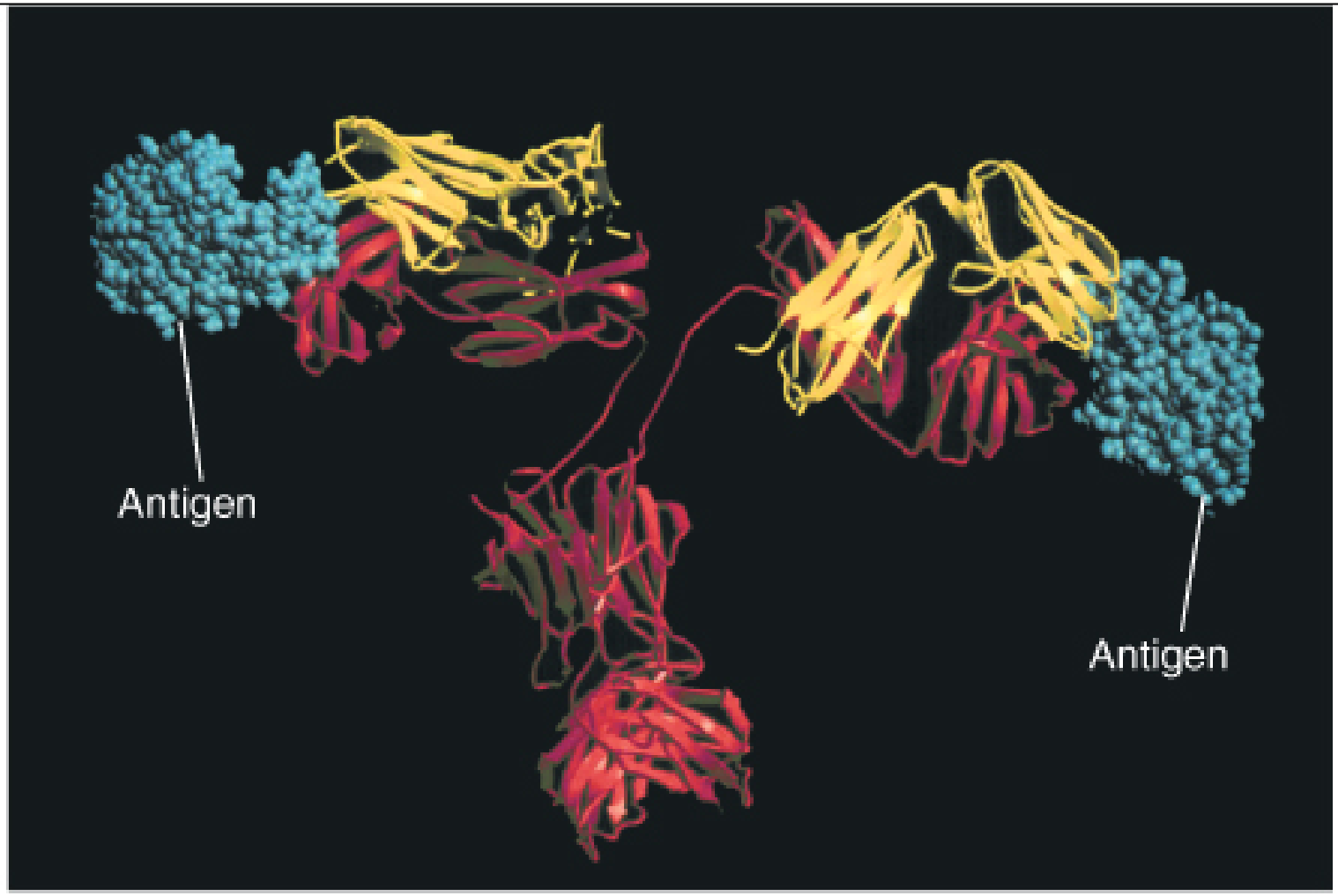
Alternative Mechanisms of Immune Diversity

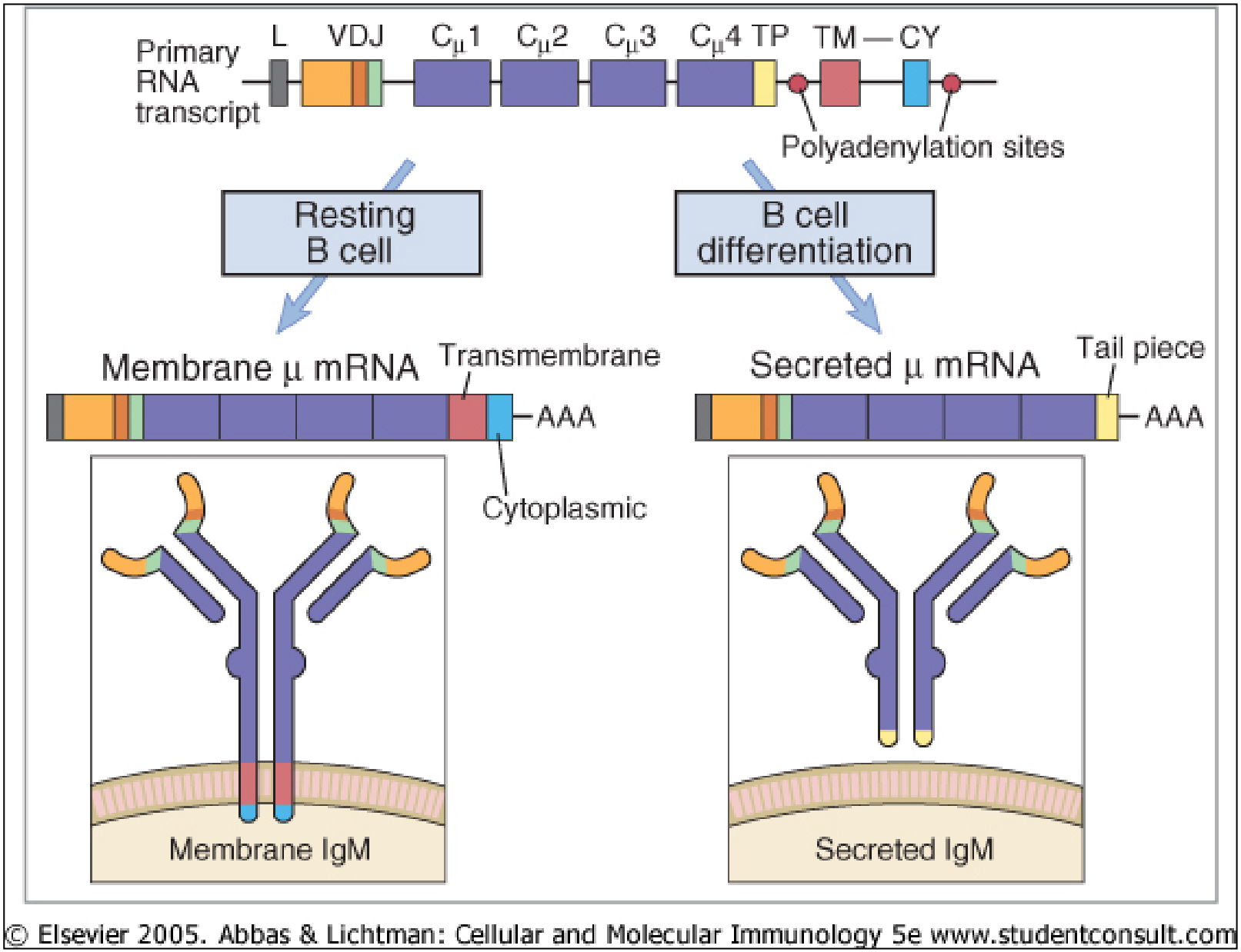
ADAPTIVE IMMUNITY

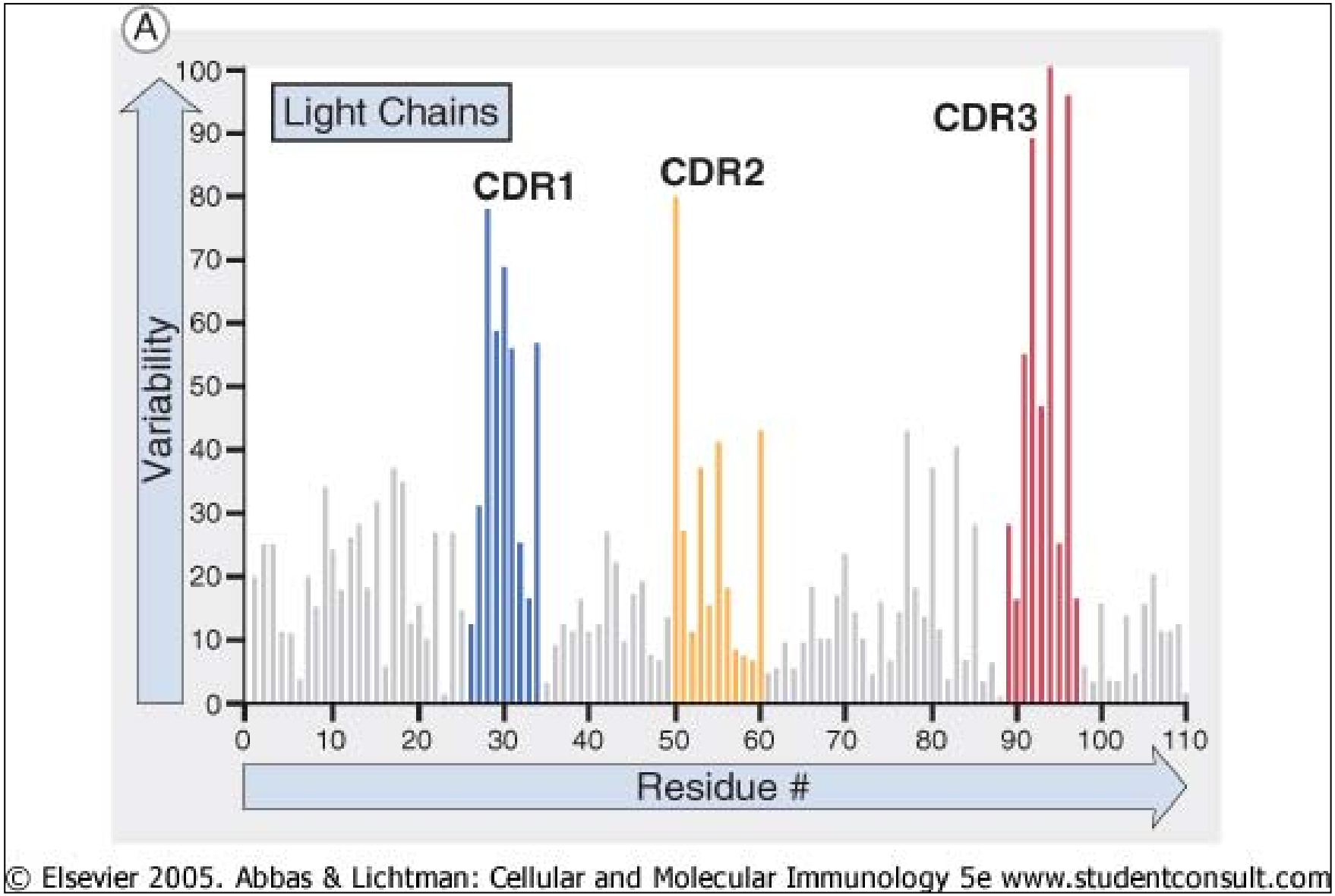
INVOLVES REARRANGEMENT OF GERMLINE GENETIC ELEMENTS AND SELECTION AT THE SOMATIC LEVEL

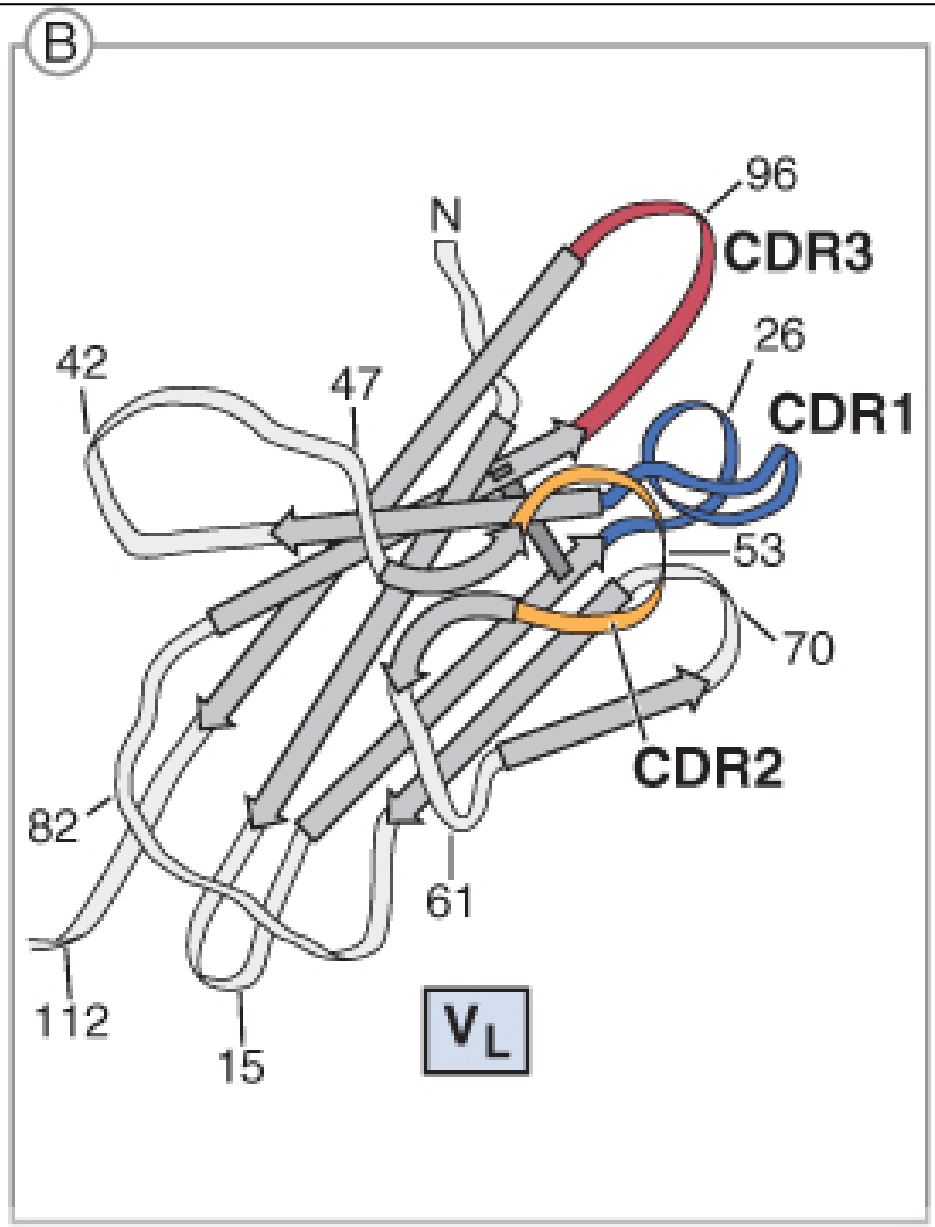
INNATE IMMUNITY

DOES NOT INVOLVE GENETIC REARRANGEMENT









Organization of Ig Gene Loci

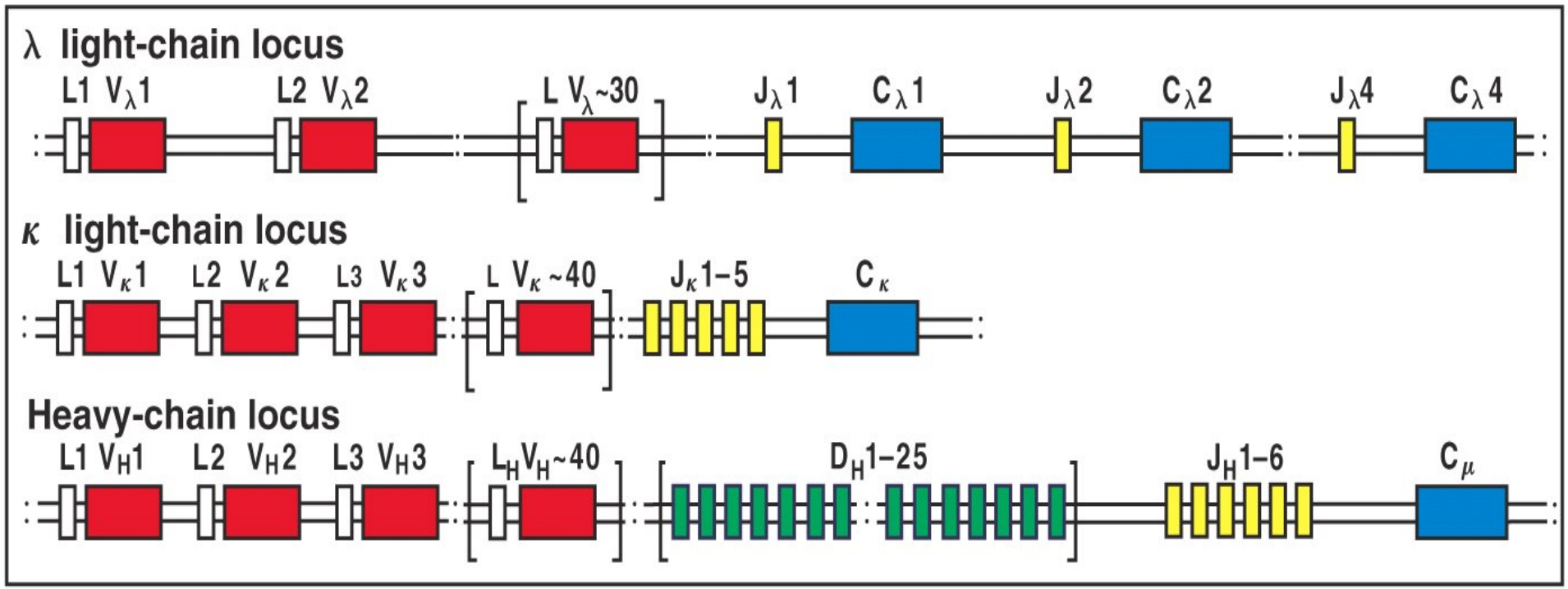
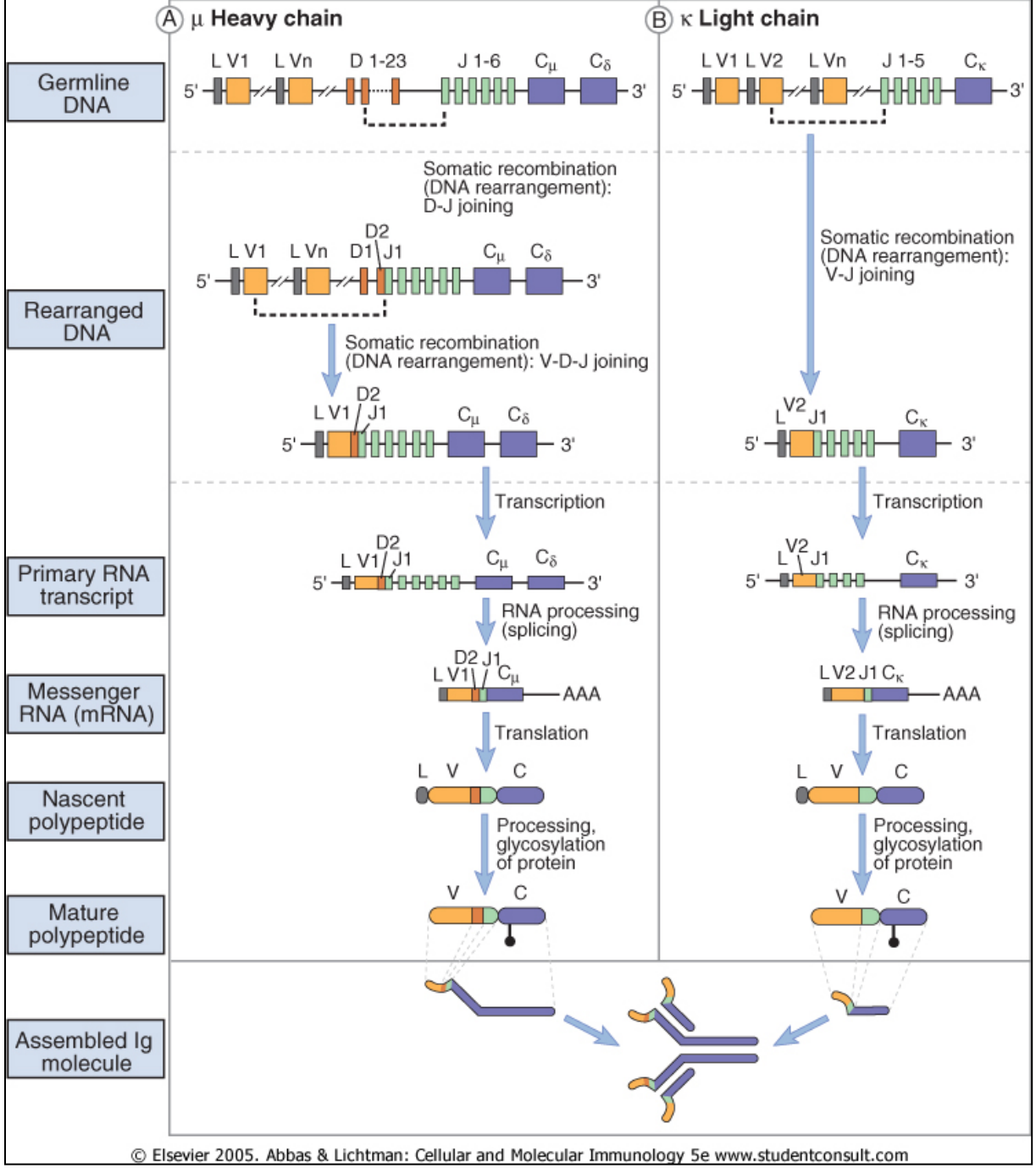
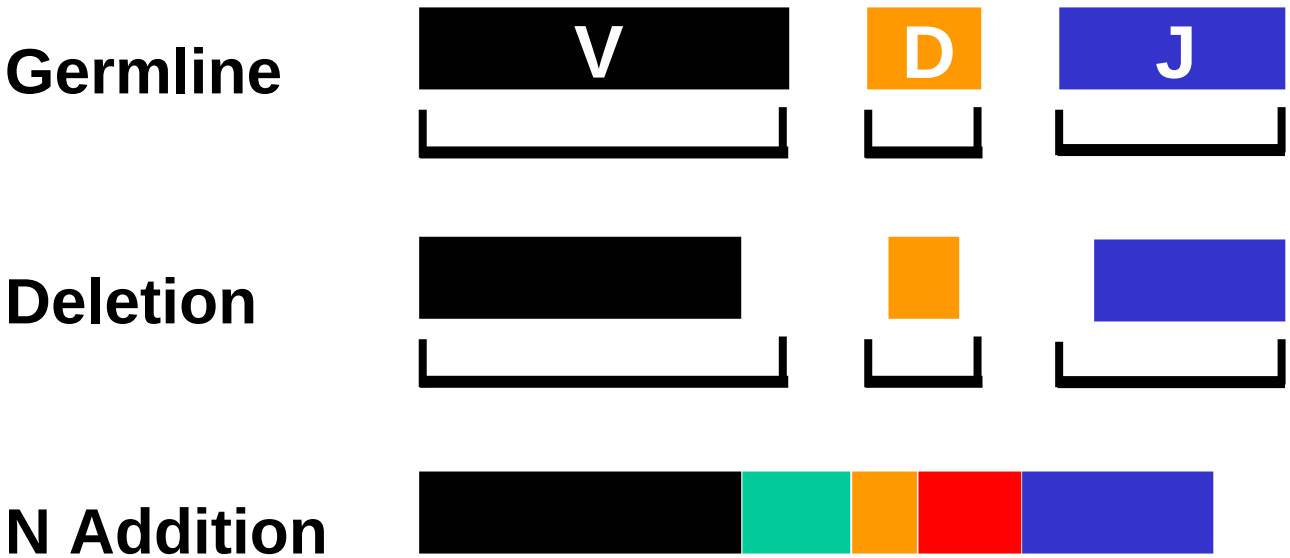


