

Molecular Genetics

Separating Mixtures

2 techniques:

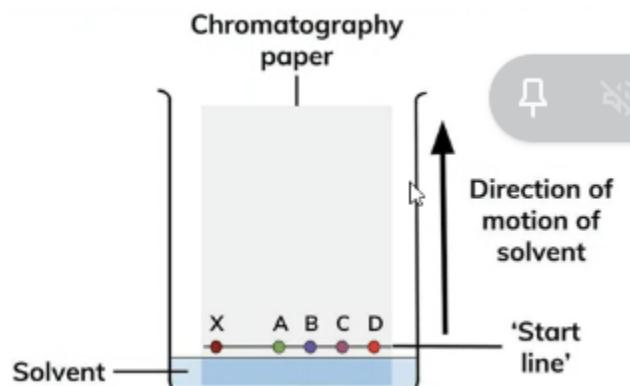
1. Chromatography
 - Paper chromatography
 - Column chromatography
2. Electrophoresis
 - Paper electrophoresis
 - Gel electrophoresis

1. Chromatography

- Used to separate organic compounds from mixtures on the basis of their solubility in one or more solvents passed through a solid support medium
- Solid support medium: paper, column of powdered material
- Involves separation on the basis of slight difference in physical properties (ie. molecular mass, solubility in organic solvent, and ionic charge at different pH values)

Paper chromatography

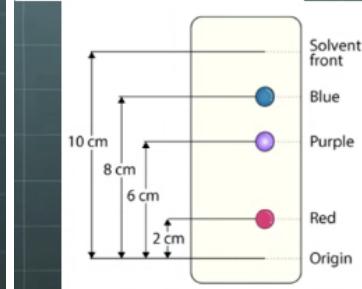
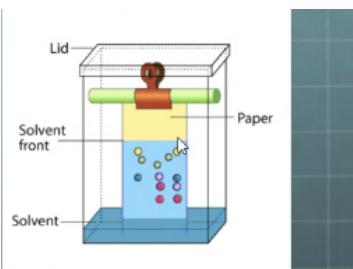
- A drop of mixture is applied to the chromatography paper
- The paper is usually immersed in a non-polar solvent (ie. acetone)
- The solvent flows up the paper by capillary action
- As the solvent flows past the mixture, it differentially dissolves the compounds in the mixture dragging them upward for different distances



• In this way, slight structural differences in the compounds of the mixture are used to permit their separation

• If the solvent is non-polar, the compounds in the mixture which are also non-polar will travel highest up the paper (since they are more soluble)

• Polar molecules are held back in the paper



Solubility of functional groups

• Carboxyl group

Most polar

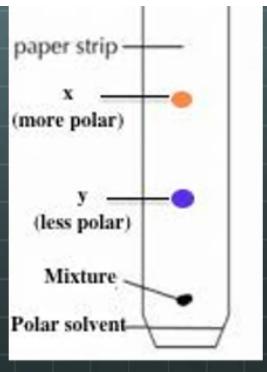
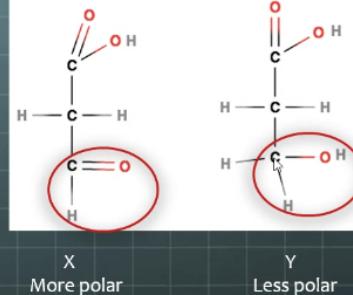
• Aldehyde group

Increasing polarity

• Ketone

Least polar

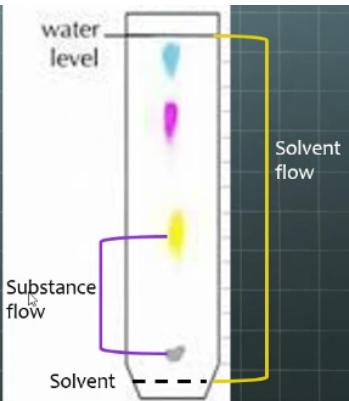
• alkyl



• Rate of flow (R_f)

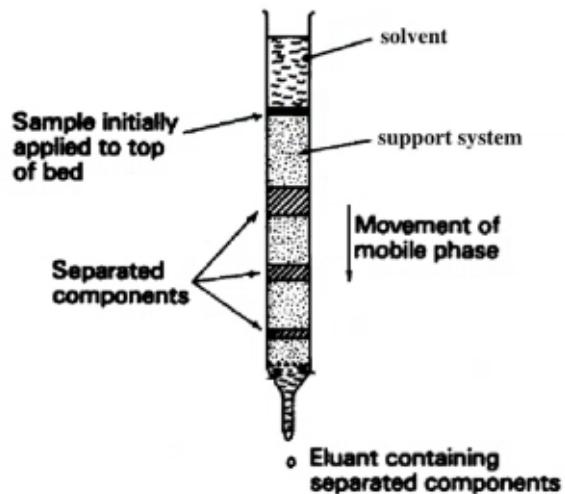
$$= \frac{\text{distance substance flow}}{\text{distance solvent flow}}$$

- In a non-polar solvent, R_f decreases with polarity
- In a polar solvent, R_f increases with polarity
- R_f is sometimes useful for identification of unknown substances



Column chromatography

- A powder or resin is packed into a glass column as the support medium
- The mixture is applied to the top and then a solvent is passed down the column
- The compounds in the mixture move down the column at different rate depending on their
 - Solubility in the solvent
 - Molecular mass
 - Ionic charges
 - Affinity towards the support medium

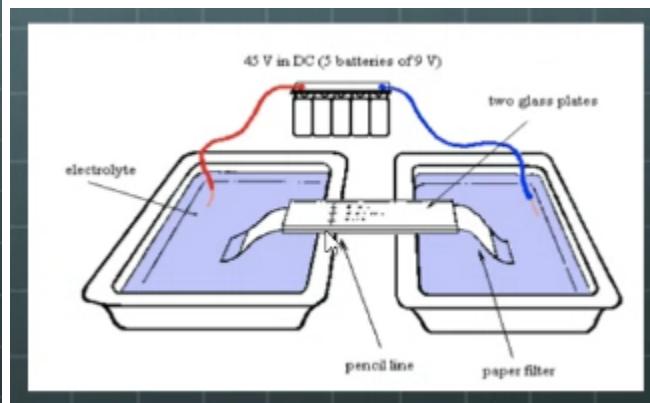


2. Electrophoresis

- Is used to separate the compounds of a mixture
- Depends upon the ability of charged molecules to migrate when placed in an electric field
- Used to determine the number, amount and mobility of components in a given sample or to separate them

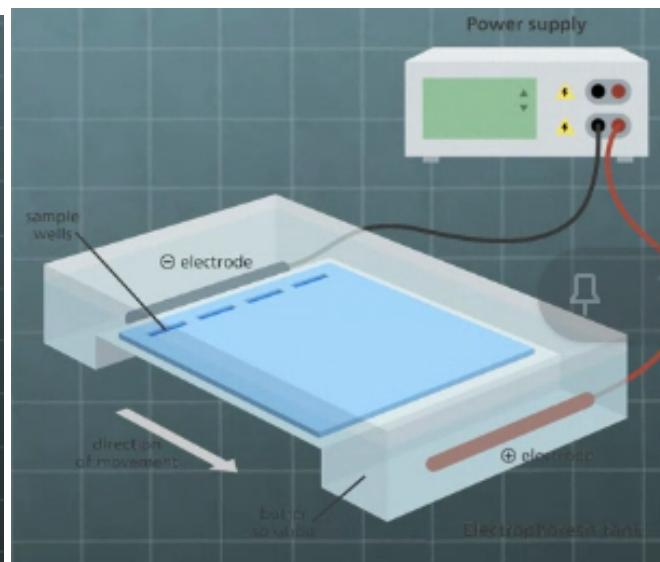
Paper electrophoresis

- Is used for separating relatively small molecules (ie. amino acids, nucleotides)
- A sheet of paper is moistened with the buffer solution
- The ends of the paper are immersed in separate reservoirs containing buffer and in which the electrodes are fitted
- The mixture sample is placed in the middle of the paper strip
- Upon passing electric current, the ions in the sample migrate toward oppositely charged electrodes



Gel electrophoresis

- Separates macromolecules on the basis of their rate of movement (size) through a gel under the influence of an electric field
- Commonly used in labs to separate charged molecules like DNA, RNA, and proteins
- Electrodes are attached to both ends
- When current is applied, one end of the gel has a + charge and the other is -
- The agarous gel consists of a permeable matrix, like a sieve



- After the current has been applied for about 2 hours, the gel is removed and a stain is added
- Smaller molecules migrate through the gel more quickly, travelling further
- The lowest bands in the gel are the smallest fragment with the greatest negative charge
- Results are run again against standard macromolecules of known molecular mass for comparison



Gel electrophoresis with DNA

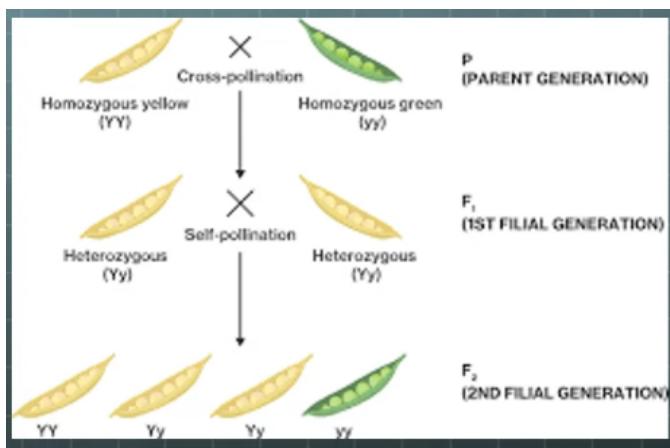
- Can distinguish DNA fragments of different lengths
- DNA is negatively charged, therefore, when an electric current is applied, DNA will migrate towards the positively charged electrode
- Shorter strands of DNA move more quickly through the Agarose gel
- Restriction enzymes are used to cut the DNA in very specific area (specific base)
- Can be used to determine relatedness between species and DNA fingerprinting (crime scene, paternal test)

Discovery of DNA

1. Gregor Mendel (1866)
2. Friedrich Miescher (1869)
3. Frederick Griffith (1928)
4. Avery-MacLeod McCarty (1944)
5. Erwin Chargaff (1950)
6. Alfred Hershey & Martha Chase (1952)
7. Rosalind Franklin (1952)
8. James Watson & Francis Crick (1953)

Gregor Mendel - sample or smthn

- Performed an experiment on pea plants
- Showed hereditary molecule is passed through generations
- Cross between true-breeds results in a 3:1 ratio in the next generation
- Discovered dominant and recessive genes
- Concluded some invisible factor provides visible traits in predictable way



Include 3 slides + mebe short vid

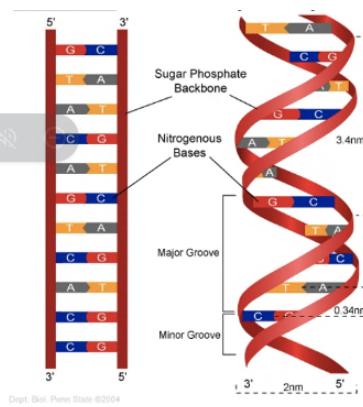
In your groups, you will:

- Read the provided material
- Research on the internet
- Prepare a short presentation (5-10 minutes) for our class on your topic
- Add your Powerpoint slides to our class Google slides (max 3 slides per group) in the order listed on the previous slide
- Your presentation should cover:
 - Who conducted the experiment
 - What was the experiment
 - When (what year) was this experiment conducted
 - Why was this experiment important
 - How does this experiment lead to the discovery of DNA

DNA Structure, RNA and Semi-conservative model

DNA Review

- Has shape of helix
- Is about 2 nm in diameter
- 2m of it in a nucleus!!
- Makes a complete helical turn every 3.4 nm



3 Main Components of DNA

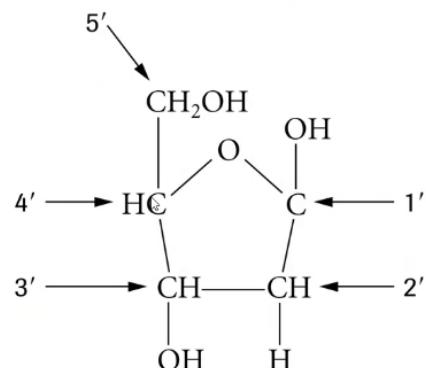
- deoxyribose sugar
- a phosphate group
- nitrogenous base
 - adenine
 - guanine
 - thymine
 - cytosine

↳

Pyrimidines = single rings TC, Purines = double rings AG

- DNA is composed of many nucleotides held together by phosphodiester bonds (therefore it is a polymer)
- Sugar – Phosphate – Sugar - Phosphate

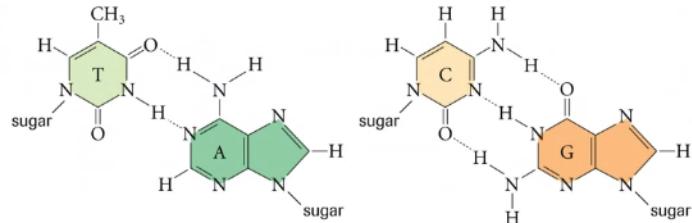
Structure of deoxyribose



Double Helix

- DNA consists of two antiparallel strands of nucleotides
- Bases of one strand are paired with bases in the other strand
- Nitrogenous base pairs are arranged above each other, perpendicular to the axis of the molecule
- A Purine always bonded to a pyrimidine
 - Adenine with Thymine
 - Guanine with Cytosine
 - Termed complimentary base pairing
 - Fundamental to the storage and transfer of genetic information

- Purines – A & G
- Pyrimidines – T & C (also U in RNA)
- bases are bonded together by hydrogen bonds
- right-handed helix (clockwise turn) makes complete turn every 10 nucleotides



AT
GC

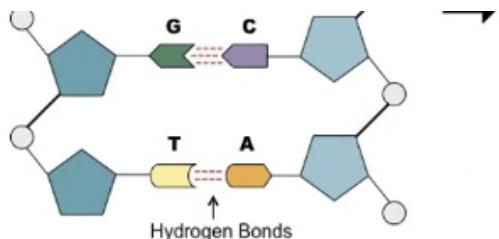
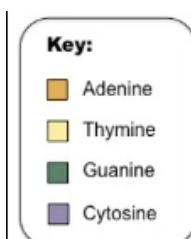
A ≡ T



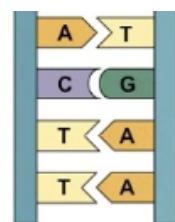
G ≡ C

DNA strands run antiparallel

- One strand runs in the 5' to 3' direction while the other strand runs in the 3' to 5'.
- The 3' end terminates with the hydroxyl group of the deoxyribose sugar.
- The 5' end terminates with a phosphate group



