GENETICS, INHERITANCE AND CANCER

NOTES

SECOND EDITION

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READY-TO-STUDY
HIGH-YIELD NOTES

FOR THE TIME-POOR
MEDICAL, PRE-MED,
USMLE OR PA STUDENT

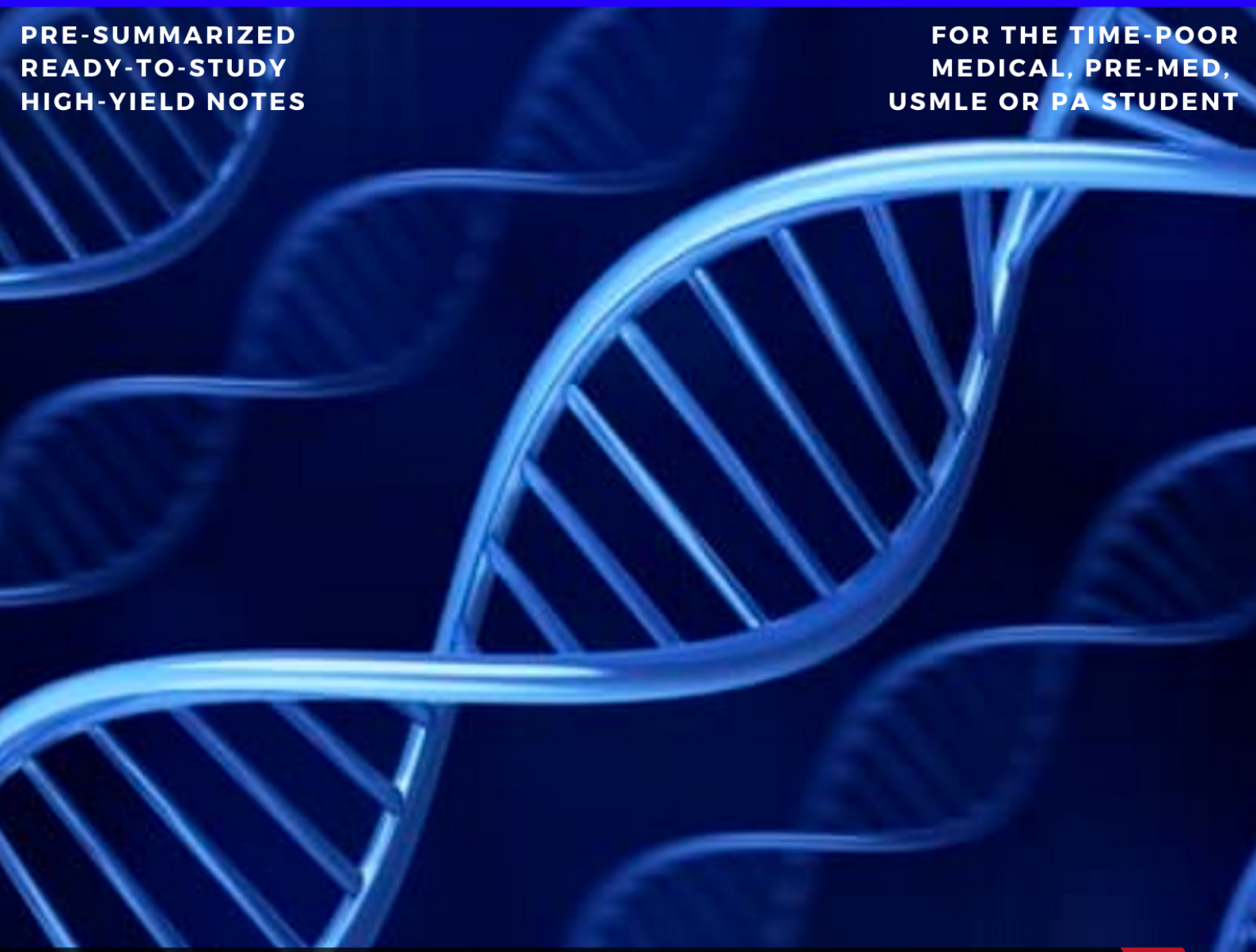

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Table Of Contents:

What's included: Ready-to-study summaries of clinical genetics, inherited conditions, and oncology presented in succinct, intuitive and richly illustrated downloadable PDF documents. Once downloaded, you may choose to either print and bind them, or make annotations digitally on your ipad or tablet PC.

Free Bonus: Oxford Handbook Of Oncology (For further reference & understanding)

File List:

- **Genetics Notes:**
  - DNA, RNA & Protein Synthesis
  - Chromosomes & Chromosomal Anomalies
  - Autosomal Recessive Inheritance
  - Autosomal Dominant Inheritance
  - Mendelian Genetics
  - Complex Mendelian Genetics
  - Genetics of Development & Sex Differentiation
  - Population Genetics & Evolution
  - Extraction & Manipulation of DNA
  - Future Genetic Therapies & Technologies
  - SUMMARY OF SIGNIFICANT GENETIC CONDITIONS
  - AUT DOM - Achondroplasia & Hypochondroplasia
  - AUT DOM - Myotonic Dystrophy
  - AUT DOM - Retinoblastoma
  - AUT REC - Phenylketonuria
  - CHROMOSOMAL - Downs Trisomy 21 Syndrome
  - CHROMOSOMAL - Edwards Trisomy 18
  - CHROMOSOMAL - Klinefelters XXY
  - CHROMOSOMAL - Supermale XYY
  - CHROMOSOMAL - Turner's Syndrome XO
  - XLinked & Mitochondrial Disorders
  - XLINKED REC - Colour Blindness
  - XLINKED REC - Hypophosphataemic Rickets
- **Cancer Physiology Notes:**
  - Intro to Neoplasia
  - Cancer Cell Death & Cellular Ageing
  - Basic Cancer Pathogenesis
  - The Genetics of Cancer
  - Cancer Risk Factors
  - Intro to Clinical Oncology

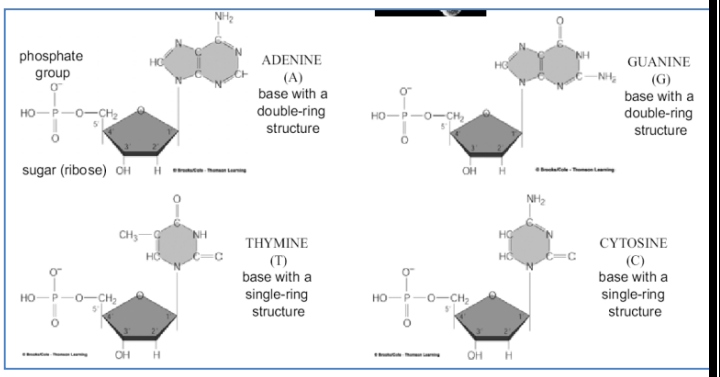
DNA, RNA, Protein Synthesis

DeoxyriboNucleic Acid – The Basics:

- DNA contains the genetic blueprints for proteins of a cell/organism.
- DNA is replicated before cell division.
- Genetic blueprints are encoded in the **nucleotide sequence**

Constituents:

- DNA (and RNA) are **nucleic acids** – DeoxyriboNucleic Acid
- A DNA molecule = 2 **complimentary polynucleotide** chains (DNA Chains/Strands)
- Within each polynucleotide chain, there are thousands of genes (which each encode a certain protein) made up of a specific sequences of nucleotides.
- **Each nucleotide** contains**:**
  - 1x Deoxyribose (5C sugar ring) (has a hydroxyl group on the 3' carbon)
  - 1x Phosphate Group (on the 5' carbon of the deoxyribose)
  - 1x Nitrogen-containing Base - **Either**:
    - Adenine (A) (Purine -OO)
    - Guanine (G) (Purine -OO)
    - Thymine (T) (Pyrimidine -O)
    - Cytosine (C) (Pyrimidine -O)

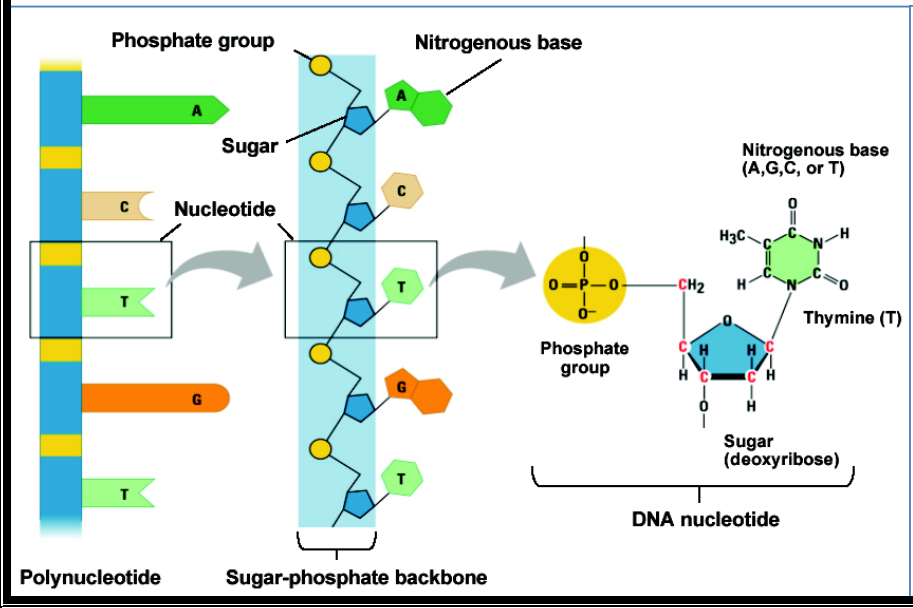


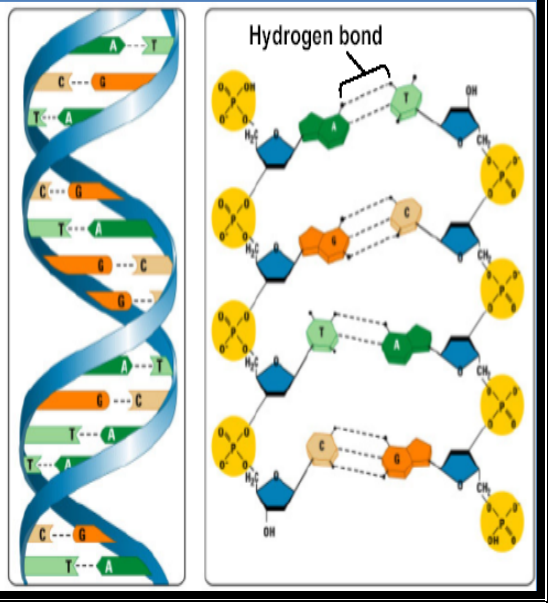
Nucleotide Pairing Arrangement:

-Each chain of DNA is held together by *hydrogen bonds* between the base pairs.
-Only specific pairs of nucleotides can bond to each other:

- **A = T** (2 *H* bonds →lower mtp) [T is replaced by U in RNA]
- **G ≡ C** (3 *H* bonds →higher mtp)

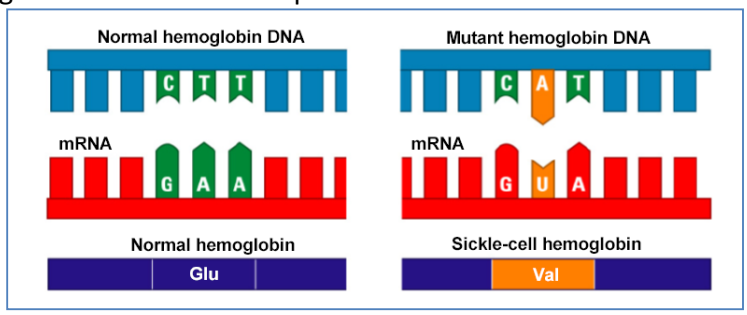
NOTICE: **double-ringed** bases always **bond to single-ringed** bases (keeps strands equidistant)
-These *H-bonds* can be broken (and the DNA ***denatured***) by **raising** the **temperature** or the **pH**





Mutations:

-Any change in the nucleotide sequence of DNA = mutation

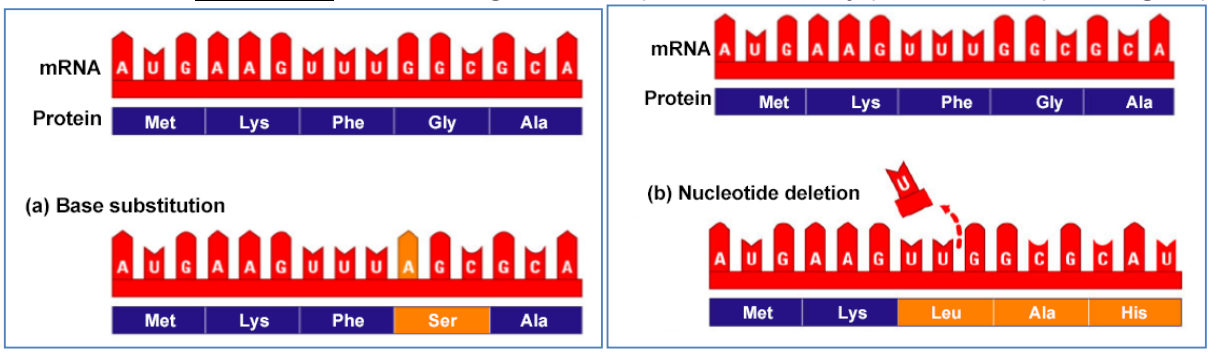


-DNA can be damaged (mutated) by various mutagens in **2 ways:**

a) Substitution of a nucleotide. (results in changes to the amino acids in proteins)

b) Insertion/Deletion of a nucleotide. (causes a '*frameshift*' →changes protein)

Mutagens: **UV** / Ionising Radiation (**Gamma & X-Rays**) / chemicals (**carcinogens**)

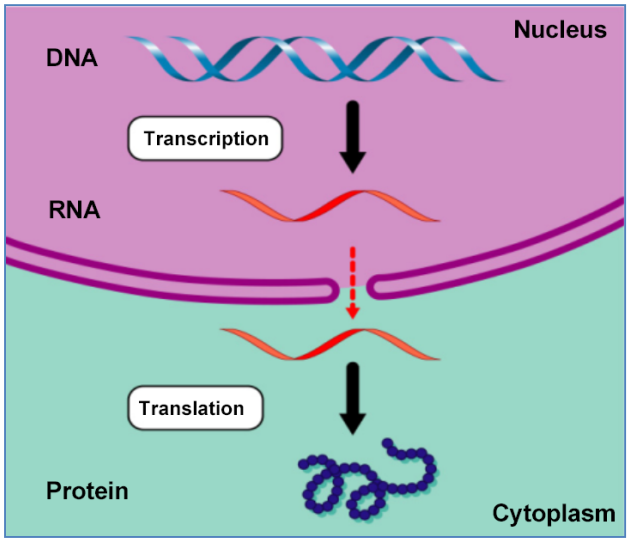


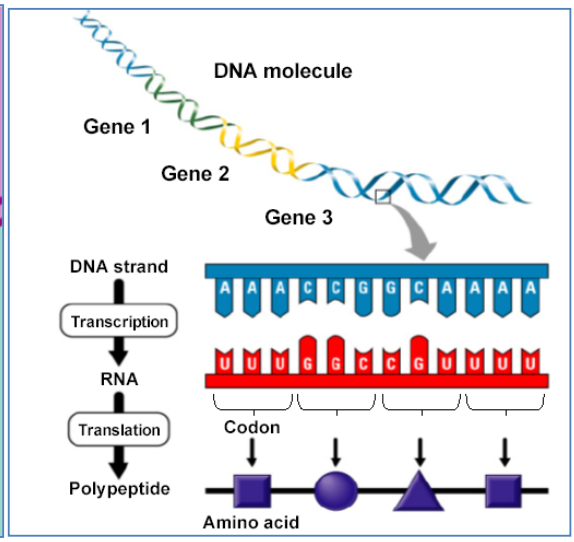
Polarity & Antiparallel:

- Every DNA chain has **Polarity:**
  - Has a 5' Phosphate (key)
  - And a 3' Hydroxyl (keyhole)
- The 2 chains in a DNA molecule are **antiparallel** and are wound into a **double helix.**

Genes To Proteins:

- An organism's genotype is the sequence of nucleotides in its DNA.
- A specific gene (nucleotide sequence) dictates production of a specific protein.
- Protein synthesis happens through **1. Transcription, then 2. Translation**



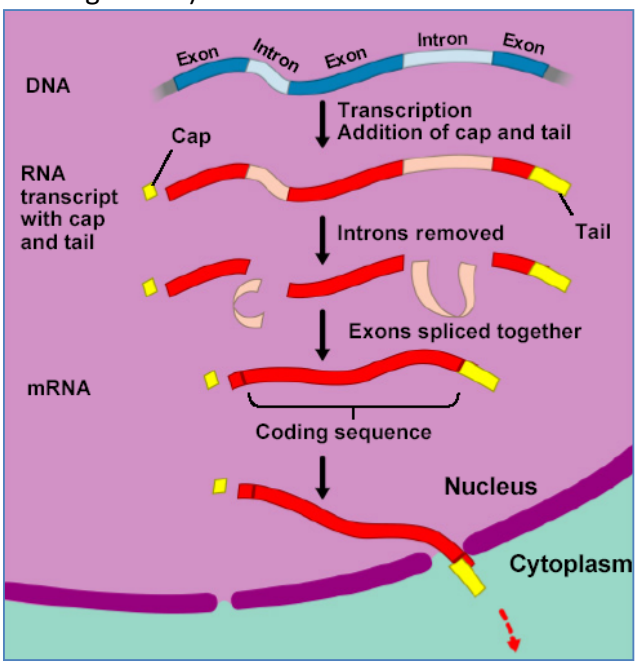


Transcription –in the **Nucleus** - converts **DNA to RNA.** (more specifically, **hnRNA** [**heteronuclear**] – the primary transcript)

Before entering cytosol, the **hnRNA** is **processed** by nuclear enzymes:

- **Introns Removed** (non-coding regions of the hnRNA)
- **Splicing Exons Together** (coding regions)
- **Addition of a <u>GTP-Cap (Gcap)</u> and a <u>Poly-A-Tail</u>**

-Produces **mRNA** (messenger RNA) which then leaves the nucleus into the cytosol.



Translation – in the **cytosol** – a **ribosome** converts **mRNA into an Amino Acid Sequence** (polypeptide/protein)

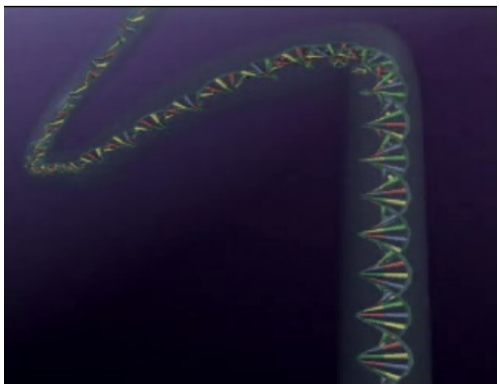
- The amino acids (and sequence) within the protein are determined by triplets of bases (**Codons**)
- **Each codon** (sometimes multiple) relates to **a specific Amino Acid.**
- **Protein synthesis** always starts with **AUG (methionine),** a '**start codon'**

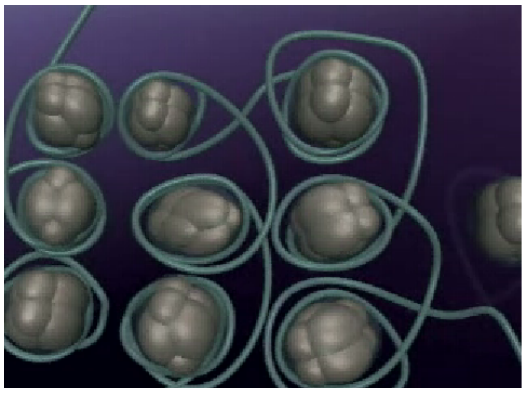
Second base

First base	U	C	A	G	Third base
U	UUU, UUC Phenylalanine (Phe)	UCU, UCC, UCA, UCG Serine (Ser)	UAU, UAC Tyrosine (Tyr)	UGU, UGC Cysteine (Cys)	U
	UUA, UUG Leucine (Leu)		UAA Stop	UGA Stop	C
			UAG Stop	UGG Tryptophan (Trp)	A
					G
C	CUU, CUC, CUA, CUG Leucine (Leu)	CCU, CCC, CCA, CCG Proline (Pro)	CAU, CAC Histidine (His)	CGU, CGC, CGA, CGG Arginine (Arg)	U
			CAA, CAG Glutamine (Gln)		C
					A
					G
A	AUU, AUC, AUA Isoleucine (Ile)	ACU, ACC, ACA, ACG Threonine (Thr)	AAU, AAC Asparagine (Asn)	AGU, AGC Serine (Ser)	U
	AUG Met or start		AAA, AAG Lysine (Lys)	AGA, AGG Arginine (Arg)	C
					A
					G
G	GUU, GUC, GUA, GUG Valine (Val)	GCU, GCC, GCA, GCG Alanine (Ala)	GAU, GAC Aspartic acid (Asp)	GGU, GGC, GGA, GGG Glycine (Gly)	U
			GAA, GAG Glutamic acid (Glu)		C
					A
					G

DNA Organisation/Storage Inside Nucleus:

1. DNA helices are long, thin and delicate and must be tightly packed in order to fit within the nucleus.
2. **Portions of DNA** helices are **wrapped twice around** special proteins called **histones, forming** circular **nucleosomes**.
3. **Nucleosomes** are **packed tightly into** a "thread", forming a **fibre** known as **chromatin**.
4. This fibre is then coiled again. "string"
5. This **chromatin** "string" is then **coiled again and again** into a "rope" known as a **Chromosome** seen under light microscope.

1. 

2. 

3. 

4. 

5. 

DNA Replication:

- When a cell is getting ready to divide (**Interphase**), its **DNA replicates**.

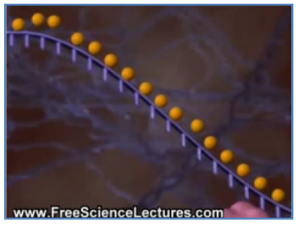
1. Chromosomes uncoil from their structure into paired DNA strands.
2. The two strands are separated by an enzyme called **Helicase** at the **Origin of Replication (ORI).**
3. Each nucleotide is then bound by a **Single-Stranded DNA Binding Protein** which keeps the strands from re-anhealing. (coming back together)
4. One of the DNA strands encodes the "leading strand" (new strand), which is laid down continuously from its 5' to 3' end (towards the Helicase). This is done by an enzyme called **DNA Polymerase III** which moves towards the **helicase.**
5. The other DNA strand (which runs in the opposite direction) encodes the "lagging strand" (other new strand), which must also be laid down from its 5' to 3' end (this time away from the Helicase).
6. This is first initiated by **RNA Primase** which lays down an **RNA Primer**.
7. Another **DNA Polymerase III** then attaches to the **RNA Primer** and lays down a short length of DNA (away from the Helicase) called an **Okazaki Fragment** (100 - 200 Nucleotides long) until it reaches another RNA Primer further down the line. Once the **DNA Polymerase III** reaches the 2nd RNA Primer it dissociates.
8. **DNA Polymerase I** then **replaces** the **RNA Primer** (between the 2 Okazaki fragments) **with DNA**.
9. **DNA Ligase** then links the 2 Okazaki fragments together.

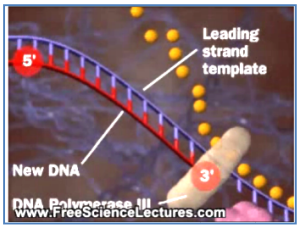
****Note: DNA Polymerase proofreads** as it adds new nucleotides****

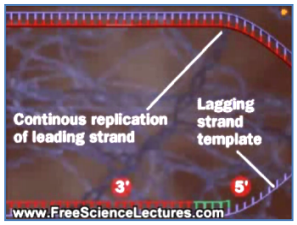
1. 

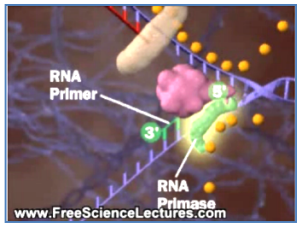
2. 

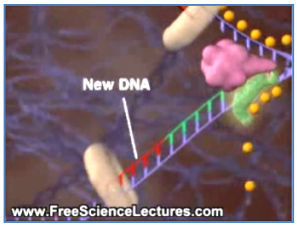
3. 

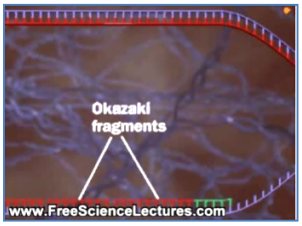


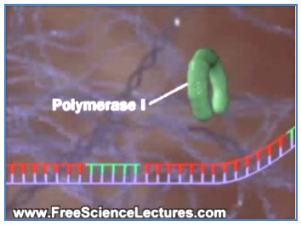
4. 

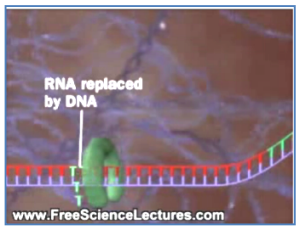
5. 

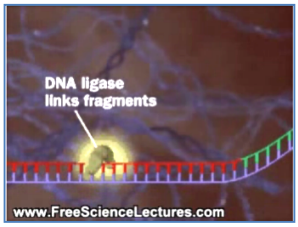
6. 

7. 

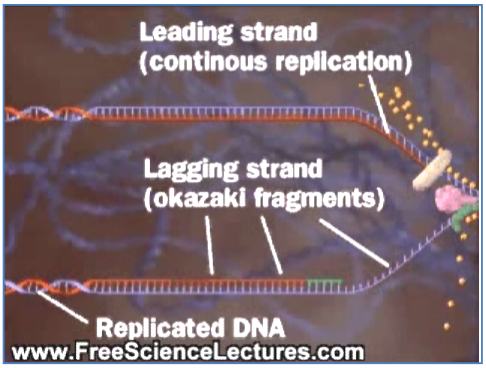


8. 

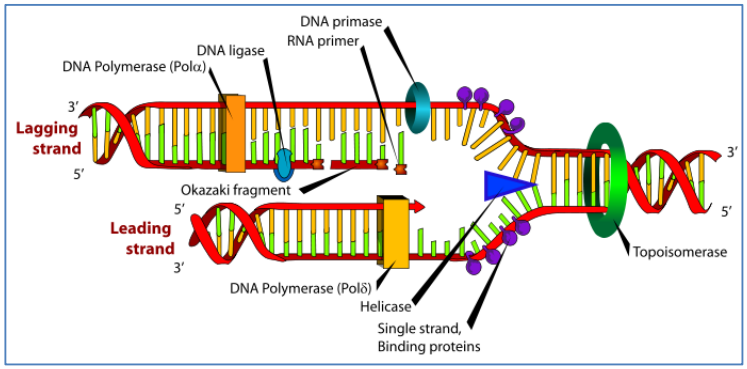


9. 

2 Schematic Overviews

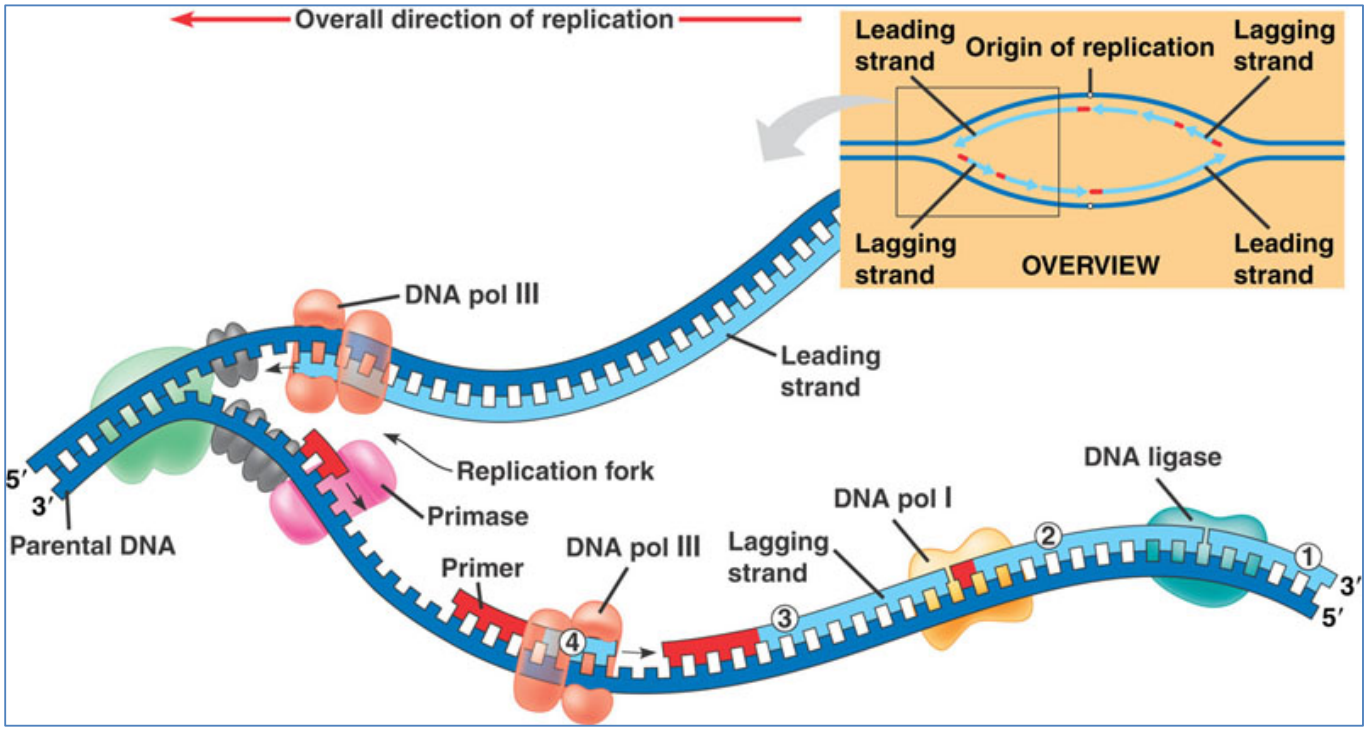


Overview of Above

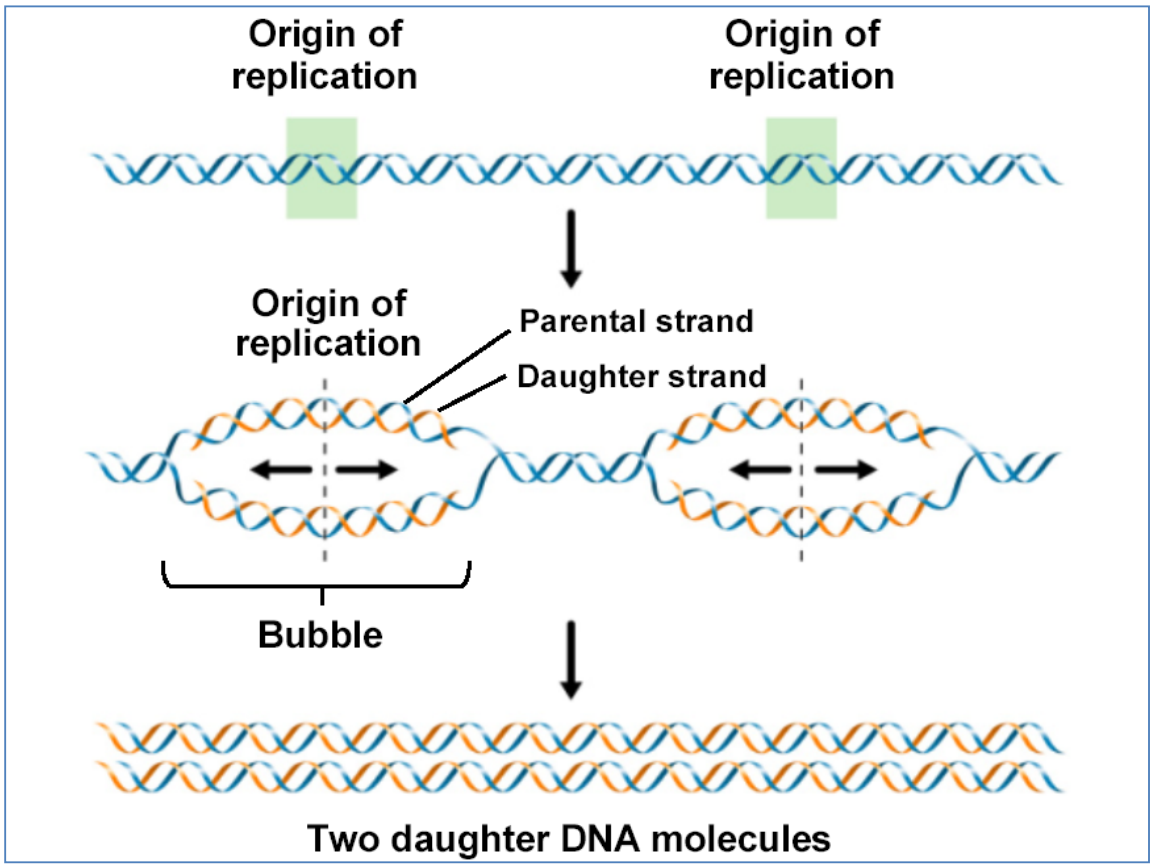


NB. **Topoisomerase** uncoils the DNA to stop it tangling.

2x Helicases can bind at one ORI and unzip the DNA in both directions, forming a 'replication bubble'



There can be multiple ORIs, and therefore multiple replication bubbles. (increases replication rate)



Transcription:

Transcription is the process of **making RNA from a DNA** template.

- Requires **DNA** (containing a particular **gene**, aka **transcription unit**)
- **Transcription Factors**
- **RNA polymerase**
- **ATP**

1. Begins with a **strand of DNA** (made up of different important regions)
    a. **Transcription unit** (The gene/length of DNA **for the desired protein**)
    b. **TATA box -** a DNA sequence found in the ***promoter region*** of a gene (usually the binding site of transcription factors [or in DNA packing; histones])
2. Several **complexes** known as **transcription factors** bind to the TATA box (**allow** the successful **binding of RNA polymerase** to the DNA)
3. **Initiation Phase:** **RNA polymerase binds** to the DNA @ the *promoter* & other transcription factors complete the mature transcription complex.
4. **Energy** must be **added** to the system (**Via ATP**) for transcription to begin.
5. **RNA polymerase** then **splits** the **DNA** into 2 strands **along its** own **length**. (most transcription factors are released after transcription has begun)
6. **Elongation Phase:** **RNA polymerase** then moves along the transcription unit, **synthesising an RNA Template** from the strand of DNA. As it moves along, it **constantly unzips** forward DNA **and rezips DNA** strands left behind.
7. **Termination Phase:** When the **terminator region** is reached, the **RNA Polymerase dissociates** from the DNA and the newly formed **strand** of **RNA is released**.

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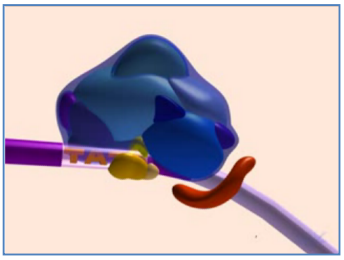

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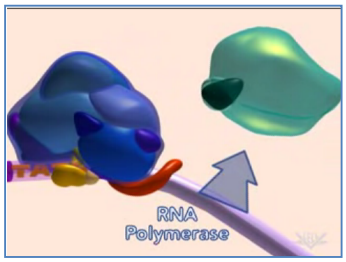

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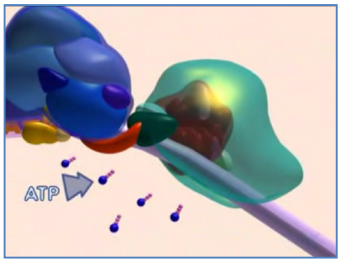

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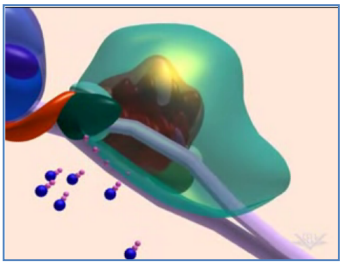

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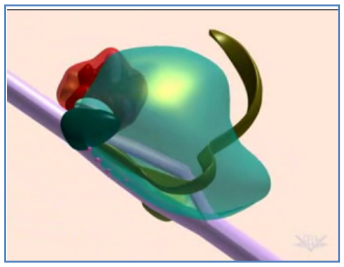

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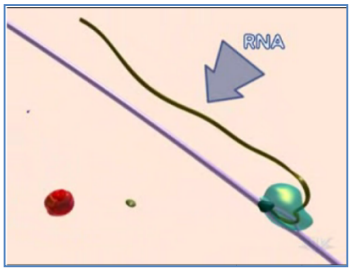

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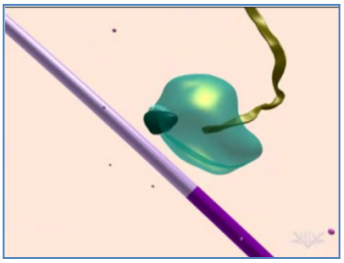
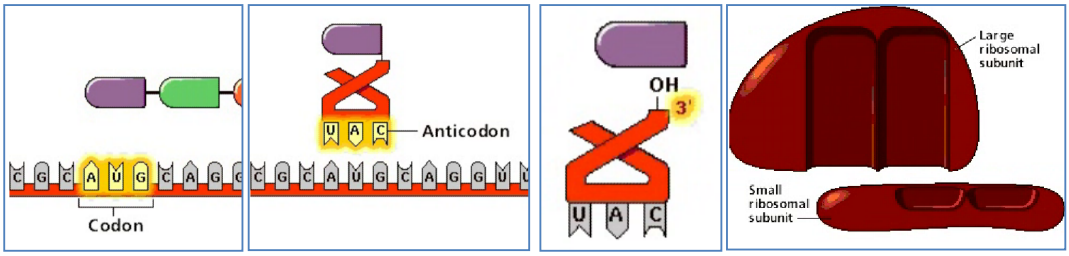


Translation:

- Proteins are long chains of **Amino Acids**
- There are **20 common kinds** of amino acids.
- The **sequence** of amino acids in the chain determines the function of that protein.
- The instructions for making proteins are 'coded' in DNA → transcripted to mRNA →translated into proteins.
- Each amino acid is specified by a group of 3 bases on the mRNA called **codons.**
- In addition to mRNA, there are other crucial components:
    - mRNA
    - Twisted strands of RNA called **transfer-RNA (tRNA)** match specific amino acids to corresponding base codons on the mRNA.
        - The 3 bases on the tRNA = **anticodon**
        - An amino acid is first bound to the 3' end of the tRNA by an **AminoAcyl Synthetase**.
    - **Ribosomes** made of **ribosomal-RNA** (rRNA) + protein = the workbench for protein synthesis. **-**Protein synthesis also occurs from the 5' end of the mRNA to the 3' end.
        - Small subunit
        - Large subunit



1. **Initiation:** Formation of the **initiation complex** begins when the small r-subunit recognises and binds to a sequence of bases in the *leader sequence* on the mRNA.
2. A tRNA carrying the anticodon of the **start codon (AUG -** carrying **methionine)** binds to the mRNA.
3. The initiation complex is completed when the large r-subunit covers the 'start' tRNA by joining to the small r-subunit and the mRNA. The 'start' tRNA fits snugly into the **'P-Site'** of the complex. The other site (**'A-Site'**) is empty.
4. **Elongation:** -Begins when a 2nd tRNA (matching the mRNA codon) and its attached amino acid settles into the **A-Site.**
5. The 1st amino acid is transferred from its tRNA onto the 2nd amino acid via enzyme forming a peptide bond between them.
6. The pair of amino acids are now attached to the 2nd tRNA. The 1st tRNA leaves the P-site.
7. The ribosome moves down the mRNA to a 3rd codon. The tRNA bearing the di-peptide now occupies the P-Site.
8. The empty A-Site then attracts another tRNA (bearing another amino acid). The di-peptide is then transferred to the 3rd amino acid and the 2nd tRNA is released. The ribosome then continues down the mRNA, increasing the length of the polypeptide.
9. **Termination:** Translation is terminated when the ribosome encounters one of three **stop codons** (UAA, UAG or UGA) at the end of the gene.
10. A **Release Factor** recognises the stop codon, and settles into the A-Site.
11. An enzyme releases the polypeptide chain from its tRNA via hydrolysis. This causes everything, including the ribosome, to dissociate from the mRNA.

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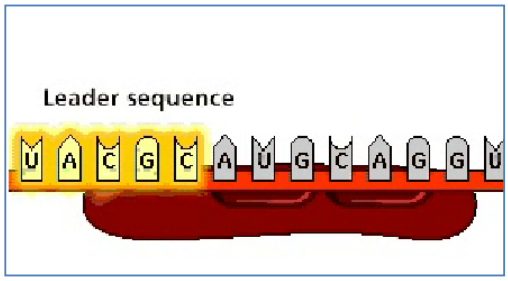

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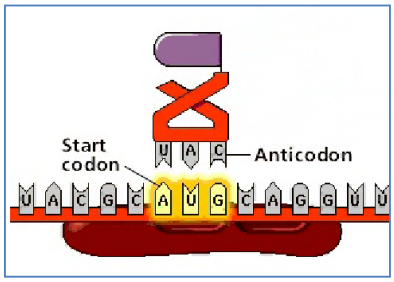

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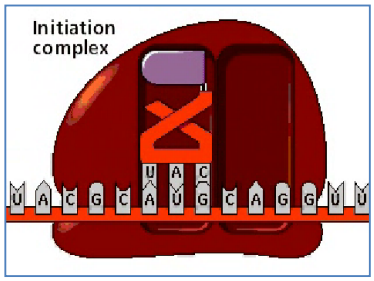

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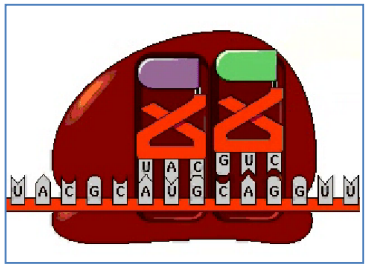

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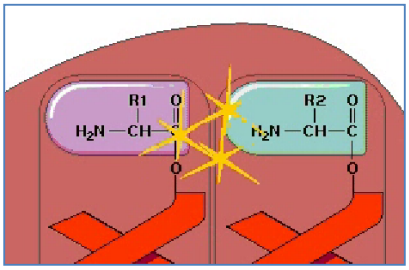

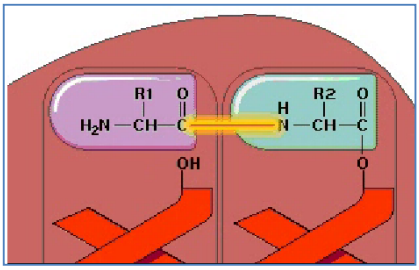

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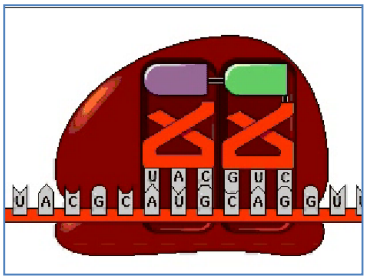

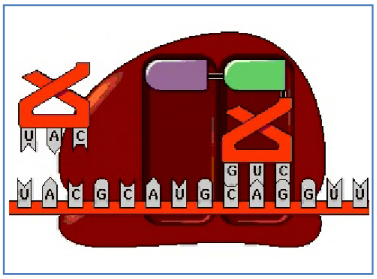

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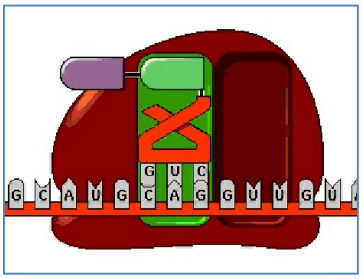

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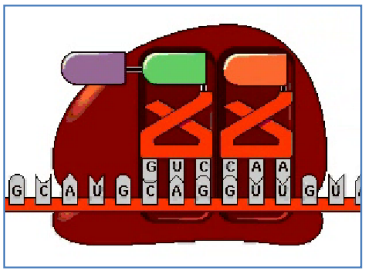

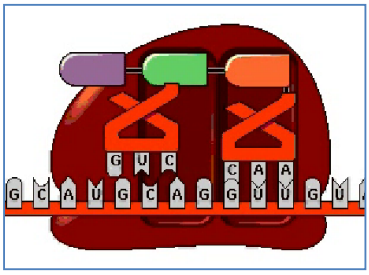

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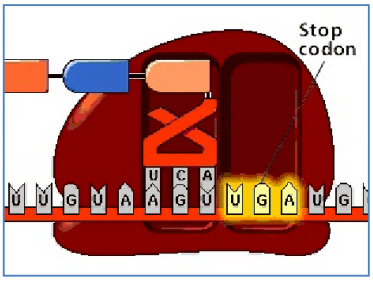

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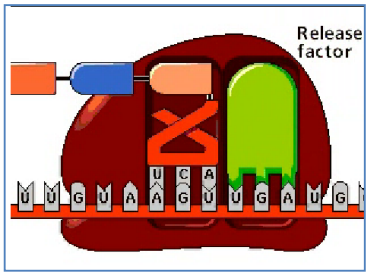

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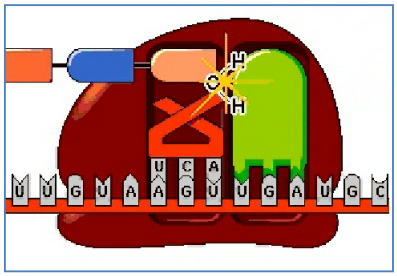
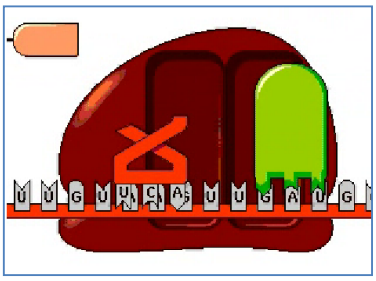
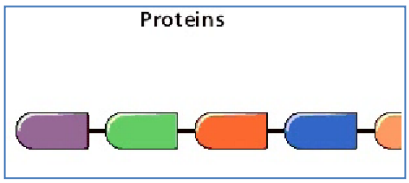
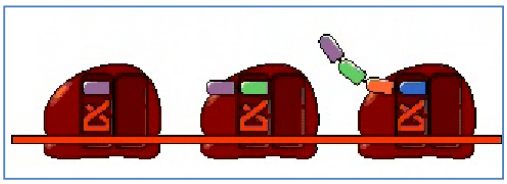






Resulting Polypeptide (protein)

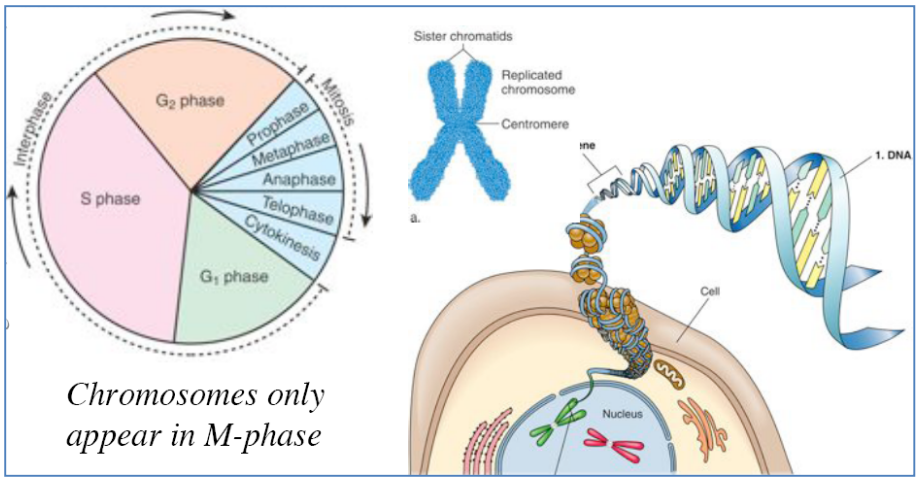


Ribosome moves along mRNA

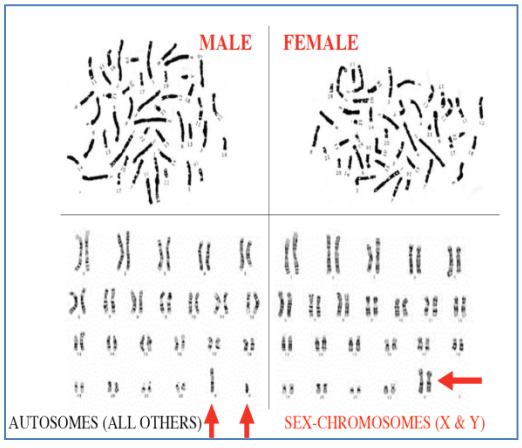
Chromosomes & Disease: Genetic Cockups!

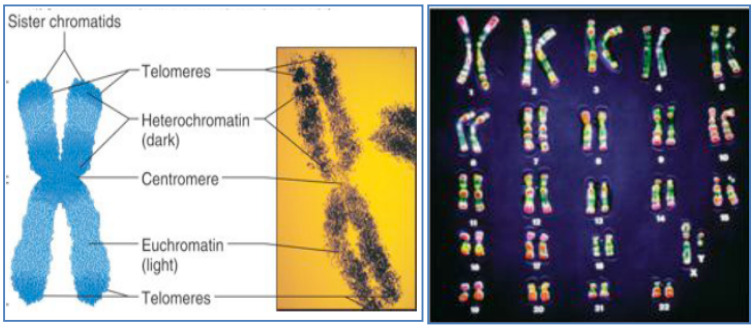
Human Chromosomes:

- **What are they?**
  - Highly compacted **linear** pieces of DNA
  - Contain many different genes
  - Only visible during M-Phase once the replicated chromosomes have condensed.
  - Vary in size; 50-250Mbase-pairs

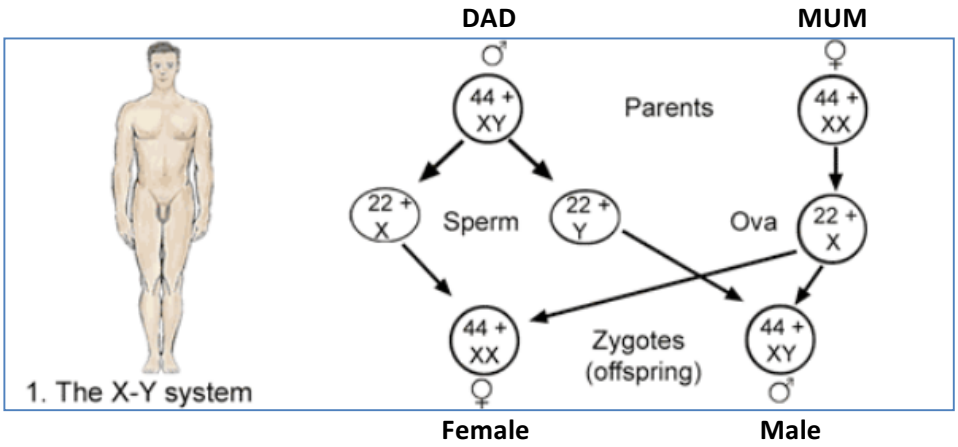


- **Chromosome Number:**
  - Varies between species
  - Similar species have similar chromosome numbers
    - Eg. Chimps – 48 , Humans – 46
  - Chromosome number ≠ sophistication
- **Cell Function:**
  - Normal people are born with 2x sets of 23 chromosomes
    - 1 set from mum
    - 1 set from dad
  - **2 Sex Chromosomes:**
    - X & Y
    - XX = Female
      - X from Dad
      - X from Mum
    - XY = Male
      - X from Mum
      - Y from Dad
  - **44 Autosomes (all others):**
  - **Genes:**
    - Sequences of DNA that encode proteins.
    - Different genes have different functions.
      - Some genes are expressed in all cell types.
      - Most cells only express genes relevant to their function.
  - Pairs of identical chromosomes are paired & numbered by length & banding patterns



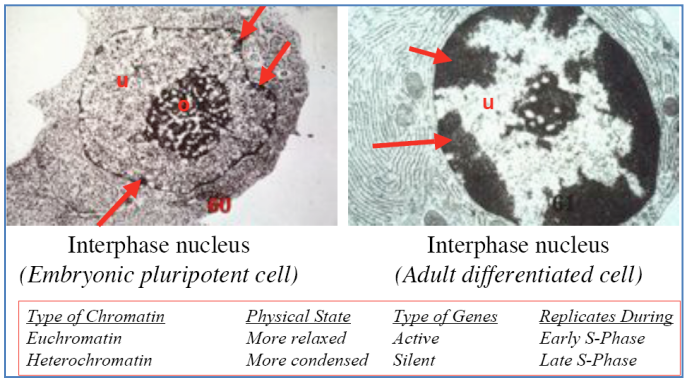


Human Sex Determination:



Chromosome Nomenclature:

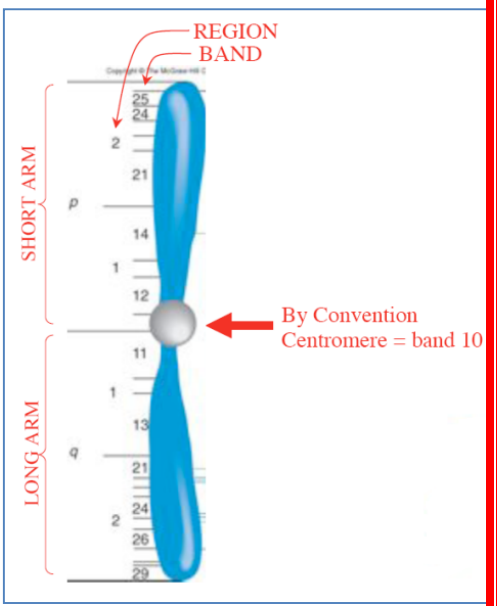
- **Bands**
  - Chromosomes show distinct banding patterns
  - Due to **gene rich** and **gene poor** regions
  - Regions = **heterochromatin (dark)** and **euchromatin (light)**
    - **Heterochromatin (Dark):**
      - Genes that have been turned off and packed away
      - Genes that the cell doesn't need for its specific functions
    - **Euchromatin (Light):**
      - Genes that are unpacked and readily transcribed into proteins
      - These genes are critical to the cell's specific function.



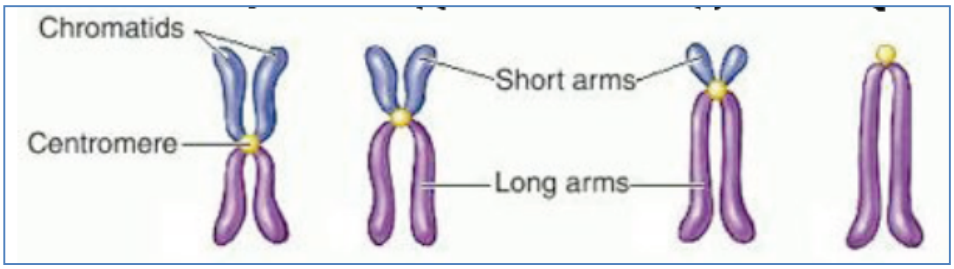
Interphase nucleus *(Embryonic pluripotent cell)* | Interphase nucleus *(Adult differentiated cell)*

Type of Chromatin	*Physical State*	*Type of Genes*	*Replicates During*
Euchromatin	*More relaxed*	*Active*	*Early S-Phase*
Heterochromatin	*More condensed*	*Silent*	*Late S-Phase*

- **Chromosome Charting:**
  - **Arms**
    - Long = 'q'
    - Short = 'p'
  - **Regions**
    - 2 regions/arm
    - Divided evenly
  - **Bands**
    - Centromere = Band 10
    - Counts upward both ways toward the centromere
  - **Centromere**
    - Where the 2 sister chromatids are anchored together
  - **Telomere**
    - Repeated sequences of non-coding DNA at the Very ends of each chromosome arm.



