BCR Editing and Transcriptional Regulation

Professor Aguilera

AID is essential for Switching, Hypermutation and Gene Conversion

A V(D)J recombination

RAG1/RAG2

VDJ Sµ Cµ Cδ Cy3 Cy1 Cy2b Cy2a Cε C γ Cα

B Somatic hypermutation

Lesion (AID?)

Repair (AID?)

VDJ vV VDJ

C Gene conversion

VDJ Sµ Cµ Cδ Se Ce

D Class switch recombination

VDJ Cε + Cδ
Current Model for Switch Recombination

- Heavy chain genes in IgM-expressing cell
- UNG creates abasic sites; AP-endonuclease/fyrase creates nicks on both S region strands

Current Model for Somatic Hypermutation

- AP site error-prone bypass
- Replication
- Gap filled by C/A/G/T
- Long patch repair:
- Mutations from C/G and A/T
- AP site
- Original dNTP
- Mutated dNTP
- How do mutations from A/T arise?
- MMR?
- dG glycosylase?
Ig receptor and co-receptors needed for activation

Co-ligation leads to phosphorylation of CD19 leading to signaling

CD21 bind the complement system and activates B cells

Cross-linking of all three receptors leads to activation of somatic hypermutation

anti-IgM anti-CD19 and anti-CD21

B-cell (BL2) analyze after 90 min

Look at mutation of Ig V region or C region by sequencing PCR products

*Nature Immunology* 3, 815 - 821 (2002)
AID-dependent somatic hypermutation occurs as a DNA single-strand event in the BL2 cell line
Table 1. Induction of mutation in the BL2 cell line by aggregation of surface receptors

<table>
<thead>
<tr>
<th>Receptor cross-linking</th>
<th>Mutation frequency</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>V region (x10^4)</td>
<td>C region (x10^-4)</td>
<td>V region (x10^4)</td>
</tr>
<tr>
<td>No stimulation</td>
<td>0.96</td>
<td>—</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td>(1/10400)</td>
<td></td>
<td>(3/40768)</td>
</tr>
<tr>
<td>IgM</td>
<td>0.75</td>
<td>—</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td>(1/13312)</td>
<td></td>
<td>(4/40768)</td>
</tr>
<tr>
<td>IgM + CD19</td>
<td>1.3</td>
<td>—</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td>(2/14976)</td>
<td></td>
<td>(4/32448)</td>
</tr>
<tr>
<td>IgM + CD21</td>
<td>3.2</td>
<td>—</td>
<td>2.9</td>
</tr>
<tr>
<td></td>
<td>(5/15808)</td>
<td></td>
<td>(10/32864)</td>
</tr>
<tr>
<td>IgM + CD19 + CD21</td>
<td>5.4</td>
<td>1.0</td>
<td>7.0</td>
</tr>
<tr>
<td></td>
<td>(9/16640)</td>
<td>(1/9975)</td>
<td>(34/48256)</td>
</tr>
</tbody>
</table>

Mutations in a 416-bp fragment of the rearranged V4-39-J5 gene and a 399-bp fragment around the C,1 exon were analyzed; the mutation frequency is expressed as mutations per base pair. The error frequency of the PCR amplification was estimated.

Note that little mutation is generally observed at nearby CH region — important structure.

Table 2. Percentage nucleotide substitution in mutations generated by IgM-CD19-CD21 cross-linking in the BL2 cell V4-39-J5 gene

<table>
<thead>
<tr>
<th>From</th>
<th>To</th>
<th>A</th>
<th>T</th>
<th>G</th>
<th>C</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td></td>
<td>—</td>
<td>0.9%</td>
<td>4.6%</td>
<td>1.4%</td>
<td>6.9%</td>
</tr>
<tr>
<td>T</td>
<td>3.1%</td>
<td>—</td>
<td>2.7%</td>
<td>7.8%</td>
<td>13.6%</td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>26.3%</td>
<td>4.8%</td>
<td>—</td>
<td>7.5%</td>
<td>38.6%</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>10.6%</td>
<td>24.2%</td>
<td>6.1%</td>
<td>—</td>
<td>40.9%</td>
<td></td>
</tr>
</tbody>
</table>

