

BIO240 Lecture Notes Chapters 2 and 3

Chapter 2:

I. The Genetic Material

A. DNA composition and structure – nucleotide components

[Figures 2.8 through 2.10]

1. **nitrogenous bases;**
pyrimidines - single ring; cytosine and thymine (uracil in RNA)
purines - double ring; adenine and guanine
2. **pentose sugar** (or 5-carbon sugar)
ribose (RNA)
deoxyribose (DNA)
3. **phosphate group:** mono, di and triphosphate groups possible

[Definitions: nucleoside - nitrogenous base and pentose sugar

nucleotide - nitrogenous base, pentose sugar and phosphate group

important trinucleotides include ATP, GTP (guanosine triphosphate)]

B. Polynucleotides: nucleotides linked by phosphate groups (phosphodiester bonds)

dinucleotides, trinucleotides, etc.

oligonucleotides < 20 nucleotides

polynucleotides > 20 nucleotides

C. DNA Complex structure: strands and helices.

Watson-Crick model: [Figure 2.13]

1. Two long polynucleotide chains coiled around a central axis, forming a right-handed double helix
2. **Two chains are antiparallel: their C-5' to C-3' orientations run in opposite directions**
3. The bases of both chains are flat structures lying perpendicular to the axis; they are stacked on one another approximately 3.4Å (0.34nm) apart, located on the inside of the helix.
4. **The nitrogenous bases of opposite chains are paired to one another as the result of hydrogen bonds; in DNA only A=T and G≡C bonds are possible.**
5. Each turn of the helix is 34Å long and runs the length of 10 bases each turn of the chain.
6. In any segment of the molecule, alternating larger major grooves and smaller minor grooves are apparent along the axis.
7. The double helix measures 20Å in diameter.

[Hydrogen bonds are weak electrostatic bonds between nucleotide bases. Individually weak but cumulatively very stabilizing]

II. Chromatin and Chromosome Structure

Prokaryotes: Single looped major chromosome (plus one or more smaller looped or linear chromosomes). DNA less complexed with protein and NOT membrane bound.

DNA loop is very large compared to prokaryotic cell [Fig. 2.19]. Chromosomes are **supercoiled** [Fig 2.20] to compact into tight bundle. DNA also further compacted into **looped domains** [Fig. 2.22].

Eukaryotes: diploid or (2N) equals 2 haploid gametes joined.

C value: measure of amount of DNA in haploid genome of eukaryotes (Table 2.4).

C value paradox: amount of DNA in species genome is not necessarily correlated with structural complexity of organism.

Chromosomes: linear double-stranded DNA molecule complexed with about twice as much protein as DNA (by weight). DNA plus complexed proteins is **chromatin**.

Chromatin structure: DNA and histone proteins and non-histone proteins.

Histone proteins: Five major types all of which are highly conserved across all eukaryotic genomes: H1, H2A, H2B, H3, H4.

Two amino acid differences in H4 between cows and peas.

Non-Histone proteins: not highly conserved and may vary widely between species, cell types within single species, and within same cells at different times.

Thought to have a role in higher order structures of chromatin.

Euchromatin: cycles through very condensed and highly diffuse stages through cell cycle. Presumably contains transcriptionally active genes requiring access to DNA.

Heterochromatin: tend to remain highly condensed.

Constitutive: structural in nature, usually always condensed in both homologs.

Facultative: usually condensed but may incorporate transcriptionally active regions, e.g. Barr bodies.

Chromatin packing: Human DNA would be about 2.3m long if laid out end to end.

Nucleosomes (Level 1): DNA wound around octamer histone complex of H2A, H2B, H3, and H4 (two each) forming a nucleosome [Fig. 2.24].

Winding around nucleosomes achieves an approximate 6 X compaction.

10-nm nucleofilaments (Level 2): Linked nucleosomes create a “beads-on-a-string” level of structure to compacting chromatin, **10-nm nucleofilaments** [Fig. 2.24b]. Nucleosomes, in turn, linked by DNA strands called **linker DNA strands** [Fig. 2.24c].

