Molecular Biology
DNA REPLICATION

- Bacteria and viruses enabled scientists to learn about DNA.

- **Griffith**:
  - worked with bacteria that caused pneumonia in mammals
  - worked on 2 strains of bacteria:
    1) smooth strains: encased in a capsule and therefore pathogenic
    2) rough strain: lacked a capsule and therefore could be destroyed by the host immune system
  - his experiment showed transformation: when DNA from one source is assimilated into another source, causing a “transformation”/change in genotype and phenotype
  - he didn’t know it was DNA that was being transferred

- **Avery**: first to suggest that DNA was the agent being transferred.
- **Most scientists didn’t embrace Avery’s idea and thought that proteins were the source of genetic information.**

- **Hershey and Chase**:
  - Proved DNA, not proteins, was responsible for transferring genetic information in their work with bacteriophage/phages—viruses that infect bacteria
  - A virus is just DNA or RNA in a protein coat called capsid.
  - Knew that both proteins and DNA contained the element CHON; proteins also have S and DNA also has P.
  - Using 2 different radioactive tags (\(^{32}\)P for the bacteriophages’s DNA and \(^{35}\)S for the bacteriophage’s protein coat)
  - Incorporated the tags into the T2 bacteriophage that infects E. coli bacteria
  - Then let the bacteriophage infect E. coli and saw that P appeared in the E.coli → proved that the T2 bacteriophage injected its DNA into the host.

Phages: viruses that infect bacteria.

\(^{35}\)S: radioactive substance was given to batch 1; it's found in protein.

\(^{32}\)P: radioactive substance was given to batch 2; it's found in DNA.
- **Chargaff**: first scientist to notice that the percentages of the nitrogenous bases A and T in DNA were very close in number (~30%) and so were the C and G (~20%).
- **Chargaff's rules**: A=T and C=G equalities
- **Watson and Crick**:
  - Discover DNA's true shape: double helix.
  - By using X-ray crystallography images taken by Rosalind Franklin (a colleague of Wilkins)
  - Finally were able to build the correct model of DNA with phosphate and sugars (deoxyribose) along the sides the ladder and the N-bases along the rungs
  - Purine adenine always paired with pyrimidine thymine; other purine guanine always paired with the pyrimidine cytosine
- DNA to replicate → H-bonds between the N-bases must first be broken.

- Each chain acts as a template for the formation of a new complementary strand.
- One at a time, nucleotides line up along the template strand according to the base-pairing rules.

- **Semiconservative model**: when a double helix replicates, each of the 2 daughter molecules will have one old strand and one new strand.
DNA replication was first studied in *E.coli* bacteria because they have a single circular chromosome called the **nucleoid** (5 million base pairs) and they can undergo DNA replication and divide in less than an hour.

- **Human** has 46 chromosomes, 6 billion base pairs, and DNA takes few hours to replicate and divide.

**Origin of replication**: in prokaryotes DNA replication begins here, it proceeds in both directions along the circular DNA molecule/nucleoid until the entire molecule is copied.

- In eukaryote, DNA is linear and there are hundreds to thousands of origins of replication.
- The reason for having so many origins of replication is to increase the speed copying the very long eukaryotic DNA.

The opening up of the 2 strands of DNA form bubbles that eventually fuse together forming 2 daughter DNA strands.

**Replication forks**: point where each bubble forms along the DNA is shaped like the letter Y.

**DNA polymerase**: enzyme that add nucleotides to the DNA strand; it pairs up the correct N-base of the nucleotide to its complementary partner along the template strand.

**Nucleoside triphosphate**: energy that resembles ATP and drive this reaction; They are basically nucleotides, but instead of 1 phosphate group, they have 3 phosphate groups like ATP.

- This reaction is exergonic.
- The 2 DNA strands are **antiparallel** in that their sugar-phosphate backbones run in opposite directions.
- We number the carbon of deoxyribose 1’- 5’.
- At one end called the 5’ end a phosphate group is attached.
- The other end called the 3’ terminates with a hydroxyl (−OH) group.
- With DNA replication, the growing strand grows in its 5’→3’ direction ONLY!

**Leading strands**: new daughter strand/complementary strand use the 3’→5’ template to grow off from.

**Lagging strand**: new daughter strand/complementary strand that grows the other side of the replication fork and opposite to the leading strand in a 5’→3’ direction with the aid of DNA polymerase.

**Okazaki fragment**: short segments on the lagging strand.
DNA ligase: enzyme that links Okazaki fragments.

Eventually, all replication bubbles fuse to form two new strands of DNA on the inside.

A replication bubble showing old DNA strands in blue, and newly synthesized DNA strands in red. The new strand is made only in the 5' to 3' direction.