Two-hundred-and-ninety-eight independent base substitutions targeted in 416 bp of the VDJ sequence were analyzed from a total of 340 mutations that included ten deletions and 32 insertions. Percentage substitution was corrected for the base composition of the V segment. Transitions: 62.9%; transversions: 37.1%.
Similar mutation rates were detected at G1 and G2/M
But not during active DNA replication (S)

<table>
<thead>
<tr>
<th>Cell cycle fraction</th>
<th>Unfractionated</th>
<th>G1</th>
<th>S</th>
<th>G2/M</th>
</tr>
</thead>
<tbody>
<tr>
<td>FACS profile</td>
<td></td>
<td>96%</td>
<td>3%</td>
<td>1%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>90 min incubation</th>
<th>24 h incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutation frequency</td>
<td>6.8 x 10^-4 (42/61568)</td>
<td>6.2 x 10^-4 (40/64064)</td>
</tr>
<tr>
<td></td>
<td>5.4 x 10^-4 (22/40768)</td>
<td>6.7 x 10^-4 (25/37440)</td>
</tr>
<tr>
<td></td>
<td>1.8 x 10^-4 (4/21632)</td>
<td>n.d. (25/37440)</td>
</tr>
<tr>
<td></td>
<td>6.5 x 10^-4 (25/38272)</td>
<td>1.2 x 10^-4 (5/41600)</td>
</tr>
</tbody>
</table>

Mutation frequency is expressed as mutations per base pair.

Somatic Mutation occurs on one strand in G1 phase of cell cycle

<table>
<thead>
<tr>
<th>a</th>
<th>b</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Diagram a" /></td>
<td><img src="image2.png" alt="Diagram b" /></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>c</th>
<th>d</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image3.png" alt="Diagram c" /></td>
<td><img src="image4.png" alt="Diagram d" /></td>
</tr>
</tbody>
</table>
Mutation does not require de novo mRNA synthesis

**Table 4. Induction of mutation in the BL2 cell V4-39-Jμ5 gene in the presence of actinomycin D**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Mutation frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unstimulated</td>
<td>$0.7 \times 10^{-4}$ (2/29536)</td>
</tr>
<tr>
<td>90 min stimulation</td>
<td>$5.8 \times 10^{-4}$ (18/30784)</td>
</tr>
<tr>
<td>90 min stimulation with actinomycin D</td>
<td>$6.2 \times 10^{-4}$ (24/38688)</td>
</tr>
</tbody>
</table>

Mutation frequency is expressed as mutations per base pair.

Disruption of the AID gene to test role of AID in Somatic Mutation

**Diagram a**

Exons

1

<table>
<thead>
<tr>
<th>Exons</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exons</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1kb

First allele

Second allele

3.1 kb

3.2 kb

3.0 kb

3.2 kb

4.1 kb

**Diagram b**

<table>
<thead>
<tr>
<th>123.2</th>
<th>AID-129</th>
<th>64-15</th>
<th>64-6</th>
<th>AID-04</th>
</tr>
</thead>
<tbody>
<tr>
<td>PARP</td>
<td>AID</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Diagram c**

<table>
<thead>
<tr>
<th>BL2</th>
<th>AID-17</th>
<th>AID-15</th>
<th>Ramos</th>
</tr>
</thead>
<tbody>
<tr>
<td>PARP</td>
<td>AID</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**Mutation of AID disrupts Somatic Mutation**

Table 5. Frequency of mutations induced in the V4-39-J\textsubscript{H}5 gene of the AID\textsuperscript{−−} BL2 clone before and after transfection with AID-expressing vectors