30-nm chromatin fibers (Level 3): nucleosomes of 10-nm nucleofilaments associate with one another to form a 30-nm chromatin fiber the exact structure of which is, as yet, undetermined [Fig. 2.25a]. One possible model is the “solenoid model” [Fig. 2.25b].

H1 histone is necessary for forming 30-nm chromatin fibers. If H1 is stripped from chromatin, the chromatin can form 10-nm nucleofilaments but not 30-nm chromatin fiber.

Compaction from 10-nm nucleofilaments into 30-nm chromatin fibers achieves an additional 6X compaction.

Looped Domains (Level 4): chromatin fibers are looped in lengths of variable size (tens to hundreds of kilobases) extending from main chromosome axis.

Loops are anchored to a filamentous structural framework of (nonhistone) protein inside the nuclear envelope.

Coiling and folding (Level 5): Looped domains coil into the approximate 700 nm width of the chromosome arm [Fig. 2.27].

M-phase condensation (Level 6): The highest level of compaction is

obtained during cell division, specifically by Metaphase (or Metaphase I).

Centromeres: DNA sequences near (or constituting) spindle fiber attachment sites (kinetochore). Same function in all eukaryotic cells but no common sequence across eukaryotes.

Telomeres: linear ends of chromosomes.

Telomeres tend to associate with inside of nuclear envelope and with one another.

Telomeres of a species share a common sequence, which sequence is necessary for replication of telomeric regions of chromosomes.

Telomeres may be linear or may form a looped structure (which is correct form is currently being debated) [Fig. 2.30].

III. The Genetic Intermediary: RNA – composition and structure.

A. Similarities:

1. same general molecular structure of DNA nucleotides
2. RNA nucleotides pair with DNA nucleotides using strict pairing rules

B. Differences:

1. **ribose** instead of deoxyribose
2. **uracil** in place of thymine, so A-U and G-C
3. **single-stranded**

IV. Types of RNA serve distinct functions:

A. ribosomal RNA (rRNA) - largest molecules of RNA and about 80% of RNA in cell; ribosomes are sites of gene expression, or translation of genetic messages to cell

B. messenger RNA (mRNA) - carries transcribed message from DNA template to ribosomes

C. transfer (tRNA) - carries amino acids to ribosome during translation, smallest RNA molecules

D. Other types:

1. small nuclear RNA (snRNA) - involved in processing mRNA in nucleus
2. telomerase RNA - involved in replication of DNA at ends of

chromosomes

3. antisense RNA - involved in gene regulation

Chapter 3: DNA Replication

I. General Overview:

A. DNA replicates *semi-conservatively*, that is each new double helix of DNA is comprised of one entirely newly synthesized strand and its template strand that was part of the original double helix [Fig. 3.1].

B. First, the double helix unzips and DNA polymerases match the original nucleotides with their complements (A-T, G-C). Replication using DNA polymerase is bidirectional from the 3' to 5' end of the original strands, thus replicons are built from 5' to 3' (i.e. the new bases are added to the 3' ends at the 5' connection of the new base, then the new base has only a 3' end unattached, etc) [Fig 3.4].

C. DNA polymerases I, II, and III, all have exonuclease abilities, i.e. the ability to remove bases just added and reattach correct bases if necessary.

1. **DNA polymerase I** is believed to be essential to removing RNA primers used to initiate replication and then to fill in the gaps left when the primer is removed.
2. **DNA polymerase II** is believed to be a repair mechanism for damaged DNA.
3. **DNA polymerase III** is believe to be the primary polymerase responsible for replication and elongation of DNA replicons

D. Continuous and discontinuous synthesis occurs because DNA strands are antiparallel [Figures 3.6 and 3.8].

E. Self-Correction: Exonuclease activities allow each replicon to undergo a quality control check and correction of most misplaced bases.

