Neurobiology I

10/24

Today: start of engineering life

How can we use genes + proteins to get stuff done

Neuron: nervous system made of neurons

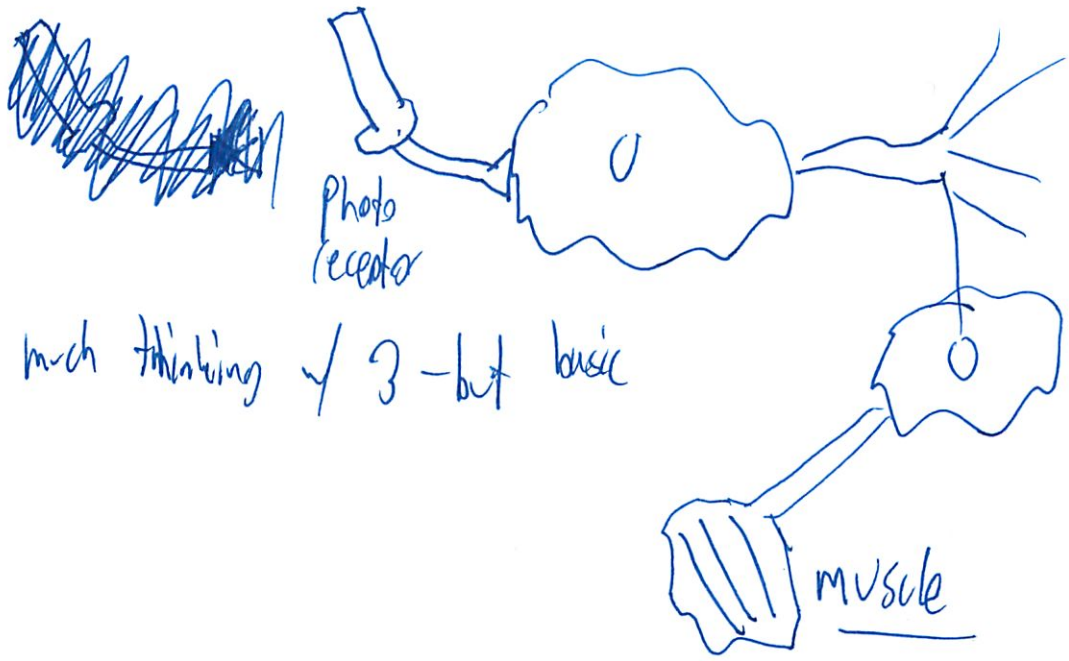
10^{12} in humans

Make connections ~10^3 connections per neuron

So 10^{15} connections

Many shapes + sizes

Some recieve signals from outside



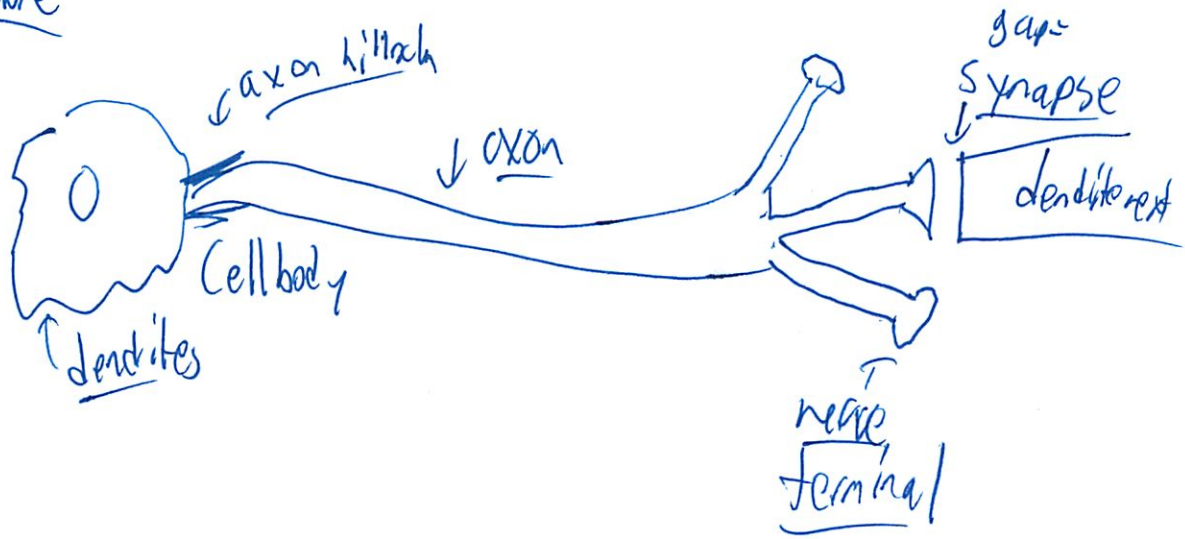
not much thinking w/ 3 - but basic

Receptor

Light Receptor very exact - in light condition
can see 1 neuron

Sand Receptor very sensative as well

Basic Structure



How long are axons?
typicall cell [redacted] 10-20 microns μ
Can be up to 1m
[redacted] giraffe/whales up to 1m

1.) How do receptors transduce signals?
2) How do electrical signals propagate along axons?
3) How do signals transmit across synapse to neurons/muscles?

4) How does pattern of connection give rise to connections
5) How do those patterns arise during development
6) Change during learning?

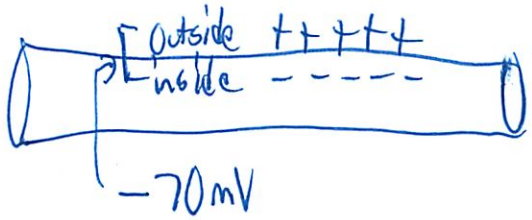
Goal of 7.012 → rewire neurons

7.) How does all this give rise to consciousness?
- we don't know

Transmitting Signals Along an Axon

Very fat axon
like in a squid

measure electric potential inside vs outside



-70mV - do we care?

but across 3nm

electric field - current drop over a difference

$$\frac{-70mV}{3nm} = \frac{.07\,V}{3\times10^{-7}cm} = 200{,}000\ V/cm$$

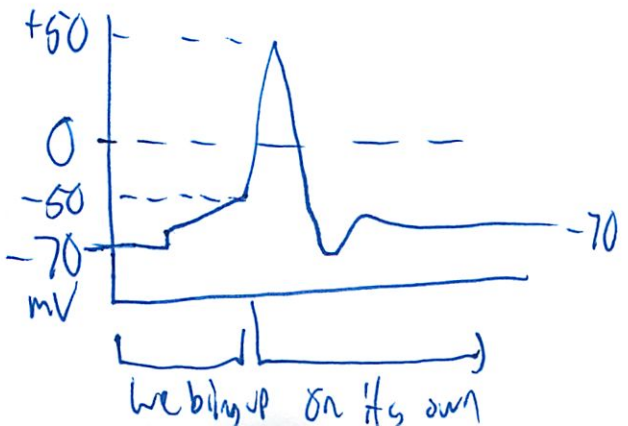
if some dipole moment

- can feel voltage change

-200,000 → 200,000

400,000 V/cm change

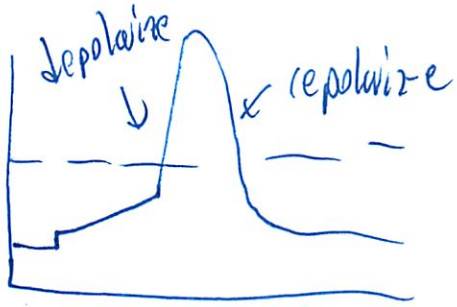
- stuff can change shape as a result of that



= action potential

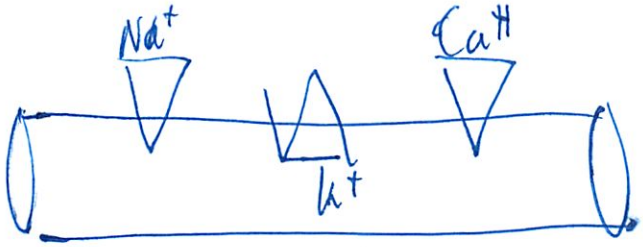
(5)

How does it do that?



Charge Seperation

Charge - Specific ions ⊕ or ⊖

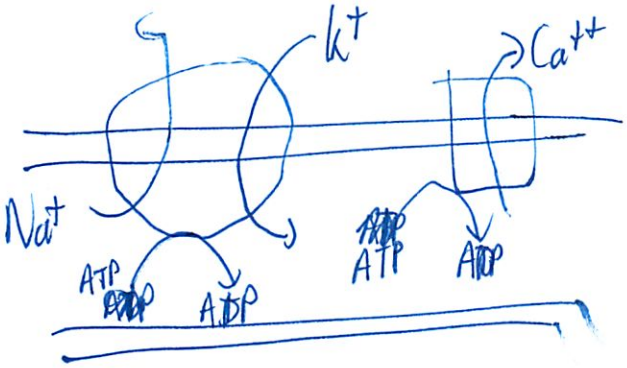


	Inside	Outside
Na^+	12 mM	145 mM
K^+	139 mM	4 mM
Ca^{++}	0.1 μM	2 mM

How did we get ions on one side or other
Something must be moving ions in/out

6

Membrane Pumps



Moving molecules against concentration gradient

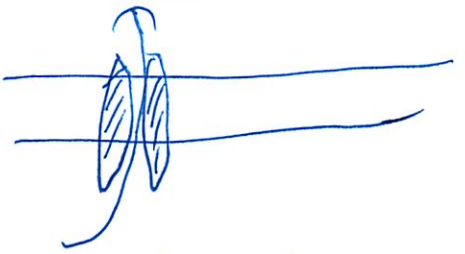
Must pay in ATP

↑ $Na^+ - K^+$ antiporter (ATP driven)

↑ Ca^{++} Uniporter (ATP driven)

(7) Resting Channel

K^+



passive, resting
K^+ channel

└ barn door left open!

At ~~the~~ start

no electric cost to leave - neutral
Higher concentration inside
So favors leaving
So leaves!
Letting ⊕ escape so inside grows ⊖

8

Still favorable from concentration

but growing unfavorable from electrical gradient

---

at some points these offset

−70 mV is where these balance

└ electrical equilibrium potential

How much potassium leaked out?

Math ---

Need to move $\frac{1}{100,000}$ of potassium ions to do this

So $1^{39} \rightarrow 1^{38}$ (potassium cation)

Action Potential

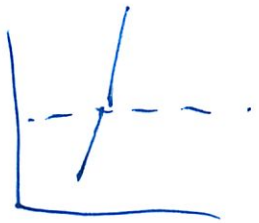
another protein sodium channel

9

normally closed
What causes it to open?
electrical potential
−70mV → −50 mV ← what causes this? exogenous?

Sodium rushes in
- favorable! Concentration
- favorable! electic gradient

Charge rushes in
depolarizes



at 0 no more electrical potential
but concentration still favorable

up to when they balance → at +50mV
└ equilibrium potential

(10)

How restore?

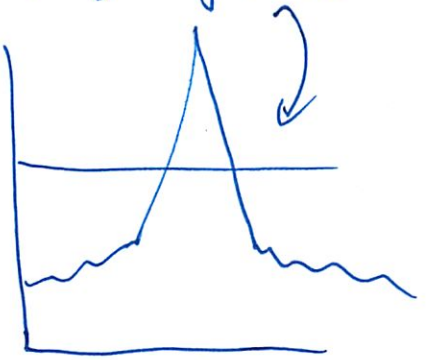
pump → too slow

Potassium channel!

Potassium channel

Opens at +50 mV

Voltage gated



Causes it to go back to −70ms

So together

Voltage Na^+ opens

↓

Voltage k^+ opens

Voltage Na^+ inactivates

(.5ms)

---

all this in 1ms

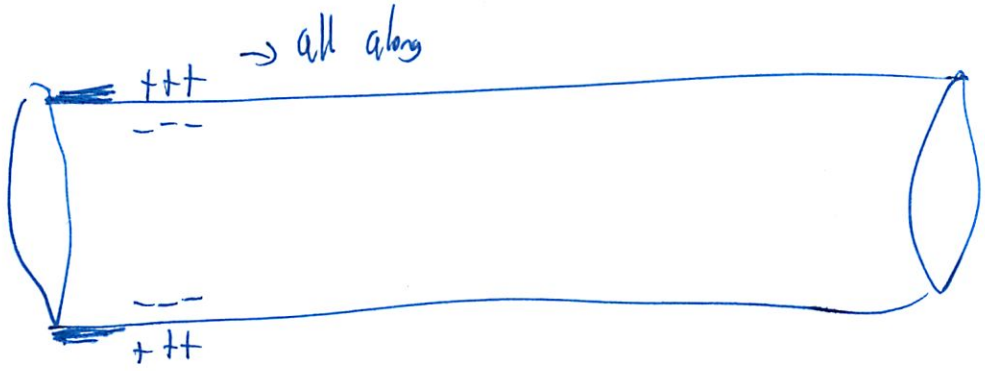
(11)

Shown how patch of membrane works

Need to get signal working along axon

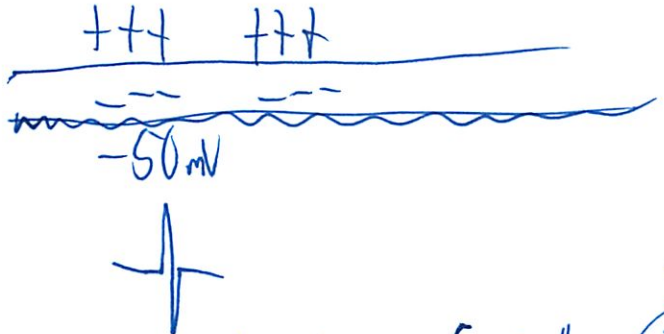
Propagation

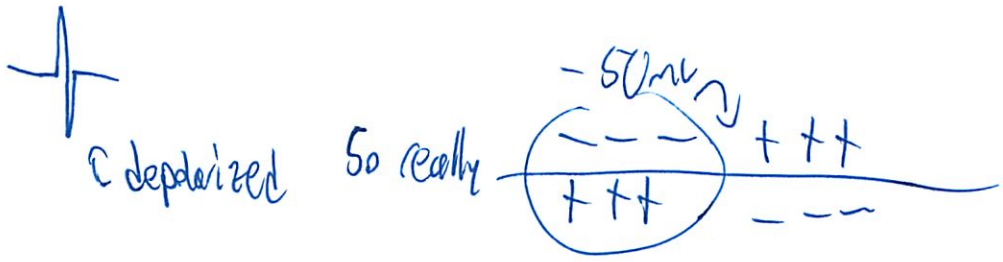
along axon



If depolarize at start to -50mV

Suppose down the road





depolarized So really

(12)

Nan nearby we are at -50mv

Which causes a reaction there

So ~~charges~~ chain reaction!

At end → send back the other way?

Opening voltage gate sodium channels

Inactive for a while at some point

Unidirectionally all charge

Send action potential down 1 axon in 1 direction

help Na^+ channel inactivation helps

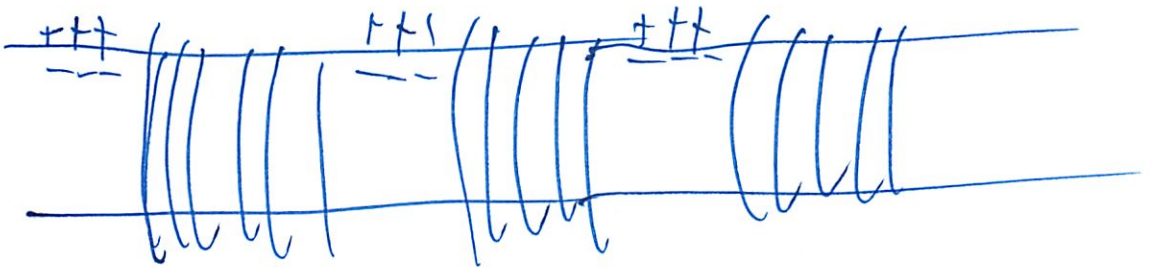
Speeding up

it takes time to send potential

but what if wrap insulator around axon

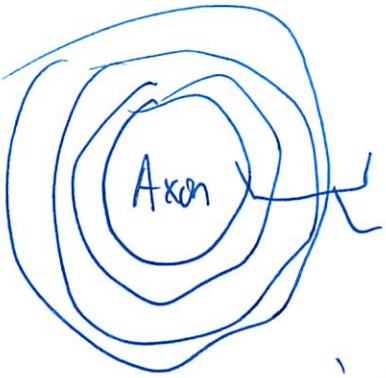
(13)

Still feels charge pulled
but w/o action potential



So jumps across insulator
Saltatory conduction
faster by 100 fold

But humans don't have rubber



- just lipid bylayers
Uses spaces w/ little nodes (Ranvier: correct)

(14)

Multiple Sclerosis attacks this myelin insulator

This slows down your movement

Since slows Conduction

Neurobiology 2

10/26

(on video - watching 10/31)

Last time cell like a ~~the~~ wire

1. Membrane potential

2. Ionic Concentration gradient

Stored energy ready to go
Like a battery

Resting channels

Open at all time, passive

↑ K will always flow
if sep charges
- but no change in electrical potential Once
until electrical offset offsets
conc gradient (-70mv)

(2)

Action Potential

Sodium channel opens

Sodium rushes in to ↓ conc gradient

Then sodium reaches +50 peak - must work against electrical gradient then

Voltage gated Potassium opens, rushes out of the cell back to resting potential

Then channels shut down

Why do we believe there is any of this

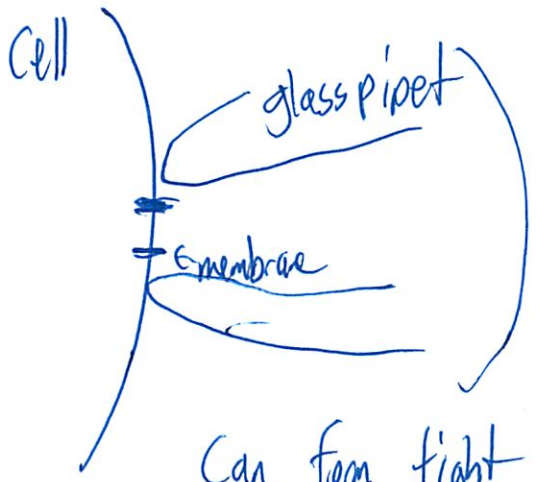
diff concentration of ions in squid axon

Could study bulk properties

must be mv molecular channels there

(3)

Patch clamping Cool trick



Can form tight seal around membrane
+ yank
pulls off patch of membrane

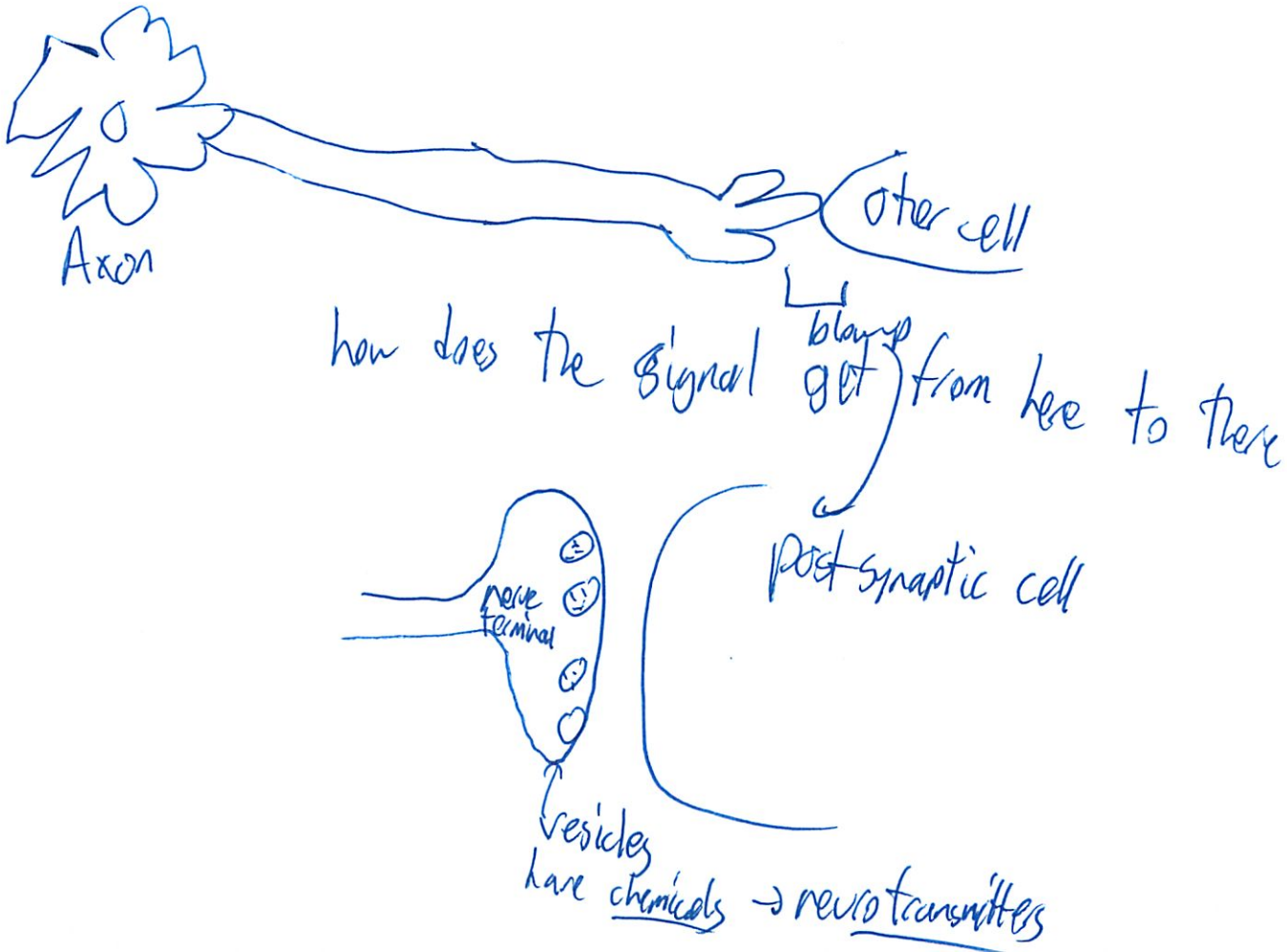
Then can study flow of current through patch

find conductance? 2 channels - quantal (sp)
can be open or closed

Can -70, -50, +50

Can draw singular molecular properties
activate / inactivate fire
which ions flow / don't flow through

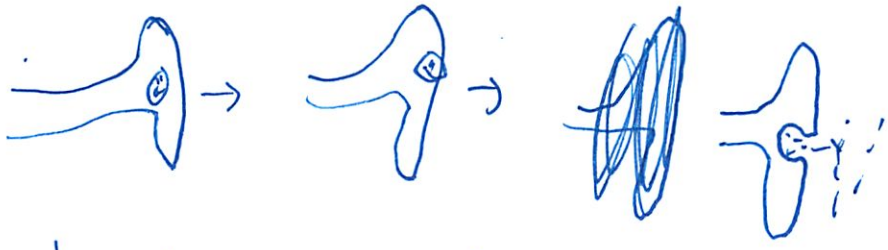
Signaling b/w Nerve cell + another cell



(5)

When action potential comes down

Vesicles fuse w/ membrane + pour out content



How does this actually happen?

Input is electricity

have a cytoskeleton - provides structure

What fraction of ions moving earlier $\frac{1}{100,000}$

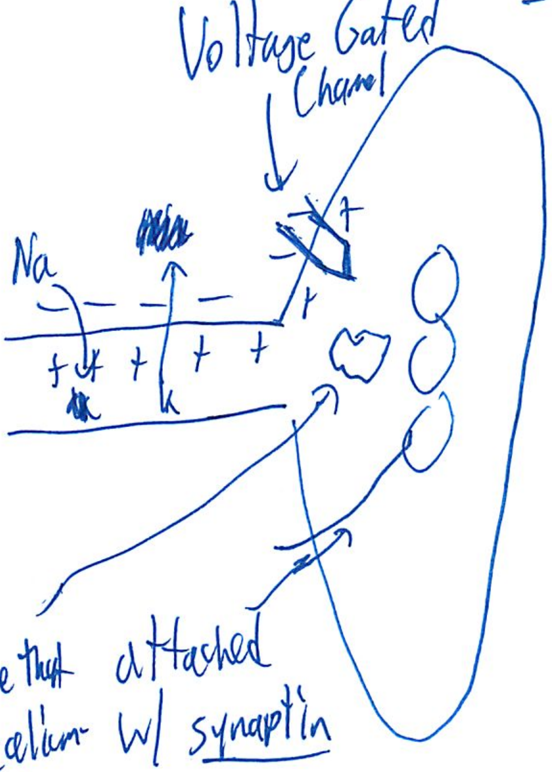
no way to tell - not sitting in membrane in vesicles
- it can't read it directly

must get into cell somehow

Can we use another voltage gated channel for another ion?

6

- uses other ion



Calcium Ca^{++}

- outside 2mM
- inside .5 μM

? 4000x more outside than inside

So voltage gated calcium channel

Calcium comes rushing in!

Nerve terminal notices this!

calcium dep protien kinase - puts phospate groups on things

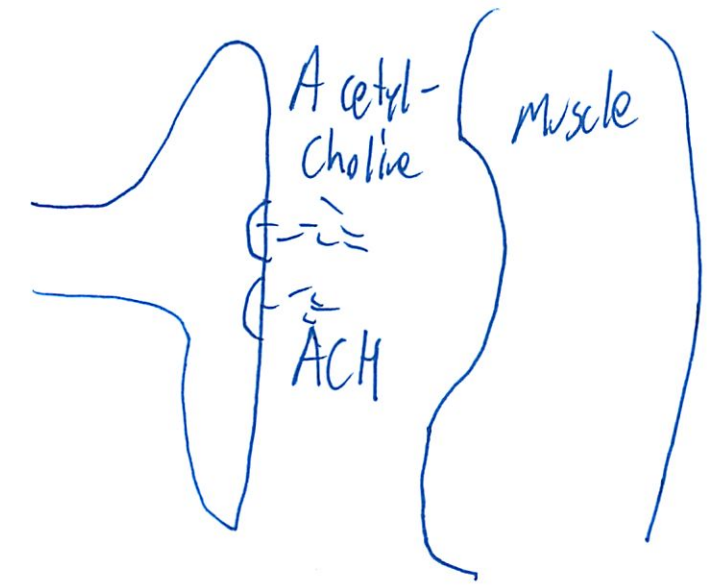
want to stick phosphate group on Synaptin - ℗

Then synaptin chases its form + releases ~~mem~~ vesicles which are then free to go to the membrane + fuse + spill its guts

(1)

Other side of nerve terminal

Neuromuscular junction



Wan want to trigger next cell

Turn Chem signal back into ~~electrical~~ electrical cell

So membrane protien that is a Ach receptor

(8)

Now how to transduce into signal:

Action potential

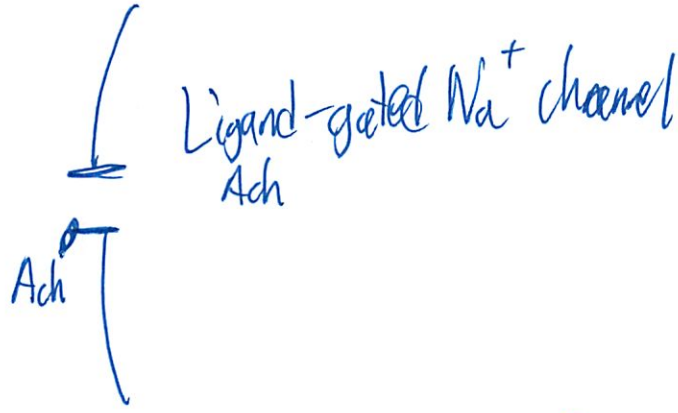
Need to get ions into cell

But only work if Ach is present

↳ Ach-gated channel

To trigger action potential → want to make more (+)

So can open Na^+ channel



When Ach binds, Na^+ flows in

So get whole action potential mechanism
But offset w/ Ligent gated Na^+ channel

(9)

Ions flow in
−70 → −50
muscel contracts

But how to relax muscel?
Must get rid of Ach
Pump it out
Can we recycle? → reuptake it?

Could have enzyme that breaks it down
by Acetylcholinesterase (AchE)

So signal not experienced for too long

While muscel active → holding hand steady
its continuing to release AchE

Must be all Ach production at high enough
rate to keep up

(10)

If AchE inhibited → couldn't release muscel

↑ nerve gas
tokyo subway
Sarin gas

Toxins + Drugs

- Nerve Gas → Sarin
↓
AchE
↓
rigid paralysis

- Tetrodo-toxin (sodium)
Blocks voltage gated sodium channels
Get to -50 + nothing
flaccid paralysis
fugo (esp) puffer fish sushi

(11)

- Curare
  - poison arrows S. American groups
  - irreversibly binds to Ach receptor
  - blocks Ach binding to AchR

- Alpha Bungaro toxin (snakes)
  - irreseblly binds AchR
  - Good Things

Good things to avoid!

Nerve-Nerve Synapses

Before — neuron → (muscle) only 1 neurotransmitter Ach

1 to 1

now — every AP → Contraction
(action potential)

This is a bit more complicated ☺

Complicated dibitic trees

? could be 1000 neuron synapses on a single cell

Must somehow integrate all these inputs
if action potential method on dendrite

ligand-gated sodium channel
$-70 \rightarrow -50$

but <u>not</u> action potential
Voltage gated channels <u>not</u> present on dendrite

So when Na^+ rushes in → it gets a bit more ⊕
Just some time: A bit more ⊕ still

(13)

100 at same time

even more ☺

Need enough so ~~enough~~ gets to -50 at start of axon

then action potential!

Depends on timing

~~more~~ lots of mechanisms resetting it to -70

(Only a transient depolarization)

Analog computer!

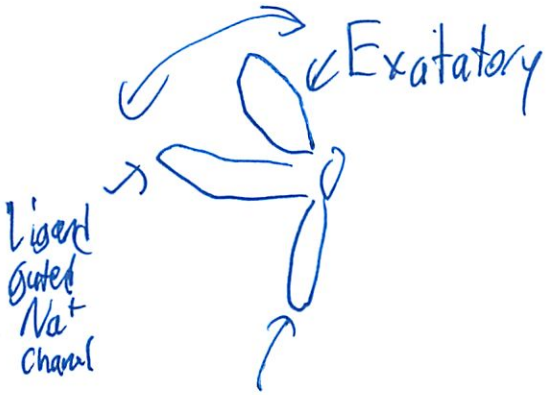
depends on voltage at start of axon

Only control if/when fire → not the magnitude of

(24)

Add ⊕ charge → excitatory

Ligand gated sodium channel



Inhibitory

Neurotransmitter binds to ligand gated Chanel at its own but here claide⁻ rides in ligand Cl^- Chanel gated

Cald write compter Simulation

This is one of 10^{12} cells doing this

15

Wide set for
- excitatory neurotransmitters
  - ie glutamite
- inhibitory transmitters
  - Glycine
- whole range

MSG is a neurotransmitter
- food meat taste

Nitros oxide is neurotransmitter
Leads to summation of signal

7.012

11/2

Immunology 1

(2 min late)

Stem cells - rel undifferentiate cells
Can grow into other cells
(re study)

Hematopoieti system

Whole series of cell types
Specalized cells
2 arms: humoral = soluble substance
↳ fluids
cellular = cellular response

Immunity responsible for ( )
Parents not having their kids vaccinated
These diseases are almost wiped at in US

(2)

Edward Gerard = vaccine

injected a small amt of cow pox

and small pox

Showed could avoid

(I liked video better → could slow down!)

Viruses small subcellular particles

Virus encoded proteins

foreign towards normal body

immune System went to get rid of

↳ antibody molecules

inactivating the infectability of virus molecule

bacteria neutralized

antibodies bind to antibody determinants

Coat the bacteria

macrophages recognizes antibody coted molecule,

Consumes it

(3)

= phagocytosis

τ to eat phage

τ cellular process

Antibody molecules that specifically recognize

Body recognize specific ones → others recognize other things

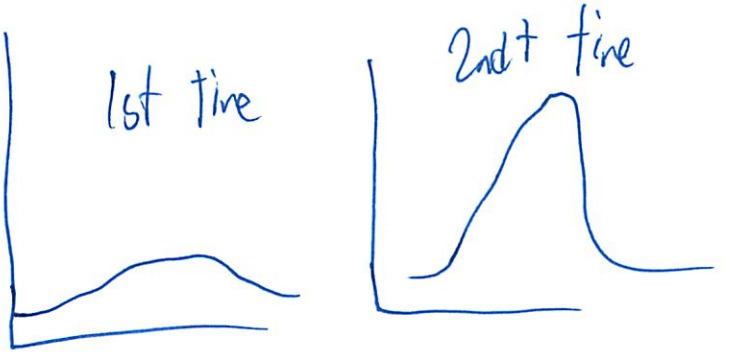
Plot concentration logarithmically

Want specific antibody molecules

titer - [illegible]

immune system remembers exposure

wants response much faster



(4)

Can release antibody molecules
But billion of them
Each Specific for something

SV40
- causes Cytopathic (bubbles) effect
kills cells

- Small cell in middle of each plaque
- but each plaque is larger than a cell
- erodes layer in monolayer
- diameter of plaque could be 100x larger
- decendents of original

Virus Stock
put virus particles on petri dish
initial high concentration of virus
can try to dilute
till countable # of plaques

(5)

(he goes fast)

So can calc initial concentration of virus particles

Diff inds exposed

How concentrated is the antibody in their serum

C - must add a lot of serum

Neutralize plaque forming units

A - dilute sirum

Started very concentrate

then neutralize serum by 100 have

same effective

So can see how much antibody in serum

Antiserum blood

Clotted

Supernatent fluid above

Anti = w/ antibodies; neutralizing virus particles

(6)

Now can we functionally gage concentration of neutralizing antibodies

Exposed to 2nd, unrelated viral agent

No benefit to previous, unrelated infection

No cross immunity

- memory
- long term memory
- specificity

Serum

Specific antigen binding site

Diagram of antibody

heterotetrames 2 heavy + 2 light

disulfide bonds

so covalently in fact

(7)

Specalized antigen binding site

~~part~~ both part from light site + part from heavy site being foreign ( )

kinase has dozens of epitopes

└ subdomains

each can serve as an antigen

more or less amonagenic (sp) - able to provoke an immune respons

↑ epitopes

Antigen (missed)

hand in glove complementarity

Collab venture of heavy + light chain

lots of antibody molecules floating around

part of region same

different

(8)

Variable regions - vary from 1 antibody to the next
↳ the details of their amino acid structure

each version can have a billion copies of each

looks like 2 palms of a hand

---

pic of antibody binding site w/ an antigen

How are antigens made?

cells responsible → plasma cells

thousands a second

Endoplasmin ~~particulum~~ reticulum

Very specalized

Does each plasma cell make 1 or multiple species?

(9)

Cancer cells → ~~mute~~ linear decendents/monoclonal
- tumors

"transformation"

but liver or polyclonal
monoclonal
↓ ↓↓↓↓
↓
O

---

multiple myeloma

Usually millions of antibodies
migrate as smear - since each slightly diff

immunoglobulin

but looks like only making 1 kind of antibody
tells why people die from m. myeloma
Antibodies crowed out by 1

(10)

So 1 cell is reproducing a great deal

So original cells only make 1 antibody as well too

Each plasmid cell makes its own antibody

Multiple myeloma → one plasmid cell reproduces uncontrolably

Lets look at normal immune response

Antigenic determinant

antibody binds to virus particle

B cells are precursors to plasmid cells

B cell knows its recognizing antigen

So undergoes clonal expansion

* provoked by antigenic determinant

(11)

Also memory cells

Undergo expansion too
retreat into bone marrow
sit there for 10 - 30 years
reemerge when 2nd exposure

Myeloma cells start expanding uncontrolably
not due to antigenic determinant
Since ~~cells mutated~~ —
mutated genes

Monoclonal antibody

take advantage of our above knowledge
~~drawn~~ (he never explained)

Next time ← how to get rid of problem
w/ monoclonal antibodies

7.012 Immunology

The immune system has many different "arms". We will focus on its **humoral** and **cellular** arms.

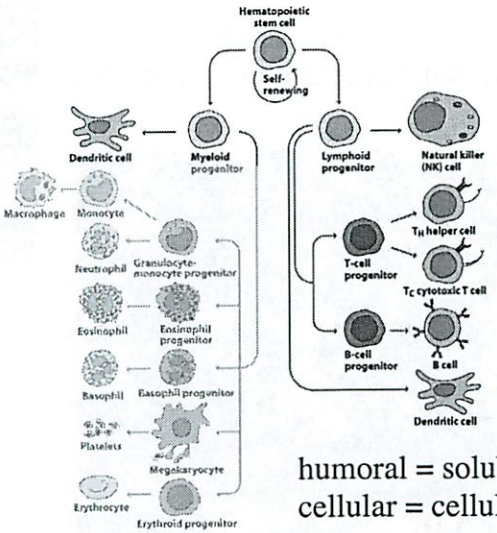


Figure 2-2
Kuby IMMUNOLOGY, Sixth Edition
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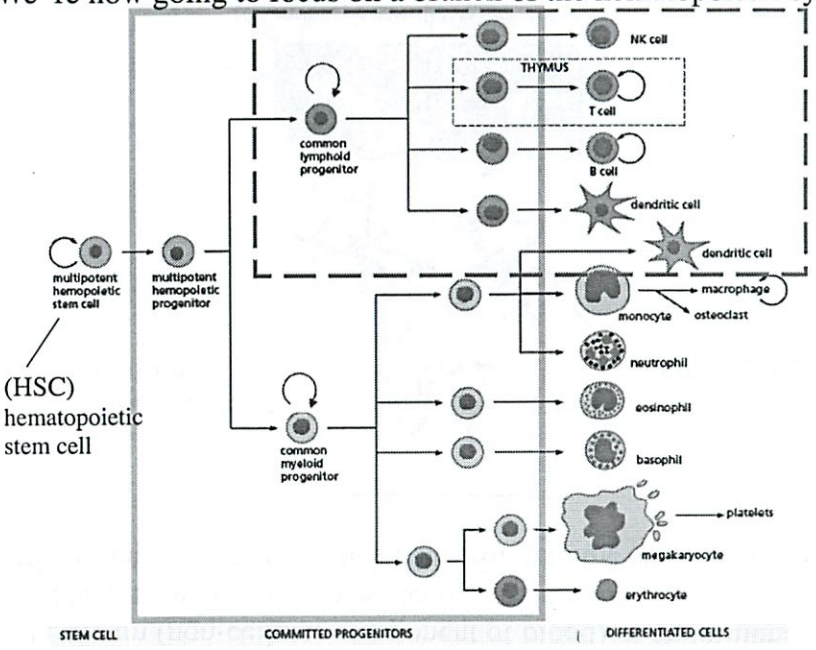
humoral = soluble substances
cellular = cellular responses

We're now going to focus on a branch of the hematopoietic system.



(HSC) hematopoietic stem cell

What is the immune system responsible for? For example:

These infectious diseases have been largely eradicated in industrialized countries through large-scale **vaccination** programs. **Vaccination** gives long-term immunity against these infectious agents. It mimics natural exposures to various infectious agents. Immunity implies that the immune system has a long-term memory of such exposure.

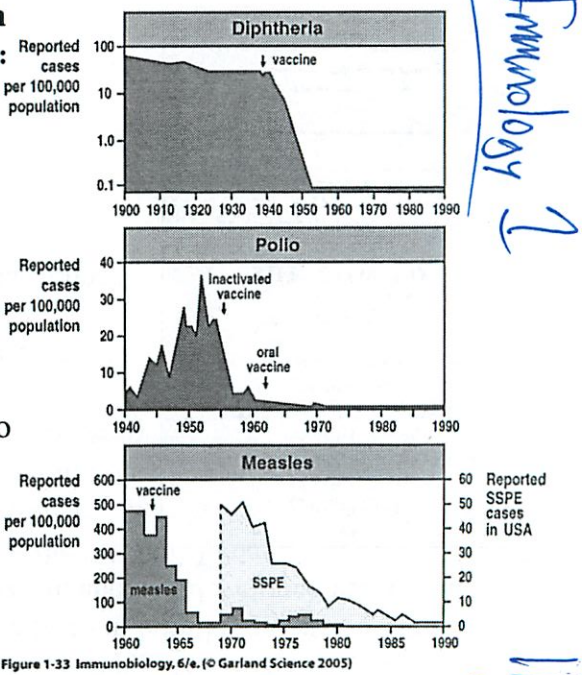


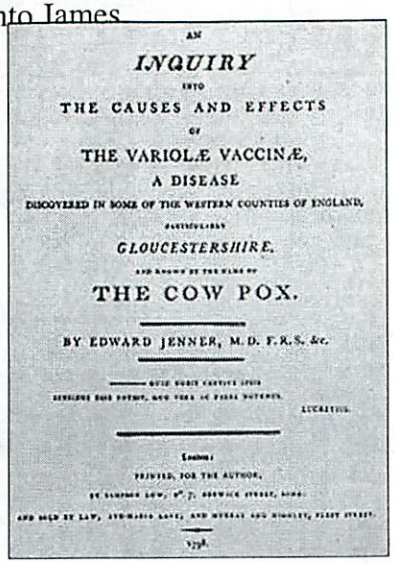
Figure 1-33 Immunobiology, 6/e. (© Garland Science 2005)

L22 Immunology 1

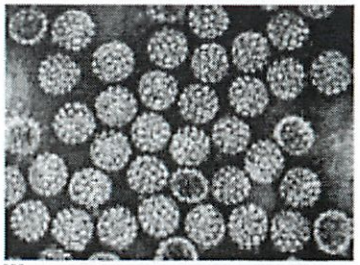
4/11

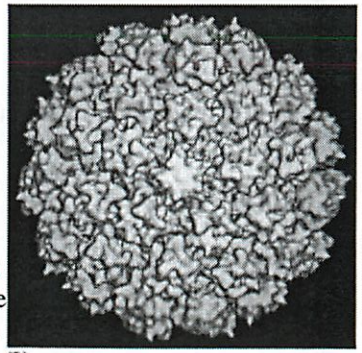
Edward Jenner and the first vaccination.
(vacca in Latin = cow)

Jenner decided to try out a theory he had developed. A young boy called James Phipps would be his guinea pig. He took some pus from cowpox blisters found on the hand of a milkmaid called Sarah. She had milked a cow called Blossom and had developed the tell-tale blisters. Jenner 'injected' some of the pus into James. This process he repeated over a number of days gradually increasing the amount of pus he put into the boy. He then deliberately injected Phipps with smallpox. James became ill but after a few days made a full recovery with no side effects. It seemed that Jenner had made a brilliant discovery.


AN
INQUIRY
INTO
THE CAUSES AND EFFECTS
OF
THE VARIOLÆ VACCINÆ,
A DISEASE
DISCOVERED IN SOME OF THE WESTERN COUNTIES OF ENGLAND,
GLOUCESTERSHIRE,
THE COW POX.
BY EDWARD JENNER, M.D. F.R.S. &c.
LUCRETIUS.
PRINTED, FOR THE AUTHOR,

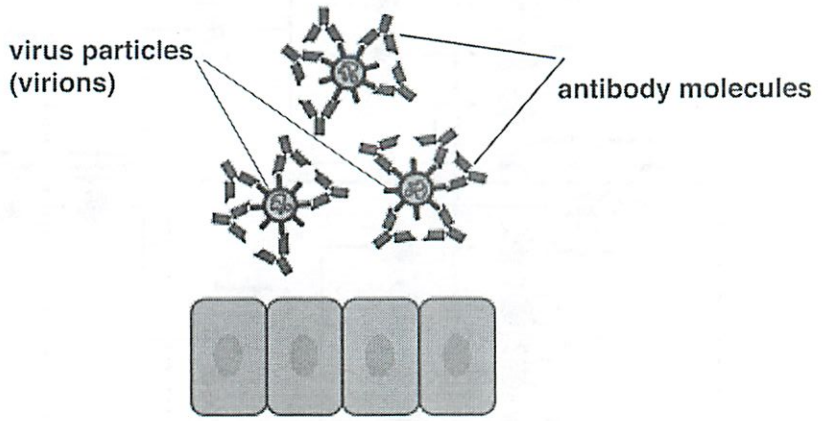
We can be infected by a of infectious agents. Here are some virus particles -- virions -- of the sort that we encountered earlier this semester. The capsid proteins of the virus particles represent **antigens** that may be recognized as foreign proteins by the immune system. Their "foreignness" is critical to this recognition. Since proteins are composed of amino-acids, this foreignness must derive from amino-acid sequences that are present in the virus but not in the infected host.


(A)


(B)

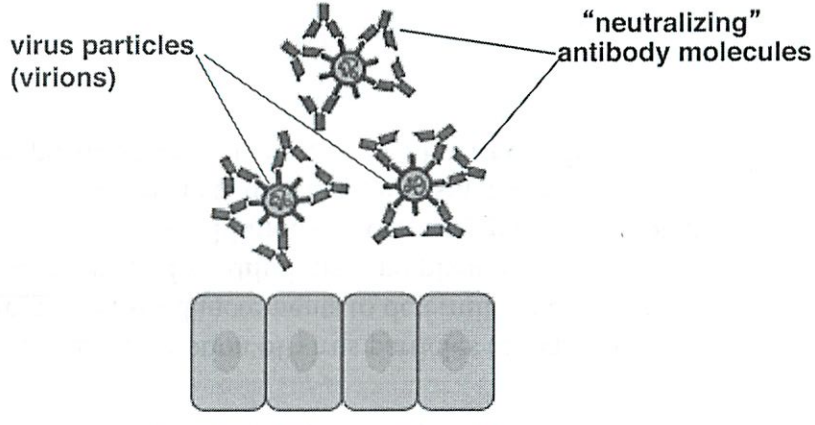
Viruses are only one of a series of infectious agents that can infect us -- also bacteria, fungi, mycobacteria, and larger parasites such as various types of worms.

In the serum (non-cellular component of blood) of an immune individual, soluble antibody molecules bind to the surface of virus particles and prevent them from adsorbing to the surface of target cells.

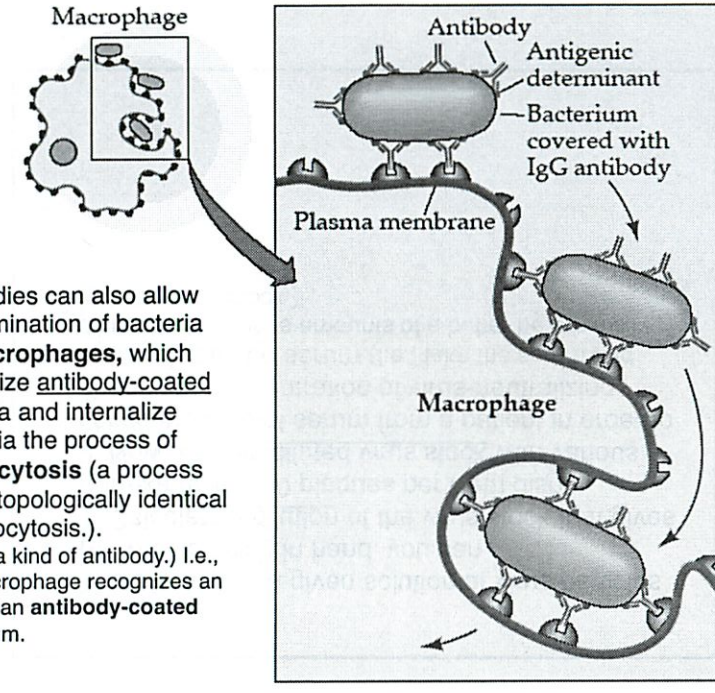


(A) antibody prevents viral adsorption

The serum that contains these virus-binding antibodies is termed an **antiserum** and the virus particles, once bound by antibody molecules, are said to be **neutralized** by the antiserum.

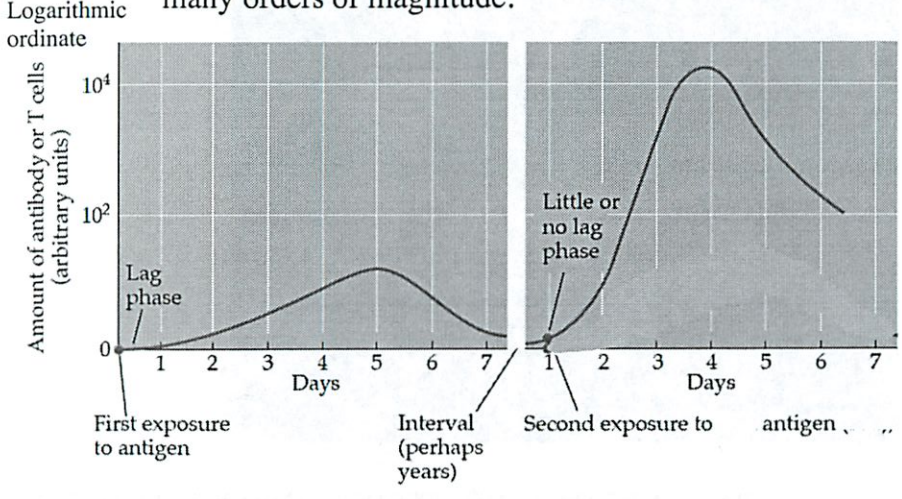


(A) antibody prevents viral adsorption



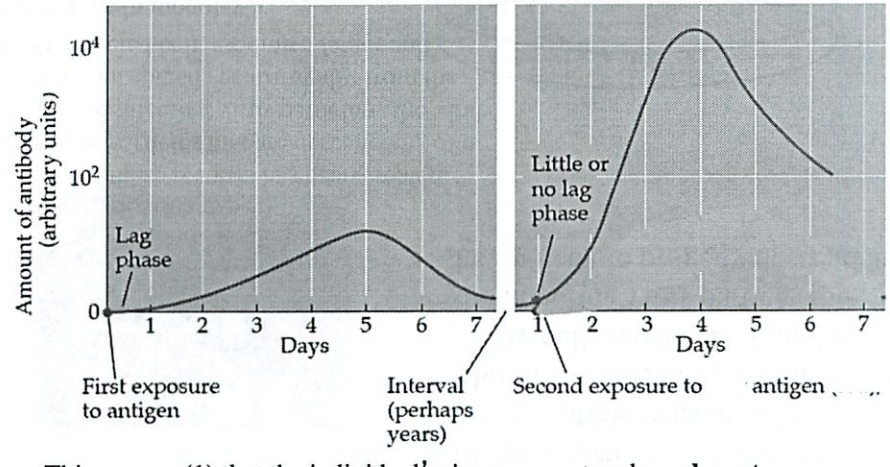
Antibodies can also allow the elimination of bacteria by **macrophages,** which recognize antibody-coated bacteria and internalize them via the process of **phagocytosis** (a process that is topologically identical to endocytosis.).
(IgG is a kind of antibody.) I.e., the macrophage recognizes an engulfs an **antibody-coated** bacterium.

Antiserum titer, i.e., antibody concentration (note log scale on ordinate) can vary enormously over many orders of magnitude.



Assume for a moment that the infectious agent is SV40 and that the **titers** of SV40-neutralizing antibodies in an individual's serum are being measured.

These graphs indicate that the **2nd time** that an individual is exposed to SV40, s/he will produce far higher titers of anti-viral antibodies than the 1st time, and do so more rapidly.



This means: (1) that the individual's immune system has a **long-term memory** of this earlier exposure; and (2) that associated with this memory is a **heightened ability** to respond effectively to the infectious agent. .

How can we **measure antibody titer**? To begin, we measure viral titer: e.g., when SV40 infects a permissive host cell (which allows it to replicate), it creates a cytopathic effect (i.e., cell killing) and after 2 days cells die.

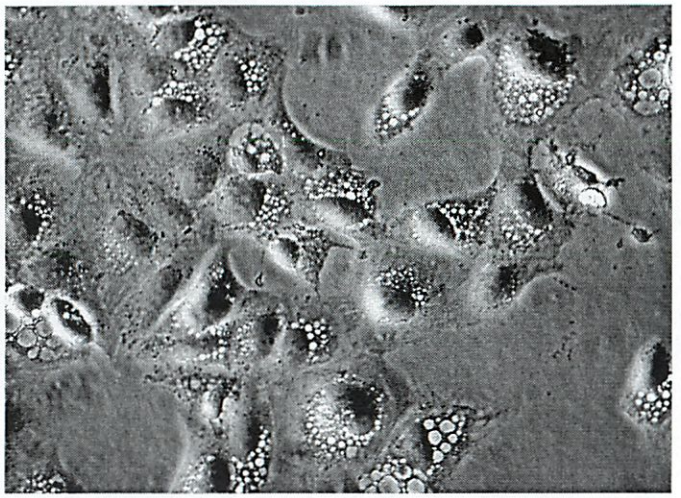
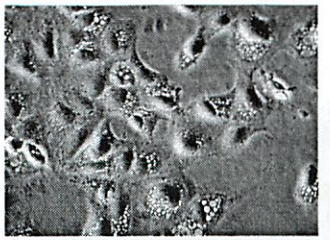


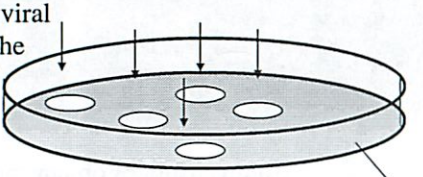
Figure 3.10b *The Biology of Cancer* (© Garland Science 2007)

How can one measure the concentration of "live" (biologically active) virus particles in a fluid?

If a virus has a **cytopathic effect** on cells, can introduce a solution of virus particles onto a monolayer of susceptible cells, and look for **plaques --** holes in the monolayer where cells have been killed by the infecting virus particle and its immediate progeny descendants.

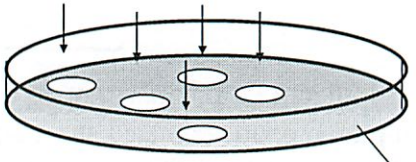


Each one of these plaques has been created by the virus particles that are descendants of a single initial infecting virus particle -- the viral infection then spreads centrifugally from the initially infected cell, eroding a larger and larger plaque in monolayer.



One can take a solution of virus particles (a "virus stock) and dilute it in ten-fold increments to determine the virus titer.

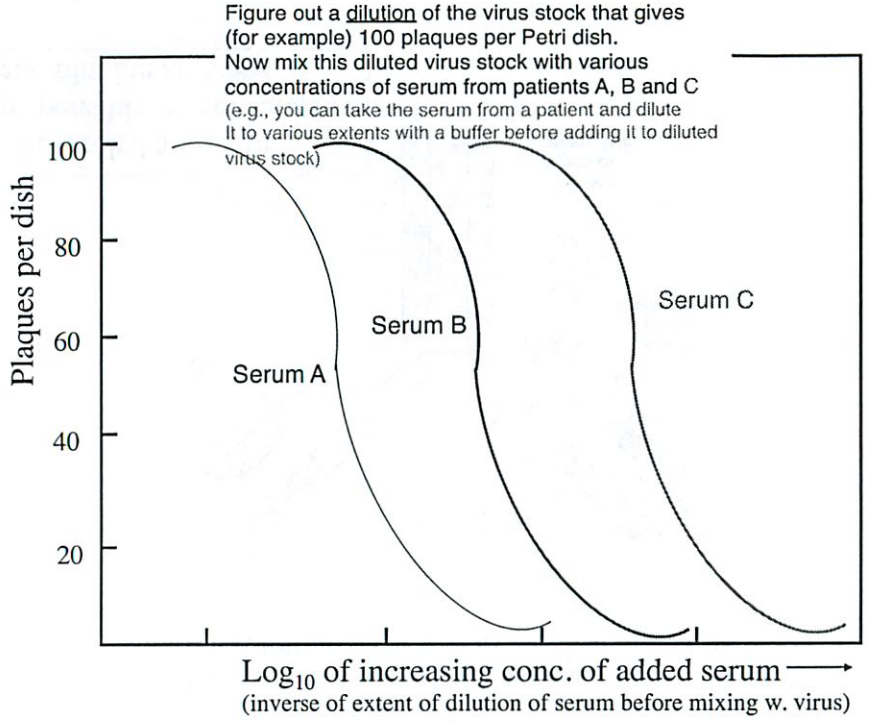
a. too many 10-fold dilutions - no plaques;
b. too few 10-fold dilutions -- too many plaques to count)
c. At an intermediate dilution, get a small but reasonable no. of plaques to count, e.g., 10 to 30 per Petri dish.

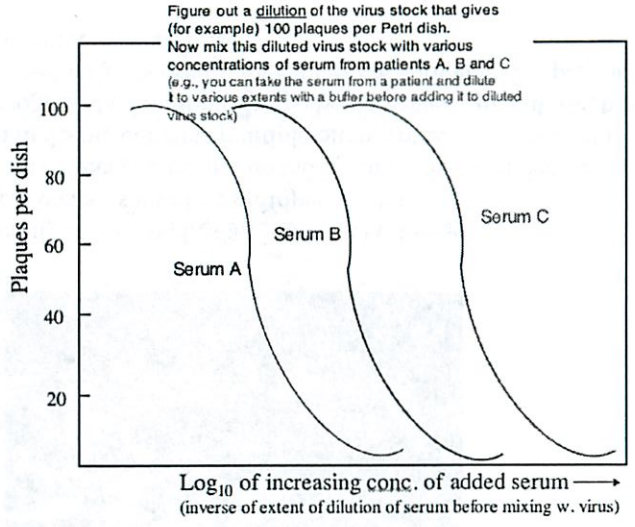


Each one of these plaques has been created by the virus particles that are progeny of a singe initial infecting particle

Therefore, with a given solution of virus particles (a "virus stock") in hand, you can

1. Calculate a dilution of the virus stock that gives (for example) 100 plaques per Petri dish.
2. Now mix this diluted virus stock with various concentrations of serum from a patient in order to gauge the concentration of virus-neutralizing antibodies in the serum (I.e., take the serum and dilute it with various amounts of a buffer before mixing with the virus stock.)



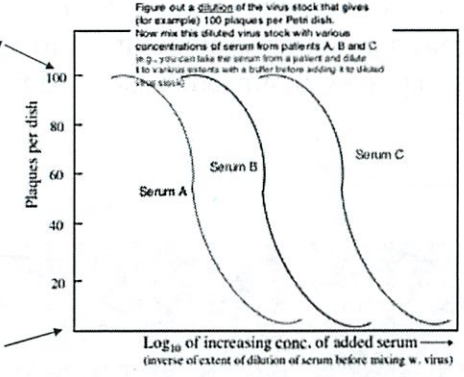


dilute serum by successive factors of 10

Serum A is able to inactivate 50% of virus infectivity at a low concentration while a far higher concentration of serum is required of Serum C is required for 50% inactivation of virus infectivity.

Full infectivity

No infectivity

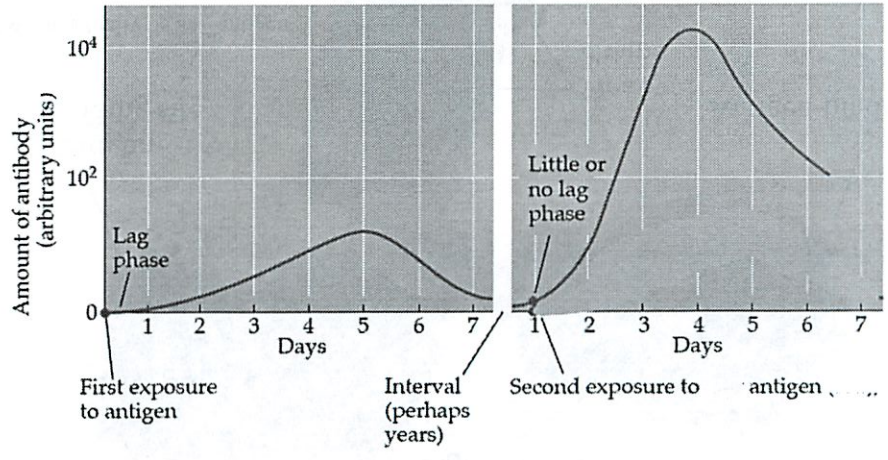


Look at when 50% of the Virus is neutralized

dilute serum by successive factors of 10

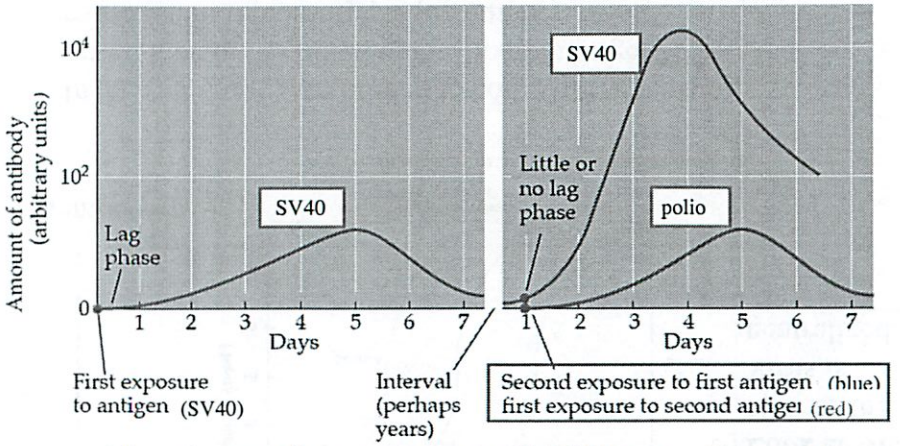
In the **red curve**, we needed to add ~100 times more serum than in the **black curve** in order to get 50% reduction in infectivity/plaque number.
Therefore, the concentration of anti-viral neutralizing activity is 100x higher in the red serum than in the black serum.

These concentrations of neutralizing antibodies in a patient's serum (prepared at various times) can therefore be measured over many orders of magnitude.



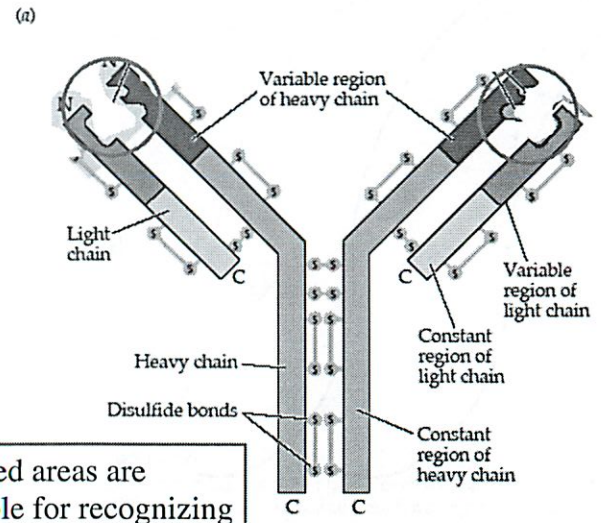
Antibodies in the serum = anti-viral-neutralizing activity

Imagine now that this individual becomes infected with a second, unrelated viral agent (e.g., polio) years later.



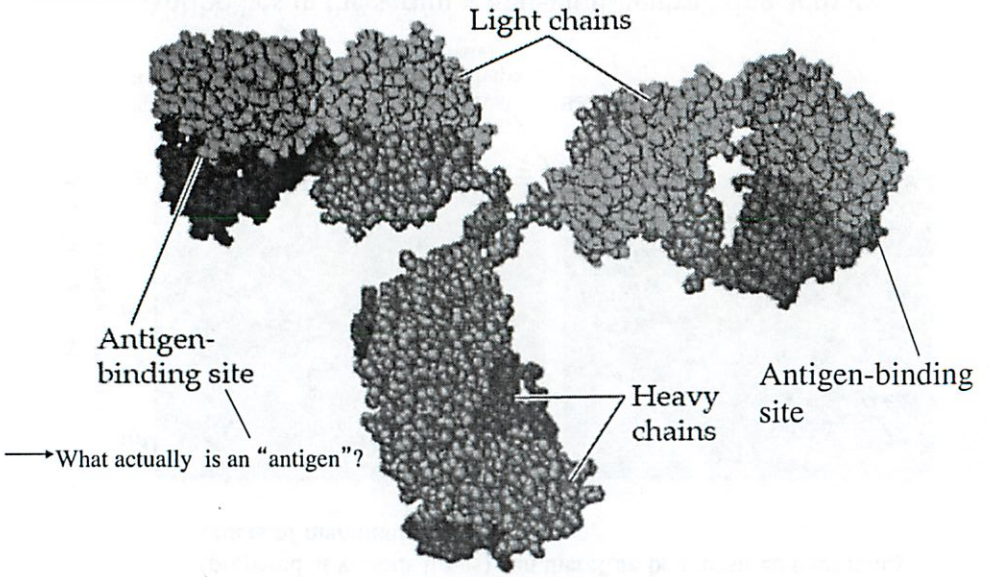
Note that previous exposure to SV40 has no effect on the response of the immune system to the second infectious agent years later. Hence, the response is specific to each agent.

What are antibody molecules?
Heterotetramers -- 2 heavy + 2 light chains

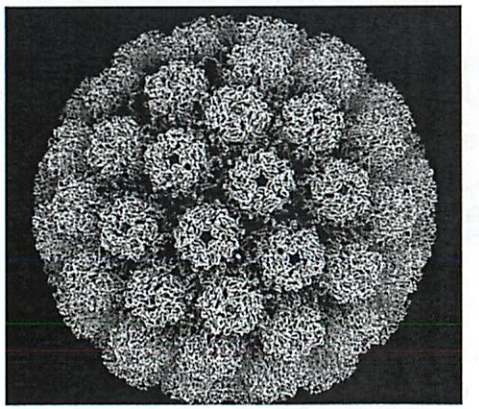


The circled areas are responsible for recognizing and binding antigens

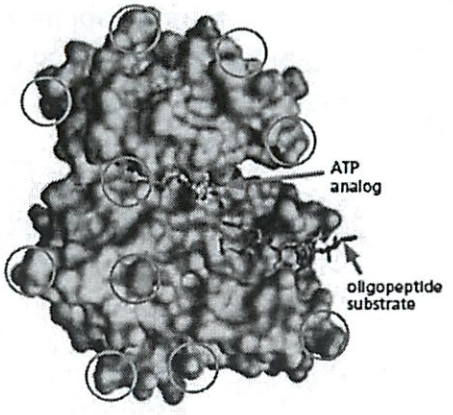
What is present in the serum? Here is the structure of the most common antibody molecules in the serum. Space-filling model of an antibody molecule.



Reconstructed image of SV40 virus virion (distant relative of HPV)

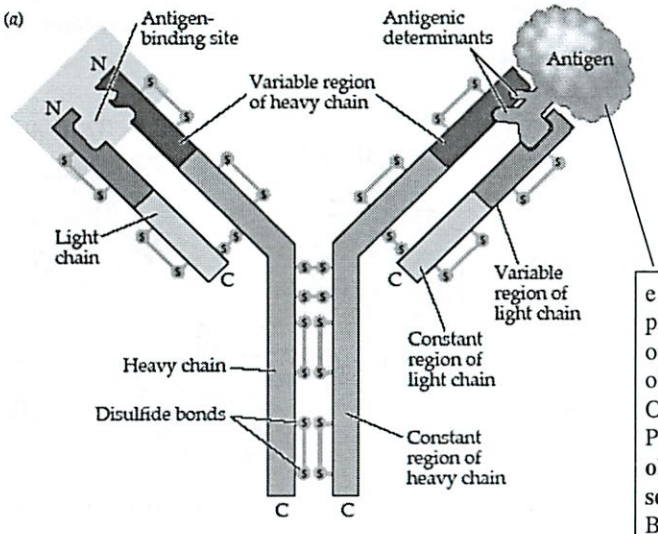


Terms/concepts:
An **antigen** is an entity that **provokes an immune response**.
An antigenic protein contains multiple **epitopes**, each being an oligopeptide that can be recognized and bound by an antibody molecule.
Since each protein molecule contains multiple oligopeptides, it may contain multiple epitopes and be recognized by multiple antibody molecules. In real life, however, only a minority of randomly chosen oligopeptides are antigenic, i.e. provoke the formation of an antibody against them.



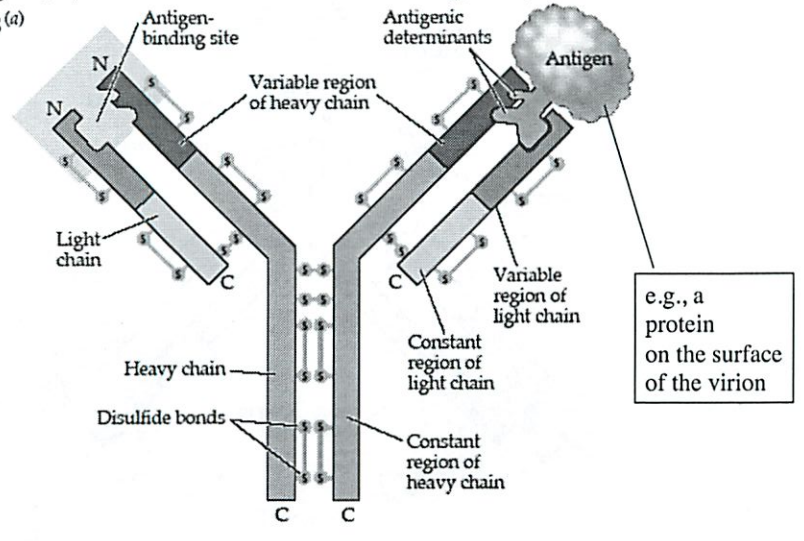
This kinase molecule carries dozens of potential **epitopes** ◯ , on its surface,each one of which might in principle function as an antigen to provoke an immune response. In fact, as we will see, oligopeptides from inside the protein can also, in principle, be antigens.

What are antibody molecules?



e.g., a protein on the surface of the virion
Or, more Precisely, an **oligopeptide sequence** Bound by The antibody

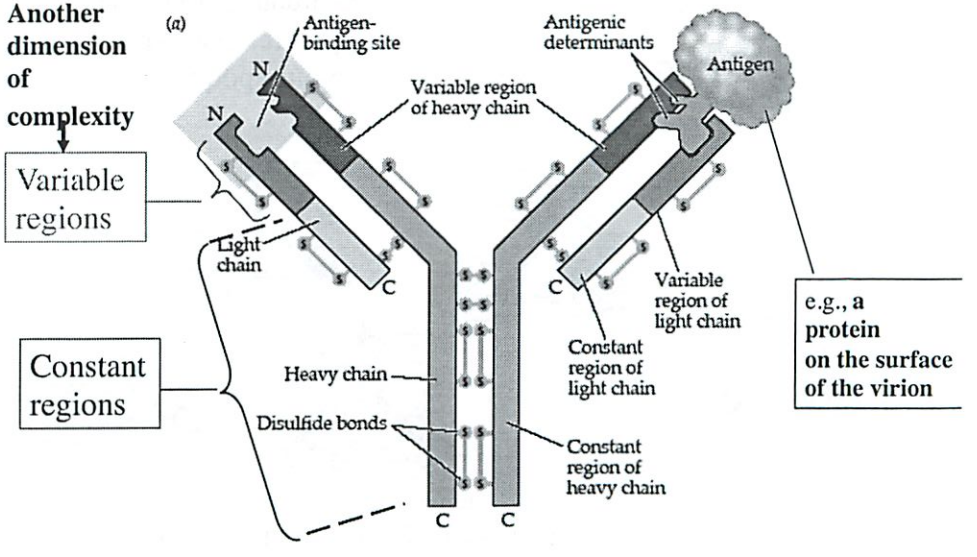
What are antibody molecules? This antibody molecule is a heterotetramer, composed of two identical "heavy" (H) chains and two identical "light" (L) chains



e.g., a protein on the surface of the virion

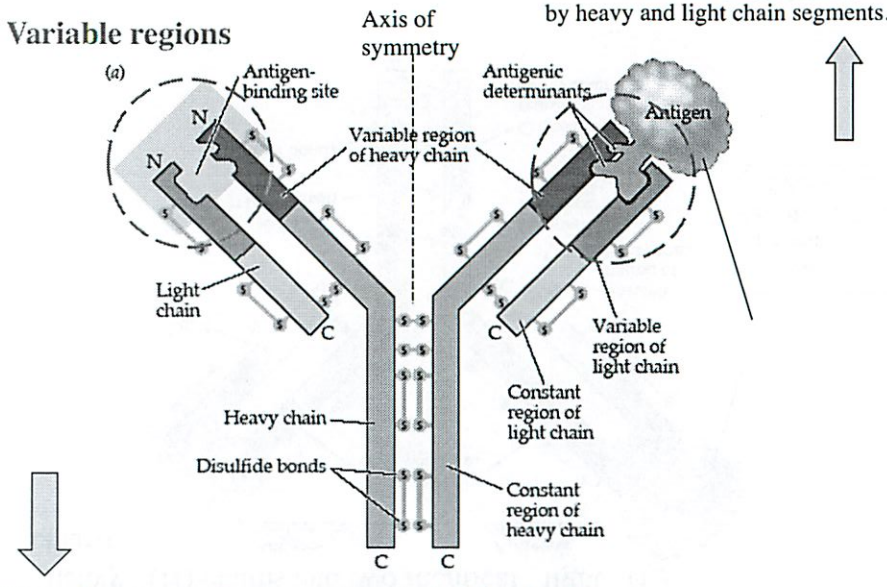
What are antibody molecules? This antibody molecule is a heterotetramer, composed of two identical "heavy" (H) chains and two identical "light" (L) chains

Another dimension of complexity



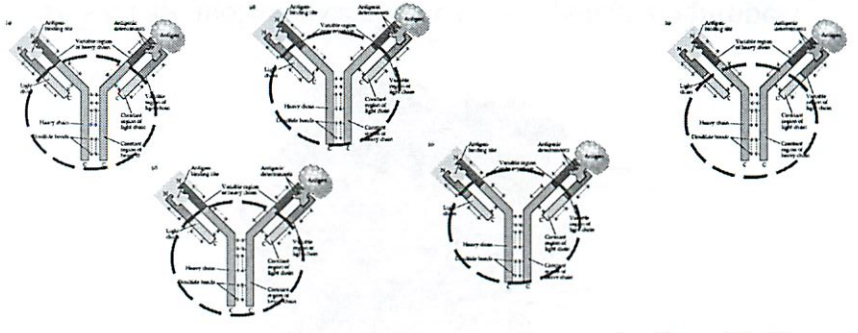
e.g., **a protein on the surface of the virion**

Note that each antigen-binding domain is formed cooperatively by heavy and light chain segments.

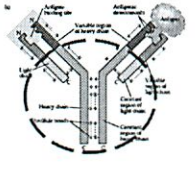


Note that the antibody molecule is **bivalent,**
i.e., has two antigen-binding domains (which are identical to one another).

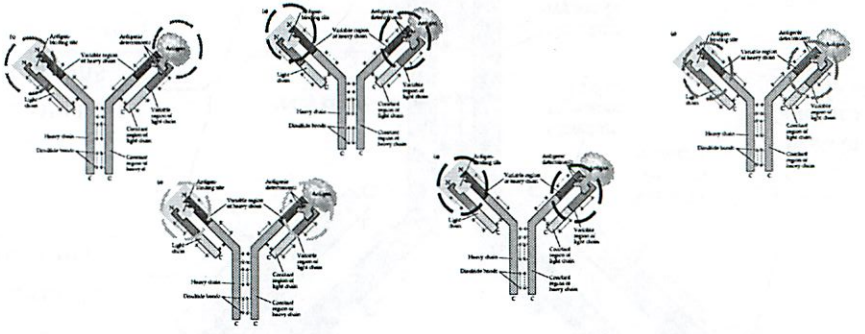
The serum of an individual contains millions of distinct antibody molecules, each with its own antigen-binding domain.



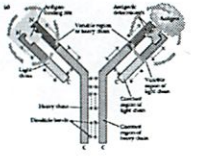
Conversely, the **constant regions** of the depicted antibodies are all **identical**



The serum of an individual contains millions of distinct antibody molecules, **each with its own** antigen-binding domain. Each antibody species is present in millions/ billions of molecular copies in the serum.



Each antibody molecule (="species") has **its own variable region** (and therefore can recognize **its own specific antigen**).



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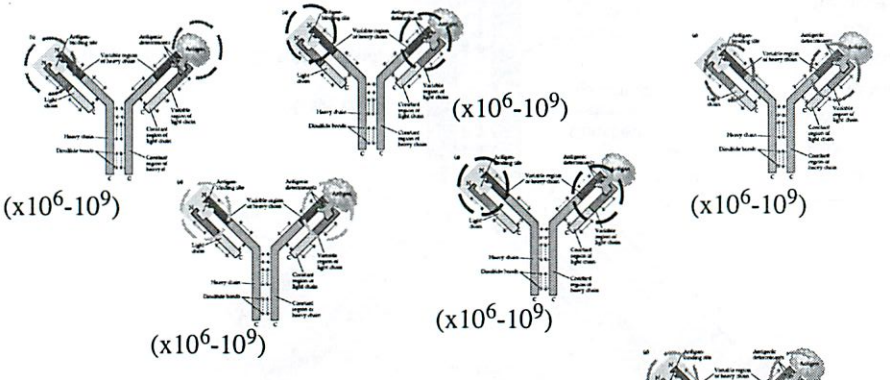


Each antibody molecule ("species") has **its own variable region** (and therefore can recognize its own specific antigen).

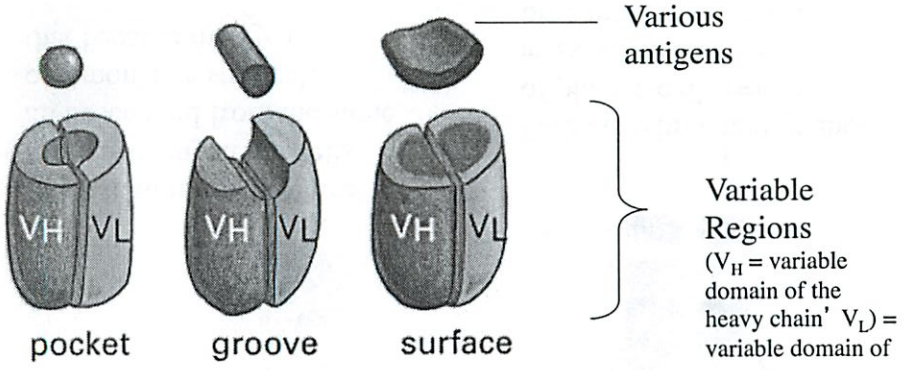


Figure 24–35. Molecular Biology of the Cell, 4th Edition.