<table>
<thead>
<tr>
<th>BL2 subclone (phenotype)</th>
<th>Mutation frequency</th>
<th>Before stimulation</th>
<th>After 3 stimulations</th>
</tr>
</thead>
<tbody>
<tr>
<td>60-64 (AID\textsuperscript{−−})</td>
<td>0</td>
<td>(0/11648)</td>
<td>0</td>
</tr>
<tr>
<td>60-64-8 (AID-transfected)</td>
<td>0</td>
<td>(0/9568)</td>
<td>4.6 × 10\textsuperscript{−4}</td>
</tr>
<tr>
<td>60-64-15 (AID-transfected)</td>
<td>1.0 × 10\textsuperscript{−4}</td>
<td>(1/9568)</td>
<td>3.8 × 10\textsuperscript{−4}</td>
</tr>
<tr>
<td>60-129 (AID\textsuperscript{−−})</td>
<td>0</td>
<td>(0/9152)</td>
<td>0</td>
</tr>
<tr>
<td>60-129-2 (AID-transfected)</td>
<td>0</td>
<td>(0/9568)</td>
<td>3.4 × 10\textsuperscript{−4}</td>
</tr>
<tr>
<td>60-129-10 (AID-transfected)</td>
<td>1.0 × 10\textsuperscript{−4}</td>
<td>(1/9568)</td>
<td>4.2 × 10\textsuperscript{−4}</td>
</tr>
<tr>
<td>BL2 (not transfected)</td>
<td>1.0 × 10\textsuperscript{−4}</td>
<td>(1/9568)</td>
<td>11.5 × 10\textsuperscript{−4}</td>
</tr>
</tbody>
</table>

Mutation frequency is expressed as mutations per base pair.

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**Table 3. Frequency of cells harboring mutations in one or two V4-39-J\textsubscript{H}5 DNA strands after mutation was induced in the G1 phase**

<table>
<thead>
<tr>
<th></th>
<th>Frequency of mixed V sequence cells</th>
<th>Frequency of homogenous V sequence cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>90 min after stimulation\textsuperscript{a}</td>
<td>10/70</td>
<td>0/70</td>
</tr>
<tr>
<td>24 or 48 h after stimulation\textsuperscript{a}</td>
<td>9/115</td>
<td>6/115</td>
</tr>
<tr>
<td>Control\textsuperscript{b}</td>
<td>11/117</td>
<td>6/17</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Direct amplification of the BL2 cell V region was done at the one-cell (90 min), two-cell (24 h) or four-cell stage (48 h).
\textsuperscript{b}Control represents amplification from two mixed BL2 cells that differed at nucleotide position 57 of their V4-39 gene; this controlled for unequal PCR amplification of two V\textsubscript{H} alleles. The number of cells harboring mutations per total number of cells analyzed is indicated. See Fig. 2 and text for details.
Controlling somatic hypermutation in immunoglobulin variable and switch regions

Robert W. Maul and Patricia J. Gearhart

Fig. 2. Two regions of hypermutation. The x-axis depicts the 30-kb heavy chain locus containing a promoter (oval), start of transcription (arrow), VDJ gene (box), enhancer (oval), start of intronic transcription (arrow), switch region (hexagon), and Cj gene. The y-axis shows the frequency of mutation (inches), which are centered around the VDJ gene and S region.

Fig. 3. Model for distribution of enzymes in the Sμ region. The large oval circle represents the region forming R-loops. RNA polymerase II molecules (circles) pile up in this region and transcription is slowed. AID proteins (stars) associate with RNA polymerases and pause as well to deaminate dC at a high frequency. Further downstream, AID falls off and mutation is reduced. DNA polymerase η (ovals) is active in the 50 end, but not in the single strand R-loop region, which reduces the frequency of A:T mutations.
Collateral Damage from Antigen Receptor Gene Diversification

Chromosomal translocations that juxtapose antigen receptor genes and oncogenes are frequently associated with lymphoid malignancies. In this issue, Robbiani et al. (2008) show that activation-induced deaminase (AID), an enzyme involved in antigen receptor gene diversification, generates DNA double-strand breaks (DSBs) in oncogenes, and Tsai et al. (2008) propose that AID and the recombinase-activating gene (RAG) endonuclease may collaborate to generate off-target DSBs.
Aberrant switch recombination events can lead to chromosome translocations.

