AP 19-21 Review

Viruses & Biotechnology
Viruses

• You need to know:
  • The components of a virus
  • The differences between lytic and lysogenic cycles.
Viruses: A virus consists of a nucleic acid surrounded by a protein coat.

- Smaller than ribosomes
  - Smallest can be less than 20 nm across
- Genomes can be double or single stranded DNA or RNA.
- The capsid is a protein shell that surrounds the genetic material.
- Some viruses have viral envelopes that surround the capsid and aid the viruses in infecting their host.
- Bacteriophages, or phages, are viruses that infect bacterial cells.
Viral Envelope

Reverse Transcriptase

Bacteriophage

Protein coat

Nucleic acid

DNA

Tail

Fibres

Influenza

Protein coat

Nucleic acid

RNA

Viral Envelope
Viruses reproduce only in host cells

- Viruses have a limited **host range**:
  - Human cold virus only infects the upper respiratory tracts of humans.
- Viral reproduction occurs only in host cells.
- 2 variations of bacteriophage reproduction
  - Lytic cycle
  - Lysogenic cycle
Viruses Lytic vs. Lysogenic

• The **lytic cycle** ends in the death of the host by rupturing (lysis) it.
  • Phage injects its DNA into host cell and takes over the host’s ‘machinery’ to make more viral DNA and protein coats.
  • These components self assemble, & eventually many new copies of the virus ‘burst out’ through the cell (causing it to lyse).
1. Attachment
2. Entry of phage DNA and degradation of host DNA
3. Synthesis of viral genomes and proteins
4. Assembly
5. Release
Viruses Lytic vs. Lysogenic

- **Lysogenic Cycle**: Bacteriophage’s DNA becomes incorporated into the host cell’s DNA and is replicated along with the host cell’s genome.
  - Viral DNA is known as a **prophage**.
  - Under certain conditions, the prophage will enter the **lytic cycle**.
The phage injects its DNA. Phage DNA circularizes. Occasionally, a prophage exits the bacterial chromosome, initiating a lytic cycle.

New phage DNA and proteins are synthesized and assembled into phages. Phage DNA integrates into the bacterial chromosome, becoming a prophage.

The cell lyses, releasing phages. The bacterium reproduces, copying the prophage and transmitting it to daughter cell.

Cell divisions produce population of bacteria infected with the prophage.
Retroviruses

- **RNA viruses** that use the enzyme reverse transcriptase to transcribe DNA from an RNA template.
  - DNA then integrates into the chromosome of host animal cell.
  - Host then transcribes **prophage** into RNA which can be used to create viral proteins and assemble new viruses which can be released to infect more cells.
    - HIV
Glycoprotein
Reverse transcriptase
Viral envelope
Capsid
RNA (two identical strands)
HIV

HOST CELL

Viral RNA
Reverse transcriptase
RNA-DNA hybrid
DNA

NUCLEUS

Proivirus
Chromosomal DNA

RNA genome for the next viral generation
mRNA

New virus

0.25 μm

HIV entering a cell

Membrane of white blood cell

HIV leaving a cell

New HIV leaving a cell

Figure 19.8
Animation: HIV Reproductive Cycle
Right-click slide / select “Play”
Viruses... Kind of: Viroids & Prions

- **Viroids** are circular RNA molecules several hundred nucleotides in length that infect plants.
  - Affect systems that affect plant growth.
- **Prions** are misfolded, infections proteins that cause the misfolding of normal proteins they contract in various animal species.
  - Mad cow disease is caused by prions.
DNA Technology & Genomics

- You must know:
  - Terminology
  - Steps in cloning
  - How PCR is possible
  - How gel electrophoresis works
DNA cloning yields multiple copies of a gene of other DNA segment—Vocab

• Process of manipulating genes and genomes.
  • Genetic Engineering

• Process of manipulating organisms or their components for the purpose of making useful products.
  • Biotechnology

• DNA that has been artificially made, using DNA from different sources—often different species.
  • Recombinant DNA

• Producing multiple copies of a specific segment of DNA.
  • Gene cloning
DNA cloning yields multiple copies of a gene of other DNA segment—VOCAB

