Generation of Antigen Receptors

A. Antibody and T cell receptor gene rearrangement

B. RNA splicing changes the Ig expressed

C. Changes in BCR genes after Ag activation

Janet Stavnezer – AS8-1053
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Antibodies consist of heavy and light chains

IgG

Two identical Ag-combining sites formed by variable regions of H+L chains

Two identical heavy chains

Two identical light chains: Light chains come in two isotypes - lambda (\(\lambda\)) and kappa (\(\kappa\)).

Not known if these two isotypes have specific different functions, but seems unlikely.
Antibody (BCR) and T Cell Receptor (TCR) Gene Rearrangement
Genes for BCRs and TCRs are created by recombination of gene segments.

What problem does this mechanism solve? This mechanism solves the problem of generating diversity for antigen-binding, while maintaining a constant region that can bind to defined receptors and provide effector function.

Dreyer and Bennett in 1965 proposed there were many antibody V genes, but only very few C genes. At this time it was thought - one gene, one polypeptide, so this was an heretical idea.

Opposing idea to Dreyer + Bennett - Clonal selection was established already, so others postulated there were many (10,000 - 10^6) genes for antibodies that encoded both V and C regions together, and that B cells expressed one gene for L and one for H chain.

But this hypothesis had problems accounting for the limited diversity of C regions.
Tonegawa’s experiment (1976) showed that Ig lambda (\(\lambda\)) light chain genes are in different segments in non-B cells but joined in antibody-synthesizing cells.
Provided first evidence for Dreyer and Bennett’s hypothesis.

**Tonegawa’s experiment:**

1. Isolate \(\lambda\) chain mRNA from plasmacytoma (myeloma) cells that make lots of one Ig.
2. Copy mRNA into cDNA with reverse transcriptase from retrovirus.
3. Hybridize the cDNA with Southern blots of genomic DNA from
   i) mouse embryos - too young to make antibodies and
   ii) with DNA from mouse plasmacytoma cells making antibodies containing \(\lambda\) or \(\kappa\) light chains.

\[\text{Ig } \lambda \text{ chain mRNA} \quad \overset{\text{Reverse transcriptase + dNTP’s}}{\longrightarrow} \quad \overset{\text{AAAAA}}{\longrightarrow} \quad \overset{\text{TTTTT}}{\longrightarrow} \quad \text{32P-labeled cDNA copy of } \lambda \text{ chain mRNA}\]
Tonegawa’s Southern blotting experiment showed that Ig λ light chain genes are rearranged in a plasmacytoma that expresses λ but not in a plasmacytoma that expresses κ light chains.

Figure 10.11  Gel blotting analysis of the DNA fragments carrying λ genes in the cells of a BALB/c embryo (B), H2020, a plasmacytoma, which produces λ1 chains (A), and MOPC-321, which synthesizes kappa chains (C). The DNA fragments were hybridized with radioactive cDNA from H2020. A fragment containing the gene (8.6 kb) and fragments containing the V1 and V12 genes (4.8 and 3.5 kb) are found in the embryo DNA as well as in both the λ-secretting (A) and κ-secretting (C) plasmacytoma DNA. However, the λ secreting plasmacytoma (A) also shows an additional fragment (7.4 kb) that contains both the V and C genes. The embryonic, non-rearranged pattern often coexists with the differentiated pattern in differentiated cells. Presumably the embryonic pattern persists in the No. 16 chromosome that is not being expressed. The close homolog V1 and V12 accounts for them both bind to the V11 probe. [From C. Brack et al., Cell 15:1, 1978 © M.I.T.]

Tonegawa’s

Experiment:

Origin →

λ+  κ+

A B C

8.6 kb
7.4 kb
4.8 kb
3.5 kb
Maps of cloned λ light chain genes in embryonic DNA and in λ-expressing plasmacytoma (myeloma) cells showed that joining occurs within the λ variable region - between the V and J gene segments. Maintains reading frame of codons. No other recombination; and introns do not change.
Three Ig gene families in humans (and mice) are encoded in 3 separate gene clusters located on 3 different chromosomes.

The 3 gene families have different structures from each other. L and H chain loci have many V genes and a few J genes, (except mouse has only a few V\(\lambda\) genes). The H chain locus also has many D genes.

