Stem Cells 3 + Virology

11/21

(2 min late)

As more time goes on - more specialized
can't put in wrong place

Must watch histo-compatability
Can't keep in immune-suppressed state for some time

Could we grow in vitro?
But how do we cause cells to grow differently
Generate ES cells
Implant in the embryo

Inducing agents → contextual signals
how to make ES cells from own tissue?
try to recreate one using a normal cell
Can introduce adult cell nucleus into an egg (w/ nucleus removed)
diploid

(2)

Does genome DNA change during stem cell differentiation?

Yes → on B cells
(; not T;)
No → elsewhere!

How plastic is the ~~xx~~ mammary gland cell?
Can we turn stuff on and off?

Yes → got it to assume program of early fertilized egg - not mamary gland
Add chemicals to mimic sperm to have it divide
Add hormones to fake pregancy

Could we do this to regenerate some of our cells?
(we already did a P-set on this...)

- Yes - just don't let process go to completion

Lots of problems:

Getting eggs - pay a young woman
- expensive + painful
- ethical

Very Finicky

(3)

Lots of tries

Often the cloned org grows very big + dies

We don't know why ...

Virology

Virus is small partical

much smaller than cell it effects

Carries DNA

invades cells

gets cells to produce more virus progeny

Can only live w/ cells

diff kinds

rod-like

loose helical

Spherical

Virion = virus particle

9

Very small / efficient genome
down to 1 protien even!

And you can encode a large DNA w/.

Nucleocapsid
lipid bylayer
not just RNA + protien

~~Icosahedral~~ structure
Icosahedral

Carry double + single stranded
DNA RNA

capsid are encoded by viral genome

Can spontaneously form nucleocapsids

Can be immunogenic
- host can form antibodies
  - like Gardisal prevents against HPV

Sticking out of lipid bylayer are transmembrane spikes
- allow virus to tether themself to cell
- allow ____ to be introduced to cell

retrovirus RNA → DNA
- are diploid – 2 identical copies of genome

Dengue virus
- mosquito is million times bigger in real life
  - (silly picture!)
- virus tether themselves to normal protiens

Bacteriophages

Plate on lawn of bacteria

Then initially infected cell kills neighbor cells

Overlay w/ a bit of agar (Cisp)

So it moves not far

Can see how many units of virus originally

Same w/ eukaryots virus like polio

kills cell in 10 hrs

Cytopathic - kills host cells

So can generate quantification of a solution of virus - a virus stock

Only 1 % may be active

Pretty sloppy when making progeny

We want to know how many actives we have

(1)

Note time after adsorption – sticking to the cell

Some get in via fusion w/ cell membrane
allows virus nucleoplasmic core into host cell
⊖ charge, so hard to do
Very evolved proteins

Pinocytosis engulfs external pro(?)

The double stranded virus DNA could be immediately transcribed
Or only after a ~~second~~ replication

Nucleocapsoid core coats itself w/
plasma membrane
- steals a patch of it
ie HIV

(8)

But how many viruses are there?

diff classes of DNA

Diagram of how virus replicates

Class 1 Virus very parasitic on host cell
so only needs a small # of genomes

episome = non chromosome DNA
? of host cell

Class 2
Single stranded DNA
like feline parvovirus
can't transcribe DNA when single stranded
so transitions to double stranded

Class 3
RNA genomes
Single or double stranded
but not as stable as DNA
must bring in its own RNA polymerase
Virus encoded
can use double stranded RNA to make mRNA

(9)

Class IVa! most cold viruses

Single stranded RNA

has poly A just like mRNA

Can jump onto ribosome and be treated like normal

needs to make RNA based RNA polymerase

Converts viral RNA from single → dable stranded

Everything in cytoplasm → not nucleus

Next the Class IVb + Class V

7.012

11/26

Viruses Cont

(Via video on 11/29)

Viruses = nucleic acids

classified according to their genome

DNA + RNA genomes

- ~~Sno~~ dsDNA
- Single DNA
- Single RNA
  - pAs in cytoplasm
  - translated
  - "pos-stranded RNA"
    - same polarity as mRNA
    - not complementary strand "(-) stranded RNA"

~~- Can't rely on RNA~~

Class 3 Double RNA

Must bring its own RNA polymerase

(2)

Class 5 single ⊖ RNA

Most common

Complementry to coding strand

Must carry a RNA-dependent RNA polymerase

Class 6 single RNA + DNA

Retro virus

inc HIV

details tady

Reverse transcription RNA → DNA

At first we assumed virus has cytotoxic effect on cell
- actually kills cell
- many have this, inc cold virus

Use this as a way to measure virus activity
know how many plaque forming units present
(review what virus plaques are)

(3)

But remember viruses sloppy
actually # of particles may be 100x higher
physically affected
can't actually make changes

Peyton Rocks
Chicken
Sarcoma (?sp) - tumor
connected tissue
not epithelia (?sp) - skin

grinding it up - passed through filter
put in young chicken
hen gets sarcoma

Suggested one could transfer phenotype
of a tumor through a filter!

(4)

This is not a cytoplasmic effect

Transforms cell to neoplastic (cancerous growth state)

temperate interaction

allows cell to survive in a diff state

HIV push through plasma membrane

Can go on forever w/o killing the cell

Rous virus cells pile up on each other

↳ foci (live)

Plaque = dead

monolayer = 1 cell thick

when touch one another stop growing

↳ contact inhibition

Cancer cells lose this

Can see w/ naked eye

(5)

So descendents of original cells continue to be transformed
└ direct lineal descendents
└ so must be heretical
~~Viral~~ genome └ viral genome passed down

SV 40 DNA is replicated extra-chromosome
not associated w/ chromosome
ind replication

How does it do that?

1968 → SV 40 Rat cell experiment
- isolated DNA + broke up + put on sucrose gradient
- for centrifugal analysis
- RNA broken up, DNA survives
- Form 1 = closed, 1 nick, can't unwind itself
- Form 2 = single nick, so can relax
- Where does mass of DNA go?

(better organized lecture)

(6)

green is nucleus - where it goes when normal but only detected in normal DNA

done at high pH - so H bonds broken

so could not separate

∴ SV40 DNA must be linked w/ cell DNA

└ integrated w/ DNA of host chromosome

ensured viral DNA perpetuated

SV40 usually not integration

non-homologous recombination ← took VA

= recomb b/w DNA seq w/no seq homology

└ same origin

Some accident 1/1000

But Rous has a ssRNA

└ single stranded

So how does it end up in decendents?

also carries transforming gene

neoplastic = cancerous transformation

(7)

Howard Temin when viral RNA goes into cell
↳ reverse transcription

people thought he was crazy!
then integrated into DNA
here this is a certain part
functionally equiv to host cell gene
key step was to find enzyme to do that

retro since opposite of conventional direction
could be translating RNA
but instead reverse transcribe
entering RNA not imm. transcribed
(but not in polio?)

sheds no light on transformation

(8)

Transformation

Avian leukosis virus (ALV)

from 1 chicken to another

Very similar to Rous sarcoma virus

but does not cause transformation

Ras sarcoma - a ! off change!

Same 3 genes to let virus replicate

SRC encodes this transformation

↳ causes important

Where did we get this?

↳ on rare occasion get

happens pleiotropic - single gene has multiple downstream effects

↳ recalibrates multiple regulatory effects

32:00

made a nucleic acid probe

reacted w/ SRC seq

(9)

By reverse transcribing Src genome
made ⊖ stranded copy cDNA copy
got rid of viral RNA

Then hybridized to mutant Rous virus
(which was missing Src)

The Src cDNA wouldn't bind to anything
So they could isolate that and use it to check
for Src sequences!

So where else is this seq?

Looked in virus + non virus infected seq

But it hybridized to normal chicken DNA!

quail + turkey + ducks as well!
also a sponge!
in all animal DNA!

Suggests behaves like a cell gene
Been preserved in all genomes
So must have done / are doing some important function
Can't get rid of or animal dies (won't reproduce)
The further away on tree, the more diverse the genes

Src gene is in normal cells
So Ras Sarcoma stole this gene from somewhere
didn't invent it

So <u>proto-oncogene</u> present in normal DNA
but can be copied / transduced by
virus + carried around by virus

Almost all ~~gen~~ retroviruses have some of these
~20-40 of them
"stolen" proto onco genes

(IV)

Not part of normal ~~gene~~ viruses

Since break virus's ability to replicate

Only 1- offs

Accidents that we discov this

Why do we keep proto onco genes around?

They are required for normal functioning!

But how does Src gene transform cells at bio chem level?

Src makes a Src protien

acts as a kinase

- an enzyme that takes 3rd Phosphate off ATP gives to protien phosphate

(12)

Antibody that immunieprecipitate Scr protien

So Antibody became phosphorilated

Lgot a protien

Only in lab

(kinda confused) this was inside cell
antibodies usually only outside cell

400-500 kinases in cells

Src protien unusually phosphorilation

Tyr

So Tyr-kinase = Tk

rare in normal cells

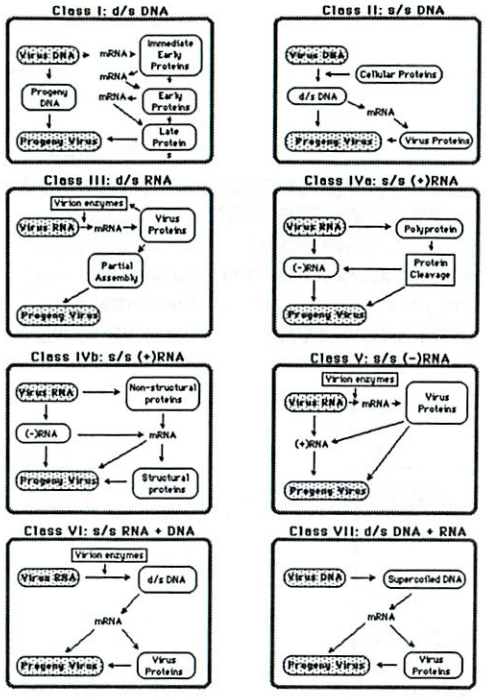
usually stimulates growth of cell

When Scr active lots of these signals

So Catalytic

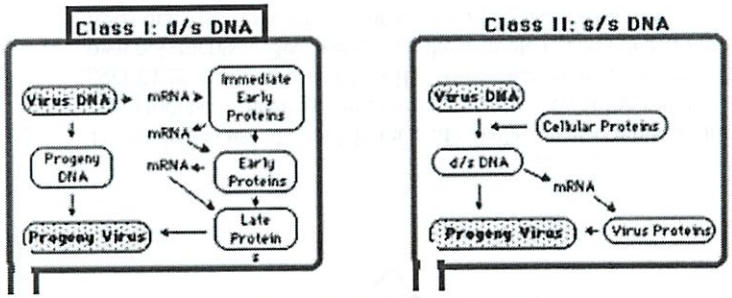
Tumor Virology 7.012

Viruses classified according to their nucleic acid genomes



Viruses classified according to the structures of their genomes

Let's look at these one at a time.

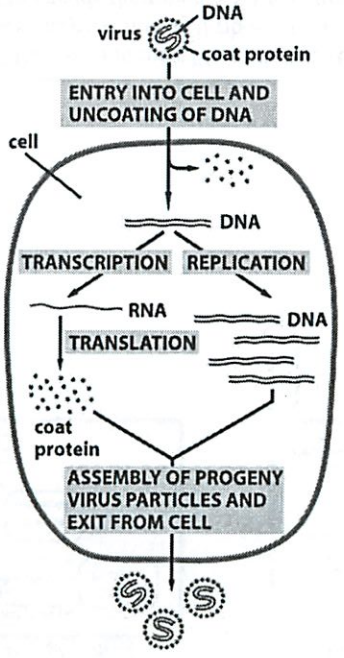


Keep in mind that most viruses like to **minimize the number of enzymes and proteins** they encode and maximize their use of host-cell enzymes and proteins.

These viruses also replicate in the nucleus and, like the Class I viruses, rely on the host for all of their synthetic functions. ***Why do they make dsDNA?***
Example: feline parvovirus.

These viruses replicate in the nucleus. They rely on the host for DNA polymerase, RNA polymerase, and protein synthesis. **Example:SV40 virus**

Many dsDNA viruses (replication similar to that of host genome; "Class I viruses)

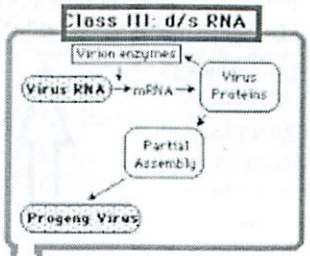


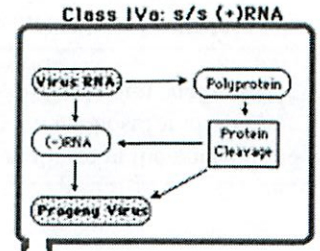
In these class of viruses, there is almost total parasitism on the host cell for DNA, RNA and protein synthesis.

Figure 3.3 *The Biology of Cancer* (© Garland Science 2007)

11/23 11/26

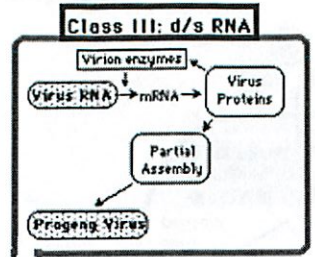
Viruses classified according to the structures of their genomes

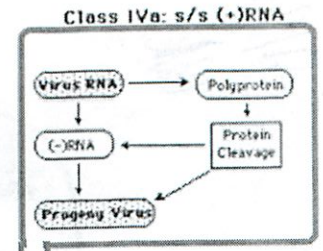




These viruses use no DNA in their life cycle and also replicate in the cytoplasm. Their genomes are (+) strand, i.e., <u>of the same polarity as mRNA.</u> Like Class III viruses, they must encode their own polymerase. Because they make a single strand (+) genomic RNA that also serves as an mRNA, and because translation initiates at only one site on mRNA, they make viral polyproteins. ***Why?*** **Example: poliovirus, most cold viruses (rhinoviruses)**

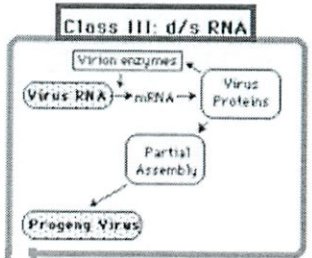
Viruses classified according to the structures of their genomes

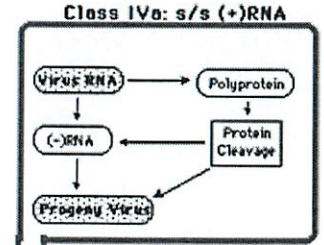




These viruses do not use DNA at all in their life cycle and replicate in the cytoplasm. Like all viruses, they use host-cell ribosomes to make their proteins. ***Why do the viruses need to encode their own RNA polymerases?*** **Example:reovirus**

Viruses classified according to the structures of their genomes





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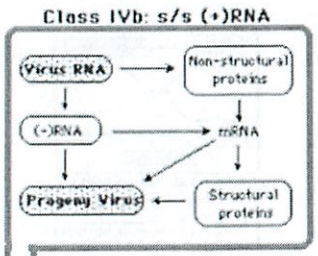
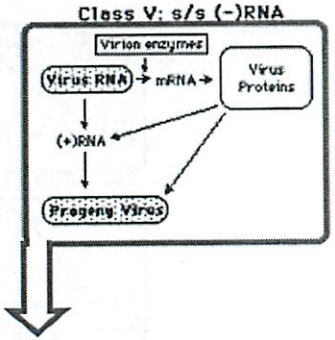
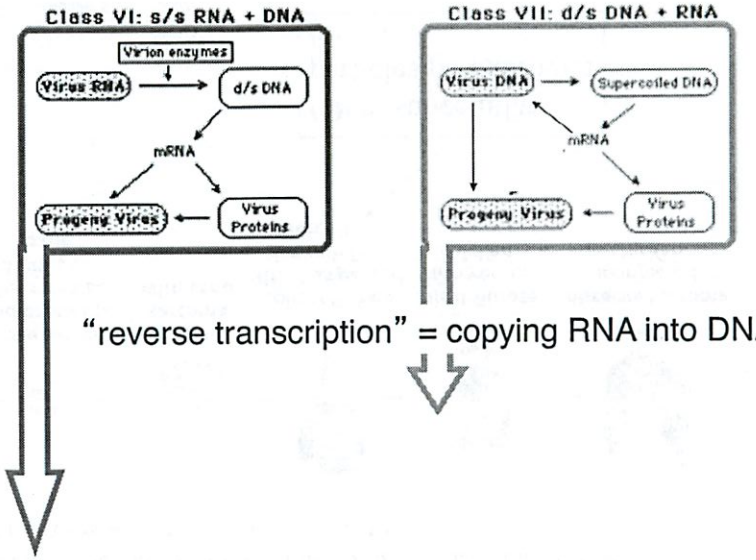




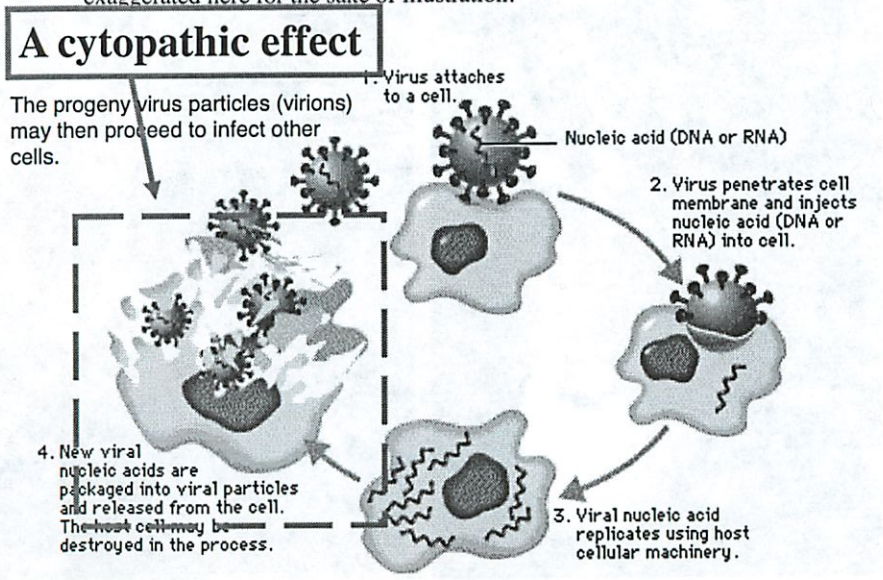
These viruses have (-) RNA genomes, I.e., of the strand **complementary** to viral mRNA. Like Class III and IV viruses, they must encode their own polymerase. Because their (-) strand RNA genome cannot be translated, <u>they must carry an RNA-dependent RNA polymerase in their virions and bring it into the infected cell (virion enzymes).</u> ***Why?*** **Examples: rabies, measles viruses**

Viruses classified according to the structures of their genomes



These viruses, like the Class IV viruses, have (+) genomes. However, they dispatch their RNA genomes into the nucleus, where it is **reverse-transcribed** into dsDNA, which then serves as the template for RNA pol II, making progeny genomes. **Example: HIV**

The basic scheme: a viral lytic cycle. 2 things: (1) "lytic" means that the viruses lyses (kills) the cell. (2) the size of the virus relative to the cell is exaggerated here for the sake of illustration.



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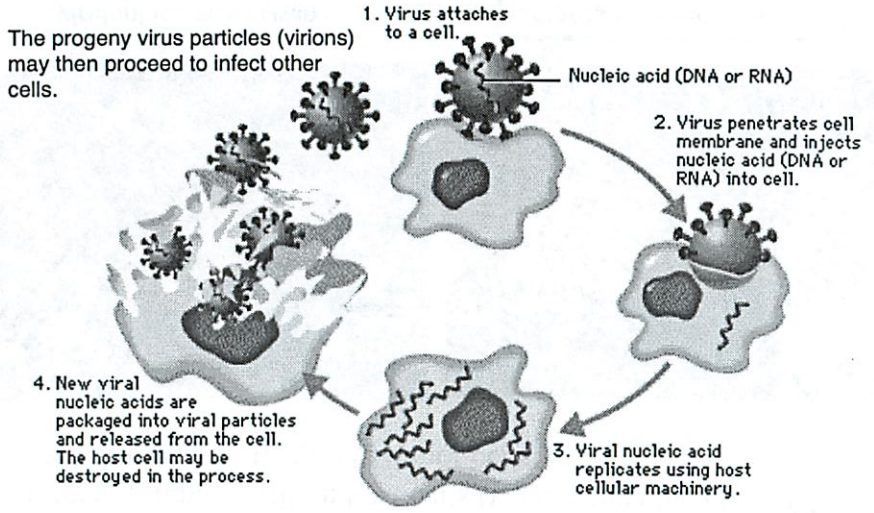
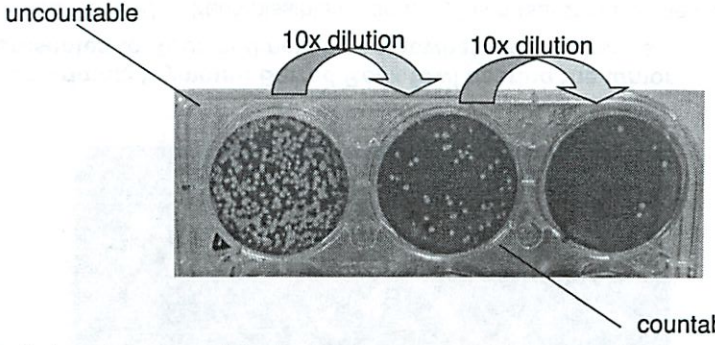


Use of the cytopathic effect to quantify virus concentrations:



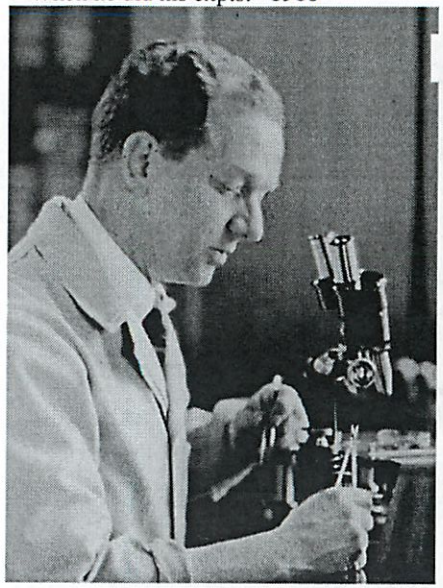
This biological assay generates plaque-forming units per ml. (PFU/ml.)

Viral lytic cycles make possible **plaque assays:** Infect a cell monolayer with a solution (a "viral stock") of virus particles. Then overlay the infected monolayer with some agar, to ensure that the progeny particles from an initially infected cell can only infect nearby cells (and not spread to infect distant cells in the monolayer. Each resulting plaque is the consequence of a single virus particle infecting a cell and the progeny of this infection infecting and killing nearby cells, thereby eroding a hole in the cell monolayer that eventually becomes visible to the naked eye.

This plaque assay is a way of **quantifying** the number of infectious virus particles (**virions**) in the initial stock. Plaque assays are often done by serially diluting the virus stock (e.g., by factors of 10) so that at one dilution or another there will be a countable number of plaques (e.g., the middle wells here).

Peyton Rous

When he did his expts. ~1910



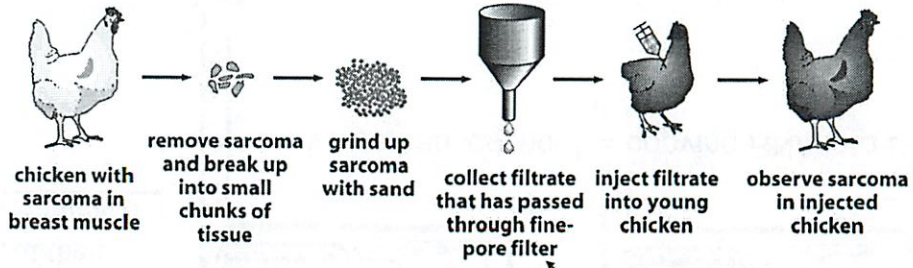
~1965, when he got his Nobel

Figure 3.1 *The Biology of Cancer* (© Garland Science 2007)

The original Plymouth Barred Rock fowl bearing the tumor presented to Rous and held by somewhat arthritic hands

.Rous, P. 1910. A transmissible avian neoplasm (sarcoma of the common fowl). *J. Exp. Med.* 12:696-705.

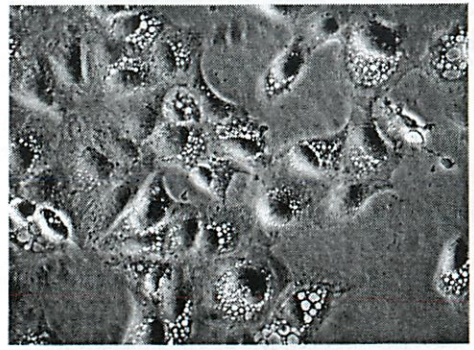
This is Rous' s experiment. (By the time he did this in 1909/10, it was known that bacteria were infectious agents that did not pass through fine-pored filters whereas viruses would; indeed this ability to pass through a filter represented the **operational definition** of a virus (since neither bacteria nor viruses were visualizable at the time).



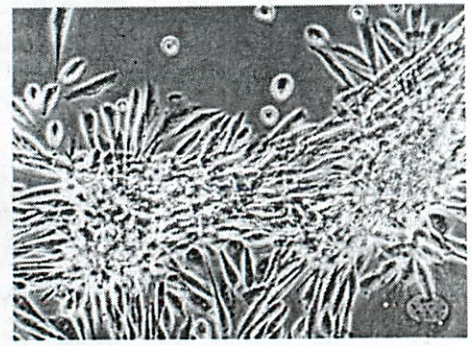
(Only subcellular particles pass through filter.)

Figure 3.2 *The Biology of Cancer* (© Garland Science 2007)

In fact, certain tumor viruses, like Rous sarcoma virus (RSV) instead of rapidly killing host cells (via a cytopathic effect/CPE) instead transform them into tumor cells.



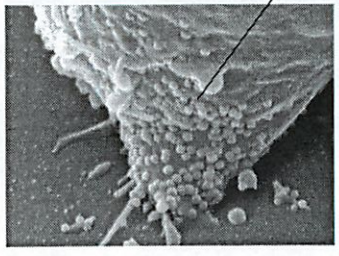
virulent interaction with host: **cytopathic effect**, cell death



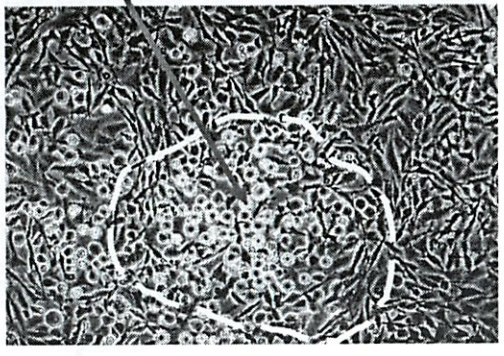
temperate interaction with host (I.e.,cells survive), in addition:cell become transformed into cancer cells

Figure 3.10b *The Biology of Cancer* (© Garland Science 2007)

In addition to budding progeny virus particles from an infected cell, Rous sarcoma virus can transform an infected cell, i.e. convert it from a normal growth state to a transformed state, yielding a colony of transformed cells, which is called a **focus.**



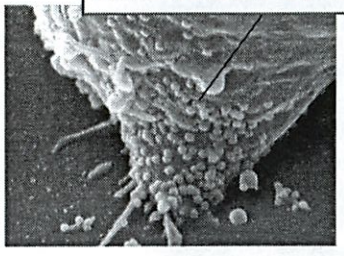
By implication, the virus establishes a chronic infection in a cells (rather than killing it).



A focus of transformed cells:
These cells will form tumors if implanted in proper host

Figure 3.5 *The Biology of Cancer* (© Garland Science 2007)

Hence, not all interactions between a virus and its host cell lead to a cytopathic effect and the death of the cell



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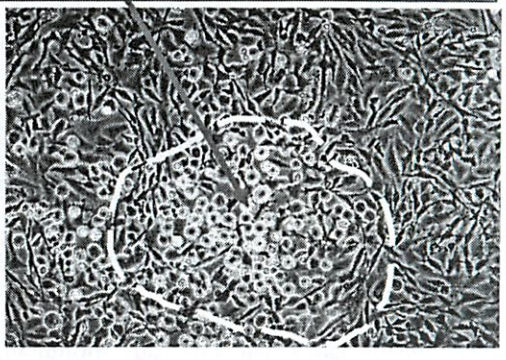

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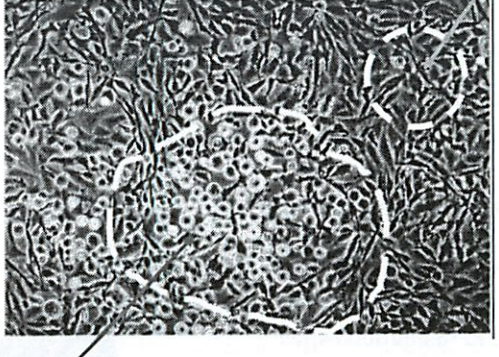
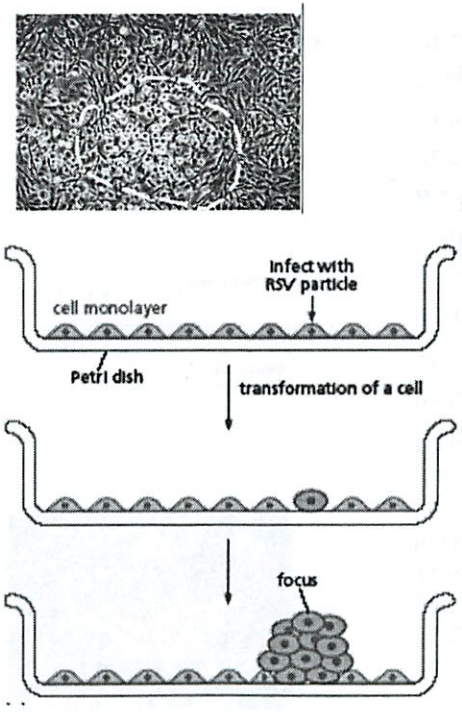
A focus of transformed cells



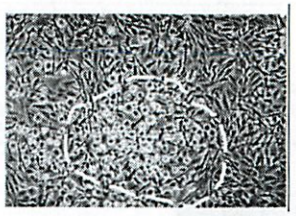
These normal cells fill up the bottom of the plate and stop growing when they touch one another, forming a "confluent monolayer".

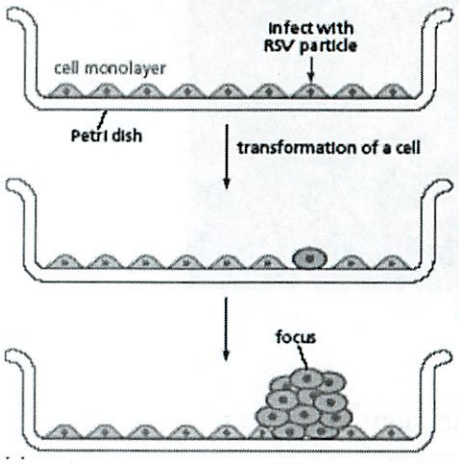
These transformed cells continue to grow after they touch one another and consequently pile up into a layer of cells many cells thick



Schematically, a focus looks like this: The normal cells in the Petri dish fill up the plate and stop growing once they touch each other, forming a cell monolayer. The transformed cells continue to proliferate even after they've touched one another, and therefore pile up on top of one another.

All the transformed cells in a focus are the descendants of an initially infected, transformed cell.





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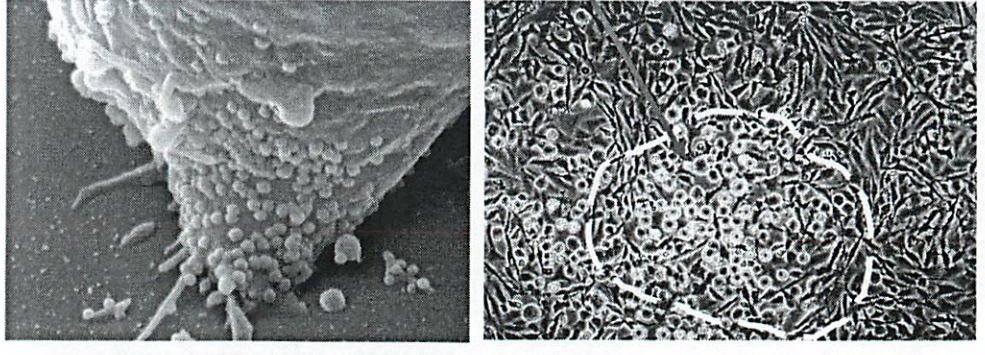
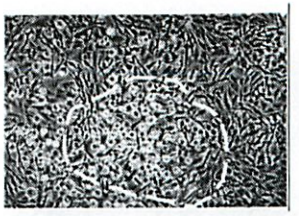
This fact indicates that there is a **stable heritability** of the transformed phenotype, i.e., the descendants of the initially transformed cell <u>are also transformed.</u>

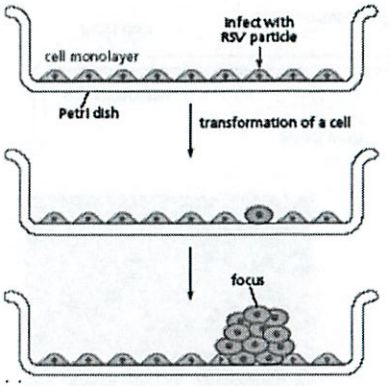
How does the viral genome (which was responsible for transforming the initially infected cell) **perpetuate itself** in all of the lineal descendants of the initially Infected, tranformed cell?



(Chromosomes are transmitted systematically to both daughter cells during mitosis, but how do viral genomes become systematically passed from mother to daughter cell at mitosis??)

Figure 3.5 *The Biology of Cancer* (© Garland Science 2007)





<u>All the transformed cells in a focus are the descendants of an initially infected, transformed cell.</u>

This fact indicates that there is a stable heritability of the transformed phenotype, i.e., the descendants of the initially transformed cell are also transformed.

If an infecting virus uses a viral gene to transform an infected cell (such a viral transforming gene is often called an **oncogene**), and the oncogene is required not only to induce the transformed cell phenotype but all to **maintain** it, this dictates that the viral genome (containing a viral oncogene) must be transmitted to and perpetuated by the descendants of the initially transformed cell.

The descendants of an SV40 transformed cell continue to be transformed.

How does the viral genome, which ostensibly carries the viral oncogene responsible for cell transformation, persist in the descendant cells? The SV40 viral genome, seen here, has no connection with the mitotic apparatus that is specialized to allocate chromosomes to both daughter cells during mitosis.

SV40 genomic DNA

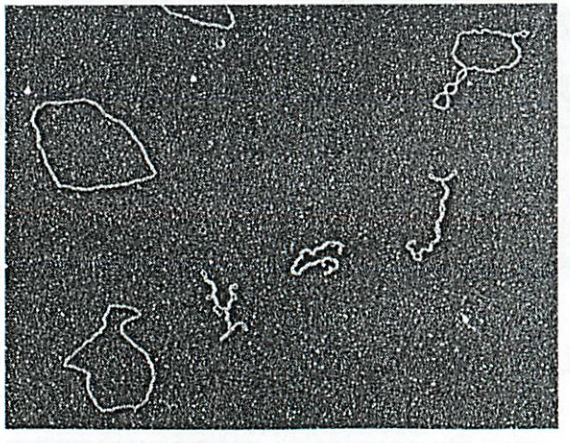
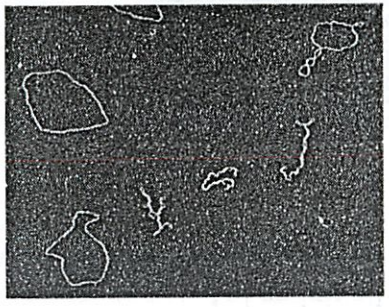


Figure 3.11 *The Biology of Cancer* (© Garland Science 2007)

The descendants of an SV40 transformed cell continue to be transformed.

How does the viral genome, which ostensibly carries the viral oncogene responsible for cell transformation, persist in the descendant cells? The SV40 viral genome, seen here, has no connection with the mitotic apparatus that is specialized to allocate chromosomes to both daughter cells during mitosis.

SV40 genomic DNA

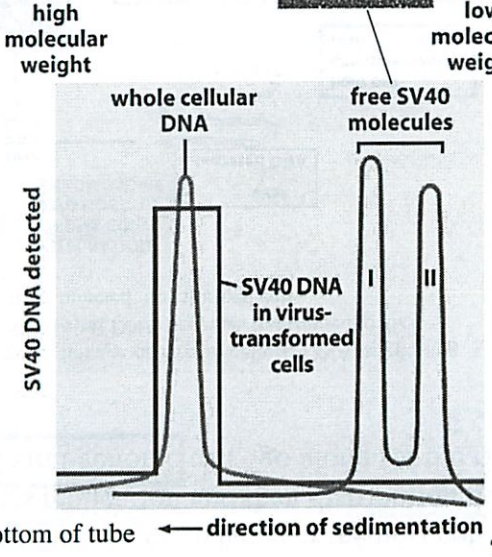


As a consequence, this non-chromosomal DNA (=episomal) **will be lost** sooner or later during successive rounds of cell growth and division.

Figure 3.11 *The Biology of Cancer* (© Garland Science 2007)

Prepare DNA from an SV40 transformed cell and centrifuge it under alkaline conditions (under which all non-covalent bonds are broken).

Use radioactive SV40 DNA as a probe to analyze the various fractions of the centrifuge tube to determine where in the tube the complementary SV40 DNA is located. This analysis indicates that it co-sediments with the high mol. wt. cellular DNA, not the low mol. wt. viral DNA.



Since SV40 sequences co-sediment w. cell DNA, they must be physically linked to cell DNA; therefore are <u>integrated</u>.

Figure 3.15 *The Biology of Cancer* (© Garland Science 2007)

The descendants of an SV40 transformed cell continue to be transformed.

How does the viral genome, which ostensibly carries the viral oncogene responsible for cell transformation, persist in the descendant cells? The SV40 viral genome, seen here, has no connection with the mitotic apparatus that is specialized to allocate chromosomes to both daughter cells during mitosis.

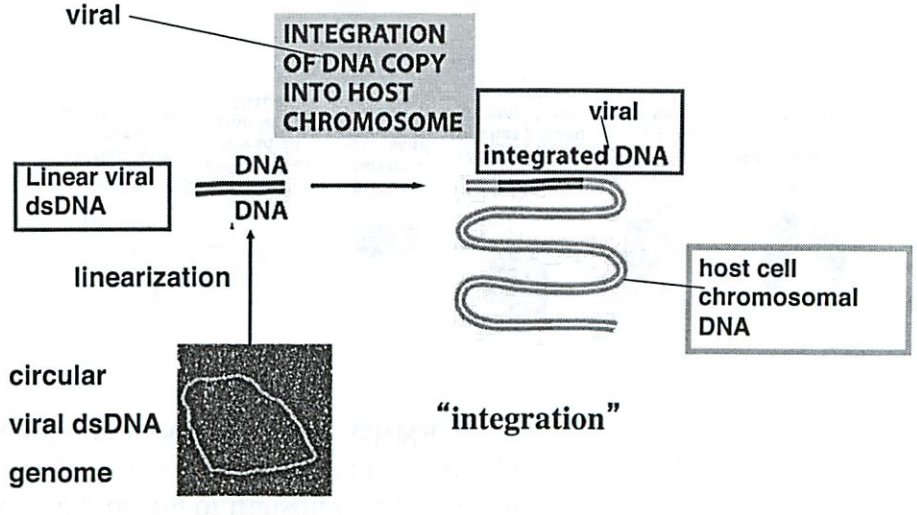
SV40 genomic DNA

As a consequence, this non-chromosomal DNA (=episomal) **will be lost** sooner or later during successive rounds of cell growth and division.

How does the SV40 genome <u>hang on</u> and perpetuate itself in the lineal descendants of a cell that was initially infected by and transformed by an SV40 virus particle??

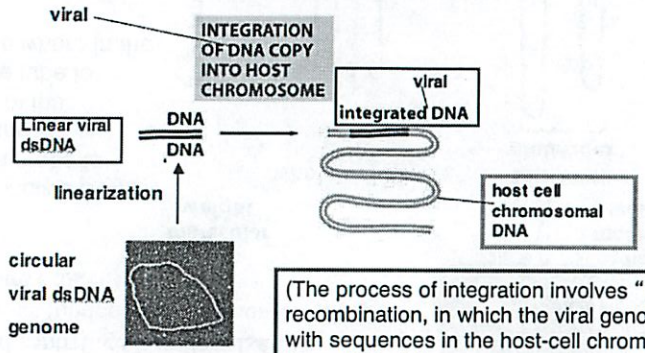
Figure 3.11 *The Biology of Cancer* (© Garland Science 2007)

This suggests physical linkage of viral to cellular DNA, suggesting A model of how SV40 viral DNA becomes transmitted to the progeny of an initially infected, transformed cell



Now, following integration, whenever the host cell chromosomal DNA undergoes replication and then distribution during mitosis, the integrated viral genome can "go along for the ride"!

This suggests physical linkage of viral to cellular DNA, suggesting
A model of how SV40 viral DNA becomes transmitted to the progeny of an initially infected, transformed cell



(The process of integration involves "illegitimate" recombination, in which the viral genome recombines with sequences in the host-cell chromosome with which it has limited if any sequence homology.)

The illegitimate recombination between the SV40 genome and the genome of an infected rodent cell is a rare event that occurs in 1 in 10^3 or 10^4 infected cells. It is not part of the virus' s growth cycle,
But the problem is even more complex in the case of RNA tumor viruses, e.g., the Rous sarcoma virus discovered by Peyton Rous.

How does a virus with a ssRNA genome perpetuate itself in the descendants of an initially infected, transformed cell?

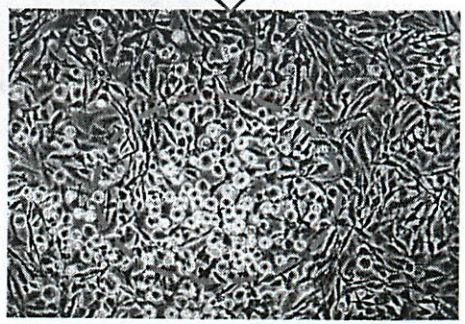
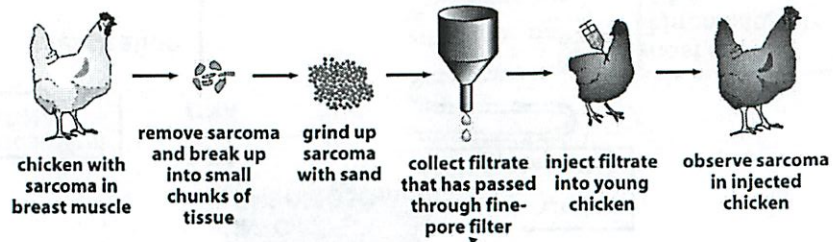


Figure 3.6 *The Biology of Cancer* (© Garland Science 2007)

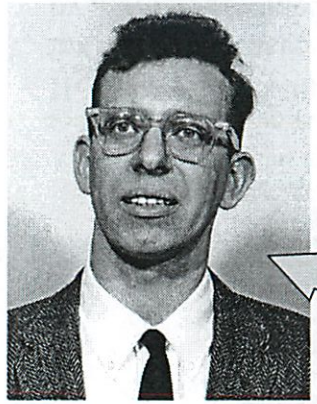
But the problem of transmitting the genomes of Rous Sarcoma Virus (RSV) is even more complex:
its genome is constructed of **ssRNA**.



(Only subcellular particles pass through filter.)

Figure 3.2 *The Biology of Cancer* (© Garland Science 2007)

Howard Temin ca. 1970



As a graduate student, Howard Temin, then a graduate student at Caltech, wrestled with the problem. He came up with an unorthodox solution that landed him in the scientific wilderness.

How does a virus with a ssRNA genome perpetuate itself in the descendants of an initially infected cell?

A focus of RSV-transformed cells

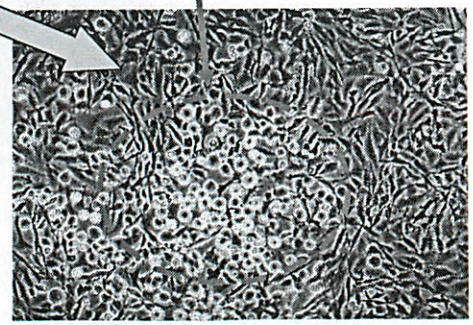
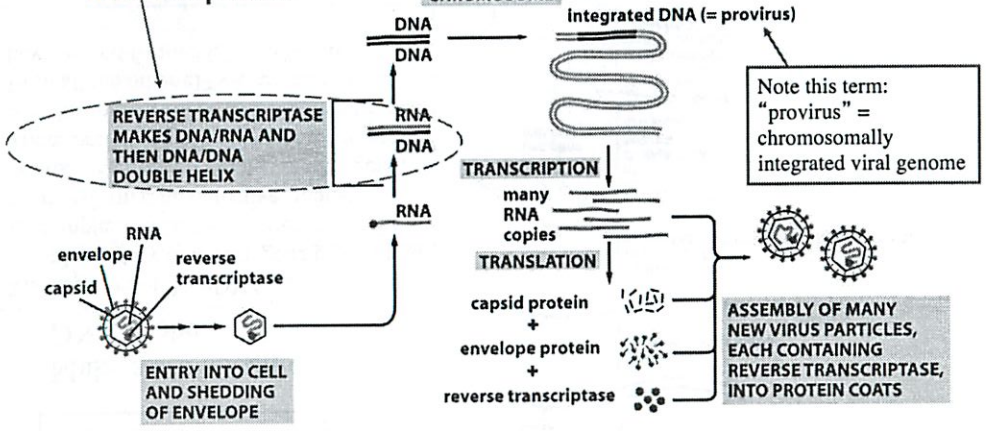


Figure 3.6 *The Biology of Cancer* (© Garland Science 2007)

Temin's weird Idea:reverse Transcription!

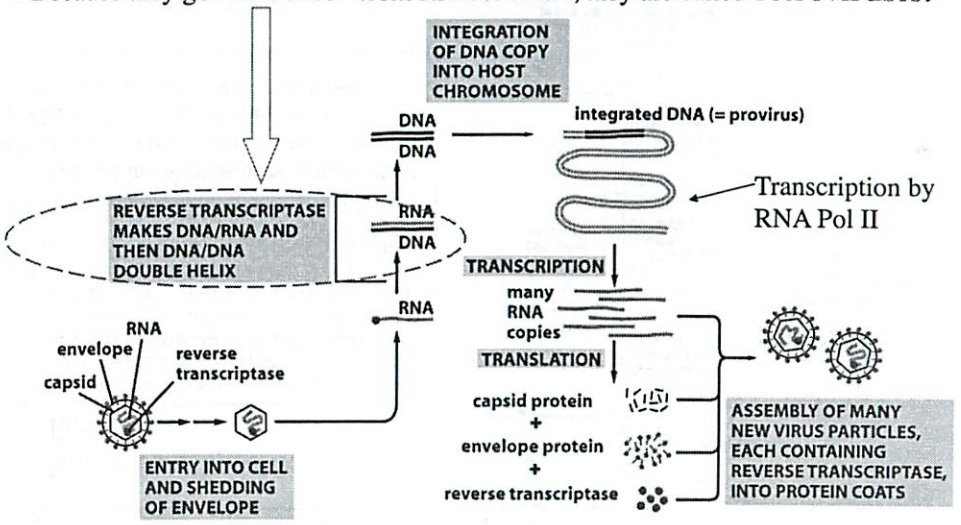
The essence of the idea: that RSV perpetuates itself by making a dsDNA copy of its ssRNA genome. This copy was then integrated into the host cell genome, which afforded it the ability to be transmitted to the descendants of an initially infected host cell.



Note this term: "provirus" = chromosomally integrated viral genome

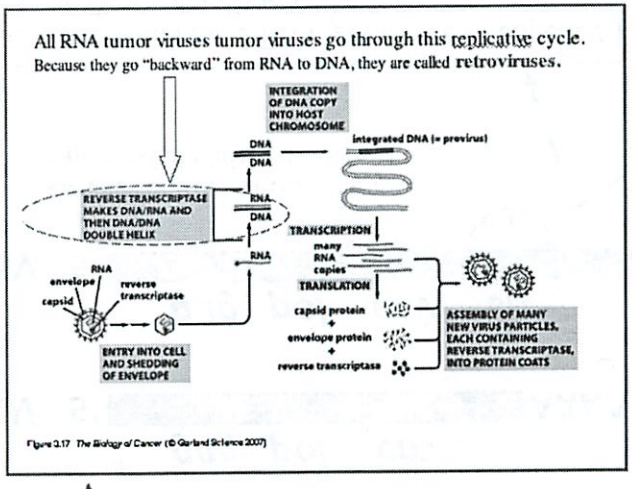
Figure 3.17 *The Biology of Cancer* (© Garland Science 2007)

All RNA tumor viruses tumor viruses go through this replicative cycle.
Because they go "backward" from RNA to DNA, they are called **retroviruses.**



Transcription by RNA Pol II

Figure 3.17 *The Biology of Cancer* (© Garland Science 2007)

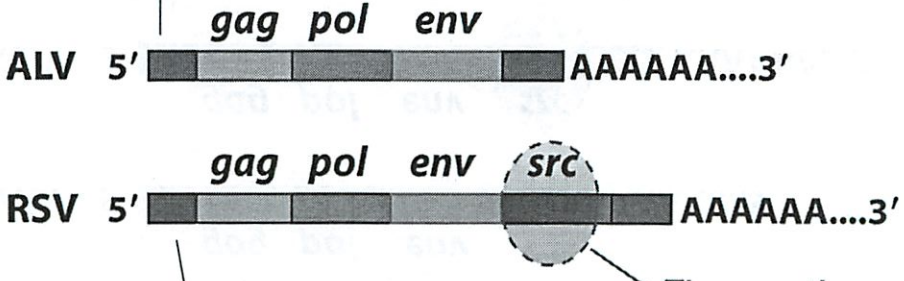


This tells us how the viral genome (with its cancer-inducing oncogene)is perpetuated in the genome of the descendants of an initially infected cell.

But it **still doesn't tell us** how the viral oncogene **transforms** these cells.

Analysis the genomes of chicken retroviruses revealed the organization of the common ALV chicken virus (ALV), which had three genes: *gag* (encodes capsid); *pol* (encodes reverse transcriptase & integrase), and *env* (encodes glycoprotein spikes).

Genome of widespread avian leukosis virus (ALV) **cannot** transform infected cells



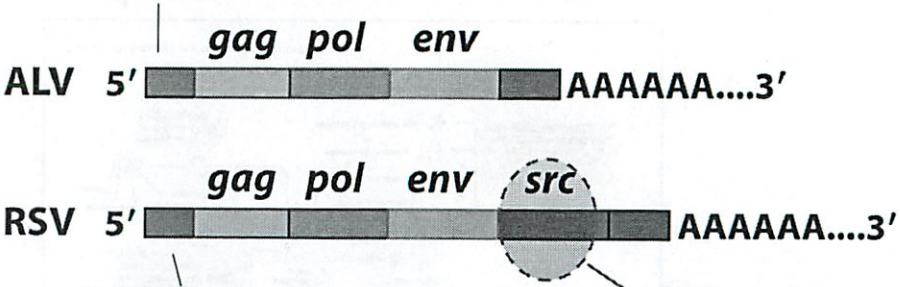
Genome of Rous sarcoma virus **can** transform infected cells

The genetic difference, apparently encoding the viral transforming activity.

Figure 3.19 *The Biology of Cancer* (© Garland Science 2007)

ALV (avian leukosis virus) is common in chicken coops.
RSV (Rous sarcoma virus) has been isolated only once over a period of 100 years! Therefore RSV is the outlier/exception.

Genome of widespread avian leukosis virus (ALV) **cannot** transform infected cells



Genome of Rous sarcoma virus **can** transform infected cells

The genetic difference, apparently encoding the viral transforming activity.

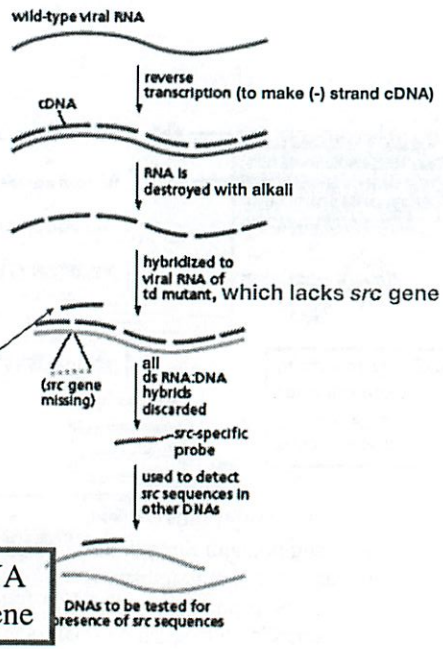
Question: Where did RSV get its *src* oncogene?

Figure 3.19 *The Biology of Cancer* (© Garland Science 2007)

Question: Where did RSV get its *src* oncogene?

Make a *src*-specific DNA probe:

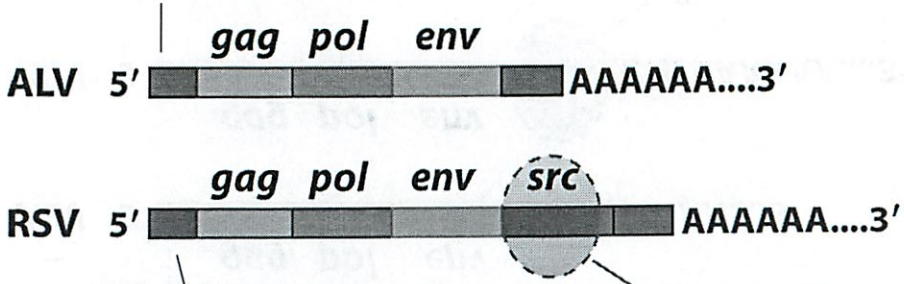
The segment of the cDNA that is complementary to the *src* gene cannot find its complement in the RNA genome of a td (transformation defective) mutant (whose genome has deleted the *src* gene. Therefore, the cDNA against the *src* gene remains unhybridized and can be retrieved from all the other cDNA segments that have indeed formed DNA:RNA hybrids.



Strategy: Make a radiolabelled DNA probe that is specific for the *src* gene

Analysis the genomes of chicken retroviruses revealed the organization of the common ALV chicken virus (ALV), which had three genes: *gag* (encodes capsid); *pol* (encodes reverse transcriptase & integrase), and *env* (encodes glycoprotein spikes).

Genome of widespread avian leukosis virus (ALV) **cannot** transform infected cells



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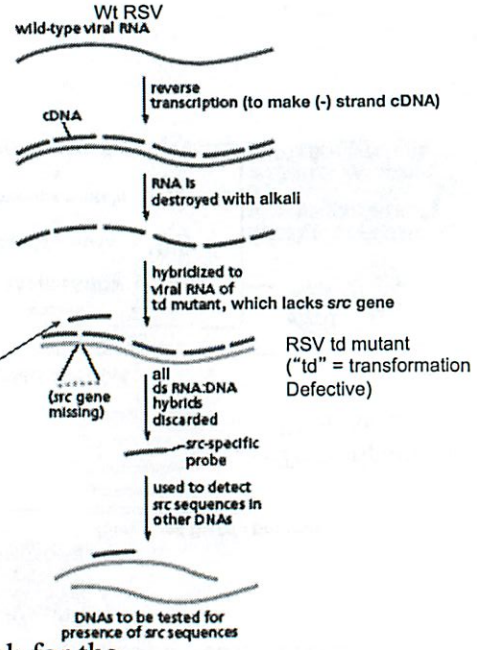
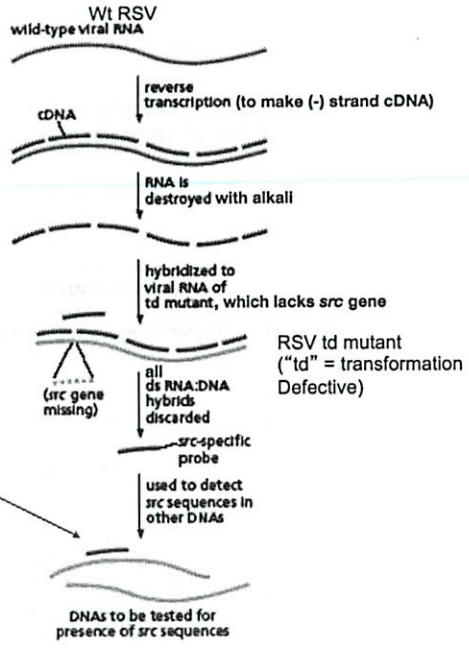
If this *src* cDNA is radiolabeled, i.e., was synthesized in the presence of radioactive dNTP precursors, it can be used as a **probe** to detect complementary DNA sequences in other DNAs of interest.



This probe can then be used to search for the presence of *src* sequences in other DNAs.

Question: Where did RSV get its *src* oncogene?

Make a *src*-specific DNA probe:



This probe can then be used to search for the presence of *src* sequences in other DNAs.

In fact, the RSV *src* probe hybridizes to normal chicken DNA, i.e., DNA of chicken cells that have never been infected by RSV.

What does melting temperature indicate? The more mismatched the hybrid is, the lower the temperature at which the hybrid denatures -- i.e., a less heating is required to pull apart the two strands in the hybrid (the probe DNA strand and the genomic DNA strand to which it is hybridized/annealed).

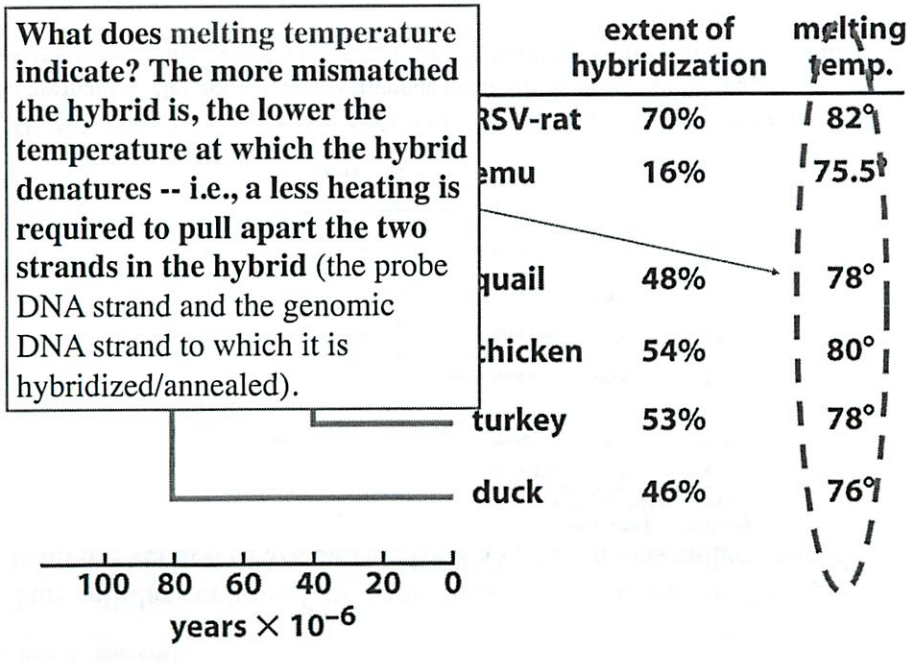
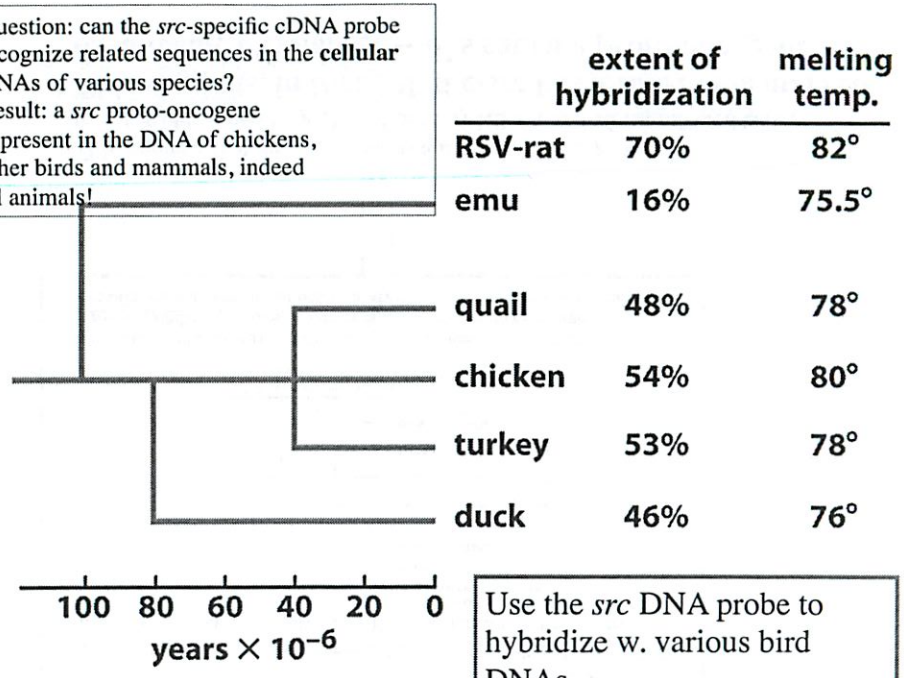


Figure 3.21 *The Biology of Cancer* (© Garland Science 2007)

Question: can the *src*-specific cDNA probe recognize related sequences in the **cellular** DNAs of various species?
Result: a *src* proto-oncogene is present in the DNA of chickens, other birds and mammals, indeed all animals!

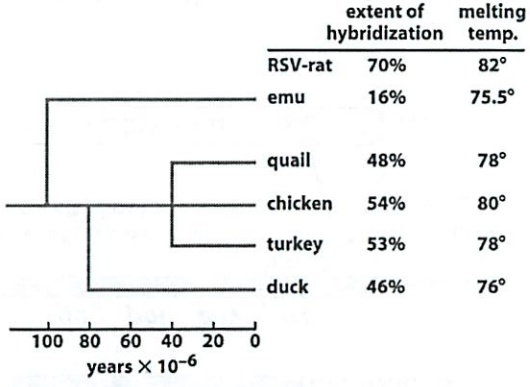


Use the *src* DNA probe to hybridize w. various bird DNAs

Figure 3.21 *The Biology of Cancer* (© Garland Science 2007)

Answer: a *src* proto-oncogene is present in the DNA of chickens, other birds and mammals, indeed all animals!

This cellular version of *src* came to be called "c-*src*" to distinguish it from the version of *src* carried by RSV, which was called "v-*src*".



The behavior of c-*src* was reminiscent of -- indeed identical to -- the behavior of a normal cellular gene, whose sequence is largely conserved over large evolutionary time periods, but which slowly diverges with the passage of time -- over millions of years.

Figure 3.21 *The Biology of Cancer* (© Garland Science 2007)

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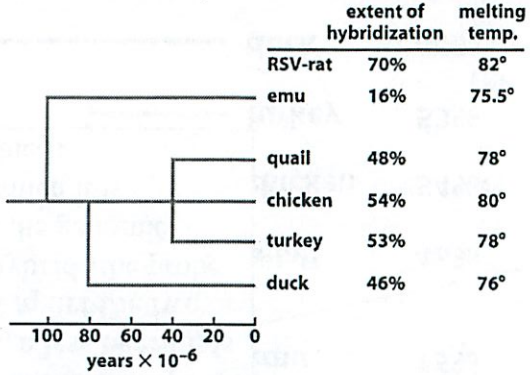
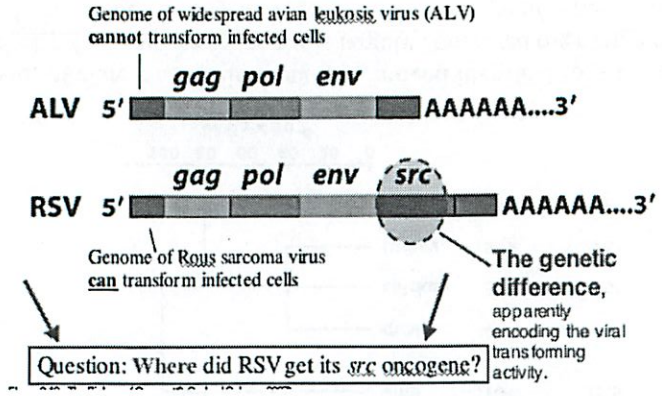
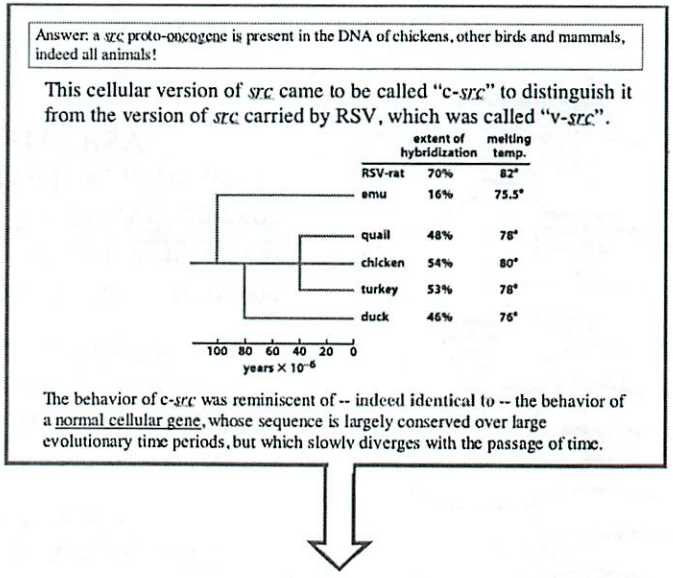


The behavior of c-*src* was reminiscent of -- **indeed identical to** -- the behavior of a normal cellular gene, whose sequence is largely conserved over large evolutionary time periods, but which slowly diverges with the passage of time.

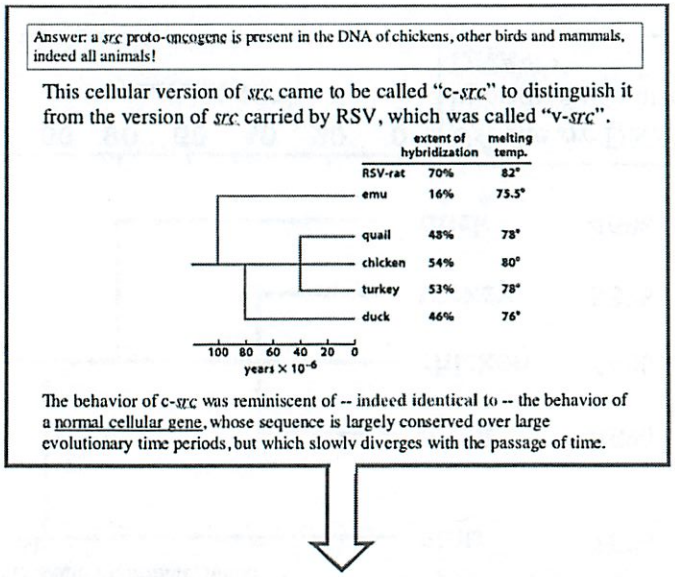
Figure 3.21 *The Biology of Cancer* (© Garland Science 2007)



Now we know the answer: the gene was copied/stolen **from the genome of a normal chicken cell!**



This suggests that the c-*src* gene is a normal cellular gene that was **kidnapped by RSV** and used by RSV to transform infected cells. .



This suggests that the c-*src* gene is a normal cellular gene that was kidnapped by RSV and used by RSV to transform infected cells.

This suggests, in turn, that c-*src* has a role in normal cell/ organismic physiology -- it's called a proto-oncogene.

In fact, a variety of other retroviruses in a variety of mammalian and avian species have picked up host cell proto-oncogenes. (Homologues of each of these proto-oncogenes is present in the genomes of all vertebrates, i.e.,as part of the shared genetic repertoire of vertebrates.

Name of virus	Viral oncogene	Species	Major disease	Nature of oncoprotein
Rous sarcoma	*src*	chicken	sarcoma	non-receptor TK
Y73/Esh sarcoma	*yes*	chicken	sarcoma	non-receptor TK
Fujinami sarcoma	*fps*[b]	chicken	sarcoma	non-receptor TK
UR2	*ros*	chicken	sarcoma	RTK; unknown ligand
Myelocytomatosis 29	*myc*	chicken	myeloid leukemia[c]	transcription factor
MH Hill virus 2	*mil*[d]	chicken	myeloid leukemia	ser/thr kinase
Avian myeloblastosis E26	*myb*	chicken	myeloid leukemia	transcription factor
Avian myeloblastosis E26	*ets*	chicken	myeloid leukemia	transcription factor
Avian erythroblastosis E54	*erbA*	chicken	erythroleukemia	thyroid hormone receptor
Avian erythroblastosis E54	*erbB*	chicken	erythroleukemia	EGF RTK
3611 murine sarcoma	*raf*[e]	mouse	sarcoma	ser/thr kinase
SKV770	*ski*	chicken	endotheliome (?)	transcription factor
Reticuloendotheliosis	*rel*	turkey	immature B-cell lymphoma	transcription factor
Abelson murine leukemia	*abl*	mouse	pre-B-cell lymphoma	non-receptor TK
Moloney murine sarcoma	*mos*	mouse	sarcoma, erythroleukemia	ser/thr kinase
Harvey murine sarcoma	H-*ras*	rat, mouse	sarcoma	small G protein
Kirsten murine sarcoma	K-*ras*	mouse	sarcoma	small G protein
FBJ murine sarcoma	*fos*	mouse	osteosarcoma	transcription factor
Snyder–Theilen feline sarcoma	*fes*[f]	cat	sarcoma	non-receptor TK
McDonough feline sarcoma	*fms*	cat	sarcoma	CSF-1 RTK
Gardner–Rasheed feline sarcoma	*fgr*	cat	sarcoma	non-receptor TK
Hardy–Zuckerman feline sarcoma	*kit*	cat	sarcoma	steel factor RTK
Simian sarcoma	*sis*	woolly monkey	sarcoma	PDGF
AKT8	*akt*	mouse	lymphoma	ser/thr kinase
Avian virus S11	*sea*	chicken	erythroblastic leukemia[g]	RTK; unknown ligand
Myeloproliferative leukemia	*mpl*	mouse	myeloproliferation	TPO receptor
Regional Poultry Lab v. 30	*eyk*	chicken	sarcoma	RTK; unknown ligand
Avian sarcoma virus CT10	*crk*	chicken	sarcoma	SH2/SH3 adaptor
Avian sarcoma virus 17	*jun*	chicken	sarcoma	transcription factor
Avian sarcoma virus 31	*fos*	chicken	sarcoma	transcription factor[h]
A542 sarcoma virus	*mef*	chicken	sarcoma	transcription factor
Cas NS-1 virus	*cbl*	mouse	lymphoma	SH2-dependent ubiquitylation factor

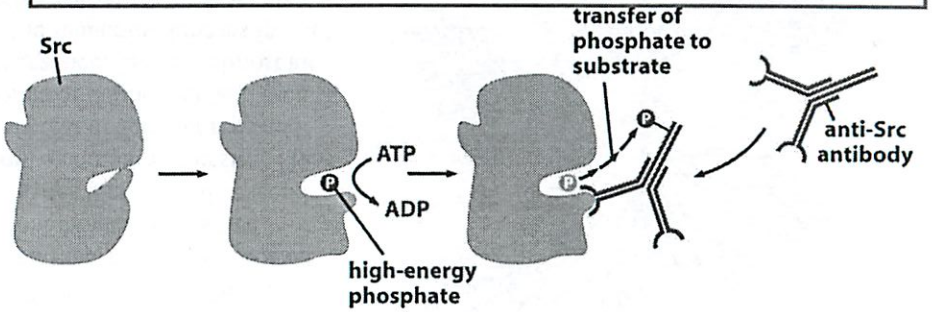
Abbreviations. CSF, colony-stimulating factor; EGF, epidermal growth factor; G, GTP-binding; PDGF, platelet-derived growth factor; RTK, receptor tyrosine kinase; ser/thr, serine/threonine; SH, src-homology segment; TK, tyrosine kinase; TPO, thrombopoietin.

