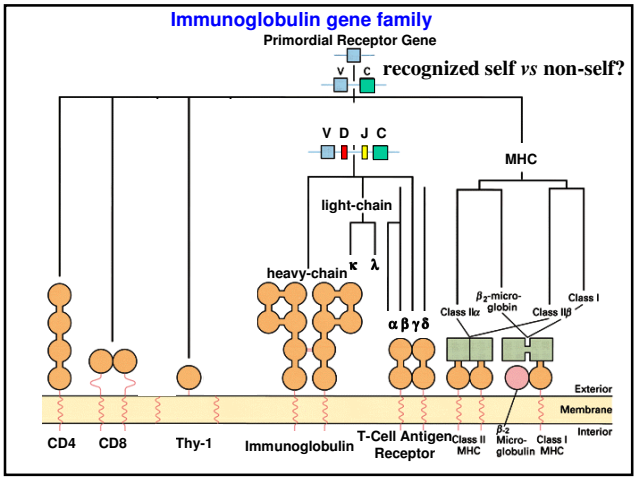
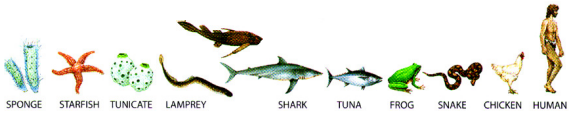
Lecture 2

Immunoglobulin Isotype Class Switching



Big-Bang Theory of Immunology



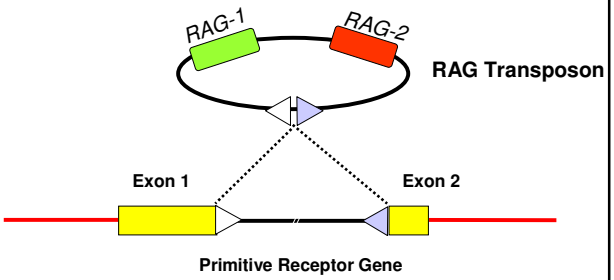
B/T Lymphocytes

450 million years ago

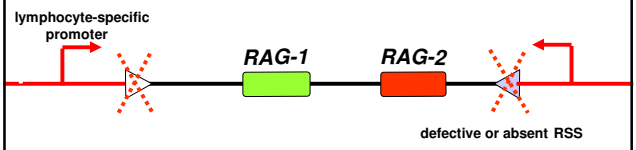
How was the recombination system created?

How do you control such a system?

Transposable elements created the first antigen receptor genes

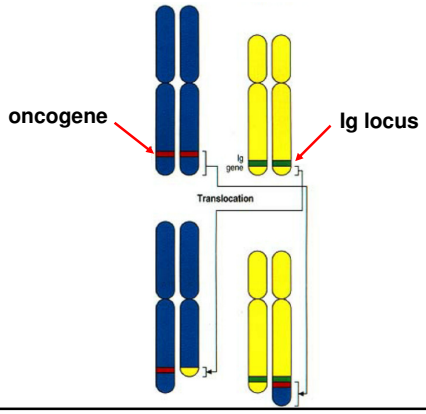


The RAG genes become part of host's genome

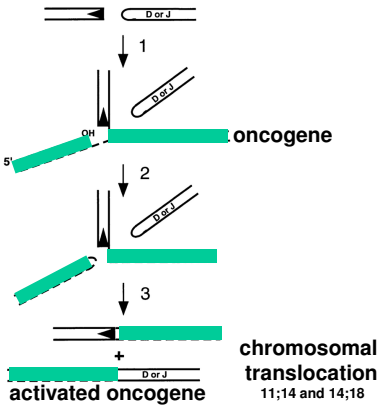


Furthermore, the RAG genes had to come under the control of lymphocyte-specific promoters

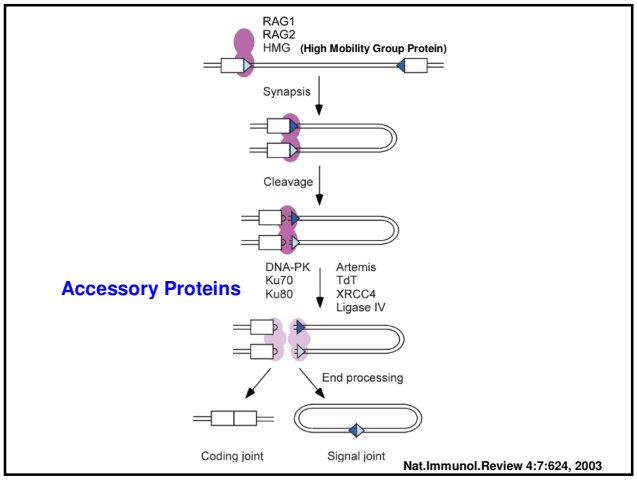
Lymphocyte tumors (lymphomas) generally contain abnormal rearrangements involving the Ig and TCR loci



Oncogenic translocation probably result from RAG transposition



Hiom, K., Melek, M. and Gellert, M. (1998). Cell **94**:463-470



Nat.Immunol.Review 4:7:624, 2003

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

- Normal birth and growth but immunodeficient
- Have no Mature B/T cells
- No V-(D)-J recombination
- Higher levels of precursor-T cells (Thy-1+, IL-2R+)
- Higher levels of NK+, macrophages, and granulocytes
  probably filling a "void" left by loss of lymphocytes

Important question:

- Do the RAGs bind and cut the RSS?

