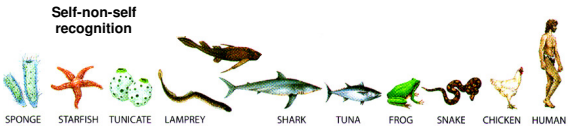
Lecture 1

Lymphocytes and Antigen Receptors

posted at http://utminers.utep.edu/raguilera

All organisms have some sort of immune system

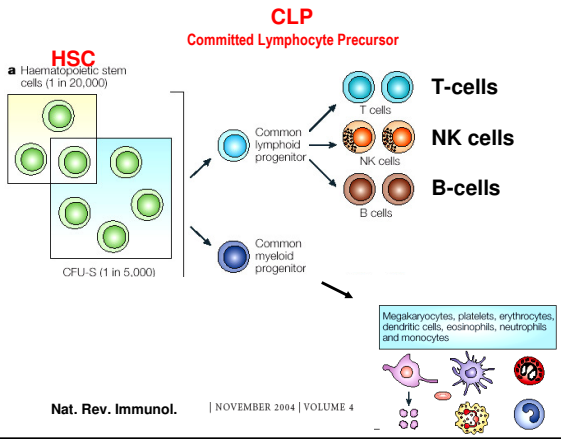


B/T Lymphocytes

450 million years ago

Insects also have a sophisticated native immune system composed of specialized cells and hundreds of antimicrobial response peptides/proteins

All lymphocytes arise from a common precursor



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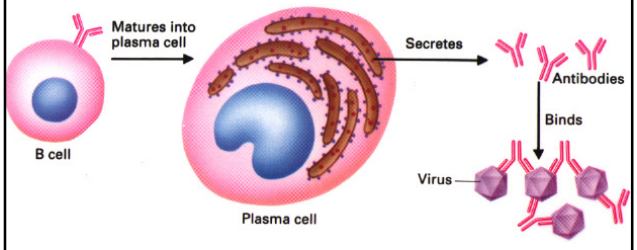
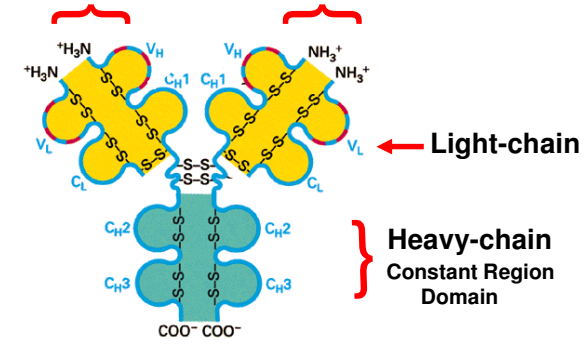
B-lymphocytes produce antibodies



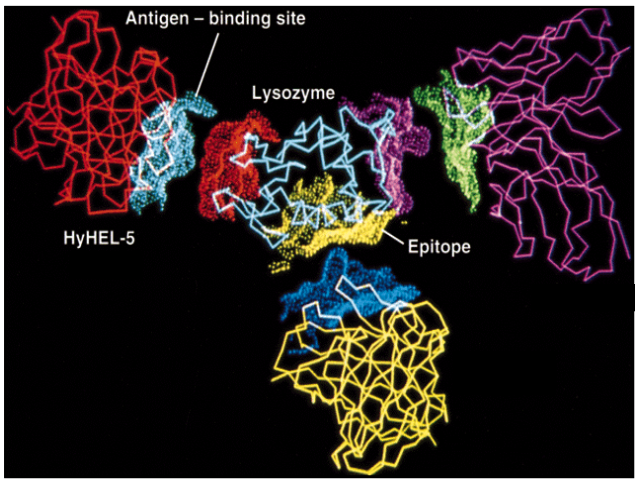
Immunoglobulin (Ig) Molecule



- 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

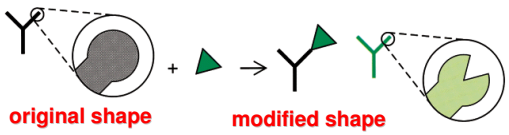


Theories used to explain antibody diversity

1930s-50s-Many theories were proposed to explain how so many different antibodies can be made to a "Universe" of different antigens