To make a functional antibody variable region gene, one V, one D (H chain only), and one J gene join. Any gene segment can be chosen from the same gene family.
Discovered by two graduate students (D. Schatz and M. Oettinger) who transduced a library of genomic DNA fragments from lymphoid cells into non-lymphoid cells. They found a fragment that could induce V(D)J recombination in non-lymphoid cells. It encoded two genes, *rag1* and *rag2*.

Two proteins, Rag1 and Rag2, work together to initiate V(D)J recombination.
To make a functional antibody kappa gene, one V and one J gene segment recombine, retaining the 3’ J segments in the genome.

After the recombination diagrammed in blue has occurred, the cell will express Vk40-Jk2. RNA splicing will delete Jk3-Jk5-Ck intron, and the cell will express mRNA with the Jk2 segment spliced to Ck.
Transcription and splicing of κ mRNA from joined (recombined) or κ light chain gene

Recombined gene in κ-expressing B cells:

Primary transcript

Splicing occurs between L and V and between J and C exons.

Splicing diagram

Translation

Processing
Recombination of multiple gene segments results in a large number of H and L chain V region sequences.

**Number of functional gene segments in human immunoglobulin loci**

<table>
<thead>
<tr>
<th>Segment</th>
<th>Light chains</th>
<th>Heavy chain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>κ</td>
<td>λ</td>
</tr>
<tr>
<td>Variable (V)</td>
<td>40</td>
<td>30</td>
</tr>
<tr>
<td>Diversity (D)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Joining (J)</td>
<td>5</td>
<td>4</td>
</tr>
</tbody>
</table>

**Mouse Ig gene segments (functional)**

<table>
<thead>
<tr>
<th>Segment</th>
<th>Light chains</th>
<th>Heavy chain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>κ</td>
<td>λ</td>
</tr>
<tr>
<td>V</td>
<td>~60</td>
<td>3</td>
</tr>
<tr>
<td>D</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>J</td>
<td>4</td>
<td>2</td>
</tr>
</tbody>
</table>

**Number of functional gene segments**

- Light chains: 200, 120, ~5520 (1.8 million)
- Heavy chain: 240, 2860 (0.7 million)
BCR rearrangement occurs at defined stages of B cell development in the bone marrow.

The process is highly regulated.
Stages of B cell development

- **Pro-B**
  - Stem cell
  - H-chain genes: Germline
  - L-chain genes: Germline
  - Surface Ig: Absent
  - Pre-pro-B cell
  - D-J rearranging
  - V-DJ rearranging
  - Late pro-B cell
  - VDJ rearranged
  - Large pre-B cell
  - μ chain transiently at surface as part of pre-B-cell receptor. Mainly intracellular
- **Pre-B**
  - Stem cell
  - H-chain genes: Germline
  - L-chain genes: Germline
  - Surface Ig: Absent
  - Pre-pro-B cell
  - D-J rearranging
  - V-DJ rearranging
  - Late pro-B cell
  - VDJ rearranged
  - Large pre-B cell
  - μ chain
  - Pre-B receptor
  - Small pre-B cell
  - V-J rearranging
  - Intracellular μ chain
  - Immature B cell
  - IgM
  - Mature B cell
  - IgD

In fetal liver and bone marrow

B cells migrate to spleen.
Clonal Selection Theory

The fact that B cell receptor (and T cell receptor) gene recombination occurs prior to antigen exposure fits with the clonal selection theory.

How?
Answer:

The BCRs and TCRs are expressed prior to exposure to Ag.

Each cell expresses a different BCR (or TCR), and therefore has a different Ag specificity.

Ag activates only B cells that bind it, and only these cells proliferate and produce Abs (which are specific for the Ag).
Ig gene segment recombination occurs in an ordered progression.

<table>
<thead>
<tr>
<th>Rearrangement</th>
<th>Stem cell</th>
<th>Pre pro-B cell</th>
<th>pro-B cell</th>
<th>Large pre-B cell</th>
<th>Small pre-B cell</th>
<th>Immature B cell</th>
<th>Mature B cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>D–J_H</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>IgM</td>
<td>IgM IgD</td>
</tr>
<tr>
<td>V_H–DJ_H</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V_κ–J_κ</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V_λ–J_λ</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

Figure 7-7 part 1 of 2 Immunobiology, 6/e. (© Garland Science 2005)
Allelic Exclusion

Unlike most genes, only one allele of each antibody locus is expressed, i.e. only one H chain and one L chain allele is expressed in each B cell.

Allelic exclusion is essential to prevent production of unselected antibodies, which could be autoreactive at worst, and wasteful at best.