- Derived from bacteria, these products are used to cut strands of DNA at specific locations
  - Restriction Enzymes
- Place on the DNA where restriction enzymes cut.
  - Restriction sites
- The resulting pieces of DNA made by the restriction enzymes.
  - Restriction Fragments
- Single-stranded end of DNA restriction fragment that is able to form hydrogen bonds with complimentary single-stranded pieces of DNA.
  - Sticky end
- The enzyme capable of sealing sticky ends together.
  - ligase
5 steps of cloning

1. Identify & Isolate gene of interest & appropriate cloning vector (plasmid)
2. Cut both with the same restriction enzyme
   I. Why is this important?
      I. Ensures matching sticky ends
3. Join the pieces of DNA. Form recombinant plasmids by mixing the plasmids with DNA fragments.
   I. How can these be sealed together?
      1. LIGASE
4. Get the vector into a host cell.
   I. What process have we learned where bacteria take up genetic material from their environment?
      1. Transformation
5. Select for cells that have been transformed.
   1. Antibiotic resistance gene or reporter gene (fluorescent proteins).
Cloning into a plasmid
**Gene Cloning Process**

1. **Isolation of plasmid DNA and DNA containing gene of interest**
2. **Gene inserted into plasmid**
3. **Plasmid put into bacterial cell**
4. **Cells cloned with gene of interest**
5. **Identification of desired clone**
6. **Various applications**

**Applications:**
- Gene for pest resistance inserted into plant
- Gene used to alter bacteria for cleaning up toxic waste
- Protein dissolves blood clots in heart attack therapy
- Human growth hormone treats stunted growth
- Copies of gene isolated and transferred to other organisms

**Outcome:**
- Recombinant bacterium
- Recombinant DNA (plasmid)
After cloning

- **Nucleic acid hybridization:**
  - Synthesize a radioactive tracer (tracker) probe complimentary to a known sequence in the gene of interest
- Can be used to generate a **genomic library**: made up of 1000’s of recombinant plasmid clones, each containing a piece of the original genome being studied.
  - A cDNA library is made up of complementary DNA made from mRNA and reverse transcriptase.
• **PCR—Polymerase Chain Reaction** is a method used to greatly amplify a particular piece of DNA without the use of cells. PCR is used to amplify DNA when the source is impure or small (crime scene).
PCR

- So how does we do it?
  - We recreate the environment within a cell in a test tube, including:
    1. **Template DNA**
       - That we want to amplify
    2. **PCR Buffer**
       1. containing a mixer of salts that creates an environment similar to that inside the cell
    3. **dNTPs** $\rightarrow$ Adenosine, Thymine, Cytosine, Guanine
       1. DNA building blocks
    4. **Forward and Reverse Primers**
       1. Need to tell enzyme where to start to replicate
       2. binds to DNA at regions on either side of the region that we want to replicate
    5. **Polymerase Enzyme** $\rightarrow$ Taq
PCR

5. Polymerase Enzyme → Taq
   • Enzyme that replicates DNA
   • Taq comes from bacteria Thermophilus aquaticus
     • Where do you think it likes to live?
       • hot water
     • So why use Taq and not our own polymerase?
       • Only difference between Taq and the polymerase in your cell is that Taq can be heated to 96°C without being destroyed, where our polymerase is destroyed at temperature around 50°C. What temperature does it like? - 37°C
PCR Denaturing

• Step 1: Denaturing is the first step in PCR, in which the DNA strands are separated by heating to 95°C.
PCR Primers

- Primers range from 15 to 30 nucleotides, are single-stranded, and are used for the complementary building blocks of the target sequence.

- A primer for each target sequence on the end of target DNA is needed.
  - Allows both strands to be copied simultaneously in both directions.

TTAACGGCCTTAA ... TTTAAAACCGGTT
AAATTGCCGGAAATT ...........>

and

< ............ AAAATTGGCCAA
TTAACGGCCTTAA ... TTTAAAACCGGTT
PCR Primers

• The primers are added in excess so they will bind to the target DNA instead of the two strands binding back to each other.
PCR Annealing

- **Annealing** is the process of allowing two sequences of DNA to form hydrogen bonds.
- The **annealing** of the target sequences and primers is done by cooling the DNA to 55°C.
PCR Taq DNA Polymerase

- Taq stands for Thermus aquaticus, which is a microbe found in 176°F hot springs in Yellow Stone National Forest.

