Chapter 7: DNA Replication, Transcription & Translation; Mutations & Ames test

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DNA is the genetic material

- Hereditary information is carried by DNA
  - Griffith/Avery transformation experiments
  - Hershey-Chase viral labeling experiment

- DNA is organized into chromosomes
  - In bacteria, generally one per cell, circular in shape
  - Humans: 46 linear chromosomes (23 homologous pairs)

- Each chromosome contains many genes
  - Gene is a functional unit, like a word in a sentence.
  - One definition: one gene carries info to make one protein

Components of DNA

- Phosphate group
- Sugar
- Base

All three together = a nucleotide

DNA structure

- Double stranded
- Backbones:
  - Alternating sugar (deoxyribose) / phosphate
- Strands held together by hydrogen bonds
  - Between the bases
- Adenine / Thymine
- Guanine / Cytosine
- Antiparallel
  - 5' & 3' ends
- Wrapped in a helix

DNA structure linked to DNA function

- Information is encoded in sequence of the bases
- Complementary base pairing of strands that are NOT covalently bound: suggests mechanism for REPLICATION
DNA’s jobs (information transfer)

1. Direct its own replication so each daughter cell gets an exact copy of the parent’s genome

2. Direct all cellular activity by expressing genes as RNA
   - Transcription into messenger RNA (mRNA)
   - Translation of mRNA into protein

Information transfer: “The Central Dogma”

- DNA to DNA = replication
- DNA to RNA = transcription
- RNA to protein = translation

DNA Replication

Replication is **Semiconservative**:
- One strand from the original molecule is always “conserved” in each new DNA copy

1. DNA double helix denatures (strands separate)
2. Each strand serves as the **template** for synthesis of a new second strand
3. **DNA polymerase** adds complementary nucleotides
   - 1,000 per second!!!
   - Corrects its own mistakes (proofreading)

Semiconservative Replication

- DNA strands separate
- DNA polymerase (enzyme) synthesizes complementary (new) strands, using the nucleotide sequence of the original molecule as a template
- Two DNA molecules result
  - Each double stranded (ds) DNA has one old and one new strand

DNA replication in prokaryotes begins at a specific location: **origin of replication**
- Plasmids must have an “origin” to survive

Replication proceeds simultaneously in both directions away from the origin
- The moving point where replication is actually occurring is called the **replication fork**

Nucleotides can only be added to the 3’ end

- 3’ carbon on deoxyribose has –OH group to which new nucleotide gets attached
- 5’ carbon has phosphate group, no extension of the DNA strand there
DNA is synthesized
5' → 3'

- Remember the strands run antiparallel
- Replication "up" one strand, "down" the other

To copy the 3' → 5' strand: Polymerase makes a continuous long "Leading Strand" in the antiparallel 5' → 3' direction

To copy the 5' → 3' strand: Polymerase must make a 3' → 5' strand (IMPOSSIBLE)

Solution:
- **Lagging strand** DNA synthesis:
  - DNA polymerase must jump forward and backward as the helix unwinds
  - Short, discontinuous fragments of DNA are made 5' to 3' even though the DNA is unwinding in the other direction
  - These are called **Okazaki fragments**
    - Lagging strand synthesis is enzymatically complex (many steps involved)

DNA → RNA
Transcription

- Unwound regions of DNA can also be "copied" as RNA
- **RNA polymerase**
  - **Properties of RNA:**
    - Single-stranded (but often folded up)
    - Uracil instead of thymine (U still pairs with A)
    - Ribose instead of deoxyribose in backbone

★ Nucleotides:
DNA vs. RNA

- A, U, G, C: Phosphate group (SAME)
  - Sugar: Ribose instead of deoxyribose (2' OH group)
  - Uracil instead of thymine

Transcription
• Unwound regions of DNA can also be "copied" as RNA
• RNA polymerase
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RNA types

1. **rRNA (ribosomal)**: rRNAs bind to proteins to form the protein-producing ribosome “factories” (70S in prokaryotes)
2. **mRNA (messenger)**: mRNAs carry the protein-coding information from DNA to the ribosomes. mRNA sequence determines amino acid sequence of protein.
3. **tRNA (transfer)**: tRNAs translate the coded message of mRNAs into a different chemical polymer: proteins!