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

Other important findings:

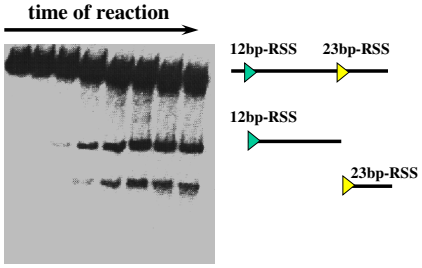
- RAG mediated cleavage requires intact RSS heptamer and nonamer sequences
- Cleavage products were identical to those detected *in vivo*- blunt 5' phosphorylated signal-ends and coding-ends contain hairpins (closed covalently)
- Recombinant Rag-1 and Rag-2 proteins plus house-keeping proteins are sufficient to mediate recombination *in vitro*

- 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:

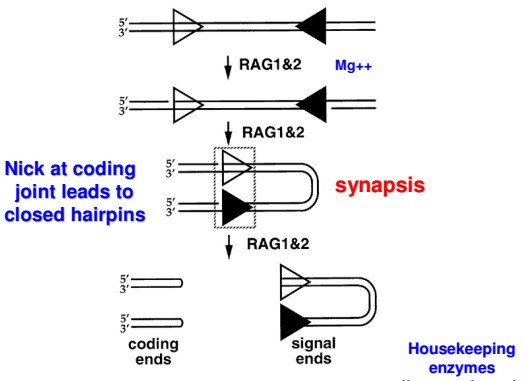
Can the *in vitro* system perform all the steps seen *in vivo*?

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



Abnormal recombination can cause lymphoid tumors

DNA REPAIR 5 (2006) 1234–1245

Known oncogenic loci

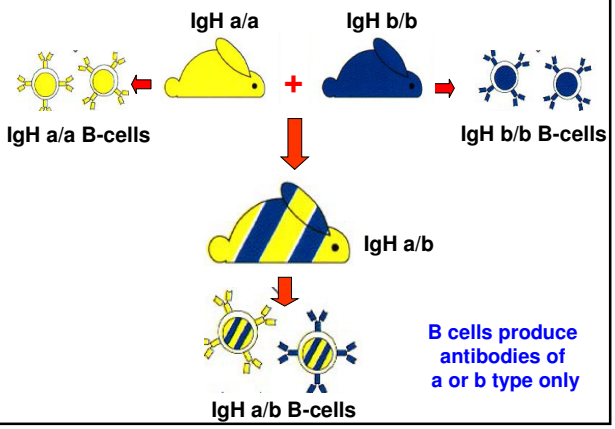
Recombination Zone Configuration	R (%)	Relative Efficiency
12 + 23	9.0	20,000
LMO2 + 23	0.34	760
12 + Ttg-1	0.017	38
12 + Hox11	< 0.00041	< 0.9
Hox11 + 23	< 0.00047	< 1
12 + SIL	0.012	27
SIL + 23	< 0.00036	< 0.8

High incidence of lymphocyte neoplasms are due to abnormal recombination

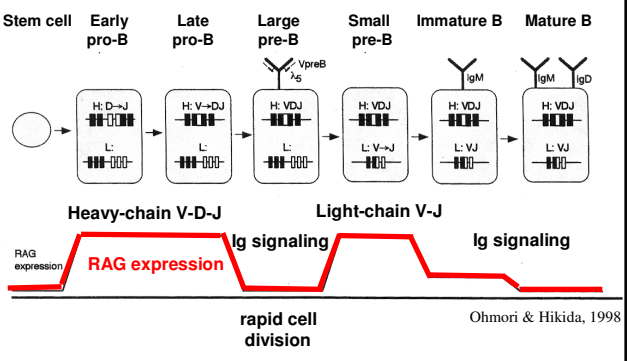
Positive and Negative Control of Recombination

- Positive control is necessary to activate the RAG genes at a specific stage of lymphocyte differentiation-the Pro-B/Pro-T cell stage
- Negative control is essential to maintain integrity of functionally rearranged genes by preventing rearrangements that could destroy functional genes or lead to aberrant rearrangements-oncogene activation
- Negative control is also necessary to prevent the production of lymphocytes with more than one specific receptor-to prevent anti-self reactivity- Allelic Exclusion

