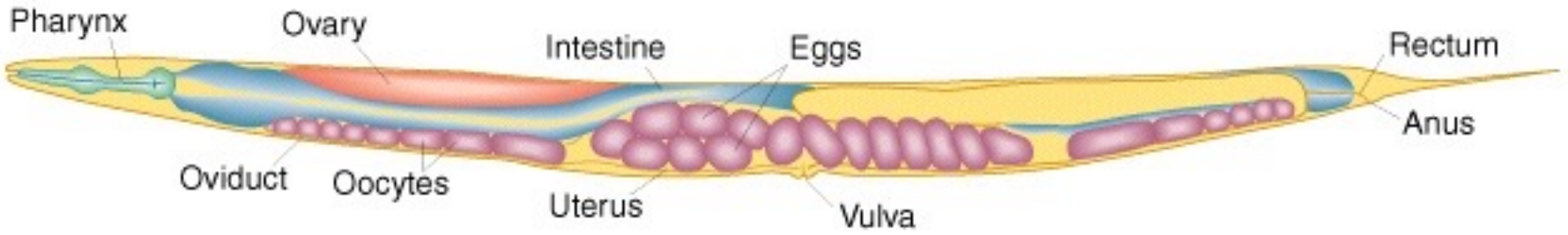
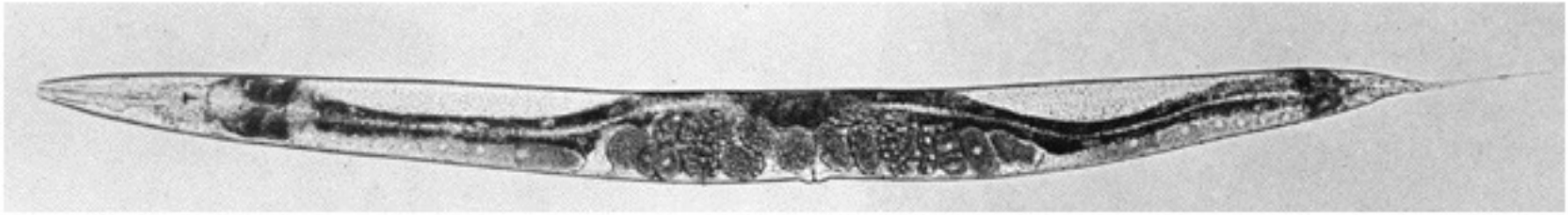
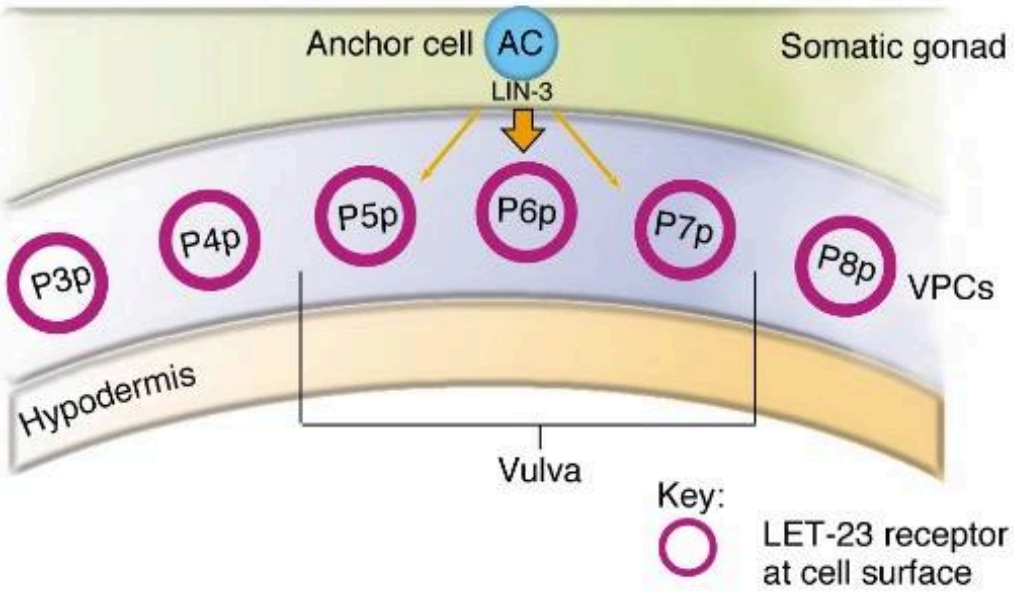
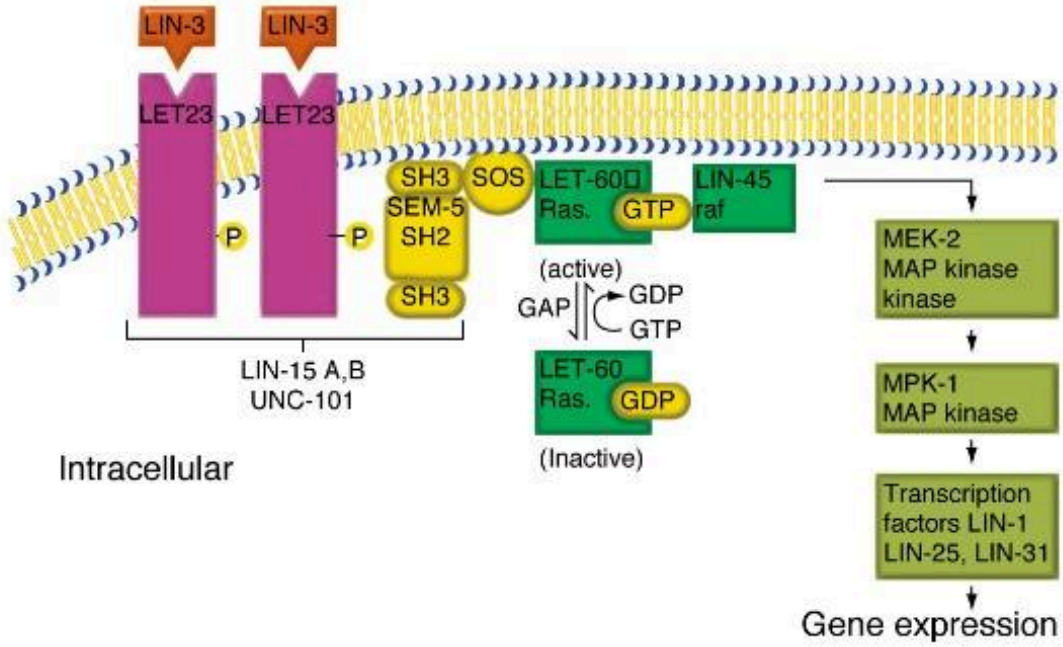

Pharynx
Ovary
Intestine
Eggs
Rectum
Anus
Oviduct
Oocytes
Uterus
Vulva



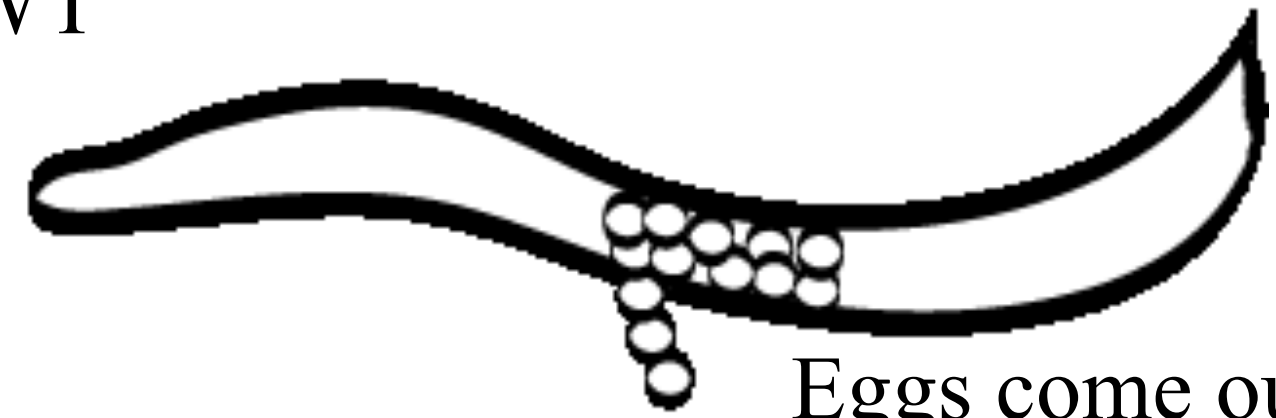
(a)



(c) Extracellular

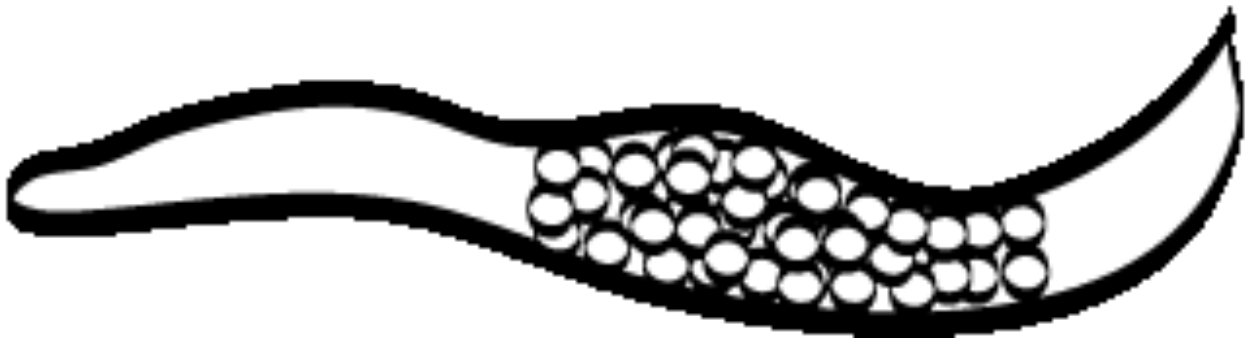


WT



Eggs come out of Vulva

Vulvaless mutant



There is also a multi-vulva phenotype, which allows egg laying and has multiple openings to the environment


vulvaless
phenotype

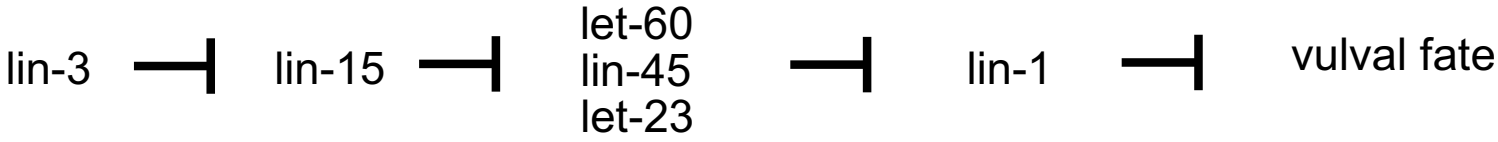
Constructing an epistasis pathway for the vulva

Vul = let-23
lin-3
let-60
lin-45

Muv = lin-15
lin-1

lin-1 epistatic to let-23
lin-1 epistatic to lin-3
lin-1 epistatic to let-60
lin-1 epistatic to lin-45

let-23 epistatic to lin-15
lin-15 epistatic to lin-3
let-60 epistatic to lin-15
let-45 epistatic to lin-15



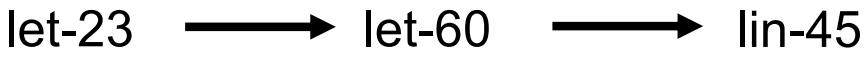
Dominant mutations and epistasis

Epistasis relationships can only be determined between mutations that have different phenotypes. Dominant alleles of mutations that have a similar LOF phenotype can be used to determine epistasis relationships between these genes.

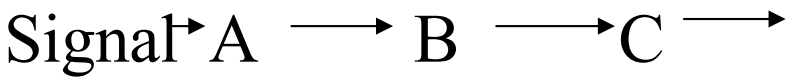
ras = Let-60 GOF = Muv

lin-45; let-60 GOF = Vul

let-23; let-60 GOF = Muv



Ordering genes with the same loss-of-function phenotype



A- = B- = C- = off

How to order genes A, B and C?

Method 1: Constitutive mutations: Gene is on regardless of upstream regulation

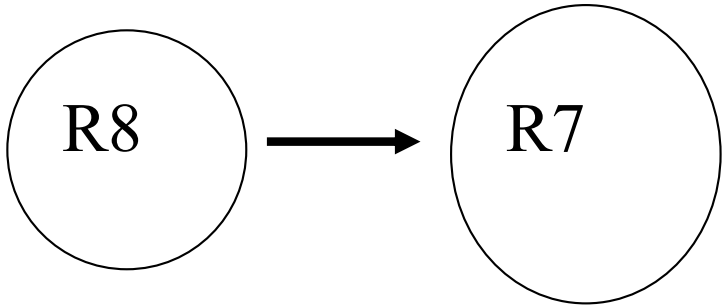
B^c = On

A- B^c = On

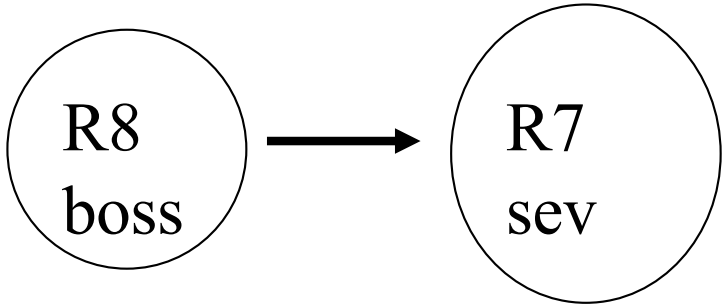
B^c C- = Off

Ordering genes with the same loss-of-function phenotype

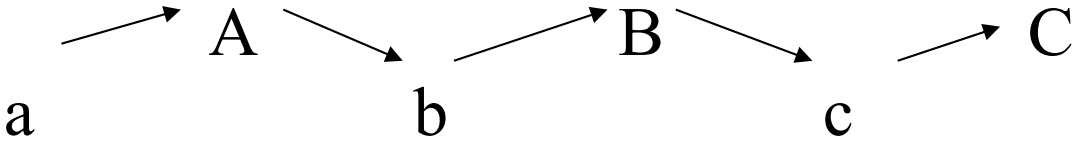
Method 2: site of action in cell signaling



Boss acts in R8
Sev acts in R7



Ordering genes with the same loss-of-function phenotype



Method 3: Molecular epistasis

a- B expression off C expression off
a regulates b and c

b- A expression on C expression off
b regulates c, but not a

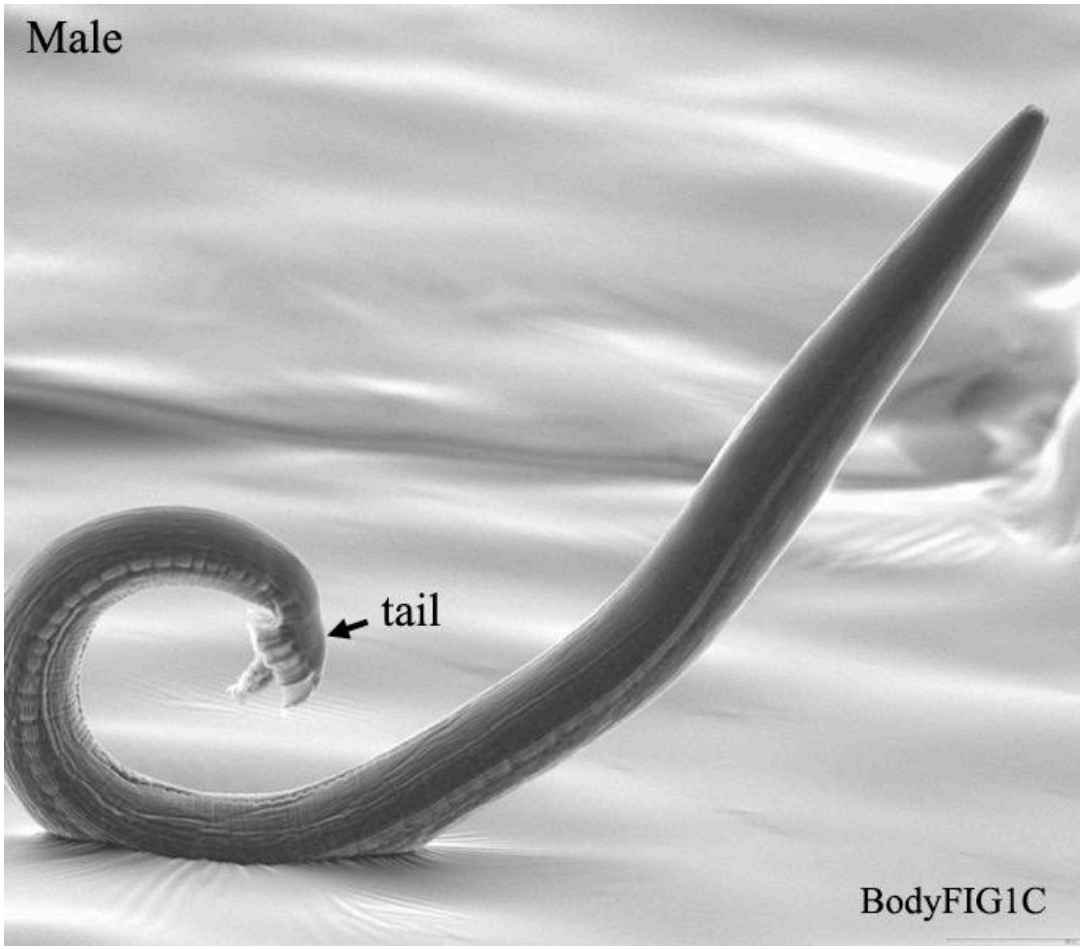
c- A expression on B expression on
c does not regulate a or b

Can also use molecular epistasis to order other types of regulatory pathways, such as splicing or phosphorylation

Direct screens for cell death muta

C. elegans


Hermaphrodite
BodyFIG1A
HEAD
TAIL
Image source: Juergen Berger & Ralph Sommer, Max-Planck Institute for Developmental Biology


Male
tail
BodyFIG1C

Worm Genetics

XX = hermaphrodite
Makes both eggs and sperm;
Is able to self fertilize internally

XO = male
Able to mate with
and fertilize
hermaphrodites

XX

self progeny
XX

XX x XO

self progeny
XX

cross progeny
1/2 XX; 1/2 XO

So how do you tell the difference between self and cross progeny?

Dumpy (*dpy-5* I) hermaphrodite x wt male

self progeny

Dpy hermaphrodite

cross progeny

1/2 nonDpy hermaphrodite; 1/2 nonDpy male

Apoptosis plays many important roles

- Histogenic cell death: up to a half of the neurons normally die during development of parts of the brain.

- Phylogenic cell death: the loss of the vertebrate tail during human fetal development.

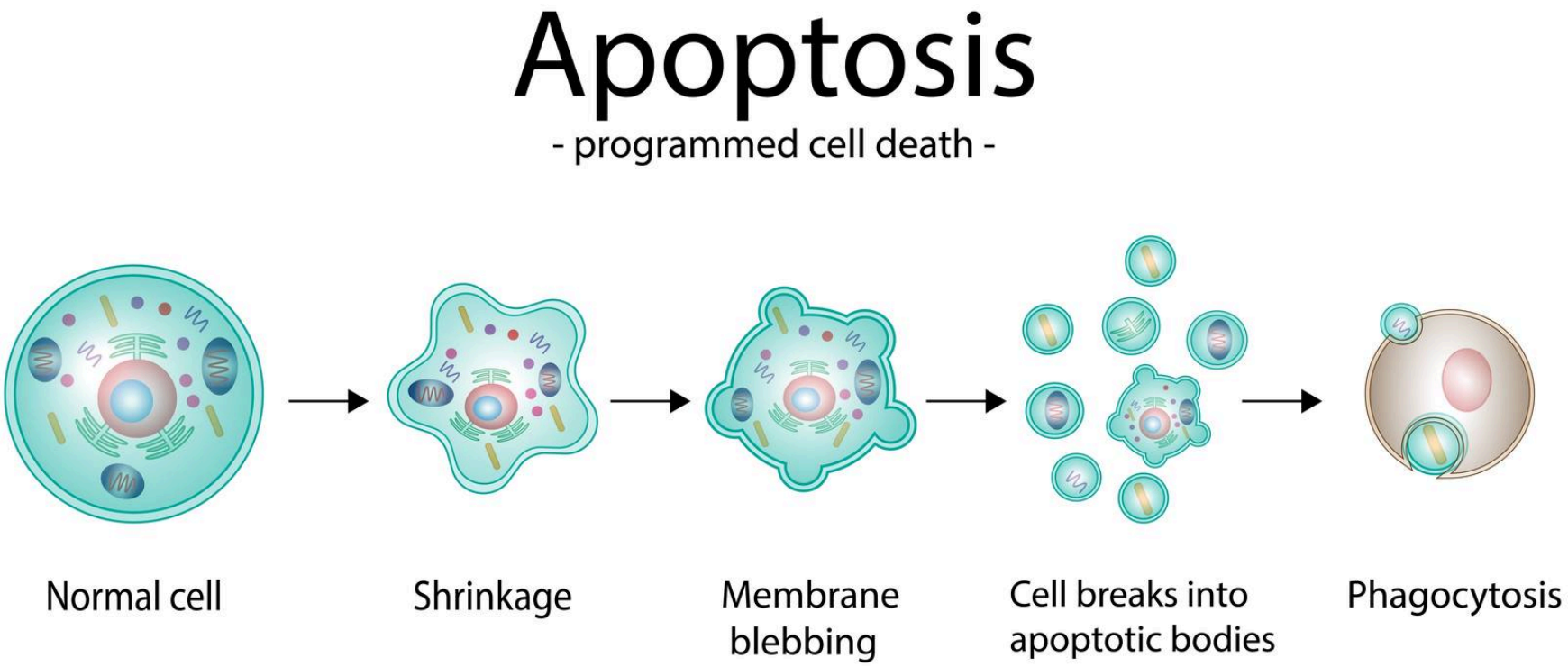
- Morphogenic cell death: the loss of mesenchyme between the digit.

- Cancer: damaged precancerous cells are removed by programmed cell death

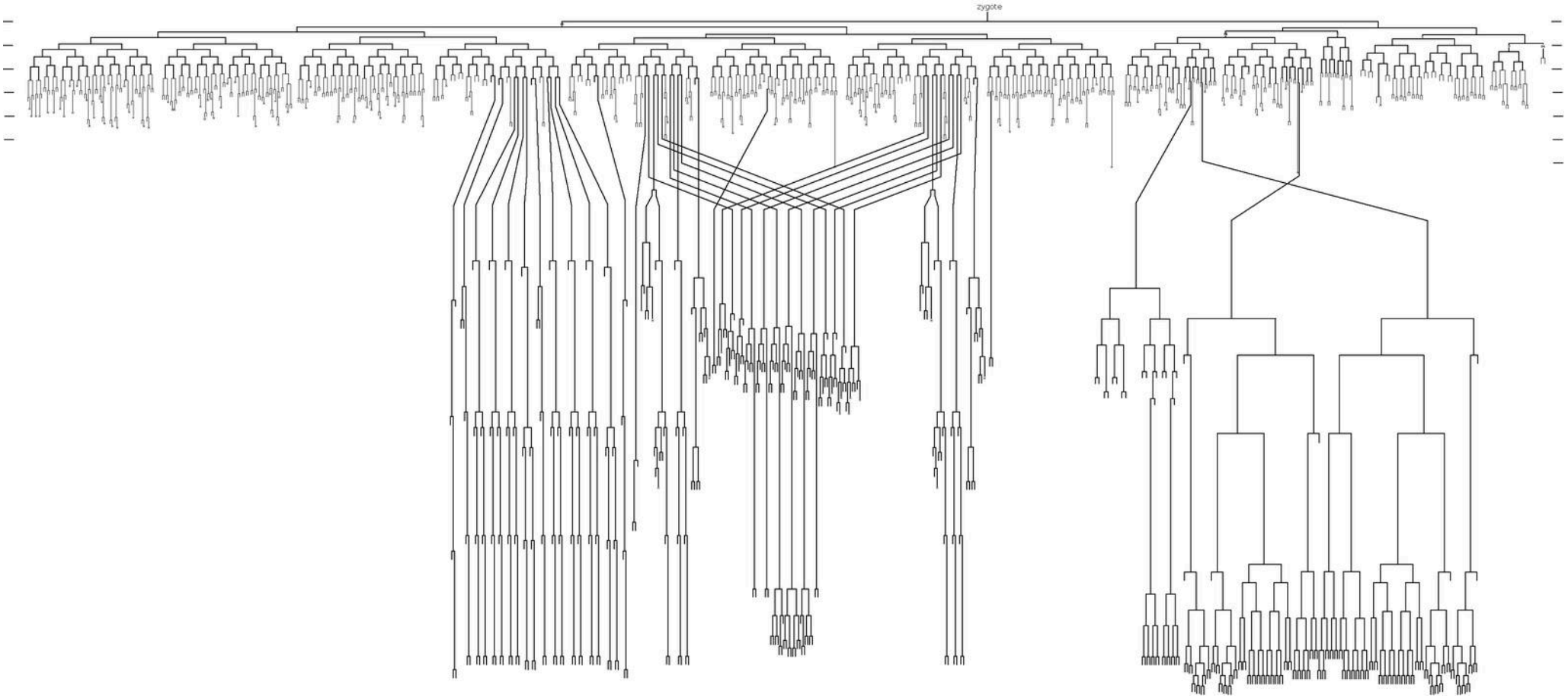
- Programmed cell death in *C. elegans*: more than 10% of the cells produced during development die.

Hallmarks of apoptosis

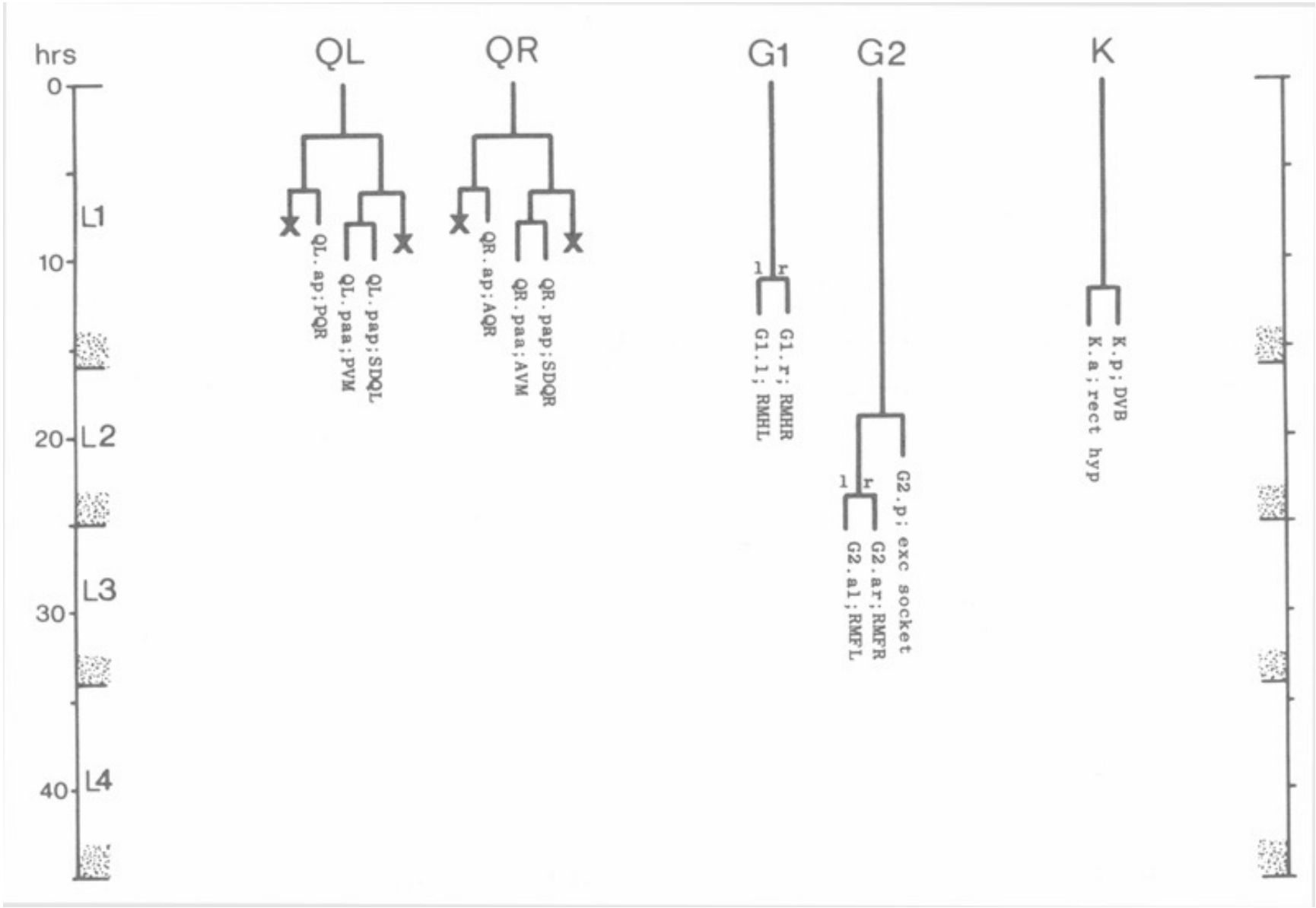
- Nucleus condensation.
- DNA fragmentation.
- Phagocytosis



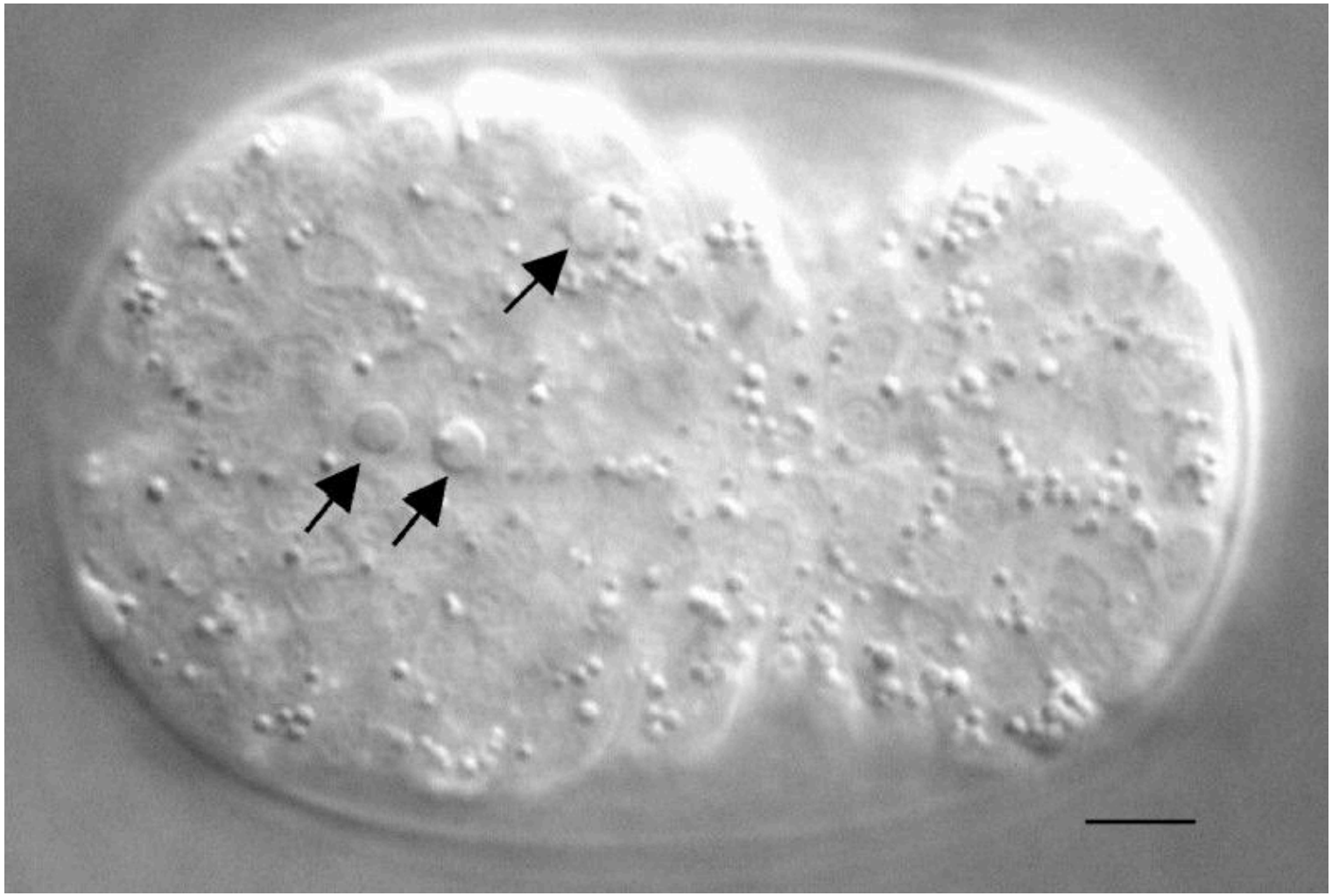
C. elegans develops from an invariant lineage



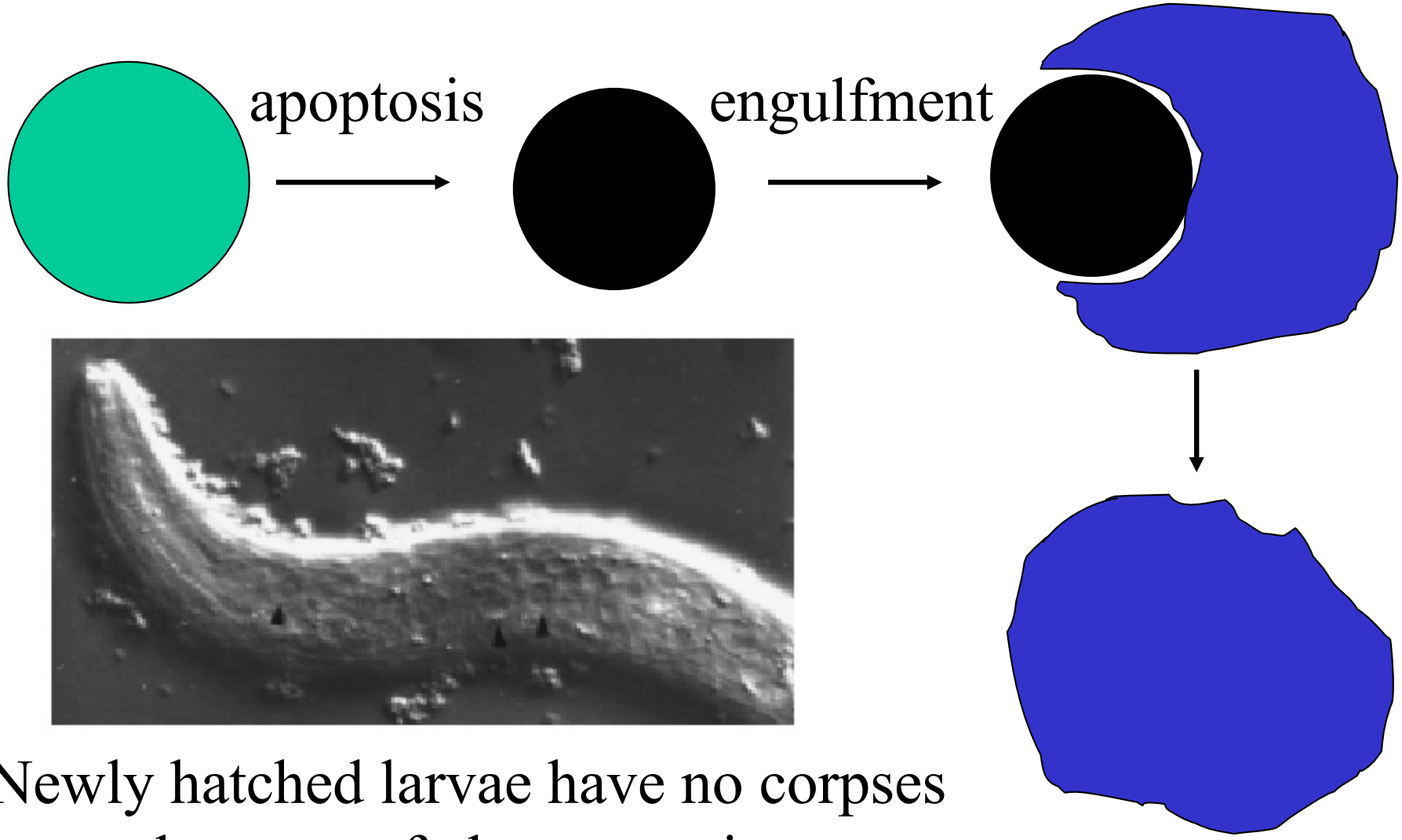
131 cells undergo apoptosis or programmed cell death in the *C. elegans* hemaphrodite.
The cell deaths are indicated by Xs in the lineage.