Selective Theory: Single cells express a large number of receptors that upon contact with antigen would induce the cell to release more of the "selected" receptor

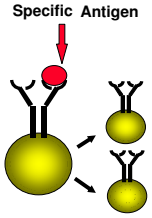
Instructional Theory: Antigens would serve as molds to "instruct" the creation of a specific antibody



Correct Hypothesis:

Clonal Selection Hypothesis: 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)



Ig receptors genes did not follow 1-gene/1-protein theory

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

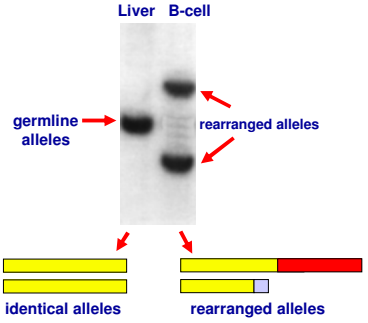
Since one gene encodes one protein (generally), this would mean that cells would need more genes than potentially encoded by genome

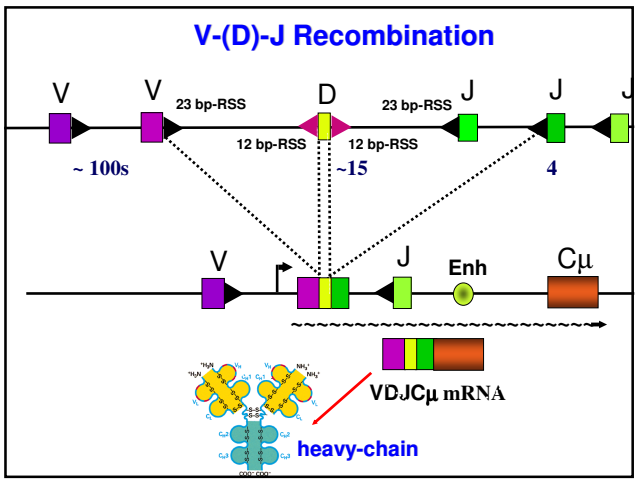
The answer to this problem resulted in a Nobel Prize

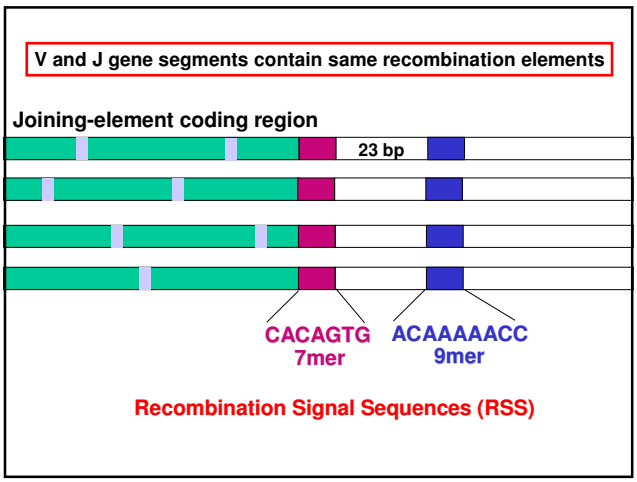
1987 Nobel Prize
Susumu Tonegawa

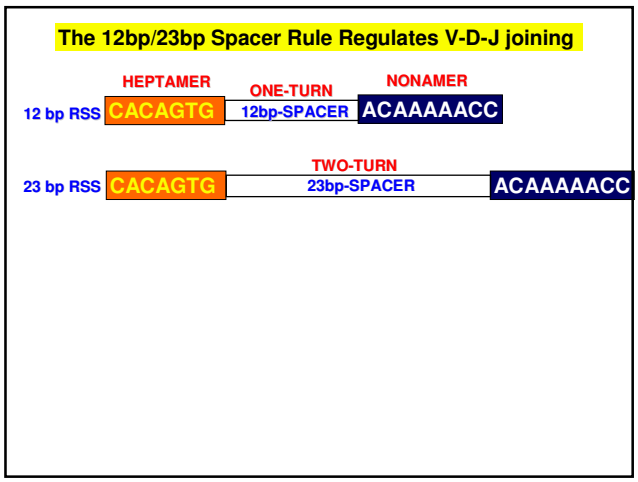
Using light-chain mRNA as probes was able to demonstrate that the variable region and the constant regions were "rearranged" in B-cell tumors (plasmacytomas)