Phenomenon of Allelic Exclusion

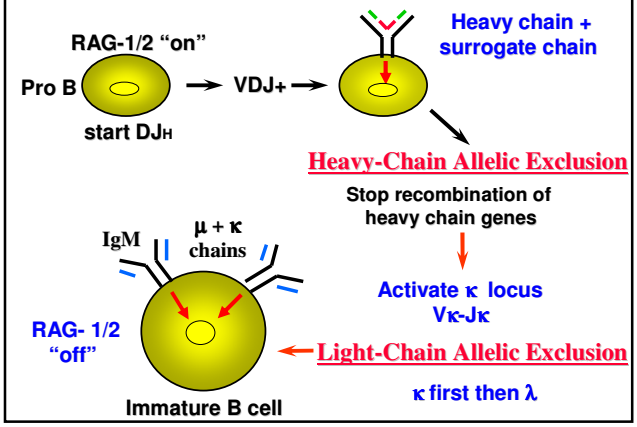


Ordered rearrangement and Allelic Exclusion



Down regulation coincides with pre-B cell receptor and expression of functional Ig on surface of B cells

Allelic Exclusion is Regulated by Signal Transduction



There are several antibody isotypes

see Fig. 4.30

	Immunoglobulin								
	IgG1	IgG2	IgG3	IgG4	IgM	IgA1	IgA2	IgD	IgE
Heavy chain	γ_1	γ_2	γ_3	γ_4	μ	α_1	α_2	δ	ε
Molecular weight (kDa)	146	146	165	146	970	160	160	184	188
Serum level (mean adult mg ml^{-1})	9	3	1	0.5	1.5	3.0	0.5	0.03	$5x10^{-5}$
Half-life in serum (days)	21	20	7	21	10	6	6	3	2

highest lowest

◆Different Ig isotypes are needed for surveillance at different sites in the body

The different Ig isotypes have different effector functions

Functional activity	IgM	IgD	IgG1	IgG2	IgG3	IgG4	IgA	IgE
Neutralization	+	–	++	++	++	++	++	–
Opsonization	–	–	+++	*	++	+	+	–
Sensitization for killing by NK cells	–	–	++	–	++	–	–	–
Sensitization of mast cells	–	–	+	–	+	–	–	+++
Activates complement system	+++	–	++	+	+++	–	+	–

Distribution	IgM	IgD	IgG1	IgG2	IgG3	IgG4	IgA	IgE
Transport across epithelium	+	–	–	–	–	–	+++ (dimer)	–
Transport across placenta	–	–	+++	+	++	+/–	–	–
Diffusion into extravascular sites	+/–	–	+++	+++	+++	+++	++ (monomer)	+
Mean serum level (mg ml^{-1})	1.5	0.04	9	3	1	0.5	2.1	$3x10^{-5}$

see Fig. 4.30

Human Ig Isotypes Differ in Structure and Function

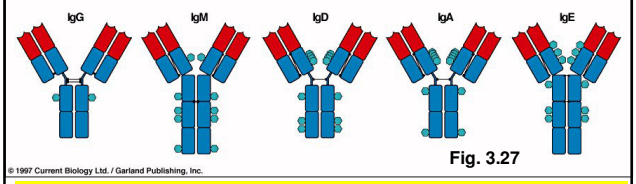
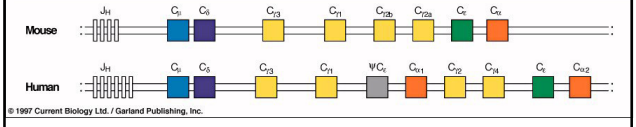