Each variable region **recognizes its own particular antigen**

Yet another depiction of the antigen-combining sites of antibody molecules (below)

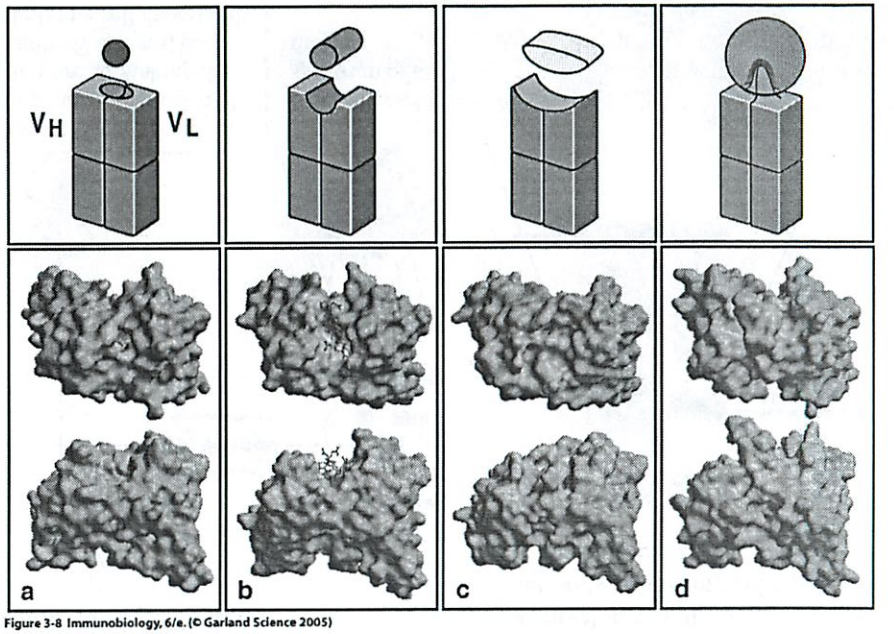
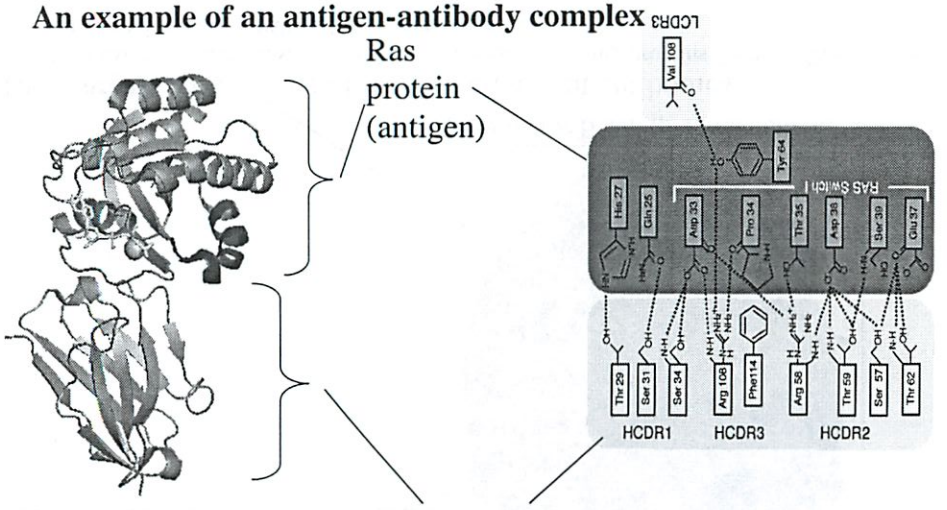


Figure 3-8 Immunobiology, 6/e. (© Garland Science 2005)

An example of an antigen-antibody complex

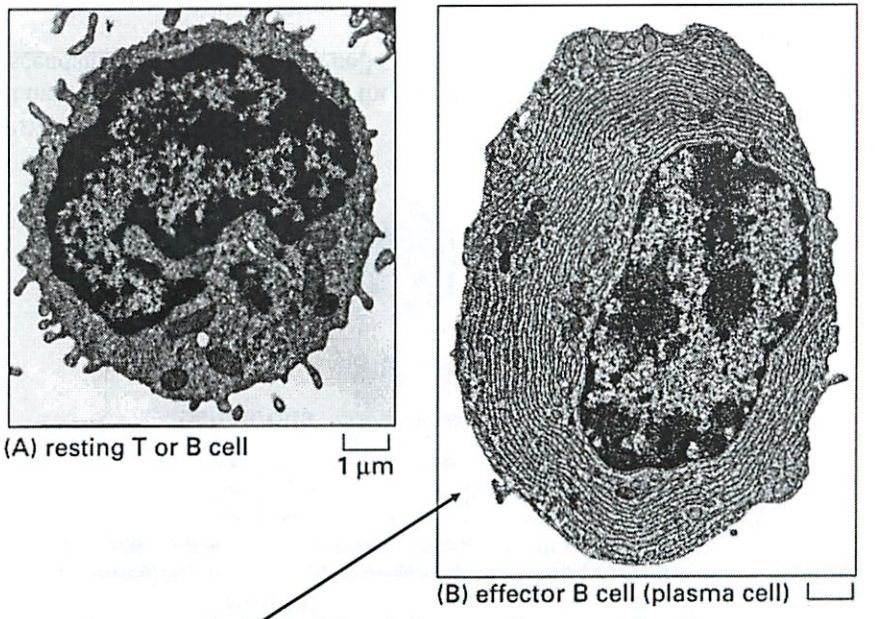


Ras protein (antigen)

Antigen-binding domains of antibody molecule

(Various types of non-covalent interactions between the protein enable specific recognition)

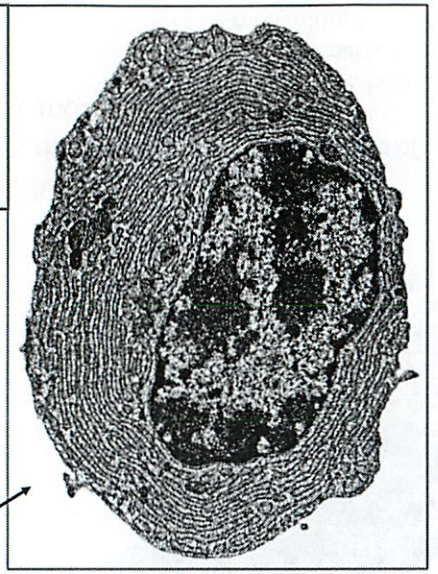
Courtesy of T. Rabbitts



These are the cells in the blood that crank out antibodies

Note their extensive endoplasmic reticulum (ER) for processing proteins destined for secretion. Secreting proteins is essentially all these **plasma cells** do.

Does one plasma cell make multiple kinds of antibodies, OR does each plasma cell make **its own** specific, specialized antibody?

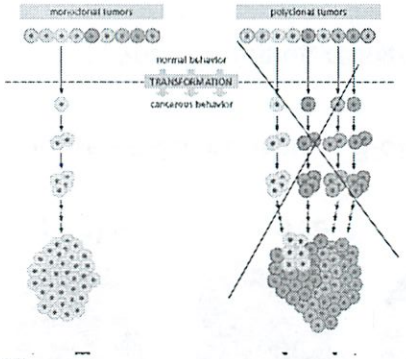


(B) effector B cell (plasma cell)

These are the cells in the blood that crank out antibodies

Note their extensive endoplasmic reticulum (ER) for processing proteins destined for secretion. Secreting proteins is essentially all these **plasma cells** do.

Does one plasma cell make multiple kinds of antibodies, or does each plasma cell make its own specific, specialized antibody?



Remem
All the
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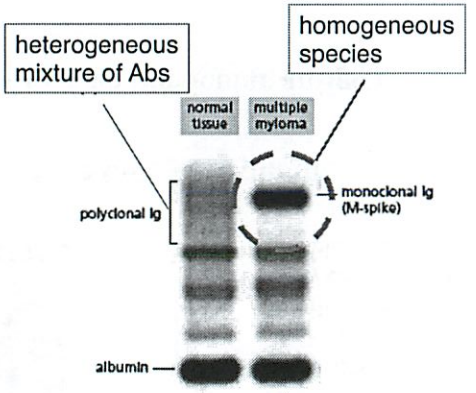
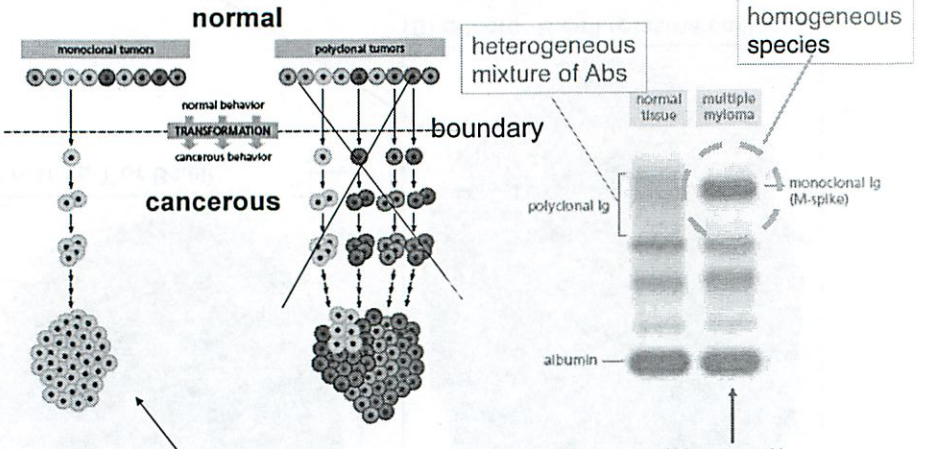
In these tumors, there are billions of myeloma cells, all descended from the same common ancestral cell that became malignant



Patients with a malignancy of plasma cells (=multiple myeloma)make one predominant antibody

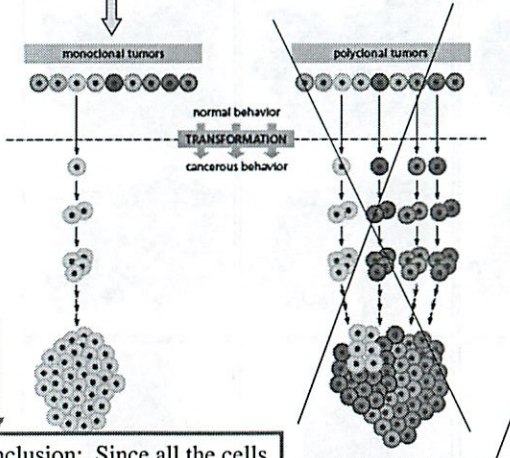
Does one plasma cell make multiple kinds of antibodies, or does each plasma cell make its own specific, specialized antibody?



Tumors are **monoclonal**, i.e.,
All the cancer cells in a tumor are the lineal descendants of a single founder cell.

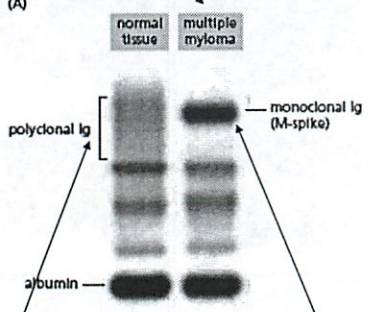
Patients with a malignancy of plasma cells (=multiple myeloma)make one predominant antibody

Human tumors are Monoclonal growths



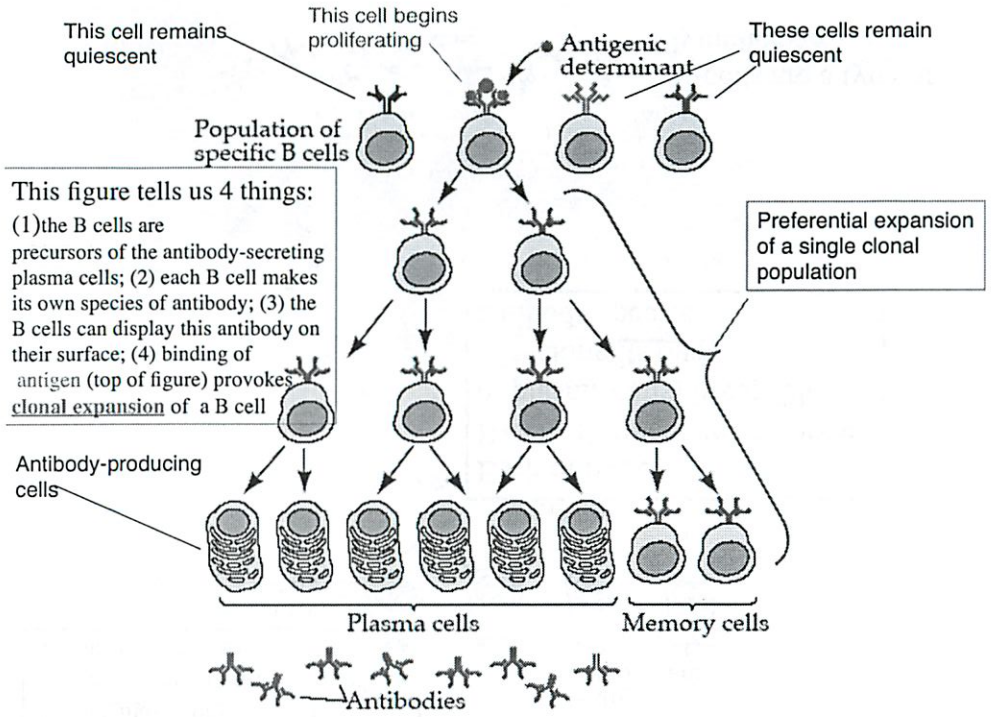
Conclusion: Since all the cells in a tumor are ~identical, and since they are all making the same antibody species, each normal plasma cell makes only one antibody

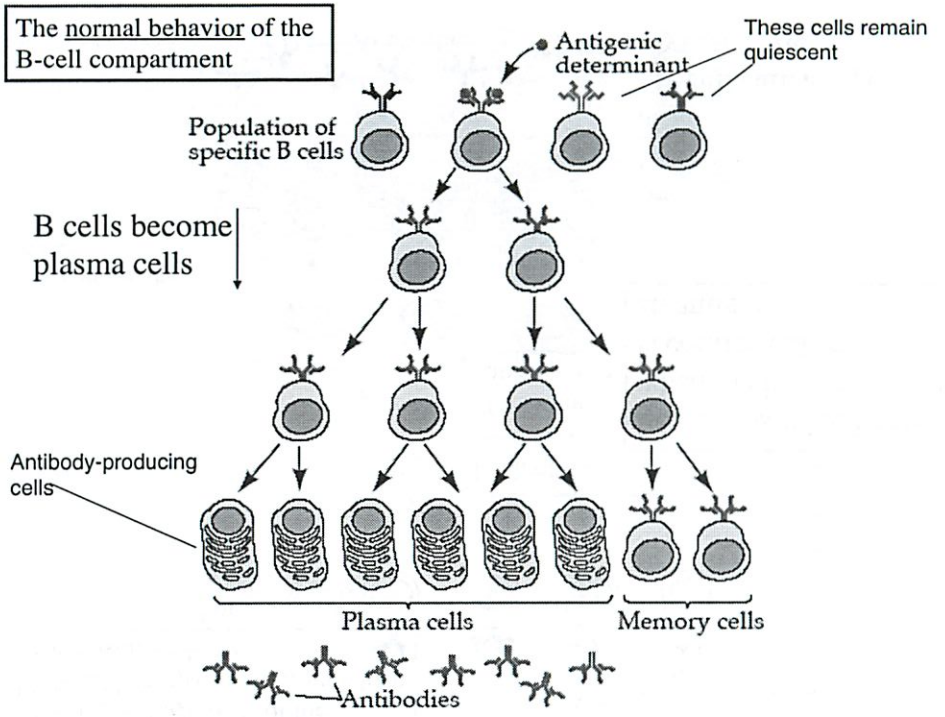
Plasma/serum of patient w. myeloma, a tumor of antibody-producing cells (= plasma cells)

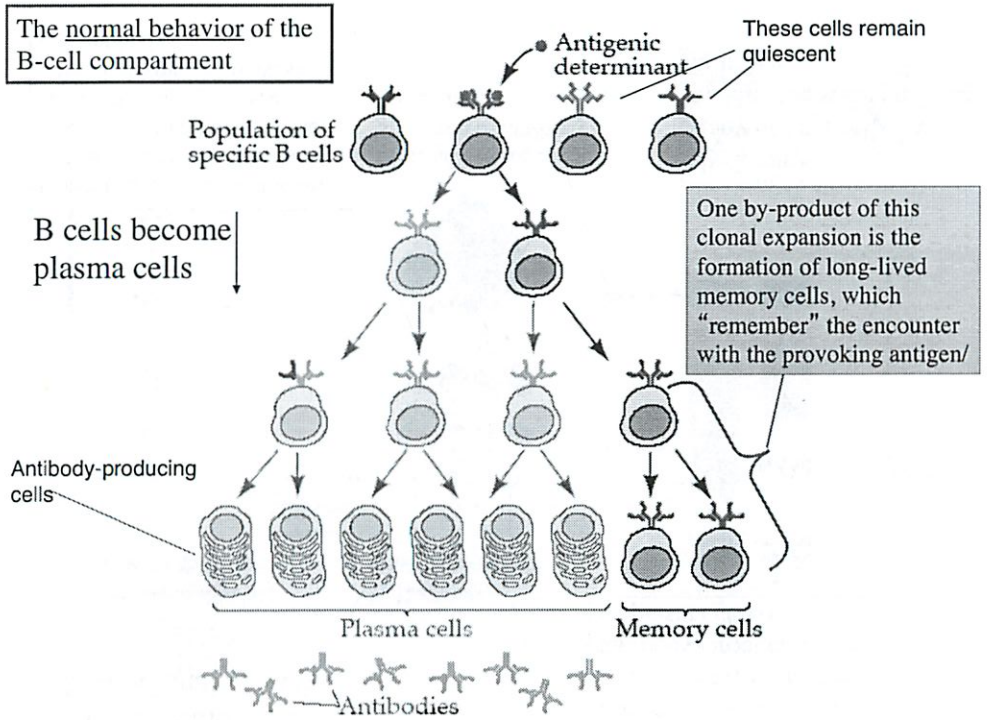


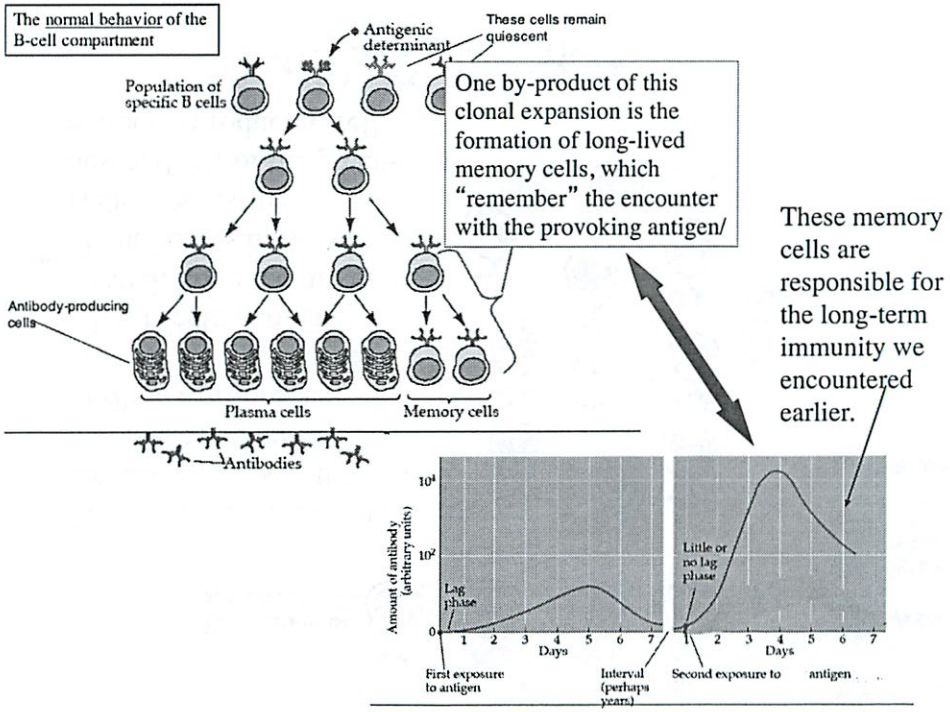
Normal heterogeneous spectrum of antibody molecules

Myeloma:
Many plasma cells are making the same antibody molecule

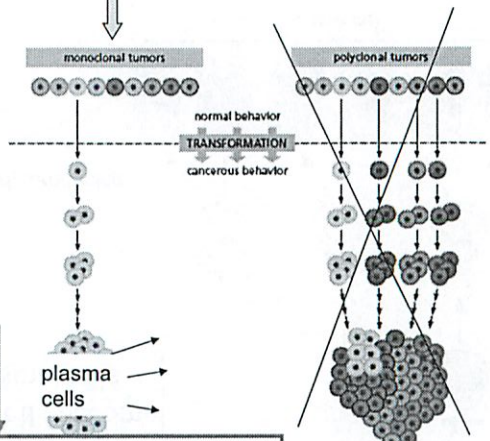

This cell remains quiescent
This cell begins proliferating
Antigenic determinant
These cells remain quiescent
Population of specific B cells
This figure tells us 4 things:
(1)the B cells are precursors of the antibody-secreting plasma cells; (2) each B cell makes its own species of antibody; (3) the B cells can display this antibody on their surface; (4) binding of antigen (top of figure) provokes clonal expansion of a B cell
Preferential expansion of a single clonal population
Antibody-producing cells
Plasma cells
Memory cells
Antibodies


The normal behavior of the B-cell compartment
Antigenic determinant
These cells remain quiescent
Population of specific B cells
B cells become plasma cells
Antibody-producing cells
Plasma cells
Memory cells
Antibodies


The normal behavior of the B-cell compartment
Antigenic determinant
These cells remain quiescent
Population of specific B cells
B cells become plasma cells
One by-product of this clonal expansion is the formation of long-lived memory cells, which "remember" the encounter with the provoking antigen/
Antibody-producing cells
Plasma cells
Memory cells
Antibodies

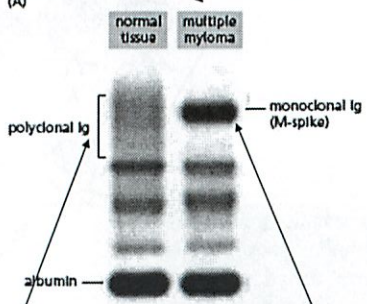

The normal behavior of the B-cell compartment
Antigenic determinant
These cells remain quiescent
Population of specific B cells
One by-product of this clonal expansion is the formation of long-lived memory cells, which "remember" the encounter with the provoking antigen/
Antibody-producing cells
Plasma cells
Memory cells
Antibodies
These memory cells are responsible for the long-term immunity we encountered earlier.
Amount of antibody (arbitrary units)
Lag phase
Days
First exposure to antigen
Interval (perhaps years)
Little or no lag phase
Second exposure to antigen

Human tumors are
Monoclonal growths



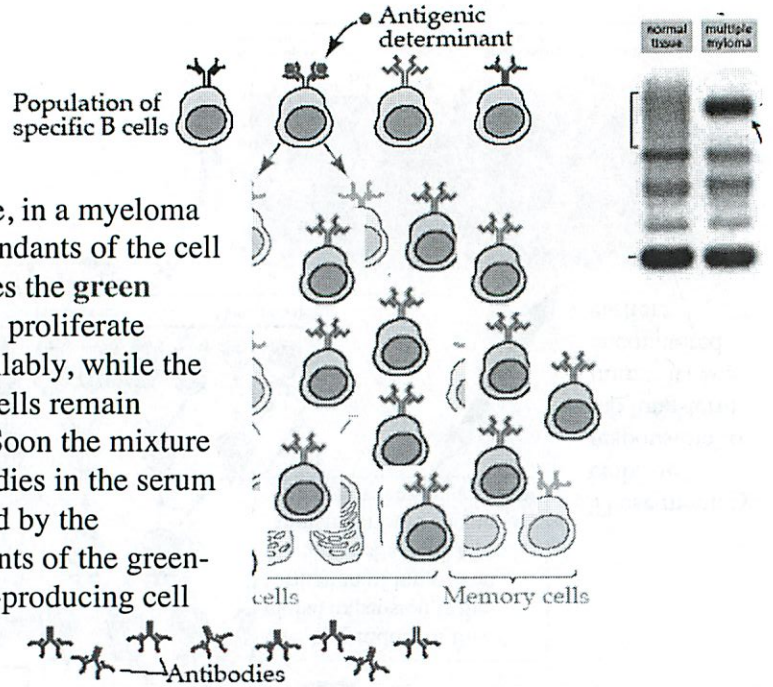
Conclusion: Since all the cells in a tumor are ~identical, and since they are all making the same antibody species, each normal plasma cell makes only one antibody

Plasma/serum of patient w. myeloma, a tumor of antibody-producing cells (= plasma cells)



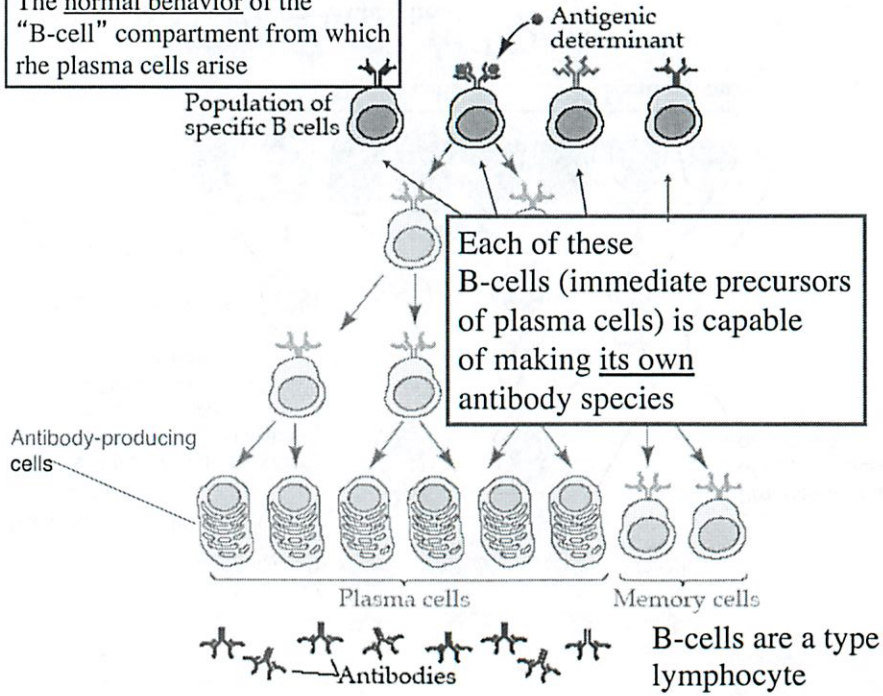
Normal heterogeneous spectrum of antibody molecules

Myeloma:
Many plasma cells are making the same antibody molecule



Therefore, in a myeloma the descendants of the cell that makes the **green antibody** proliferate uncontrollably, while the other B cells remain normal. Soon the mixture of antibodies in the serum is dictated by the descendants of the green-antibody-producing cell

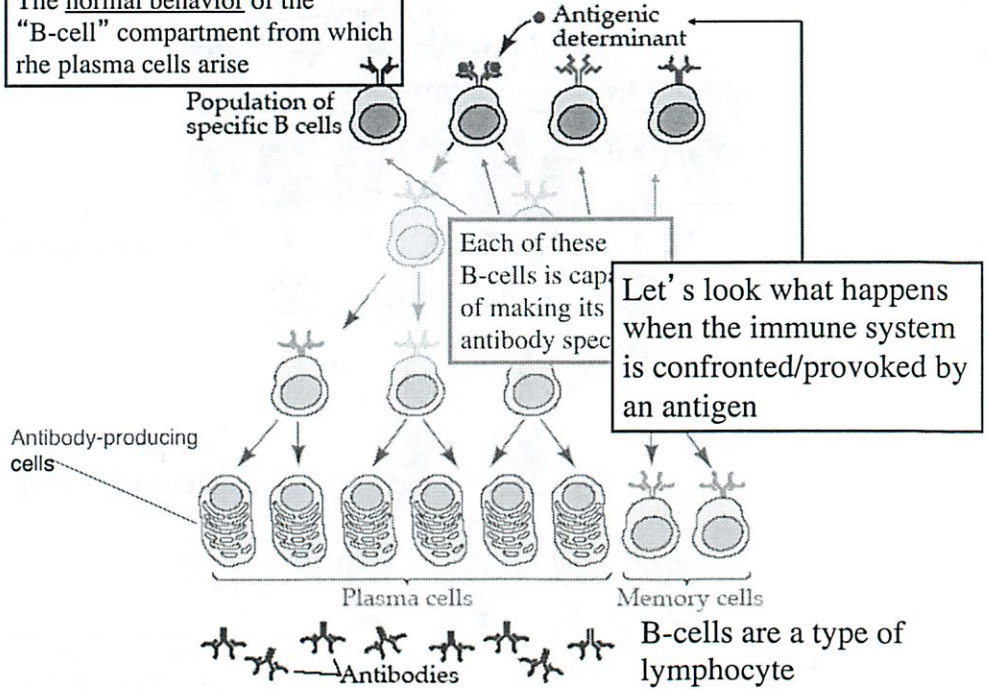
The normal behavior of the "B-cell" compartment from which rhe plasma cells arise



Each of these B-cells (immediate precursors of plasma cells) is capable of making its own antibody species

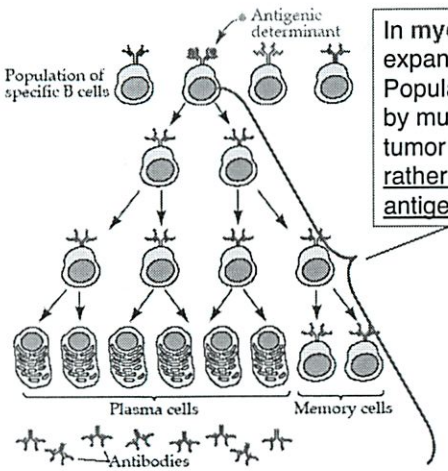
B-cells are a type of lymphocyte

The normal behavior of the "B-cell" compartment from which rhe plasma cells arise



Let's look what happens when the immune system is confronted/provoked by an antigen

B-cells are a type of lymphocyte



In **myeloma**,the preferential expansion of a single clonal Population of B-cells is driven by mutant genes carried by the tumor cells, i.e., oncogenes, rather than being driven by antigen exposure.

2,012

11/5

(2 min late)

Immunology 2

antiserum

high affinity - binds well

antibody + antigen → causes production of immunological memory

Myeloma cell endless rounds of proliferation

Antiserum can contain antibodies against a bunch

So want pure : monoclonal antibody

↳ it + decendents only B-cells

1. immunize mouse
2. Want b cells to make antibody we want
3. These ↑ in # → clonal expansion

Want a pure monoclonal population

(2)

Antigen is oligopeptides

Immune system should attack foreign

↳ Otherwise autoimmune disease

is trait: tolerance

4. Try to immortalize each of B-cells so they grow indefinitly in culture

~~Want to immortalize those B-cells~~

How: In patice: cell fusion

fuse w/ a neloma cell ← heterokaion

- 2 diff nuclei in same cytoplasm

In theory: immortalizing gene added

hybridoma

have phenotype of immortalization

(His lectures are so hard to follow!)

(3)

5. Place each hybridoma cell in a microwell

Need to find the one that makes the antibody of interest

↓

the supernatent

Can have them marked w/ color when recognize

Then put culote of cells in a flask

All cells are then genetically identical

- many copies of that antibody

---

2 monoclonal antibodies

blue + green die

binds cell to cell junctions

Protiens that drive Breast Cancer cells

Dramatically reduces relapses

(4)

Cell service receptor over expressed in patients w/ breast cancer

---

Major Conceptual Problem:

How does the immune system know how to make So many distinct variable regions?

Over the course of evolution → shared diseases have we developed antibodies for each?

We are exposed to agents our family forefathers haven't

But look at the spectrum of an antibody

bivalent bind two at a time

Variable domain has 3 parts/subdomains

- is on heavy chain
- V, D, J — O — Cγ1

V, D, J: Variable domain

Cγ1: Constant domain

(5)

Lots of stuff in DNA?

No - would need millions of genes for ab fibodies

but only have 19,000

Evolution anticipates?

No - we make antigens to synthetic

One long region of DNA

locus since longer than a gene

~40 alt V domains

each person has a slightly diff #

each w/ a leader seq

So stick into secretary pathway

So can be secreted out of a cell

(6)

Stocastic choice

this fused to that, fused to that

:

Undergoes DNA rearrangement

randomly fuses segments

deletes intervening segments

One V segment fused w/ D segment

heavy $40 \times 25 \times 6 = 6000$ distinct combos

light $40 \times 5 = 200$

but heavy and light $6000 \times 200 = 1.2 \cdot 10^6$

Bivalent so 2 pairs of hands

Random Stocastic fusions

Explains how each B cell produces a diff antibody — adaptive immune system

(V)

Slide that shows how chains are built over time

1. DNA rearrangement w/ deletion
2. RNA splicing

Other processes that lead to further diversification

One condition: make good antibody

So get junction of diversity

One codon can be left out

from sloppiness of fusions

So 10^{14} diff alternatives

Enzyme directed to antigen aid

Mutigenic

focuses on variability domain

makes point mutations in variable

Can create antibodies that bind even better w/ antigens

(8)

don't want it to mutate other genes

esp producing ∞ cells (cancer)

10^{16} (cartilage)

IgG IgM IgD IgA1 IgE

antigen binding site

But what if have specific antibodies required

All types of diff things possible

Can then be joined w/ other constant regions

IgM can float around as a pentamer

V DJ recombination

└ combinatorial fusions

fused to $\mu, \delta, \gamma, \varepsilon, \alpha$ regions

(9)

not fuse → but splice

↳ called class switching

delete at intervening DNA
(see slide)

Can be splice VDJ Cα
makes IgA

Same antigen site
but diff antigen recognizing domains

Been portraying as secreted (missed)

But can't work if red antibody molecule floats away

How does B-cell know how to make more

Must be tethered to cell surface

IgM initially makes a cell surface receptor

Then it can serve as a sensor if clonal expansion is necessary

(10) L Signal transduction

Other reasons to disqualify
rearrange to (7
if makes antibody that recognizes normal protein
L autoreactive

So want to eliminate those B cells ahead of time
to enable tolerance
to recognize self/non-self

Differing functions
each has diff properties
Opsonization - bacteria totally coated in antibodies
natural born killers - can attract their attention
so they kill the cell
mast cell make us sneeze

11

When ~~baby~~ born get antibodies from mom
Umbilical
Same w/ breast feading
Why not to use formula

IgA good at being transported trans placentally

Lumen cavity of gut

all recognize same antigen
Just diff functions

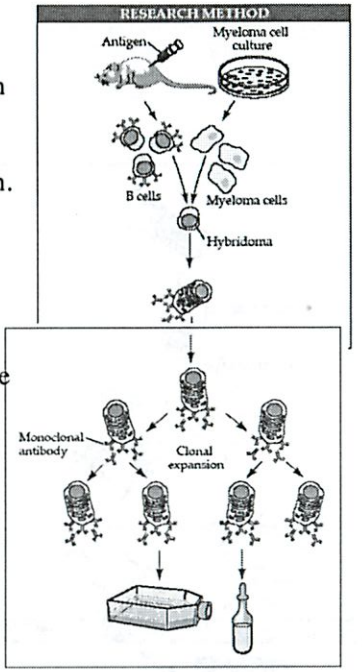
(These lectures are so hard to understand
- he goes so fast)

How to make a monoclonal antibody (MoAb) Overview:

a. A mouse that has been immunized with a certain antigen contains a variety of antibody molecules that have been produced against that antigen, i.e., are reactive with that antigen.

- In addition to these antigen-specific antibodies, the serum of a mouse contains millions of others unrelated antibody molecules.

c. A **monoclonal antibody** is a solution of antibody molecules that are all identical to one another and therefore all recognize the same epitope of the same antigen. (This contrasts with the serum of a mouse, which contains a vast array of antibodies above).



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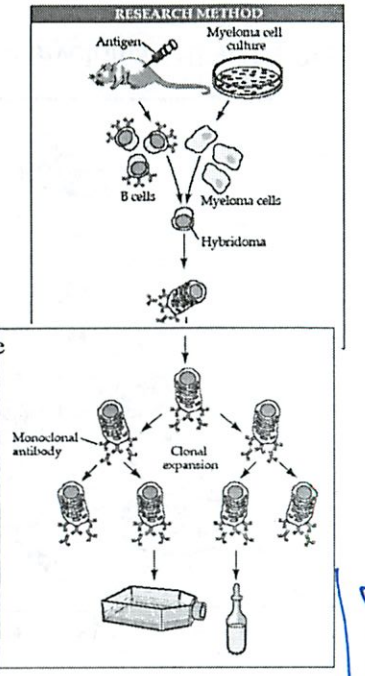
c. A **monoclonal antibody** is an solution of antibody molecules that are all identical to one another and therefore all recognize the same epitope of the same antigen. (This contrasts with the serum of a mouse that contains a variety of antibodies above).

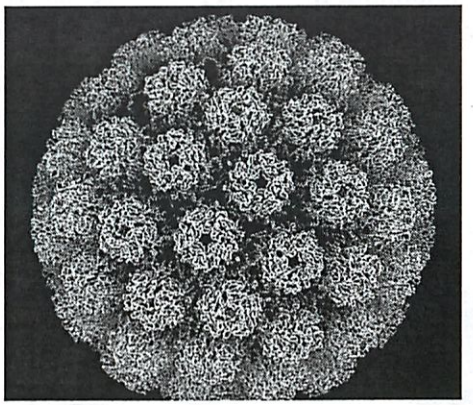
d. The various B-cell clones that have expanded in response to the antigen stimulation will be represented by B-cell populations in the spleen.



L23 Immunology 2

Reconstructed image of SV40 virus virion (distant relative of HPV)

Let's say that we want to make a monoclonal antibody against the SV40 virion

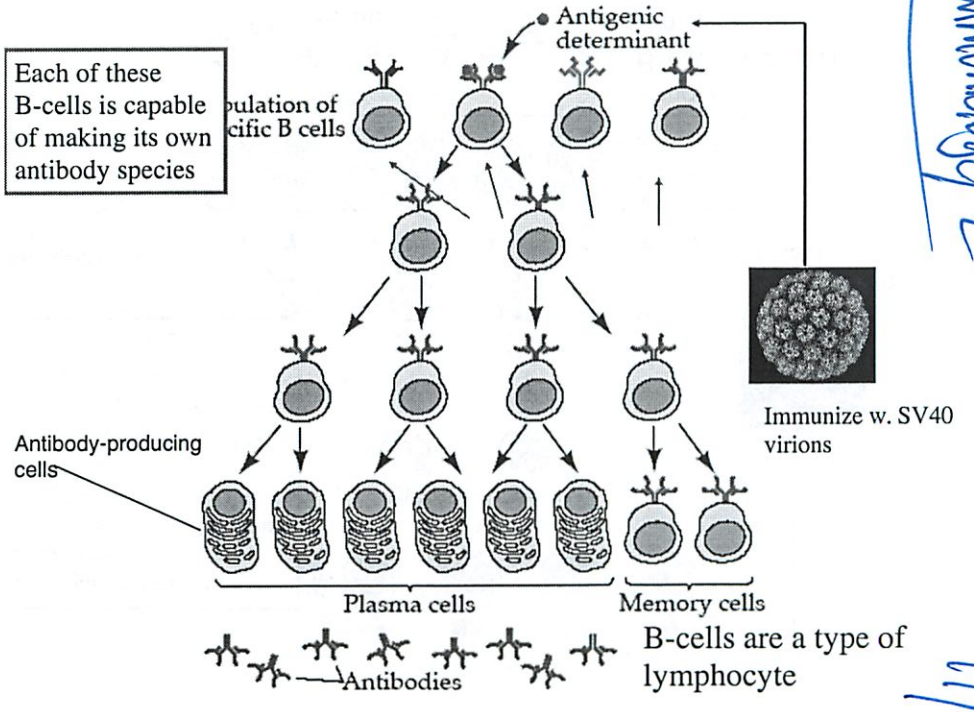


Terms/concepts:

An **antigen** is an entity that **provokes an immune response**.

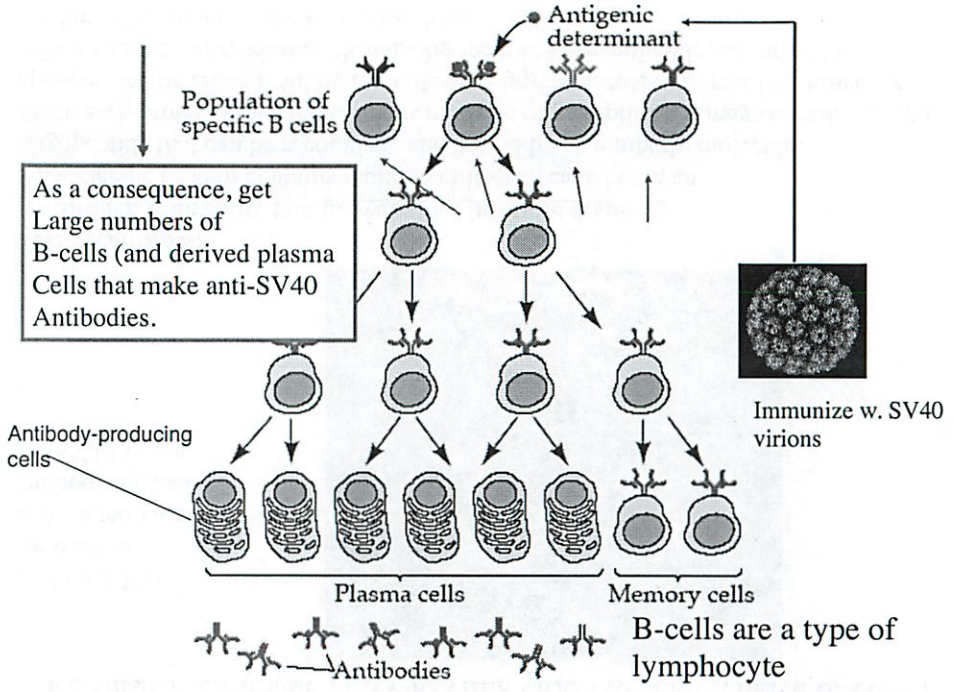
An antigenic protein contains multiple **epitopes**, each being an oligopeptide that can be recognized and bound by an antibody molecule.

Since each protein molecule contains multiple oligopeptides, it may contain multiple epitopes and be recognized by multiple antibody molecules. In real life, however, only a minority of randomly chosen oligopeptides are antigenic, i.e. provoke the formation of an antibody against them.



B-cells are a type of lymphocyte

11/18/15



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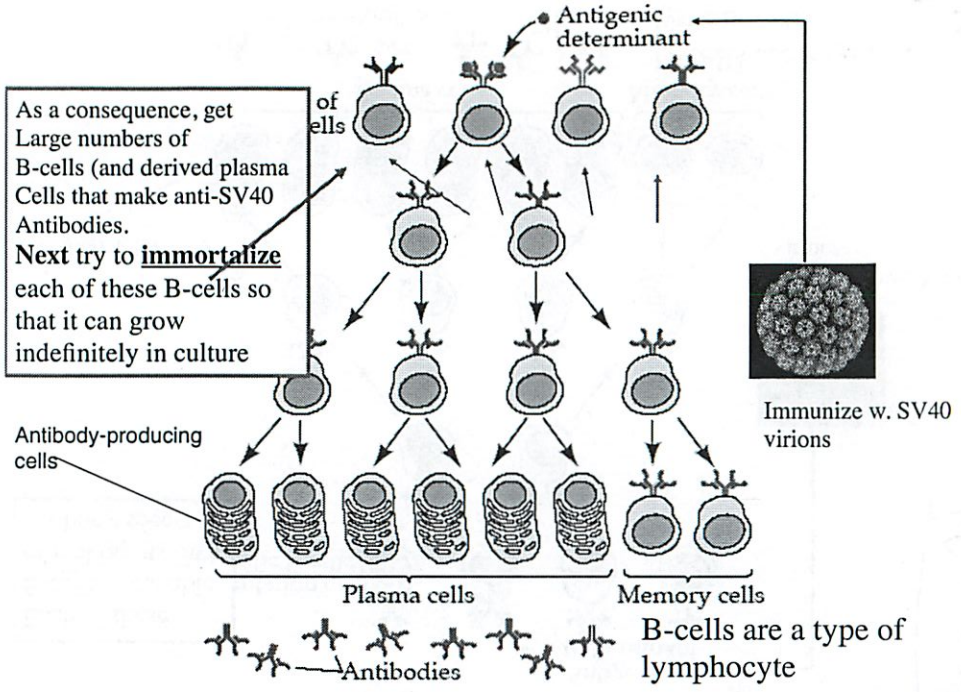


B-cells are a type of lymphocyte

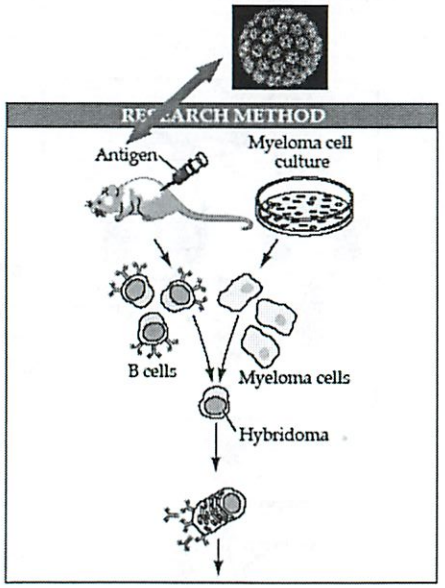
How to make a monoclonal antibody (MoAb) Overview:

1. Take the B cells from the spleen of a mouse that has been immunized with a certain antigen.

2 **How to immortalize?**

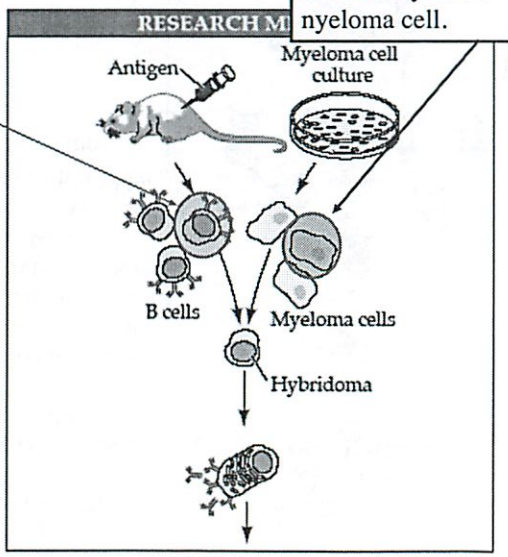
Fuse each B cell with a myeloma cell*. As a consequence, each B cell will continue to make its own antibody, but now the oncogenes from the myeloma cells will enable the fused hybrid cell# to proliferate indefinitely.

*this particular myeloma cell does not make its own antibody molecules. However, it contains oncogenes that enable the resulting hybrid cell to proliferate Indefinitely and behave like a myeloma cell. Therefore, by making this fusion, one creates a hybrid cell that behaves like a myeloma cell and, **in addition**, secretes the antibody of the B-cell to which it has been fused.



This cell donates the ability to produce a specific antibody

This cell donates the ability of a cell to indefinitely like a myeloma cell.



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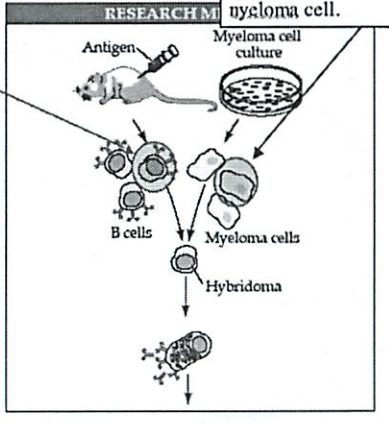
A hybridoma is a fused cell arising from a myeloma cell and a B-cell

This experiment will yield thousands of hybridoma cells, each hybrid cell arising from the fusion of a single myeloma cell (from a myeloma cell line) with a distinct B-cell prepared from the spleen of an SV40-immunized mose.. Since each B-cell in the spleen is likely to make a distinct antibody, each of these hybridomas will make a distinct antibody.

This cell donates the ability to produce a specific antibody

This cell donates the ability of a cell to indefinitely like a myeloma cell.



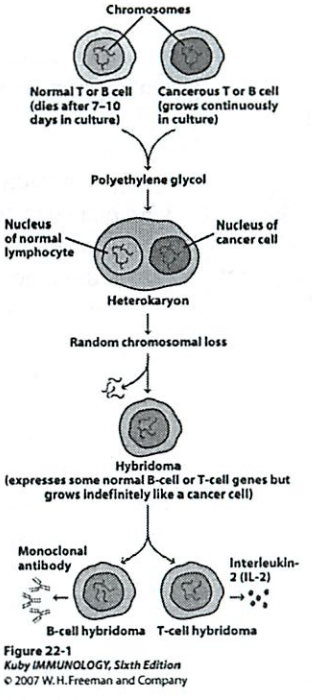


Figure 22-1
Kuby IMMUNOLOGY, Sixth Edition
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How to make a monoclonal antibody (MoAb) Overview:

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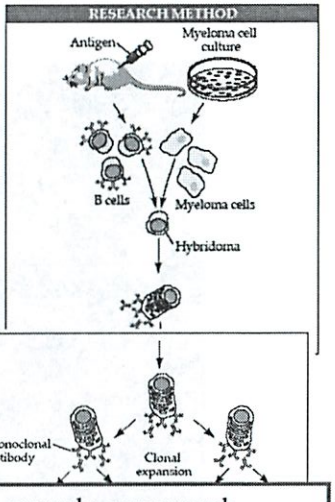
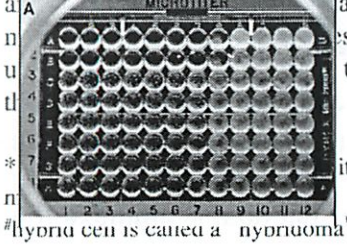
3. Place each of these hybridoma cells in a microwell, and then screen all the wells for those few that produce an antibody of interest.
4. Isolate the cells from a well producing a and its cells to n se cells and thus u that recognizes t

* its own antibody n

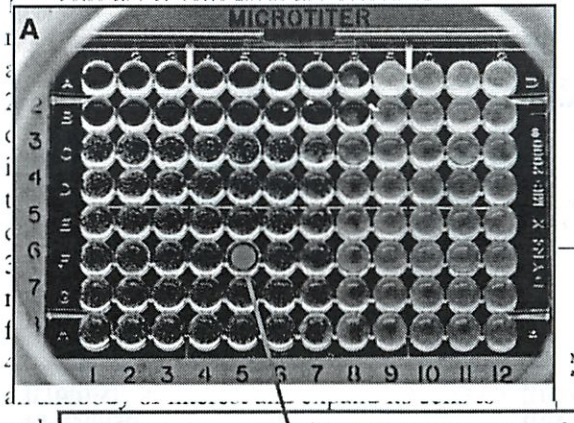
#hybrid cell is called a "hybridoma"

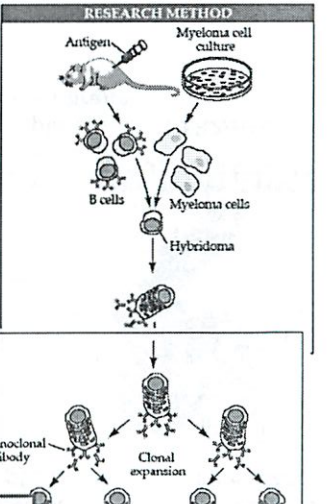




For example, screen each of the wells for the presence of antibody molecules that can recognize & precipitate the antigenic protein that was used in the initial immunization.

How to make a monoclonal antibody (MoAb) Overview:





For example, screen each of the wells for the presence of antibody molecules that can recognize & precipitate the antigenic protein that was used in the initial immunization. (Let's say that when there is an antibody that recognizes the antigen of interest, the well turns red. **Retrieve** the cells from this well, expand them in culture, and determine that they all make the same MoAb, which reacts with the antigen of interes

#hybr

For example, a monolayer of epithelial cells has been stained here with two monoclonal antibodies (MoAbs), one coupled to a blue dye, the other to a green dye. The blue MoAb recognizes a cell-to-cell adhesion protein, the green MoAb recognizes a cytoskeletal protein.

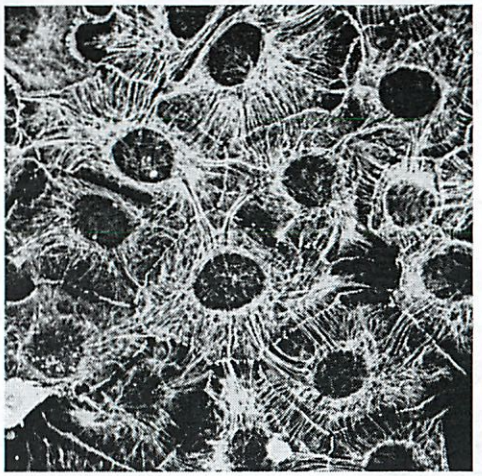


Figure 1.14b *The Biology of Cancer* (© Garland Science 2007)

Here, **therapeutic MoAbs** have been made that recognize a cell-surface receptor that is overexpressed in breast cancer cells or lung cancer cells. In the case of br. Ca Herceptin can reduce or prevent recurrence (relapse) of certain breast cancers.

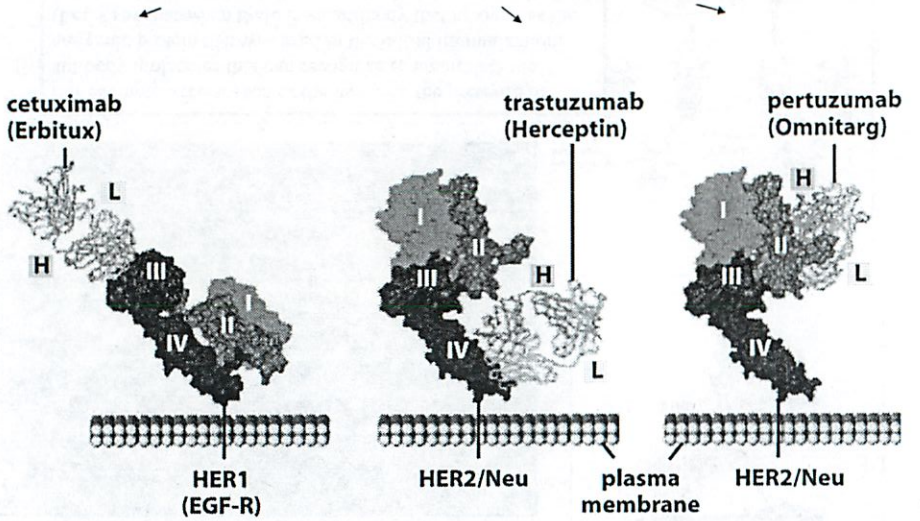


Figure 15.38b *The Biology of Cancer* (© Garland Science 2007)

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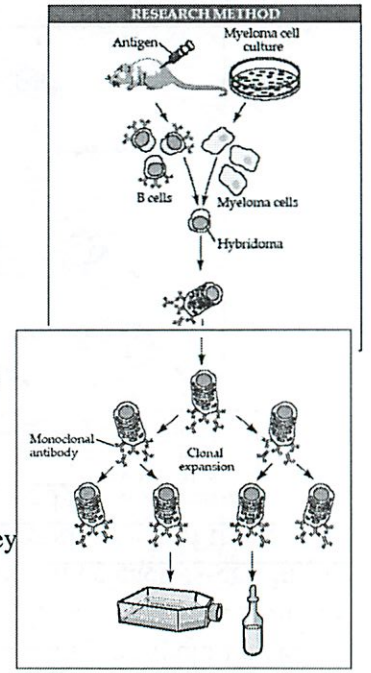
3. Place each of these fused cells in a microwell, and then screen all the wells for those few that produce an antibody of interest.
4. Isolate the cells from a well producing an antibody of interest and expand its cells to make unlimited numbers of these cells and thus unlimited amounts of antibody molecules that they secrete and that recognizes the antigen/protein of interest.



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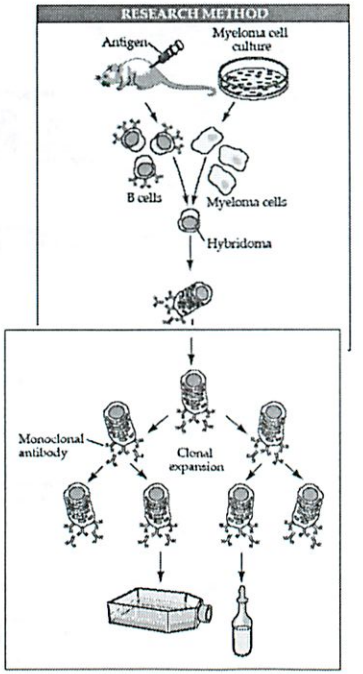
4. Isolate the cells from a well producing an antibody of interest and expand its cells to make unlimited numbers of these cells and thus unlimited amounts of antibody molecules that recognize the antigen/protein of interest.
This solution of antibody molecules, all identical to one another, will be termed a "**monoclonal antibody**".



A major conceptual problem:

If each of the subpopulations of B cells in the immune system makes its own specific antibody molecule (each having its own **variable region**),, and there are thousands, even millions of distinct subpopulations of B cells in the immune system, **how does the immune system as a whole know how to make so many distinct variable regions?**

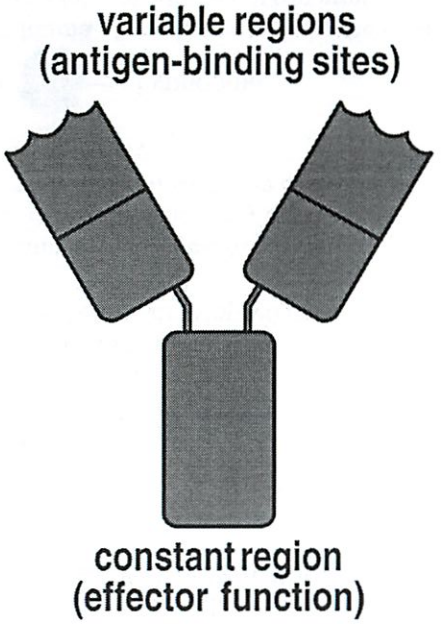
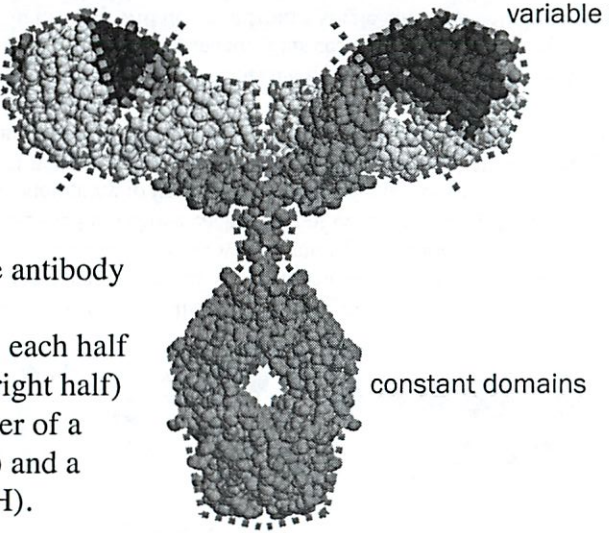


Figure 1-16 Immunobiology, 6/e. (© Garland Science 2005)

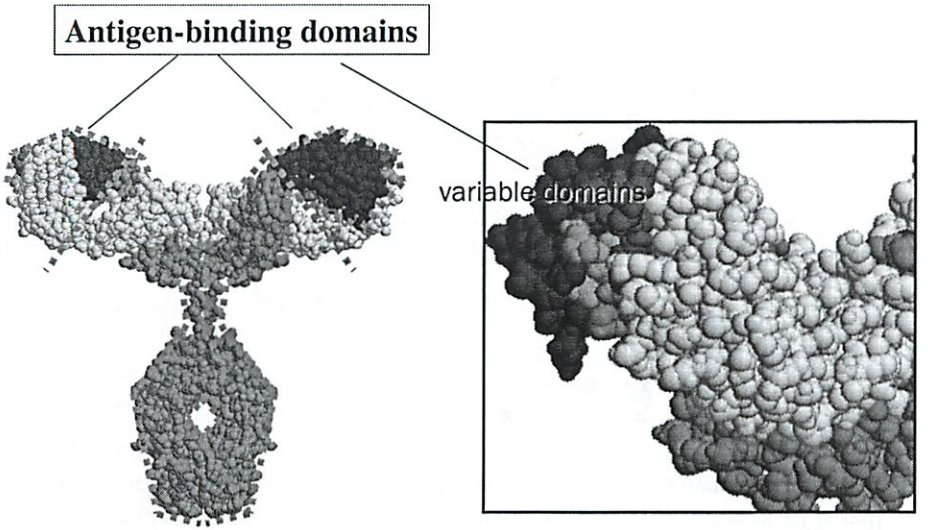
The structure of an antibody molecule



Recall that the antibody molecule is a **tetramer;** each half (left half and right half) is a heterodimer of a light chain (L) and a heavy chain (H).

(Padlan, EA. *Mol Immunol* 31 : 169 - 217, 1994)

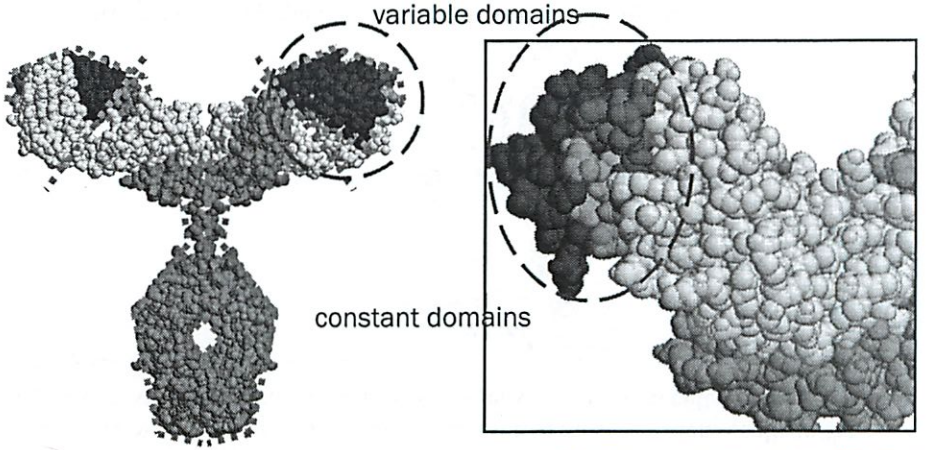
The left and right arms both have antigen-binding domains. Hence the antibody molecule is "bi-valent", I.e. it can bind two antigen molecules simultaneously.



(Padlan, EA. *Mol Immunol* 31 : 169 - 217, 1994)

There are gene segments in the cellular genome that encode the sequences found in the antigen-combining "variable" domains of antibody molecules. Each variable domain is composed of three subdomains, termed V, D, and J. The V (variable), D (diversity), and J (junctional) gene segments encode the diverse antigen-binding surface.

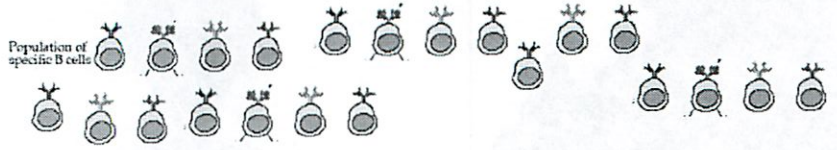
(You may ask how a "variable" domain can contain/include a variable sub-domain. This dumb & confusing nomenclature is a historical artifact and we're stuck with it.)



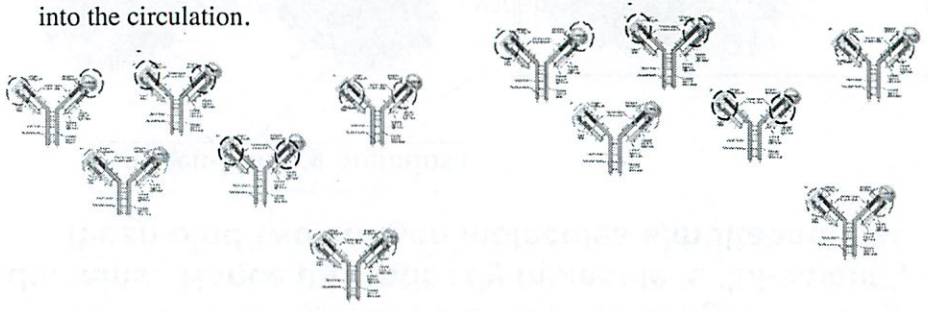
(Padlan, EA. *Mol Immunol* 31 : 169 - 217, 1994)

Let's review in some detail the steps needed to make a monoclonal antibody.

a. In the spleen (and bone marrow) of a mouse there are normally millions of B-cell populations, each B cell having developed the ability to make its own particular antigen-specific B cell.

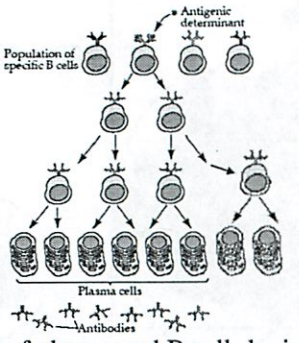


In this mouse, many of these B cells are, on occasion, differentiating/maturing into plasma cells, and the latter are secreting millions of antigen-specific antibodies into the circulation.

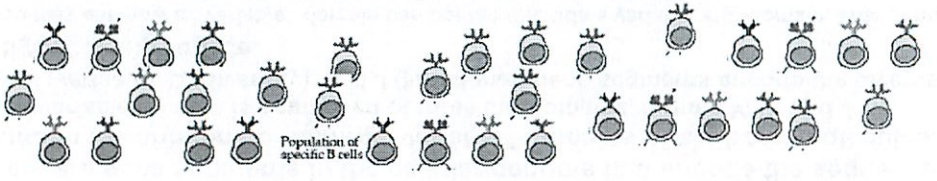


b. We would like to make a monoclonal antibody that recognizes specifically a protein of particular interest to us, e.g. this protein -- ●

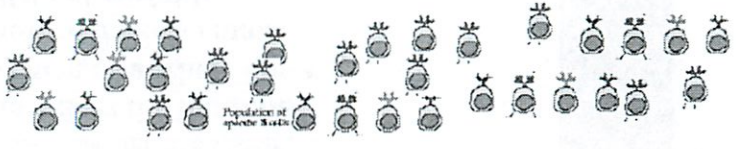
Accordingly, we repeatedly inject this protein into a mouse, which results in the **clonal expansion** of the subpopulation of B-cells (and plasma cells) that makes an antibody recognizing this antibody



This distorts the overall population of plasma and B cells by increasing the representation of the ● cells in the population.



c. Now, we would like to take each one of the B-cells from this very heterogeneous population and "immortalize" it, i.e., change it so that it can be propagated in tissue culture, such as a flask or Petri dish.

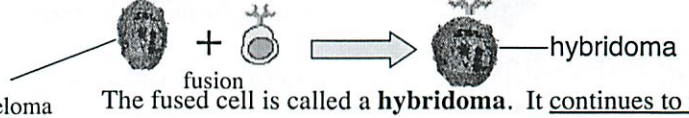


To do so, we take a line of myeloma cells that has two characteristics:

i. The myeloma cells have (for one or another reason) stopped making their own antibody molecules.

ii. The myeloma cells carry several dominant oncogenes, which can influence the behavior of the normal B-cells to which they are fused.

So we take myeloma cells and fuse each of them with one of the B-cells from the repeatedly immunized mouse.



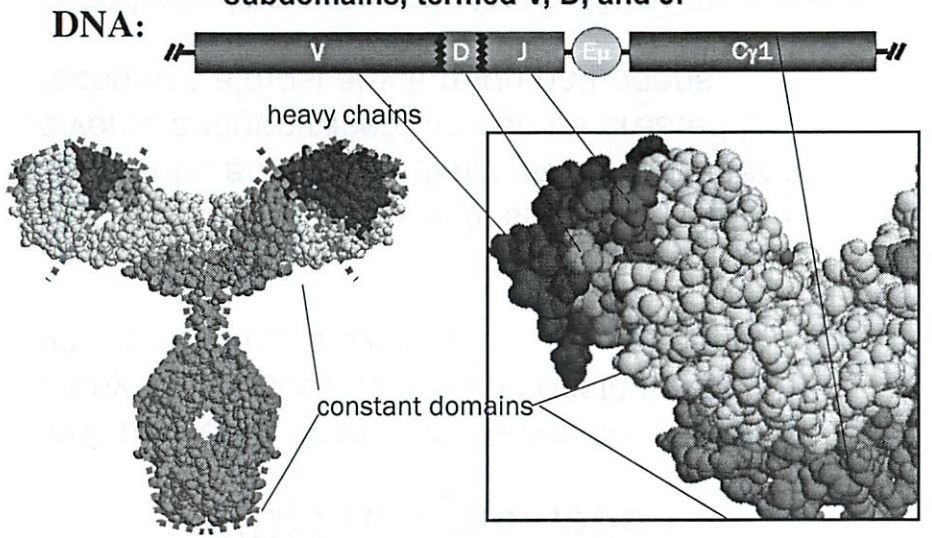
The fused cell is called a **hybridoma**. It <u>continues to make</u> the same antibody as its B-cell parent. In addition, however, it can grow vigorously in culture and proliferate indefinitely (unlike normal B-cells.)

d. Imagine now that each one of the cells in this figure is a hybridoma (instead of the originally drawn collection of B-cells).

Put each one of these hybridoma cells (there will be many tens of thousands of them) in a separate well of a microtiter place (e.g.,. a rectangular plastic tray in which there are 96 wells). Allow the cells in each of these well to multiply and then withdraw the supernatant fluid (the medium above each group of cells) and assay the fluid from each one of these wells, attempting to find the well that contains cells making antibody molecules that react with the antigen of choice -- the antigen ● that was used previously to repeatedly immunize the mouse in order to expand the number of B-cells making antibody against this antigen.

Once the well is identified that has the antibody of interest, the cells in this well can be isolated and their number expanded. This cell population will be <u>monoclonal</u> (since all the cells in the well will descend from a single ancestral cell that was originally placed in the well), and all of the antibody molecules in the supernatant medium above these cells will be <u>**identical**</u> to one another, since these antibodies are being made by a monoclonal population of antibody-secreting cells. Hence, you've made a **monoclonal antibody!**

There are gene segments in the cellular genome that encode the sequences found in the antigen-combining "variable" domains of antibody molecules. Each variable domain is composed of three subdomains, termed V, D, and J.



(Padlan, EA. *Mol Immunol* 31 : 169 - 217, 1994)

If each of the subpopulations of B cells in the immune system makes its own specific antibody molecule (each having its own **variable region**),, and there are thousands, even millions of distinct subpopulations of B cells in the immune system,
how does the immune system as a whole know how to make so many distinct variable regions?

Are there thousands/millions of **variable region**-encoding genes in the genome? In the human genome is there a variable gene segment for each variable region of each antibody species present in the blood plasma?

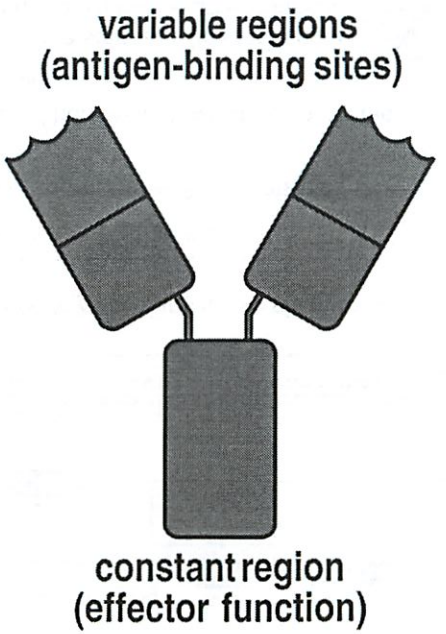


Figure 1-16 Immunobiology, 6/e. (© Garland Science 2005)

If each subpopulation of B cells in the immune system makes its own specific antibody molecule (each having its own **variable region**),, and there are thousands, even millions of distinct subpopulations of B cells in the immune system,
how does the immune system as a whole know how to make **so many** distinct variable regions?

Are there thousands/millions of **variable region**-encoding genes in the genome? Is there a set of 3 variable gene segments (V+D+J) for each variable region in the human genome?

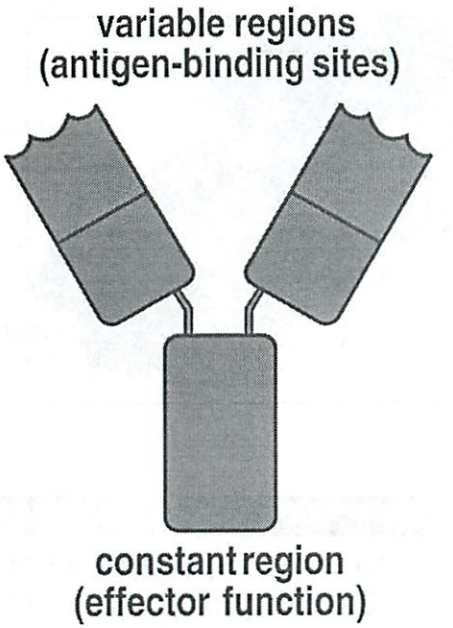


Figure 1-16 Immunobiology, 6/e. (© Garland Science 2005)

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how does the immune system as a whole know how to make so many distinct variable regions?
Are there thousands/millions of **variable region**-encoding genes in the genome? Is there a variable gene for each variable region in the human genome?

Has evolution <u>anticipated</u> our encounters with thousands of infectious agents by designing antibody-encoding genes that can recognize millions of epitopes??

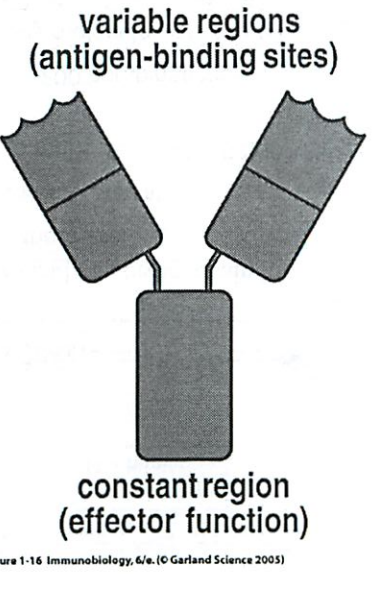


Figure 1-16 Immunobiology, 6/e. (© Garland Science 2005)

The **Actual Structure** of the Antibody-encoding Genes!

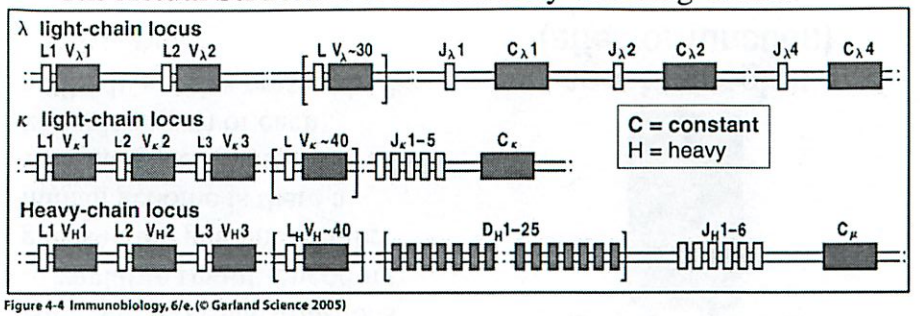


Figure 4-4 Immunobiology, 6/e. (© Garland Science 2005)

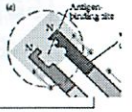
[There are two alternative light chain loci (κ & λ) and one heavy chain locus.

For example, in the case of the Heavy chain locus,, there are

40 alternative V_H segments + 25 alternative D_H segments + 6 alternative J_H segments.

In addition, there is only one C segment (denoted here as C_μ), which encodes the constant region of the resulting antibody molecule.

(The "L" segments in front of each V_H segment is a standard "leader" seque that ensures that the N-terminus of the resulting protein will be able to inserte the membrane of the rough ER in anticipation of its being secreted.)



The **Actual Structure** of the Antibody-encoding Genes

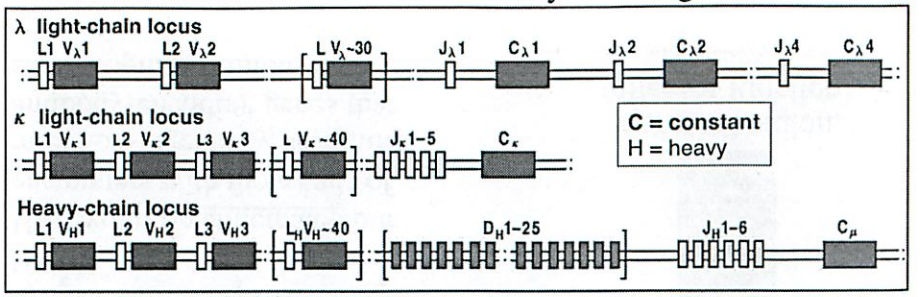


Figure 4-4 Immunobiology, 6/e. (© Garland Science 2005)

[There are two alternative light chain loci (κ & λ) and one heavy chain locus.

In the case of the Heavy chain locus, for example, there are

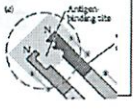
40 alternative V_H segments + 25 alternative D_H segments + 6 alternative J_H segments

These segments can associate **combinatorially** to make 40x25x6 = 6,000 distinct combinations.

One of the light chain loci (e.g., κ chain) has 40x5 = 200 combinations

Since the antigen-recognizing domain of an antibody molecule is made c variable regions, one from the H and the other from the L chain, this mea

therefore, 6,000x200 = 1.2×10^6 distinct domains can be made!



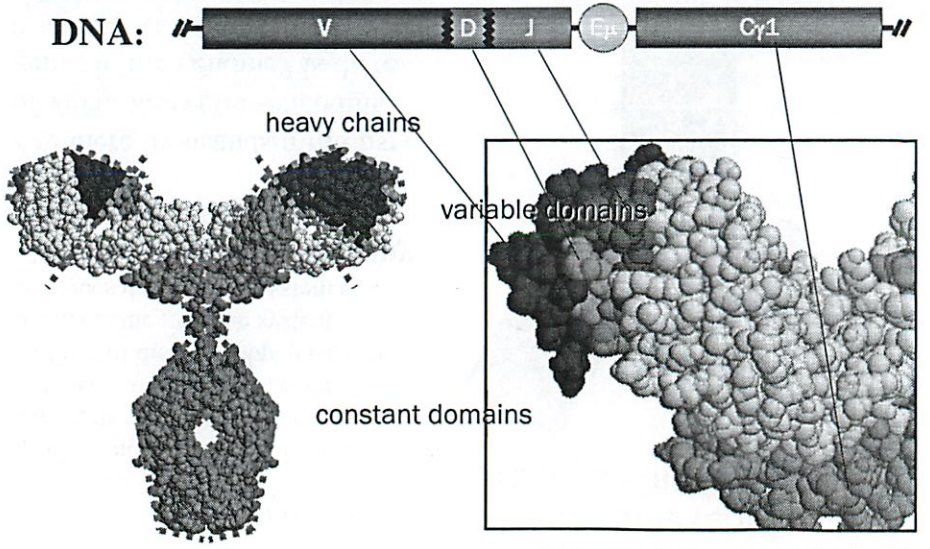
V(D)J recombination

- Indispensable for the differentiation of B- and many T-lymphocytes, which constitute the adaptive immune system*.