Figure 4-4 Immunobiology, 6/e. (© Garland Science 2005)



Junctional Diversity is Associated with Nucleotide Deletions and Additions

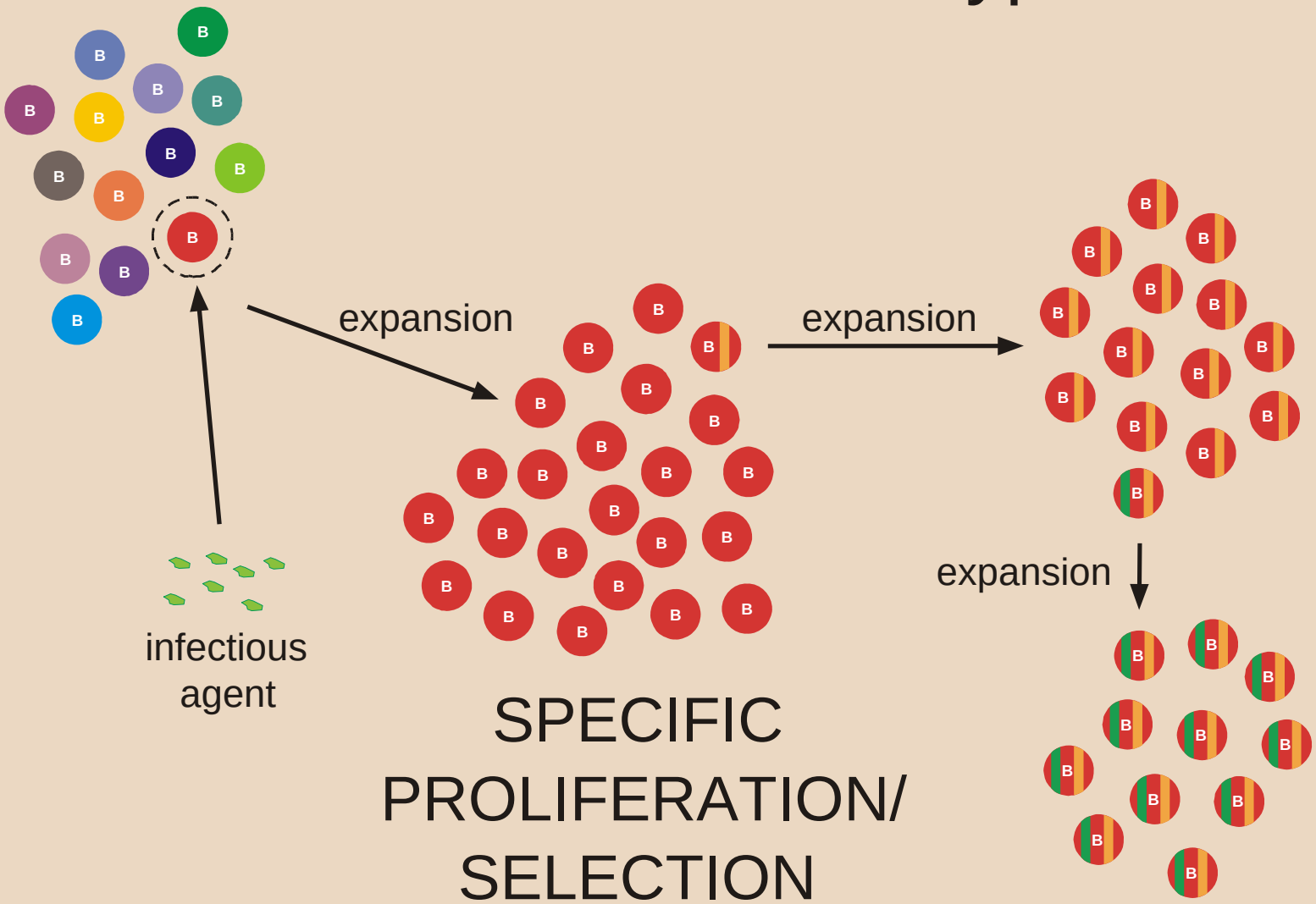


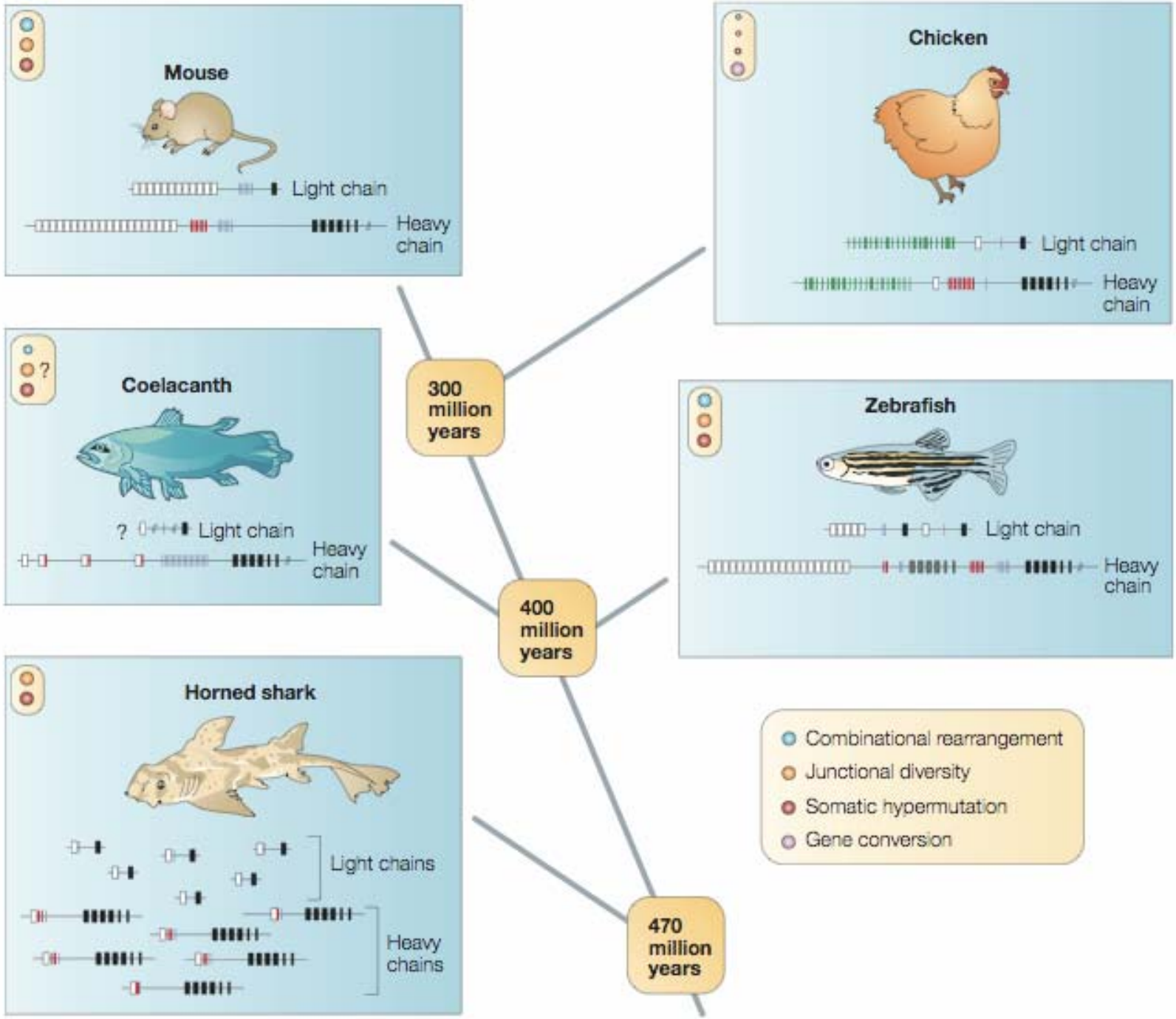
EXAMPLE

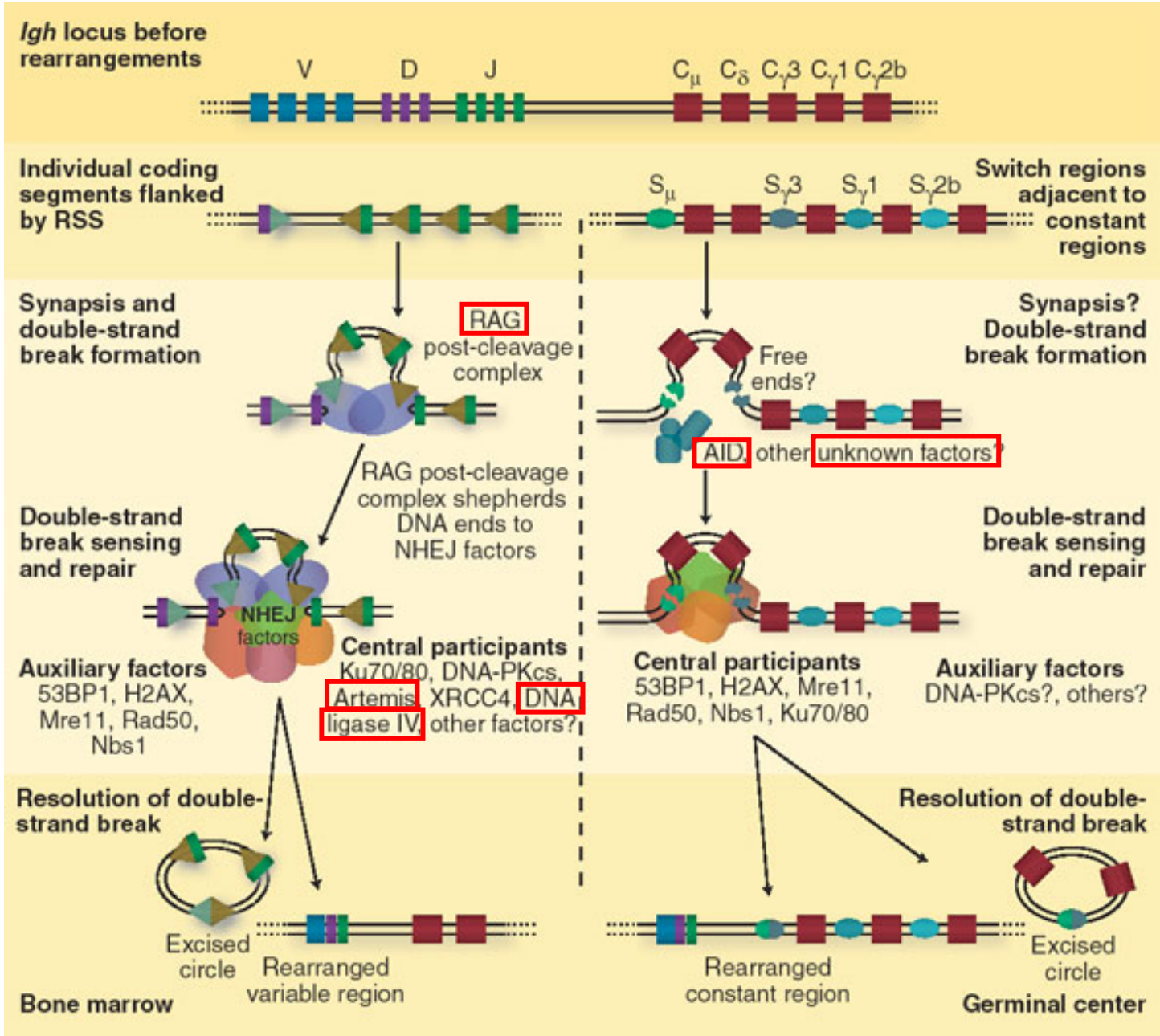
TYR-TYR-CYS-ALA-ARG GLU-ALA-PHE-SER ALA-GLY-LEU-TYR

TYR-TYR-CYS-GLY-ALA-SER-THR-PHE-ASP-MET-GLY-LEU-TYR

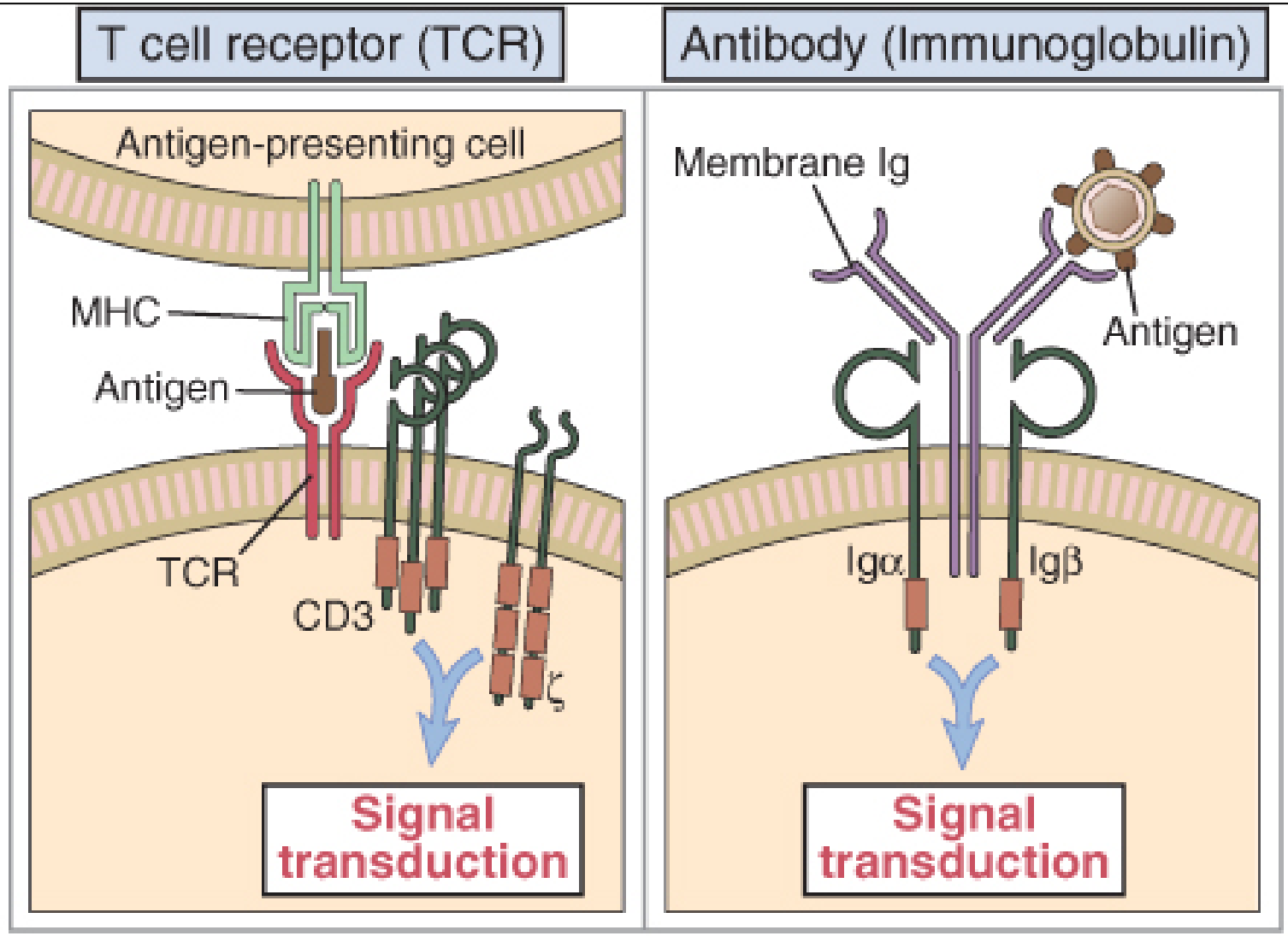
Clonal Selection and Somatic Hypermutation




Mouse
Light chain
Heavy chain
Chicken
Light chain
Heavy chain
Coelacanth
?
Light chain
Heavy chain
300 million years
Zebrafish
Light chain
Heavy chain
400 million years
Horned shark
Light chains
Heavy chains
470 million years
Combinational rearrangement
Junctional diversity
Somatic hypermutation
Gene conversion


Igh locus before rearrangements
V
D
J
C_μ C_δ $C_\gamma3$ $C_\gamma1$ $C_\gamma2b$
Individual coding segments flanked by RSS
S_μ $S_\gamma3$ $S_\gamma1$ $S_\gamma2b$
Switch regions adjacent to constant regions
Synapsis and double-strand break formation
RAG post-cleavage complex
Synapsis? Double-strand break formation
Free ends?
AID, other unknown factors?
RAG post-cleavage complex shepherds DNA ends to NHEJ factors
Double-strand break sensing and repair
NHEJ factors
Double-strand break sensing and repair
Auxiliary factors
53BP1, H2AX, Mre11, Rad50, Nbs1
Central participants
Ku70/80, DNA-PKcs, Artemis, XRCC4, DNA ligase IV, other factors?
Central participants
53BP1, H2AX, Mre11, Rad50, Nbs1, Ku70/80
Auxiliary factors
DNA-PKcs?, others?
Resolution of double-strand break
Excised circle
Rearranged variable region
Bone marrow
Resolution of double-strand break
Rearranged constant region
Excised circle
Germinal center