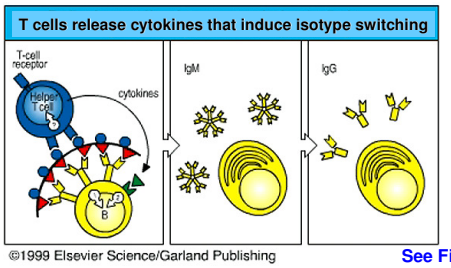
Fig. 3.27

Arrangement of the Mouse and Human Ig Constant Region Exons

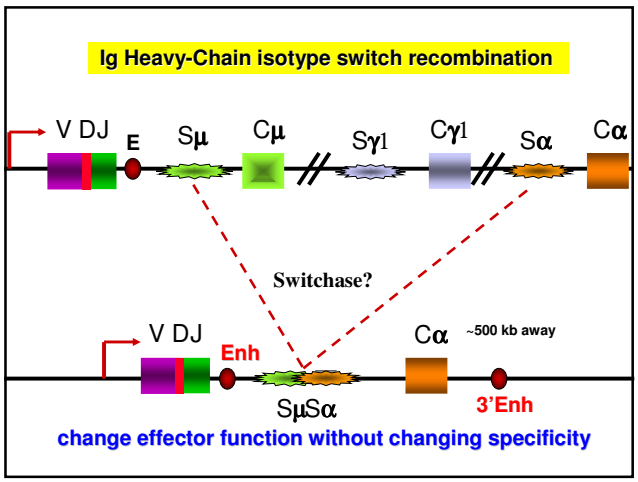


see Fig. 4.30

T cells release cytokines that induce isotype switching

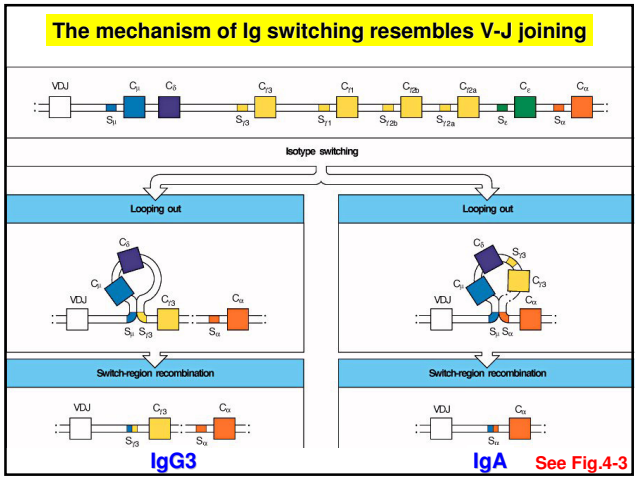


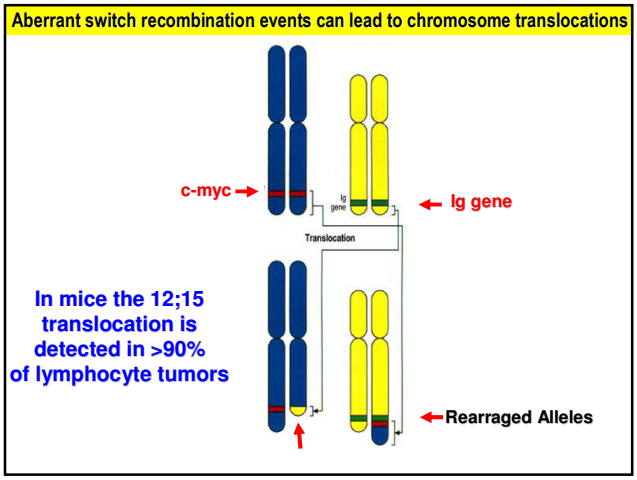
See Fig. 7.9



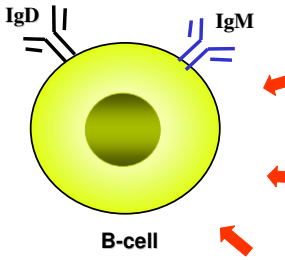
Isotype Switch Recombination

- Preserves original antigen-specific binding domain by keeping VDJ and exchanging downstream CHexons
- Switch recombination takes place in G-rich DNA regions upstream of each CH isotype -DNA is generally looped out
- Switch-recombinase is different than RAGs and has only recently been discovered
- As with V-(D)-J joining additional DNA repair factors such are necessary for repair of recombination products





Induction of Ig Class Switch Requires Several Signals



Signal 1

Antigen + T cell contact or Bacterial LPS

Induce Proliferation

Signal 2

Lymphokine or INFγ or TGF-β

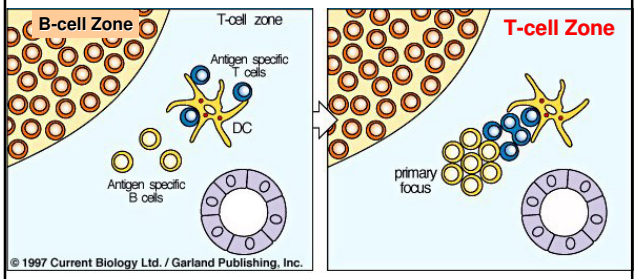
Prime Chromatin for Switching?

Signal 3

Induction of switch factors/enzymes?

