

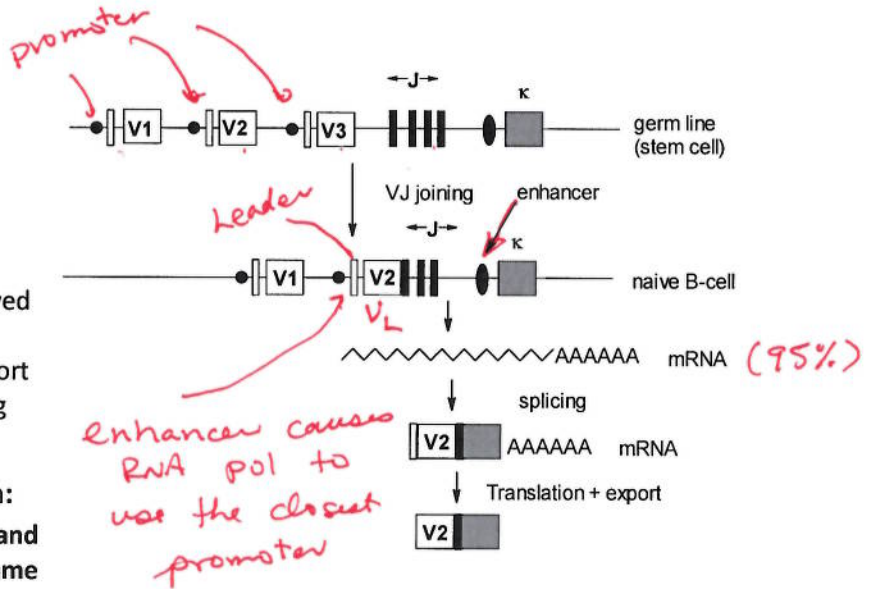
**Lecture 11: Expression of Ab, mRNA Editing, T-cell Independent Antigens, Regulation**

**Questions to be answered:**

1. If each V segment has its own promoter why is there only one transcript from the rearranged gene?
2. Why do secreted and membrane bound antibodies have the same specificity (and sequence)?
3. Why do IgM and IgD on naïve B-cells have the same specificity (and sequence)?
4. What shuts down antibody production?
5. Can you make antibodies to antigens that don't contain proteins?

**Expression of Ig Genes**

1. Promoter selection is obtained by proximity to enhancer sequences, which enhance the level of transcription from the closest promoter, hence **only rearranged genes are expressed.**
2. Unused V/D/J segments are removed from mRNA by splicing.
3. Leader (signal) peptide signals export to rough ER. This is removed during transport across ER membrane.