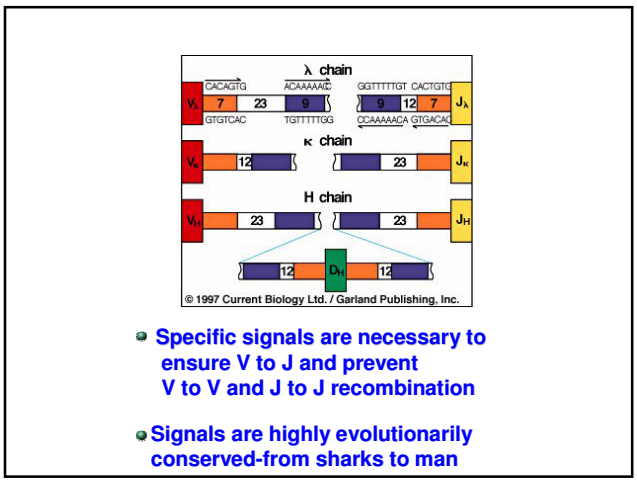
Southern Analysis of Immunoglobulin Gene Alleles




V-(D)-J Recombination
V
V
23 bp-RSS
D
23 bp-RSS
J
J
J
12 bp-RSS
12 bp-RSS
~ 100s
~15
4
V
J
Enh
Cμ
VDJCμ mRNA
heavy-chain


V and J gene segments contain same recombination elements
Joining-element coding region
23 bp
CACAGTG
7mer
ACAAAAACC
9mer
Recombination Signal Sequences (RSS)


The 12bp/23bp Spacer Rule Regulates V-D-J joining
HEPTAMER
ONE-TURN
NONAMER
12 bp RSS
CACAGTG
12bp-SPACER
ACAAAAACC
TWO-TURN
23 bp RSS
CACAGTG
23bp-SPACER
ACAAAAACC


λ chain
κ chain
H chain
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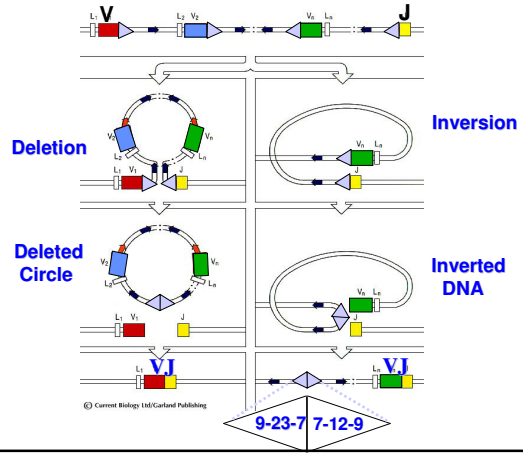
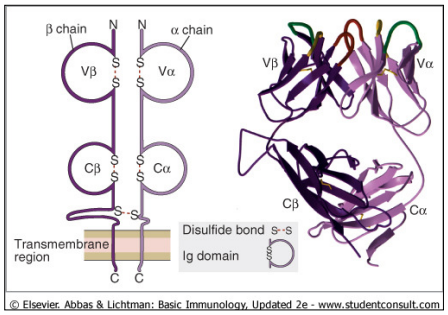
Specific signals are necessary to ensure V to J and prevent V to V and J to J recombination
Signals are highly evolutionarily conserved-from sharks to man

Recombination can proceed via deletion or inversion

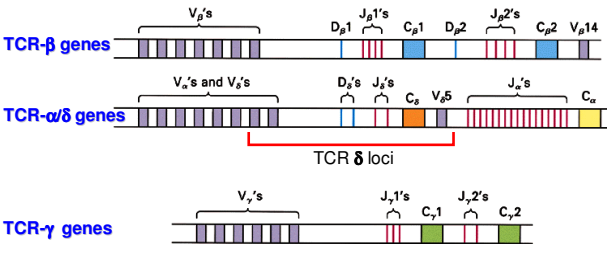


T-cell Antigen Receptors Resemble Antibodies



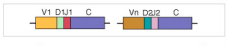
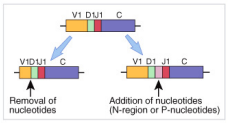
T-cell Antigen Receptor Genes

