The Cell: An Introduction to Its Anatomy, Physiology and Genetics

The size of a cell varies from as small as 200 nanometers (one billionth of a meter) up to the size of an ostrich egg.

The minimal size of any cell is dependent on the content of the cells.

The primary contributors to the size of a cell are macromolecules like DNA (the "brains" of the cell) and protein (the ultimate signal coded for in the DNA).

Both macromolecules (large molecules) are required for control of cellular activity and to sustain cellular activity.

The size of the average cell in the human body is between 0.5 micrometers and 20 micrometers (one millionth of a meter; the old unit was micron). The diameter of the average red blood cell is between 8 and 10 micrometers.

The Potential Size of The Cell is Dependent upon Two Characteristics

1) the relationship between the nucleus (NOOK lee uss) and the cytoplasm (SIGH toe plazum), e.g., a lymphocyte (LIMM foe sight) has a large nucleus and little cytosol (SICH toe soll).

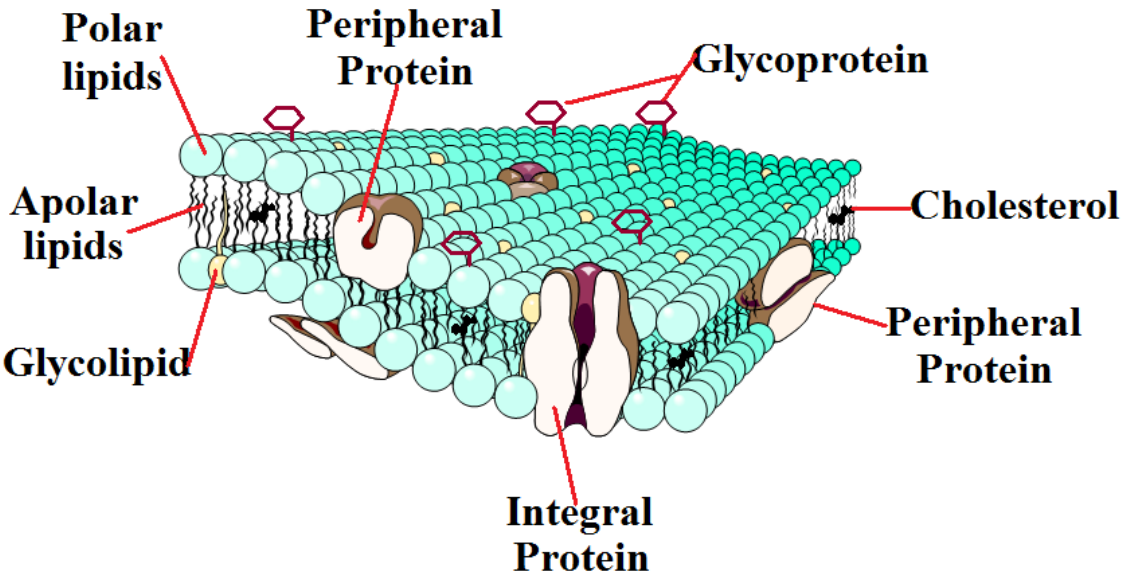
This cell synthesizes many useful proteins, e.g., antibodies, but does not have the cytosol to retain them for very long, hence, the synthesized compounds are released into the general circulation.

On the other hand, monocytes have a large nucleus and a large volume of cytosol. These cells function, among many, as macrophages (MACK row fa juz). These cells synthesize lytic substances that work intracellularly on phagocytized (fa GOE si tized) micro-organisms, cellular debris, excretions or anything else the body does not want or need.

2) The amount of surface for nutrient and/or waste transport. The more surface area on a cell, the more nutrients the cell may take up and the more waste that may be efficiently excreted. The more a cell takes up and utilizes, the larger the cell.

Any Body Cell Has Three Parts: Membrane Systems; Cytoplasm (Cytosol); Nucleus.

Part I - Membrane Systems



This membrane has three-dimensional structure, Figure, above.

Note that it is a lipid (fat) bilayer (two layers) membrane, i.e., there are two layers of lipid that surround the cell.

The outer-most and inner-most of the two layers are polar (hydrophilic [high droe FILL ick] - water loving - or lipophobic [lye poe FOE bick] - fat fearing) lipids. This is important for they must interact with the aqueous solvent of which bodies and cells consist: water. Water is a polar molecule.

The two middle-most regions of this lipid bilayer are apolar (hydrophobic - water fearing -- or lipophilic - fat loving). It is this middle region that gives the membranes a powerful way of separating the cell "innards" from the outside and from other cells, allowing different cells to "bunch together" to form different kinds of tissues and, hence, organs and organ systems and organisms.

The outer layer of phospholipid in a typical cell membrane is primarily phosphatidylcholine (PC or lecithin) and sphingomyelin; the inner layer is primarily phosphatidylethanolamine and phosphatidylserine. The value of this is that the outer layer is a bit more rigid and the inner layer is more flexible, much like taking two sheets of corrugated aluminum roofing and layering them, then bending them. With the aluminum roofing, the inner layer seems to extend beyond the edges of the outer layer when it's bent. By making the inner layer of a membrane more

flexible, it bends, as it were, to retain its alignment with the outer layer without extending beyond the outer layer.

Cholesterol (ko LESS turr all) works with the rigidity of the membrane.

There is also protein associated with the cell membrane: peripheral proteins that are attached to either the inner layer or the outer layer of the membrane that act as receptors for molecules that are unable to get through the membrane and integral proteins that are completely inserted through the membrane. The latter proteins are often-times ion channels, as their outer layer is hydrophobic and more interactive with the cell membrane, while the center portion is hydrophilic.

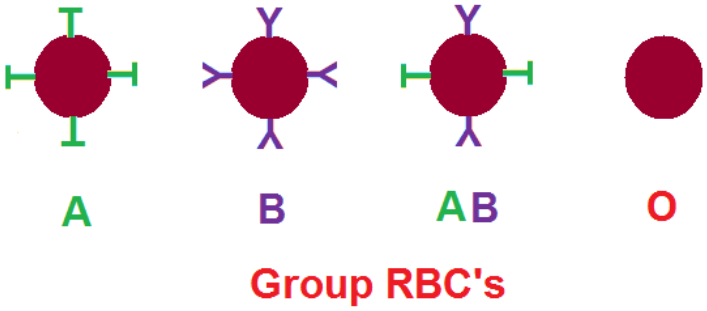
This hydrophilic region allows ions to traverse the cell membrane to regulate ion balance inside and outside the cell.

Glycoproteins (GLY koe PRO teens; a combination of carbohydrate and protein) are also found on the surface of the membrane. As a general rule, these compounds are the compounds used for cell recognition or cell identification.

One use of glycoproteins by the body is as the antigens on the surfaces of red blood cells -- RBC. The illustration, above, shows that different glycoproteins provide RBC's with unique identifying markers. RBC that are of the Group A persuasion have only that glycoprotein on their surfaces; those with Group B have only the Group B glycoprotein; those that are Group AB, have both glycoproteins; those with Group O lack both glycoproteins. See Figure, below:

Glycolipids are also found in the membrane. They tend to stabilize the structure of the membrane.

Cholesterol is found in cell membranes, as well. As a general rule, the more cholesterol in the membrane, the more rigid the membrane; the converse is equally true.

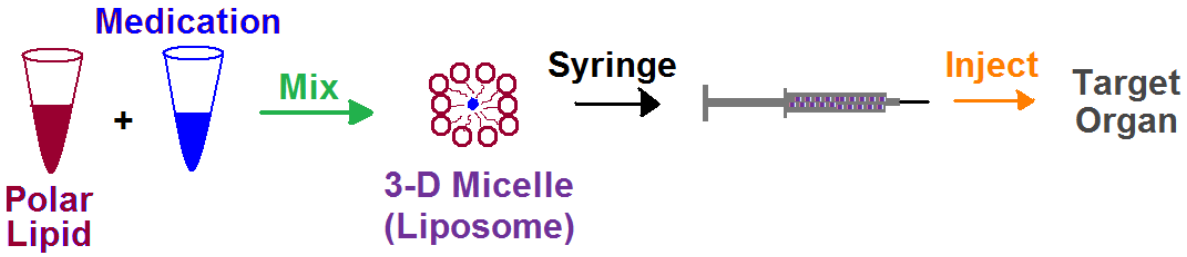


Inside the cell, underneath the cell membrane is a system of microtubules that acts as the cytoskeleton. These microtubules provide a framework to give a shape to the cell. They also act as the cell's irrigation system - more on this, later.

It is estimated that at least 85% of the population secretes soluble blood group substances in saliva, gastric juice, milk, seminal fluid, urine, ovarian cyst fluid and amniotic fluid. Indeed,

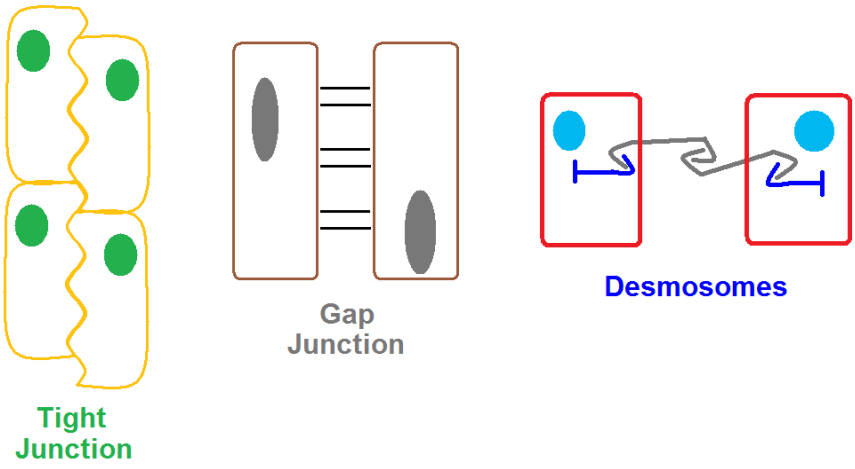
before the invention of DNA testing, it was by these substances that people were determined to be at the scene of a crime.

Pharmacologists, biochemists and physiologists have been studying the chemical properties of the various cell membranes in the human body in hopes of understanding how to use the information to make a carrier (called a liposome - fat sac or fat pocket) that will take a specific drug from a syringe, through the blood without enzymatic modification to a specific target cell or tissue:



The figure, above, shows graphically how this, theoretically, occurs. A polar lipid that has the same characteristics as the membrane to which it is to migrate and traverse is mixed with the drug it is to carry. The polar regions of the lipid rearrange around the drug in such a manner that the apolar regions bind with the drug so that a three-dimensional cage is formed around the drug. This cage is called a micelle (MYE cell; liposome). The liposomes are then injected into the blood, travel to the target organ and keep the drug safe from blood enzymes that might inactivate it prior to getting to the target organ, tissue or tumor. There has been some success with this in the lab.

Cells are connected to each other by one of three Intercellular Connections: Tight Junctions, Gap Junctions or Desmosomes. The Figure, right, illustrates each type of connection.



Tight junctions occur by fusing membranes. These are commonly found in the intestine and blood-brain barrier. It is this kind of connection in the central nervous system that makes it so difficult to get drugs to cross the blood-brain barrier and, hence, to treat disorders of the nervous system.

Gap junctions occur between cells to connect them by narrow channels and are separated by small spaces (synapses). These are common in the nervous system.

Desmosomes consist of filaments that penetrate the cell membrane and cement, hook or suture cells together to form solid, "crumbly" tissues like the liver and kidney.

Cell Membrane Functions

Cell membranes control the passage of substances across themselves: Selectively (allow some materials to cross without difficulty, e.g., water) and Semipermeably (SE mye PER mee abb lee; restricts the passage of other compounds, e.g., glucose and proteins).

The permeability of the cell membrane depends on a number of conditions:

1) Membrane thickness: the thicker it is, the longer it takes the compound to cross the membrane;

2) The size of the materials: tiny molecules like urea easily pass through the membrane, while slightly larger molecules, like glucose will not and very large molecules like proteins simply won't cross the membrane;

3) Lipid solubility: like dissolves like, i.e., if the compound is soluble in lipid, it will cross the membrane easily; conversely, if the molecule is polar, it will not cross;

4) Electrical charge: the time of crossing increases or decreases based upon the charge of the material AND the membrane;

5) Active transport systems: more on this coming up;

6) Binding sites: more on this coming up.

Transmembrane Movement

Passive Movements

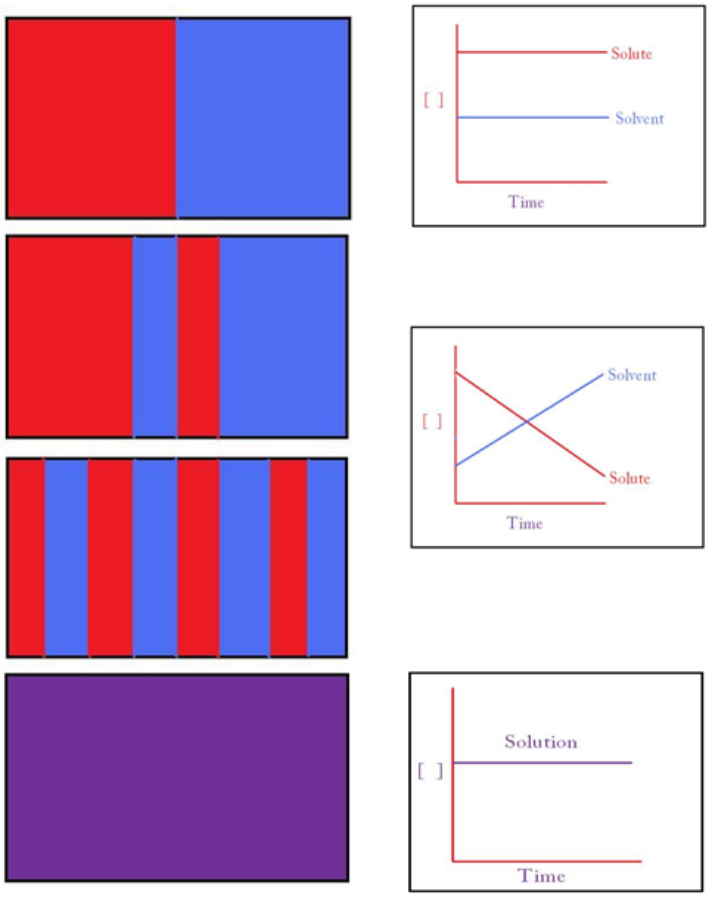
Passive movements are caused by pressure or concentration changes WITHOUT the use of energy.

The first passive movement to be examined is DIFFUSION (dye FEW zhun). By definition, diffusion is the net movement of solute from a region of higher solute concentration to a region of lower solute concentration. The difference between the two regions is called the Concentration Gradient.

The figure, right, illustrates this concept of solute movement. One of the best analogies is that of placing a dye tablet in a beaker of water. At first, there is no movement of the dye into the water and there is no concentration gradient, i.e., there is only dye tablet and water.

As time goes by, though, the dye begins release some of itself into the surrounding water - now there are 3 regions: the dye tablet, the clear water and the region in the water that has varying concentrations of dye (the concentration gradient).

As more time goes by, the dye is uniformly distributed throughout the water, making a homogeneous solution.



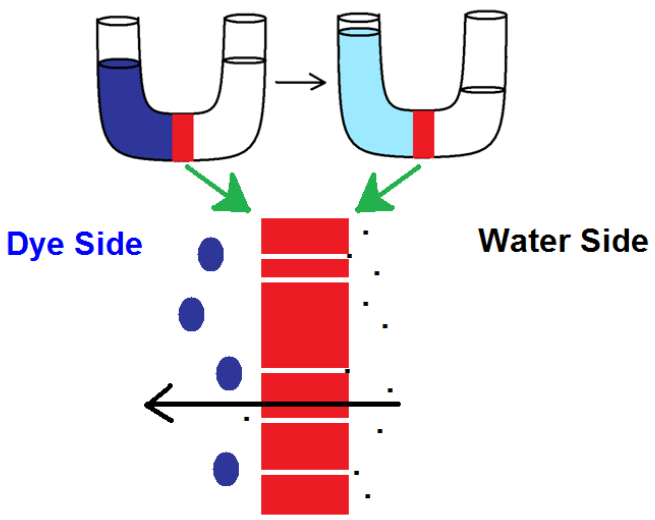
Facilitated diffusion is defined as diffusion assisted by integral proteins in the membrane which act as carriers, e.g., glucose. Facilitated diffusion has a rate that is faster than diffusion, proper.

The rate of facilitated diffusion is proportional to the concentration gradient, i.e., if there is a lot more of a substance outside a cell than inside the cell, then the rate is rapid. It is proportional to the amount of carrier available, i.e., if there are only 5 carriers and 500 molecules to be carried, then the rate of uptake will be very slow. Conversely if there are 5000 carriers and 500 molecules, then the rate of uptake will be very rapid.

The rate of facilitated diffusion depends on how quickly the carrier and substance combine, e.g., insulin. Insulin catalyzes the rapid binding of glucose to the glucose transporter to drive glucose inside the cell. This is called enhancement and greatly increases the efficiency of glucose uptake into our cells.

Osmosis is DIFFERENT from diffusion. Osmosis is defined as the movement of water from a region of higher water concentration to a region of lower water concentration across a semi-permeable membrane until there are equal water concentrations on either side of the membrane.

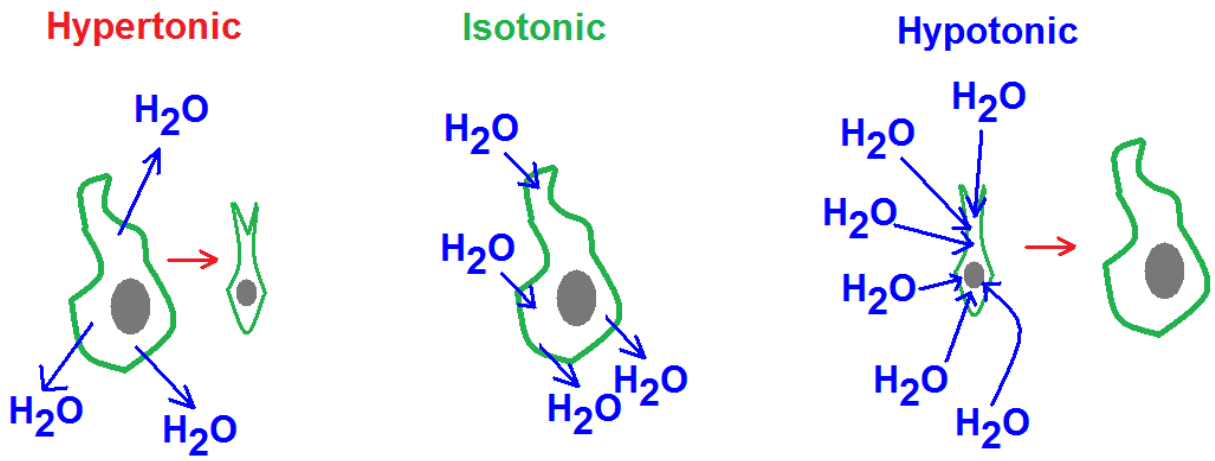
The Figure, right, illustrates the movement of water across a semi-permeable membrane to equalize, if you will, the water concentrations on either side of the membrane.



Note in the expansion of the semi-permeable membrane that the reason that the sugar does not cross the membrane is because it is too large to pass through the pores in the membrane, while the water is not.

Osmotic pressure is defined as the pressure needed to stop the flow of water across the membrane; KEY: the greater the concentration of solute, the greater the osmotic pressure. The converse is equally as true.

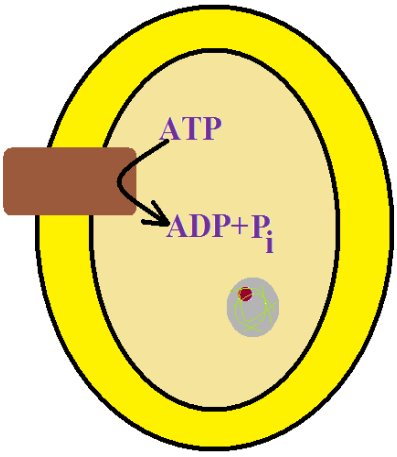
There are three kinds of solutions that involve osmosis: Hypertonic (high purr TON ick), Hypotonic, Isotonic. Hypertonic solutions contain little water in this medium; lots of solute, i.e., concentrated compared to where it is being added. Hypertonic solutions cause crenation (kree NA shun), i.e., shrinking of the cell. One useful application of hypertonic solutions is to infuse these solutions into a patient who has received head trauma to reduce the swelling (edema) of the brain and reduce the damage to the brain. Hypotonic solutions contain a lot of water in this medium; little solute, i.e., dilute compared to where it is being added. Hypotonic solutions cause lysis (LYE siss), i.e., destruction of cells. Hypotonic solutions may be used to rapidly rehydrate a dehydrated patient. Isotonic solutions contain concentrations of solute and solvent identical to that to which it is being added. Isotonic solutions cause no change in cellular size. Image below illustrate these three solutions' effects on cells:



Transmembrane Movement

Active Movements

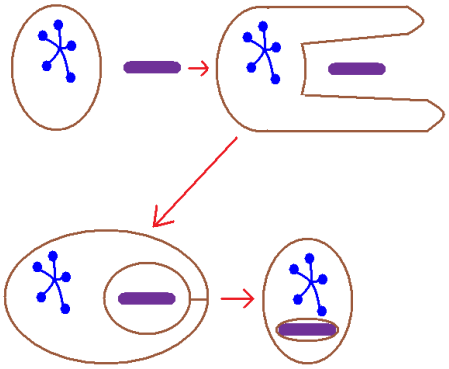
Active movements are defined as those movements that are caused by the release of energy to move material across the membrane from low concentration to high concentration, i.e., AGAINST or ACROSS a concentration gradient.



These movements require energy in the form of ATP (Adenosine TriPhosphate; uh DENN o sin tri PHOS phate). We use up to 40% of the ATP we synthesize daily for active transport. Considering that we synthesize about 4 pounds of ATP per day, that comes to 1.6 pounds of ATP a day we use in these movements. The simplest application of cellular ATP hydrolysis is illustrated by the ATP'ase at right.

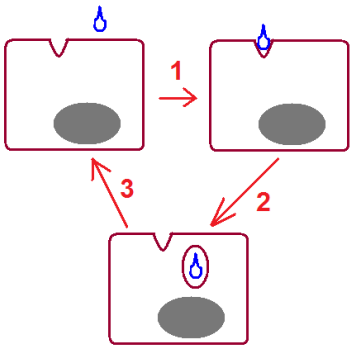
Active movements require integral proteins, e.g., Glucose/Sodium (Na^+) transporter.

The first active movement to be discussed is endocytosis. There are 2 sub-categories under this heading: phagocytosis (FAGG oh sigh TOE siss; cell eating) and pinocytosis (PEE noe sigh TOE siss; cell drinking).



Phagocytosis is initiated by the recognition that something is where it's not supposed to be, e.g., a micro-organism.

The cell responding (a white blood cell called a PMN; PolyMorphoNuclear cell, aka neutrophil) in the image at right, extends pseudopodia (sue doe POE dee uh; false feet) around the organism[s] or particle and then encloses the "object" with the pseudopodia to form a phagosome (FAGG oh some; an eating sac or eating pocket). The phagosome is internalized and it differentiates and fuses with a lysosome (LYE so some) and, presto!, the "object" is hydrolyzed.

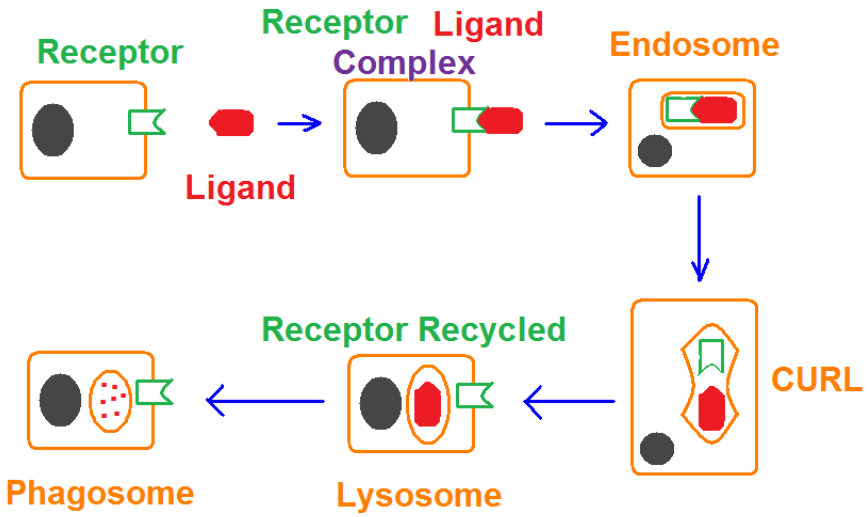


Pinocytosis is similar to phagocytosis, image at left. Cell drinking begins with a water droplet "foraging" around on the surface of a cell. Once the water finds a small invagination (inn VAJ i NA shun), it falls into it and causes the membrane to change shape so

that the pinosome (drinking sac or drinking pocket) is internalized and the water is sent to the appropriate compartment in the cell. Once the water is internalized and utilized, the process begins anew.

Receptor-Mediated Endocytosis

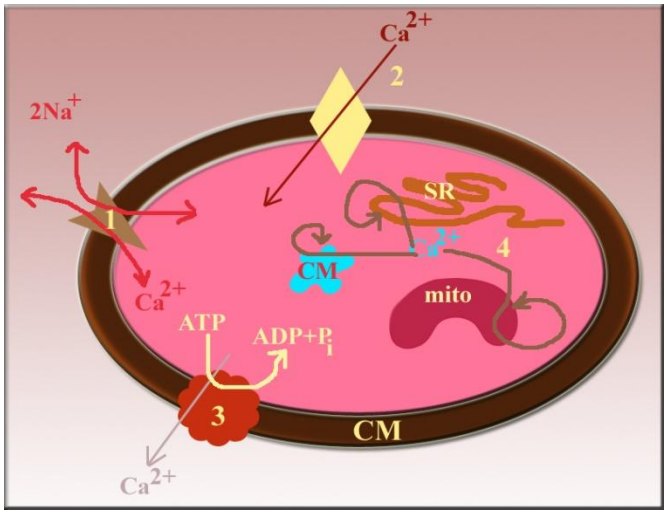
Receptor-Mediated Endocytosis (image below right) requires that the substance to be internalized by the cell have its own cell-bound receptor.



