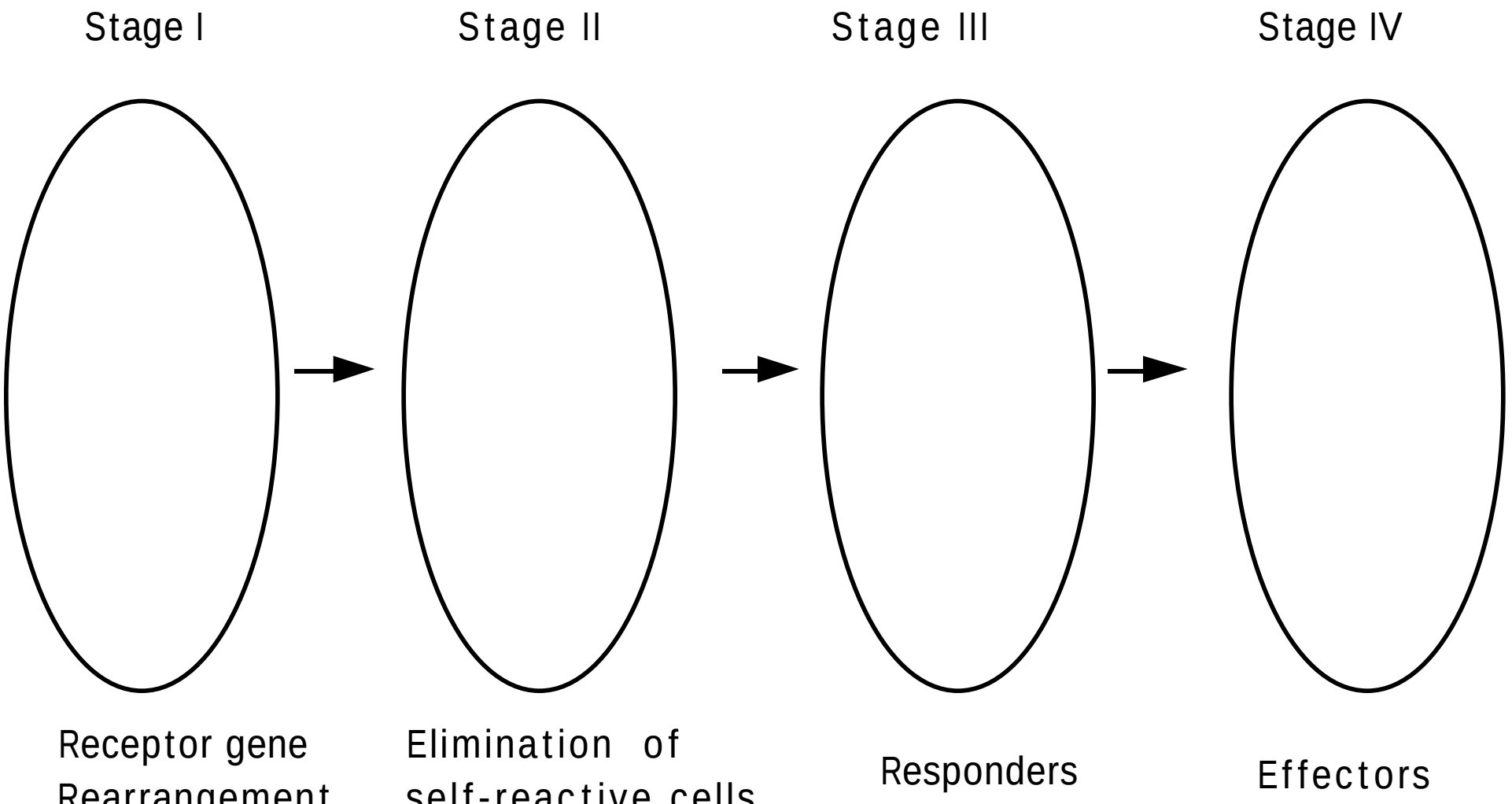
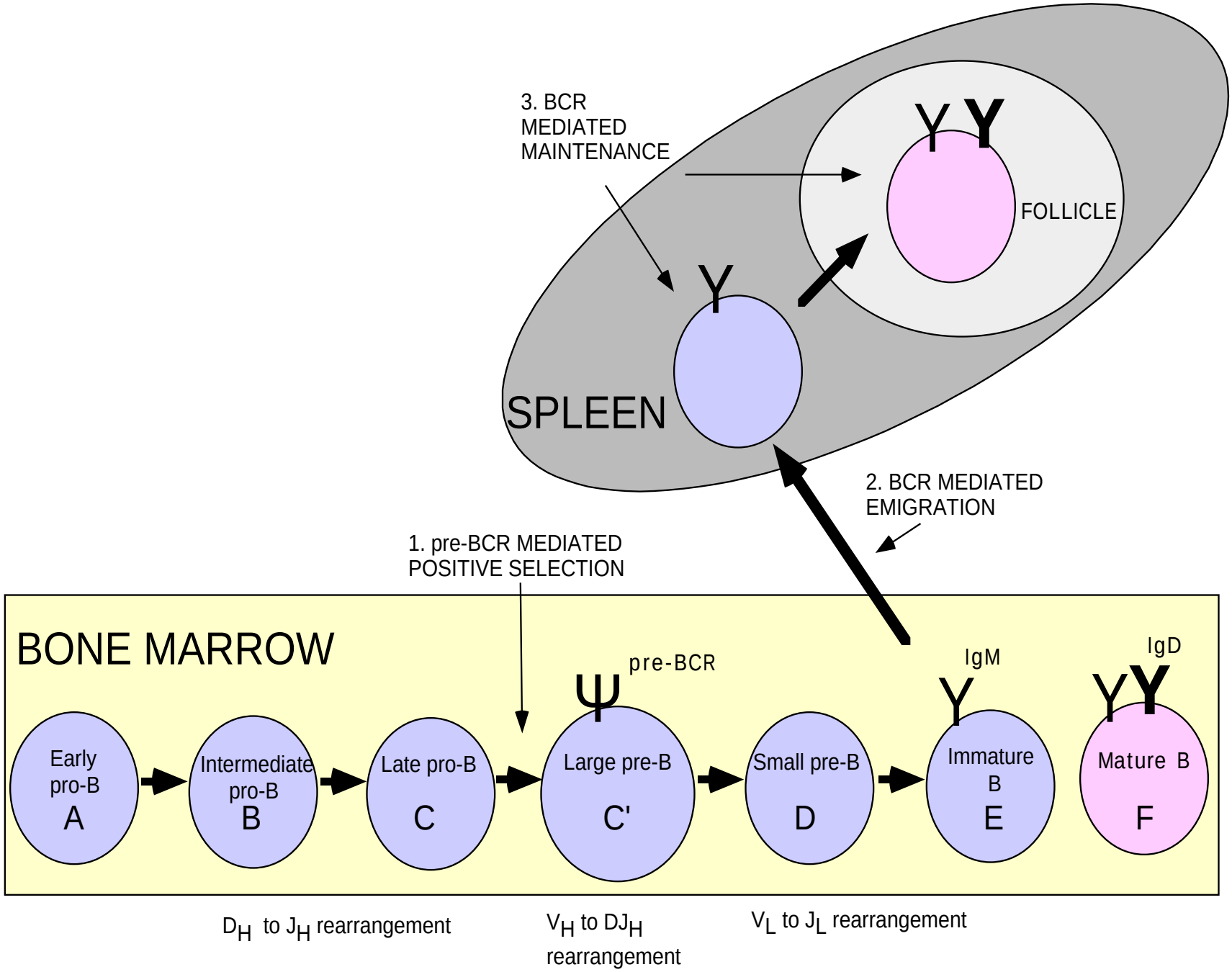
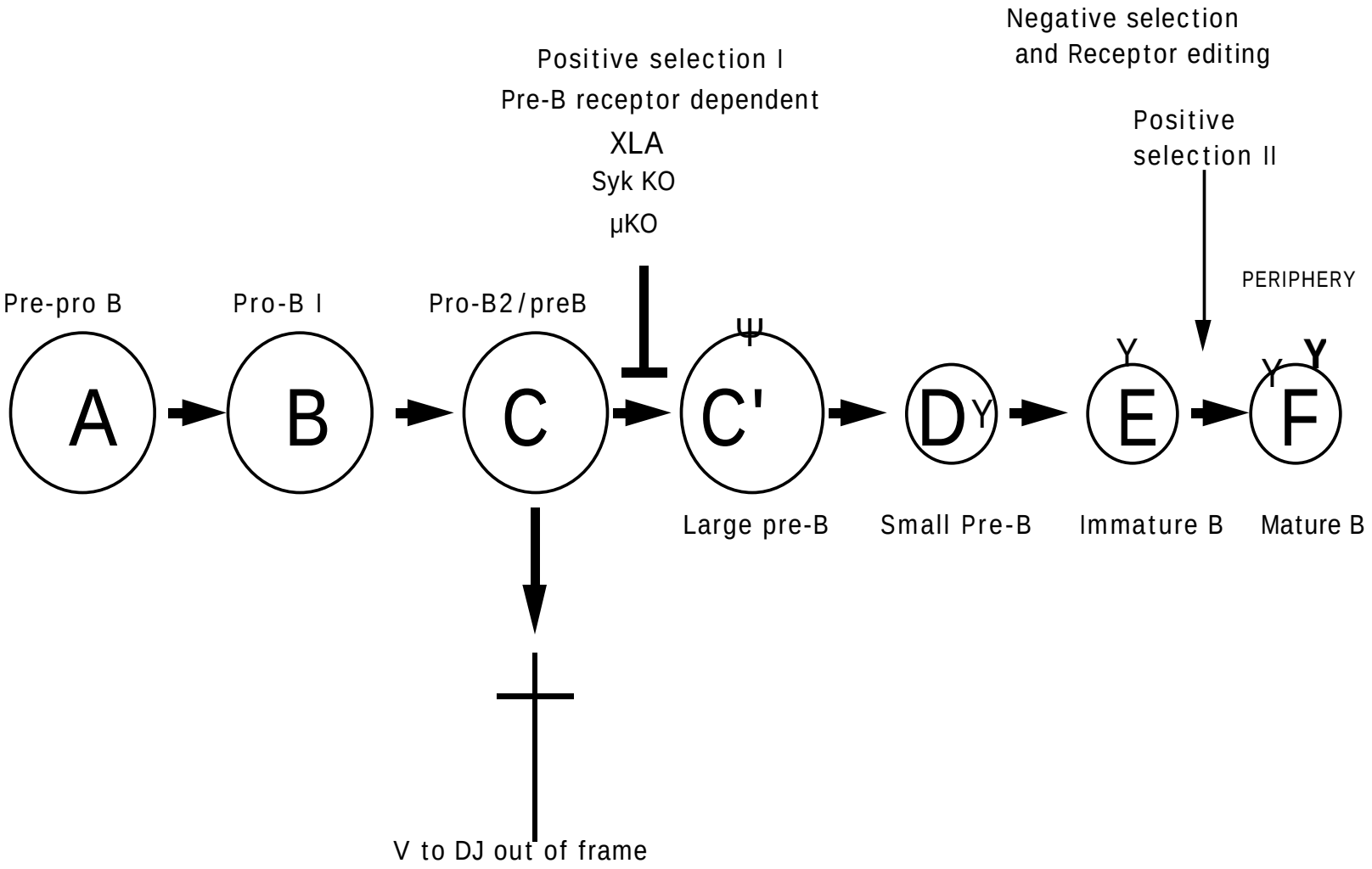
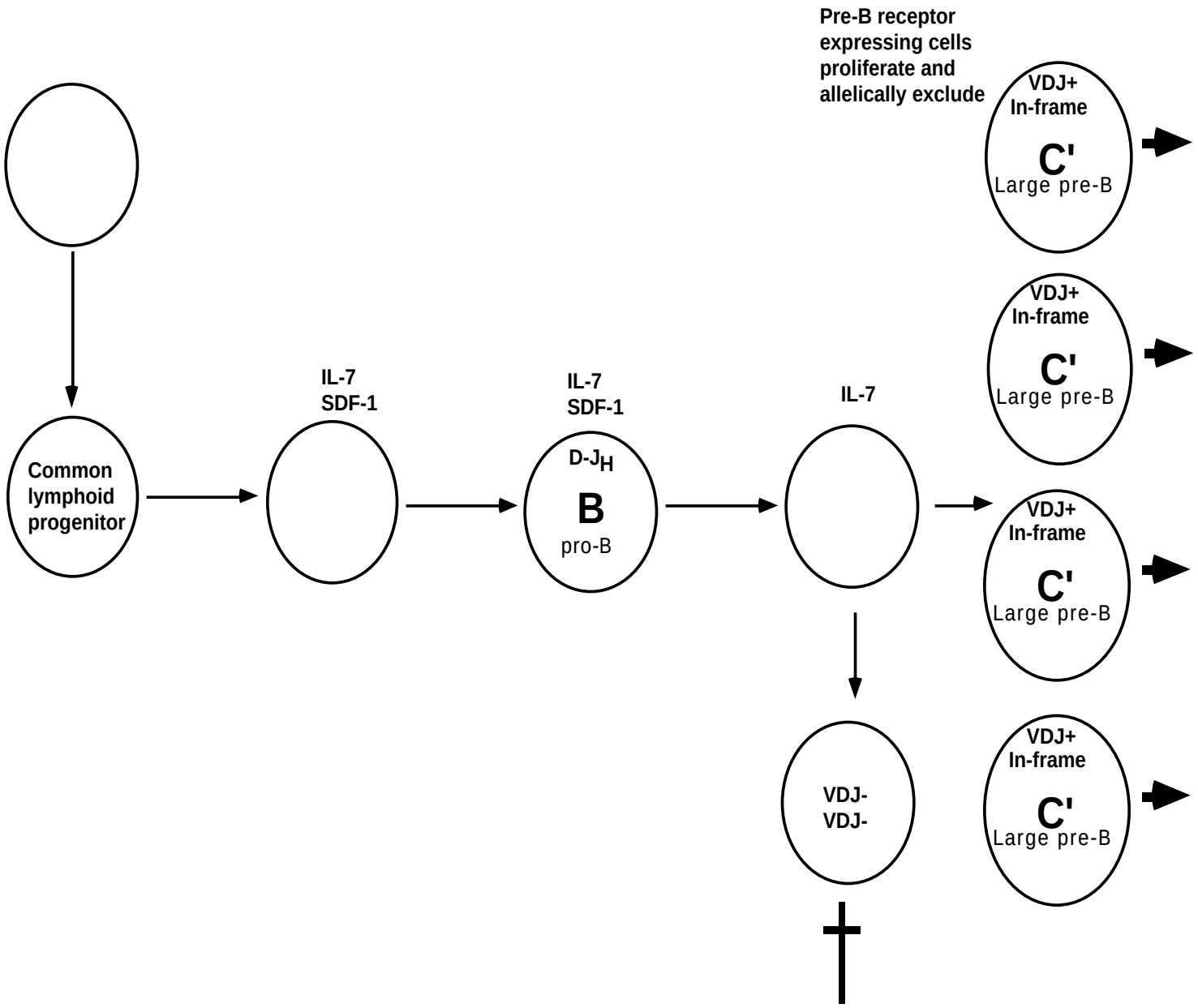
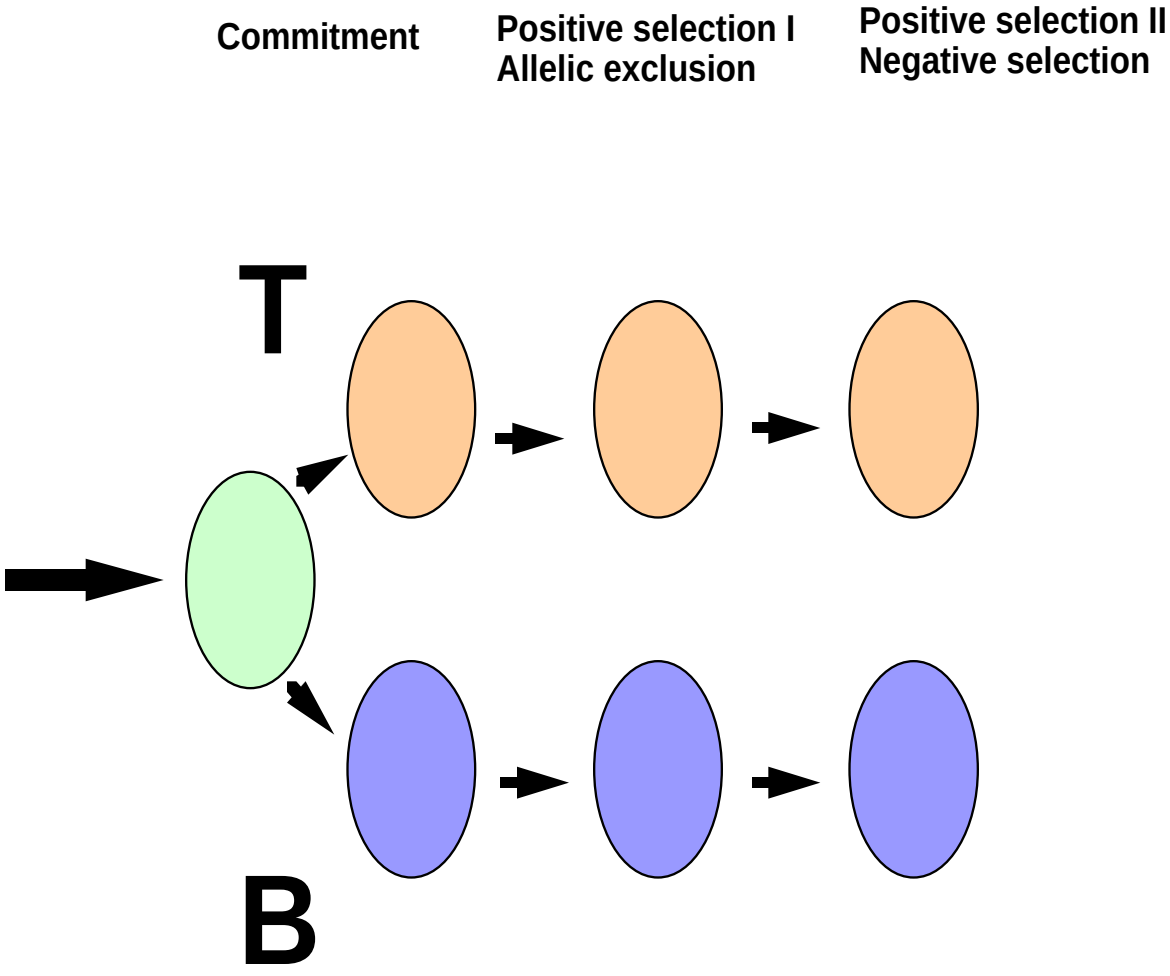

