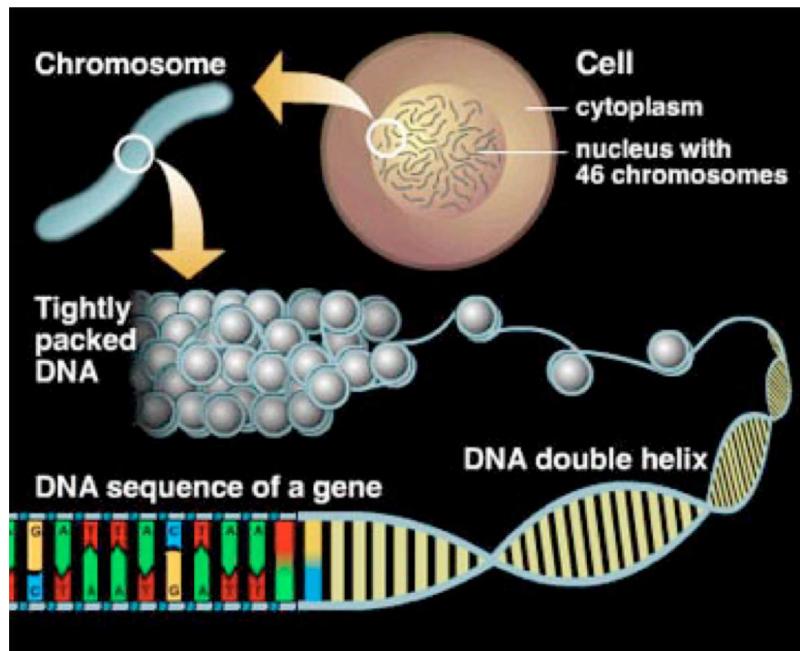
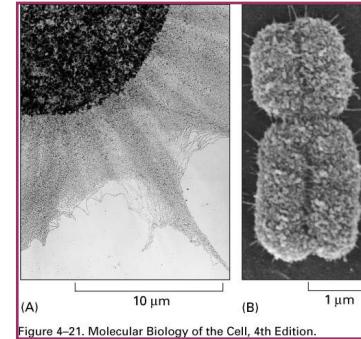




Nuclear composition in cell

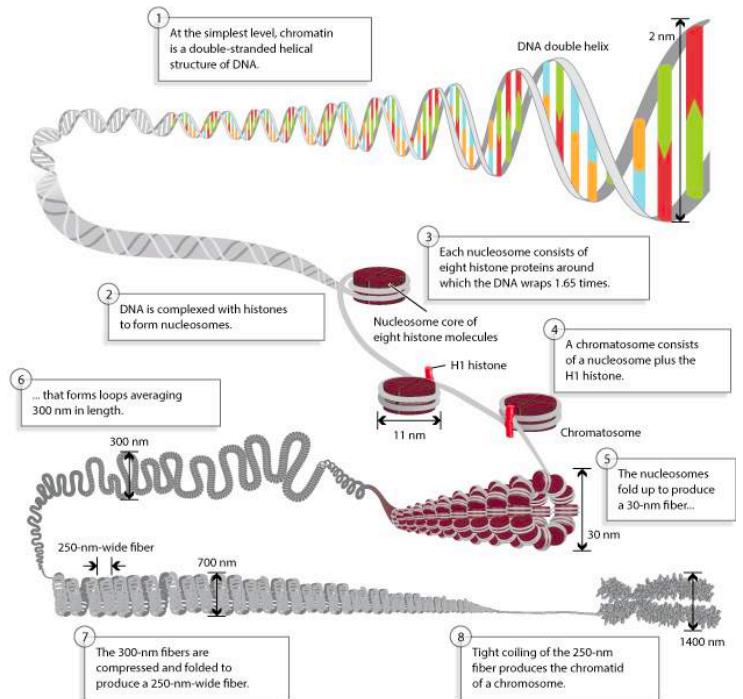


Packaging of DNA into Chromosomes



Challenges of Packaging DNA

- How to get 2 meters of DNA into nucleus of 6 μm
- Packaging accomplished with help of proteins
- Must be compacted in manner that still allows for it to be accessed by enzymes that govern replication, transcription, and repair



© 2013 Nature Education Adapted from Pierce, Benjamin. *Genetics: A Conceptual Approach*, 2nd ed.

Genome and Chromosomes

	Genome size (base pairs)	Chromosome number (<i>n</i>)
<i>Amoeba dubia</i>	670,000,000,000	Several hundred
Trumpet lily (<i>Lilium longiflorum</i>)	90,000,000,000	12
Mouse (<i>Mus musculus</i>)	3,454,200,000	20
Human (<i>Homo sapiens</i>)	3,200,000,000	23
Carp (<i>Cyprinus carpio</i>)	1,700,000,000	49
Chicken (<i>Gallus gallus</i>)	1,200,000,000	39
Housefly (<i>Musca domestica</i>)	900,000,000	6
Tomato (<i>Lycopersicon esculentum</i>)	655,000,000	12

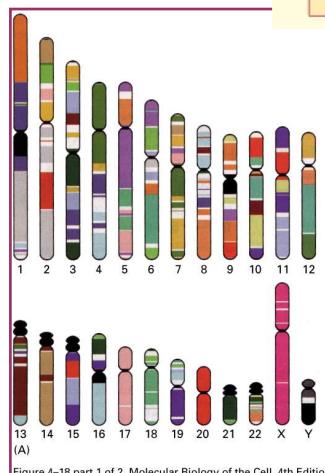


Figure 4–18 part 1 of Molecular Biology of the Cell, 4th Edition.

Five levels of chromosomal packaging

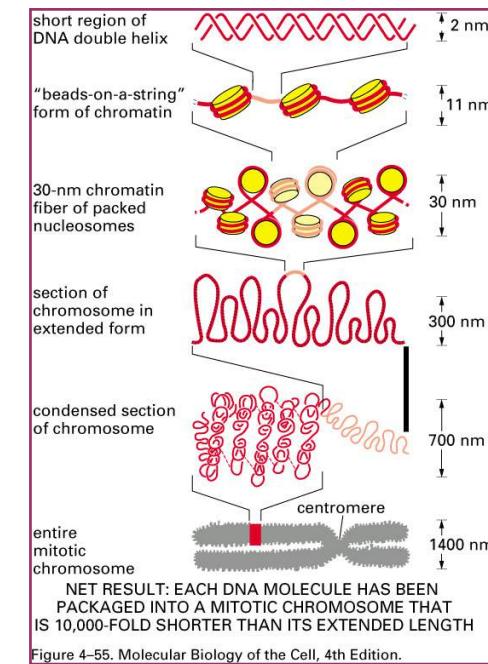


Figure 4–55. Molecular Biology of the Cell, 4th Edition.

Packaging of DNA into Chromosomes

DNA is packaged into a set of chromosomes

- DNA divided into set of chromosomes
- Chromosome= single DNA molecule and proteins associated with it

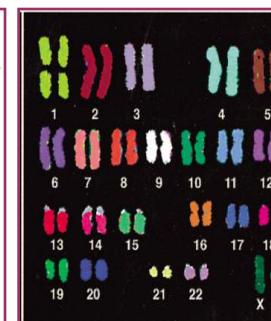
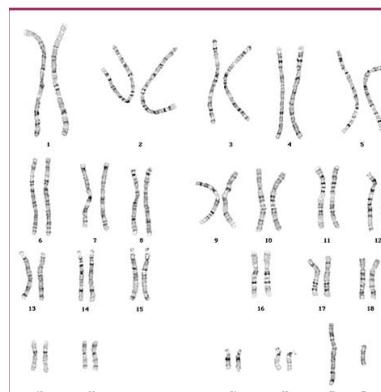
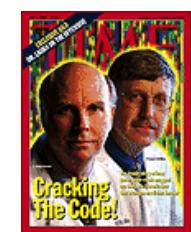
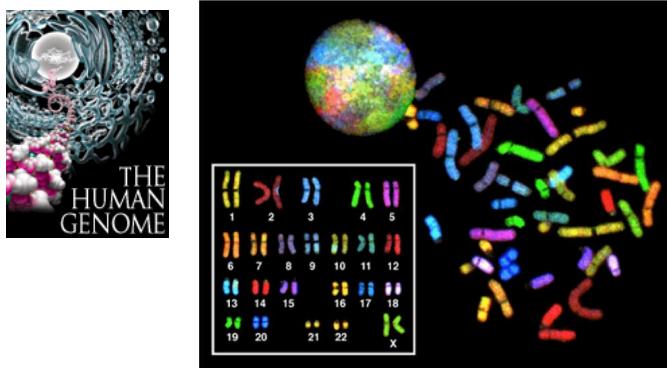


Figure 4–10. Molecular Biology of the Cell, 4th Edition.

What is the Human Genome Project?



3 billion bases
30,000 genes

<http://www.genome.gov/>

- An organism's genome consists of all its genes.
- The Human Genome Project is a multinational research project to determine the sequence of all 3×10^9 base pairs and hence all human genes roughly 30000 genes.

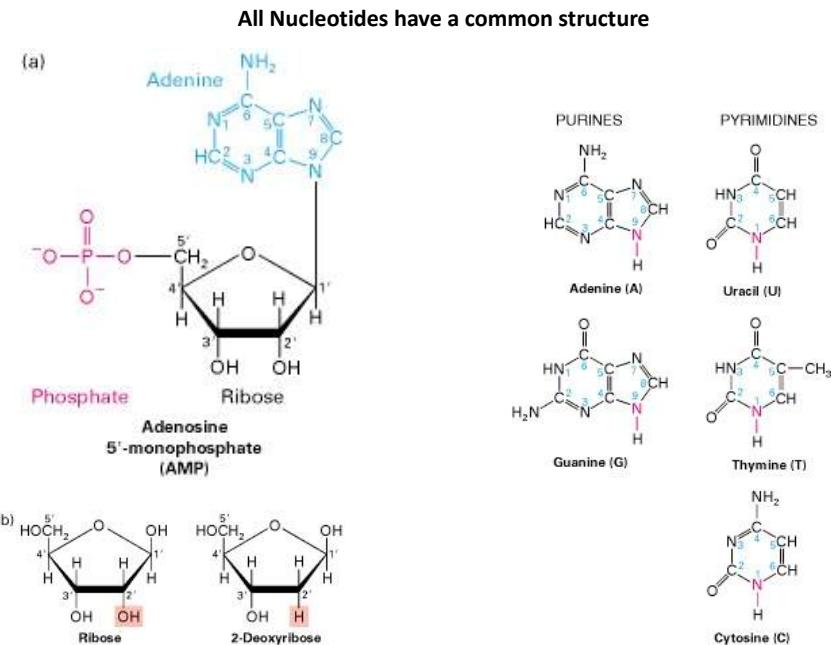
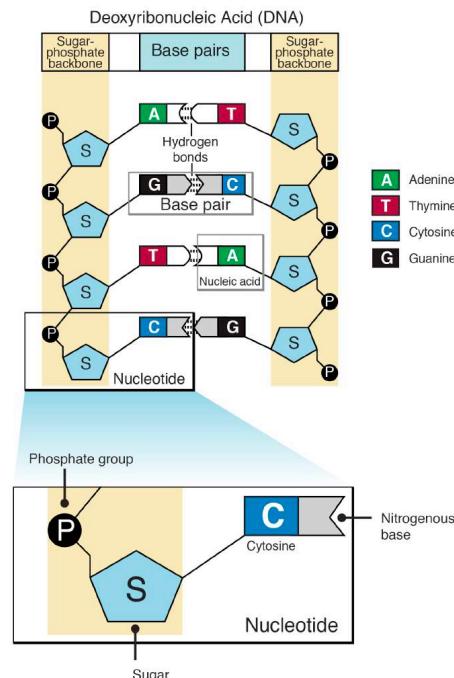
Three main components of Nucleic acid are:

- (1) phosphate (PO_4) groups;
- (2) five-carbon sugars; and
- (3) nitrogen-containing bases

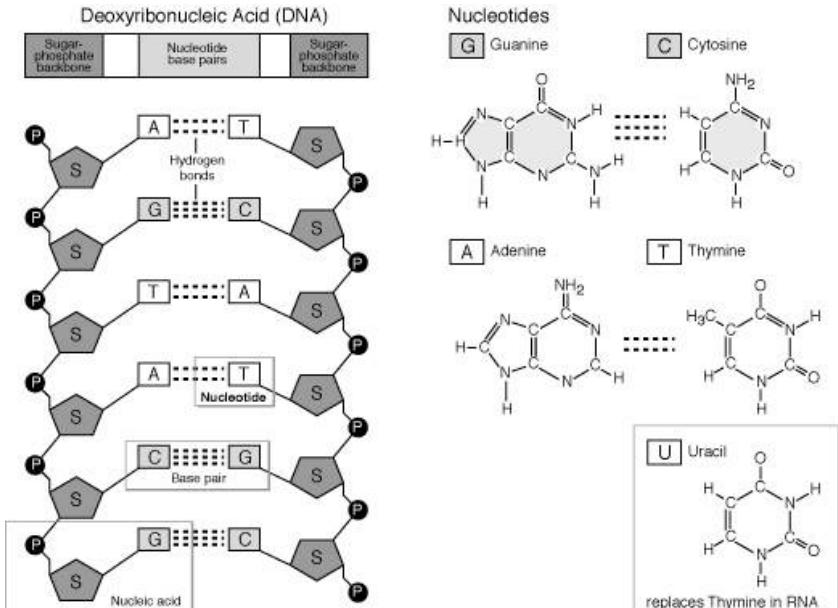
called purines (adenine, A, and guanine, G) and pyrimidines (thymine, T, and cytosine, C; RNA contains uracil, U, instead of T).

From the roughly equal proportions of these components, DNA and RNA molecules are made of repeating units of the three components.

Each unit, consisting of a sugar attached to a phosphate group and a base, is called a nucleotide.



Primary structure of Nucleic Acids



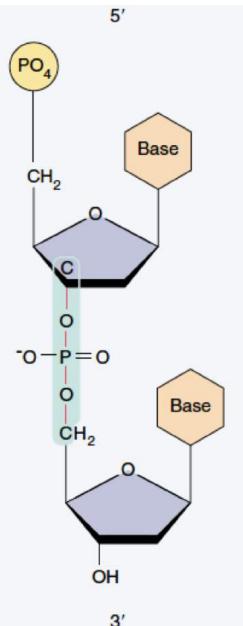
Source: image from the National Human Genome Research Institute (NHGRI)

The reaction between the phosphate group of one nucleotide and the hydroxyl group of another is a dehydration synthesis, eliminating a water molecule and forming a covalent bond that links the two groups.

- The linkage is called a phosphodiester bond because the phosphate group is now linked to the two sugars by means of a pair of ester ($P-O-C$) bonds.

- The two-unit polymer resulting from this reaction still has a free 5' phosphate group at one end and a free 3' hydroxyl group at the other, so it can link to other nucleotides.

- In this way, many thousands of nucleotides can join together in long chains.



Erwin Chargaff showed that nucleotide composition of DNA molecules varied in complex ways, depending on the source of the DNA.

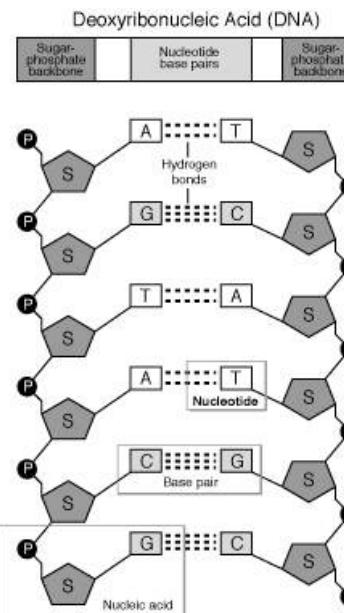
Chargaff's Analysis of DNA Nucleotide Base Compositions

Organism	Base Composition (Mole Percent)			
	A	T	G	C
<i>Escherichia coli</i> (K12)	26.0	23.9	24.9	25.2
<i>Mycobacterium tuberculosis</i>	15.1	14.6	34.9	35.4
Yeast	31.3	32.9	18.7	17.1
Herring	27.8	27.5	22.2	22.6
Rat	28.6	28.4	21.4	21.5
Human	30.9	29.4	19.9	19.8

Chargaff observed an important regularity in double stranded DNA: the amount of adenine present in DNA always equals the amount of thymine, and the amount of guanine always equals the amount of cytosine.

Chargaff's rules:

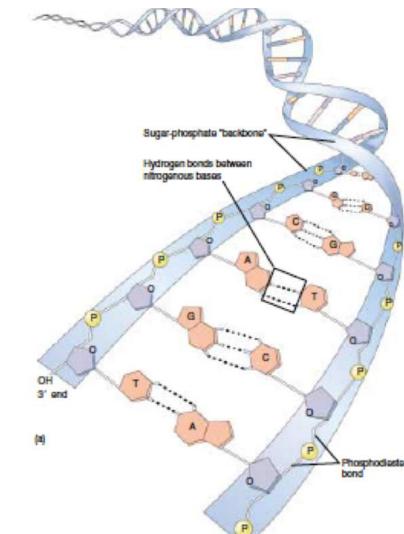
1. The proportion of A always equals that of T, and the proportion of G always equals that of C: i.e $A = T$, and $G = C$.
2. It follows that there is always an equal proportion of purines (A and G) and pyrimidines (C and T).



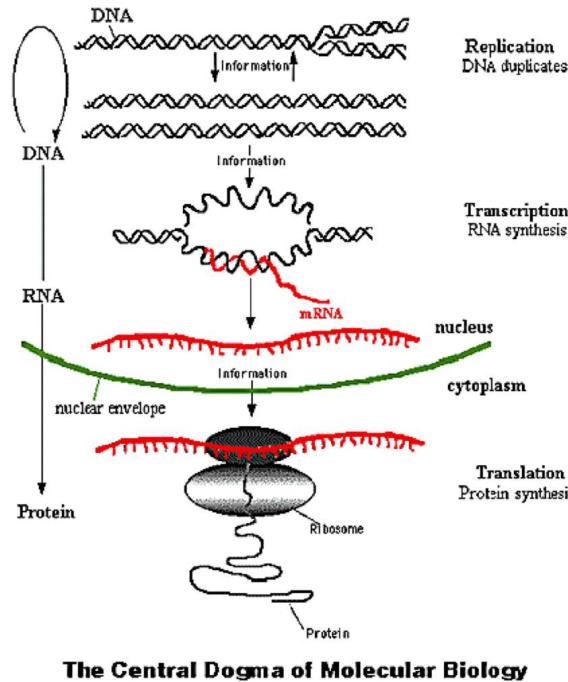
James Watson and Francis Crick, at Cambridge University, worked out structure for the DNA molecule



James Watson and Francis Crick deduced the structure of DNA in 1953 from Chargaff's rules and Franklin's diffraction studies.



DNA is a double helix. In a DNA duplex molecule, only two base-pairs are possible: adenine (A) can pair with thymine(T), and guanine (G) can pair with cytosine (C). An A-T base-pair has two hydrogen bonds, while a G-C base-pair has three.



Essentials of Genetics and outcome of genetic engineering

- Why does a commercial dairy cow produce four times as much milk as most other mammals?
- Why do we look like our cousins?
- Why do roses come in so many different colors?

The answers to these and other questions about the diversity of living things involve processes that occur at the level of genes.

Genetics is concerned with genes that are constructed of DNA, the basic building blocks of life.

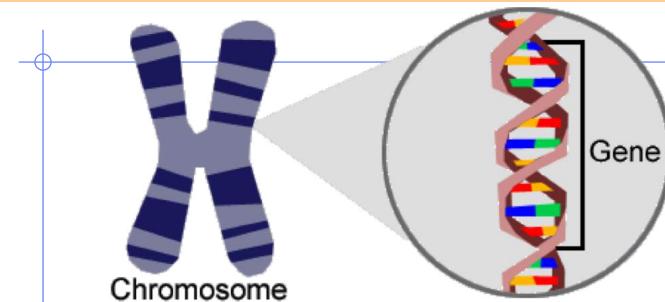
Genetic engineering is concerned with altering the DNA within particular plants or animals in order to create more healthy or altered products.

The code is universal

- Since all living organisms...
 - ◆ use the same DNA
 - ◆ use the same code book
 - ◆ read their genes the same way

				Second base				
				U	C	A	G	
First base (5' end)	U	UUU [Phe]	UCU [Ser]	UAU [Tyr]	UGU [Cys]			
	UUC	UUC [Phe]	UCC [Ser]	UAC [Tyr]	UGC [Cys]			
	UUA	UUA [Leu]	UCA [Ser]	UAA Stop	UGA Stop			
	UUG	UUG [Leu]	UCG [Ser]	UAG Stop	UGG [Trp]			
C	C	CUU [Leu]	CCU [Pro]	CAU [His]	CGU [Arg]			
	CUC	CUC [Leu]	CCC [Pro]	CAC [His]	CGC [Arg]			
	CUA	CUA [Leu]	CCA [Pro]	CAA [Gln]	CGA [Gln]			
	CUG	CUG [Leu]	CCG [Pro]	CAG [Gln]	CGG [Gln]			
A	A	AUU [Ile]	ACU [Thr]	AAU [Asn]	AGU [Ser]			
	AUC	AUC [Ile]	ACC [Thr]	AAC [Asn]	AGC [Ser]			
	AUA	AUA [Met or start]	ACA [Thr]	AAA [Lys]	AGA [Arg]			
	AUG	AUG [Met or start]	ACG [Thr]	AAG [Lys]	AGG [Arg]			
G	G	GUU [Val]	GCU [Ala]	GAU [Asp]	GGU [Gly]			
	GUC	GUC [Val]	GCC [Ala]	GAC [Asp]	GGC [Gly]			
	GUA	GUA [Val]	GCA [Ala]	GAA [Glu]	GGA [Glu]			
	GUG	GUG [Val]	GCG [Ala]	GAG [Glu]	GGG [Glu]			

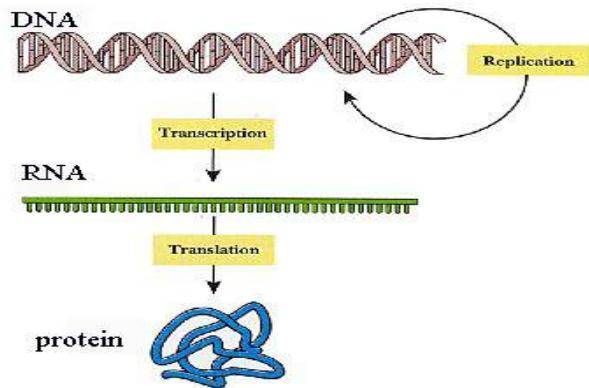
What is a Gene?



A gene is a sequence of DNA on a chromosome that codes for protein.

- The gene is the basic unit of heredity.
- A gene is a stretch of DNA which codes for a specific protein.

Why would altering DNA affect our characteristics/traits?



- DNA codes for the proteins that determine our traits.
- Proteins perform, regulate, or influence all our bodily functions.

What is genetic engineering?

- Genetic engineering is deliberate addition, deletion, or intentional mutation of an organism's DNA sequence to produce a desired result.
- Genetic engineering means manipulation or alteration of DNA sequences.
- Genes, mostly from other, often totally unrelated species are inserted in the genetic "master program".
- Genes from e.g. fish, scorpions, bacteria and viruses have been inserted into food plants in genetic engineering projects.

What are Genes?

- Genes are at the very heart of life.
- Genes constitute the blueprint of an organism.
- They decide all the properties and all the capabilities of an organism.
- We are defined by our genes and how they interact with the environment.
- In computer terms, genes are the master program of life.
- In biological terms this master program is called the hereditary substance, the chromosomes.
- It is constituted by chains of so called DNA molecules that carry the "code words" or instructions of the master program.
- We are just a vehicle for the reproduction of DNA.

Questions and concerns?

- How can we apply our understanding of DNA to manipulate specific genes to produce desired traits?
- How can we use genetic engineering practice to address current problems facing humanity?
- What are moral and ethical problems related to its implementation?

Genetic engineering is so new and astonishing that people are still trying to figure out the pros and cons. (advantages and disadvantages).

Thank you

SBL100-Lecture

Introduction to genetic engineering part II

Send questions to:

Prof. Shilpi Minocha
sminocha@bioschool.iitd.ac.in
011-2654 8433



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Genetic engineering

Genetic

Genes

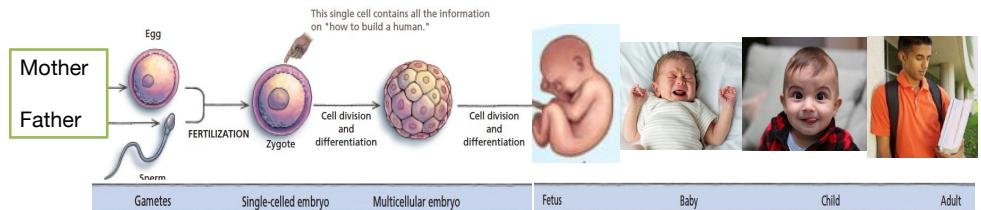
DNA

Genetics

The Inheritance of Traits

- Most of us recognize similarities and resemblances between our birth parents and among members of our family.
- In order to understand how our parents' traits were passed to us among siblings, we need a basic understanding of the human life cycle.

A life cycle is a description of the growth and reproduction of an individual.



- All human beings are produced from the fusion of a single sperm cell produced by the male parent, and a single egg cell produced by the female parent.
- After the egg and sperm, or gametes, fuse at fertilization the resulting single, fertilized cell (the zygote) divides dozens of times to produce daughter cells.

- Each of these daughter cells also divides dozens of times.
- The cells in this resulting mass then differentiate into specialized cell types, which continue to divide and organize to produce the various structures of a developing human.
- We are made up of billions of individual cells, all of them the descendants of that first product of fertilization.
- Each normal sperm and egg contains information about "how to build a human."
- A large portion of that information is in the form of genes — segments of DNA that contain specific pieces of information about the traits of a human.

A Special Case—Identical Twins

Occasionally two children of the same set of parents share 100% of their genes.

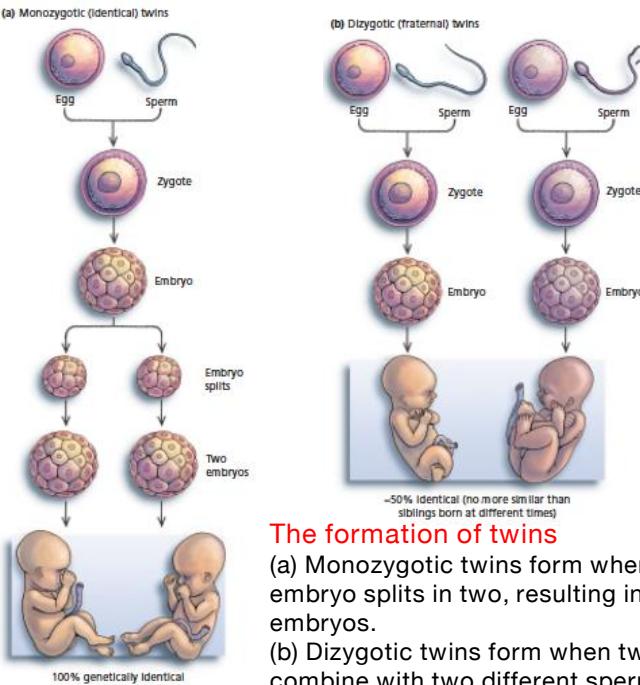
Identical twins are the result of a single fertilization event—the fusion of one egg with one sperm giving rise to two offspring.

After fertilization, the fertilized egg cell grows and divides, producing an embryo made up of many daughter cells containing the same genetic information.

Monozygotic twinning occurs when cells in an embryo separate from each other. If this happens early in development, each cell or clump of cells can develop into a complete individual, yielding twins who carry identical genetic information.

In the United States, approximately one person in 150 is an identical twin.

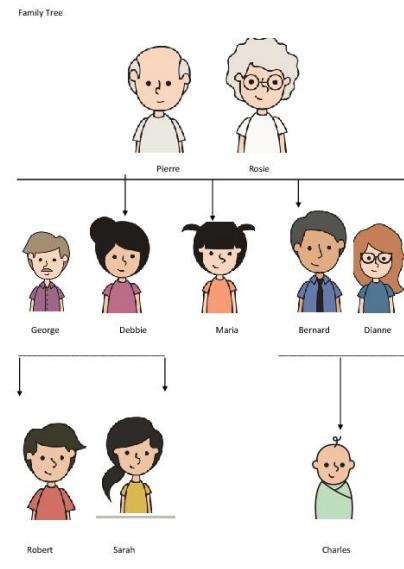
Dizygotic twins occur when two separate eggs fuse with different sperm. The resulting embryos, which develop together, are genetically not similar.



The formation of twins

- (a) Monozygotic twins form when a newly fertilized embryo splits in two, resulting in two identical embryos.
 (b) Dizygotic twins form when two different eggs combine with two different sperm cells, resulting in two different embryos.

Gene inheritance



Donna's English 2017

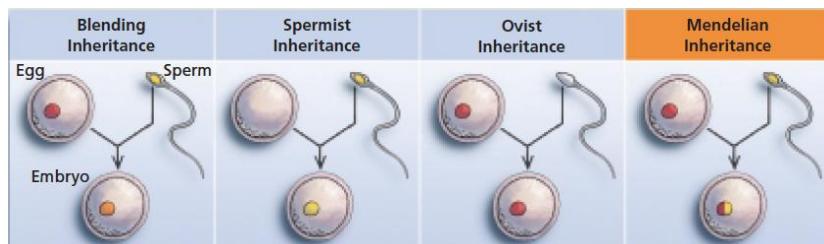
Gene inheritance

Gregor Mendel the first person to accurately describe the inheritance of genetic traits. Mendelian pattern of inheritance.

- Mendel was born in a poor family Austria in 1822.
- Being poor, he entered a monastery to obtain an education.
- Later, Mendel attended the University of Vienna; studied math and botany.
- but was unable to pass the examinations.
- Mendel returned to the monastery and began his experimental studies of inheritance in garden peas.
- Mendel studied close to 30,000 pea plants over a 10-year period



able to control the types of mating that occurred by hand-pollinating the peas' flowers.

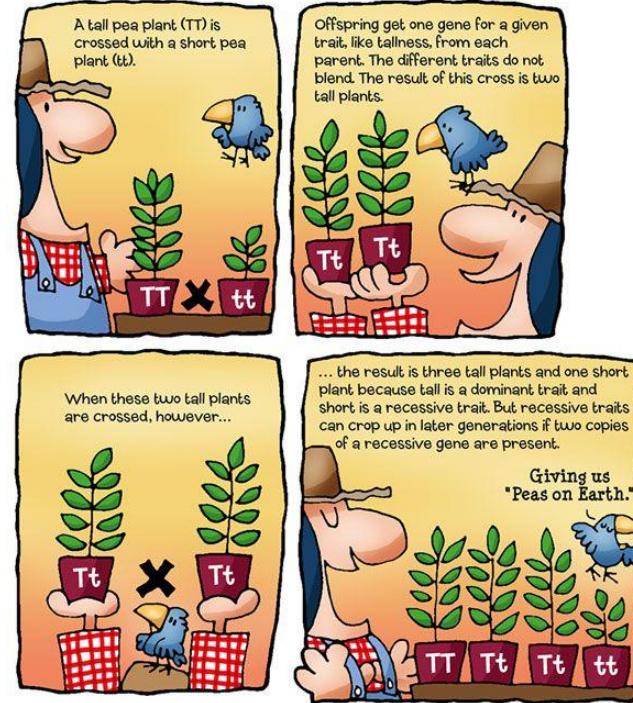


Mendel worked with seven characteristics of pea plants:

plant height, pod shape and color, seed shape and color, and flower position and color.