Eventually, all replication bubbles fuse to form two new strands of DNA on the inside.
- **DNA replication:**
  - **DNA helicase:** first breaks the H-bonds to unzip and unwind the double helix.
  - **Single-strand binding proteins:** hold open the unwound DNA molecule.
  - **Primase:** arrives on the template to “prime”/start building the daughter strand. It is a unique enzyme because it doesn’t add DNA nucleotides to the daughter strand, but instead adds about 10 RNA nucleotides in a 5’ → 3’ direction.
  - **RNA primer:** the pieces after the RNA primase finished building the daughter strand.
  - Each of these RNA primers is eventually replaced with DNA by a second different DNA polymerase.
  - When the primers are removed in the lagging strand, the gaps get filled in by the 2nd DNA polymerase and DNA ligase.
  - **DNA ligase:** last enzyme that links the Okazaki fragments of the lagging strand.
- With DNA replication, errors in matching up base pairs is extremely rare—one error per billion nucleotides.
- **DNA polymerase** is able to proofread mistakes that are made during replication. It often immediately replaces a nucleotide if the wrong one is added then resumes replication.
- Chemicals, radioactivity, x-rays, and UV can change/cause mutations to existing DNA.
- **Nuclease:** repair enzymes that correct the damaged DNA; it cuts out the damaged DNA segment.
- **DNA polymerase** filled the gap; **DNA ligase** sealed the segments together.
- No enzymes are able to replace the last fragment on the very end of the DNA molecule → repeated replications of DNA produce shorter and shorter DNA molecules.

To solve this problem, eukaryotes have telomeres on the ends of their DNA.
- **Telomeres:** segments at the ends of a DNA molecule that do not contain genes, repeat the sequence TTAGG 100’s to 1,000’s of times, and protect the organism’s genes/DNA from being eroded away through successive rounds of DNA replication.
- While the telomeres do shorten with increased divisions, the genes that code for traits are unaffected.
- **Telomerase:** enzyme that helps to lengthen the telomere ends, but it only presents in germ line cells that form eggs and sperm.
- Scientists are beginning to question if an organism’s life span is limited by the length of telomeres because eventually the shortening DNA will hit genes.
**PROTEIN SYNTHESIS**

- **Garrod:**
  - Suggested that a specific gene codes for the making of a specific enzyme. If a person lacked a particular enzyme (due to mutation), this accounted for symptoms of inherited diseases.
  - His idea lacked support and he was ahead of his time

- **Beadle and Tatum:**
  - Began research using mutants of red bread mold called *Neurospora*
  - His ideas was one gene controlling one enzyme
  - Found that the wild type *Neurospora* could grow on minimal media/agar (salt, sugar, and one vitamin).
  - 3 mutant strains called auxotrophs could not survive on minimal media because they lacked a gene that coded for a particular enzyme in the pathway to making the final product- the amino acid arginine.
  - Came up with the “one gene, one enzyme hypothesis” → later was refined to the “one gene, one protein/polypeptide hypothesis.”

- Genes do not build proteins directly.
- The bridge between DNA and protein synthesis is RNA.
- RNA is always a single strand
- RNA has ribose sugar, instead of deoxyribose and has the nitrogenous base uracil in place of thymine.
- **Transcription** involves using DNA as a template to build mRNA.
  - This messenger then gets modified before leaving the nucleus → then arrives at a ribosome.
  - Once mRNA is at the ribosome, translation can occur.
  - DNA/RNA’s message is written in the “language” of 4 nucleotides whereas proteins are written in the “language” of 20 amino acids. ∴ the message needs to be translated between the 2 languages. It is also during this translation that amino acids are linked together forming protein/polypeptide.
  - Only one half of DNA is used in the making of mRNA.
  - mRNA is complementary rather than identical to its DNA template.
- **The codons of the mRNA are translated into the amino acids above.**
• The genetic code is nearly universal and shared by everything from bacteria to plants and animals→ this shared language provides evidence of a common ancestor of all modern organisms.

• Because the genetic code is the same in all organisms, we can insert human genes into bacteria and make many important proteins for medical uses (ex: insulin and HGH).

• Transcription:
  - Making of RNA
  - RNA polymerase: enzyme responsible for pulling DNA apart and bringing in RNA nucleotides
  - 3 types of RNA:
    1) mRNA (messenger)
    2) rRNA (ribosome)
    3) tRNA (transfer)
    - Each type of RNA is built by a different RNA polymerase
    - Broken into three parts: initiation, elongation, and termination.
  1) Initiation:
    - Transcription factors: collection of proteins that bind to the TATA box on the DNA molecule.
    - TATA box: promoter region of the DNA molecule and it is where RNA polymerase will bind.
  2) Elongation:
    - RNA polymerase moves along the DNA molecule using the 3’→5’ as the template so that it can grow in its 5’→3’ direction.
    - As RNA polymerase moves along the DNA template it brings in the correct complementary RNA nucleotides.
    - After RNA polymerase moves past a region of the DNA molecule, it reforms the double helix.
    - Usually there are several RNA polymerases on a DNA molecule at a time→ this increases the number of mRNA molecules being made.
  3) Termination:
    - RNA polymerase keeps adding RNA nucleotides to the growing mRNA until it transcribes a terminator (AAUAAA) and the mRNA is cut free from the enzyme.