Mechanism for allelic exclusion
One allele is recombined at a time, and if successfully recombined, the protein product is expressed along with the surrogate light chain, and signals to the cell to differentiate past the stage at which recombination occurs. This stops further recombination. Called feedback mechanism, but is it?
Ordered and regulated V-(D)-J recombination results in allelic exclusion

V-(D)-J recombination must maintain an open-reading frame, and about 2/3 of recombination events are out-of-frame. This creates non-functional alleles.
A productively rearranged Ig VDJ-Cμ gene is expressed immediately as a protein (μ chain) by the developing B cell.

The Ig μ H chain associates with a surrogate L chain on the B cell surface.

**Surrogate L chain** consists of two proteins: VpreB and λ5.

Expression of the pre-B receptor is required for normal progression:
- KO of VpreB or λ5: fewer B cells and they are autoreactive.
- K/λ light chain
1. V-D and V-J recombination occur on one allele at a time.

Expression of H chain protein on B cell surface or on an internal membrane (in preB receptor) sends signals via phosphorylation of associated protein chains (Igα, Igβ) with Immuno-Tyr Activation Motifs (ITAMs), resulting in progression of proB cell to preB cell stage and termination of V-D joining. No ligand required.

2. One kappa allele has DNA methylation (5me-CpG), a repressive DNA mark, so only one allele is accessible to the RAG proteins during the pre B cell stage. (Unknown if also true for for H chain recombination.)
Key points-1

1. Tonegawa showed that antibody genes are encoded in pieces that recombine during B cell development.

2. There are many V, D and J gene segments but few C gene segments.

3. V-(D)-J recombination occurs within the variable region of L chains and H chains and therefore must be accurate enough to maintain reading frame.
4. V-(D)-J recombination occurs in an ordered and regulated way during development of B cells in the bone marrow. This generally results in formation of only one functional H chain gene and one functional L chain gene in any one B cell (allelic exclusion).

5. Joining of coding sequences out of reading frame does not stimulate cells to progress, and they try to rearrange the second allele. If no correct joining occurs, B cells die in the bone marrow during development (during the recombination process).

6. V(D)J recombination occurs prior to Ag-activation of lymphocytes.
Mechanism of V-(D)-J recombination

When V and J gene segments were cloned, it was noticed that on the 3’ side of the V gene segments, and on the 5’ side of the J gene segments were conserved sequences: a heptamer (7-mer), an unconserved spacer, and a nonamer (9-mer).

Recombination only occurs between signal sequences with 12 and 23 bp spacers. This prevents $V_H$-$J_H$ joining and D-D joining.
RAG1 & 2 recognize RSSs and introduce DSBs between coding segment and heptamer sequence.
RAG1 and 2 create DNA breaks to initiate V-(D)-J recombination

V gene RSS

Rag1/Rag2 0H

SS DNA nick

Transesterification forms hairpin on coding ends and blunt ds break on signal (RSS) ends.

Hairpin is nicked by Artemis+DNA-PKcs.

Nicked hairpin results in overhangs, with palindromic sequence, called P nts.
The mechanism of V(D)J recombination results in the introduction of lots of diversity at the junctions between:

V and D,
D and J,
V_L and J_L gene segments.
Several ubiquitously expressed proteins are also important for V-D-J recombination:

Artemis is required for hairpin opening. DNA-PKcs functions with Artemis, activating Artemis to become an endonuclease. Other proteins involved in non-homologous end joining (NHEJ) also essential for joining step.

People and mice lacking Rag1 or 2, Ku70, Ku80, DNA-PKcs or Artemis have severe-combined immuno-deficiency (SCID), with no (or very few) B or T cells. They are also radiation sensitive (except Rag mutants).

Mutation of DNA ligase IV-XRCC4 (also required for NHEJ) also causes life-threatening general DNA repair problems.
Terminal deoxynucleotidyl Transferase (TdT)

TdT is an additional enzyme involved in H chain but much less in L chain joining.

TdT inserts untemplated nts (N nucleotides) at D-J and at V-D junctions, further increasing diversity. TdT is also an exonuclease, deletes P nts.

Not required for V-D-J recombination.

Only very low levels present in pre-B cells undergoing L chain gene joining.

Absence reduces diversity of BCR and TCR.
CDR3 is encoded by the V-N junction, the N nucleotides, the D gene, the D-N junction and the N-J junction.