	Flower Colour	Plant Height	Seed Colour	Seed Shape	Pod Colour	Pod Shape	Flower Position
Dominant Trait							
Recessive Trait							

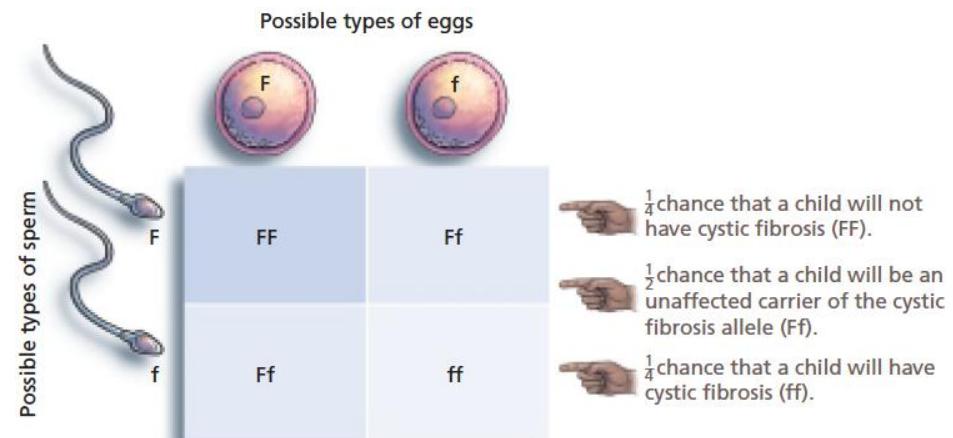
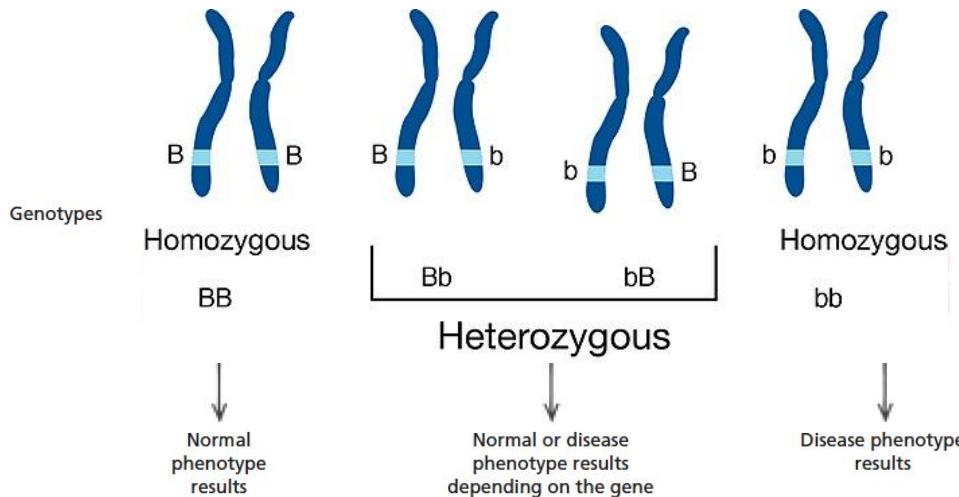
- Mendel's patient, scientifically sound experiments showing that both parents contribute equal amounts of genetic information to their offspring went largely unnoticed.
- Mendel eventually gave up his genetic studies.
- Mendel started his focus on running monastery until his death in 1884.
- His work was independently rediscovered by three scientists in 1900; only then did its significance to the new science of genetics become apparent.
- Mendelian traits identified in humans are the result of genes with mutant alleles that result in some type of disease or dysfunction.
- genetic composition of an individual is called genotype.
- the physical traits of an individual is called phenotype.



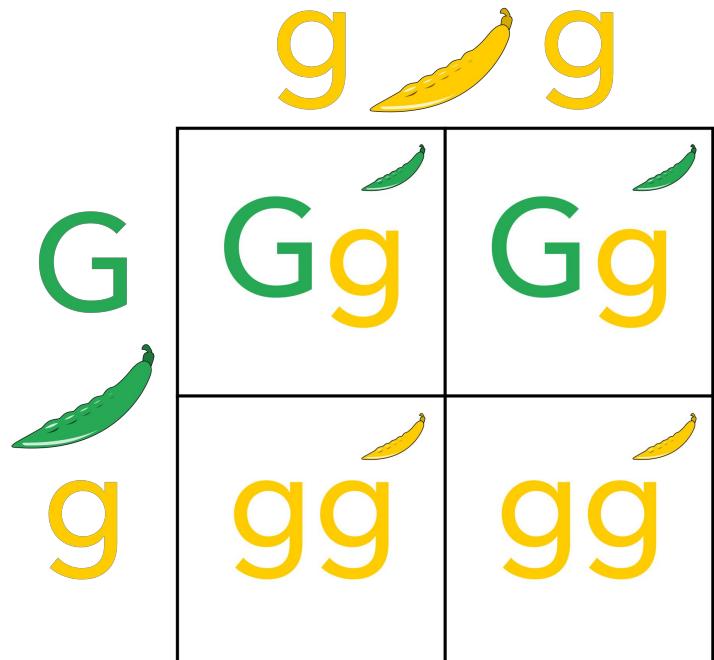
Punnett square is a tool to follow inheritance of the alleles for different types of genes .

A Punnett square is a table that lists the different kinds of sperm or eggs parents can produce relative to the gene or genes in question and then predicts the possible outcomes of a cross , or mating, between these parents.

Suppose letters F and f to represent the functional and dysfunctional allele.



every offspring of two carriers has a chance of being affected



		ABO alleles inherited from the mother		
		A	B	O
ABO alleles inherited from the father	A	A	AB	A
	B	AB	B	B
	O	A	B	O

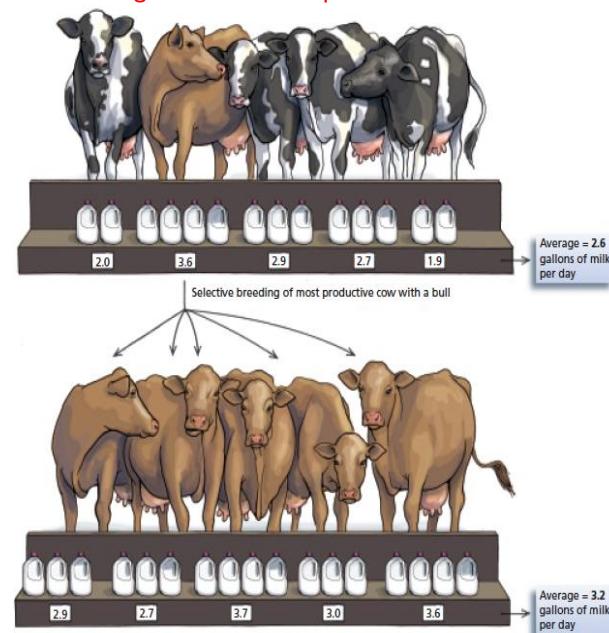
<https://www.ncbi.nlm.nih.gov/books/NBK2267/>

importance of genes in determining the value of quantitative traits.

e.g. farmers who wish to increase their dairy herd's milk production

- (a) Change the herd's environment by changing the way the cows are reared, housed, and fed; or
- (b) change the herd genetically by choosing only the offspring of the best milk producers for the next-generation herd.

The technique of controlling the reproduction of individual organisms to influence the phenotype of the next generation is known as artificial selection.



physical Analogy consider a pair of shoes and pair of chromosomes because the two shoes are similar in size, shape, and style, but are not exactly similar.

If 23 students are asked to take off their shoes and place them in a row across the front of the classroom, and they arrange their shoes so that the left shoe is on the left, and the right shoe is on the right, the students could then separate all of the left shoes from the right shoes, just as meiosis separates homologous chromosomes.

The students could continue making different combinations of left and right shoes for a very long time, because there are 2^{23} (over 8 million) possible ways to line up these pairs of shoes.

Same is true with chromosomes

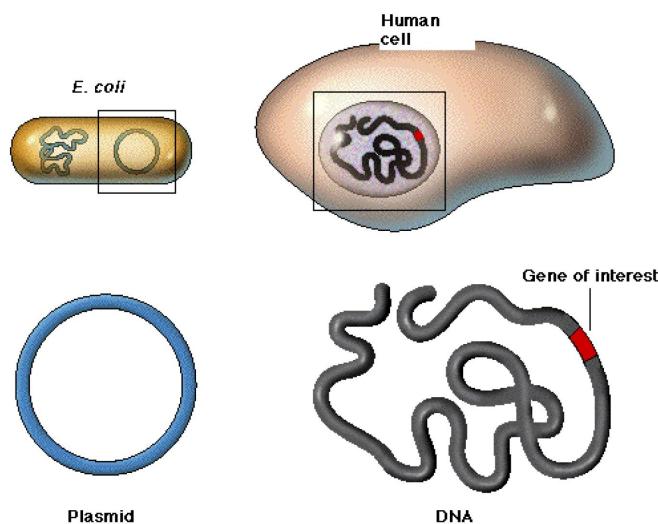
2^{23} (over 8 million) genetically different gametes are possible.

$$1 \times 8 \text{ million} \times 1 \times 8 \text{ million} = 64 \text{ trillion}$$

Together our parents could have made over 64 trillion genetically different children, and we are only one of the possibilities.



How genetic modification can be used?



How genetic modification is used to produce insulin in bacteria?

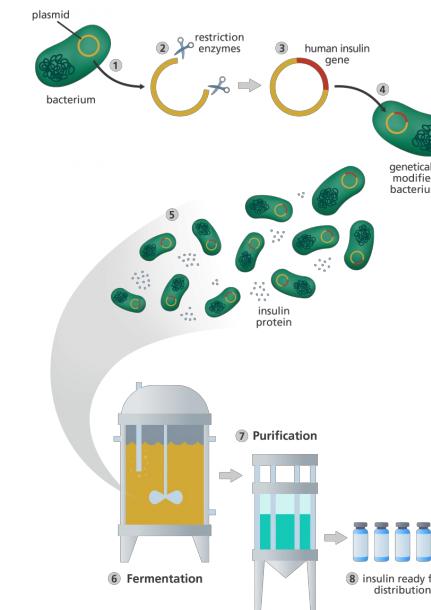
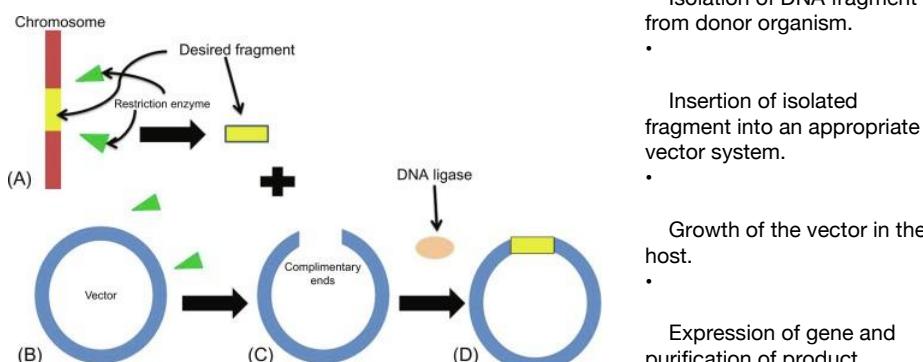


Image credit: Genome Research Limited



from Enosh Phillips, New and Future Developments in Microbial Biotechnology and Bioengineering, 2019

Generalized view of the steps involved in genetic engineering.

- (A) Using the specific restriction enzyme (RE) to cut the desired fragment containing the gene of interest from the chromosome.
- (B) Taking an appropriate vector by cutting the same RE to create complementary ends.
- (C) Mixing the desired gene (DG) fragment and RE-digested vector with ligase enzyme.
- (D) Joining ligase with the DG and the vector, which is then multiplied and expressed in the appropriate host.

References:

- An Introduction to Genetic Engineering: 2008, Dr Desmond S. T. Nicholl Cambridge University Press.
- Genetic Engineering, Edited by Idah Sithole-Niang, InTech, 2013.
- Molecular Cell Biology: Harvey Lodis et al; 7th Edition, 2013, Macmillan
- Molecular Biology of the Gene: 5th Edition, James D. Watson et al; 2004; Pearson Benjamin Cumming

SBL100-Lecture

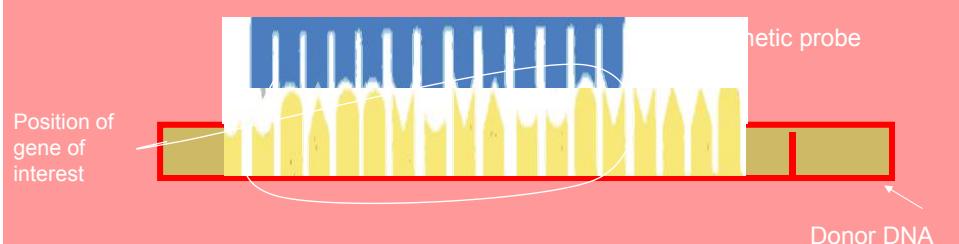
Introduction to genetic engineering Part III



1. Isolation

(a) **Isolation** of a specific gene from donor e.g. human

- Cells broken open
- Genetic probe added
- Reveals position of the gene of interest



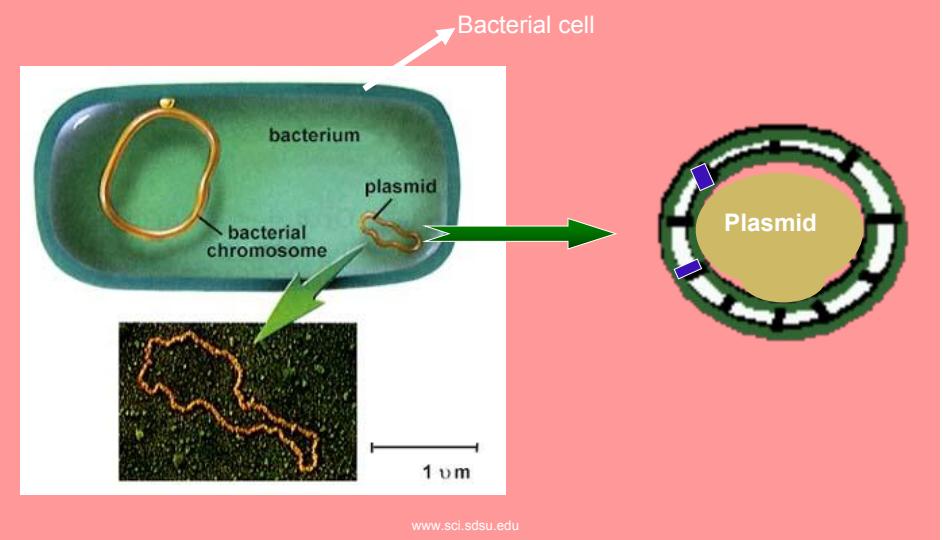
"genetic engineering" is a techniques in which DNA may be cut, rejoined, its sequence determined, or the sequence of a segment altered to suit an intended use.

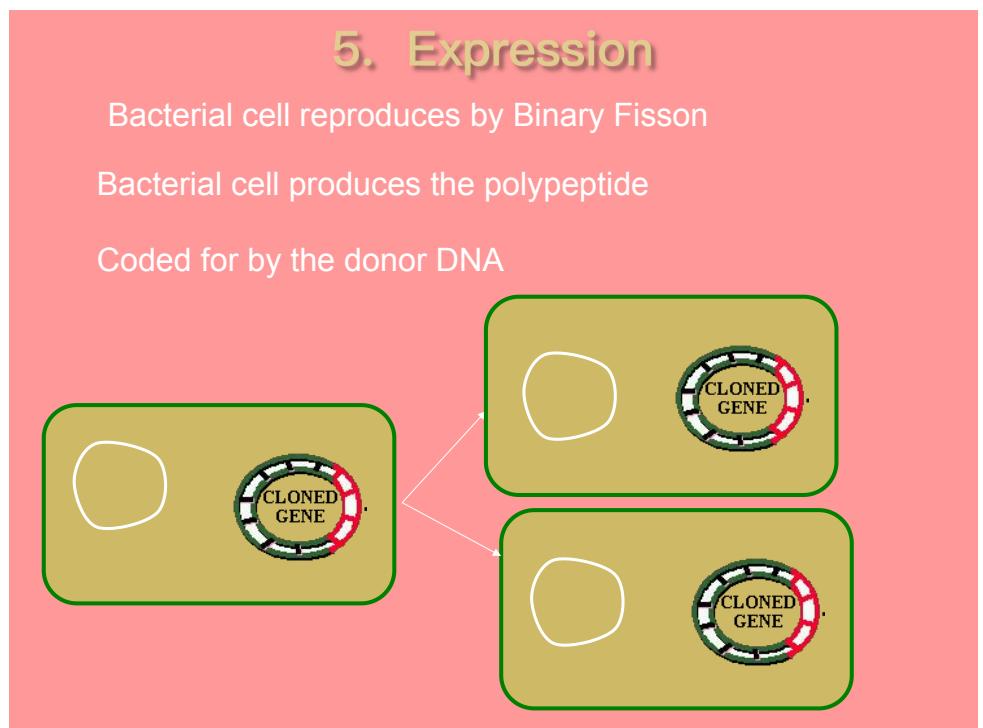
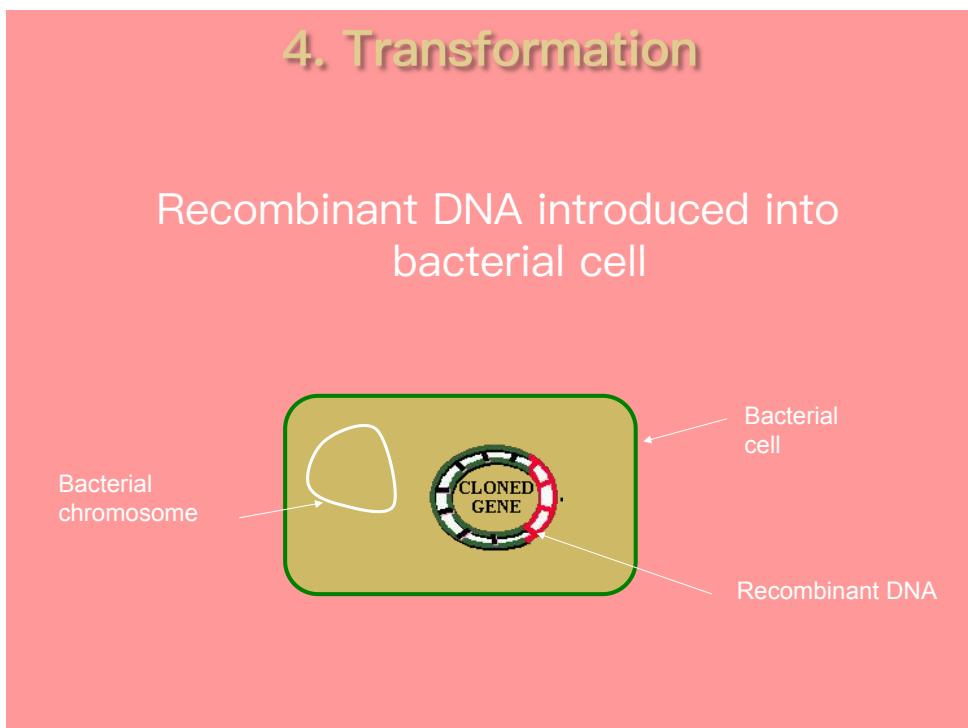
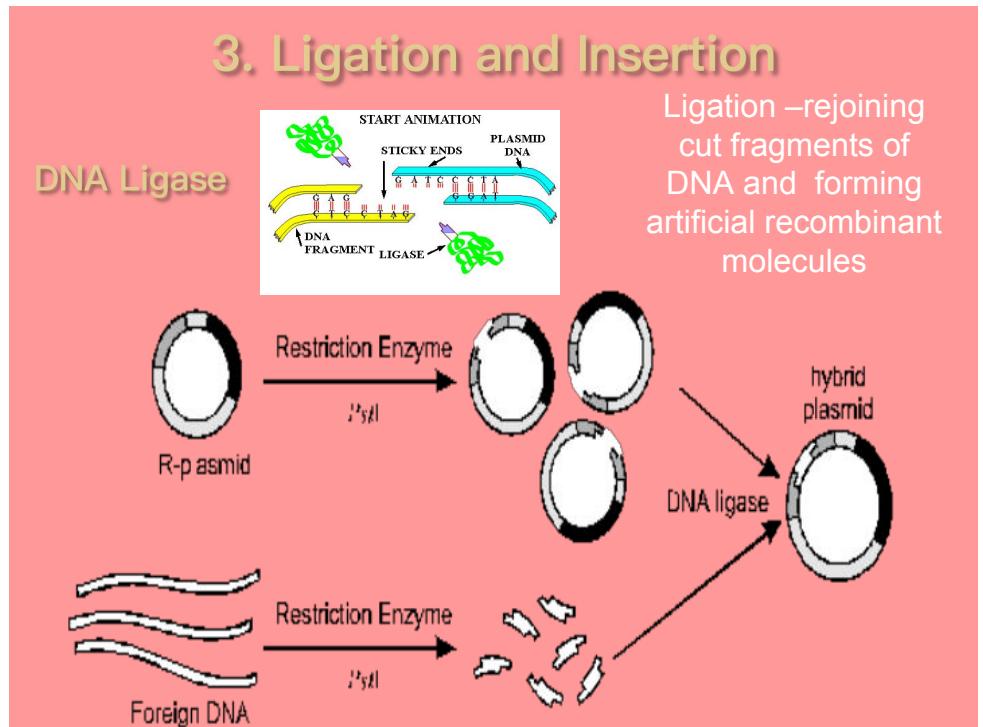
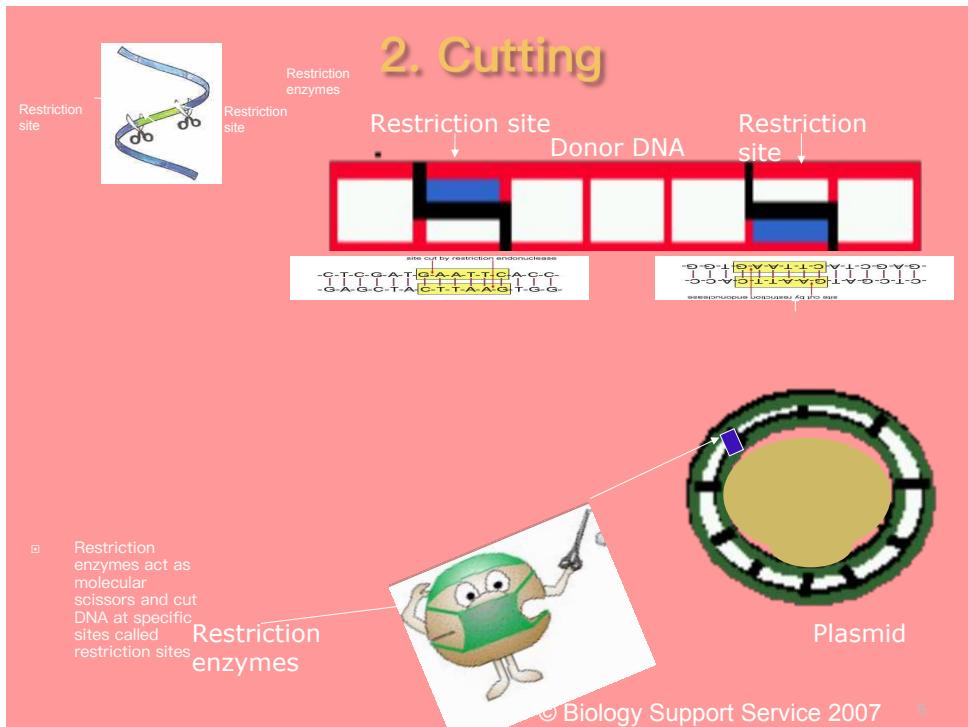
5 Stages involved in GE

1. Isolation
2. Cutting
3. Ligation and Insertion
4. Transformation
5. Expression

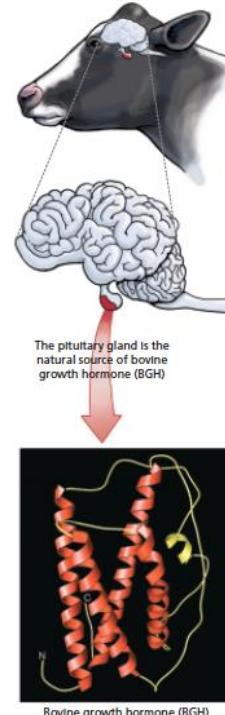
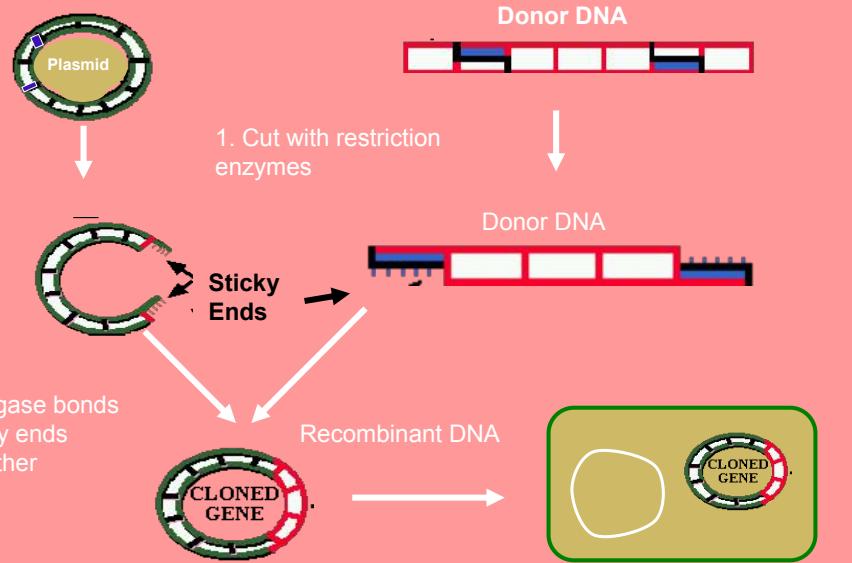
1. Isolation

(b) **Isolation** of plasmid from a bacterial cell





Summary of steps



Approaches of genetic engineering

During 1980s, genetic engineers at the Monsanto Corporation began to produce recombinant bovine growth hormone (rBGH) by manipulating the DNA sequence (gene) that carries the instructions for, or encodes, the growth hormone protein.

Growth hormone acts on many different organs to increase the overall size of the body.

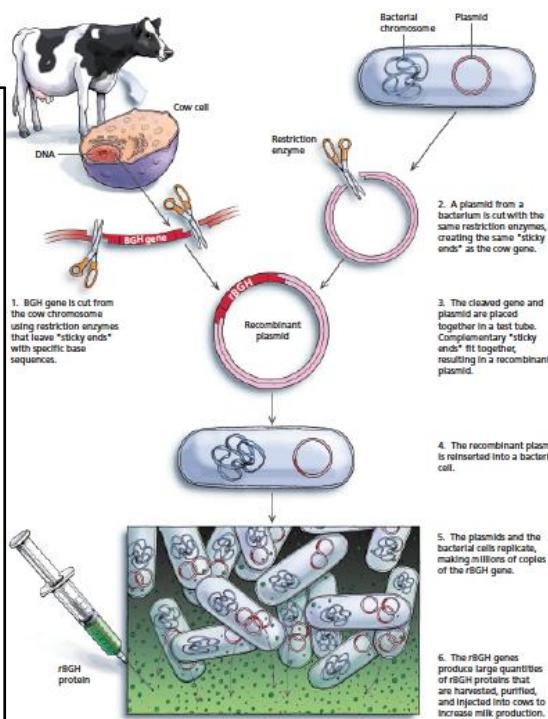
Before the advent of genetic technologies, growth hormone was procured from the pituitary glands of slaughtered cows and then injected into live cows.

However, harvesting the growth hormone from the pituitary glands of cows and humans is laborious, and many cadavers are necessary to obtain small amounts of the protein.

Genetic engineers at Monsanto realized that they could produce large quantities of bovine growth hormone in the laboratory, inject dairy cows, and increase milk production.

A case study: How engineers produced

- The gene is sliced from cow chromosome on which it resides by exposing the cow DNA.
- Once the gene is removed from the cow genome it is inserted into a bacterial structure called a plasmid .
- A plasmid is a circular piece of DNA that can replicate itself.
- When the cut plasmid and the cut gene are placed together in a test tube they reform into a circular plasmid with the extra gene incorporated.
- The bacterial plasmid has now genetically engineered to carry a cow gene.
- rBGH is called a recombinant gene because removed from its original location cow genome and

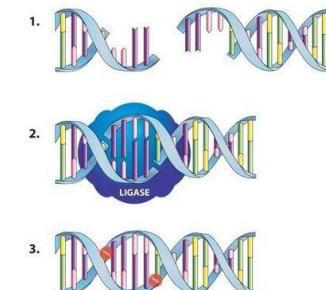


restriction enzymes are highly specific molecular scissors. cut DNA at specific sequences:

TAT C G T A C G AAC
ATA G C A T G C TTG

TAT C
ATA G
Sticky end

GT A C G A A C
CTT G
Sticky end

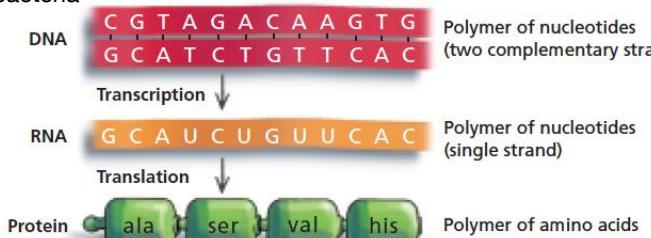


TAT C G T A C G BGH gene C G T A C G A A C
ATA G C A T G C BGH gene G C A T G C TTG

The first step in the production of the rBGH protein is to transfer the BGH gene from the nucleus of a cow cell into a bacterial cell.

Bacteria with the BGH gene will then serve as factories to produce millions of copies of this gene and its protein product—making many copies of a gene is called cloning the gene.

Once inside the cell, plasmids replicate, themselves, as does the bacterial cell, making thousands of copies of rBGH gene. Using this procedure, scientists grow large amounts of bacteria



The cloned BGH gene into bacterial cells, now produce the protein encoded by the gene.

The process of protein synthesis is also referred to as gene expression, since proteins are synthesized when the genes that encode them are turned on.



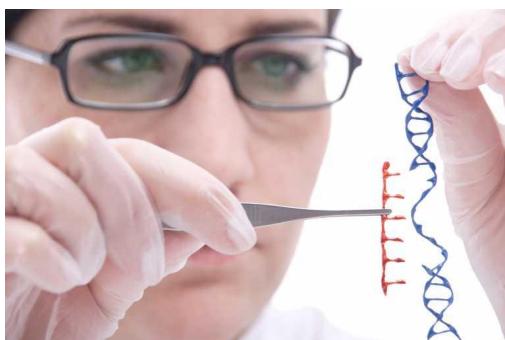
The scientists were then able to break open the bacterial cells, isolate the BGH protein, and inject it into cows.

Close to one-third of all dairy cows in the USA now undergo daily injections with rBGH, produced 20-30% more milk.

10.04.23

SBL100-Lecture

Introduction to genetic engineering Part IV



FDA Regulations

FDA a governmental organization which ensures the safety of all domestic and imported foods and food ingredients.

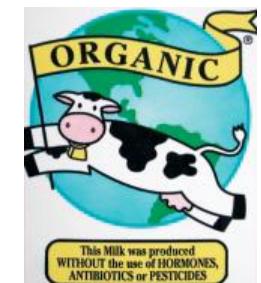
The manufacturer of any new food that is not Generally Recognized As Safe (GRAS) must obtain FDA approval before marketing its product.

Adding substances to foods also requires FDA approval.

According to both the FDA and Monsanto, there is no detectable difference between milk from treated and untreated cows and no way to distinguish between the two.

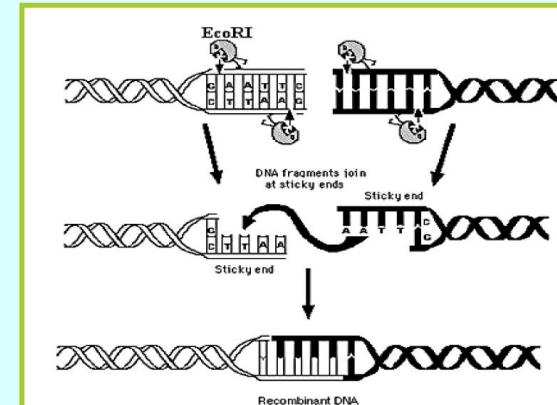
milk from r BGH treated cows was deemed safe for human consumption by FDA in 1993.

distributors of milk from untreated cows label their milk as "hormone free"



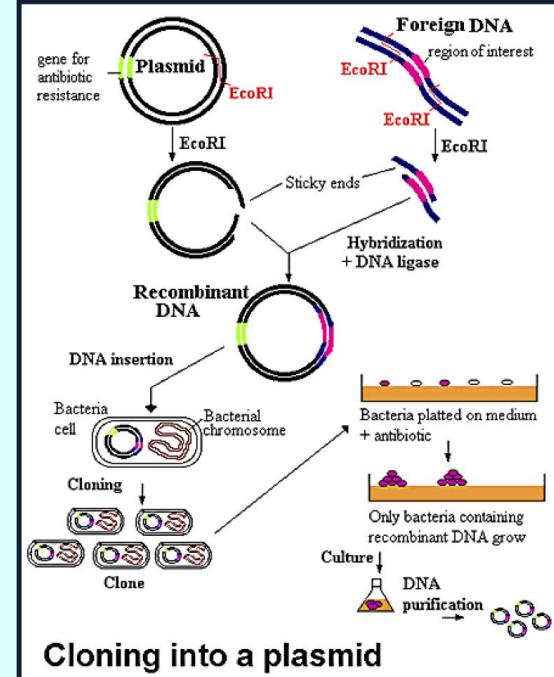
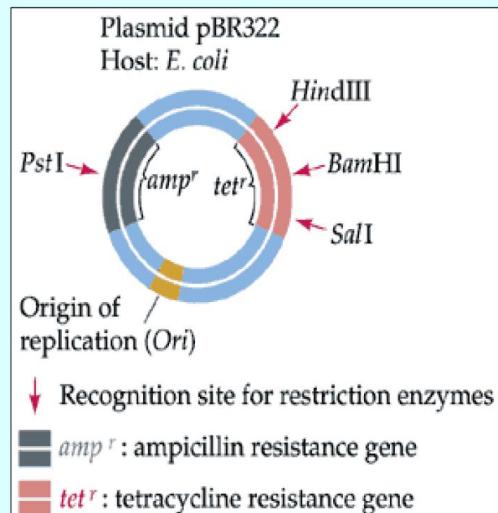
In humans, studies indicate milk from cows treated with rBGH may contain elevated levels of insulin-like growth factor-1 (IGF-1), which can increase the risk of breast cancer, colon cancer and other types of cancer.