Cell corpses can be observed by Nomarski optics

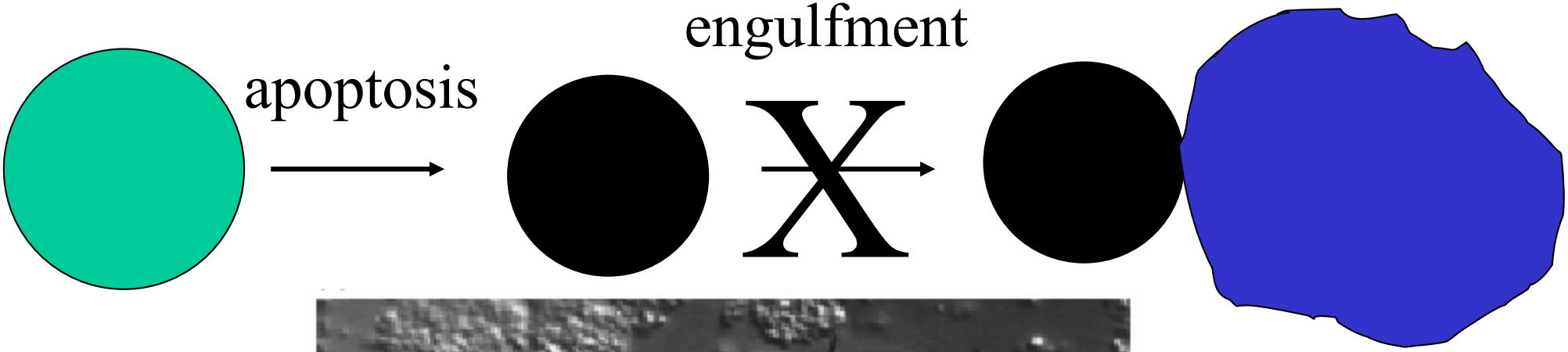


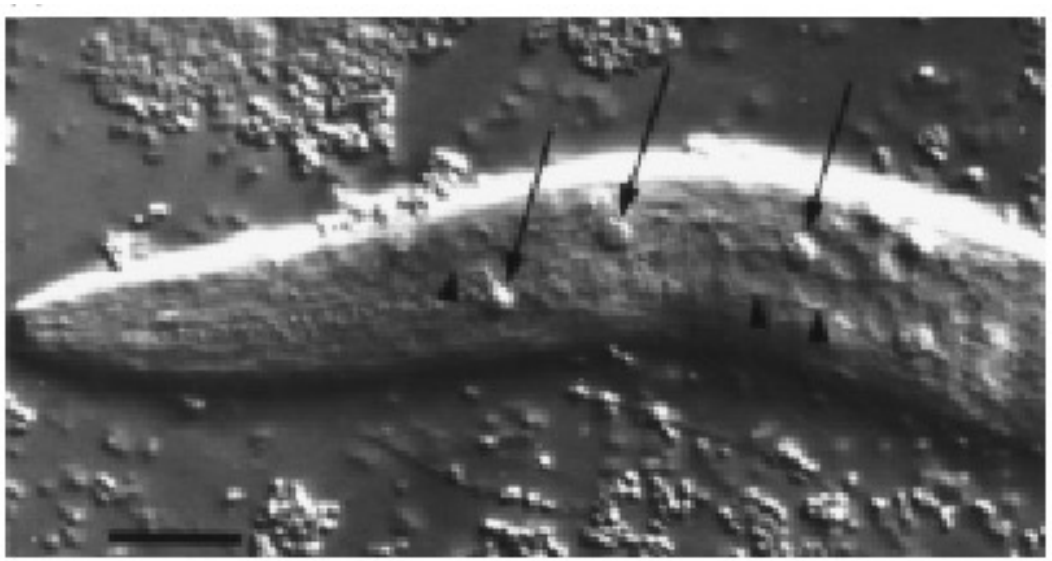
Cell death mutants defective in different stages



Newly hatched larvae have no corpses because of phagocytosis.

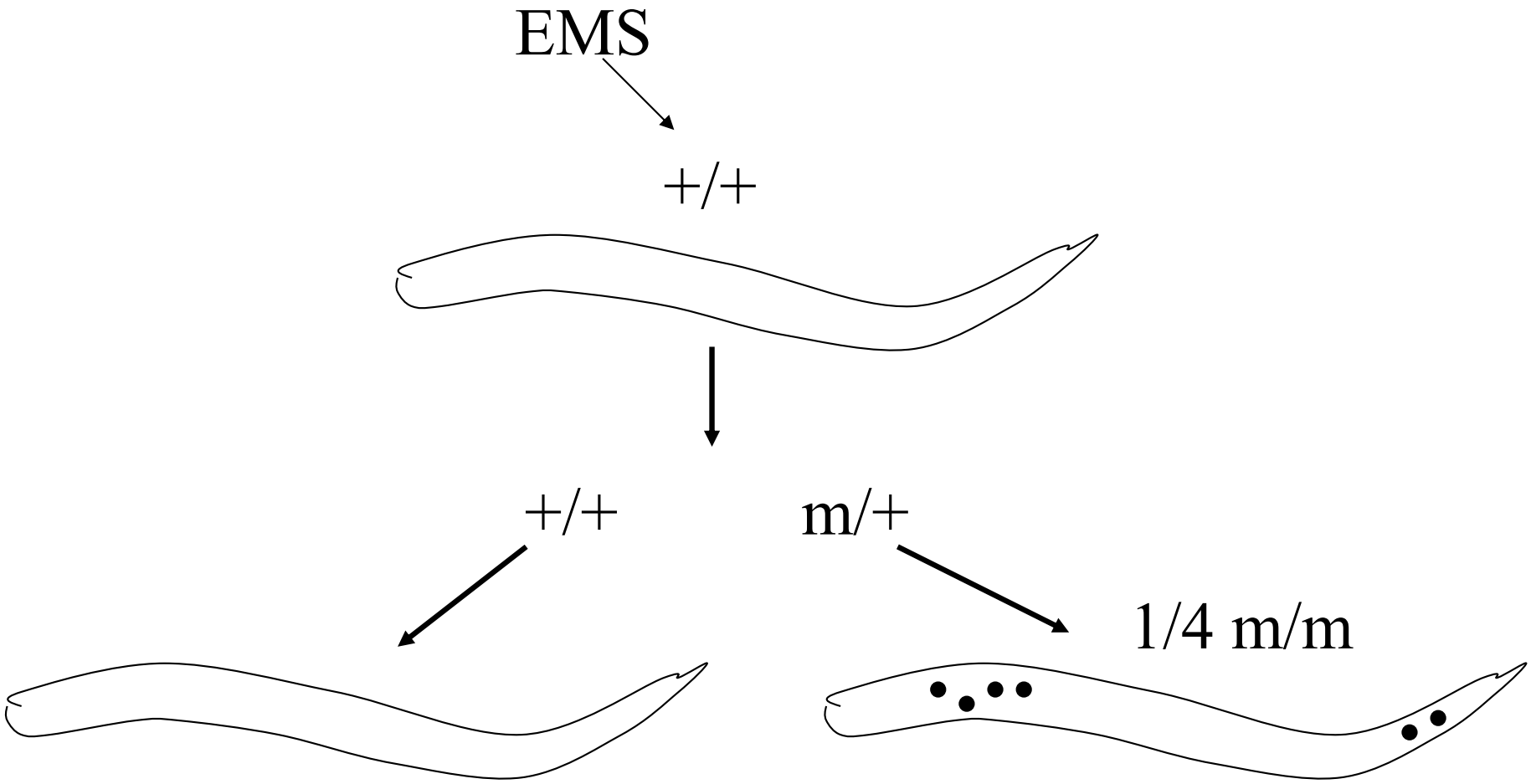
Disruption of engulfment genes results in persistent corpses.



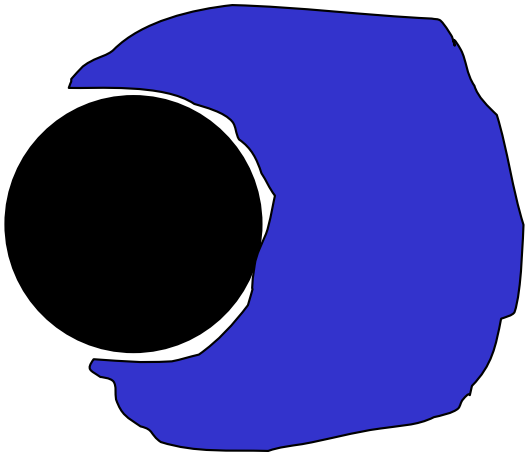


Newly hatched larvae have persistent corpses if there is a defect in phagocytosis.

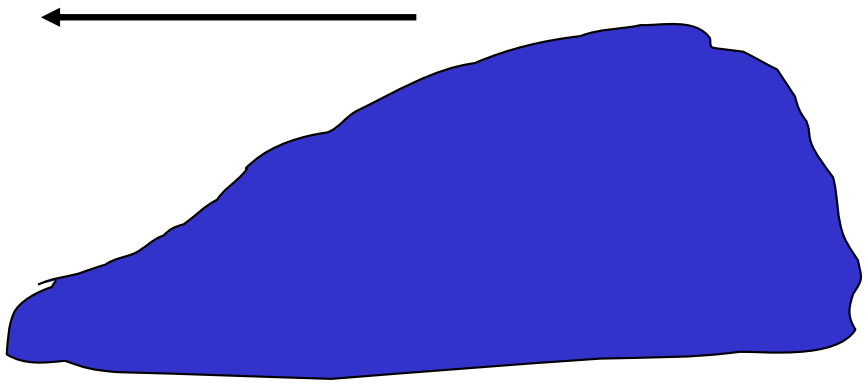
In screens for engulfment mutants Ed Hedgecock identified the *ced-1* and *ced-2* genes.
Additional screens by the Horvitz lab identified the *ced-5, ced-6, ced-7, ced-8, ced-10,* and *ced-12* genes.



The engulfment *ced* genes function in phagocytosis and often cell migration. Regulate cell movement.



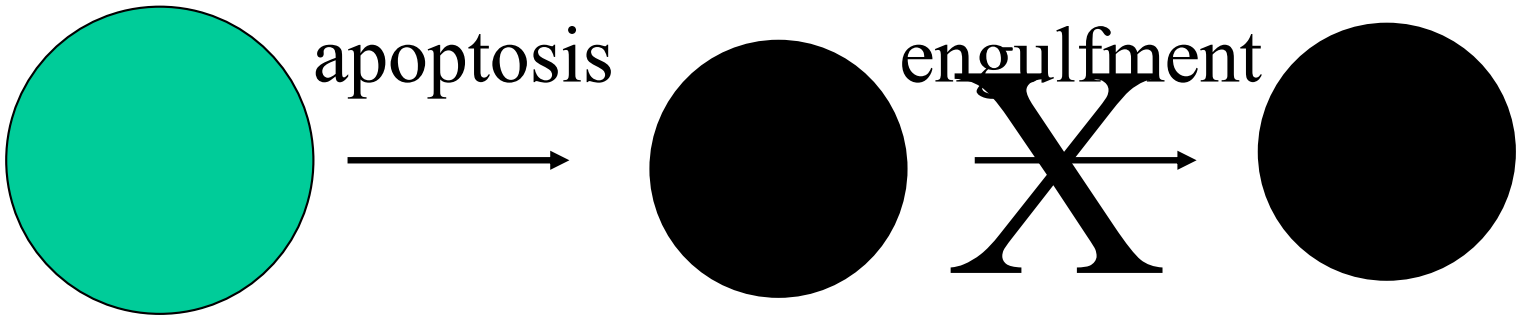
phagocytosis



cell migration

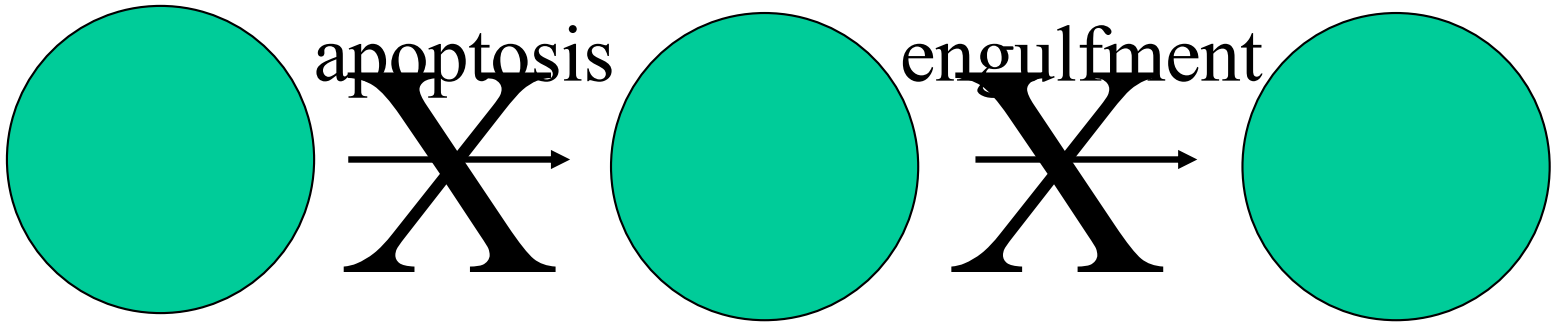
Screens for apoptosis mutants took advantage of the persistent corpses in *ced-1* mutants.

Engulfment mutant



persistent corpse

Engulfment; apoptosis double mutant

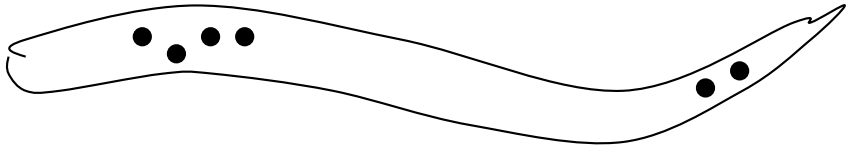


no persistent corpse

Screens for the loss of cell corpses in *ced-1* mutants identified genes involved in apoptosis.

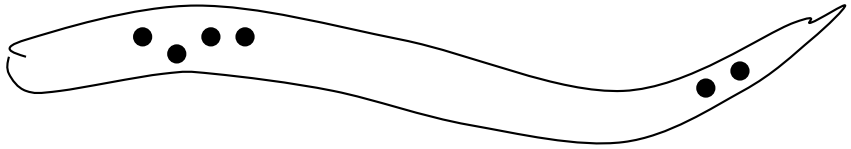
EMS

ced-1/ced-1

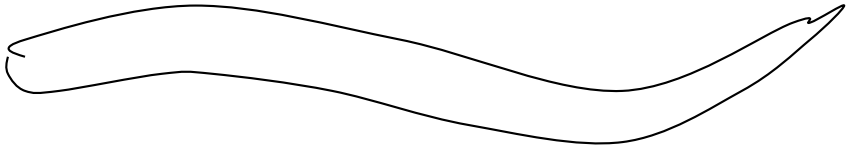


ced-1/ced-1

ced-1/ced-1; m/+



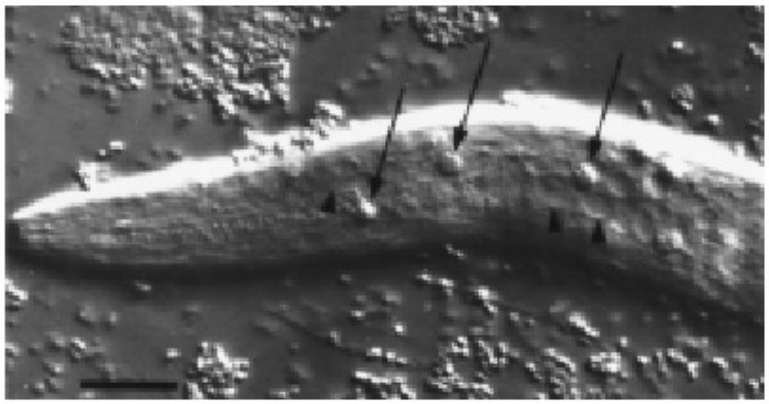
1/4 *ced-1/ced-1; m/m*



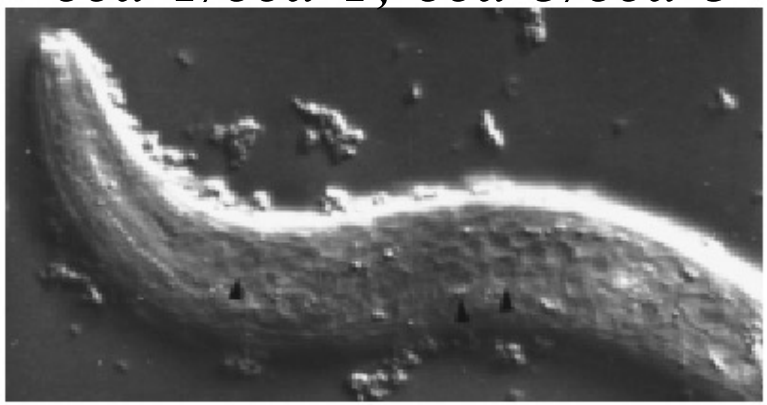
Screen for newly hatched larvae lacking cell corpses

In *ced-3(lf), ced-4(lf), ced-9(gf)* and *egl-1(lf)* mutants all 131 cells that normally die survive.

ced-1/ced-1

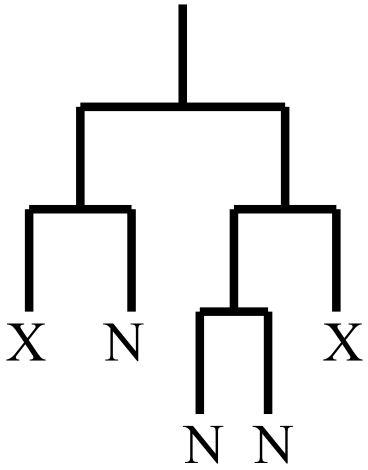


ced-1/ced-1; ced-3/ced-3



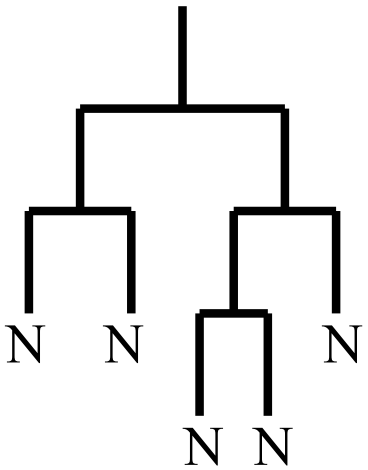
+/+

Q neuroblast



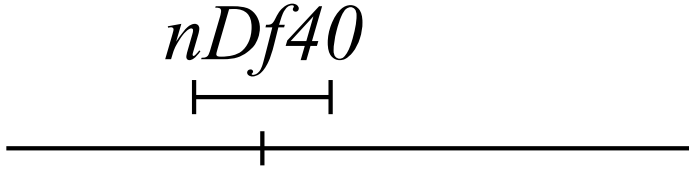
ced-3/ced-3

Q neuroblast



Arrowheads indicate extra cells

n1950 is a dominant mutation in *ced-9*



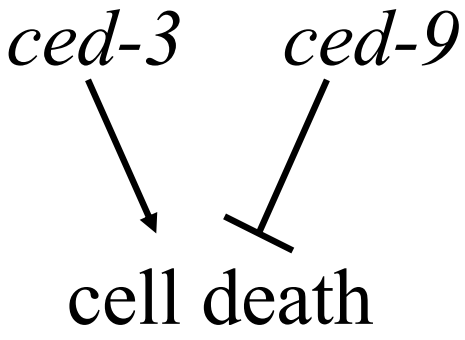
nDf40/+ wild type
n1950/+ many cells that should die survive
Therefore gain-of-function mutation

Loss of function and gain-of-function alleles of *ced-9* have opposite phenotypes.

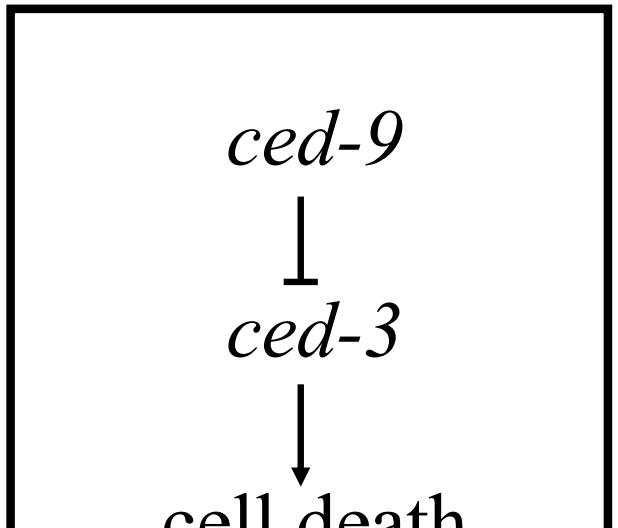
ced-9(gf) disrupts apoptosis
ced-9(lf) is recessive lethal
because of widespread cell death

ced-3 promotes apoptosis
ced-9 inhibits apoptosis

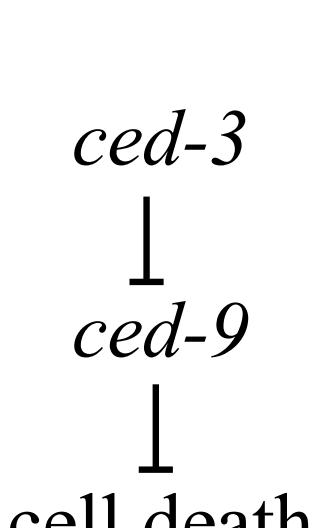
Models



Prediction: *ced-3(lf); ced-9(lf)* intermediate phenotype



Prediction: *ced-3(lf); ced-9(lf)* animals survive and have no apoptosis



Prediction: *ced-3(lf); ced-9(lf)* animals die because of extensive apoptosis

Cell death: a switch pathway

The downstream gene is epistatic in double (null) mutants in a switch pathway. This in contrast to the situation in a biosynthetic pathway, in which the upstream gene is epistatic in terms of the phenotype produced.

egl-1 ⊣ ced-9 ⊣ ced-4 → ced-3 → cell death

ON	**OFF**	**ON**	**ON**	**cell death**
OFF	**ON**	**OFF**	**OFF**	**cell survival**

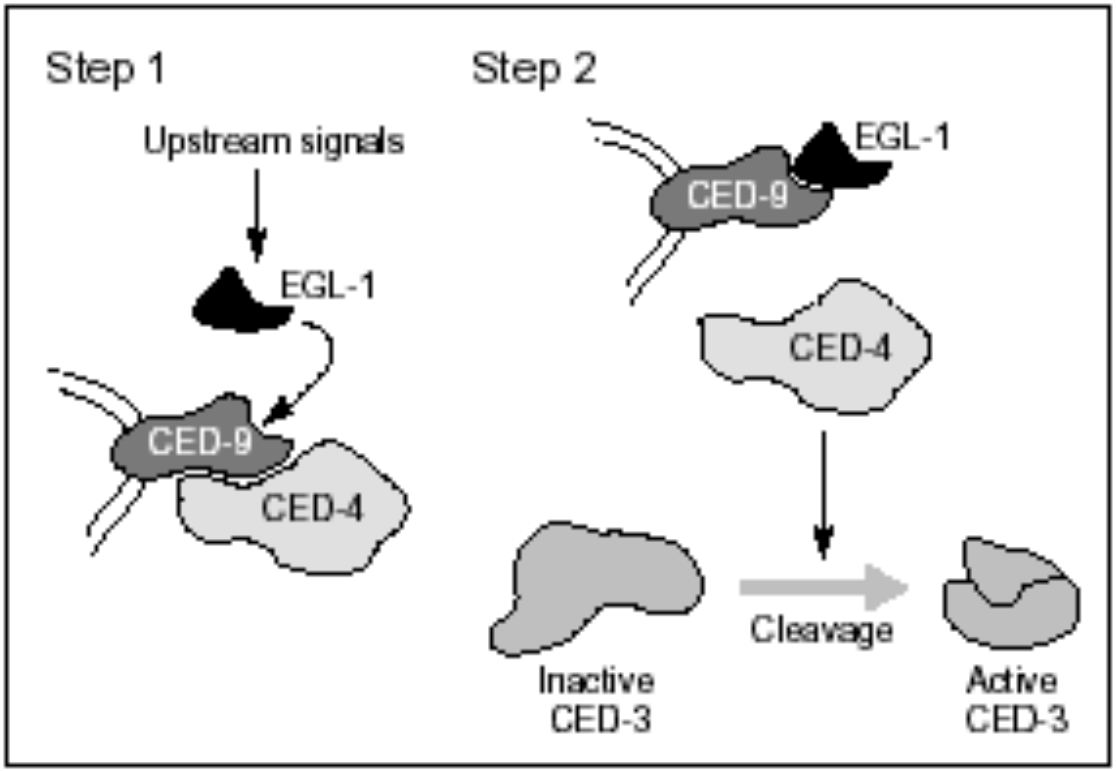
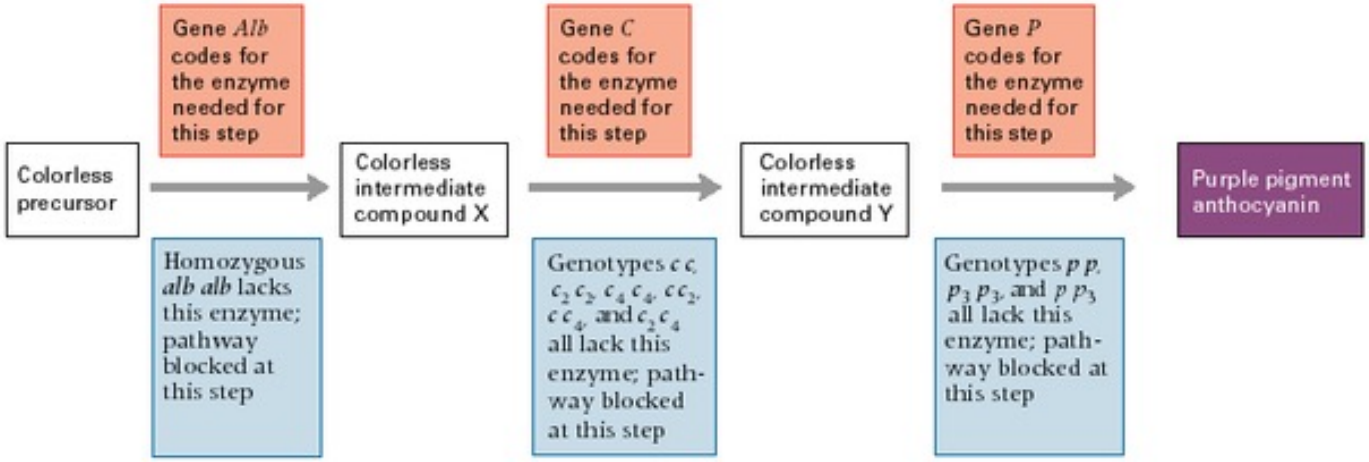
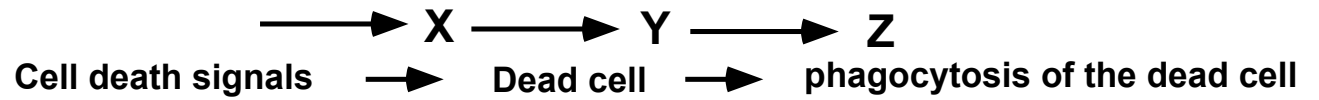


FIGURE 3. A model for the activation of programmed cell death in *Caenorhabditis elegans*. (a) Step 1: upstream signals lead to the production or activation of the EGL-1 protein. EGL-1 binds to CED-9, shown localized to cell membranes, leading to the release of CED-4 from CED-9. Although shown as a monomer, CED-9 likely functions as a dimer, as do other Bcl-2 family proteins[7]. (b) Step 2: free CED-4 then promotes the proteolytic cleavage of the pro-enzyme form of CED-3; p17 and p13 subunits derived from this processing assemble into the active form[27] (active caspases are thought to be tetramers, consisting of dimers of such a heterodimer; reviewed in Ref. 65). It is possible that CED-3 is complexed with CED-4 before EGL-1 mediates the release of CED-4 from CED-9.

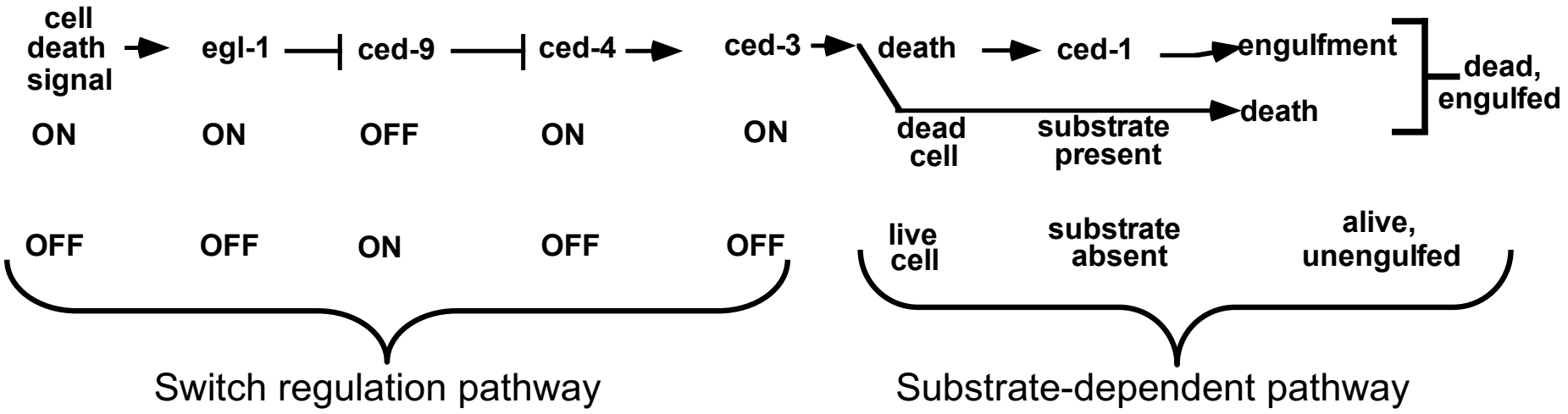


Assembly-metabolic and switch genetic pathways

1) Assembly-metabolic pathways are substrate-dependent. An obligate series of sequential steps is required to generate a final outcome. Each step generates a product that is acted on by the next step in the pathway.



The dead cell acts as a substrate for the action of genes involved in pahgocytosis. So in an epistasis experiement in which a mutation that blocks cell death is combined with one that blocks phagocytosis the phenotype is that of the upstream mutation, which in this case is cell survival.



