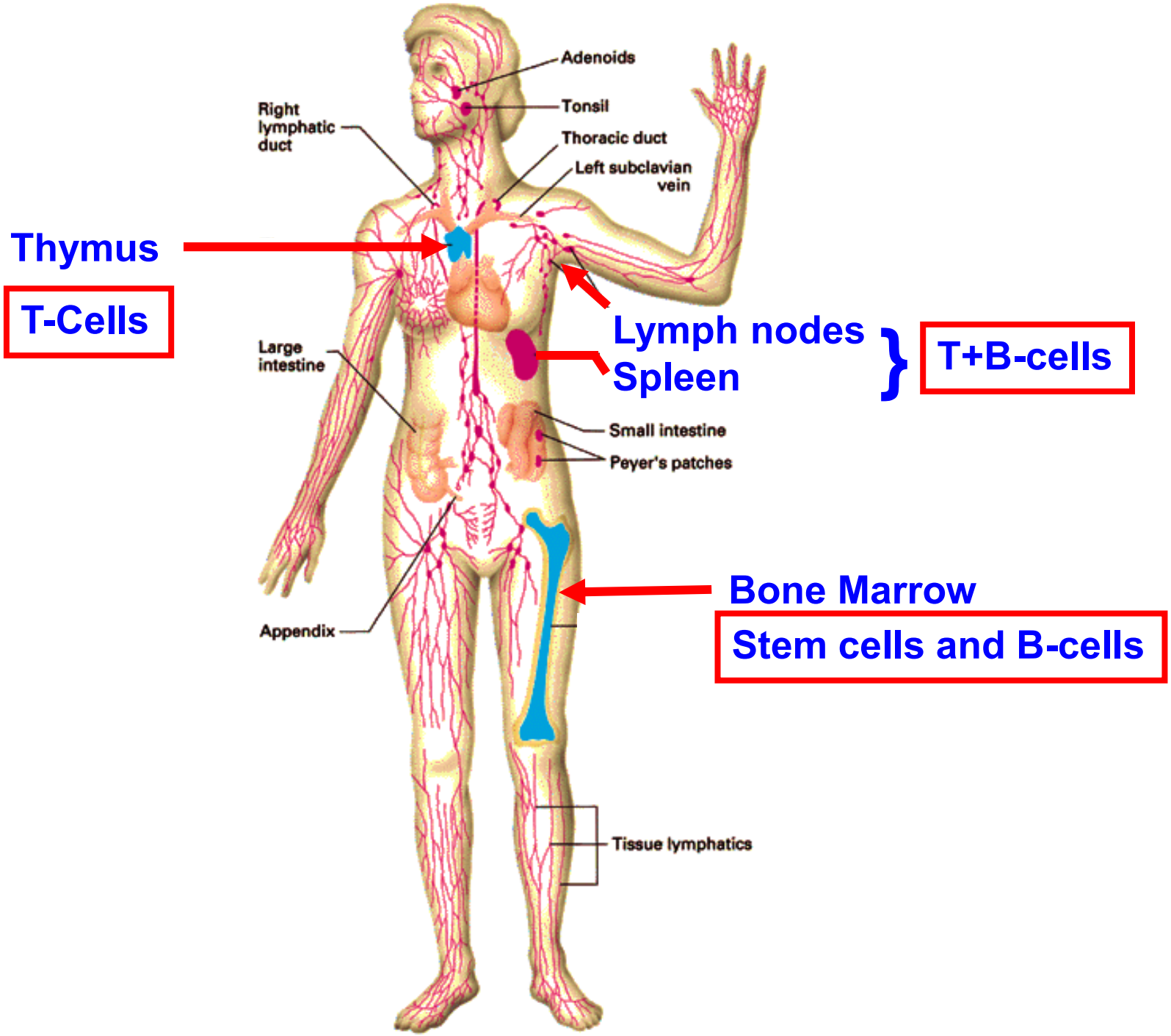
Class Lecture will be posted till 2/28 at:
http://utminers.utep.edu/raguilera/

email:raguilera@utep.edu

Lymphocytes and Antigen Receptors

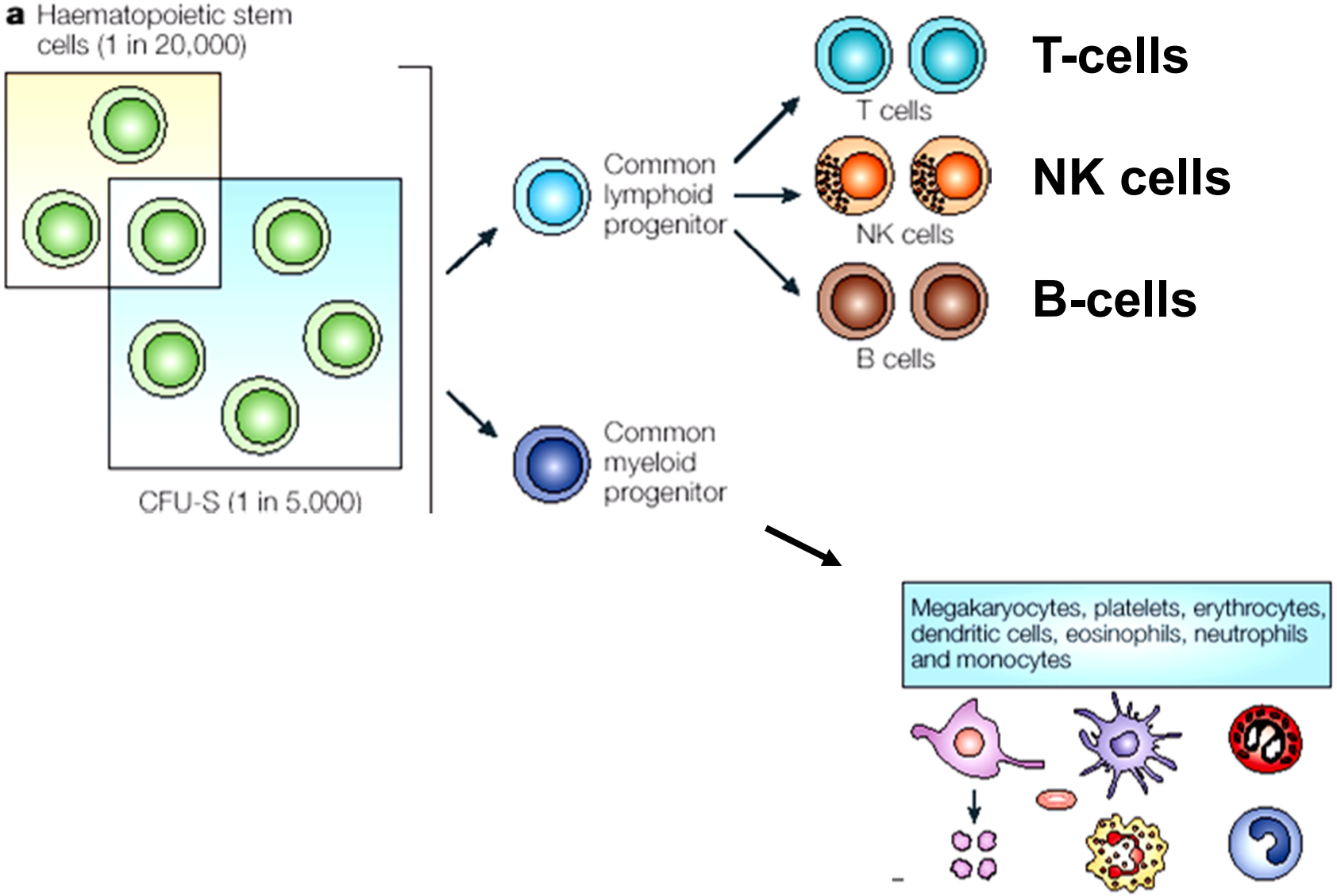

Adenoids
Tonsil
Right lymphatic duct
Thoracic duct
Left subclavian vein
Thymus
T-Cells
Lymph nodes
Spleen
T+B-cells
Large intestine
Small intestine
Peyer's patches
Bone Marrow
Stem cells and B-cells
Appendix
Tissue lymphatics

Nat. Rev. Immunol. | NOVEMBER 2004 | VOLUME 4

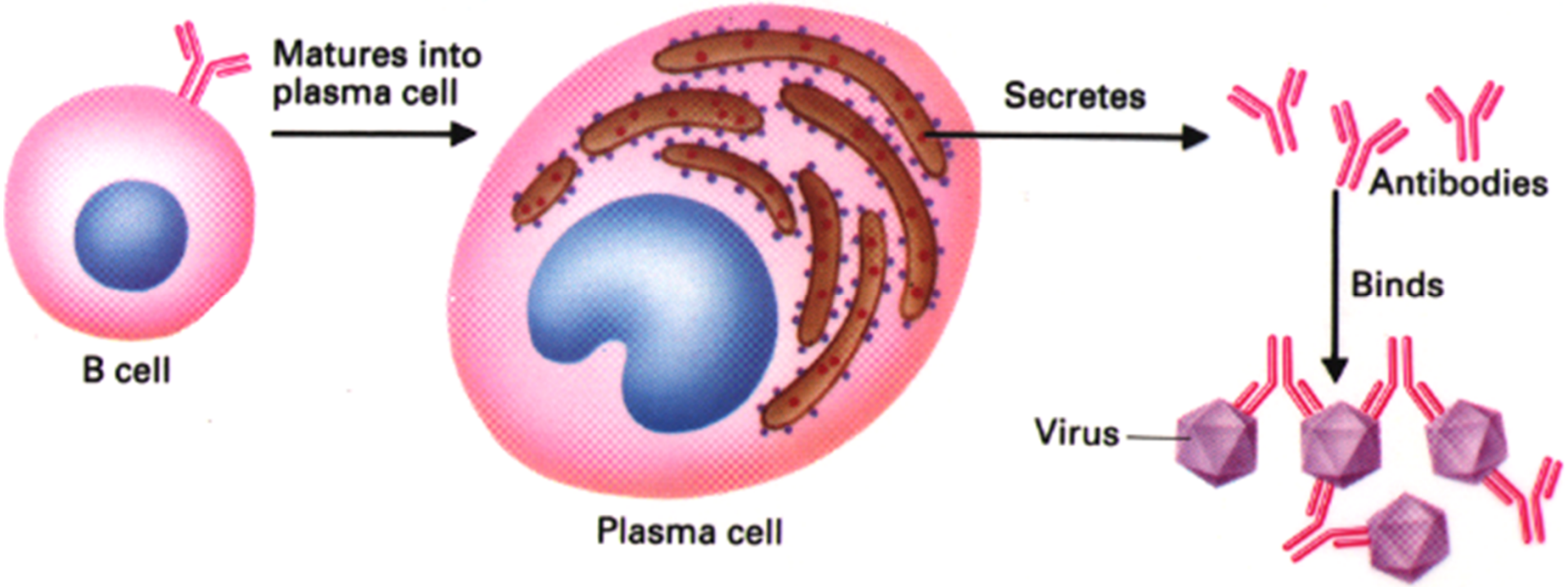
CLP

Committed Lymphocyte Precursor

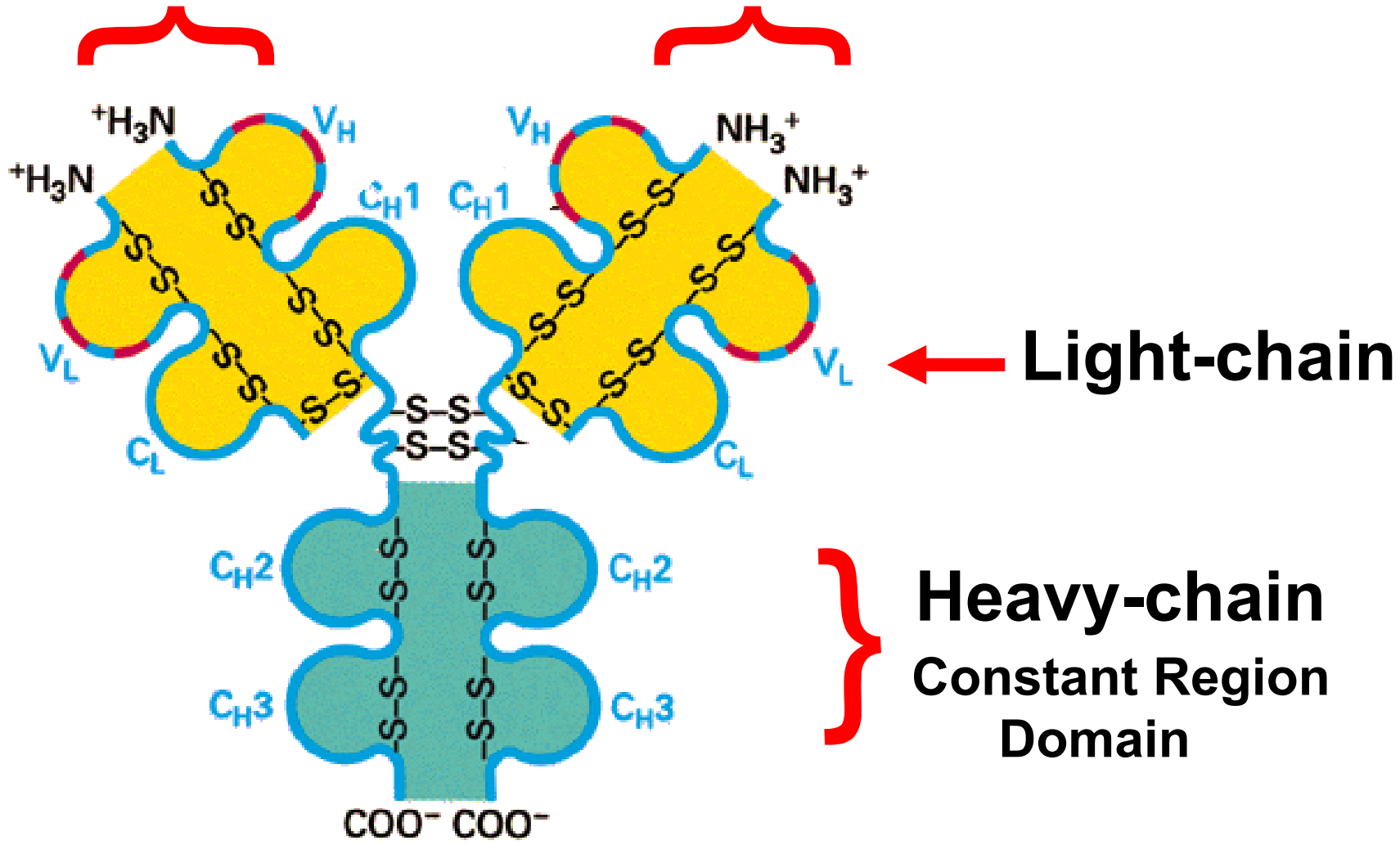
STEM CELL



B-lymphocytes produce antibodies



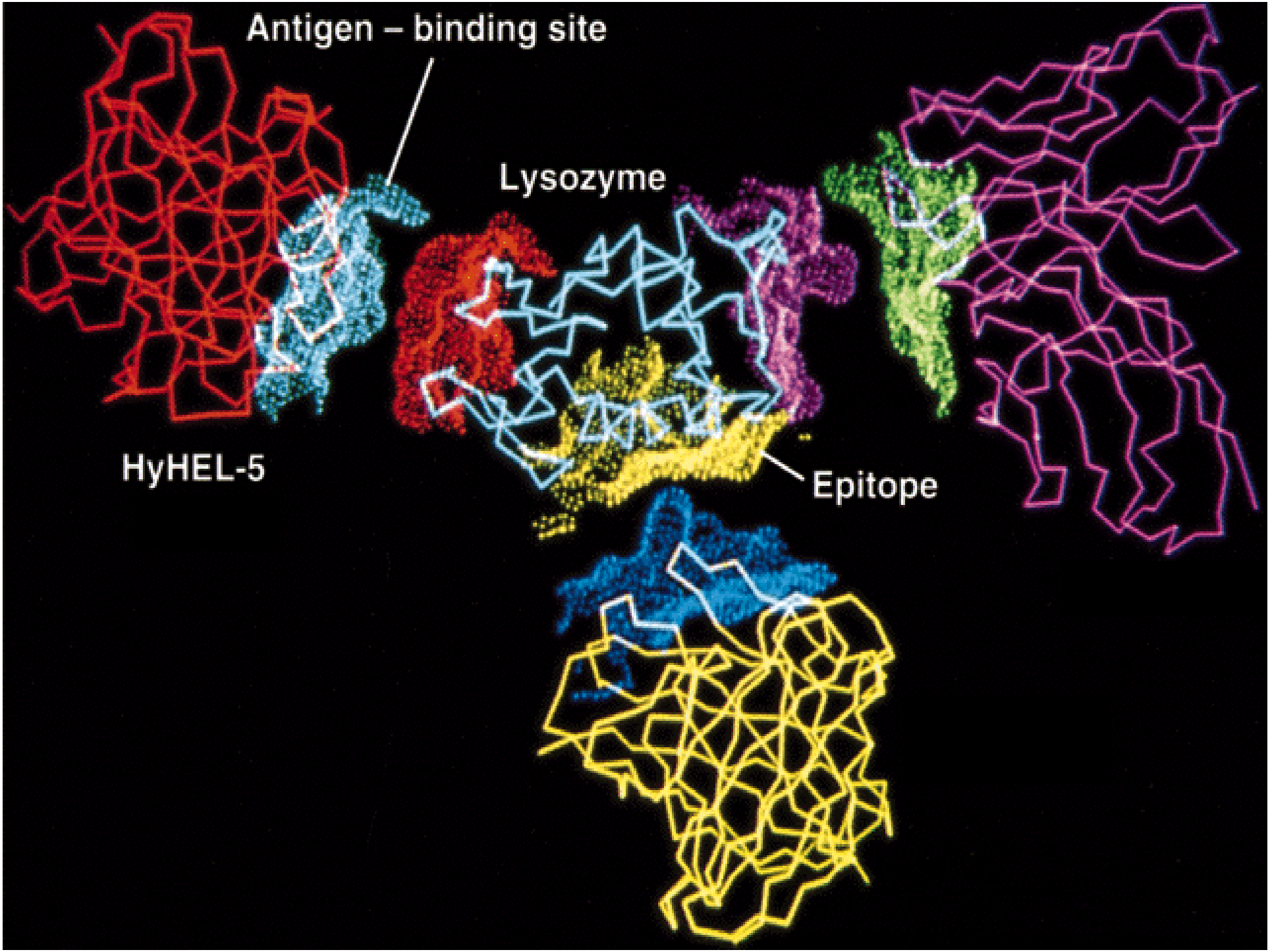
Immunoglobulin (Ig) Molecule



- **Theoretically, antibodies (Abs) can be produced to just about any foreign substance and are highly specific**

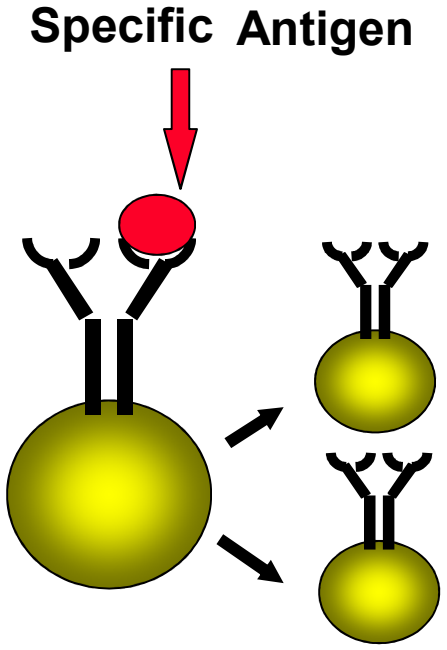
Ex.

An antibody can distinguish one protein from another by a single amino acid difference


Antigen – binding site
Lysozyme
HyHEL-5
Epitope

An individual cell expresses a specific receptor that recognizes a unique antigen-specificity determined prior to the presence of antigen

Binding of antigen to receptor induces proliferation with each daughter cell producing the same antibody specificity (to activating antigen)



Antibody genes could not follow 1 gene = 1 protein theory
Why?

To produce the billions of different antibodies necessary to combat disease, billions of antibody genes must have evolved to encode this information

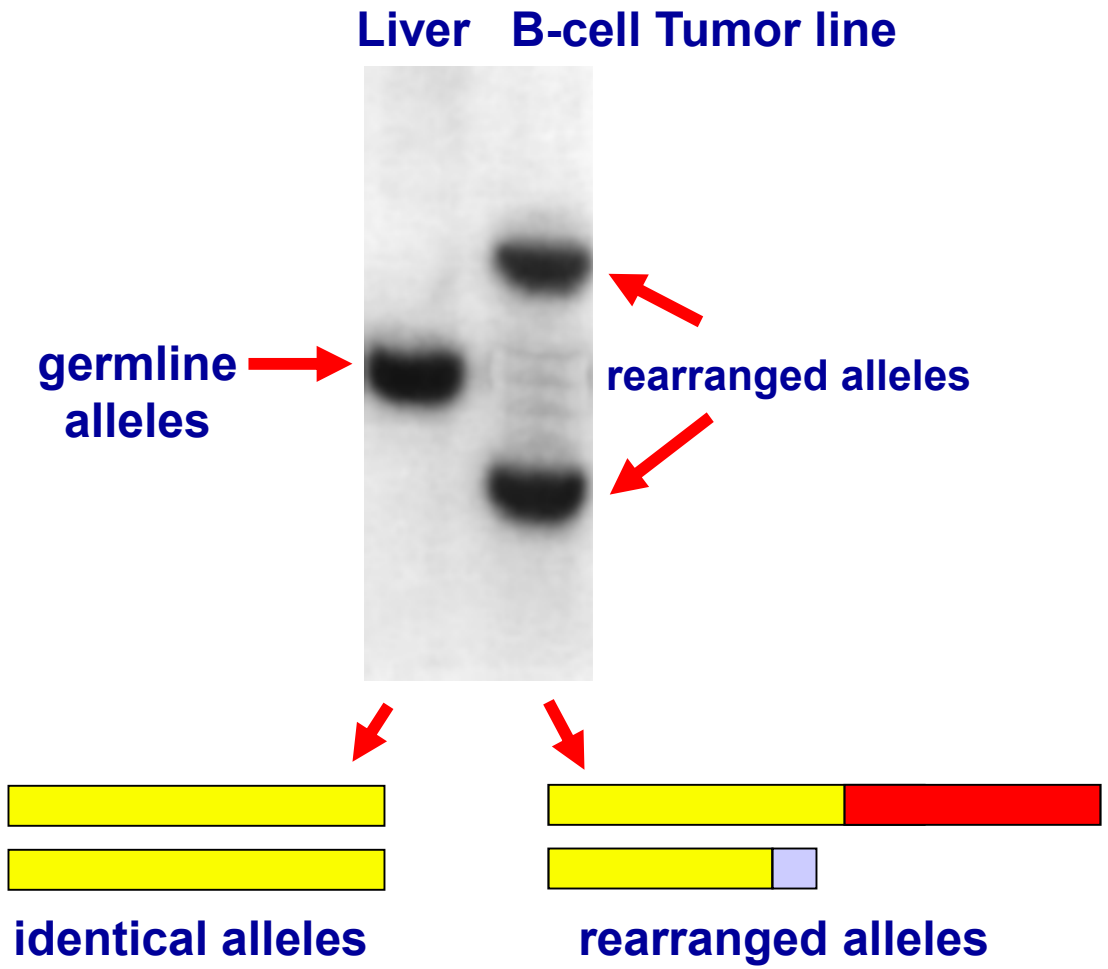
Since one gene encodes one protein (generally), this would mean that cells would need more genes than potentially encoded by genome

The answer to this problem resulted in a Nobel Prize

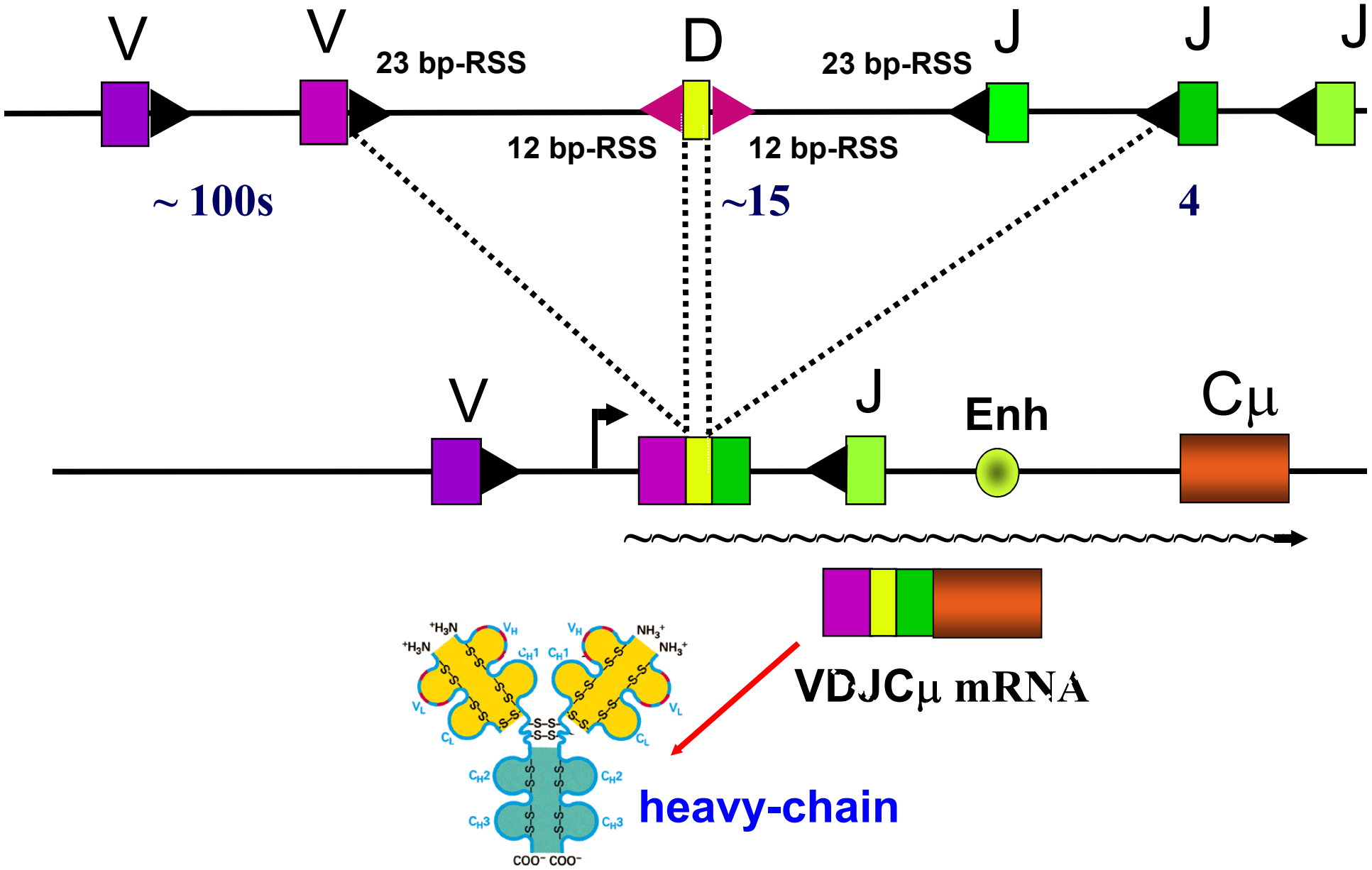
1987 Nobel Prize
Susumu Tonegawa

Using light-chain mRNA as probes was able to demonstrate that the variable region and the constant regions were “rearranged” in B-cell tumors

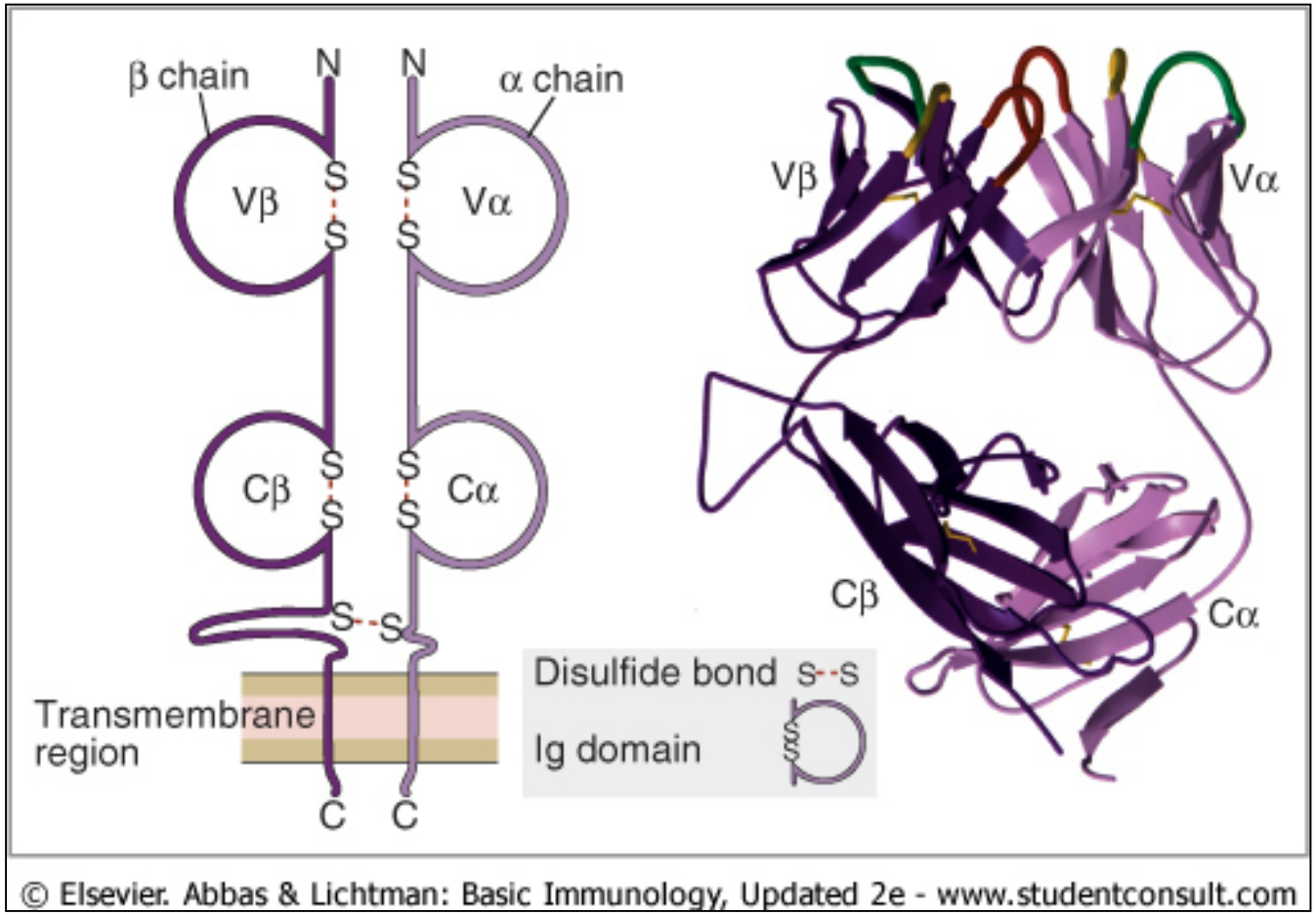
Southern Analysis of Immunoglobulin Gene Alleles



V-(D)-J Recombination

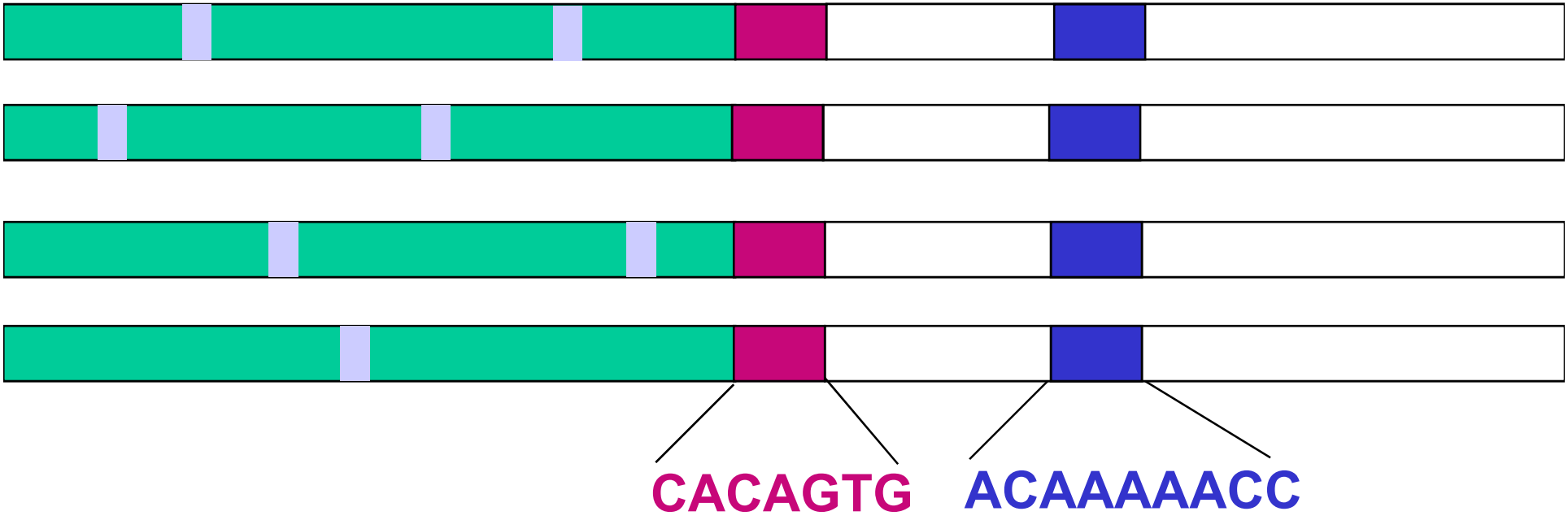


T-cell Antigen Receptors Resemble Antibody molecules

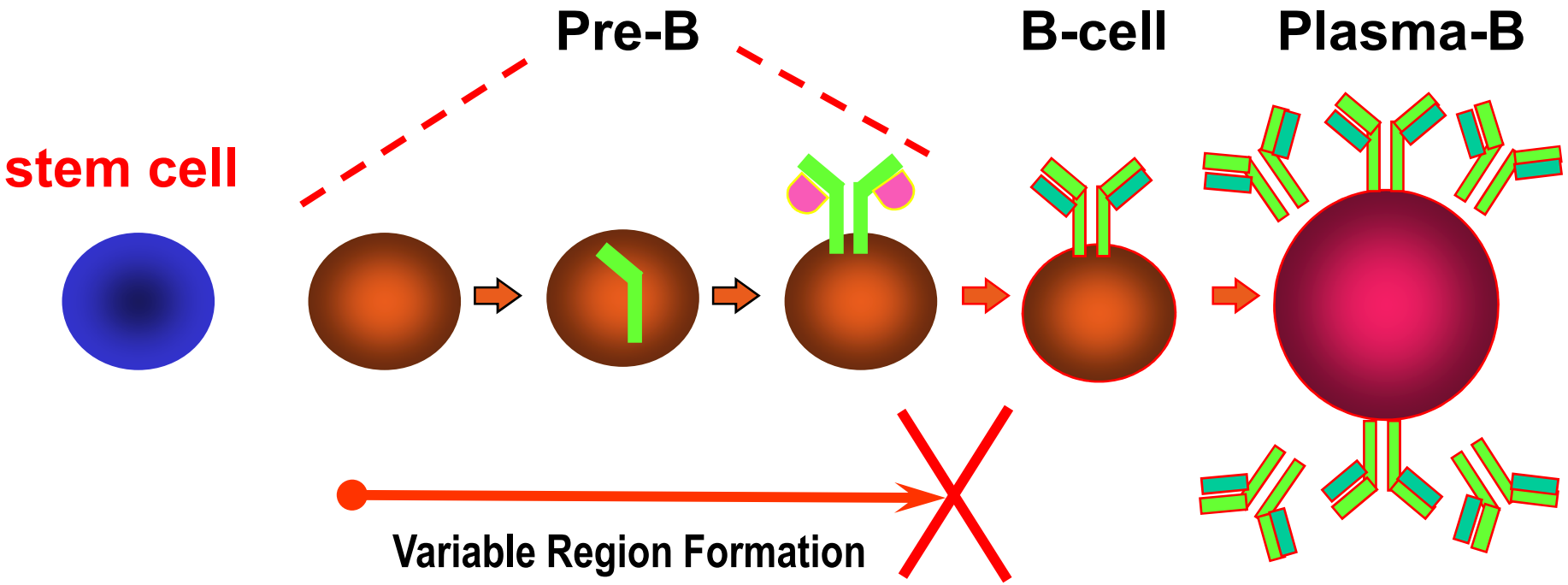


Specific sequences are recognized by recombinase enzymes

Joining-element coding region



Recombinase is expressed early during B-Lymphocyte differentiation



“Chance Favors a Prepared Mind”

Louis Pasteur

An improbable experiment leads to an "incredible" result

- A student performs a series of flawed experiment that leads to the discovery of the V-(D)-J recombinase
- The premise of the experiment was that a single recombinase gene was responsible for V-(D)-J joining
- Reasoned that a recombinase gene could be transferred from lymphocyte DNA to a cell that does not contain this activity such as fibroblasts

This experiment should never have worked!

Why?

Lymphocyte-specific genes should not be expressed in non-lymphoid cells - also unreasonable to believe that one gene product could do everything

David Schatz, 2001

Original *RAG-1* Germline Clone Contained Another Gene

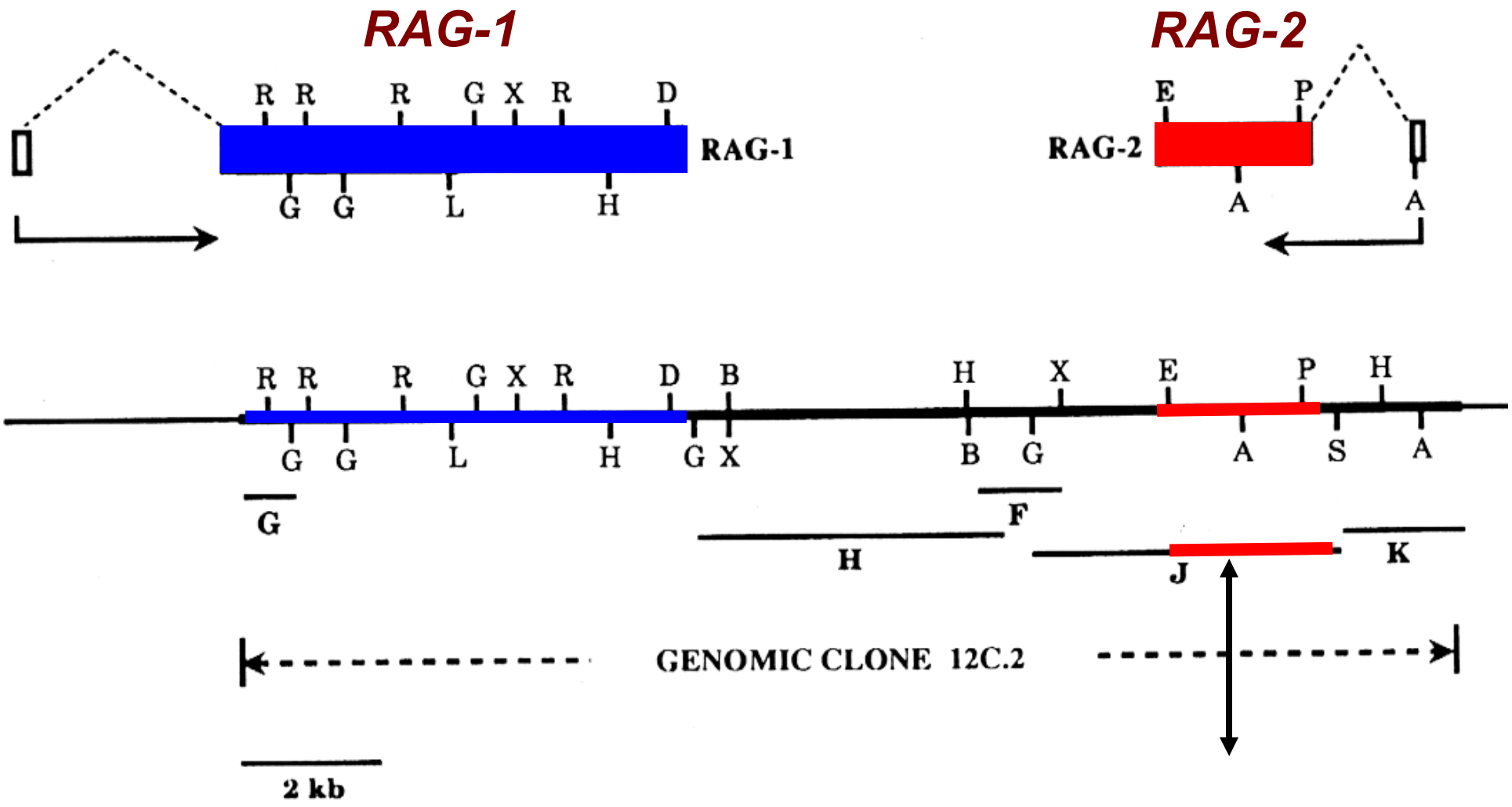
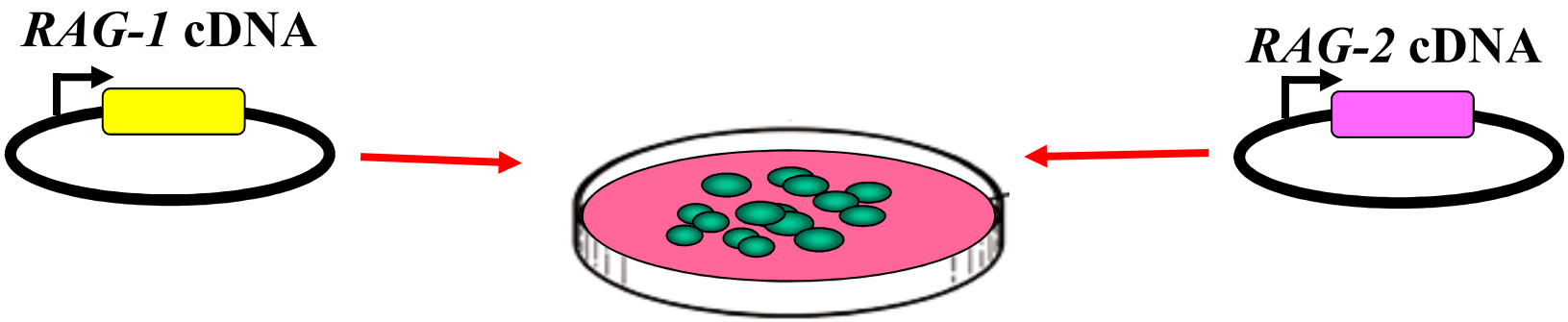


Fig.1 Oettinger, *et al.*, 1990

Eventually cloned two genes, RAG-1 and RAG-2 that do it all



Fibroblasts
(no recombination)

High level recombination

RAG $^{-/-}$ "knockout" Mutant Mice

- Normal birth and growth but immunodeficient
- Have no Mature B/T Lymphocytes
- No V-(D)-J recombination

Characterization of the Recombination Mechanism *in vitro*

van Gent, D.C. *et al*.,. (1995). Initiation of V(D)J recombination in a cell-free system. Cell, 81:925-934

Martin Gellert's Laboratory at NIH makes initial and consistent discoveries which lead to the establishment of *in vitro* rec. systems

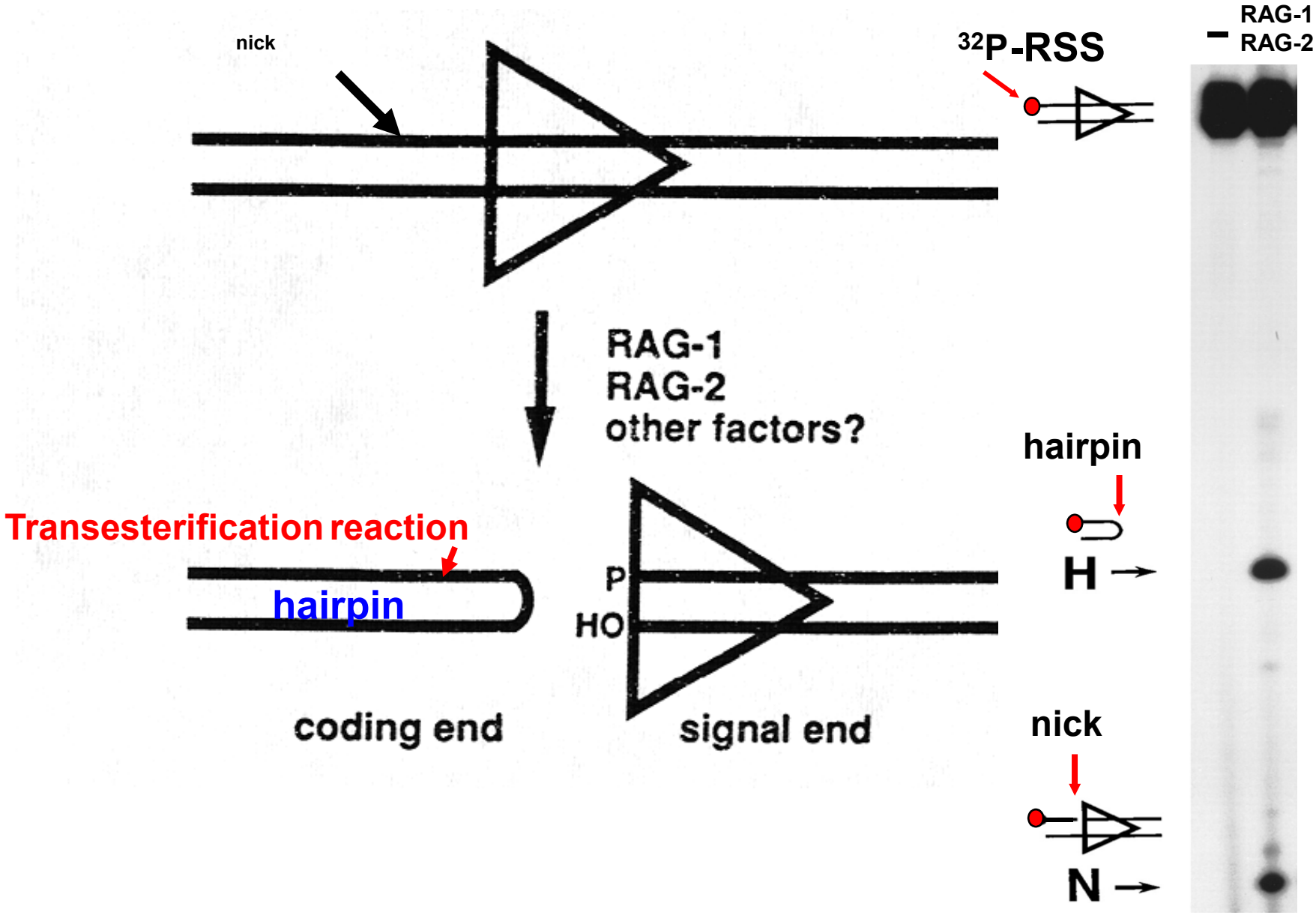
Other important findings:

- **RAG mediated cleavage requires intact RSS heptamer and nonamer sequences**

- **Cleavage products were identical to those detected *in vivo*- blunt 5' phosphorylated signal-ends and coding-ends contain hairpins (closed covalently)**

- **Recombinant Rag-1 and Rag-2 proteins plus house-keeping proteins are sufficient to mediate recombination *in vitro***

RAG Mediated Cleavage



- **Recombinant Rag-1 and Rag-2 were subsequently shown to mediate all the cleavage steps *in vitro***

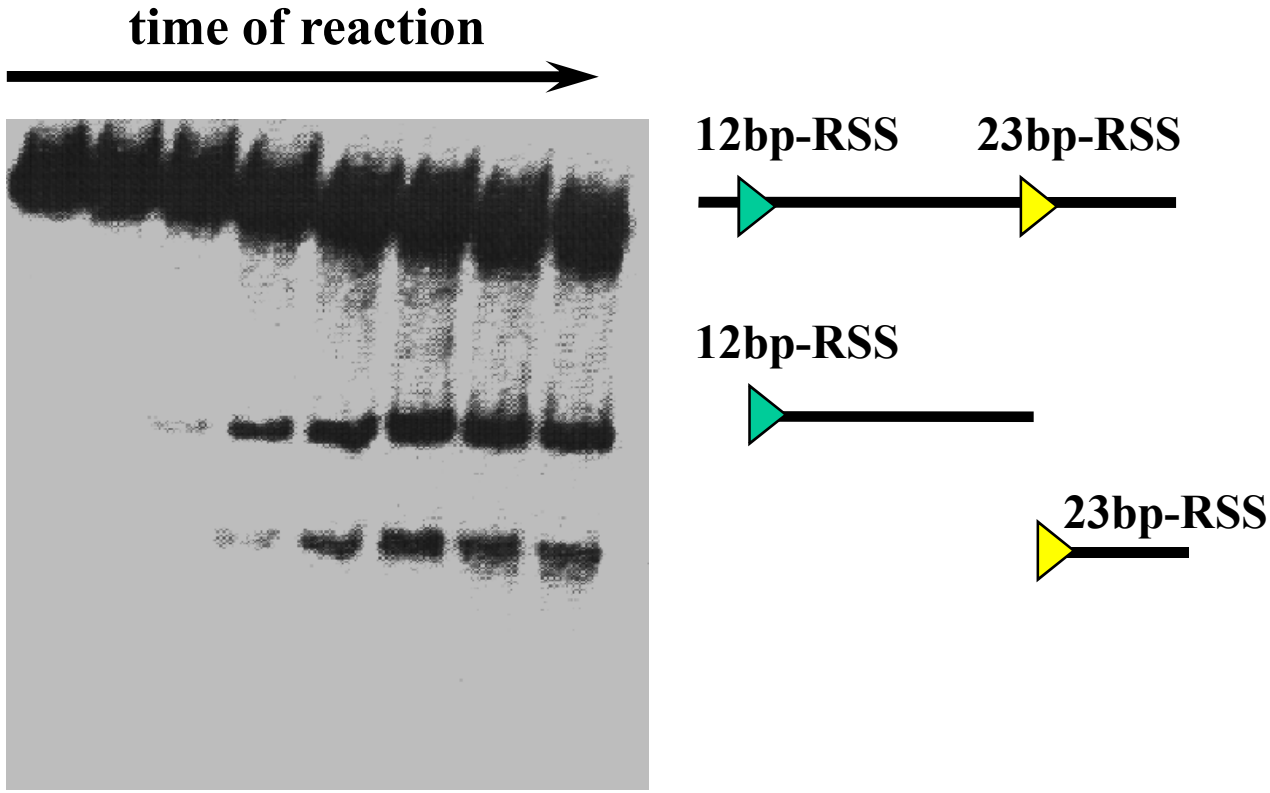
- **Rag-1 and Rag-2 forms a large stable cleavage complex that requires an intact heptamer and nonamer**

These results lead to the following question:

Can the *in vitro* system perform all the steps seen *in vivo*?

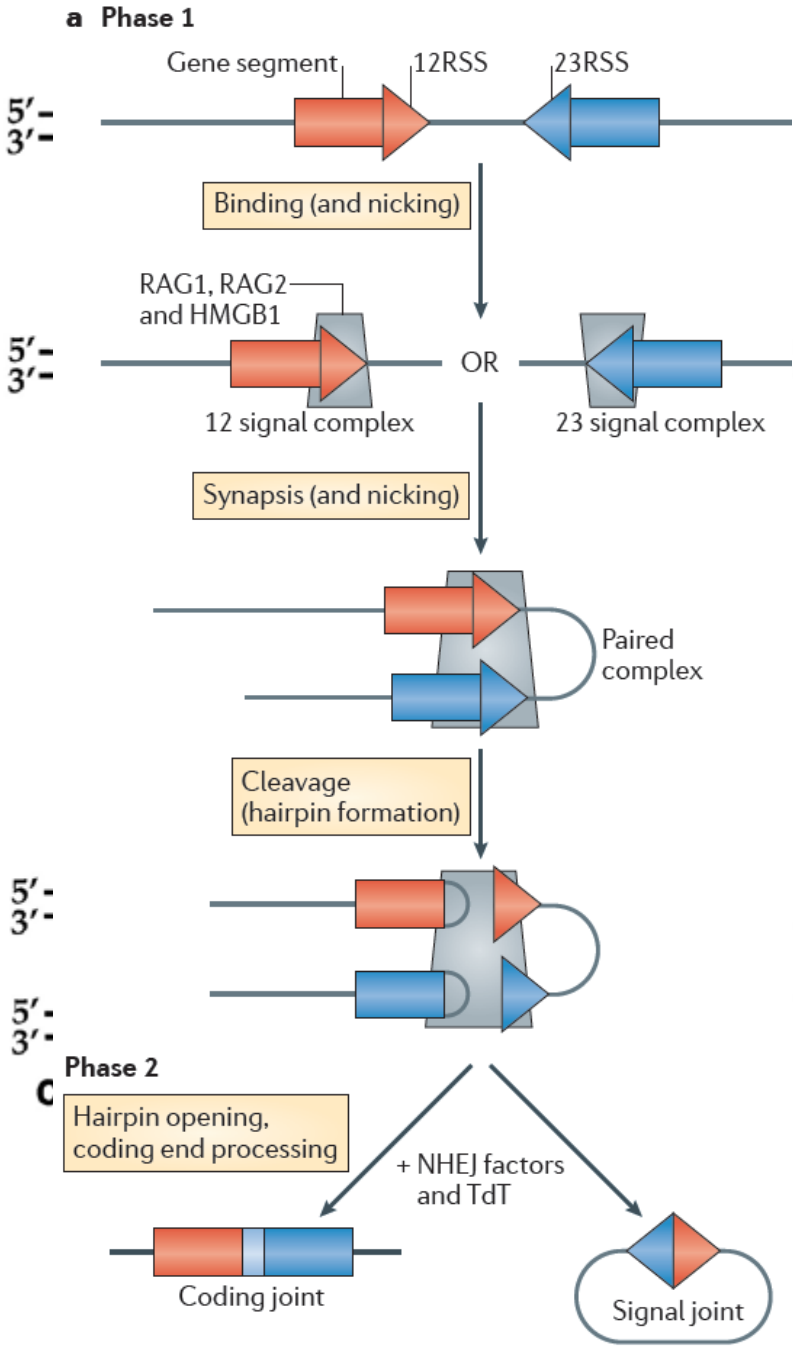
Initiation of V(D)J recombination *in vitro* obeying the 12/23 rule

Eastman, Q.E., Leu, T.M, and Schatz, D. G. Nature, **380**:85-88, 1996



For efficient cleavage, needed RAG-1+RAG-2 + Nuclear Extract (additional factors)

Simultaneous Cleavage at both RSSs



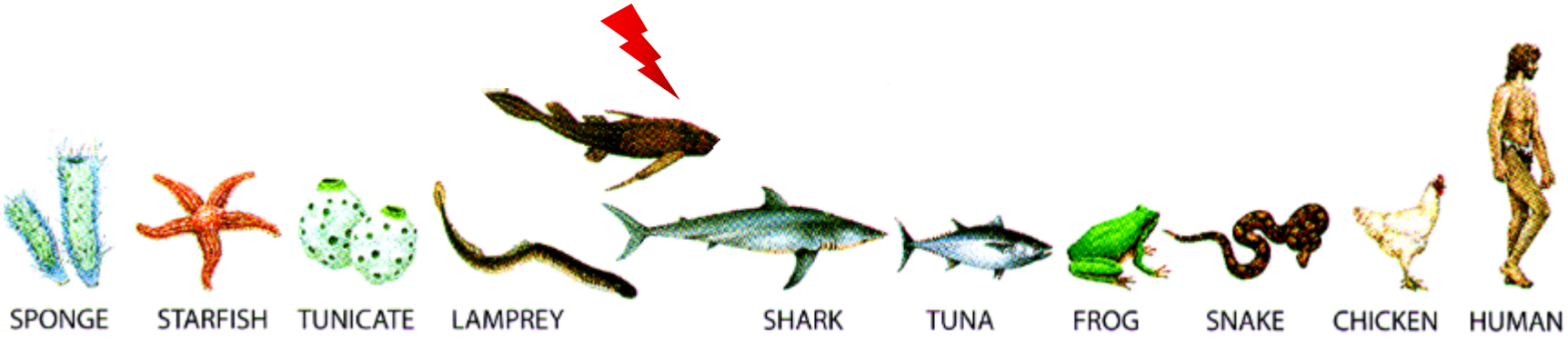
synapsis

Housekeeping enzymes ligate and repair breaks

Why should you care about recombination?

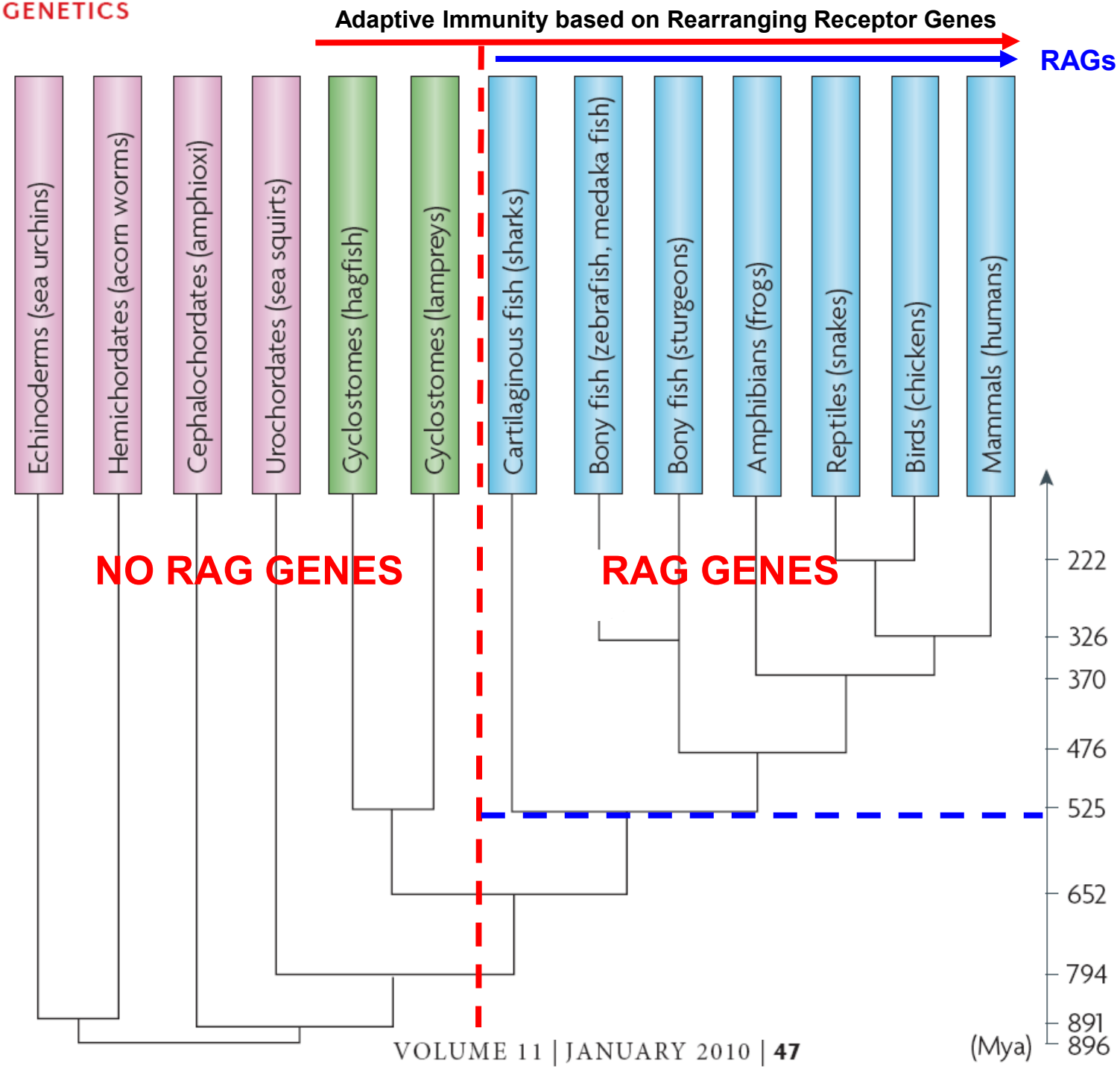
No recombination
No advanced Immune System
No life as we know it

Big-Bang Theory of Immunology



Immune System Cells (B-/T-lymphocytes)

450 x 10^6 years ago


Adaptive Immunity based on Rearranging Receptor Genes
RAGs
Echinoderms (sea urchins)
Hemichordates (acorn worms)
Cephalochordates (amphioxi)
Urochordates (sea squirts)
Cyclostomes (hagfish)
Cyclostomes (lampreys)
Cartilaginous fish (sharks)
Bony fish (zebrafish, medaka fish)
Bony fish (sturgeons)
Amphibians (frogs)
Reptiles (snakes)
Birds (chickens)
Mammals (humans)
NO RAG GENES
RAG GENES
222
326
370
476
525
652
794
891
896
(Mya)

So where did RAG genes come from?

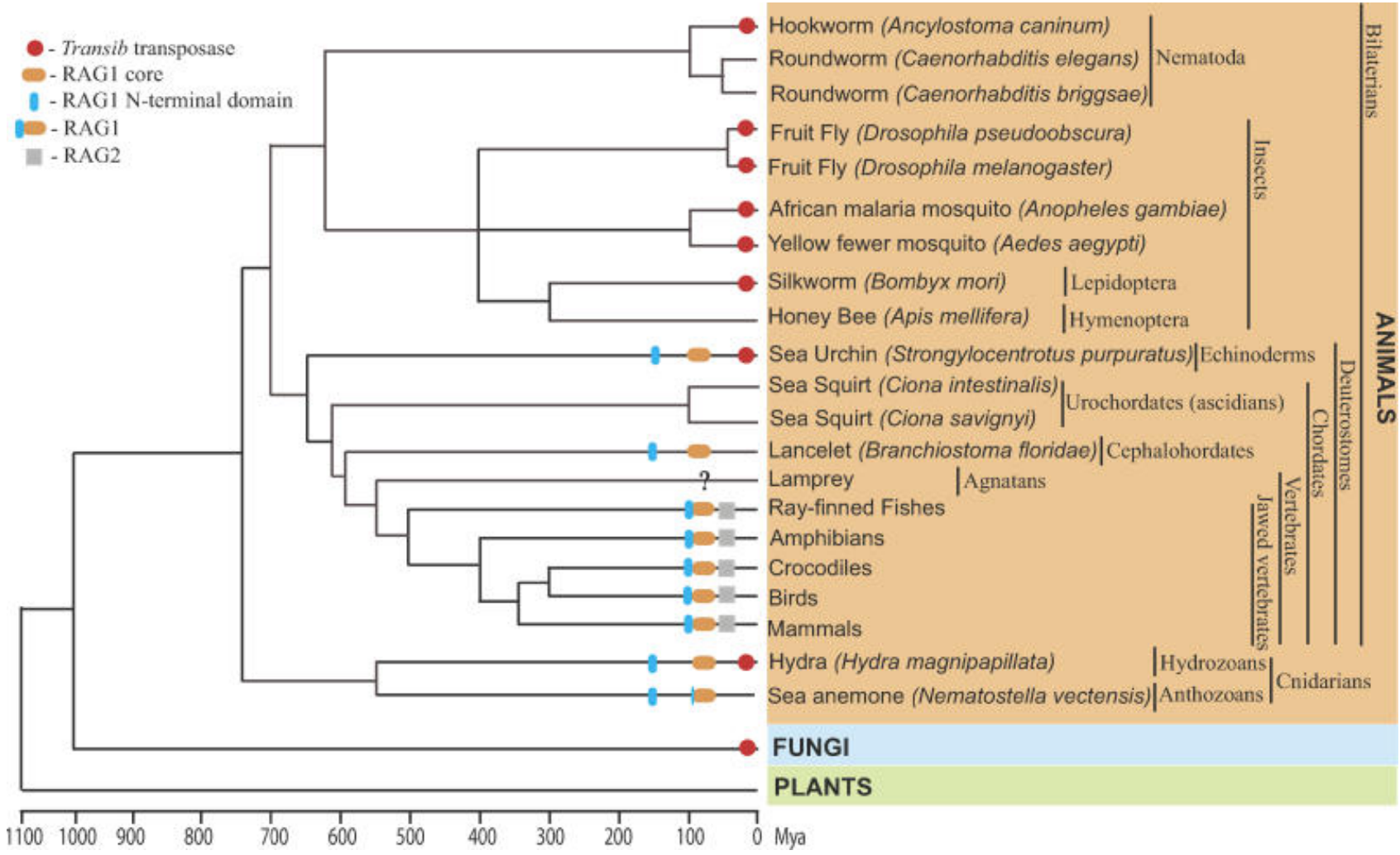
Available online at www.sciencedirect.com
ScienceDirect
Biochemical and Biophysical Research Communications 369 (2008) 818–823
BBRC
www.elsevier.com/locate/ybbrc

Molluscan mobile elements similar to the vertebrate Recombination-Activating Genes

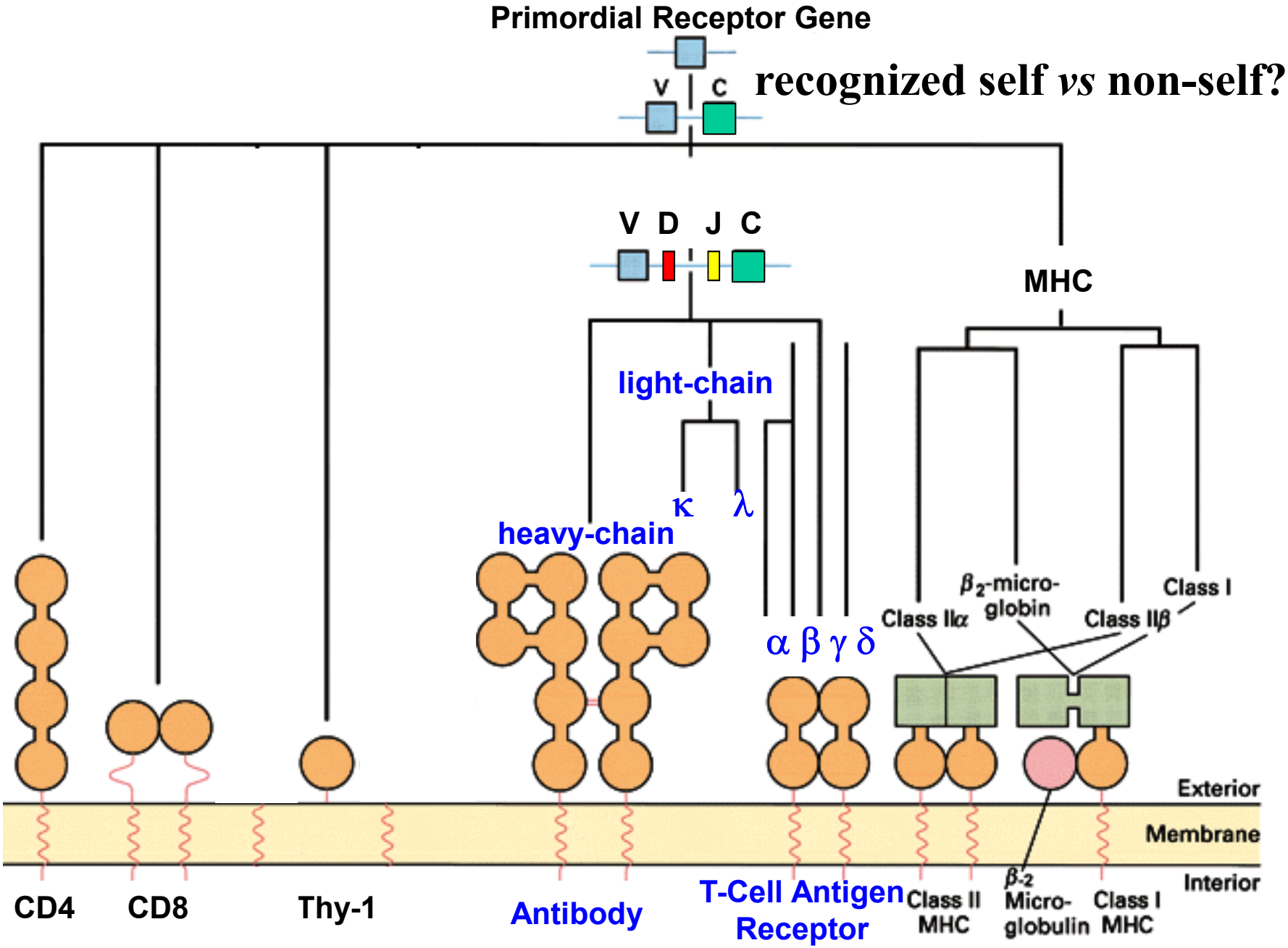
Yuri Panchin [a], Leonid L. Moroz [b,c,*]

RAG1 Core and V(D)J Recombination Signal Sequences Were Derived from *Transib* Transposons

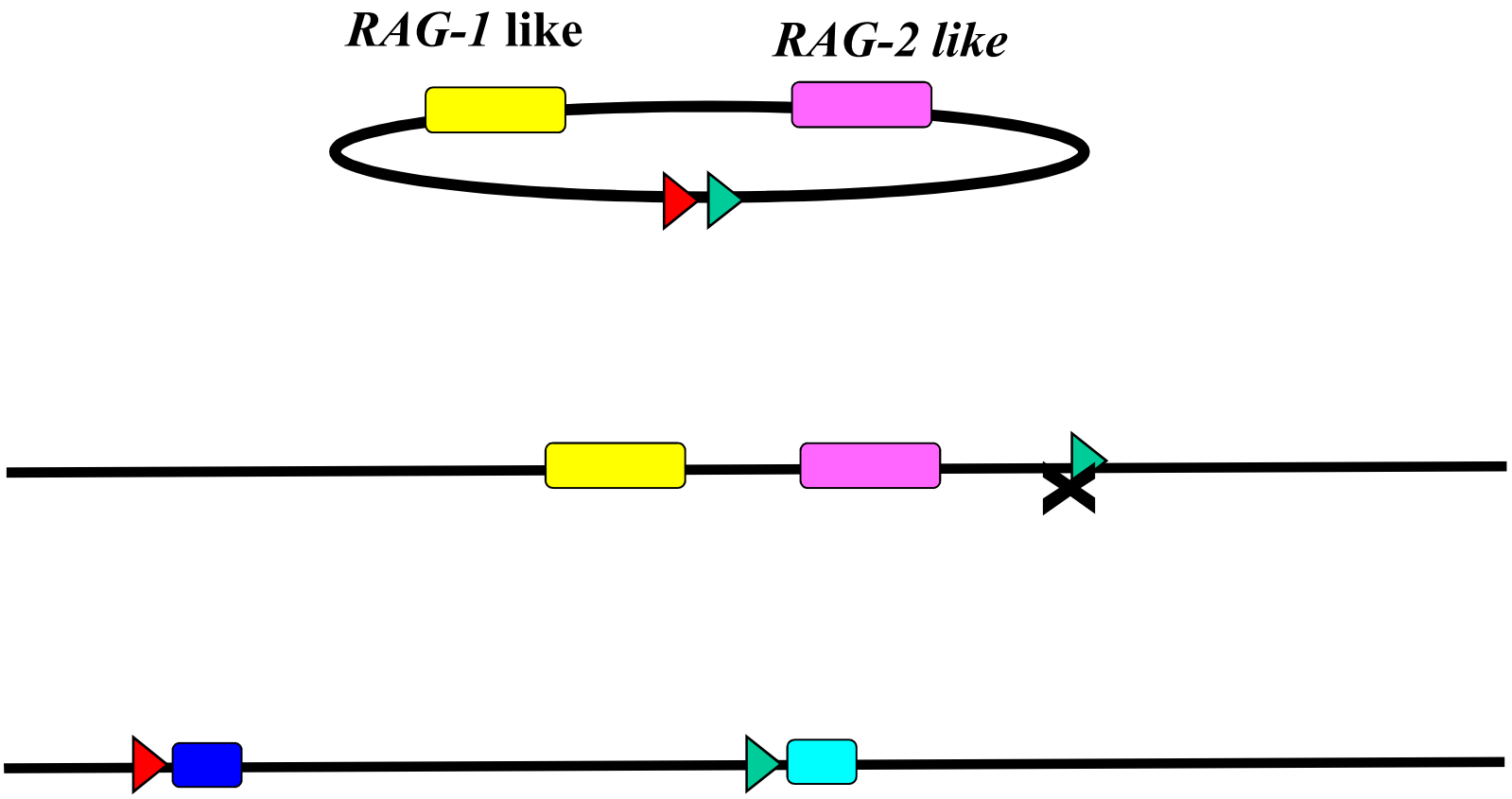
Vladimir V. Kapitonov*, Jerzy Jurka*



Immunoglobulin gene family

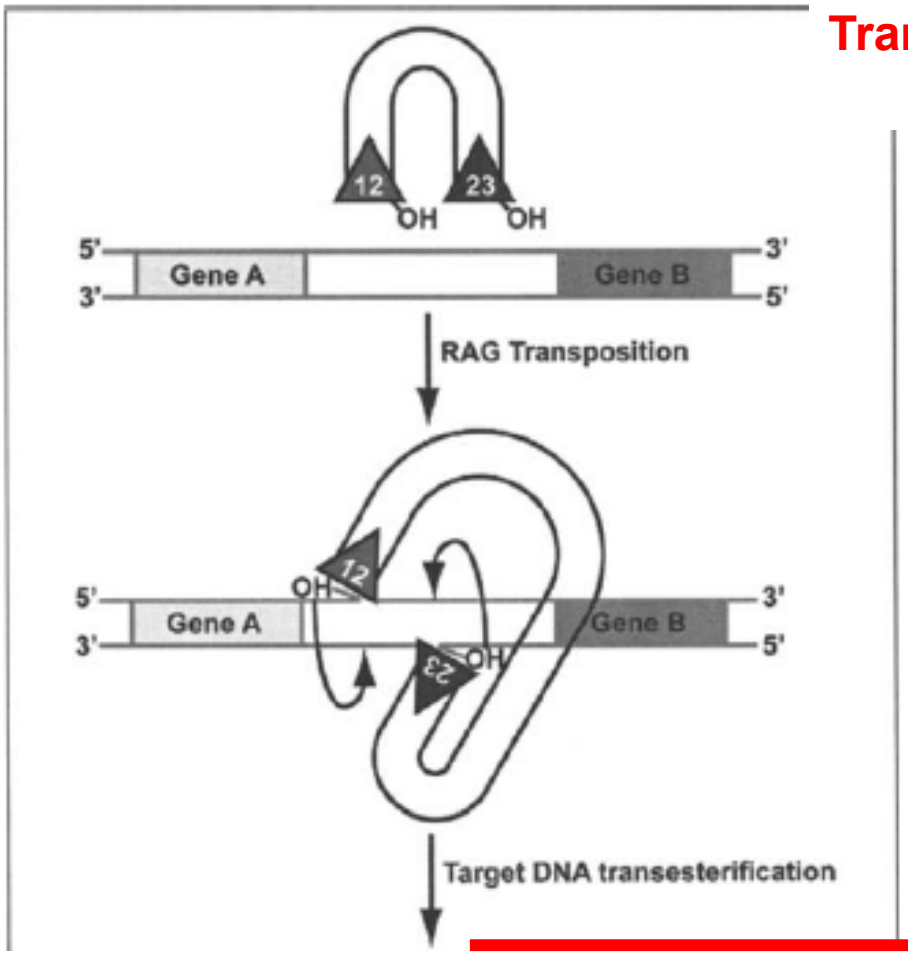


Acquisition of RAGs and Recombination Signals by Ancient Organism



Ancient Immunoglobulin-like genes

Translocations mediated by RAG mediated "transposon-like" activity



Adam G.W. Matthews and Marjorie A. Oettinger

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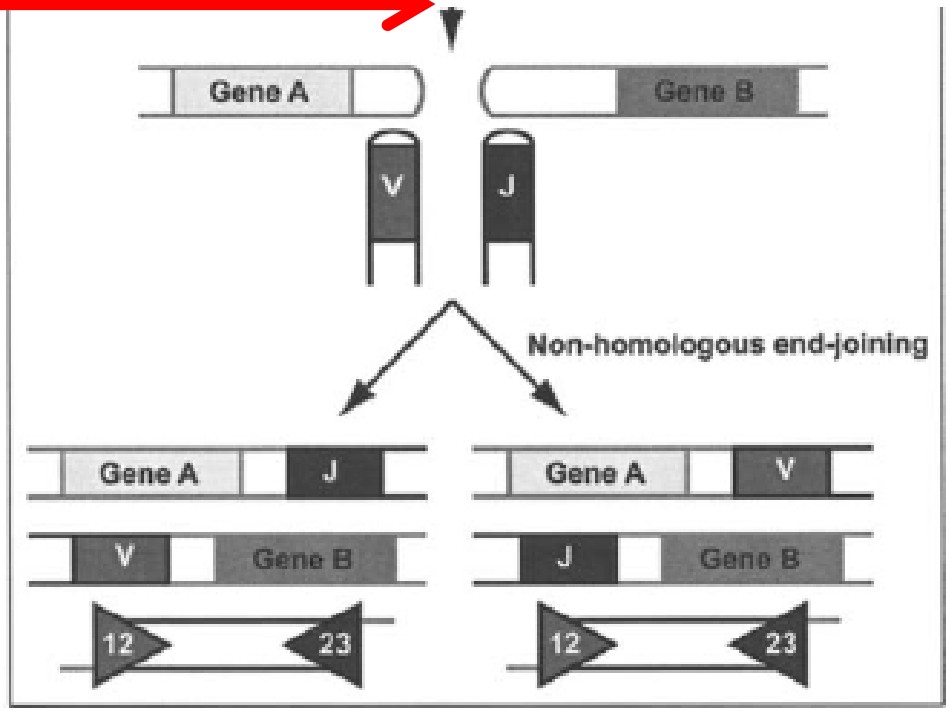
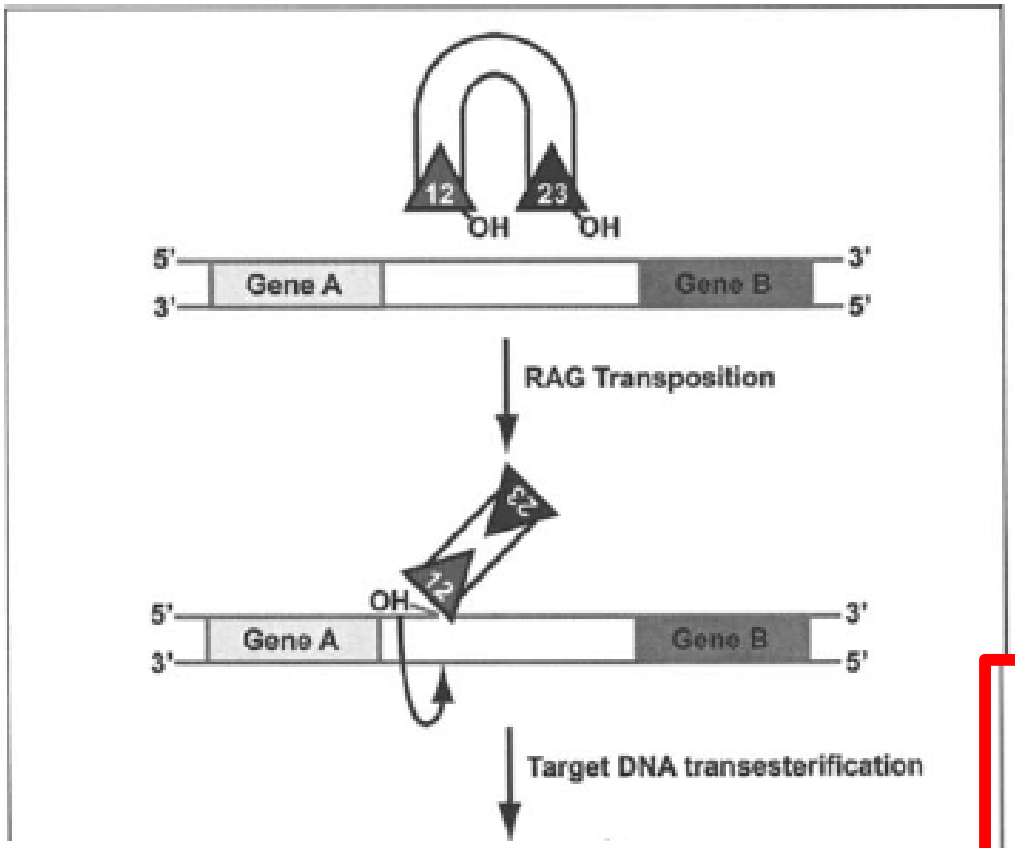
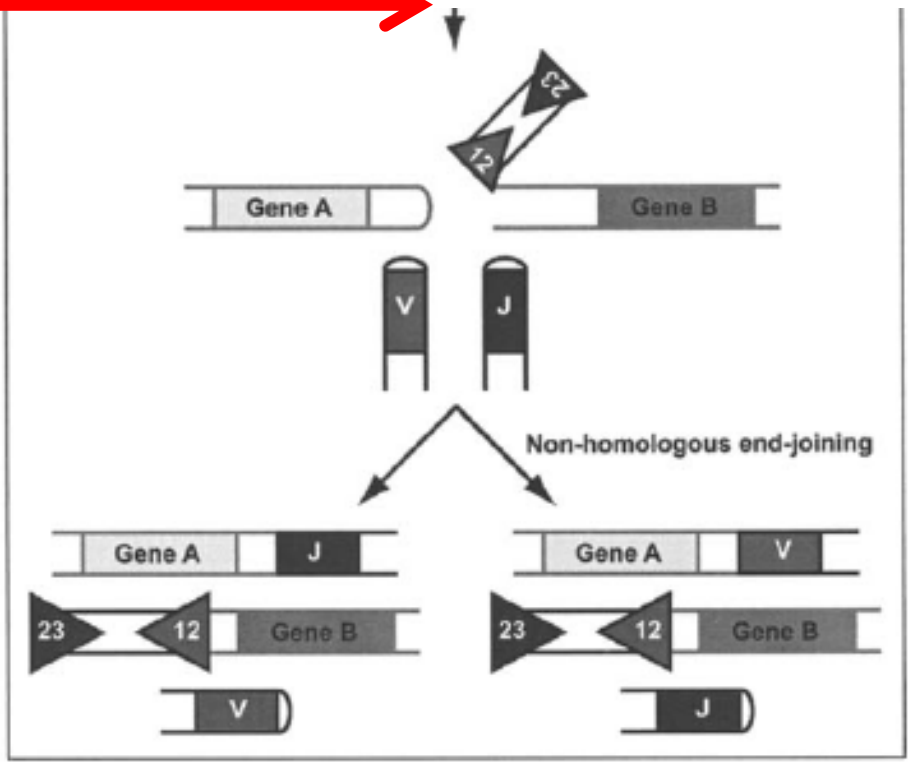




