Learning Outcome B8

- Explain how mutations in DNA affect protein synthesis

Student Achievement Indicators

- Give examples of two environmental mutagens that can cause mutations in humans.
- Use examples to explain how mutations in DNA change the sequence of amino acids in a polypeptide chain, and as a result may lead to genetic disorders.

Learning Outcome B6

- Describe recombinant DNA

Student Achievement Indicators

- Define recombinant DNA
- Describe a minimum of three uses for recombinant DNA

What are Mutations?

- Change in the sequences of bases within a gene
- Can lead to malfunctioning proteins within a cell

Causes

- Errors in replication
- Mutagens
- Transposons

Causes of Mutations

Errors in Replication

- Rare source of mutation
- DNA polymerase carries out replication - adds nucleotides and proof reads new strand again template strand.
- Usually mismatched pairs are replaced with the correct nucleotides.
- Typically there is one mistake for everyone nucleotide pair replicated.

Causes of Mutations

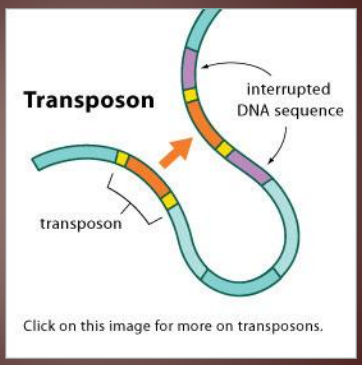
Mutagens

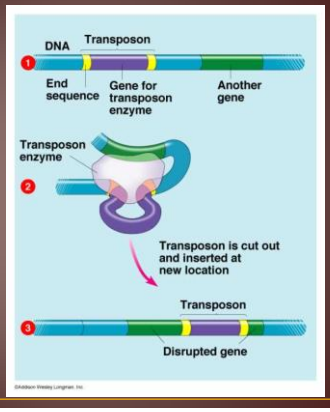
- Environmental influences
- Include radiation and certain organic materials such as pesticides, chemicals in cigarettes, UV light etc…
- Mutations due to mutagens are rare because DNA repair enzymes monitor and repair irregularities.

Causes of Mutations

Transposons

- Specific DNA sequences that have the ability to move within and between chromosomes.
- This movement may alter neighboring genes either by increasing or decreasing expression.
- This is known as "jumping genes" because the movement of a gene may impact expression and protein function.