For L chains, CDR3 is encoded by the V-J junction. So less diverse than in H chains. As in H chains, J encodes Framework 4 (FR4).
N regions sometimes also contain P nucleotides generated by opening the hairpin asymmetrically (if they have not been excised by TdT).
CDR3 is the largest and most variable CDR loop, and usually the most important for Ag binding.
RAG1 and RAG2 together recognize RSS’s and initiate recombination.

RAG1 and 2 nick DNA adjacent to the heptamer, create a hairpin with the coding ends and a blunt DSB at the signal ends (RSS).

Hairpin ends are opened asymmetrically, creating palindromic (P) sequences.

Terminal deoxynucleotidyl transferase (TdT) can insert random nts at the hairpin ends, and can delete nts from DNA ends, often resulting in loss of obvious P nts and further variability of coding sequences.

Junctional diversity greatly increases diversity at CDR3, the center of the Ag-combining site. Results in $\sim 10^{11} - 10^{13}$ different V regions.
T cell receptor genes have a similar plan and recombination mechanism as do BCR genes. All the same enzymes are involved, including RAGs, Artemis, DNA-PK and TdT.

**Human**

What is a major difference between BCR and TCR genes that can be seen in this diagram?
Comparison of BCR and TCR Structure

TCR α and β chains are like Ig light chain dimer or Fab fragment but with added transmembrane terminus.
TCR recognition of MHC/peptide

- TCR $\alpha$ and $\beta$ chains bind to amino acid residues of both the MHC molecule and the peptide.

- Center of contact with the peptide in the MHC groove is with the CDR3's from each chain ($\alpha$ and $\beta$).
TCR α genes are like Ig κ genes, except many more Jα’s (>50). TCR β genes have D genes, but plan is like λ genes.

Note that V-J joining can leave unjoined J segments. This is also true for IgH and L chain genes.

TdT inserts nts in both α and β TCR genes at V-J, V-D and D-J junctions. This further increases diversity at these junctions (CDR3) relative to the BCR.
Antibody and TCR variable regions have nearly infinite variety. They are so diverse that if one finds two different B or T cells expressing the same V-D-J join, they are considered to be from the same clone.

There are more possible receptors than cells:
\(~2 \times 10^8\) B cells in the mouse; \(~2 \times 10^{10}\) B cells in humans.

<table>
<thead>
<tr>
<th>Element</th>
<th>Immunoglobulin</th>
<th>(\alpha:\beta) T-cell receptors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>H</td>
<td>(\kappa+\lambda)</td>
</tr>
<tr>
<td>Variable segments (V)</td>
<td>40</td>
<td>70</td>
</tr>
<tr>
<td>Diversity segments (D)</td>
<td>25</td>
<td>0</td>
</tr>
<tr>
<td>D segments read in three frames</td>
<td>rarely</td>
<td>(-)</td>
</tr>
<tr>
<td>Joining segments (J)</td>
<td>6</td>
<td>5((\kappa)) 4((\lambda))</td>
</tr>
<tr>
<td>Joints with (N)- and (P)-nucleotides</td>
<td>2</td>
<td>50% of joints</td>
</tr>
<tr>
<td>Number of V gene pairs</td>
<td>(1.9 \times 10^6)</td>
<td></td>
</tr>
<tr>
<td>Junctional diversity</td>
<td>(~3 \times 10^7)</td>
<td></td>
</tr>
<tr>
<td>Total diversity</td>
<td>(~5 \times 10^{13})</td>
<td></td>
</tr>
</tbody>
</table>

Figure 4-13 Immunobiology, 6/e, (© Garland Science 2005)
γδ T cells have much less diverse TCRs than αβ T cells

TCR δ genes reside within the TCR α gene locus - between Vα and Jα gene segments. Vα-Jα joining deletes δ genes.

TCR γ genes reside on a different chromosome. 
γ chain associates with the δ chain to form the γδ TCR.

Mechanism for determination of αβ vs γδ lineage is an active area of investigation.

Fig 4.15 © 2001 Garland Science
TCR genes rearrange in the thymus, using same mechanism as Ig genes.

Important difference between BCR and αβ TCR - diversity of TCR is most highly concentrated within the CDR3, due to greater numbers of J gene segments, and due to the longer duration of TdT expression in thymocytes than in developing B cells.

Earliest genes to rearrange are γ and δ genes, then β and then α genes. γδ cells exit the thymus before αβ cells.