Restriction Enzyme



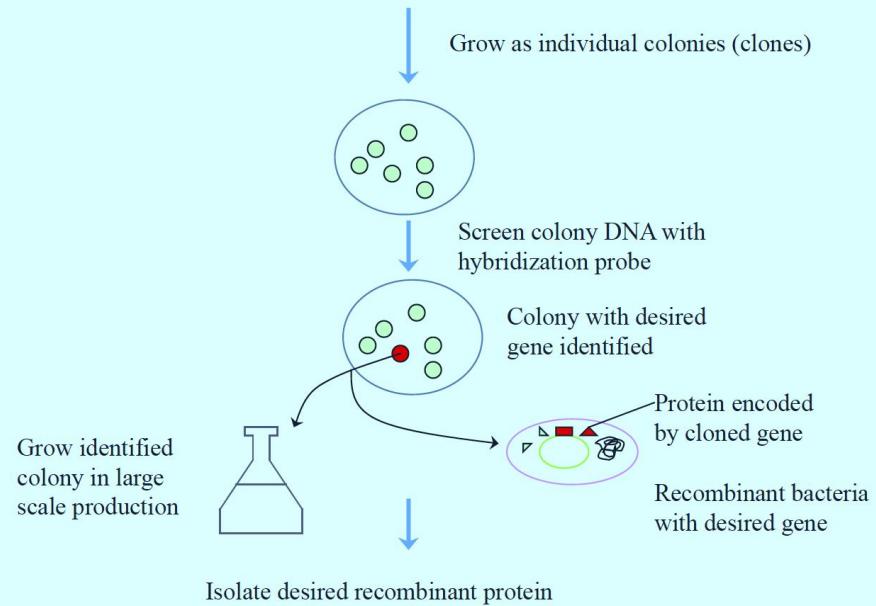
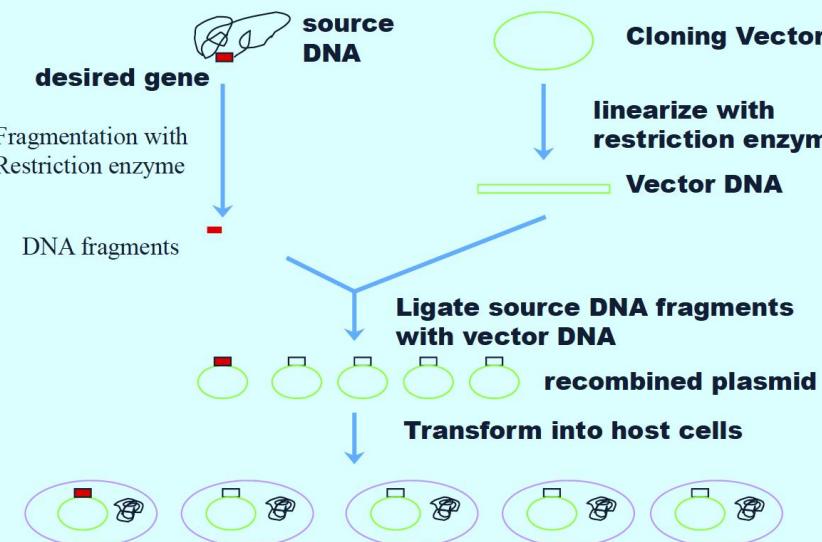
Restriction Enzyme Action of EcoRI

Vector - Plasmid



Clone = A group of cells that contain the same recombinant DNA molecule

Overall Cloning Process



Production of Recombinant Proteins and Peptides

Generic Name	Brand Name	Therapeutic Use
Human insulin	Humulin (Lilly) Novolin (Novo Nordisk)	Insulin dependent diabetes
Human growth	Protopin (Genentech) Humatropin (Lilly) Nutropin (Genentech)	Growth hormone deficiency in children; growth retardation in chronic renal disease
Hepatitis B vaccine	Engerix-B (SmithKline Beecham)	Hepatitis B prevention
Interferon alfa-2a	Recombivax HB (MSD) Roferon-A (Hoffman)	Hairy cell leukemia; AIDS related Kaposi's sacroma

Production of Recombinant Proteins and Peptides

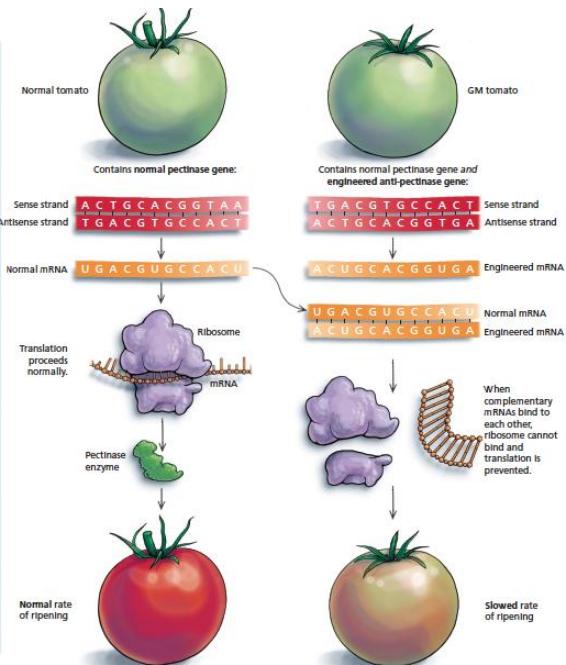
Generic Name	Brand Name	Therapeutic Use
Muromonab-CD3	Orthoclone OKT 3 (Ortho)	Acute allograft rejection in renal and cardiac transplant patients
Epoetin alfa	Epogen (Amgen)	Anemias of chronic renal disease, AIDS and cancer chemotherapy
Interferon beta-1b	Betaseron (Bertex)	Multiple sclerosis
Dornase alfa	Pulmozyme (Genetech)	Cystic fibrosis
Imiglucerase	Cerezyme (Genzyme)	Type 1 gaucher's disease

Genetic Engineers Can Modify Foods

Whether you realize it or not, you have been eating genetically modified foods now.

- Improving yield of crop plants is the driving force behind majority of genetic engineering.
- Yield can be increased when plants are engineered to be resistant to pesticides and herbicides, drought, and freezing.
- People believe that improving farmers' yields may help decrease world hunger problems.
- Crop plants are genetically modified to increase their shelf life, yield, and nutritive value.
- The first genetically engineered fresh produce was tomatoes, in store in 1994.
- These tomatoes were engineered to soften and ripen more slowly.
- It taste better and increases shelflife in grocery without overripe and mushy.
- An enzyme called pectinase mediates the ripening process in tomatoes.
- This enzyme breaks down pectin, a naturally occurring substance found in plant cells.

- In tomatoes, engineers insert a gene that produces an mRNA transcript complementary to the mRNA produced by transcription of a pectinase gene.
- In dsDNA, the strand that codes for a protein is called the sense, and its complement is called the antisense.
- When antisense version of the pectinase gene is transcribed, it produces an mRNA that is complementary to the mRNA from the normally transcribed pectinase gene.
- When GE antisense gene base pairs with its naturally occurring pectinase complement, ripening is slowed.
- Thus, less of the pectinase enzyme is produced and ripening occurs more slowly.



due to uncontrolled population increase, it will demand an increase in yield of crop plants in order to feed all the world's people.

- Genetic engineers are able to increase the nutritive value of crops.
- Engineers have increased amount of β -carotene in rice, a staple food for world's people.
- This Golden rice will help decrease blindness because cells require β -carotene in order to synthesize vitamin A, required for vision.

When a gene from one organism is incorporated into the genome of another organism, a transgenic organism is produced.

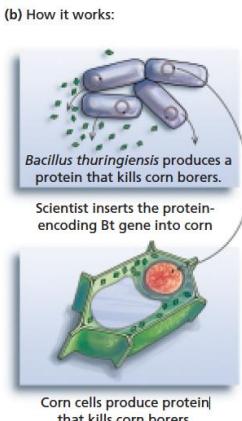
A transgenic organism is commonly referred to as a genetically modified organism or GMO.



Golden Rice has been genetically engineered to produce more β -carotene

To remove farmer's reliance on pesticides, agribusiness companies have engineered plants that are genetically resistant to pests.

For example, corn plants have been engineered to kill the corn borer.



Scientists transferred a gene from the soil bacterium *Bacillus thuringiensis* (Bt) into corn.

The Bt gene encodes proteins that are lethal to corn borers but not to humans. Close to one-half of all corn currently grown in the United States is engineered with this gene.

How Are Crops Genetically Modified?

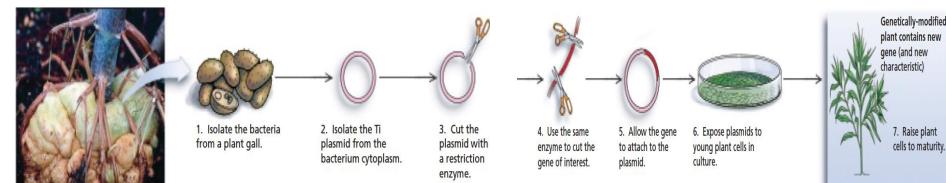
To modify crop plants, the gene must be able to gain access to the plant cell, which means it must be able to move through the plant's rigid outer cell wall.

In nature, *Agrobacterium tumefaciens* bacterium infects plants and causes tumors called galls. The tumors are induced by a plasmid, called Ti plasmid (for Tumor inducing).

Moving genes into other agricultural crops such as corn, barley, and rice are done by using a device called a gene gun.

A gene gun shoots tungsten-coated pellets covered with foreign DNA into plant cells.

A small percentage of these DNA genes may be incorporated into the plant's genome.



Some examples of Genetic engineering

Flavr Savr Tomato



Normally, tomatoes are picked while green and transported many miles before being sprayed with ethylene to ripen them.

This prevents damage and perishing on



The Flavr Savr tomato is a genetically engineered tomato which has a gene inserted to extend shelf-life by slowing down the rotting process.

The Flavr Savr tomato was the first GM fruit to be sold in the World.

Is it better to spray tomatoes with ethylene than genetically engineer them?

Some examples of Genetic engineering

Genetically engineered rice which contains a gene from carrots (or other vegetable) which causes the rice to contain the building blocks for vitamin A production in the body.



Some people think that GM crops like this one promote the use of GM foods to people that are not in the position to say no.

Golden Rice



Vitamin A deficiency causes blindness and death.

125 million children suffer from vitamin A deficiency. Most of these children live in developing countries where rice is the staple food.

Too much vitamin A causes other health problems.

Some examples of Genetic engineering

Originally created in an attempt to show levels of pollution in rivers.

Native to India and Bangladesh. None have survived in American rivers.



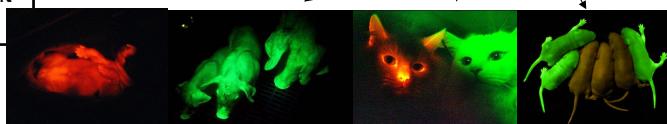
Zebra fish with a gene inserted from jellyfish or coral to make them fluoresce.



The first genetically engineered organism to be sold as a pet.

How does this fish benefit us?

They can reproduce, but it is illegal to do so!



Other fluorescent organisms

Some examples of Genetic engineering



Goats which produce spider silk in their milk!

The gene transferred from a spider causes the goats to produce an extra protein in their milk which can be extracted and spun into spider silk thread.



Spiders cannot be farmed as they are cannibalistic – they eat each other!

Superspuds

Could the Protato face the same opposition as Amflora?

Amflora

Potato created for the starch industry.

Used antibiotic resistance marker gene.

Fear that the genes could escape into the environment.

It was proposed that the waste potato was fed to livestock.

This caused outrage from some European countries, why?



Spider-Goat

Spider silk is stronger than steel, lightweight, and very elastic.

It holds its strength between -40°C and 220°C.

Spider silk could be used to manufacture;

- replacement ligaments
- wound covering (it has antiseptic properties and vitamin K which helps with blood clotting!)
- optical communications
- bullet proof clothing
- waterproof clothing!!

About 75% of spider goats are euthanised as there are strict controls meaning that they cannot leave the facility where they are created.

Why create a life to destroy it?

Some examples of Genetic engineering

AquAdvantage Salmon

This fish has not been consumed by humans yet – is it safe?

What affect could the AquAdvantage salmon have on wild salmon if it escaped?



A gene which controls the growth hormone from one breed of salmon is inserted into the DNA of another. This causes it to grow much quicker than 'normal' salmon.

Could this gene be transferred to humans if we eat it? What could happen if this occurred?

AquAdvantage salmon
Normal salmon

Some examples of Genetic engineering

Banana Vaccine

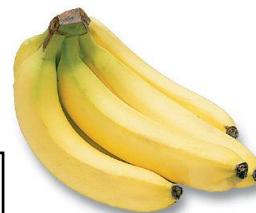
Bananas, carrots, potatoes and lettuce have all been genetically engineering to deliver vaccines for diseases.

The banana has been the most successful in testing.

When the gene has been inserted into the banana, its cells produce virus proteins (not the infectious part).

When you eat the banana you ingest these proteins and the body produces antibodies against them – this is exactly what happens with a vaccine.

Is there a risk of people taking the vaccine without realising they are doing so?



The virus' genes are transferred to the banana cells and become a permanent part of that banana's genetic code.



Some examples of Genetic engineering

Venomous cabbage

Cabbage which has been genetically engineered to include the gene for Scorpion venom.

This reduces the use of chemical pesticides sprayed on crops.

The venom is poisonous to caterpillars – it acts as a pesticide.

The toxin has been altered - it does not kill human cells



What if the toxin mutates and alters again?

What affect will the toxin have on the biodiversity of the area?



- A Blue Rose is a genetically modified Rose.



- Transgenic fruit obtained from pear and apple.

Genetic Engineered papaya, SunUp! That is resistant to the Papaya ringspot virus (PRSV)



Homegrown papaya that is affected by the Papaya ringspot virus (PRSV)





Using Modern DNA and cross fertilization techniques; the Dolion a cross between a lion and a dog.



- Dolly the sheep is the world's most famous clone.
- Dolly was born 5 July 1996 to three mothers (one provided the egg, another the DNA and a third carried the cloned embryo to term).

Genetic Engineers Can Modify Humans

The genetic modifications may one day include replacing defective or nonfunctional alleles of a gene with a functional copy of the gene.

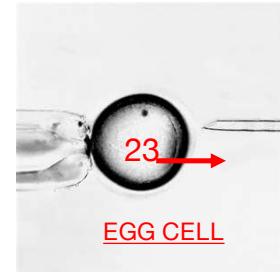
If this happens, it might be possible for physicians to diagnose genetic defects in early embryos and fix them, allowing the embryo to develop into a disease-free adult.

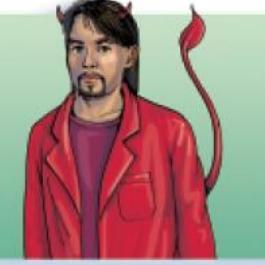
- Step 4: The egg is then charged with electricity to start mitosis.
- Step 5: Its then put into a surrogate mother so it can grow.
- Its going to be genetically identical to the parent of the body cell.
- But it will be a baby.
- Any Plants and animals can be cloned.



How could you clone a human?

- Step 1: An egg is removed from a female human
- Eggs are haploid: 23 chromosomes.
- The nucleus of the egg is removed and is thrown away.
- Step 2: A body cell is removed from another person.
- The nucleus of the body cell is removed
- Body cells are diploid: 46 chromosomes.
- Step 3:
- The nucleus of the diploid body cell is put into the egg.
- This egg no longer needs to be fertilized since it has all 46 chromosomes.



 Some reasons why the work of genetic engineers is important	 Some reasons why the work of genetic engineers is controversial
<ul style="list-style-type: none">GM animals and crops may make farms more productive.GM crops may be made to taste better, last longer, or contain more nutrients.Genetic engineers hope to cure diseases and save lives.	<ul style="list-style-type: none">GM crops encourage agribusiness, which may close down some small farms.GM animals and crops may cause health problems in consumers.GM crops might have unexpected adverse effects on the environment.Present research might lead to the unethical genetic modification of humans.

Are genetic engineers doing more good than harm? This chart lists some of the pros and cons of genetic engineering

Ethical issues raised by genomics (ELSI) (Ethical legal, societal implications)

- Individual's genome holds key to disease susceptibility
- Potential for misuse recognized by founders of Human Genome Project



Genetic modification of humans

- Once we know the genes responsible for particular diseases, should we "cure" the diseases?
- Should we also modify genes responsible for traits such as height or beauty?
- Should we allow the cloning of human beings?



Protesters at a World Trade Organization meeting in Seattle. These people are concerned about how GMOs may affect humans and the

SBL100-Lecture

Introduction to Cancer

Part I

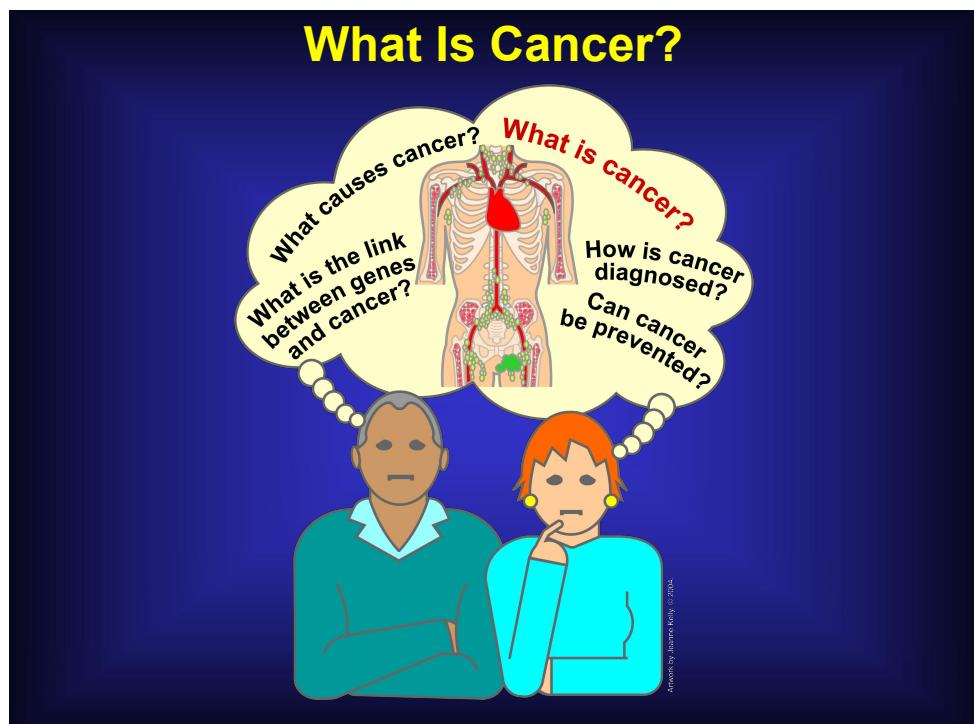
References:

- Lehninger g Principles of Biochemistry. 5th Edition. 2008. David L. Nelson and Michael M.Cox.
- Molecular Cell Biology. 5th Edition. 2004. Lodish, Berk, Matsudaira, Kaiser, Krieger, Scott, Zipursky and Darnell.
- Biology: Science for Life, 4/E Colleen M. Belk, University of Minnesota, Virginia Borden Maier, ISBN-10: 0321767829 • ISBN-13: 9780321767820 ©2013
- An Introduction to Genetic Engineering, Third edition,2008, By Desmond S. T. Nicholl
- Biology, by Raven and Johnson, sixth edition 2002
- Nickell, J. and Fischer, J.F. (1999). Crime Science: Methods of Forensic Detection. Kentucky: The University Press of Kentucky.
- Riciu , A. (2011, November 26). Pituitary Dwarfism Causes, Symptoms, Diagnosis and Treatment. Retrieved from <http://www.doctortipster.com/6928-pituitary-dwarfism-causes-symptoms-diagnosis-and-treatment.html>
- Acknowledgement:
- Internet sources, teaching slides on slideshare, scitable and others ppts from Prof Ashok and Prof CS Dey

Today:
We learnt about genetic engineering implications

Home assignment:
Advantages and disadvantages of genetic engineering

Next class:
Cancer biology



Cancer

- Cancer is one of the most common diseases in the world after heart disease.
- 1 in 4 deaths are due to cancer
- **Lung cancer** is the most common cancer in men
- **Lung and Breast cancer** is common in women
- There are over 100 different forms of cancer
- Every year 18 million people are diagnosed.
- More than 22 million peoples are living with cancer
- An estimated 9.6 million deaths in 2018.
- The most common cancers are:
- Lung (2.09 million cases)
- Breast (2.09 million cases)
- Colorectal (1.80 million cases)
- Prostate (1.28 million cases)
- Skin cancer (non-melanoma) (1.04 million cases)
- Stomach (1.03 million cases)
- \$200 billion: The overall costs of cancer, according to the National Institutes of Health (NIH).

<http://www.who.int/mediacentre/factsheets/fs297/en/>

WHO-2018

	Incident cases	
Lung	1 368 524	14.5%
Prostate	1 276 106	13.5%
Colorectum	1 026 215	10.9%
Stomach	683 754	7.2%
Liver	596 574	6.3%
Bladder	424 082	4.5%
Oesophagus	399 699	4.2%
Non-Hodgkin lymphoma	284 713	3%
Kidney	254 507	2.7%
Leukaemia	249 454	2.6%
Other cancers	2 892 790	30.6%
Total Cases	9 456 418	100%

Male

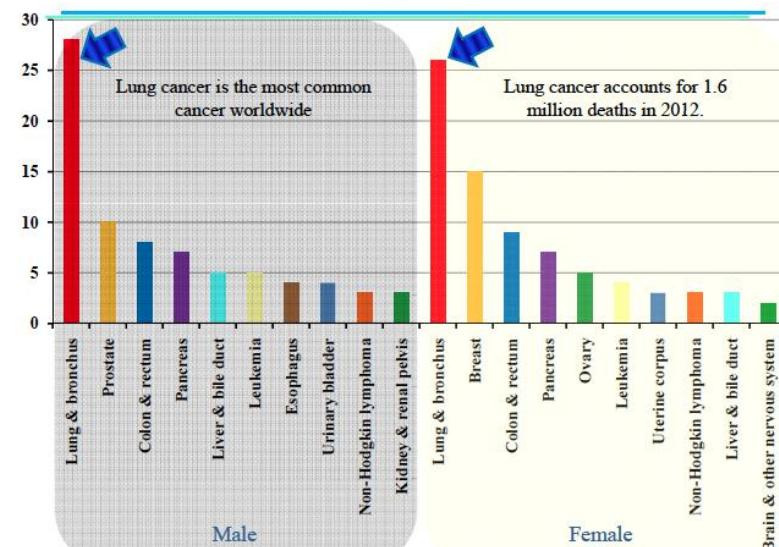


	Incident cases	
Breast	2 088 849	24.2%
Colorectum	823 303	9.5%
Lung	725 352	8.4%
Cervix uteri	569 847	6.6%
Thyroid	436 344	5.1%
Corpus uteri	382 069	4.4%
Stomach	349 947	4.1%
Ovary	295 414	3.4%
Liver	244 506	2.8%
Non-Hodgkin lymphoma	224 877	2.6%
Other cancers	2 482 031	28.8%
Total Cases	8 622 539	100%

Female



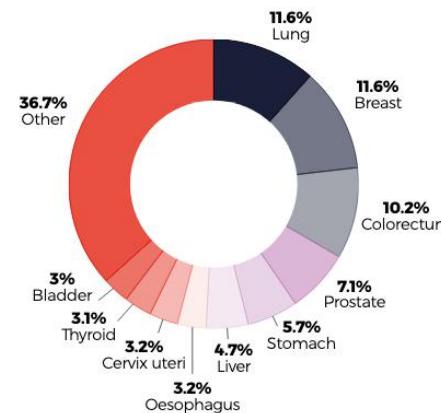
Most dangerous Cancer



<http://www.cancer.org/acs/groups/content/@research/documents/webcontent/acspc-042151.pdf>

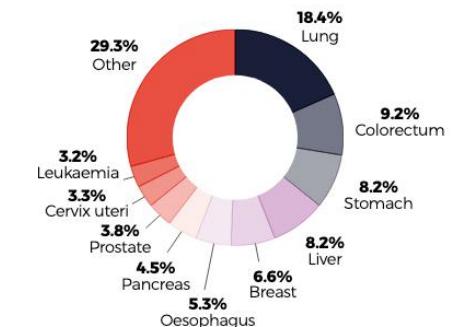
Fig. 1.2. Distribution of cases and deaths by the leading 10 cancer types in 2018 for both sexes.

Incidence



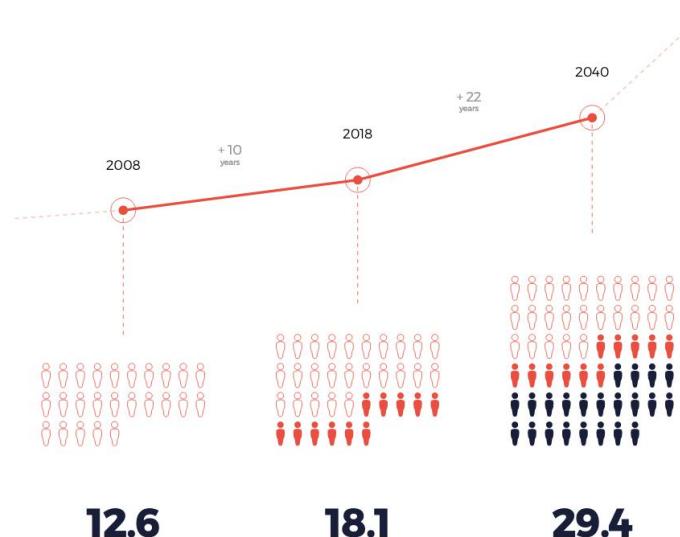
18.1 million cases

Mortality



9.6 million deaths

Fig. 1.6. Estimated global burden of cancer in 2018 and that in 2040 according to United Nations population projections.



Cancer Therapy Vol 8, page 58

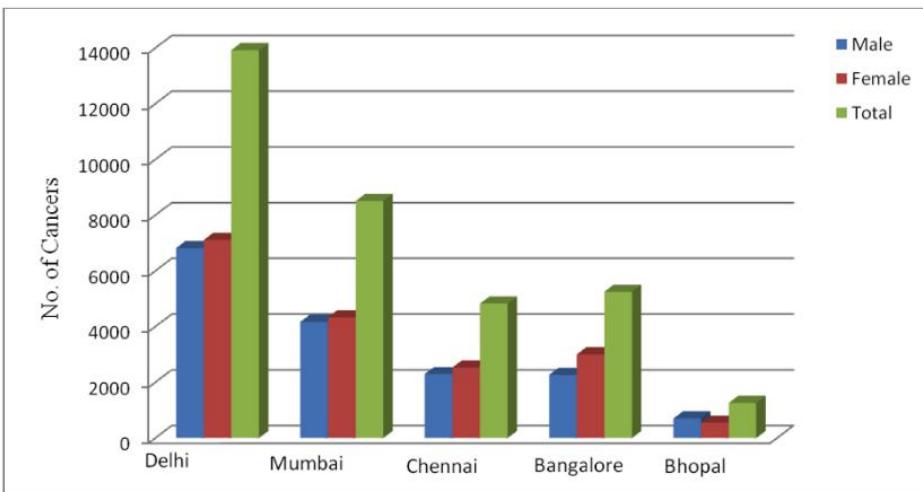


Figure 2: Cancer prevalence in five metropolitan cities of India [Marimuthu, 2008].

Cancer scenario in India

- Estimated number of people living with the disease: around 2.25 million
- Every year, new cancer patients registered: Over 11,57,294 lakh
- Cancer-related deaths: 7,84,821

Risk of developing cancer before the age of 75 years

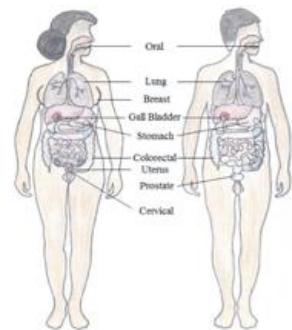
Male: 9.81%
Female: 9.42%

Total deaths due to cancer in 2018

- Total: 7,84,821
- Men: 4,13,519
- Women: 3,71,302

Risk of dying from cancer is 7.34% in males and 6.28% in females.

	MEN	WOMEN
1	ORAL	BREAST
2	LUNG	ORAL
3	STOMACH	CERVIX
4	COLORECTAL	LUNG
5	ESOPHAGUS	GASTRIC



The top five cancers in men and women account for 47.2% of all cancers; these cancers can be prevented, screened for and/or detected early and treated at an early stage.
ICMR-2018

Cancer

- **Cancer** is a disease of multicellular organisms in which there is an uncontrolled proliferation of cells.
- The cardinal features of cancer are **growth, invasion and metastasis**.
- The term **metastasis** is given to the formation of secondary tumors at sites **distant** from the primary tumor.
- No morphological or biochemical change has been identified that is present in all cancer cells and has not been seen in any normal cell.

What is Cancer?

Cancer is a disease that begins when a single cell escapes from the regulation of its own division.

Cell division is the process a cell undergoes in order to make copies of itself. This division is normally regulated so that a cell divides only when more cells are required, and when conditions are favorable for division.

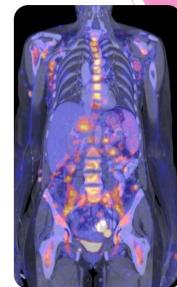
A cancerous cell is a rebellious cell that divides without instructions from the body.

Cancer is a group of diseases characterized by the uncontrolled growth and spread of abnormal cells. it can result in death.

- As genes switch on and off, they determine when and how fast the cell will grow and divide, when it will stop dividing, and even when it will die. Cancer can result when controls over cell division are lost...

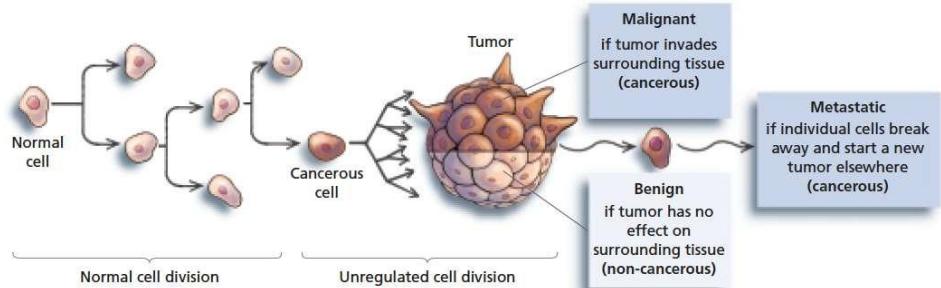


WHY IS CRAB () THE SYMBOL OF CANCER?

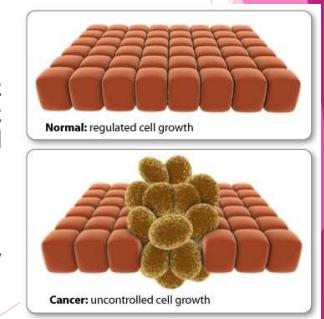


- “Cancer” is the Latin word for crab.
- In its natural habitat, a crab is a fast, resilient decapod crustacean that springs to action and moves in multiple directions.
- Similarly **cancer** spreads from the place at which it first arose as a primary tumor to distant locations in the body.