II. DNA Replication Process (prokaryotes as model):

A. Unwind DNA strand

1. **DnaA** protein binds at repeating sequences of 9 nucleotides;
2. **DNA helicases (DnaB [and DnaC?])** both insert into open helix and further destabilize helical conformation;
3. Single-stranded binding proteins (**SSBPs**) artificially stabilize open structure while replication takes place.
4. **gyrases** may release molecular-mechanical tension which builds downstream from replication fork as (unwound) areas compress helix

B. RNA primer laid down

1. **DNA polymerase** must hook up **with a 3' hydroxyl group** to snap a DNA nucleotide and begin building from 5' to 3' of new, complementary strand – SO a primer must be laid down on each template strand;
2. **DNA primase** lays down an **RNA primer** to which DNA polymerase may bind.

C. DNA polymerization

1. **DNA polymerase III** attaches to primer and polymerizes DNA strand continuously on one strand but discontinuously on complement because constrained to read from 3' to 5' and build 5' to 3'.

D. Filling in gaps

1. Discontinuous polymerization means multiple RNA primer sites on one complementary strand;
2. **Okazaki fragments:** Once primers are removed, disconnected DNA sequences are called Okazaki fragments and must be connected by filling in space where primer was with proper DNA nucleotides and then binding covalent bond spine of fragments and filled gap sequences together.
 - a. **Excision of RNA** and filling in with DNA nucleotides accomplished by DNA polymerase I;
 - b. **Ligation** of Okazaki fragments with filled gap sequences accomplished by **DNA ligase**.

E. Proof-reading: DNA polymerases I and III recognize nucleotide mismatches, remove and replace them with correct nucleotides due to **3' to 5' exonuclease activity**.

III. Eukaryotic DNA Replication: Differences between eukaryotic replication and prokaryotic replication are sourced in the greater length of eukaryotic DNA chromosomes, the linear nature of eukaryotic chromosomes, and the complexing of histone proteins with the DNA of eukaryotic chromosomes.

eukaryotes must have multiple replication origin sites

mammalian cells may have upwards of 25,000 different **replicons**

each replication site characterized by specific DNA sequences that initiates the replication covering a specific area of the genome (**autonomously replicating sequences - ARS**)

complex proteins called **origin recognition complexes (ORC)** bind to the ARSs during G1 phase of Interphase in preparation for replication during S phase (this structure of ORC bound to ARS is called a **pre-replication complex** or **pre-RC**)

changes within DNA sequence of ARS or in complex of the ORC each will prevent initiation of replication specific kinases bind to pre-RC and both

allow

DNA polymerase to bind and initiate replication, and also inhibit reformation of another pre-RC complex at that site until the cell cycle has been completed

IV. Eukaryotic enzymes (15 or more DNA polymerases):

DNA polymerase α - fills role of RNA primase (creating RNA primer) in prokaryotes

lays down the RNA primer and a few DNA nucleotides thereafter, then drops off of template.

DNA polymerase δ - replaces DNA polymerase α and synthesizes DNA strand at 100

times the rate that the α form could; has 3' to 5' exonuclease activity; fills role of DNA polymerase III in prokaryotes. Apparently, REPLICATES EITHER LEADING OR LAGGING STRAND, but which is unknown.

DNA polymerase ϵ - same as δ form but replicates whichever strand is NOT replicated by as δ form.

DNA polymerase γ - mitochondrial genome replication, although encoded within the nuclear genome.

DNA polymerases β and ζ – Apparently, involved in DNA repair.

Telomerase - allows ends of linear chromosomes to be replicated where RNA primer must be removed and there is no 3' - OH group to which DNA polymerase may bind - creates linear sequence on open side of terminal Okazaki fragment which folds over on itself creating a 3' - OH group opposite complementary strand terminal end; DNA polymerase may then fill the gap between the telomerase created sequence and the terminal DNA strand and ligase may link the strands **[Figs. 3.14 and 3.15]**.