- **Chromosome Shapes:**
    1. **Metacentric:**
        - Arms of similar length
    2. **Submetacentric:**
        - Centromere at approx 1/3
    3. **Acrocentric:**
        - Centromere very close to telomere
    4. **Telocentric:**
        - Centromere at the telomere

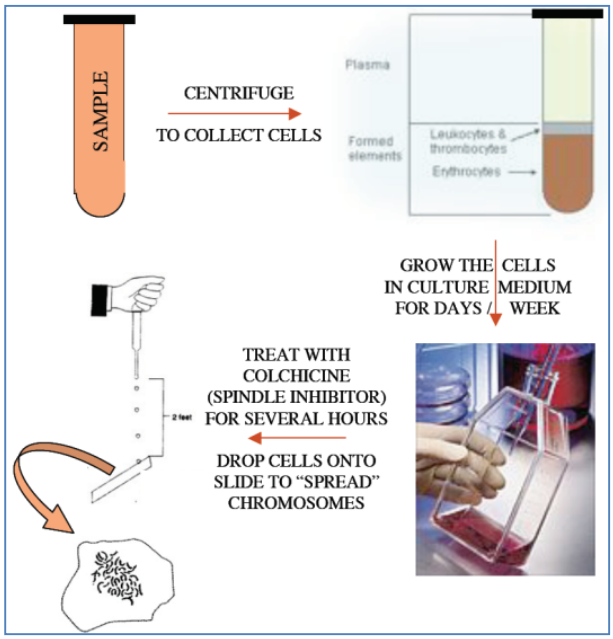


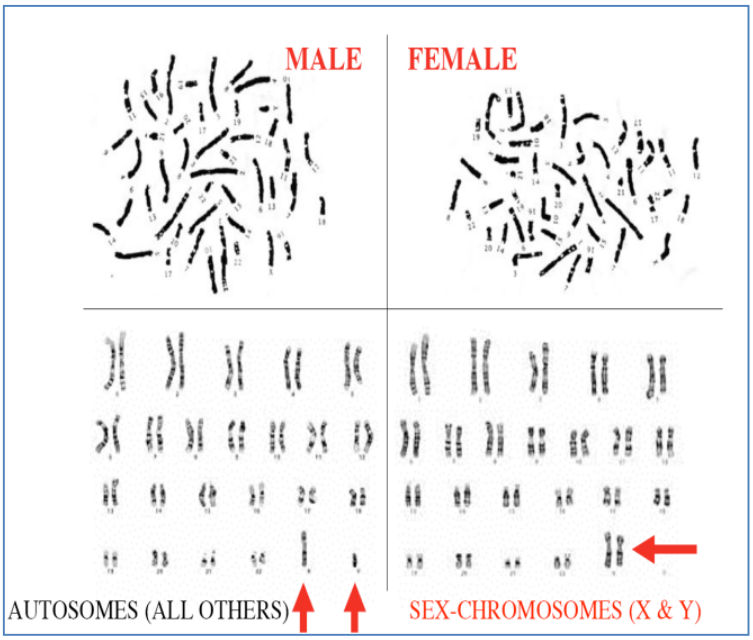
- **Writing Chromosomes:**
    - 46, XX = normal Female
    - 46, XY = normal Male
    - 47, XX, +10 = abnormal Female - extra 10th chromosome.
    - 45, XY, -22 = abnormal Male – missing 22nd chromosome.
    - More:

+	Gain
–	Loss
cen	Centromere
del	Deletion
der	Derivative chromosome
dup	Duplication
ins	Insertion
inv	Inversion
inv ins	Inverted insertion
mar	Marker chromosome
mat	Maternal
pat	Paternal
r	Ring
rob	Robertsonian translocation
t	Translocation
tel	Telomere
ter	Terminal (chromosome end)

Extracting Chromosomes – G-Banding Ideogram:

- Extract cells
- Centrifuge - Collect cells
- Grow cells in culture – amplify numbers
- Treat with spindle-inhibitor → arrests cells in **metaphase**
- Treat with **Protease** and stain with **Giemsa.**
- Drop cells onto slide – flattens cell → spreads chromosomes
- Analyse by computer – pair identical chromosomes.
- Result: **Human Karyotype**



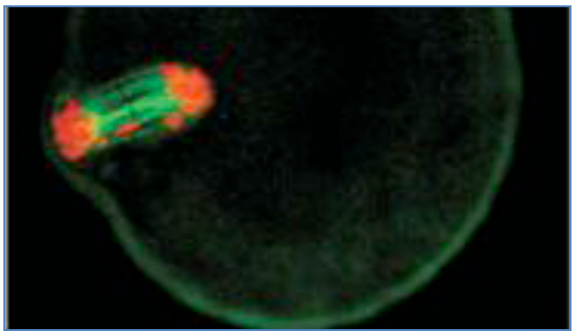


Origins of Abnormal Karyotypes:

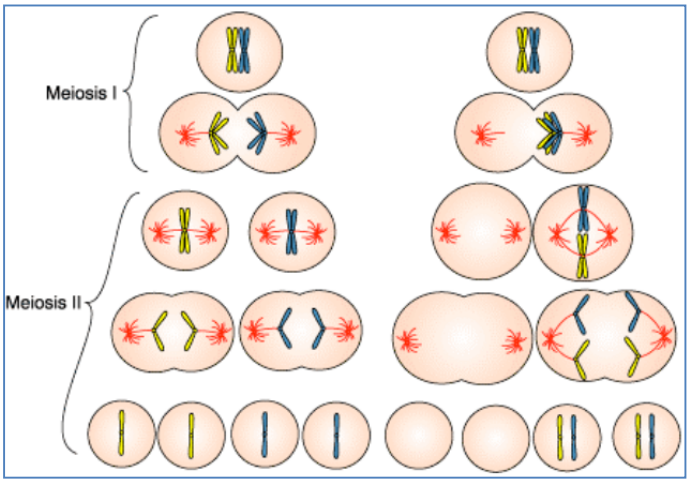
- **Errors in Cell Division**
  - During Meiosis of Mitosis
  - Gains/losses of parts of chromosomes
  - Rearrangements between chromosomes
  - Gains/losses of whole chromosomes
  - **2 Classes:**
    - **Constitutional Chromosome Abnormalities**
      - During Gametogenesis
      - Found in every cell of the body
    - **Acquired Chromosome Abnormalities**
      - During Embryogenesis
      - Found only in clusters of cells
      - Individuals are a 'mosaic' of normal & abnormal cells

5 Main Causes of Abnormal Karyotypes:

1. **Anaphase Lag**
   - During anaphase
   - One chromosome fails to migrate to the pole of the spindle
   - That chromosome then fails to be enclosed by the new nuclear envelope
   - Chromosome is lost and gets degraded
   - Results in 1x normal cell + 1x cell with a missing/extra chromosome
   - Leads to **monosomies & trisomies**



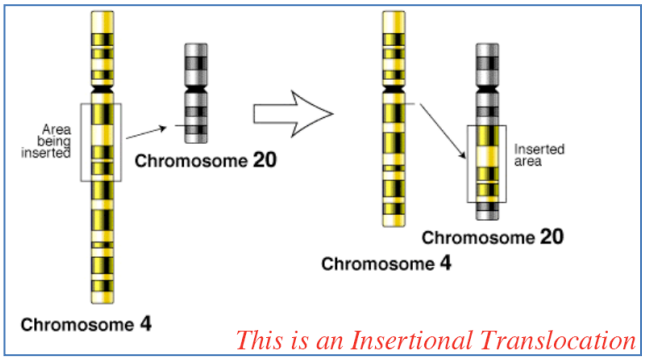
2. **Chromosome Mis-Segregation (Nondisjunction)**
   - Error during meiosis
   - Where chromosomes aren't divided equally among the gametes.
   - Results in either: gametes with extra or missing chromosomes.
   - If such gametes are fertilized → **mosomies & trisomies**



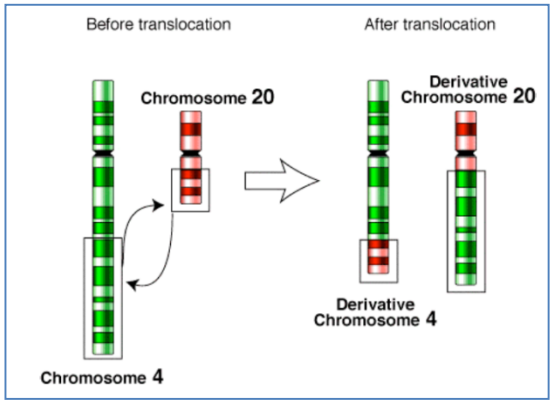
3. **Replication Failure (Meiotic/Mitotic)..and..**
4. **Dispermy**
   - Results in polyploidy.
   - How this can happen:
     - Error in Gametogenesis
       - Chromosomes duplicate but the germ cell fails to divide → diploid gamete.
     - Error at Fertilisation
       - Eg. Dispermy → 2 sperm simultaneously fertilise egg. →triploid embryo
     - Error in Embryogenesis
       - Errors during early mitotic cell divisions
       - Chromosomes duplicate by the embryonic cell/s fail to divide →triploid mosaic embryo
5. **Incorrect DNA Repair**
   - Cellular DNA damage happens regularly
   - Usually DNA repair mechanisms work well
   - Sometimes, DNA damage is incorrectly repared.

Classes of DNA Damage:

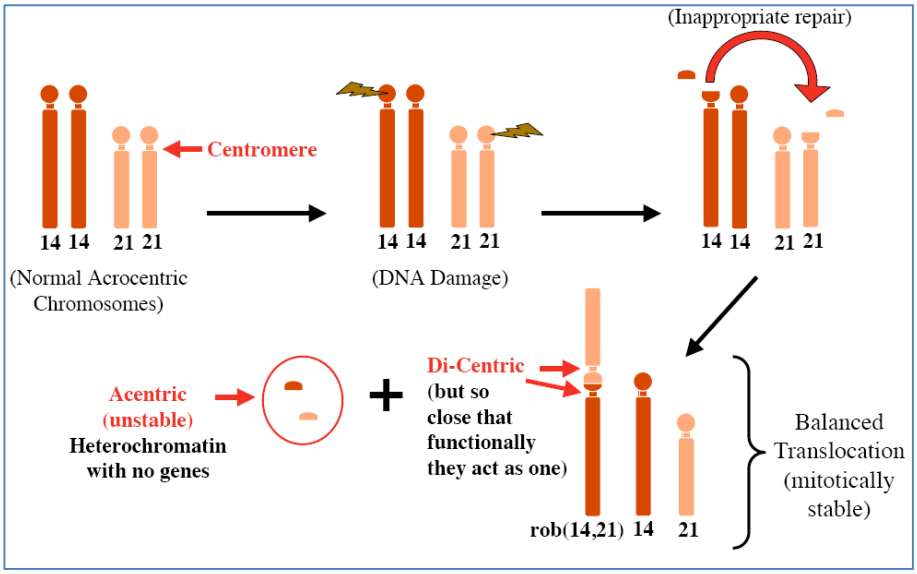
- **Translocations:**
  - Insertional:



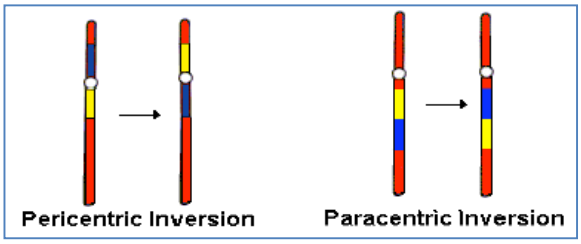
  - Reciprocal:



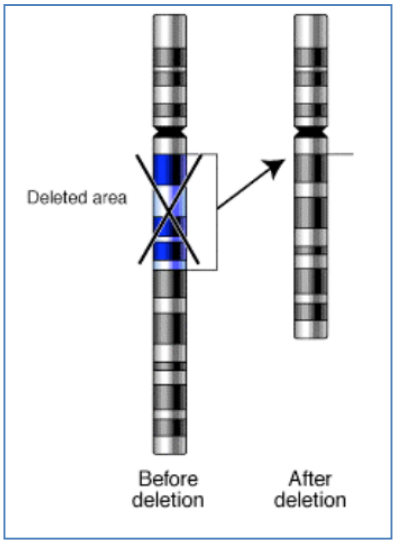
  - Robertsonian:
    (Centric Fusions of Acrocentric Chromosomes)



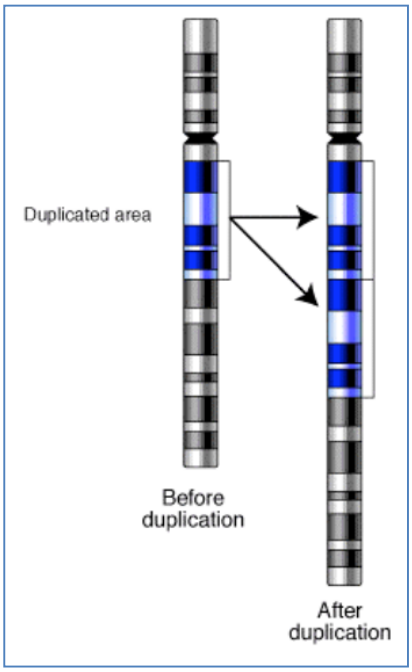
- **Inversions:**
  - A segment of the chromosome has been flipped
  - No gain / loss of genetic material
  - If breakpoints don't disrupt genes →no abnormality
  - 2 Kinds:
    - Pericentric
    - Paracentric



- **Deletions:**
  - A region of a chromosome is deleted
  - Deletion size: proportional to: Severity of abnormality.
  - Eg. William's syndrome = DNA loss on Chr. 7



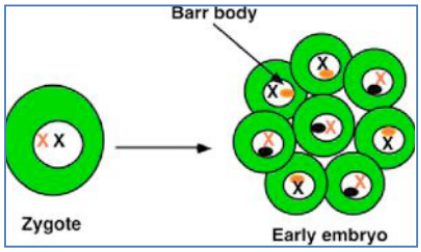
- **Duplications:**
  - A region of a chromosome is doubled
  - If duplication occurs outside a coding region & doesn't result in a frameshift →no abnormalities.

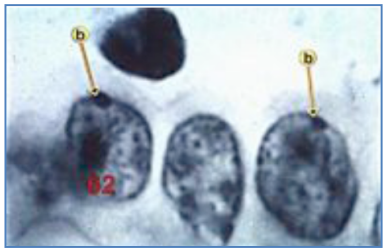


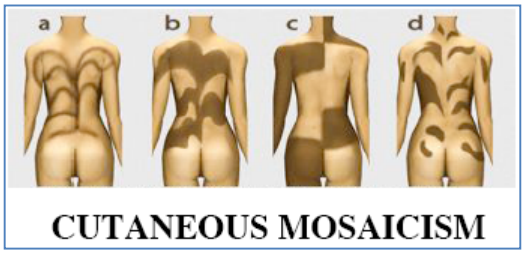
Aneuploidy Vs. Polyploidy

- **Aneuploidy:**
  - The addition/loss of a chromosome from the **normal (euploid)** 23pairs.
  - **Common cause: Nondisjunction** – failure of chromosomes to separate properly during meiosis
  - Generally manifests as a **trisomy -** 3 sets of a chromosome.
    - Most trisomies are lethal except on the small chromosomes.
      - Eg. Chr.21 – Down's Syndrome;
      - Chr.18 – Edward's Syndrome.
  - Rarely as a **monosomy** – loss of a chromosome.
    - All embryonically lethal – except on sex chromosomes.

  - **Aneuploidy of Sex Chromosomes:**
    - **Males** must cope with a single X-chromosome.
    - Evolution developed a mechanism: dosage compensation
    - **Females** only use one of their X-chromosomes.
    - One X-chromosome in each cell is randomly hypercondensed (inactivated) →**Barr Bodies.**
    - Therefore all females are **mosaics.** (eg. Dermal dysplasia)
      - **Barr Bodies:** areas of heterochromatin







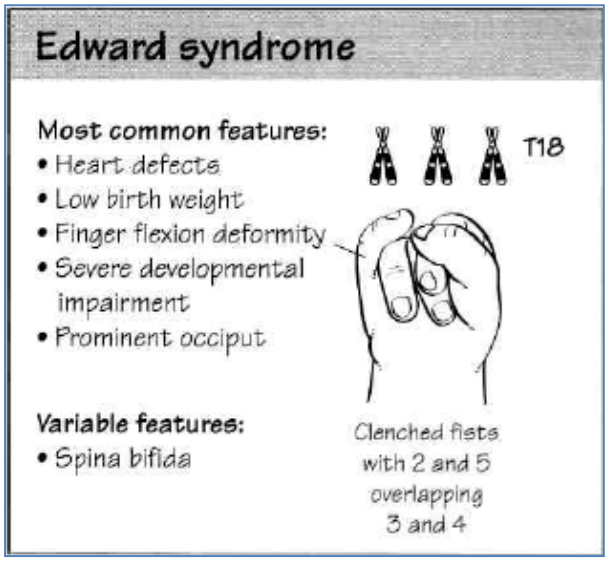
- **Polyploidy:**
  - Addition of whole *sets* of chromosomes.
  - Eg. 3 copies of every chromosome (or 4, or 5, etc)
  - Almost always embryonically lethal & results in spontaneous miscarriage.

➢ **Spontaneous miscarriage/abortion:**
  - Most of early miscarriages are due to genetic abnormality

Overview of Common Genetic Diseases

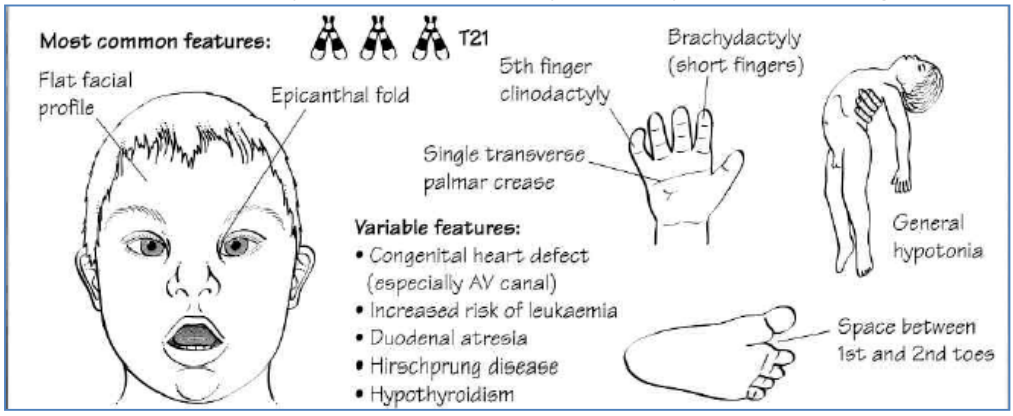
47, XY, +18: Edward's Syndrome

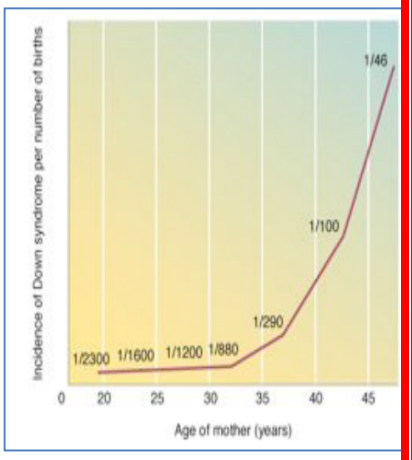
- Trisomy 18
- Rarely survive beyond infancy



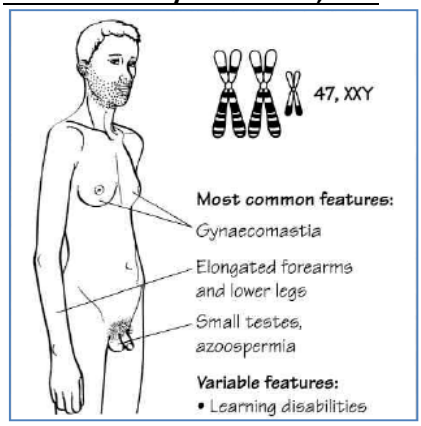
47, XY, +21: Down's Syndrome

- Trisomy 21
- Risk of Down's syndrome increases exponentially with maternal age.

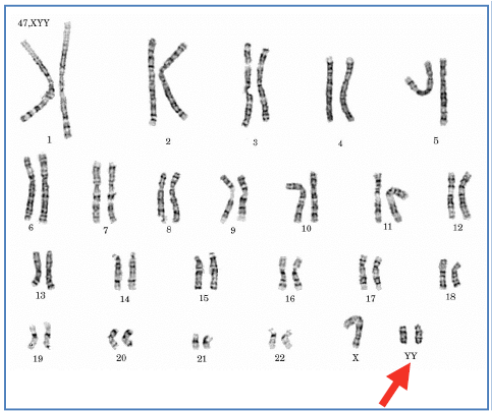


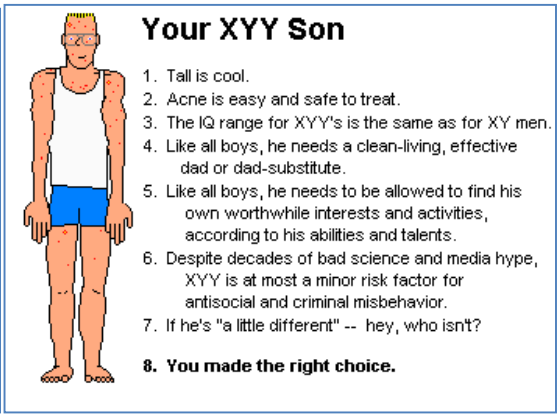


Klinefelter's Syndrome 47, XXY



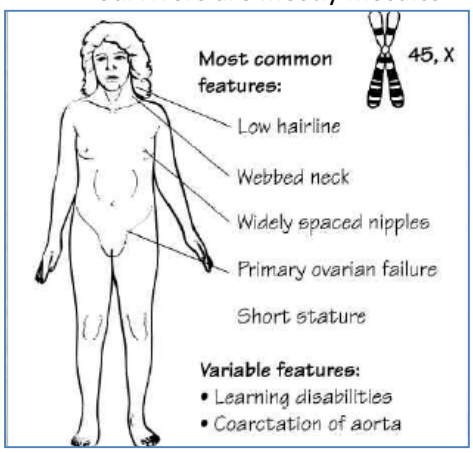
Diplo-Y (Super-Male) 47,XYY.



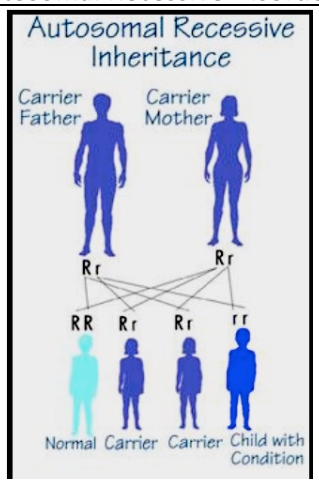


Turner's Syndrome:

- Most die during gestation
- Survivors are mostly mosaics

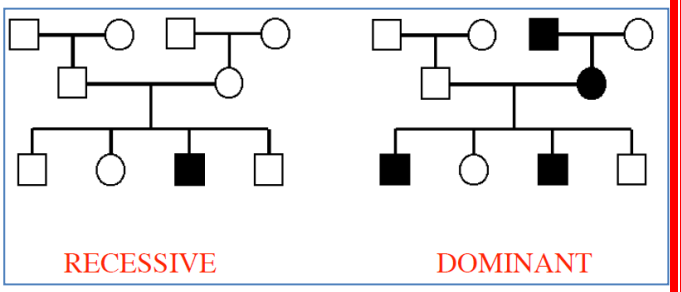


Autosomal Recessive Disorders



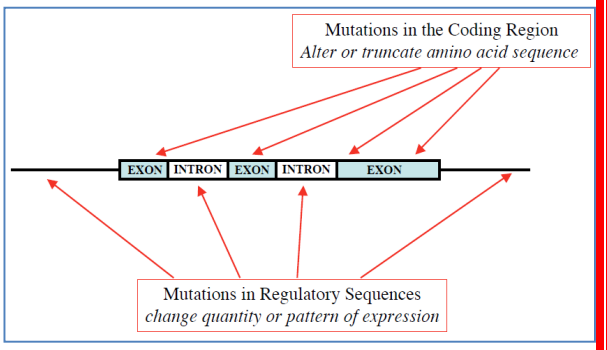
Is a Mutation Dominant Or Recessive?

- **Broad Generalisations:**
  - **Dominant**
    - Affect structural proteins
    - Gain-of-function
  - **Recessive**
    - Affect enzymes
    - Loss-of-function



What Determines The *Effect* of a Mutation?

- **Coding Regions:**
  - Alter/truncate *amino acid sequence*
  - Therefore altering *protein shape & function.*
- **Regulatory Sequences:**
  - Change *quantity or pattern of expression*.
  - May even stop expression completely.



Notation for Mutations:

- **@ DNA Level:** **Eg. (76 A>T)**
  - Capitals
  - Number = first nucleotide affected
- **@ RNA Level:** **Eg. (76 a>u)**
  - Lower-case
  - Number = first nucleotide affected
- **@ Protein Level:** **Eg. (T.26.P)**
  - Capitals
  - Letter 1: first amino acid changed.
  - Number: position of amino acid in mature protein (codon number)
  - Letter 2: what the 1st amino acid changed to.

Classes of Gene Mutations:

- **Single Base Substitutions:**
  - Errors in DNA replication/repair
    - **Synonymous Mutations** → (silent – change occurs in wobble position. Ie. Same amino acid)

```
GAT ATG AGG TGG GGG TTA CAC TTT TGT AAT …     NORMAL
    Met Arg Trp Gly Leu His Phe Cys Asn …

GAT ATG AGG TGG GGG GTA CAC TTT TGT AAT …     SYNONYMOUS (SILENT)
    Met Arg Trp Gly Leu His Phe Cys Asn …     SUBSTITUTION
                                                (116T>G)
```

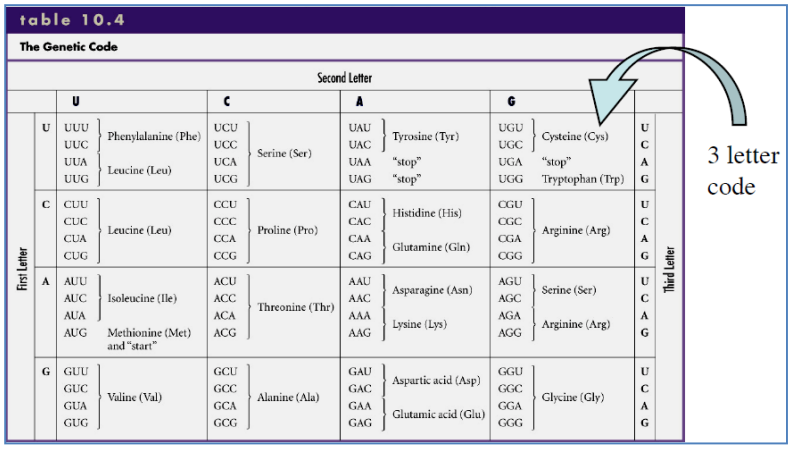


table 10.4

The Genetic Code

First Letter	Second Letter: U	Second Letter: C	Second Letter: A	Second Letter: G	Third Letter
U	UUU Phenylalanine (Phe); UUC Phenylalanine (Phe); UUA Leucine (Leu); UUG Leucine (Leu)	UCU Serine (Ser); UCC Serine (Ser); UCA Serine (Ser); UCG Serine (Ser)	UAU Tyrosine (Tyr); UAC Tyrosine (Tyr); UAA "stop"; UAG "stop"	UGU Cysteine (Cys); UGC Cysteine (Cys); UGA "stop"; UGG Tryptophan (Trp)	U C A G
C	CUU Leucine (Leu); CUC Leucine (Leu); CUA Leucine (Leu); CUG Leucine (Leu)	CCU Proline (Pro); CCC Proline (Pro); CCA Proline (Pro); CCG Proline (Pro)	CAU Histidine (His); CAC Histidine (His); CAA Glutamine (Gln); CAG Glutamine (Gln)	CGU Arginine (Arg); CGC Arginine (Arg); CGA Arginine (Arg); CGG Arginine (Arg)	U C A G
A	AUU Isoleucine (Ile); AUC Isoleucine (Ile); AUA Isoleucine (Ile); AUG Methionine (Met) and "start"	ACU Threonine (Thr); ACC Threonine (Thr); ACA Threonine (Thr); ACG Threonine (Thr)	AAU Asparagine (Asn); AAC Asparagine (Asn); AAA Lysine (Lys); AAG Lysine (Lys)	AGU Serine (Ser); AGC Serine (Ser); AGA Arginine (Arg); AGG Arginine (Arg)	U C A G
G	GUU Valine (Val); GUC Valine (Val); GUA Valine (Val); GUG Valine (Val)	GCU Alanine (Ala); GCC Alanine (Ala); GCA Alanine (Ala); GCG Alanine (Ala)	GAU Aspartic acid (Asp); GAC Aspartic acid (Asp); GAA Glutamic acid (Glu); GAG Glutamic acid (Glu)	GGU Glycine (Gly); GGC Glycine (Gly); GGA Glycine (Gly); GGG Glycine (Gly)	U C A G

3 letter code

- **Missense Mutations** → (change not in wobble position. Ie. Codes for different amino acid)
  - **Conservative:** The new amino acid is chemically similar to its predecessor.
    - May preserve protein function
  - **Non-Conservative:** The new amino acid is chemically different to its predecessor.
    - Generally screws protein function

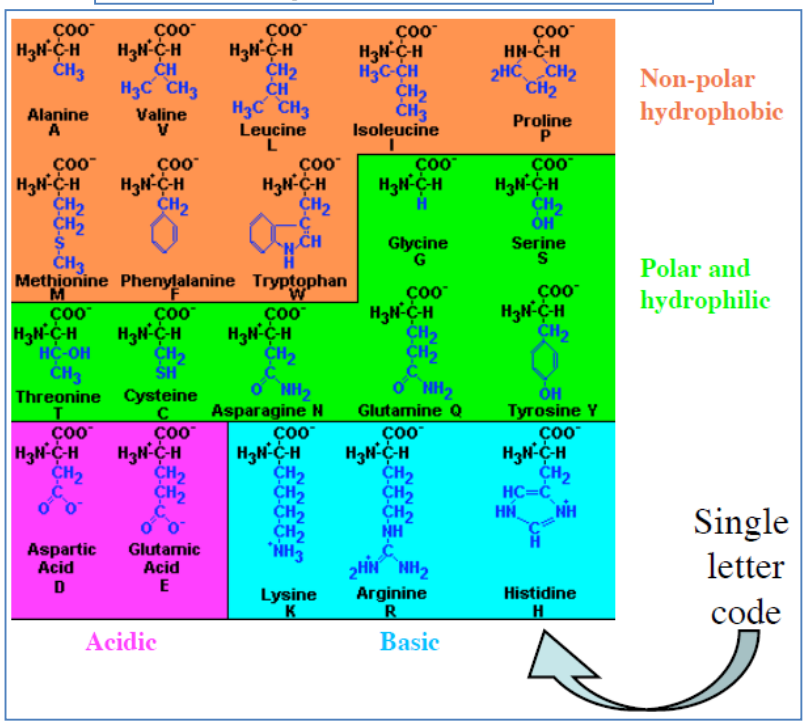
Example: C77Y

The codon for the amino acid cysteine (Cys = C in single letter code) at position 77 in the mature protein has been changed to a Tyrosine (Tyr = Y). This produces a protein with an altered amino acid sequence.

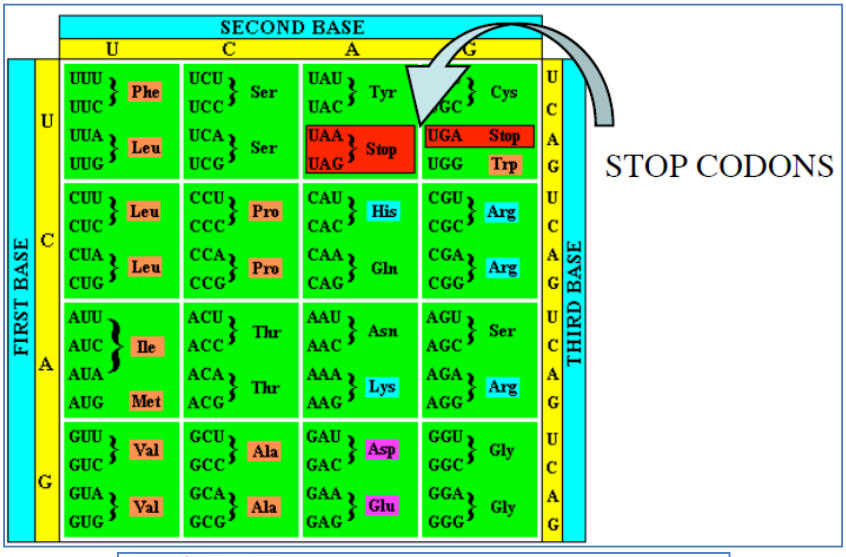
position 77

NORMAL – WSTSTCPGGRHR –

MUTANT – WSTSTYPGGRHR –



- **Nonsense Mutations** → (Code for a *stop codon.* Ie. Can truncate protein)



Example: K385X

The codon for the amino acid lysine (Lys = K in single letter code) at position 385 in the mature protein has been changed to a stop codon (STOP = X). This produces a truncated protein.

position 385

NORMAL – MGHSTKSTTAAW –

MUTANT – MGHSTXSTTAAW –

- **Insertions:**
  - One or more extra nucleotides
  - May alter splicing if in introns.
  - May cause frameshift

Example: 5385insC

a C is inserted at nucleotide position 5385 in the genomic DNA. This can cause a shift to the reading frame of the mRNA, producing a truncated protein.

position 5385

NORMAL – CCTATTTTAATC –

MUTANT – CCTATCTTTAATC –

- **Deletions:**
  - Total erasure of 1 or more bases.
  - Up to millions of BP's
  - May encompass multiple genes.
  - May cause frameshift

Example: 6174delT

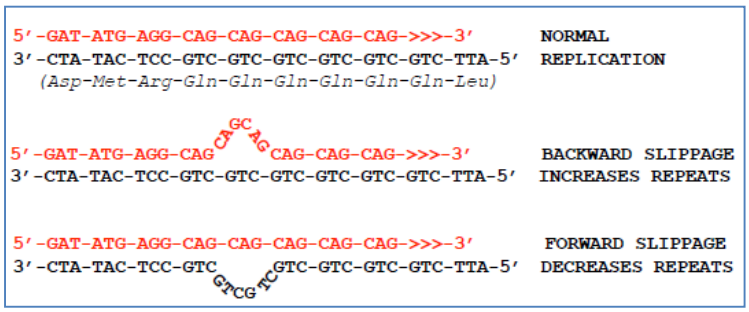
a T is deleted at nucleotide position 6174 in the genomic DNA. This can cause a shift to the reading frame of the mRNA, producing a truncated protein.

position 6174

NORMAL – GCAATTCATGAC –

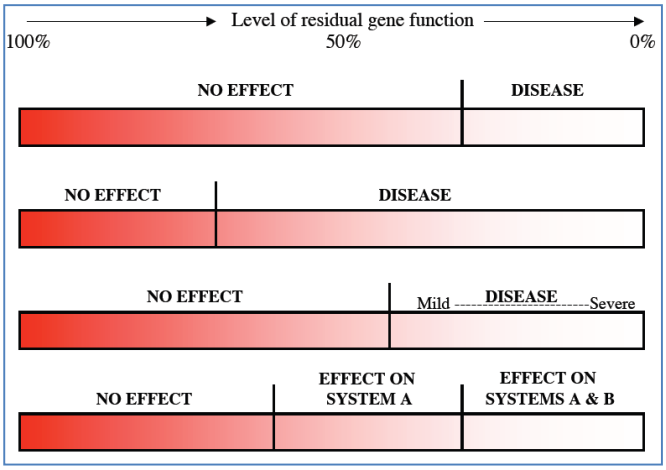
MUTANT – GCAATCATGAC –

- **Dynamic Mutations:**
  - Tandem repeats that change size each cell cycle
  - Either increase/decrease



Levels of Gene Function → Disease:

- Some have a high threshold – only show symptoms with significant loss of gene function.
- Some have a low threshold – show symptoms with little loss of gene function
- Some symptoms vary in severity (proportional to amount of loss of gene function)
- Some effect different body systems @ different thresholds.

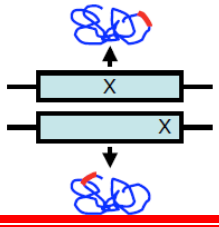


Classic Autosomal Recessive Disorders:

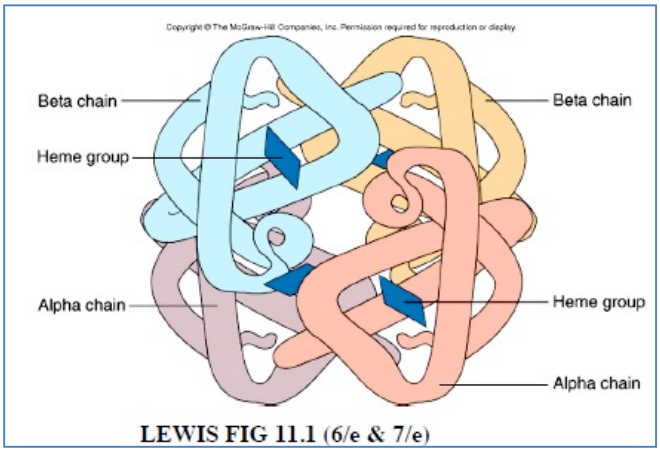
1. Phenylketonuria — CELL METABOLISM
2. Thalassemia — BLOOD CELL FUNCTION
3. Sickle Cell Disease — BLOOD CELL FUNCTION
4. Haemochromatosis — BLOOD CELL FUNCTION
5. Cystic fibrosis — MEMBRANE FUNCTION

- **Metabolism:**
  - **Phenylketonuria:** (similar to Maple-Syrup Urine Disease)
    - Enzyme that breaks down phenylalanine isn't functioning.
      - Normally converts phenylalanine to another amino acid & tyrosine
    - Phenylalanine & its breakdown chemicals (from other enzyme routes) accumulate in blood.
    - **Symptoms:**
      - Mousy odour to urine
      - Vomiting
      - Rashes
      - Irritability
      - Small head
      - Severe brain damage – if left too long untreated.

    - **Diagnosis:**
      - Heel-prick test – newborn screening
    - **Management:**
      - Manage diet – avoid high protein foods
      - Maintain blood-phenylalanine levels (some is needed for normal growth)
      - Offspring of affected mothers – mental retardation – elevated phenylalanine inhibits foetal neural development.
    - **Compound Heterozygotes:**
      - Patients with 2 different defective alleles, both contributing to the disease phenotype.
      - Compound **heterozygotes** of ***recessive*** disorders are **usually affected.**

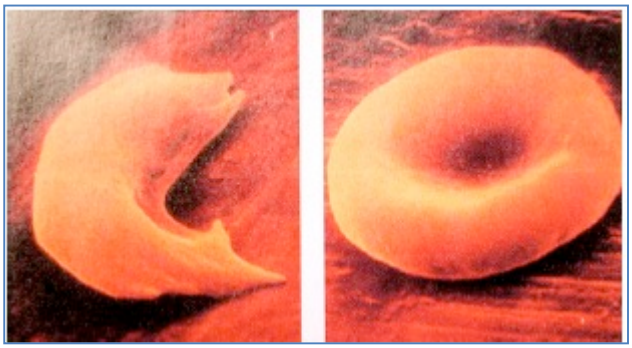


- **Blood Cell Function:**
  - **Thalassemia:**
    - Absence of synthesis of one of the **globin chains**
    - Usually via deletions that block globin protein production
    - Alpha-thalassemia = absence of alpha-globin (more common in Asians)
    - Beta-thalassemia = absence of beta-globin (more common in Caucasians)
    - **Symptoms:**
      - Babies become anaemic
      - Become pale
      - Don't sleep well
      - No appetite
      - If untreated – usually fatal (usually die between 1 & 8yrs)



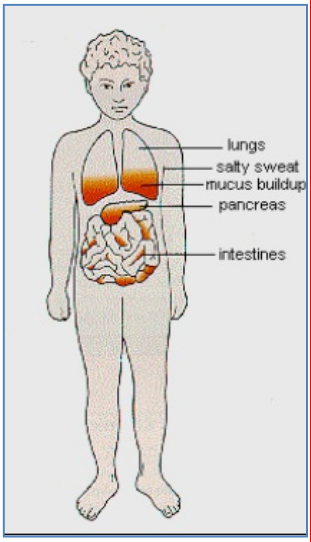
LEWIS FIG 11.1 (6/e & 7/e)

  - **Sickle Cell Disease:**
    - Mutant allele codes for faulty beta-chain haemoglobin subunit.
    - Mis-shapen red blood cell
    - More common areas affected by malaria – malarial parasites grow poorly in homozygous & sickle cell carriers.



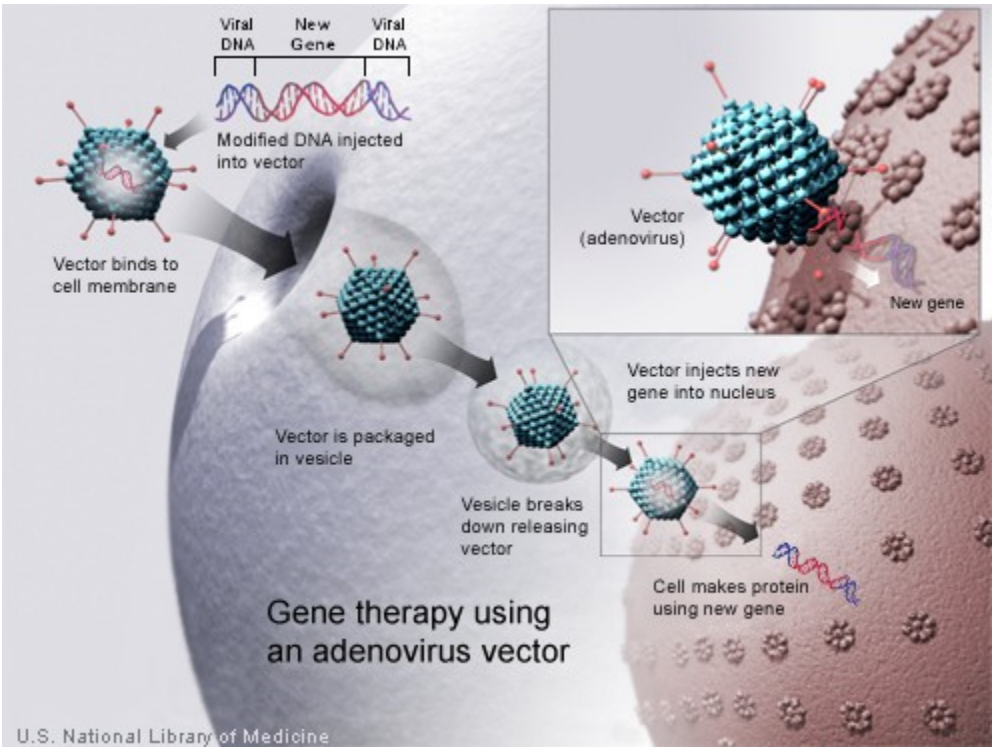
  - **Haemochromatosis:**
    - Iron Overload – hyperabsorption of iron.
    - Caused by mutations in the **HFE Gene.**
    - Iron builds up in organs (particularly: heart/liver/pancreas) and cause failure.
    - **Symptoms:** (usually men age 30-50 / women 50+)
      - Joint pain
      - Fatigue
      - Abdominal pain
      - Heart problems
      - Symptoms often occur after irreversible organ damage.
    - **Long-Term Effects:**
      - Arthritis/Liver disease/Heart abnormalities/Impotence/Early menopause/Bronze or grey complexion/Possible diabetes

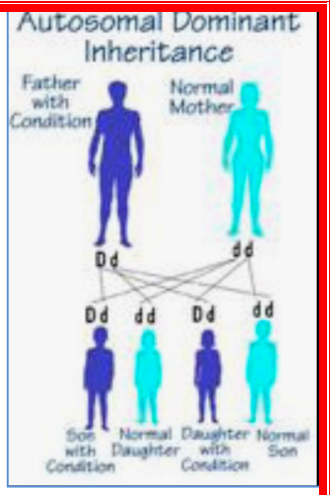
- **Membrane Function:**
  - **Cystic Fibrosis:**
    - Defective Cl^- Transporter in some cells (mostly exocrine glands)
    - Defective gene = CFTR
    - ***Q arm of Chromosome 7***
    - **Lungs:**
      - Thicker mucus secretion
      - Ciliated cells can't move mucus up trachea
      - Mucus + dust + particles + bacteria →trapped in lungs →frequent lung infections.
    - **Pancreas:**
      - Pancreatic duct clogs up – formation of cysts – eventually become fibrous
      - Hinders proper digestion (no pancreatic enzymes →duodenum)
    - **Intestine:**
      - Not enough digestive enzymes – mainly for fats
      - Malnourishment
      - Fatty stools
    - **Reproductive Ducts:**
      - Male vas-deferens blocks → sterility
    - **Sweat Glands:**
      - Secrete salt to induce H_2O flow but salt isn't actively reabsorbed.
      - Results in salt-residue on skin & salt deficit in body.
    - **Treatment:**
      - Enzyme tablets with meals
      - Electrolyte fluid – replenish lost NaCl
      - Vitamin supplements
      - Percussion – clear lungs



Gene Therapy:

- Using a genetically modified vector (viruses) to insert therapeutic genes into cells with defective genes.





Autosomal Dominant Disorders

Expression & Penetrance:

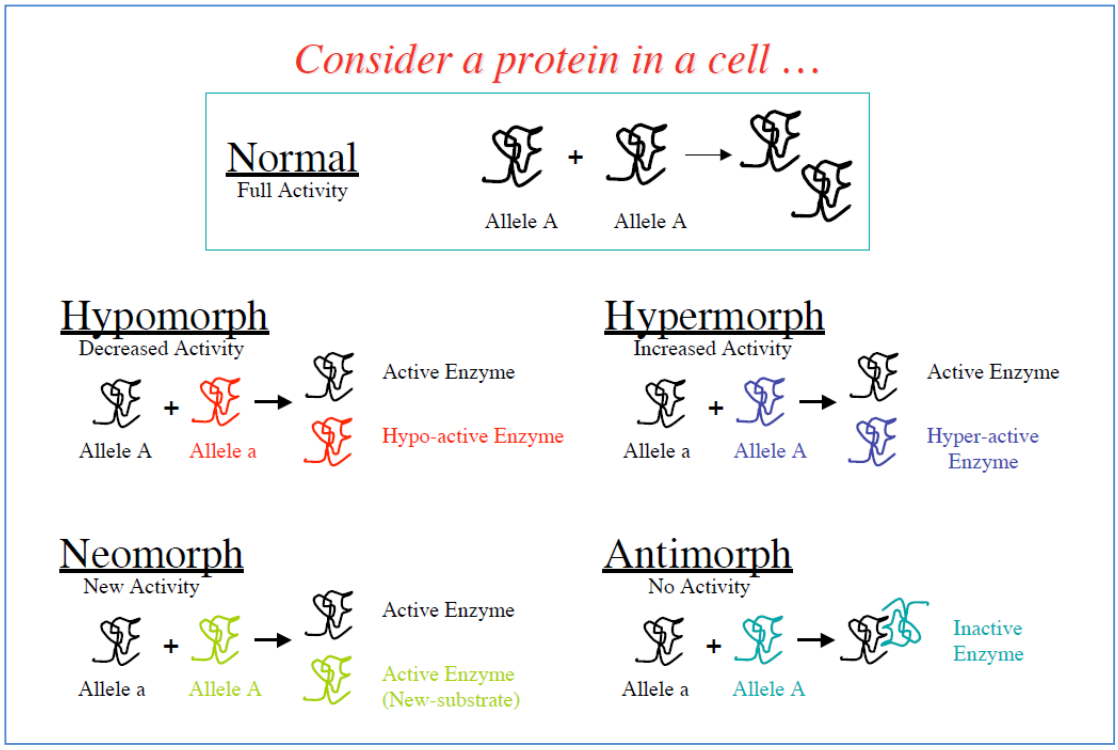
- **Expression:**
  - Severity/mildness of the **phenotype**
  - Influenced by environmental factors & mutations in other genes.
- **Penetrance:**
  - The chance that someone with the abnormal **genotype** will develop the abnormal **phenotype.**
  - Some diseases are 100% penetrant, others may only manifest in a fraction of carriers.

Anticipation:

- The **increasing disease severity** or **decreasing age of onset** from **generation to generation.**
- Anticipation **OCCURS ONLY IN** diseases associated with **NUCLEOTIDE-REPEAT-EXPANSION**.
- **Paternal Transmission:**
  - Anticipation generally occurs through **paternal transmission**
  - Arises from **instability of** the CAG **repeat** during **spermatogenesis.**

4 Types of Alleles:

- **Hypomorphs**
  - An allele that produces a **reduced level/activity of a protein**
- **Hypermorphs**
  - An allele that produces an **increased level/activity of a protein**
- **Neomorphs**
  - An allele with a **new activity** or **novel protein** product
- **Antimorphs**
  - An allele whose activity or product **antagonises the activity** of the normal gene product.



NB: Most mutant alleles are **dominant**. Except: Hypomorphs

Examples of Autosomal Dominant Disorders:

- **Growth-Factors:**
  - **Achondroplasia:**
    - "No Chondrocyte Proliferation"
    - A form of inherited dwarfism
    - **Phenotype:**
      - **Abnormal bone growth** – short stature + limb/cranial/facial disproportions.
      - Normal intelligence & lifespan
    - **Mutation** in the '**fibroblast growth-factor receptor**' gene:
      - **Normal function:** negative regulator of bone growth.
      - **Mutated function:** *Hypermorphic* – too much negative regulation → puts the 'handbrake' on bone growth.
    - **Most** (80%+) dwarfs **have normal parents** – ie. Their dwarfism is due to new mutation.
    - **Genotype: Aa**
      - AA Genotype – not compatible with life.
      - $Affected_{Heterozygous}$ + $Affected_{Heterozygous}$ = 2/3 chance of affected offspring.
      - $Affected_{Heterozygous}$ + Normal = ½ chance of affected offspring.

	A	a
A	AA	Aa
a	Aa	aa

2x Affected

	A	a
a	Aa	aa
a	Aa	aa

Normal x Affected

  - **Hypochondroplasia:**
    - "Low Chondrocyte Proliferation"
    - **Phenotype:**
      - Similar to, but milder than achondroplasia
      - Short stature
      - Disproportionate limbs
      - Normal facial features
      - Phenotype only becomes noticeable at toddler/school-age
    - **Mutation:**
      - Also in the '**fibroblast growth-factor receptor**' gene:
      - Same gene – but different mutation
      - **Normal function:** negative regulator of bone growth.
      - **Mutated function:** *Hypermorphic* – too much negative regulation → puts the 'handbrake' on bone growth.

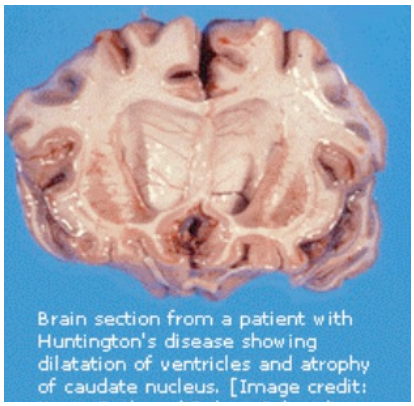
- **Neural Function:**
  - **Huntington's Disease:**
    - Degenerative brain disease.
    - **Mutation:**
      - A Dynamic Mutation
      - **Defective Gene:** Huntington Protein
        - Huntington protein is widely expressed & vital for normal function
        - On **Chromosome 4**
        - **An expansion of a run of glutamine repeats in the protein.**
        - Highly expressed in brain.
      - **Normal:** 10-26 repeats
      - **Intermediate:** 27-35 repeats (offspring is at risk due to 'slippage')
      - **Affected:** 36-121 repeats
      - Onset age depends on # of repeats in mutation.
    - **Phenotype:**
      - Onset age generally between 30-50yrs (*well after reproductive age*)
      - Intellectual decline
      - Unsteady gait
      - Involuntary movements
      - Slurred speech
      - Impaired judgement
      - Difficulty swallowing.
    - **Perpetuation:**
      - Offspring of affected parent = 50% chance of inheritance:
      - **Penetrance:** 100% - ie. If you have enough repeats, you WILL get the disease.
      - **Expression:** Age of onset decreases as # of repeats increases.
        - However – severity of symptoms (post-onset) is constant.

```
5′-GAT-ATG-AGG-CAG-CAG-CAG-CAG-CAG->>>-3′      NORMAL
3′-CTA-TAC-TCC-GTC-GTC-GTC-GTC-GTC-GTC-TTA-5′  REPLICATION
  (Asp-Met-Arg-Gln-Gln-Gln-Gln-Gln-Gln-Leu)

5′-GAT-ATG-AGG-CAG CAGC AG CAG-CAG-CAG->>>-3′      BACKWARD SLIPPAGE
3′-CTA-TAC-TCC-GTC-GTC-GTC-GTC-GTC-GTC-TTA-5′  INCREASES REPEATS

5′-GAT-ATG-AGG-CAG-CAG-CAG-CAG-CAG->>>-3′      FORWARD SLIPPAGE
3′-CTA-TAC-TCC-GTC      GTCG TC GTC-GTC-GTC-TTA-5′  DECREASES REPEATS
```

No. Repeats	Median age of onset
39 repeats	66 yrs
40 repeats	59 yrs
→ 41 repeats	54 yrs
42 repeats	49 yrs
43 repeats	44 yrs
44 repeats	42 yrs
45 repeats	37 yrs
46 repeats	36 yrs
47 repeats	33 yrs
48 repeats	32 yrs
49 repeats	28 yrs
50 repeats	27 yrs

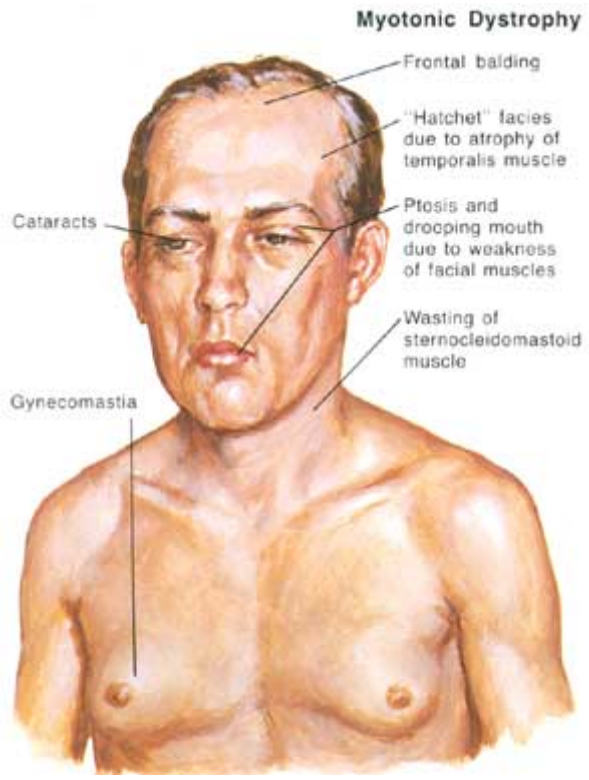
	A	a
a	Aa	aa
a	Aa	aa

Normal x Affected

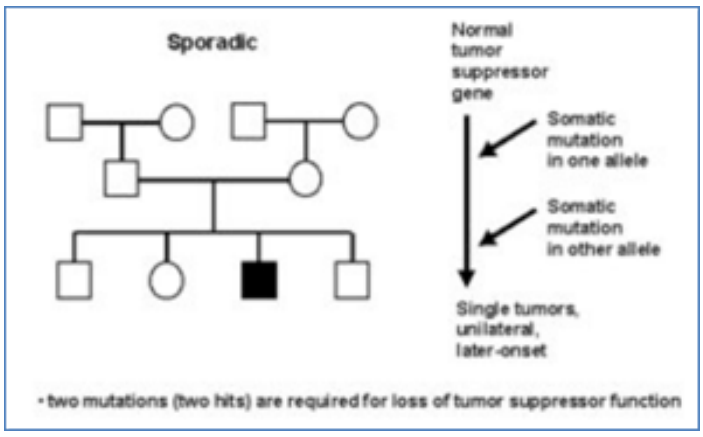


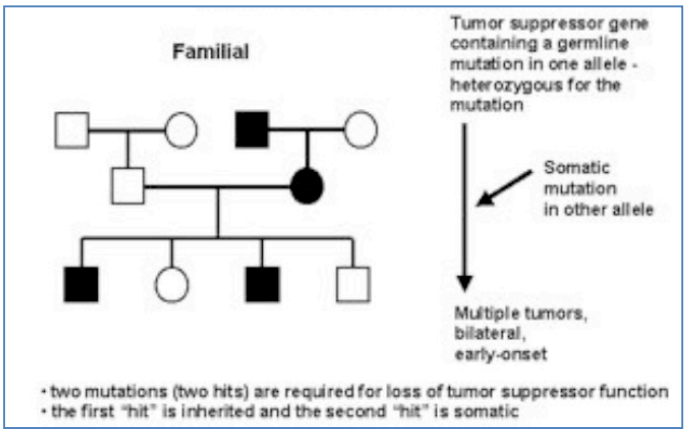
Brain section from a patient with Huntington's disease showing dilatation of ventricles and atrophy of caudate nucleus. [Image credit:

- **Myotonic Dystrophy**
  - Also a **repeat-sequence mutation.**
  - Affects skeletal & smooth muscle/eyes/heart/endocrine system/central nervous system.
  - May be mild/classical/congenital (severity)
  - **Mutation: Expansion of** CTG trinucleotide **repeat** in the DMPK gene on **chromosome 19**.

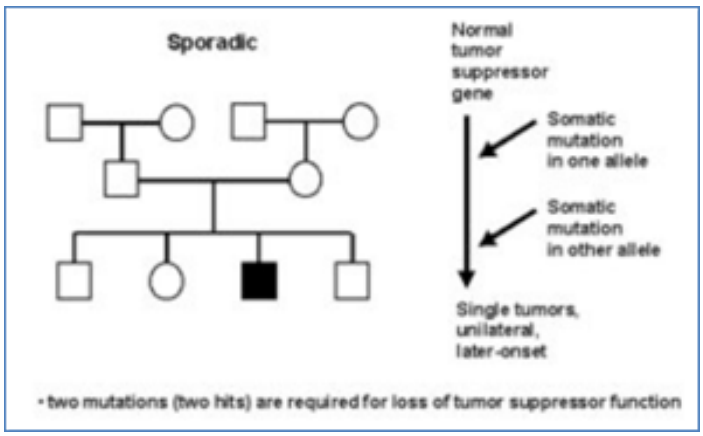


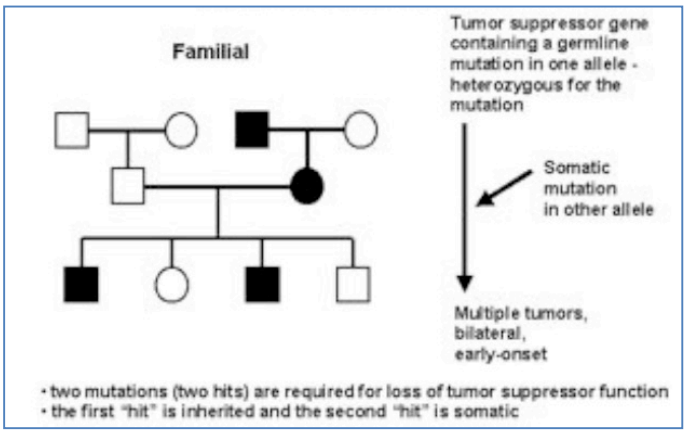
- **Tumour-Suppression:**
  - **Retinoblastoma**
    - Malignant Tumour in the retina of one/both eyes. (unilateral/bilateral)
    - Arises from inactivation of **both alleles** for the RB1 gene (tumour suppressor gene)
    - Can have multiple foci in the one eye.
    - Onset: Infancy / early childhood.
    - 1/3 cases are due to **inherited predisposition:**
      - **Autosomal dominant**
      - Generally only the '*predisposition*' is inherited, & the other functional allele makes up for the deficiency.
      - **However,** when a **mutation** in the **functional allele** occurs, the tumour/s develop.
      - This is known as **The 2 Hit Hypothesis:**





- **Neurofibromatosis**
  - Formation of benign lumps around neural tissues.
  - **Is an example of "Pleiotrophy":**
    - When a single gene mutation influences multiple phenotypic traits.
    - Tumours can grow anywhere on the body – can affect other bodily tissues.
  - **2 Types:**
    - **Type 1 NF (NF1 Protein):**
      - 'Common' type
      - Neurofibromas under skin
      - Enlargement/deformation of bones
      - Scoliosis
      - Tumours in brain/on cranial nerves/on spinal cord
      - Learning disabilities are common.
    - **Type 2-Acoustic-NF (NF2 Protein):**
      - Very rare
      - Multiple tumours on cranial+spinal nerves
      - Hallmark: tumours on auditory nerves → hearing loss in teens.
  - **NF1 & NF2** are **different proteins** of different genes of **different chromosomes**.
    - However they are **both tumour-suppressor genes.**
    - Mutations in either – cause partial/complete loss of protein function.
  - **Inherited Predisposition:**
    - **Autosomal dominant**
    - Generally only the '*predisposition'* is inherited, & the other functional allele makes up for the deficiency.
    - **However,** when a **mutation** in the **functional allele** occurs, the tumour/s develop.
    - This is known as **The 2 Hit Hypothesis:**

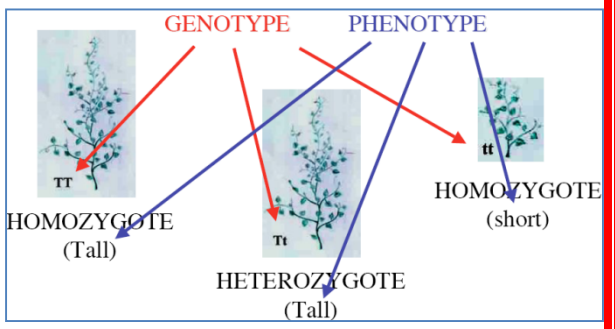




Mendelian Genetics I

Genetic Nomenclature:

- **Gene**
  - Unit of hereditary that occupies a locus (specific position in the genome) & conveys a phenotype.
- **Locus**
  - The position of a gene within a genome
  - Ie. What chromosome & relative to other genes?
  - Loci = plural
- **Alleles**
  - Different forms of a gene that result in observable differences in phenotype
- **Dominant**
  - An allele that fully manifests its phenotype in an individual with 2 different alleles at the corresponding loci of homologous chromosomes.
- **Recessive**
  - An allele that only manifests its phenotype when both the corresponding loci of homologous chromosomes contain that allele.
- **Homozygous**
  - Having a **pair of identical alleles** at the corresponding loci of homologous chromosomes.
- **Heterozygous**
  - Having a **pair of dissimilar alleles** at the corresponding loci of homologous chromosomes.
- **Genotype**
  - The genetic makeup of an organism
  - The combination of alleles present
- **Trait (Phenotype)**
  - The visible characteristics of an organism resulting from its Genotype.
  - The observable properties of the heritable character of an organism



Mendel

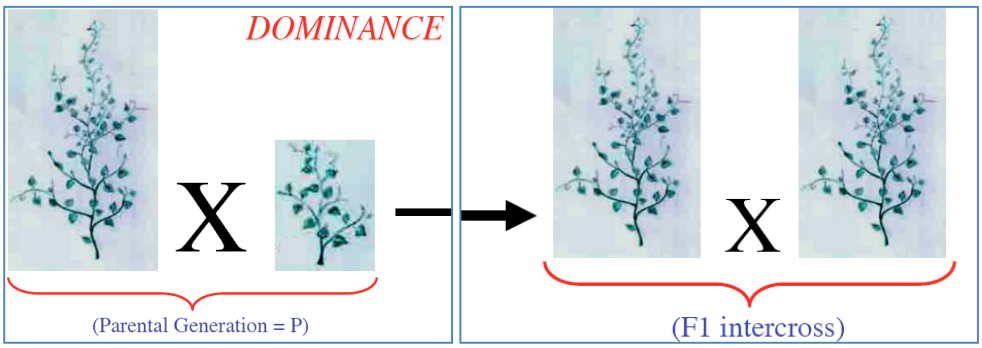
- Austrian monk
- Discovered the universal laws of heredity
- Crossed different varieties of pea plants with discrete traits.
- **Mendel's Logic:**
  - Everyone has 2 parents
  - Therefore everyone receives a 'factor' from each parent.
  - (today we call these 'factors' genes)

Mendel's Laws of Inheritance:

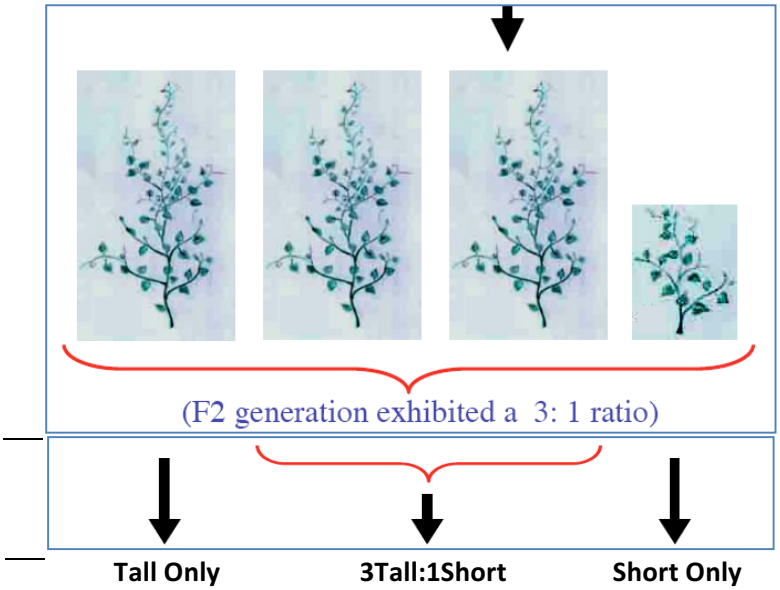
1. **Law of Segregation:**
   a. **Alternative versions of genes (alleles) are responsible for variations in inherited traits.**
      i. Eg. Each human has genes for eye-colour, but there are variations as to the specific colour.
   b. **An organism inherits 2 alleles for each physical trait – one from each parent.**
      i. During fertilisation, one allele comes from each parent.
      ii. May be two of the same alleles – Homozygous
      iii. May be two different alleles - Heterozygous
   c. **The 2 alleles for each trait segregate during gamete production.**
      i. During gametogenesis, each gamete only contains one allele for each gene.
      ii. It is up to chance which allele ends up in which gamete.
2. **Law of Independent Assortment: (Inheritance Law)**
   a. Different traits are inherited independently of each other.
   b. Exception: some genes are linked to each other & some are 'sex-linked'

Mendel's Experiments:

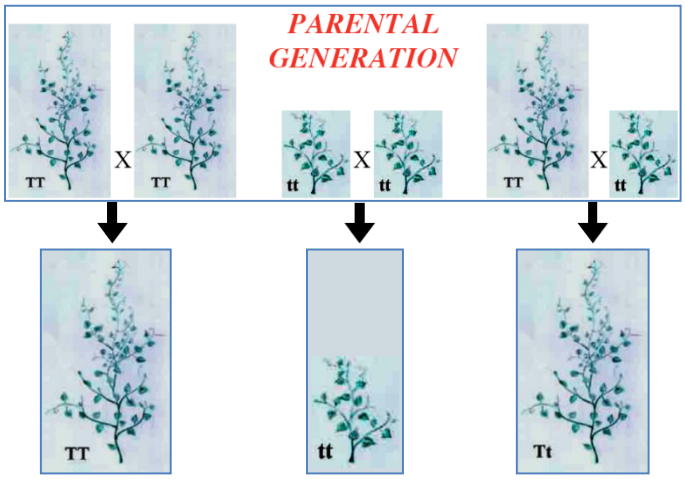
- **Dominance:**
  - Pure-breeding Tall plants + pure-breeding short plants = All hybrids were tall
  - It didn't matter which parent contributed which gamete →the 'Tall' allele isn't sex-linked.