The substance to be internalized is called a substrate (remember enzymes?).

When the receptor (very specific - binds only one substance or one kind of substance based upon its R group – much like enzymes) binds the substrate, there is a change in the shape of this complex and it is called a receptor-ligand complex.

The receptor-ligand complex is internalized into an endosome (an inside pocket or inside sac) which undergoes differentiation to a CURL (Compartment of Uncoupling of Receptor and Ligand). The receptor is recycled to the membrane for re-use while the ligand in its endosome fuses with a lysosome to cause the destruction of the particle.



The Last Endocytic Movements – Ca^{2+} Transport Mechanisms (Image at Left)

Sodium:Calcium Exchange: 1 (See "1" in the graphic; "CM" = cell membrane; "mito" = mitochondrion; "CM" = calmodulin; "SR" = sarcoplasmic reticulum) -- Requires two sodium ions to go the opposite direction for every calcium ion that goes into or out of the cell, respectively.

Calcium:ATP'ase Efflux: 3 (See "3" in the graphic above bottom of page) -- an energy driven mechanism (at the expense of ATP) that removes calcium ions from our cells

Calcium Sequestration: 4 (See "4" in the graphic above bottom of page) -- an intracellular mechanism by which our cells sequester calcium ions by "tying" them up in the mitochondrion of the cell, the sarcoplasmic reticulum or by calmodulin

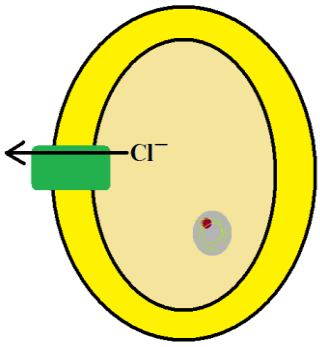
Receptor Mediated Calcium Ion Influx Mechanism: 2 (See "2" in the graphic above bottom of page) -- The calcium ion channel we <u>can</u> control is the receptor-mediated calcium ion influx mechanism.

We can turn this channel off using calcium ion channel blockers such as verapamil (Calan or Isoptin) which effects both smooth and cardiac muscle, diltiazem (Cardizem) which effects both smooth and cardiac muscle or nifedipine (Procardia) which effects smooth muscle.

We can turn this channel on, as well, with drugs like nitrendipine (lowers blood pressure), nimodipine (causes cerebrovascular dilation) or amlodipine (lowers blood pressure).

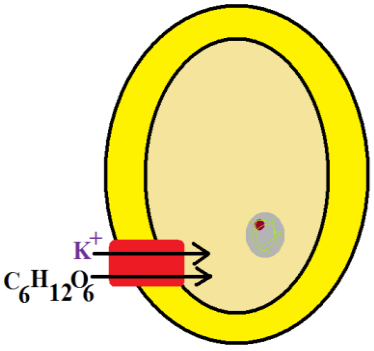
Transporter Organization

Transporters to move ions, atoms or compounds back-n-forth through the cell are organized in one of three ways: uniports (YOO nee ports), symports (SIMM ports) and antiports (AUNT ee ports).

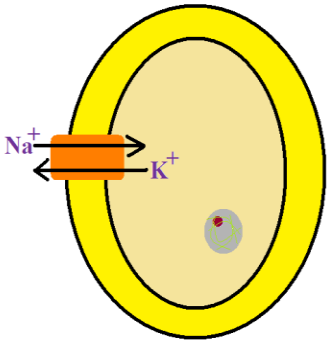


Uniports are transporters that transport a single molecule across a membrane. This is either facilitated diffusion or active transport. One example of a uniport is a potassium ion uniport that moves potassium ions out of cells. Image at immediate right is of a chloride ion uniport.

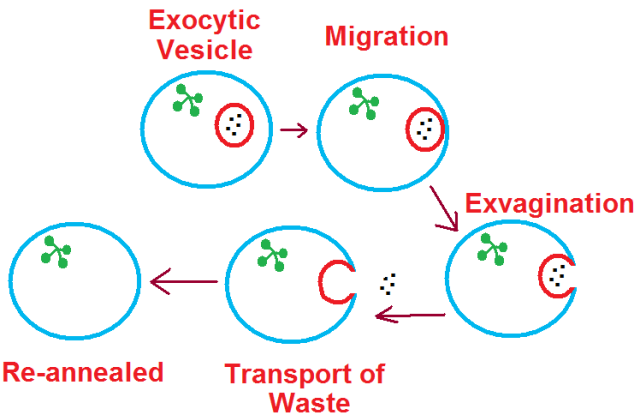
Symports are transporters that transport 2 different molecules that have to be bound to the symport in such a manner that the two particles are transported in the SAME direction. One example of a symport is the sodium/glucose symport system in the small bowel that requires that both be bound for the adequate uptake of glucose from the lumen into the bowel endothelium. Image at right is of a potassium/glucose symport mechanism



Antiports do the opposite of symports, i.e., they transport two molecules in DIFFERENT directions. A good example of an antiport is the ADP-ATP translocase that takes ATP from inside the mitochondrion and puts it in the cytosol and brings ADP from the cytosol into the mitochondrion so that it may be used to re-form ATP. Image at right is of a sodium-potassium ion anti-port mechanism.

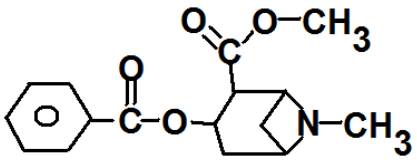


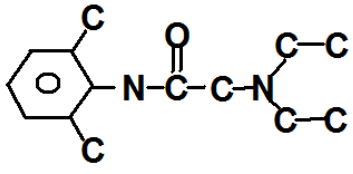
The next active transport mechanism to be discussed is exocytosis (getting something out of the cell). Exocytosis occurs when a cell has an excretion or a secretion it wishes to release. The exocytic vesicle containing the secretion or excretion migrates to the cell membrane where it fuses with the membrane, exvaginates and "dumps" out the particles, e.g. hormone, enzyme, waste. This is how pancreatic enzymes are dumped into the GI tract. Image at right is illustration of exocytosis. Remember, this transport requires ATP, too.

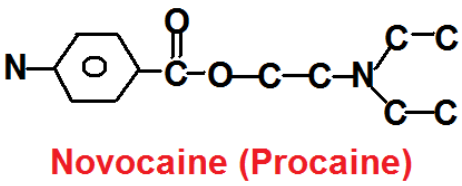


Local Anesthetics and Membranes

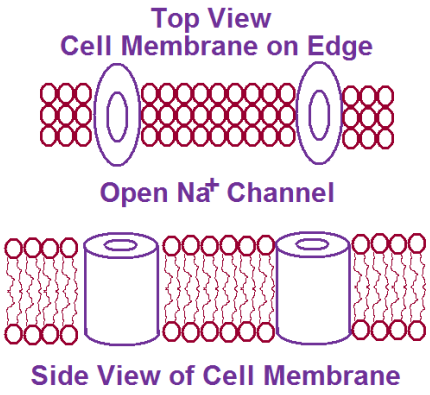
How local anesthetics work (members of the "caine" family in the graphic at right). Note that the "caines" all have a benzene ring, a carbonyl carbon (C=O) and a tertiary nitrogen (N with three functional groups bound to it). With non-judicial use of these drugs, all are hyper-allergenic, including benzocaine, a common drug in over the counter (OTC) sunburn preparations. Imagine a bad sunburn complicated by an allergic dermatitis caused by injudicious use of benzocaine.



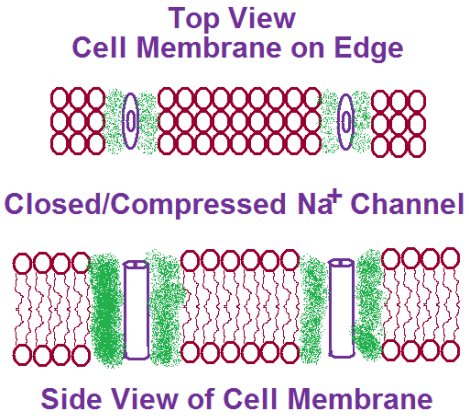




All of the "caines" block sodium ion channels so that sodium ions can not get into the cell from the outside. This causes anesthesia, i.e., a lack of ionic movement cuts off the electrical activity we know as feeling pain.



The "caines" also work by being lipid soluble (top graphic, left, is “normal”). The "caines" pass into the membranes and then "swell" (image at right; green indicates “caine” in membrane). With this swelling, the sodium ion channels are compressed shut, which blocks the sensation of pain, causing the sensation of numbness. Further information on how the "caines" block the sodium ion channel directly is covered in the neurological portion of the course.



Part II -- The Cytosol

The cytosol is also known as the cytoplasm. This is the primary region of the cell where reactions occur.

There are two types of metabolic reactions that we're interested in: catabolism (reactions that destroy nutrients for energy production) and anabolism (reactions that cause our bodies to store up nutrients for future use, i.e., "bulking up"). “Amphibolic” reactions are metabolic reactions that can go either way: catabolically or anabolically, depending on the body’s/cell’s needs.

The cytosol contains subcellular organelles (oar gan ELLS; little organs smaller than the cell). These organelles guarantee compartmentalization, i.e., that there are specific areas in the cytosol where specific reactions will occur in such a manner that there will be no interference from any other [competing] reactions.

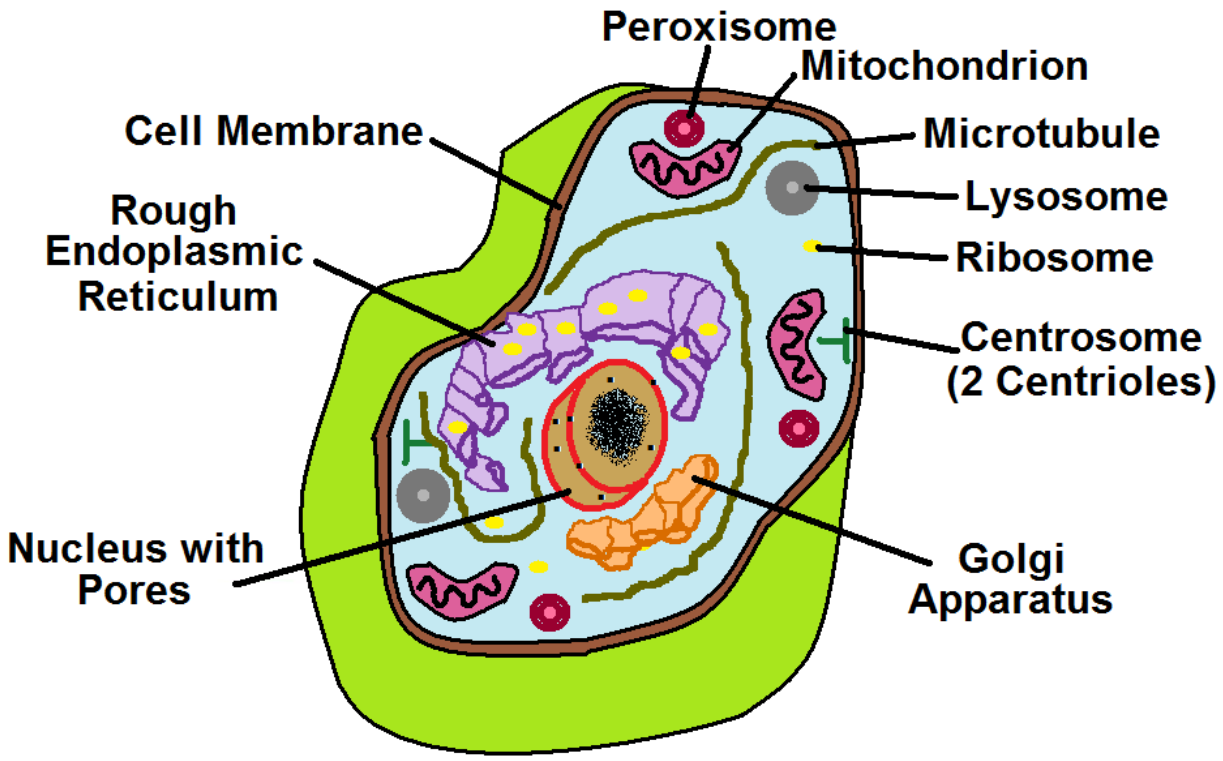
These organelles are permanent and are metabolically active. The latter means that the reactions of the Embden-Myerhof-Parnas pathway (EMP or glycolysis), hexose monophosphate shunt, electron transport and protein synthesis occur in either the cytosol or in the specific subcellular organelle.

These organelles are also self-reproducing. This is of significance, for example, in muscle tissue or in heart muscle tissue: as we exercise to improve our muscle tone or aerobic capacity, these tissues must be able to grow with us. So must the subcellular organelles to provide us with the necessary biochemical pathways to support the new growth and activity of our bodies.

The cytosol also contains inclusions: for storage (e.g., glycogen), for waste (e.g., urates) and/or for raw materials for cellular activity (e.g., fat, pigment granules). In addition, the cytosol contains salts that help maintain the ionic environment of the cell and maintain the pH of the cell.

Enzymes, bound (which allows for enzyme orientation in the case of sequential reactions; bound to internal membranes and/or to filaments) and free (dissolved in the cytosol) are located here, as well.

The Subcellular Organelles



Smooth Endoplasmic Reticulum not Illustrated Here

Rough Endoplasmic Reticulum: For protein synthesis; high numbers of these in antibody-producing cells, liver cells, pancreatic cells.

Smooth endoplasmic reticulum: For steroid synthesis and complex carbohydrate synthesis; in liver (detoxification center).

Ribosomes: protein synthesis; bound ribosomes to the endoplasmic reticulum; free in cytosol (remember, too, that the consistency of the cytosol is like jelly - NOT water); proteins are synthesized on ribosomes, then transported in the tubular endoplasmic reticulum to the Golgi apparatus

Golgi Apparatus: located near nucleus; used in protein packaging; synthesis of glycoproteins, glycolipids, mucus; proteins come in one side of Golgi and go out the other; high levels in liver and pancreas.

Lysosomes: Suicide sac of the cell; has powerful hydrolytic enzymes; implicated in cell death and digestion due to increased intracellular release; implicated in rheumatoid arthritis; very acidic contents. Gold therapy for rheumatoid arthritis: 1) inhibits lysosomal enzymes directly by stabilizing lysosomal membranes; 2) lymphocyte responses to mitogens/antigens are inhibited by gold in culture; 3) monocyte activity decreases after gold therapy, all of which lead to reduced joint erosion.

Peroxisomes: produce hydrogen peroxide; catalase is also present which hydrolyzes hydrogen peroxide to oxygen and water; protects the rest of the cell from the toxicity of the hydrogen peroxide; found primarily in the liver and kidney.

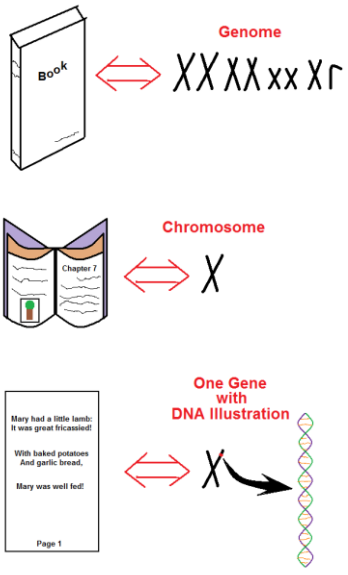
Mitochondrion: "powerhouse of the cell"; double membrane system; ATP synthesis, urea synthesis, lipid oxidation; it's the ONLY organelle to contain its own DNA, BUT still requires nuclear DNA to function; there is a theory that mito were originally bacteria that got trapped in the primordial goo from which cells arose. Contain 5 high energy protein complexes necessary for ATP synthesis.

Microtubules: used to make the cytoskeleton; aka microfilaments; used for irrigation system of cell; used in cell division.

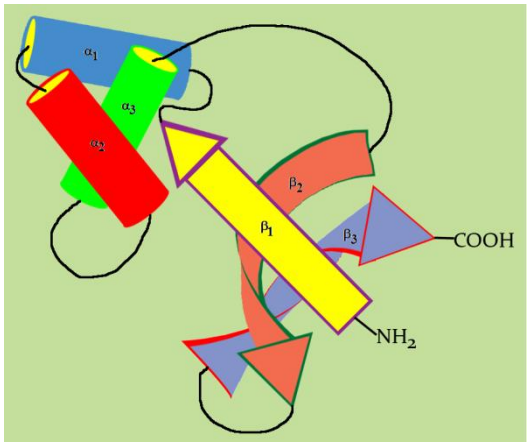
Centrosome: consists of 2 centrioles; used in cell division; used to form cilia and flagella which move materials across cell surfaces (lungs) or propel cells in fluid (sperm).

The Nucleus: The nucleus is the "brains" of the cell. It provides the direction of cellular activity. The nucleus is surrounded by an envelope with pores. The nuclear envelope contains the nucleolus (new KLEE oh luss) which consists of ribonucleoprotein) and karyolymph (CARE ee oh limph; the cytosol, if you will, of the nucleus).

The nucleus also stores the DNA, isolating it from the rest of the cell in eukaryotes. The figure, right, illustrates a simplified way in which to view DNA, i.e., from a book analogy. The book represents the entire genome (collection of chromosomes); one chapter represents one chromosome; one page represents one gene (DNA sequence of the gene).



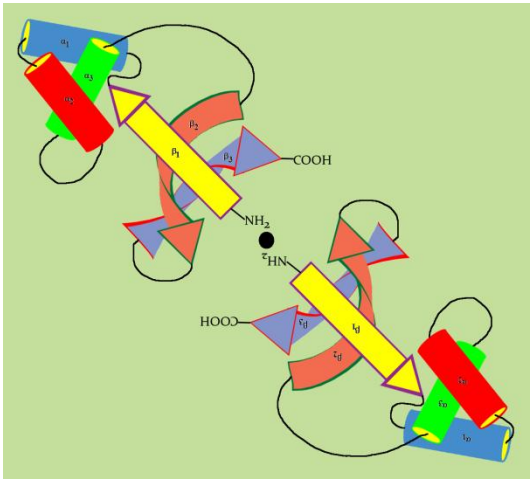
Regulatory Proteins: DNA Binding for Transcriptional Control



There are three motifs of DNA binding proteins and all three of these bind to DNA: ALL three motifs are capable of positive/negative regulation of transcription, 1) Helix-turn-helix, 2) Zinc (metallo) fingers and 3) Leucine zippers.

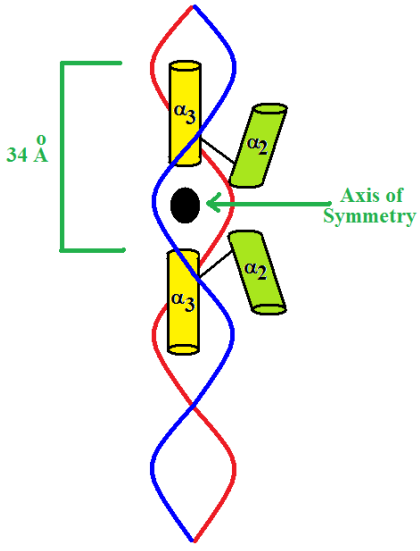
There are some general rules that are applicable to these DNA binding proteins:

1. They have a high affinity binding (binds tightly and favorably) to a specific site on the DNA.
2. They have a low affinity for the rest of the DNA.
3. Only a "small bit" of these proteins may directly contact the DNA.
4. The styles of binding sites for all 3 motifs increase cooperative binding and increase the affinity of the protein of/for/with DNA -- analogy: cooperative binding of oxygen onto hemoglobin as you learned in A&P II.



Helix-turn-helix Motif

Typically this motif has 3 antiparallel β-pleated sheets (flat arrows) and 3 α-helices (cylinders; Figure at top right). Each sub-unit plays a different role in the binding of this protein with DNA: The α_3 subunit interacts with the major groove over about 5 base pairs (bp's). β_3 interacts with another helix-turn-helix protein as it dimerizes (Figure middle right; black dot is axis of symmetry between the two β_3 sub-units).

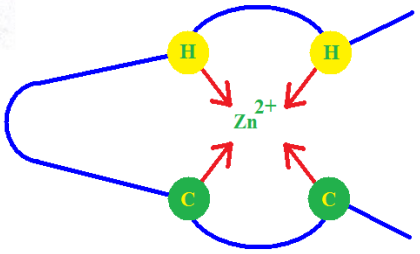


Therefore, 2 α_3 subunits then interact with the major groove to regulate this section of DNA. α_3 and α_2 sub-units are perpendicular to each other to maintain the position

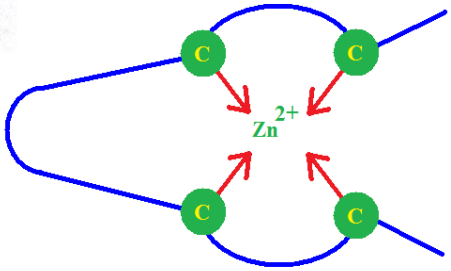
of α_3. The figure on the bottom right of the previous page shows the binding of two dimers in two regions of DNA. Note that the rest of the protein is not shown to simplify this illustration.

Zinc Finger Motifs

These were the second DNA binding proteins to be studied. Studies revealed in a protein called TFIIIA (Transcription Factor IIIA) that it has 9 Zn^{2+} ions, each of which are complexed with coordinate covalent bonds by 2 Cys residues close to each other and by 2 His residues 12-13 residues later. This protein is known as a Cys-His Zn Finger or a C_2H_2 Zn Finger (Figure at top right). The "C"'s stand for cysteine residues and the "H"'s stand for the histidine residues.



Of the forms of Zn fingers known, a change occurs in steroid receptors and thyroid hormone receptor families: the 2 his residues are replaced with 2 cys residues. This is a Cys-Cys Zn Finger or C_4 Zn Finger (Figure immediately right).



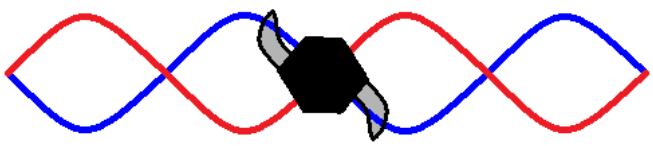
It is important to note that in all known Zn fingers, the Zn^{2+} is in tetrahedral geometry (review Chem 121).

A variation of the C4C4 finger is the C3HC4 RING finger, so called due to its appearance of two fingers turned back on each other forming a ring. RING fingers are found in BRCA-1, the gene identified as being the cause in familial breast cancer -- this is under controversial discussion, at this time. It is possible that the same RING finger style may be in BRCA-2, a gene implicated more in familial ovarian cancer than breast cancer -- again, this is under discussion at this time.

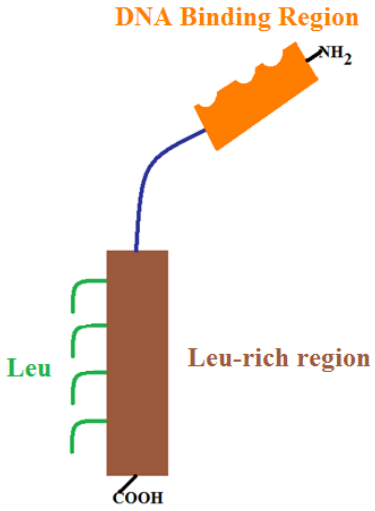
Proteins with Zn fingers lie on a face of the helix with its fingers (grey in image, below; black hexagon is protein-DNA interface) "stuck" into the major groove. Each finger spans 5 bp's (some sources suggest 2-5 bp's) as does the helix in the helix-turn-helix binding motif.

How Important Are Zn Finger Proteins?

Vitamin D receptor proteins have 2 Zn fingers. If ONE mutation occurs in either finger, this receptor will NOT bind vitamin D and Vitamin D resistant rickets manifests.

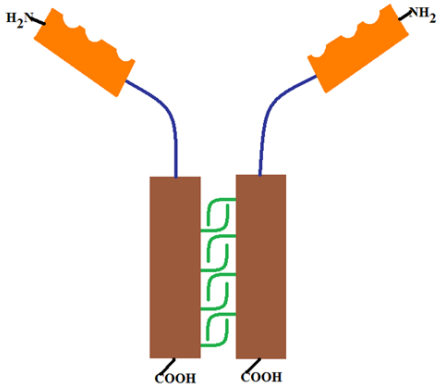


Zn fingers seem to regulate CNS development. Zn fingers may function between DNA-RNA hybrids. Zn fingers may be oncoproteins in leukemogenesis (development of leukemias). Under normal conditions/structures, hematopoietic cells develop into blood cells. When abnormal development occurs, this lead to cancers of the blood (leukemias).



Zn (RING) fingers may be required for viral "growth", indeed, it now appears that Zn fingers are involved with HIV. Zn fingers may prevent inappropriate major histocompatability class (MHC) II expression (more on MHC in BIOL 251), e.g., immunoregulatory genes which are responsible for causing multiple sclerosis as well as insulin dependent diabetes mellitus (IDDM).

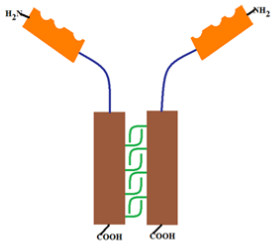
Leu Zipper Motif



This motif is found in the C-terminus of enhancer binding proteins. The leu zipper has α-helical conformation and every 7 residues there is a leu. This occurs for 8 turns with 4 repeats, i.e., leu is found at numbers 1, 8, 15 and 22 (Figure at top right).

This motif is monomeric until it's needed to bind with/to DNA, next slide. The green "zipper teeth" in the figure at right represent the leu residues. When two leu zipper monomers dimerize, these two leu-rich regions zip together into a coiled-coil which appears to increase the association of the DNA binding sites with their DNA target sites, next slide. Note that the "zipper" is located on the C-terminus of the proteins and the binding region is in the N-terminus of the proteins.