T-lymphocytes express an antigen receptor that also undergoes site specific rearrangement using exactly the same recombination signals

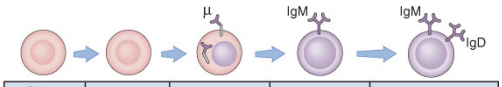


Diversity of Antigen Receptor Genes

- **Form Heterodimers** (Ig H+L and TCRαβ & TCRγδ)
- **Random Gene Assortment**
- **Flexible Recombination**
  Addition and Deletion of bases at junctions + reading frame changes
- **Somatic Hypermutation (B-cells)**
  Alteration the rearranged V genes generally confined to hypervariable regions leads to "selection" of higher affinity antibodies

	Immunoglobulin		T cell receptor	
	Heavy chain	κ	α	β
Number of V gene segments	45	35	45	50
Number of diversity (D) gene segments	23	0	0	2
Number of joining (J) gene segments	6	5	~50	12
Mechanism				
Combinatorial diversity: Number of possible V-(D)-J combinations	 Ig: ~10^6		TCR: ~3×10^6	
Junctional diversity: Total potential repertoire with junctional diversity	 Ig: ~10^{11}		TCR: ~10^{16}	

Rearrangement proceeds in an ordered fashion during B-cell Differentiation

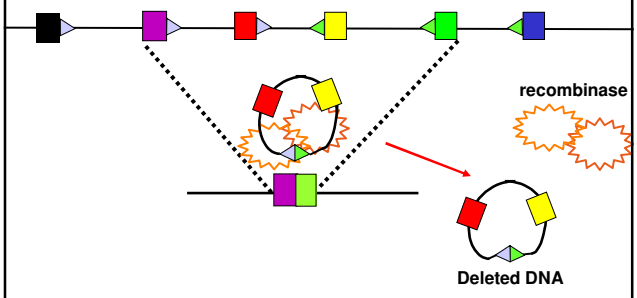


	Stem cell	Pro-B	Pre-B	Immature B	Mature B
Ig DNA, RNA	Germline DNA	Germline DNA	Recombined H chain gene (VDJ); μ mRNA	Recombined H chain gene, κ or λ genes; μ and κ or λ mRNA	Alternative splicing of primary transcript to form C_μ and C_δ mRNA
Ig expression	None	None	Cytoplasmic μ and pre-B receptor-associated μ	Membrane IgM (μ+κ or λ light chain)	Membrane IgM and IgD

What factors might be involved in VDJ Recombination?

- **Sequence-Specific DNA Binding Factors?**
- **Site-Specific Cleavage Activity?**
- **DNA Ligase Activity?**
- **Other "House-keeping" Factors (DNA Repair)?**

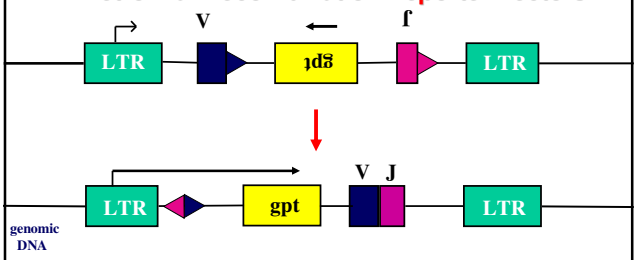
What are the "Recombinase" factors that recognize the Recombination Sequences (RSS)?