• Modification:
  - Before mRNA can leave the nucleus, it gets modified/RNA processing.
  - The 5’ end of the mRNA gets a 5’ cap- which is just a modified form of guanine.
  - The 3’ end of the mRNA molecule gets a poly-A tail: made of 30-200 adenines.
  - The functions of adding the “cap and tail” molecules to the ends of the mRNA are to protect the mRNA attach itself to a ribosome.
  - Spliceosome: modified mRNA by RNA splicing.
    - RNA splicing: cut and paste job of the mRNA molecule.
    - The mRNA molecule starts out very long, the spliceosome “cuts” out the introns.
  - Introns: noncoding segments that do not code for amino acids
    - Once all the introns are cut out, spliceosome pastes together the exons: coding segments (expressed).
Now mRNA is altered and has left the nucleus, it arrives at a ribosome.  

Ribosomes are made up of 2 parts:  
1) Large subunit  
2) Small subunit  
- The 2 subunits are made of rRNA and protein.  
- Only when the 2 subunits come together and mRNA arrives is the ribosome “functional”.  
- Each ribosome has 3 binding sites for tRNA:  
  1) P site (peptidyl-tRNA binding site): holds the growing protein  
  2) A site (aminoacyl-tRNA binding site): holds the tRNA bearing the next amino acids  
  3) E site (exit site): the “empty” tRNA exits from the ribosome.

The function of tRNA is to transfer amino acids from the cytoplasm to a ribosome.  
- Within the cytoplasm of the cell are all 20 amino acids.  
- tRNA molecules are not all identical.  
- Laid out flat, a tRNA looks like a clover leaf, when allowed to go into its natural configuration, tRNA is L shaped.  
- Anticodon: a segment on the end of the tRNA that varies from one tRNA to the next; it will bind to mRNA’s codon according to the base pairing rules.  
- On the other end of the tRNA molecule (3’ end) is where a specific amino acids binds.  
- If one tRNA variety existed for each of the mRNA’s codons, there would be 61 tRNAs; but there are only 45 because of wobble effect.  
  - Wobble effect: anticodons can recognize 2 or more different codons because the 3rd base of the codon is not very strict. Ex: GGU, GGC, GGA, and GGG all code for the amino acid glycine.  
  - Wobble effect explains why the synonymous codons for a given amino acid can differ in their 3rd base, but not usually in their other bases.
Translation:
- Has 3 stages: initiation, elongation, and termination
- All 3 stages require translation factor
- GTP (guanosine triphosphate): a molecule similar to ATP is used to energize 2 of these 3 stages

1) Initiation:
- Brings together mRNA, tRNA bearing the first amino acid of the polypeptide and the 2 subunits of a ribosome.
- Start codon on mRNA is AUG → anticodon on tRNA has to be UAC.
- Codon and anticodon forms a weak hydrogen bond.
- tRNA brings in the amino acid methionine
- once the start codon is initiated, the large ribosomal subunit completes the initiation complex
- then the tRNA is in the P site of the ribosome and ready to accept the next tRNA bearing an amino acid in the A site

2) Elongation:
- Next tRNA comes into the A site where its anticodon forms a H-bond with codon of mRNA
- Then a covalent peptide bond forms between the amino acid in the P site and the newly arrived amino acid in the A site
- The formation of the peptide bond moves the growing polypeptide chain to the newly arrived tRNA
- causes the tRNA in the P site to move or translocate to the E site
- tRNA in the A site to translocate to the P site.
- The cycle repeats.

3) Termination:
- Elongation of the growing polypeptide continues until a stop codon (UAA, UAG, and UGA) is encountered on mRNA
- Release factor: a protein that frees the polypeptide and the ribosome, mRNA, and tRNA complex breaks apart.
- This translation process takes place in less than a minute.
- There are several ribosomes working on translating the same message at the same time.
- Polyribosome: a string of ribosomes making many proteins
- Once the polypeptide is made, it begins to fold spontaneously into the secondary and tertiary structures with the aid of chaperone proteins.
- Attachments may be made like the addition of glycol- or lipo-.
- Or, 2 or more tertiary structures may be joined to form a tertiary structure.
- Occurs at the Golgi apparatus