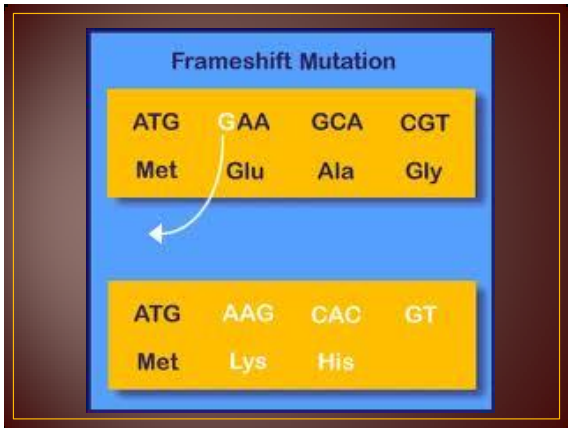
Types of Mutations

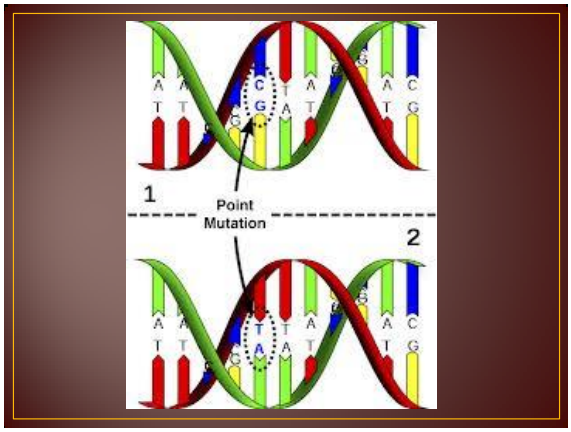
Frame Shift Mutation

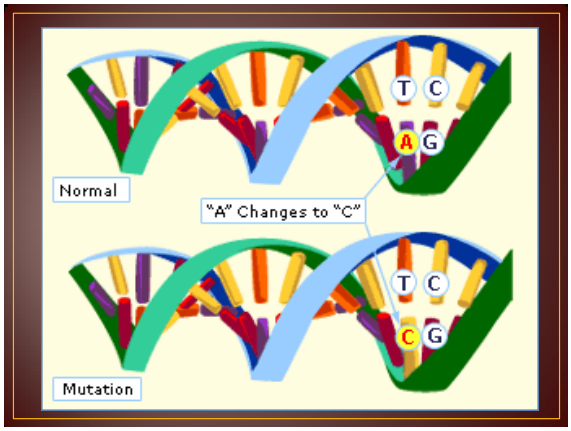
- Insertion and deletion of a nucleotide

Point Mutation

- Involves substitution of a nucleotide into a sequence
- *Example* - UAC become UAU, no change because both amino acids code for tyrosine
- Known as a silent mutation
- UAC – UAG creates the stop codon or a dysfunctional protein