[a]Not all viruses that have yielded these oncogenes are indicated here.
[b]Ortholog of the mammalian *fes* oncogene.
[c]Also causes carcinomas and endotheliomas.
[d]Ortholog of the mammalian *raf* oncogene.
[e]Ortholog of the avian *mil* oncogene.
[f]Ortholog of the avian *fps* oncogene.
[g]Also causes granulocytic leukemias and sarcomas.
[h]Functions as a transcriptional repressor.

Table 3.3 *The Biology of Cancer* (© Garland Science Adapted in part from S.J. Flint, L.W. Enquist, R.M. Krug et al., Principles of Virology. Washington, DC: ASM Press, 2000. Also in part from G.M. Cooper, Oncogenes. Boston: Jones and Bartlett Publishers, 1995.

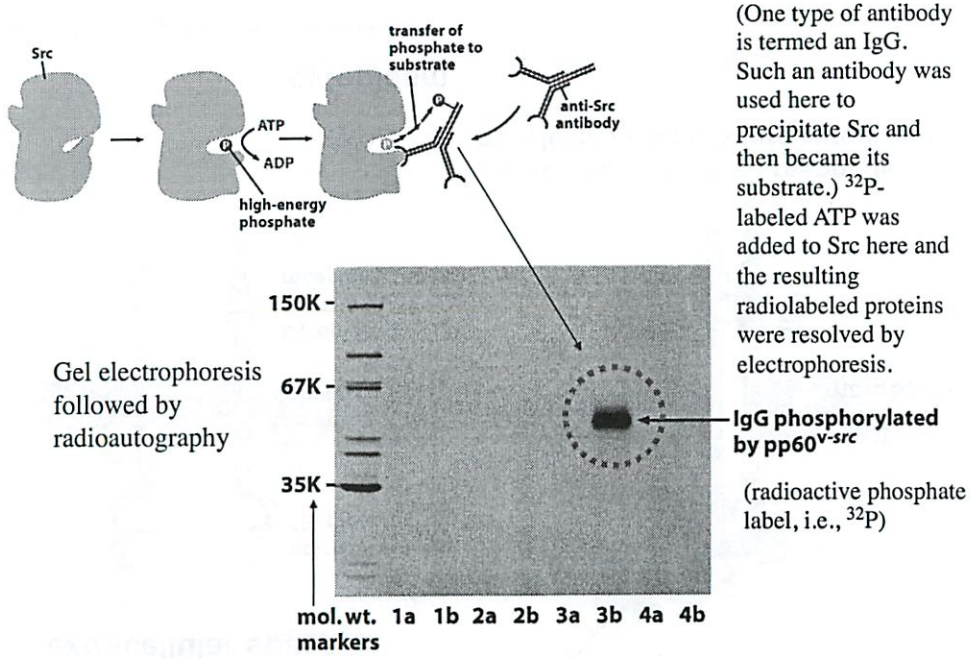
How does the *src*-encoded oncoprotein function **biochemically**?

How does the Src oncoprotein (made by the RSV *src* oncogene) function biochemically? It functions as a tyrosine **kinase** (like the tyrosine kinases that are part of various growth factor receptors).



While the anti-Src antibody molecule can bind and immunoprecipitate the Src protein, it can, as it happens, also serve as a substrate for phosphorylation by the Src tyrosine kinases.

Figure 5.5a *The Biology of Cancer* (© Garland Science 2007)



Gel electrophoresis followed by radioautography

(One type of antibody is termed an IgG. Such an antibody was used here to precipitate Src and then became its substrate.) ^{32}P-labeled ATP was added to Src here and the resulting radiolabeled proteins were resolved by electrophoresis.

IgG phosphorylated by pp60$^{v\text{-}src}$

(radioactive phosphate label, i.e., ^{32}P)

Figure 5.5b *The Biology of Cancer* (© Garland Science 2007)

This autoradiogram shows the spectrum of proteins that are phosphorylated on tyrosine residues in plain NIH3T3 mouse cells or in NIH3T3 mouse cells that have been transformed by the introduction of a *src* oncogene. This evidence indicates (1) that Src functions as a tyrosine kinase; and (2) that is phosphorylates **multiple substrates** within a cell.

Cells are lysed and then their proteins are resolved by gel electrophoresis.

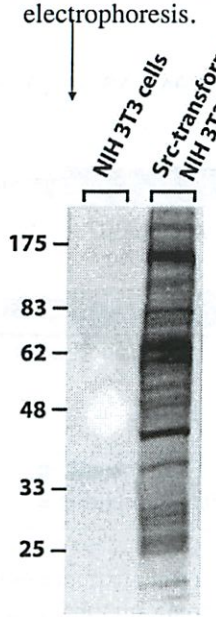


Figure 5.7a *The Biology of Cancer* (© Garland Science 2007)

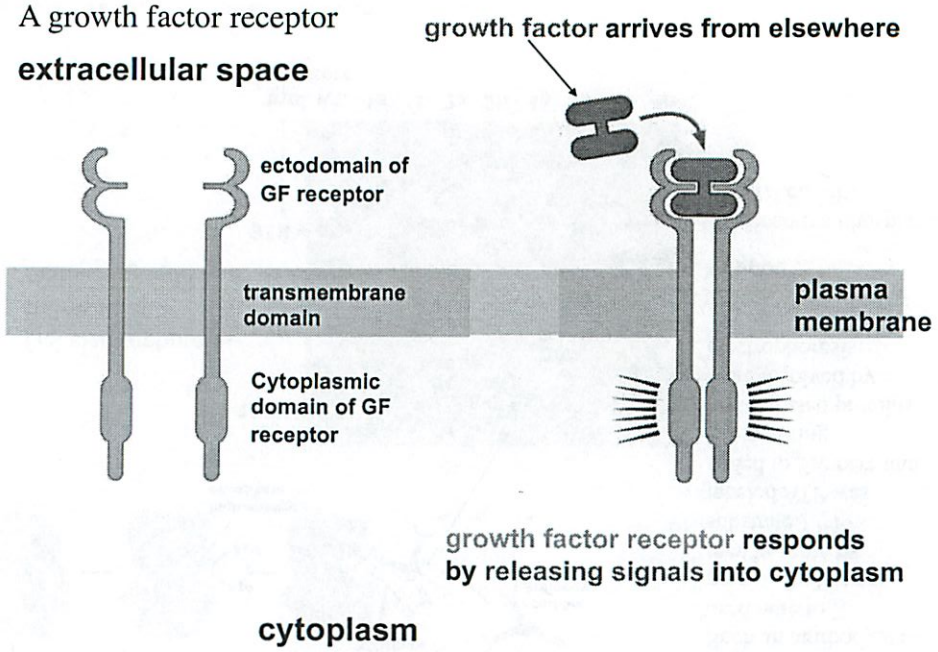
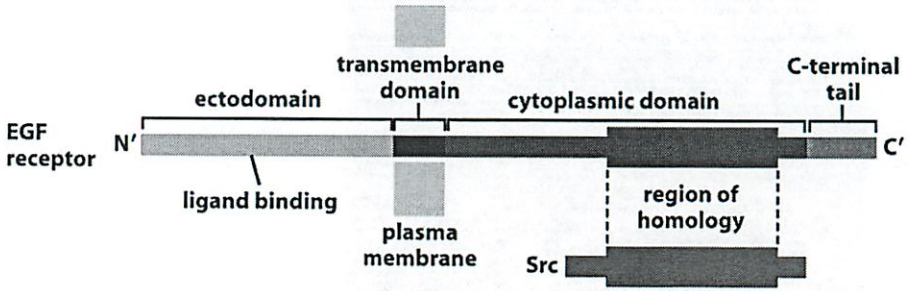


Figure 5.12b *The Biology of Cancer* (© Garland Science 2007)

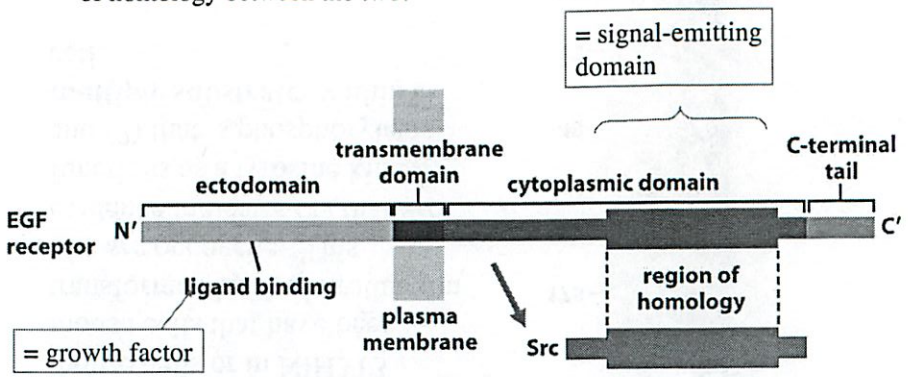
Comparison of a typical growth factor receptor, e.g., the epidermal growth factor (EGF) receptor, with Src shows that there is a region of homology between the two.



Structural **homology** suggests **functional similarity**; therefore, it is likely that the EGF receptor signals via its Src-homologous domain, i.e., via its tyrosine kinase domain.

Figure 5.9a *The Biology of Cancer* (© Garland Science 2007)

Comparison of a typical growth factor receptor, e.g., the epidermal growth factor (EGF) receptor, with Src shows that there is a region of homology between the two.



Homology = sequence relatedness -- therefore evolved from a common (evolutionary) ancestral gene.

Figure 5.9a *The Biology of Cancer* (© Garland Science 2007)

Actually, there are quite a few kinases in the mammalian genome -- almost 500! Of these, a small clade (90) are tyrosine kinases (TKs).

This tree plots different kinases as a function of their sequence similarity (closer) or lack of similarity (farther). The fact that all the TKs can be located on one branch of this tree indicates that they all descend from a common, ancestral TK gene that underwent repeated cycles of duplication and then divergence. Indeed, almost all of the remaining kinases in our cells (=ser & thr kinases) also appear on this tree indicates a common ancestral kinase for all of these various enzymes.

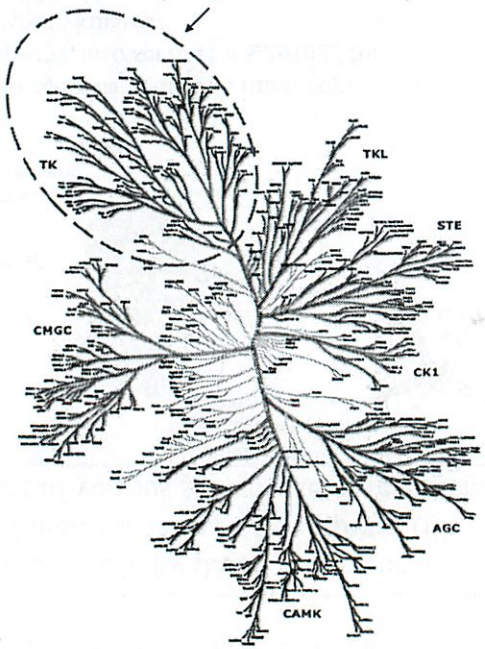
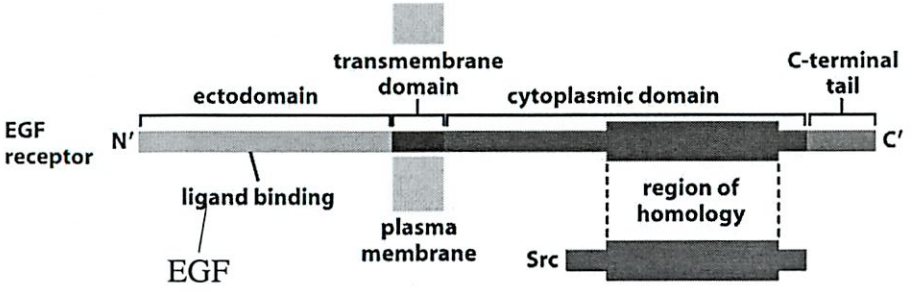


Figure 16.12 *The Biology of Cancer* (© Garland Science 2007)



These similarities suggest another idea: Perhaps the way by which an oncoprotein like Src signals is to **mimic the growth-promoting signals that are released by a growth factor receptor.** Perhaps the Src oncoprotein releases a steady stream of these growth-promoting signals, in contrast to the normal EGF receptor, which only releases such signals once it is stimulated to do so by binding its ligand, EGF.

Figure 5.9a *The Biology of Cancer* (© Garland Science 2007)

Both the Src protein & the signal-emitting domain of the GF receptor are signal-emitting tyrosine kinases. (derived evolutionarily from a common ancestral gene/protein)

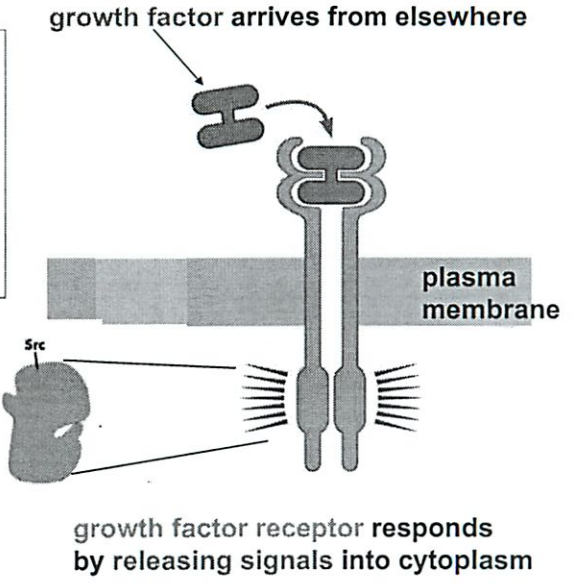


Figure 5.12b *The Biology of Cancer* (© Garland Science 2007)

But Rous sarcoma virus doesn't cause human cancer. How are proto-oncogenes and oncogenes involved in Human cancer? It's best to backtrack to the beginnings of the cancer research field!

7.012 11/30

Virology 3: Cancer

Before: tumor viruses

Now: Cancer

Ras sarcoma virus (retrovirus)

Src gene "stolen" (proto-onco) from normal use to cancer causing role

kinase - extracts Phosphates puts on it onto another

tyr-kinase which are rare and regulate cell proliferation

But human cancer is very different than our retrovirus example

~~90%~~ not viral based

But is anything similar

Do they ~~copy~~ replicate the growth factor of cells?

Cells can't proliferate on their own

must recieve signal from neighbors

└ mitogenic signal

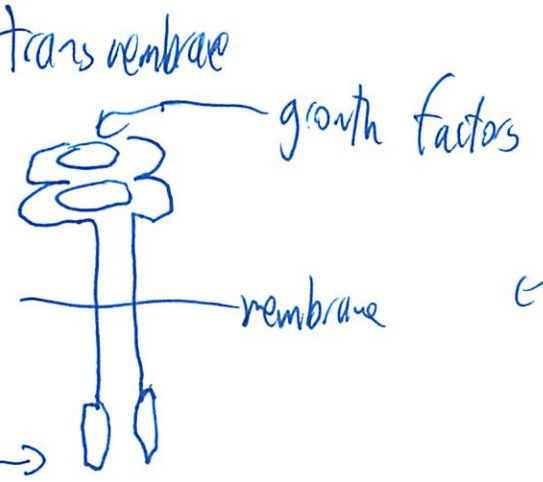
(2) Normally

vs { growth = physically ↑ in size of cell
proliferation = split in 2

must grow 2x as large before splitting

But this class growth = both

GF receptors



← activates when pieces drawn together

region of homology w/ Sac protein

Sac may not be involved directly
but is somehow related

that is the growth Factor Tyr-kinase
Cytoplasmic section

(3)

Lots of diff kinases
Tyr small %
Diversified over time

But doesn't tell us about how non-virus cancer gets triggered

* Cancer cell controls own destiny
  - which is diff than most cells

Tumors are chaos
- just have 1 goal: make more copies of themselves
- are monoclonal growth
  - only 1 cell has run away growth
- note: are complicated conversion steps (series) from normal cell to cancer cell
  - can take up to 40 years

(4)

Can speed it up w/ red meat

Why is this so complicated?

Evolution has created our bodies to make this hard on purpose!

Artist drawing

Prof: little representation of reality

in situ cancer

vs invasive cancer

- send out pioneer cells
- metastisis (isp)
- Causes 90% of deaths from Cancer

Don't understand why some tumors metastizie

What causes cancer?

At first; most thought accident / pre ordained fate

1795: First link of occupation and cancer

Cause → etiologic

1915: Katsusabro Yamagiwa painted coal tar onto ears of rabbits

First induced cancers

1917: Noticed rats in sugar family had worms in stomach → caused cancer

But then displagia - not maligerancy

Since rats living totally on sucrose

Lack of vitamens

Discreted whole notion infectious agents were a cause

(6)

Tobacco use

DbD gave at cigeretts

Lung cancer lag time

130k Americans die every year from cigeretts

$780,000,000

a few year shorter lives

Prof: most interesting thing in this course for your practical life → Don't smoke!

Marajana deaths far lower

4-5k die from second hand smoke

Nicoitean harder to kick than Heroine

Best way to cut cancer deaths is preventing smoking

Prof: When he was growing up we all thought we died of bad luck

But how does this actually cause cancer?

(7)

Way it causes cancer: mutates DNA in our cells

How mutagenic is a compound?

Mutagenic potency

Come into body inactive

Then some things in our liver convert things to be chemically active

More mutagenic = more carcinogenic

bottom left = more potent / more concentrated mutagens and carceogens

aflatoxin grows on wheat that is poorly stored

many cancerogens don't come from chemicos they come from nature!

Cells that are exposed to a mutagen
lost contact inhibition

take at their DNA
put it into normal cells → transfection

do these cells grow uncontrobably?

Yes / No ?

↑ carried in DNA ↑ not carried in DNA

So what is the nature of this DNA?

Oncogene
found to be closly related to a normal gene / proto-oncogene

Some how onco transforms cell
proto onco does not transform

but they look very similar! subtile diff?

(9)

So tried to narrow down the cause of the difference

make certain pieces recombinant

Was narrowed down to 350 bp

was a single point mutation

Showed cancer cell was a mutant cell

(Same had previously also been picked up of retrovirus)

but this was a normal mutants

So this new cell binds GTP

Can also hydrolize GTP

input

↓

Signal transduction/protein

↓

output

signal processing gene!

(10)

Binary switch

Switches from off to on

via exchange of GDP to GTP

GTP-ase

GTP → GDP

hydrolizes

releases Gamma Phosphate

turns itself off after several seconds

A point mutated Ras protient

Causes a single amino acid mutation

but it can no longer turn itself off!

(11)

What if you get a diff mutation?

Prents it from signaling at all possibly

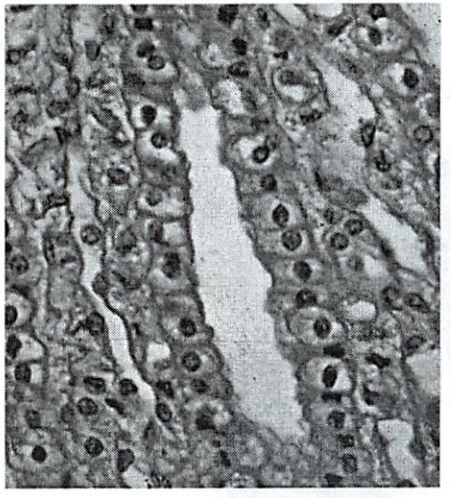
So strong selection for mutations that prevent turn off because a lot of growth stimlitory signaling

the otes don't have an advantage

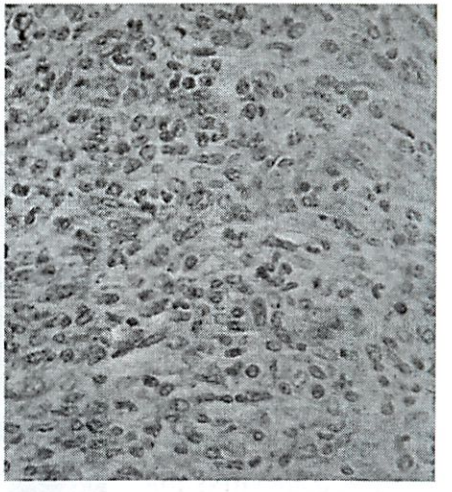
Ken

Cancer Part I. 7.012

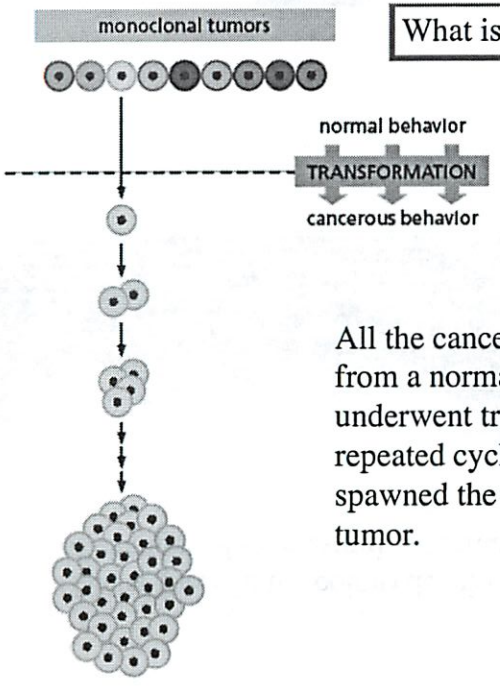
What is cancer?



Normal tissue -- well ordered



A tumor - chaos

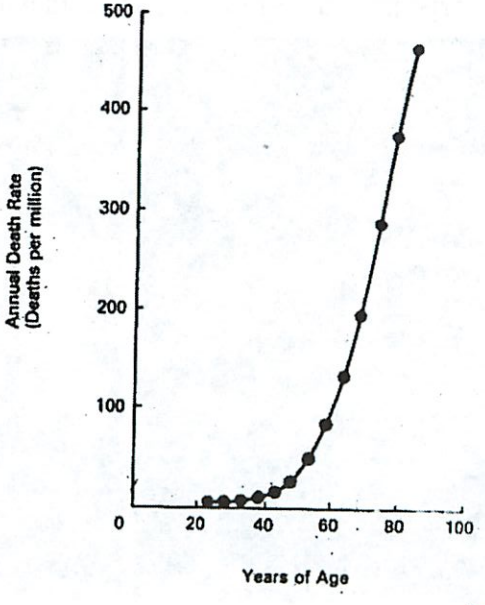


What is cancer?

All the cancer cells in a tumor descend from a normal cell ancestor that underwent transformation and, through repeated cycles of growth and division, spawned the billions of cells in a tumor.

What is cancer?

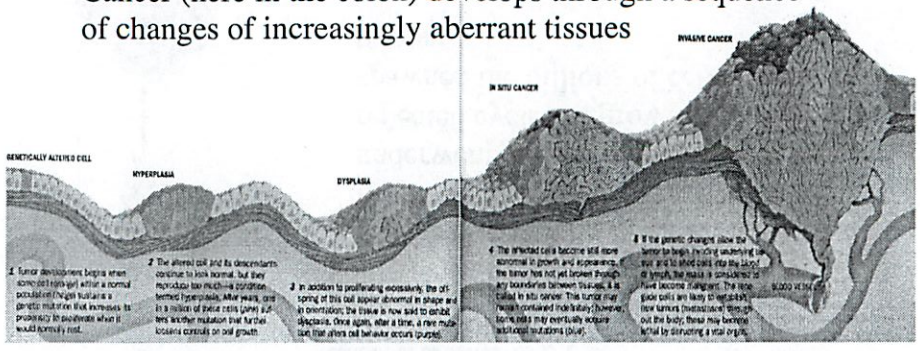
FIGURE 1.1
Annual rate of death from colon cancer in the United States. (From J. Cairns, *Cancer: Science and Society*, 1978.)



Cancer takes a long time to develop.

What is cancer?

Cancer (here in the colon) develops through a sequence of changes of increasingly aberrant tissues

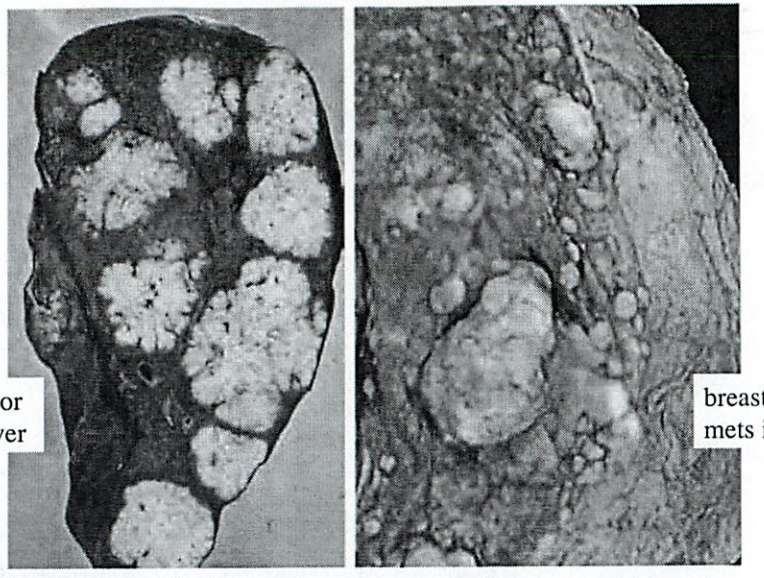


Scientific American

What causes Cancer??

1st clues:
1795, London:
Chimney sweeps get (otherwise rare) scrotal cancer.
(In modern Terms: hence,coal tars are carcinogens

What is cancer?



colon tumor mets in liver

breast tumor mets in brain

Invasive cancer cells in a primary tumor often metastasize (spread) to seed new tumor colonies in distant tissues (metastases).

Painted coal tars on the ears of rabbits and got skin cancer after 6 months. First time that cancer was induced experimentally with a chemical carcinogen.

Katsusaburo Yamagiwa, Tokyo, 1915

Figure 2.21a *The Biology of Cancer* (© Garland Science 2007)

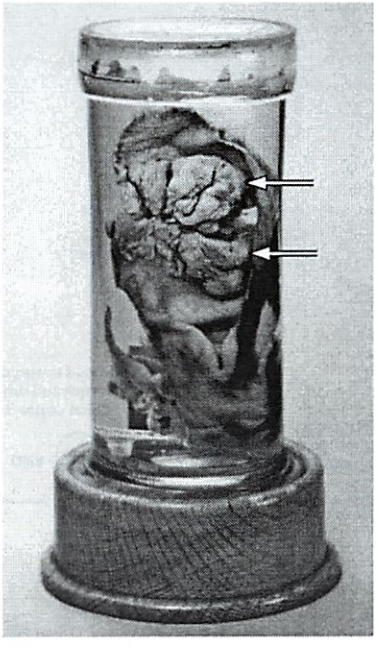


Figure 2.21b *The Biology of Cancer* (© Garland Science 2007)

1930s: tar constituents are purified and identified chemically

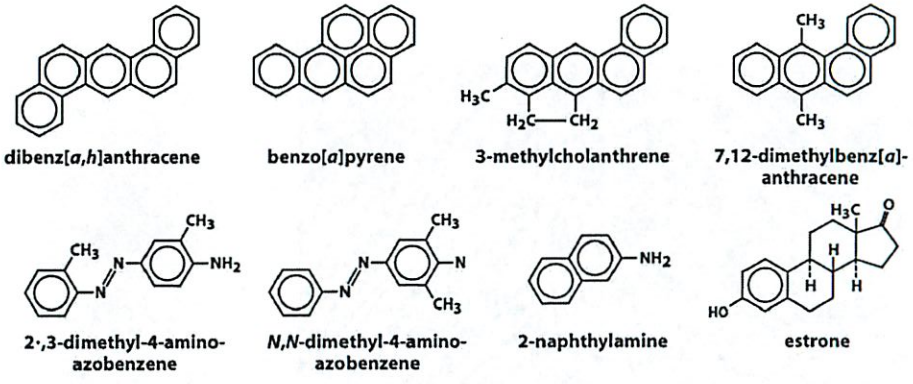
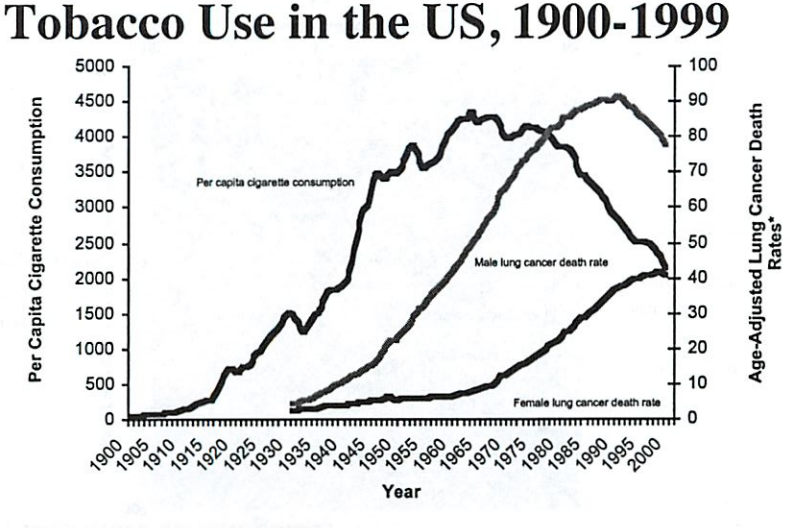


Figure 2.22 *The Biology of Cancer* (© Garland Science 2007)

1950: tobacco use, and thus **tobacco tars** implicated in lung cancer.



They subsidize tobacco!

Bruce Ames: Develop a test to quantify the **mutagenic potency** of various chemical Compounds

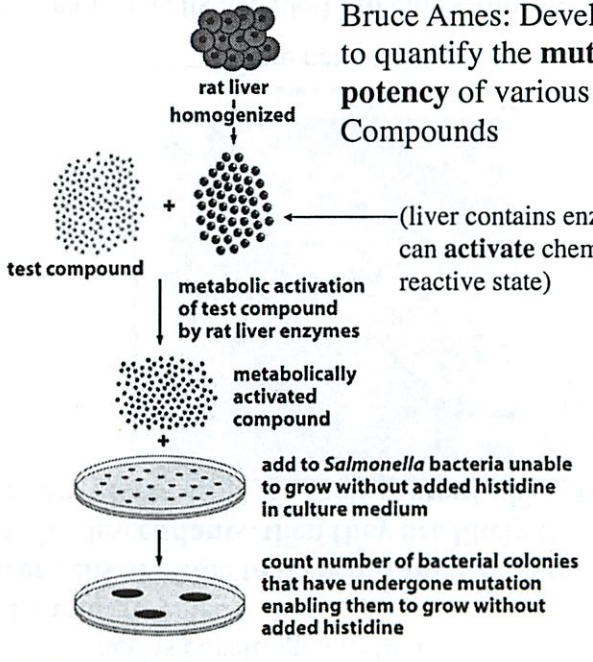
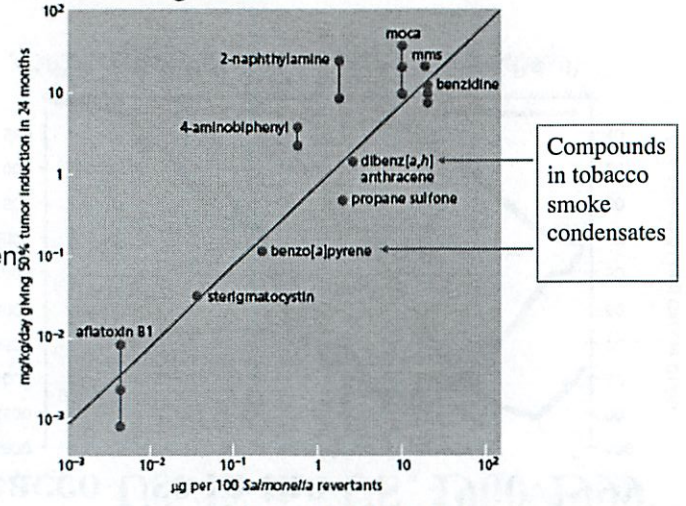


Figure 2.24 *The Biology of Cancer* (© Garland Science 2007)

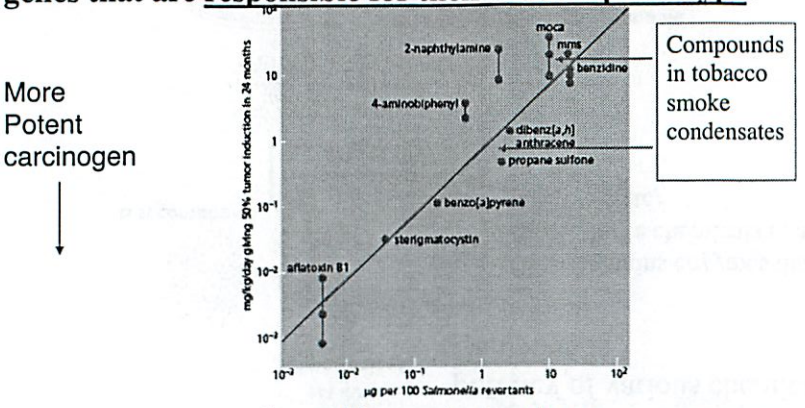
Causes of cancer: compounds that are more mutagenic are also more carcinogenic!



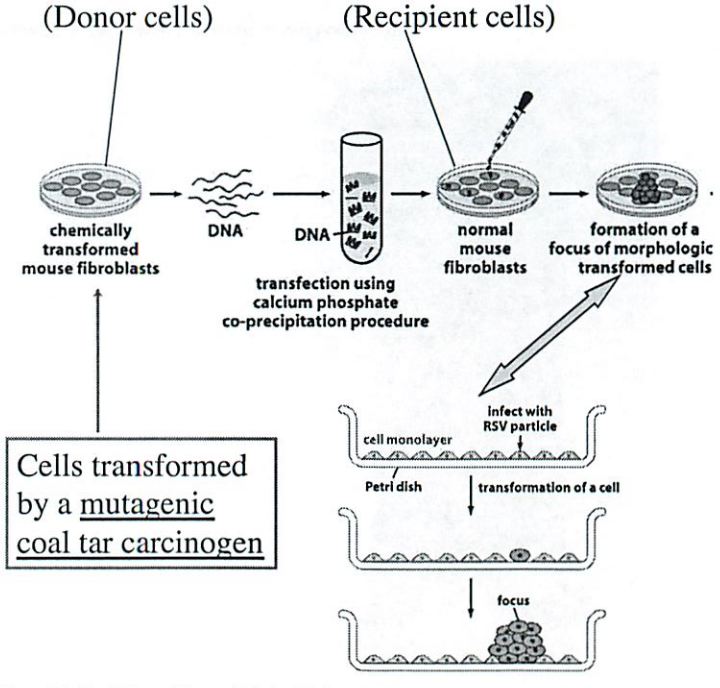
Logic:

1. If mutagens act as carcinogens, then cancer cells are likely to be mutant cells.

2. If cancer cells are able to transmit their phenotype from one cell to its descendants, then they are likely to carry mutant genes that are responsible for their mutant phenotype.



Inference: **cancer cells are likely to carry mutant genes.**



Note that this time the focus of transformed cells has **not been** caused by a retrovirus infection. Instead it's been caused by introduction of DNA from a cancer cell. (no viruses around)

Descendants of a normal recipient cell that received DNA (via transfection) from a chemically transformed donor cell

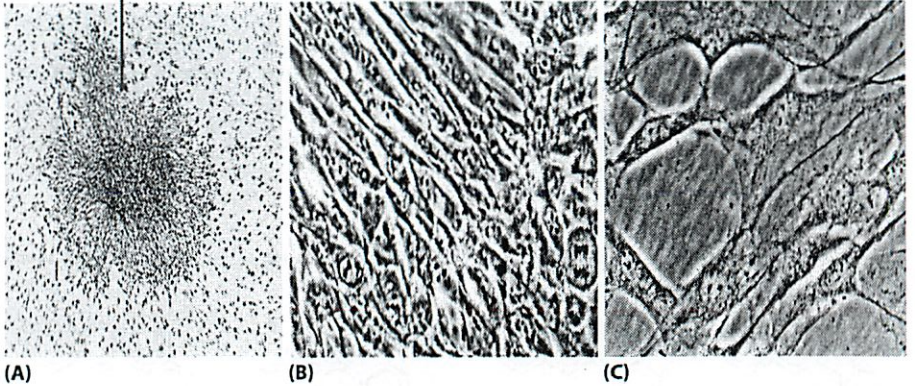


Figure 4.2 *The Biology of Cancer* (© Garland Science 2007)

Figure 4.3 *The Biology of Cancer* (© Garland Science 2007)

Goal: prove that the cells in a focus of transformed cells in a Petri dish are really cancer cells!

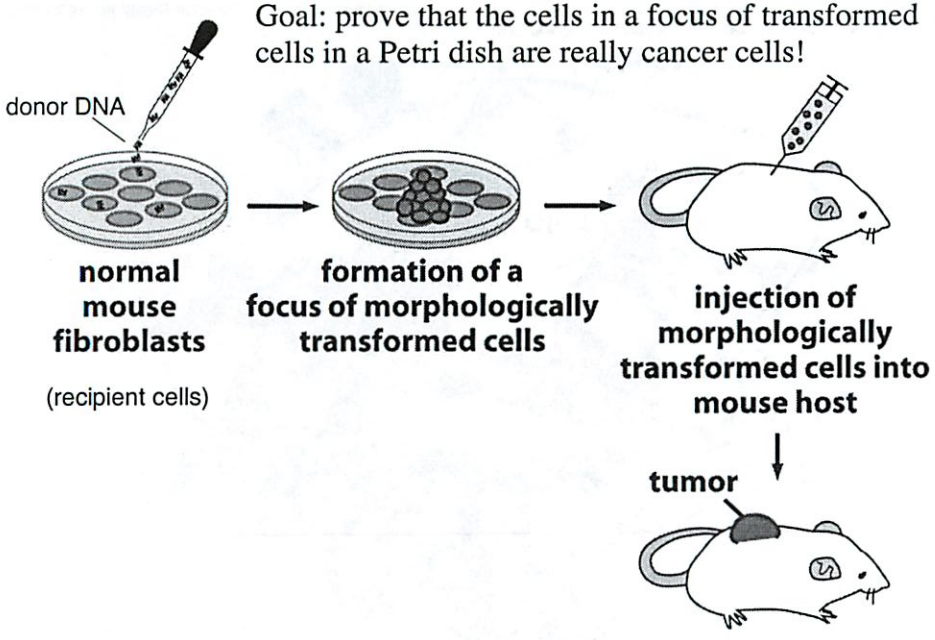
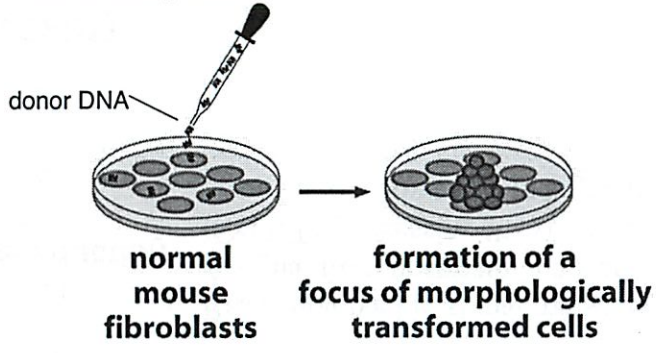
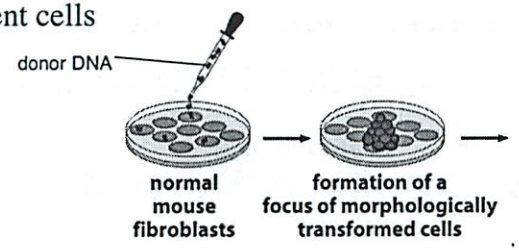


Figure 4.2 (part 2 of 2) *The Biology of Cancer* (© Garland Science 2007)

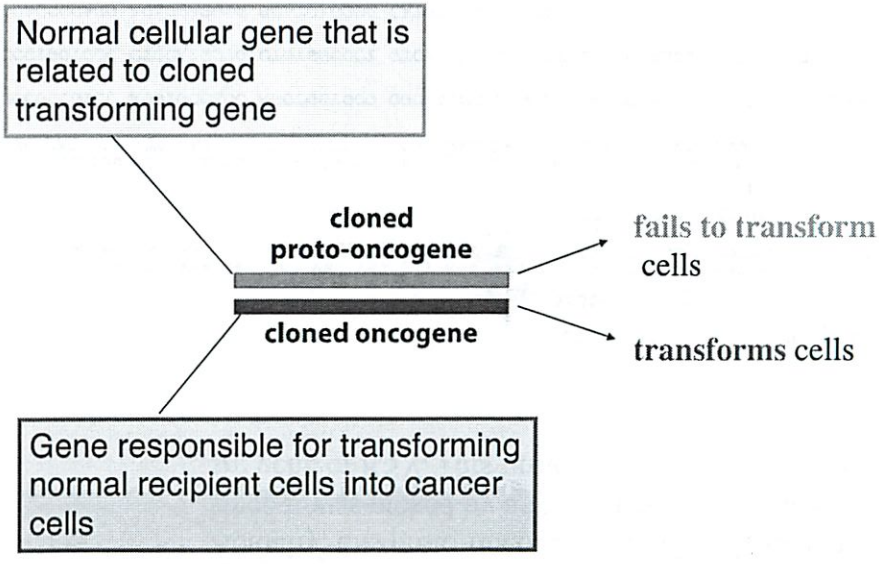
Proceed to **clone** the gene within the donor DNA that is responsible for the transformation of the recipient cells



Proceed to **clone** the "oncogene" within the donor DNA that is responsible for the transformation of the recipient cells



Find that the cloned oncogene is **closely related** to a gene present in the normal human genome.
Call the normal gene a "proto-oncogene".
(Indeed, the two genes are almost identical in sequence.)



Where is the critical difference between the two genes??

Sequence both genes
to determine differences

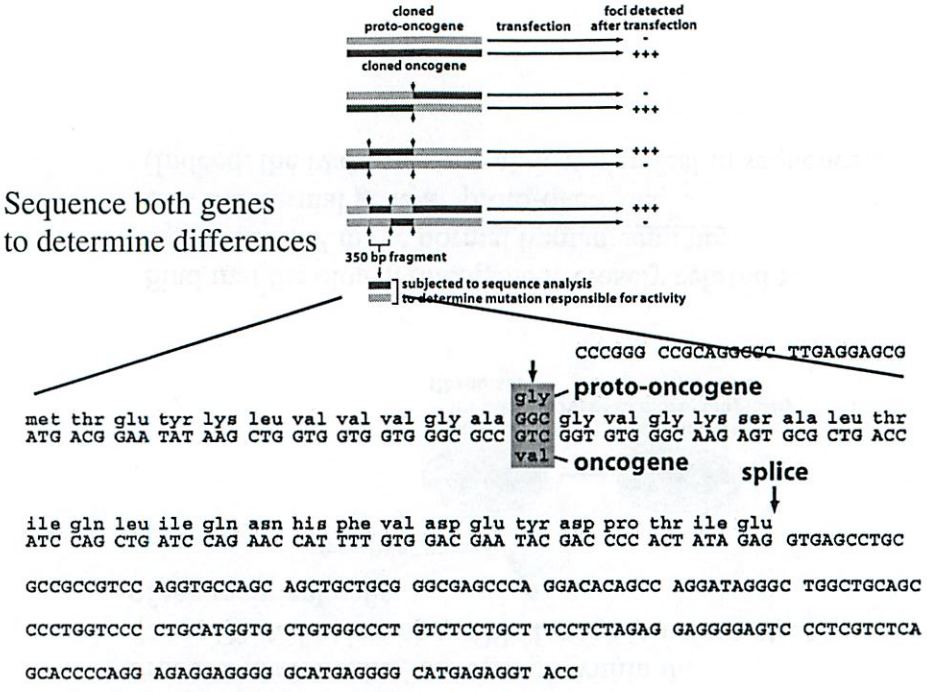


Figure 4.10 *The Biology of Cancer* (© Garland Science 2007)

Actually, this proto-oncogene was already known, since it was picked by a retrovirus, just like the *src* gene of RSV. This proto-oncogene is called *ras*.

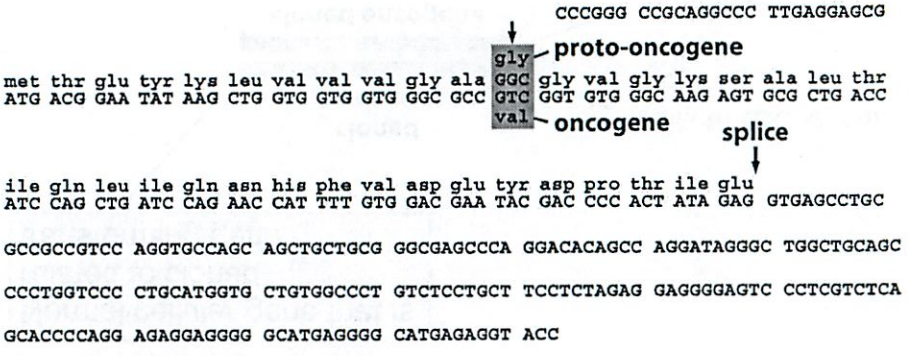


Figure 4.10 *The Biology of Cancer* (© Garland Science 2007)

Product of the *ras* gene:
A G protein!

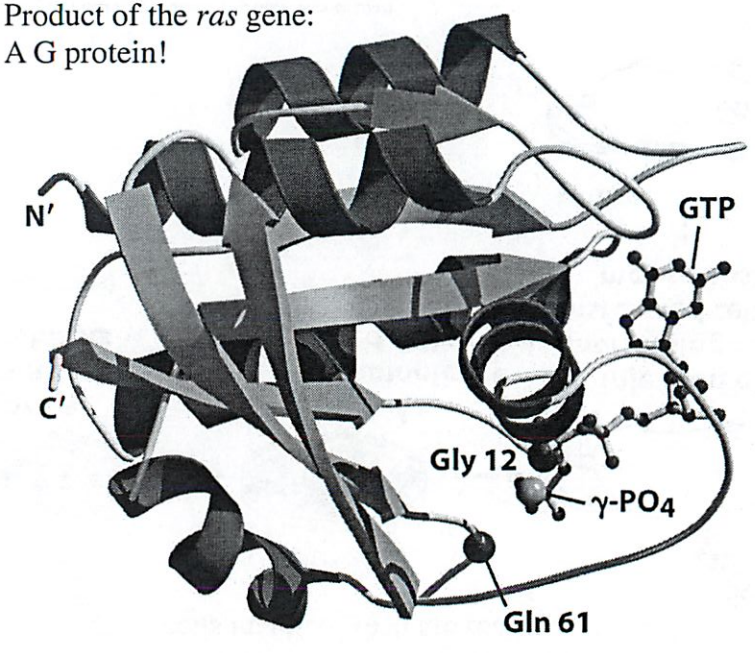
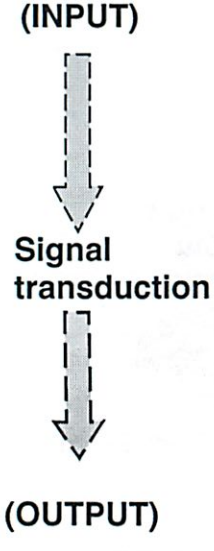


Figure 5.31 *The Biology of Cancer* (© Garland Science 2007)



Ras is involved in <u>signal transduction</u> -- receiving A signal from upstream in a signaling pathway, (INPUT), processing the signal, and then releasing a signal to a downstream target (OUTPUT).

(INPUT)(upstream)

Signal transduction

(OUTPUT)(downstream)

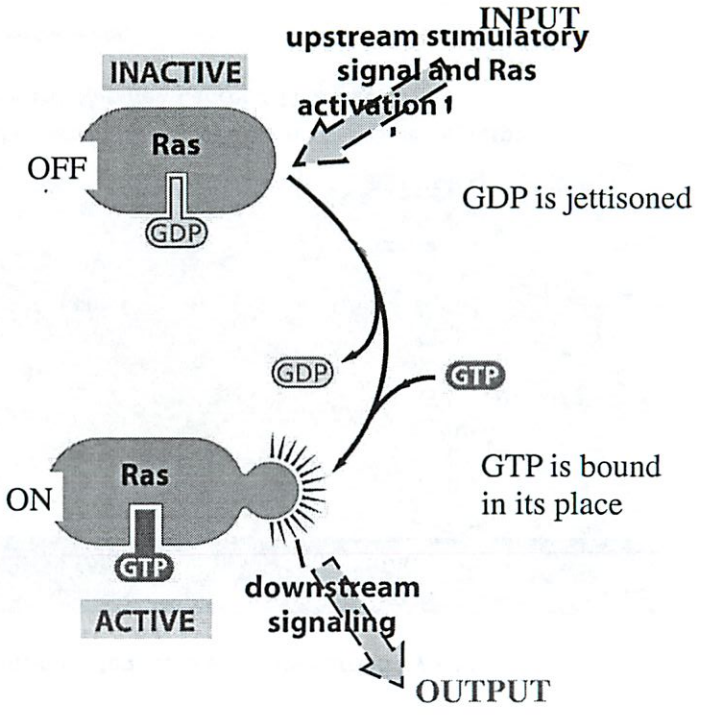
In fact a ligand-activated growth factor receptor is the usual source of upstream signals.

Ras is involved in signal transduction -- receiving A signal from upstream in a signaling pathway, (INPUT), processing the signal, and then releasing a signal to a downstream target (OUTPUT).

(INPUT)

Signal transduction

(OUTPUT)



Ras operates like a binary switch

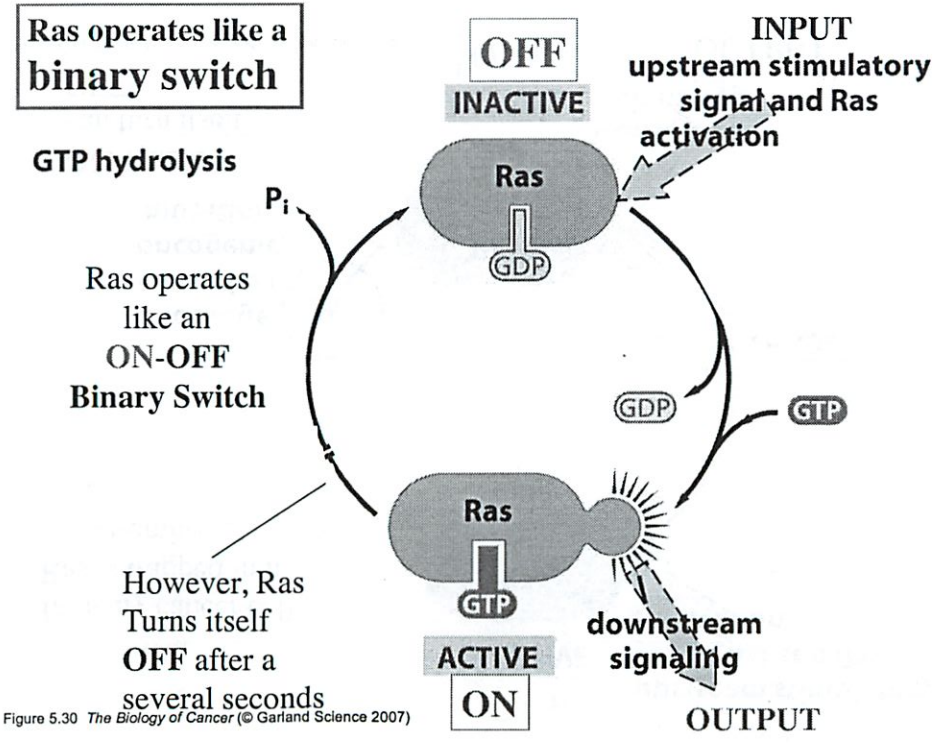


Figure 5.30 *The Biology of Cancer* (© Garland Science 2007)

What happens in many cancers?

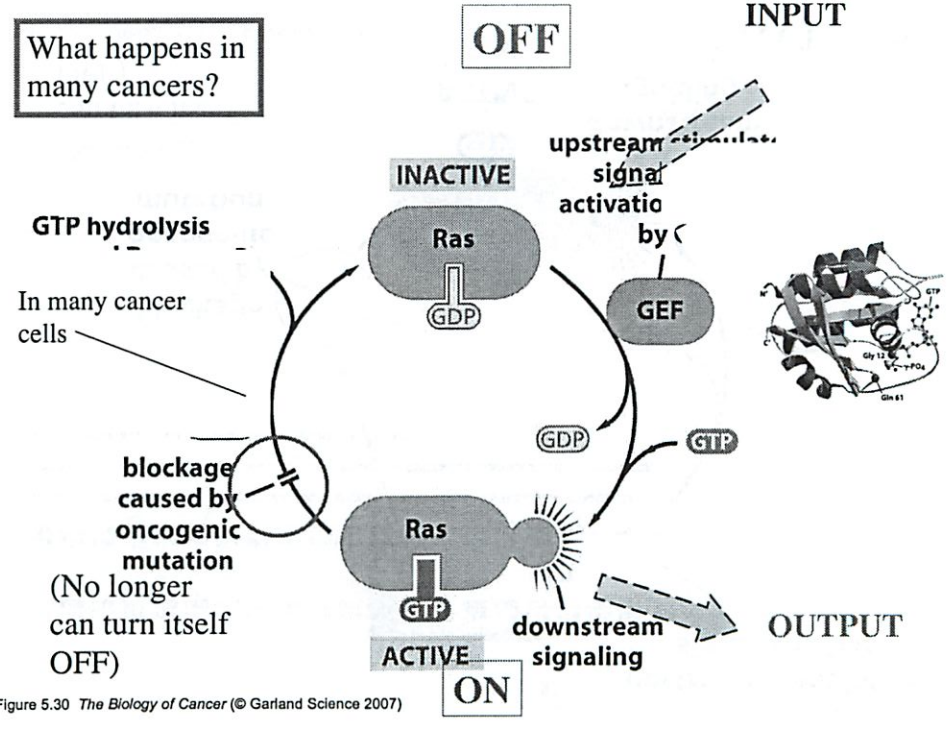


Figure 5.30 *The Biology of Cancer* (© Garland Science 2007)

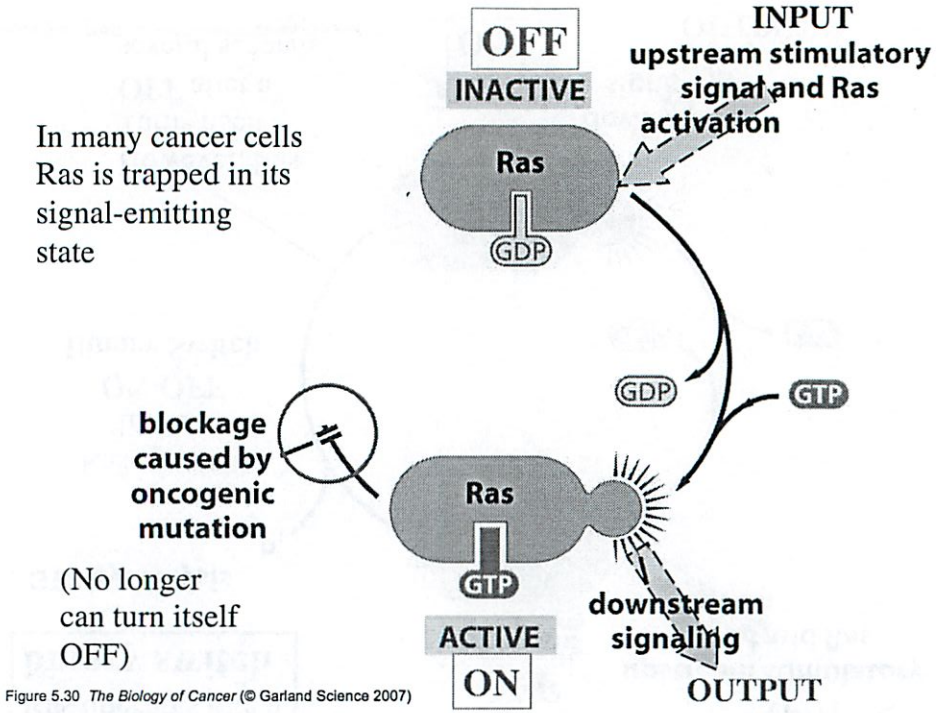


Figure 5.30 *The Biology of Cancer* (© Garland Science 2007)

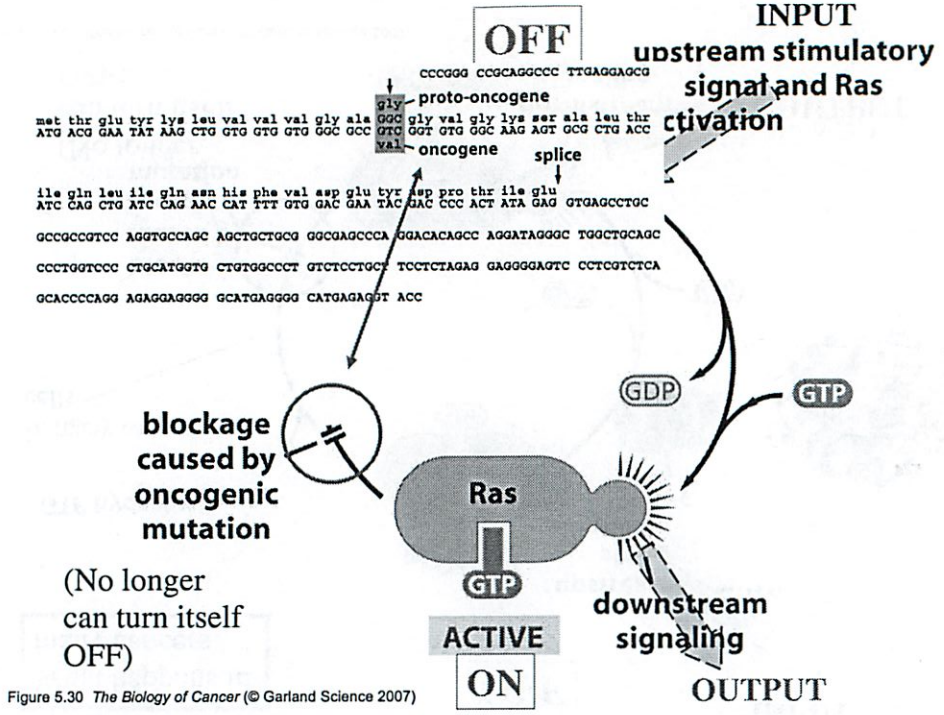


Figure 5.30 *The Biology of Cancer* (© Garland Science 2007)

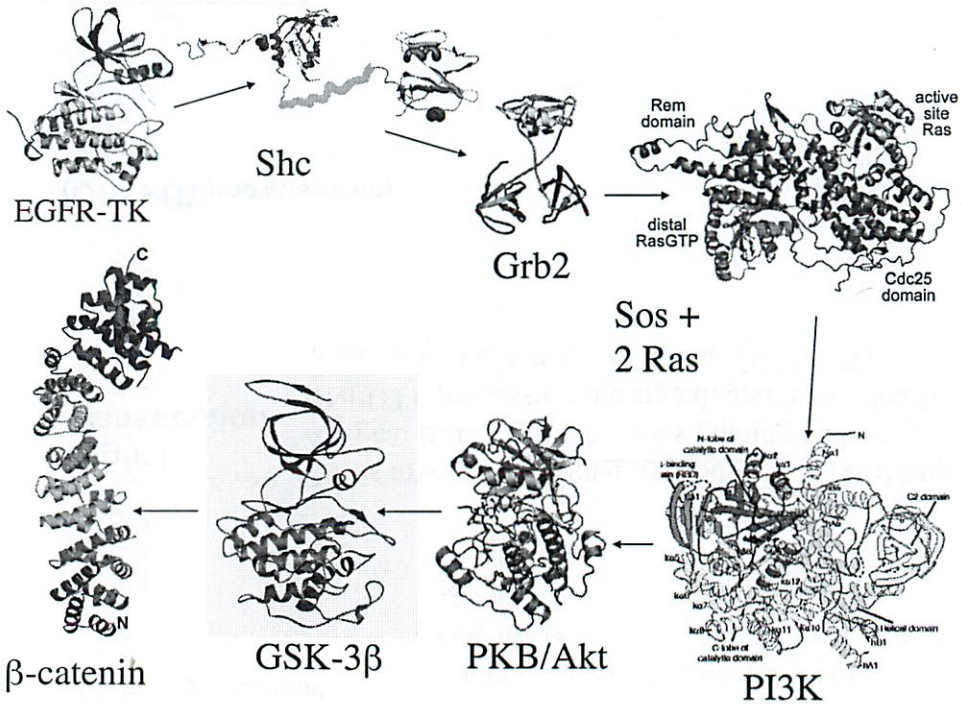


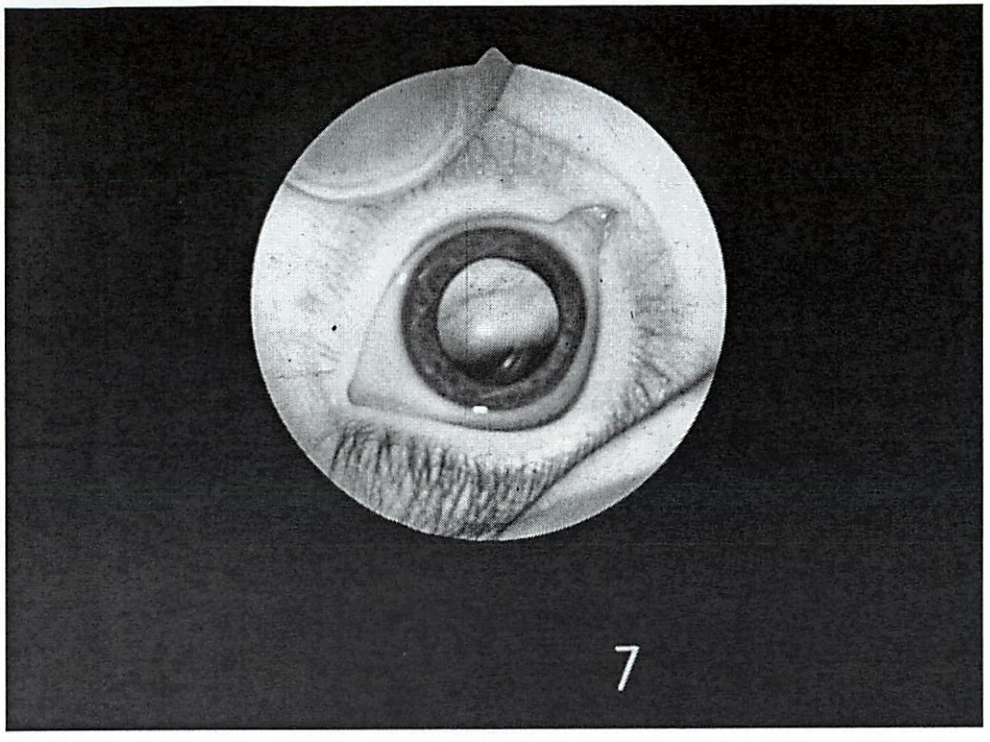
Table 4.2 A list of point-mutated *ras* oncogenes carried by a variety of human tumor cells

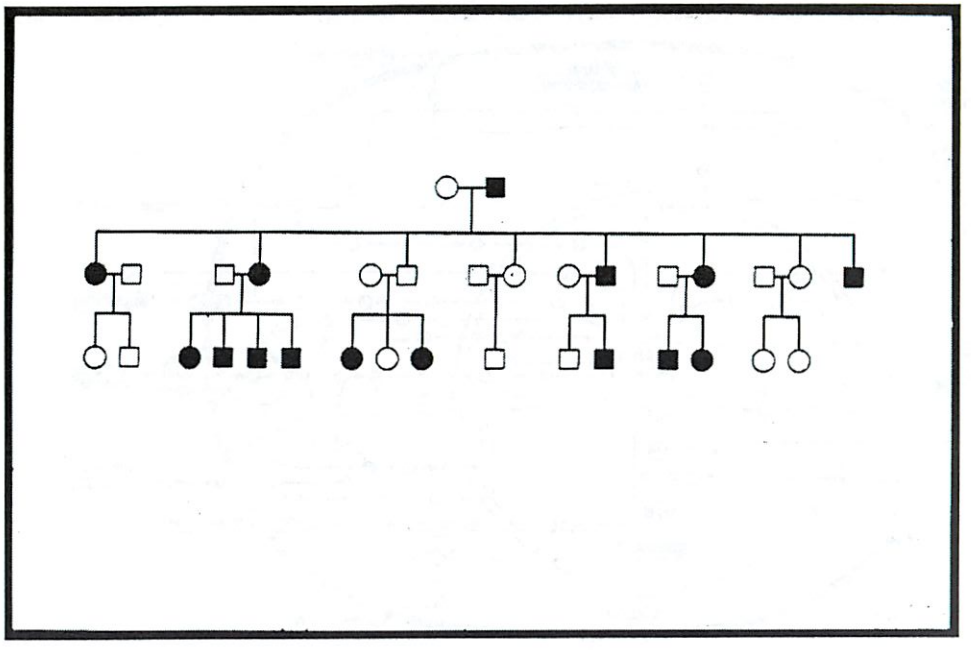
Tumor type	Proportion (%) of tumors carrying a point-mutated *ras* gene[a]
Pancreas	90 K
Thyroid (papillary)	60 (H, K, N)
Thyroid (follicular)	55 (H, K, N)
Colorectal	45 (K)
Seminoma	45 (K, N)
Myelodysplasia	40 (N, K)
Lung (non-small-cell)	35 (K)
Acute myelogenous leukemia	30 (N)
Liver	30 (N)
Melanoma	15 (K)
Bladder	10 (K)
Kidney	10 H

[a]H, K, and N refer to the human *H-RAS*, *K-RAS*, and *N-RAS* genes, respectively.

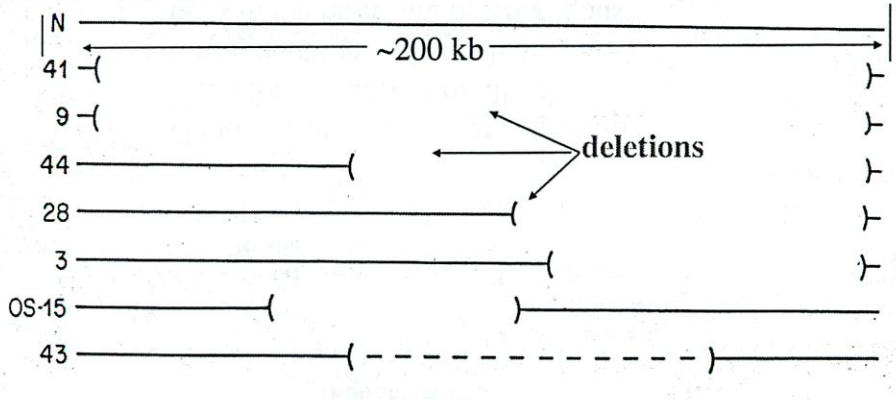
Adapted from J. Downward, *Nat. Rev. Cancer* 3:11–22, 2003.

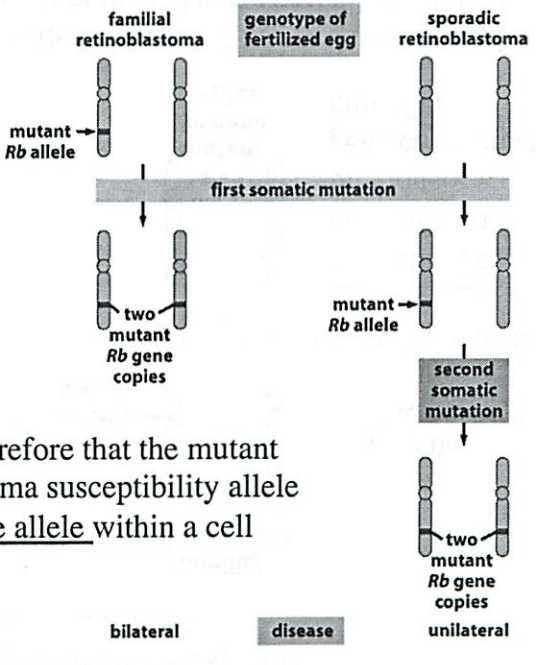
Table 4.2 *The Biology of Cancer* (© Garland Science 2007)





Mutations that affect the *Rb* (retinoblastoma gene) **kill** the gene (see these deletions) rather than potentiating its function.

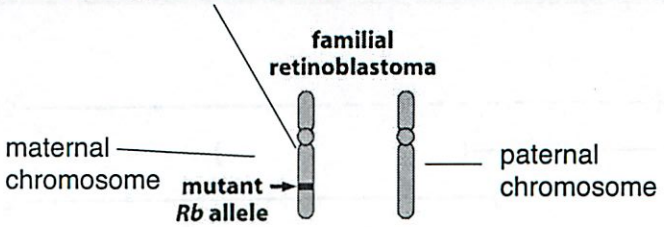




Imagine, therefore that the mutant Retinoblastoma susceptibility allele is a recessive allele within a cell

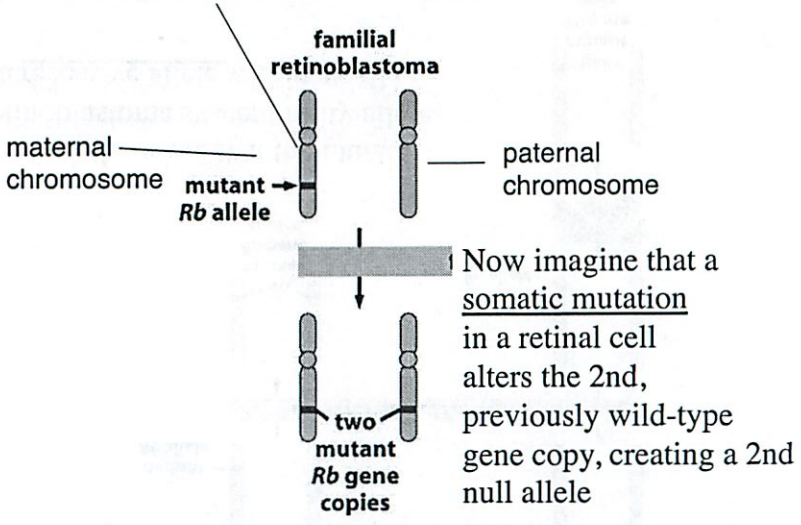
Figure 7.7 *The Biology of Cancer* (© Garland Science 2007)

Mutant null allele passed through sperm or egg,
i.e., a mutant germ-line allele



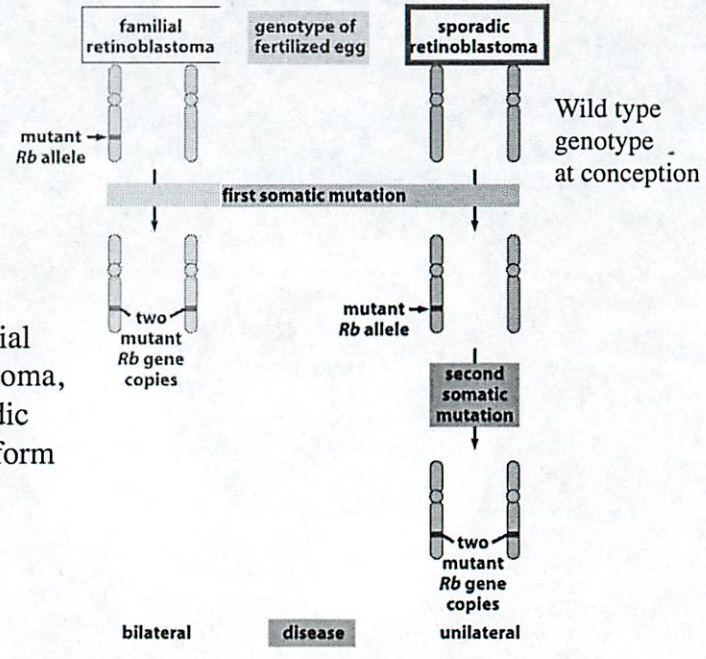
Genotype of organism <u>at conception</u>.
Therefore, genotype of all cells
throughout the body, including all
cells in the retina are heterozygous
at *Rb* locus.