Stage I
Stage II
Stage III
Stage IV
Receptor gene Rearrangement
Elimination of self-reactive cells
Responders
Effectors

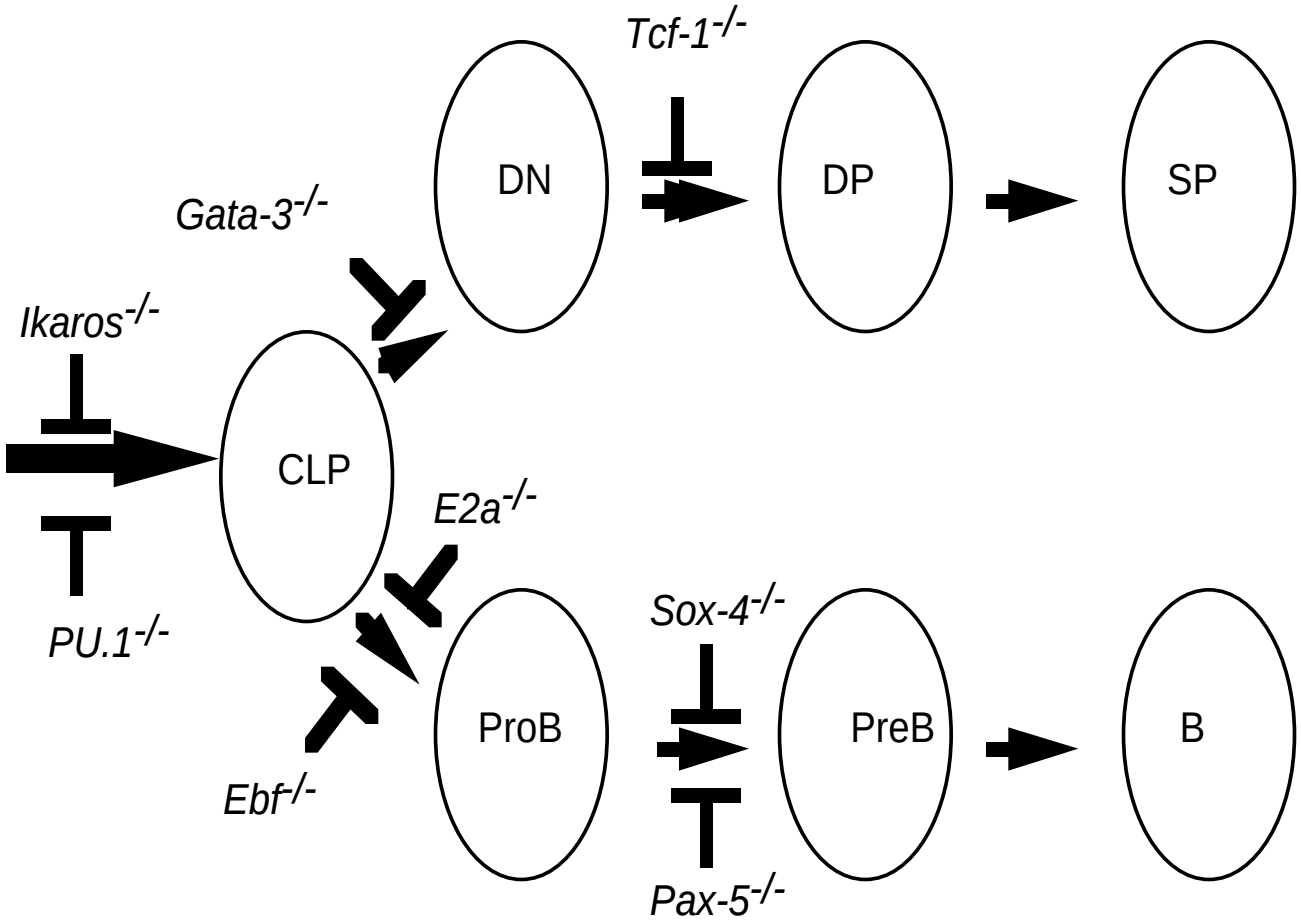

3. BCR
MEDIATED
MAINTENANCE
FOLLICLE
SPLEEN
2. BCR MEDIATED
EMIGRATION
1. pre-BCR MEDIATED
POSITIVE SELECTION
BONE MARROW
pre-BCR
IgM
IgD
Early
pro-B
A
Intermediate
pro-B
B
Late pro-B
C
Large pre-B
C'
Small pre-B
D
Immature
B
E
Mature B
F
D_H to J_H rearrangement
V_H to DJ_H
rearrangement
V_L to J_L rearrangement


Negative selection
and Receptor editing
Positive selection I
Pre-B receptor dependent
XLA
Syk KO
μKO
Positive
selection II
PERIPHERY
Pre-pro B
Pro-B I
Pro-B2/preB
A
B
C
C'
D
E
F
Large pre-B
Small Pre-B
Immature B
Mature B
V to DJ out of frame