  - **However:** Tall-Short Hybrid + Tall-Short Hybrid = 3:1 Mixture of Tall:Short offspring.

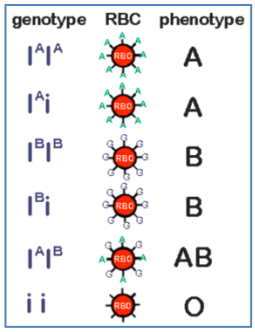


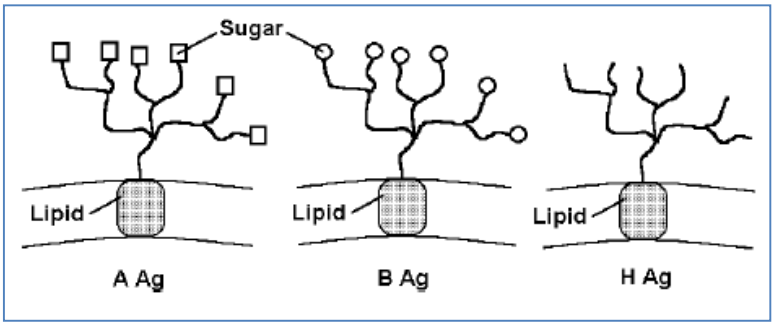
  - **F2 Gens were self-pollinated:**
    - Plant 1: Produced only tall plants
    - Plant 2: Produced a 3:1 Mixture of Tall:Short offspring
    - Plant 3: Produced a 3:1 Mixture of Tall:Short offspring
    - Plant 4: Produced only short plants
  - **Mendel's Conclusion:**
    - There are genes in gametes that determines physical characteristics
    - There are variations of these genes (alleles) that result in different traits.
    - Some of these variations (alleles) are dominant over others.



Exceptions to Mendel's Laws:

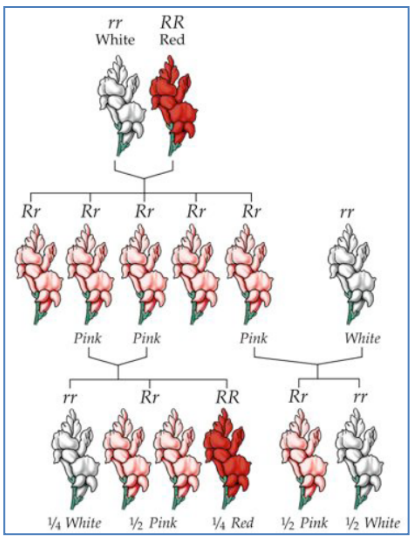
- Some phenotypic traits are determined by 2 or more genes.
- Many genes have **multiple alleles**
- Many alleles exhibit a seemingly identical phenotype, but are subtly different.
- Some allele combinations show **incomplete dominance**
- Some allele combinations show **co-dominance.**
    - **Multiple Alleles:**
        - There can be 3, 4, 5 or more different forms of a gene (alleles).
        - However, there can only ever be **2 alleles/organism**
        - Eg. Blood type:
            - There are 3 different alleles for blood @ the ABO Locus:
                - **I^A (type A), I^B (Type B), and i (Type O)**
                    - I^A Codes for enzyme that attaches the 'A' sugar to the Red Blood Cell
                    - I^B Codes for enzyme that attaches the 'B' sugar to the Red Blood Cell.
                    - i Codes for an inactive enzyme





    - **Codominance:**
        - **Eg. Blood Type:**
            - The 'i' allele is recessive – only expressed when homozygous.
            - The 'A' allele is dominant over 'i' allele.
            - The 'B' allele is dominant over 'i' allele.
            - **But neither 'A' or 'B' are dominant over each other.**
            - **When 'A' and 'B' are heterozygous, they are both expressed equally.**

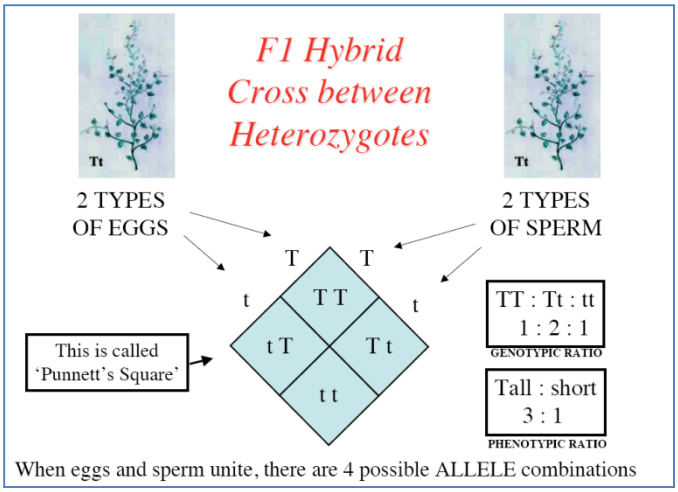
    - **Imcomplete Dominance:**
        - Blending of traits.
        - **Eg. Plants:**
            - Homozygous **RED +** Homozygous **WHITE =** Heterozygous **PINK**
            - Heterozygous **PINK +** Heterozygous **PINK = 1WHITE : 2PINK : 1RED**
            - Heterozygous **PINK +** Homozygous **WHITE = 1PINK : 1WHITE**

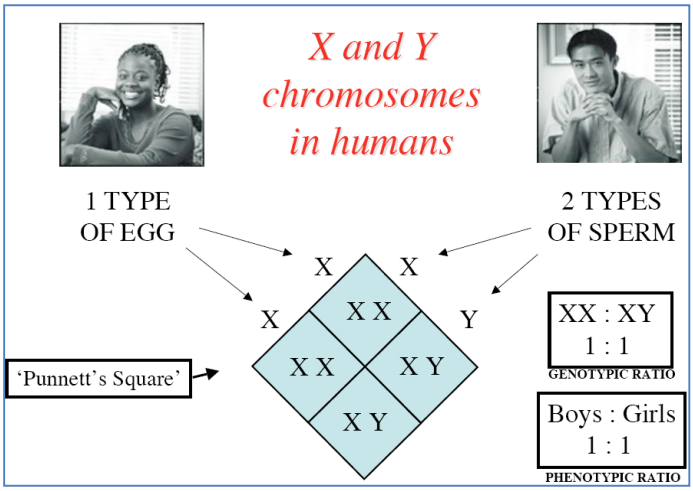


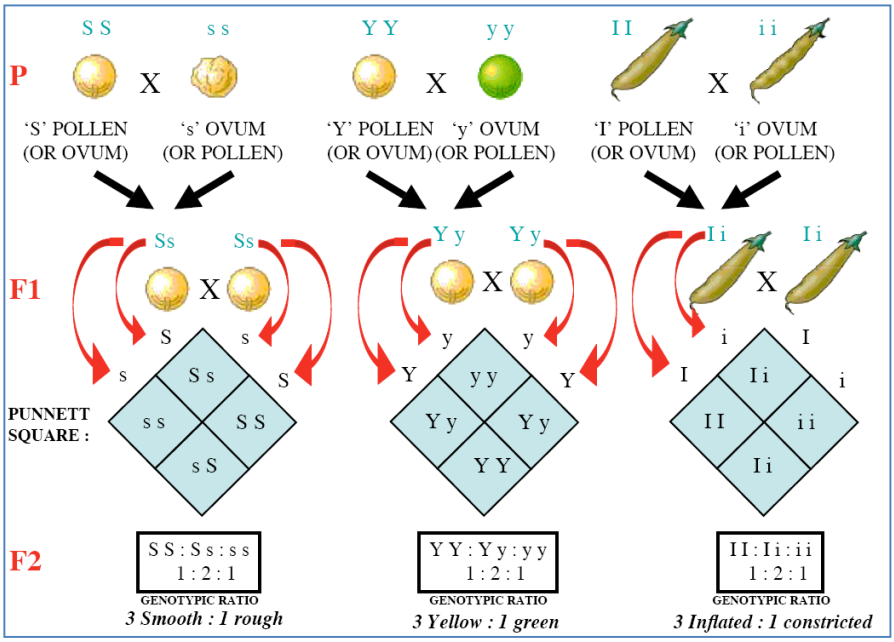
SideNOTE: Sex Linked Genes:

- Genes located on one of the sex chromosomes (X or Y) but not the other.
- Since, typically the X chromosome is longer, it bears a lot of genes not found on the Y chromosome, thus most sex-linked genes are X-linked genes.
- Typically, X-linked traits show up more in males than females because typical XY males only have one X chromosome, so if they get the allele on their X chromosome, they show the trait.
- If a typical XX female is a carrier, 50% of her sons will get that X chromosome and show the trait.
- In order for an XX female to exhibit one of these X-linked traits, most of which are recessive mutations, she would have to have two copies of the allele (X'X'), which would mean that her mother would have to be a carrier and her father have the trait so she could get one allele from each of them.

Punnet's Squares:







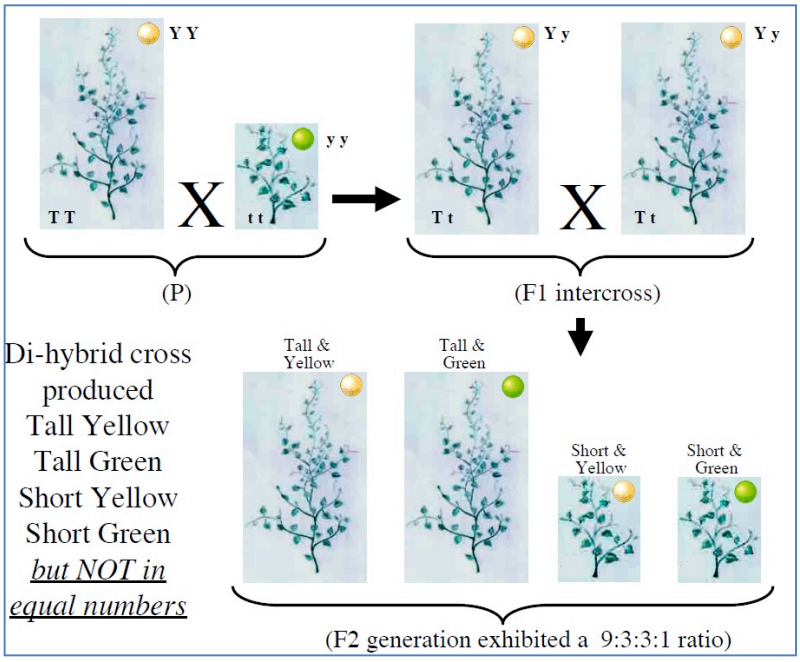
Mendelian Genetics 2

Mendel's Laws of Inheritance: (from last week)

1. **Law of Segregation:**
    a. **Alternative versions of genes (alleles) are responsible for variations in inherited traits.**
        i. Eg. Each human has genes for eye-colour, but there are variations as to the specific colour.
    b. **An organism inherits 2 alleles for each physical trait – one from each parent.**
        i. During fertilisation, one allele comes from each parent.
        ii. May be two of the same alleles – Homozygous
        iii. May be two different alleles - Heterozygous
    c. **The 2 alleles for each trait segregate during gamete production.**
        i. During gametogenesis, each gamete only contains one allele for each gene.
        ii. It is up to chance which allele ends up in which gamete.
2. **[This week] Law of Independent Assortment: (Inheritance Law)**
    a. Different traits are inherited independently of each other.
    b. Ie. Alleles at multiple loci separate independently.
    c. Exception: some genes are linked to each other & some are 'sex-linked'

What Happens With 2 or More Characterists?

- **Di-Hybrid Cross:**
    - A **cross** between **two F1 offspring** of **two individuals** that differ in **two traits**
- **Eg**. Pure-breeding Tall & Yellow plant (dominant) + Pure-breeding Short & Green plant.
- All F1. Offspring would be heterozygous, and exhibit the Tall & Yellow phenotypes.
- However, when heterozygous F1 plants were interbred = **Di-hybrid cross produced:**
    - **9x** Tall & Yellow
    - **3x** Tall & Green
    - **3x** Short & Yellow
    - **1x** Short & Green

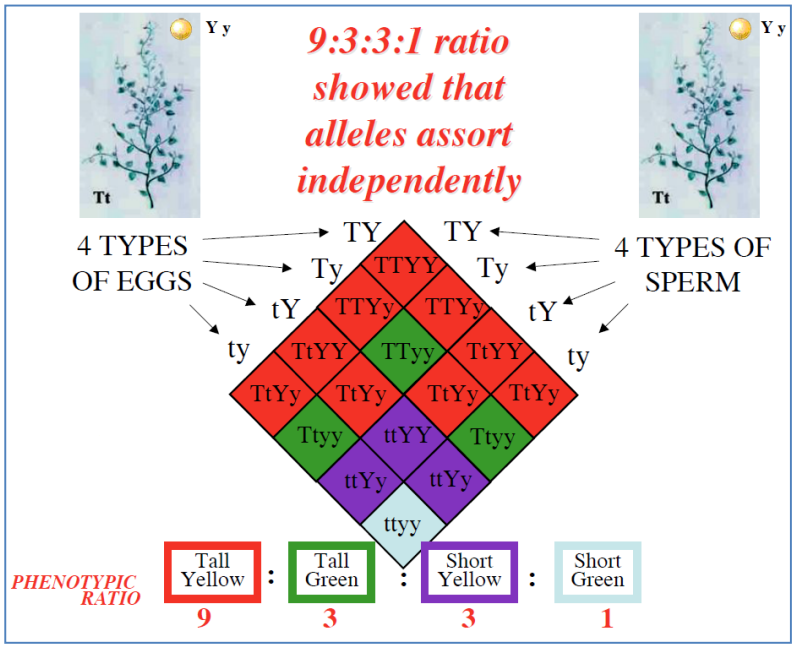


According to Mendel's 2nd law, given 2 characteristics, the following gametes are equally likely:

- TY
- Ty
- tY
- ty

- Therefore there are **16 possible allele combinations**
    - But only **4 different possible phenotypes**.
        - Tall & Yellow
        - Tall & Green
        - Short & Yellow
        - Short & Green

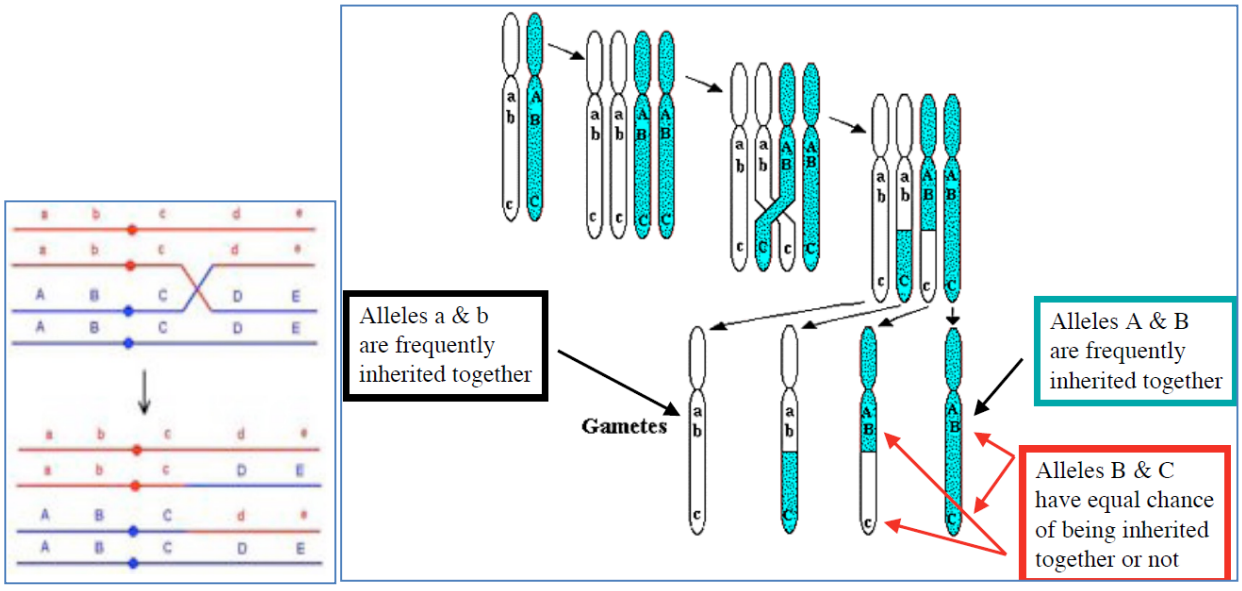
- **Multiple Allele Punnett Square**



- On average, the 4 phenotypes occurred in a 9:3:3:1 ratio.
- **This 9:3:3:1 ratio proved that:**
  - Both alleles showed **simple dominant/recessive** relationships
  - Both alleles **separated independently** in meiosis (gametogenesis).

BUT HANG ON!!!

- If a chromosome has hundreds (or thousands) of genes, it's not possible for all alleles to assort independently.
- **Turns out Mendel didn't get it quite right.**
- Genes **don't always assort independently:**
  - Many alleles show **linkage** due to their location **on the same chromosome.**
  - Gene pairs only assort independently if:
    - They are located on different chromosomes
    - They are located far apart on the same chromosome.
- But what about those alleles on the **same chromosome** that don't show **linkage?**
  - **Answer: *Meiotic Recombination:***
    - Phenomenon of **'crossing over'** in **Meiotic Prophase**
    - Ensures chromosomes in gametes are a unique combination of genes.
  - However: Gene pairs on a chromosome located close to each other are less likely to assort independently.
    - Because the chance of a 'cross-over' between them is lower.

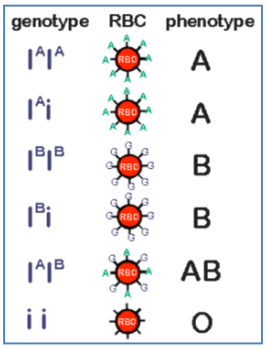


Gene Mapping:

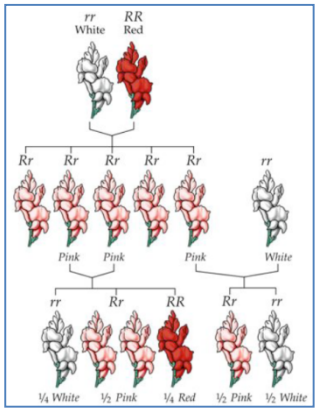
- The probability of crossing over between 2 genes is roughly proportional to their distance apart on the chromosome.
- The greater the distance between the genes, the higher the probability of a cross-over between them.
- The Distance is measured in **genetic map units (m.u.)** or **centimorgans (cM)**
- **1mu** = the distance between genes that give a 1% probability of meiotic recombination.
- **A Recombinant Frequency** (RF) of 1% = 1m.u.
- **Linked Genes** = **those < 50cM apart.** (ie. Less than 50% chance of recombination)

Deviations from Simple Dominant/Recessive Relationships:

- **Last Week:**
  - **Codominance:**
    - Equal expression of both phenotypes
    - **Eg. Blood Type:**
      - The 'i' allele is recessive – only expressed when homozygous.
      - The 'A' allele is dominant over 'i' allele.
      - The 'B' allele is dominant over 'i' allele.
      - **But neither 'A' or 'B' are dominant over each other.**
      - **When 'A' and 'B' are heterozygous, they are both expressed equally.**



  - **Imcomplete Dominance:**
    - Blending of traits.
    - **Eg. Plants:**
      - Homozygous **RED +** Homozygous **WHITE =** Heterozygous **PINK**
      - Heterozygous **PINK +** Heterozygous **PINK = 1WHITE : 2PINK : 1RED**
      - Heterozygous **PINK +** Homozygous **WHITE = 1PINK : 1WHITE**



- **This Week:**
  - **Epistasis:**
    - Where alleles in one gene masks the phenotypic effects of another gene.
    - Eg. Coat colour in Labradors:
      - **At locus 1:** Black (B) is dominant over brown (b)
      - **At locus 2:** Normal coat colour (brown/black) (E) is dominant over yellow (e).
    - When locus 2 is homozygous yellow (e), it masks the effects of the "black" gene at locus 1.
      - 'e' is said to be **'epistatic'** over 'B'

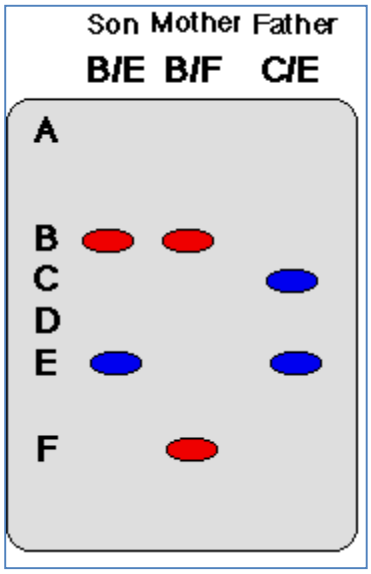
GENOTYPE	PHENOTYPE
eeBB	= Yellow
eeBb	= Yellow
Eebb	= Chocolate
EEbb	= Chocolate
EeBb	= Black
EEBb	= Black
EeBB	= Black
EEBB	= Black

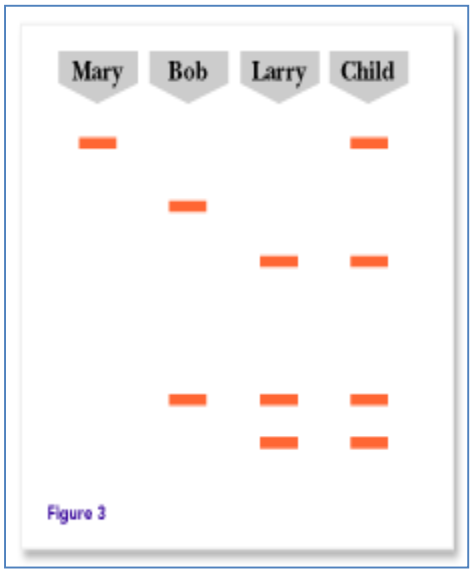
Mutated Alleles:

- Allele Mutations happen in 1 of 3 places:
  - **In the Coding Region of a Gene:**
    - Results in an altered protein sequence
    - May or may not retain some function
  - **In the Non-Coding (Intronic) Region:**
    - Results in incorrect splicing of hnRNA →mRNA
  - **In the Regulatory Sequences:**
    - Results in incorrect or failure of gene expression

Gene Testing:

- Paternity Testing/Forensic Testing/etc.
- **VNTRs (Variable Number of Tandem Repeats)**
  - Short DNA sequences repeated in a head-to-tail fashion.
  - Many different VNTRs are found throughout the human genome.
  - Different people vary as to the numbers of repeats at specific VNTR loci.
  - This variability is used for DNA fingerprinting.
  - Some alleles will be the same amongst some people.
  - However, a complete DNA fingerprint looks at up to 13 different loci.
  - This ensures the probability of a profile matching *by chance* is very low.





Complex Medical Genetics 1

NB: Complex Genetics is about determining the chances (risk) of developing a trait.

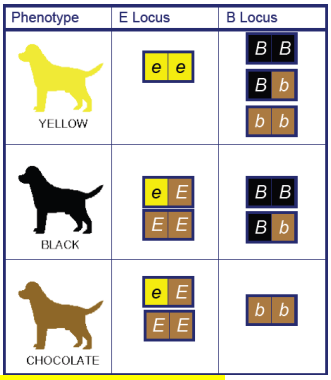
Key Terms:

- **Alleles:** 2 or more variations of a gene that convey different phenotypes
- **Dominant:** An allele that will result in phenotype
- **Recessive:** An allele that will only result in phenotype if homozygous
- **Linkage:** Where 2 genes are located close together on the same chromosome and are inherited together.
- **Recombination:** Process of gene swapping (re-assortment of traits) that occurs in meiosis.
- **Segregation:** The separation of the two members of a chromosome pair from each other at meiosis

Two Locus Traits:

- **Eg. Coat Colour in Labradors:**
  - 3 Colours:
    - Yellow
    - Chocolate
    - Black
  - **At locus 1:** Black (B) is dominant over brown (b)
  - **At locus 2:** Normal coat colour (brown/black) (E) is dominant over yellow (e).
  - **But:** When locus 2 is homozygous yellow (e), it masks the effects of the "black" gene at locus 1.
    - 'e' is said to be **'epistatic'** over 'B'
    - **NB: Epistasis:** Where alleles in one gene masks the phenotypic effects of another gene.

GENOTYPE	PHENOTYPE
eeBB	= Yellow
eeBb	= Yellow
Eebb	= Chocolate
EEbb	= Chocolate
EeBb	= Black
EEBb	= Black
EeBB	= Black
EEBB	= Black



NB: Q: What parental genotype could produce a Labrador litter containing all 3 coat colours?
A: Both parents must be heterozygous at both Loci. (one parent may be homozygous 'e-e')

Multilocus Traits:

- Phenotypes that are controlled by more than 2 genes.
- Some are controlled by so many genes, that the effects of individual genes are very small.
- Sometimes, effects of individual genes are so small that they can't be demonstrated individually – must be measured using statistics.

Gene/Environment Interactions:

- Where the phenotype/trait is only apparent in a certain environment.
- **Eg. Phenylketonuria:**
  - A single gene mutation resulting in:
    - Lack of phenylalanine hydroxylase
    - Extreme sensitivity to phenylalanine
    - Phenylalanine intake → severe retardation = **ie. A 'Genetic Cause**/Diease'
  - **But** in a phenylalanine-free environment, the mutation *has no phenotype!*
    - Phenylalanine intake → poisoning = **ie. An 'Environmental Cause'**
- **A Genetic** or **Environmental "Cause":** Will result in increased expression of the trait (all else being equal)

Complex Genetic Traits:

- Traits resulting from the interplay of **multiple factors** (genetic & environmental) that each have relatively small effects.
- **Either Quantitative:** This week
  - Metric
  - A trait that is continuous & measurable in infinitesimal increments.
    - Height
    - Weight
    - Blood pressure
    - Enzyme Levels
- **Or Qualitative:** Next Week
  - Categorical
  - A trait that is 'all or nothing' – 'it either *is* or it *isn't*'

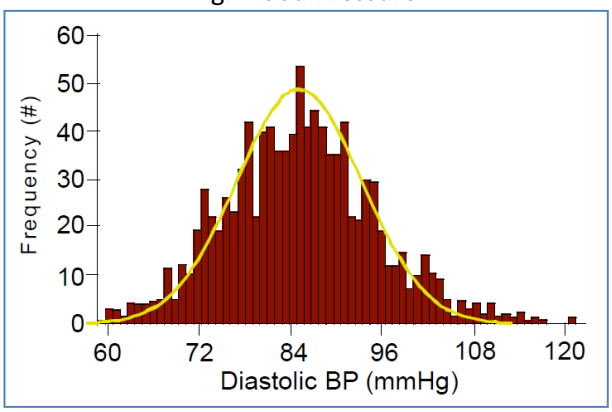
- **NB: Complex Genetics is about determining the chances (risk) of developing a trait.**
- **Risk of Developing a Trait – 2 Parts:**
  - **Susceptibility:**
    - The Genetic Component
    - One's innate (genetic) tendency (predisposition) to develop a trait.
    - Eg. Type 2 Diabetes: 98% heritability – but is also triggered by environmental factors.
  - **Liability:**
    - The Total Risk
    - Incorporates all sources of variation, including environmental (and other external) factors.

- **Sources of Variation in Phenotype:**
  - **Genetic**
  - **Environmental Factors**
  - **Stochastic Factors (chance)**

THIS WEEK: Complex QUANTITATIVE Traits:

- Metric
- A trait that is continuous & measurable in infinitesimal increments.
  - Height
  - Weight
  - Blood pressure
  - Enzyme Levels
- **Genetic Transmission:** Absence of clear-cut segregation & Mendelian ratios.
- **Distribution of Phenotypes → Normal Distribution:**

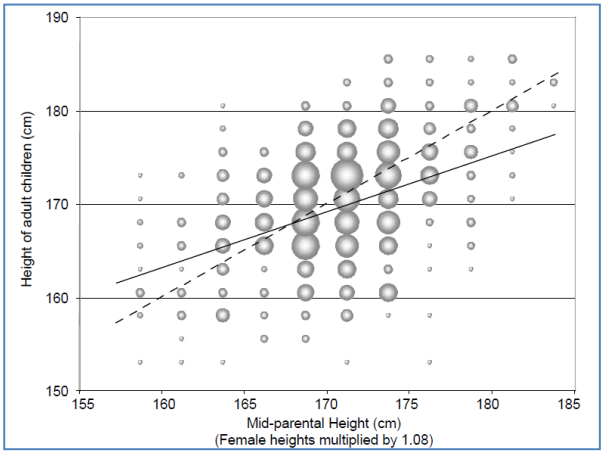
Eg. Blood Pressure



  - Therefore, statistical approaches are used in Predicting Traits.
  - However, there are problems with using a statistical approach:
    - Eg. Regression to the Mean...

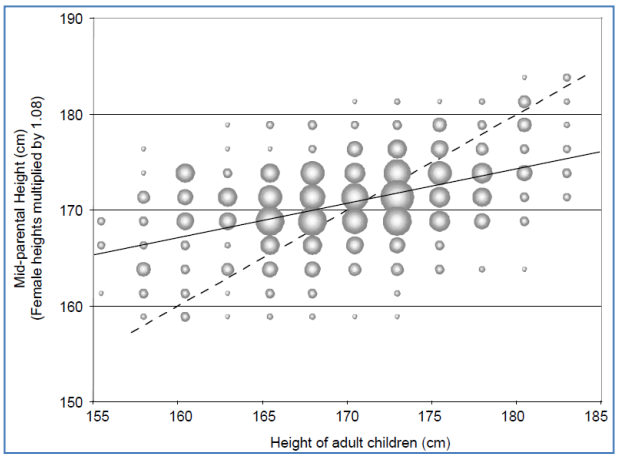
- **Regression To The Mean:**
  - The tendency of values at the 'extreme' ends of a data set to be more mediocre upon remeasurement.
  - Why?
  - **Explanation 1: Remeasuring Extreme Values:**
    - All measurements consist of 2 components:
      - The "Real" Value:
      - The Error
    - Extreme measurements occur because *both* components are acting in the *same direction.*
    - *However,* when remeasured, the "real" value won't change, but the *Error* is *unlikely* to be as *extreme again.*
    - Therefore the result will *more likely* be more mediocre than the initial measurement.
  - **Explanation 2: Statistical Artefact:**
    - Any distortion in a recorded graph/dataset that is not present in the corresponding studied population. Artefacts can be introduced inadvertently by hardware or software, or by an operator (eg. Measuring/Graphing techniques).

  - **Eg. Graphing Techniques: Height of Children Vs. Mean Height of Parents**
    - This graph shows that tall children most frequently have tall parents.
    - However, the distribution of children of tall parents is not symmetrical – It is skewed towards the mean – lowering the mean. (same with children of short parents)
    - Why?:
      - Problems of remeasuring extreme values.
      - Limits of the biological system – ie. A 18ft tall body isn't possible due to bone strength & gestation.

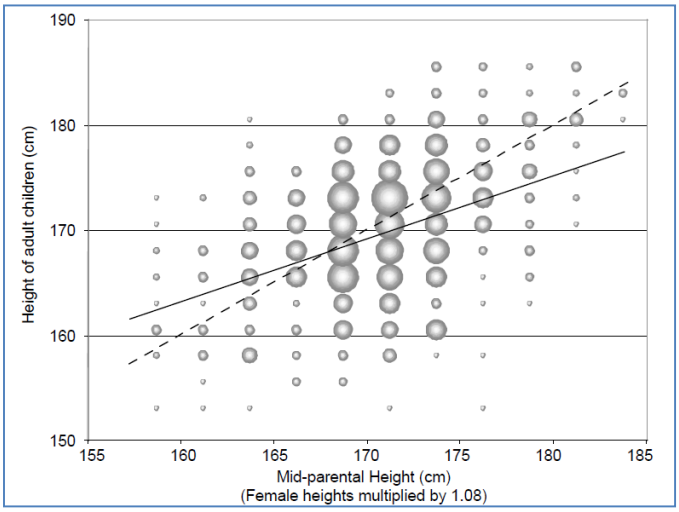


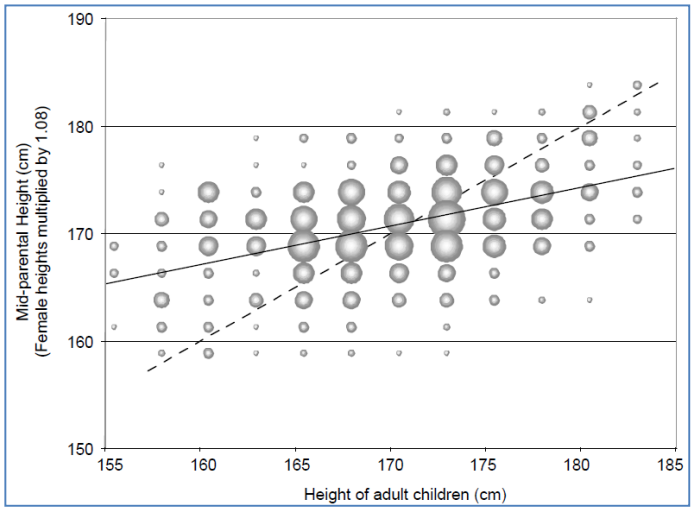
Dashed Line = Expected Values: Where Children should = Mean of Parents' heights.
Full Line = Line of Best Fit: Children are less exceptional than their parents at both ends → Regression to the Mean.

- However, If we plot the same data on a graph where the X & Y axis are swapped, we get a different 'line of best fit'.



- **THIS HAS TO BE A STATISTICAL ARTEFACT.**





- Why does this happen?
  - Because **the graphing program** that produces the line of best fit assumes the X-axis (dependent variable) is correct → **only takes into account errors on the Y-axis.**
- **Therefore: To Accurately "FIT" Graph Values that Regress to the Mean:**
  - The 'theoretical trend' actually lies *between the 2 fit-lines from each data set.*
  - *Ie. The result of a 2Dimensional Line Fit.*

Phenocopies:

- The production of a phenotype, which closely resembles a phenotype that normally results from a specific gene mutation.
- **Causes:**
  - **Environmental:**
    - Depends on phenotype.
    - Eg. Height:
      - Nutrition
      - Infection
      - Trauma
  - **Stochastic (Chance):**
    - Luck of the draw.
  - **Genetics:**
    - Eg. Short Stature:
      - Turner's Syndrome
      - Noonan Syndrome
      - Silver-Russel Syndrome
      - Achondroplasia
    - All have the same phenotypes.

Predicting the Adult Height of Children:

- **Boys:**
  - Average of Parents' Heights + 6cm
  - Error: +/- 10cm
- **Girls:**
  - Average of Parents' Heights – 6cm
  - Error: +/- 8.5cm

Eugenics:

- The 'improvement' of humanity by altering its genetic composition.
- By encouraging breeding of those that have 'desirable' traits/genes & discouraging breeding between those with 'undesirable' traits/genes.
  - Just know: It wouldn't work because most of the time, 'desirable' traits/genes are the result of complex genetic combinations which can't be selected for by breeding. – And it is morally wrong.

Complex Medical Genetics II

Complex Genetic Traits:

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- **Either** **Quantitative:** last week
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- **Sources of Variation in Phenotype:**
  - **Genetic**
  - **Environmental Factors**
  - **Stochastic Factors (chance)**

Heritablity:

- The proportion of a trait's phenotypic variation that's attributable to *genetic factors*
- Ie. The contribution of genetics to a trait.

Complex QUALITITIVE Traits:

Definition:

- Phenotypes that are **discontinuous** in nature & distribution.
- Genetic transmission preserves classes, but don't segregate according to typical Mendelian Ratios.
- Examples:
  - Diabetes
  - Multiple Sclerosis
  - Asthma
  - Neural Tube Defects (eg. Spina Bifida)
  - Cancer

Explanation for Non-Mendelian Inheritance of Complex Genetic Traits:

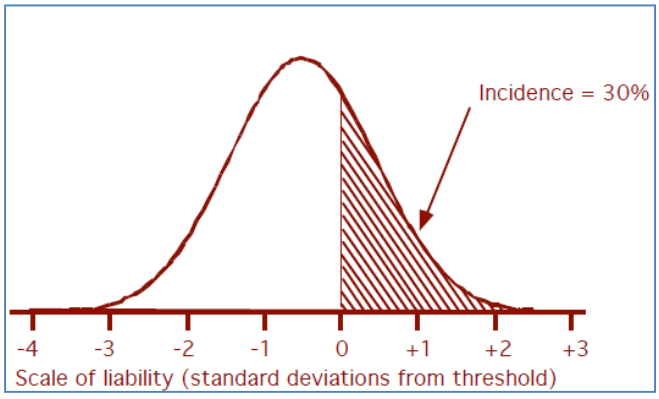
- If multiple genetic factors contribute to the trait, each factor must be inherited discretely (Mendelianly).
- However, because there are multiple factors, the overall *'trait'* itself doesn't follow "genetic rules"

Sources of "Complexity" in Genetic Traits:

- More than One Genetic Factor contributing to the Trait.
- Environmental Factors can have the same effect as Genetic Factors.

Assessing Risk of Complex Genetic Diseases:

- Because there are multiple factors at play, a **statistical approach** is **required** to **assess risk** of disease.
- **Falconer's Threshold Model:**
  - Liability (tot. Risk) is *normally distributed* in the general population.
  - A *threshold* exists, above which disease appears.
  - The area under the curve beyond threshold shows *Incidence.*
  - This model can predict the risk of complex qualitative diseases, using a statistical approach.



Heritablity:

- The proportion of a trait's phenotypic variation that's attributable to *genetic factors*
- Ie. The contribution of genetics to a trait.

Calculating Heritability & Risk:

- **Heritability:**
  - **Need:**
    - Incidence of the trait in the *general population* (%)....&
    - Incidence of the trait in *nth°degree relatives* (%)
    - The 'r' values for the *nth°degree* relatives.
    - Falconer's Tables (used to find 'X_g', 'X_r' & 'a' values)
    - These 2 Equations:

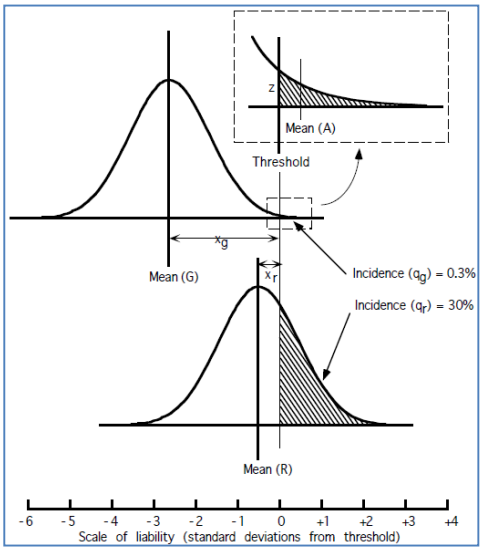
$$b = (x_g - x_r,)/a \quad \textbf{AND} \quad h^2 = b/r$$

  - **Steps:**
    1. From the statistics, find the 2 incidences of the trait:
       - General Population eg. 0.3%
       - nth°degree relatives of affected people eg. 30.0% (**aka: Concordance**)
    2. Using Falconer's Tables, Determine the 'X' values for both of the above:
       - X_g = 2.748

q %	x	a
0·30	2·748	3·050

       - X_r = 0.524

q %	x	a
30·0	0·524	1·159



NB: 'X' values = distance between Threshold & the Mean (in standard deviations)

    3. Using Falconer's Tables, Determine the 'a' values for the Incidence of the General Population
       - 'a' = 3.050

q %	x	a
0·30	2·748	3·050

    4. Using the First Equation, Determine 'b' (the regression coefficient)
       - $b = (X_g - X_r)/a$
         $= (2.748 - 0.524)/3.050$
         $= 0.784$
    5. Determine the 'r' value for the specific *nth°degree relative*:
       - Genetically Identical: Monozygotic Twins → r = 1
       - 1st Degree: Siblings OR Children → r = 0.5
       - 2nd Degree: Grandchildren → r = 0.25
    6. Using the 'b' & 'r' values found, determine heritability:
       - $h^2 = b/r$
         $= 0.784/1 = 0.784 = 78.4\%$ heritability

- **Risk:** (ie. finding the *incidence* of the disease in *nth°degree relatives*...from...)
  - **Need:**
    - Incidence of Disease in General Population — eg. 0.3%
    - Heritability of the disease — eg. 30.0%
    - Falconer's Tables
    - The 'r' values for *nth°degree* relatives. — Eg. r = 1

  - **Steps:**
    1. Using the Figure for Heritability, and the 'r' value for the *nth°degree* relative, find 'b':
       - Eg. $h^2 = b/r$

         $0.784 = b/1$

         $1 \times 0.784 = b = 0.784$
    2. Using the 'b' value, the 'X_g' value, and the 'a' value, calculate the 'X_r' value:
       - $b = (X_g - X_r)/a$

       $0.784 = (2.748 - X_r)/3.050$

       $3.050 \times 0.784 = 2.748 - X_r$

       $X_r = 2.748 - (3.050 \times 0.784)$

       $X_r = 0.466$
    3. Using the 'X_r' Value and Falconer's Tables, determine the incidence (ie. The *risk*):
       - $X_r = 0.466$

         Therefore q% (ie. Incidence) = 32%

         Ie. If you are an identical twin of Someone affected with this disease, You have a 32% risk of getting the Disease.

         NB: the +2% is probably due to rounding errors and is very close to the original 30%

q %	x	a
30·0	0·524	1·159
31·0	0·496	1·138
32·0	0·468	1·118

Questions from the Clinic

7. My identical twin sister has got SLE. What are the odds that I will get it too? (Heritability is 70%, prevalence in the community is 0.3%).

10. What is the heritability of type 1 diabetes? (Risk in first degree relatives 6%, prevalence in community 0.3%).

Genetics of Development & Sex Determination

Human Development:

- Begins at fertilisation
- During embryogenesis, Mitosis is essential for development.
- During Mitosis, the daughter cells are *identical* copies of the parent cell.
- So: *How do the cells in a developing embryo become the many different & specialised cells of the body?*

Genetically Controlled Developmental Processes:

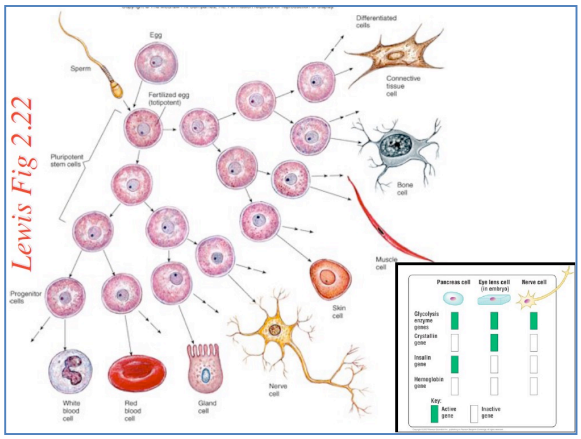
- **Cell Proliferation**
  - Cell reproduction/division
- **Cell Specialisation/Differentiation**
  - The gain/loss of specialised functions/characteristics of a cell.
- **Cell-Cell Interactions**
  - Coordinating the action of cells relative to their neighbours
- **Cell Movements**
  - Rearrangement of cells into structured tissues/organs

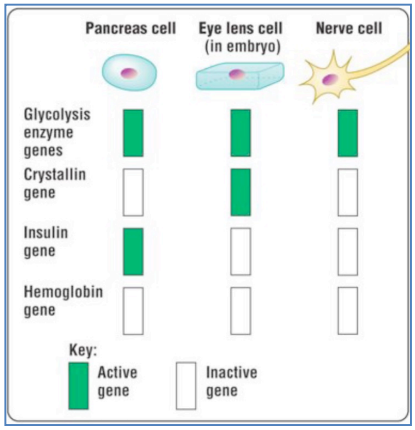
Cell Determination & Differentiation:

- Takes Totipotent cells → turns them into Terminally Differentiated cells with specific functions.
- Require a complicated series of alterations in **gene expression**.

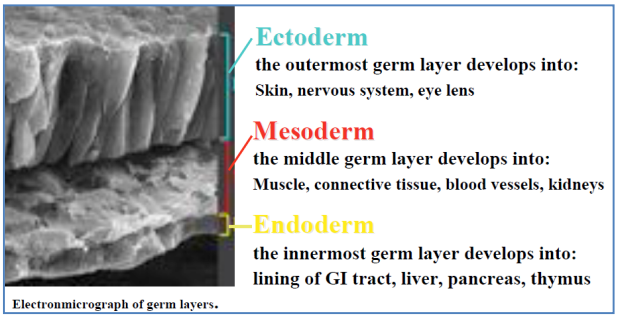
Cell Lineage:

- Plays an important role in **Germ Layer Formation**
- Embryonic stem cells take many different paths of "lineage" that will lead to different specialised cell types.
  - **Eg.Early embryogenesis** – before morulla stage, cells can become any human cell→**totipotent**
  - -at the morulla stage, the cells are determined to become either a primary germ layer or trophoblasts; but have not yet differentiated.
  - **The primary germ layers** are **pluripotent=**determined, but for multiple possible pathways.
- All cells express the genes necessary for compatibility with life.
  - However, different cells express only the genes relevant to their function.

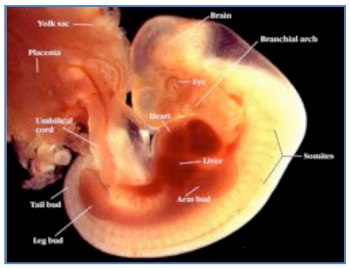




3 Primary Germ Layers

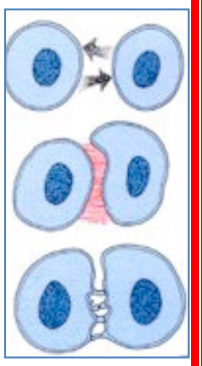


- **HOWEVER:** Lineage isn't the only thing.
  - **And** it doesn't account for differentiation of an embryo at **4 weeks**
  - **By week 4 of development**: all partitions of cells are set aside and are in their final positions.



- **So HOW do the GERM LAYERS (wk 2) transform into the EMBRYO (wk 4)**
  - **Ans –** MORPHOGENS:

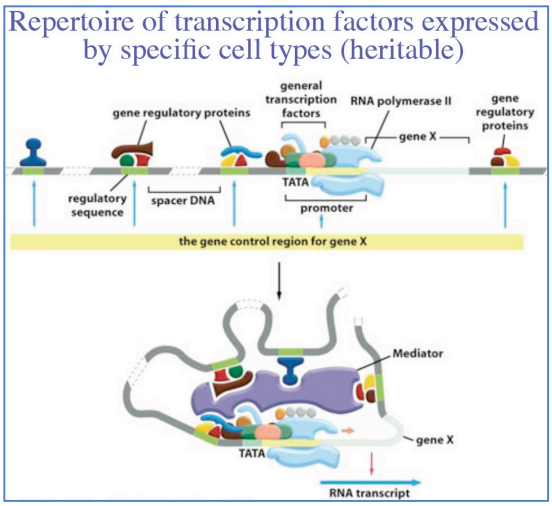
MORPHOGENS – Transmission of 'Spatial Information':

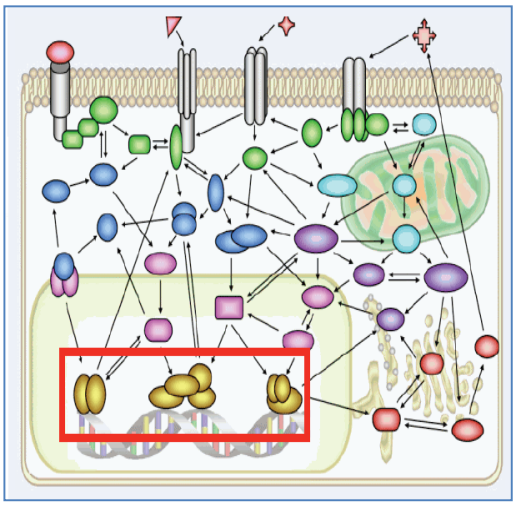


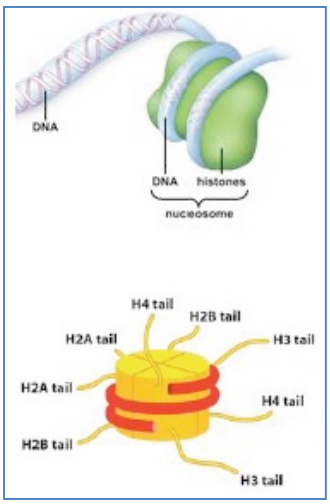
- Substances that influences differentiation & growth of embryonic cells
- **3 Modes of Transmission: -** via -
  - **1. Diffusible Molecules**
    - Ligand secreted by inductor → binds to receptor on reacting cell.
  - **2. Extracellular Matrix Signalling**
    - Ligand travels along the extracellular matrix → binds to reacting cell
  - **3. Direct Contact – Gap Junctions**
    - Ligand travel from inductor cell → reacting cell via Gap Junctions

Chemistry of Differentiation in Cells:

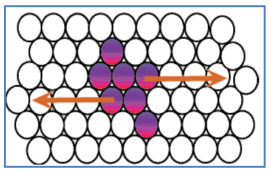
- **Cell Receives a Signal – either:**
  - Positional cues
  - Hormones
  - Cell-Cell interactions (morphogens)
- **Signals result in activation/inactivation of specific TRANSCRIPTION FACTORS:**
  - Results in expression of different subsets of genes by cells.
  - Such genes expressed will be relevant to that cell's function.
- **Once Differentiated, How does a cell (& its daughter cells) remember what it is?**
  - Answer: Modifications of 'tails' on the **histones** 'opens'/'closes' that part of the genome.
  - **During Mitosis** half of the histones in the genome are passed to each daughter cell.
    - Therefore a cell's 'job' is heritable.



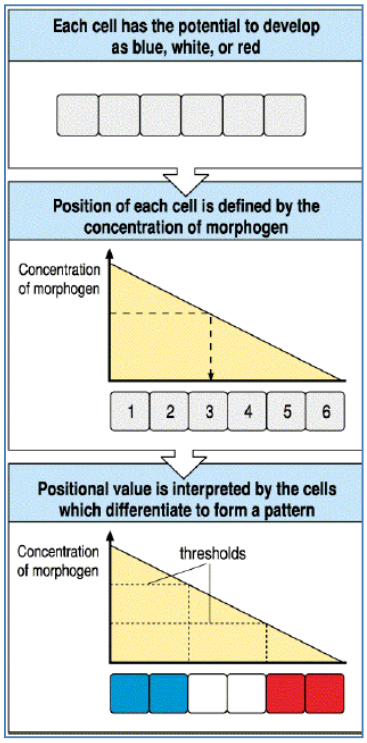




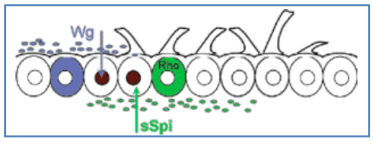
- **Morphogens are Proteins Secreted by Populations of 'ORGANISER' Cells:**
  - 'Organiser' cells act in a Paracrine manner – secrete morphogens into the Extra-Cellular-Matrix.



  - Stem Cells (targets) are sensitive to:
    - Morphogen Concentration
      - Ie. The cell is determined by reaching a certain threshold of morphogen.



    - Morphogen Combinations
      - Ie. Stem cells may receive multiple different morphogens from different 'organisers'
      - This can result in a completely different cell fate.

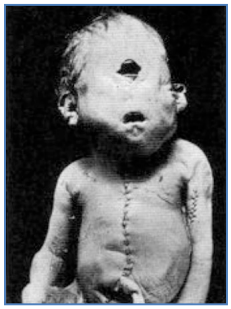


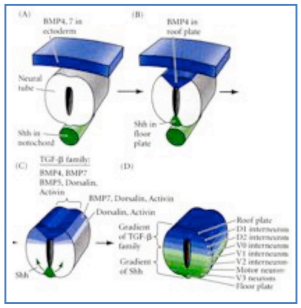
Other Signals Controlling Cell Identity:

- **Cellular Induction:**
  - The prompting of a cell to differentiate into a specific cell type – seen above.
- **Time Restriction:**
  - The time window when a reactant cell is receptive to the inductor's signal. – after which it follows a default/other pathway.
- **Space Restriction:**
  - The Paracrine nature of cellular induction ensures inductor & reactant cells must be very close.
- **Reciprocal Inductions:**
  - When the 'Inductor' is induced to differentiate due to a signal from the reactant cell.
- **Sequential Inductions:**
  - A series of reactions where the Reactant differentiates into an Inductor, and induces other reactant cells – etc. etc.

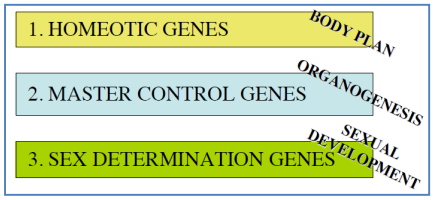
Induction Error – Cyclopia

- Genetic origin
- One cause: mutation in the 'Sonic Hedgehog' gene → encodes the morphogen responsible for the development of 2 cerebral hemispheres.
- Therefore, only 1 cerebral hemisphere develops, along with a single optic lobe.