Mutant allele passed through sperm or egg,
i.e., a mutant germ-line allele



Now imagine that a
<u>somatic mutation</u>
in a retinal cell
alters the 2nd,
previously wild-type
gene copy, creating a 2nd
null allele

Now, that retinal cell has two mutant copies of *Rb*
gene. If both alleles are null alleles, total loss of Rb function.



Non-familial
retinoblastoma,
i.e., sporadic
(random) form
of disease.

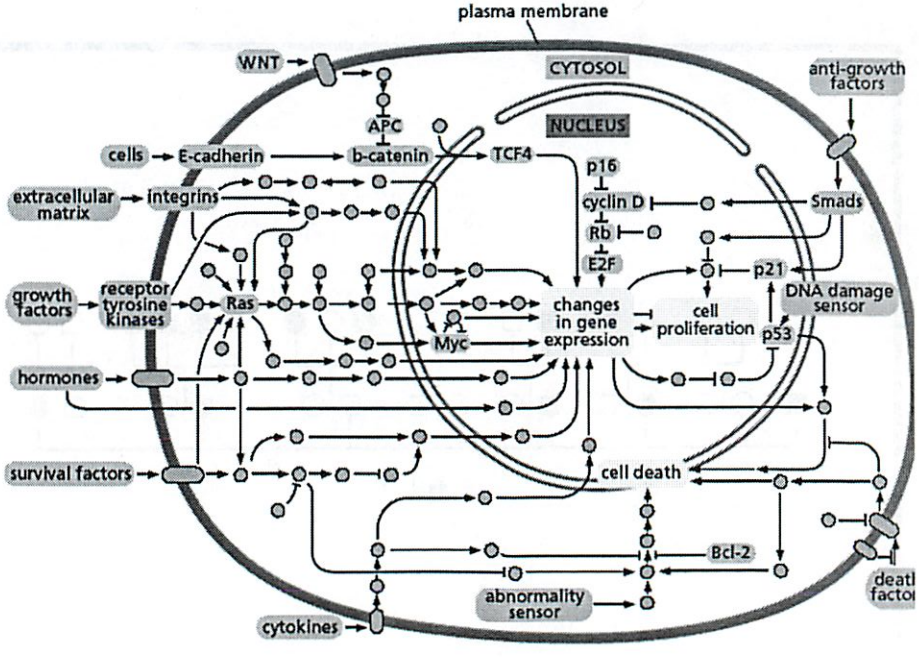
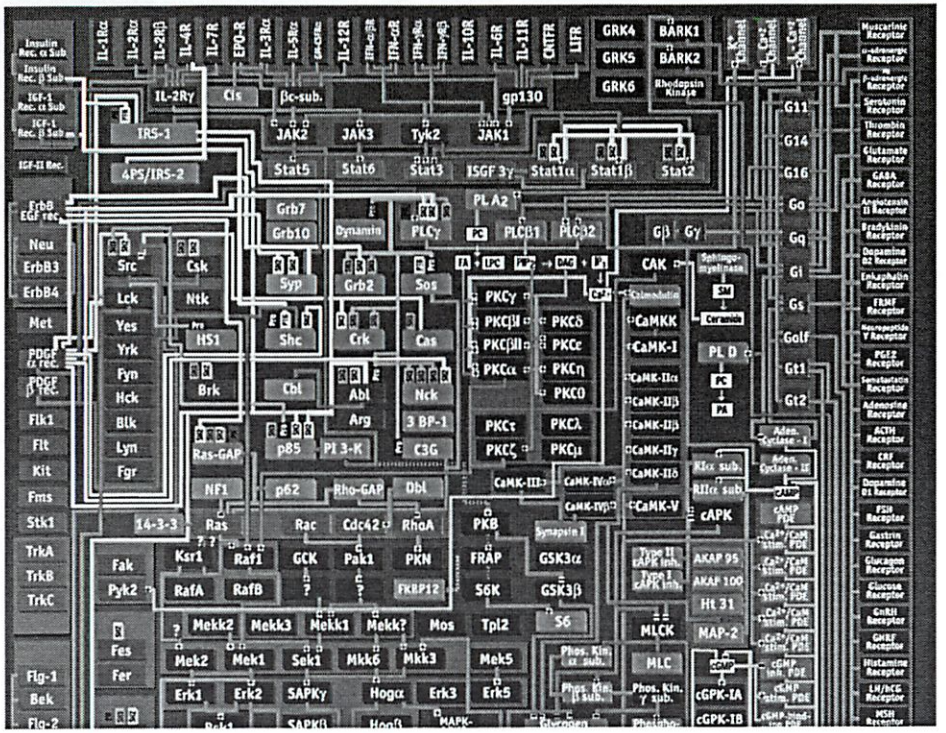


Figure 7.7 *The Biology of Cancer* (© Garland Science 2007)


Insulin Rec. α Sub.
Insulin Rec. β Sub
IGF-1 Rec. α Sub
IGF-1 Rec. β Sub
IGF-II Rec.
ErbB EGF rec.
Neu
ErbB3
ErbB4
Met
PDGF α rec.
PDGF β rec.
Flk1
Flt
Kit
Fms
Stk1
TrkA
TrkB
TrkC
Flg-1
Bek
Flg-2
IL-1Rα
IL-2Rα
IL-2Rβ
IL-4R
IL-7R
EPO-R
IL-3Rα
IL-5Rα
GM-CSFR
IL-12R
IFN-αβR
IFN-γR
IFN-βR
IL-10R
IL-6R
IL-11R
CNTFR
LIFR
GRK4
GRK5
GRK6
BARK1
BARK2
Rhodopsin Kinase
Ca2+ Channel
K+ Channel
Na+ Channel
Muscarinic Receptor
α-adrenergic Receptor
β-adrenergic Receptor
Serotonin Receptor
Thrombin Receptor
Glutamate Receptor
GABA Receptor
Angiotensin II Receptor
Bradykinin Receptor
Dopamine D2 Receptor
Endothelin Receptor
FRMF Receptor
Neuropeptide Y Receptor
PGE2 Receptor
Somatostatin Receptor
Adenosine Receptor
ACTH Receptor
CRF Receptor
Dopamine D1 Receptor
FSH Receptor
Gastrin Receptor
Glucagon Receptor
Glucose Receptor
GnRH Receptor
GHRF Receptor
Histamine Receptor
LH/hCG Receptor
MSH Receptor
IL-2Rγ
Cis
βc-sub.
gp130
IRS-1
4PS/IRS-2
JAK2
JAK3
Tyk2
JAK1
Stat5
Stat6
Stat3
ISGF 3γ
Stat1α
Stat1β
Stat2
Grb7
Grb10
Dynamin
PLCγ
PC
PLCβ1
PLCβ2
PL A2
Gβ
Gγ
G11
G14
G16
Go
Gq
Gi
Gs
Golf
Gt1
Gt2
Src
Csk
Syp
Grb2
Sos
CAK
Sphingo-myelinase
SM
Ceramide
Lck
Ntk
Yes
Yrk
Fyn
Hck
Blk
Lyn
Fgr
HS1
Shc
Crk
Cas
PKCγ
PKCβII
PKCβI
PKCα
PKCδ
PKCε
PKCη
PKCθ
PKCι
PKCζ
PKCλ
PKCμ
Calmodulin
CaMKK
CaMK-I
CaMK-IIα
CaMK-IIβ
CaMK-IIβ
CaMK-IIγ
CaMK-IIδ
CaMK-V
CaMK-III
CaMK-IVα
CaMK-IVβ
PL D
PC
PA
Brk
Cbl
Abl
Arg
Nck
3 BP-1
C3G
Ras-GAP
p85
PI 3-K
NF1
p62
Rho-GAP
Dbl
14-3-3
Ras
Rac
Cdc42
RhoA
PKB
FRAP
GSK3α
GSK3β
S6K
S6
Synapsin I
Adeny. Cyclase - I
Adeny. Cyclase - II
cAMP
cAMP PDE
Ca2+/CaM stim. PDE
Ca2+/CaM stim. PDE
Ca2+/CaM stim. PDE
Ca2+/CaM stim. PDE
Ca2+/CaM stim. PDE
cGMP Inh. PDE
cGMP Stim. PDE
cGMP-stimulated PDE
Rlα sub.
RIIα sub.
cAPK
Type II cAPK Inh.
Type I cAPK Inh.
AKAP 95
AKAP 100
Ht 31
MAP-2
MLCK
MLC
cGMP
cGPK-IA
cGPK-IB
Phos. Kin. α sub.
Phos. Kin. β sub.
Phos. Kin. γ sub.
Fak
Pyk2
Fes
Fer
Ksr1
Raf1
RafA
RafB
GCK
Pak1
PKN
?
FKBP12
Mekk2
Mekk3
Mekk1
Mekk?
Mos
Tpl2
Mek2
Mek1
Sek1
Mkk6
Mkk3
Mek5
Erk1
Erk2
SAPKγ
Hogα
Erk3
Erk5
SAPKβ
Hogβ
MAPK-

2012
Case 2

Clonal expansion is like Darwinian evolution

multi-stage tumor expansion

Many mutations - happen at random

though most break cell

so clonal expansion does not help

Ras

input
↓
signal transduction
↓
output

on/off cycle

since GTP-ase

hydrolizes to GDP (so inactivates)

in order of seconds

(2)

But can block turn off
So too much growth stimulating protein

~~Blocking~~

If blocked turn on - cell would not expand
So likely <u>not</u> to cause massive clonal expansion

Play a normal role in tissue

Codon 12 - Freq mutation
that carrys on

The other ones not marked do have mutations
but those cells don't expand

Codon 12 is what GTP → GDP

Ras plays a role in a complex signaling cascade
we are simplifing

(3)

Retinoblastoma

Only in children up to 7 ← since cells have specialized

~10,000 cases/year in US

Often in familial form

Was fatal historically

- But now passed on to their children
  - They used to die

Much diff than Ras onco gene

Delete many things in the gene

Lots of null-alleles

But these are advantageas for cell to proliferate

- Opposite as previous!

Those deleted genes usually inhibit growth

- defeactive ~~brea~~ braking gene
  - called tumor suppression genes

(4)

Are actually more ~~breakin~~ braking cells than growth promoting cells!

Usually Dad's fault

Hetero - cell normal

bA in retina for some reason will type last cell now homozygous in retina

So often in both eyes

---

also a sporadic form

after formation of zygote

2 mutations

1st $\frac{1}{10^6}$

2nd even more improbable

So very improbable

So usually only in 1 eye

(5)

Therapy

mortality ↑ from heart disease has plateaued, but cancer is unchanged

1. medicines
2. not smoking
3. change in diet

People living longer so cancer + alzithers more common

└ dabble edge sword

Cancer is much more complicated than heart disease

Some cancers ↓

~~[illegible]~~ refrigeration + food preservatives

Vaccines like HPV

(he don't belive in Organic)

but some flat

(6)

easiest is to prevent these diseases in the first place

---

often the more ya look the more ya find

incidence higher when people go to dermatologist more

So much of this much of the rise might be

due to ∩ diagnostic

---

breast cancer

had a "crisis"

but mortality flat/down

much better detection tools

would never have been recognized before

but who should one treat

since many tumors never expand to be

more serious

(7)

Microarray

(missed)

Can put into groups

Use molecular tools to stratify tumors into subclasses

→ Signal induction cascades

Can inhibit in diff places

Mitogenic - proliferation permitting

- Often receptors over exposed on surface

~30% of breast cancers

Causes them to fire w/o reason

HER2 protien over exposed

Can test how many extra copies

Causes cells to proliferate more

Not amplified → better prognosis

So makes a big difference

Can make something which blocks HER2 from functioning

Must stay outside cell → too big

Can only bind to receptor outside cell

If try to kill cells by irating

trying to induce suicide (apiptosis)

iop

Adding Herceptin is more powerful than radiation On its own

So reduces chance by about half of another Cancer somewhere else in the body.

(9)

Blast crisis (missed)

↑ early cells

leads to death

Karyotype Collection of chromosomes in cells

note the translocation

(reciprical translocation)

When not homologous

Called Philadelphia chromosone (Ph)

---

formed fusion protien

Can make diff kinds

Shl → Tyr kinase

important in driving proliferation

tried to block in 90s

(he lost me)

(10)

but Tyr-kinase of Abl is very similar

To the other tyrosine-kinase

those play critical roles

Can't ~~with~~ shut those down!

Gleevec

Specifically inhibited Tyr-kinase of Abl

Also 2 more - but they play minor roles

fits very well into enzyme cavity of protein

Specific for that kinase - but not others!

% of inhibition of kinase

highly specific

if give early, can help them live 10 years more

must keep giving

(11)

Some individuals develop resistance to drug

So one thing on side has point mutation
sticks out + blocks gleevec

Expands since selectivly advantageous for cell!

Leads to clinical relapse

So we need another drug that doesn't get
blocked → second-like drug

Several (~7) possible mutation

Note Gleevec kills transit amplifying cells but
fail to kill cancer stem cells
those survive
then when Gleevec stopped, they come back

(12)

Also a number of Th inhibitors → Tarceva

Plus more in development

So chemical space

only a limited # of things can be generated

10^{16} possible total drugs

10^6 discovered

7.012

2012

2nd cancer lecture

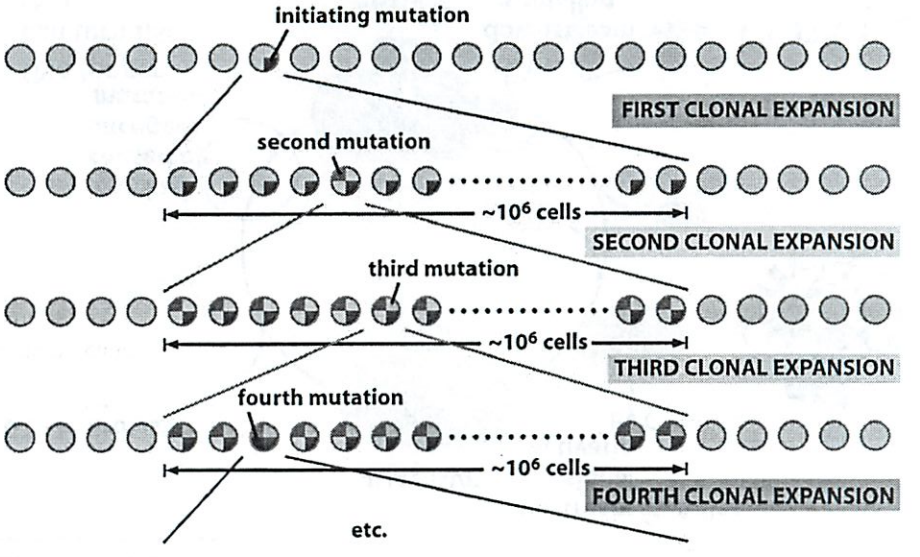


Figure 11.12 *The Biology of Cancer* (© Garland Science 2007)

To review:

Product of the *ras* ge
A G-protein!

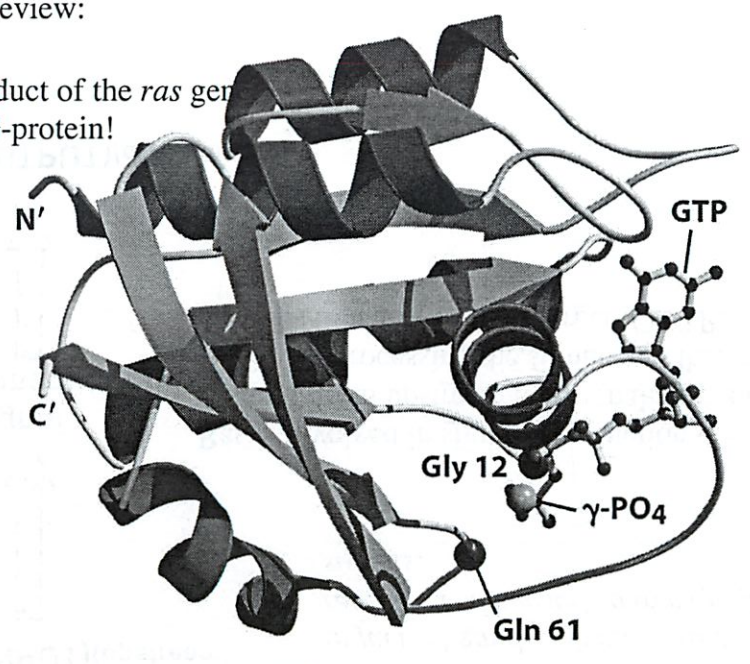
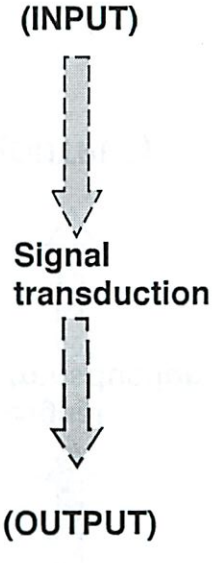


Figure 5.31 *The Biology of Cancer* (© Garland Science 2007)



Ras is involved in signal transduction -- receiving A signal from upstream in a signaling pathway, (INPUT), processing the signal, and then releasing a signal to a downstream target (OUTPUT).

h 121

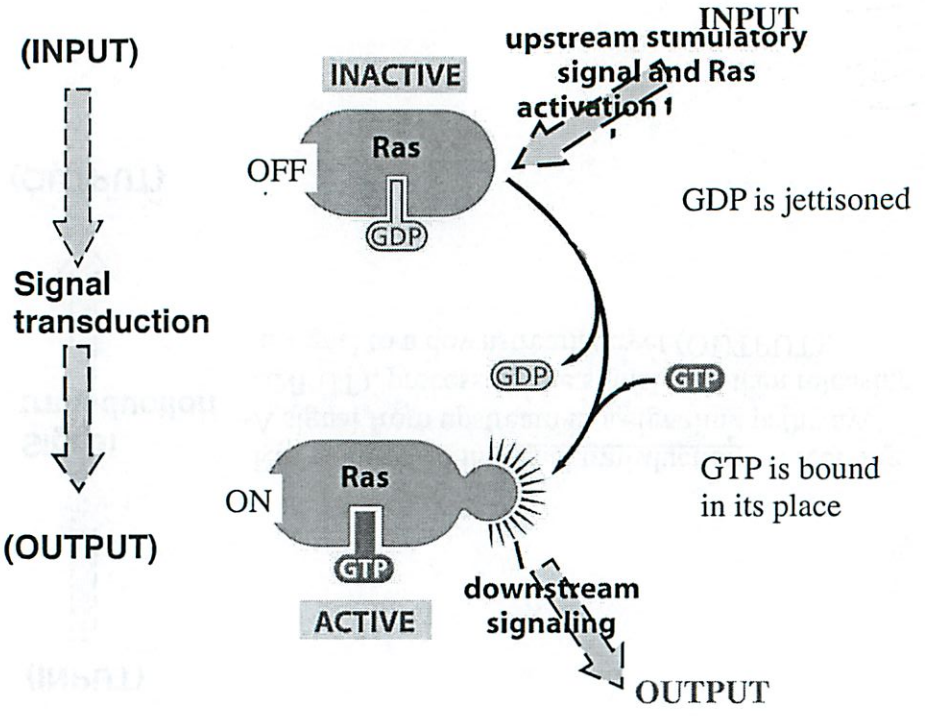
(INPUT)(upstream)

In fact a ligand-activated growth factor receptor is the usual source of upstream signals.

Signal transduction

Ras is involved in signal transduction -- receiving A signal from upstream in a signaling pathway, (INPUT), processing the signal, and then releasing a signal to a downstream target (OUTPUT).

(OUTPUT)(downstream)



Ras operates like a binary switch

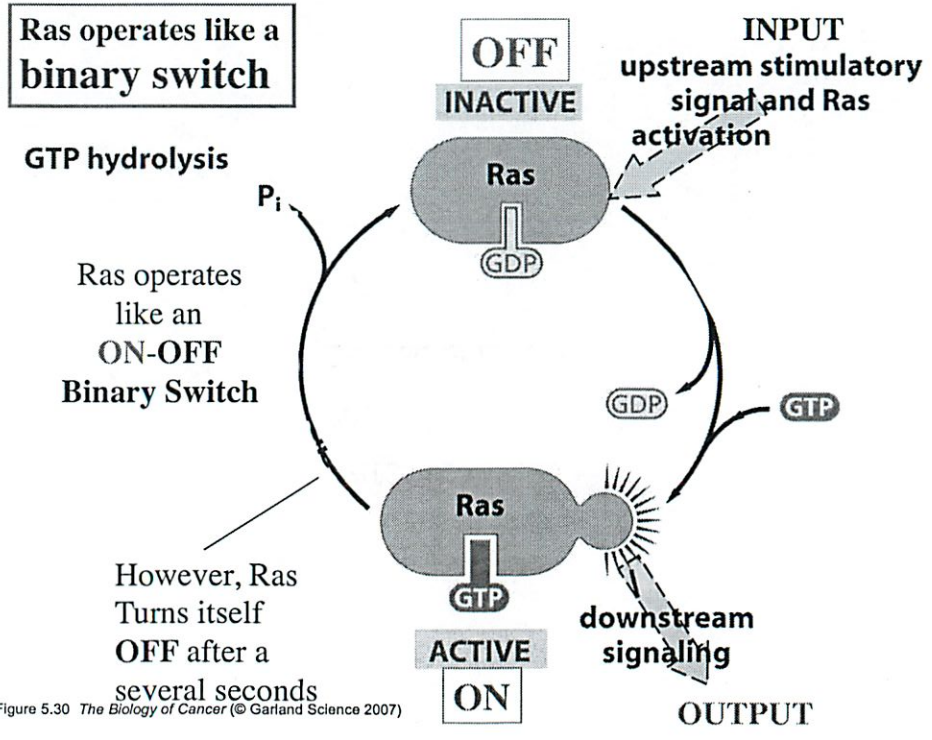


Figure 5.30 *The Biology of Cancer* (© Garland Science 2007)

What happens in many cancers?

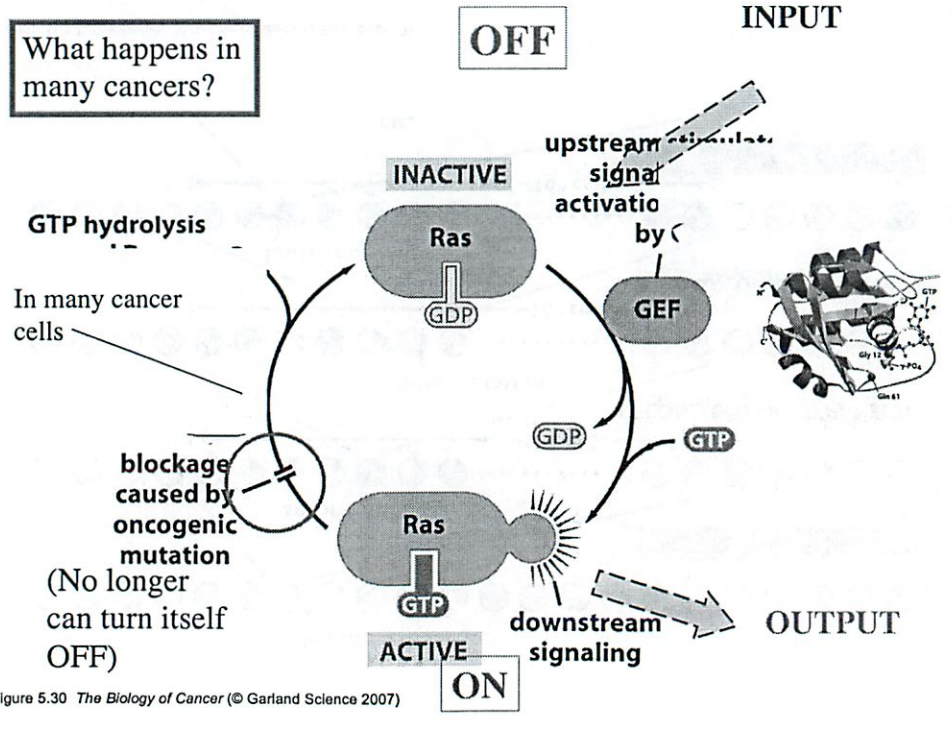


Figure 5.30 *The Biology of Cancer* (© Garland Science 2007)

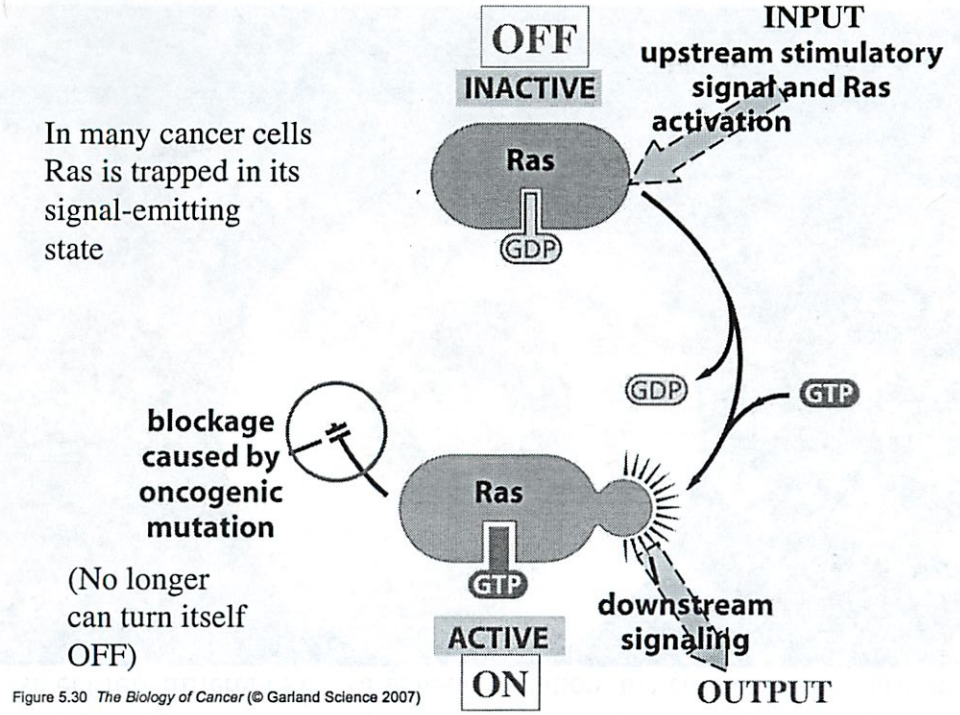


Figure 5.30 *The Biology of Cancer* (© Garland Science 2007)

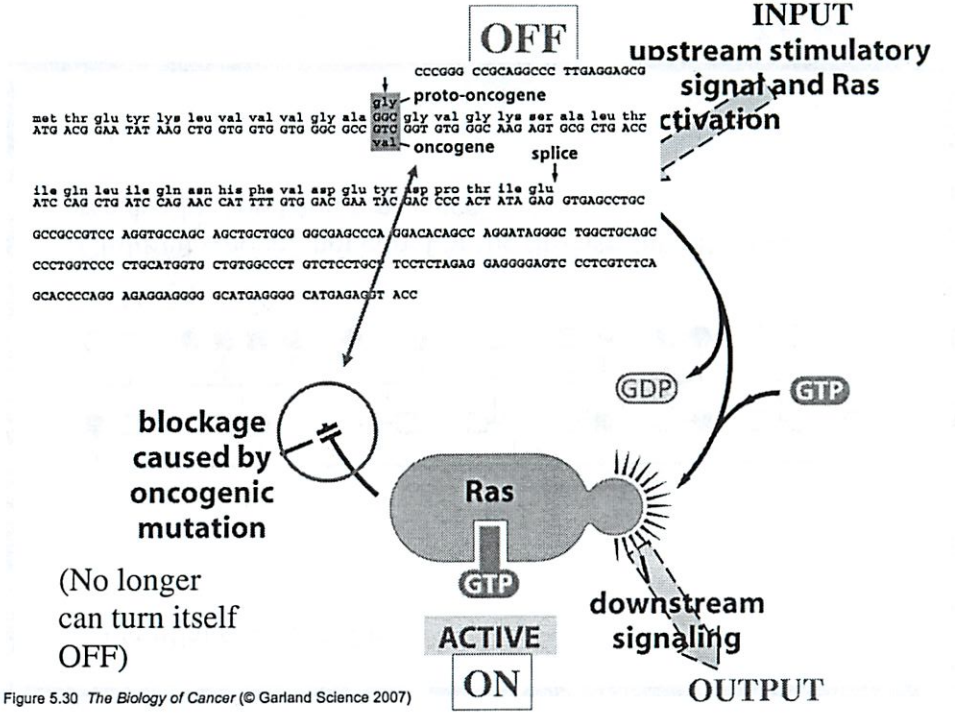


Figure 5.30 *The Biology of Cancer* (© Garland Science 2007)

Table 4.2 A list of point-mutated *ras* oncogenes carried by a variety of human tumor cells

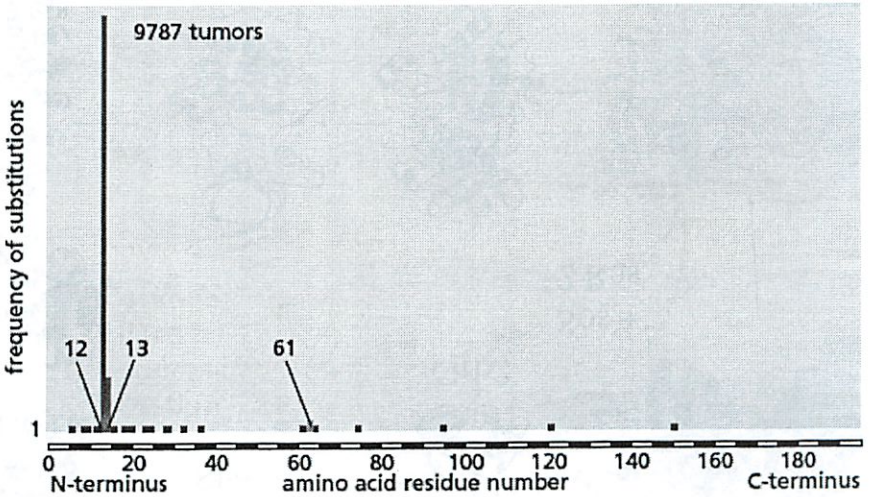
Tumor type	Proportion (%) of tumors carrying a point-mutated *ras* gene[a]
Pancreas	90 K
Thyroid (papillary)	60 (H, K, N)
Thyroid (follicular)	55 (H, K, N)
Colorectal	45 (K)
Seminoma	45 (K, N)
Myelodysplasia	40 (N, K)
Lung (non-small-cell)	35 (K)
Acute myelogenous leukemia	30 (N)
Liver	30 (N)
Melanoma	15 (K)
Bladder	10 (K)
Kidney	10 H

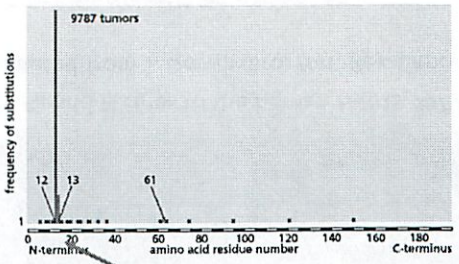
[a]H, K, and N refer to the human *H-RAS*, *K-RAS*, and *N-RAS* genes, respectively.

Adapted from J. Downward, *Nat. Rev. Cancer* 3:11–22, 2003.

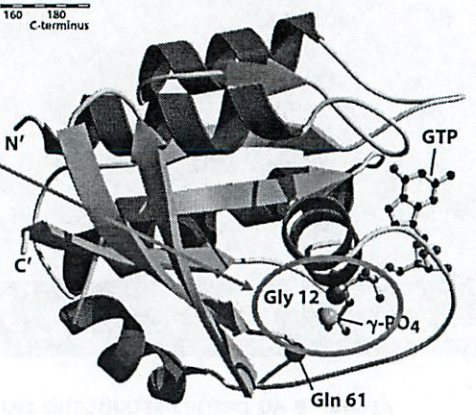
Table 4.2 *The Biology of Cancer* (© Garland Science 2007)

Recurring mutations in codon 12 of *RAS* in human tumors.



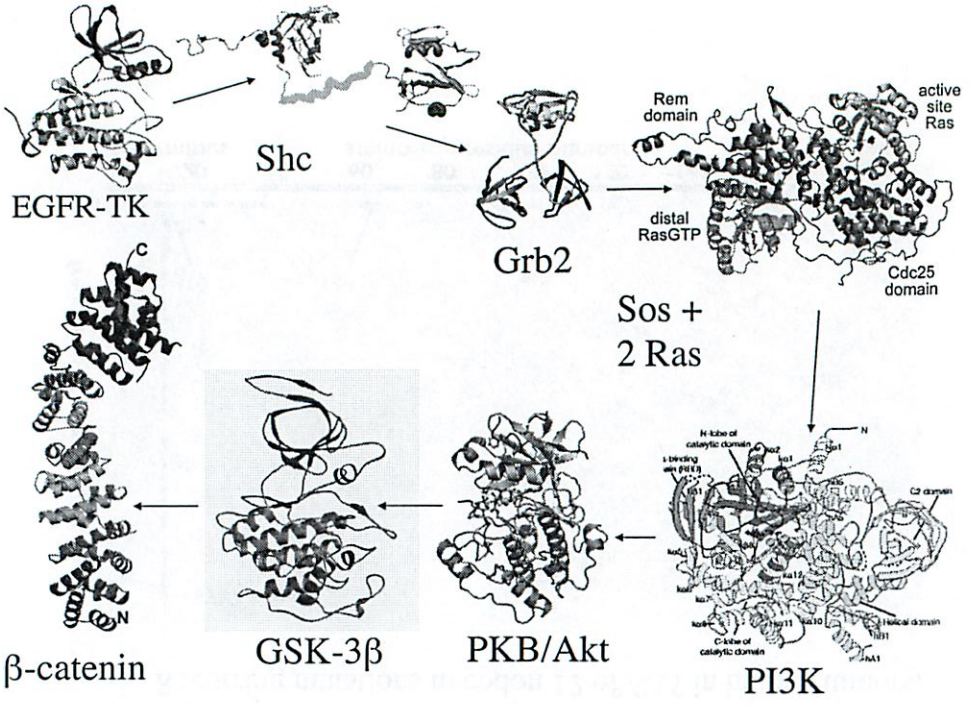


How can we rationalize the repeated finding of codon 12 mutations in muman cancers?

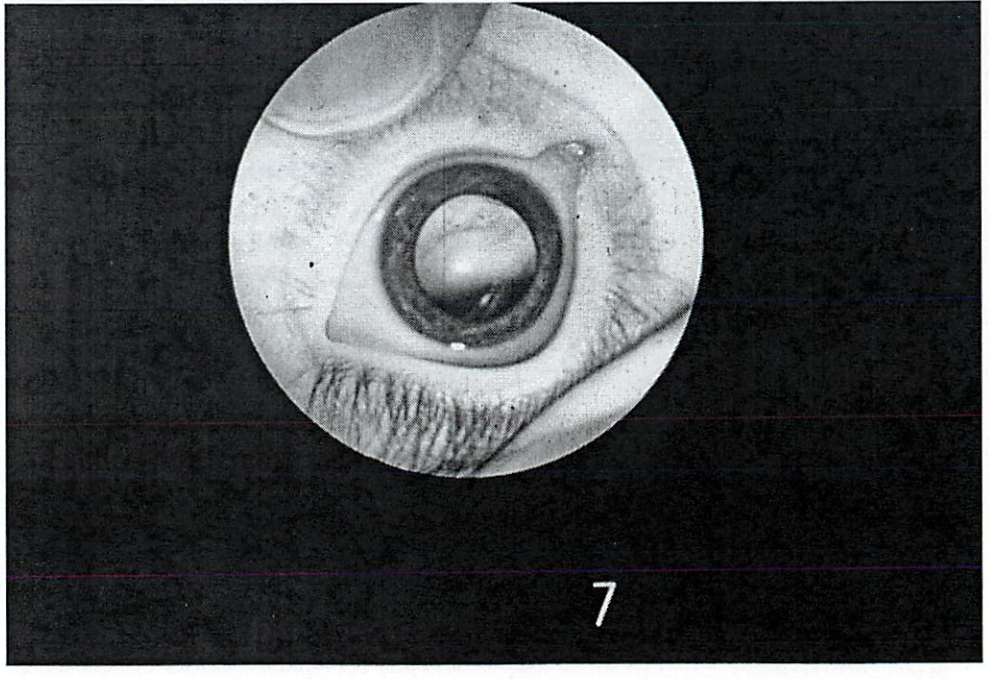


The vast majority of the point mutations affect a.a. residue 12, which normally encodes glycine.

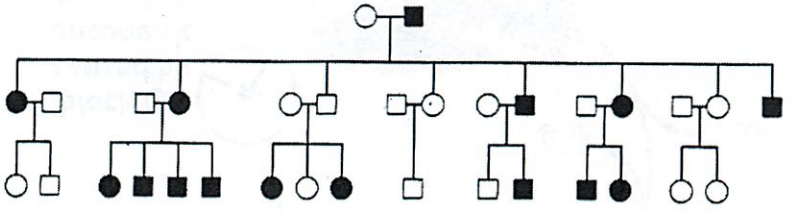
Figure 5.31 *The Biology of Cancer* (© Garland Science 2007)



An entirely different kind of genetic mutation: the case of retinoblastoma

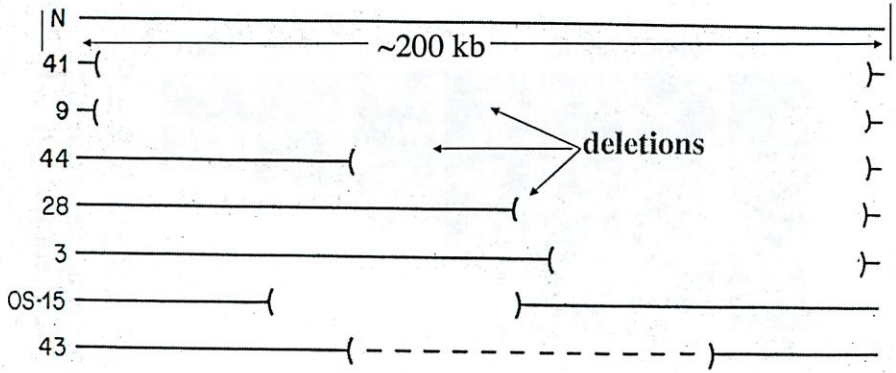


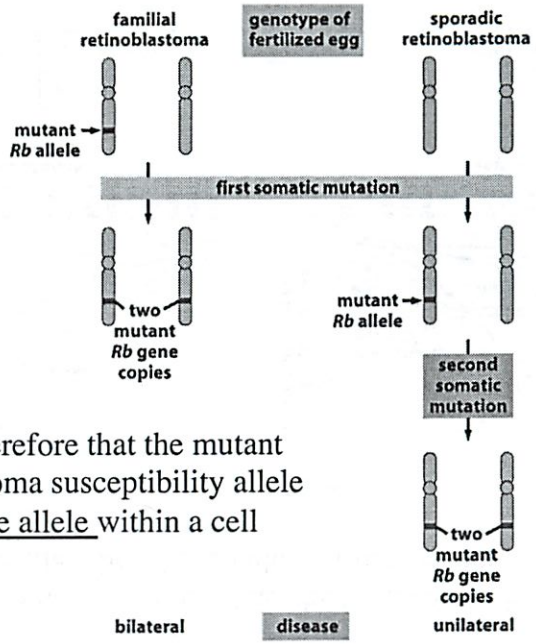
Familial susceptibility:



Children who are not cured of the disease cannot however reach adulthood and reproduce.

Mutations that affect the *Rb* (retinoblastoma gene) **kill** the gene (see these deletions) rather than potentiating its function.

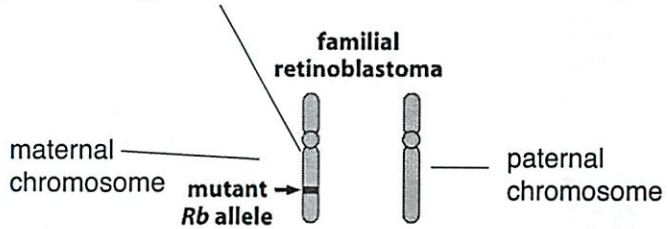




Imagine, therefore that the mutant Retinoblastoma susceptibility allele is a recessive allele within a cell

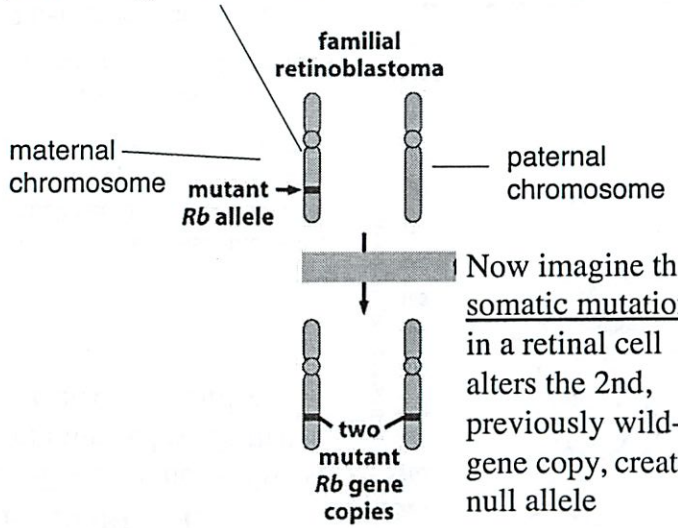
Figure 7.7 *The Biology of Cancer* (© Garland Science 2007)

Mutant null allele passed through sperm or egg, i.e., a mutant germ-line allele



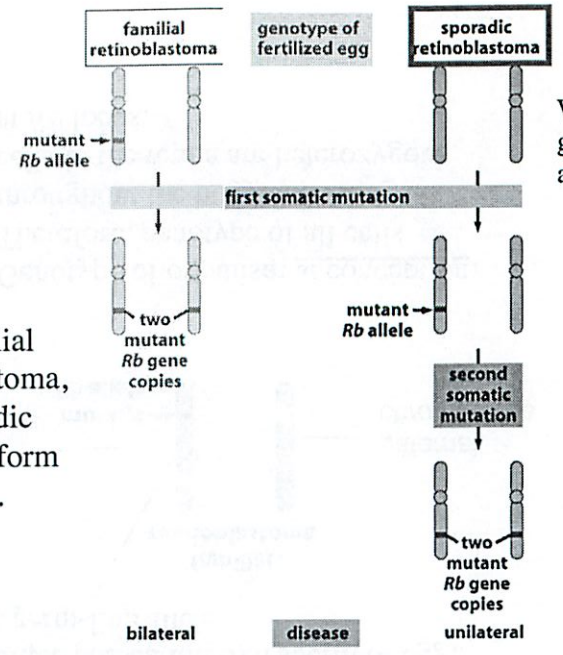
Genotype of organism at conception. Therefore, genotype of all cells throughout the body, including all cells in the retina are heterozygous at *Rb* locus.

Mutant allele passed through sperm or egg, i.e., a mutant germ-line allele



Now imagine that a somatic mutation in a retinal cell alters the 2nd, previously wild-type gene copy, creating a 2nd null allele

Now, that retinal cell has two mutant copies of *Rb* gene. If both alleles are null alleles, total loss of Rb function.



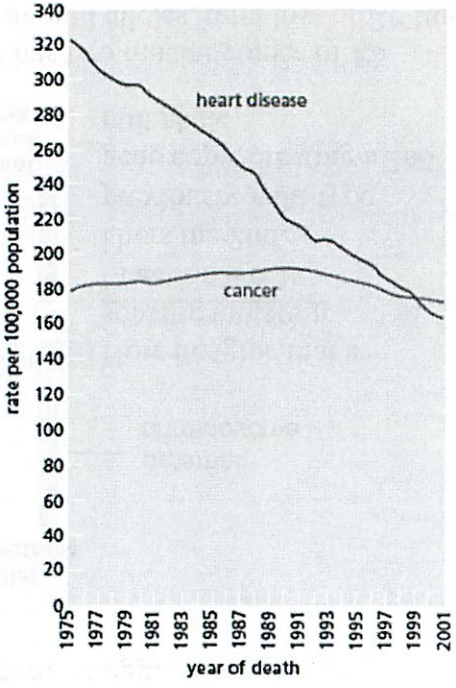
Non-familial retinoblastoma, i.e., sporadic (random) form of disease.

Figure 7.7 *The Biology of Cancer* (© Garland Science 2007)

Therapy

THE PROBLEM:

Mortality from heart disease has plummeted while that from cancer has hardly changed



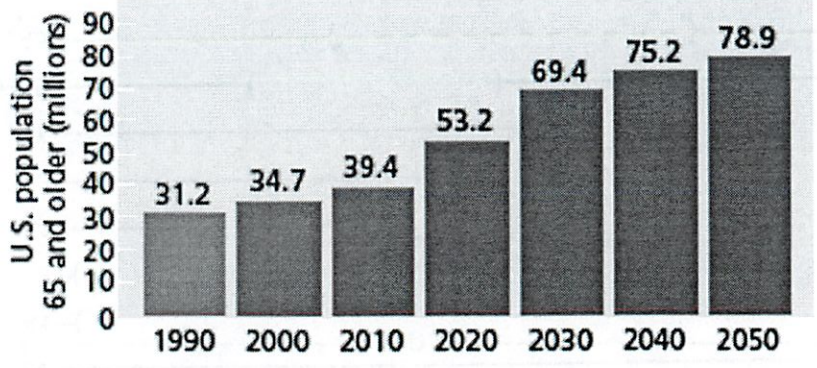
Age-adjusted death rates the rate at which people of a certain age die in a population -- which compensates for the facts that

(1) Many diseases occur at different rates at different ages

(2) Different populations have differing distributions of old and young people.

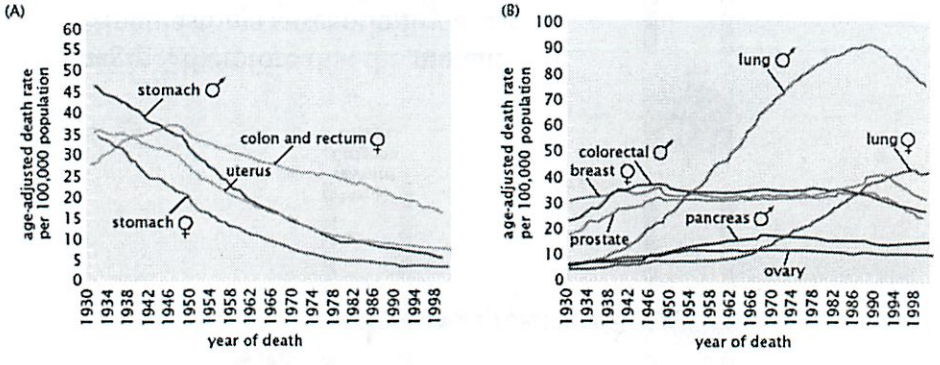
An aging population. Therefore, more diseases of the old

(A)



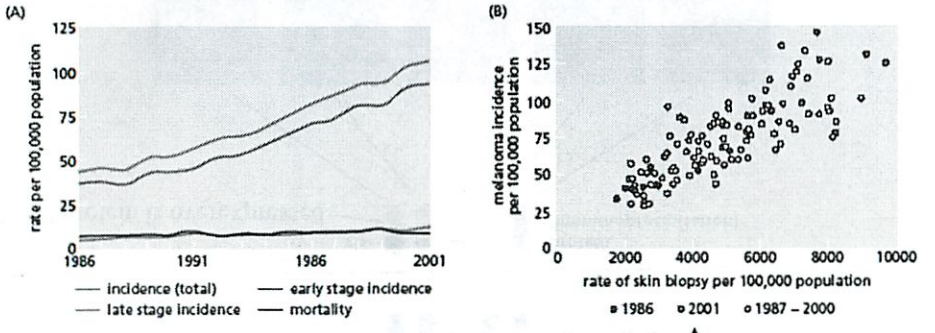
(C)

Some age-adjusted deaths from cancer have declined while others have held constant or increased

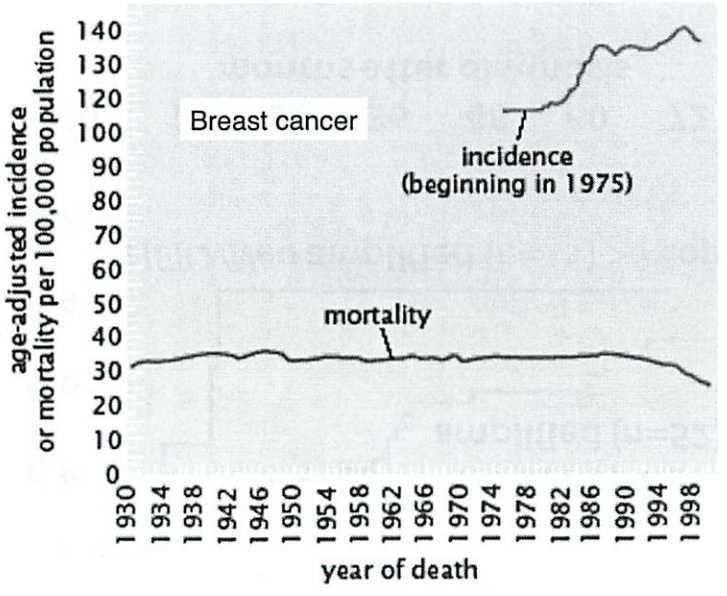


Incidence of disease is very hard to interpret, i.e., we don't really know how often certain diseases strike.

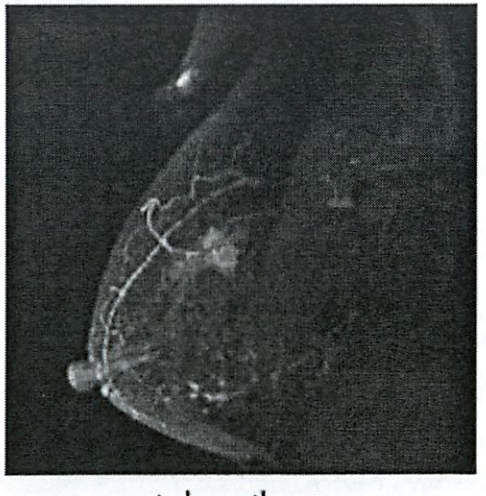
The case of melanoma incidence



The more you look, the more you find!

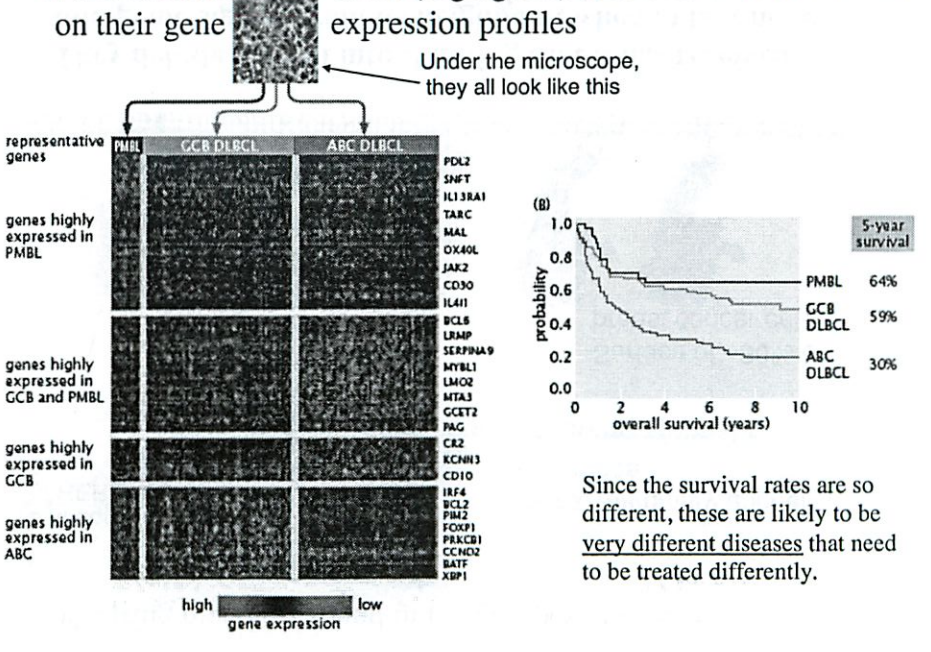


The better you can search, the more you find



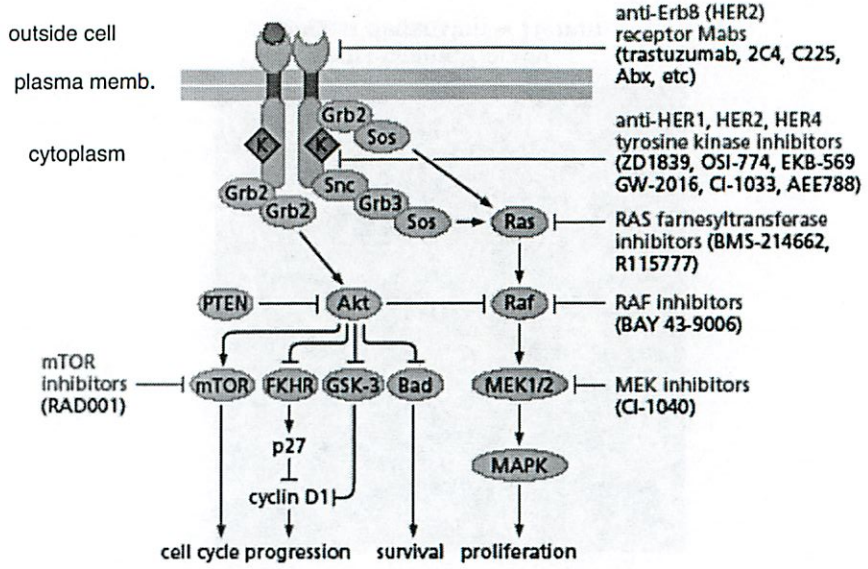
post-chemotherapy
longest dimension – 16 mm

Superficially similarly appearing tumor behave very differently if one stratifies them (segregates) them into subclasses based on their gene expression profiles



Since the survival rates are so different, these are likely to be very different diseases that need to be treated differently.

Alternative: Develop drugs that inhibit oncoproteins



Note that monoclonal antibodies can only be used to perturb proteins that are expressed on the **outside** of the cell

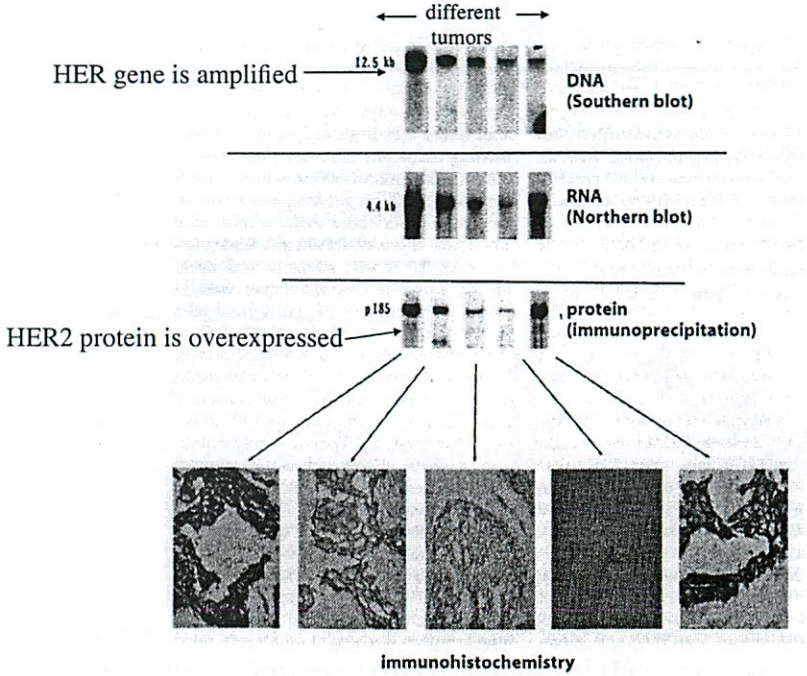
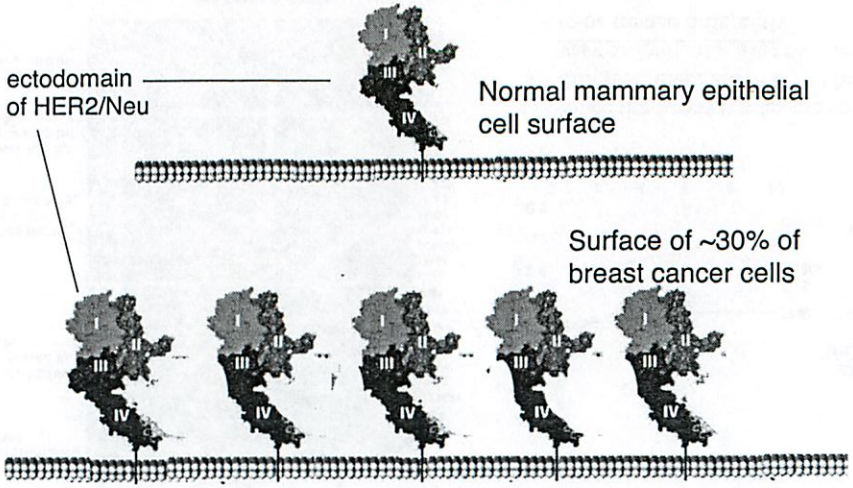


Figure 4.6c *The Biology of Cancer* (© Garland Science 2007)

Misfiring receptors found in many types of cancer



They delude the cell into thinking that it has received mitogenic signals from its neighbors, when in fact none has been received.

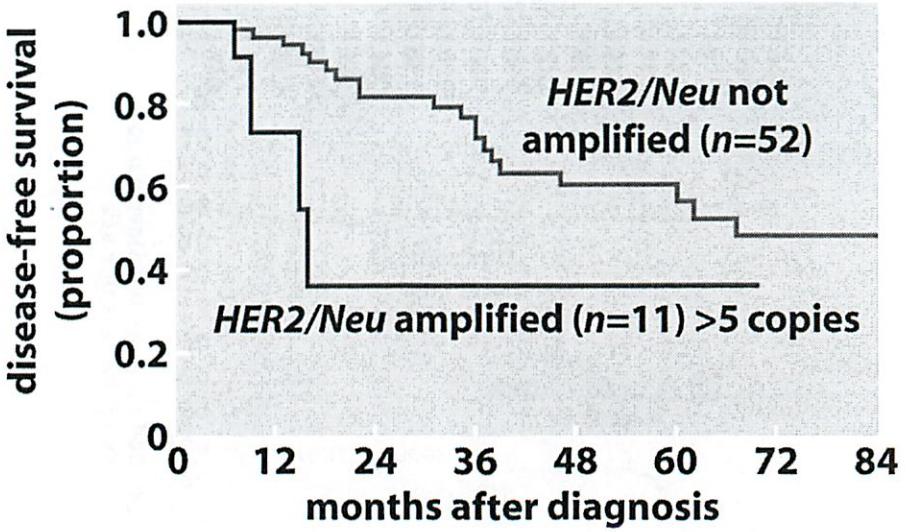


Figure 4.6b *The Biology of Cancer* (© Garland Science 2007)

Monoclonal antibody

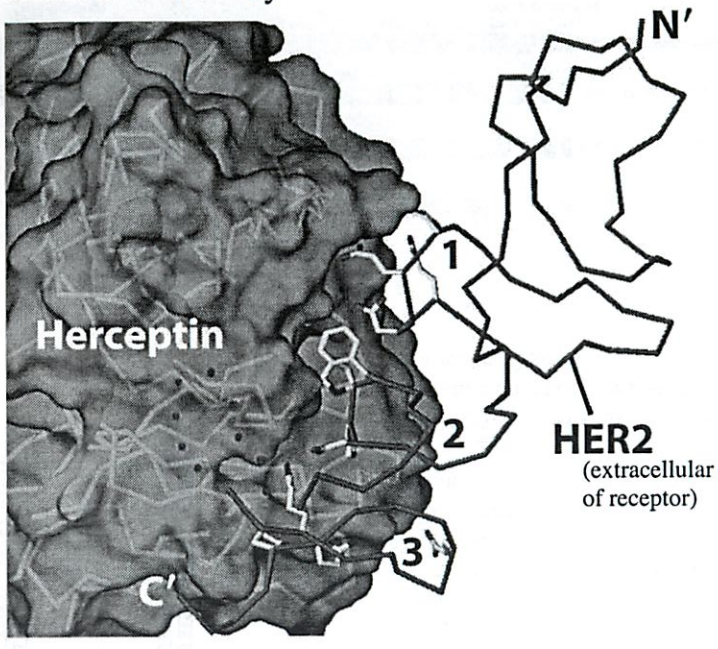


Figure 15.35b *The Biology of Cancer* (© Garland Science 2007)

Ectodomains of EGF-R and its cousin HER2/Neu

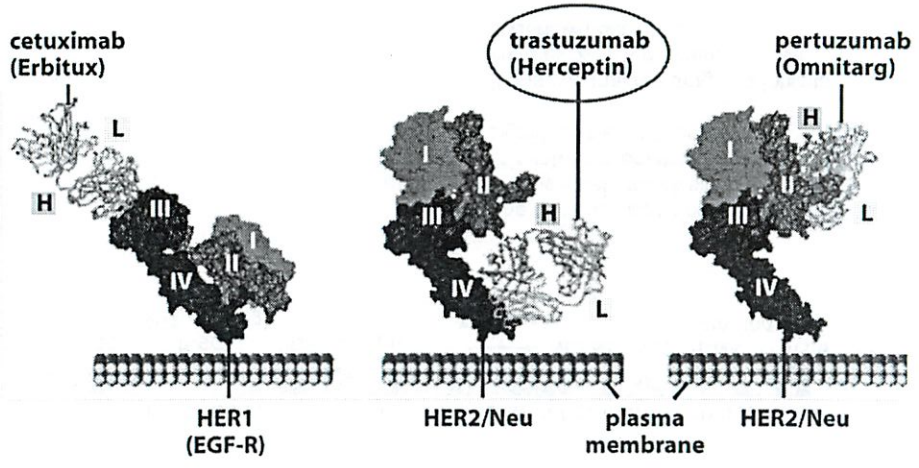
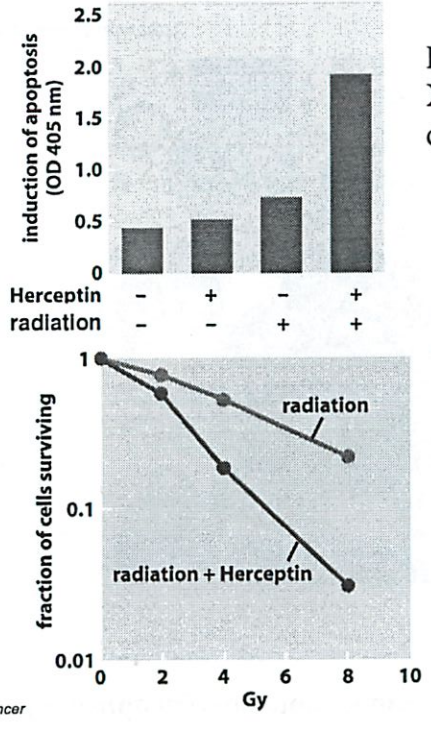


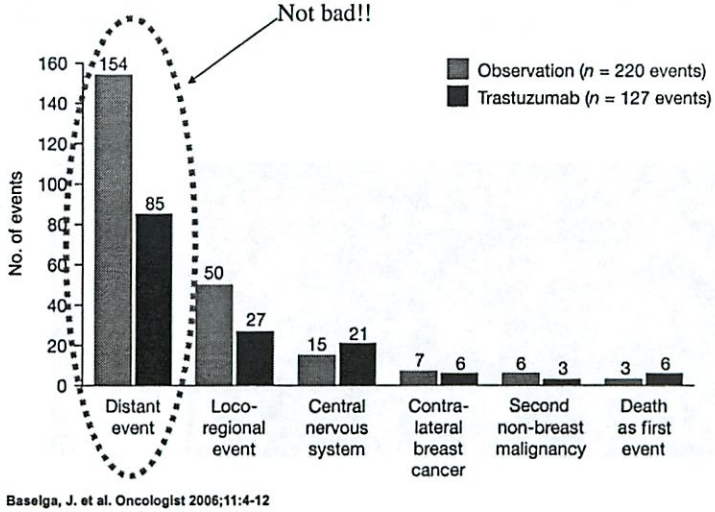
Figure 15.38b *The Biology of Cancer* (© Garland Science 2007)



Herceptin potentiates X-ray killing of cancer cells

Figure 15.37c *The Biology of Cancer*

Figure 3. Frequency of efficacy end point events in the Herceptin(R) Adjuvant (HERA) trial [8]

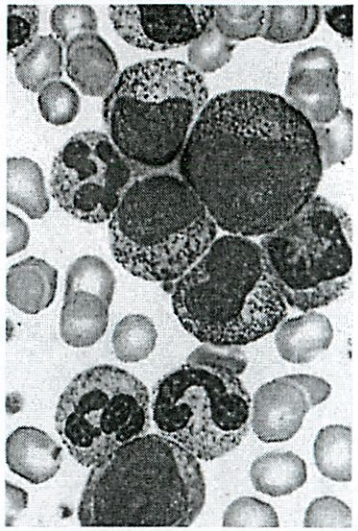


Baselga, J. et al. Oncologist 2006;11:4-12

The Oncologist

Take the case of **chronic myelogenous leukemia**

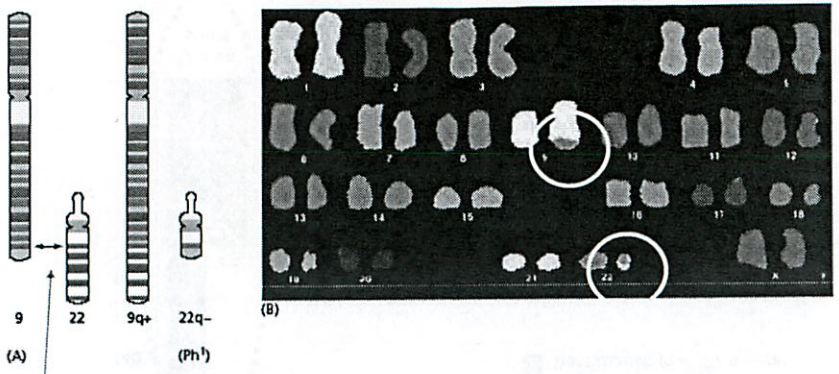
myelogenous -- arising in the bone marrow



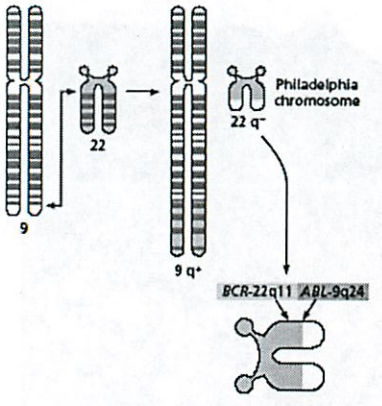
Large numbers of abnormally appearing cells in the circulation

Figure 2.8d *The Biology of Cancer* (© Garland Science 2007)

Want about inhibiting **intracellular** oncoproteins?
The case of chronic myelogenous leukemia

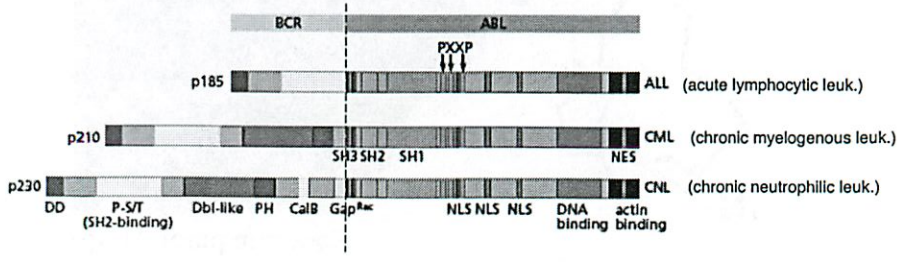


Reciprocal translocation between chromosomes 9 and 22, which depends upon non-homologous recombination between these two chromosomes.



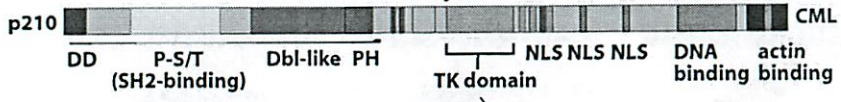
The reciprocal translocation causes the fusion of two previously unaffiliated genes, one from chr. 22, the other from chr. 9,resulting in the formation of a fusion gene that encodes a novel fusion protein

(actually 3 alterative fusion proteins, depending on where the fusion between the 2 genes occurs.)



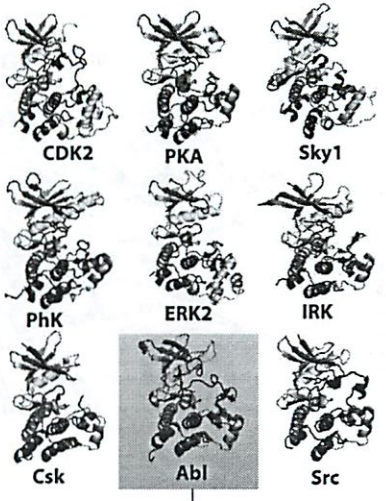
Let's focus on the Bcr-Able fusion protein made in chronic myelogenous leukemia (CML)

It has a number of functionally distinct domains, labeled here.



The tyrosine kinase domain **(TK)** is most critical, since it is involved in emitting oncogenic signals to the CML cell.

If one can make a drug that inhibits firing by the TK domain, one can shut down Bcr-Abl signaling



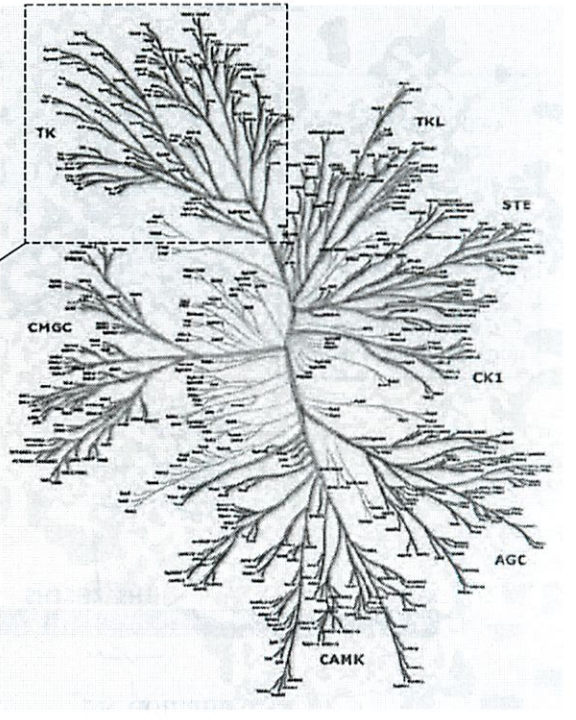
But there's a major problem, since the Abl **tyrosine kinase** domain is only one of many TK's encoded in human genome. A drug that shuts down the Abl TK may shut down many others. This lack of specificity may cause many undesired side-effects.