- By recombining a relatively small set of gene segments, an exponentially greater number of diverse antigen receptors can be created to recognize almost any foreign pathogens.

*The adaptive immune system is the arm of the immune system that can respond to external challenges/stimuli by mounting some type of antigen-specific response.

The V (variable), D (diversity), and J (junctional) gene segments encode the diverse antigen-binding surface



(Padlan, EA. *Mol Immunol* 31 : 169 - 217, 1994)

There two alternative light chain loci (κ & λ) and one heavy chain locus are organized slightly differently. The **light chain** variable region is encoded by a V and a J segment fused together in the DNA (through **deletion of intermediary DNA sequences**). The **heavy chain** variable region is encoded by a V, a D, and a J segment fused together (also through **deletion of intermediary DNA sequences**).

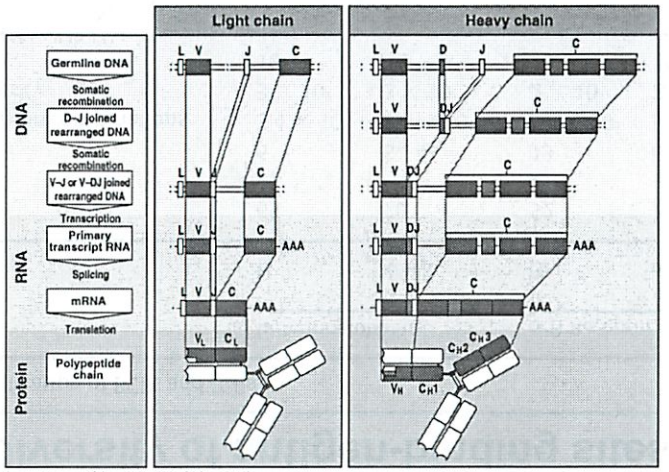


Figure 4-2 Immunobiology, 6/e. (© Garland Science 2005)

These segments (after fusion) make one-half of the antigen-binding site.

These segments (after fusion) make the other half of the antigen-binding site.

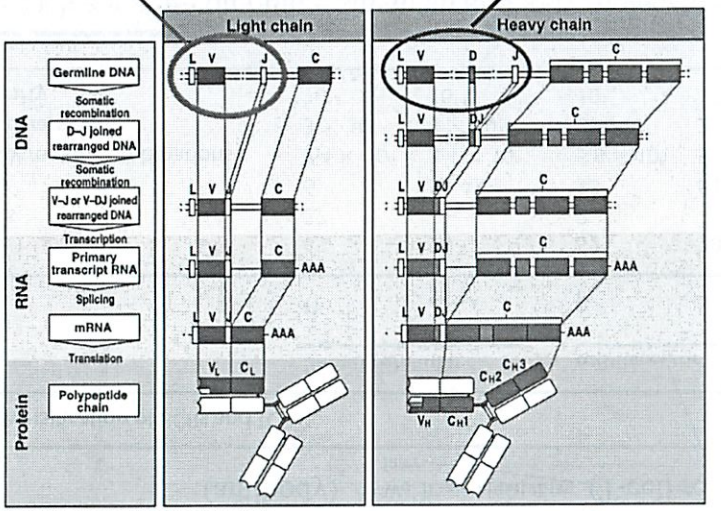


Figure 4-2 Immunobiology, 6/e. (© Garland Science 2005)

After the variable region pre-mRNA has been created by transcription of the fused V+J (or V+D+J) DNA)segments , the process of **RNA splicing** ensures that the variable region segment is joined (in the resulting pre-mRNA) with the nearest downstream **C region** pre-mRNA **segment**.

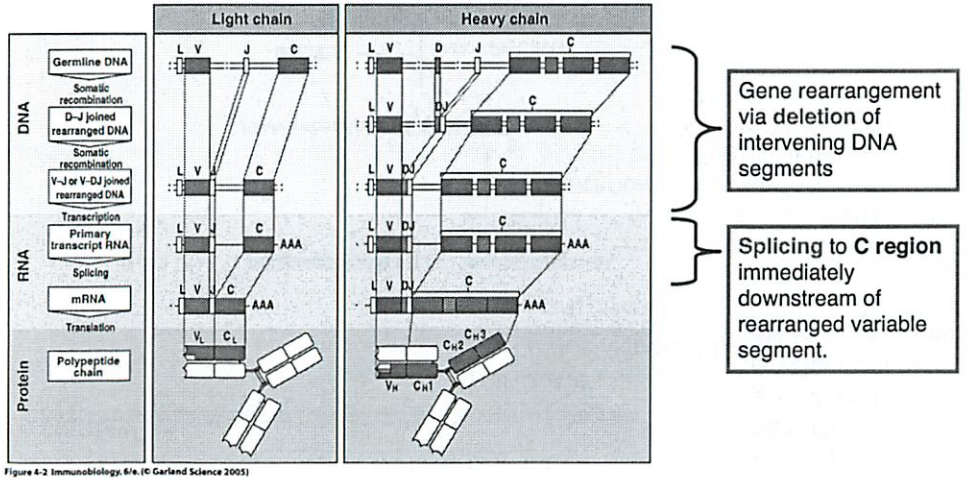


Figure 4-2 Immunobiology, 6/e. (© Garland Science 2005)

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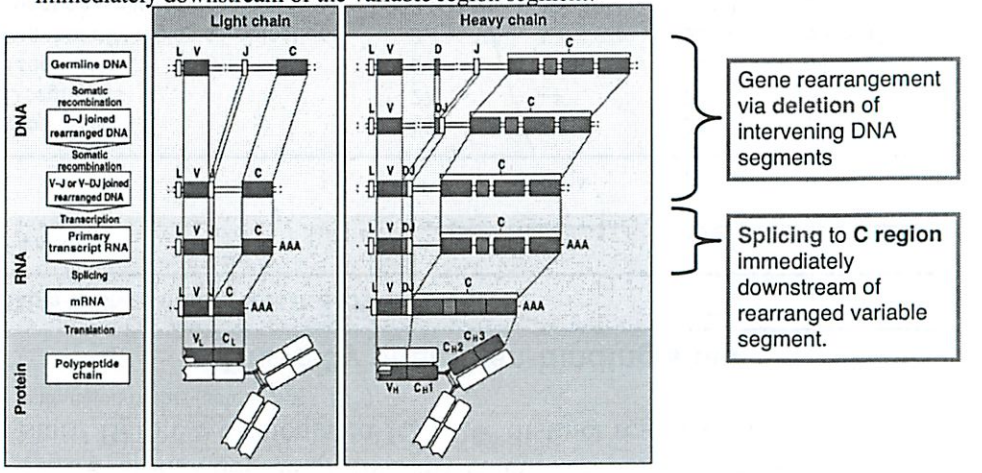
In the light chain locus, this **C region** segment is either the κ & λ -encoding segment. In the heavy chain locus, this nearest (leftmost in this diagram) **C region** is initially the "μ" constant chain. However, later on, as the immune response develops, this leftmost μ region may be deleted from the DNA, placing another one of the heavy chain **C regions** immediately downstream of the variable region segment.



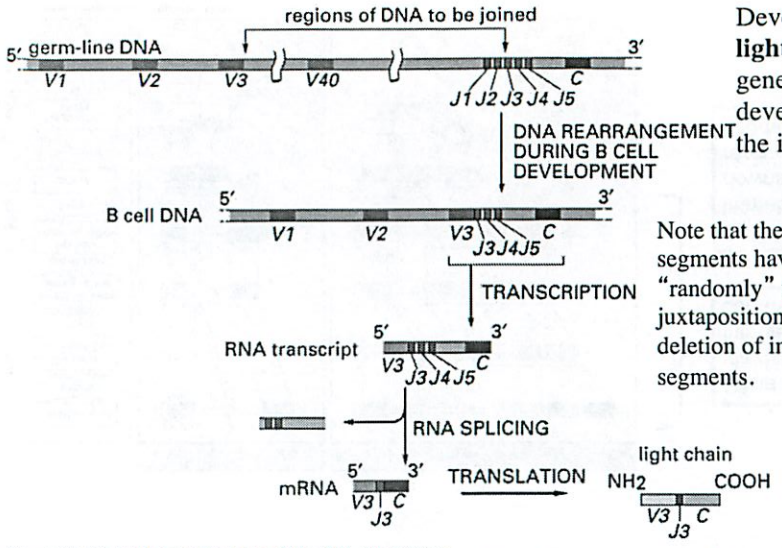


Figure 24–37. Molecular Biology of the Cell, 4th Edition.

Development of a **light chain** gene during development of the immune system

Note that the *V3* and *J3* segments have been chosen "randomly" for juxtaposition/fusion via deletion of intervening DNA segments.

Note that upon transcription of the fused *V3* and *J3* segments the resulting transcript is spliced to the nearest downstream C segment, thereby skipping over (deleting) the *J4* and *J5* segments, which are still present in the DNA.

Diversity of antigen-binding sites

Table 1. Diversification of BCRs and TCRs

Element	Immunoglobulin		α:β Receptor	
	H	κ + λ	β	α
V segments	65	70	52	70
D segments	27	—	2	—
J segments	6	5κ 4λ	13	61
Number of V region combinations	3.4×10^6	3.4×10^6	5.8×10^6	5.8×10^6
Junctional diversity	3×10^7	3×10^7	2×10^{11}	2×10^{11}
Total diversity	10^{14}	10^{14}	10^{18}	10^{18}

This arises because the fusion of V-D-J (heavy chain) and V-J (light chain) DNA segments is sloppy, yielding a variety of fused genes. (Cells that generate an out-of-frame fusion are eliminated.)

Market et al., PLoS Biol. 2003

However, this degree of diversity calculated before is only a start! Before we calculated 1.2×10^6 distinct regions. (The no. calculated in this table is 3.4×10^6.)

Diversity of antigen-binding sites

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Total diversity	10^{14}	10^{14}	10^{18}	10^{18}

Two **alternative** light chains -- either κ or λ

Market et al., PLoS Biol. 2003

Diversity of antigen-binding sites

(antibody) We'll talk about this later (T-cell receptor)

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Junctional diversity	3×10^7	3×10^7	2×10^{11}	2×10^{11}
Total diversity	10^{14}	10^{14}	10^{18}	10^{18}

Since each antigen-combining site in an antibody molecule is formed from one heavy (H) and one light (L) variable region, The total no. of possibilities is the product of these two numbers.

Market et al., PLoS Biol. 2003

Diversity of antigen-binding sites
(antibody)

Table 1. Diversification of BCRs and TCRs

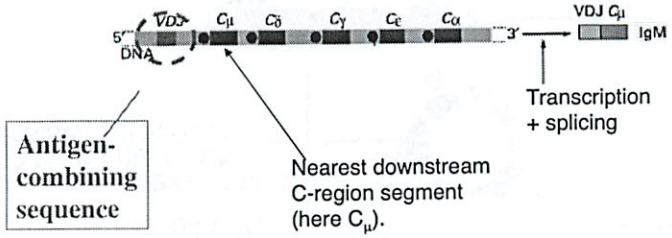
Element	Immunoglobulin	
	H	κ + λ
V segments	65	70
D segments	27	—
J segments	6	5κ 4λ
Number of V region combinations	3.4×10^6	3.4×10^6
Junctional diversity	3×10^7	3×10^7
Total diversity	10^{14}	10^{14}

In truth, there is an **additional source** of diversity: There is an enzyme that operates on the DNA segments encoding the variable regions and that purposely introduces point mutations into these segments, leading to further diversification. This enzyme works to deaminate cytosine residues, thereby making them effectively thymidine residues. (Process termed the creation of "hypervariability".)

Market et al., PLoS Biol. 2003

Let's return to the constant region of the heavy chain genes. After V-D-J recombination, these genes look like this.

Class switching



Initially, following *VDJ* recombination in a heavy region locus, when the resulting antigen-combining variable region segment is transcribed, it becomes joined via RNA splicing to the **nearest constant** region segment downstream. This constant region segment is C_μ (constant μ), and results in the formation of an IgM molecule following translation of the resulting spliced mRNA.
μ --> M, α -->A, γ -->G

Various antibody classes: The red variable regions may all be identical and recognize the same antigen. The **blue** constant regions differ, allowing these antibody molecules to have multiple alternative functions.

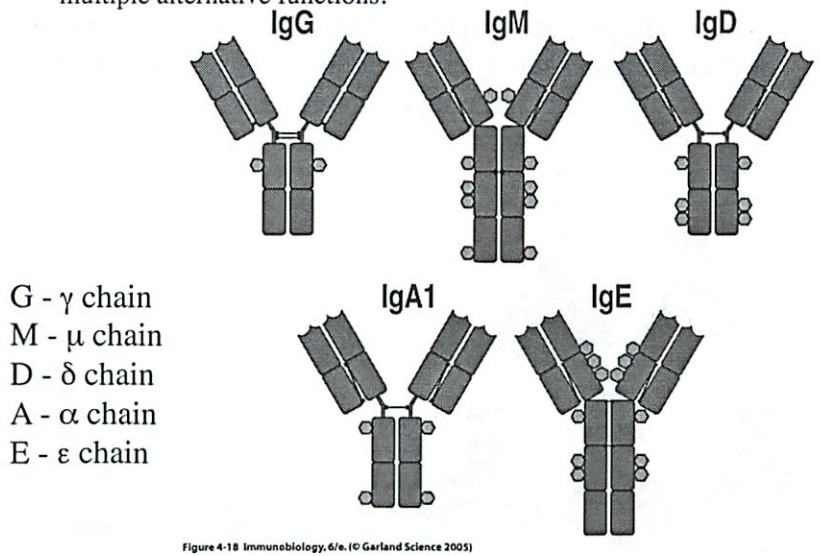


Figure 4-18 Immunobiology, 6/e. (© Garland Science 2005)

Here is the unusual structure of secreted IgM molecules. Note that this structure allows this assembly to bind simultaneously 10 identical antigen molecules -- great crosslinking potential!

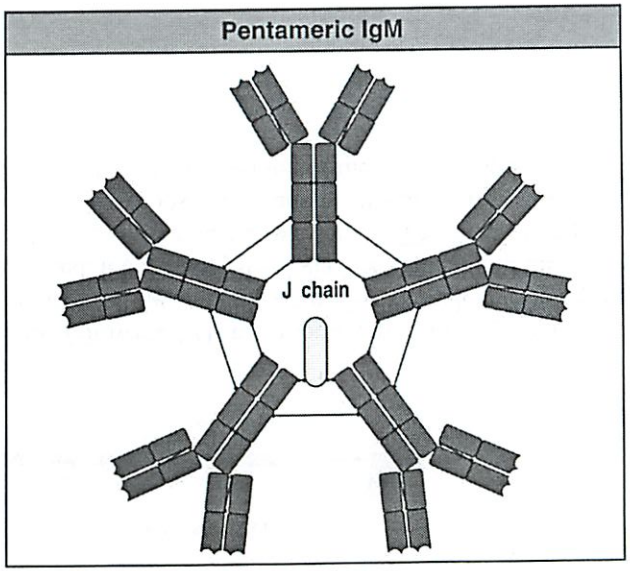
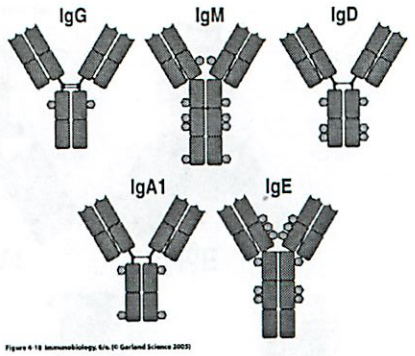


Figure 4-23 part 1 of 3 Immunobiology, 6/e. (© Garland Science 2005)

The differences between these are due to the incorporation of different **heavy chains;** light chains are the same

G - γ chain
M - μ chain
D - δ chain
A - α chain
E - ε chain



The use of fusion of a heavy chain **variable** segment (resulting from VDJ recombination) to various **constant region** segments reflects the process of **class switching** (i.e., switching from making an IgM to making an IgG molecule while keeping the same variable region segment)

Class switching

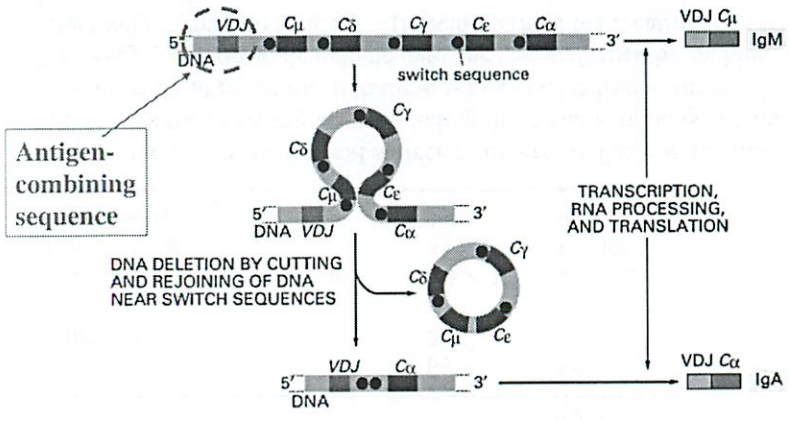


Figure 24–41. Molecular Biology of the Cell, 4th Edition.

Before the class switching the antigen-combining site was joined (following RNA splicing) to a C_μ (constant μ), segment, yielding an IgM molecule. Afterward, as the immune response develops, the C_μ DNAsegment (as well as other constant region DNA segments) may be deleted from the genome. Now, in the example depicted here, the variable(VDJ) region becomes spliced to a C_α segment, yielding an IgA antibody with the same antigen-binding specificity.

The first step:

Class switching

Antigen-combining sequence

Initially, following *VDJ* recombination in a heavy region locus, when the resulting antigen-combining variable region segment is transcribed, it becomes joined via RNA splicing to the **nearest constant** region segment downstream. This constant region segment is *Cμ* (constant μ), and results in the formation of an IgM molecule following translation of the resulting spliced mRNA.

VDJ already rearranged

Class switching (H chains): (enabling one already-developed antigen-combining segment to become allied with another distinct constant region)

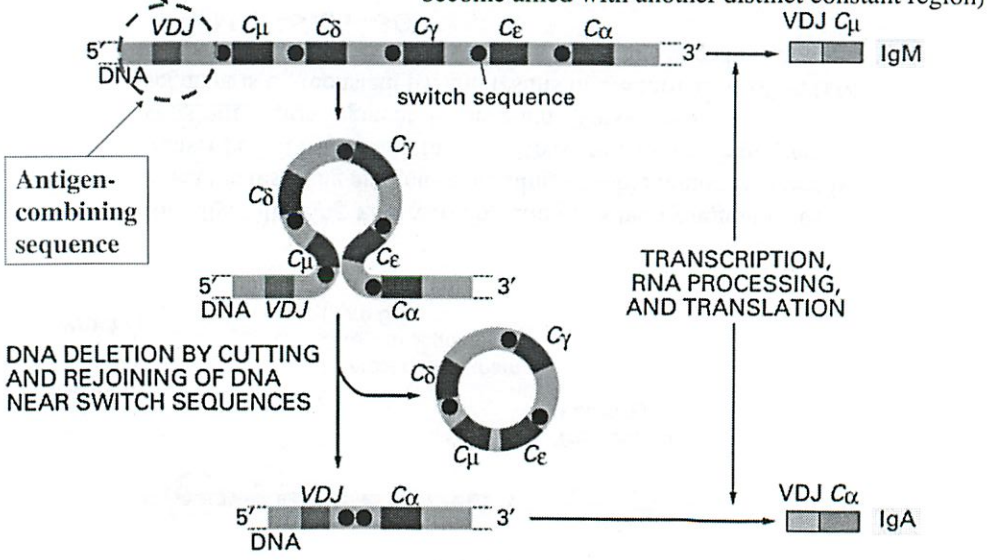


Figure 24–41. Molecular Biology of the Cell, 4th Edition.

To review: The immune system has many different "arms". We will focus on its **humoral** and **cellular** arms.

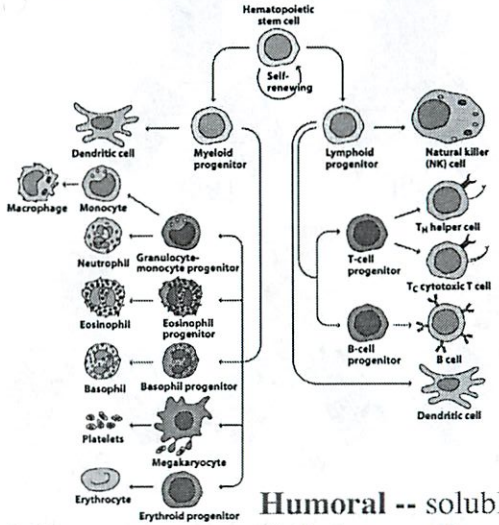
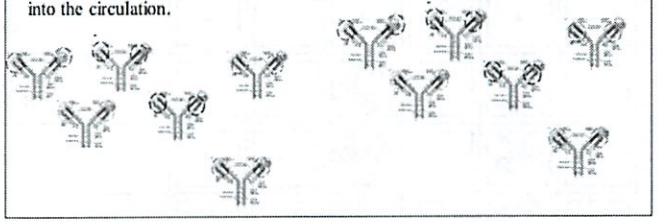


Figure 2-2
Kuby IMMUNOLOGY, Sixth Edition
© 2007 W.H. Freeman and Company

Humoral -- soluble antibodies
Cellular -- cell-mediated responses

Let's **review** in some detail the steps needed to make a monoclonal antibody.
a.In the spleen (and bone marrow) of a mouse there are normally millions of B-cell populations, each B cell having developed the ability to make its own particular antigen-specific B cell.

In this mouse, many of these B cells are, on occasion, differentiating/maturing into plasma cells, and the latter are secreting millions of antigen-specific antibodies into the circulation.

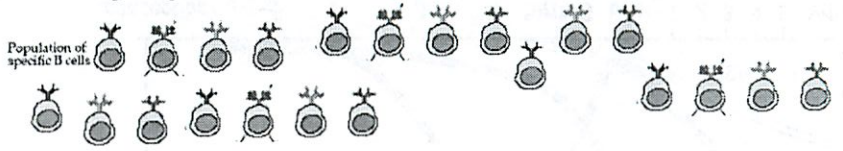


There are at least two reasons why a B-cell or B-cell clone will be **eliminated** early in its development:

1. It fails to make (via gene rearrangement) a functional antibody
2. It makes an antibody that reacts with **Self,** i.e. the body's own native proteins -- the issue of **Tolerance** (self vs. non-self)

Let's **review** in

In the spleen (and bone marrow) of a mouse or human there are normally millions of B-cell populations, each B cell having developed the ability to make its own particular antigen-specific B cell.



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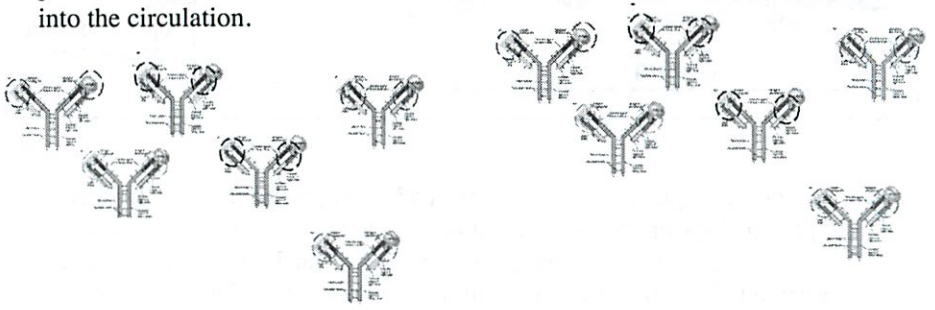


Therefore, class-switching allows the immune system to make a number (~8) of antibody classes, which share in common identical antigen-combining V regions but have distinct **C regions.**

G - γ chain
M - μ chain
D - δ chain
A - α chain
E - ε chain

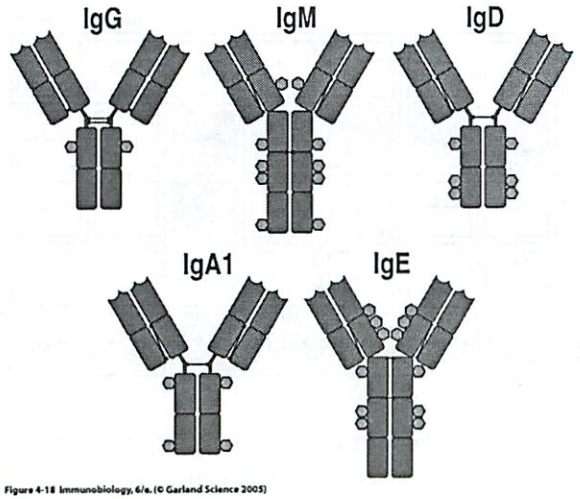


Figure 4-18 Immunobiology, 6/e. (© Garland Science 2005)

While all having the same variable regions, these various classes of an antibody, being fused to different constant regions, have **differing functions**.

Functional activity	IgM	IgD	IgG1	IgG2	IgG3	IgG4	IgA	IgE
Neutralization	+	–	++	++	++	++	++	–
Opsonization	+	–	+++	*	++	+	+	–
Sensitization for killing by NK cells	–	–	++	–	++	–	–	–
Sensitization of mast cells	–	–	+	–	+	–	–	+++
Activates complement system	+++	–	++	+	+++	–	+	–

Figure 9-19 part 1 of 2 Immunobiology, 6/e. (© Garland Science 2005)

FYI: "Opsonization" means coating a particles, such as a bacterium, with antibody molecules, enabling phagocytes to gobble up the particle. "Mast cells" can recognize an antibody-coated particle or cell and release toxic compounds in response. "Complement" is a group of proteins that punches holes in the membranes of antibody-coated cells.

So, to review: Various antibody classes: The **red** variable regions may all be identical and recognize the same antigen. The **blue** constant regions differ, allowing these antibody molecules to have multiple alternative functions.

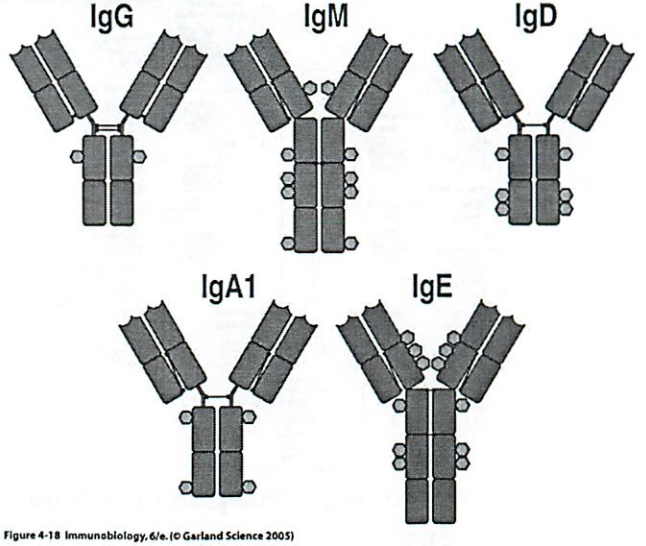


Figure 4-18 Immunobiology, 6/e. (© Garland Science 2005)

While all having the same variable regions, these various versions of an antibody, being fused to different constant regions, have differing functions. They're found in different compartments in the body.

Distribution	IgM	IgD	IgG1	IgG2	IgG3	IgG4	IgA	IgE
Transport across epithelium	+	–	–	–	–	–	+++ (dimer)	–
Transport across placenta	–	–	+++	+	++	+/–	–	–
Diffusion into extravascular sites	+/–	–	+++	+++	+++	+++	++ (monomer)	+
Mean serum level (mg ml^{-1})	1.5	0.04	9	3	1	0.5	2.1	3×10^{-5}

Figure 9-19 part 2 of 2 Immunobiology, 6/e. (© Garland Science 2005)

FYI: "extravascular" means the portions of tissues outside of the circulatory system, i.e., outside of blood vessels.

The immune response develops progressively. IgG molecules are transferred via the placenta during gestation and via milk during breastfeeding. Following exposure to novel antigens early in life, IgM molecules are initially produced; however, as the immune system and immune responses develop, these IgM molecules are progressively changed over to IgG and then IgA responses, etc.

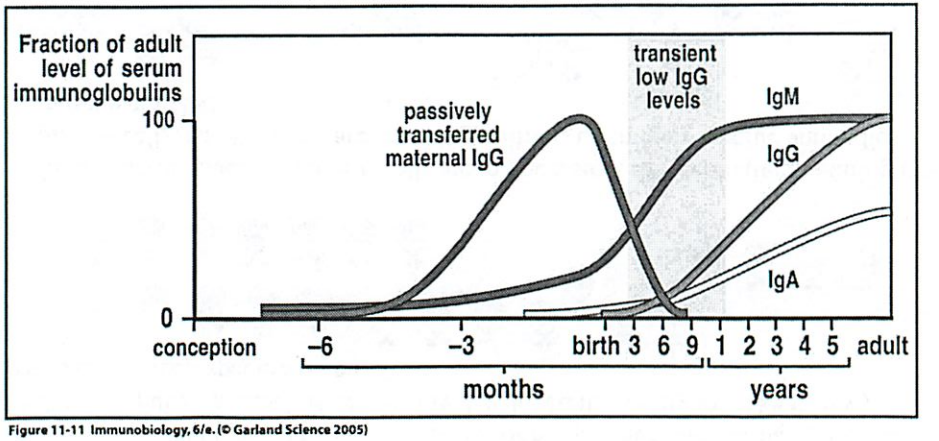


Figure 11-11 Immunobiology, 6/e. (© Garland Science 2005)

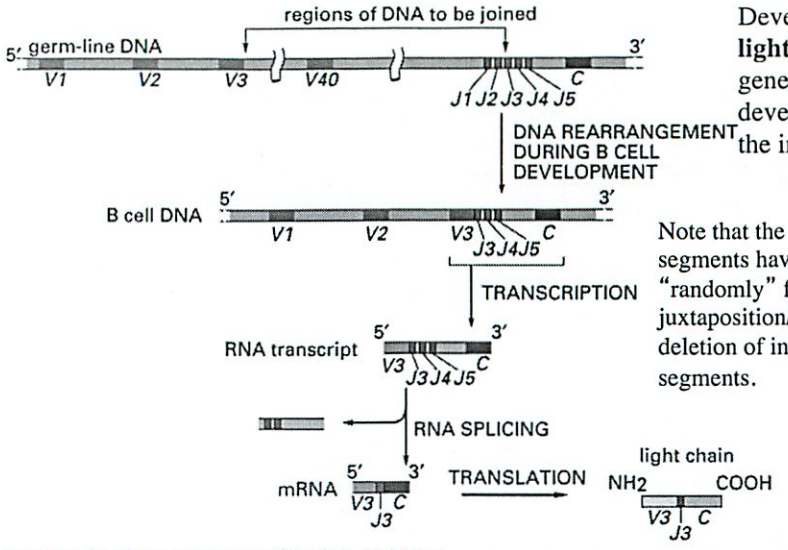
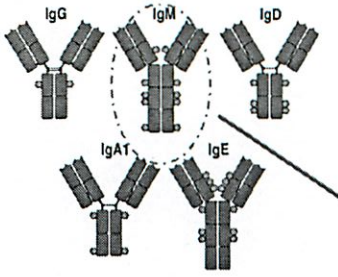


Figure 24–37. Molecular Biology of the Cell, 4th Edition.

Development of a **light chain** gene during development of the immune system

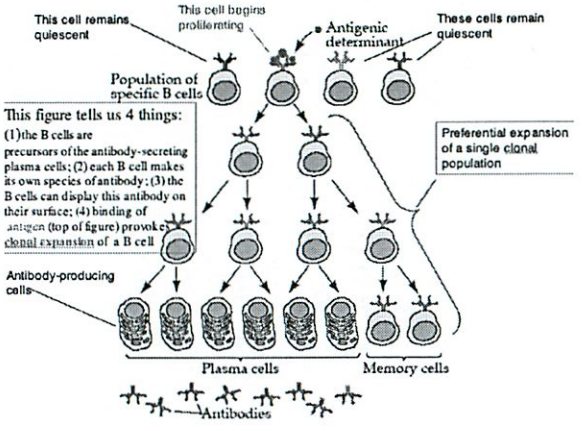
Note that the *V3* and *J3* segments have been chosen "randomly" for juxtaposition/fusion via deletion of intervening DNA segments.

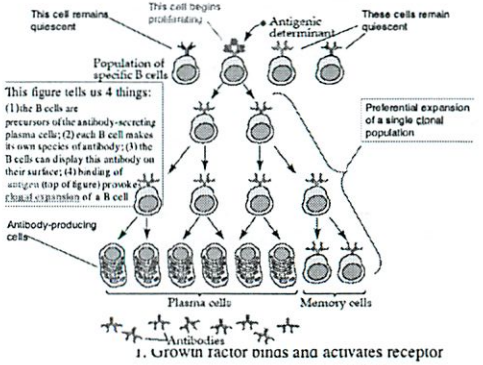
Note that upon transcription of the fused *V3* and *J3* segments the resulting transcript is spliced to the nearest downstream C segment, thereby skipping over (deleting) the *J4* and *J5* segments, which are still present in the DNA.



This "class switching" helps to explain a puzzle that was implicit in our earlier depiction of clonal expansion. If exposure to an "antigenic determinant" provokes clonal outgrowth, how can a B-cell sense the presence of an antigen in its surroundings? Answer: The initially displayed antibody molecule is a cell-surface,

IgM transmembrane protein is initially configured like a growth factor receptor. (Later on it becomes secreted.)





After B cells have undergone a VDJ & VJ recombination, they display the initial product of this recombination - a form of IgM -- on their surface. This IgM functions much like a growth factor receptor, I.e., when it binds its "ligand", it becomes activated and sends a stimulatory signal into the cytoplasm that stimulates clonal expansion and leads ultimately to descendants (plasma cells) that secrete soluble IgM and, following class switching, other antibody types -- e.g., IgG, IgA, IgE, etc.

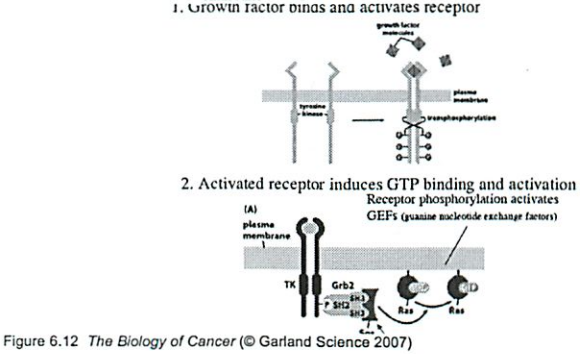
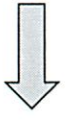


Figure 6.12 *The Biology of Cancer* (© Garland Science 2007)

Next problem: How can the immune system monitor the various compartments in the body to determine whether novel antigens (and thus foreign infectious agents) have invaded the body and should be attacked and neutralized?

In the case of certain viruses and bacteria, they may release soluble protein antigens that can be recognized by the immune system, e.g., stimulate the clonal expansion of B-cell clones.

But what if a virus has invaded a cell and is multiplying within the cell??

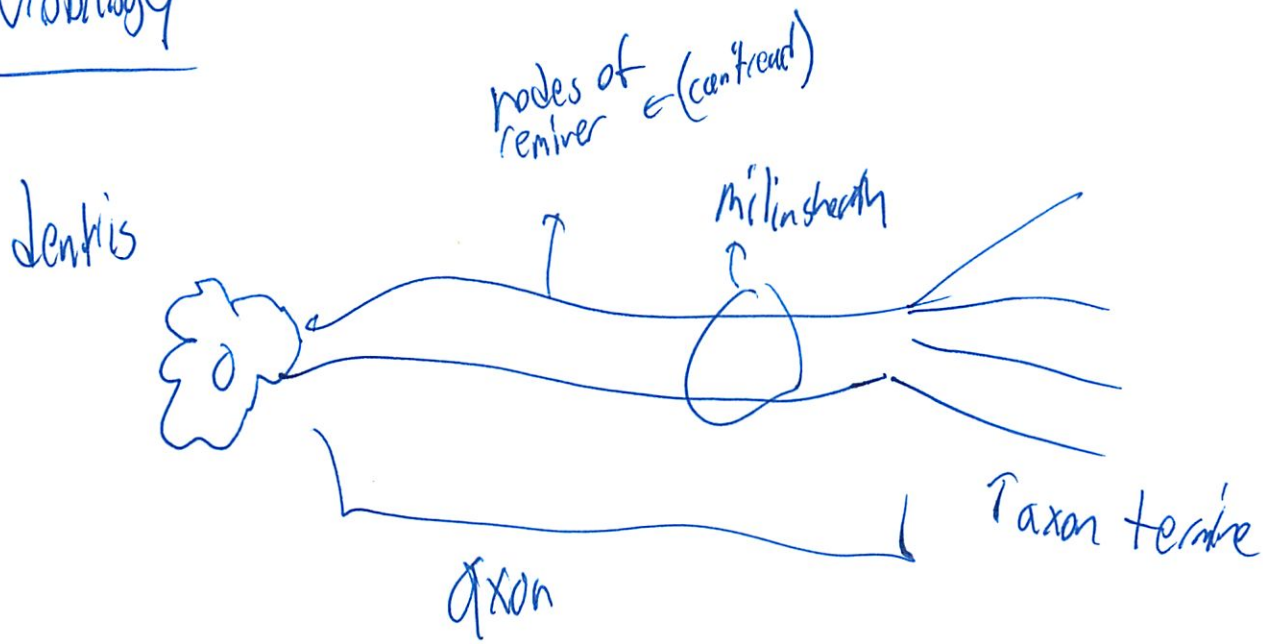


7.012 Recitation

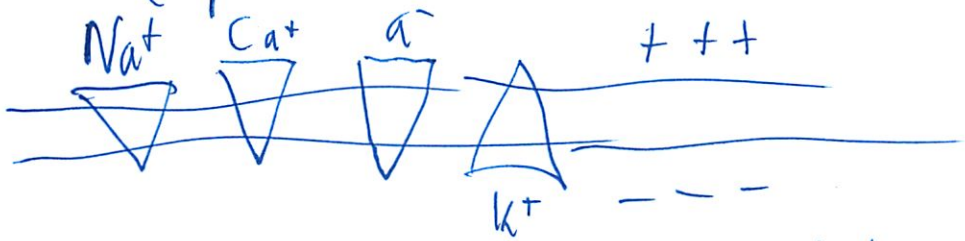
11/6

(5 min late)

Neurobiology

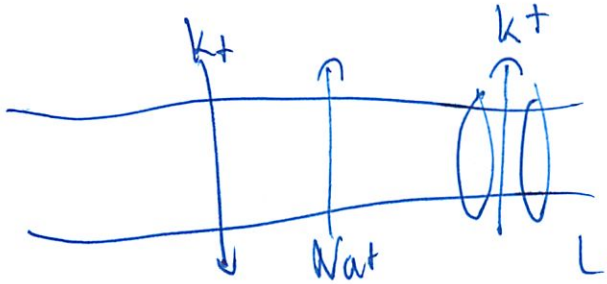


Membrane potential



1. Chemical

2. Electrical



Na/k+ pump

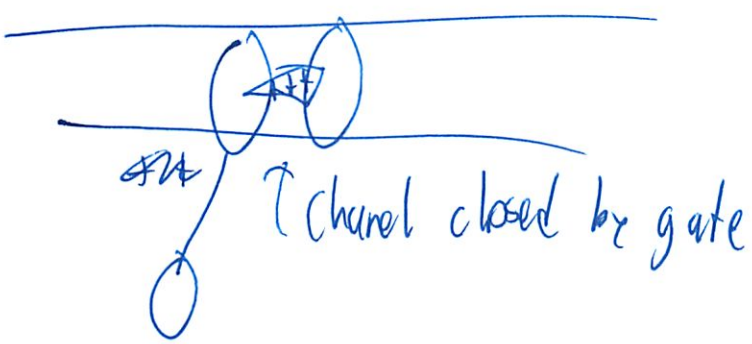
K+ Resting Channel

ATP → ADP

Ions - Na+ k+

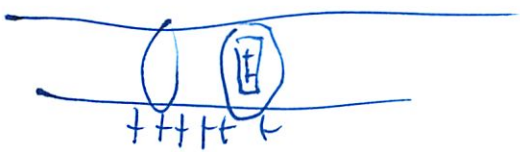
Ch - Voltage Gated

How sensitive to voltage



As +++ builds inside cell
gate moves inside potien
Since gate = (+)

Threshhold = property of channel



(3)

Sodium rushes in

favorable electrically + chemically

favorable:

high → low conc

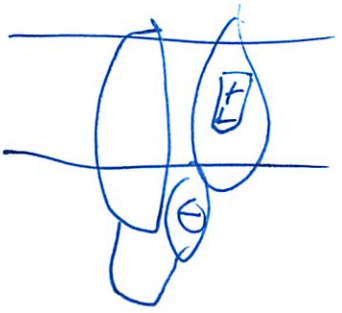
away from same charge, towards neutral

inactivation

not same as closed

repel ⊖ charged ball

gate comes back down



ball is slow

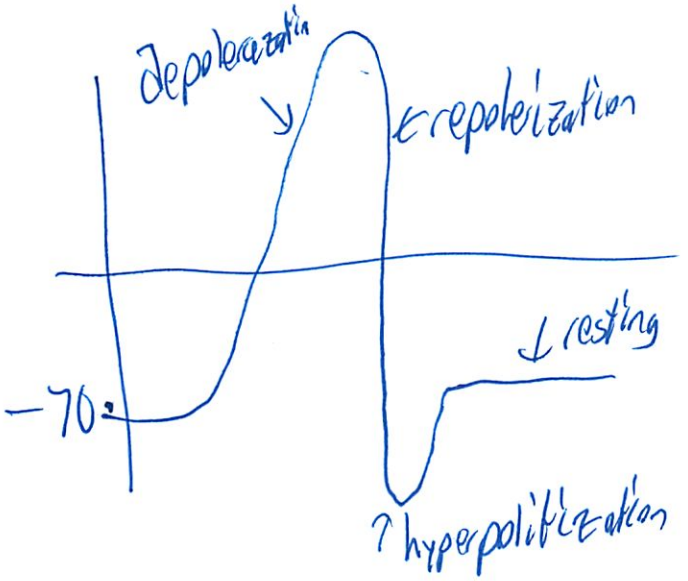
Could let out a bit too much potassium

↳ hyperpolarization

let out too much potassium

(4)

On p-set must know what is high/low concentration

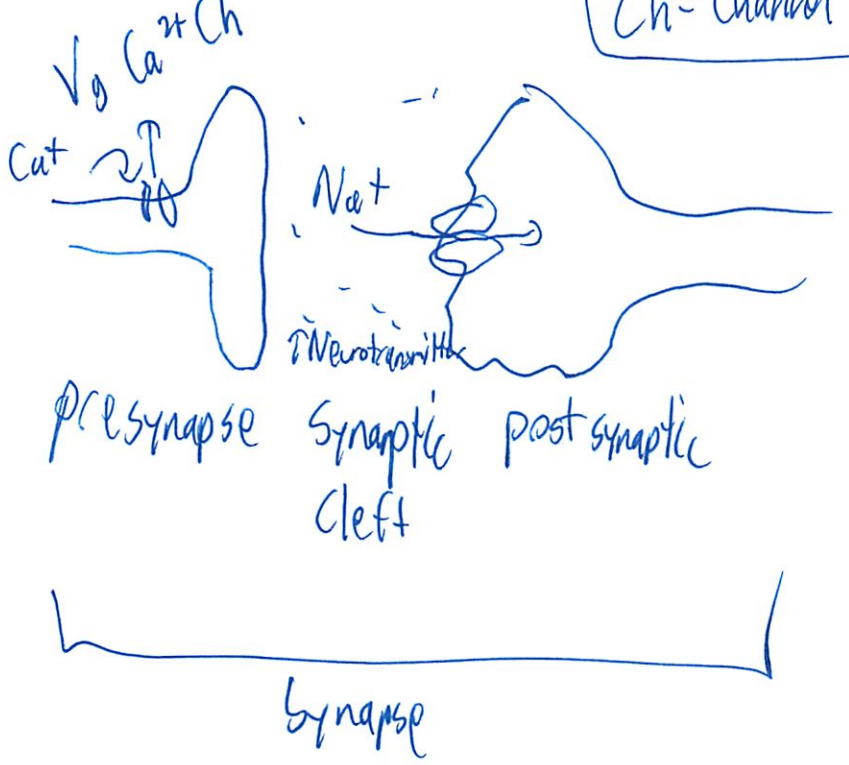


Action potential jumps down

(need to review this stuff a bit more slowly)

(5)

Vg = Voltage gated
Ch = Channel



Gate opens; Voltage gated calcium channel
Calcium moves in
That pulls vesicle to membrane
Fuses
Releases neurotransmitter into cleft

NT bind to channels in post synaptic
Ligand gated
The NT

(6)

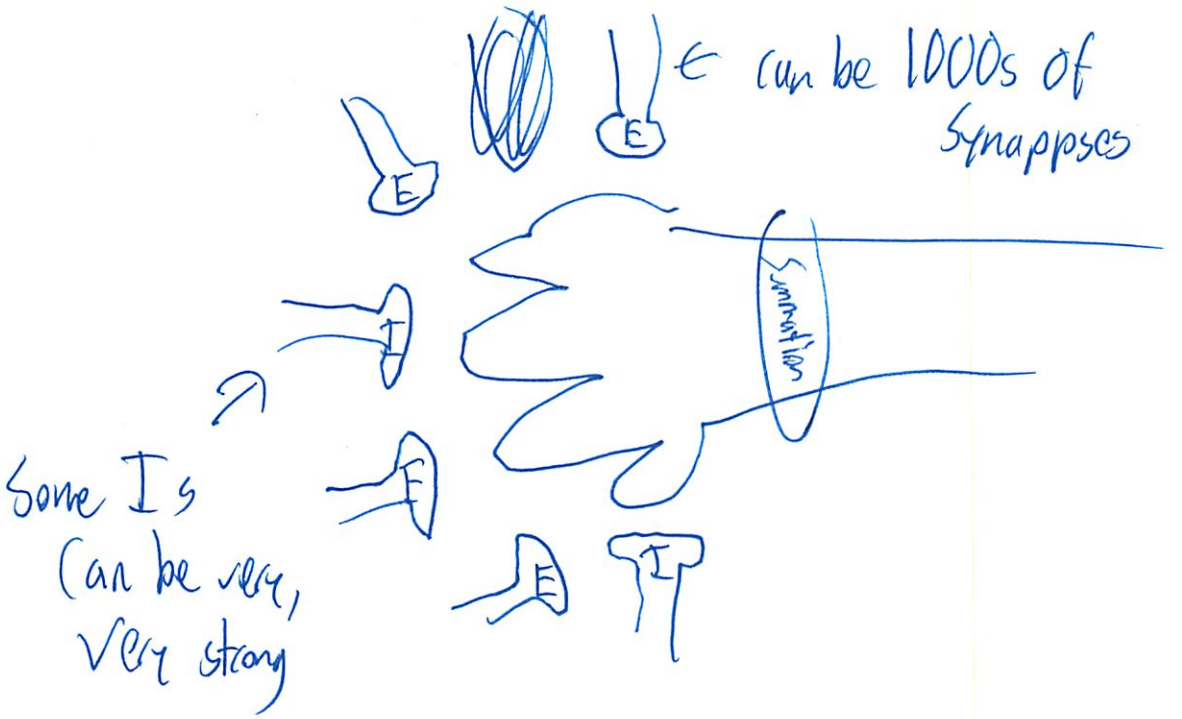
Each Neuron makes a specific 1 neurotransmitter

Summation
must be large enough to reach summation

excitatory more likly to fire — Na^+ in, Ca^{2+} in
inhibitory less likly to fire — K^+ out, Cl^- out

? diff direction than saw before

and makes sense Cl^- in



E can be 1000s of synapses

Some I's can be very, very strong

1

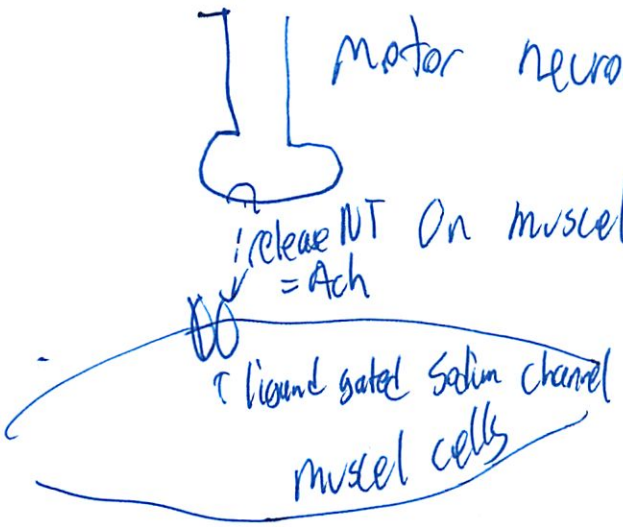
Neurotransmitters

CNS

typically, but not absolutly!

Dopamine - E
GABA - I
Serotonin - E

Ach in muscle opens sodium Ch → E
" nerve systems opens cloride Ch → I



ligand Opens channel
Sodium rushes in
Muscel can contracts

(8)

Once NT in cleft - must remove or have signaling
reuptake

1. Reuptake
2. Degrade

Acetylcolinsetease - destroys Ach

Bacholinum - toxin
bacteria when don't cook food properly
basis for Botox

Botox - inhibits interaction
vescles can't fuse
Can't release Ach
So get fluid paralysis
↳ muscles relaxed
opposite: rigid paralysis

(9)

Pico - competes for binding for channel
↳ sp but can't open
flaccid paralysis

tetanis - rigid ~~paral~~ paralysis
↳ inhibitor of Ach-ese
So NT constantly signaling
all muscles contracted
breaks the back

When ya learn - new synapses are formed
If don't continue to stimulate it → ya lose it

(10)

Immunology

2 parts: Innate - general protection of the body

no specificity

no memory

- Macrophages
- Neutrophils
- DC
- Mast cells
- Natural killer cells

} will anything that is unknown

Adaptive

Humoral - production of antibodies mediated by b cells

Cell mediated - T cells (mediated by)

11

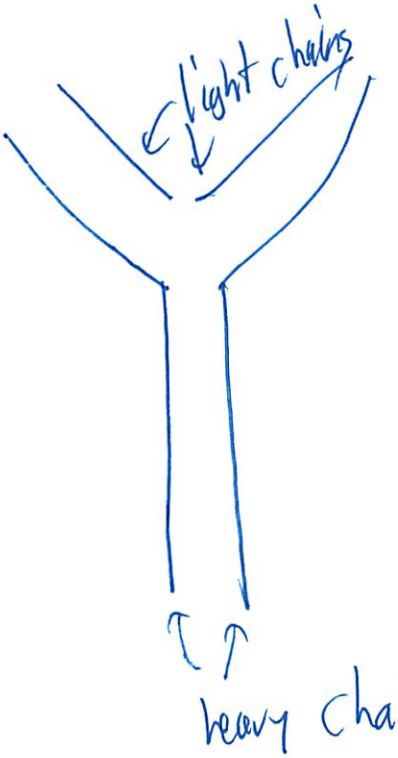
Antibody - Protien that binds to antigens

quatinary

band w/ disulfage bridges

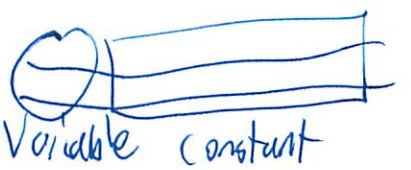
band by Cys

↑ qv on final!



both heavy + light have some constant seq

and variable regions



12

Antigen - protien

Antibody recognizes domains of the antigen

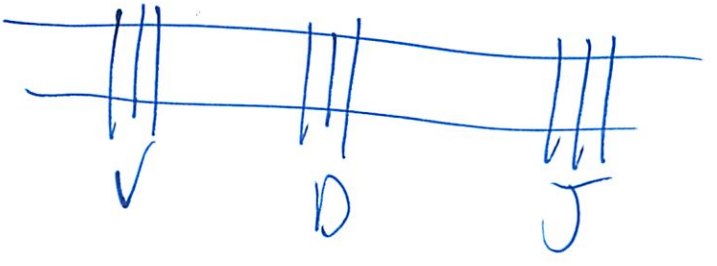
So can be multiple antibodies for the same antigen

Polyclonal - come from diff clones
└ antibodies

V(D)J Recombination

Not splicing!

Are domains

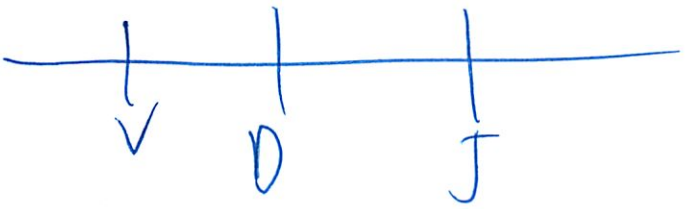


heavy chain

light chain - same w/o D region

(13)

So pick one V, one D, one J



So have a unique DNA seq

It gets transcribed, spliced, mutated

(adds to variety)

Diff segments mixed together & other antibodies

Only 1 way that we have diversity

But w/ all of this we have enough combos that we can do anything

(14)

Somatic Hypermutation

tendency of cells to cause mutations in DNA of cells of antibodies

call these mutations formed adds in variation

What type of cells is this happening in?

B-cells

○ Immature

↓ VDJ

○ Mature

2012 7.012 Recitation 12

Summary of Lectures 19

A neuron receives signals at its dendrites and sends signals down its axon. A synapse occurs wherever the axon terminus of one neuron meets the dendrite of another neuron (or a muscle cell). At a synapse, the electrical signal of an action potential is converted to the chemical signal of a neurotransmitter, and then this is converted back into an electrical signal in the post-synaptic cell. Action potentials are the characteristic changes in membrane potential that propagate down the length of axons, unidirectionally at each point on the membrane from the hillock to the terminus. The axons of motor neurons are coated in a myelin sheath that allows action potentials to travel down axons faster by allowing them to jump from node to node between patches of insulation.

An action potential begins when a threshold membrane potential is reached at the axon hillock, and voltage-gated Na+ channels are induced to open. Once Na+ rushes in, the inside of the cell becomes positive, and this induces the voltage-gated K+ channels to open. Thus K+ rushes out, restoring the membrane potential back to the Nernst potential for K+. Action potentials do not vary by amplitude; the maximal membrane potential is always the Nernst potential for Na+. Instead, action potentials vary in frequency.

Molecules can move across membranes through pumps, channels, and transporters. Integral membrane proteins like these cross the membrane via transmembrane domains. Pumps are ATPases that set up the concentration gradients of ions across cell membranes. A membrane potential is only set up by ions that move freely across the membrane through open channels, creating one side of the membrane that is more positive. This movement of ions does not dissipate the concentration gradient because the number of ions that move to generate a membrane potential is very small. Most cell membranes only contain open K+ channels and thus have a membrane potential of –70mV, which is very close to the equilibrium potential for K+.

Ions are free to move across the membrane through open ion channels. Two forces act to dictate this movement – the concentration gradient and the membrane potential. Ions move down their concentration gradients through channels, and ions move towards the side of the membrane that harbors the opposite charge. The equilibrium potential for an ion is the membrane potential at which the two opposing forces on the ion are equal.

Questions:

1a) Explain what is meant by the term "resting membrane potential". Is this resting membrane potential exclusively present in neurons? **Explain**.

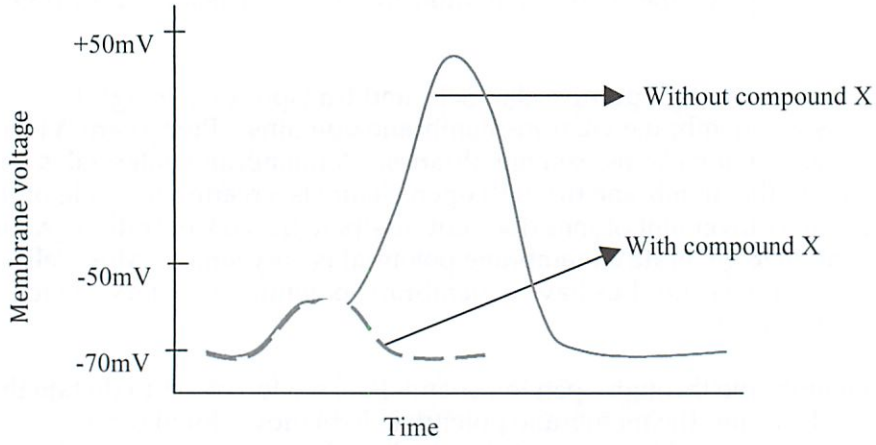
b) List the distinct protein complexes that are found in the plasma membrane of nerve cells and are essential in establishing and maintaining the **resting membrane potential**. For each protein complex you list, include what ion(s) move through that protein complex and in which direction that ion moves (into the cell or out of the cell) to maintain the resting membrane potential.

Protein complexes	*Ion(s) moved*	*Net direction of ion movement*

c) Once the resting membrane potential has been established, which protein complex or complexes are essential to generate an **action potential** (*both the depolarization and repolarization phases*). For each protein complex you list, include what ion(s) move through that protein complex and in which direction that ion moves (into the cell or out of the cell) to generate an action potential.

Protein complexes	*Ion(s) moved*	*Net direction of ion movement*

d) The compound X alters the action potential by interfering with one of the protein complexes listed above.



Which protein complex is most likely affected by this compound? **Explain** your reasoning.

7.012 Section 14 - 2012

Summary from Lectures 20 & 21

Neurotransmitters, synapses and neuromuscular junctions: A neuron receives signals at its dendrites and sends signals down its axon. A synapse occurs wherever the axon terminus of one neuron meets the dendrite of another neuron (or a muscle cell). At a synapse, the electrical signal of an action potential is converted to the chemical signal of a neurotransmitter, and then this is converted back into an electrical signal in the post-synaptic cell. This occurs because, when an action potential reaches the axon terminus of a pre-synaptic cell, voltage-gated Ca++ channels open, and intracellular calcium induces the exocytosis of neurotransmitters into the synaptic cleft. The exocytosed neurotransmitters then bind to receptors in the dendrites of the post-synaptic cell. These ligand-gated ion channels are then opened, leading to changes in membrane potential that are summed at the axon hillock. If the sum of all these changes is greater than threshold, the post-synaptic neuron will fire an action potential, first at the hillock and then at each subsequent location down the axon to the axon terminus.

Neuromuscular junctions are synapses where a nerve cell contacts a muscle cell. The neurotransmitter that is released from the neurons at neuromuscular junctions is Ach (acetylcholine). The release of enough Ach will trigger the muscle cell to contract. Ach is cleared from the synapse by an enzyme that cleaves Ach called Ach esterase.

Nerve-nerve synapses use many different neurotransmitters. Some neurotransmitters are excitatory; their receptors allow the flow of ions that causes the inside of the post-synaptic cell to become more positive, making the cell closer to the threshold needed to fire an action potential. Other neurotransmitters are inhibitory; their receptors allow the flow of ions that causes the inside of the post-synaptic cell to become more negative, making the cell farther from the threshold needed to fire an action potential.

There can be many, many inputs to a postsynaptic cell, and the summation of all of these inputs occurs across the cell body. If the excitatory inputs are sufficient to depolarize the membrane at the axon hillock, the voltage-gated Na+ channels at the axon hillock will open and an action potential will be generated.

Neuronal circuits: Aplysia, a sea slug, offers a distinct advantage by eliciting a *visible* (measurable) response (*siphon-mediated gill withdrawal reflex*) to a stimulus, which can be studied directly. The response is triggered by *several* electrical synapses firing simultaneously, and thus can be used to study *habituation, sensitization* and *classical conditioning*. Hence this animal is considered a good model in neurobiology.

Habituation is the decrease in behavioral response to a stimulus after repeated exposure to that stimulus over a period of time. In the case of habituation in Aplysia, it was found that the decrease gill withdrawal reflex was a result of diminished release of neurotransmitter from the pre-synaptic neuron, possibly due to progressive inactivation of calcium ion channels. In the habituated animal, fewer pre-synaptic vesicles are released for the same stimulus. In addition, the size of the vesicles is seen to *decrease in habituation*.

Sensitization is the progressive amplification of a response following repeated administrations of a stimulus. In the case of *sensitization* in Aplysia, it was found that the increase gill withdrawal reflex was a result of increased release of neurotransmitter from the pre-synaptic neuron, due to an increase in both vesicle *number and size*.

Short-term memory, which usually lasts for a few minutes, involves covalent bonding of pre-existing proteins leading to alterations in the strength of already existing connections. By contrast, long-term memory requires the activation of nuclear components that may ultimately result in the alteration of synaptic connections or alterations in the type or amount of neurotransmitters or their corresponding receptors.

Neurons connect to form circuits, and in some cases, these neurons grow and innervate areas based on guidance cues/signals. In other cases, neurons project axons in a more random fashion, and those whose axons make useful connections survive whereas those failing to make useful connections die.

Questions

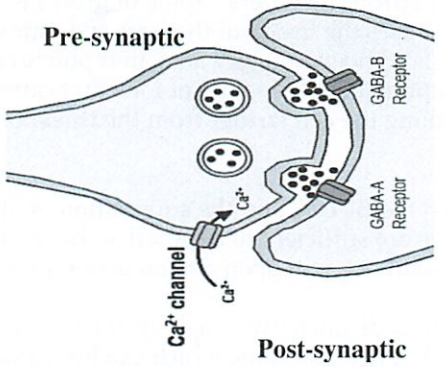
1. Dopamine is one of major neurotransmitters in the mammalian brain that regulates mood, cognition and locomotion. Dopamine acts on two types of receptors: the D1 receptor is an inhibitory ligand-gated channel, the D2 receptor activates the G proteins, and is excitatory. The released neurotransmitter is taken back into the presynaptic cell, for re-use.

a) On what part of the neuron are the dopamine receptors localized?

b) The D1 receptor is inhibitory and transports K+ ions. Would K+ be moved into or out of the postsynaptic cell? Explain the mechanism underlying this inhibitory effect.

c) The D2 receptor is excitatory, and its ion targets are believed to include Ca2+. Would Ca2+ be moved into or out of the postsynaptic cell? Explain the mechanism underlying this excitatory effect.

2. GABA is a major inhibitory neurotransmitter in central nervous system (CNS). It acts by binding to **GABA-A receptors that are chloride channels and GABA-B receptors that activate K+ channels via G proteins.**



K+ concentration is high inside the neuron, while Ca^{2+}, Na^{+} and Cl^{-} ion concentrations are high outside. Passage of Na^{+} into the neuron is responsible for an action potential.

a) In what direction will ions flow when the GABA-A receptor is activated – *in or out* of the neuron?

b) How does this flow alter the likelihood of an action potential in the post-synaptic neuron? **Explain.**

3. In Aplysia, or sea slug, the axonal connections of all the neurons have been traced.
In experiment 1, you tap the mantle of this organism 60 times, once every 2 sec, at a stretch and look at its gill withdrawal reflex. In experiment 2, you give 10 taps, once every 2 sec, wait for an hour before giving the next 10 taps. You repeat this 6 times every day for a couple of days. You then stop for a few days and repeat the experiment again. You find that although the total number of taps is same in both the experiments, the response of the organism is different in these two experiments.

a) Which of these two experiments would likely result in **short-term-habituation**? Explain what **short-term-habituation** is and why this habituation lasts only for a few minutes?

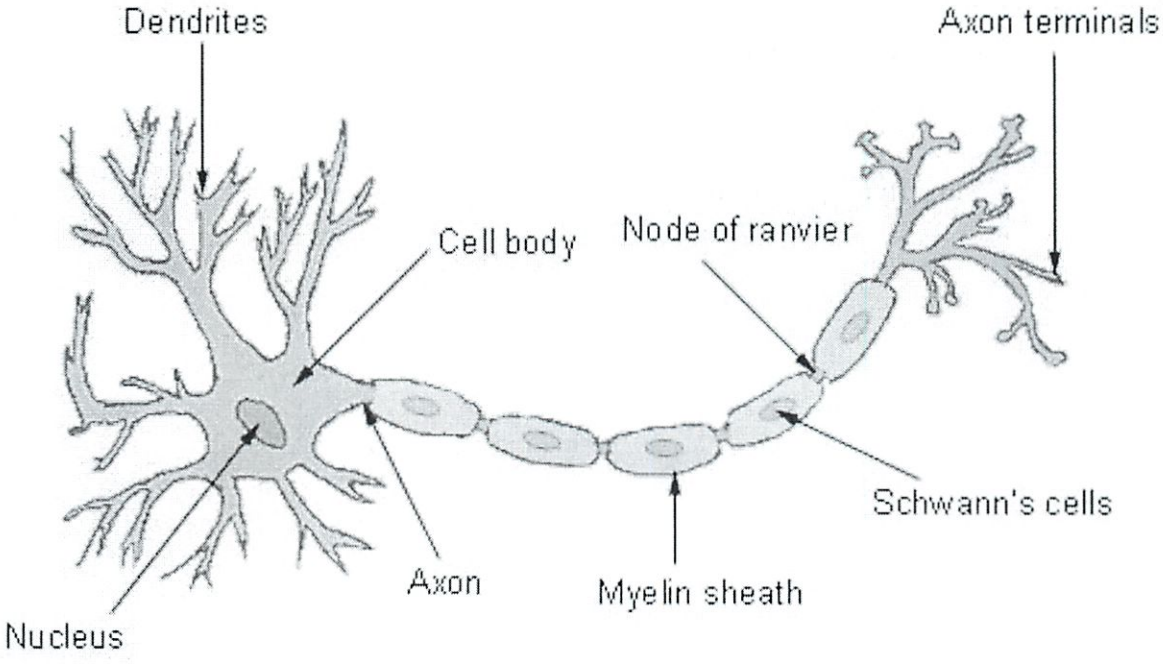
b) For the experiment you **did not select** while answering part (i), explain why the final response is an example of **long-term-habituation**.

c) What does the fact that **long-term-habituation** occurs tell you about the plasticity of neuronal circuits?

11/6
11/8

Samin Houshyar
R27 & R28
Section 13 & 14

I. Neurobiology
   a. Neuron Anatomy



   b. Membrane potential
      i. Ions

      ii. Protein pumps and channels

   c. Resting potential

d. Action Potential
    i. Depolarization

        1. Threshold

    ii. Repolarization

    iii. Hyperpolarization

e. Experimental Techniques
    i. Patch clamping & Giant Squid Axon

f. Synapses
    i. Neuro-neuro synapse
        1. Components
            a. Neurotransmitters

            b. Channels

            c. Ions

2. Types of synapses

    a. Inhibitory

    b. Excitatory

3. Synaptic math!

ii. Neuro-muscular synapse
    1. Components
        a. Neurotransmitters

        b. Channels

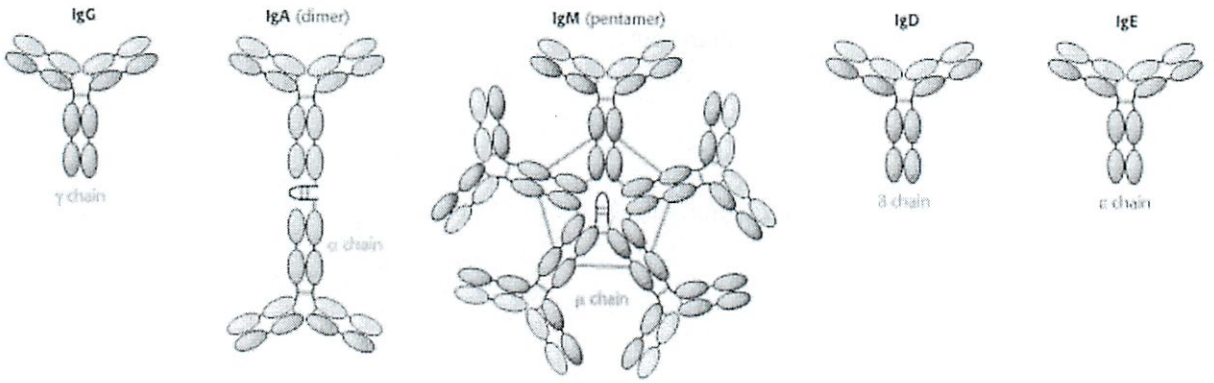
        c. Ions

    2. Toxins
        a. Botulinium toxin – inhibitor of vesicles fusing to membrane

        b. Tetradotoxin – blocks voltage gated sodium channels

        c. Curare – competitive inhibitor of Ach

II. Immunology
- a. The innate immune system
  - i. Non-cellular components
    1. Skin
    2. Saliva
    3. Mucosa
    4. Stomach acid
    5. Tears
    6. Etc.
  - ii. Cellular component
    1. Macrophages
    2. Dendritic cells
    3. Neutrophils
    4. Natural killer cells
- b. Adaptive Immune system
  - i. Humoral
    1. B-cells
       - a. Immature
       - b. Naïve
       - c. Plasma
       - d. Memory
    2. Antibody Structure



3. Antibody production
    a. VDJ Recombination **(*NOT the same thing as splicing*)**

    b. Somatic hypermutation

4. Clonal Selection

5. Controls over clonal selection
    a. Clonal deletion

ii. Cell-mediated
    1. T-cells
        a. T-helper cells (CD4+)
            i. Regulatory role
            ii. Antigen presenting cells (APCs)

b. T-cytotoxic (CD8+)
   i. Viral infections

iii. Infections: Specificity and memory of the immune system

1. Examples
   a. Malaria – infects _____
   b. Cholera – infects _____
   c. Tuberculosis – infects _____
   d. Smallpox – infects _____
   e. Flu – infects _____
   f. HIV – infects _____

iv. Autoimmune diseases
   1. Myasthisia gravis
   2. Rheumatoid arthritis
   3. Multiple sclerosis
   4. ALS (Stephen Hawking)
   5. Diabetes type I

v. Transplants

vi. Vaccinations

7.012
Immunology 3

11/7

(3 min late)

Immune response develops as a child

Some period before it is active

Want some exposure

But we keep washing our hands

Ligid = bind to receptor

How can immune system know what's going on inside cell

Hands carry diff intracellular polypeptides

Protiens made into cytoplasm

Then some r/b cleaved into oligopeptides

Pumped into endoplasmic reticulum

Loaded onto MHC Class I molecule which transport

Oligopeptides to surface

Where they are displayed

(2)

Cell could be infected by virus which is transmitted intracellular

Viral protiens are also displayed on cell surface

Immune system recognizes this

Sees viral oligopeptides

Immune system needs to kill

Proteasome like a garbage disposal

(in that they chop up I think)

All cellular protiens are subject to this

Early warning system for immune system

MHC Class I

Hydrogen bonds

w/ stick of oligo peptide

Can display a bunch of diff oligopeptides

But can't display everything

So collection of MHC Class I

(3)

Diff have diff hands

But anyone of us has limited ability to display them all

Smallpox → not effectively presented

MHC can't present that set of oligopeptides

But could this wipe out the human race?

Each of us has a diff set of MHC Class 1

ensures diversity & continuity of species

Diff allelic versions of gene

MHC Class I are most polymorphic on the genome!

Identical twins have same MHC I

Can transfer b/w

But b/w others people → if transplant

Immune system attacks

This is what makes kidney transplant hard

(4)

Blood has less MHC Class I → so can transplant organs

Must supress immune system of reciepient

How do we do that pharmalogically?

Without over supressing it.

T-cell | before b-cells → Humoural system

T Elvibs

Now Cellular immune response

Cytotoxic t-cell = T_C

Can recognize other cells in body, attack, & kill

has a t-cell receptor on the surface

TcR quite similar to antibody cells

but never secreted -

always teathered to T_C

(5)

B+ _____ is same as in b-cells

Recognizes viral oligopeptides

Tc has T-Cell receptor

Recognizes Oligopeptide and MHC Class I

L but recognizes a certain one
(green on slide)
only

Good way to get rid of virus infected cell
before Virus grows + reproduces

Can also recognize cancer cells?

Antibody molecule is transmembrane protein (check)

(6)

T-cell receptor looks like antibody molecule
- but very specialized
- always on a T cell

Cytoplasmic cytotoxic T lymphocyte (CTL)

CTL has recognized cell

Formed synapse

Cytotoxic granules sent to the other cell

Much better to get rid of infected cell
- and be 1 short - since have many

Viruses try to evade being killed

Force the cell to shut down synthesis of MHC Class I

Cell has been prevented this from warning immune system

(7)

But our immune system clever too

Natural killer cell (NK) no T-cell receptor just kill

kills cells w/ too little MHC Class I on surface!

"this cell looks suspicious"

Many cancer cells do this as well!

Down regulate MHC to avoid immune attack

NK goes after it injects cytotoxic granules

Macrophages gobbles up cellular garbage

dendritic cell digests this

cleaves it into oligopeptide antigens

loads onto MHC Class II

load pushes to surface

presenting oligopeptides from other cells

white blood cells → professional antigen presentation cells

junk collectors that are constantly showing the junk they just found

---

the two MHC classes are pretty similar

---

but why do B cells call a certain class of T-cells # T_H (T-helpers)

(9)

t-cells have TCR

Dendritic cell looks for T_H w/ the garbage they picked up

---

like shopkeepers in Demacus

Shopkeeper - female

dendritic - male - like street walker
it hopes to find a shopkeeper

Shopkeepers turn him down
till find one that fits w/ t cell repetor
"they fall in love"
(weird story)

form an embrace (I can't get anymore vivid)
results in the activation
t-helper cell gets very excited
I found a soul mate

(10)

Then t-helper cell goes to b-cell
happens to be displaying same oligopeptide
receptor

Then b-cell gets activated
(Remember we've had 2 interactions)
Activation of b-cell
Prelude to B-cell making antibodies

Why so complicated:
immune system must keep a strict quality
Control

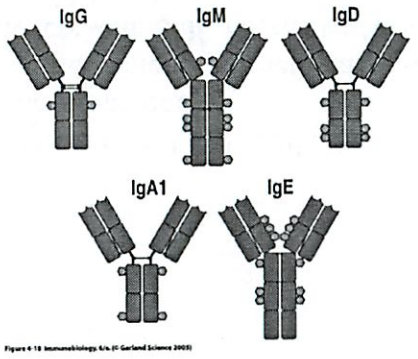
Return to earlier figure
Stimulation of b-cell actually much
more complicated than shown

Before i Gross oversimplification

Next time Self vs non Self

The differences between these are due to the incorporation of different **heavy chains;** light chains are the same

G - γ chain
M - μ chain
D - δ chain
A - α chain
E - ε chain



The use of fusion of a heavy chain **variable** segment (resulting from VDJ recombination) to various **constant region** segments reflects the process of **class switching** (i.e., switching from making an IgM to making an IgG molecule while keeping the same variable region segment)

Class switching

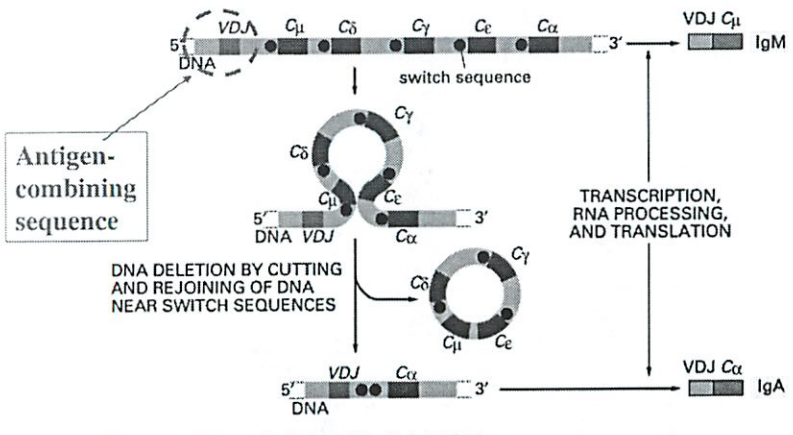
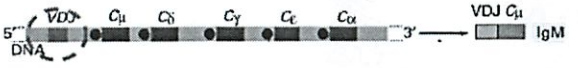


Figure 24–41. Molecular Biology of the Cell, 4th Edition.

Before the class switching the antigen-combining site was joined (following RNA splicing) to a C_μ (constant μ), segment, yielding an IgM molecule. Afterward, as the immune response develops, the C_μ DNAsegment (as well as other constant region DNA segments) may be deleted from the genome. Now, in the example depicted here, the variable(VDJ) region becomes spliced to a C_α segment, yielding an IgA antibody with the same antigen-binding specificity.

The first step:

Class switching



Antigen-combining sequence

Initially, following *VDJ* recombination in a heavy region locus, when the resulting antigen-combining variable region segment is transcribed, it becomes joined via RNA splicing to the **nearest constant** region segment downstream. This constant region segment is *Cμ* (constant μ), and results in the formation of an IgM molecule following translation of the resulting spliced mRNA.

VDJ already rearranged

Class switching (H chains): (enabling one already-developed antigen-combining segment to become allied with another distinct constant region)

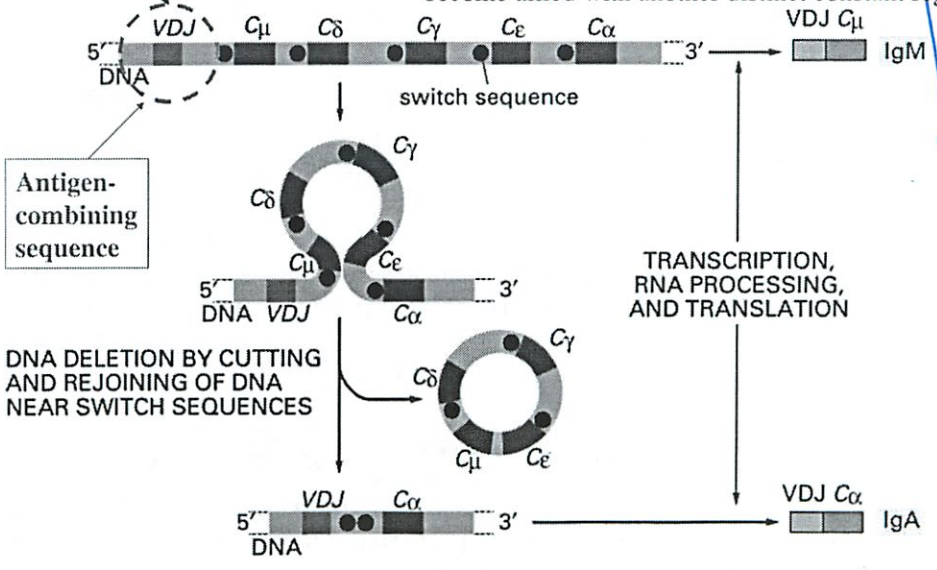


Figure 24–41. Molecular Biology of the Cell, 4th Edition.

To review: The immune system has many different "arms". We will focus on its **humoral** and **cellular** arms.