(a)

Genotype	Phenotype	
	Viable?	Programmed cell death
Wild-type	Yes	Normal
ced-9(lf)	No	Excess
ced-4(lf)	Yes	Reduced
ced-3(lf)	Yes	Reduced
egl-1(lf)	Yes	Reduced
ced-4(lf);*ced-9*(lf)	Yes	Reduced
ced-9(lf);*ced-3*(lf)	Yes	Reduced
ced-9(lf);*egl-1*(lf)	No	Excess

egl-1 ⊣ *ced-9* ⊣ *ced-4* *ced-3* → Programmed cell death

(b)

Overexpressed gene	Genotype	Cell killing
ced-3	*ced-4*(+)	+++
ced-4	*ced-3*(+)	+++
ced-3	*ced-4*(lf)	++
ced-4	*ced-3*(lf)	+/–

ced-4 → *ced-3* → Programmed cell death

(c)

Genotype			
ced-4	*ced-9*	*ced-3*-induced killing	
+	lf	++++	*ced-9* protection
+	+	+++	
lf	lf	++	No *ced-9* protection
lf	+	++	

ced-9 ⊣ *ced-4* → *ced-3*

(d)

Genotype	*egl-1*-induced killing
Wild-type	+++
ced-3(lf)	–
ced-4(lf)	–
ced-9(gf)	–

egl-1 ⊣ *ced-9* ⊣ *ced-4* → *ced-3*

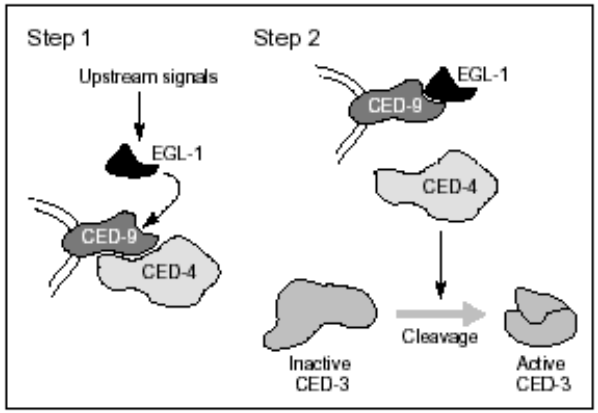


FIGURE 2. Interactions among *egl-1*, *ced-9*, *ced-4* and *ced-3*. Genetic epistasis experiments and studies in which *egl-1*, *ced-9*, *ced-4* and *ced-3* have been overexpressed to kill cells in different genetic backgrounds have suggested an order of action of these genes[10,15,29]. The relative efficiency of cell killing is represented by the number of plus (+) signs, as interpreted from data in Refs 10, 29. (a) Loss-of-function mutations in *ced-4* or *ced-3* suppress the lethality caused by loss of *ced-9* function, suggesting that *ced-9* functions upstream of *ced-4* and *ced-3*. By contrast, a loss of function mutation in *egl-1* does not suppress *ced-9* (lf) lethality, suggesting that *egl-1* functions upstream of *ced-9*. (b) The overexpression of *ced-3* or *ced-4* can kill cells in wild-type *Caenorhabditis elegans*. In the absence of *ced-4* activity, overexpressed *ced-3* can still kill, albeit at a slightly reduced efficiency. By contrast, in the absence of *ced-3* activity, *ced-4* is almost incapable of killing cells. These results suggest *ced-4* acts upstream of *ced-3* to activate programmed cell death. (c) Endogenous *ced-9* activity protects cells from programmed cell death induced by overexpression of *ced-3*, because killing is more efficient in a *ced-9*(lf) background than in a *ced-9*(+) background. While elimination of *ced-4* function reduces the killing activity of *ced-3*, this residual *ced-3* activity is no longer inhibited by *ced-9*, suggesting that *ced-9* functions to protect against programmed cell death, at least in part, by negatively regulating *ced-4*. (d) The overexpression of *egl-1* can also kill cells. This killing is blocked by loss-of-function mutations in *ced-4* or *ced-3* or by a gain-of-function mutation in *ced-9*, suggesting that *egl-1* acts upstream of these genes.

Genotype	Phenotype	
	Viable?	Programmed cell death
Wild-type	Yes	Normal
ced-9(lf)	No	Excess
ced-4(lf)	Yes	Reduced
ced-3(lf)	Yes	Reduced
egl-1(lf)	Yes	Reduced
ced-4(lf);*ced-9*(lf)	Yes	Reduced
ced-9(lf);*ced-3*(lf)	Yes	Reduced
ced-9(lf);*egl-1*(lf)	No	Excess

ced-4 promotes ced-3 activity

Overexpressed gene	Genotype	Cell killing
ced-3	*ced-4*(+)	+++
ced-4	*ced-3*(+)	+++
ced-3	*ced-4*(lf)	++
ced-4	*ced-3*(lf)	+/–

ced-4 → *ced-3* → Programmed cell death

ced-9 protects, but only in the presence of ced-4.

Genotype			
ced-4	*ced-9*	*ced-3*-induced killing	
+	lf	++++	*ced-9* protection
+	+	+++	
lf	lf	++	No *ced-9* protection
lf	+	++	

ced-9 ⊣ *ced-4* → *ced-3*

The problem of the immune system: How to create infinite diversity with limited components in order to counter a continuously (and more quickly) evolving foe?

Mature B and T cells are able to recognize foreign antigen. They either neutralize these molecules or destroy the cells that synthesize them. B and T cells recognize foreign antigens using receptors on their cell surface. It is estimated that we can each make more than 10^9 different receptors. However, there are only about 10^5 genes in the genome. So how are we able to do it?

1. Combinatorial rearrangements in somatic cells

2. Removal or addition of nucleotides during the above rearrangements

3. Increased mutation rate at specific loci during an immune response

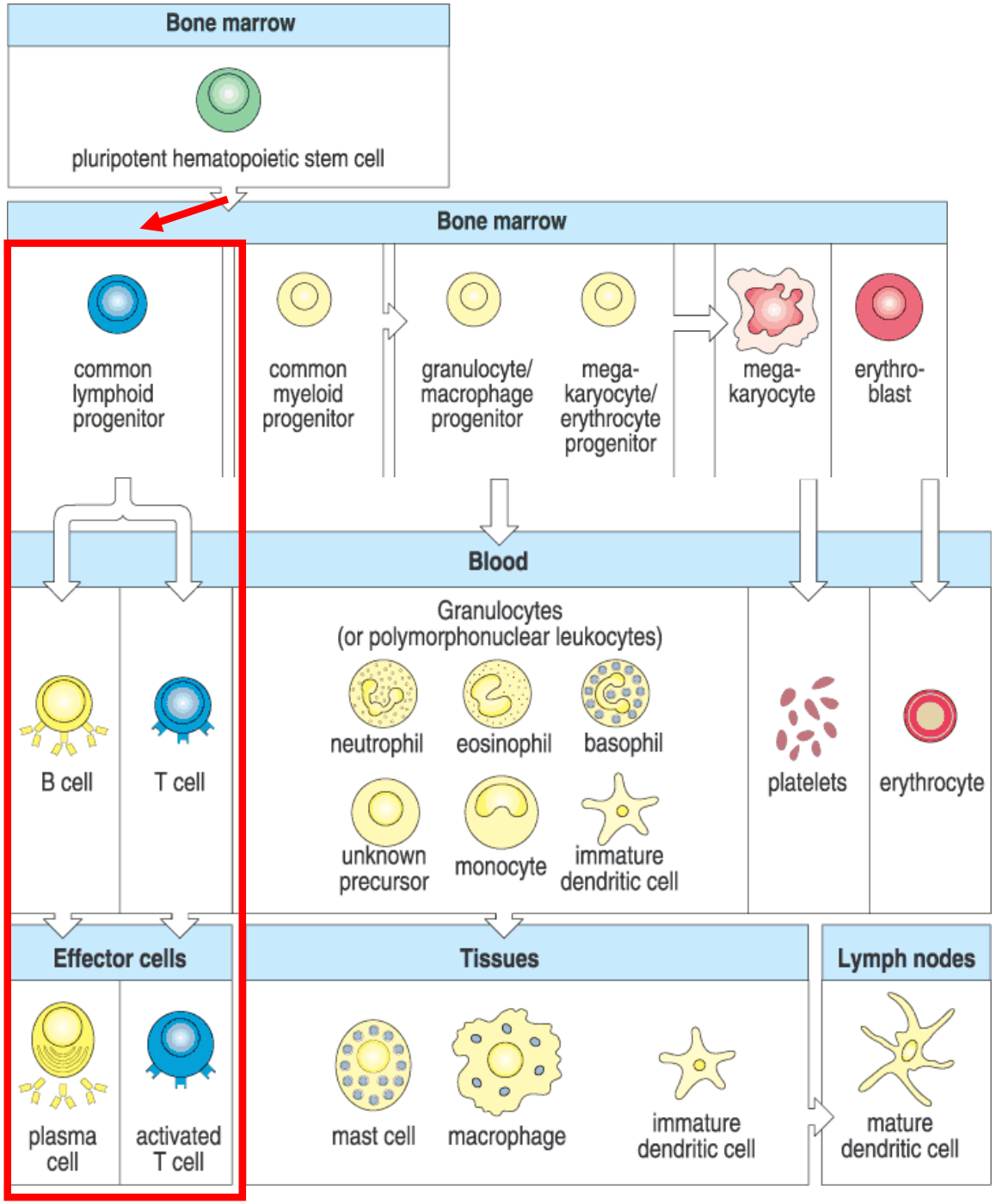
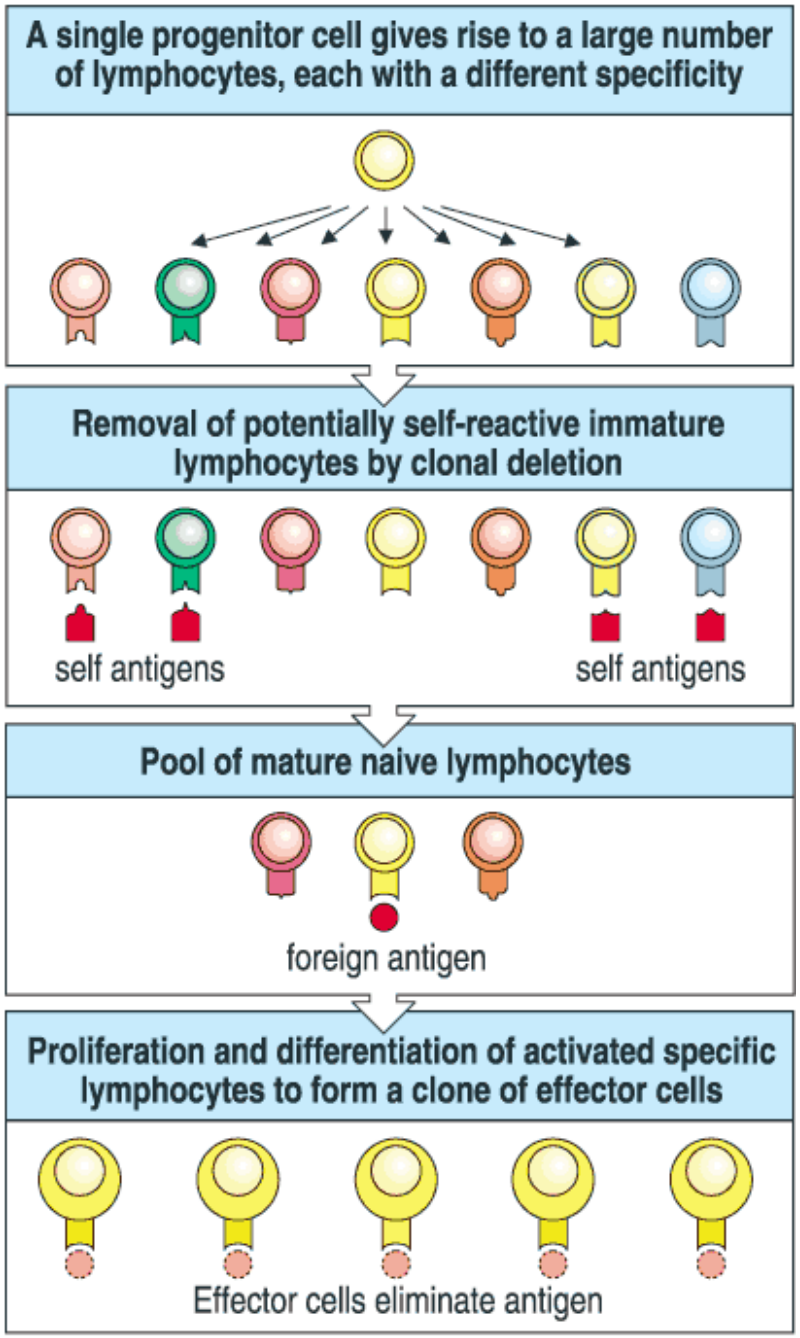


Fig 1.3 part 2 of 2 © 2001 Garland Science

All the cellular elements of blood, including the lymphocytes of the adaptive immune system, arise from hematopoietic stem cells in the bone marrow. These pluripotent cells divide to produce two more specialized types of stem cells, a common lymphoid progenitor that gives rise to the T and B lymphocytes responsible for adaptive immunity, and a common myeloid progenitor that gives rise to different types of leukocytes (white blood cells), erythrocytes (red blood cells that carry oxygen), and the megakaryocytes that produce platelets that are important in blood clotting. The existence of a common lymphoid progenitor for T and B lymphocytes is strongly supported by current data. T and B lymphocytes are distinguished by their sites of differentiation-T cells in the thymus and B cells in the bone marrow-and by their antigen receptors. Mature T and B lymphocytes circulate between the blood and peripheral lymphoid tissues. After encounter with antigen, B cells differentiate into antibody-secreting plasma cells, whereas T cells differentiate into effector T cells with a variety of functions. A third lineage of lymphoid-like cells, the natural killer cells, derive from the same progenitor cell but lack the antigen-specificity that is the hallmark of the adaptive immune response
(not shown). The leukocytes that derive from the myeloid stem cell are the monocytes, the dendritic cells, and the basophils, eosinophils, and neutrophils. The latter three are collectively termed either granulocytes, because of the cytoplasmic granules whose characteristic staining gives them a distinctive appearance in blood smears, or polymorphonuclear leukocytes, because of their irregularly shaped nuclei. They circulate in the blood and enter the tissues only when recruited to sites of infection or inflammation where neutrophils are recruited to phagocytose bacteria. Eosinophils and basophils are recruited to sites of allergic inflammation, and appear to be involved in defending against parasites. Immature dendritic cells travel via the blood to enter peripheral tissues, where they ingest antigens. When they encounter a pathogen, they mature and migrate to lymphoid tissues, where they activate antigen-specific T lymphocytes. Monocytes enter tissues, where they differentiate into macrophages; these are the main tissue-resident phagocytic cells of the innate immune system. Mast cells arise from precursors in bone marrow but complete their maturation in tissues; they are important in allergic responses.



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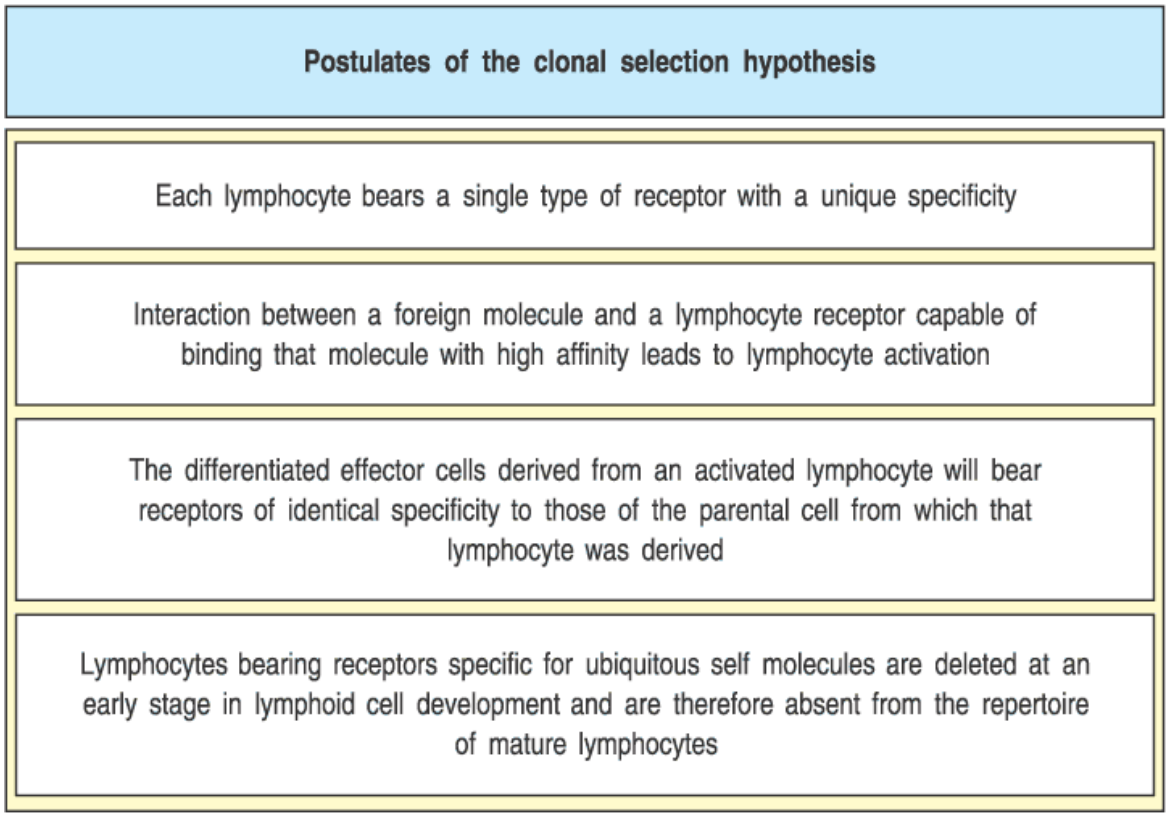
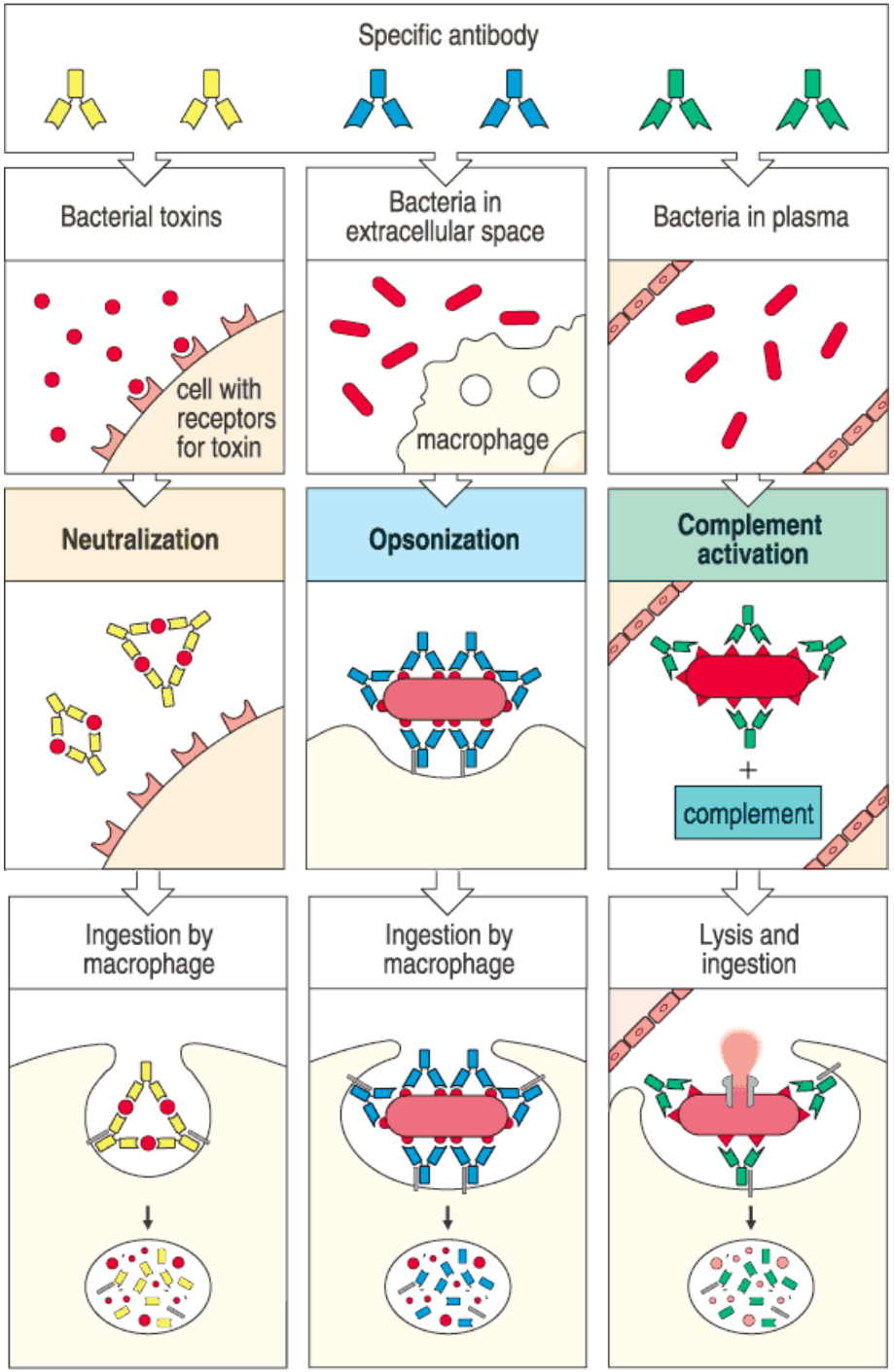


Fig 1.15 © 2001 Garland Science

Each lymphocyte progenitor gives rise to many lymphocytes, each bearing a distinct antigen receptor. Lymphocytes with receptors that bind ubiquitous self antigens are eliminated before they become fully mature, ensuring tolerance to such self antigens. When antigen interacts with the receptor on a mature naive lymphocyte, that cell is activated and starts to divide. It gives rise to a clone of identical progeny, all of whose receptors bind the same antigen. Antigen specificity is thus maintained as the progeny proliferate and differentiate into effector cells. Once antigen has been eliminated by these effector cells, the immune response ceases.

The immune system protects against four classes of pathogen		
Type of pathogen	**Examples**	**Diseases**
Extracellular bacteria, parasites, fungi	*Streptococcus pneumoniae* *Clostridium tetani* *Trypanosoma brucei* *Pneumocystis carinii*	Pneumonia Tetanus Sleeping sickness PC Pneumonia
Intracellular bacteria, parasites	*Mycobacterium leprae* *Leishmania donovani* *Plasmodium falciparum*	Leprosy Leishmaniasis Malaria
Viruses (intracellular)	Variola Influenza Varicella	Smallpox Flu Chickenpox
Parasitic worms (extracellular)	*Ascaris* *Schistosoma*	Ascariasis Schistosomiasis

Fig 1.23 © 2001 Garland Science



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The left panels show antibodies binding to and neutralizing a bacterial toxin, thus preventing it from interacting with host cells and causing pathology. Unbound toxin can react with receptors on the host cell, whereas the toxin:antibody complex cannot. Antibodies also neutralize complete virus particles and bacterial cells by binding to them and inactivating them. The antigen:antibody complex is eventually scavenged and degraded by macrophages. Antibodies coating an antigen render it recognizable as foreign by phagocytes (macrophages and neutrophils), which then ingest and destroy it; this is called opsonization. The middle panels show opsonization and phagocytosis of a bacterial cell. The right panels show activation of the complement system by antibodies coating a bacterial cell. Bound antibodies form a receptor for the first protein of the complement system, which eventually forms a protein complex on the surface of the bacterium that, in some cases, can kill the bacterium directly. More generally, complement coating favors the uptake and destruction of the bacterium by phagocytes. Thus, antibodies target pathogens and their toxic products for disposal by phagocytes.

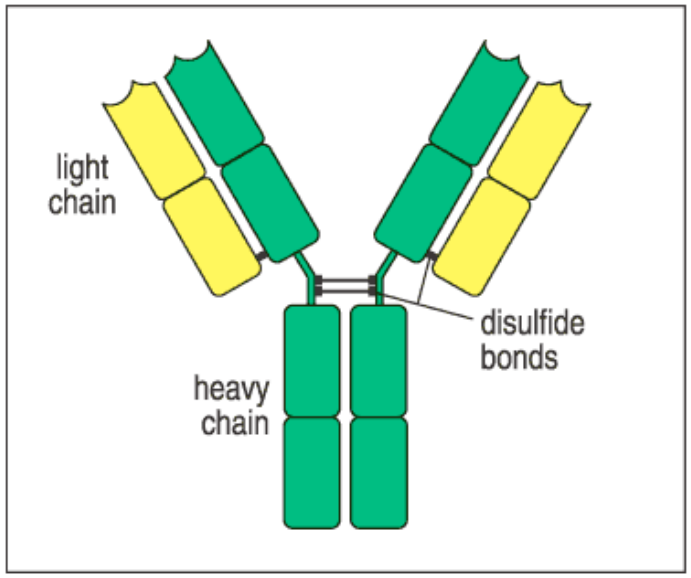
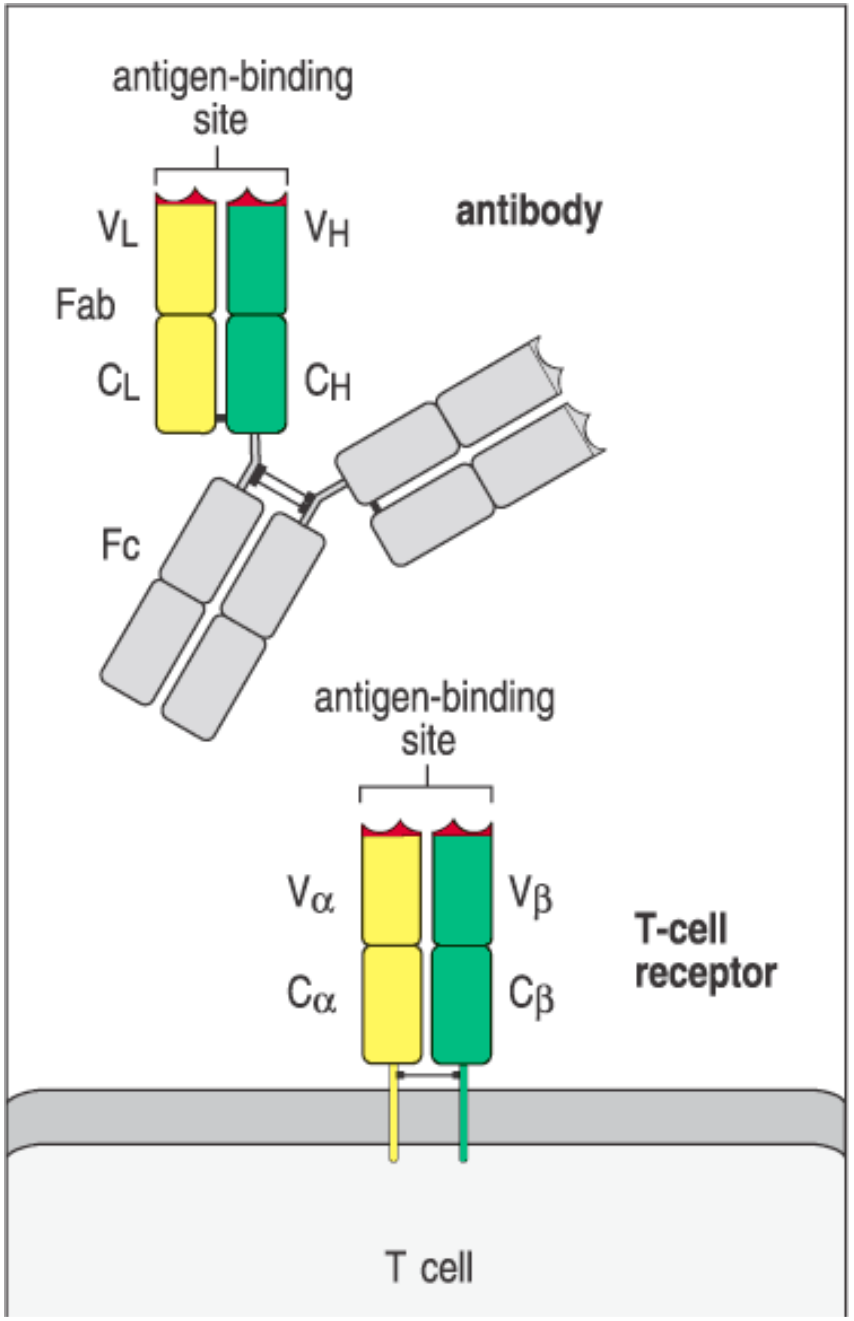
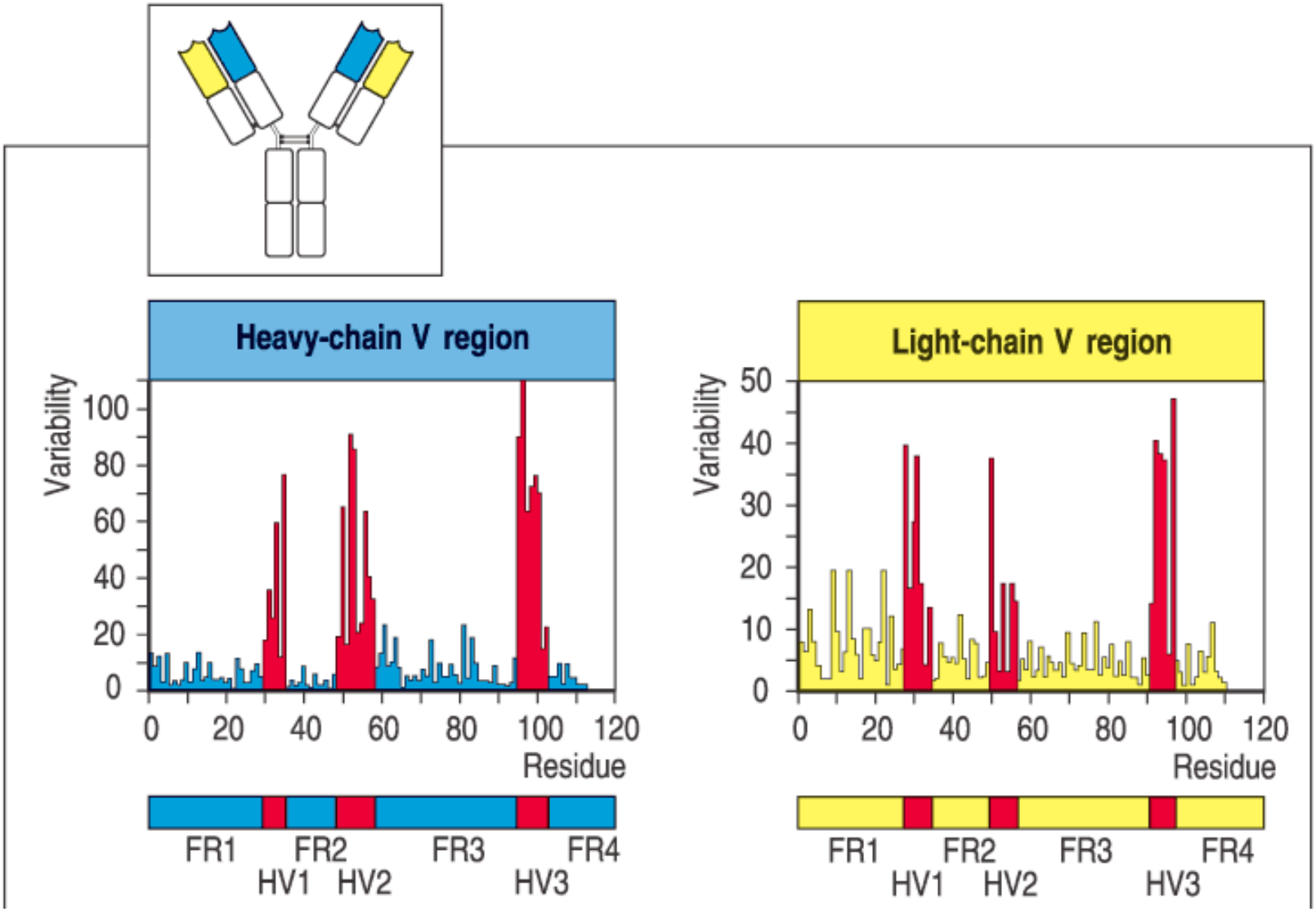


Fig 3.2 © 2001 Garland Science

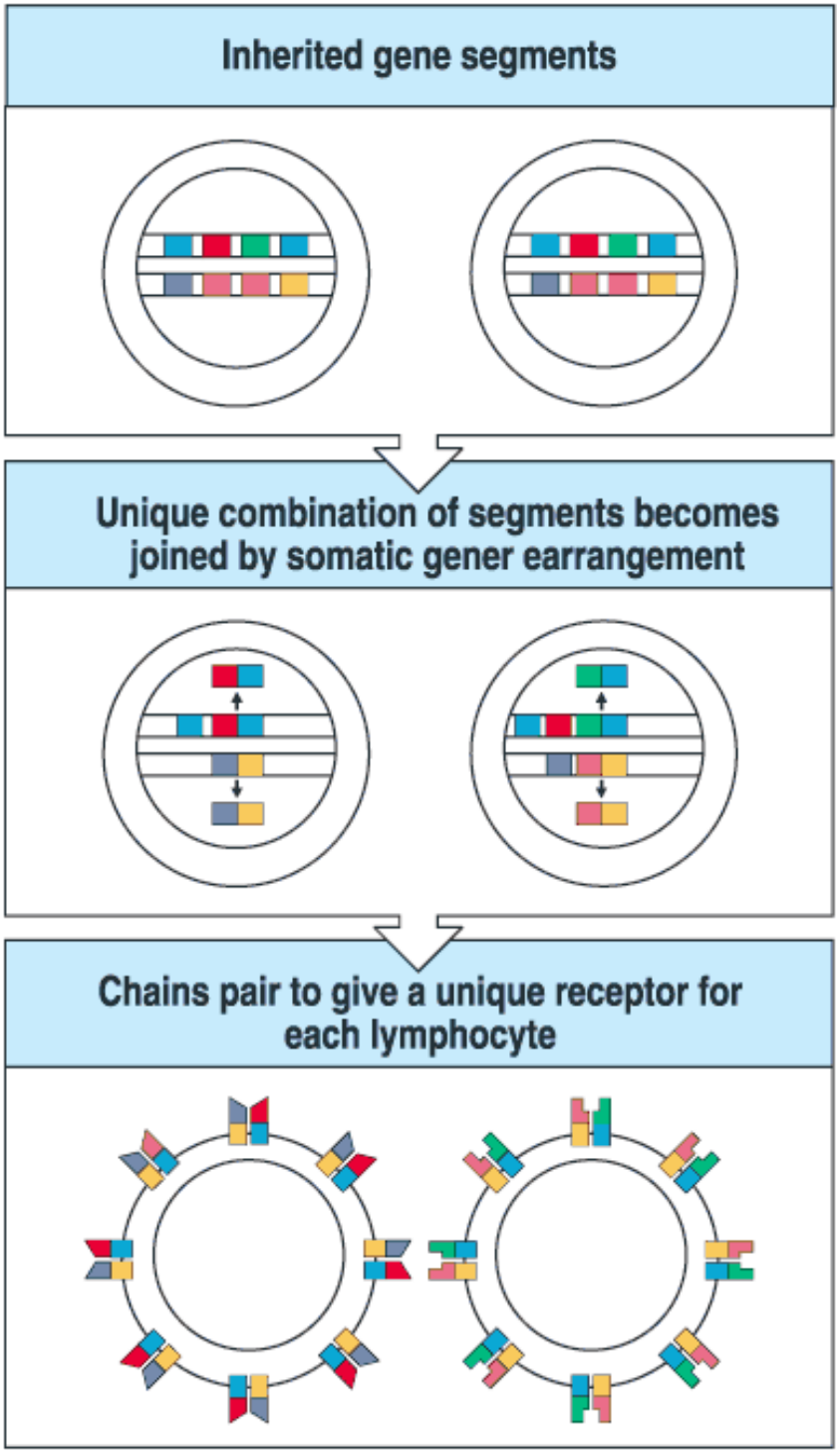
Each immunoglobulin molecule is made up of two heavy chains (green) and two light chains (yellow) joined by disulfide bonds so that each heavy chain is linked to a light chain and the two heavy chains are linked together.

