REVIEW ARTICLE

B cell development through V(D)J recombination and class-switch recombination

Aya F. Ahmed*

Department of Zoology, Biotechnology program, Faculty of Science, Port Said University, Port Said, Egypt

Received: March 31, 2021; **accepted:** April 30, 2021.

In mammalian bone marrow, pro-B/pre-B cells undergo V(D)J recombination process which includes rearrangement of variable (V), diversity (D), and joining (J) gene segments of the immunoglobulin genes to generate the primary immunoglobulin repertoire. Upon activation by antigen, B lymphocytes undergo additional two genomic modification processes, somatic hypermutation (SHM) and immunoglobulin class switch recombination (CSR) to enhance the affinity of immunoglobulin function. Activation-induced cytidine deaminase (AID) enzyme is an essential factor which mediates cytosine deamination reaction in CSR and SHM processes. These processes utilize base excision repair (BER) and the mismatch repair (MMR) pathways which are critical pathways in processing base modification resulting from AID action. Non-homologous end joining (NHEJ) is crucial for repairing double strand break (DSB) after BER and MMR activities. In this review, we summarize the V(D)J recombination and class switch recombination processes and discuss DNA repair pathways which are involved during V(D)J recombination and class switch recombination.

Keywords: Base excision repair; mismatch repair pathway; DNA repair; Ku70/80 heterodimer.

***Corresponding author:** Aya F. Ahmed, Department of Zoology, Biotechnology program, Faculty of Science, Port Said University, Port Said, Egypt. Phone: +20 10 11029770. Email: ayafayez1510@yahoo.com.

Introduction

Production of antibody diversity by the immune system depends on the capability of B and T cells to alter their genomes. Three genomic alterations in the immunoglobulin heavy-chain (IgH) and light-chain (IgL) loci enhance B cells to create the diverse repertoire of immunoglobulins, which include V(D)J recombination, class switch recombination (CSR), and somatic hypermutation (SHM). Developing B-lineage cells in the bone marrow assembles the exons that encode IgH and IgL variable regions through a process known as V(D)J recombination. V(D)J recombination involves assembly of variable (V), diversity (D), and joining (J) segments of the V exon of the immunoglobulin genes. Adjacent recombination signal sequence (RSS) exists at the 3' end of each V, J segment and at both ends of each D segment of each gene segment (V, D, and J). Recombination activating genes, RAG-1 and RAG-2, encode two proteins which identify RSS and introduce double-stranded breaks at both strands of DNA at the RSS forming double-stranded breaks (DSB), then the regular machinery for repairing DSBs (by no homologous end-joining) swings into action (figure 1A) [1, 2]. These processes allow production of IgM by B cells which migrate to secondary lymphoid organs. After immunization, IgM B cells undergo further antigen-driven immunoglobulin-gene diversification through

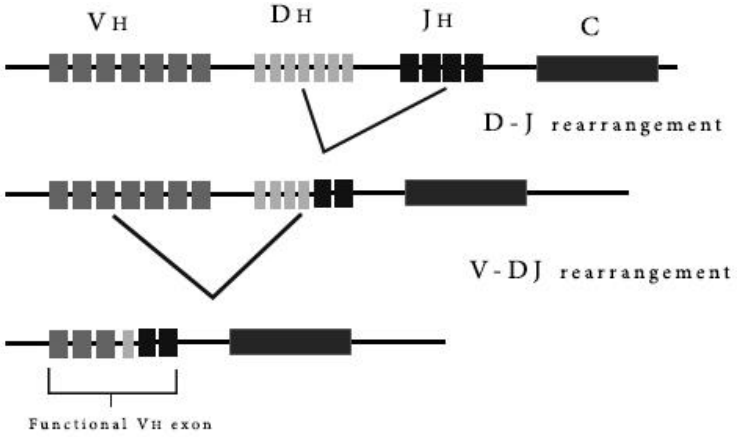
somatic hypermutation (SHM) and class-switch recombination (CSR) in secondary lymphoid organs. Somatic hypermutation process involves accumulation of point mutations in the antibody V-region of both the heavy and light chains to alter the specificity of the antibody and produce high-affinity antibodies. Activation-induced cytidine deaminase (AID) enzyme is sufficient to generate hypermutation in the antibody V-region during SHM process. AID enzyme is an essential factor in SHM and CSR, which catalyzes conversion reaction of deoxycytidine residues to deoxyuridine on target DNA [3, 4].