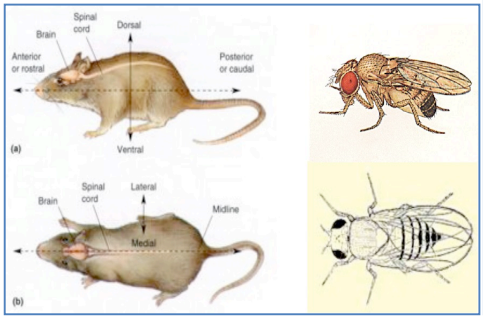


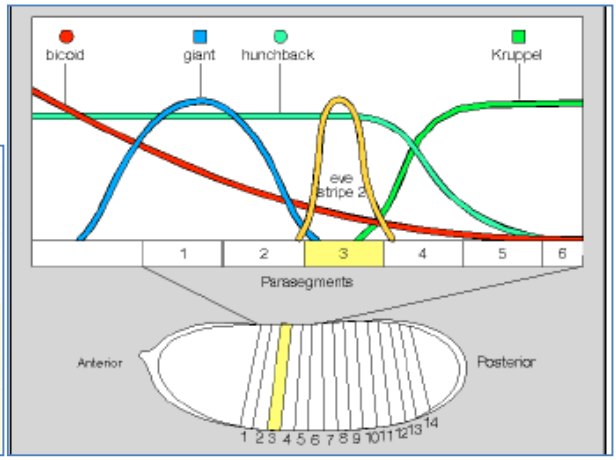
Genetics of Development:

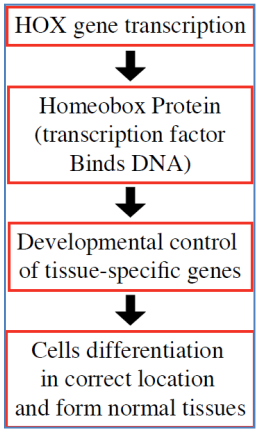


- Body Plan & Master Control Genes are highly conserved across vast evolutionary distances – even prehistoric

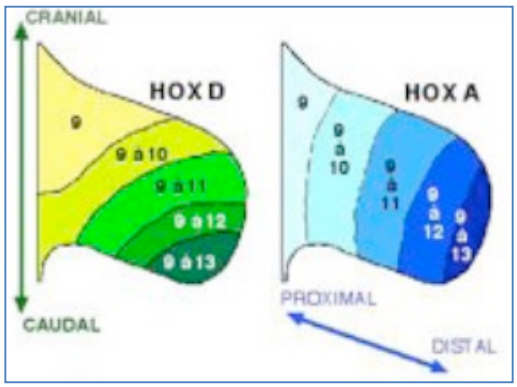
1. **Body Plan Formation – The Homeobox (HOX) Genes:**
   - **'Body Plan'**
     - The fundamental layout for all living things is laid down early in development.
     - Ultimately determined by the many **Homeobox Genes.**
   - **'Homeobox (HOX) Genes'**
     - The genetic component of 'body plan' formation.
     - Mammals have 39 HOX genes
   - **Segmentation:**
     - Early human embryos are hard to distinguish from other vertebrates.
     - The embryo is 'segmented' according to the basic 'body plan'. (similar to fly larva).
     - These 'segments' consist of cell masses – **somites** – which develop into the **Ribs, Vertebrae & Back Muscles.**
     - Specific 'segments' are determined by unique combinations of Homeobox Gene Expression.
       - Ie. Cells exposed to different combinations of Homeobox Proteins differentiate differently.
       - **How:** Hox Gene Transcription→Hox Protein (a transcription factor) Binds DNA → This controls the transcription of Tissue-Specific Genes → Cells Differentiate into Specific Tissues.



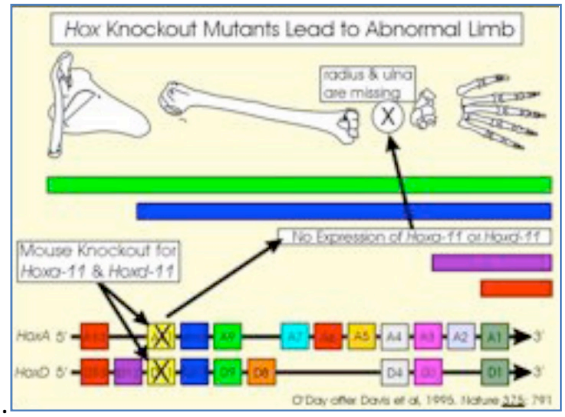




- **Eg. Hox D Expression in Limb Development:**
  - Different Hox Genes begin expression at different times are expressed for different durations.
  - Ie. HoxD9 & D10 are expressed earliest + continue throughout development.
  - However, HoxD13 is expressed later in the development of digits.

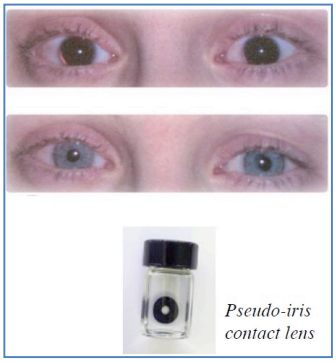


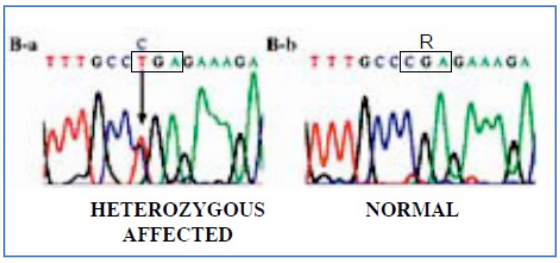
- **Egs. Of Hox D Mal-Expression in Limb Development:**
  - 1. If a 'knockout' (deletion/mutation) occurs in one of the Hox D genes, the part of the limb under its control will fail to develop completely.
  - 2. Slight errors in the Hox D genes responsible for finger development may lead to one of many forms of '*Syndactyly*'.

1. 

2. 

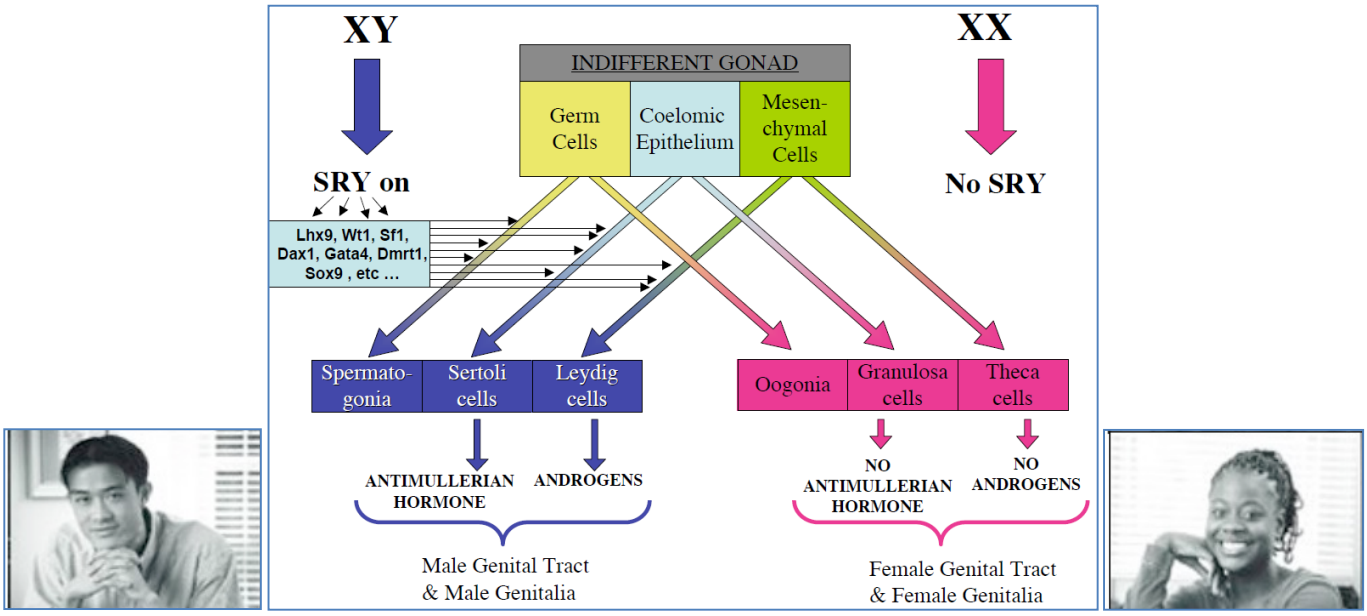
2. **Organogenesis – Master Control Genes:**
    - An organ = many different types of cells organised in a specific fashion for optimum function.
    - Organogenesis is tightly controlled by genetic factors during embryogenesis.
    - These Genetic Factors: **Master Control Genes**
    - **Master Control Genes:**
        - Trigger a cascade of gene expression → directs cells in a coordinated program of **proliferation and differentiation.**
        - **Eg. The Master Control Gene of the Eye:**
            - Pax6
            - The master control gene of ocular and neural tube development across all species.
            - Mutation in this gene → complete loss of iris (Aniridia)
                - In mice, (loss of function) mutations in *both* alleles → no eyes at all!
                - Therefore, in humans, there must be a 50% reduction in function.
                    - AKA - Haploinsufficiency
            - Insertion of this gene into tissue → formation of extra eye tissue
            - Pax6 is expressed in the Foetal Eye during development.



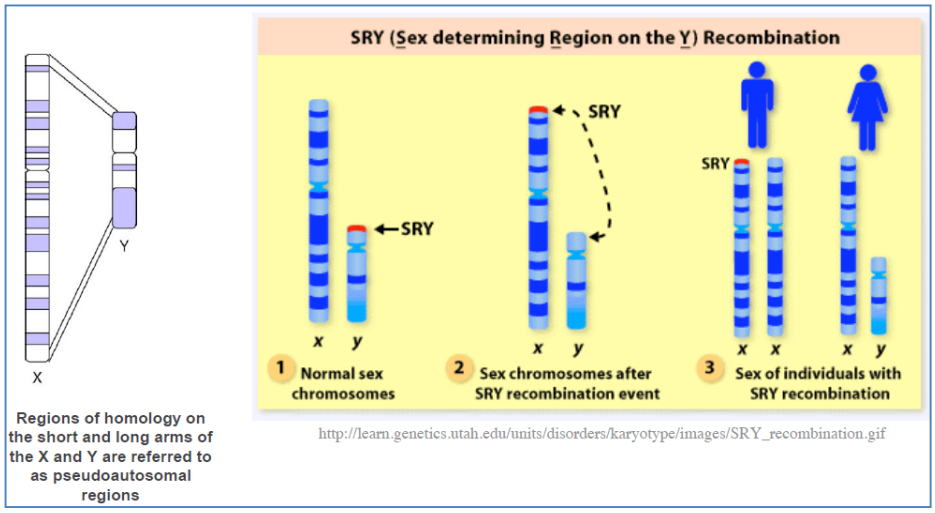


3. **Sex Determination – The SRY Gene:**

- **Gender Genotypes:**
  - Male = XY
  - Female = XX
- However:
  - XXY individuals are Male.
  - And XO individuals are Female
- Therefore:
  - There must be a ***dominant gene*** on the ***Y Chromosome*** that confers the ***Male Phenotype***.
  - This gene is known as the **SRY Gene** and encodes the **'testis determining factor'** (a transcription factor protein)**.**
  - In early development, the presence of the **'testis determining factor'** instructs the gonads to develop into testes. Testes then secrete **Testosterone** → development of male secondary characteristics.
  - Without this transcription factor (testis determining factor) the ***default*** **pathway is female**.

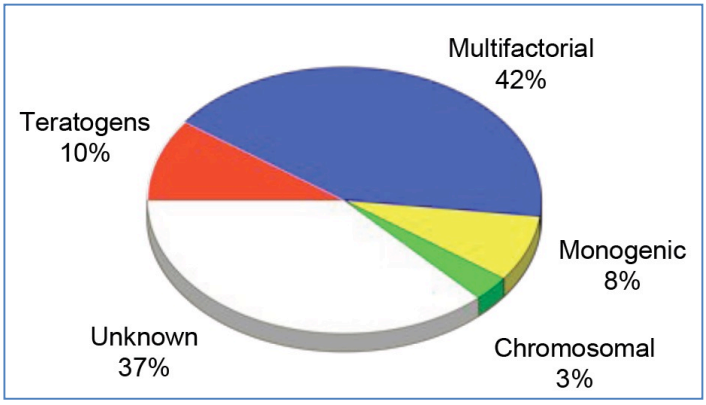


- **Anomalies in Sex Determination:**
  - XY Females & XX Males
  - **How?:**
    - Chromosome rearrangement – where the SRY gene translocates from the Y-Chromosome to the X-Chromosome.



  - **NB:** The SRY gene sits near the top of the Y Chromosome next to the pseudoautosomal region

Causes of Birth Defects:



Teratogens:

- Agents that disrupt normal cellular development during embryogenesis:

Drugs

Thalidomide	**limb defects**
Cocaine	**stroke, nervous system**
Cigarettes	**poor growth, low birth weight**
Alcohol	**fetal alcohol syndrome (FAS)**

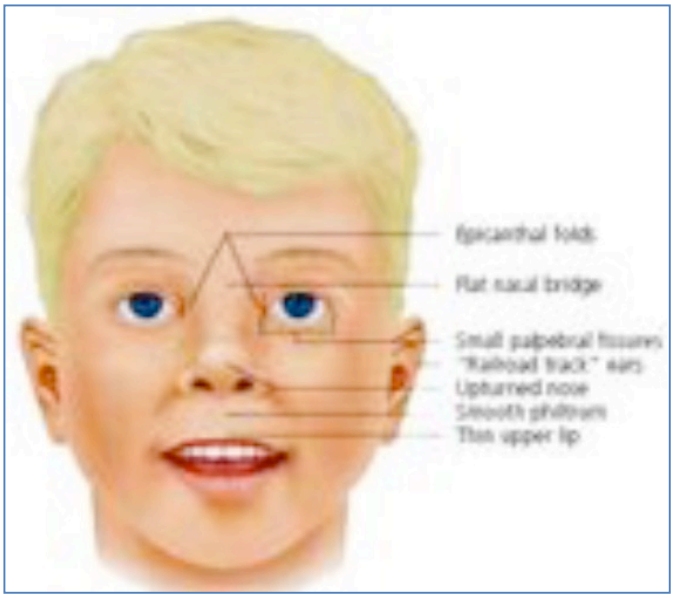
Nutrients

folic acid deficiency	**neural tube defects**
vitamin C excess	**scurvy, immune effects**

Viral infection

rubella	**deafness, cataracts, heart**
herpes	**death, CNS damage, skin lesions**
HIV	**HIV infection, prematurity**

Eg. Foetal Alcohol Syndrome:



Congenital Malformations:

- Anatomical/structural abnormalities
- Result of abnormal embryogenesis
- Due to either genetic / environmental factors

Critical Periods in Development:

- Organs develop at different stages during embryogenesis.
- Different organs are at their most vulnerable at different stages of embryogenesis.
- Organs are most vulnerable to teratogens during organogensis – ie. Elevated cell division.

Population Genetics & Evolution

Darwin's Theory of Evolution:

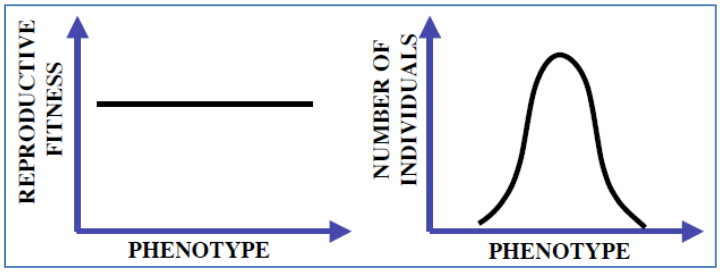
- Observation of species changing in appearance & behaviour over time.
- Process in which **Genetic Variation** in a Population Changes **Over Time**.
- Darwin provided an mechanism as to ***how*** evolution it happens:
  - **'Natural Selection'**

The Theory of Natural Selection & Evolution:

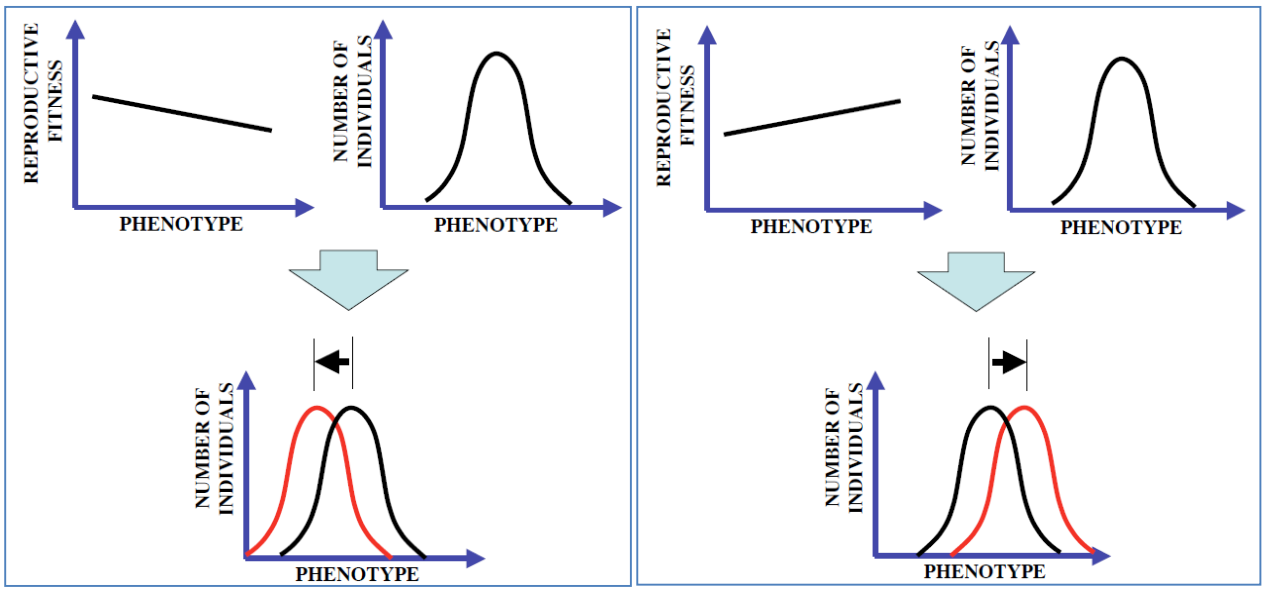
- **Based on 3 Premises:**
  - **I.** **Individuals** in a population **are Unique** (Vary from one another)
  - **II.** The **Variation** between Individuals **is Heritable**.
  - **III.** **Number** of **Offspring** of Individuals **is More Than The Environment Can Support**
    - Ie. Inevitably many will die.
- **The Result:**
  - Only a selection of offspring survive to reproduce
  - Survival ("Reproductive Fitness) depends on inherited traits
    - Those with traits best suited to the environment survive. (have a 'high reproductive fitness')
  - Unequal survival & reproduction → Favourable traits passed to subsequent generations.
  - **Mutations which impair survival** and/or reduce reproductive fitness **reduce in frequency**.
  - **Mutations which increase survival** and/or improve reproductive fitness **increase in frequency**.
  - **Neutral Mutations randomly drift in frequency by chance.**

Evolution By Natural Selection:

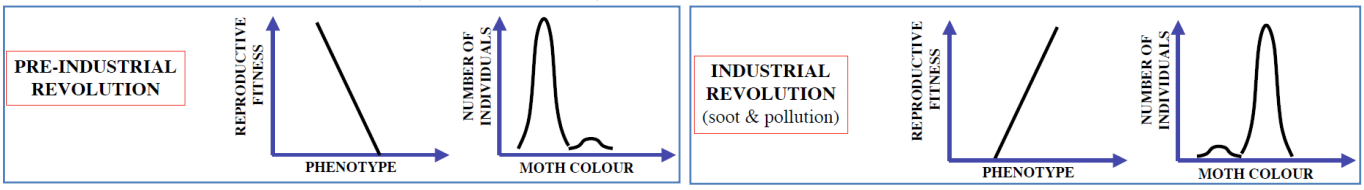
- When All Phenotypes have similar Reproductive Fitnesses, the Phenotype distribution will be static:



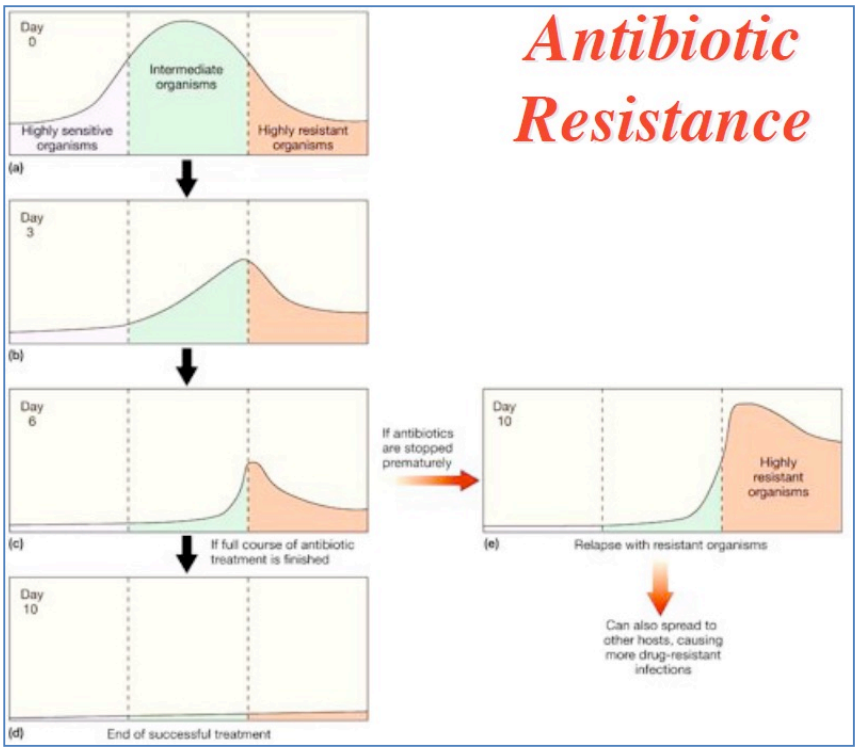
- When the Reproductive Fitness Favours a Specific Phenotype, the Phenotype distribution will shift towards that Phenotype over a consecutive Generations:



- **Eg. Light & Dark Moths:**
  - Pre-Industrial Revolution:
    - Dark Moths more visible against light Lichen-covered Trees – Lower Reproductive Fitness
    - Light Moths prevailed. – Pop. Increased
  - Post-Industrial Revolution:
    - Light Moths more visible against dark sooty-covered Trees – Lower Reproductive Fitness
    - Dark Moths prevailed. – Pop. Increased

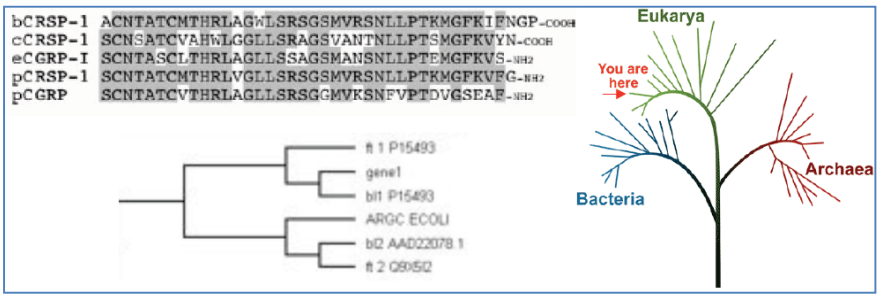


- **Eg. Antibiotic Resistance:**
  - Bacterial infections contain a spectrum of low-resistance & high-resistance.
  - When a person takes antibiotics, the drug selects out the vulnerable bacteria.
  - If the full course of antibiotics isn't taken, the highly resistant bacteria that are left repopulate.
  - Any subsequent antibiotics will be ineffective.



Mapping Evolution:

- Now with DNA analysis, we don't need to look at specific traits
- Instead, we can just look at gene sequences & how they have changed over time/across species.
- Ie. Genes of similar species have similar genotypes.
- Allows us to form "gene-trees":



Population Genetics & Evolution:

- Ie. How **changes in ALLELE FREQUENCIES** vary under the influence of:
  - Systematic Forces (Eg. Natural Selection)
  - Random Forces (Eg. Neutral Mutation)
- NB: Selection occurs at the level of the PHENOTYPE, not the GENOTYPE
- NB: When talking about Allele Frequency, we look at the GENOTYPE and include the incidence of recessive alleles as well as dominant alleles.
- There is a Mathematical Relationship between Allele Frequencies & Genotype Frequencies:
  - AKA: The Hardy Weinberg Principle:

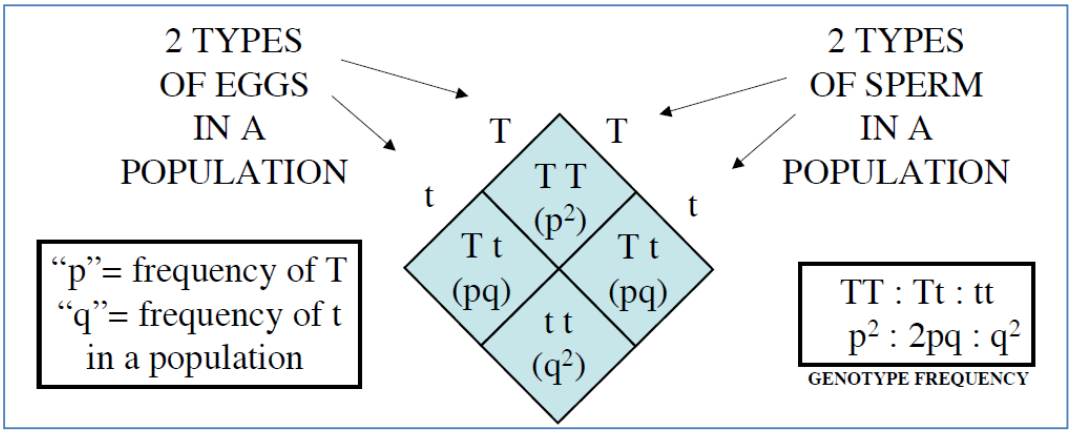
Hardy Weinberg Equilibrium:

- Let 'p' = The Frequency of 'T' in a population. (as a %age)
- Let 'q' = The Frequency of 't' in a population. (as a %age)
  - In the next generation, the GENOTYPE Frequencies Ratio will be:

$$TT : Tt : tt$$
$$p^2 : 2pq : q^2$$

- **For 2 Alleles:**
  - Frequencies (%) of Alleles: $p + q = 1$
  - Therefore: $(p + q)^2 = 1^2 = 1$
  - Therefore: $(p + q)^2 = (p^2 + 2pq + q^2) = 1$

- **For 3 Alleles:**
  - Frequencies (%) of Alleles: $p + q + r = 1$
  - Therefore: $(p + q + r)^2 = 1^2 = 1$
  - Therefore: $(p + q + r)^2 = (p^2 + 2pq + 2pr + 2qr + q^2 + r^2) = 1$



- **5 Conditions:**
  - **1. The population is large**
    (reduces genetic 'drift')
  - **2. Mating is random**
    (no preference for type of 'mate')
  - **3. No mutation of alleles**
    (no spontaneous mutations)
  - **4. No migration occurs**
    (Immigration/Emmigration)
  - **5. No selection**
    (all genotypes have same reproductive fitness)

- **Hardy Weinberg Equilibrium:**
  - Allele Frequencies created at Fertilisation will be preserved in the next generation.
  - Allele Frequencies will remain unaltered, generation after generation.
  - **Useful for: estimating** the **Genotype Frequencies from Allele Frequencies** in different populations.

- **Eg. HWE Demonstration:**

Consider the following genotype frequencies at the M-N blood type locus:

BLOOD TYPE	GENOTYPE	NUMBER OF PEOPLE
M	L^ML^M	1787
MN	L^ML^N	3039
N	L^NL^N	1303

Q: If the assumptions of the Hardy Weinberg Equation are met by this population, what will be the Genotype Frequencies of the Next Generation? (If population size remains static)

1. • Total number of alleles is 2x (1787+3039+1303) = 12258
2. • Total number of L^M alleles is 2x (1787) + 3039 = 6613
   • Frequency of the L^M allele is 6613/12258 = 0.5395
3. • Total number of L^N alleles is 2x (1303) + 3039 = 5645
   • Frequency of the L^N allele is 5645/12258 = 0.4605
4. • Because L^N and L^M are the only two alleles of this gene, Frequency of the L^M + frequency of the L^N = 1

   (0.5395) + (0.4605) = 1

   p + q = 1
5. the genotypic ratios in the next generation :

   $L^ML^M : L^ML^N : L^NL^N$

   $(p^2 : 2pq : q^2)$

A: Genotype Frequencies:

6. $(0.5395)^2 : 2(0.5395 \times 0.4605) : (0.4605)^2$

   0.2911 : 0.4968 : 0.2121
7. $L^M L^M$: 0.2911 x Population Size = 0.2911 x 6129 = 1784.2

   $L^M L^N$: 0.4968 x Population Size = 0.4968 x 6129 = 3044.8

   $L^N L^N$: 0.2121 x Population Size = 0.2121 x 6129 = 1300.0
8.

BLOOD TYPE	GENOTYPE	NUMBER OF PEOPLE	EXPECTED
M	L^ML^M	1787	1784.2
MN	L^ML^N	3039	3044.8
N	L^NL^N	1303	1300.0

-Results are very close to initial population: Pop. Is in Hardy Weinberg Equilibrium.

- Examples of Populations comprised of 10,000 individuals that are in H-W equilibrium:

(Freq A=p; Freq a=q)	AA	:	Aa	:	aa
1. p=0.9, q=0.1	8100	:	1800	:	100
2. p=0.8, q=0.2	6400	:	3200	:	400
3. p=0.6, q=0.4	3600	:	4800	:	1600
4. p= 0.5, q=0.5	2500	:	5000	:	2500

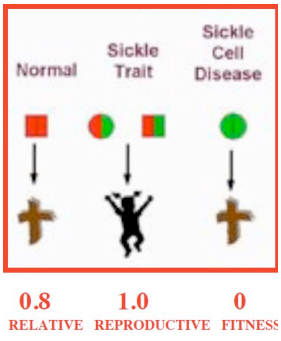
- **Eg.2.**

  - Consider the metabolic disorder PKU.
  - Approximately 1/10,000 people are affected
  - Homozygous affected individuals occur at a frequency of 0.0001
  - Call the frequency of all disease alleles in the population = q,
    then $q^2 = 0.0001$,
    therefore $q = 0.01$
    and as $p + q = 1$, $p = 0.99$
  - So we would expect that the frequency of heterozygous carriers of a defective PKU allele to be:
    (freq "Pp" genotype) = $2pq = 2 \times 0.01 \times 0.99 = 0.0198$
    or approximately 1 in 50

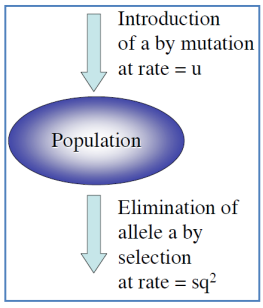
- **<u>Exceptions to Hardy Weinberg Equilibrium:</u>**
  - **<u>Non-Random Mating:</u>**
    - **Assortive Mating:**
      - Couples usually don't know the genotype of their mate.
      - However, some phenotypic traits are often a major influence when choosing a mate.
        - (eg. Hair colour, skin colour, intelligence)
    - **Consanguineous Mating:**
      - Marriage between related individuals
      - Ie. Narrows the 'gene pool'.

  - **<u>Unequal Survival:</u>**
    - Different Phenotypes have different survival rates.
    - Ie. Natural Selection
    - **By either: Selective Advantage/Selective Disadvantage**

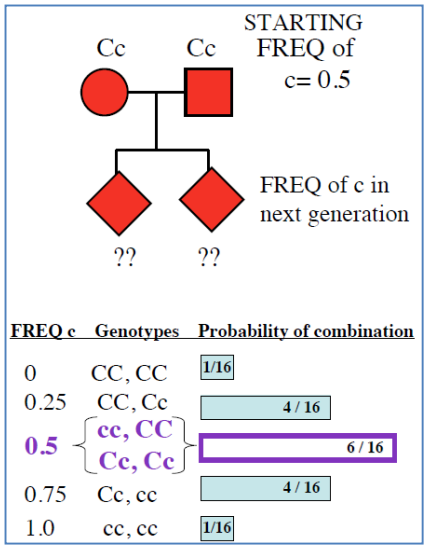
    - **Eg. Heterozygote Advantage: Sickle Cell Allele & Malaria Resistance:**
      - If Homozygous – Sickle Cell disease causes Selective Disadvantage.
      - If Normal – Susceptible to Malaria → Mild Selective Disadvantage
      - If Heterozygous – Resistant to Malaria → Mild Selective Advantage



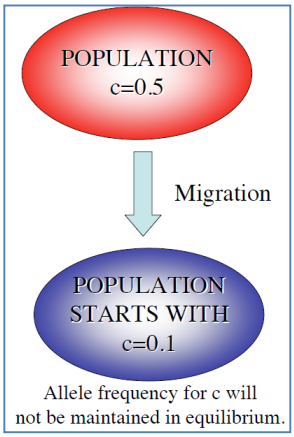
    - **Mutation-Selection Balance:**
      - Sometimes Deleterious Alleles are maintained simply by Dynamic Equilibrium.
      - Ie. The allele is being lost at the same rate that it is being maintained via spontaneous mutation.



- **Population Subdivision:**
  - **PANMICTIC Populations:** Where any member is able to mate with any other.
  - **HOWEVER –** most populations aren't PANMICTIC – in reality, they're subdivided by:
    - Geographical Factors
    - Ecological Factors
    - Economic Factors
    - Cultural Barriers
    - Age Barriers
    - Other Social Barriers
  - **Founder Effect:**
    - When a small sub-population of people become isolated
    - Can produce a special type of genetic drift: The Founder Effect
    - Where genetic diversity is much reduced compared to original population.
  - **Random Genetic Drift:**
    - When Population sizes get small, the random nature of Mendelian segregation leads to random drifting of population allele frequencies.
    - **Eg.** Cosider 2 'Cc' individuals that produce 2 children:
      - The pop. Frequency of 'c' = ½
      - Although the chance of the 2 offspring maintaining the allele frequency has the highest probability, the combined probability that it will be altered, is much higher.
      - Hence, 'drift' occurs:



- **Migration:**
  - Movement of people = movement of genes.
  - If the allele frequencies differ between locations, H-W. Eq. will be disrupted.
  - However, if migration stops, H-W. Eq. will be restored within One Generation.



Tools of Molecular Biology

Manipulation of DNA:

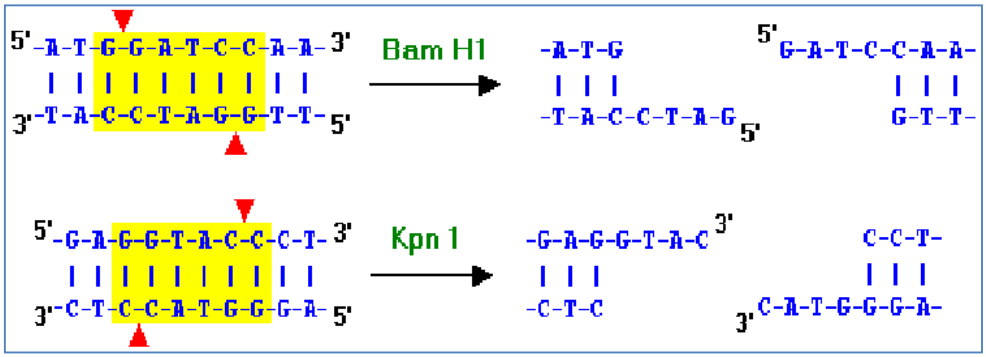
Cutting

- **Endonucleases/Restriction Nucleases/Restriction Enzymes**
  - Enzymes that cleave the sugar-phosphate backbones of double-stranded DNA.
    - **Found in bacteria** – defends it from viruses by chopping up the foreign DNA.
  - Cut within a stretch of **4, 5, 6 & 8** base-pairs.
  - These stretches of DNA are called **Recognition Sequences/sites.**
    - The length of a recognition sequence determines its scarcity. (a 6 base pair recognition sequence will occur once every 4^6 bases simply by probability.)
    - Such endonucleases are therefore rarer than 4base pair endonucleases
    - **Isoschizomer** are endonucleases have the same recognition site as each other.
  - Produce **sticky ends** (overhangs) that are **either 5' or 3'**
  - OR **blunt ends** (no overhang).

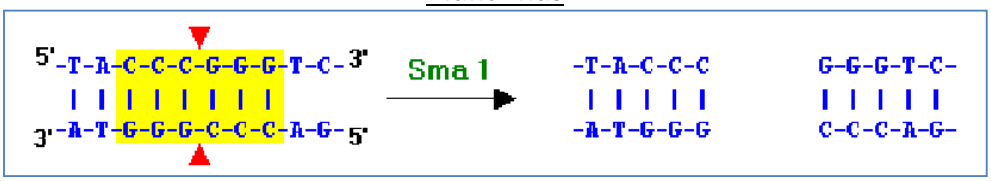
Recognition Sequences

Enzyme	Recognition Sequence
BamH I	GGATCC CCTAGG
Not I	GCGGCCGC CGCCGGCG
Sau3A I	GATC CTAG
Sac I	GAGCTC CTCGAG
Sst I	GAGCTC CTCGAG
Hinf I	GANTC CTNAG
Xho II	PuGATCPy PyCTAGPu

Sticky Ends



Blunt Ends



- **Exonucleases**
  - Enzymes that cleave nucleotides **one at a time from a terminus of a polynucleotide chain**
  - I.e. can remove overhanging nucleotides from sticky ends produced by endonucleases.
  - Produce **blunt ends**

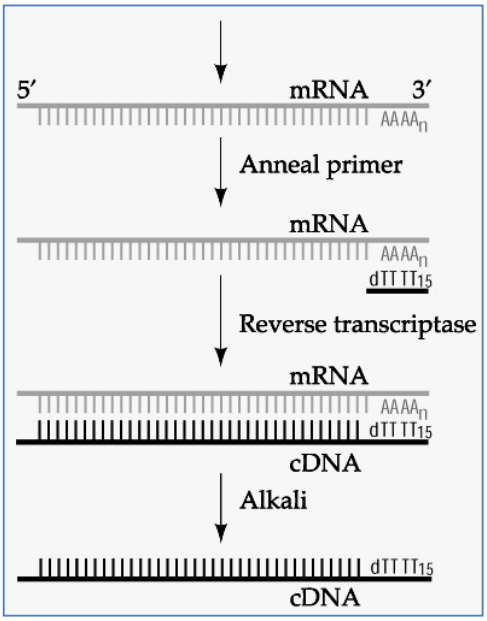
Rejoining (ligating)

- **DNA Ligase**
  - Enzymes seen in DNA synthesis – **reseal the gaps in DNA backbone** between okazaki fragments.
  - Can be used to insert fragments of DNA into host DNA to form **recombinant DNA** molecules.
  - **Seals** the gap between **annealed compatible sticky-end overhangs** OR **blunt-ends**. *(**Uses ATP**)*
  - A host cell containing recombinant DNA molecules will obliviously replicate & transcribe the DNA.
  - This can be exploited when cloning DNA for protein synthesis.

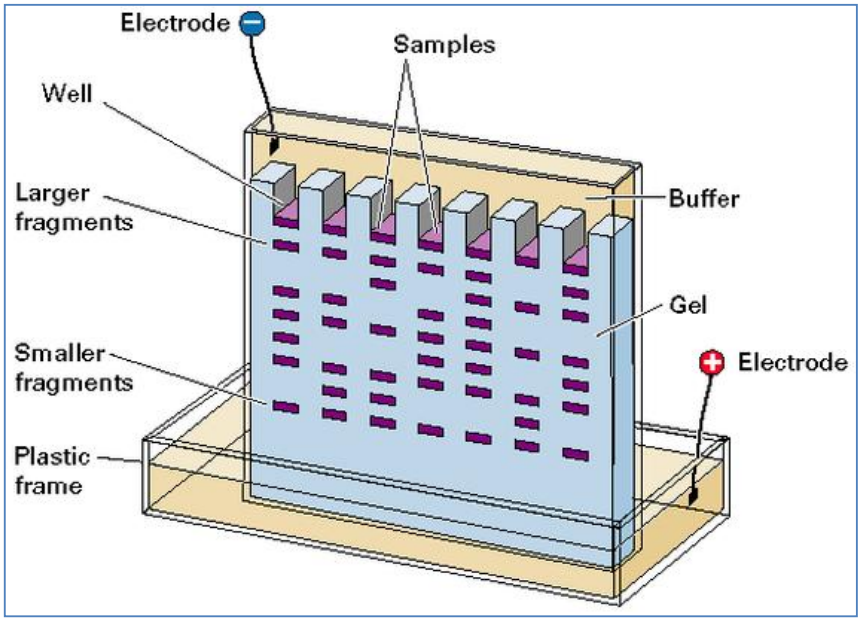
Extraction & Separation

- **Extraction of mRNA for Plasmid Cloning or PCR.**
  - Lyse eukaryotic cells that are responsible for producing desired protein (Contain desired gene)
  - Solution will contain DNA, RNA, mRNA, Proteins & membrane
  - Centrifuge to get rid of membrane & other insolubles.
  - Add Phenol – dissolves proteins. Then decant phenol-protein solution.
  - Add DNAse to break down any DNA.
  - Isolate mRNA from RNA by adding metal 'poly-T-beads'. Poly-A tail of mRNA sticks to beads.
  - Magnetise beads and wash away residual RNA.
  - Add hot water to break H-bonding between poly-T-beads and poly-A-tails, and pour off solution
  - Solution now contains the mRNA coding sequence for a protein. (without introns or junk DNA)
- **Convert mRNA gene to cDNA (complimentary-DNA)**
  - **Convert to DNA (cDNA)** using **reverse transcriptase –** creates an mRNA/cDNA hybrid molecule.
  - Then add **RNAse,** which chews up the mRNA strand, leaving a single cDNA strand
  - Then add **DNA Polymerase**¸ which synthesises the 2ns strand of cDNA.
  - Clone using plasmids, or PCR
- **Extraction of DNA for Sequencing**
  - Aqueous extraction of DNA, RNA & Proteins from lysed cells + buffer & EDTA
  - Centrifuge to get rid of membrane & other insolubles.
  - Add Phenol – dissolves proteins. Then decant phenol-protein solution.
  - Add RNAse to break down any RNA
  - EtOH causes DNA to precipitate → then centrifuge.
- **Gel Electrophoresis**
  - After a DNA molecule has been cleaved using a restriction nuclease, the fragments may need to be separated.
  - Separates DNA fragments of different lengths by applying a voltage across the gel.
  - In the gel, shorter molecules move faster than longer ones.
  - DNA is negatively charged → migrate towards positive electrode.
  - The samples are labelled with **ethidium bromide,** a UV-florescent die so bands are visible.
  - The DNA bands can then be transferred onto a piece of nitrocellulose, for safekeeping.

Convert mRNA gene to cDNA



Gel Electrophoresis



DNA Sequencing

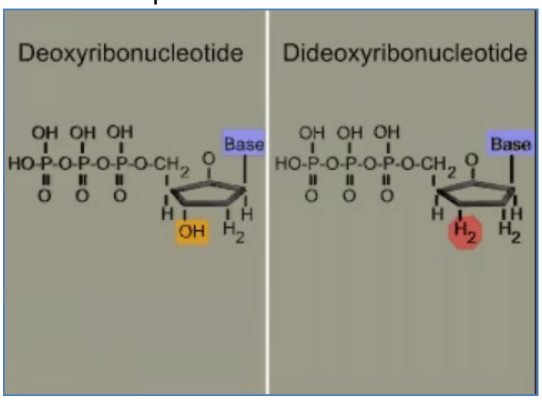
- **What can it tell us?:**
  - Location & length of genes
  - Identify specific gene sequences
  - Start & stop codons
  - Protein binding domains
  - Splicing domains
  - Predict amino acid sequence
  - Protein structure & function
- **Methods**
  - **Pyrosequencing**
    - Coupling each DNA synthesis reaction to a reaction that produces ATP. Presence of ATP causes an enzyme (luciferase) to give off a burst of light which is then read by a computer.
    - This involves alternating the addition & degradation of each of the 4 nucleotides for each reaction until a burst of light is detected.

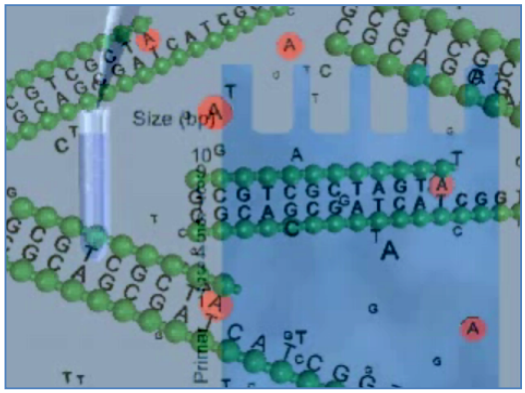
$DNA + d(A/T/G/C)TP \rightarrow DNA_{+1} + PP_i$

$PP_i + AMP + PEP \rightarrow Pyruvate + ATP + P_i$

ATP+Luciferase = *light*

  - **Using DiDeoxyRiboNucleotides**
    - Single stranded DNA used as a template for synthesis.
    - DNA synthesis is started by adding a primer
    - The DNA is mixed with free dNTPs (deoxyribonucleoside triphosphates) which become the complimentary strand.
    - ***However***, it is also mixed with traces of fluorescently labelled ddNTPs (dideoxyribonucleoside triphosphates) – [don't have the 3' hydroxyl group]
    - Therefore, once a ddNTP has been incorportated into the cDNA strand, DNA synthesis stops.
    - This occurs randomly, with different ddNTPs being incorporated in different places, resulting in millions of terminated cDNA strands of different lengths.
    - These strands are separated by size by electrophoresis, read by a laser, and sequenced by a computer.

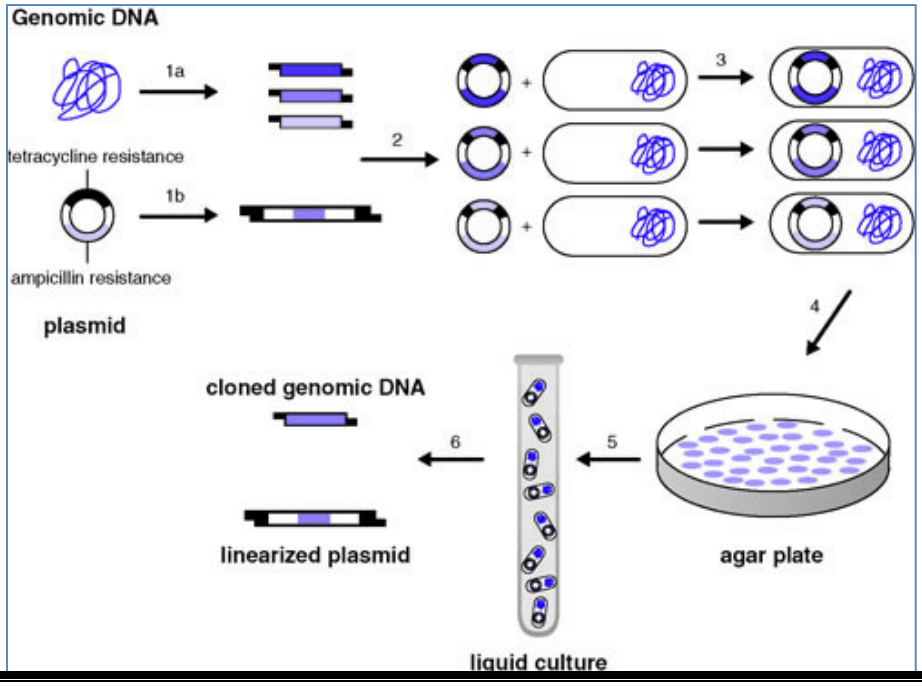




DNA Amplification/Cloning

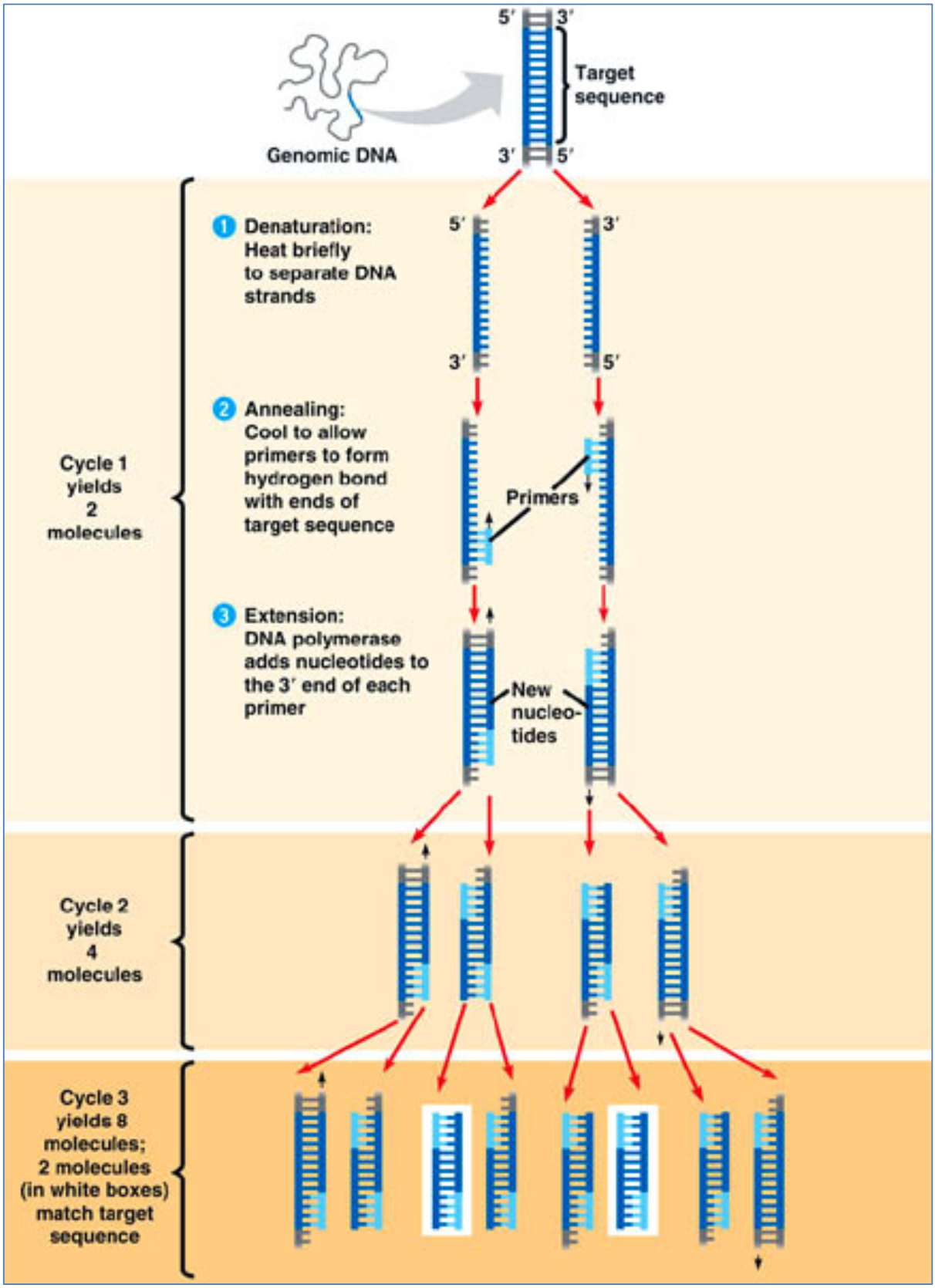
Plasmid/Vector cloning

- **Transformation**
  - Some bacteria can take up small, circular DNA molecules (plasmids) through 'transformation'.
  - Plasmids are only a few thousand nucleotides long.
  - **5 characteristics of plasmids:**
    - Has an ORI (origin of Replication)
    - Has a selectable marker (often an antibiotic resistance gene)
    - Has a polylinker/multiple cloning site (MCS)
    - Must be transformable
    - Must be isolatable from chromosomal DNA
- **Insertion**
  - A strand of DNA (gene) can be inserted into a laboratory plasmid.
  - First, the plasmid & the gene are 'cut' with the same endonucleases.
  - This forms compatible sticky ends and both the DNA & plasmid can **hybridize** via H-bonding.
  - DNA ligase then reseals the backbone, forming a recombinant plasmid.
  - The recombinant plasmid is then mixed with bacteria & is taken up into the bacteria.
- **Replication**
  - The bacteria are put on an agar plate containing antibiotics.
  - Those that don't contain the antibiotic resistance gene (plasmid) will die, leaving those that do.
  - These plasmids are replicated autonomously & are distributed to each daughter cell in mitosis.
  - Therefore, recombinant DNA can be easily copied inside rapidly dividing bacteria.
- **Extraction & Purification – To check for mutations in replicated genomic DNA**
  - Some bacteria are then lysed and plasmid DNA is extracted from the rest of the cell contents.
  - The inserted DNA fragments are removed from the plasmids using restriction enzymes.
  - The mixture of fragments and plasmid DNA is separated by size via gel electrophoresis.
  - This leaves the purified genomic DNA to be screened for mutations.
- **Protein Synthesis:**
  - If there are no mutations in the genomic DNA (eg. The gene that translates insulin), the colony of cells where the plasma DNA was extracted will be scaled up and used for commercial protein (insulin) manufacture.



Polymerase Chain Reaction

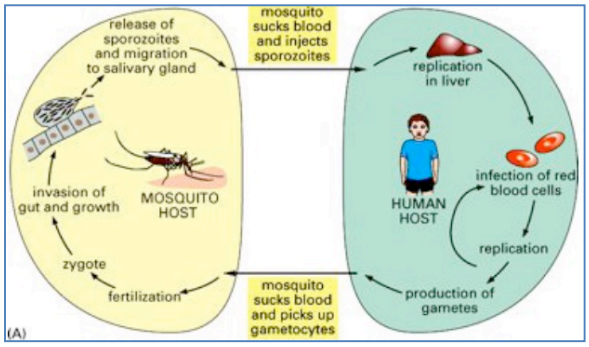
- **Things Required for PCR:**
  - Small template of DNA containing target sequence.
  - 2 specific oligonucleotide primers for each 3' end of the 2 target strand sequences.
  - Taq-Polymerase: A heat-stable DNA polymerase
  - dNTPs : Deoxynucleotide triphosphates.
  - A solution of buffer containing $MgCl^2$.
- **Pre-Steps:**
  - Extract mRNA gene from cells.
  - Convert mRNA gene to cDNA (complimentary-DNA)
- **Steps:**
  - **Denaturation**
    - 2 strands of template DNA are separated at 94°C
    - During this stage, the taq-polymerase is added.
    - Taq-polymerase isn't denatured as it is thermostable.
  - **Annealing of a primer**
    - Solution is cooled to 40-65°C and the primers bind to the 3' ends of the 2 DNA strands.
  - **Extension via heat-resistant DNA Polymerase**
    - Solution temp is increased to 74°C, and the taq-polymerase adds dNTPs from the primers in a 5' → 3' direction.
  - Repeating these steps for 20-30 cycles yields about 1 billion template strands, which will be visible on an agarose gel.
- **Extraction of Target Sequence Strands.**
  - The target strands are separated from the remaining longer-strands via gel-electrophoresis.
  - **What for?**
    - Insertion into bacteria → protein synthesis
    - Pathology screening – identifying species & even strains of pathogens
    - Forensic & Paternity DNA analysis & testing.



Future Genetic Therapies & Technologies

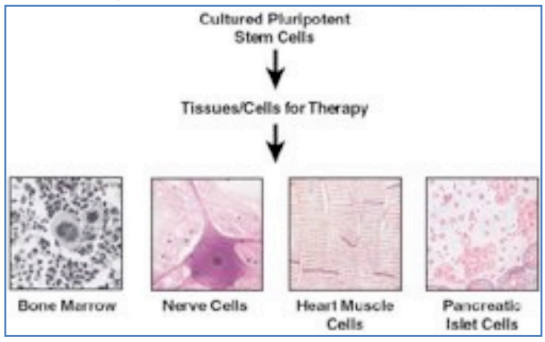
Knowing The Enemy:

- In the past: Immunisation was the method of disease control.
- In the future, Analysis of Pathogen (& Host) Genomes will lead to:
  - New Insecticides (Vector Control) – eg. Making the vector incapable of carrying the parasite/germ.
  - New Drug Therapies: ie. Drugs that target metabolic enzymes of that Pathogen → dies
  - New Vaccines

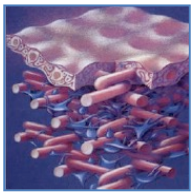


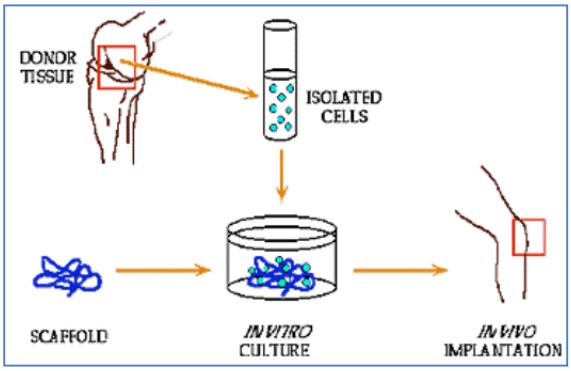
Replacement Parts:

- Made-to-order body parts
- Made of your own cells → genetically identical to you →No rejection
- Organ Donation will become Archaic
- **Stem Cells:** (from embryos/cord blood/adult stem cells)



- **Tissue Engineering:**
  - Biomaterial Scaffolding:
    - Convey signals to surrounding tissues to promote regeneration.
  - Cells Alone:
    - Without a biomaterial → form tissues
  - Cells Grown on a Biomaterial Scaffold:
    - Scaffold acts as a framework for developing tissues.

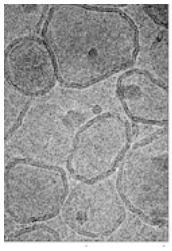


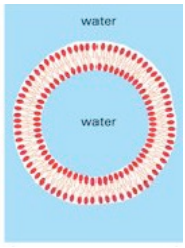


Gene Therapy:

- Ways of Correcting Gene Defects
- Recessive disorders (eg. Haemophilia / Cystic Fibrosis) are good candidates.
- Dominant disorders are more difficult to treat with gene therapy – because you need to *remove* alleles.
- **Germline Gene Therapy:**
    - Corrections/extra genes added to germ cells
    - Currently ethically unacceptable.

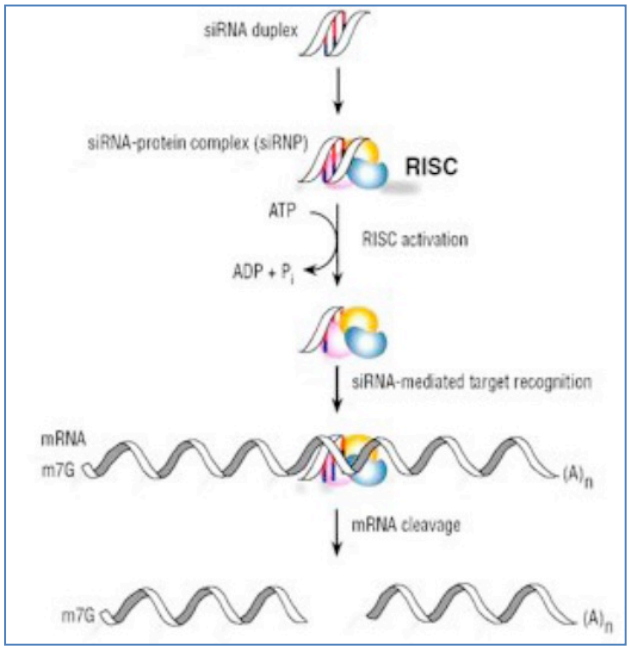
- **Somatic Gene Therapy:**
    - Involves inserting a 'normal' gene into cells → cells produce the missing gene-product
    - → alleviate/eliminate symptoms
    - **How:**
        - **Mechanical/Chemical:**
            - **Liposomes –** (soap bubbles)
                - Small manufactured vesicles containing the gene to be expressed
                - Cells fuse with them, and their contents (genes) are released
                - Some of this DNA may be integrated into a chromosome and expressed



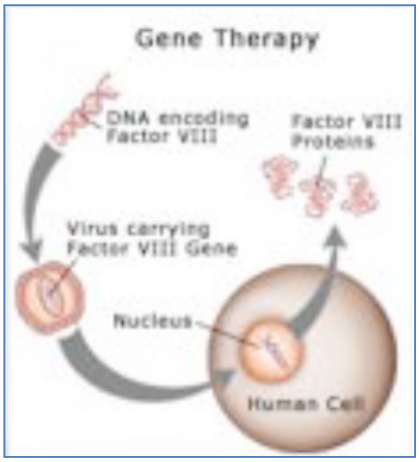


- **Microprojectiles:**
    - Tiny inert metal spheres coated in DNA (the needed genes) are 'shot' into living tissues.
    - Some of this DNA may be integrated into a chromosome and expressed

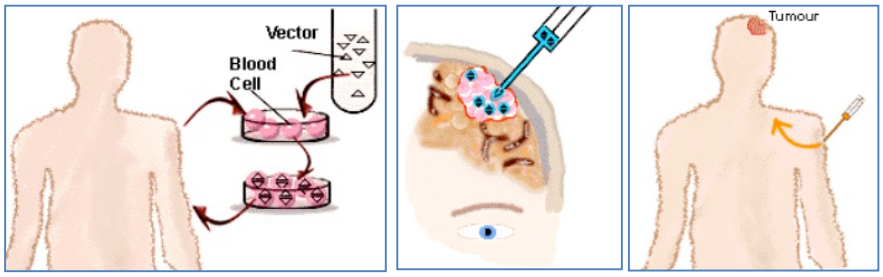
- **siRNA (Small Interfering RNA):**
    - Good for Dominant Traits or any disease associated with 'gain of function' mutations.
    - – are small 21-23bp RNA molecules that bind to any synonymous mRNA strand, and cleaves it in two. (similar concept to endonucleases)
    - Cleavage of 'mutated' mRNA → less synthesis of mutated proteins.