The Fab fragment of antibody molecules is a disulfide-linked heterodimer, each chain of which contains one immunoglobulin C domain and one V domain; the juxtaposition of the V domains forms the antigen-binding site (see Section 3-6). The T-cell receptor is also a disulfide-linked heterodimer, with each chain containing an immunoglobulin C-like domain and an immunoglobulin V-like domain. As in the Fab fragment, the juxtaposition of the V domains forms the site for antigen recognition.





A variability plot derived from comparison of the amino acid sequences of several dozen heavy-chain and light-chain V domains. At each amino acid position the degree of variability is the ratio of the number of different amino acids seen in all of the sequences together to the frequency of the most common amino acid. Three hypervariable regions (HV1, HV2, and HV3) are indicated in red and are also known as the complementarity-determining regions, CDR1, CDR2, and CDR3. They are flanked by less variable framework regions (FR1, FR2, FR3, and FR4, shown in blue or yellow).



The diversity of lymphocyte antigen receptors Is generated by somatic gene rearrangements.

Different parts of the variable regions of antigen receptors are encoded by sets of gene segments. During a lymphocyte's development, one member of each set of gene segments is joined randomly to the others by an irreversible process of DNA recombination. The juxtaposed gene segments make up a complete gene that encodes the variable part of one chain of the receptor, and is unique to that cell. This random rearrangement is repeated for the set of gene segments encoding the other chain. The rearranged genes are expressed to produce the two types of polypeptide chain. These come together to form a unique antigen receptor on the lymphocyte surface. Each lymphocyte bears many copies of its unique receptor.

The other allelic copy of the receptor, encoded on the homologous chromosome, is silenced. Cells express only one receptor type.

*THE MOLECULAR BA$IS OF ANTIBODY FORMATION: A PARADOX**

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Communicated by Norman Davidson, July 15, 1965

When a foreign toxin or pathogen enters the circulatory system of a higher organism, it triggers the division of cells which are capable of making antibodies to react with the foreign substance.[1] The main circulating antibody which is produced in response to such a challenge is referred to as γ-globulin. Although perhaps as many as 10,000 different kinds of γ-globulin molecules may be produced, they all have remarkably similar structural properties including a common molecular weight of 150,000. As detailed knowledge of the structure of these molecules has accumulated, an apparent genetic paradox has arisen which will be the subject of this communication.

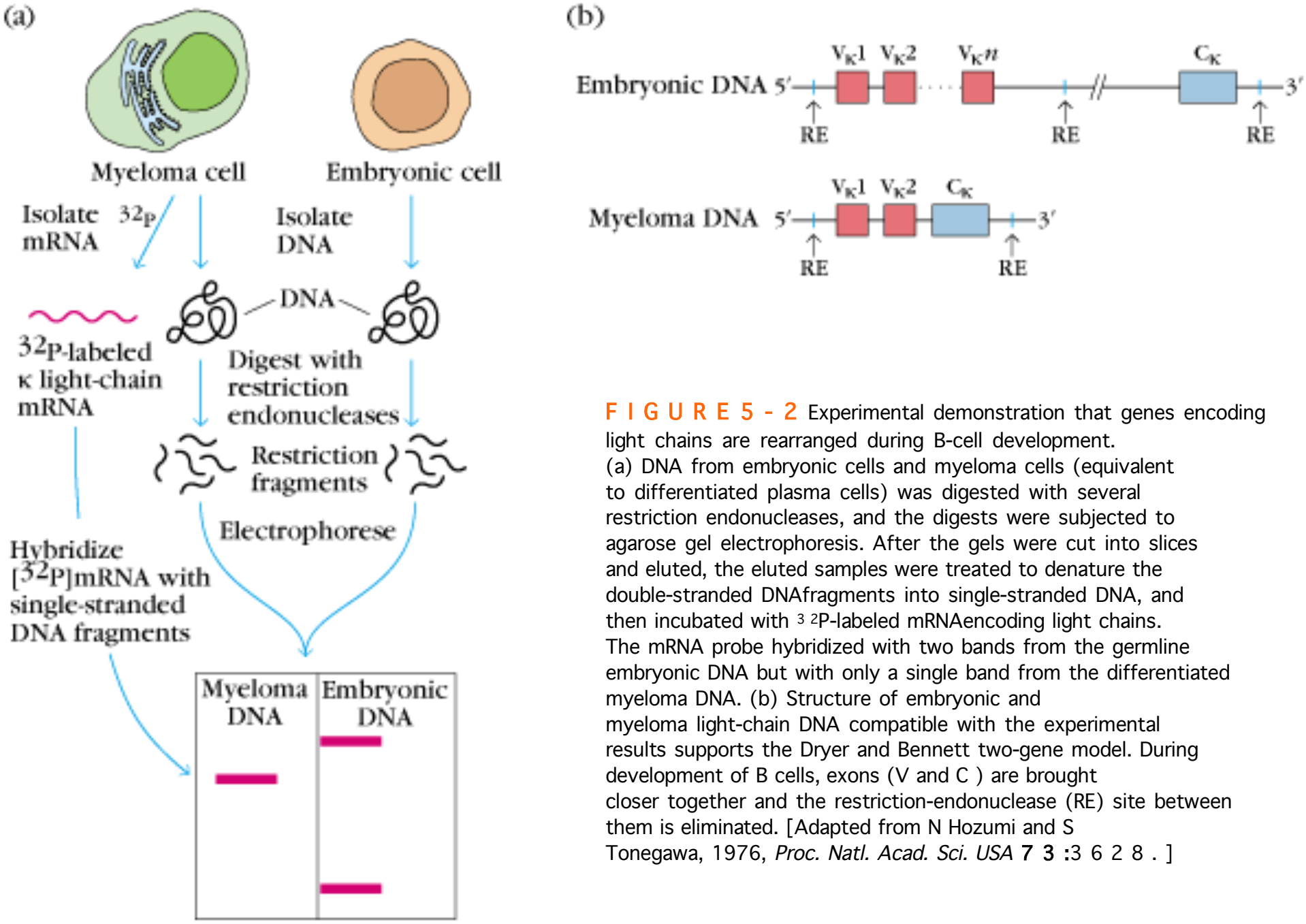


FIGURE 5-2 Experimental demonstration that genes encoding light chains are rearranged during B-cell development. (a) DNA from embryonic cells and myeloma cells (equivalent to differentiated plasma cells) was digested with several restriction endonucleases, and the digests were subjected to agarose gel electrophoresis. After the gels were cut into slices and eluted, the eluted samples were treated to denature the double-stranded DNAfragments into single-stranded DNA, and then incubated with ^{32}P-labeled mRNAencoding light chains. The mRNA probe hybridized with two bands from the germline embryonic DNA but with only a single band from the differentiated myeloma DNA. (b) Structure of embryonic and myeloma light-chain DNA compatible with the experimental results supports the Dryer and Bennett two-gene model. During development of B cells, exons (V and C) are brought closer together and the restriction-endonuclease (RE) site between them is eliminated. [Adapted from N Hozumi and S Tonegawa, 1976, *Proc. Natl. Acad. Sci. USA* **73**:3628.]

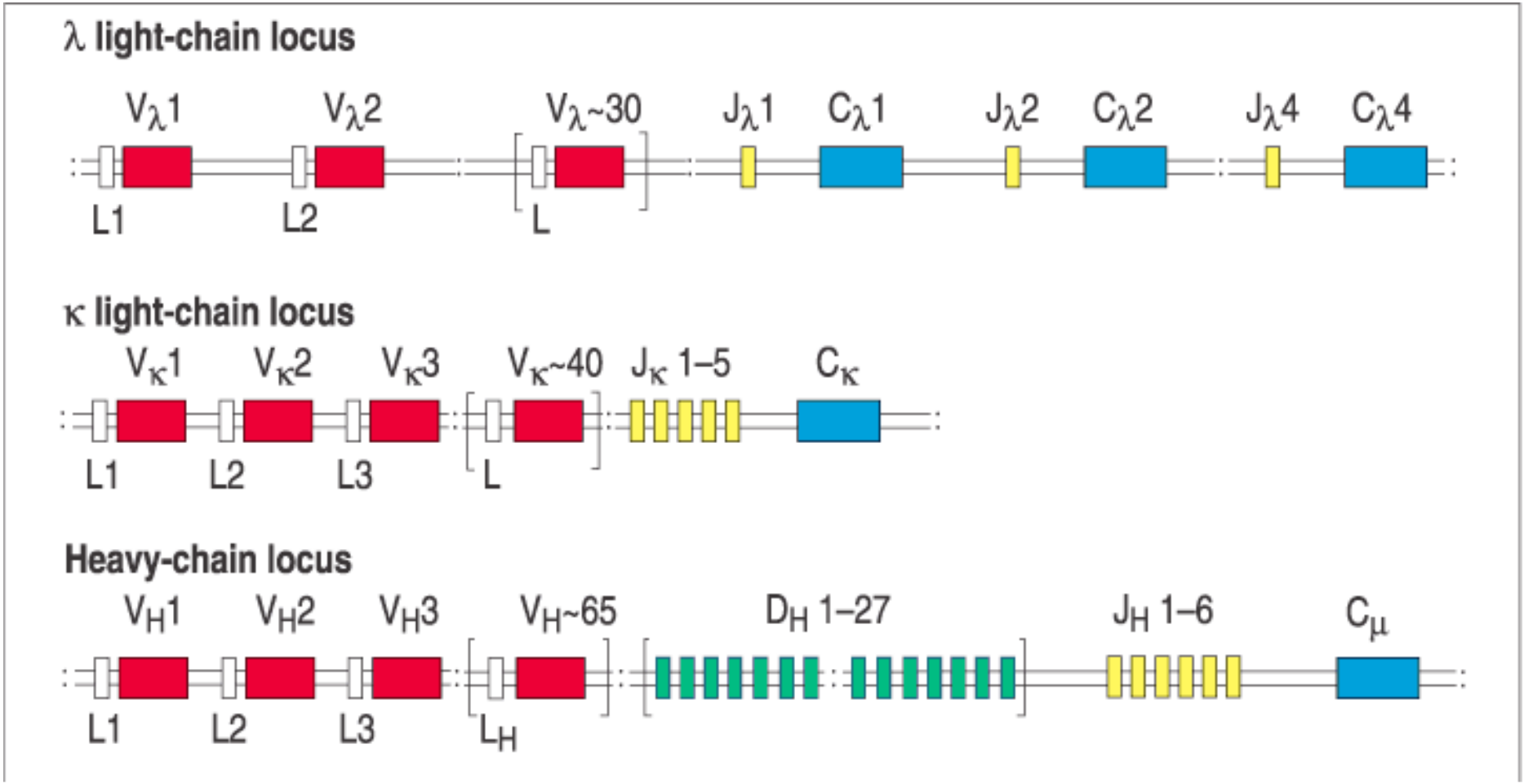


Fig. 4.4 The germline organization of the immunoglobulin heavy- and light-chain loci in the human genome.

The genetic locus for the l light chain (chromosome 22) has about 30 functional Vl gene segments and four pairs of functional Jl gene segments and Cl genes. The k locus (chromosome 2) is organized in a similar way, with about 40 functional Vk gene segments accompanied by a cluster of five Jk gene segments but with a single Ck gene. In approximately 50% of individuals, the entire cluster of k V gene segments has undergone an increase by duplication (not shown for simplicity). The heavy-chain locus (chromosome 14) has about 65 functional VH gene segments and a cluster of around 27 D segments lying between these VH gene segments and six JH gene segments. The heavy-chain locus also contains a large cluster of CH genes that are described in Fig. 4.18. For simplicity we have shown only a single CH gene in this diagram without illustrating its separate exons, have omitted pseudogenes, and have shown all V gene segments in the same orientation. L, leader sequence. This diagram is not to scale: the total length of the heavy- chain locus is over 2 megabases (2 million bases), whereas some of the D segments are only six bases long

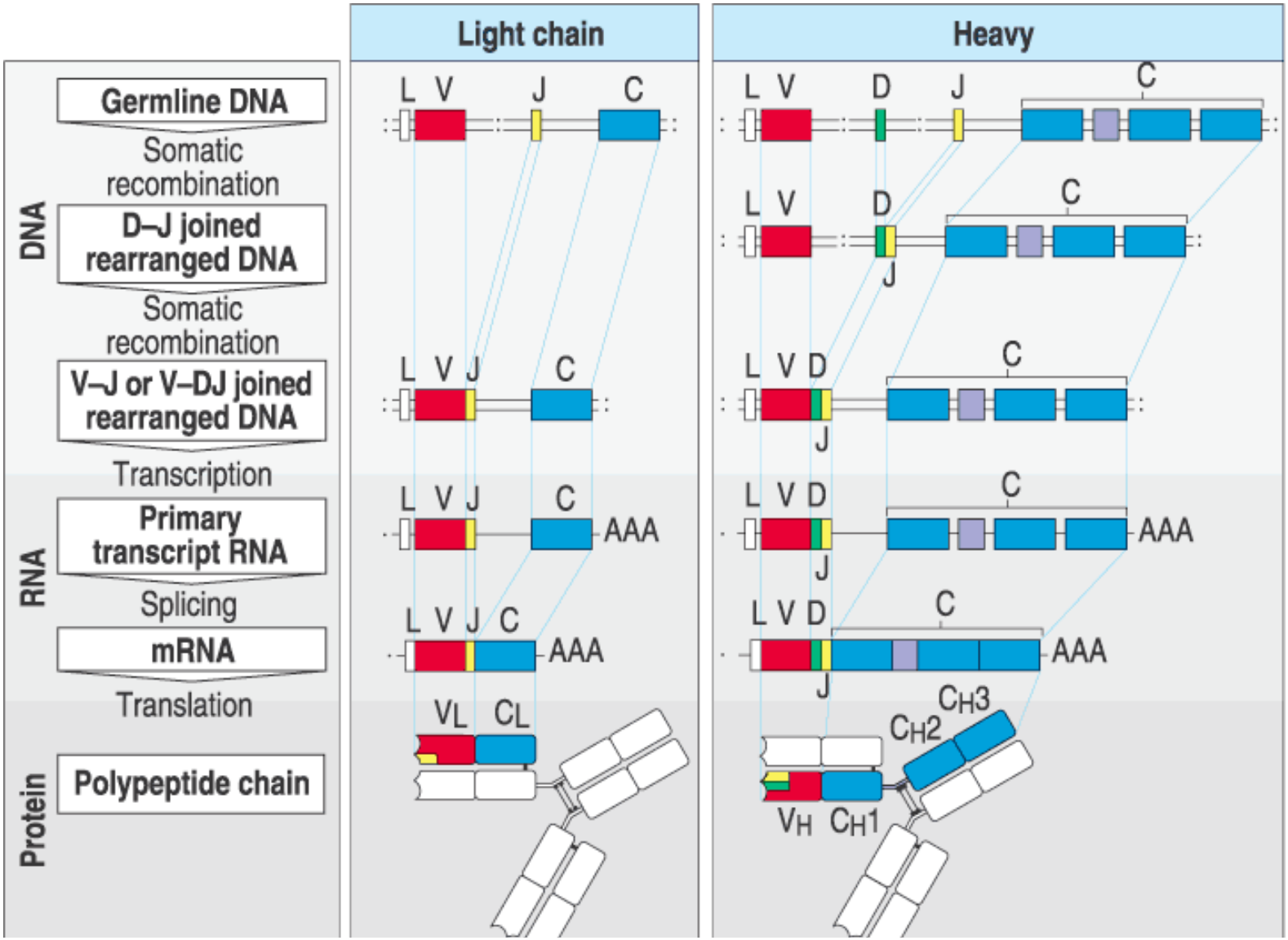


Fig. 4.2 V-region genes are constructed from gene segments.

Light-chain V-region genes are constructed from two segments (center panel). A variable (V) and a joining (J) gene segment in the genomic DNA are joined to form a complete light-chain V-region exon. Immunoglobulin chains are extra-cellular proteins and the V gene segment is preceded by an exon encoding a leader peptide (L), which directs the protein into the cell's secretory pathways and is then cleaved. The light-chain C region is encoded in a separate exon and is joined to the V-region exon by splicing of the light-chain RNA to remove the L-to-V and the J-to-C introns. Heavy-chain V regions are constructed from three gene segments (right panel). First, the diversity (D) and J gene segments join, then the V gene segment joins to the combined DJ sequence, forming a complete VH exon. A heavy-chain C-region gene is encoded by several exons. The C-region exons, together with the leader sequence, are spliced to the V-domain sequence during processing of the heavy-chain RNA transcript. The leader sequence is removed after translation and the disulfide bonds that link the polypeptide chains are formed. The hinge region is shown in purple.

Sources of diversity during immunoglobin gene rearrangement

1. Germline diversity in the sequences of the V, D, and J segments within an individual and within the population.
2. Combinatorial joining of individual V, D, and J segments to form a single heavy or light chain.
3. Combinatorial joining of the different heavy and light chains synthesized.
4. Removal or addition of random numbers of nucleotides at the junctions of the V, D, and J recombination regions.
5. Somatic hypermutation during the proliferative phase of an immune response.

Number of functional gene segments in human immunoglobulin loci

Segment	Light chains		Heavy chain
	κ	λ	H
Variable (V)	40	30	65
Diversity (D)	0	0	27
Joining (J)	5	4	6

Antibody diversity is generated in four main ways. Two of these are the consequences of recombination. The third is due to the different possible combinations of a heavy and light chain in the complete immunoglobin molecule. The fourth is a mutational process that occurs in mature B cells, acting only on rearranged DNA encoding the V regions.

The gene rearrangement that combines two or three gene segments to form a complete V-region exon generates diversity in two ways. First, there are multiple different copies of each type of gene segment, and different combinations of gene segments can be used in different rearrangement events. This combinatorial diversity is responsible for a substantial part of the diversity of the heavy- and light-chain V regions. Second, junctional diversity is introduced at the joints between the different gene segments as a result of addition and subtraction of nucleotides by the recombination process. A third source of diversity is also combinatorial, arising from the many possible different combinations of heavy- and light-chain V regions that pair to form the antigen-binding site in the immunoglobin molecule. The two means of generating combinatorial diversity alone could give rise, in theory, to approximately 3.5×10^6 different antibody molecules. Coupled with junctional diversity, it is estimated that as many as 10^{11} different receptors could make up the repertoire of receptors expressed by naïve B cells. Finally, somatic hypermutation introduces point mutations into the rearranged V-region genes of activated B cells, creating further diversity that can be selected for enhanced binding to antigen.

There are multiple copies of the V, D, and J gene segments, each of which is capable of contributing to an immunoglobin V region. Many different V regions can therefore be made by selecting different combinations of these segments. For human κ light chains, there are approximately 40 functional Vκ gene segments and 5Jκ gene segments, and thus potentially 200 different Vκ regions. For λ light chains there are approximately 30 functional V_λ gene segments and 4 J_λ gene segments, yielding 120 possible V_λ regions. So in all, 320 different light chains can be made. For the heavy chains of humans, there are 65 functional V_H gene segments, approximately 27 D_H gene segments and $6J_H$ gene segments, and thus around11,000 different possible V_H regions (65 X 27 X 6 = 11,000). As both the heavy- and light-chain V regions contribute to antibody specificity, each of the 320 different light chains could be combined with each of the approximately 11,000 heavy chains to give around 3.5×10^6 different antibody specificities. The ability to create many different specificities by making many different combinations of a small number of gene segments is known as combinatorial diversity.

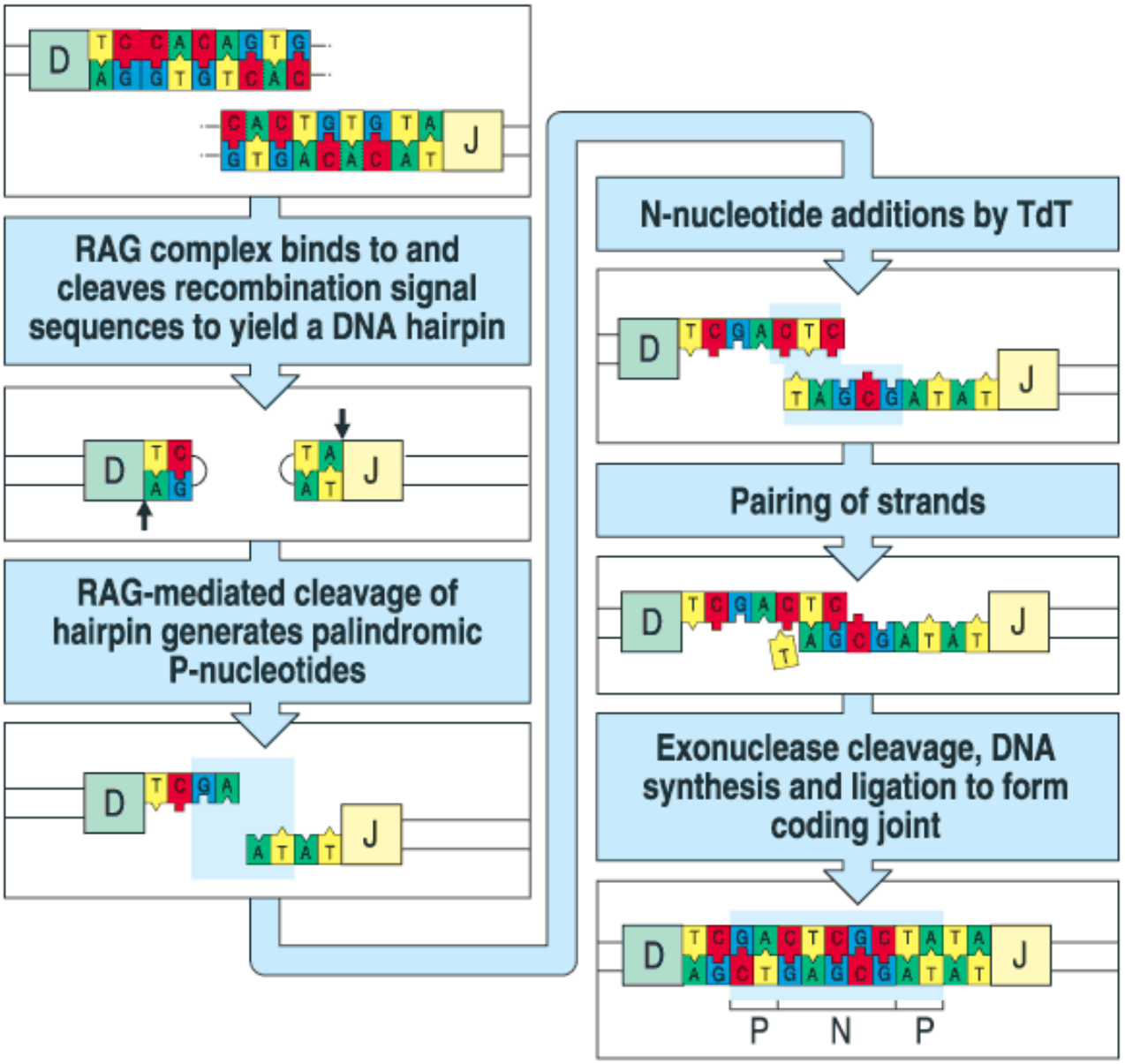


Fig 4.8 © 2001 Garland Science

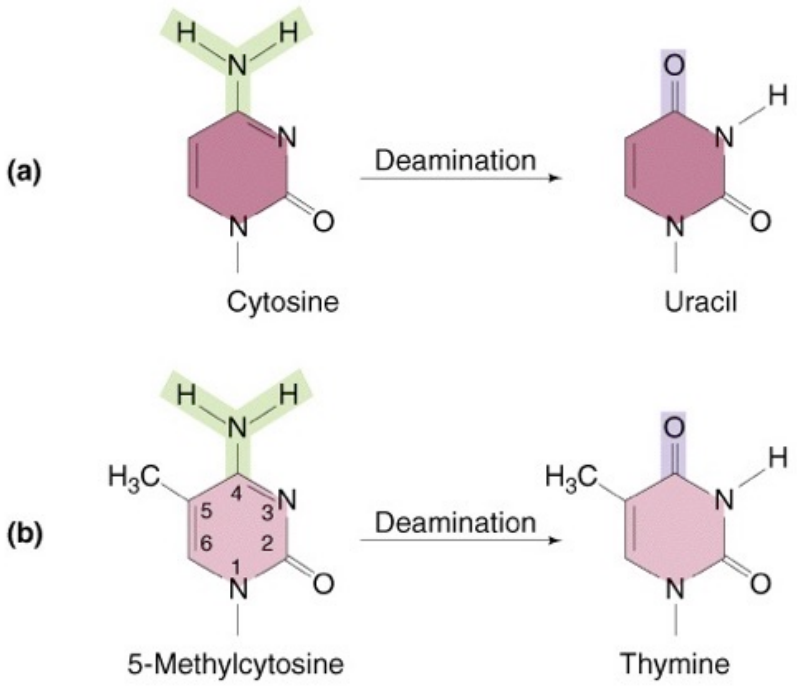
Fig. 4.8 The introduction of P- and N-nucleotides at the joints between gene segments during immunoglobulin gene rearrangement.

The process is illustrated for a DH to JH rearrangement; however, the same steps occur in VH to DH and in VL to JL rearrangements. After formation of the DNA hairpins (see Fig. 4.7), the two heptamer sequences, as indicated by the outline, are ligated to form the signal joint (not shown here), while RAG proteins cleave the DNA hairpin at a random site to yield a single-stranded DNA end. Depending on the site of cleavage, this single-stranded DNA may contain nucleotides thatwere originally complementary in the double-stranded DNA and which therefore form short DNA palindromes, as indicated by the shaded box in the third panel. Such stretches of nucleotides that originate from the complementary strand are known as P-nucleotides. For example, the sequence GA at the end of the D segment shown is complementary to the preceding sequence TC. Where the enzyme terminal deoxynucleotidyl transferase (TdT) is present, nucleotides are added at random to the ends of the single-stranded segments (fourth panel), indicated by the shaded box surrounding these nontemplated, or N, nucleotides. The two single-stranded ends then pair (fifth panel). Exonuclease trimming of unpaired nucleotides and repair of the coding joint by DNA synthesis and ligation leaves both the P- and N-nucleotides present in the final coding joint (indicated by shading in the bottom panel). The randomness of insertion of P- and N-nucleotides makes an individual P-N region a valuable marker for following an individual B-cell clone as it develops, for instance in studies of somatic hypermutation (see Fig. 4.9).

Activation induced cytosine deaminase

Deamination promotes somatic hypermutation

Not always restricted to immunoglobulin loci. Thus....B cell lymphomas



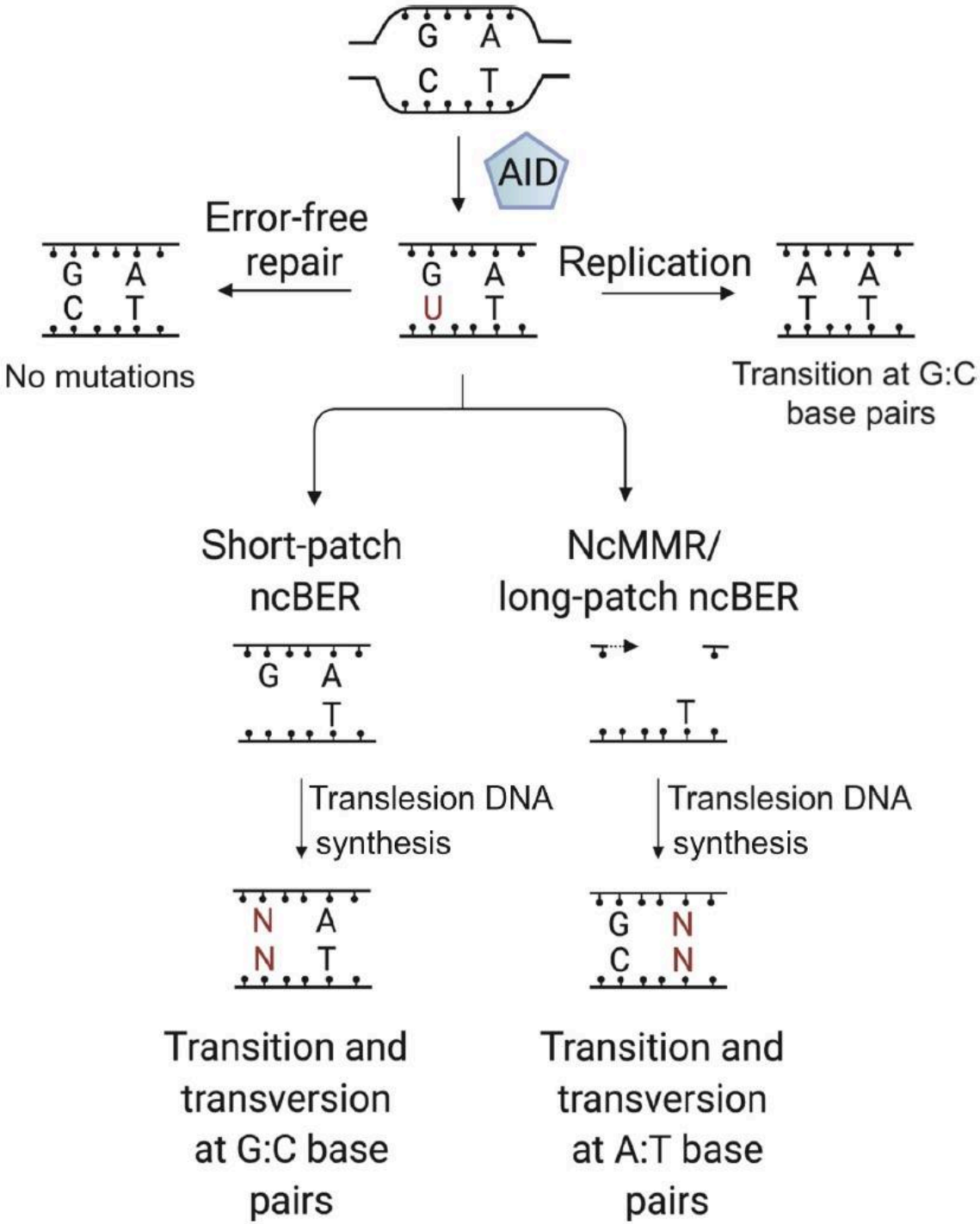


Figure 1. Multifaceted Uracil Lesion Processing in Somatic Hypermutation (SHM). AID-Induced dU:dG mismatches can be resolved by various pathways, leading to a diverse mutation spectrum at G:C and A:T base pairs in immunoglobulin variable (Ig V) regions. AID-induced uracil lesions can be repaired faithfully by canonical BER and MMR, leading to no SHM. Replicating over uracils by replicative polymerases generates transition mutations at G:C base pairs.

UNG, and to a lesser extent, SMUG1, from the BER pathway, detect and excise uracils from DNA, creating abasic sites. Replicating over abasic sites by translesion synthesis polymerase from short-patch ncBER pathway generates transition and transversion mutations at G:C base pairs.

Noncanonical MMR pathway or long-patch BER can excise single stranded DNA surrounding either the dU:dG mismatch, or the abasic site, respectively, to initiate patch excision that leads to the engagement of translesion synthesis polymerases, generating both transition and transversion mutations at A:T base pairs. Abbreviations: AID, Activation-induced Cytidine Deaminase; ncBER, noncanonical base excision repair; ncMMR, noncanonical mismatch repair; SMUG1, single-strand selective monofunctional uracil DNA glycosylase; UNG, uracil DNA glycosylase.