Class-switch recombination process includes recombination between switch regions which are highly repetitive GC-rich sequences that lie upstream of all immunoglobulin C-region genes, with the exception of the δ C-region gene (Figure 1b). Class-switch recombination process involves transcription through mammalian S regions [5]. Researchers demonstrated that generation of ssDNA R-loop substrates for the cytidine deaminase action of AID is processed by transcription through mammalian S regions. In S-region, the AID-generated dU:dG mismatch is processed by the base excision repair (BER) and mismatch repair (MMR) pathways, which generate staggered DSB. Accordingly, both Uracil-DNA glycosylase (UNG) deficiency [6] and MMR deficiency [7, 8] result in CSR defects and immunodeficiency. Class-switch recombination ends are demonstrated to be repaired by non-homologous end joining (NHEJ) which depends on X-ray cross-complementing protein 6/5 (XRCC6/5) [9, 10]. The NHEJ process starts with binding of a protein complex, XRCC6/5 (Ku70/80) heterodimer to DNA end. The association of a DNA end with the Ku heterodimer creates a scaffold for attraction of the other NHEJ enzymes. The DNA-Ku scaffold attracts the DNA-dependent protein kinase catalytic subunit (DNA-PKCS) to the DSB to form the active protein kinase complex DNA-PK. After capturing both DNA ends together by this kinase, these non-ligatable DNA termini need to be processed before the final ligation reaction can take place. The nucleases and polymerases enzymes are essential to either remove or fill-in single-stranded, noncompatible overhangs. Finally, ligation reaction of the processed DNA ends is catalyzed by the ligase IV/XRCC4 complex. These reactions are enhanced by XLF/Cernunnos protein [21]. In this review, we discuss V(D)J recombination and CSR processes with their regulatory mechanisms. The interplay between AID enzyme and DNA repair pathways and how this interplay enhances genomic stability during B cell development are also discussed.

VDJ recombination

Developing B-lineage cells in the bone marrow or fetal liver undergo V(D)J recombination process which includes production of antigen receptor diversity after assembly of gene segments V (variable), D (diversity), and J (joining)at the antigen receptor loci. The Ig receptor gene segments are flanked by RSSs. The RSS normally consists of a highly conserved heptamer motif (5'-CACAGTG-3’) and nonamer sequence (5'-ACAAAAACC-3’) separated by a spacer sequence of 12 or 23 nucleotides [11, 13]. The heptamer sequence is identified to be the critical recognition element. The first three nucleotides of the heptamer are identified as the highest sequence conservation which is essential for recombination. The nonamer sequence has a few highly conserved positions which are dispensable for recombination. V(D)J recombination occurs only between two gene segments flanked, respectively, by 12-(12RSS) and 23-(23RSS) bp spacers containing RSSs [1]. V(D)J recombination consists of two phases, cleavage and joining stages (Figure 1) [14, 15]. Cleavage stage is initiated by assembly of a hetero-tetrameric recombination activating gene 1 and 2 (RAG1 and RAG2) complex on a 12- or 23-RSS [16] and captures a complementary RSS. At the 5' end of the heptamer, the DNA is unwound [17], then RAG1 and RAG2 introduce a single-strand DNA nick at the heptamer-coding sequence [18-20]. After nicking the DNA at the heptamer coding border, RAGs process a trans-esterification reaction, in which the free 3' hydroxyl group