NATURE



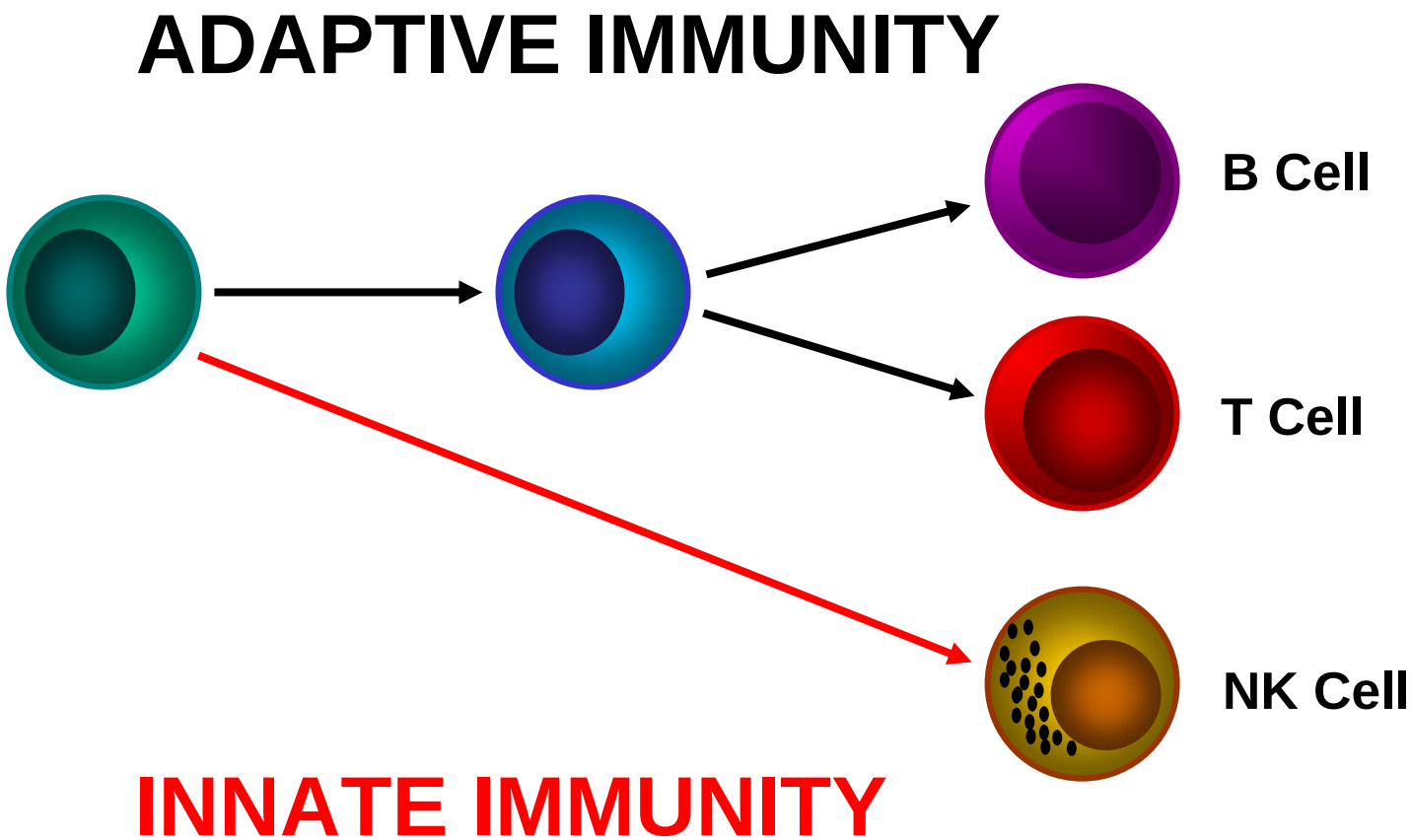
ADAPTIVE IMMUNITY

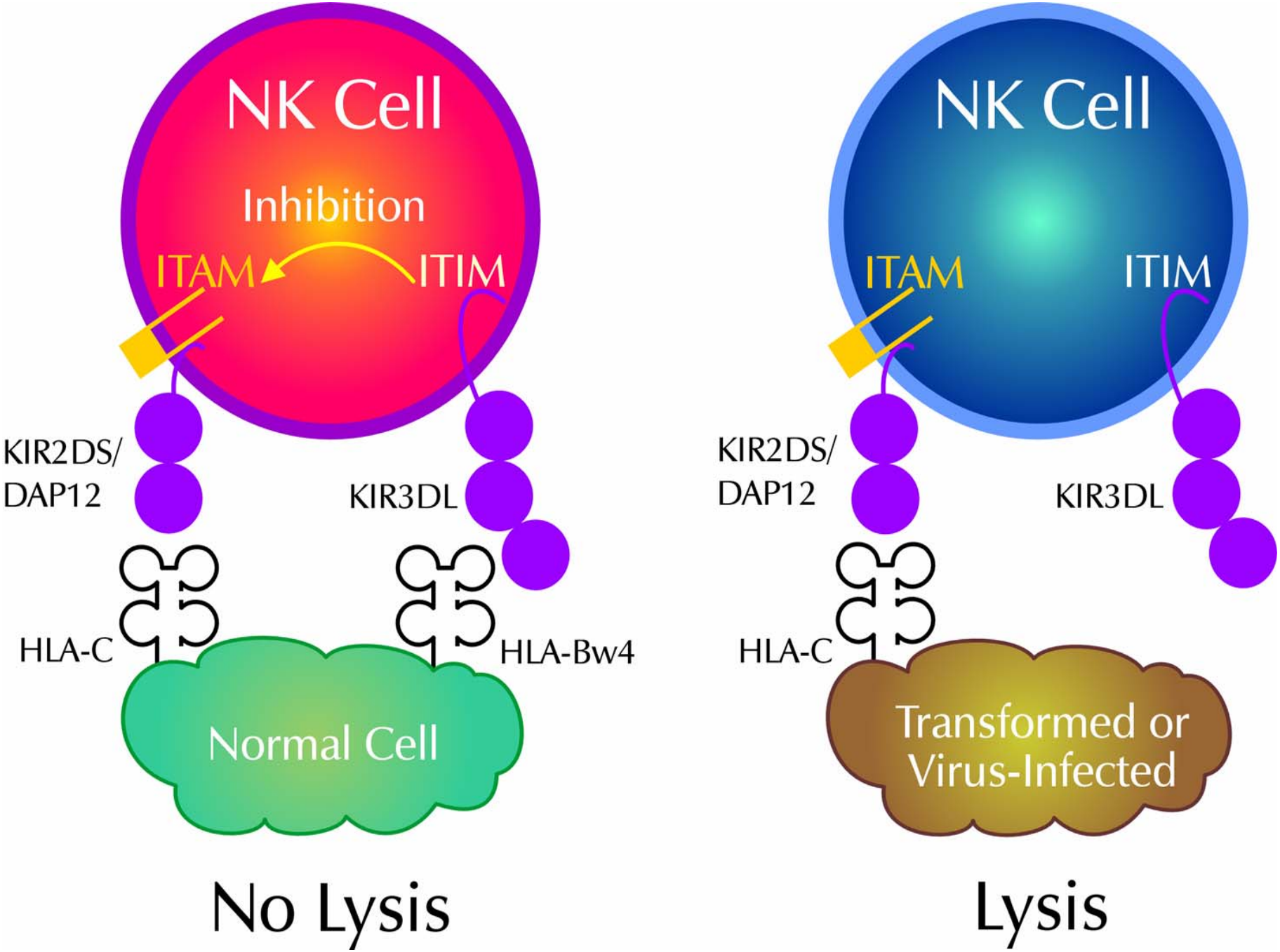
INVOLVES REARRANGEMENT OF GERMLINE GENETIC ELEMENTS AND SELECTION AT THE SOMATIC LEVEL

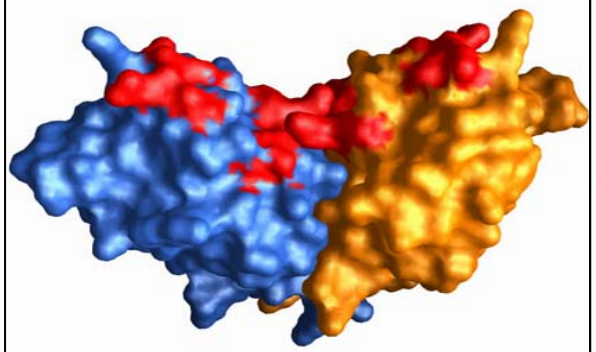
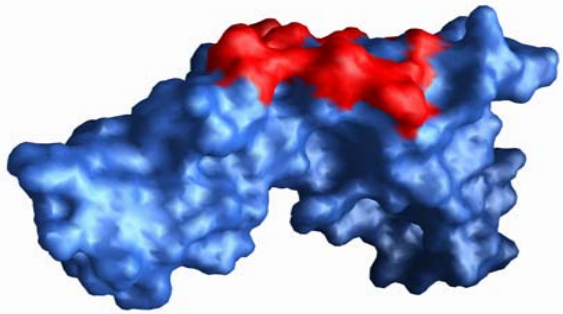
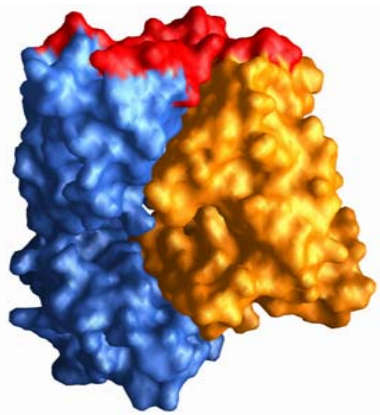
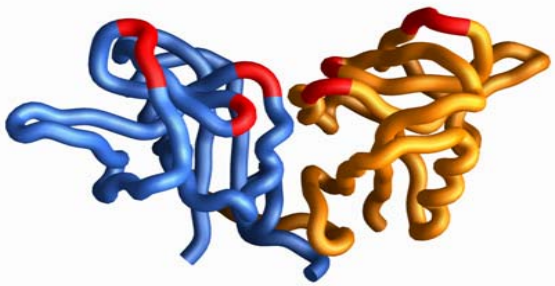
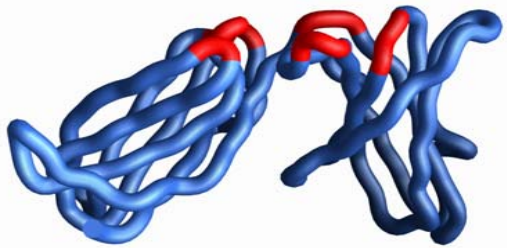
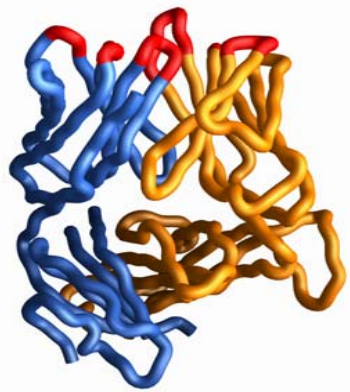
INNATE IMMUNITY

DOES NOT INVOLVE GENETIC REARRANGEMENT

NK, B AND T CELLS SHARE A COMMON LINEAGE



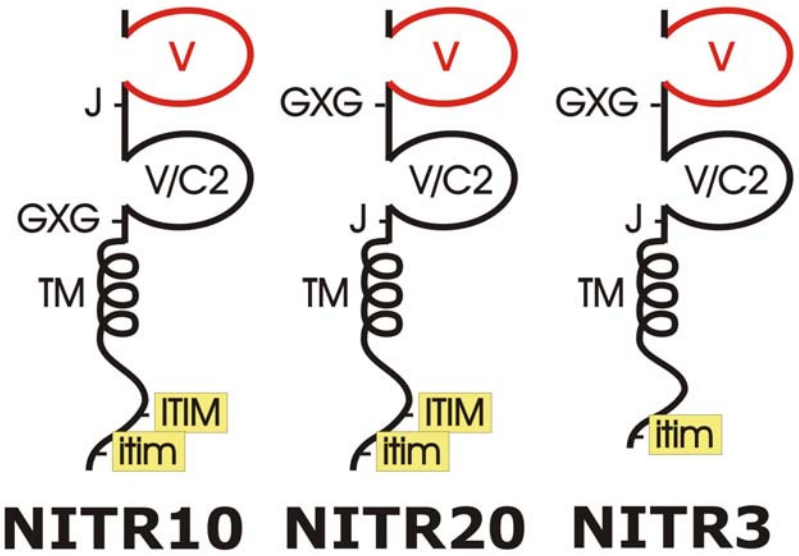

NK Cell
Inhibition
ITAM
ITIM
KIR2DS/
DAP12
KIR3DL
HLA-C
HLA-Bw4
Normal Cell
No Lysis
NK Cell
ITAM
ITIM
KIR2DS/
DAP12
KIR3DL
HLA-C
Transformed or
Virus-Infected
Lysis

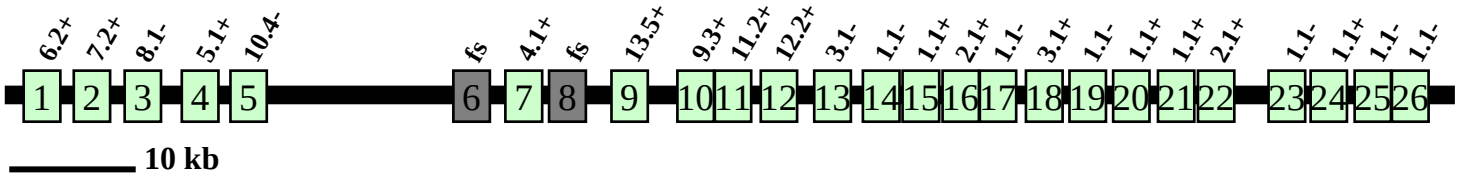
NKG2D ligand binding site	KIR ligand binding site	TCR ligand binding site
		
		

NITR Genes in Pufferfish

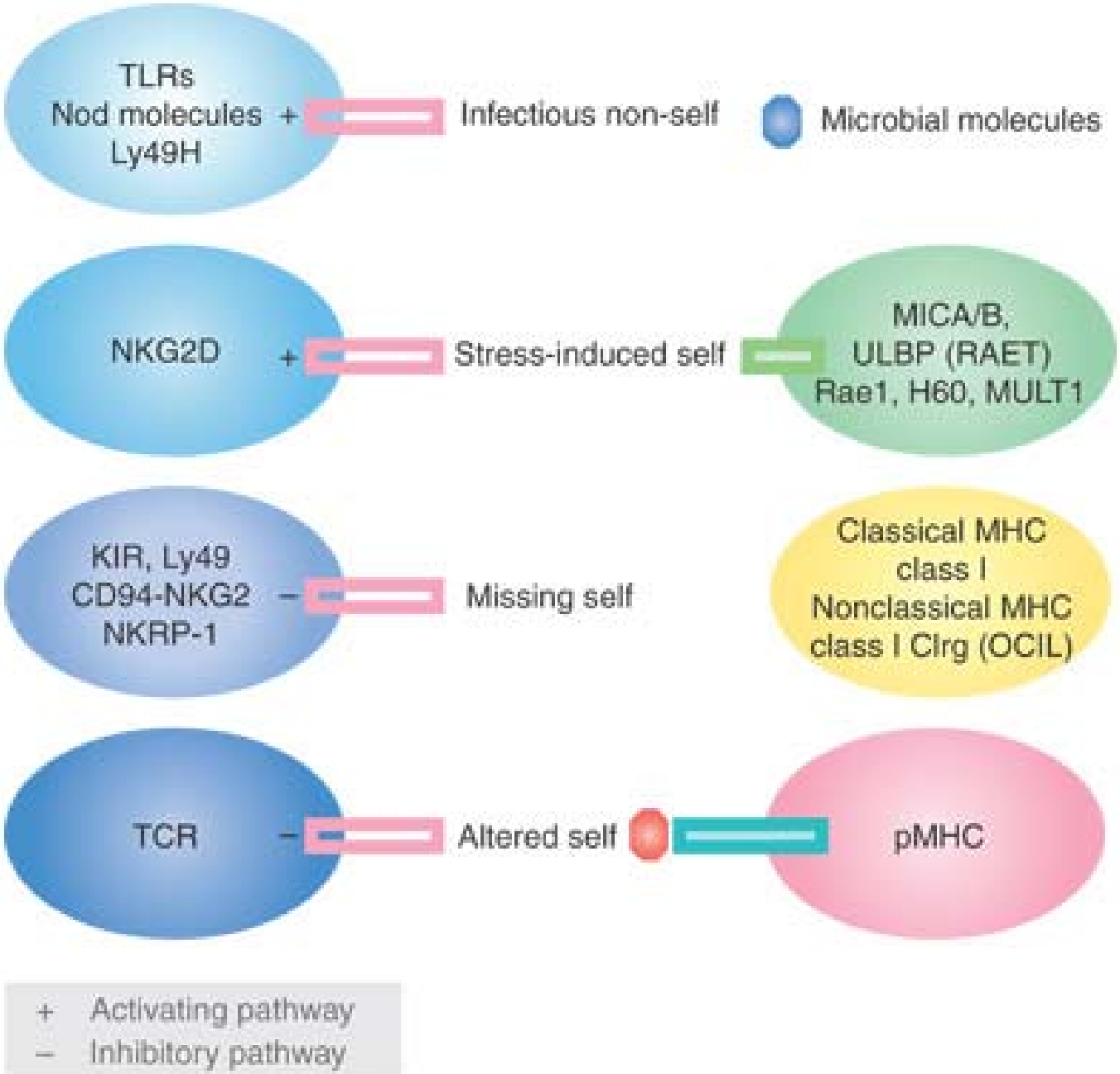
Rast et al., 1997, *Immunity* **6**:1
Strong et al., 1999, *PNAS* **96**:15080

- Unique innate/adaptive relationship
  (diversified V domains in innate receptors)