Antiparallel DNA Strands



DNA Ladder

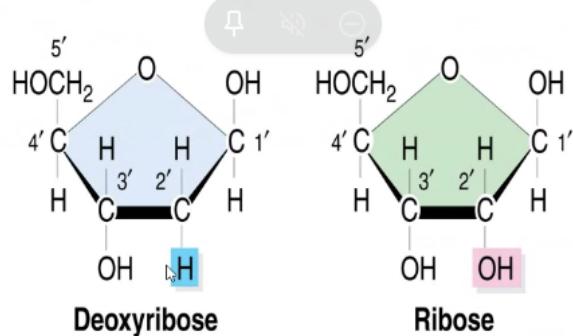


Double Helix

- It would take someone typing 60 words/min for 8 hours a day for 50 years to type out the human genome
- Junk dna → when you dont know what it does, just label it junk :)

RNA

- Single stranded polymer of nucleotides
- Ribose sugar + phosphate + nitrogenous base



A = U

G = C

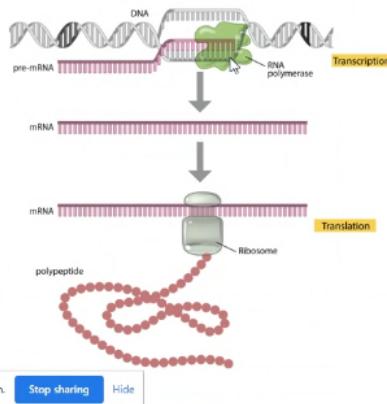
1. rRNA – ribosomal RNA
2. mRNA – messenger RNA
3. tRNA – transfer RNA

Ribosomal RNA = rRNA

- 200 – 300 nucleotides
- Large complex molecule made up of both double and single helices
- Made by DNA in the nucleus
- Found in cytoplasm
- Makes up the framework of ribosomes

Messenger RNA = mRNA

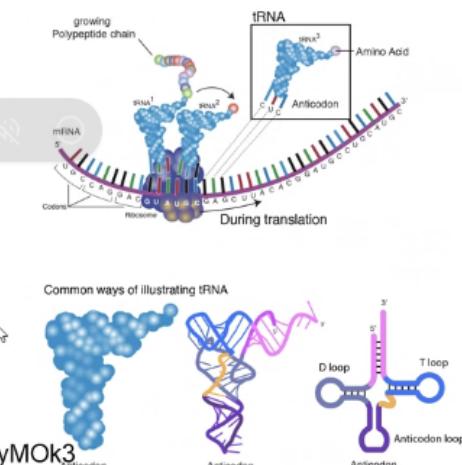
- 1000 nucleotides
- Long single-stranded molecule
- Mirror copy of part of one strand of the DNA helix
- Enters the cytoplasm where it associates with the ribosomes and acts as a template for protein synthesis
- Short lived, easily broken down (exists only a few minutes)



Transfer RNA = tRNA

- 80 nucleotides linking together
- Found in cytoplasm
- Helps decode mRNA sequence into protein
- Function at specific sites in the ribosome during protein synthesis

<https://www.youtube.com/watch?v=1THyMOK3VwU>



||||||||||||||||||||||||||||||||||||||||||||

Homework:

- Complete dna v rna table → on test
- Gizmo
 - Questions prob on test as well

Next Unit:

- Human body systems

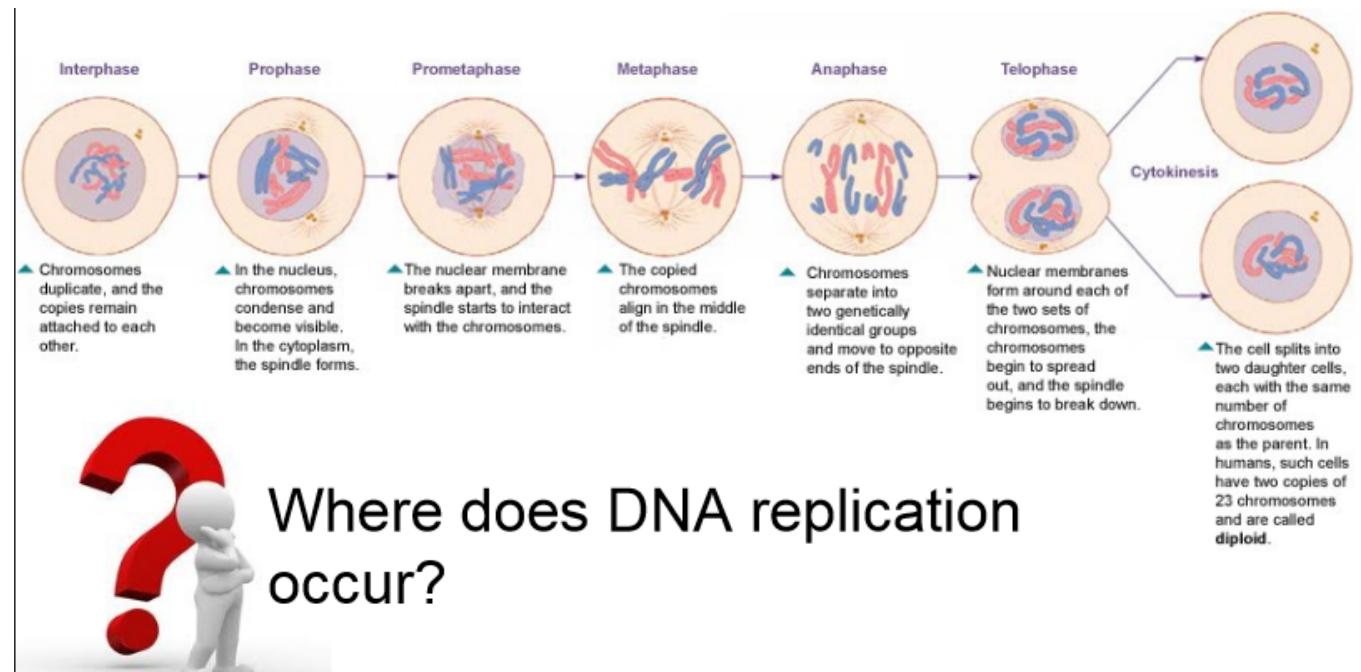
Last Unit:

- Population dynamics

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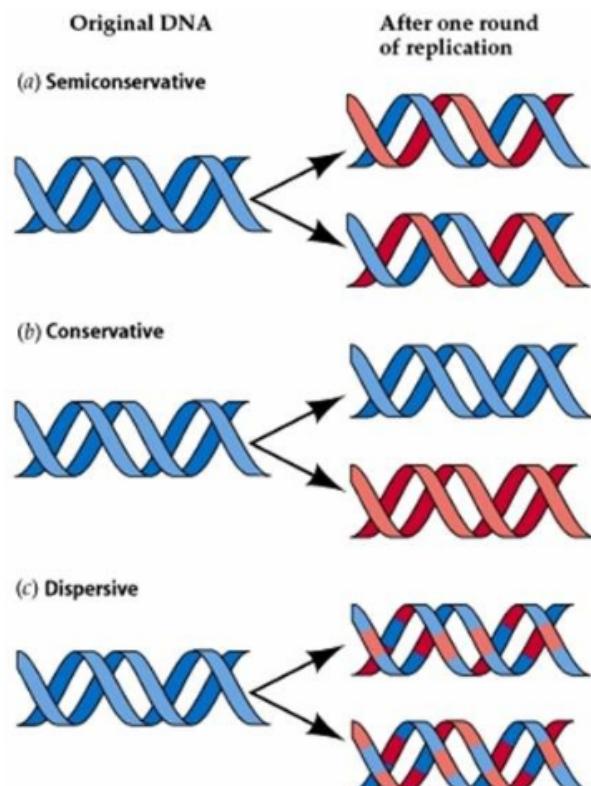
DNA Replication

Review



Replication Theories

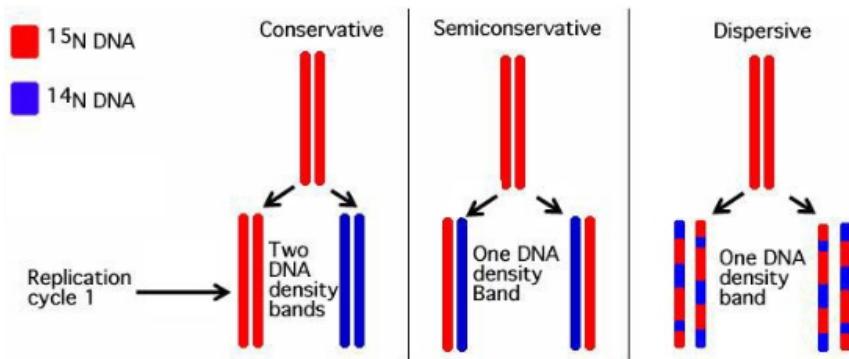
- **Conservative**
 - Both parental strands stay together after replication
- **Dispersive**
 - Parental and daughter DNA are interspersed in both strands following replication
- **Semiconservative**
 - Double-stranded DNA contains one parental and one daughter strand following replication



Meselson and Stahl

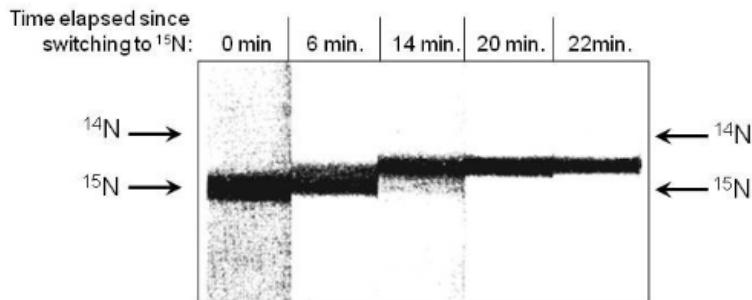
- Grew bacteria for many generations in ^{15}N medium to incorporate it into all nitrogen containing molecules of cells (ie. DNA)
- Bacteria transferred to medium containing ^{14}N for one round of replication
- Lighter isotope is incorporated into any newly synthesized DNA

Hypothetical Predictions

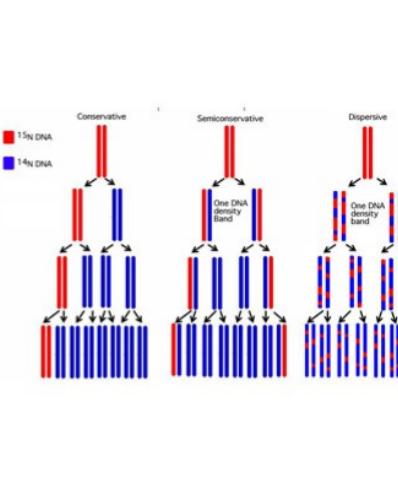


- After 1st round of replication: Found intermediate density of DNA → semiconservative or dispersive

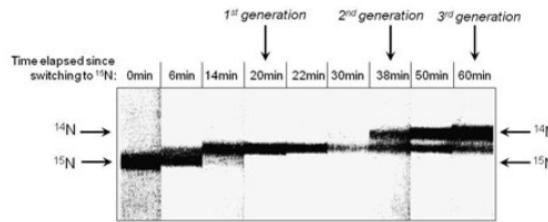
Experimental Results



- To distinguish between semiconservative and dispersive, a second round of replication is needed
- If DNA replication is semiconservative, two densities will be observed
- If replication is dispersive, a intermediate density will be observed



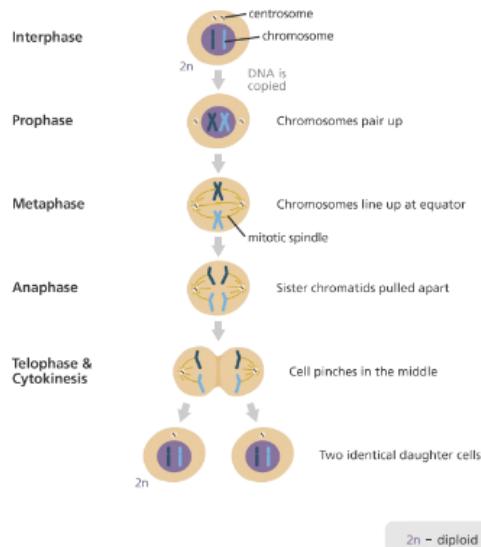
- After 2nd replication: found two different densities



- This indicates it's semiconservative model

DNA replication

- Occurs in the nucleus
- DNA is replicated during interphase
- genetic material is divided equally between two daughter nuclei via mitosis
- Cytokinesis splits it into two new cells
- Requires a series of enzymes which carry out different jobs in completing the process

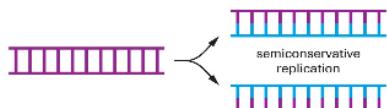


Key Enzymes

Specific enzymes work together to expose the DNA template strands.

- **DNA helicase:** separate the parental double helix by breaking the hydrogen bonds between complimentary bases
- **Single-stranded binding proteins (SSBs):** keep unwound parental DNA apart by blocking the hydrogen bonding
- **Polymerase:** Catalyzes the synthesis of a new DNA strand
- **DNA ligase:** joins 3' end of each new DNA fragment to the 5' end of the growing chain
- **DNA Gyrase** – enzyme relieves tension of unwinding helix

- DNA cannot be simply pulled apart due to hydrogen bonding
- Two parent DNA strands must be unravelled and kept separate.
- Replication is initiated in two steps:
 1. Single-stranded binding proteins unwind a short stretch of DNA double helix
 2. Helicases attaches and break the hydrogen bonds between bases, pulling the two strands apart
- Each strand then acts as a template to build the complimentary strand



- When separated, a replication bubble forms

Building Complementary strands

- In prokaryotes
 - DNA polymerase I, II, III are the three enzymes known to function in replication and repair
- In eukaryotes
 - Several different types of DNA polymerase are at work
 - enzyme that builds the complementary strand using the template strand as a guide in prokaryotes is **DNA polymerase III**
- Primase attaches to parental strand and generates a short strands of RNA primers
- DNA polymerase III attaches and add complementary nucleotides to each strand
- The nucleotides are drawn from the pool of free-floating nucleotides surrounding the existing strand
- New strand is synthesized in 5' → 3' direction
- This results in "leading" and "lagging" strand

Leading Strand

DNA polymerase III...