- **Viral Vectors:**
  - The desired gene is inserted into a virus's genome
  - The host is then infected with the virus
  - The virus's DNA enters the nucleus → produces the desired proteins.



- **Administration Methods:**
  - **Ex Vivo:** Cells a removed, cultured in the lab with a Vector, then reinserted.
  - **In Situ:** Affected Tissue is Directly exposed to the Vector.
  - **In Vivo:**Vector is introduced generally & the Vector hones in on affected cells

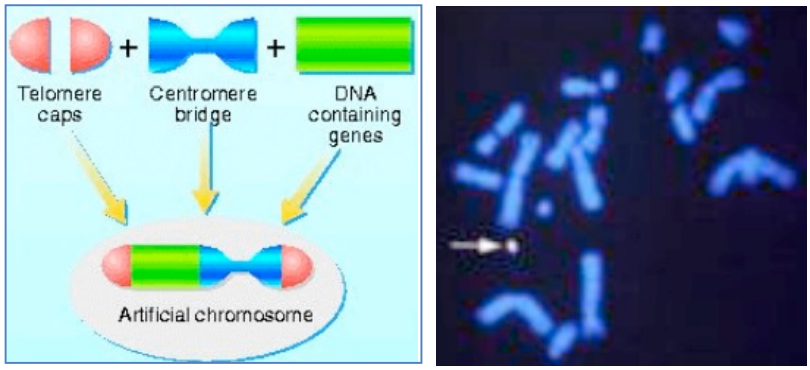


Ex Vivo In Situ In Vivo

- **Problems with Viral Vectors:**
  - Repeat administration builds up an immune response
  - Infection of the virus itself must be contained
  - Viral insertion into Oncogenes (tumour suppressor genes) can cause oncogenesis (cancer)

Human Artificial Chromosomes:

- Replacement Chromosomes are being developed to treat specific genetic conditions
- Will be stably passed down during cell division
- Very promising off-the-shelf gene therapy.
- The biggest problem at the moment is introduction into cells. (but it will come!)



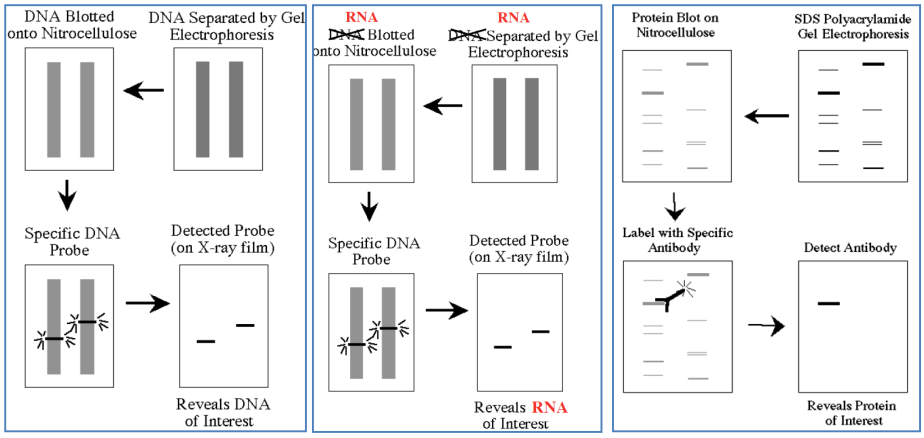
Prevention Better Than Cure:

- All Diseases have a genetic component
- Such 'predisposition' alleles for almost any trait will be identified
- The effectiveness of some treatments is influenced by genetic factors also.
- Therefore, in order to *prevent* and/or *treat* a disease effectively, the Patient's Genome must be known.
- Therefore, soon it will be commonplace for everyone to know their Genome Sequence.

- **Screening For Predisposition Alleles:**
  - –As opposed to sequencing someone's *entire* genome –(not cost effective yet)

How:

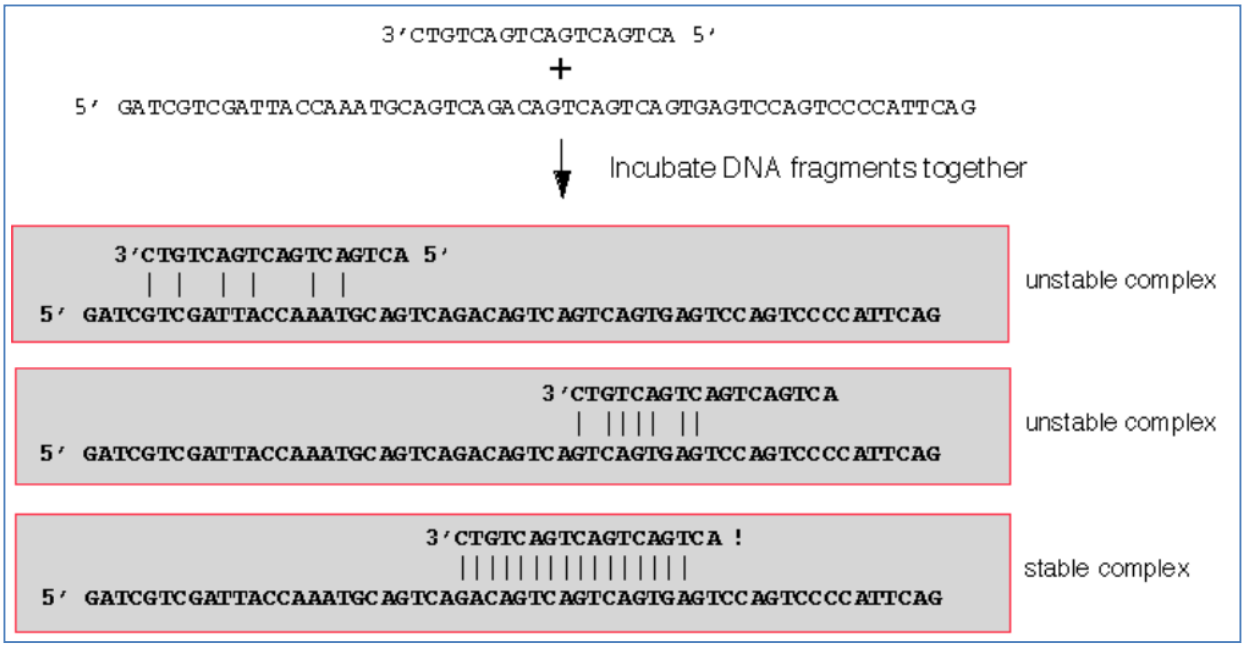
- ***The Old-Fashioned Way = 'Blotting':***
  - Southern Blotting (DNA) – Looking for a single known *DNA sequences* in a genome.
  - Northern Blotting (mRNA) – Looking for specific *gene expressions* of DNA sequences.
  - Western Blotting (Protein) – Looking for



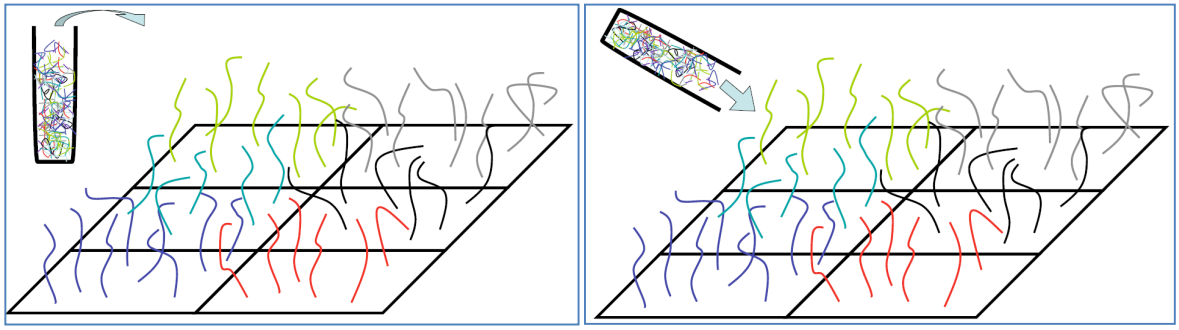
Southern Blotting Northern Blotting Western Blotting

- ***The New Way = DNA MicroArray Technologies:***
  - Slides with its surface covered with thousands of pieces of immobilised DNA
  - DNA pieces laid out in rows & columns
  - (typically glass/nylon/silicon)

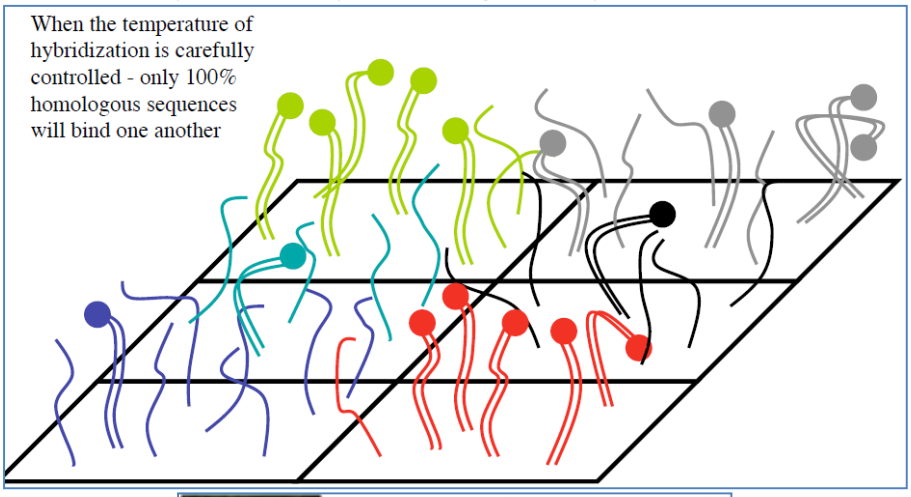
  - **How: *DNA Hybridisation*:**
    - The fundamental mechanism in which 2 *complementary* single strands of DNA anneal to each other (under the right thermal conditions).

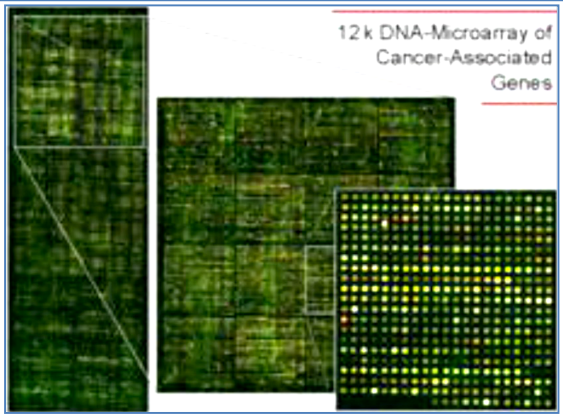


- **So *What Happens* on a Microarray?:**
  - Each square of an array contains known sequences of DNA bound to the surface.
  - These may correspond to:
    - Known Genes that are on human chromosomes
    - mRNA transcripts known to be produced in human cells.
    - Known (disease-causing) *mutations* in genes on human chromosomes
  - Then a mashed mixture of a person's cells (of a specific tissue) is purified for DNA.
  - This mixture of DNA is fluorescently marked,
  - Then it is washed across the surface of the array at a temperature ideal for 100% homologous sequence pairing.

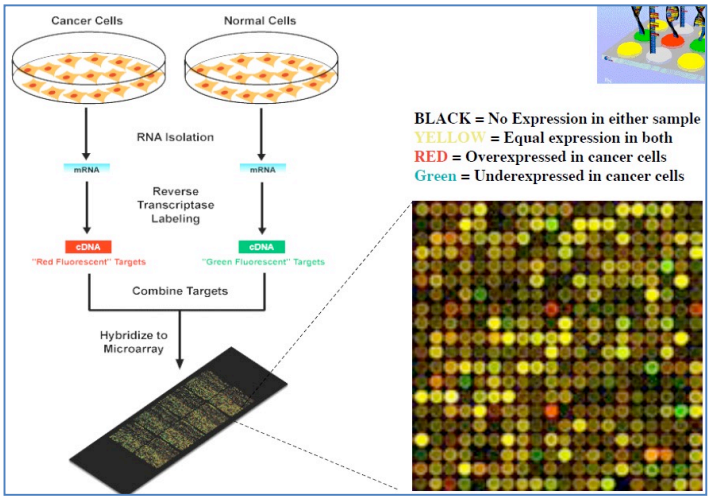


  - Finally, the end result is scanned for the fluorescent markers on each square
  - The presence of the marker on a specific square indicates the presence of that sequence in the patient's original sample.

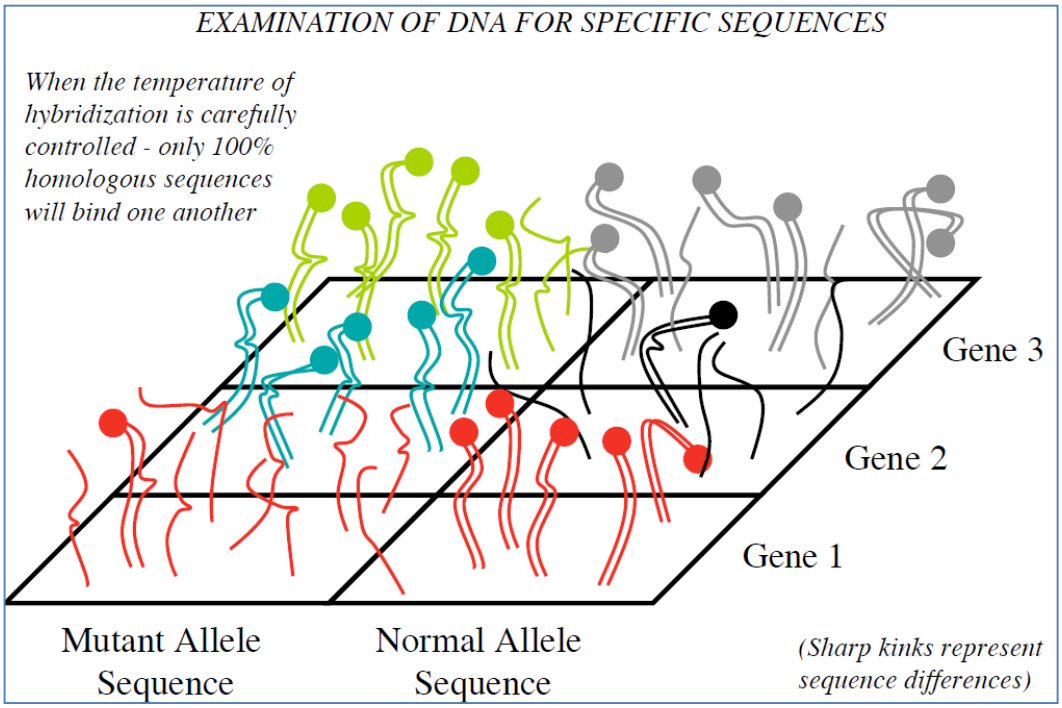




- **Applications:**
  - **Expression Profiling:**
    - Analysing which *known* Genes are being *expressed* in particular tissues:
      - *mRNA* extracted from a patient's tissue of interest.
      - *mRNA* is converted to cDNA & then fluorescently labelled.
      - The Labelled cDNA is washed across the array (Containing the normal expression profile of that tissue type)
      - Any abnormalities in expression will be detected.
    - Analysing the difference in expression between Cancerous & Normal Cells:



  - **Mutation Profiling:**
    - See if there are any *known* disease-causing-*Mutations* in particular tissues.
      - The Array contains synthetic strands of *normal genes* and *common mutations of those genes*.
      - Nucleic DNA extracted from a patient's cells is fluorescently marked.
      - The labelled DNA is washed across the array.
      - Any mutations in the patient's genome will fluoresce the 'mutant' column and any normal genes will fluoresce the 'normal' column.
        - Fluorescence of a single column suggests homozygosity.
        - Fluorescence of multiple columns suggests heterozygosity.



System: Genetics

Classic Autosomal Recessive Disorders:

Phenylketonuria: (similar to Maple-Syrup Urine Disease)

- Enzyme that breaks down phenylalanine isn't functioning.
  - Normally converts phenylalanine to another amino acid & tyrosine
- Phenylalanine & its breakdown chemicals (from other enzyme routes) accumulate in blood.
- **Symptoms:**
  - Mousy odour to urine, Vomiting, Rashes, Irritability
  - Severe brain damage – if left too long untreated.
- **Diagnosis:**
  - Heel-prick test – newborn screening
- **Management:**
  - Manage diet – avoid high protein foods
  - Maintain blood-phenylalanine levels (some is needed for normal growth)
  - Offspring of affected mothers – mental retardation – elevated phenylalanine inhibits foetal neural development.
- **Compound Heterozygotes:**
  - Patients with 2 different defective alleles, both contributing to the disease phenotype.
  - Compound **heterozygotes** of ***recessive*** disorders are **usually affected.**

Thalassemia:

- Absence of synthesis of one of the **globin chains**
- Usually via deletions that block globin protein production
- Alpha-thalassemia = absence of alpha-globin (more common in Asians)
- Beta-thalassemia = absence of beta-globin (more common in Caucasians)
- **Symptoms:**
  - Babies become anaemic
  - Become pale
  - Don't sleep well
  - No appetite
  - If untreated – usually fatal (usually die between 1 & 8yrs)

Sickle Cell Disease:

- Mutant allele codes for faulty beta-chain haemoglobin subunit.
- Mis-shapen red blood cell
- More common areas affected by malaria – malarial parasites grow poorly in homozygous & sickle cell carriers.

Haemochromatosis:

- Iron Overload – hyperabsorption of iron.
- Caused by mutations in the **HFE Gene.**
- Iron builds up in organs (particularly: heart/liver/pancreas) and cause failure.
- **Symptoms:** (usually men age 30-50 / women 50+)
  - Joint pain
  - Fatigue
  - Abdominal pain
  - Heart problems
  - Symptoms often occur after irreversible organ damage.
- **Long-Term Effects:**
  - Arthritis/Liver disease/Heart abnormalities/Impotence/Early menopause/Bronze or grey complexion/Possible diabetes

Cystic Fibrosis:

- Defective Cl^- Transporter in some cells (mostly exocrine glands)
- Defective gene = CFTR
- ***Q arm of Chromosome 7***
- **Lungs:**
  - Thicker mucus secretion
  - Ciliated cells can't move mucus up trachea
  - Mucus + dust + particles + bacteria →trapped in lungs →frequent lung infections.
- **Pancreas:**
  - Pancreatic duct clogs up – formation of cysts – eventually become fibrous
  - Hinders proper digestion (no pancreatic enzymes →duodenum)
- **Intestine:**
  - Not enough digestive enzymes – mainly for fats
  - Malnourishment
  - Fatty stools
- **Reproductive Ducts:**
  - Male vas-deferens blocks → sterility
- **Sweat Glands:**
  - Secrete salt to induce H_2O flow but salt isn't actively reabsorbed.
  - Results in salt-residue on skin & salt deficit in body.
- **Treatment:**
  - Enzyme tablets with meals
  - Electrolyte fluid – replenish lost NaCl
  - Vitamin supplements
  - Percussion – clear lungs

Examples of Autosomal Dominant Disorders:

Achondroplasia:

- "No Chondrocyte Proliferation"
- A form of inherited dwarfism
- **Phenotype:**
  - **Abnormal bone growth** – short stature + limb/cranial/facial disproportions.
  - Normal intelligence & lifespan
- **Mutation** in the '**fibroblast growth-factor receptor**' gene:
  - **Normal function:** negative regulator of bone growth.
  - **Mutated function:** *Hypermorphic* – too much negative regulation → puts the 'handbrake' on bone growth.
- **Most** (80%+) dwarfs **have normal parents** – ie. Their dwarfism is due to new mutation.

Hypochondroplasia:

- "Low Chondrocyte Proliferation"
- **Phenotype:**
  - Similar to, but milder than achondroplasia
  - Short stature
  - Disproportionate limbs
  - Normal facial features
  - Phenotype only becomes noticeable at toddler/school-age
- **Mutation:**
  - Also in the '**fibroblast growth-factor receptor**' gene:
  - Same gene – but different mutation
  - **Normal function:** negative regulator of bone growth.
  - **Mutated function:** *Hypermorphic* – too much negative regulation → puts the 'handbrake' on bone growth.

Huntington's Disease:

- Degenerative brain disease.
- **Mutation:**
  - **Defective Gene:** Huntington Protein
    - Huntington protein is widely expressed & vital for normal function
    - On **Chromosome 4**
    - **An expansion of a run of glutamine repeats in the protein.**
    - Highly expressed in brain.
- **Phenotype:**
  - Onset age generally between 30-50yrs (*well after reproductive age*)
  - Intellectual decline, Unsteady gait, Involuntary movements, Slurred speech, Impaired judgement, Difficulty swallowing.
- **Perpetuation:**
  - Offspring of affected parent = 50% chance of inheritance:
  - **Penetrance:** 100% - ie. If you have enough repeats, you WILL get the disease.
  - **Expression:** Age of onset decreases as # of repeats increases.
    - However – severity of symptoms (post-onset) is constant.

Myotonic Dystrophy

- Also a **repeat-sequence mutation.**
- Affects skeletal & smooth muscle/eyes/heart/endocrine system/central nervous system.
- May be mild/classical/congenital (severity)
- **Mutation: Expansion of** CTG trinucleotide **repeat** in the DMPK gene on **chromosome 19**.

Retinoblastoma

- Malignant Tumour in the retina of one/both eyes. (unilateral/bilateral)
- Onset: Infancy / early childhood.
- 1/3 cases are due to **inherited predisposition:**

Neurofibromatosis

- Formation of benign lumps around neural tissues.
- **Is an example of "Pleiotrophy":**
  - When a single gene mutation influences multiple phenotypic traits.
  - Tumours can grow anywhere on the body – can affect other bodily tissues.
- **2 Types:**
  - **Type 1 NF (NF1 Protein):**
    - 'Common' type
    - Neurofibromas under skin
    - Enlargement/deformation of bones
    - Scoliosis
    - Tumours in brain/on cranial nerves/on spinal cord
    - Learning disabilities are common.
  - **Type 2-Acoustic-NF (NF2 Protein):**
    - Very rare
    - Multiple tumours on cranial+spinal nerves
    - Hallmark: tumours on auditory nerves → hearing loss in teens.
- **NF1 & NF2** are **different proteins** of different genes of **different chromosomes**.
  - However they are **both tumour-suppressor genes.**
  - Mutations in either – cause partial/complete loss of protein function.
- **Inherited Predisposition:**
  - **Autosomal dominant**
  - Generally only the '*predisposition'* is inherited, & the other functional allele makes up for the deficiency.
  - **However,** when a **mutation** in the **functional allele** occurs, the tumour/s develop.
  - This is known as **The 2 Hit Hypothesis:**

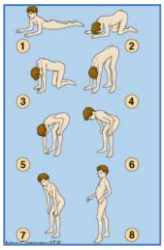
X-Linked Disorders

Colour Blindness:

- X-Linked Recessive
- Commonest Form: Red-Green Colour Blindness
- Caused by unequal crossover of the Red & Green Opsin Genes (on the X-chromosome) during GAMETOGENESIS.

Muscular Dystrophy:

- X-Linked Recessive
- Dys-trophy = "Faulty Nourishment"
  - It was thought that to be a malnourishment problem.
- Muscles weaken & waste away
- Caused by **mutations** in the **Dystrophin Gene**
- **Duchenne Muscular Dystrophy:**
  - Gower's Manoeuvre to get off the floor
  - Waddling gait
  - Enlarged Calves
  - Scoliosis
  - Eventually – wheelchair
  - Reduced pulmonary function by 20yrs – usually fatal.
    - Cardiac failure ... or
    - Respiratory failure



- **Milder Form: Becker Muscular Dystrophy:**
  - Onset in 20's
  - Immobility by 20th year of onset.
  - Hypertrophy of calves
  - Toe-walking

Haemophilia:

- X-Linked Recessive
- **Inability to form blood clots**
- Caused by **no Clotting Factor 8** (80%, severe) **or Factor 9** (20%, mild)
- Generally affects **Males**
  - Females exhibit very mild symptoms.
- Results in **severe Haematomas:**

- **Treatment:**
  - **Old:** **Blood Transfusions**
  - Infusions of **blood-derived** clotting factors
  - **Heat-treated blood-derived** clotting factors
  - **Recent:** **Recombinant** clotting factors
  - Approx. Once every 3 weeks.

Kennedy's Disease:

- **AKA: Spinal & Bulbar Muscular Atrophy (SBMA)**
- **X-Linked DOMINANT**...**but**...**Sex-Limited** – Phenotype seen mainly in males.
    - *Most sources say X-linked Recessive....
- **Cause:** expansion of **triplet-repeats** in the **Androgen Receptor Gene.**
- **Symptoms:**
    - Progressive neuromuscular disease
    - Onset: 30-50yrs
    - Muscle cramps
    - Twitching of muscles
    - Weakening of muscle.
    - Wasting of muscle

GENETICS Pathology:
ACHONDROPLASIA & HYPOCHONDROPLASIA

Examples of Autosomal Dominant Disorders:

- **Growth-Factors:**
  - **Achondroplasia:**
    - **Autosomal Dominant**
    - "No Chondrocyte Proliferation"
    - A form of inherited dwarfism
    - **Phenotype:**
      - **Abnormal bone growth** – short stature + limb/cranial/facial disproportions.
      - Normal intelligence & lifespan
    - **Mutation** in the '**fibroblast growth-factor receptor**' gene:
      - **Normal function:** negative regulator of bone growth.
      - **Mutated function:** *Hypermorphic* – too much negative regulation → puts the 'handbrake' on bone growth.
    - **Most** (80%+) dwarfs **have normal parents** – ie. Their dwarfism is due to new mutation.
    - **Genotype: Aa**
      - AA Genotype – not compatible with life.
      - $Affected_{Heterozygous}$ + $Affected_{Heterozygous}$ = 2/3 chance of affected offspring.
      - $Affected_{Heterozygous}$ + Normal = ½ chance of affected offspring.

	A	a
A	AA	Aa
a	Aa	aa

2x Affected

	A	a
a	Aa	aa
a	Aa	aa

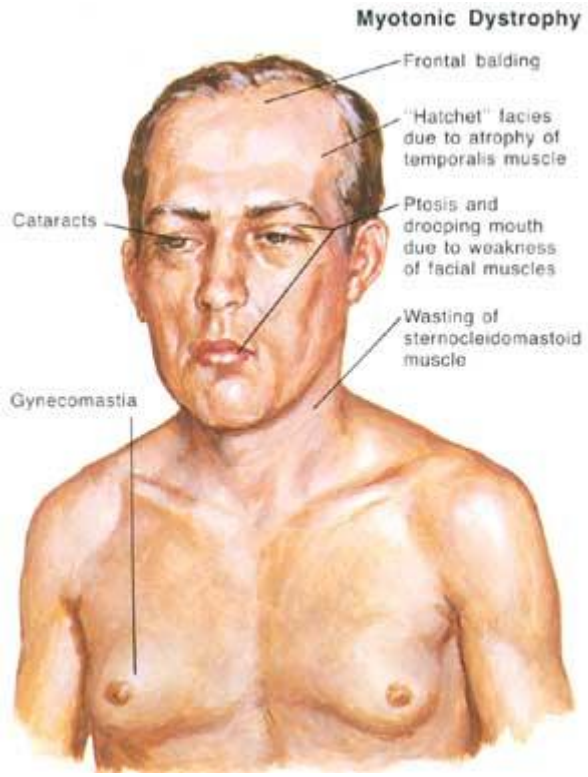
Normal x Affected

  - **Hypochondroplasia:**
    - "Low Chondrocyte Proliferation"
    - **Phenotype:**
      - Similar to, but milder than achondroplasia
      - Short stature
      - Disproportionate limbs
      - Normal facial features
      - Phenotype only becomes noticeable at toddler/school-age
    - **Mutation:**
      - Also in the '**fibroblast growth-factor receptor**' gene:
      - Same gene – but different mutation
      - **Normal function:** negative regulator of bone growth.

- **Mutated function:** *Hypermorphic* – too much negative regulation → puts the 'handbrake' on bone growth.

GENETICS Pathology:
MYOTONIC DYSTROPHY

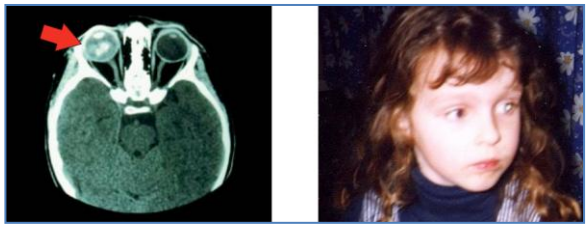
- **Myotonic Dystrophy**
  - Also a **repeat-sequence mutation.**
  - Affects skeletal & smooth muscle/eyes/heart/endocrine system/central nervous system.
  - May be mild/classical/congenital (severity)
  - **Mutation: Expansion of** CTG trinucleotide **repeat** in the DMPK gene on **chromosome 19**.



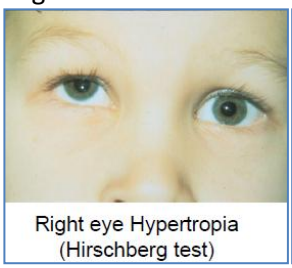
GENETICS Pathology:
RETINOBLASTOMA

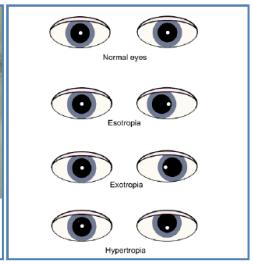
Clinical Features:

- **What is it?**
  - ***Malignant Tumour of the Retina***.
  - **NB:** If the Tumour travels down the Optic Nerve, the prognosis is poor.
  - Occur Unilaterally *OR* Bilaterally
- **Pathogenesis – "2 Hit Hypothesis":**
  - Arises from inactivation of **both alleles** for the RB1 gene (tumour suppressor gene)
    - **One Inactivation is Inherited (Autosomal Dominant) → Predisposition**
    - **The other is Acquired (Random Hit) → Cancer**
- **Typically Begins to form During Foetal Development (When Retinoblasts are rapidly dividing)**
  - However, the disease usually manifests in Infancy/Early-Childhood.
  - Almost always evident by 5yrs of age.
- **Clinical Manifestations:**
  - Often, a hyper-reflective retina **("*Leukocoria*")** in flash photography is diagnostic.

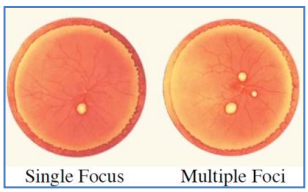


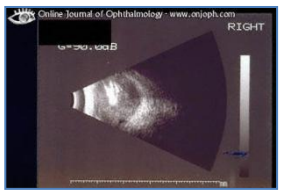
  - Tumour may also cause ***Strabismus*** (Eyes don't look in the same direction).
    - NB: Hirschberg test – check for the 'reflection dot' in the centre of the pupil.

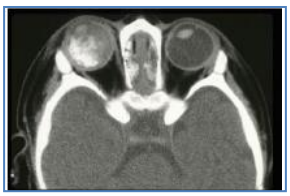




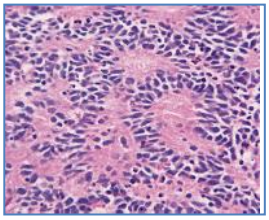
  - **Other Less Common Signs Include:**
    - Vision Problems
    - Eye Pain
    - Redness of the Sclera (white part of the eye)
    - Pupil is Unreactive to Bright Light → Photophobia
- **Hereditary RB Patients are at Higher Risk for *Other Cancers*:**
  - **Osteosarcoma** (Bone)
  - **Soft Tissue Sarcomas** (Muscles/Tendons/Ligaments/Fatty Tissue)
  - **Melanoma** (Melanocytes:skin)
  - **Why?** – Because every cell of their body has already lost 1x RB-Allele (Ie. Already had the 1st 'Hit')
  - **Therefore** – Regular Screening required for early detection.
- **Diagnosis:**
  - Opthalmoscope
  - Ultrasound
  - MRI/CT







- **Pathology:**
  - Histopathology – Presence of *'Homer Wright Rosettes*'. (In both familial & sporadic)
  - Molecular Pathology – Ie. Genetic Testing for sporadic or familial.



- **Treatment:**
  - **If Unilateral:**
    - Enuculation & Prosthesis (Removal of the eye)
  - **If Bilateral:**
    - Radiation
    - Photocoagulation/Laser Ablation (small tumours)
    - Cryotherapy (Freezing – for small peripheral tumours)
    - Chemotherapy (if high risk of metastasis)
- **Prognosis:**

• 5 year survival
  >90% if tumour is intraocular at diagnosis
  <10% if tumour is extraocular at diagnosis

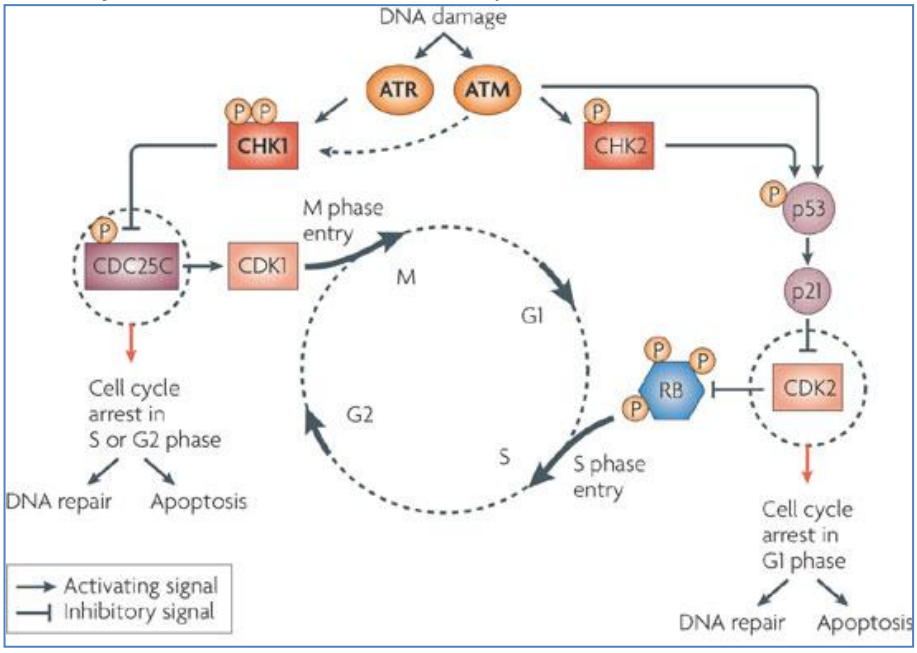
• Sporadic (~60%)
  – 5% will develop another Ca by age 50

• Familial / germline (~40%)
  – 50% risk of Ca by age 50
  – additional tumours in eye and elsewhere
  – ~10% cases show family history

- **Prevention?**
  - NO Way
  - Genetic Testing for Families Affected → Early Detection
  - To Prevent Passing Defective Genes to the Next Generation, Pre-Implantation Genetic Diagnosis or Amniocentesis may be considered. → Abort affected foetuses.

<u>Genetics of Retinoblastoma:</u>

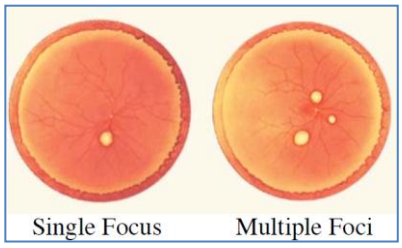
- **Retinoblastoma = Mutation in the *'RB1'* gene on *Chromosome 13*.**
  - ***'RB1 Gene'*** **=** A Tumour Suppressor Gene.
  - **RB Normally Functions to:** control the Cell-Cycle transition from **G1 to S-Phase.**



- **Inheritance: 30% of Retinoblastoma are due to an *Inherited Disposition*:**
  - **At The Genetic Level –** The ***Predisposition*** is Inherited ***Autosomal-Dominantly***:

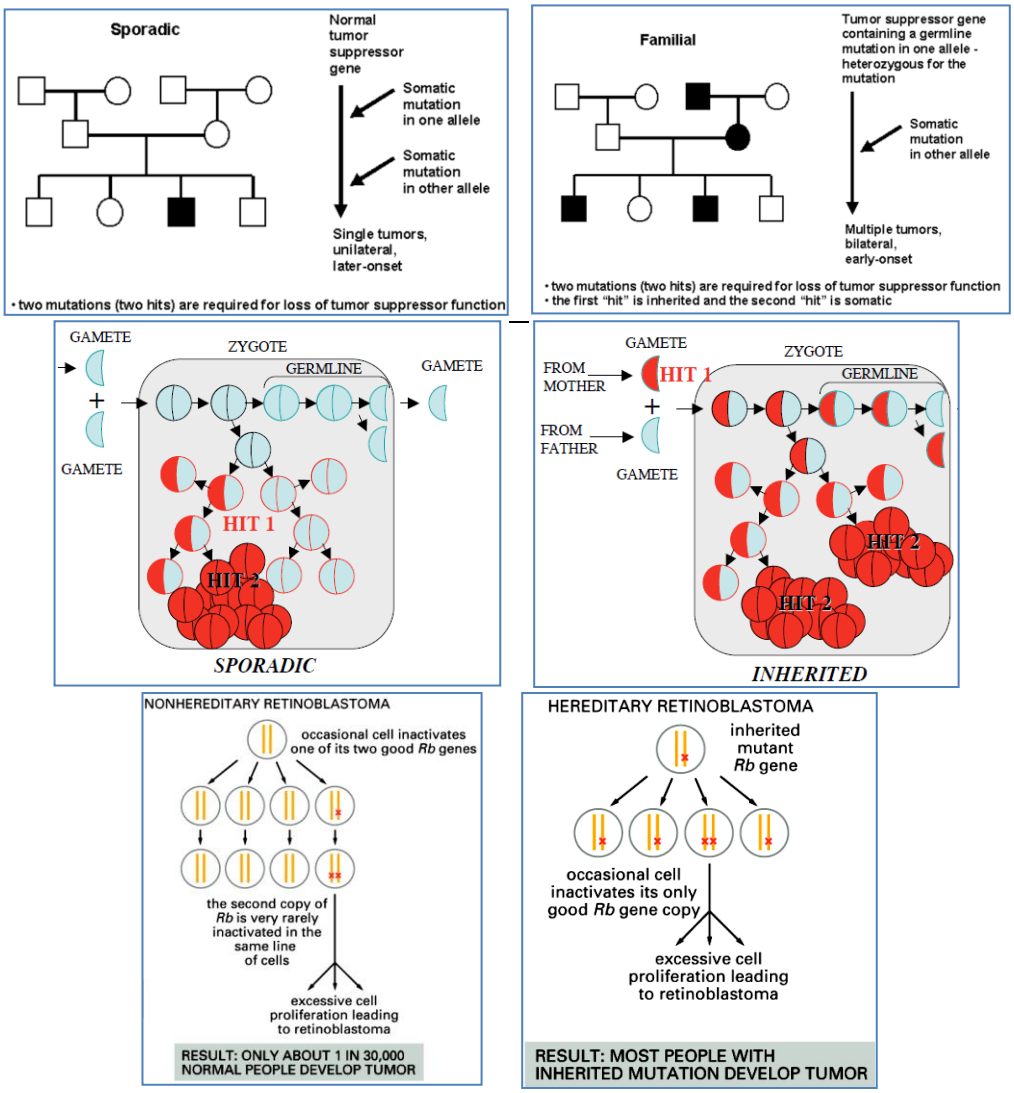
        - Ie. You only need to be Heterozygous to be *Predisposed*.
    - **At The Cellular Level –** The ***Disease*** is ***Autosomal-Recessive***:
        - Ie. You need to be Homozygous for defective RB-Gene;
        - OR Exhibit ***'Loss of Heterozygosity'*** in favour of the Mutated RB-Gene.

- **Typical Manifestations of *Inherited Retinoblastoma*:**
    - Bilateral Retinoblastomas (Early Onset);
    - And/Or Multiple Foci in One Eye (Early Onset).
    - NB: Single Foci cases are typically sporadic, and have (Late Onset).



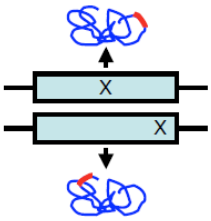
<u>Knudson's 2-Hit Hypothesis for Tumour-Suppressor Genes: Applies to Retinoblastoma:</u>

- **Sporadic** – 2 Mutations ('Hits') are required for Retinoblastoma.
- **Familial** – All Retinoblasts already have the 1st hit → only require 1 more 'Hit' for Retinoblastoma.
- **NB:** One of the "*Hits*" can be a ***<u>"Loss of Heterozygosity"</u>*** in Favour of the Mutated Gene**.**



GENETICS Pathology:
PHENYLKETONURIA

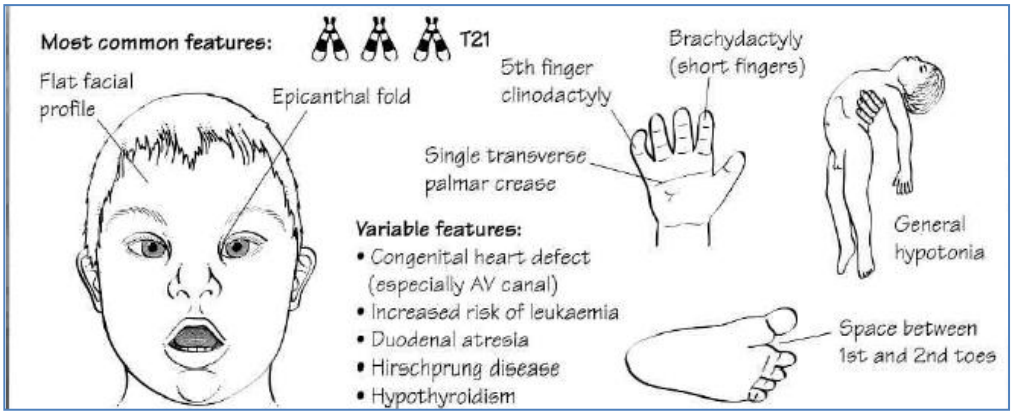
- **Phenylketonuria:** (similar to Maple-Syrup Urine Disease)
  - Enzyme that breaks down phenylalanine isn't functioning.
    - Normally converts phenylalanine to another amino acid & tyrosine
  - Phenylalanine & its breakdown chemicals (from other enzyme routes) accumulate in blood.
  - **Symptoms:**
    - Mousy odour to urine
    - Vomiting
    - Rashes
    - Irritability
    - Small head
    - Severe brain damage – if left too long untreated.
  - **Diagnosis:**
    - Heel-prick test – newborn screening
  - **Management:**
    - Manage diet – avoid high protein foods
    - Maintain blood-phenylalanine levels (some is needed for normal growth)
    - Offspring of affected mothers – mental retardation – elevated phenylalanine inhibits foetal neural development.
  - **Compound Heterozygotes:**
    - Patients with 2 different defective alleles, both contributing to the disease phenotype.
    - Compound **heterozygotes** of ***recessive*** disorders are **usually affected.**

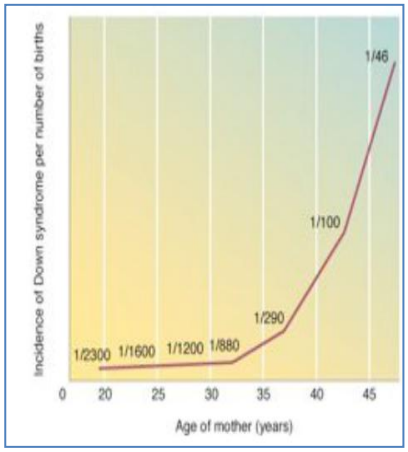


GENETICS Pathology:
DOWNS TRI-21

47, XY, +21: Down's Syndrome

- Trisomy 21
- Risk of Down's syndrome increases exponentially with maternal age.

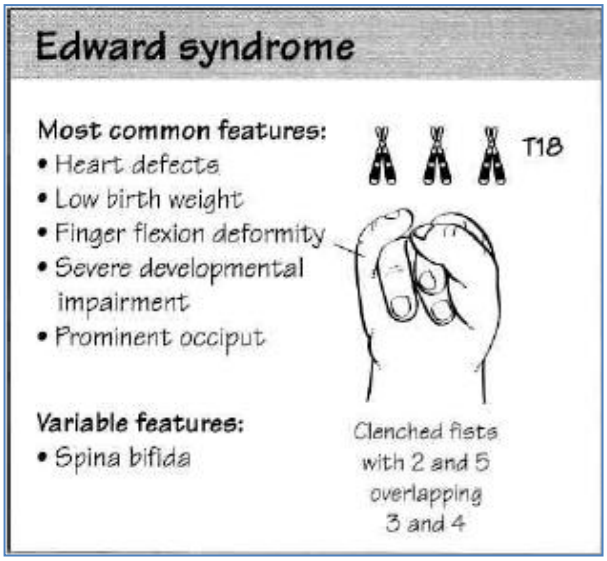




GENETICS Pathology:
CHROMOSOMAL - EDWARDS TRI-18

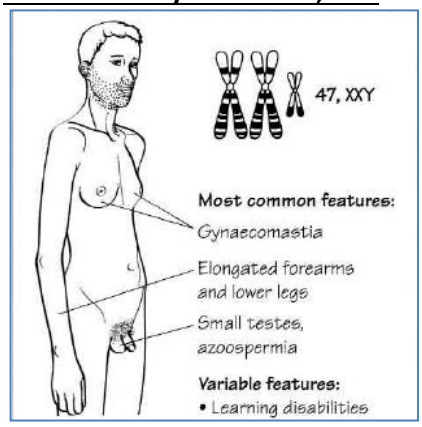
47, XY, +18: Edward's Syndrome

- Trisomy 18
- Rarely survive beyond infancy



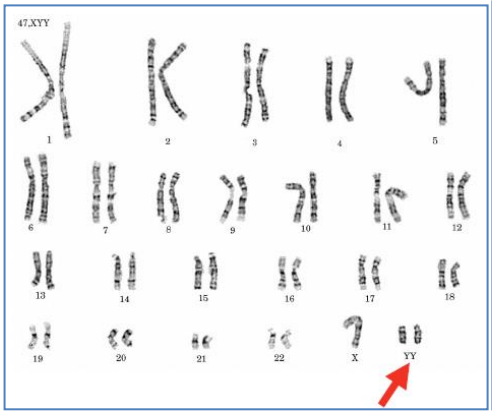
GENETICS Pathology:
KLINEFELTERS XXY

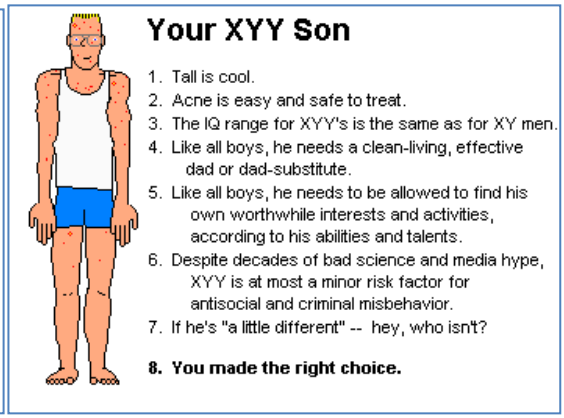
Klinefelter's Syndrome 47, XXY



GENETICS Pathology:
SUPERMALE XYY

Diplo-Y (Super-Male) 47,XYY.

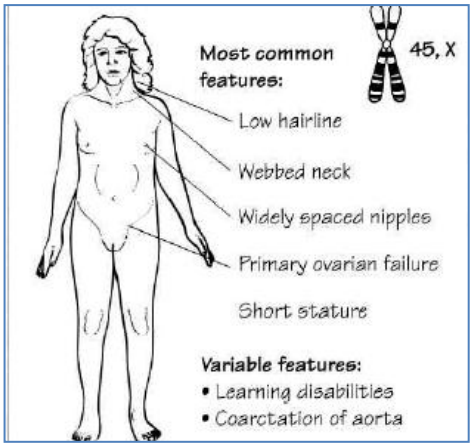




GENETICS Pathology:
TURNERS XO

Turner's Syndrome:

- Most die during gestation
- Survivors are mostly mosaics



X-Linked & Mitochondrial Disorders

Fundamentals:

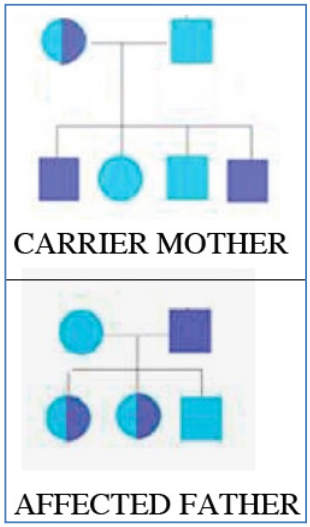
- Females have 2 'X'-Chromosomes
- Males have 1 'X' & 1 'Y'-Chromosome.
- Unlike Autosomes, X & Y Chromosomes share no common sequence & carry completely different genes.

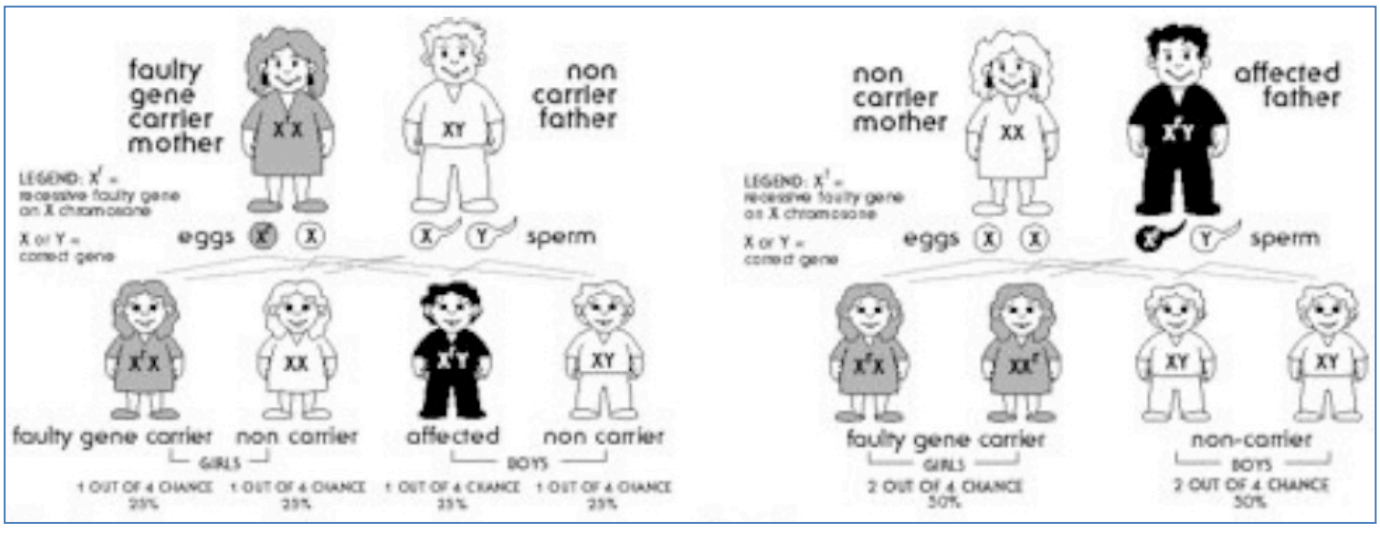
X-Linked Traits:

- **X-Chromosome** contains both **Dominant & Recessive alleles**
  - **In Females:**
    - Dominant/recessive rules apply
    - & can be either heterozygous/homozygous
    - Females may be mildly affected – By:
      - Mosaicism
      - Random Inactivation of the 'Good-X'
      - Spontaneous mutation
      - Etc...
  - **In Males:**
    - All inherited X-linked alleles are expressed (due to having only 1 X-chrmomosome)
    - Therefore all X-Linked alleles act 'dominantly.'
    - Males are neither Homozygous nor Heterozygous for X-linked traits.
      - Instead they are **Hemizygous.**

X-Linked Inheritance:

- Differs from autosomal inheritance – **Sex** of the individual **determines** their **phenotype**.
  - **Maternal:**
    - Carrier Mother + Non-Carrier Father:
      - 25% chance of having an affected child
      - ≈0% of daughters likely to be affected
      - 50% of daughters likely to be carriers
      - 50% of sons likely to be affected
  - **Paternal:**
    - Non-Carrier Mother + Affected Father:
      - ≈0% chance of having an affected child
      - ≈0% of daughters likely to be affected
      - 100% of daughters likely to be carriers
      - ≈0% of sons likely to be affected
- **NB:** The only form of inheritance that **'Skips a Generation'.**
  - From **Grandfather → Grandson** (**through** a carrier **mother**)



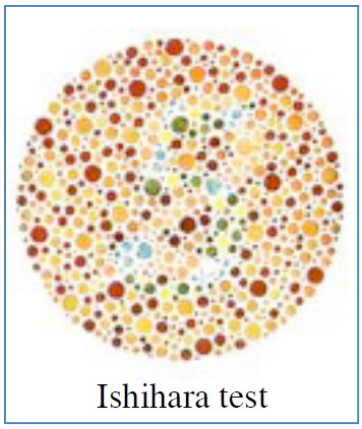


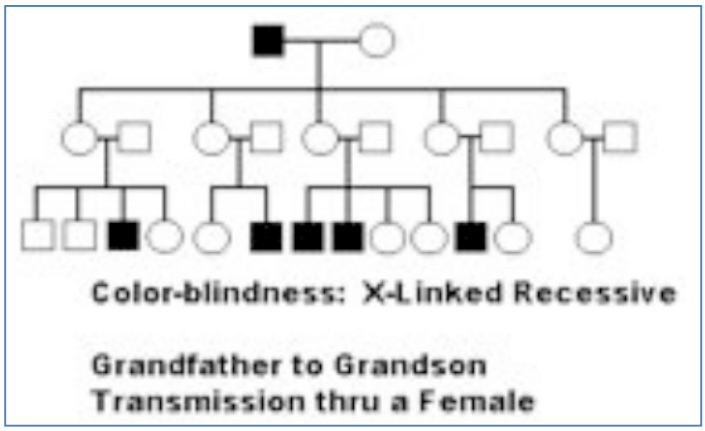
Sex-Specific Genetic Terms:

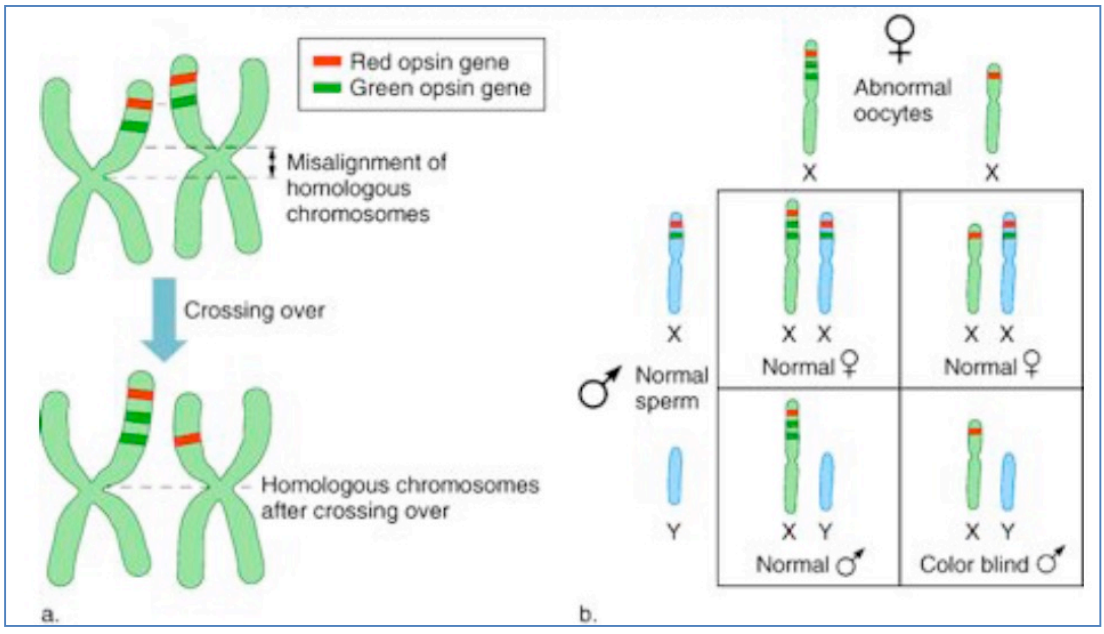
- **Sex LINKED Traits:**
  - Any Alleles on the sex chromosomes.
  - May be Dominant/Recessive (in females)
  - Eg. Colour Blindness
- **Sex LIMITED Traits:**
  - Alleles may be X-Linked/Autosomal
  - Phenotype is only expressed in one of the sexes.
  - Eg. Kennedy's Disease
- **Sex INFLUENCED Traits:**
  - Alleles may be X-Linked/Autosomal
  - Alleles are dominant in one sex, but recessive in the other.
  - Eg. Baldness

Examples of Human X-Linked Disorders:

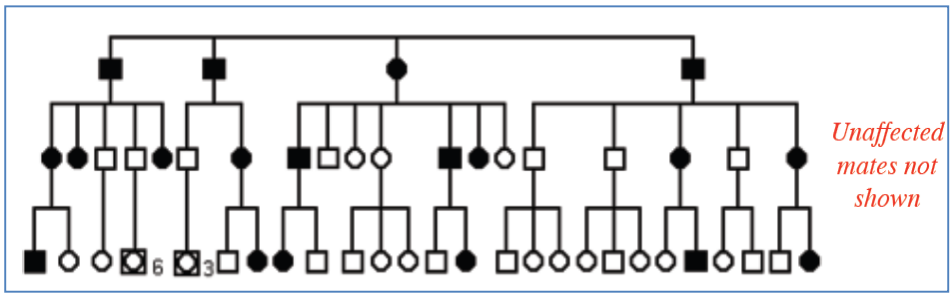
- **Colour Blindness:**
  - X-Linked Recessive
  - Commonest Form: Red-Green Colour Blindness
  - Caused by unequal crossover of the Red & Green Opsin Genes (on the X-chromosome) during GAMETOGENESIS.