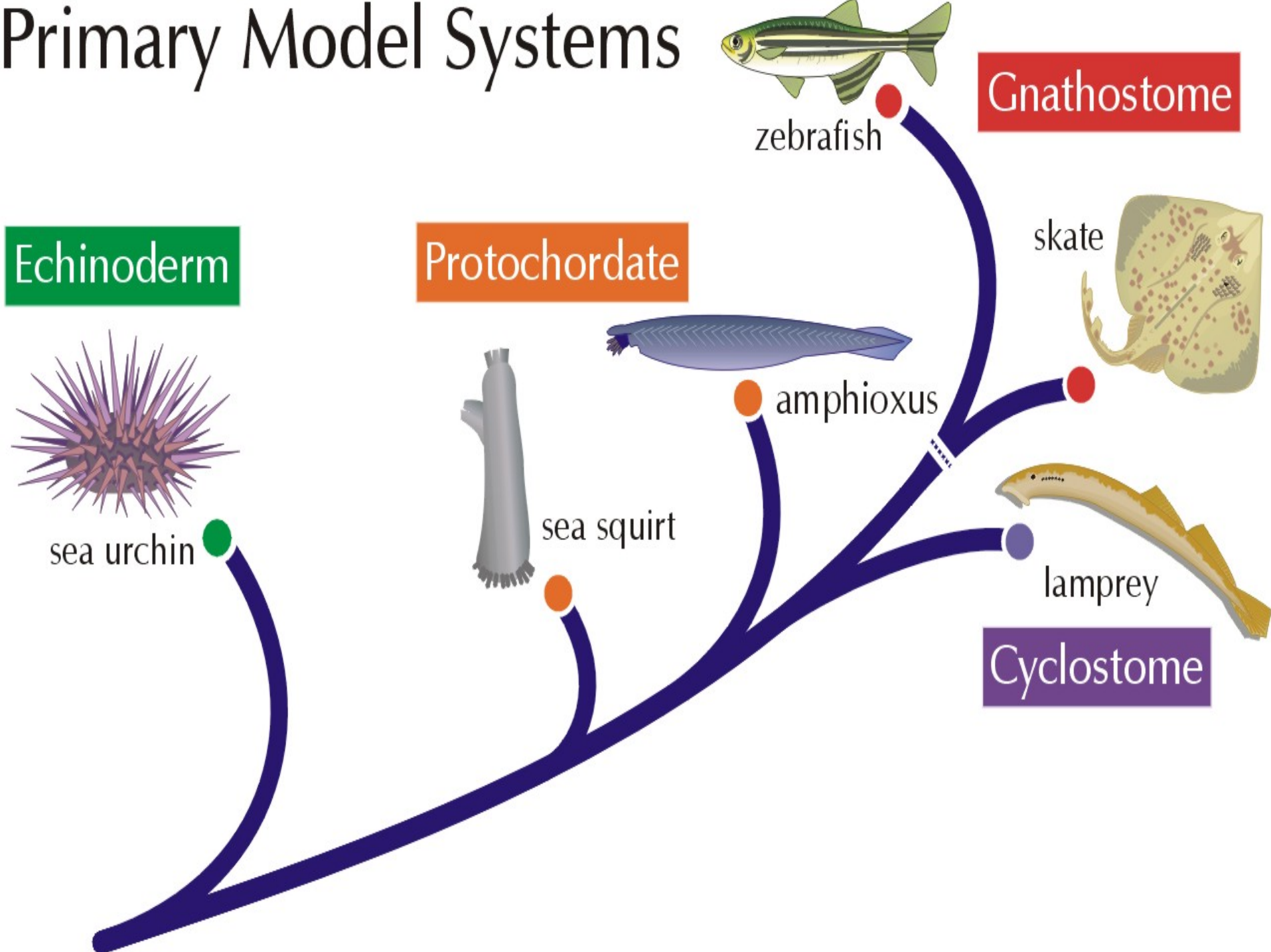




Structural Variation in Zebrafish NITRS

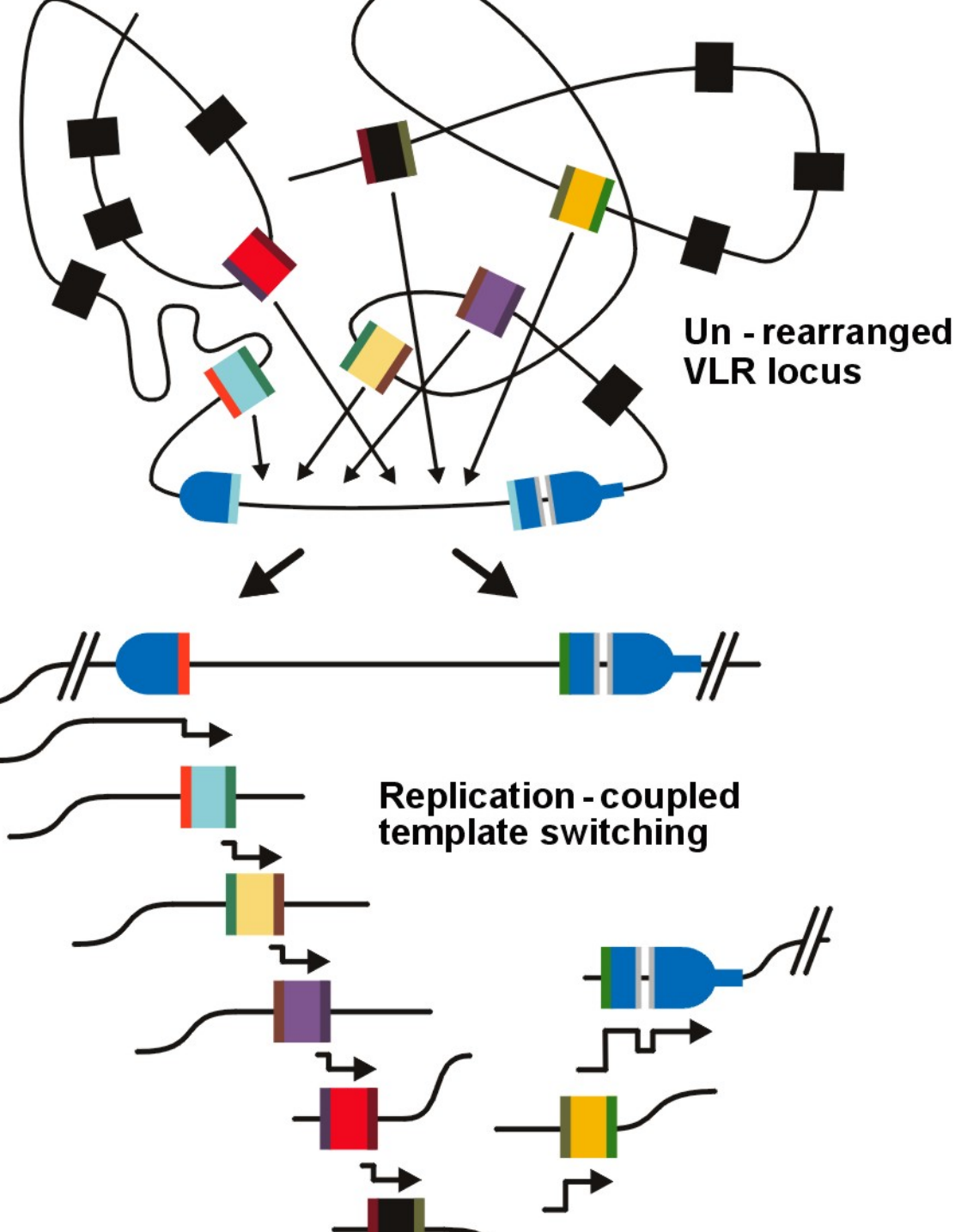

TLRs
Nod molecules
Ly49H
+
Infectious non-self
Microbial molecules
NKG2D
+
Stress-induced self
MICA/B,
ULBP (RAET)
Rae1, H60, MULT1
KIR, Ly49
CD94-NKG2
NKRP-1
–
Missing self
Classical MHC
class I
Nonclassical MHC
class I Clrg (OCIL)
TCR
–
Altered self
pMHC
+ Activating pathway
– Inhibitory pathway

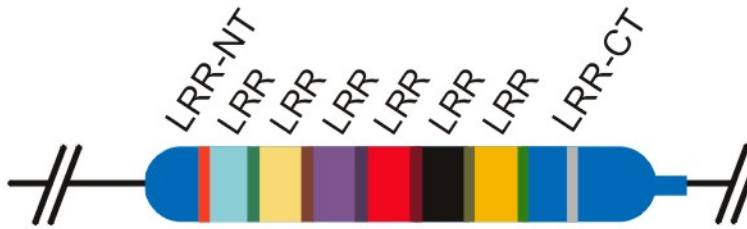
Primary Model Systems



Immunity In Jawless Vertebrates

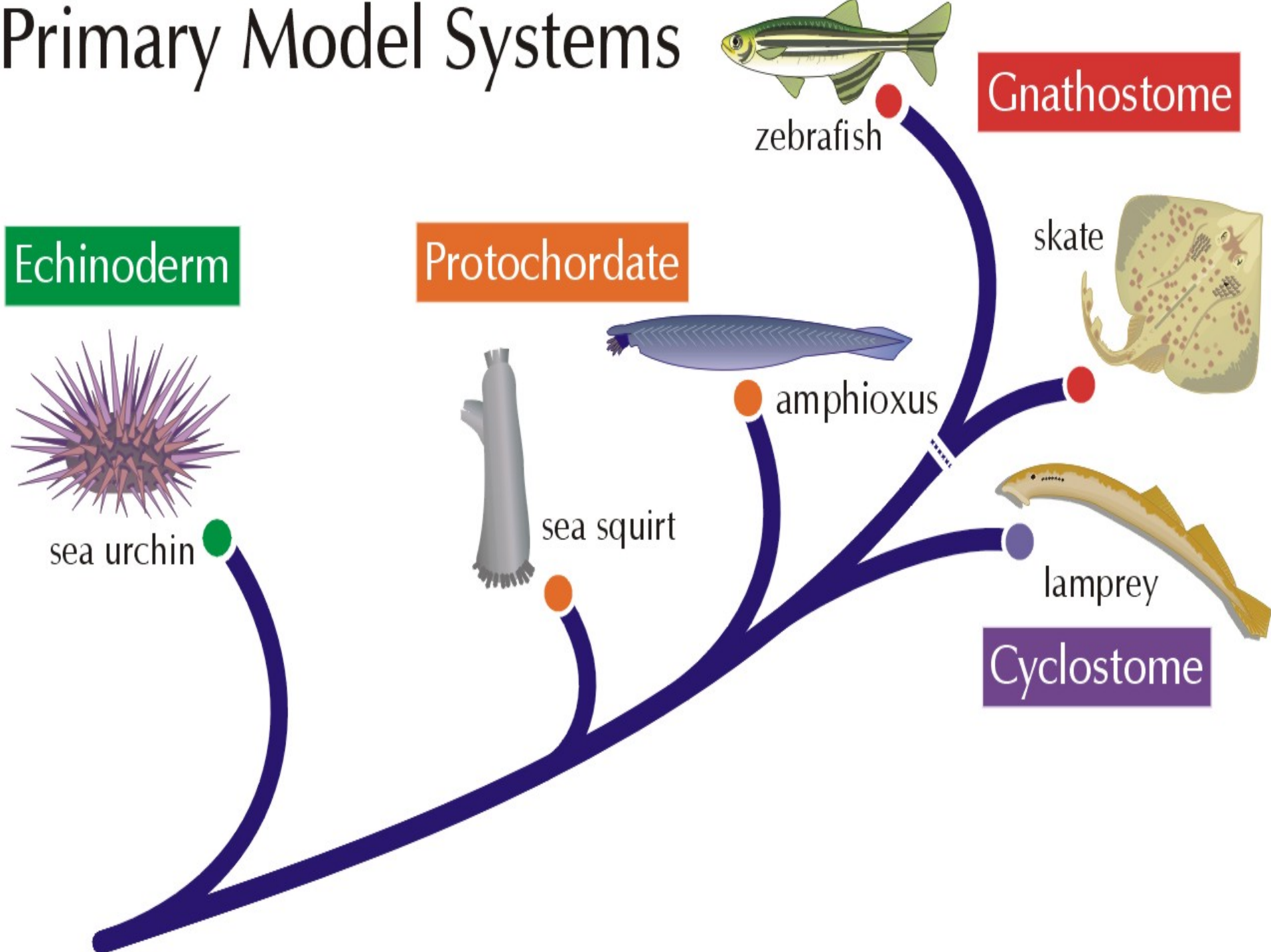
- No MHC I or MHC II
- No immunoglobulin or TCR
- No RAG or TdT
- Lymphocytes
- Specific humoral immune response
- VLRs
- Allograft rejection



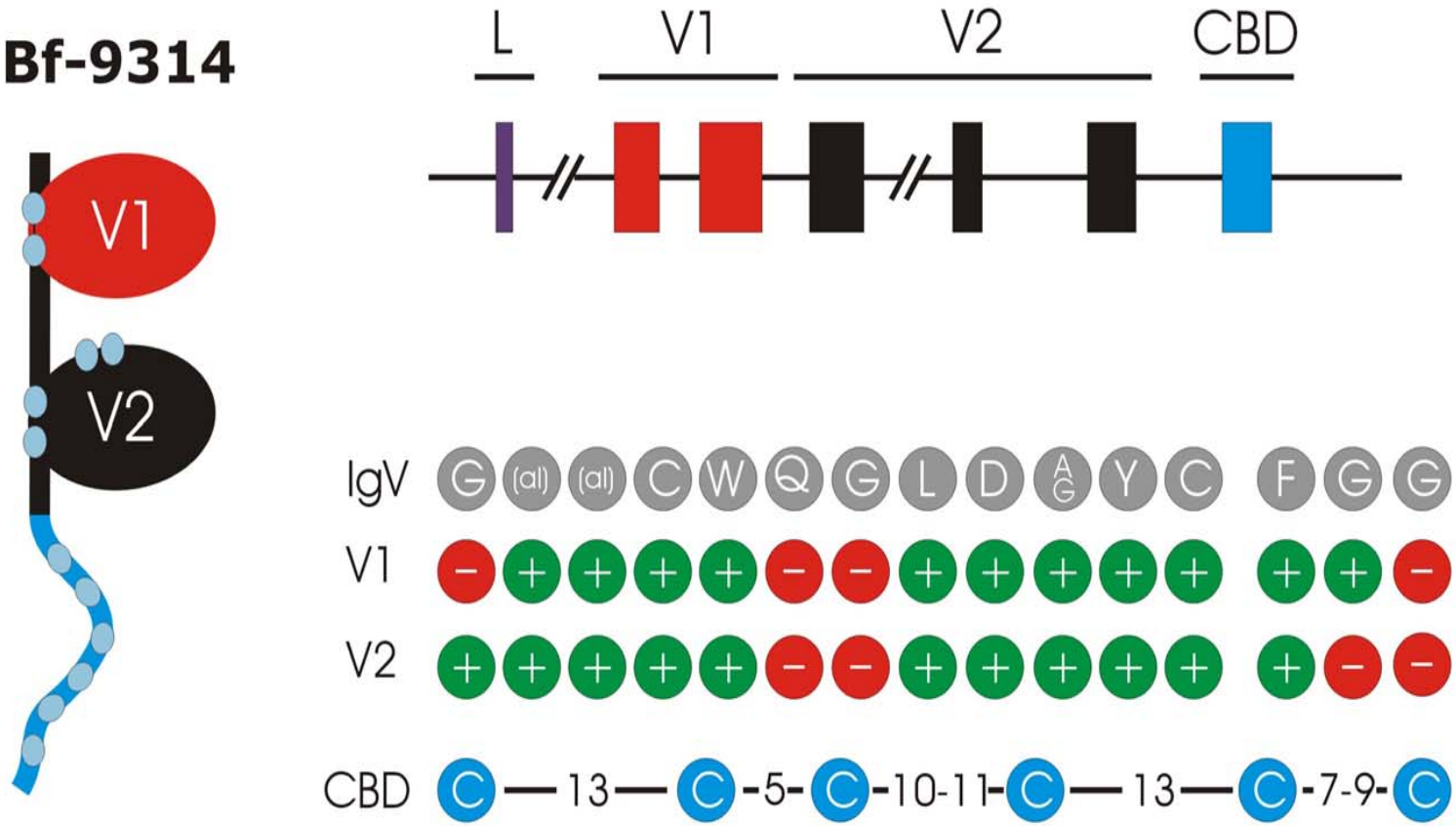


Mature VLR gene

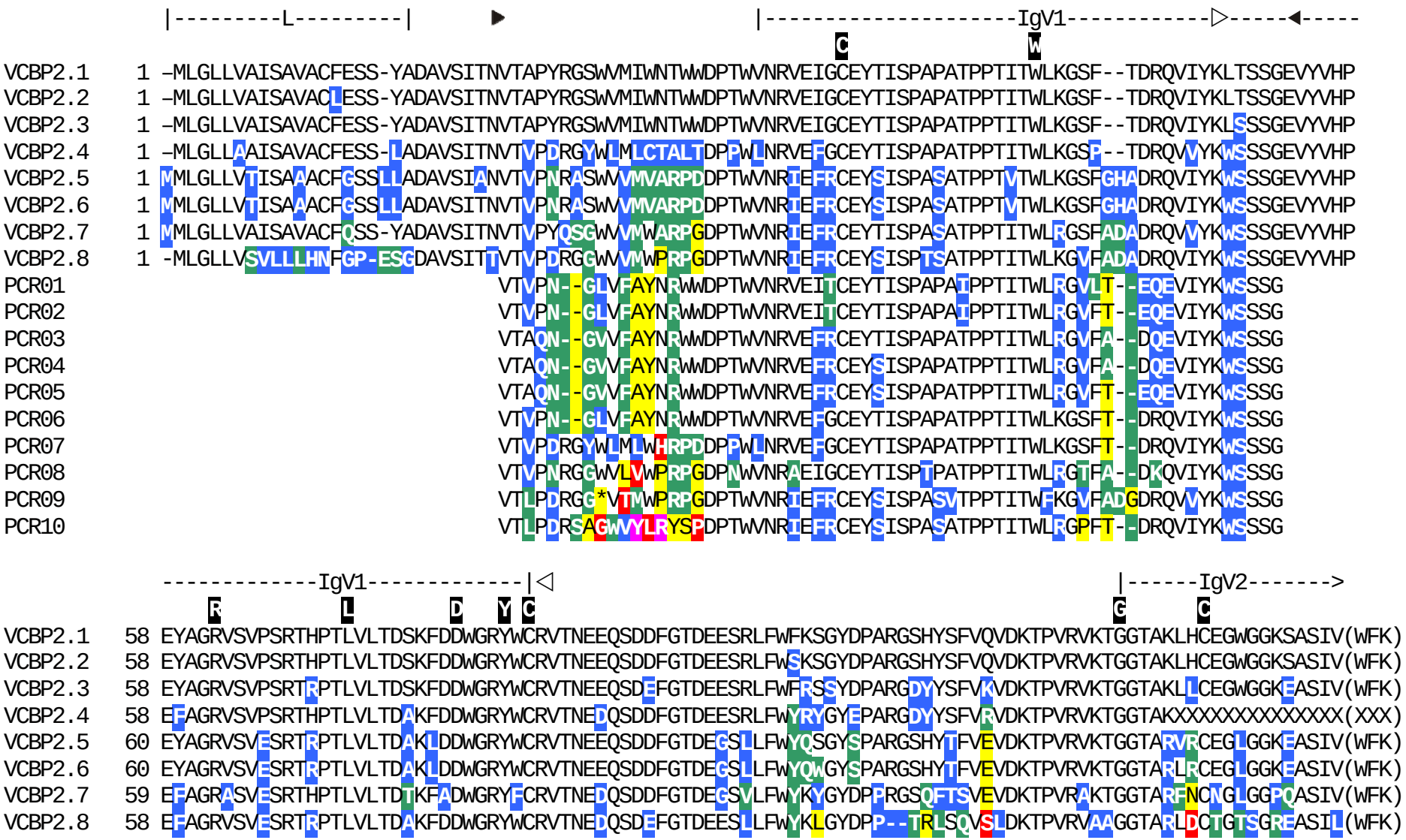
Primary Model Systems



V region-containing chitin binding protein (V-CBP)



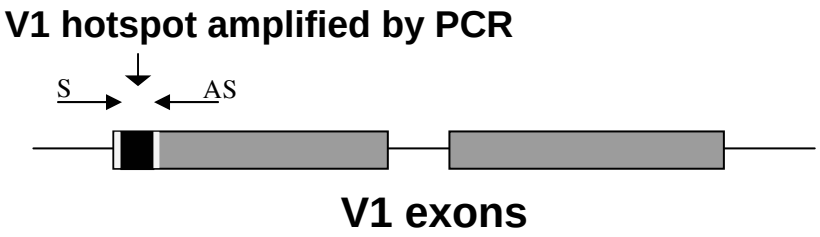
VCBP Diversity



Cannon, Haire, Litman. Nature Immunology 2002; 3:1200-1207

1	2	3	4	5	6	7	8	9	10	11	12	BAC	Sequence	#aa	cDNA
X													VTVPNRA---SWVVMVARPDDPTWVNRIEFR	28	+
X	X	X	X	X	X	X		X		X		X	VTVPDRG---GWVVMWPRPGDPTWVNRIEFR	28	+
X		X									X		VTVPNRG---SWVVMRPRLED-----RAEFR	23	+
X						X							VTVPDRS--AGWVMLWYRPDDPTWEHRIEFR	29	+
X				X									VTLPDRS---GWVYLRYSP-DPTWVNRIEFR	27	+
	X												VTVPERG---GWVVMWPRPGDPTWVNRIEFR	28	
	X	X	X		X						X		VTVPDRS--ASYIMLWHRPDDPTWVNRIEFR	29	+
	X												VTAPYRG---SWVMIWNTWWDPTWVNRVEIG	28	+
		X											VTVPDRG---GWVVMWPRPGDPTWVNRVEFR	28	
		X	X		X								VTVPDR---EYWLMLWHRPDDPTWVNRAEIG	28	
			X										VTVPDRG---HWVMMWPRPGDPTWVNRIEFR	28	
			X	X			X	X	X		X		VTVPNRG---GWVLVWPRPGDPNWVNRAEIG	28	+
			X						X				VTVPDRG---GWVVMWARPGDPTWVNRIEFR	28	
				X									VTVPDRG---GWVVMWPRPGDPTWVNQIEFR	28	
				X									VTVPDR---EYWLMLWHRPDDPTWVNRVEFG	28	
				X									VTAQN-----GVVFAYNRWWDPTWVSRVEFR	26	
				X								X	VTAQN-----GVVFAYNRWWDPTWVNRVEFR	26	+
				X									VTAQN-----GVVFAYNRWWDPTWVNRVEFG	26	
				X									VTAQN-----GVVFAYNRRWDPTWVNRVEFR	26	
					X								VTVPDRG---GWVVMWPRPGDPTWVNRAEIG	28	
					X								VTVPDRG---GWVVMWPRPGDPTWVSRIEFR	28	
					X								VTVPDR---DYWLMLWHRPDDPTWVNRAEIG	28	
					X								VTVPDRS--ASYIMLWHRPDDPTWVNRTEFR	29	
						X							VTVPDRG---GWVMMWPRPGDSTWVNRIEFR	28	
						X							VTVPDRG---GWVMMWPRPGDPTWVNRIEFR	28	
						X		X					VTVPDRG---GWVVMWPRPGDPTWVNWIEFR	28	
						X							VTVPDRS--AGWVMLWYRPDDPTWEHRMEFR	29	
						X		X				X	VTVPDRSAAAGWVYLRYSP-DPTWVNRIEFR	30	
						X							VTVPYHN---YYVLAADRPWDPTWVNRVEIG	28	
							X		X				VTVPYQS---GWVVMWARPGDPTWVNRIEFR	28	
							X						VTVPDRS--ASYIMLWHRPDDPTWVNRVEFR	29	
							X						VTVPDRS--ASYIMLWHRPDDPTWVNRVEFG	29	
								X					VTVPNRG---SWVLMWPRPGDPNWVNRIEIR	28	
								X					VTVPYQS---GWVVMWPRPGDPTWVNRIEFR	29	
								X					VTVPDRS--ASYIMLWHRPDDPTWEHRIEFR	28	
									X				VTVRDRG---GWVVMGARPGDPTWVNRIEFR	28	
									X				VTVPDRG---GWVMIWARPGDPTWVNRIEFR	28	
										X	X		VTVPNRM---GWVVVSPRWGDPTWVNRAEIG	28	+
										X			VTVPNRG---SWVVMVARPEDPTWVNRIEFR	28	+
											X		VTVPDRS--AGWIMLWHRPDDPTWVNRIEFR	29	+
												X	VTVPDRG---GWVVMWPRLGDPTWVNRIEFR	28	
												X	VTVPDRG---GWVVMWPRLGDPTWVNRVEFR	28	
												X	VTVPYHN---YYVLAADRPWDPTWVNRVEIR	28	