- Taq produces an enzyme called DNA polymerase, that amplifies the DNA from the primers by the polymerase chain reaction, in the presence of Mg.
PCR Cycles

Initial Mix

Room Temperature

Forward Primer
Reverse Primer
Deoxynucleotides

AGCT
PCR Cycles

1st cycle - Denaturation Step

T=94°C - 1 min

Taq DNA polymerase
PCR Cycles

1st cycle - Annealing Step

T=55°C - 1 min
1st cycle - Elongation Step

T=72°C - 3 min
PCR Cycles Review

- Denaturation: 94° - 95°C
- Primer Annealing: 55° - 65°C
- Extension of DNA: 72°
- Number of Cycles: 25-40
Polymerase Chain Reaction (PCR)

Template DNA

Denature (96°C)

Primer Annealing

Extension (72°C)

Single PCR cycle

5’ – ACGTACGTAGCGATGCTAGCTGACACTGACTG – 3’
3’ – TGCATGCATCGCTACGATCGACTGTGACTGAC – 5’

5’ – ACGTACGTAGCGATGCTAGCTGACACTGACTG – 3’
3’ – TGCATGCATCGCTACGATCGACTGTGACTGAC – 5’

5’ – GTACG – 3’
3’ – TGCATGCATCGCTACGATCGACTGTGACTGAC – 5’

5’ – ACGTACGTAGCGATGCTAGCTGACACTGACTG – 3’
3’ – TGCATGCATCGCTACGATCGACTGTGACTGAC – 5’

5’ – GTACGTAGCGATGCTAGCTGACTGACACTGACTG – 3’
3’ – TGCATGCATCGCTACGATCGACTGTGACTGAC – 5’
Polymerase Chain Reaction (PCR)

Template DNA

2nd PCR cycle

Denature (96°C)

Primer Annealing

Extension (72°C)
Polymerase Chain Reaction (PCR)

35 cycles

$1 \rightarrow 2 \rightarrow 4 \rightarrow 8 \rightarrow 16 \rightarrow \cdots \rightarrow 34,359,738,368$
Animation 20-3 Polymerase Chain Reaction
PCR Requirements

- Magnesium chloride: .5-2.5mM
- Buffer: pH 8.3-8.8
- dNTPs: 20-200µM
- Primers: 0.1-0.5µM
- DNA Polymerase: 1-2.5 units
- Target DNA: ≤ 1 µg
DNA Amplification Using Polymerase Chain Reaction

Reaction mixture contains target DNA sequence to be amplified, two primers (P1, P2) and heat-stable Tag polymerase.

Reaction mixture is heated to 95°C to denature target DNA. Subsequent cooling to 37°C allows primers to hybridize to complementary sequences in target DNA.

When heated to 72°C, Tag polymerase extends complementary strands from primers.

First synthesis cycle results in two copies of target DNA sequence.

Second synthesis cycle results in four copies of target DNA sequence.

Source: DNA Science, see Fig. 13.
DNA Technology allows us to study the sequence, expression, and function of a gene.

**Gel Electrophoresis:**
- Separates DNA & Proteins on the basis of size and charge.
- Uses electrical current.
- Negative charged phosphate draws the DNA toward a positive pole.
- Gel allows smaller molecules to move more easily, allowing the DNA fragments to be separated by size.
DNA Technology allows us to study the sequence, expression, and function of a gene

- Southern Blotting
  - Combines gel electrophoresis and nucleic acid hybridization
  - Allows researchers to find specific human genes
  - Specific enough to differentiate between alleles
    - Ie. Normal Hb & sickle cell.
DNA Technology allows us to study the sequence, expression, and function of a gene

DNA Microarray assays:

- Allows genome-wide studies of gene expression.
  1. Small amounts of ssDNA fragments representing different genes are fixed to a glass slide in a tight grid, termed a DNA chip.
  2. All mRNA from the cell being tested are isolated and converted to cDNA by reverse transcriptase, and tagged.
  3. The cDNA binds to the ssDNA on the chip, indicating which genes are “on” in the cell (actively producing mRNA).

- The dye alerts the researcher, allowing them to see differences in gene expression.
  - As in the difference between the genes being expressed in noncancerous tissues and cancerous tumor cells.
Genomes and Their Evolution

• Scientists use bioinformatics to analyze genomes and their functions.
  • Bioinformatics is the use of computers, software, and mathematical models to process and integrate the incredible volume of data from these sequencing projects.
  • In addition to DNA sequences, protein interactions are analyzed in an approach called proteomics.