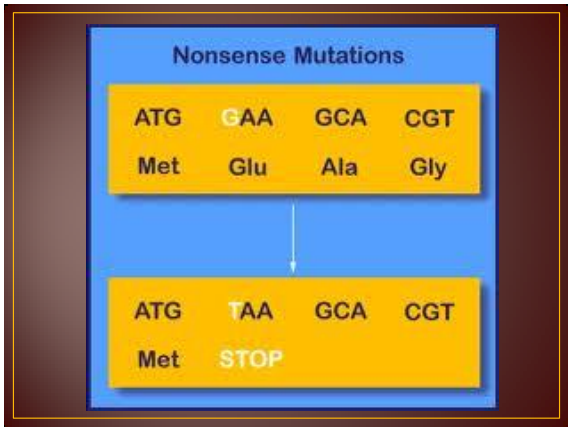


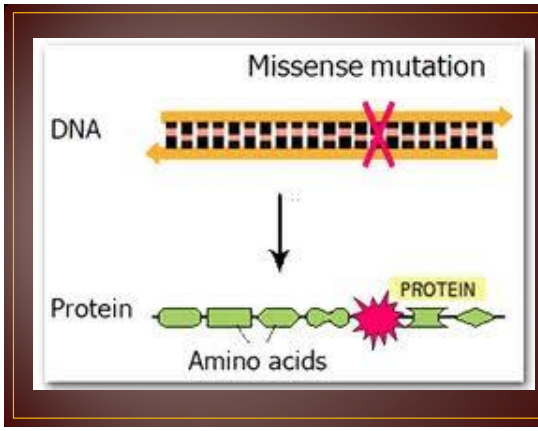


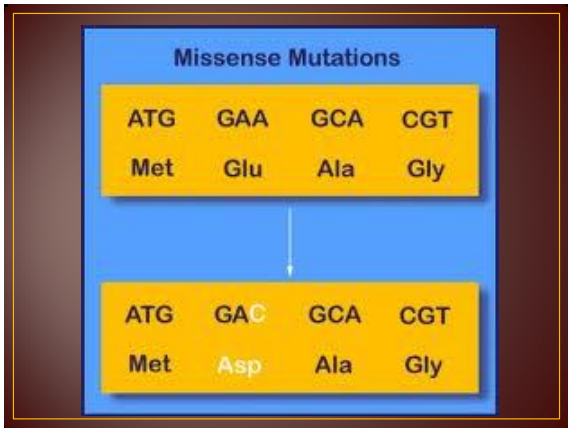
Types of Mutations

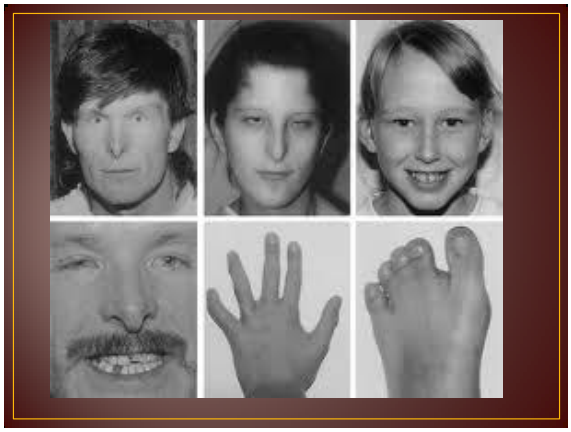
Nonsense Mutation
- Will stop protein synthesis

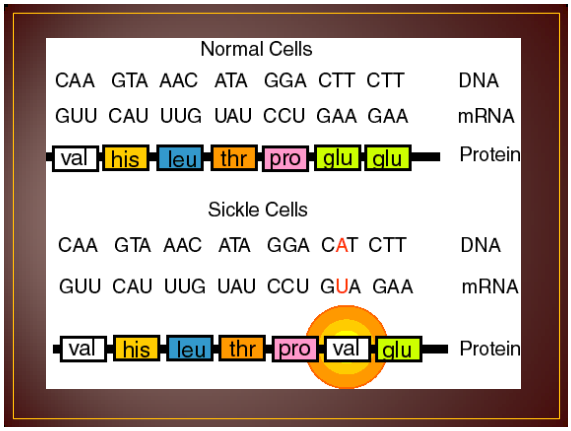
Missense mutation
- Affects the shape of a protein by substituting in another base
- Affect is on function and appearance
- Example - sickle cell animal
- Change in amino acid sequence creates the protein valine instead of glutamate which affects the protein hemoglobin
- It has a different shape which changes the shape of the red blood cells
- These misshaped RBC's causes clogs in small blood vessels and can cause damage to major organ systems.

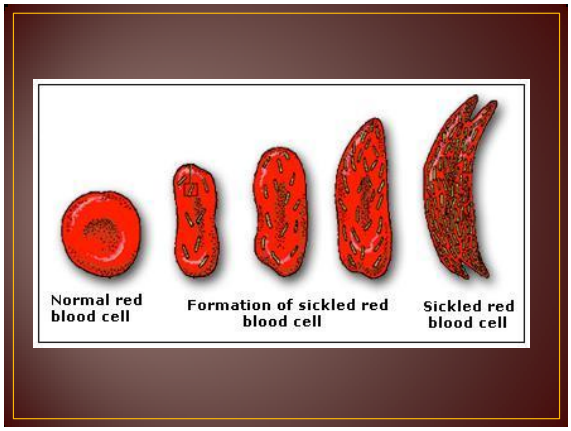


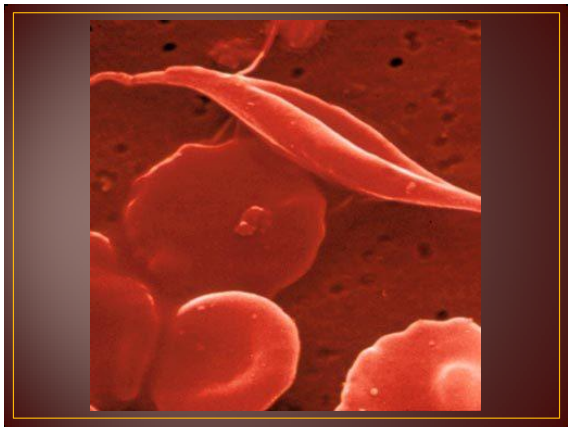


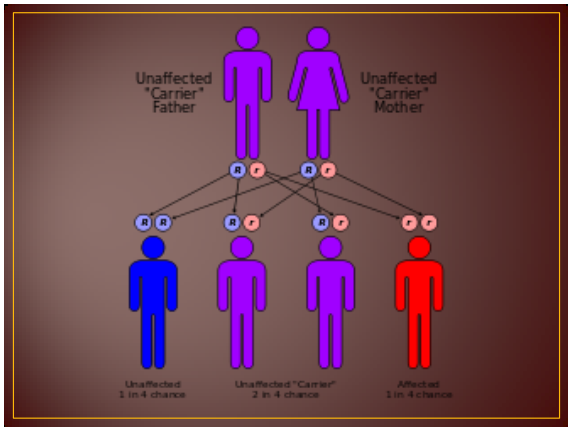

Missense Mutations
ATG GAA GCA CGT
Met Glu Ala Gly
ATG GAC GCA CGT
Met Asp Ala Gly