Functions only under certain conditions:

- It synthesizes DNA in the 5' to 3' direction, therefore adding free **deoxyribonucleoside triphosphates** to a 3' end of an elongating strand
 - Requires an initial starting 3' end to commence elongation
 - An **RNA primer** of 10 – 60 base pairs of DNA are annealed to the template strand since DNA polymerase III cannot initiate a new complementary strand by itself
 - Primer is synthesized by enzyme **primase**
- RNA primer marks initiation sequence
- DNA polymerase III can start elongation by adding free deoxyribonucleotide triphosphates to the growing complementary strand
- Free bases in nucleoplasm used by DNA polymerase III to build complementary strands
- DNA polymerase III uses energy derived from breaking the bond between the first and second phosphate to drive dehydration synthesis (condensation rxn) that adds complementary nucleotide to elongating strand
- Extra two phosphates recycled by cell

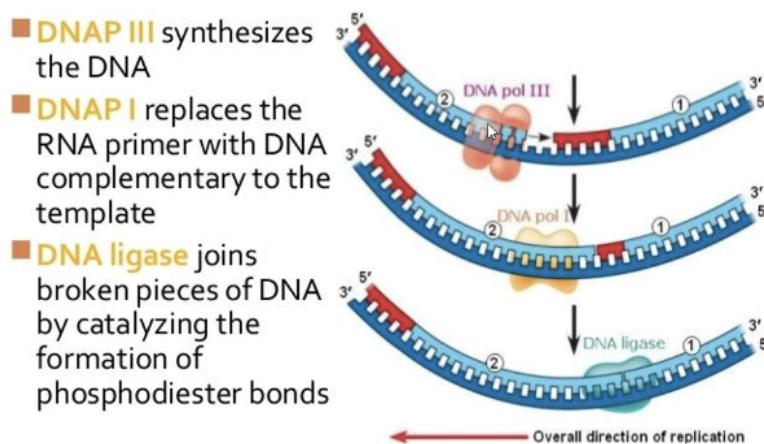
*Since DNA always synthesized in 5' to 3' direction, and the templates strands run antiparallel, only one strand is able to be built continuously.

*This is the strand which uses the 3' to 5' template strand and is called the **leading strand** and is build towards the replication fork.

Lagging Strand

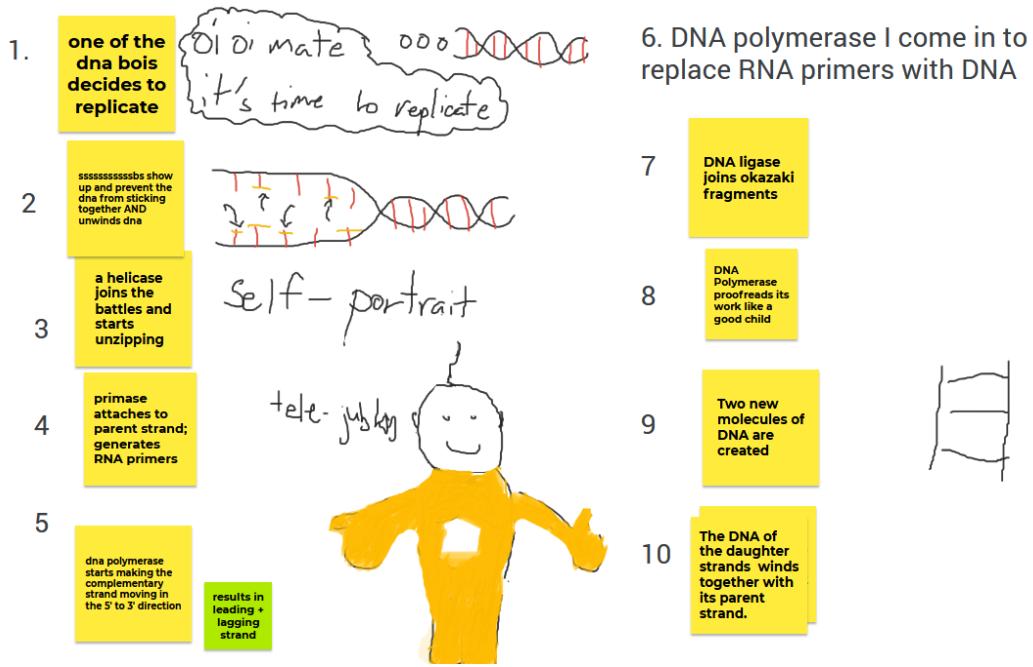
- The other strand is synthesized discontinuously in short fragments and is called the **lagging strand**
- Okazaki fragments** are short fragments of DNA that are a result of the synthesis of the lagging strand during DNA replication (eukaryotes 100-200 nucleotides in length, 1000-2000 in prokaryotic)
- DNA ligase** joins the Okazaki fragments into one strand by the creation of a phosphodiester bond
- The two double stranded DNA molecules are produced and twist into a helix automatically

Lagging - Okazaki Fragments



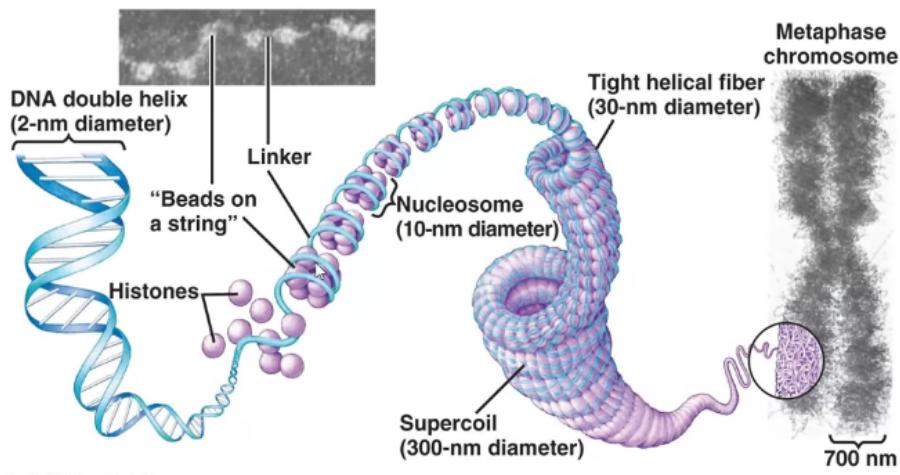
Ensuring quality control of new DNA strands - Dna repair

- As complementary strands are built, DNA polymerase III and DNA polymerase I proofread newly synthesized strands
- When mistakes occur, either enzyme can function as **exonuclease** which backtracks past the nucleotide on the end of the strand that is incorrectly paired to a nucleotide, excises it, and continues adding nucleotides to the complimentary strand



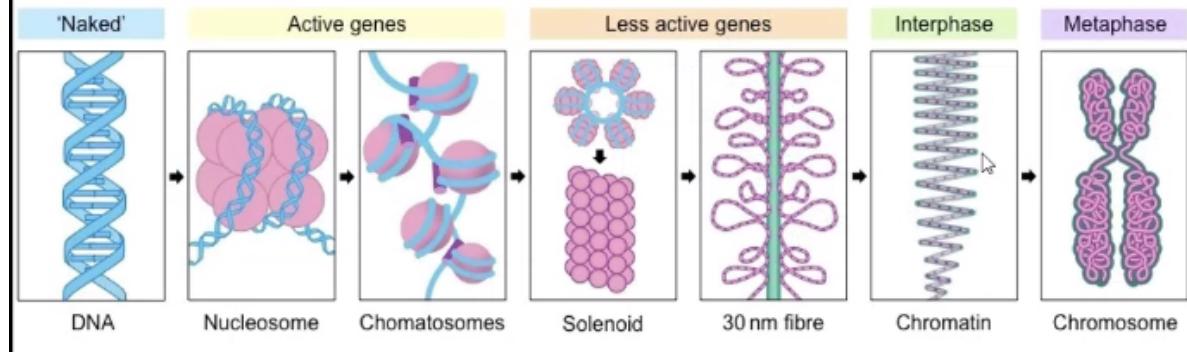
Eukaryotic DNA Organization

- DNA is coiled around a special protein called histones
- Positively charged histones allow negatively charged DNA to wrap tightly around them
- They are grouped into units of 8 histones called **nucleosome**
- Eight nucleosomes are coiled into cylindrical fibres known as chromatin fibres or **solenoids**
- When the cell enters the reproductive stage, the solenoids can be further supercoiled to form the X-shaped chromosomes that are visible during the metaphase stage of mitosis.



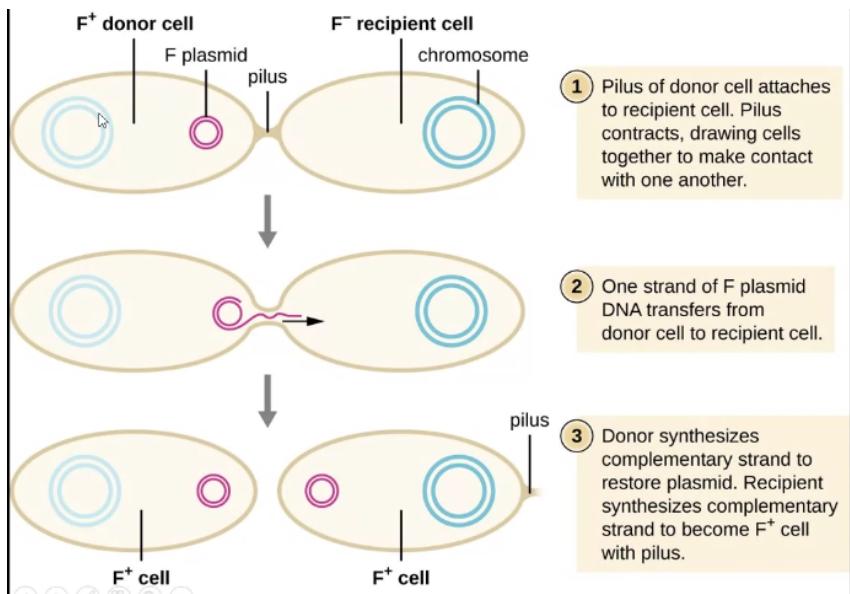
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- During interphase, DNA is in the form of loosely packed nucleosomes or solenoids



Prokaryotic DNA

- Genomes in prokaryotes are smaller and circular in structure
- Lack of membrane/nucleus → less tightly packed → more accessible
- Usually has one replication origin and one bubble
- Plasmid (smaller circular pieces of DNA) float throughout the cell
- During conjugation, plasmids can exit cell and enter another
- Two neighbouring bacteria can pass plasmids into the other
- Recipient will incorporate new plasmid into its genome

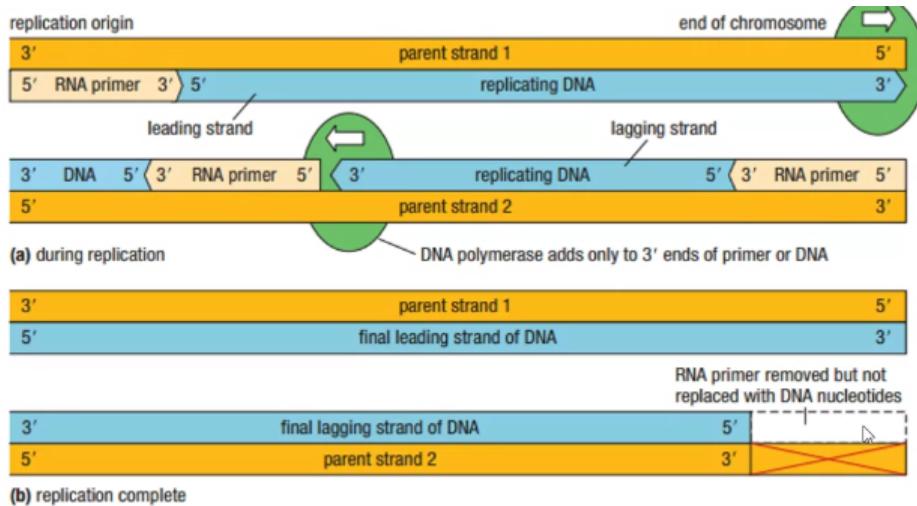


Prokaryotic DNA Organization

- Coiling technique
- Circular DNA twists (like a circular elastic band) until the coils bunch on top of each other forming a tight ball – **supercoiling**

DNA Loss During Replication

- Occurs on the lagging strand
- Normally, RNA primers are removed by DNA polymerase and the Okazaki fragments are joined together
- This does not happen to the primer on the final Okazaki fragment since there is no DNA adjacent to the 5' end
- RNA primer is still removed, but not replaced



Since the last sequence of the parent strand is not copied, the lagging strand is shorter.

If this occurs every replication cycle, the DNA will get shorter and can lead to loss of important genes.

Telomeres

- To prevent loss of important genetic information, telomeres are found at the ends of eukaryotic chromosomes
- Telomere is a repetitive, non-coding sequence found at the end of chromosomes
- In many species the code is:
 - 5' – TTAGGG – 3'
- This code can be repeated as many as 2000 times
- After many replications, the telomeres are completely lost
- Cell senescence: occurs when cells with missing genetic information do not function properly – lose ability to grow, metabolize, divide
- Hayflick limit – the total number of times that a normal cell can divide
 - Human: 50 times
 - Different for diff spp, not related to how long spp can live
- Special case: germ line cells that produce gametes have telomerase that adds more DNA to restore lengths
 - Go over exceptions to telomerase

Telomere & Aging

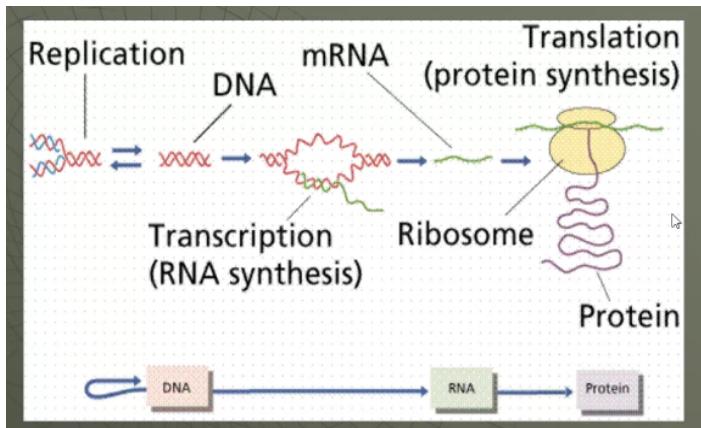
- Cells divide at different rate so they reach senescence at different time
- As we age, more cells will reach this stage, putting a strain on the healthy cells to function at optimum level
- Can telomere be the answer to the fountain of youth?
 - Dietary supplements claiming to increase telomerase activity or decrease degradation

Telomeres & Cancer

- Cancer cells do not reach cell senescence
- They have large quantities of telomerase
- New cancer therapy targets and inhibit the telomerase within cancer cells
- Other treatments may decrease size of telomeres in potentially cancerous cells
 - But these cells seem to be able to avoid death by increasing telomerase activity
- Can telomerase from cancer cells be used on healthy cells to prolong life?