2 types of ribosomes:
1) Free ribosomes: free in the cytosol, mostly make proteins that remain in the cytosol and function there.
2) Bound/attached ribosomes: attached to the endoplasmic reticulum, make proteins of the endomembrane system (nuclear envelope, ER, Golgi, lysosomes, vacuoles, and plasma membrane) and proteins that get released from the cell.
- Translation always begins on free ribosomes and the growing polypeptide may signal the ribosome to attach to the ER.
- Once at the ER, the protein usually goes into the cisternal space of the ER where it will get folded and then get secreted from the cell.
- Mutation types:
  a) Segment of chromosome
  b) Entire chromosome (nondisjunction)
  c) Entire set (polyploidy)
• **Mutations**: changes in the genetic material of a cell.
• **Point mutations**: changes in just one or a few base pairs. Ex: sickle cell anemia—a mutation of just 1 base pair in the gene that codes for hemoglobin.
• **Types of Point Mutation**:
  A) **Base-pair substitutions**:
  - Replacing 1 nucleotide and its partner with another pair of nucleotides.
  1) **Silent mutations**: codes for the same amino acids due to the wobble effect.
  2) **Missense mutations**: codes for a different amino acid.
  3) **Nonsense mutation**: codes for a stop codon.
  B) **Insertions and Deletions**: the gain or loss of one more nucleotide pairs
  1) **Frameshift causing extensive missense**: one nucleotide lost or gained changes every amino acid after it due to a shift in the reading frame.
  2) **Frameshift causing immediate nonsense**: one nucleotide lost or gained codes for a stop codon.
  3) **Insertion or deletion of 3 nucleotides**: no extensive frameshift: results in the loss or gain one amino acid and the protein.
• Mutations can occur during DNA replication, during the cell cycle, etc.
• **Mutagens**: mutation caused by an outside and/or environmental factor. Ex: physical and chemical agents like cell phones, plastics in the microwave, microwave popcorn, burn food, diet food (aspartame), red meat, plastic water bottle, etc.

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**VIRUSES & BACTERIA**

- Tiny in contrast to bacteria
- Are nucleic acid (DNA or RNA) enclosed in a **capsid**—a protein coat
- Some have a viral envelope that helps them infect their host
- These viral envelopes are usually derived from the host cell to help the virus be unnoticed by the immune system—made up of phospholipids and glycoprotein.
- Some viruses carry a few enzymes in their capsids.
- Come in many shapes like rod shaped or more complex in structure.
- Most complex viruses called bacteriophage/phage infect bacteria.
- Can ONLY reproduce within a host cell
- When a person with a cold sneezes, the viruses released are not “activated” until a host, namely another person, gets the virus into their own body because viruses lack enzyme for metabolism and cannot make their own protein.
- Scientists classify viruses as nonliving things.
- **Host range**: each type of virus can only infect a limited range of host cells
Viruses are able to identify their hosts by protein receptors on the surface of the host cells that form a “lock & key” fit with the receptors on the surface of the virus.

Some viruses’ host range is so small it includes only one species.

Rabies can infect raccoons, skunks, dogs, and humans.

Most viruses that infect eukaryotes are tissue specific. Ex: cold viruses infect ONLY the cells of the respiratory tract. HIV infects ONLY helper-T cells (CD4).

Once the virus is inside the host cell, it takes control of the host to reprogram the cell to make copies of the viral DNA or RNA and viral protein.

The host provides the nucleic acids, enzymes, ribosomes, RNA, amino acids, ATP, etc for the making of viral parts.

- **Lytic cycle:**
  - A viral cycle in which during the last stage of infection 100s → 1000s of viruses burst free from the host cell causing the host cell to lyse.
  - First destroys the host’s DNA and then destroys the host, and then the viruses are free to go infect other healthy cells.
  - **Virulent virus** (virulent=very infectious): a virus that reproduces by this lytic cycle
  - Virulent because of host destruction and the process is very fast, 20-30 minutes.
  - Luckily for host cells, many have evolved to protect themselves from viral infections. Ex: some bacterial hosts have mutated their surface receptors and are no longer recognized by a particular bacteriophage.
  - Some host cells have enzymes that are able to break down viral DNA or RNA.
  - But just as hosts evolve to protect themselves from viruses, viruses also evolve to counteract this.

- **Lysogenic cycle:**
  - A cycle when a virus infects a host cell but doesn’t destroy the host
  - The viral DNA does not destroy the host’s DNA.
  - The viral DNA becomes incorporated into the host’s DNA.
  - **Provirus/prophage:** the viral DNA is a part of the host’s DNA
  - It doesn’t interfere with the normal functioning of the host cell.
  - Every time the host cell reproduces, the viral DNA gets copied as well.
  - This can go on for years until the lysogenic cycle is triggered to switch to the lytic cycle
  - Viruses are free to infect more cells
  - The “trigger” varies but is often physical or emotional stress
  - Some prophage genes in the lysogenic cycle can alter the phenotype of the host cell.
  - Ex: the bacteria that causes scarlet fever would be harmless to humans if it were not for the provirus genes in the bacteria that cause the host bacteria to make toxins.
Most viruses have RNA as their genetic material.

There are different types of RNA viruses that work differently within the host cell.