Normal Cells
CAA GTA AAC ATA GGA CTT CTT DNA
GUU CAU UUG UAU CCU GAA GAA mRNA
val his leu thr pro glu glu Protein
Sickle Cells
CAA GTA AAC ATA GGA CAT CTT DNA
GUU CAU UUG UAU CCU GUA GAA mRNA
val his leu thr pro val glu Protein


Normal red blood cell
Formation of sickled red blood cell
Sickled red blood cell




Unaffected "Carrier" Father
Unaffected "Carrier" Mother

Cloning

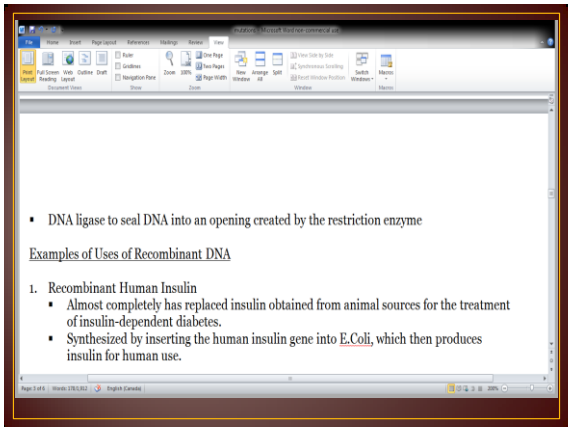
- Is the production of identical copies of an organism through asexual reproduction
- Human twins are clones because one embryo is separated and it becomes two individuals.
- This is known as natural cloning

Gene Cloning

- Is the production of many identical copies of a gene
- Used to compare normal genes to mutated genes

Recombinant DNA

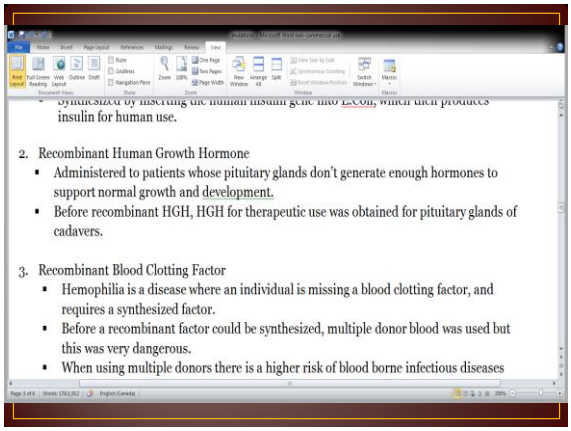
- A method of cloning
- Involves DNA from two sources
- Example – human and bacterial cell
- Use a vector
- Vector is a piece of DNA that can be manipulated in order to add foreign DNA.
- Plasmid is a common vector
- Plasmids are small accessory rings of DNA that are not part of the bacterial chromosome and are capable of self-replicating.
- Two enzymes are needed to introduce foreign DNA to vector DNA.
- Restriction enzymes are used to cleave DNA
- DNA ligase to seal DNA into an opening created by the restriction enzyme