Mediate Switch Recombination

Switch recombination requires T-cell contact and cytokines



T cells interact with APC and become activated "armed"

Ag specific B cells become trapped in the T cell zone where they encounter primed T cells - leading to clonal expansion

Specific B-T interactions lead to B-cell proliferation and switch recombination

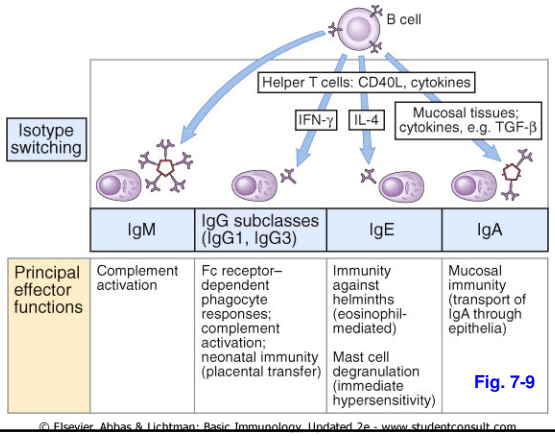


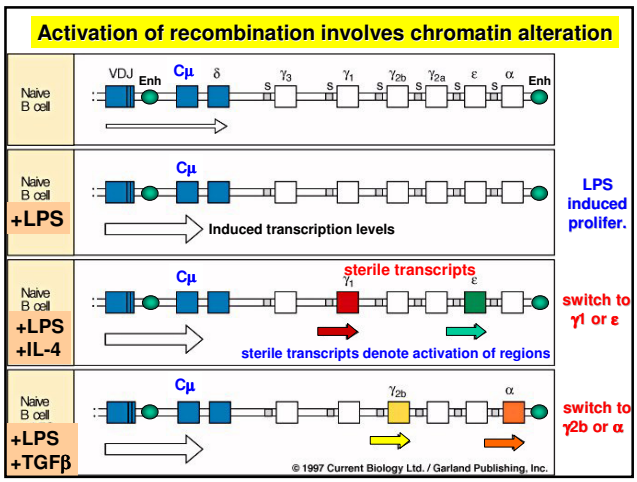
Fig. 7-9

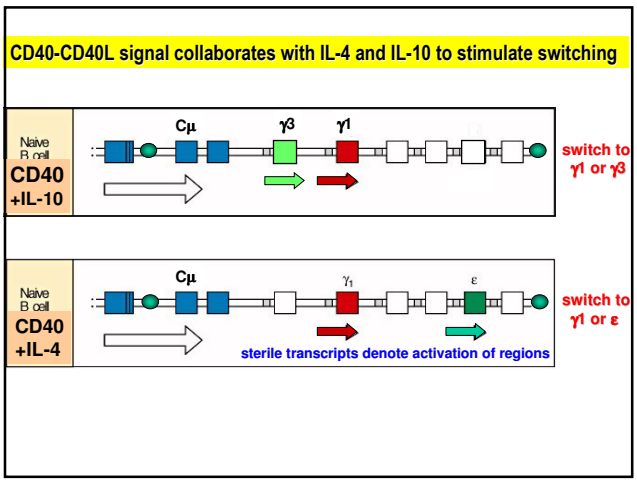
Various Cytokines Regulate the Switching Process

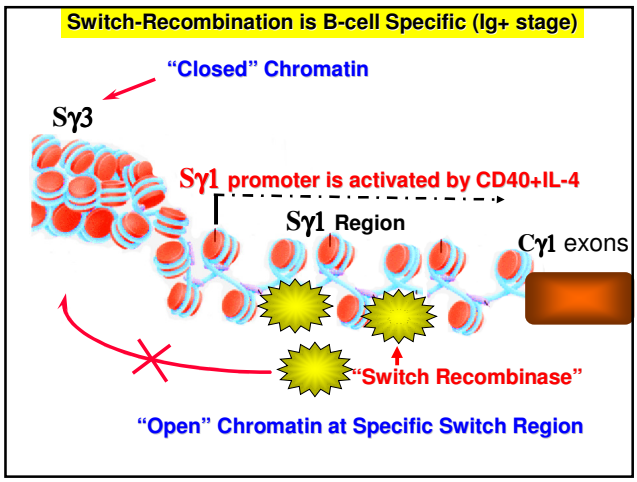
Role of cytokines in regulating Ig isotype expression

Cytokines	IgM	IgG3	IgG1	IgG2b	IgG2a	IgA	IgE
IL-4	Inhibits	Inhibits	Induces		Inhibits		Induces
IL-5						Augments production	
IFN-γ	Inhibits	Induces	Inhibits		Induces		Inhibits
TGF-β	Inhibits	Inhibits		Induces		Induces	