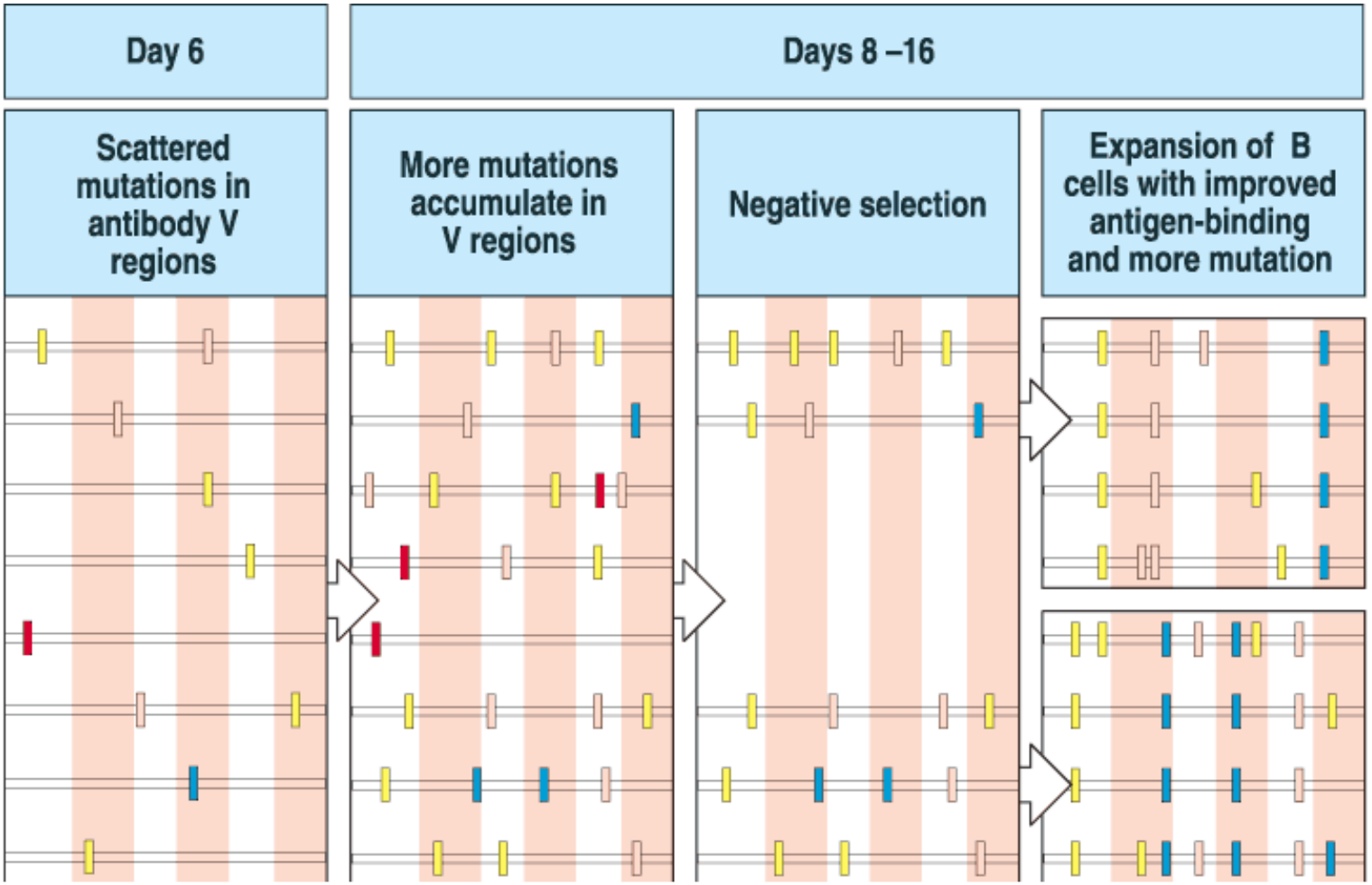


Fig. 4.9 Somatic hypermutation introduces variation into the rearranged immunoglobulin variable region that is subject to negative and positive selection to yield improved antigen binding.

In some circumstances it is possible to follow the process of somatic hypermutation by sequencing immunoglobulin variable regions at different time points after immunization. The result of one such experiment is depicted here. Within a few days of immunization, it is found that the variable regions within a particular clone of responding B cells have begun to acquire mutations (first panel). Each variable region is represented by a horizontal line, on which the positions of the mutations are represented by vertical bars. These may be silent (yellow bars), neutral (pink bars), deleterious (red bars), or positive (blue bars). Over the course of the next week, more mutations accumulate (second panel). Those B cells whose variable regions have accumulated deleterious mutations and can no longer bind antigen die, a process of negative selection (third panel). Those B cells whose variable regions have acquired mutations that result in improved antigen binding are able to compete effectively for binding to the antigen, and receive signals that drive their proliferation and expansion, along with continued mutation (fourth panel). This process of mutation and selection can actually go through multiple cycles (not shown for simplicity) during the second and third weeks of the germinal center reaction. In this way, over time, the antigen-binding efficiency of the antibody response is improved.

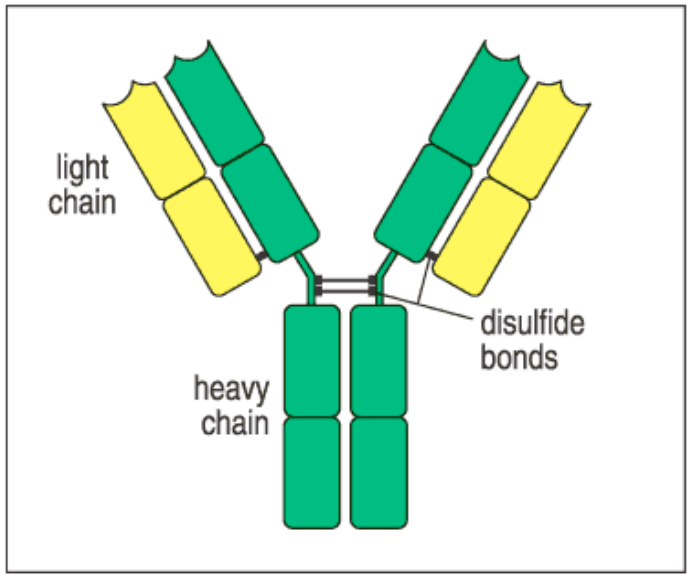
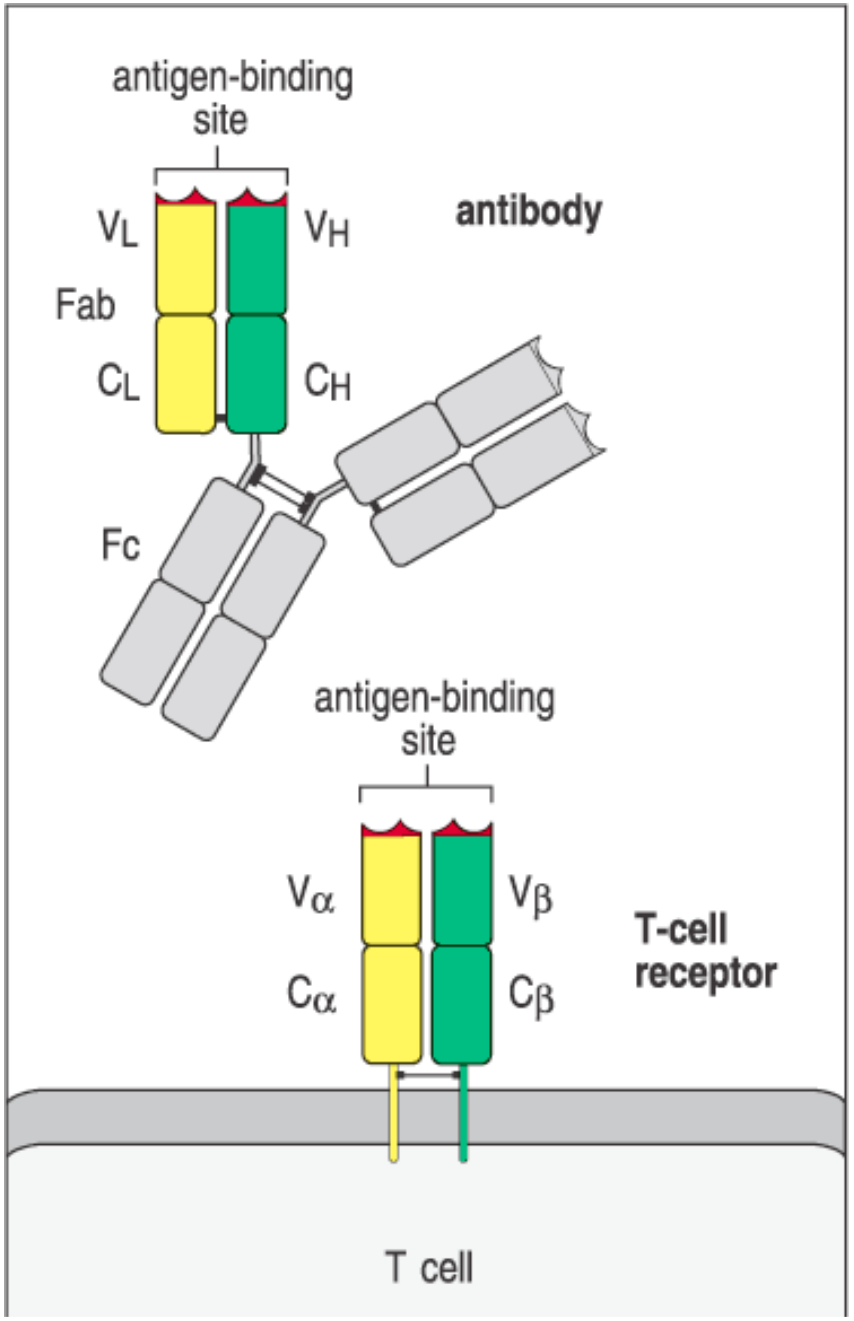


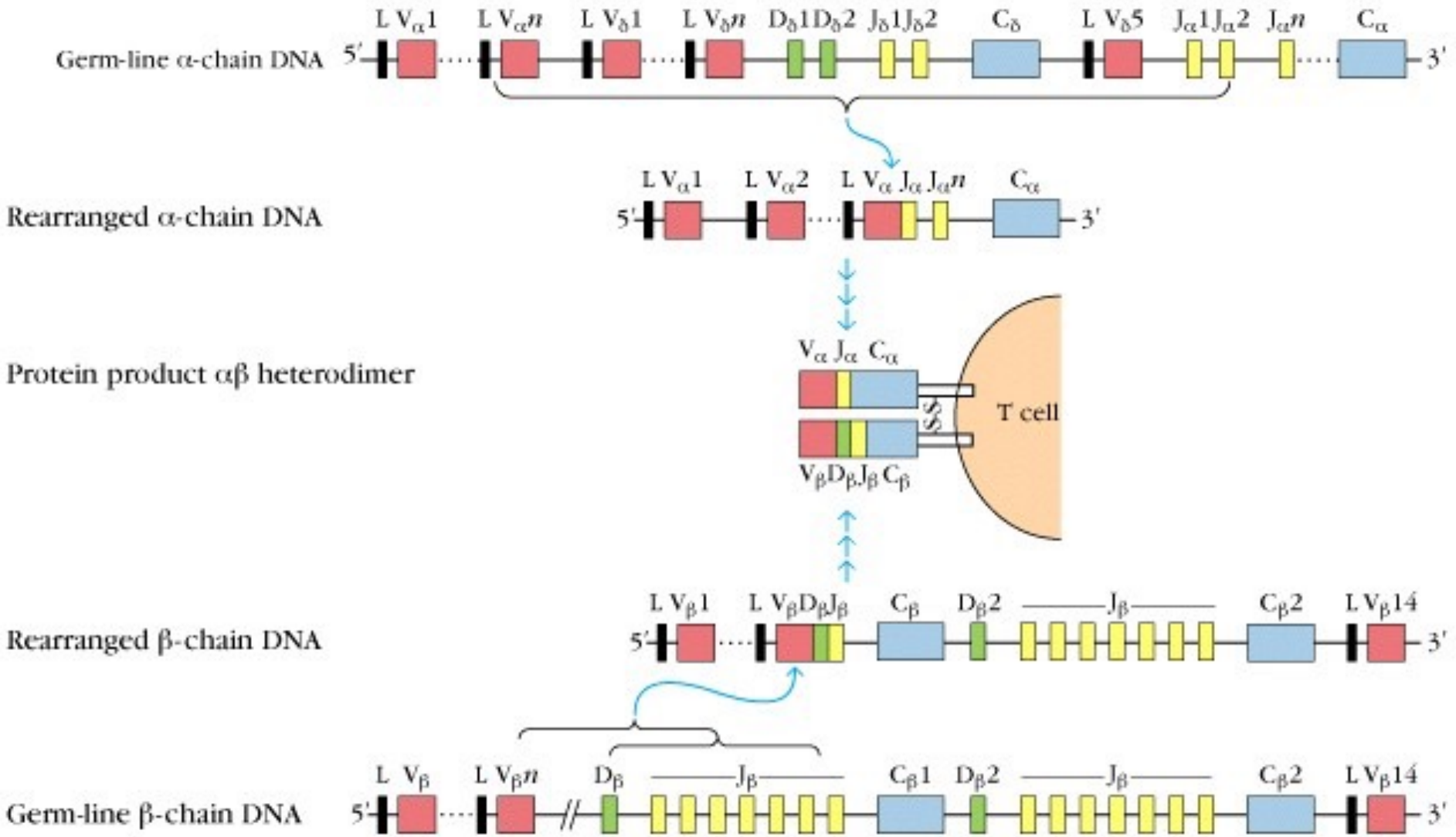
Fig 3.2 © 2001 Garland Science

Each immunoglobulin molecule is made up of two heavy chains (green) and two light chains (yellow) joined by disulfide bonds so that each heavy chain is linked to a light chain and the two heavy chains are linked together.

The Fab fragment of antibody molecules is a disulfide-linked heterodimer, each chain of which contains one immunoglobulin C domain and one V domain; the juxtaposition of the V domains forms the antigen-binding site (see Section 3-6). The T-cell receptor is also a disulfide-linked heterodimer, with each chain containing an immunoglobulin C-like domain and an immunoglobulin V-like domain. As in the Fab fragment, the juxtaposition of the V domains forms the site for antigen recognition.



T cell rearrangement creates a diversity of antigen-binding receptors



Cytotoxic T cells kill virally infected target cells

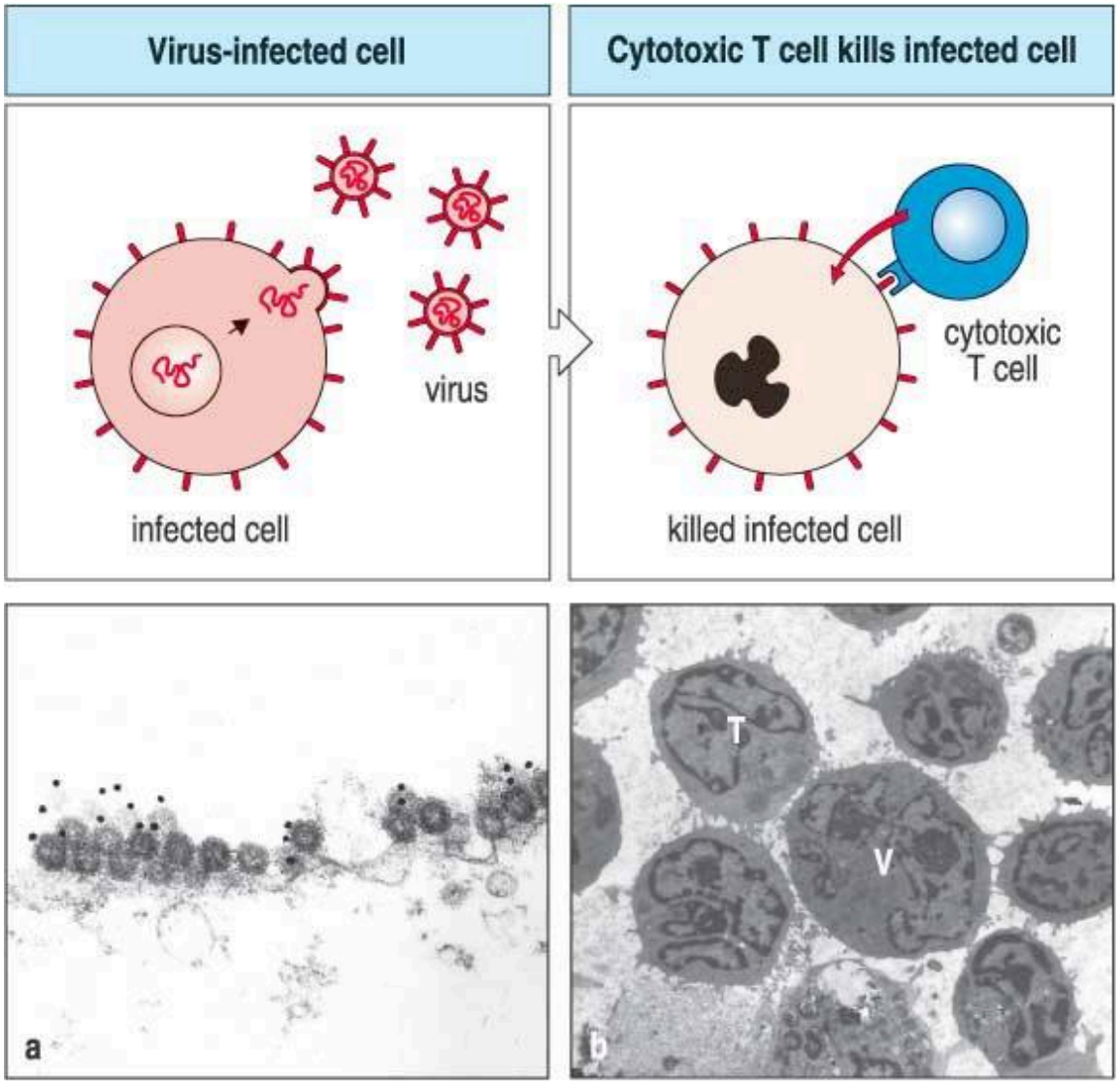
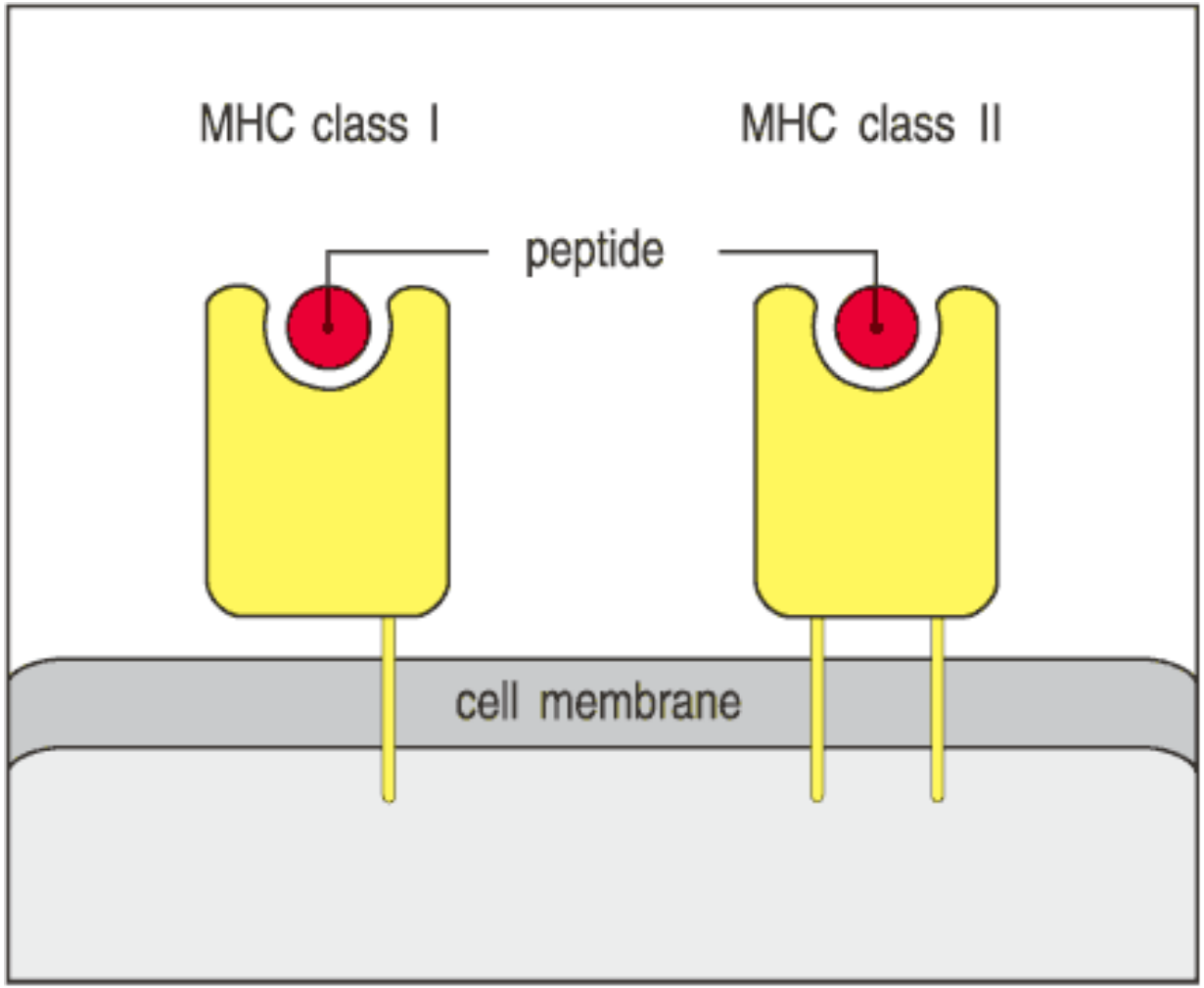


Fig 1.25 © 2001 Garland Science

Cells infected by viruses are recognized by specialized T cells called cytotoxic T cells, which kill the infected cells directly. The killing mechanism involves the activation of enzymes known as caspases, which cleave after aspartic acid. These in turn activate a cytostolic nuclease in the infected cell, which cleaves host and viral DNA. Panel a is a transmission electron micrograph showing the plasma membrane of a cultured CHO cell (the Chinese hamster ovary cell line) infected with influenza virus. Many virus particles can be seen budding from the cell surface. Some of these have been labeled with a monoclonal antibody that is specific for a viral protein and is coupled to gold particles, which appear as the solid black dots in the micrograph. Panel b is a transmission electron micrograph of a virus-infected cell (V) surrounded by cytotoxic T lymphocytes. Note the close apposition of the membranes of the virus-infected cell and the T cell (T) in the upper left corner of the micrograph, and the clustering of the cytoplasmic organelles in the T cell between the nucleus and the point of contact with the infected cell.



MHC molecules are membrane proteins whose outer extracellular domains form a cleft in which a peptide fragment is bound. These fragments, which are derived from proteins degraded inside the cell, including foreign protein antigens, are bound by the newly synthesized MHC molecule before it reaches the surface. There are two kinds of MHC molecule-MHC class I and MHC class II-;with related but distinct structures and functions.

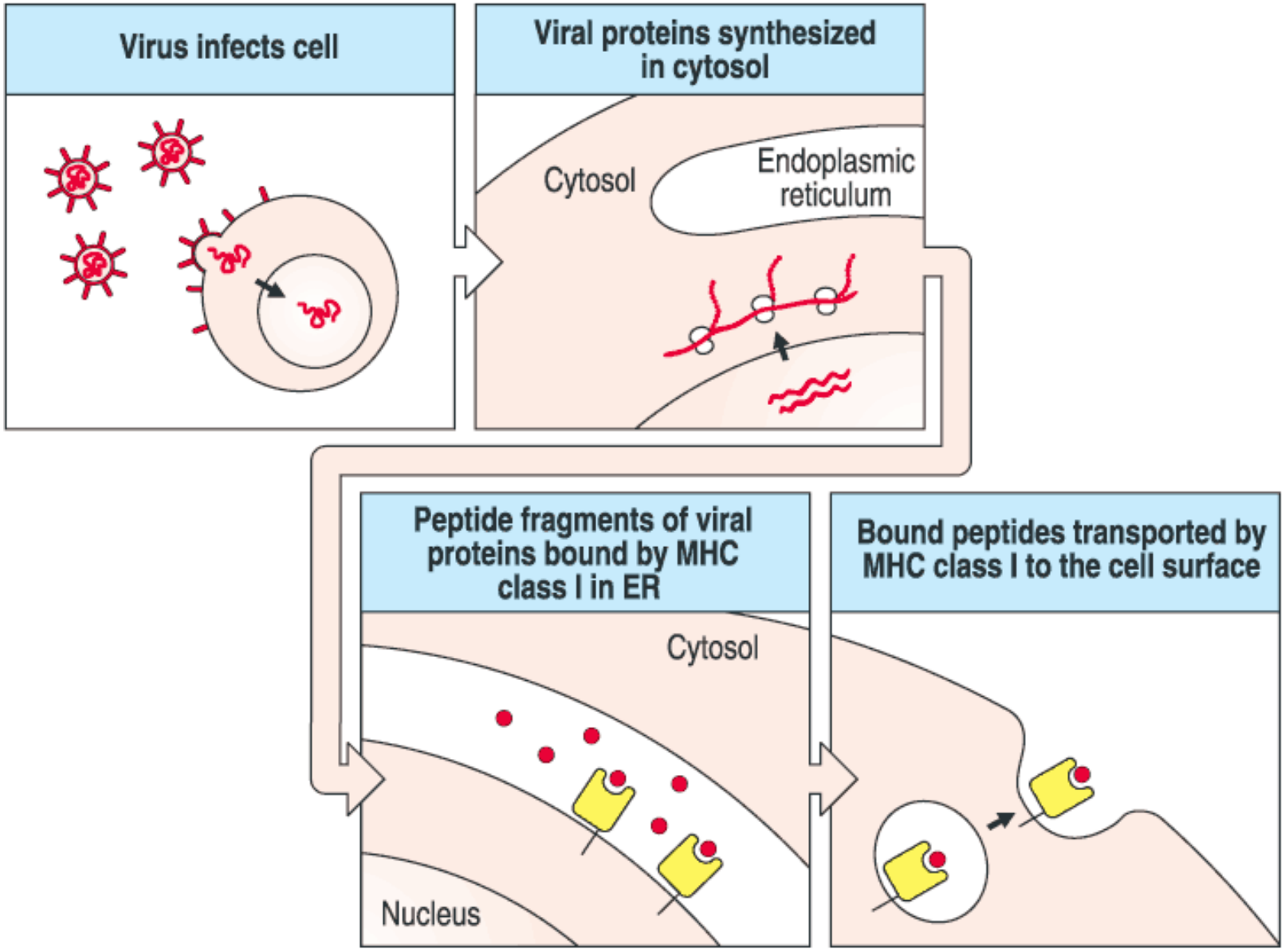
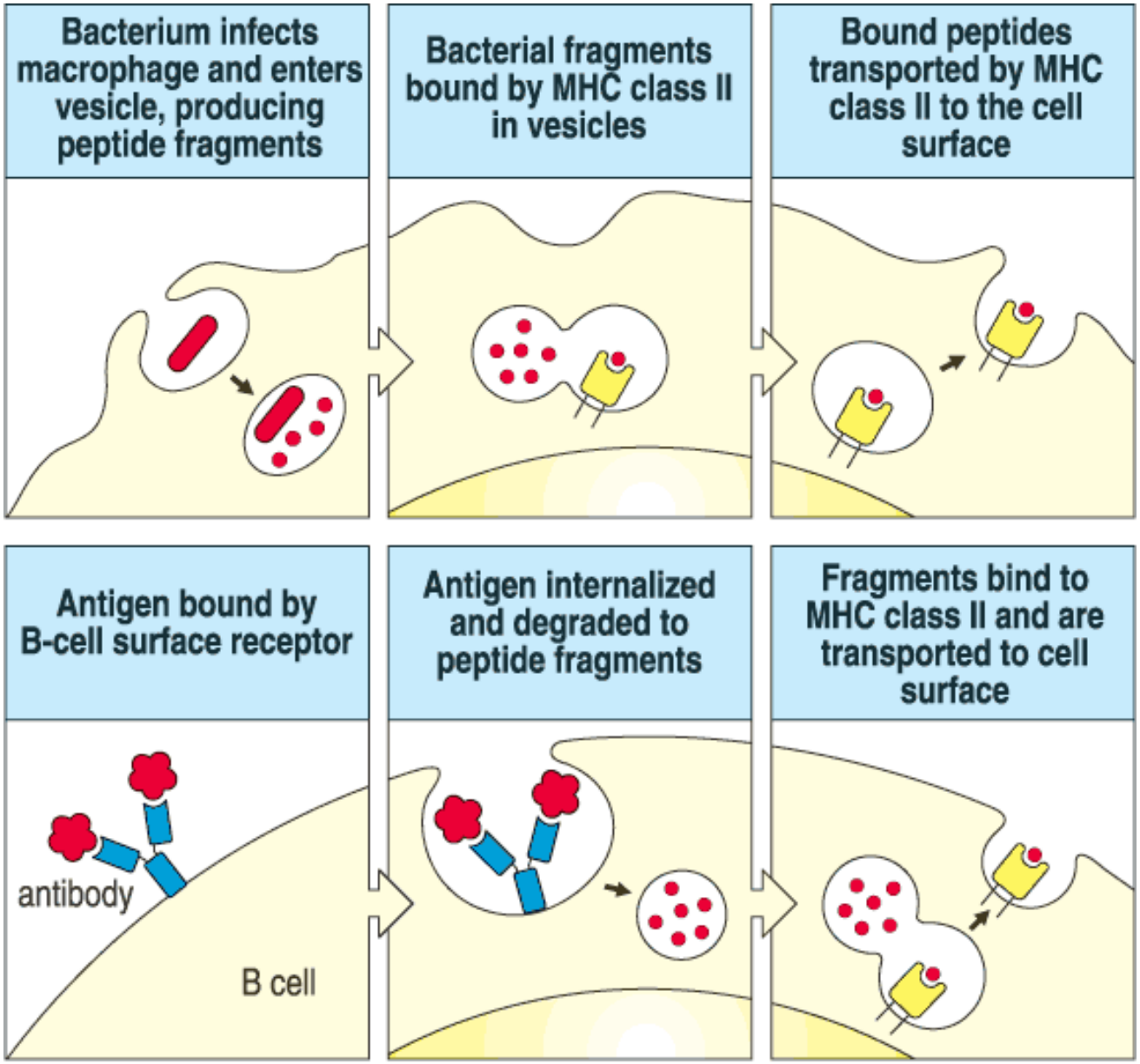
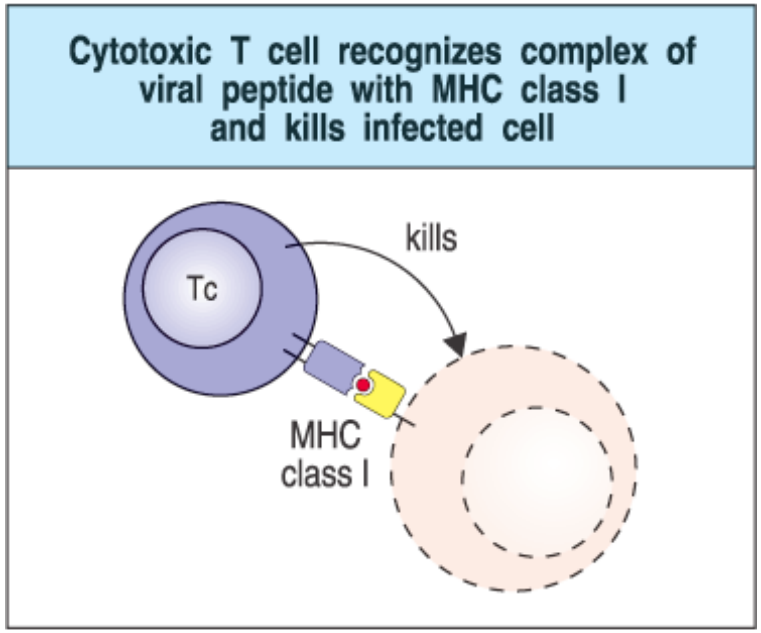


Fig 1.28 © 2001 Garland Science

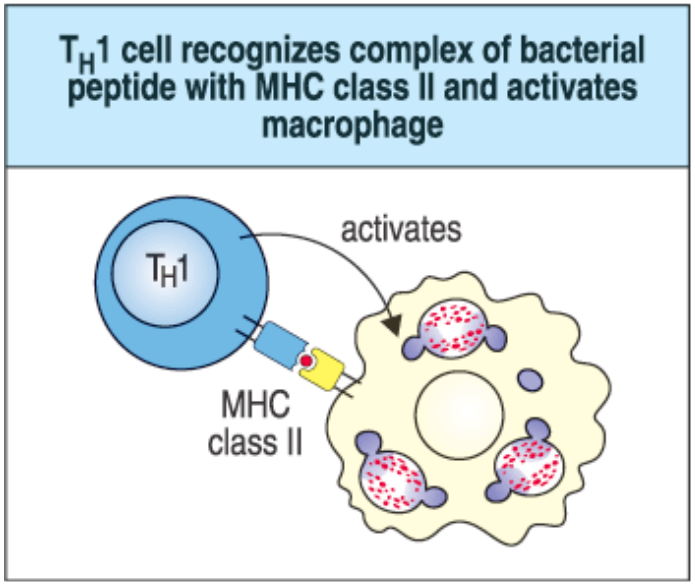
In cells infected with viruses, viral proteins are synthesized in the cytosol. Peptide fragments of viral proteins are transported into the endoplasmic reticulum (ER) where they are bound by MHC class I molecules, which then deliver the peptides to the cell surface.

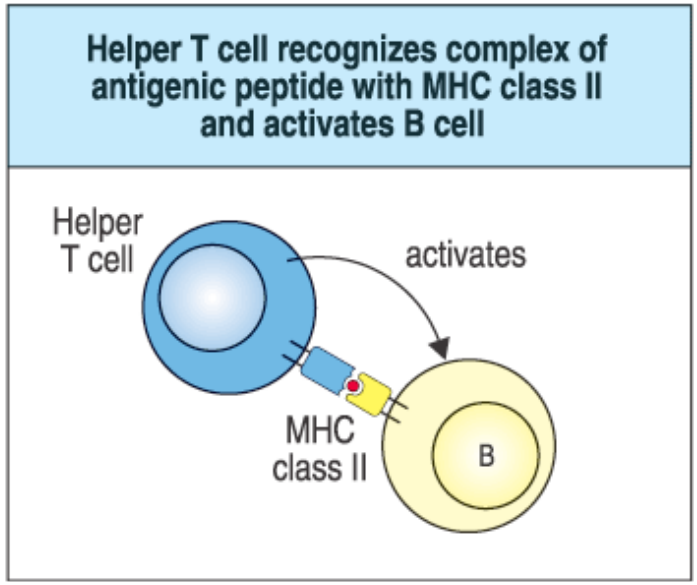


Some bacteria infect cells and grow in intracellular vesicles. Peptides derived from such bacteria are bound by MHC class II molecules and transported to the cell surface (top row). MHC class II molecules also bind and transport peptides derived from antigen that has been bound and internalized by B-cell antigen receptor-mediated uptake into intracellular vesicles (bottom row).