Ishihara test

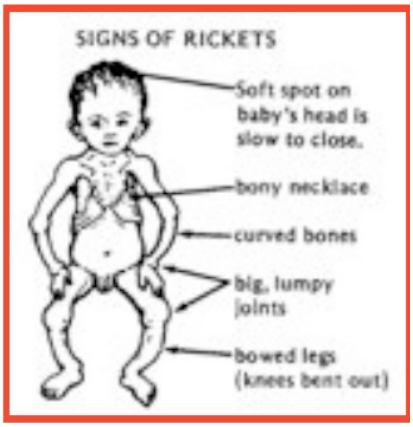




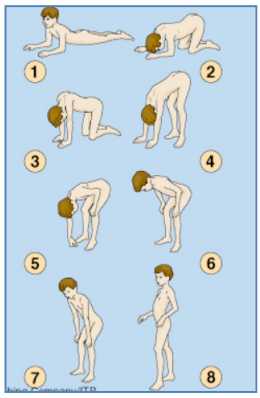
- **Hypophosphatemic Rickets:**
  - X-Linked DOMINANT
  - Can be passed on from either parent → offspring
    - But: Affected father can't pass it to the son.
  - Heterozygosity/Homozygosity/Hemizygosity$_{(males)}$ = Affected
  - Females have 2x the chance of getting it than Males
    - Ie. By having 2 'X' chromosomes, they effectively 'line up' twice.



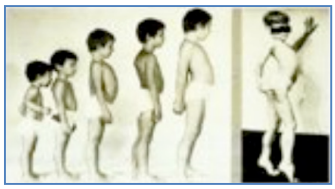
  - **Main Causes:**
    - **#1. Insufficient Vit. D:**
      - Poor dietary intake
      - Inadequate UV light exposure
      - Malabsorption of Calcium, Phosphate & Fat-Soluble Vitamens (ADEK)
    - **#2. Abnormal Vit. D Metabolism:**
      - Liver & Renal Disease
      - Heriditary
      - Unresponsiveness to Vit. D.
    - **#3. Abnormal Renal-Tubular Absorption of Phosphate:**
      - Genetic Hypophosphatemic Rickets
      - Tumour-related



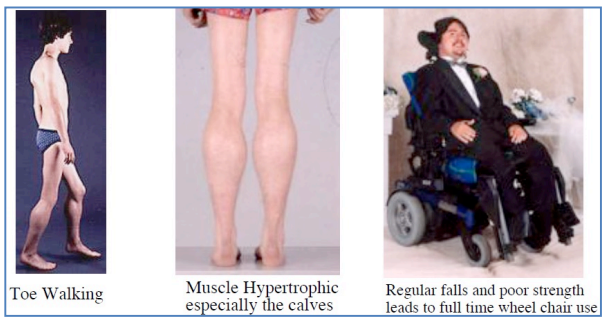
- **Muscular Dystrophy:**
  - X-Linked Recessive
  - Dys-trophy = "Faulty Nourishment"
    - It was thought that to be a malnourishment problem.
  - Muscles weaken & waste away
  - Caused by **mutations** in the **Dystrophin Gene**



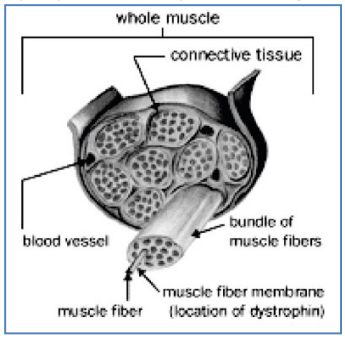
  - **Duchenne Muscular Dystrophy:**
    - Gower's Manoeuvre to get off the floor
    - Waddling gait
    - Enlarged Calves
    - Scoliosis
    - Eventually – wheelchair
    - Reduced pulmonary function by 20yrs – usually fatal.
      - Cardiac failure ... or
      - Respiratory failure
    - Females almost always have at least one good dystrophin gene.
      - Even with random x-inactivation, it's usually not a problem
      - However – female carriers can have heart problems – must have regular checkups.

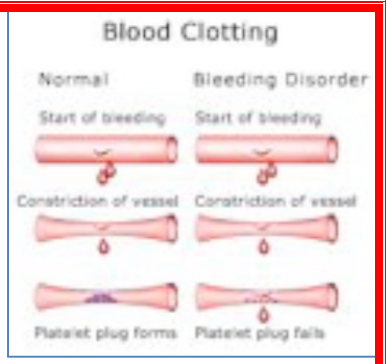


  - **Milder Form: Becker Muscular Dystrophy:**
    - Onset in 20's
    - Immobility by 20th year of onset.
    - Hypertrophy of calves
    - Toe-walking



  - **The Dystrophin Gene:**
    - Longest gene in the body
    - Mutations in this gene cause BMD & DMD
    - Dystrophin= a thin sheath just under a striated muscle cell's membrane
      - Is thought to play a role in preventing membrane damage during contraction

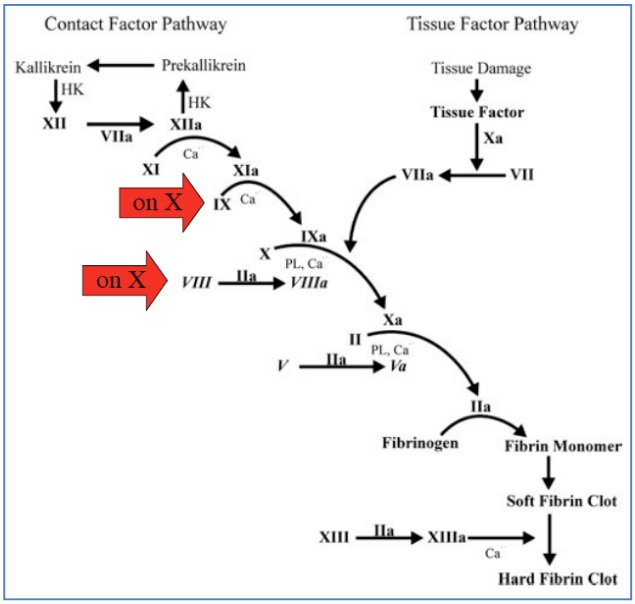




- **Haemophilia:**
  - X-Linked Recessive
  - **Inability to form blood clots**
  - Caused by **no Clotting Factor 8** (80%, severe) **or Factor 9** (20%, mild)
  - Generally affects **Males**
    - Females exhibit very mild symptoms.
  - Results in **severe Haematomas:**

  - **Treatment:**
    - **Old:** **Blood Transfusions**
    - Infusions of **blood-derived** clotting factors
    - **Heat-treated blood-derived** clotting factors
    - **Recent:** **Recombinant** clotting factors
    - Approx. Once every 3 weeks.

  - Factor 8 Deficiency – more severe due to where it interferes with the Blood Clotting Cascade:



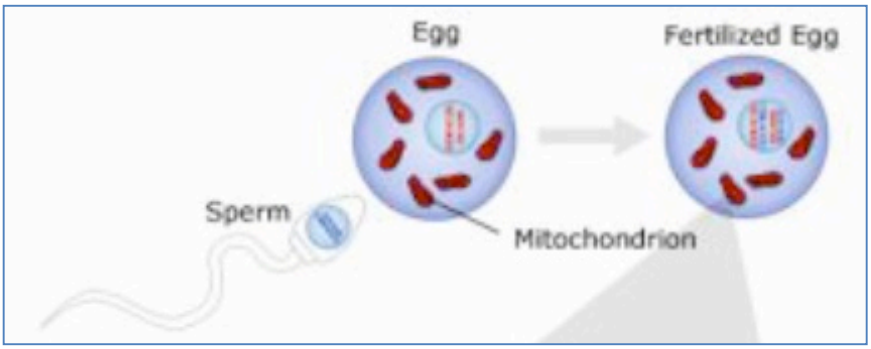
don't need to know this cascade

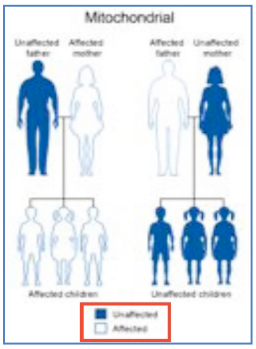
- **Kennedy's Disease:**
  - **AKA: Spinal & Bulbar Muscular Atrophy (SBMA)**
  - **X-Linked DOMINANT...but...Sex-Limited** – Phenotype seen mainly in males.
    - *Most sources say X-linked Recessive....
  - **Cause:** expansion of **triplet-repeats** in the **Androgen Receptor Gene.**
  - **Symptoms:**
    - Progressive neuromuscular disease
    - Onset: 30-50yrs
    - Muscle cramps
    - Twitching of muscles
    - Weakening of muscle.
    - Wasting of muscle

Mitochondrial Genetics:

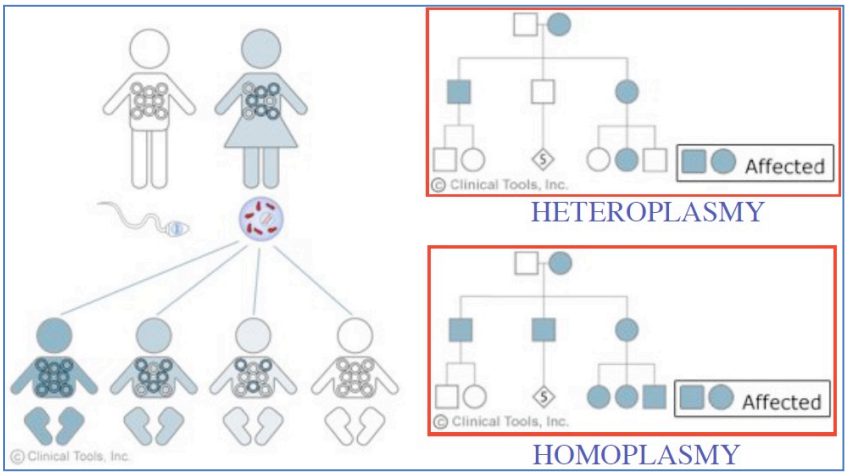
- **Mitochondria:**
  - Power-stations of the cell
  - Thought to have evolved from endosymbiotic bacteria.
  - Encode their own Ribosomes + tRNAs
  - Make their own Proteins
    - However they **import some nuclear proteins**
    - Such proteins have **mitochondrial signal peptides** – targets mitochondria.
    - Therefore – **many defects** are **result from nuclear mutations.**
      - (not exclusively inherited maternally)
  - Replicate **'clonally'** by **Binary Fission**
  - Each cell contains many mitochondria:
    - Not all are of the same lineage
    - Ie. Different strains
    - Therefore – there may be 'good' mitochondria...& mutated mitochondria.

- **Mitochondrial Genome: "Extranuclear DNA"**:
  - Not affiliated with the 'human-nuclear-genome'.
  - **Single Circular Chromosome**
  - **Codes for specific mitochondrial enzymes**.
  - **Mutations** in the mitochondrial genome sometimes occur.
    - Often involve **brain** – due to high energy demands.
    - Only **tissues** with **high %age** of **diseased mitochondria** will be impaired.
    - Disease often results in the **accumulation of acids** (metabolic intermediates) in body.
      - Lactic acid is a big one.
  - The mitochondrial chromosome is **inherited almost exclusively from the MOTHER**.
    - Vary rarely do sperm contribute mitochondria to an egg.



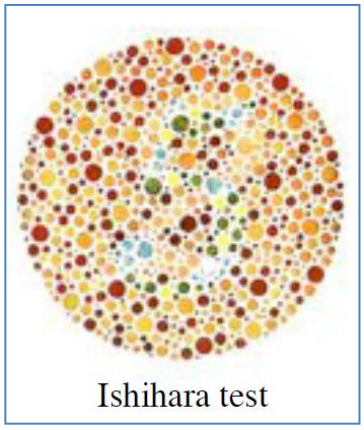


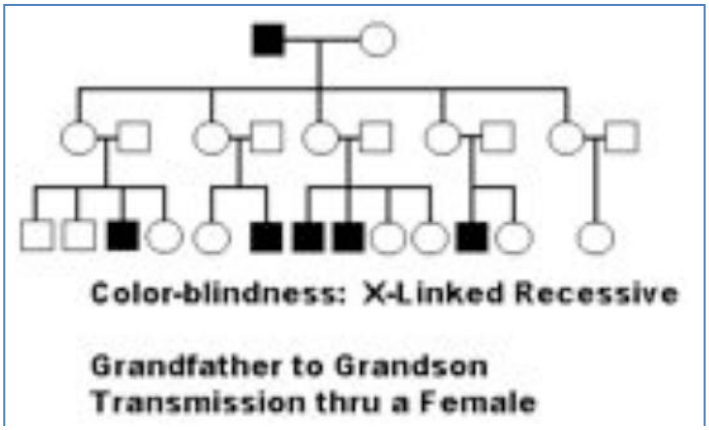
- **Mitochondrial Disease Inheritance:**
  - **Heteroplasmy:** Some 'good' & some 'bad' (mutated) mitochondria
  - **Homoplasmy:** All 'good' OR all 'Bad' (mutated) mitochondria.

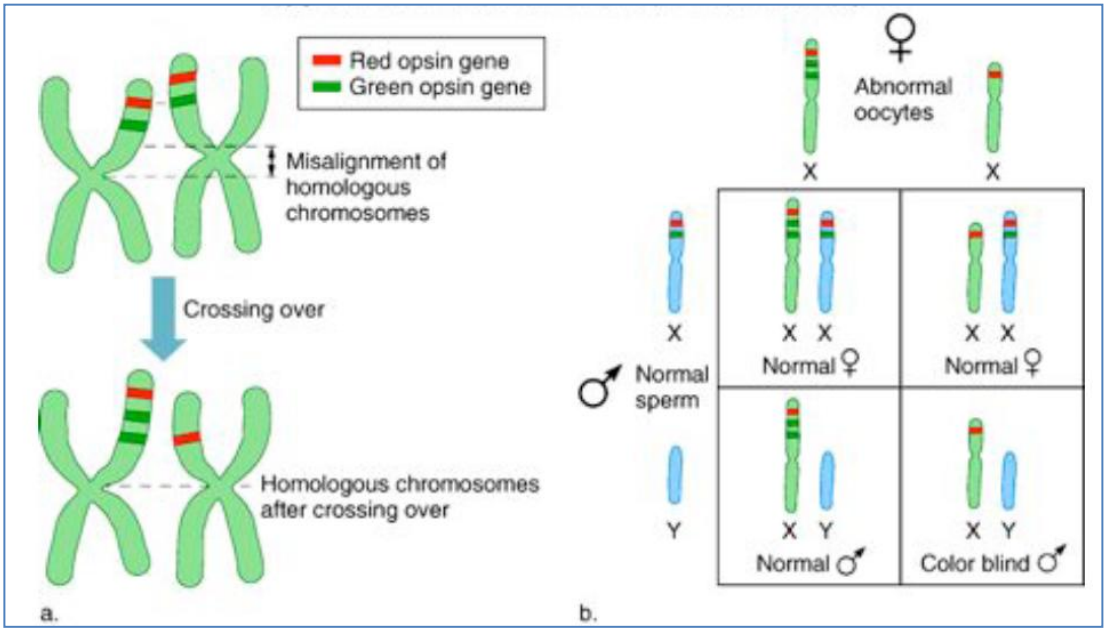


GENETICS Pathology:
XLINKED REC - COLOUR BLINDNESS

- **Colour Blindness:**
  - X-Linked Recessive
  - Commonest Form: Red-Green Colour Blindness
  - Caused by unequal crossover of the Red & Green Opsin Genes (on the X-chromosome) during GAMETOGENESIS.

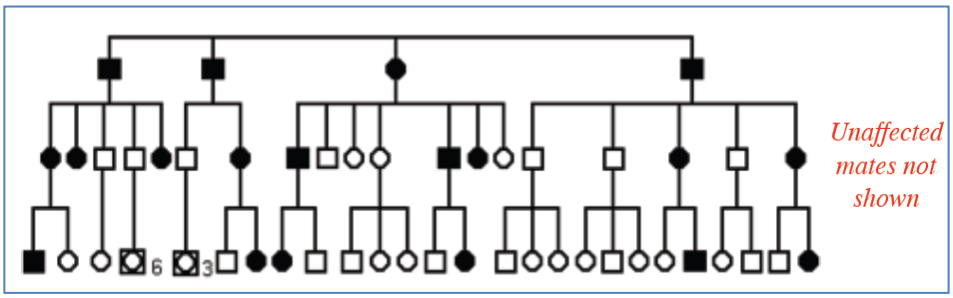




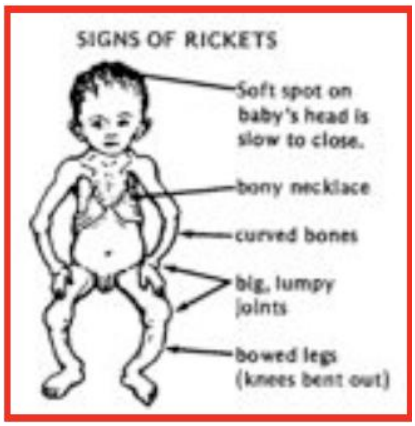


GENETICS Pathology:
XLINKED REC - HYPOPHOSPHATAEMIC RICKETS

- **Hypophosphatemic Rickets:**
  - X-Linked DOMINANT
  - Can be passed on from either parent → offspring
    - But: Affected father can't pass it to the son.
  - Heterozygosity/Homozygosity/Hemizygosity$_{(males)}$ = Affected
  - Females have 2x the chance of getting it than Males
    - Ie. By having 2 'X' chromosomes, they effectively 'line up' twice.



  - **Main Causes:**
    - **#1. Insufficient Vit. D:**
      - Poor dietary intake
      - Inadequate UV light exposure
      - Malabsorption of Calcium, Phosphate & Fat-Soluble Vitamens (ADEK)
    - **#2. Abnormal Vit. D Metabolism:**
      - Liver & Renal Disease
      - Heriditary
      - Unresponsiveness to Vit. D.
    - **#3. Abnormal Renal-Tubular Absorption of Phosphate:**
      - Genetic Hypophosphatemic Rickets
      - Tumour-related



Introduction to Neoplasia

Terminology of Cancer:

- **Oncology:**
  - "The study of tumours or neoplasms"
  - NB: "Oncos" = Tumour (in Greek)

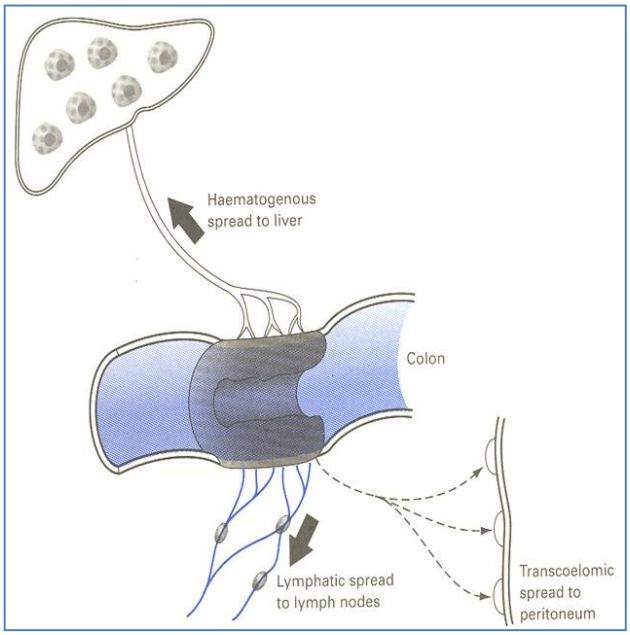
- **Tumour:**
  - NB: *Originally* meant "Swelling due to inflammation";
  - However, *today* it is associated with ***any Neoplasia/Cancer/Growths***, and **includes** both **Malignant & Benign** neoplasms.

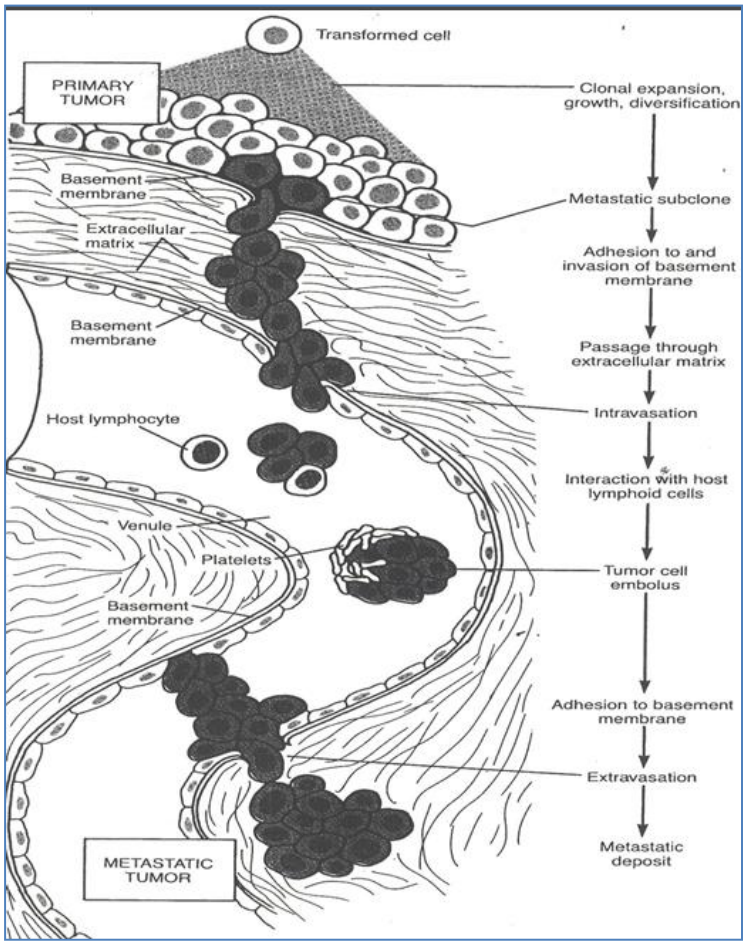
- **Neoplasia (Neoplasm) – (NB: Phil Summers prefers "Neoplasm" to "Tumour"):**
  - "New Growth" (in Greek)
    - "Neo" = New
    - "Plasia" = Growth
  - "An ***Abnormal Mass of Tissue*** which ***grows faster than normal tissues,*** & then ***maintains growth rate*** even ***after cessation of the stimuli*** which evoked the change"
  - **Consist of 2 Basic Components:**
    - 1. Proliferating Neoplastic Cells (Make up the cellular component of the neoplasm)
    - 2. Supportive Connective Tissue & Blood Vessels (Non-Cellular components of neoplasms)
  - **Arise from the Clonal Expansion of a single cell that has undergone Neoplastic Transformation:**
    - Ie. Loss of Some/All Specialised Functions; or Acquisition of *New* Biological Functions.
  - **Neplastic Transformation can be Caused By:**
    - Chemical Agents (Eg. Carcinogens/Free Radicals/Etc)
    - Physical Agents (Eg. UV Light/Ionising Radiation/etc)
    - Biological Agents (Eg. Viruses/Chronic Inflammation/etc)

- **Cancer:**
  - A common term for all **MALIGNANT Tumours**.
  - Cancers = A Collection of diseases Characterised by ***Uncontrolled Growth of Cells*** leading to Invasion of Surrounding Tissues & Spread (Metastasis) to other parts of the body.

- **Anaplasia:**
  - Ie. Highly De-Differentiated
  - **Exhibit *Pleomorphism***:
    - Variation in Size & Shape of Cells & Their Nuclei.
    - Large Cells of Irregular Size
  - **Abnormal Nuclear Morphology:**
    - Disproportionately Large
    - Abundant Chromatin → Dark-Staining (Hyperchromic) Nuclei
    - Multinucleated Cells
  - **Loss of Polarity:**
    - The Orientations of Anaplastic cells are Disturbed (Ie. The lose polarity)
    - Cells grow in a Disorganised Pattern
  - **Formation of '*Tumour Giant Cells*':**
    - Very Large Cells
    - Huge Polymorphic Nucleus (Sometimes multiple)
      - Have a Low *Cytoplasm:Nuclear* Ratio.
    - (Not to be confused with Langhan's Giant Cells derived from macrophages)

- **Metastasis:**
  - Ie. Metastases are 2^{o} Neoplasms which are Anatomically Discontinuous with the Primary Neoplasm, but consist of Cells which Originated in the Primary Neoplasm.
    - NB: Only certain subclones of the 1^{o} Tumour possess the right combination of Gene Products to be Metastatic.
  - **Mechanisms of Invasion & Metastasis:**
    - **1. Progressive Infiltration/Invasion of Extracellular Matrix:**
      - Detachment of Neoplastic Cells
      - Can produce Collagenase → Destruction of Extracellular Matrix & Surrounding Tissues → Necrosis.
      - Don't Recognise Anatomical Boundaries.
      - ***Invasiveness*** = A Reliable Indicator of Malignancy.
    - **2. Vascular Dissemination (Intravasation):**
      - Malignant cells make it to the bloodstream.
      - Expression of Adhesion Molecules for attachment to endothelium.
    - **3. Adhesion to Basement Membrane → Extravasation:**
      - Cell adheres to Basement Membrane of Vessels & Migrates out into surrounding tissue.
      - Some preferentially spread to certain sites (eg. Prostatic Carcinoma often goes to bone; Lung Carcinomas end up in Brain & Adrenals)
  - **Pathways of Spread/Metastasis:**
    - **1. Direct Seeding** – of body cavities & surfaces. (Cells fall off & spread)
    - **2. Via Lymphatics** – (Often results in secondary tumours in Lymph Nodes)
    - **3. Via Blood Vessels (Haematogenous Spread) –** (Liver, Lungs & Kidneys are most at risk)





Classification & Naming of Neoplasms:

- **NB: Neoplasms are classified on the basis of whether they are *Benign* or *Malignant*:**
- **Benign:**
    - **Naming:**
        - **Cell-of-Origin + Suffix: "-oma":**
            - Eg. Benign Neoplasm of Fibroblasts - ***Fibroma***
            - Eg. Benign Neoplasm of Chondrocytes (Cartilage) - ***Chondroma***
            - Eg. Benign Neoplasm of Osteoblasts - ***Osteoma***
            - Eg. Benign Neoplasm of Smooth Muscle – ***Leiomyoma***
            - Eg. Benign Neoplasm of Striated Muscle - ***Rhabdomyoma***
            - Eg. Benign Neoplasm of *Glandular* Epithelium – ***Adenoma***
                - (Ie. Sweat-Gland Adenoma, Hepatic Adenoma, Renal Adenoma, Etc.)
            - Eg. Wart-like/Finger-like Projections – ***Papilloma***
                - (Eg. On Skin – the common wart)
            - Eg. Benign Neoplasm Projecting above Mucosal Surface – ***Polyp***
                - (Eg. Gastric Polyp, Colonic Polyps)
        - **Exceptions to the "oma" rule:**
            - ***Lymphoma*** – Malignant carcinoma of Lymphoid Cells
            - ***Melanoma*** – Malignant carcinoma of Melanocytes in the skin (Also in skin & eye).
            - ***Seminoma* –** Malignant carcinoma of Testicular Germ Cells
            - ***Teratomas* –** Can be Malignant or Benign; Can Consist of a Variety of Cell Types.
    - **Histological Features of Benign Neoplasms:**
        - **- Show Low Levels of Anaplasia:**
            - Maintain a Near-Normal level of Differentiation
        - **- Slow Growth Rate:**
            - Slow (Months-Years)
        - **- NOT Metastatic:**
            - Neoplasms Remain Localised as a Discrete Lesion.
            - Often encased in Fibrous Capsule.
            - Ie. Still obey Anatomical Boundaries.

- **Malignant:**
  - **Naming:**
    - **Cells of *Mesenchymal (Connective)* Tissue or *Soft Tissue* + Suffix: "-Sarcoma":**
      - Eg. Malignant Neoplasm of Fibrous Tissue – ***Fibrosarcoma***
      - Eg. Malignant Neoplasm of Smooth Muscle – ***Leiomyosarcoma***
    - **Cells of Epithelial Tissue + Suffix: "-Carcinoma":**
      - Eg. Malignant Neoplasm of Glandular Epithelia – ***Adenocarcenoma***
      - Eg. Malignant Neoplasm of Squamous Cells – ***Squamous-Cell Carcinoma***.
  - **Histological Features of Malignant Neoplasms:**
    - **- Are Highly Anaplastic:**
      - Ie. Highly De-Differentiated
      - **Exhibit *Pleomorphism***:
        - Variation in Size & Shape of Cells & Their Nuclei.
        - Large Cells of Irregular Size
      - **Abnormal Nuclear Morphology:**
        - Disproportionately Large
        - Abundant Chromatin → Dark-Staining (Hyperchromic) Nuclei
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        - Very Large Cells
        - Huge Polymorphic Nucleus (Sometimes multiple)
          - Have a Low *Cytoplasm:Nuclear* Ratio.
        - (Not to be confused with Langhan's Giant Cells derived from macrophages)
    - **- Rapid Growth Rate:**
      - Weeks
      - Frequent Mitosis → Many Histologically-visible Mitotic Figures in a High-Power Field.
    - **- Are Metastatic:**
      - Ie. Metastases are 2^{o} Neoplasms which are Anatomically Discontinuous with the Primary Neoplasm, but consist of Cells which Originated in the Primary Neoplasm.
        - NB: Only certain subclones of the 1^{o} Tumour possess the right combination of Gene Products to be Metastatic.
      - **Mechanisms of Invasion & Metastasis:**
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          - Can produce Collagenase → Destruction of Extracellular Matrix & Surrounding Tissues → Necrosis.
          - Don't Recognise Anatomical Boundaries.
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          - Malignant cells make it to the bloodstream.
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          - Cell adheres to Basement Membrane of Vessels & Migrates out into surrounding tissue.
          - Some preferentially spread to certain sites (eg. Prostatic Carcinoma often goes to bone; Lung Carcinomas end up in Brain & Adrenals)
      - **Pathways of Spread/Metastasis:**
        - **1. Direct Seeding** – of body cavities & surfaces. (Cells fall off & spread)
        - **2. Via Lymphatics** (Often results in secondary tumours in Lymph Nodes)
        - **3. Via Blood Vessels** (Liver, Lungs & Kidneys are most frequent victims)
          - (AKA: Haematogenous Spread)

Summary of Differences Between Benign and Malignant Neoplasms:

Comparisons between Benign & Malignant Tumours		
Characteristics:	**Benign:**	**Malignant:**
Naming (Suffix)	**Suffix:** "-Oma"	**Suffix:** "-Sarcoma"
Differentiation/ Anaplasia	Well Differentiated (Ie. Near-Normal level of Differentiation) Structure is often Typical of Original Tissue. (Ie. Only Mildly Anaplastic)	Lack of Differentiation (Ie. Are *De-Differentiated*) Structure is often Atypical of Original Tissue (Ie. Highly Anaplastic)
Growth Rate	Grow slowly over Months-Years	Grow Quickly and Erratically
Local Invasion	Usually a Cohesive, Well-Demarcated Mass. (Ie. Remain localised as a Discrete Lesion) Often Encased in a Fibrous Capsule DO NOT Invade/Infiltrate surrounding tissue.	Locally Invasive Infiltrates Surrounding Tissue Some Produce Collagenase → Destruction of Surrounding Tissue → Necrosis. Don't respect anatomical boundaries
Metastasis	No Metastasis.	Metastasis (Most likely: Large, Undifferentiated Neoplasms)

Naming Tumours:

- **Exceptions to the "oma" rule:**
  - ***Lymphoma*** – Malignant carcinoma of Lymphoid Cells
  - ***Melanoma*** – Malignant carcinoma of Melanocytes in the skin (Also in skin & eye).
  - ***Seminoma*** – Malignant carcinoma of Testicular Germ Cells
  - ***Teratomas*** – Can be Malignant or Benign; Can Consist of a Variety of Cell Types.

TABLE 7–1 Nomenclature of Tumors

Tissue of Origin	Benign	Malignant
COMPOSED OF ONE PARENCHYMAL CELL TYPE		
Tumors of Mesenchymal Origin		
Connective tissue and derivatives	Fibroma Lipoma Chondroma Osteoma	Fibrosarcoma Liposarcoma Chondrosarcoma Osteogenic sarcoma
Endothelial and Related Tissues		
Blood vessels Lymph vessels Synovium Mesothelium Brain coverings	Hemangioma Lymphangioma Meningioma	Angiosarcoma Lymphangiosarcoma Synovial sarcoma Mesothelioma Invasive meningioma
Blood Cells and Related Cells		
Hematopoietic cells Lymphoid tissue		Leukemias Lymphomas
Muscle		
Smooth Striated	Leiomyoma Rhabdomyoma	Leiomyosarcoma Rhabdomyosarcoma
Tumors of Epithelial Origin		
Stratified squamous Basal cells of skin or adnexa Epithelial lining of glands or ducts Respiratory passages Renal epithelium Liver cells Urinary tract epithelium (transitional) Placental epithelium Testicular epithelium (germ cells)	Squamous cell papilloma Adenoma Papilloma Cystadenoma Bronchial adenoma Renal tubular adenoma Liver cell adenoma Transitional-cell papilloma Hydatidiform mole	Squamous cell carcinoma Basal cell carcinoma Adenocarcinoma Papillary carcinomas Cystadenocarcinoma Bronchogenic carcinoma Renal cell carcinoma Hepatocellular carcinoma Transitional-cell carcinoma Choriocarcinoma Seminoma Embryonal carcinoma
Tumors of Melanocytes	Nevus	Malignant melanoma

Understanding Grading & Staging of Tumours:

- **Why?**
  - Assessment of Prognosis
  - Assessment of likely response to treatment.
  - Provides a basis for communication between treatment centres worldwide.

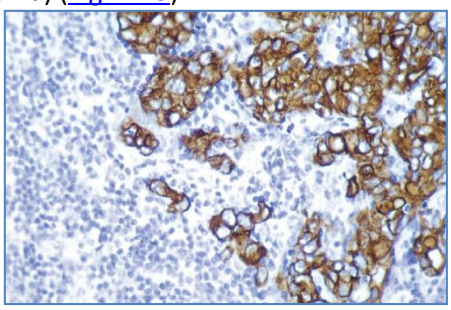
- ***Grading* Malignancy of Neoplasms:**
  - **NB: Classification is based on Histological Appearance:**
    - Level of Anaplasia (De-Differentiation)
    - Level of Mitosis (Ie. Number of Mitotic Bodies in a High Power Field.)
  - **– Grade 1:**
    - Well Differentiated (Low Anaplasia)
    - Few Mitosis (<1 Mitotic Body per High-Power Field)
  - **– Grade 2/Grade 3:**
    - Intermediates.
  - **– Grade 4:**
    - Poorly Differentiated (Highly Anaplastic)
    - Abundant Mitosis (>6 Mitotic Bodies per High-Power Field)

- ***Staging* Neoplasms:**
  - **NB: Criterion Vary for each Specific Form of Neoplasm, but Typically Include:**
    - Size of Primary Lesion
    - Extent of Spread to Regional Lymph Nodes.
    - Presence/Absence of Blood-Borne Metastases.
  - **The 'TNM' System:**
    - **T – (Size of Primary Tumour):**
      - T0 = In Situ Lesion
      - T1 = Less than 5cm Diameter
      - T2 = More than 5cm Diameter
      - T3 = Bigger
      - T4 = Even Bigger
    - **N – (# of Regional Lymph Nodes Involved):**
      - N0 = No Lymph Node Involvement
      - N# = # number of Lymph Nodes Involved.
    - **M – (Metastases?):**
      - M0 = No Metastases
      - M1, M2, M3 = Presence of Metastases.

- **Combining Grading & Staging:**
  - **Expressed in the following Format:**
    - Eg. Grade 1 – T1 (≈2cm), N0, M0. (Ie. Relatively Benign)
    - Eg. Grade 4 – T3 (≈7cm), N2, M1. (Ie. Highly Malignant)

Laboratory Diagnosis of Cancer:

- **Immunohistochemistry:** The use of specific antibodies identify cell products or surface markers of Neoplasms:
  - *Categorization of De-differentiated Malignant Tumors:*
    - In many cases, different malignant tumors resemble each other because of dedifferentiation.
      - However, they must be accurately identified because often their treatment and prognosis are different.
    - Tumors that can't be distinguished by routine Hematoxylin-&-Eosin (H&E)–Stained Tissue Sections often have the same Intermediate Filaments as their Original Tissue, which can be distinguished by Specific Antibodies.
      - Eg. Cytokeratins, (detected by immunohistochemistry), point to an epithelial origin (carcinoma) (Fig. 7-49)



      - Eg. Desmin is specific for neoplasms of muscle cell origin.
  - *Detection of molecules that have prognostic or therapeutic significance:*
    - Eg. Immunohistochemical detection of Estrogen & Progesterone receptors in breast cancer cells is of prognostic and therapeutic value because these cancers are susceptible to anti-estrogen therapy.

- **Flow Cytometry:** Used in conjunction with Immunohistochemistry - To Identify Cell Characteristics:
  - Eg. Cell Surface (Membrane) Antigens
  - Eg. DNA Content of Tumour Cells.

- **Molecular Diagnosis:**
  - For Diagnosis & Prognosis of Malignant Neoplasms:
    - Eg. FISH (Fluorescence In-Situ Hybridisation)
    - Eg. PCR (Polymerase Chain Reaction) & DNA Analysis to check for Genomic Mutations.
  - NB: Also useful for Diagnosis of *Hereditary Predispositon to Cancer*.
    - (Eg. BRCA1/2 & RB Gene Mutations)

- **Tumour Markers:**
  - Biochemical Assays for Tumour-Associated Enzymes, Hormones & Other Tumour Markers IN THE BLOOD can *Contribute* (But not definitively) to the Diagnosis of Cancer in some instances.
  - Common Markers Include:
    - CEA → Carcino-Embryonic Antigen (many neoplasms)
    - PSA & PSMA → Prostate Specific Antigen (Prostate Cancer)
    - HCG → Human Chorionic Gonadotropin (In some Testicular Cancers)

GLS Questions

– Regarding: *NON-Histological* Detection of Neoplasms:

1. **Sometimes, poorly differentiated malignant neoplasms are difficult to categorize based on histological examination (in haematoxylin and eosin-stained tissue sections).**

- **What substance present in neoplastic cells and detected using immuno-histochemical methods identifies the neoplasm as being of epithelial origin?**
  - Certain characteristics from the cell of origin are preserved
    - Intermediate filaments are often characteristics of their cell of origin → cytokeratins are intermediate filaments of epithelial cells

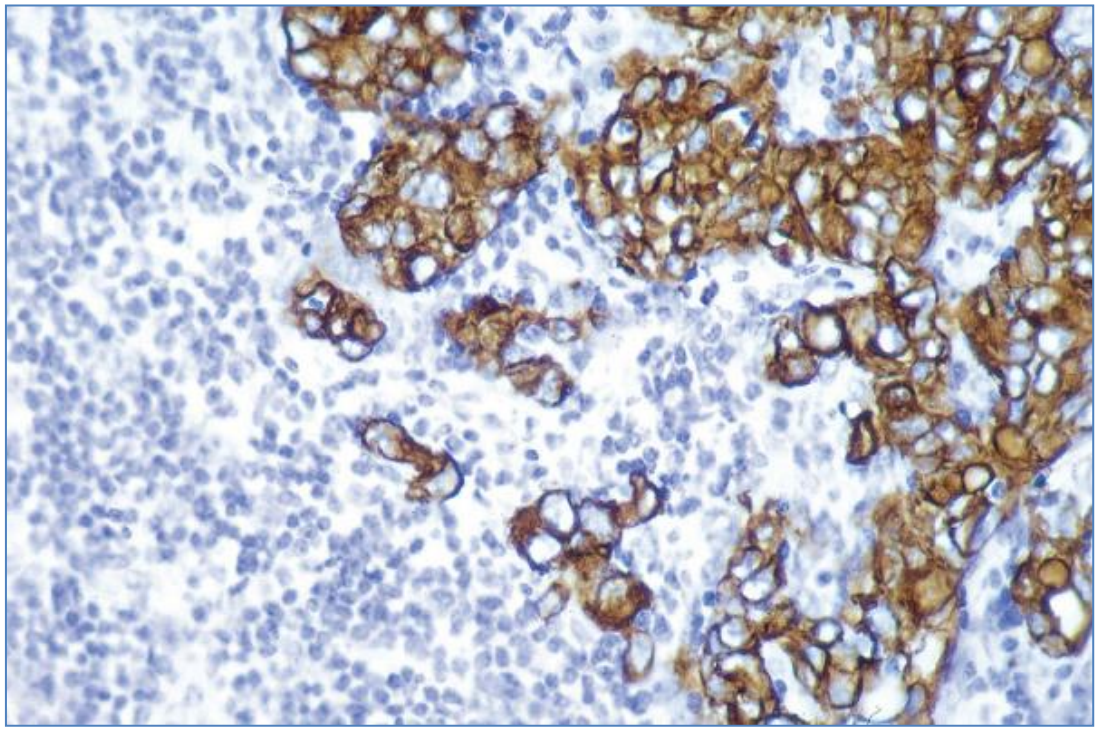


FIGURE 7-49 Anti-cytokeratin immunoperoxidase stain of a tumor of epithelial origin (carcinoma).

2. **One well- studied gene that is a common target for genetic alterations in human tumours is the p53 gene. What is the function of p53 and how do mutations of this gene potentially lead to the development of neoplasms?**
   - *Function*: tumour suppressor gene
     - p53 accumulates when DNA is damaged → arrests cell cycle
     - Prevents mutated cells from replicating
       - ⇨ allows time to repair
       - ⇨ triggers apoptosis (via caspases)
   - Acts through cell cycle inhibitor (p21)
   - If absent → potential for damaged/ mutated cells to replicate - ↑ in oncognes

   (Robbins 292, 31)

Cancer, Cell Death & Cellular Ageing

Growth Characteristics of Normal Cells:

- Subject to **contact inhibition**
- Limited **lifespan**
- **Anchorage dependant**
- **Growth-factor** dependant
- **Able** to **apoptose**.

Growth Characteristics of Tumour & Cancer Cells:

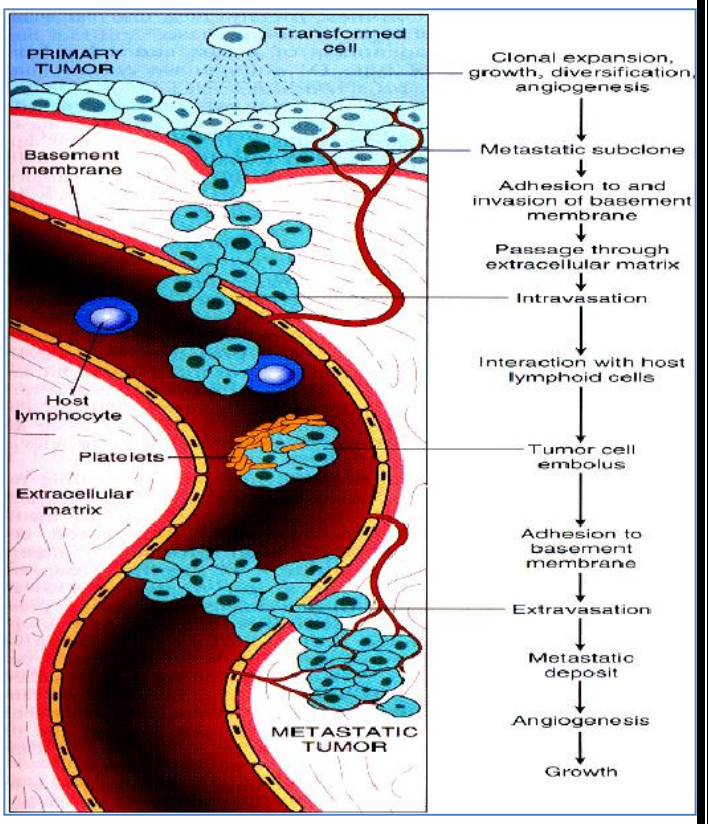
- **Not** subject to **contact inhibition**
- Unlimited **lifespan**
- **Anxhorage *In*dependant**
- **Unresponsive** to **growth-inhibitors**
- **Unable** to **apoptose.**
- **Differentiate** independently.
    - Differentiated tumours = **teratomas**.
    - May form teeth, hair, bone, nails, toes, brain matter etc.

General Characteristics of BENIGN TUMOUR CELLS:

- **Lower mitotic index than cancerous tissue.**
- **Well-defined capsule**
- **NOT INVASIVE**
- **Well differentiated** – still exhibit characteristics of their normal cells of origin.
- **NOT METASTATIC**

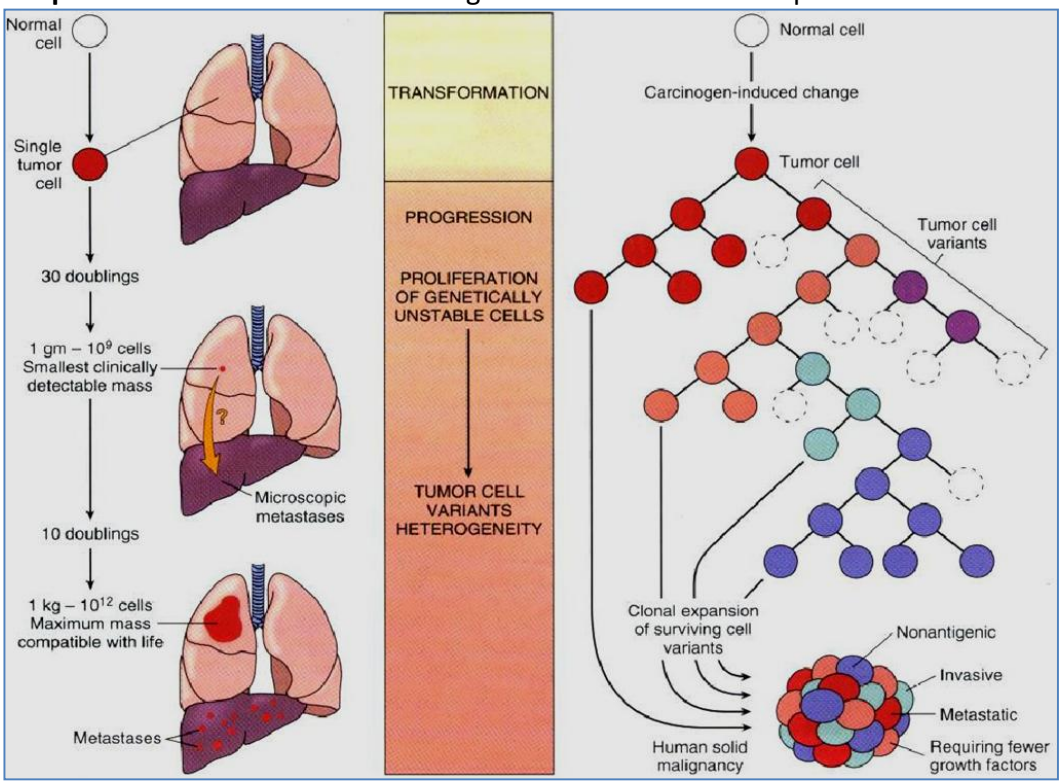
General Characteristics of METASTATIC CANCERS:

- **Abnormally high mitotic rate**
- **Show signs of de-differentiation**
    - have features of *primordial stem-cells*
- **Disordered growth patterns**
    - Grow as a chaotic mass in all directions
- **Can be Metastatic (Colonise distant tissues)**
    - Cells can break away from primary tumour & travel through blood/lymph.
    - Establish new tumours (secondaries) called **metastases.**
- **Show gross genetic abnormalities.**
    - Aberration in chromosome number
    - Deletions, translocations in genome
- **Grow in the absence of growth factors.**
- **Are Immortal**
    - Escape cellular ageing (senescence)
    - Many also don't apoptose
- **Malignant phenotype is heritable.**
    - Cancer cells propagate through many mitotic divisions without losing cancerous features.



Cause of Cancer

- Genetic **mutations** that are **non-lethal** to the cell.
- Results from **mutagens**:
  - Chemicals
  - Radiation
  - Carcinogens
  - Free-Radicals
  - Microbes (viruses)
  - Inherited.
- **DNA damage** = dysregulated growth patterns →uncontrolled proliferation.
  - **Damage** to **regulatory genes:**
    - Results in **loss / gain** of **function** of:
      - **DNA repair Genes**
      - **Cell Ageing Genes**
      - **Protooncogenes**
      - **Growth-inhibiting (anticancer) Genes**
      - **Apoptosis Genes**
- **Clonal expansion:** cancers arise from a single cell with uncontrolled proliferation.



Defects in DNA Repair Genes:

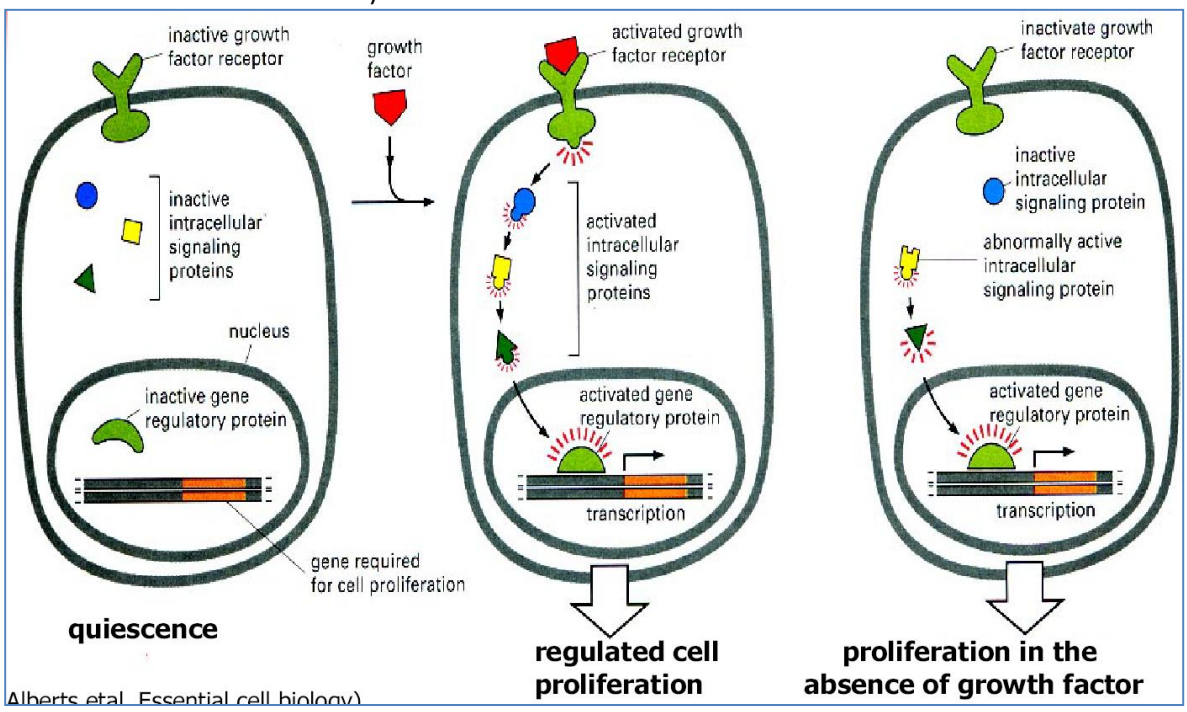
- Genetic mutations happen all the time.
- However, DNA is repaired by the cell.
- Defective DNA repair can lead to uncorrected mutations → cancer

Defects in Cell Ageing Genes:

- Cellular age is determined by the number of divisions.
- When cells age, they enter **senescence:** a terminal non-dividing state.
- Mutations that enable the cell to avoid senescence → cancer.

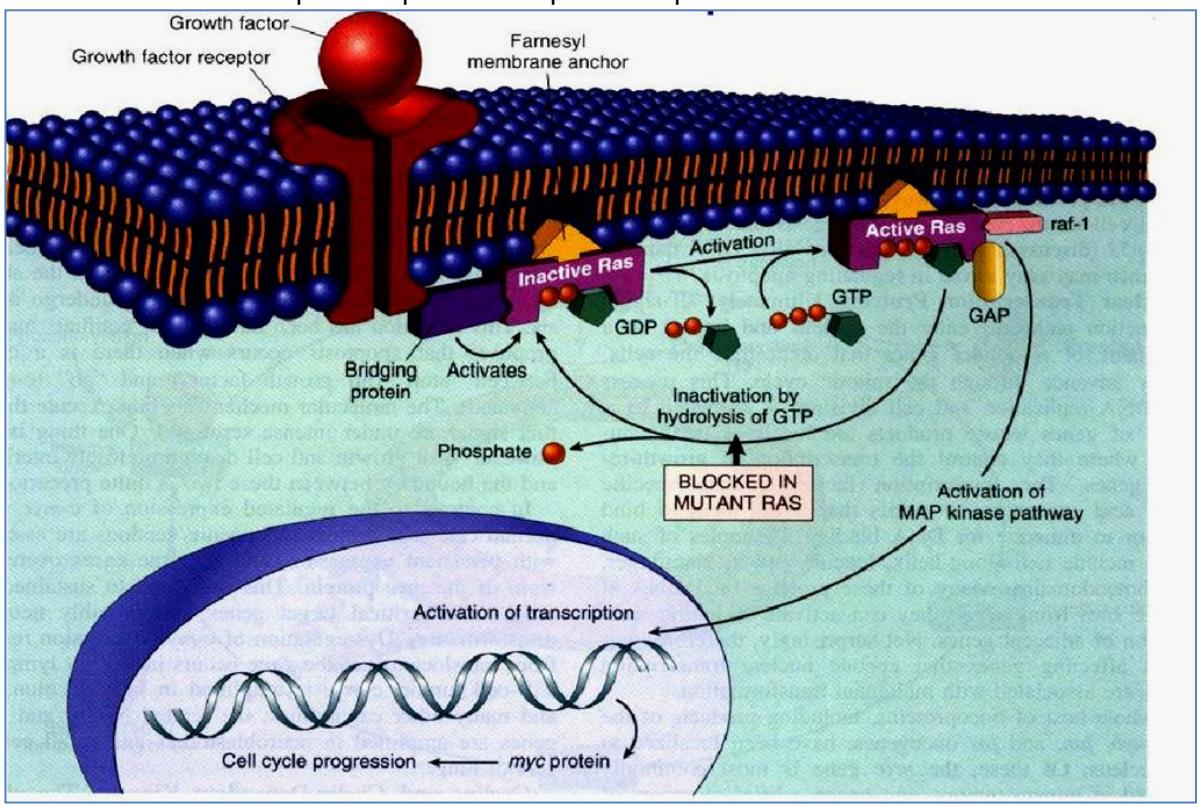
Gain of Function in a Proto-oncogene:

(**Proto-oncogene**: a normal gene that can become an **oncogene** due to mutations or increased expression. An **oncogene** is a protein encoding gene, which — when deregulated — participates in the onset and development of cancer. **Proto-oncogenes** code for proteins that help to regulate cell growth and differentiation →such as Ras)



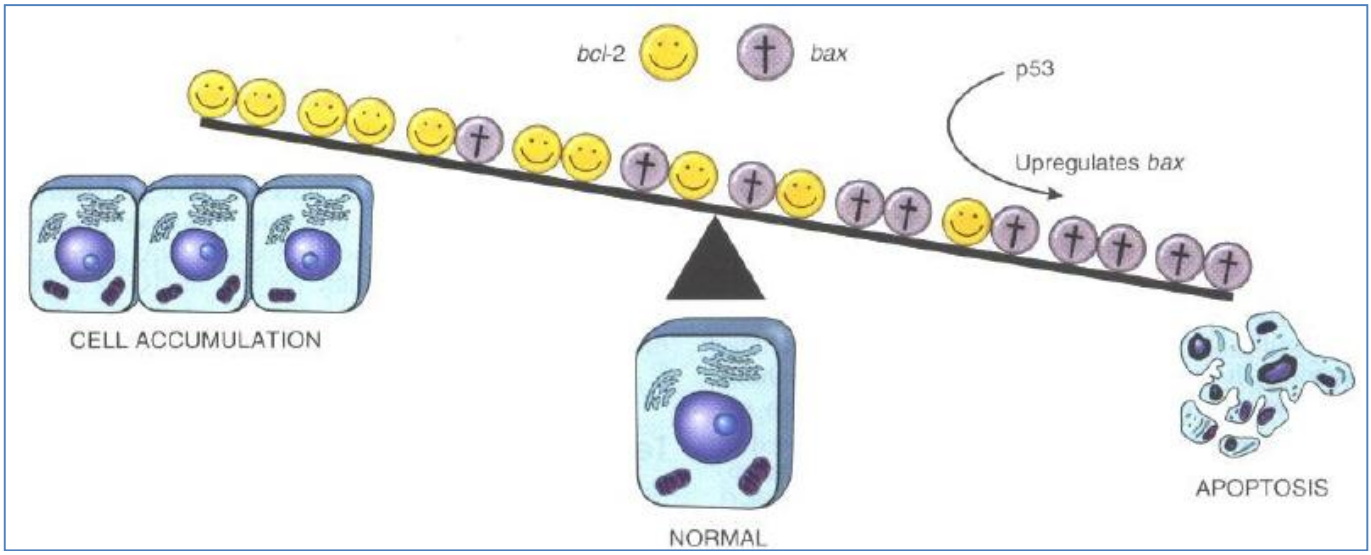
Oncogenic activation of Ras:

- Active Ras protein loses its hydrolysing ability and cannot be turned off.
- Results in over-transcription of proteins required for proliferation.



Abnormal Expression of Apoptosis-Regulating Genes: (Bcl-2)

- Over-expression of **anti-apoptotic regulators**
- Under-expression of **pro-apoptotic regulators (Bax & Bak) → initiates the CASPASE CASCADE**
    - Leads to abnormalities in regulation of cell proliferation.

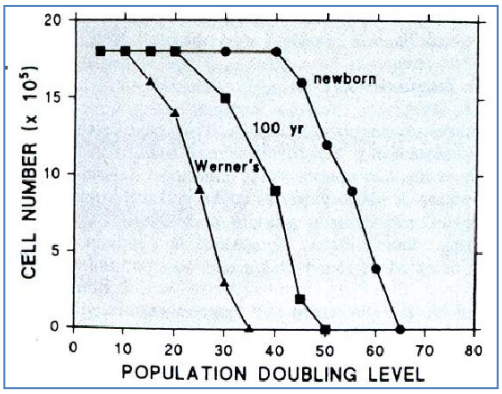


Cell Ageing

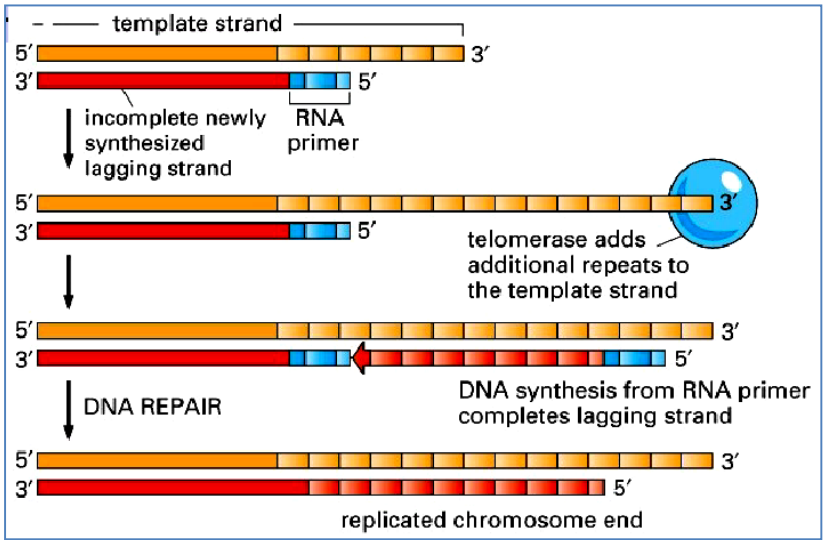
- Progressive alterations in structure → loss of functional capacity (senescence) ending in death.