IL-10 + CD40 stimulates switching to IgG1 and IgG3



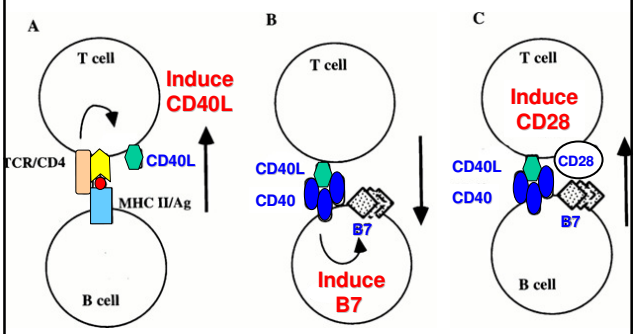




CD40

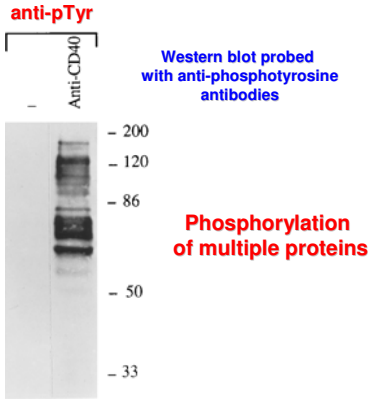
- Receptor molecule expressed by B cells and a few other cells
- anti-CD40 antibodies + IL-4 induces proliferation and class switching to Cγ1 and Cε
- anti-CD40 + IL-10 induces IgA, IgM and IgG synthesis
- CD40 x-linking induces CH region sterile transcripts and leads to rapid activation of protein kinases and phosphorylation of multiple intracellular proteins
- Targeted disruption of CD40 leads to profound defects in Germinal Center formation and serum Ab levels-due to defective Ig isotype-class switching.

B-T interaction *via* MHCII/Ag lead to induction of CD40-CD40L interactions



Additional signaling molecules are subsequently activated like B7-CD28

Crosslinking of CD40 leads to rapid phosphorylation of multiple proteins



Hanissian and Geha (1997). Immunity 6: 379-387.

CD40 Ligand (CD154)

- Expressed nearly exclusively by CD4+ T helper cells located in lymphoid follicles where helper function and isotype class-switching takes place.
- Induced after TH activation
- Involved in B cell activation leading to proliferation and differentiation into Ab-secreting cells
- Humans with defective CD40-CD40L show immuno-deficiency due to inability to induce isotype-switching

Inhibition of CD40 signaling affects Ig class switching

Genes	IgM	IgG2a	IgG1	IgG2b	IgG3	IgA	IgE
CD40	=	↓ 8X	↓ 18X	↓ 2X	↑ 2X	↓ 3X	↓ 44X
CD40L	=	↓ 12X	↓ 24X	↓ 6X	↓ 2X	↓ 3X	↓ >220X

Lorenz and Radbruch (1996). Curr. Tops. Micro. Immunol., 217:151-69 (REVIEW).

Proposed Functions of the Switch Recombinase Complex

- Specifically Recognize Switch Recombination Signals (SRS) Probably not
- Cleave Switch Recombination Signals not
- Ligate Recombined DNA not

Comparison of Sμ motif distribution between mammalian species

Switch motifs	Switch μ Region[a]			
	Mouse	Human	Pig	Shrew
TGAGC	44	182	219	43
TGGGN[b]	34	239	209	28
TGGGG	13	10	12	1
TGGGC	8	205	125	13
TGGGA	6	5	5	3
TGGGT	7	19	67	11
TAGGG	2	3	0	0
TAGGA	0	0	0	0
TGAGC TGAGC	12	40	49	12
TGAGC TGGGN	10	50	91	22
TGGGN TGAGC	11	73	115	12
TGGGN TGGGN	1	80	18	1

[a]Nucleotide sequences used for this comparison were obtained from the National Center for Biotechnology Information.
[b]N denotes any nucleotide at this position.

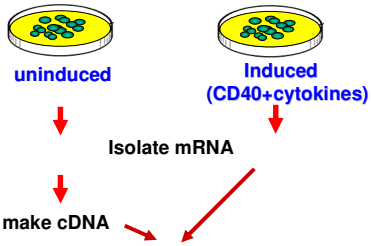
Lyon and Aguilera, 1997

Cloning of a putative "switch" factor

Muramatsu, M., *et al.*, (1999). Specific expression of activation-induced cytidine deaminase (AID), a novel member of the RNA-editing deaminase family in germinal center B cells. J. Biol. Chem. ***274***, 18470–18476.