The peptide:MHC class I complex on virus-infected cells is detected by antigen-specific cytotoxic T cells. Cytotoxic T cells are preprogrammed to kill the cells they recognize.





On recognition of their specific antigen on infected macrophages, TH1 cells activate the macrophage, leading to the destruction of the intracellular bacteria (left panel). When helper T cells recognize antigen on B cells, they activate these cells to proliferate and differentiate into antibody-producing plasma cells (right panel).

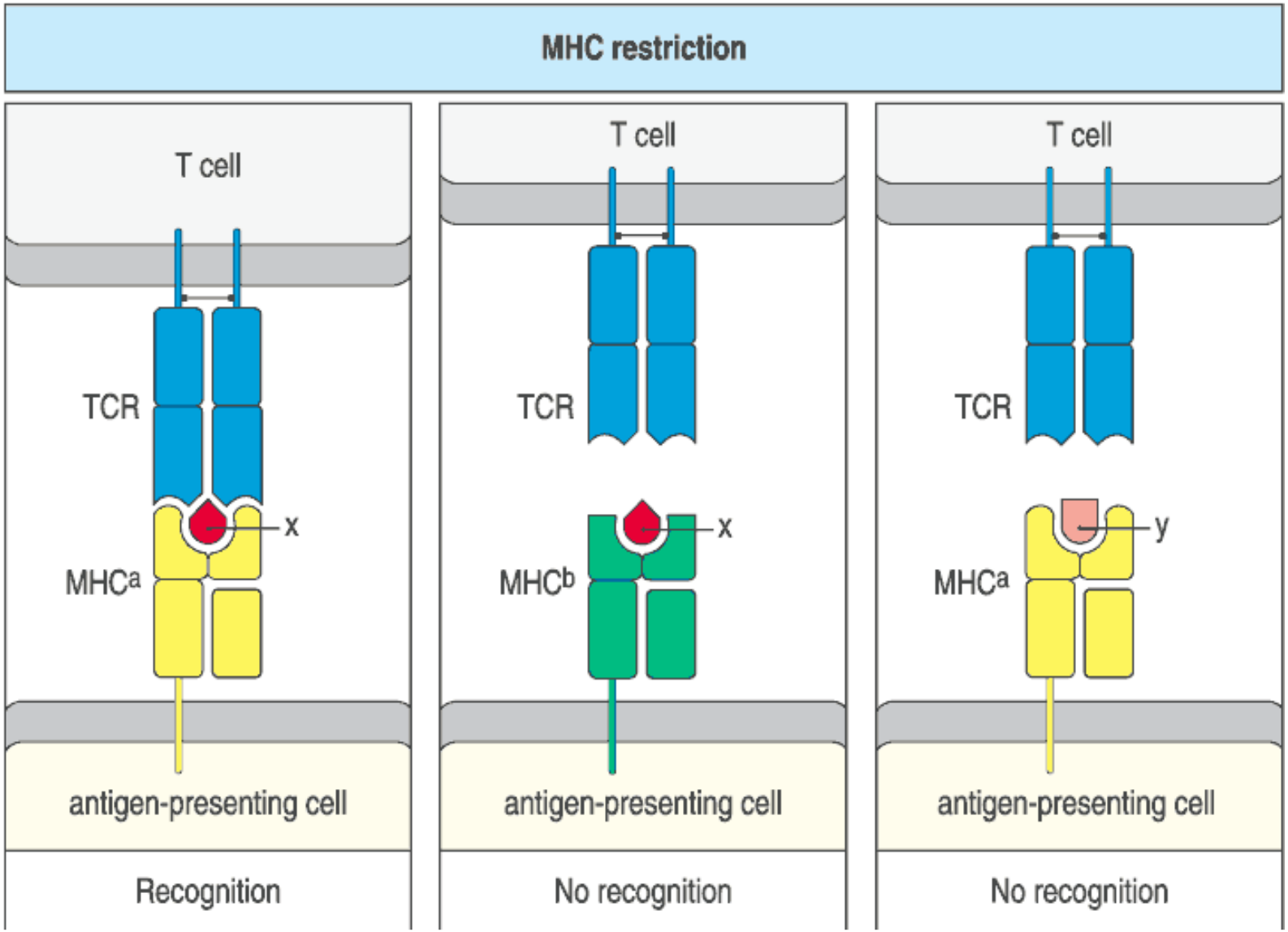


Fig. 5.16 T-cell recognition of antigens is MHC restricted.

The antigen-specific T-cell receptor (TCR) recognizes a complex of antigenic peptide and MHC. One consequence of this is that a T cell specific for peptide x and a particular MHC allele, MHCa (left panel), will not recognize the complex of peptide x with a different MHC allele, MHCb (center panel), or the complex of peptide y with MHCa (right panel). The co-recognition of peptide and MHC molecule is known as MHC restriction because the MHC molecule is said to restrict the ability of the T cell to recognize antigen. This restriction may either result from direct contact between MHC molecule and T-cell receptor or be an indirect effect of MHC polymorphism on the peptides that bind or on their bound conformation.

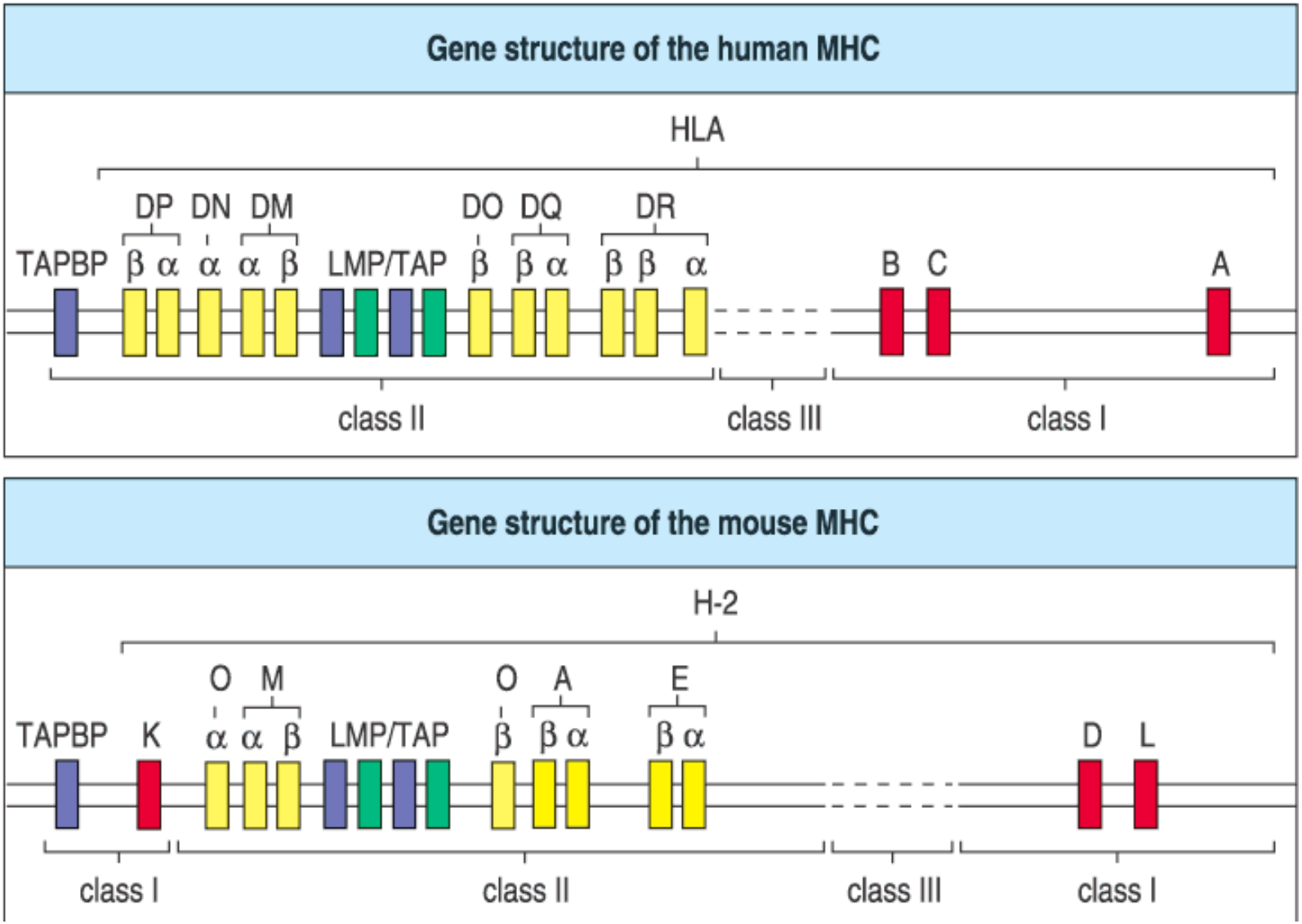


Fig. 5.10 The genetic organization of the major histocompatibility complex (MHC) in human and mouse.

The organization of the principal MHC genes is shown for both humans (where the MHC is called HLA and is on chromosome 6) and mice (in which the MHC is called H-2 and is on chromosome 17). The organization of the MHC genes is similar in both species. There are separate clusters of MHC class I genes (shown in red) and MHC class II genes (shown in yellow), although in the mouse an MHC class I gene (H-2K) appears to have translocated relative to the human MHC so that the class I region in mice is split in two. In both species there are three main class I genes, which are called HLA-A, - B, and -C in humans, and H2-K, -D, and -L in the mouse. The gene for b2-microglobulin, although it encodes part of the MHC class I molecule, is located on a different chromosome, chromosome 15 in humans and chromosome 2 in the mouse. The class II region includes the genes for the a and b chains of the antigen-presenting MHC class II molecules HLA-DR, -DP, and -DQ (H-2A and -E in the mouse). In addition, the genes for the TAP1:TAP2 peptide transporter, the LMP genes that encode proteasome subunits, the genes encoding the DMa and DMb chains, the genes encoding the a and b chains of the DO molecule (DNa and DOb, respectively), and the gene for tapasin (TAPBP) are also in the MHC class II region. The so-called class III genes encode various other proteins with functions in immunity (see Fig. 5.11).

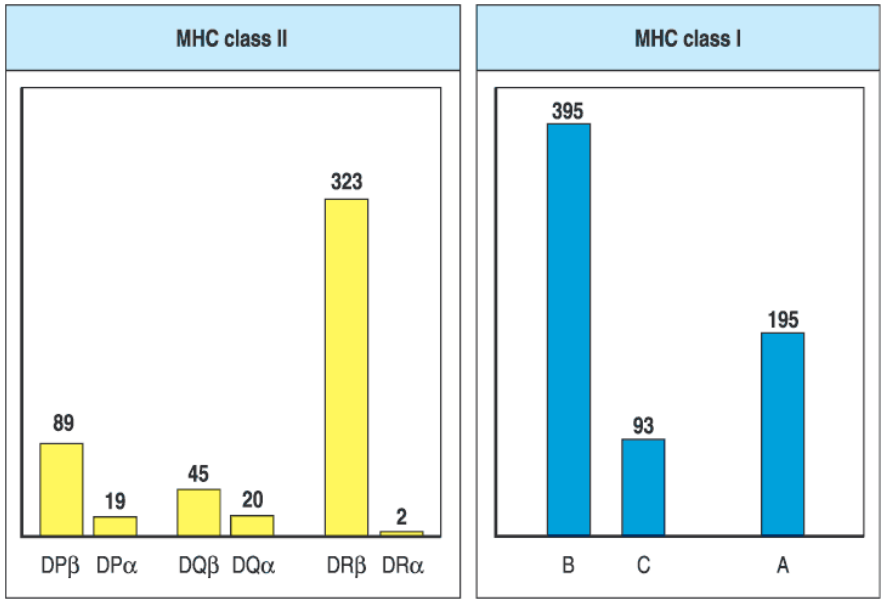


Fig 5.12 © 2001 Garland Science

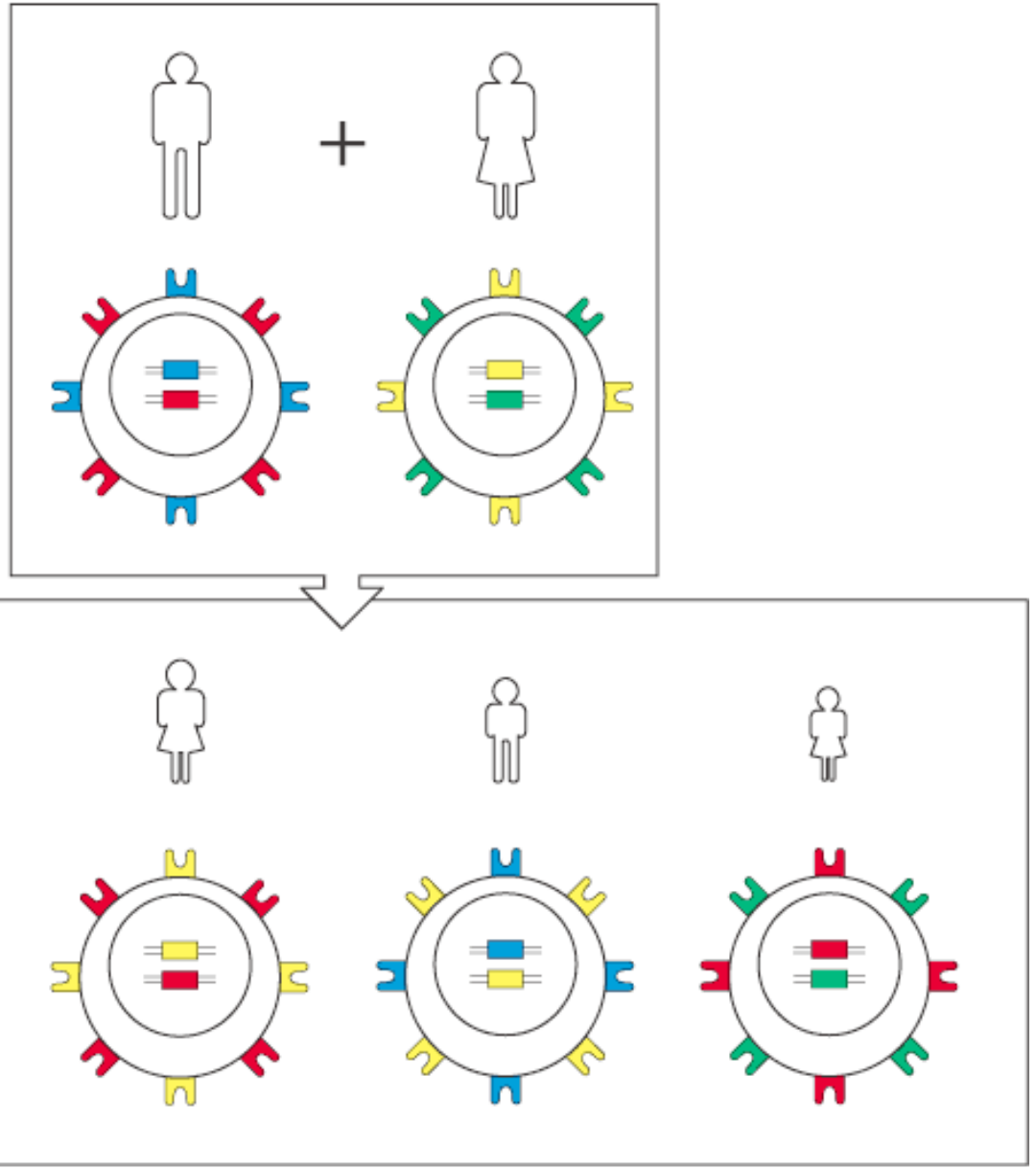


Fig. 5.12 Human MHC genes are highly polymorphic.

With the notable exception of the DRa locus, which is functionally monomorphic, each locus has many alleles. The number of different alleles is shown in this figure by the height of the bars. The figures are the numbers of HLA alleles currently officially assigned by the WHO Nomenclature Committee for Factors of the HLA System as of August 2000.

MHC expression is codominant. All alleles present are expressed

The MHC is so polymorphic that most individuals are likely to be heterozygous at each locus. Alleles are expressed from both MHC haplotypes in any one individual, and the products of all alleles are found on all expressing cells. In any mating, four possible combinations of haplotypes can be found in the offspring; thus siblings are also likely to differ in the MHC alleles they express, there being one chance in four that an individual will share both haplotypes with a sibling. One consequence of this is the difficulty of finding suitable donors for tissue transplantation.

Why is there such a high level of MHC diversity?

Parasites that can avoid recognition by host MHC will have an advantage in infection. Hosts can minimize this possibility by expressing a diversity of MHC alleles.

Negative frequency-dependent selection (rare allele advantage). When a new pathogen arises, if it is invisible to the host MHC, animals that carry these MHC will die and those that carry rare, resistant alleles will survive. This allele will subsequently increase in the population. An evolving pathogen that is now invisible to this new (common) allele will again select for individuals carrying rare, resistant alleles, continuing the cycle. Thus, no one allele is ever optimal for long at preventing pathogen infection.

Dominance and overdominance. Heterozygotes recognize recognize a wider variety of antigens derived from multiple pathogens and so have a higher relative fitness than either homozygote. Because MHC alleles are codominant, high levels of heterozygosity limits the possibility that you will carry alleles that fail to recognize the pathogen.

Optimizing your (or rather your offspring's) MHC allele diveristy. Some (perhaps many) animals are able to sense MHC diversity in potential mates (olfaction). Greater diversity is often preferred.

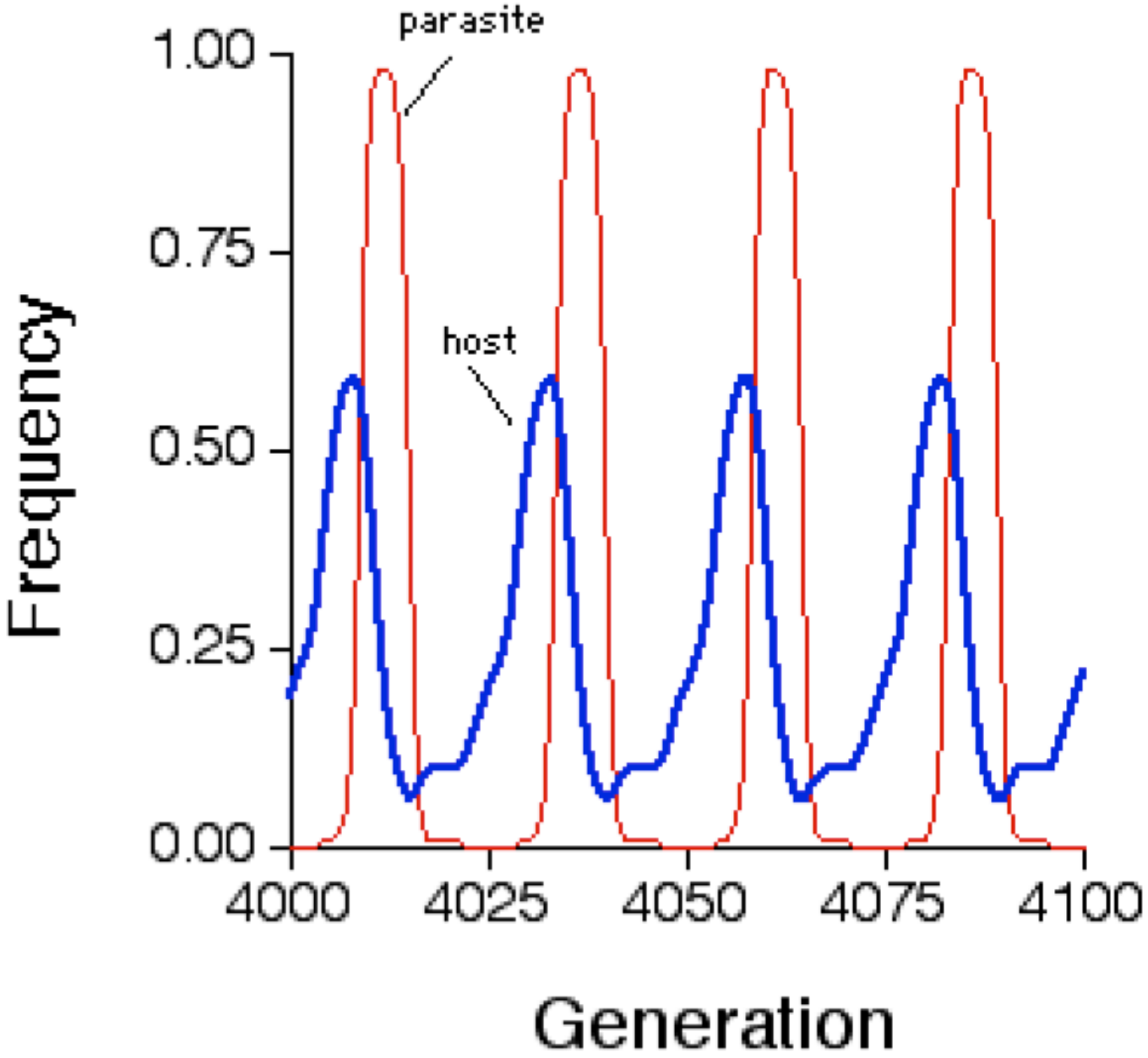
Host-parasite evolution: an evolutionary arms race

Host:

Evolve resistant genotypes

Parasite:

Evolve to overcome resistant genotypes



MHC heterozygosity confers a selective advantage against multiple-strain infections

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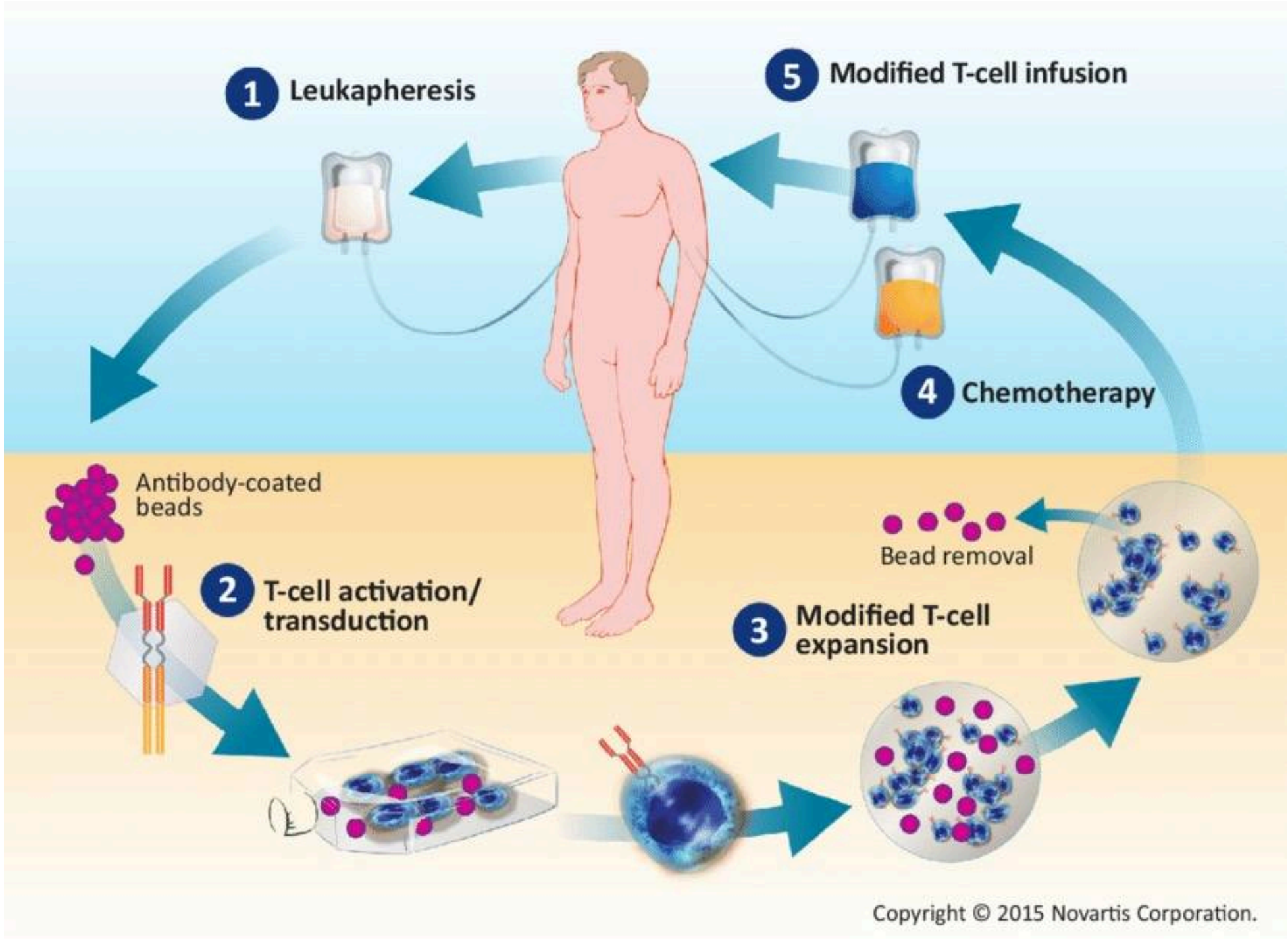
Department of Biology, University of Utah, Salt Lake City, UT 84112

Edited by John J. Mekalanos, Harvard Medical School, Boston, MA, and approved May 28, 2002 (received for review January 4, 2002)

Genetic heterozygosity is thought to enhance resistance of hosts to infectious diseases, but few tests of this idea exist. In particular, heterozygosity at the MHC, the highly polymorphic loci that control immunological recognition of pathogens, is suspected to confer a selective advantage by enhancing resistance to infectious diseases (the "heterozygote advantage" hypothesis). To test this hypothesis, we released mice into large population enclosures and challenged them with multiple strains of *Salmonella* and one of *Listeria*. We found that during *Salmonella* infections with three avirulent strains, MHC heterozygotes had greater survival and weight than homozygotes (unlike sham controls), and they were more likely to clear chronic *Salmonella* infection than homozygotes. In laboratory experiments, we found that MHC heterozygosity enhanced the clearance of multiple-strain *Salmonella* infections. Yet, contrary to what is widely assumed, the benefits of heterozygosity were due to resistance being dominant rather than overdominant, i.e., heterozygotes were more resistant than the average of parental homozygotes, but they were not more resistant than both. The fact that MHC heterozygotes were more resistant to infection and had higher fitness than homozygotes provides a functional explanation for MHC-disassortative mating preferences.

create an incidental heterozygote advantage because of heterosis, i.e., a spurious effect resulting from masking recessive deleterious mutations that may accumulate in inbred lines (12). Fourth, experimental studies have only tested single strains of pathogens or parasites, whereas the evidence for heterozygote advantage in human populations has been with genetically polymorphic infections (6, 8). Thus, the critical tests of the MHC-heterozygote advantage hypothesis have not been performed.

Another problem is that when heterozygotes show an advantage in population studies (6–8), it is generally interpreted as "heterozygote superiority" or "overdominance" (i.e., heterozygotes are assumed to have higher fitness than both parental homozygotes). Yet, heterozygotes may have a selective advantage over homozygotes on average if resistance is merely dominant (i.e., if heterozygotes inherit the resistance of the most resistant parental homozygote); and resistance is generally dominant, at least in tests with single pathogens (5). Therefore, for clarity, we will use "heterozygote advantage" in the broad sense when heterozygotes have a fitness advantage over homozygotes on average (like population studies), which may be due to dominance or overdominance, and only use heterozygote superiority (or overdominance) in the narrow sense when heterozy-


1 Leukapheresis
5 Modified T-cell infusion
4 Chemotherapy
Antibody-coated beads
2 T-cell activation/ transduction
Bead removal
3 Modified T-cell expansion
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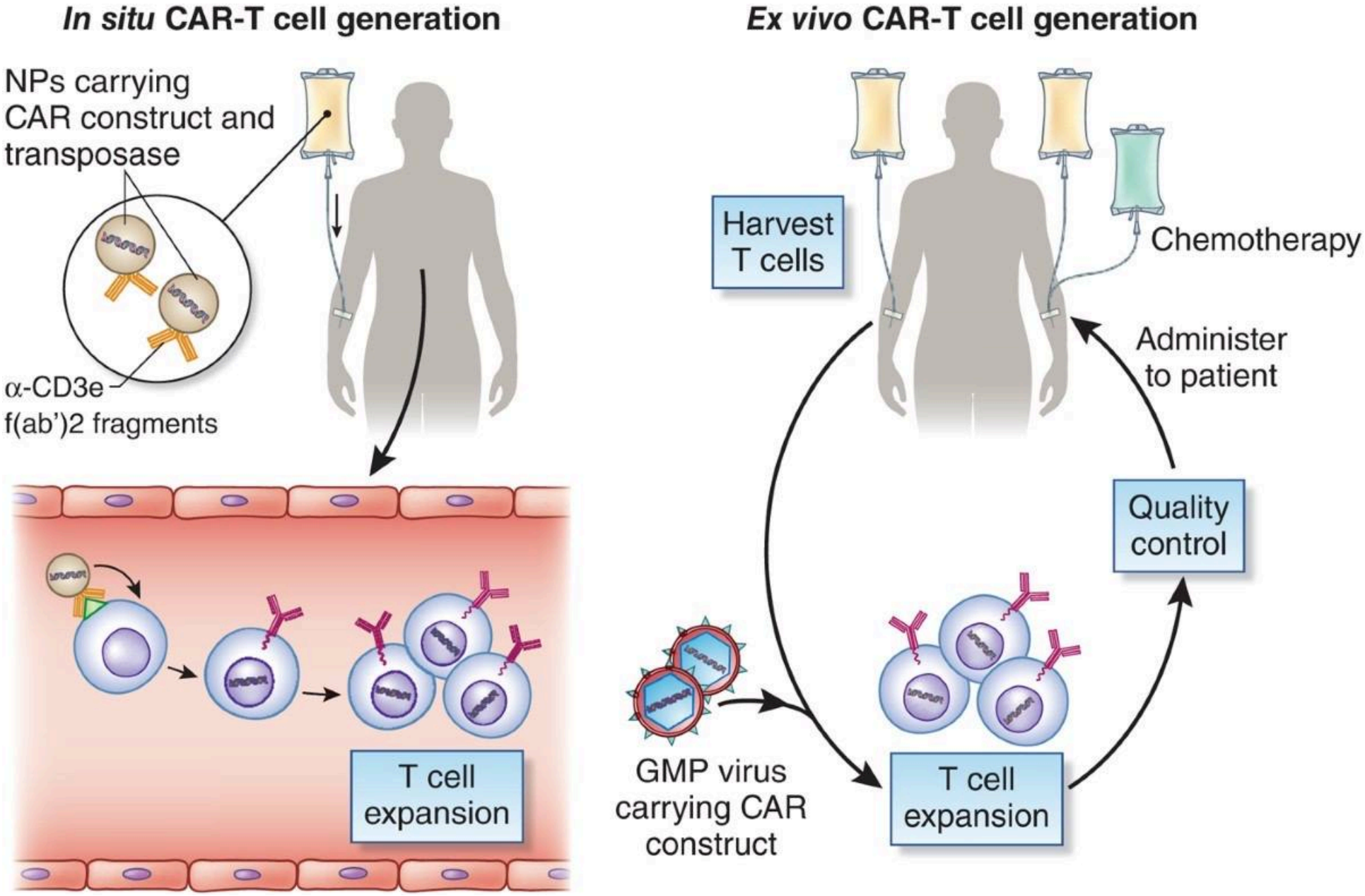
These are autologous T-cells. Allogenic T cells (from a donor) must first have their endogenous T-cell receptor removed using Crispr/HR



