Gene Expression, Viral and Bacterial Genetics, and Biotechnology

Eukaryotic Gene Regulation
- Methods of Controlling Gene Expression
  - Pre-transcriptional control
    - Regulatory genes can promote or inhibit the transcription of certain genes.
    - Activators and repressors are used to influence how easily RNA polymerase will attach to a promoter
    - Very similar to systems in prokaryotes
  - Post-transcriptional control
    - Processing of mRNA by removal of introns
  - Post-translational control
    - Modification of protein (often at the Golgi)
  - Short interfering RNA’s (siRNAs), short fragments of RNA
    - block mRNA transcription (by binding to original DNA)
    - block mRNA translation (by binding to mRNA)
    - degrade RNA (by combining with enzymes)
- Genetic, chemical, and other environmental influences are constantly altering the regulation of gene expression throughout an organism’s life

Viral Genetics
- Viruses are parasites of cells
  - They can not reproduce alone because they have no metabolism... must find a host
  - Viruses are specific to the types of cells they parasitize.
    - EX: bacteriophages, or phages for short, only attack bacteria.
- Structure
  - Viruses have a nucleic acid (either DNA or RNA)
  - Capped: protein coat that surrounds the nucleic acids
  - Some viruses have an envelope to help penetrate a host cell
    - Envelope is made of phospholipids and proteins

Bacteriophage Reproduction
- Bacteriophage: viruses that infect bacteria
- There are 2 basic cycles that bacteriophages follow:
  1. Lytic cycle
    - The virus inserts itself into the host cell
    - The viral DNA “takes charge” and uses the host cell to make copies of the virus
    - The viruses evert from the cell, killing the cell in the process (the cell lyses)
    - New viruses infect other cells and repeat the process.
  2. lysogenic cycle
    - The virus incorporates its DNA into the host DNA but then sits dormant.
    - The dormant virus is called a prophage (or provirus if the virus is not a bacteriophage)
    - It remains inactive until some trigger, usually from the environment, causes the virus to begin the lytic cycle.
    - When the viral DNA leaves the bacterial chromosome, sometimes pieces of bacterial DNA go with it (transduction)

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Other Viruses
- Animal viruses reproduce in multiple ways
- Viruses infect primarily vertebrates
- Insects often act as vectors (intermediates) by transferring viruses to vertebrates

- Retroviruses - viruses that use RNA to carry their genetic information instead of DNA.
- Reverse transcriptase - retroviruses contain an enzyme that copies their RNA into DNA.
  - This DNA can then be integrated into the host cell's DNA and the lysogenic cycle can proceed.
  - HIV is an example of a retrovirus.

Other Viruses
- Viruses are classified based on their genetic material:
  - DNA
  - RNA

- HIV (Human Immunodeficiency Virus)
- Prions
- Viroids
- Prions are infectious proteins that cause degenerative brain diseases
- Prion disease is the most common cause of spongiform encephalopathies in humans

Viroids
- Viroids are circular molecules of RNA only a few 100 nucleotides long
- Infect plants
- Viroids do not code for proteins but use host cell genes to replicate and cause errors in plant genetic regulatory systems
- Symptoms of infection:
  - Abnormal development
  - Stunted growth

Prions
- Prions are infectious proteins that cause degenerative brain diseases
- Transmission:
  - Most likely transmitted through food
  - Incubation period of at least 10 years
  - Virtually indestructible
- Method of infection:
  - Prions are misfolded proteins that seem to cause normal proteins to misfold. Proteins begin to aggregate into chains which interfere with normal functioning.
- Examples:
  - Mad cow disease
  - Scrapie (sheep)
  - Kuru (humans)
Bacterial Genetics
- Bacteria are prokaryotes
  - they do not have a nucleus or organelles
  - they do have ribosomes
- In some bacteria, a capsule (sticky outer coat) surrounds the cell wall for protection. The capsule helps to "glue" bacteria to surfaces within human body.
- Bacterial shapes
  - Bacilli - rod shape
  - Cocci - spherical
  - Spirilla - spiral or corkscrew shaped

How bacteria get new genes
- There are 3 ways that bacteria swap DNA:
  1. Transformation
  2. Transduction
  3. Conjugation
- All of these increase genetic diversity

1. Transformation - when a bacteria takes up foreign DNA fragments through its cell wall and expresses that DNA
   - EX: Griffith & Avery experiment w/mice

2. Transduction - when viruses transfer not only viral DNA but bacterial DNA to a host via infection
   - Types
     1. General transduction - during the lytic cycle, fragments of bacterial DNA are inserted into phage capsids
     2. Specialized transduction - as a cell leaves the lysogenic cycle and enters the lytic cycle, prophage (or provirus) DNA may take some bacterial DNA with it. This DNA will be replicated along with viral DNA and passed on to all phages
How bacteria get new genes

3. **Conjugation**: direct transfer of DNA between two bacteria
   - Also called sex-duction (sexual reproduction)
   - Donor "male" extends cytoplasmic tubes called pilli which attach to the "female" to create a mating bridge
   - Plasmids are transferred from "male" to "female" and may be incorporated into the female’s bacterial chromosome
   - The ability to be "male" (form a sex pil and donate DNA) results from a special piece of DNA called an F-factor
   - Cells with the F-factor are called F+ and cells without it are called F-
   - After conjugation, both cells are F+
   - The "female" becomes "male" and can carry on the process

**Gene Regulation in Bacteria**

- Gene regulation has been studied the most in bacteria.
- 2 major types of systems
  1. **Repressible systems**: "on" unless a co-repressor is present
  2. **Inducible systems**: "off" (not producing proteins) unless an inducer is present

**Gene Regulation in Bacteria - The Trp Operon**

Remember:
- If a gene is "off", the protein will not be produced because the repressor is active
- If the gene is "on", the protein will be produced because the repressor is inactive due to the inducer

The Trp operon is an example of a repressible system:
- This gene is normally "on" and producing tryptophan because the repressor is inactive
- When tryptophan levels get too high, it acts as a co-repressor and binds to the repressor, making it active
- This stops transcription
**Gene Regulation in Bacteria - The Lac Operon**

Remember:
- If a gene is "off", the protein will not be produced because the repressor is active.
- If the gene is "on", the protein will be produced because the repressor is inactive due to the inducer.