Pre-B receptor expressing cells proliferate and allelically exclude
Common lymphoid progenitor
IL-7
SDF-1
IL-7
SDF-1
D-J$_H$
B
pro-B
IL-7
VDJ+
In-frame
C'
Large pre-B
VDJ+
In-frame
C'
Large pre-B
VDJ+
In-frame
C'
Large pre-B
VDJ+
In-frame
C'
Large pre-B
VDJ-
VDJ-

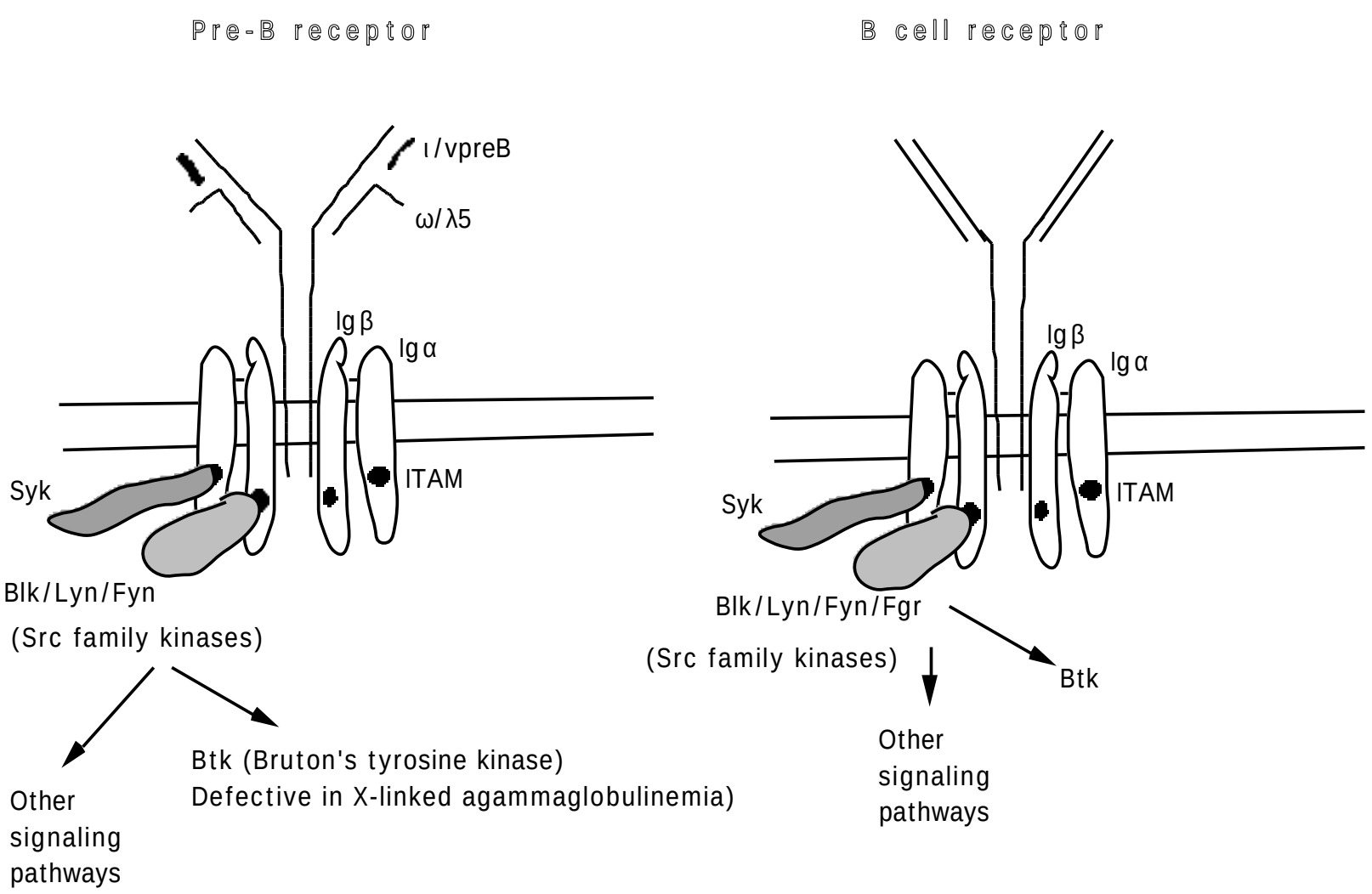

Commitment
Positive selection I
Allelic exclusion
Positive selection II
Negative selection
T
B

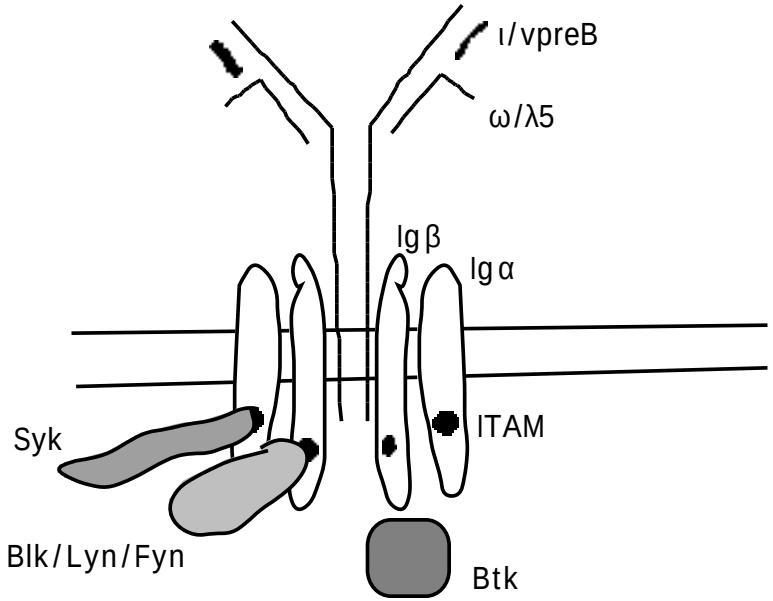
Transcriptional regulation of early lymphoid development



Entry versus commitment

- Commitment implies irreversibility and in wild type B cells has occurred when Ig H-chain gene rearrangement is initiated
- Certain transcription factors such as EBF and E2A are required to turn on genes required early in B cell development
- In the absence of Pax-5 cells “enter” the B lineage but remain highly plastic

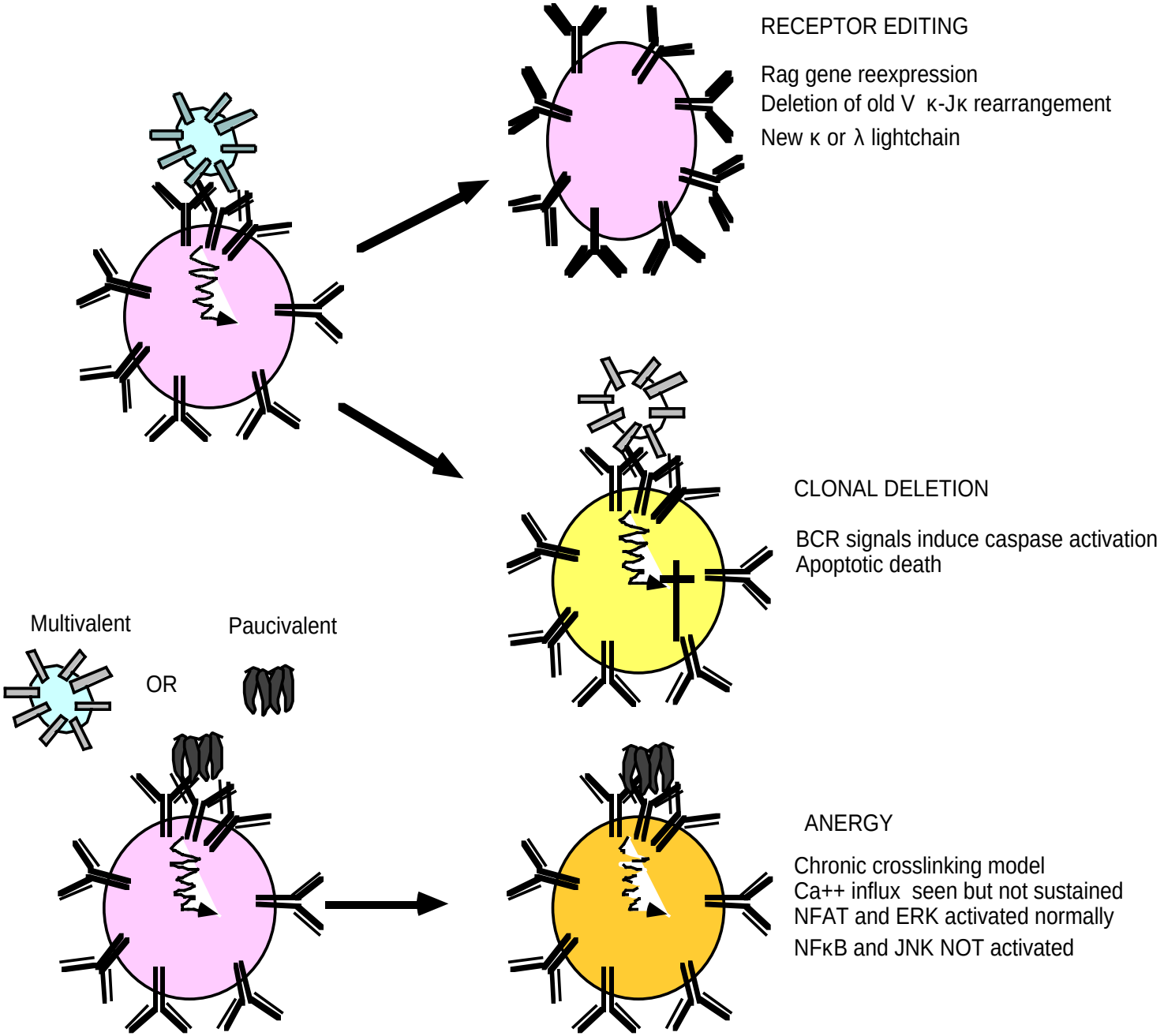

Pre-B receptor
ι/vpreB
ω/λ5
Igβ
Igα
ITAM
Syk
Blk/Lyn/Fyn
(Src family kinases)
Other
signaling
pathways
Btk (Bruton's tyrosine kinase)
Defective in X-linked agammaglobulinemia)
B cell receptor
Igβ
Igα
ITAM
Syk
Blk/Lyn/Fyn/Fgr
(Src family kinases)
Btk
Other
signaling
pathways