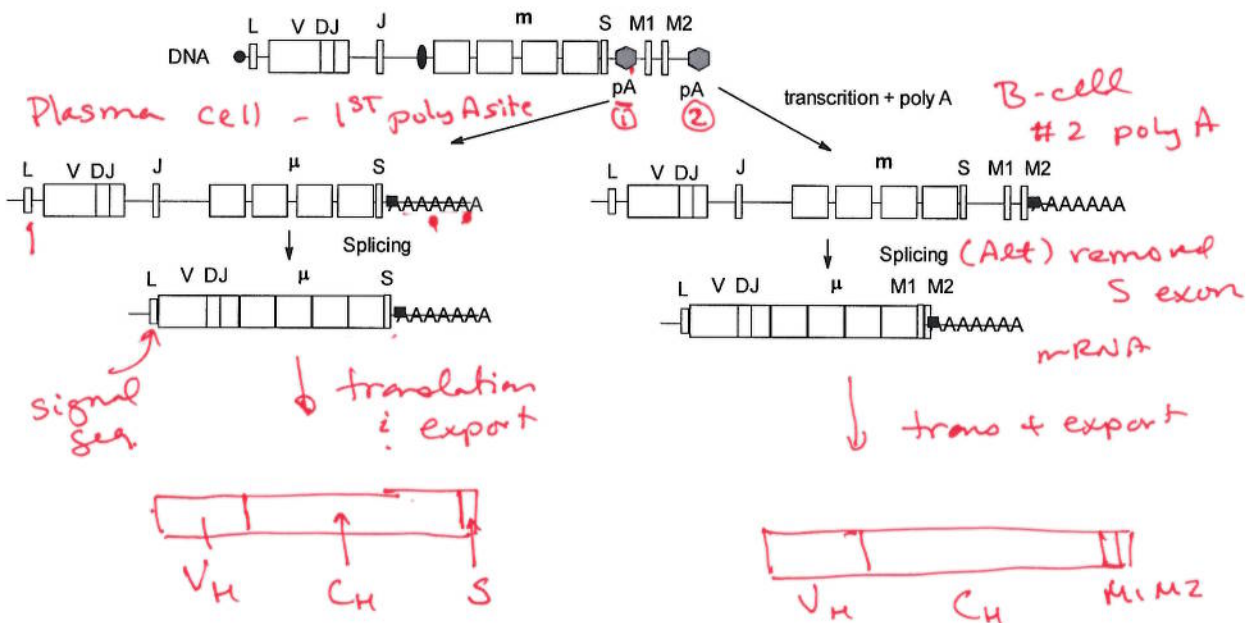
**Events that Rely on polyA addition:**

- i. Production of Membrane Bound and Soluble Immunoglobulin with the same specificity.

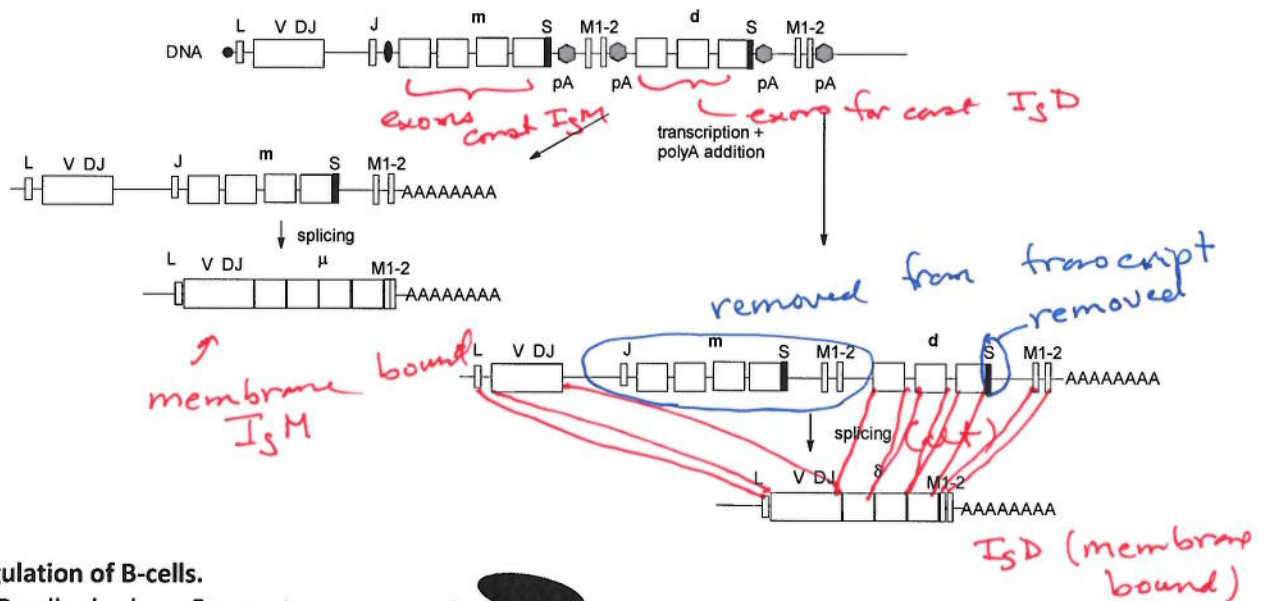
The 3' end of every constant region contains the following elements:

1. S exon codes for the carboxy-terminal sequence found in soluble IgM, a hydrophilic sequence.
2. polyA: poly A cleavage and addition sites
3. M1, M2 exons that code for the membrane domain. Hydrophobic sequences.
4. 2<sup>nd</sup> polyA site.

Alternate use of the two polyA sites results in the expression of soluble IgM ( $\mu$ 4-S) or membrane bound ( $\mu$ 4-M1-M2) IgM. The same events would occur for all other antibody isotypes.