Seems to be basic (alkaline -- positively charged) amino acids.
Slips into major groove of DNA



Leu "zipped up"

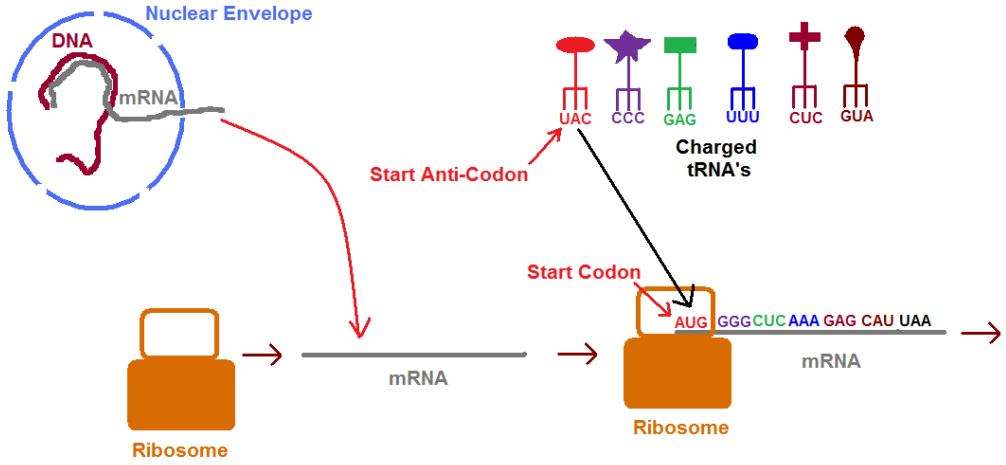
The binding region of the leu zipper seems to consist of basic amino acids (positively charged amino acids, Figure bottom right). This works out reasonably well, since the phosphate backbone is negatively charged and affords good binding to basic amino acids.

ALL three motifs are capable of positive/negative regulation of transcription.

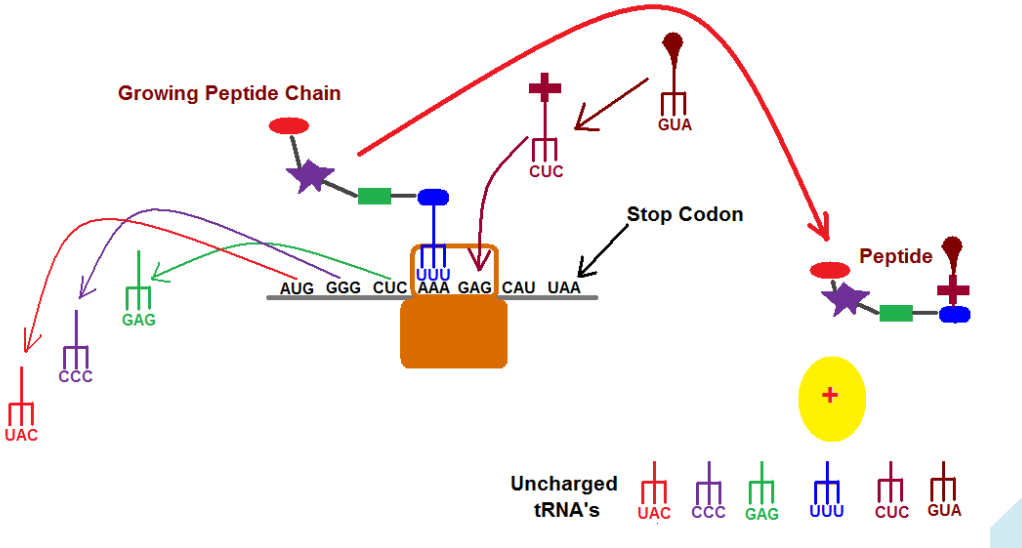
TRANSCRIPTION

DNA is the blueprint for the cell, coding for everything that that cell will do throughout its lifetime. There is one bit of a problem, however. That is the fact that DNA is 1) isolated from the rest of the cell, 2) it's too large to leave the nucleus and 3) as the cell is designed, the DNA is incapable of directly running the show. To solve this problem, there are enzymes in the nucleus that "read" the DNA in such a manner that the DNA is used as a template for the synthesis of another macromolecule, RNA. When the DNA is used as a template for RNA synthesis, this is called transcription. Transcription is very superficially indicated in the graphic, below, by the grey line in the nuclear envelope.

TRANSLATION



The mRNA is small enough that it is able to leave the nucleus through the nuclear pores and go out into the cytosol. This movement of the RNA is the "message" sent by the DNA to initiate many events in the cell, hence this form of RNA is called messenger RNA (mRNA). mRNA binds with ribosomes on the rough endoplasmic reticulum (rER). This is illustrated in the immediately above graphic with the orange ribosome preparing to bind to the grey



mRNA. When it binds on the rER, it initiates a cascade of events called protein synthesis or translation.

In short, the ribosome acts as a sort of zipper handle, sliding on the mRNA. As the ribosome reads a set of three (3) nucleotides in the mRNA sequence, it interprets this triplet to code for a single amino acid. As each triplet is read, another form of RNA transfers the specific amino acid to the mRNA-ribosome complex to perpetuate translation. This type of RNA is called transfer RNA (tRNA). This process is illustrated in the graphic at the bottom of the previous page.

This process of amino acid addition is called translation, or protein synthesis. Each amino acid has its own tRNA; tRNA's are constantly re-cycled for re-use during translation. As the ribosome continues to read more of the mRNA, more amino acids are brought in by the corresponding tRNA and the peptide chain grows. Eventually, the ribosome reaches a triplet sequence known as the stop sequence on the mRNA. When the ribosome reaches this sequence, the peptide is "snipped" from the mRNA-ribosome complex, packaged, processed and transported to the proper cellular locale. Again, illustrated in the graphic at the bottom of the previous page.

Normal Cell Division

The nucleus regulates cell growth and reproduction, as well. The cell cycle consists of 4 discrete periods – coming shortly. Cell division occurs when the cells reproduce themselves. Somatic cell division (body cell division) occurs when a parent cell produces 2 identical daughter cells.

The division of the nuclear material is called mitosis; the cytoplasmic division is called cytokinesis. The daughter cells have the same number and the same kind of chromosomes (KROME uh somes) as does their parent cell.

Reproductive cells also undergo division of its/their nuclear material. This is called meiosis (my OH siss); cytokinesis (sigh to kunn EE siss)also occurs. When a parent cell divides by meiosis, haploid cells are formed. It is by this mechanism that spermatogenesis (spur ma toe GEN uh siss) occurs in the testes and oogenesis (oh oh GENN uh siss) in the ovaries.

There are two (2) successive nuclear divisions in meiosis: reduction division (meiosis I) and equatorial (or equational) division (meiosis II).

In terms of the reproductive cell divisions, the sex cells are called gametes (GAMM eets). In the female they are also called ova; in the male, sperm. Union/fusion of gametes is called fertilization and forms a zygote.

Somatic cells contain 46 chromosomes (2N), which are also equal to 23 pairs of chromosomes for ALL activities of the cell. In a sense, 23 chromosomes are duplicate. "N" or "n" describe the number of different chromosomes within the nucleus. Somatic cells contain 2 sets of each

chromosome. These cells are called diploid (DYE ploid) cells and are identified, as well, by 2N or 2n. In diploid cells, 2 chromosomes in a pair are called homologous chromosomes. Cells that contain 2N chromosomes contain 22 pairs that are autosomal (regulate the body) and 1 pair of sex chromosomes (X and X or X and Y for female and male, respectively). The chromosome number does NOT double in meiosis, rather, it halves producing haploid cells: N, n or 23 chromosomes.

Cell Reproduction

Mitosis and cytokinesis of somatic cells "runs" from approximately (varies by cell and by study) 1-2 hours in length up to more than 30 hours. Cell division occurs and the cells reproduce themselves.

Cell Cycle

The 4 Phases of Cell Growth and Reproduction (Mitosis) are summarized in the table, below, and in the following images.

G_1 phase	S phase	G_2 phase	Mitosis and cytokinesis
~ 8-10 hours in length	~ 6-9 hours in length	~ 2-6 hours in length	~ 1-2 hours in length
Growth phase	Synthesis phase	Growth phase	Cell division
Increased metabolism occurs; Gaps in DNA synthesis develop.	Chromosomes replicated.	Increased metabolism occurs; Gaps in DNA synthesis filled in.	Cells reproduce
Cells that will NOT divide, again, are stopped in this phase, e.g., nerve cells (not 100% true, any more).	Once a cell is in this phase, it is committed to replicate.	Cell volume increases about two-fold greater than it was in G_1.	
These first three phases are collectively known as Interphase. During these three phases, chromosomes replicate, centrosomes and centrioles replicate, RNA synthesis and protein synthesis increase. Once the chromosomes are capped off in interphase, they are ready to undergo division via either: mitosis (all cells in the human) or meiosis (only the immature sex cells in the human, i.e., spermatogonia and oogonia in the male and female, respectively).			

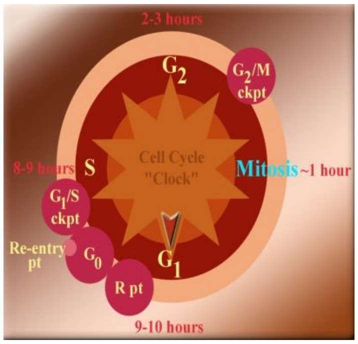
Between G_1 and S phase are four important stages: the "R" point, the G_0 phase, the re-entry point and the G_1/S checkpoint. These stages are summarized in the table below, as well as in the graphics following.

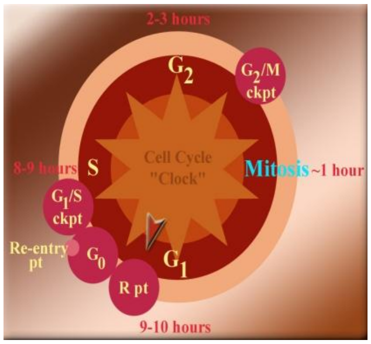
R point	G_0 phase	Re-entry point	G_1/S checkpoint
Restriction point: is a decision point; cell decides to grow or quiesce [for later stimulation to regain entry into the growth cycle].	Cells that come here are not proliferative; are viable; have metabolic activity; quiescent; cancer cells avoid this stage; Recently, research has shown that some nerve cells that enter here actually DO re-enter interphase.	The point where previously quiescent cells are stimulated to leave G_0 and re-enter the cell cycle.	A transition point; 1) to make certain enough time has passed since last mitosis, OR 2) cell is big enough to cause DNA synthesis, THEN go to S phase (uses a protein kinase).

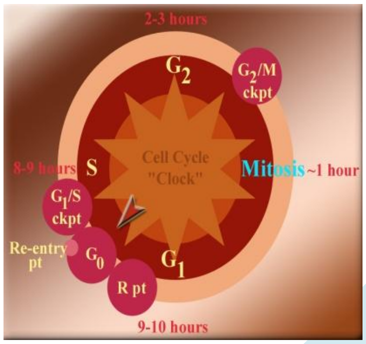
G_2/M checkpoint is between S phase and mitosis. This is another transition point in cell division where: 1) DNA synthesis is required to be completed and 2) When DNA repair is done in this stage, the cell goes on to mitosis (M phase) (again, uses a protein kinase for this function).

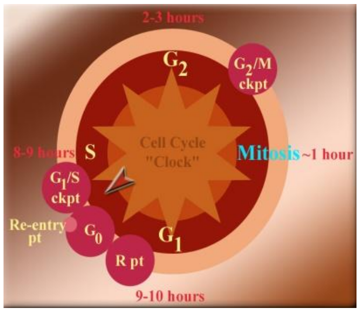
Remember from previous courses that there are four stages in mitosis: prophase (P), metaphase (M), anaphase (A) and telophase (T). The approximate time ratio in minutes for each stage is P: M: A: T -- 12: 1: 1: 6 – again, this varies by cell-type and study.

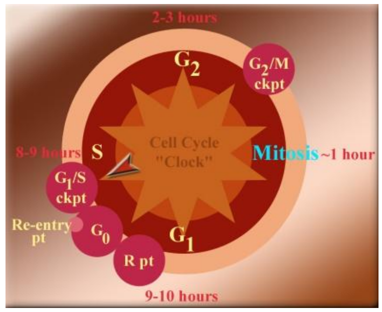
The images, below, summarize the sequential phases of mitosis and cytokinesis - note “clock hand”:

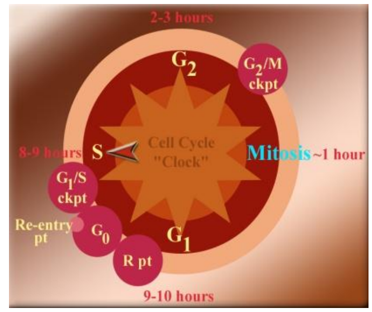


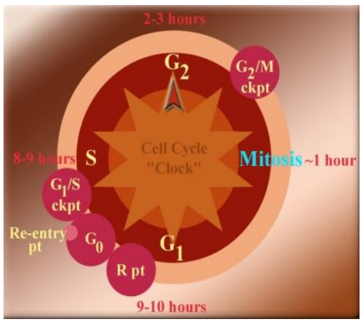


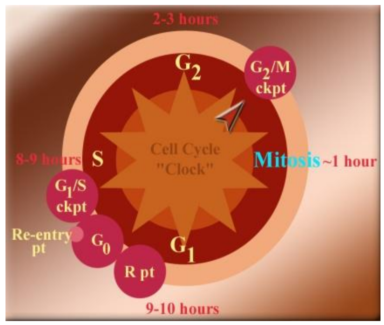


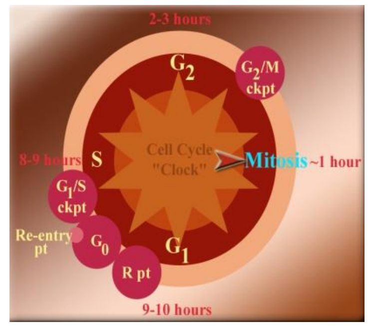










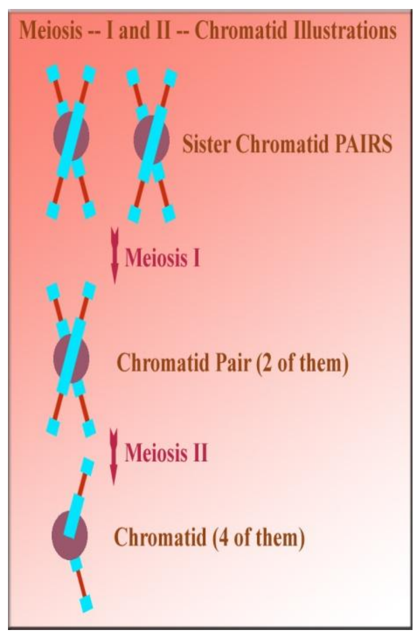


Chromosomes

Anatomy and Nomenclature

A lower hierarchical method of naming chromosomes is summarized in the following table and graphic illustrations.

Sister chromatid pairs	Chromatid pair	Chromatid
a.k.a.	a.k.a.	A.k.a.
--tetrad (4 strands of double stranded DNA) --bivalent (1 pair) --chromosome pair --homologous chromosomes	--dyad (2 strands of double stranded DNA) --monovalent (1/2 pair) --chromosome	--monad (1 strand of double stranded DNA) --hemivalent (1/4 pair) --hemichromosome

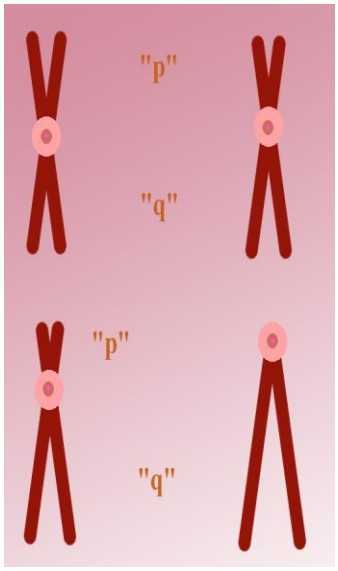


Chromosomes contain genes. Each identical gene site is called a *locus*. Plural is *loci*. Alternate forms of the same gene are called *alleles*.

HAPLOIDY is N number of chromosomes, i.e., 1/2 the number of chromosomes and come from successful meiosis; DIPLOIDY is 2N number of chromosomes, i.e., all the chromosomes that are supposed to be there and come from successful mitosis. In the human, haploidy is 23 chromosomes and diploidy is 46 chromosomes or 23 pairs of chromosomes.

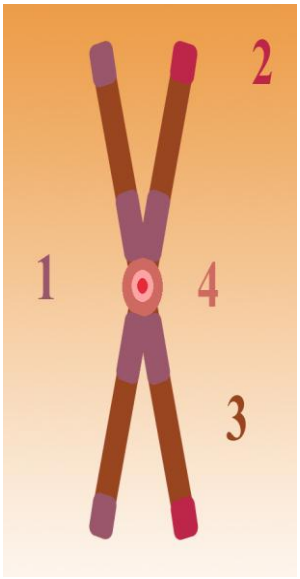
Centromere Location

When describing chromosomes, particularly those in metaphase, it is helpful to describe them in terms of the location of the centromere on the chromosome. Figure, right, illustrates how the location of the centromere may be used to name chromosomes. As you can see in this figure, we also have names for the two arms (one above and one below the centromere): "p" for petite arm or the short arm (by convention this arm is ABOVE the centromere) and the "q" arm or long arm ("q" comes after "p" in the alphabet; by convention it is the arm BELOW the centromere).



Top Left: metacentric – center of chromosome; Top Right: submetacentric – slightly off ("above") center of chromosome; Bottom Left: acrocentric – closer to the "top" of the chromosome than the center; Bottom Right: telocentric – AT the "top" of the chromosome.

The Metaphase Chromosome



1) Heterochromatin (NOTE: numbers in text correspond to numbers in graphic at right): The heterochromatin is dense, compact chromatin. It contains very low numbers of protein coding genes, i.e., genetically inert (sort of like a "spacer"). It does NOT effect the phenotype and is capable of being stained. Repeated sequences within the heterochromatin with high frequencies are called satellite DNA. Heterochromatin contains telomeres (at the ends of the arms of the chromosomes) and centromeres (at the junction of the arms). There are two types of heterochromatin: constitutive and facultative.

Constitutive Heterochromatin: Constitutive heterochromatin is associated with centromeric regions that are compact in interphase. They are also known as chromocenters.

Facultative Heterochromatin: Facultative heterochromatin condenses ONLY at a specific stage. It is generally switched OFF, but can be reversed. Facultative heterochromatin is used as a switching mechanism during development.

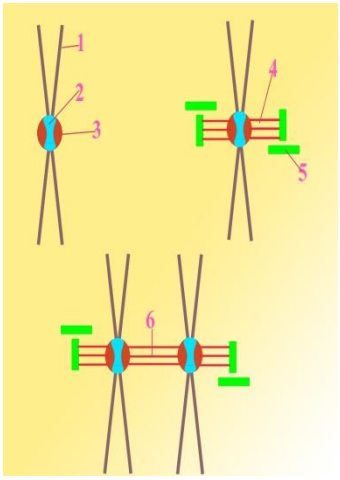
2) Telomeres: The very ends of the facultative heterochromatin are called telomeres. They give the chromosomes stability by "capping off" the ends of the chromosomes. There are 4 telomeres per chromosome. They are added by the enzyme, telomerase.

Telomerase: In short, there is a sequence (single stranded; SS) of nucleotides that codes for the telomeres. It must be added onto the chromosomes under the influence of DNA polymerase or DNA ligase. Once the SS DNA sequence is added to the chromosome, telomerase (which has a built-in RNA sequence that is used as a complimentary DNA template) elongates the telomere as a longer strand of SS DNA. The chromosome with the elongated SS DNA (telomere) sequence then is acted upon by DNA polymerase with nucleotides to fill in the SS telomere with a complimentary strand of DNA and the chromosome is "capped off".

Note in the figures the base sequences in the RNA and in the SS DNA. What differences do you observe?, e.g., what bases are present in DNA but not RNA and *vice versa*? Once the chromosomes are capped off, they are ready to undergo division via either mitosis (all cells in the human) or meiosis (only the immature sex cells in the human, i.e., spermatogonia and oogonia in the male and female, respectively).

3) Euchromatin: The chromatin between the heterochromatin is called euchromatin. It is loose, uncoiled chromatin. It contains very high numbers of protein-coding genes, i.e., genetically reactive ("key"). It effects phenotype, but does not take stain after it compacts in mitosis or meiosis.

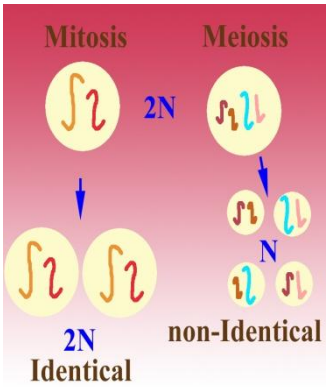
4) Centromere: The centromere is also sometimes known as the kinetochore. The kinetochore is really granules within the centromere used to attach to spindle fibers (kinetochore fibers). There are 2 kinetochores per 1 centromere. The centromere also attaches to sister chromatids. It is highly compact, organized chromatin and attaches, as well, to spindle fibers. This region of the chromosome does contain proteins. The proteins help protect the centromeric region resistant to DNA'ase (an enzyme used to hydrolyze DNA).



Kinetochore – illustrated at right; keyed, below.

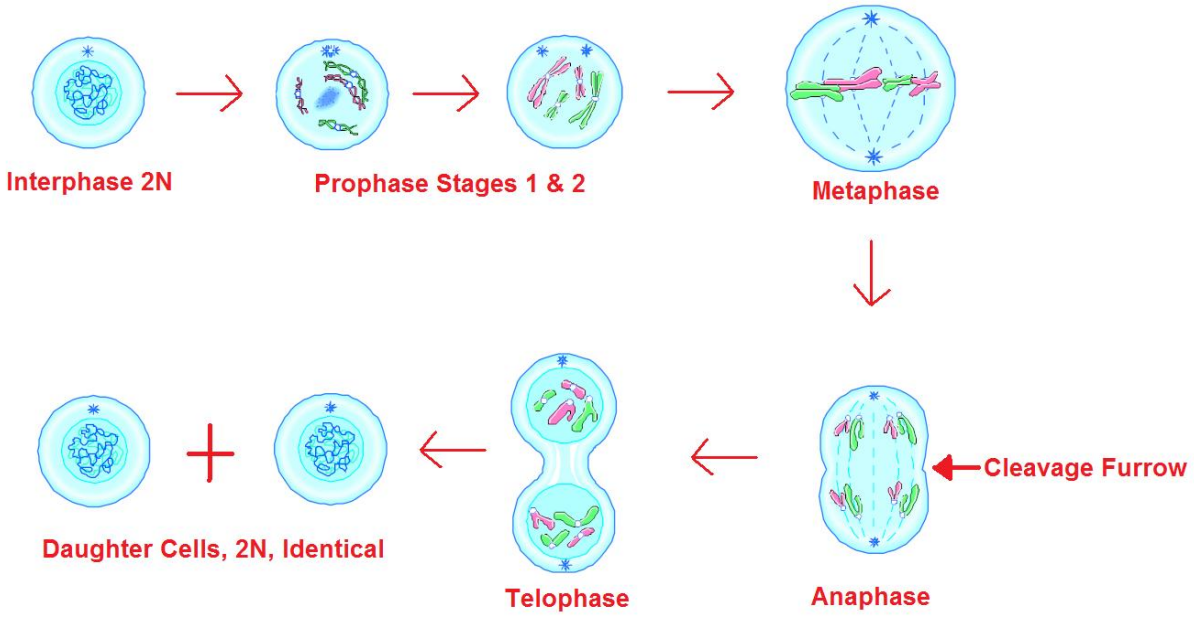
1. Chromosome
2. Centromere
3. Kinetochore
4. Kinetochore fibers
5. Centrioles
6. Kinetochore fibers between sisters

Mitosis vs Meiosis



Summary: Cells undergoing mitosis have daughter cells that are genetically identical with identical chromosomes (2N); Cells that undergo meiosis have daughter cells that are non-identical and have only half the number of chromosomes (N) of the parent cells (2N), Figure at right.

Mitosis



The graphic, above, illustrates an elementary version of mitosis by phase. Remember that Interphase is a combination of G_1, S and G_2 phases.

During Prophase, the nuclear membrane (envelope) is lost, the nucleolus is lost, organization of DNA is gained, centrioles move to the "poles" of the cell and the mitotic spindle forms with the chromosomes arranged parallel to the centrioles.

During Metaphase, the centrioles are aligned properly at each pole and the chromosomes are aligned perpendicular to the poles at the equator.

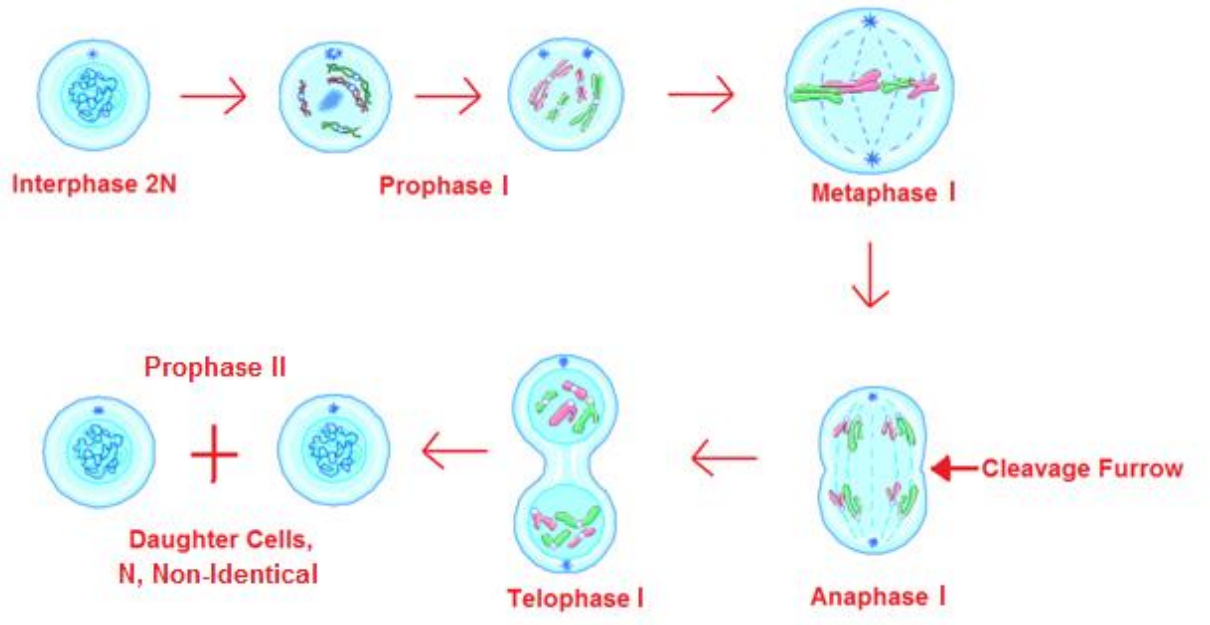
In Anaphase, the cleavage furrow is the first indicator of cytokinesis; chromosomes are pulled apart towards the poles.