• Systems Biology aims to model the behavior of entire biological systems, and is enhanced by bioinformatics.
  • This has many applications, including medical ones—for example, in the understanding and treatment of cancer.
Gene Density and Noncoding DNA

- Humans and other mammals have the lowest gene density, or number of genes, in a given length of DNA.
- Multicellular eukaryotes have many introns within genes and noncoding DNA between genes.
How many genes does the human genome have?

• Only about 20,000 genes...
Evolution

• How might genes with novel functions evolve?

• Duplication events can lead to the evolution of genes with related functions, such as those of the alpha-globulin and beta-globulin gene families.

• Mutations and transpositions can occur, and nonfunctional, pseudogenes may be found in clusters. Ultimately, new genes with new functions may occur.
Evolution of Genes with Related Functions: The Human Globin Genes

• The genes encoding the various globin proteins evolved from one common ancestral globin gene, which duplicated and diverged about 450-500 million years ago

• After the duplication events, differences between the genes in the globin family arose from the accumulation of mutations
Figure 21.14

Ancestral globin gene

Duplication of ancestral gene

Mutation in both copies

Transposition to different chromosomes

Further duplications and mutations

α-Globin gene family on chromosome 16

β-Globin gene family on chromosome 11

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Subsequent duplications of these genes and random mutations gave rise to the present globin genes, which code for oxygen-binding proteins.

The similarity in the amino acid sequences of the various globin proteins supports this model of gene duplication and mutation.

### Table 21.2 Percentage of Similarity in Amino Acid Sequence Between Human Globin Proteins

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<th>β-Globins</th>
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<td>α</td>
<td>ζ</td>
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<td>α-Globins</td>
<td>—</td>
<td>58</td>
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<tr>
<td>ζ</td>
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<tr>
<td>β-Globins</td>
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<td>38</td>
</tr>
<tr>
<td>ε</td>
<td>37</td>
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</table>
Evolution of Genes with Novel Functions

- The copies of some duplicated genes have diverged so much in evolution that the functions of their encoded proteins are now very different.

- For example, the lysozyme gene was duplicated and evolved into the gene that encodes α-lactalbumin in mammals.

- Lysozyme is an enzyme that helps protect animals against bacterial infection.

- α-lactalbumin is a nonenzymatic protein that plays a role in milk production in mammals.
Comparing Developmental Processes

• Evolutionary developmental biology, or evodevo, is the study of the evolution of developmental processes in multicellular organisms.

• Genomic information shows that minor differences in gene sequence or regulation can result in striking differences in form.
Widespread Conservation of Developmental Genes Among Animals

• Molecular analysis of the homeotic genes in *Drosophila* has shown that they all include a sequence called a **homeobox**

• An identical or very similar nucleotide sequence has been discovered in the homeotic genes of both vertebrates and invertebrates

• Homeobox genes code for a domain that allows a protein to bind to DNA and to function as a transcription regulator

• Homeotic genes in animals are called **Hox genes**
Figure 21.18

Adult fruit fly

Fruit fly embryo (10 hours)

Fly chromosome

Mouse chromosomes

Mouse embryo (12 days)

Adult mouse
Adult fruit fly

Fruit fly embryo (10 hours)

Fly chromosome
Figure 21.18b

Mouse chromosomes

Mouse embryo (12 days)

Adult mouse
• Related homeobox sequences have been found in regulatory genes of yeasts, plants, and even prokaryotes
• In addition to homeotic genes, many other developmental genes are highly conserved from species to species
• Sometimes small changes in regulatory sequences of certain genes lead to major changes in body form
• For example, variation in Hox gene expression controls variation in leg-bearing segments of crustaceans and insects
• In other cases, genes with conserved sequences play different roles in different species
Figure 21.19

Thorax  Genital segments  Abdomen

Thorax  Abdomen

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Comparison of Animal and Plant Development

• In both plants and animals, development relies on a cascade of transcriptional regulators turning genes on or off in a finely tuned series.
• Molecular evidence supports the separate evolution of developmental programs in plants and animals.
• *Mads-box* genes in plants are the regulatory equivalent of *Hox* genes in animals.