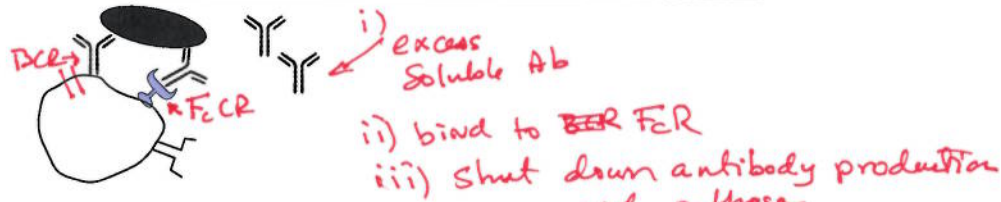
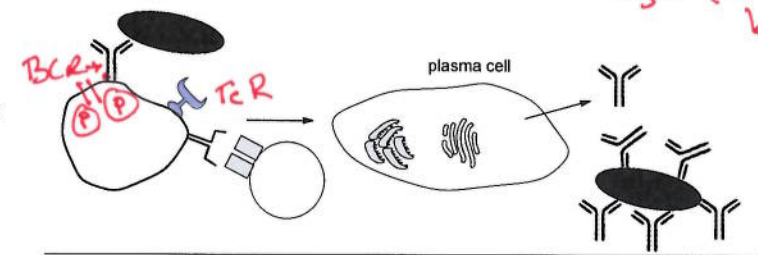


**ii. Co-expression of IgM and IgD:** These two isotypes are expressed at the same time in the mature B-cell. The two constant domain gene segments are adjacent to each other on the chromosome. The 3' end of the  $\mu$  gene and the 3' end of the  $\delta$  gene have two polyadenylation sites. Alternative use of two of these sites leads to expression of membrane bound IgM or IgD.



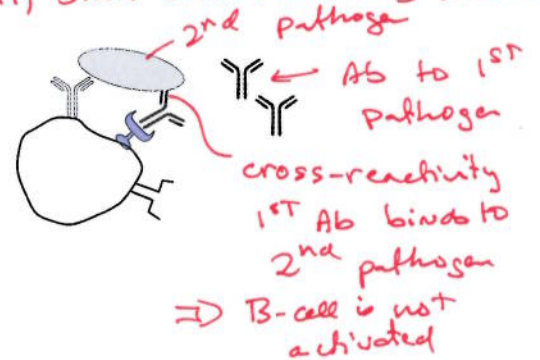
**Regulation of B-cells.**

- i) B-cells also have  $F_c$  receptors.
- ii) Binding of antibody to  $F_c$  receptors attenuates ability of the B-cell to become active due to dephosphorylation of activated BCR-antigen complex.
- iii) This mechanism is in place to turn off B-cell activation when pathogen is cleared by the acquired system.



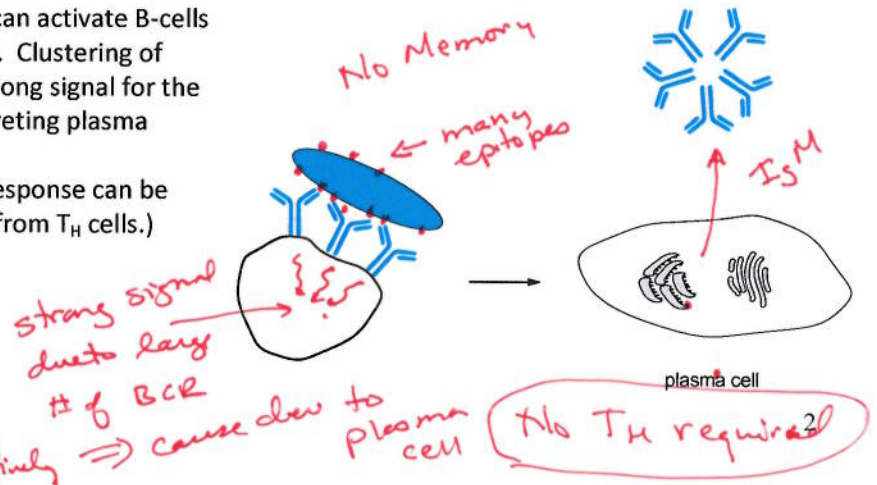
**"Original Antigenic Sin"**

- i) Exposure to pathogen generates antibodies to that pathogen (black antibodies)
- ii) If original antibodies are present they can prevent the production of antibodies to a closely related pathogen that has similar epitopes as the original pathogen (light gray). Making the first antibodies is the "sin", it prevents the production of subsequent antibodies.