A



B

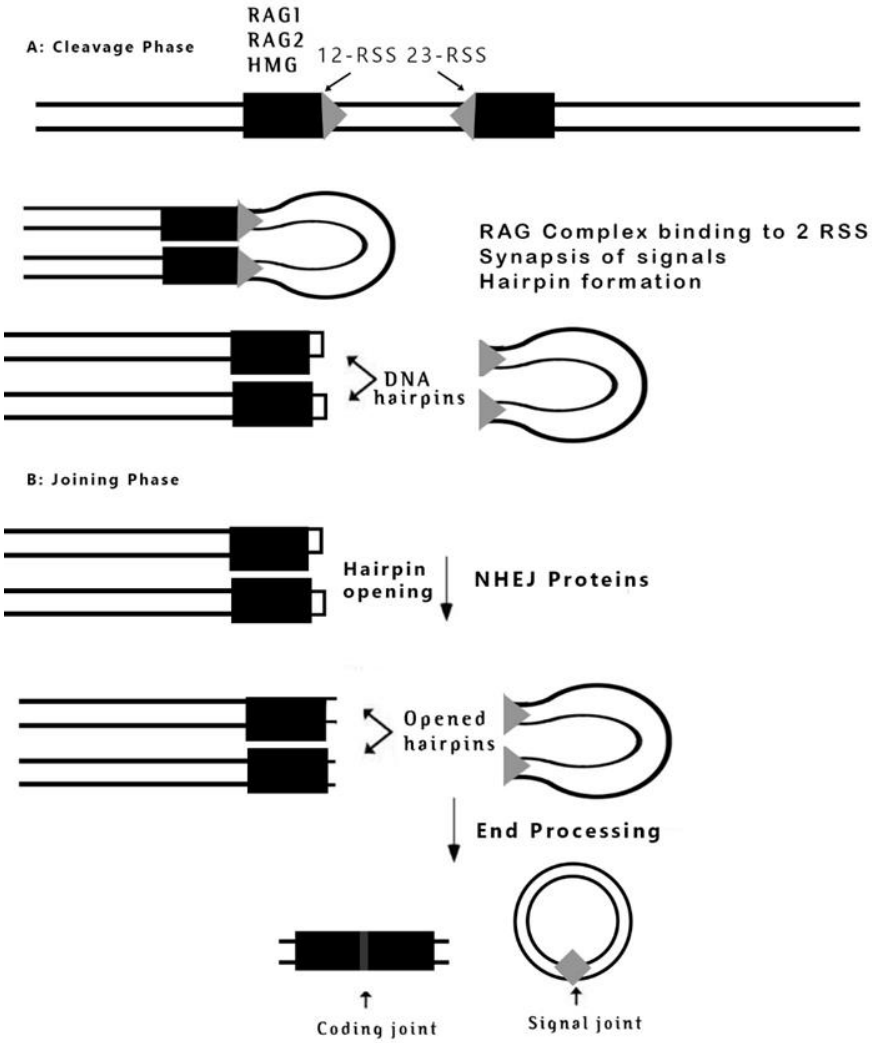


Figure 1. V(D)J recombination. **A.** the first step includes combine one D gene with one J gene. Next, a V gene is combined with the DJ gene to create a VDJ unit. **B.** adjacent recombination signal sequence (RSS) exists at the 3' end of each V, J segment and at both ends of each D segment of each gene segment (V, D, and J). Heterotetrameric complex RAG1+RAG2 binds to either a 12-RSS or 23-RSS. This complex is synapsed with complementary RSS. The RAG enzyme then nicks one DNA strands of each RSS and stimulates hydroxyl group to attack the other DNA strand through trans esterification reaction. These reactions generate hair pinned coding ends and blunt signal ends which repaired by NHEJ repair pathway [14, 15].

attacks the opposite DNA strand [21], resulting a pair of sealed hairpins at the coding ends, and blunt signal ends [22].

The non-homologous end joining (NHEJ) proteins are recruited to the coding ends and then these proteins open and join the coding ends to generate imprecise coding joints containing added nucleotides [23]. The Ku heterodimer is well demonstrated to start the repair phase via assembly to the RAG-generated breaks and is required for both signal and coding joints [24–26]. Ku recruits DNA-protein kinase catalytic subunit (DNA-PKcs) which is required for coding joint formation. DNA-PKcs serves as a scaffold for other repair factors such as Ku heterodimer [27], Ligase IV–XRCC4 [28–30], and Artemis [31]. Inactivation of DNA-PKcs may result in a defect in coding joint formation and abnormal B- and T-cell development [32]. DNA-PKcs recruits and activates the hairpin-opening nuclease Artemis enzyme [33, 34], which is defined by its nucleolytic activities. Then, these enzymes could further contribute to coding end processing [35]. *In vivo* data showed accumulation of coding ends in developing lymphocytes from Artemis-deficient mice. These studies have reflected the Artemis' role as the hairpin-opening nuclease [36]. The final ligation step to form both signal and coding joints is processed by the Ligase IV–XRCC4–Cernunnos/XLF complex [37, 38]. Ligase IV deficient cells showed impairment in V(D)J recombination in a human pre-B cell line [37, 39]. Productive assembly of IgH and IgL V-region exons allows the expression of IgH and IgL chains as cell-surface IgM by newly generated B cells. IgM B cells migrate to secondary lymphoid organs, for example, the spleen and lymph nodes, where they can undergo antigen-driven immunoglobulin-gene diversification through somatic hypermutation (SHM) and class-switch recombination (CSR) [40].