From Gene to Protein

Protein synthesis - overview



Protein review

- ◆ Determine the physical characteristics of organisms
- ◆ Ex. antibodies, enzymes, hormones
- ◆ Drive cellular processes

Link between Genes and Proteins

- ◆ In 1909, Garrod suggested that phenotypes are expressed due to the presence of certain enzymes
- ◆ He assumed that individuals had particular disorders due to their inability to produce a certain type of enzyme

Garrold assumed that the production of enzyme was related to the genes in the DNA.

Alkaptonuria is a hereditary condition whereby the urine is black. Individuals with this disorder do not have the enzyme to break apart alkaption.

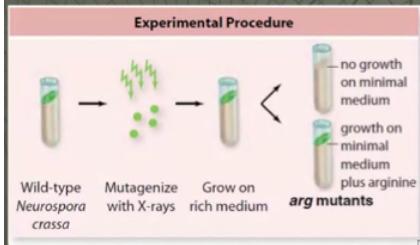


Beadle and Tatum's Experiment

- ◆ Determine if there was a one-to-one relationship between genes and proteins
- ◆ Hypothesis: mutation in a gene that codes for a specific enzyme will result in no production of that enzyme
- ◆ Explored Garrold's idea by studying bread mold 'Neurospora Crassa'



- Created mutant *N. crassa* deficient in producing essential nutrients using X-rays



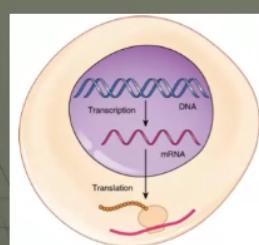
Minimal Medium: contains sugar and inorganic salts. Bacteria need to have enzymes to produce molecules needed in their diet.
Complete Medium: contains all 20 amino acids, bacteria do not need enzymes

- Mutated mold is mated with non-mutant bacteria to create spores
- Spores are grown in complete and minimal media
- When culture is transferred to minimal medium – forces cells to synthesize enzymes
- If no growth in minimal medium, it means the mutation has affected its ability to make enzymes or complex molecules
- To figure out which enzyme/vitamin is affected, different combinations can be added to the growth medium. If growth is observed after adding an enzyme or vitamin, it indicates that that's the missing enzyme/vitamin.

- Beadle & Tatum concluded that one gene codes for one enzyme. This relationship was updated to the **one-gene/one-polypeptide hypothesis** since not all proteins are enzymes.

Messenger between DNA and Proteins

- Scientists know:
 - Genes are located in the chromosome within the DNA
 - Proteins are synthesized in the cytoplasm
 - RNA is found in both nucleus and cytoplasm
- Conclusion: RNA could be synthesized in the nucleus and transported to the cytoplasm to synthesize protein



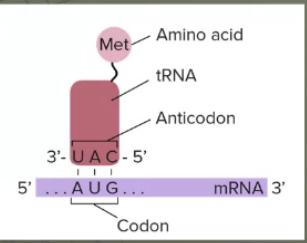
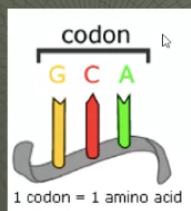
Protein synthesis overview

- ◆ **Transcription** involves the copying of information in DNA into mRNA
- ◆ **Translation** involves ribosomes using mRNA as blueprint to synthesize a protein composed of amino acids.

The genetic code

- ◆ A set of rules determining how genetic information in the form of a nucleotide sequence is converted into an amino acid sequence of a protein
- ◆ RNA has 4 nucleotides (A, U, G, C) and 20 amino acids

- ◆ The genetic code consists of a combination of 3 nucleotides called **codon**
- ◆ Each codon codes for an amino acid



Interpreting codons

| Second base of codon | | | | Third base of codon | | | | |
|----------------------|-----|-----|-----|---------------------|-----|-----|-----|-----|
| First base of codon | | U | C | A | G | U | C | |
| U | UUU | Phe | UCU | | UAU | Tyr | UGU | Cys |
| | UUC | | UCC | Ser | UAC | | UGC | |
| | UUA | | UCA | | UAA | | UGA | |
| | UUG | Leu | UCG | | UAG | | UGG | Trp |
| C | CUU | | CCU | | CAU | His | CGU | U |
| | CUC | Leu | CCC | Pro | CAC | | CGC | C |
| | CUA | | CCA | | CAA | Gln | CGA | A |
| | CUG | | CCG | | CAG | | CGG | G |
| A | AUU | | ACU | | AAU | Asn | AGU | U |
| | AUC | Ile | ACC | | AAC | | AGC | C |
| | AUA | | ACA | Thr | AAA | Lys | AGA | A |
| | AUG | Met | ACG | | AAG | | AGG | G |
| G | GUU | | GCU | | GAU | Asp | GGU | U |
| | GUC | Val | GCC | | GAC | | GGC | C |
| | GUA | | GCA | Ala | GAA | Glu | GGA | A |
| | GUG | | GCG | | GAG | | GGG | G |

Figure 7 The genetic code, written in the form in which the codons appear in mRNA: The AUG initiator codon, which codes for methionine, is shown in green; the three terminator codons are shown in red. The triplet sequences are in the 5' to 3' order.

Table 3 Amino Acids and Their Abbreviations

| Amino acid | Three-letter abbreviation | Amino acid | Three-letter abbreviation |
|---------------|---------------------------|---------------|---------------------------|
| alanine | Ala | leucine | Leu |
| arginine | Arg | lysine | Lys |
| asparagine | Asn | methionine | Met |
| aspartic acid | Asp | phenylalanine | Phe |
| cysteine | Cys | proline | Pro |
| glutamic acid | Glu | serine | Ser |
| glutamine | Gln | threonine | Thr |
| glycine | Gly | tryptophan | Trp |
| histidine | His | tyrosine | Tyr |
| isoleucine | Ile | valine | Val |

Start codon: AUG

Stop codon: UAA, UAG, UGA

Characteristics of Codons

1. **Genetic code is redundant:** more than one codon can code for a particular amino acid

| First Base | U |
|------------|--|
| U | UUU phenylalanine UUC phenylalanine UUA leucine UUG leucine |

UUU and UUC both code for the amino acid phenylalanine.

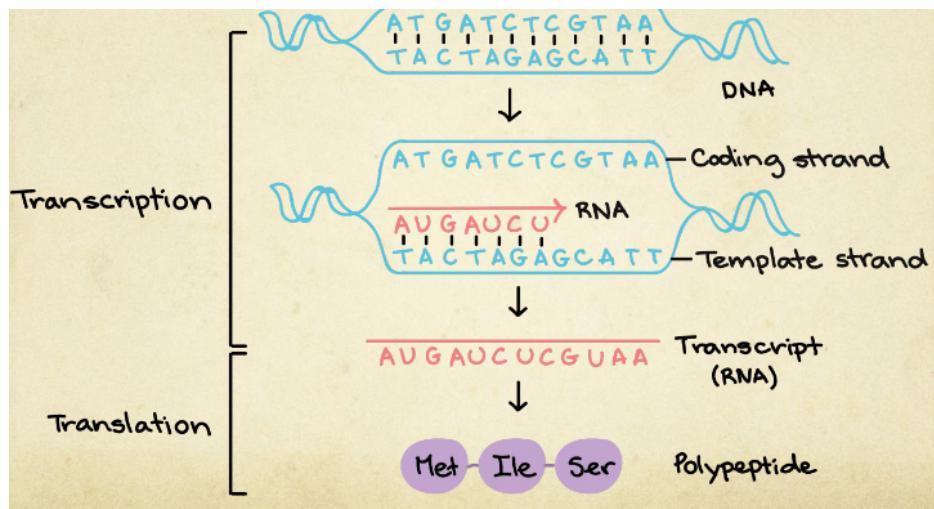
UUA and UUG both code for the amino acid leucine.

2. **Genetic code is continuous:** the mRNA is read continuously without any spaces or pauses. There is always a start and an end to the translation process.

3. **Genetic code is universal:** most organisms build their proteins using the rules of the triplet codon.

Protein Synthesis

Overview

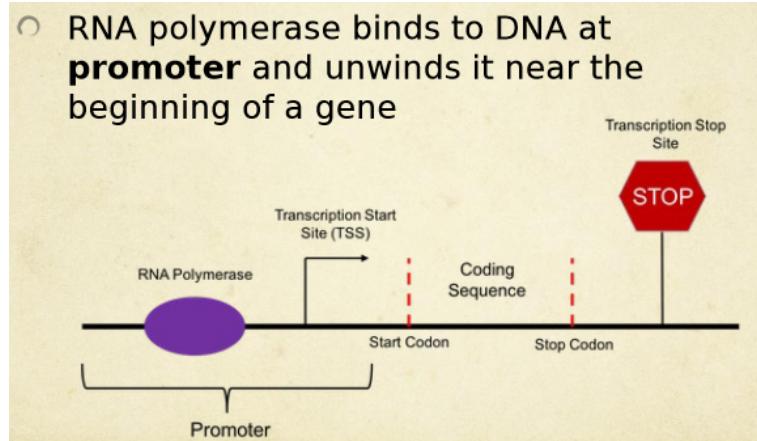


Transcription

- DNA code is chemically rewritten as an RNA code
- DNA → RNA
- Occurs within the nucleus
- 3 steps:
 1. Initiation
 2. Elongation
 3. termination

Step 1 - Initiation

- RNA polymerase binds to DNA at **promoter** and unwinds it near the beginning of a gene



- **Promoter** - a nucleotide sequence that lies just before a gene and allows for the binding of RNA polymerase.
- A key element of the promoter is the TATA box, a section of the DNA with a high percentage of thymine and adenine bases
 - Prokaryotes: TATAAT
 - Eukaryotes: TATA
- A = T only has a double bond, requiring less energy to open up the DNA strand
- This serves as recognition for the RNA polymerase indicating where to bind

Step 2 - Elongation

- RNA polymerase build the single-stranded RNA molecule
 - Strand synthesizes in 5' → 3'
 - DNA strand that is **not copied** is called the coding strand. It contains the same sequence as the mRNA except for uracil.
 - RNA elongates as nucleotides are added
 - DNA double helix reforms as RNA polymerases pass
-
- Coding strand 5' ATGATCTCGTAA 3'
 RNA 5' U G A U C G 3'
 Template strand 3' T A C T A G A G C A T T 5'
- Genes that undergo transcription have many RNA polymerases transcribing at the same time

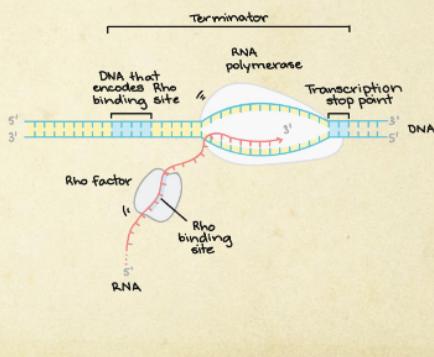
Step 3 - Termination

- Transcription is terminated when RNA polymerase recognizes a termination sequence
 - An example of a termination sequence is a string of adenine which transcribes into uracils (polyuracil). Nuclear proteins bind to polyuracil and stop transcription
- RNA polymerase is free to bind to another promoter
- The end product of this step is a **pre-mRNA strand**
- The strand still needs to undergo some modifications

○ In bacteria:

1. Rho-dependent termination:

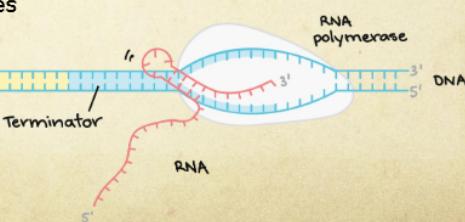
- RNA contains a binding site for Rho factor (protein).
- Rho factor binds to the sequence and starts "climbing" up the transcript towards RNA
- A sequence called transcription stop point causes RNA polymerase to pause
- When the protein catches up to the RNA polymerase, Rho pulls the RNA transcript and the template DNA apart



○ In bacteria:

2. Rho-independent termination:

- RNA polymerase approaches end of the gene at a region with lots of C and G nucleotides
- RNA transcribed folds back on itself, the complementary G & C bases bind together forming a hairpin
- A stretch of poly-U region follows, forming a weak interaction with template
- Hairpin & poly-U stalls polymerase and produces enough instability for the enzyme to fall off



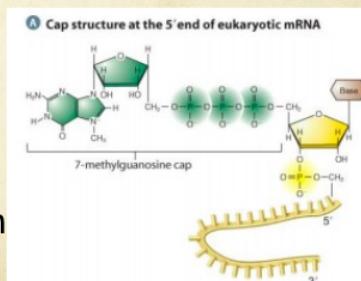
Post Transcriptional Modifications

Step 1 - Capping

There are two modifications that need to be made to the pre-mRNA before it leaves the nucleus.

1. Capping

- ◊ A 5' cap consisting of seven G's is added
- ◊ This will serve as the attachment site for mRNAs to ribosomes to allow translation



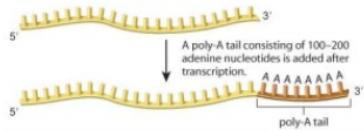
Step 2 - Tailing

2. Tailing

❖ Poly-A polymerase adds a poly(A) tail (chain of 50~250 adenine nucleotides) on the 3' end

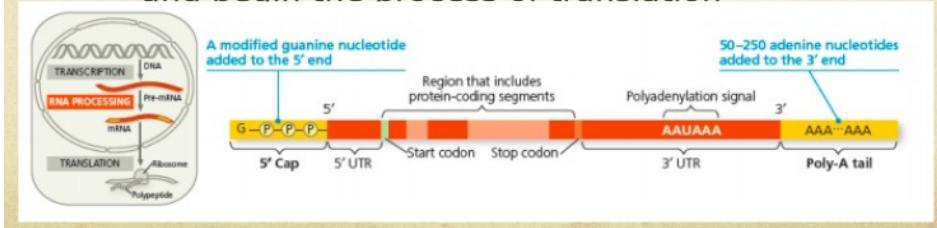
❖ The tail enables mRNA to be translated efficiently and protects it from RNA-digesting enzymes in the cytosol

④ Addition of a poly-A tail at the 3' end of eukaryotic mRNA



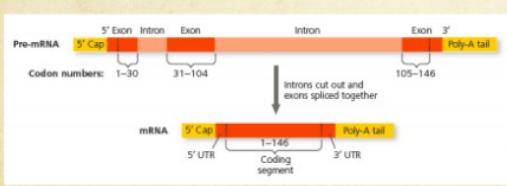
Benefits of Modifications

1. Facilitate the transport of the mRNA into the cytoplasm
2. Protect the mRNA from degradation by enzymes
3. Enables ribosomes to bind to the 5' end and begin the process of translation



RNA Splicing

- The average length of an mRNA is ~8000 nucleotides long, but only 1200 nucleotides are required to make a protein
- The mRNA contains regions of **introns** and **exons**



Introns: non-coding sequence not required for protein synthesis

Exons: coding sequence required for protein synthesis

- There are small signals between the introns and exons that indicate where the splicing should occur
- The process involves snRNP (small nuclear ribonucleoprotein) and other proteins forming a complex (**spliceosome**) at the signal and splice out the introns

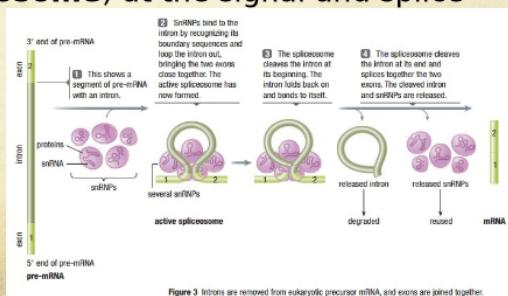


Figure 3: Introns are removed from eukaryotic precursor mRNA, and exons are joined together.