Telophase, the last phase, completes cytokinesis and chromosome separation to form two identical daughter cells that are 2N, as was the mother cell. These new cells will undergo Interphase as necessary to continue the cell cycle.

Meiosis

We have to expand upon your fundamental knowledge of meiosis. As you learned in pre-req BIOL courses, there are two cycles of PMAT in meiosis: PMAT I and PMAT II. Prophase I is the stage we must expand. It consists of 7 distinct, more or less, stages: preleptotene, leptotene, zygotene, zygopachytene, pachytene, diplotene and diakinesis. The only stage with which you need be familiar is diakinesis. During diakinesis there is continued compaction of chromosomes; spindle fibers form; the nucleus loses its nuclear envelope; the cell moves into metaphase 1.

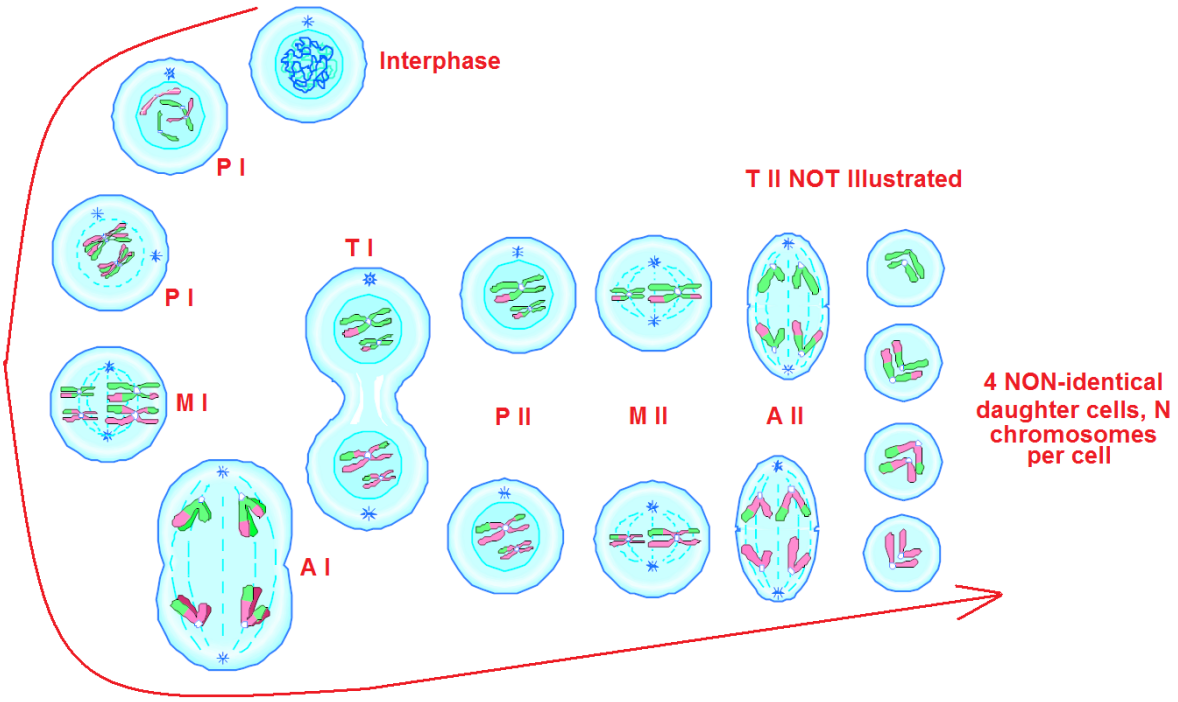
Meiosis I – Reduction Division



If the above diagram looks familiar, that's about right – there's not much difference between Meiosis I and MiTosis, with the exception that this stage of Meiosis reduces the chromosome number to half that of the parent cell (N from 2N). The idea of Meiosis I is to reduce the chromosome count to half of what it was.

Meiosis II -- Equatorial (Equational) Division

Meiosis II functions to double the number of daughter cells, yet maintain N numbers of chromosomes, Figure, below.



The figure above ties Meiosis I with Meiosis II (P = Prophase; M = Metaphase; A = Anaphase; T = Telophase; I = one; II = two). If you look carefully at the outcome, each of the four cells are unique.

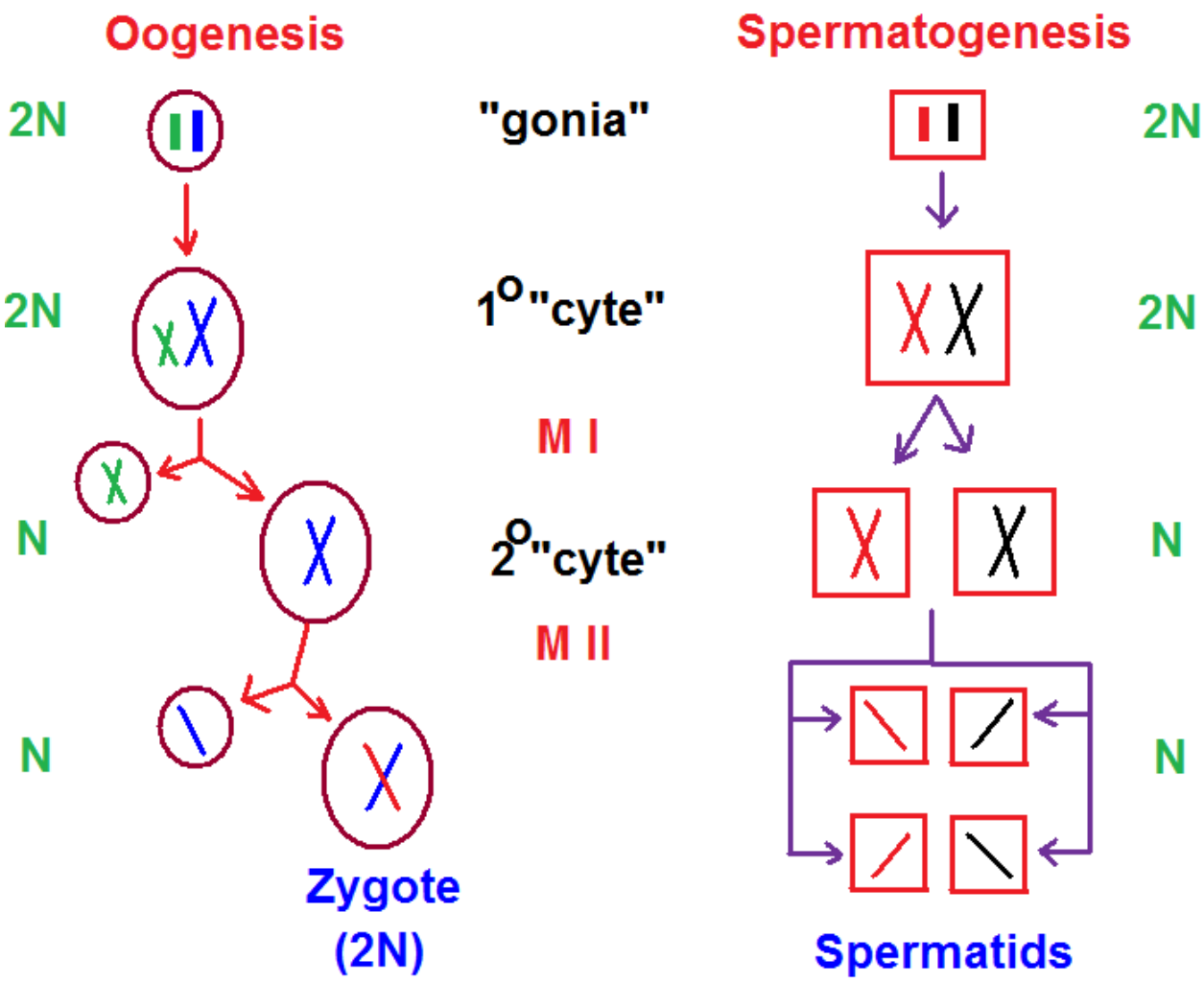
Applications of Cell Division

In the previous section, we discussed how the cell or cells undergo[es] reproduction. In this section, we will discuss some of the applications of the cell reproductive cycle to human. We want to tackle the production of gametes in both the male and female of our species: spermatogenesis and oogenesis, respectively.

Spermatogenesis

The production of spermatozoa begins with spermatogonia. These cells have 2N chromosomes and are a stem cell-type cell before birth. Spermatogonia (spur matt oh GOAN ee uh) undergo mitosis and are arrested in prophase I at birth. At this point, they are differentiated into primary spermatocytes. The spermatocytes will remain dormant until the onset of puberty.

At puberty, under the influence of hormones, the 2N primary (1° spermatocyte) spermatocytes undergo meiosis I to form 2 secondary (2°) spermatocytes. These new immature sperm cells have N chromosomes. The 2° spermatocytes then undergo meiosis II division to form 4 early spermatids which will develop into late spermatids and, then, spermatozoa. When the N



spermatozoa fertilizes an N oocyte, the resulting zygote will be 2N. Note that from 1 spermatogonium, 4 spermatozoa are produced.

Oogenesis

The production of oocytes parallels that of the production of spermatozoa. Oogenesis starts with an oogonium (oh oh GOAN ee um; stem cell-type) before birth. The oogonium is arrested in prophase I (now a primary [1°] oocyte) after undergoing mitosis until puberty kicks in.

Once puberty causes the release of various hormones, the primary oocyte (2N), or primary follicle as it is sometimes known, undergoes meiosis I. In this case, there are two different kinds of cells produced: a polar body and a secondary (2°) oocyte, or Graafian (GRAPH ee ann) follicle (N). The function of the polar body remains a mystery although it may or may not undergo meiosis II.

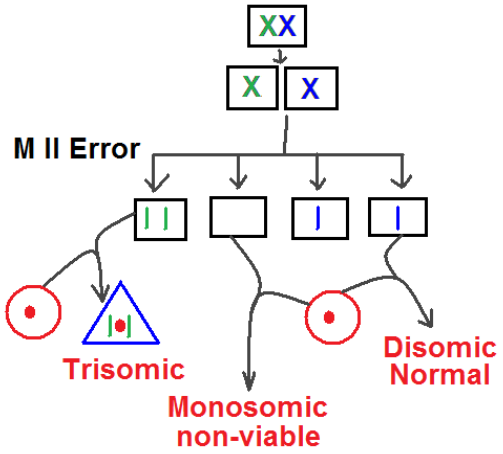
The Graafian follicle is ovulated following maturation (ovulated secondary oocyte). IF the Graafian follicle is fertilized by a spermatozoa, then the fusion product (sperm (N) + Graafian follicle (N)) "triggers" the fertilized oocyte to undergo meiosis II. The products of this division are 1 more polar body and the zygote (2N). If fertilization does not occur, the Graafian follicle is shed. Both Oogenesis and Spermatogenesis are summarized in the graphic at the top of the previous page. Colored bars re[resent chromosomes; “cytes” mean either oocytes or spermatocytes, etc.

Compare and Contrast: One note of significance is that spermatogenesis (production of spermatozoa) occurs in the male reproductive tract PRIOR to entering the epididymis. The process of sperm maturation, spermiogenesis (spur mee oh GENN uh siss), occurs IN the epididymis.

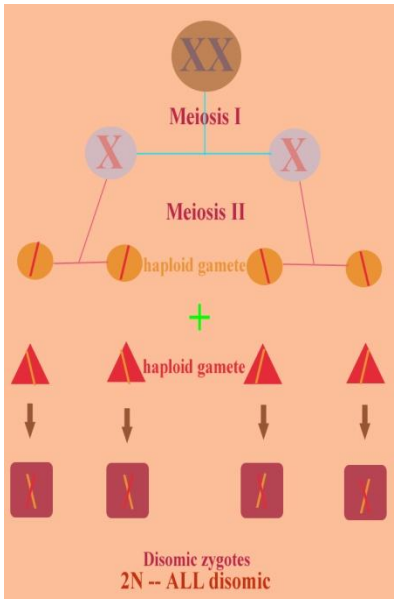
4 Chromosome Movements that are Significant Pre-, Peri- and Post-Meiosis.

Dysjunction	Non-dysjunction	Translocation	Reciprocal Translocation
Separation of a tetrad in anaphase I to 2 dyads; in anaphase II to 2 monads.	Error in separation; failure of a pair of chromosomes to separate at meiosis; move to the same pole in anaphase; if in anaphase I = primary event; if in anaphase II = secondary event.	Movement of a chromosome segment to a different genomic site; may occur within 1 chromosome OR between non-homologous chromosomes.	Exchange of segments between 2 NON-homologous chromosomes.
NORMAL	NOT normal	NOT normal	NOT Normal

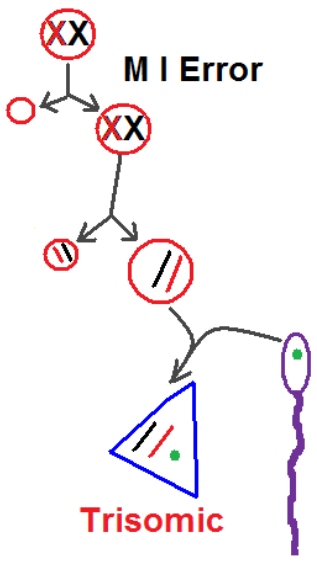
Dysjunction: NORMAL -- Bottom line: all secondary oocytes and spermatozoa are supposed to have N numbers of chromosomes. A graphical illustration of this process is on the top left of the next page.



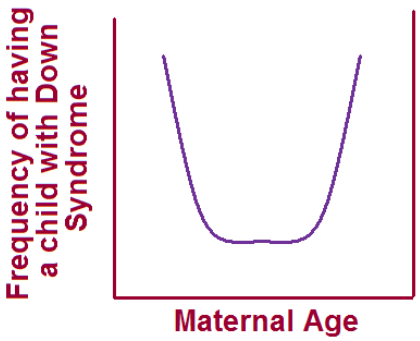
NON-Dysjunction: ABNORMAL -- The results, whether the error occurs in meiosis I or II are trisomic zygotes (three copies of the chromosome), disomic (normal; 2 copies of the chromosome) or monosomic (one copy of the chromosome). The



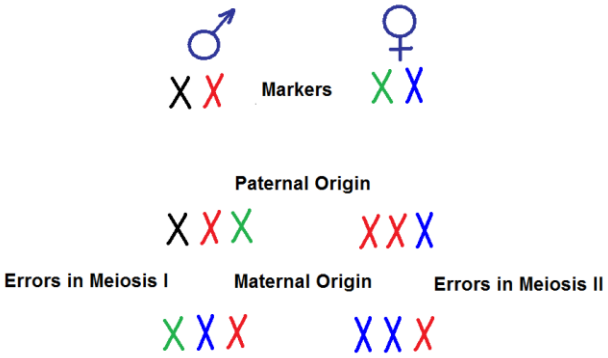
latter tends to be a lethal "combination"; the former not necessarily lethal, but causes severe developmental abnormalities. The disomic individual is the one we refer to as "normal". A graphical illustration of this process is on the top right (Meiosis I error in oogenesis) of this page; an illustration of non-dysjunction in spermatogenesis during Meiosis II is on the bottom right of the previous page.



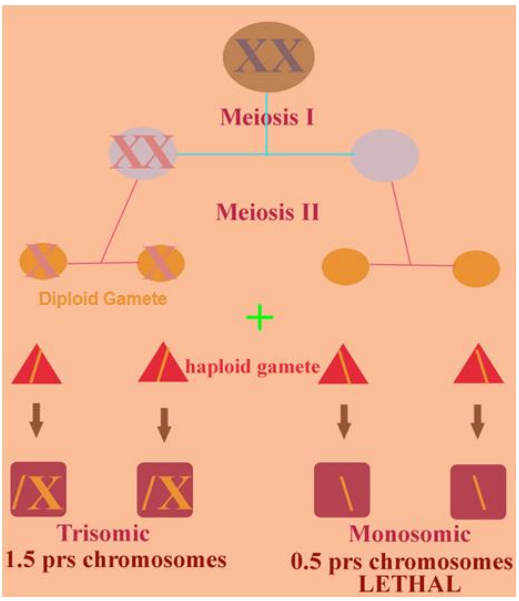
Of interest are three topics regarding non-dysjunction: 1) errors in meiosis I and II occur in both genders, 2) studies have shown that for women the ages of greatest frequency of having a child with Down Syndrome are the very young and the older mother, i.e., a "U" shaped curve (at left) and 3) recent research has shown that at least a third of all cases of children born with trisomy 21 are traceable to the paternal side of that family, i.e., 1/3 d of these cases are paternal in origin. Equivalent paternal age studies have not been performed.

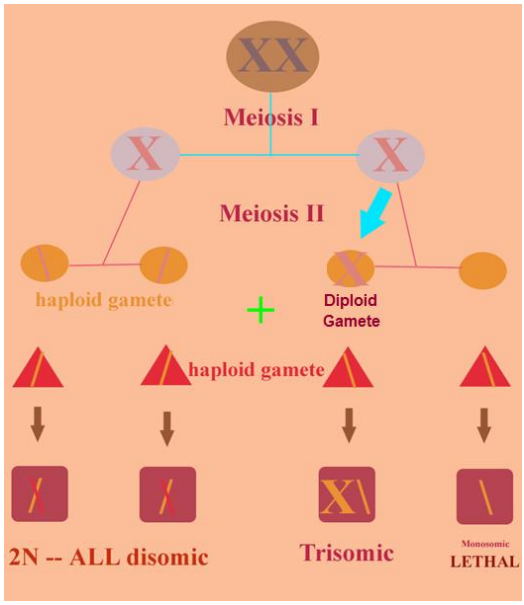


Bottom line: I suspect that in the next 50 years, or so, research will show that the likelihood of a male or a female providing a sperm or ovum with the potential to cause trisomy 21 - and possibly other trisomic disorders - will be equal, as will the source of the extra chromosome in children with other trisomies. This is supported by new techniques that use genetic markers to identify the “source” of the genetic abnormality. One elementary example if the use of markers in Down Syndrome (Trisomy 21) to identify, a) the parental source and b) the site of meiosis error is in the illustration at right. The black and red “x’s” are the markers for the father while the green and blue “x’s” are the maternal markers; Meiosis I errors are on the left of the graphic and errors in Meiosis II are on the right of the graphic.

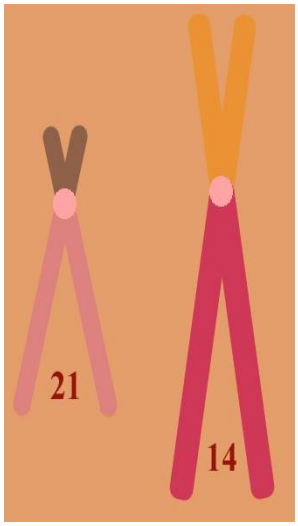


Primary Translocation: ABNORMAL -- Primary (Meiosis I – Illustrated at right) and Secondary (Meiosis II – Illustrated at immediate lower left) events in Non-dysjunction. In short, for some reason, mostly unknown, the chromosomes seem to be sticky and do not want to separate. One instance of stickiness that is known to occur is that chronically alcoholic women who get pregnant tend to give birth to babies that have trisomies, i.e., there is something about the chronically high dose of grain alcohol that makes their chromosomes (seems to be one pair of chromosomes rather than all of the chromosomes) not want to "unstick" from each other. Given time and funding, I suspect that we'll find the same sort of thing happening in the male of our species, as well.



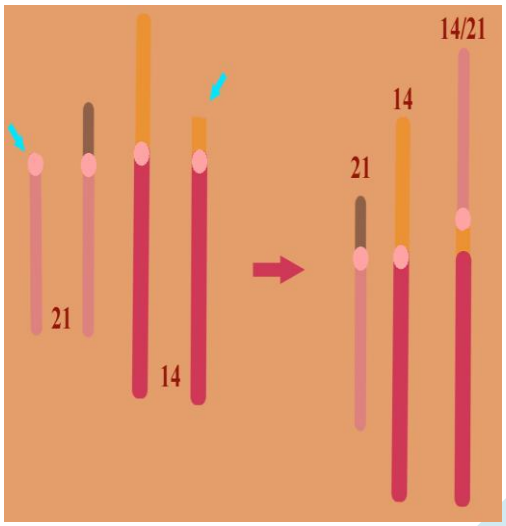


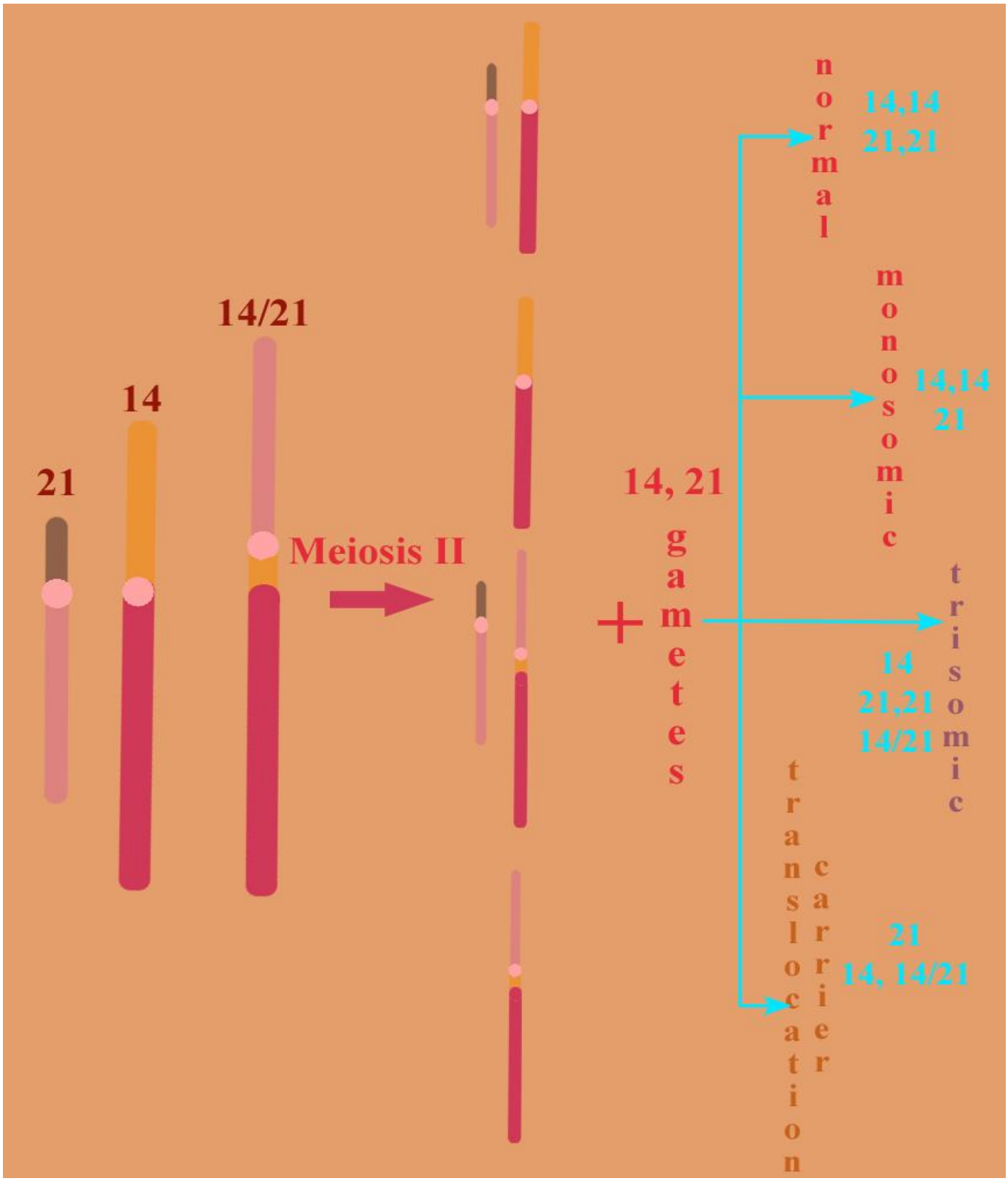
Reciprocal Translocation: ABNORMAL – Stage 1 (bottom left image, this page)-- “normal" acrocentric chromosomes 14 and 21 in the human. Sometimes the "p" arms are lost (more or less) on one of the two of these chromosomes leaving a short 21 and a short 14. The two short chromosomes then anneal to make a sort of "normal" appearing chromosome to our cells.



Reciprocal Translocation: ABNORMAL – Stage 2 (immediate bottom right image) -- This hybrid chromosome is called chromosome 14/21, and tends to act as a 21st chromosome.

Reciprocal Translocation: ABNORMAL – Stage 3 (top of following page) -- If a male or female carry the





genetic combination 14, 21, 14/21 as their genetic makeup, they will produce 4 gametes in meiosis II that will have 14, 21 and 14 and 21, 14/21 and 14/21 as the genetic makeup.