In mice the 12;15 translocation is detected in >90% of lymphocyte tumors.
AID Is Required for the Chromosomal Breaks in c-myc that Lead to c-myc/IgH Translocations

David F. Robbiani,1 Anne Bothmer,1 Elsa Callon,1 Bernardo Rojas-San-Martin,1,6 Yair Dorsett,1

SUMMARY

Chromosomal translocation requires formation of paired double-strand DNA breaks (DSBs) on heterologous chromosomes. One of the most well-characterized oncogenic translocations juxtaposes c-myc and the immunoglobulin heavy-chain locus (IgH) and is found in Burkitt’s lymphomas in humans and plasmacytomas in mice. DNA breaks in IgH leading to c-myc/IgH translocations are created by activation-induced cytidine deaminase (AID) during antibody class switch recombination or somatic hypermutation. However, the source of DNA breaks at c-myc is not known. Here, we provide evidence for the c-myc promoter region being required in targeting AID-mediated DNA damage to produce DSBs in c-myc that lead to c-myc/IgH translocations in primary B lymphocytes. Thus, in addition to producing somatic mutations and DNA breaks in antibody genes, AID is also responsible for the DNA lesions in oncogenes that are required for their translocation.

Cell 135, 1028–1038, December 12, 2008

Deletion of the c-myc oncogene promoter stops translocations

Figure 1. The Myc<sup>Δ</sup> Allele Is Protected from Translocating to the IgH

(A) Schematic representation of wild-type (Myc<sup>+</sup>) and mutant (Myc<sup>Δ</sup>) c-myc alleles. The dashed bracket indicates the region deleted and replaced by a loxP site (triangle) in the Myc<sup>Δ</sup> allele.

(B) Genotyping summary from the indicated Myc<sup>Δ+</sup> crosses.

(C) RNA-FISH showing biallelic versus monoclonal c-myc transcription in wild-type (WT) versus Myc<sup>Δ+</sup> cells, respectively.

(D) Diagram showing the location of the primers used in the PCR assay to detect derivative chromosome 12 (der12, black arrow) and derivative chromosome 15 (der15, green arrow) c-myc/IgH translocations. The table summarizes the number of allele-specific der12 and der15 c-myc/IgH translocations in activated Myc<sup>Δ+</sup> B cells, as determined by sequencing. Data are from three independent experiments.
14;18 Translocation is the most common rearrangement in Human Lymphomas
(all Follicular Lymphomas)

Bcl-2 (B-cell lymphoma 2)

Any of these 4 DNA ends can join with any one of the others

NHEJ

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 #1”, solid downward arrows) is independent of the RAG cleavage at the paired Jb/Jb signal pair (denoted by “RAG complex #2”), and the work done 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 few DNA-end problem rather than a six DNA-end problem. NHEJ is the pathway for repairing the ends, based on the new findings from the present study, which are shown in black.

Receptor Editing

Strong ligation of IgM by self antigen

Arrest of B-cell development and continued light-chain rearrangement: low cell-surface IgM

2/18/2014
Re-rearrangement of κ-light chain results in new receptor specificity

- If the new receptor is still self-reactive, the B cell undergoes apoptosis.
- If the new receptor is no longer self-reactive, the immature B cell migrates to the periphery and matures.