- Steps:
 1. snRNPs recognize and form complementary base pairs with mRNA sequence at the junctions of the intron and adjacent exons
 2. Other snRNPs are recruited and causes the intron to loop out and bring the exon ends close together
 3. Active spliceosome has been formed at this point and introns are removed to the exact base
- Alternative splicing occurs when exons are joined in different combinations to produce different mRNAs from a single DNA gene → increase number & variety of proteins encoded by a single gene
 - Ex. human have 20 000 genes but produce ~100 000 proteins

Eukaryotes vs Prokaryotes

Table 1 Comparison of Transcription in Eukaryotes and Prokaryotes

| Variable | Prokaryotes | Eukaryotes |
|-------------------|---|--|
| location | Transcription occurs throughout the cell. | Transcription takes place in the nucleus. |
| enzymes | A single type of RNA polymerase transcribes all types of genes. | Different RNA polymerases are used to transcribe genes that encode protein (RNA polymerase II) and genes that do not encode protein (RNA polymerase I, III). |
| elongation | Bases are added quickly (15 to 20 nucleotides per second). | Bases are added slowly (5 to 8 nucleotides per second). |
| promoters | The promoters are less complex than those in eukaryotes. | The promoters are immediately upstream of protein-coding genes, and they are more complex than those in prokaryotes. |
| termination | A protein binds to the mRNA and cleaves it, or the mRNA binds with itself. | Nuclear proteins bind to the polyuracil site and terminate transcription. |
| introns and exons | There are no introns. | There are both introns and exons. |
| product | Transcription results in mRNA ready to be translated into protein by ribosomes. | Transcription results in pre-mRNA, which must be modified to protect the final mRNA from degradation in the cytosol and to remove introns. |

Translation

Key players

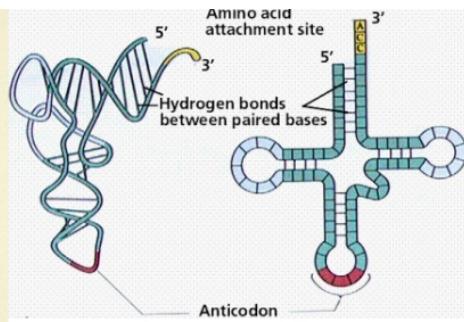
- mRNA
- tRNA
- Ribosome / rRNA

Recall tRNA

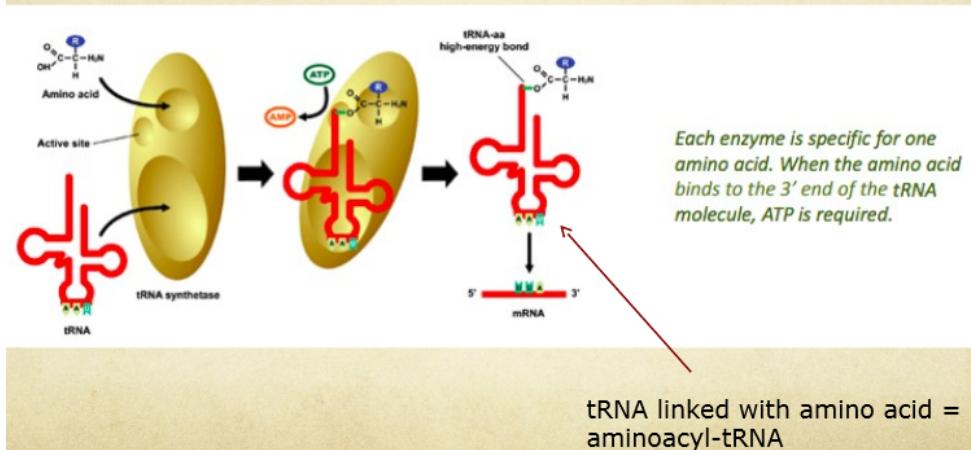
- Transfer RNA – an RNA molecule that links the codons on mRNA to the corresponding amino acid for protein synthesis
- Each tRNA has 2 functional regions:
 - **Anticodon loop**: sequence of three nucleotides that are complementary to an mRNA codon
 - **Acceptor stem**: single-stranded region where an amino acid is attached

tRNA structure

- Anticodon binds to complementary mRNA sequence
- Aminoacyl-tRNA synthetase attaches amino acid to the acceptor stem

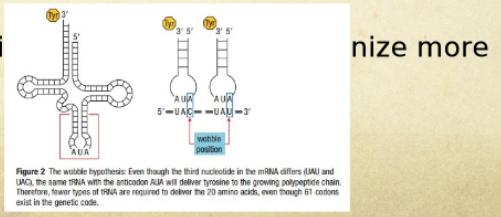


tRNA synthase



Wobble Hypothesis

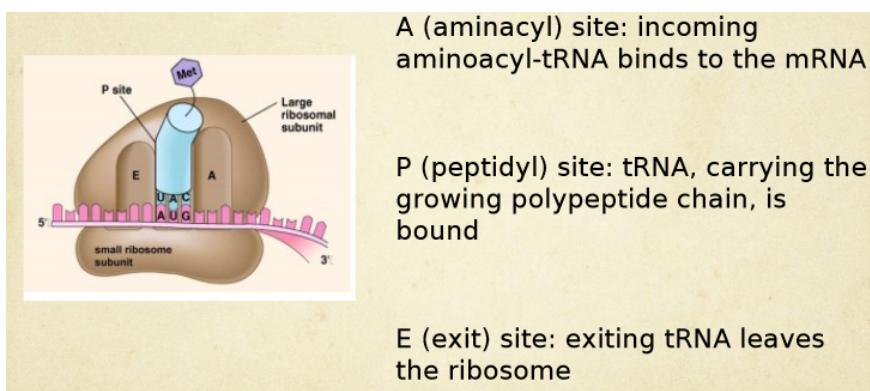
- There are more than one codon for one amino acid
- Only the first two bases of the codon have a precise pairing with the bases of the anticodon of tRNA, while the pairing between the third bases of codon and anticodon may Wobble (sway/move unsteadily)
- Wobbling permits more than one codon



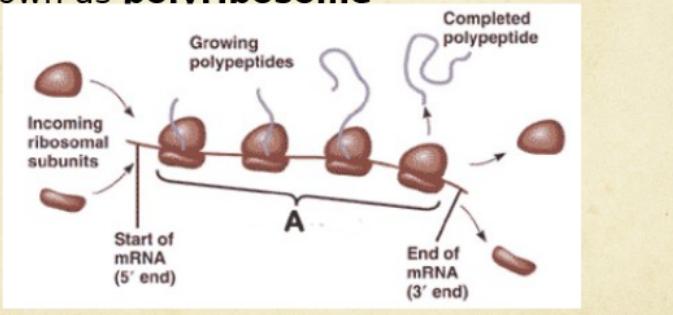
*Wobbling permits a single tRNA to recognize more than one codon

Ribosomes

- A cell structure composed of proteins and rRNA that provides the site where protein synthesis occurs
- 2 subunits: large and small subunits
- Large subunit contains a binding site for mRNA and 3 sites for binding of tRNA
- Small subunit ensures correct pairing between anticodon and codon



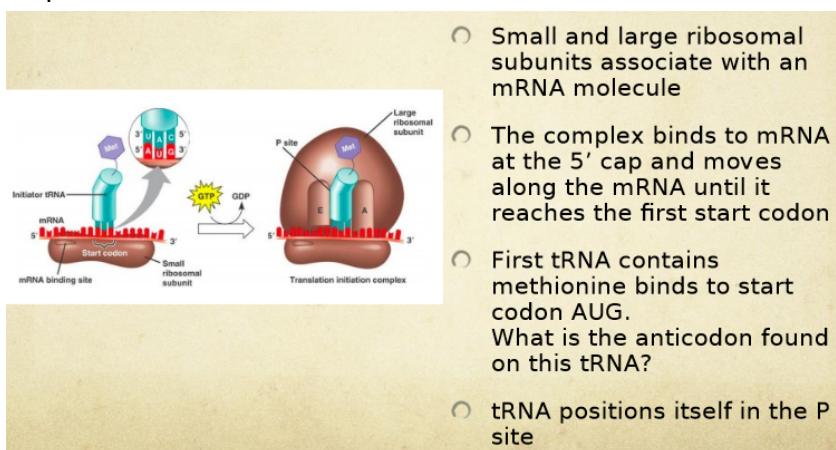
There are multiple ribosomes that attach themselves to the mRNA simultaneously. This is known as **polribosome**



Back to Translation

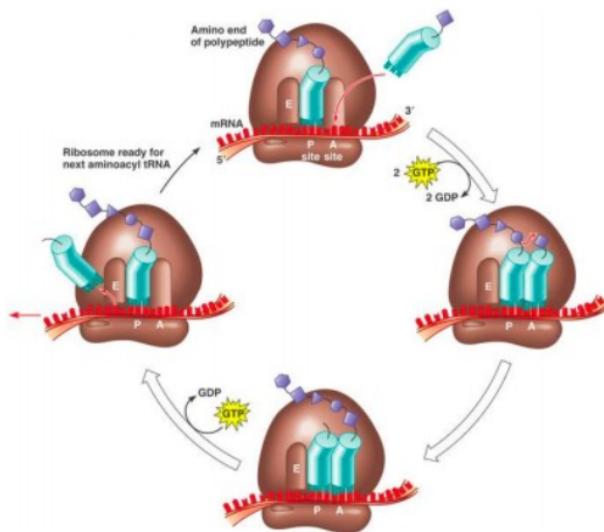
- RNA code is translated into a polypeptide chain
- mRNA → amino acid → polypeptide chain
- Occurs in the cytoplasm
- 3 steps:
 1. Initiation
 2. Elongation
 3. termination

Step 1 - Initiation



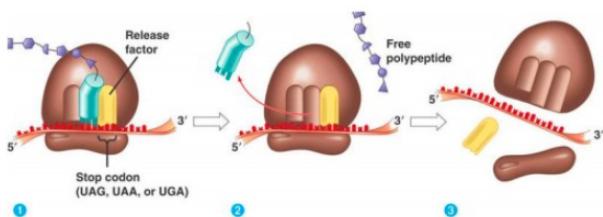
Step 2 - Elongation

- Codons are read in sets of three
- Second tRNA with appropriate anticodon binds to the codon in A site
- The amino acid (Met) is cleaved from the tRNA in the P site and forms a peptide bond with the amino acid on the tRNA in A site. The bond is catalyzed by peptidyl transferase
- Ribosome moves along the mRNA to the next codon. The two tRNAs remain bound to their codon. This shifts both tRNAs down one site to sites E and P.
- An appropriate tRNA moves into A site and steps are repeated
- Empty tRNA in E site released



Step 3 - Termination

- When A site of ribosome arrives at the first stop codon, the process switches to termination
- Stop codons: UAG, UAA, UGA
- Release factor (a protein) binds to the A site
- Polypeptide chain is released from the tRNA at P site, but no amino acid is present at A site
- Ribosomal subunits separate and detach from the mRNA
- Empty tRNA is released



Eukaryotes vs Prokaryotes

Table 1 A Comparison of Translation in Prokaryotes and Eukaryotes

| Variable | Prokaryotes | Eukaryotes |
|-------------|---|---|
| location | <ul style="list-style-type: none"> mRNA is translated by ribosomes in the cytosol as it is being transcribed from DNA | <ul style="list-style-type: none"> mRNA can only be translated after exiting the nucleus to interact with ribosomes in the cytosol some translation occurs in mitochondria and chloroplasts |
| initiation | <ul style="list-style-type: none"> mRNA bases pair directly with a ribosomal binding site, just upstream of the start codon mRNA 5' cap is involved | <ul style="list-style-type: none"> complex of Met-tRNA, with small ribosomal subunits, binds to an mRNA 5' cap and scans until it encounters the start codon |
| elongation | <ul style="list-style-type: none"> 15 to 20 elongation cycles per second | <ul style="list-style-type: none"> 1 to 3 elongation cycles per second |
| termination | <ul style="list-style-type: none"> stop codon appears and a release factor binds so that the polypeptide is released | |
| polysomes | <ul style="list-style-type: none"> mRNA strand can be translated by multiple ribosomes simultaneously, even as it is being transcribed from DNA | <ul style="list-style-type: none"> mRNA strand can be translated by multiple ribosomes simultaneously, but only in the cytosol |

Polypeptide to Protein

- ◆ The completed polypeptide travels through the ER to the Golgi
- ◆ The polypeptide at the end of translation is not functional. It must be folded into the correct conformation/shape
- ◆ Reactions such as removal of amino acids from ends or addition of molecules (sugar) to the chain are required to activate the polypeptide
- ◆ Some polypeptides are assembled together to form a single functioning protein
- ◆ The Golgi packages it into a vesicle
- ◆ Vesicle moves to the membrane and is released by exocytosis.