Transcription: DNA → RNA

Prokaryotic Transcription

- In eukaryotes, transcription is more complex: noncoding regions called introns must be spliced (cut) out of RNAs before they are finished
- **Prokaryotes DO NOT have introns**

Eukaryotes: transcription in nucleus; translation in cytoplasm

All 3 kinds of RNA are involved in translation

1. **rRNA**: crucial part of the ribosome which catalyzes synthesis of new proteins
2. **mRNA**: the blueprint; information-carrying molecule which dictates the amino acid sequence of a new protein
3. **tRNA**: tRNA (transfer RNA) translates nucleotide sequences into amino acid sequences

tRNA recognizes the Genetic Code

mRNA sequences code for amino acids:

Three nucleotides = One amino acid

**Codon** = 3 nucleotides
The Genetic Code

- Each amino acid may have multiple codons (degenerate)
- Some codons do NOT code for an amino acid; they mean STOP (end of protein)
- AUG = methionine = START (all mRNAs start being translated with AUG)

The Genetic Code

- Nearly the same in ALL organisms (from bacteria to humans)
- This makes genetic engineering possible
  - DNA from one organism can be put into another and will be expressed normally

tRNA

- Amino acid bound specifically matches the sequence of the anticodon
- Accurate base pairing of the anticodon with the codon of mRNA brings correct amino acid to the growing polypeptide (protein) chain
PROKARYOTES:

Many ribosomes can simultaneously be acting on the same mRNA molecule (polyribosomes) AND transcription and translation in cytoplasm; can occur simultaneously.

Mutations

• Change in DNA
• **Point mutation**: substitution of a single base

Point mutation can cause a change in amino acid sequence of a protein.

Mutations: **Frameshift**

• Frameshift mutations: deletion or insertion of nucleotides
  – Alter the amino acid sequence of *entire protein* from that point on

• NOT if in multiples of 3
  • Insertion or deletion of 3/6/9 etc. nucleotides will insert or delete one/two/three amino acids but other amino acids will remain the same

Point mutations can be *silent* (no change in amino acid sequence) because the genetic code is degenerate.

Point mutations can also produce a **stop codon**, making truncated protein.
Ultraviolet radiation

- Formation of pyrimidine dimers (such as T-T)
- Such damaged DNA cannot be replicated or transcribed normally

DNA repair

- Some bacteria are able to repair DNA damage & prevent permanent mutations
- Multiple mechanisms exist
  - **Light repair**: bonds between thymidine dimers are broken
    - Called "light" because expression of the enzyme involved is induced by light
  - **Dark repair**: see next slide

Enzymes that act on DNA

- **Nuclease**:
  - Cuts DNA backbone (breaks covalent bonds)
  - Many kinds, depends on where they work in a DNA strand, whether they cut one strand or both...
- **Polymerase**:
  - Synthesizes new DNA polymer (adds nucleotides)
- **Ligase**:
  - Pastes together broken DNA backbones (forms covalent bonds; opposite of nuclease activity)

Studying mutations: The Ames Test

- In eukaryotes, mutations can disrupt control of cell division, ultimately causing cancer
- Generally, mutagen = carcinogen
  - (a chemical that causes mutations may also cause cancer)
- Important question: how to determine if a chemical is a potential carcinogen?
Ames test

- One could expose lab animals to a chemical and wait years, looking for cancer to develop (expensive, slow)
- Or, screen for mutagenesis in bacteria! (fast, cheap)

*Ames test*

- Positive test: further study needed
- Negative test: chemical is probably safe