Clonal Deletion

---

**Primary and Secondary V(D)J Recombination during B Cell Development**

<table>
<thead>
<tr>
<th>Pro B</th>
<th>Pre B</th>
<th>Immature B</th>
<th>Periphery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mature B</td>
</tr>
<tr>
<td>D→D_{JH}</td>
<td>V→DJ_{H}</td>
<td>productive</td>
<td>μκκ</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Secondary Vκ to Jκ</td>
<td>Receptor Editing</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Vκ→Jκ</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Non-productive or κ deletion</td>
<td></td>
</tr>
</tbody>
</table>

IgH Gene Rearrangement

<table>
<thead>
<tr>
<th>Pre B</th>
<th>Immature B</th>
<th>Periphery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>μκκ</td>
<td>μκκ</td>
</tr>
</tbody>
</table>

IgL Gene Rearrangement

<table>
<thead>
<tr>
<th>Immature B</th>
<th>Periphery</th>
</tr>
</thead>
<tbody>
<tr>
<td>μκκ</td>
<td>μκκ</td>
</tr>
</tbody>
</table>
**κ light-chain variable region gene recombination**

1st rearrangement

2nd rearrangement

**Secondary Light-Chain Rearrangements**

The organization of the κ light chain locus allows for secondary rearrangements that can remove a previously rearranged VJκ

Apparently signaling through the Ig receptor can modulate RAG re-expression depending on strength of signal
V gene replacement through weak RSS homologies

Trials and Tribulations with VH Replacement.

Can replace one V with another.)

Important Genes/Proteins Expressed During B-cell Differentiation

Fig. 6.7

<table>
<thead>
<tr>
<th>Protein</th>
<th>Function</th>
<th>Time of expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>RAG-1</td>
<td>Recombination</td>
<td></td>
</tr>
<tr>
<td>RAG-2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tdt</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vp26</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Igα</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Igβ</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VB</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TAP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BSAP</td>
<td>Transcriptional regulation</td>
<td></td>
</tr>
</tbody>
</table>

2/18/2014 30
**Transcription Factors Known to Regulate Critical Steps in B-cell Differentiation**

**Approximate time when a particular factor is critical:**

<table>
<thead>
<tr>
<th>Progenitor</th>
<th>Pro-B</th>
<th>Pre-B</th>
<th>Naïve B</th>
<th>GC B</th>
<th>Plasma B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ikaros</td>
<td>PU.1</td>
<td>E2A</td>
<td>BSAP</td>
<td>NF-κB</td>
<td>Ets-1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Blimp-1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C/EBPβ</td>
</tr>
</tbody>
</table>

**Approximate Expression Pattern:**

- **Ikaros**
- **PU.1**
- **E2A, Ets-1, Oct-2, OCA-B, TF3E, BSAP**
- **active NF-κB**

**Targeted disruption of BSAP disrupts B-lymphopoiesis and V-D-J joining**

- **A**
  - Pro-B and Pre-B cells
  - WT
  - BSAP−/−

- **B**
  - BSAP−/−
  - VH → DJ

- **C**
  - pro-B cell development
  - Normal: BSAP+/+ pro-B cells

Receptor Editing and Marginal Zone B Cell Development Are Regulated by the Helix-Loop-Helix Protein, E2A

Melanie W. Quong,1 Annica Martensson,2 Anton W. Langerak,3 Richard R. Rivera,1 David Nemazee,2 and Cornelis Murre 1

J. Exp. Med. Volume 199, Number 8, April 19, 2004 1101–1112

E2A Transcription factor promotes λ light chain rearrangement

3-83 Ig transgene reacts against self (MHC H-2K\(^{kb}\))
And transgenic mice have monoclocal B cells in the permissive background (H-2K\(^d\))

When crossed to the non-permissive background B cells undergo receptor editing to replace light chains with another kappa or a lambda
**3-83Tg IgHa/IgLk**

B10D2 Not autoreactive Non-deleting background

**Deleting MHC (Kd)**

A

B10D2 E2A wt B6:B10D2

B10D2 E2A +/- B6:B10D2

B10D2 E2A +/- B6:B10D2

C

3-83Tg B10D2

3-83Tg E2A wt B6:B10D2

3-83Tg E2A +/- B6:B10D2

**Figure 7.** Model indicating the potential roles of E-proteins during B cell maturation.
Most lymphocyte-specific genes are regulated by a complex array of cis regulatory elements