Mutations

Introduction

- Every normal cell carries a full complement of genetic material
- A mutation can occur in:
 - a somatic (body) cell
 - a germinal (reproductive) cell – can be transmitted to offspring

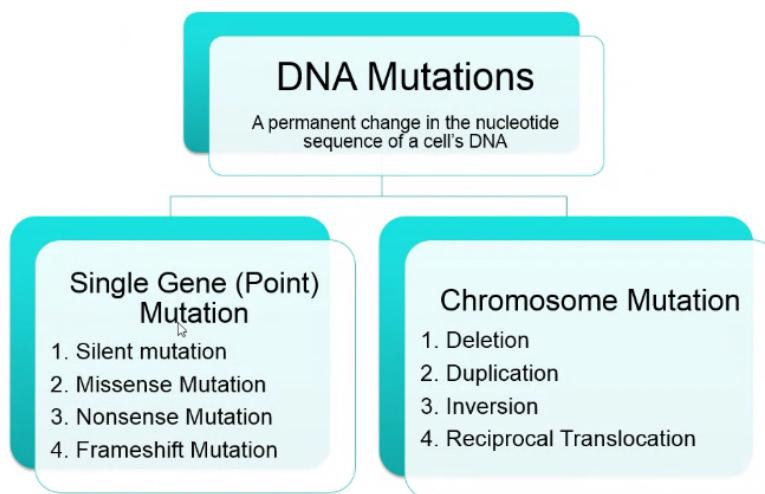
Mutations are not all bad

- mutations are random changes in genetic material
- most mutations that are detectable are detrimental
- some mutations provide variation, allowing for adaptation to the environment (can be favorable)
- The majority of mutation are neutral
- some mutations cannot be detected
- Mutation is only beneficial or negative in a given time and situation

Example of beneficial mutations

- In 1848, Edleston (naturalist) recorded the first sighting of a dark peppered moth
- By 1900, in areas around English cities, as much as 98% of the moth population were dark
- Industrial Revolution: factories burning coal for fuel made tree trunks dark and bare

Types of mutations

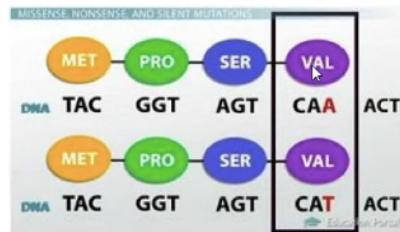


Single Gene mutations

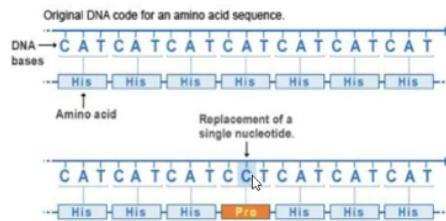
- Single gene mutations may also be categorized according to how they affect the amino acid sequence of a protein.
 1. Silent mutations
 2. Missense Mutation
 3. Nonsense Mutation

Silent mutations

- Involves the replacement of one nucleotide with another nucleotide
- does not result in a change in the amino acid sequence of the protein
- due to the redundancy of the genetic code
- or a change in the code on the introns.

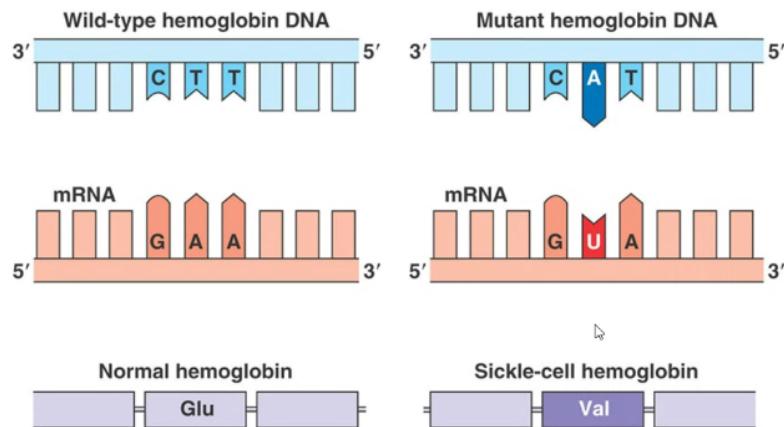


Missense Mutation



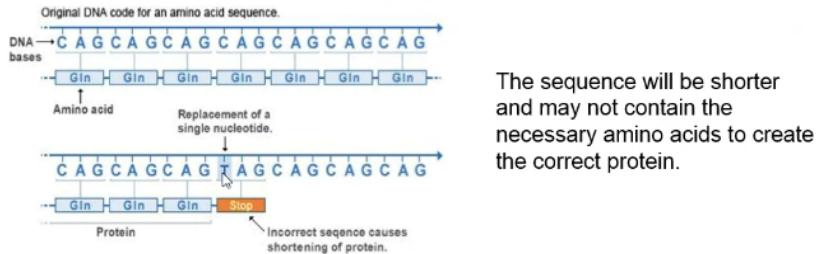
- Substitution of one amino acid resulting in a different amino acid
- E.g. sickle cell anemia
- Only affects one base pair on the DNA or one codon of mRNA.
- Can be called a base pair substitution in this case.

- Sickle cell anemia



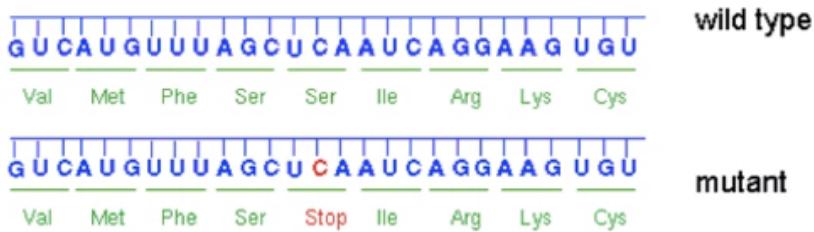
Nonsense mutations

- a mutation that converts a codon for an amino acid into a stop codon (usually lethal to the cell)
- Also called a chain termination mutation
- UGA, UAA and UAG are the stop codons!



Frameshift Mutations

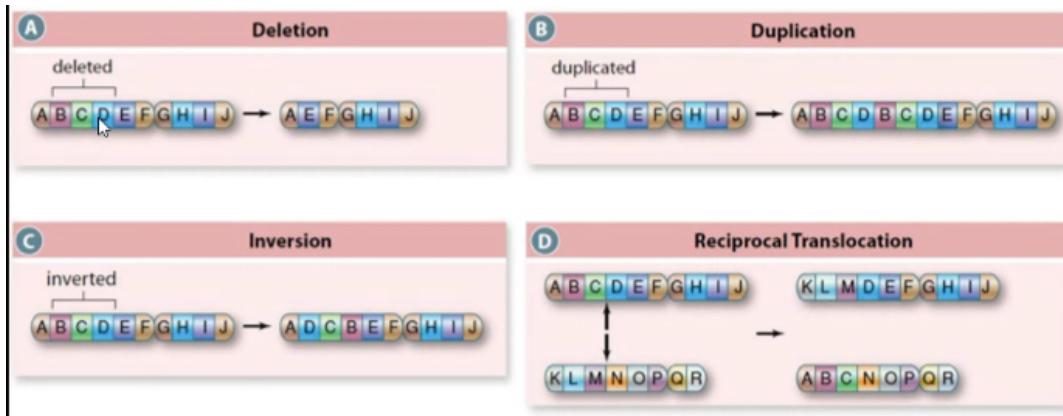
- occurs when the reading frame is changed.
- Caused by an insertion or deletion of a nucleotide
- Changes the remainder of the code.



Chromosomal mutations

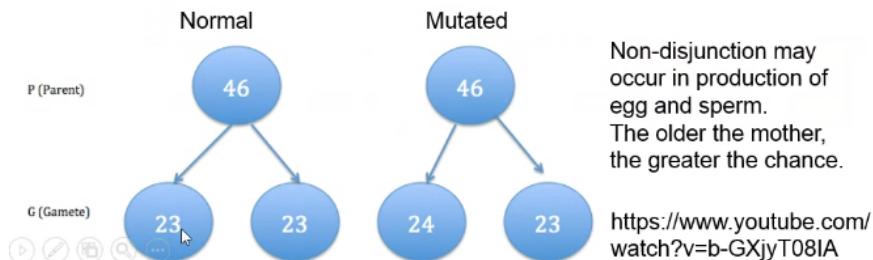
There are 4 main types of chromosome mutations that may affect many genes.

1. **Deletion:** A series of nucleotides are deleted from the chromosome
2. **Duplication:** a series of nucleotides are duplicated on the same chromosome
3. **Inversion:** a group of nucleotide sequences are inverted in the chromosome
4. **Reciprocal Translocation:** a group of nucleotides from one chromosome are exchanged with the nucleotides of a different chromosome.



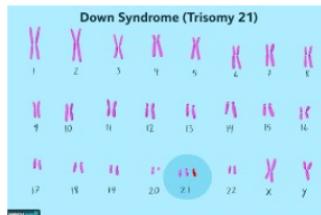
Changes in Chromosomes

- Extra chromosomes may be passed or missed
- Gametes are haploid, one from each parent.



Mutation example - Down Syndrome

- Down syndrome
 - 47 chromosomes (have extra 21st chromosome)
 - Features: flat, broad face, squint eyes, furrowed and protruding tongue, low IQ

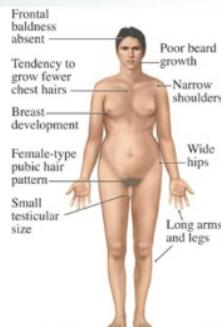


Mutation example - Klinefelter's Syndrome

Examples

■ Klinefelter's Syndrome

- 44 autosomes + XXY = 47 chromosomes
- 44 autosomes + XXXY = 48 chromosomes
- XX is usually female, but there's a Y so all patients are males
- Features: male with small testes, no sperm in the ejaculate, abnormal breast development
- The greater number of X the individual has, the more marked is the condition

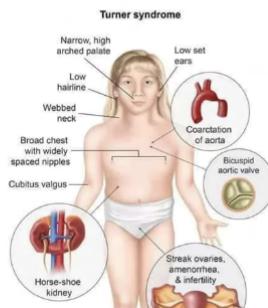


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Mutation Example - Turner's Syndrome

■ Turner's Syndrome

- 44 autosomes + X = 45 chromosomes
- Missing a X chromosome
- Features: small female, sexually immature



Mutation Example - XYY Syndrome aka Super human male

■ XYY Syndrome

- Aka. Super human male
- 44 autosomes + XYY = 47 chromosomes
- Features: aggressive, antisocial male

What causes mutation

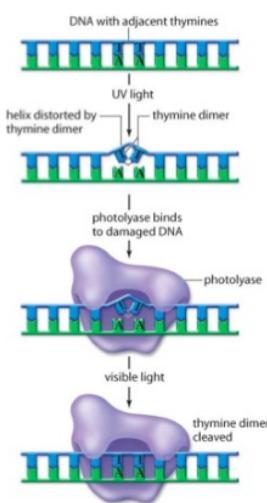
- Spontaneous mutations
 - occur under normal conditions.
 - May involve mispairing during replication
- Induced mutation
 - caused by mutagen that directly alters the DNA within a cell.
 - Common mutagens
 - Chemical: formaldehyde, carbon monoxide
 - Radiation: UV, x-rays

DNA Repair

- If mutations are detrimental and accumulate too quickly, the cell needs to repair the mutation so that certain proteins can be restored
- Two methods of repair:
 - Specific repair mechanism
 - Non-specific repair mechanism

Specific repair mechanisms

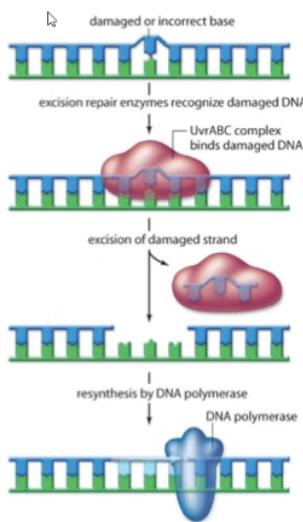
- Photorepair is a specific mechanism to repair damage to DNA caused by exposure to UV radiation
- A photolyase enzyme recognizes the damage, binds to the dimer and uses visible light to cleave the dimer.



Non specific repair mechanisms

Non-Specific Repair

- Excision repair enzyme can repair a variety of damage
- It binds to damaged DNA and cuts out the damaged strand
- DNA polymerase comes in to fill in gap



Controlling Gene Expression

Control mechanics

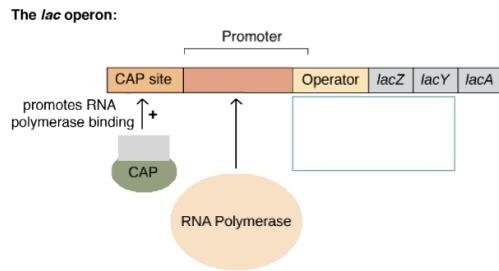
- Determine when to make more proteins and when to stop making more
 - Ex. insulin is only required when glucose level in blood is high, not made all the time
- Cell has mechanisms to control transcription and translation
- Genes need to be turned on and off as they are needed
- Housekeeping genes** are genes that are always needed, and are constantly synthesizing proteins (switched on)

Prokaryotic Control Mechanisms

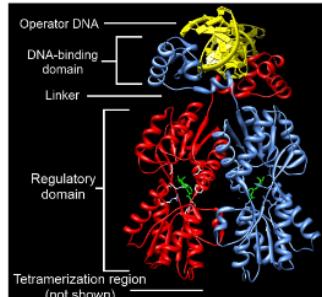
- Gene expression in prokaryotes is regulated by the concentration of lactose and tryptophan
- Use negative feedback control

The Lac Operon

- **Lac operon** is a cluster of three genes that contain the DNA sequences coding for protein involved in the metabolism of lactose
- Consists of:
 - Promoter (site where transcription begins)
 - Operator (sequence of bases controlling transcription)
 - Coding regions (for enzymes used to metabolize lactose)

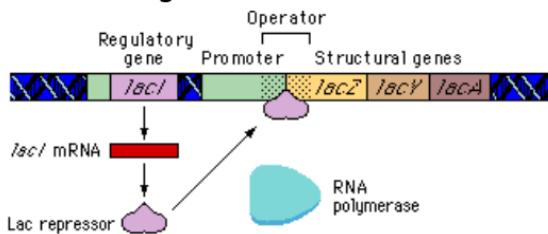


- Upstream of operon contains a gene coding for repressor protein (*lac I*) which detects the concentration of lactose in the environment
 - This protein is always present since lactose is a main source of energy



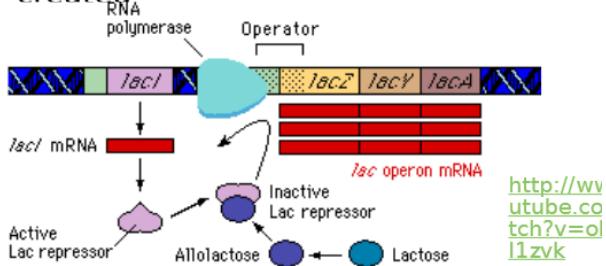
- Absence of lactose

- Lac repressor is active
- Binds to operator, preventing binding of RNA polymerase
- Stops lactose metabolizing enzymes from being made



- Presence of lactose

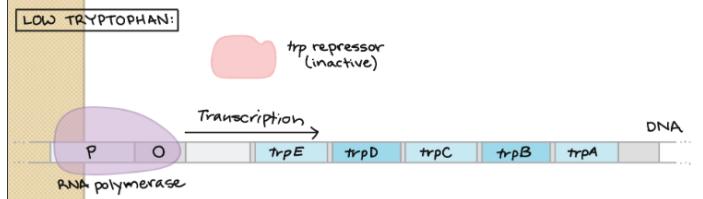
- Some lactose binds to the repressor, making it inactive
- RNA polymerase binds to promoter for transcription
- Lactose metabolizing enzymes are created