- **On a Cellular Basis:**
    - **Changes in Structure & Function:**
        - **Decrease in:**
            - rate of mitochondrial oxidative phosphorylation
            - nucleic acid synthesis
            - synthesis of proteins (structural/enzymes/receptors/transcription factors)
            - effectiveness of DNA repair mechanisms
        - **Increase in:**
            - incorrectly folded proteins
            - irregularly shaped nuclei.
        - **Changes in organelle structure & function**

- **Senescence:**
    - Irreversible arrest of cell division in G1 phase.
    - Non-responsive to mitogens
    - Abnormalities in morphology, metabolism & functions
    - Increased resistance to apoptosis
    - Correlation between # of divisions & senescence.
        - Suggests that # of divisions is limited & decreases with age.



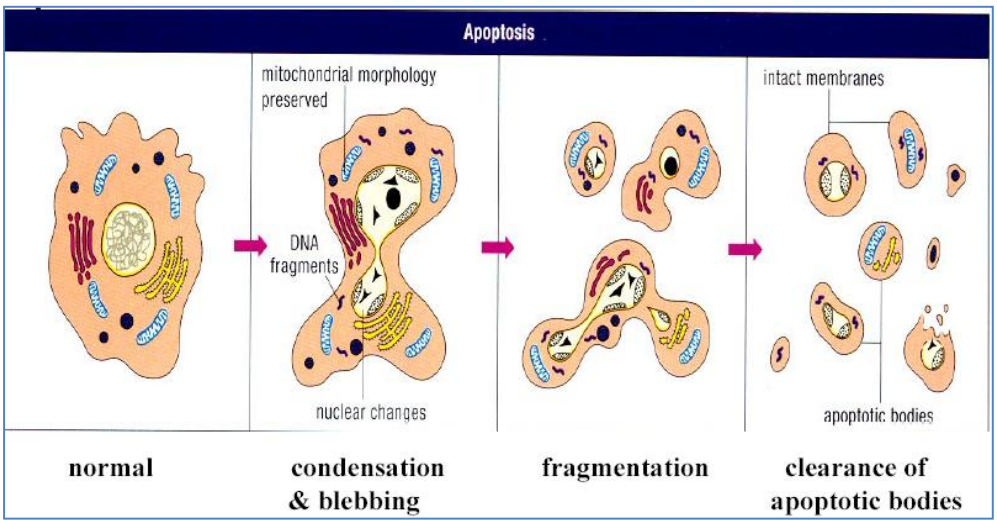
- **Cellular Clocks (the cause of senescence??):**
  - **Telomere Replication:**
    - **Telomere:** *The non-coding end-region of a chromosome-protects the start of the **coding sequence** from shortening during successive replications.*
    - However, each time DNA replication takes place, the telomere itself gets shorter.
    - **Telomerase** counters this by adding repeats to the template strand, allowing telomere elongation on the new DNA strand.
    - Unfortunately, **telomerase activity decreases with # of cell divisions**.

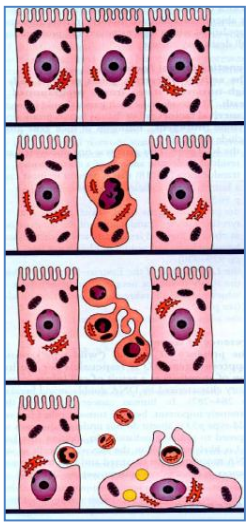


Cell Death:

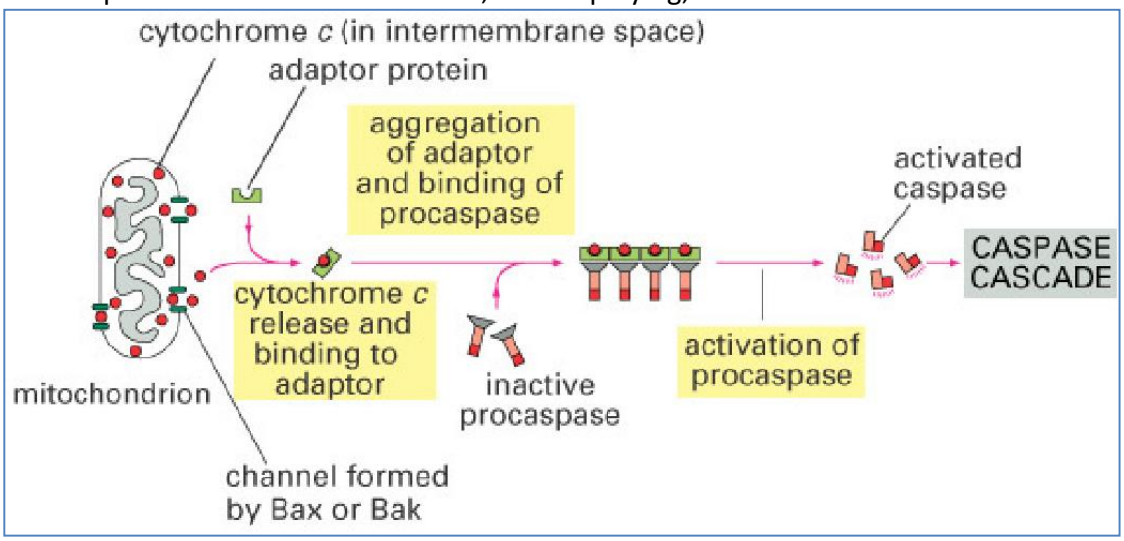
- **Apoptosis**

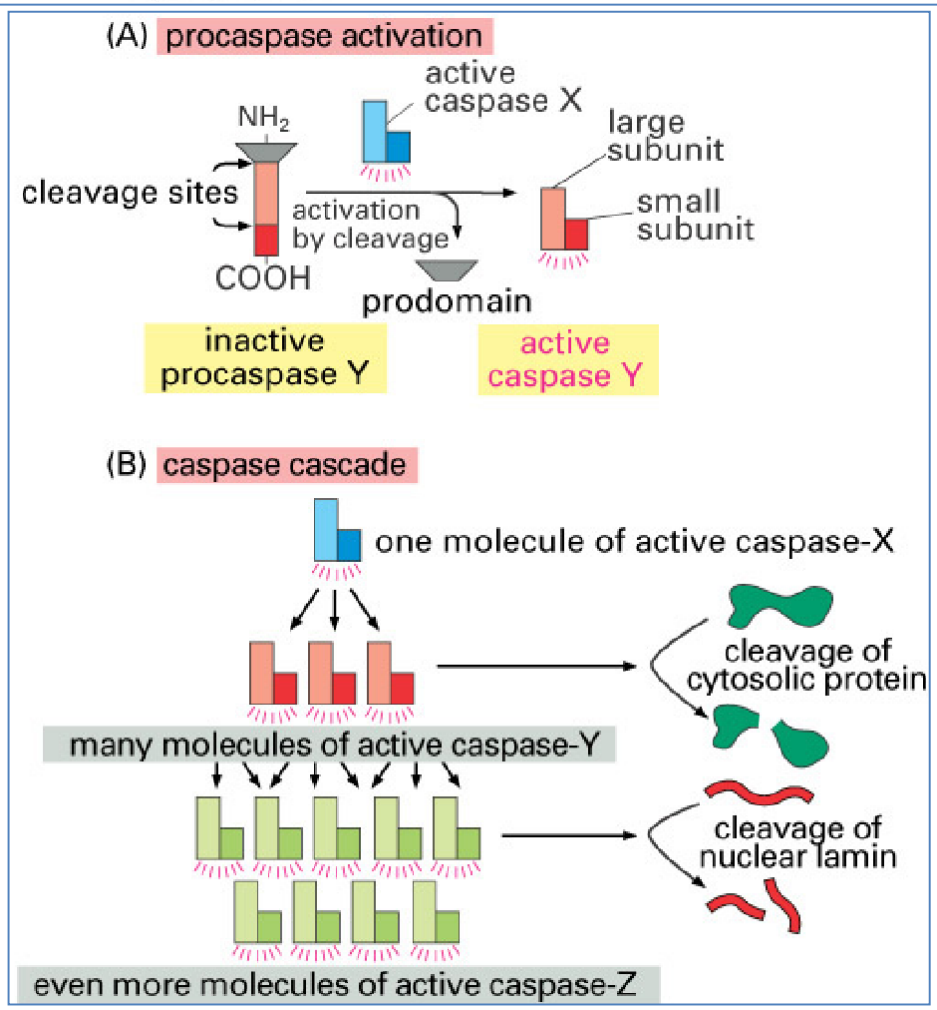
- Cells that undergo apoptosis shrink & condense, dying neatly without damaging neighbours.
  - Normal process
  - Regulated
  - Energy dependent
  - Enzyme dependent (requires gene expression)
  - Ordered disassembly
  - Minimal inflammation
  - Minimal scarring
  - Apoptotic bodies attract phagocytes (eg. Macrophages) and are engulfed/phagocytosed.





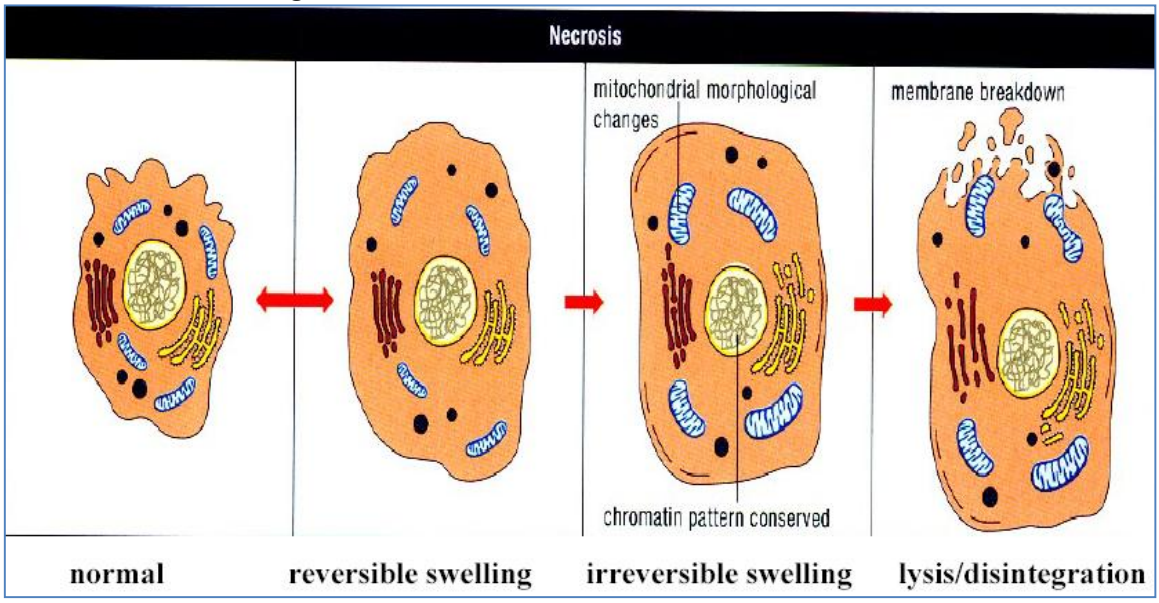
- **Regulated by CASPASES**:
  - Proteolytic enzymes that cut up proteins & nuclear laminin. (nuclear envelope)
- Initially caspases are created as inactive enzymes called **procaspases**.
- The activation of procaspases is regulated by the **Bcl-2** protein family **(Bax & Bak)**.
- Bax & Bak increase outer-mito-membrane permeability → releases **cytochrome-C** into cytosol
- **Cytochrome-C** then binds to **adapter proteins→activates procaspases→acitive caspases**
- Active **caspases activate other procaspases** →causes an explosive chain reaction (cascade).
- Caspase cascade → is destructive, self-amplifying, and irreversible.





- **Necrosis**

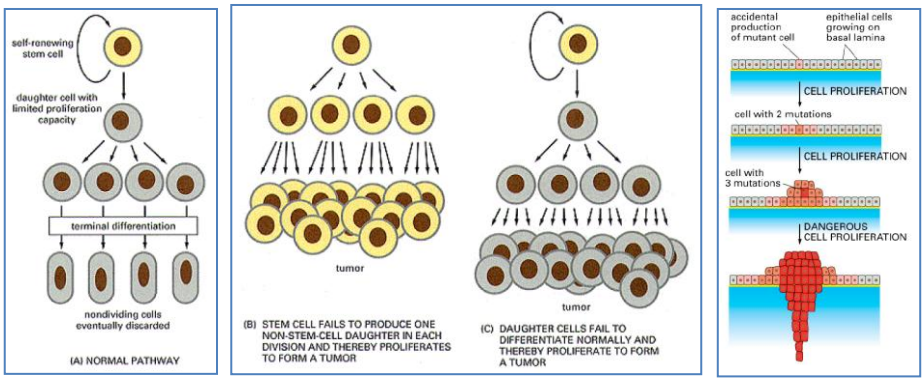
- Cells that die from acute injury typically swell & burst, spilling their contents.
    - Abnormal process (pathophysiological)
    - Unregulated
    - Energy independent
    - Enzyme independent (doesn't require gene expression)
    - Chaotic destruction
    - Activates inflammatory response
    - Results in scarring.



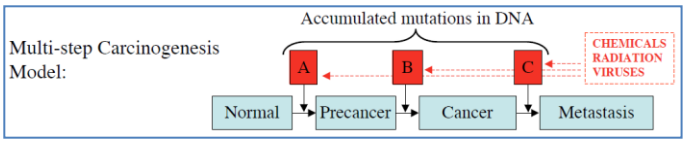
ONCOLOGY Pathology:
CANCER PATHOGENESIS

Review Of Carcinogenesis:

- **Cancer Cells are *"Clonal"*:**
  - **Cancer cell populations arise from a Single Progenitor Cell**



- **Cancer is a Genetic Disease at its Fundamental Level, But with an Environmental Component:**
  - **Results from an *Accumulation* of *Mutations* in DNA.**



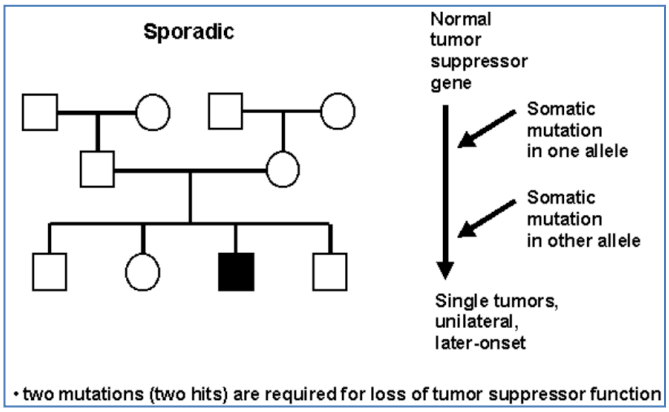
Involves Activation of *Proto-Oncogenes* and/or Inactivation of *Tumour-Suppressor Genes*:

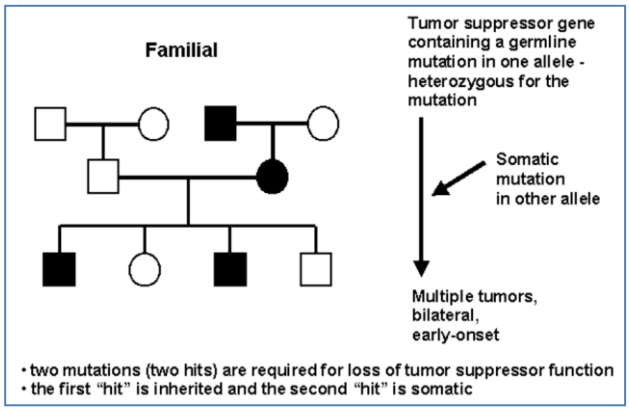
- **1. Gatekeepers (*AKA: PROTO-ONCOGENES*):**
  - **(Genes that Control Cellular Proliferation)**
    - **Normal Functions of Proto-Oncogenes:**
      - Growth Factors Production
      - Growth Factor Receptors
      - Cell Cycle & Apoptotic Proteins
      - Transcription Factors
  - **However, in *Tumours,* Proto-Oncogenes are *Mutated* to '*Oncogenes'*:**
    - Oncogenes → Uncontrolled Cell Division.
  - **ACT DOMINANTLY – (only 1 mutated Proto-oncogene is required for cancer)**
- **2. Caretakers (*AKA: TUMOUR-SUPRESSOR GENES*):**
  - **(Genes that Maintain Genome Integrity)**
    - **Normal Functions of Tumour Suppressor Genes:**
      - DNA-Damage Sensing Pathways (**P53** & RB)
      - DNA-Repair Pathways (BRCA1 & 2)
      - Cell Cycle Regulation
      - Apoptosis (if DNA damage is irreparable)
      - Telomere Shortening (Ie. Limits number of possible divisions)
      - **Ie. The *Brakes of the Cell Cycle*.**
  - **However, in *Tumours*, Tumour Suppressor Genes are *Mutated*.**
    - → No Brakes on the cell cycle → Cancer-Cell Division is Unopposed.
  - **ACT RECESSIVELY (KNUDSON'S 2-HIT) – (Requires *2 "Hits"* OR *Loss of Heterozygosity* for Cancer).**
  - **(NB: Abnormal P53 Function (** The Most Common Feature of Cancer Cells) → Cancer):**
    - NB: 50-60% of ALL Tumours Show *Loss of P53 Function.*
    - Cells with Abnormal P53 cannot Arrest the Cell Cycle if DNA Damage is present.
      - → Cells Replicate with Damaged Chromosomes.

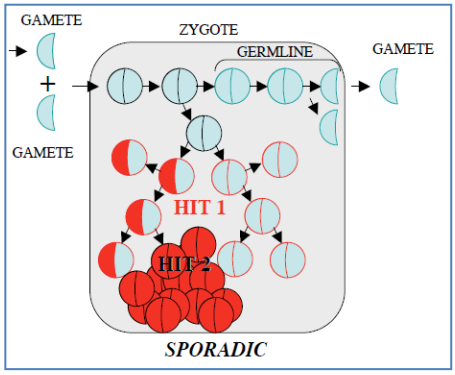
- **3. Landscapers:**
    - **(Cell-Cell Interaction Genes = Ie. Genes involved in Cell-Cell Adhesion & Cell Movement)**
    - **NB: If Defective –** Enables cancer cell to ***Metastasise*** to other tissues.

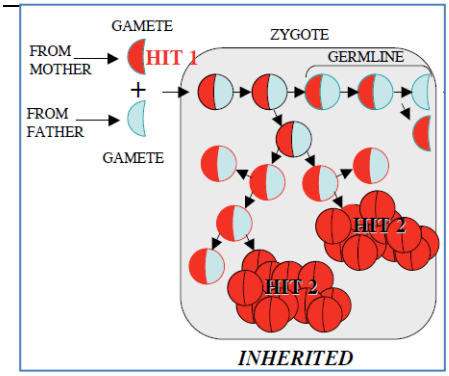
Knudson's 2-Hit Hypothesis:

- **Sporadic** – 2 Mutations ('Hits') in a Tumour Suppressor Gene are required for Cancer.
- **Familial** – All Body Cells already have the 1st hit → only require 1 more 'Hit' for Cancer.
- **NB:** One of the "*Hits*" can be a ***"Loss of Heterozygosity"*** in Favour of the Mutated Tumor Suppressor Gene.









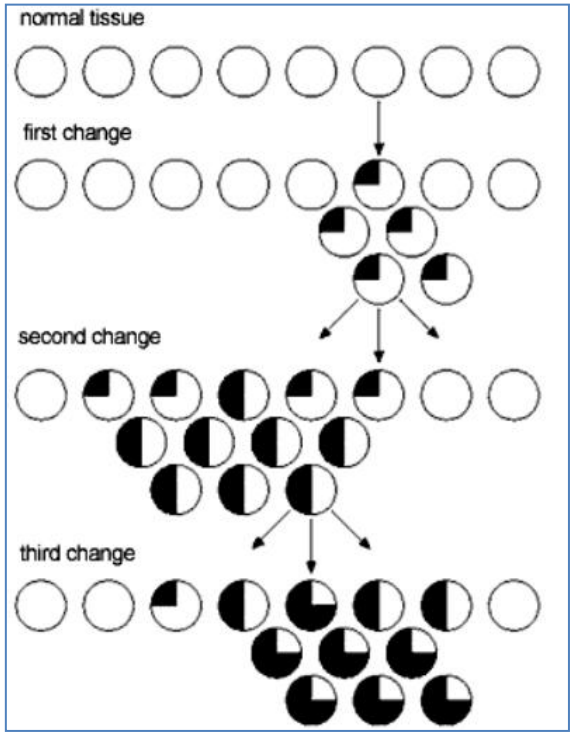
The Genetic Basis of Cancer

Why do people die of Cancer ?

- Cancer cells Infiltrate Organs/Tissues → Prevent organs from functioning normally.
- Cancer is a disease of misbehaving cells that grow in the wrong place and the wrong time.
- **Stats – For your interest only:**
  - Mortality:
    - ~10% caused by the 1° tumour
    - ~90% attributable to metastasis and only
    - (Depends on type of cancer)
  - In about ~50% of surgically removed cancers reappear on average.
  - Metastatic disease is only curable in around ~10% of cases. (by Chemotherapy/Radiation Therapy)

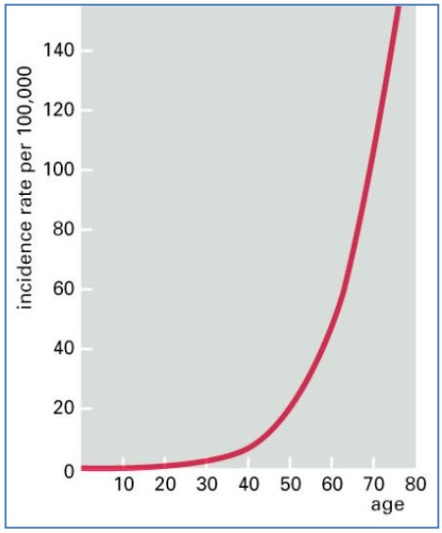
Cancer - A Game of Probabilities?:

- **Fact: Millions of Cells Divide every Second:**
  - Therefore billions of DNA bases are copied every second.
  - Therefore, even with a *low* mutation rate, Many Mutations WILL Occur.
- **So Why Doesn't *Everybody* have Cancer?:**
  - Cancer Requires *at least* 4 Consecutive Mutations *AND* only in Certain Genes.
  - Therefore, the chance of this happening to any 1 cell is almost *Zero %*.
- **So How Does Cancer Defy the Odds?**
  - **Cancer is common because of the *Combination* of the following 2 Factors:**
    - **A) Some Mutations *Enhance* the rate of Cell Proliferation:**
      - → Expanded Population of Cells in which Mutation is More Likely.
    - **B) Some Mutations cause *Genome Instability*:**
      - → Acceleration in Mutation Rate

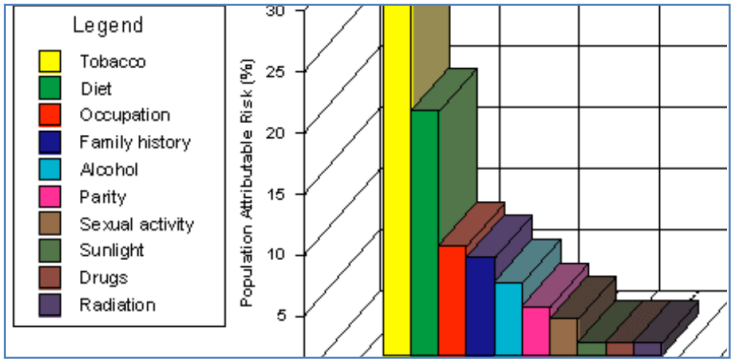


Carcinogenesis is a Multi-Step, Multi-Factorial Process:

- **Typically occurs over long periods of time - Hence, Cancer Risk increases with AGE.**



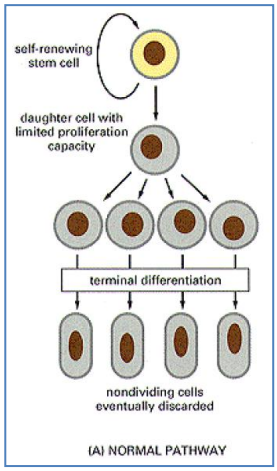
- **Cancer has an Environmental Component:**
  - Agents that increase Mutation Rates → Increase risk of Cancer.
  - NB: Some agents (eg. Tobacco & Alcohol) are Synergistic.

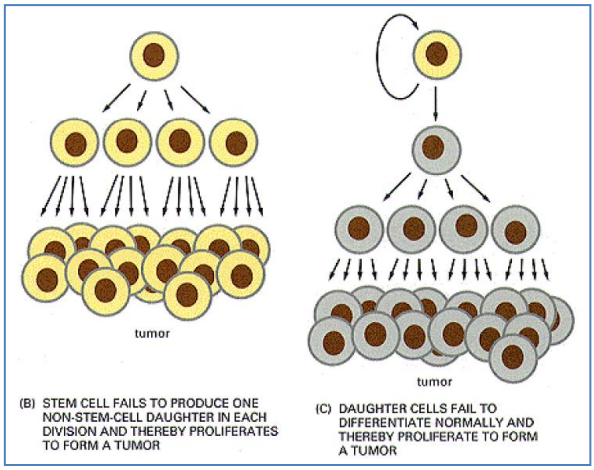


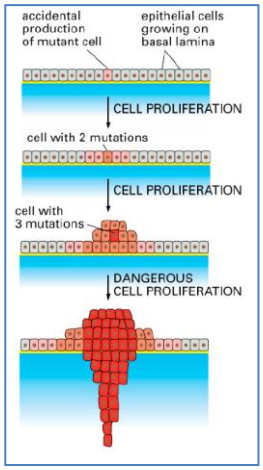
- **Viruses are also known to Cause Cancer:**
  - **NB:** Viruses typically infect ***Resting*** Cells (Ie. Those which aren't dividing)
    - But the cell needs to be replicating DNA for the Virus to Reproduce.
  - Therefore, Viruses produce proteins which ***Inhibit RB*** → *Force the Cell to divide*.
    - However, the P53 protein can '*sense*' disrupted RB-Pathway and Induce Apoptosis.
  - Therefore, Viruses also produce proteins which ***Inhibit P53*** → Avoid Apoptosis.

VIRUS	ONCOGENE	FUNCTION
Human Papilloma	E6, E7	Inactivate p53 & Rb
Adenovirus	E1A, E1B	Inactivate p53 & Rb
Simian Virus 40 (SV40)	Large T antigen	Inactivate p53 & Rb
Epstein-Barr	BZLFI, EBNA5	Binds p53, activates myc
Hepatitis B	HBX	Binds p53

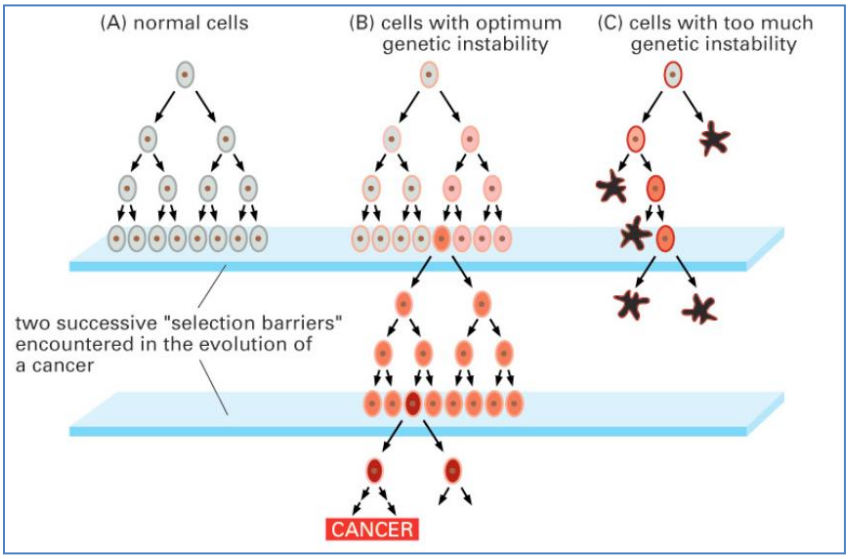
- **Cancer Cells are *"Clonal"*:**
  - **Cancer cell populations arise from a Single Progenitor Cell**
    - Either: Stem Cells fail to produce Daughter Cells in each division → Tumour.
    - OR: Daughter Cells Differentiate Abnormally → Tumour
    - (NB: May be a combination of both)

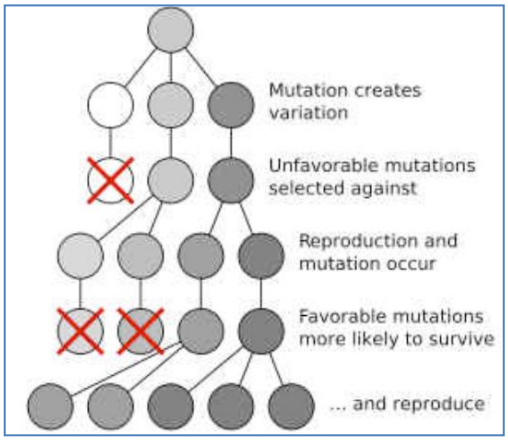






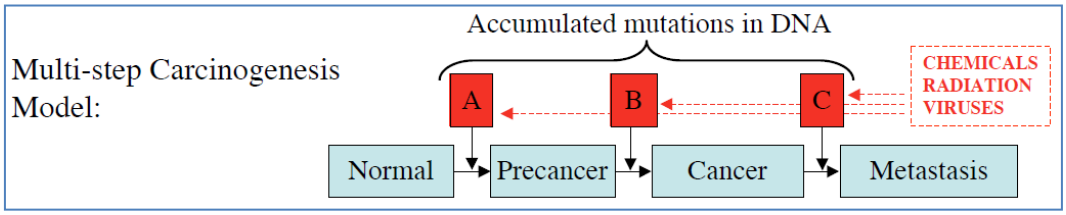
- **Cancer Cells Undergo Selection for Specific Traits:**
  - **Traits Include:**
    - Increased Proliferation Rates → ↑Mutation Rate
    - Increased genome instability → ↑Mutation Rate
    - Tissue invasion & Metastasis
  - **NB:** Cancer cells that are *Too* Genetically Unstable aren't compatible with life → are *Selected Out*.
    - Conversely, mutations causing faster growth will *'Out-Compete'* normal cells.



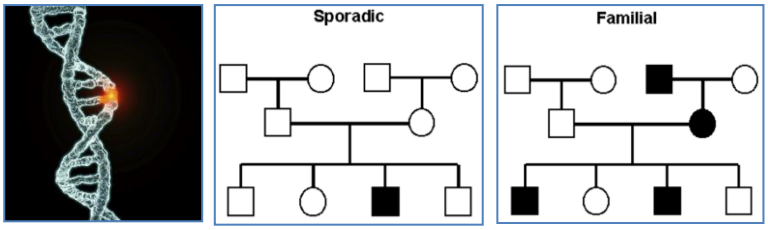


Cancer is a Genetic Disease at its Fundamental Level:

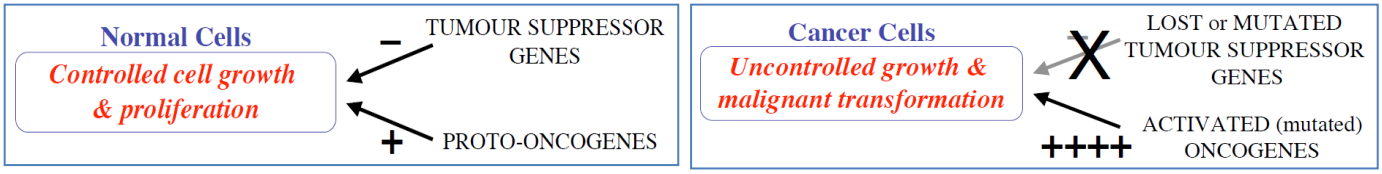
- **Typically results from an Accumulation of Mutations in DNA.**
  - Most cancers often require 4 or more *Sequential Somatic Mutations* of *Several Particular Genes*.



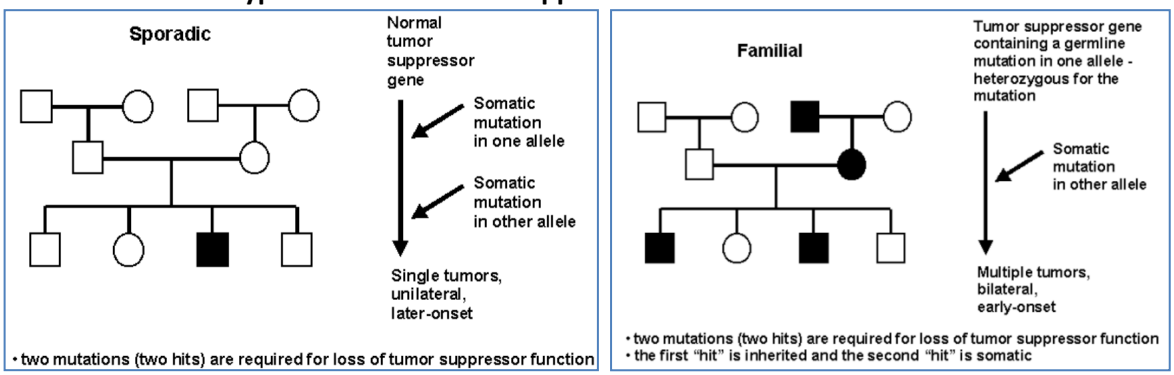
- **May be Hereditary (Inherited Genetic Predisposition) or Non-Inherited (Spontaneous/Sporadic):**



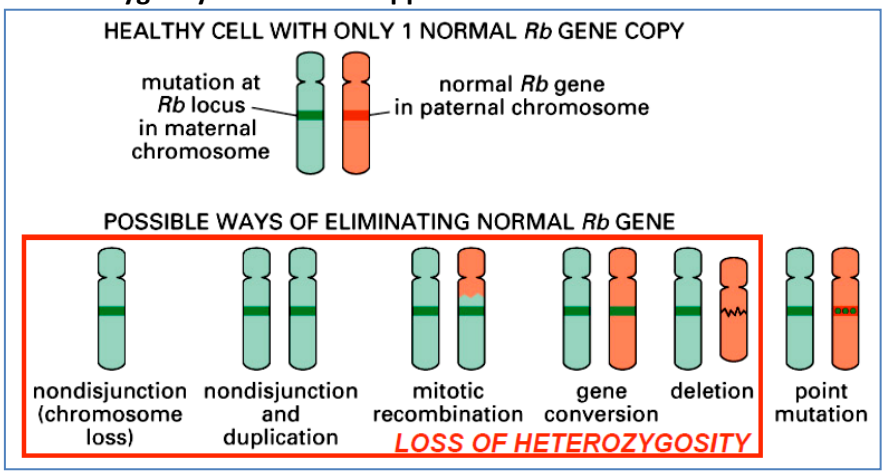
- **Involves Activation of Proto-Oncogenes and/or Inactivation of Tumour-Suppressor Genes:**



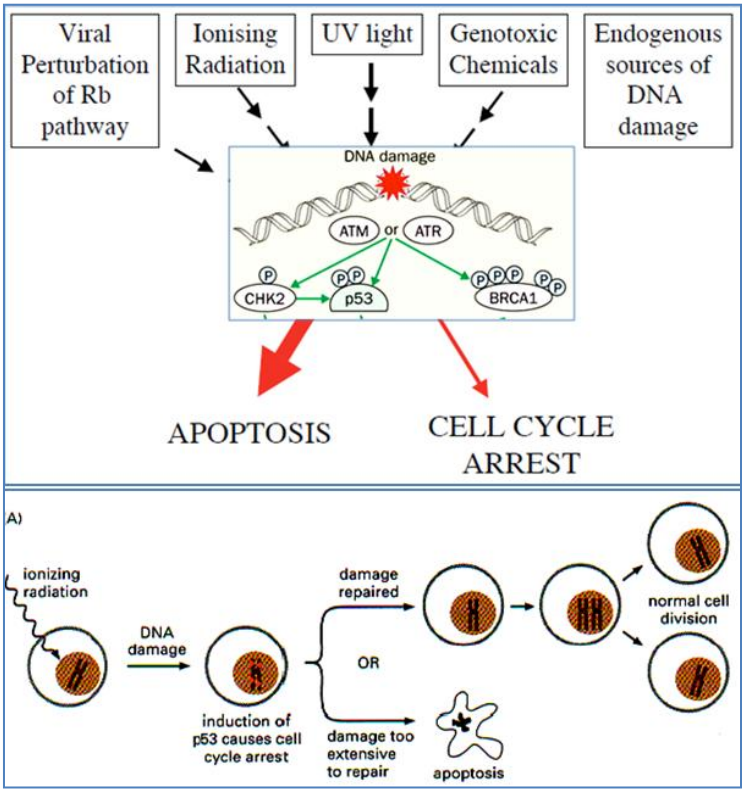
  - **Knudson's 2-hit hypothesis for Tumour Suppressor Genes:**

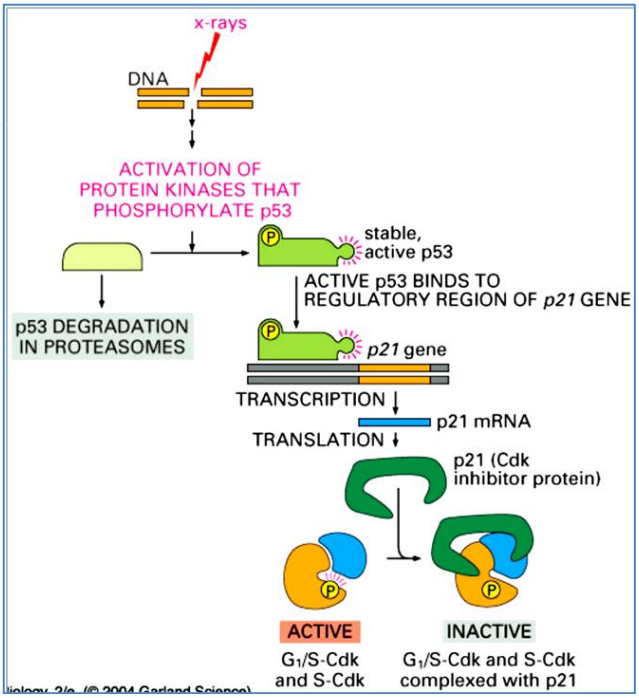


  - **Loss of Heterozygosity for Tumour Suppressor Genes:**

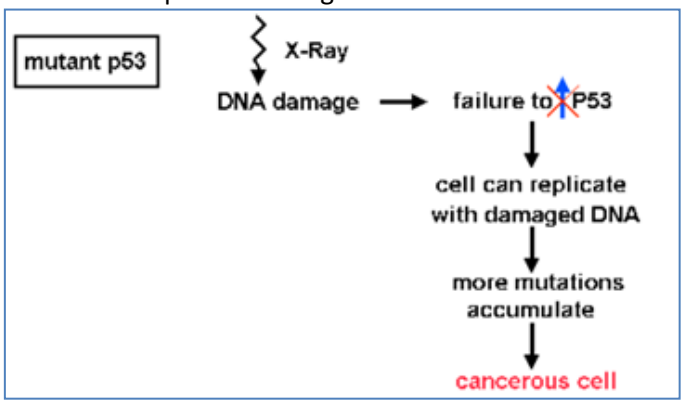


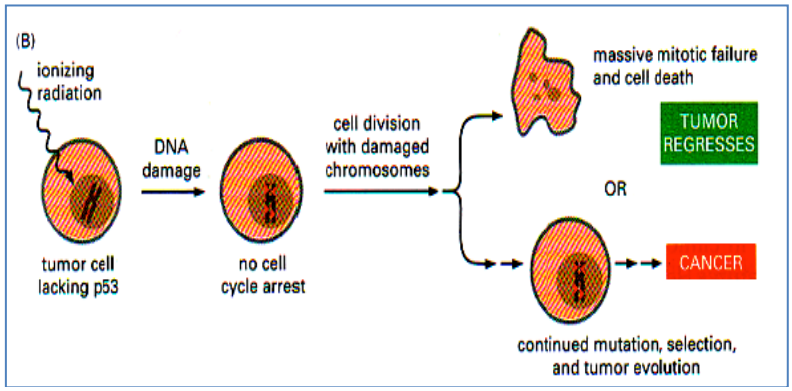
- ***P53 is the Gene Most Frequently Mutated In Cancer:**
  - **Normal Function of P53:**
    - "*The Guardian of the Genome*"
    - Function of P53 is to '*Sense*' any DNA Damage or Potentially DNA-Damaging Stimuli. (NB: DNA Damage activates ATM or ATR, which activate P53):
    - → P53 Arrests Cell-Cycle (By triggering P21 Transcription, which Inactivates S-Cyclin-CDK → Stops DNA Replication)



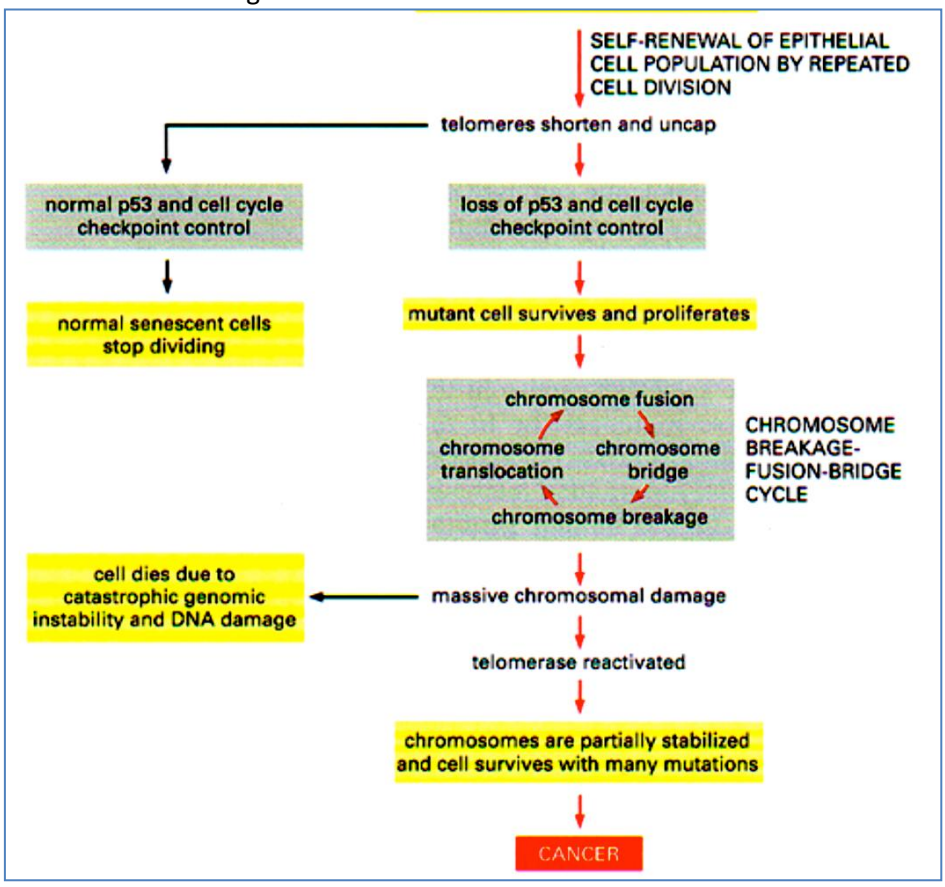


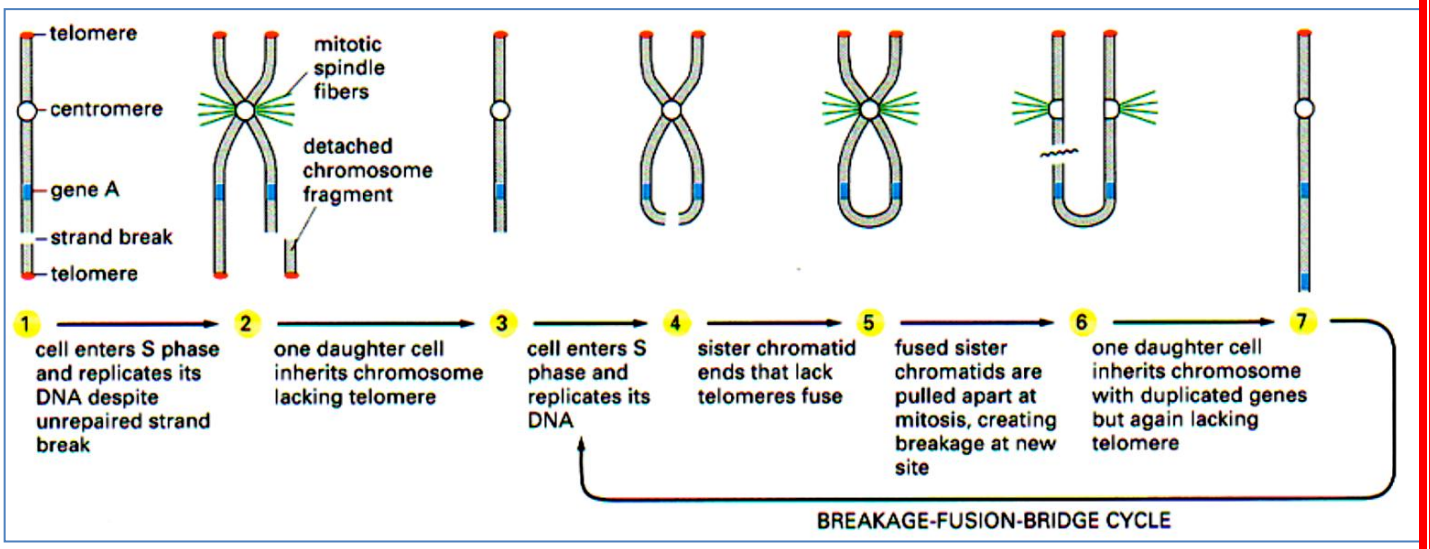
  - **Abnormal P53 Function → Cancer:**
    - NB: 50-60% of ALL Tumours Show *Loss of P53 Function.*
    - Cells with Abnormal P53 lose the ability to Arrest the Cell Cycle even if DNA Damage is present. → Cells Replicate Damaged Chromosomes.





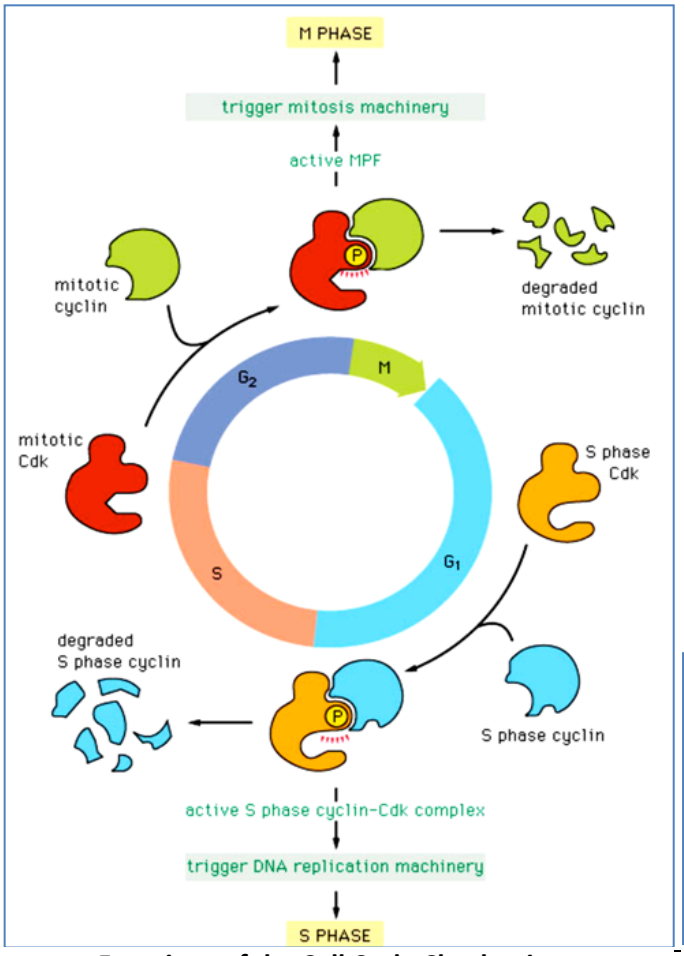
- **Chromosome Instability – "*The Chromosome Breakage-Fusion-Bridge Cycle*":**
  - **(Ie. Defective P53 also contributes to Generation of Abnormal Karyotypes) –** (In addition to being unable to arrest the cell cycle if DNA damage occurs)
  - **If DNA Repair Mechanisms are Defective:**
    - If Defective P53 *Doesn't Recognise* Random DNA Strand Break → Cell Replicates with Defective Chromosomes.
  - **→ Accelerates Accumulation of Mutations in Cancer Cells:**
    - Daughter Cell Inherits a Chromosome without a Telomere.
    - When the Daughter Cell enters S-Phase, the ends of the replicated Chromosomes Fuse Together → Forming a 'Bridge'.
    - When the Sister Chromatids are pulled apart by the spindle fibres in Anaphase, a *Different Random DNA Break* occurs due to mechanical force.
    - Now *BOTH* Daughter Cells inherit chromosome without telomeres.

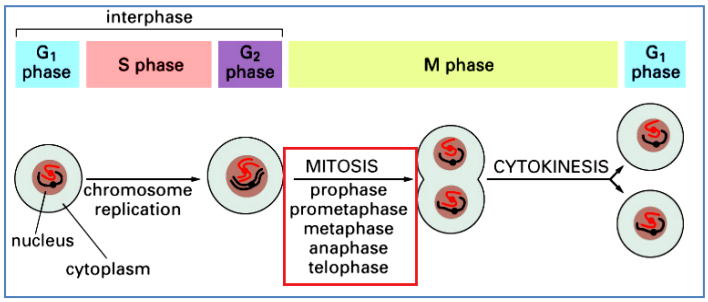




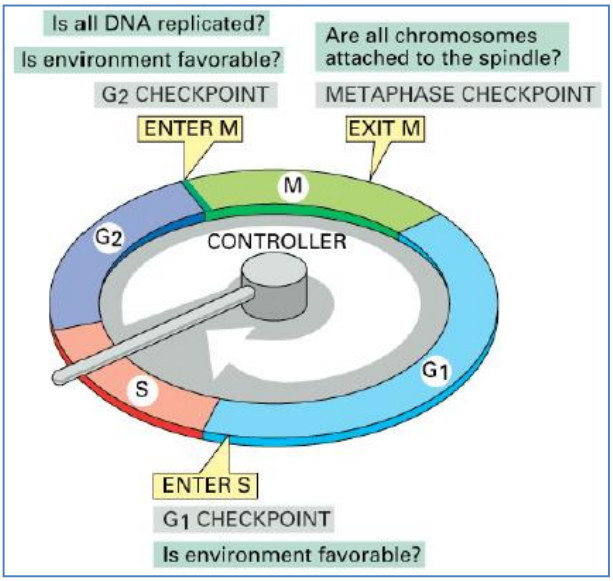
Cell-Cycle Checkpoints & Cancer:

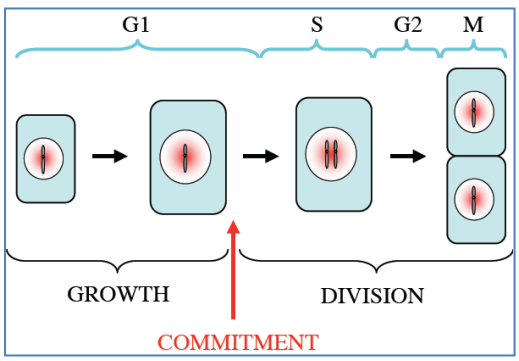
- **Cyclin-CDK Complexes:**
  - = Kinases which Phosphorylate (& activate) Cellular Machinery required for the process of Cell Division.





- **Functions of the Cell Cycle Checkpoints:**
  - **G1-S Phase Checkpoint:**
    - Should the Cell **Commit** to Cell Division?
    - Is the Environment Favourable?
    - **NB:** This is the most commonly *Dysregulated Checkpoint* in cancer.
  - **G2-M Phase Checkpoint:**
    - Is *All* the DNA Replicated Properly?
    - Is the Environment *Still* Favourable?

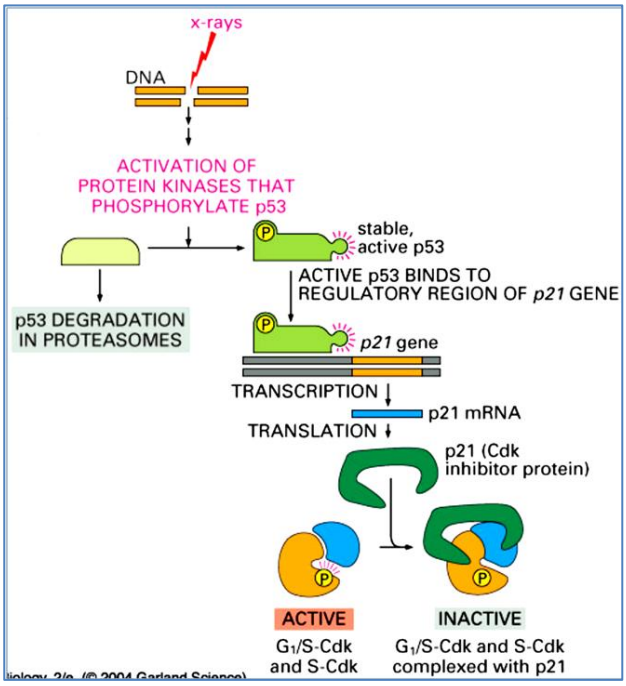


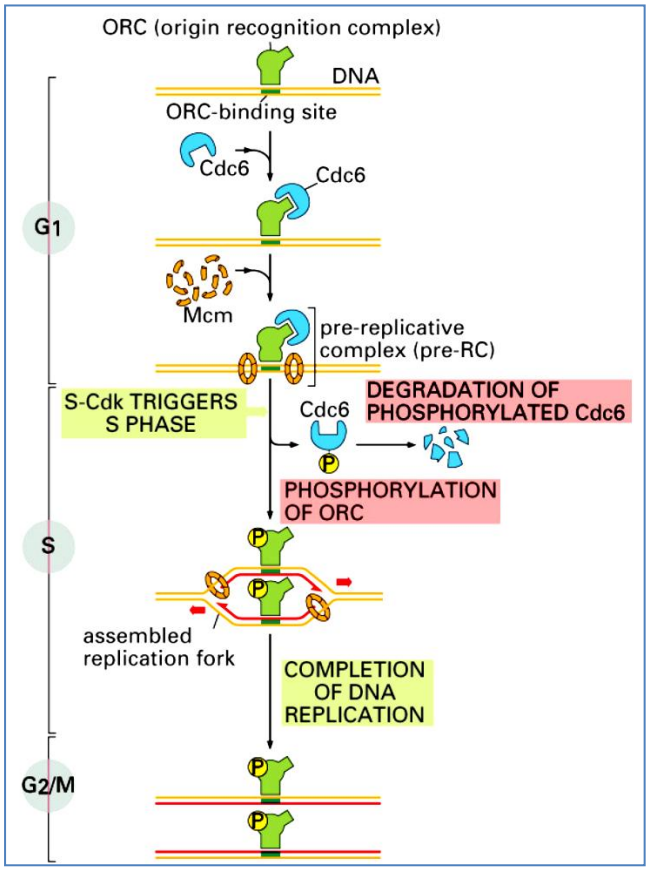


  - **Hence, Dysregulation of the Cell-Cycle is KEY to Tumour Formation.**

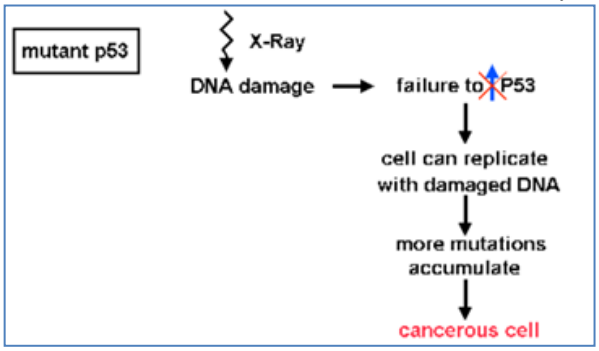
The TWO Cell-Cycle *Checkpoints* Most Commonly Dysregulated in Cancer:

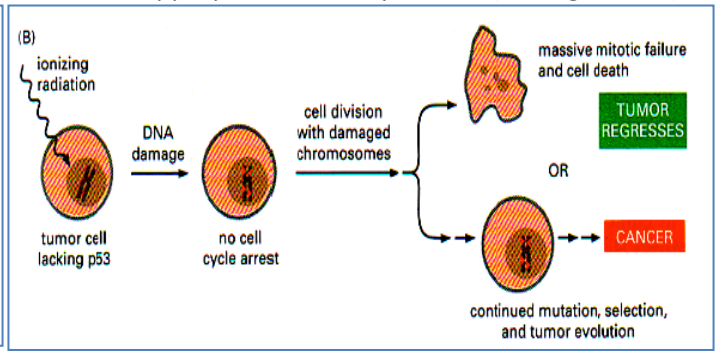
- **The G1/S-Phase →**
  - Should the Cell **Commit** to Cell Division?
  - Is the Environment Favourable?
  - **NB:** This is the most commonly *Dysregulated Checkpoint* in cancer.
  - **Normal Function:**
    - **If DNA is Good:** Active S-Cyclin-CDK Phosphorylates & Removes the Inhibitory Cdc6-Protein on DNA-Replication Machinery
      - → Ie. Triggers DNA Replication.
    - **If DNA is Damaged** – S-Cyclin-CDK is Inactivated by **P21**(Inhibitor Protein) as a product of **P53** → Delays DNA Replication until it is repaired.





- **Dysfunction: **Defective P53 is the Most Common Feature of Cancer Cells:**
  - **Defective P53** → Loss of *'DNA Damage Sensor'* → No Transcription of P21 Inhibitory Protein → No Inhibition of S-Cyclin-CDK → Inappropriate DNA Replication (damaged DNA).