Reciprocal Translocation: ABNORMAL -- you will find 4 possible combinations of gametes and the zygotes they may produce in the following table and previous graphic:

Gamete 1	14, 21	14,0	14/21, 21	14/21
Gamete 2	14, 21	14, 21	14, 21	14, 21
Zygote	14, 14, 21, 21	14, 14, 21, 0	14, 14/21, 21, 21	14, 14/21, 21
Comment	Normal, disomic	monosomic 21	Trisomic for 21	Disomic, carrier for 14/21

NOTE: This sort of translocation is called Robertsonian Translocation.

Trisomy 21 explains about 95% of cases of Down Syndrome. Parents of children with TRUE trisomy 21 have much reduced odds of having another child with Trisomy 21 the second or more children they have. Parents with the 14/21 carrier state have much greater odds of having many children with Down Syndrome over several generations, hence, the carrier (14, 21, 14/21) is the contributor to FAMILIAL Down Syndrome. It also follows that NOT ALL Tri 21 are truly 21, 21, 21: they may also be 14, 14/21, 21, 21.

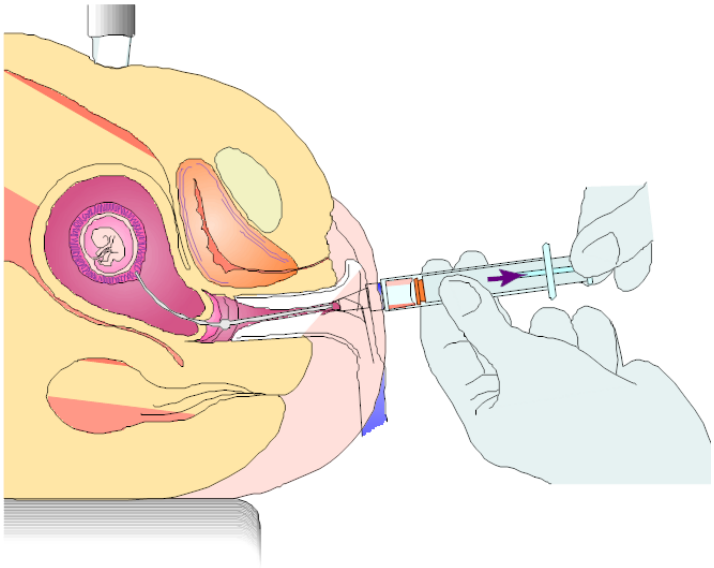
Karyotyping

How may one determine the presence of one, two or three or more sets of chromosomes for one individual? How does one determine the gender of an unborn baby? The techniques involved are either Chorionic Villus Sampling or by Amniocentesis.

All of these may initially be studied by karyotyping. Karyotyping is the process of taking some cells, synchronize them all so that they reach metaphase at the same time, introduce a drug called colchicine to kill the cells, then examine the chromosomes microscopically.

Now this sounds easier than it is. Once the cells are dead, one must find a good field to photograph. Once the photo is developed and enlarged, the chromosomes are cut out and laid in the order of biggest to smallest (1st to 23d chromosome pairs) and by the location of the centromere and satellite regions on the chromosomes. That was the “old” way; nowadays, computers are used for karyotyping, making the work much easier.

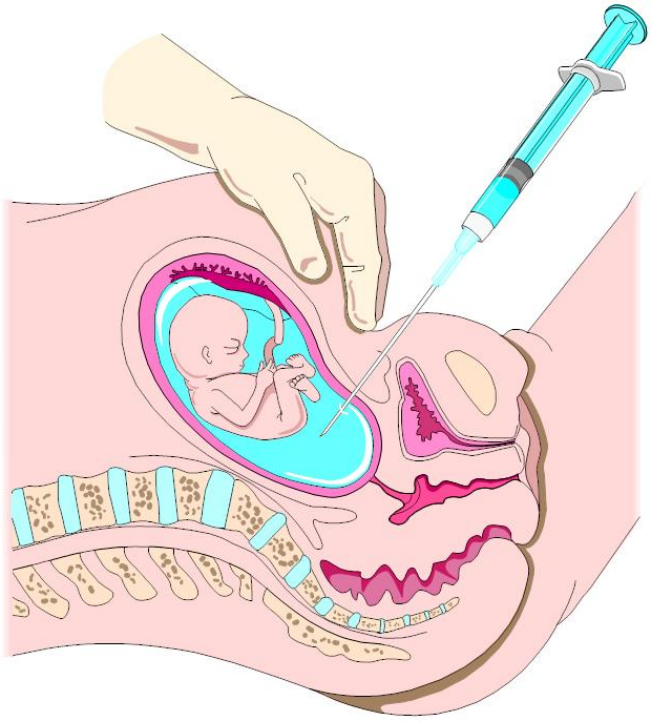
Chorionic Villus Sampling



The significance of these chorionic villi, besides fetal well-being, is that extra-placental villi samples may be obtained between 8-12 weeks of gestation. This is called chorionic villus sampling. The graphic at right shows the approximate procedure. An endoscope or aspiration needle is inserted through the vagina into the cervix, aided by ultrasound so that the aspiration needle may obtain a sample of these villi from the chorion.

Although this is riskier to the fetus than amniocentesis, cells may be immediately karyotyped and anomalies detected sooner so that the parents may make decisions regarding the pregnancy.

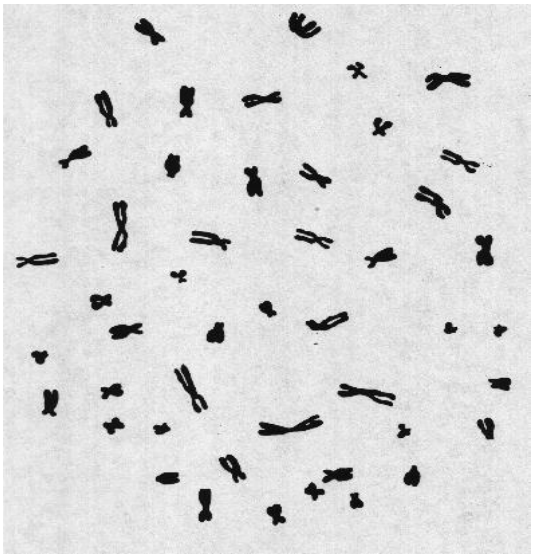
Amniocentesis



The graphic at right illustrates amniocentesis: the removal of amniotic fluid for diagnostic purposes. In general, this is coupled with sonography for placental localization so that it is not inadvertently damaged. The needle and syringe are held at 90° to the abdominal wall and inserted into the amniotic sac. A sample is withdrawn for diagnostic studies.

There is general agreement in the literature that 16 weeks of gestation is adequate for this procedure, although there are some references that indicate that amniocentesis **may be performed** at 14 weeks of gestation. The drawback to amniocentesis is that it takes several weeks to get back the results of karyotypes.

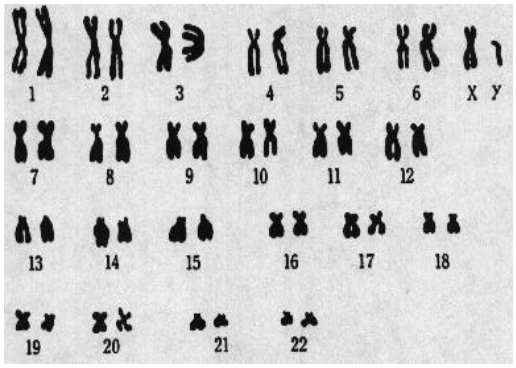
Karyotyping after Obtaining Samples

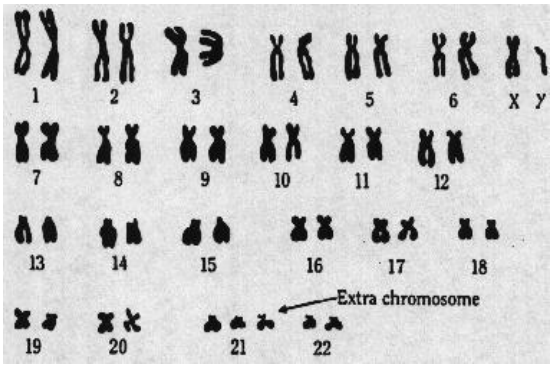


Can you tell if this set of chromosomes at right is normal? Trisomic? Male? Female? NO! You have to cut and paste them onto a karyotyping form to examine and analyze them to answer this burning question. Again, computers have made this process much easier than it was even 15 years ago.

Male Karyotypes

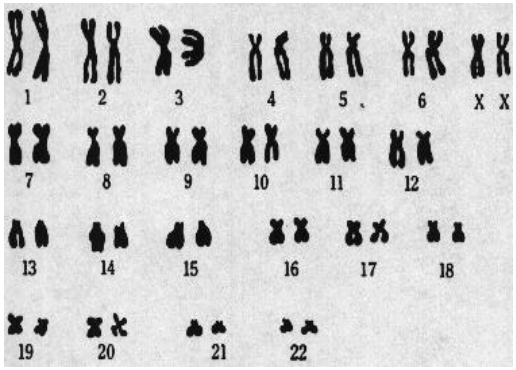
The two images, below, illustrate both normal (on the left) and Trisomy 21 karyotypes (on the right):

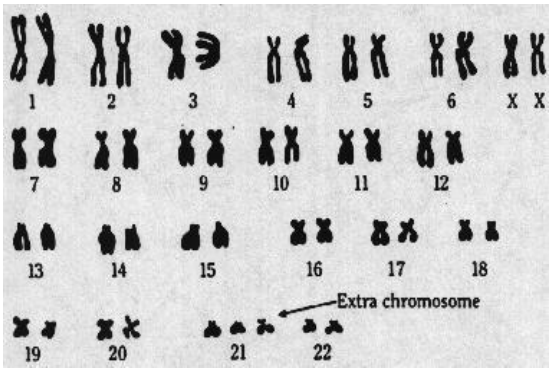




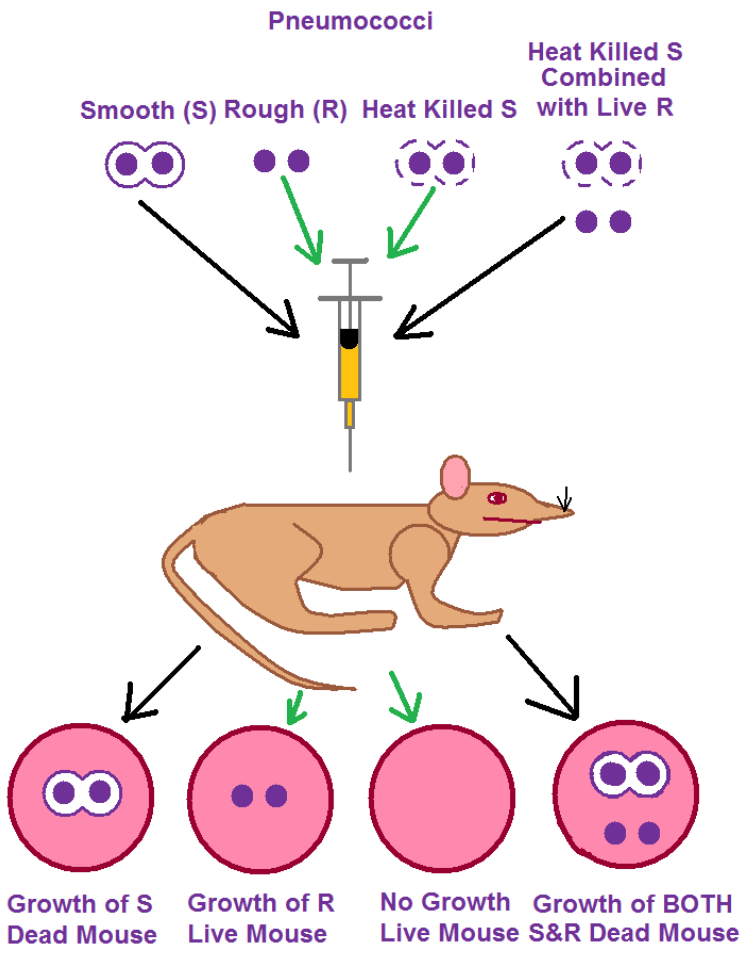
Female Karyotypes

The two images, below, illustrate both normal (on the left) and Trisomy 21 karyotypes (on the right):





Griffith's Transformation



The first evidence that DNA was responsible for transmitting genetic information was shown by Griffith in 1928, LONG before Watson and Crick showed the secondary structure of DNA to be a double strand of DNA in an α-helix. Griffith's transformation, as his experiment has become known, demonstrated conclusively that DNA was "the stuff of heredity" (Figure at right).

Griffith began his experiment by astutely observing that when some strains of *Streptococcus pneumoniae* were injected into mice, they didn't die, while other strains of the same bacterium caused the mice to die. As Griffith delved further into this mystery, he noticed that there was a big difference between the two bacterial strains: one had a capsule around itself (he called this the smooth or "S" strain) and the other did not (he called this the rough or "R" strain).

He then took some of the smooth bacterium (this was the one that had previously killed the mouse) and heat-killed it. This dead bacterium was injected into a mouse and the mouse lived.

Up to this point, whenever Griffith had injected bacteria into the mouse, he had always been able to culture it. After injecting the heat-killed bacteria, he was unable to re-isolate any bacteria.

This was a positive thing, for it conclusively demonstrated that the bacteria was, indeed, dead.

It was the next step in Griffith's experiments that turned the heredity world on its head: Griffith took BOTH heat-killed smooth bacteria and live rough bacteria and injected them

simultaneously into another mouse. The mouse died and when Griffith isolated bacteria from this animal, he observed the growth of both S and R strains of the bacteria in culture.

Since the S strains were previously heat-killed, the only other answer to explain this phenomenon was that the R strains of the bacterium had taken up [some of] the genetic material and begun synthesizing and releasing a capsule based upon that genetic information. While elegant, and conclusive, as well as analytical, in 1928, Griffith's transformation was crude by current standards of DNA identification.

How are nucleic acid and protein samples analyzed? They are first separated on a polyacrylamide (or agarose) gel during electrophoresis. Remember that electrophoresis is the separation of molecules in a solid/formed matrix/support by molecular size (or charge) driven by an electrical charge through an electrical field.

The gels are placed in a gel holder or tank and a buffer solution is poured into the tanks and slightly over the gels. The samples are carefully placed in their wells with micro-pipets. Electrodes are attached to each end of the gel and the current is turned on. Depending on the gel, its thickness and length, and the buffer, the gels are run for a pre-determined period of time.

Once the gels have been completed, they are incubated with a piece of film. Since the fragments are labeled with radioactive phosphorus (^{32}P), the film will be exposed wherever there are radioactively labeled bands.

The film is exposed and viewed over a light box. Alternatively (for those researchers who have good funding), gels may be scanned by computer and viewed on-screen for analysis.

With several questions now answered about DNA and its structure, there is a burning question regarding techniques of identifying either specific DNA regions or individuals by their DNA sequences.

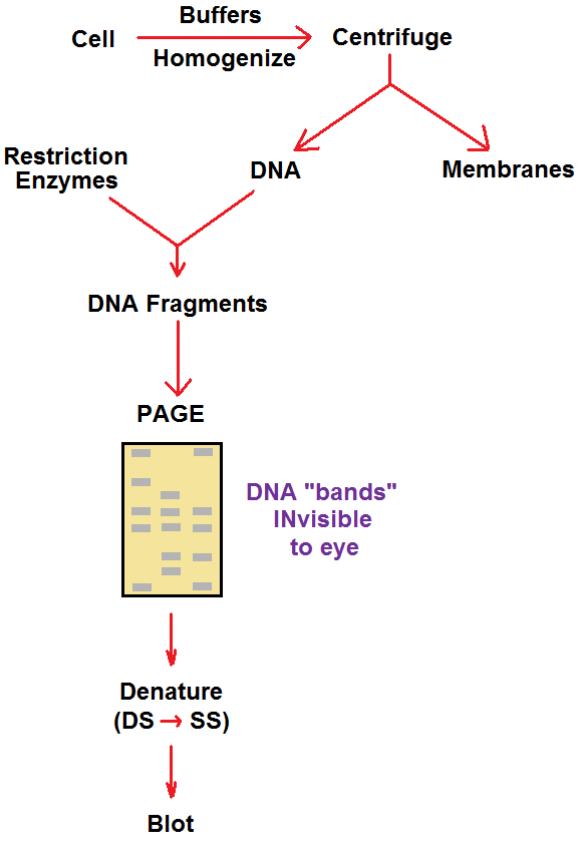
About 25% of the human genome is in many different alleles (identical genes with different sequences). These are called polymorphisms.

Each is an inherited pattern and segregate according to Mendel's rules (coming up in the future). These **restriction fragment length polymorphisms (RFLP's)** may be used to identify diseases, criminal acts or the release of suspects for lack of correct evidence, *ad nauseum*. Only identical twins have identical RFLP's, but NOT identical fingerprints or retinal scans.

VNTR's (variable numbers of tandemly repeated units) comprise very unique RFLP's. They serve as "molecular finger prints" of an individual. Both of these fragments may be studied by Southern blot and PCR.

Southern Blot

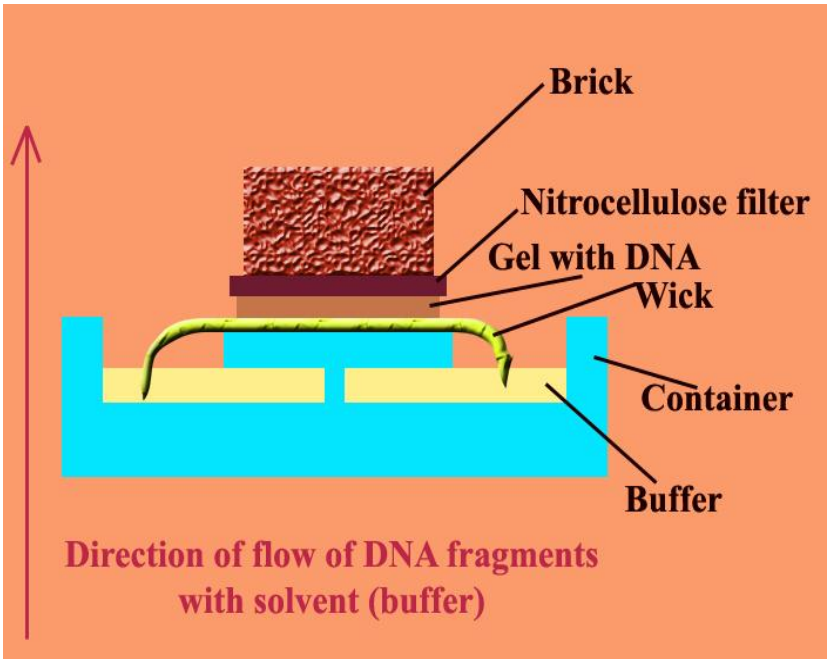
Cells are mixed with appropriate buffers and homogenized. The gimish is centrifuged to give various cell fractions, which include DNA (Figure at right).



The DNA is digested by restriction enzymes to fragments and submitted to PAGE.

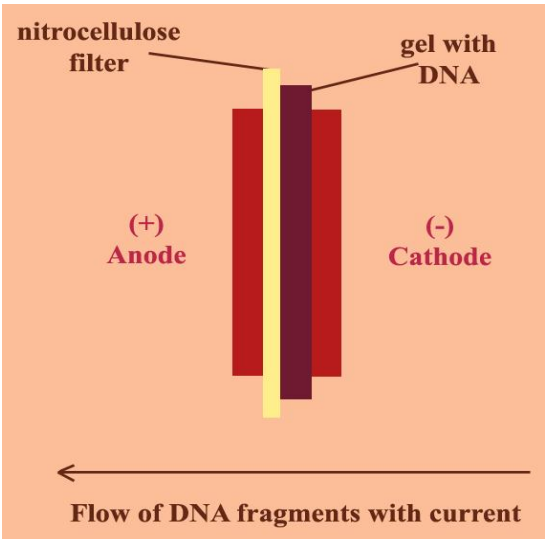
There are NO fragments visible to the human eye in this gel -- yet. The gel is treated in such a manner that the DS DNA is denatured to SS DNA and then the gel is blotted onto a nitrocellulose filter.

Buffer is poured into a "pan" with a stand in its center (Figure lower right). A wick (some just use paper towels) is laid over the stand, upon which the gel is placed. A nitrocellulose filter is placed on top of the gel and a brick (or some heavy weight) is placed on top of the filter. The solvent (buffer) flows up the wick and under the pressure of the brick, causes the fragments to be transferred to the nitrocellulose filter.



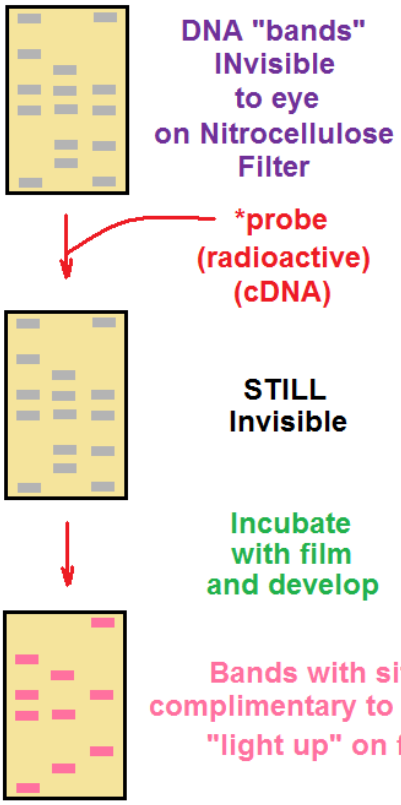
The second way in which material is transferred from the PAGE gel to a nitrocellulose filter: by electrophoresis (figure upper right, following page). The gel with the SS DNA is placed between two electrodes with a nitrocellulose filter placed on the positively charged electrode (anode) side. Electrophoresis is run as described earlier. The DNA

(negatively charged) is attracted to the anode and the SS DNA is transferred to the nitrocellulose filter.



There are STILL no bands visible on the nitrocellulose filter, yet, Figure, middle right, below.

A radioactively (32P) labeled "probe", a specific sequence of DNA complimentary to what you are looking for (cDNA -- complimentary DNA) is added to the nitrocellulose filter and incubated. Once incubation is complete, the irradiated filter is incubated with a piece of film and, then, developed. The only bands, which show up on the autoradiograph, are those bands that are complimentary to the probe and light up on the film. Those fragments may be identified on the nitrocellulose filters by matching it up with the film; the fragments may then be studied as desired.



Northern and Western Blots

Two other blots are of significance, as well: the Northern and Western blots. While the techniques to do these blots are similar, they examine DIFFERENT macromolecules. The Northern blot (named for geography rather than someone's name) examines RNA fragments. The probe for the Northern blot is radioactively labeled cDNA (cloned DNA – copied DNA).

The Western blot (more warped geneticist's humor) looks for proteins, Figure, upper right, following page. The probe for the Western blot is radioactively labeled antibodies (more on this in the future in advanced courses). The Western blot has gained notoriety as it is the confirming test to positively diagnose people who are infected with HIV.

Polymerase Chain Reaction

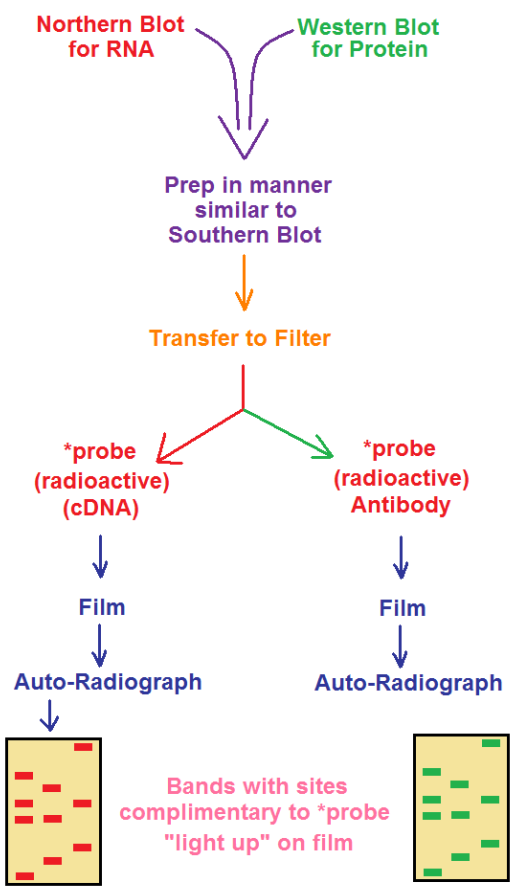
What happens, though, if there isn't enough DNA in a sample to be studied adequately? A technique called the PCR (polymerase chain reaction) was developed just for this purpose

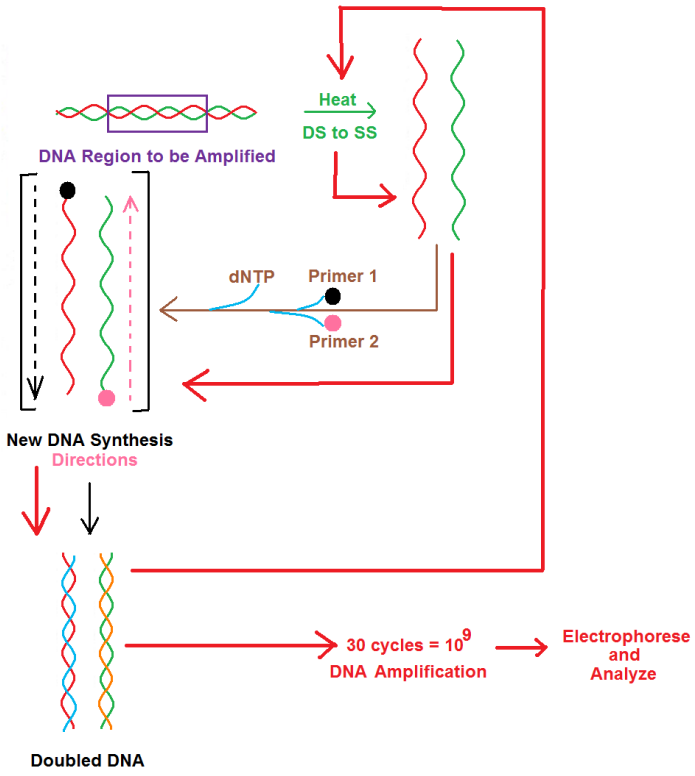
(Figure lower right). In brief, a fragment of DS DNA is heated to denature it. To the two strands of SS DNA, two primers are added, as well as DNA Pol and dNTP's. As this chain reaction gets going, after 20 cycles, this small amount of, previously unstudyable, DNA is amplified one million fold! After 30 cycles, this DNA is amplified a billion-fold! This technique has proven useful in numerous criminal trials.

The PCR is also used 1) to detect viruses of a "sneaky" nature (e.g., HIV), 2) prenatally, e.g., to identify genetic defects, 3) to detect polymorphisms, 4) in tissue typing to reduce tissue rejections, 5) in old DNA samples, e.g., bacteria from pyramids in amber to track evolutionary changes and 6) in forensics, e.g., to identify perpetrators and separate them from innocent suspects.

How does one begin to use this information with technology to make "DNA Identifications"? One way is to actually fracture the strands of DNA. There are four classes of enzymes necessary to accomplish this task: Exonucleases, endonucleases, topoisomerases and restriction enzymes (summarized in the following table, next page).

Restriction enzymes are the most commonly used in this sort of venture. Restriction enzymes are of bacterial origin and have very specific "clipping sites" in the DNA molecules.

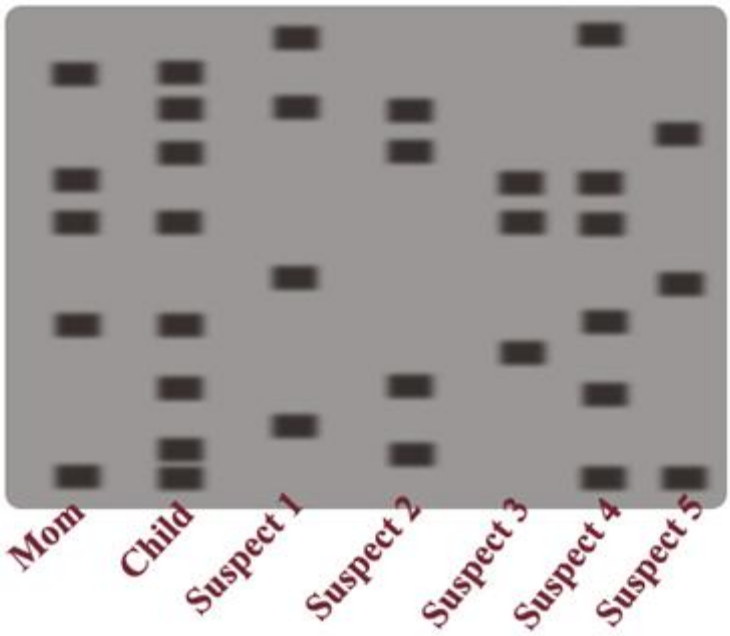




As a general rule, the biological function of restriction enzymes is to cause the destruction of foreign DNA, e.g., viral DNA.

Exonucleases: remove a single nucleotide from the end of the nucleotide chain	Endonucleases: "cuts" strands from within leaving 3'-OH and 5' phosphate ends; very specific	Restriction enzymes: A special class of enzymes -- recognizes a target sequence in the DNA; used to detect paternity, criminal presence,	Topoisomerases: remove supercoiling as replication progresses (think overwound rubber band), e.g., DNA gyrase

Restriction enzymes "clip" at unique sites that contain these specific nucleotide sequences – the details are unimportant and are better explained in Human (or General) Genetics courses. The point is that each human has very unique DNA when it comes to DNA analysis using restriction enzymes. There are Two examples in the use of restriction enzymes that are most commonly used (at least as far as the general public is concerned): Paternity and Criminal Presence.

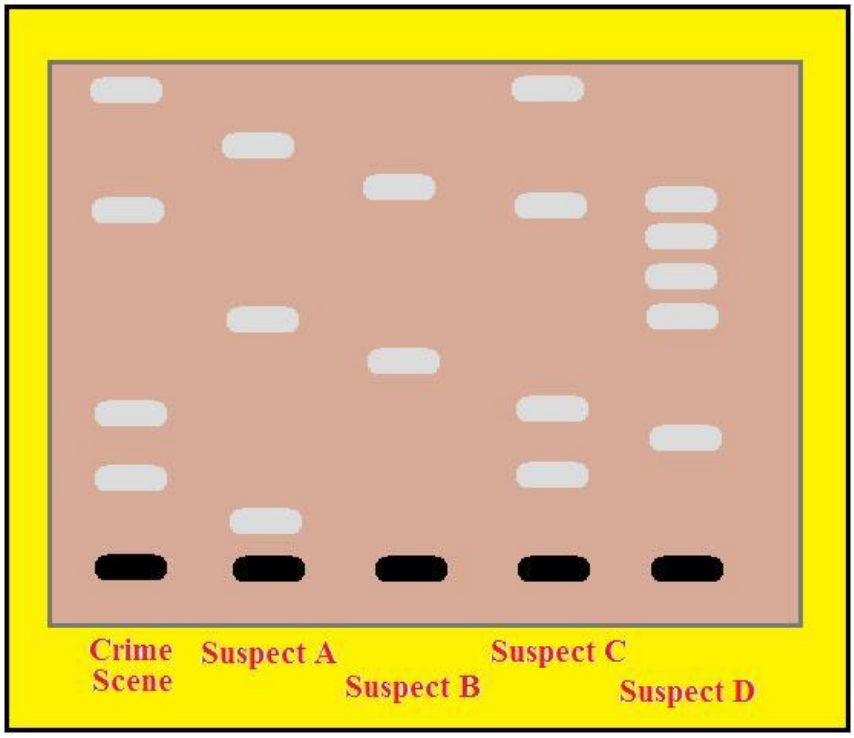


A representation of the electrophoretic patterns of the DNA for 7 people is shown at right: a female, one child and 5 possible fathers.

While this is an over-simplification, it nevertheless illustrates how paternity may be determined using restriction enzymes. When one matches up the bands from mom to child, it's pretty easy to see which bands came from "dad" – and when you match those remaining bands, it's easy to see that "dad" is Suspect #2.

ASIDE: By the way, when it comes to paternity, identical twins are not entirely identical: they have unique fingerprints and their retinal maps are unique, as well. Their DNA, however, is close enough that without special techniques it's difficult to tell them apart. Each twin inherited half of his or her genetic information from each parent. While each twin will have his or her own unique DNA, each will also have sequences identical to each parent.

Restriction enzymes to include or exclude three individuals from having been present at a crime scene are illustrated at middle right. The right lane is the crime scene DNA, the 2d through 5th lanes are the DNA from the evidence, i.e., from each of four suspects. Note that the patterns of the evidence and the DNA from Suspect C match. Suspects A, B and D may go home and Suspect C will be convicted.



The number of DNA fragments depend upon at least three features:

1) the DNA itself (how was the sample cared for; how old is the sample; is there a mixture of DNA's),

2) the restriction enzymes used for the separation (ya hafta use the same restriction enzymes for every technique in the "batch" you are running) and

3) the technique used by the individuals running the gels (good technique gives good results; bad technique gives bad results; ya gotta run quality control with your samples).

Mendelian Genetics

Mini-Glossary

- *Genetic locus*: chromosomal location of the two copies of a gene.
- *Allele*: One member of a pair or series of genes that occupies a specific position on a specific chromosome.
- *Pleitropism*: 1 gene that provides for 2 or more phenotypes.
- *Dominant*: refers to the phenotype, NOT to the genotype; tells us that the mutation in this type of gene presents clinically with only a single dose, i.e., heterozygous; this is at the gene level.
- *Recessive*: refers to phenotype, NOT to the genotype; tells us that the mutation in this kind of gene presents clinically with a double dose, i.e., homozygous; this is at the gene level. <u>It is, therefore, inappropriate to refer to GENES as dominant or recessive: genes are either expressed or NOT expressed.</u>
- <u>Sickle cell anemia</u>: recessive trait: homozygous. BUT, sickle cell gene is expressed with one dose, too, which produces carriers with hemoglobin S (HbS; $\alpha_2\beta_2^S$) and HbA ($\alpha_2\beta_2$) that may cause sickling when exposed to low pO_2: heterozygous; this is expressed at the BIOCHEMICAL LEVEL. A recessive trait may, therefore, be termed <u>codominant</u> at the biochemical level of gene <u>product</u> (HbS [sickle hemoglobin] and HbA [adult hemoglobin]) or <u>dominant</u> under changed environmental conditions (heterozygous sickling).
- If a patient has a disease that is demonstrable to follow Mendelian rules, in all probability, the disease -- regardless how involved the disease is -- comes from 1 gene.

Introduction

There are many aspects to genetics. The aspect with which we have an interest is how or why we are the way we are. There are many complicated ways in which to examine genetics, but the simplest manner is still that which Gregor Mendel developed in 1865. Mendel began his work by observing that pea plants had different characteristics from other pea plants. The same has been observed for a number of other plants, most notably the petunia.

Mendel's Laws

During Mendel's work with plants, he developed three laws:

1. Law of Unit Inheritance: genetic factors keep their own identity and do not blend/merge/fuse in a hybrid, i.e., each gene has its own individual identity.

2. Law of Segregation: 2 alleles of one particular pair of genes are never found in the same reproductive cell, but always segregate between multiple gametes (1/2 to one cell and 1/2 to another).

3. Law of Independent Assortment: That different chromosomes conglomerate to reproductive cells in a manner that requires no dependence on other chromosomes (1/2 of chromosomes go to 1 cell and 1/2 to another BUT don't follow other chromosome halves in a dependent manner).

To understand Mendel's work, we must accept that there is one genetic characteristic that is expressed, or is dominant, and one genetic characteristic that that is not expressed, or is recessive.

Also remember that genes, as a general rule (and particularly as applied to humans) come in pairs. The idea here is that if a gene that is expressed (gives a dominant phenotype) is mixed with another gene that is expressed, then the phenotype is expressed.

If a gene that is not expressed is mixed with another like gene, then the phenotype is expressed.

If, however, a gene that is expressed is mixed with a gene that is not expressed, then the characteristic that is expressed is mostly the dominant characteristic (with some "leaking" of the recessive trait).

The only manner in which recessive traits may be observed is having both recessive traits combined.

When gametes undergo meiosis, they reduce their numbers of chromosomes by half so that when chromosomes rearrange, the, i.e., the zygote, have the right number, i.e., a pair, of chromosomes.

Geneticist R.C. Punnett many years after Mendel's death developed the Punnett Square as he noticed this phenomenon. His method greatly assisted in understanding Mendel's results and methods.

To understand how the Punnett square works, let us examine two families of petunias: one that is all red and one that is all white. Let us assign the following characteristics to the red petunias: RR (the upper case letter says this gene [phenotype!] is “dominant”), where the R is the "code" for the red color.

Let us assign the following characteristics to the white petunias: ww (the lower case letter says this gene [phenotype!] is “recessive”), where the w is the "code" for the white color.

It is easy to see if the red petunias reproduce only with themselves, that the genetics will stay the same, i.e., all flowers will be RR.

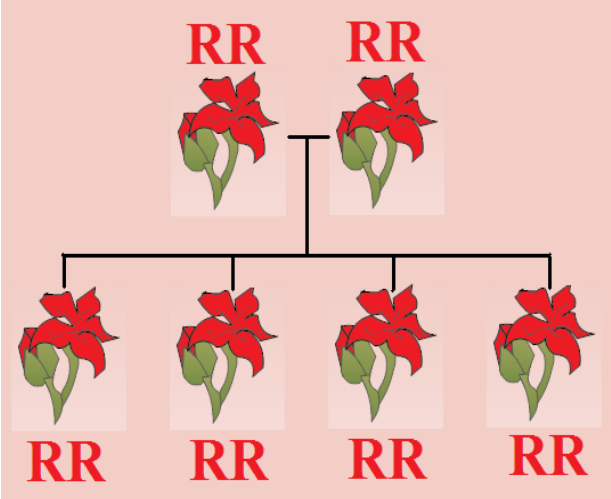
The same will happen with the ww. Let's look at Punnett's square to determine how this works:

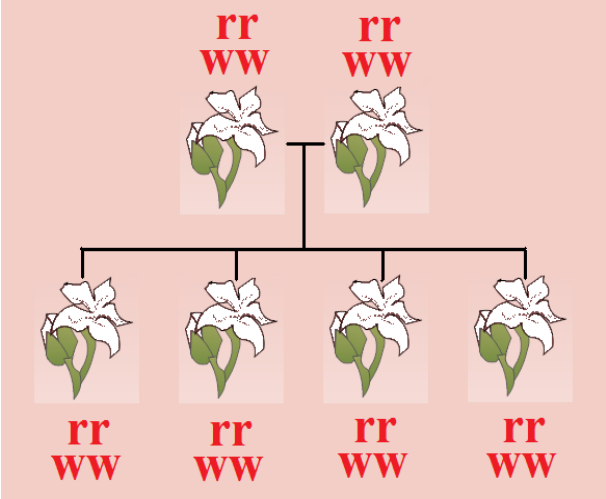
Gametes	R	R		Gametes	w	w
R	RR	RR	F_1	w	ww	ww
R	RR	RR		w	ww	ww
Color	red	red		Color	white	white

Each gene pair separated in half, then rearranged following reproduction. All RR plants are red; all ww plants are white. In both cases, the offspring receive one chromosome from each parent, which aligns appropriately to give the expected characteristic. The genetic characteristics in the two Punnett squares, above, are for the parent (P) generation.

The genetic characteristics that are expressed after parental conjugation are expressed in the family (F), first generation (1), F_1.

Q&D: Mendelian Genetics – Pure Red Flowers (image below left); Q&D: Mendelian Genetics – Pure White Flowers (NOTE: “rr” = “ww” to match text; Image below right))

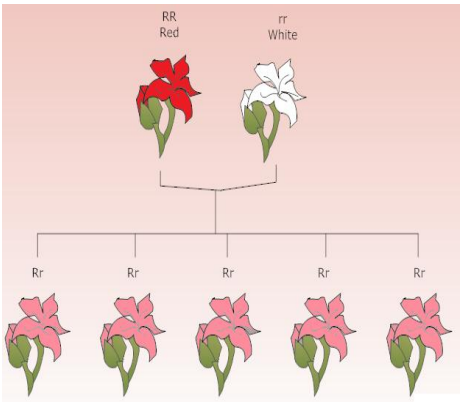




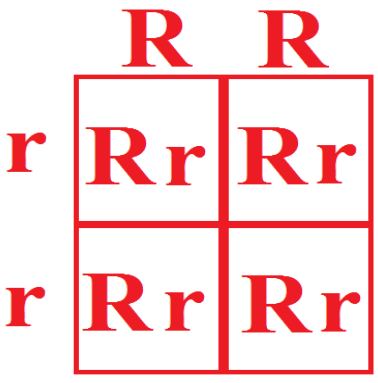
What would happen if we were to cross the F_1 RR generation with the F_1 ww generation?

Gametes	R	R		Gametes	R	R
w	Rw	Rw	F_2	w	wR	wR
w	Rw	Rw		w	wR	wR
Color	pink	pink		Color	pink	pink

The F_2 generation so conceived consists of the expressed gene (R) and the unexpressed gene (w). According to what has been previously discussed, this generation ought to be red (R is expressed). In reality, though, they are pink. The genetic makeup of F_2 is Rw; this is called its GENOTYPE. The color that is expressed is pink and is called the PHENOTYPE.



Q&D: Mendellian Genetics (Image left) et Punnett Square (Image right) – Pink Flowers (NOTE: "r" = "w")

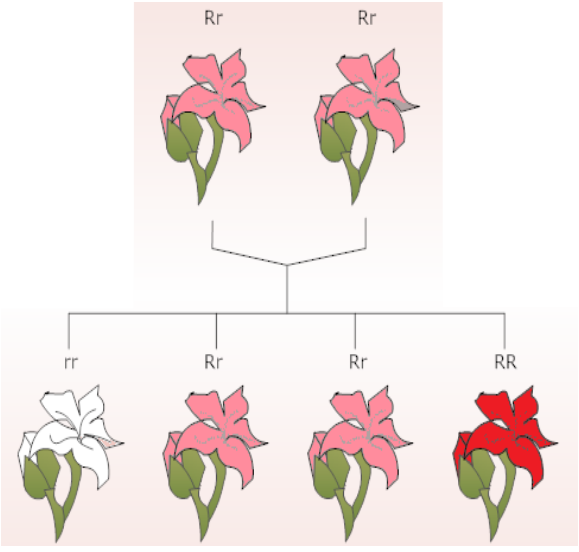


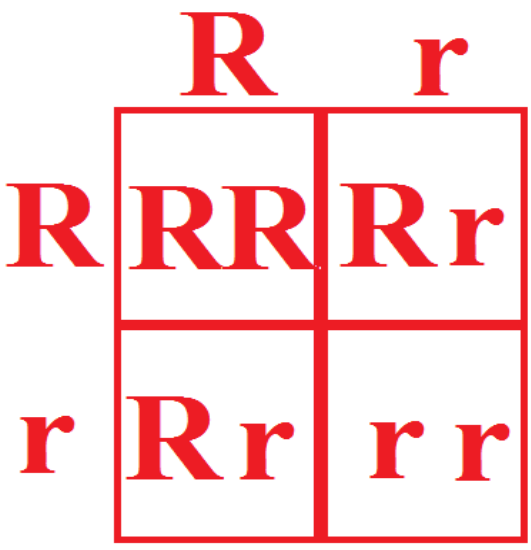
What would happen if we were to cross the F_2 Rw with the F_2 wR generation?

Gametes	R	w		Gametes	w	R
w	Rw	ww	F_3	R	wR	RR
R	RR	Rw		w	ww	wR
Color	pink /red	White/pink		Color	Pink/white	Red/ pink

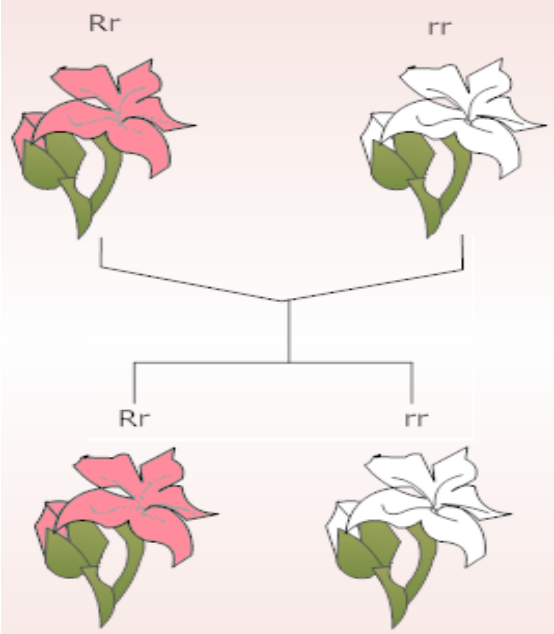
Now, we know that of the F_3 generations, 1 (or 25%) will be red, 2 (or 50%) will be pink and 1 (or 25%) will be white. Another point, however, is that 75% of the F_3 generation has the gene that is expressed (R). It appears, then, that when blending the two F_2 generations, one would have a 3:1 ratio of dominant to recessive traits. Genotypes like RR and ww are homozygous; Rw or wR (identical, by the way) are called heterozygous.

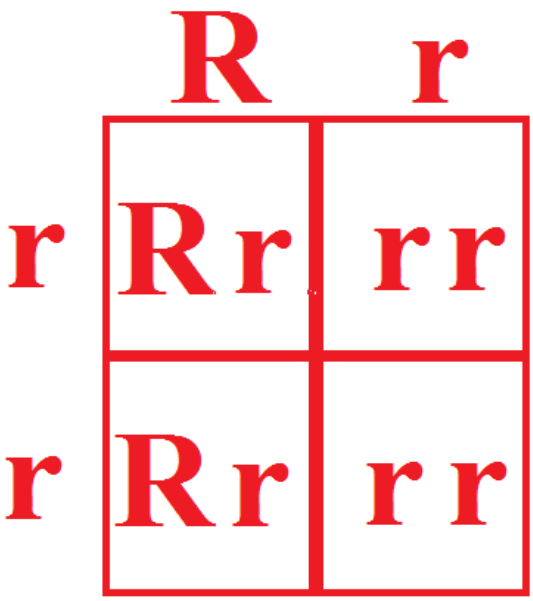
Q&D: Ibid – Multihybridized Flowers (NOTE: "r" = "w") – Mendelian (Image below left) and Punnett Square (Image below right).





Q&D: Ibid (NOTE: “r” = “w” from previous slide)





We can apply the same kinds of concepts to humans. Let us take a tall family, coded TT, and a short family, coded ss, and apply Mendel's ideas through Punnett's square:

Gametes	T	T		Gametes	s	s
T	TT	TT	F_1	s	ss	ss
T	TT	TT		s	ss	ss
trait	tall	tall		trait	short	short

The tall family will all have tall members in F_1 (TT) and the short family will have all short members in F_1.

What, though, would happen if a TT mated with an ss?

Gametes	T	T
s	sT	sT
s	sT	sT
trait	tall	tall

All members if this mating (F_2) will be tall (sT and Ts): the tall gene is expressed. The short gene is partially expressed, as well. These people will be taller than their short parent, but be shorter than their tall parent.

What if two F_2 people mate?

Gametes	T	s
T	TT	sT
s	sT	ss
trait	tall	tall and short

Notice that the F_3 follows the same pattern that the petunias followed: 3:1 dominant traits to recessive traits, hence, 25% will be tall, 50% will be shorter (but, relatively speaking, tall) and 25% will be short. Overall, 75% will be tall and 25% short.

What about applying this concept to various genetic diseases? This is very easy. Let's use phenylketonuria (PKU; an inborn error in metabolism that blocks appropriate metabolism of phenylalanine -- an amino acid -- but increases the levels of toxic metabolites which causes retardation) as our example. PKU is an autosomal recessive mutation. This means it is NOT sex-linked and both recessive traits must be present to have the metabolic error (a double dose of the genes that are not normally expressed).

Let's assign "P" as the expressed, normal, gene and "p" as the unexpressed, abnormal, gene and go back through the Punnett squares as we have done with the previous examples:

Gametes	P	P		Gametes	p	p
P	PP	PP	F_1	p	pp	pp
P	PP	PP		p	pp	pp
trait	Normal	Normal		trait	PKU	PKU

In the first case, we looked at the combination of homozygous genes that are expressed. The phenotype is normal phenylalanine metabolism.

In the second case, above, we looked at the combination of homozygous genes that are not normally expressed as a single dose. The phenotype is abnormal phenylalanine metabolism, i.e., PKU.

Let's combine PP with pp to make the second-generation offspring:

Gametes	P	P
p	Pp	Pp
p	Pp	Pp
trait	Normal Phe metabolism	

All F_2 are heterozygous Pp. The phenotype is normal phe metabolism, BUT each offspring is a CARRIER for PKU.

Let's combine two Pp offspring:

Gametes	P	p
P	PP	Pp
p	Pp	pp

In this combination, the genotypes are 25% PP, 50% Pp and 25% pp. The phenotype is 75% normal phenylalanine metabolism and 25% PKU.

Listed below in the table are selected hereditary traits in humans along with the letter that is used to code for the genotype.

Trait	Letter	Trait	Letter
Curly hair	C	Near/far sighted	G
Dark brown hair	H	Normal hearing	E
Brown eyes	B	Large eyes	S
Male pattern baldness	M	Migraines	A

Note that all the letters are upper case: these are dominant traits.

Listed below are selected hereditary traits in humans along with the letter that is used to code for the genotype. Note that the letters are lower case to represent recessive traits.

Trait	Letter	Trait	Letter
Straight hair	c	Normal vision	g
All other hair colors	h	Deafness	e
Blue or gray eyes	b	Small eyes	s
Have hair	m	No migraines	a

It is important to also remember that the expression of various genotypes is based upon probability. For our purposes, probability (P) is defined as the following:

$$P = \frac{Frequency\ that\ X\ happened}{Frequency\ that\ X + Y + Z + n\ happened}$$

If X is guaranteed to happen every time, then the probability is 1. If X is guaranteed to happen 1 out of 2 times, then the probability is (1)/(2) = 0.5. If X is guaranteed to happen one out of eight times, then the probability is (1)/(8) = 0.125.

This is a nice simple introduction into probability. Is it always this simple? No. The reason it is not always this simple is because we have not taken into account the probability of X happening progressively. To determine if X will happen progressively, one must multiply the probability of X happening at all times itself the number of times you wish X to occur. Let's use sex of offspring as our example and the Punnett square (a means of getting to the frequency of "X" happening:

Gametes	X	Y
X	XX	XY
X	XX	XY

By phenotype, when a man and a woman mix their chromosomes, 50% of the offspring will be female (XX) and 50% will be male (XY).

BUT, what if you wanted to determine the probability of a family having 5 boys in a row?

$$P = \frac{1*1*1*1*1}{2*2*2*2*2} = \frac{1}{32}$$

The probability of having a boy is 1/2. For the probability of five of them to be born in a row, one must multiply 1/2 times itself 5 times. Hence, the probability is not real good that two parents will have an all-male basketball team in the family, i.e., 1 out of 32 times this will happen.