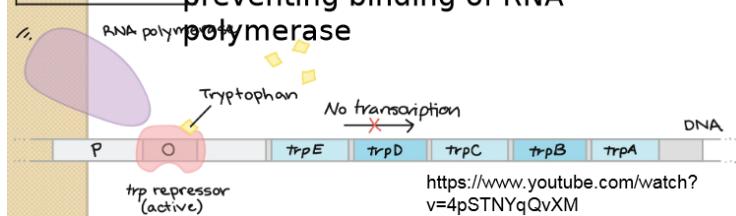
The Trp Operon

- Tryptophan is an amino acid used to build proteins
- Can be obtained from environment (saves energy!) or synthesized
- Consists of:
 - Promoter
 - Operator
 - Trp repressor coding genes

- Absence of tryptophan:
 - Cell needs to make its own tryptophan
 - Repressor protein is inactive, does not bind to operator
 - RNA polymerase bind to promoter and transcribes genes for tryptophan biosynthesis enzyme
 - Results in synthesis of tryptophan



- Presence of tryptophan:
 - Cell does not need to make tryptophan
 - Tryptophan binds to repressor
 - Repressor binds to operator, preventing binding of RNA polymerase



<https://www.youtube.com/watch?v=4pSTNYqQvXM>

Eukaryotic Control Mechanisms

- More steps, do not use operon systems
- Four categories:
 - **transcriptional** (controls transcription from DNA to mRNA)
 - **posttranscriptional** (controls the removal of introns)
 - **translational** (controls rate that mRNA is activated through ribosomes)
 - **posttranslational** (affects the rate proteins can leave the cell)

Transcriptional Regulation

- Most common type of regulation
- DNA is wrapped around histone blocking the promoters from proteins initiating transcription
- Examples:
 - Type I: activator molecule binds to promoter which signals complex to change shape
 - Type II: activator molecule binds to sequence, signals addition of acetyl to histone, loosening histone's association with DNA, allows promoter to become accessible

Post-Transcriptional Regulation

- Regulate through changes in pre-mRNA processing and rate at which mRNA are degraded
- Examples:
 - Alternative splicing: results in different mRNA by removing different combinations of introns. Depending on the protein being made, regions of introns and exons differ
 - Masking proteins: bind to mRNA, keep them inactive. Ex. animal eggs are kept inactive until fertilized.

Translation Regulation

- Occurs during protein synthesis by a ribosome
- Specific enzymes can add or delete adenine in the poly-A tail of mRNA molecules
- The change in length of poly-A tail may increase or decrease the time required to translate the mRNA into a protein

Post-Translational Regulation

- Three methods:
 - Processing: Proteins require processing mechanisms (removes specific regions) to be activated.
 - Chemical modification: addition or deletion of certain chemical groups affecting protein function. Can be used to put the protein “on hold”
 - Rate of degradation: short-lived proteins are tagged. Adding or removing tags can shorten or extend the functional life of protein

Regulation Cancer

- Lack regulatory mechanisms
- The probability of DNA experiencing a mutation increases as one ages
- Exposure to potential mutagen has a cumulative effect in the number of mutations in the genome
- Benign tumour: mass of slowly growing cells, remains in place, does not return once removed, not life-threatening
- Malignant tumour: hard to remove, require chemotherapy and radiation

QUIz

10 mc

2 short answers

5 fill in blanks

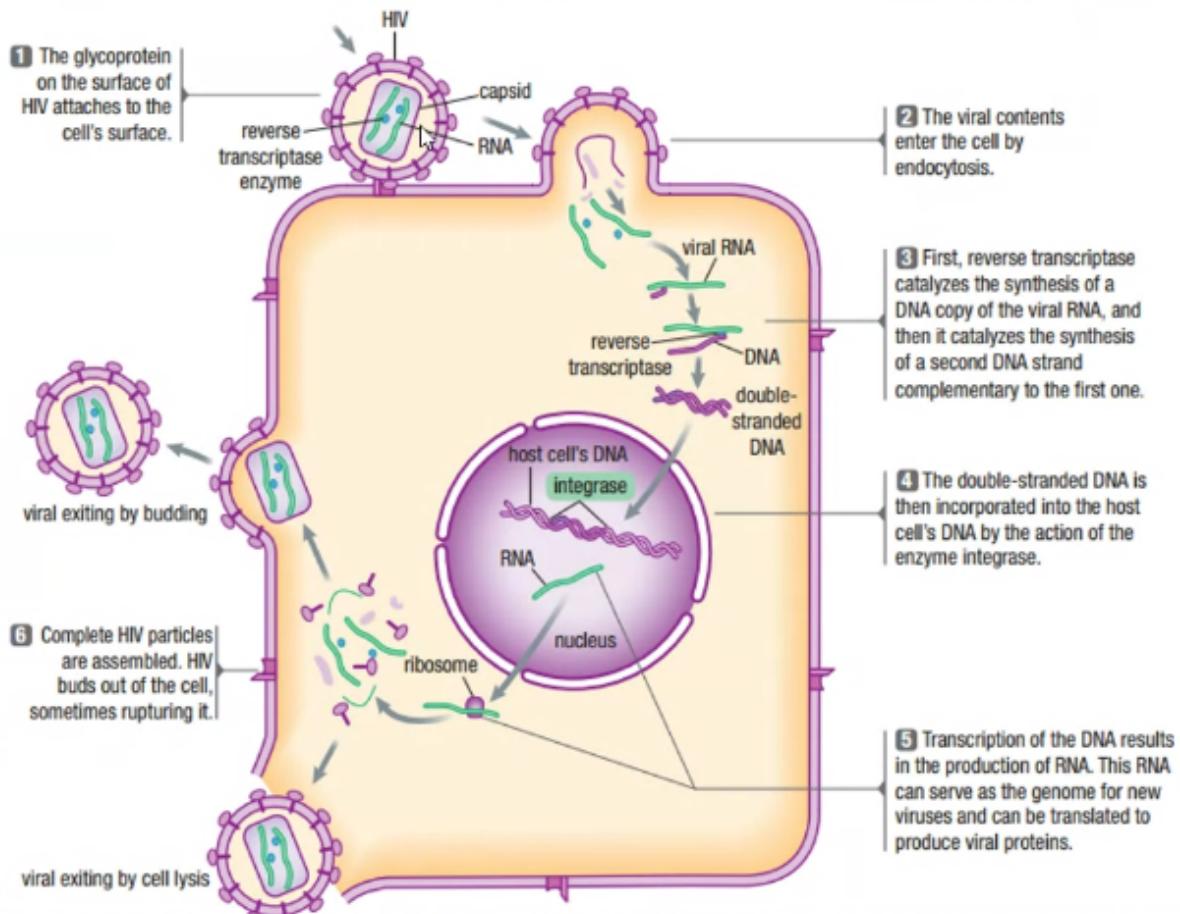
Viruses

- Each year, the flu is caused by a different strain of the virus
- Not considered alive since they cannot replicate themselves and depend on other organisms
- Some have an envelope that's leftover from host
- Have a protein code called **capsid**
- Over 4000 species
- Usually infect only a single species or a few closely related species or just one organ system
- Example: influenza A virus
 - Contains only 8 genes but can code for 11 proteins
 - High replication error creating mutations

- If virus has DNA, they follow the same method of DNA replication we learned
- If (retro)virus has RNA, it codes for reverse transcriptase enzyme which synthesize complementary DNA strand
 - Example: HIV (human immunodeficiency virus)

Virus Replication

- Steps:
 1. Retrovirus infects host cell
 2. RNA and reverse transcriptase are injected into the host
 3. The reverse transcriptase uses the host's cell material to create complementary molecule of single stranded DNA
 4. The reverse transcriptase directs the synthesis of the second strand to create a double-stranded DNA
 5. The enzyme integrase incorporates DNA into the host cell genome



Using viruses as vectors

- Scientists use virus to insert new genetic material into existing cell genome
- Insulin has been inserted into sunflower using this method and extracted from plant cells for therapeutic purpose
- **Transduction** – use of viral vector to insert genetic material into a host genome
 - Harmful material is removed and replaced with desired genetic material
 - Commonly used virus: retroviruses, adenoviruses, and herpes viruses

Genome, Viruses, Biotechnologies

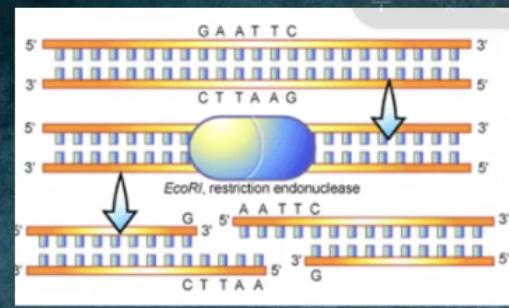
We will look at the following:

- Recombinant DNA technology
- Gene Cloning
- Polymerase Chain Reaction (PCR)
- DNA Sequencing

Recombinant DNA Technology

- Recombinant DNA: a molecule of DNA composed of genetic material from different sources.
- Prokaryotic organisms have many different restriction enzymes that help them protect themselves against viral DNA
- Restriction enzymes cleave viral DNA so that it can no longer replicate within the organism
- Ex. Restriction endonuclease

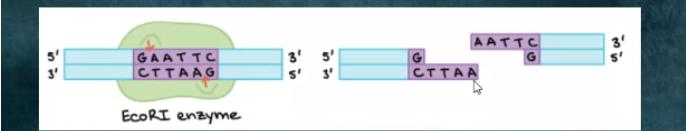
- Restriction endonuclease recognizes specific nucleotide sequence and cleaves the double stranded DNA
- The enzyme will cut at the restriction site of the target sequence



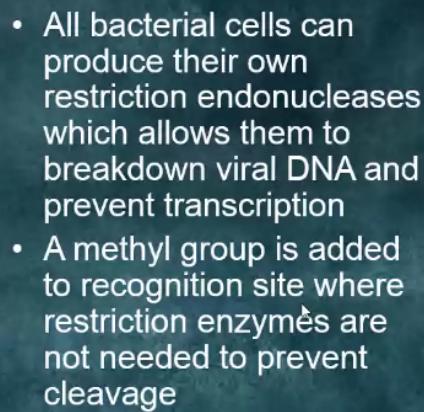
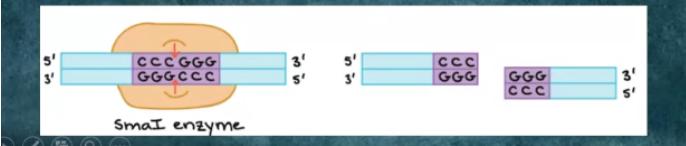
Characteristics of Restriction Enzyme:

- 1) **Sequence Specificity:** Each enzyme recognizes a specific sequence which it cuts on every DNA
- 2) **Staggered cuts:** sticky ends are left on either strand of the DNA

- Sticky ends: the ends of the DNA have an overhang that allows them to bind to a complimentary overhang

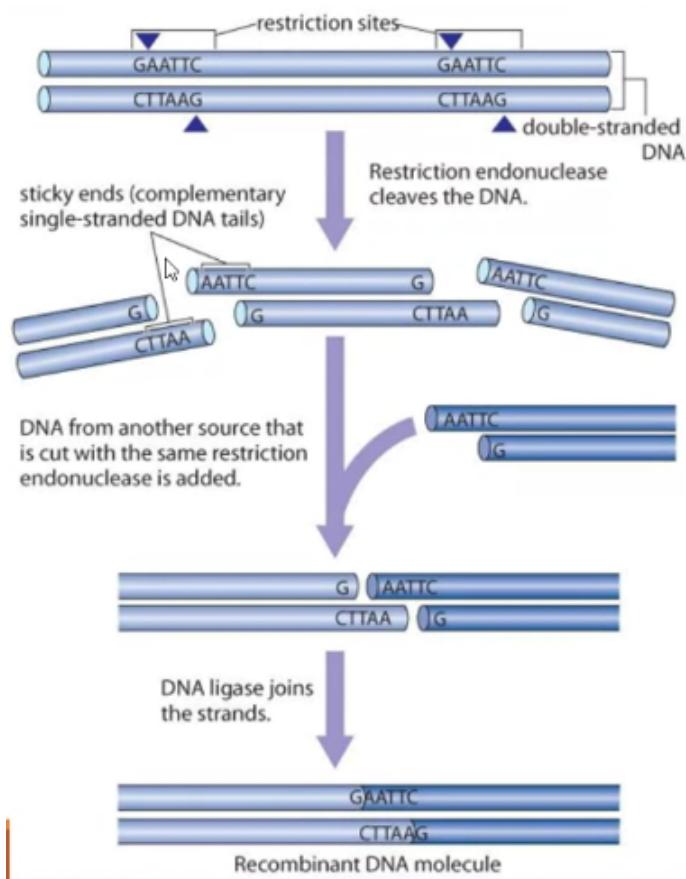
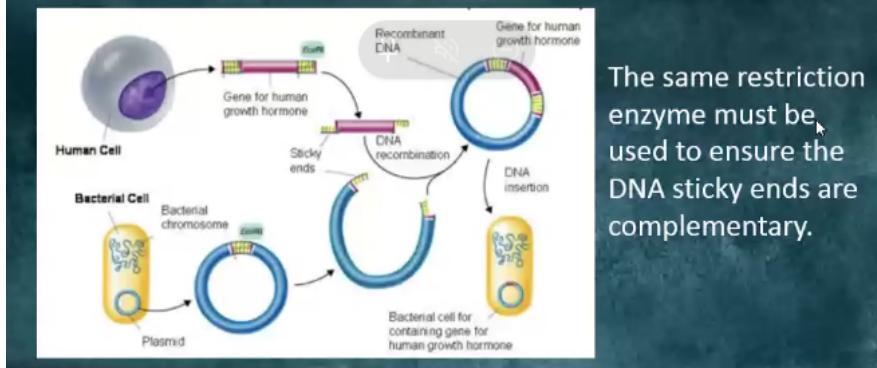


- Blunt ends: the ends of the DNA are fully base paired when cut by a restriction endonuclease



- Features of bacterial cells and restriction endonuclease are useful in recombinant technology
 1. Enzymes can produce sticky ends
 2. By adding a methyl group to a recognition site, it will not be destroyed
 3. Most bacterial strains contain plasmids which can be used as vector to carry genes of interest

- Restriction endonuclease enzymes are used to combine DNA of interest to a plasmid
- Sticky ends of DNA can combine to any other DNA that have a complementary sticky end



Step 1: Restriction enzymes are used to cleave the target sequence of DNA of interest

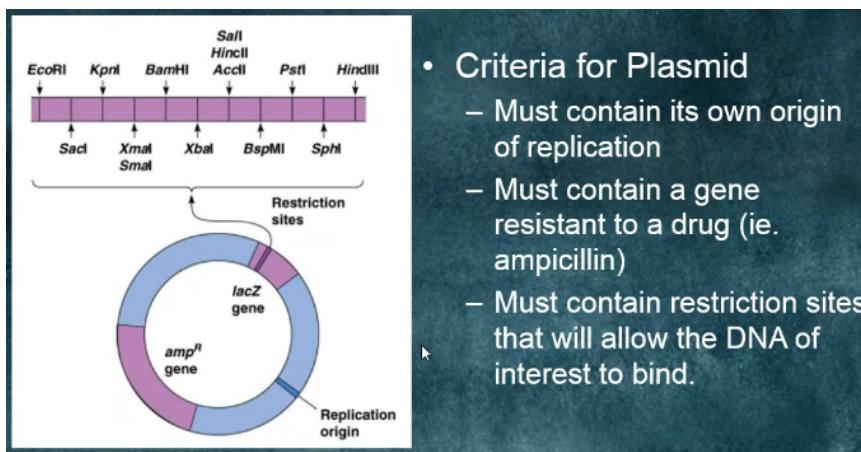
Step 2: When the target DNA sequence is removed, it produces a DNA fragment with sticky ends.