An alternative type of "transposition"



14;18 Translocation is the most common rearrangement in Human Lymphomas (all Follicular Lymphomas)

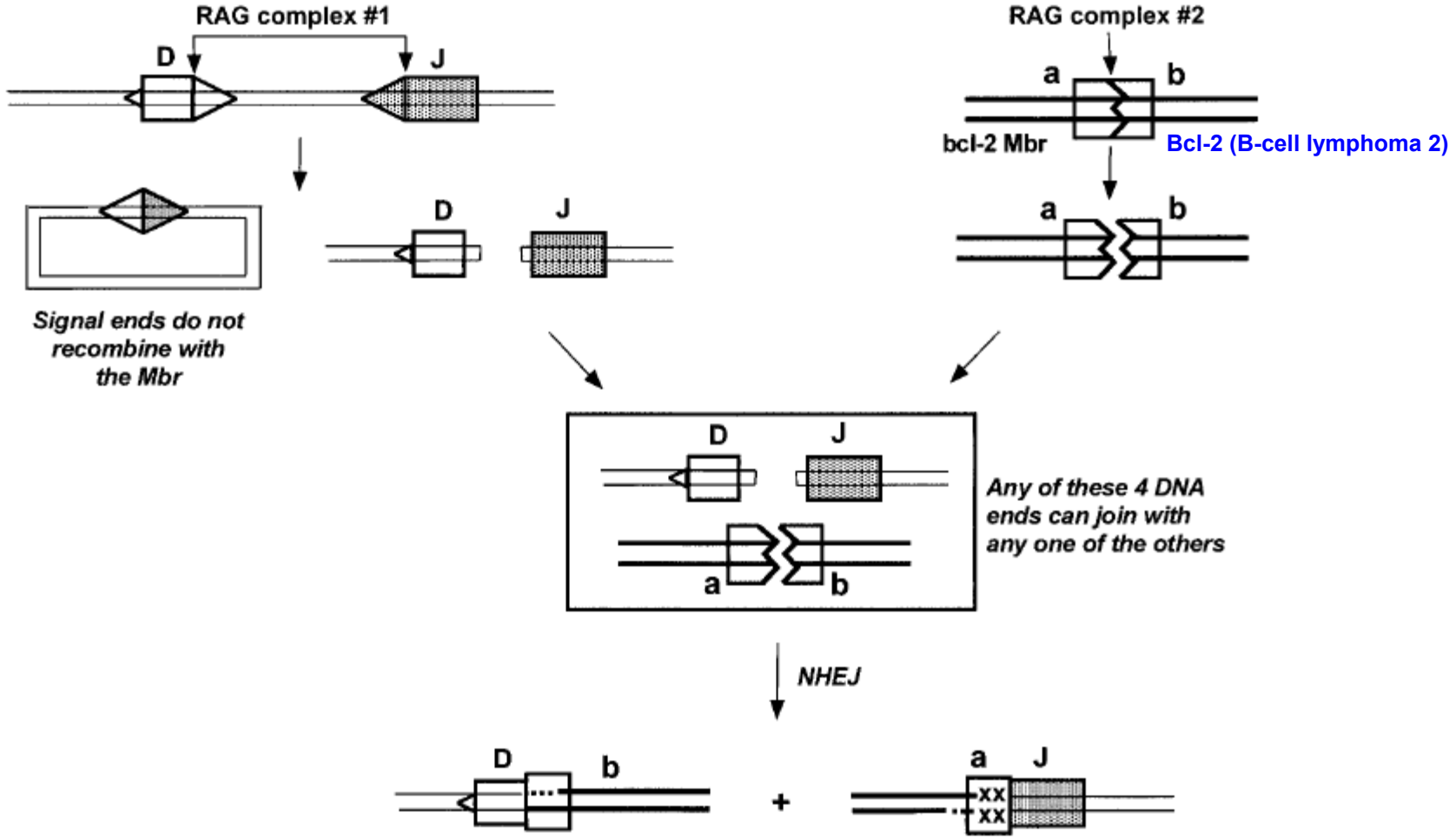


FIG. 5. Biochemical model for the mechanism of the t(14;18) chromosomal translocation. The aspects of the mechanism depicted by the two question marks in Fig. 1 are significantly clarified by this study. Our previous work indicated that the RAG cleavage at the Mbr (denoted by "RAG complex #2" and downward arrows) is independent of the RAG cleavage at the paired 12/23-signal pair (denoted by "RAG complex #1"), and the work here has confirmed and extended this point (30). Because the signal ends do not recombine with the Mbr, they may already be joined; alternatively, they may still be bound by the RAG complex that cleaved them (1). In either case, they are unavailable to recombine with the Mbr. This reduces the translocation intermediate step to a four-DNA-end problem rather than a six-DNA-end problem. NHEJ is the pathway for rejoining the ends, based on the new findings from the present study, which are shown in italics.

Human Severe Combined Immunodeficiency

Clinical Immunology (2010) 135, 183–192

available at www.sciencedirect.com

Clinical Immunology

www.elsevier.com/locate/yclim

REVIEW

More than just SCID—The phenotypic range of combined immunodeficiencies associated with mutations in the recombinase activating genes (RAG) 1 and 2

Tim Niehues [a,*], Ruy Perez-Becker [a], Catharina Schuetz [b]

[a] *HELIOS Klinikum Krefeld, Center for Child and Adolescent Health; Krefeld, Germany*
[b] *Department of Pediatrics and Adolescent Medicine, University Hospital Ulm, Germany*

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Severe Combined Immune deficiencies in Humans due to V-(D)-J recombination deficiencies

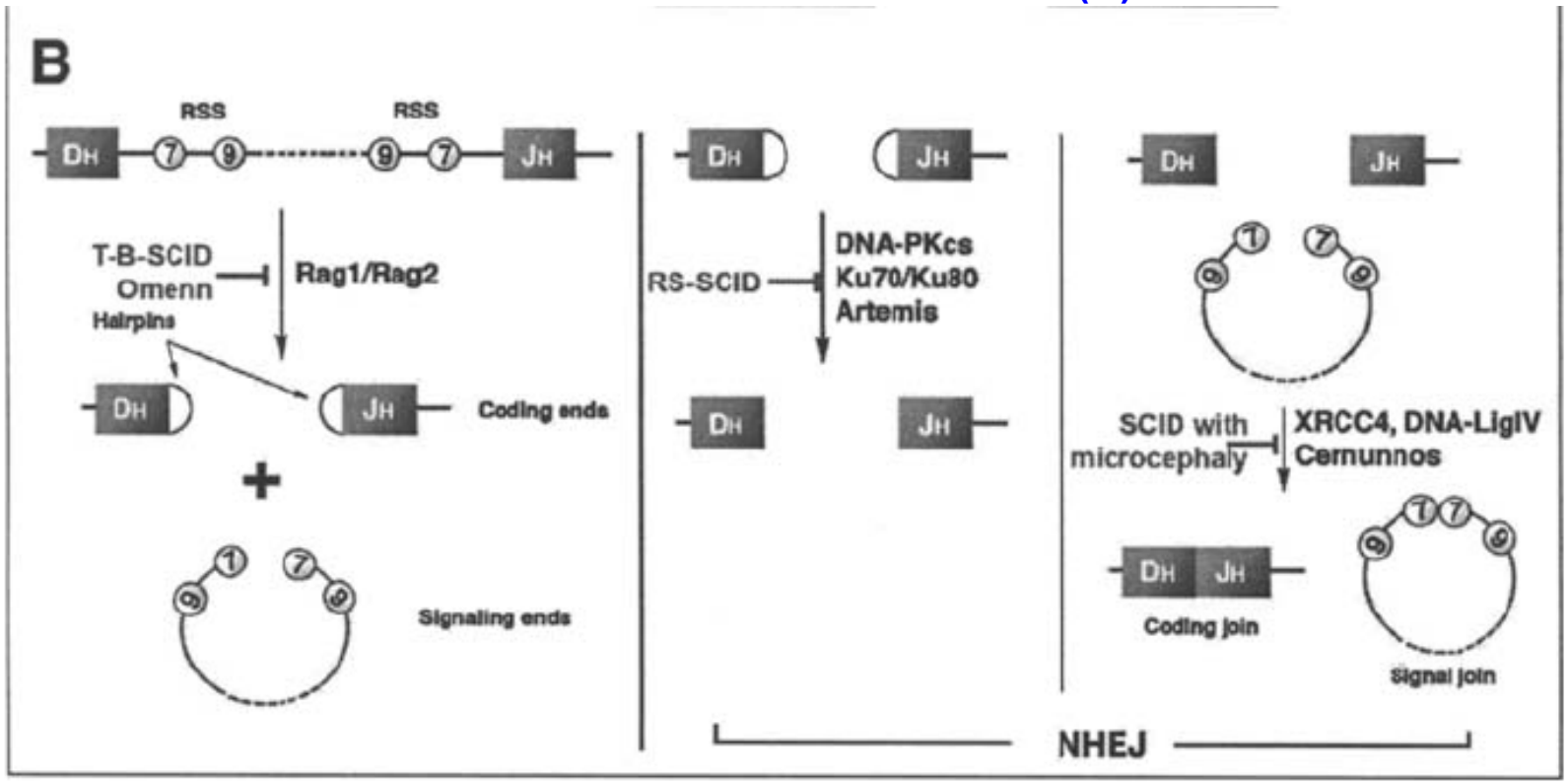

Figure 1. V(D)J recombination. A) Organization of IgH locus and rearrangement process. B) The V(D)J recombination reaction and faulty steps in SCIDs.

Table 1. Gene defects in T-B- SCID and RS SCIDs

Gene	Mutation	Radiosensitivity	Immune Defect	Growth Delay	Microcephaly	Cancer Predisposition	Ref
Rag1, Rag2	Null	No	T-B-SCID	No	No	No	11
	Hypomorphic	No	Omenn	No	No	No	15
Artemis	Null	Yes	RS-SCID	No	No	?	45
	Hypomorphic	Yes	RS-SCID	No	No	Yes	107
	Hypomorphic	Yes	Omenn	No	No	No	17
DNA-LigIV	Hypomorphic	Yes	μ-SCID	Yes	Variable	Yes	77
Cernunnos	Hypomorphic?	Yes	μ-SCID	Yes	Yes	?	82