The Search for the Recombinase Complex

- Pre-B cell lines were created by Abelson Murine Leukemia transformation of mouse bone marrow cells *in vivo+in vitro*
- In early 1980s, retroviral vectors were developed in the laboratory of David Baltimore to study the mechanism of V-(D)-J recombination
- These retroviral vectors revealed that V-(D)-J recombination can be reproduced in the Pre-B cell lines, but not other cells
- These results lead to a frantic search for the V(D)J recombinase

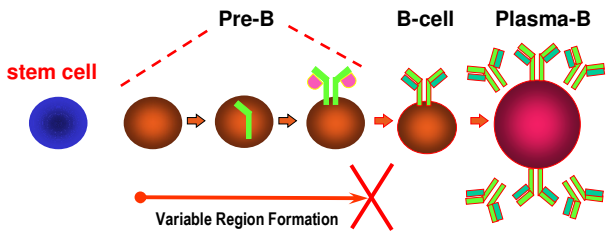
Retroviral Recombination Reporter Vectors



Rearrangement by Inversion Confers Drug Resistance

gpt = xanthine-guanine phosphoribosyltransferase gene

Recombinase must be expressed early during B-Lymphocyte differentiation



Retroviral vectors were found to rearrange only in pro/pre-B cell lines but not in non-lymphoid or mature B cells

"Chance Favors a Prepared Mind"
Louis Pasteur

An improbable experiment leads to an "incredible" result

- A student performs a series of flawed experiments 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

Retroviral vectors used to detect site-specific recombination (inversion)

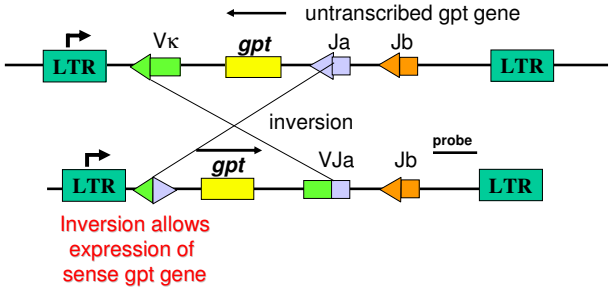


Fig. 1 Schatz and Baltimore (1987)

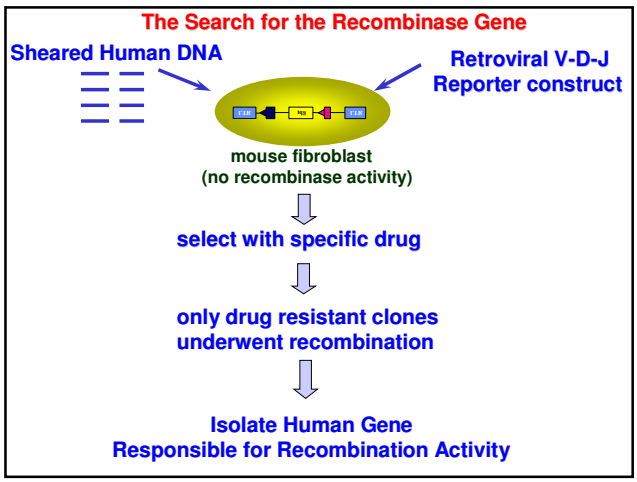
Table I.

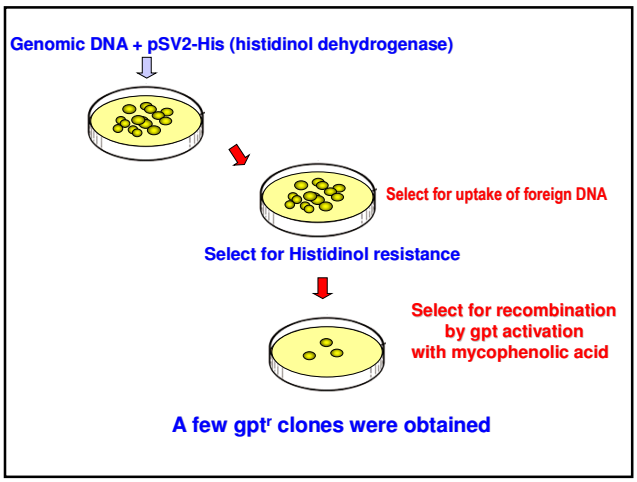
Developmental Stage Specificity of V(D)J Recombination Activity