Step 3: The other DNA of interest must be cut with the same restriction enzyme to produce complementary sticky ends.

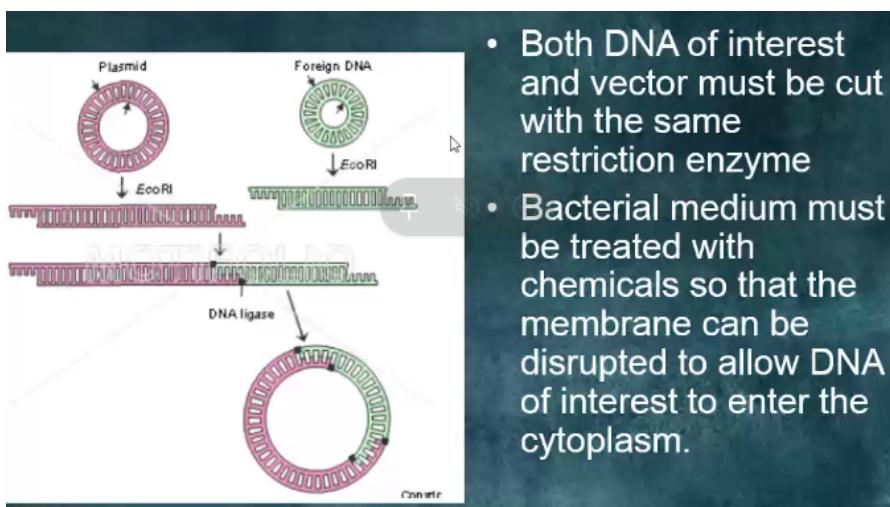
Step 4: Both DNA restriction fragments are incubated with DNA ligase so that a covalent bond can be formed between the fragments.

Gene cloning in Bacteria

- Recombinant DNA can be used to determine particular function of a gene of interest
- Recombinant DNA can be used to clone a gene of interest. A vector is required
- A vector can be a plasmid (from prokaryotic cells) that can carry the human gene of interest
- Vectors contain origins of replication that are independent of the bacterial DNA

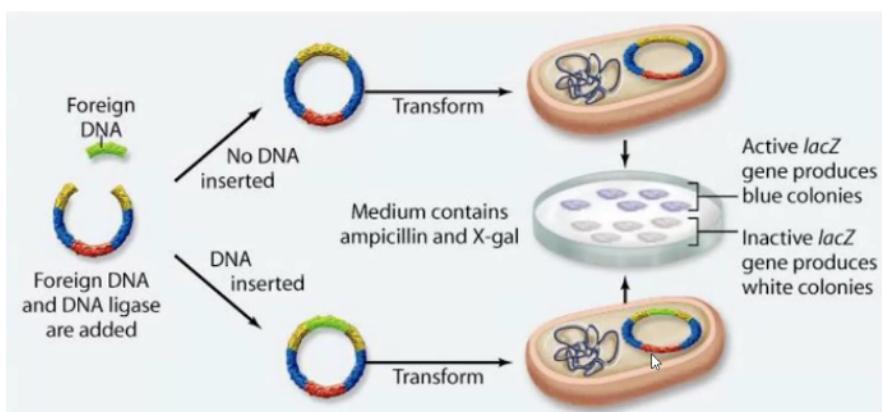


- Criteria for Plasmid
 - Must contain its own origin of replication
 - Must contain a gene resistant to a drug (ie. ampicillin)
 - Must contain restriction sites that will allow the DNA of interest to bind.



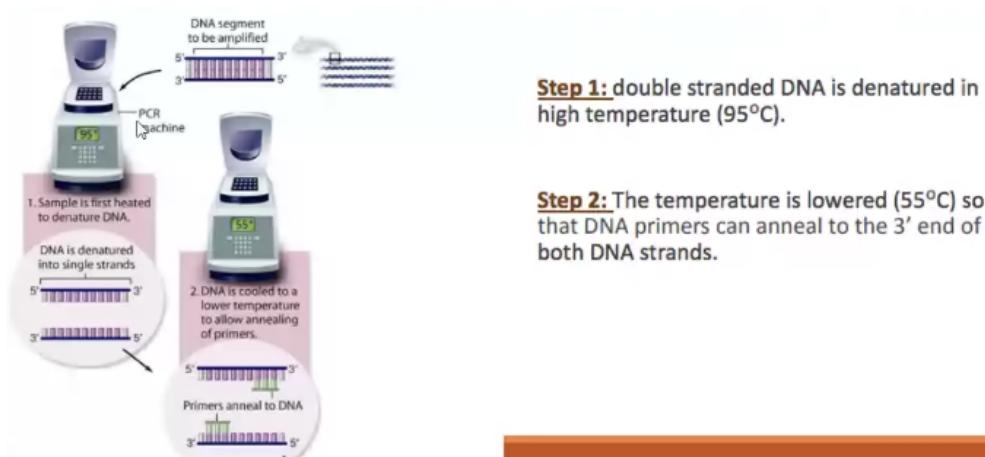
- Both DNA of interest and vector must be cut with the same restriction enzyme
- Bacterial medium must be treated with chemicals so that the membrane can be disrupted to allow DNA of interest to enter the cytoplasm.

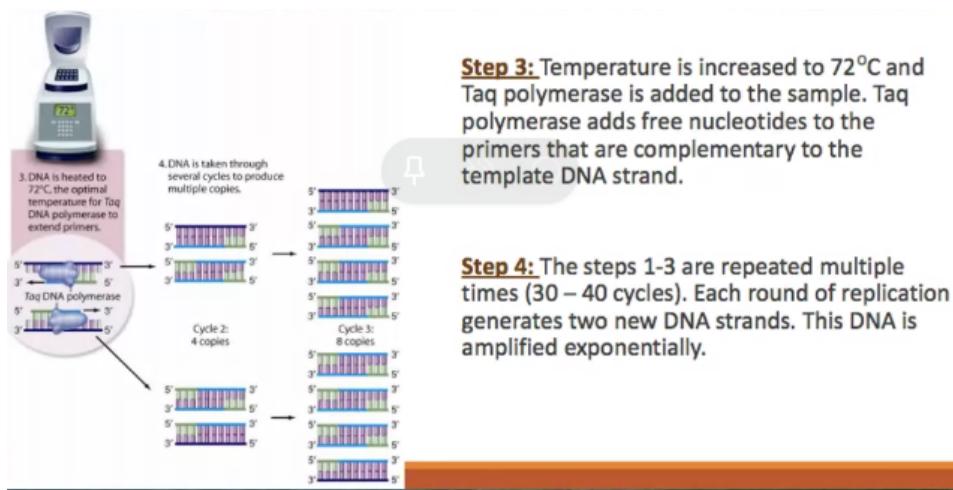
- To determine whether the gene has been integrated, bacteria are grown in ampicillin and an X-gal (sugar)
- Active lacZ gene breaks down bacterial colonies, turning them blue
- The colonies that have integrated the gene are transferred and grown in a separate culture



The Polymerase Chain Reaction - PCR

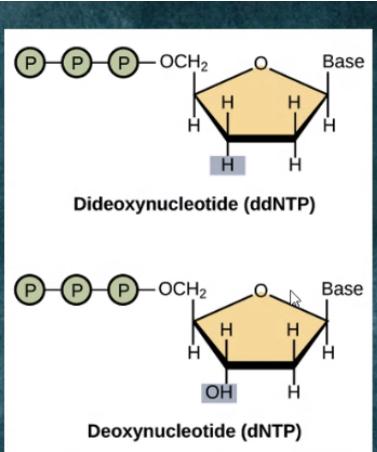
- PCR is a biotechnology used by scientists to produce large amounts of DNA (ie. DNA amplification)
- Enables scientists to purify a small fragment of DNA for analysis purposes





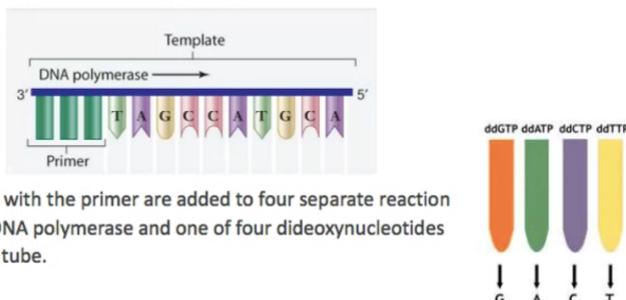
DNA Sequencing

- Refers to any process that can identify the sequence of the bases along a fragment of DNA
- One of the processes being used is known as chain termination sequencing
- A prime-single stranded nucleotide chain, DNA polymerase, nucleotides and a low concentration of the dideoxynucleotide variants are used
- Dideoxynucleotides resembles normal DNA nucleotides except a hydroxyl group is missing at carbon 3 position



- Once the ddNTP is attached to a growing DNA strand, the DNA polymerase cannot add any more
- The DNA fragment eventually breaks off
- All the fragments formed are then separated by gel electrophoresis
- The gel is then read from bottom to top to obtain a nucleotide sequence of the new DNA strand
- This strand is complementary to the original DNA fragment

Step 1: DNA is denatured into s.s stranded DNA and the radioactively labelled primer is added to a reaction tube. This provides a 3'-OH for replication.



Step 2: S.S DNA annealed with the primer are added to four separate reaction tube along with dNTPs. DNA polymerase and one of four dideoxynucleotides is added to each reaction tube.

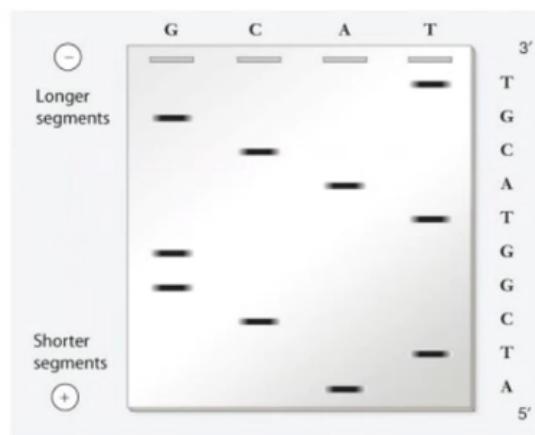
Step 3: The reaction proceeds and DNA polymerase begins to add the nucleotides onto the growing DNA chain. Due to the dideoxynucleotides, different fragments are produced.

| | | | | | |
|------------------|------------------------------|---|--|--|--|
| Reaction for ddG | 5' ————— A T C G | | | | |
| | 5' ————— A T C G G | | | | |
| | 5' ————— A T C G G T A C G | | | | |
| Reaction for ddC | 5' ————— A T C | | | | |
| | 5' ————— A T C G G T A C | | | | |
| Reaction for ddA | 5' ————— A | → | | | |
| | 5' ————— A T C G G T A | | | | |
| Reaction for ddT | 5' ————— A T | | | | |
| | 5' ————— A T C G G T | | | | |
| | 5' ————— A T C G G T A C G T | | | | |

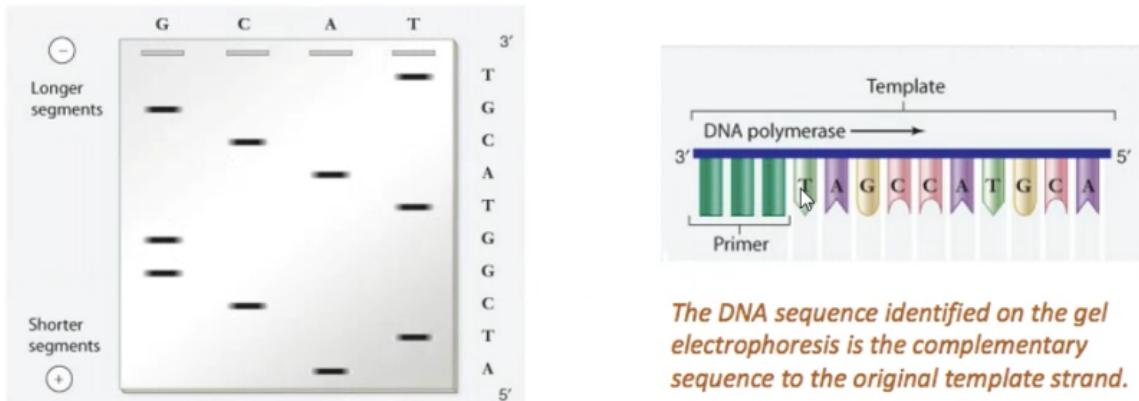
The corresponding radioactive dideoxy will be found at the end of each newly synthesized DNA fragment in the reaction tube.

Step 4: Each of the reaction tubes are separated on the gel electrophoresis with a polyacrylamide gel. Depending on the size of the DNA fragments, they will travel across the gel at different speed towards the positive end of the gel.

Once the fragments have run along the gel, it is exposed to X-ray film through autoradiography. This highlights the radioactive tags of dideoxynucleotides.



Step 5: Scientists then compare the fragments within the gel and analyze the length of the fragments along with their radioactive labels. Through comparison, scientists are able to identify the location of each nucleotide within the DNA sequence.



The DNA sequence identified on the gel electrophoresis is the complementary sequence to the original template strand.

New Sequencing Methods

- New and efficient methods were developed for the Human Genome Project
- Old method was limiting as scientists could only read 300 nucleotides at a time

New Method:

- More bases can be read
- Dideoxynucleotides are labelled with their own colour of dye tags
- All dideoxynucleotides are added to one reaction tube only
- In gel electrophoresis, only one lane is required and a laser lights up the tags in the gel
- Photodetectors are used to identify the colour and the fragments can be analyzed