Cloning of induced genes by subtractive hybridization

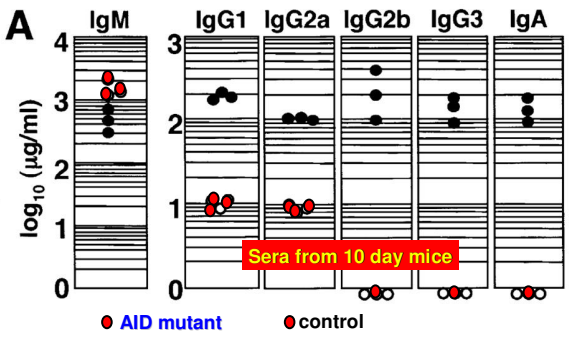
B-cell line (CH12F3.2) that can be induced to switch



Isolate unique cDNAs that do not hybridize to un-induced RNA
Isolated a gene called
Activation-Induced Cytidine Deaminase (AID)

Muramatsu, M., *et al*., (2000). Class switch recombination and hypermutation require activation-induced cytidine deaminase (AID), a potential RNA editing enzyme. Cell, **102, 553–563,**

AID Mutant mice Fail to Switch



AID appears to be an RNA editing Enzyme

"**AID appears**

to play a role in DNA cleavage because (1) AID deficiency does not affect germline transcription of target DNA

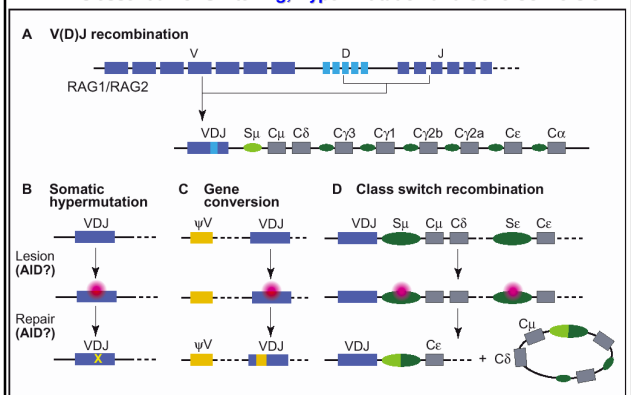
(2) AID induction augments class switching of CH12F3-2 cells, which are known to express germline transcripts even without stimulation

(3) AID deficiency does not affect VDJ recombination, which includes the end joining repair system. ''

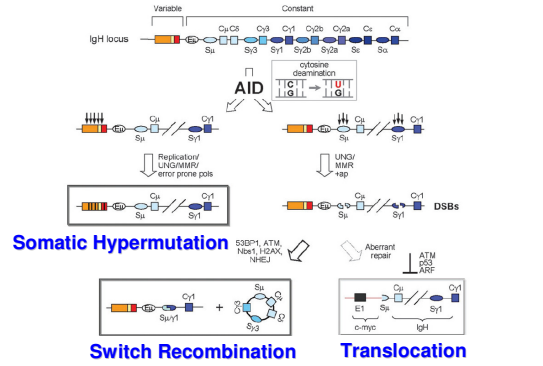
HOW DOES AID WORK ?

AND WHAT DOES IT DO?

AID is essential for Switching, Hyper-mutation and Gene Conversion



Current Model for Switch Recombination



Carcinogenesis vol.28 no.12 pp.2427–2433, 2007
doi:10.1093/carcin/bgm201
Advance Access publication September 4, 2007

Oncogenic events triggered by AID, the adverse effect of antibody diversification

Fig. 1. Molecular events triggered by AID. Mature B cells harbour IgH genes (top diagram) that comprise a 5′ portion that has been productively rearranged by V(D)J recombination (orange, yellow and red boxes) coding for the antigen recognition variable region of the antibody molecule, and a number of constant regions (blue boxes), preceded by highly repetitive switch regions (blue ovals). Antibody diversification by SHM and CSR is triggered by AID deamination of dC residues (arrows) on the variable or switch regions of the Ig genes, thereby generating dU:dG mismatches on DNA. In SHM the dU:dG mispairing can be replicated over, recognized by UNG, or processed by MMR factors or error-prone polymerases, leading to nucleotide substitutions. In the case of CSR, the dU:dG mismatches introduced by AID in switch regions are recognised by UNG or MMR to produce a substrate for an apyrimidinic endonuclease (ap) that leads to the generation of DSBs. CSR requires switch region DSBs be brought in close proximity, which involves DNA damage response factors including H2AX, ATM, p53-binding protein 1 and Nijmegen syndrome protein 1 and be ligated by non-homologous end joining. CSR results in the deletion of the intervening DNA sequence and the replacement of the Cμ constant region by a downstream region (CSR to Cγ1 is shown). In addition, AID-triggered DSBs can also be substrates for pro-oncogenic chromosomal translocations, such as c-myc–IgH translocations found in Burkitt lymphoma. Surveillance pathways mediated by ATM, p53 and ARF provide protective mechanisms against the generation or spreading of AID-promoted chromosome translocations.

AID is partially responsible for B cell tumors

Majority of human lymphomas are B cell derived

Lymphomas generally have translocations involving the Ig loci and a known oncogene such as:

c-myc in Burkitt lymphoma
BCL1 in mantle lymphoma
BCL6 in diffuse large B cell lymphoma
BCL2 in follicular lymphoma

Injection of mice with Pristane (2,6,10,14-Tetramethylpentadecane) induces lymphomas due to induction of c-myc/Ig translocations- originally shown in mid 1980's

These translocations were not detected in AID mutant mice treated with pristane

AID-dependent somatic hypermutation occurs as a DNA single-strand event in the BL2 cell line.