Cell type	Growth under Selection (recombination)	Rec. Freq. (event/cell div.)
Lymphocyte precursor (Ba/F3 cell line)	None	$<1/2x10^6$
Pre-B cells (38B9, PD31)	**YES**	**$1/1x10^3$**
B-Cell (IgM Positive) (Wehi 231)	None	$<1/4x10^6$
Fibroblast (NIH 3T3)	None	$<1/2x10^7$

As expected, Pre-B cell lines contain a specific recombinase activity

Schatz and Baltimore (1987)





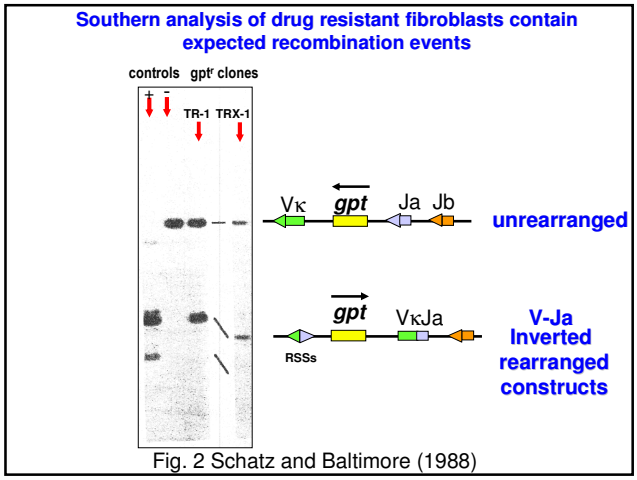


Fig. 2 Schatz and Baltimore (1988)

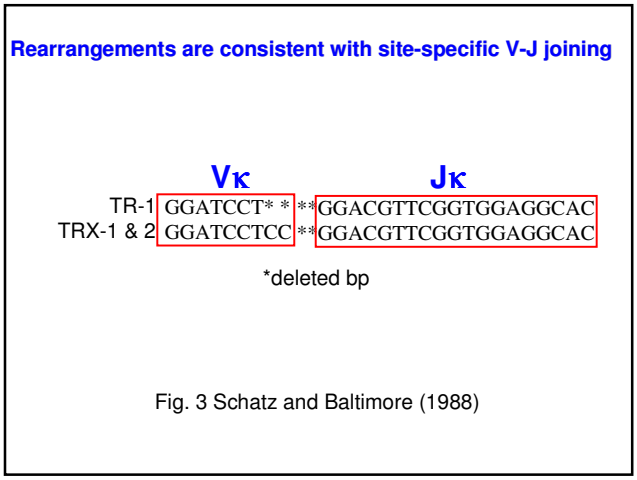


Fig. 3 Schatz and Baltimore (1988)

Identification of V-(D)-J Recombinase Gene

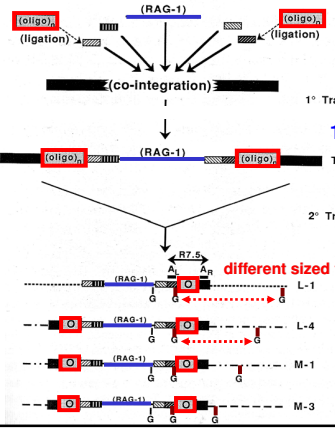
Human DNA

3TGR cells
(Fibroblasts w/ LTR Recombination Vector)

Stable Transfer of Recombination Activity

What gene caused this effect?

How do you isolate a human gene within a sea of mouse and human genes?