**T-cell Independent Antigens:**

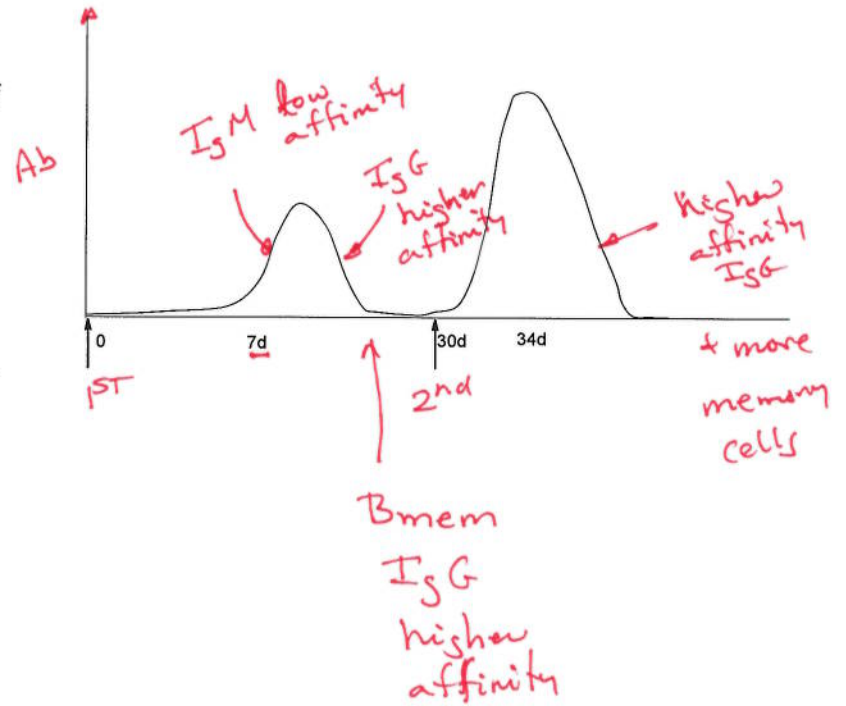
- i) Repeating epitopes on pathogen can activate B-cells to differentiate into plasma cells. Clustering of BCR can provide a sufficiently strong signal for the cell to differentiate into IgM secreting plasma cells.
- ii) No direct contact with  $T_H$  cells, (response can be aided by proliferation cytokines from  $T_H$  cells.)
- iii) No class switching
- iii) No affinity maturation
- iv) No memory cells.



low affinity  
high avidity  
∴ can bind effectively ⇒ cause due to plasma cell

**Secondary B-cell Response:**

- i) B & T<sub>H</sub>-memory cells can live for decades
- ii) Clonal expansion during activation of B-cells produces large number of memory cells.
- iii) Formation of antigen-B-cell/T-cell complex in 2° organs enhanced by larger number of cells.
- iv) Activation process faster.
- v) Heavy chain type (isotype) and high affinity are preserved on memory B-cells.



## Summary of B-Cell Development, Activation, and Maturation:

	Pro- B	Pre-B	Immature B	Naive B	Activated B	Plasma B	Memory B
H-Chain genes	DJ	VDJ	VDJ	VDJ	VDJ	VDJ	VDJ
L-Chain genes/proteins	-	VpreB /λ5	VJ	VJ	VJ	VJ	VJ
RAG1/RAG2	+	+	+	-	-	-	-
TdT	+	+	-	-	-	-	-
Affinity Mat	-	-	-	Low Aff.	+ (AID)	High Aff.	High Aff.
Class Switch.	-	-	-	-	+ (AID)	-	-
Membrane Isotype	-	μ	μ	μ+δ	μ+δ or α,ε,γ alone	none	μ+δ or α,ε,γ alone
Secreted Ig	-	-	-	-	none	High affinity μ,α,ε,γ	-

### Review Guide on B-Cells

Describe the steps in B-cell development from stem to memory & plasma cell.