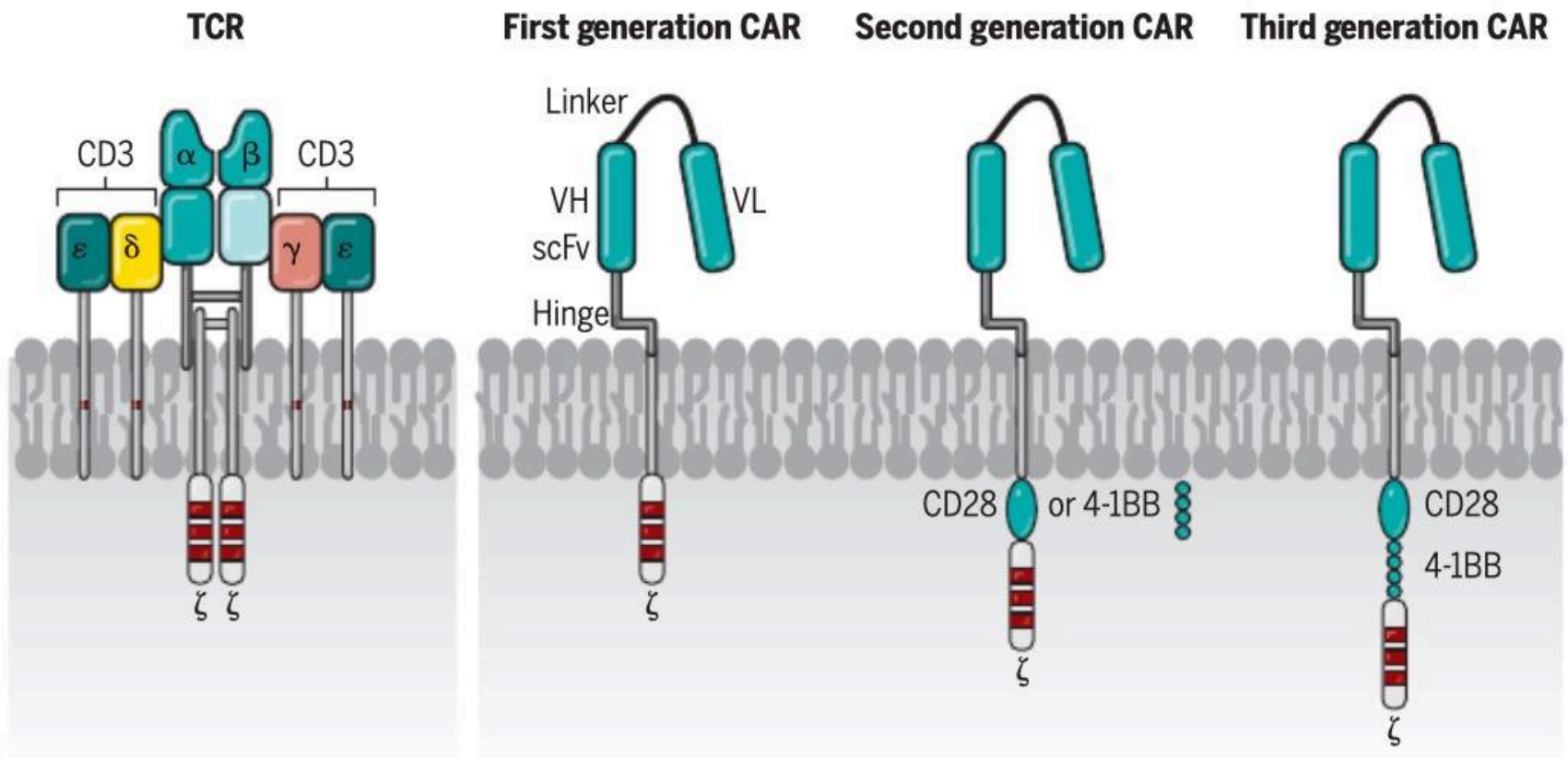
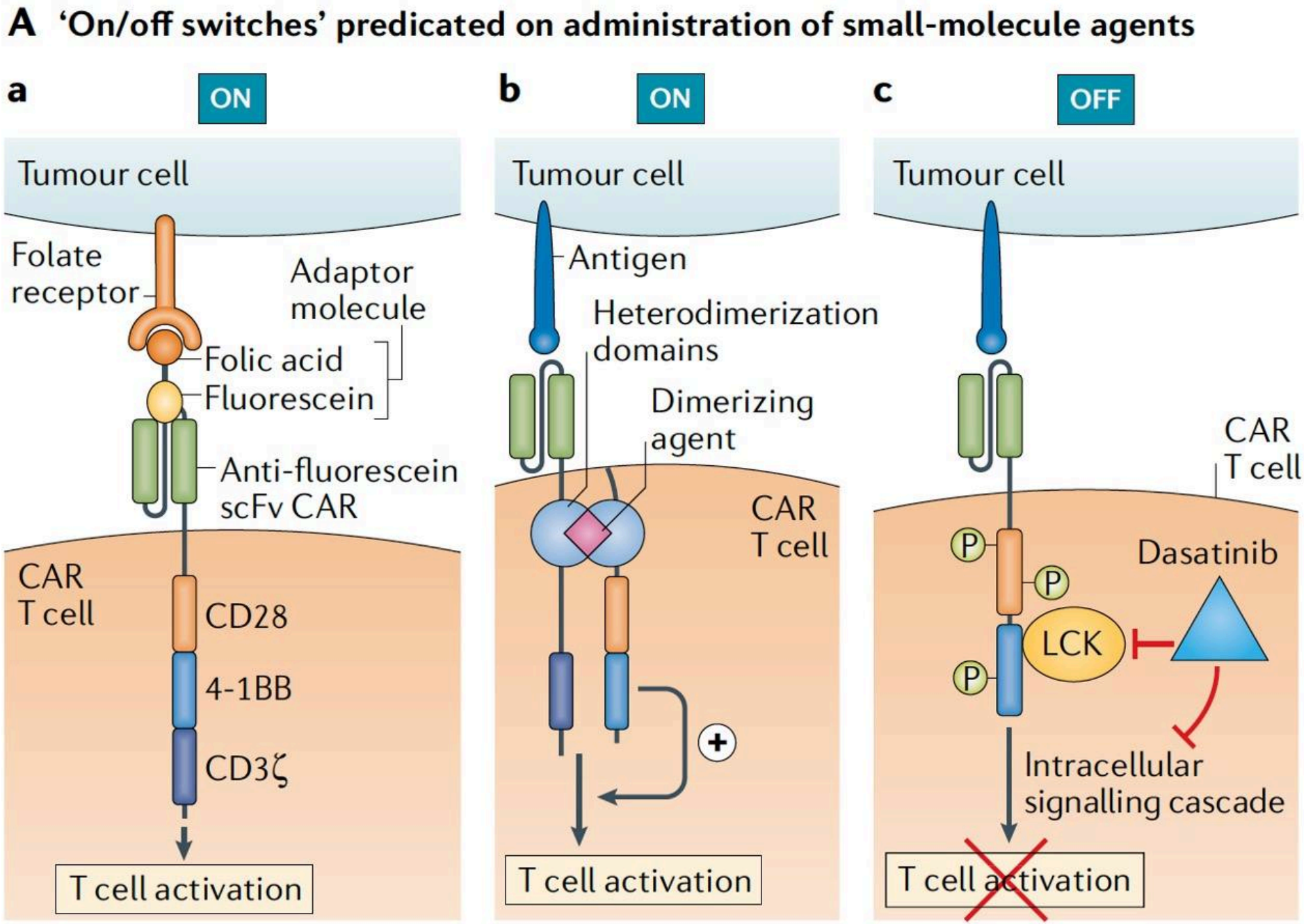


Fig. 1. Engineered T cells: design of TCR versus CAR T cells. T cells can be redirected to have specificity for tumors by the introduction of (**left**) transgenic TCRs (T cell receptors) or (**right**) CAR (chimeric antigen receptor) proteins. CARs are fusion proteins composed of an extracellular portion that is usually derived from an antibody and intracellular signaling modules derived from T cell signaling proteins. First-generation CARs contain CD3ζ, whereas second-generation CARs possess a costimulatory endodomain (e.g., CD28 or 4-1BB) fused to CD3ζ. Third-generation CARs consist of two costimulatory domains linked to CD3ζ. scFv, single-chain variable fragment; VH, variable heavy chain; VL, variable light chain.

More complex T-cell engagers



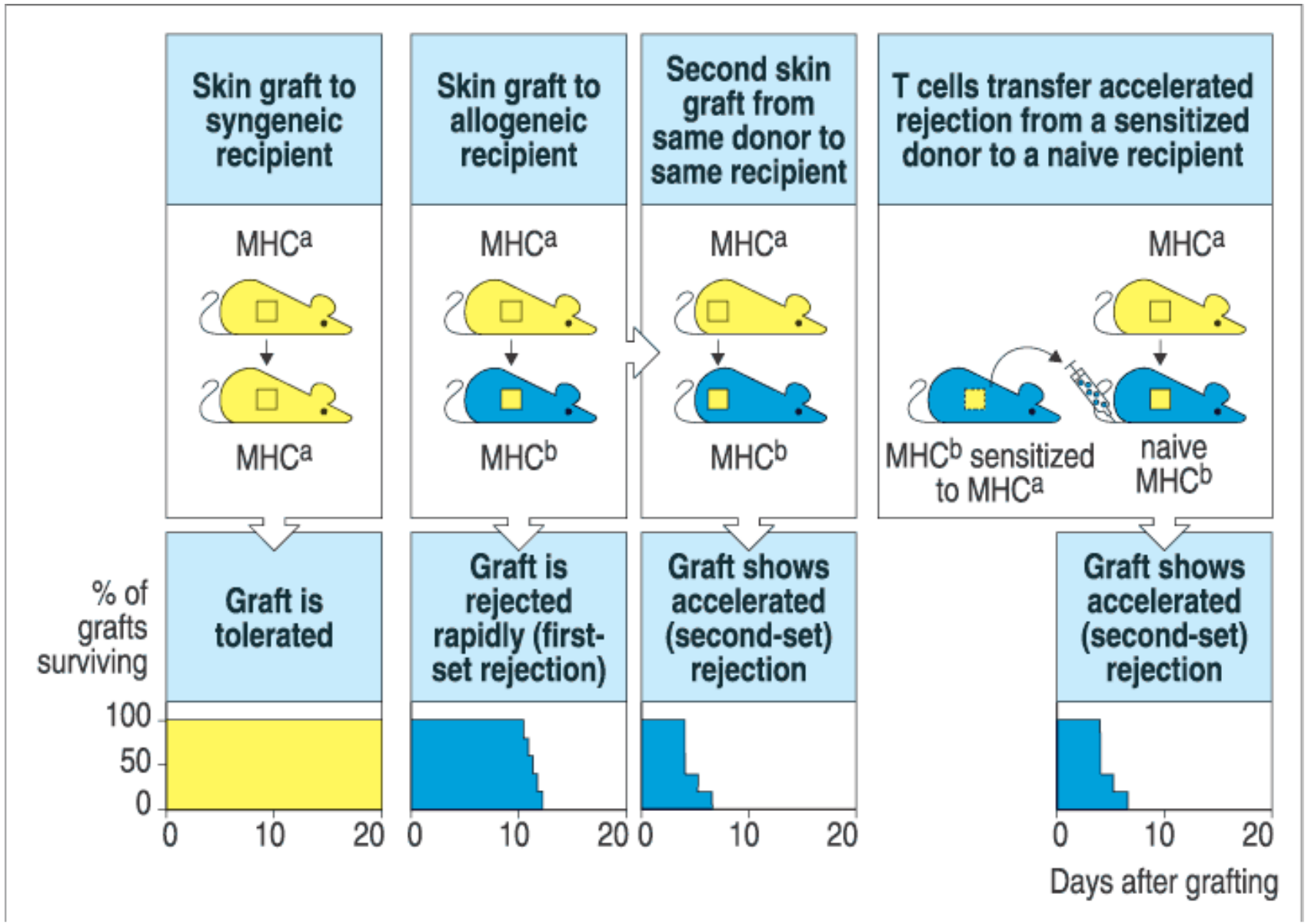


Fig. 13.22 Skin graft rejection is the result of a T cell-mediated anti-graft response.

Grafts that are syngeneic are permanently accepted (first panels) but grafts differing at the MHC are rejected around 10-13 days after grafting (first-set rejection, second panels). When a mouse is grafted for a second time with skin from the same donor, it rejects the second graft faster (third panels). This is called a second-set rejection and the accelerated response is MHC-specific; skin from a second donor of the same MHC type is rejected equally fast, whereas skin from an MHC-different donor is rejected in a first-set pattern (not shown). Naive mice that are given T cells from a sensitized donor behave as if they had already been grafted (final panels).

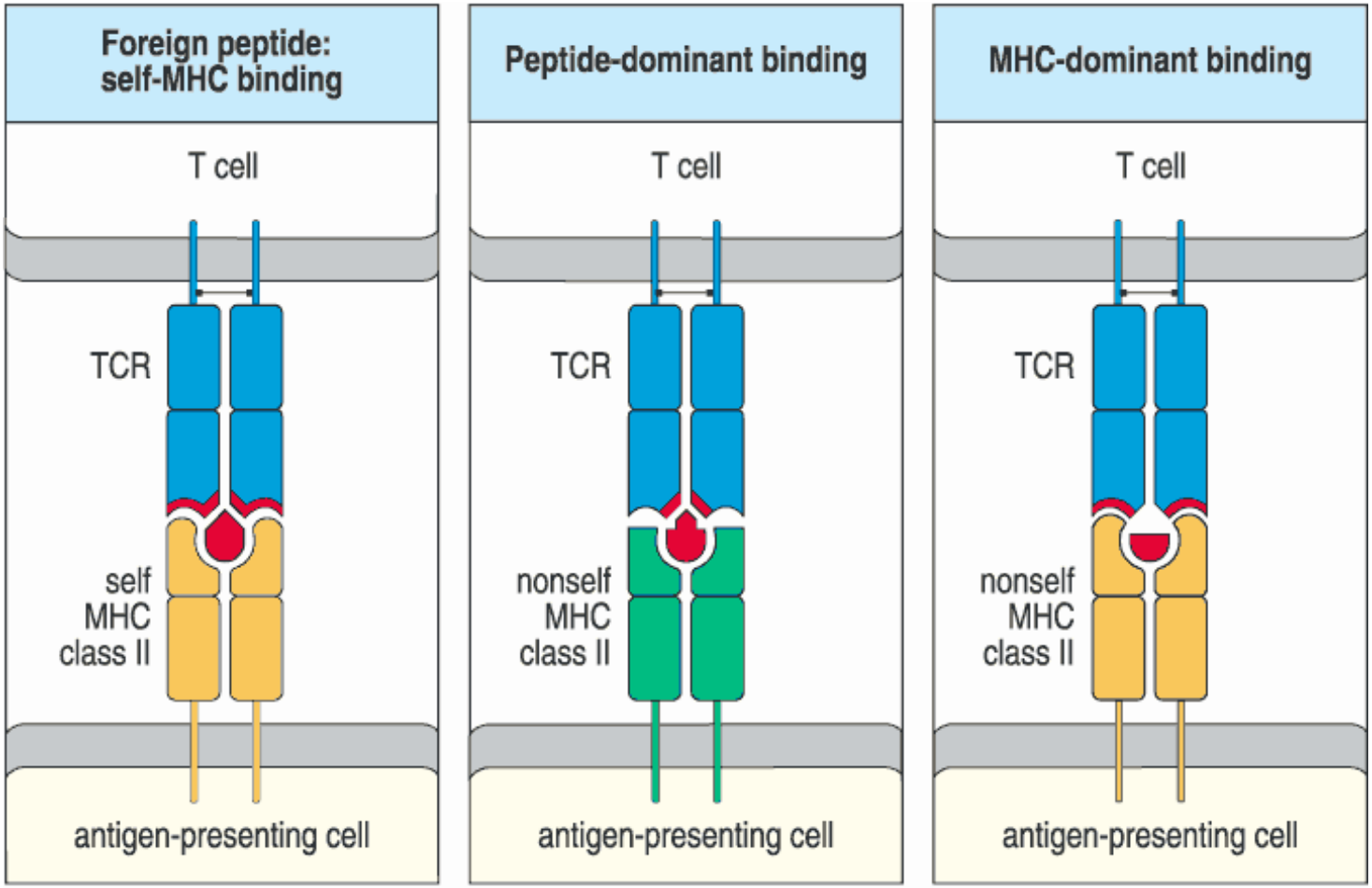
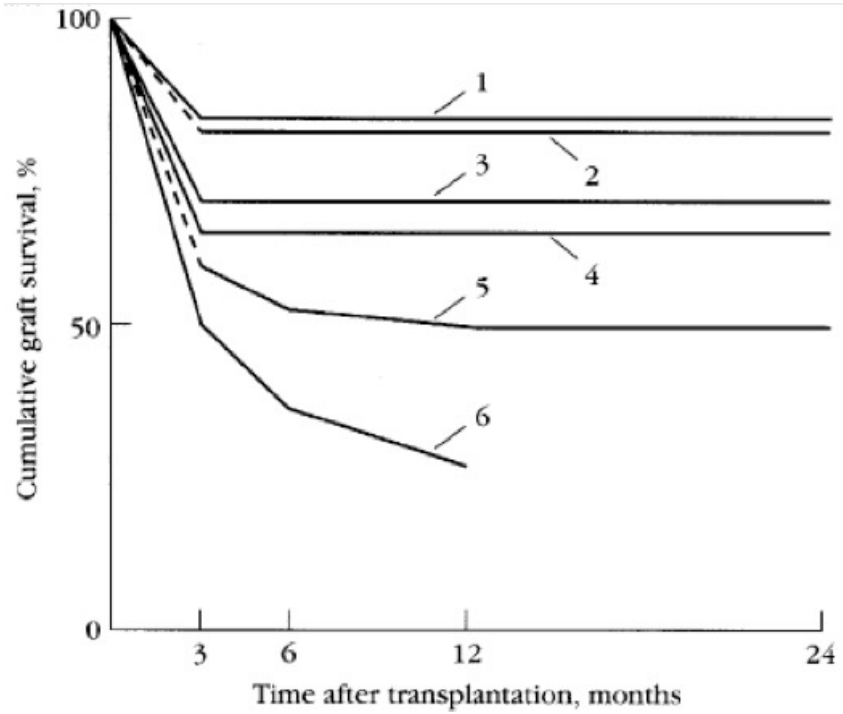


Fig. 5.17 Two modes of cross-reactive recognition that may explain alloreactivity.

A T cell that is specific for one peptide:MHC combination (left panel) may cross-react with peptides presented by other, nonself (allogeneic), MHC molecules. This may come about in either of two ways. Most commonly, the peptides bound to the allogeneic MHC molecule fit well to the T-cell receptor (TCR), allowing binding even if there is not a good fit with the MHC molecule (center panel). Alternatively, but less often, the allogeneic MHC molecule may provide a better fit to the T-cell receptor, giving a tight binding that is thus less dependent on the peptide that is bound to the MHC molecule (right panel).

Mismatching within the MHC loci between donors and recipients leads to graft rejection



Curve no.	HLA mismatches (no.) A,B	D
1	0	0
2	1 or 2	0
3	3 or 4	0
4	0	1 or 2
5	1 or 2	1 or 2
6	3 or 4	1 or 2

FIGURE 23-9

The effect of HLA-A, -B and -D antigen matching on survival of kidney grafts. Mismatching of HLA-A or HLA-B antigens has little effect on graft survival unless HLA-D is also mismatched. [Adapted from T. Moen et al., 1980, *N. Engl. J. Med.* **303**:850.]

One solution to graft rejection is to use immunosuppressive drugs. These have the drawback that the patient develops a generally weakened immune system, and is thus more susceptible to other infectious diseases.

Some genetic solutions to graft rejection

1. Clone yourself. Your mini-me MHC loci will be identical to your own.

2. If it is your kid that needs a donor, carry out in vitro fertilization and do genotyping at the 4-8 cell stage to identify those embryos that are MHC matched (1/4). Bone marrow stem cells from the in vitro-conceived kid will act as a MHC matched donor. As described in the newspaper article, this approach is actually being used to treat kids with genetic diseases of the immune system. Of course this approach is not perfect. The donor will be MHC matched, but will show differences from the recipient kid at other loci in the genome due to meiotic recombination--the donor is MHC matched, but is not a clone!!

The patients immune system may destroy the new bone marrow (graft rejection)

Graft versus host disease (the patients body is perceived as foreign and is destroyed by the newly introduced immune system)

Young Lion King star Shannon Tavarez loses leukemia battle

By DAILY MAIL REPORTER
Last updated at 7:28 PM on 2nd November 2010

Comments (0) | Add to My Stories

The little girl who starred in The Lion King on Broadway and won the hearts of many including celebrities like Alicia Keys and Rihanna has died.

Shannon Tavarez lost her battle with leukemia yesterday afternoon at Cohen Children's Medical Center on Long Island, New York.

Katharina Harf, co-founder of the bone marrow donor center DKMS confirmed the news that the 11-year-old succumbed to her illness despite receiving groundbreaking treatment.

Shannon had beat out hundreds of other hopefuls in her very first open audition to play the part of young lion Nala in the musical.

She split the role with another girl but performed four shows a week for six months.

Shannon received an umbilical-cord blood transplant in August that doctors hoped would save her life. This procedure was performed an alternative to the more customary bone marrow transplant.

Dr Larry Wolfe who cared for Shannon said that they failed to find a perfect bone marrow match for the young performer.

Searching for a match for Shannon was particularly overwhelming as her mother is African-American and her father is Hispanic from the Dominican Republic.

Brave: Shannon Tavarez, 11, has lost her battle with leukemia after a suitable bone marrow donor was not found

Patients from mixed ancestry have a more challenging time finding good bone marrow matches because there aren't as many people from those groups signed up as potential donors.

Adult somatic cells can be made to differentiate into pluripotent Stem cells through the expression of four genes.

Induced pluripotent stem cells (iPS cells)

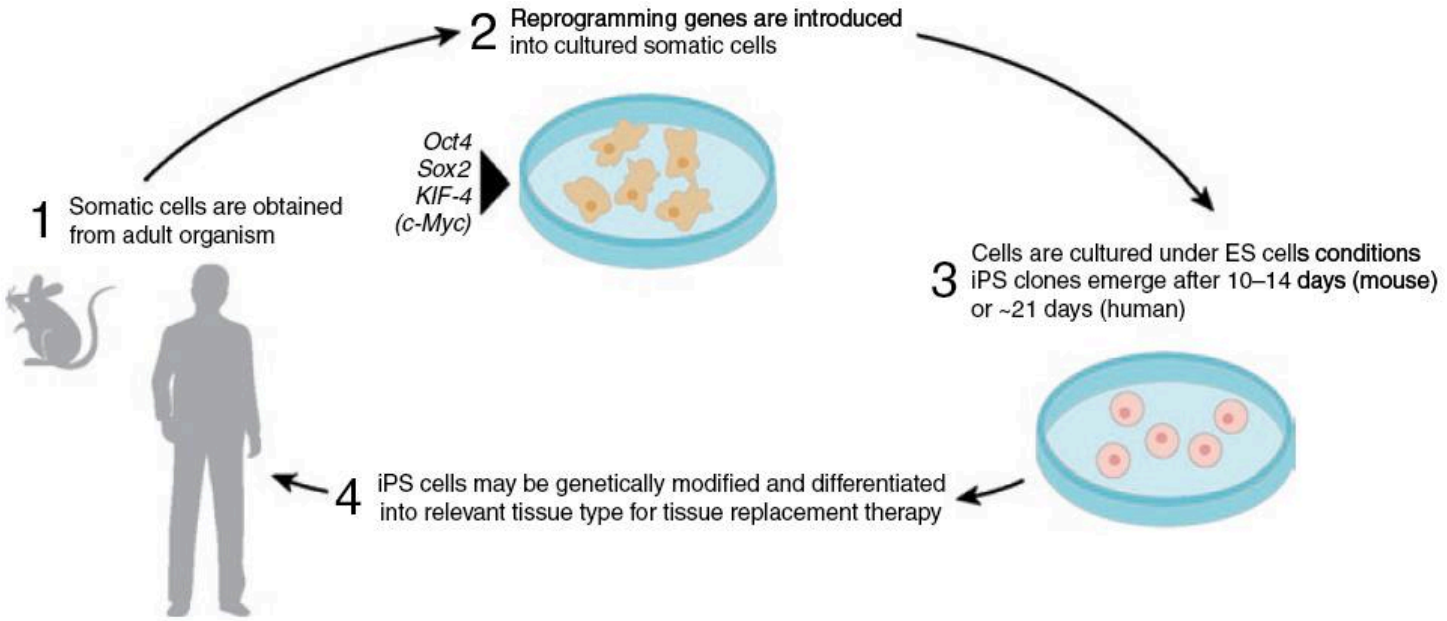
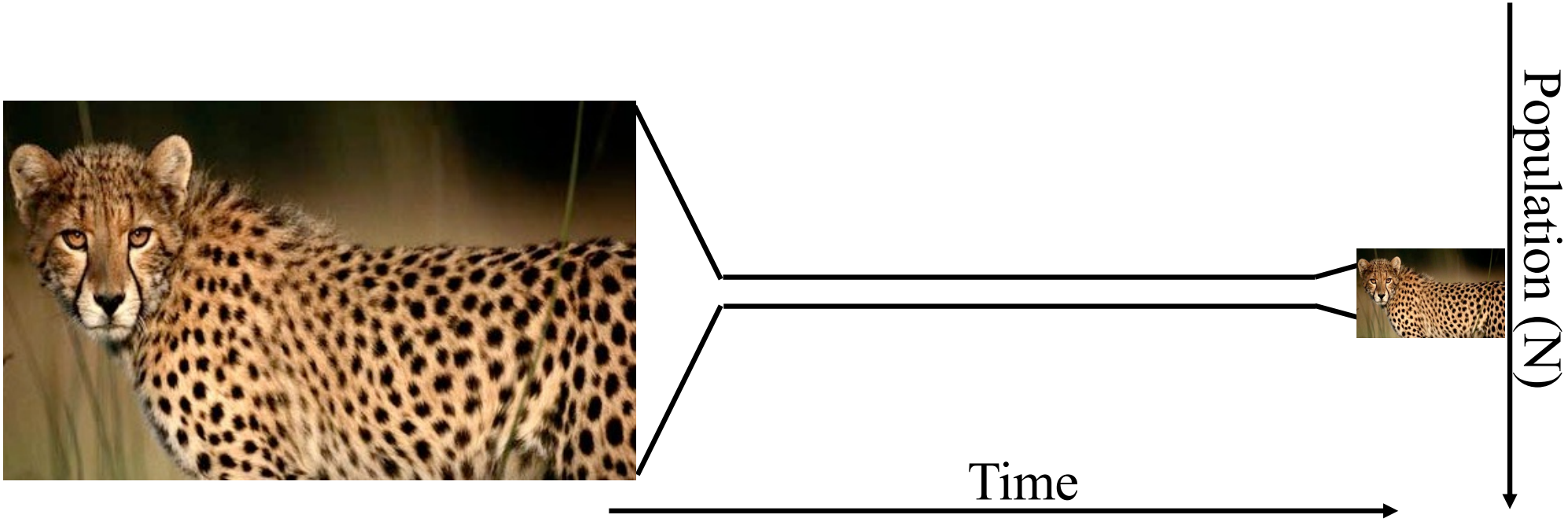


Figure 1 Schematic representation of direct nuclear reprogramming. Somatic cells are obtained from adult organisms. The reprogramming factors are introduced *in vitro*. Under ES cell culture conditions, iPS cells arise. This pluripotent cell population may be differentiated into various cell types and thus provides opportunity for disease specific investigations and enabling regenerative medicine strategies. ES, embryonic stem; iPS, induced pluripotent stem.

Endangered species, disease resistance and MHC diversity



Population bottleneck results in loss of MHC diversity. A novel infectious agent (or just a new variant of an old one) may lead to the death of all individuals in the population.

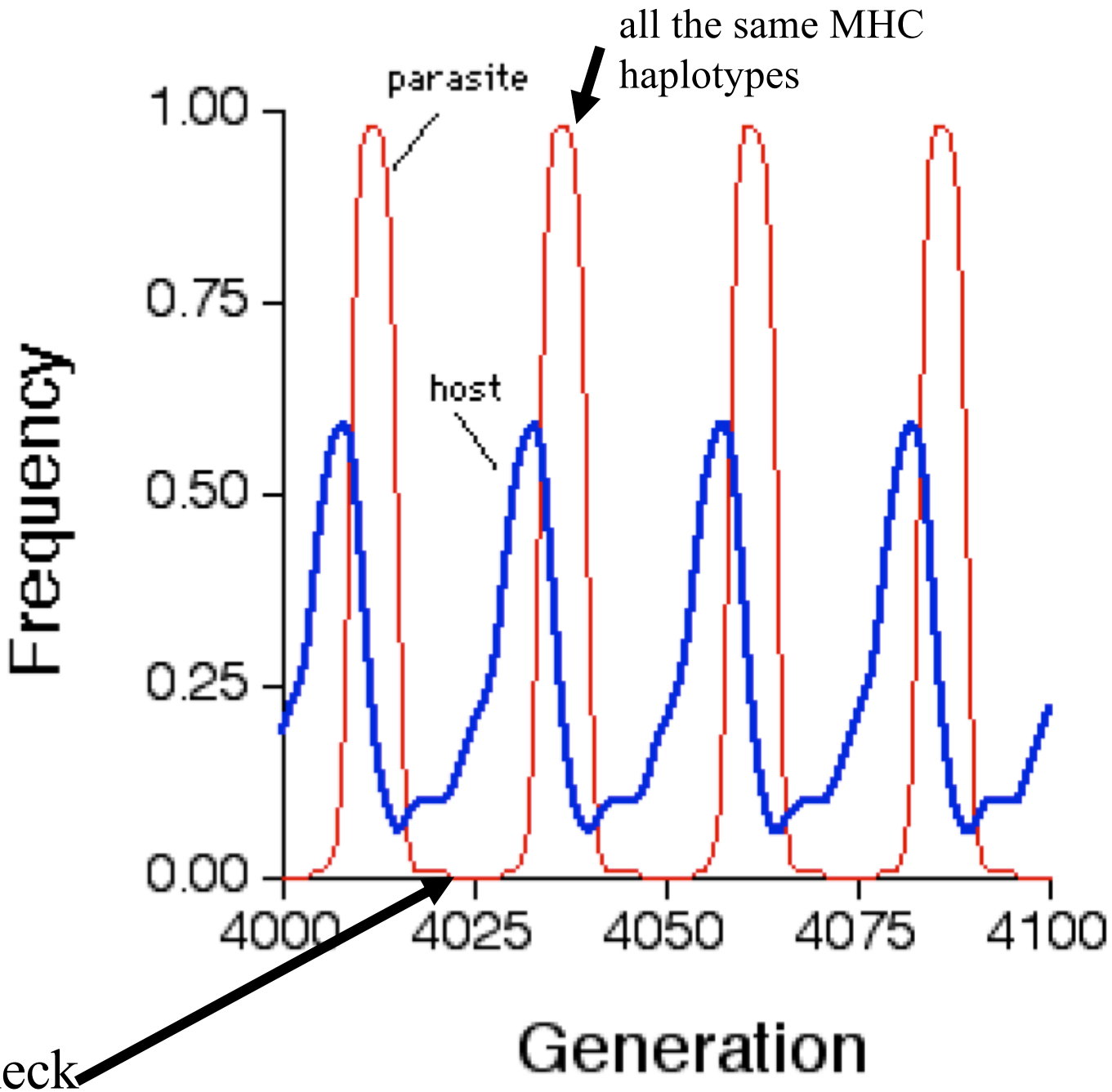
Host-parasite evolution: an evolutionary arms race

Host:

Evolve resistant genotypes

Parasite:

Evolve to overcome resistant genotypes



Cheetahs are so inbred that unrelated individuals accept skin grafts from each other

SANTA CRUZ, CA -- The cheetah, spotted embodiment of feline grace and power, races across African plains. The pocket gopher, small and squat, scurries among tunnels in the American West. Unbeknownst to either cheetah or gopher, research at the University of California, Santa Cruz, has unveiled a common bond between them -- and has bolstered one side of a hot cheetah debate.

That bond is remarkable genetic similarity between individuals within each species. Many scientists suspect that one reason behind the decline of cheetahs is their lack of genetic variation--a degree of sameness close to that seen in inbred laboratory mice. Key evidence came from a surprising 1985 experiment in which unrelated captive cheetahs accepted skin grafts from each other, a sign that their immune systems were genetically almost identical. However, in recent years some ecologists have questioned the validity of that work.

The UCSC study provides an independent check. Researchers M. A. Sanjayan and Kevin Crooks used pocket gophers in the first attempt to repeat the 1985 experiment on wild animals. Just as with the cheetahs, gophers in populations with little genetic variation accepted skin grafts from one another. However, gophers from a population with higher genetic diversity rejected skin grafts from their neighbors--an experimental control that the 1985 study lacked.

These results led Sanjayan and Crooks, both Ph.D. candidates in biology, to conclude that the cheetah study was accurate. Consequently, cheetahs and other genetically impoverished species, such as California sea otters and elephant seals, may have little variability among their immune systems and thus may be more vulnerable to outbreaks of disease.

"Genetic variation is the stuff of evolution," says Sanjayan. "It allows a population to adapt effectively to changes in the environment. You exist at a price if you have low genetic variation. It's like having lots of clones of yourself."

If animals within a population are similar to each other immunologically, Sanjayan adds, "That doesn't bode well for disease resistance. If a new disease arises, either they'll all be resistant or they'll all be wiped out. You may not get a middle response, where at least some individuals make it. That's something you'd expect with other spec

SAVE THE TASMANIAN DEVIL

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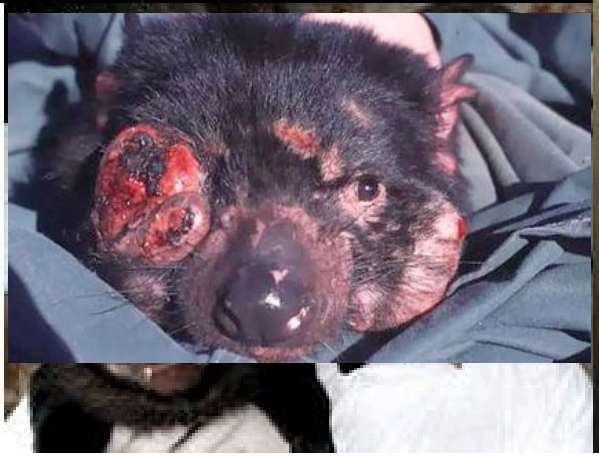
ABOUT THE TASMANIAN DEVIL

The Tasmanian devil, the largest living marsupial carnivore, earned its common name because of its blood-curdling nocturnal screams. But their true nature belies this reputation. Read more



Tasmanian devil facial tumour disease threatens the existence of the Tasmanian devil

A SPECIES AT RISK

The first signs of Tasmanian Devil Facial Tumour Disease (DFTD) were observed in 1996. It's a new and fatal condition in Tasmanian devils, characterised by cancers around the mouth and head. Tasmanian devils with the disease usually die within three to eight months of the lesions first appearing. Spotlight surveys indicate a decline in sightings of more than 50% statewide. Help now

HOW YOU CAN HELP

FIND OUT HOW >>

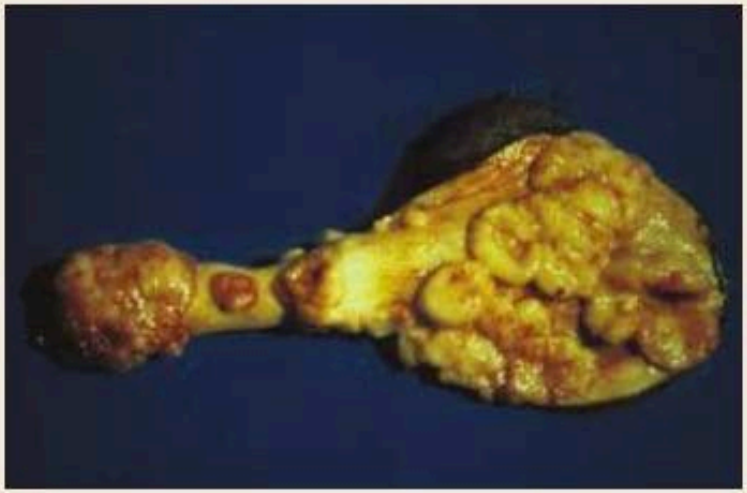
Transmission of a fatal clonal tumor by biting occurs due to depleted MHC diversity in a threatened carnivorous marsupial

Hannah V. Siddle[†], Alexandre Kreiss[‡], Mark D. B. Eldridge[§¶], Erin Noonan[‖], Candice J. Clarke[††], Stephen Pyecroft[‖], Gregory M. Woods[‡], and Katherine Belov[†‡‡]

A cancer as a new, parasitic, species of animal

Box 1 | **Dog venereal cancer: a highly degenerate mammal**

Mammals are continually sloughing off cells, but these cells usually die once they are separated from the organism. Even the most aggressive and invasive cancer cells usually die when their host dies. However, there is a fascinating exception: canine transmissible venereal sarcoma (CTVS) is an infectious disease of dogs that is caused by a pathogenic lineage of cancerous cells[70]. This cell lineage is transmitted from one dog to another, usually during coitus. Once on a new host, the cells reproduce to form a tumour-like growth, usually around the genitals, but occasionally on the skin and in and around the mouth. The figure shows a primary venereal cancer in the dog, involving the penis and surrounding tissue. The cell lineage can then be transmitted to another host. There are no obvious differences in susceptibility between breeds of dogs, and the cells can even be transmitted to foxes. It is this continuity of the cell lineage (as opposed to merely continuity of an infectious virus) that distinguishes CTVS from other transmissible cancers (for example, human cervical cancer). Even without treatment, tumours usually regress after 1–3 months, and if regression is complete, then the host is immune to subsequent re-infection. CTVS can be found in many parts of the world, and in some regions is the most common dog tumour. It is thought to have originated only once and spread worldwide, and a LINE retrotransposable element insertion upstream of the c-*MYC* oncogene[71] was presumably important in its genesis. This 'naturally occurring allograft' has become a true pathogen, even a highly degenerate mammal. Image courtesy of Wilfried T. Weber, Pennsylvania, USA.