Link oligos to genomic DNA after RE digestion

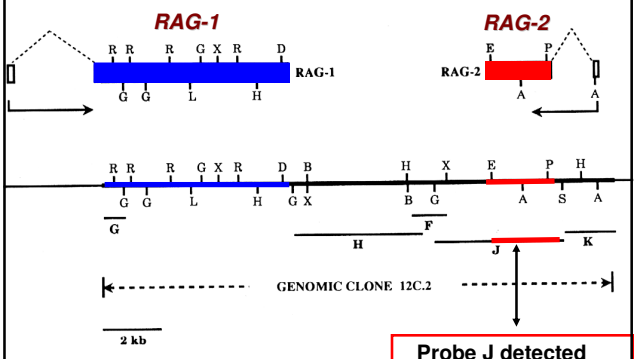
1° transfectant

different sized fragments on 3' side

2° transfectants

Fig. 2
Schatz *et al.*, 1989

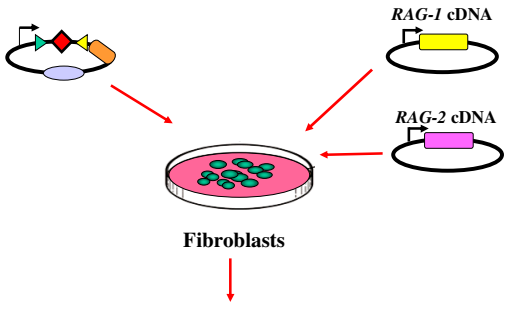
Original *RAG-1* Germline Clone Contained Another Gene



Probe J detected 2.2 kb mRNA by Northern in pre-B cells

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

RAG-2 and *RAG-1* are sufficient for high level recombination



High level recombination

Table I *RAG-1* and *RAG-2* Act Synergistically

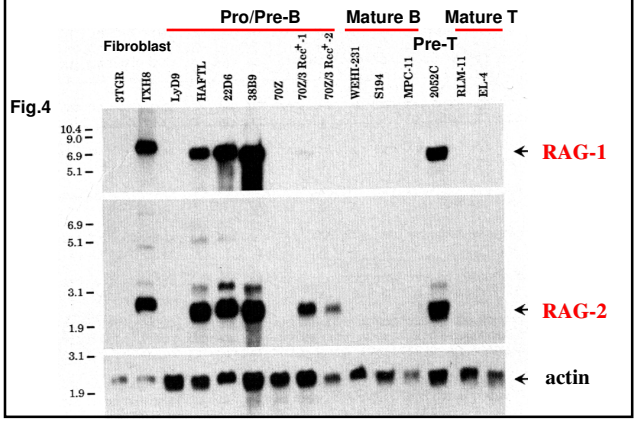
Cell line	DNA	Amp^r	Oligo+ Cam^{r}Amp^r	*R*
3TGR	0	184,000	0	0
3TGR	RAG-1	60,400	0	0
3TGR	RAG-2	50,000	0	0
3TGR	RAG-1 + RAG-2	70,600	490	0.7
NIH 3T3	RAG-1 + RAG-2	193,600	2,166	1.1
NIH 3T3	Human RAG-1 + mouse RAG-2	73,200	372	0.5

*Total number of independent transfections

Efficient Rearrangement only found when both *RAG-1* and *RAG-2* were cotransfected into fibroblasts

Oettinger, *et al.*, 1990

Expression of the *RAG-2* is Pro/Pre-Lymphocyte Specific

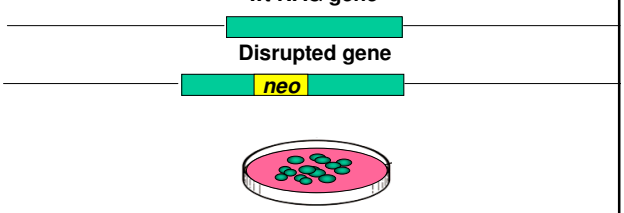


Targeted Disruption of *RAG-1* and *RAG-2* yields conclusive proof that the RAG genes are essential for V-(D)-J recombination

Shinkai, Y., *et al.*, (1992).
RAG-2-deficient mice lack mature lymphocytes owing to inability to initiate V(D)J recombination. Cell 68:855-867.

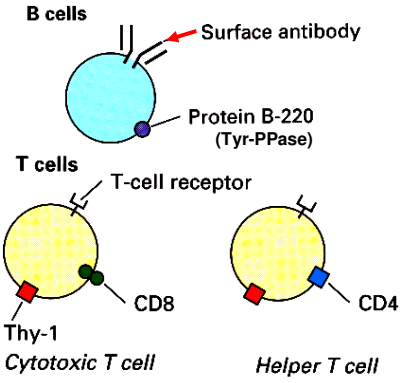
Mombaerts, P., *et al.*, (1992).
RAG-1-deficient mice have no mature B and T lymphocytes. Cell 68:869-877.