- **Retroviruses** (retro=backwards): viruses have RNA and use reverse transcriptase—an enzyme within the virus itself to make DNA.
- The DNA is then integrated into the host’s DNA as a provirus where it will be replicated over and over again in a lysogenic cycle.
- This is why HIV infected individuals can appear unaffected for a so long (10+ years).
- Instead of lysing the cell early on, the HIV viruses can bud off from the host cell and go infect other T-cells then at any time switch to the lytic cycle and destroy helper T-cells enough that there is almost a total loss of immunity and one no longer just has HIV, but AIDS.
- **HIV**: a difficult antigen for the body to destroy because it keeps mutating and the immune system cannot keep up with it.
- The reason for all the mutations of RNA viruses is because they don’t have the proofreading steps of DNA replication.
- **AZT**: a drug interferes with action of reverse transcriptase; it controls HIV.
- **Vaccines**: dead or weakened forms of pathogens (viruses or bacteria).
- Since they are dead or weakened, they cannot cause disease, but instead stimulate the immune system to mount a defense in the event of a “live” attack

**Dr. Jenner**
- Made the first vaccine
- Noticed that milkmaids who contracted cowpox (a mild disease that usually infects cows) were resistant to smallpox (a disease that often resulted in death).
- Then scratched a “small farm boy” named James Phipps with a needle containing fluid from the sore of a milkmaid who had smallpox.
- The boy was later exposed to smallpox, but he didn’t get sick.
- The cowpox and smallpox viruses are so similar that the immune system cannot distinguish them
- **Vaccine**: can be used before one gets a viral or bacterial infection and are usually given before one gets an infection.
- **Antibiotics**: cannot be used on viral infections; they are used after one gets a bacterial infection; they cannot be used on viral infections since they kill bacteria by inhibiting enzyme (most viruses lack enzyme). Ex: penicillin interferes with the enzymes that build cell wall.

Viruses seem to always be emerging because of several reasons:

a) RNA viruses mutate often → this is why there is a flu shot almost every year for the RNA influenza virus.

b) New viral diseases arise as viruses increase their host range. Ex: Hanta was spread from deer mice in SW United States to humans who inhaled the dust containing traces of urine and feces from the mice.

c) Increased travel, blood transfusion technology, sexual promiscuity, and intravenous drug use have increase viral disease cases.

d) New roads are cleared and man explores once isolated plants, animals, and tribes, viruses are spread.

e) With plant viruses, they can also be spread by insects that act as carriers and by farmers who inadvertently transmit the viruses on their gardening tools. Agricultural scientists are now breeding viral resistant crops that resist many viruses.

**Viroids**: smaller than viruses and are circular pieces of RNA that only infect plants. They can only replicate inside a host plant; when they do replicate, they disrupt plant cell metabolism and stunt plant growth.

**Prion**: not nucleic acids like viroids but are proteins; they caused mad cow disease. It is a misfolded protein normally present in brain cells. When it gets into a cell containing the normal form the protein, it converts the normal protein to a prion version causing degenerative brain diseases.
- Bacteria:
  - Are prokaryote
  - Are asexually
  - Divide by binary fission about every 20 min
  - Most are genetically identical to the parent cell
  - Due to the short generation times creating large numbers of bacteria, new mutations arise often and bring about genetic variety.
- 3 ways to produce variety in bacterial DNA (aside from mutation):

  1) Transformation:
     - The alteration of a bacterial cell’s genome by the uptake of foreign DNA from the environment.
     - Ex: Griffith experiment—heat killed smooth cells transformed harmless rough cells into pathogenic pneumonia that killed the host mouse.
     - With his experiment, the live R cells took up a piece of DNA from the heat-killed S cells, which enabled the R cells to get a capsule.
     - Having this capsule disabled the mouse’s immune system to destroy the pathogenic bacteria and the mouse died.
     - This transformation occurred because some bacteria are able to uptake closely related DNA in the environment and incorporate it into their genome.
     - Not all bacteria can pick up foreign DNA; they can be stimulated to pick up DNA with the help of heat shock and Ca++→ this is used to stimulate bacteria to incorporate foreign/naked DNA that code for protein such as insulin for diabetics.

  2) Transduction:
     - When bacteriophage carry bacterial genes from one host to another.
     - 2 types:
       i) Generalized transduction: involves the lytic cycle
       ii) Specialized transduction: involves the lysogenic cycle