Figure 16.13a *The Biology of Cancer* (© Garland Science 2007)

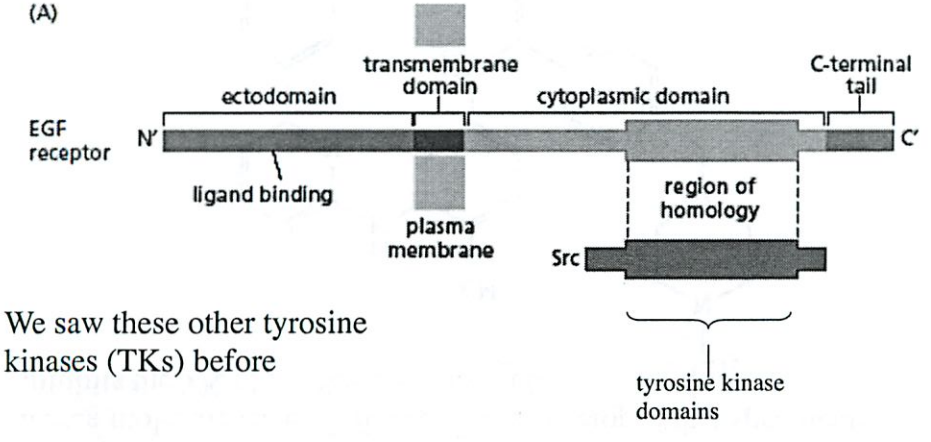
In fact, the human genome encodes almost 600 structurally related kinases, obviously evolved from a single evolutionary ancient Kinase (through the process of repeated gene duplications followed by sequence divergence of duplicated genes).

The tyrosine kinase branch of the "kinome tree" contains almost 100 distinct, structurally related TK's.

(The remainder are threonine/serine kinases that attach phosphate groups to those amino-acid residues.)

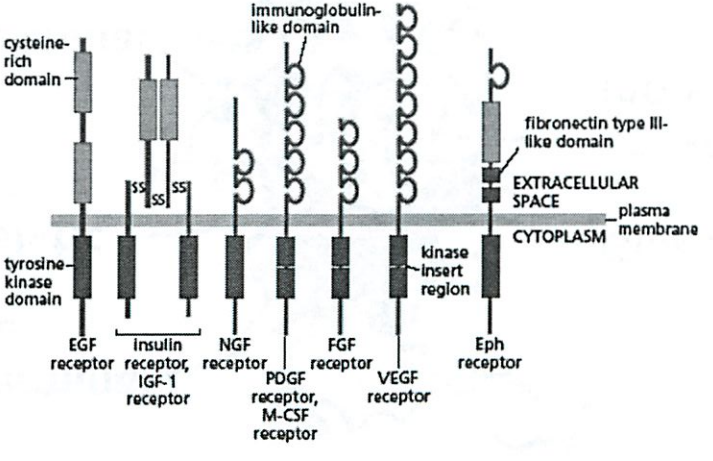


Here's an example of another tyrosine kinase, in this case used as the signal-emitting domain of the EGF (epidermal growth factor) receptor; yet another TK is the Src oncoprotein of Rous sarcoma virus.

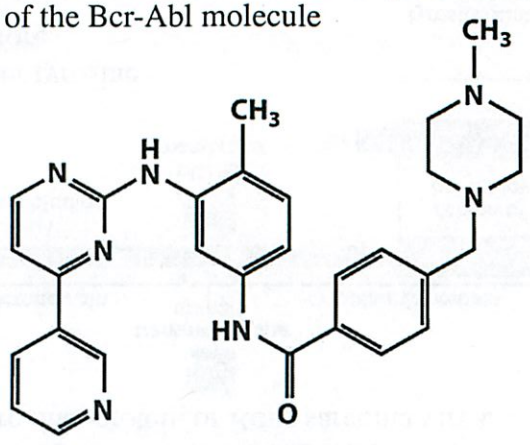


We saw these other tyrosine kinases (TKs) before

Here's a small sampling of growth factor receptors that use their tyrosine kinase domains to release growth-stimulatory signals



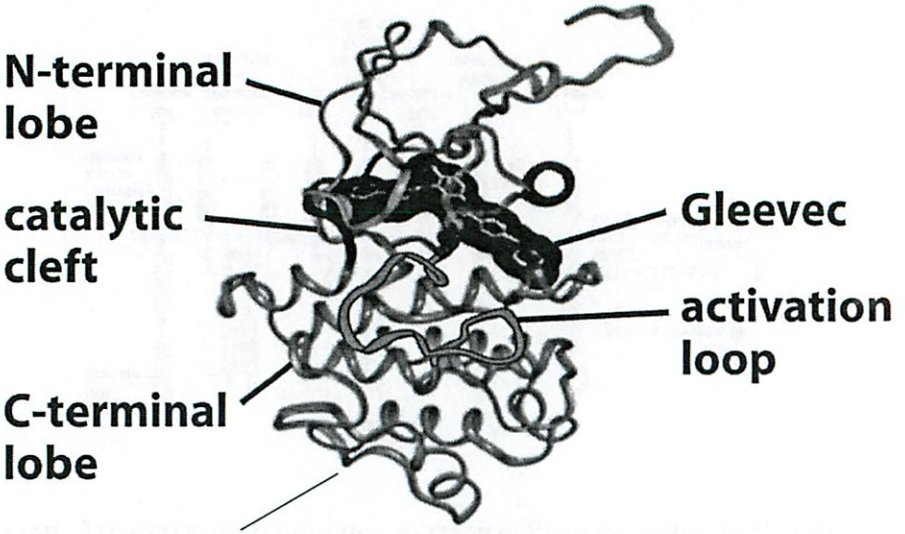
In fact, by screening many structurally related drug compounds, a drug molecule called "Gleevec" was developed that **specifically inhibits** the TK of the Bcr-Abl molecule



Gleevec®
(imatinib mesylate)

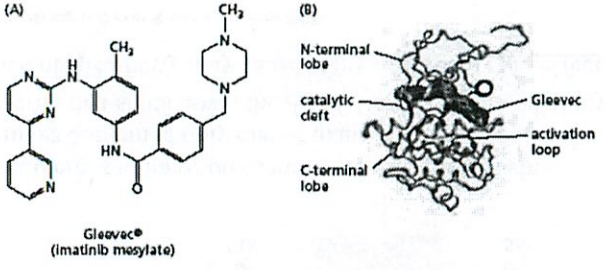
Figure 16.10a *The Biology of Cancer* (© Garland Science 2007)

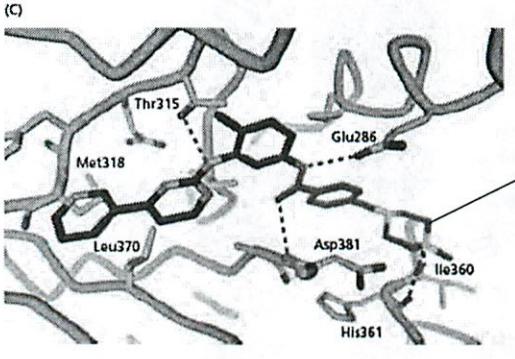
Gleevec fits into the catalytic cleft of the Bcr-Abl tyrosine kinase domain, preventing ATP from entering into the catalytic site



3D structure of the TK domain of the Bcr-Abl fusion protein

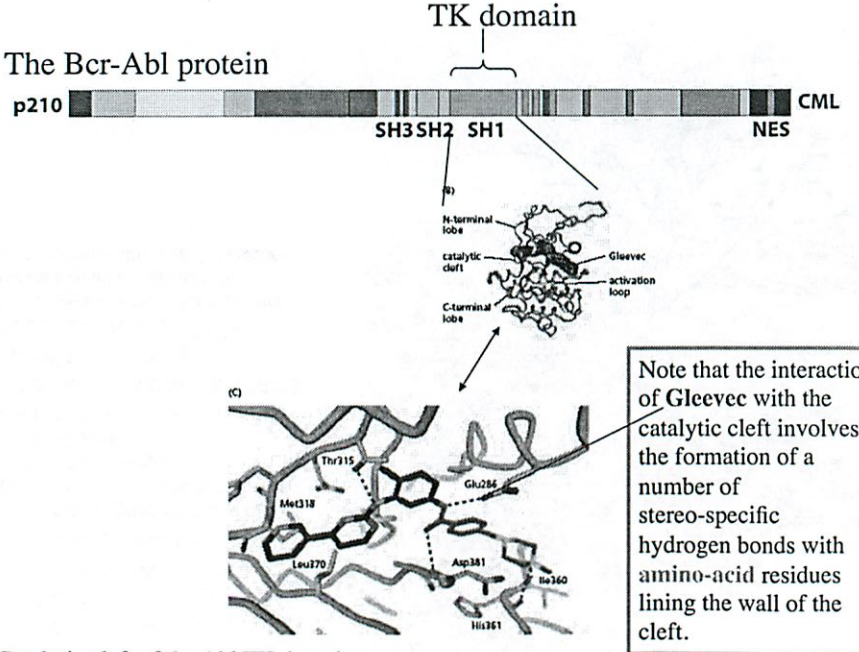
Figure 16.10b *The Biology of Cancer* (© Garland Science 2007)





Note that the interaction of **Gleevec** with the catalytic cleft involves the formation of a number of stereo-specific hydrogen bonds with amino-acid residues lining the wall of the cleft.

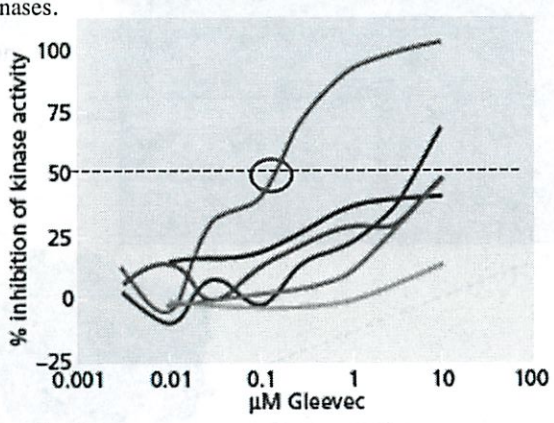
Catalytic cleft of the Abl TK domain of the Bcr-Abl fusion protein



Note that the interaction of **Gleevec** with the catalytic cleft involves the formation of a number of stereo-specific hydrogen bonds with amino-acid residues lining the wall of the cleft.

Catalytic cleft of the Abl TK domain of the Bcr-Abl fusion protein

Gleevec achieves a 50% inhibition of the firing of the Abl (= c-Abl) TK at a concentration of ~01. μM, whereas other TK's are only inhibited at **much higher** (100x)concentrations! Hence, Gleevec should work against Abl when applied at al concentration that does not affect the other kinases.

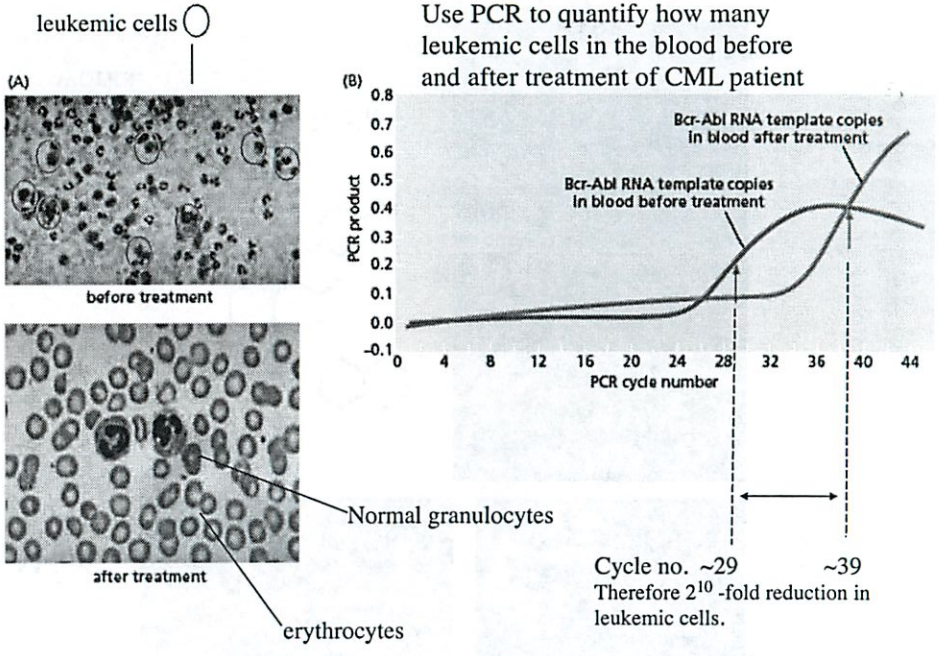


Various tyrosine kinases (TK's)

- c-Abl 0.1 μM
- Flt-4 >10 μM
- c-Fms 4.6 μM
- KDR >10 μM
- FGFR-1 >10 μM
- c-Src >10 μM

TK of Rous sarcoma virus

Note: the concentrations given after each TK are those required to achieve 50% inhibition of firing by this TK.

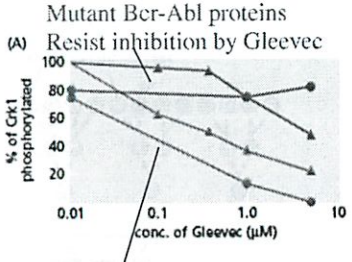


Use PCR to quantify how many leukemic cells in the blood before and after treatment of CML patient

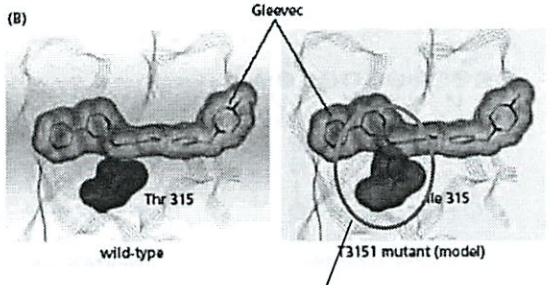
Cycle no. ~29 ~39

Therefore 2^{10} -fold reduction in leukemic cells.

After a while, some patients' tumors become **resistant** to Gleevec inhibition(and their CML reappears. . When they do so, often the Bcr-Abl proteins in the reappearing CML cells show amino-acid replacements that sterically block Gleevec binding!

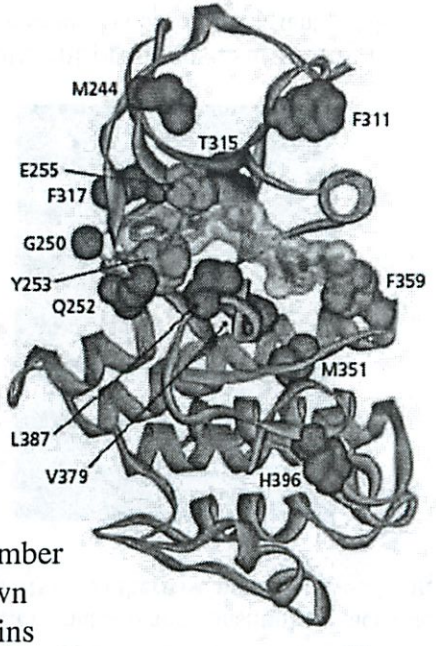


Unmutated Bcr-Abl fusion proteins continue to be inhibited by Gleevec



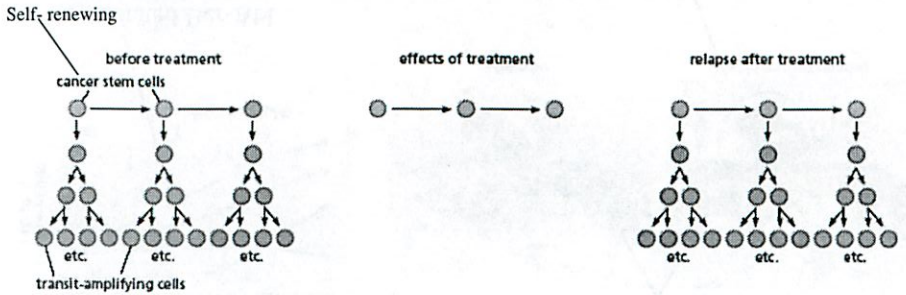
Here the threonine-to-isoleucine Replacement introduces a sterically bulky a.a. side-chain that blocks Gleevec binding

In fact, amino-acid substitutions in a number of sites in the TK domain of Bcr-Abl generate Gleevec-resistant mutant proteins. These residues are shown here as **red balls.**



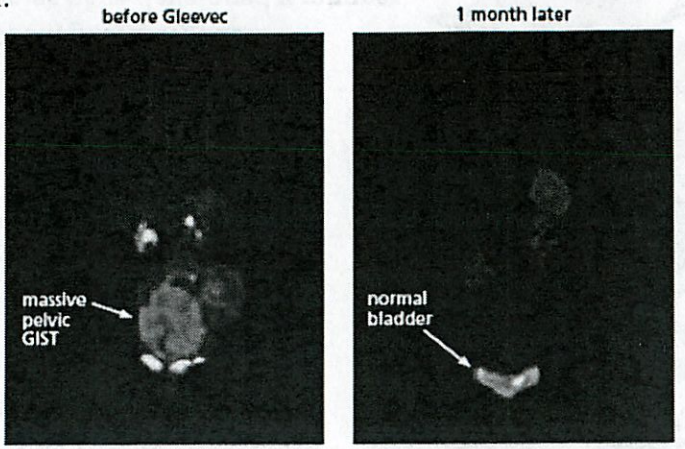
Since Gleevec was developed, pharmaceutical companies have developed a number of additional drugs that shut down Gleevec-resistant Bcr-Abl proteins and succeed in causing remission of disease.

A major problem with Gleevec and similar drugs: they kill the transit-amplifying cells in a CML cell population but fail to kill the cancer stem cells.

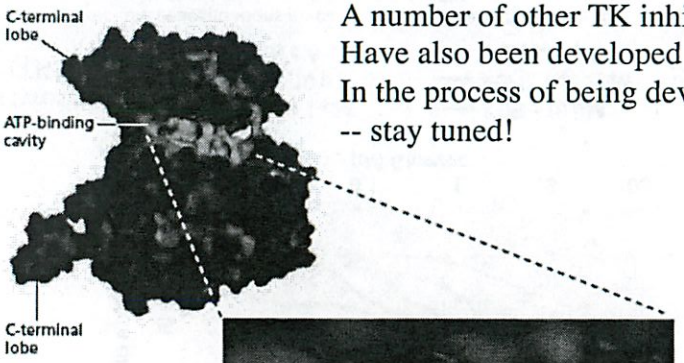


Hence, the moment that Gleevec treatment is halted, the tumor grows back.

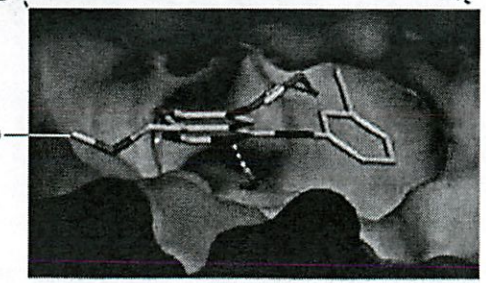
In fact, Gleevec actually shuts down 3 distinct tyrosine kinases -- those associated with Bcr-Abl, the PDGF (platelet-derived growth factor) receptor, and the Kit growth factor receptor. Kit is mutated and constitutively activated in gastrointestinal stromal tumors (GISTs), an otherwise untreatable abdominal tumor.



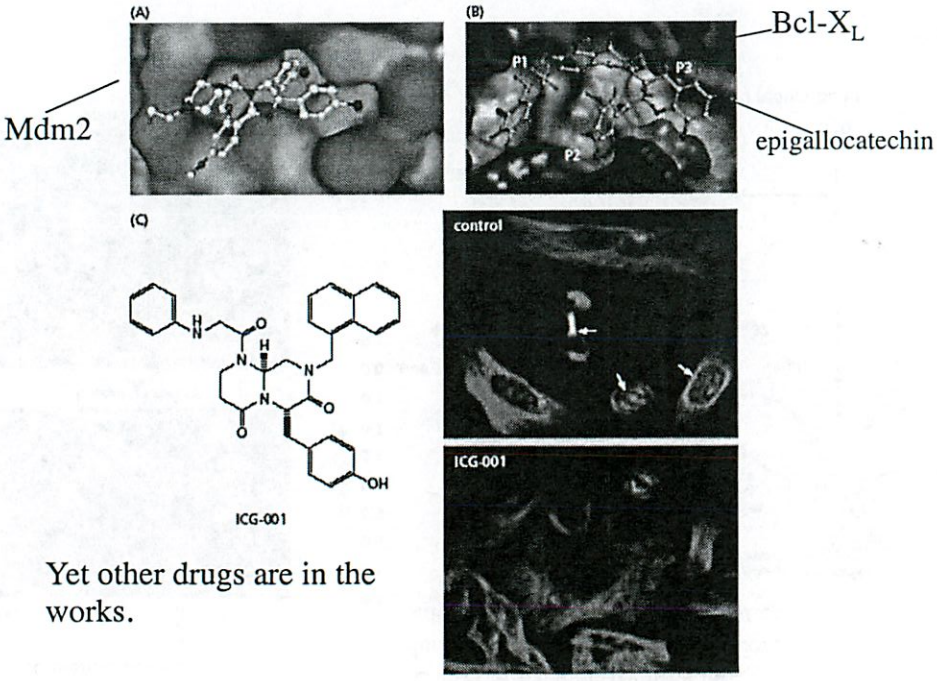
In many patients, Gleevec treatment produces astounding results.
(Unfortunately, after some months, their tumors become resistant to Gleevec.)



A number of other TK inhibitors Have also been developed or are In the process of being developed -- stay tuned!

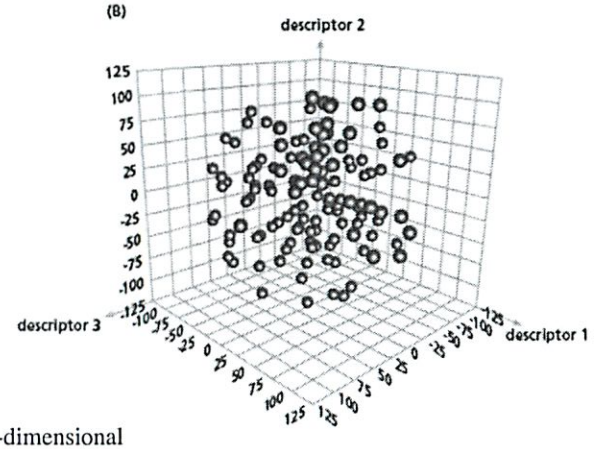


e.g., the drug Tarceva knocks out the EGF (epidermal growth factor) receptor that is activated in many lung cancers.

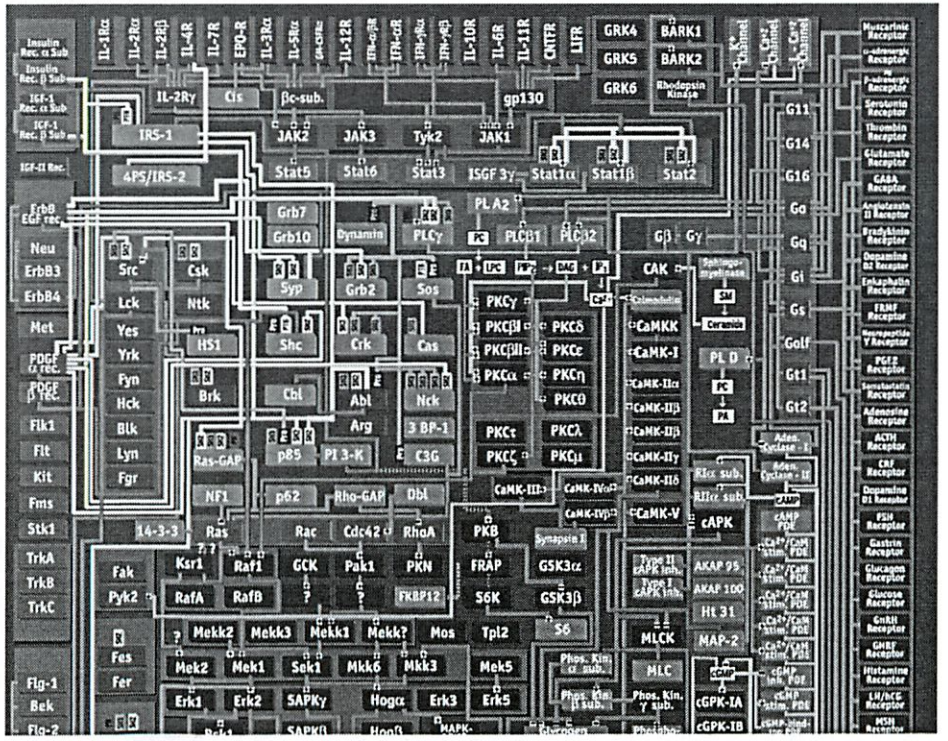


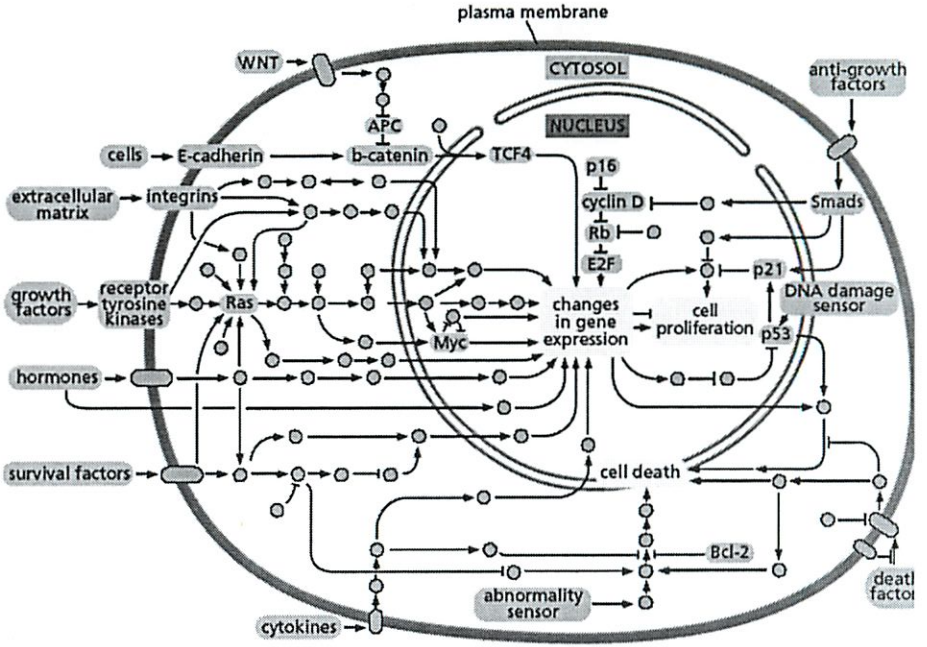
Yet other drugs are in the works.

And only small corners of "chemical space" -- which defines the total universe of drugs molecules that might be synthesized (perhaps as many as 10^{16}) have been explored to date!



This image presents a 3-dimensional depiction of "chemical space". In fact, real chemical space has many dozens of dimensions, each defined by the presence of a distinct functional group in a complex organic molecule made by synthetic organic chemistry





7.012 Recitation 18 - 2012

Summary of Lecture 29 & 30:

Viruses: Viruses are particles that consist of a protein coat surrounding a genome. This genome encodes the few proteins that a virus needs, such as the coat proteins and any other proteins necessary to get inside the host cell and make copies of its genome. Viruses that have no lipid bilayer surrounding their coats typically dock onto some protein on the surface of cells and inject their genomes into the host. Viruses that have a lipid bilayer surrounding their coats typically fuse their own membranes with the host membrane, such that the entire viral particle is absorbed into the host cell. Once a virus is inside its host, it can create many new viral particles. To this end, a virus takes over the host machinery and uses it to make lots of coat proteins and lots of viral genomes. The genomes are then packaged into the coats and the new viral particles escape from the host cell. The viruses escape either by lysing the cell or budding off from the cell.

Viral genomes can be single-stranded RNA, single-stranded DNA, double-stranded RNA, or double-stranded DNA. The DNA viruses use the host cell DNA polymerase to replicate their genome and show a much lower mutation rate. The RNA viruses can either have a plus (+) stranded RNA genome or a minus (-) stranded RNA genome. The genome of the plus stranded RNA viruses has the same polarity as the mRNA of the host cell unlike the genome of the minus (-) stranded RNA viruses which has the opposite polarity. Therefore the genome of the plus stranded RNA virus can be directly translated by the host cell translation machinery to make the viral proteins that can be used for the replication of viral genome and making new viral particles. In comparison, the minus stranded RNA viruses need to bring in their own viral proteins at the time of infection that are used to read the minus strand viral RNA genome as a template to make a complementary plus strand RNA that can be then translated by the host cell translation machinery to make the viral proteins. RNA viruses in general show a higher mutation rate compared to the DNA viruses.

Retroviruses: These are viruses whose genomes are RNA strands that are converted to DNA upon entry into the cell. Retroviral genomes contain a gene that encodes the enzyme Reverse Transcriptase (RT). Reverse transcriptase is a DNA polymerase that reads a strand of RNA as a template, and synthesizes the complementary strand of DNA. Retroviruses use RT to convert their RNA genomes into DNA such that these pieces of DNA can now randomly integrate into the host cell's genome. In this way, the virus hides out in a chromosome and gets replicated and passed on to all daughter cells of the original cell it infected.

Tumor suppressors genes and proto- oncogenes: These are normal genes that work in a regulated fashion in a normal cell to properly control the cell cycle. The wild-type function of a tumor suppressor gene is to inhibit the cell cycle in any cell that is not supposed to be actively growing and dividing. Both homologous versions of a tumor suppressor gene must loose their function to transform a normal cell to a cancerous type. The wild-type function of an oncogene is to promote the cell cycle in any cell that is supposed to be actively growing and dividing. One of the two homologous versions of an oncogene must gain a function or increase its function for a cell to become cancerous. Normal cellular counterparts of the oncogenes are called the proto- oncogenes. Some of these genes are carried by oncogenic viruses and are designated as v-oncogenes. The v– oncogenes can be linked to potent promoters that lead to their inappropriate and high level expression, leading to deregulated cell division. One example is the Rous sarcoma virus (RSV). This retrovirus infects the chickens, thereby causing them to acquire tumors. Here the viral genome contains a gene that it has stolen at some point from a host cell. This gene is an oncogene called src that is involved in cell signaling. The virus carries a mutant version of src that produces an overactive form of the normal cellular kinase src. When RSV infects a cell, the mutant src is transcribed and translated, creating an overactive cell signaling protein that promotes growth and division in chicken cells to form tumors. Other examples include the avian leukemia virus that causes leukemia and human papilloma virus responsible for cervical cancer.

Cancer Viruses: Viruses can also cause cancer. RSV is a type of retrovirus that infects chickens, thereby causing them to acquire tumors. The way that RSV causes tumors is that the viral genome contains a gene that it has stolen at some point in history from a host cell. This gene is an oncogene called src and it is involved in cell signaling. The virus carries a mutant version of src that produces an overactive form of the normal cellular kinase src. When RSV infects a cell, this mutant src is transcribed and translated,

creating an overactive cell signaling protein that tells the chicken's cells to grow and divide. Thus the chicken's cells form tumors.

Questions:

1. Why do some people think that viruses are alive and some people don't?

2. The following sequence is a short viral gene from a double-stranded DNA virus that actually encodes three different proteins. The sequence shown is from the transcription start site to the transcriptional end site. The upper strand of this gene is used as a template in transcription.

```
5'-TACTCTATCGCTTTAGCGGTATGCTATCAGAGCCATGCATGCATC-3'
3'-ATGAGATAGCGAAATCGCCATACGATAGTCTCGGTACGTACGTAG-5'
```

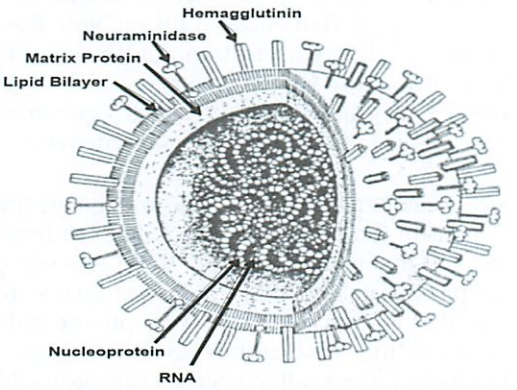
i. How do you know that it is true that these three short proteins are not produced from alternative splicing of a single transcript?

ii. How many amino acids long would each of the three proteins be that are produced from this gene?

iii. What is a major advantage for the virus to using this strategy?

iv. What is a major disadvantage for the virus to using this strategy?

3. Influenza virus is an RNA virus that does not replicate via a DNA intermediate. The virus typically infects vertebrate epithelial cells. The following is a schematic of the influenza virus.



Influenza virus is unable to make more viral RNA within the host cells using exclusively the host cell proteins.

a) Explain why this is so.

b) Explain how the virus overcomes this issue and replicates its genome in the host.

4. Based on your answer to question 3 above, would you classify Influenza virus as a plus stranded / minus stranded RNA virus?

5. Viruses can also cause cancer. One such example is the Rous sarcoma virus (RSV) that causes sarcoma, a cancer of connective tissues. The virus does so by inserting itself near the cellular c-Src gene, a non-receptor tyrosine kinase.

i. How does RSV convert the c-src to its mutated form?

ii. How does the conversion of c-src to its mutated form help the virus?

7.012 Recitation

12/4

Exam 3 back today

Avg = 70

P-Set 7 due today

OH 7:30-9:30 today

Viruses

Lytic virus infects

assembles very fast

lyses hosts

acute infection

ie cold + flu

fast

Lysogenic virus integrates its genome into the host + stays dormant

virus will start expressing gene in weeks + years

Can make virus particals slowly - stay lysogenic

or switch to lytic cycle ← Or

(1)

ie HIV

Each virus infects a very particular type of cell

Cold sores → virus in high production mode

Viral structure

All viruses have a protien coat

↙ some have bilipid membrane

= aka capsid

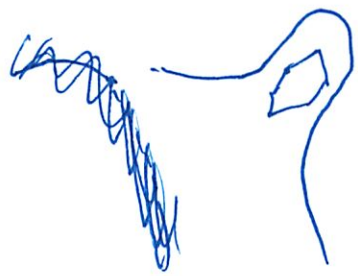
↑ affects how it functions

no membrane → lyse cell to get at

w/ membrane → bud out

how it exits cell

Can kill cell by too much budding out but this is not this chat



HIV assembles Glycoproteins

(3)

Replication Cycle

1. Genome
2. Protien

1. ds DNA

   ← want to travel as light as possible

   → host DNA pol ← uses host for both
   → host RNA pol ←
   Ribosone

   pol. = polymerase

2. ss DNA

   ↓ host DNA pol

   Then make sure strand that came in
   gets packaged up to new capsid
   Don't need to worry ~~about~~ about

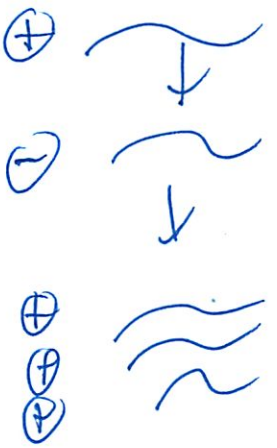
(4)

* always need host ribosome
can't make own protien

3. ⊕ ssRNA (sense) = mRNA

⊕ ~~~ RNA

- RNA dep RNA pol ← makes RNA from RNA
  - encoded already
  - ~~virus~~



4. ⊖ ssRNA (anti-sense)

⊖ ~~~

brings RNA dep RNA polymerase
- must bring along
- can't encode since this is not coding strand

⊖ ~~~ [RDRP]

↓

⊕ ~~~ = translate

↓

⊖ ≈≈≈

(5)

↙ don't really worry about

5. dsRNA

our bodies don't get infected - plants

RNA - dep RNA polymerases

↑ sometimes bring, sometimes encode

6. Retrovirus

Typically are membrane bound (5)

Those genes responsible for making virus and the enzymes it brings along

→ Reverse transcriptase

→ ~~Integrates~~ Intagrase

→ Protease

two identical ⊕ stranded RNA

Reverse Transcriptase brought in → dsDNA → integrates into host genome

Why do this instead of translating + moving on
The longer ya infect your host the more
Children ya can affect.

Cancer Viruses

Single layer of cells 
Lie ~~epithelial~~ epithelial cells

Then over time, due to mutations you get
Some outgrowth 

They have a very specific make up + structure
but when primary tumor have changed
- more ~~[illegible]~~ round
- less differentiated
- etc

Induces ~~blood~~ blood vessle to come to the tumor
- Angiogenesis

this helps tumor grow
and lets cells spread by traveling through the vessle

↑ metastasis

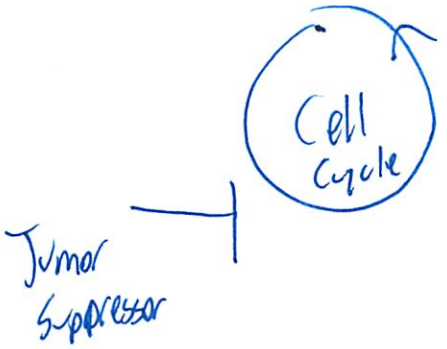
blood + empathic vessle

don't need to know → tumor secegrets VEGF that causes vessle production
↳ abnormal vessle
very difficult to target w/ medicine

Carcinoma - cancer of epithenerial cells
- skin, breast
etc
don't need to really know

(8)

Genetic alterations (missed)



← proto oncogenes
└ promote cell division
when its required
not just when right signal sent

Mutations | ISO → go into abnormal cell cycle

need loss of function
to ~~inhibit~~ no longer inhibit cell cycle
recessive

Oncogene

gain of function
dominant

Will find both types of mutations in cancer cells
progressive differentiation
mutiple rounds

---

know diff oncogenes + protooncogenes

(9)

Carcinogen - causes cancer

Mutigen - causes mutation

≠

some overlap
but not total

So what is a carcogen that can't cause mutation
↳ estrogen hormone
excessive amts causes a lot of cell division
esp in late tern pregenancies

*Since cell already has a mutation
↳ Only really activated when hormone levels ↑ during pregancy

(10)

Test for carcogenicity

take potential carcogen

insert into mouse

wait some weeks

look for tumor

Test for mutagenicity → Ames Test

1. Start w/ mutant bacteria his −

⊖his media

Then expose this to your mutagen
- UV
- compound X

(11)

Plate on minimal media

See how many colonies grow

2

1000 2

Called revertant mutations

mutant → wild type

Then look at potency of

If have a mutant, get more

→ 100

101

Modified Ames Test

X → treat w/ liver enzymes

responsible for major chemical modifications

Can cause a lot of revertant modifications

X* → 500

~~Rat food~~ goes through liver

but can also add chemicals that make potent mutagens

(12)

trying to see how ~~[illegible]~~ many mutations it causes

~~have very spec~~

Exam 3

Avg 74.75

83-100 A

71-82 B

59-70 C

47-58 D

< 47 F

Me 72

L with I was above avg

But close

(B)

Ames Test Review WP

Possible mutagen will cause a high # of revertants

his ⊖ → his ⊕

how much can mutagen cause the

mutation his ⊖ → his ⊕

not perfect, some ~~[illegible]~~ false pos + false negs

Samin Houshyar
R27 & R28
December 4, 2012

12/4
12/6

I. Viruses
   a. Viral Life Cycles
      i. Lytic

      ii. Lysogenic

   b. Structure

      i. Membrane present

      ii. No Membrane

   c. Genomes & Viral Replication
      i. dsDNA

ii. ssDNA

iii. (+)ssRNA

iv. (-)ssRNA

v. dsRNA

vi. Retroviruses

II. Cancer

a. Cancer Progression

i. Normal tissue → hyperplasia → neoplasia → primary cancer → metastasis

ii. Angiogenesis

b. Cancer Types
    i. Carcinoma

    ii. Sarcoma

    iii. Blood cancers
        1. Leukemia

        2. Lymphoma

c. Genetic Alterations
    i. Chromosomal Abnormalities: Duplications, Deletions, Translocations
    ii. Genetic Mutations: Substitutions, Deletion/Addition

II. Carcinogenicity Assay

III. Mutagenicity Assay: Tests for potency of a mutagen

a. Revertant Mutation

b. Standard Ames Test

c. Modified Ames Test

IV. Cancer Genes

a. Tumor Suppressor Genes (Loss of Function)

b. Oncogenes (Gain of Function)

i. Proto-oncogenes

V. Viral Cancer

a. RSV – retrovirus carrying oncogene.

i. Src tyrosine kinases: c-src & v-src

ii. What was the origin of that oncogene?

b. HPV – causes cervical cancer in women.

i. E7 --| pRB

ii. E6 --| p53

VI. Examples

a. RB – TSG, causing retinoblastoma; has sporadic and familial forms. What is the difference between these two?

b. Ras – Oncogene, found mutated in 80% of all human tumors.
    i. Weinberg's Experiment: Start with immortal cell lines. Isolate genes from bladder cancer cells and transfect their genes into these cells. Abnormal cells were observed and they had the ability to cause cancer in nude mice. Isolated gene and determine sequence: Ras!
    ii. Was the Ras normal?

    iii. How did Ras transform cells?

c. BCL-2

d. p53

e. Apc

f. Brca

g. CML

h. Her-2 mutations causing breast cancer

VII. Therapy

a. Drugs

i. Directly (ex. Taxol)

ii. Indirectly (ex. Anti VEGF)

b. Gene Therapy – delivery problems

c. RNAi – delivery problems

d. Immunotherapy, ex. Herceptin

7.012 12/5

AIDS

(2 min late)

review

Clonal expansion

in response to antigen

lots of cells involved

T_H recognize antigen dendritic cell

Would activate + proliferate

Look for B cell that also exposes

Activates B cell

Which differentiates to plasma cell

B cells ~~have~~ are IgM at for

~~MHC~~ receptor sticking at

if recognizes, internalizes receptors

presents MHC

So diff B cells have diff oligopeptides presented

T_H looking for match

(2)

Dendritic picks up everything ← promiscuous

B cell only presents oligopeptide it recognized w/ cell surface antibody ← selective

---

So T_H plays critical role in mediating expansion also essential messenger

Can also aid in development of other types of cells

T_H can also activate T_C

↑ second role

T_H can also activate macrophage

↑ goes in wild frenzy destroys anything nearby

So T_H has 3 functions

1. Humoral B
2. Cellular T_C
3. Cellular Macrophage ↑ren

(3)

AIDS Immune System

Mutant genes can be inherited that encode mutations in immune system

- ind can't respond to certain infectious disease
- immune system is critically important to prevent opportunistic infections
- Syndrome always a collection of disease traits which are associated w/ 1 condition

Inborn Deficiency

- Can use gene therapy to fix adenosine deaminase (ADA) deficiency
  - w/ retrovirus to deliver ADA
  - but 1-2 got lymphoma - since got retrovirus integrated randomly + activated proto onco gene

(4)

AIDS

- lots of infections present in young gay men in San Francisco
- Some common some rare
- all are infectious viruses
- important key role of immune system to combat infectious virus
- acquired
- ~~Became~~ began killing them off in large #s
- found to be a retrovirus
  - never found as etiologic factor in cancer
  - but became applicable here!
  - HIV is what causes it
- long term disease

(5)

A whole series of immunologic defects associated w/ AIDS

~40 million people living w/ AIDS

~5 million infections / year

HIV+ Hemophiliacs got it much earlier

Since recieving blood transfusions

defective in blood clotting, need blood transfusions

infectious agent found in blood

Association of AIDS + HIV

Younger hemophelicas

less since their blood screened in blood

So virtually no one gets HIV from blood

Since 1980

Very good diagnostics tools

(16)

Also back then blood commonly gotten from the homeless who used Herion w/ shared needles

---

Phases of Disease

(see slide)

HIV

retrovirus

Human Immunodefecency Virus

big fight if HIV causes AIDS

Caused hundreds of thousands of people to die

integrates into DNA

Control of transcription important to look at

How will immune system know cell infected then?

It can't

So HIV can hide out in undetectable

(1)

Hiv originated in monkeys

Who were eaten → spread to humans

Then spread through fucking/prostitution

Dendritic cells actually the cause

pick up HIV particles

Present these to T_H cells

Or Dendritic cell itself infected

and that lets virus reproduce

Viral vector

Virus must physically tether to surface of cell

like w/ CD4 antigen (on T_H)

tethering precides introdtion of nuclear core

Viral envelope fuses w/ cell

introduces core into cell

8

CD4 = critical receptor

But lots more genes

Can make up to 10 proteins

Very complex regulatory network

alt splicing + alt reading frame

When T_H activated → NF-kB

(missed) goes to nucleus, causes transcription

HIV responds to NF-kB

to turn on transcription of pro virus

So virus can be latent / undetectable

(missed)

(9)

Chart of relative levels

Why does infectious viral level bounce around?

Immune system recognizes viral glycoprotein spikes

But HIV has sloppy reverse transcriptase

So generates new slightly diff seq

Then immune system can't recognize anymore

Until it recognizes again

New back + forth see-sawing

Cat + mouse

→ Here virus goes right to core of defence system

like going to core of Death Star

So inverse relationship HIV + antibodies

(missed

(10)

Lots of these can be used as good places for therapeutic intervention

In addition each represents a drug target at least in principal

Multiple inhibitory drugs

But for some virus alters reverse transcriptase to be drug resistant

$1/10^5 - 10^6$

But all 4 drugs concombently

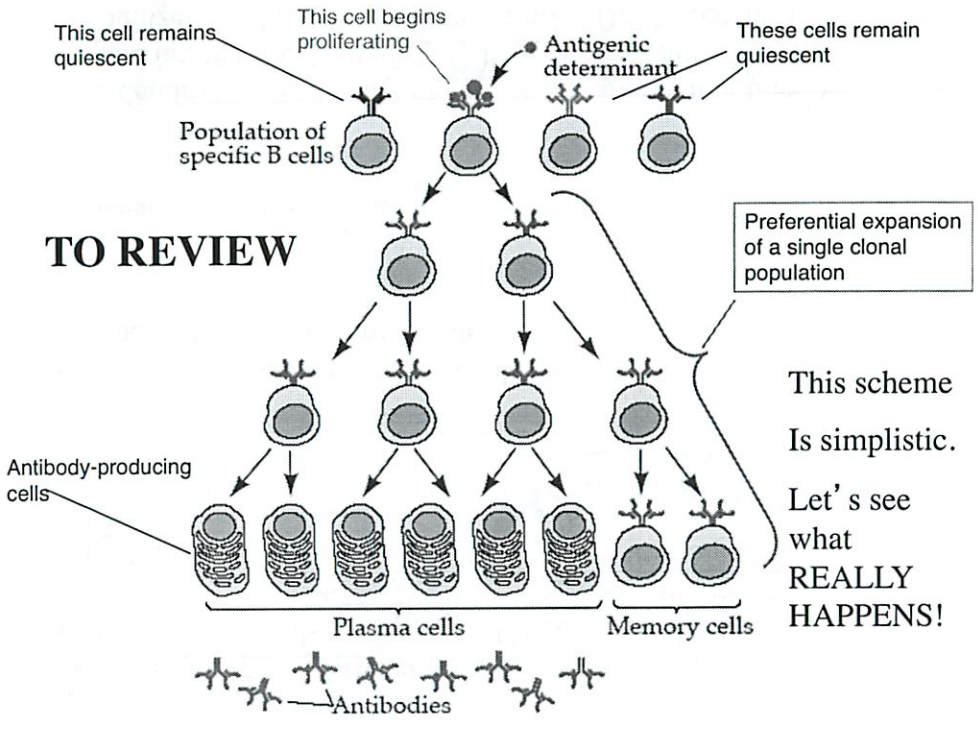
This has prettymuch put HIV at of business

Rare that it has resistance to all 4

$10^{-6} \cdot 10^{-6} \cdot 10^{-6} \cdot 10^{-6}$

keeps HIV under control but not cured

Stop drugs and it comes back

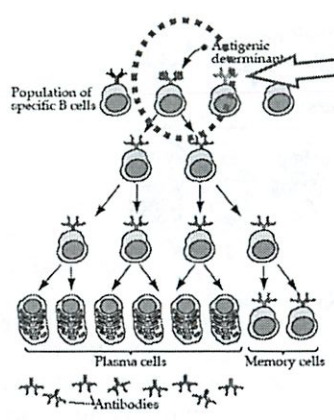


TO REVIEW

This scheme

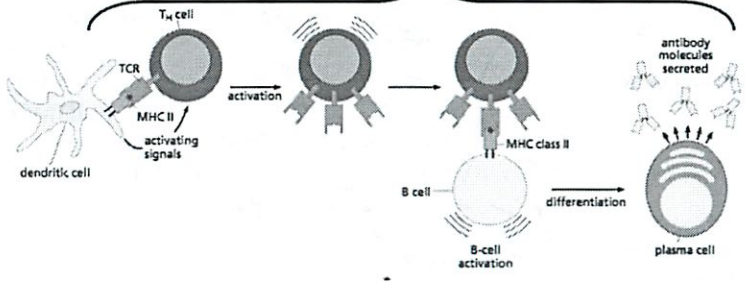
Is simplistic.

Let's see what REALLY HAPPENS!



This process of stimulating the **clonal expansion** of a B cell and the subsequent differentiation of the descendant B cells into antibody-secreting plasma cells

really involves multiple steps and multiple cell types



Meanwhile, and independent of this, B cells have been developing their own sets of antibody molecules, each that recognizes a specific oligopeptide antigen. Initially, these antibody molecules are displayed on the surface of the B cell (as IgM molecules), and if they encounter a cognate antigen on some virus particle (an antigen bound by their cell-surface IgM molecule), this results in the internalization of the antigen (by endocytosis), its degradation into oligopeptides, it introduction into the endoplasmic reticulum (ER), its loading on MHC class II molecules, and transport back to the surface of the B cell.

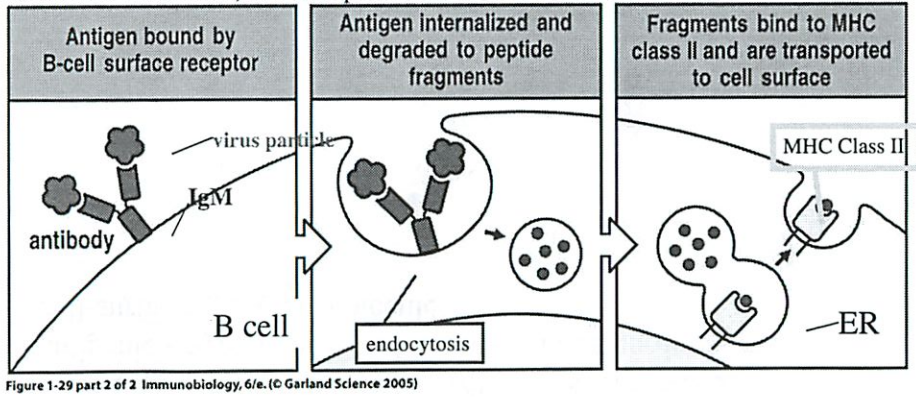


Figure 1-29 part 2 of 2 Immunobiology, 6/e. (© Garland Science 2005)

Remember, here's how the B-cell makes a living

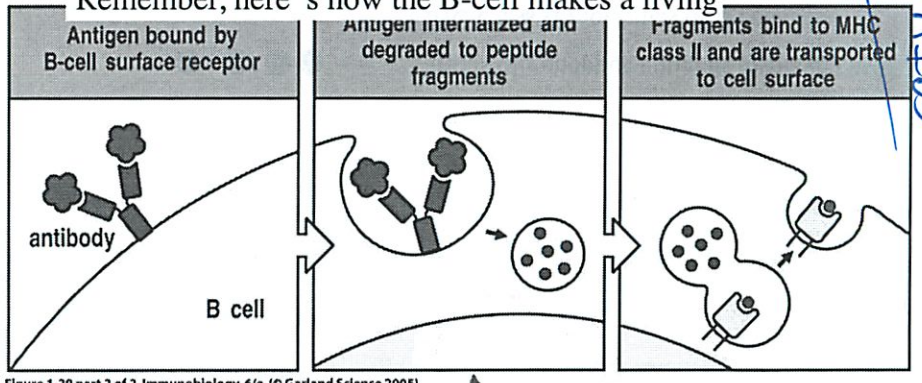
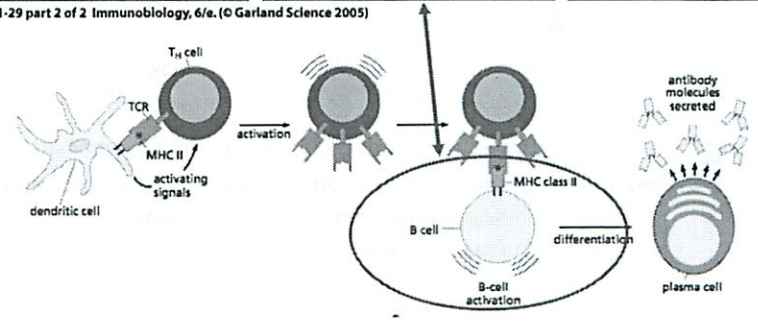


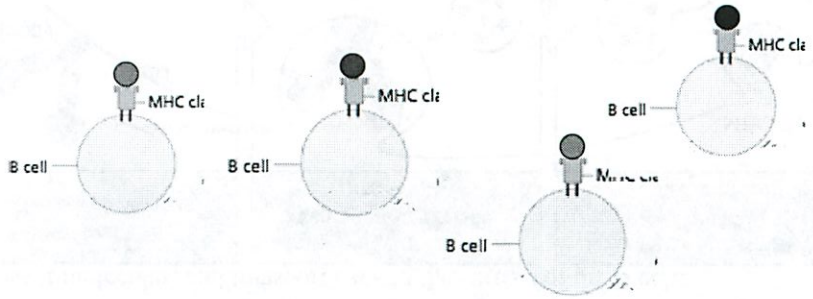
Figure 1-29 part 2 of 2 Immunobiology, 6/e. (© Garland Science 2005)



Lec 23 HIV/AIDS

12/5

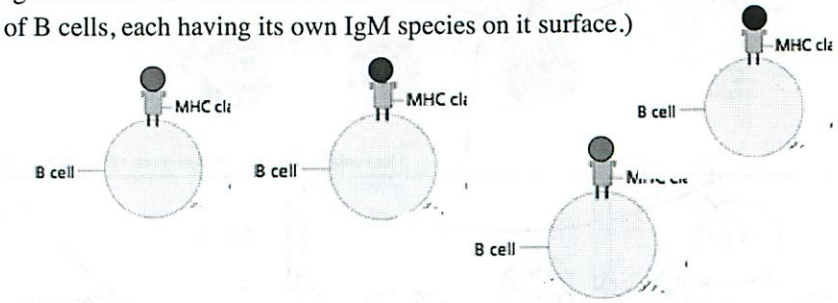
Remember, there will be thousands of different kinds of B cells, each displaying (via its surface MHC class II molecules) an antigenic fragment of something it captured earlier with its cell-surface antibody molecule



The activated/aroused T_H cell will wander among these thousands of B cells, looking for one that happens to display a peptide recognized by its T-cell receptor (TCR).

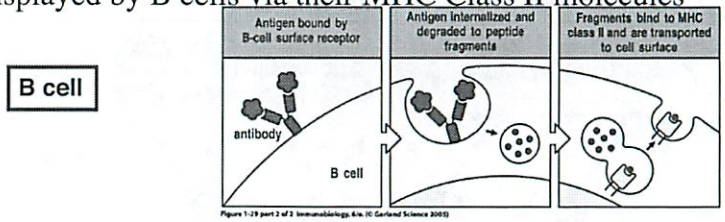
● ● ●● — Various oligopeptide antigens displayed by MHC Class II molecules of B cells.

(Just for clarification, each B cell will have generated its own antigen-recognizing IgM cell-surface molecule -- the initial protein product of the antibody gene rearrangement process. Each B cell will therefore display hundreds, even thousand of identical IgM molecules on its surface. There will be thousands, even millions of B cells, each having its own IgM species on it surface.)

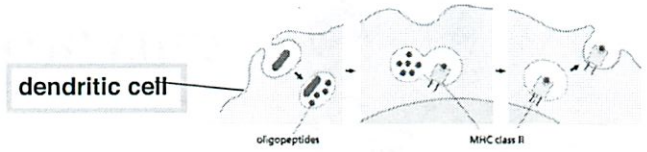


● ● ●● — Various oligopeptide antigens displayed by MHC Class II molecules of B cells.

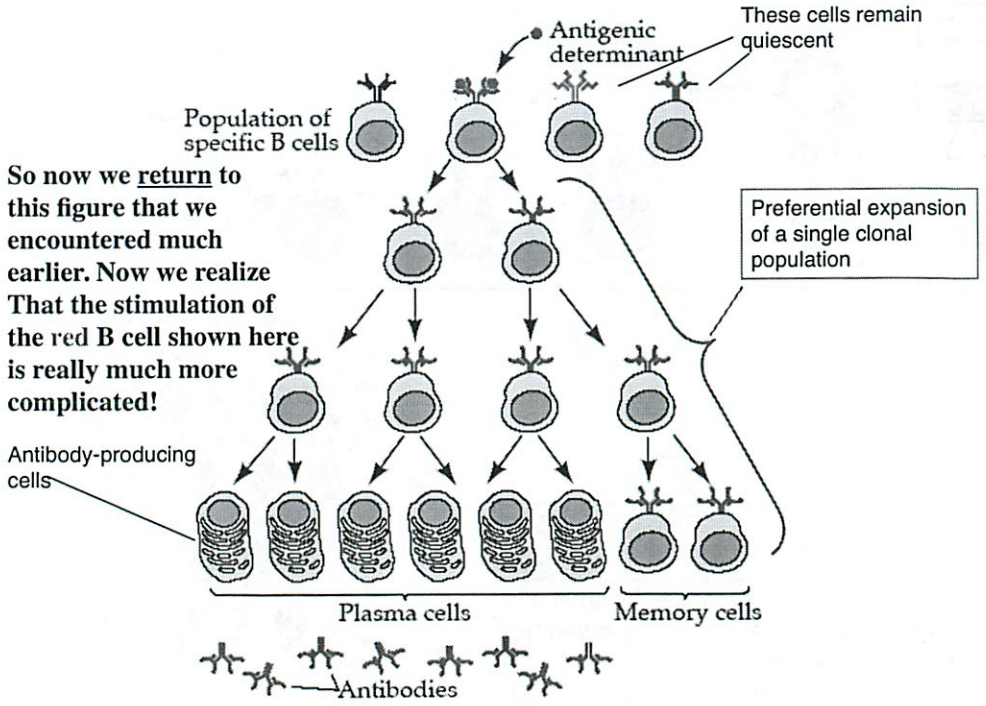
Note an **important difference** between the oligopeptide antigens displayed by B cells via their MHC Class II molecules

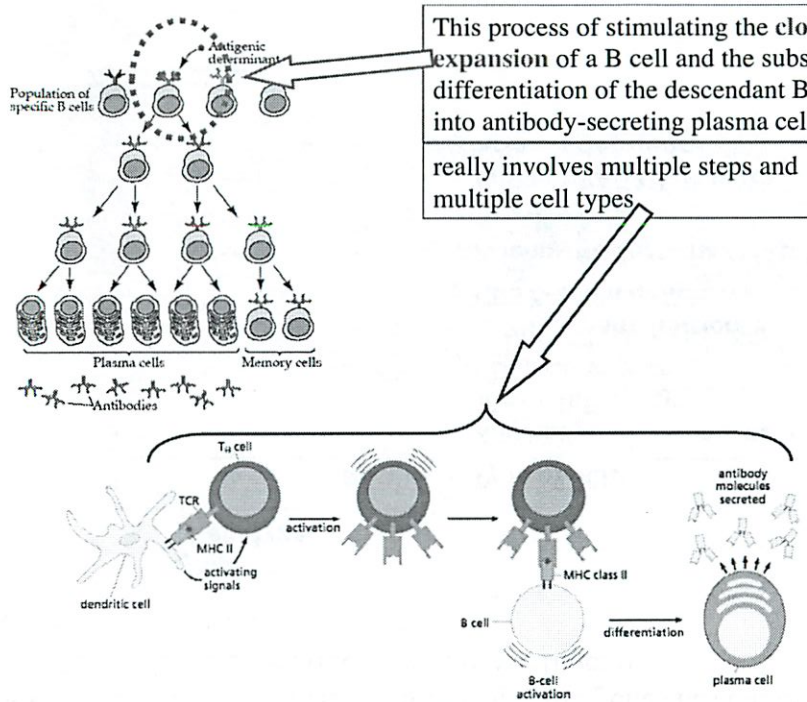


and the oligopeptide antigens displayed by dendritic cells via their MHC Class II molecules



The dendritic cells display any piece of garbage that they've picked up; the B cells will only display fragments of particles recognized by their cell surface antibody (IgM) receptors



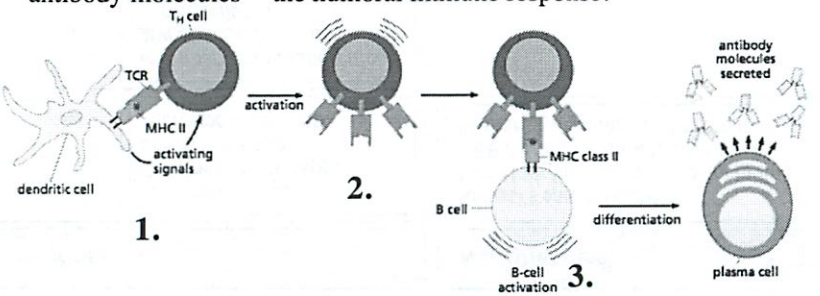


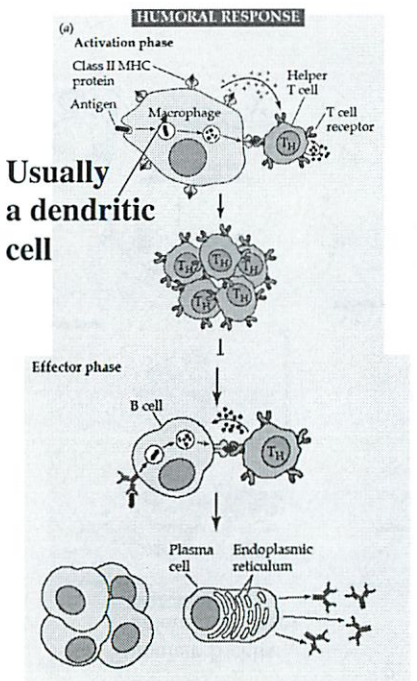
To summarize/recapitulate the whole process:

1. Dendritic cell scavenges particles and carries them to the lymph node where it presents oligopeptide fragments via its MHC Class II to helper T cells

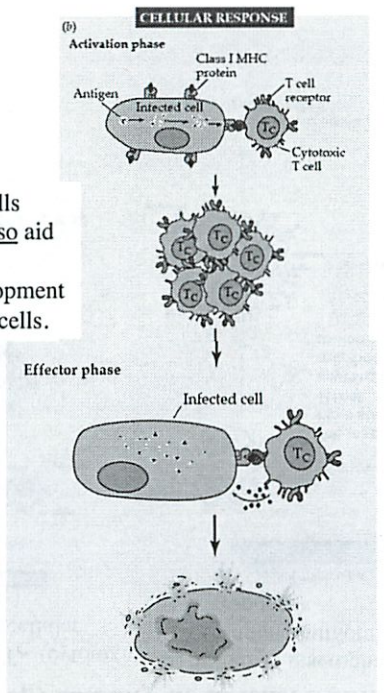
2. If a helper T cell recognizes the presented oligopeptide antigen, it becomes activated and looks around for a B cell that may also display the same oligopeptide antigen (via MHC class II) on its surface.

3. If it finds such a B cell, it causes the B cell to become activated, causing the B cell to mature into a plasma cell and to release large amount of soluble antibody molecules -- the humoral immune response!

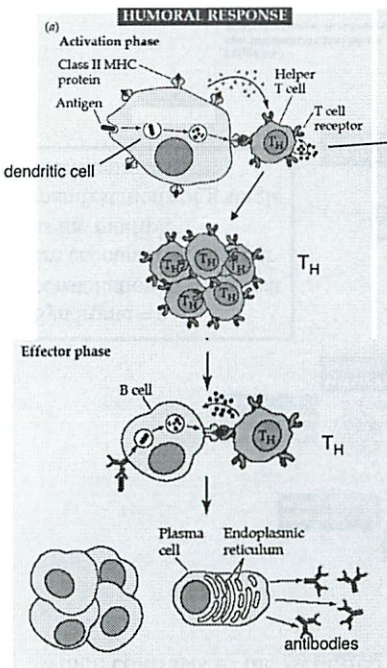


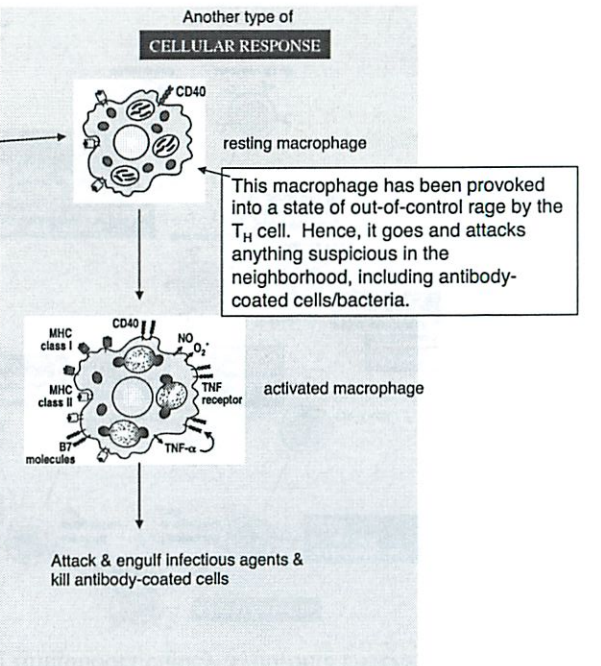


Usually a dendritic cell



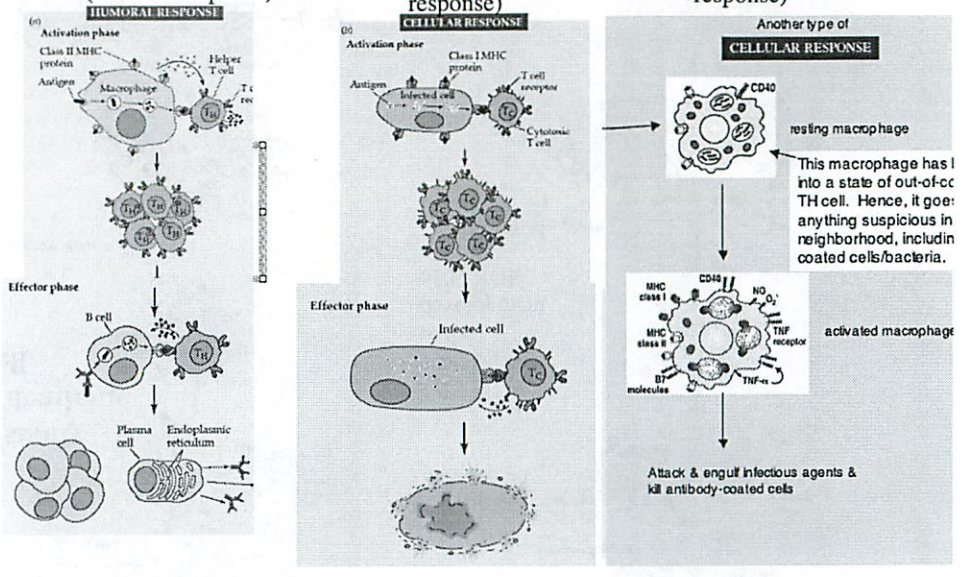
T_H cells can <u>also</u> aid in the development of T_C cells.





This means that the helper T cells (T_H) actually do at least 3 things;

1. Stimulate B cells (humoral response)
2. Stimulate T_C (cytotoxic T-cells (cellular response)
3. Stimulate macrophages (another humoral response)



These are some of the inborn (i.e., inherited) immunodeficiency syndromes, which contrasts to the Acquired Immunodeficiency Syndrome (AIDS)

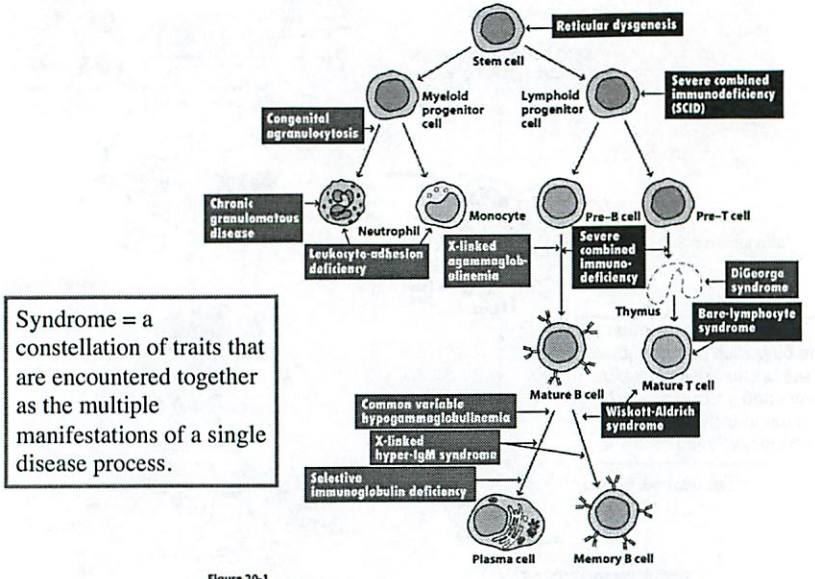


Figure 20-1
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Syndrome = a constellation of traits that are encountered together as the multiple manifestations of a single disease process.

For example, germ-line inactivation of specific genes has either limited or wide-ranging effects on immune function.

(SCID = severe combined immunodeficiency)

Lymphocyte phenotype			
T	B	NK	Type of SCID
−	+	−	X-linked IL-2Rγ–chain deficiency JAK-3 deficiency CD45 deficiency
−	+	+	IL-7R α-chain deficiency CD3 δ-chain deficiency
−	−	−	Adenosine deaminase (ADA) deficiency
−	−	+	*RAG1* or *RAG2* deficiency Artemis deficiency

Figure 20-3b
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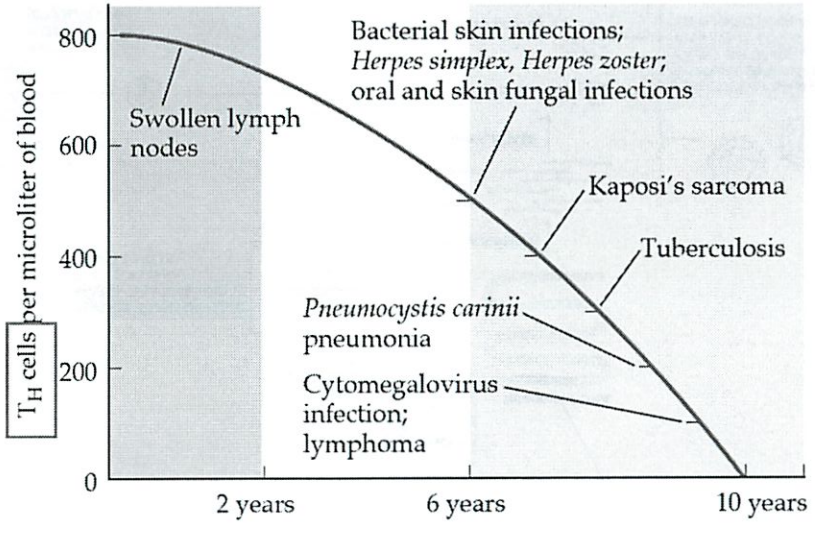
Infections		Malignancies
Parasites	*Toxoplasma* spp. *Cryptosporidium* spp. *Leishmania* spp. *Microsporidium* spp.	Kaposi's sarcoma - HHV8 Non-Hodgkin's lymphoma, including EBV-positive Burkitt's lymphoma Primary lymphoma of the brain
Intracellular bacteria	*Mycobacterium tuberculosis* *Mycobacterium avium intracellulare* *Salmonella* spp.	
Fungi	*Pneumocystis carinii* *Cryptococcus neoformans* *Candida* spp. *Histoplasma capsulatum* *Coccidioides immitis*	
Viruses	Herpes simplex Cytomegalovirus Varicella zoster	

Figure 11-30 Immunobiology, 6/e. (© Garland Science 2005)

The clinical manifestations of AIDS indicate defects in multiple arms of the immune system.