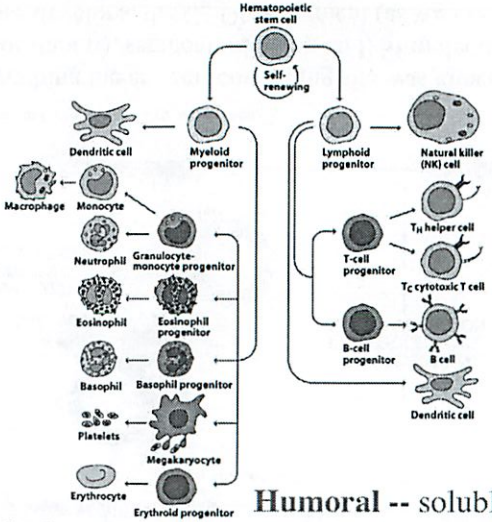
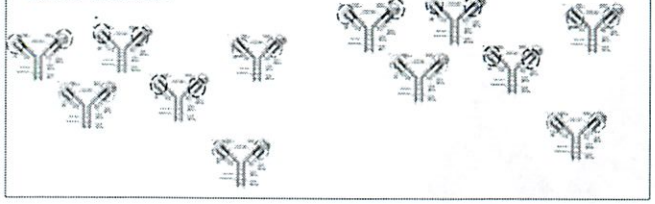


Figure 2-2
Kuby IMMUNOLOGY, Sixth Edition
© 2007 W.H. Freeman and Company

Humoral -- soluble antibodies
Cellular -- cell-mediated responses

Let's **review** in some detail the steps needed to make a monoclonal antibody.
a.In the spleen (and bone marrow).of a mouse there are normally millions of B-cell populations, each B cell having developed the ability to make its own particular antigen-specific B cell.

In this mouse, many of these B cells are, on occasion, differentiating/maturing into plasma cells, and the latter are secreting millions of antigen-specific antibodies into the circulation.

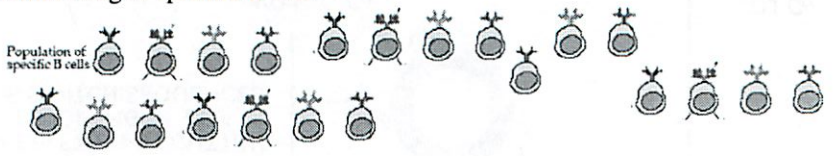


There are at least two reasons why a B-cell or B-cell clone will be **eliminated** early in its development:

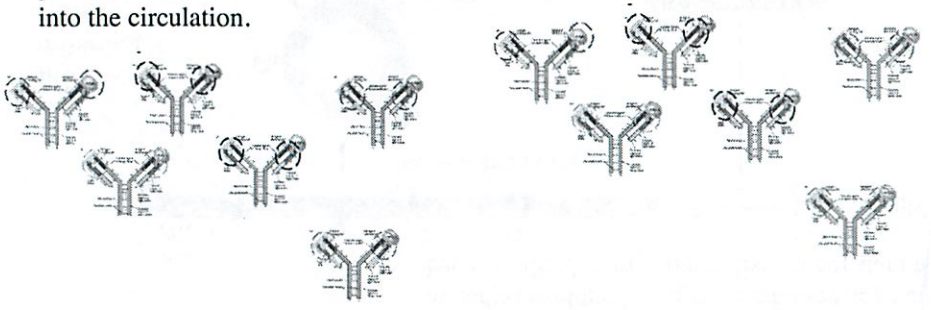
1. It fails to make (via gene rearrangement) a functional antibody
2. It makes an antibody that reacts with **Self,** i.e. the body's own native proteins -- the issue of **Tolerance** (self vs. non-self)

Let's **review** in

In the spleen (and bone marrow) of a mouse or human there are normally millions of B-cell populations, each B cell having developed the ability to make its own particular antigen-specific B cell.



In this mouse, many of these B cells are, on occasion, differentiating/maturing into plasma cells, and the latter are secreting millions of antigen-specific antibodies into the circulation.



Therefore, class-switching allows the immune system to make a number (~8) of antibody classes, which share in common identical antigen-combining V regions but have distinct **C regions.**

G - γ chain
M - μ chain
D - δ chain
A - α chain
E - ε chain

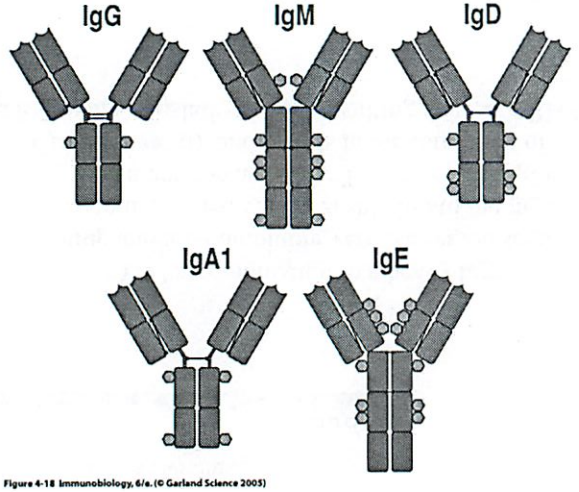


Figure 4-18 Immunobiology, 6/e. (© Garland Science 2005)

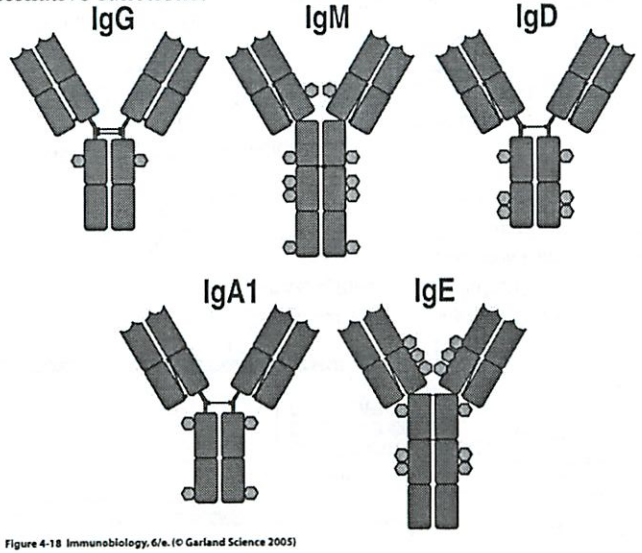
While all having the same variable regions, these various classes of an antibody, being fused to different constant regions, have **differing functions**.

Functional activity	IgM	IgD	IgG1	IgG2	IgG3	IgG4	IgA	IgE
Neutralization	+	–	++	++	++	++	++	–
Opsonization	+	–	+++	*	++	+	+	–
Sensitization for killing by NK cells	–	–	++	–	++	–	–	–
Sensitization of mast cells	–	–	+	–	+	–	–	+++
Activates complement system	+++	–	++	+	+++	–	+	–

Figure 9-19 part 1 of 2 Immunobiology, 6/e. (© Garland Science 2005)

FYI: "Opsonization" means coating a particles, such as a bacterium, with antibody molecules, enabling phagocytes to gobble up the particle. "Mast cells" can recognize an antibody-coated particle or cell and release toxic compounds in response. "Complement" is a group of proteins that punches holes in the membranes of antibody-coated cells.

So, to review: Various antibody classes: The red variable regions may all be identical and recognize the same antigen. The **blue** constant regions differ, allowing these antibody molecules to have multiple alternative functions.



G - γ chain
M - μ chain
D - δ chain
A - α chain
E - ε chain

Figure 4-18 Immunobiology, 6/e. (© Garland Science 2005)

While all having the same variable regions, these various versions of an antibody, being fused to different constant regions, have differing functions. They're found in different compartments in the body.

Distribution	IgM	IgD	IgG1	IgG2	IgG3	IgG4	IgA	IgE
Transport across epithelium	+	–	–	–	–	–	+++ (dimer)	–
Transport across placenta	–	–	+++	+	++	+/–	–	–
Diffusion into extravascular sites	+/–	–	+++	+++	+++	+++	++ (monomer)	+
Mean serum level (mg ml^{-1})	1.5	0.04	9	3	1	0.5	2.1	3×10^{-5}

Figure 9-19 part 2 of 2 Immunobiology, 6/e. (© Garland Science 2005)

FYI: "extravascular" means the portions of tissues outside of the circulatory system, i.e., outside of blood vessels.

The immune response develops progressively. IgG molecules are transferred via the placenta during gestation and via milk during breastfeeding. Following exposure to novel antigens early in life, IgM molecules are initially produced; however, as the immune system and immune responses develop, these IgM molecules are progressively changed over to IgG and then IgA responses, etc.

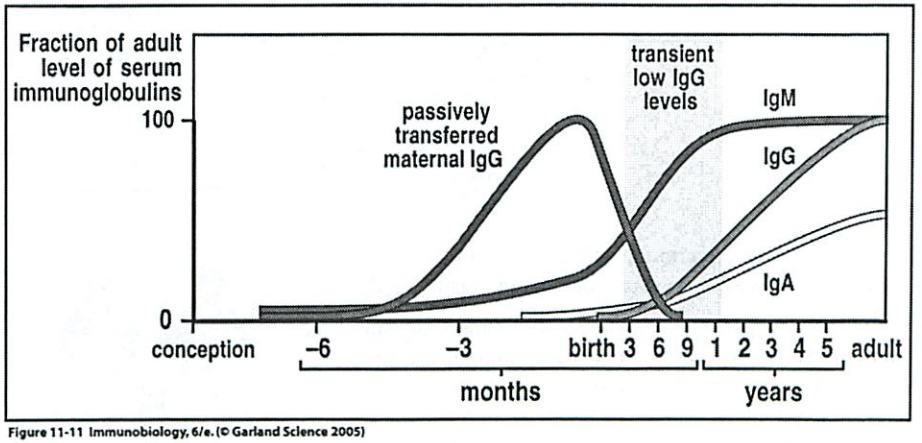


Figure 11-11 Immunobiology, 6/e. (© Garland Science 2005)

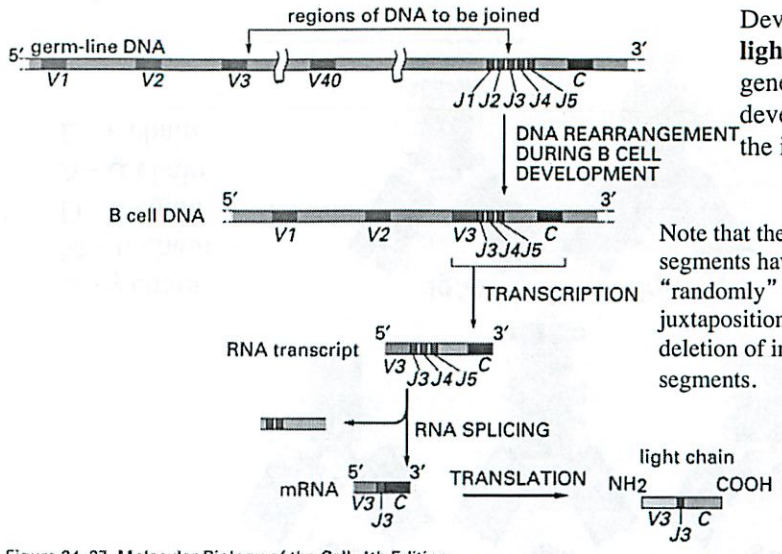
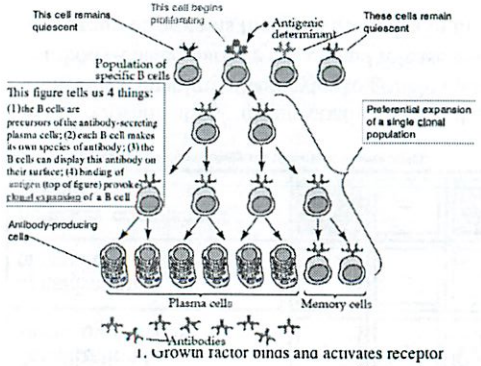


Figure 24–37. Molecular Biology of the Cell, 4th Edition.

Development of a **light chain** gene during development of the immune system

Note that the *V3* and *J3* segments have been chosen "randomly" for juxtaposition/fusion via deletion of intervening DNA segments.

Note that upon transcription of the fused *V3* and *J3* segments the resulting transcript is spliced to the nearest downstream C segment, thereby skipping over (deleting) the *J4* and *J5* segments, which are still present in the DNA.



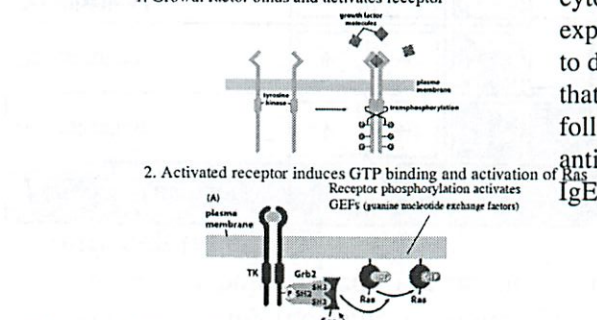
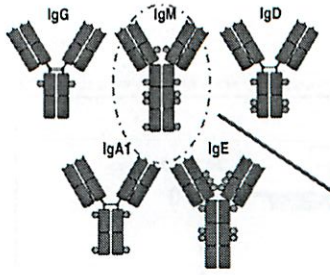


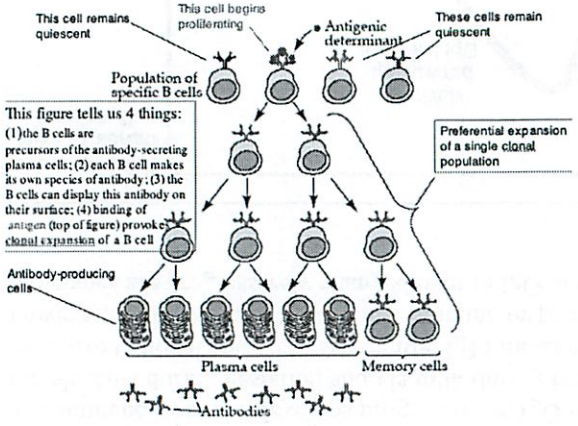
Figure 6.12 *The Biology of Cancer* (© Garland Science 2007)

After B cells have undergone a VDJ & VJ recombination, they display the initial product of this recombination - a form of IgM -- on their surface. This IgM functions much like a growth factor receptor, I.e., when it binds its "ligand", it becomes activated and sends a stimulatory signal into the cytoplasm that stimulates clonal expansion and leads ultimately to descendants (plasma cells) that secrete soluble IgM and, following class switching, other antibody types -- e.g., IgG, IgA, IgE, etc.



This "class switching" helps to explain a puzzle that was implicit in our earlier depiction of clonal expansion. If exposure to an "antigenic determinant" provokes clonal outgrowth, how can a B-cell sense the presence of an antigen in its surroundings? Answer: The initially displayed antibody molecule is a cell-surface,

IgM transmembrane protein is initially configured like a growth factor receptor. (Later on it becomes secreted.)

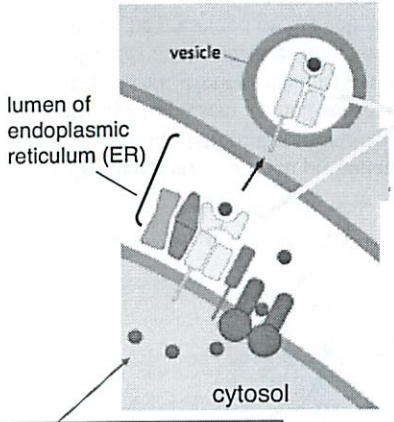


Next problem: How can the immune system monitor the various compartments in the body to determine whether novel antigens (and thus foreign infectious agents) have invaded the body and should be attacked and neutralized?

In the case of certain viruses and bacteria, they may release soluble protein antigens that can be recognized by the immune system, e.g., stimulate the clonal expansion of B-cell clones.

But what if a virus has invaded a cell and is multiplying within the cell??

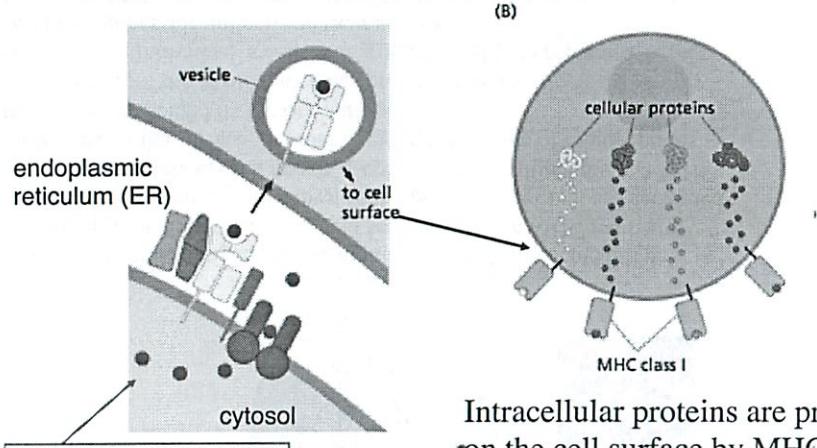
Major question:
How can the immune system know what's going on inside cells? (including the presence of viral proteins inside infected cells).
Intracellular proteins are **digested into oligopeptides**, imported into the ER, where they are loaded onto antigen-presenting MHC Class I molecules



MHC (major histocompatibility complex) Class I molecule

Oligopeptide fragments of intracellular proteins

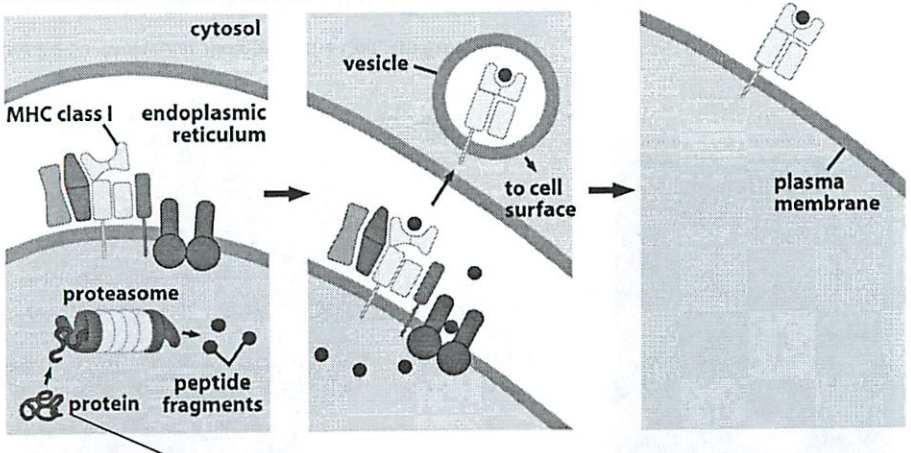
Major question:
How can the immune system know what's going on inside cells? (including the presence of viral proteins inside infected cells).
Intracellular proteins are digested into oligopeptides, imported into the ER, where they are loaded onto antigen-presenting MHC Class I molecules which carry them to and present them on the cell surface.



Peptide fragments of intracellular proteins

Intracellular proteins are presented on the cell surface by MHC Class I antigens

Here's a more detailed depiction of how cells (normal and virus-infected) display intracellular antigens on the cell surface via their MHC class I molecules.



Intracellular proteins are chopped up in the cell's garbage disposals --the **proteasomes** in the cytoplasm; the resulting oligopeptides are loaded onto the MHC Class I molecules in the lumen of the endoplasmic reticulum (ER) and then transported to the cell surface.

MHC Class I

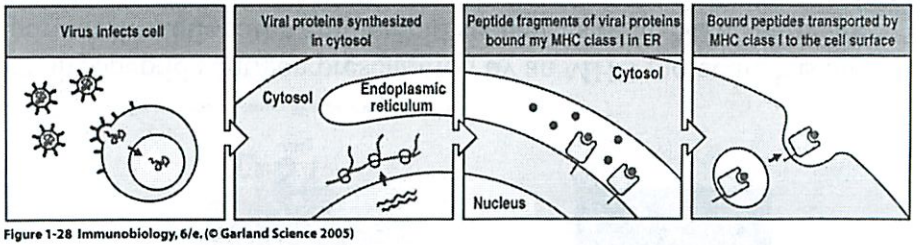


Figure 1-28 Immunobiology, 6/e. (© Garland Science 2005)

This presentation of intracellular proteins occurs routinely for all cellular proteins. When a cell is infected by a virus, the virus-encoded proteins (in the form of oligopeptide fragments) are also presented by the same system on the cell surface.

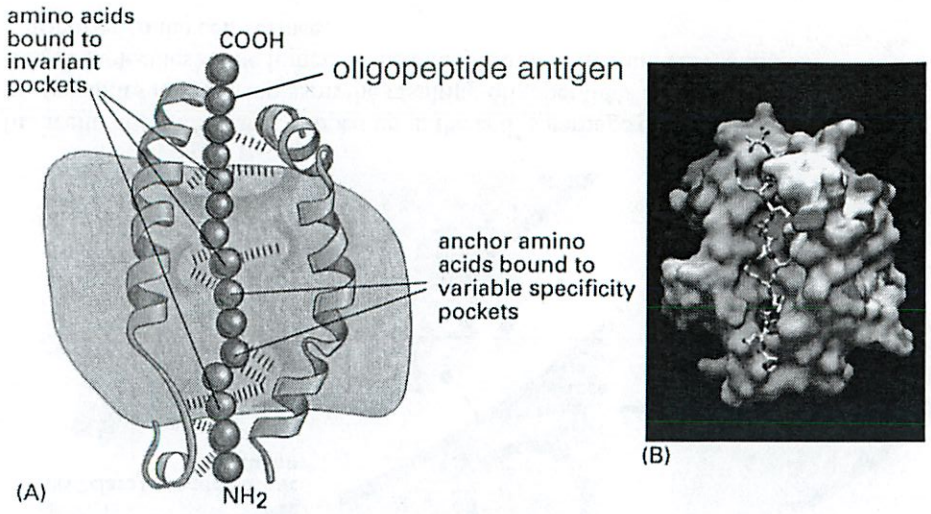


Figure 24–53. Molecular Biology of the Cell, 4th Edition.

This is the "palm of the hand" of an MHC molecule that it uses to present oligopeptides to the outside world, I.e., to the immune system, which can "see" antigens presented in this way.

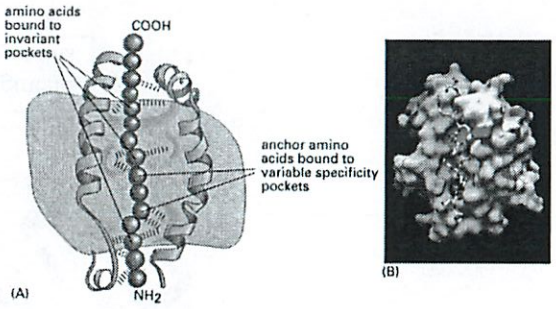
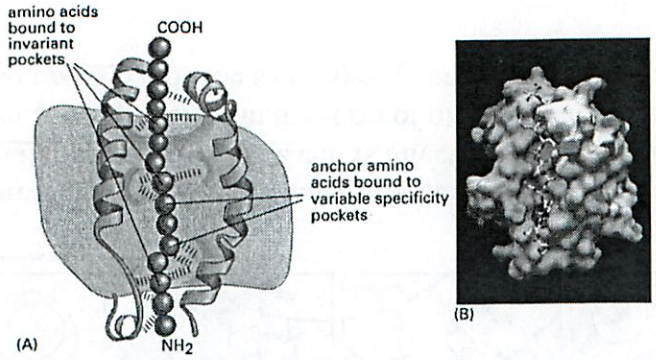


Figure 24–53. Molecular Biology of the Cell, 4th Edition.

This oligopeptide antigen presentation by an MHC molecule has an important limitation: the amino-acid sequences of an MHC palm are only able to present a small subset of the oligopeptides that are generated within a cell. Hence, each person's cells display multiple distinct MHC molecules on the surface, in order to extend the range of oligopeptides that can be presented on the cell surface.

However, all of the MHC molecules presented on the surface of an individual's cells will still not be able to present certain oligopeptide antigens on their surface.



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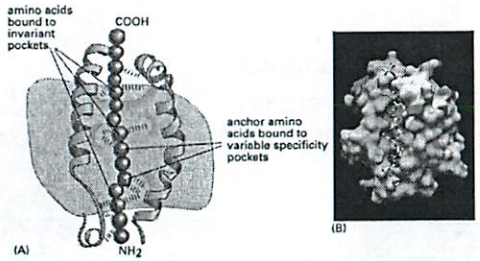

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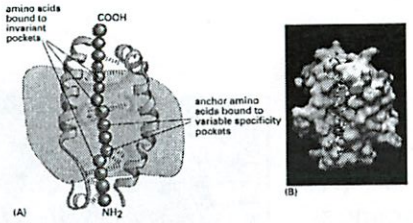
This oligopeptide antigen presentation by an MHC molecule has an important limitation: the amino-acid sequences of an MHC palm are only able to present a small subset of the oligopeptides that are generated within a cell. Hence, each person's cells display multiple distinct MHC molecules on the surface, in order to extend the range of oligopeptides that can be presented on the cell surface.

However, all of the MHC molecules presented on the surface of an individual's cells will still not be able to present certain oligopeptide antigens on their surface.

What will happen when that individual is infected by a novel pathogen (e.g., a virus) **none** of whose oligopeptides can be recognized by that individual's MHC molecules? That virus can fly "under the immunological radar", multiply unhindered, and kill the individual.



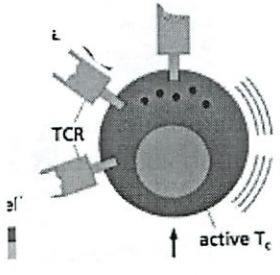
However, all of the MHC molecules presented on the surface of an individual's cells will still not be able to present certain oligopeptide antigens on their surface.
What will happen when that individual is infected by a novel pathogen (e.g., a virus), none of whose oligopeptides can be recognized by that individual's MHC molecules?
That virus can fly "under the immunological radar", multiply unhindered, and kill the individual.

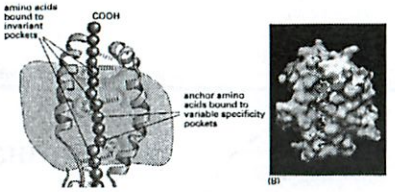
What will happen if every individual in the species displays the **identical set** of MHC antigen-binding molecules?
The novel pathogen will spread unhindered through the species and **wipe out** the species!! boo hoo

For this reason, the MHC-encoding genes within a species are highly **polymorphic,** and each individual displays different combinations of MHC-encoding alleles, ensuring that the MHC molecules of at least some members of the species will be able to bind and present the pathogen's oligopeptides, enabling immunological defense against this virus.

A cytotoxic T lymphocyte (T_c, sometimes: CTL) will display on its surface an antibody-like molecule called a **T-cell receptor** (TCR).
This T-cell receptor is an antibody-like mol much like the cell-surface IgM protein of the B cells.
(B cells and T cells are the two major classes of lymphocytes.)
The T-cell receptor is the product of a series of gene rearrangements just like the VDJ/VJ recombination occurring in B cells. Hence, there are millions of T lymphocytes, each with its own Cell-surface receptor, each able to Recognize it's own antigen.
(The T-cell receptor never progresses To a secreted, soluble form.)

oligopeptide





For this reason, the MHC-encoding genes within a species are highly **polymorphic,** and each individual displays different combinations of MHC-encoding alleles, ensuring that the MHC molecules of at least some members of the species will be able to bind and present the pathogen's oligopeptides, enabling immunological defense against this virus.

The MHC molecules themselves are proteins, and therefore one person's MHC molecules, displayed on the surface of his/her cells will be different from another's (unless they are identical twins). Hence, these MHC molecules can themselves function as antigens. As a consequence, if you take cells from one person and put them in another's body, these transplanted cells will be recognized as being **foreign** by the recipient's immune system and will be attacked and eliminated (just as if they were an invading pathogen). Hence these cells will be histo-incompatible! ("histo" = tissue). MHC = major histocompatibility antigen.
The MHC molecules that routinely display oligopeptides on the surfaces of cells throughout the body are called "Class I MHC molecules".

A cytotoxic T lymphocyte (T_c, sometimes: CTL) will display on its surface an antibody-like molecule called a **T-cell receptor** (TCR).
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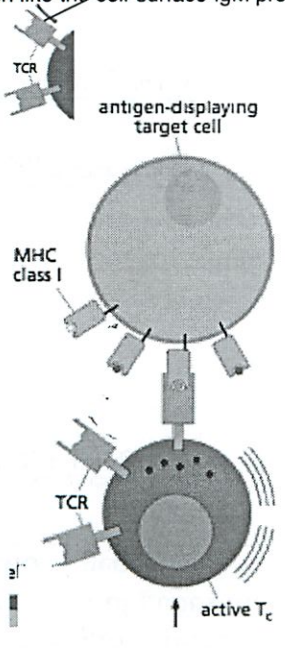
(The T-cell receptor never progresses To a secreted, soluble form.)

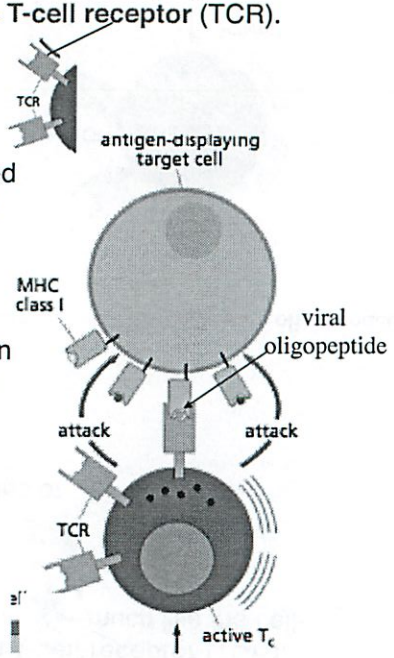
T cells can use their TCR to recognize Antigens displayed on other cells via the MHC Class I molecule and attack those cells. This class of T cells is called a "cytotoxic T cell, or simply Tc.



A cytotoxic T lymphocyte (T_c, sometimes: CTL) will display on its surface an antibody-like molecule called a T-cell receptor (TCR).

The T_c will use its TCR to recognize an oligopeptide antigen that is being displayed on the surface of another cell (a target cell) by that cell's MHC class I molecules.
If the recognized antigen is recognized as being foreign, this will provoke an attack by the T_c.

Once the T_c recognizes the target cell's antigen being displayed, it will attack the target cell and kill it.
For example, if the target cell has been infected by a virus and is presenting a viral oligopeptide on its surface, the T_c may kill the target cell before the virus has had a chance to multiply extensively within the target cell, thereby aborting the viral replication cycle



Cytotoxic T lymphocytes display a "T-cell receptor" on their surfaces. The TCR is encoded by a separate set of genes that also undergo rearrangement just like the antibody genes!!

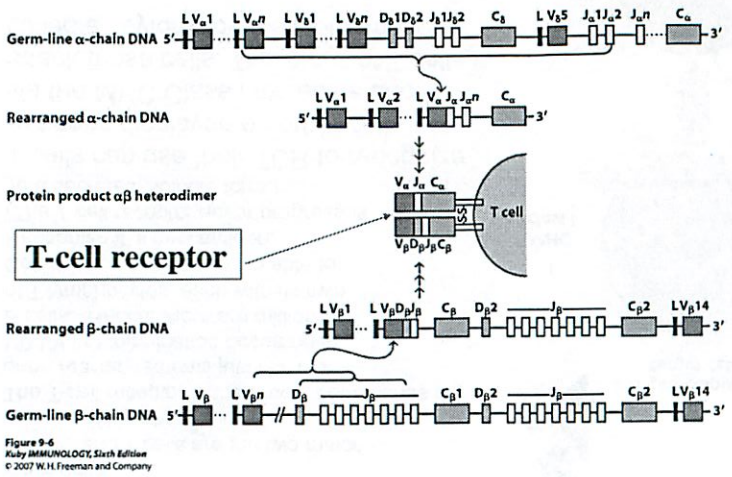


Figure 9-6
Kuby IMMUNOLOGY, Sixth Edition
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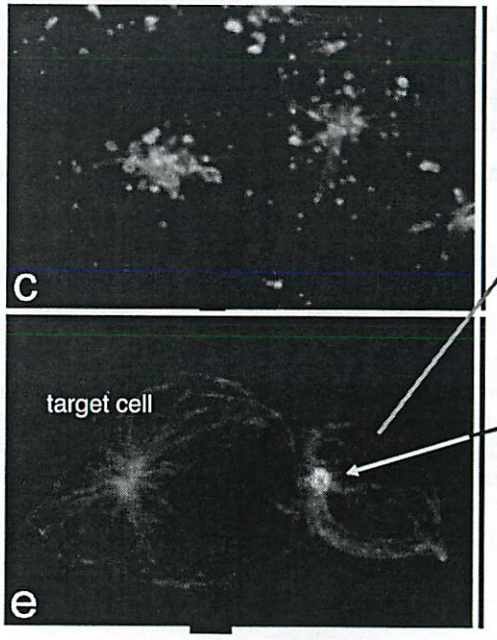


The cytotoxic granules within a cytotoxic T lymphocyte (CTL) are usually scattered around the cell (above). However, when the CTL recognized a target cell (below) that needs to be killed, it forms a tight connection with the latter and concentrates all of its cytotoxic granules close to the target cell in order to discharge them and kill its target (below)!

To review: (cytotoxic T cell is abbreviated either CTL or T_c)

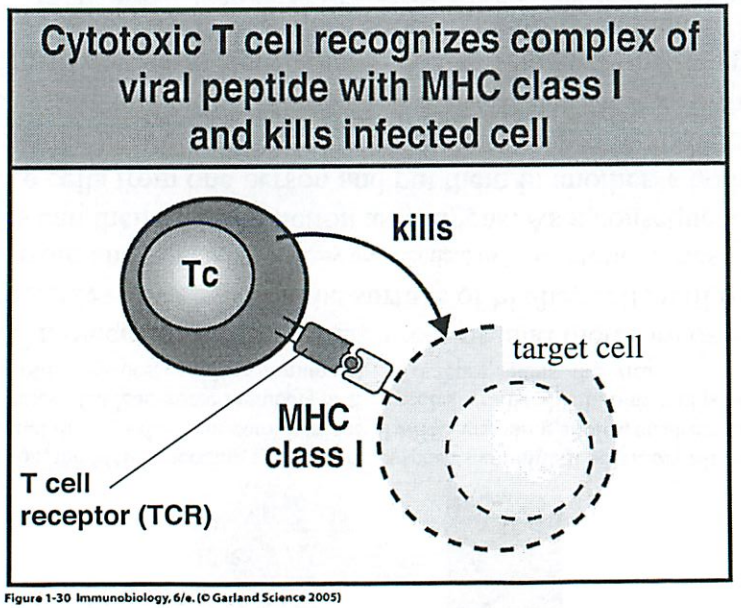
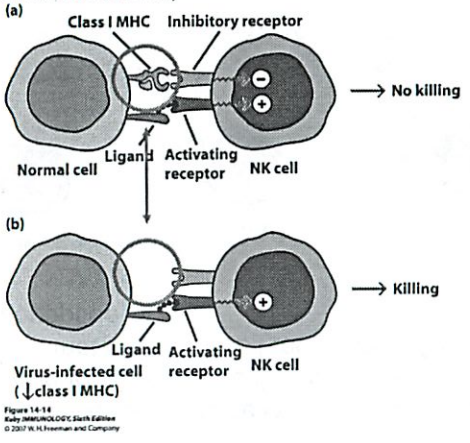


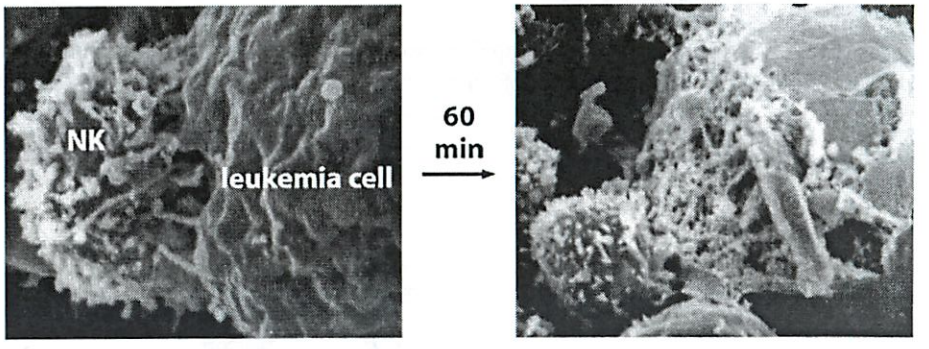
Figure 1-30 Immunobiology, 6/e. (© Garland Science 2005)

A number of viruses attempt to evade the immune system by forcing the down-regulation of the host MHC Class I proteins of the virus-infected cell (thereby avoiding display of viral oligopeptide fragments on the cell surface!). However, the immune system has a **counter-response**: It has natural killer (NK) cells that recognize and preferentially kill virus-infected cells that have abnormally low levels of MHC Class I molecules on their surface.

(Note that natural killer cells do not recognize specific viral antigens and do not have antigen-specific T-cell receptors on their surface. They simply recognize the abnormal condition of absent MHC Class I molecules.)

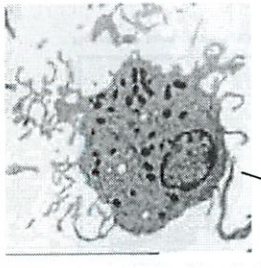


This NK cell is killing a leukemia cell
that apparently has tried to escape immune recognition by downregulating its cell-surface class I MHC molecules



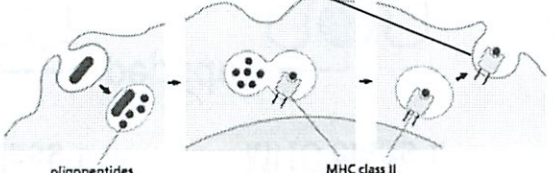
Not a pretty sight!

Figure 15.12e *The Biology of Cancer* (© Garland Science 2007)

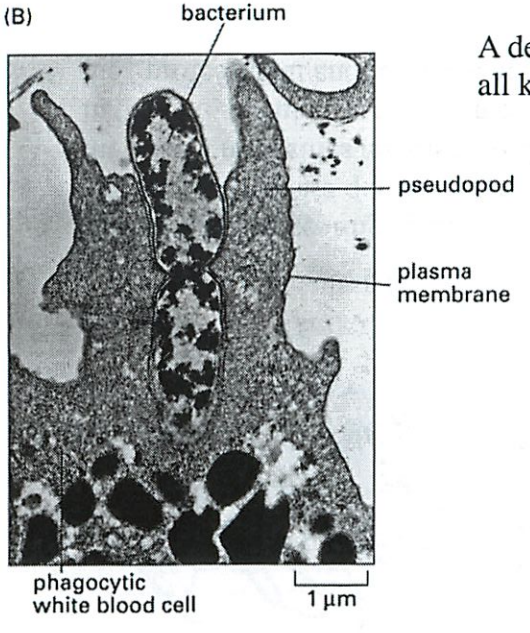


Now we encounter a second type of MHC receptor -- MHC Class II, used largely by "professional antigen-presenting cells"

A dendritic cell will scavenge the particles (including infectious agents) in the tissues, digest them, and present oligonucleotide digestion products on its surface through its MHC class II "hands"



A dendritic cell phagocytoses particles like a macrophage; however, a dendritic cell is more effective in subsequently presenting antigens to the immune system.



A dendritic cell will gobble up all kinds of garbage!

Figure 24–24 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

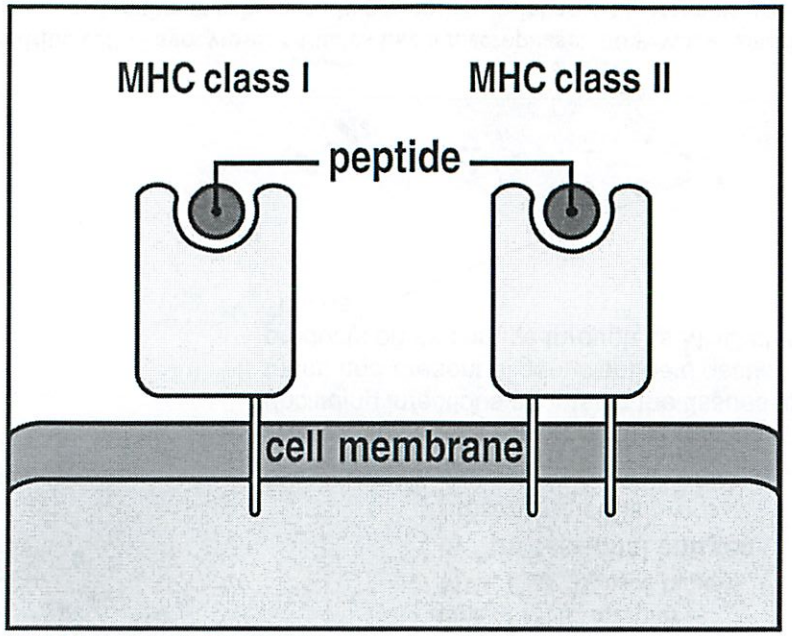
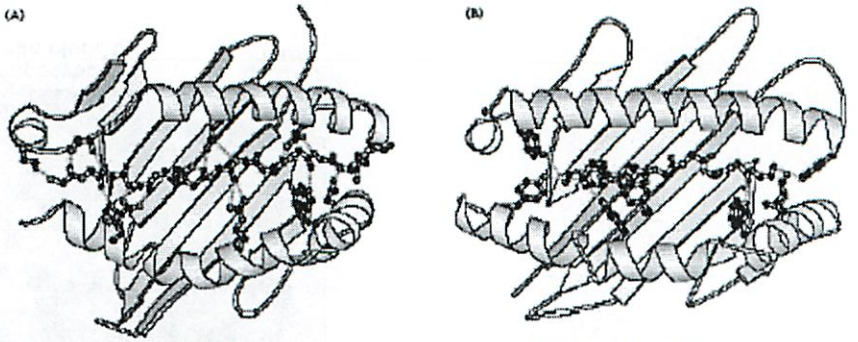


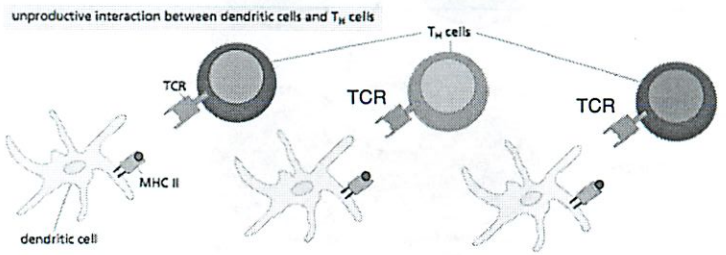
Figure 1-27 Immunobiology, 6/e. (© Garland Science 2005)



MHC class I MHC class II

The antigen-presenting "palms" of the MHC class I and class II proteins are quite similar to one another. However, the class I molecules are displayed by virtually all the cell types in the body, while the class II molecules are displayed by the "professional antigen-presenting cells", e.g., dendritic cells.

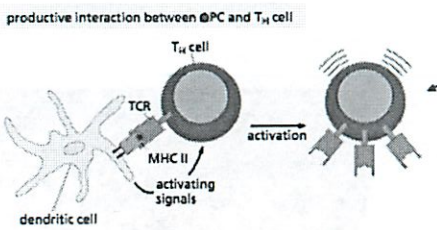
After displaying a peptide, the dendritic cell will then cruise around (in lymph nodes), looking for T cells (specifically helper T cells (T_{H}))
-a 2nd class of T cells -- that display T-cell receptors (TCRs)
-that recognize its oligopeptide.



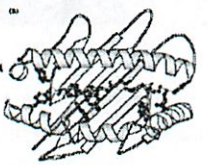
More often than not, the dendritic will fail to find a T_H cell that has a receptor that recognizes its oligopeptide •.

(Remember that a T cell receptor (TCR) is an antibody-like molecule on the surface of the T cell that can come in millions of different versions, each capable of recognizing a different antigen presented in this case by an MHC Class II molecule.

On rare occasion, however, the dendritic cell will happen to encounter a helper T cell (T_H) whose T-cell receptor(TCR) recognizes the oligopeptide antigen. This will cause the dendritic cell to activate the **T_H cell.**



(Actually, the TCR recognizes more than the oligopeptide antigen: it recognizes this oligopeptide + the nearby residues of the palm of the MHC Class II molecule)



The shop owners
Are all T helper
Cells, each w. her
Own T cell receptor.

stores

Store owners
Each store
w. her own
Thelper cell
In frontg

TCR TCR TCR ← owners

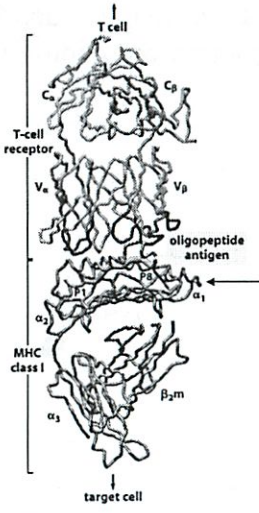
street

← owners

Imagine a
dendritic cell
parading down
A narrow street
Lined on each
Side by small shops
In front of which the
(female) shop owners
Are waiting, hoping
For business

stores

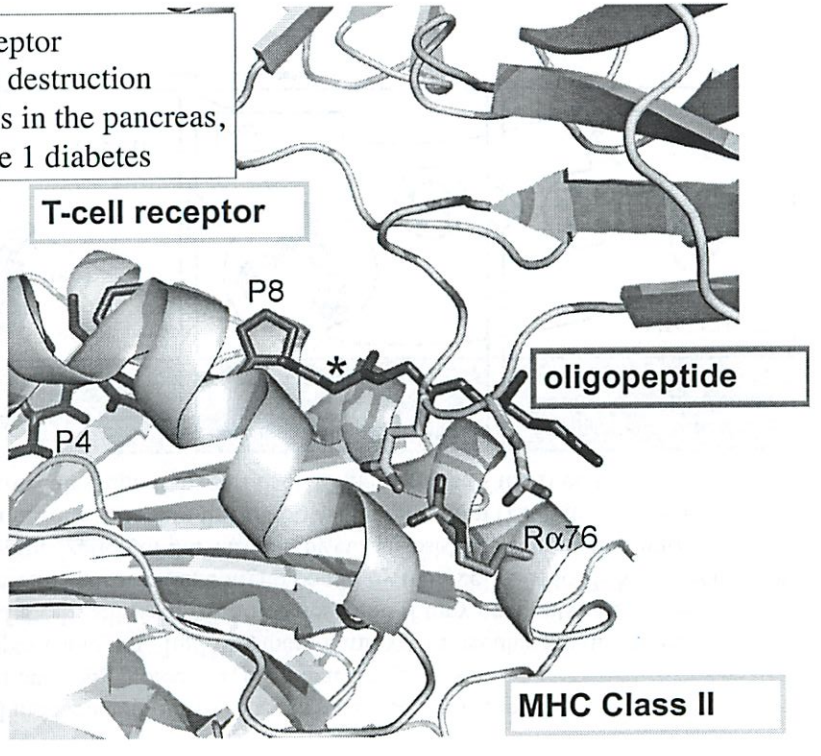
Here's what this encounter actually looks like. Above you see the T-cell receptor (TCR) expressed by a T lymphocyte; it recognizes a specific oligopeptide antigen that is carried in the "palm" of the MHC class I molecule displayed on the surface of a potential target cell.



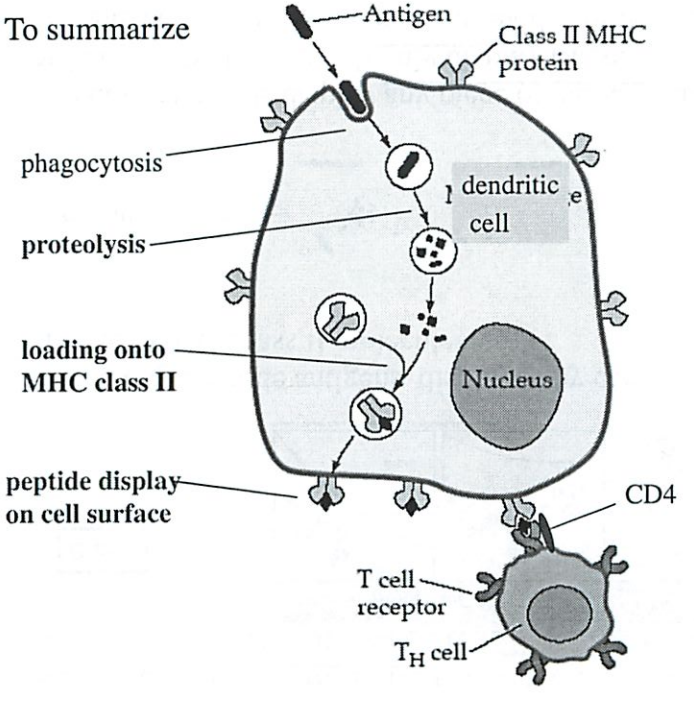
The "palm of the MHC Class I molecule

Figure 15.18b *The Biology of Cancer* (© Garland Science 2007)

A T-cell receptor
that triggers destruction
of the b-cells in the pancreas,
creating type 1 diabetes



To summarize



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.

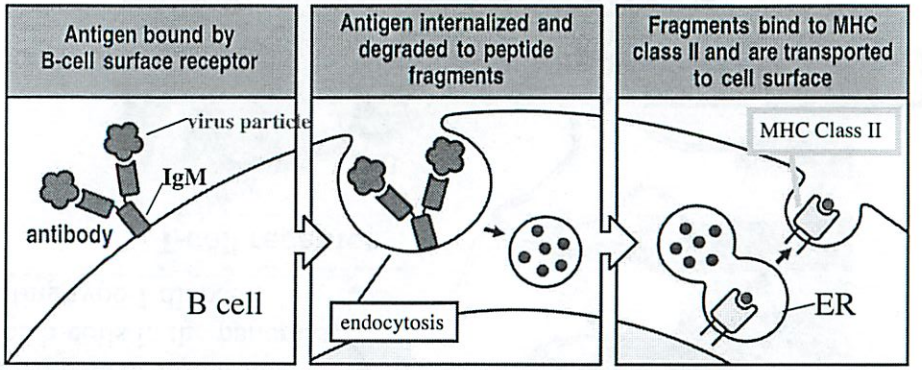


Figure 1-29 part 2 of 2 Immunobiology, 6/e. (© Garland Science 2005)

We go back to the B cell displaying an oligopeptide antigen.

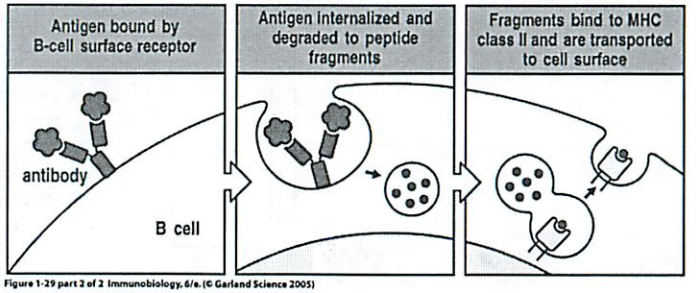
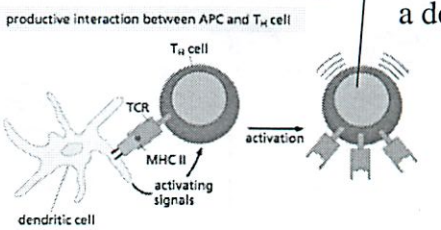


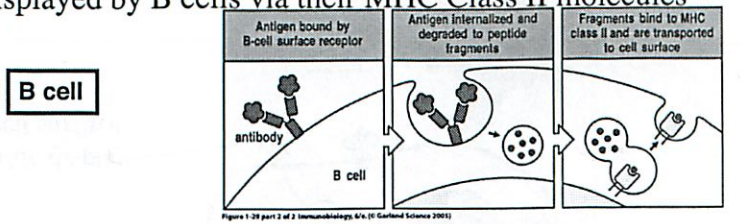
Figure 1-29 part 2 of 2 Immunobiology, 6/e. (© Garland Science 2005)

and the T helper cell (T_H) cell that was previously activated by a dendritic cell.

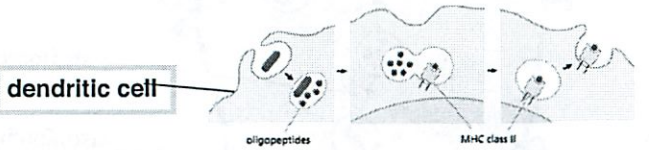


The activated T_H cell will now cruise around looking for a B cell that **also displays** this particular antigenic oligopeptide on its MHC Class II molecules

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

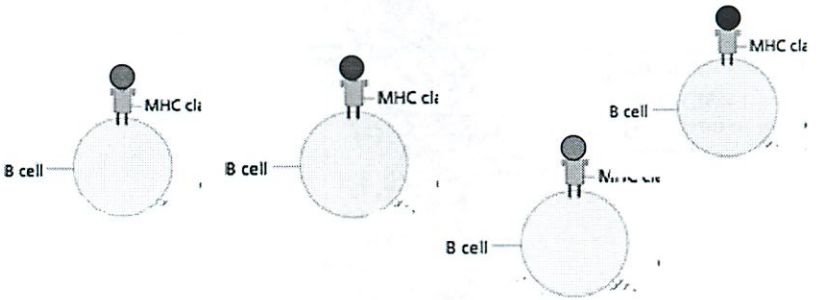


and the oligopeptide antigens displayed by dendritic cells via their MHC Class II molecules



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

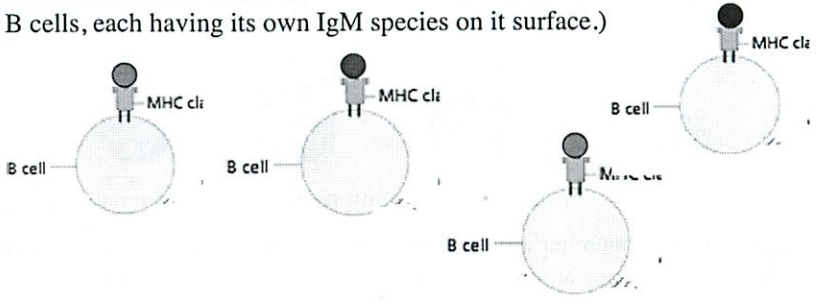
Remember, there will be thousands of different kinds of B cells, each displaying (via its surface MHC class II molecules) an antigenic fragment of something it captured earlier with its cell-surface antibody molecule



The 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.

On rare occasion, the helper T cell (T_H) will find a B cell that displays on its Surface (via its MHC class II molecules) the antigen that is recognized by the T-cell receptor of the helper T (T_H cell). Now things get really interesting. The two hook up (!!!) and the T_H cell sends a signal to the B cell to activate it. The B cell now begins to multiply like mad and to secrete in Large amounts soluble antibody molecules, creating the humoral Immune response.

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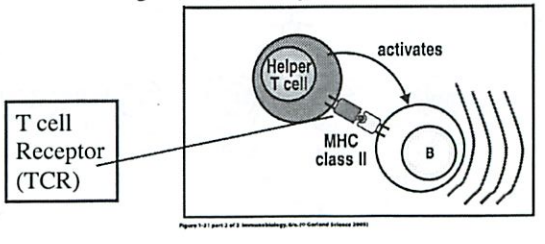
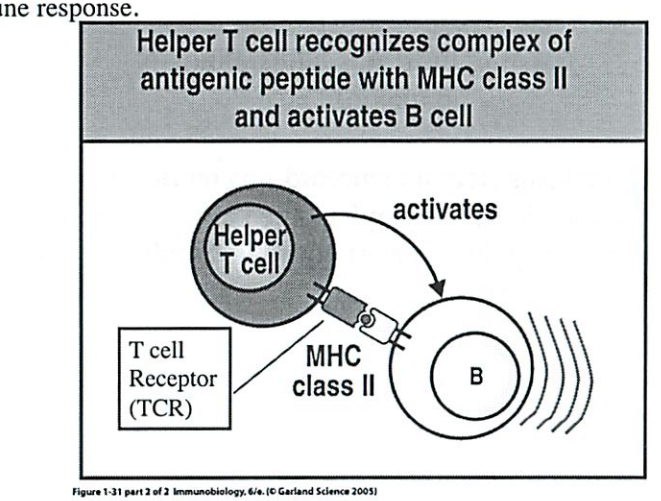
In effect, the helper T cell (T_H) will have recently encountered a dendritic cell that presented some scavenged oligopeptide antigen on its surface and is recognized by the T-cell receptor on the surface of the T_H cell. **Now,** the activated, excited T_H cell will cruise around, looking for a B cell that presents on its surface **the same oligopeptide antigen** (once again presented by MHC II protein. If it encounters such a B-cell, it says" What a coincidence! I just encountered the SAME oligopeptide being presented by a dendritic cell. Now I meet you! This is fabulous! So the two couple and the B cell gets excited and proliferates and starts making antibodies (by becoming a plasma cell).





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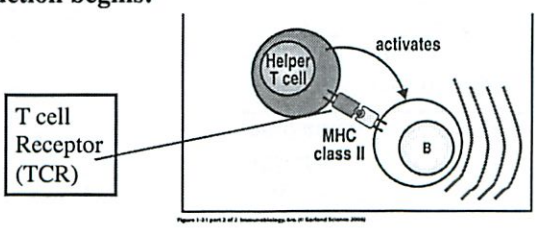
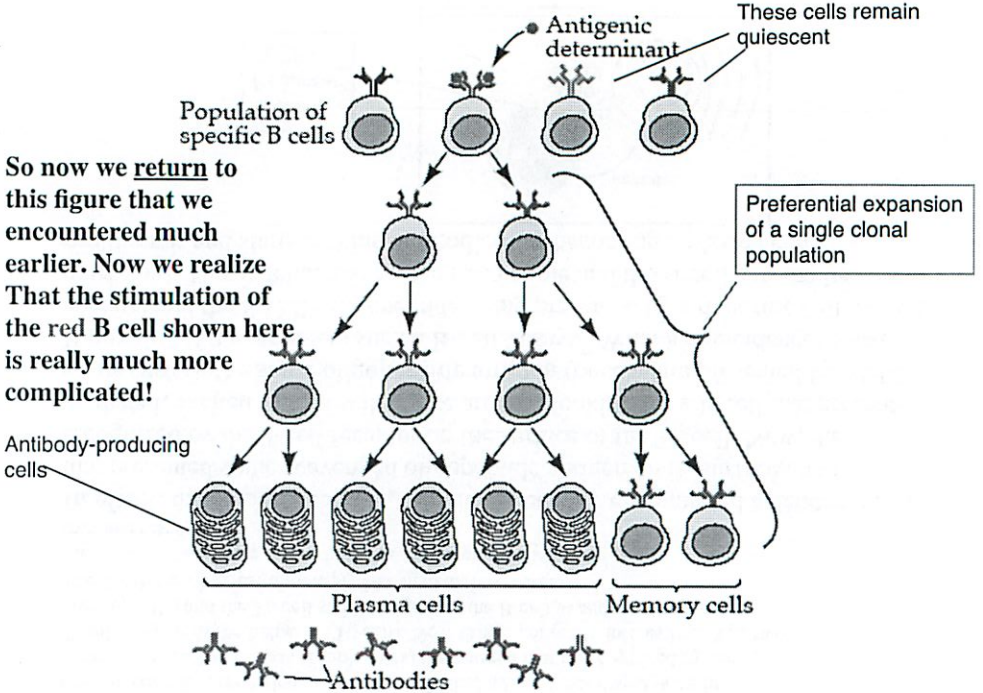
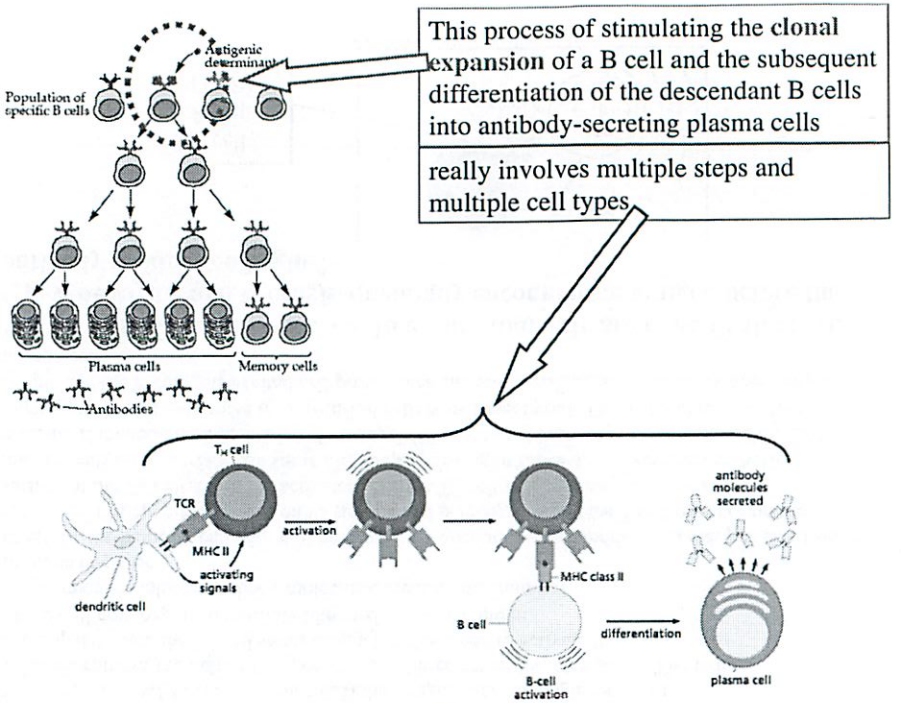
Why is this so complicated? --> To ensure that only after two distinct cell types (dendritic and B-cells)sequentially encounter an antigen before the antibody production begins!





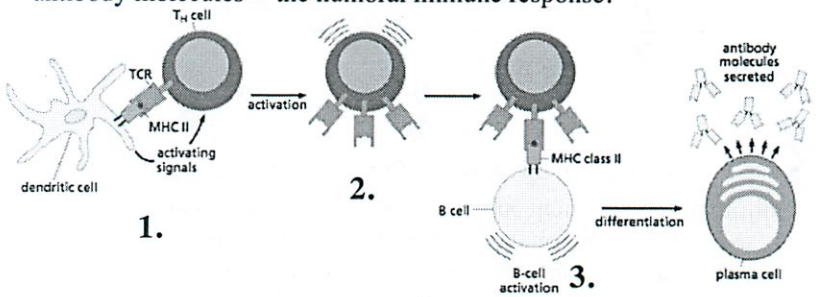


To summarize/recapitulate the whole process:

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!



Finally, the question of tolerance! Why does the immune system not attack the cells displaying oligopeptide fragments of normal cell proteins on their surfaces?

The problem of
distinguishing **self** from **non-self**

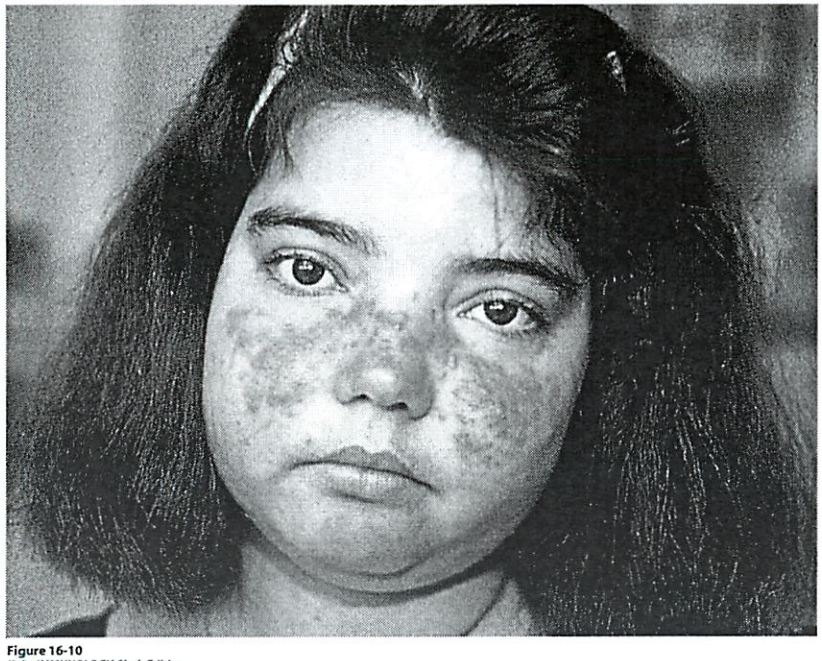


Figure 16-10
Kuby IMMUNOLOGY, Sixth Edition

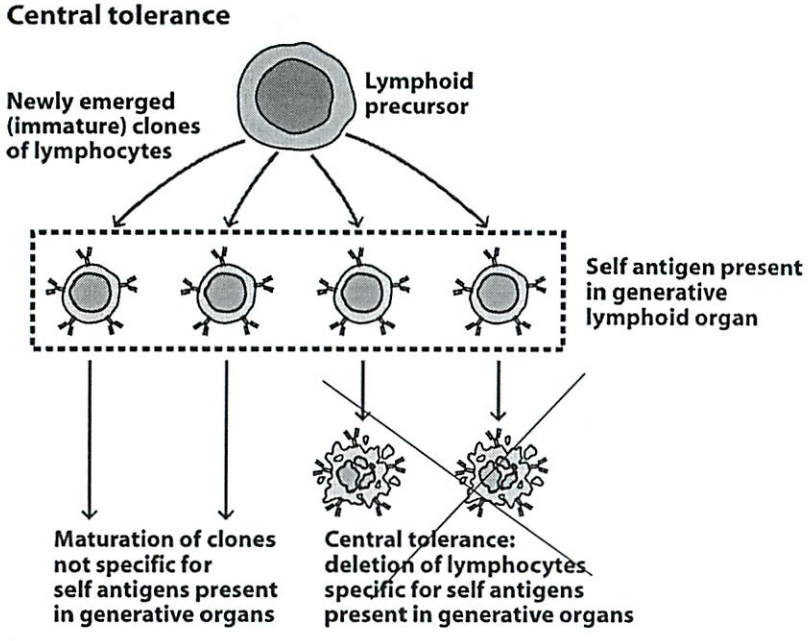


Figure 16-1a
Kuby IMMUNOLOGY, Sixth Edition

Is this important? Multiple auto-immune diseases are caused by the breakdown of **immunologic tolerance!**

TABLE 16-1 Some autoimmune diseases in humans

Disease	Self antigen	Immune response
	ORGAN-SPECIFIC AUTOIMMUNE DISEASES	
Addison's disease	Adrenal cells	Auto-antibodies
Autoimmune hemolytic anemia	RBC membrane proteins	Auto-antibodies
Goodpasture's syndrome	Renal and lung basement membranes	Auto-antibodies
Graves' disease	Thyroid-stimulating hormone receptor	Auto-antibody (stimulating)
Hashimoto's thyroiditis	Thyroid proteins and cells	T_H1 cells, auto-antibodies
Idiopathic thrombocyopenia purpura	Platelet membrane proteins	Auto-antibodies
Insulin-dependent diabetes mellitus	Pancreatic beta cells	T_H1 cells, auto-antibodies
Myasthenia gravis	Acetylcholine receptors	Auto-antibody (blocking)
Myocardial infarction	Heart	Auto-antibodies
Pernicious anemia	Gastric parietal cells; intrinsic factor	Auto-antibody
Poststreptococcal glomerulonephritis	Kidney	Antigen-antibody complexes
Spontaneous infertility	Sperm	Auto-antibodies
	SYSTEMIC AUTOIMMUNE DISEASES	
Ankylosing spondylitis	Vertebrae	Immune complexes
Multiple sclerosis	Brain or white matter	T_H1 cells and T_C cells, auto-antibodies
Rheumatoid arthritis	Connective tissue, IgG	Auto-antibodies, immune complexes
Scleroderma	Nuclei, heart, lungs, gastrointestinal tract, kidney	Auto-antibodies
Sjögren's syndrome	Salivary gland, liver, kidney, thyroid	Auto-antibodies
Systemic lupus erythematosus (SLE)	DNA, nuclear protein, RBC and platelet membranes	Auto-antibodies, immune complexes

Table 16-1
Kuby IMMUNOLOGY, Sixth Edition

The immune system either eliminates lymphocytes that recognize self-antigens or makes them "anergic", I.e., functionally unable to respond to self-antigens that they may happen to recognize.

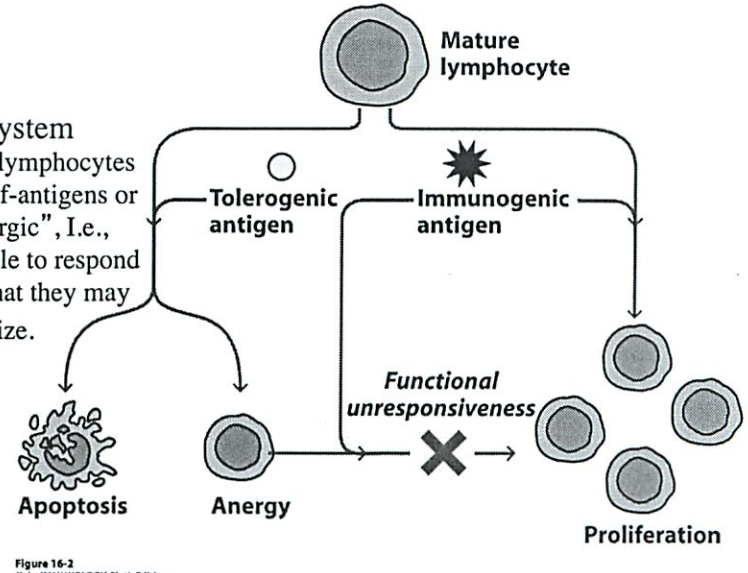


Figure 16-2
Kuby IMMUNOLOGY, Sixth Edition

tolerogenic = inducing immune tolerance

Bio Review

11/7

Nervous System

Textbook Chap 12 & 13

How do animals coordinate their bodies so quickly? Send messages around the body?

Neurons Specialized cells that transmit + recieve signals

Central Nervous System (CNS)
brain + nerve cords

Peripheral Nervous System (PNS)
to + from CNS

nerve axons that are bundeled together

Cell body organelles + nucleus remain in cell body

dendrites recieve messages

axon transmit message & longer

Myelin sheath insulation for axion
produced by Schwann cells

exceptions

Sensory neurons collect from sensors

motor neurons deliver messages

interneurons interconnect neurons

(get to the signal the transmission - that is what is important)

Synapse sites of information transmission

action potential what axon transmits

neurotransmitters over synapse

(3)

Glial cells - ~~produce~~ protect & support neurons

---

130 Action Potentials | Chemical + eletical signals

Membrane potential

ions - small charged atoms

resting have electrical potential
since uneven Na^+ K^+

Cell membrane prevents crossing

electrochemical gradient

membrane potential = potential energy across a membrane

if cell at rest → resting potential

↑ ; if membrane potential = 0 ?

No!

(9)

Na^+ K^+ Cl^-

↑ higher in cell

↑ higher out of cell

Na^+ Cl^-

K^+

Study w/ electrophysiology
w/ micro electroids

resting potential −60mV → −80mV

inner more neg charged than membrane

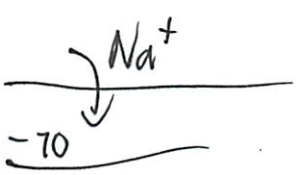
technically a gap → −70mV

(5)

Ion channels allow specific ions through

happens through electro gradient

always towards O right

So 

voltage-gated - at a certain voltage

ligand-gated - in presense of certain molecules

No

↑ so becomes more negitive inside

ion pump active mechanism

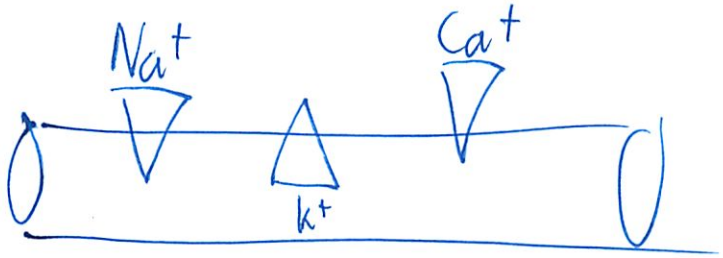
Sodium potassium

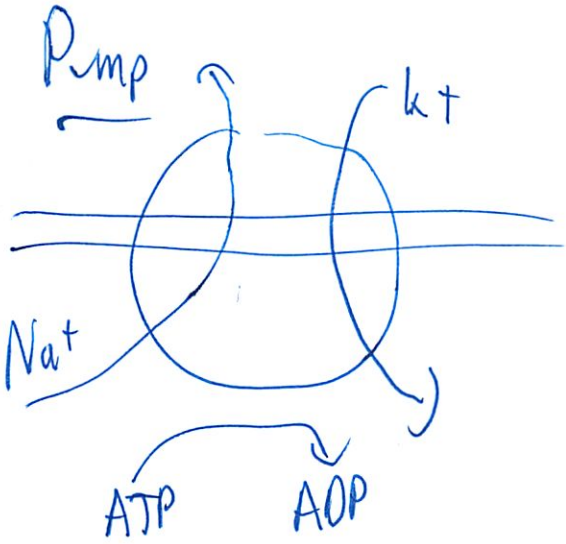
Sodium at
potassium in
} against conc gradients

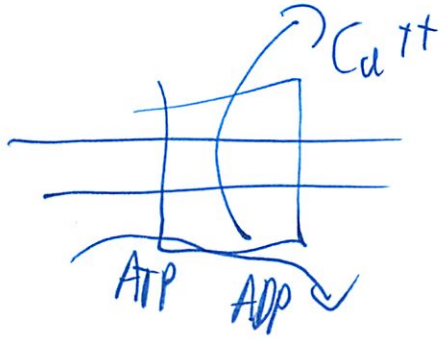
(6)

Ok lets get diration straight!

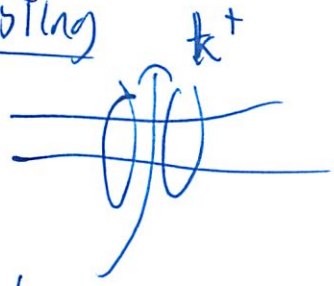
Class notes







Resting



k escapes, letting inside grow ⊖

'cuz + wants to go to +?

(7)

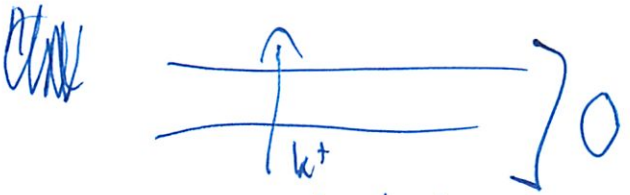
Still favorable from concentration

gradient unfavorable from electrical

balance at −70 mV

So understand this

It's the concentration



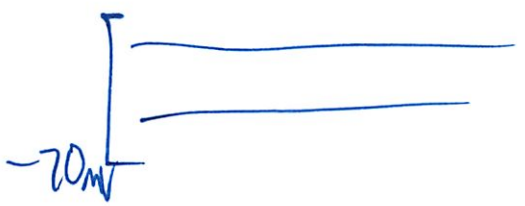
no electrical cost to leave

high concentration of K inside

(; how do we even get here;)

So it leaves

until



now electrically hard to leave

(8)

So this means K^+ ~~want~~ wants to go towards ⊖
Harder for it to go towards ⊕
Which is consistent w/ my knowledge

But how do we know about concentrations?
Table in notes
But does not add up!!

Textbook

hyperpolarization - membrane potential becomes more ⊖ neg caused by open K^+ channels which allow K^+ to flow out removing + from interior

graded potential

depolarization - opposite of hyperpolarization
like light mag $-50V \Rightarrow -40mV$

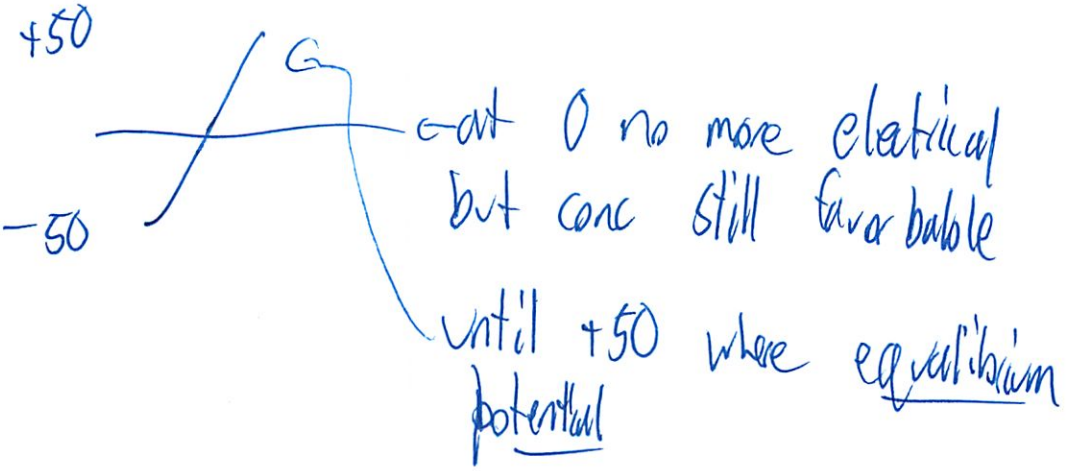
(1)

* Sodium channels open
allow lots of (+) to move in

So conc. → move in ✓
charge → move in ✓

Notes

Rapidly charge moves in



(10)

Then at +50 Potassium Voltage gated opens

- Potassium rushes back in ← elsewhere Sam rushes out think its out
  till −70mV - since electical favorable till 0
  ~~at +70~~

(but why does it go back to −70 where electical + conc unfavorable ?
Concentration pushes out
or is conc temp flipped ?

Book

(1) Note at rest Vngated potassium + Sodium - potasium pumps maintain resting

(2) Is the arival of a Stimulus
(3) Depolarization
(5) Potassium channel closes

(11)

I still don't 100% get it

So in last step k moves out

~~So a bunch~~

So a bunch of k rushes in

Until -70

Then slowly leaves

Recitation

hyperpolarization

let out too much potassium

Ok so during fall Potassium rushes out

it rushing out makes it more $\ominus$ inside

So at $+50$ Sodium rushes at -50

- conc favors k leaving
- until -70 mV

(12)

Review

Start w/ ~~[illegible]~~ −70 mV

K wants to leave conc
but can't since charge

Exogenous to −50 mV

Sodium channel opens

Sodium rushes in

~~[illegible]~~ conc whole time Favorable

~~[illegible]~~ Favorable charge −50 → 0

Voltage Gated ✓ Unfav charge 0 → 50

) balance at +50

Then potassium channel opens

potassium rushes out

Conc: whole time favorable

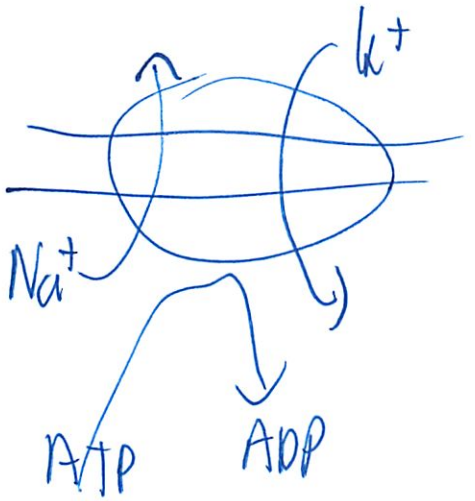
Charge 50 → 0 Favorable

0 → −50 Unfavorable

) balance at −70

(13)

Running in log → pump



I guess that is resting

Not really clear how this plays in "Maintain resting potential"

All or nothing again

Moves down

Oh I think I get this now!