PreTCR receptor has a β chain and preTCRα chain.

γδ genes show limited diversity and the γδ T cells are found in specific places in the body at front line of defense. They are thought to be more primitive than αβ T cells. They are in the intestinal epithelium and skin.
Key Points: TCR Genes

Like BCR genes, TCR genes are encoded in V, D and J gene segments that recombine during T cell development (occurs in thymus).

Like BCR genes, TCR genes rearrange in an ordered, regulated pattern, and are generally allelically excluded.

Like BCR genes, TCR gene segments have RSS’s adjacent to them and observe the 12-23 spacer rule.

TCR’s have even more diversity in CDR3 than BCR’s.

TCRs are only expressed on cell surfaces, never secreted.
B. RNA splicing of the IgH gene changes the constant region gene that B cells express.
All antibody classes can be expressed as both membrane-bound and secreted forms.

Resting naïve cells express membrane-bound IgM (and IgD). Upon activation, B cells begin to increase synthesis of IgM and secrete it. Membrane-bound and secreted Ig differ in the C terminus of H chain.

Membrane bound form has transmembrane hydrophobic domain. Difference is due to alternative transcription termination and processing.
IgH chains can have different constant (C) regions, but express the identical variable region (VDJ segment). IgM is expressed first after H and L chain genes have recombined, then IgD.

Heavy chain genes in IgM/IgD expressing mouse B cell

IgD expression requires alternative RNA transcription termination and processing.

IgM and IgD with the same VDJ gene are co-expressed on naïve B cells.
Alternative transcription termination/RNA splicing regulate the expression of IgM vs IgD and membrane-bound vs secreted Ig.

Naïve resting B cells express both membrane-bound IgM and membrane-bound IgD.

IgD function still unclear. It signals less well and cannot substitute for the µ heavy chain during development.

Upon B cell activation, IgD expression is lost, and membrane bound IgM levels increase.

Next, some B cells differentiate to become plasma cells, loaded with endoplasmic reticulum and capable of synthesis and secretion of abundant IgM.
Light chains do not differ in membrane-bound and secreted Ig.

IgG

C regions
Two related processes alter the \textit{IgH} gene and protein sequences after Ag activation of B cells:

\textit{CSR} = class switch recombination;
\textit{SHM} = somatic hypermutation of \textit{V} genes.
Antibody Class (or Isotype) Switching

Naive B cells express IgM and IgD.

After B cells are activated by immunization or infection, they switch from IgM and IgD to IgG, IgE or IgA expression.

This switch improves the ability of the antibody to eliminate pathogens.
CSR allows a B cell that is activated by antigen binding (and co-stimulatory signals) to switch to expressing one of these downstream $C_H$ genes.

B cells can switch to any of the downstream $C_H$ genes, depending on the antigen and co-stimulatory signals.

Initiates 2-3 days after infection or immunization.
Class switch recombination (CSR)
Changes the antibody H chain constant region, which changes the class (isotype) of the antibody. This changes the effector function of the antibody.

The antibody class determines which Fc receptors it can bind to, its ability to bind and activate complement, its ability to pass through epithelial membranes, and the antibody stability.

Certain antibody classes are better for resistance to extracellular bacteria; other classes for viruses, or parasites.
CSR occurs by an intrachromosomal deletion between switch (S) regions located 5' (upstream) of each $C_H$ gene.

Heavy chain genes in IgM/IgD expressing cell

Heavy chain genes in IgE-expressing cell

AID (Activation-induced cytidine deaminase) is required for CSR and for SHM.

AID KO mice were created by T. Honjo’s group.
AID initiates CSR by deamination of dC residues

AID (activation-induced cytidine deaminase)

(M. Neuberger et al)
Ung excises the dU base during CSR

AID

Base Excision Repair (BER)

Uracil-DNA Glycosylase (Ung2)

Ung\(^{-/-}\) B cells: 95% reduced CSR

(Rada, Petersen and Neuberger)
AID initiates CSR by deamination of dC residues

**AID**
(Activation-induced cytidine deaminase)

Base Excision Repair (BER)

Uracil-DNA Glycosylase (Ung)

AP Endonuclease

DNA pol β

**Ung−/−** B cells:
95% reduced CSR

Initiates class switch recombination if occurs on both strands.
Transcription of recombining S regions is required for CSR.

Heavy chain genes in IgM/IgD expressing cell

Heavy chain genes in IgE-expressing cell

Switch recombination

AID (Activation-induced cytidine deaminase) is required for CSR and for SHM.