- DNA ligase to seal DNA into an opening created by the restriction enzyme

Examples of Uses of Recombinant DNA

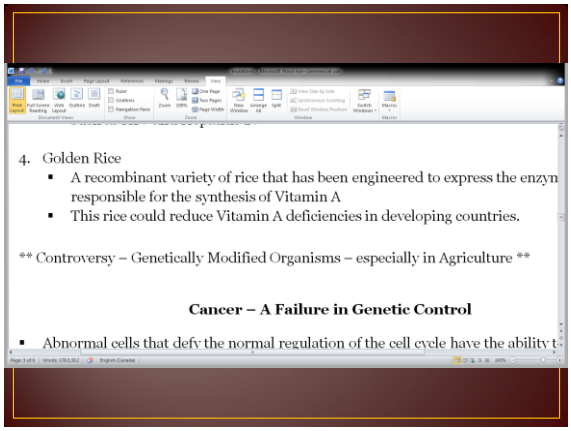
1. Recombinant Human Insulin
   - Almost completely has replaced insulin obtained from animal sources for the treatment of insulin-dependent diabetes.
   - Synthesized by inserting the human insulin gene into E.Coli, which then produces insulin for human use.



insulin for human use.

2. Recombinant Human Growth Hormone
   - Administered to patients whose pituitary glands don't generate enough hormones to support normal growth and development.
   - Before recombinant HGH, HGH for therapeutic use was obtained for pituitary glands of cadavers.

3. Recombinant Blood Clotting Factor
   - Hemophilia is a disease where an individual is missing a blood clotting factor, and requires a synthesized factor.
   - Before a recombinant factor could be synthesized, multiple donor blood was used but this was very dangerous.
   - When using multiple donors there is a higher risk of blood borne infectious diseases



4. Golden Rice
   - A recombinant variety of rice that has been engineered to express the enzyn responsible for the synthesis of Vitamin A
   - This rice could reduce Vitamin A deficiencies in developing countries.

** Controversy – Genetically Modified Organisms – especially in Agriculture **

Cancer – A Failure in Genetic Control

- Abnormal cells that defy the normal regulation of the cell cycle have the ability t

Cancer – A Failure in Genetic Control

- Abnormal cells that defy the normal regulation of the cell cycle have the ability to invade and colonize other areas.
- Normal cells exhibit contact inhibition which means when they come into contact with neighboring cells they stop dividing.
- Cells that begin to proliferate abnormally lose contact inhibition and form tumors.
- These cells pile on top of one another and grow in multiple layers.
- As long as a tumor stays clustered in a single mass it is considered to be benign.
- Benign means non-cancerous

Cancer – A Failure in Genetic Control

- When cells invade surrounding tissues they are cancerous.
- Cancers cells can travel through blood, lymph and can start secondary tumors elsewhere in the body.
- Known as metastic tumors
- Cancer is said to have metastasized, if it spreads to other tissue.
- Metastic cancer is more difficult to treat and the remission rate is much lower.

Characteristics of Cancer Cells

- Cancers cells are genetically unstable
- A cell acquires a mutation that allows it to continue to divide
- Eventually one of the progeny (daughter cells) will acquire another mutation and gain the ability to form a tumor.
- Further mutations occur and the most aggressive cells become the dominant cells in the tumor.
- Metastic tumor cells undergo multiple mutations and also tend to chromosomal aberrations and rearrangements.
- Cancer cells do NOT correctly regulate the cell cycle
- They normal controls of the cell cycle do not operate to stop the cycle and allows cells to differentiate.

Characteristics of Cancer Cells

- Cancers cells tend to be non-specialized.
- Rate of cell division and the number of cells increase.
- Cancers cells escape the signal for cell death.
- Genetic damage and other problems with the cell cycle initiate apoptosis.
- Apoptosis is programmed cell death.
- Cancers cell do not respond to internal signals to die and they continue to divide despite genetic damage.
- Cells from the immune system can detect an abnormal cell and swill send signals to that cell inducing apoptosis.
- Cancer cells ignore these signals.
- Normal cells have a built in number of times they can divide before they die.
- Normal cells stop entering the cell cycle because the telomeres become shortened.