These infections are largely "opportunistic infections", i.e., infections by agents with which we normally co-exist, which are normally kept under control, and that take the "opportunity" to launch full-fledged infectious cycles when immune defenses are compromised.



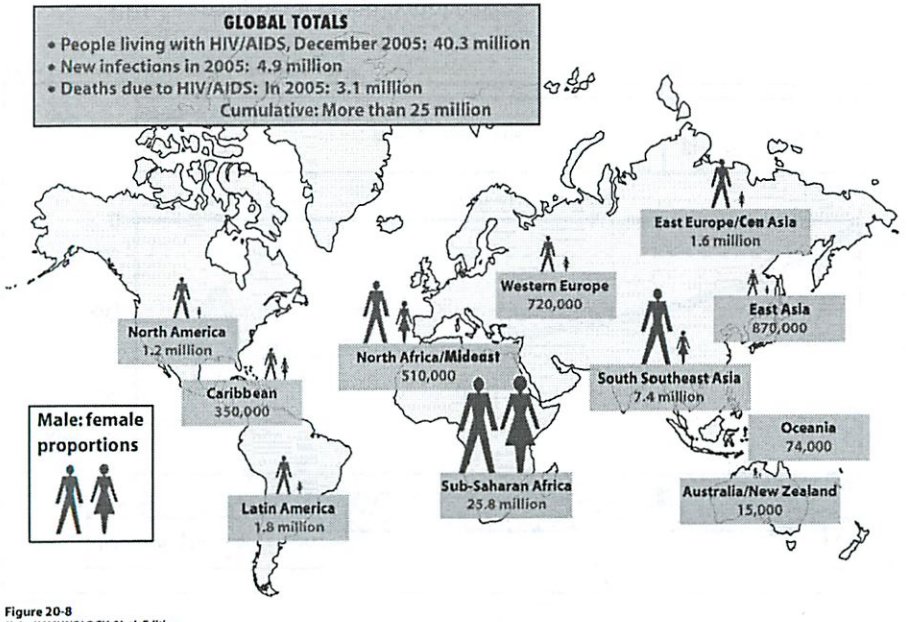


Figure 20-8
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These various clinical manifestations of AIDS (largely opportunistic infections) are the consequences of the breakdown of multiple components of the immune response. (This indicates how important T_H cells are.)

cytokine =
A growth factor
Like protein
That signals
Between
Various types
Of immune cells

TABLE 20-4 Immunologic abnormalities associated with HIV infection

Stage of infection	Typical abnormalities observed
	LYMPH NODE STRUCTURE
Early	Infection and destruction of dendritic cells; some structural disruption
Late	Extensive damage and tissue necrosis; loss of follicular dendritic cells and germinal centers; inability to trap antigens or support activation of T and B cells
	T HELPER (T_H) CELLS
Early	No in vitro proliferative response to specific antigen
Late	Decrease in T_H-cell numbers and corresponding helper activities; no response to T-cell mitogens or alloantigens
	ANTIBODY PRODUCTION
Early	Enhanced nonspecific IgG and IgA production but reduced IgM synthesis
Late	No proliferation of B cells specific for HIV-1; no detectable anti-HIV antibodies in some patients; increased numbers of B cells with low CD21 and enhanced Ig secretion.
	CYTOKINE PRODUCTION
Early	Increased levels of some cytokines
Late	Shift in cytokine production from T_H1 subset to T_H2 subset
	DELAYED-TYPE HYPERSENSITIVITY
Early	Highly significant reduction in proliferative capacity of T_H1 cells and reduction in skin-test reactivity
Late	Elimination of DTH response; complete absence of skin-test reactivity
	T CYTOTOXIC (T_C) CELLS
Early	Normal reactivity
Late	Reduction but not elimination of CTL activity due to impaired ability to generate CTLs from T_C cells

Table 20-4
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AIDS in hemophiliacs receiving HIV-contaminated blood:

An important source of infection early on and a demonstration of the fact that the infectious agent is blood borne.

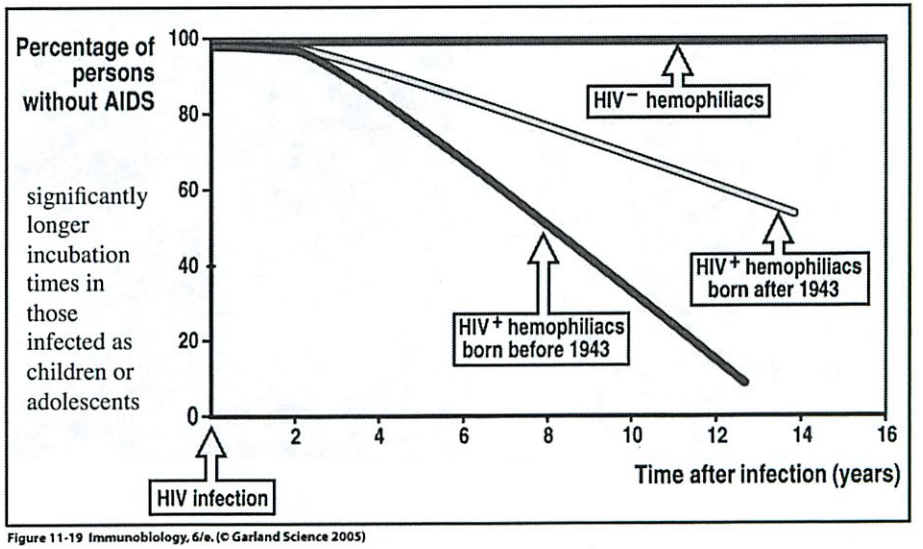


Figure 11-19 Immunobiology, 6/e. (© Garland Science 2005)

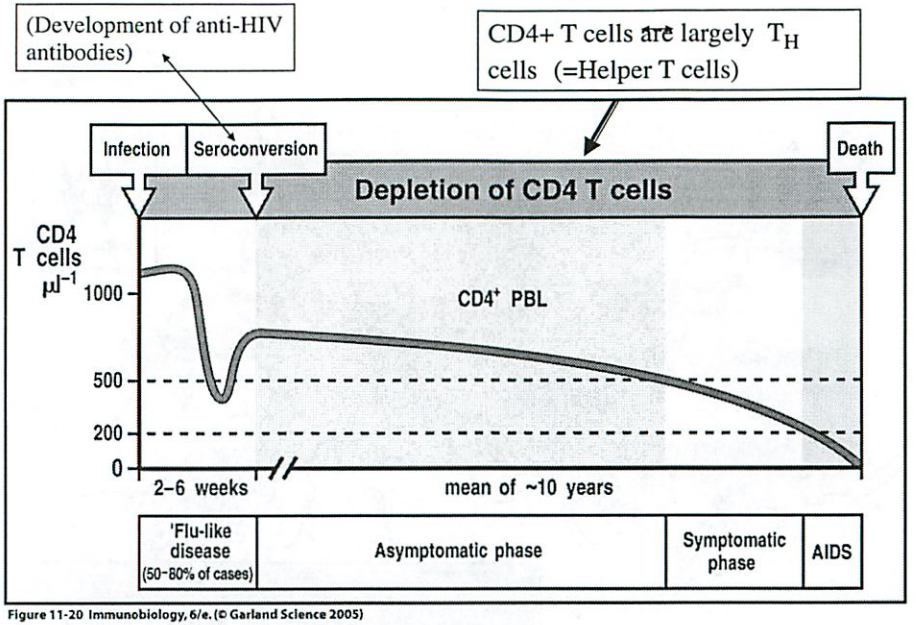
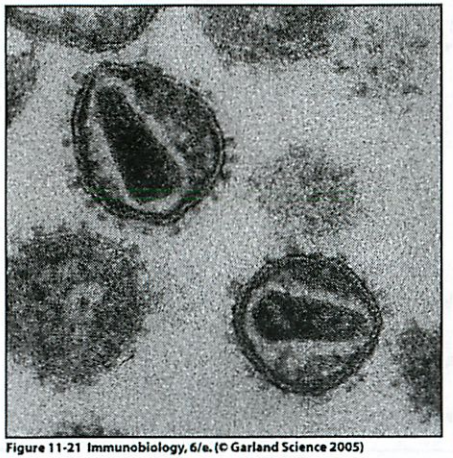


Figure 11-20 Immunobiology, 6/e. (© Garland Science 2005)

"PBL" = peripheral blood lymphocytes

A retrovirus that resembles HIV except that it's much more complicated



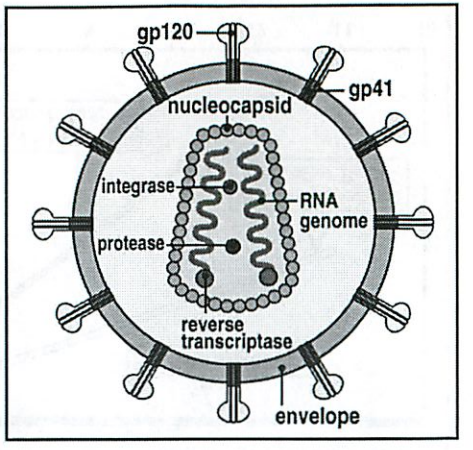
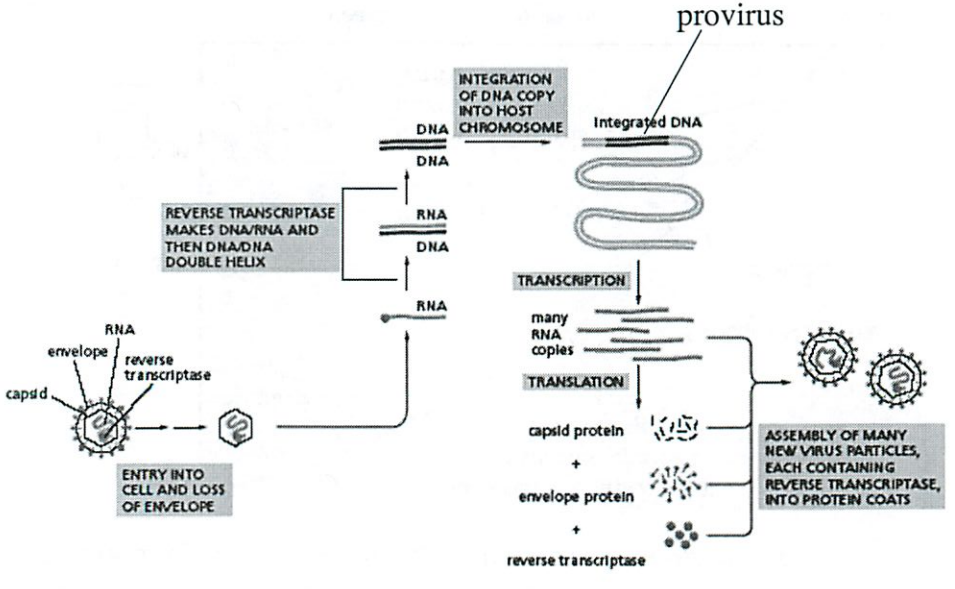
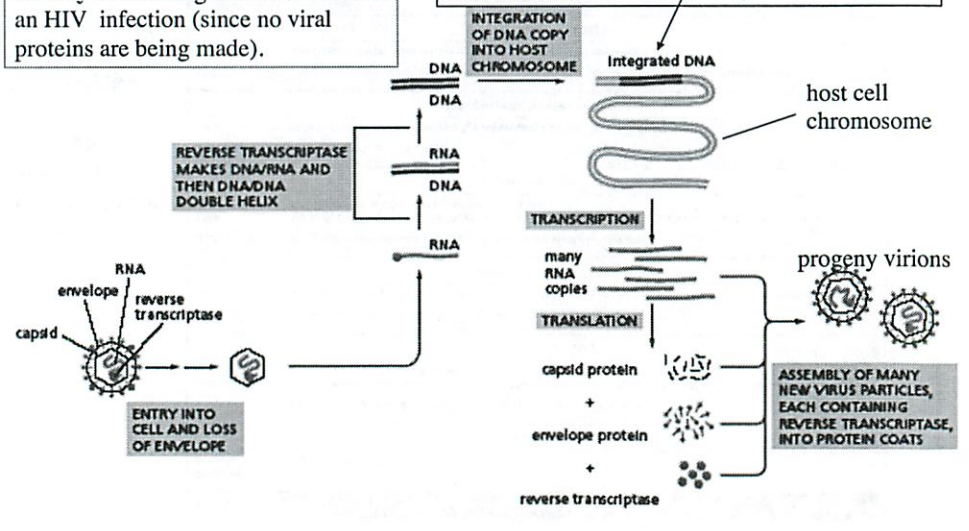


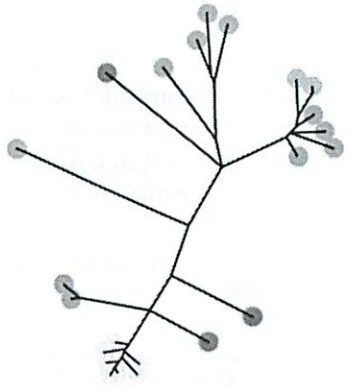
Figure 11-21 Immunobiology, 6/e. (© Garland Science 2005)



Note that if the proviral transcription is repressed, the immune system has no way of knowing that a cell harbors an HIV infection (since no viral proteins are being made).

Note that the integrated provirus may either be transcribed or be repressed (I.e., not transcribed).



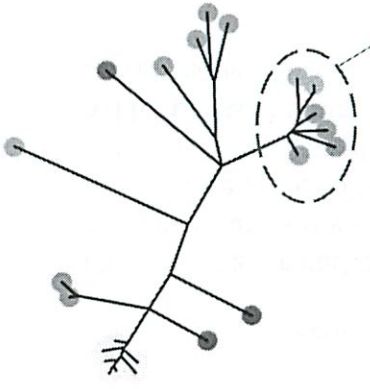


HIV-1 M group
HIV-1 O group
SIV chimpanzee
SIV mandrill
SIV Sykes' monkey
SIV African green monkey
SIV sooty mangabey
HIV-2

HIV originated in monkeys and was carried via inter-species transmission into humans 50-70 years ago somewhere in Central Africa.

How can one know how these viruses are related to one another?
By sequencing their genomes and determining how similar their genomes are.

Figure 25–38. Molecular Biology of the Cell, 4th Edition.



HIV-2 is a relatively non-pathogenic strain that progresses very slowly

HIV-1 M group
HIV-1 O group
SIV chimpanzee
SIV mandrill
SIV Sykes' monkey
SIV African green monkey
SIV sooty mangabey
HIV-2

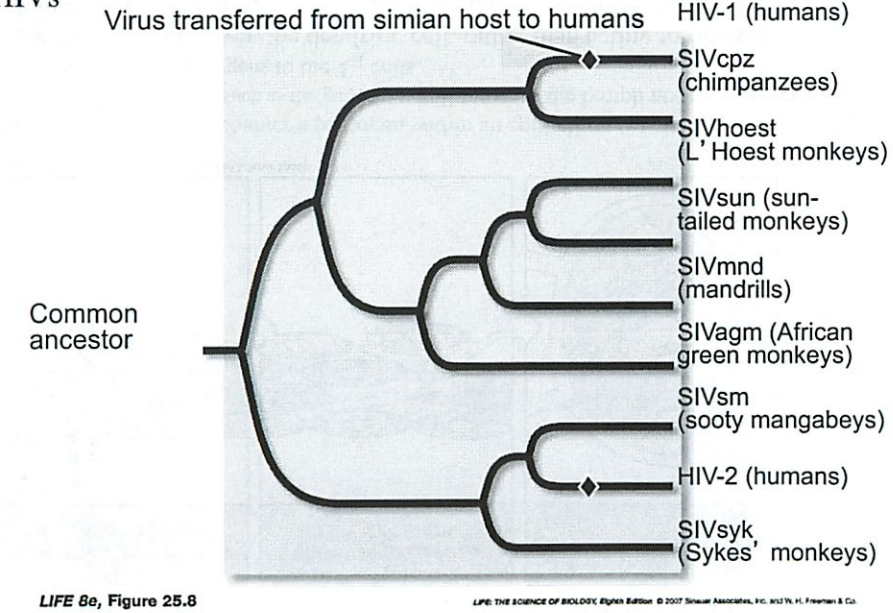
HIV originated in monkeys and was carried via inter-species transmission into humans 50-70 years ago somewhere in Central Africa.

How can one know how these viruses are related to one another?
By sequencing their genomes and determining how similar their genomes are.

Figure 25–38. Molecular Biology of the Cell, 4th Edition.

Figure 25.8 Phylogenetic Tree of Immunodeficiency Viruses

Another way of depicting the relationships between the various SIVs & HIVs



LIFE 8e, Figure 25.8

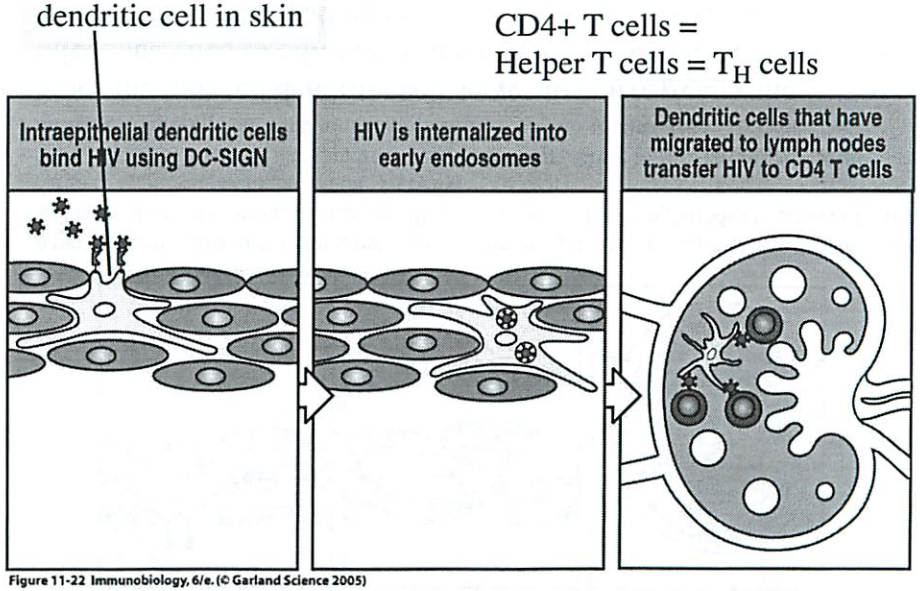


Figure 11-22 Immunobiology, 6/e. (© Garland Science 2005)

After dendritic cells encounter a pathogen within an epithelium (a layer of cells forming the skin or the lining of tubes such as the gut), they will move to the lymph nodes to present their cargo of captured pathogens to the T_H cells.

CD4+ T cells = Helper T cells = T_H cells

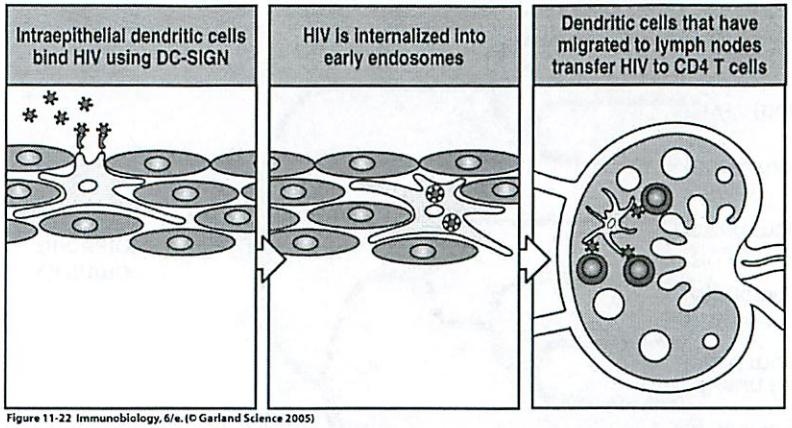


Figure 11-22 Immunobiology, 6/e. (© Garland Science 2005)

After dendritic cells encounter a pathogen within an epithelium (a layer of cells forming the skin or the lining of tubes such as the gut), they will move to the lymph nodes to present their cargo of captured pathogens to the T_H cells.
Note that here, however, the dendritic cell, rather than acting to **present oligopeptide antigens** to the T_H cell, functions to present HIV virions to the CD4+ T_H cells. Oops.

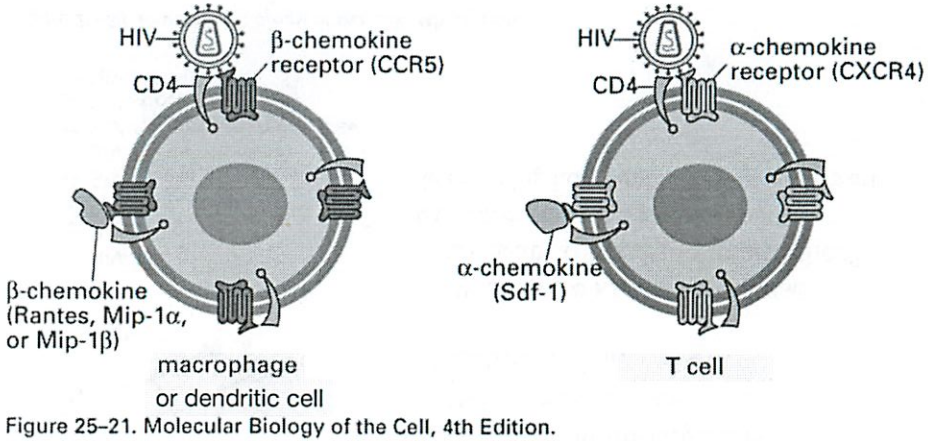


Figure 25–21. Molecular Biology of the Cell, 4th Edition.

Like other viruses, HIV adsorbs to the surface of host cells by attaching to normal host host cell-surface proteins such as growth factor receptors . The CD4 protein is used by T_H cells to recognize and bind the MHC Class II proteins used by the professional antigen-presenting cells -- the dendritic cells, macrophages and B cells.

CD4+ T cells = Helper T cells = T_H cells

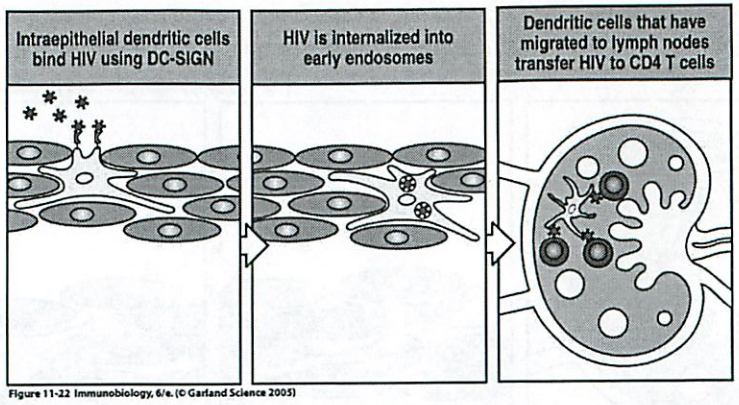
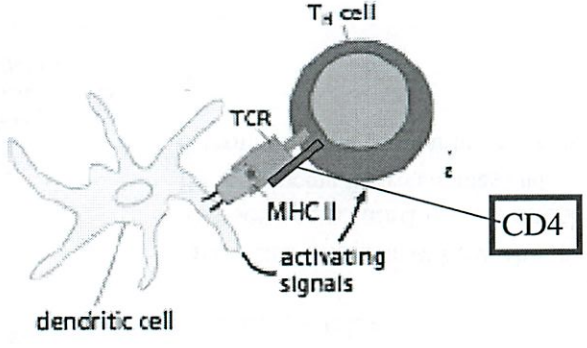


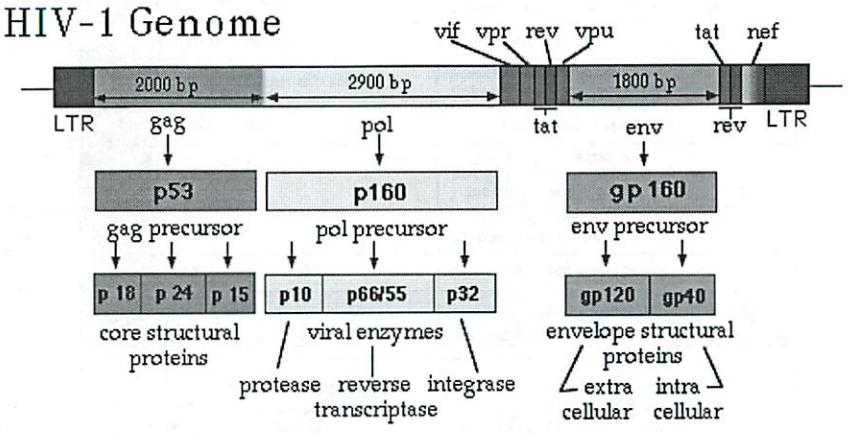
Figure 11-22 Immunobiology, 6/e. (© Garland Science 2005)

After dendritic cells encounter a pathogen within an epithelium (a layer of cells forming the skin or the lining of tubes such as the gut), they will move to the lymph nodes to present their cargo of captured pathogens to the T_H cells.
Note that here, however, the dendritic cell, rather than acting to **present oligopeptide antigens** to the T_H cell, functions to present HIV virions to the CD4+ T_H cells.
Since the infected dendritic cell serves to convey/carry the infectious virus to other cells in the body, the dendritic cell serves as a **vector** for this pathogen. (pathogen = disease-causing agent)



Or, to look at an earlier figure, when the T-cell receptor (TCR) recognizes an oligopeptide antigen ● presented by the dendritic cell, the CD4 protein, which is physically aligned with the TCR, is used by the T_H cell to recognize the basic **MHC Class II** structure (independent of whatever ● oligopeptide antigen may happen to be bound to the MHC Class II protein).

Another more detailed view of the viral genome:



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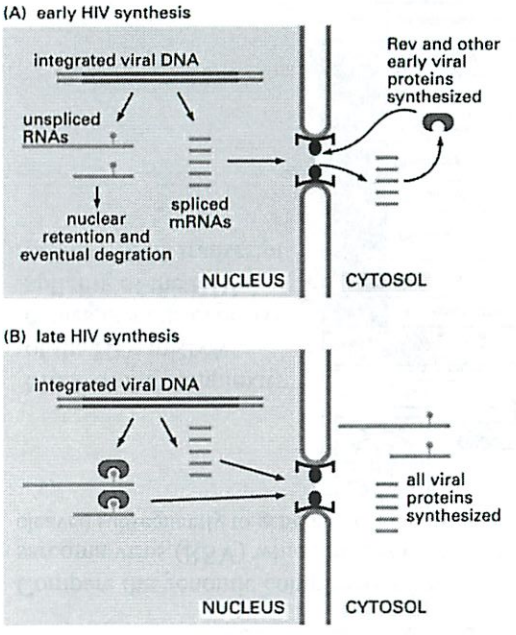
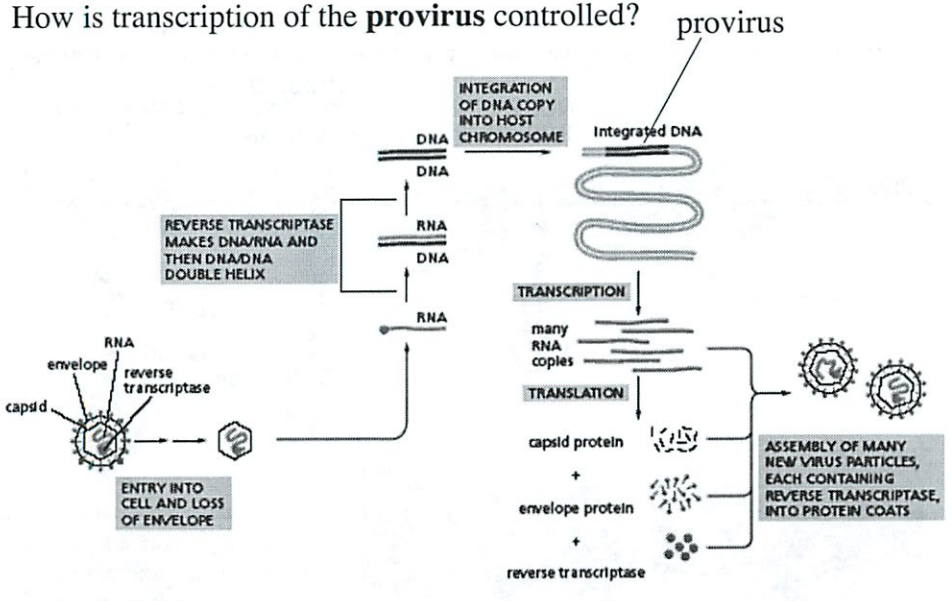


Figure 7–97. Molecular Biology of the Cell, 4th Edition.

For example, among other functions, the virus can control which of its mRNAs is selectively exported from the nucleus to the cytoplasm and which is retained in the nucleus.

How is transcription of the **provirus** controlled?



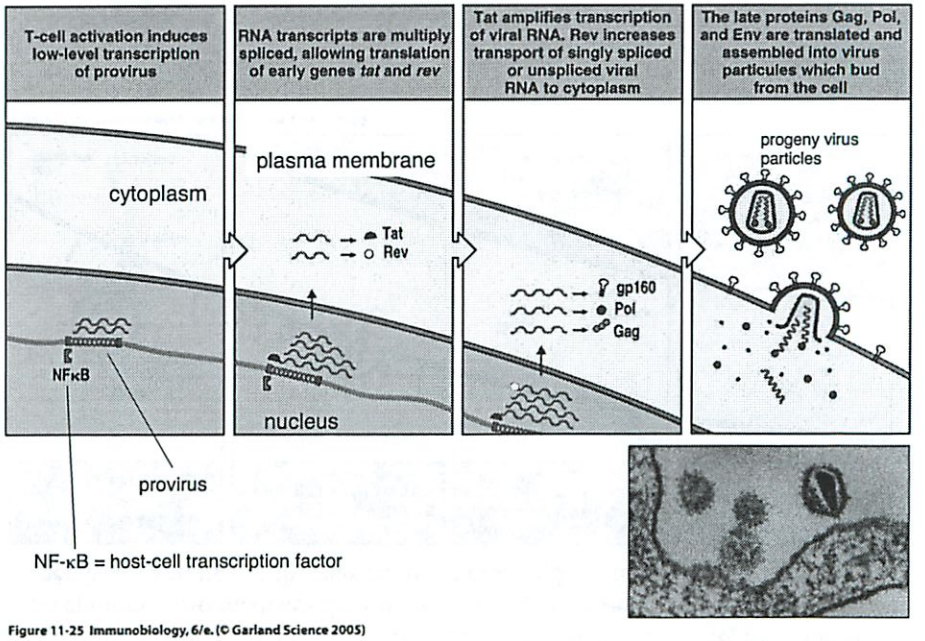
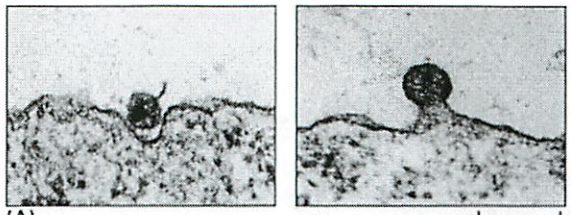
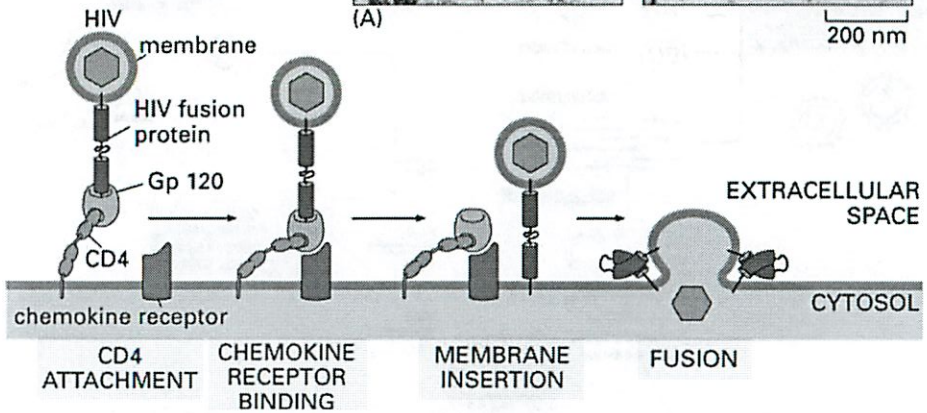


Figure 11-25 Immunobiology, 6/e. (© Garland Science 2005)

Actually the HIV glycoprotein spike binds to the CD4 protein as the primary anchoring site (primary cell-surface receptor) and then to a 2nd "co-receptor", in this case a receptor for a growth factor-like molecule termed a chemokine.





Previously we termed this entire process the "adsorption" of the virion to the cell surface.

Figure 13–16. Molecular Biology of the Cell, 4th Edition.

In fact, the virion glycoprotein spike, in the case of HIV germed "gp120" (gp = glycoprotein), not only mediates attachment to the CD4 receptor + the co-receptor but also mediates the subsequent fusion between the virion membrane and that of the plasma membrane of the infected cell.

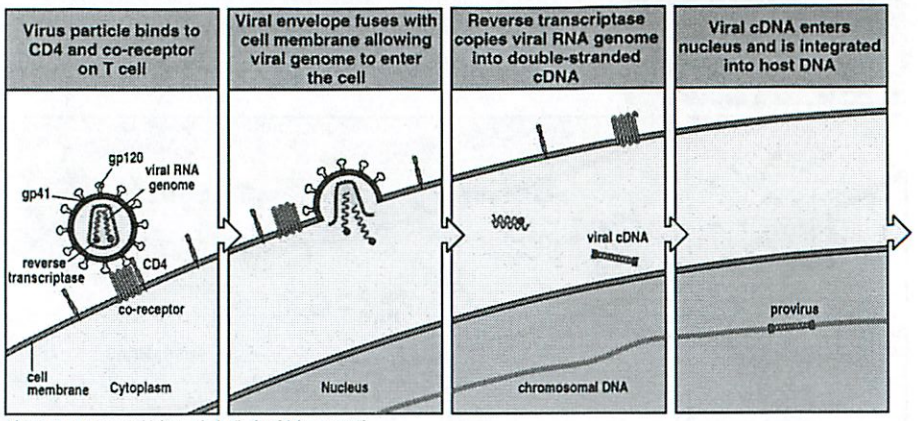
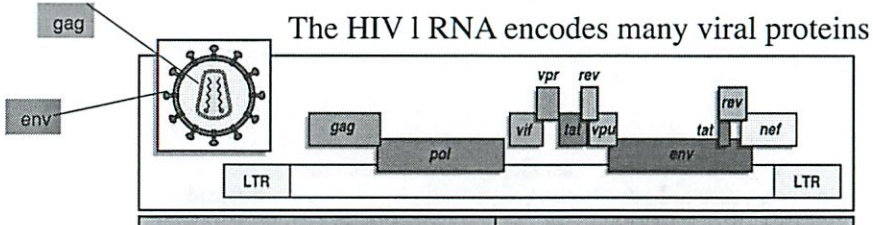


Figure 11-23 Immunobiology, 6/e. (© Garland Science 2005)

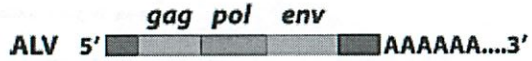
The HIV l RNA encodes many viral proteins



Gene		Gene product/function
gag	Group-specific antigen	Core proteins and matrix proteins
pol	Polymerase	Reverse transcriptase, protease, and integrase enzymes
env	Envelope (viral glycoprotein)	Transmembrane glycoproteins. gp120 binds CD4 and CCR5; gp41 is required for virus fusion and internalization
tat	Transactivator	Positive regulator of transcription
rev	Regulator of viral expression	Allows export of unspliced and partly spliced transcripts from nucleus
vif	Viral infectivity	Affects particle infectivity
vpr	Viral protein R	Transport of DNA to nucleus. Augments virion production. Cell cycle arrest
vpu	Viral protein U	Promotes intracellular degradation of CD4 and enhances release of virus from cell membrane
nef	Negative-regulation factor	Augments viral replication *in vivo* and *in vitro*. Decreases CD4, MHC class I and II expression

RT & protease

Figure 11-24 Immunobiology, 6/e. (© Garland Science 2005)



Compare the genomic complexity of the ALV retrovirus (the precursor of Rous sarcoma virus (RSV) which makes essentially **three** proteins (some of which are cleaved subsequently to generate several distinct proteins).

Much of the complexity of the HIV mRNA comes from alternative splicing of the HIV primary RNA transcript.

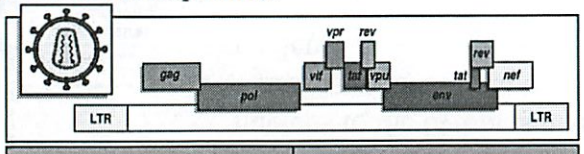


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Figure 11-24 Immunobiology, 6/e. (© Garland Science 2005)

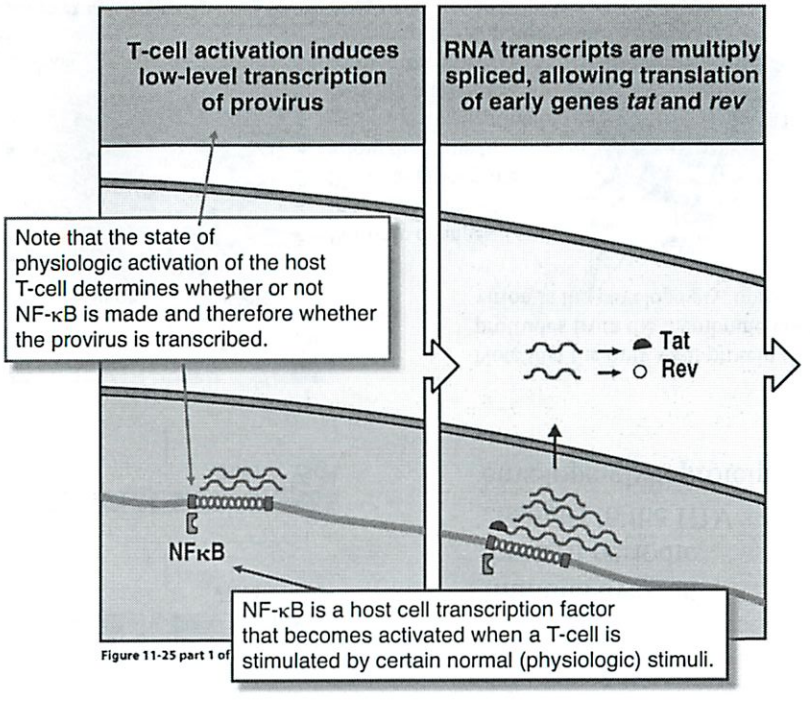


Figure 11-25 part 1 of

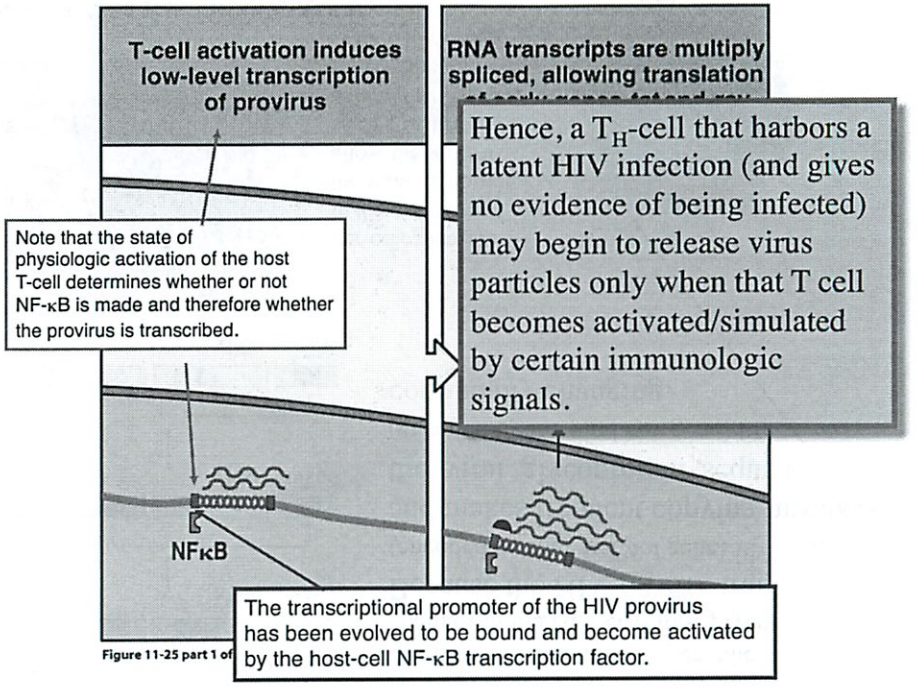


Figure 11-25 part 1 of

Hence, a T_H-cell that harbors a latent HIV infection (and gives no evidence of being infected) may begin to release virus particles only when that T cell becomes activated/simulated by certain immunologic signals.

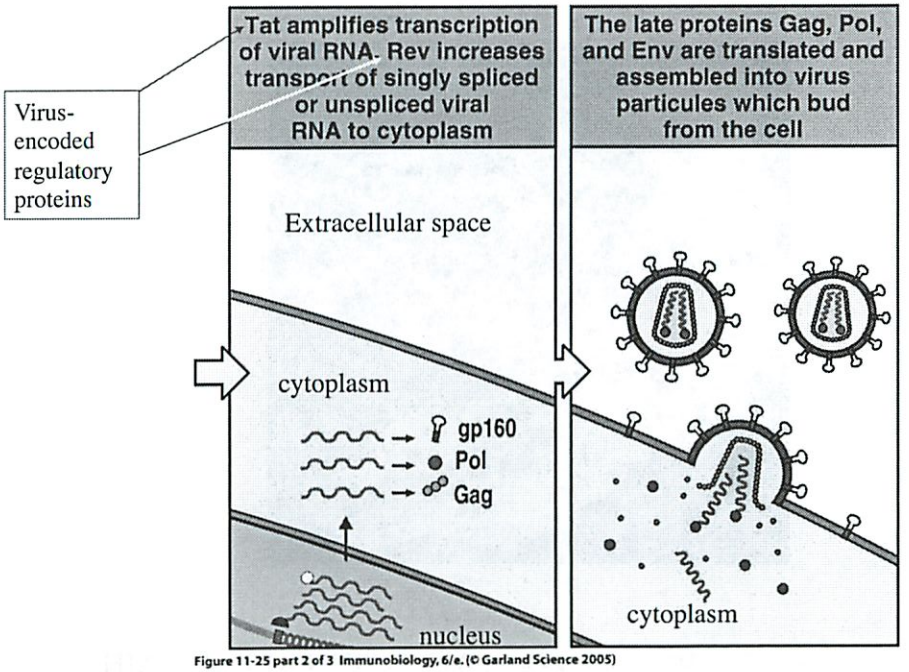
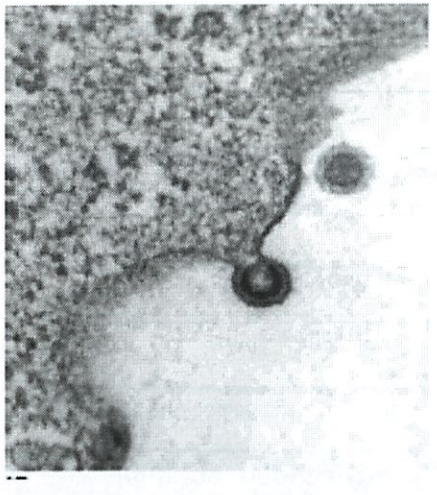


Figure 11-25 part 2 of 3 Immunobiology, 6/e. (© Garland Science 2005)



Progeny virus particles budding from HIV-infected cell

HIV virions emerging via budding from a virus-infected cell

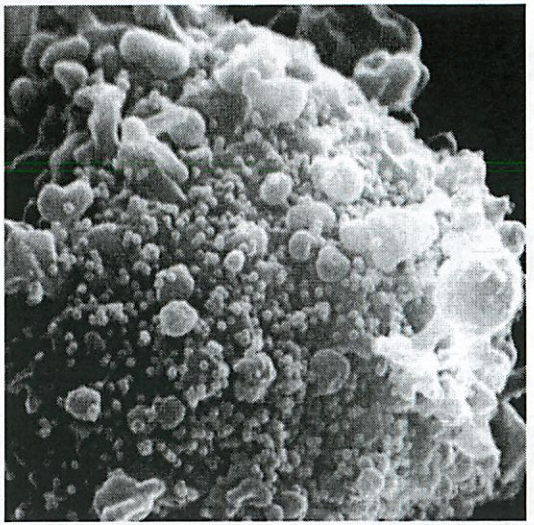


Figure 20-10
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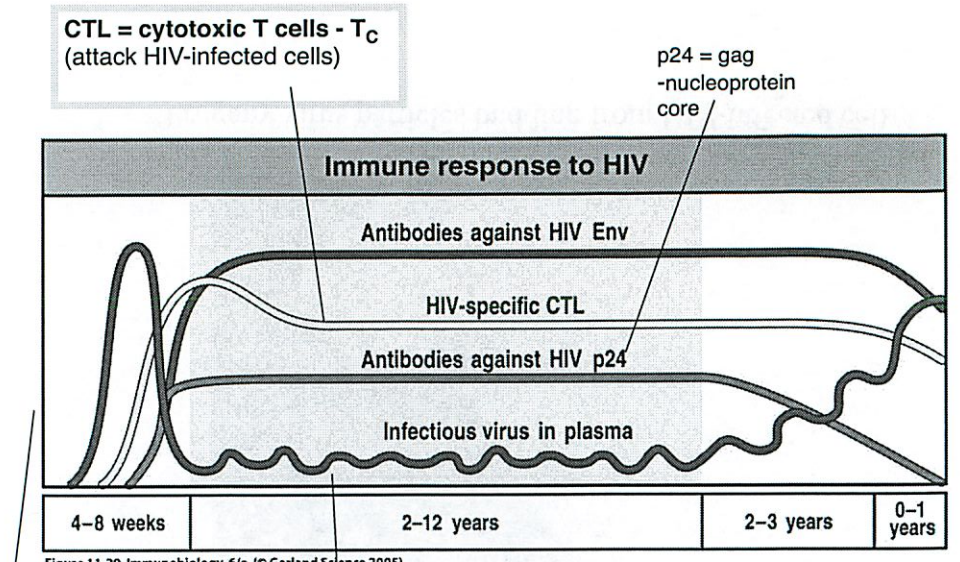
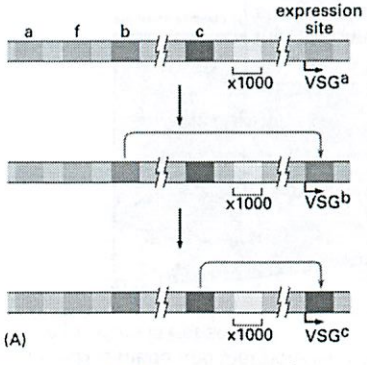


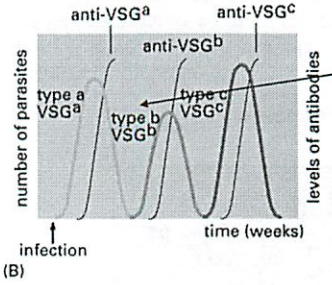
Figure 11-29 Immunobiology, 6/e. (© Garland Science 2005)

log scale

Note cyclic appearance and disappearance of viral titers -- a seesaw battle between the virus and immune system



Immune evasion through periodic changes in the HIV envelope glycoprotein



Periodic changes in the structure of the viral glycoprotein allow the virus to escape neutralization by existing antibodies, forcing the immune system to develop new ones.

Note that the only viral protein that protrudes from the membrane-coated virion is the envelope glycoprotein

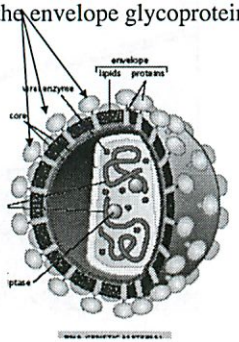
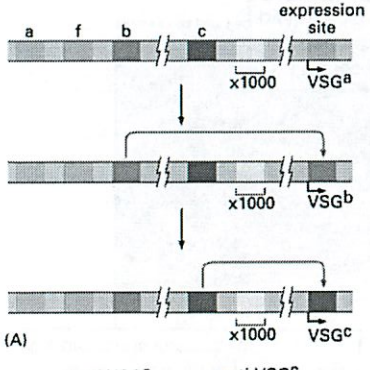
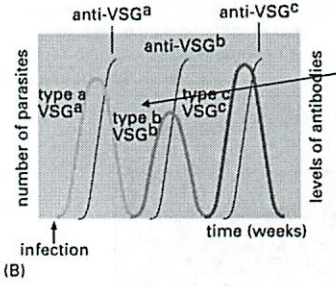


Figure 25–37. Molecular Biology of the Cell, 4th



Immune evasion through periodic changes in the HIV envelope glycoprotein:
Because the HIV reverse transcriptase (encoded by the viral *pol* gene) is "sloppy" and makes frequent copying mistakes, the viral glycoprotein sequences (encoded by the viral *env* gene) are continually changing.



Periodic changes in the structure of the viral glycoprotein allow the virus to escape neutralization by existing antibodies, forcing the immune system to develop new ones.

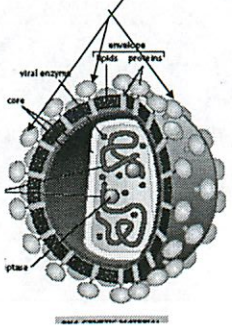
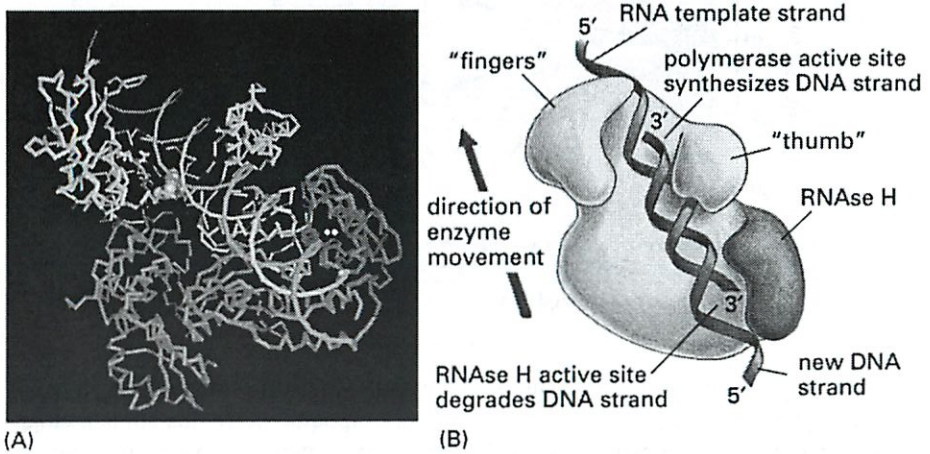


Figure 25–37. Molecular Biology of the Cell, 4th

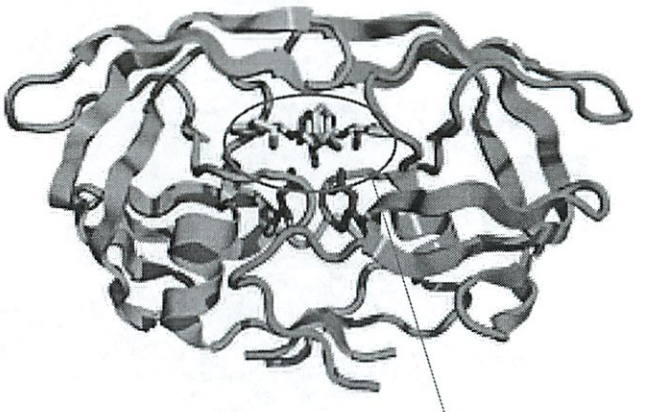
For example, drugs can be developed to antagonize the viral reverse transcriptase

The viral reverse transcriptase enzyme



(A) (B)

Figure 5–74. Molecular Biology of the Cell, 4th Edition.



HIV protease together with inhibitory drug at active site.

TABLE 20-5 Some anti-HIV drugs in clinical use

Generic name (other names)	Typical dosage	Some potential side effects
REVERSE TRANSCRIPTASE INHIBITORS: NUCLEOSIDE ANALOGUE		
Didanosine (Videx, ddI)	2 pills, 2 times a day on empty stomach	Nausea, diarrhea, pancreatic inflammation, peripheral neuropathy
Emtricitabine (Emtriva, FTC)	1 pill, 1 time a day	Headache, diarrhea, nausea, rash
Lamivudine (Epivir, 3TC)	1 pill, 2 times a day	Usually none
Stavudine (Zerit, d4T)	1 pill, 2 times a day	Peripheral neuropathy
Zalcitabine (HIVID, ddC)	1 pill, 3 times a day	Peripheral neuropathy, mouth inflammation, pancreatic inflammation
Zidovudine (Retrovir, AZT, ZDV)	1 pill, 2 times a day	Nausea, headache, anemia, neutropenia (reduced levels of neutrophil white blood cells), weakness, insomnia
Pill containing lamivudine and zidovudine (Combivir)	1 pill, 2 times a day	Same as for zidovudine
Abacavir (Ziagen)	2 pills, 1 time a day	Nausea, vomiting, diarrhea, lactic acidosis (severe liver disease)
Tenofvir (Viread)	1 pill, 1 time a day	Nausea, vomiting, increased risk of bone breakage

Table 20-5 part 1
Kuby IMMUNOLOGY, Sixth Edition

TABLE 20-5 Some anti-HIV drugs in clinical use

Generic name (other names)	Typical dosage	Some potential side effects
PROTEASE INHIBITORS		
Indinavir (Crixivan)	2 pills, 3 times a day on empty stomach or with a low-fat snack and not within 2 hours of didanosine	Kidney stones, nausea, headache, blurred vision, dizziness, rash, metallic taste in mouth, abnormal distribution of fat, elevated triglyceride and cholesterol levels, glucose intolerance
Nelfinavir (Viracept)	3 pills, 3 times a day with some food	Diarrhea, abnormal distribution of fat, elevated triglyceride and cholesterol levels, glucose intolerance
Ritonavir (Norvir)	6 pills, 2 times a day (or 4 pills, 2 times a day if taken with saquinavir) with food and not within 2 hours of didanosine	Nausea, vomiting, diarrhea, abdominal pain, headache, prickling sensation in skin, hepatitis, weakness, abnormal distribution of fat, elevated triglyceride and cholesterol levels, glucose intolerance
Saquinavir (Invirase, a hard-gel capsule; Fortovase, a soft-gel capsule)	6 pills, 3 times a day (or 2 pills, 2 times a day if taken with ritonavir) with a large meal	Nausea, diarrhea, headache, abnormal distribution of fat, elevated triglyceride and cholesterol levels, glucose intolerance
Atazanavir (Reyataz)	2 pills, 1 time a day	Must be used with at least two other drugs
Fosamprenavir calcium? (Lexiva)	2 pills, 2 times a day	Appetite loss, malaise, diarrhea, nausea, vomiting
FUSION INHIBITORS		
Enfuvirtide (Fuzeon, T-20)	Subcutaneous injection 2 times daily	Soreness at injection site, dizziness, loss of sleep, numbness in feet and legs

Table 20-5 part 3
Kuby IMMUNOLOGY, Sixth Edition

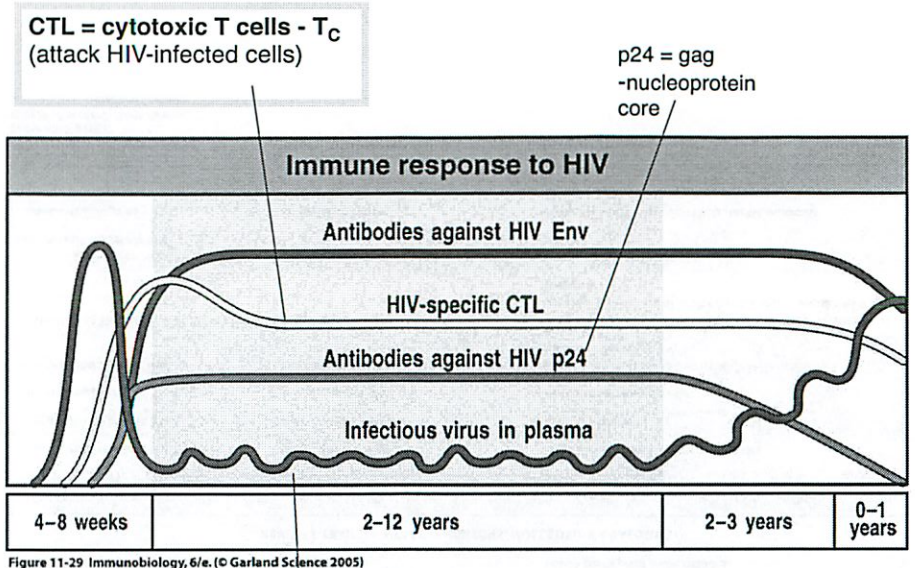
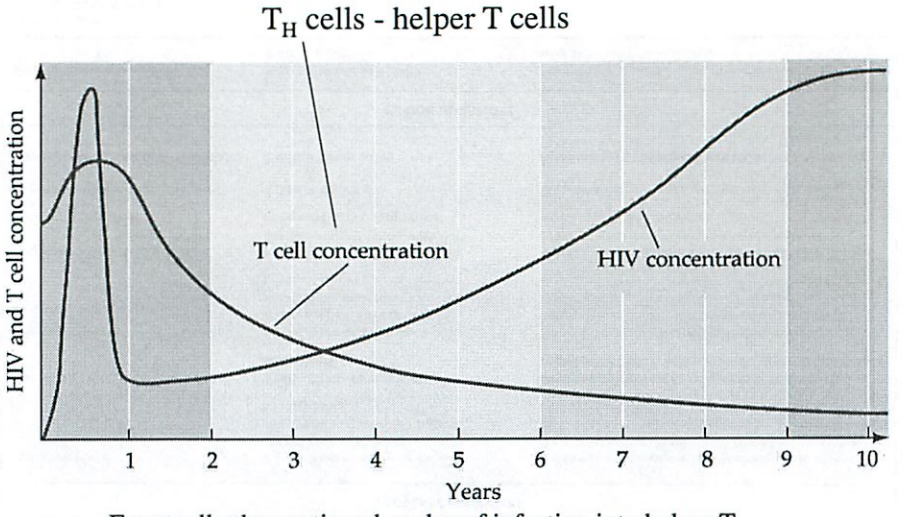


Figure 11-29 Immunobiology, 6/e. (© Garland Science 2005)



Eventually the continued cycles of infection into helper T cells results in their depletion and the resulting increase in viral titer in the serum as the immune system loses its ability to suppress viral replication.

Here is a more quantitative rendering of the time course of the disease. Note that the anti-HIV antibody is antibody against all viral proteins, and that "AIDS" only occurs clinically when the immune system becomes totally crippled.

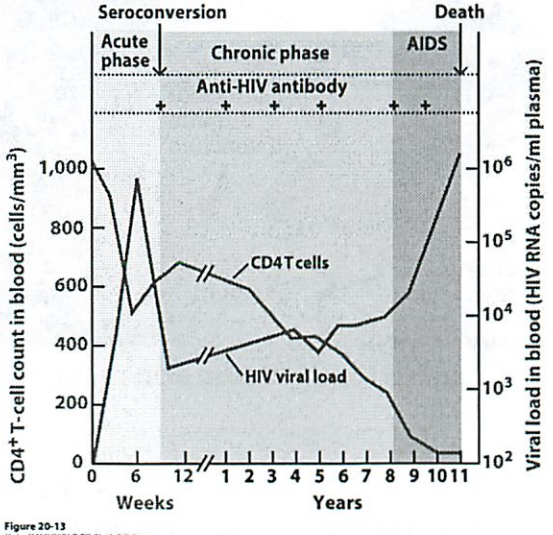


Figure 20-13
Kuby IMMUNOLOGY, Sixth Edition
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These four steps of the viral life cycle represents potential points for therapeutic intervention. Accordingly, each of these represents a drug target.

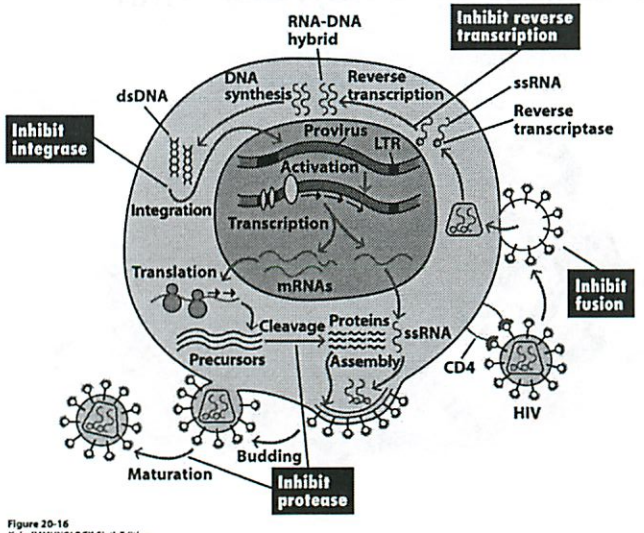

Figure 20-16
Kuby IMMUNOLOGY, Sixth Edition
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Effects of combination anti-HIV drug therapy on the no. of viral

RNA molecules in the plasma of a virus-infected individual

(One of these drugs may shut down the viral reverse transcriptase, while another may shut down the viral protease needed for the post-translational processing of the viral Gag polyprotein that cleaves it into the individual nucleocapsid proteins.)

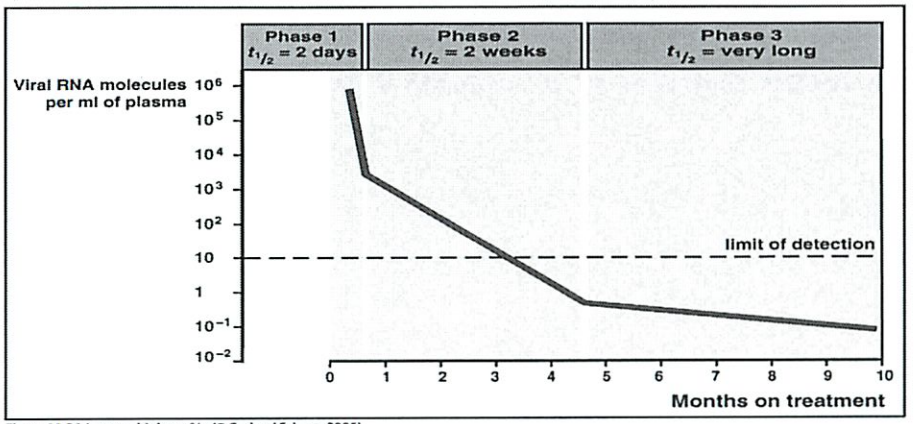

Figure 11-26 Immunobiology, 6/e. (© Garland Science 2005)

Effects of the use of combination drug therapy on the death rate and infectious complication rate of HIV-infected Individuals.

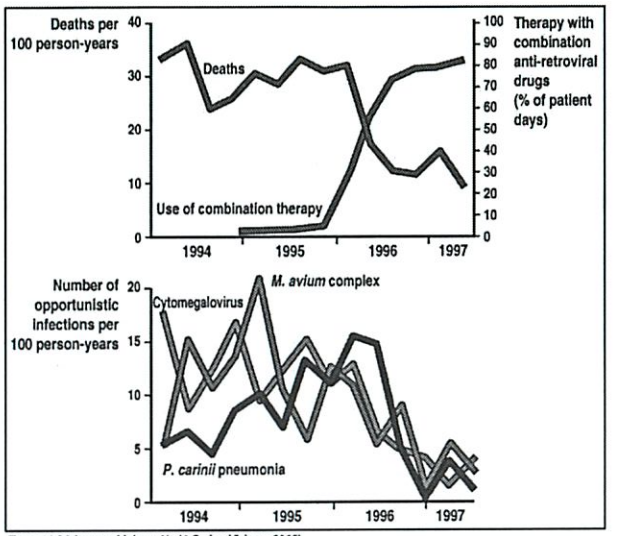

Figure 11-28 Immunobiology, 6/e. (© Garland Science 2005)

Or, to put this into perspective, for a population of young People:

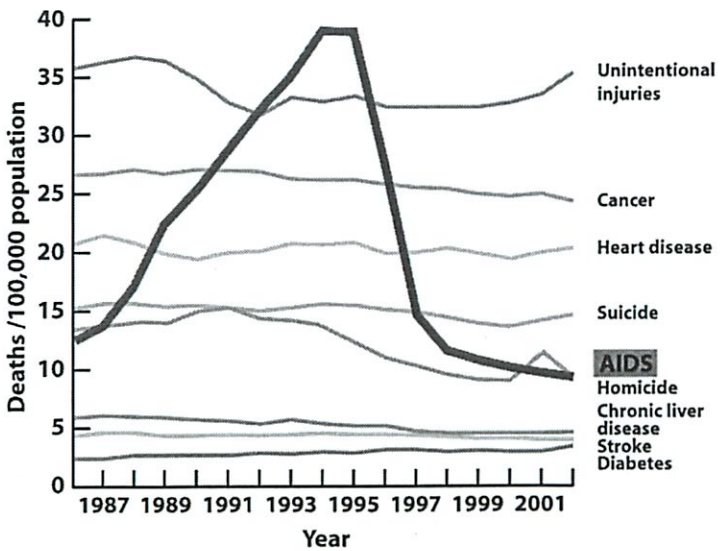

Figure 20-7
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TABLE 20-6 Why AIDS does not fit the paradigm for classic vaccine development

Classic vaccines mimic natural immunity against reinfection generally seen in individuals recovered from infection; there are no recovered AIDS patients.
Most vaccines protect against disease, not against infection; HIV infection may remain latent for long periods before causing AIDS.
Most vaccines protect for years against viruses that change very little over time; HIV-1 mutates at a rapid rate and efficiently selects mutant forms that evade immunity.
Most effective vaccines are whole killed or live attenuated organisms; killed HIV-1 does not retain antigenicity, and the use of a live retrovirus vaccine raises safety issues.
Most vaccines protect against infections that are infrequently encountered; HIV may be encountered daily by individuals at high risk.
Most vaccines protect against infections through mucosal surfaces of the respiratory or gastrointestinal tract; the great majority of HIV infection is through the genital tract.
Most vaccines are tested for safety and efficacy in an animal model before trials with human volunteers; there is no suitable animal model for HIV/AIDS at present.

SOURCE: Adapted from A. S. Fauci, 1996, An HIV vaccine: breaking the paradigms, *Proceedings of the Association of American Physicians* 108:6.

Table 20-6
Kuby IMMUNOLOGY, Sixth Edition
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Doing
PSet 7

12/5
9:20P

1a.) IDK
did we ever discuss?

oh lowest mutation → the double stranded
2 copies

b) 33% A

So 33% T/U

16.5% C 16.5% G

c) Clever
Need to be very good on the diff types

retrovirus RNA
reverse transcriptase
replicates as part of DNA
Onco genes does not always play apart
to be a retrovirus...

(2)

2) Rouse Sarcoma has + w/ src ?
thought it copies over...

---

from reading it seems

gag pol env is ALV
gag pol env src is RSV

2a) So RNA directed DNA polymerase
makes DNA
not made from DNA

c) Src and ?
Ras
? where is this from?
Most Comon oncogene in cancer

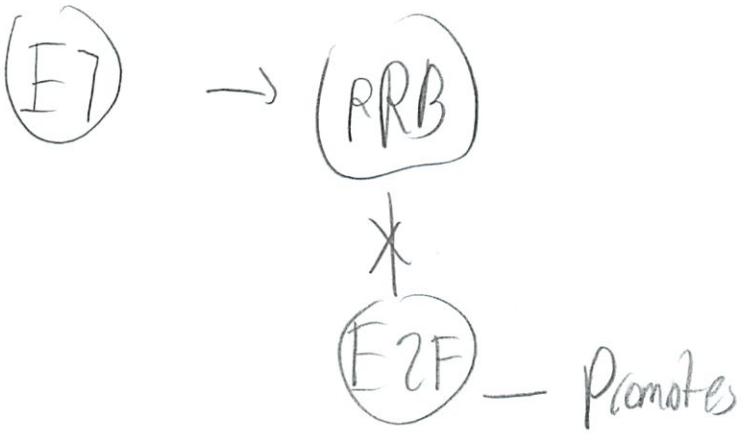
(3)

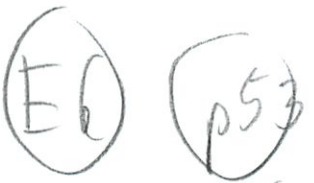
d) tumor supression gene

anti-onco gene

protects cell from 1 step to tancer

if reduced / leads to cancer





remove blah

transcription factor Control

promote or blocks

binding to promote ↓ cell growth

(4)

↑ E7 ↑ E7-pRP

↓ pRB - E2F

↑ E2F - promoter

So ↓ promoter to DNA

So ↓ transcription

2e) How do we know if dominant?

dimer formed from 2 structurally similar monomers - can be strong or weak

I see in b - deletion so nope

c) intron deletion who cares

3d) Looked up on WP before!

(6)

4) a) Another one of these

dom? No from 1, 2

X linked

Dad Mom

X Y x x ← affected dad

all daughters carriers ← not true
no sons ← or that

Dad Mom

x Y x X

half sons have X Y

half daughters carriers

Dad Mom

x Y X X

all Sons have

all daughters carriers

(6)

b) Ah they told us here -/-
but blood cells have +/-
Why?

Beats me
Tumor cells have
Unless tumor has changed

c) +/- blood
but not

d) Rb tumor supression gene
pRB inhibits E2F
pRB deactivated
E2F activated - stimulates cell division

(8)

5, a) Did we ever talk about radiation therapy?

b) Didn't do chemo either!

Pratice learning as you go
Might be better!
But more confusing!

(8)

Jeff

12/6 midnight

Envelope vs non envelope?

E7 = oncogene

Can look up online!

2e) Not sure right way on question

4) Autosomal dominant

recessive; new guy would have to have which might be unlikely

b) tumor cell had a mutation

heterozygous originally

then homo for disease allel

a mutation

caused cell to become cancer cell

(9)

c) Need 2 copies of allele to have cancer
if protoonco gene
(he thinks that is property of protooncos)

5b) Chem effects normal cells in same way
damages normal cells
all symptoms of dying cells normally

---

* Must get a lot better w/ pedigrees!

Own
fix
12:30A
12/6

---

* ~~+/- is p~~

top pedigree is predisposition
+/- is actually at a risk for

(c)

Shaded = black + will get cancer

4 is rare exception, has gene, doesn't get cancer

because not full mutation

---

if 1 copy mutated

Chance 2nd copy will be mutated is high

---

if recessive - even harder to reason about

C is rare exception

Name Michael Plasmide Section 27 TA Hashyar

2012 7.012 Problem Set 7

Please print out this problem set and answer the questions on the printout.
Answers to this problem set are to be turned in at the box outside 68-120 by 4.00 PM, Thursday ~~Dec 6th~~.

15

Question 1
You are studying the following four different viruses.

- Type A is an **enveloped, minus stranded** RNA virus.
- Type B is an **enveloped, plus stranded** RNA virus (no viral proteins are packaged in the virion).
- Type C is an **enveloped, plus stranded retrovirus,** reverse transcriptase is packaged in the virion
- Type D is a **non-enveloped double stranded** DNA virus.

a) Which of these viruses (*Type A/ B/ C/ D*) is likely to have the **lowest mutation rate? Explain** why you selected this option.

Type D since it has 2 strands, which means mutations are likely to be corrected on its own

b) You analyze the genome of each virus and are surprised to find that each has 33% adenine (A) in its genome.

i. Based on this information, you can predict the % of remaining bases (*T/ G/ C/U*) in the genome for which virus(es)?

Type D 33% T 16.5% C 16.5% G | Type A,B,C No, since no pairs on others side

ii. In the table below, give the percentage of each appropriate base (*T/ G/ C/ U*) found in the genome of the virus(es) you selected in part (i).

└ only 1

VirusType	*Base*	*A*	*T*	*G*	*C*	*U*
D	*% in the viral genome*	33	33	16.5	16.5	0

−1 What!? So silly!

c) You successfully transduce a eukaryotic cell line with each of the above viruses in four separate plates. You isolate the viruses from the infected cells in each plate and use them to infect fresh eukaryotic cells that are being incubated with **actinomycin D** *(inhibits transcription by blocking only the host RNA polymerase)* or **anisomycin** (*host ribosome inhibitor*). Complete the following table for each of the treatments.

Treatment	**Virus**	**Virus formed (*Yes/No*)?**
Actinomycin D	Type A	Yes
	Type B	Yes
	Type C	Yes
	Type D	No
Treatment	**Virus**	**Virus formed (*Yes/ No*)?**
Anisomycin	Type A	No
	Type B	No
	Type C	No
	Type D	No

d) Which of the above virus(s) (*Type A/ B/ C/ D*) **must integrate** its genome in the host cell? Give all possible options and explain why you selected each.

D; must use host DNA polymerase + RNA polymerase

C; retrorse transcriptase so then like D since DNA ~~plus must get an oncogene~~

└ Oncogene not required

A, B, C come w/ own (or transcribe) own RNA polymerase

Name_____ Section_____ TA_____

Question 2

The discovery that cancer could be caused by a virus was a major one. However, the subsequent discovery that Rous Sarcoma Virus (RSV), a cancer-causing virus discovered in chickens, encoded a mutant form (v-src) of a normal cellular gene (c-src) was even more surprising. Rous sarcoma virus (RSV) is a retrovirus that also has a + stranded RNA genome that encodes four genes; gag (encodes the capsid protein), pol (encodes the reverse transcriptase), env (encodes the envelope glycoprotein) and src (encodes a tyrosine kinase enzyme).

a) Given the information, reverse transcriptase is considered which of the following?

- A DNA directed RNA polymerase
- A RNA directed DNA polymerase
- A RNA directed RNA polymerase

b) Why is it essential that the RSV encodes Reverse transcriptase?

So it can make more of it, so its children can also carry reverse transcriptase so it can convert the RNA to DNA

c) What are two major classes of genes involved in the development of cancer? For each, describe the type of mutation that is associated with cancer, and how this mutation would promote tumor formation.

Src - If ALV lands at right spot it includes Src which is a Tyr kinase which mimics the growth promoting signals that are released by a growth factor recepto

Ras - If mutation, can block Ras from turning off (GTP-δ DP) leaving it to keep growth signal on.

d) The Human papilloma virus (HPV) has been implicated as a risk factor for cervical cancer. The E7 protein of HPV binds to pRB protein preventing it from binding to the host transcription factor E2F, which is now free to bind to the promoters of genes that promote cell cycle. In contrast, another HPV protein, namely E6 binds to p53 targeting it for destruction by proteosomes thus removing the block on the host cell's entry into the cell cycle.

i. Would you classify E7 as an oncogene or a tumor suppressor gene? **Explain** why?

Oncogene it allows E2F to promote the cell cycle causing cell growth

ii. Would you classify E6 as an oncogene or a tumor suppressor gene? **Explain** why?

Oncogene - adding it increases chance of cancer since it removes block on cell growth

Name_____ Section_____ TA_____

Question 2 continued

e) Each of the five genes given below, when mutated, can result in a transformed phenotype in the mutant cells. In the final column, give the phenotype (*normal or transformed*) of a diploid cell that has the two alleles given. ***Note:*** *A description of each gene is given.*

ras: encodes a protein, which is active in its GTP bound form and inactive in its GDP bound form. When active it promotes cell division.
cyclin D: encodes a protein that interacts with a CDK (cyclin dependent kinase), and promotes cell division.
erb-B2: encodes an epidermal growth factor receptor which is active when dimerized. It promotes cell division when activated.
p16: encodes a protein that inhibits cyclin-dependent kinase. – inhibits cell division
WT1: encodes a protein that inhibits progression through the cell cycle.

Gene	Class	Status of allele 1	Status of allele 2	Phenotype
ras	Proto-oncogene	Mutation such that protein cannot hydrolyze GTP to GDP	Wild-type	Transformed
Cyclin D	Proto-oncogene	Mutation that results in deletion of entire gene	Wild-type	Normal
erb-B2	Proto-oncogene	Mutation such that the receptor protein constitutively dimerizes	Mutation that results in the deletion of 120 base pairs in intron 5	Transformed
p16	Tumor suppressor	Point mutation that results in truncated protein of 20 amino acids	Wild-type	Transformed –1
WT1	Tumor suppressor	Mutation in promoter that prevents RNA polymerase from binding	Mutation that results in the deletion of 4 base pairs in the coding region 20 base pairs after the start codon	Transformed

Question 3

Cancer is caused by the accumulation of two or more mutations in the same cell that affects its proliferation and survival.

a) Why does a person's chance of having cancer increase with age?

Cancer takes time to develop. Also more cells created as get older (in total, not per year) so mutation chance ↑ed

→ mutations can lie dormant for awhile

b) Cell lines are often used to test the oncogenic potential of viruses. If cancer is a multi-step process, why can the introduction of a single active viral oncogene transform these cells?

Since this one oncogene induces the production of a bunch of cells, which form a tumor over time as cells transform as in a different environment.

Name_____ Section_____ TA_____

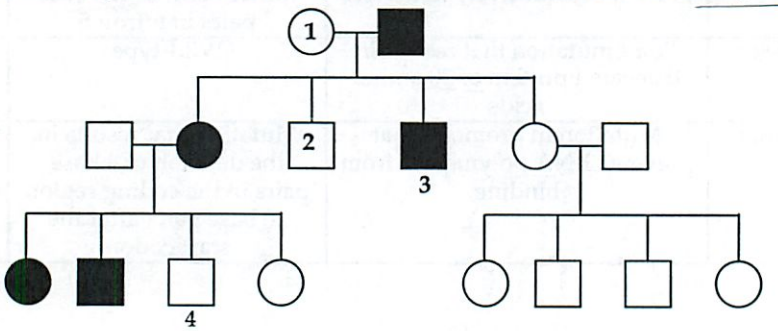
Question 3 continued

d) Briefly describe what an Ames test is and how it may be used to evaluate the mutagenic potential of a chemical agent. Do you think you can evaluate the mutagenic potential of any carcinogen using Ames test (*Yes/ No*)? Justify your answer.

Test for mutagenicity
1. start w/ mutant bacteria his⁻ in media with his⁺
2. Expose to mutagen
3. Plate on minimal media, see how many colonies grow
└ these are the mutated ones,
No. Lots of false positives/negs

Question 4

The following human pedigree shows the mode of inheritance of the predisposition to colon cancer.
Please note: *The shaded squares and circles represent the individuals who eventually develop cancer.*



a) Looking only at the pedigree, the predisposition to this disease appears to have what mode of inheritance? autosomal dominant

b) If you check the genotype of the tumor cells from individual 3, you find that they are homozygous for the disease allele (-/-). However, if you check the genotype of the blood cells from this individual, you find that they are heterozygous for the disease allele (+/-). **Explain** why the genotype with respect to the disease allele in the blood cells is different from that in the tumor cells isolated from individual 3.

The cell was heterozygous at first. Then a mutation caused it to go homozygous for the disease allele, thus becoming a tumor cell.

c) For individual 4, the blood samples are heterozygous, carrying both the wild-type allele and a mutant allele of the gene associated with this type of cancer. However, this individual **did not develop** cancer. **Explain** why. Tumor cells have developed a different type of mutation. Or don't have all the steps needed for mutation

d) One example of a tumor suppressor gene is the Retinoblastoma (Rb) gene . The wild-type pRB protein binds and inhibits the activity of the transcription factor E2F. At the appropriate time in the cell cycle, pRB is phosphorylated (inactive state) and E2F becomes available to act as a transcription factor that stimulates cell division. If a cell has lost the functional pRB and E2F proteins, would you expect cell division (*Yes/ No*)? **Explain** your choice.

No. E2F needed to stimulate cell division
Assuming cell won't divide w/o it

Name_____ Section_____ TA_____

Question 5

a) Radiation therapy can be used to treat tumors. Briefly **explain** how radiation therapy works to treat a tumor.

Radiation kills cells by damaging their DNA so they stop dividing or die

b) Chemotherapeutic drugs often have side effects such as diarrhea, constipation, mouth sores, hair loss, nausea, and blood-related side effects.

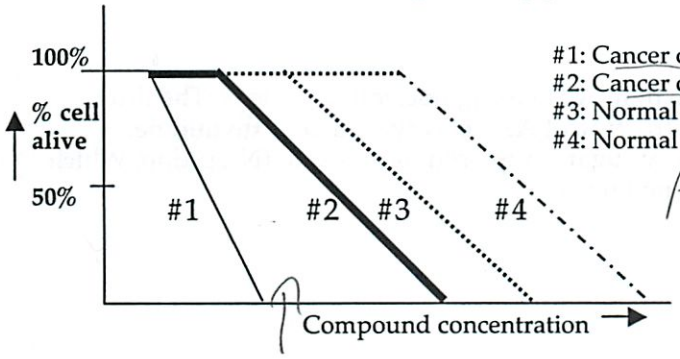
i. Chemotherapeutic drugs have a wide range of structures and functions, yet many elicit the same side effects. **Explain** why the side effects are the same for a variety of different drugs.

it kills cells that divide rapidly, All affect cell division or DNA Synthesis + function in some way.

ii. Describe what is meant by the "therapeutic window" of a drug used in chemotherapy, and how it relates to the side effects seen in a patient.

The range of dosages that treat disease effectively but still stay safe. The higher you go the more side effects one is likely to have.

iii. Prior to being used for treatment, each chemotherapeutic drug is extensively screened. During drug screening you identify two compounds A and B that have the potential to kill cancer cells and normal cells as shown by the following graph. Which compound (*A*/*B*) is a better candidate for cancer treatment? **Explain** why you selected this option.



#1: Cancer cells + Compound A
#2: Cancer cells + Compound B
#3: Normal cells +Compound B
#4: Normal cells + Compound A

A, at the concentration indicated by the arrow all cancer cells are dead while all normal cells are alive

iv. **Explain** how the use of following drugs may prevent cancer cell growth and /or cell proliferation.

Drug	Target of drug	How is cancer cell growth and / or proliferation prevented?
Vincristine	Microtubule inhibitor	Prevents cells from splitting - so harder for cancer to spread
VEGF inhibitor	Inhibits blood vessel formation	Prevents blood vessels from reaching tumor and letting tumor spread

c) Her-2 receptor is encoded by the Her-2 proto-oncogene and is a member of the epidermal growth factor (EGF) family of receptor tyrosine kinases. Her-2 gene amplification is correlated with aggressive forms of breast cancer that respond better to treatment with herceptin than other non-aggressive forms of breast cancer. **Explain** why this is so.

Herceptin is a monoclonal antibody that interferes with the HER2 receptor. Since the agressive forms have Her-2 amplification those are treated better by Herceptin.

Name_____ Section_____ TA_____

Question 6 (This question is optional and will NOT be graded)
Human immunodeficiency virus (HIV) is a retrovirus. Its genome is a single (+) stranded RNA that is packaged with the reverse transcriptase enzyme within a protein capsid. This is further packaged into an envelope that is derived from the plasma membrane of the host cell in which the virus had replicated. The surface of the envelope is covered with the envelope glycoprotein, called gp120.

a) HIV specifically infects the T- helper (T_H) cells of the human immune system. If HIV enters the host cell by means of host receptor recognizing a viral protein, what would be the most likely interacting proteins during HIV infection?

b) Why the HIV infected cells remain undetected by the host immune system for several years?

c) Some individuals are resistant to HIV infection even after repeated exposure. Assuming that these individuals express a normal level of the functional receptor that you have recognized above, how can you explain their resistance to HIV?

d) In recent years, therapies have been developed to fight AIDS using nucleotide analogs. The drug used to combat AIDS is Azidothymine (AZT). The structure of AZT is very similar to thymidine. However, in AZT the 3'-OH group on the deoxyribose sugar is replaced by an azido (N_3) group. Which process of the life cycle of HIV do you think is inhibited by AZT?

Solution key- 2012 7.012 Problem Set 7

Question 1

You are studying the following four different viruses.

- Type A is an **enveloped, minus stranded** RNA virus.
- Type B is an **enveloped, plus stranded** RNA virus (no viral proteins are packaged in the virion).
- Type C is an **enveloped, plus stranded retrovirus,** reverse transcriptase is packaged in the virion)
- Type D is a **non-enveloped double stranded** DNA virus.

a) Which of these viruses (*Type A/ B/ C/ D*) is likely to have the **lowest mutation rate? Explain** why you selected this option.

Type D. This is the only type of virus from the list that depends solely on host DNA polymerase for replication. DNA polymerase, but not RNA polymerase, has proofreading capacity (3' -> 5' exonuclease activity) that would lower the rate of mutation.

b) You analyze the genome of each virus and are surprised to find that each has 33% adenine (A) in its genome.

i. Based on this information, you can predict the % of remaining bases (*T/ G/ C/U*) in the genome for which virus(es)?
Type D.

ii. In the table below, give the percentage of each appropriate base (*T/ G/ C/ U*) found in the genome of the virus(es) you selected in part (i).

Virus Type	*Base*	*A*	*T*	*G*	*C*	*U*
D	*% in the viral genome*	*33%*	*33%*	*17%*	*17%*	*0%*

c) You successfully transduce a eukaryotic cell line with each of the above viruses in four separate plates. You isolate the viruses from the infected cells in each plate and use them to infect fresh eukaryotic cells that are being incubated with **actinomycin D** (*inhibits transcription by blocking **only** the **host** RNA polymerase)* or **anisomycin** (*host ribosome inhibitor*). Complete the following table for each of the treatments.

Treatment	**Virus**	**Virus formed (*Yes/No*)?**
Actinomycin D	Type A	*Yes*
	Type B	*Yes*
	Type C	*No*
	Type D	*No, this virus uses the host RNA polymerase to express the viral genes*
Treatment	**Virus**	**Virus formed (*Yes/ No*)?**
Anisomycin	Type A	*No*
	Type B	*No*
	Type C	*No*
	Type D	*No*

d) Which of the above virus(s) (*Type A/ B/ C/ D*) **must integrate** its genome in the host cell? Give all possible options and explain why you selected each.

Type C. This virus brings in its own reverse transcriptase at the time of infection. This reverse transcriptase is used to make a cDNA copy of the viral genome, which gets integrated into the host genome and stays as a provirus.

Question 2
The discovery that cancer could be caused by a virus was a major one. However, the subsequent discovery that Rous Sarcoma Virus (RSV), a cancer-causing virus discovered in chickens, encoded a mutant form (v-src) of a normal cellular gene (c-src) was even more surprising. Rous sarcoma virus (RSV) is a retrovirus that also has a + stranded RNA genome that encodes four genes; gag (encodes the capsid protein), pol (encodes the reverse transcriptase), env (encodes the envelope glycoprotein) and src (encodes a tyrosine kinase enzyme).

a) Given the information, reverse transcriptase is considered which of the following?

- A DNA directed RNA polymerase
- **A RNA directed DNA polymerase**
- A RNA directed RNA polymerase

b) Why is it essential that the RSV encodes Reverse transcriptase?
This enzyme is used to make a cDNA copy of the viral genome that can integrate into the genome of the host cell. The host cell will not have an viral RNA directed RNA polymerase enzyme that can carry out these functions. So the virus needs to bring it during infection.

c) What are two major classes of genes involved in the development of cancer? For each, describe the type of mutation that is associated with cancer, and how this mutation would promote tumor formation.
Oncogenes: Mutations that activate or maintain growth/ division promoting properties of a protein. Mutations that increase expression of a growth/division promoting proteins.
Tumor Suppressors: Mutations that inactivate proteins that restrict cell growth/division. Mutations that prevent expression of proteins that restrict cell growth/division.

d) The Human papilloma virus (HPV) has been implicated as a risk factor for cervical cancer. The E7 protein of HPV binds to pRB protein preventing it from binding to the host transcription factor E2F which is now free to bind to the promoters of genes that promote cell cycle. In contrast, another HPV protein, namely E6 binds to p53 targeting it for destruction by proteosomes thus removing the block on the host cell's entry into the cell cycle.

i. Would you classify E7 as an oncogene or a tumor suppressor gene? **Explain** why?
It is an oncogene since it binds to and inhibits the function of the RB tumor suppressor gene product.

ii. Would you classify E6 as an oncogene or a tumor suppressor gene? **Explain** why?
It is an oncogene since it binds to and inhibits the function of the p53 tumor suppressor gene product.

Question 2 continued

e) Each of the five genes given below, when mutated, can result in a transformed phenotype in the mutant cells. In the final column, give the phenotype (*normal or transformed*) of a diploid cell that has the two alleles given. ***Note:*** *A description of each gene is given.*

ras: encodes a protein, which is active in its GTP bound form and inactive in its GDP bound form. When active it promotes cell division.
cyclin D: encodes a protein that interacts with a CDK (cyclin dependent kinase), and promotes cell division.
erb-B2: encodes an epidermal growth factor receptor which is active when dimerized. It promotes cell division when activated.
p16: encodes a protein that inhibits cyclin-dependent kinase.
WT1: encodes a protein that inhibits progression through the cell cycle.

Gene	Class	Status of allele 1	Status of allele 2	Phenotype
ras	Proto-oncogene	Mutation such that protein cannot hydrolyze GTP to GDP	Wild-type	*transformed*
Cyclin D	Proto-oncogene	Mutation that results in deletion of entire gene	Wild-type	*Normal*
erb-B2	Proto-oncogene	Mutation such that the receptor protein constitutively dimerizes	Mutation that results in the deletion of 120 base pairs in intron 5	*Transformed*
p16	Tumor suppressor	Point mutation that results in truncated protein of 20 amino acids	Wild-type	*Normal*
WT1	Tumor suppressor	Mutation in promoter that prevents RNA polymerase from binding	Mutation that results in the deletion of 4 base pairs in the coding region 20 base pairs after the start codon	*Transformed*

Question 3

Cancer is caused by the accumulation of two or more mutations in the same cell that affects its proliferation and survival.

a) Why does a person's chance of having cancer increase with age?
Cancer is a multi- step process that involves accumulation of many mutations in both tumor suppressor genes and oncogenes ***in the same cell.*** *These mutations accumulate throughout the life of a cell, either spontaneously or by exposure to carcinogens, by replication mistakes or by chromosomal translocations. Since the chances of accumulation of these mutations increase over time, the chances of an individual having cancer also increases with age.*

b) Cell lines are often used to test the oncogenic potential of viruses. If cancer is a multi-step process, why can the introduction of a single active viral oncogene transform these cells?
Unlike the normal cells, the cell lines are immortal i.e. they can divide indefinitely and they are made so through the accumulation of mutations. Therefore the introduction of a single viral oncogene into a cell line essentially reflects the addition of one more mutation to a series of mutations that were preexisting in the cell line. Hence the transformation of a cell line by adding a single active viral oncogene does not contradict the statement that "cancer is a multi- step process".

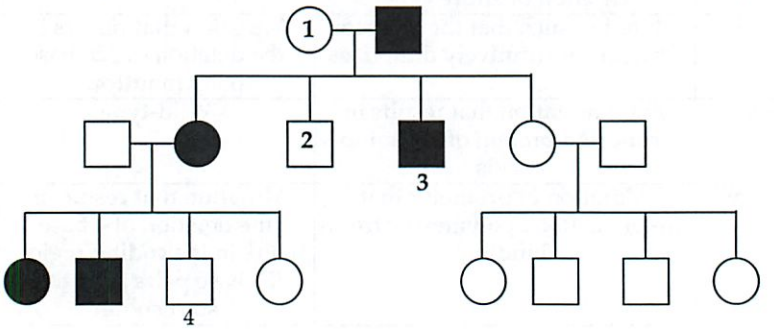
Question 3 continued

d) Briefly describe what an Ames test is and how it may be used to evaluate the mutagenic potential of a chemical agent. Do you think you can evaluate the mutagenic potential of any carcinogen using Ames test (*Yes/ No*)? Justify your answer.

The test employs a specific histidine minus (his-) strain of bacteria. A mutation in a gene(s) required for synthesis of the amino acid histidine prevents this strain from growing on solid media that lacks histidine. However, a compensatory mutation in this gene(s) may cause the His- bacterial strain to "revert" to a his+ strain that can grow on a medium lacking histidine. In Ames test you incubate the His- bacteria suspension with the test compound for a specified time interval. You then plate the bacteria on a petri-plate that contains solid culture medium lacking histidine. Only those bacteria that have undergone reversion from his- to his+ will grow and form colonies. The number of His+ colonies formed represents the mutagenic potential of the test compound. This test will not be able to pick up those cancer causing compounds that are non- mutagenic in their native state but are converted to mutagenic intermediates by different enzymes in the body. Such compounds are often called pro-mutagens and remain undetected by standard Ames test.

Question 4

The following human pedigree shows the mode of inheritance of the predisposition to colon cancer.
Please note: *The shaded squares and circles represent the individuals who eventually develop cancer.*



a) Looking only at the pedigree, the predisposition to this disease appears to have what mode of inheritance?

Autosomal dominant

b) If you check the genotype of the tumor cells from individual 3, you find that they are homozygous for the disease allele (-/-). However, if you check the genotype of the blood cells from this individual, you find that they are heterozygous for the disease allele (+/-). **Explain** why the genotype with respect to the disease allele in the blood cells is different from that in the tumor cells isolated from individual 3.

This indicates that this cancer results from the loss of both alleles of a tumor suppressor gene, not the acquisition of an oncogene. Thus the apparent autosomal dominant mode of inheritance represents the inheritance of ***predisposition*** *to this type of cancer. The cancer phenotype is correlated with being a carrier of the disease allele as the chance of a subsequent mutation in the good copy of the disease allele is very high. The retinal cells have undergone a loss-of heterozygosity (LOH) of the tumor suppressor gene and therefore they show the development of cancer. In comparison, the blood cells are still having one functional copy of the Rb gene and hence are heterozygous.*

c) For individual 4, the blood samples are heterozygous, carrying both the wild-type allele and a mutant allele of the gene associated with this type of cancer. However, this individual **did not develop** cancer. **Explain** why.

This individual was lucky enough and did not have the 2nd mutation that would have resulted in the LOH of the tumor suppressor gene and therefore the development of cancer. In other words it means that the pedigree is showing incomplete penetrance. This individual may get the disease later.

Question 4 continued

d) One example of a tumor suppressor gene is Retinoblastoma (Rb) gene . The wild-type pRB protein binds and inhibits the activity of the transcription factor E2F. At the appropriate time in the cell cycle, pRB is phosphorylated (inactive state) and E2F becomes available to act as a transcription factor that stimulates cell division. If a cell has lost the functional pRB and E2F proteins, would you expect cell division (*Yes/ No*)? **Explain** your choice.

pRB inhibits cell division by binding and inactivating E2F which otherwise promotes cell division. Therefore a loss of function of both pRB and E2F in the same cell would not increase cell division and hence would not lead to tumor growth.

Question 5

a) Radiation therapy can be used to treat tumors. Briefly **explain** how radiation therapy works to treat a tumor.

Radiation therapy works by massively damaging the DNA of the rapidly dividing cancer cells. With extensive DNA damage, cells will often initiate a pathway for apoptosis or programmed cell death.

b) Chemotherapeutic drugs often have side effects such as diarrhea, constipation, mouth sores, hair loss, nausea, and blood-related side effects.

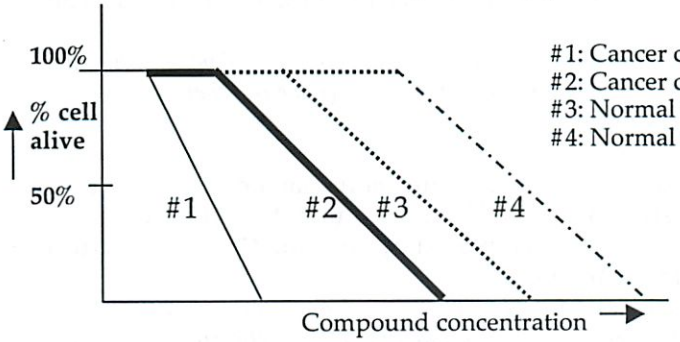
i. Chemotherapeutic drugs have a wide range of structures and functions, yet many elicit the same side effects. **Explain** why the side effects are the same for a variety of different drugs.

Most of these chemotherapeutic agents target the cancer cells since they are actively dividing cells compared to the normal cells. However, normal cells that are actively dividing, such as hair cells, blood cells and gut cells, are also targeted by these treatments resulting in hair loss and nausea. Therefore, they all result in very similar side-effects.

ii. Describe what is meant by the "therapeutic window" of a drug used in chemotherapy, and how it relates to the side effects seen in a patient.

The therapeutic window is a measure of the difference between the concentration of a drug that is required to kill the cancer cells (effective dose) and the concentration of the drug that affects normal cells. A drug with a wider therapeutic window will have fewer side effects at the effective dose.

i. Prior to being used for treatment, each chemotherapeutic drug is extensively screened. During drug screening you identify two compounds A and B that have the potential to kill cancer cells and normal cells as shown by the following graph. Which compound (*A/B*) is a better candidate for cancer treatment? **Explain** why you selected this option.



#1: Cancer cells + Compound A
#2: Cancer cells + Compound B
#3: Normal cells +Compound B
#4: Normal cells + Compound A

Compound A will be a better choice since it has a larger therapeutic index i.e. The effective concentration of compound A that is required to kill the cancer cells is far less compared to the concentration needed to kill the normal cells.

ii. **Explain** how the use of following drugs may prevent cancer cell growth and / or cell proliferation.

Drug	Target of drug	How is cancer cell growth and / or proliferation prevented?
Vincristine	Microtubule inhibitor	*Prevents the formation of mitotic spindle and hence inhibits cell proliferation*
VEGF inhibitor	Inhibits blood vessel formation	*Prevents supply of nutrients and removal of waste thus contributive to cell death.*

Question 5 continued

c) Her-2 receptor is encoded by the Her-2 proto-oncogene and is a member of the epidermal growth factor (EGF) family of receptor tyrosine kinases. Her-2 gene amplification is correlated with aggressive forms of breast cancer that respond better to treatment with herceptin than other non-aggressive forms of breast cancer. **Explain** why this is so.

Some less aggressive forms of breast cancer are not associated with an overexpression of the Her-2 gene, so they are not sensitive to Herceptin. Often the aggressive forms of breast cancer are associated with an amplification of the Her-2 gene. The amplification of the Her-2 gene correlates with the increased expression of receptor on cell surface, which increases the proliferation signal that is critical for tumor development. Herceptin is a monoclonal antibody that works on both the extracellular and the intracellular domains of the HER-2 receptor. It does so by binding to the Her-2 receptors that are expressed on the surface of cancerous cells thereby flagging these cells for destruction by the immune system. At the same time Her-2 blocks the downstream signaling by the Her-2 receptors thereby preventing tumor proliferation.

Question 6 (This question is optional and will NOT be graded)

Human immunodeficiency virus (HIV) is a retrovirus. Its genome is a single (+) stranded RNA that is packaged with the reverse transcriptase enzyme within a protein capsid. This is further packaged into an envelope that is derived from the plasma membrane of the host cell in which the virus had replicated. The surface of the envelope is covered with the envelope glycoprotein, called gp120.

a) HIV specifically infects the T- helper (T_H) cells of the human immune system. If HIV enters the host cell by means of host receptor recognizing a viral protein, what would be the most likely interacting proteins during HIV infection?

The gp120 protein on the surface of HIV envelop binds to the CD4 receptor on the surface of T helper cells and this ligand-receptor binding event is the first step of infection. However an additional interaction between the gp41 protein of HIV and chemokine receptor (CCR 4 or 5) on the surface of host cell is needed for the virus to enter the host cell.

b) Why do HIV infected cells remain undetected by the host immune system for several years?

The viral genome gets converted into cDNA that integrates into the host genome and stays as a provirus. In this dormant form it is not detected by the immune system.

c) Some individuals are resistant to HIV infection even after repeated exposure. Assuming that these individuals express a normal level of the functional receptor that you have recognized above, how can you explain their resistance to HIV?

The gp41 protein on the surface of virus binds to a chemokine receptor (CCR) on the surface of T helper cells. If a person shows a homozygous mutation for the CCR gene (CCR-/ CCR-) he/she will not have the chemokine receptor and will not contract AIDS even after repeated exposure to HIV.

d) In recent years, therapies have been developed to fight AIDS using nucleotide analogs. The drug used to combat AIDS is Azidothymine (AZT). The structure of AZT is very similar to thymidine. However, in AZT the 3'-OH group on the deoxyribose sugar is replaced by an azido (N_3) group. Which process of the life cycle of HIV do you think is inhibited by AZT?

AZT is a thymidine analogue (a nucleotide used in the synthesis of DNA. Therefore AZT interferes with the synthesis of DNA from RNA by reverse transcriptase. This enzyme incorporates AZT more effectively into the growing DNA chain and this blocks the further elongation of the chain because the growing end has no 3'-OH group on the deoxyribose sugar. So the viral concentration decreases over time with response to the treatment.

7.012 Recitation 19 - 2012

Summary of Lecture 31 & 32:

Cancer: This is a disease of old age. This is caused by the accumulation of multiple mutations over time in a cell. Most of these mutations are the somatic mutations and hence not passed by the affected person to the next generation. But there are some familial forms of cancers too.

Tumor suppressors genes and proto- oncogenes: These are normal genes that work in a regulated fashion in a normal cell to properly control the cell cycle. The wild-type function of a tumor suppressor gene is to inhibit the cell cycle in any cell that is not supposed to be actively growing and dividing. Both homologous versions of a tumor suppressor gene must loose their function to transform a normal cell to a cancerous type. The wild-type function of an oncogene is to promote the cell cycle in any cell that is supposed to be actively growing and dividing. One of the two homologous versions of an oncogene must gain a function or increase its function for a cell to become cancerous. Normal cellular counterparts of the oncogenes are called the proto- oncogenes. Some of these genes are carried by oncogenic viruses and are designated as v-oncogenes. The v– oncogenes can be linked to potent promoters that lead to their inappropriate and high level expression, leading to deregulated cell division. One example is the Rous sarcoma virus (RSV). This retrovirus infects the chickens, thereby causing them to acquire tumors. Here the viral genome contains a gene that it has stolen at some point from a host cell. This gene is an oncogene called src that is involved in cell signaling. The virus carries a mutant version of src that produces an overactive form of the normal cellular kinase src. When RSV infects a cell, the mutant src is transcribed and translated, creating an overactive cell signaling protein that promotes growth and division in chicken cells to form tumors. Other examples include the avian leukemia virus that causes leukemia and human papilloma virus responsible for cervical cancer.

Retinoblastoma: This is a cancer of the retina. In Familial retinoblastoma, multiple tumors in the retinas of both eyes occur in the first weeks of infancy when the fetus inherits from one of its parents a chromosome that has its RB locus deleted or otherwise mutated. So in this form of the disease, a germline mutation plus a somatic mutation of the second allele leads to the disease. In sporadic retinoblastoma a single tumor appears in one eye sometime in early childhood before the retina is fully developed and mitosis in it ceases. In this form, both inherited RB genes are normal and a single cell must be so unlucky as to suffer a somatic mutation (often a deletion) in both in order to develop into a tumor. Such a double hit is an exceedingly improbable event, and so only rarely will such a tumor occur.

Cancer therapy: Most cancer patients are treated with some combination of surgery, radiation, chemotherapy or immunotherapy. Radiation and chemotherapy have the disadvantage of destroying healthy as well as malignant cells and thus can cause severe side- effects. Drug design is a very expensive process. Drugs must be specific (i.e. they can't inhibit other proteins in addition to their targets or else they will cause side effects), must work at a low concentration (so that the amount that needs to be taken by the patient is feasible), and must not be metabolized by the patient either too quickly (so that taking the drug is ineffectual), too slowly, or into toxic byproducts.

Questions:

1. Why is cancer predominantly a disease of old age? What about the usual cause of cancer makes it more common in older people?

2. Why are the mutations that cause cancer generally not passed on to one's offspring? Why are almost all cancers sporadic as opposed to familial/inherited?)

3. Describe the basic principal behind the standard Ames test. How is this different from modified Ames test?

4. What does it mean that a tumor is almost always clonal (or monoclonal)?

5. Why is it that cancer cells containing mutations in genes encoding ECM (extracellular matrix) proteins are more dangerous than cancer cells with normal ECM genes?

6. Weinberg's famous experiment: Ras was the first oncogene to be discovered. Ras is part of a cell signaling pathway. The input for this pathway is an extracellular protein growth factor, and the output is to induce transcription of genes necessary for the cell cycle to occur. Ras is a GTPase that is active in the GTP-bound form but inactive in the GDP-bound form. Ras was discovered in the Weinberg lab via the following experiment. Human tumor DNA was cut into pieces, and each different piece was put into a different mouse cell. The mouse cells were then grown in Petri plates. Only the mouse cell that took up the mutant allele of the oncogene could grow and divide enough to form a colony of cells.

i. Do you think that the mouse cells had their own versions of Ras before the experiment began? If yes, do you think that the mouse versions of Ras were wild-type or mutant?

i. In this experiment, it seems that there was only one mutation necessary to make the mouse cells over-proliferate. We know, however, that cancer results from an accumulation of mutations. Why then did this experiment work?

ii. If a patient had a tumor that was caused in part by mutations in Ras, do you think it would be a good therapeutic decision to treat the cancer patient with a drug that targets and inhibits Ras?

iii. Do you think it would be a good therapeutic decision to provide this cancer patient with a wild-type copy of the Ras gene?

iv. Do you think that this experimental technique would work to identify tumor suppressor genes? Why or why not?

7. Retinoblastoma is caused by a mutation in the retinoblastoma tumor suppressor gene. There are several mechanisms, which can cause a cell to lose its normal gene and thus be predisposed to develop into a tumor. These may result in a "loss of heterozygosity" or "LOH".

i. What do you mean by LOH?

ii. Many clinicians and scientists are currently trying to develop cancer treatments that are more specific and targeted than chemotherapy. If a patient had a tumor that was caused in part by mutations in Rb do you think it would be a good therapeutic decision to provide this cancer patient with a wild-type copy of the Rb gene?

8. Consider a patient who has CML, and answer the following questions.

i. Would the Philadelphia chromosomal translocation be present in all of the cells in the patient's body?

ii. Would the Philadelphia chromosomal translocation be present in all of the cells in the patient's blood system?

iii. How many independent times did the Philadelphia chromosomal translocation occur in the patient?

iv. Could the patient pass CML onto his/her kids?

9. For each of the following medicines/treatments, answer these three questions:
i. Which disease/condition does this treatment work for?
ii. What is the target of this treatment?
iii. Why does this treatment work?
- Herceptin (an antibody directed against the Her2 growth factor receptor expressed in breast cells):
- Gleevec:

7.012 Recitation

12/6

Antiviruses

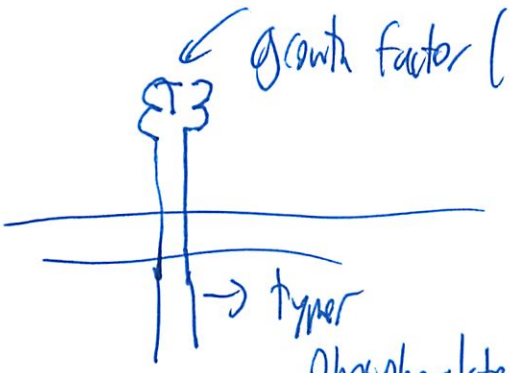
Cancer Viruses

RSV - Rous Sarcoma Virus

ALV ← typical

RSV

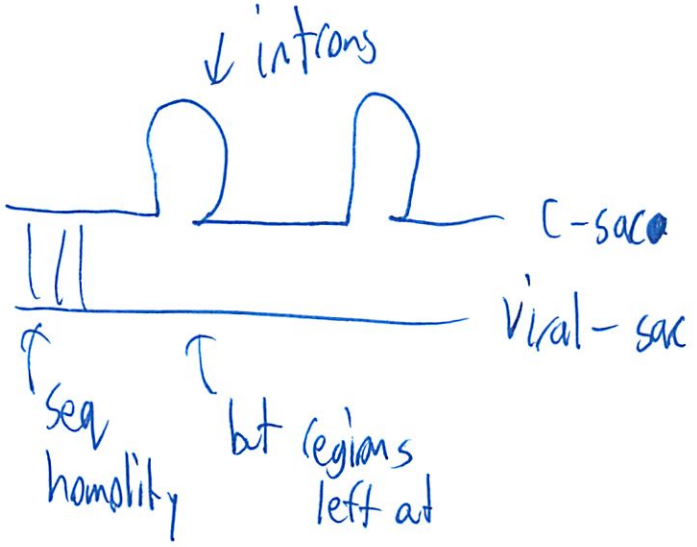
Src - (eceptor tyricine kinases



phosphorylate each other

Should only happen in presence of growth factor but viral Src just tyricine expression

(2)



So when virus infected gene, it inserted its genome. It already had a viral mutation that virus inserted before at just the right place

So 2 ind random probabilites both must happen

1. mutation C-src → v-src
2. ~~Knocks~~ virus inserts at just the right place

Allows virus to ~~[illegible]~~ have children
Since in DNA of host cell

(3)

HPV

Causes cervical cancer in women

E7 ⊣ pRB

E6 ⊣ p53

↑ both tumor-suppressor protiens

↑ both oncogees

Is now a vaccine for.

Ras

Bob Wineberg found
1st human Oncogene

1st non-viral/non-infectias cancer
immortal cell line - just keep dividing

(4)

Telomerase - extends DNA

~~Won't~~ divide goes ↓ as ya age

Cells won't divide since lose DNA

So ya turn on telomerase

Some cancer cells do it

So these cells will divide ∞ as long as nutrients + space

↳ transformed cells

Cells should stop growing when touch each other

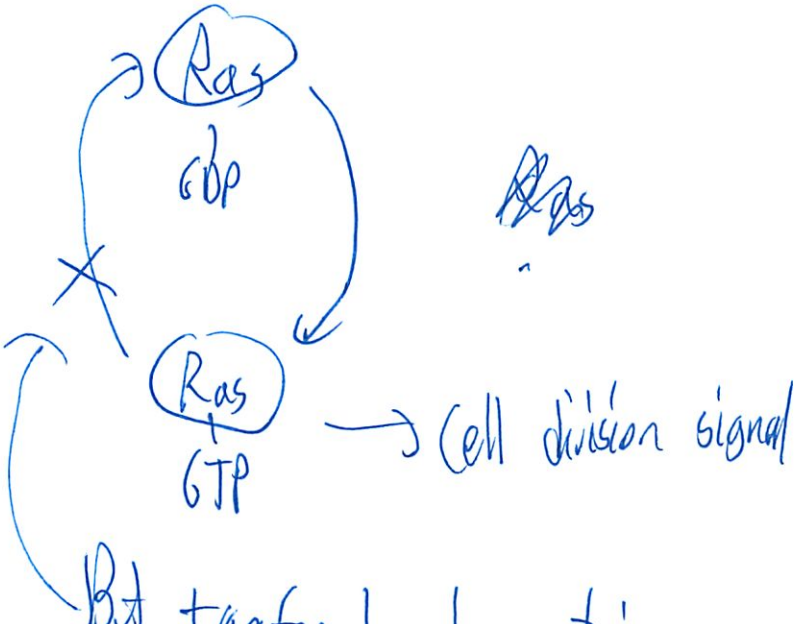
(5)

Bob cut up parts of Bladder Cancer
See what part leads to mutation / transform cells
Then see what is in that part / the mutation

G-proteins

Ras is one

Normally GDP → GTP → GDP
(active = sends signals for cell division



But transformed loses this
Can't become inactive in the future
Is a mutant Ras - but mutation only shows up at certain time

(6)

Retinal Blastoma

familial – already have 1 mutated copy
Sporadic – late in adult life

pRB is tumor suppression gene

†pRB †pRB

Need 2 events – ind. in each eye

1. Lose a pRB †⊖ †pRB
2. Must lose other pRB †⊖ †⊖

Takes a long time to get 2 events

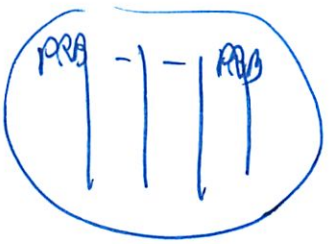
But familial # 1 happens from genes
So only need #2
Often occurs as child since lots of cell division there

Each eye is ind

So familial - commonly in both eyes

but sporadic - eyes somewhat ind

↑ Since mitotic recombination



↑ not in class - too complex
actually common to get

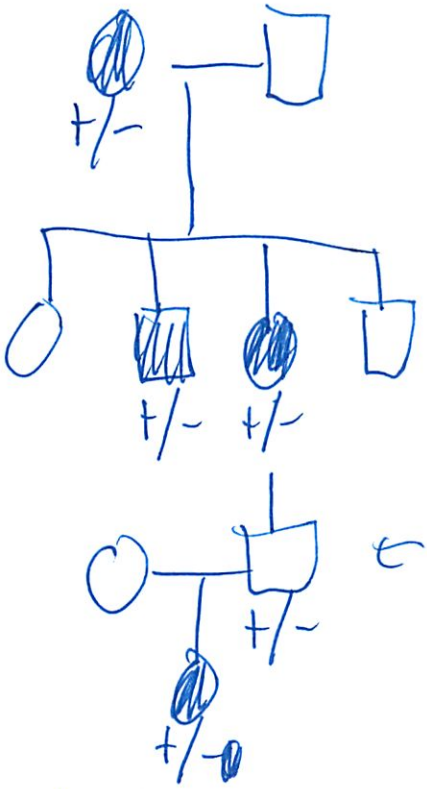
So if get familial 2nd mutation likely

But still happens ind. in each eye

(8)

These cancers show up as autosomal dominant

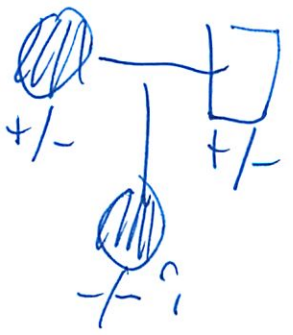
What's up w/ the family trees?



← mutation didn't happen (mitotic recomb)
Could still get it later

Why do people get it even though heterozygous
Since mutation - (mitotic recombination)

But could you have



pRB is essential for dev
So the fetus will abort
baby will die

(9)

Same as w/ Ras

Both Appears autosomal dominant

But, always autosomal recessive

tumor suppression

↑ need both copies lost to have it

CML

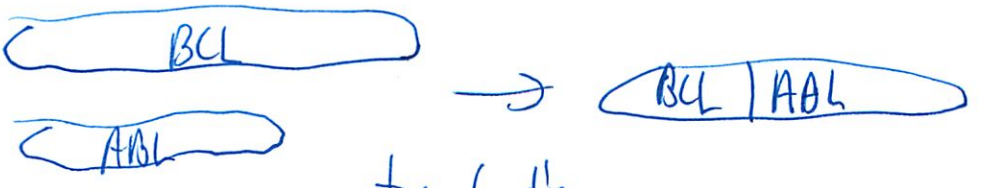
B-cell cancer

Later in life

Rel. easy to cure

But it is no longer a single mutation

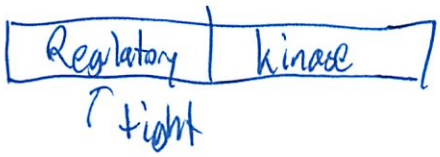
2 chromosome



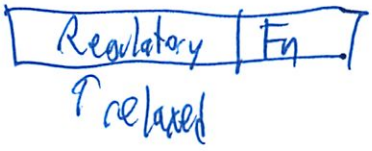
translocation

(10)

ABL has 2 domains



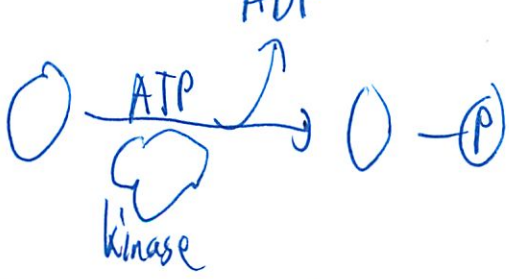
BCL also



So combo

Since we lose strict regulatory region
this combo sends lots of growth signals
So get cell division / cancer!

Has good drug: Gleevac



Gleevac binds to ATP kinase receptor

(10)

So inhibits ATP binding
it can no longer phosphorilate
So our picture does not happen

Therapies for Cancer

- Radiation
- Chemo - broad/non specific - any dividing cell
- Surgery - still biggest/most sucessful
  - remove majority of mass
- gene therepy
- small moleules + immunotherepy
  ie bleevec, Herceptin

When pulled apart in mitosis

So cell stops dividing
Very expensive since from rare plant
Can we artificially synthaze it?

(12)

- Radiation
  - damages DNA
  - forces cells to die

- All of these must do pretty early in life

- Gene Therapy -
  - replace copy of Tumor Suppression gene
  - but how do ya get delivery to work
  - still very much R+D

Diff cancers spread to diff parts

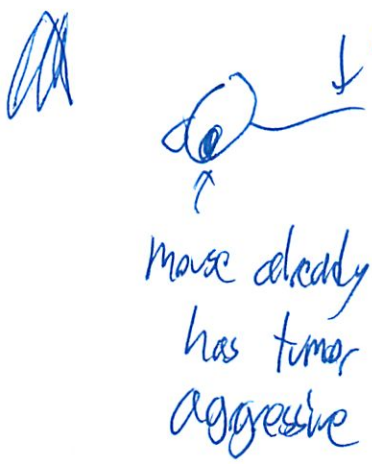
Breast cancer

↳ brain, lung, bone

So have preferred destination

Could go anywhere theoretically

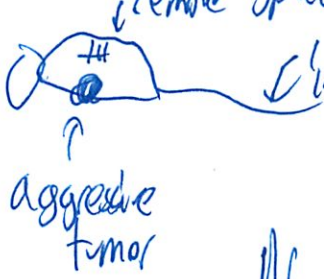
(13)



So then get lots of cancer in the lung

No cancer in lung

But



No cancer again!

So what is it w/ spleen cell?
We don't know!

Cancer is a systemic disease

(14)

Also tumor carrying mice have huge spleen

So what is it? We don't know

Bio 12/7

L34 Prions

(Video on 12/9)

Divia

Do subject eval

(Is she reading off a script)

Features of Immune System

1. Self vs non-self (foreign) ← good at selecting + recognizing
2. Memory ← vaccine or 2nd exposure provide life long immunity
3. Specificity ← certain viruses
4. Antigen (anything foreign)

- Proteins
- DNA

Live infectious pathogens
- bacteria
- Viruses
- fungi
- parasites

Can infect cells that they recognize
Use host cell machinery to replicate

(2)

Prions

A 3rd class of infectious pathogens
- Unprecidented/unique

Cause invariable fatal brain disease
diff names in each group
Can spread between species
- Zoonotic transmission

So very dangerous

All appear similar
- spongy mass
- protien aggregates

Called Transmissible spongiform encephalopathy (TSE)

Sheep → lose fur
bloody lesion

(3)

Mad Cow Disease / Bovine Spongiform Disease

Scrapy → Sheep

Kuru → Humans

Creutzfeld-Jacob Disease

(easy to understand since she moves very slowly)

Was on 24

In humans

1958 - Alps + Gadjusek

Fore tribe → New Papua Guinea, Australia

- in children
- shivering
- uncontrolled laughing
- dimentia

but since they had ritual cannibalism it passed

(1)

Significant ↓ when they stopped that

Thats why called Mvv

Found that when injected to Chimps
Saw they died in 2 years
So saw a slow growing virus
But key observation was the inter species spread

Stanly Prusner

1. Started by taking brain tissue from Scrapy Sheep
2. Infected normal hamsters
3. In a few months they subcured
4. Same w mice
   - used as experimental model

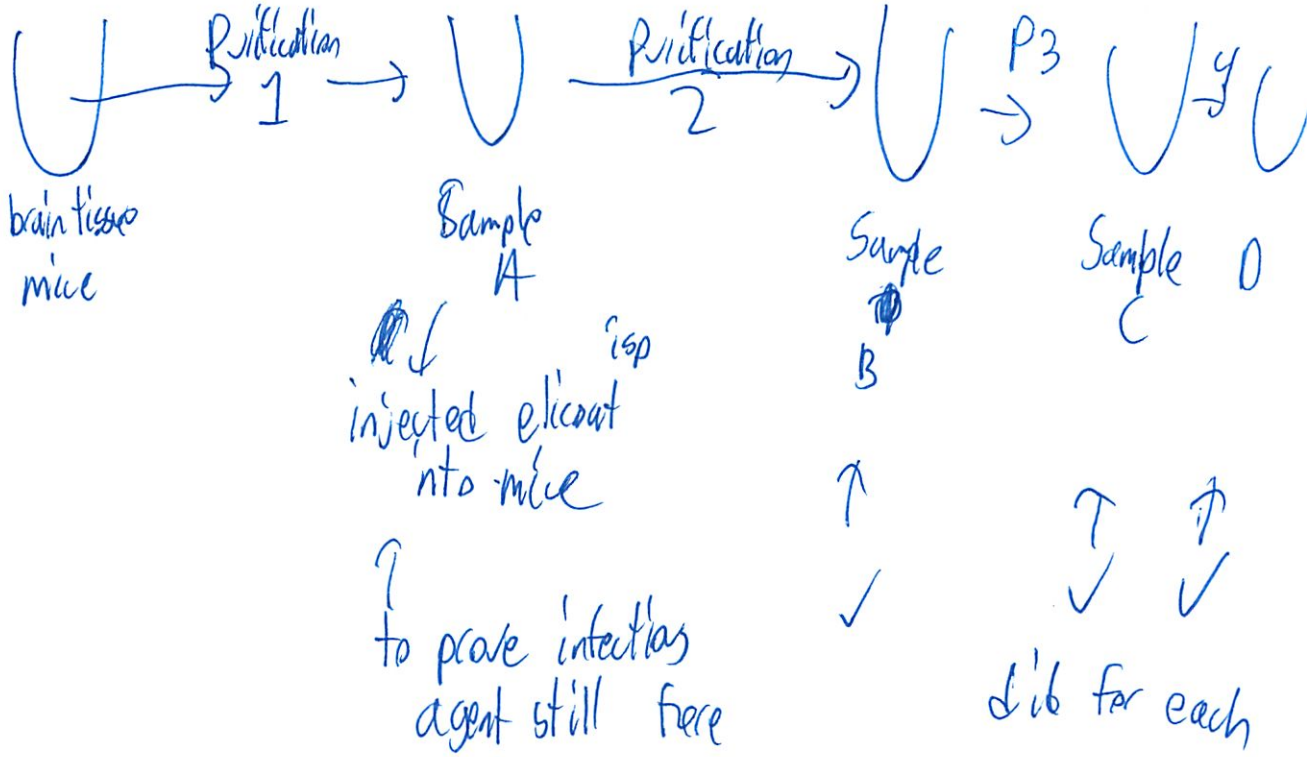
(5)

Found spreads b/w same species → faster
and b/w species (slower)

All show same histopathology (sponge form appearance)

2.

brain tissue from mice
multiple rounds of purification



So infectious agent present in all samples

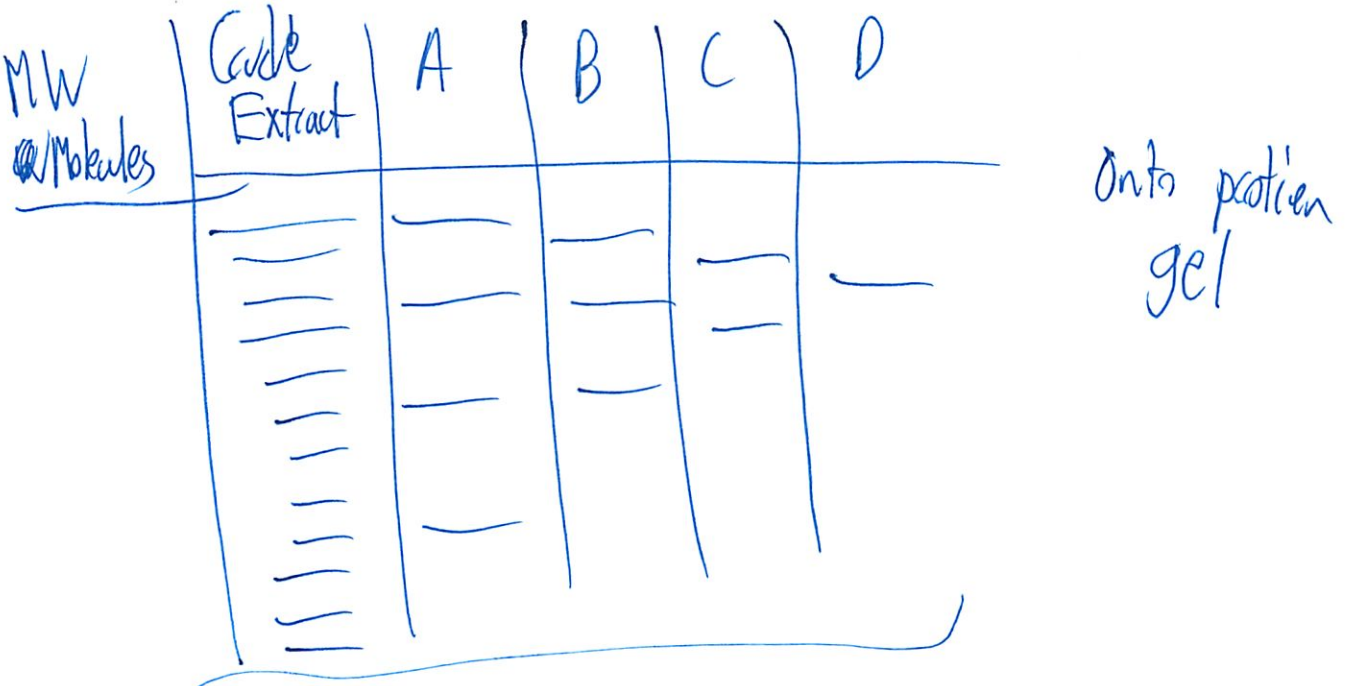
(6)

The more the sample was purified, the more infectious it was

So infectious ability ↑ w/ each step of purification

Found no traces of nucleic ~~acid~~ acid

Is it a protein?



Principle is same as gel electrophoresis
higher mol weight migrate slower

↑ lots of proteins ↑ decreased a great deal . ↑ just 1 band

(7)

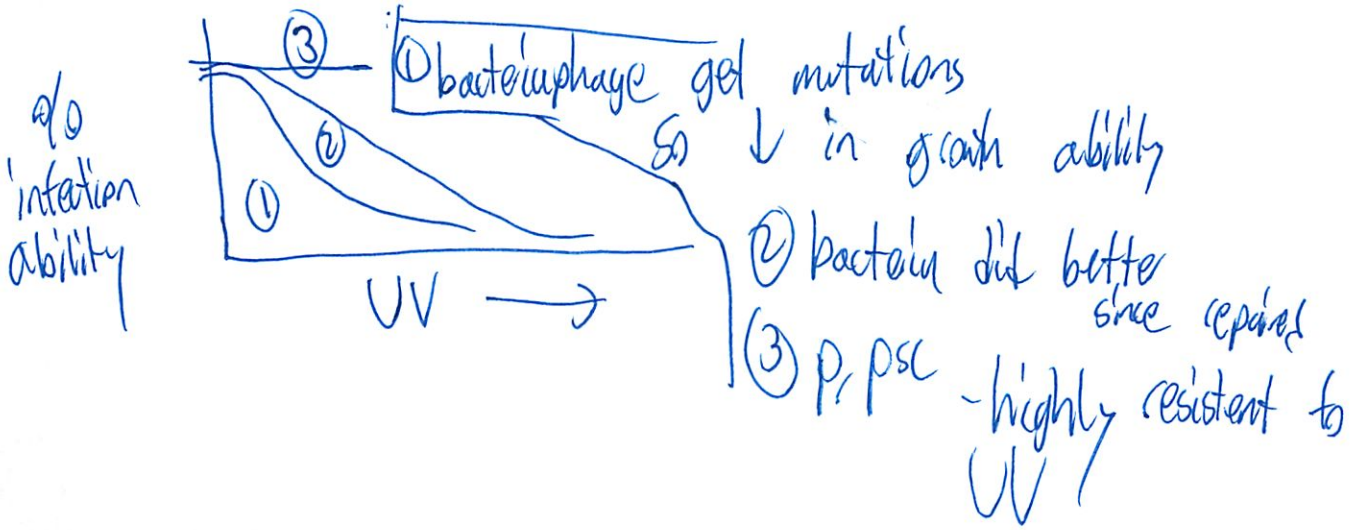
Was a protein
called $P_r P^{sc}$

not nucleic acid
not living putagens

3rd set of experiments

took $P_r P^{sc}$ and bacteriophages
↖ viruses that infect bacteria
↖ no repair mechanisms

bacteria
↑ follow central dogma of bio
do have repair mechanisms



% infection ability

① bacteriophage get mutations so ↓ in growth ability
② bacteria did better since repairs
③ $P_r P^{sc}$ - highly resistant to UV

(9)

Experiment #4

eluted the protein

↓

Sequenced it

↓

primary amino acid

↓

normal cell protien

Pr^{PC}

identical amino acid seq

but same gene Pr^{NP}

but diff conformations

When exposed to protiase

└ break down proteins as ↑ concentration

Pr^{PC} is sensative to protiase

Pr^{SC} is <u>not</u> sensative

(9)

Identical primary structure and encoded by same gene

but PrP^{Sc} is UV+ protease resistance

Since PrP^{C} is α helix

PrP^{Sc} is β helix

Experiment #5

PrP^{Sc} into mouse

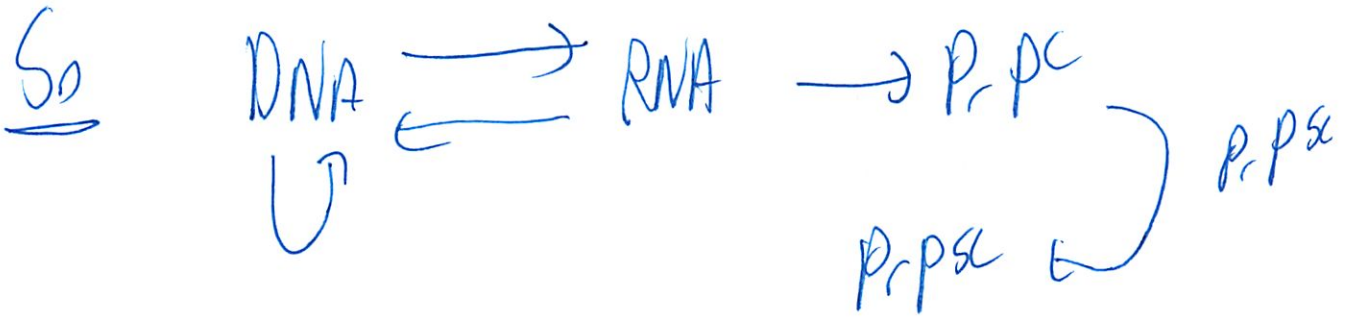
When inject X amt

Can recover 1000x amts

so infectious agent

It is replicating w/o nucleic acid

(11)

So 

Infected tissue having accumulation of PrPSC

PrPSC is

PrPSC is binding to + altering the Concentration of PrPC and turning it to PrPSC

L Prion Hypothesis

No one accepted this at first but was right at end of day

(11)

Diff ways for it to spread

- cirtistic canabalism
- through hospital probes
  heat and formaldihide don't kill $Pr P^{sc}$!
- appear spontaneously

Are humans that are poly immune from it
  L have mutation in PrPNP
  Some disease resistant feder

Is a PrP knock-out mice
  Injected $Pr P^{sc}$ will not cause a disease

Prions have benefital use
  naturally expressed in certain yeast
  not killing cuiver cell
  esp if in non-fungal agent
  can activate other non-dormant gene, help gene survive & pass on traits

(17)

if remove prions → yeast dies

[redacted]

Season 9 of 24

Jack Bower has Prion disease

how do we save him?

1. So find donor in acquired prion disease resistant factor

I think → PrC

2. Find compatible donor that is $Pr.P^{C-}/Pr.P^{C-}$ and use their cells for gene therapy

7.012 Lecture 34
12/7/12
PRIONS

By

Diviya Sinha

Email: dsinha@mit.edu

Building 68-120c

Online subject evaluations are now open

http://web.mit.edu/subjectevaluation

- You have until **Monday, Dec 17 at 9 AM.**
- Please evaluate **ALL subjects** in your list.
- Please write comments.

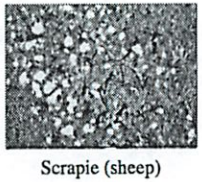
Your feedback is read and valued!

PRIONS (PrP^{Sc})- infectious pathogens

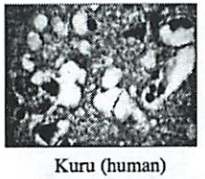
1. They cause **brain/ neurodegenerative diseases** in multiple species with similar histopathology.

2. These diseases can spread between individuals of the same species and also from one species to other.

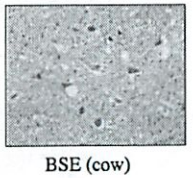
3. These diseases are classified as transmissible spongiform encephalopathy or **TSE** and **they are all fatal.**



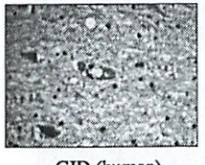
Scrapie (sheep)



Kuru (human)



BSE (cow)

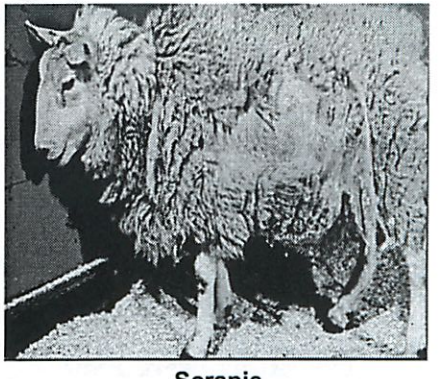


CJD (human)

Figure shows similar neuropathologies in TSE of different species

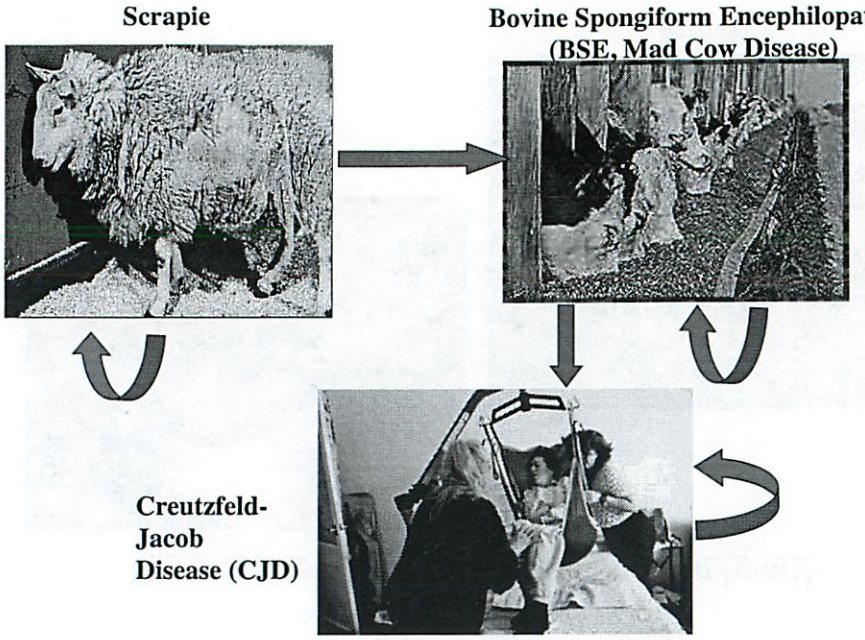
TSE and their implications for Human health

BSE or mad cow disease



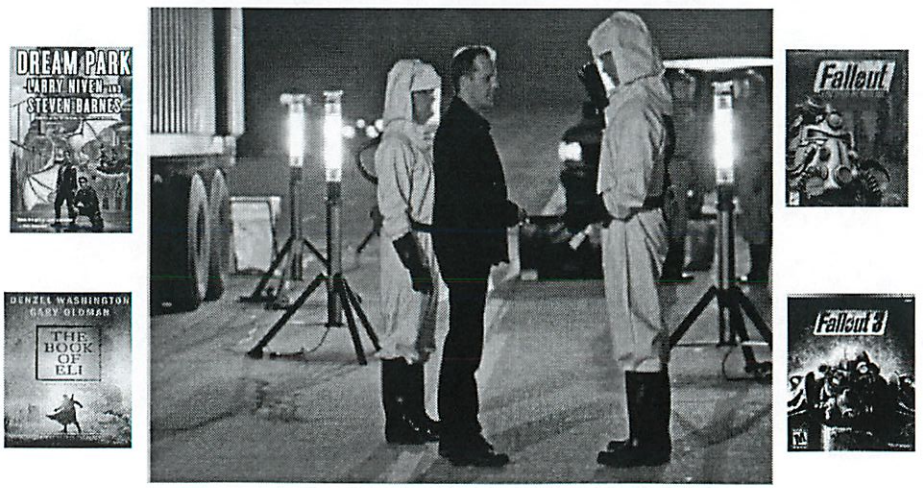
Scrapie

Transmissible Spongiform Encephalopathies



Discovery of TSE

Reflection of prion diseases in media



1. Kuru discovered in **1950** in the **Fore tribe of Papua New Guinea.**

Symptoms: The affected individual showed violent shivering, uncontrolled laughing sickness, loss of coordinated movement, dementia followed by death.

Cause: ritualistic cannibalism

Researched further by **Gajdusek & Bloomberg** who showed its interspecies transfer and were awarded the **Nobel Prize in 1976.**

Causative agent: The disease was thought to be caused by a **slow growing virus** that could cross species barrier.

Results from Stanley Prusiner's experiment

1. Prions spread between members of the same species (faster) and also from species to species (slower). Prion **pathogens reside in the brain tissue** of the affected species. All affected species show the same histopathology.

2. Prions are **not live** pathogens. They are **are resistant to UV treatment** unlike conventional pathogens. So they are no nucleic acids. Instead they are **infectious protein** particles (PrP^{Sc}).

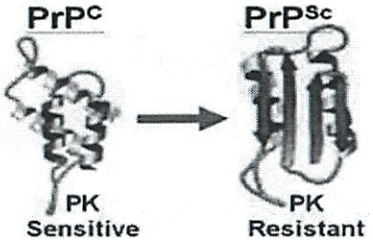
3. Comparing PrP^{SC} with PrP^{C}

1. Both PrP^{SC} and PrP^{C} have **identical primary structure.**

2. They are **encoded by the same gene, PrNP.**

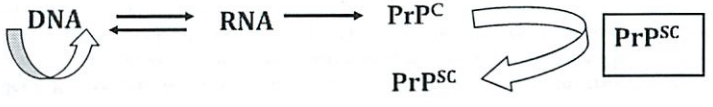
3. The **PrP^{SC} is UV and protease resistant and represents a highly stable pathogenic form** unlike its normal cellular counterpart.

4. They both have **different conformations.**

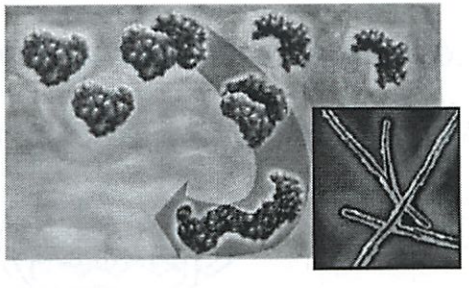


4. Replication of PrP^{Sc}:

Prions (PrP^{SC}) can **replicate within the host cell to increase their number** by binding to and altering the conformation of PrP^{c}.



Infected tissue showing accumulation of PrP^{Sc}



The Prion Hypothesis (Stanley Prusiner)

Scrapie and other TSEs are caused by a protein-only infections agent (a "prion"). The disease-causing protein is an altered form of a cellular protein, which can cause the cellular protein to adopt the altered conformation. In this way, the prion can "replicate."

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Volume 117 >> Issue 48 : Tuesday, October 7, 1997 PDF of This Issue

U.S. Neurologist Wins Nobel Prize for Discovery of Prions

By Rick Weiss
The Washington Post
WASHINGTON

Stanley B. Prusiner, a maverick American scientist who for two decades endured derision from his peers as he tried to prove that bizarre infectious proteins could cause brain diseases like "mad cow disease" in people and animals, Monday was awarded the ultimate in scientific vindication: the Nobel Prize in Medicine or Physiology.

Prusiner, a 55-year-old neurologist at the University of California San Francisco, was cited by the Swedish Nobel committee "for his pioneering discovery of an entirely new genre of disease-causing agents and the elucidation of the underlying principles of their mode of action."

Pathogenetic Features of Prion Diseases

Disease	Host	Mechanism of Pathogenesis
Kuru	Fore people in New Guinea	Infection through ritualistic cannibalism
Creutzfeldt-Jakob disease		
Iatrogenic	Humans	Infection from prion-contaminated human growth hormone, dura mater grafts, and so forth
New variant	Humans	Infection from bovine prions?
Familial	Humans	Germ-line mutations in the PrP gene
Sporadic	Humans	Somatic mutation or spontaneous conversion of PrP^{C} into PrP^{Sc}?

Note: Iatrogenic means transmission of the disease through PrP^{SC} contaminated surgical/ hospital instruments.

RT QUESTION MORE.

⌂ + News + Cannibals survived brain disease epidemic thanks to a mutation +

Cannibals survived brain disease epidemic thanks to a mutation

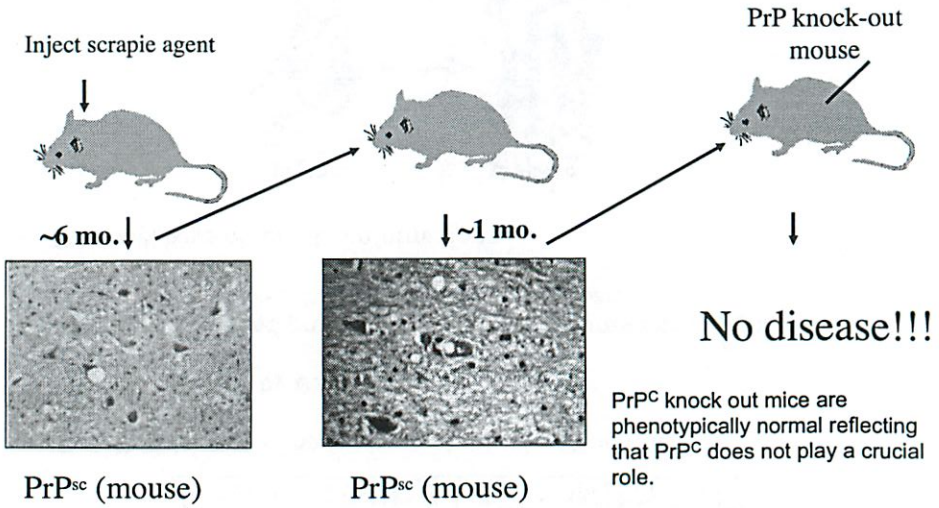
Published: 20 November, 2009, 17:40
Edited: 5 October, 2010, 23:25

In 51 survivors of the epidemic and their descendants, they've discovered a previously-unknown variant of a section of PRNP. None of the victims had the mutation. Moreover, the bloodlines of the mutation carriers suffered from kuru about six times less than bloodlines of those without it.