(4)

Synapses

b/w nerve cells

(skipping to highlights)

Immunology Review

11/7

Textbook Chapter 18

triggers a response against patogens

patogens foreign cells that cause infection

innate immunity simple basic levels

like exoskeleton (skin)

leukocytes detect microbes

phagocytic

ex macrophage

adaptive immunity recognize cells

humoral - production of anti body

cell-mediated - T-cells

Can rearrange immunoglobulin genes

8

Lymphocytes in bone marrow + thymus

Lymphatic system liquid network that passes throughout body
passes through lymphatic nodes

Immunization immune response through inoculation
of an antigen dained from a specific pathogen

Vaccine introduce less harmful strand

16.4 Innate

not specalized attacks
(?did we study at all?)
going to skip for now

(3)

Adaptive

Research

T + B cells generate immune response

Antigen foreign substance

T cells structured simpler

T cell receptor

α, β polypeptide chains

Constant + variable region

T cell - host cell engulf pathogen

destroys pathogen

allows antigen to bind to MHC

(seems weird order + no pictures)

(9)

Lots of diversity

- recombination
- transcription
- processing
- translation

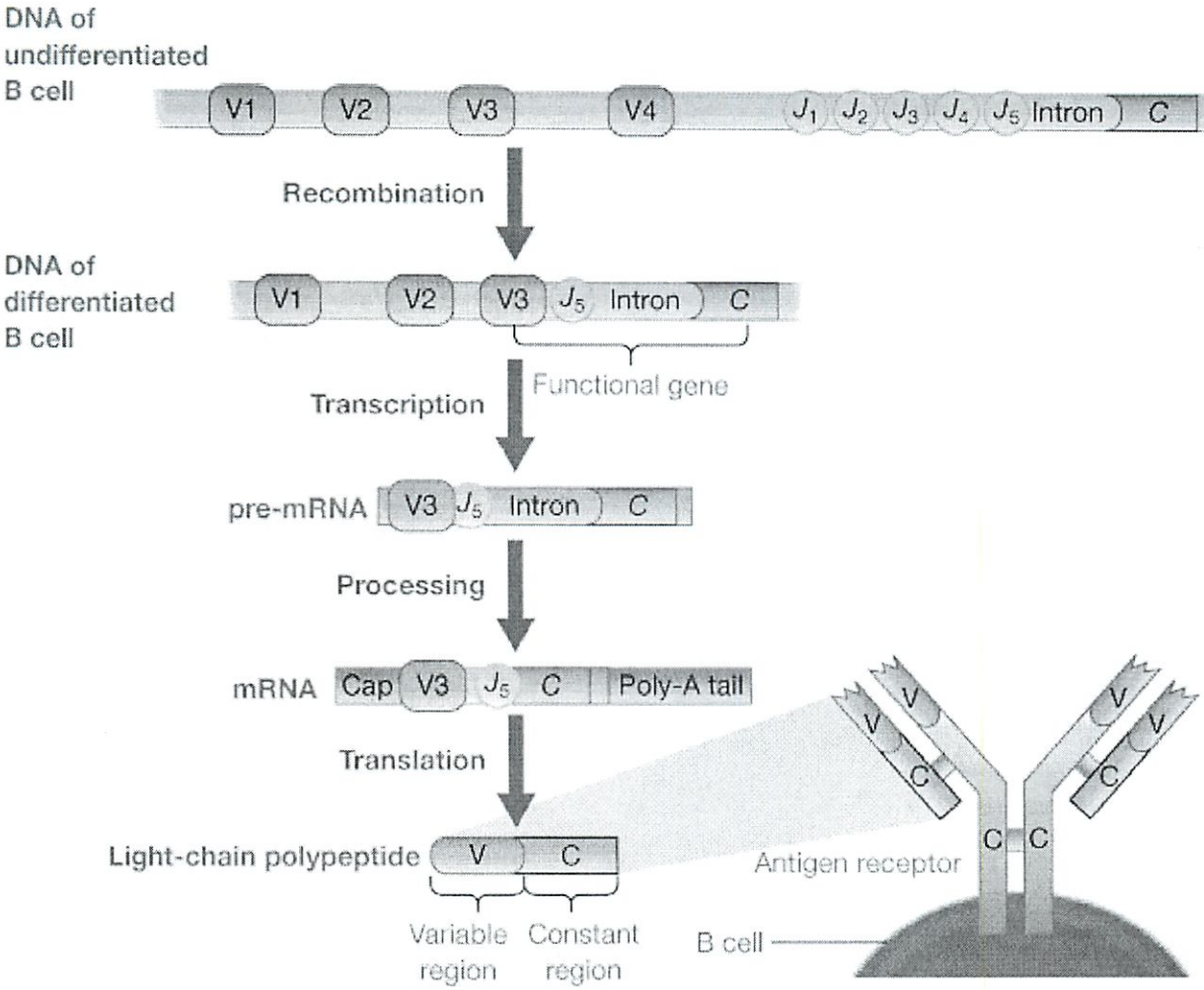
(going fast)

Clonal selection

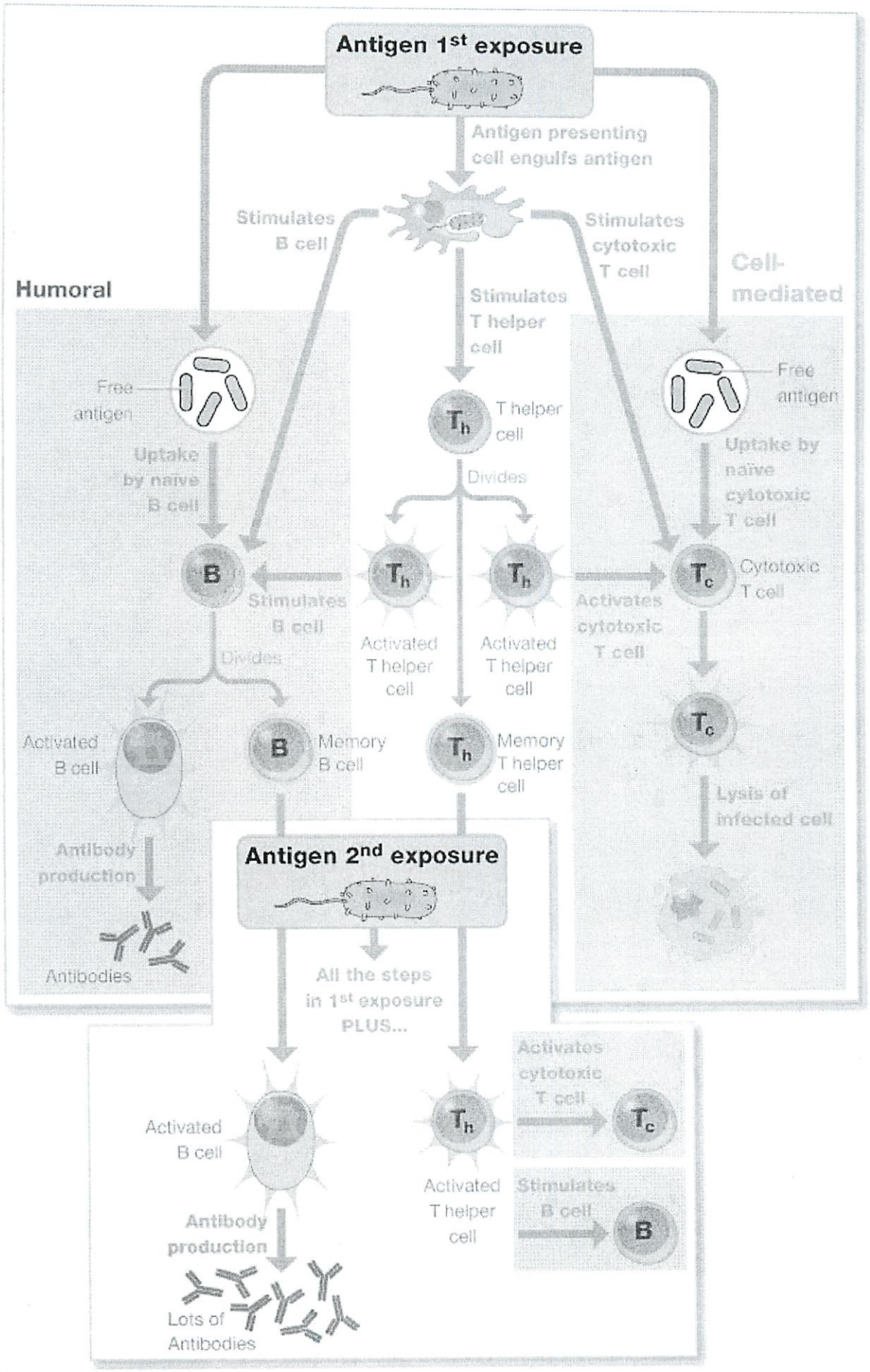
"lock + key"

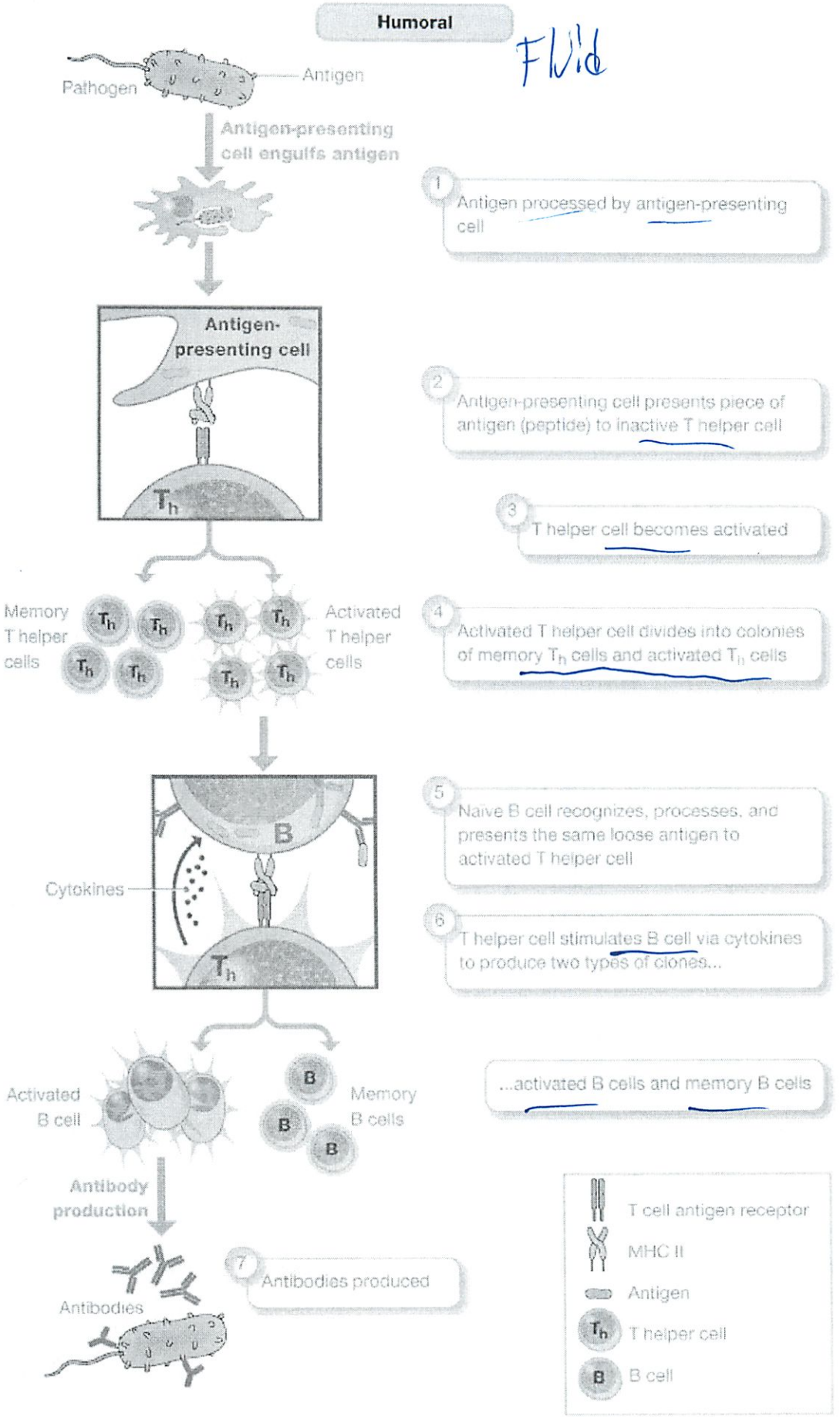
(nice chart!)

5

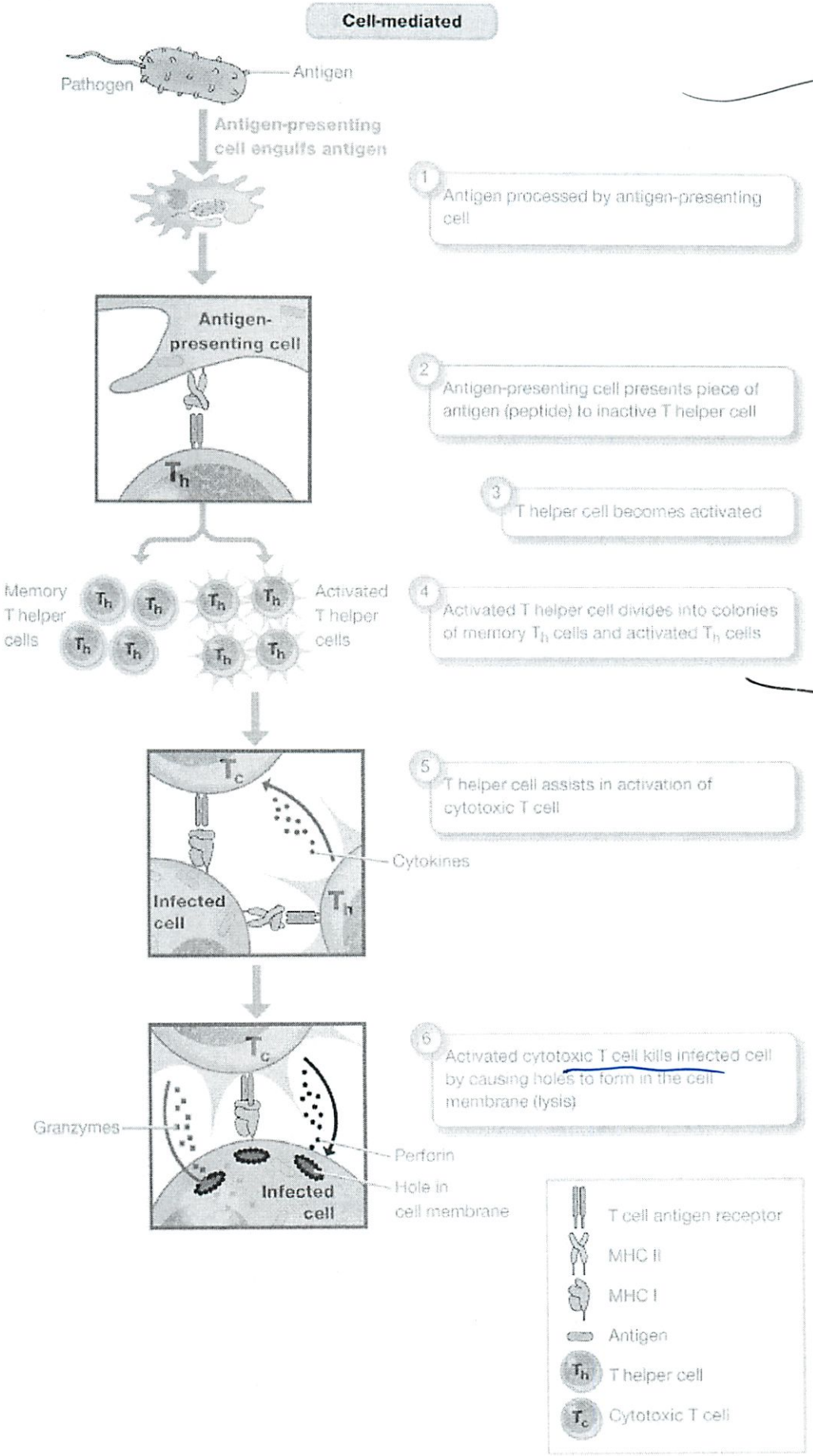

DNA of undifferentiated B cell
V1
V2
V3
V4
J_1 J_2 J_3 J_4 J_5 Intron C
Recombination
DNA of differentiated B cell
V1
V2
V3
J_5 Intron C
Functional gene
Transcription
pre-mRNA
V3 J_5 Intron C
Processing
mRNA
Cap V3 J_5 C Poly-A tail
Translation
Light-chain polypeptide
V C
Variable region
Constant region
V
C
Antigen receptor
B cell

6


Antigen 1st exposure
Antigen presenting cell engulfs antigen
Stimulates B cell
Stimulates cytotoxic T cell
Stimulates T helper cell
Humoral
Cell-mediated
Free antigen
Uptake by naive B cell
Th T helper cell
Divides
Free antigen
Uptake by naive cytotoxic T cell
B
Stimulates B cell
Th
Th
Activates cytotoxic T cell
Tc Cytotoxic T cell
Divides
Activated T helper cell
Activated T helper cell
Activated B cell
B Memory B cell
Th Memory T helper cell
Tc
Lysis of infected cell
Antibody production
Antigen 2nd exposure
Antibodies
All the steps in 1st exposure PLUS...
Activates cytotoxic T cell
Activated B cell
Th
Tc
Activated T helper cell
Stimulates B cell
B
Antibody production
Lots of Antibodies


Humoral
Pathogen
Antigen
Antigen-presenting cell engulfs antigen
1 Antigen processed by antigen-presenting cell
Antigen-presenting cell
T_h
2 Antigen-presenting cell presents piece of antigen (peptide) to inactive T helper cell
3 T helper cell becomes activated
Memory T helper cells
Activated T helper cells
4 Activated T helper cell divides into colonies of memory T_h cells and activated T_h cells
B
Cytokines
5 Naive B cell recognizes, processes, and presents the same loose antigen to activated T helper cell
6 T helper cell stimulates B cell via cytokines to produce two types of clones...
Activated B cell
Memory B cells
...activated B cells and memory B cells
Antibody production
Antibodies
7 Antibodies produced
T cell antigen receptor
MHC II
Antigen
T helper cell
B cell

8



Same?

this seems only diff (no B cells)

(9)

mast cells part of wound healing

(not really defined in the chap...)

MCH 1 vs MCH 2 ✓ yes basically

T cells ↑ professional antigen presenting cell

169 Innate Immunity

Many students may feel overwhelmed with the complexity of the immune system. However, just think of all the roles required in a modern army. There are the infantry, analogous to the cells that patrol the body and attack foreign objects directly. Then there are cells that fight infection by destroying infected body cells, somewhat like a demolitions expert. There are mobile cells, akin to the cavalry, and cells that stay put, guarding vital entryways into the body. There are even nonliving defenses, somewhat like drones or chemical traps tripped by pathogens, resulting in their demise. This complexity allows us to fight off not only the sheer number of pathogens we encounter every minute of existence but more importantly the diversity of those pathogens, which have evolved equally sophisticated tactics to foil our defenses.

Innate Immunity

Animals directly interact with their surrounding environment, exposing themselves to **pathogens**, which are disease-causing microorganisms and viruses. The immune system includes both passive defenses that shield us from pathogens trying to enter *the body*, and immune responses that actively fight infections. The immune system can be divided into the innate and adaptive immune systems, based on the specificity of the defenses involved. **Innate immunity** is present from birth and is general, defending against all pathogens in the same way. **Adaptive immunity** is specific, involving specialized attacks tailored to specific pathogens that have invaded the body in the past. Adaptive immunity has an immunological memory, with memory cells that stand ready to defend against a specific pathogen if it ever invades again. Innate immunity has no such immunological memory. Innate immunity is discussed here.

Like all animals, invertebrates exhibit innate immunity.

The effectiveness of the innate immunity system can be directly observed in insects. For initial protection, the hard, chitinous exoskeleton of insects creates a physical barrier that inhibits or prevents the entry of pathogens. To protect openings in the exoskeleton, such as the anus, the intestinal cells also secrete the enzyme **lysozyme**, which can cleave the cell walls of microorganisms, destroying them.

In addition to the exoskeleton and lysozyme as barriers to microbial invasion, specific cellular features also play a major role in the innate immune system of insects. Insects and other invertebrates, such as mollusks, contain cells called hemocytes in their blood (hemolymph). There are a variety of hemocytes, with some performing immune functions such as **phagocytosis**, the ingestion and digestion of pathogens by immune cells. The immune cells that accomplish phagocytosis are generally called **phagocytes**. Phagocytes generally move and capture pathogens using pseudopods (Figure 1; Figure 3), which are extensions of their cytoplasm and cell membrane. Once they surround a pathogen, they ingest it into an endocytic vesicle (Figure 2). This vesicle then fuses with lysosomes, which secrete digestive enzymes that destroy the pathogen. Any undigested remains of a pathogen leave the phagocyte through exocytosis.

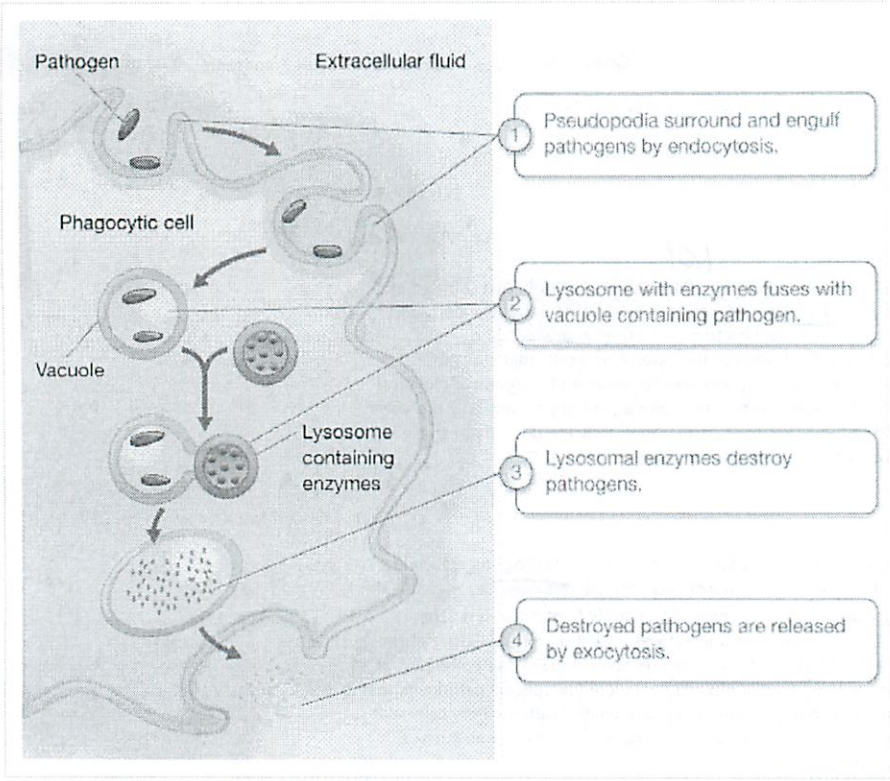


Figure 1: Phagocytosis in invertebrate cells.
Hemocytes engulf microbes through the process of phagocytosis, degrading these foreign bodies into simpler macromolecules and components. Using pseudopodia, the hemocyte surrounds the pathogen to produce a vacuole that fuses with a lysosome. Enzymes inside the lysosome digest the pathogen into smaller components and release the material outside the cell.

Hemocytes can also secrete antimicrobial peptides that bind to and destroy the cell wall of bacteria. Scorpine, a particular antimicrobial peptide found in scorpion venom, has the capacity to circulate throughout the body, thereby increasing the chances of interacting with migrating microbes.

Research efforts have long attempted to design an effective treatment for malaria, a mosquito-borne disease caused by the protozoon *Plasmodium*. Malaria causes almost one million deaths around the world each year. A research group at the University of Maryland extracted a chemical from the fungus *Metarhizium anisopliae*. This chemical is known to be lethal to mosquitoes and is therefore a promising new preventative measure for malaria. Using the extract to kill mosquitoes would decrease the spread of malaria, yet this approach would also artificially select for resistant mosquitoes. The research group attempted to generate a genetically modified fungal extract that did not kill mosquitoes but instead prevented the malaria parasite from completing its life cycle within the mosquito. Comparative analysis of the original fungal extract and the genetically modified fungal extract showed that the genetically modified extract significantly decreased the number of malaria parasites in mosquitoes.

How does the body identify pathogens?

How do innate immunity systems detect the presence of a pathogen? Cells of the innate immune system have receptors that recognize molecules on the outer surface of pathogens. For example, Toll-like receptors (TLRs) are proteins that recognize specific pathogen components. Recognition triggers intracellular signal transduction cascades that allow the cells to react and combat the infection. In many insect species, TLRs recognize molecules in microbial cell walls. The TLRs trigger a signal cascade that results in the secretion of antimicrobial peptides.

Vertebrate innate immune cells possess several different types of Toll-like receptors. For example, type 3 Toll-like receptors (TLR3), located in the endocytotic vesicles of phagocytes, bind specifically to the double-stranded RNA characteristic of many viruses (Figure 2). Other white blood cells possess type 4 Toll-like receptors (TLR4). These are located on the leukocyte cell membrane and detect lipopolysaccharide molecules characteristic of Gram-negative bacteria.

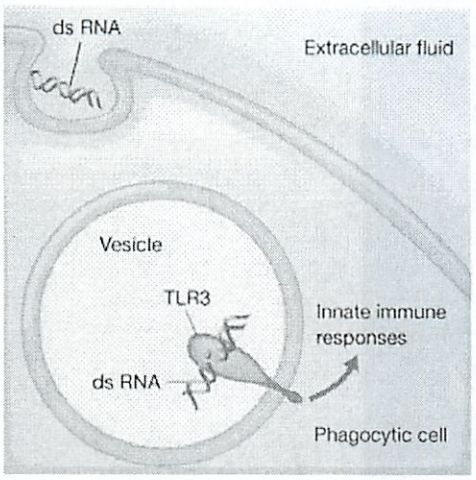


Figure 2: Detection of pathogens using the Toll-like receptor (TLR) protein.
Analogous to the Toll receptor protein in invertebrates, mammals possess different types of Toll-like receptors (TLRs) that bind to specific components of the pathogen. For example, the type 3 Toll-like receptor (TLR3), specifically located in endocytotic vesicles, binds to a virus-specific double-stranded RNA.

Test Yourself

What are the possible molecular differences between the Toll-like receptors that allow them to have specific targets?

Submit

Skin, mucous membranes, and bodily secretions all serve as barrier defenses in vertebrates.

Similar to the innate immune system of invertebrates, vertebrates also possess physical and structural barriers that prevent or slow the invasion of pathogens. For example, the epithelial cells of our skin undergo apoptosis shortly after they are created from stem cells at the base of the epidermis. These dead cells form a thick "armor" that the pathogens must penetrate before they even reach any living cells. In addition, the outer layers of skin are periodically shed, along with any microbes trapped within. Sebaceous glands in mammals also secrete a protective oily film over the skin surface that inhibits bacterial growth.

The mucous membranes are also a formidable barrier for pathogens to cross, despite the fact that these line openings in the skin. All mucous membranes produce mucus, a thick fluid containing glycoproteins and water that serves to trap airborne particles and microbes. The mucus membranes of the nose have cilia as well as mucus. These cilia are hair-like structures that trap and move airborne particles. Pathogens caught in the cilia of the respiratory system are expelled and deposited into the esophagus, where they are destroyed by the highly acidic conditions of the stomach.

The direct exposure of the eyes to the external environment could be an easy portal for entry, but tears continuously cleanse and protect the eyes from microbes and other foreign matter. Similarly, saliva washes pathogens from the mouth; the pathogens are swallowed and destroyed in the stomach.

Innate cell-based immunity in vertebrates involves a variety of white blood cells.

Phagocytosis is an important aspect of vertebrate innate immunity as well. Vertebrates possess a variety of phagocytic cells involved in innate immunity. The smaller, mobile phagocytic cells, called **neutrophils**, are activated by signals produced by injured cells at the site of infection or inflammation. Larger phagocytic cells, called **macrophages**, either move around the body or remain sedentary in a particular organ until pathogens appear nearby. **Monocytes** have the ability to develop into wandering macrophages during an immune response. Phagocytosis in all these cells occurs in much the same way as in the invertebrate hemocyte depicted in Figure 1, using pseudopods (Figure 3) and intracellular digestion with lysosomes.

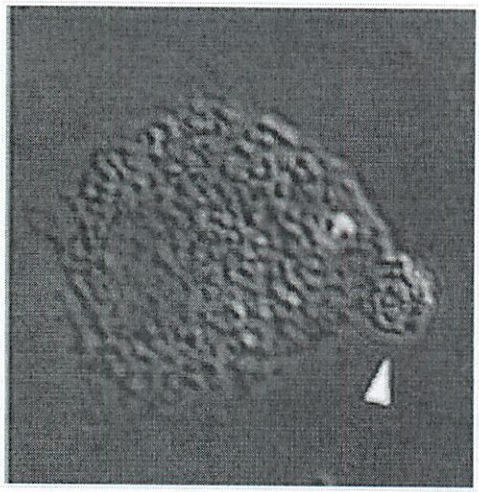


Figure 3: A macrophage pseudopod.
This macrophage is phagocytosing a liposome. The arrow is pointing to the pseudopod that was formed to enclose the liposome.

Dendritic cells are specialized phagocytes located in the skin and lymph nodes. They are very mobile cells with many arms, somewhat resembling a spider. This allows them to move and engulf pathogens wherever the need is greatest. **Eosinophils** are another type of leukocyte; they play an important role in innate immunity by releasing chemicals that attack larger parasites, such as worms. **Natural killer cells** search the body for infected or malfunctioning body cells. Body cells that are trouble in some way indicate this to the immune system by displaying certain membrane proteins. When natural killer cells find these cells, they degrade the cell membrane of the infected body cell, causing it to lyse. Natural killer cells may also induce the infected cell to undergo apoptosis, as they do with cancer cells. Natural killer cells are therefore not phagocytes, as they do not engulf and digest pathogens. Rather, they kill the pathogens by killing cells that the pathogens have infected.

The lymphatic system serves as a circulatory network that drains excess interstitial fluid from capillary beds. The fluid could also transport pathogens throughout the host's body. The lymphatic system has therefore evolved lymph nodes, which work as somewhat of a "security checkpoint." Macrophages position themselves in the narrow passageways of the lymph nodes, engulfing any pathogens present in the lymphatic fluid. The lymph nodes also contain resident and migrating dendritic cells, and they also produce and harbor cells of the adaptive immune system.

The complement system and inflammatory response are innate, chemical-based responses to infection.

Do vertebrates also use chemical immune defenses, similar to the antimicrobial peptides of invertebrates? Vertebrates actually have a wide variety of innate, chemical-based immune defenses. **Interferons** are protein secretions generated during infection. As the name implies, these proteins interfere with or prevent the reproduction of viruses within the local region of invasion, preventing further spread. The mechanisms employed by interferons have long fascinated scientists, and the study of these mechanisms has resulted in a handful of new antiviral therapies.

Another chemical component employed by mammalian innate immunity involves the **complement system**, a collection of proteins circulating in the blood. The presence of certain pathogens activates the complement cascade, producing a variety of proteins. For example, the C3b complement protein attaches to pathogens and produces a coating that makes it easier for phagocytes to engulf the pathogen. Several complement proteins also join together to make the **membrane attack complex**. This cylinder-shaped protein complex inserts itself into the microbial cell membrane, creating a pore through which cytoplasm leaks from the microbe. Finally, complement proteins also bind to mast cells, triggering the inflammatory response described next.

Why do wounds swell right after injury? The **inflammatory response** serves as a protective mechanism that impedes pathogen spread during an infection or after body tissue is damaged. The inflammatory response is initiated by **mast cells** at the site of the injury or infection. These mast cells secrete **histamine**, a chemical that induces blood vessels to dilate and become more permeable in the affected area. Blood leaks from the capillaries, flooding the affected tissue with complement and interferons. Platelets can also secrete histamine, increasing the inflammation response as they reach the affected area. The massive increase in blood flow to the affected area causes the redness, pain, swelling, and increase in temperature characteristic of inflammation (Figure 4).

The first leukocytes to arrive generally are neutrophils. The neutrophils squeeze through the walls of the blood vessels to enter the affected tissue, phagocytizing any pathogens they encounter. They are followed by monocytes, which develop into macrophages. In the later periods of the inflammatory response, the

neutrophils die and are totally replaced by the much more effective macrophages, which not only phagocytize pathogens but also engulf dead tissue cells and other debris. The accumulation of dead neutrophils and macrophages over a period of days produces pus that is absorbed once the infection has ended.

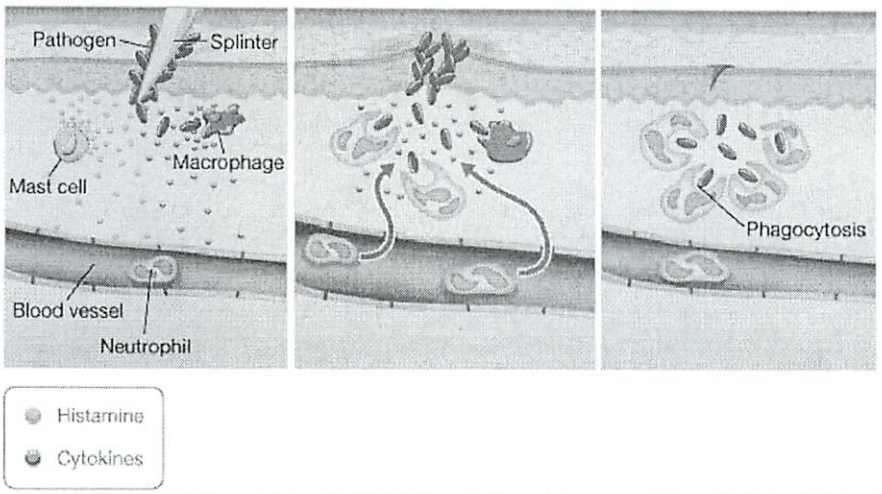


Figure 4: Inflammation as an immune response to infection.
The inflammatory response serves as a protective mechanism of the body that triggers the production of chemicals that leads to tissue repair. Histamine (orange) induces blood vessels to widen, facilitating the diffusion of other molecules for healing. Macrophages and neutrophils also secrete cytokines (purple) that increase the circulation of blood. The phagocytic cells engulf any microbes that may have entered the site of injury.

Test Yourself

What causes the swelling within and around an injured tissue?

Submit

Fever is an abnormally high body temperature that is often triggered by the inflammatory response. Infections often trigger the release of cytokines by a wide variety of white blood cells. **Cytokines** are small protein hormones that perform a variety of functions in the body. Those produced by immune cells often regulate the innate and the adaptive immune response. Neutrophils produce a fever-inducing cytokine in response to toxins released by many bacteria. Higher body temperatures make interferons function more effectively, inhibit the growth of some pathogens, and speed up the repair mechanisms of the body. In extreme cases of infection, such as septic shock, fever could become lethal, as extreme body temperature begins to cause brain damage.

CAREERS

The Need for Immunologists Has Increased with the Spread of Bioterrorism

One major incident associated with the September 2001 terrorist attack in the United States involved the introduction of anthrax spores in envelopes mailed to several federal offices across the country. This event has resulted in the development of rapid treatment and prevention methods that could be employed in future bioterrorist attacks. The pathogens commonly used in these heinous crimes include microbial strains that could be easily transmitted across various substrates and that have rapid infection rates in the human population. Potential pathogens for bioterrorist attacks include anthrax and salmonella. The federal government has supported research into techniques for the rapid detection of various pathogens. More importantly, research efforts also focus on the production of vaccines and treatment schemes for infections caused by potential bioterrorist attacks.

CAREERS

Notes on

IN THIS MODULE

- Innate Immunity
- Summary
- Test Your Knowledge

P-Set 5

Doing

11/7

1. SNP

Wasn't this last exam?

WP: Variation when single nucleotide differs

Often occur in non coding regions

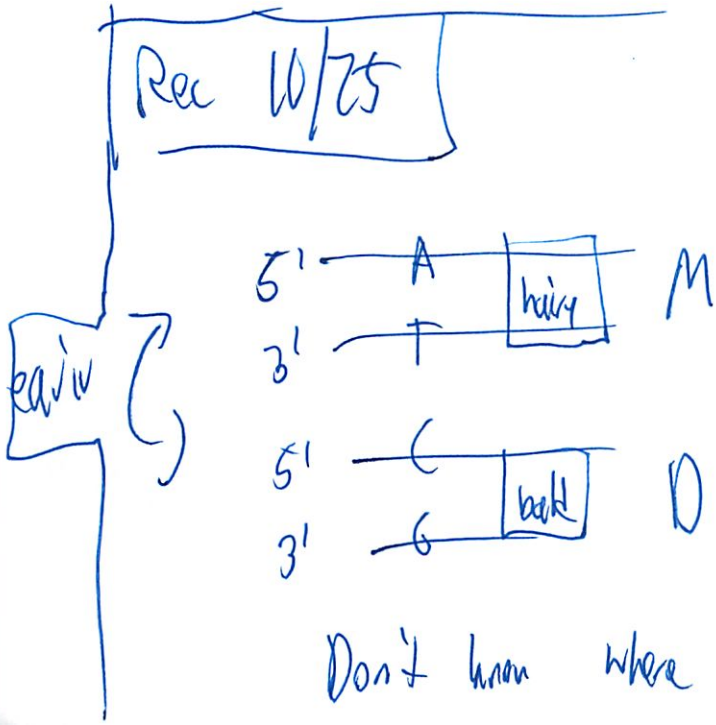
Oh so are naturally occurring

Then do some sort of correlation

Is gene correlated w/ SNP?

* Comparing b/w cohorts in genome wide association studies

Rec 10/25



Don't know where gene is or pattern of inheritance

(2)

But know C → often correlates w/ bald

Physically close → so low chance

Not all Cs have problem

¿Only if have from M/D likely to have problem as well?

Actually do w/ SNP array

Why 2 letters? Have not seen before?

Oh 2 sets of SNPs

PheA is gene we want to investigate?

(A) Still bad at answering

(3)

2 b) depends on action potentials' exact defn

2c) Ah ok the normal concentration is maintained by pumps

K^+ channel always open?

don't think so

Conflicting reports...

2d) GABA — ? or both?

is both

1) always and voltage gated

(kinda going fast so can check)

3. Excitory vs inhibitory

(4)

Jeff

1. Any progeny w/ kids has one kids

Sex linked recessive

must have 2 mutent X to be have disease

Go 1, 2, 4 could work

1, 2, 8 Y from Dad

Could get disease from mom who has 0 or 1 diseased

X-linked dominant 1, 2, 5 doesn't tell us gets disease from dad Could or couldn't from mom

father has or mother has 1 or 2

look at 1, 2, 8

8 gets Y from Dad

8 would have to get one diseased from mom - but she has 0

(X) not possible

3, 4, 10 Consistent

8, 9, 14 consistent

(✓) possible

Autosomal dom

1 has 1 or 2 diseased

2 has 0 diseased

4, 5, 7, 8 consistent

Shared has 1 diseased

(5)

✓ Consistent

recessive autosomal

✓ Consistent

Now check SNPs

SNP correlates Trait

Trait ⟶✕ SNP

no correlation

look at diseased parent and child

1, 2, 4

SNP 1 so got C from Dad
G from Mom

SNP 2 got G from Dad
A from Mom

* If no cross over → SNPs 1+2 travel together

(6)

~~SNP~~ we indicators
to identify which allele from which parent

C_1 seems to be determinator

from 3, 4, 10 → 3 given as only D allele
10 is showing
So 10 must be I
So dominant

We know X linked dom (X)
So autosomal dom (✓)

Only way we can find
make SNP will only have I if X linked
~~Since in~~
So X-linked easily thrown out

6

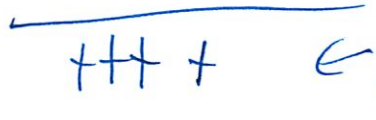
Notes

Potassium open - both ways
always open

V.G K^+
partially open vs fully open
↑ rest state ↑ at the +50

The Na^+ in and K^+ out
means that for some time

+50mV



2a) a just cross
c) b/c Charge gradient
Charge factor as well

(8)

14

(i) Charge

what I like brick wall is solid since
hard its made of brick :)

(9)

11/8
On own

Don't know anything on this
Mostly guess to get it done

Somatic relating to the body

Or non-germ cells

Diff heavy chains

IgG γ
IgM μ
IgD δ
IgA1 α
IgE ϵ

4e)

(10) We never leave!

IgM → largest
1st to appear
970 kDa
10 binding sites
Spleen produces
produced by b cells

IgG most abundant
Secreted by plasma B cells
150 kDa

e iii) tricky!

Name Michael Plasmeier Section 27 TA Hoshyar

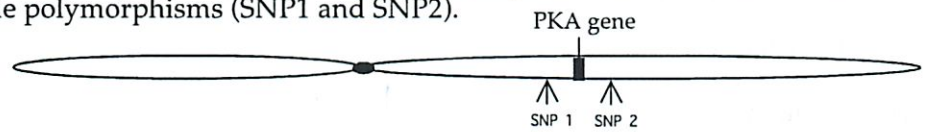
2012 7.012 Problem Set 5

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 Nov 8th.

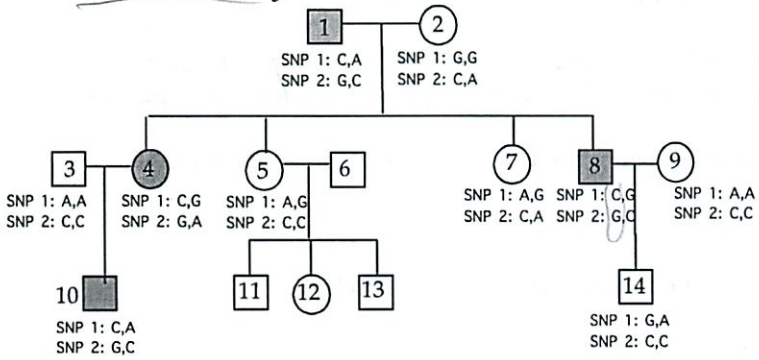
12/17

Question 1
A single nucleotide polymorphism (SNP) is a DNA sequence variation occurring when a single base pair in the genome differs among members of a species or paired chromosomes in an individual. By convention this base pair change is represented as one nucleotide — A, T, C, or G — of the base pair.

The chromosomal position of the PKA gene is diagrammed below, as is the location of two single nucleotide polymorphisms (SNP1 and SNP2).



The pedigree shows the inheritance of SNP1 and SNP2 associated with the PKA gene. The affected individuals are shaded. Also listed are the alleles of SNP 1 and 2 for some individuals. ***Note:*** *Assume individual 3 does not carry the disease allele.*



The two letters identify the alleles of the SNP that would be found on the "top" strand of each of the two homologous chromosomes. For example, "SNP 1: C,A" indicates that on one of the homologous chromosomes the top strand would contain a C (that is, the chromosome would have a C/G base pair in this position), while on the other chromosome the top strand would contain an A (that is, the chromosome would have an A/T base pair in this position).

a) What is the most likely **mode of inheritance** for this disease? Autosomal dominant 1/1

b) Individuals 5 and 6 have no affected children. What is the **genotype** for individual 6 at the PKA locus? *Note: Use the symbol 'A' or 'X^A' to represent the allele for the dominant phenotype and 'a' or 'X^a' for the allele for the recessive phenotype.*

aa 1/1

c) Individuals 5 and 6 have no affected children. Can you predict the genotype for individual 6 at SNP1 and SNP2 loci (*Yes*/(*No*))? **Explain** why you selected this option.

Presence of SNP indicates correlation w/ gene not the other way around! 0/1

d) Which allele (or alleles) of SNP 1 and SNP2 is linked to the mutant PKA gene in this family? ***Note:*** *Assume no recombination.*

SNP1: C **SNP2:** G 1/1

e) Briefly describe how you can use a SNP microarray to determine the SNP genotype of an Individual.

The microarray lets one quickly find out what SNP one has at particular positions. If the complement binds, the strand must match. One SNP or individual pair can TAGC across the columns. 1

Name_____ Section_____ TA_____

Question 2

a) Circle **all** the correct options from below. The resting membrane potential of a neuron is determined by…

i. ions that can travel freely through channels in the resting neuron
but don't since matched

ii. ions that require ATP to cross the resting membrane

iii. unequal distribution of different ions across the neuronal membrane

b) Circle **all** that apply. An action potential is generated by the passage of ions through…

i. **only** the resting ion channels
not only

ii. voltage-gated ion channels

iii. G-protein coupled receptors

iv. **only** the sodium potassium ATPase pump

4/4

c) Under resting conditions the Na^+, $Ca^{2+}$and Cl^- concentrations are high outside the neuron, K^+ concentration is high inside and this is maintained by the action of specific channels and pumps.

i. What feature of the plasma membrane prevents the free diffusion of ions across it?

The electric charge

ii. Is the resting membrane potential observed exclusively in a neuron?

No, could be in other cells too

iii. Complete the following table for the two channels/pumps that establish and maintain the resting membrane potential.

Channels/pumps	Ions passing through them	Default state (open/closed).	Is the ion transport active or passive? Explain.
Na^+ Channel	Na^+	Closed	passive - Sodium rushes in when open
K^+ Channel permanent	K^+	Open	passive - K rushes out when concentration + electrical gradient allow

9/11

that → was right

Name_____ Section_____ TA_____

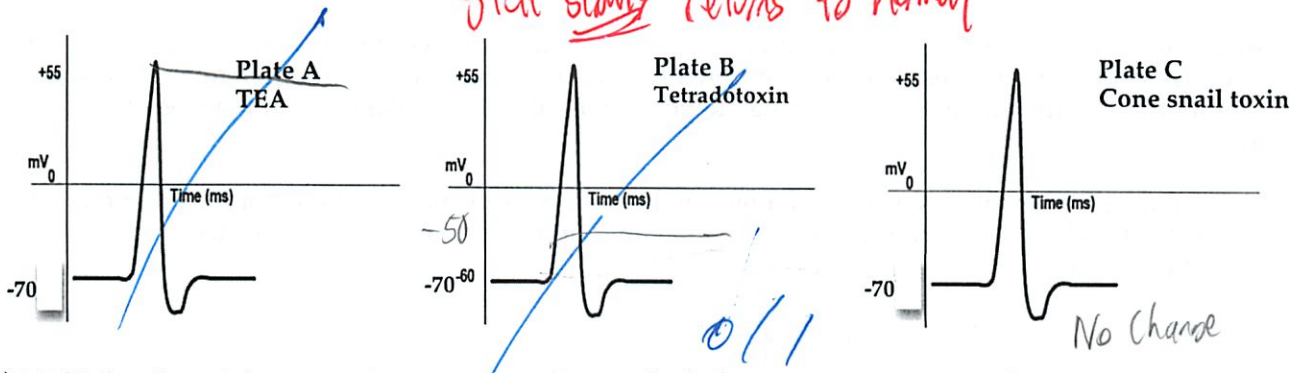
Question 2 continued

d) Different neurotoxins are very often used to study neuronal function. You culture a GABA (an inhibitory neurotransmitter) secreting neuron in the presence of the following neurotoxins in three separate petri-plates (A-C)

- **A:** Neuron is treated with tetraethylammonium (TEA), which **inhibits voltage gated K^+ channels.**
  Stuck at high voltage
- **B:** Neuron is treated with tetradotoxin, which **inhibits voltage gated Na^+ channels.**
  Stuck at low
- **C:** Neuron is treated with cone snail neurotoxin, which **inhibits voltage gated Ca^{++} channels.**
  not used - pumped out

A normal action potential in a GABA secreting neuron that has been stimulated **in the absence of any neurotoxin** has been drawn in each panel below. Sketch the alteration in action potential following the treatment of the neuron with each neurotoxin. *Note: If there is no change please write "NO CHANGE" on the graph.*

Stuff slowly returns to normal



e) Multiple sclerosis is an autoimmune disorder in which the immune system of the patient attacks and destroys the myelin sheath of a neuron. These patients show a very slow propagation of action potentials along the axons of their neurons. **Explain** why is this so.

The lack of insulation makes the electrical differences more pronounced.

f) A functional neuron may receive both excitatory and inhibitory signals from multiple neurons at the synaptic junctions. In a post- synaptic neuron, where are the signals from all the pre- synaptic excitatory or inhibitory synapses integrated and the decision to fire an action potential made? **Circle** the correct option from the following choices. **Explain** why you selected this option.

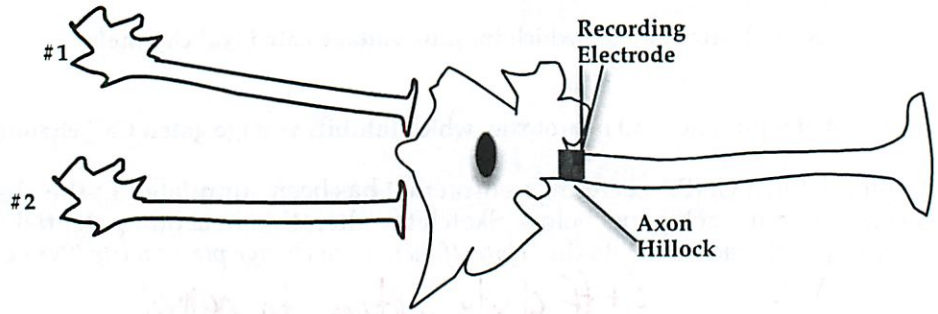
Cell Body *Axon Hillock* *Myelin Sheath* *Synaptic Cleft*

This is what controls if the excitatory postsynaptic the synapse potential "fires" by causing it to depolarize to threshold for an action potential to be generated

Name_____ Section_____ TA_____

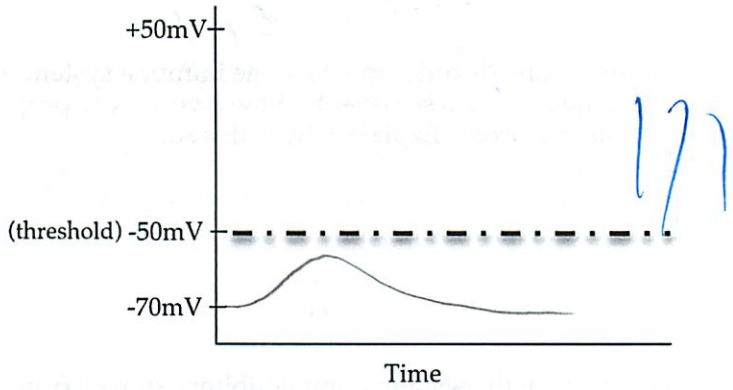
Question 2 continued

g) The following question refers to an experimental design depicted below. There are **two excitatory pre-synaptic neurons** that independently converge on a post-synaptic neuron. The two pre-synaptic neurons can be stimulated individually. In the absence of any stimulation, the recording electrode in the post-synaptic neuron measures the membrane potential as -70mV.

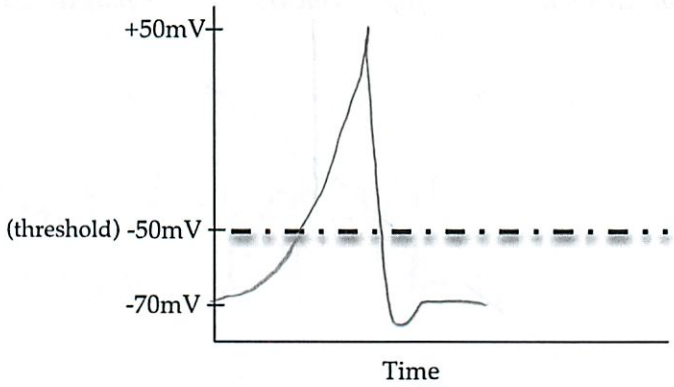


If **only one** excitatory pre-synaptic neuron is stimulated, you record a deviation from -70mV with the recording electrode in the post-synaptic neuron, but you do not record an action potential. If **both** the excitatory pre-synaptic neurons are stimulated, you record an action potential in the post-synaptic neuron.

i. On the graph below sketch the changes in the post-synaptic neuronal membrane potential, as measured by the recording electrode, when **only one** excitatory pre-synaptic neuron is stimulated.



ii. On the graph below sketch the changes in the post-synaptic neuronal membrane potential when **both** the pre-synaptic neurons are stimulated.



Name_____ Section_____ TA_____

Question 3

Dopamine is one of the neurotransmitters in the mammalian brain that regulates mood, cognition and locomotion. Dopamine is commonly associated with the reward system of the brain. Dopamine can be an excitatory or inhibitory neurotransmitter, depending on the dopaminergic receptor that it binds to. It is derived from the amino acid tyrosine. At dopaminergic synapses, the released neurotransmitter is taken back into the pre-synaptic cell for re-use.

a) Beginning with the stimulation of the pre-synaptic neuron, list the steps, in order, that result in a release of neurotransmitter. Include any relevant channels, ions and molecules specific for this process.

1. action potential on pre-synaptic neuron (Na, K)
2. Reaches voltage gated calcium channel - calcium ions rushing in
3. Enzyme binds Calcium w/ Synaptin
4. Calcium dep protien kinase puts Phosphate group on Synaptin
5. Synaptin changes form and release vesicles
6. Vesicles go to membrane, fuse, + spill neurotransmitter (Dopamine)

b) Clozapine, an anti- psychotic medication interferes with the binding of dopamine to the receptor. You are studying an excitatory dopaminergic synapse where the post-synaptic cell has receptors for dopamine. If you treat these neurons with dopamine plus Clozapine, would the likelihood of generating an action potential in the post- synaptic neuron increase, decrease or not change relative to the treatment with dopamine alone? **Explain.**

The excitatory can not bind - so likelihood of generating an action potential decreases

1/1

c) The most extensively studied effect of cocaine on the central nervous system is the blockade of the the protein that binds to dopamine and pumps it out of the synaptic cleft back into the pre-synaptic neuron. What effect would cocaine have at an excitatory dopaminergic synapse?

It would cause it to be left there allowing dopamine to build up in the cleft, - making people feel happy

1/1

d) Serotonin (5-hydroxytryptamine, 5-HT) is an excitatory neurotransmitter. It acts by binding to several HT receptor subtypes. The 5-HT3 receptor is a Na^+ channel whereas the 5-HT-2 receptor is a G-protein-coupled receptor, which leads to the opening of Ca^{2+} ion channels.

i. As the amount of serotonin is increased, **circle** the option that may change: *Amplitude of action potential/ frequency of action potential/ threshold potential.* Provide an **explanation** for the option that you have circled.

Causes Ca^{2+} to enter cell, more positive
makes action potential more likely - as we get closer to threshhold - less of a gap it has to make up

Name_____ Section_____ TA_____

Question 3 continued

ii. Complete the following table for each of the treatments. Assume that serotonin is present at these synapses. ***Note:*** *Consider each treatment independently.*

Treatment	**Action potential in the post-synaptic neuron is *more likely or less likely* to occur compared to untreated synapses? Explain your choice.**
Prozac, which inhibits the re-uptake of serotonin from the synapse	faces serotonin to stay, keeping action potential more likely
Kentasarin blocks the binding of 5-HT3 receptor to 5-HT	prevents from binding - so less likly to have action potential (excitory)

2/2

Question 4

a) The immune system is comprised of different cell types such as the *mast cells, macrophages, helper-T (T_H), cytotoxic-T (T_C), memory B and plasma B cells*. From the choices provided, list **all** the cell type(s) that would…

i. Participate in the **innate immune response**. mast + macrophages

ii. Bind directly to an **antigen circulating** in the blood stream. ~~Antigen presenting cells (macrophages), plasma B cells~~

iii. Secrete large amount of antibody in response to an infection. ~~plasma B cells, memory B cells~~

iv. Provide **protective immunity** against **second exposure** to the **same antigen**. memory B, Tc

v. Show rearrangement of specific gene(s). ?? Tc, memory B

0/2

b) The diverse array of both TcR and antibodies is generated by DNA rearrangement. In addition to the DNA rearrangement, name the **three major processes** that contribute to the generation of the TcR and antibody diversity.

~~transcription~~ recombination
processing hypermutation
translation class switching

c) Circle **all** correct options from the following choices. The **innate immune response**…

i. Occurs **only** following the **first exposure to an antigen.**

ii. Occurs **only** following the **subsequent exposure the same antigen.**

iii. Occurs in response to **all infections**.

iv. Is **non-specific** unlike the adaptive immune response.

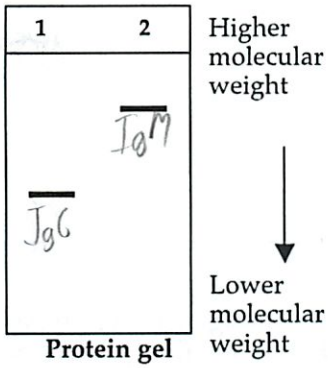
Name_____ Section_____ TA_____

Question 4 continued

d) You have purified a novel protein, which you call **Protein R.** You want to develop antibodies against this protein. You inject Protein R into a rabbit and after a month you draw some blood from this rabbit and determine that the rabbit's immune system has produced antibodies against Protein R. You wait for one month and then inject Protein R again into the same rabbit. You observe a stronger immune response with the second injection than you did in response to the first injection. Why is the primary immune response **slower** and **weaker** compared to the secondary immune response?

The second time around it has more memory against Protein R which causes a stronger response. This is absent the 1st time around

e) During the primary and secondary response to an antigen, the B cells of the immune system **produce membrane bound (i.e. IgM class)** and **secreted (i.e. IgG) antibodies**. You isolate the antibodies produced against Protein R, resolve them on a **protein gel** based on their molecular weight and obtain a profile as shown in the schematic below.



i. Which class of antibodies (*secreted IgG/ membrane bound IgM*) is present in lane 1 of the protein gel?

ii. Identify the B cell-type (*mature/ memory/ plasma cells*) that is responsible for producing antibodies shown in…

- Lane 1 of the protein gel. plasma cells
- Lane 2 of the protein gel. mature B cells

iii. If you compare the structure of the IgM and IgG antibodies that are produced against Protein R…

- would you expect these antibodies to have the **same or different <u>variable</u>** regions? **Circle** the correct option and **explain** why you selected this option.

Same – same among all types so recognizes R 1/1

- would you expect these antibodies to have the **same or different <u>constant</u>** regions? **Circle** the correct option and **explain** why you selected this option.

different – so have different functions

Name_____ Section_____ TA_____

Question 4 continued

f) Complete the table for the following cell types.

Cell types	Cell-surface proteins participating in the cell-cell interactions *(CD4/ CD8/MHC-I/ MHC-II/TcR/antibody)*	Briefly describe their role in the humoral immune response
T_H cells	TcR CD4	Antigen presenting cell present antigen peptide to T_H cell which becaos active + divides into memory T_h and activated T_h cells
Antigen presenting cells (APC) Professional	MHC 2 MHC1- others	Grab antigens Present antigen to T_h
Macrophages	MCH 2	A possible antigen presenting cell

g) All somatic cells types in our body are said to have the same set of genes although each cell type may express a unique set of genes that provides it with unique functions. However, the genome of the mature T and the B cells is slightly different compared to the genome of the remaining cells in an individual. **Explain** why this may be so.

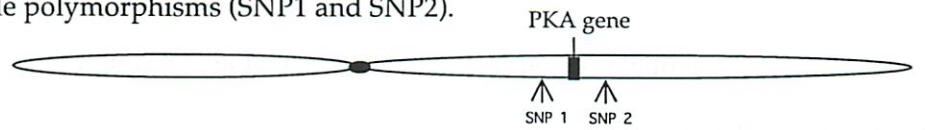
The variable region is different
V (D) J stats w/ many combos
Are recombined
→ Delete some genes in the middle during recombination
Fusion is sloppy

Solution Key- 2012 7.012 Problem Set 5

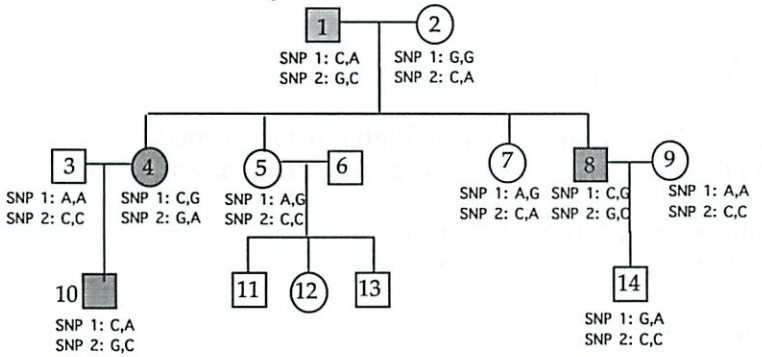
Question 1

A single nucleotide polymorphism (SNP) is a DNA sequence variation occurring when a single base pair in the genome differs among members of a species or paired chromosomes in an individual. By convention this base pair change is represented as one nucleotide — A, T, C, or G — of the base pair.

The chromosomal position of the PKA gene is diagrammed below, as is the location of two single nucleotide polymorphisms (SNP1 and SNP2).



The pedigree shows the inheritance of SNP1 and SNP2 associated with the PKA gene. The affected individuals are shaded. Also listed are the alleles of SNP 1 and 2 for some individuals. ***Note:*** *Assume individual 3 does not carry the disease allele.*



The two letters identify the alleles of the SNP that would be found on the "top" strand of each of the two homologous chromosomes. For example, "SNP 1: C,A" indicates that on one of the homologous chromosomes the top strand would contain a C (that is, the chromosome would have a C/G base pair in this position), while on the other chromosome the top strand would contain an A (that is, the chromosome would have an A/T base pair in this position).

a) What is the most likely **mode of inheritance** for this disease? *Autosomal dominant*

b) Individuals 5 and 6 have no affected children. What is the **genotype** for individual 6 at the PKA locus?

Genotype of #6 is "aa"

c) Individuals 5 and 6 have no affected children. Can you predict the genotype for individual 6 at SNP 1 and SNP2 loci (*Yes/ No*)? **Explain** why you selected this option.

No you cannot predict the genotype of individual 6 at SNP1 and SNP2 since this individual is coming into the family and hence may have a SNP genotype that is different from the individuals in this family.

d) Which allele (or alleles) of SNP 1 and SNP2 is linked to the mutant PKA gene in this family? *Note: Assume no recombination.*

SNP1: *C* **SNP2:** *G*

e) Briefly describe how you can use the SNP microarrays to determine the SNP genotype of an Individual.

DNA microarrays are small, solid supports onto which the oligonucleotides that represent the known SNPs in human genome are immobilized, or attached, at fixed locations. The supports themselves are usually glass microscope slides, but can also be silicon chips or nylon membranes. The DNA is printed, spotted, or actually synthesized directly onto the solid support. On the micro arrays the fluorescent- tagged genomic sample of interest in layered and allowed to hybridize with the oligonucleotides that have complementary sequence. The arrays are read through a laser detector to identify the SNP genotype of the individual test sample.

Question 2

a) Circle **all** the correct options from below. The resting membrane potential of a neuron is determined by…

i. ions that can travel freely through channels in the resting neuron

ii. ions that require ATP to cross the resting membrane

iii. unequal distribution of different ions across the neuronal membrane

b) Circle **all** that apply. An action potential is generated by the passage of ions through…

i. **only** the resting ion channels

ii. voltage-gated ion channels

iii. G-protein coupled receptors

iv. **only** the sodium potassium ATPase pump

c) Under resting conditions the Na^+, $Ca^{2+}$and Cl^- concentrations are high outside the neuron, K^+ concentration is high inside and this is maintained by the action of specific channels and pumps.

i. What feature of the plasma membrane prevents the free diffusion of ions across it?
It is hydrophobic and therefore prevents the free diffusion of ions across it.

ii. Is the resting membrane potential observed exclusively in a neuron?
It is a feature of all cells.

iii. Complete the following table for the two channels/pumps that establish and maintain the resting membrane potential.

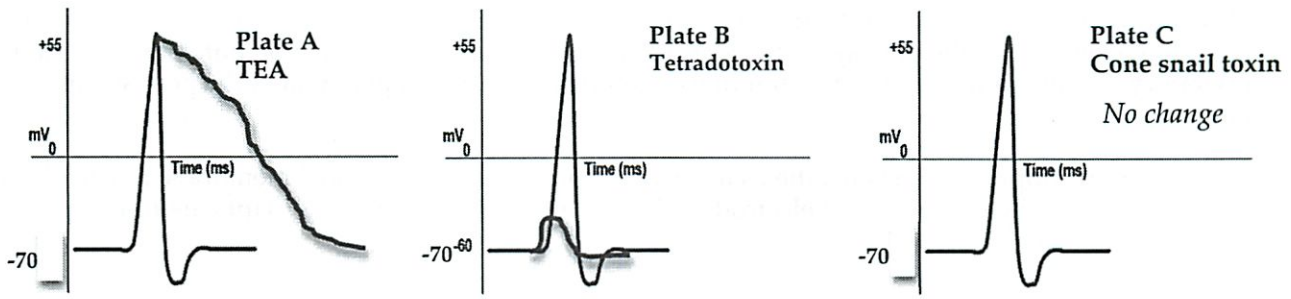
Channels/pumps	**Ions passing through them**	**Default state (open/closed).**	**Is the ion transport active or passive? Explain.**
Open K+ channels	*K+*	*Open*	*The ion transport is passive since the K+ ions move from the inside of the cell (region of higher concentration) to the outside of the cell (region of lower concentration) down their concentration gradient*
Na+/ K+ ATPAse pump	*Na+ and K+*	*Open*	*It is active since both the Na+ and K+ ions move against their concentration gradient and this requires energy in the form of ATP.*

Question 2 continued

d) Different neurotoxins are very often used to study neuronal function. You culture a GABA (an inhibitory neurotransmitter) secreting neuron in the presence of the following neurotoxins in three separate petri-plates (A-C)

- **A:** Neuron is treated with tetraethylammonium (TEA), which **inhibits voltage gated K^+ channels.**
- **B:** Neuron is treated with tetradotoxin, which **inhibits voltage gated Na^+ channels.**
- **C:** Neuron is treated with cone snail neurotoxin, which **inhibits voltage gated Ca^{++} channels.**

A normal action potential in a GABA secreting neuron that has been stimulated **in the absence of any neurotoxin** has been drawn in each panel below. Sketch the alteration in action potential following the treatment of the neuron with each neurotoxin. *Note: If there is no change please write "NO CHANGE" on the graph.*



e) Multiple sclerosis is an autoimmune disorder in which the immune system of the patient attacks and destroys the myelin sheath of a neuron. These patients show a very slow propagation of action potentials along the axons of their neurons. **Explain** why is this so.

The Na+ ions can leak out in the absence of insulation provided by the sheath. This results in slow conduction of impulse along the length of axons i.e. slow action potentials.

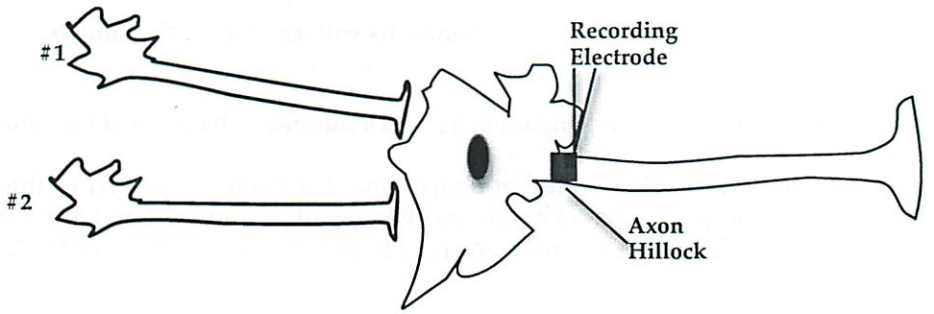
f) A functional neuron may receive both excitatory and inhibitory signals from multiple neurons at the synaptic junctions. In a post- synaptic neuron, where are the signals from all the pre- synaptic excitatory or inhibitory synapses integrated and the decision to fire an action potential made? **Circle** the correct option from the following choices. **Explain** why you selected this option.

Cell Body *Axon Hillock* *Myelin Sheath* *Synaptic Cleft*

The voltage gated Na+ channels are responsible for the depolarization phase of the action potential. These channels are not found in the cell body or the dendrites of the neuron. Instead they are located along the entire length of the axon starting from axon hillock.

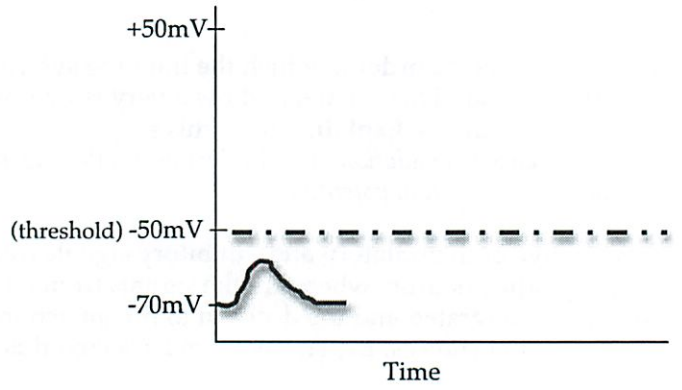
Question 2 continued

g) The following question refers to an experimental design depicted below. There are **two excitatory pre-synaptic neurons** that independently converge on a post-synaptic neuron. The two pre-synaptic neurons can be stimulated individually. In the absence of any stimulation, the recording electrode in the post-synaptic neuron measures the membrane potential as -70mV.

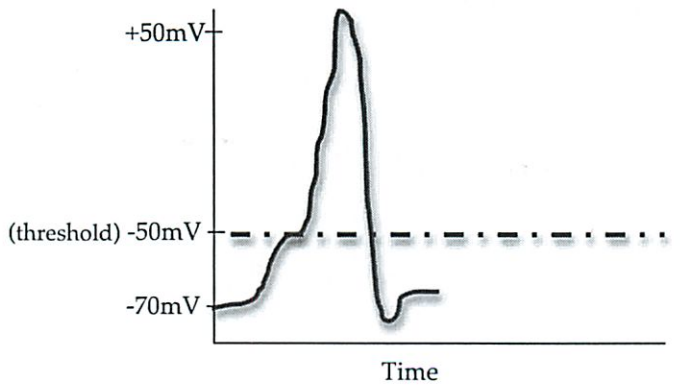


If **only one** excitatory pre-synaptic neuron is stimulated, you record a deviation from -70mV with the recording electrode in the post-synaptic neuron, but you do not record an action potential. If **both** the excitatory pre-synaptic neurons are stimulated, you record an action potential in the post-synaptic neuron.

i. On the graph below sketch the changes in the post-synaptic neuronal membrane potential, as measured by the recording electrode, when **only one** excitatory pre-synaptic neuron is stimulated.



ii. On the graph below sketch the changes in the post-synaptic neuronal membrane potential when **both** the pre-synaptic neurons are stimulated.



Question 3

Dopamine is one of the neurotransmitters in the mammalian brain that regulates mood, cognition and locomotion. Dopamine is commonly associated with the reward system of the brain. Dopamine can be an excitatory or inhibitory neurotransmitter, depending on the dopaminergic receptor that it binds to. It is derived from the amino acid tyrosine. At dopaminergic synapses, the released neurotransmitter is taken back into the pre-synaptic cell for re-use.

a) Beginning with the stimulation of the pre-synaptic neuron, list the steps, in order, that result in a release of neurotransmitter. Include any relevant channels, ions and molecules specific for this process.
Stimulation of the pre-synaptic neuron generates an action potential, which travels along the length of axon to reach the axon terminus. The depolarization of the membrane at the axon terminus activates the voltage gated Ca^{2+} channels through which the Ca^{2+} ions flow from outside to inside. These Ca^{2+} ions bind to and activate Ca^{2+} dependent kinases, which phosphorylate the synapsin protein present on the surface of vesicles containing neurotransmitters. This triggers the fusion of vesicles with the axon terminus membrane, causing a release of the neurotransmitter into the synaptic cleft.

b) Clozapine, an anti- psychotic medication interferes with the binding of dopamine to the receptor. You are studying an excitatory dopaminergic synapse where the post-synaptic cell has receptors for dopamine. If you treat these neurons with dopamine plus Clozapine, would the likelihood of generating an action potential in the post- synaptic neuron increase, decrease or not change relative to the treatment with dopamine alone? **Explain.**
The binding of dopamine to D2 receptors has an excitatory effect. Treatment of the neuron with clozapine will interfere with dopamine binding and decrease the likelihood of an action potential in the post-synaptic neuron.

c) The most extensively studied effect of cocaine on the central nervous system is the blockade of the the protein that binds to dopamine and pumps it out of the synaptic cleft back into the pre-synaptic neuron. What effect would cocaine have at an excitatory dopaminergic synapse?
Cocaine will have an excitatory effect on the post-synaptic neuron since it prevents the re-uptake of dopamine. So dopamine can stay in the synaptic cleft for a longer time period, it can bind to the dopamine receptors located on the cell body of the post synaptic neuron to cause an excitatory effect on the postsynaptic neuron.

d) Serotonin (5-hydroxytryptamine, 5-HT) is an excitatory neurotransmitter. It acts by binding to several HT receptor subtypes. The 5-HT3 receptor is a Na^{+} channel whereas the 5-HT-2 receptor is a G-protein-coupled receptor, which leads to the opening of Ca^{2+} ion channels.

i. As the amount of serotonin is increased, **circle** the option that may change: *Amplitude of action potential/ frequency of action potential/ threshold potential.* Provide an **explanation** for the option that you have circled.
Frequency of action potential may increase with an increase in the amount and duration of the stimulus unlike the amplitude of action potential and threshold potential, which are always constant.

ii. Complete the following table for each of the treatments. Assume that serotonin is present at these synapses. *Note: Consider each treatment independently.*

Treatment	**Action potential in the post-synaptic neuron is *more likely or less likely* to occur compared to untreated synapses? Explain your choice.**
Prozac, which inhibits the re-uptake of serotonin from the synapse	*More likely. Serotonin will be available for a longer period to bind to the receptors located on the surface of the post-synaptic neuron.*
Kentasarin blocks the binding of 5-HT3 receptor to 5-HT	*Less likely. Receptor will not be available to bind to serotonin*

Question 4

a) The immune system is comprised of different cell types such as the *mast cells, macrophages, helper-T (T_H), cytotoxic-T (T_C), memory B and plasma B cells*. From the choices provided, list **all** the cell type(s) that would…

i. Participate in the **innate immune response**. *Mast cells & macrophages*

ii. Bind directly to an **antigen circulating** in the blood stream. *Memory B cells*

iii. Secrete large amount of antibody in response to an infection. *Plasma B cells*

iv. Provide **protective immunity** against **second exposure** to the **same antigen**. *Memory B& T_H*

v. Show rearrangement of specific gene(s). *Memory and plasma B cells, T_C and T_H cells.*

b) The diverse array of both TcR and antibodies is generated by DNA rearrangement. In addition to the DNA rearrangement, name the **three major processes** that contribute to the generation of the TcR and antibody diversity.

1.Somatic hypermutations, which affect the variable regions of the gene.
2 .Terminal transferase activity which results in adding nucleotides to the V, J and D joining points thus producing junctional; diversity.
3. Alternative splicing

c) Circle **all** correct options from the following choices. The **innate immune response**…

i. Occurs **only** following the **first exposure to an antigen.**

ii. Occurs **only** following the **subsequent exposure the same antigen.**

iii. Occurs in response to **all infections**.

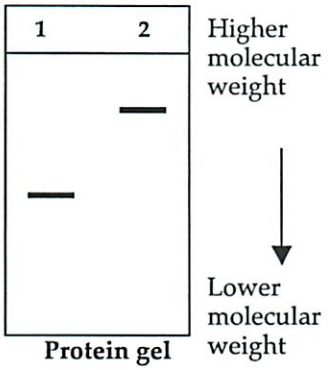
iv. Is **non-specific** unlike the adaptive immune response.

d) You have purified a novel protein, which you call **Protein R.** You want to develop antibodies against this protein. You inject Protein R into a rabbit and after a month you draw some blood from this rabbit and determine that the rabbit's immune system has produced antibodies against Protein R. You wait for one month and then inject Protein R again into the same rabbit. You observe a stronger immune response with the second injection than you did in response to the first injection. Why is the primary immune response **slower** and **weaker** compared to the secondary immune response?

During the primary immune response, the memory B cells, against the specific antigen, are generated. These B cells express surface IgM molecules against the specific antigen. Furthermore, they can also proliferate to form more memory B cells and plasma cells that produce the IgG antibody to counteract the antigen infection. During the secondary immune response, the memory B cells generated during the primary immune response immediately start proliferating to generate more of their own kind and also plasma cells to counteract the viral infection thus making the response faster and stronger compared to primary response.

Question 4 continued

e) During the primary and secondary response to an antigen, the B cells of the immune system **produce membrane bound (i.e. IgM class)** and **secreted (i.e. IgG) antibodies**. You isolate the antibodies produced against Protein R, resolve them on a **protein gel** based on their molecular weight and obtain a profile as shown in the schematic below.



i. Which class of antibodies (*secreted IgG/ membrane bound IgM*) is present in lane 1 of the protein gel?
Secreted IgG antibody

ii. Identify the B cell-type (*mature/ memory/ plasma cells*) that is responsible for producing antibodies shown in...
- Lane 1 of the protein gel. *Plasma B cells*
- Lane 2 of the protein gel. *Mature & memory B cells*

iii. If you compare the structure of the IgM and IgG antibodies that are produced against Protein R...

- would you expect these antibodies to have the **same or different <u>variable</u>** regions? **Circle** the correct option and **explain** why you selected this option.

They will have the same variable regions both for the heavy and light chains which will join together to form the same antigen binding site that binds to the antigen.

- would you expect these antibodies to have the **same or different <u>constant</u>** regions? **Circle** the correct option and **explain** why you selected this option.

They will have different constant regions since these regions are different in different class of antibodies and are added by class switching. The constant region of IgM will allow it to be a membrane protein whereas those of IgG will allow this to be a secreted protein.

f) Complete the table for the following cell types.