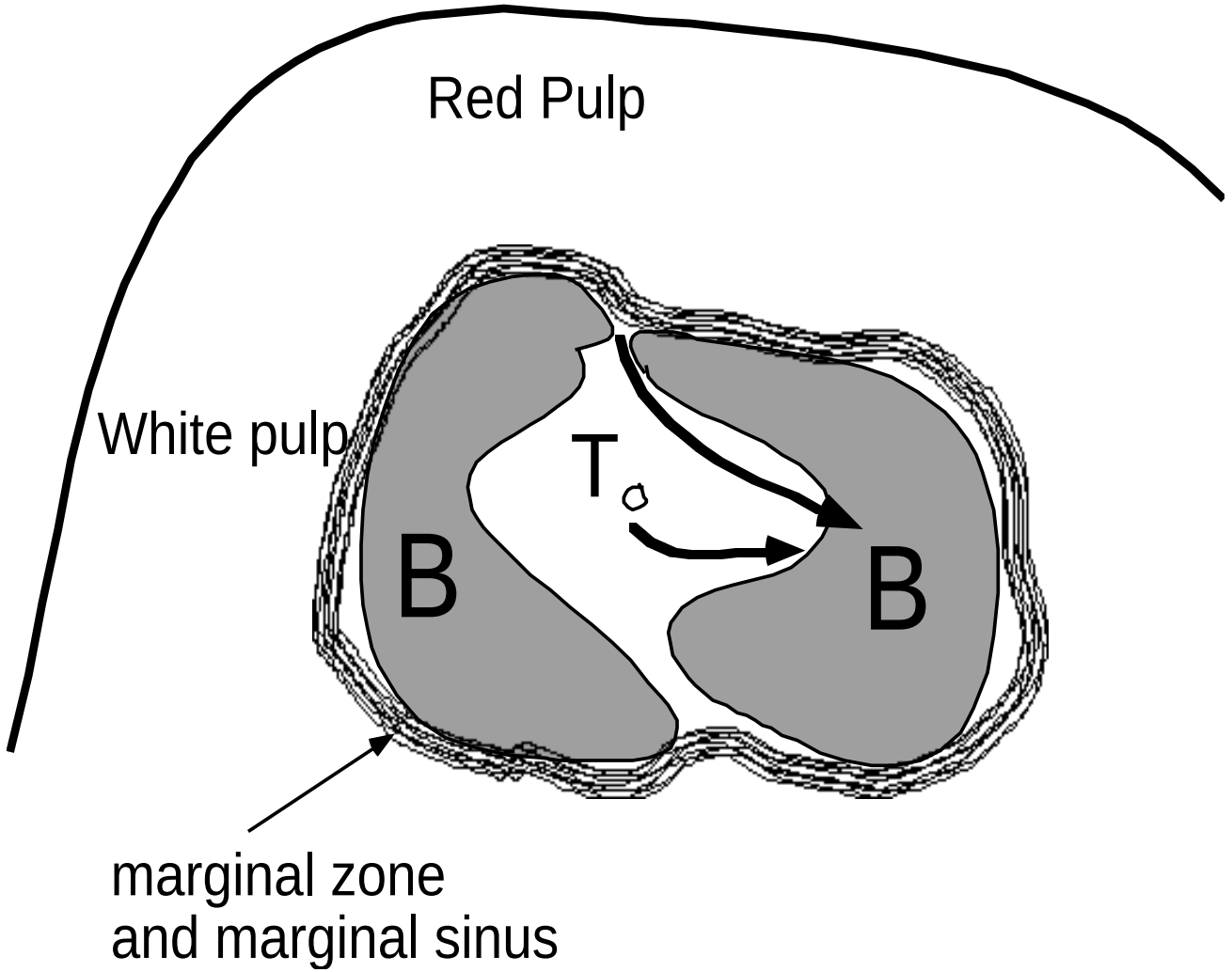


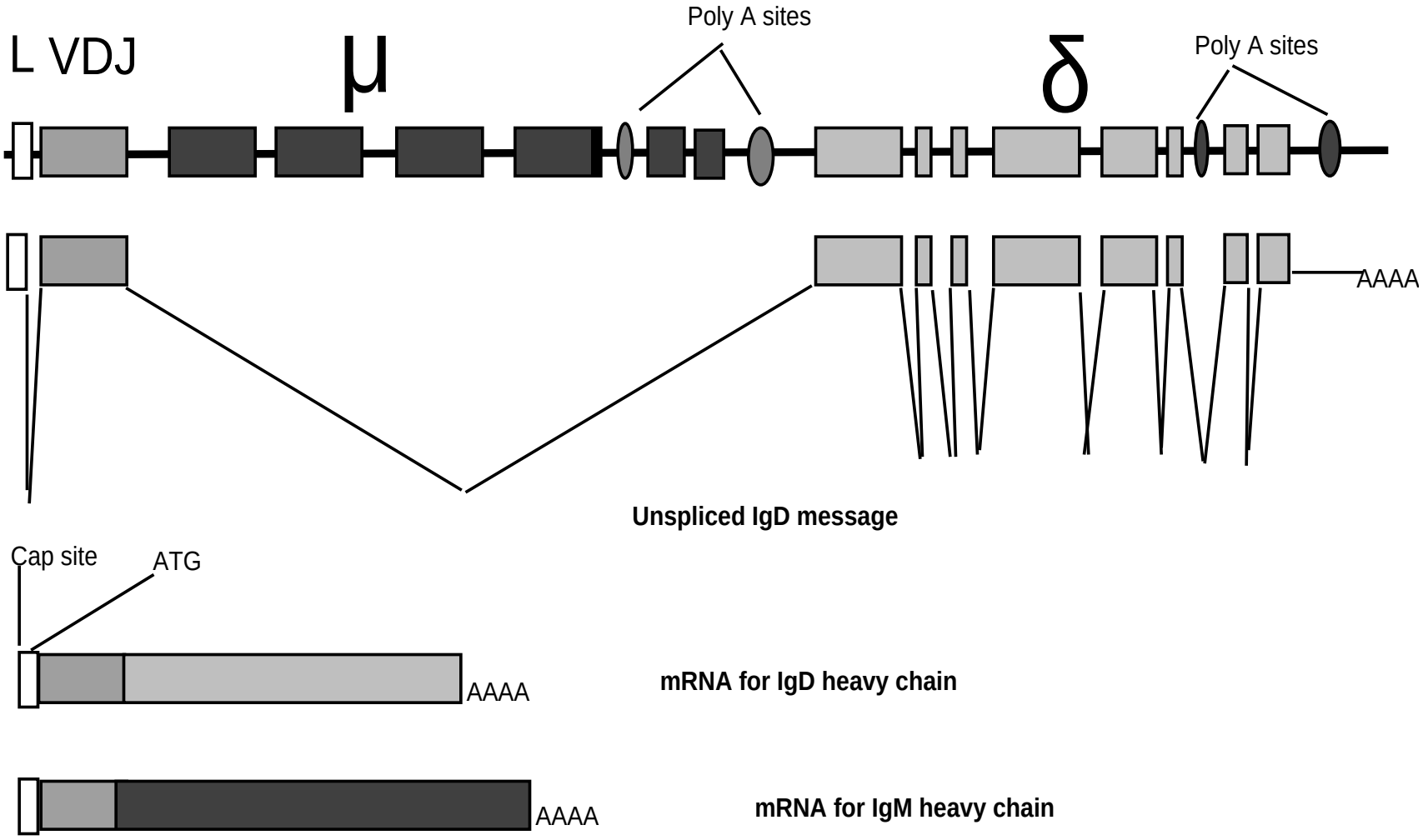
1)Survival
2) Proliferation
3) Allelic exclusion
4) Induction of κ
rearrangement
5) Shut off of surrogate
light chain expression

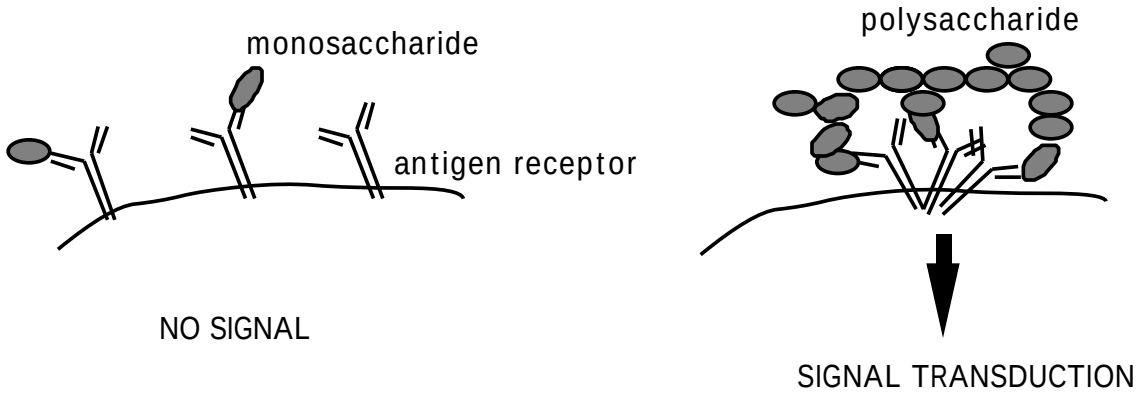
B cell tolerance

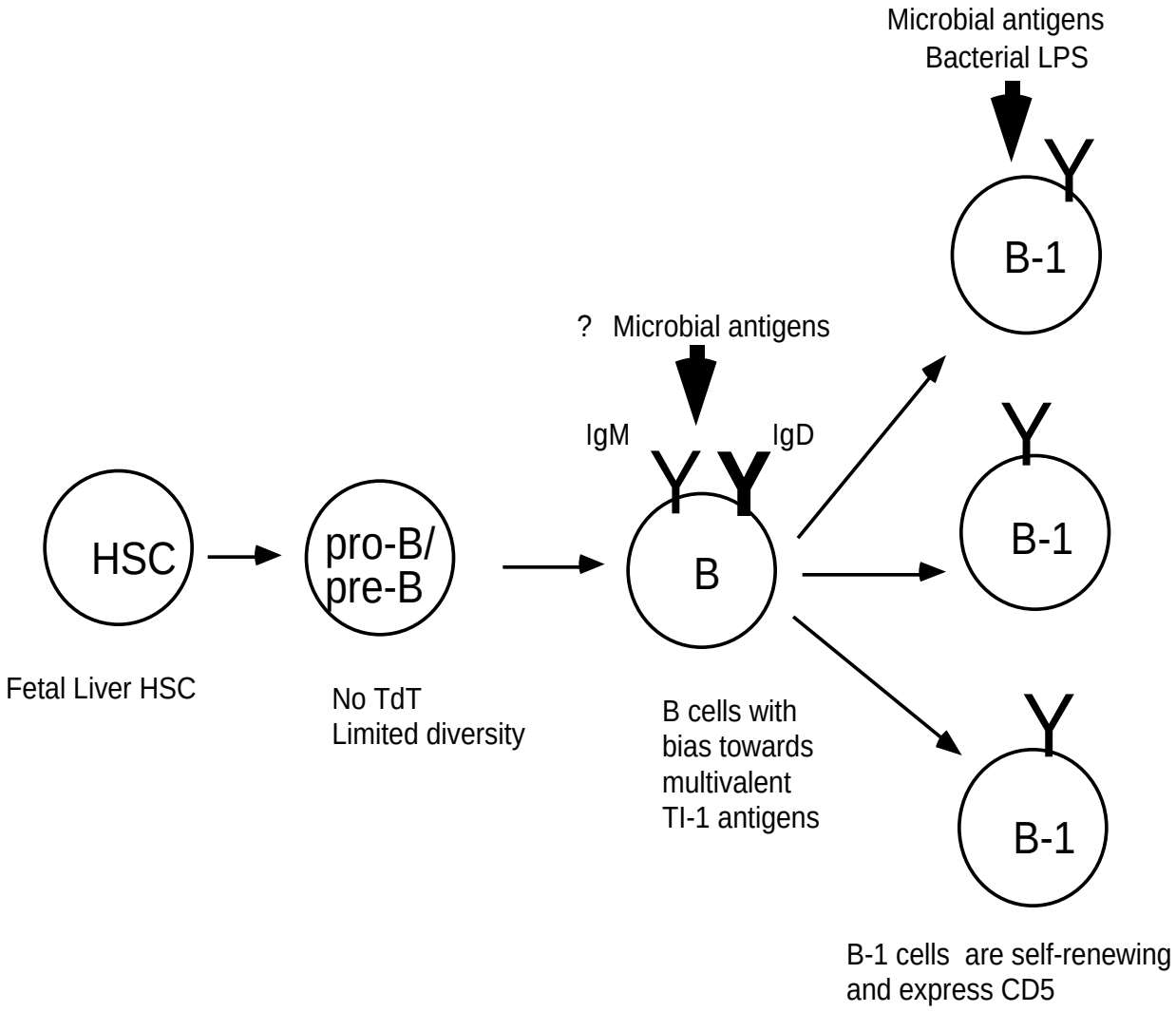
See Immunobiology, by Janeway,C., Travers, P., Walport, M. and Capra, J., Garland Publishing, 5th edition, 2001 & Cellular and Molecular Immunology by Abbas, A., Pober, J., and Lichtman, A., W B Saunders; 4th edition.