In their work, published in The New England Journal of Medicine, Mead and colleagues say that the mutation is an acquired prion disease resistance factor, which underwent positive selection during the decades of epidemic.

"I hope it will become a textbook example of how evolution happens," said Mead.

The PrP knock-out mouse



PrP^{C} knock out mice are phenotypically normal reflecting that PrP^{C} does not play a crucial role.

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Susan Lindquist accepts the National Medal of Science from President Obama, November 30 '09

Still image from a computer simulation showing how a string of shapes can fold itself up into an arbitrary shape. Image: Center for Bits and Atoms

Reconfigurable robot a step toward something that can become almost anything.

Precisely engineering 3-D brain tissues

November 30, 2012

Scientists discover

Prions play key role in yeast survival and evolution

For the first time, researchers find prions in wild strains of yeast, and show they can help the organisms withstand stress.

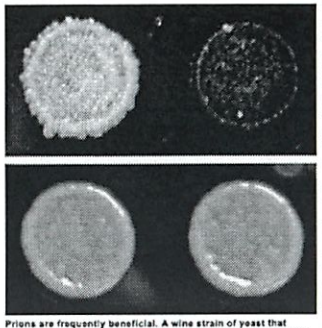
Anne Trafton, MIT News Office

February 16, 2012

Share

Misfolded proteins called prions are best known for causing neurodegenerative disorders such as Creutzfeldt-Jakob disease and mad cow disease. However, a new study by scientists at MIT's Whitehead Institute finds that they can also play a much more beneficial role.

The research team, led by Susan Lindquist, has shown that in yeast, prions awaken dormant stretches of genes that can help the yeast survive environmental stresses. Furthermore, those new traits can be passed on to offspring, contributing to evolution in an unexpected way.



Prions are frequently beneficial. A wine strain of yeast that naturally contains prions — in this case formed by a particular transcription factor — can grow in the presence of an antifungal drug (left), but becomes susceptible to the drug when the prions are eliminated (right). The prions did not affect growth in the absence of the drug (bottom).
Images: R. Halfmann, D. F. Jarosz, S. K. Jones, A. Chang, A. K. Lancaster, S. Lindquist, and *Nature*

related

Susan Lindquist

Whitehead Institute for Biomedical Research

ARCHIVE: "Scientists ID secret to infectious protein"

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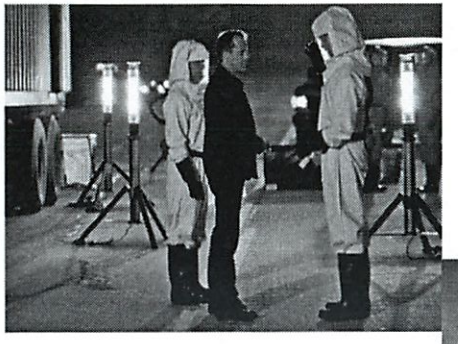
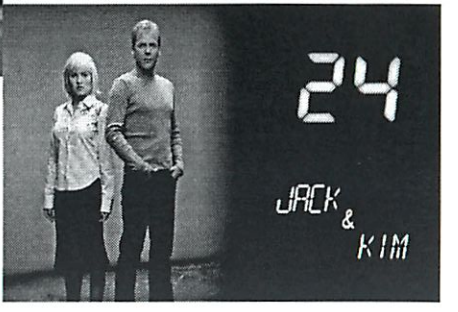


1. Find a compatible donor that has a mutation in the acquired prion disease resistant factor use his cells for gene therapy.

2. Find a compatible donor that is PrP^{C-}/PrP^{C-} and use his cells for gene therapy.



Bio

Molecular Evolution

12/15

How are different species related?

Build a table

Can build a Phylogenetic tree

Can also look at DNA

Coding DNA vs Junk DNA

Organisms have mutations

Coding seq are improved through evolution

↳ selection for beneficial = selection mutation 3%

Junk DNA - no selection = neutral mutation 97%

So mutates more rapidly

Histone H4 is similar in humans and corn

Fibrinopeptides - not critical so changes a lot

Very different principles flies + human eyes
but share similar genes!

Can take mamalian eye genes put on
el bow of fly → get an eye!
These genes are interchangable!

Realize → common ancestor
Not that eyes were seperate

The further the two are
Can compare the ribosomal DNA

We diverged from bactera ~1.5 billion years ago!
3000 species ~~in~~ in tree of life
Sea anemone
we have similar genes to it

(3)

A lot of recombination - autosomal

How can we learn about our origins

Depections from bones

As humans adred

Neanderthals

500,000 years ago

Very human like

How do we know if they are similar?

Y chromosone + mitocondria DNA never recombines

↑ male ↑ female

decendent through that gender

$10^3 - 10^4$ mtDNA in each nucleus

Can evolve rapidly

Regions polymorphic

(4)

How close are we to Neanderthals?

People sequenced bones

30,000 years or more get fragments - very stable

Worried skin of archiologists got on it

So retind our branch chart

Nieanderthals never reinvented their tools

Same for 100,000 years

Humans reinvented every tools 1000 years

4% of our DNA from Nieanderthau

So same species

for European, Asian dicsent, not Africa

Some mixing had to have occured

---

knuckle bone in a cave
(missed)

(5)

Old species: lots of polymorphic variation

Since new species small in #, so less polymorphic variation

Age of species is time since bottleneck

Can measure time since bottleneck by genetic diversity

The more diverse, the older the species.

In 4 generations, $\frac{1}{4}$ Y DNA survive ; 237

$\frac{1}{4}$ mtDNA survive

8 " , only 5.6% of each survive

ideal population is often 1 Y DNA + 1 mtDNA

just statistically...

(6)

See how closly people in Europe + Asia are related
Africans are longer chats

So all of us are from Africa
descended from mitochandrial eve
L all mtDNA descended from her
was in small breeding population

Africans much more diverse

!Xhoisan Bushman 100,000 years ago
most people 50,000 years ago

Can plot a flow of where people went using mtDNA
Inbreed population look very Similar

Finish dominated (missed)

(7)

Can look at ancestors of Europeans
Even have names - lol!

How long have we been wearing clothes
Can look at lice
60 - 70,000 years ago
Created home for lice on body

Domisticated animals
domisticated twice for many

Priests = cohans
Only natural sons can ~~do~~ be priests
Romans put them at of business
Cohen is a family name
So all modern male Cohens should have same ancestor
But how often does Mrs. Cohen have a side relationship
~5 - 10%

(8)

Apparently ~~you~~ genetic canslers can't ~~the~~ tell people

Did study on this

70% have same Y-chromosone - Cohens

15% non Cohens have that Y-chromosone

in 100 generations Mrs. Cohen is Faithful

Lemba tribe

all the males in 1 of 4 castes has Cohen Y-chromosone

So 300-400 years a Mr. Cohen "joined the tribe"

Gene flow in Indian castes

Upper caste = European more so

Gene from lactose 8,000 years ago

Table 25.1 Eight Vertebrates Ordered According to Unique Shared Derived Traits (Part 2)

How are organisms related to one another? (Phylogeny)

The traditional way of determining how organisms are related to one another is to group them according to shared traits (phenotypes).

TABLE 25.1

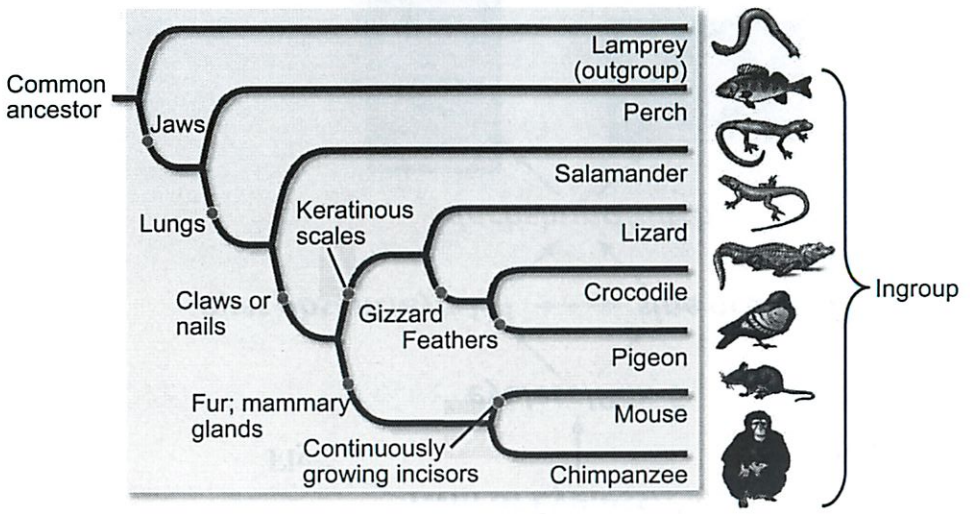
Eight Vertebrates Ordered According to Unique Shared Derived Traits *(Part 2)*

	DERIVED TRAIT[a]			
TAXON	FEATHERS	FUR	MAMMARY GLANDS	KERATINOUS SCALES
Lamprey (outgroup)	–	–	–	–
Perch	–	–	–	–
Salamander	–	–	–	–
Lizard	–	–	–	+
Crocodile	–	–	–	+
Pigeon	+	–	–	+
Mouse	–	+	+	–
Chimpanzee	–	+	+	–

[a]A plus sign indicates the trait is present, a minus sign that it is absent.

LIFE 8e, Table 25.1 (Part 2)

Figure 25.3 Inferring a Phylogenetic Tree



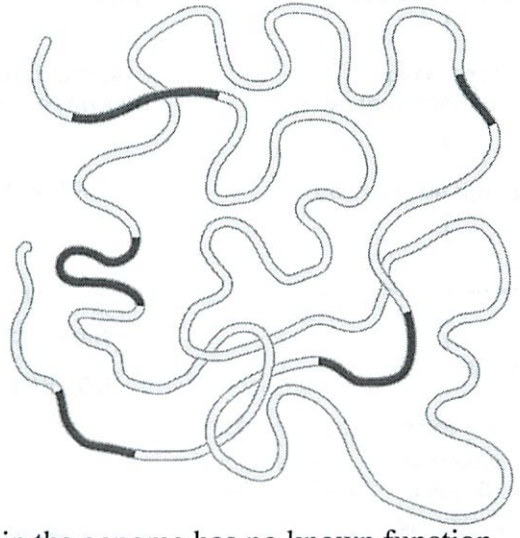
LIFE 8e, Figure 25.3

L 35 Molecular Evolution

Coding DNA

Junk DNA

But there is another way! It depends on the fact that more closely related organisms have more recent common ancestors!



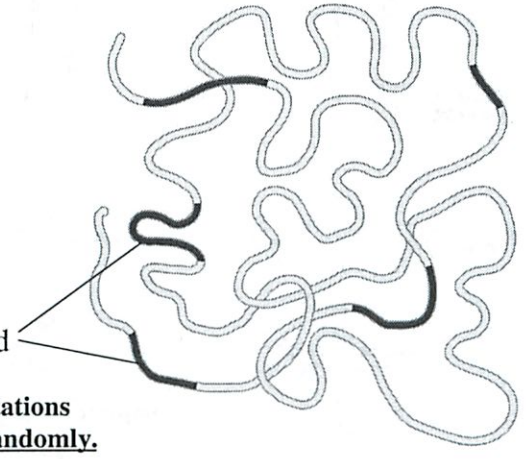
Most of the DNA in the genome has no known function

Figure 1.4 *The Biology of Cancer* (© Garland Science 2007)

Imagine that the sequences for the **coding** DNA are the result of hundreds of millions of years of evolutionary optimization. In that case, the great majority of mutations affecting the sequence of the coding DNA and the structure of its encoded proteins are likely to be **deleterious** -- deviations from the already-optimized. (i.e. you can't get any better than the best!) Hence, mutations affecting coding DNA are likely to compromise the fitness of an organism and will be selected against.

Coding DNA

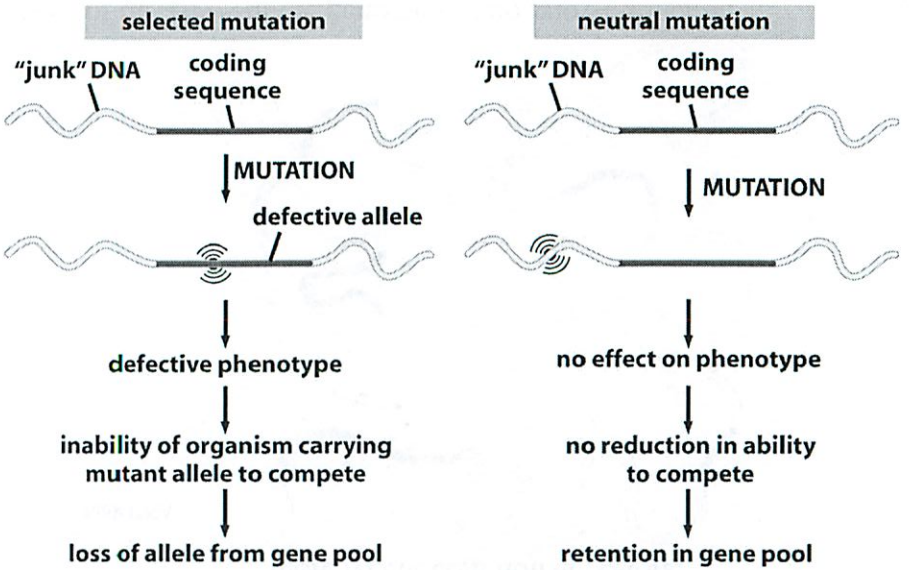
Junk DNA



Remember that mutations strike the genome randomly.

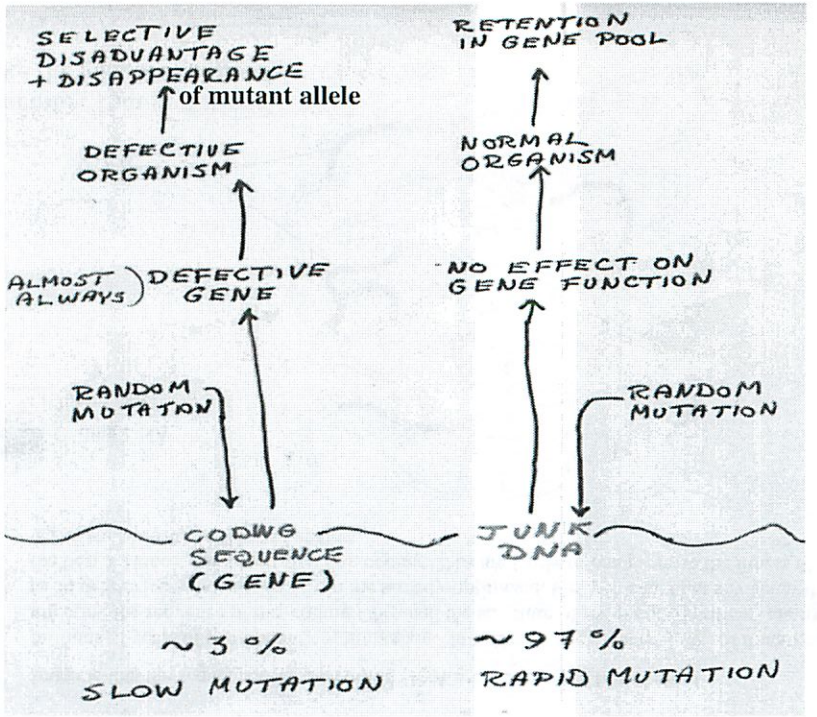
Figure 1.4 *The Biology of Cancer* (© Garland Science 2007)

12/10



(hence, coding sequences do not evolve rapidly and are therefore highly "conserved" over long evolutionary time periods.)

(formation of a genetic "polymorphism" that does not affect phenotype)



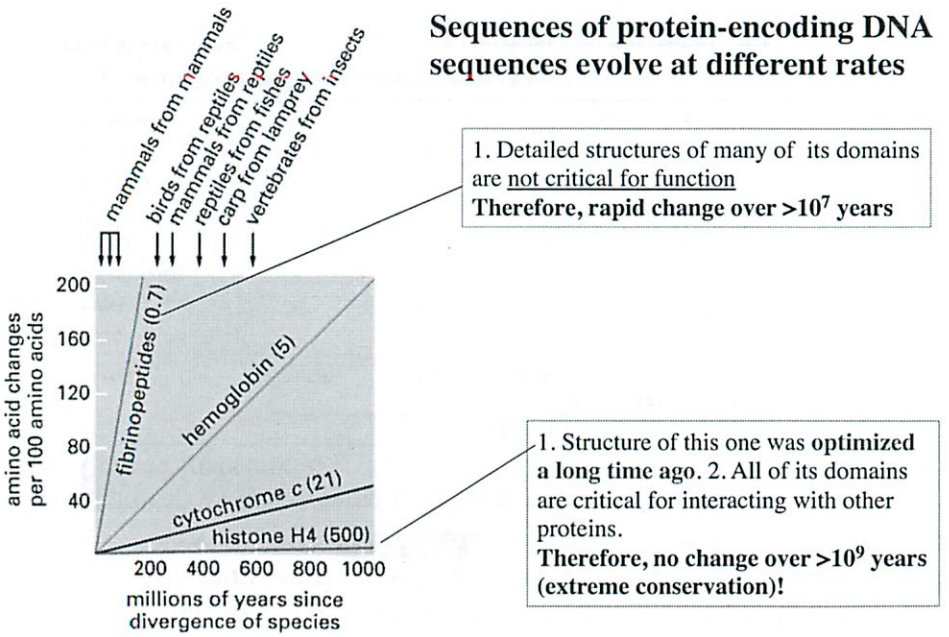


Figure 5–1. Molecular Biology of the Cell, 4th Edition.

Extreme example of evolutionary conservation: The same master genes control eye development in flies and humans (even though the eyes look entirely different!)

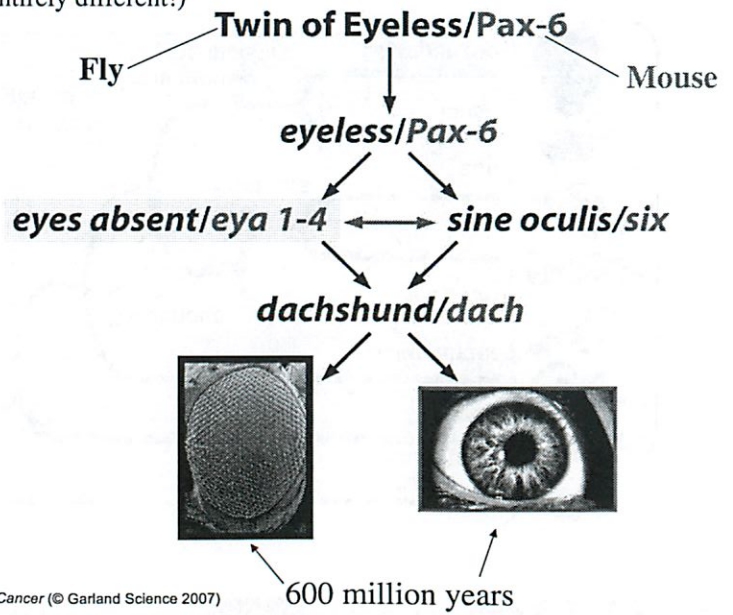
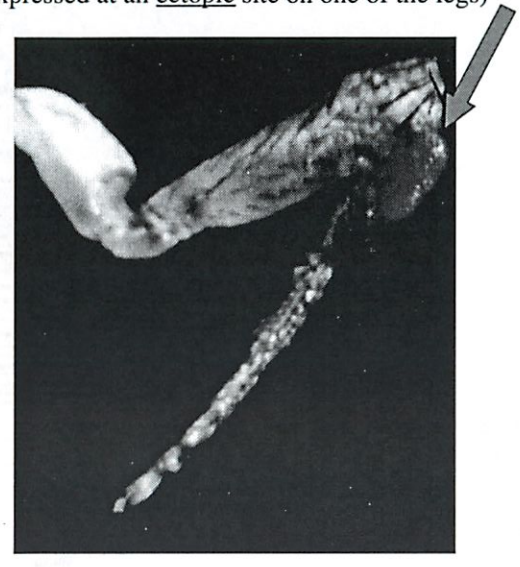


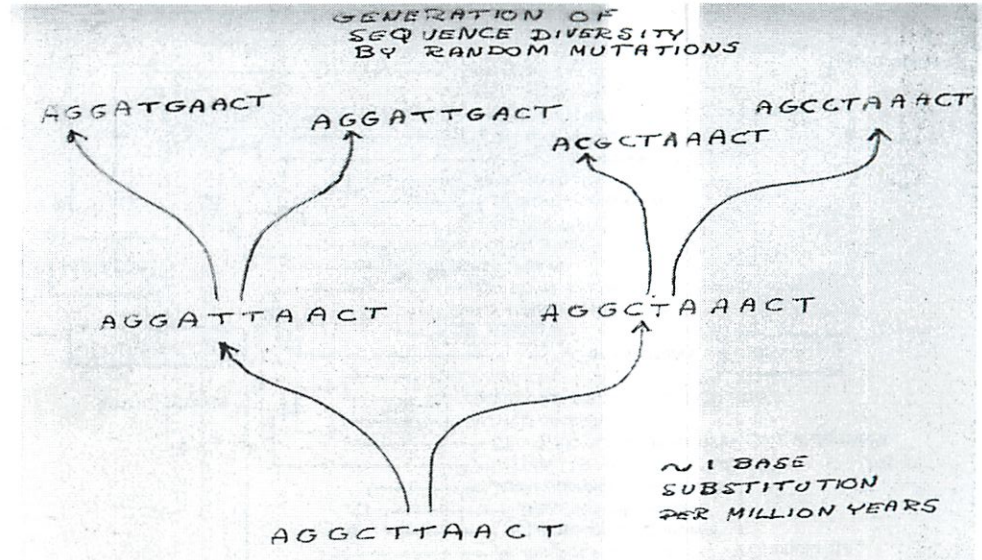
Figure 1.7a *The Biology of Cancer* (© Garland Science 2007)

Express the mouse *Pax6* gene (which specifies eye formation in mammals) on the leg of a fly --> get a fly eye!
(in this case, expressed at an ectopic site on one of the legs)



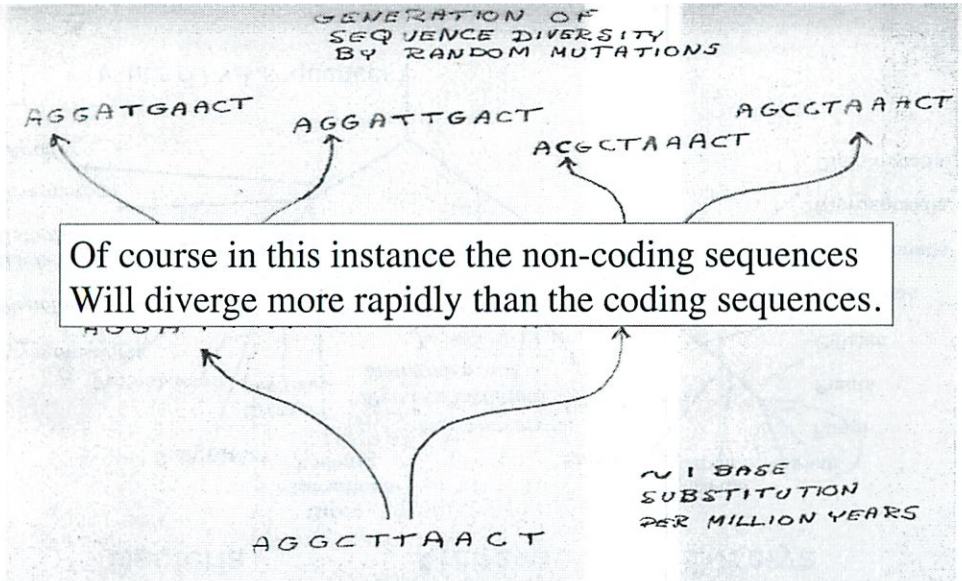
(ectopic = a biologically or anatomically inappropriate site)

Figure 1.7b *The Biology of Cancer* (© Garland Science 2007)



Over the course of time, due to random mutations, gene sequences randomly drift apart (diverge) unless sequence changes compromise fitness.

Therefore, a comparable (homologous) DNA sequence in two organs will be more divergent in more distantly related organisms.



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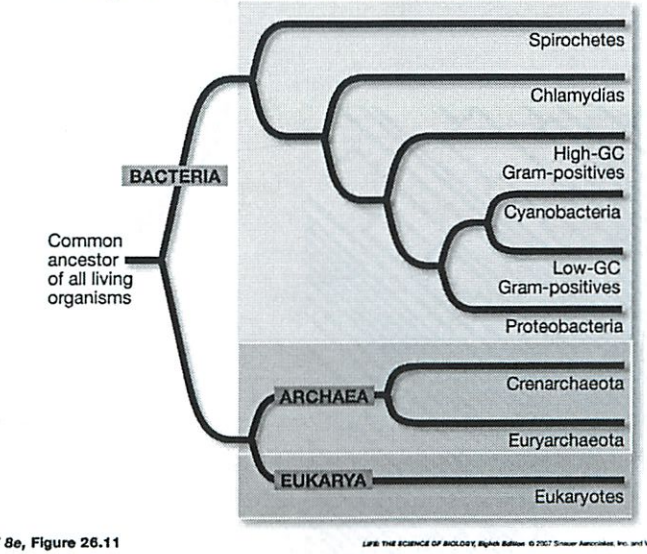
Therefore, a comparable (homologous) DNA sequence in two organs will be more divergent in more distantly related organisms.

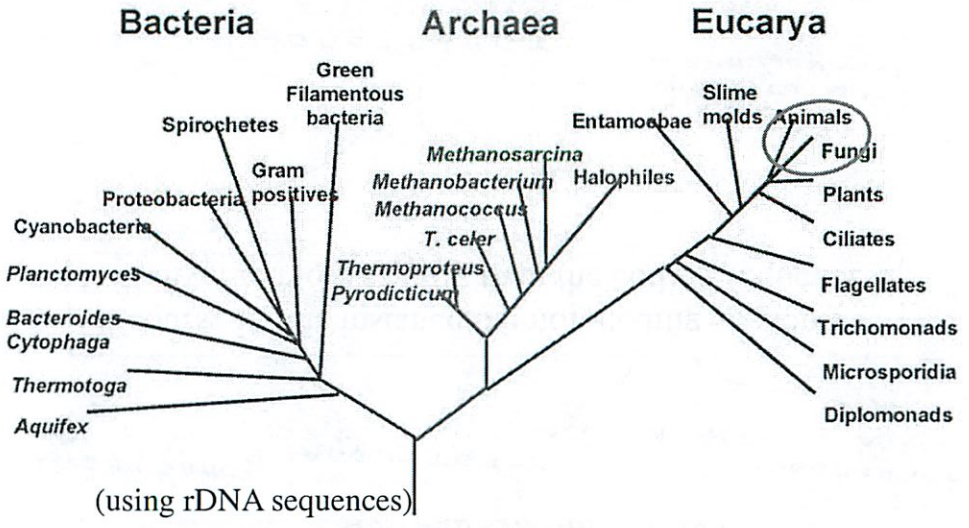
26.11 Two Domains: A Brief Overview

Compare the ribosomal RNA/DNA sequences of all cellular organisms on the planet I.e., study the divergence of a gene that is present in the cells of all organisms.



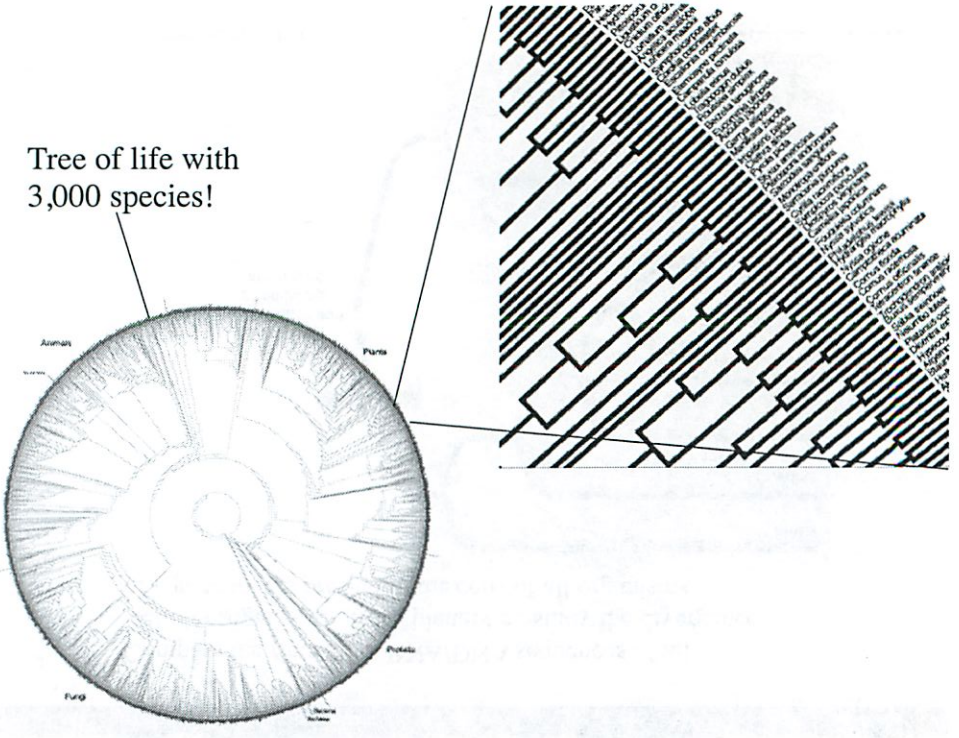
LIFE 8e, Figure 26.11

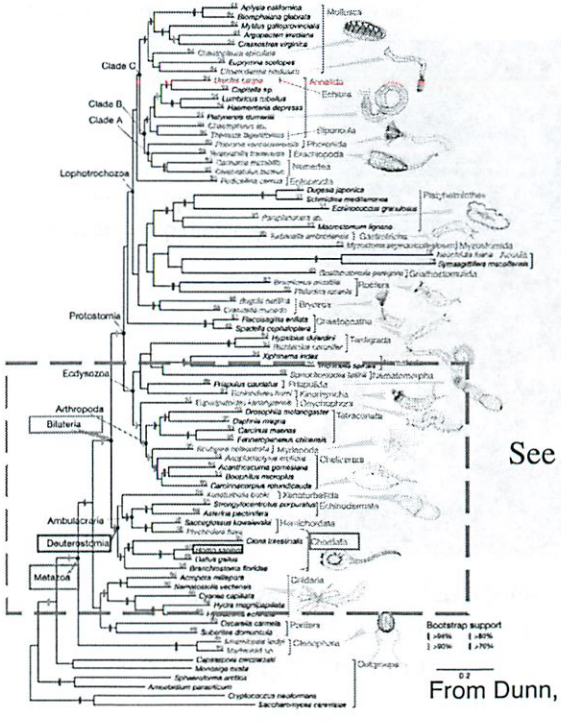
Phylogenetic Tree of Life



(using rDNA sequences)

Tree of life with 3,000 species!



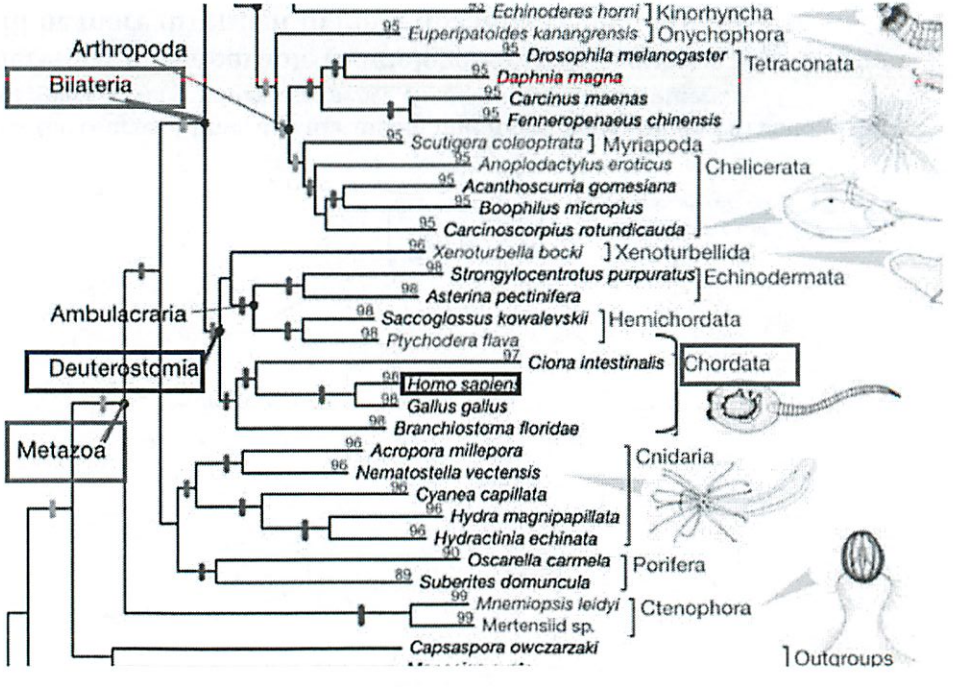


See next slide

Metazoa

(Probably the most definitive representation of phylogeny to date.)

From Dunn, CW et al. Nature. 2008 452:745-9.

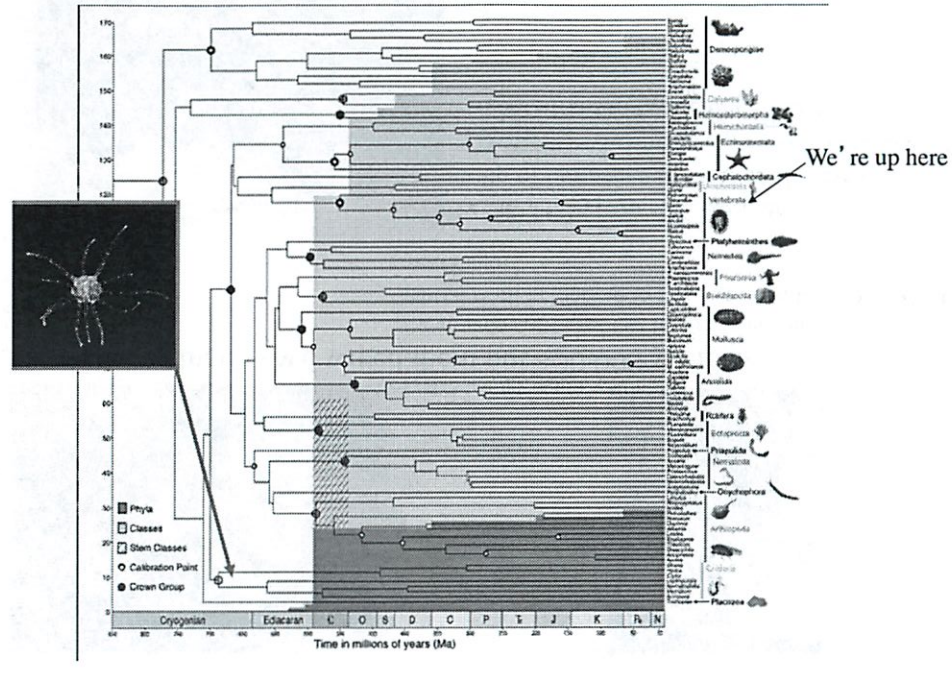


Several inches wide →

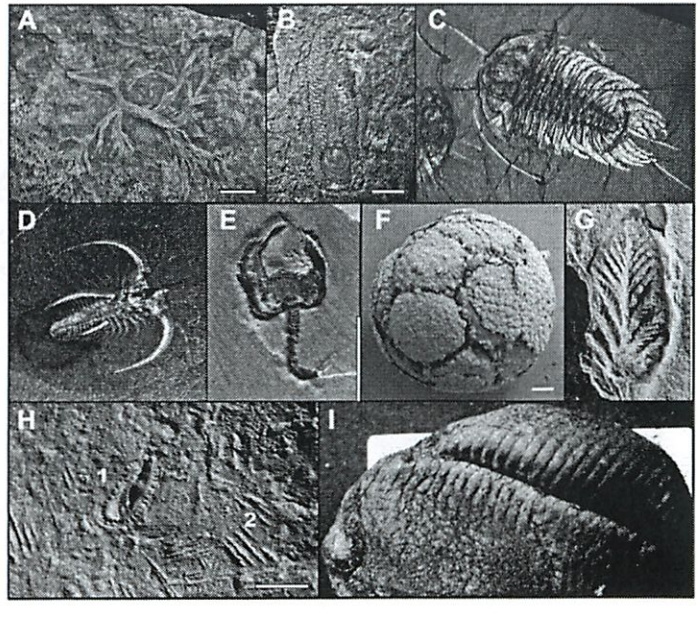
Or just sequence whole genomes!

Compare the **sea anemone** with other animals and see what the genome of their last common ancestor looked like, even though such creatures have been extinct for 600 or 700 million years.
The sea anemone, for example, has about 18,000 genes, while humans have about 20,000. This implies that the common ancestor had about the same number of genes, between 18,000 and 20,000. Many of the anemone's genes lie on its 30 chromosomes in patterns similar to the patterns of related genes on the 46 chromosomes of humans.
Roughly 80 percent of eumetazoan genes even further back in time to before the origin of animals (since related genes are found in fungi, plants, slime molds and other non-animals). Only 20 percent of the ancestral eumetazoan genes seem to be unique to animals, I.e., were "invented" when multi-cellular animals arose. .

And metazoan evolution started long before we imagined!



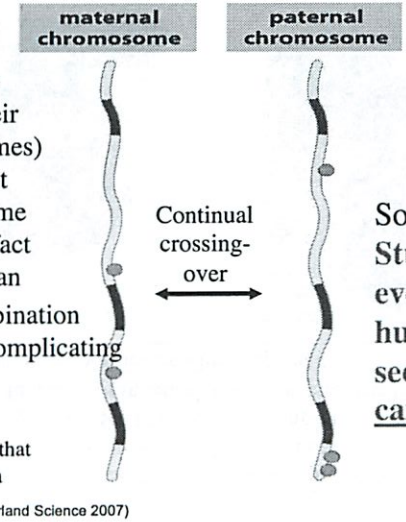
Cambrian and pre-cambrian (edicaran) fossils



How can you study the divergence of DNA sequences **within a species** (e.g., ours) if the 2 copies of a gene are continually swapping sequence information?
(which confounds studies of sequence divergence) ?????

Mom and Dad have different polymorphic DNA markers* on their chromosomes (autosomes) But analyses of descent over long periods of time is complicated by the fact that these autosomes can **recombine.** Recombination mixes up sequences, complicating analysis.

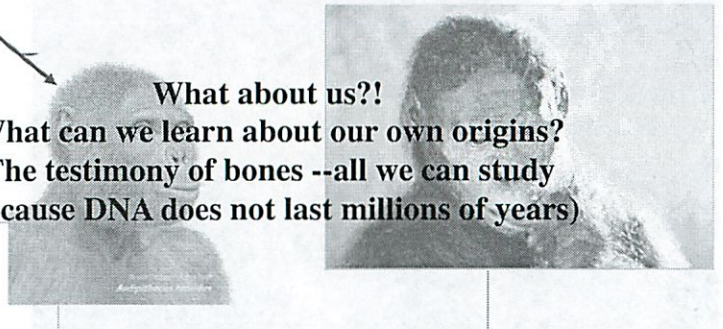
*Polymorphisms are slightly different nucleotide sequences that generally do not affect function



Solution: **simplify**! **Study the evolution of human (or other)DNA sequences that cannot recombine!**

Figure 1.6 *The Biology of Cancer* (© Garland Science 2007)

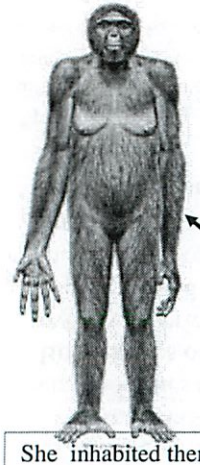
The partial skeleton of a female who lived **4.4 million years** ago is the culmination of 17 years of excavation and research. **Ardi** -nicknamed for her species, Ardipithecus ramidus - stood about -4 feet tall, weighed 110 pounds, and was less like a chimpanzee - than many scientists had expected.



What about us?!
What can we learn about our own origins?
The testimony of bones --all we can study
(because DNA does not last millions of years)

She inhabited then-wooded regions of Ethiopia. Her skeleton raised surprising questions about how key human traits evolved.

They eventually unearthed 47 bones of a skeleton - nearly 40% of a hominid, or humanlike creature, that lived around **3.2 million** years ago. Based on its small size, and pelvic shape , they concluded it was female and named it 'Lucy".



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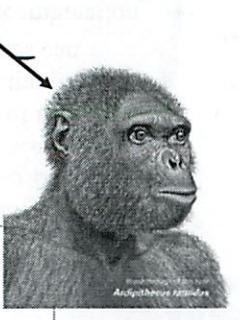

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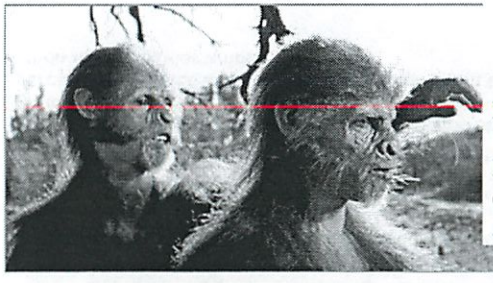


Homo habilis

By **three million years** ago, the world of Lucy and Australopithecus afarensis had vanished. Hidden forces were transforming the Earth's climate, with devastating consequences for the African landscape.

In addition, **three million years** ago:
In East Africa, a hominid called *Paranthropus boisei* became specialised so that it could eat tough-to-chew but more abundant plant foods such as nuts, roots and tubers.

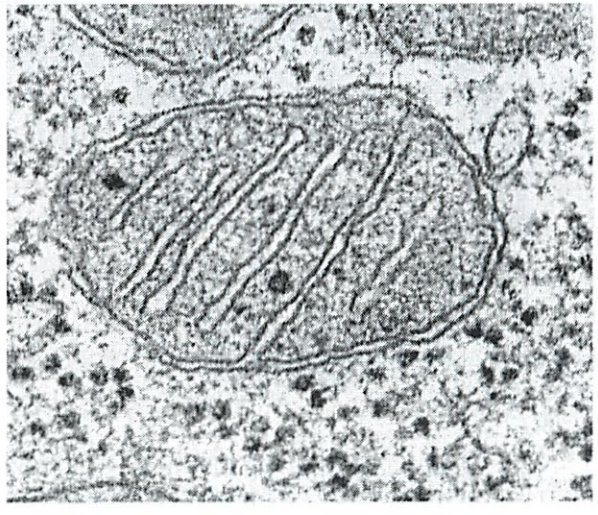
By **two million years** ago, a new species of Homo appeared - the first species we would truly recognise as human. *Homo ergaster* evolved during an accelerated period of global cooling and drying that cleared more and more tropical rainforest from Africa and created a desert in the northern half of the continent.

Neanderthals -- we wiped them out ~30,000 years ago

the remains of the oldest human ever found in Europe - a partial skull belonging to a young male who lived **780,000 years** ago.

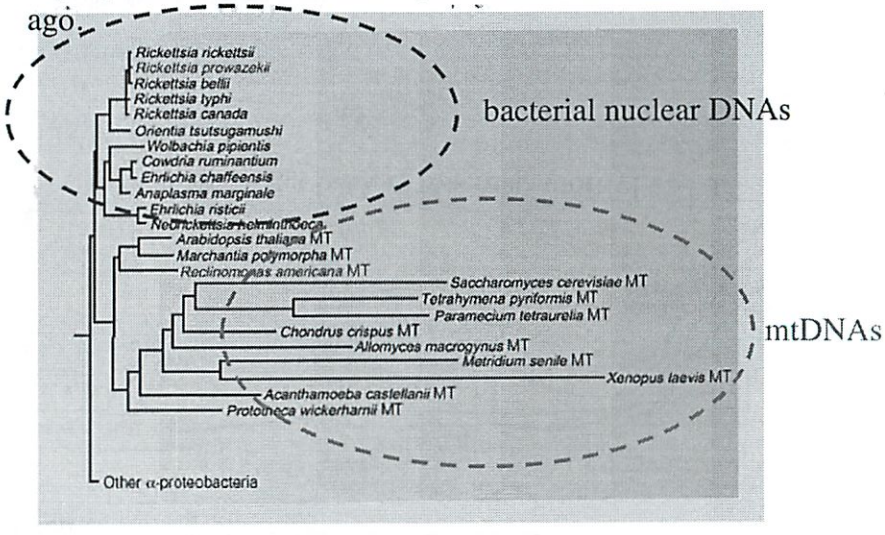
By **500,000 years** ago, another group of humans had reached Boxgrove in West Sussex, England. *Homo heidelbergensis* was not afraid to tackle big animals.

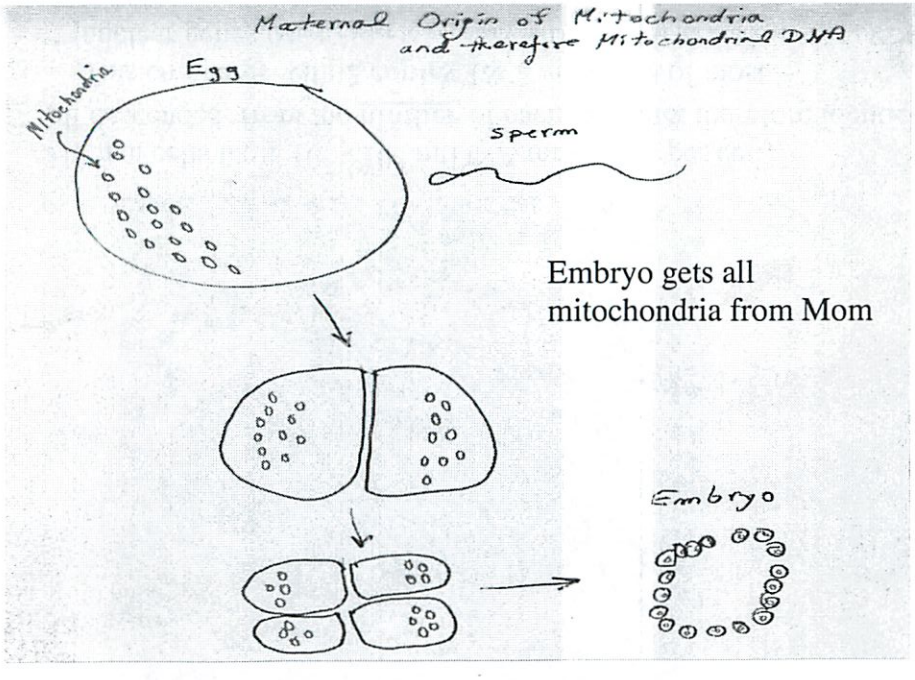
How can we learn about our distant ancestors by studying Contemporary/modern DNA?



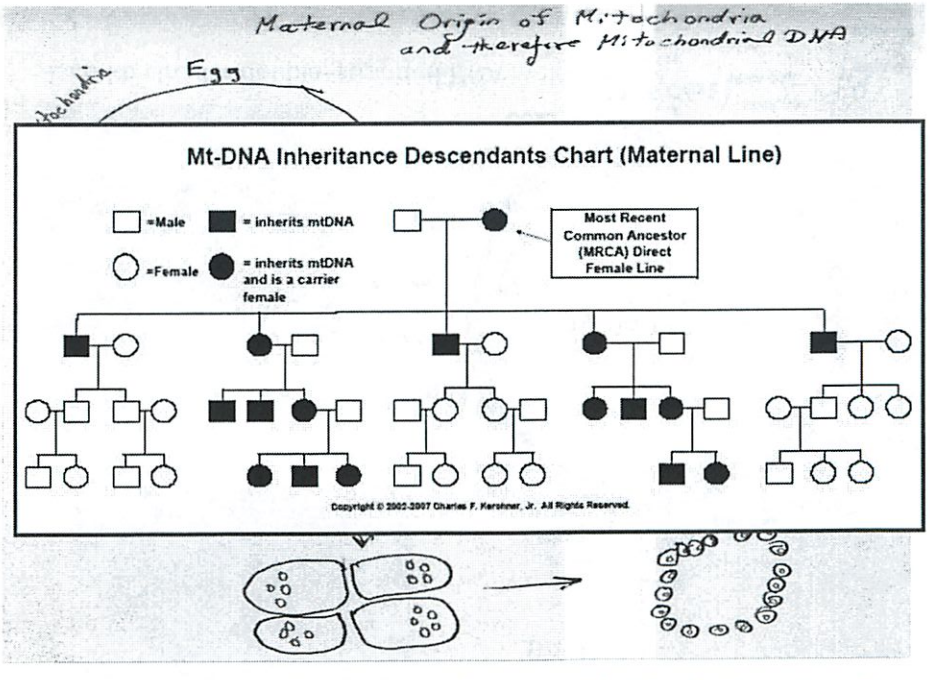
Study **mitochondria** and their DNA (mtDNA)

Mitochondria have their own DNA, which is related to that of certain modern bacteria. Similar organisms colonized the "cytoplasm" of the first eukaryotic cells ~1.5 billion years ago.





In mammals, 99.99% of mitochondrial DNA (mtDNA) is inherited from the mother.



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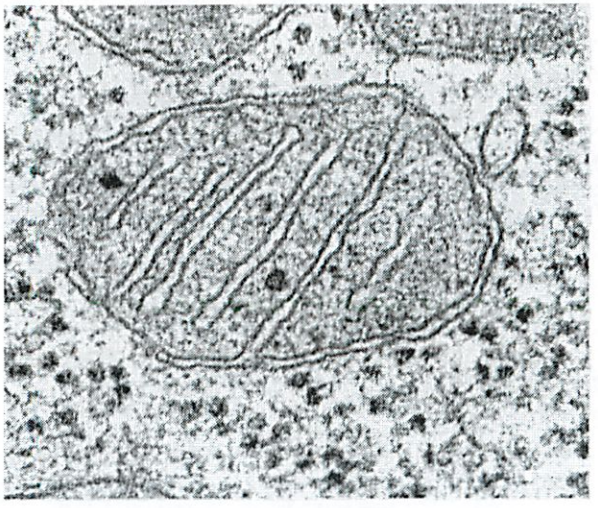
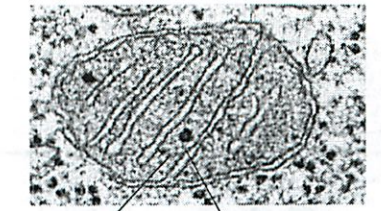
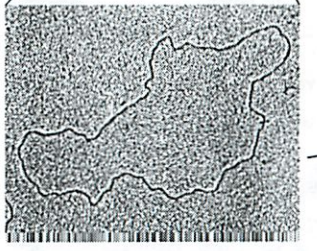


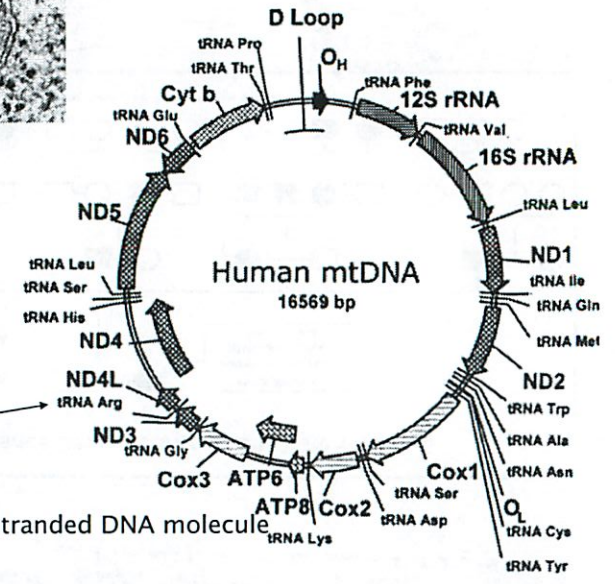
Human cells have 10^3 - 10^4 mtDNA molecules per cell,
all descended from the **mother** of each human& therefore identical

This compares with **2 copies** (& 2 versions) of most nuclear genes.(Much easier to get many mtDNA copies for sequence analyses.)



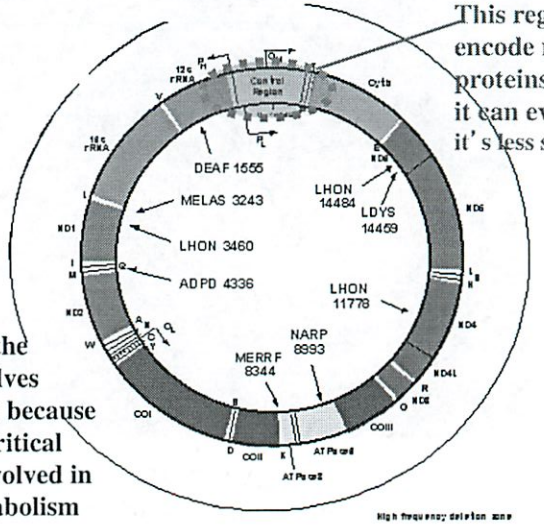


mtDNA



16.6 kb circular double-stranded DNA molecule

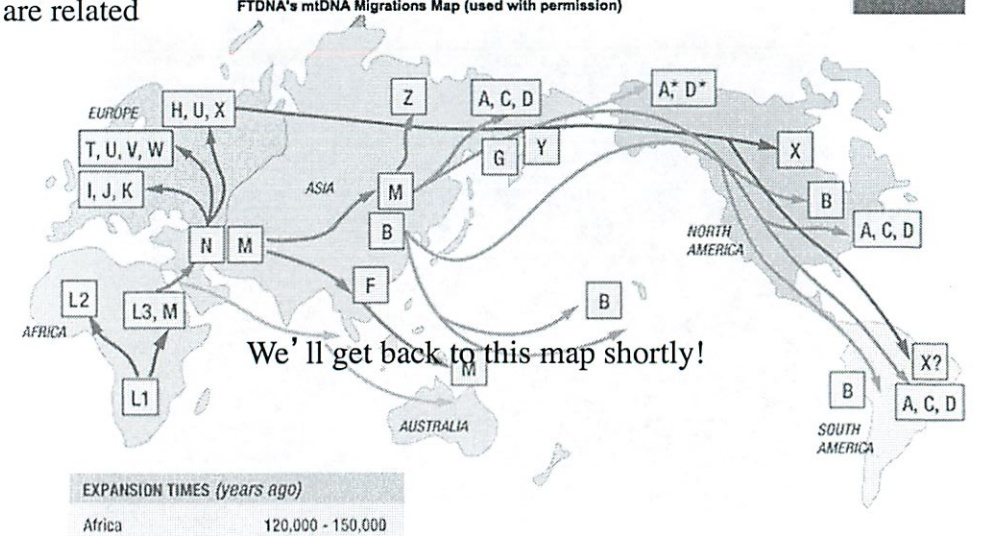
Human mtDNA encodes 13 polypeptides involved in respiration and oxidative phosphorylation,
2 rRNAs and a set of 22 tRNAs that are essential for protein synthesis in the mitochondria



This region does not encode mitochondrial proteins; therefore it can evolve rapidly (because it's less subject to selection)

The rest of the genome evolves more slowly because it encodes critical enzymes involved in energy metabolism

Analyze how different mtDNAs in different human populations are related

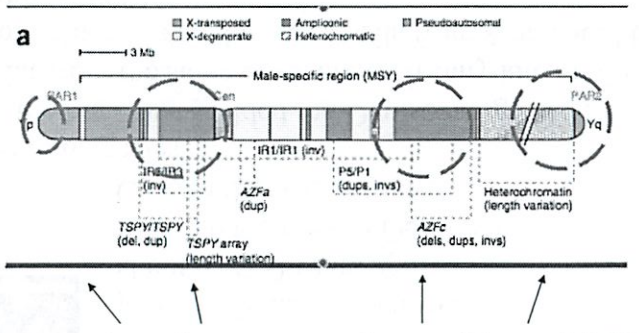


We'll get back to this map shortly!

EXPANSION TIMES *(years ago)*	
Africa	120,000 - 150,000
Out of Africa	55,000 - 75,000
Asia	40,000 - 70,000
Australia/PNG	40,000 - 60,000
Europe	35,000 - 50,000
Americas	15,000 - 35,000
Na-Dene/Esk/Aleuts	8,000 - 10,000

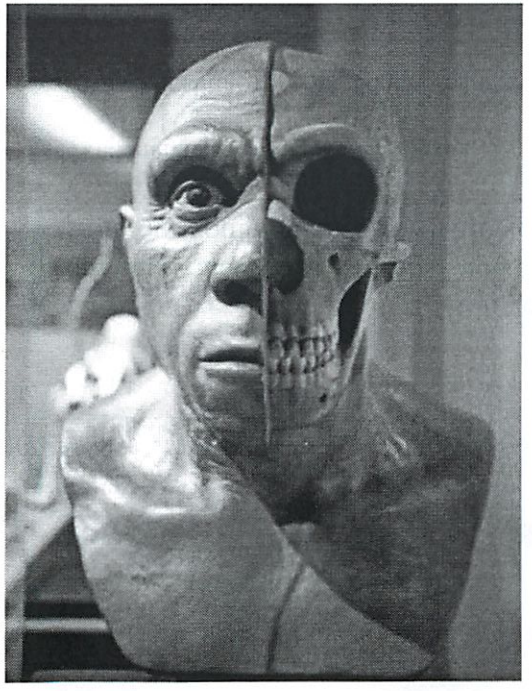
Yet **another human DNA** sequence that does not recombine!

High mutation rates have driven extensive structural polymorphism among human Y chromosomes



Four regions show great polymorphic variability

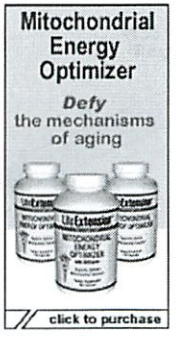
Y chromosome is only transmitted from father to son.



How distantly related are we to Neanderthals?

Solution: How different is their mtDNA from ours?

Mitochondria to the rescue!



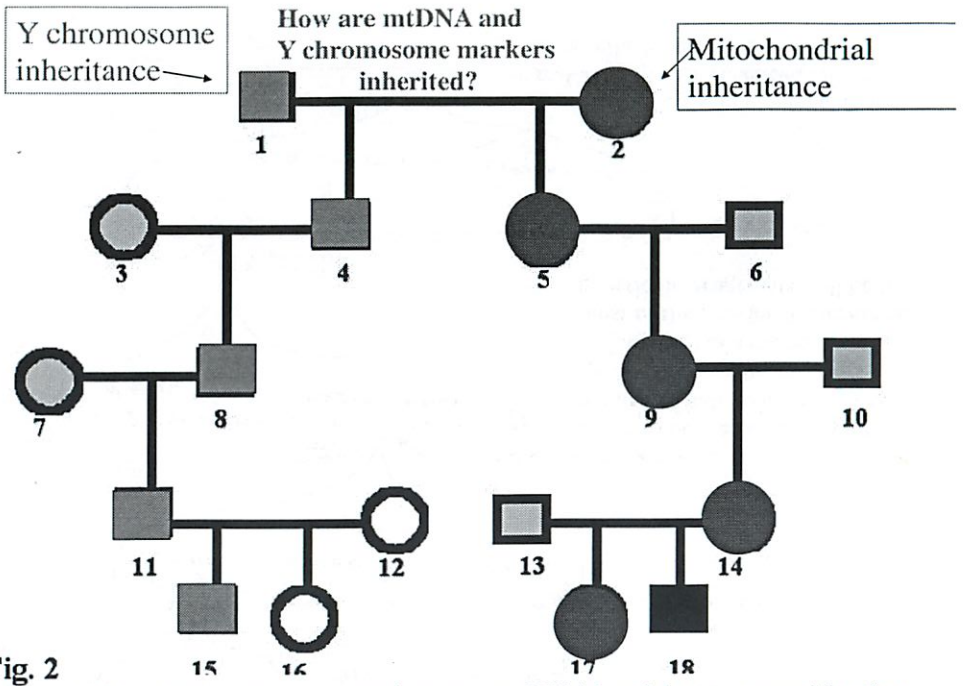
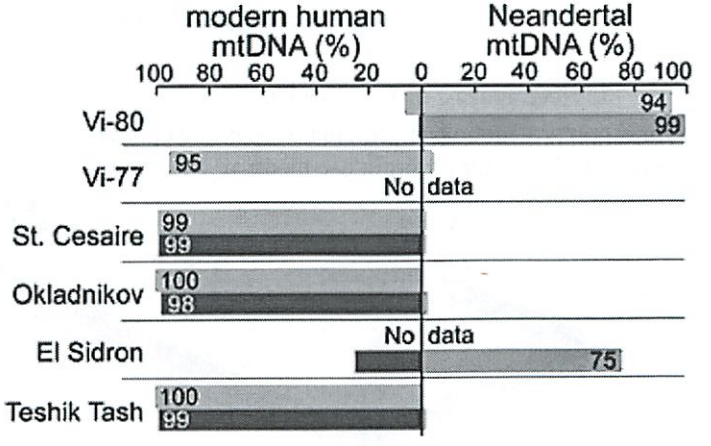
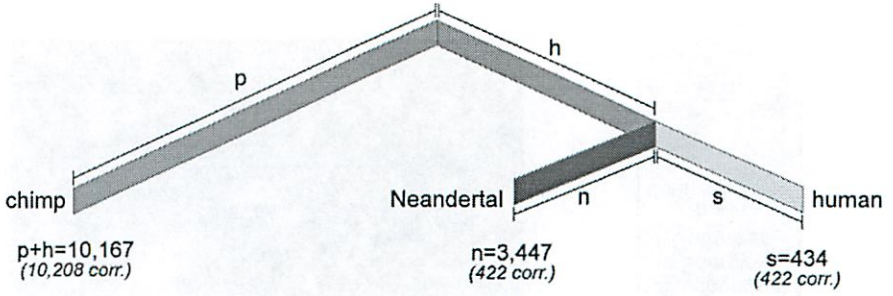


Fig. 2 **Neither Y chromosome markers nor mtDNA is subject to recombination**

Some mtDNA is preserved in the bones of Neanderthals. One possible artifact: Contamination of Neanderthal mtDNA by DNA of modern humans, e.g., paleontologists who have handled the bones!

Degree of contamination of Neanderthal bone DNA by modern human DNA (I.e., mitochondrial DNA). Therefore choose sample w. minimum amount of modern human mtDNA.

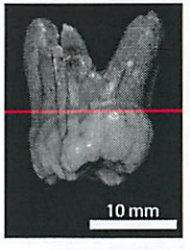




Answer: we're about 5-6 million years away from our common ancestor w. chimps* and about 400,000 years from our common ancestor with Neanderthal.

*known mostly from paleontology

Max Planck Institute for Evolutionary Anthropology



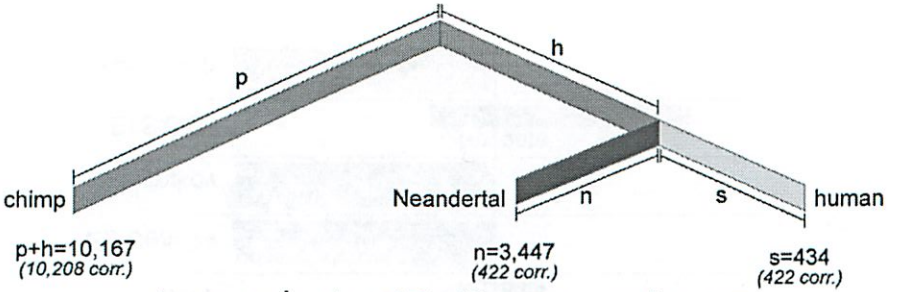
Denisovan molar! (also found knuckle bone, toe bone

juvenile female who lived about 41,000 years ago, found in the remote Denisova Cave in the Altai Mountains

A new hominid species!

Currently, the bone that yielded the Denisovan genome, and a single molar from the same cave, are their only known fossil remains, but other archaic human fossils from Asia could bear traces of this group.

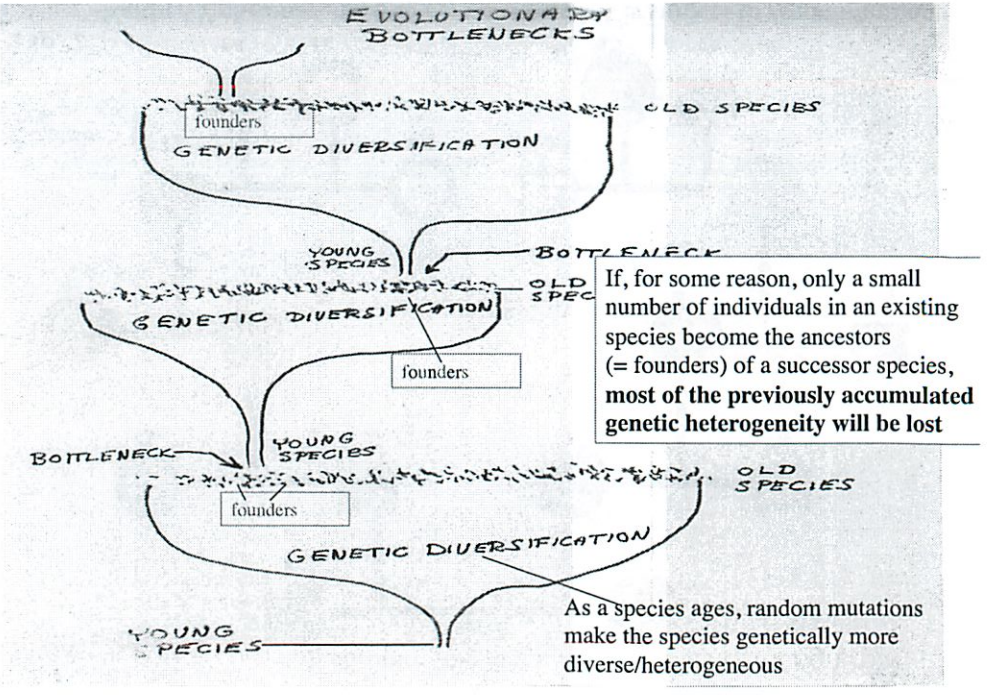
6% of the DNA of Melanesians and Australian aborigines derive from Denisovans



Answer: we're about 5-6 million years away from our common ancestor w. chimps* and about 400,000 years from our common ancestor with Neanderthal.

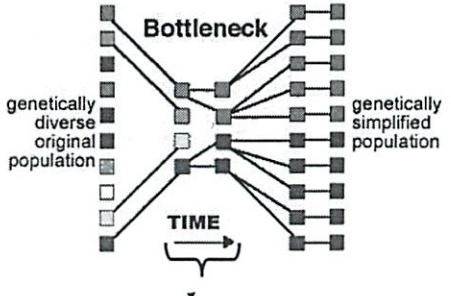
*known mostly from paleontology

outside Africa owe up to 4% of their DNA to Neanderthals. One explanation might be that humans migrating out of Africa mated with Neanderthals, probably resident in the Middle East, before their offspring fanned out across Europe and Asia.



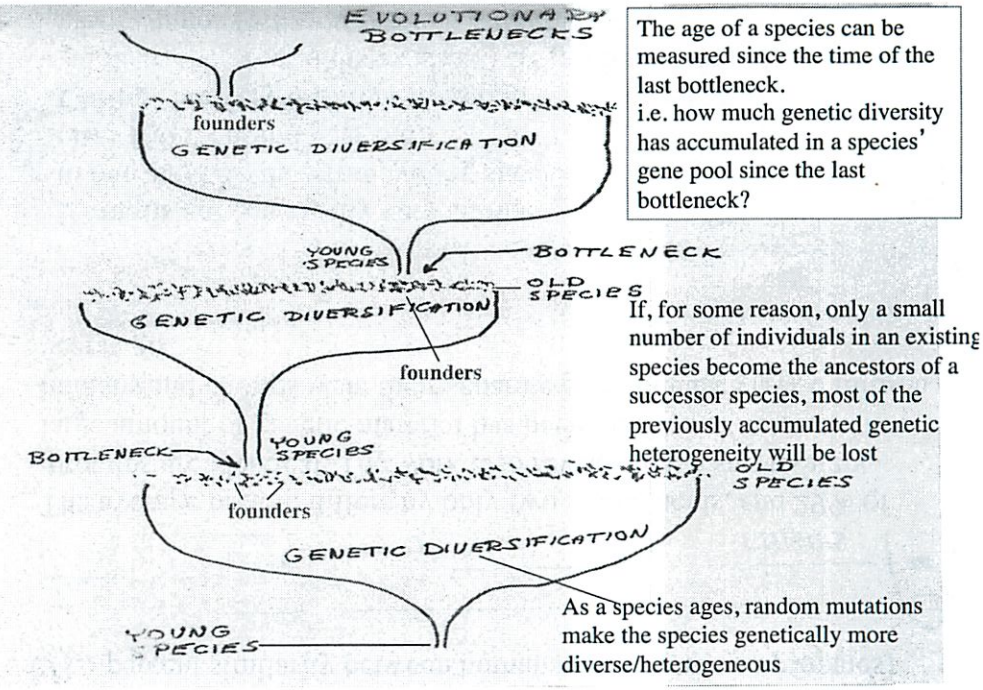
Non-adaptive evolutionary events: sampling effects

You might think that all evolutionary processes are by their very nature adaptive, but that is not the case.
Accidents can "select" a small subset of organisms from the larger population. Founder effects and evolutionary bottlenecks occur when a new population is based on a small, randomly selected group of individuals.



These individuals become the "founders" of the new, descendant population

Although humans seem to vary a great deal in physical appearance, we are actually a genetically homogeneous species. Chimpanzees, our nearest evolutionary relatives, exhibit variation in 1 of 5 mitochondrial control region nucleotides, whereas humans exhibit variation in only 1 of 17.[27] Similarly, chimpanzees show considerably more varia-



The age of a species can be measured since the time of the last bottleneck.
i.e. how much genetic diversity has accumulated in a species' gene pool since the last bottleneck?

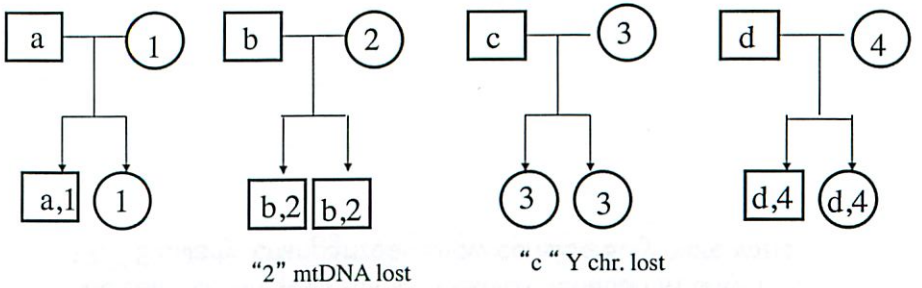
If, for some reason, only a small number of individuals in an existing species become the ancestors of a successor species, most of the previously accumulated genetic heterogeneity will be lost

As a species ages, random mutations make the species genetically more diverse/heterogeneous

What happens during an evolutionary bottleneck to the genetic diversity of a species?

For example, what happens to the preexisting genetic diversity of the **mtDNA** or the **Y chromosomes** in small populations of interbreeding inviduals?