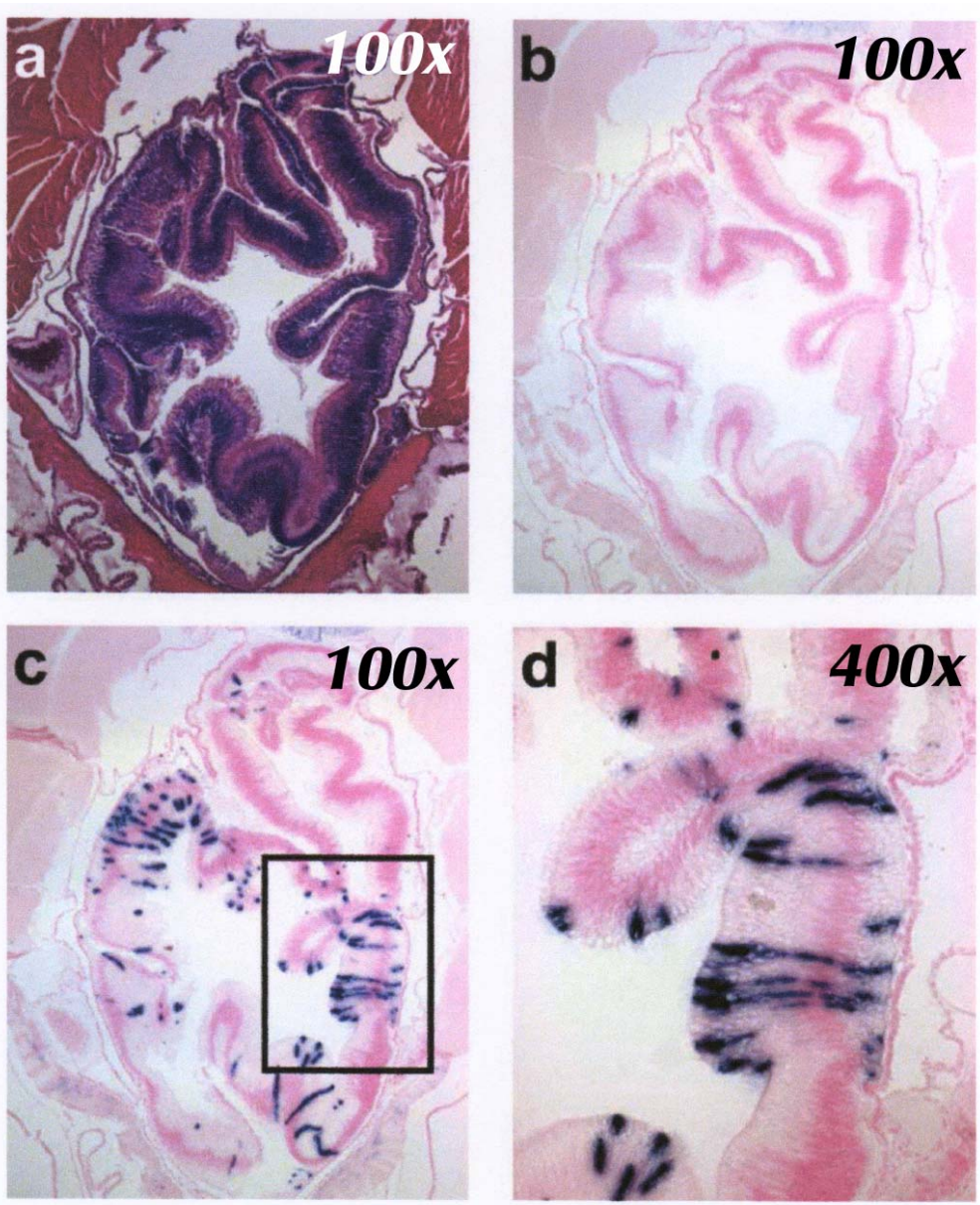
Estimation of VCBP V1 domain hotspot haplotype diversity via 454 sequencing technology



Initial data from pilot run:

1) 6800+ sequencing events
2) Sequences >105bp were kept producing 2789 sequences, which were stacked if they were 99-100% identical
3) 243 unique bins were created, each representing a unique allelic/haplotypic variant

In Situ Hybridization



Identification of diversified genes that contain immunoglobulin-like variable regions in a protochordate

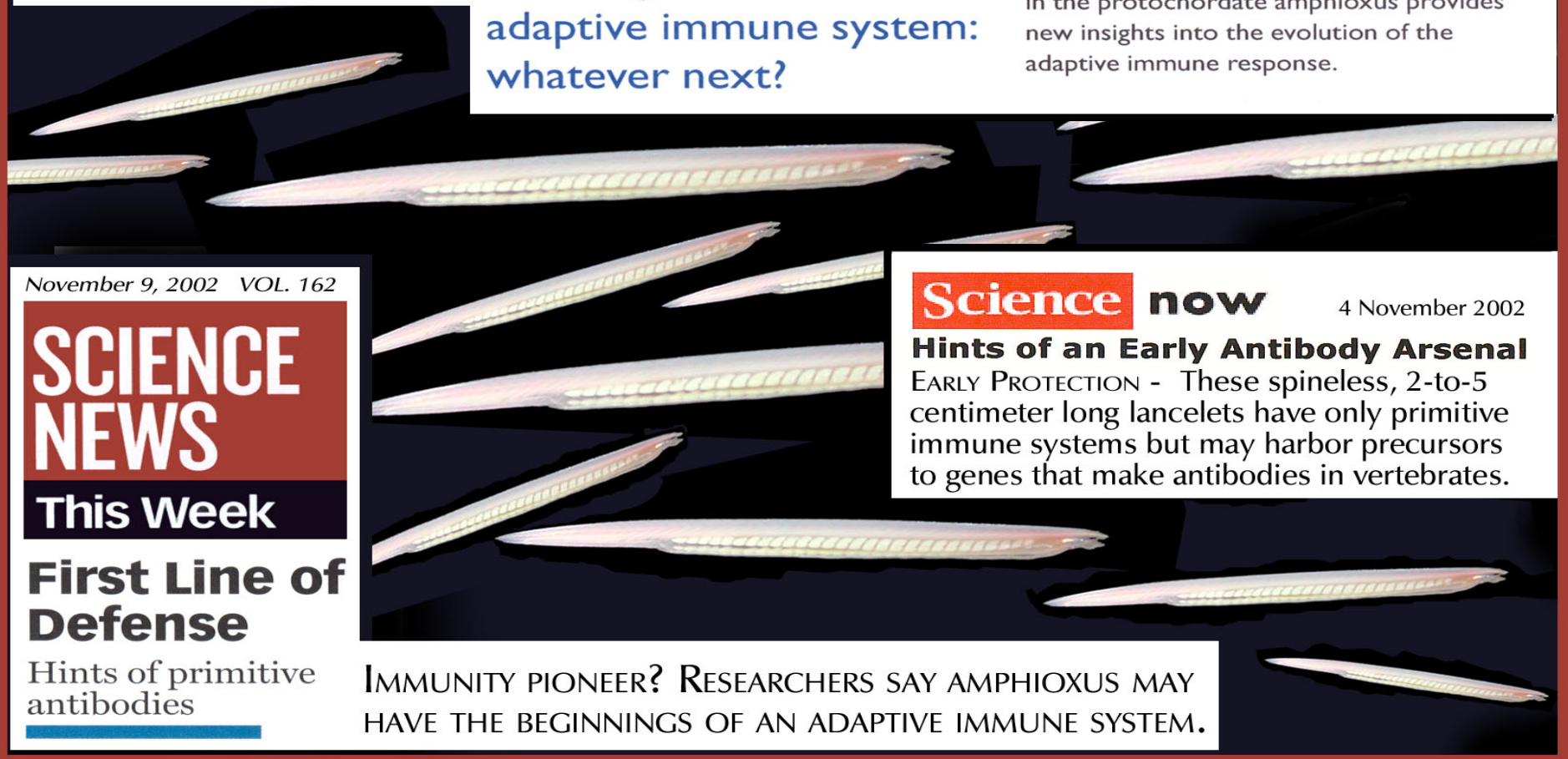
John P. Cannon[1,2], Robert N. Haire[3] and Gary W. Litman[1–3]

Published online 4 November 2002; doi:10.1038/ni849

News & Views

The origins of the adaptive immune system: whatever next?

The discovery of a V-like Ig multigene family in the protochordate amphioxus provides new insights into the evolution of the adaptive immune response.



November 9, 2002 VOL. 162

SCIENCE NEWS

This Week

First Line of Defense

Hints of primitive antibodies

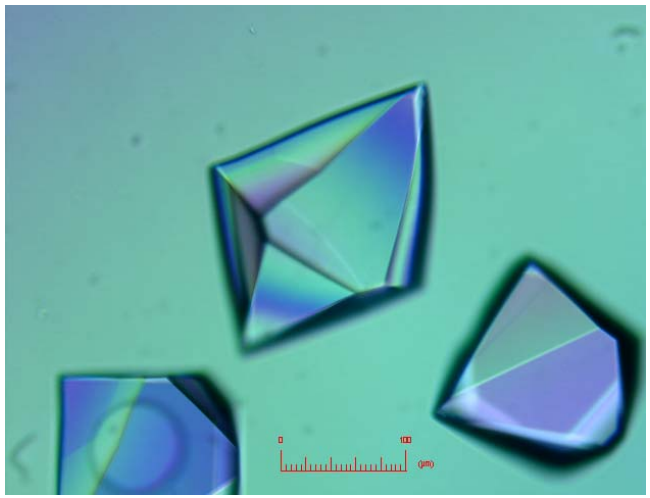
Science now 4 November 2002

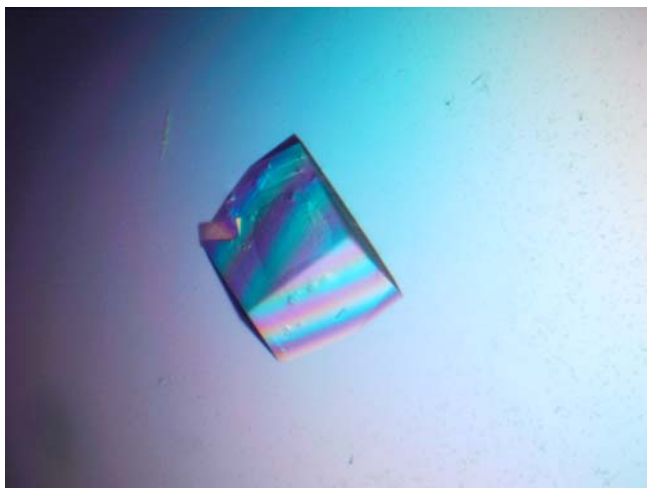
Hints of an Early Antibody Arsenal

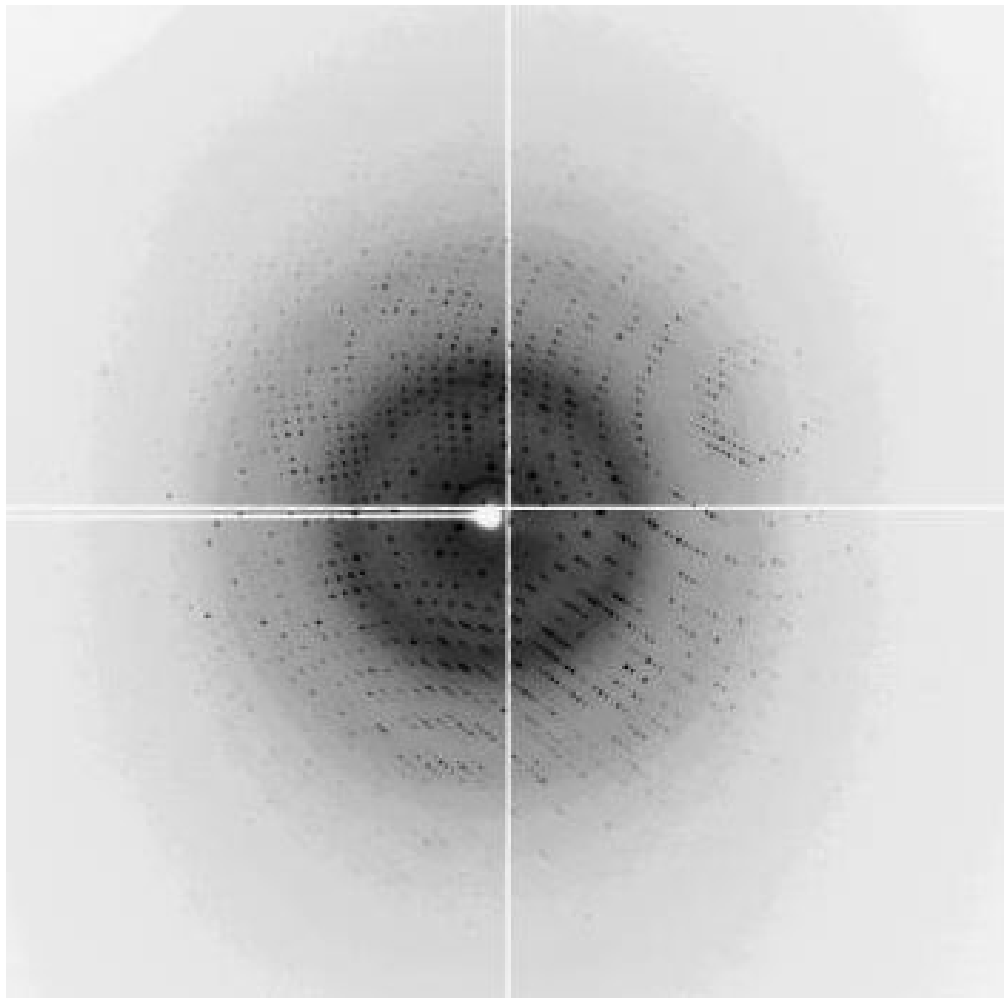
Early Protection - These spineless, 2-to-5 centimeter long lancelets have only primitive immune systems but may harbor precursors to genes that make antibodies in vertebrates.

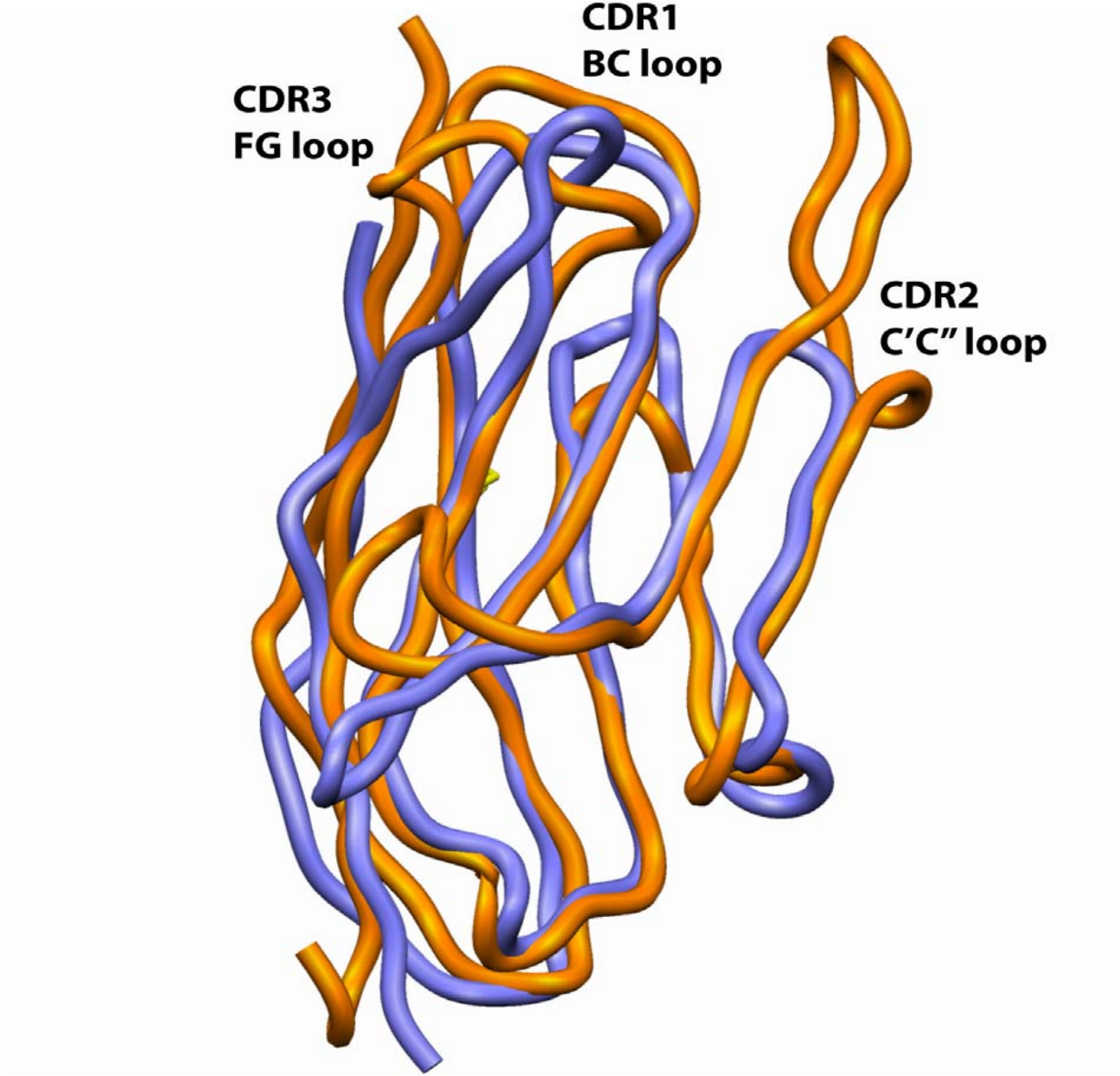
Immunity pioneer? Researchers say amphioxus may have the beginnings of an adaptive immune system.

VCBP3 Crystals

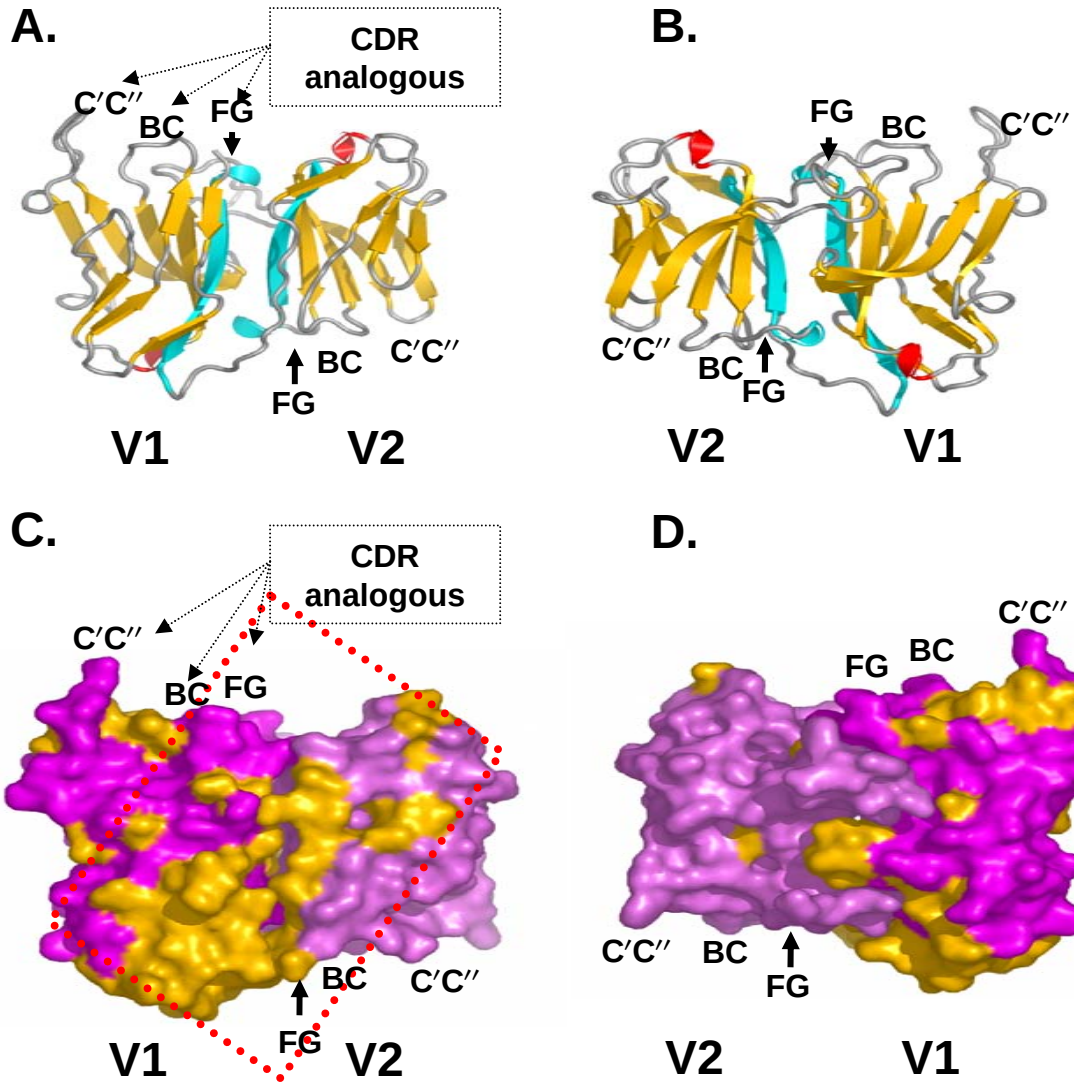


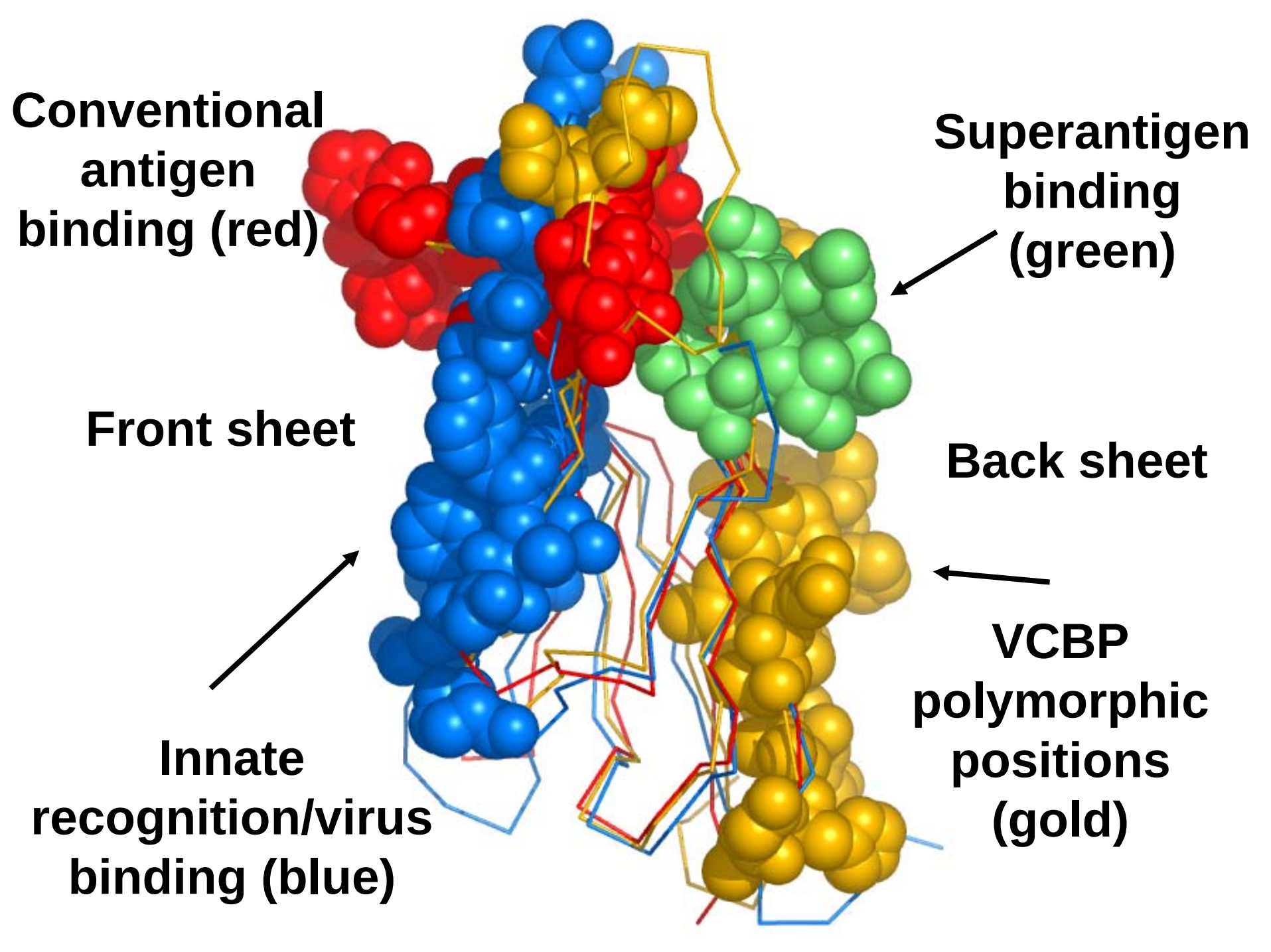




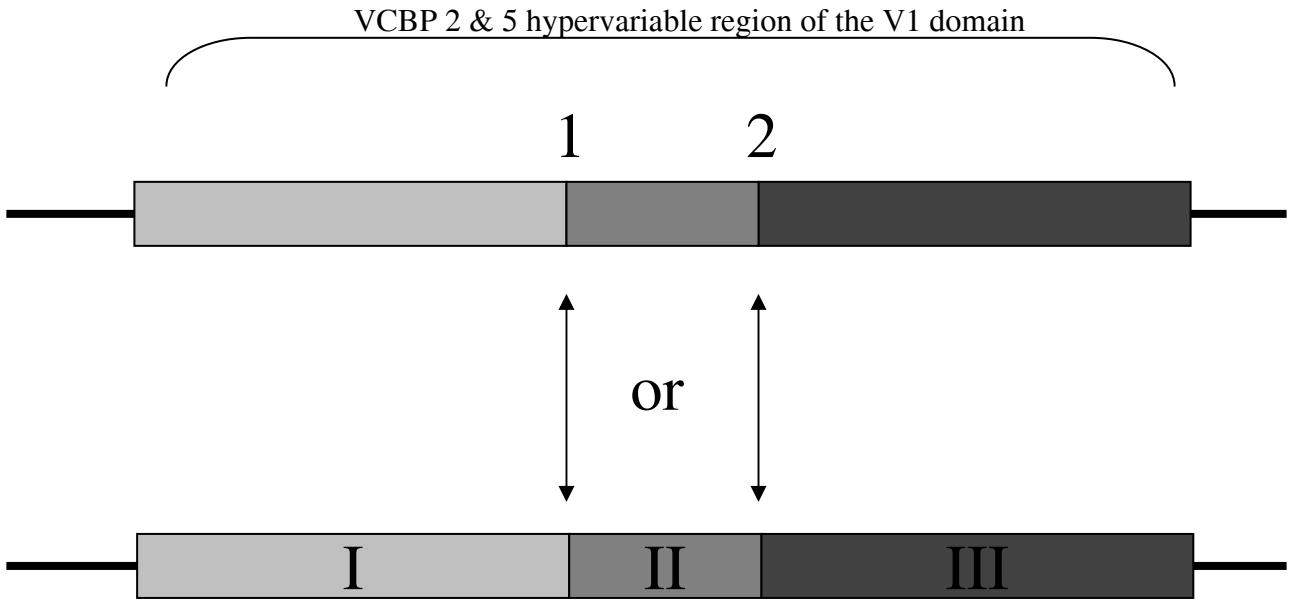


Hernandez Prada, Haire, Allaire, Jakoncic, Stojanoff, Cannon, Litman, Ostrov.
Nature Immunology 2006; 7:in press

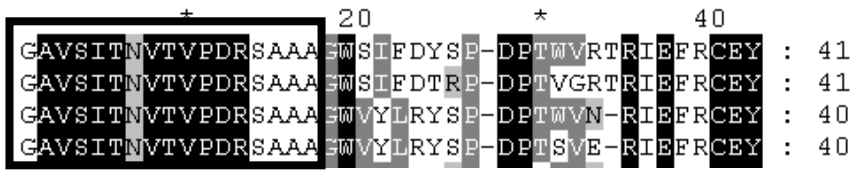

A.
CDR analogous
C′C″
BC
FG
BC
C′C″
FG
V1
V2
B.
FG
BC
C′C″
C′C″
BC
FG
V2
V1
C.
CDR analogous
C′C″
BC
FG
BC
C′C″
FG
V1
V2
D.
C′C″
FG
BC
C′C″
BC
FG
V2
V1


Conventional antigen binding (red)
Superantigen binding (green)
Front sheet
Back sheet
Innate recognition/virus binding (blue)
VCBP polymorphic positions (gold)

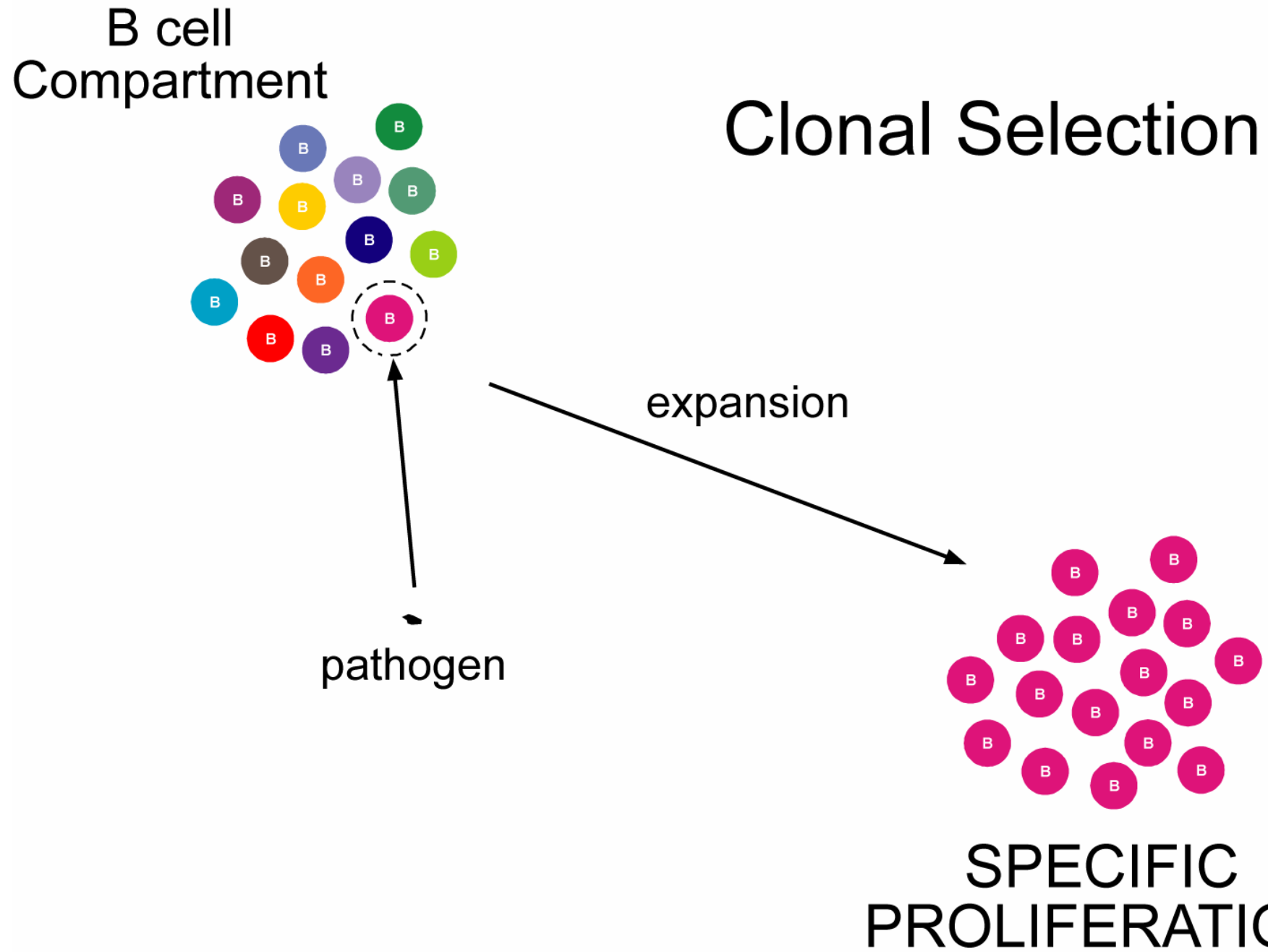
Extensive screening of the hotspot region implicates two sites for meiotic strand exchange within the VCBP 2 and 5 hypervariable regions

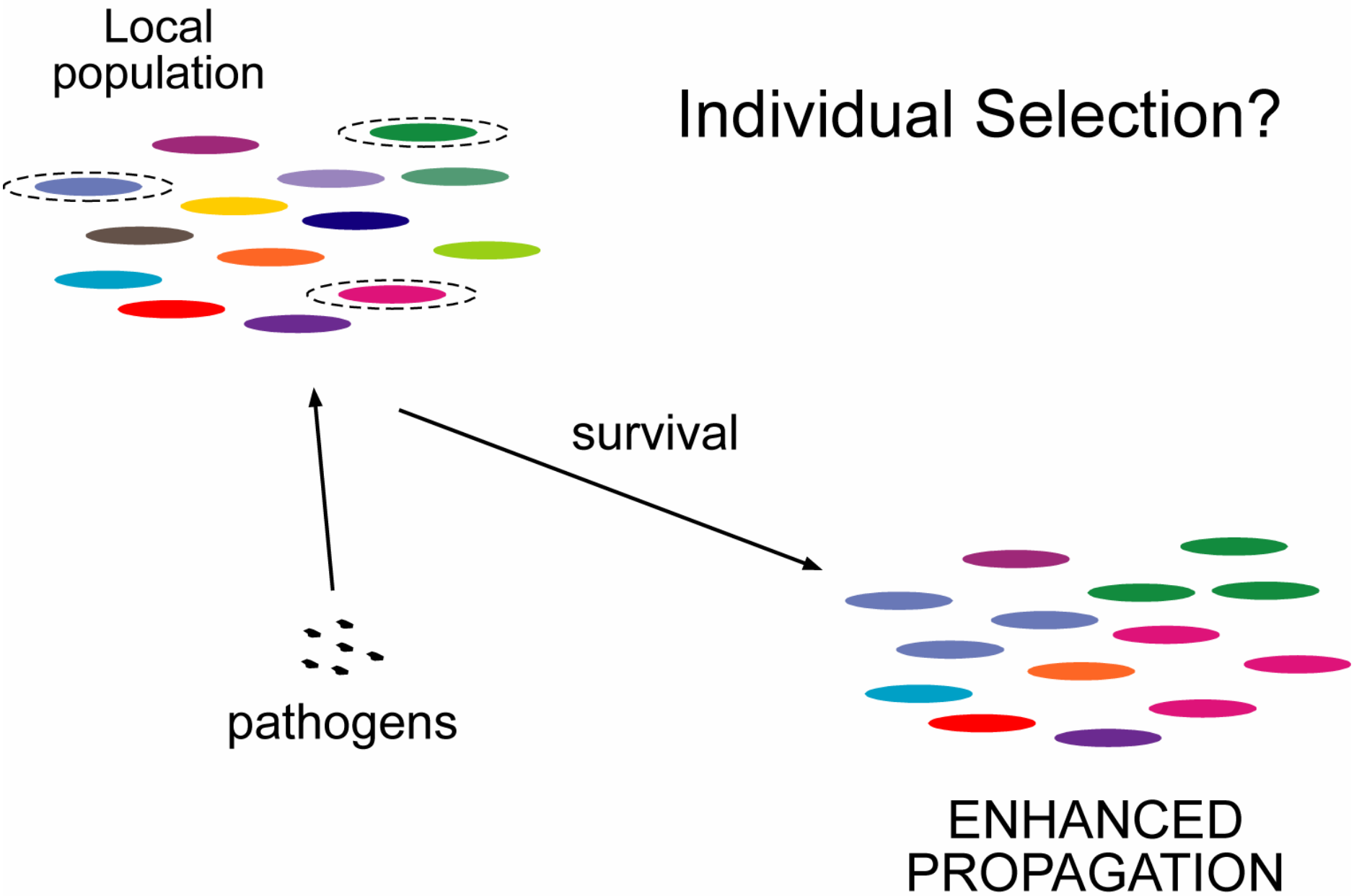


Example of 4 different haplotypes sharing a specific “block I” type.

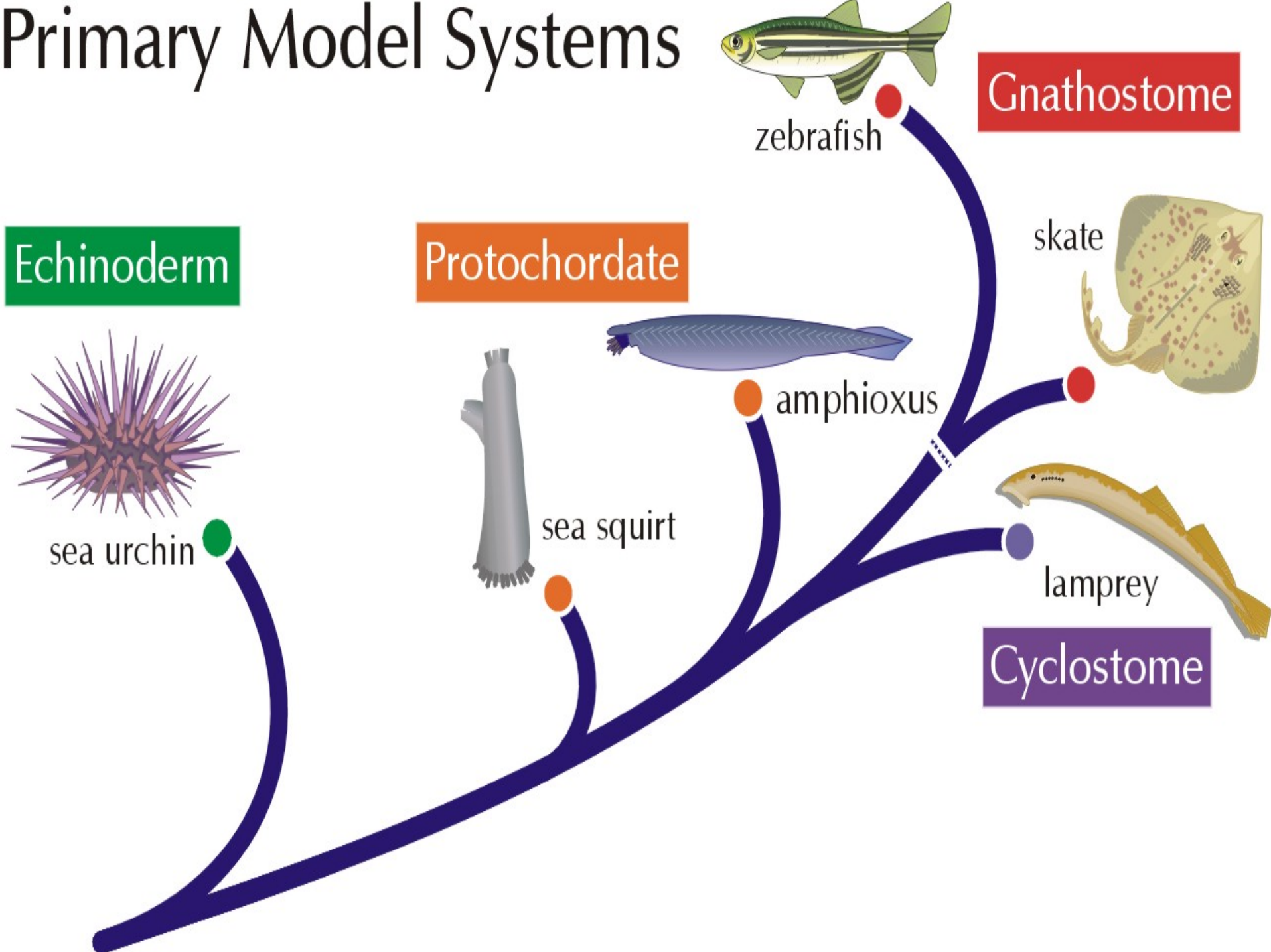


Clonal Selection




Local population
Individual Selection?
survival
pathogens
ENHANCED PROPAGATION

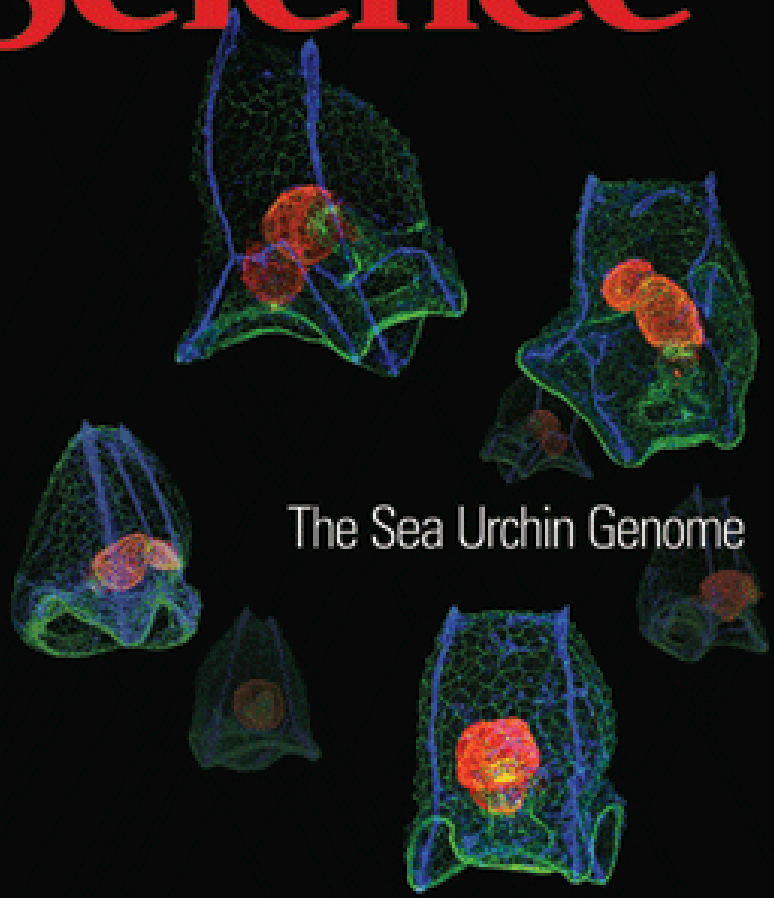
Primary Model Systems




Charles Hollahan

10 November 2006 | $10

Science

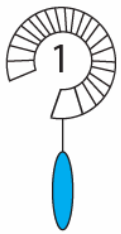


The Sea Urchin Genome

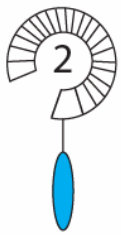
AAAS

Human and Mouse Toll-like Receptors

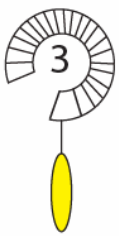
H Human only
M Mouse only
Colors indicate family relatedness



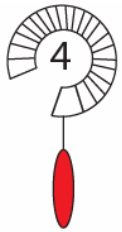
Lipoproteins



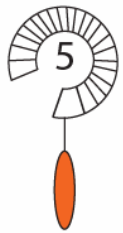
Lipoproteins
Peptidoglycan
Lipoteichoic Acid



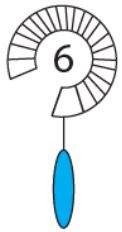
dsRNA



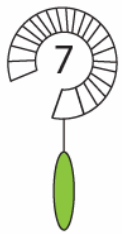
LPS



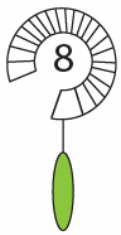
Flagellin



Lipoproteins

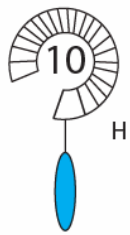


ssRNA

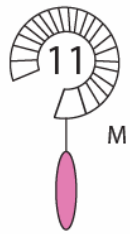


ssRNA

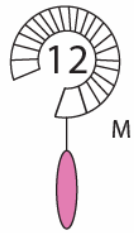
CpG DNA



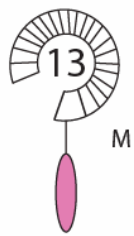
?



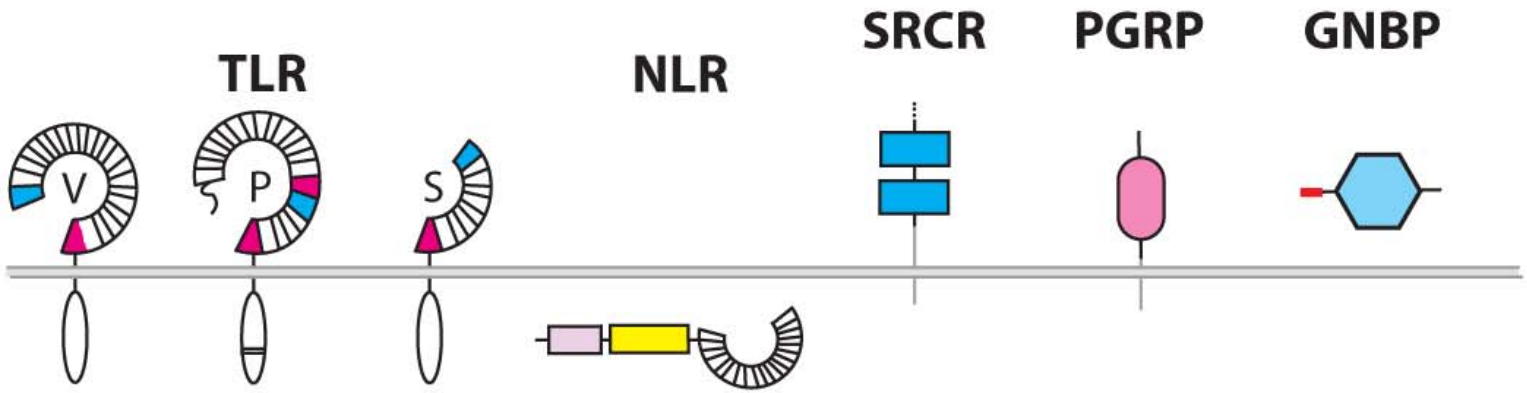
Profilin



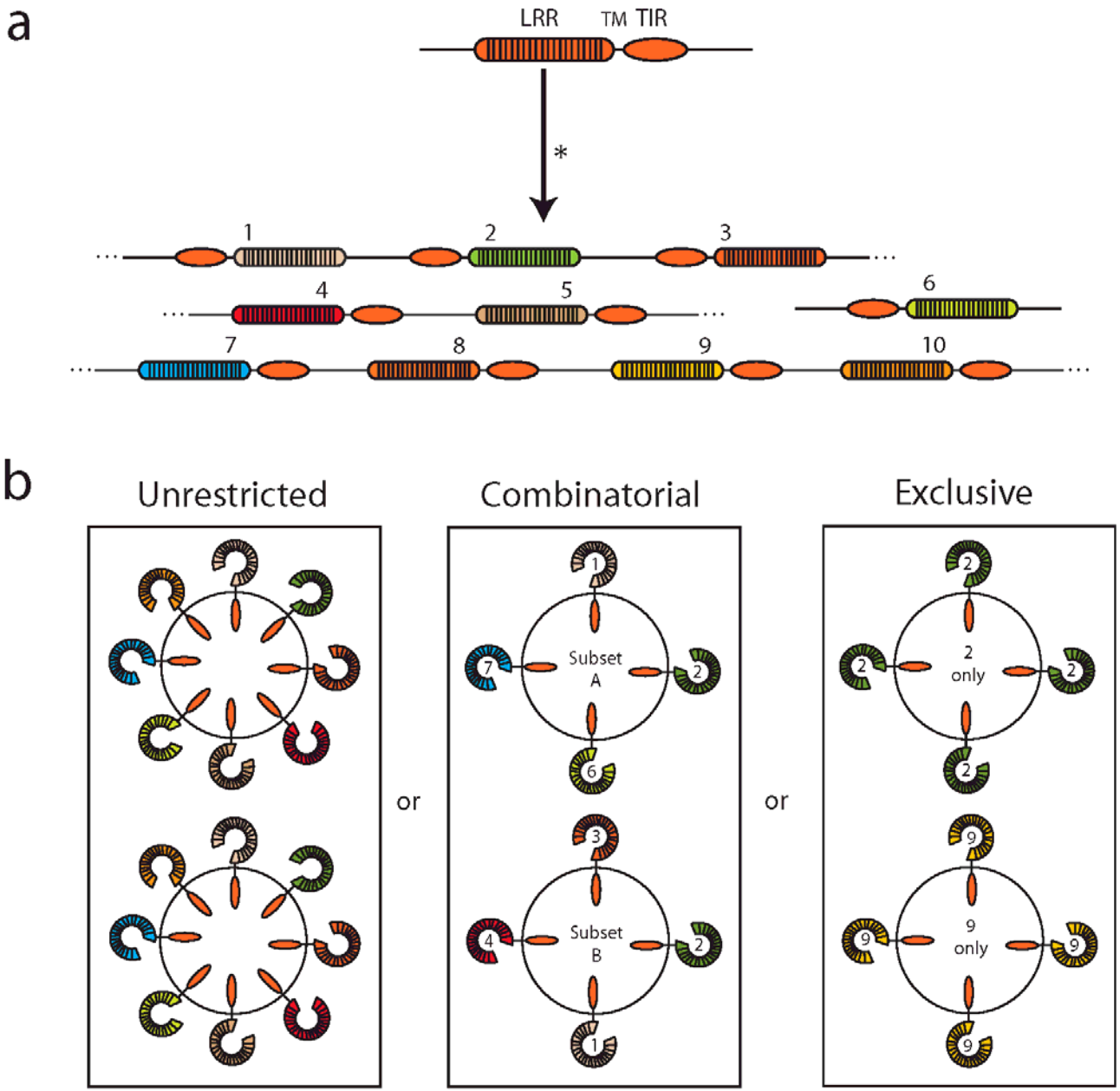
?



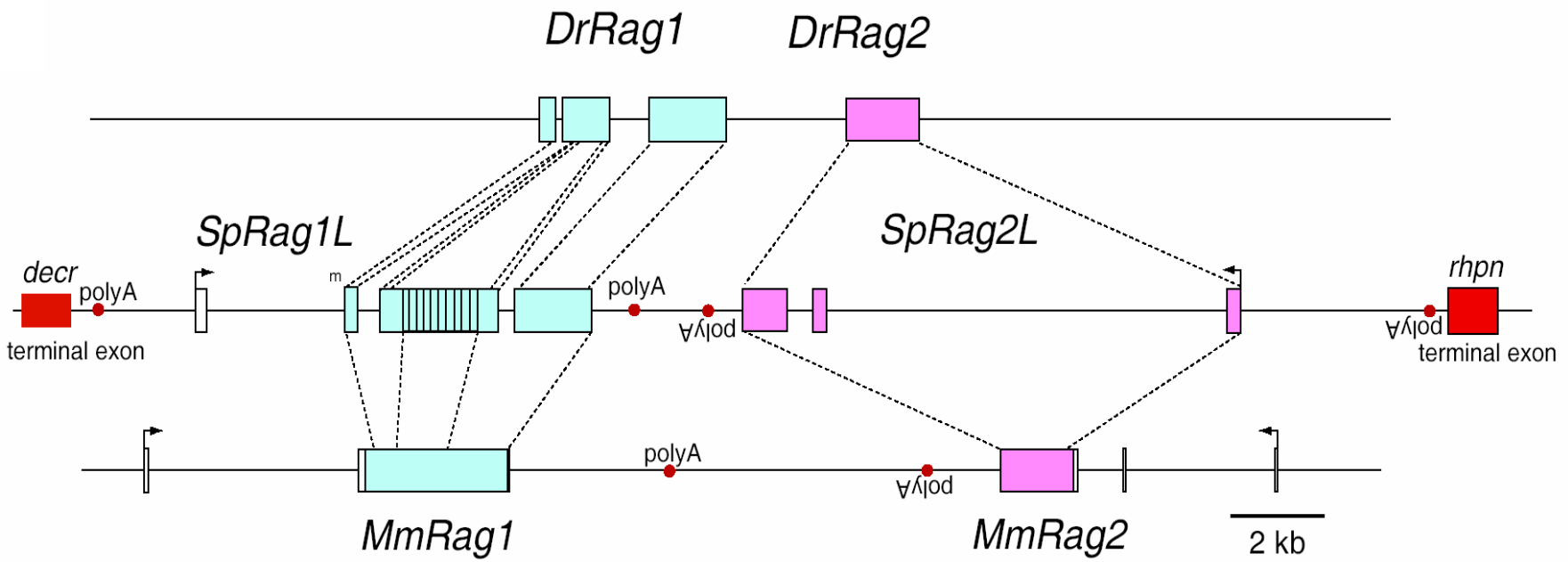
?



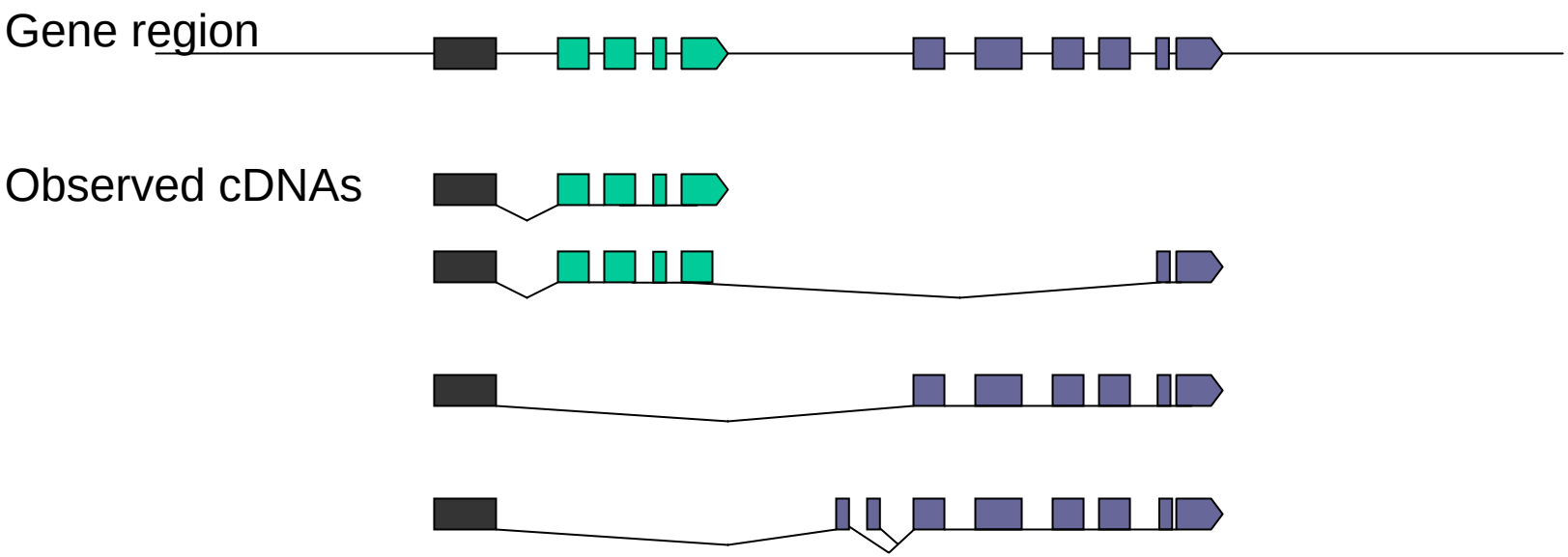
	TLR			NLR	SRCR	PGRP	GNBP
H.s.	*10(+1ψ)*	*0*	*0*	*20*	*81(16)*	*6*	*0*
C.i.	*3*	*0*	*0*	*0*	*22(8)*	*6*	*0*
S.p.	***214***	***3***	***5***	***203***	***1095(218)***	***5***	***3***
D.m.	*1*	*8*	*0*	*0*	*14(7)*	*15*	*4*
C.e.	*0*	*1*	*0*	*0*	*3(1)*	*0*	*0*


a
LRR
TM
TIR
*
1
2
3
4
5
6
7
8
9
10
b
Unrestricted
Combinatorial
Exclusive
or
or
Subset A
Subset B
2 only
9 only

Sea urchin rag-like genes

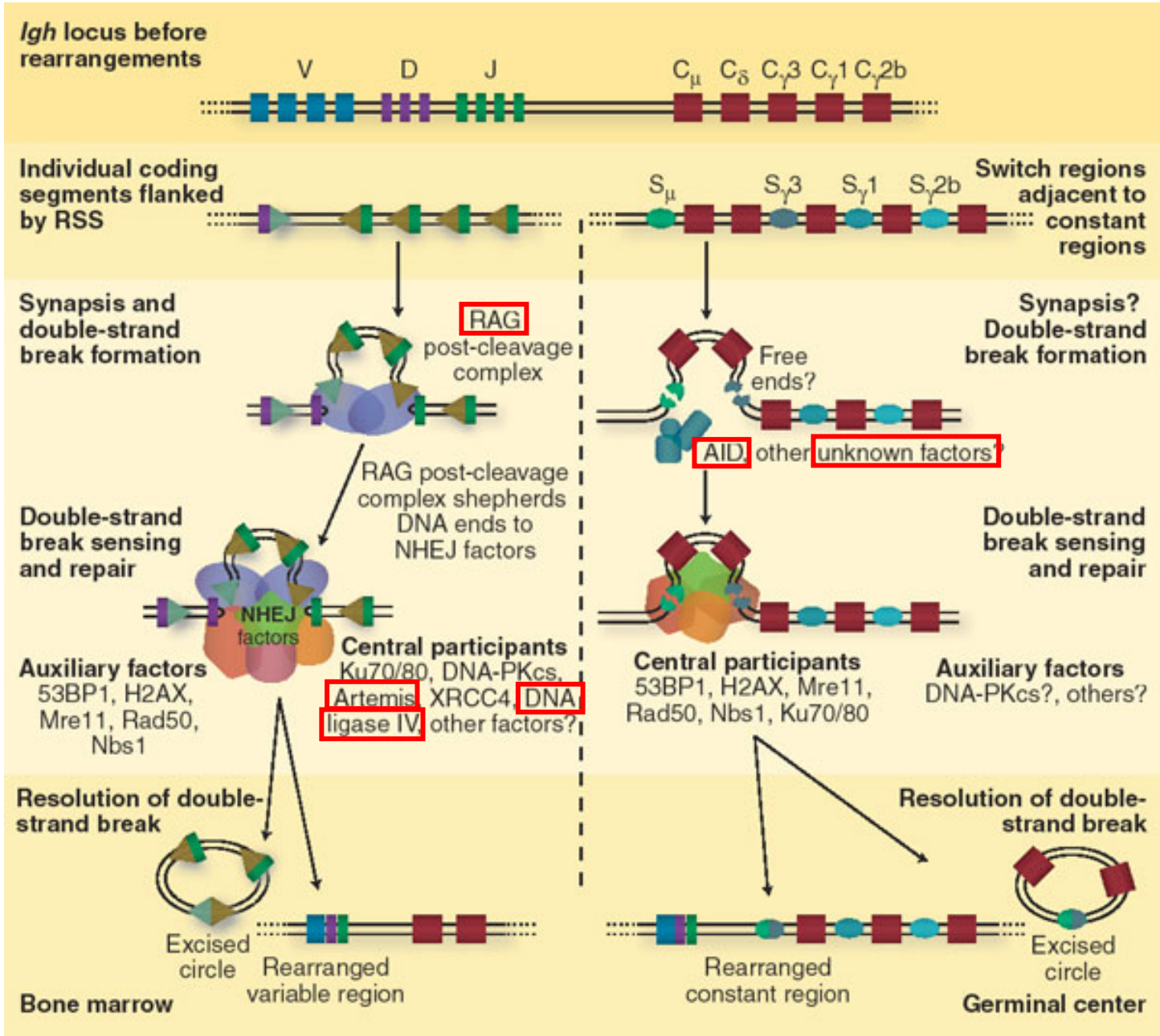


Amphioxus expresses two distinct Pol-mu sequences from same genetic region

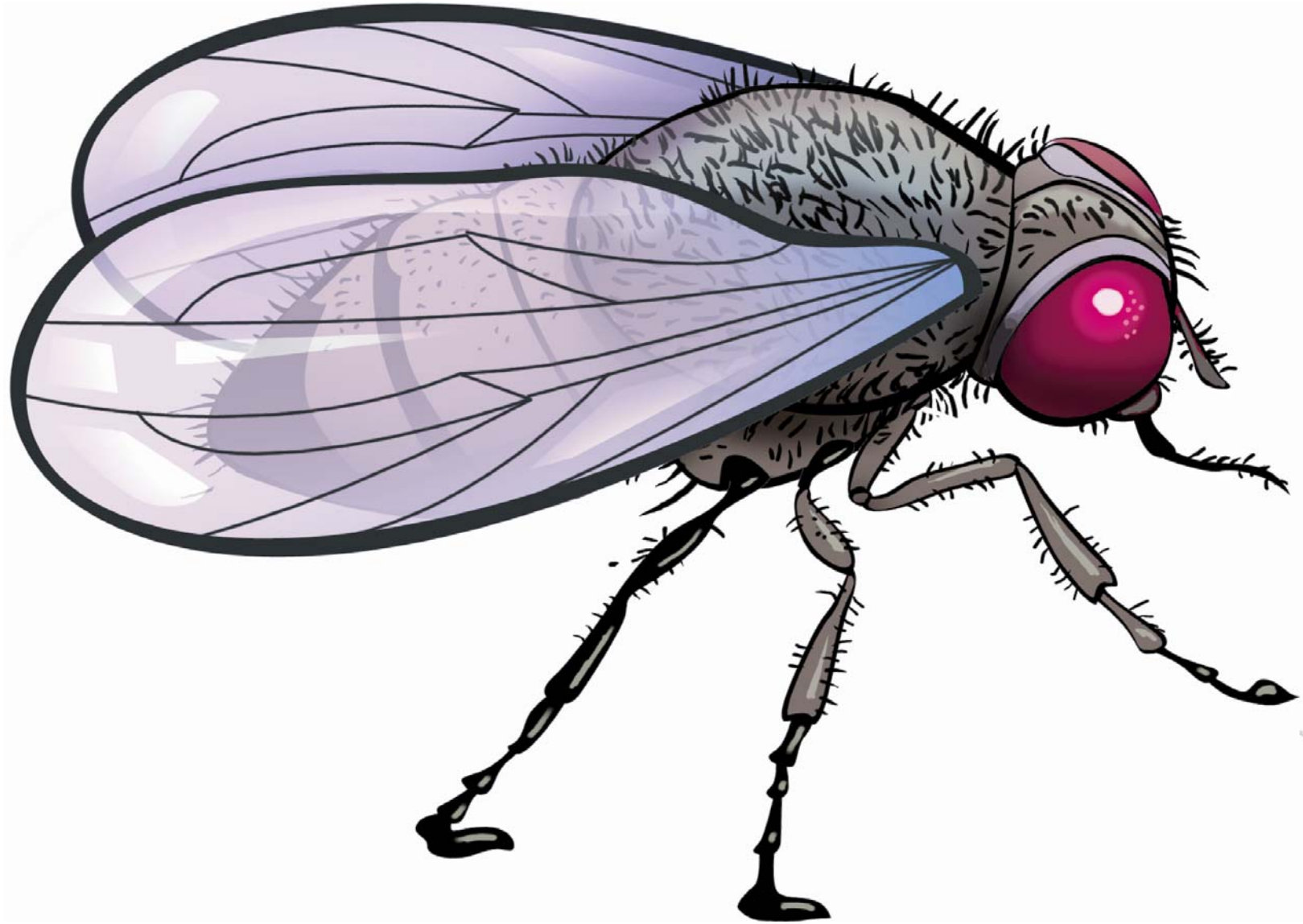


Each Pol-mu scaffold contains a single locus that generates two different gene products. The second gene product is more similar to vertebrate TdT by blast.

