active site
- DNA topoisomerase covalently attaches to a DNA phosphate, thereby breaking a phosphodiester linkage in one DNA strand
- the two ends of the DNA double helix can now rotate relative to each other, relieving a accumulated strain
- the original phosphodiester bond energy is stored in the phosphotyrosine linkage, making the reaction reversible
- spontaneous re-formation of the phosphodiester bond regenerates both the DNA helix and the DNA topoisomerase

**Topoisomerase II**

- two circular DNA double helices that are interlocked
- a type II DNA topoisomerase makes a reversible covalent attachment to opposite DNA strands, interrupting the orange double helix and forming a protein gate
- the topoisomerase gate opens and shuts to let a second DNA helix pass
- of the covalent attachment of the topoisomerase restores an intact double helix
- two circular DNA double helices that are separated

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Figure 5-22 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Figure 5-24 Molecular Biology of the Cell 5/e (© Garland Science 2008)
Effective class of cancer chemotherapeutic drugs: DNA topoisomerase II inhibitors:

- Increase stability of topoII-DNA cleavage complexes
- High levels of transient DNA breaks
- Become permanent double-stranded breaks when replication machinery or helicases attempt to traverse DNA:topoII block in DNA
- Too many breaks: cell death

DNA topoisomerase II inhibitors demonstrate some anti-cancer activity as single agents, but are now most often used in combination therapies.

As an unintended consequence of treatment with DNA topo II–targeting drugs, some patients develop chromosome rearrangements: MLL gene fusions which cause leukemia.
Eukaryotic chromosomal DNA replication differs from prokaryotic DNA replication in three ways:

- It is compartmentalized within the nucleus, partitioning it from the site of synthesis of replication proteins and precursors and from extracellular stimuli that may trigger initiation of synthesis;
- It begins at multiple origins, activated throughout S-phase in a precise and temporally regulated manner;
- The nucleosomal proteins must be duplicated along with the DNA to maintain proper chromosomal organization.

Replication occurs once during the cell cycle in eukaryotes.

Replication timing:
Early replicating: euchromatin; gene-rich; highly expressed.
Late replicating: heterochromatin; gene-poor; not expressed.

Each chromosome has many replication origins.
The mechanisms of eukaryotic chromosome duplication ensure that patterns of histone modification can be inherited.

Models for inheritance of heterochromatin during DNA replication:

(A) H2A-H2B dimer with parental H3-H4 tetramer

(B) H2A-H2B dimer with H3-H4 tetramer and CENP-A H3-H4

Parental nucleosomes with modified histones

Only half of the daughter nucleosomes have modified histones

Parental pattern of histone modifications re-established by reader–writer complexes that recognize the same modifications they catalyze

Histone modification

Heterochromatin proteins

Nucleosomes

NEW HETEROCHROMATIN PROTEINS ADDED TO PROPERLY MODIFIED HISTONES

Heterochromatin

Euchromatin

Heterochromatin

Euchromatin

Heterochromatin

Euchromatin

Heterochromatin

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Histone modification

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Telomeres function to protect single-stranded chromosome ends – left by removal of primer RNA at the ends of replicated linear chromosome – from recombination, fusion, and from being recognized as damaged DNA. Telomere shortening can trigger replicative senescence in human cells.

The 3’ end of the template DNA strand has no room for a primase to synthesize a primer.

Telomerase is a riboprotein containing an RNA template for synthesizing the G-rich telomere sequence.

Telomerase is not expressed in all cells. It is primarily expressed in germ cells, stem cells and cancer cells.

Many cycles of telomere synthesis and DNA replication by Pol α elongates 3’ ends.
5. Slipped mispairing during DNA replication is a likely mechanism for triplet repeat expansion. The wild-type genes associated with triplet repeat disorders contain variable but relatively low numbers of tandem (adjacent) trinucleotide units. Individuals with genes carrying repeats above a threshold number are affected.

Model for trinucleotide repeat expansion: DNA replication slippage

DNA polymerase pauses while replicating through the triplet-repeat tract and transiently dissociates. During reassociation, a misalignment between template and nascent strand would result in unpaired repeat sequences either on the template or on the newly synthesized strand. If the mismatch is not repaired, the unpaired sequence on the newly synthesized strand will cause expansion of the repeat tract following the next round of DNA replication.

Telomere ends are protected from degradation and repair by a unique folded structure.
The tendency of these tandem arrays to increase in size from generation to generation explains one of the hallmarks of these disorders – anticipation – which is the progressive increase in disease severity and decrease in age of onset seen with successive generations in an affected family.

The preponderance of disorders involving nucleotide triplets probably reflects the expansion of arrays located in coding regions resulting in a dominant and readily recognizable phenotype.

These repeats all form unusual nucleic acid secondary structures that may play a role in their instability and in some cases the disease pathology (e.g., forming a barrier to DNA or RNA polymerase, affecting protein binding or nucleosome position, etc.)

Research into the mechanism and consequences of expansion may lead to therapeutic approaches to this group of disorders.
Further Reading: