

DNA Mutations and Elementary Cancer Genetics :

An Introduction

Definitions

- *Wild-type DNA* is typical DNA found in nature.
- *Mutagenesis* is the production of a mutation.
- A *mutation* is any change that presents in the DNA of a cell. Mutations are chance activities and occur spontaneously in all nuclear material.
- Mutations are neither good nor bad (like emotions) and are simply a natural occurrence of cellular activities.
- Most mutations impair cell function rather than increase useful cell functions, therefore, mutations are thought of as being harmful.
- Mutations can not be predicted, nor can the effects be predicted - caveat: some ARE predictable, i.e., the ones we recognize and have studied extensively.
- Genetic changes are not as a result of a mystical punishment as thought around the turn of the century.
- (One of the biggest myths was that "God" was punishing a woman who had a child with Down Syndrome. Of course, men were the ones who pushing this idea. We now know that at least a third of all cases of children with Down Syndrome are paternal in origin. As research unfolds, I expect that we'll find that it will come out to 50/50 between parents.)

- Those compounds that interact with DNA and increase the frequency with which bases are altered or which causes the likelihood of mutation are called *mutagens*.
- Even with incredible minimal exposure to mutagens, there is still a small likelihood that a gene may mutate. This is called a *spontaneous mutation*. The frequency of spontaneous mutations vary greatly between genes and organisms. When this frequency increases, it is assumed that some mutagen is causing it.

The table, below, summarizes different types of mutations.

Type of Mutation	Example
Wild type ("normal")	DNA is in cell nuclei.
Point	DNA is in cell nuclei (NO period)
Insertion	DNA is NOT in cell nuclei.
Gene duplication	DNA is in IN cell nuclei.
Gene duplication with point mutation	DNA is in <u>AN</u> cell nuclei.
Chromosome duplication	DNA is in cell nuclei. <u>DNA is in cell nuclei.</u>
Translocation	DNA is cell nuclei in.
Inversion	DNA is in cell <u>ielcun.</u>
Frameshift*	DN Ai sin cel lnu clei.

*removal of or insertion of bp sequences that alter the reading of the DNA sequence.

- In terms of mutations of hereditary and somatic forms, or of dominant and recessive natures, when the mutation is passed onto the offspring it is said to be inherited, hereditary, genetic for lower organisms.
- For MAN there are two sort of mutations:
 - hereditary, which occurs ONLY in sex cells: sperm and ova (includes BRCA-1 and BRCA-2); on the sex chromosomes (23d pair of chromosomes; X and Y for either XX (female) or XY (male)) or
 - somatic mutation, which consists of most mutations; on the autosomes (22 pairs of chromosomes that code for the body)
- These occur in all other cells.
- Somatic mutations are NOT hereditary and are NEVER passed onto progeny.
- Hence, there is a genetic and an environmental element involved in cancer.
- It is of significant interest that mutation, aging and cancer are tightly linked by somatic mutations.

Remember, too, that the genotype is the genetic information (the DNA sequence) while the phenotype is the expression of the genetic information.

If alleles are identical, they are homozygous; if not identical, they are heterozygous.

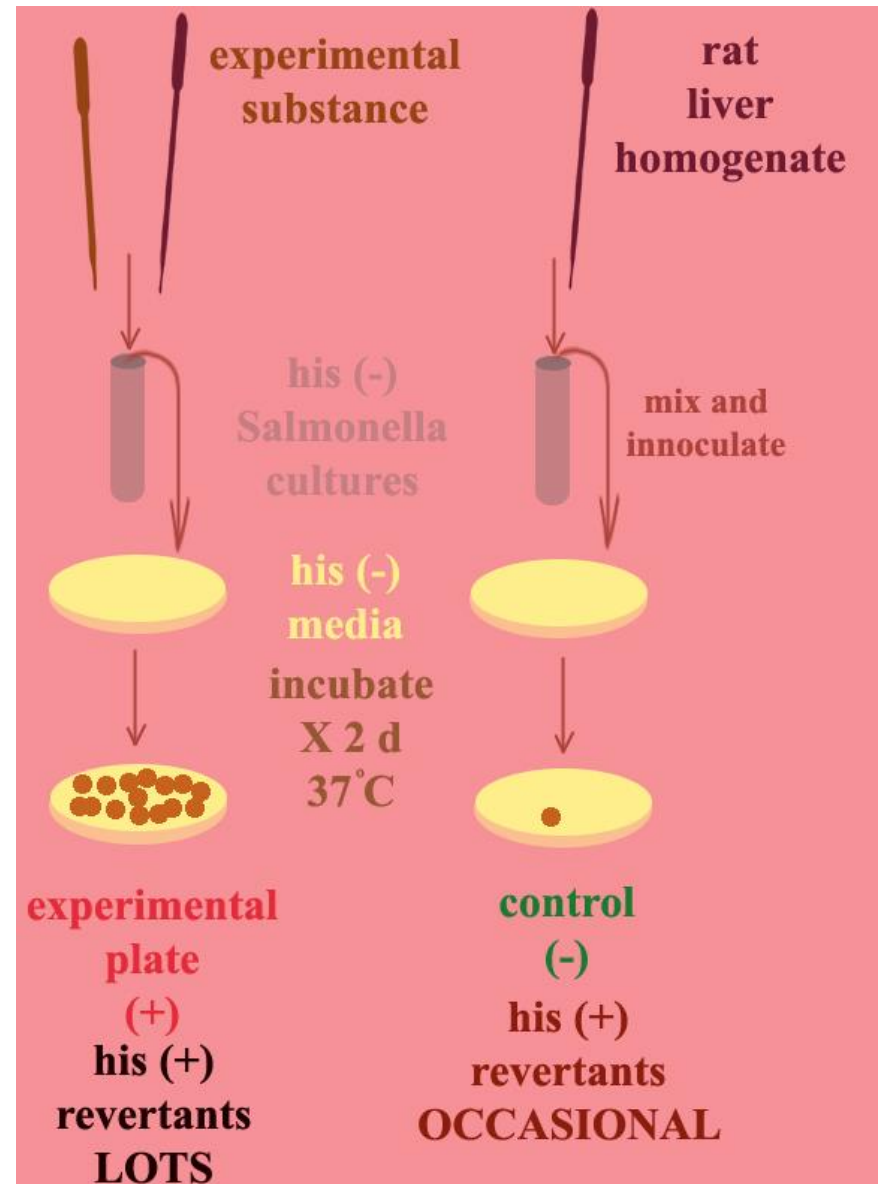
The allele expressed as phenotype is said to be dominant; the allele NOT expressed as phenotype is said to be recessive. (This last sentence is not altogether true as we'll see in later lectures.)

A deviation from homozygous to heterozygous is a mutation, too.

- A *carcinogen* is an agent that causes cancer.
- A *forward mutation* causes DNA to change from wild type to a mutant form. This is to what mutations refer.
- A *reverse mutation* is a mutation that causes reversion from mutant to wild-type. This form of mutation is also known as *revertant*.
- A *pseudorevertant mutation* is a "double mutant". These grow under wild type conditions and are not easily distinguishable from wild types. They are generally due to one gene "out-regulating" another.

Ames Test

- A great example of applied mutational studies is the Ames Test.
- This test uses a strain of *Salmonella typhimurium* that lacks the ability to synthesize its own histidine (his-).
- The way it works is as follows: under normal, wild-type conditions, this micro-organism has the ability to synthesize his (his+). In the presence of dextrose (glucose), electrolytes and suitable medium, it grows well.
- The his- variant when grown in the presence of identical medium under identical conditions won't grow as does the his+ wild-type variant.
 - The "trick" to this test is to take the his- variant and grow it in the presence of glucose, electrolytes, rat liver homogenate and varying concentrations of suspected mutagen.
 - If the suspected mutagen is a mutagen, it will cause the his- to mutate to either a true revertant or a pseudo-revertant and the bacterium will grow.
 - The growth will be proportional to the concentration of mutagen/carcinogen in the medium.



- One example of a chemical that causes mutations is sodium nitrite (NaNO_2). Sodium nitrite is found in spinach, naturally, and is a food preservative that is added to keep the meat colored red and to reduce spoilage.
- The problem with sodium nitrite is that when it reacts with stomach acid (HCl) it forms nitrous acid (HNO_2). The nitrous acid kills cells and increases mutations. The way that this works is several-fold.
 - First nitrous acid oxidizes the amine on Adenine to a ketone, causing the synthesis of hypoxanthine (HX). HX hydrogen bonds with C. The problem, here, is that A and T generally double hydrogen bond and G and C triple hydrogen bond.
 - By altering the hydrogen binding "sites", as it were, there are only two hydrogen bonds between HX and C. The DNA is altered due to this altered bonding, i.e., is mutated.
 - Secondly, nitrous acid oxidizes the amine on C to a ketone to form U. Remember that U is generally in RNA and binds with A during transcription/translation.
 - Because of this mutation, i.e., forms U=A base pair rather than T=A base pair, the U HAS to be changed to T. The mechanism is one of those previously studied in DNA Replication and Repair.

- Other chemicals include nitrosamines (related to sodium nitrite), e.g., pesticides, herbicides, 2d hand smoke.
- Indeed it is WELL known that the more one smokes, the faster one dies. Current research is also showing that second-hand smoke is emerging as a killer, as well.
- Mutations are identical whether they are spontaneous (occur as a result of no known cause) or induced (due to something from the environment).

- The simplest kind of mutations are called *point mutations*: a change in 1 base pair. These include the following mutations:
- Transition mutation: Those that occur with the highest frequency occurs where the point mutated is accomplished by changing 1 Pu (purine) for another Pu; or 1 Pyr (pyrimidine) for another Pyr. Those that occur with the least frequency are to change 1 Pu for a Pyr; or 1 Pyr for a Pu.
- Missense mutation: these are point mutations that lead to amino acid changes in the codon, e.g., HbA and HbS (hemoglobin A in healthy individuals and hemoglobin S in those with sickle cell disorder).
- A variant of this mutation is the temperature sensitive (Ts) mutation: This occurs if the new protein is active at one temperature (generally 30°C) and inactive at another (generally between 40°C and 41°C).
- Nonsense mutation: A point mutation that produces stop codons from a "normal" amino acid codon. **These are also known as chain termination mutations and there are three sequences known: Amber (UAG), Ochre (UAA) and Opal (UGA) - you learned UAA as one of the stop codons back prior to translation.** These are the ONLY stop codons produced as a point mutation. Amber is usually denoted Am, Ochre as Oc and Opal as Opal.
- Neutral Mutation: A point mutation in a codon that does not change the amino acid or the phenotype.

- More complicated mutations may add, delete or move pieces of, or complete, chromosomes. There are four of these that we are interested in studying:
- Deletions: occur with a loss of base pairs in the range of 100's to 1000's of bp's. These are not able to revert to wild-types as are point mutations.
- Duplications: occur when a piece of DNA is added to a chromosome that contains an identical sequence of DNA. This suggests having "spares" of chromosomes in case of some sort of catastrophe at the nuclear level. It also suggests that this "night" increase the survival of the organism.
- Translocations: Occurs when a piece of DNA is moved from 1 new site to a new site, e.g., 14/21 in Robertsonian translocation, previously discussed.
- Inversions: Occurs where a piece of DNA is snipped out, turned around and replaced in the same spot. This mutation alters the genotype and phenotype, as well. These sequences are valuable since they do NOT recombine during meiosis so they don't pair up since they are different. This mutation seems to permit gene preservation spanning multiple generations.

- In 1984, 25% of Americans were predicted to have cancer during their lifetimes. 20% were predicted to succumb to the cancer.
- In 1995, one-third to 40% of Americans were predicted to succumb to cancer.
 - This number didn't change in 2016 (last year for which data available)
- Cancer diagnoses are quickly increasing in response to the ever enlarging senior population.

Definitions

- Cancer is out of control growth in animal cells. It is also the generic term applied to the numerous diseases that result from the growth of tumors.
- A tumor is a mass of cells that have accumulated at a particular site. If these cells migrate, then the cancer is said to have metastasized. If these tumors cause diseases or death, the tumors are said to be malignant. Cells that do not cause death or disease are said to be benign and do not metastasize.

- Leukemias are cancers of blood cells;
- Sarcomas are cancers of bone and muscle cells: connective tissue tumors.
- Adenomas are cancers of glandular tissues;
- Carcinomas are cancers of the skin and membrane cells.

- Metastasis is the activity undertaken by malignant cells as they migrate to other parts of the body. These cells produce new tumors at the new sites.
- A carcinogen is something that causes cancer.
- A cancer-promoting agent or sensitizing agent is a compound that is not carcinogenic, but stimulates the formation of tumors in tissues which have been exposed, previously, to carcinogens.
- Oncogenic viruses are viruses which cause cancers in animal tissues when the cell expresses the viral genes.
- Provirus is viral genetic material incorporated into animal cells' DNA but is not expressed, YET.
- Reverse transcriptase is an enzyme enclosed with the genetic material in an RNA-containing virus. This enzyme is used to synthesize DNA.
- The oncogenic hypothesis is an idea that postulates that viral genes became part of animal cells' DNA one million years ago. These genes tend to NOT be expressed normally, but may be induced to begin the out of control reproduction of cells into a tumor.
- A prophage is an unexpressed phage genome integrated in bacterial DNA.
- Lysogenic bacteria are bacteria that contain prophages.

Genetic "Slang" Nomenclature

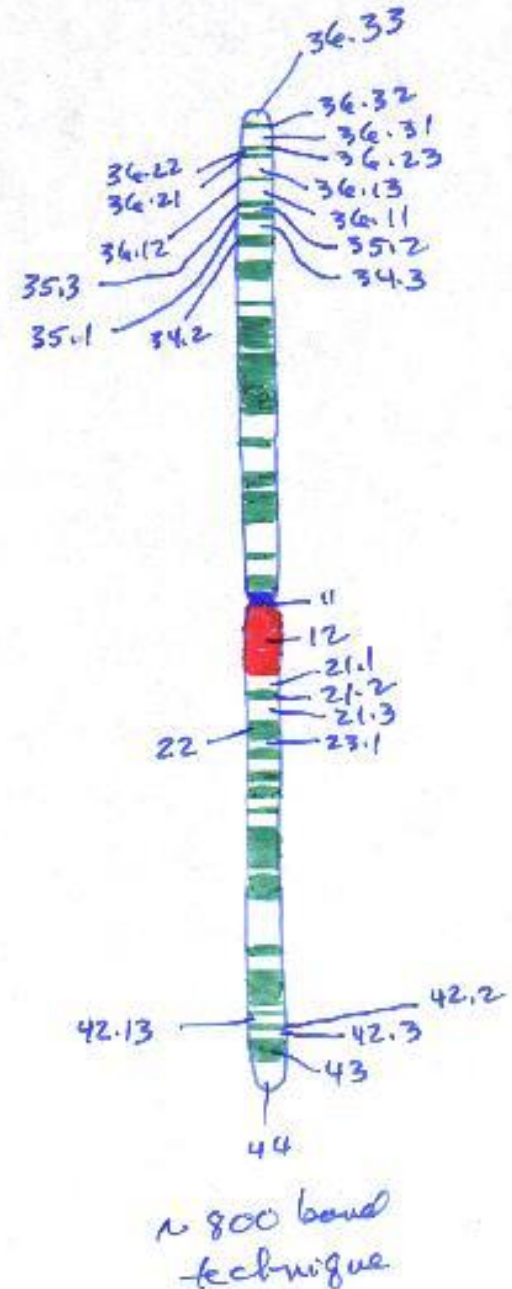
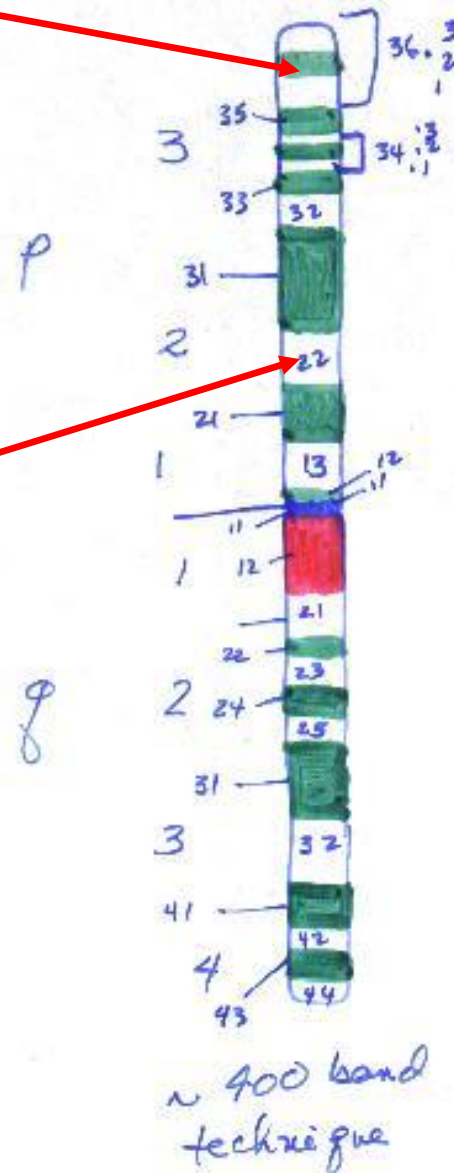
- A normal male and female are 46, XY and 46, XX, respectively.
- A minus or plus sign placed **in front of** the chromosome number indicates monosomy or trisomy, respectively, e.g., 47, XY, +21 is a male with Down Syndrome.
- A plus or minus sign placed **behind** the chromosome number indicates added or missing chromosomal material on one of that chromosome's arms (remember p = small arm; q = long arm), e.g., 46, XY, 9q+ is a male with added genetic material on the long arm of chromosome 9.

INSIDE -- Chromosome Banding

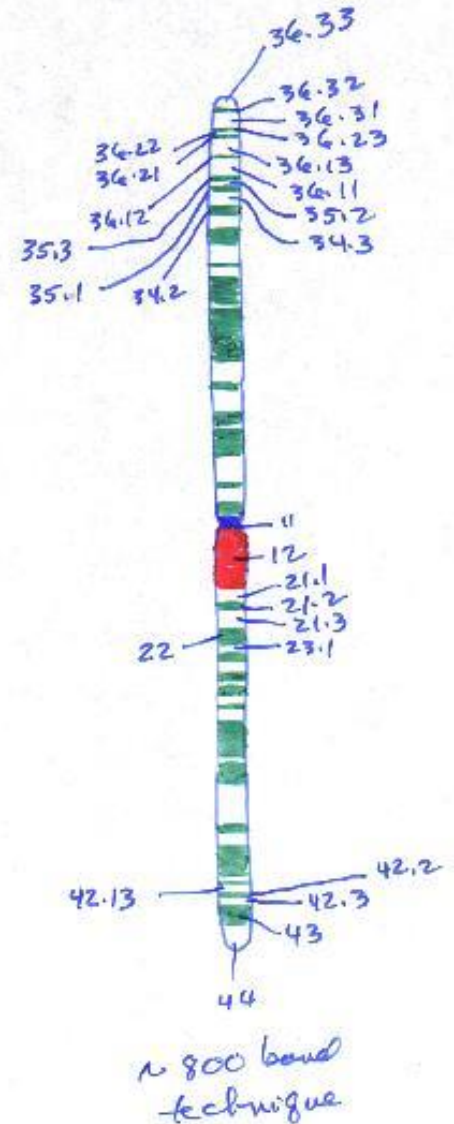
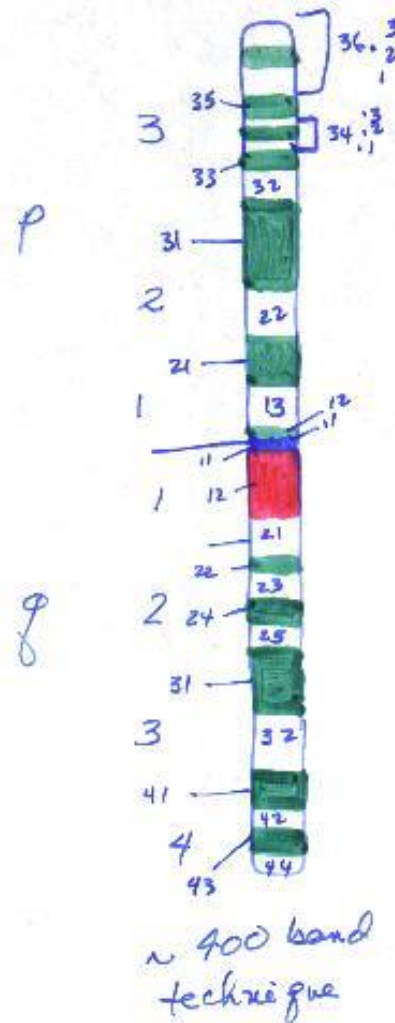
- To band, if you will, a chromosome one does the following.
- The most frequently and easiest cells obtained are lymphocytes. They require 3 days for growth. If other cells are used, they are usually thymus cells, gonadal cells, skin fibroblasts, bone marrow cells, amniotic fluid cells, chorionic villus cells or tumor cells if necessary. Bone marrow cells do NOT require a mitogenic agent for growth; tumor cells require 1-2 weeks to grow.
- All cells are synchronized with methotrexate (a blocker of cell growth; it is also a dihydrofolate reductase inhibitor) with the exception of bone marrow cells.
- The block (methotrexate) is removed and the cells are allowed to grow in tissue culture for the times mentioned above for specific cells.
- The growth of the cells is stopped in late prophase or metaphase with colchicine. The killed cells are lysed with hypotonic solution, fixed and smeared on a microscope slide.

- Traditionally, there are two simple stains to observe the banding on these chromosomes:
- 1) Quinacrine HCl and fluorescent microscopy
- 2) Giemsa and visual microscopy.
- Both methods give about 400-800 bands per chromosome.

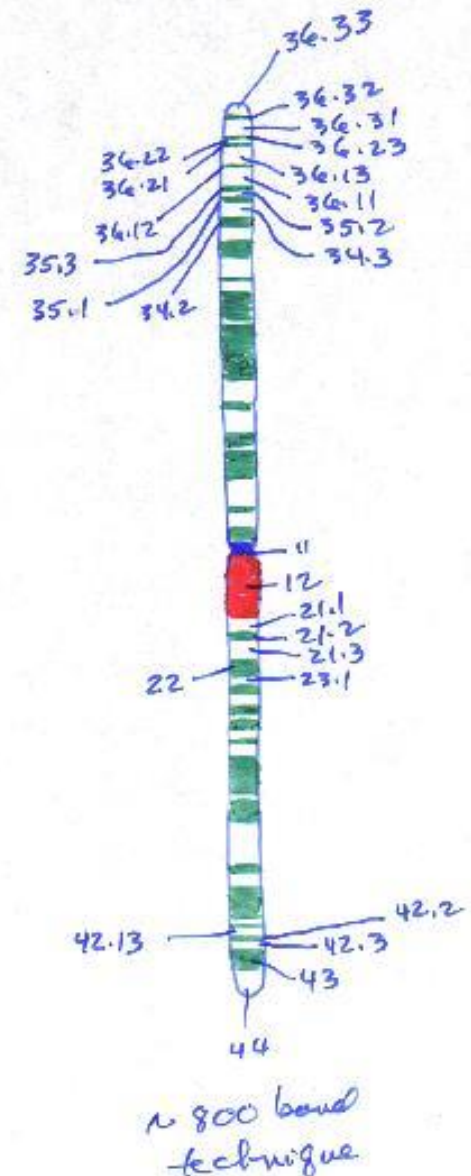
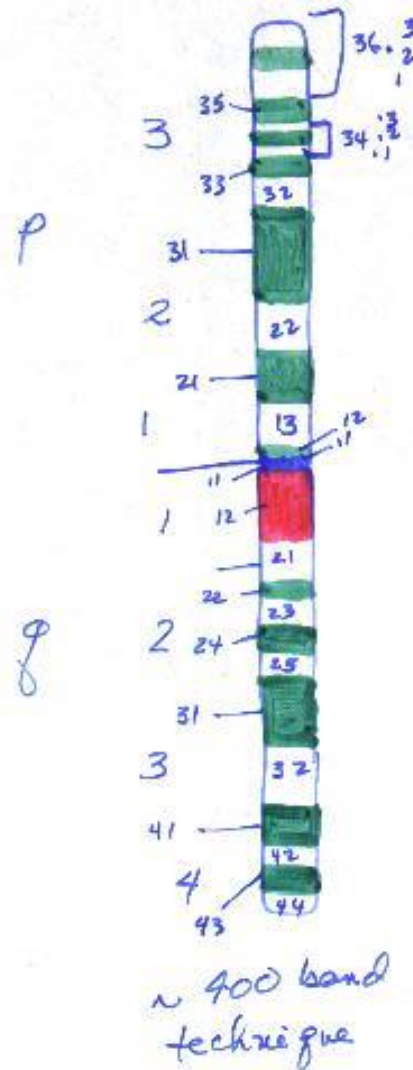
- The dark/colored bands are regions where the chromosome took the stain. The region that appears to be a "non-stain" is negatively stained. Variably shaded bands have staining characteristics that are in between the negatively stained regions and the stained regions.



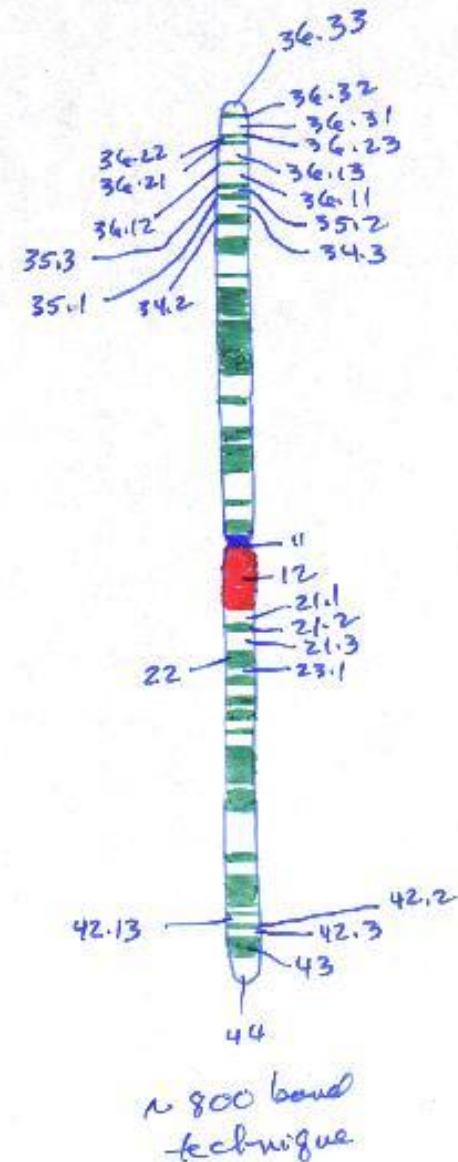
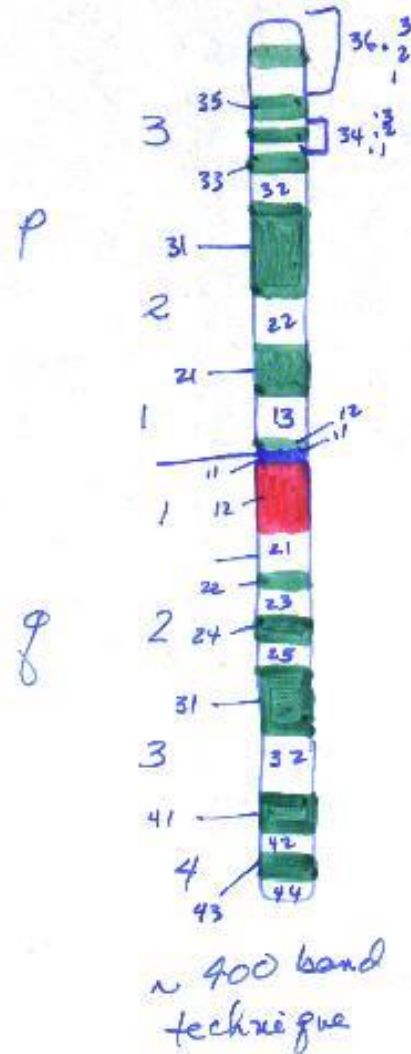
- Note that on the far left there is a "p" and a "q". Those, of course, represent the short and long arm of the chromosome, as we learned earlier.
- On the left of the left graphic, note that there are some numbers that are staggered above and below a line through the centromere.
- On the "p" arm, they number 1,2,3; on the "q" arm, they number 1,2,3,4. These are the number of base regions on this chromosome.
- Notice that between these numbers are numbers like 13, 21, 22, 31, 32, 33, 34, 35, 36. Using 13 as the example, this says that we are looking at the third sub-region in region 1.



- At the top of the left graphic, note that there are decimal numbers: 34.1, 34.2, 34.3. Using 34.2 as an example, this means that we're looking at the second band in the 4th subregion of the 3d region of this chromosome. These can be all further subdivided and identified. The majority of these sub-divisions are beyond the scope of this course.



- Suffice it to say that if you came upon a description of something on a chromosome like 13p24.5 that it means that you are on the 13th chromosome, short arm, 5th band in the 4th sub-region of the 2d region.
- In other words, you now have an address for a specific gene on the chromosome.



End of INSIDE

- A technique called FISH (fluorescent *in situ* hybridization) has been developed to study/identify specific genes on the chromosome. A short (2-3 kbp) DNA sequence is chosen to use as the probe. The DNA is nick translated with biotin or digoxigenin (detergents) and heat denatured. The SS strands are mixed with denatured chromosomes (denatured by heat and formamide) grown under the usual conditions. The DNA's reanneal and are mixed with fluorochrome reagents. The samples are examined under fluorescent microscopy.
- This technique identifies structural rearrangements and marker genes. The study of interphase chromosomes does not appear to be 100% perfected. Hence, findings from FISH must be confirmed by standard cytogenetics' methods.

- It is also possible to identify breaking points in the chromosome:
- e.g., 46, XX, **t(14:21)(q12;p12)** is a female with a reciprocal (exchange) **translocation ("t")** between the **long arm of 14 in region 1, band 2 (breaking point)** and the **short arm of 21 in region 1, band 2 (its breaking point)**, respectively.
- If one wishes to be even more specific in identifying a particular point about the chromosomal band in the chromosomal region, one need only add a "dot" and the sub-band, e.g., 14p13.3. Explained this means chromosome 14, short arm, third sub-band of 3d band in region 1.

Additional Chromosomal Nomenclature:

- 46, XY, dup(6p) is a male with the duplication of the short arm of chromosome 6.
- 46, XX, del(2)(q23) is a female with deletion from chromosome 2, in the long arm in the 3d band in region 2.
- 46, XY, inv(15)(p13;q15) is a male with inversion on chromosome 15 that is between the top of the short arm to the bottom of region 1 from the long arm, i.e., this whole region is turned upside down and the p arm is now part of the q arm and *vice versa*.

- 46, X,r(X) is a female with one normal X and one ring X chromosome.
 - A ring chromosome consists of chromosomes that have fused together as a result of the loss of (deletions) the tips of the chromosomes. This is often lost resulting in monosomy in at least some of the individual's cells.
- 46, X, i(Xp) is a female with one normal X chromosome and an isochromosome of the short arm of the X chromosome.
 - An isochromosome forms instead of dividing the long way, it divides the short way, i.e., instead of splitting the "X" down the middle, it divides across the "X" leaving, temporarily, a "V" shaped piece of genetic material that then swings down to the traditional meiotic shaped chromosome. Progeny with isochromosomes typically are of the X chromosomes and produces/mimics several trisomies, e.g., Turner's Syndrome.

- Cancer is the out of control growth of cells and the unregulated reproduction of these cells in higher animals [and plants, but they have no role in this course].
- Cancer is used to generically describe diseases of humans that are caused by collections of these tumors.
- Benign tumors do not produce disease, although in some cases, e.g., fibroadenoma of the breast, the benign neoplasm has been known to differentiate into a malignant mass.

In the table below is a summary of three different breast masses and some of the characteristics that go along with them:

Characteristic	Fibroadenoma	Fibrocystic Disease	Carcinoma
Age	35±20 YOA ¹ 32.5±22.5 YOA	34.5±14.5 YOA 42.5±17.5 YOA	55±25 YOA 55±30 YOA
(Usual) Sites	Both breasts	Both breasts	One breast
Number of Masses	1 [or more] ²	1 or more	One
Properties	firm, rubbery spherical or plate shaped masses	soft to firm spherical masses	Irregularly or star- shaped masses that feel like rocks
Mobility	Yes	Yes	No - won't move
Skin Retraction	No	No	YES, generally
Pain/Discomfort	No	Yes	No
Delineation	Yes	Yes	NO: irregularly shaped
Changes with menstrual cycle	No	Yes	No

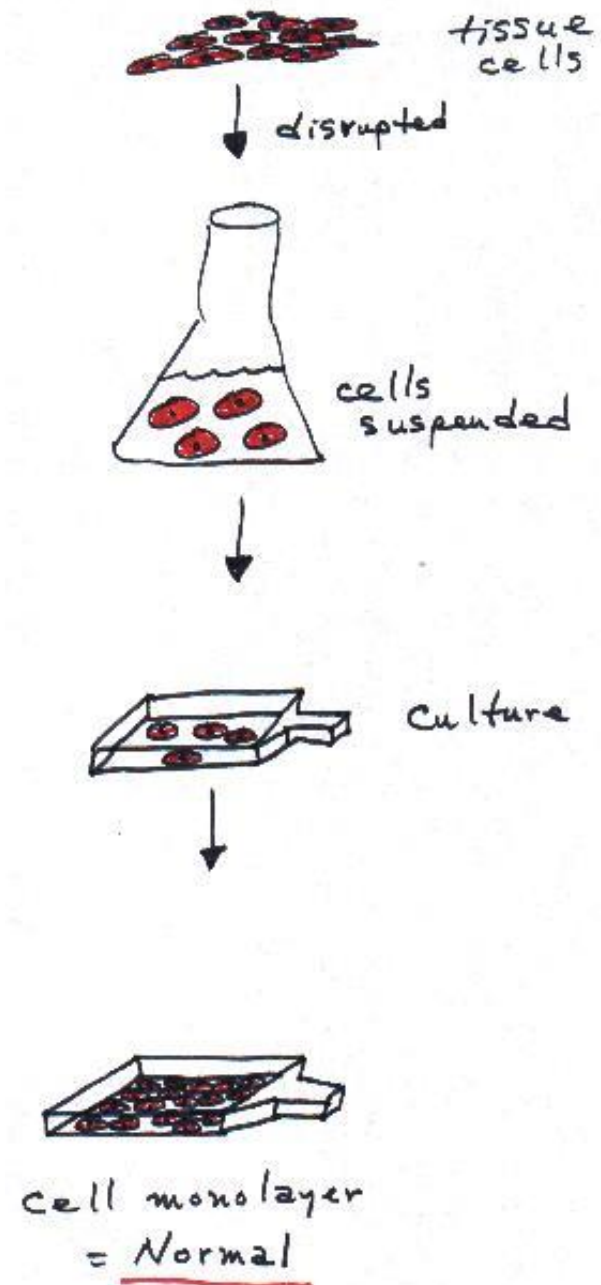
¹Years Of Age; ²1 mass is more common, although more are possible.

- Cancer is not an inherited disease - with the exception of some rare forms of diseases, e.g., breast cancer regulated by BRCA-1 and ovarian cancer regulated by BRCA-2.
- This is in spite of the fact that multiple members of the same blood line have died of cancer; it also does not mean that other, future, members of the blood line are necessarily predisposed to neoplastic disease.
- Genetic events are the major formation of cancer development. The vast majority of genetic events that alter DNA to cause cancer occur throughout the lifetime of the patient in that patient's somatic tissues. Since these transformations occur in somatic tissues, they may not be transmitted to the progeny.
- Thus, while these events are genetic, they are not inherited.

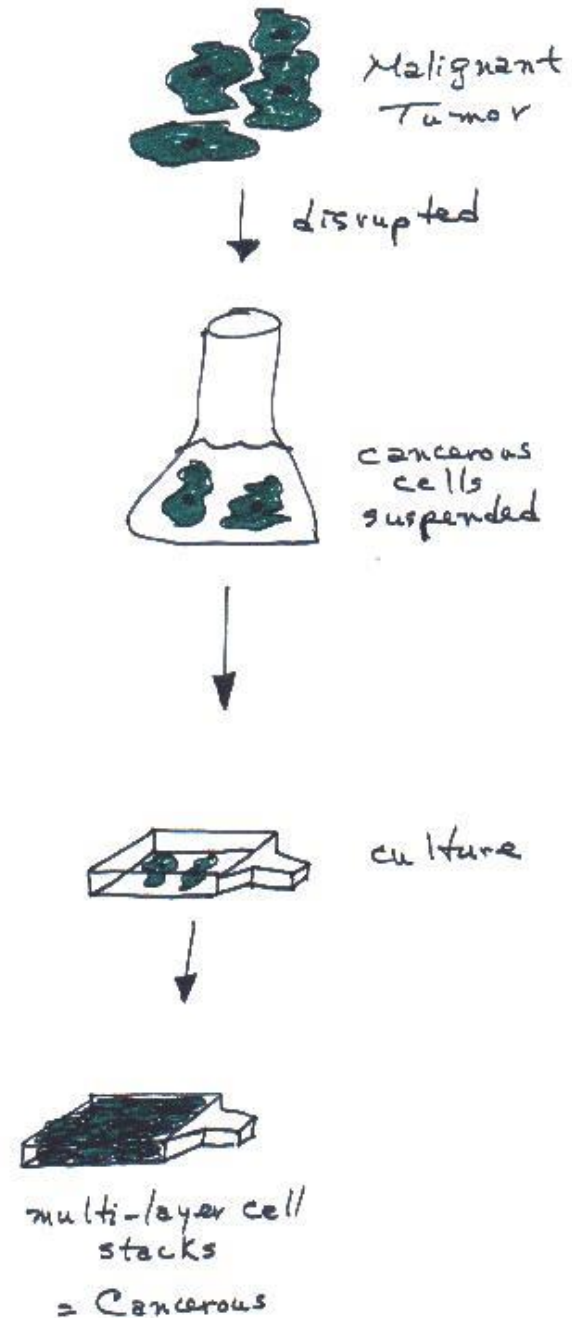
- If these sorts of mutations take place in germ line cells (sperm, ova), then these genes are transmitted from one generation to the next group of progeny which DOES cause a predisposition to getting cancer.
- The progeny will have a high frequency of DNA-coded, very specific, cancers. It appears that for the progeny in these kinds of blood lines that inheritance of only one mutated allele is all that is required to produce cancer. Virtually 100% of the progeny that inherit the gene will develop cancer.
- There also seems to be groups of progeny that present with a more scattered clustering of various cancer types. These types appear to be, for example, breast and colon (not inclusive) cancers. The off-spring of these individuals have a significant increase of each offspring's risk to develop cancer. Although many pieces of information continue to be unraveled, the genetic "drive" for this "familial" cancer production remains to be elucidated.

- One issue to emphasize, however, is that if the mutations occur in somatic cells, the person will not transmit that gene to his or her offspring, i.e., the offspring will NOT inherit cancer. If the mutations occur in the germ line cells (sperm, ova), then the offspring[s] will inherit the cancer and pass it along, as well, when they reproduce.

- Cells destined to become cancerous have been mutated in one or more "parts" of that cell's DNA. These mutations force the cell[s] to not respond to external/internal messages that regulate growth and reproduction, normally. These cells are also different in that they are "stickier" than "normal" cells. If one grows normal human cells in tissue culture in plastic Petri dishes or flasks, normal cells grow over the medium until they are ONE cell thick.

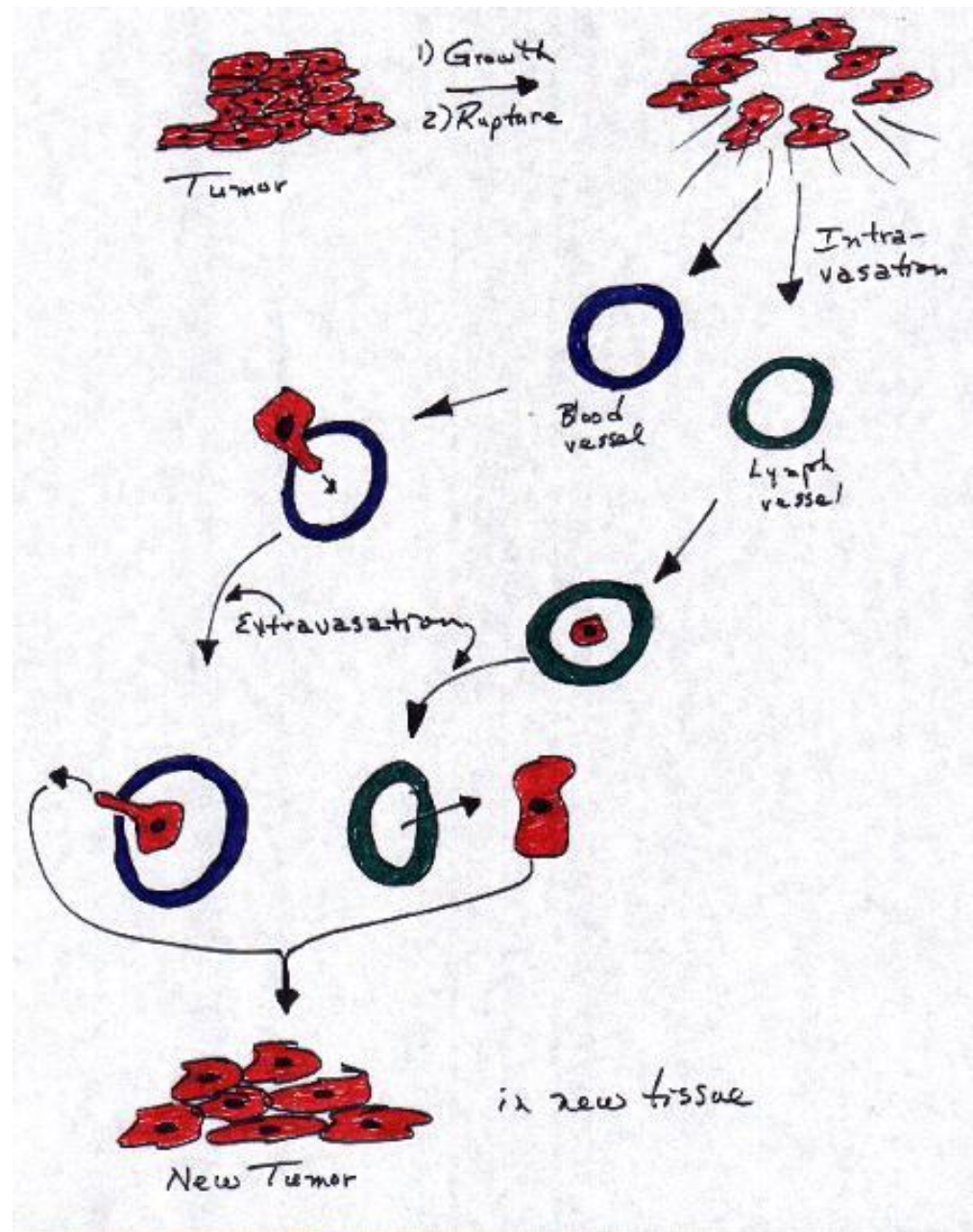


- Cancer cells, however, will not only grow into one layer, but they will continue to grow over themselves, piling all over themselves in thick, uneven piles.



- An important note to remember is that not all DNA mutations result in cancer. Only if the mutations occur in gene regions that control growth and reproduction with the cell have the opportunity to form a tumor. One example of how small a mutation may be was identified in 1984: bladder cancer cells were demonstrated to differ from the normal bladder cells by a change in one (1) base pair!

- A very tiny tumor has on the order of more than one million cells BEFORE one may detect it (e.g., breast cancer takes 6 years for x-ray detection, and it is only the size of a very small pin head on the film), while very large tumors may contain on the order of 10 billion cells or more. In the case of malignant cells, some break off and spread to other parts of the body to set up house keeping there. This is called metastasis.
- Eventually, these tumors will eradicate bodily organs and the patient will die.
- Perspective: chemotherapy and inroads of treatments



Carcinogens: Switches of Disease and Death

- If animals in the laboratory are subjected to x-radiation or other forms of ionizing radiation (review your notes on nuclear chemistry), their somatic cells and germ line cells show mutations readily in their DNA.
- More often than not, these animals develop neoplasms (new growths; tumors). Additionally, the higher the radiation dose, the more the number of tumors that these animals develop.
- Furthermore, the higher the dose of radiation, the more animals that were effected.
- Finally, the more massive the radiation, the more of the animals that die of cancer.

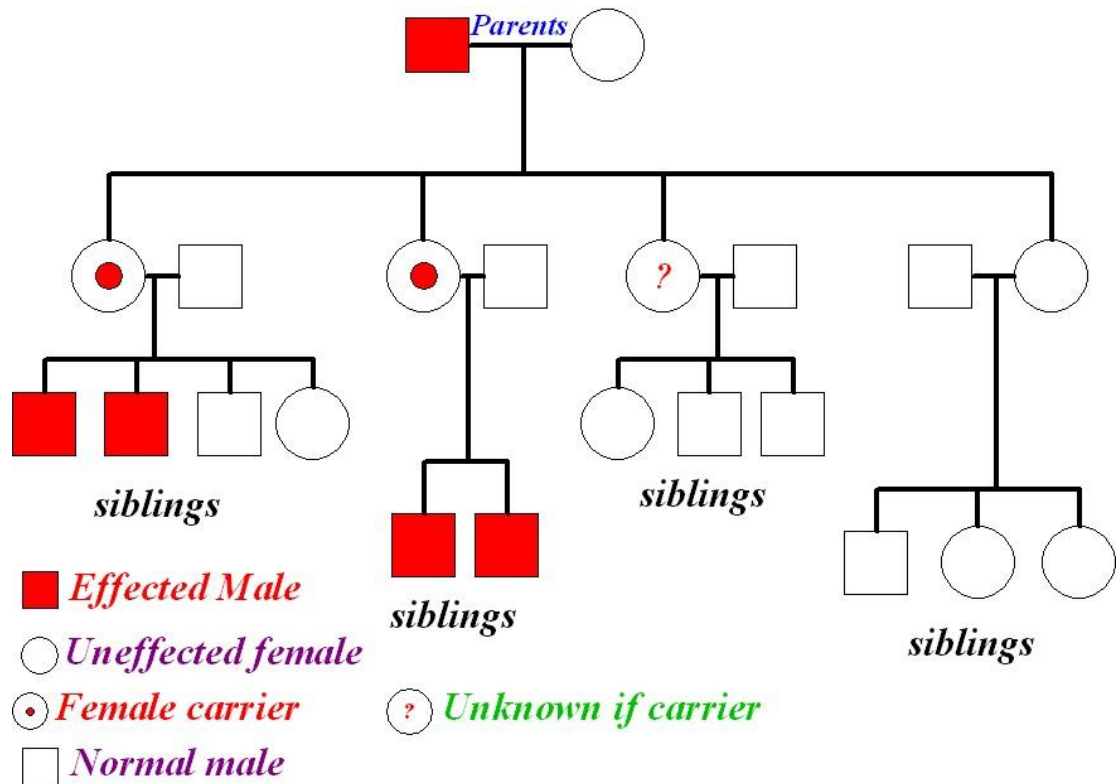
There have been many associations of carcinogens with various tumors/cancers over the years as shown in the table, below:

Carcinogen	Tissue Effected	Cancer
Atomic bomb blasts in Hiroshima and Nagasaki	Blood; bone	Leukemias; osteogenic sarcoma
Polycyclic hydrocarbons in oils, tars, soot (chimney sweeps)	Scrotum	Scrotal cancer
Cigarette smoke (first/second hand)	Lung; larynx	Lung cancer*; laryngeal
Vinyl chloride (PVC; industry workers)	Liver	Hepatoma
As (gold miners)	Skin	Skin; bronchial
Glues, varnishes	Bone marrow	Leukemia
Isopropyl oil (isopropylene manufacture)	Nasal cavity	Nasal/Sinus cancer
Mustard gas	Bronchi; larynx; nasal sinuses	Bronchial; laryngeal; nasal cancer
Naphthylamines	Bladder	Bladder cancer

- *Dr. Matthew Meselson at Harvard: "The continued high consumption of cigarettes in spite of their being the cause of nearly all lung cancer (plus a large proportion of pulmonary heart diseases, bronchitis and emphysema) results in part from ignorance or disbelief of the facts, especially among young people."
- Bottom line: the more cigarettes one smokes the sooner that individual will die. The more second hand smoke inhaled by others, the greater their chances of getting lung cancer and succumbing to an earlier death.
- More and more, health care professionals are finding that those who smoke, particularly in light of the strong evidence of death associated with smoking, are committing slow suicide.

Cancer - Heredity: Oxymoron or Fact of Life?

- As a general rule, cancers that develop in human beings are not categorized as genetic diseases since they do not fit the necessary criteria:
- 1)** Must be a pattern of inheritance that is predictable over a number of generations. They must obey Mendel's laws (coming up in future lectures).
- An example, however, that illustrates X-linked transmission of a genetic trait is demonstrated in the Figure, right.



X-Linked Disorder -- From Dad to Daughters to Grandsons

- **2)** There must be some form of chromosome anomaly, e.g., loss or gain of a chromosome or rearrangement of chromosomal material.
- **3)** There must be a way to assign a biochemical defect to a particular gene, e.g., HbA vs HbS.

- Cancers are NOT contagious, nor does one contract cancer by eating a plant that has a tumor.
- Furthermore, it is impossible to "catch" cancer from an animal: cancer is species specific and, while cancers may be transferred between species in the carefully controlled environment of the laboratory, this does not happen in the chaotic environment of the "real world".
- Additionally, to transfer a cancer between species in the lab requires that much more than one million cells be used for this transfer. This will not happen in the "real world".

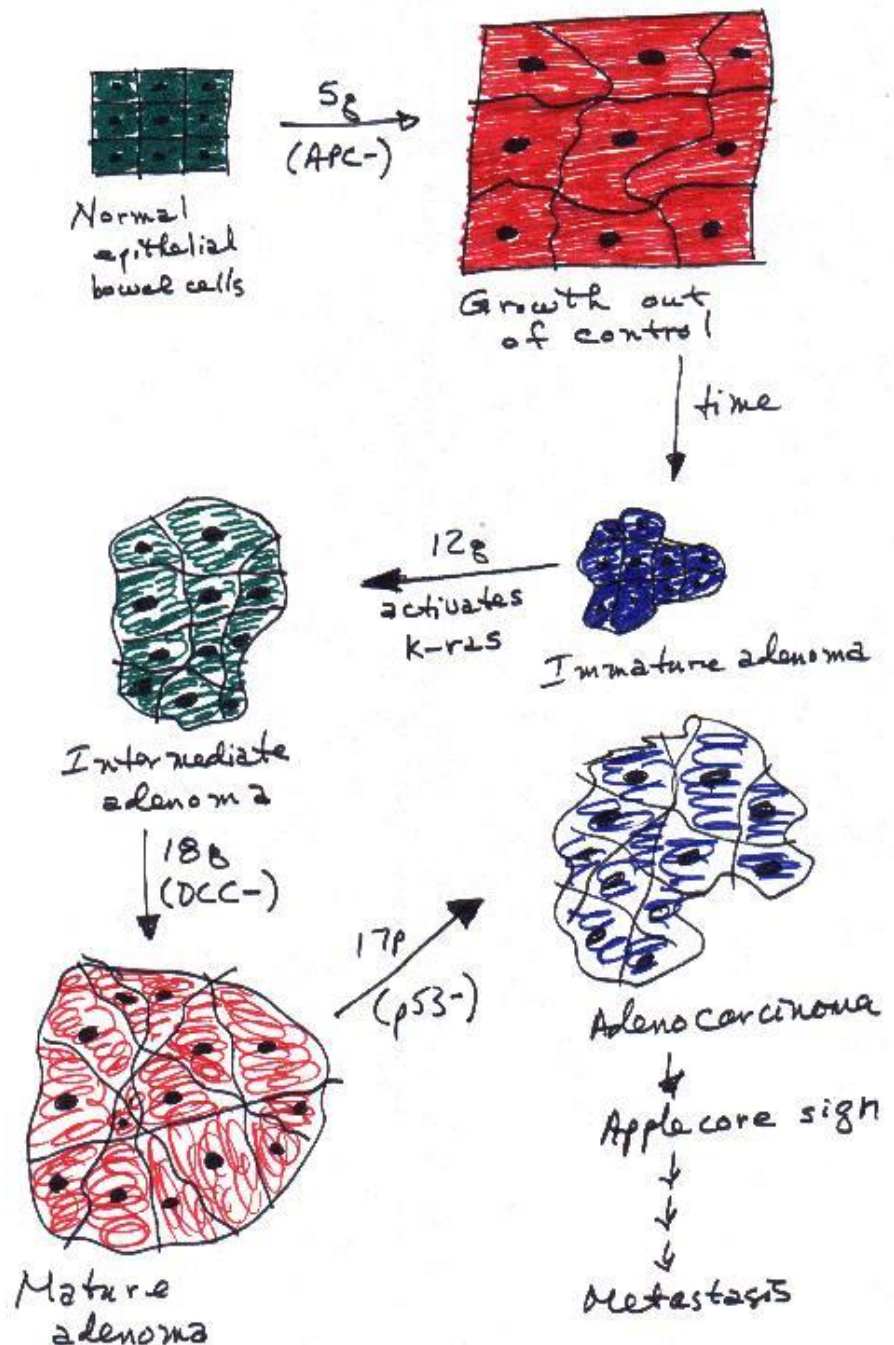
- Somatic cell mutations do not transfer to future progeny. The best evidence for this is the fact that survivors of Hiroshima and Nagasaki, while they had much higher rates of leukemias and osteogenic sarcomas compared with the un-irradiated group had offspring with no increase in genetic defects or genetic diseases.
- In spite of this data, however, one must use caution: the results of reproduction, i.e., no increase in "mutants" in their offspring, may merely represent that any germ line mutations may have been too slight to observe in the population surveyed in Japan.

- Although the idea to be conveyed here is that cancer development depends on genetic activities, it does not follow that cancer is 100% inherited - although in rare cases, some patients are definitely "aimed" towards developing cancer as a result of a genetic disease. An example that may differentiate between genetic and hereditary follows:
- People who are born with fair skin are born with fair skin as a result of the genetic combination in their skin cells.
- Fair skin, then, is inherited.
- People who have fair skin are very susceptible to the effects of the sun, i.e., they burn easily.
- It is well known that the earlier one with fair skin gets burned, the greater the risk of developing malignant melanoma.
- The cancer is not inherited, nor are these people predisposed to malignant melanoma: the over-exposure to/of sun is controllable by the person.
- Hence malignant melanoma as a response to over-exposure by sun is NOT inherited from one's parents - refer to above for apparent oxymoron.

- Additionally, the biggest "susceptibility factor" to ANY genetic mutation seems to be that some people's cells are more "open to the idea of mutation" than others' cells.
- In spite of mutations that occur, it still takes many years to develop the disease, e.g., BRCA takes about 6 years (non-invasive (*in situ*)) for it to be detected by either manual palpation or mammography (Kent Skogerson, M.D., *personal communication*).

- Some cells require a number of mutations to occur before "carcinogenetic" changes occur to progress the development of [a] tumors. This is called the "multi-hit concept of carcinogenesis". An excellent example of this concept is the development of colorectal cancer.
- The following figure illustrates the cascade of events that causes the development of cancer from the loss of three genetic products and the activation of one gene product.

- In brief, normal bowel epithelial cells grow over time as they are supposed to.
- At some point in time, chromosome 5q loses APC (adenomatous polyposis coli – tumor suppressor gene) which starts a period of growth that has no control. As time continues, this rapidly growing mass differentiates into an immature adenoma.
- Chromosome 12q activates k-ras (proto-oncogene - to be defined shortly) which causes the conversion of the immature adenoma into an intermediate adenoma.
- Once the intermediate adenoma stage has been reached, chromosome 18q lacking DCC (Deleted in Colorectal Carcinoma) causes the intermediate adenoma to form a mature adenoma.
- Chromosome 17p, after losing p53 (a tumor suppression factor - probably the most important one there is), allows the mature adenoma to differentiate into an adenocarcinoma of the bowel.
- When visualized on barium enema, often times it appears as an applecore shadow on the bowel, hence "applecore sign".
- If not caught, it will metastasize with time.





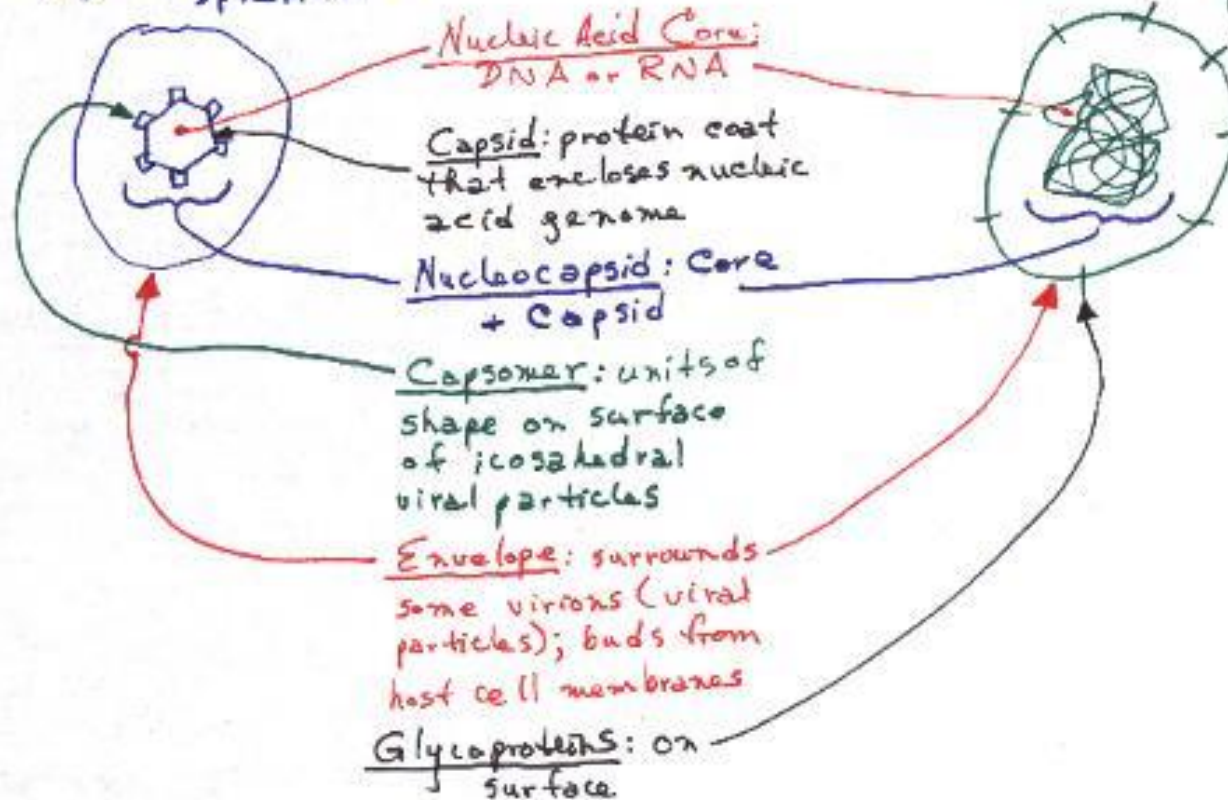
- When visualized on barium enema, often times it appears as an **applecore shadow** on the bowel, hence "**applecore sign**". If not caught, it will metastasize with time.
- Used under "Fair Use" copyright laws from:
 - <http://radiographics.rsnajnl.org/cgi/content/full/22/6/1369/F1A>
 - **Clare J. Roche, MB, MRCPI, DMRD, FRCR, David P. O'Keeffe, FRCR, FRCPI, FFRRCSI, W. Kit Lee, MD, Vinay A. Duddalwar, MB, FRCR, William C. Torreggiani, MB, MRCPI, FRCR, FFRRCSI and John M. Curtis, MB, FRCR ; Selections from the Buffet of Food Signs in Radiology¹** ¹ From the Department of Radiology, University College Hospital, Newcastle Rd, Galway, Ireland (C.J.R., D.P.O.); the Department of Medical Imaging, Geelong Hospital, Victoria, Australia (W.K.L.); the Department of Radiology, Aberdeen Royal Infirmary, Aberdeen, Scotland (V.A.D.); the Department of Radiology, Tallaght Hospital, Dublin, Ireland (W.C.T.); and the Department of Radiology, University Hospital, Aintree, Liverpool, England (J.M.C.). Presorted as an education exhibit at the 2001 RSNA scientific assembly. Received March 28, 2002; revision requested April 29 and received June 13; accepted June 17. **Address correspondence to C.J.R. (e-mail: clareroche@hotmail.com). Radiographics. 2002;22:1369-1384**

Oncogenic Viruses -- Part 1

- Oncogenic viruses are cancer-causing viruses. The first of these identified is called the Rous sarcoma virus (R-src: "sarck").
- It was first identified in 1911 by Peyton Rous. Rous showed the oncogenic effects of this virus by injecting them into chickens and producing tumors. These viruses are species specific, i.e., are NOT transmittable between species.

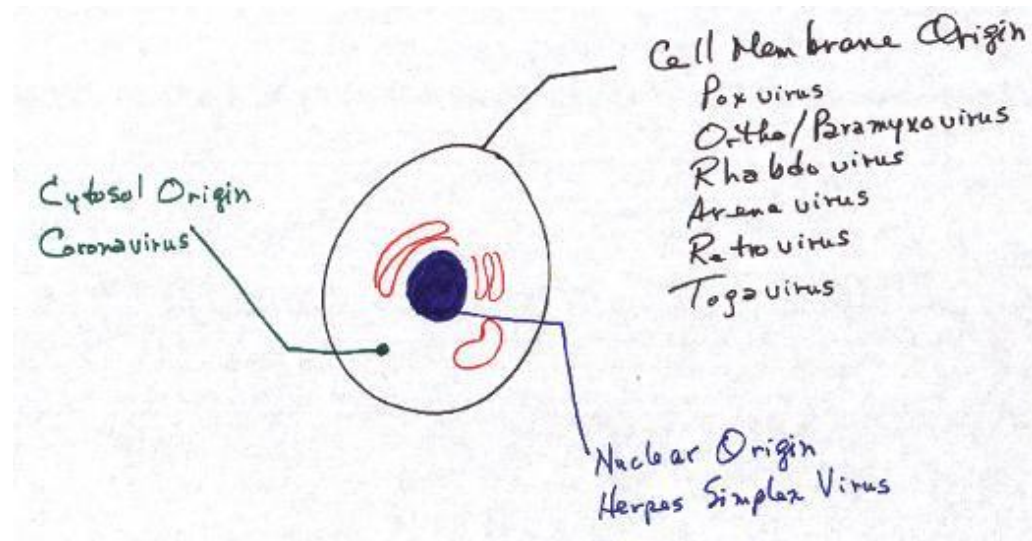
Icosahedral Symmetry:
12 vertices; 21 pentagonal faces; appears spherical

Helical Symmetry:
Genetic Material in a helix; supported by a protein capsid



- Some basic information about viruses to follow this topic:

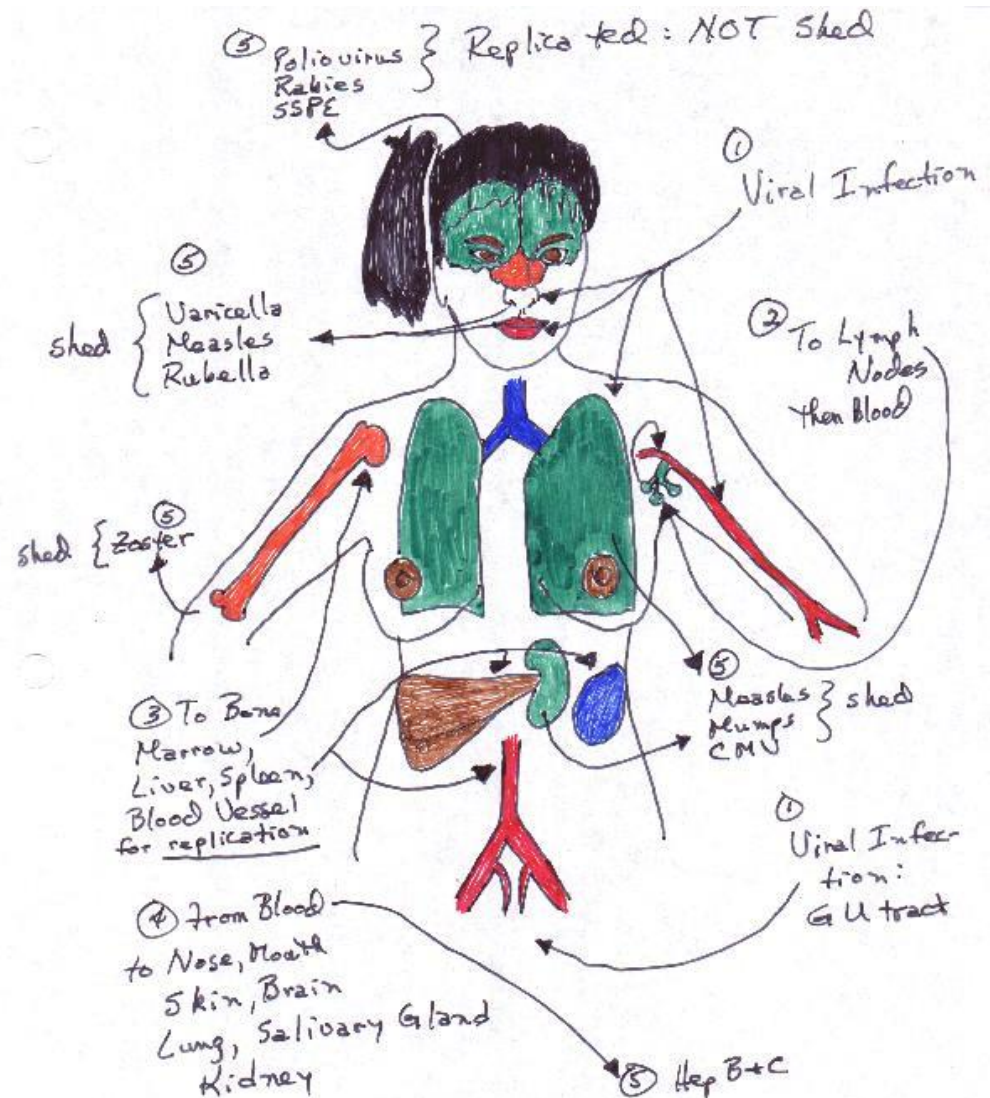
The Cellular Site of Origination of Viral Envelopes



- Herpes viruses, responsible for cold sores and genital sores, bud from the nuclear membrane of eukaryotic cells;
- Coronavirus, responsible for some of the common colds, buds off the cytosol;
- Poxvirus, responsible for smallpox,
- Orthomyxovirus, responsible for influenza viruses,
- Paramyxovirus, responsible for mumps and measles,
- Rhabdovirus, responsible for rabies,
- Arenavirus, responsible for Lassa fever,
- Retrovirus, responsible for AIDS and some leukemias, and
- Togavirus, responsible for rubella, yellow fever, dengue fever, bud off the cell membrane.

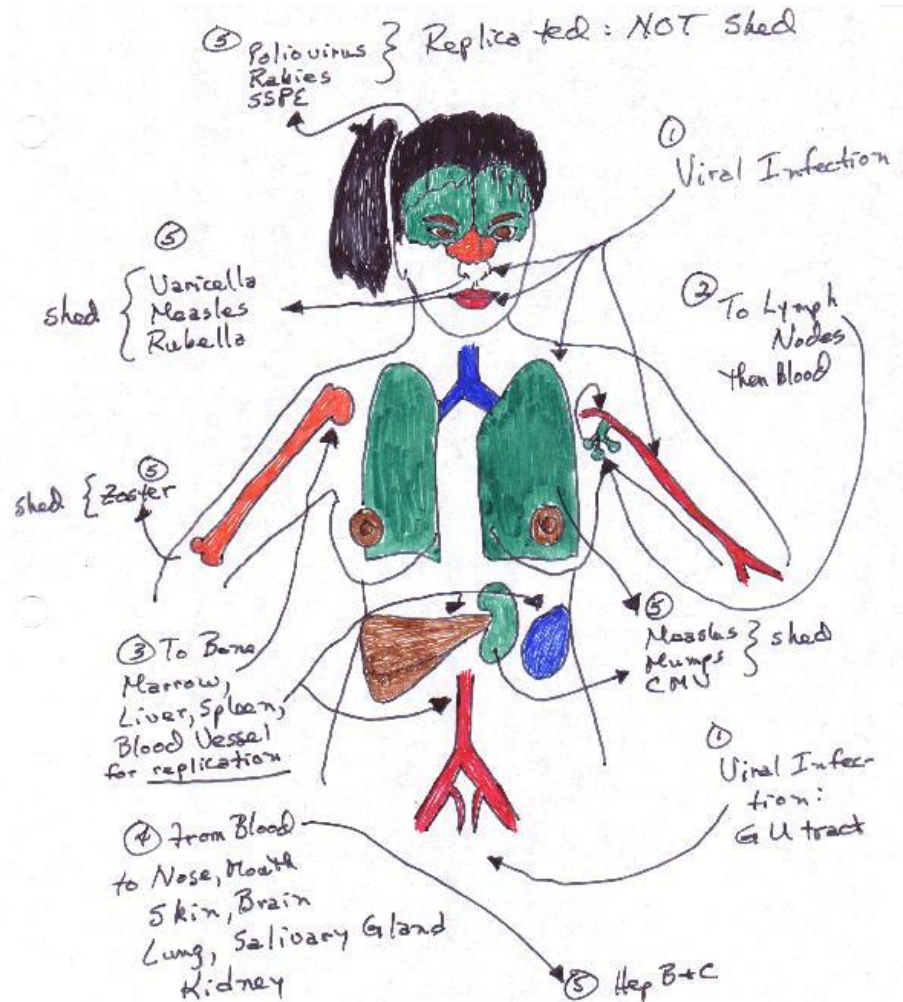
Viral Entry into The Human Body and Replication

- As a general rule, one must be infected with the virus. This may be accomplished by inhaling the particle, ingesting the particle or injecting the particle -- this includes genito-urinary tract viral infection, as well. Following infection, the viruses are transported to the lymph nodes, then the blood. From there they go to bone marrow, the liver, spleen and back to blood vessels for replication. From the blood the viral particles travel to the nose, mouth, skin, brain, lung, salivary gland and kidney.

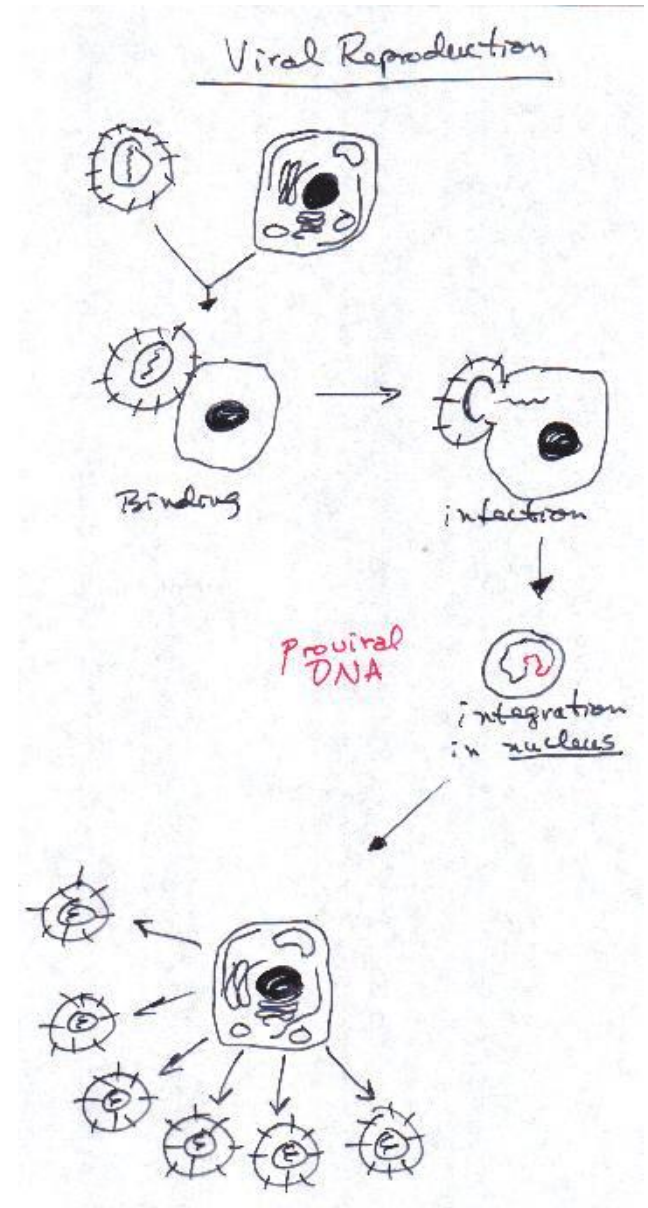


Viral Entry into The Human Body and Replication

- From the blood, viral particles are excreted -- shed -- and may cause diseases like Hepatitis C. From nasal and oral secretions, the viruses of varicella, measles, rubella are shed. From the skin, Varicella virus is shed. From the lungs and kidneys, the measles' and mumps' viruses, along with cytomegalovirus, are shed. The brain does not shed viruses. Rather it allows replication of some viruses like poliovirus, rabies virus and SSPE (a long term complication of the hard measles that causes hardening of the grey and white matter in the brain 5-15 years after infection; SSPE = subacute sclerosing panencephalitis).

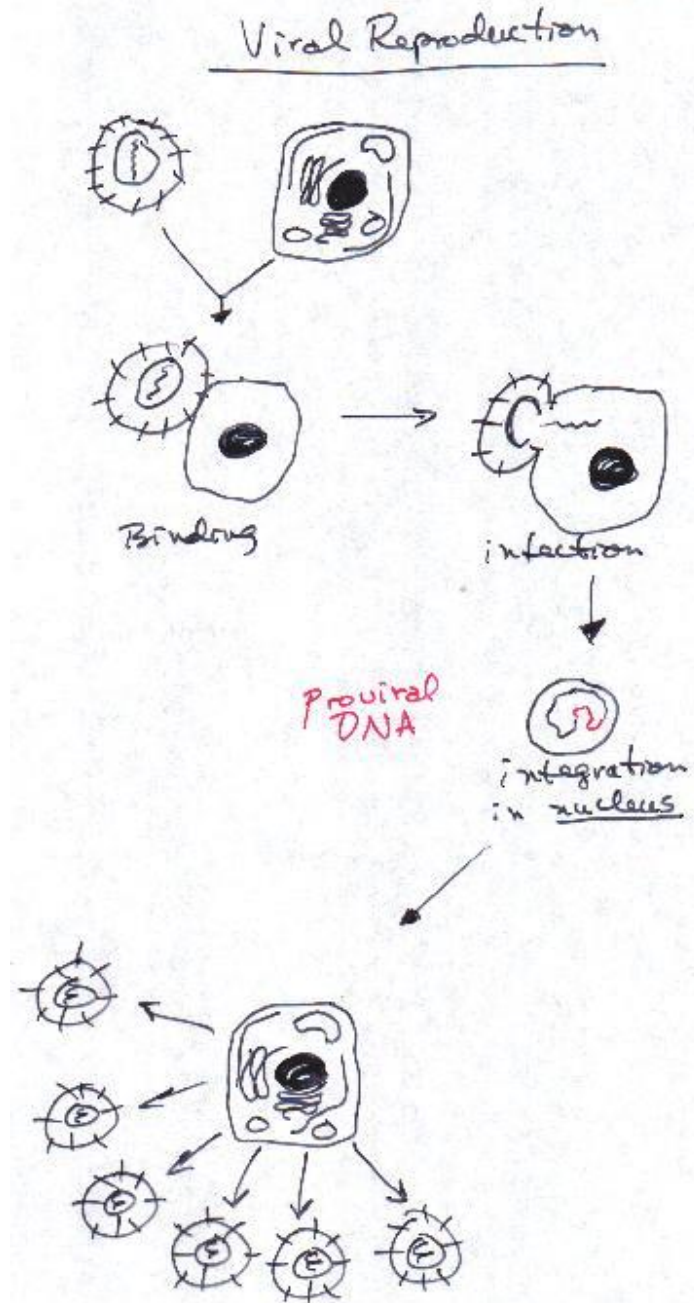


- Oncogenic viruses are different from many other viruses in that they typically do not destroy the cell in which they are "working"; conversely, viruses like HIV are very cytotoxic (coming up). There are two mechanisms that may happen following infection of a cell by an onco-virus:
(1) viral reproduction – right.
- The results of the first is that new virions are produced to infect cells.

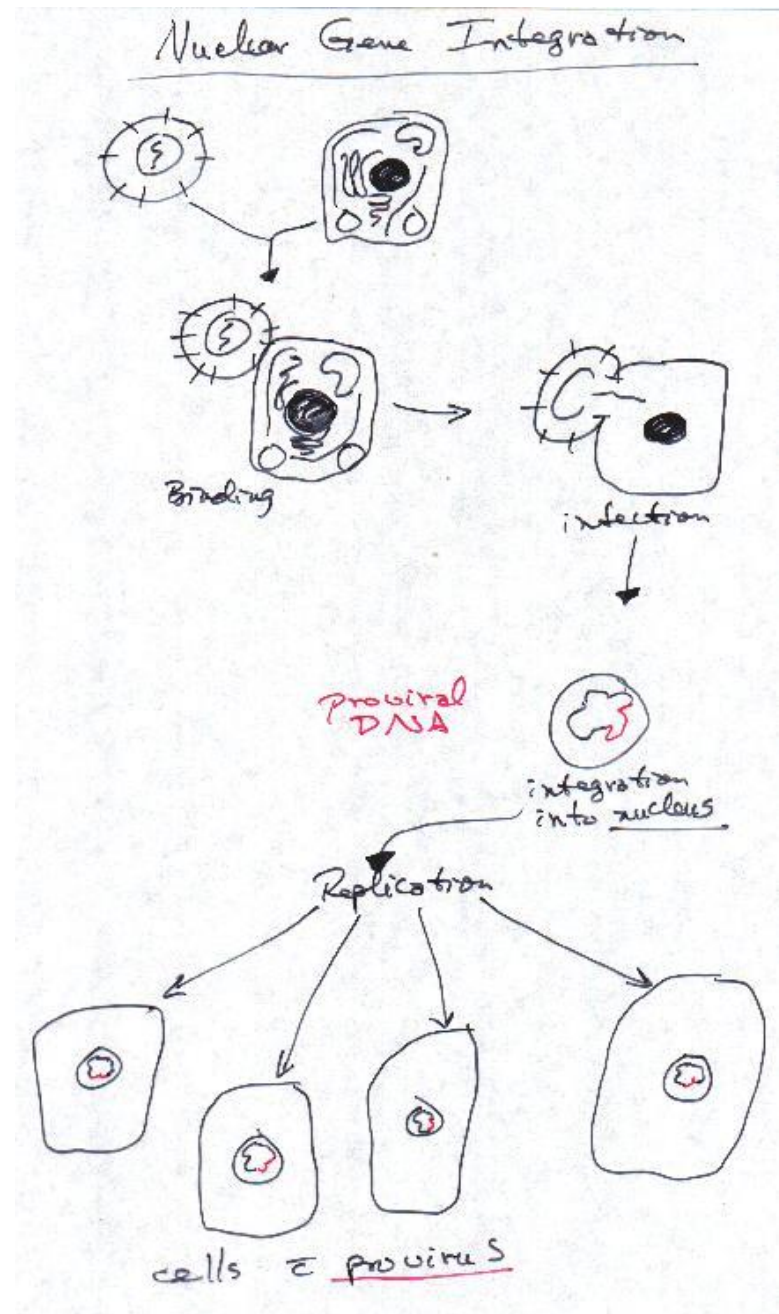


Viral reproduction has essentially 4 phases/stages:

1. **Viral binding:** in this phase, the virus binds to the cell in preparation for the next stage.
2. **Infection:** during this stage, the viral and cellular membranes fuse, forming a single continuous membrane. At the same time, the viral genome is dumped into the cell.
3. **Nuclear integration:** during this phase, the proviral DNA is integrated into the cellular DNA in the nucleus. This DNA will be "read" by the intracellular machinery to make more viral particles.
4. **Viral expectionation:** in this stage, mature virions are released from the cell into the general circulation. This allows more cells to become infected, perpetuating the reproduction of the virus.

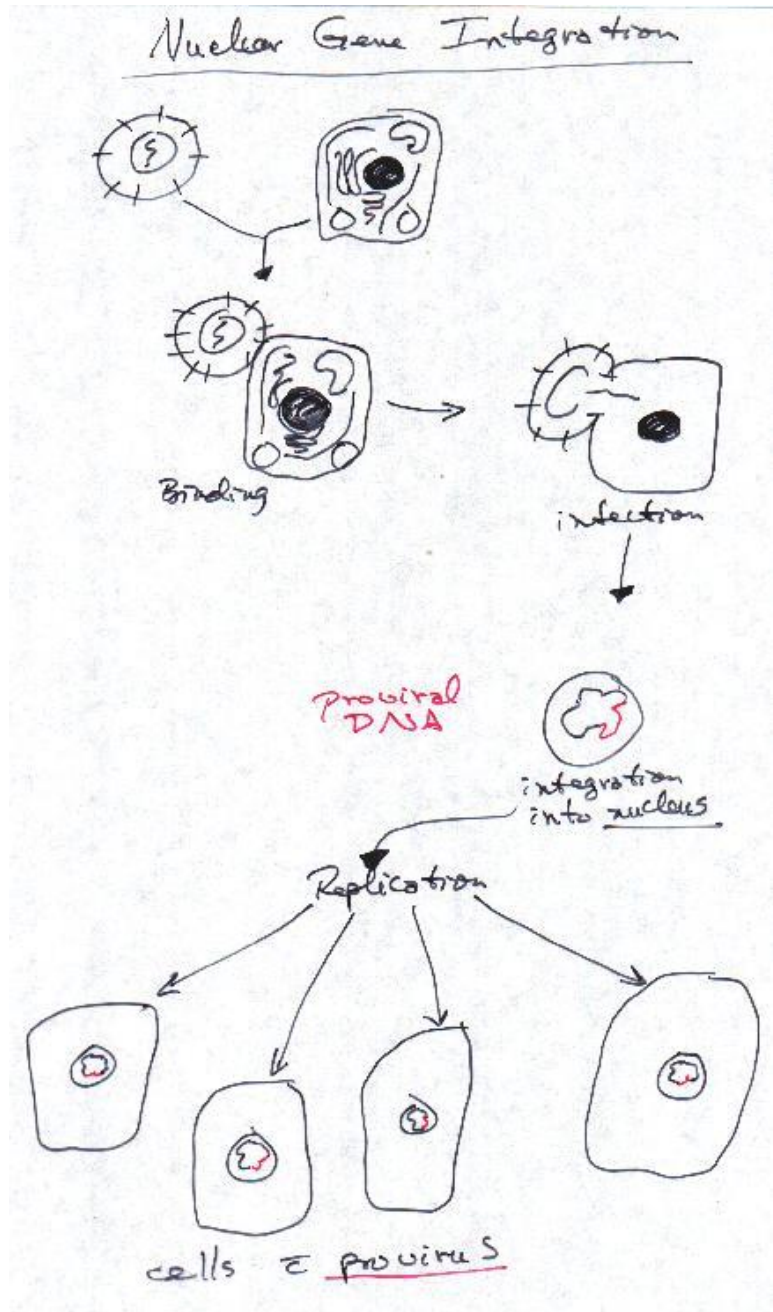


- or **(2)** nuclear gene integration – right.
- The result of the second is that the proviral DNA is replicated and passed along to new generations during mitosis which are likewise able to produce new virus for cell infection and tumorigenesis.



Nuclear gene integration has 4 stages, as well:

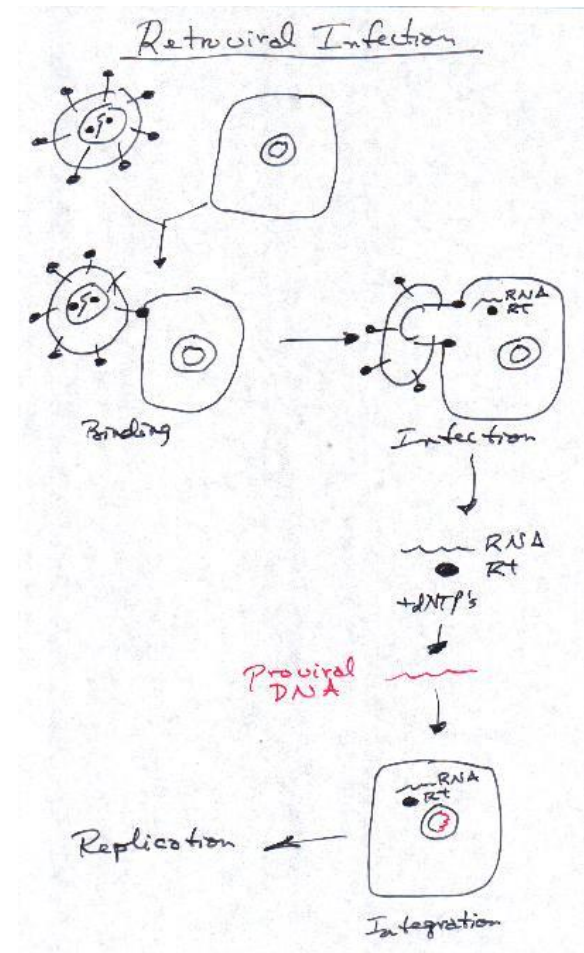
1. Binding, as above.
2. Infection, as above.
3. Nuclear integration, as above.
4. Replication: in this phase, the cells replicate. Each new cell has a copy of the proviral DNA in its genome. As time continues, more of these cells are produced.



- Although numerous viruses of the oncogenic type contain DNA, many also contain RNA.
- In order to infect cells, these viruses carry an enzyme, reverse transcriptase, RT, that is "dumped" into the cell with the RNA.
- The two, RNA and RT, are used to synthesize DNA by reverse transcription.
- This DNA is then integrated into the cell's DNA.

Retroviral Infection of A Cell

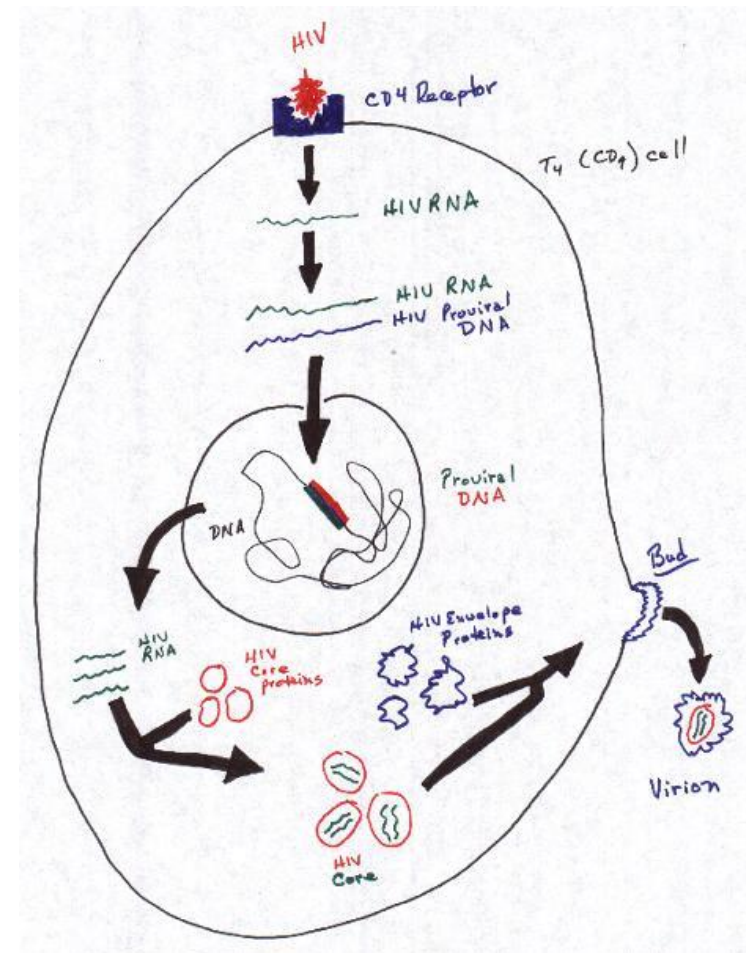
- Retroviral infection resembles previously discussed nuclear gene integration. The difference, here, is that a retrovirus dumps RNA and RT into the cell once the cell is infected. The RNA and RT utilize dNTP's to synthesize a complimentary strand of proviral DNA that will be integrated into the cell's DNA. The cell, then, will replicate and will also synthesize numerous copies of the retrovirus to perpetuate viral infection.



A Generic View of The Life Cycle of HIV -- The "Ultimate" Retrovirus, Although

NOT -- To Date -- **Known to Be An Oncovirus**

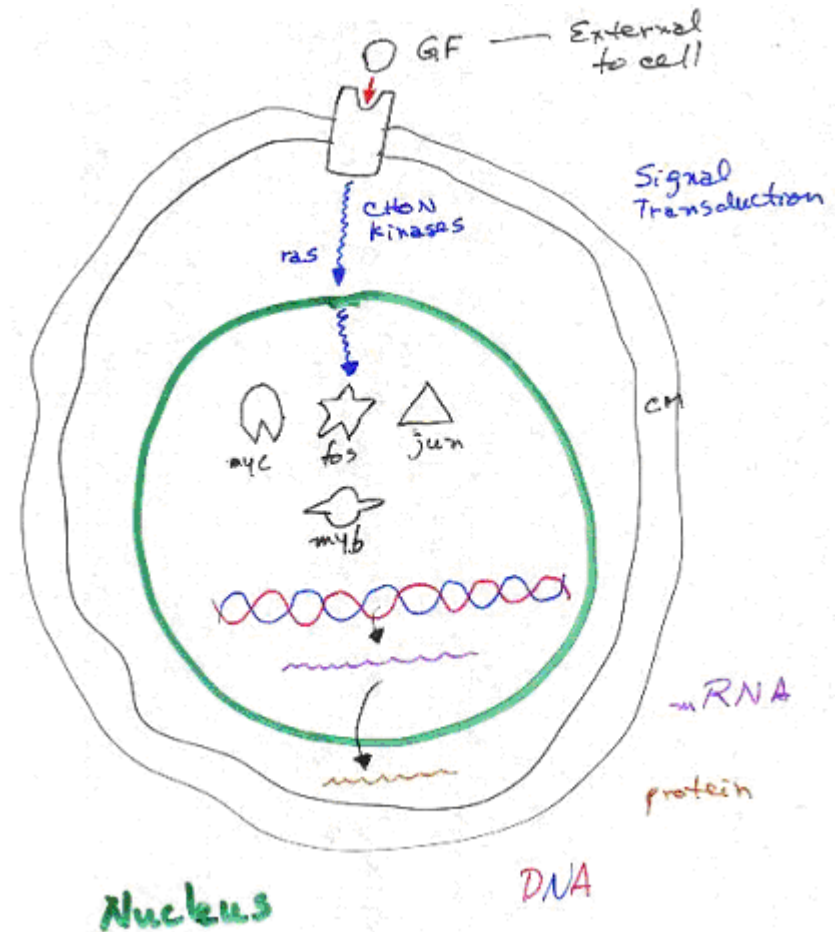
- The life cycle of HIV in a generic sense. HIV infects a T₄ cell and goes through the normal infective, synthetic processes we've discussed, previously. A point to remember is that the proviral DNA is integrated by an enzyme called integrase into the cellular DNA. Our cellular machinery then reads this new DNA and causes the increased output of more viral particles. As indicated, retroviruses bud from the cell membrane. The Figure only shows 1 virion being budded. It's important to remember that each T₄ cell buds thousands of HIV particles before the cell is ultimately destroyed.



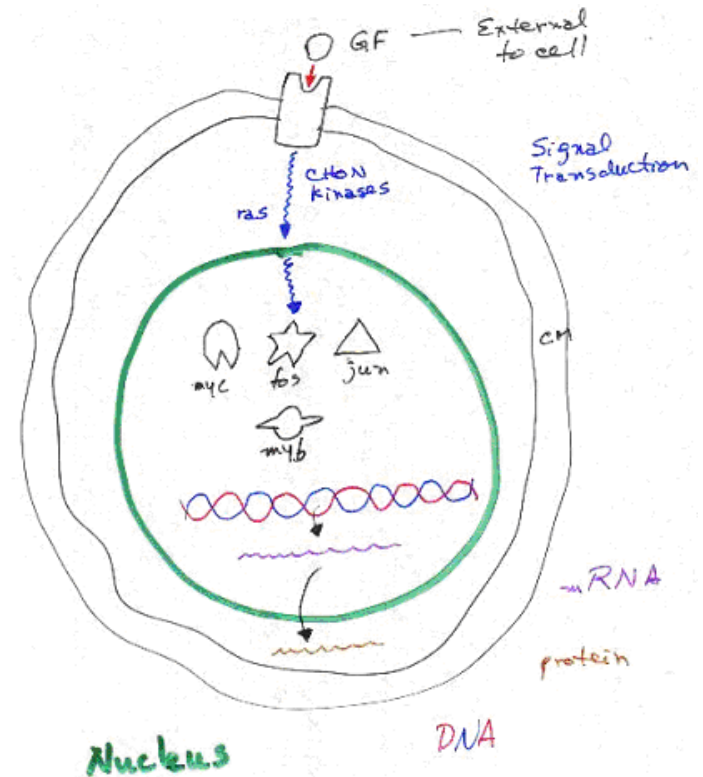
Oncogenes: Normal Human Genes Gone Awry or Virally Transmitted Genes?

- It has been suggested that millions of years ago, viruses transmitted their genes into animal cells and were integrated into the cellular DNA of these cells.
- These altered cells also passed their genetic material (with the proviral genes) on down the "tree". Assuming that these genes are never transcribed, the effects of this information will never be felt.
- However, if a "sensitizing material" contacts these cells, transcription may be triggered and turn out cells which grow and reproduce without regulation.

- A very crude rendition of normal regulator proteins.
- *fos*, *jun*, *myc*: DNA binding proteins that regulate transcription and, hence, effect translation indirectly.
- *c-myc*, is a DNA-binding protein that appears to regulate cell growth and differentiation and is a proto-oncogene.
- A normal gene which, when altered by mutation, becomes an oncogene that can contribute to cancer. Proto-oncogenes may have many different functions in the cell. Some proto-oncogenes provide signals that lead to cell division. Other proto-oncogenes regulate programmed cell death (apoptosis).
- The defective versions of proto-oncogenes, known as oncogenes, can cause a cell to divide in an unregulated manner. This growth can occur in the absence of normal growth signals such as those provided by growth factors. A key feature of oncogene activity is that a single altered copy leads to unregulated growth.
- proto-oncogene is an oncogene in its non-cancerous state, it is a normal gene
- Look over this site:
<http://web.indstate.edu/thcme/mwking/oncogene.html>



- A growth factor, external to the cell, acts through its receptor to turn on protein (CHON) kinases/ras in the cytosol which signals for the nuclear synthesis/release of myc, fos, jun and myb (all of these are DNA binding proteins that regulate transcription and, hence, translation indirectly). The DNA is then transcribed as necessary and translation is commenced.



Quick aside

- **Cis-acting elements** - DNA sequences in the vicinity of the structural portion of a gene that are required for gene expression
- Genes that have sequence modules in common that control the coordinate regulation.
- **Trans-acting factors** - factors, usually considered to be proteins, that bind to the cis-acting sequences to control gene expression
- Source: <http://www.cc.ndsu.nodak.edu/instruct/mcclean/plsc731/cis-trans/cis-trans6.htm>

END OF QUICK ASIDE

- Proto-oncogenes are from normally functioning cellular regulators of cellular processes.
- They are the counter part to oncogenes (**cis acting**).
- The name, proto-oncogene is confusing.
- They are found in different compartments of the cell.
- Proto-oncogenes are expressed during various phases of the cell reproductive cycle.
- They serve to sequentially direct the cell through a systematic reproductive cycle.
- The table below summarizes some examples of proto-oncogenes:

Classification	E.g.	Chromosome	Function
Growth factor	sis	22q12-q13	PDGF sub-unit
GF receptor	src	20q11	Tyr kinase
Signal transducers	H-ras	11p15	GTP-binding protein
	trk	1q32	Protein kinase
DNA-binding proteins	myb	6q22	DNA binding

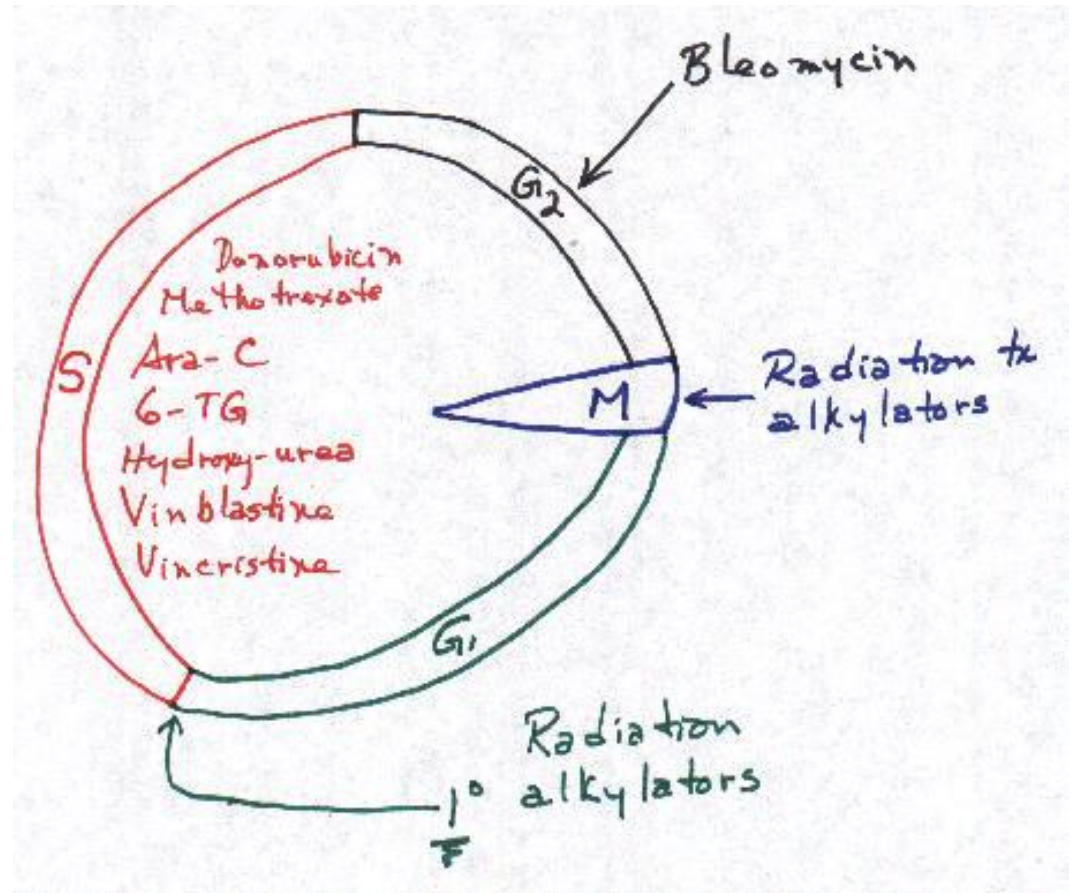
- The cell cycle is regulated externally by mitogens, growth factors, hormones, lymphokines, all of which operate through cellular receptors.
- Much of the growth and reproduction of the cell is regulated by growth factors, e.g., platelet-derived growth factor (PDGF), epidermal growth factor (EGF), steroids.
- Each of these operates through receptors: steroids via nuclear steroid receptors and the rest via membrane bound receptors on the surface of the cell.
- When a growth factor binds to its cellular receptor, it initiates a cascade of events which are collectively called signal transduction.
- Included in this process is the activation of protein kinases, which are responsible for altering the activity of various proteins by phosphorylating or dephosphorylating them.
- The end result of this cascade is to effect transcription in such a manner as to influence cell growth and reproduction.
- Examples of genes that encode DNA-binding proteins responsible for altering transcription are myc (8q24)(trans), fos (14q24-q31)(trans), jun (proto-oncogene)(trans). fos interacts with jun to influence transcription.

- The risk of mutations which activates the potential oncogenic activity of proto-oncogenes, while obviously not easily or frequently activated, suggests that many safeguards exist so as to reduce proto-oncogenic stimulation of oncogenes.