  3) Conjugation:
     - Bacterial version of sex in which one bacterial cell transfers DNA to another bacterial cell.
     - Plasmids are the source of this DNA
     - It is a one way process in which the “male” makes a temporary cytoplasmic bridge called sex-pilus and transfers some DNA to a female.
     - “Maleness” comes about due to the presence of a special piece of DNA called the F plasmid (F=fertility).
     - Having the F factor enables the bacteria to be the donor of DNA.
     - Plasmids: small pieces of self-replicating DNA in bacteria that is separate from the nucleoid.
     - If the F factor is in the plasmid, it is called the F-plasmid—consists of 25 genes, most of which are required to make sex pili.
     - Geneticists use the symbol F+ to denote a cell that contains the F plasmid and it is a “male”.
     - Cells lacking the F plasmids are F- and “female”
     - But F- can become F+ when 2 cells conjugate.
     - If a bacterium didn’t transfer just a plasmid by conjugation but transferred part of the nucleoid, then a F+ cell incorporates the F plasmid into its own nucleoid→ creating a Hfr cell.
     - Now with conjugation, this “male” Hfr cell will first undergo replication, and then transfer part of the DNA (containing both a part of the original F plasmid and some genes from the bacterial chromosome/nucleoid) to the F- cell.
     - Then crossing over will occur between homologous regions of the newly transferred genes and the bacterial chromosome oft eh “female” cell.
- **R-plasmid**: some plasmids carry genes that make them resistant to antibiotics.
- When bacteria containing specific R plasmids are posed to a specific antibiotic, they survive.
- R-plasmids code for enzymes that are able to break down antibiotics such as tetracycline or penicillin.
- These antibiotics don’t destroy the bacteria.
- By natural selection, an increasing number of bacteria become resistant to antibiotics → makes treating bacterial infections more difficult.
- **Transposons**: transposable piece of DNA that can move from one location to another; it can move within the nucleoid, between the nucleoid and plasmids or from one plasmid to another plasmid. Sometimes, called “jumping genes” ← misleading because while some transposons do “jump” from one location to another, others just make a copy and the copy gets inserted elsewhere.
- **Barbara McClintock**: first person to identify transposons in breeding experiments with Indian corn in the 1940s, noticed changes in the color of corn kernels that could only be explained by “mobile” genetic elements capable of moving from one location to another in the genome.
- **Metabolic pathways**: the making or breaking of molecules. Ex: if an *E. coli* bacterium is deprived of one of the amino acids tryptophan from its environment in the colon, it needs to make its own to survive.
- Cells can regulate the numbers of specific enzymes made. If tryptophan accumulates in a cell, the cell shuts down the making of this amino acid by **feedback inhibition** — negative feedback inhibition because it is maintaining homeostasis.
- The blocking of this metabolic pathway actually takes place at the level of stopping transcription of mRNA coding for these enzymes in the pathway.
- The 5 genes on the DNA molecule that code for the transcription of mRNA and later translated into tryptophan are grouped together on a chromosome.
- This segment of DNA is transcribed by one promoter site where RNA polymerase can bind to and begin transcription.
- **Operator**: segment of DNA after the promoter that can “switch on” or “switch off” the making of these enzymes; it controls the access of RNA polymerase to the genes.
- The **promoter** + the **operator** + the **genes** they control = operon.
- There are 2 types of operons:
  1) **Repressible operon**: turned off when a small molecule binds to its regulatory protein
  2) **Inducible operon**: turned on when a small molecule binds to its regulatory protein.
- **Repressible operon:**
  - Tryptophan (trp) operon is an example since it is turned off by an allosterically binding molecule.
  - When no molecule is bound to the operator the trp operon is on.
  - Located just upstream of the operon is a regulatory gene that makes a regulatory protein.
  - If a lot of tryptophan is present in the cell, this activates the repressor which then binds to the operator and the operon is off.
  - Usually function in anabolic pathways (the making of something)

- **Inducible operon:**
  - The lac operon is an example since it is turned on when a small molecule binds allosterically to its regulatory protein, removing it from the operator.
  - It makes the enzymes that hydrolyze lactose.
  - When a bacterium is exposed to milk, this signals the bacterium that it needs the make enzymes to break it down.
  - Usually function in catabolic pathways (the breaking down of something)

- DNA exists in its loose “stretched out” form called chromatin during interphase of the cell cycle.
- During prophase of mitosis and meiosis I, the chromatin condenses into X-shaped structures or replicated chromosomes.
- Eukaryotic DNA is also combined with a large amount of proteins called histones.
- There are 5 types of positive charged histones that the negative charged DNA molecule wraps itself around.
- DNA is negative due to the phosphate groups.
- **Nucleosome:** combination of DNA wound around histones and has appearance of “beads on a string”
DNA TECHNOLOGY

- **Recombinant DNA**: DNA from 2 different sources that is combined.
- **Genetic engineering**: direct manipulation of genes for practical purposes.
- **In vitro** = “in glass” or in a test tube or flask
- **Biotechnology**: manipulation of organisms to perform practical tasks or provide useful products. Ex: using microbes to make wine and cheese; selective breeding of livestock or crops; moving genes from a mammal to a plant.
- **Gene cloning**: making well-defined gene-sized pieces of DNA in multiple identical copies.
- **Restriction enzyme/restriction endonuclease**: enzyme that occurs naturally in bacteria that protect the bacteria against intruding DNA from other organisms. They cut foreign DNA at “very specific” points along the DNA strand.
- **Restriction site**: a specific restriction enzyme recognizes specific short nucleotide sequences in DNA. Most are palindrome and 4-8 nucleotides in length.
- The restriction enzyme has to cut **covalent phosphodiester** and H-bonds of both strands and the cuts produce fragments of DNA with single stranded DNA called “sticky ends”.
- Since one type of restriction enzyme always cuts at the same restriction site, the same restriction fragments are always produced.
- Target sequence may occur many times in a DNA molecule → produces many fragments.
- The sticky ends of the fragments easily form H-bonds with their complimentary base pairs → this can bring about a recombinant DNA molecule if it is from another source.
- **DNA ligase** seals the strands together, forming covalent phosphodiester bonds.

Cloning vector: the plasmid that will be cut with a restriction enzyme and that can carry a piece of foreign DNA into a cell and replicate there. Ex: *E.coli* bacterial plasmids are the most commonly used.