Class switch recombination

Class switch recombination (CSR) is a process by which the constant region in the immunoglobulin heavy chain is rearranged to convert one class of immunoglobulin (such as IgM) to another (such as IgG). CSR involves the interconnection between switching regions which are extremely frequent GC-rich sequences of 1-10 KB in length that lie upstream of all immunoglobulin C-region genes with the exception of the δ C-region gene [42] (Figure 2). Recent reports demonstrated that CSR are initiated by AID activity. Moreover, AID is a putative RNA-editing enzyme which catalyzes conversion of deoxycytidine (C) to deoxyuridine residues (U) [42]. The deamination reaction involves direct nucleophilic attack at position 4 of the pyrimidine ring of cytosine by Zn^{2+} coordinated to AID. *In vitro* experiments have shown that AID prefers sequences that conform to WRC motifs (W = A, T; R = A, G) [43], which are highly enriched in S regions in the form of AGCT [44]. Some reports demonstrated that complete deletion of AID hotspot motifs reduces CSR efficiency [45]. AID expression is limited to activated B cells [46] and AID deficient mice have shown completely defective in SHM and CSR [47]. Therefore, patients with HIGM2 harboring inactivating AID mutations lost the ability to undergo through SHM and CSR [48].

Several pathways are identified as key players in stimulation of AID gene expression. CD40 ligand (CD40L):CD40 interaction is an essential pathway which stimulates AID transcription and increases AID expression after signaling NF-κB pathway [49]. Several types of cytokines such as B cell-activating factor (BAFF), IL-4, TGF-β1, and a proliferation-inducing ligand (APRIL) are produced by immune cells (e.g., macrophages and dendritic cells) also stimulate expression of AID gene.

In mouse B cells, AID expression is induced by IL-4 which stimulates Stat6 and protein kinase A (PKA)/CREB pathways [50]. Also, the p38MAPK/CREB and JNK/AP-1 pathways, which are stimulated by BAFF, play critical roles in AID gene expression [51]. In replicating B cells, the cyclooxygenase 2/prostaglandin E2 pathway induces AID expression [52]. Other transcription factors, such as IRF-8 [53], Pax5 [54], Sp1/3 [55],

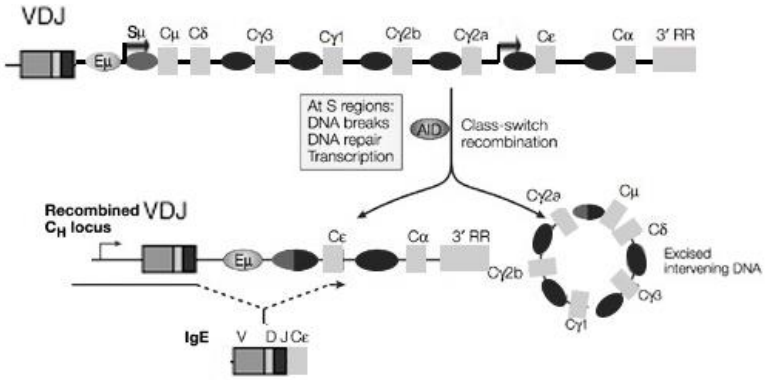


Figure 2. Class switch recombination (CSR). DNA breaks are introduced into switch (S) regions after the activity of the enzyme activation-induced cytidine deaminase (AID) and these DNA breaks undergo DNA repair process. CSR process proceed for repositioning the constant region genes and deleting the interstitial sequence as an episomal circuit [42].