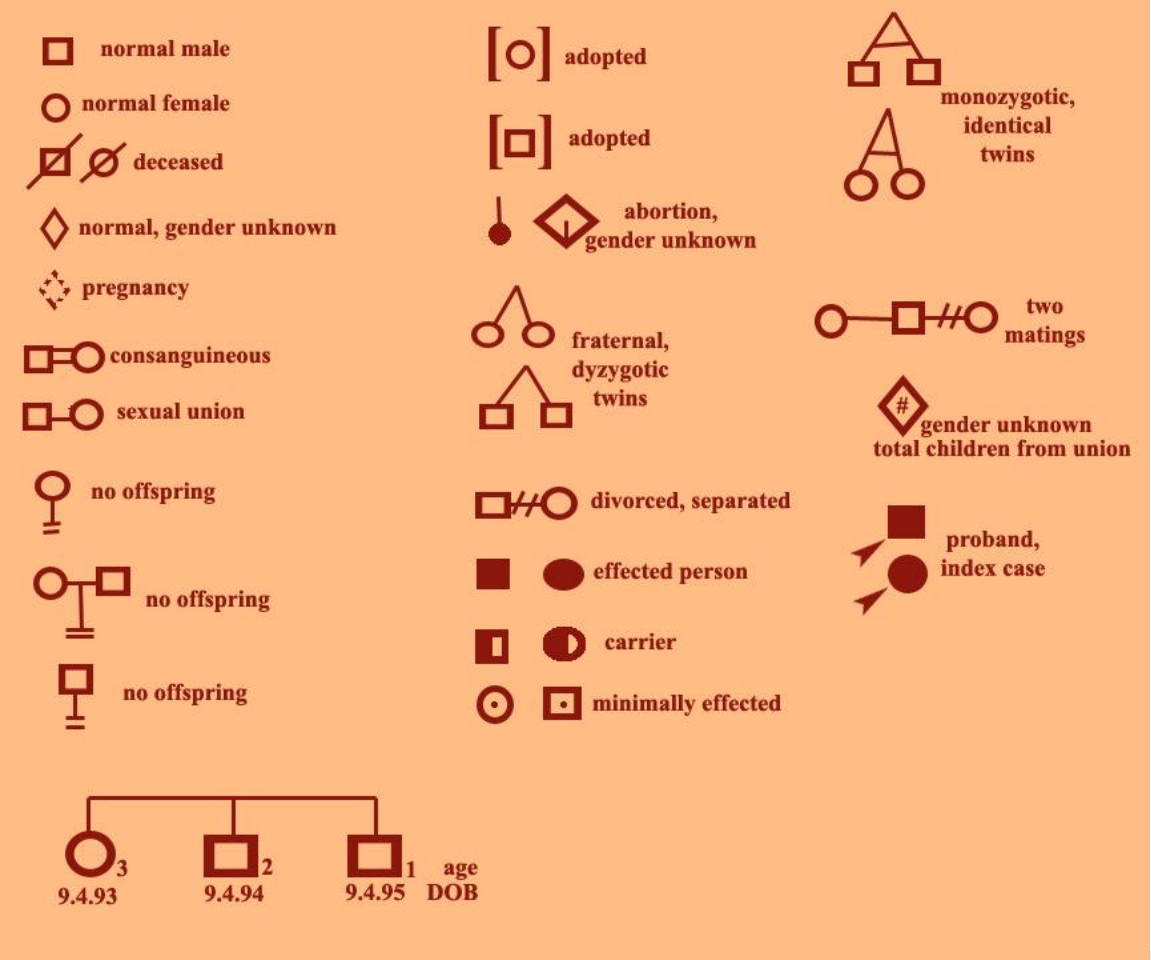
Genetic Disorders

There are three categories of Genetic disorders we are interested in:

- **chromosomal,**
- **simply inherited disorders (Mendelian) and**
- **multifactorial disorders**.

Chromosomal disorders are defined as a loss, addition or abnormal arrangement of chromosomes (monosomy, trisomy). **Simply inherited disorders** are subdivided into autosomal and X-linked disorders. These two classes of disorders may be further sub-divided into dominant and recessive. In each case, the disorder comes from a SINGLE mutant gene. The last case, the **multifactorial disorders**, involve polygenic interactions with multiple exogenous/environmental factors. The inheritance risk with these disorders is less than with Mendelian disorders.

Before we look at the various genetic disorders, we must examine how to identify which steps



are necessary to learn more about the "possible genetic disorder". This requires a detailed family history. It also requires developing a family tree. The code for following a family tree is presented, above, on the bottom of the previous page.

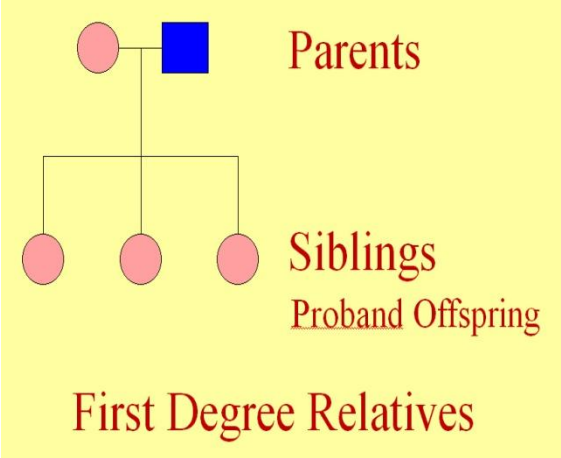
Proband or Index Case

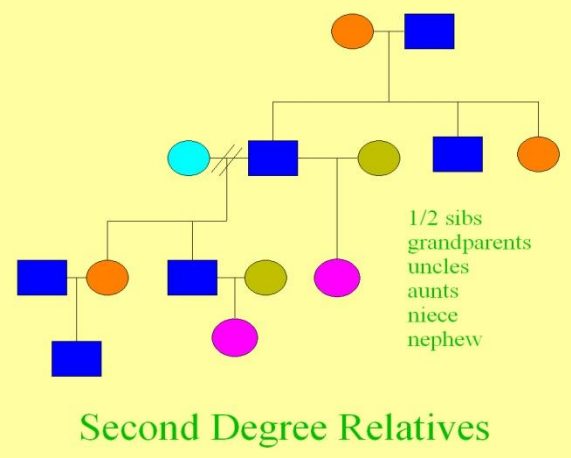
When taking the family history, it is necessary to identify as many family members as possible. It starts with the "proband" or the "index case". This is the person with the disorder. This individual is marked on the family tree with an arrow.

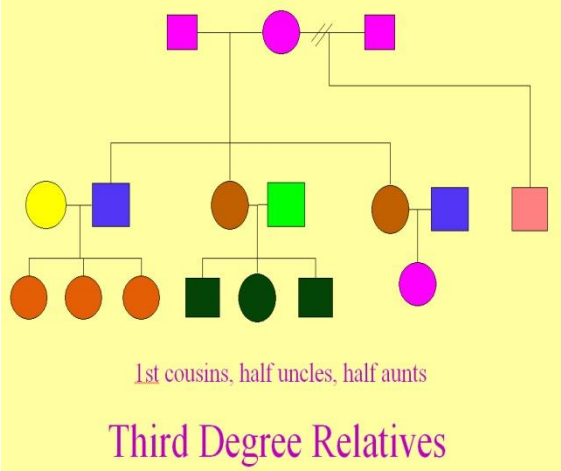
Intrafamilial Relationships

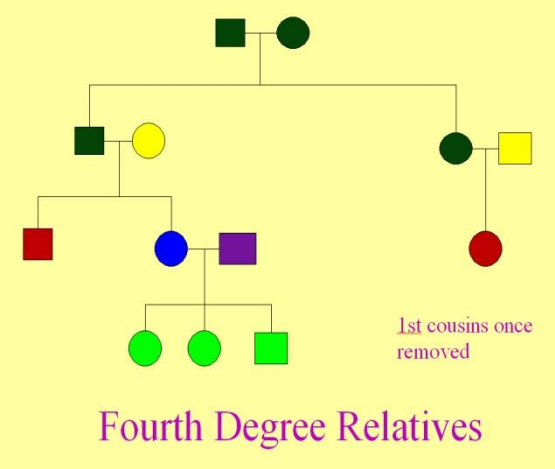
Primary (1°) relatives	Secondary (2°) relatives	Tertiary (3°) relatives	Quaternary (4°) relatives	Pentanary (5°) relatives
First degree	Second degree	Third degree	Fourth degree	Fifth degree
Parents, siblings, offspring of the proband	Half-sibs, grandparents, uncle, aunt, niece, nephew	1st cousins, half uncles, half aunts	1st cousins once removed	2d cousins
Share half of genes	Share a quarter of genes	Share an eighth of genes	Share a sixteenth of genes	Share a thirty-second of genes

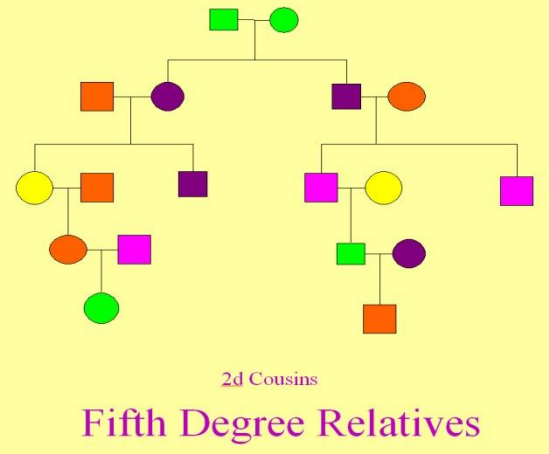
The images following illustrate the intrafamilial relationships described in the above table using family trees:


Parents
Siblings
Proband Offspring
First Degree Relatives


1/2 sibs
grandparents
uncles
aunts
niece
nephew
Second Degree Relatives


1st cousins, half uncles, half aunts
Third Degree Relatives


1st cousins once removed
Fourth Degree Relatives


2d Cousins
Fifth Degree Relatives

Some of the information to obtain from these individuals whewn building a family tree is as follows:

1. All names used by each person. Their date of birth (DOB) with current age. How old were people when they died. What caused their death. Name or describe the disease/defect/deficiency. What gender were they.

2. Give the survey to the rest of the family:

a. Anyone else got it?

b. Anyone else have a trait that proband does not, but is known to be part of the same defect?

c. Anyone else in the family have another trait that is genetic (to confirm hereditary disease even though may not be involved with proband's defect)?

d. Anyone else with rare disease -- or died from it? -- may help to identify defects in family members that may be related to the index case.

e. Any consanguinity in the marriage? This rules out (R/O) homozygous recessive traits).

f. Any common last names in families of mating pairs -- consanguinity may be unknown to proband.

g. What is the ethnic origin? Some ethnic groups have increased chances of genetic disease. Examples are listed in the table, below:

Ethnic group	Disease
African American	Sickle cell anemia
Ashkenazi Jews	Tay-Sachs
Chinese	Glucose-6-phosphate dehydrogenase deficiency
Mediterranean	β-thalassemia
Northern Europe	Cystic fibrosis
Scandinavians	α_1-antitrypsin deficiency

The two simplest classes of genetic disorders will be examined first. In a nutshell, each of us has 46 chromosomes: 23 pairs. 22 of these pairs are called autosomes and the last pair is called the sex chromosomes. The abnormalities that fall into the chromosomal category include variations in the number of chromosomes, e.g.:

Chromosome type	Genetic description	Name of Disorder
Autosome	Trisomy 21	Down Syndrome
Sex	47, XXY	Klinefelter's Syndrome
Sex	45, X	Turner's Syndrome

Genetic disorders that fit into the multi-factorial classification may best be described by the following. They are not inherited in standard Mendelian manner. The inheritance risks are less than those with Mendelian inheritance risks to siblings (sibs) and children. The risks of recurrence increases with increased members of the family being effected. The risks of recurrence decrease with increasing distance between family members, approaching zero for third degree family members.

Consanguinity (mixing the same blood; in-breeding) increases the risk of recurrence.

Pedigrees (family trees) may superficially resemble Mendelian characteristics in SMALL families.

The gene may express greater in one gender than the other, e.g., male pattern baldness.

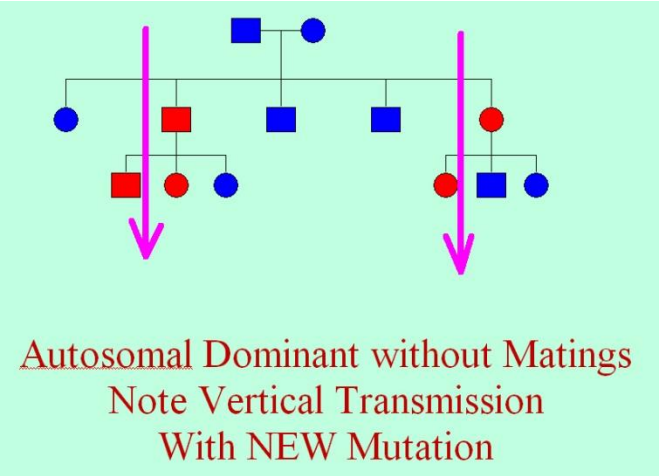
The last classification of genetic disorders, the simply inherited disorders (Mendelian-type) is more detailed and will be subdivided into autosomal disorders and X-linked disorders. In autosomal disorders, the genes are situated on all BUT the X or Y-chromosomes. When two alleles -- B and b, for example -- occupy each locus of a chromosome pair, there are three possible combinations:

BB	Bb	bb
Homozygous	Heterozygous	Homozygous
Trait is dominant	Trait is dominant	Trait is recessive
Double dose of "B"	Single dose of "B"	"No" dose for "B"; double dose for "b"

Autosomal Dominant Disorders

These disorders are transmitted from 1 generation to the next by both sexes. Both sexes are at equal risk of being effected. It is vertically transmitted. Three or more male-to-male transmittances prove autosomal dominant transmission. The chance to pass along the gene is 50% per conception.

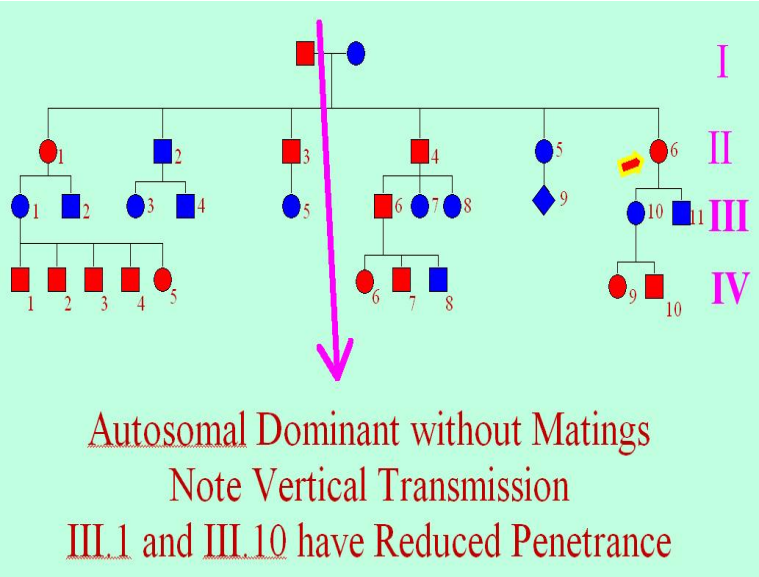
It may present as "reduced penetrance", i.e., it may appear to "skip" a generation. It is identified after the effected offspring is born from an "uneffected" parent OR by molecular biology techniques.



The risk of the child to have the clinical disease is equal to the product of 0.5 (for 50% chance to inherit the gene) and the percent of carriers with the disease (penetrance). Age of onset varies making it difficult to determine age of risk or if the patient is beyond the age of risk. A unique case MAY be due to a new mutation.

Children of minimally effected parents may be severely effected. New mutations seem to happen more often in reproductive cells of fathers who are of older age (5-7 years older than the general population of paternally inherited mutations, approximately 30 vs. 37 years of age). With "new mutations", R/O reduced penetrance and "mistaken"/extramarital paternity (Figure above right).

It may be possible that a defect is NOT autosomal dominant (may be a phenocopy: nongenetic conditions that mimic a specific genotype) or the defect may be similar to but genetically different with a different pattern of transmittance. Double-check this family's history very carefully!

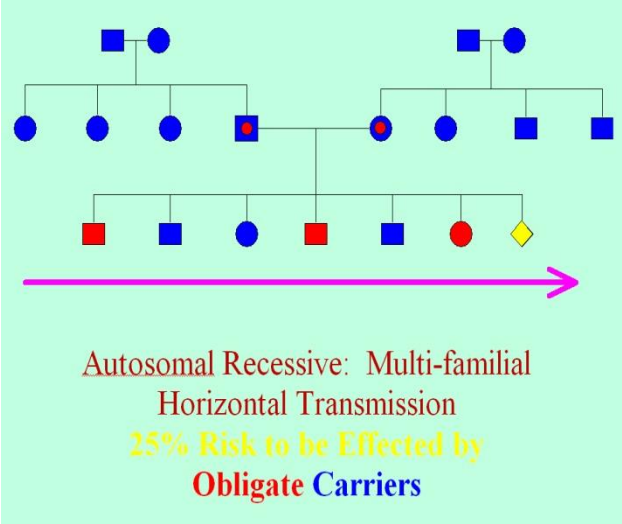


Autosomal dominant disorders are "generally" not due to enzyme defects (most biochemical defects

are substrate-limited and NOT enzyme limited, hence, even with 50% of functioning enzymes, the reactions will continue to "run" as "ordered").

Note in the Figure (bottom right, previous page), that there are 4 generations of a family represented. Note also that III.1 (offspring 1 in the third generation) and III.10 show reduced penetrance -- one of their parents had the gene/effect, they don't express the effect, but their own offspring do.

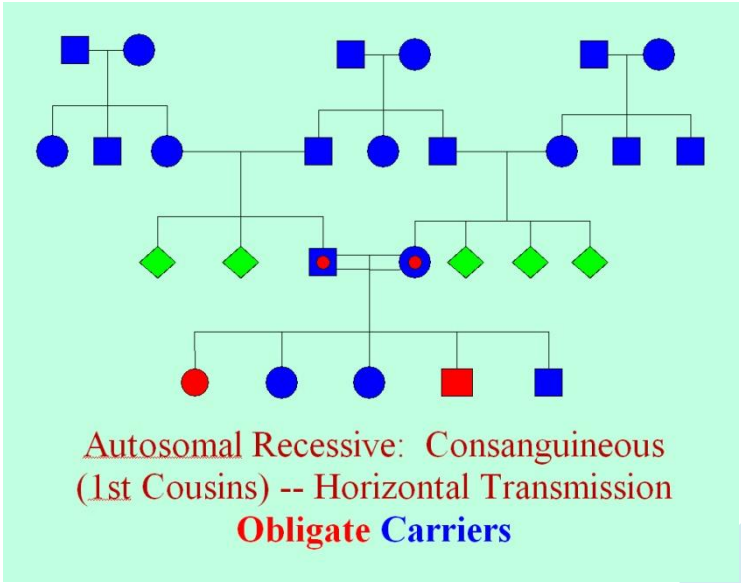
Autosomal Recessive Disorders



Those not clinically effected/symptomatic are heterozygotic and usually identified AFTER a child with the disorder is born. These parents are called obligate carriers. Disorders are found only in sibs. Males and females are at equal risk. No other relatives are effected with the EXCEPTION of in-bred families.

When both parents are carriers, the risk of having an effected child is 25% per conception; 50% for having a child who will be a carrier of the gene; 25% chance for having a child with homozygous "normal" inheritance. Sibs without the disorder born to people with the disorder have a 67% risk of being a carrier of the gene. Carrier testing MAY be available and, if so, identifies carriers.

In general: the risk of children born to individuals with the disorder is NOT very high (EXCEPT in consanguinity, image at lower right) due to low population frequency of carriers. All offspring of people with the disorder, though, are carriers.



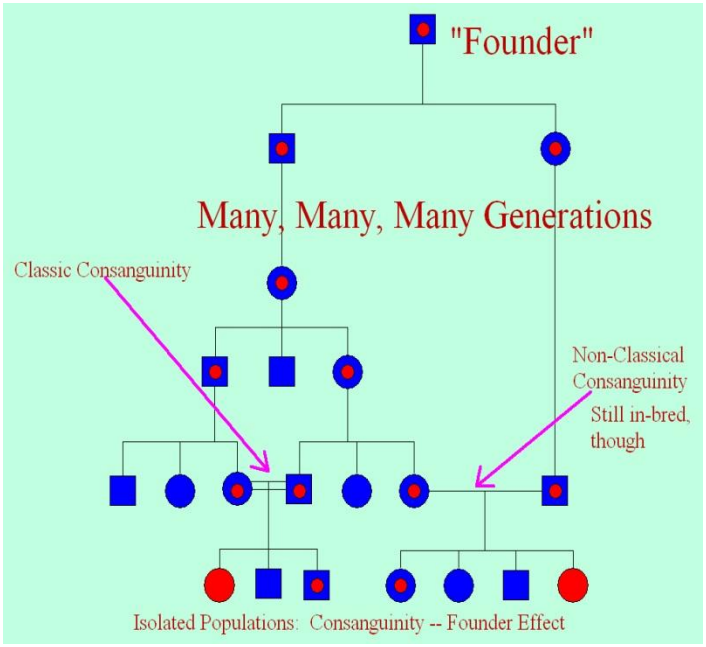
When 2 parents with the same mutation on the same gene reproduce, all offspring will have the same disorder. Alternatively, when two parents with different mutations on different genes reproduce, no child will have the disorder. This is called assortative mating, e.g.,

albinism: two different genes with two different mutations cause this.

The rarer the disorder, the more likely consanguinity exists somewhere. An increased frequency of consanguinity is not detected if the recessive disorder is common, e.g., sickle cell anemia and PKU. Spotty cases of autosomal recessive disorders are observed now because of people having small families.

Vertical transmission does NOT occur, but HORIZONTAL transmission does:

Many couples who are carriers may have children without the disorder. These carriers go undetected/unidentified. Autosomal recessive disorders are frequently associated with enzyme defects.



Isolated Populations – Consanguinity

Vertical transmission does NOT occur, but HORIZONTAL transmission does: Figure (at right) represents what happens in very small, isolated populations as you would expect to find in Switzerland, for example.

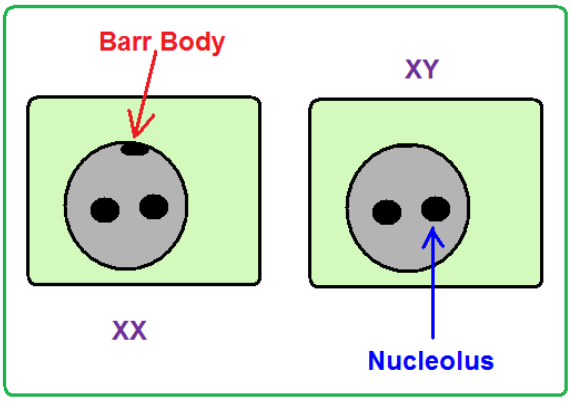
X-linked Disorders -- Dominant/Recessive Combined

Disorders come from genes on the X-chromosomes. A female may be heterozygous or homozygous since she has 2 X-chromosomes. X-linked dominant and X-linked recessive refer ONLY to expression in females. A male has only 1 X chromosome, therefore, he is hemizygous for X-linked traits. Males express the trait regardless of whether the trait is dominant or recessive since they only have 1 X chromosome. Males transmit the X chromosome to ALL female offspring, therefore, females are called obligate carriers.

Fathers do NOT transmit the trait to their sons. To make a male baby, the father provides the Y chromosome; the mother provides the X chromosome. **THE feature of X-linked transmittance/inheritance is total, complete lack of male-to-male gene passing.**

Females have 2 X-chromosomes. One would think that this would translate to twice as much protein information in the female, BUT this is NOT the case: 1 X chromosome is inactivated **(called lyonization after Mary Lyon who discovered this phenomenon).**

After cellular differentiation, 1 of the 2 X chromosomes inactivates and condenses to form a Barr body. Barr bodies can be observed in cell nuclei/nuclear membranes (figure at right) that stain darker than the rest of the chromatin. If the Barr body is stained with a fluorescent stain, it will be the brightest spot in the nucleus.



Inactivation of one of the X-chromosomes is a random activity: each cell may inactivate paternal- or maternal-derived X-chromosomes with equal probability. After inactivation, the SAME X remains inactivated throughout all following cell generations. Hence, a female at any one time in her cells has half of her father's X and half of her mother's X expressed.

One Exception: The tip of the X chromosome is homologous to the Y chromosome. This allows XY recombination and pairing during meiosis. This region is NOT lyonized.

A "Twist" on Lyonization

Sven Bocklandt [1, 3, 4] , Steve Horvath [1, 2], Eric Vilain[1] and Dean H. Hamer[3], Extreme skewing of X chromosome inactivation in mothers of homosexual men. **Human Genetics** 118:6 (691) 2006. [(1) Department of Human Genetics, University of California, Los Angeles, CA, USA; (2) Department of Biostatistics, University of California, Los Angeles, CA, USA; (3) Laboratory of Biochemistry, National Cancer Institute, Bethesda, MD, USA; (4) Gonda 5524, 695 Charles Young Drive South, Los Angeles, CA 90095-7088, USA]

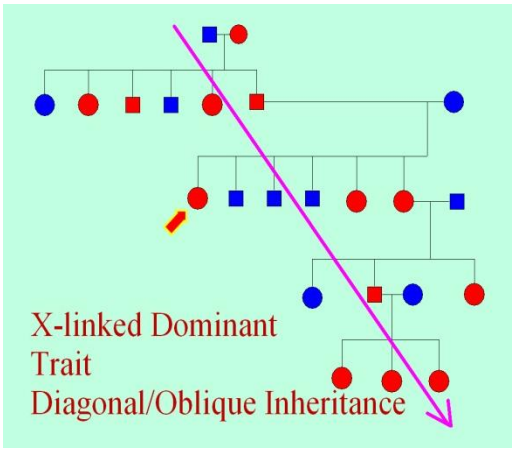
97 mothers of homosexual men; 103 age-matched control women without gay sons.

The number of women with extreme skewing of X-inactivation was significantly higher in mothers of gay men (13/97=13%) compared to controls (4/103=4%) and increased in mothers with two or more gay sons (10/44=23%).

Findings support a role for the X chromosome in regulating sexual orientation in a subgroup of gay men.

Remember that the tip of the X is analagous to the Y – what does lyonization of this part of the X mean?

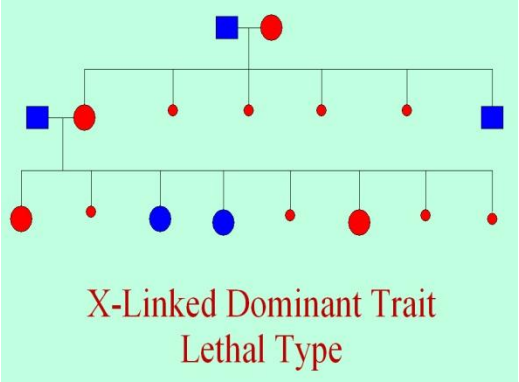
Women may be carriers of X-linked disorders if they have at least: 1 son with the disorder OR 1 brother with the disorder OR 1 uncle on mom's side with disorder OR 1 sister with a son who has the disorder.



Due to germline mosaicism, prenatal diagnosis needs to be offered to parents following the birth of a child with the disorder before another pregnancy is contemplated. Mosaicism occurs when two or more populations of cells that have slightly different genetics are present. Recurrence rates are very low.

There is an increased risk to produce offspring with abnormal karyotype[s] if mosaicism occurs in reproductive cells. This increased risk is greater than the risk associated with Mendelian inherited disorders.

Germ cell mosaicism apparently explains cases in which 2 offspring of normal parents are effected with a dominant condition. One child may be diagnosed with a milder disorder later in life, while the other is diagnosed earlier in life with a more severe clinical presentation -- to the point of being lethal.

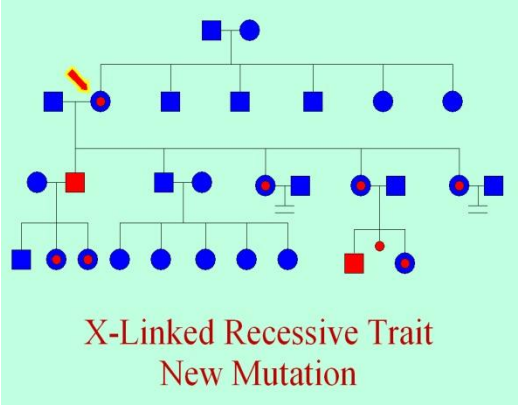


X-Linked Dominant Trait

X-linked dominant disorders (Figure at top right) are rare. If they occur in sons, they may be lethal. Effected women (with X-linked dominant disorder) have twice as many female offspring than males and have an increased frequency of abortion due to lethal transmittance (Figure middle right) to male zygote/embryos/fetuses.

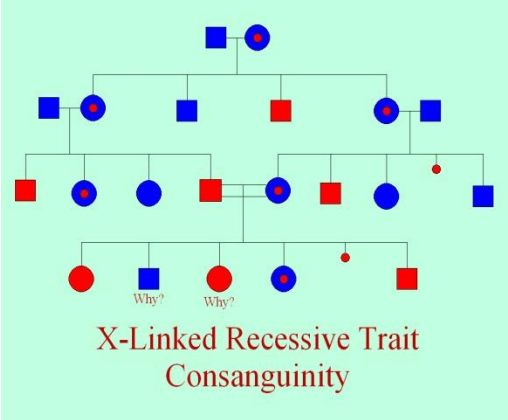
X-Linked Recessive Trait

A female carrier of an X-linked recessive trait has 50% chance of having female offspring as carriers and 50% chance of male offspring having the disorder. X-linked transmission -- whether dominant or recessive -- is diagonal or oblique



Consanguineous matings, as well, may present as x-linked recessive (Figure top right of next page).

Note that IV.2 is uneffected and IV.3 is effected in the Figure in this instance. Can you think of why this is?



Selected Hereditary Diseases of Humans

Alkaptonuria

Alkaptonuria is inherited as an autosomal recessive disorder. It has been mapped to chromosome 3 and is a disorder of homogentisate oxidase. Its classical diagnosis is based upon the fact that the urine turns brown/black on standing -- particularly if the urine is alkaline or has alkali added to it. It leads to a dark pigmentation of ligaments, cartilage, fat, skin and urine called "ochronosis". Ochronosis is a dark blue discoloration that is easiest observed in regions of skin that overly cartilage. This discoloration usually is present in/by the 3d and 4th decades of life. Alkaptonuria also causes degenerative joint disease (arthritis) of the spine and peripheral joints. Although the disease makes one miserable, there does not seem to be a reduced life expectancy.

Since this disease is, relatively speaking, benign, it is probably not necessary to reduce the amounts of phe and trp in the diet. Large doses of vitamin C seem to reduce oxidation/polymerization of homogentisate (in the test tube). Arthritis is treated with anti-inflammatory drugs.

Recently (Feb. 2006), there have been some studies that have suggested that, while NSAID's work nicely to reduce inflammation, they may also be hindering prostaglandin-mediated osteoblastic repair of the bony surfaces – some are now advocating the use on non-NSAID's to mediate the pain of arthritis.

2009: some clinicians and researchers are suggesting that a combination (unstandardized as of yet) of acetaminophen and ibuprofen will provide narcotic levels of pain relief without addiction and other side effects of narcotics.

Alzheimer's Disease -- 1 Form

Alzheimer's is inherited in a confusing manner: there seems to be at least 3 genes involved in this disease (21pter-q21; 14 (early onset) and 19 (late onset of this disease). Additionally, there is an incredible amount of reduced penetrance. It could be inherited autosomal dominant OR recessive. The involved protein is β-amyloid protein. This disease causes 50-70% of the cases of senile dementia. β-amyloid protein forms the core of plaque formation outside nerve cells and plays a role in the formation of neurofibrillary tangles in 2 regions of cells in the brain. Alzheimer's alters language skills, personality and causes seizures.

NOTE: there seems to be some sort of relationship with Down Syndrome: 1) patients with trisomy 21 who live to be 40 YOA show Alzheimer's pathology; 2) Alzheimer's patients report a higher incidence than expected of 1st degree relatives with trisomy 21 -- interesting the relationship with 14 and 21: perhaps Robertsonian translocation is involved, here, as well???????

Although determination of apolipoprotein E_4 levels is available for diagnostic testing, the results are unreliable. To date, the only way to ascertain Alzheimer's is at autopsy by examining brain tissue samples. Death by Alzheimer's is approximately 8-10 years after onset of the disease. The best therapy remains as managing depression and anxiety and other symptoms with symptomatic treatment.

α_1-antitrypsin (α_1-AT; aka A1PI) Deficiency

This disorder is inherited autosomal recessive from 14q. The protein effected is α_1-AT.

The lack of this protein increases the risks of premature COPD in smokers. (α_1-AT is produced in the liver and travels to the lungs where it inhibits elastase activity -- if elastase is not inhibited, this causes small airway destruction; with smokers, it causes COPD.)

For those lacking α_1-AT secondary to the inherited disorder, there is commercially available protein available for replacement therapy.

To determine whether one has this disease, fetal DNA testing may be performed, RFLP's may be used, fetal blood levels of α_1-AT may be taken by periumbilical blood sampling (PUBS) in the last half of gestation and arterial blood gases may be utilized, as well.

The best therapy is to quit smoking, provide symptomatic treatment and treat infections to any part of the respiratory system aggressively.

Cystic fibrosis

This disease is inherited autosomal recessive on 7q31-32. The protein involved is the cystic fibrosis transmembrane regulator (CFTR). This protein works with a chloride channel, but it is uncertain as to how.

Although most of us are familiar with this disorder causing lung problems, it also causes GI disturbances (bulky, greasy stools), lots of gas and infertility in more than 95% of effected males (no vas deferens develops).

Cor pulmonale develops in advanced cases; this is of poor prognosis.

To some degree these symptoms may be treated with enzyme capsules to replace those not secreted by the pancreas. This patient may also need antacids. Diagnostic testing includes sweat chloride testing, probing for CFTR and a fecal test to test for presence of pancreatic enzymes. NOTE: 1 in 22 is a carrier of this disorder.

Hereditary Fructose Intolerance

This disorder is inherited as an autosomal recessive disorder. There is a fructose-1-phosphate (F-1-P) aldolase deficiency. This disease causes hypoglycemia with increased accumulation of F-1-P in tissues.

The patient fails to thrive and has nausea and vomiting (N/V), jaundice, an enlarged liver, which may develop into liver failure, proteins and amino acids in the urine and tyr in the urine, as well.

Diagnostic testing is to "pre-load" the patient with fructose and observe for hypoglycemia and hypophosphatemia.

Therapy is to discontinue cane sugar from the diet. Patients must double check over the counter (OTC) medications for sucrose additions as tablet binders. If the diet is discontinued, the patient has an increased risk of growth failure; on the diet, the patient will grow relatively normally.

Homocystinuria

This disease is inherited autosomal recessive. There is a deficiency in cystathionine-β-synthetase. The life expectancy of a patient with this disorder is reduced in the untreated patient and in the pyridoxine (B_6)-unresponsive patient. It causes retardation, arachnodactyly (spider fingers -- long, slender, curved), osteoporosis, dislocated optic lenses, high risk to throw clots (idiopathic), may have seizures, MI, CVA and PE.

Diagnostic testing is to detect homocystinuria and cyanocobalamin levels. Therapy is aimed at two groups: Group 1 is vitamin responsive and their urinary homocystine excretion is reduced with doses at or greater than 200 mg of B_6 every day; Group 2 is vitamin-unresponsive and must be treated by dietary modifications: reduce met in diet and increase cys in diet.

Maple Syrup Urine Disease (MSUD)

This disease is inherited autosomal recessive; 5 forms are known.

The most severe form involves the protein α-keto acid decarboxylase/acyl CoA dehydrogenase. The urine smells like maple syrup or burned sugar.

Diagnostic testing examines the levels of branched chain amino acids (BCAA) and alloisoleucine in urine.

Therapy includes dietary restrictions on BCAA. If caught within 10 days after birth, the child will undergo normal growth/development. BCAA's need to be monitored regularly.

Phenylketonuria (PKU)

PKU is inherited as an autosomal recessive disorder. Classical PKU is on 1p; atypical PKU is on chromosome 4.

Classic PKU is caused by a deficiency in phenylalanine hydroxylase.

PKU causes mental retardation, hyperactivity, eczema; it is associated with blond, blue-eyed and fair-skinned individuals.

The urine of patients with untreated PKU smells like a "mouse". Blood tests are now mandated within 2-3 days after a child is born. Urinary testing may also be undertaken.

Therapy is to reduce phe in the diet. If the patient follows the diet, there will be normal development; if the patient does not follow the diet, mental retardation will set in.

It is important to remember to titrate the phe levels carefully: phe is necessary to regulate the fever centers of the brain. Too little and the child has a fever constantly; too much and the child becomes irreversibly mentally retarded.

Tay-Sachs

Tay-Sachs is inherited autosomal recessive.

The protein effected is β-N-acetylhexosaminidase A.

It is common in eastern European Jews.

Onset of the disease occurs at about 3-6 months of age. The infant develops hypotonia, hyperacusia (abnormally sensitive hearing) and retardation. Death usually occurs by age 2-3 years. Diagnostic testing is available. Therapy seems to be symptomatic.

α-thalassemia

This disease is inherited in a manner consistent with autosomal recessive characteristics.

It seems to be on 16p. With inactivation of 3 of the 4 α-globin chains, a form of hemoglobin known as HbH forms. This causes hemolytic anemia. It is caused by the formation of a

tetramer of beta subunits. The new tetramer has very high oxygen affinity and, hence, doesn't want to release oxygen to the cells. NOTE: inactivation of all 4 of the α-globin chains = a stillborn baby. This is called hydrops fetalis.

This disorder causes jaundice, hepatosplenomegaly. Diagnostic testing includes reticulocyte counts, MCV (mean corpuscular volume) and detection of lots of hypochromia on a peripheral blood smear.

Prenatal screening includes electrophoresing parental Hb to identify the presence of the thalassemic Hb.

Therapy does not include iron: it is needless and it may be toxic -- increase folate intake.

β-thalassemia

This disorder is likewise inherited as α-thalassemia, but on 11p. The gene effected is the β-globin gene. There are two variations of this disorder: major and minor.

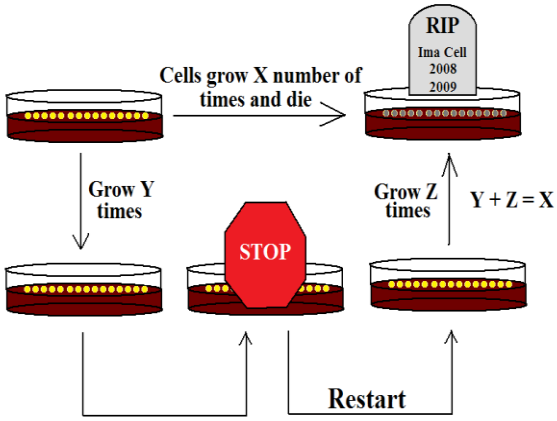
Major: is the most common cause of transfusion-dependent anemia in childhood. The patients are normal at birth but develop anemia by their first year as fetal Hb (HbF) levels drop off. (HbF is a tetramer of $\alpha_2\gamma_2$; HbA_2 (to be discussed in a bit) is a tetramer of $\alpha_2\delta_2$.) Without treatment, patient develops a massively enlarged spleen and liver, develops enlarged medullary cavity with thinned cortex, prominent forehead and maxilla and pathologic fractures. May cause RBC sickling. Diagnostic testing includes looking for reduced MCV, elevated HbA_2 OR F -- normal Hb, HbA, is a tetramer of 2 alpha sub-units and two beta subunits ($\alpha_2\beta_2$) -- and electrophoresing Hb. Therapy consists of blood transfusions with iron chelation, bone marrow transplants. Hb must be maintained at or above 11 mg%. Splenectomy reduces transfusions. Pneuimmune vaccine before, after or without splenectomy and PCN after splenectomy reduce infection by *Streptococcus pneumoniae*.

Minor: usually asymptomatic. There seems to be no response to iron therapy.

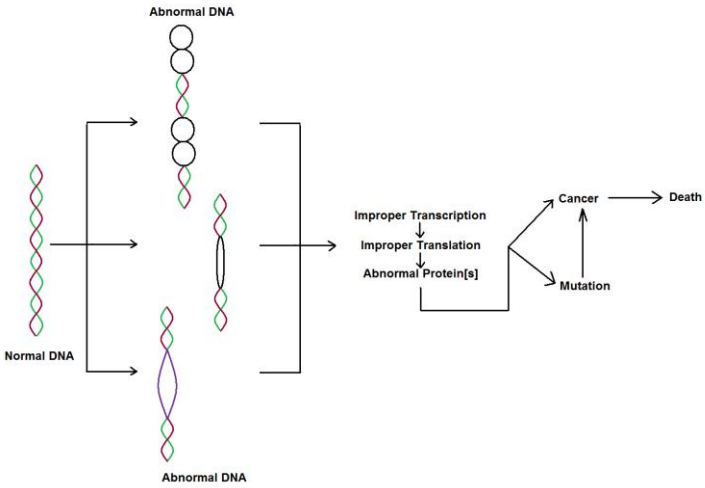
Genetic counseling needs to be approached sensitively

Aging Theories

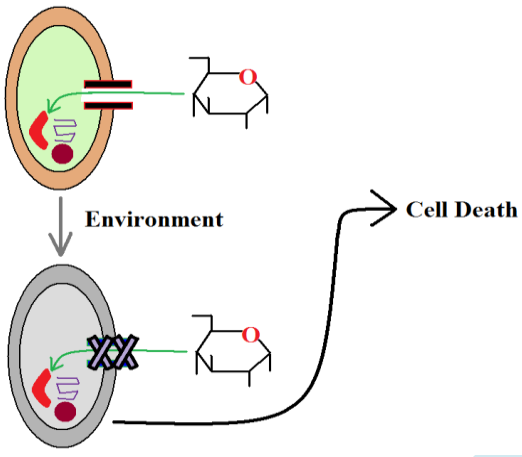
There are 8 aging theories for discussion. In all likelihood, though, each one is correct to a point on its own merit and all are fully correct when "mixed" together, i.e., when they are all combined. Another way of viewing this is that individually, there are problems with all of these theories. Together, they make pretty good sense.



Program Theory (image at right): When cells are grown in culture, they will replicate "X" number of times and then die. This has been shown in embryonic (emm bree AWN ick) cells grown in culture, then taking clones and doing the next experiment: start and stop the growth of these cells and see how many times they will replicate. Interestingly enough, no matter how many times the cells were started and stopped, when the calculations were complete, the cells in both cases replicated the same number of times.

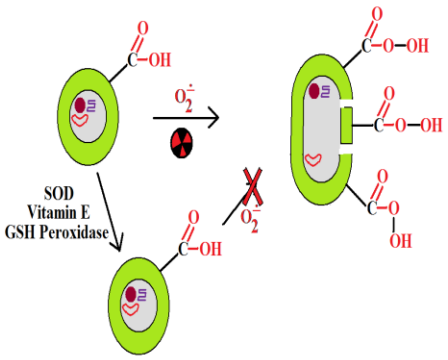


Error Theory (image at right): Normal DNA under normal conditions alters its structure as we age. Due to these alterations, the DNA is not read correctly so that transcription and translation are malfunctioning which leads to a malfunctioning (abnormal) protein that either directly causes cancer or indirectly causes cancer through a mutation. Either way, the cell dies.

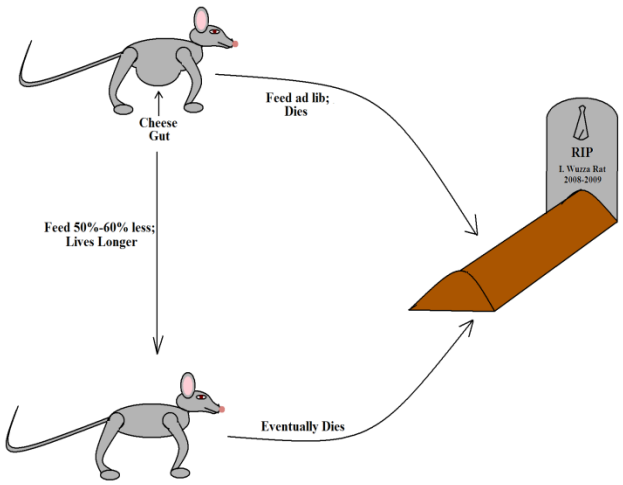


Cellular Theory (image at right): Under normal cellular "wear and tear", the cells become debilitated and do not function as well while we age. It tends to support the idea that if we increase our metabolic processes, the increase causes our cells to age faster and reduce our life span. Probably a part of this is correct, however, the reduction in life span we think about probably

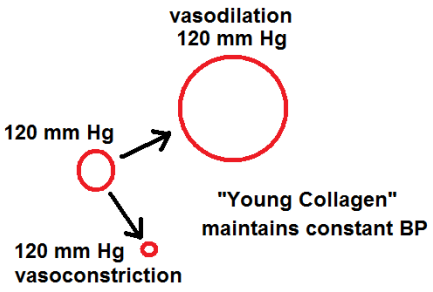
amounts to only a few years, at most, on the far end of the life span, i.e., we're probably not gonna notice the loss of those few years.



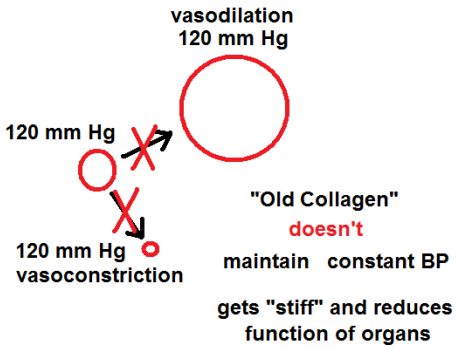
Free Radical Theory (image at right): When lipids in our cell membranes are exposed to free radicals, e.g., superoxide anion (O_2^-) or radiation, the free carboxyl groups are oxidized to COOOH groups from the COOH groups normally present. The COOOH groups are called lipid peroxides. These lipid peroxides are quite reactive and will cause the cell membrane to rupture, causing the demise of the cell. We do know that when cells are exposed to free radicals or radiation in a test tube and we've added Vitamin E or superoxide dismutase (SOD; enzyme that hydrolyzes superoxide to water and oxygen) or glutathione peroxidase (GSH peroxidase; another enzyme that protects against lipid peroxidation), that the life span of the cells are prolonged. The key is to remember that, thus far, it only works in the test tube.



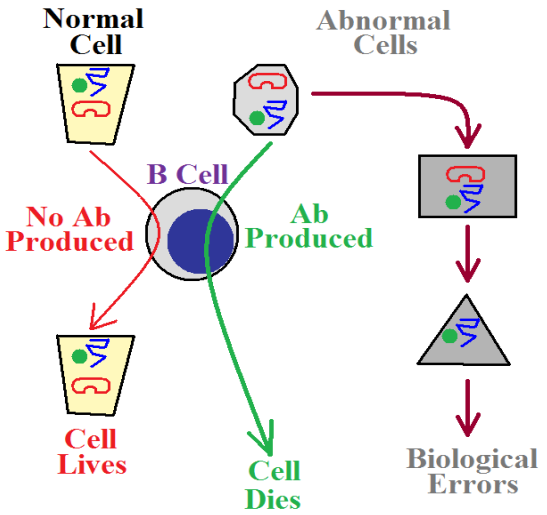
Nutritional Model Theory (image immediately above right): If an animal is fed 50-60% energy less than what it will normally obtain on its own that it will live longer and be healthier. This is the only model that actually works by itself - BUT! Remember: animals are much different from humans so it may not apply to humans. In addition, just because one has a great deal of lean muscle mass, as opposed to adipose tissue, this does not mean that that person will live longer than someone with the opposite physical characteristics: it also depends on one's genetic make-up..



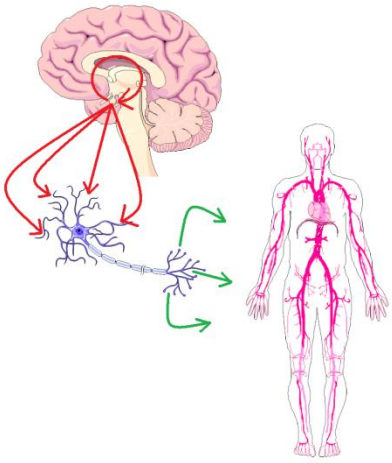
Collagen Theory of Aging (image right): As we age, our collagen (CALL uh junn) in our bodies gets older. When that happens the old collagen gets stiff and does not act as flexibly, causing problems, e.g., causes hypertension by not expanding to accomodate the flow

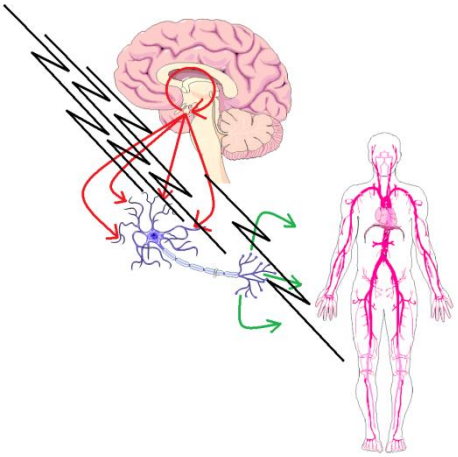


of blood through the vessels, stiff collagen causes organs to malfunction as they seem to be "crispy" and hinder metabolic reactions.



Mutating Autoimmune Theory of Aging (image at right): Normal cells have normal functions and secrete normal proteins in, on or through the cell membrane. None of which ought to cause any sort of immune response. When, though, these cells mutate with time, they secrete foreign proteins in on or through the cell membrane which DOES solicit an immune response by the body. This response shuts down the cell. Alternatively, this theory also suggests that whole cells mutate over time and cause biological errors leading to the demise of the organism.





Neuroaging Theory: As we age (normal in left image; aging in right image), we undergo thalamo-hypothalamo-pituitary (thuh LAMM oh HIGH poe thuh LAMM oh pi TOO uh tear ee; THP) Axis and neuronal degeneration. The THP axis is the "natural pacemaker" for all cellular aging and the concurrent effects on physiological processes. As we age, then there are alterations in hormonal release (lowered levels as we age) and effect (reduced numbers of receptors and/or increased peripheral resistance to the hormone by its target cells). All of these effects lead to the decline in cell function we call "aging" throughout the organism.

Experimental

Listed below in the table are selected hereditary traits in humans along with the letter that is used to code for the genotype:

Trait	Letter	Trait	Letter
Curly hair	C	Near/far sighted	G
Dark brown hair	H	Normal hearing	E
Brown eyes	B	Large eyes	S
Male pattern baldness	M	Migraines	A

Note that all the letters are upper case: these are dominant traits.

Listed below are selected hereditary traits in humans along with the letter that is used to code for the genotype. Note that the letters are lower case to represent recessive traits.

Trait	Letter	Trait	Letter
Straight hair	c	Normal vision	g
All other hair colors	h	Deafness	e
Blue or gray eyes	b	Small eyes	s
Have hair	m	No migraines	a

One parent has the following genotype: CCHhBBmmGNGNEessaa. Describe this parent by describing the phenotype. Note: GN is the gene for near-sightedness.)

One parent has the following genotype: cchhbbMMggEESSAA. Describe this parent by describing the phenotype.

These two parents mate. Using the Punnett Square, below, determine the genotypes (first box) and phenotypes (second box) of the F2 offspring:

(Box 1) Gametes→↓		

Child 1	Child 2
Child 3	Child 4

What percent of F_2 will have to wear glasses to correct for near-sightedness?

For the next exercise, refer to the following table:

Trait	Letter	Trait	Letter
Roll tongue	R	Polydactylism (many fingers)	M
Earlobes attached	Q	Hypertension	H
Gender	XY or XX	Broad lips	L
Dimples	D	Normal color vision	C
Widow's peak	W	Coarse body hair	S
Freckles	F	Disease resistance	G
Group A Blood	B^A	Rh factor	O
Group B Blood	B^B	Brachydactylism (webbed fingers)	P
Group O Blood	B^O	Feet with normal arches	N

Upper case letters, of course, remind us that these traits are dominant. Lower case letters would tell us that the traits are recessive (either not expressed unless homozygous or when ARE expressed are the opposite of the dominant trait).

Based upon what you know about yourself (and perhaps others in your family), record in the space below your genotype next to your phenotype. (If you are not certain if you are homozygous or heterozygous, simply record your genotype as, e.g., F — if you do not know if your freckles are homozygous or heterozygous).

Using the information from this experiment and what you know about your family, develop a 4-5 generational family tree in the space below depicting one of the traits you've identified above and determine how it has been genetically passed down from generation to generation.

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4) Marieb: Human Anatomy and Physiology Laboratory Manual, Brief Version, Second Edition. (Benjamin Cummings: Menlo Park) © 1987.