Cell types	**Cell-surface proteins participating in the cell-cell interactions** *(CD4/ CD8/MHC-I/ MHC-II/TcR/antibody)*	**Briefly describe their role in the humoral immune response**
T_H cells	*CD4 and TcR*	*Recognize the antigen presented by the APC through MHC-II molecules. Secrete cytokines that are immuno- modulatory molecules and promote the proliferation of the memory B cells specific for an antigen.*
Antigen presenting cells (APC)	*MHCII*	*Internalize and process the antigen and present processed antigenic fragments on thir surface through MHC-II so that they are recognized by specific T_H cells.*
Macrophages	*Tail or Fc portion of the antibody molecule bound to the antigen.*	*Function as an APC. Engulf and degrade the antigen that is coated by the IgG molecules secreted by the plasma B cells.*

g) All somatic cells types in our body are said to have the same set of genes although each cell type may express a unique set of genes that provides it with unique functions. However, the genome of the mature T and the B cells is slightly different compared to the genome of the remaining cells in an individual. **Explain** why this may be so.

The TcR gene in the T cells and the Antibody / Immunoglobulin gene in the B cells have undergone DNA rearrangement which accounts for why their genome is different compared to the genome of the other somatic cells in the same individual.

7.012
Recitation

11/8

(3 min late)

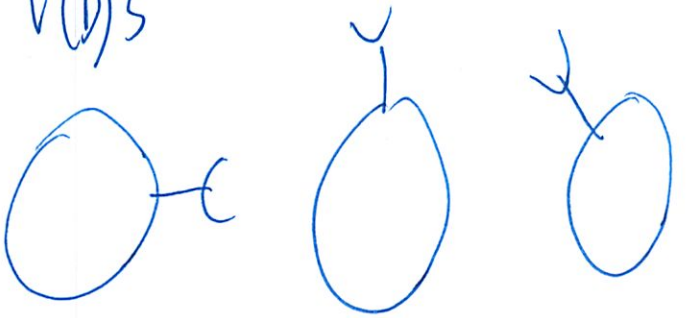
~~[illegible]~~ (wrong class)

V(D)J Recomb
VDJ heavy chain
VJ light chain

immature B-cell

↓

V(D)J

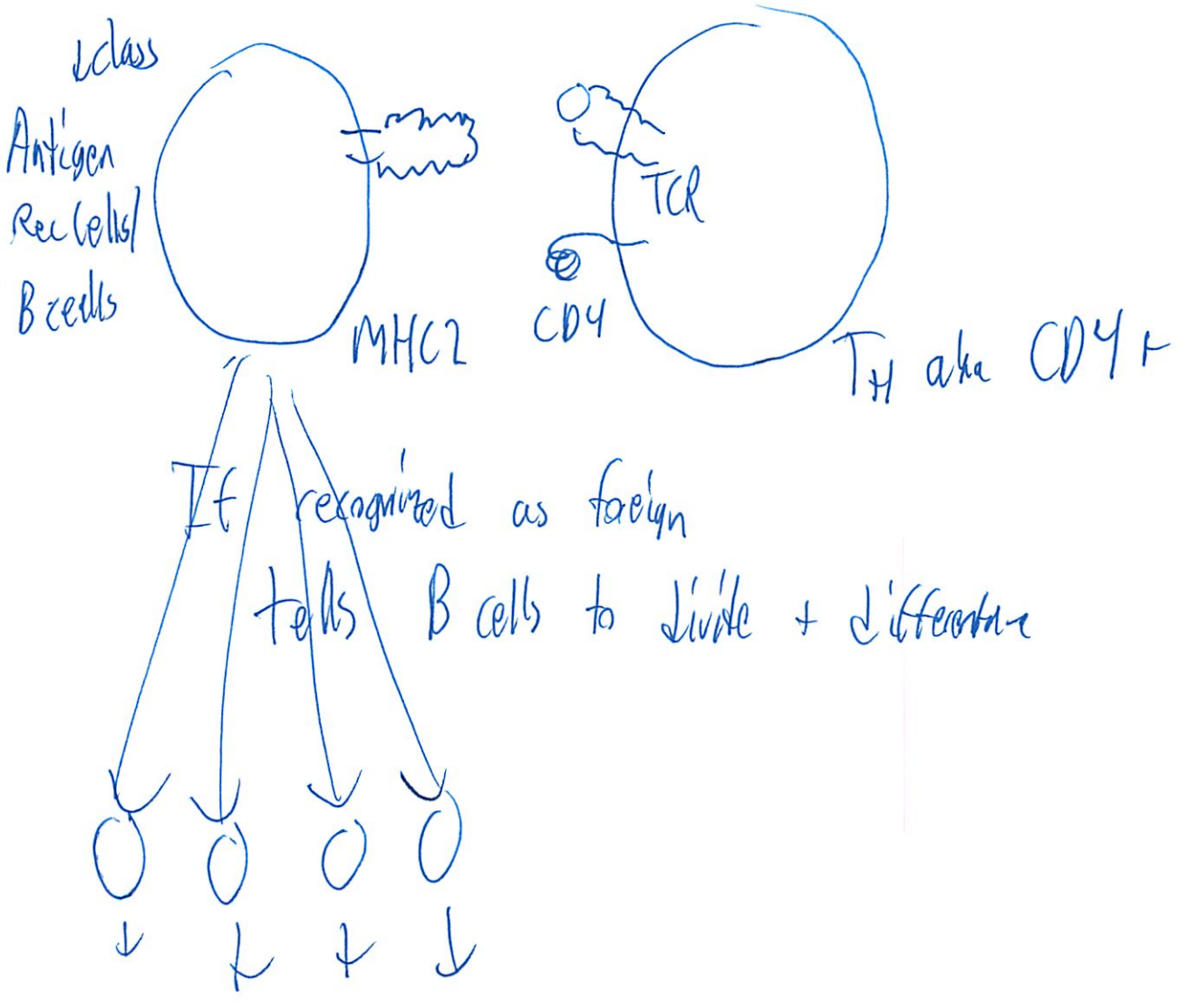


each recognizes a specific antigen

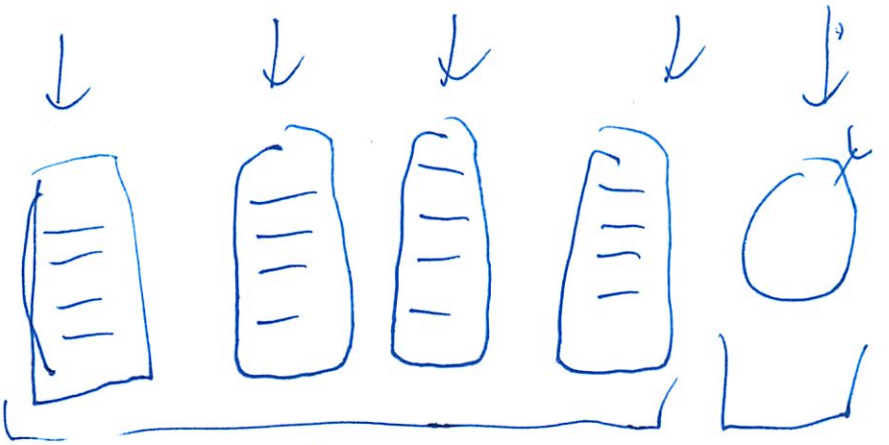
mature/naive
lymph nodes
↑ location in body
↳ under chin, arms, legs
vessels / bumps

(2)

Then presenter makes MHC 2 poke out of layer



(3)



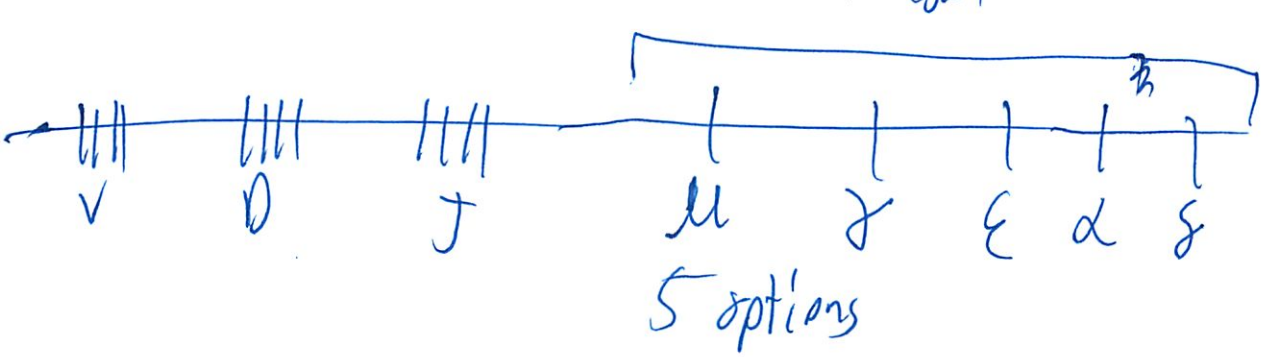
Lines = antiplasmic reticulum = makes plenty of secreting

plasma B cells
tons of them
Can't keep
no space in blood + lymph nodes

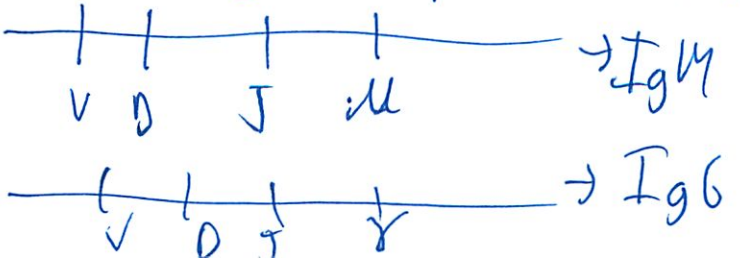
memory B cells

Control/activation by T cell is required

Classes of antibodies



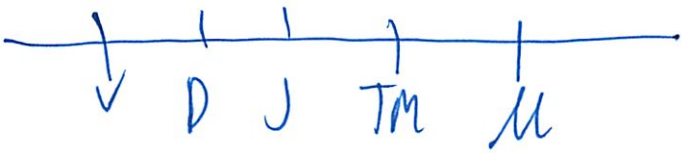
alternate splicing - only take L chain



(4)

Region in membrane must be bilipid / hydrophobic
"transmembrane"

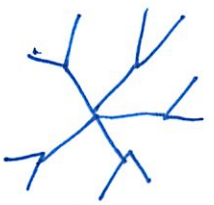
In original splice in TransMembrane
So it can be stuck in the region in IgM



Cells almost always make IgM

So IgG is slightly shorter than IgM

Secreted as a pentone

IgM 

IgG 

(5)

If mutation so non functional
Body will kill them

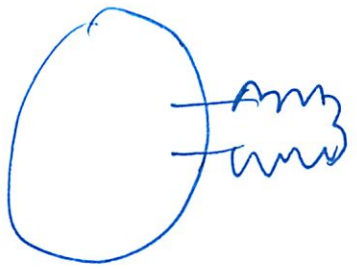
Clonal deletion
if self recognition happens

How does T cell know that is a foign peptide
T-Helper activated
Then goes to find B cell

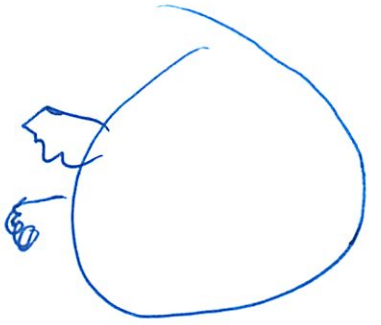
Macrophage engulfs bacteria
breaks it up
inside a phagosome

puts peptides on MHC II

6



MΦ = Macrophage



Inactive T_H Cell

Antigen Presenting Cells

- B cells
- MΦ
- DC

} MHC II

form complex if binding

This will also divide

get active T_H and memory cells

This finds B cells

- IgM as Antibody Receptorer – has TM — can also be secreted as Pentamer
- IgE Worm infections

IgA dymer can pass through stomach lining

IgD mothers milk + placenta

Change for what ya want

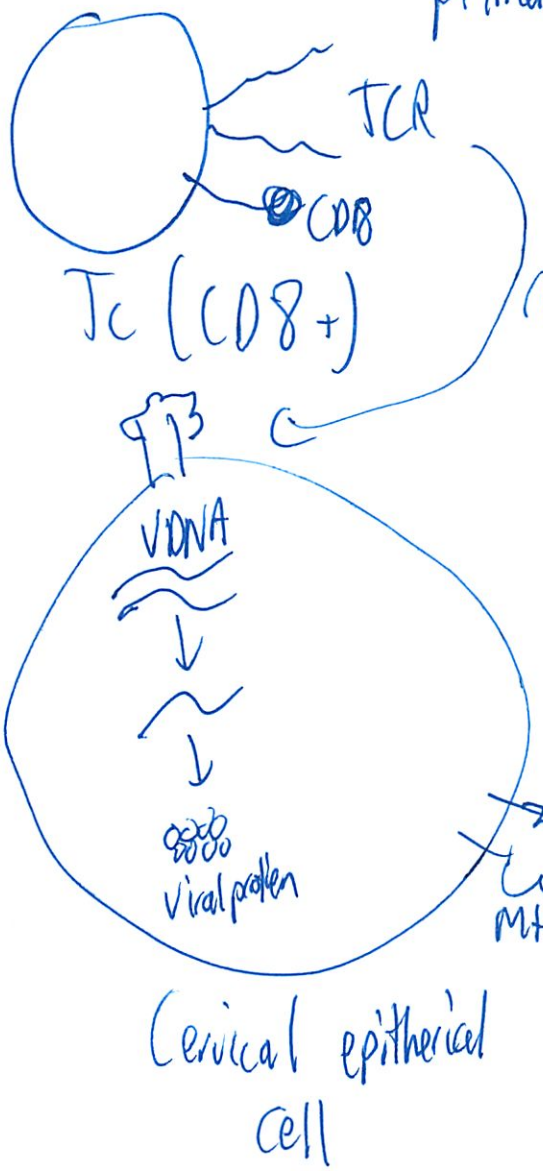
Or where infection is

during splicing process of DNA

6

Cell Mediated Immunity

primarily for viral infections



Whatever protien cell makes
Piece of it is shown on MHC I
Constantly happening - up to 30%
of cell's protien

takle intercelle infections
MHC I in all

TCR Under go recombination

So clonal deletion to remove body (non foreign) cells

L in thymus

expos every cell

if a T cell recognizes self
They are killed

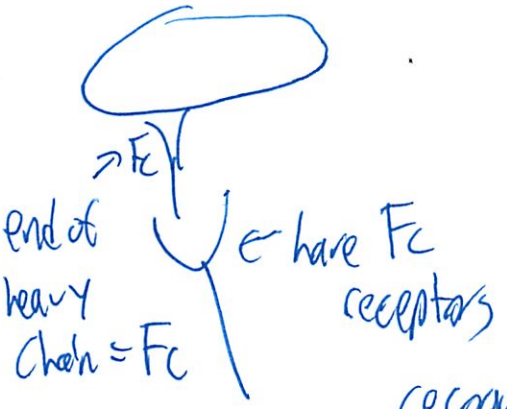
Humoral vs Cell mediated

Could go after some infections

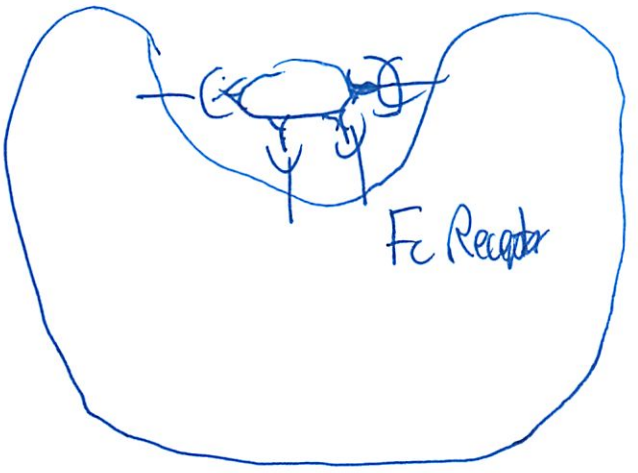
Antibody Could cause { neutralize
Opsonize - marks for fagocite to come + distroy
toxic Complements (activates)

9

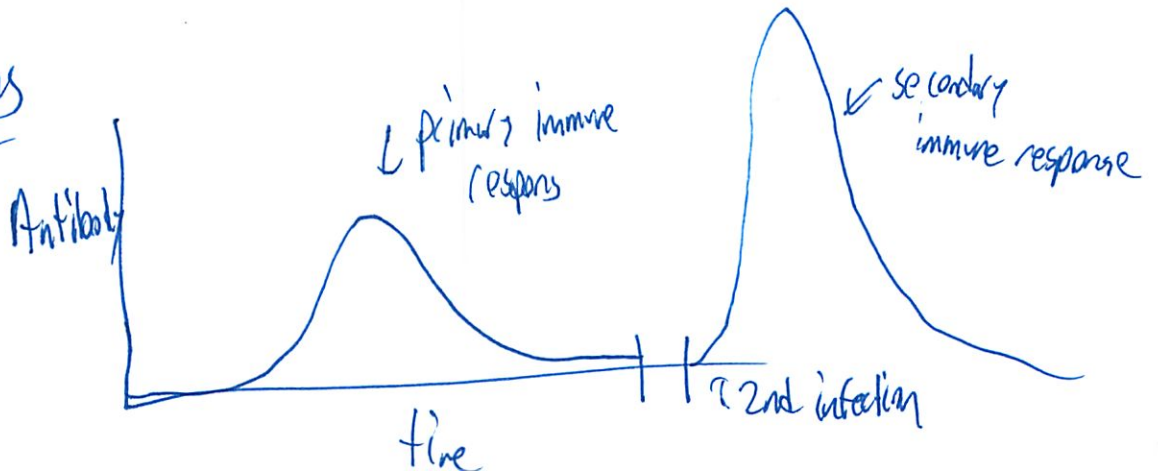
Fabosite normally eats anything it can find



recognizes Antibody bound
So easier for fabosite to eat



Infections



(10)

Almost immediate

No lag

Sign of memory + specificity

Chicken pox

everything ya have vaccinations for

polio

Vaccinations

diff types

- live, weakend ← humoral and cell mediated but 10% chance remote back
- dead agent
- protien ← weaker HPV) humoral -

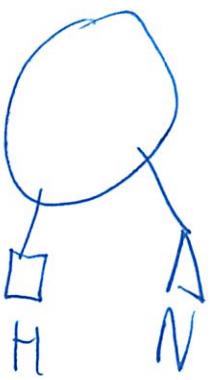
Cell mediated - must have protien production

(11)

flu is fast changing agent

Series of proteins it ships over + over

based on global patterns



Transplants

What needs to match?

tolerance of donated molecules

MHC I

TCR has not been taught to not recognize this

Thymus has not been updated

(12)

Doctors look for MHC match

Look at genome

Identical twins ✓

Parent → Child ✓

Child → Parent ✗ ~~threshold~~

has half MHC from other patient

Lots of alt splicing

Want someone as close as possible

Infections

Malaria infects red blood cells

don't have MHC.I since no nucleus

So hard to immune recognition

Colera affects stomach epithelial cells

kills host so fast -> can't generate a response

(23)

Turbolcous — ? recent

takes out [illegible] respotory

Small pox

Skin cells

HIV

Helper T cells

Auto Immune Diseases

Thymus does not work properly

7.012 Recitation 15 - 2012

Main concepts from Lectures 22-24

The immune system protects the body from foreign entities that have invaded it, such as bacteria and viruses. It does so by making proteins called antibodies, which recognize and direct an attack against foreign entities, which are often called antigens. A person's body produces billions of different antibodies. Many of these randomly generated antibodies have the potential to recognize proteins that are made in one's own body. The immune system however has a way of distinguishing antibodies that act against "self" antigens from that against "non-self / foreign" antigens. It does so by destroying or preventing the proliferation of any antibody-producing cell that recognizes a self-made antigen.

Our bodies make billions of different antibodies. These are proteins and thus are encoded by genes. However, our genomes contain less than 30,000 genes. So it is not possible that we would have a different gene to encode for each different antibody that we generate. The explanation for this is that there is a cluster of segments of genes (called V, D, and J segments) in the section of the genome that encodes antibodies. Every antibody-producing cell rearranges these DNA segments to join one V segment to one D segment to one J segment, thereby creating one gene that makes one antibody. Every different antibody-producing cell rearranges this cluster of DNA segments differently. Thus all antibody-producing cells contain only one gene that encodes an antibody, but every cell contains a different arrangement of that gene thus leading to antibody diversity. Other than cells of immune system, every cell in your body contains the exact same DNA as every other cell because no such rearrangement occurs.

The immune system provides humoral and innate immunity. There are three important cell types that play critical roles in the humoral arm of the immune system – phagocytic cells, B cells and helper T cells (T_H). When a phagocytic cell encounters a foreign particle in the body, it eats that particle and displays pieces of the particle on its surface using the cell-surface display protein MHC class II. A T_H cell has a protein on its cell surface called T cell receptor (TCR). Every T_H cell has a different TCR that recognizes a different antigen (because every T_H cell rearranges the TCR gene in the genome differently, in a mechanism similar to VDJ joining). If a T_H cell encounters a phagocytic cell displaying the antigen recognized by its own TCR, then that T_H cell undergoes clonal expansion. The many T_H cells of this kind then go searching for a B cell that recognizes the same antigen as that T_H cell. If the T_H cells find the right B cell, then that B cell undergoes a clonal expansion. These B cells make secreted antibody that recognizes and attack the foreign antigen.

The immune system displays memory; we know this because the second time the immune system encounters an antigen, the response of B cells and T cells is faster and stronger. The principle behind vaccination is to expose an organism to some of the antigens of a harmful foreign particle that will spark the immune system's memory in case the actual entire foreign particle ever invades that organism.

Another type of immunity is the innate immune response. This response is however not antigen – specific and does not generate immune memory. This commences immediately upon pathogen entry and involves phagocytes and cytotoxic T cells (Tc). If the phagocytes cannot rapidly eliminate pathogen, inflammation is induced with the synthesis of cytokines and acute phase proteins.

Questions:

1. Viral infections can be treated using anti-viral drugs, or they can be prevented in the first place through the use of vaccines. Some vaccines are just injections of viral particles that have been inactivated in some way (such as extreme heat). Other vaccines are injections of a single viral protein that has been purified and produced using recombinant DNA techniques. How does a vaccine work?

2. Each one of your gametes contains 3 X 10^9 base pairs of DNA in its nucleus. How many base pairs of DNA are contained within:
a) Each nerve cell?
b) Each antibody-producing white blood cell?
c) Each red blood cell?

3. The cellular arm of the immune system employs cytotoxic T lymphocytes (Tc) and natural killer cells. The TC cells can recognize the viral infected body cells.

a) What proteins are involved in the presentation of the antigen to the Tc lymphocytes?

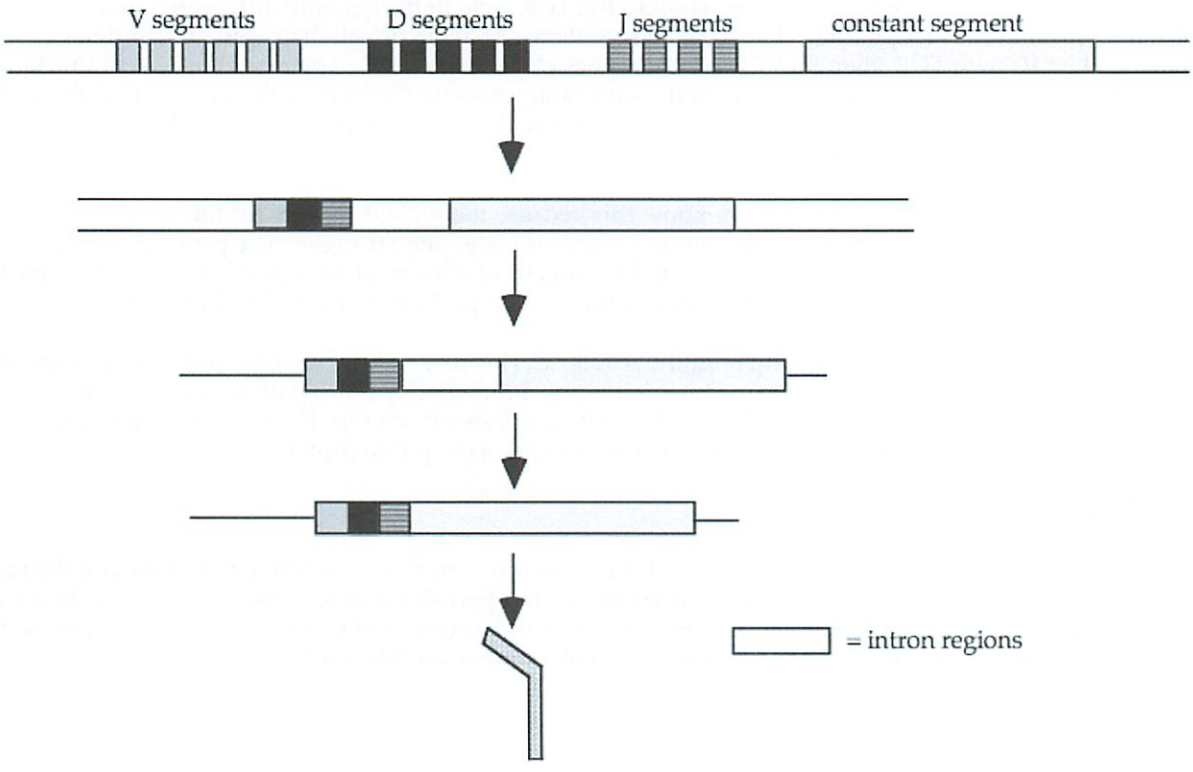
b) Explain why the Tc lymphocytes do not recognize an infected cell if the virus is latent i.e. viral capsid protein does not occur.

4. Shown below is a schematic of the production of a heavy chain polypeptide for an antibody. At the top is the chromosomal arrangement found in an immature B cell, at the bottom is shown the heavy chain polypeptide.

i. Label the process indicated by each arrow. Choose the one best option for each from:
homologous recombination , transcription , translation, translocation, ligation, DNA rearrangement, splicing

ii. Indicate on the diagram below where you would expect to find each of the following components:
Promoter (*), Transcriptional terminator (1), start codon (2), stop codon (3)

iii. Indicate on the diagram below the variable and the constant region of the heavy chain and the N and C terminus of this polypeptide.



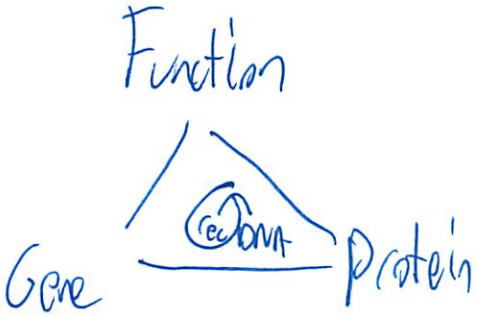
Lecture

Genomics

11/9

(watching video 11/14)

Last lecture on → (normally would have been earlier)



→ Big Picture: Genomics

before: individual processes + pathways

before: 5 years 1 gene

now: look at entire big picture at same time

Where do hyp come from?

└ from explatory review of lots of data

Still on going

Not in the textbooks

(2)

Contents of Human Genome

1990 HGP → seq 3 bill bases

——— ~50-100k bases

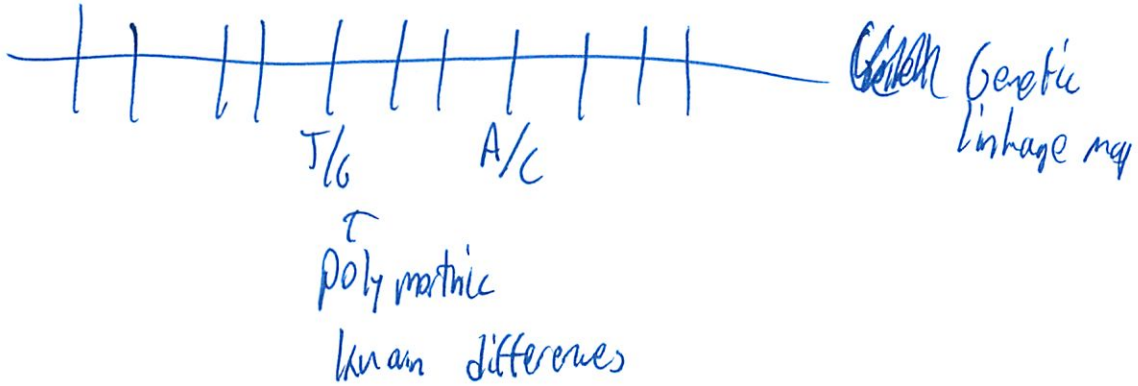
— — — ← Fragment sequence

↑ map back (genetic linkage mapping)

hierarchical / top down

Bio came together + set goals

Started w/ landmarks



(3)

Had the whole assembly line
flourcent sequencing

6 diff centies all doing in parallel
1/3 at MIT - largest single contributor

Finished announced 2003-2004

Contents of Genome

Facts circa 2000

Genes ~ 100k

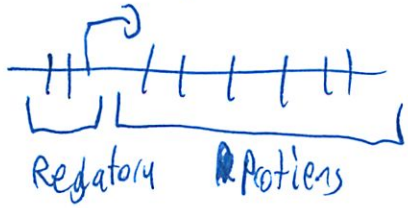
almost all genes encoded protiens

Very few RNA only (~10)

→ like tRNA, rRNA

weird exceptions

regulatory seq small compared to protien regions



(4)

littered w/ transposons

"jumping fragments" on evolutionary timeline

half of DNA ~50%

Called Junk, useless, parasites, selfish...

What is true today

~21,000 protien coding genes

Many can be cut up + rearranged

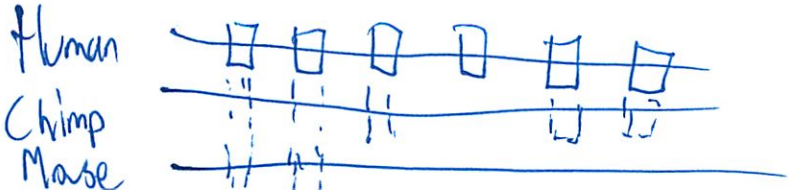
How do we get this #?

mRNA → cDNA → sequence

map back to genome

but how do we know we've found all the mRNAs?

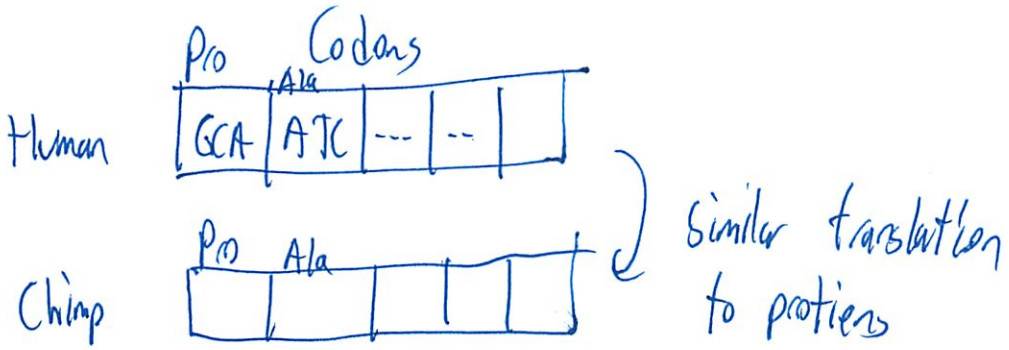
evolutionary comparison



(5)

Can line up bits and see if consistent w/ evolution

Steady stuff encodes protiens
- distinctive patterns



We diff 1½% from chimps

But do we expect a 1 bp deletion
No, it would screw up the protien

Howabat 3 bp deletion?
Is more common!
→ So know that lots of 3 bp deletions means it is probably encoding for protiens!

(6)

Intergenic DNA has lots of gaps
↳ So we expect its not protien

Looking for protien-like ~~replication~~ patterns

And we can confirm since we have the RNAs for those

How do we know not unique protiens in humans?
We know that has not really been the case
Power of evolutionary analysis

(He comments how can people not believe in evolution!)

Lots of RNA that didn't encode protiens
↳ 4,000 long intergenic non coding RNAs
lots of short non coding RNAs

RNAs - many nucleate cell protien + RNA complexes
like telomerase

(7)

RNA often functions importantly in the cell
many we don't understand well
Complex machinery

Regulation

exons + regulator

Completly wrong!

how much of genome is evolutionarily Conserved

Will find stretches of conversation

Some protien coding

but also many that are not protien coding

Some pieces very well Conserved

Can add up amt of conservation in human genome

but protien coding 1.5%

evolutionary conserved 6%

The majority of evolution conserved fragments are not protiens

almost shurly regulatory sequences

---

development seq have the most
↳ very important that these are right!

So regulatory seq conserved

We know a bunch of these non-protien Conserved
↳ learning what their functions are

When did these gene patterns arise?

Can have a chart about where things are Common and when things changed
(see slide)

Very few protien coding seq in last 60 mil years
Mostly new regulatory seq

* this is how we get diff animals
must look at whole genome to see

---

transposons

more favorable view today

Suppose wanted to regulate 10 genes

Could have evolution on all 10 elements

but that it develops in 1 gene and then it spreads through genome

(proto viral)

always moving DNA around genomes

evolution selects

So important distributions of innovation

most are still useless though

(10)

We can see new seq have arrived via a transposon & have some distinctive pattern

Signatures (sig)

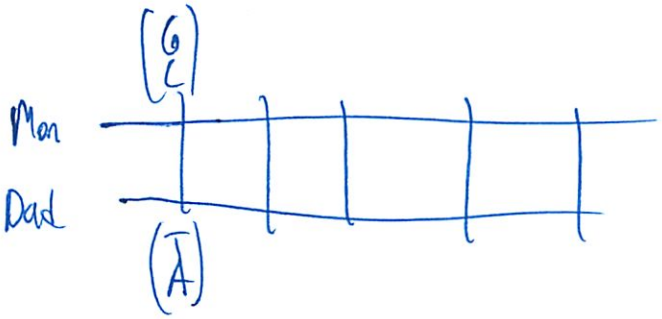
Very new view

Can use traces to see pattern of evolutionary change

So can see when transposons diverged

Human Genetic Variation

Humans differ by 1/1000 bases



? 1/1000 chance different

So 3 mill differences

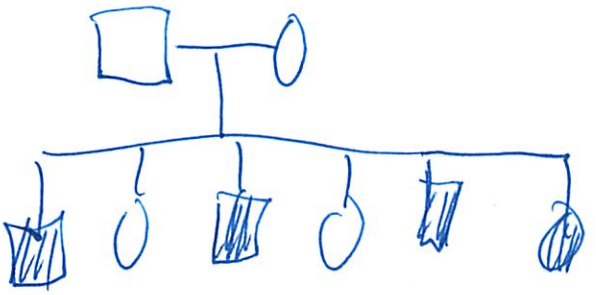
↳ where heterozygous sites

(11)

So easy to trace inheritance for family

Can trace these genetic markers

Useful for simple mendelian diseases



Many diff mutations

tracing the difference in families

Same linkage, but specific mutation diff

(12)

Complex polygenic diseases

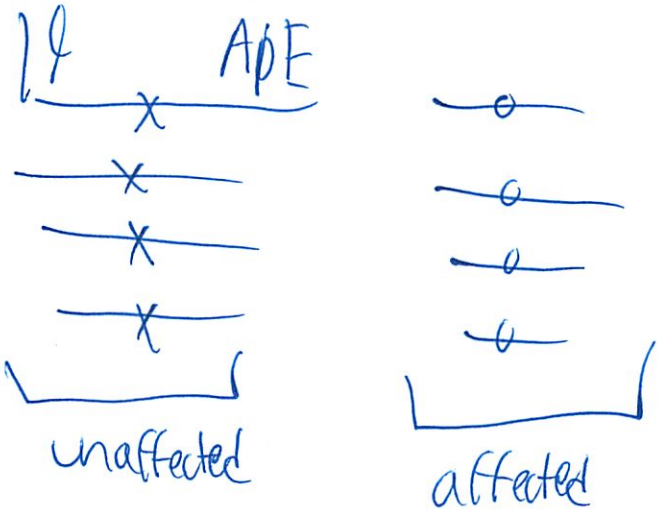
rather than linkage approach

could be that 3 mill diffs is cause of disease

just look at affected vs unaffected people

↳ not a family

look at differences



but never that nice

just a higher risk of

50% vs 53%

↳ but big if large denominator

(13)

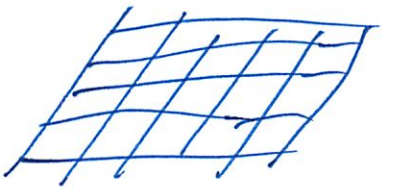
See what is correlated w/ what disease

- tons of examples
- Mapping by association/correlation using common genetic variants in the population
  - might need 5-10 mil genetic diffs to see
  - but this is possible today!
- oligonucleotides on DNA chips (Affy)
  See which spots light up
  each spot (5 mill) has diff probes
- ~~tons~~ 1,500 genes have been found w/ correlation of risks of diseases!
- ~~can also use for RNA~~ expression

(14)

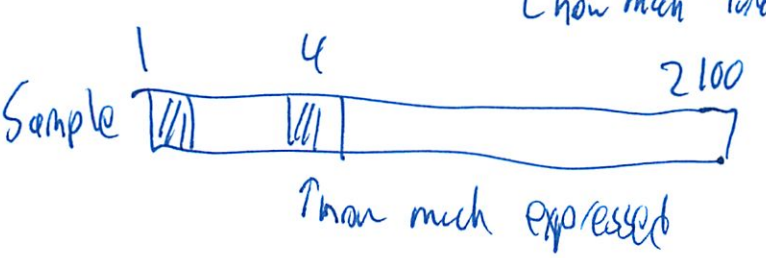
RNA Variation / Expression

Chip that reads RNA



Wash your RNA over it

Can measure how much RNA from each gene



Have a len 21000 vector of your expression of

If find different cancers will show up diff on chips

(even though ~~Strata 2~~ look similar in microscope)

(15)

Which genes turned on

Takes 3 mins for PC to say diff

It's the big picture that matters!

Important for finding patterns!

7.012
Development

11/14

(1 min late) - growth + proliferation
- differentiation

Progressive Differentiation

Commitment to a fate
how reversable is this?
progressive

Gene expression array
Cells from diff tisses vs diff genes
how a gene is transcribed

Analyze tissue w/ what genes they expressed

house keeping genes expressed everywhere
basic hw of all cell types in the body

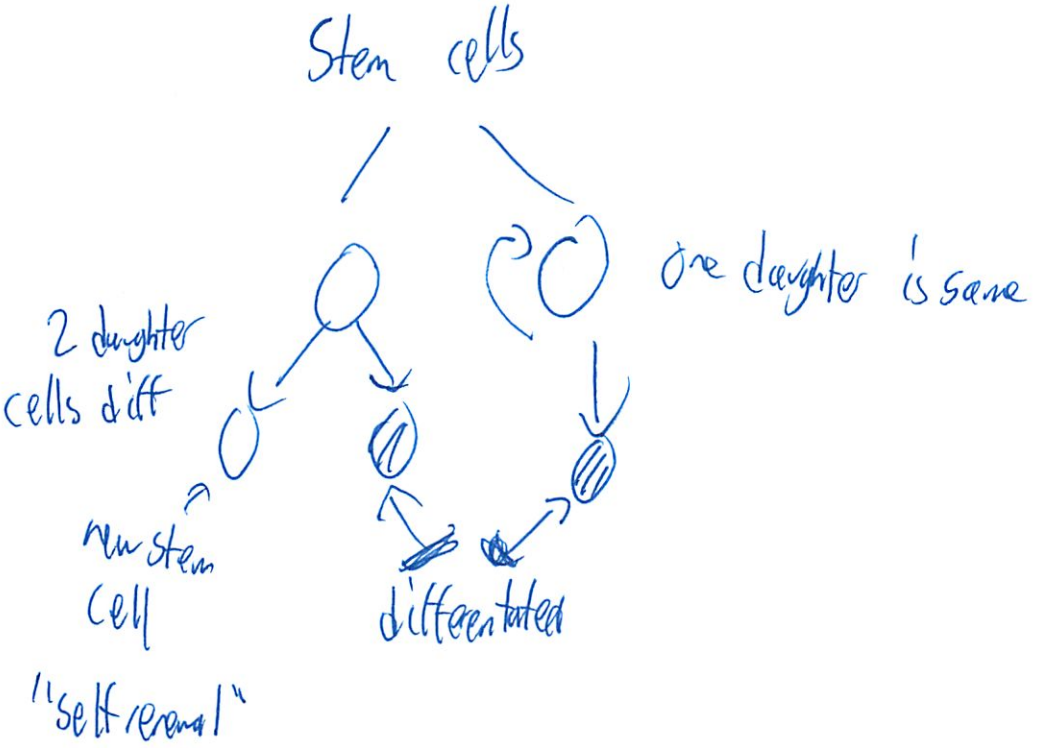
2

1000 specialized genes
mostly the brain
and no wave else

Diff lung genes strongly expressed in some cases

Stem cells

adult



Then 2nd slide → more complicated version of this

(3)

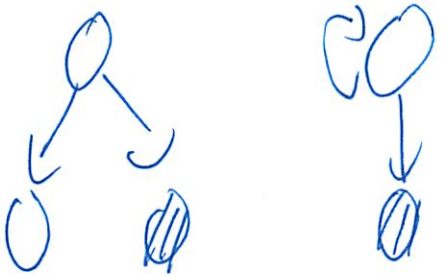
post-mitotic differentiated cells

Can't divide again

One division → large # of proginey
for stem cells

Progenator = transit-amplifying cells
around for a limited amt of time
may be part of the decision making progress

Asymmetic division



We don't really know why
Divides in certain place → Stem cell niche

※ contextual signals matter

(4)

Sometimes can divide symetrically

esp early on in org's life

Need a bunch of stem cells

two essential properties of stem cells

Cell can have distinct alt. differentiated progeny

Stem cells → oligopotential

↳ can have

Pluripotent - can generate a variety of tissue layers

(more generalized than above)

* two keys

1. Can replicate themselves
2. Can differentiate

Some can split off further to more specialized cells ...

(5)

Mesenchymal stem cells generate connecting tissue
recruited through blood to wound sites
rebuild damaged tissue

Cells in bone marrow have decide what to do
through context

hematopoiesis = formation of blood

Self renewal in small intestine
Some 300 g of cells that fall off everyday

Stem cells at the bottom (in crypt)
At least 3 diff specialized stem cells that could be created

Why in crypt
progeny move up and at and to lumina
Only stem cells stay

Why are getting rid of those
lots of junk in intestine
Genome possibly being damaged

(6)

So constant stream of new cells

Wasting cells is not that much of a problem really

500 x more mitosis than cells in body at any 1 time

---

lose dead skin in shower

but body making new cells

Villae ↑ surface area, so can absorb nutrients

Large intestine → reasorb water in lumina of gut

So poly solid waste

Otherwise diarrea

if loose too much water → could die!

Crypt has mucins

protect stem cells from contents of lumina

---

we make 3 x 10^11 cells per day

(9)

Diagram of how cell is organized

All transgenic amplifying cells doing most of the work!

---

adenomatous polyposis coli (APC)

required to make the cells die

otherwise they would accumulate in the crypt

now cells can sit around + accumulate

↳ creates a polyp

individuals w/ mutant APC have lots of polyps

Can be a road to getting cancer

1/500 polips

Mormons in UT have a large genome records

and descended from a few ancestors

and have a lot of kids

(8)

Colon cancer

from eating fast food
Whole series of intermediary steps
1st mutation is loss of APC
Can accelerate by going to fast food every day

Mammary gland

Stem cell lines mammary glands

hair follicles stem cells

adult stem cells

pluripotent cells

not todipodent - (make everything)

Can make everything except the placenta

(9)

Embryonic Stem Cell Derivation

Early in embryo

Inner Cell Mass
Put in petri dish
Allow cells to differentiate themselves

In vitro - on glass
in vivo - on tissue

blastocyst early embryo

We can make ES cells from a black coated mouse
into white coated mouse
See what happens in uterus

Mouse has spots!
also in a bunch of other tissue
└ which we can't see

(10)

Cells responding to contextual signals

Participate as equals.

Chimera

- like the person w/ the body of a horse and the face of a man

7.012 Recitation

11/15

Missed / traveling

11/15

Samin Houshyar
Recitation 16
R27 & R28

I. Using Genomic Data to for Diagnosis and Treatment

II. Stem Cells

What is a stem cell?

Two properties define a stem cell: ability to renew self and generate new cell types

Types of division:

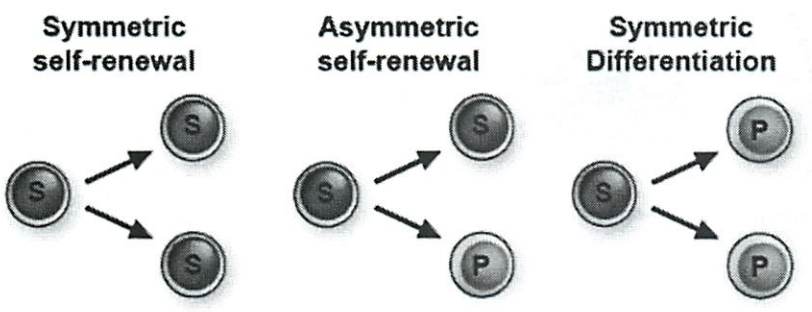
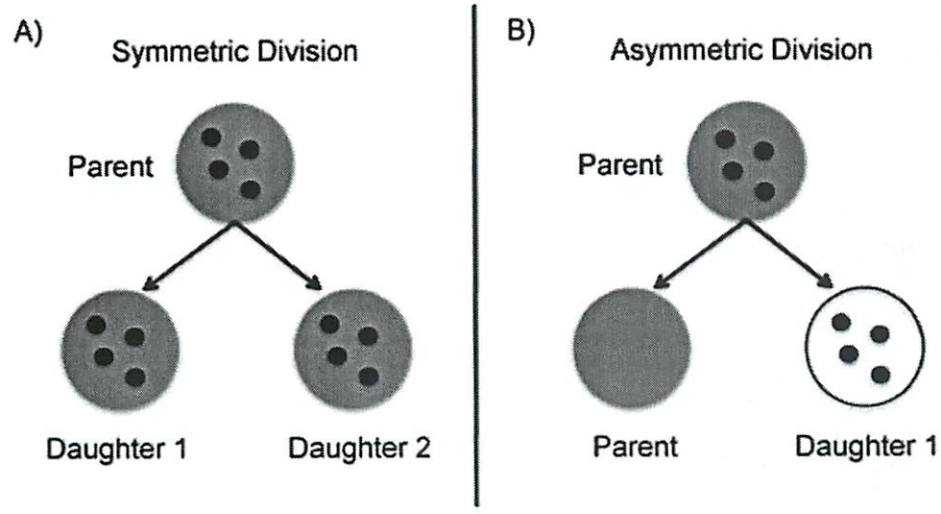


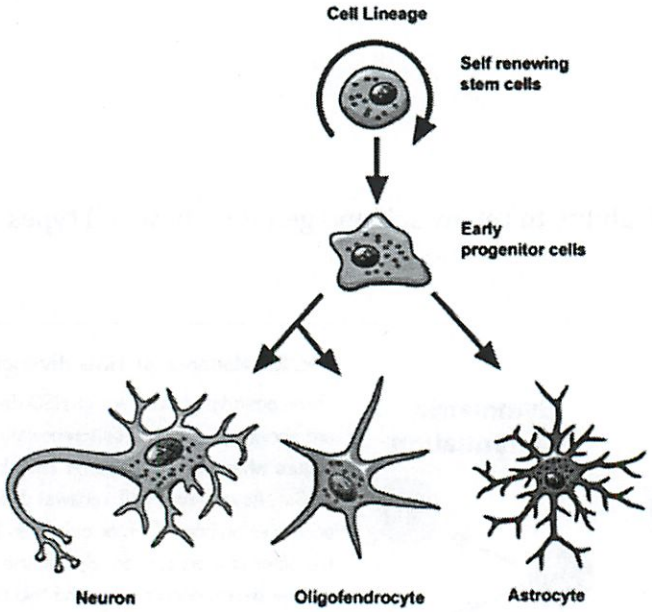
Fig. 3. Manners of HSC division
Three postulated manners of HSC division are shown. Symmetric self-renewal division occurs when the two daughter cells both are HSCs. Asymmetric self-renewal division occurs when one daughter cell is an HSC and the other is a progenitor. Symmetric differentiation occurs when the two daughter cells both are progenitors. It is very important to show that these events actually take place and to determine what controls these divisions.

How can a cell undergo asymmetric division?

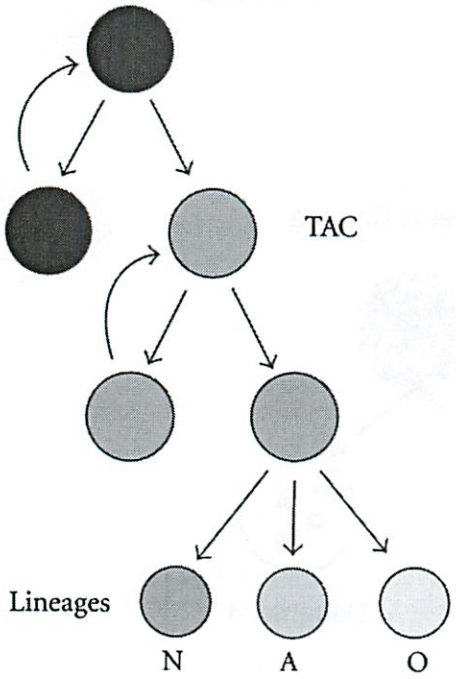


Differentiation

What is the difference between committed and differentiated?



Transient Amplifying Cells



Potency

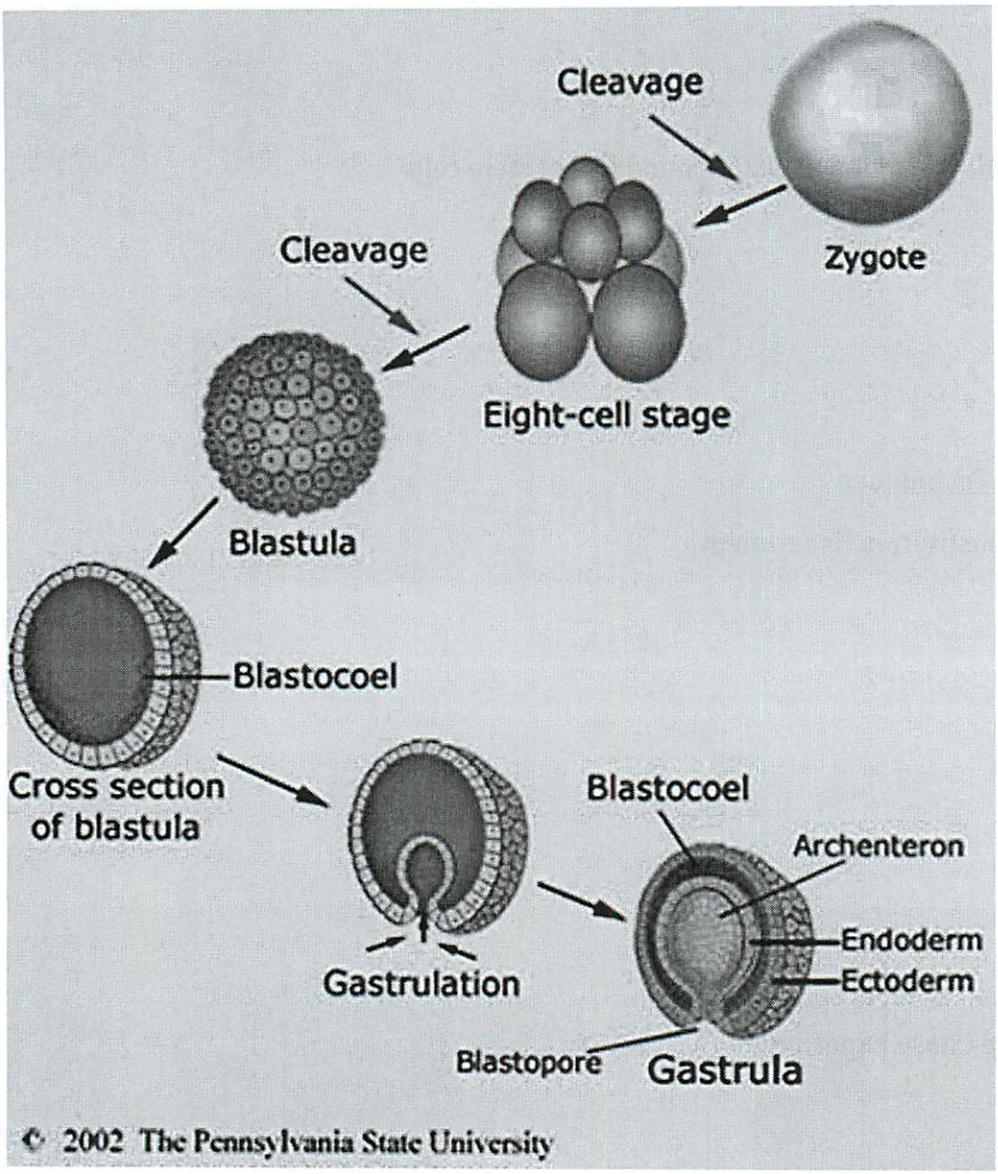
Stem Cell Niche – determines potency and fate of stem cells

Experimental Techniques

1. Reconstitution Experiment

2. Pulse Chase Experiment

III. Development



Embryonic vs. Adult Stem Cells

Induced Pluripotent Stem Cells (iPS)

IV. Cloning

Somatic Cell Nuclear Transfer

Dolly the Sheep

Reproductive vs. Therapeutic Cloning

Chimeras

Lecture

Stem Cells 2

11/16

(watching videos 11/19)

Adult vs embryonic stem cells

Switch back + forth

HSC - hematopoietic stem cell

branches off + specializes

based on contextual signals

Multipotent = can create a variety of cell types

Till + McCulloch

irradiate mice - wipe out bone marrow

dies in a few days

needs platelets to prevent hemorrhaging

but can save if w/ stem cells (bone marrow graft)

shows cells can settle down quickly and start normal function

Syngenic = same genetic bg

allogeneic = different " "

must be tolerant of same histocompotability antigens

had to get fully compatible in humans

and spleen → form colonies in + on spleen

clonal outgrowths

all decendents of single cell

generated all these types of hemapoietic cells

↳ looks diverse

but how do we know all I found?

(3)

If lightly irradiate donor

So some chromosomes slightly

So effects are random + unpredictable

Each donor cell diff type of chromosome

mark not likely

Lprivate mark

special, unique mark

Some ↑ might be 10% shorter
chromosome

but we look at spleen cell in mouse

Yes!

So must be from some cell

We can also take spleen from 2nd mouse
and put in 3rd mouse

(9)

Once agents rescue mouse
Can do this dam to run
So stem cells can be self renewing
and generate other cells

Asymetic → one just like mom (identical)
and other starts differentiating

Symmetric → both daughters become identical stem cells
So net gain of 1
must be symetic if pool ↑

Always happens w needs of org
not random

Eye is oligopotential adult stem cells
One retena stem cell can produce all these cells

9

Oligopotential mensenchymal stem cell
produces bone + muscle cell

---

reflects how systems originally evolved

Ontogeny - origin + development of an organism

Phylogeny - study of evolution of organisms
through molecular sequencing

---

tumors abnormal stem cells
at one time thought all cells in tumor
were equivilent

but can seperate by fluroent makers
2 cell surface antigens
CD44↑ ⟶ CD24

(e)

High levels of CD24 make
Can make monoclonal antibody
+ tag w/ a die

Minority + majority cells
put in immunial compromised math

Xenograph = graph cells from 1 species into another

So compromise their immune cells so don't reject the foreign tissue

So must be phenotypical homogenity within tumor
Some cells seed new tumor
Others don't
even though all genetically identical

(7)

So transfer from normal tissue to tumor
↳ gives new tumor
like a tumor stem cell!

So some like self renewing stem cell
others are transit amplifying cells

So linage of cancer cells
very similar to normal tumor
not all cells are = to each other
Metastis moves from ~~current~~ primary tumor cell to foreign tissue
~~not all cells are = to each other~~
and forms a new tumor
responsibly for 90% of deaths
the distant colonies

8

Cancer stem cell can make new tumor
w/ tumor initiating cells
(must have

[redacted]

& understanding hierarchy to understand
how it operates

[redacted]

Embryotic Stem Cells (ES)

Can inject in blastocys of another mouse

Can get a spotted mouse

proves done black cells insinate into white embryo
began to respond to contextual signals around

Called Chimera

(9)

Some offspring of these totally black
↳ got into spotted mouse's sperm/genes

ES cell is pluripotent
forms lots of diff type of tissues

ES cells
if put under skin
will form a tumor
w/ variety of cell types
some not-so well formed start of
certain variety of cells

As differentiation proceed cells narrow their potential

Nice comparison chart

To summarize: Embryonic stem (ES) cells versus adult stem cells (such as an HSC)

ES	Adult
• Isolated from early embryos • Can expand indefinitely in culture • Can give rise to all cell types in the body	• Isolated from adult tissue • Can not be expanded in culture • Can only give rise to same tissue

11

Early in embrogenisis already a segregation

Can trace very early on

Respond to cues from neighbor

? From where sperm enter eggs?

We don't fully understand how...

Some cells still not fully decided

Can change them early on

ectopic improper physical location

but we don't understand how these linage work

Some cells killed off if ~~don't~~ not needed

like the webbing b/w peoples hands

(12)

Or diff zones of development of fly larva

hox genes

Order of genes along chromosome
is colinear w/ order of fly

Same set of genes in humans too!

So we come from same worm-like ancestor

Eternal Youth

Lots of Degenerative Diseases
could be reversed if add new fresh cells

taking from others is histone incompatable

So want to be able to use stem cells

(13)

But also we want ES cells from ourselves

Can create a fertilized egg identical to somatic (of the body)

Cells of a patient and donor egg to develop into an early-stage blastocyst (embryo) with inner cell mass where could prepare ES cells.

If take nucleus out of fertilized egg

Put in skin cell nucleus instead

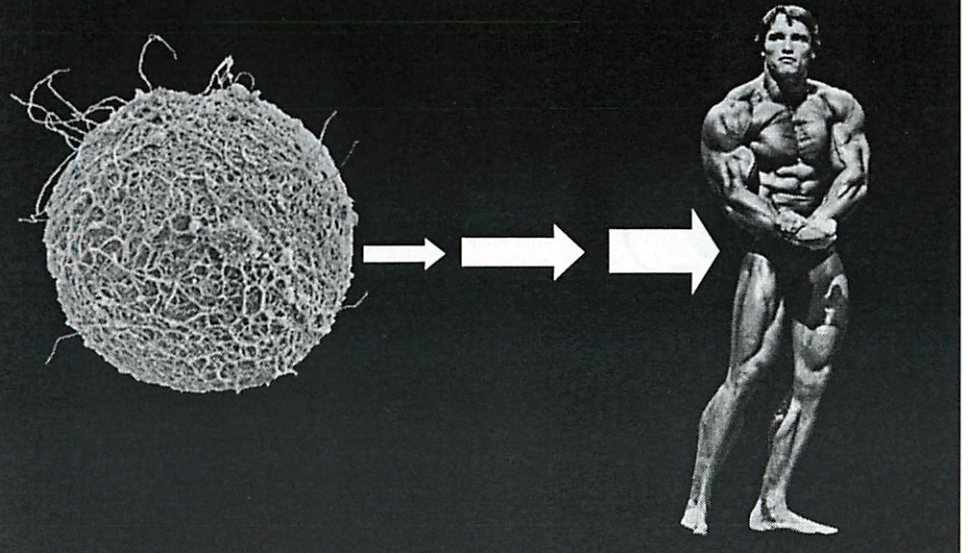
Cytoplasm of egg forces nucleus to become egg cell nucleus

So w/ Dolly the Sheep

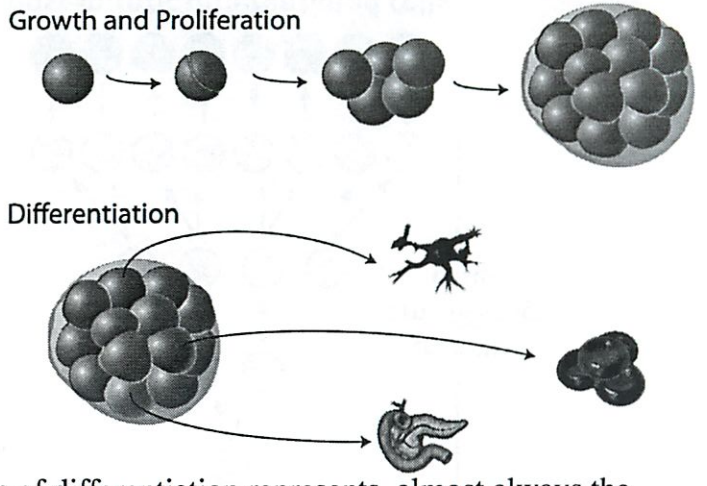
Embryo | diploid
- full cell already

haploid - need 2 → sperm + egg

Major question: How do you go from a fertilized egg to a future Governor of California?

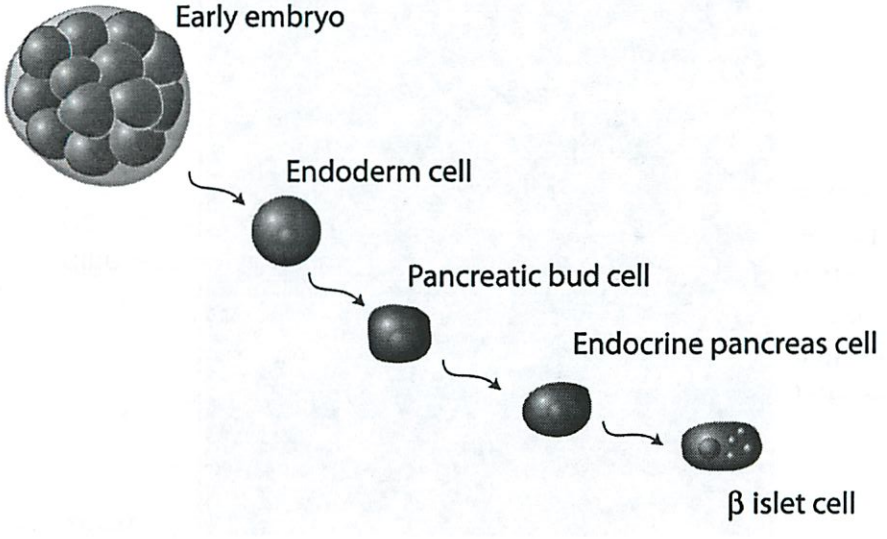


Development Has Two Aspects

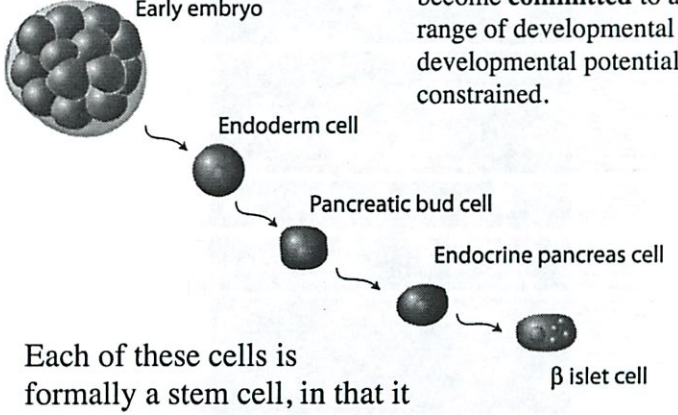


The process of differentiation represents, almost always the **acquisition of tissue-specific, specialized traits**, which is achieved by changes in gene expression rather than changes in the structure of the DNA genome and its nucleotide sequences.

Progressive Differentiation



Progressive Differentiation



As this differentiation proceeds, the cells become **committed** to an increasingly narrow range of developmental options, i.e., their developmental potential becomes increasingly constrained.

Each of these cells is formally a stem cell, in that it can self-renew and can also spawn more differentiated daughters.

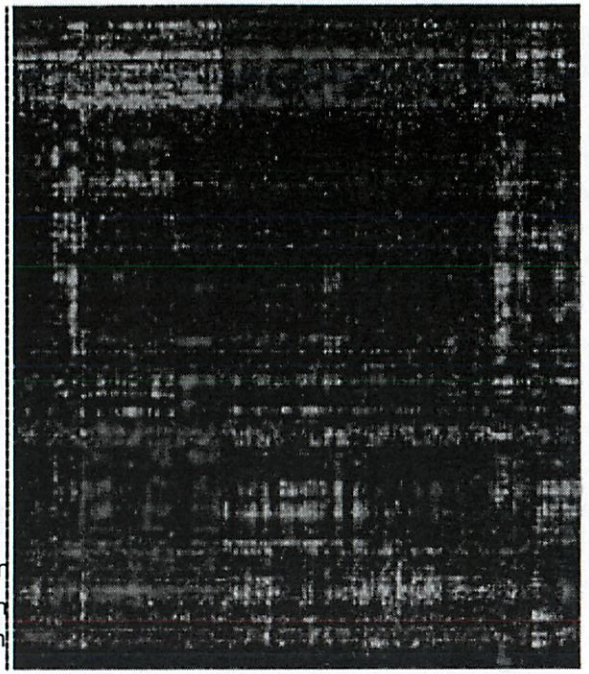
11/4

DNA microarray

cells from different tissues

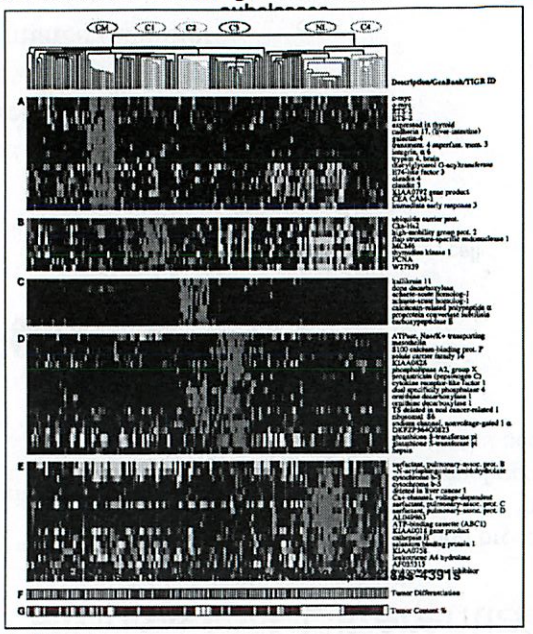
different genes

high expression
avg. expression
low expression



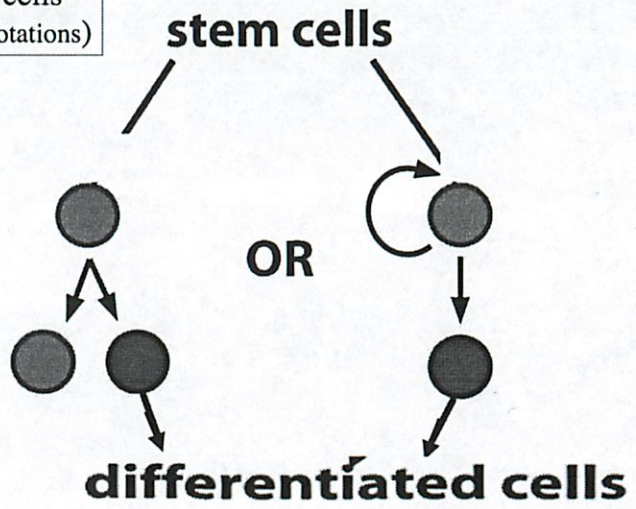
Different cell types express different sets of genes

Gene expression clusters and histologic differentiation within lung adenocarcinoma



Clinical Cancer Research

The behavior of stem cells
(two equivalent graphic notations)



Note that the two daughters are different

Figure 11.16a *The Biology of Cancer* (© Garland Science 2007)

Hierarchical organization of many normal tissues

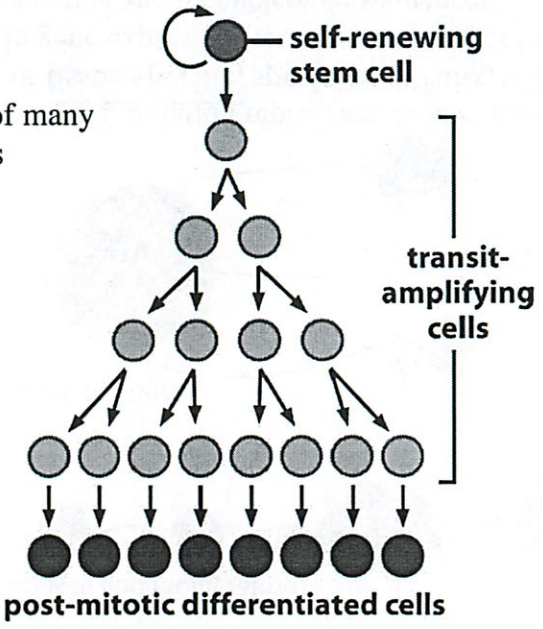
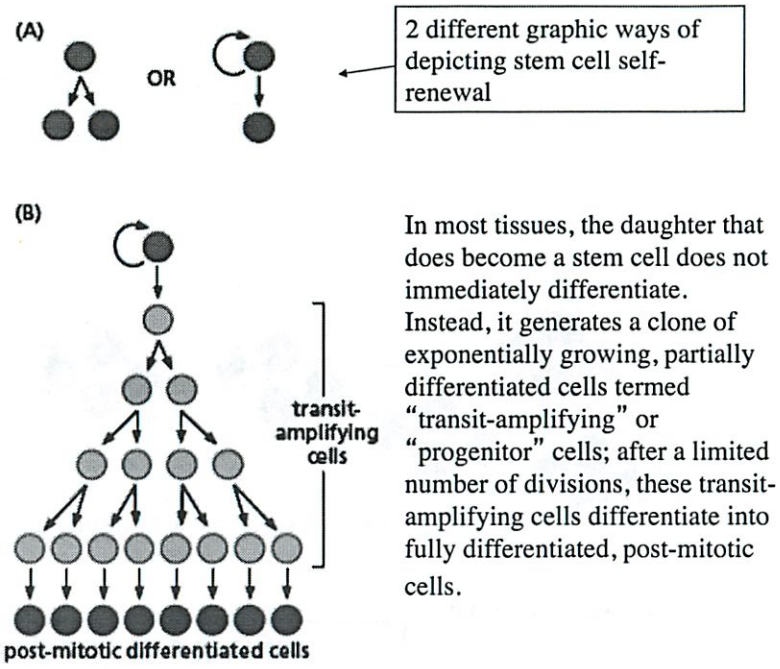


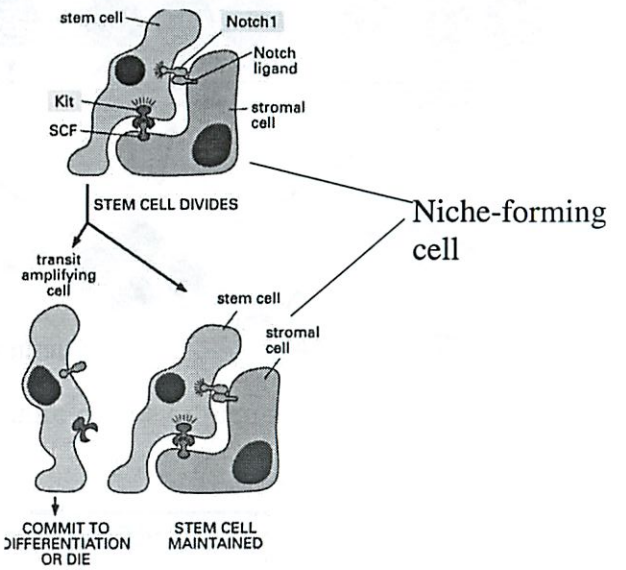
Figure 11.16b *The Biology of Cancer* (© Garland Science 2007)



2 different graphic ways of depicting stem cell self-renewal

In most tissues, the daughter that does become a stem cell does not immediately differentiate. Instead, it generates a clone of exponentially growing, partially differentiated cells termed "transit-amplifying" or "progenitor" cells; after a limited number of divisions, these transit-amplifying cells differentiate into fully differentiated, post-mitotic cells.

Stem cell asymmetric divisions are likely due to contact with certain Other cells that form the stem cell "niche".



Niche-forming cell

Figure 22-36. Molecular Biology of the Cell, 4th Edition.

Symmetric vs.
Asymmetric divisions

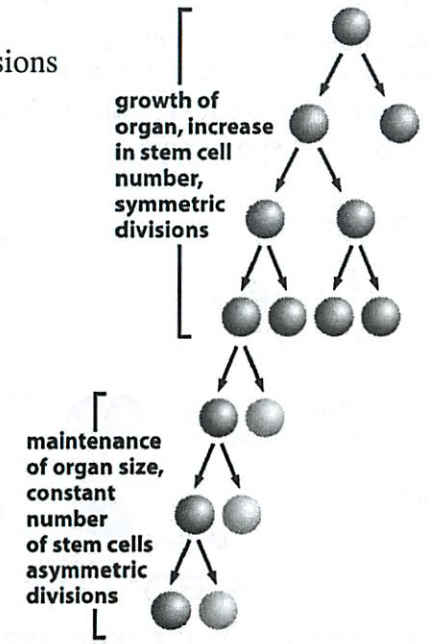
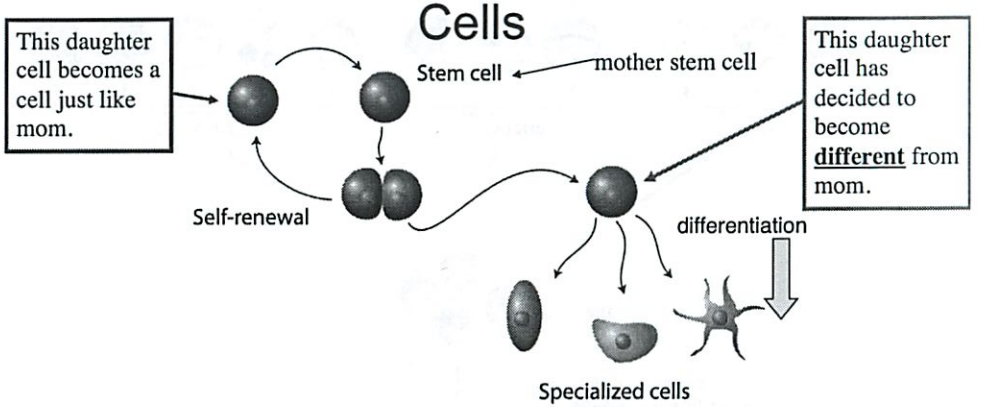


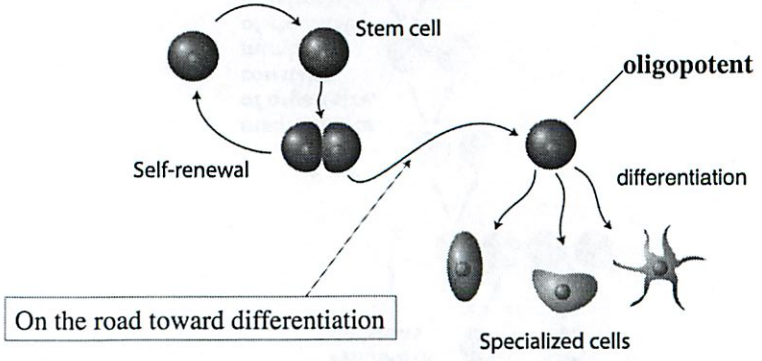
Figure 12.3c *The Biology of Cancer* (© Garland Science 2007)

Two Essential Properties of Stem Cells



By self-renewing (making daughter-cell copies of itself), a stem cell can ensure that the **pool** of stem cells remains constant in a tissue. (pool = collection of similar cells)

Two Essential Properties of Stem Cells

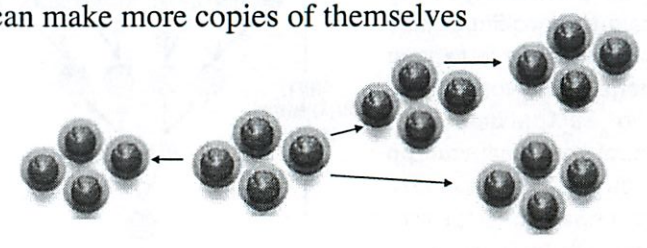


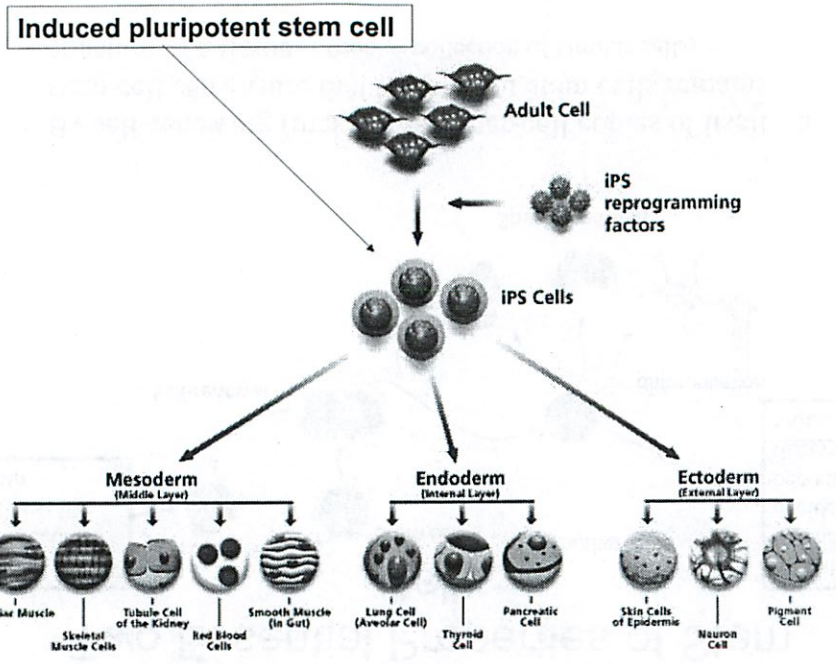
Note that one of the daughter cells of the stem cell has undertaken to differentiate, and having done so, can spawn at least 3 distinct types of differentiated descendant cells. (Such a cell is termed "**oligopotent**" because its has the potential to spawn several (oligo-) distinct differentiated cell types.