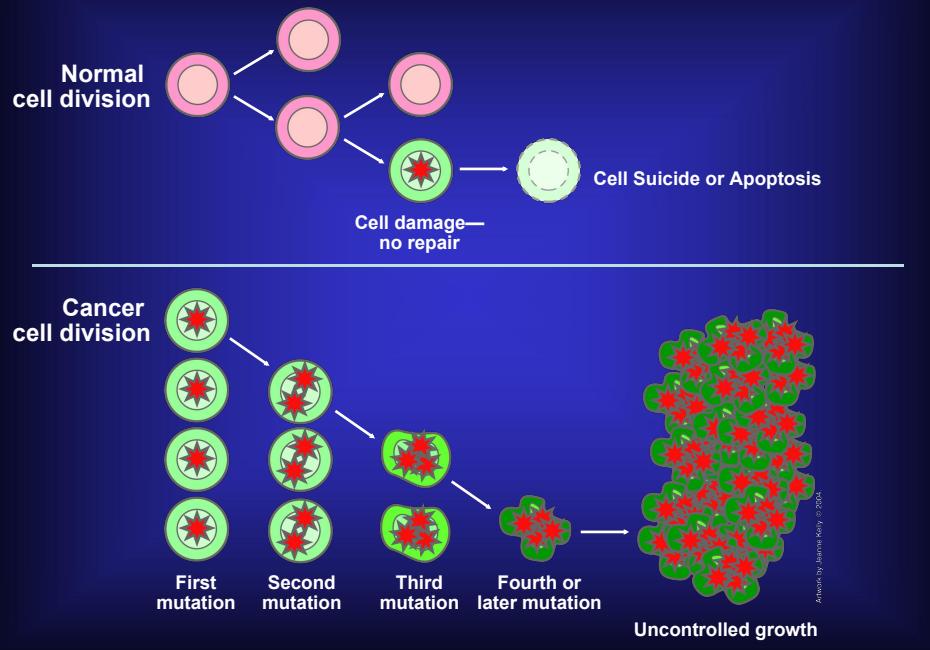
Background / Rationale



- Cancer is uncontrolled cell growth.
- Cancer progression is a multistep process that begins with abnormal cells with malignant potential or neoplastic characteristics and proceeds with tumor growth, stromal invasion, and metastasis.
- However, despite of numerous efforts, effective therapeutic inventions against many cancer types have not been achieved.



Loss of Normal Growth Control

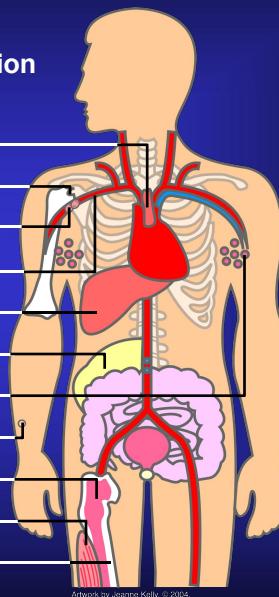


Naming Cancers

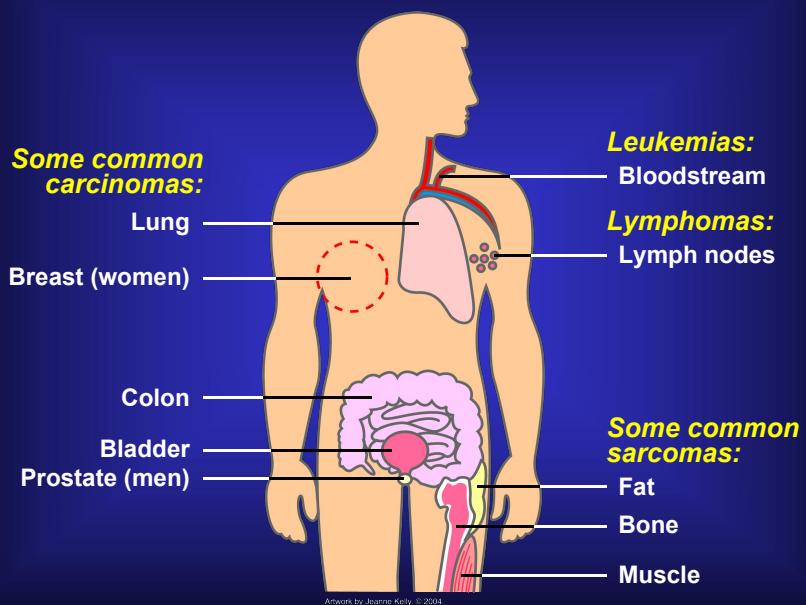
Cancer Prefixes Point to Location

Prefix **Meaning**

adeno-	gland
chondro-	cartilage
erythro-	red blood cell
hemangio-	blood vessels
hepato-	liver
lipo-	fat
lympho-	lymphocyte
melano-	pigment cell
myelo-	bone marrow
myo-	muscle
osteo-	bone



Different Kinds of Cancer



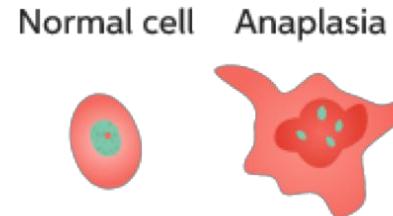
Cancer types: classified by tissue of origin

- **Carcinoma** – cancer of skin or in tissues that line or cover internal organs.
- **Sarcoma** – cancer of bone, cartilage, fat, muscle, blood vessels, or other connective or supportive tissue.
- **Leukemia** – cancer of blood-forming tissue such as the bone marrow and causes large numbers of abnormal blood cells.
- **Lymphoma and myeloma** – cancers of the cells of the immune system.
- **Central nervous system cancers** – cancers of the tissues of the brain and spinal cord.

More than 80% of human cancers are carcinomas, because Most cell proliferation in the body occurs in epithelia. Epithelial tissues are most frequently exposed to the various forms of physical and chemical damage that favor the development of cancer.

Common Terminologies in Cancer Biology

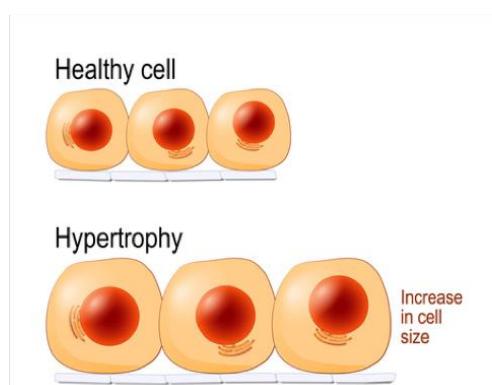
- **Anaplasia:** Loss of differentiation of cells and/or tissues (Greek: Ana – backward; plasia – formation)
 - **Loss of polarity:** Normally the nuclei of epithelial cells are oriented along the basement membrane
 - **Pleomorphism:** variation in size and shape of the tumor cell
 - Nuclei to cytoplasmic ratio – nuclei are enlarged, increased from normal 1:5 to 1:1
 - **Anisonucleosis** – variation in size and shape of nuclei.



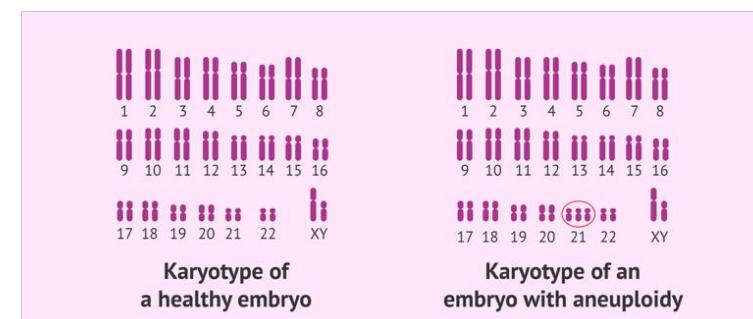
Hyperplasia: Increased number of cells in a tissue
Hyper – over, plasia – formation.



Hypertrophy: Increase in the size of a tissue
hyper – over; trophy -



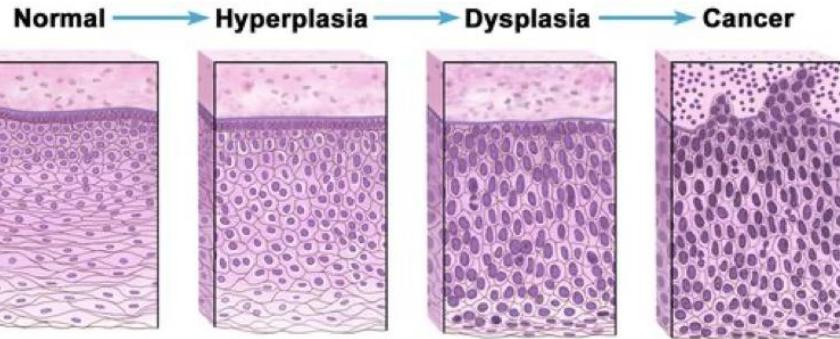
Aneuploidy: Possessing an abnormal number of chromosomes



Around 90 % of the tumors have cancer cells with extra or missing chromosomes

Dysplasia: Abnormal tissue development

Normal Cells May Become Cancer Cells



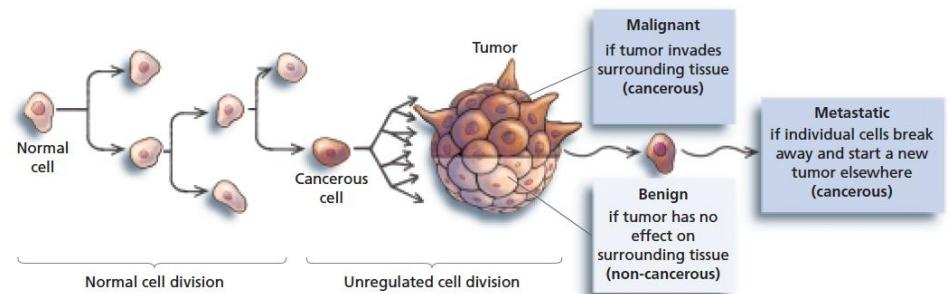
Cancer and metastasis

Tumor: Unregulated cell division that leads to a pile of cells that form a lump.

A tumor is a mass of tissue that has no apparent function in the body.

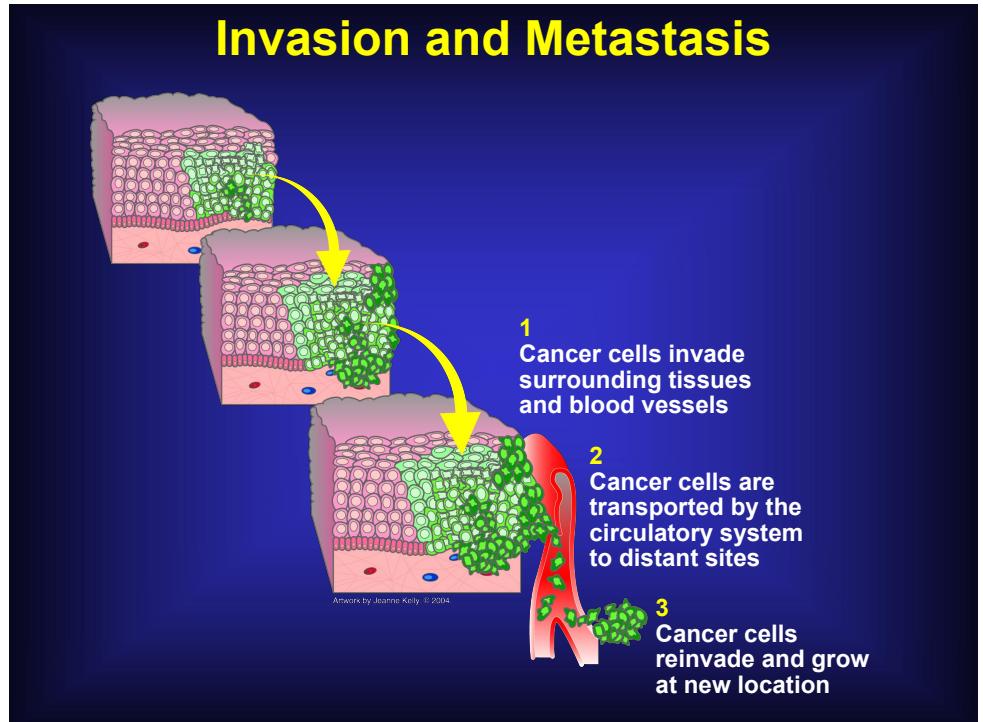
Tumors that stay in one place and do not affect surrounding tissues are said to be benign

Cells from the original tumor can break away and start new cancers at distant locations, this process is called metastasis

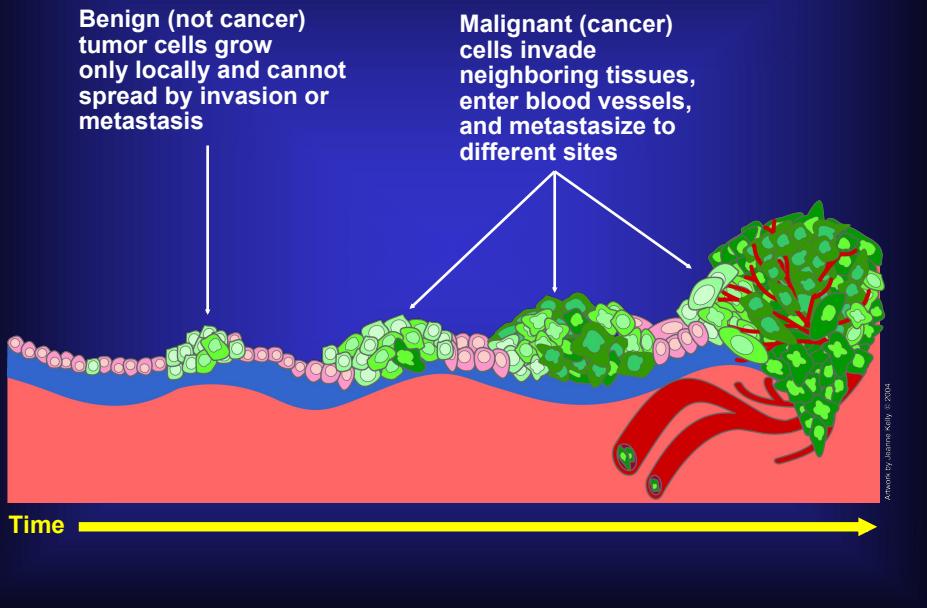


Malignancy

- A term for diseases in which abnormal cells divide without control and can invade nearby tissues.
- Malignant cells can also spread to other parts of the body through the blood and lymph systems.
- There are several types of malignancy
 - Carcinoma
 - Sarcoma
 - Leukemia
 - Lymphoma



Malignant versus Benign Tumors



SBL100-Lecture

Introduction to Cancer

Part II

Cancer cells differ from normal cells in three ways:

- (1) they divide when they should not;
- (2) they invade surrounding tissues; and
- (3) they move to other locations in the body.

- The purpose of cell division is to heal wounds, replace damaged cells, and help tissues and organs grow.
- Normal cells are programmed to divide a certain number of times— usually 60–70— and then they die.
- Cancer cells do not obey these life-span limits, instead they are immortal.
- This is because cancer cells can activate a gene that is usually turned off after early development.
- This gene produces an enzyme called telomerase . This enzyme, only active early in development and in cancer cells, allows cells to divide without limit. Cells with active telomerase enzyme are immortal.

Cancer Cells Versus Normal Cells

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TABLE 9.2

Cancer Cells Versus Normal Cells

Cancer Cells	Normal Cells
Nondifferentiated cells	Differentiated cells
Abnormal nuclei	Normal nuclei
Do not undergo apoptosis	Undergo apoptosis
No contact inhibition	Contact inhibition
Disorganized, multilayered	One organized layer
Undergo metastasis and angiogenesis	

Normal: regulated cell growth

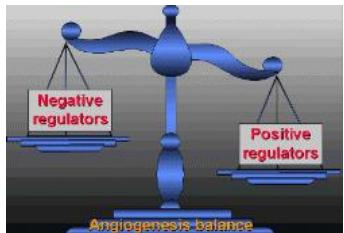
Cancer: uncontrolled cell growth

The diagram shows two types of cell growth. 'Normal' growth is represented by a single layer of rounded pink cells, each with a nucleus. 'Cancer' growth is represented by a dense, irregular cluster of green and pink cells, many of which are elongated and have multiple nuclei. Below the table, there are two rows of small illustrations. The first row shows a single pink cell next to a green cell, with the caption 'Large number of irregularly shaped dividing cells'. The second row shows a single pink cell next to a green cell, with the caption 'Large, variably shaped nuclei'. The third row shows a single pink cell next to a green cell, with the caption 'Small cytoplasmic volume relative to nuclei'. The fourth row shows a single pink cell next to a green cell, with the caption 'Variation in cell size and shape'. The fifth row shows a single pink cell next to a green cell, with the caption 'Loss of normal specialized cell features'. The sixth row shows a single pink cell next to a green cell, with the caption 'Disorganized arrangement of cells'. The seventh row shows a single pink cell next to a green cell, with the caption 'Poorly defined tumor boundary'.

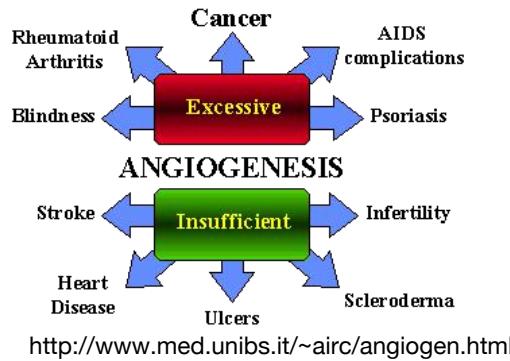
What is Angiogenesis?

It is growth of new capillary blood vessels in the body. It is an important natural process used for healing and reproduction. The body controls angiogenesis by producing a precise balance of growth and inhibitory factors in healthy tissues.

Abnormal blood vessel growth leads to cancer.



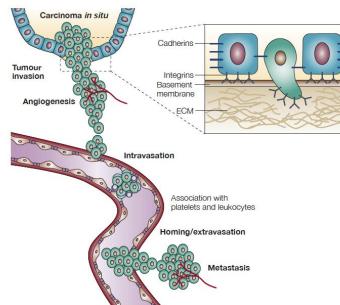
<http://bcove.me/erpvo6ia>



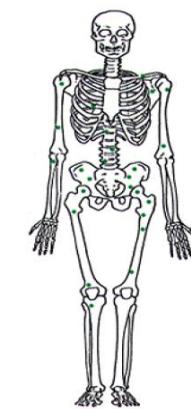
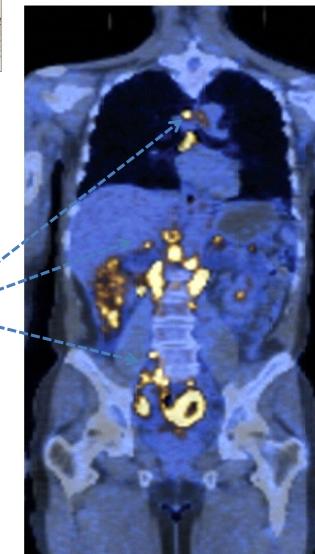
Acquired capabilities that contributes to the deadly behaviour of metastatic cells:

- ability to move through, and thereby invade, other tissues.
- metastatic cells have to induce angiogenesis to escape the limits that passive diffusion of nutrients and oxygen impose on tumour growth. angiogenesis provides a gateway for tumour cells to enter the circulation, which facilitate the migration and invasion of tumour cells.
- metastatic cells have to survive in various foreign microenvironments before they colonize their target organ, and they have to survive

What is Metastasis? It is process by which a tumor cell leaves the primary tumor, travels to a distant site via the circulatory system, and establishes a secondary tumor.



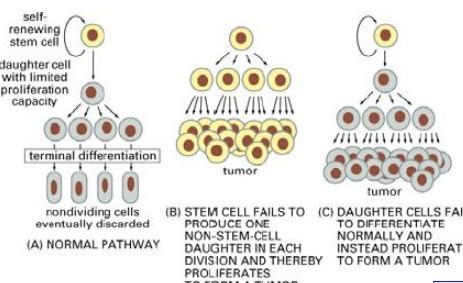
Metastatic tumors



Malignant tumors typically give rise to metastases, making the cancer hard to eradicate.

(From Union Internationale Contre le Cancer, TNM Atlas: Illustrated Guide to the Classification of Malignant Tumors, 2nd ed. Berlin: Springer-Verlag, 1986.)

Cancerous growth often depends on defective control of cell death, cell differentiation, or both

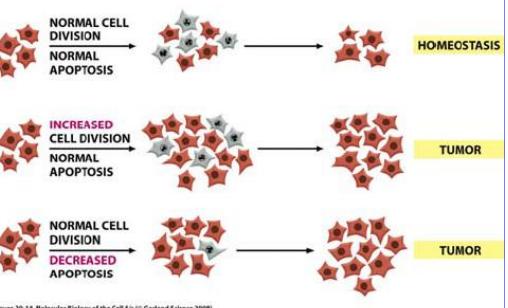


Apoptosis is needed to destroy cells

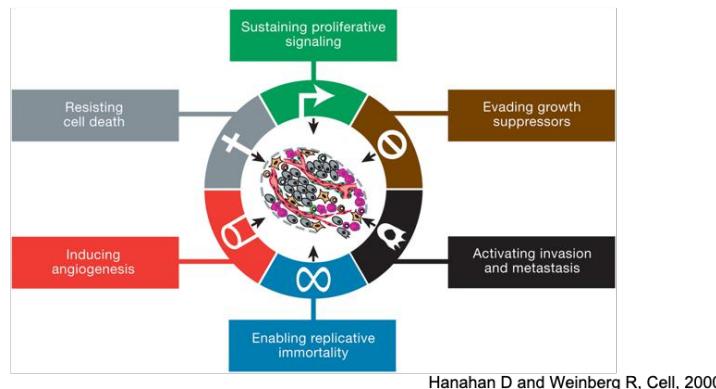
Examples: Cells infected with viruses
Cells of the immune system
Cells with DNA damage
Cancer cells



Increased cell division & decreased apoptosis



Hallmarks of Cancer



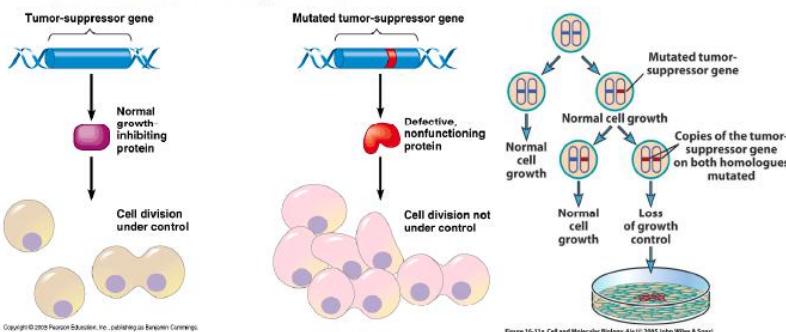
Hallmarks of Cancer

- We foresee cancer research developing into a logical science, where the complexities of the disease, described in the laboratory and clinic, will become understandable in terms of a small number of underlying principles.
- Rules that govern the transformation of normal human cells into malignant cancers
- A small number of molecular, biochemical and cellular traits – acquired capabilities – shared by most and perhaps all types of human cancers.
- Our faith in such simplification derives directly from the teachings of cell biology that virtually all mammalian cells carry a similar molecular machinery regulating their proliferation, differentiation and death.

Hanahan D and Weinberg R, Cell, 2000

Tumor suppressor

- Genes in the body that can suppress or block the development of cancer
- Need TWO bad copies before problems occur- **recessive**
- Homozygous loss of p53 is found in 65% of colon cancers, 30–50% of breast cancers, and 50% of lung cancers.

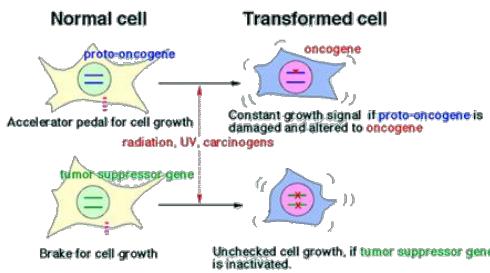


Oncogenes & Proto-oncogenes

- **Oncogene** - Genes that promote cell growth and/or motility, which when upregulated or deregulated, empower cells with the cancerous properties of unregulated growth and motility.
- Can transform healthy cells.
- **Proto-oncogenes** - Proto-oncogenes are a group of genes that cause normal cells to become cancerous when they are mutated.
- Mutations in proto-oncogenes are typically dominant in nature.
- Often, proto-oncogenes encode proteins that stimulate cell division.
- First oncogene was discovered in chicken (Rous Sarcoma Virus)

TUMOR SUPPRESSOR GENES VERSUS PROTO ONCOGENES

TUMOR SUPPRESSOR GENES	PROTO ONCOGENES
Protective genes that help to control the cell growth	Normal genes which, when altered by mutation, become oncogenes that can contribute to cancer
Mutations alter the gene products that inhibit the progression of the cell cycle, causing the development of tumors	Mutations alter the gene products in such a way to increase their expression, which cause cancers by increasing cell division
Suppress cell division	Activate cell division
Inactivation (loss of function) causes cancers	Activation (gain of function) causes cancers
Cancer development is recessive since both copies of alleles have to be mutated to develop cancer	Cancer development is dominant since a mutation of a single copy can cause cancers



1. Three Nobel Prizes for a chicken virus



Rous (1879–1970)
1966 Nobel Prize
"Rous sarcoma virus"

- In 1910 Peyton Rous extracted material from a cancer tumor in a hen and injected it into a healthy chicken.
- The chicken developed cancer, and he concluded that cells from the hen's tumor contained an infectious substance, a virus, that transmits cancer.
- However, the study could not be replicated in mammals and was long overlooked.
- When research showed that viruses can operate by affecting the genetic material of normal germ cells, interest in Rous' discovery was reignited.

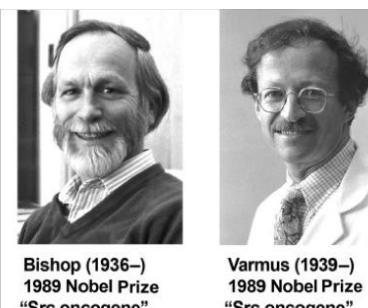
2. Three Nobel Prizes for a chicken virus



Howard Temin

- ❖ At the beginning of the 1960s, Howard Temin, a young virologist at the University of Wisconsin in Madison, came up with the "**provirus hypothesis**"
- ❖ A discovery that was more intuitive than based on solid experiments.
- ❖ According to the provirus hypothesis, a DNA provirus is synthesized after an RNA virus has entered a host cell.
- ❖ Temin's provirus hypothesis was heavily attacked, because it was in conflict with the central dogma
- ❖ The enzyme, which became known as reverse transcriptase, was not localized in the cells but in the viruses: the RNA tumor viruses were called retroviruses.

3. Three Nobel Prizes for a chicken virus



Bishop (1936–)
1989 Nobel Prize
"Src oncogene"

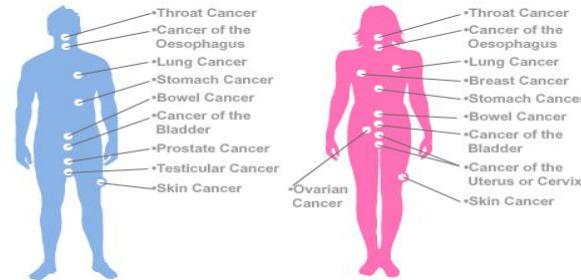
Varmus (1939–)
1989 Nobel Prize
"Src oncogene"

- ❖ Varmus and Bishop showed that nearly-identical versions of cancer-causing genes (so-called oncogenes) carried by retroviruses, viruses that integrate themselves into the DNA of infected cells, are present in the genome of normal, uninfected cells in a wide range of species
- ❖ Normal cells carried within them the seeds of cancer in the form of genes they called proto-oncogenes.

Stages of Cancer Spread

- Stage 1: Confined to organ of origin
- Stage 2: Locally invasive
- Stage 3: Spread to lymph nodes
- Stage 4: Spread to distant sites

WHICH PARTS OF THE BODY ARE AFFECTED BY CANCER?



- ▶ Almost all the major parts of our body may be affected by **cancer**.
- ▶ Size of cancer cells:
 - ▶ One million cancer cells = head of a pin
 - ▶ One billion cancer cells = a small grape

3. Three Nobel Prizes for a chicken virus



Bishop (1936–)
1989 Nobel Prize
“Src oncogene”



Varmus (1939–)
1989 Nobel Prize
“Src oncogene”

- ❖ Proto-oncogenes are conserved and are present across species, from yeast to fish to humans.
- ❖ Only when a proto-oncogene is altered through the rearrangement of the cell's chromosomes or through cumulative mutations--mutations at several different sites in the gene are required--does it trigger uncontrolled cell growth and division.
- ❖ “Unified theory of cancer”

Cancer Staging

- The extent to which cancer has spread is called its stage.
- It's important for doctors to determine the cancer stage in order to design the best course of treatment (prognosis).
- Cancer stage is determined during diagnosis and depends on the size, type and location of the cancer.
- Tumor grade is a term used to refer to the appearance and behavior of cancer cells (how likely they are to grow and spread).

What does a pathologist look for examining biopsy tissue?

Normal	Cancer	
		Large number of irregularly shaped dividing cells
		Large, variably shaped nuclei
		Small cytoplasmic volume relative to nuclei
		Variation in cell size and shape
		Loss of normal specialized cell features
		Disorganized arrangement of cells
		Poorly defined tumor boundary

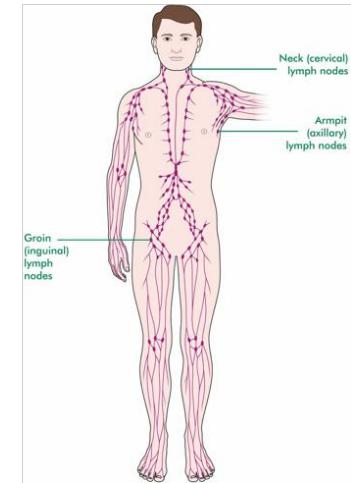
Source: www.mayoclinic.org © 2008.

Cancer Staging

- **TNM** is the most common tumor staging system used to describe cancer.
- **T—Tumor** represents the size and magnitude of the primary tumor and has the following subsets:
 - **TX.** TX means there's no information about the primary tumor, or it can't be measured..
 - The proper use of X is to denote the absence or uncertainty of assigning a given category (T, N, or M) when all reasonable clinical or pathologic maneuvers have been used in staging
 - **T0.** Primary tumor cannot be found.
 - **Tis** means that the cancer cells are only growing in the layer of cells where they started, without growing into deeper layers. This may also be called *in situ* cancer or pre-cancer.
 - **T1, T2, T3, T4:** These denote the size and progression of the primary tumor, increasing in progression with each number, and may be further broken down into subsets.

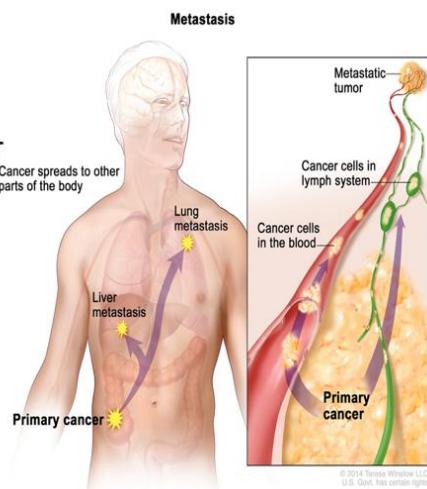
Cancer Staging

- **N—Node** represents the specific number of lymph nodes that also have cancer, and it has the following subsets:
 - **NX.** Presence of cancer in nearby lymph nodes is unmeasurable.
 - **N0.** There is no cancer in the lymph nodes.
 - **N1, N2, N3.** These denote the number and location where cancer-containing lymph nodes have been detected, increasing in progression with each number.



Cancer Staging

- **M—Metastasis** represents distant metastasis, or whether or not the cancer has spread to other areas, and it includes the following subsets:
 - **MX.** Rate of metastasis is unmeasurable.
 - **M0.** Cancer has not metastasized to other areas.
 - **M1.** Cancer has metastasized to other areas.



Cancer Staging

TUMOR		NODES				METASTASIS	
T	Tumor	Local tissues	Organ	N	Nodes	M	Metastases
		0	1	1	0	0	0
		1	2	2	1	1	1
		2	3	3	2	2	2

T = Primary tumor; the number equals size of tumor and its local extent. The number can vary according to site.
T0 = Broad flat tumor
T1 = Tumor <2 cm size
T2 = Lesion 2-5 cm
T3 = Skin and/or chest wall involved by invasion

N = Lymph node involvement; a higher number means more nodes are involved.
N0 = No axillary nodes involved
N1 = Mobile nodes involved
N2 = Fixed nodes involved

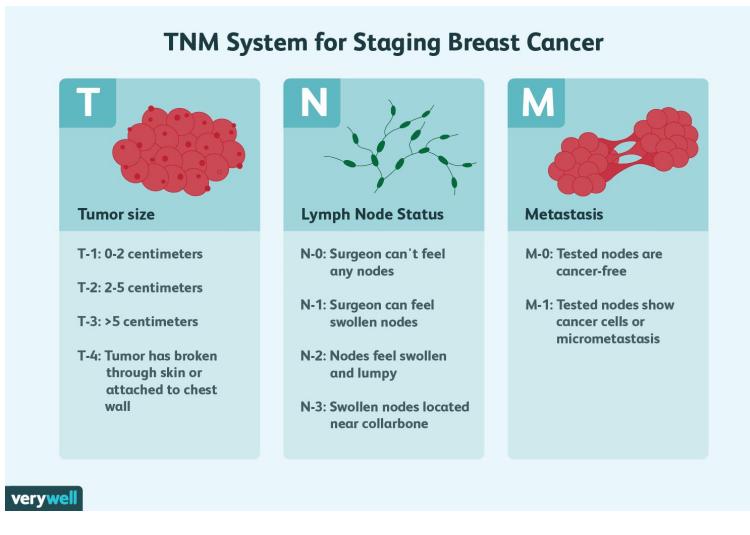
M = Extent of distant metastases.
M0 = No metastases
M1 = Demonstrable metastases
M2 = Suspected metastases

Fig. 9-3. Tumor Staging by the TNM System
Example of staging for breast cancer.

Mobile lymph nodes are those that can be easily moved, while fixed lymph nodes are stuck to an internal structure. Mobile nodes are generally benign (non-cancerous), while fixed nodes are commonly seen with cancer.

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Cancer Staging



- **Stage 0** refers to cancer in situ, or cancer that's limited to the place it started.
- **Stage 1** cancer hasn't spread far into nearby tissues or other parts of the body.
- **Stage 2** is used to designate larger tumors and those that have spread deeply into nearby tissues and lymph nodes.
- **Stage 3** is similar to stage 2, but with increased severity.
- **Stage 4** is an advanced stage of metastatic cancer in which the primary cancer has spread to distant organs and areas of the body.

Stage of the tumor	TNM
STAGE 0	Tumor in situ
STAGE I	T1-2 N0 M0
STAGE II	T1-2 N1 M0 T3 N0 M0
STAGE III	T1-2 N2-3 M0 T3 N1-3 M0 T4 N0-3 M0
STAGE IV	T1-4 N0-3 M1 (or every cancer with distant metastasis)

Textbook & Readings:

- Alberts B. et al., **The Molecular Biology of the Cell** Garland Science Press, ISBN 0-8153-3218-1 is recommended.
- Robert A. Weinberg, **The Biology of Cancer** Garland Science Press, ISBN 0-8153-4078-8.
- Lauren Pecorino, **Molecular Biology of Cancer**, Oxford University Press. ISBN 978-0-19-921148-7.
- M. Molls, P. Vaupel, C. Nieder, M.S. Anscher. **The impact of tumor biology on cancer treatment and multidisciplinary strategies**, Springer. ISBN 978-3-540-74385-9.
- Yi Lu, R. I.Mahato, **Pharmaceutical perspectives of cancer therapeutics**, Springer. ISBN 978-1-4419-0130-9.

History of Cancer

- The oldest credible evidence of cancer in mammals consists of tumor masses found in fossilized dinosaurs and human bones from prehistoric times
- a recent large-scale study that screened by fluoroscopy over 10,000 specimens of dinosaur vertebrae for evidence of tumors and further assessed abnormalities by computerized tomography
- Out of several species of dinosaurs surveyed, only Cretaceous hadrosaurs (duck-billed dinosaurs), that lived 70 million years ago, harbored benign tumors (hemangiomas and osteoblastoma but 0.2% of specimens exhibited malignant metastatic disease



Guy B Faguet, Int J Cancer 2015

History of Cancer

- 2,000 year old mummy
- Bone cancer
'osteosarcoma'
- Nasopharyngeal Cancer
- Oldest description of cancer – 3000 BC – ancient Egyptian textbook on trauma surgery – 8 cases of tumors
- "There is no treatment"



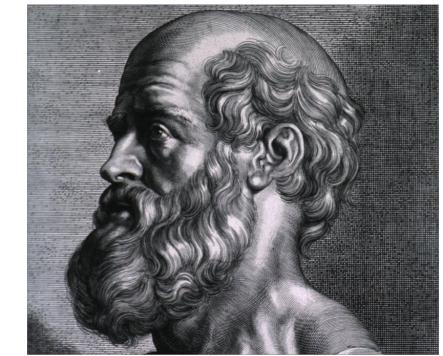
Origin of the word Cancer

- In Greek, these words refer to a crab, most likely applied to the disease because the finger-like spreading projections from a cancer called to mind the shape of a crab
- The Roman physician, Celsus (28-50 BC), later translated the Greek term into **cancer**, the Latin word for crab
- Galen (130-200 AD), another Greek physician, used the word **oncos** (Greek for swelling) to describe tumors.



Origin of the word Cancer

- Hippocrates used the terms carcinos and carcinoma to describe non-ulcer forming and ulcer-forming tumors



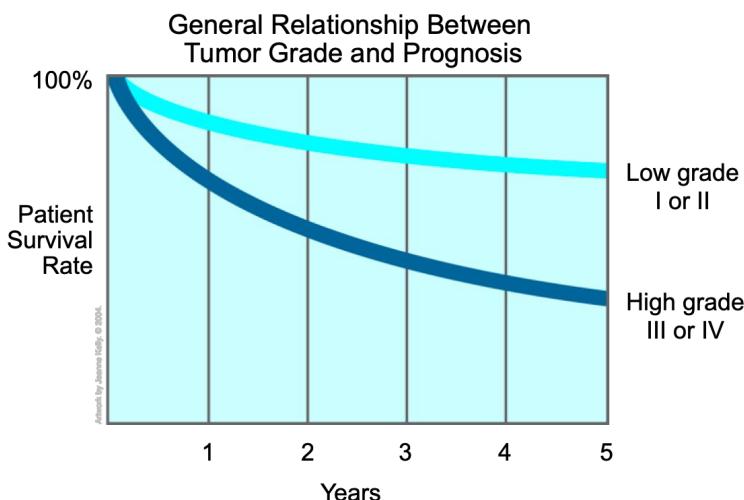
Hippocrates – The father of medicine

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- This method not only allowed a better understanding of the damage cancer had done but also aided the development of cancer surgery.



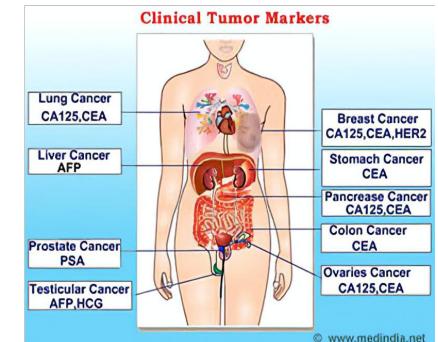
What is the relationship between tumor grade and patient survival?



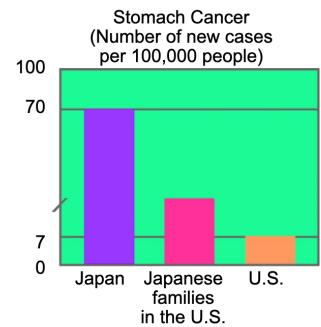
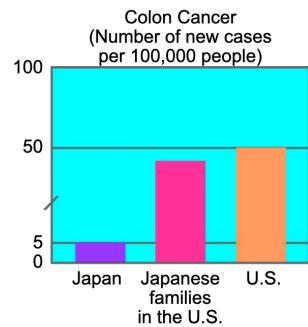
Tumor Markers

- Tumor cell markers (biologic markers) are substances produced by cancer cells or that are found in plasma, cell membranes, in the blood, CSF, or urine
- Hormones
- Enzymes
- Genes
- Antigens
- Antibodies

CEA – Carcinoembryonic Antigen
AFP – Alpha-fetoprotein
PSA – Prostate Specific Antigen
CA-125 (Mucin-16)
HCG – Human Chorionic Gonadotropin



Is the incidence of these cancers due to gene behavior or environmental risk?



Environmental Risk Factors

Increased

- Tobacco
- Radiation
 - Ionizing
 - UV
- Alcohol
- Diet
- Obesity
- Occupational Hazards

Decreased

- * Exercise
- * Proper Diet

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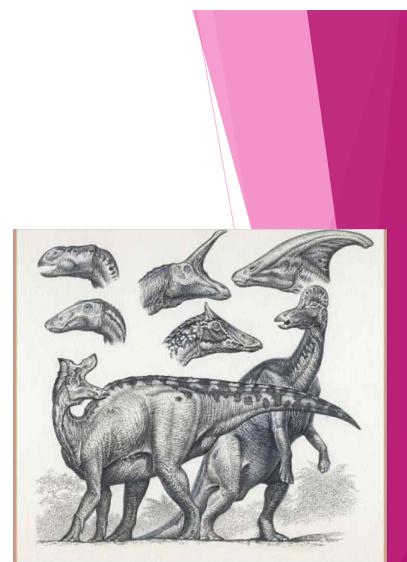
SBL100-Lecture

Introduction to Cancer

Part III

History of Cancer

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Guy B Faguet, Int J Cancer 2015

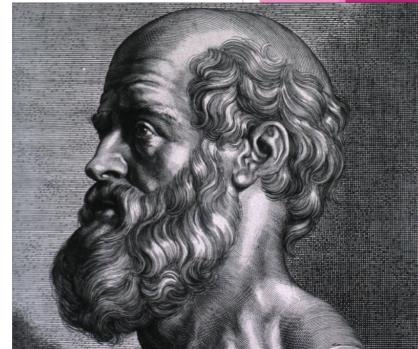
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Stages of Cancer Spread

- Stage 1: Confined to organ of origin
- Stage 2: Locally invasive
- Stage 3: Spread to lymph nodes
- Stage 4: Spread to distant sites

WHICH PARTS OF THE BODY ARE AFFECTED BY CANCER?

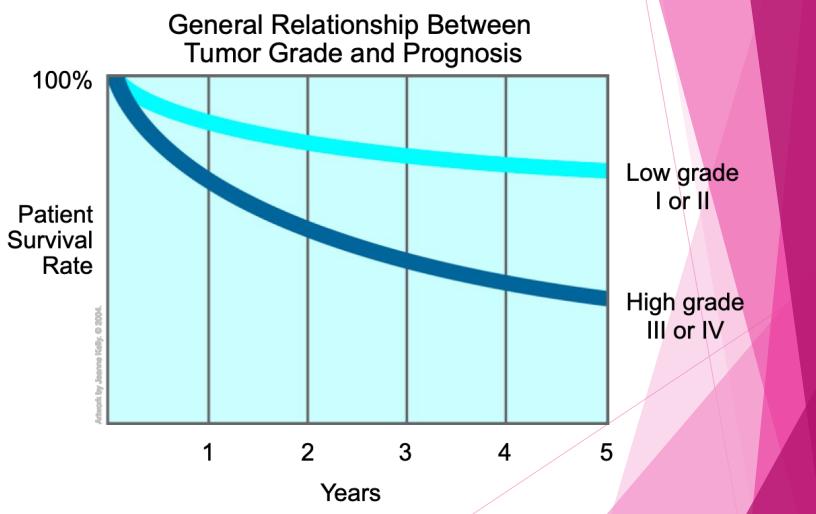


► Almost all the major parts of our body may be affected by **cancer**.

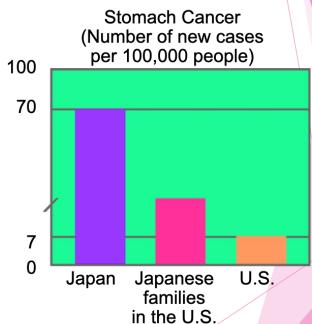
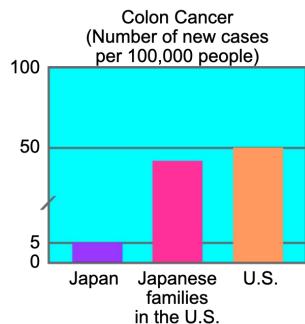
► Size of cancer cells:

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- One billion cancer cells = a small grape

What is the relationship between tumor grade and patient survival?



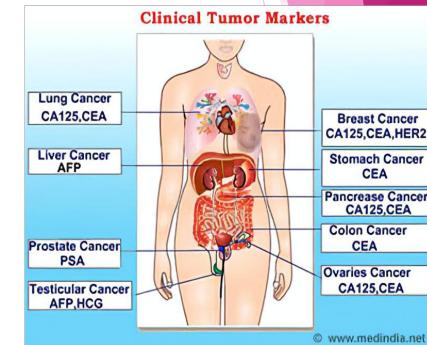
Is the incidence of these cancers due to gene behavior or environmental risk?



Tumor Markers

- Tumor cell markers (biologic markers) are substances produced by cancer cells or that are found in plasma, cell membranes, in the blood, CSF, or urine
- Hormones
- Enzymes
- Genes
- Antigens
- Antibodies

CEA – Carcinoembryonic Antigen
AFP – Alpha-fetoprotein
PSA – Prostate Specific Antigen
CA-125 (Mucin-16)
HCG – Human Chorionic Gonadotropin



Environmental Risk Factors

Increased

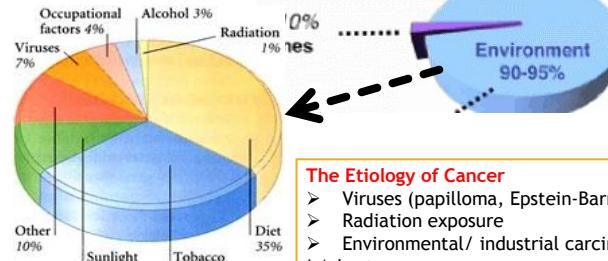
- Tobacco
- Radiation
 - Ionizing
 - UV
- Alcohol
- Diet
- Obesity
- Occupational Hazards

Decreased

- * Exercise
- * Proper Diet



CAUSES OF CANCER



The Etiology of Cancer

- Viruses (papilloma, Epstein-Barr, hepatitis B, retrovirus)
- Radiation exposure
- Environmental/ industrial carcinogens
 - * Asbestos
 - * Aromatic amines
 - * Bischloromethyl ethers
 - * Beta-naphthalene and benzedrine
 - * Polycyclic hydrocarbons
 - * Drug-induced cancers (alkylators such as melphalan and cyclophosphamide)
 - * Nickel
 - * Vinyl chloride
 - * Isopropyl alcohol
- Diet and nutrition
- Tobacco and alcohol consumption
- Immunodeficiency syndromes: HIV is associated with Kaposi's sarcoma,
- Genetic susceptibility

Cancer is caused by external factors, such as tobacco, infectious organisms, and an unhealthy diet, and internal factors, such as inherited genetic mutations, hormones, and immune conditions etc..

Chemicals



- ▶ Alcohol
- ▶ Asbestos
- ▶ Wood dust
- ▶ Rubber, plastics, dyes
- ▶ Tobacco

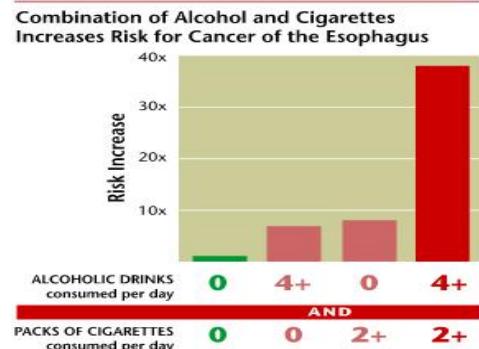
Smoking

- ▶ Single biggest cause of cancer
- ▶ 25-40% smokers die in middle age
- ▶ 9 in 10 lung cancers
- ▶ Known to cause cancer in 1950



- Tobacco
 - Multipotent carcinogenic mixture
 - Linked to cancers of the lung, lower urinary tract, aerodigestive tract, liver, kidney, pancreas, cervix
 - Linked to myeloid leukemia

Smoking and alcohol



Life style



Highly caloric diet
Rich in fat
Low physical activity
Smoking /drinking
Tobacco

Environmental risk factors

- Ultraviolet radiation
- Causes basal cell carcinoma, squamous cell carcinoma, and melanoma
- Principal source is sunlight
- Ultraviolet A (UVA) (Longer wavelength – skin aging) and ultraviolet B (UVB) – shorter wavelength and skin burning.
- Promotes skin inflammation and release of free radicals



Carcinogens

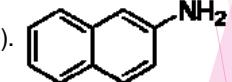
Any agent that provoke the development of cancer is called carcinogenic.

Carcinogenesis is linked to mutagenesis (i.e. cause mutations)

Carcinogens are classified into 2 classes

- 1) Chemical carcinogen: Typically cause simple local changes in nucleotide sequence and
- 2) Radiations such as **x- Rays**: Typically cause chromosomal breakage and Translocation & **UV Rays**: Cause specific DNA base alteration i.e. Point mutation

Oncogene : genes whose products play important roles in the regulation of biochemical activities within cells, including those activities related to cell division



2-Naphthylamine is used to make azo dyes (R-N=N-R').

It is found in cigarette smoke and suspected to be contributory to the development of bladder cancer. It is activated in the liver but quickly deactivated by conjugation to glucuronic acid.

In the bladder, glucuronidase re-activates it by deconjugation, which leads to the development of bladder cancer.

[On average, each cigarette smoked shortens lifespan by 11 minutes and smokers who die of tobacco-related disease lose, on average, 14 years of life.]

Cancers Develop in Slow Stages from Mildly Aberrant Cells

Lung cancer does not begin to rise until after 10 or 20 years of heavy smoking.

Leukemias in Hiroshima and Nagasaki did not show rise until about 5 years after the explosion of atomic bombs;

Industrial workers exposed for a limited period to chemical carcinogens do not usually develop the cancers characteristic until 10, 20, or even more years (Fig 6).

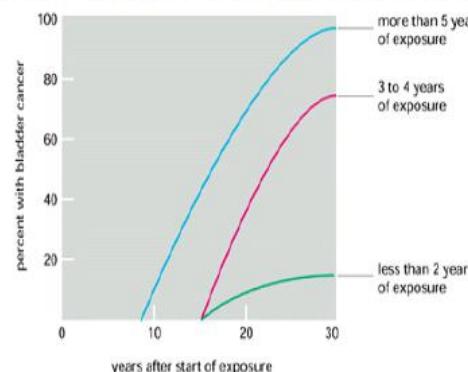
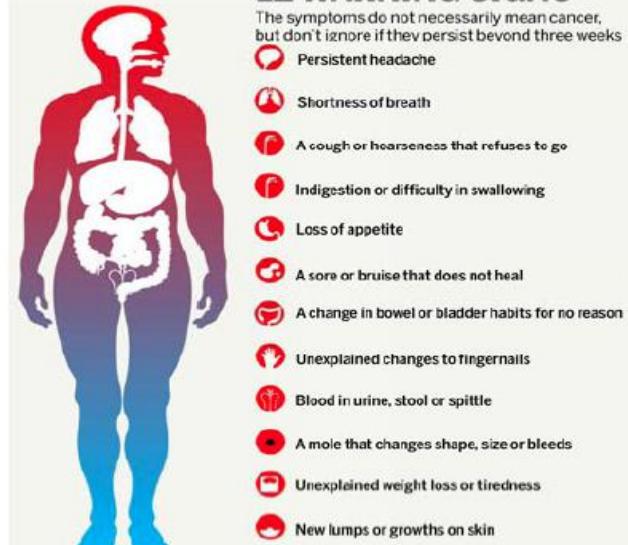


Figure 6. Delayed onset of cancer following exposure to a carcinogen. The graph shows the length of the delay before onset of bladder cancer in a set of 78 men who had been exposed to the carcinogen 2-naph-thylamine, grouped according to the duration of their exposure. (Modified from J. Cairns, Cancer: Science and Society. San Francisco: W.H. Freeman, 1978. After M.H.C. Williams, in Cancer, Vol. III (R.W. Raven, ed.). London: Butterfield, 1958.)

Clinical symptoms or signs of cancer

12 WARNING SIGNS

The symptoms do not necessarily mean cancer, but don't ignore if they persist beyond three weeks



Persistent headache

Shortness of breath

A cough or hoarseness that refuses to go

Indigestion or difficulty in swallowing

Loss of appetite

A sore or bruise that does not heal

A change in bowel or bladder habits for no reason

Unexplained changes to fingernails

Blood in urine, stool or sputum

A mole that changes shape, size or bleeds

Unexplained weight loss or tiredness

New lumps or growths on skin

Cancer diagnosis and treatment

- The earlier cancer diagnosis, the better the chance of its being cured.
- Some cancer – such as skin, breast, mouth, testicles, prostate, and rectum -- may be detected by routine self-exam or other screening measures.
- Cancer diagnosis begins with a thorough physical exam and a complete medical history.
- Followed by Laboratory studies of blood, urine, and stool can detect abnormalities that may indicate cancer.
- When a tumor is suspected, imaging tests such as X-rays, computed tomography (CT), magnetic resonance imaging (MRI), ultrasound, and fiber-optic endoscopy examinations help doctors determine the cancer's location and size.
- To confirm the diagnosis of most cancers , a biopsy needs to be performed in which a tissue sample is removed from the suspected tumor and studied under a microscope to check for cancer cells.

Physical Diagnosis - Melanoma



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A = Asymmetry, one half the mole does not look like the other half.



B = Border, irregular scalloped or poorly circumscribed border.



C = Color, varied from one area to another; shades of tan, brown, black, or sometimes white, red, or blue.



D = Diameter, larger than 6 mm (the diameter of a pencil eraser).

The Gold Standard of Diagnosis - Biopsy

- ❖ A biopsy is the surgical removal of a small piece of tissue for microscopic examination, which will tell whether a tumor is actually present and if so, whether is malignant or benign.
- ❖ There are three ways tissue can be removed for biopsy:
 - Endoscopy
 - Needle biopsy
 - Or surgical biopsy

Endoscopy

By using a thin lighted tube, doctor is able to look at areas inside the body, see what's going on, take pictures, and remove tissue or cells for examination, if necessary.(colonoscopy/bronchoscopy)

Needle Biopsy

The doctor takes a small tissue sample by inserting a needle into the abnormal area

Surgical Biopsy: excisional biopsy when the doctor removes the entire tumor, often with some surrounding normal tissue. While An incisional biopsy when the doctor removes just a portion of the tumor. If cancer is found to be present, the entire tumor may be removed immediately or during operation. (local/regional/general anesthesia)

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SBL100-Lecture

Introduction to Cancer

Part IV

Next step is to determine the “aggressiveness”of the cancer or how fast the cancer is growing after the diagnosis of cancer the doctor want to learn the stage, or extent, of the disease. This process is referred to as “staging” and tells the doctor how far the cancer has spread in body. Treatment based on the results of staging.

The four common stage of cancer are:

- Stage1: In situ : Early cancer that has not spread to neighboring tissue.
- Stage 2: Local: Cancer is found only in the organ where it started to grow
- Stage 3: Regional: Cancer has spread to the surrounding tissues or lymph nodes.
- Stage 4: Distant: Cancer has spread to other organs and systems of the body.

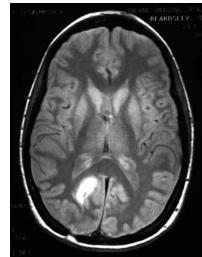
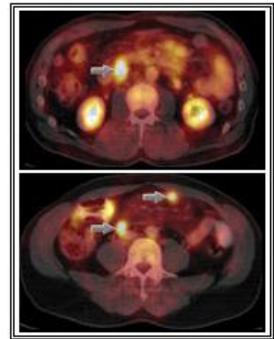
Staging determines the extent of the disease.

Staging is performed using a number of methods such as Imaging studies (ultrasound, magnetic resonance imaging (MRI), and computed tomography (CT scan), x- rays, various blood tests, bone marrow biopsy, and surgery.

Imaging

- ▶ **CT** computed tomography scan
- ▶ **PET** positron emission tomography
- ▶ **SPECT** (single photon emission computed tomography)

- ▶ Patients are injected with a small amount of radioactive material and then have to lie in a machine that captures the gamma ray emissions from the material. The images that are created represent thin 'slices' through the body. Computer software is then used to combine the slices to make a virtual three dimensional model that can be viewed from any angle
- ▶ **MRI** magnetic resonance imaging



Modern diagnostic imaging technologies provide the ability to discriminate tissues down to a millimetre, using magnetic resonance imaging (MRI) and X-ray computed tomography (CT), while the range of positron emission tomography (PET) and single photon emission tomography (SPECT) are a few millimetres.

1. BIOPSY

A biopsy involves removing a piece of tissue to be examined in a laboratory. The examination and analysis of cells, enables cancer cells to be identified and be differentiated from non-cancerous cells and a more definitive diagnosis to be made.

There are many different types of biopsies:

- Needle biopsy, CT-guided biopsy, ultrasound-guided biopsy, bone biopsy, bone marrow biopsy, liver biopsy, kidney biopsy, aspiration biopsy, prostate biopsy, skin biopsy and surgical biopsy.

2. BLOOD TEST

Blood tests can be extremely useful as they evaluate levels of essential molecules such as sugars, fats, proteins and DNA and enable doctors to associate abnormal levels with specific cancers.

Complete Blood Counts (CBC) are commonly used as all-encompassing measurements of the body's health; they measure size, number, and maturity of blood cells in a specific volume of blood.

3. COMPUTED TOMOGRAPHY (CT)

Computed Tomography (CT) scans are an important diagnostic tool to determine the presence of a cancer and its particular stage of development. CT scans involve taking a series of images of the body with an x-ray to create a 3-dimensional (3D) picture of the inside of the body.

These three-dimensional pictures will be configured to form a cross-sectional view that can display any abnormalities and abnormalities. These can then be analyzed to determine the existence and/or stage of the cancer.

5. POSITRON EMISSION TOMOGRAPHY (PET)

Positron Emission Tomography (PET) involves injecting radioactive tracer into the veins of a patient. The tracer commonly used is a radioactive form of glucose. This is effective since cancer, maintaining the continuous division of cells, increases the metabolism of glucose.

PET imaging allows physicians to examine the way glucose is metabolized in the body, and thus may be an indicator of cancer.

6. ULTRASOUND

Ultrasound testing involves using sound waves to create images of internal body parts. It is extremely accurate at visualizing blood flow and finding abnormalities in blood vessels.

There are multiple types of ultrasound procedures, depending on the type of cancer, consisting mainly of Guided Biopsy/Fine Needle Aspirate (FNA) and Contrast Enhanced Ultrasound.

The Guided Biopsy/FNA is a procedure, which combines biopsies with ultrasounds depending on abnormalities seen in the ultrasound.

4. MAGNETIC RESONANCE IMAGING (MRI)

Magnetic Resonance Imaging (MRI) involves using magnetic fields and radio waves, registering signals from water molecules and forming intricate images of specific areas of the body.

This form of imaging may not be recommended if a patient has a heart pacemaker, metal heart valve replacements, aneurysm clips or any other implants that had metal fragments in his/her eyes.

Like the CT scan, it involves minimal preparation before the scan, which lasts no more than an hour, and allows an immediate return to daily activity.

7. X-RAY

An x-ray involves electromagnetic radiation to image the inner processes of the body.

In an x-ray denser materials, which have a lower absorption rate, show up as a lighter color, while air, fat and muscle show up as darker grays and blacks.

X-rays can be used to find, image, and analyze the progress of a tumor in low-doses of radiation.

High doses of radiation actually have a therapeutic ability to destroy cancerous cells.

8. THERMOGRAPHY

A thermograph involves digitized infrared imaging capable of differentiating metabolic activity and circulation within the veins of cancerous and non-cancerous cells.

Cancer cells, with higher metabolic activity produce higher levels of cell production and growth, hence display increased circulation by both utilizing existing blood vessels to greater capacity and forming new ones.

9. SURGERY

Surgery may be done to further examine a tumor or abnormal area. If less invasive procedures to remove tissues, such as biopsies, do not produce enough information, an open surgical exploration might be performed.

Surgeons typically make an incision and look at the abdominal area to further determine the location and size of the tumor. In addition, doctors may completely remove a tumor in question to analyze it and further determine its stage and growth (cancerous or noncancerous).

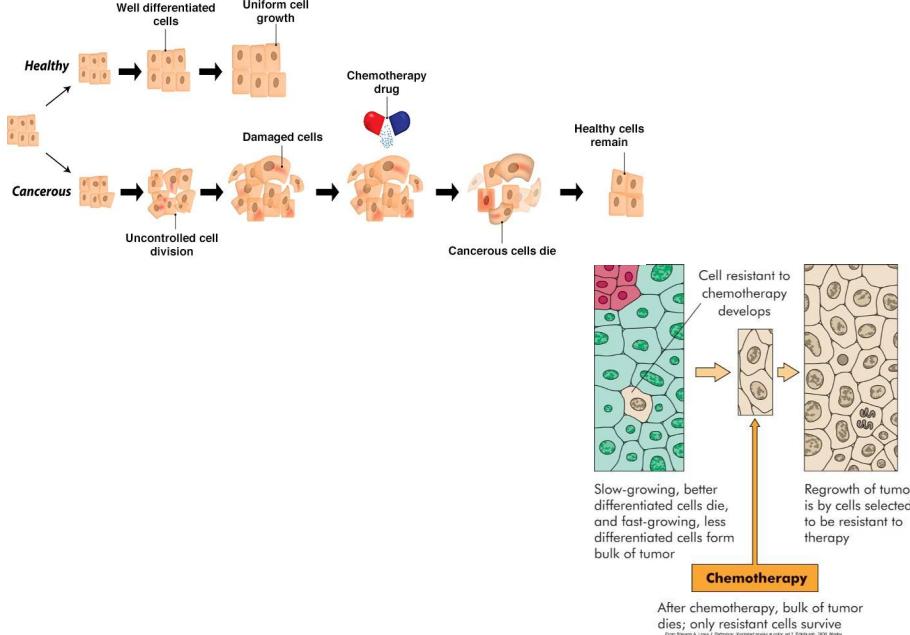
Clinical Manifestations of Cancer

- Anemia
 - A decrease of hemoglobin in the blood
 - Mechanisms
 - Chronic bleeding resulting in iron deficiency, severe malnutrition, medical therapies, or malignancy in blood-forming organs
- Leukopenia and thrombocytopenia
 - Direct tumor invasion to the bone marrow causes leukopenia and thrombocytopenia
- Infection
 - Risk increases when the absolute neutrophil and lymphocyte counts fall

Cancer Therapy

- **Surgery:** Depending on the [type of cancer](#), carcinoma may be treated with the surgical removal of cancerous tissue, as well as some surrounding tissue. Minimally invasive surgical treatment methods may help to reduce healing time and reduce the risk of infection after surgery.
- **Radiation therapy:** Radiation therapy may be used in combination with surgery and/or chemotherapy. Advanced radiation therapies use image guidance before and during treatment on target tumors, and are designed to help spare healthy tissues and surrounding organs.
- **Chemotherapy:** Chemotherapy treats carcinoma with drugs designed to destroy cancer cells, either throughout the whole body, or in a specific area. In some cases, chemotherapy may be used in combination with other treatments, such as radiation therapy or surgery.

Chemotherapy



Cancer Therapy: Systemic treatment

➤ Chemotherapy

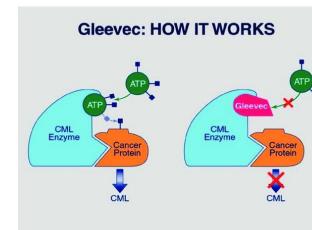
- Cytotoxic drugs + body defenses
- Single agent
- Combination chemotherapy
- E.g. Cisplatin, Paclitaxel, Etoposide

➤ Typical chemotherapy or radiation therapy has severe drawbacks.

- **Gleevec:** Inhibits the receptor protein kinase activity of *Abl*, one of the receptor oncogenes (cancer gene) in Chronic myelogenous Leukemia.
- Revolutionized treatment of this disease.

➤ Immunotherapy

- **passive:** Antibodies against a protein that is unique or overexpressed on cancer cells, can kill the cells. Example: Herceptin.



Emerging Treatments : Immunotherapy and Molecular Targeting

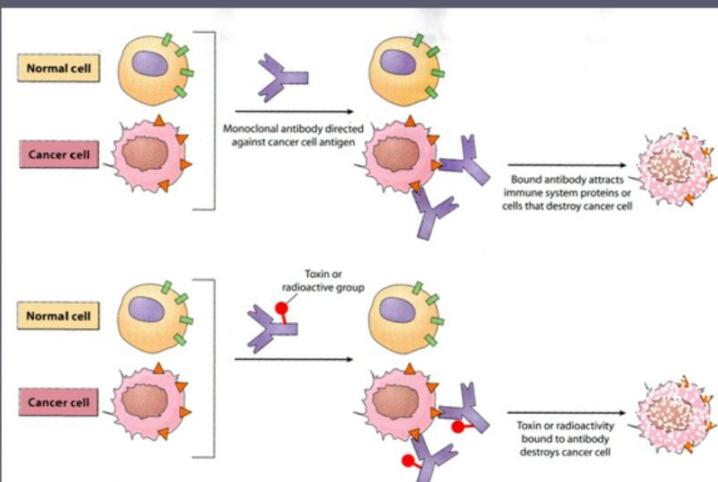


Figure 11-14 Two Ways of Using Monoclonal Antibodies for Cancer Treatment. Monoclonal antibodies can selectively target cancer cells by binding to tumor-specific antigens located on the outer cell surface. (Top) After monoclonal antibodies become selectively bound to cancer cells, the antibody's presence triggers an attack by other cells or proteins of the immune system. (Bottom) Antibodies can also be used as delivery vehicles for radioactive groups or other toxic substances. Linking them to monoclonal antibodies allows such substances to be concentrated at tumor sites without accumulating to toxic levels elsewhere in the body.

Cancer Therapy: New Approaches

Cancer Vaccines

- Majority used for treatment not prevention.
- May offer method that can enhance the immune response against cancer
- At this time, cancer vaccines are only available in clinical trials.
- Dendritic cell (DC) vaccines: DC are immune cells; recognize, process and present to T-cells. DC is less in number; DC is prepared from isolated monocytes from patients blood and introduced back to patient for massive response from T cells.
- Tumor cell vaccine: tumor isolated from patients; antibodies generated and introduced to patient.
- DNA vaccine: genetically engineered DNA inserted in patients cell and injected in the body so that the cell prepares the protein inside the patient.

Gene therapy

- Replaces a faulty gene or adds a new gene in an attempt to cure disease or improve body's ability to fight disease (cancer)
- modified version of the herpes simplex 1 virus which kills melanoma cells

Emerging Treatments : Immunotherapy and Molecular Targeting

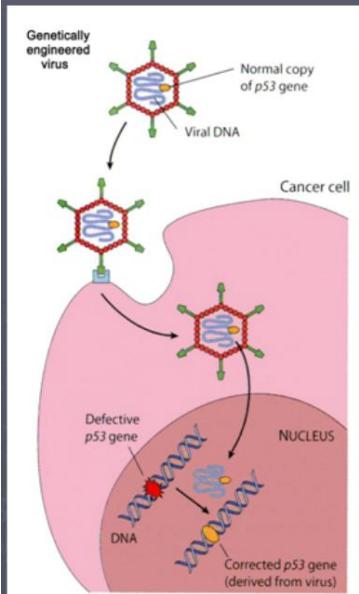


Figure 11-17 Strategy for Using Gene Therapy to Repair a Defective Cancer Cell Gene. Many human cancers exhibit defects in the *p53* gene. If these defects could be corrected, restoration of the *p53* pathway might cause cancer cells to self-destruct by apoptosis. Viruses engineered to contain a normal copy of the *p53* gene have therefore been used in gene therapy experiments to infect tumors and insert the normal *p53* gene into the DNA of cancer cells.

Emerging Treatments : Immunotherapy and Molecular Targeting

Table 11-3 Examples of Possible Targets for Anticancer Drugs

Target Protein	Pathway or Function	Drugs Approved*
ErbB2 receptor	Growth factor receptor	Herceptin
EGF receptor	Growth factor receptor	Iressa, Erbitux, Tarceva
FGF receptor	Growth factor receptor	
PDGF receptor	Growth factor receptor	
VEGF	Angiogenesis signaling	Avastin
Bcr-Abl kinase	Apoptosis signaling	Gleevec
Src kinase	Ras-MAPK pathway	
Raf kinase	Ras-MAPK pathway	
Ras	Ras-MAPK pathway	
Cyclin-dependent kinases	Cell cycle progression	
PI 3-kinase	PI3K-Akt pathway	
Hsp90	Stabilizes growth signaling proteins	
Mdm2	Apoptosis inhibitor	
Bcl2	Apoptosis inhibitor	
Matrix metalloproteinases	Invasion/metastasis/angiogenesis	
Proteasome	Targeted protein degradation	Velcade
Telomerase	Limitless replicative potential	

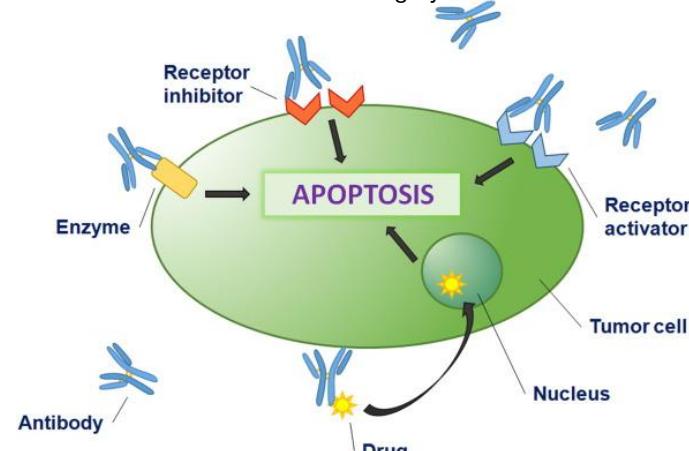
*Drugs listed in this column have already been approved for treating cancer patients.

Antibodies

- ▶ Target specific antigen
- ▶ Specificity is relative
- ▶ Various mechanisms of action
 - ▶ Complement activation
 - ▶ antibody-dependent cell-mediated cytotoxicity ADCC
 - ▶ Calcium entry
- ▶ May synergize with chemotherapy
- ▶ Expected or unexpected toxicities



Direct tumor cell killing by antibodies



This event may be triggered by antibodies binding to a tumor cell surface receptor, leading to its activation and, consequently, apoptosis. Finally, an antibody may bind to an enzyme, leading to signaling abrogation, neutralization and cell death. Conjugated antibody therapies are based around delivering a payload – for example, a drug, toxin, small interfering RNA or radioisotope – to a tumor cell.

<https://www.sciencedirect.com/science/article/pii/S2214647415000215#f0010>

Small molecules

- ▶ Target oncogene product
- ▶ Inhibit signaling at key steps
- ▶ Safer than chemotherapy
- ▶ Specific side effects
- ▶ Specificity is often relative



Side Effects of Cancer Treatment

- Gastrointestinal tract
- Bone marrow
- Hair and skin
- Reproductive tract

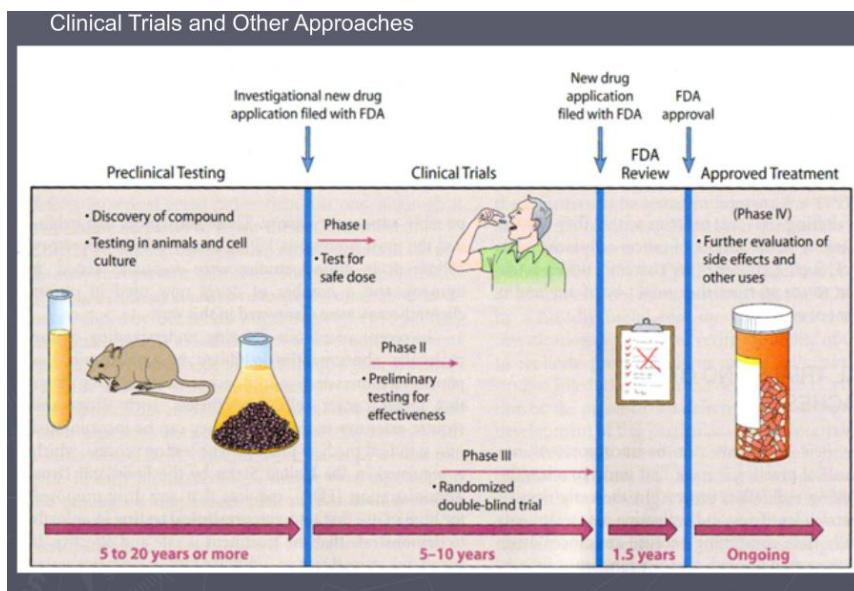
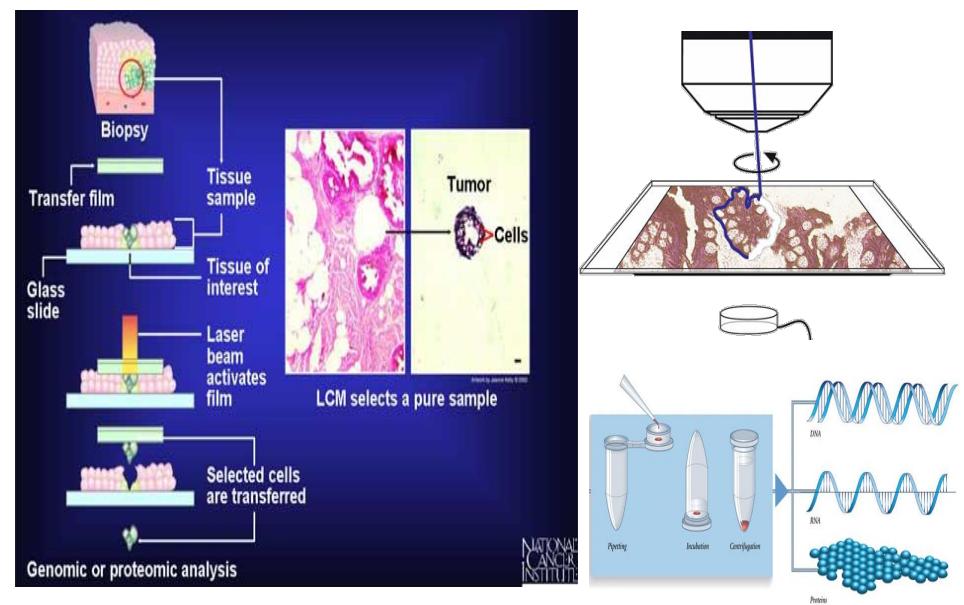


Figure 11-19 Typical Timeline for Developing a New Cancer Drug. Developing a new cancer treatment takes many years and requires numerous steps, including preclinical laboratory and animal testing, clinical trials in cancer patients, and FDA approval. [Adapted from J. A. Zivin, *Sci. Amer.* 282 (April 2000): 70.]

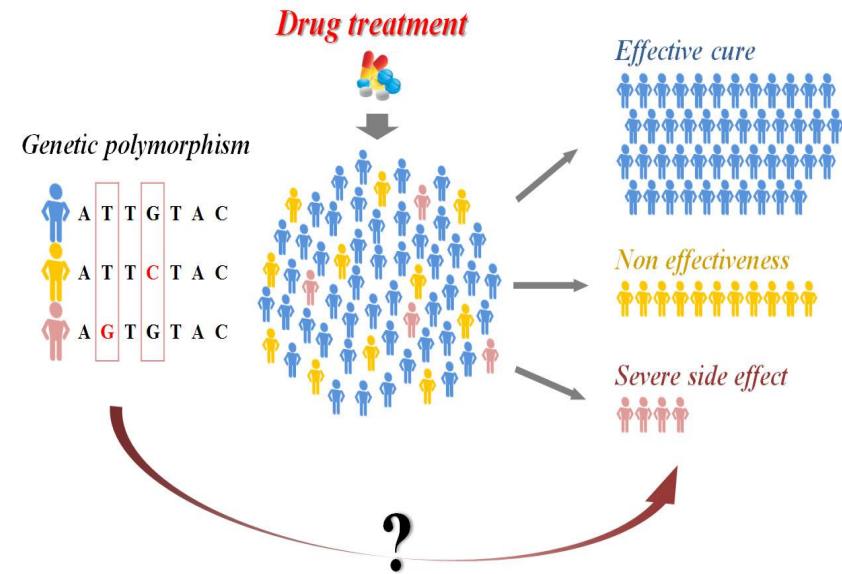
Laser capture microdissection



Pharmacogenomics: drug therapies tailored to individuals

- Design therapies based on the individual's genome
- Subtle, but important, differences in genomes
 - Linked to differences in how one responds to drugs
- Identify those who will suffer harmful side effects from particular drugs and who will respond best
- Customized therapy or personalized medicine

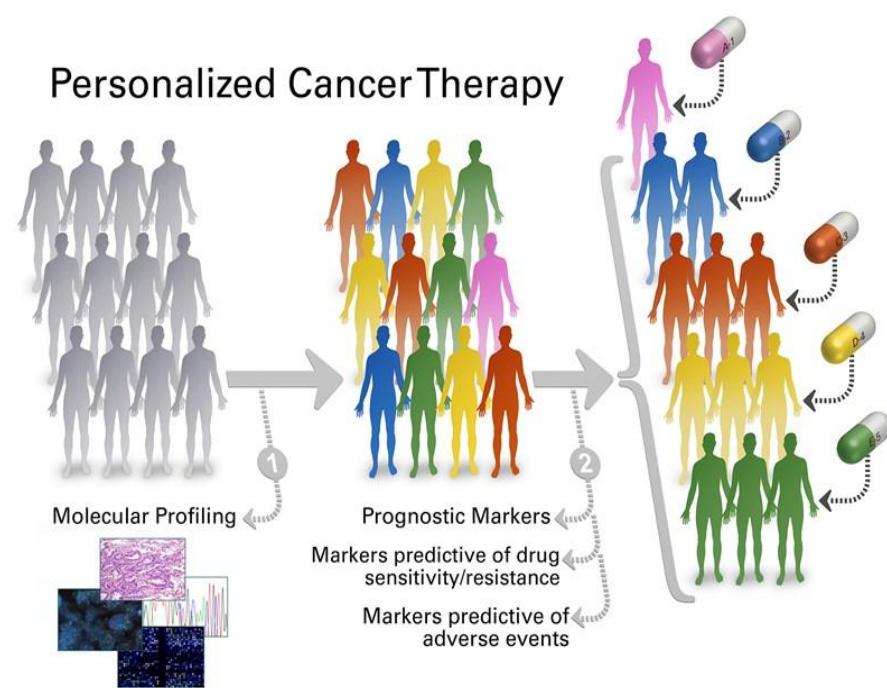
Pharmacogenomics



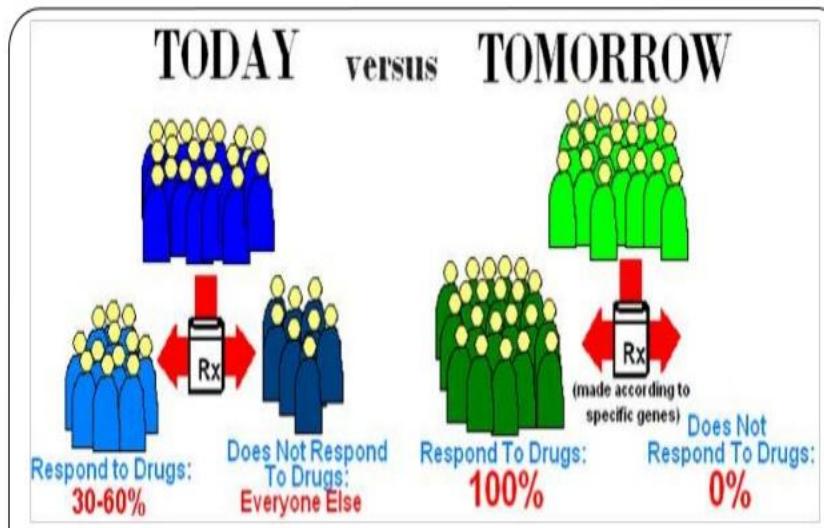
Pharmacogenomics



Personalized Cancer Therapy



Pharmacogenomics



Summary

- Cancer cells proliferate defying normal controls; invades and colonize surrounding tissues (malignant); gives rise to secondary tumors, or metastases; harder to eradicate surgically.
- mutations have important role in cancer.
- phenomenon of tumor progression, takes many years
- It is mainly disturbance of balance between cell division (mitosis) and cell death (apoptosis)
- Thus many factors contribute to the development of cancer, and since some factors are avoidable features of environment, a large proportion of cancers are in principle preventable.
- To cure the disease requires an understanding of the special properties of cancer cells that enable them to evolve, multiply, and spread.
- Drugs for differentiation/ program cell death are good approaches.
- To become malignant, tumor cells must cross basal laminae; antibodies can be designed that interfere with this ability. Drugs can be designed to maintain function of suppressor genes.

Textbook & Readings:

- Alberts B. et al., **The Molecular Biology of the Cell** Garland Science Press, ISBN 0-8153-3218-1 is recommended.
- Robert A. Weinberg, **The Biology of Cancer** Garland Science Press, ISBN 0-8153-4078-8.
- Lauren Pecorino, **Molecular Biology of Cancer**, Oxford University Press. ISBN 978-0-19-921148-7.
- M. Molls, P. Vaupel, C. Nieder, M.S. Anscher. **The impact of tumor biology on cancer treatment and multidisciplinary strategies**, Springer. ISBN 978-3-540-74385-9.
- Yi Lu, R. I.Mahato, **Pharmaceutical perspectives of cancer therapeutics**, Springer. ISBN 978-1-4419-0130-9.

SBL100

Part 1

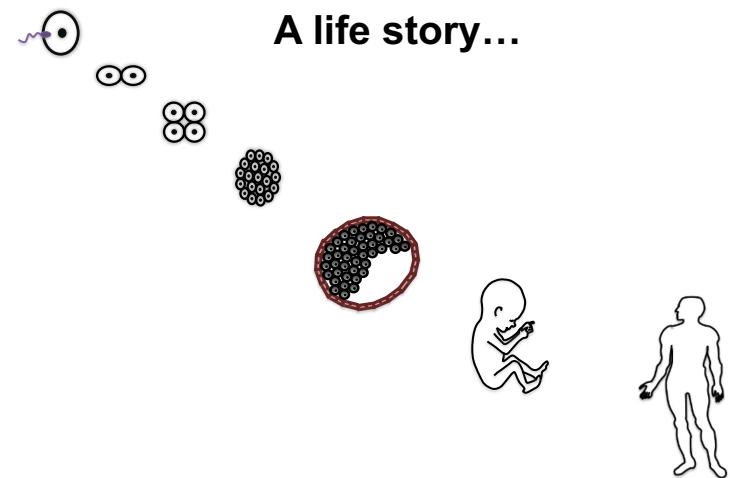
Stem Cells

Today: We learnt basic introduction about Cancer and some therapy

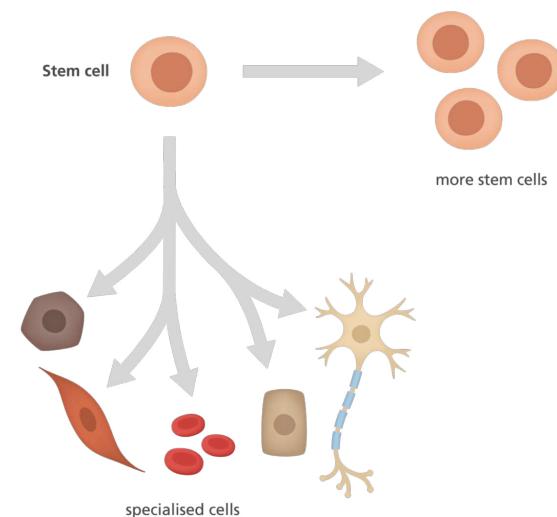
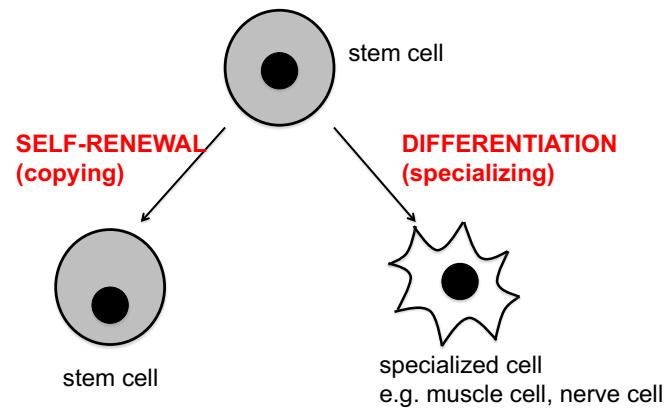
Assignment: what are the measures can be taken to prevent cancer

Next class: Stem cells

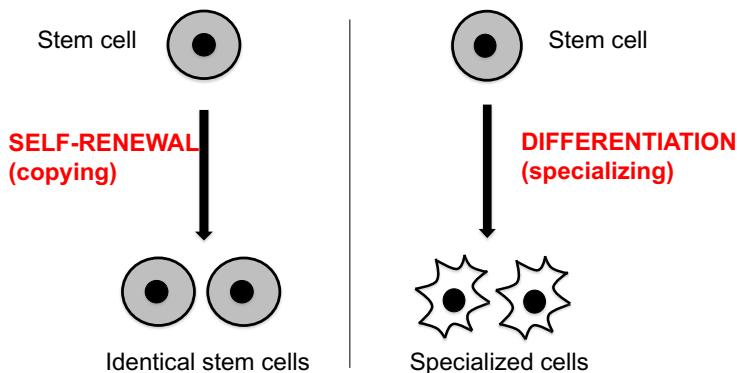
What is a STEM CELL?



What is a stem cell?



What is a stem cell?



Skin Shedding



30000 to 40000 cells per minute
Epidermis – Completely replaced every 28 days
30 90 mg per hour of skin is shed

Definition?

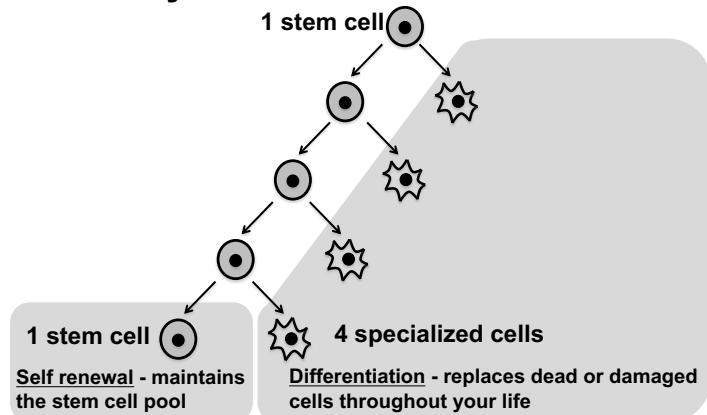
Stem Cell: "functionally defined as having the capacity to self-renew and the ability to generate differentiated cells".

(Doug Melton, 2013, Essentials of Stem Cell Biology, 3rd Ed)

Self Renewal: Cell capable of dividing and giving rise to one or more cells like the parent

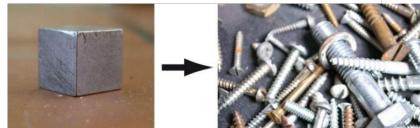
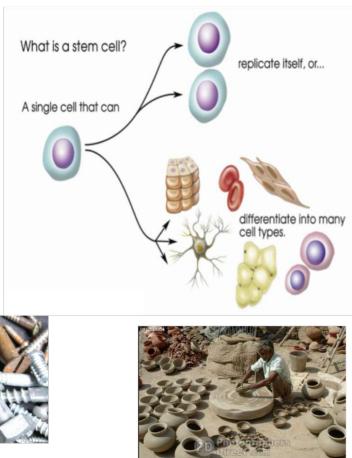
Differentiation: loss of developmental potential and acquisition of specialized traits of a mature cell type

Why self-renew AND differentiate?



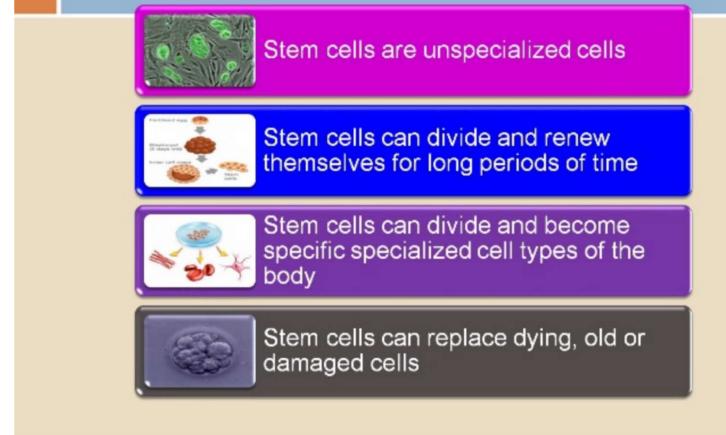
Stem cells

A reserve cell with the capacity to grow and multiply to replace dead or damaged adult cells.

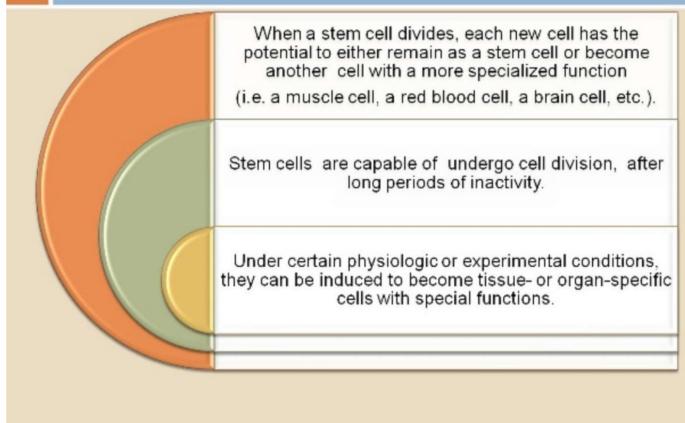


- A cell that has the ability to continuously divide and differentiate (develop) into various other kind(s) of cells/tissues

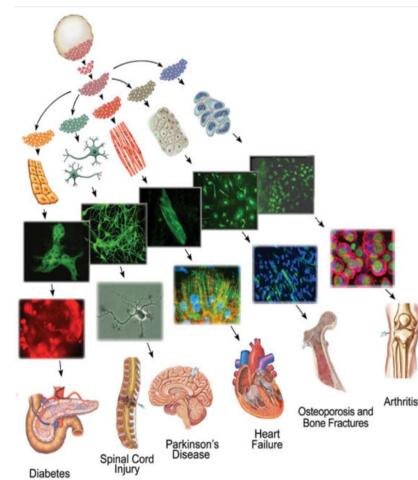
General properties of Stem Cells



Unique features of stem cells



WHY STEM CELLS ??



Many disease or disorder can be cured

- Tissue repair
- Heart disease
- Cancer
- Arthritis
- Parkinson's disease
- Diabetes
- Leukemia

Stem cell therapy.

Numerous diseases and damaged organs could potentially be treated with cell therapy.

- Treatment of neural diseases such as Parkinson's disease, Huntington's disease and Alzheimer's disease.
- Repair or replace damaged neurons.
- Repair of damaged organs such as the liver and pancreas.
- Treatments for AIDS.



Embryonic Stem Cells
blastocyst - a very early embryo



tissue stem cells
fetus, baby and throughout life



Embryonic stem cells come from a five to six-day-old embryo. They have the ability to form virtually any type of cell found in the human body.

Adult stem cells are undifferentiated cells found among specialised or differentiated cells in a tissue or organ after birth. Based on current research they appear to have a more restricted ability to produce different cell types and to self-renew.

Where are stem cells found?

Stem cells exist in both embryos and adults

In embryos

- stem cells function to generate new organs and tissues.

In adults

- they function to replace cells during the natural course of cell turnover.

Stem Cell Jargon

Potency

A measure of how many types of specialized cell a stem cell can make

Totipotent vs Pluripotent

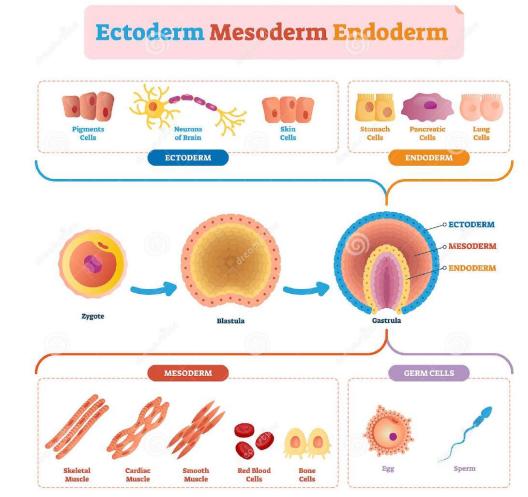
Totipotency

The ability of a cell to make any type of cell in the body



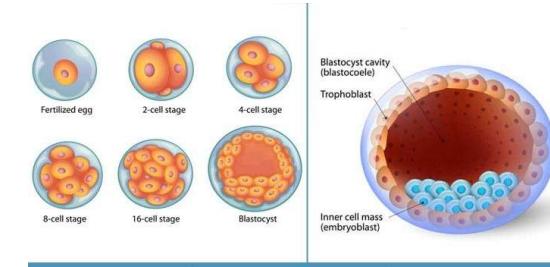
Differentiation

- As the stem cells divide, they are impacted by their spatial positions
- Results in the formation of three germ layers
 - Ectoderm
 - Mesoderm
 - Endoderm



Pluripotency

The ability of a cell to differentiate into cells from any three germ layers

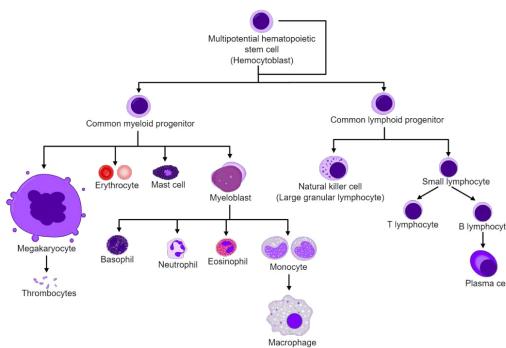


Embryonic Stem Cell (ESC) or
Induced Pluripotent Stem Cell
(iPSC)

Multipotency

The ability of a cell to differentiate into limited range of cell types

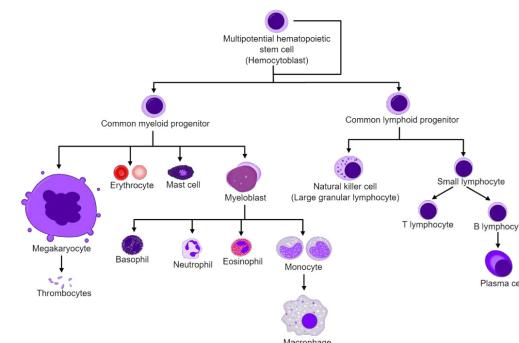
Mesenchymal or
Hematopoietic Stem
Cell



Oligopotency

The ability of a cell to differentiate into limited number of cell types

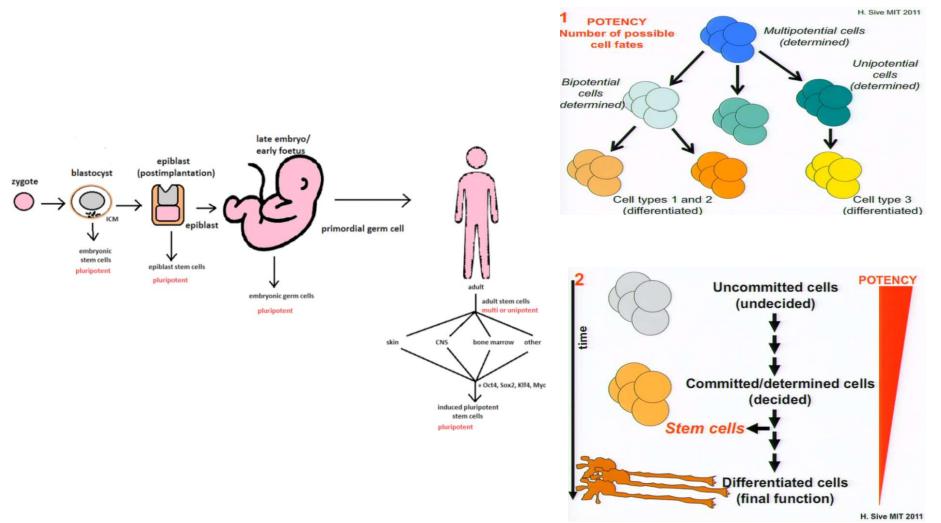
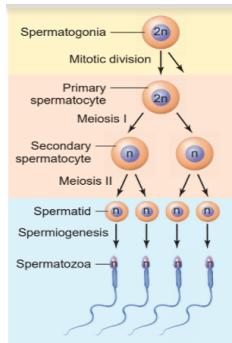
Myeloid or Lymphoid
Progenitor



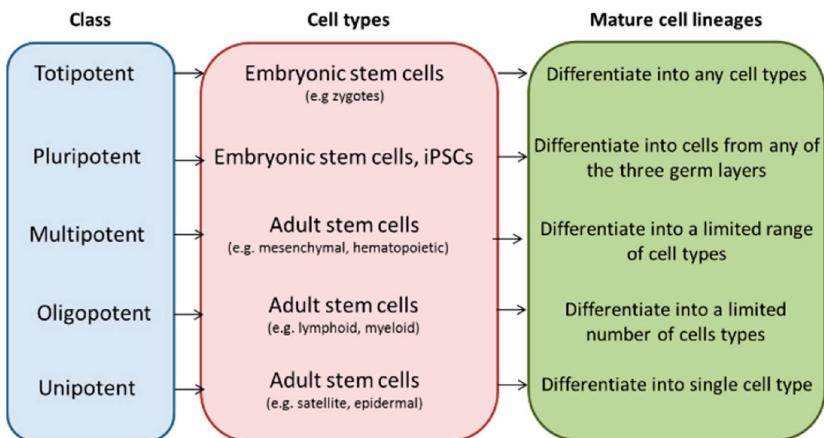
Unipotency

The ability of a cell to differentiate into single cell type

A germ line stem cell
(producing sperm)
Epidermal stem cell
(producing skin)



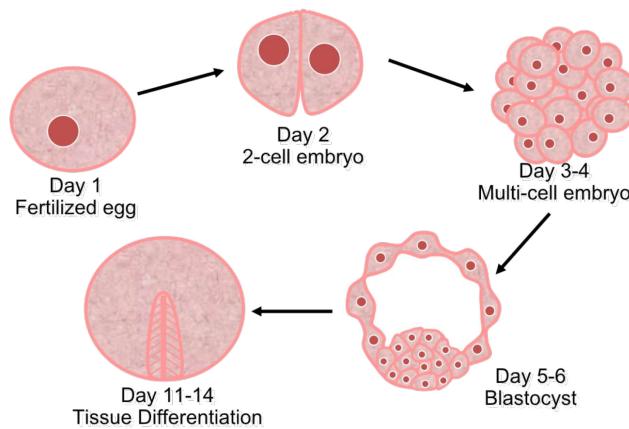
STEM CELLS CLASSIFICATION



Types of Stem Cells

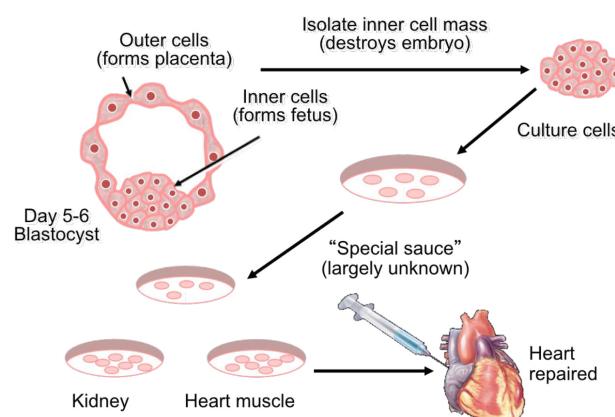
- Embryonic Stem Cells
- Adult Stem Cells
- Induced Pluripotent Stem Cells

Stages of Embryogenesis

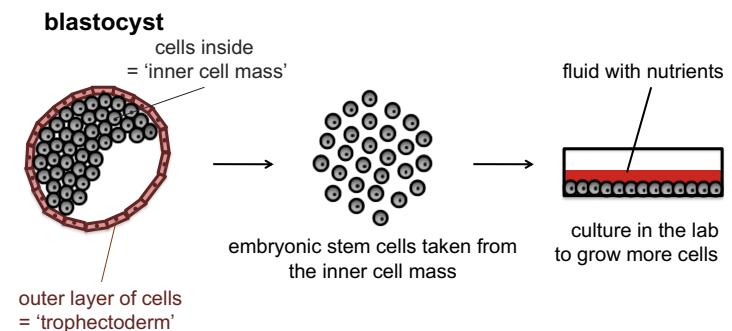


Types of stem cell:
1) **Embryonic stem cells**

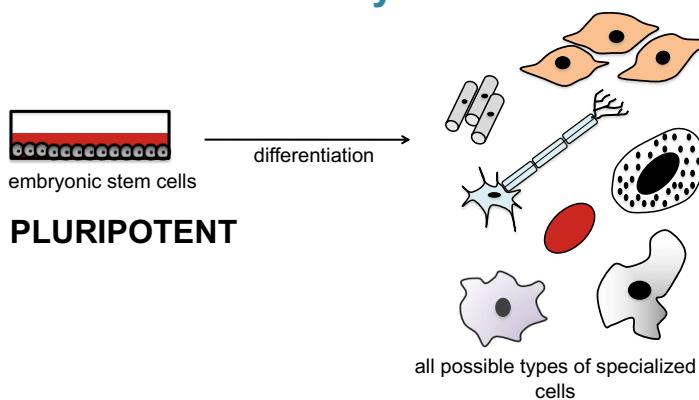
Derivation and Use of Embryonic Stem Cell Lines



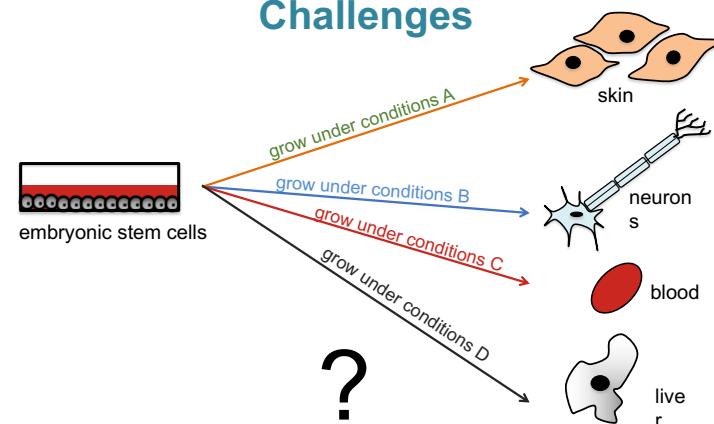
Embryonic stem (ES) cells: Where we find them



Embryonic stem (ES) cells: What they can do

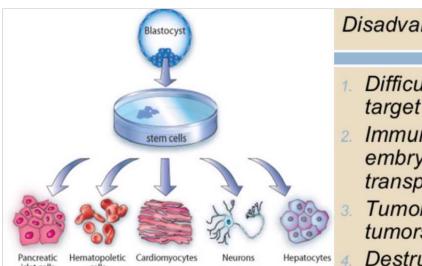


Embryonic stem (ES) cells: Challenges



Advantages of Embryonic Stem Cells

1. Flexible - have the potential to make any cell.
2. Immortal –can provide an endless supply of cells.
3. Availability - embryos from in vitro fertilization clinics.

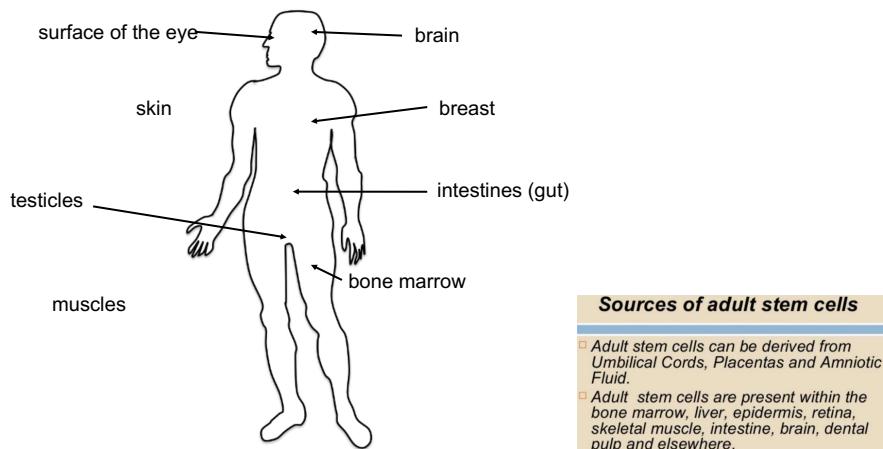


Disadvantages of Embryonic Stem Cells

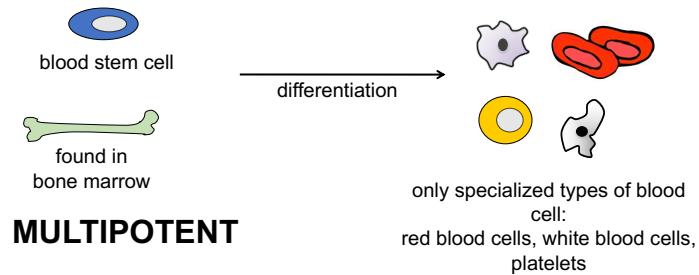
1. Difficult to differentiate uniformly into a target tissue.
2. Immunogenic - cells from a random embryo donor may be rejected after transplantation
3. Tumorigenic - capable of forming tumors.
4. Destruction of developing human life.

Types of stem cell: 2) Adult stem cells

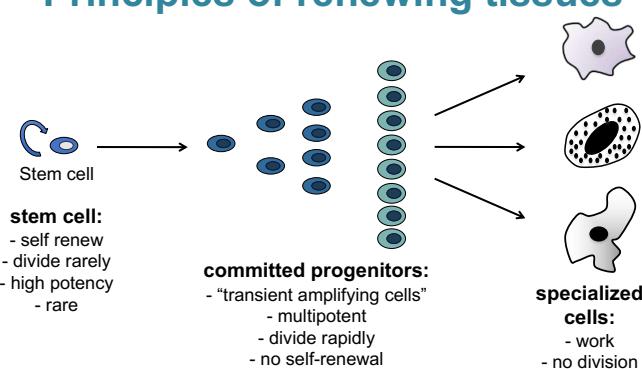
Tissue stem cells: Where we find them



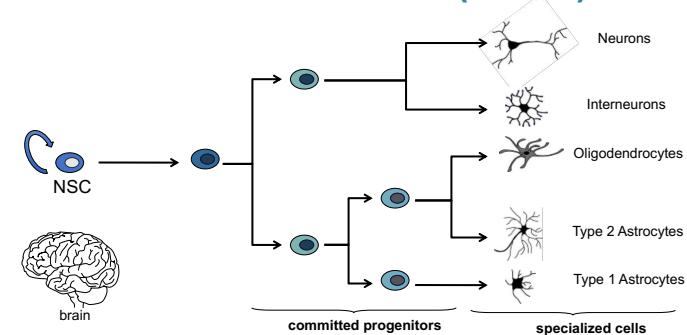
Tissue stem cells: What they can do



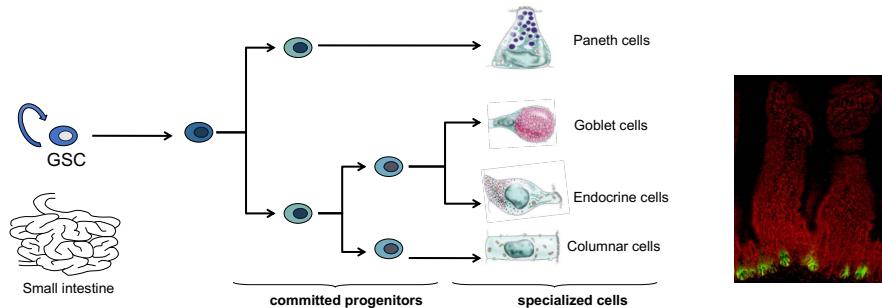
Tissue stem cells: Principles of renewing tissues



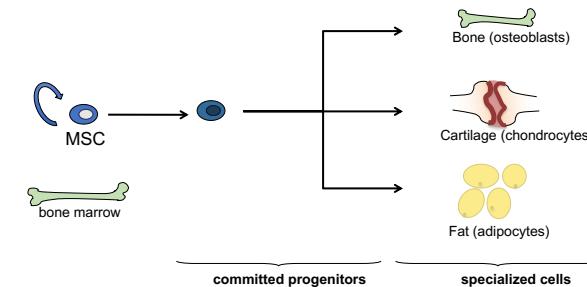
Tissue stem cells: Neural stem cells (NSCs)



Tissue stem cells: Gut stem cells (GSCs)



Tissue stem cells: Mesenchymal stem cells (MSCs)



Advantages of Adult Stem Cells

1. Adult stem cells from bone marrow and umbilical cords appear to be as flexible.
2. Somewhat specialized.
3. Not immunogenic - recipients who receive the products of their own stem cells will not experience immune rejection.
4. Relative ease of procurement - some adult stem cells are easy to harvest (skin, muscle, marrow, fat)
5. Non-tumorigenic-tend not to form tumors.
6. No harm done to the donor.

Disadvantages of Adult stem cell

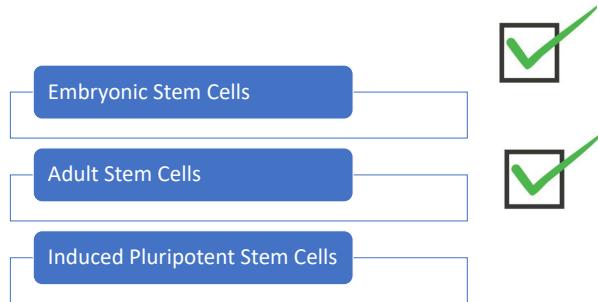
1. Limited quantity - can sometimes be difficult to obtain in large numbers.
2. Finite - may not live as long as embryonic stem cells in culture.
3. Less flexible - may be more difficult to reprogram to form other tissue types

SBL100

Part 2

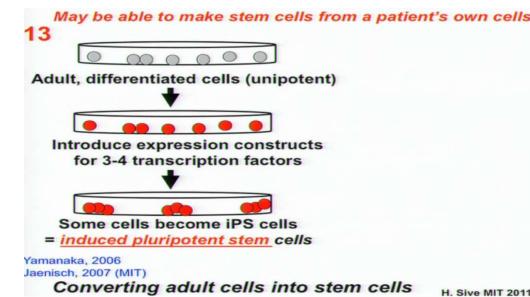
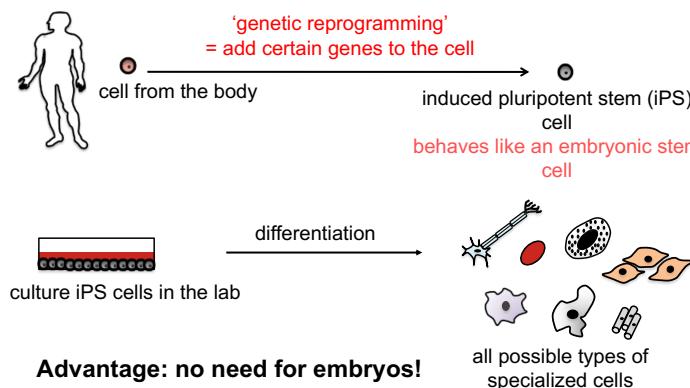
Stem Cells

Types of Stem Cells



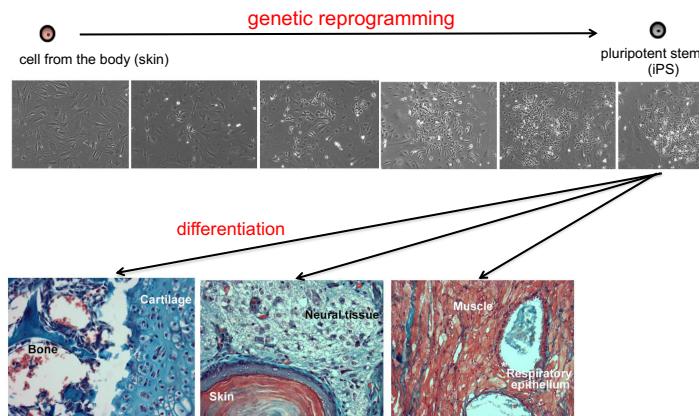
Types of stem cell:
3) Induced pluripotent (iPS) stem cells

Induced pluripotent stem cells (iPS cells)

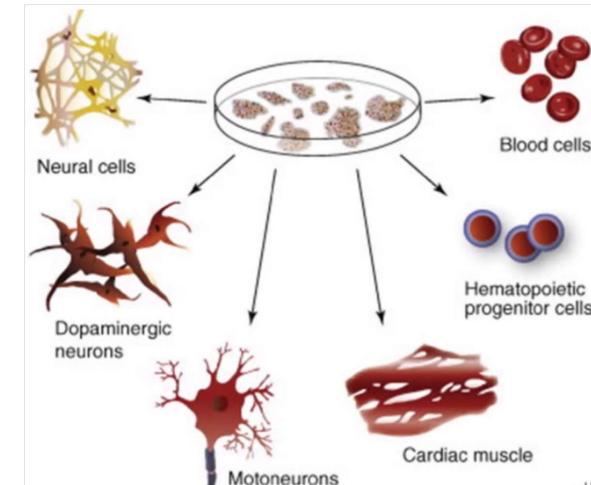


- Therapeutics:**
- Idea: Inject stem cells to repair damaged organ
 - Need stem cells with correct potency
 - Adult stem cells are rare, say around 0.01 % only
 - So use of iPSC are the clever technology to have Pluripotent SC
 - Turned differentiated cells in lab by genetic reprogramming,
 - So very very less chance for rejection

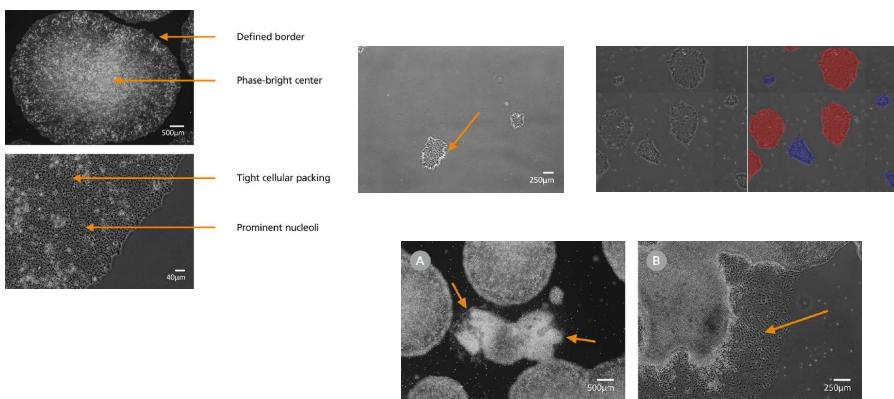
Induced pluripotent stem cells (iPS cells)



Induced Pluripotent stem cells



Induced pluripotent stem cells (iPS cells)



Shinya Yamanaka



The Nobel Prize in Physiology or Medicine
2012 with Sir John Gurdon

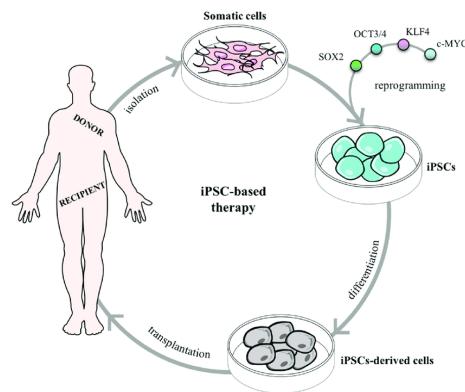
"for the discovery that mature cells can be
reprogrammed to become pluripotent"

Physician to scientist – due to lack of cure for
certain diseases

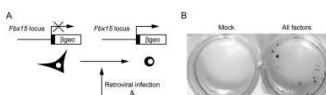
24 Factors to begin with...

- 24 Candidate Factors
- Group 1: Oct-3/4, Sox2, Nanog
- Group 2: Tcf1, Stat3, c-Myc, ERas...
- Group 3: ECAT1, ESG1, Fbx15...

Yamanaka Factors - OSKM



Fbx15 – Specifically expressed in mouse ES cells.



Takahashi & Yamanaka, Cell, 2006,

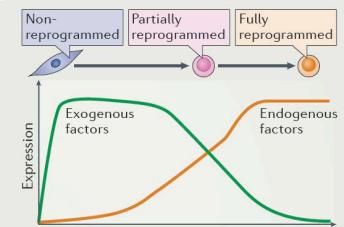
OCT3/4
SOX2
KLF4
c-MYC

Box 1 | Identifying and screening for pluripotency factors

One of the most important details in the generation of induced pluripotent stem cells (iPSCs) was the identification of the key pluripotency factors. To establish the list of candidate genes (see Supplementary information S1 (table)), we first used *in silico* subtraction to identify cDNAs that are specifically enriched in embryonic stem cells (ES cells) in comparison to somatic cells. Using this sequence information, we obtained full-length sequences of cDNAs encoding novel genes that were designated ES cell-associated transcripts (ECATs)⁴.

Yoshimi Tokuzawa, a former graduate student in the Yamanaka laboratory, was a key person in the discovery of iPSCs. She generated knockout mice and ES cells with the disruption of one of the ECATs — F-box only protein 15 (*Fbxo15*) — by using the promoter trap strategy⁵. In this case, β -galactosidase (β -geo) disrupted the gene, and its expression could be used as a reporter of *Fbxo15* promoter activity, as cells expressing β -geo would be resistant to genetin (G418).

As FBXO15-null mutant mice developed normally and were fertile, we could readily isolate FBXO15-null mouse embryonic fibroblasts (MEFs). As expected, with no expression of FBXO15 in somatic cells, MEFs were not resistant to G418 treatment, but FBXO15- β -geo ES cells could survive even in an exceptionally high concentration of G418, suggesting that the endogenous *Fbxo15* promoter was strongly and tightly regulated (see the figure). We then used these FBXO15- β -geo MEFs as a system to screen for reprogramming factors. This system was based on transducing various combinations of candidate genes and evaluating the ability of MEFs to survive G418 treatment (see the figure).



<https://www.nature.com/articles/nrm.2016.8>

Comparison of Embryonic v/s Adult Stem Cells

Embryonic stem (ES) cells Adult stem cells

almost all the cell types of an adult organism.

limited range of cells within a tissue type,

from the inner cell mass of the blastocyst.

within most organs, e.g. in bone marrow, brain, liver, etc

Totipotent and pluripotent stem cells –

multipotent stem cells

Cord blood stem cells



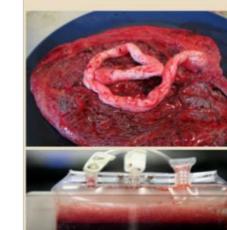
- The umbilical cord and placenta are rich sources of stem cells.
- Cord blood collection is a safe, simple procedure that poses no risk to the mother or newborn baby.

Cord blood stem cells

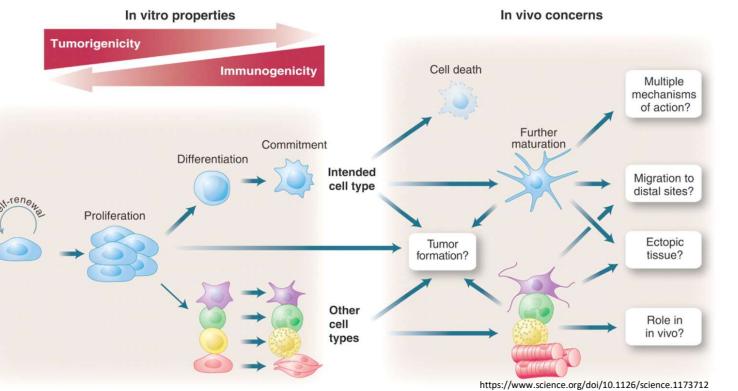
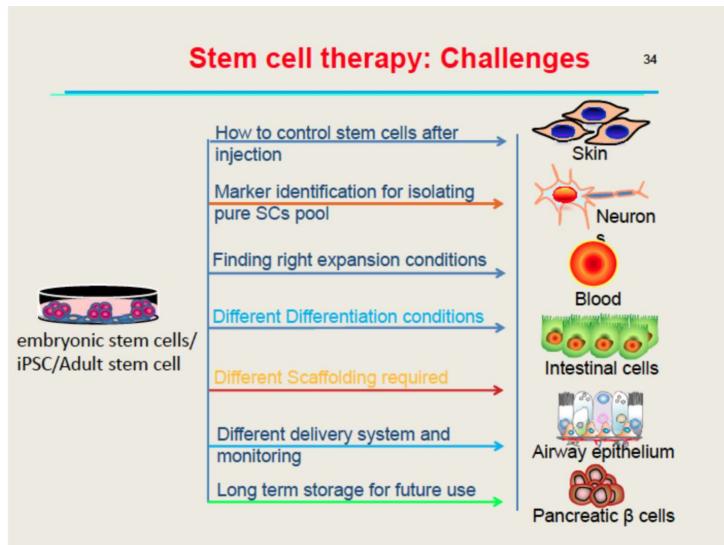
- Cord blood stem cells can grow into blood forming cells, immune system cells or other types of cells.
- Cord blood stem cells have been used to treat 70 different diseases, including leukemia, lymphoma, and inherited diseases.



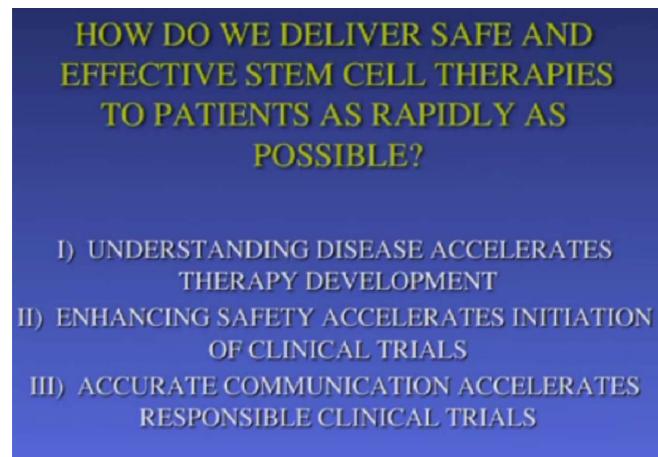
Cord-blood banking



- Cord blood banks collect and store the blood within the umbilical cord and placenta after the birth of a baby.
- The stem cells are separated from the rest of the blood and stored frozen in liquid nitrogen.
- There are 2 types of banks i.e. public and family cord blood banks.



Risk assessment of stem cell-based products. The potential for tumor formation (tumorigenicity) represents a concern correlated with the self-renewal of undifferentiated cells, whereas cells at other levels of maturation may also pose risk. Many stem cell-based therapies will not consist of a pure, homogeneous target cell population, which raises additional questions about risks that nontarget cells may present, as well as their physiologic role after administration. Ectopic tissue formation and migration from the site of transplantation are also concerns, particularly when stem cell-based products are introduced to anatomically sensitive sites. Additionally, differentiation of stem cell-based products that are allogeneic with respect to the recipient results in increased immunologic incompatibility due to expression of foreign nonself antigens. Death of large proportions of the transplanted cell population, not unique to stem cells, may constitute further risk.



Dream is to get Stem cell population for every organ of our body
 Dream is to get repaired any damaged organ or limb
 Just inject correct stem cells to get any organ repaired.



Stem cell based therapy

36

Paolo macchiarini (scaffolding, seeding) and Harvard bioscience

windpipe

- Artificial trachea grown from her own stem cells on a 3-inch-long frame of plastic fibers
- Bone marrow stem cells seeded on scaffold and grown in a bioreactor before transplantation
- Hannah died after three months of the surgery
- Though controversies exist but this story suggest the future use of stem cells for therapy

Bone marrow cancer

Haemophilia

Brain tumour

Skin wounds and burns

Muscular Dystrophy

Stem cells in Plastic Surgery

- Example: *Correction of a deformity by autologous fat transfer:*
- Current- injection of large numbers of heterogeneous cells
- manually- primitive- unpredictable

Recent advancement in stem cells

24 July 2015, University of Texas used silk fibers to provide salivary gland stem cells with a 3D scaffold on which to grow a matrix of salivary gland stem cells.

The achievement is significant because "salivary gland stem cells are some of the most difficult cells to grow in culture and retain their function.

<http://uthscsa.edu/hscnews/singleformat2.asp?newID=5099>



There are currently no treatments for dry mouth, where the salivary glands do not produce enough saliva.

Artificial blood grown in a lab from stem cells is one step closer to being available to people with complex blood types for whom it is difficult to find matching donors.

UK's NHS (National Health Service) Blood and Transplant manufactured blood may be used in clinical trials with humans within 2 years

http://www.nhsbt.nhs.uk/news-and-media/news-articles/news_2015_06_25.asp



Stem cell therapy.

Numerous diseases and damaged organs could potentially be treated with cell therapy.

- ❖ *Treatment of neural diseases such as Parkinson's disease, Huntington's disease and Alzheimer's disease.*
- ❖ *Repair or replace damaged neurons.*
- ❖ *Repair of damaged organs such as the liver and pancreas.*
- ❖ *Treatments for AIDS.*

Stem cell transplantation (SCT)/ Bone marrow transplantation (BMT).

- *When a patient's bone marrow fails to produce new blood cells, for whatever reason, he or she will develop anemia, persistent infections and bleeding problems.*
- *In order to restore blood cell production a patient may be given Stem cell transplantation (SCT) for healthy stem cells.*
- *Stem cell transplants are used to treat malignant diseases, mainly leukemia, lymphoma or myeloma which involve the bone marrow.*

Cancer treatment

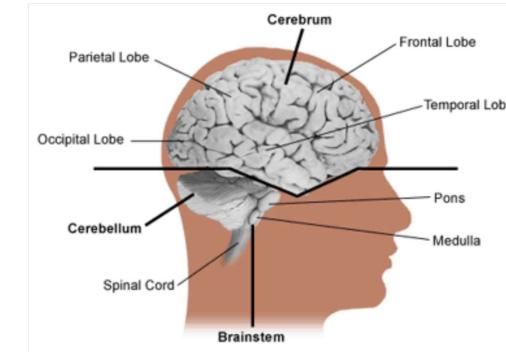
- *Intense chemotherapy damages a person's bone marrow.*
- *A chemo-cancer patient is left vulnerable to infection, anemia and bleeding because of the depletion of fresh blood cells.*
- *Transplanting bone marrow tissue into a chemo-cancer patient may be carried out.*

Skin tissue repair

- *skin, is readily cultured to provide replacement tissue for burns victims.*
- *Healthy skin cells from the patient can be grown rapidly in vitro to provide self-compatible skin grafts.*

POSSIBLE USES OF TISSUE DERIVED FROM STEM CELLS TO TREAT DISEASE	
Cell type	Target disease
Neural (nerve) cells	Stroke, Parkinson's disease, Alzheimer's disease, spinal cord injury, multiple sclerosis
Heart muscle cells	Heart attacks, congestive heart failure
Insulin-producing cells	Diabetes
Cartilage cells	Osteoarthritis
Blood cells	Cancer, immunodeficiencies, inherited blood diseases, leukemia
Liver cells	Hepatitis, cirrhosis
Skin cells	Burns, wound healing
Bone cells	Osteoporosis
Retinal (eye) cells	Macular degeneration
Skeletal muscle cells	Muscular dystrophy

- In China a man with Parkinson's was treated with human ES cells which turned into a tumor (teratoma) in his brain that killed him.



- The power of ESCs is also the source of their peril.

- Embryonic stem cell research requires human cells. This could create a commercial market for human cells. Some may say: "This devalues life"



\$\$\$\$
\$\$\$\$\$

♦ The buying and selling of human reproductive material may be seen by some as a devaluation of human life. Human life is invaluable. It should not be given a price tag.

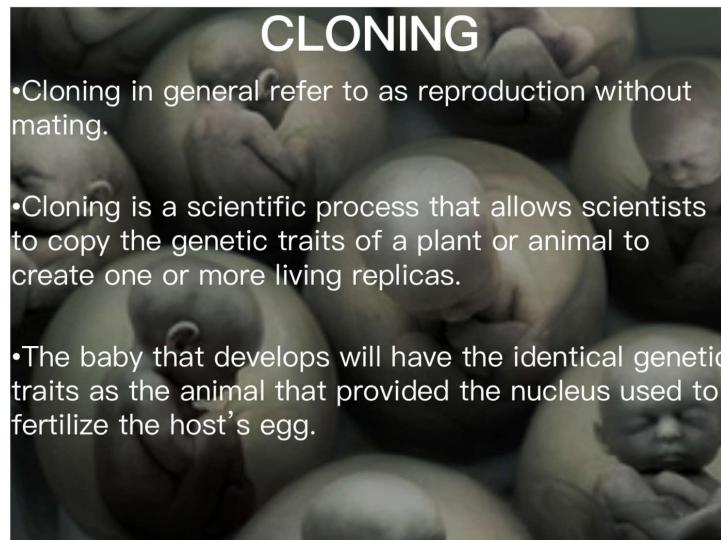
♦ The salesman in this cartoon treats the eggs, sperm and embryos he is selling in the same way that a salesman would treat kitchen appliances or used cars.