RECEPTOR EDITING
Rag gene reexpression
Deletion of old V κ-Jκ rearrangement
New κ or λ lightchain
CLONAL DELETION
BCR signals induce caspase activation
Apoptotic death
Multivalent
OR
Paucivalent
ANERGY
Chronic crosslinking model
Ca++ influx seen but not sustained
NFAT and ERK activated normally
NFκB and JNK NOT activated


Red Pulp
White pulp
T
B
B
marginal zone
and marginal sinus

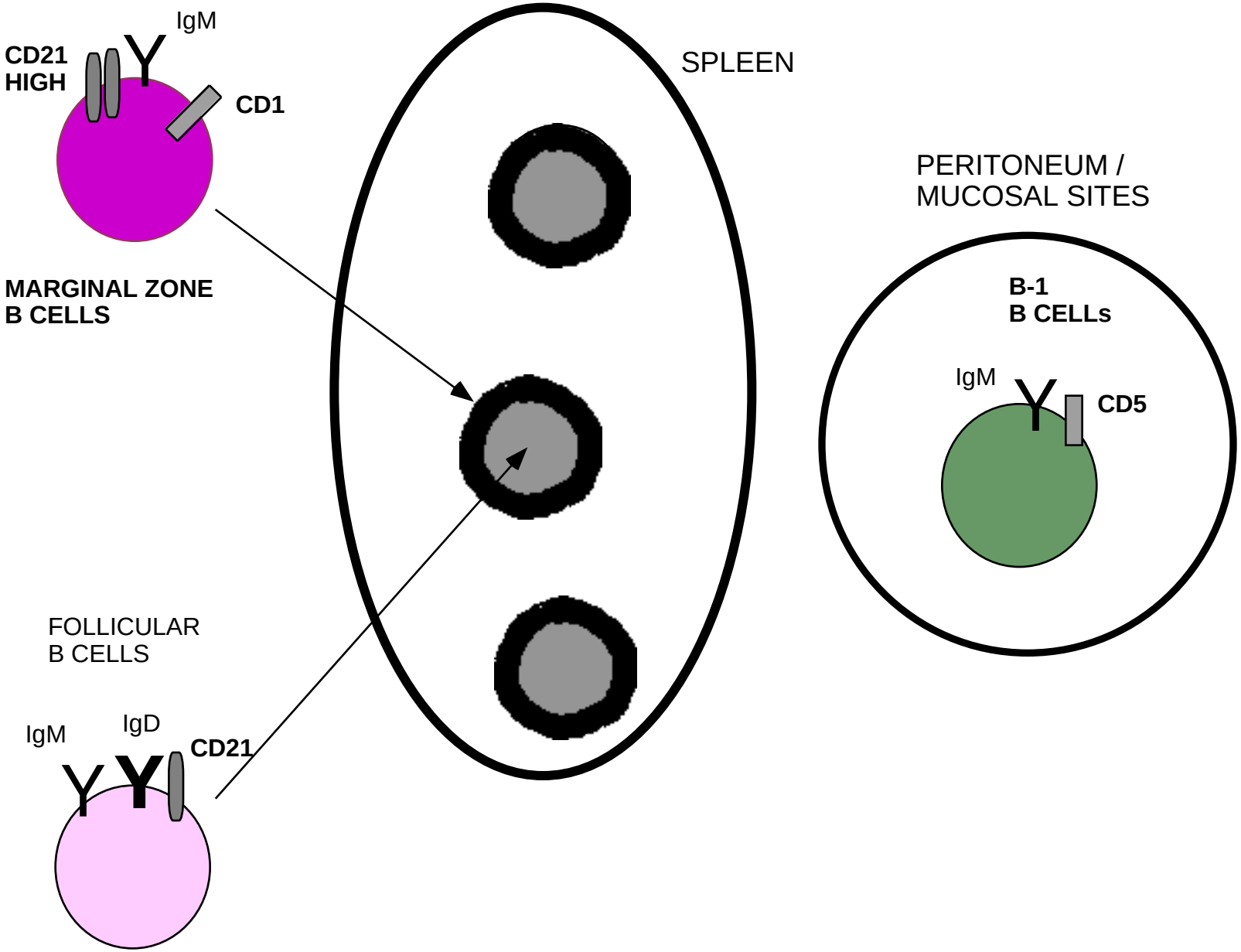

L VDJ
μ
Poly A sites
δ
Poly A sites
AAAA
Unspliced IgD message
Cap site
ATG
AAAA
mRNA for IgD heavy chain
AAAA
mRNA for IgM heavy chain


monosaccharide
antigen receptor
NO SIGNAL
polysaccharide
SIGNAL TRANSDUCTION



The B-1/CD5 B "lineage"

THREE DISTINCT TYPES OF PERIPHERAL B LYMPHOCYTES



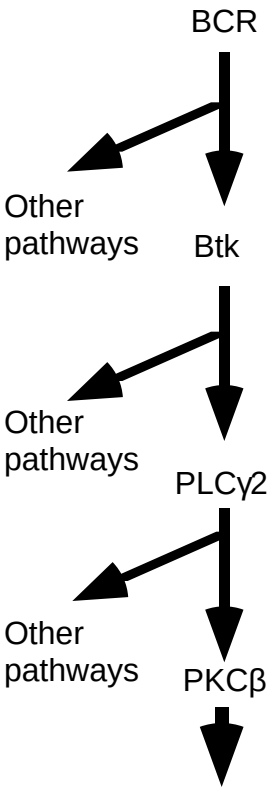
MZ and Follicular B cells

See Immunobiology, by Janeway,C., Travers, P., Walport, M. and Capra, J., Garland Publishing, 5th edition, 2001 & Cellular and Molecular Immunology by Abbas, A., Pober, J., and Lichtman, A., W B Saunders; 4th edition.

Are only “chosen” B cells selected by endogenous antigens?

OR

Do all B cells get tickled via the antigen receptor ?



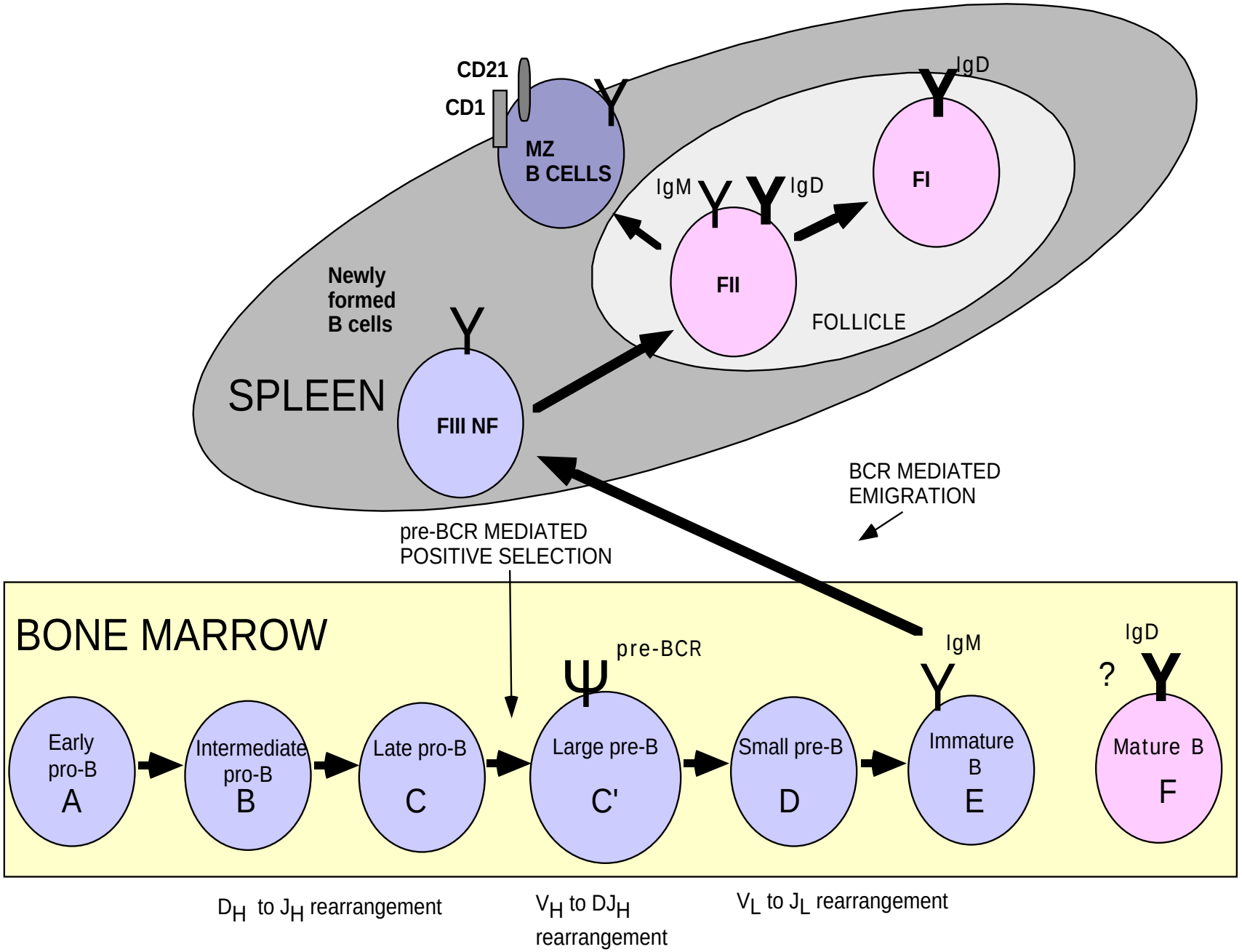
Mutation	Signal Strength	MZ	FO	B-1
BCR	"No BCR signals"	-	-	-
Btk	"Weak BCR signals"	+	-	-
PLCγ2	"Weak BCR signals"	+*	-	-
PKCβ	" Weak and Intermediate BCR signals"	+	+	-
None	"Weak, Intermediate, and Strong BCR signals"	+	+	+