The Lac operon is an example of an inducible system.

- The gene is normally "off". If an inducer (allo-lactose) is present, the gene will turn "on" and produce enzymes that will digest lactose.

**Biotechnology**

- Biologists utilize their knowledge of genetics, inheritance, and DNA to manipulate it for useful purposes.
  - Human Genome Project
    - locate, map, and sequence every gene of the human genome (over 50,000 genes)
  - Medicine
    - Diagnosis and treatment of genetic diseases
    - Gene therapy - replacing mutated genes with healthy genes
    - Producing human proteins and drugs
    - Human DNA is spliced into bacterial DNA to create bacteria that produce human proteins
  - Modifying plants and animals
    - Creation of transgenic crops and animals for resistance to pests, faster growth, etc...
  - Science of identification
    - Determine familial relationships
    - Determine evolutionary relationships

**DNA Sequencing**

- Machines are capable of automated DNA sequencing.
- They are loaded with four nucleotides (A, T, C, G) that fluoresce when they bind with a complementary base.
- The DNA being sequenced is fed through the machine and the machine reads the glowing bases in order.
- The human genome and the genomes of many other organisms have been sequenced in this way.

**Gene Therapy**

- Gene therapy - an attempt to change the DNA in a human to "correct" it.
- Scientists have attempted to do this by creating a recombinant virus capable of changing the DNA in specific human cells.
- One of the first trials with gene therapy involved children with SCID (bubble boy syndrome)
  - Several of the children cured developed leukemia.

**Restriction Enzymes**

- Restriction enzymes cut the DNA at predictable sequences (called restriction sites).
- This produces what is known as restriction fragments.
- Restriction fragments have "sticky ends" that are capable of binding to other fragments that have been cut with the same restriction enzyme.

**Recombinant DNA**

- Recombinant DNA - DNA from two organisms that has been combined.
  - Methods of Natural Recombination
    - Viral transduction
  - Bacterial conjugation
  - Methods of Artificial Recombination
    - Recombinant DNA Technology
  - Artificial Recombination
    - Scientists have inserted DNA from animals into other animals, animals to plants, plants to plants, plants to bacteria, etc...
    - An organism containing recombinant DNA is known as a "transgenic" organism
    - It allows us to make bacteria that will produce human proteins, such as growth hormone or insulin.
    - This has also allowed us to make genetically modified foods that possess traits such as a longer shelf life, more/new vitamins, and disease resistance.
How to make recombinant bacteria - let’s make bacteria that glows!
1. The “gene of interest” (for phosphorescence) is cut out using restriction enzymes.
2. A bacterial plasmid is cut using the same restriction enzymes.
   - This produces sticky ends ends of DNA that will bind with each other.
   - The plasmid also contains a gene for antibiotic resistance.
3. The gene of interest and the plasmid are mixed. The enzyme ligase will permanently bind the sticky ends to each other.
4. The bacteria is tricked into taking up the plasmid (transformation) from the environment.
5. The bacteria will now express the protein (it glows!).
6. The bacteria that are recombinant can be selected for by growing the bacteria on petri dishes with antibiotics.

How to make recombinant DNA
- Gel electrophoresis: a process that allows us to identify individuals for crime or paternity cases, among other uses.
- Steps - Overview

Gel Electrophoresis
- Gel electrophoresis: a process that allows us to identify individuals for crime or paternity cases, among other uses.
- Steps
1. DNA must first be chopped up into specific pieces by restriction enzymes.
   - The locations of these sequences vary from person to person.
   - The variation in sizes of the fragments from each person are called restriction fragment length polymorphisms (or RFLPs).
2. The DNA fragments are placed into wells in an agarose gel which is covered in a buffer.
3. Electricity is then applied.
   - DNA is negatively charged, causing the fragments to migrate to the + end.
   - Smaller fragments move farther than larger ones, creating bands of DNA at different locations.
4. The bands are then stained for observation.
Polymerase Chain Reaction (PCR) - a process that makes many copies of DNA in a short period of time.
- Useful if only a tiny amount of DNA is found at a crime scene.
- Process is very similar to DNA replication in a cell.

Steps
1. Strand of DNA to be copied is placed in a test tube with the following:
   - primers to signal where to start copying
   - extra nucleotides
   - Taq polymerase (a DNA polymerase that is stable at high temps).
2. The DNA is heated up to separate the DNA strands (instead of helicase) and the DNA is copied.
3. It cools down and then heats back up again, allowing an exponential amount of DNA to be made.

Cloning
- Isolate DNA and desirable gene that codes for "signature".
- Nucleus with altered DNA
- Embryo
- Insert modified DNA
- Unfertilized pig egg cell
- Remove nucleus
- Cell nucleus
- Cytoplasm
- Pig cell

Signature of cell