Enhancer promoter elements silencer
TATA/Inr exon 1 exon 2

mRNA

Elements involved in the transcriptional regulation of Ig and TCR genes

Promoters

*cis*-acting DNA elements are position and orientation dependent
~ 10 - 50 bp upstream of gene

Enhancers Silencers
positive acting negative acting

Enhancers and silencers are *cis*-acting elements that can exert effect from a large distance and are orientation independent
Lymphocyte-Specific transcription factors (TFs) are required to activate several lymphocyte-specific genes.

Promoters and Enhancers Collaborate for Optimal Tissue-Specific Expression

Closed - inactive chromatin is heavily methylated (CpG)

A model of interaction of Promoter and Enhancer Elements
Unrearranged V-genes are generally not expressed

No or low level sterile transcripts

1000x increase in expression

Why?

Enhancer within Cµ intron
Active only in B cells
Similar to viral enhancers but B-cell specific
no enhancer--no expression

Multiple lymphocyte and non-lymphocyte specific factors interact with the Ig promoter and enhancer regions

Ernst and Smale (95)

Not all TFs are lymphocyte-specific
RAG Gene Regulation

Cloning of the RAG-1 and RAG-2 Promoter Regions

RAG Cosmid Clone

RAG-1

RAG-2

~10 kb

~5 kb
RAG-luciferase reporter plasmid

Analysis of Promoter Regions

Transfect cell lines

test cell extract for luciferase activity

The RAG-2 promoter exhibits cell-type specificity
Two distinct factors interact with the BSAP binding site

**B-cells**
- Pro/Pre-B
- B
- Plasma

**T-cells**
- Pre-T
- mature-T
- Thymus

BSAP

WT

BSAP

c-Myb
Mutation of the core BSAP site leads to a decrease in promoter activity

Fold Luciferase Activity

Pre-B cell

WT   BSAPm

WT   BSAPm

RAG-2 promoter construct

BSAP and SP1 synergistically activate the RAG-2 promoter

Fold Promoter activity

RAG-2 promoter vector

RAG-2 promoter vector

+Sp1 +BSAP  +Myb

+Sp1  +BSAP  +Myb

+Sp1  +BSAP  +Myb

Transcription factor cDNAs vectors transfected into fibroblast cell line
Summary of features of the RAG-2 promoter:

1) BSAP interacts with and activates the promoter in B-cells

2) The GA-box plays a critical role in the regulation of the promoter for B- and T-cell expression

3) Sp1 and Sp3 bind and activate the promoter via the GA-box

4) BSAP and Sp1 synergistically activate the promoter

5) c-Myb transcription factor interacts within BSAP binding site and also collaborates with Sp1 to activate the promoter

Apart from promoters, additional elements are required for RAG expression.

A putative LCR region controls lymphocyte-specific expression of both promoters.

Analysis of transgenic mice with RAG region deletions:

- **B/T cells**
  - RAG-1
  - RAG-2

- **B cells**
  - RAG-1
  - RAG-2

- **no B/T cells**
  - RAG-1
  - RAG-2
Upstream region affects the expression of both RAG genes

Using these transgenic systems, two groups identified essential elements required for lymphocyte-specific expression of both RAG-1 and RAG-2 in mice

Loops-out model for coordinated regulation

Yu et al., 1999

In absence of anti-silencer, silencer is dominant

A cis element in the recombination activating gene locus regulates gene expression by counteracting a distant silencer

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Shh! Shh! It’s, oh, so quiet

Dimitris Kioussis

The regulated expression of *Rag* genes in lymphocytes ensures the expression of a single antigen receptor on the lymphocyte surface. It now seems that a complex 'ballet' of activating and silencing elements controls the precise timing of *Rag* expression in thymocytes.