- One such mechanism of proto-oncogenic "inhibition" is tumor suppressor genes. The following table provides some examples of these tumor suppressor genes:

Tumor suppressor	Chromosome	Function	Germ line mutation disease	Somatic cell mutation disease
RB	13q14	Transcription regulator	Retinoblastoma	Prostate carcinoma
APC	5q21	Cell adhesion	Adenomatous polyposis coli	Colon carcinoma
P53	17p13	Regulates transcription and cell cycle		BRCA; colon carcinoma
WT1	11p13	Regulates transcription	Wilms tumor (childhood renal carcinoma)	Nephroblastoma

- It is of interest that p53 (and other suppressors) regulate the cell cycle, for there are various cancer chemotherapeutic agents/regimens that work at their optimal activities during various stages of cell growth:



Seven Danger signs of Cancer

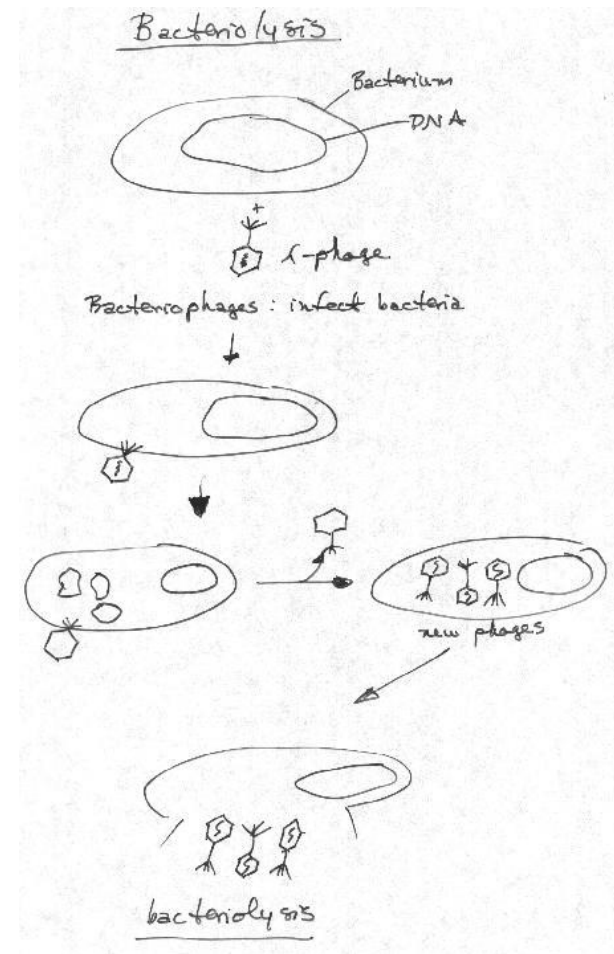
- 1. Change in bowel or bladder habits
- 2. Asore that does not heal
- 3. Unusual bleeding or discharge
- 4. Thickening lump in the breast or elsewhere in the body
- 5. Indigestion or trouble swallowing
- 6. Obvious changes in a wart or mole
- 7. Nagging cough or hoarseness

New Terms and Definitions

- Selection: phenotypic variation due to genotype, e.g., bacterium with gene for resistance to TET can be distinguished from bacterium lacking the gene by growing in media with TET (agent of selection). Necessary for BIOL 251.
- Expression: selection of the gene requires this; observed by phenotype; Necessary for BIOL 251.
- Bacteriophage: viruses associated with prokaryotes; Necessary for BIOL 251.
- Lytic phages: make many numbers/copies of themselves as they LYSE their host cell; Necessary for BIOL 251.
- Temperate phages: enter non-lytic prophage state; nucleic acid replication of phage linked to host cell DNA replication; Necessary for BIOL 251.
- Lysogenic: prophage infected bacteria; due to a physiological signal that can initiate lytic cycle and cause bacterial death; Necessary for BIOL 251.
- Prokaryotic DNA replication: linked to the cell membrane (mesosomes); this provides separation of the membrane which binds the DNA to “hold it” in place for replication; Necessary for BIOL 251.
- Two ends of DNA: 5' and 3' : 5' = phosphate end (P) and 3' = -OH end

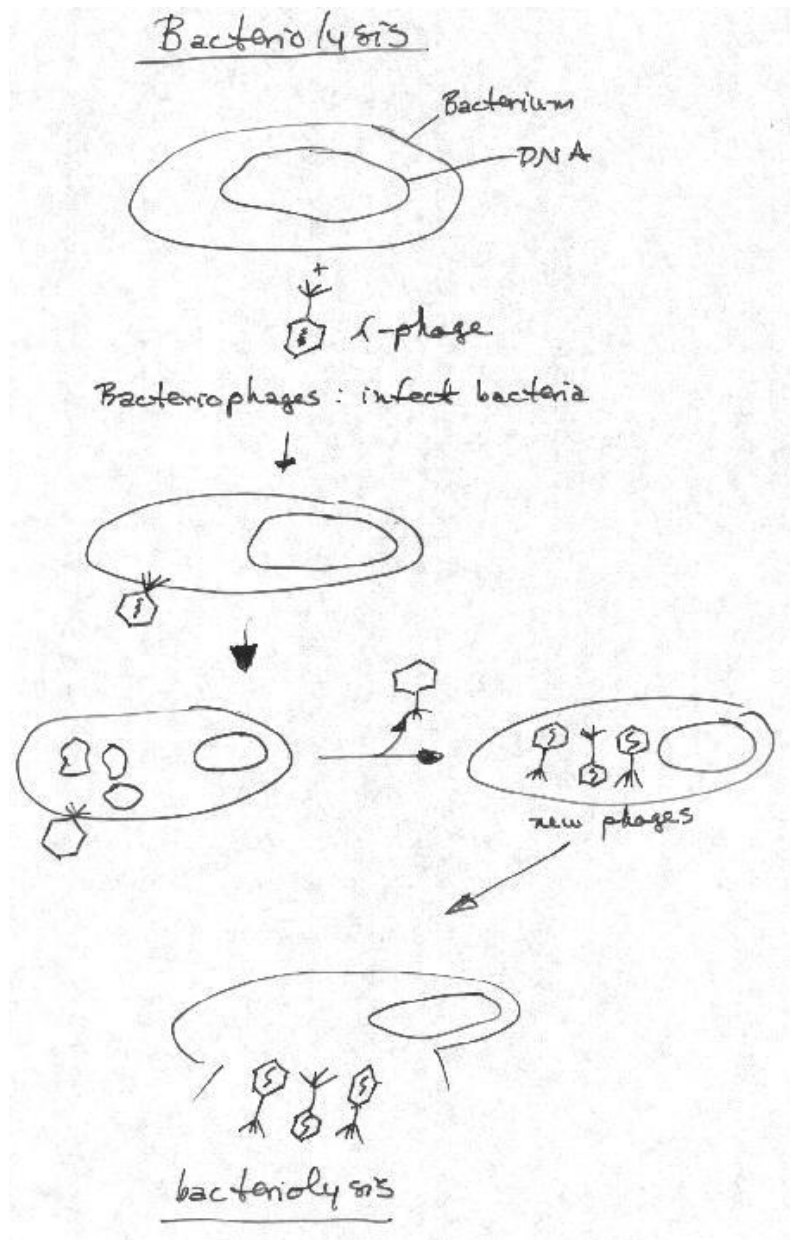
Lysogenic Bacteria: Shifting to an Analogy Using Bacteria

- Oncogenic viruses also infect bacteria. Just as with human cells, when oncoviruses infect bacteria, there are two directions bacteria may take: **(1)** bacteriolysis (destruction of the bacteria with concomitant release of infective viruses).

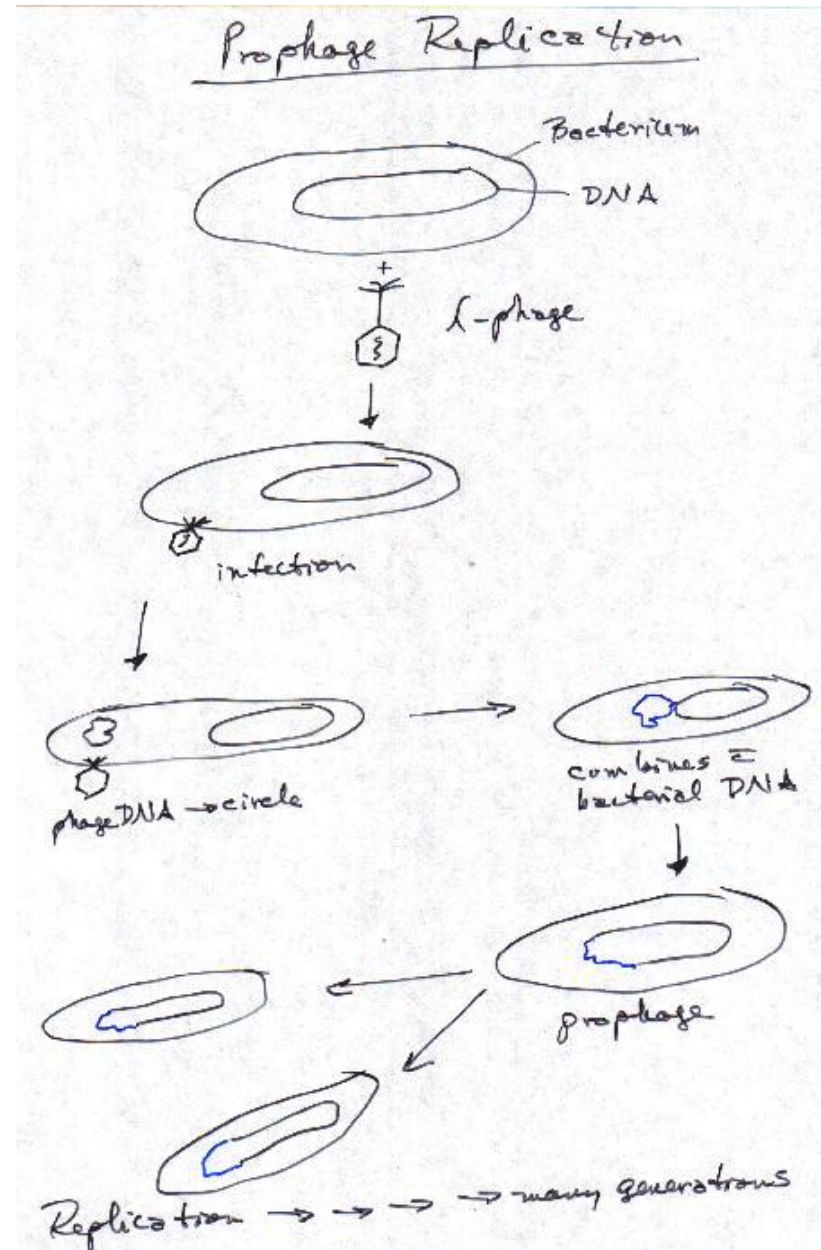


- A phage, in generic terms, is a virus capable of infecting bacteria.
- If the prophage is simply transported in the bacterial DNA for replication and reproduction, this bacterium is then called a lysogen.
- In many cases, the prophage will not be expressed.
- If, however, the prophages are exposed to ionizing radiation or some other "sensitizing agent", then the prophage DNA is expressed, new phages released and the bacteria are lysed in the process.

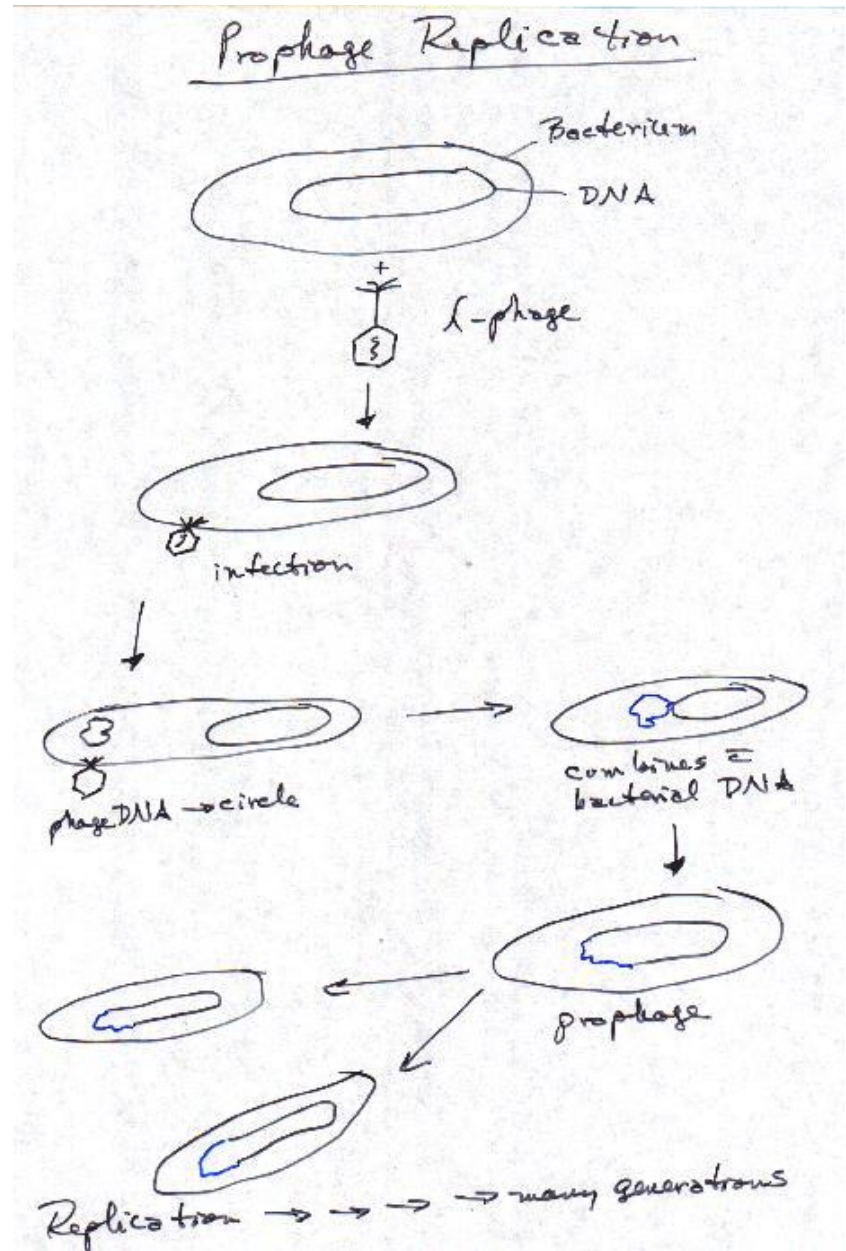
- Bacteriolysis occurs when a bacteriophage (virus that infects a bacterium) infects a bacterium.
- An example of this bacteriophage is λ -phage. As the graphic shows, this bacteriophage literally binds with its feet, then compresses itself to open a channel in its stalk to eject its genome into the bacterium. The phage is then released, new phages are synthesized by the bacterium and the bacterium lyses, releasing more bacteriophages.



- or (2)
replicating
prophage DNA
for future
progeny



- In prophage replication, infection and genomic ejection are identical as in bacteriolysis. In this case, however, the circular DNA ejected by the phage combines with the bacterial DNA. This prophage is now replicated during normal bacterial reproduction for many generations.



Future Goals

- Inherited predispositions to cancers have not been elucidated well. It remains to be determined the significance of genes in common cancers such as BRCA, colon cancer or prostate cancer (it has been estimated that if all men live to be 95, there would be 100% prostate cancer rate among men).
- It is well known that there are some familial clusterings of cancers -- this tends to suggest some sort of common cancer initiating allele or commonly inactivated allele that regulates some part of the cell cycle. It will be of interest to observe the development of new molecular biology techniques in further elucidating the cause (and, hence, the treatment of) of cancer.