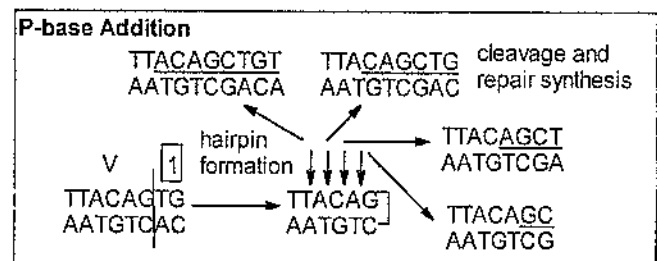
- Checkpoints, location, mechanism, order, and their purpose
- Allelic exclusion and its importance
- Enzymes/proteins involved & what they recognize

B-cell development:

- VpreB, λ5
- Rag1/Rag2 : Recombination signal sequences 1+2 or 2+1 pairing rules.
- TdT (terminal transferase)

Diversity generation:

- Combinatorial joining of segments to make V-exons
- P bases, N bases, imprecise joining
- Pairing of heavy and light chains due to C<sub>L</sub> C<sub>H1</sub> interactions



### Summary of Diversity:

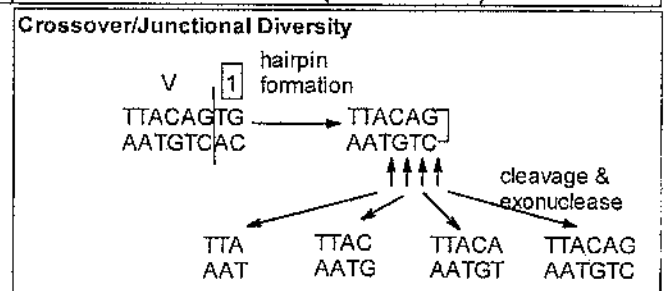
Mechanism of Diversity	Heavy Chain	Light Chain
Combinatorial V-D-J and V-J:	$300 \times 12 \times 4 = 1.4 \times 10^4$	$300 \times 4 = 1.2 \times 10^3$
P base (V-D-J), (V-J) (x3/joint)	$\times 9$	$\times 3$
Junctional (Crossover) diversity (x3/joint)	$\times 9$ (VDJ)	$\times 3$ (VJ)
N-base addition (TdT) (V-D-J)	$\times 9$	$\times 1$
# Chains	$\sim 1.0 \times 10^7$	$\sim 1.0 \times 10^4$
Estimated Diversity	$1.0 \times 10^{11}$ (#HC x #LC)	

Production of heavy and light chain proteins

- Promoter/enhancer interactions
- polyA addition and splicing: co-express of IgM & IgD, production of soluble antibody.

B-cell Activation:

- BCR + CR/C3b
- MHC → TCR/CD4
- CD40 ← CD40L
- B7 → CD28
- Cytokine receptors ← IL2, IL4, IL5