The Ground Rules:
Stem cells can do two things:

pluripotent stem cells

→ 1. They can make more copies of themselves

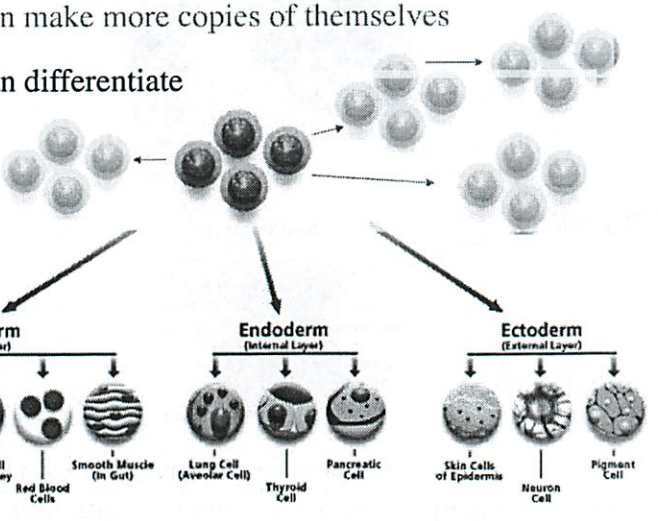


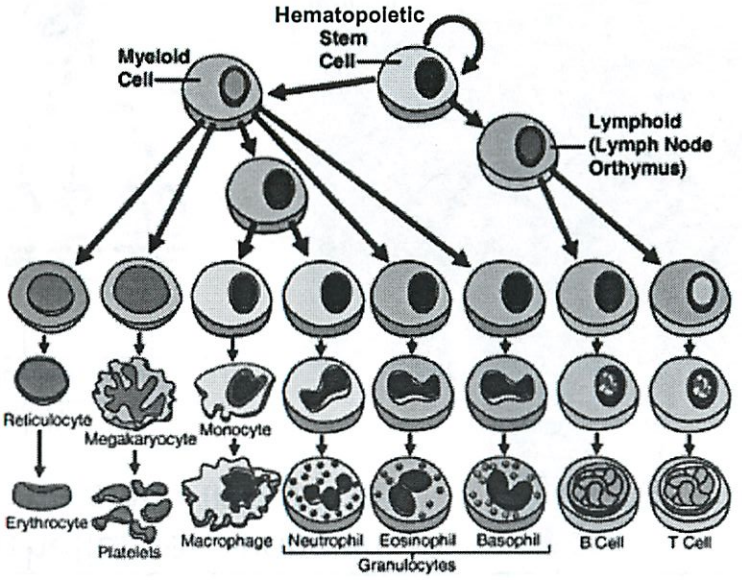


The Ground Rules:
Stem cells can do two things:

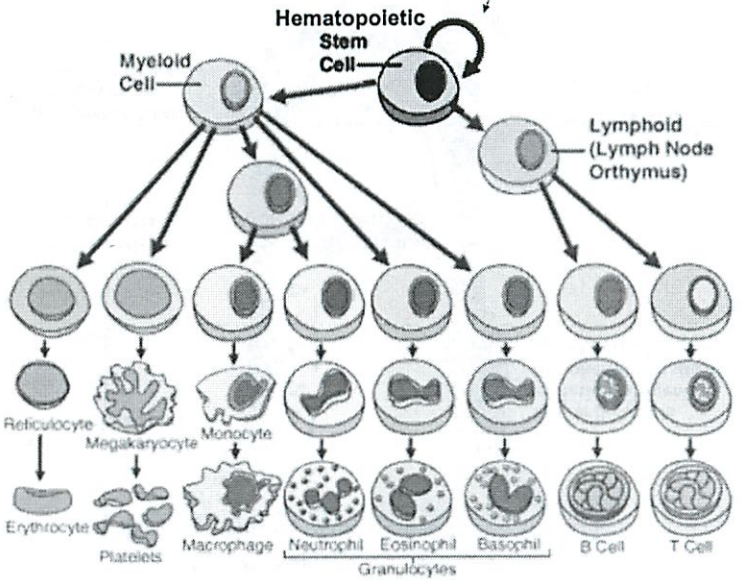
1. They can make more copies of themselves

→ 2. They can differentiate

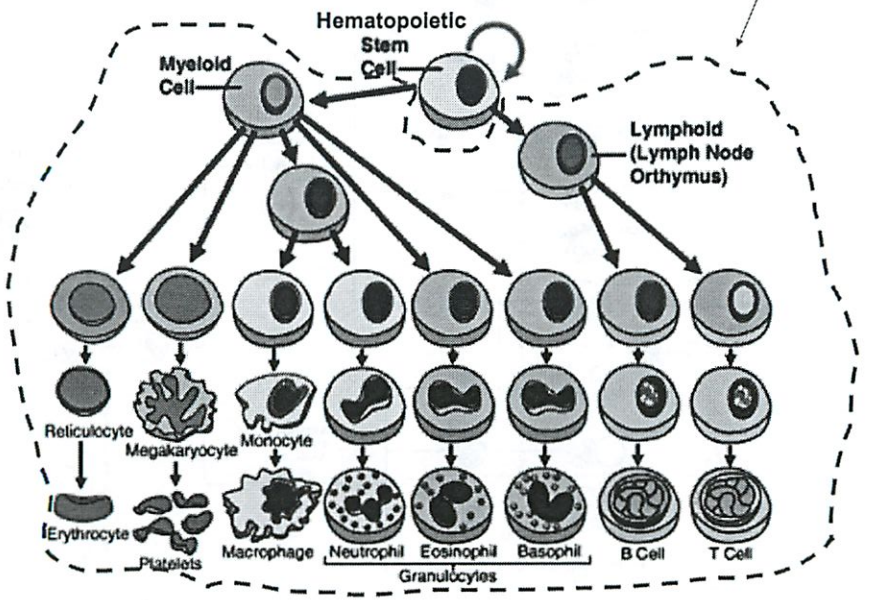


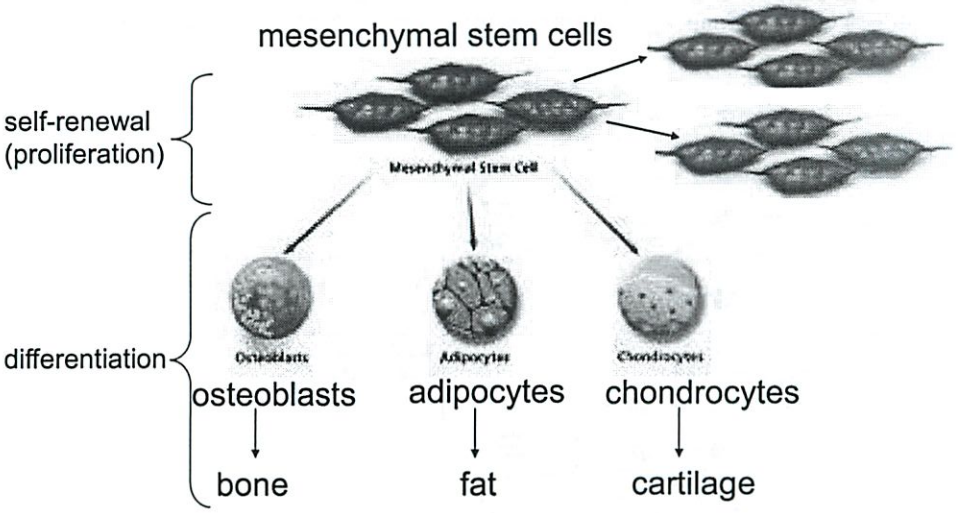


Once again, the dichotomy between **self-renewal**

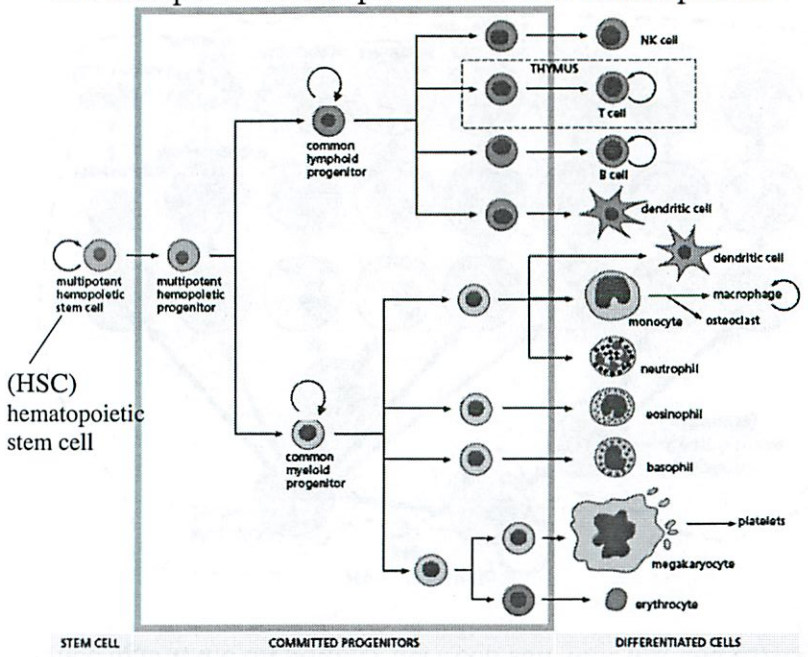


Once again, the dichotomy between self-renewal and **differentiation**

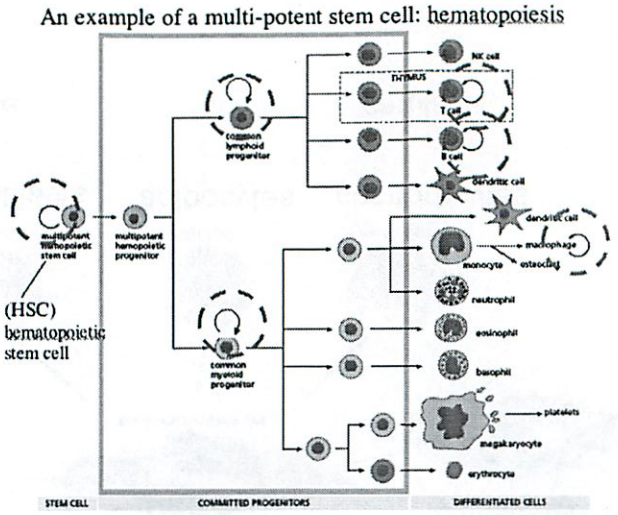




An example of a multi-potent stem cell: hematopoiesis



An example of a multi-potent stem cell: hematopoiesis

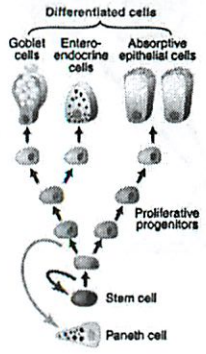


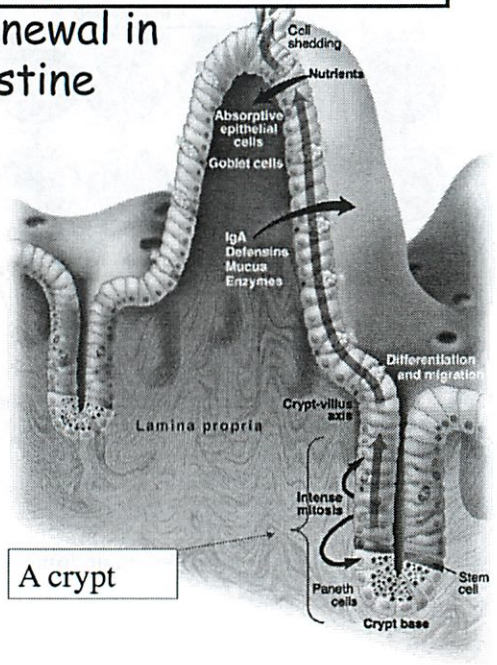
They indicate that when a mother cell divides, one of its two daughters can assume a phenotypic state identical to the mother state (i.e., **self-renewal)** while the other cell may enter into a new phenotypic state, e.g., one leading toward differentiation.

Another example of an oligopotent adult stem cell: the gut

Epithelial self-renewal in the small intestine

One stem cell type makes at least 3 kinds of differentiated progeny





A crypt

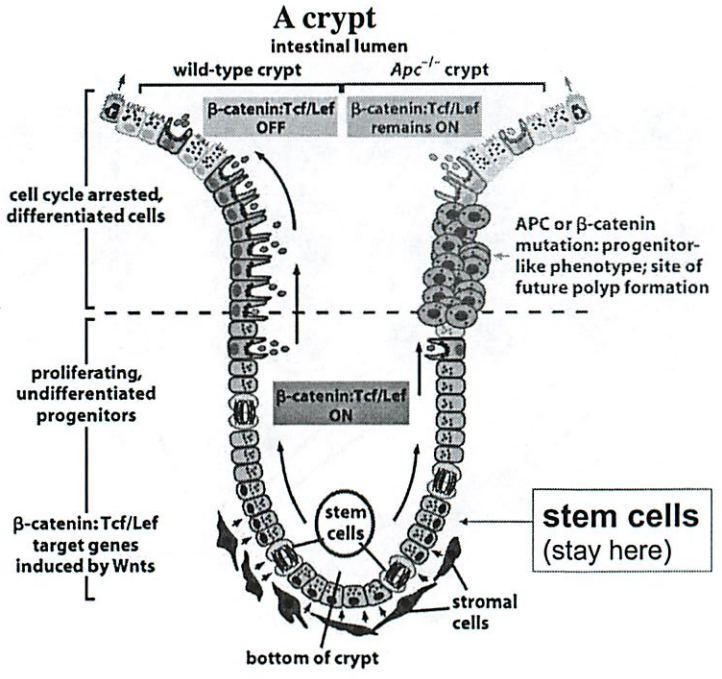


Figure 7.24a *The Biology of Cancer* (© Garland Science 2007)

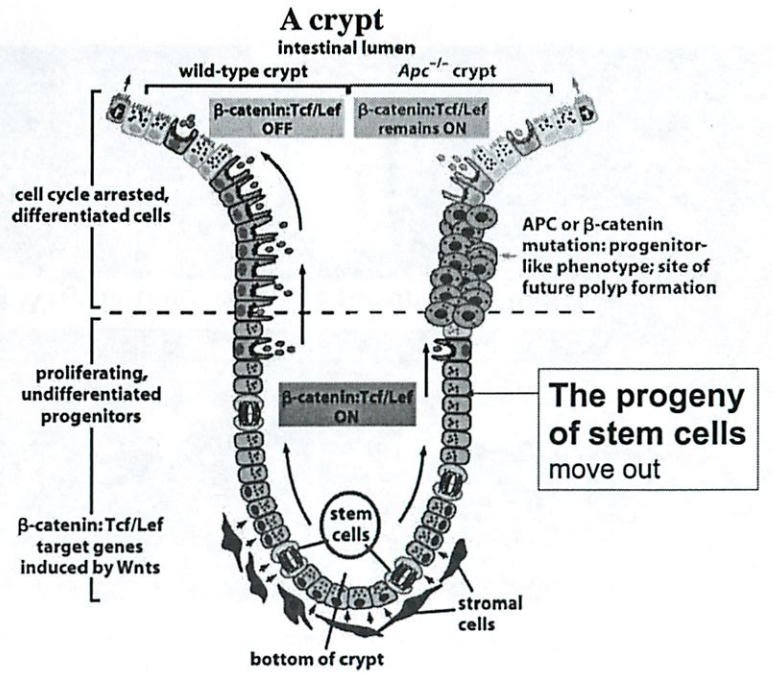
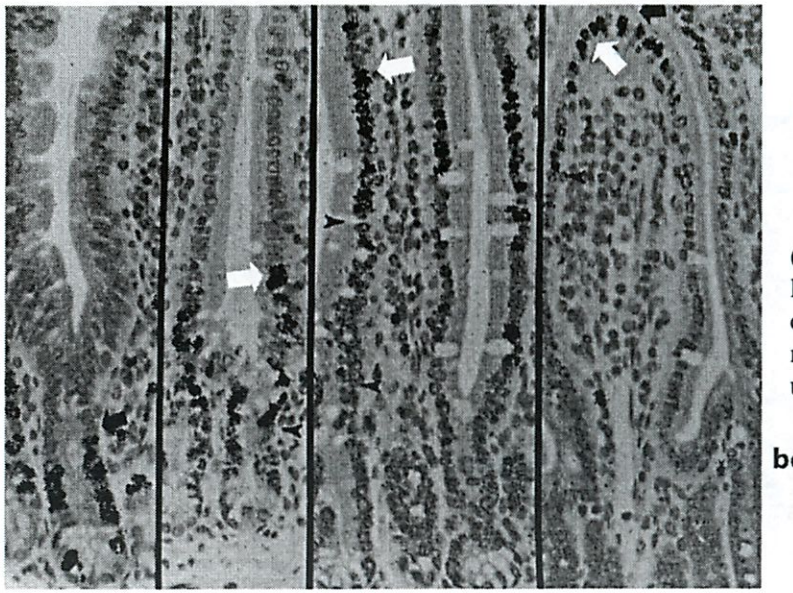


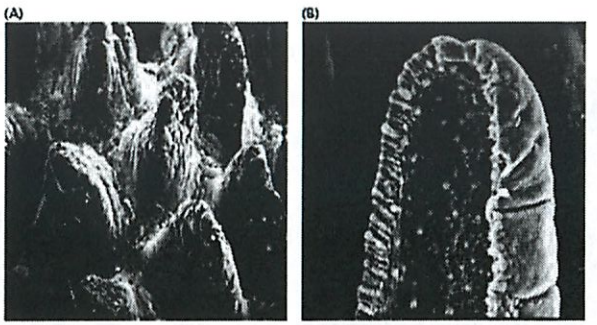
Figure 7.24a *The Biology of Cancer* (© Garland Science 2007)



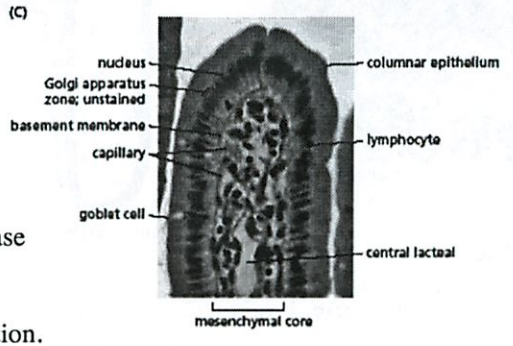
Pulse label -- give mouse ^{3}H-thymidine for 40 minutes, then take it away. Detect labeled cells with radioautography -- applying a photographic emulsion to tissue slices.

Figure 17.2c *The Biology of Cancer* (© Garland Science 2007)

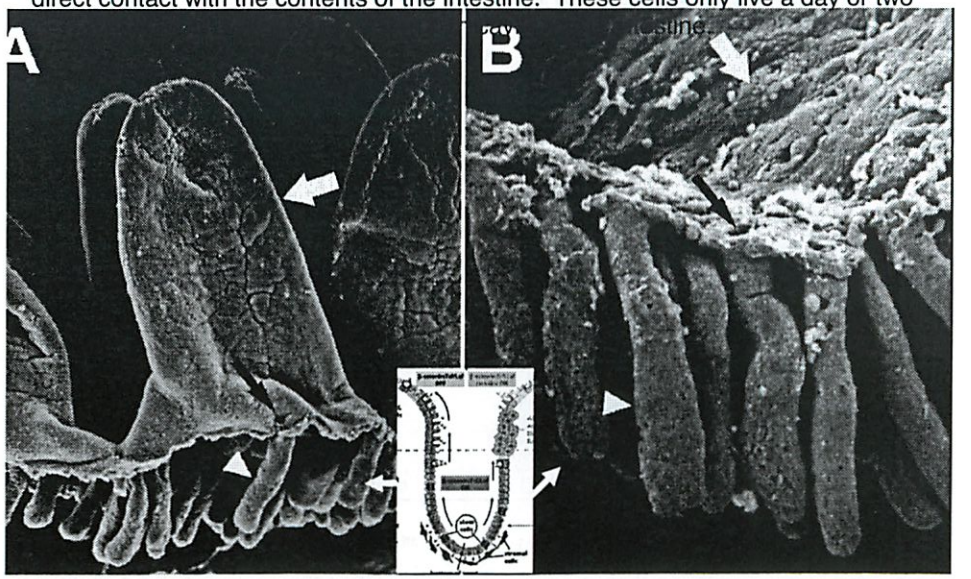
Both the small intestine/duodenum *(left)* and the large intestine/colon *(right),* have crypts -- deep pits *(white arrowheads).* At the bottom of these crypts are stem cells that continually generate progenitor cells that move up the walls of the crypts and then up the sides of the villi *(fingerlike-projections, arrow left panel)* and in direct contact with the contents of the intestine. These cells only live a day or two



Villi protruding into the lumen of the small Intestine (increase surface area of epithelium to maximize absorption.



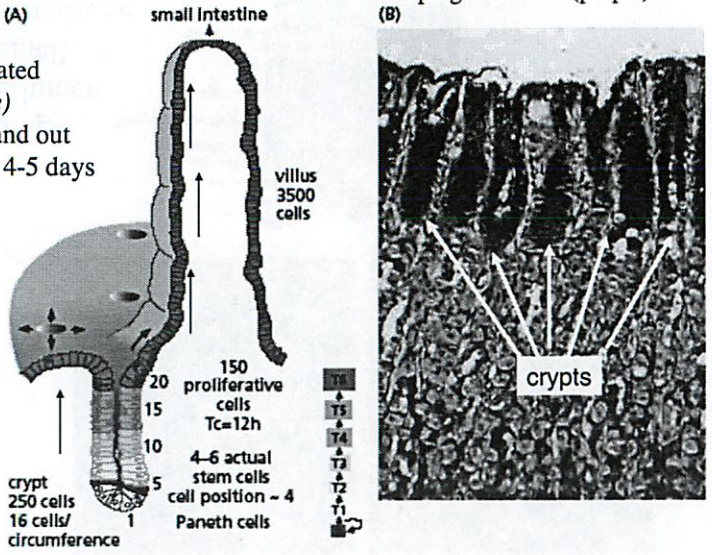
The outer epithelial cell layer is continuously turning over



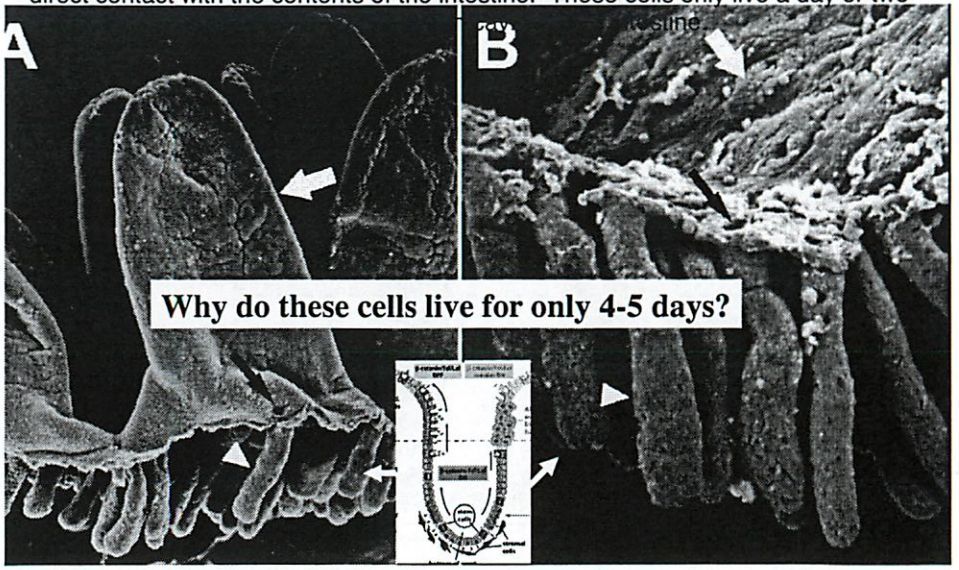
Each of us makes and sheds ~300 gm. Of epithelial cells in gut every day! (1 gm = ~10^9 cells)

Stem cells sit at the bottoms of crypts, protected by a thick plug of mucins (purple).

Differentiated cells *(blue)* move up and out and die in 4-5 days



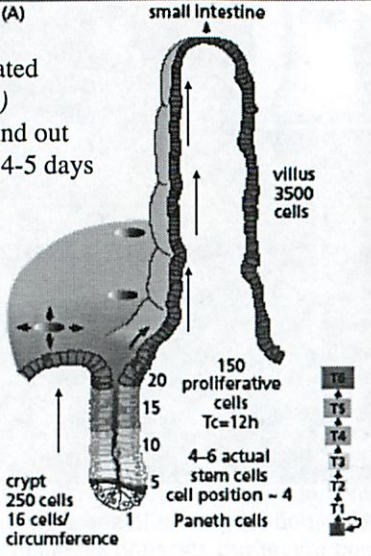
Both the small intestine/duodenum *(left)* and the large intestine/colon *(right)*, have crypts -- deep pits *(white arrowheads)*. At the bottom of these crypts are stem cells that continually generate progenitor cells that move up the walls of the crypts and then up the sides of the villi *(fingerlike-projections, arrow left panel)* and in direct contact with the contents of the intestine. These cells only live a day or two

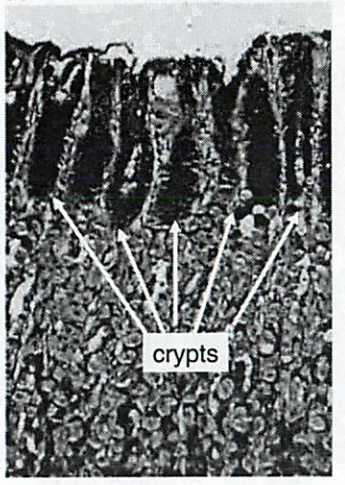


Each of us makes and sheds ~300 gm. Of epithelial cells in gut every day! (1 gm = ~10^9 cells) = 3 x 10^{11} cells per day = 10^{14} cells per year = ~6 x 10^{15} in a lifetime

Stem cells sit at the bottoms of crypts, protected by a thick plug of mucins (purple).

Differentiated cells *(blue)* move up and out and die in 4-5 days

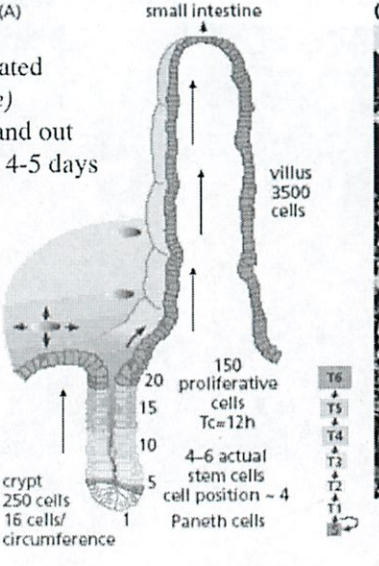


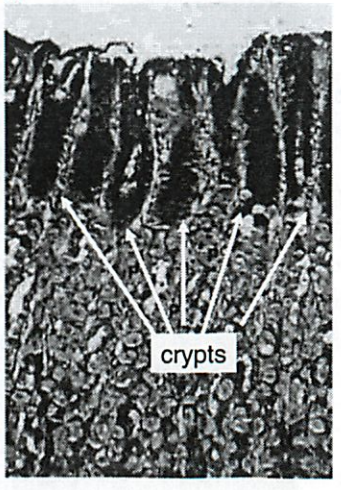


Why are the crypts lined with plugs of mucins?

Stem cells sit at the bottoms of crypts, protected by a thick plug of mucins (purple).

Differentiated cells *(blue)* move up and out and die in 4-5 days





There are yet **other ways to protect stem cells:**
Minimize the number of successive divisions that a stem cell passes through in a lifetime!

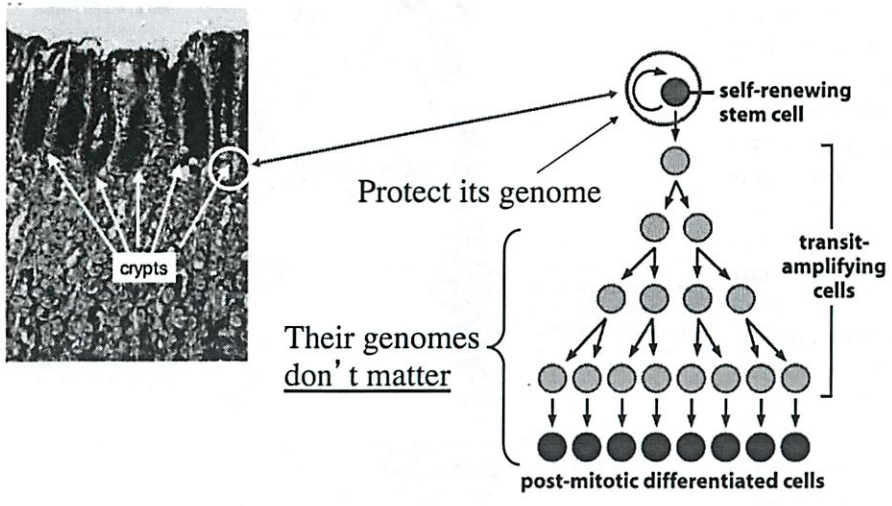
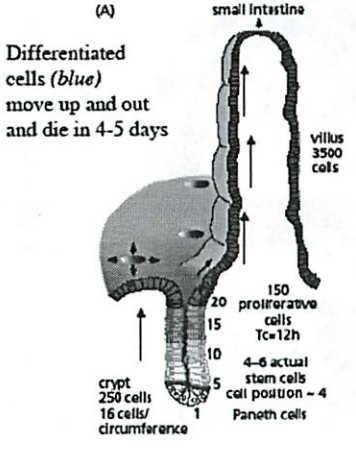


Figure 11.16b *The Biology of Cancer* (© Garland Science 2007)



Note that the rapid evacuation of differentiated cells (via their outward migration over 4-5 days) ensures that any cell that happens to sustain a mutation will soon be jettisoned anyhow, so that its mutant phenotype becomes irrelevant/moot.
This is a powerful strategy for minimizing the accumulation of mutant cells and thus the formation of intestinal tumors.

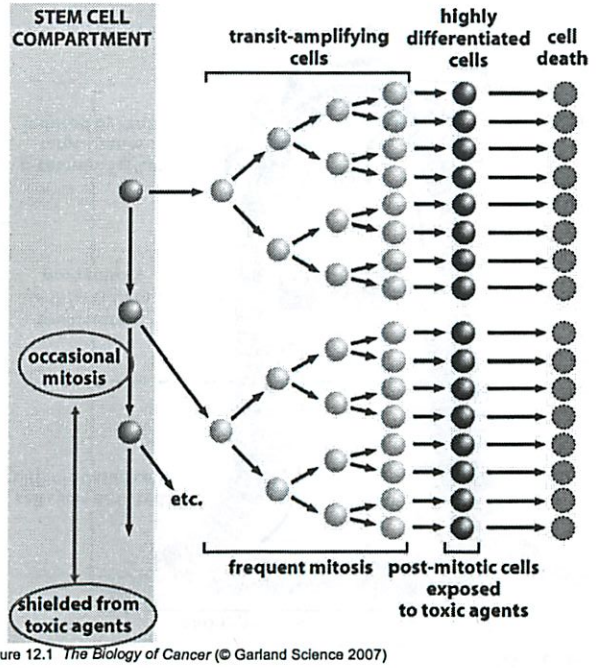
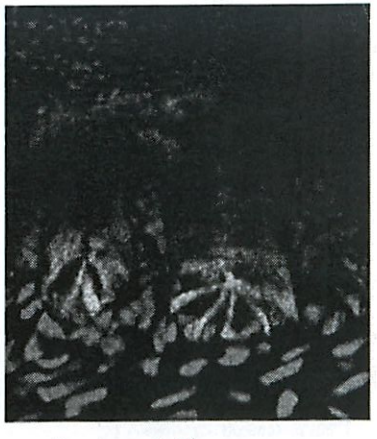


Figure 12.1 *The Biology of Cancer* (© Garland Science 2007)

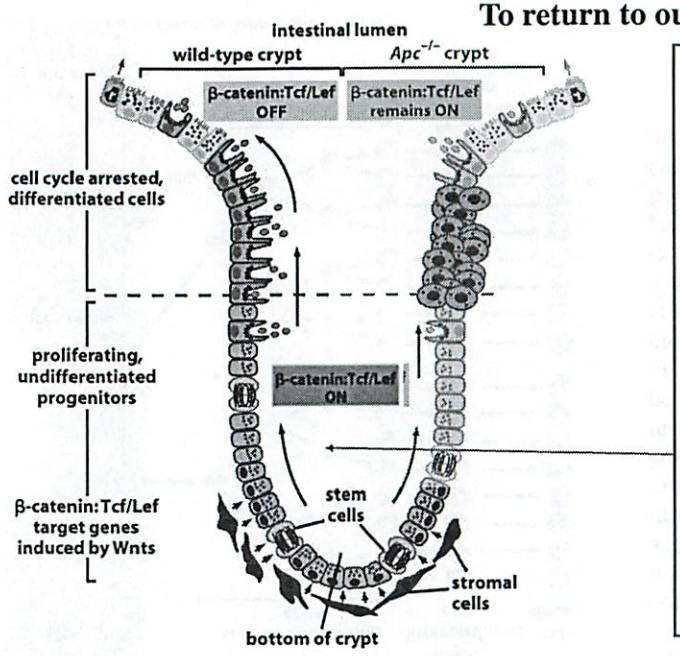
Note that the stem cells only need to divide occasionally, minimizing their accumulation of mutations due to misreplication of DNA. Hence, the genomes of the cells that remain permanently ensconced in the tissue are protected from replication-induced mutations.
(Stem cells may also be physically shielded from exposure to toxic agents, such as mutagens.)

Lgr5-Driven GFP Expression in Crypt Base Columnar Cells: a way to label stem cells



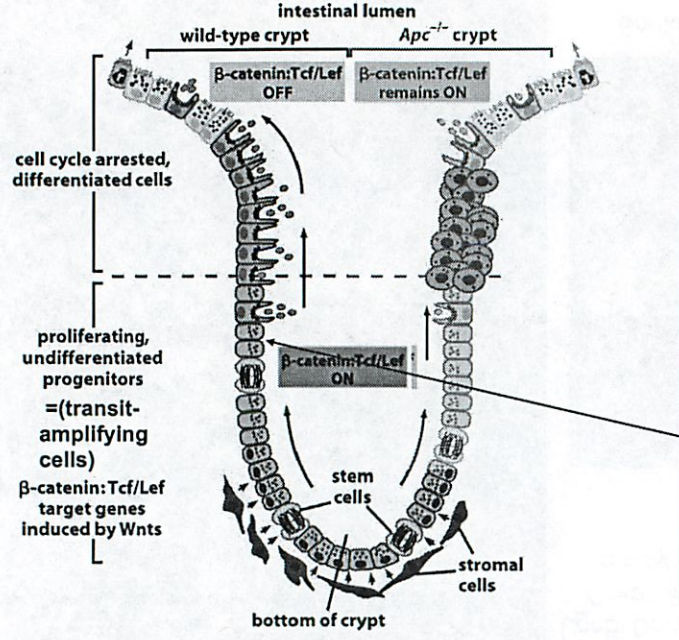
Barker & Clevers

To return to our theme:



Usually, the progeny of the stem cells, which are themselves not totally differentiated (but on the road to being so) are called "progenitor cells". Usually, the progenitor cells and their differentiated progeny spend ~4-5 days moving out from the crypt before their differentiated descendants are sloughed off into the lumen (cavity) of the gut.

Figure 7.24a *The Biology of Cancer* (© Garland Science 2007)



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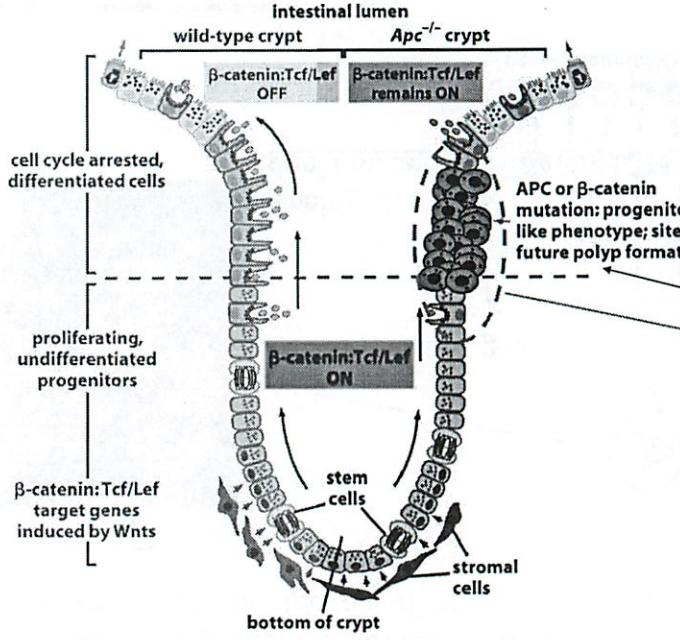
An intact APC protein is required in order to ensure that these progenitors continue their upward migration

Figure 7.24a *The Biology of Cancer* (© Garland Science 2007)



Here is an example of when things go **wrong**: The APC protein is required for the continued out-migration of progenitor cells from the crypt. If it malfunctions, cells accumulate in the crypt and eventually form an adenoma/polyp, which can, in turn develop into a carcinoma (an invasive cancer).

Figure 7.24a *The Biology of Cancer* (© Garland Science 2007)

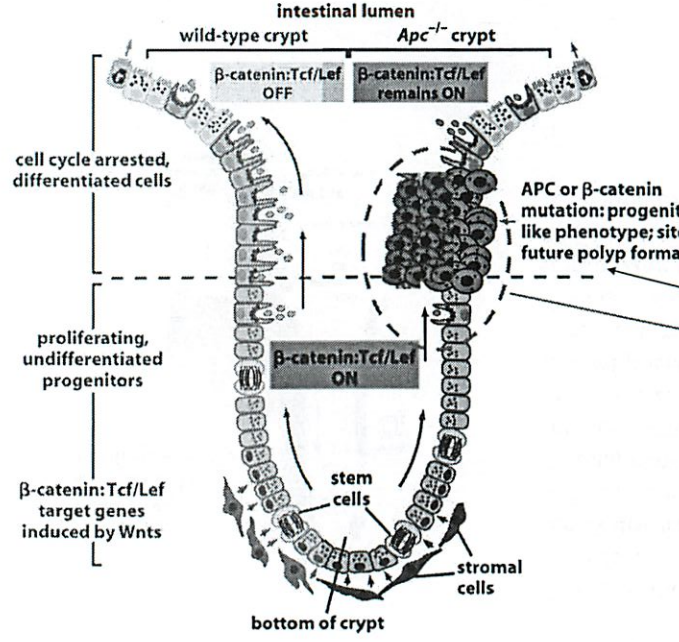


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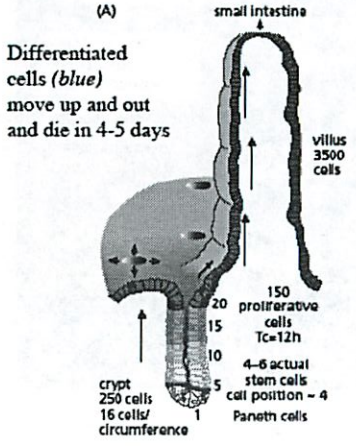
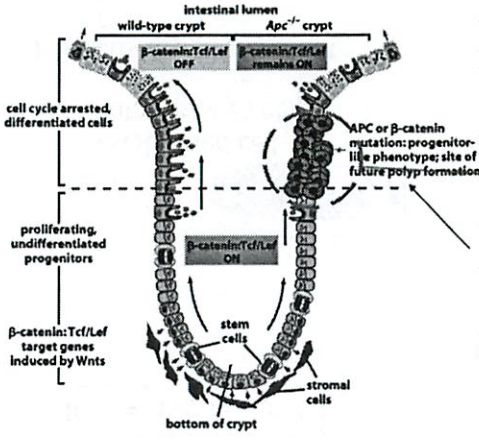
Figure 7.24a *The Biology of Cancer* (© Garland Science 2007)



Note that the rapid evacuation of differentiated cells (via their outward migration over 4-5 days) ensures that any cell that happens to sustain a mutation will soon be jettisoned anyhow, so that its mutant phenotype becomes irrelevant/moot.
This is a powerful strategy for minimizing the accumulation of mutant cells and thus the formation of intestinal tumors.

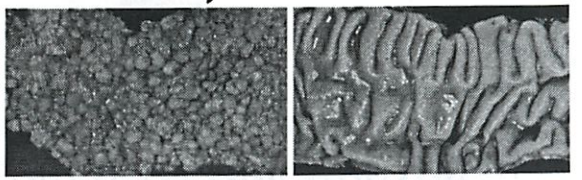
In order for intestinal tumors to arise, they must evade this forced evacuation process by protecting cells from this evacuation, i.e., by trapping cells in the crypts. The descendants of those trapped cells can accumulate additional mutations and still remain in the crypts.



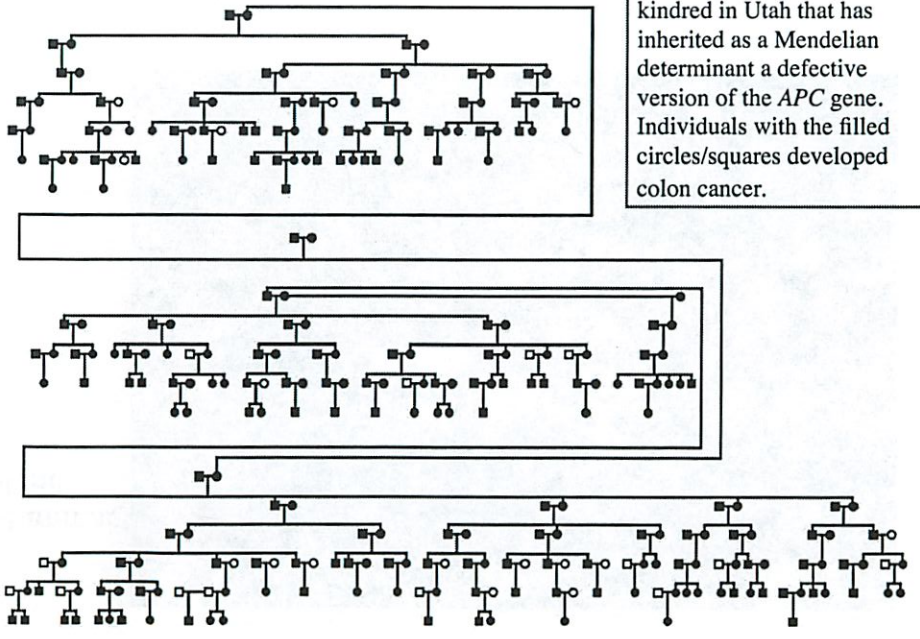
Here is an example of when things go wrong: The APC gene is required for the continued out-migration of progenitor cells from the crypt. If it malfunctions, cell accumulate in the crypt and eventually form an adenoma/polyp, which can, in turn develop into a carcinoma (an invasive cancer).

Individuals who inherit a defective version of the *APC* gene can develop hundreds of polyps in their gut, some of which can later progress to carcinomas.

"APC" = condition termed adenomatous polyposis coli



Colon of patient with APC | Normal colon



Here is an example of a large kindred in Utah that has inherited as a Mendelian determinant a defective version of the *APC* gene. Individuals with the filled circles/squares developed colon cancer.

This explains why the initial mutation in the series of mutations leading to a colonic tumor (carcinoma) is almost always an inactivation of the *APC* tumor suppressor gene.

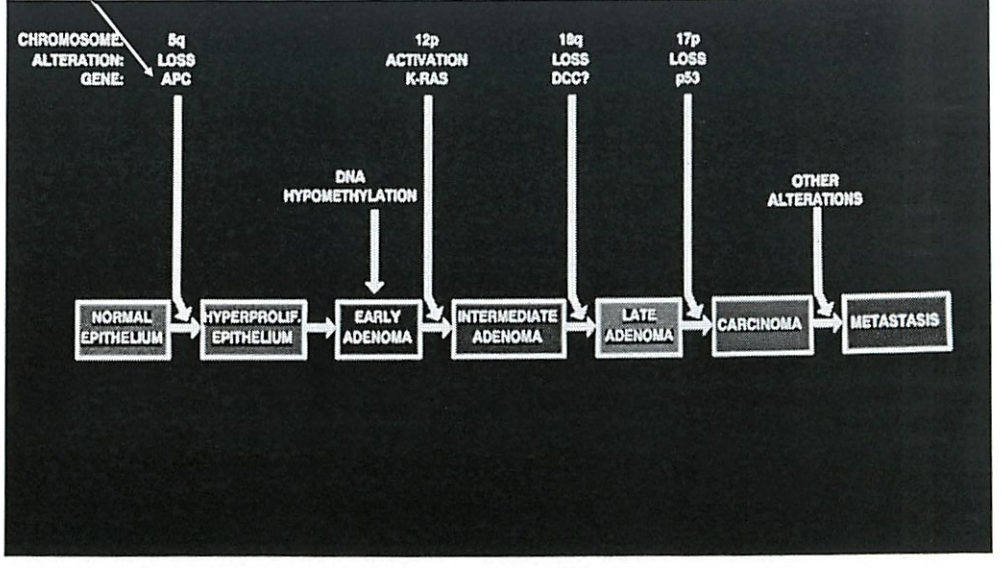


Figure 7.23 *The Biology of Cancer* (© Garland Science 2007)

Stem cells in other organs as well

Mammary gland

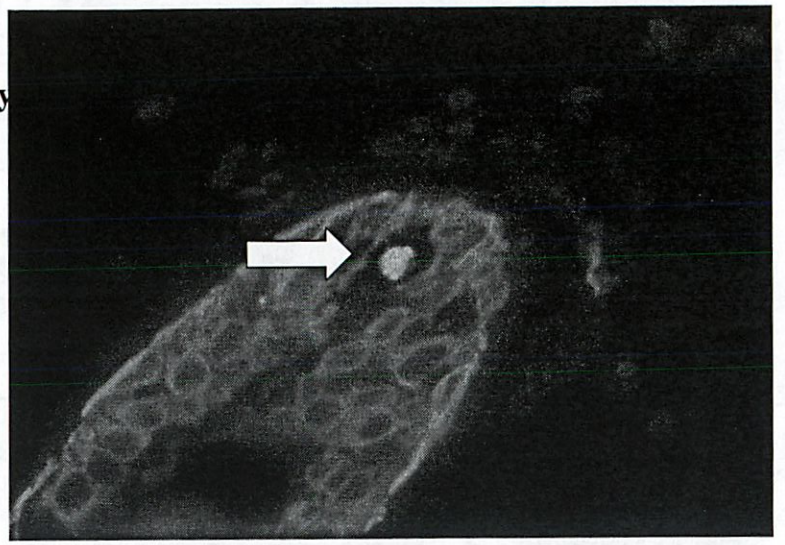


Figure 12.5e *The Biology of Cancer* (© Garland Science 2007)

Label-retaining cells at the sites of the stem cell niche

Hair follicle

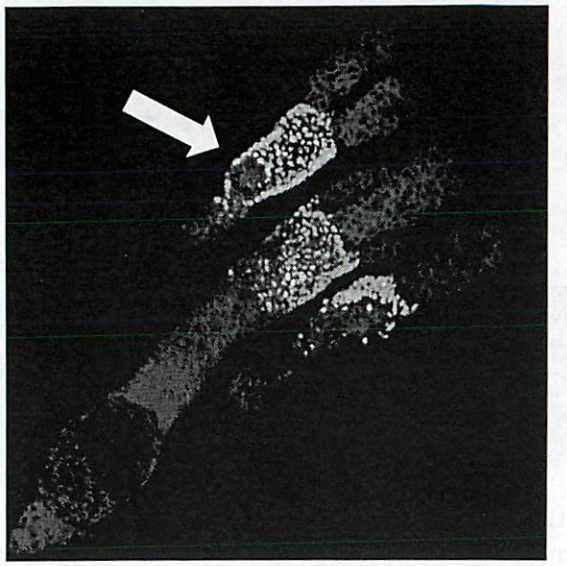
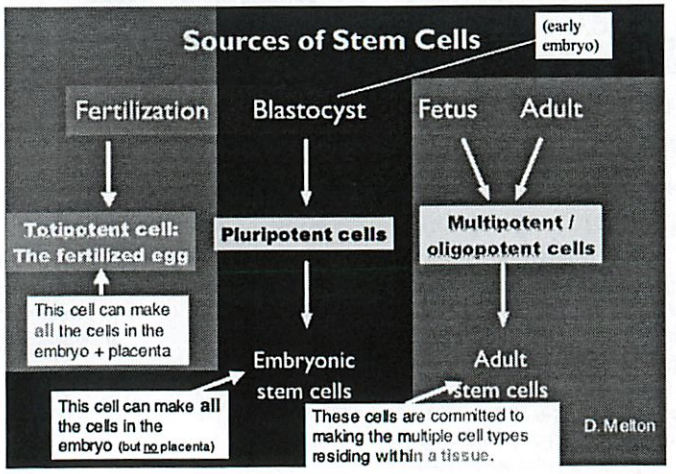


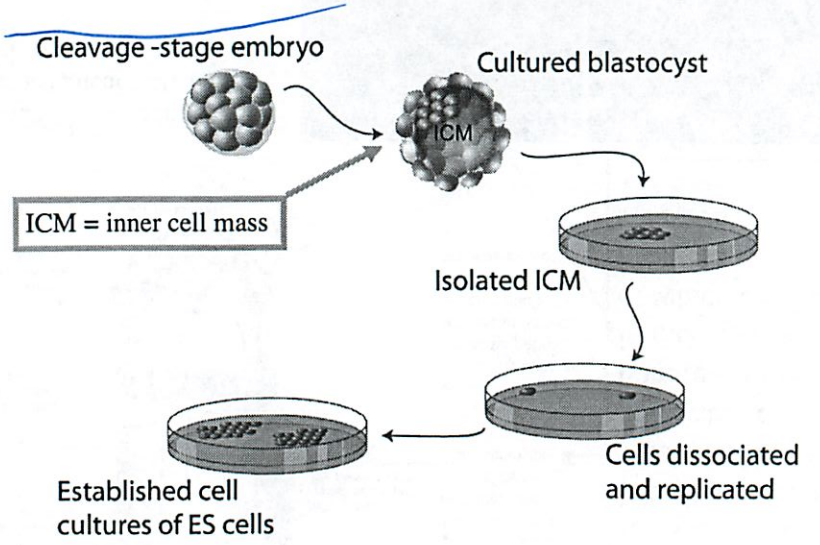
Figure 12.5f *The Biology of Cancer* (© Garland Science 2007)

Pluripotent cells = ES cells = **embryonic stem cells** (vs. adult stem cells)

Hence, as development proceeds, a cell that has the potential to make all types of cells (**totipotent**), including the entire embryo and the placenta, generates a cell that can only make cells of the embryo (**pluripotent**),which then generates a variety of "adult stem cells" that are committed to only make the cells in one or another adult tissue (**multipotent, oligopotent**). Therefore, as development proceeds, cells progressively narrow the range of cell types that they can spawn.

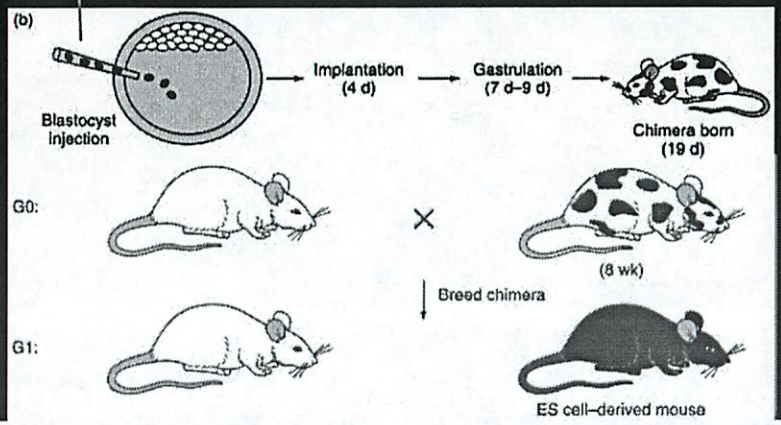


Embryonic Stem (ES) Cell Derivation



ES cells are pluripotent

How can one demonstrate that ES cells are pluripotent? Inject ES cells from a black-coated mouse into the blastocyst of a white-coated mouse. These white-coated cells will integrate into the developing embryo, creating a **chimera**, some of whose cells come from the white-coated embryo and and some from the introduced black-coated ES cells.



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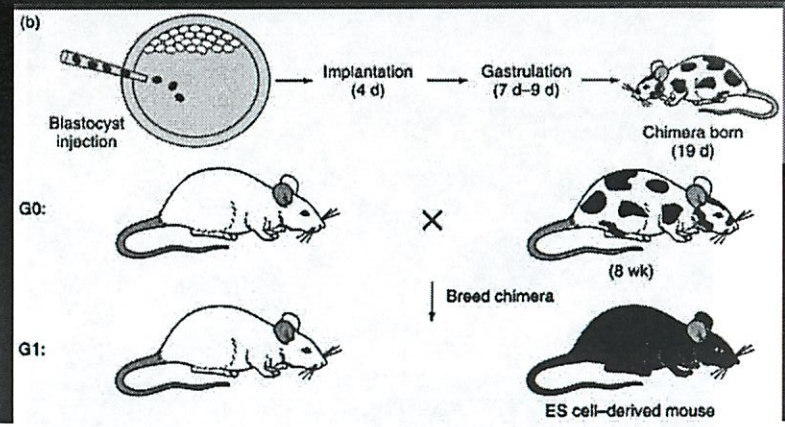
When you now breed the chimeric mouse with a white mouse, since some of the black-coated cells will have integrated into the gonads (testes or ovaries), some of the gametes will now transmit the chromosomes deriving originally from the initially introduced black-coated ES cells.



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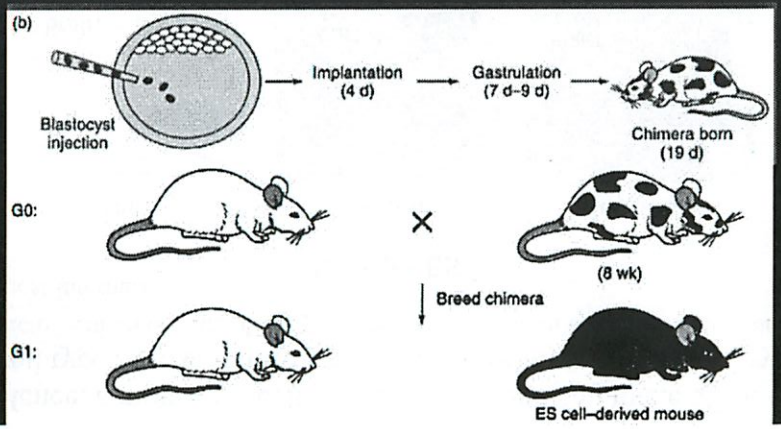
The fact that some of the offspring of the cross between a white and a chimeric animal have **black coats** indicates that the descendants of the previously introduced ES cells have become established in the gonads of the G0 chimeric mouse



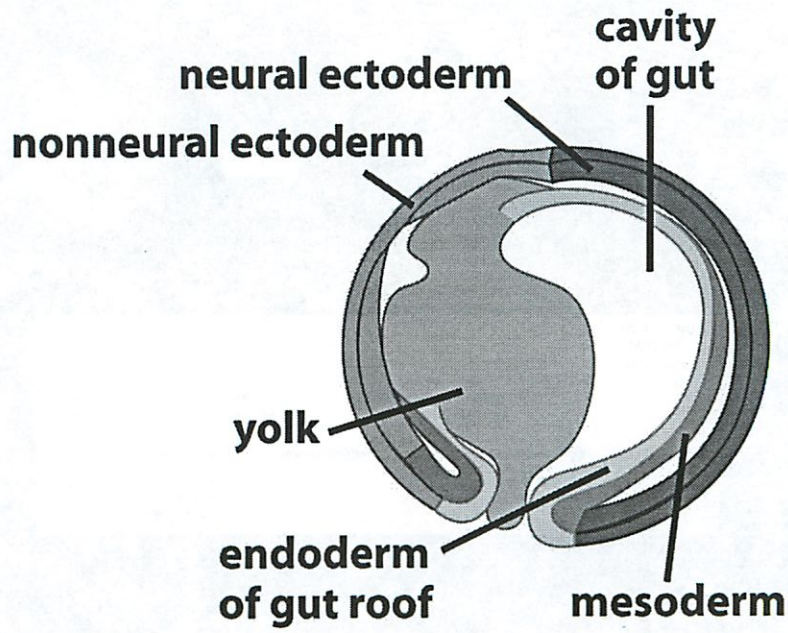
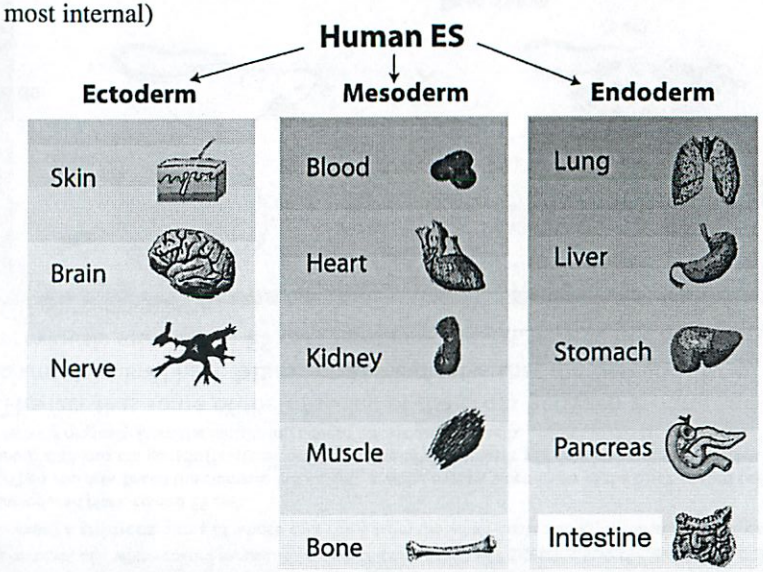
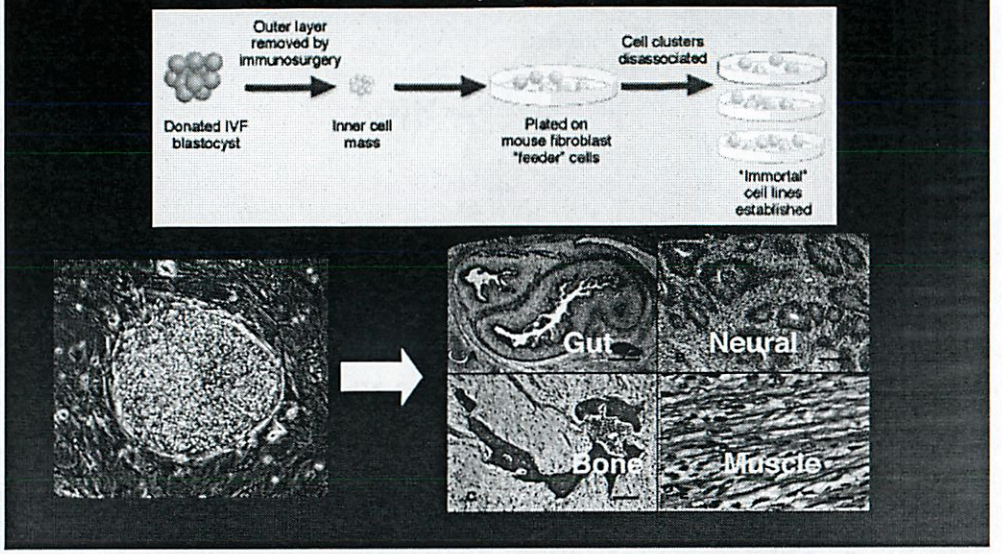


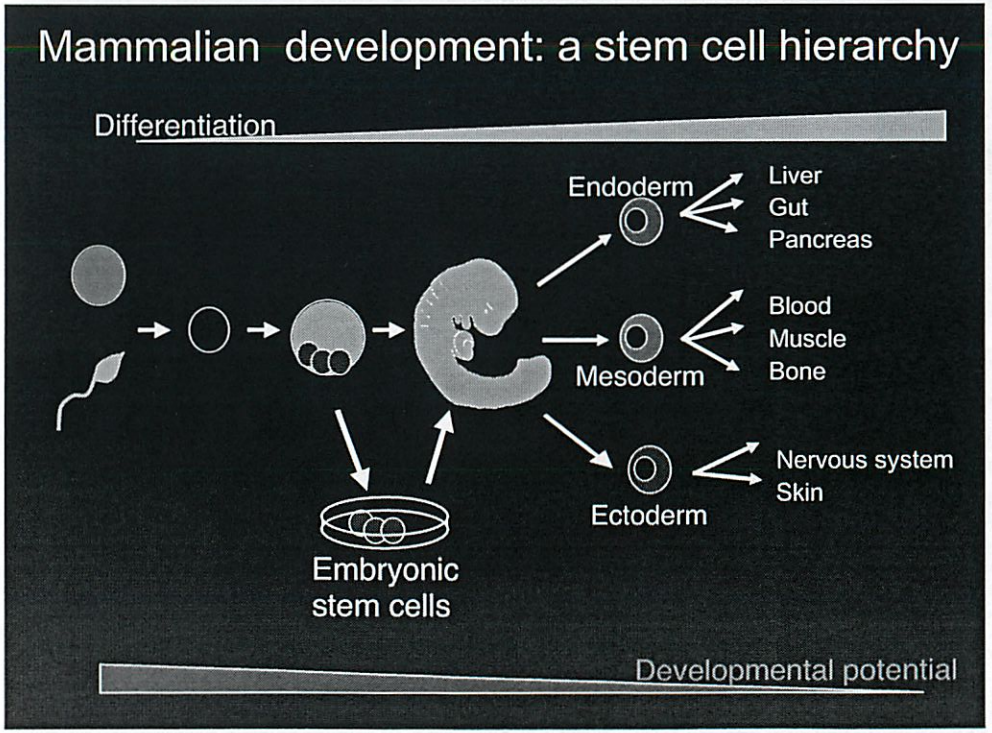
Figure 2.5a *The Biology of Cancer* (© Garland Science 2007)

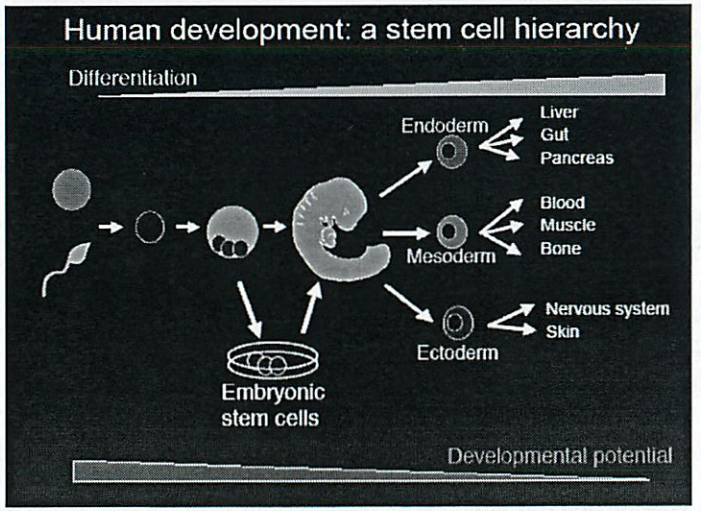
Hence, a mouse ES cell is pluripotent, and can make all of the cell types deriving from the three major layers in the embryo. (ectoderm on outside of early embryo, mesoderm in the middle, endoderm most internal)



In fact, when ES cells are injected into a host mouse they can form a (usually benign) tumor that has sectors resembling a variety of distinct normal adult tissues. (further evidence of the versatility of ES cells)





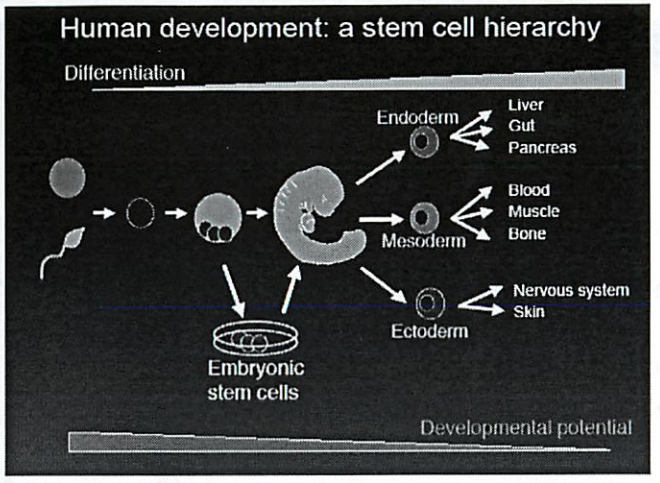


Note that as cells become increasingly differentiated, and thus increasingly committed to forming one specific tissue or another,

Differentiation

They give up the potential to differentiate into other alternative tissues, i.e., they lose their developmental potential

Developmental potential

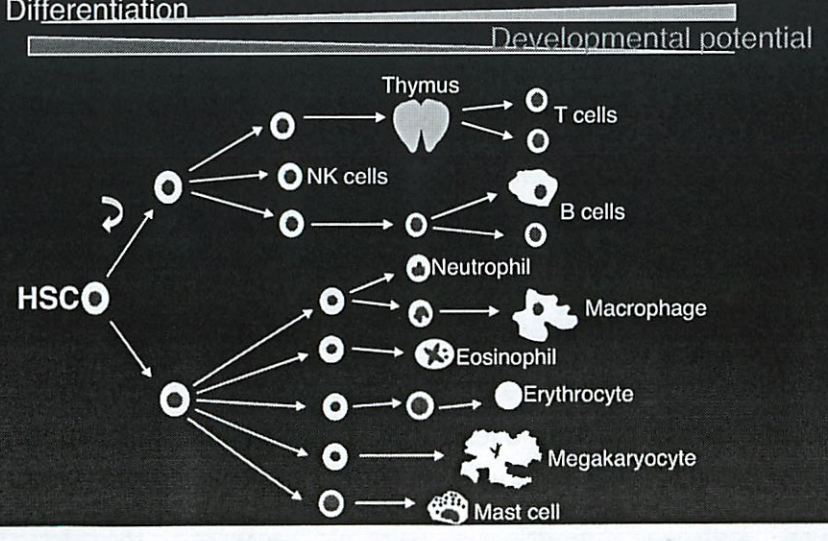


As each tissue forms, its formation is accompanied by, indeed enabled by the formation of tissue-specific stem cells, i.e., stem cells that have become committed to producing the differentiated cells of one or another tissue. Hence, the pluripotent ES cells generates a variety of multipotent or oligopotent tissue-specific stem cells.

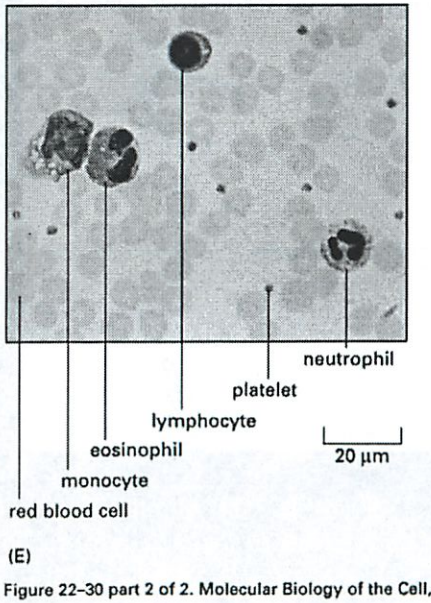
To summarize:
Embryonic stem (ES) cells versus adult stem cells (such as an HSC)

ES cells	Adult stem cells
• Isolated from early embryos	• Isolated from adult tissue
• Can expand indefinitely in culture	• Can not be expanded in culture
• Can give rise to all cell types in the body	• Can only give rise to same tissue

Back to adult stem cells:
Possibly the most versatile of the committed "adult" stem cells is the hematopoietic stem cell (HSC), which can become any of a variety of alternative cell types forming the blood and immune system.



Multipotent stem cells: hematopoietic stem cell spawns cells With distinct phenotypes



(E)

Figure 22–30 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

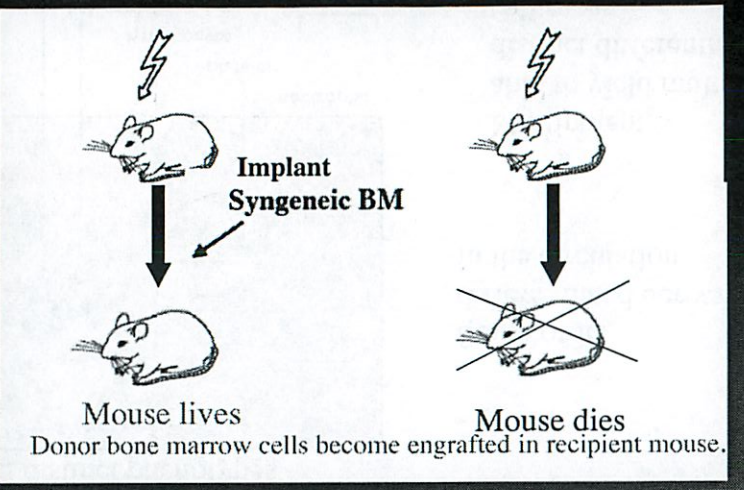
Some of its differentiated derivatives in the circulation

Multipotent = able to yield multiple distinct differentiated cell types

How can we **prove** that there is a single hematopoietic stem cell (HSC) type that can generate all of the cell types that form the blood and immune systems?

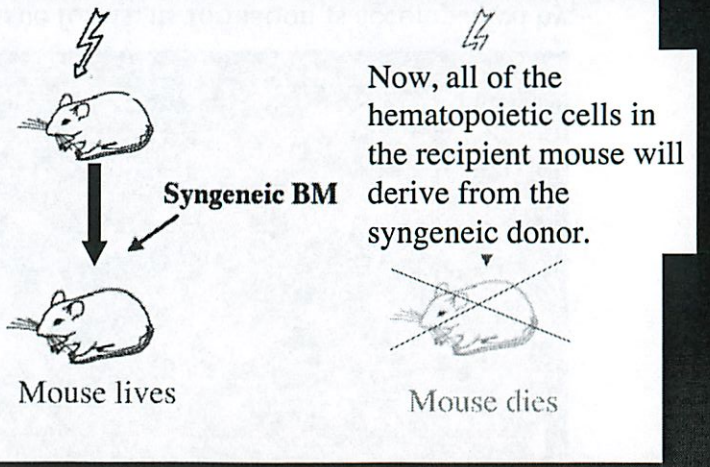
- HSC was first stem cell to be isolated and studied
- Most of what we know about stem cells comes from studies with HSCs
- First and only stem cell used in the clinic
- Discovered by Till and McCulloch in 1961

Experiment of Till and McCulloch (1961): Irradiate a mouse so that its existing hematopoietic tissues are effectively eliminated. In the absence of further intervention, such a mouse will soon die --hence the term "lethally irradiated mouse".

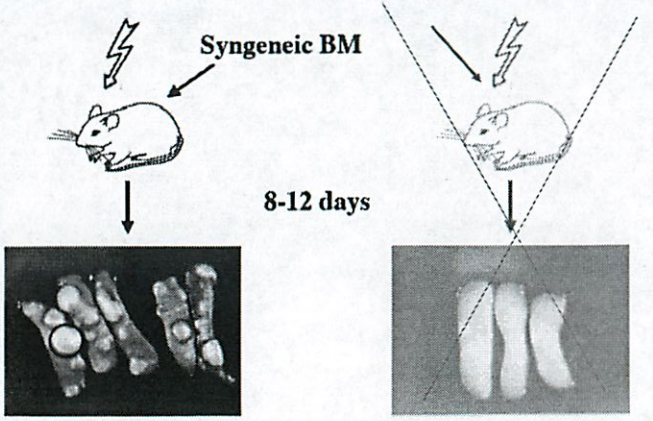


Donor bone marrow cells become engrafted in recipient mouse.

Experiment of Till and McCulloch (1961): Irradiate a mouse so that its existing hematopoietic tissues are effectively eliminated. In the absence of further intervention, such a mouse will soon die --hence the term "lethally irradiated mouse".



In addition to repopulating the bone marrow of the recipients, the donor syngeneic BMCs (=bone marrow cells) will form discrete colonies in the spleens of the transplanted recipient mice.

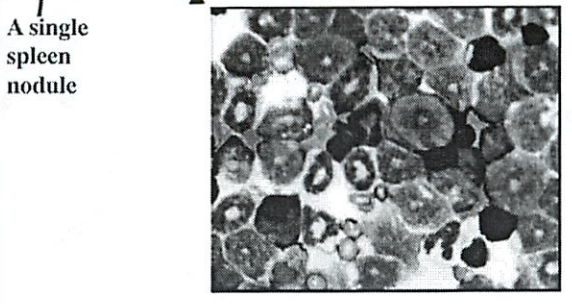


spleens of recipients

Till and McCulloch Radiat.Res. 1961

Cells within single nodules are heterogeneous

red cells, platelet progenitors and granulocytes - i.e., multiple distinct hematopoietic cell types



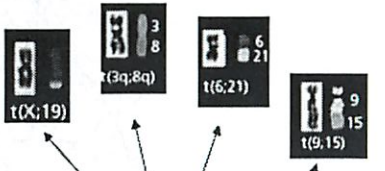
Multipotency

The spherical shape of individual spleen nodules suggested that each nodule was from a single founder cell, i.e.,that each nodule is a **clonal outgrowth** (i.e., all the cells in the nodule are descended from a single founding cell) . **How can we prove this?**

A demonstration of the clonality of a spleen nodule -- that it is a clonal outgrowth derived from a single multipotent stem cell

Weakly irradiate donor mouse (i.e., to **mark its chromosomes rather than to kill BMCs)**

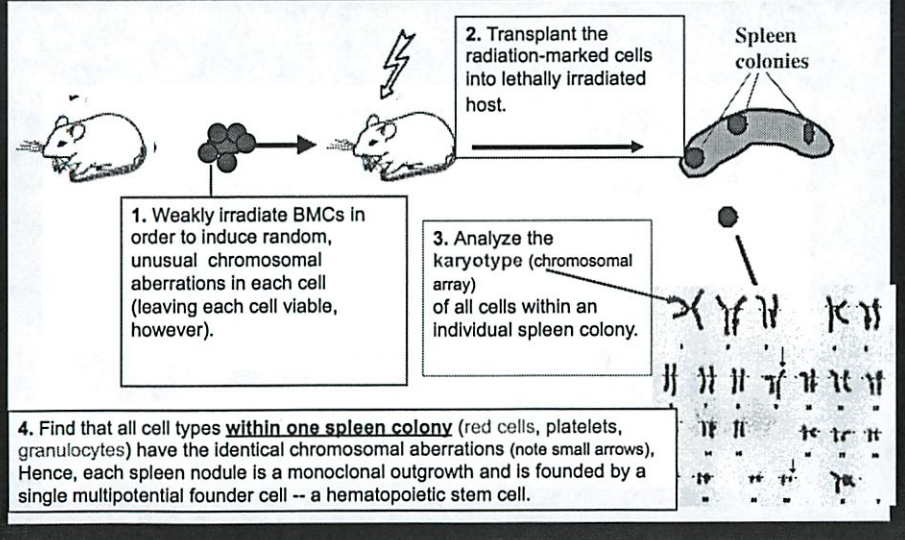
1. Weakly irradiate BMCs in order to induce random, unusual chromosomal aberrations in each cell (leaving each cell viable, however).
Motive: to mark each donor BMC with its own characteristic chromosomal mark -- each donor BMC cell is marked differently.



Following weak irradiation each bone marrow cell has a characteristic, unique chromosomal abnormality

ren

A demonstration of clonality of the clonality of a spleen nodule -- that it is a clonal outgrowth derived from a single multipotent stem cell



4. Find that all cell types **within one spleen colony** (red cells, platelets, granulocytes) have the identical chromosomal aberrations (note small arrows), Hence, each spleen nodule is a monoclonal outgrowth and is founded by a single multipotential founder cell -- a hematopoietic stem cell.

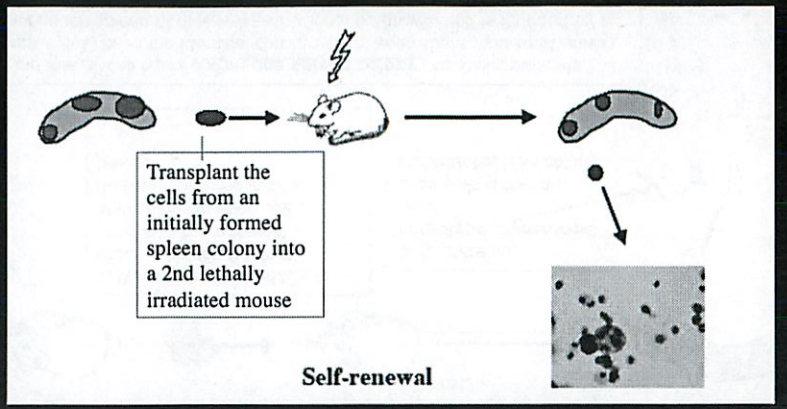
4. Find that all cell types **within a single spleen colony** (red cells, platelets, granulocytes) have the identical chromosomal aberrations (note small arrows),

Cells within single colonies are heterogeneous

Multipotency

Hence, each spleen nodule is a monoclonal outgrowth and is founded by a single multipotential founder cell -- a hematopoietic stem cell.

First demonstration of stem cell self-renewal



A single colony, upon secondary transplantation, gives rise to multiple functionally equivalent colonies. Hence, a single HSC, upon transplantation, can make more copies of itself.

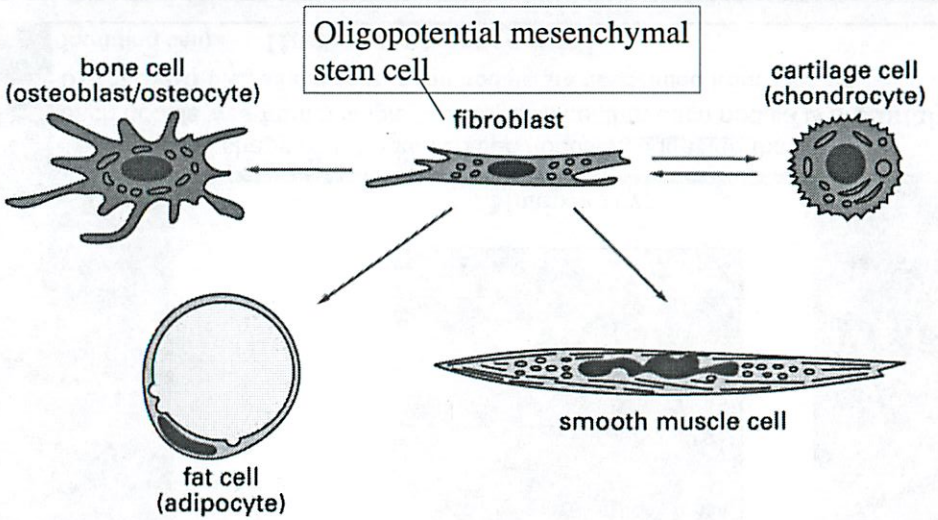
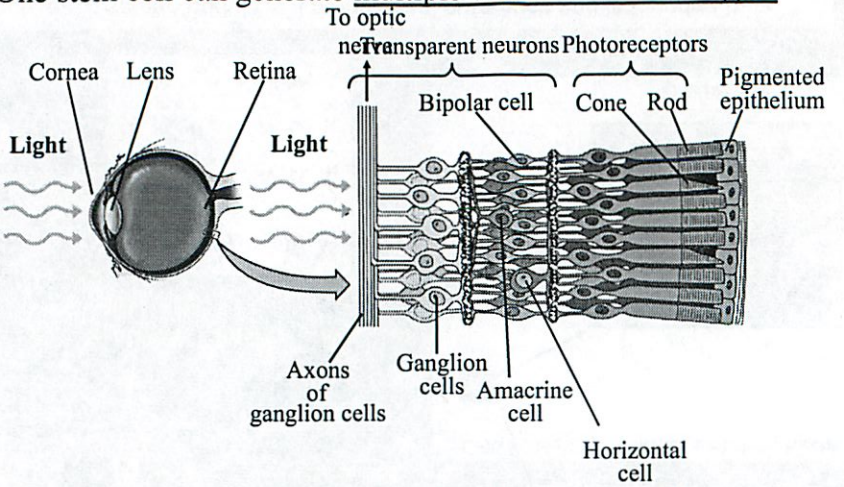


Figure 22–45. Molecular Biology of the Cell, 4th Edition.