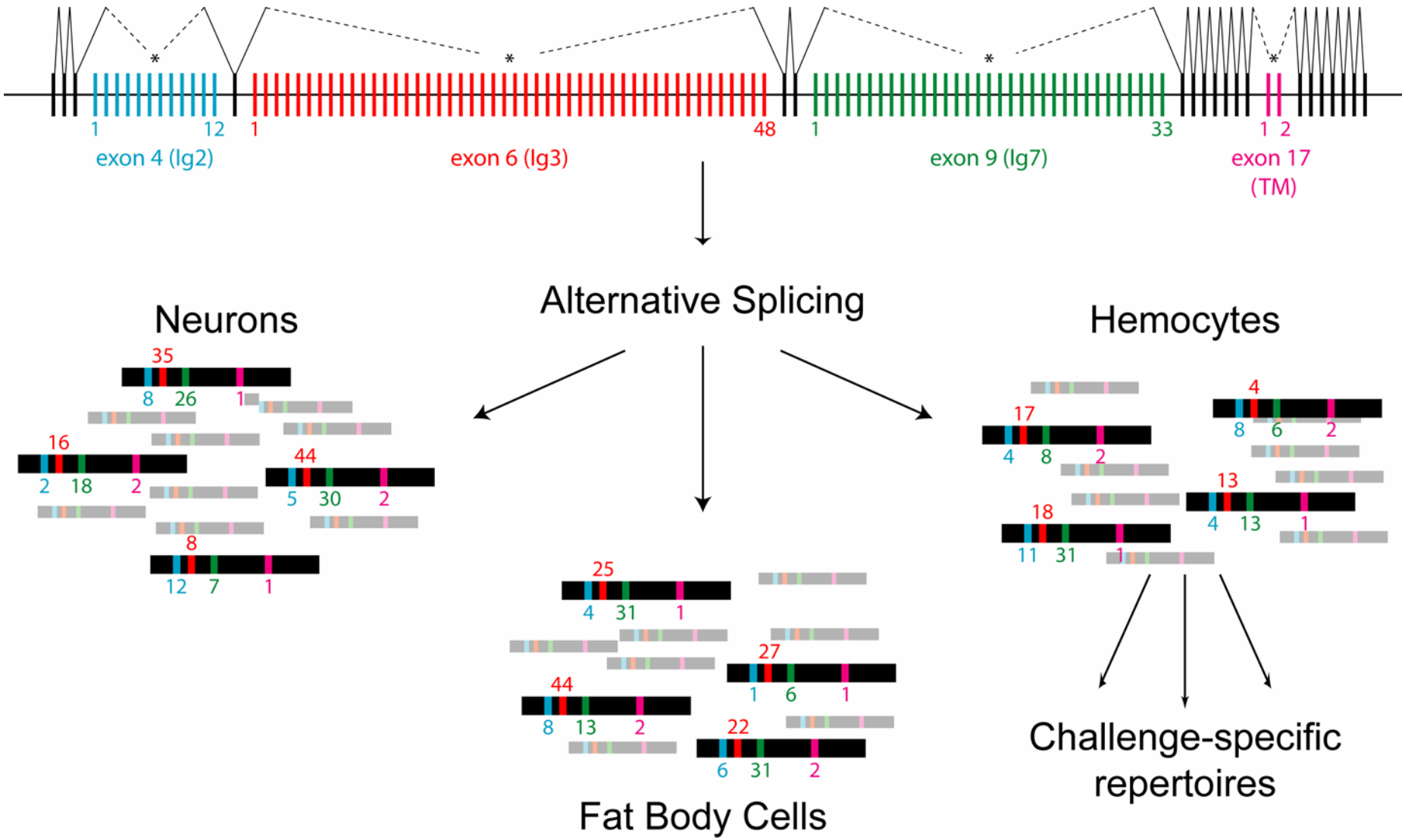
Both scaffolds contain similar gene organization, but represent distinct haplotypes, or could represent duplicated gene regions.

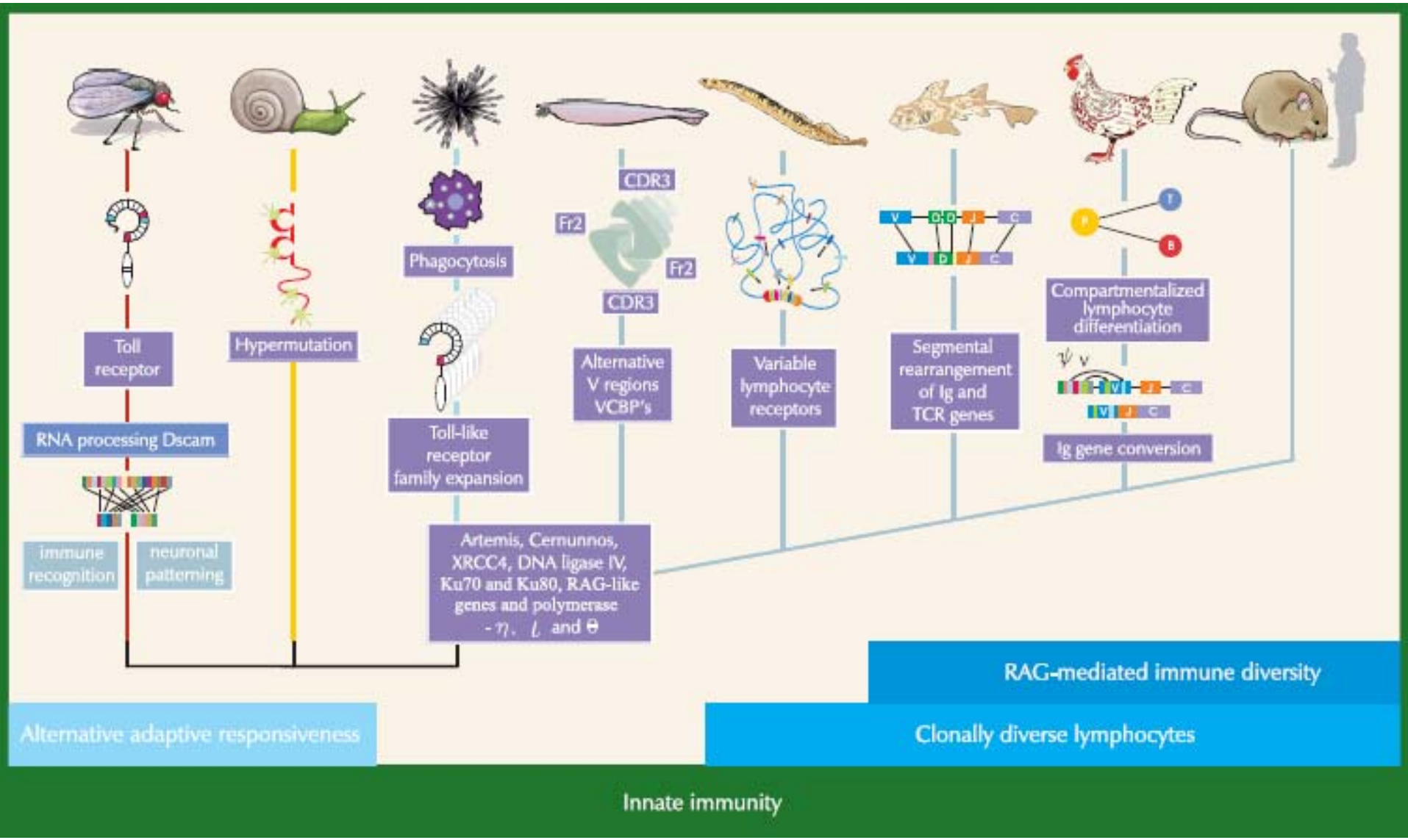

Igh locus before rearrangements
V
D
J
C_μ C_δ $C_\gamma3$ $C_\gamma1$ $C_\gamma2b$
Individual coding segments flanked by RSS
S_μ $S_\gamma3$ $S_\gamma1$ $S_\gamma2b$
Switch regions adjacent to constant regions
Synapsis and double-strand break formation
RAG post-cleavage complex
Synapsis? Double-strand break formation
Free ends?
AID, other unknown factors?
RAG post-cleavage complex shepherds DNA ends to NHEJ factors
Double-strand break sensing and repair
NHEJ factors
Double-strand break sensing and repair
Auxiliary factors
53BP1, H2AX, Mre11, Rad50, Nbs1
Central participants
Ku70/80, DNA-PKcs, Artemis, XRCC4, DNA ligase IV, other factors?
Central participants
53BP1, H2AX, Mre11, Rad50, Nbs1, Ku70/80
Auxiliary factors
DNA-PKcs?, others?
Resolution of double-strand break
Excised circle
Rearranged variable region
Bone marrow
Resolution of double-strand break
Rearranged constant region
Excised circle
Germinal center

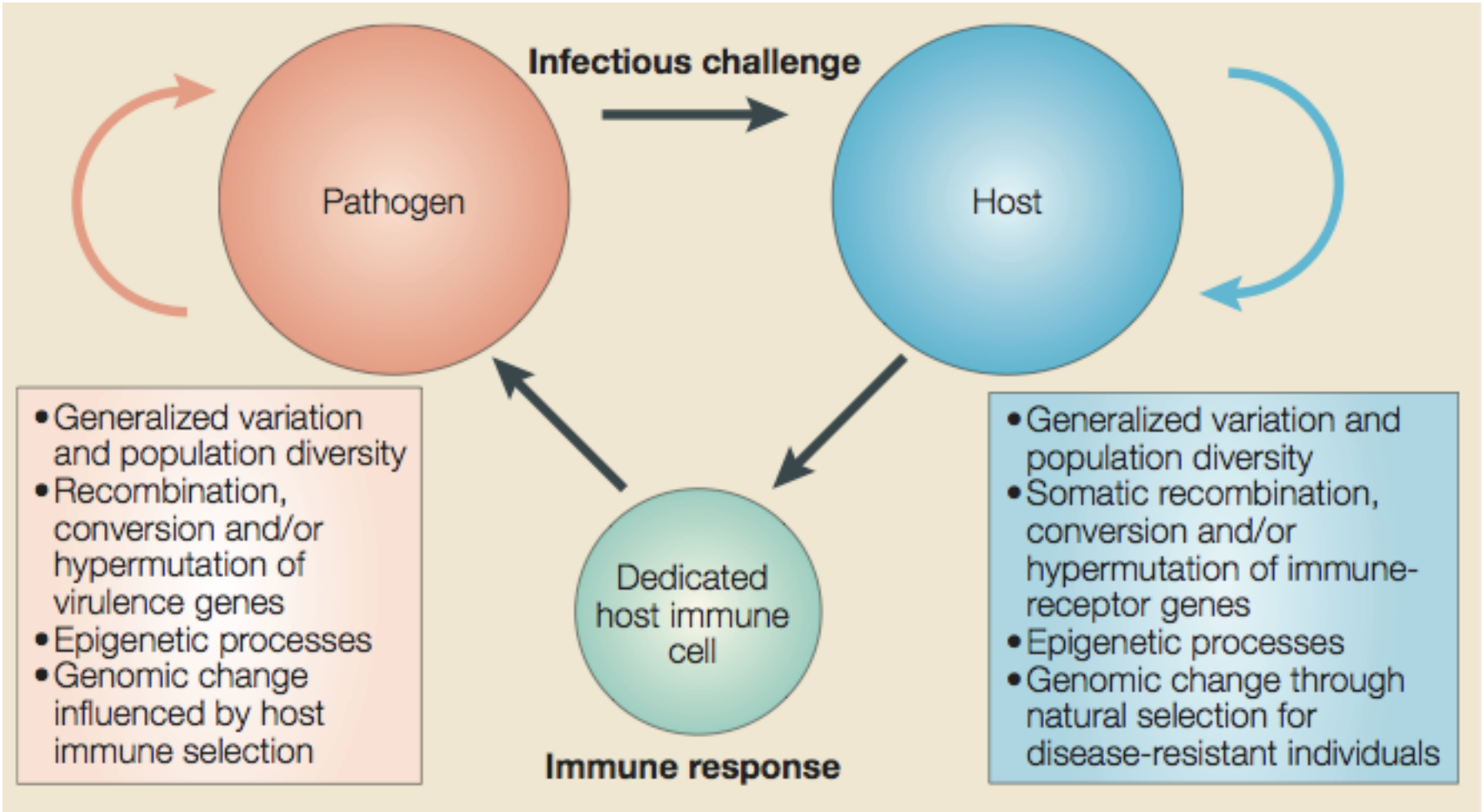
NATURE

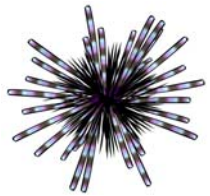


DSCAM


1
12
exon 4 (Ig2)
1
48
exon 6 (Ig3)
1
33
exon 9 (Ig7)
1 2
exon 17
(TM)
Alternative Splicing
Neurons
35
8 26 1
16
2 18 2
44
5 30 2
8
12 7 1
25
4 31 1
27
1 6 1
44
8 13 2
22
6 31 2
Fat Body Cells
Hemocytes
17
4 8 2
4
8 6 2
13
4 13 1
18
11 31 1
Challenge-specific
repertoires

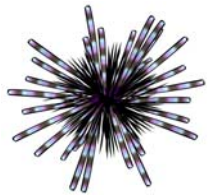

Toll receptor
RNA processing Dscam
immune recognition
neuronal patterning
Hypermutation
Phagocytosis
Toll-like receptor family expansion
CDR3
Fr2
Fr2
CDR3
Alternative V regions VCBP's
Artemis, Cernunnos, XRCC4, DNA ligase IV, Ku70 and Ku80, RAG-like genes and polymerase -η, ι and Θ
Variable lymphocyte receptors
Segmental rearrangement of Ig and TCR genes
Compartmentalized lymphocyte differentiation
Ig gene conversion
RAG-mediated immune diversity
Alternative adaptive responsiveness
Clonally diverse lymphocytes
Innate immunity


Infectious challenge
Pathogen
Host
• Generalized variation and population diversity
• Recombination, conversion and/or hypermutation of virulence genes
• Epigenetic processes
• Genomic change influenced by host immune selection
Dedicated host immune cell
Immune response
• Generalized variation and population diversity
• Somatic recombination, conversion and/or hypermutation of immune-receptor genes
• Epigenetic processes
• Genomic change through natural selection for disease-resistant individuals


INNATE IMMUNITY

ADAPTIVE IMMUNITY


GERMLINE ENCODED

VARIATION IN SOMATIC CELLS



VARIATION IN SOMATIC CELLS

VLRS	ANTIBODY/T CELL RECEPTORS



VARIATION IN SOMATIC CELLS

LRR	IMMUNOGLOBLULIN

VARIATION IN SOMATIC CELLS

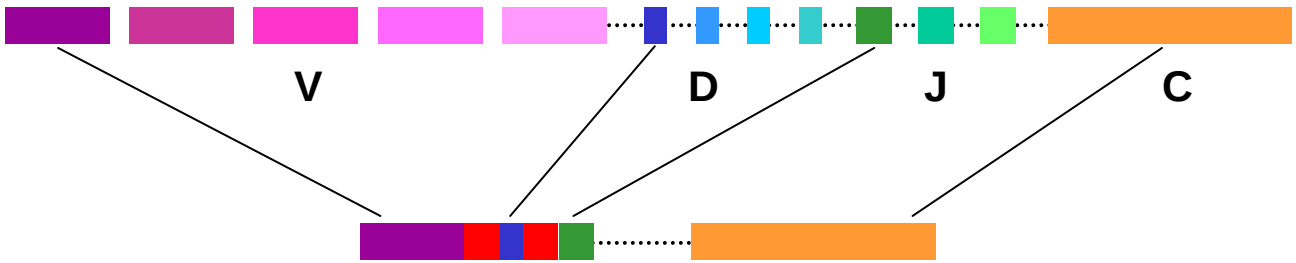
VLRS	ANTIBODY/T CELL RECEPTORS
GENE CONVERSION	SEGMENTAL REARRANGEMENT
	JUNCTIONAL DIVERSITY
	SOMATIC MUTATION
	GENE CONVERSION

VARIATION IN SOMATIC CELLS

VLRS	ANTIBODY/T CELL RECEPTORS
?	RAG TdT DRF

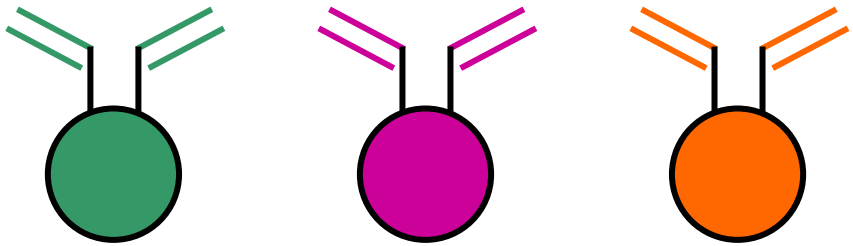
VARIATION IN SOMATIC CELLS

ANTIBODY / T CELL RECEPTOR



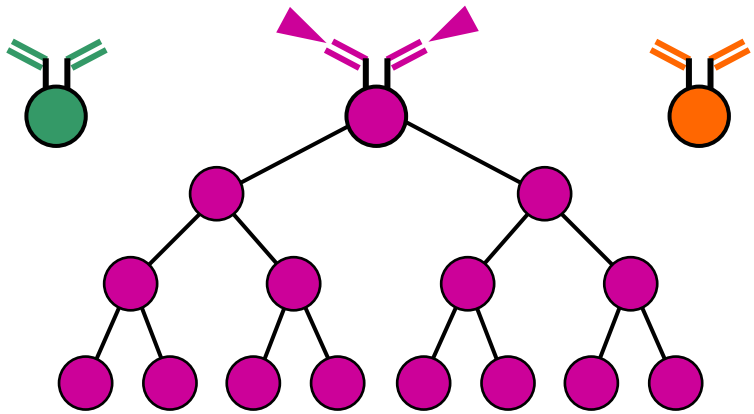
VARIATION IN SOMATIC CELLS

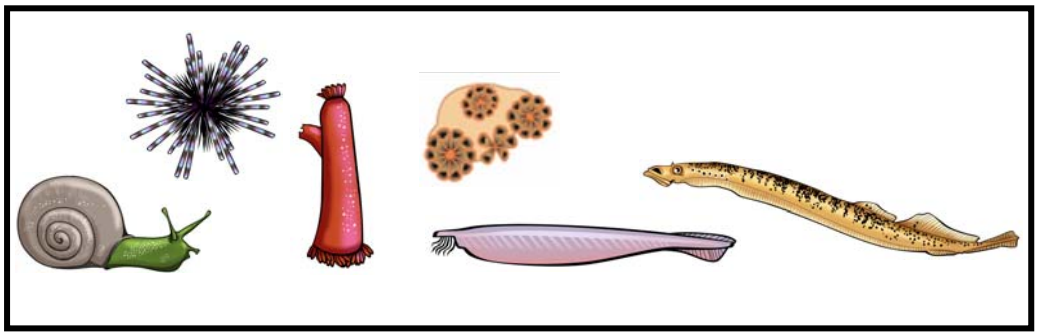
CLONAL DIVERSITY



VARIATION IN SOMATIC CELLS

CLONAL EXPANSION





INNATE IMMUNITY

ADAPTIVE IMMUNITY

- John Cannon
- Larry Dishaw
- Donna Eason
- Robert Haire
- Ronda Litman
- Gail Mueller
- Natasha Schnitker

- Chris Amemiya
- Tatsuya Ota

- Jeffrey Yoder
- Norman Miller
- Jonathan Rast
- David Ostrov
- Jose Hernandez