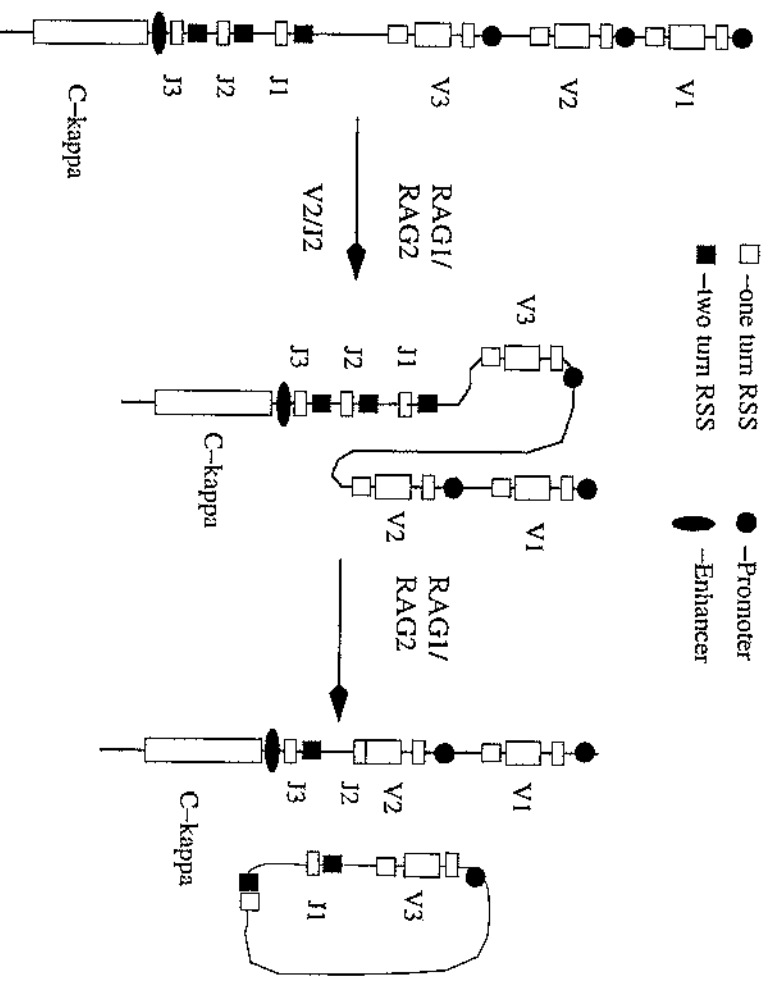
Class Switching: cytokine driven

Affinity Maturation:

- Avidity versus affinity, why maturation is important after class switch.
- Selective pressure by B-T<sub>H</sub> interactions

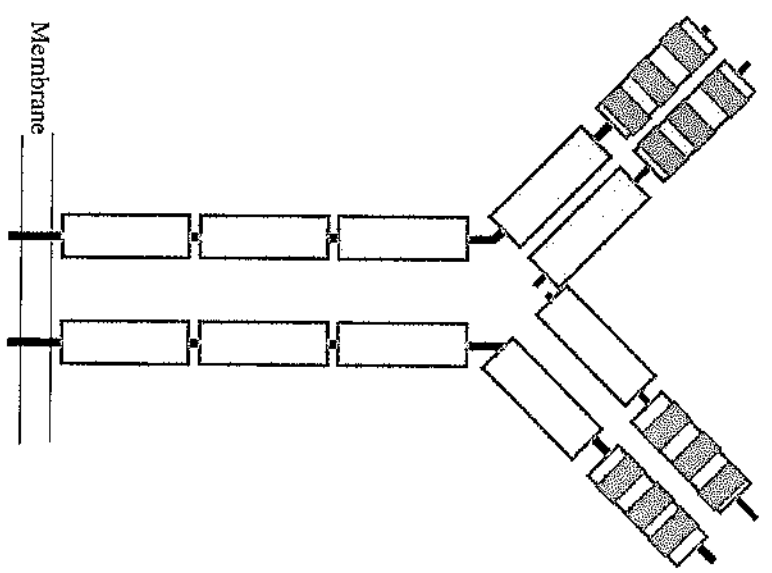
Antigenic sin, Secondary response, T-cell independent antigens