Figure 45.20 *The Retina*

Other examples: Oligopotential adult stem cells: the eye
One stem cell can generate multiple differentiated cell types



The oligopotential stem cell in the retina is not really an "adult" cell; it is a committed, lineage-specific stem cell.

Oligopotential stem cells: the brain
One stem cell can generated multiple specialized neuronal cell types

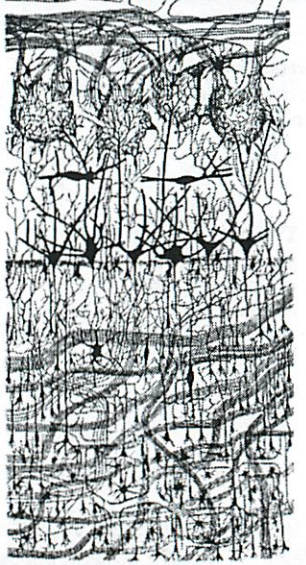
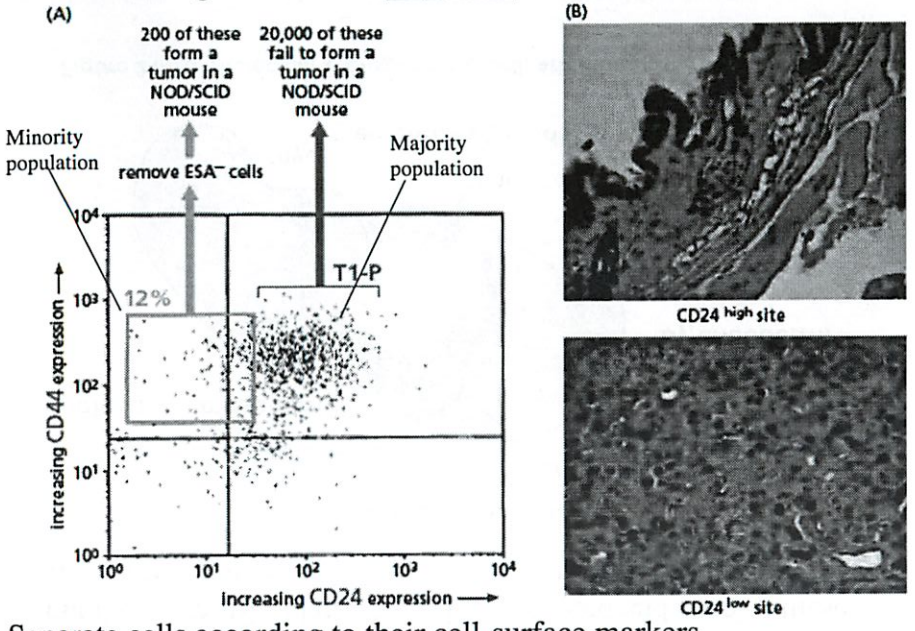


Figure 21–89. Molecular Biology of the Cell, 4th Edition.

Tumors are organized in the same way as normal tissues!



Separate cells according to their cell-surface markers

In the context of a tumor cell population

Tumor-initiating cell (sometimes called a "cancer stem cell")

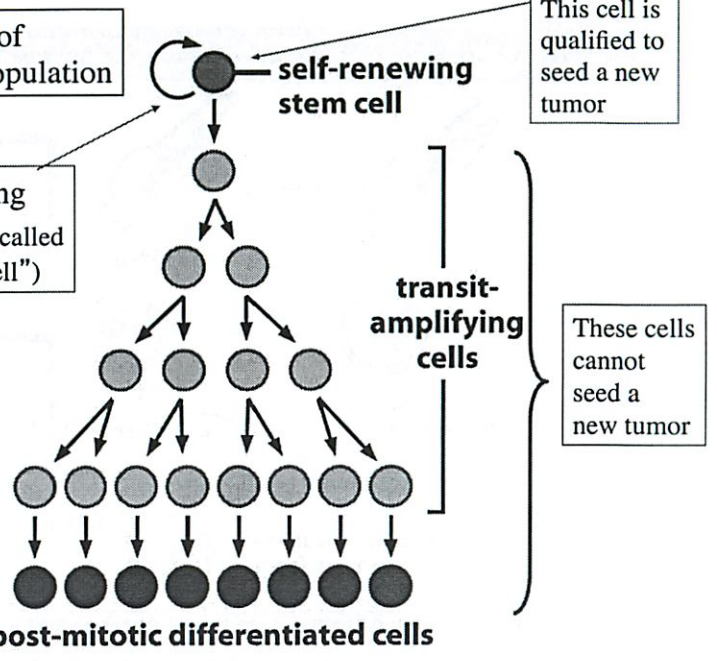


Figure 11.16b *The Biology of Cancer* (© Garland Science 2007)

Within a human breast cancer

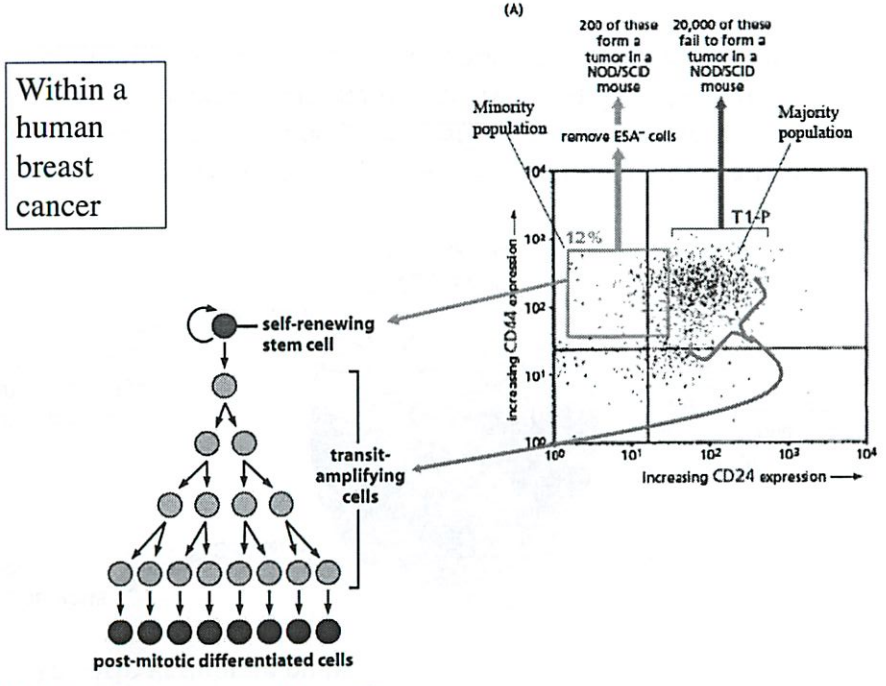
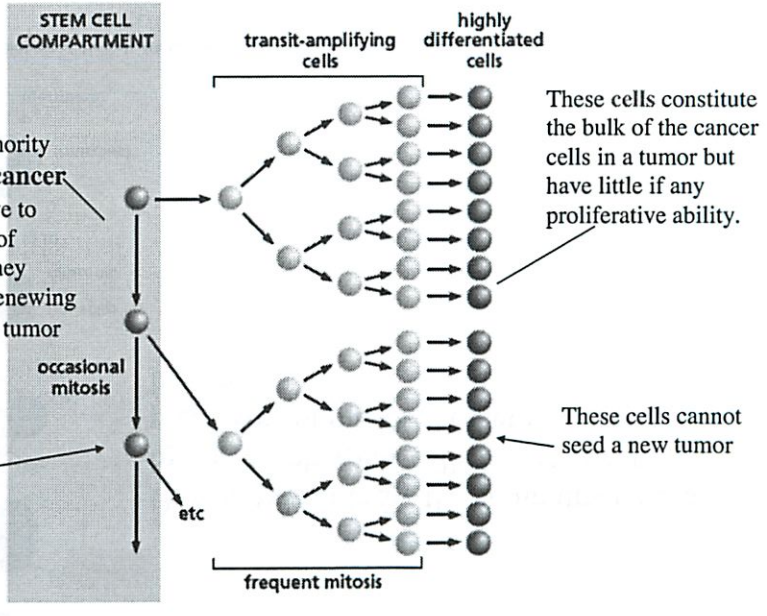


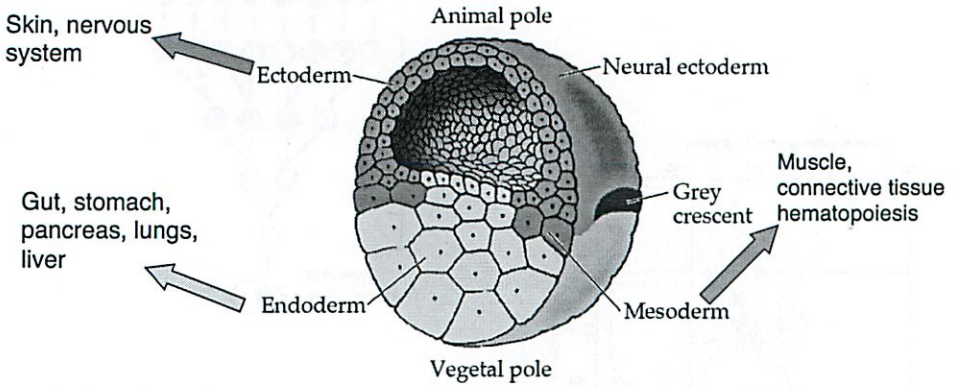
Figure 11.16b *The Biology of Cancer* (© Garland Science 2007)

In tumors, a minority population -- the **cancer stem cells** -- serve to generate the bulk of the cancer cells; they are the only self-renewing cancer cells in the tumor

These cells can seed a new tumor



Let's return now to developmental biology aka embryology
An early vertebrate embryo



In a chordate embryo (e.g., a vertebrate like us) the cells in an early embryo already are destined to form a variety of specialized cells in the future organism, i.e., they are committed to enter into one or another differentiation lineage.

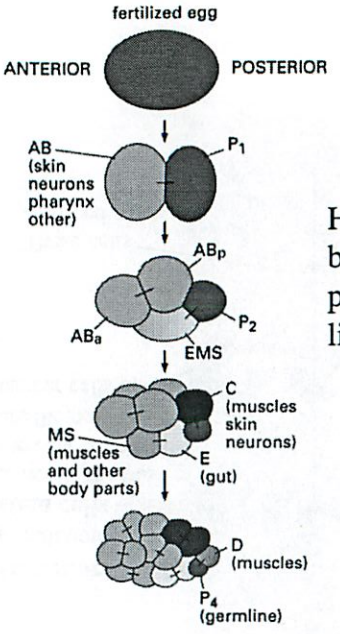


Figure 21–19. Molecular Biology of the Cell, 4th Edition.

How irreversible are the commitments made by early embryonic cells to become the precursors of one or another embryonic lineage?

Later in embryogenesis, the major cell layers have been laid out but these are still relatively undifferentiated and each of these cell layers then differentiates into specific cell lineages.

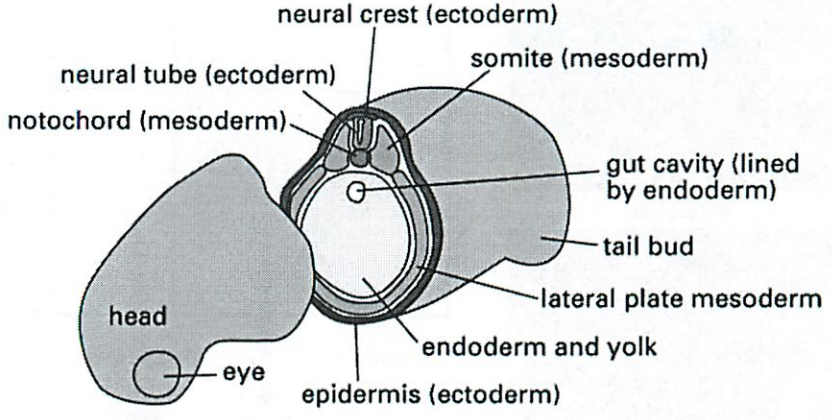
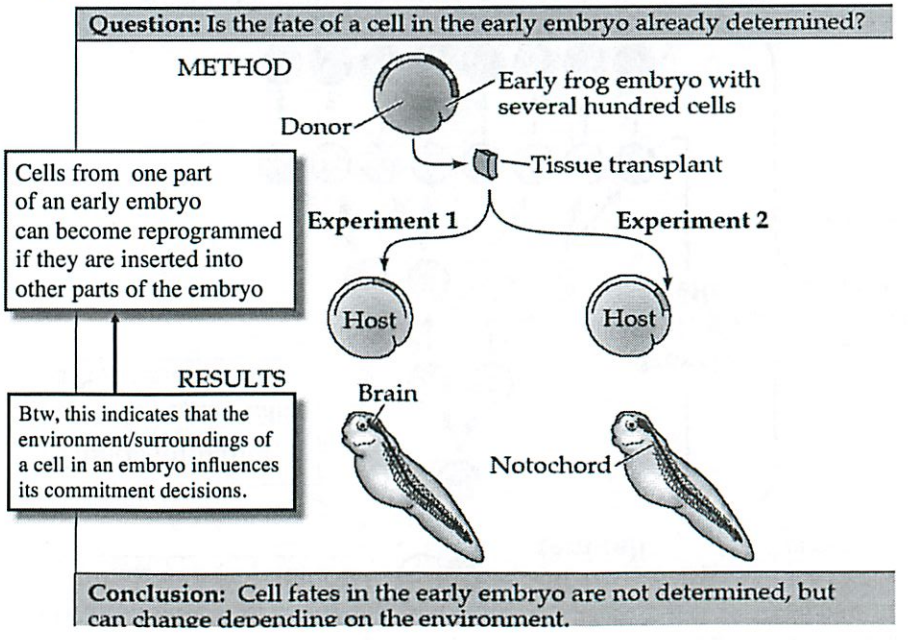
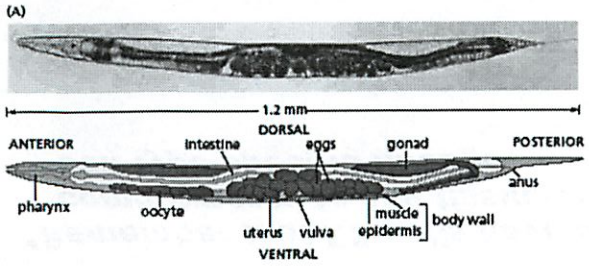


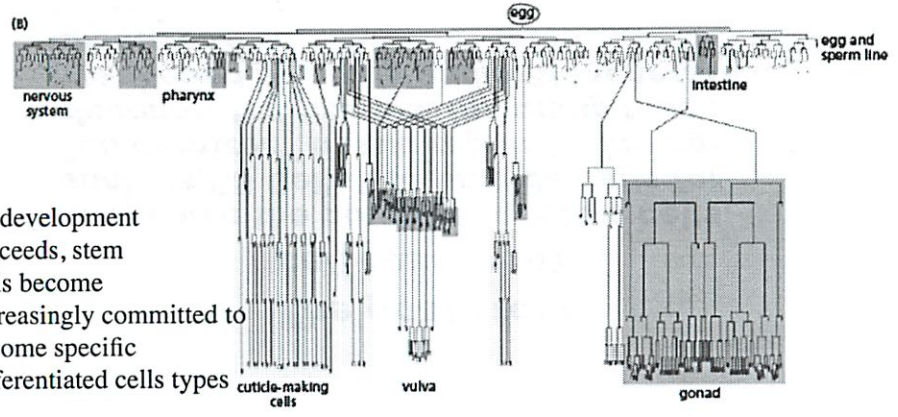
Figure 21–70. Molecular Biology of the Cell, 4th Edition.

Early embryonic cells are plastic, i.e. they are not irrevocably committed to enter one or another differentiation lineage





Such commitment decisions are made in all phyla. Cell lineages in the *C. elegans* worm



As development proceeds, stem cells become increasingly committed to become specific differentiated cells types

Remarkably, each of the genes in a Hox gene cluster is specialized to program the subsequent development of one or another early embryonic (head-to-tail oriented) segment, and the order of these genes in the Hox cluster is co-linear with the physical segments along the head-to-tail axis of the embryo itself!

More astounding, the same head-to-tail organization, mediated by similar Hox gene clusters, characterizes our own (chordate/mammalian) embryos, a vestige of the time when our common ancestor with the fly 600 MM years ago when we and flies arose from a common segmented-like worm ancestor

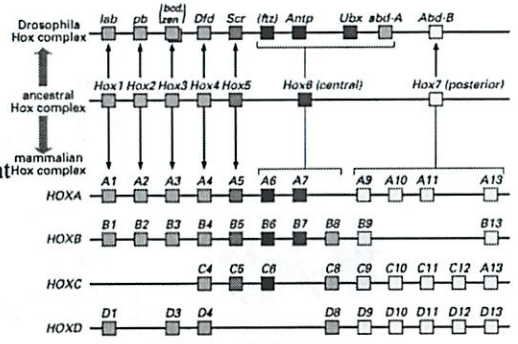


Figure 21–45 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

Figure 21–45 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

These commitments are also made along the head-tail lineage of many metazoa

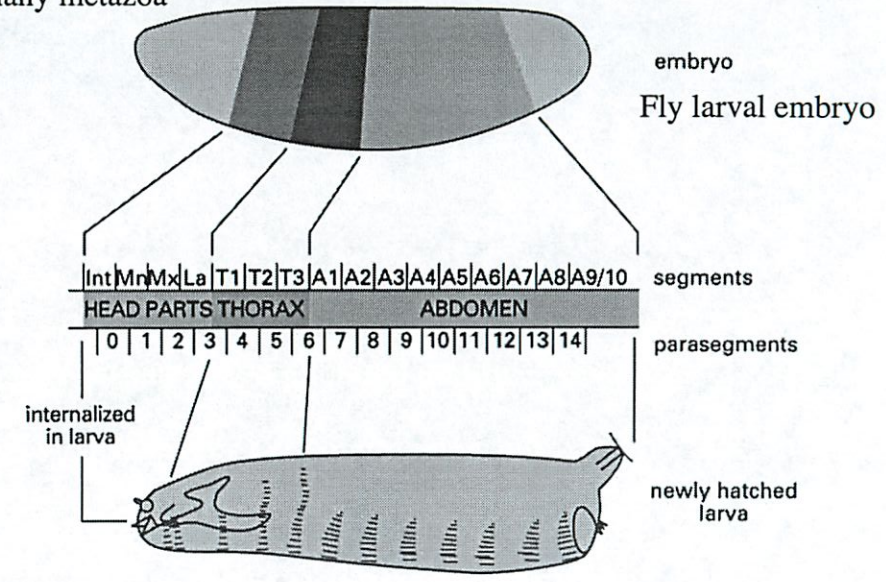
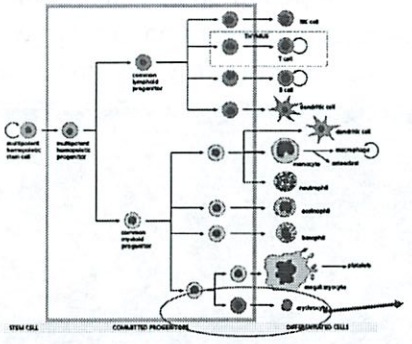


Figure 21–26. Molecular Biology of the Cell, 4th Edition.

Even in the **adult**, decisions about differentiation are continually being made in certain tissues!



Let's examine the dynamic regulation of how one arm of the hematopoietic system is regulated in the adult -- that involved in erythropoiesis, the manufacture of red blood cells/erythrocytes.

Enucleation = ejection of cell nucleus

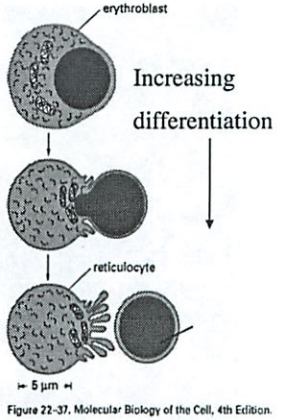
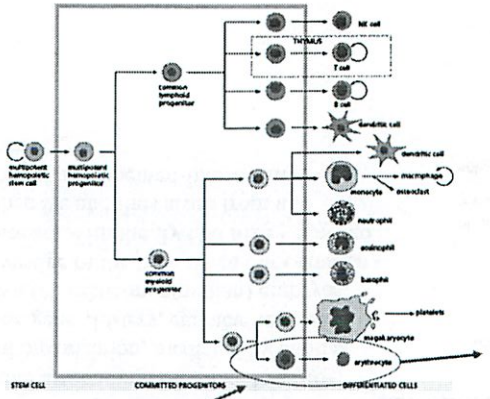


Figure 22–37. Molecular Biology of the Cell, 4th Edition.

Erythrocyte (= red blood cell) precursor



The rate of cell division and of cell production through this arm of the hematopoietic system is **regulated** in adults -- it changes in response to physiologic needs.

Enucleation = ejection of cell nucleus during red cell development

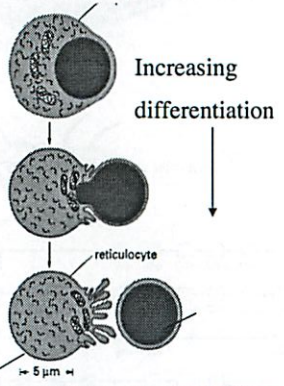


Figure 22-37. Molecular Biology of the Cell, 4th Edition.

Erythrocyte (= red blood cell) precursor

Let's turn now to a related area: The quest for eternal youth!

The Fountain of Eternal Youth-- Lucas Cranach the Elder
Will stem cell research offer what he dreamt of?
Can one regenerate damaged or aged human tissues/organs?

The goal stem cell research?

Can one use the potential of stem cells, such as ES Cells* *to provide cells for "customized" tissue repair in damaged or degenerated tissues, including those weakened by aging and/or degenerative diseases?*

***Remember that ES cells have not yet made commitments to any tissue-specific or cell-type-specific lineage.**

Degenerative Diseases

Alzheimer's – Forebrain neurons

Parkinson's – Midbrain neurons

ALS – Motor neurons

Cardiovascular diseases – Cardiac muscle cells

Type I diabetes – Pancreatic β cells

D. Melton

What is the goal stem cell research?

Using the potential of stem cells, such as ES Cells *to provide cells for "customized" tissue repair in damaged or degenerated tissues, including those weakened by aging and/or degenerative diseases.*

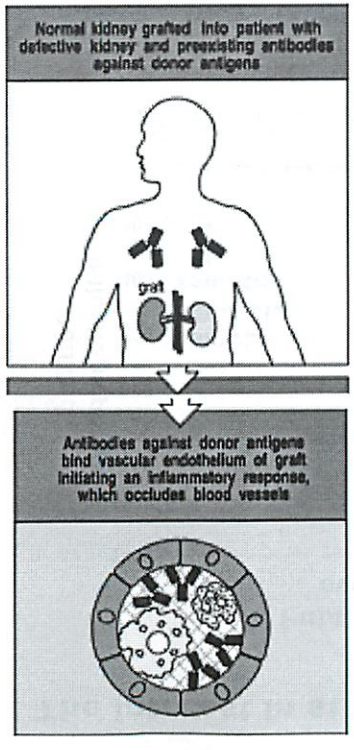
The main thrust: to replace damaged or missing cells within a tissue by introducing "fresh" cells into that tissue, including those not damaged by organismic aging.

What is the goal stem cell research?

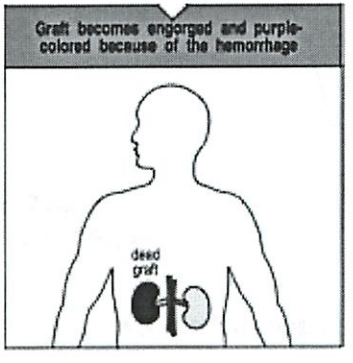
Using the potential of stem cells, such as ES Cells *to provide cells for "customized" tissue repair in damaged or degenerated tissues, including those weakened by aging and/or degenerative diseases.*

The main thrust: to replace damaged or missing cells within a tissue by introducing "fresh" cells into that tissue, including those not damaged by organismic aging.

Major problem: Most sources of tissue will derive from non-syngeneic (genetically non-identical) sources, such as organ donors. Such tissues will be histo-incompatible because they will display different sets of MHC molecules on the cell surfaces; without lifelong immunosuppression, such implanted cells will be rejected by the immune systems of the recipients.



In the absence of immunosuppressive drugs, a foreign donor organ will be rejected by the immune system.



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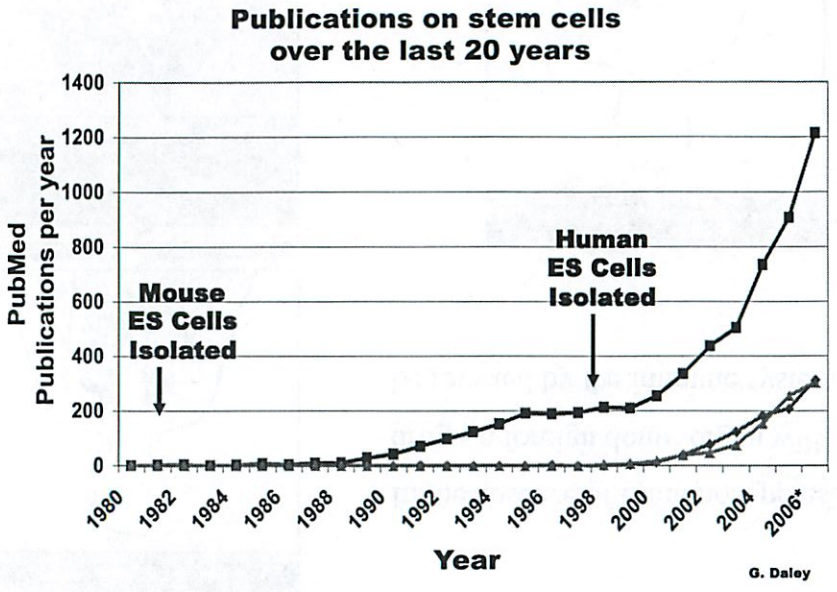
Major solution: Take cells from a patient's own body, convert them into undifferentiated stem cells of various sorts, and then reimplant them into the patient, hoping they will differentiate properly in the patient's tissues. Alternatively, the cells can be induced to differentiate *in vitro* prior to implantation.

The interest in stem cells has grown exponentially



Recall early mammalian development -- blastocysts

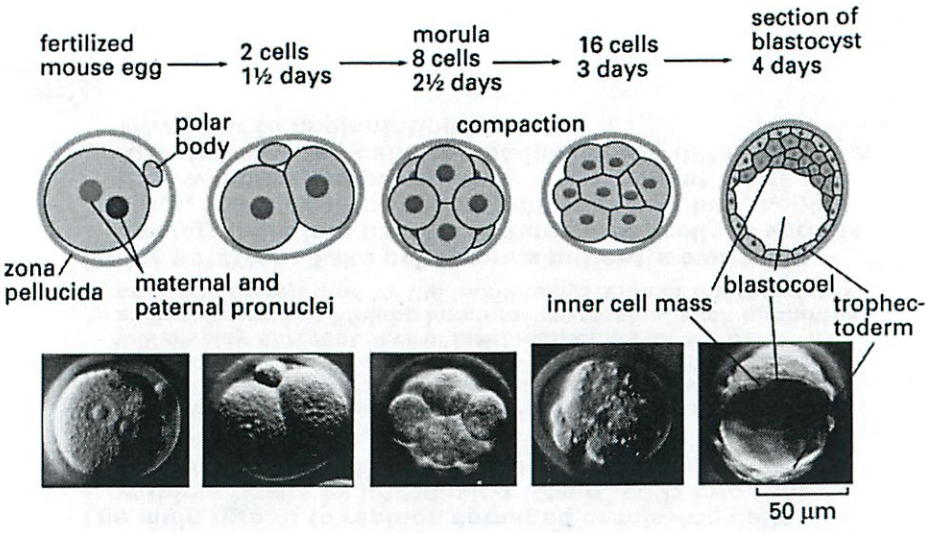


Figure 21–83. Molecular Biology of the Cell, 4th Edition.

Plasticity *in vivo* of early embryonic cells

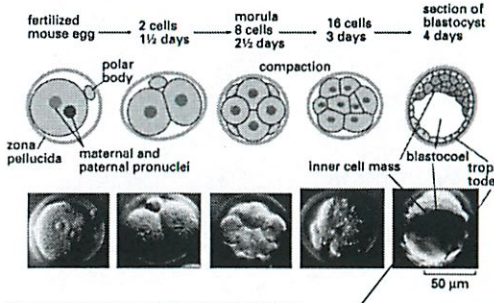


Figure 21–83. Molecular Biology of the Cell, 4th Edition.

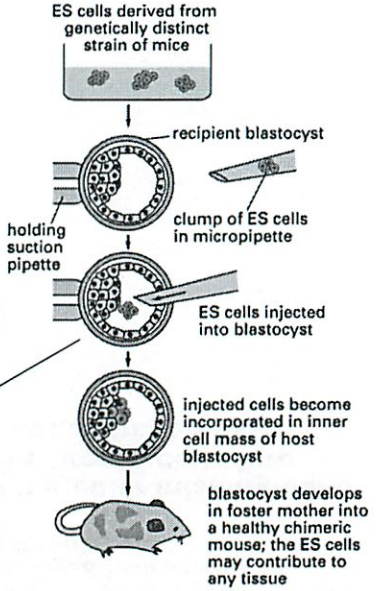
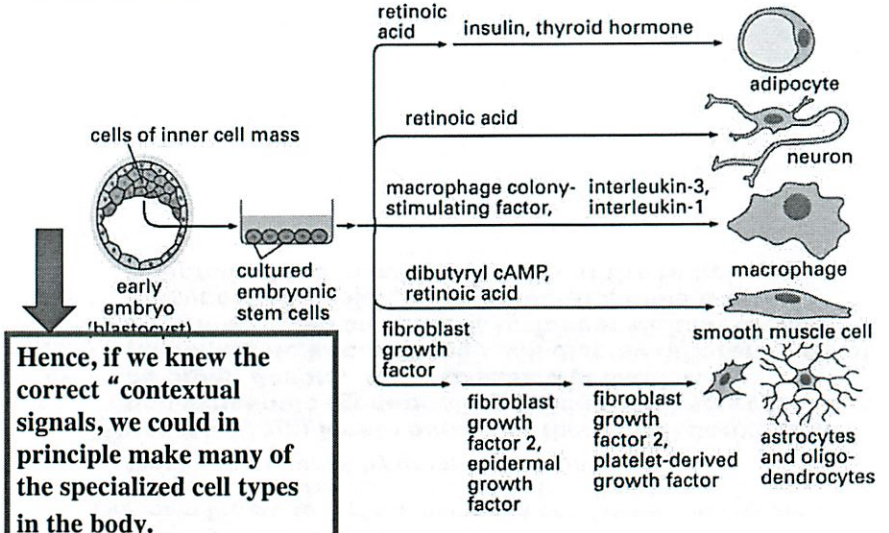


Figure 21–86. Molecular Biology of the Cell, 4th Edition.

The embryonic stem (ES) cells extracted from one inner cell mass can be injected into a blastocyst of another embryo and become incorporated into the cells of that embryo. The fact that these introduced ES cells can form any and all the organs of a mouse indicates that they are **pluripotent.** (They' d be totipotent if they could also form the placenta, which they can' t.)

Plasticity (multipotency) of ES cells prepared from the inner cell mass can also be demonstrated by inducing these **to differentiate** *in vitro* under various conditions.



Hence, if we knew the correct "contextual signals, we could in principle make many of the specialized cell types in the body.

-igure 22–57. Molecular Biology of the Cell, 4th Edition.

Plasticity (multipotency) of ES cells prepared from the inner cell mass can also be demonstrated by inducing these **to differentiate** *in vitro* under various conditions.

"engraft" -- to become established as a graft in an organism's body

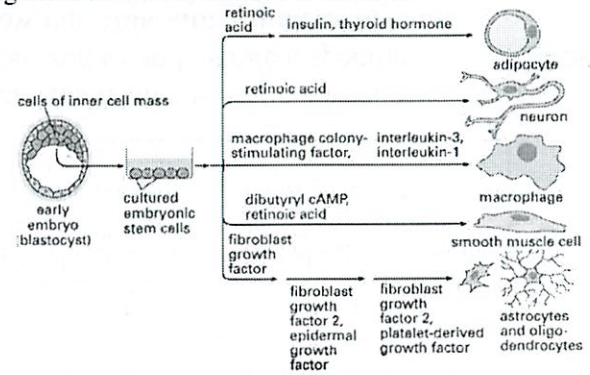
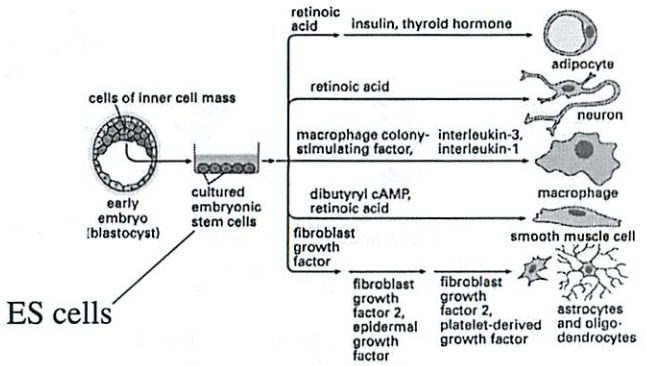


Figure 22-57. Molecular Biology of the Cell, 4th Edition.

Hence, if we knew the correct "contextual signals, we could in principle make many of the specialized cell types in the body.

If these were histocompatible with tissues from your own body, they could be transplanted in and might engraft without the need for immunosuppression!

So, how can one make ES cells that are genetically matched (identical) to all of the somatic cells in a patient's body?



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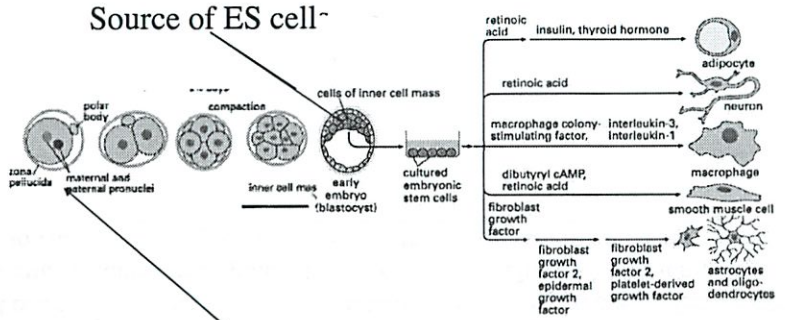

Figure 22-57. Molecular Biology of the Cell, 4th Edition.

One solution: Create a fertilized egg that is genetically identical to the somatic cells of a patient and allow that egg to develop into an early-stage (blastocyst) embryo containing an inner cell mass from which ES cells can be prepared.

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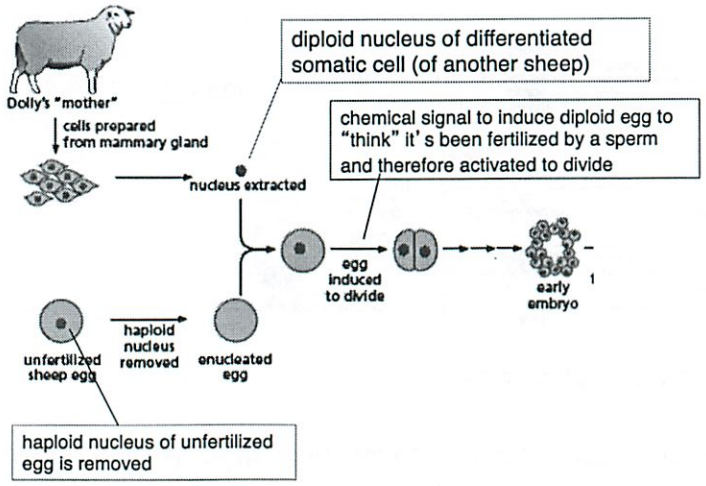
Solution: (Organismic) cloning



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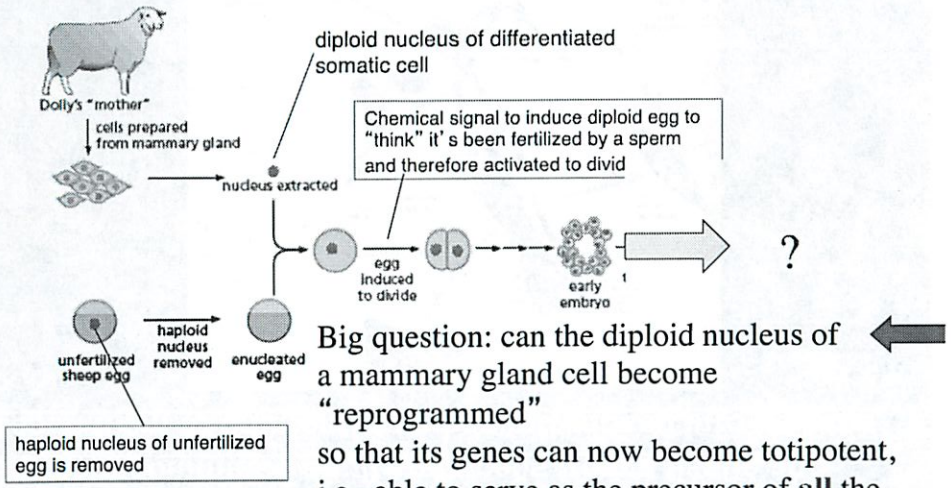

Big question: can the diploid nucleus of a mammary gland cell become "reprogrammed" so that its genes can now become totipotent, i.e., able to serve as the precursor of **all** the

Hence, the nucleus of a somatic cell has some plasticity, and when it is placed within the cytoplasm of an egg, it can be reconfigured to become equivalent to the nucleus of a fertilized egg!

Young Dolly Happy Dolly Pensive Dolly

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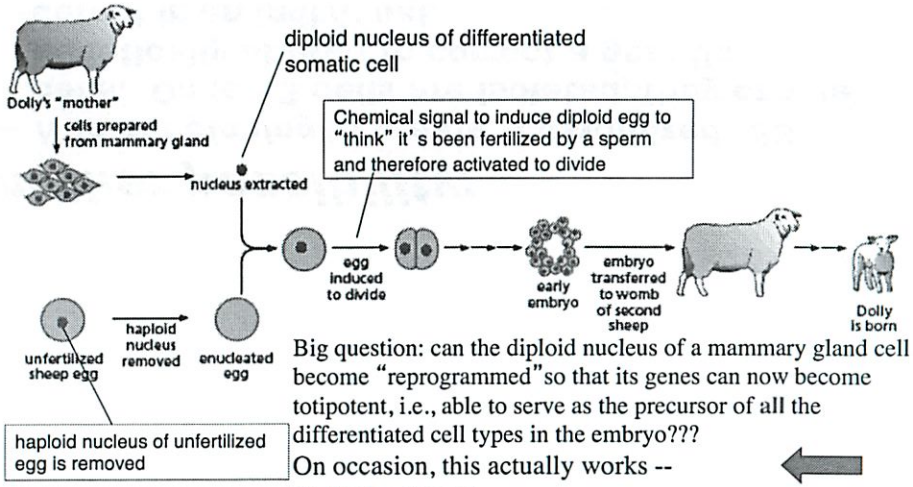
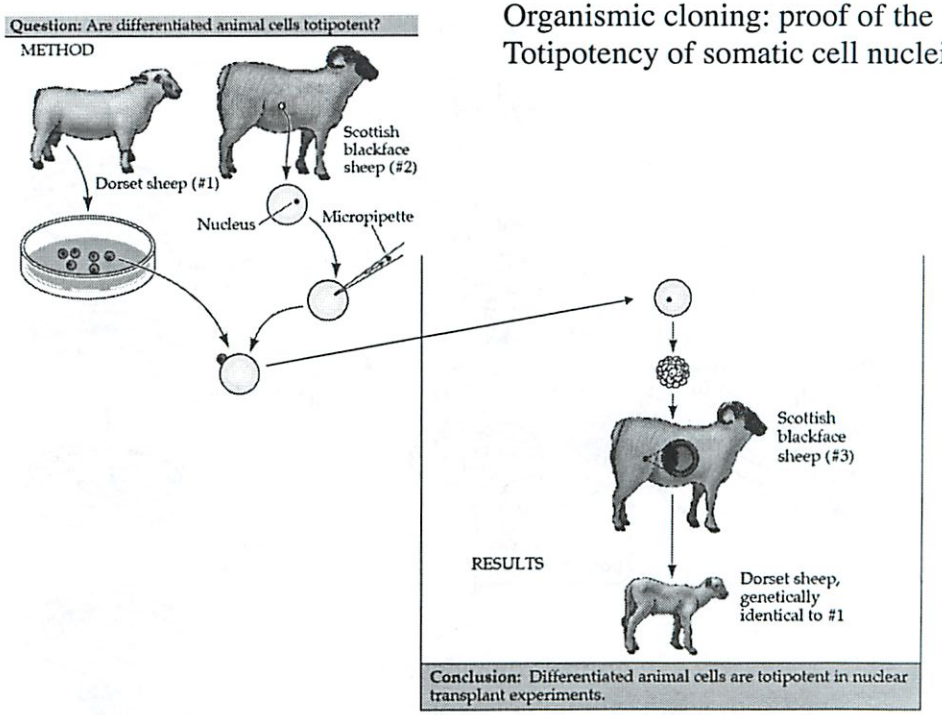


Big question: can the diploid nucleus of a mammary gland cell become "reprogrammed" so that its genes can now become totipotent, i.e., able to serve as the precursor of all the differentiated cell types in the embryo???
On occasion, this actually works -- Dolly the Sheep!

Organismic cloning: proof of the Totipotency of somatic cell nuclei

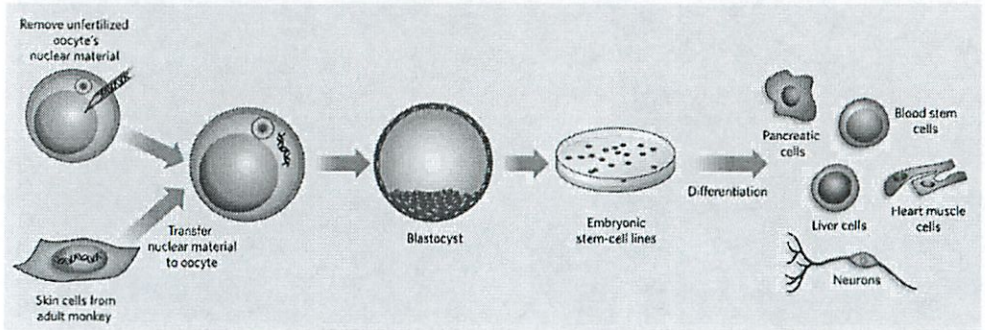


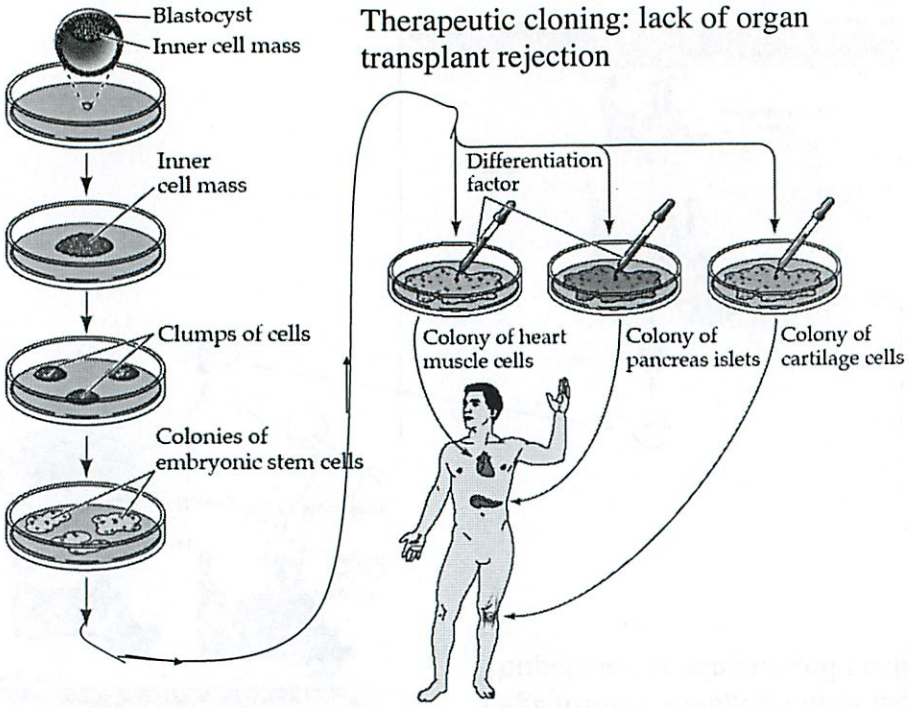
Having shown this plasticity, can one use it to generate ES cell lines from a person's somatic cells?

Advantage: the ES cells will generate histocompatible differentiated cells

Disadvantage: this will require procuring unfertilized eggs from egg donors

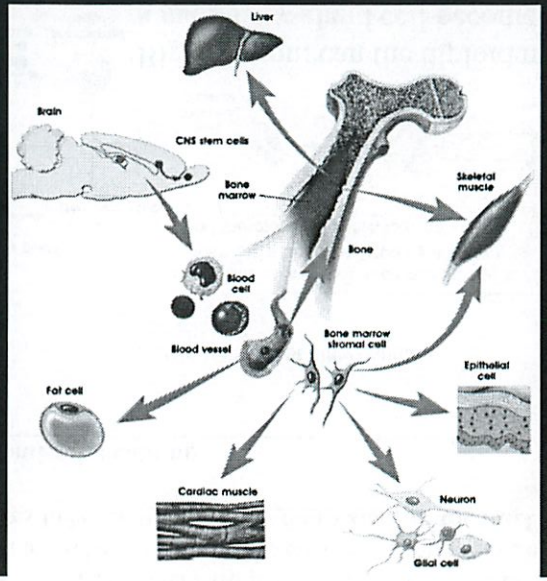
Disadvantage: this requires the formation of an early human embryo, which many feel is ethically unacceptable





Can adult stem cells learn new tricks??

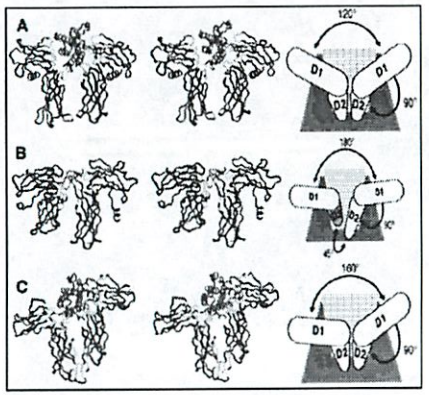
Prepare committed adult stem cells from a tissue and teach them to differentiate when introduced into a tissue.



Another possibility:

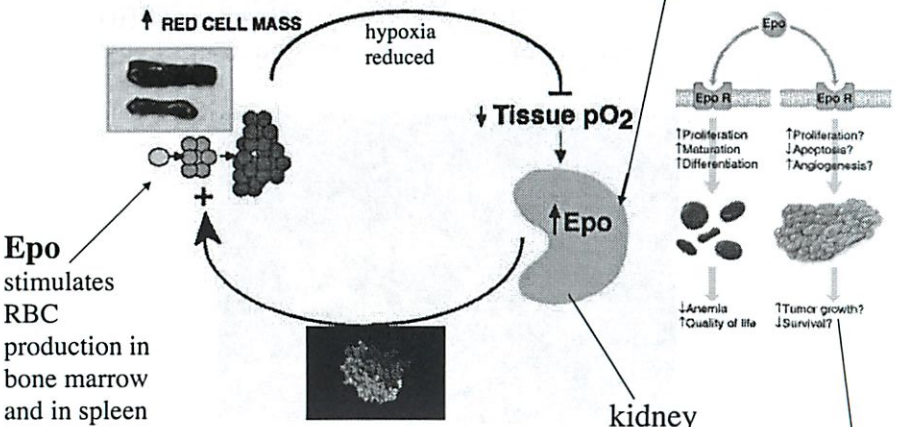
- ***Nuclear cloning*** **to create "customized" ES cells. Once ES cells are isolated, they can be genetically altered to correct a genetic defect in an individual.**

In the bone marrow, EPO (red) binds to the EPO receptor displayed on the surfaces of erythrocyte precursors and activates them biologically, causing them to proliferate and differentiate.



EPO binding to its cognate receptor, called EPO-R. This binding activates tyrosine kinase signaling in the cytoplasm of these red cell precursor cells.

Low tissue oxygen (= hypoxia) stimulates EPO synthesis in the kidney



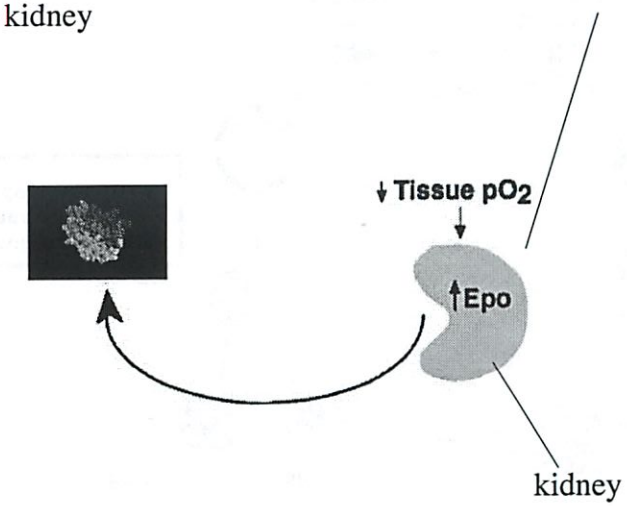
Epo stimulates RBC production in bone marrow and in spleen

kidney

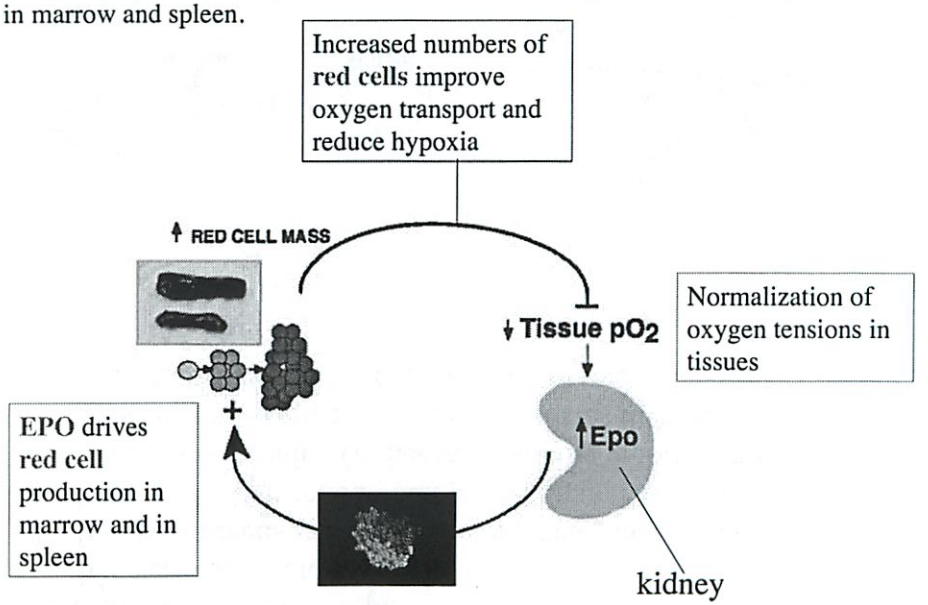
Epo production can also foster tumor growth

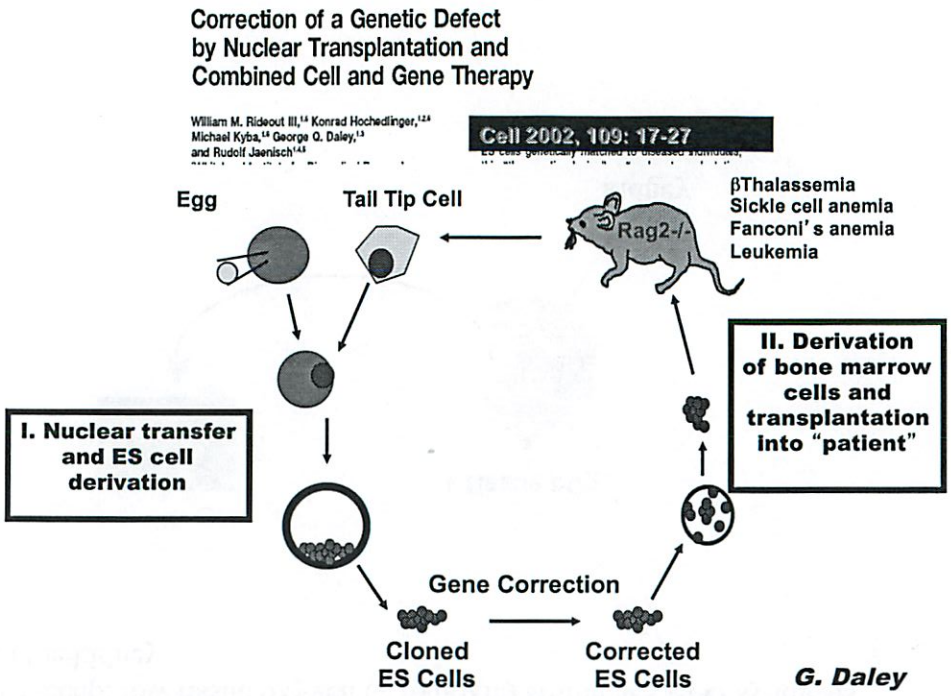
RBC= red blood cell=erythrocyte

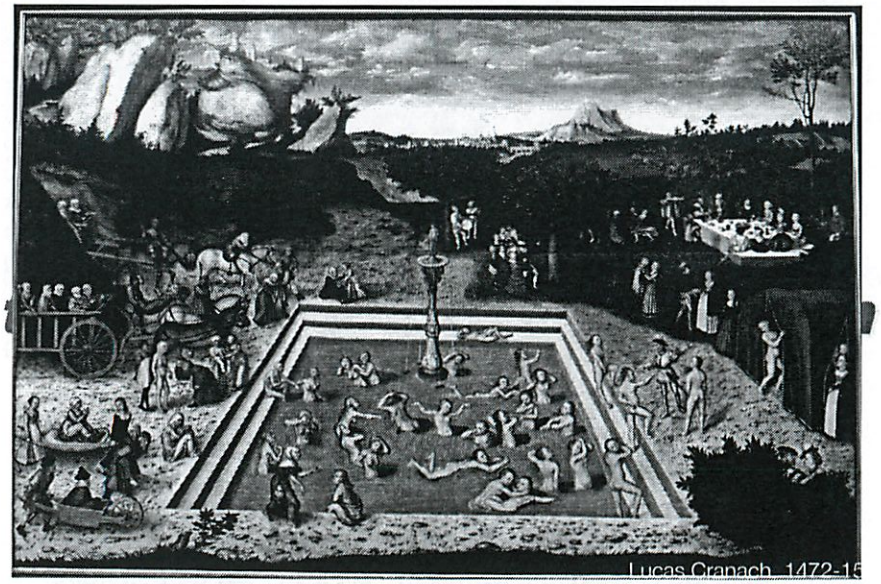
To recap: low tissue oxygen (= hypoxia) stimulates EPO synthesis in the kidney



To recap: low tissue oxygen (= hypoxia) stimulates EPO synthesis in the kidney. EPO then proceeds to stimulate erythropoiesis -- red cell production in marrow and spleen.







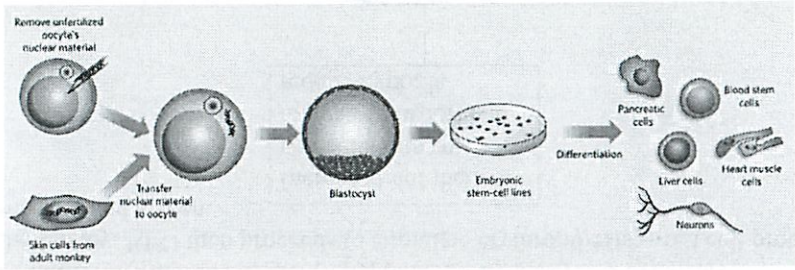
The Fountain of Eternal Youth-- Lucas Cranach the Elder

So, what's your guess?

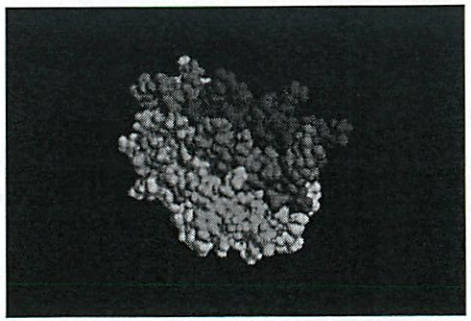
Will stem cell research offer what he dreamt of?

This all sounds great except:

1. It's very labor intensive
2. It only succeeds in a small percentage of manipulations
3. It involves destruction of an early human embryo.
4. Organismic cloning (rather than making embryonic stem cells) fails almost invariably and the organisms that are cloned are almost always defective in one way or another.

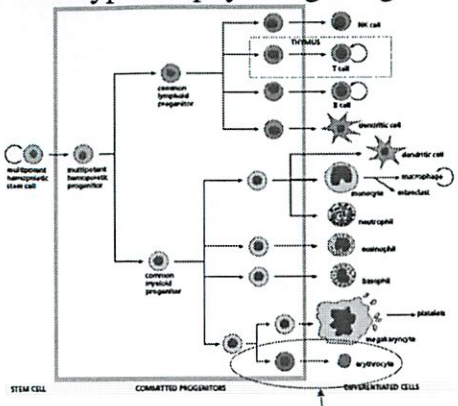


How is erythropoiesis regulated to ensure that we have a reasonable level of circulating red blood cells?
When oxygen tensions are low or when the blood is delivering inadequate levels of oxygen to the tissues

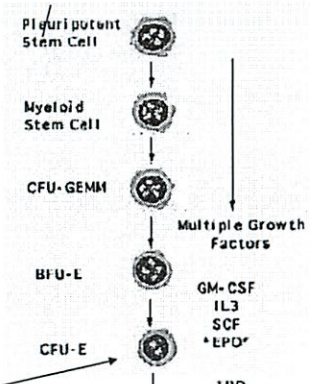


The kidney begins to crank out erythropoietin (EPO) -- a type of growth factor.
It then leaves the kidneys and pass via the blood into the bone marrow.

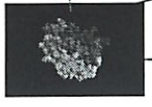
In fact, **all of the arms** of the hematopoietic system are under various types of physiologic regulation throughout life.



Detail of the last steps of erythropoiesis

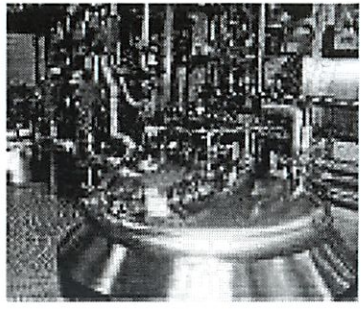


EPO acts here



red blood cell (erythrocyte)

EPO can be made on industrial scale, making $$$$$$ for a small no. of (by-now very large) biotech companies



The industrially manufactured EPO can be used to increase RBC production in anemic patients, including those who have become anemic because of hemorrhage and those who are anemic because their kidneys are failing.

Lecture

11/19

Rational Medicine

Familial Hypercholesterolemia

Put together what we learned

Is rational medicine

↑ designed - understanding of genetics + bio chem

not just stumbled on

heart attacks + cholesterol

discovered this year

Heart Disease

Hearts

- Pump blood around body
- Provides nutrients around body
- Signals → hormones distributed
- Pump waste products from tissue
- Distributes cells - red
  - white

(2)

If damaged → bad

If out of oxygen → bad

Circulatory System

Vessels 

Can get build up of material  Cross section

which causes artery

atherosclerotic plaques

Blocks vessles very bad

Brain ← blockage to brain = stroke

↑

Heart ← heart attack

↓

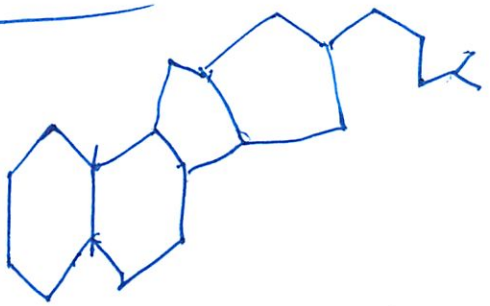
Body

(3)

Death from cardiovascular
~~1~~ 1-2 mil / year

- India + China ~~are~~ used to be beh
But rapidly catching up + exceeding

Cholesterol



Very hydrophobic
waxy
Why is it evil?

Uses

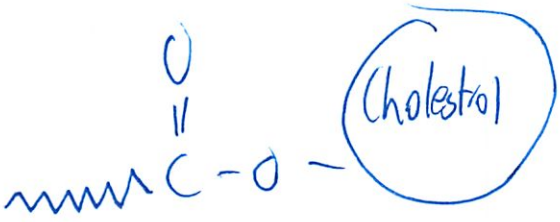
- structural role in membranes
½ lipids in membrane
stiffen membrane

(4)

Biochemical precursor to steroid hormones
- to making Vitimen D
- to making bio acids
  - secreted into digestive system
  - emulsify fats during digestion

not evil, essential!

Can put on ester tail
- slightly more soluble



From 1. Diet
- red meat
- eggs

2. Own Synthesis
CH_3COO^- → → → → Cholesterol
acetate (many steps)

mostly in liver
but lots of cells can make it

(5)

Lipoprotein Particles

~~lipid monolayer~~

(not to scale)

Packages to distribute cholestrol around body

low density lipoproteins → LDL ← ApoB100 used to target
high " " → HDL

Why is cholesterol bad?
is in plaque

lipids responsible for heart attack goes back a while

Lipid Hypothesis

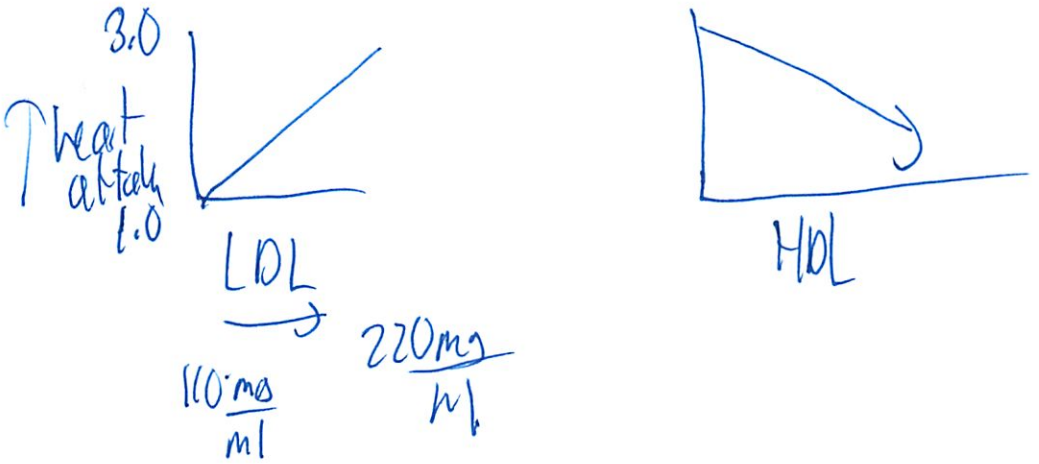
having lots of lipids leads to heart disease

(6)

1856 Virchow

1913 Fed rabbits lipids

1950 on Epidemiology
- like Framingham Heart Study



said to be protective

But this could just be a correlation

If we ↓ LDL will it ↓ heart attack risk?

Esp we want to know the mechanism

7

Genetics of FH

- Familial Hyper (a lot) cholesterol (blood) emia

- Joe Goldstein / Mike Brown

- Found simple Mendelian trait

Normal +/+

Cholesterol normal 15 mg/ml

Heart attack normal age

F_H Hets $\frac{FH}{+}$

~250

~ 15 years early

So 1 in 500

F_H Homo. $\frac{FH}{FH}$

> 600 mg/dl

at age 20

1/million

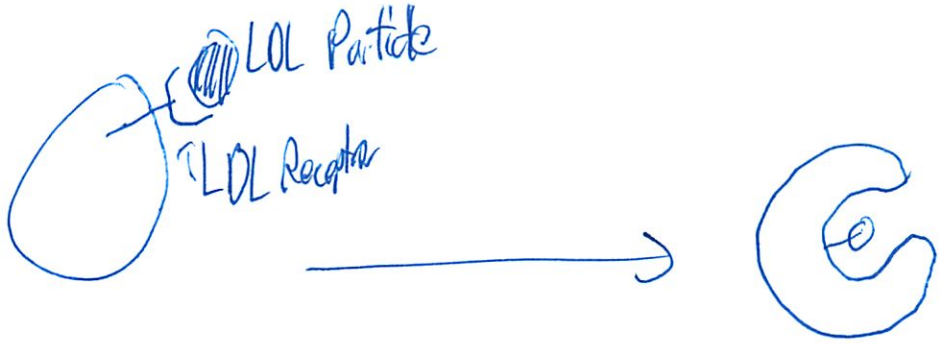
So 1/1000 · 1/1000

Found the gene as well

Gene → Protein → F_1 (code)

Encodes LDL receptor

(8)



esp Liver cells
↳ 75% are in liver cells

if no receptors
Ya are making it
But not clearing it out
Some secondary clearing

*

Synthesis of Cholesterol

acetate → -[--]-- → cholesterol

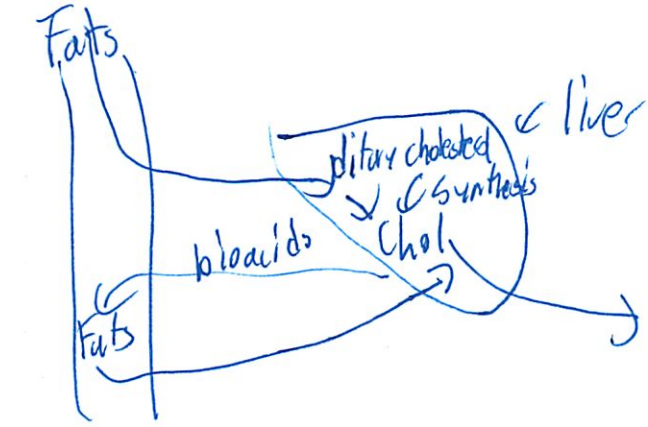
↑ key step
which regulates on amt of cholesterol
HMbCoA reductase
Committed step as well---

(4)

Bio Medical Engineering + Drug Development

Rutland Therapy for FH 2

(heart disease)



↙ liver (since most of LDL)

Digestive System

How will we ↓ LDL in blood?

1. Reduce Dietary Cholesterol
   ~10% reduction
   (not a lot)
   had to prove it has real impact

2. Force bioacids to be excreted, not recycled
   So more cholesterol turned to bio acid
   bioacid binders

(10)

~10% reduction

3. Hit key committed step

Stop HMCoAR

Statins

~50% reduction

remarkably effective

15 mil Americans

Originally designed for those w/ disease

But good for everyone

Recent Discoveries

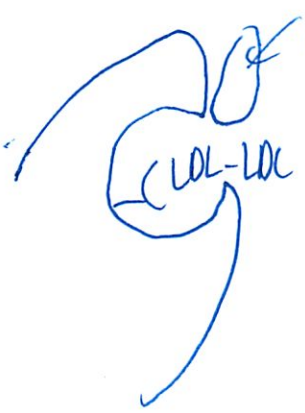
(: not on exam)

1. LDL lowering

new ways to ↓ LDL

(11)

When LDL receptors bring in LDL particles



Pcsk9 targets LDL receptors for distruction

So remove Pcsk9 so get more LDL reptors

But what if you remove all?

People found mutation

That could do it

These people healthy

Pcsk9 +0- Lower LDL

Works well

(12)

HDL Raising

4 cos that inhibit enzymes

Produces higher HDL

↓ work

So should ↓ heart attack

But 1st was higher heart attack

Correlation ≠ not causation

no genetic link!

Dozens of genetic variation → Genetic variation ↓ LDL

→ Genetic variation ↑ HDL

100,000 people

Looked at heart attack association

Confirmed ↓ LDL

no correlation HDL

(13)

Should

Epidemiologist → would be a link

Genetics → actually no link

That's why <u>rational medicine</u>

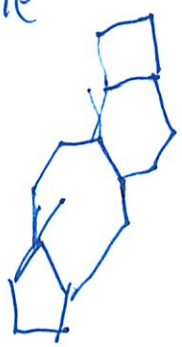
7.012
Recitation

11/20

P Set 6 Due tmo
Exam 3 Wed 11/28/12
Review Tue 11/27/12

Familial hypercholesterolemia

Cholesterol



← not to scale or side #
(~~other~~ Some 5, 6 sided)

1. Dehovo synthesis (body makes)
   acetate → HMG-COA → Melonate → ... → Chl
   ↑ step of no return, rate limiting

2. Food

(7)

Heart Disease

cholesterol deposits

Coronary artery - goes to the heart

HDL	Vary in how much cholesterol it carries	"high" - good
LDL		"low" - bad
VLDL		"very low" - bad

How is cholesterol removed from blood?

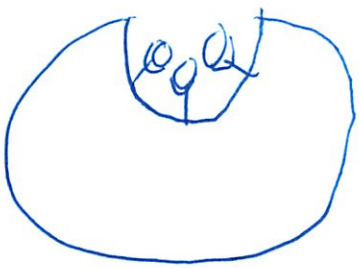
LDL receptor in liver

basis for biosalts - req for digestion

binds

(3)

Receptor mediated endocytosis



bows in

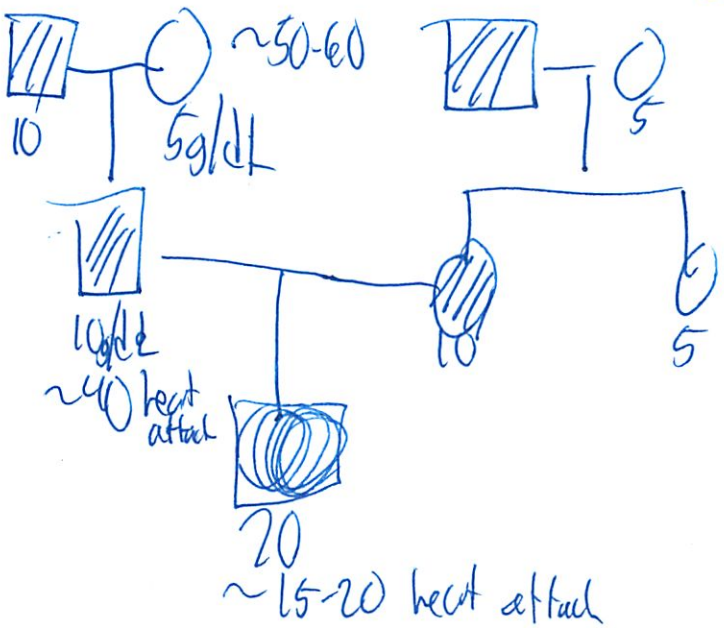
Disease + genetic basis

Case of incomplete dominance

intermediate phenotype

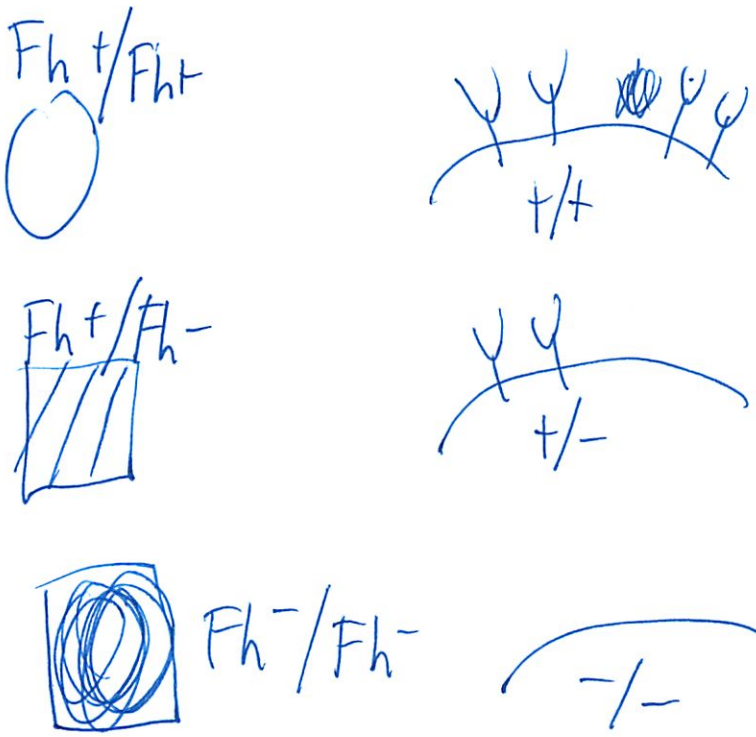
1 copy is not enough to resrve

red + white flowers = pink flowers



(4)

So a quantative relationship



? not enough receptors in the liver

Could be a faulty receptor (mutation) or missing

(5)

Treatments

1. Change of Diet
2. Statings
   inhibit rait limiting step of HMG-COA

Livers' bioacids take a lot of crogy out of the cell + uses a lot of cholesterol

Bio acids reasorbed in large intestin
If block this reuptake
more gets excreated out
So more cholesterol used in making bio acid

3. Lives transplant
4. Gene therapy

(6)

Gene therapy

Liver transplant

Only need 1/3

Will regenerate it

✓ grow in petri dish

So 1. take liver

2. fix gene for # of cells

3. Regrow

4. retransplant

But this hasn't worked

Cells become non-differentiated

(7)

In-vivo

Can put gene inside virus

Virus will put genes in each cell

But also causes cancer

Since it can insert genes anywhere

Pick a virus specific for liver

- Hepatitis

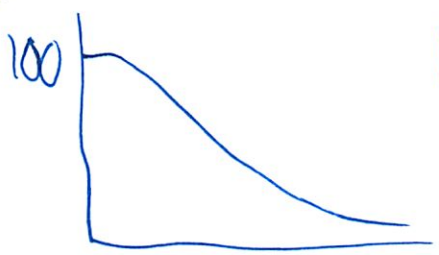
Must weaken it - so only inserts gene

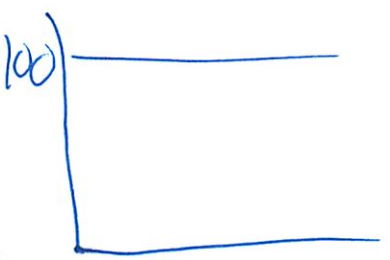
Lots of modifications

(8) Pset 6 q's

5. (missed)

Ex)





heterozygote

since half # of functional receptors

Can also just measure binding

Put radioactive sol

See how much is bound

(9)

Ex2

Wild type → full radioactivity

hetero → half ↓

homo → no

Since issue is binding

tracking amt in solution

Ex1 → measuring in medium

Wild type

Will see

2 → cell surface

wash off solution from cells

Since takes time to go in

~~[illegible]~~

So still going in

lot of radioactivity

Bio Resins

Beads that bind to bioacids + prevent

reuptake

(10)

(missed)

d) Liver promotor

each tissue has own promoter

Even each type of cell

Some promoters all ove body

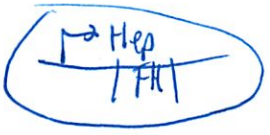
Since some genes need to be expressed

everywhere - like actin

(isp)

So where put Fh put hepastocte (sp)

(live) promoter

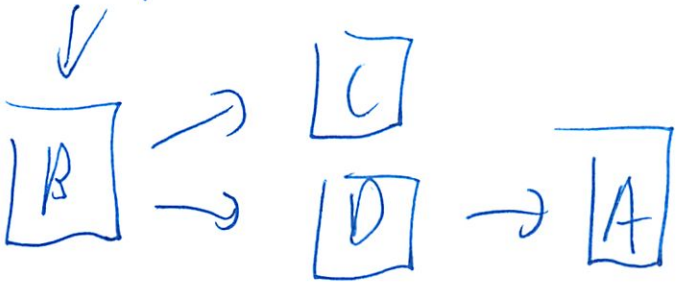


3b)

A	motor nuron	M
B	" sensory, liteal, hipo campl	M, S, L, H
C	S, L, H	
D	M, T	

(4)

Want most potent 1st



T must be a lineage

3d) ~~#2 2~~

e) Synaptic

needs nucleus - to have

not red blood cells

b, t are bad choices

lose DNA through regeneration

f) IPS

- prefer same genotype
- then put back in patient
- otherwise bad reaction

12

Human egg $30-40k
expensive!

4ci) Bcell - only 1 type of b cell

iii) Normal No → but lots of mutation from egg

ii) Yes - want cell not that exposed to env

4b) Reproductive - new org
Therapeutic - to help
Differences are on hand out

7) Find link

11/20

Samin Houshyar
Recitation 17
R27 & 28

Familial Hypercholesterolemia

Cholesterol

Synthesis:

1) *de novo* synthesis: acetate → HMG-CoA → Melanoate →→→→ Cholesterol (key enzyme: _____)
2) Food intake

Role in the body:

1. Major component of bile acids (required for digestion)
2. Structural component of cell membrane

Role of Cholesterol in heart disease

How is cholesterol carried in the body?

People's genotypes: +/+, +/FH, FH/FH. What phenotypes did Brown and Goldstein observe?

Look at uptake of cholesterol with radiolabeled LDL?

+/+

+/FH

FH/FH

Treatments for FH:

1. No hamburgers!!!! i.e. reduce cholesterol in diet
2. Reduce re-uptake of bile acids (remember Le Chatlier's principle of equilibria)
3. HMG-CoA reductase inhibitor (statins)
4. Liver transplant
5. Gene therapy