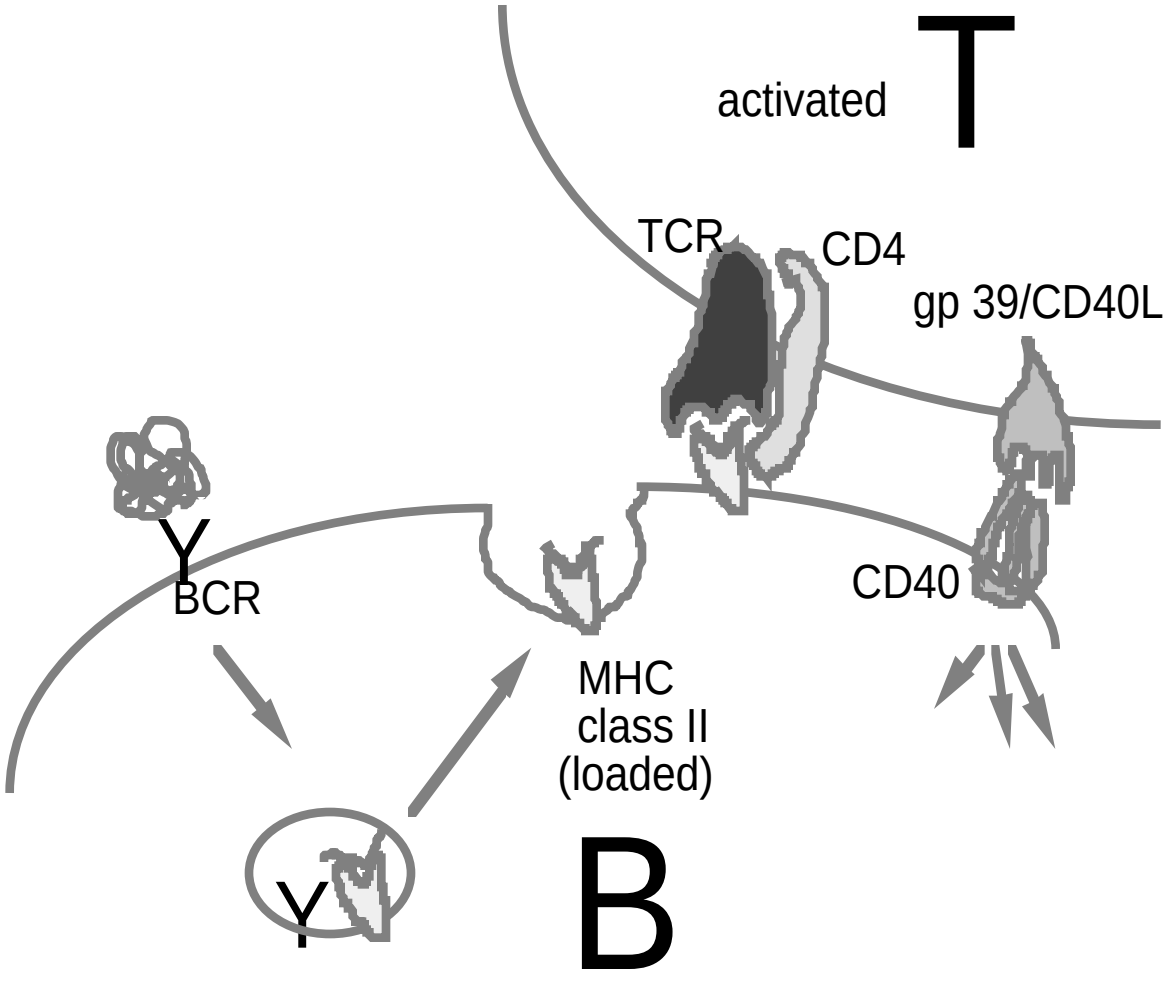
LONG-LIVED PERIPHERAL B CELL POPULATIONS

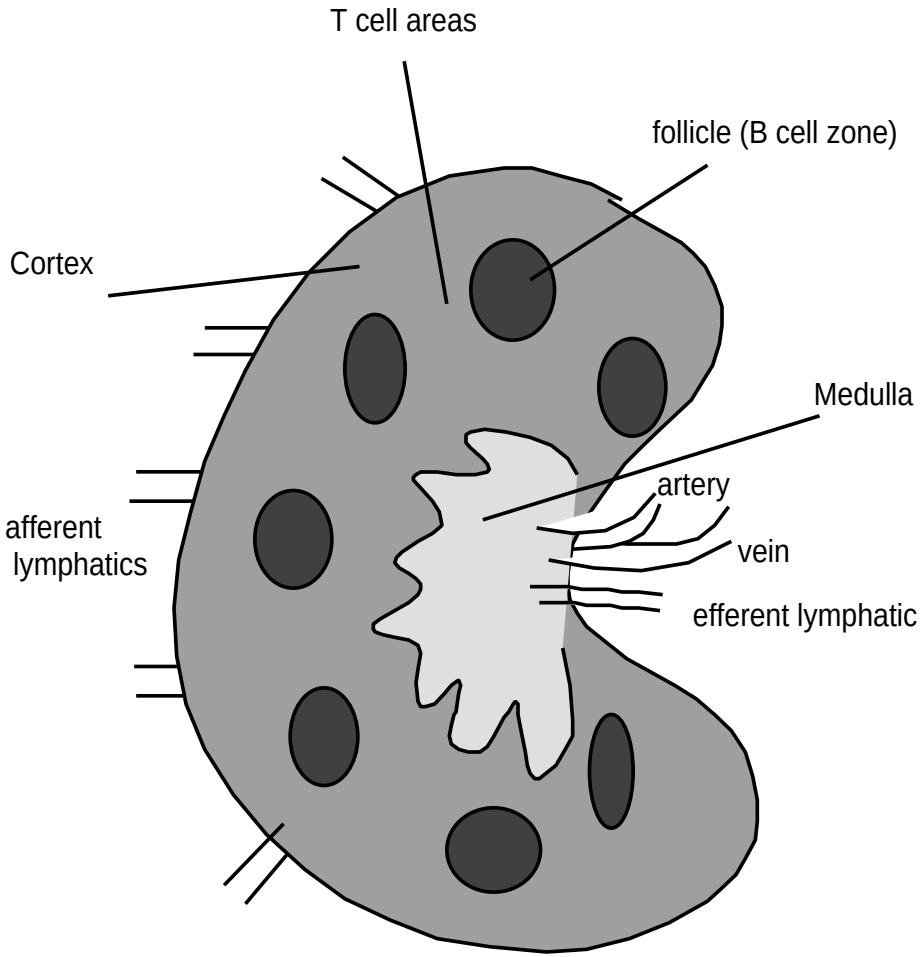
Strong BCR Signals …….B-1 cells

Intermediate strength BCR signals…….Follicular B cells

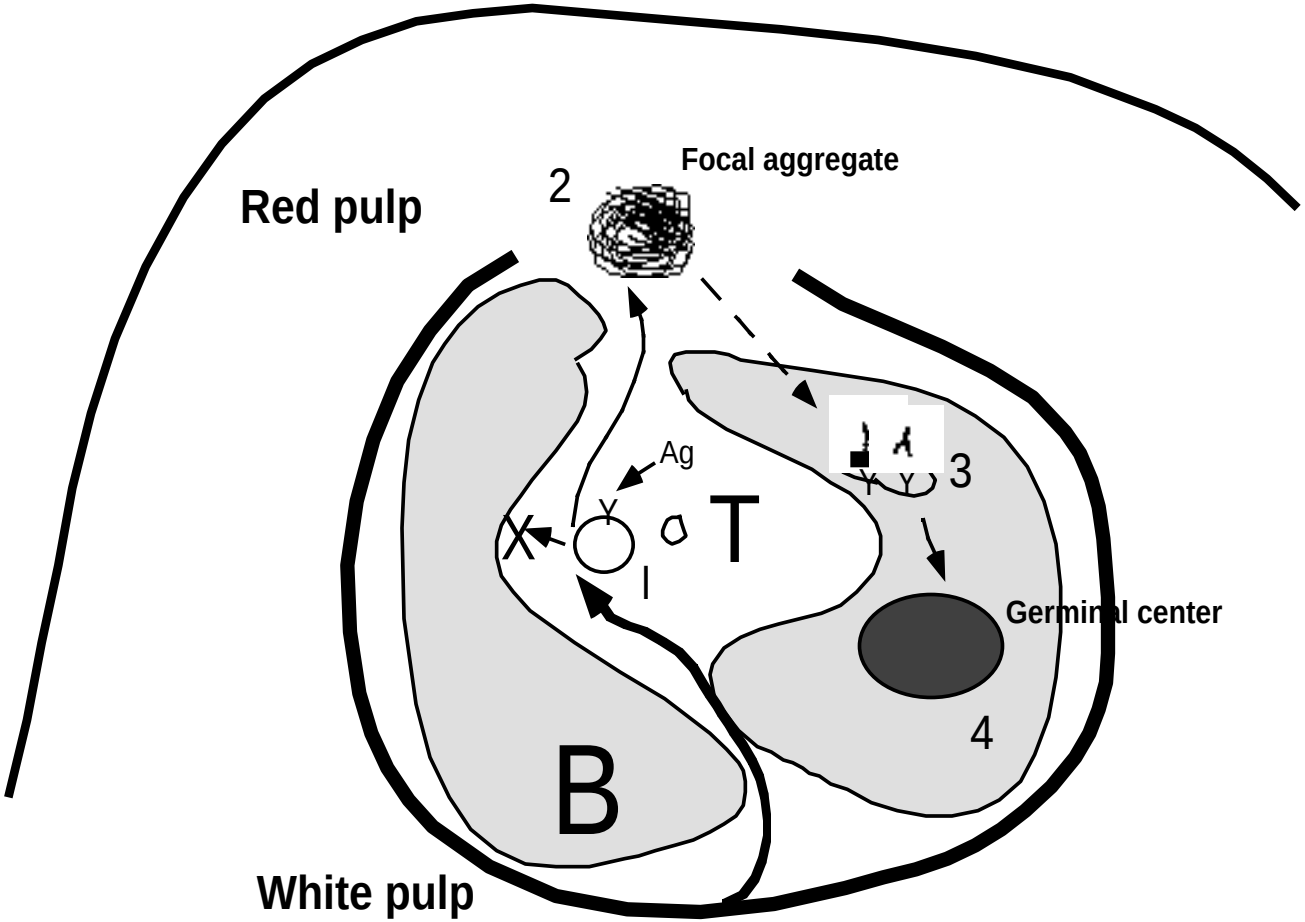
Relatively weak BCR signals …..MZ B cells

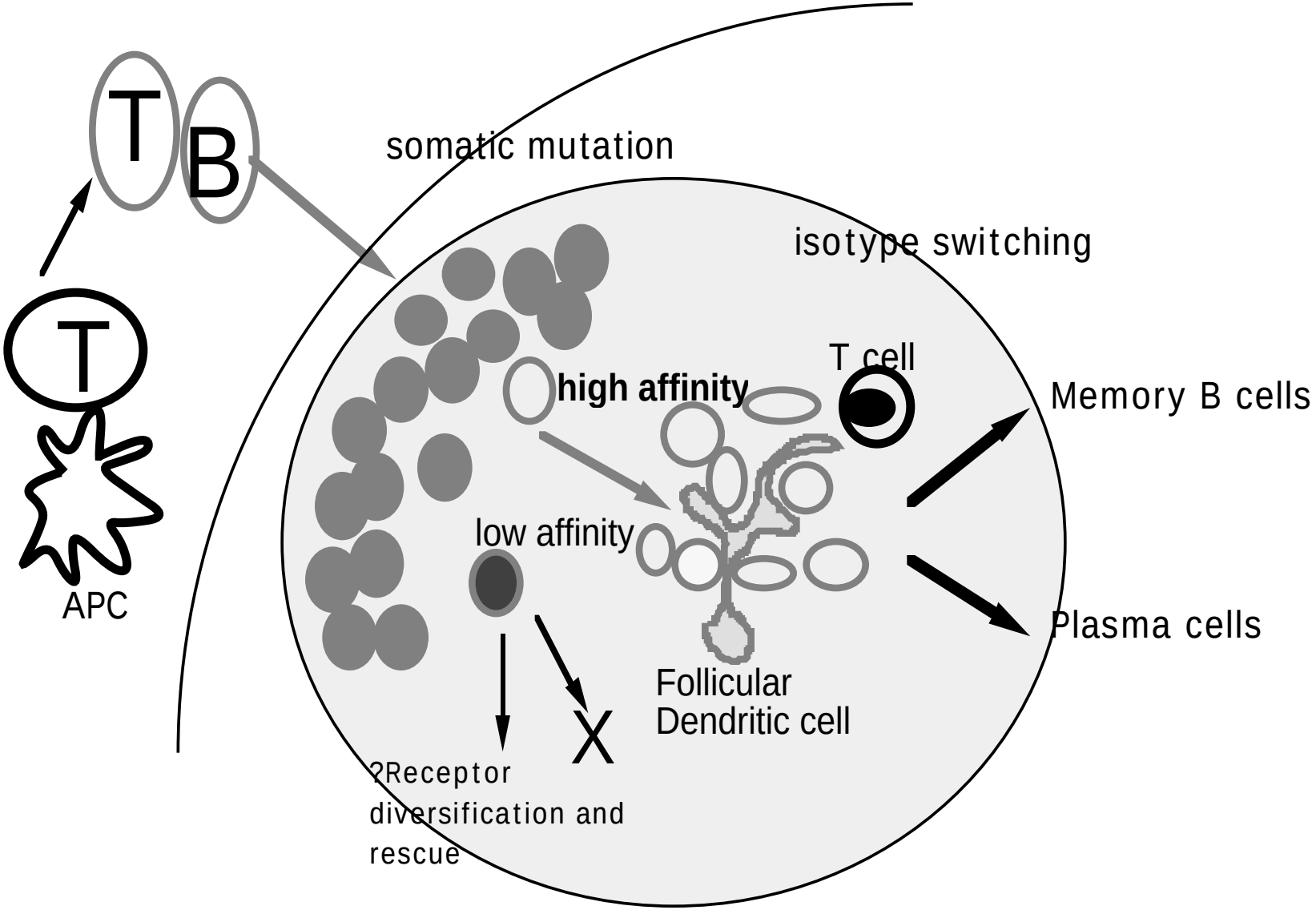

CD21
CD1
MZ
B CELLS
IgD
FI
IgM
IgD
FII
FOLLICLE
Newly
formed
B cells
SPLEEN
FIII NF
BCR MEDIATED
EMIGRATION
pre-BCR MEDIATED
POSITIVE SELECTION
BONE MARROW
pre-BCR
IgM
IgD
?
Early
pro-B
A
Intermediate
pro-B
B
Late pro-B
C
Large pre-B
C'
Small pre-B
D
Immature
B
E
Mature B
F
DH to JH rearrangement
VH to DJH
rearrangement
VL to JL rearrangement