### A. Light Chain Rearrangement in B-Cell Development

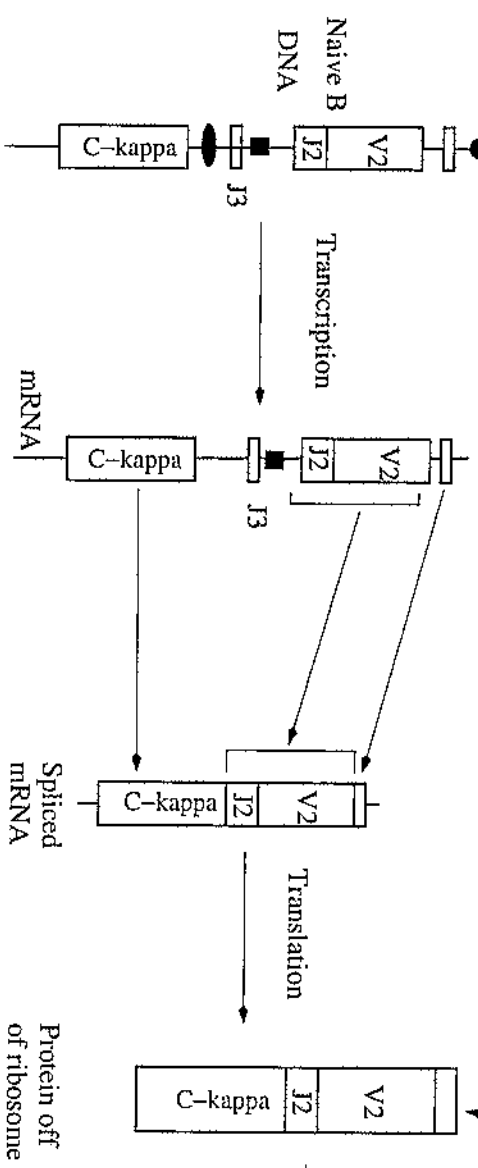


- --one turn RSS
- --two turn RSS
- --Promoter
- ◐ --Enhancer

### Membrane Bound IgM in Naive B-Cells



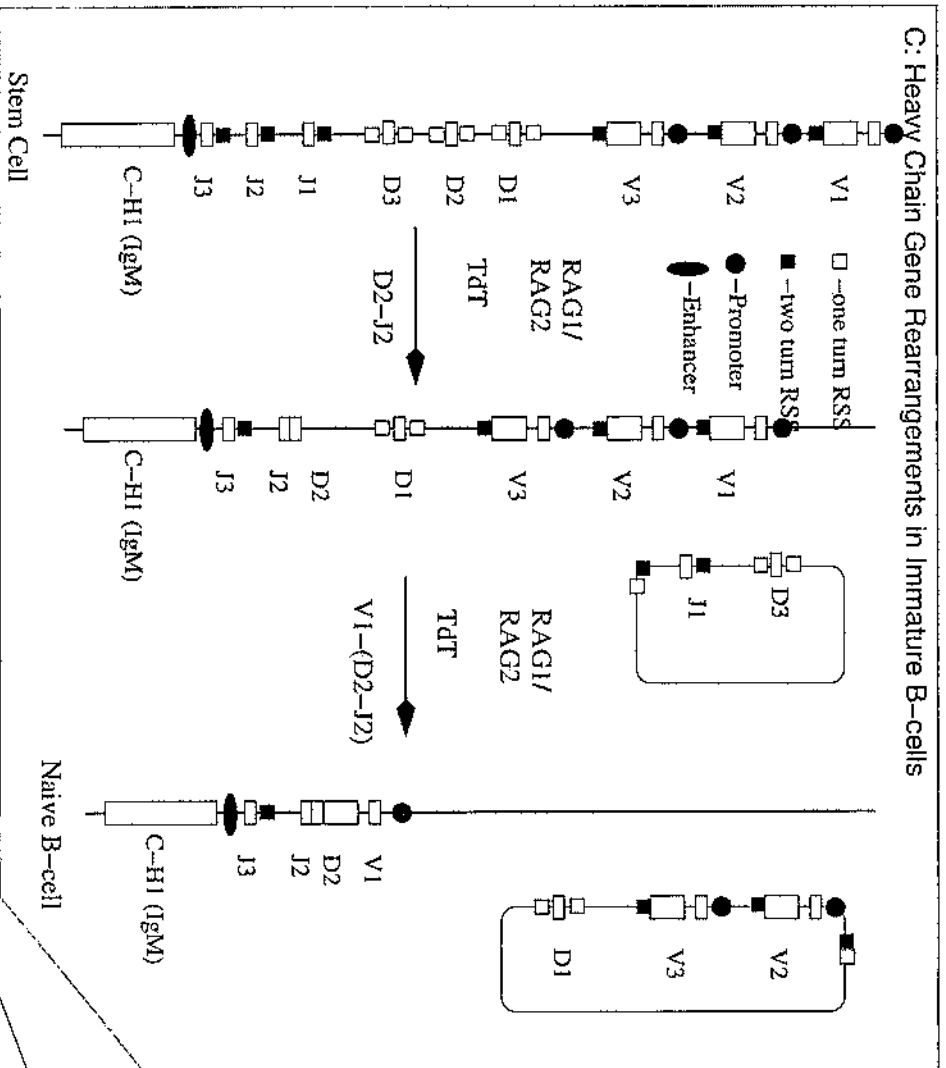
### B: Production of light chain protein



Protein off of ribosome

Light Chain ER

C: Heavy Chain Gene Rearrangements in Immature B-cells



D: Production of Membrane Bound IgM (B-cells) or soluble IgM (plasma cells).