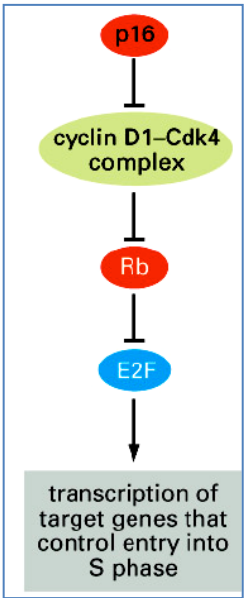


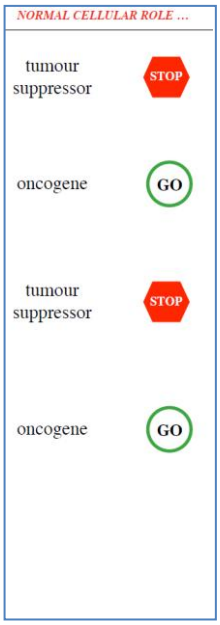


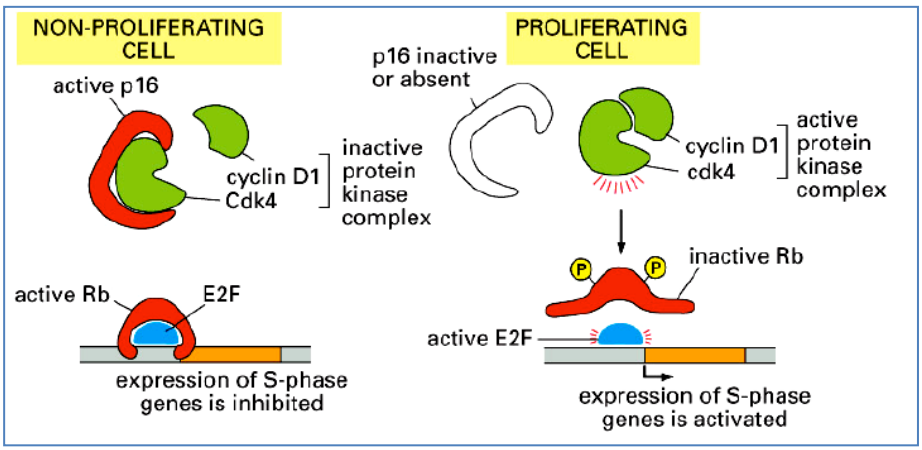
- **NB: Many Viruses *'Capture'* Oncogenes which function to Inactivate P53 (& RB):**

VIRUS	ONCOGENE	FUNCTION
Human Papilloma	E6, E7	Inactivate p53 & Rb
Adenovirus	E1A, E1B	Inactivate p53 & Rb
Simian Virus 40 (SV40)	Large T antigen	Inactivate p53 & Rb
Epstein-Barr	BZLFI, EBNA5	Binds p53, activates myc
Hepatitis B	HBX	Binds p53

- **The G2/M-Phase →**
    - Is *All* the DNA Replicated Properly?
    - Is the Environment *Still* Favourable?
    - **Normal Function:**
        - **If DNA is Good:** Active M-Cyclin-CDK Phosphorylates & Removes the Inhibitory Rb-Protein on E2F (A Gene-Regulatory Protein) responsible for Protein Synthesis.
            - → Ie. Triggers Transcription/Translation of Genes required for Cell Division.
        - **If DNA is Damaged** – M-Cyclin-CDK is Inactivated by P16(Inhibitor Protein) → Prevents Inactivation of **RB**.







- **Dysfunction: Defective RB is a Common Feature of Cancer:**
    - **Defective RB →** Constantly Phosphorylated (inactivated) RB-Protein → NO Inhibition of E2F → Inappropriate Expression of S-Phase Genes → Cell Divides before DNA has replicated properly.
    - **NB: Many Viruses *'Capture'* Oncogenes which function to Inactivate RB (& P53):**

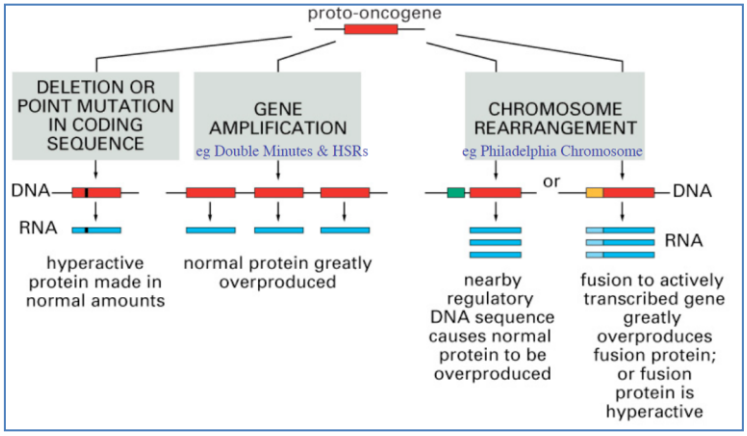
VIRUS	ONCOGENE	FUNCTION
Human Papilloma	E6, E7	Inactivate p53 & Rb
Adenovirus	E1A, E1B	Inactivate p53 & Rb
Simian Virus 40 (SV40)	Large T antigen	Inactivate p53 & Rb
Epstein-Barr	BZLFI, EBNA5	Binds p53, activates myc
Hepatitis B	HBX	Binds p53

Category Descriptions of Genes Altered in Cancer:

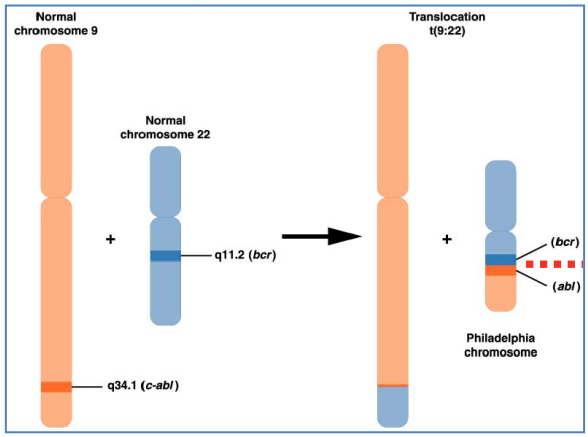
- **1. Gatekeepers (*AKA: PROTO-ONCOGENES*):**
  - **(Cancer-Susceptibility Genes = Ie. Genes that Control Cellular Proliferation)**
  - **NB:If Defective –** Directly predisposes cell to cancer by Triggering Clonal Expansion & Tumorigenesis.
- **2. Caretakers (*AKA: TUMOUR-SUPRESSOR GENES*):**
  - **(Genome-Maintenance Genes = Ie. Genes involved in Maintaining the Integrity of the Genome)**
  - **NB: If Defective –** Indirectly predisposes cell to cancer due to ↑Chance of Mutation of Gatekeepers.
- **3. Landscapers:**
  - **(Cell-Cell Interaction Genes = Ie. Genes involved in Cell-Cell Adhesion & Cell Movement)**
  - **NB: If Defective –** Enables cancer cell to Metastasise to other tissues.

Cancer Mutations Fall into 2 Categories:

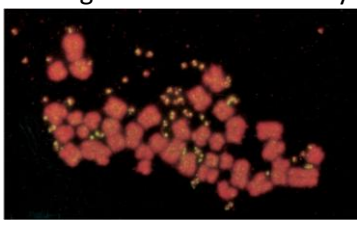
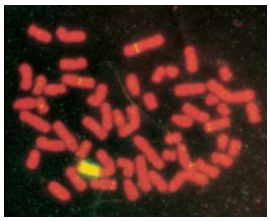
- **1. Inappropriate Activation of Proto-Oncogenes (*AKA: GATEKEEPERS*):**
  - **(Ie. A *'Gain of Function'* Mutation)**
  - **Normal Functions of Proto-Oncogenes:**
    - Secretion of Growth Factors
    - Growth Factor Receptors
    - Cell Cycle & Apoptotic Proteins
    - Nuclear Transcription Factors
  - **However, in *Tumours,* Proto-Oncogenes are *Mutated* to '*Oncogenes*':**
    - Oncogenes → Uncontrolled Cell Division.
  - **Mechanisms of Mutation:**
    - DNA Point Mutations (Single base-pair Changes) → Change critical amino acids.
    - Gene Amplifications (Ie. Duplicating a gene)→ Increase Expression Levels
      - (Eg. "Double Minute Chromosomes" & "Homogenously Staining Regions")
    - Virus → 'Capture' Oncogenes → Turn them on → Inactivate P53 / RB.
    - Chromosome Abnormalities (Deletions/Inversions/Translocations) → Cause Mis-Expression.
      - (Eg. The Philadelphia Chromosome → Most Leukaemias)



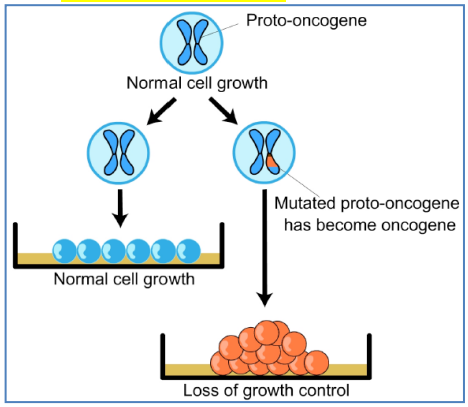
- **NB: The "Philadelphia Chromosome" *Translocation* is associated with Leukaemias:**
  - The **Bcr-Gene** from Chr-22 fuses with the **Abl-Gene** from Chr-9.
  - Bcr-Gene (A *Promoter* Gene) + Abl-Gene (A Proto-Oncogene) = Oncogene.

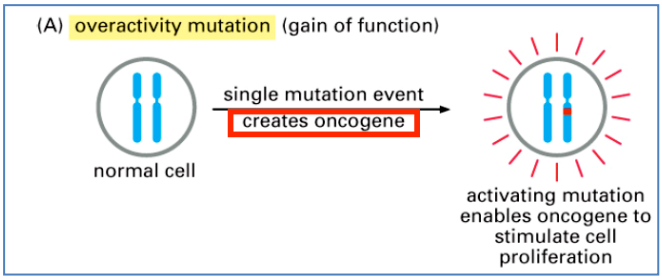


- **NB: Proto-Oncogene Amplifications are also associated with many other cancers:**
  - **I) "Double-Minute" Chromosomes:**
    - Hundreds of Small *Independant 'Mini-Chromosomes'* (usually containing proto-oncogenes) which are due to *Gene Amplification*.
  - **II) "Homogenously Staining Regions":**
    - A *Greatly Lengthened* segment of a chromosome (usually containing proto-oncogenes) due to *Gene Amplification*.
    - NB: These regions are fluorescently stainable. See below.

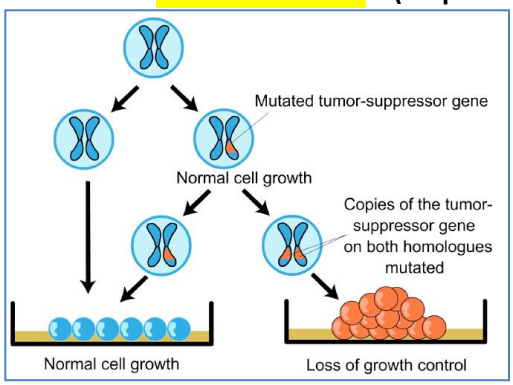
I)  II) 

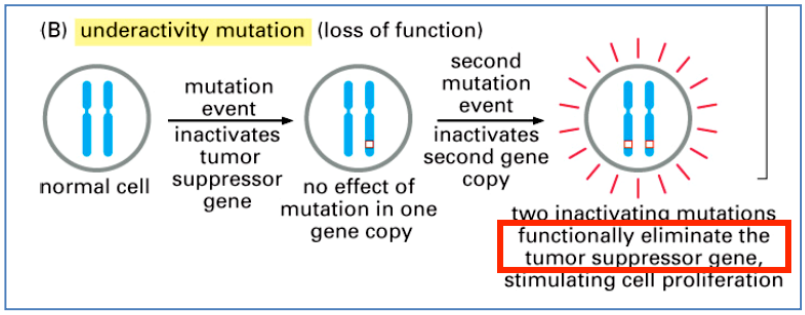
  - **ACT DOMINANTLY – (only 1 mutated Proto-oncogene is required for cancer)**





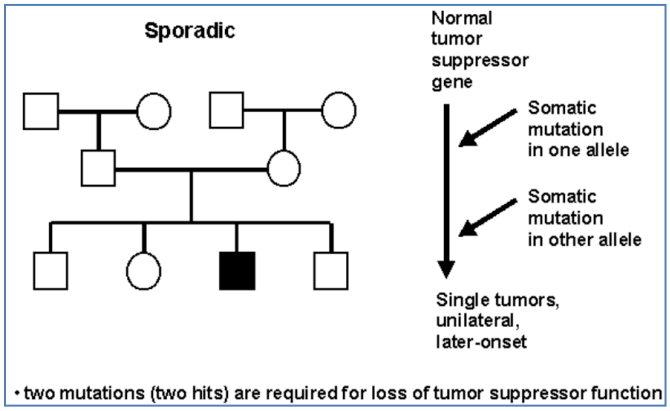
- **2. Loss of Tumour Suppressor Genes (*AKA: CARETAKERS*):**
  - **(Ie. A *'Loss of Function'* Mutation)**
  - **Normal Functions of Tumour Suppressor Genes (as *Defence Pathways* against Cancer):**
    - DNA-Damage Sensing Pathways (P53 & RB)
    - DNA-Repair Pathways (BRCA1 & 2)
    - Protein Degradation
    - Cell Cycle Regulation
    - Apoptosis (if DNA damage is irreparable)
    - Telomere Shortening (Ie. Limits number of possible divisions, then causes Senesce)
  - Genes involved in the above processes = ***Tumour Suppressor Genes*** = The *Brakes of the Cell Cycle*.
  - **However, in *Tumours*, Tumour Suppressor Genes are *Mutated*.**
    - → No Brakes on the cell cycle → Cancer-Cell Division is Unopposed.
  - **Mechanisms of Mutation:**
    - DNA Point Mutations (Single base-pair Changes) → Loss of gene function.
    - Deletion of Genes/Chromosome-Region
    - Chromosome Mis-Segregation → Loss of Whole Chromosome
    - Abnormal DNA Methylation → Loss of Gene Expression
  - **ACT RECESSIVELY – (Requires *2 "Hits"* OR *Loss of Heterozygosity* for Cancer).**

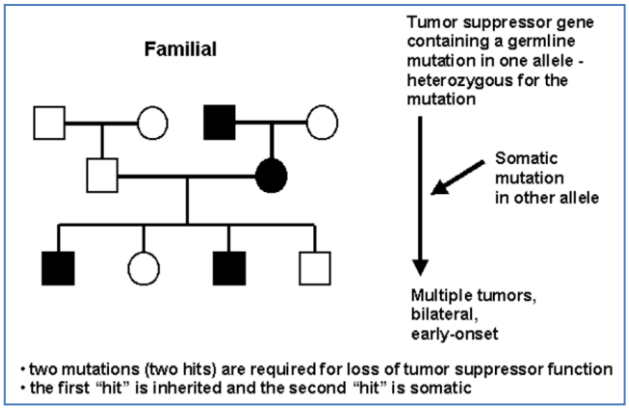


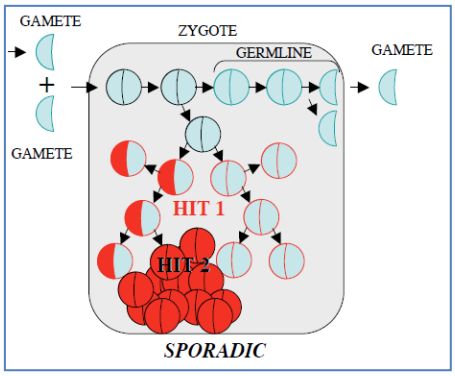


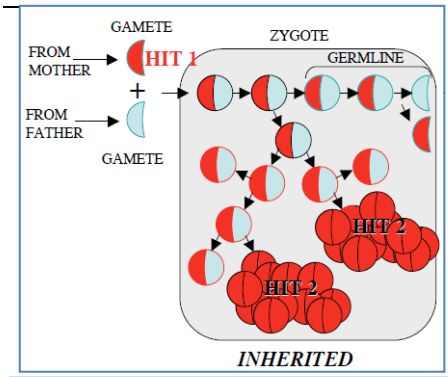
Tumour-Suppressor Genes *Act* Recessively: (Knudson's 2-Hit Hypothesis & Loss of Heterozygosity):

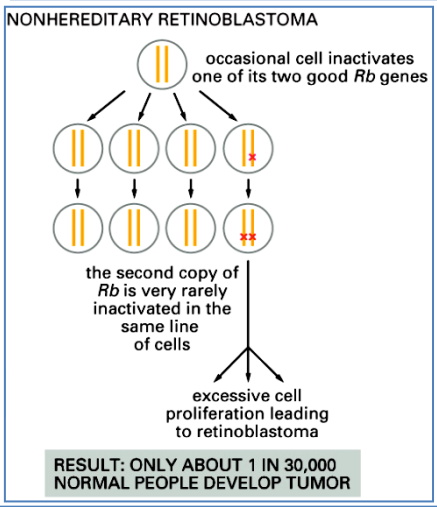
- **Knudson's 2-Hit Hypothesis:**
  - **Sporadic** – 2 Mutations ('Hits') in a Tumour Suppressor Gene are required for Cancer.
  - **Familial** – All Body Cells already have the 1st hit → only require 1 more 'Hit' for Cancer.
  - **NB:** One of the "*Hits*" can be a ***"Loss of Heterozygosity"*** in Favour of the Mutated Tumor Suppressor Gene**.**

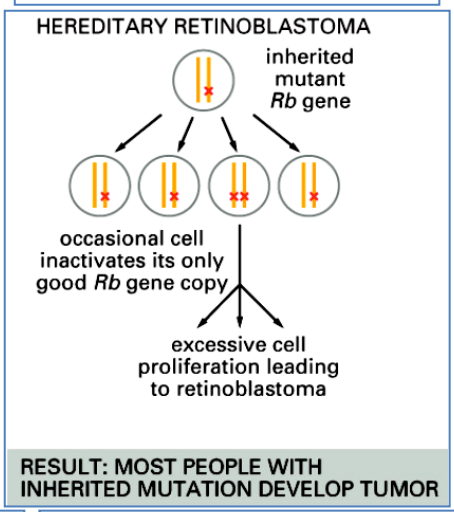


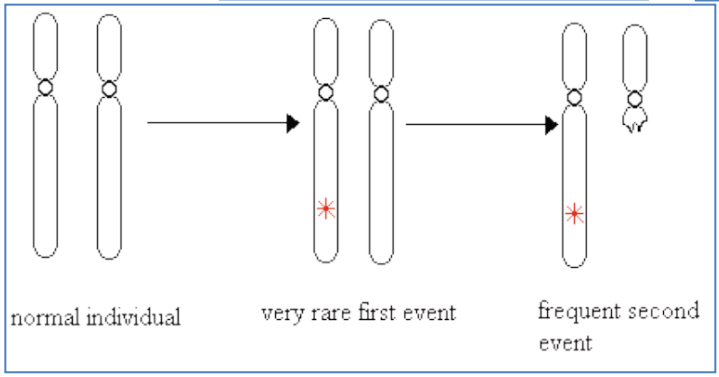


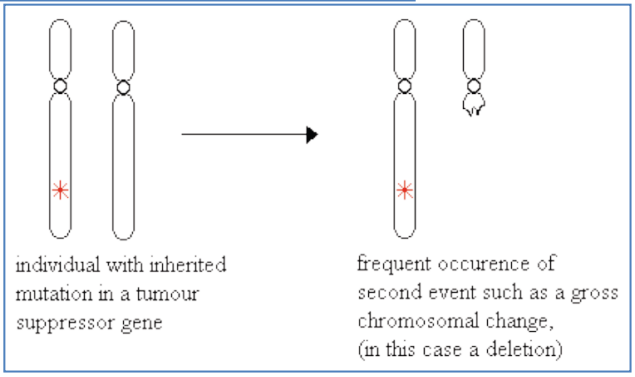




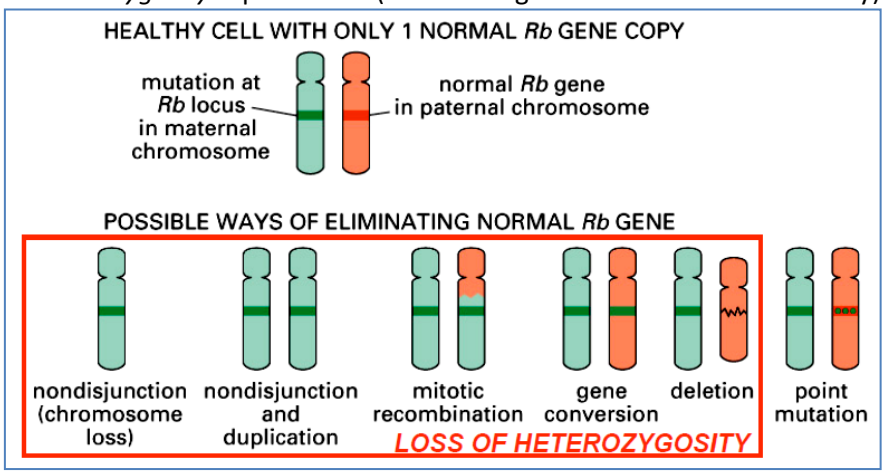


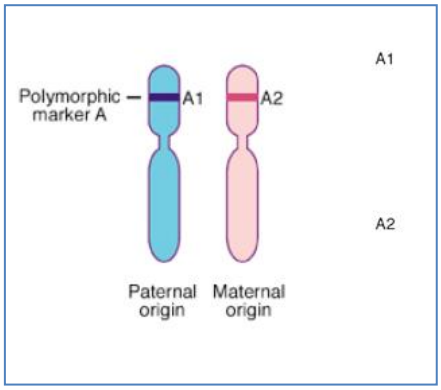


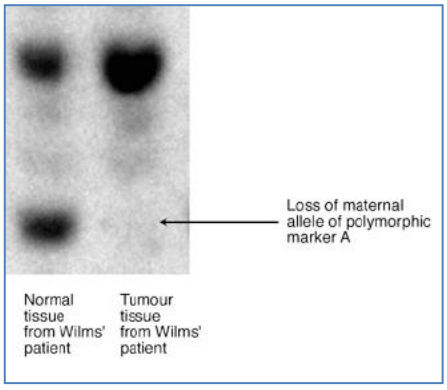




- ***"Loss of Heterozygosity"*:**
  - **Loss of Heterozygosity in a Particular Gene/Chromosome-Region = One Allele has been Lost:**
    - Ie. A Person who was previously *Heterozygous* (1x normal & 1x mutated) for a specific allele *'Loses'* the normal allele → Sole Expression of the Mutated Allele.
    - This is a problem if the remaining allele is a Non-Functional Tumour-Suppressor Gene.
  - **Alleles can be *'Lost'* by any of the following:**
    - Chromosome Loss
    - Chromosome Loss & Duplication of Abnormal Chromosome.
    - Mitotic Recombination
    - Gene Conversion
    - Gene Deletion
    - NB: *Point Mutations* in the Normal Chromosome *Also* eliminates the normal *Rb* Gene, but *Heterozygosity* is preserved. (Since both genes are mutated differently)

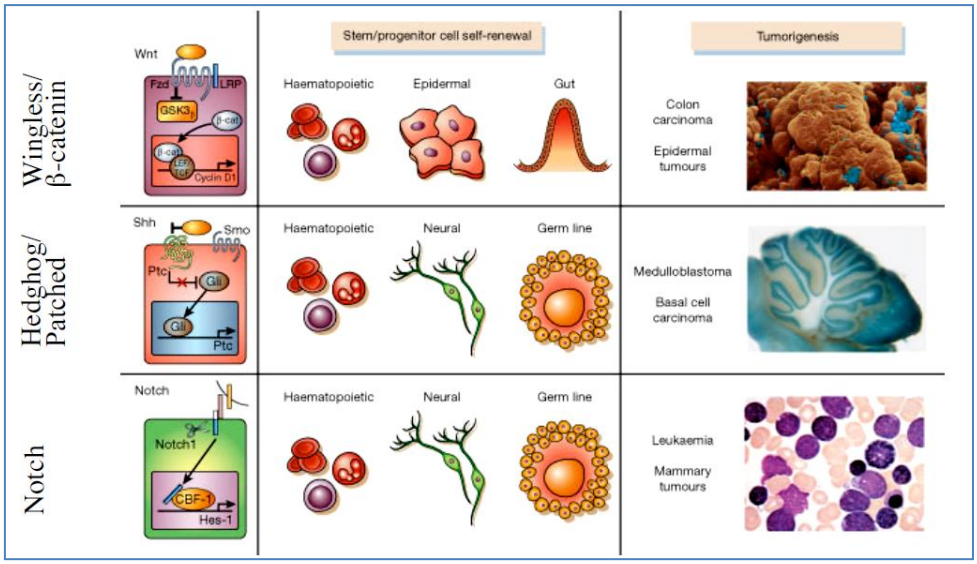




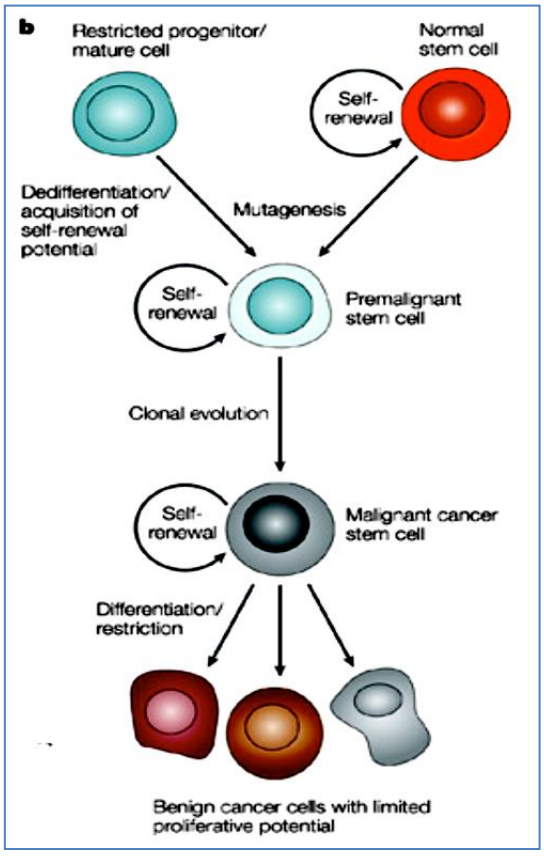


Cancer Stem Cells – The New Theory in Cancer:

- **What are Cancer Stem Cells?**
  - Rare tumour cells with the ***Ability to Self-Renew*** & give rise to ***Phenotypically Diverse Tumour***.
  - → Drive Tumourigenesis
- **Similarities between *Cancer Stem Cells* & *Normal Stem Cells*:**
  - **'Asymmetric Division':**
    - ***One* Daughter cell is for *Self-Renewal***, & Maintains its capacity for Unlimited Proliferation.
    - ***The Other* Daughter cell *Differentiates*** into *Phenotypically-Diverse* Mature Cells, & Loses its capacity for Unlimited Proliferation.
  - **Self-Renewal is Regulated by Similar Pathways:**
    - However, these pathways are *Dys-Regulated* in some Cancer Stem Cells.
    - 1. The "Wingless" Pathway
    - 2. The "Hedgehog" Pathway
    - 3. The "Notch" Pathway
  - **SCs & CSCs Divide Infrequently.**



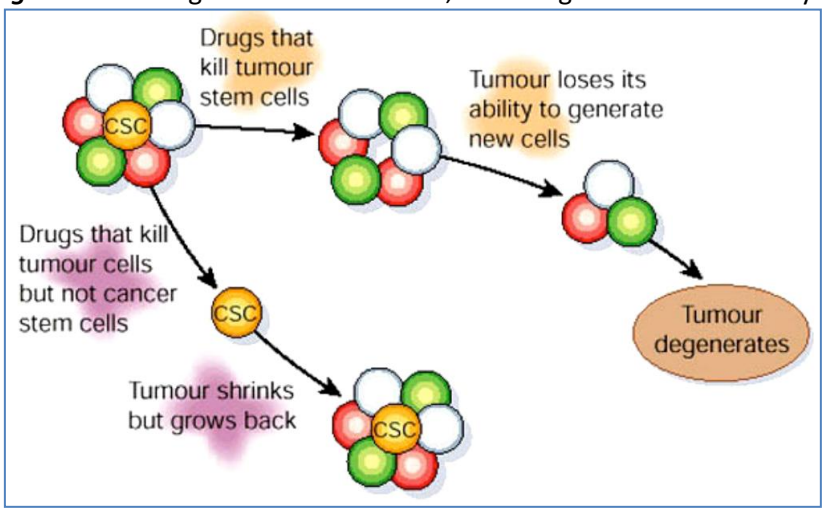
- **Where do Cancer Stem Cells Come From?**
  - CSCs are thought be the result of Multiple Mutations → ***Transformation of Normal Stem-Cells*.**



- **Differences between the Classical Theory of Cancer & the Cancer Stem-Cell (CSC) Theory:**

	Old Model (Classical):	**Cancer Stem-Cell Theory:**
Hypothesis:	Cancer is a ***Hyper-Proliferative Disease***	Cancer is a ***Stem-Cell Disorder***
Cause of Unregulated Growth:	Accumulation of ***Genetic Changes***: ➔ Proto-Oncogene Activation ➔ Inhibition of Tumour-Supp's. ➔ Inhibition of Cell Death	Disruption in the regulation of ***Stem-Cell Renewal.***
Proliferative Ability:	All tumour cells are ***Equally Tumourigenic.***	Only a ***Subset of Cancer Cells*** are CSCs, with the ability to proliferate indefinitely.

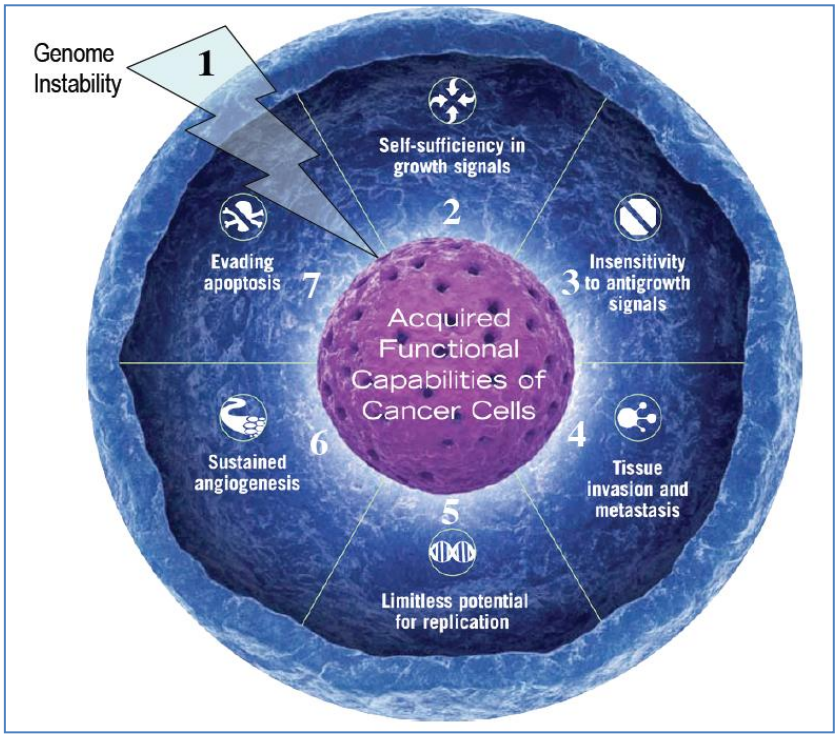
- **Therapeutic Implications of the Cancer Stem-Cell Theory:**
  - Most current drugs target *Tumour Cells*, but *Not Cancer Stem-Cells*.
  - Current drugs also target *Rapidly Dividing Cells*, but *Not quiescent Cancer Stem Cells*.
  - ***New Drugs*** – Should target Cancer Stem Cells, removing the tumour's ability to generate new cells.



- **Metastases = *Migratory Cancer Stem Cells*:**
  - Metastasis is a selective process for cells that can ***Invade, Embolize, Survive in Circulation, Adhere to Distal Capillary, Migrate into Distant Organ & Survive/Proliferate in Distant Organ***.

Summary of KEY GENETIC CHANGES in Cancer Cells:

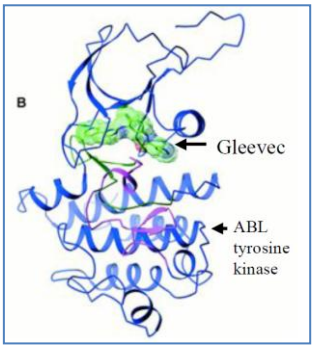
1) **Genome Instability:**
    a. Ie. Loss of checkpoint (Gatekeeper-Genes) & DNA repair (Caretaker-Genes) functions.
        i. Eg. **Failure of P53 or P21** to Inhibit S-Cyclin-CDK @ the G1-S Phase Checkpoint.
    b. Also, Chromosome Instability is a hallmark of cancer.
        i. Eg. The ***"Chromosome Breakage-Fusion-Bridge Cycle"*.**
2) **Self-Sufficiency in Growth-Signalling:**
    a. Ie. Permanent activation of Cell-Cell Growth-Signalling Cascades.
3) **Failure of Anti-Growth Signalling:**
    a. Ie. Inactivation/Failure of Tumour Suppressor Genes.
        i. Eg. **Failure of Rb Pathway** to Inhibit M-Cyclin-CDK @ the G2-M Phase Checkpoint.
4) **Tissue Invasion & Metastasis:**
    a. Ie. Loss of Cell-Adhesion Gene (Landscaper Genes) Function.
    b. →Migration from site of Primary Tumour & Invasion of Normal Tissue.
5) **Limitless Replicative Potential:**
    a. Ie. Reactivation of Telomerase → Cell Avoids *Senescence* → Cell Becomes Immortal.
6) **Sustained Angiogenesis:**
    a. Ie. Secretion of Angiogenic Growth Factors (VEGF - Vascular Endothelial Growth Factor)
    b. → Enables the cancer to support itself.
7) **Evasion of Apoptosis**
    a. Ie. Inactivation of Pro-Apoptotic Genes AND/OR Activation of Anti-Apoptotic Genes.

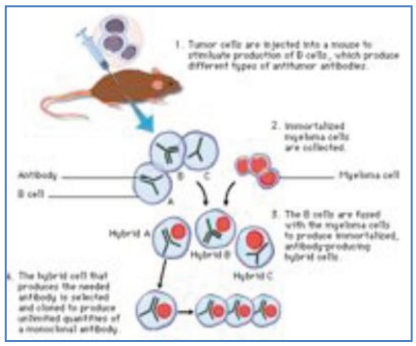


EMERGING DIAGNOSTICS AND THERAPIES FOR CANCER:

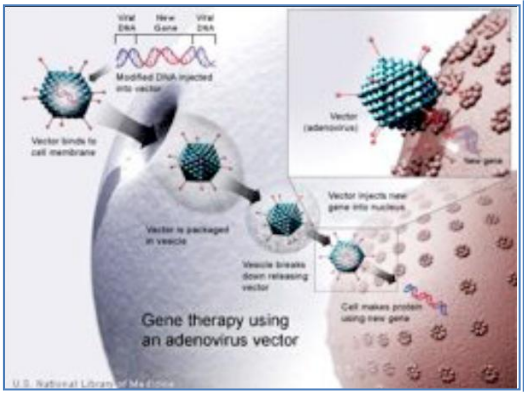
3 Emerging Technologies:

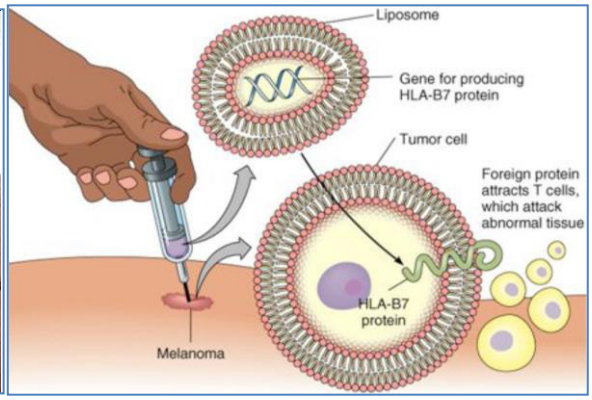
- **1. Rational Drug Design:**
  - **Eg. *Taylor-Made* Drugs** that have been *Chemically Engineered* to act specifically on its target.
  - **Eg. Monoclonal Antibodies** that have been *Chemically Engineered* to act specifically on its target:
    - Attach to Antigen → Stop its function (Eg. Prevent Growth-Factor Receptor Function)
    - Attach to Antigen → Provoke an Immune Response (Eg. Destroy Growth-Factor Receptor)
    - *Conjugated* MABs → Abs joined to Chemotherapy/Radioactive Drug → Direct Drug Delivery.





- **2. Gene Therapies:**
  - **Potential Aims:**
    - **General:**
      - Selective Killing of Ca. Cells
      - Stimulation of Immune System to kill Ca. Cells.
      - Protect surrounding (normal) tissues to harmful effects of Chemo/Radio.
    - **Oncogene Inactivation:**
      - Selectively Inhibit the *Expression* of an Activated Oncogene in Tumour Cells.
    - **Tumour Suppressor Gene Reactivation:**
      - Replace Mutated TS-Genes with Functional Genes.
  - **Potential Methods:**
    - **Gene-Therapy Vectors:**
      - Ie. Getting the DNA into cells.
      - Liposomes/Microprojectiles/Viral-Vectors.
    - **Stimulation of the Immune System:**
      - Introduction of a gene into tumour cells causing them to express a foreign protein → Alerts Immune System → Cell-Mediated Immune Response.





- **3. Microarray Assay Diagnostics:**
  - **A Screening tool for Billions of Possible Gene-Mutations associated with Thousands of Cancers.**
    - Ie. Will be able to screen everybody for every heritable cancer in 1 simple test.
  - **How does it work?**
    - 1. Each micro-well on a Micro-Array Card is filled with a Different Mutation.
    - 2. mRNA from a tissue of interest is converted to cDNA & then Fluorescently Labelled.
    - 3. The mixture of cDNA is washed over the DNA Micro-Array, allowing complimentary sequences to pair up.
    - 4. Each well is then examined for fluorescence → Indicates presence/absence of mutation.

Cancer Epidemiology

General Cancer Epidemiology:

- **What is Epidemiology?**
  - Study of Health and Disease in populations – distribution and determinants of disease in populations.
- **What is *Unique* about *Cancer* Epidemiology?:**
  - Cancer is a *Chronic Disease* – (Epi. In the past has been reserved for *Acute, Infective* Diseases)
  - Cancer has a *Long Latency Period* – (Many are asymptomatic – alters statistics)
  - Relatively speaking, Cancer is a *Rare* Disease (<5%)

Patterns of Disease:

- **Sporadic:**
  - Random cases of Disease.
- **Endemic:**
  - The base-line incidence rate of disease in a population.
- **Epidemic:**
  - When the incidence rate rises above the Endemic Rate.
- **Pandemic:**
  - When an Epidemic *Crosses Continents*.

Clinical Epidemiology:

- **Clincical Epidemiology:**
  - **= Using Statistics, Probability & Population-Based Data to Inform Medical Decisions.**
- **Important Terms:**
  - **Incidence:**
    - **General Incidence:**
      - *New* Cases/*Population* over a Period of time.
    - **Incidence Proportion – (*'Cumulative'* Incidence):**
      - *New* Cases/'*At-Risk*' Population over a Period of time.
    - **'*True*' Incidence:**
      - *New* Cases/*Average number At-Risk* over a Period of time.
      - (Ie. Takes into account *Dynamic* Populations
      - (NB: *Average Number At-Risk* is measured in "*Person-Years*" – Ie. 1 person living through 1 year = 12 people living 1mth each)
      - ***Average Number @ Risk* = (#At Risk at Start + #At Risk at End)/2**
  - **Prevalence:**
    - **General Prevalence:**
      - Total Cases/Population @ a Point in Time.
      - (NB: More useful in Chronic Disease because incidence rates are low)
    - ***'Period'* Prevalence:**
      - Proportion of population who Have/Had the disease during a period of time.
  - **Mortality:**
    - Deaths from Specific Disease/Population.
  - **Survival:**
    - Expressed as a 5-year survival rate.
    - = How many people with the disease survive past 5yrs.
  - **Lifetime Risk:**
    - = Likelihood that someone who lives to 90 will develop the disease.

Types of Study - (Used to calculate Relative-Risk/Attributable Risk/Odds Ratio):

- **Observational:**
  - **Cohort Study:**
    - Start with 2 Groups of People – *With* and *Without* Exposure to a Risk Factor.
    - Prospective – See who gets the disease.
  - **Case-Control Study:**
    - Start with 2 Groups of People – *With* and *Without* Disease.
    - Retrospective – See proportion of those with Disease & the Risk Factor.
- **Experimental:**
  - **Animal Model –** (Ie. Not ethical to purposely expose humans to possible risk factors)
  - **Following Disasters –** (Ie. Taking advantage of *Incidental Exposure* to a specific risk factor and monitoring for disease)

Causal Relationships:

- **2 Types of Causes:**
  - **Primary Causes:**
    - Necessary for disease, but not always sufficient (Eg. Genetics/Smoking/Radiation)
  - **Secondary Causes:**
    - Predisposing/Enabling/Reinforcing Factors – Ie. Anything that ↑Risk. (Eg. Age)
- **3 Types of Causal Relationships:**
  - **Direct:**
    - 'A' Causes 'B'.

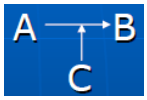
  - **Casual Network:**
    - 'A' + 'B' + 'C' Causes 'D'

    - 'A' & 'B' *both* Cause 'C'

  - **Modified:**
    - 'A' is Modified by 'C', Causing 'B'.

- **Criteria for Causal Relationship:**
  - Dose Dependency
  - Consistency
  - Specificity
  - Time Sequence
  - Coherence
  - Analogy
- **Common 'Causes' in Cancer:**
  - Geography
  - Environment
  - Occupation
  - Diet
  - Gender
  - Race
  - Culture
  - Genetics

Non-Causal Relationships:

- Correlation
- Confounding Factors – (Independent Variables with an *Actual* Variable *AND* the Outcome)
- Chance
- Selection Bias
- Incidentals

Measuring the *Strength* of an Association:

- **Risks:**
  - **Risk:**
    - ≈The Incidence Rate x Duration of Disease.
  - **Relative Risk:**
    - = Strength of the Relationship between a Risk Factor and the Disease.
    - **How? –** (Ratio of *Risk* of disease in People *With* the Risk Factor : People *Without* the Risk Factor)
    - (Given as a Ratio – Ie. 5:1 = 5x more likely)
  - **Attributable Risk:**
    - = How much of the Disease in the Population is Attributed to the Risk Factor.
    - **How? –** (Incidence with risk Factor – Incidence without risk Factor)
    - (Given as a *Difference*)
  - **Odds Ratio:**
    - = How much *More Likely* you are to get the Disease if You've been Exposed to a Risk Factor.
    - **How? –** (Ratio of *Odds* of Disease in *Those Exposed* : *Those Not Exposed*)
-
- **Probabilities:**
  - **Sensitivity Vs. Specificity:**
    - **Sensitivity –** The ability of a test to Indicate Disease when it is present.
      - Gives a probability of *False Negative*.
    - **Specificity –** The ability of a test to Indicate Non-Disease when *no disease* is present.
      - Gives a probability of *False Positives*.
  - **Positive Predictive Value (PPV):**
    - - Often used to compute *Probability Of Disease* in a Positive Test Result.
      - **If Result +ve:** Person probably has Disease.
      - **If Result -ve:** Person probably has *No* Disease.
    - **Calculating PPV:**
      - **1.** You are visited by a 65 year-old man who is worried about cancer of the prostate. His fear arises from the fact that his best mate recently died from the disease. The man reports no signs or symptoms.
        - Prevalence = 0.5% in men over 60yrs (Ie. 1 in 200 asymptomatic men).
        - Assume DRE sensitivity of 69%.
        - Assume DRE specificity of 97%.
      - What is the likelihood that you would detect prostate cancer in this man?

TEST RESULT	Present	Absent	Total
Positive	69	597	666
Negative	31	19303	19334
Totals	100	19900	20000

PPV = 69/666 = .10 = 10%

      - **2.** The man was still not convinced and requested a PSA. Let's assume that PSA has:
        - *Prior Probability* (calculated above) = 10%.
        - Assume PSA sensitivity of 80%.
        - Assume PSA specificity of 95%.

	TRUE DISEASE STATUS		
	Present	Absent	Total
TEST RESULT			
Positive	80	45	125
Negative	20	855	875
Totals	100	900	1000

PPV = 80/125 = .64 = 64%

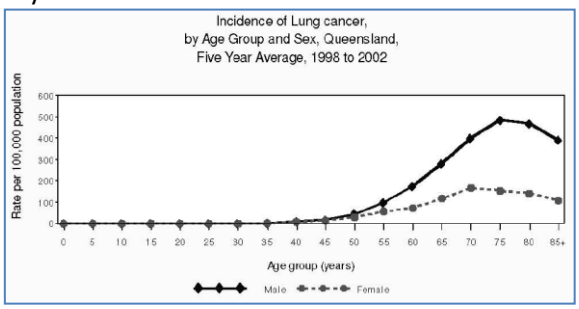
(Ie. If both DRE & PSA are *Positive*, PPV is ≈64% - Ie. Pt has a 64% chance of having Prostate Cancer.)

Cancer Prevention:

- **Queensland Cancer Council's Objectives:**
  - **Objective 1:**
    - Reduce Smoking – (Incl. Passive Smoking)
  - **Objective 2:**
    - Promote Healthy Behaviours:
      - Reducing Overweight/Obesity
      - Optimising Nutrition
      - Increasing Physical Activity
  - **Objective 3:**
    - Promote Sun-Safe Behaviours
  - **Objective 4:**
    - Reduce Alcohol Consumption
  - **Objective 5:**
    - Strengthen the role of Doctors in reducing cancer in the population through Enhanced Prevention.

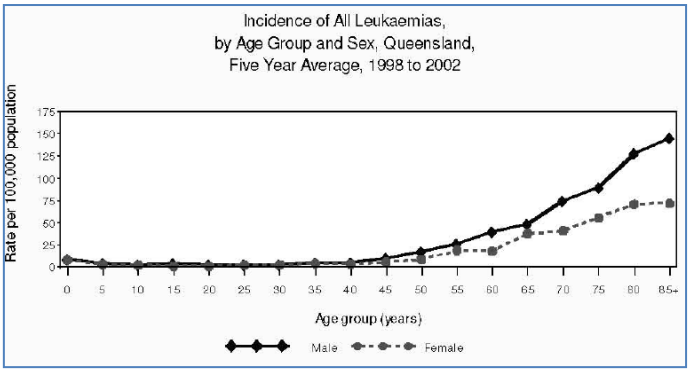
(SS) Specific Cancers – (Incidence/Mortality/Risk Factors/Age-Ranges/Gender Differences):

- **Lung Cancer:**
  - **Risk Factors:**
    - Smoking (30% lifetime risk)
    - TB
    - Asbestos
    - Radiation
    - Air-Pollution
  - **Incidence:**
    - Higher in Males
    - Higher in Low-SES
    - Higher in Rural/Remote
    - Higher in Indigenous
    - Peaks at ≈70yrs

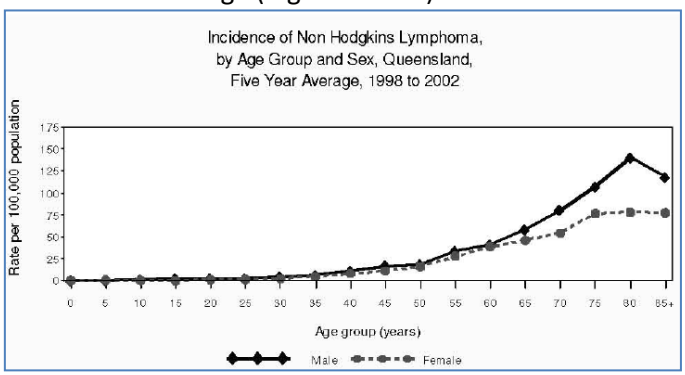


- **Oesophageal Cancer:**
  - **Risk Factors:**
    - Dietary – Nitrosamines
    - Smoking/Alcohol
    - Pre-Cancerous Lesions
  - **Incidence:**
    - Mostly from 70-80yrs
    - 3x in Males

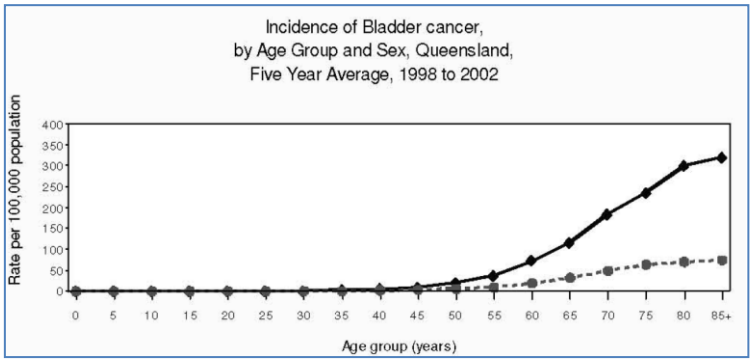
- **Lymphomas:**
  - **Hodgkins Lymphoma:**
    - **Risk Factors:**
      - (Possibly Epstein-Barr Virus)
    - **Incidence:**
      - Bimodal – Highest in Young & Elderly.



  - **Non-Hodgkins Lymphoma:**
    - **Risk Factors:**
      - Immunosuppression
      - Comorbidity
    - **Incidence:**
      - Increases with age (highest in 70^{+})



- **Bladder Cancer:**
  - **Risk Factors:**
    - Aniline Dye (used in Textile/Rubber industries)
    - Smoking
    - Chronic UTI's
    - Calculus Disease
    - Schistosomiasis
  - **Incidence:**
    - Mostly from 60-70yrs
    - 2x in Males



- **Breast Cancer:**
  - **Risk Factors:**
    - Age
    - Living in Developed Country
    - Previous Benign Disease
    - Cancer in *Other* breast
    - Early Menarche/1st Child @ 40yrs+.
    - Genetics
  - **Incidence:**
    - Highest in Women (Possible in men)
    - Increases with age
  - **Benefits of Early Detection:**
    - Lower risk of Advanced Disease → ↑Chance of Survival
    - Less Radical Surgery – (More treatment options)
    - Better Post-Diagnosis Quality of Life.
  - **Disadvantage of Early Detection:**
    - Women living longer with *Knowledge* of the disease, but not necessarily living longer overall → Un-Necessary Distress →↓Quality of Life.
- **Prostate Cancer:**
  - **Risk Factors:**
    - Age
    - Environmental/Occupational
    - Familial
    - High-Fat Diet
  - **Incidence:**
    - Has been steadily increasing – (Due to men living longer → more at higher risk)
  - **Calculating PPV:**
    - **1.** You are visited by a 65 year-old man who is worried about cancer of the prostate. His fear arises from the fact that his best mate recently died from the disease. The man reports no signs or symptoms.
      - Prevalence = 0.5% in men over 60yrs (Ie. 1 in 200 asymptomatic men).
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- **Colorectal Cancer:**
  - **Risk Factors:**
    - Environmental (Cultural/Socia/Lifestyle) – Estimated 70-80%.
      - Dietary/Nutritional Habits:
        - High Fat
        - High Meat
        - Low Fibre
        - Low Fruit
    - Genetics:
      - Familial Adenomatous Polyposis,
      - Inflammatory Bowel Disease (Ulcerative Colitis & Crohn's Disease)
      - Heriditary Non-Polyposis Colon Cancer
    - Mortality is highest in Low-SES – (Poor access to care)
  - **Incidence:**
    - Steadily rising over time.
    - Highest in Westernized Countries.
  - **Screening:**
    - Faecal Occult Blood Test is most effective.

- **Melanoma:**
  - **Risk Factors:**
    - Sun Exposure (UV Radiation)
    - Fair Skin/Red Hair
    - Family History
    - Moles
    - Geographic Location
    - Occupation
  - **Incidence:**
    - 4^{th} most common cancer in Aus.
    - 4x Incidence in Aus than rest of world.
    - 11% Lifetime Risk

- **Non-Melanoma Skin Cancer (BCC/SCC):**
  - **Risk Factors:**
    - Age
    - UV Radiation
    - Sun Exposure
    - Fair Skin/Red Hair
  - **Incidence:**
    - BCC – 66%
    - SCC – 33%
    - 66% Lifetime Risk of Skin Cancer.

- **Kidney Cancer:**
  - **Risk Factors:**
    - Smoking
    - High Protein Diet
    - Obesity
    - Hypertension
    - Asbestos/Petroleum Product/Heavy Metal Exposure.
  - **Incidence:**
    - Renal Cell Carcinoma = Most Common Kidney Cancer. (85%)
    - 2x risk in Males

- **Stomach Cancer – (Gastric Adenocarcinoma):**
  - **Risk Factors:**
    - Diet:
      - Preservatives
      - Smoked/Salted food
      - Lack of Fruit/Vet.
    - Low SES
    - Family History
    - H.Pylori Infection
    - Smoking
  - **Incidence:**
    - Mostly Men over 40yrs.
    - 2x risk in Males

- **Brain Cancer:**
  - **Risk Factors:**
    - Age
    - Family history
    - Genetic
    - Immune system disorders
    - Environmental:
      - Cell Phone Use
      - Exposure to Ionising Radiation
      - Exposure to Vinyl Chloride (Plastic, Pipes, Tobacco smoke)
    - Metastatic Disease
  - **Incidence:**
    - Australia – Highest in the world
    - Higher in Developed countries
    - Bimodal Age Distribution – (Onset common in Kids under 12 & Adults over 55yrs)

- **Cervical Cancer:**
  - **Risk Factors:**
    - HPV – Human Papillomavirus Infection
    - Smoking
    - Immunocompromise
    - Sexual History
    - Long Term Oral Contraceptives
    - Multiple Children
  - **Incidence:**
    - 5^{th} most deadly cancer in women

- **Uterine Cancer:**
  - **Risk Factors:**
    - Age
    - No/Few Pregnancies
    - History of Irregular Menstruation
    - Overweight
    - Endometrial Hyperplasia
    - HRT (Oestrogen only)
    - Family History
  - **Incidence:**
    - Highest in Post-Menopausal Women (60-70yrs)

- **Ovarian Cancer:**
    - **Risk Factors:**
        - Age
        - Family History
        - (Many births & Use of Contraceptive Pill – Are *Protective*)
    - **Incidence:**
        - 9th most common cancer in women (2.7% of all female cancers)
        - Most common in Elderly (over 60yrs)

- **Testicular Cancer:**
    - **Risk Factors:**
        - Un-descended testes
        - Previous Testicular Cancer
        - Previous Male Infertility
        - Family History
        - (NB: No evidence supporting physical injury or lifestyle risk factors)
    - **Incidence:**
        - Majority occur between 25-35yrs
        - More common in Caucasian Populations.

Intro to Clinical Oncology:

Aim of Clinical Oncology:

- **To cure the cancer if possible, and if not curable, control symptoms & improve QOL & length of life.**
- NB: Some cancers are curable, however most metastatic disease is incurable. Depends on the stages & the type of cancer.

Managing Cancer Patients:

- **Medical Oncologist:**
  - Chemotherapy
  - Hormonal therapy
- **Radiation Oncologist:**
  - External Beam
  - Brachytherapy – (Where the radiation source is placed inside/next to the area requiring treatment.)
  - Systemic radiation
- **Oncological surgeons:**
  - Definitive
  - Palliative
- **Allied Health:**
  - Dietician
  - Physio
  - Occupational
  - Social work
- **Palliative Care Doctors:**
  - Symptom Control (not necessarily terminal cases)
  - Terminal care

Overall approach to management:

1. **What type of cancer – Imaging:**
   - **Chest x-rays – obsolete**
   - **Ct-Scans**
   - **Bone Scans –** (Highlights areas where there is osteoblastic activity)
   - **PET scans –** (Uses radiolabelled glucose – so detects metabolically active tissues)
   - **MRI –** (Mainly used in Neural cancers

2. **Staging**
   - **To assess the extent of the disease (early or locally advanced or metastatic)**
   - **Different staging systems. (eg. TNM system)**
   - **Requires:**
     - History
     - Examination
     - Tumour markers (Some are specific to specific cancers, some aren't) – most useful in monitoring progression of disease, not diagnosis.
       - Eg. A-feto protein
       - B-HCG
       - CEA
       - PSA
     - Tests: FBC, LFTs bone marrow
     - CT scans, MRI, bone scans, pet scans.

3. **Is it curable? – Prognostic markers:**
   - Size of primary
   - Histological differentiation
   - Node Involvement
   - Metastasis

4. **If it is Curable:**
    - **Surgery**
    - **Chemotherapy:**
        - **Drugs that inhibit cancer cell proliferation by inhibiting DNA synthesis & Apoptosis:**
            - Platinum
            - Alkylating Agents
            - Microtubule formation
            - Topoisomerase inhibitors
        - **Side Effects:**
            - Nausea vomiting
            - Alopecia, myelosuppression
            - Infertility
            - 2nd malignancy
            - Other organs
        - **Curative:**
            - Leukaemia, Lymphoma, germ cell tumours etc.
        - **Adjuvant:**
            - Breast, colon, ovarian
    - **Biological Agents – specific for site of action:**
        - **Monoclonal Antibodies:**
            - Her2 positive breast cancer
            - EGFR positive cancers
            - CD20 (Lymphoma)
            - (Abs work by either blocking specific receptors, or immune mediated killing of cancer cells)
        - **Anti-Angiogenic Factors:**
            - VEGF (Vascular endothelial growth factor) Antibodies
            - (Starves the cancer)
        - **Small Molecules (Eg. Tyrosine kinase inhibitors)**
            - Eg. Glivec – used in CML or GI-stromal tumours.
            - Work by blocking tyrosine kinase on the intracellular domain of EGFR receptors.
    - **Hormonal Agents:**
        - Tamoxifen – blocks oestrogen/prog receptors
        - Aromatase inhibitor – blocks oestrogen production from androgen conversion in tissues.
        - Prostate Cancer treatment.
    - **Radiotherapy**
        - **Curative, Adjuvant & Palliative.**
        - **External Beam Vs Brachytherapy:**
            - Local effect on cancer by DNA damage.
            - Side Effects are local as well.
            - Normally is given in fraction
            - **External Beam:**
                - Focussed radiation exposure (non invasive)
            - **Brachytherapy:**
                - Injection of radioactive meds into the cancer
        - **Palliative care:**
            - By shrinking the cancer, then chemotherapy or radiotherapy
            - In addition to analgesics, nausea meds, physio, nutrition etc.

5. **<u>If it Isn't Curable:</u>**
    - **Aim of treatment = prolong survival**
    - **Improve and maintain QOL by symptom control**
    - **How can chemo or radio-therapy improve qol?**
        - Reduces size of metastases → can be good for symptomatic treatment.
        - Decreasing the need for sedative effect of analgesics
        - By living longer

<u>NB: "Median" survival rates:</u>

- Important when discussing prognosis
- How long the mid-person statistically who has that cancer survived.
- NB: This time differs with/without treatment.

<u>Questions for the patients:</u>

1. **Greeting and introduce yourselves – Establish rapport**
2. **What cancer:**
    a. Melanoma
    b. Metastasised to the lung (4.5x3x3cm) → removed half of L-Lung.
    c. 2008 - 9mths later, found a lump on her back (size of Tennis ball) – was the melanoma.
3. **How was it diagnosed – presenting symptoms, investigations/Scans/Biopsy**
    a. Picked up by physical examination by dermatologist because she had some sunspots on her nose.
4. **When was it diagnosed**
    a. 2006
5. **How did you and the family feel when you got the diagnosis.**
    a. Didn't know much about melanoma, was unaware that it might come back.
    b. Husband finds it hard to cope with the news.
    c. Both daughters have depression
    d. Son is fine.
6. **What stage was it:**
7. **Curable or incurable:**
    a. Incurable
8. **Prognosis – (Timeframe):**
    a. Doctors are surprised that she's still alive.
    b. Originally didn't get a definitive prognosis – just said she didn't have long.
9. **What treatments:**
    a. Surgery
    b. CAT scans every 3 months – but now every 6 months.
    c. No chemo – apparently doesn't help with melanoma.
10. **Side effects of treatment:**
    a. Restriction Physically & shortness of breath (had lung removed)
11. **How are you coping and quality of life:**
    a. Used to be fit. Now not so.
    b. Used to be very busy.
12. **Impact of this illness on life, family, etc.**
    a. Outlook on life – living more in the moment.
13. **What support systems have you used? (Commonly Cancer council of Queensland)**
    a. Just family.
    b. Not a fan of support groups