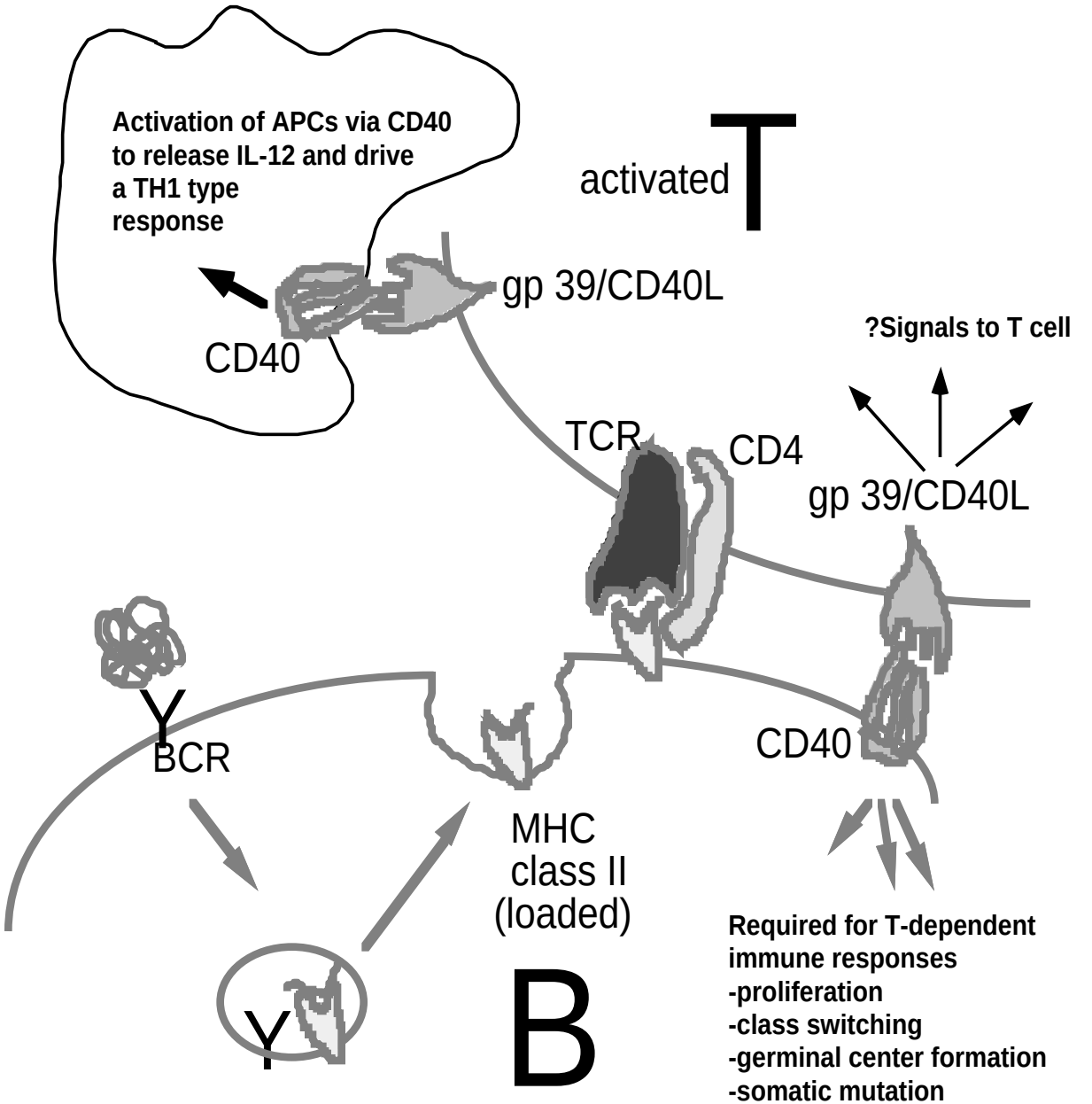

activated
T
TCR
CD4
gp 39/CD40L
Y
BCR
CD40
MHC
class II
(loaded)
Y
B

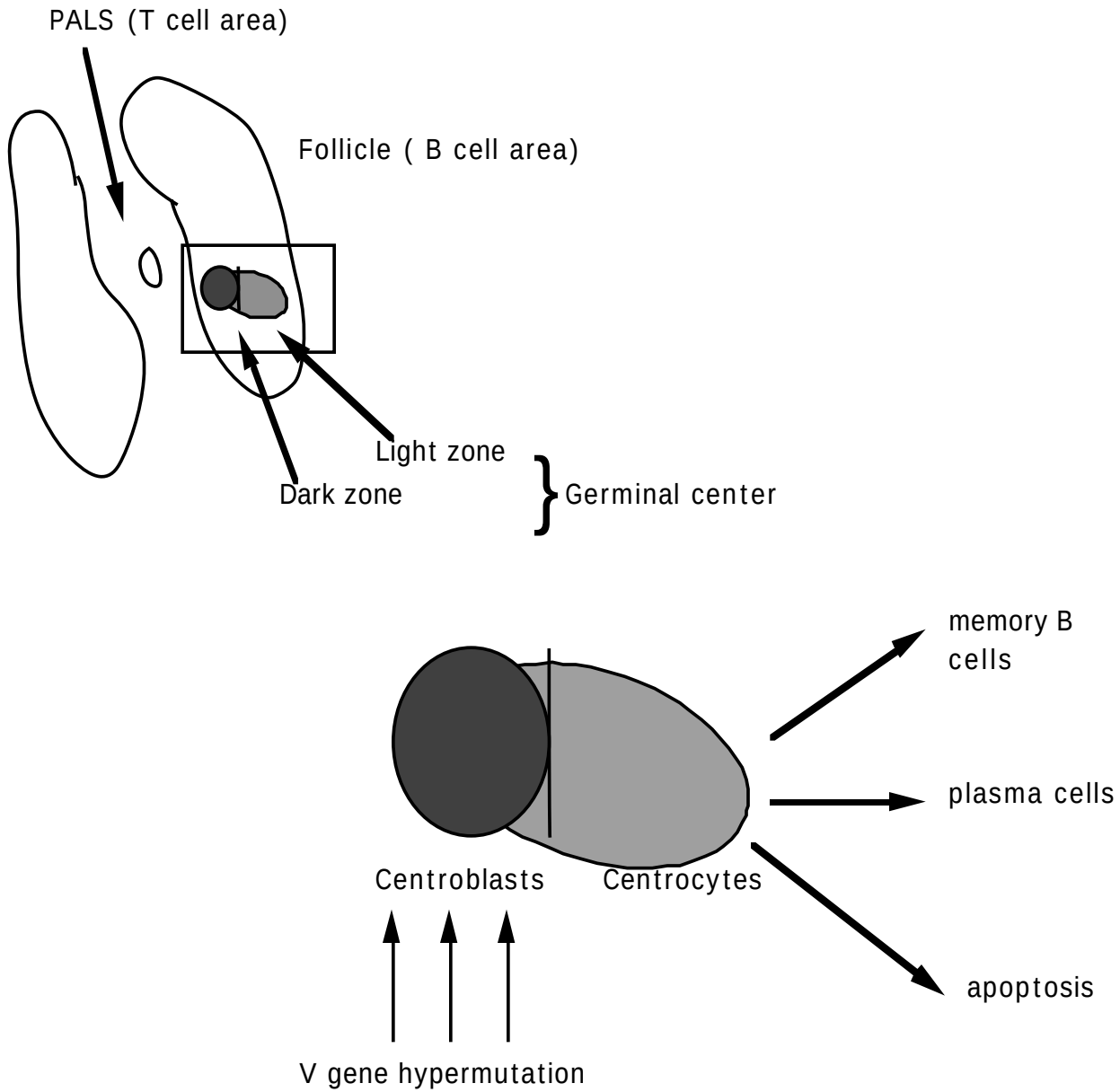


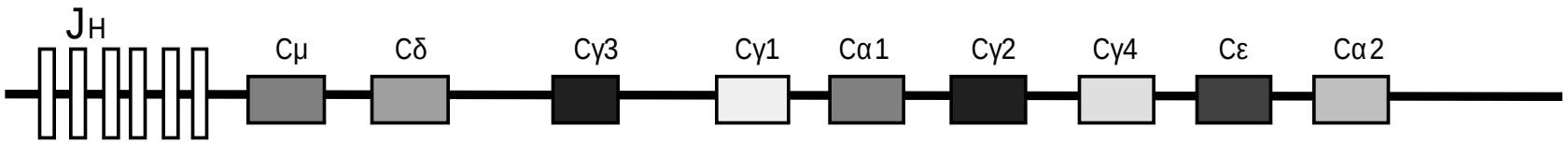
1.Dendritic cells (interdigitating) in T cell zones
2. Follicular dendritic cells in B cell areas
3. Macrophages everywhere

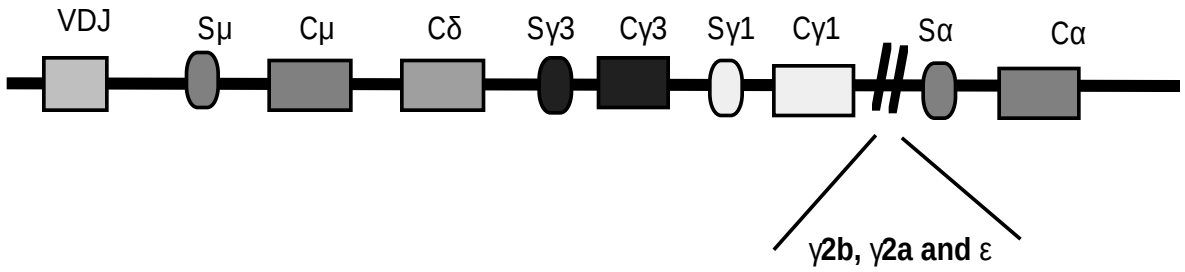

Red pulp
2
Focal aggregate
Ag
3
T
Germinal center
4
B
White pulp

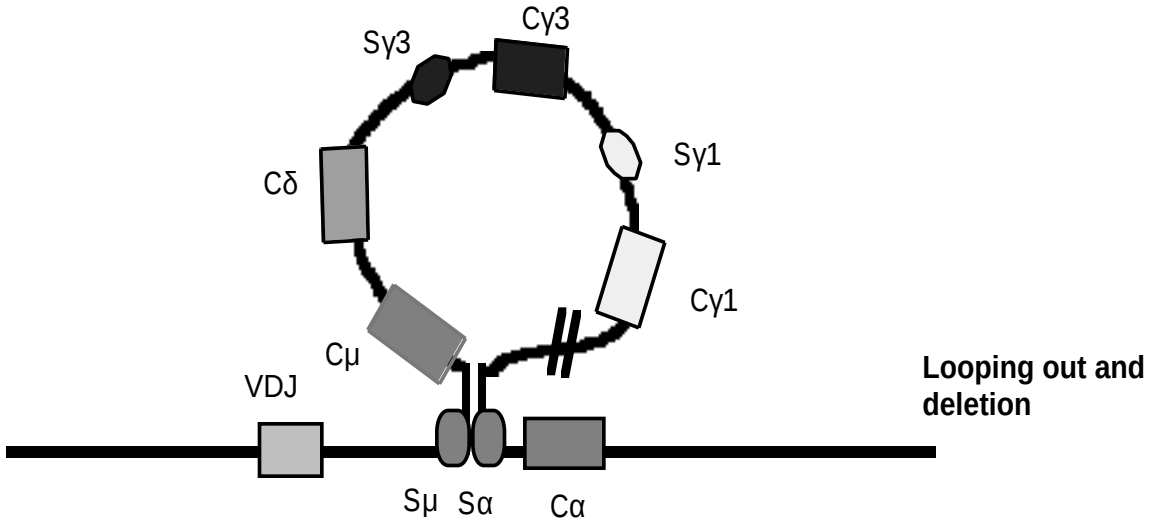

T
B
T
APC
somatic mutation
isotype switching
T cell
high affinity
Memory B cells
low affinity
Plasma cells
Follicular
Dendritic cell
X
?Receptor
diversification and
rescue