- **Step 5 on the figure on the right, we can distinguish the colony containing the gen of interest by:**
  a) Look for the gene itself
  b) Look for its protein product.
• **Nucleic acid hybridization**: a method that looks for the gene directly by probing a single-stranded nucleic acid with radiation.
  
  • If we know the nucleotide sequence of our gene, we can make a radioactive probe complementary to it, then by denaturing the H-bonds of the newly cloned DNA (from the plasmid library), the radioactive probe can “tag” the correct clone by H-bonding to its single-stranded complement.

- Prokaryotes lack introns, but eukaryotes don’t.
- Scientists must make artificial eukaryotic genes that lack introns.
  
  ^is done by allowing a cell to undergo transcription and make mRNA, then allowing spliceosome to remove introns. The mRNA is then added to a solution of reverse transcriptase which creates a strand of DNA, minus the introns—called complementary DNA (cDNA).
- Scientists sometimes use yeast—single-celled fungi that grow as easily as bacteria, have plasmid, and are eukaryotes.
- Scientists have made artificial chromosomes that are vectors containing an origin of replication, a centromere, 2 telomeres, and foreign DNA. \(\rightarrow\) These vectors are much longer than plasmid vectors enabling long pieces of DNA to be cloned.

- Another reason to use eukaryotic host cells for expressing a cloned gene is that many proteins are changed after translation by the addition of lipop- or glyco-. ∴ Host cells from an animal or plant cell culture may be necessary.
- **Electroporation**: electrical impulses that create holes in the plasma membrane of eukaryotic cells; the temporary holes then allow DNA to enter.
- Scientist can also inject DNA into a eukaryotic cell using microscopic needle.
- **Polymerase chain reaction** (PCR): used to clone impure DNA, is used to clone billions of any piece of DNA quickly (in a few hours).
  
  It starts with a special kind of DNA polymerase, a supply of nucleotides, single-stranded DNA primers, and the DNA to be cloned in a test tube.
- The DNA to be cloned is then heated to separate the strands, then the DNA primers to hydrogen bond to each strand; and DNA polymerase adds nucleotides in its 5' \(\rightarrow\) 3' direction.
- The solution is then heated again and the cycle repeats again for about 20 cycles.
- Because the DNA cloned by PCR can be in small amounts or partially degraded, it has many applications, including DNA fingerprinting in murder trials when only a small amount of DNA is left at the scene of the crime and make copies of DNA from a woolly mammoth.
- **Gel electrophoresis**: separates
DNA or protein on the basis of size and electrical charge and sorts a mixture of DNA into bands along the gel.

- **Restriction fragment analysis**: is used to compare different alleles of a gene or DNA of different individuals or species and involves treating the DNA molecules in question with restriction enzyme.
- **Gel electrophoresis** yields different banding patterns called **restriction fragment length polymorphism** (RFLPs).
- **RFLPs**: homologous chromosomes vary in length of fragments due to different cuts by restriction enzymes.
- The cuts vary since homologous chromosomes can be different alleles.
- **South Blotting**: involves putting the gel from restriction fragment analysis in a basic/alkaline solution and putting blotting paper on top of it. This transfers the DNA from the gel to the blotting paper and denatures it. The paper blot is exposed to a single-stranded radioactive nucleic acid probe that forms complementary base pairs to the DNA on the blotting paper. And then completed by exposing the radioactive probes to photographic film that yields specific DNA bands.
- **Human genome**: started in 1990, expected 100,000 genes but actually 20,000 genes, find the precise location of all an organism's genes and introns. Ex: human DNA, *E.coli*, yeast, Drosophila, and mice, then compared between organisms and confirmed evolutionary links between even distantly related organisms.
- **Biotechnology** makes enormous contributions to medicine like diagnosing diseases.
- **Human gene therapy**: may someday enable scientists to correct genetic disorders by replacing a defective allele with a functional one using recombinant DNA techniques. For it to be permanent, the cells that receive the normal allele must be ones that multiply throughout the patient's life. The new allele could be inserted into the somatic cells of the tissue affected by the disorder in a child or adult or even the germ line cells or embryonic cells.
- **Many bacteria** can extract heavy metals (Cu, Pb, Ni) from the environment, which is important as ores get depleted. Microbes can then degrade some compounds released after an oil spill. Some bacteria can detoxify organic compound into nontoxic forms helping out sewage and water treatment plants.
- **Transgenic organisms**: organisms that contain genes from another species have also been developed by injecting foreign DNA into the nuclei of egg cells or early embryos. Ex: Dairy cow have been injected with rBGH (recombinant bovine growth hormone) made by *E. coli* to raise milk production. BGH also improves weight gain in cattle.
- The vector most commonly used to move genes into plants is the plasmid from the bacterium *Agrobacterium tumefaciens*—this bacterium infects plants and causes tumors called Crown Gall disease.
- The crown gall causing plasmid is called the **Ti (tumor inducing) plasmid**.
- When scientists wish to insert a new gene into a plant by use of the Ti plasmid, they remove the genes that cause the disease and replace it with foreign DNA.
- **Ti plasmid** only infects **dicots** (beans, roses, peas, oaks, etc).
- **Monocots** plants (wheat, corn, rice, orchids, palms, etc) cannot be genetically engineered with the aid of *Agrobacterium*. They must rely on *electroporation* and DNA needles to get DNA into these plants.
- Genetically engineered plants have genes for herbicide resistance (helps farmers to control weeds and not destroy the crops) and insect resistance (makes farmers to reduce the need to apply insecticide).
- Fruits are being genetically engineered to contain genes that retard spoilage—done by blocking the production of the ethylene (ripening hormone).
THE GENETICS OF DEVELOPMENT