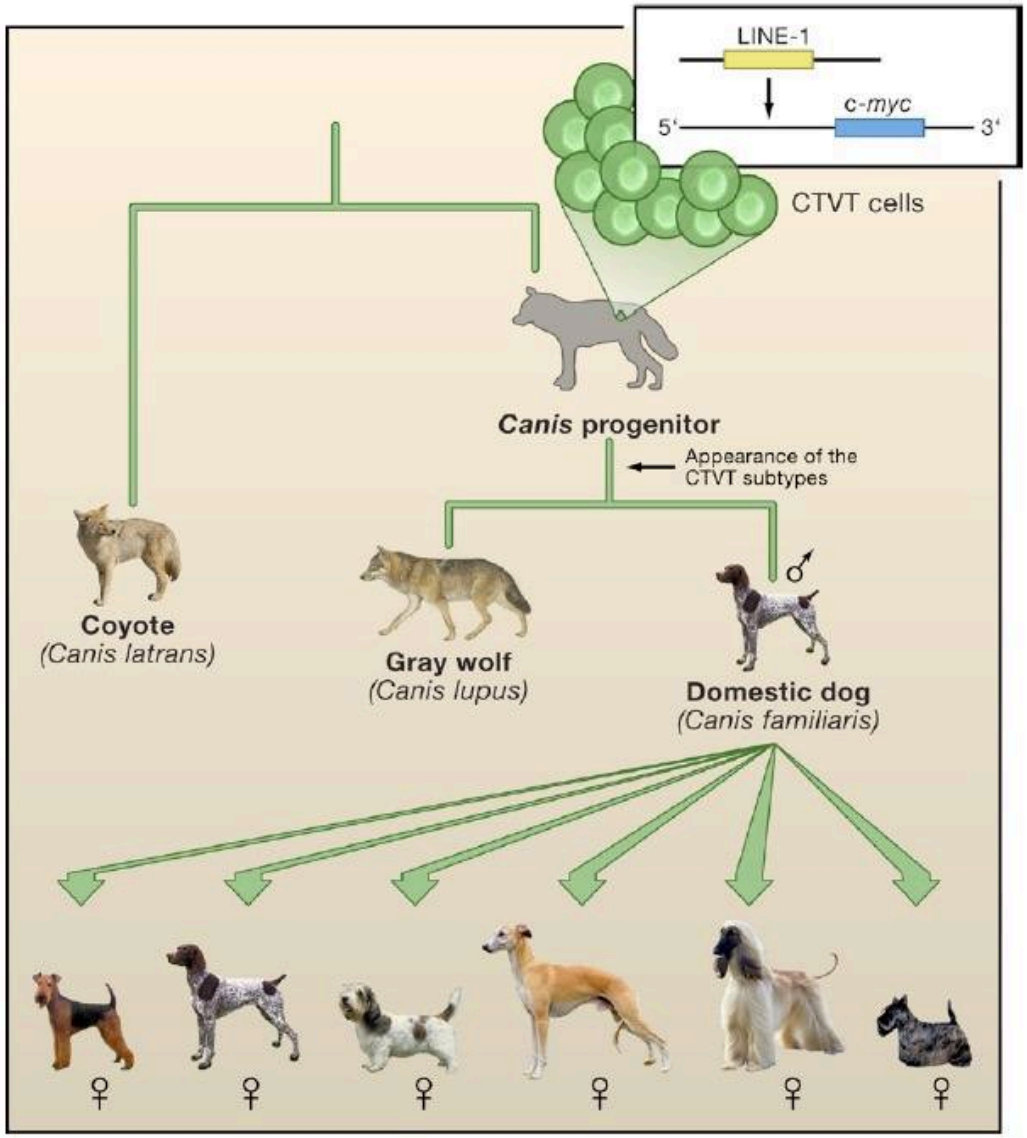
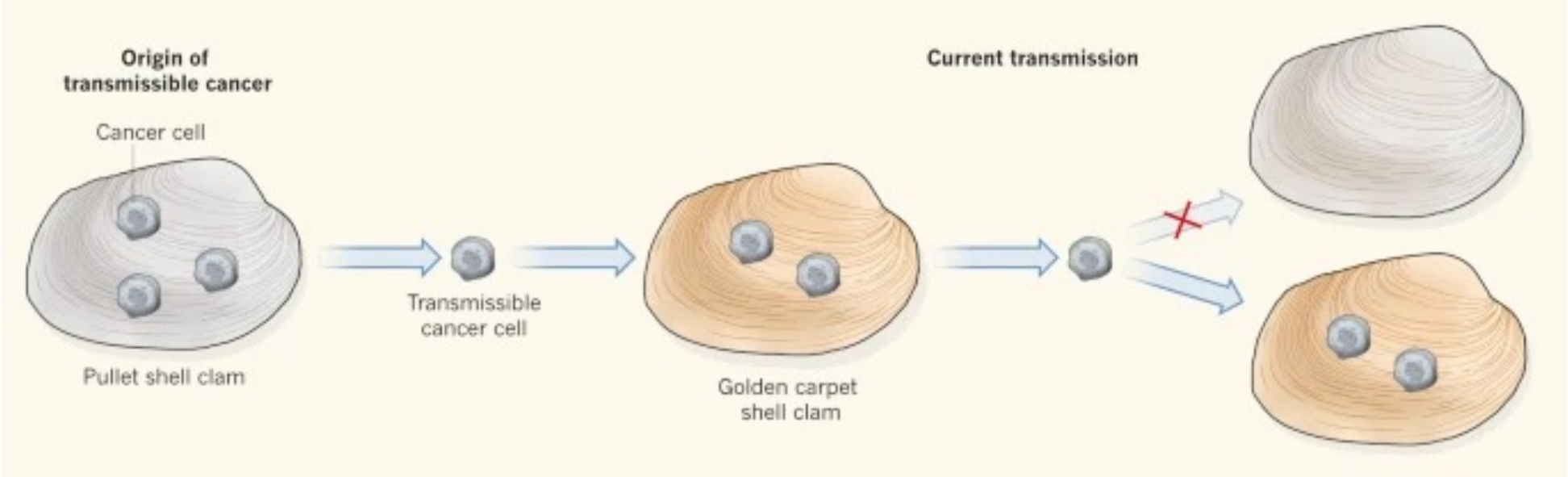


Figure 1. Ancestry of the Canine Transmissible Venereal Tumor Lineage

The canid phylogeny depicts the origins of canine transmissible venereal tumor (CTVT). The LINE-1 element was inserted upstream of c-*myc*, a proto-oncogene, in the cells of a wolf/dog progenitor. CTVT lineage divergence occurred soon after its emergence. The subtypes do not necessarily correspond to species. Once the CTVT tumor enters the dog lineage, an infected individual can rapidly transmit it (green arrows) by coitus. The tumor can potentially be transmitted among *Canis* species. Scottish terrier photo courtesy of the American Kennel Club (AKC); other dog photos courtesy of Mary Bloom, AKC. Wolf photo courtesy of Priscilla Barrett (D.W. MacDonald and P. Barrett, Mammals of Europe [Princeton University Press, Princeton, NJ, USA, 1993]). Coyote photo courtesy of Dr. Robert K. Wayne.

A transmissible cancer in clams!!

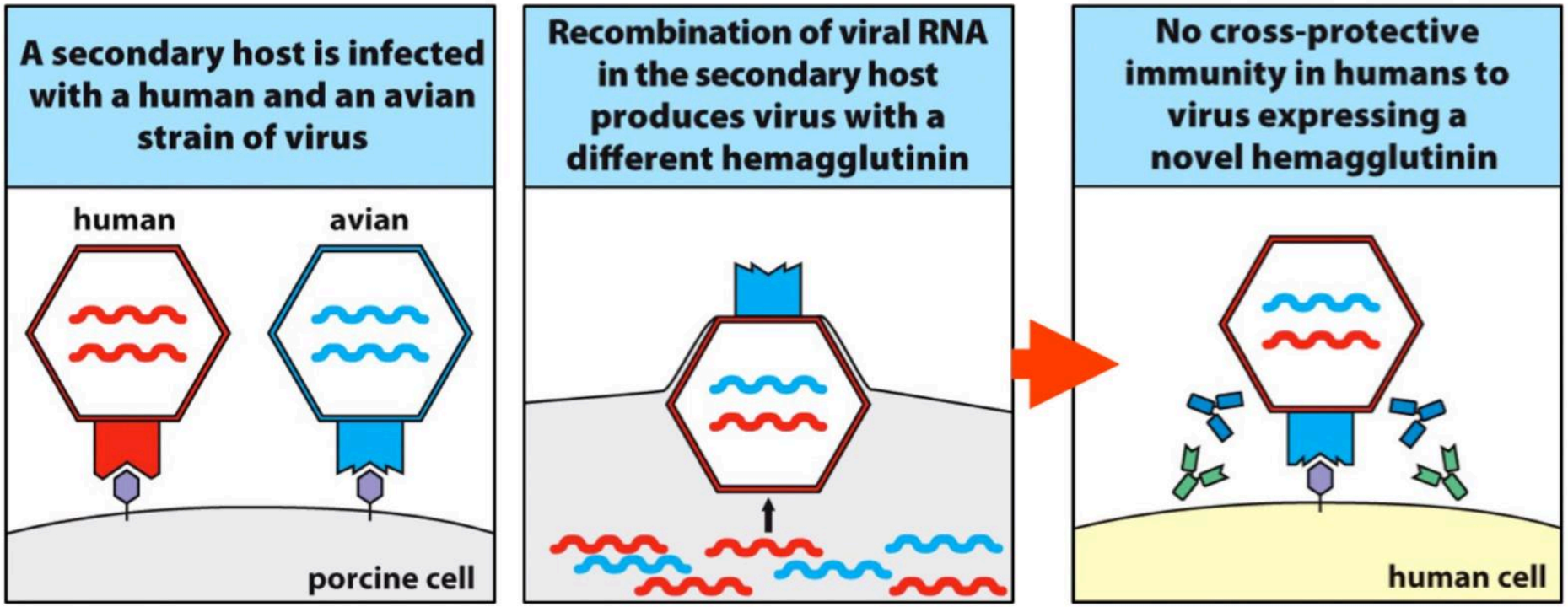


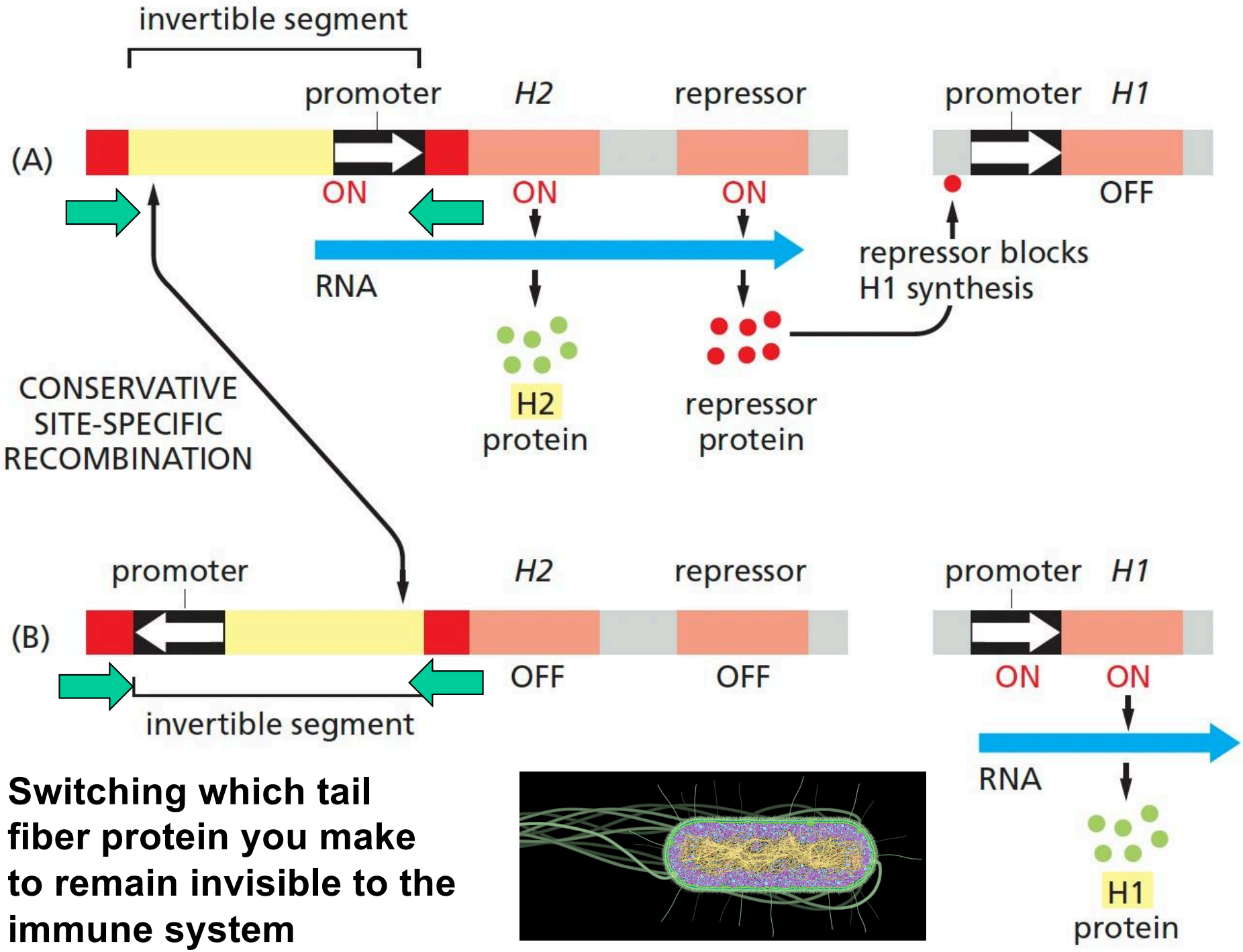
Pathogens fight back.

Prokaryote and eukaryote pathogens can actively modify their genomes to create evolutionary novelty at specific locations

Antigenic Variation:

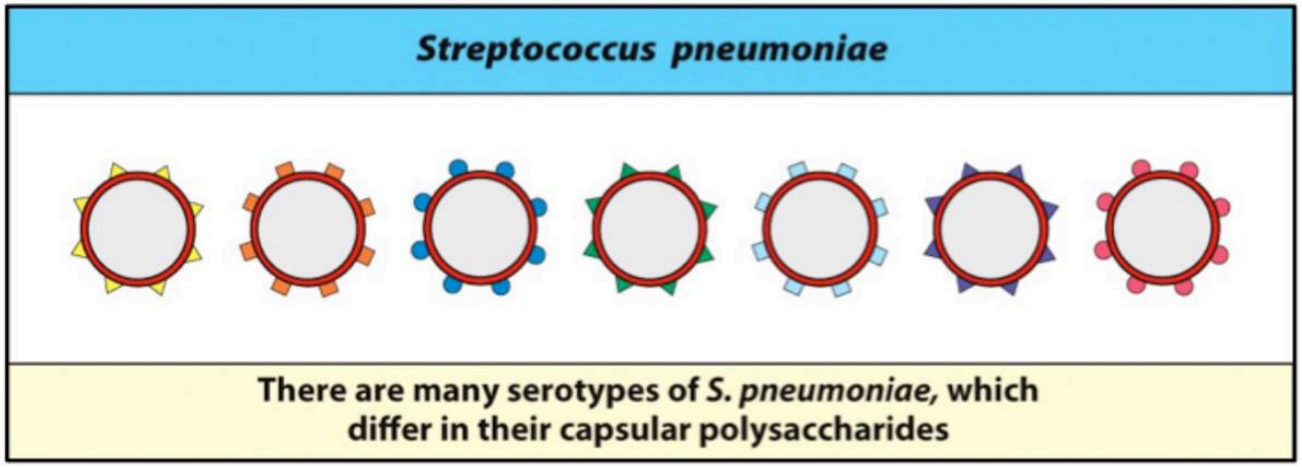
•• Antigenic Shift ••



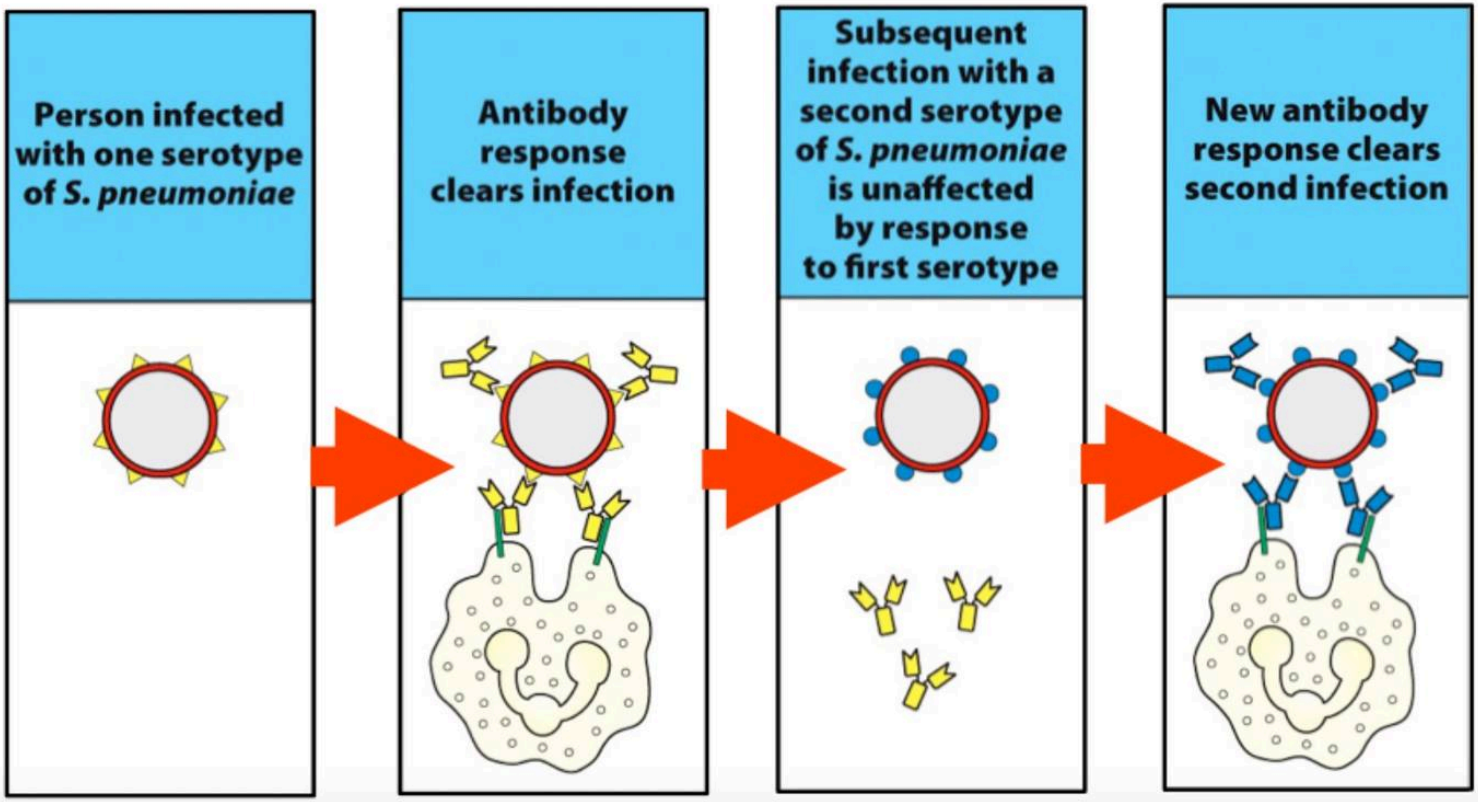


Switching which tail fiber protein you make to remain invisible to the immune system

Antigenic Variation

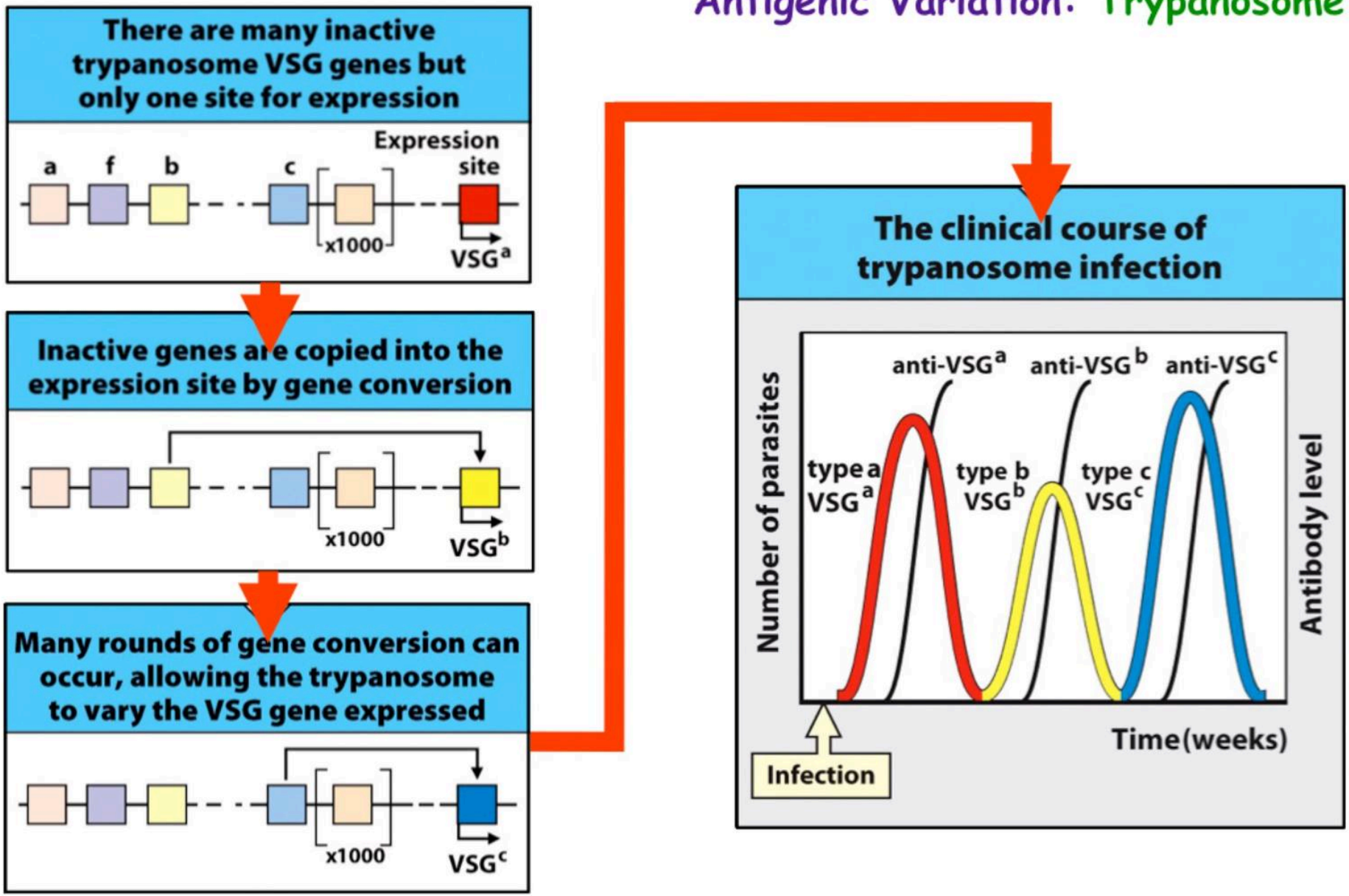


84 distinct serotypes



VSG= variant-specific gp

Antigenic Variation: Trypanosome

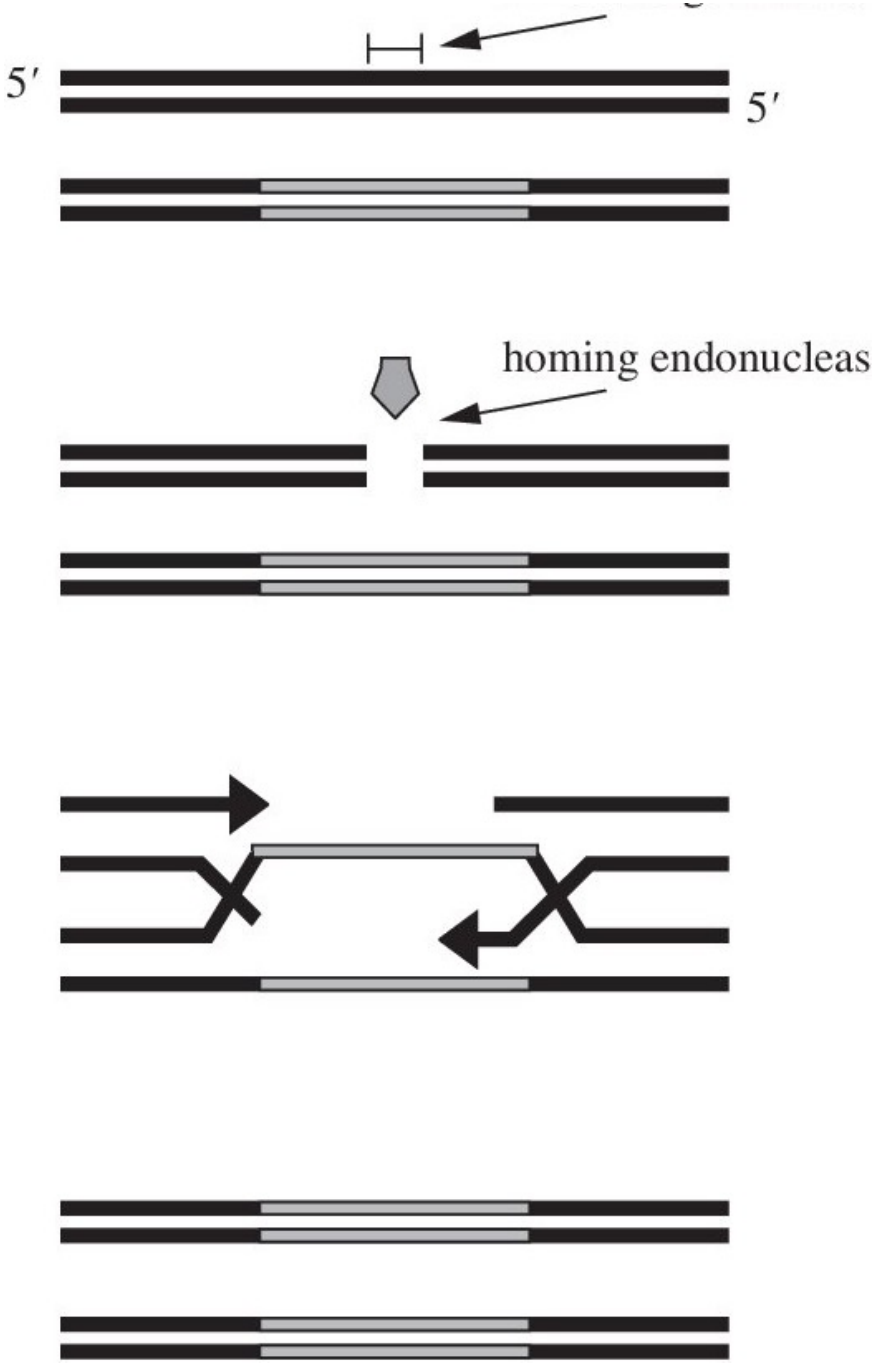


Programmed Rearrangement

Gene conversion: Following a DNA break a template DNA is used as a source for repair.

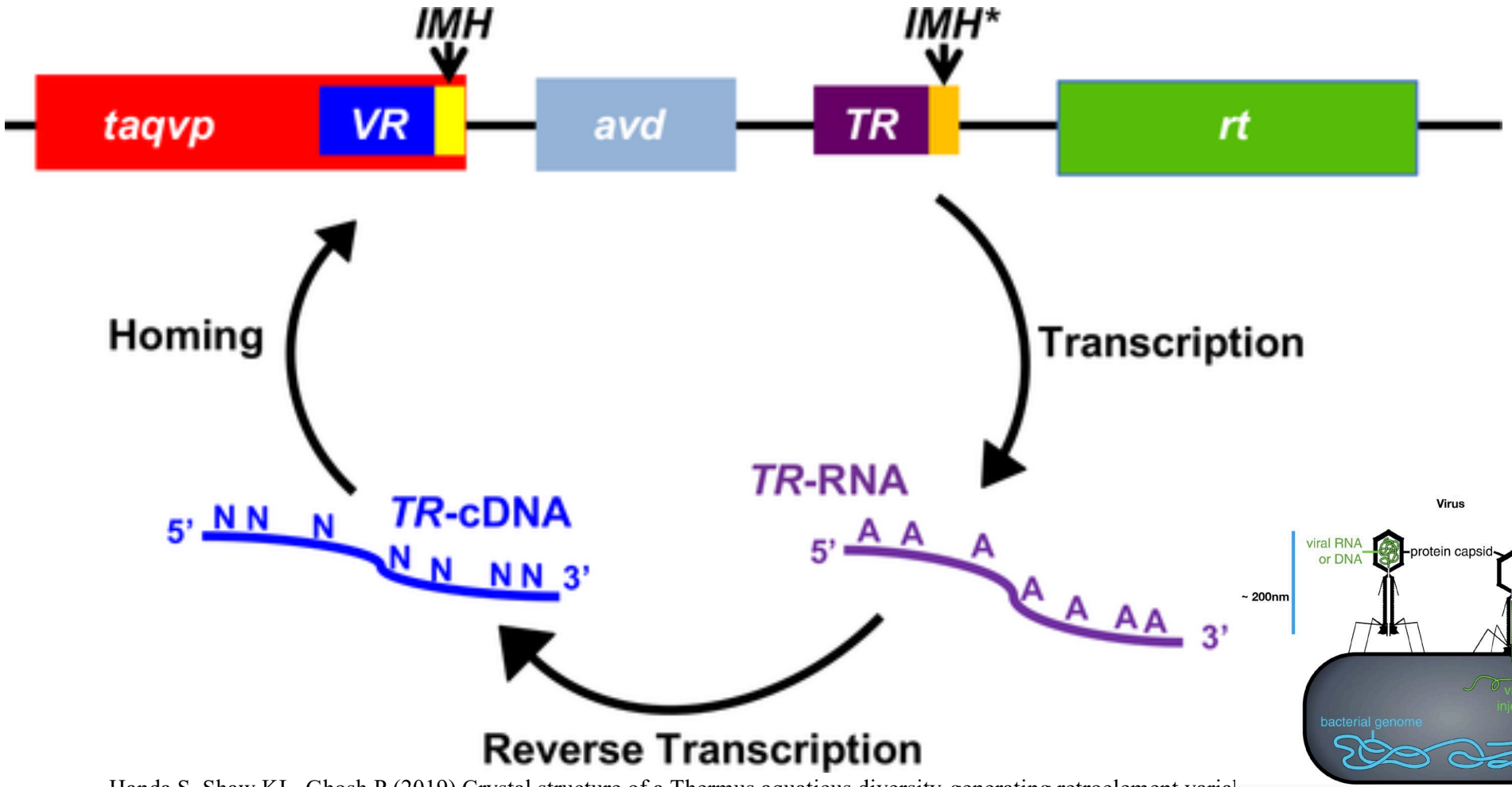
This results in "copying" of information from a donor molecule to a recipient one.

The one-way aspect of this is important because it leaves multiple silent templates available for sequential incorporation as time passes.



Diversity generating retroelement

Reverse transcriptase copies the TR-RNA into DNA. It takes every A residue and randomizes it. This generates the TR c (copy) DNA. The TR cDNA inserts itself in place of the VR region, creating a gene that carries a novel protein sequence that can bind to other receptors. 100 positions randomized over 20 amino acids. More diversity than stars in the universe. Infinite diversity (targeted mutagenesis) in a very small package



Handa S, Shaw KL, Ghosh P (2019) Crystal structure of a Thermus aquaticus diversity-generating retroelement variable protein. PLOS ONE 14(1): e0205618. https://doi.org/10.1371/journal.pone.0205618