Faili, et al **Nat Immunol.** 2002 Sep;3(9):815-21.

Immunoglobulin (Ig) gene hypermutation can be induced in the BL2 Burkitt's lymphoma cell line by IgM cross-linking and coculture with normal or transformed T helper clones

Table 1. Induction of mutation in the BL2 cell line by aggregation of surface receptors

Receptor cross-linking	Mutation frequency		
	Experiment 1[a]		Experiment 2[b]
	V region	C region	V region
No stimulation	0.96×10^{-4} (1/10400)	–	0.7×10^{-4} (3/40768)
IgM	0.75×10^{-4} (1/13312)	–	1.2×10^{-4} (4/40768)
IgM + CD19	1.3×10^{-4} (2/14976)	–	1.2×10^{-4} (4/32448)
IgM + CD21	3.2×10^{-4} (5/15808)	–	2.9×10^{-4} (10/32864)
IgM + CD19 + CD21	5.4×10^{-4} (9/16640)	1.0×10^{-4} (1/9975)	7.0×10^{-4} (34/48256)

Table 2. Percentage nucleotide substitution in mutations generated by IgM-CD19-CD21 cross-linking in the BL2 cell V4-39-J_H5 gene

From	To					
	A	T	G	C	Total	
A	–	0.9%	4.6%	1.4%	6.9%	} 20.5%
T	3.1%	–	2.7%	7.8%	13.6%	
G	26.3%	4.8%	–	7.5%	38.6%	} 79.5%
C	10.6%	24.2%	6.1%	–	40.9%	

Nat Immunol. 2002 Sep;3(9):815-21.

AID is necessary for Somatic Hypermutation

Table 5. Frequency of mutations induced in the V4-39-J_H5 gene of the AID$^{-/-}$ BL2 clone before and after transfection with AID-expressing vectors

BL2 subclone (phenotype)	Mutation frequency			
	Before stimulation		After 3 stimulations	
60-64 (AID$^{-/-}$)	0	(0/11648)	0	(0/9568)
60-64-8 (AID-transfected)	0	(0/9568)	4.6×10^{-4}	(5/10816)
60-64-15 (AID-transfected)	1.0×10^{-4}	(1/9568)	3.8×10^{-4}	(4/10400)
60-129 (AID$^{-/-}$)	0	(0/9152)	0	(0/9568)
60-129-2 (AID-transfected)	0	(0/9568)	3.4×10^{-4}	(3/8736)
60-129-10 (AID-transfected)	1.0×10^{-4}	(1/9568)	4.2×10^{-4}	(4/9568)
BL2 (not transfected)	1.0×10^{-4}	(1/9568)	11.5×10^{-4}	(23/19968)

Mutation frequency is expressed as mutations per base pair.

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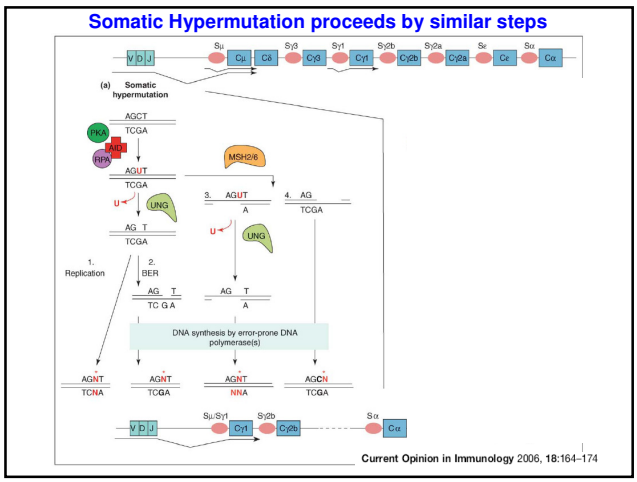
Immunoglobulin Genes: Generating Diversity with AID and UNG

Uracil-DNA Glycosylase

Ursula Storb[1] and Janet Stavnezer[2]

Somatic hypermutation and switch recombination of immunoglobulin genes require the activity of the activation-induced deaminase, AID. Recent studies of mice deficient for the uracil-DNA glycosylase UNG, which removes U from DNA, suggest that AID catalyses the deamination of dC to dU during antibody diversification.


Somatic Hypermutation proceeds by similar steps
(a) Somatic hypermutation
PKA
AID
RPA
UNG
MSH2/6
1. Replication
2. BER
DNA synthesis by error-prone DNA polymerase(s)
Current Opinion in Immunology 2006, 18:164–174