AID KO mice were created by T. Honjo’s group.
Germline (GL) transcripts are required for CSR to make substrate for AID.

Transcription directs CSR to a specific switch region and $C_H$ gene.

Transcription is induced by cytokines that direct switch to that same $C_H$ gene.

Examples:

TH2 cytokine IL-4 induces GL $\gamma 1$ and $\varepsilon$ transcripts and CSR to IgG1 (mouse) and IgE.

TH1 cytokine $\gamma$-interferon ($\gamma$IFN) induces GL $\gamma 2a$ transcripts and CSR to IgG2a (mouse).

TGF$\beta$ induces GL $\gamma 2b$ and $\alpha$ transcripts and CSR to IgG2b and IgA.
Somatic hypermutation (SHM) of the V genes initiates ~ 7 days after immunization or infection, and occurs in special sites in spleen and lymph nodes called germinal centers.

SHM improves the affinity of the antibody for antigen, by as much as $10^4$. 

**Antibody variable region (VDJ) genes undergo “somatic hypermutation” after activation by antigen.**
Somatic hypermutations are found in the first 1 kb of transcribed recombined V(D)J genes.

This localization restricts mutations to V(D)J genes and surrounding introns, and results in unmutated C genes.

Within this region, mutations are introduced into both CDRs and FRs, and can be deleterious or helpful.
Somatic hypermutation of antibody V regions

1 week after immunization or infection, nucleotide substitutions begin to be introduced into recombined V(D)J genes expressed in B cells that are activated by antigen binding.

Mutations are introduced randomly and so they can destroy the antibody, or they can increase ability of BCR to bind antigen and these are selected.

Legend

- Replacement mutations
- Silent mutations
AID initiates somatic hypermutation of V genes by deamination of dC residues

AID (activation-induced cytidine deaminase) deaminates cytidine to uracil, which is recognized by uracil-DNA glycosylase (UNG). UNG removes uracil, and AP endonuclease removes the adjacent thymine. Displacement synthesis introduces mutations at the 

Somatic hypermutation

replication

G C

A T

replication

G C

A T

replication

C G

T A

Displacement synthesis mutates A:T bp
AID: converts dC residues in Ig VDJ (H chain) and VJ (L chain) regions and in \( S \) regions to dU residues.

The dU residues are repaired by the highly active ubiquitous DNA repair pathway, base excision repair, but repair does not go to completion to restore the dC residue.

Mismatch repair proteins (Msh2-Msh6) can also bind U:G mismatches, and try to repair the U:G mismatch, but this results in introducing mutations at A:T bases during SHM and converting SSBs to DSBs during CSR. This occurs because error-prone translesion Polymerase \( \eta \) is recruited.
Key Points: CSR and SHM

CSR and SHM occur after activation of B cells in humans and mice by antigen and T cells.

Both CSR and SHM require AID, which initiates DNA lesions that during repair by BER and mismatch repair generate DSBs and mutations.

Isotype to which B cells switch is determined by type of pathogen. The pathogen determines which cytokines are produced and which downstream $C_H$ genes are transcribed.

Mutations are introduced into expressed V genes and surrounding intron sequences. B cells expressing BCR’s with increased affinity due to mutations are selected. B cells with lower affinity die by apoptosis.

After CSR and SHM, B cells can differentiate to become long-lived plasma cells, constitutively secreting abundant Ig. Alternatively, they become memory cells, awaiting the next infection by the same pathogen.
AID is preferentially but not exclusively targeted to antibody V regions and S regions that are being transcribed. There are many other genes being transcribed in B cells. What makes Ig genes the best targets? Recent papers report several proteins that interact with AID and might help to target AID.

Nonetheless, AID mutates and instigates mutations and ds breaks in numerous non-Ig genes, some of which are oncogenes.

This explains why B cell lymphomas are the most common types of non-Hodgkins lymphoid cancer.
Isotypes and Allotypes

Isotypes - related proteins encoded by different genes, i.e. different loci. Examples: $\mu$ H chain and $\alpha$ H chain

Allotypes - proteins encoded by different alleles of the same locus. Examples: proteins encoded by the H2-D or Ig $C_{\mu}$ locus from the mother’s and father’s chromosomes (e.g. H2D$^d$ and H2D$^b$ or $C_{\mu}^b$ and $C_{\mu}^a$)