Characteristics of Cancer Cells

- Telomeres are the end of chromosome that prevents them from fusing with one another.
- During each round of cell division, the telomeres become shorter and eventually are too short and this signals apoptosis.
- Cancer cells turn on the gene that code for the enzyme telomerase, which is capable of rebuilding and lengthening telomeres.
- Cancer cells appear immortal and they keep entering the cell cycle
- Cancer cells can survive and proliferate elsewhere in the body.
- Many changes that occur in order for a cancer cell to metastasize are not understood

Characteristics of Cancer Cells

- Though blood and lymph cancer cells can travel and form new tumors.
- As a tumor grows it must increase its blood supply by forming new blood vessels, this process is called angiogenesis.
- Tumor cells switch on genes that code for the production of growth factors that promote blood vessel formation.
- New blood vessels supply the tumor with nutrients and oxygen they require for rapid growth but they also rob normal tissue of nutrients and oxygen.

Proto-Oncogenes & Tumor Suppression Genes

- Proto-oncogenes codes for proteins that promote he cell cycle and apoptosis.
- They are able to accelerate the cell cycle
- These genes become mutated and this causes cancer because apoptosis does not occur and cell division continues.

Tumor Suppressor Genes

- Encodes proteins that inhibit he cell cycle and promote apoptosis.
- Stops the acceleration of the cell cycle
- When it becomes mutated cell division continues and apoptosis does not occur.
- Cells repeatedly enters the cell cycle

Causes of Cancer

Hereditary

- *Example* – retinoblastoma
- Forms eye tumors
- One copy of gene encoding retinoblastoma proteins is damaged due to chromosomal aberrations or mutations.
- One copy of the geen is normal
- In the next generation, an individual may inherit one copy of a normal retinoblastoma gene and one "bad" copy of this gene.
- The RB gene is tumor suppressor gene so as long as the normal gene produces RB proteins cancer will not develop.
- But if the normal genes becomes mutated or non-functional, the person will most likely develop cancer.
- This demonstrates that fact cancer can not be inherited but some people have a greater potential to get cancer.

Causes of Cancer

1. Environment

- Nonhereditary retinoblastoma takes longer to develop because the individual has inherited two normal genes and both must become mutated in order for cancer to develop.
- Environmental Factors that can mutate genes:
  - chemical carcinogens
  - smoking
  - UV light/radiation - caused by natural sunlight and tanning beds.
  - Viruses – Example - *Human Papilloma Virus*

Potential Cancer Treatmnents

Surgery

- Uses to remove tumors
- Danger of some cells being left behind, so usually followed with radiation or chemotherapy.

Potential Cancer Treatmnents

Radiation

- Is a mutagenic so dividing cells such as cancer cells are more susceptible to its affects than other cells
- Causes cancer cells to undergo apoptosis

Potential Cancer Treatmnents

Chemotherapy

- Used when cancer cells have spread through the body
- Kills cells by damaging DNA or interfering with DNA replication
- Wants to kill all cancer cells, hope enough normal cells can stay alive to keep functioning normally

Future Therapies

- Cancer vaccines to elicit immune responses against tumor proteins allowing the body to destroy the tumor

P35 gene Therapy

- The gene for P53 proteins can be injected directly into tumor cells
- Confines and reduces tumors by breaking up the network of new capillaries in the vicinity of the tumor

Diagnosis of Cancer

Tumor Marker Test

- Marks are normal proteins that are produced in small amounts
- Cancers cells produce these proteins in excess
- Example - PSA (prostate-specific antigen) detects prostate cancer
- PSA is normally produces by the prostate and found in the blood
- When PSA levels rise a problem with the prostate is expected
- Tests can not differentiate between benign conditions and cancer of the prostate so further testing must be done.
- Physicians use tumor marker tests to determine if the cancer is responding to therapy or if the cancer has returned.

Diagnosis of Cancer

Genetic Test

- Tests for detection of mutated proto-oncogenes or tumor suppressor genes to detect the likelihood that cancer may develop.
- Example - breast, colon, bladder and thyroid cancer.
- Genetic test for breast cancer - mutations of the BRCA1 and BRCA2 genes
- Mutations in these genes are involved in many cases of breast cancer and ovarian cancer.
- Mutations present in one of these genes, increases the risk of developing cancer by 3-7% more likely.
- Increases risk but some people will inherit the mutated gene and many not develop cancer
- May be recommended to more actively pursue screening, tests etc…