Generation of *RAG-1/2* knockouts



Homozygous neo^r Embryonic Stem Cells (ES)

Lymphocyte-cell surface receptors serve as cell "markers" which are needed for their isolation and identification

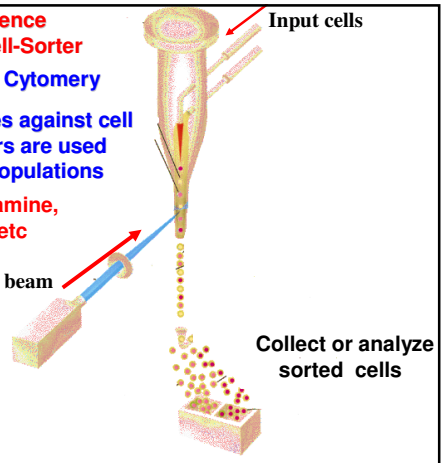


Fluorescence Activated Cell-Sorter

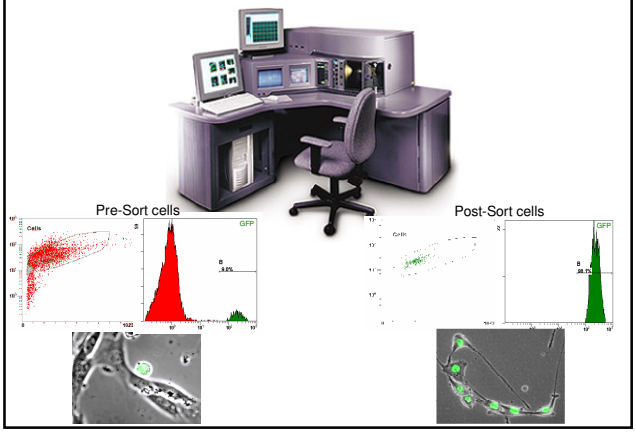
FACS or Flow Cytomery

Labeled antibodies against cell surface markers are used to isolate cell populations

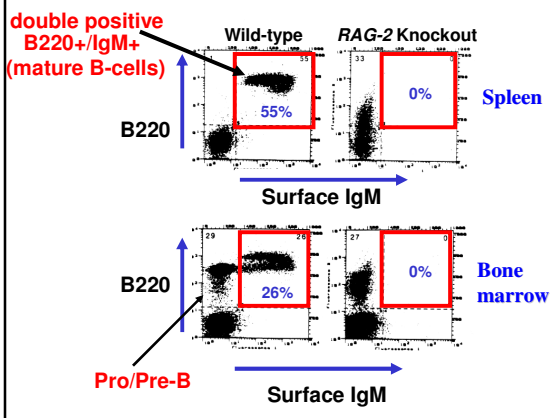
FITC, Rhodamine, Texas Red, etc

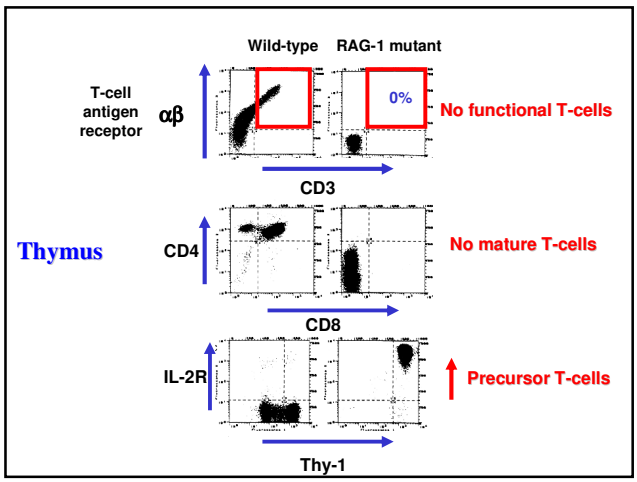


Flow Cytometer and Sorter EPICS ALTRA



FACS Analysis of Lymphocyte Populations




Wild-type
RAG-1 mutant
T-cell antigen receptor
αβ
0%
No functional T-cells
CD3
Thymus
CD4
No mature T-cells
CD8
IL-2R
Precursor T-cells
Thy-1