- Single-celled zygote undergoes mitosis giving rise to many cells that are not all the same; they have differentiated into specialized kinds of cells with specific structures and functions. Ex: some cells differentiate into muscle cells or skin cells, etc.

- These cells aren’t just mixed up randomly; they are organized into tissues; tissues are organized into organs; organs into systems; and systems form the entire organism.

- Morphogenesis: gives rise to the shape of the organism and lays out the body shape of an organism very early in embryonic development. Ex: determining which end of organism will be the head region.

- The criteria of doing research done on development of organisms:
  1) Having readily observable embryos
  2) Short generation times
  3) Small genomes
  4) Preexisting knowledge about the organism’s genes.

  Ex: Drosophila, nematode C. elegans (found in soil), the mouse, the zebra fish, plants arabidopsis (member of the mustard family).

  Using a microscope to follow cell divisions one after another, biologists are able to reconstruct the ancestry of every cell in the adult body and develop an organism’s complete cell’s lineage.

- Genomic equivalence: all cells contain the same genes. But not all genes are expressed. Ex: cells in the finger contain genes for eye color but are inactive.

- With many plants, a whole new plant can be grown from differentiated somatic cells. Ex: cells taken from the root of a carrot plant could grow into a normal adult plant. But not differentiated animal cells.

- Totipotent: the cells retain the ability to form all parts of the adult organism. Ex: stem cells and plants cells.

- To clone an animal cell, the nucleus of an unfertilized egg is removed then a somatic cell is taken from the animal to be cloned and the 2 are fused. Then normal mitotic divisions produce a morula.

- The ball of cells is then inserted into a surrogate mother’s uterus where after gestation, and animal identical to the donor is born.

- Dolly: first cloned animal (a sheep) in 1997, containing chromosomal DNA identical to that of the nucleus of the donor, many animals have been cloned since.

- Different cell types make different proteins.

- First evidence of differentiation is differentiated cells make tissues-specific proteins. Ex: muscle cells make actin and myosin and fuse to form an elongated, multinucleated skeletal muscle fiber that goes into the G₀ phase and stops the cell cycle.

- The first difference that arises amongst cells in an embryo is from the cytoplasm of the unfertilized egg cell→ influences development and is not homogenous.

- Cytoplasmic determinants: with each mitotic division, the cytoplasmic environment is different for each cell; this regulates the expression of genes affecting the fate of cells.
- The environment around the cells influences differences that arise amongst cells.
- **Induction/inductive signaling**: chemical signals between cells cause changes in nearby cells. This in turn induces target cells to differentiate into specialized cells making up an organism.
- **Cytoplasmic determinants** and inductive signaling between cells contribute to morphogenesis.
- **Spatial organization of tissues** and organs develop in their characteristic places by pattern formation—begins early in the embryo when the animal’s basic body plan is arranged.
- ^Ex: the construction of *Drosophila*’s body plan is in 3 segments: head, thorax, and abdomen. Cytoplasmic determinants are present in the unfertilized egg that provide information about the axes (dorsal back, ventral front, anterior had, and posterior tail) even before fertilization. The mother had egg polarity genes that code for cytoplasmic proteins to ensure the embryo’s axes lined up. *Drosophila*’s eggs all contain the polarity protein bicoid in their cytoplasm, which anchors on the anterior end of the embryo and tells the developing organism "this end up". ∴ If bicoid is injected at the other sites in the developing embryo, mutations arise such as 2 head regions.
- **Homeotic genes**: these genes specify the types of appendage that will form and their correct location.
- In *Drosophila*, these are 180 nucleotdies long called homeobox. Other animals have this too.
- Not all homeobox genes control the identity of body parts, but most are involved with development.
- **Apoptosis**: timely "suicide" of cells.
- During development, cell’s trigger the activation of suicide proteins in the cells destined to die. These proteins then trigger the release of enzymes like proteases (to cut up proteins) and nucleases (cut up nucleic acid).
- The target cells then shrink, their nuclei condense, and they get engulfed and digested by neighboring cells.
- **Apoptosis** is vital for normal development of the nervous system, immune system, and morphogenesis of human hands and feet-preventing webbed fingers and toes.
- It is also linked to cancer, in that normally a cell with damaged DNA should trigger apoptosis, but cancerous cells continue to survive.