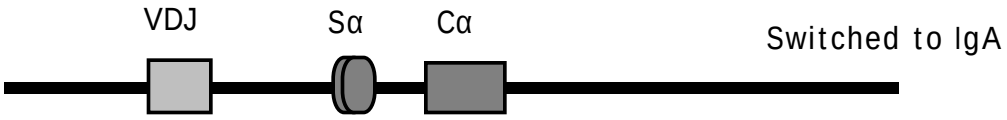

Activation of APCs via CD40
to release IL-12 and drive
a TH1 type
response
activated T
gp 39/CD40L
CD40
?Signals to T cell
TCR
CD4
gp 39/CD40L
Y
BCR
CD40
MHC
class II
(loaded)
Y
B
Required for T-dependent
immune responses
-proliferation
-class switching
-germinal center formation
-somatic mutation
CD40L mutations lead to
X-linked hyper-IgM syndrome


PALS (T cell area)
Follicle (B cell area)
Light zone
Dark zone
Germinal center
memory B cells
plasma cells
apoptosis
Centroblasts
Centrocytes
V gene hypermutation

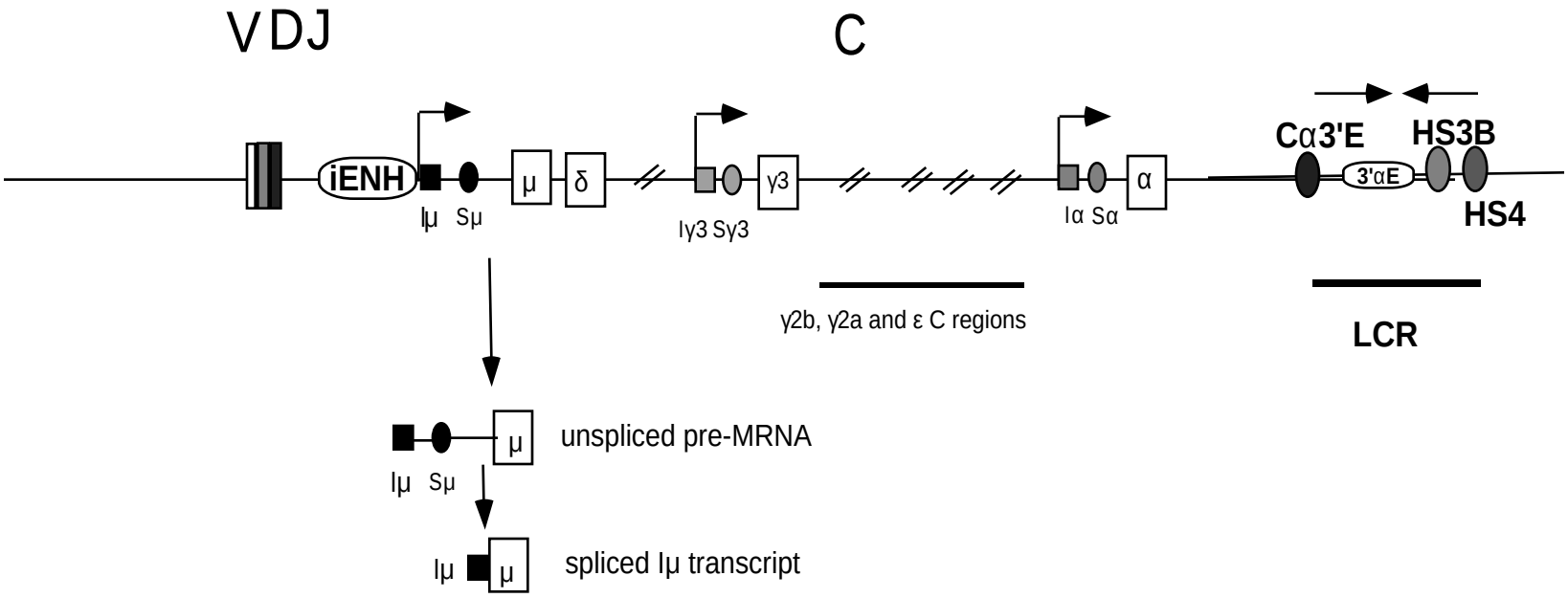

JH
Cμ
Cδ
Cγ3
Cγ1
Cα1
Cγ2
Cγ4
Cε
Cα2


VDJ
Sμ
Cμ
Cδ
Sγ3
Cγ3
Sγ1
Cγ1
Sα
Cα
γ2b, γ2a and ε


Cγ3
Sγ3
Sγ1
Cδ
Cγ1
Cμ
VDJ
Looping out and deletion
Sμ
Sα
Cα


VDJ
Sα
Cα
Switched to IgA

Switch regions and I-region promoters



Class Switching (Murine)

1. IL-4 promotes switching to IgG1 and IgE
2. TGF-β promotes switching to IgA
3. γ-IFN promotes switching to IgG2a

Somatic mutation-I

1. Point substitutions. Non-templated single base changes in rearranged H- and L-chain V region genes

2. Requires T cell help, occurs in centrocytes

3. 10^{-4} to 10^{-3} base pairs/generation

4. Bell shaped curve of mutations starts in leader intron and ends about 1.5 kb downstream

5. Hotspot motifs

6. Transitions more common than transversions

SOMATIC MUTATION -II

7. Requires enhancer

8. Mechanism:AID DEPENDENT DNA DEAMINATION

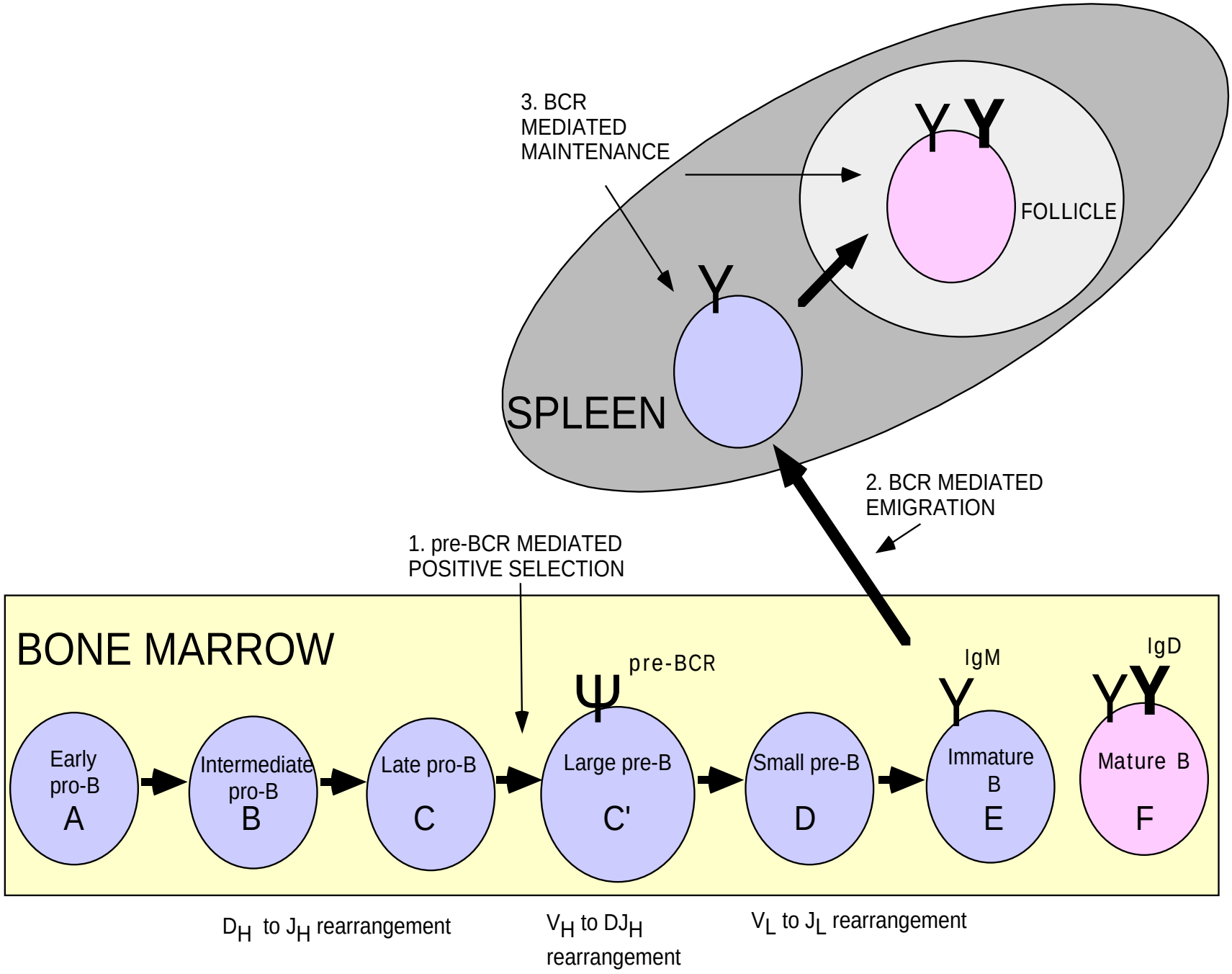
a. Cytosines converted to uracils

b. Replication or error prone repair generates mutations

9. Accessibility? ? Need for transcription??

AID required for both class switching and somatic mutation

- AID is a novel Activation induced cytidine deaminase
- Related to a protein involved in RNA editing
- Required for class switching and also for somatic mutation
- Aid-/- mice have large germinal centers
- Humans lacking AID present with


3. BCR
MEDIATED
MAINTENANCE
FOLLICLE
SPLEEN
2. BCR MEDIATED
EMIGRATION
1. pre-BCR MEDIATED
POSITIVE SELECTION
BONE MARROW
pre-BCR
IgM
IgD
Early
pro-B
A
Intermediate
pro-B
B
Late pro-B
C
Large pre-B
C'
Small pre-B
D
Immature
B
E
Mature B
F
D_H to J_H rearrangement
V_H to DJ_H
rearrangement
V_L to J_L rearrangement

For more information and examples, see Immunobiology, by Janeway,C., Travers, P., Walport, M. and Capra, J., Garland Publishing, 5th edition, 2001 & Cellular and Molecular Immunology by Abbas, A., Pober, J., and Lichtman, A., W B Saunders; 4th edition.