SBL100

Part 3

Stem Cells

Cloning



- Cloning in general refers to reproduction without mating.

- Cloning is a scientific process that allows scientists to copy the genetic traits of a plant or animal to create one or more living replicas.

- The baby that develops will have the identical genetic traits as the animal that provided the nucleus used to fertilize the host's egg.

Cloning

There are two **VERY** different types of cloning:

Reproductive cloning

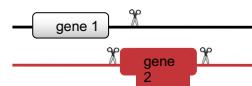


Use to make two identical individuals

Very difficult to do

Illegal to do on humans

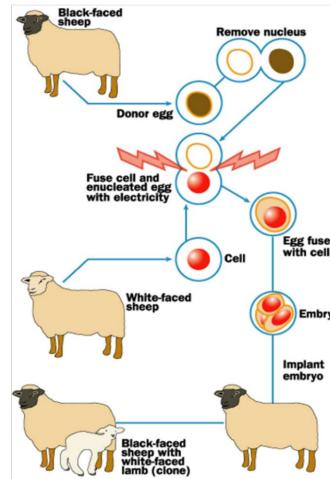
Molecular cloning



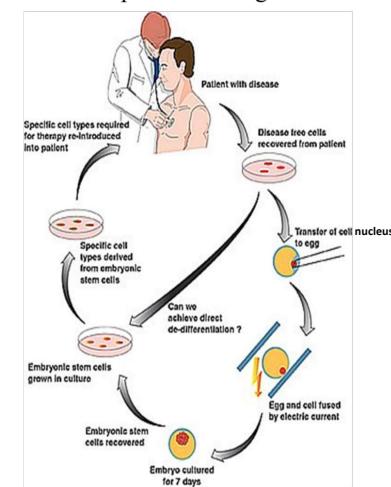
Use to study what a gene does

Routine in the biology labs

Reproductive Cloning



Therapeutic Cloning



THE THERAPEUTIC CLONING VERSUS REPRODUCTIVE CLONING

THERAPEUTIC CLONING	REPRODUCTIVE CLONING
The production of embryonic stem cells for the use in replacing or repairing damaged tissues or organs, achieved by transferring a diploid nucleus from a body cell into an egg whose nucleus has been removed	The deliberate production of genetically identical individuals; each newly produced individual is a clone of the original
Creating embryo develops under laboratory conditions	Creating embryo develops under uterine conditions
Responsible for creating embryonic stem cells to treat diseases such as diabetes and Alzheimer's disease	Important for harvesting stem cells that can be used to study embryonic development

Visit www.PEDIAA.com

Xenopus laevis vs Rana pipiens



Xenopus laevis	Rana pipiens
South African Origin	European and North American Origin
Responds to mammalian hormones (FSH, LH)	Pituitary Gland Extract
Eggs laid throughout the year	Seasonal (Spring)
Aquatic Frog	Terrestrial
One year life cycle	Four year life cycle
Resistant to many infections	More susceptible to infections

Stem Cells : A Historical Perspective



Sir John Gurdon

British Geneticist

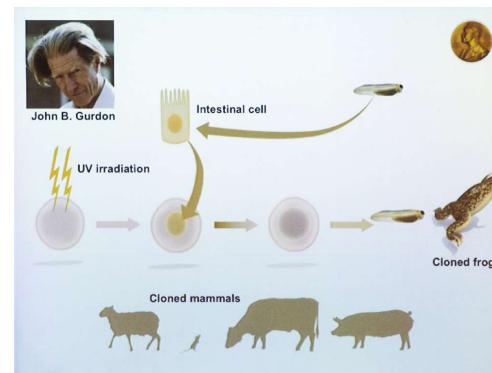
Early struggles – Removed from all science classes

Nuclear Transfer with Xenopus, Year 1962

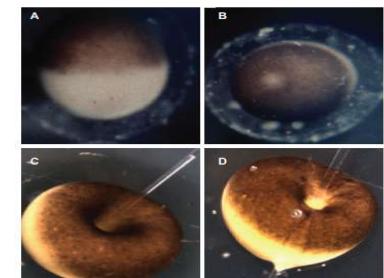
Hypothesis – “Nucleus of a specialized cell contains the complete genome”

Nobel Prize – 2012 Physiology and Medicine

“The Egg and the Nucleus: A battle for Supremacy”



Hypothesis – “Does all cell types in the body have the same set of genes?”



Nobel Prize – 2012 Physiology and Medicine

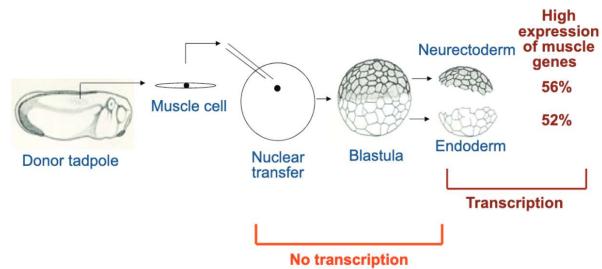


FIGURE 7. Epigenetic memory in nuclear transplant embryos. Nuclear transplant embryos derived from muscle nuclei were grown to the blastula stage, and then depleted of the mesoderm region (muscle lineage). The remaining regions (neurectoderm for nerve/skin cells and endoderm for intestine lineages) express the muscle gene marker MyoD to an excessive extent in about half of all such embryos (Ng and Gurdon 2008).

Source: <https://www.nobelprize.org/uploads/2018/06/gurdon-lecture.pdf>

Sir Martin J Evans

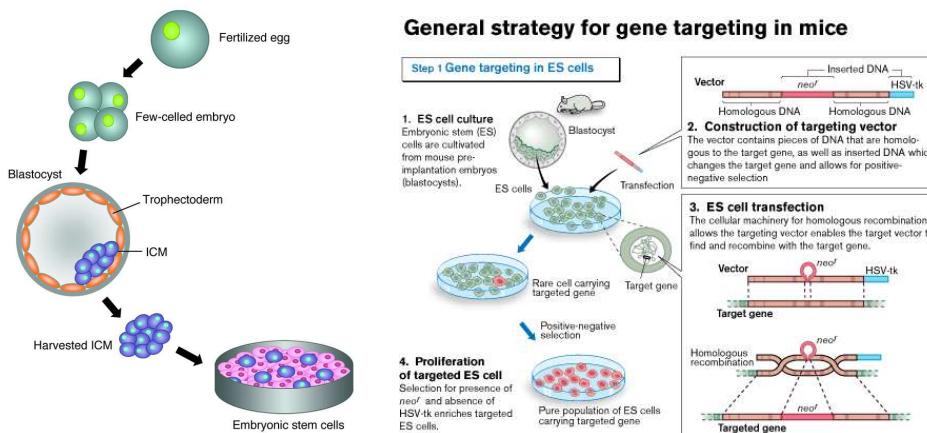
Nobel Prize – 2007 Physiology and Medicine



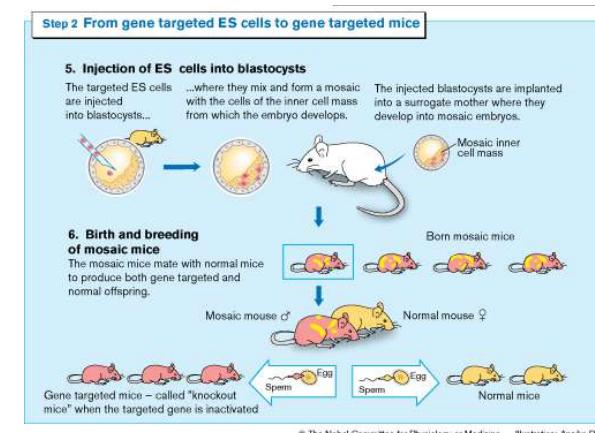
Embryonic stem cells – vehicles to the mouse germ line

For the discoveries of principles for introducing specific gene modifications in mice by the use of **embryonic stem cells**

Mario R. Capecchi, Martin J. Evans and Oliver Smithies



Mario R. Capecchi, Martin J. Evans and Oliver Smithies



Mario R. Capecchi, Martin J. Evans and Oliver Smithies

Nuclear Transfer in Mammals

Took ~40 years, 1996



Dolly – Finn Dorset Ewe
Egg donor - Scotting Black Face

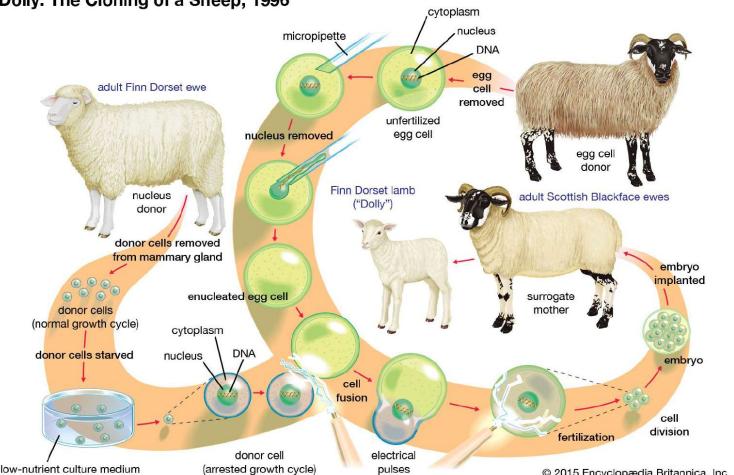


Sir Ian Wilmut



Rosslin Institute 22 Feb 1997
3000 Phone Calls

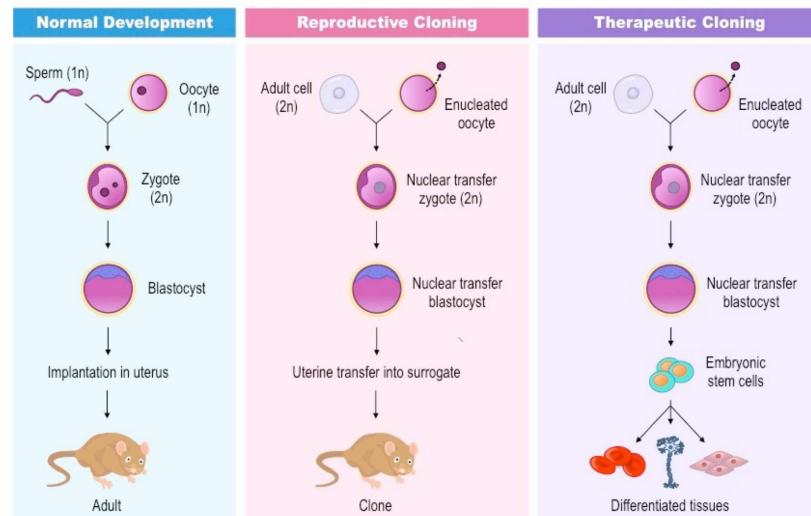
Dolly: The Cloning of a Sheep, 1996



© 2015 Encyclopædia Britannica, Inc.

Dolly's Life

- When Dolly was 1 year Old – Analysis of her DNA showed that her telomeres were shorter than would be expected of that age.
- Dolly could conceive and gave birth to 6 lambs.
- In 2000, lung infection (viral infection)
 - Jaagsiekte Sheep Retrovirus
- 2001 – Diagnosed with arthritis
- 2003 – Tumor in the lungs
- Dolly's body was donated to National Museum Of Scotland Edinburgh



FUTURE ASPECT & CONCLUSION

- Stem cells pose a bright future for the therapeutic world by promising treatment options for diseases which are considered as non-curable.
- Holds hope for curing or improving treatments for 70+ diseases

Is Stem Cell Research Ethical?

Embryonic Stem Cells - morally objectionable, because the human embryo must be destroyed during harvest.

Embryonic Germ Cells - morally objectionable when utilizing fetal tissue derived from elective abortions.

Umbilical Cord Stem Cells - morally acceptable, since the umbilical cord is no longer required once the delivery has been completed.

Placenta-derived Stem Cells - morally acceptable, since the afterbirth is no longer required after the delivery has been completed.

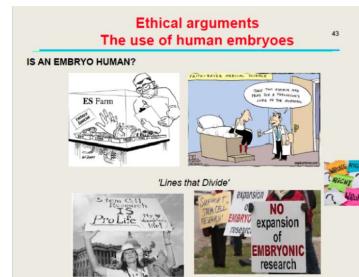
Adult Stem Cells - morally acceptable.

Should stem cell research continue?

- If the government doesn't fund research in this area, private sectors will certainly pursue stem cell research. Private sector research is market driven and not government-regulated.

Stem cell video

<https://www.youtube.com/watch?v=evH0I7Coc54>



Are human playing as God?



RELIGIOUS ISSUES

"Only God has the power to create human life."

"Playing God"

"Some people believe that cloning is similar to playing God. They believe that God should be the creator of all living and natural things."

"Reverence for life"

"God is the Creator of all life. Period."

"and His alone"

"Not Unique"

"People believe human cloning takes away from an individual being unique and stresses psychological and social development."

"Human cloning"

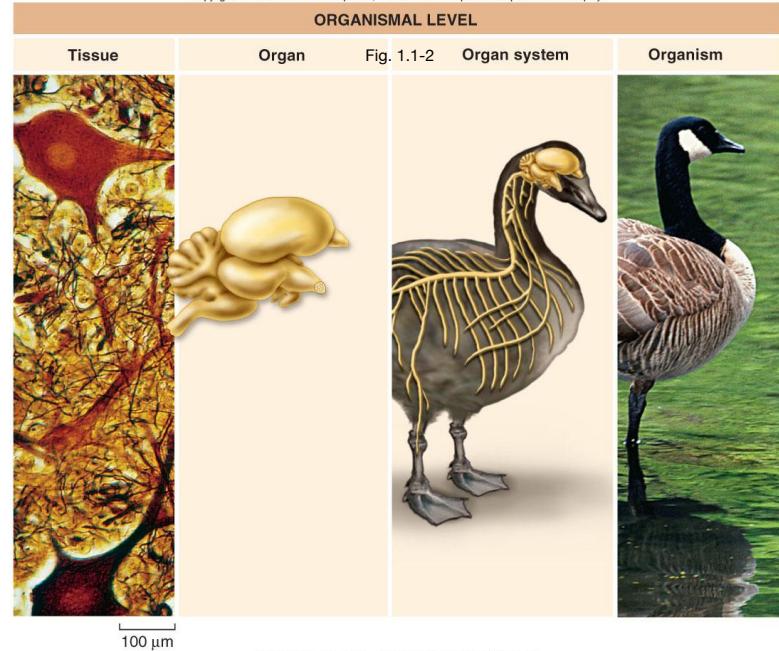
"It is believed that a human has the right for the full human development in a natural environment and that the human embryo should be left alone after the 14th day of fertilization."

"The breath of life is given to us by God - not by scientists splicing genes in a lab."

"Human cloning"

References:

- Essentials of Stem Cell Biology, 2013, Edt: Robert Lanza, Anthony Atala, Academic Press.
- Stem Cells: From Basic Research to Therapy, Federico Calegari, Claudia Waskow, CRC Press, 2014.
- Stem Cells and Cloning (2nd Edition): Kelly M Hogan, Michael A. Palladino
<http://www.nature.com/news/human-stem-cells-created-by-cloning-1.12983>
- <http://www.eurostemcell.org/films>
- Publications
- Reviews and Papers (will be cited on the slide)



SbI100

Biological Machines

Understanding functions of Biological systems are very very complex

Biological machines convert chemical (free) energy to mechanical work, information.

How do they work?

- What structural and chemical features are important?
- How is chemical energy utilized, converted into force, motion?
- What role is played by thermal energy and fluctuations?
- What is unique about the nano-scale: what difference does small size make?

Biological systems are very very complex, the misbalance at any level, gene or cells or tissues leads to various diseases.

Disease is a disorder or malfunction of the mind or body, which leads to a departure from good health.

Can be a disorder of a specific tissue or organ due to a single cause. E.g. malaria.

May have many causes.
Often referred to as multifactorial. E.g. heart disease.

Diseases are diagnosed by a doctor analysing the symptoms (physical and mental signs).

Some common diseases are Cancer, Malaria, Dengue, AIDS, Influenza, Hepatitis A,B,C, Jaundice, Tuberculosis, Schizophrenia, Alzheimer, Parkinson, Cholera, Typhoid etc.

There are still very very severe diseases which cannot be treated so far due to lack of proper understanding which results lack of proper medicines.

Speciality of Our Inner Clock

With utmost precision, our inner clock adapts our physiology to the dramatically different phases of the day.

The clock regulates critical functions such as behavior, hormone levels, sleep, body temperature and metabolism.

Our wellbeing is affected when there is a temporary mismatch between our external environment and this internal biological clock, for example when we travel across several time zones and experience "jet lag".

There are also indications that chronic misalignment between our lifestyle and the rhythm dictated by our inner timekeeper is associated with increased risk for various diseases.

2017 Nobel Prize in Physiology or Medicine

Jeffrey C. Hall, Michael Rosbash and Michael W. Young

molecular mechanisms controlling the circadian rhythm



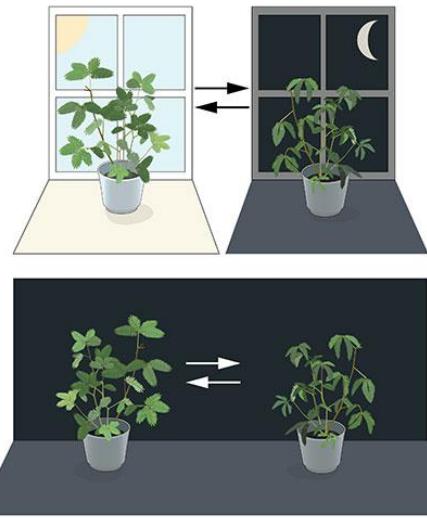
Circadian rhythms control when we're at our peak performance physically and mentally each day, keeping our lives ticking in time with Earth's day/night cycle

living organisms, including humans, have an internal, biological clock that helps them anticipate and adapt to the regular rhythm of the day. But how does this clock actually work?

Using fruit flies as a model organism, this year's Nobel laureates isolated a gene that controls the normal daily biological rhythm.



They showed that this gene encodes a protein that accumulates in the cell during the night, and is then degraded during the day.



In 18th century, Jean Jacques d'Ortous de Mairan studied mimosa plants, and found that the leaves opened towards the sun during daytime and closed at dusk.

He wondered what would happen if the plant was placed in constant darkness. He found that independent of daily sunlight the leaves continued to follow their normal daily oscillation (Figure).

Plants seemed to have their own biological clock.

The leaves continue to follow their normal daily rhythm, even without any fluctuations in daily light.

Summary of findings

- circadian rhythm, originating from the Latin words circa meaning "around" and dies meaning "day".
- circadian rhythms play in coordinating our lives with Earth's day, controlling everything from your metabolism to the timing of sleep.
- Young's lab recently identified a prevalent mutation in a human clock gene, cryptochromes 1, that lengthens the cellular clock and makes it difficult to get to bed before midnight.
- This inherited "night owl" gene is estimated to be pretty common, found in nearly 1 out of 75 of us.
- Those who have a natural "night owl" tendency – delaying school start times by even just one hour can significantly improve academic performance.

Jeffrey Hall , Michael Rosbash and Michael Young discovery

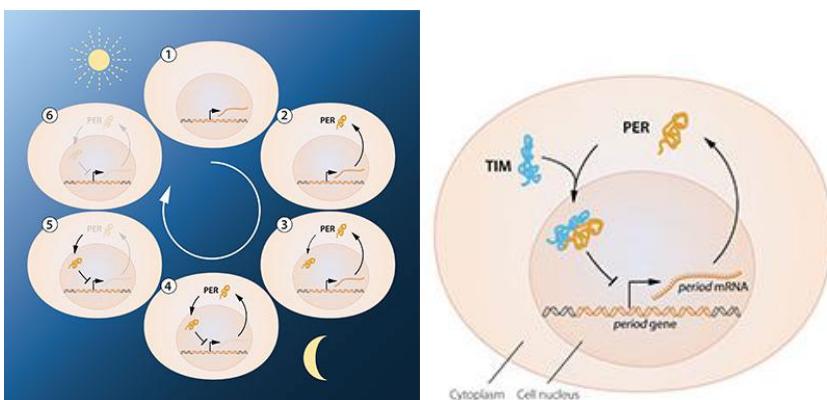
PER, the protein encoded by period, accumulated during the night and was degraded during the day. Thus, PER protein levels oscillate over a 24-hour cycle, in synchrony with the circadian rhythm.

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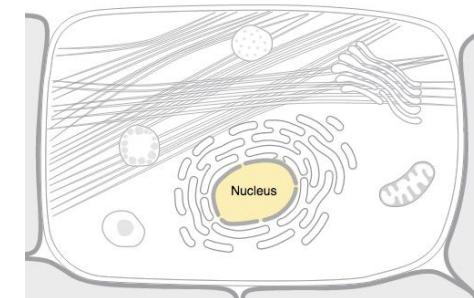
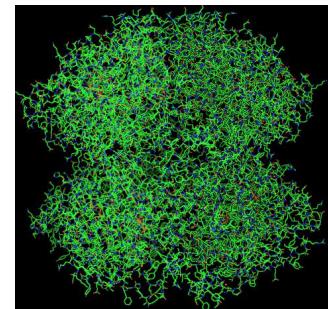
Biological Machines

The biological cycle depends on the rhythmic formation and nuclear localization of the TIM-PER complex.

Light induces the degradation of TIM, which promotes elimination of PER.



They reasoned that by an inhibitory feedback loop, PER protein could prevent its own synthesis and thereby regulate its own level in a continuous, cyclic rhythm.



PER a protein having 1224 amino acids

They hypothesized that the PER protein blocked the activity of the period gene.

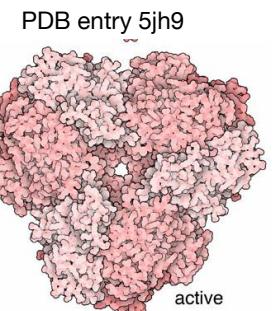
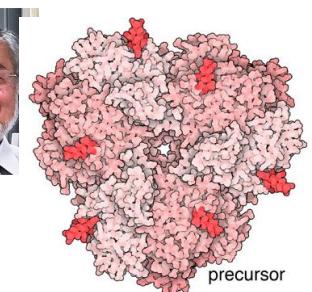
The Nobel Prize in Physiology or Medicine 2016 Yoshinori Ohsumi
Aminopeptidase 1 and Autophagy



Cells are constantly changing, building new molecules when needed and breaking down others when they are finished with them.

The [proteasome](#) and [exosome](#) systems recycle biomolecules one at a time, but the cell also has a system for bulk recycling, termed autophagy. The name means “self-eating,” and that’s just what the cell does.

It packages up the waste and delivers it to its internal digestive system, lysosomes, where everything is broken down by a soup of digestive enzymes.



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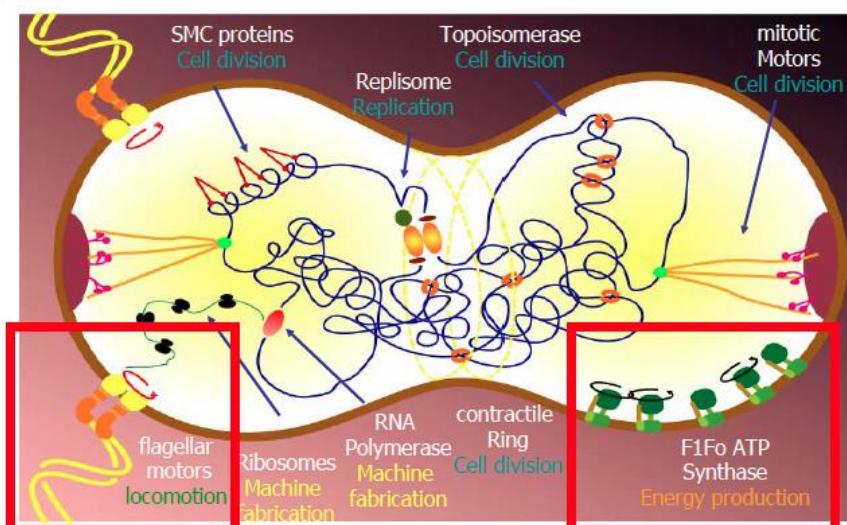
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Researchers at the Institute of Molecular Biology (IMB) in Mainz, Germany said last month that – by studying a type of worm called *C. elegans* – they've made a breakthrough in understanding why humans age. They call the aging process a quirk of evolution

Their work involves identification of the genes belonging to a process called autophagy – from Greek words auto meaning self and phagy meaning devouring – a normal physiological process related to the destruction of damaged cells in the body

Many jobs - many machines

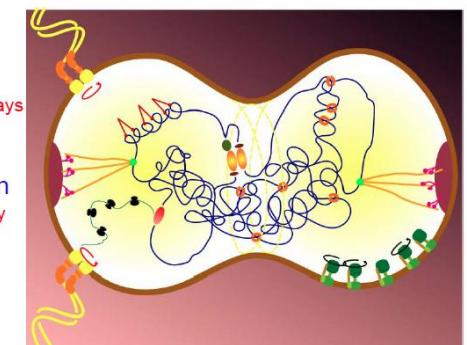


Biological Molecular Machines

Cells are not built in factories
They have to build themselves

Lots of Jobs to Do:

- Getting food and fuel Channels, pores
- Eliminating waste Metabolic pathways
- Converting raw materials to useful stuff
- Building molecular machines
- Copying and protecting genetic information
- Repairing damage Genetic machinery
- Dividing the cell
- Controlling and coordinating all these
- Etc.



There is a machine (or system of machines) for every job

There is a system for making machines (central genetic machinery)

There is a system of coordination among the machines

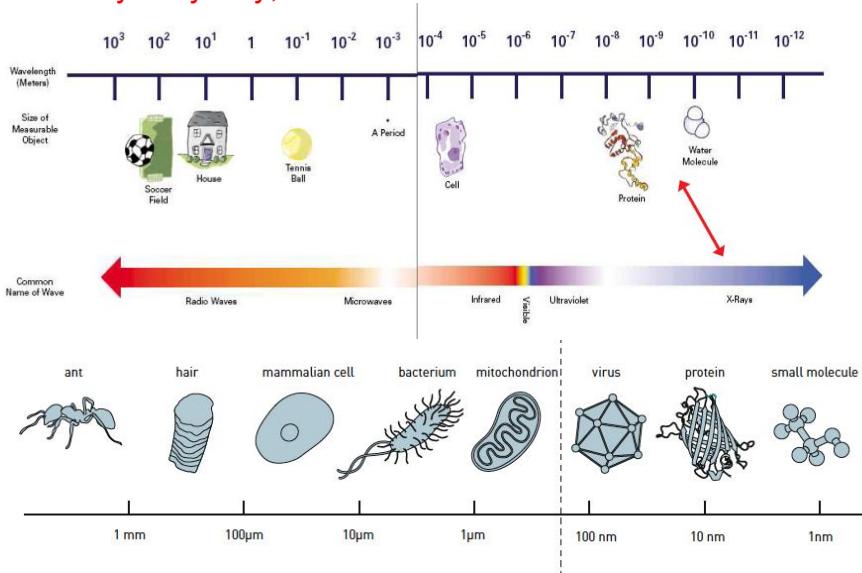
A machine is an apparatus using mechanical power having several parts, each with a definite function and together performing a particular task.

A molecular machine, is “an assemblage of parts that transmit forces, motion, or energy from one to another in a predetermined manner”.

- Living organisms are made up of numerous biological machines which are more complex than artificially constructed machines.
- biological machines are necessary for performing life functions.
- Proteins that hydrolyze ATP perform mechanical work in the cells.
- ATP is analogous to the fuel required by engines to perform work.
- The details of life are finely calibrated.

Molecular machines are highly complex and we are just beginning to understand their inner workings, till date only a few have been studied sufficiently by biologists. So we need great engineers

Main reason in failure of our not understanding the biological problems is because the cells, proteins, DNA are very very tiny,



An example of Molecular machine: Hemoglobin

- Hemoglobin is found in the Red blood cells.
- Hemoglobin in the blood carries oxygen from the respiratory organs (lungs or gills) to the rest of the body (i.e. the tissues).
- Hemoglobin is the protein that makes blood red.
- It is composed of four protein chains, two alpha chains and two beta chains, each with a ring-like heme group containing an iron atom.
- Oxygen binds reversibly to these iron atoms and is transported through blood.
- "Hemoglobin is a remarkable molecular machine that uses motion and small structural changes to regulate its action."



Noble Prize 2017 in chemistry
For discovery of Cryo-Electron Microscopy

Jacques Dubochet University of Lausanne in Switzerland, Joachim Frank at Columbia University, and Richard Henderson at the MRC United Kingdom

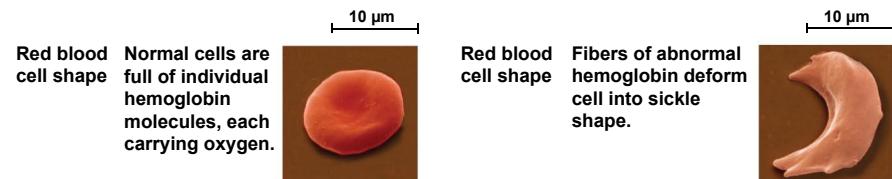
The wavelength of electrons is much shorter than that of light, it can reveal much finer detail

high-resolution, 3D images to target cancer drugs and demystify the dengue, Chikungunya and Zika virus and many many more.

- Hemoglobin structure has been explained by Max Perutz's 18-year quest.
- Hemoglobin uses iron within its protein structure to carry oxygen from the lungs to the rest of the body through the blood.
- The breathing required a protein channel to open for O₂ to access the heme.
- The coupled change of local and quaternary conformations between oxy and deoxy forms explained the cooperativity of O₂ binding.

- Some organisms like snails and crabs, on the other hand, use copper to transport oxygen, so they truly have blue blood.
- Aside from oxygen transport, hemoglobin can bind and transport other molecules like nitric oxide and carbon monoxide.
- Nitric oxide affects the walls of blood vessels, causing them to relax. This in turn reduces the blood pressure.
- Carbon monoxide, is a toxic gas. It readily replaces oxygen at the heme groups, forming stable complexes that are difficult to remove.
- This abuse of the heme groups blocks normal oxygen binding and transport, suffocating the surrounding cells.

Sickle-Cell Disease: A Simple Change in Primary Structure



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A slight change in primary structure can affect a protein's conformation and ability to function.

Sickle-cell disease, an inherited blood disorder, results from a single amino acid substitution in the protein hemoglobin, unable to transport oxygen

	Primary Structure	Secondary and Tertiary Structures	Quaternary Structure	Function	Red Blood Cell Shape
Normal	1 Val 2 His 3 Leu 4 Thr 5 Pro 6 Glu 7 Glu		Normal hemoglobin	Molecules do not associate with one another; each carries oxygen. 	
Sickle-cell	1 Val 2 His 3 Leu 4 Thr 5 Pro 6 Val 7 Glu		Sickle-cell hemoglobin	Molecules crystallized into a fiber; capacity to carry oxygen is reduced. 	

Artificial Blood

- Blood transfusions have saved countless lives.
- However, the need for matching blood type, the short life of stored blood, and the possibility of contamination are still major concerns.
- The main challenge is keeping the four protein chains of hemoglobin together; the four chains rapidly fall apart.
- To avoid this problem, novel hemoglobin molecules have been designed where two of the four chains are physically linked together. Two additional glycine residues form a link between two of the chains, preventing their separation in solution.
- There are two basic approaches to constructing an oxygen therapeutic. The first is [perfluorocarbons](#) (PFC), chemical compounds which can carry and release oxygen.
- The second is haemoglobin derived from animals, or artificially via [recombinant](#) technology, or via stem cell production of [red blood cells](#) in vitro.

References:

- Lehninger g Principles of Biochemistry. 5th Edition. 2008. David L. Nelson and Michael M. Cox.
- Molecular Cell Biology. 5th Edition. 2004. Lodish, Berk, Matsudaira, Kaiser, Krieger, Scott,Zipursky and Darnell.
- Alberts B and Lye RM (1992) Unscrambling the puzzle of biological machines: The importance of the details. *Cell* 68:415-420.
- Howard J (1997) Molecular motors: Structural adaptations to cellular functions. *Nature* 389:561-567.
- Kikkawa M (2013) Big steps toward understanding dynein. *J Cell Biol* 202:15-23.
- Porter ME and Sale WS (2000) multiple inner arm dyneins and a network of kinases and phosphatases that control motility. *J Cell Biol* 151: F37-42.
- Sowa Y and Berry RM (2008) Bacterial flagellar motor. *Quarterly Rev Biophy* 41:103-132.
- Vallee RB, Williams JC, Varma D, Barnhart LE (2004) Dynein: An ancient motor protein involved in multiple modes of transport. *J Neurobiol* 58:189–200.
- Molecular motor: NATURE | VOL 422 | 17 APRIL 2003 | www.nature.com