In a population of constant size:



"2" mtDNA lost

"c " Y chr. lost

1/4 of mtDNA genotypes are lost in a single generation

1/4 of Y DNA genotypes are lost in a single generation

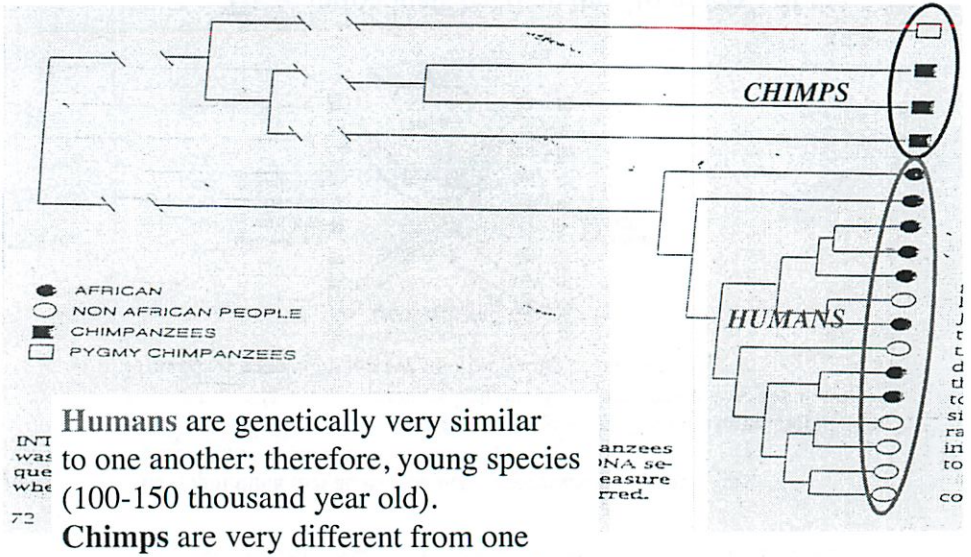
□ = Y chromosome ○ = mt DNA

Therefore, the loss in n generations is 0.75^n

On average, after about 4 generations, only 1 of the 4 (0.237) mtDNAs will survive and 1 of the 4 Y DNAs will survive!
After 8 generations, only 5.6% of mtDNAs and Y DNAs will survive!

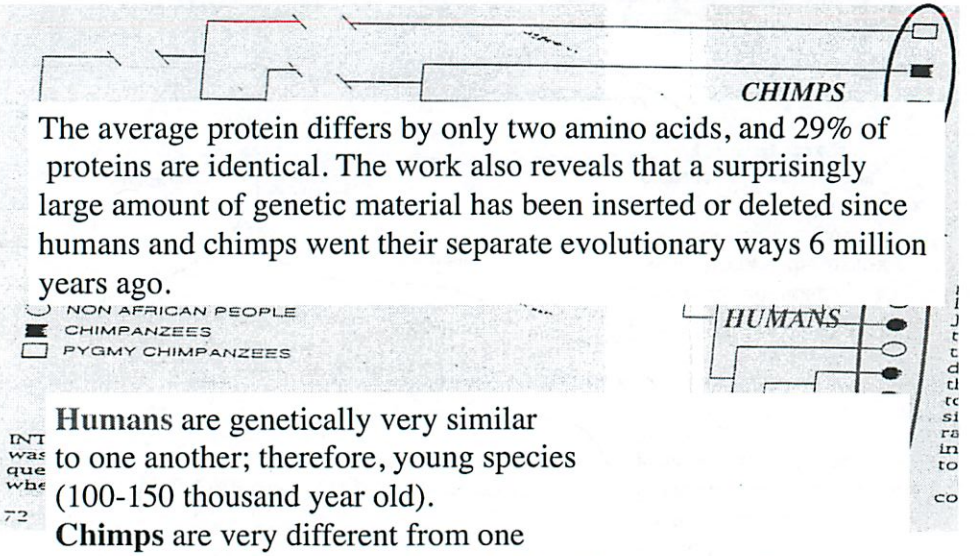
Hence, if the population size of a species stays
(1) Constant and
(2) small for a number of generations,
everyone will have
the same Y chromosome and the same mtDNA!
(because of random **genetic drift)!**

(98.5 percent similarity between humans and nonhuman primates)



Humans are genetically very similar to one another; therefore, young species (100-150 thousand year old).
Chimps are very different from one another; therefore old species (~1.5? million years old).

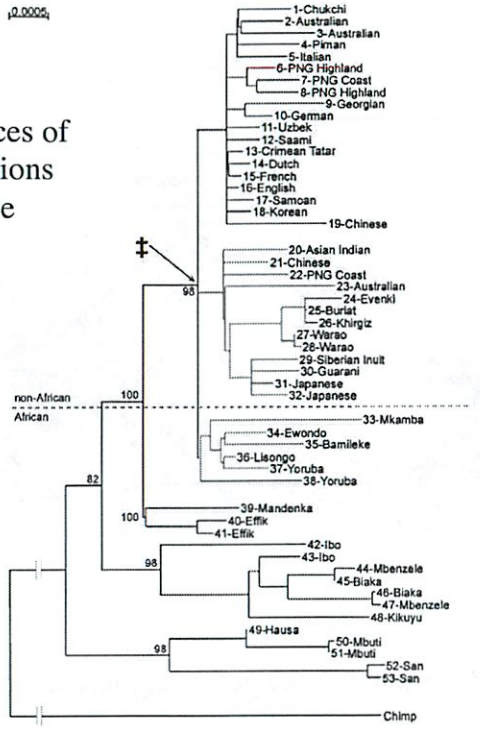
(98.5 percent similarity between humans and nonhuman primates)



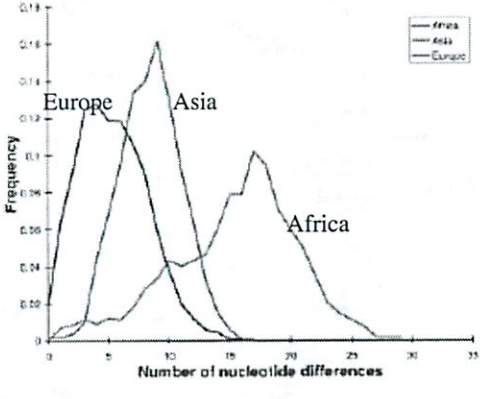
The average protein differs by only two amino acids, and 29% of proteins are identical. The work also reveals that a surprisingly large amount of genetic material has been inserted or deleted since humans and chimps went their separate evolutionary ways 6 million years ago.

Humans are genetically very similar to one another; therefore, young species (100-150 thousand year old).
Chimps are very different from one another; therefore old species (~1.5? million years old). <--*time when chimps last went through a bottleneck*

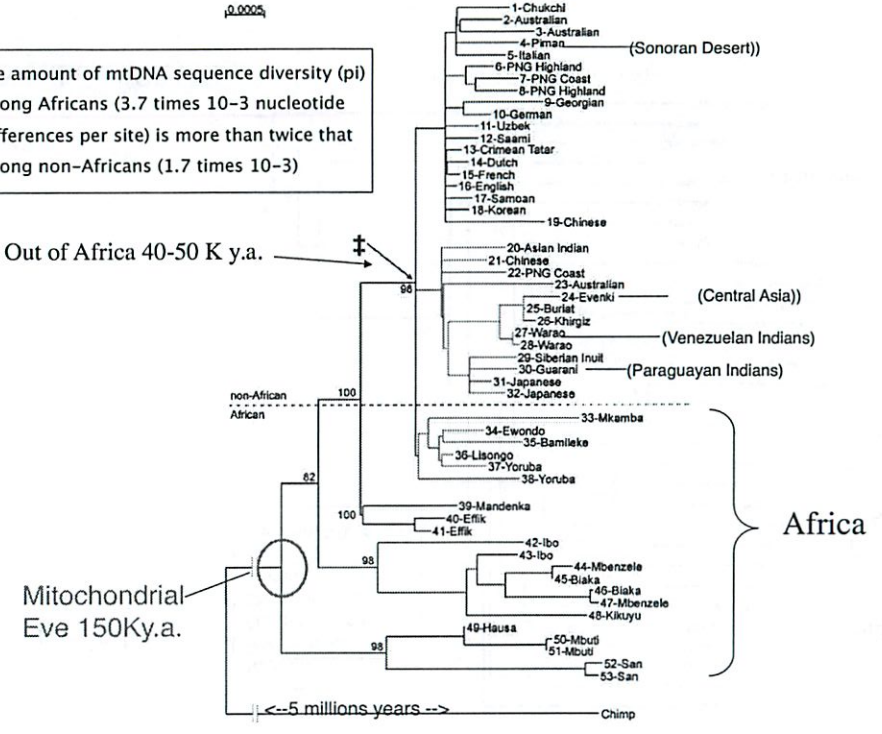
Genetic distances of human populations across the globe e.g., mtDNA Measurements
(next slide)



DNA sequence heterogeneity



The amount of mtDNA sequence diversity (pi) among Africans (3.7 times 10-3 nucleotide differences per site) is more than twice that among non-Africans (1.7 times 10-3)



Wow! Look at these genetic distances!

Far greater genetic diversity in Africa -- Therefore the human race has resided in Africa far longer than elsewhere (resulting in the accumulation of far more intraspecies genetic variability in Africa).

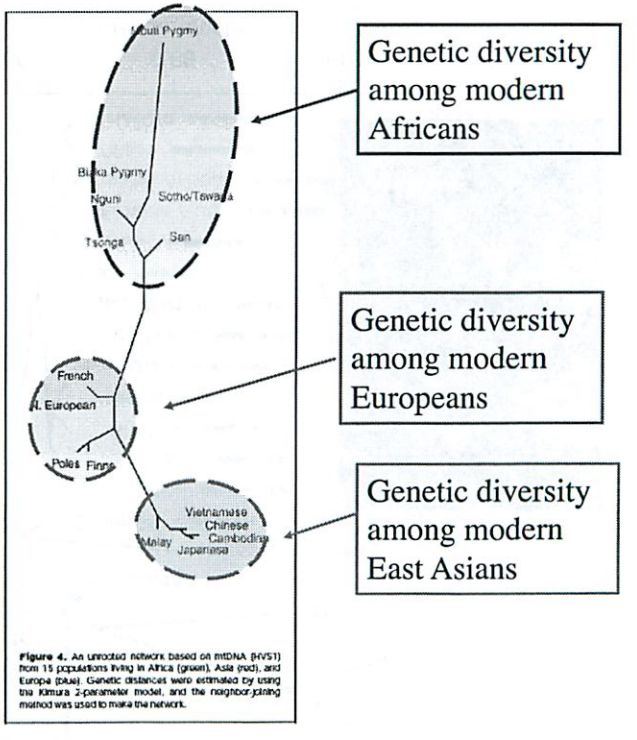
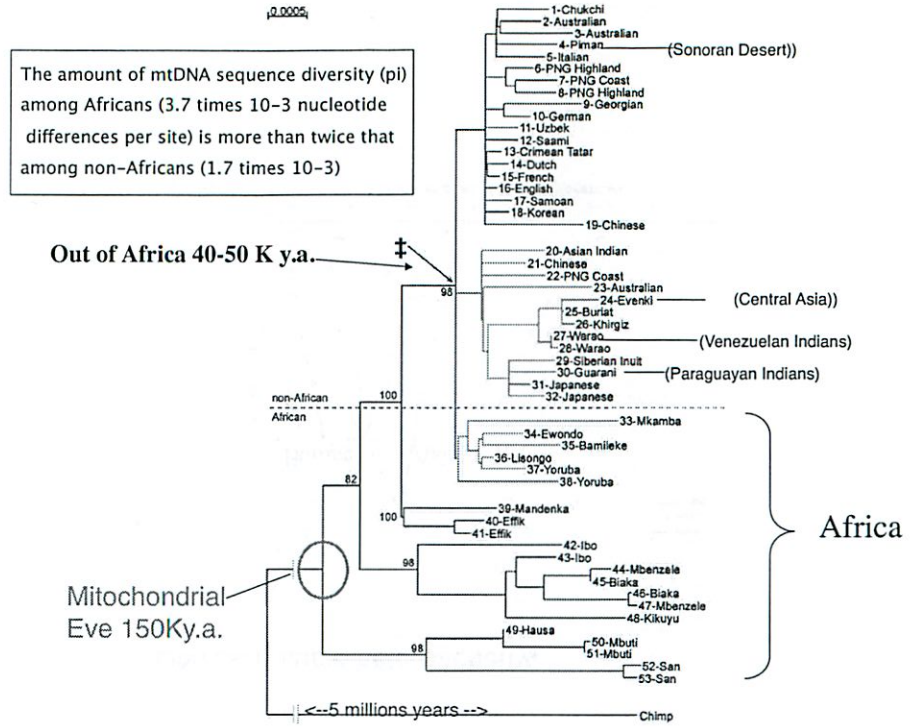
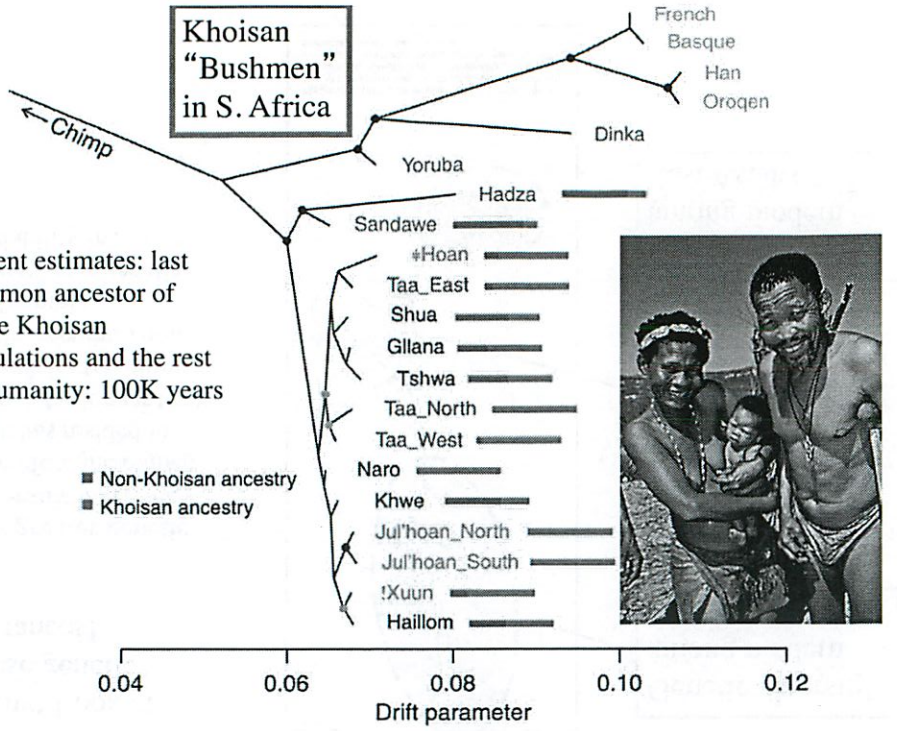


Figure 4. An unrooted network based on mtDNA (HVS1) from 15 populations living in Africa (green), Asia (red), and Europe (blue). Genetic distances were estimated by using the Kimura 2-parameter model, and the neighbor-joining method was used to make the network.

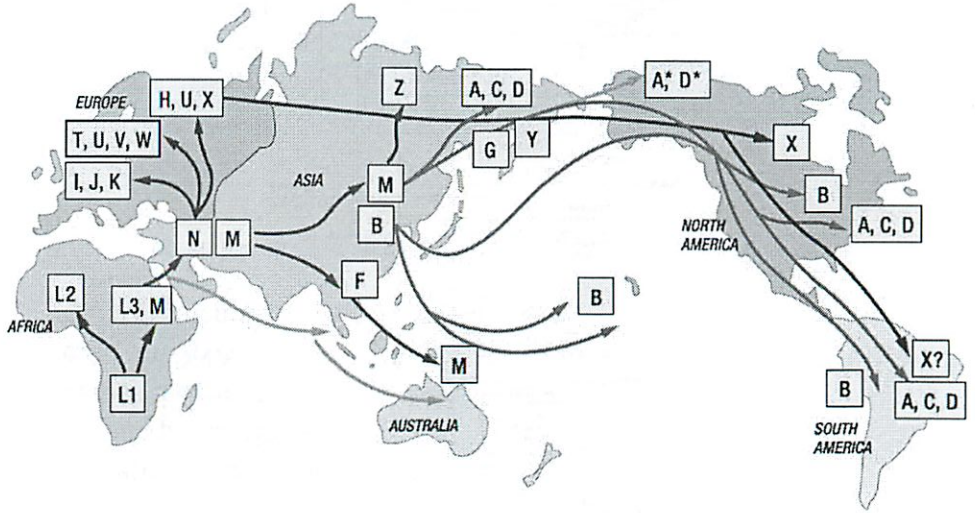
The amount of mtDNA sequence diversity (pi) among Africans (3.7 times 10-3 nucleotide differences per site) is more than twice that among non-Africans (1.7 times 10-3)



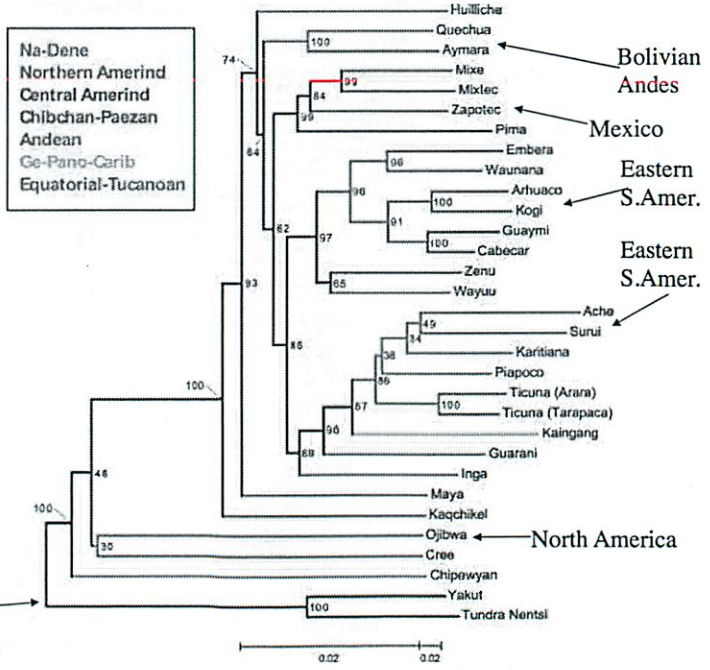


Recent estimates: last common ancestor of some Khoisan populations and the rest of humanity: 100K years ago!

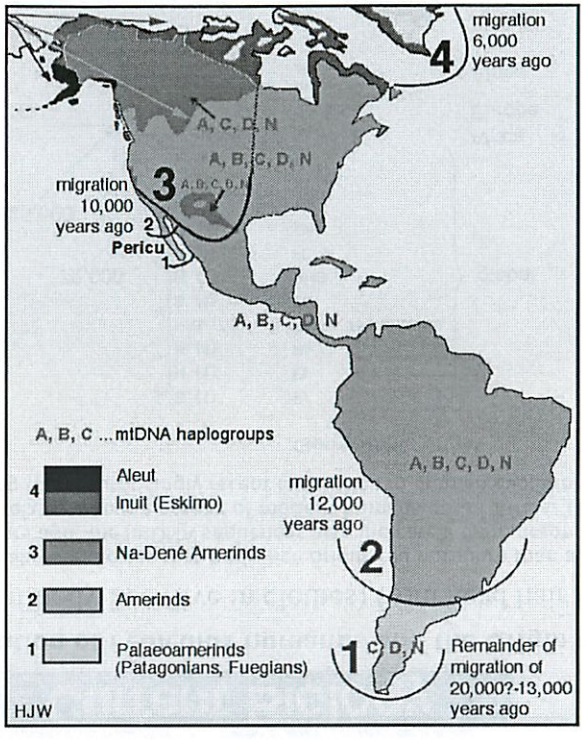
Flow of mitochondrial DNA (mtDNA) genetic types throughout the world

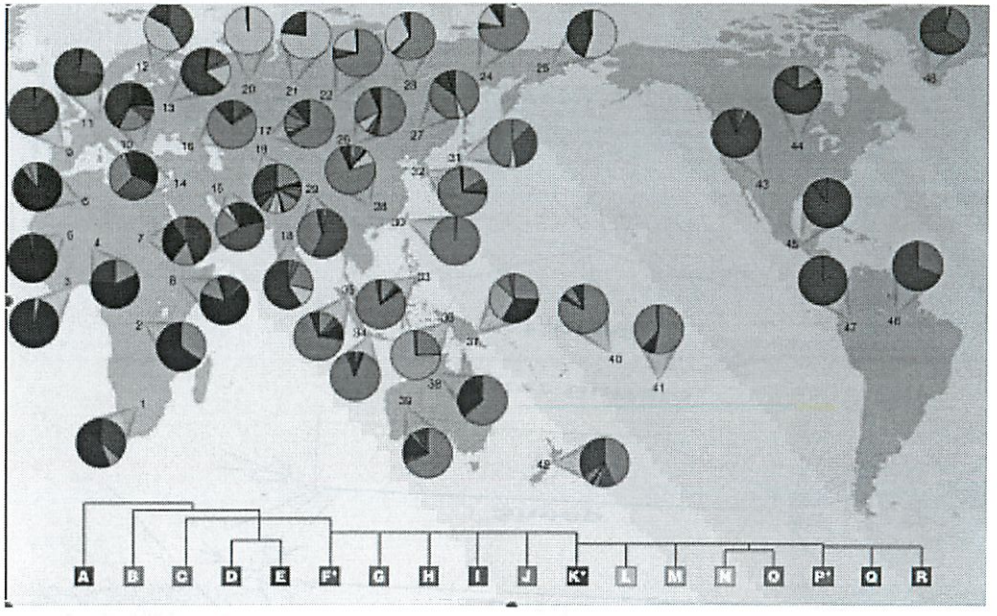


Amerindian relationships



Northeast Siberia





Distribution of Y-chromosome haplotypes across the globe

This pattern echoes the pattern of spread of mtDNA across the globe..

(A haplotype is a constellation of polymorphisms that segregate together.)

In Finland, the earliest known settlement of Homo sapiens is 10,000 years old, located in Korpilahti swamp at Antrea, Karelia. These post-Ice Age settlers from Eastern Europe followed the retreating continental glacier that covered all of Finland.

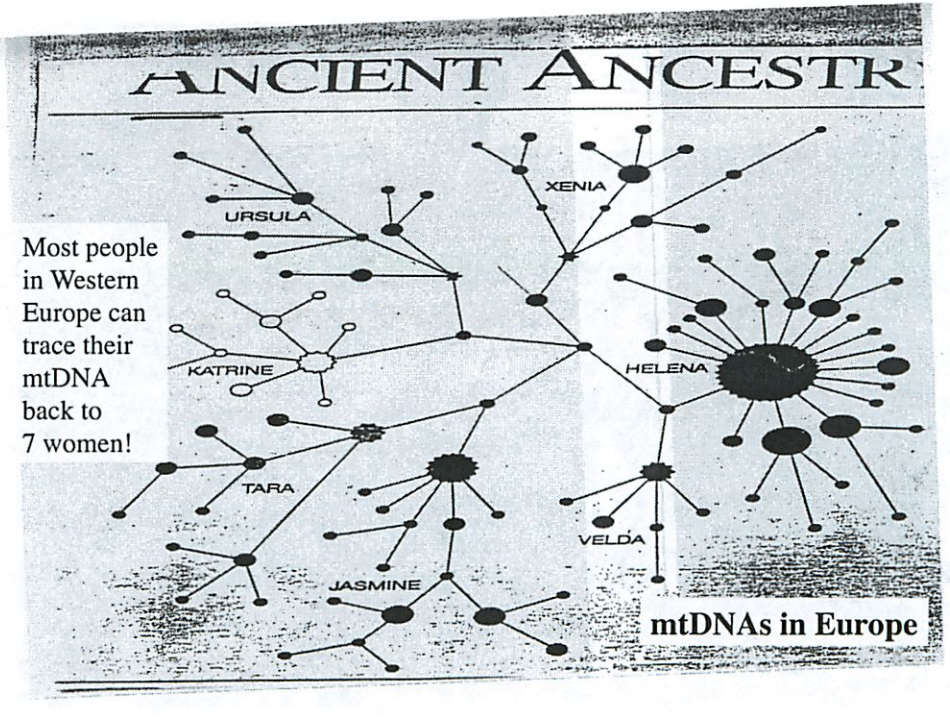
Y-DNA studies of Finland's male gene pool point to two founding populations:

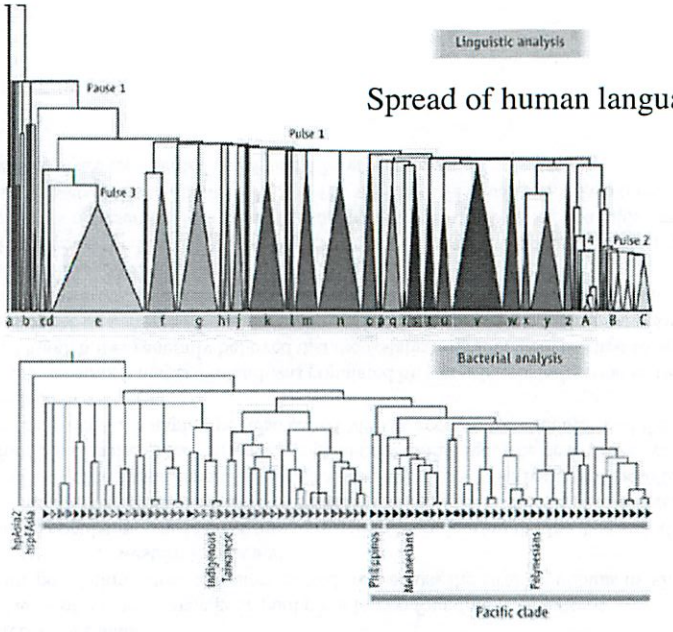
* an Asiatic population whose ancestors moved west across the Ural Mountains in what is now western Russia and
* a European population whose ancestors retreated to Iberia during the Ice Age and afterward migrated northeast into the Netherlands, Germany, Scandinavia, and Finland.

The descendants of the Asiatic population, who carry the Y-DNA N3 haplotype (also called Tat and M46), account for 59% or more of the current male gene pool in Finland. The descendants of the Iberian population, who carry the Y-DNA I1a haplotype (also called M253), account for about 29% of the current male gene pool in Finland.

Scientists are debating whether Finland was populated by a continuous migration or two founding m igrations. Although it is generally believed that the Asiatic migrants were the first to arrive, there is little agreement on the time-frame for the populating migrations. One two-migration theorist argues that the early Asiatic migration from the east peaked around 4000 years ago and the Iberian migration from the west and south peaked about 2000 years ago.

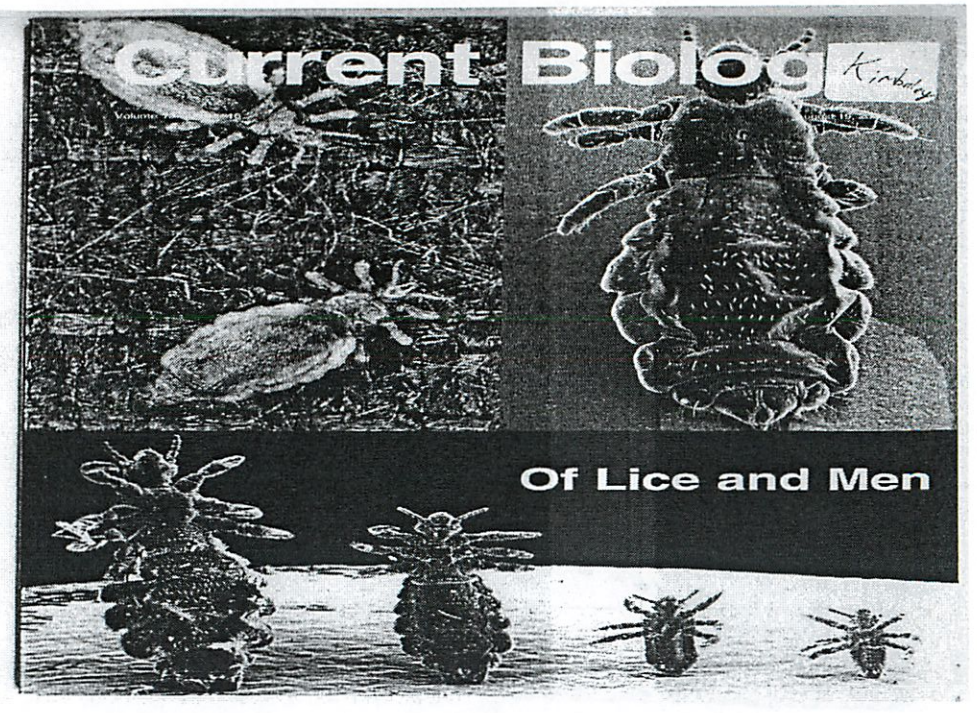
Y-DNA Frequency The I1a and N3 haplotypes are not uniformly distributed across Finland, nor are they the only haplotypes represented. In western Finland about 40% of the male population carry I1a and about 41% carry N3. In eastern Finland about 19% carry I1a and about 71% carry N3. Two other haplotypes -- R1a1 and R1b -- are represented at 7% and 4% respectively.





Spread of human languages

Phylogenetic trees for Pacific human populations. (Top) Tree derived from linguistic data by Gray et al. (Bottom) Tree based on DNA analysis of the bacterium H. pylori by Moodley et al.

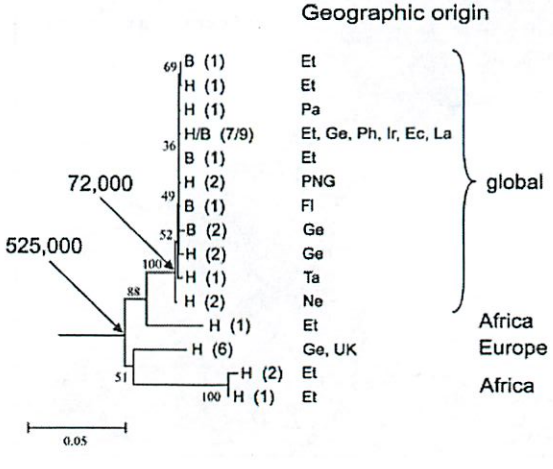


Current Biology

Molecular Evolution of Pediculus humanus and the Origin of Clothing

Divergence of body lice (live in clothes) from head hair lice.

A molecular clock analysis indicates that body lice originated not more than about 72,000 ± 42,000 years ago; the mtDNA sequences also indicate a demographic expansion of body lice that correlates with the spread of modern humans out of Africa. These result suggest that clothing was a surprisingly recent innovation in human evolution.



How often have various species been domesticated from wild animal populations?

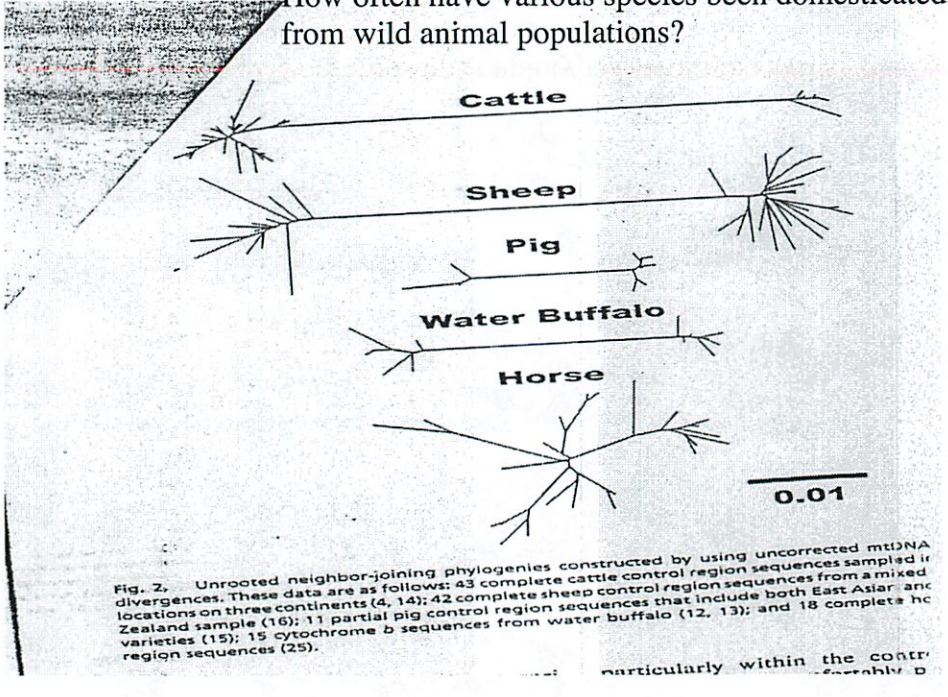
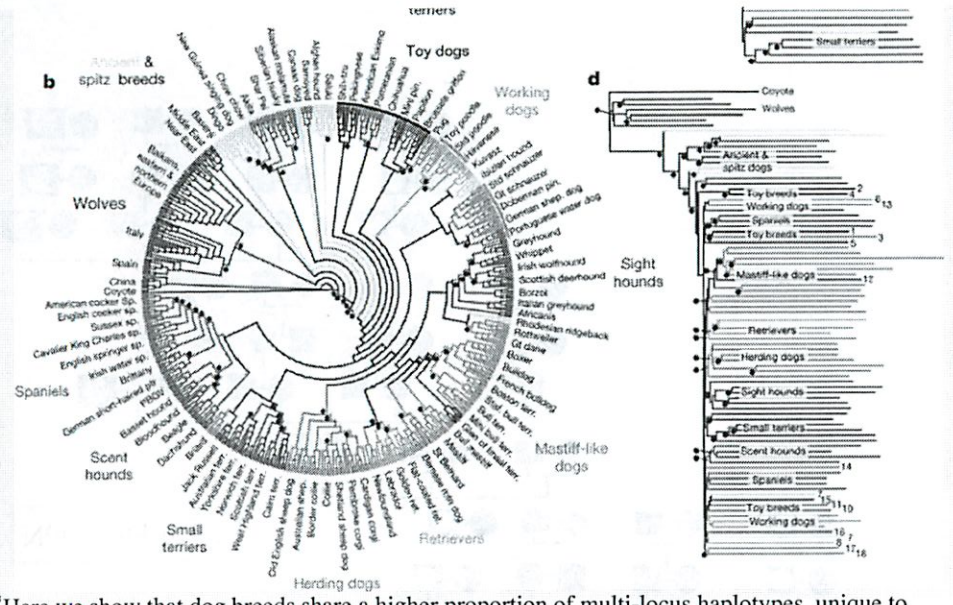


Fig. 2. Unrooted neighbor-joining phylogenies constructed by using uncorrected mtDNA divergences. These data are as follows: 43 complete cattle control region sequences sampled i locations on three continents (4, 14); 42 complete sheep control region sequences from a mixed Zealand sample (16); 11 partial pig control region sequences that include both East Asiar anc varieties (15); 15 cytochrome *b* sequences from water buffalo (12, 13); and 18 complete hc region sequences (25).

particularly within the contr

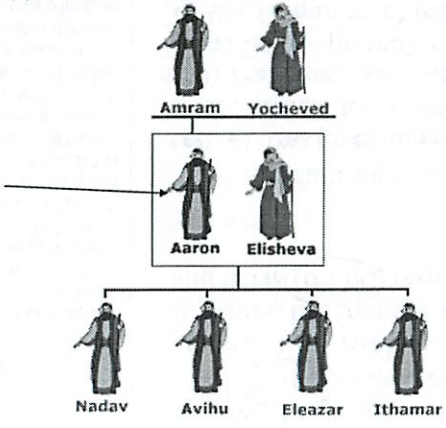


"Here we show that dog breeds share a higher proportion of multi-locus haplotypes unique to grey wolves from the Middle East, indicating that they are a dominant source of genetic diversity for dogs rather than wolves from east Asia, as suggested by mitochondrial DNA sequence data

Nature 464, 898-902, 2010

Exodus 28:1 The Lord says to Moses, "Then bring near to yourself Aaron your brother, and his sons with him, from among the sons of Israel, to ministers as priests to Me--Aaron, Nadab and Abihu, Eleazar and Ithamar, Aaron's sons."

Moses appointed Aaron as the first priest = (Cohen)



Only the naturally born son of a Cohen can become a Cohen.

Different modern depictions of the High Priest in Jerusalem
(Details of clothing given in Exodus) ; priest = cohen

(In addition to the High Priest, there were many other priests (Cohanim) helping in the Temple in Jerusalem.)

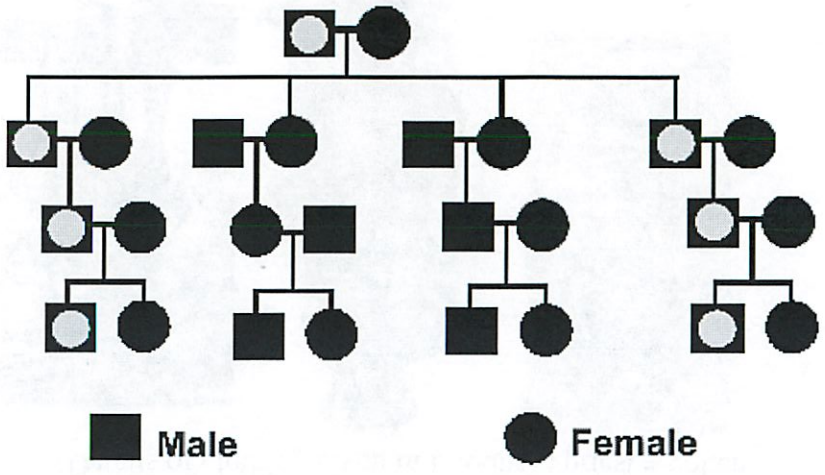
Reconstructed
Image of temple

Arch of Titus
in Rome

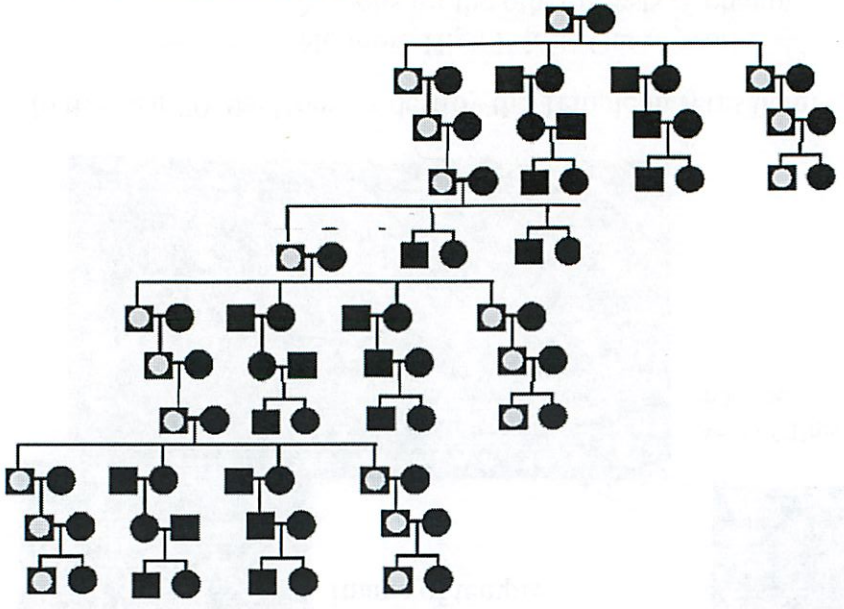
In the year 70, the Romans destroy the Temple in Jerusalem.

→ No more High Priest! Out of work.
No jobs for the other priests (Cohanim)

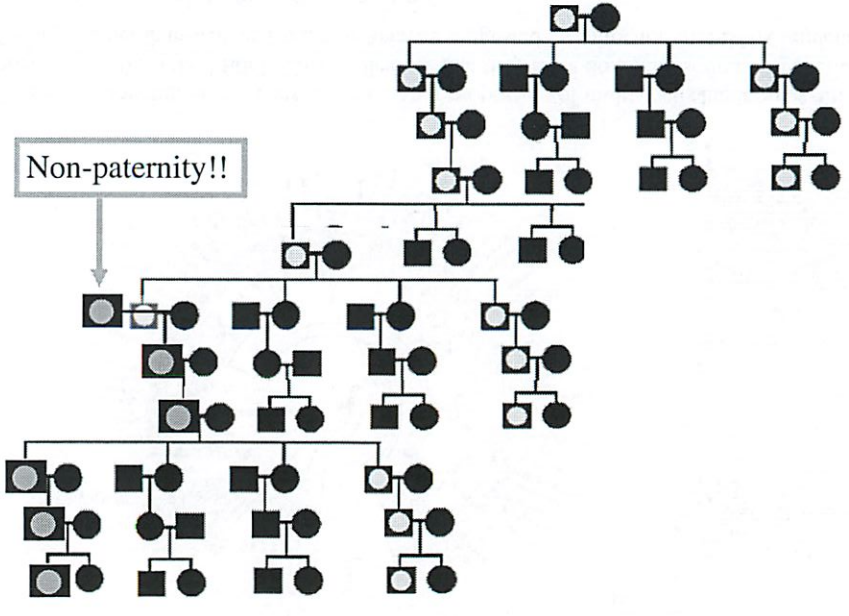
The **Y chromosome** is inherited in the male line, just like the traditional **family name**



The **Y chromosome** is inherited in the male line, just like the traditional **family name**



The **Y chromosome** is inherited in the male line, just like the traditional **family name**



1928 years later

N. African
Middle East

Priests

Central European

Commoners

Origins of Old Testament priests

According to Jewish tradition, following the Exodus from Egypt, males of the tribe of Levi, of which Moses was a member, were assigned special religious responsibilities, and male descendants of Aaron, his brother, were selected to serve as Priests (Cohanim). To the extent that patrilineal inheritance has been followed since sometime around the Temple period (roughly 3,000–2,000 years before present), Y chromosomes of present-day Cohanim and Levites should not only be distinguishable from those of other Jews[1], but — given the dispersion of the priesthood following the Temple's destruction — they should derive from a common ancestral type no more recently than the Temple period. Here we show that although

In the Ashkenazic and Sephardic Cohanim, the modal haplotype (cluster) frequencies are 0.449 (0.694) and 0.561 (0.614), respectively. For comparison, among the Ashkenazic and Sephardic Israelites, the frequencies are 0.132 (0.147) and 0.098 (0.138), respectively.

Assuming a mutation rate of 0.0021 (ref. 4), this gives an estimate of 106 generations, which for a generation time of 25 (30) years gives an estimate of 2,650 (3,180) years before present, dating the coalescence of the Cohanim chromosomes to between the Exodus and the destruction of the first Temple in 586 BC.

Cohens/Cohanim = ~3% of modern Jewish populations)

Y Chromosomes Traveling South: The Cohen Modal Haplotype and the Origins of the Lemba—the "Black Jews of Southern Africa"

The Lemba are a traditionally endogamous group speaking a variety of Bantu languages who live in a number of locations in southern Africa. They claim descent from Jews who came to Africa from "Sena." "Sena" is variously identified by them as Sanaa in Yemen, Judea, Egypt, or Ethiopia. .....

..... Interestingly, one of the Lemba clans carries, at a very high frequency, a particular Y-chromosome type termed the "Cohen modal haplotype," which is known to be characteristic of the paternally inherited Jewish priesthood and is thought, more generally, to be a potential signature haplotype of Judaic origin.

"Here, using complete sequences of the maternally inherited mitochondrial DNA (mtDNA), we show that close to one-half of Ashkenazi Jews, estimated at 8,000,000 people, can be traced back to only 4 women carrying distinct mtDNAs that are virtually absent in other populations, with the important exception of low frequencies among non-Ashkenazi Jews. We conclude that four founding mtDNAs, likely of Near Eastern ancestry, underwent major expansion(s) in Europe within the past millennium."

"Finnish Y-haplotype diversity was even lower than the Native American populations. .....A limited number of closely related founding males may have contributed to the low number of paternal lineages in the Finnish population. In contrast, high levels of genetic diversity for mtDNA and autosomal STRs may be the result of sex-biased gene flow and recent immigration to urban areas from established internal isolates within Finland.

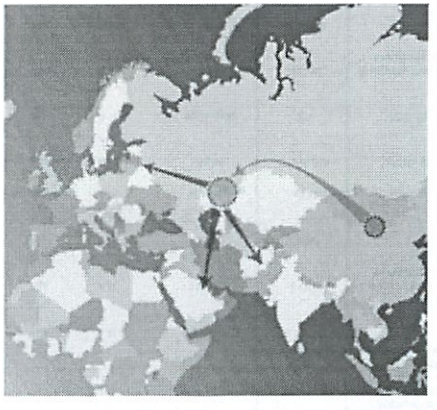
Female gene flow stratifies Hindu castes

Marriages between individuals of equal status are preferred. Matings between a man from a higher varna and a woman from a lower varna are permissible under certain circumstances, in which case the offspring tend to attain a status similar to that of their father. In contrast, marriage of a woman from a higher varna to a man of a lower varna is strongly discouraged. This suggests that women have limited but upward social mobility, whereas men have very little.

Genetic affinities among the lower castes and tribal groups of India: inference from Y chromosome and mitochondrial DNA.

Y-SNP data provides compelling genetic evidence for a tribal origin of the lower caste populations in the subcontinent.....The Indo-Europeans established themselves as upper castes among this already developed caste-like class structure within the tribes.

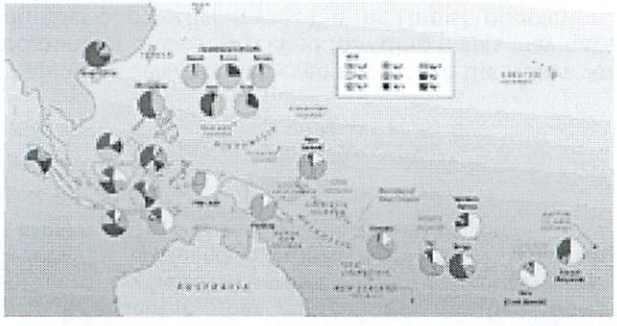
Can you drink milk? Here's why!



2006 study of polymorphisms in the lactase gene (whose product breaks down milk: " A new study suggests that tribes from the Asian steppes (blue circle) migrated to the Ural mountains, where they mixed with locals (red circle), generating a gene variant endowing lactose tolerance that Ural farmers later spread."

The Peopling of the Pacific
Ann Gibbons

Archaeologists, linguists, and geneticists struggle to understand the origins of the bold seafarers who settled the remote Pacific Islands



Although samples of Polynesians are still small, all four studies report a "striking" lack of genetic diversity within the Polynesian haplogroups, suggesting that only a few men founded the Polynesian populations

Enjoyed meeting you all!
Nice to know you!
See you around campus!

Many markers present in startling high percentages--> small founder populations

Table 1 Some Ashkenazi Jewish diseases

Disease (McKusick) number)	Biochemical defect	Gene structure known	Mode of inheritance	Chromosomal locus	Overall heterozygote frequency in Ashkenazim	Frequency of most common mutation in Ashkenazim	Biological fitness of most common homozygotes	References
Tay Sachs disease 272600	Hexosaminidase A deficiency	Yes	A.R.	15q23-q24	3–4%	80% (d)	Lethal	8,9
Gaucher disease 230800	Glucocerebrosidase deficiency	Yes	A.R.	1q21	4–6%	93.5% (d) (in population screening studies) ~70% (d) (among clinically affected patients) See text	At least 1/2 of homozygotes for the common mutation have mild or no clinical illness	7,11
Canavan disease 271900	Aspartoacylase deficiency	Yes	A.R.	17pter–p13	1.7–2%	83% (d)	Almost lethal	10
Niemann-Pick disease 257200	Sphingomyelinase deficiency	Yes	A.R.	11p15.4	1–2%	3 equally frequent mutations (d)	Lethal	(Schuchmann & Desnick, pers. comm.)
Mucolipidosis IV 252650	?	No	A.R.	?	~1%*	?	Lethal but milder variants may exist	19
Bloom syndrome 210900	?	No	A.R.	15q26.1	~1%	97% (i)	Very low	5
Idiopathic torsion dystonia 128100	?	No	A.D.	9q34	0.1–0.3%	>90% (i)	Normal? (heterozygotes)	2
Familial dysautonomia 223900	?	No	A.R.	9q31–q33	3%	75% (i)	Moderately impaired	6
PTA (factor XI deficiency) 264900	PTA deficiency (clotting factor)	Yes	A.R.	4q35	8.1%	2 equally frequent mutations (d)	Almost normal	15
Pentosuria 260800	Xylitol dehydrogenase deficiency	No	A.R.	?	2.5–3%	?	Normal	20

A.R., Autosomal recessive; (d), direct estimate; *, uncertain estimate; A.D., autosomal dominant; (i), indirect estimate.

7.012 Recitation 20 - 2012

Summary of Lectures 33-35:

HIV: HIV is a retrovirus that infects the T_H cells of our immune system. HIV gets into our T_H cells by docking onto a protein called CD4 that our T_H cells have on their surface. Our T_H cells have CD4 on their surfaces because CD4 helps T_H cells recognize the MHC class II molecules on the surface of macrophages, which is the job of a T_H cell. However the HIV virus has evolved to have a glycoprotein on its surface that binds to CD4, thus targeting HIV to T_H cells. This glycoprotein also has the ability to fuse the lipid bilayer of HIV to the cell membrane of our T_H cells, thus dumping the contents of the HIV virus into our T_H cells. The HIV virus harms our T_H cells, thereby depleting our immune system and therefore our ability to fight the virus. HIV also mutates very quickly due to it having a reverse transcriptase that is highly mutagenic. This allows the HIV to be constantly changing the amino acid makeup and the shape of its viral proteins so that our immune system cannot gain immunity to the HIV.

Prions: These are the infective proteinaceous particles. The diseases caused by prions are caused by the defective proteins. The defects arise not from the mutations in the genes that express these proteins, but from errors in the folding of these proteins into the proper three dimensional conformation. The protein with the altered conformation then seems to induce a change in the conformation of the normal protein counterpart so that it also becomes abnormal. The altered proteins have profound effects on its function in the cell. There is a long period of several years between the onset of the disease and the manifestations of the disease symptoms. Prions unlike the bacteria, viruses or nucleic acids cannot be altered or killed through UV irradiation. The transmissible spongiform encephalopathies (TSE), scrapie, kuru, mad cow disease and chronic wasting disease are some examples of prion related diseases.

Molecular evolution: Phylogeny is a branch of biology that explains how are organisms related to one another. The traditional way of determining how organisms are related to one another is to group them according to shared traits (phenotypes). However, another way is to look at the mutations in the coding and non-coding regions between the genome of different organisms: organisms that are closely related will have mutations predominantly in the non-coding regions and will have preserved coding regions since the mutations in the coding regions are deleterious. Over the course of time, due to random mutations, gene sequences randomly drift apart (diverge) unless sequence changes compromise fitness. Therefore, a comparable (homologous) DNA sequence in two organisms will be more divergent in more distantly related organisms. The non-coding sequences will diverge more rapidly than the coding sequences. One can also create an evolutionary tree based on comparative sequencing of 16S or 18S ribosomal RNA. The evolutionary distance between groups of organisms in the tree is proportional to the cumulative horizontal distance between the end of a branch and the node that joins the two groups.

The genetic distance of the human population across the globe can be measured by comparing the sequence of the mitochondria DNA that is always derived from the mother and has a minimum chance of recombination. Similarly the migration of males can be tracked by analyzing the Y chromosome.

Questions

1. HIV can lie dormant in a person's body for many years without causing any noticeable symptoms. Explain how an unrelated infection that activates the humoral response pathway may lead to the development of a full – blown HIV infection.

2. The polymerase that copies the genome of the HIV virus is very error-prone. Why does this make it difficult for the body to mount an immune response against HIV?

3. Diseases can either be inherited or caused by infections by different pathogens. However prions are the exception. What are prions?

4. Do these mutations in genome contribute to progression of prion diseases?

5. A black African tribe, called the Lemba, has an oral history that traces the Lemba lineage to one of the "Lost" tribes of Israel. Explain how and why you might be able to use the Y chromosome of Lemba males to confirm whether the Lemba are of Jewish descent.

7.012 Recitation

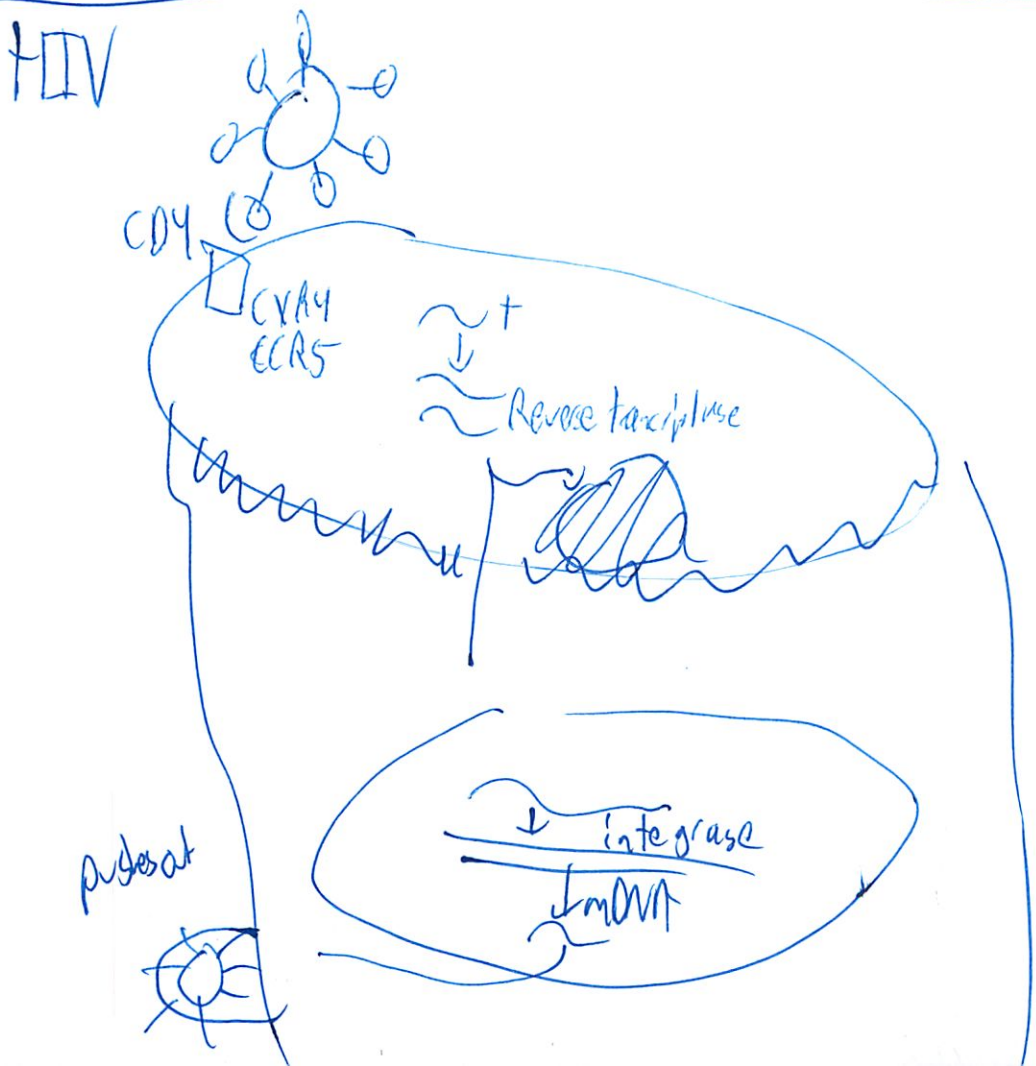
12/11

(50 min late, 5 min left)

↳ just copied board

HIV
Plans
Molecular Evolution
Subject Eval
Final Exam

HIV

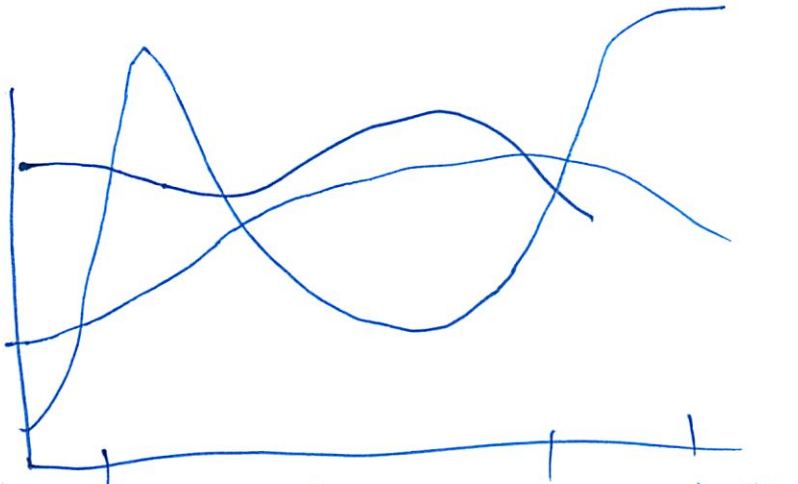


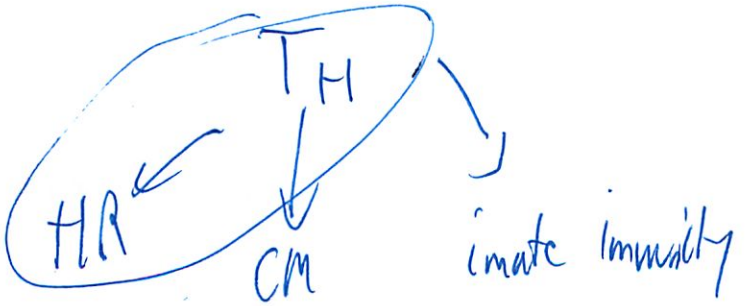
(2)

- Excessive Budding
- CTL mediated
- ↓ HIV antibodies

Disease

- fungal growth main (opportunistic)
- Kaposi's sarcoma

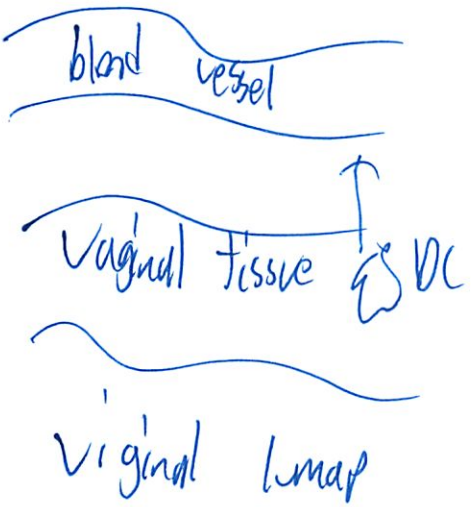




(3)

Modes of Transmission

- Blood
- Mother-fetus
- Sex

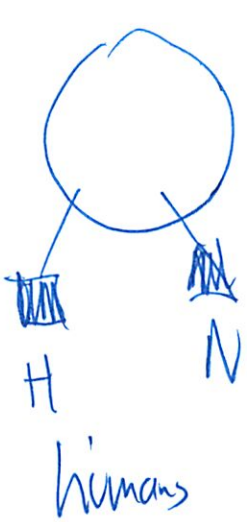


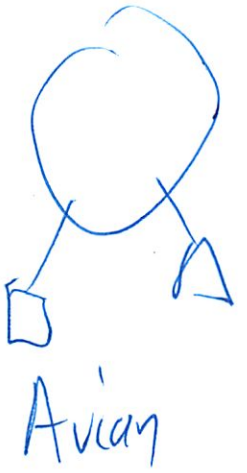
Prions

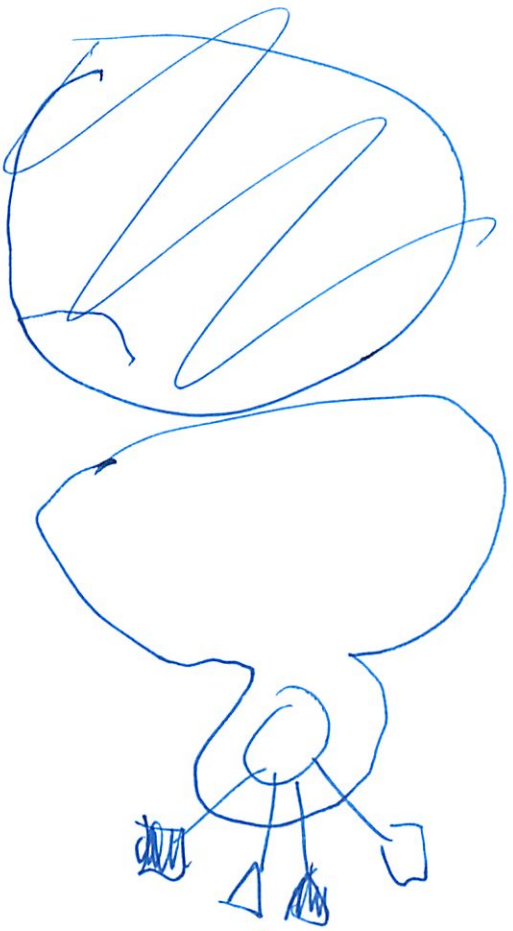
BSE, kuru, Scrapies

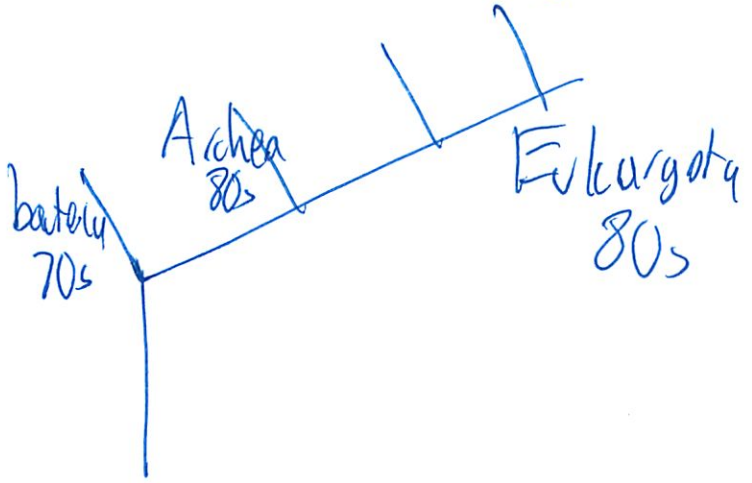
→ transmitted across species

(4)









(5)

Taxonomy

Genetics
DNA
rRNA

Tracing Linage

Paternal
Maternal
Y chromosome
mitchondrial

2012

12/12

L36 Final Lecture

Lander is back

Today: New things/future of bio

Cool things

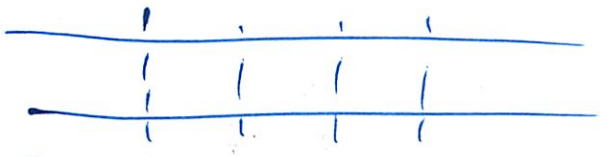
Not on final

- Human History
- RNAs
- Cell Programming

Human History

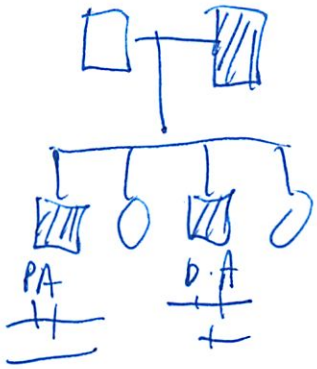
$3 \cdot 10^9$ ——— Human Genome

1 letter in a thousand is the polymorphism rate

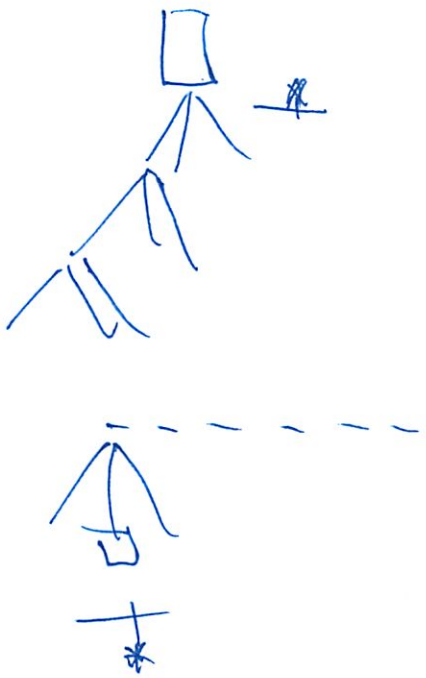


talked about how spelling difference is in genetics

(2)



Can use spelling differences to compare people
Can also tell when the difference arose



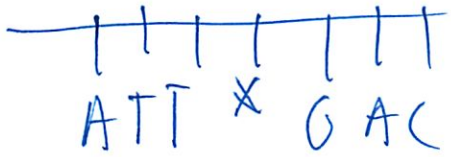
Same spelling difference
But how do we know not happened ind?

:Look for mutations next to it:

(3)

Freq of polymorphism $\frac{1}{1000}$

So in 10,000 bp find ~10 mutations



if mutation arose elsewhere → would have different spelling differences in the immediate vicinity

So the cycil cell anaemic arose only once

But won't genetic recomb mess this up

1% recombination

So 1 million bases

10,000 generations before any crossover in an 10000 BP

This is 200,000 years w/ 20 years age to baby

So can look 50k years w/o risk

(4)

So we can look at recent human evolution.

Sickle cell anemia

Mutation Hemoglobin β

Freq in Africa

Hetrozygoge have protection from malaria

1 thing good

2 things bad

So rose to high freq quickly

└ positive selection

└ One of our best examples...

Lactose tolerance

adult mammals don't drink milk

Mutations in Europeans + East Africa

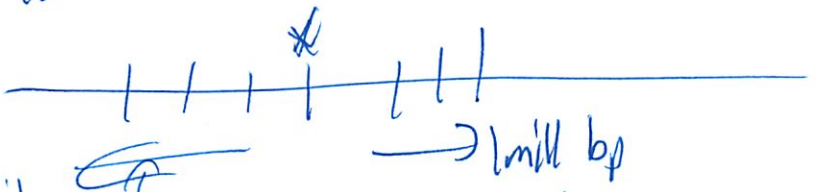
Since extra nutrients

from domesticated animals

positive selection 6000 years ago

(5)

Can look at mutation



nearby markers = haplotype

So we know arose from 1 mutation

Huge chunk of identical DNA
over time recombination will shorten
↳ its a clock, like radioactive decay

Random Selection always happens
Chance of it might arise by accident
But freq is high, and happens quickly
↳ So positive selection

* Find mutation at high freq of population
and on a long haplotype

(6)

There are 300 regions of strong positive selection

Positive selection for skin pigmentation

- light skin in Europe
- thicker hair in Asia
- immune system soped up in Africa

So why are we picking at human history?

Ancient History

humans migrated from Africa ~100,000 years ago

Spread across world

Did they split up into groups?

Can trace back the gene genologies

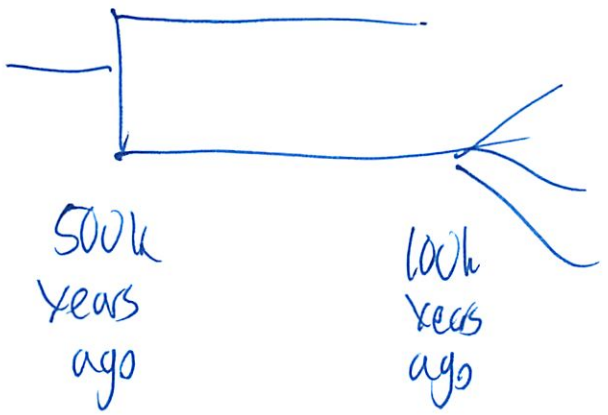
(7)

How do populations split + mix?

Are we the decendents of Neanderthals ---
Look at scraps of their DNA

No connection shown in mitochondrial DNA

But when tools got bette
Saw some spelling diff more common
So we cold not have mated
(i head correct?)



What is a species?

(8) RNA

DNA → RNA → Protein

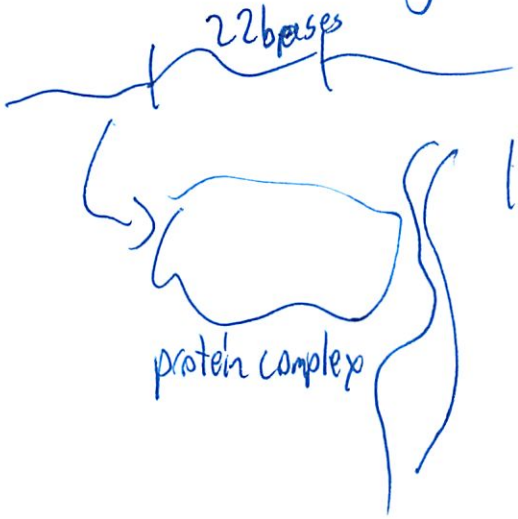
↳ is a <u>vast</u> over simplification

RNA is actually pretty complicated

↳ Not some temporary sticky note

Do a lot of other things besides encoding proteins

1. Short Non-coding RNAs



looks for mRNA that matches it

↳ if it finds mRNA that matches our short RNAs, it distroys the mRNA

So changes the regulation

"Short interfering RNAs"

Could make virus that carries short interfering RNA - shut down genes - equivilent of a mutant

(9)

Built a library at the Broad Institute

2. Long non coding RNAs

Several kilo bases long 1-2000 bases long

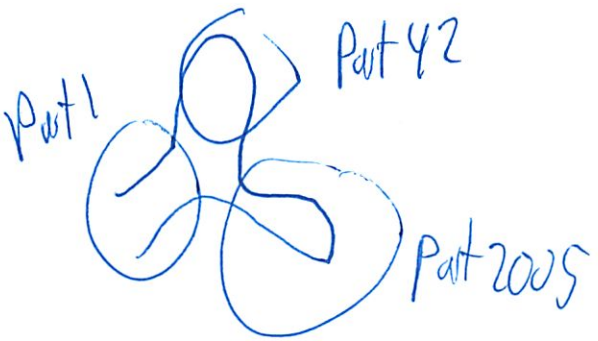
never transmitted to protiens

7 cases known a while back

now 5000 cases

↳ genes that make RNA not transmitted to protien

- They act as binders to several protiens



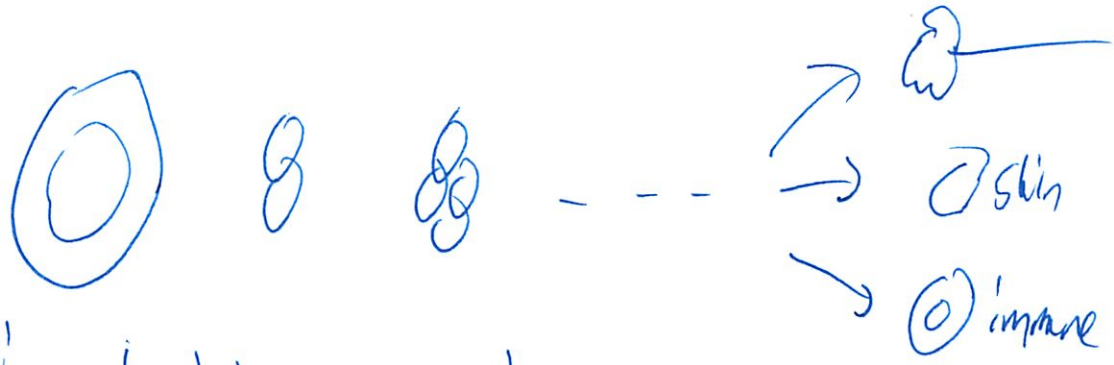
Each part can bind multiple protiens

telomerase = long scaffolding

(10) (not paying attention that well - tired!)

Development + Reprogramming

Cell divides + divides



View is this was a 1 way process

Can't reverse process

└ lawyers can't become artists

But in 60s took at nucleus from frog that could reprogram cell back

We could do it in frogs, but not mammals

Why?

Had to be something in the cell

Though brute force could turn things back!

4 genes alone would suffice!

(11)

This is indeed pluripotent cells

Oct 4, (missed)

Summary

Gone from simple to complicated

This is continuing to move at a fast pace!

Lots of doctors are being overturned