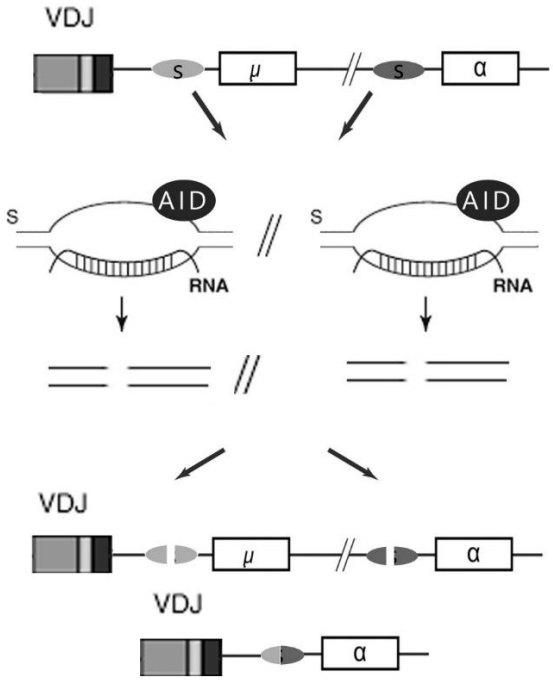


Figure 3. Model of class switch recombination (CSR). CSR requires AID enzyme activity which deaminates a cytosine to create an uracil. Transcription by RNA polymerase II (RNA Pol II) through S region is an essential factor which provides the single-stranded DNA template for AID enzyme activity. Uracil, which produced by AID enzyme, then is processed through BER or MMR pathways [42].

E2A (E47), are identified to be critical in stimulation AID gene expression [56].

Chromatin immunoprecipitation studies indicated that AID expression in cells that are undergoing CSR is directly related to the transcribed S-region of the immunoglobulin (Figure 3) [57]. Transcriptional intervening (I) promoter and an I exon exist upstream of all C genes except δ C-region gene. Non-coding germline transcripts are produced after selective activation of I promoters and initiate at the I exon and undergo downstream of the corresponding heavy chain constant region gene (CH gene) [58] yielding ssDNA substrate for AID enzyme action [59].

After binding of AID to the S region, protein kinase A is recruited and phosphorylates AID enzyme at Ser38 of the N-terminal region. The phosphorylated AID creates a binding site for RPA [60] which stimulates the deamination activity of AID and converts C into U. Then, a DSB is resulted if two Us are near in opposite strands after BER and MMR pathway activities [61]. In S regions, the first protein complex assemble to DSB is Mre11-Rad50Nbs1 complex which recruits the protein kinase ataxia-telangiectasia mutated (ATM). ATM activates and phosphorylates several DNA damage response proteins involving H2AX, mediator of damage checkpoint protein 1 (MDC1) [62], breast cancer 1 (BRCA1), p53 binding protein 1 (53BP1), ubiquitin ligases RNF8, RNF168, and receptor-associated protein 80 (RAP80) [63]. DSB in different S regions are repaired by NHEJ repair pathway. The inference that CSR ends are repaired by NHEJ was initially supported by *in vivo* and *in vitro* studies which demonstrated that Ku70 or Ku80 deficient mice showed severely defect in CSR process [64]. Furthermore, the importance of DNA DSBs in CSR is indicated by the finding that CSR is abolished, or substantially impaired in B cells that are deficient in Ku70, Ku80, or DNA-PKcs.

Switching deaminated DNA to DSBs by BER and MMR

The BER and MMR pathways mediate the conversion of deaminated cytidines into DSBs. Base excision repair (BER) pathway is essential for recognition and repairing the base modifications including Us in the DNA [65, 66]. In the BER pathway for CSR, the first step is processed by Uracil-(N)-glycosylase (UNG). The UNG gene encodes two isoforms UNG1 and UNG2 that differ by their N-terminal sequence [67]. UNG2 is the major DNA glycosylase involved in U removal during CSR and SHM and is active on both single-stranded (ss) and double-stranded (ds) DNA. Inactivating mutations of UNG may result in severe blockade of CSR and defect in DSB formation at S regions [68]. Indeed, UNG deficient mice display a slow removal of U and display an about 20-fold increased risk of developing B-cell lymphomas [69]. UNG2 excises the irregular Us bases on the sugar-phosphate backbone creating an abasic site (Figure 4). Then, apurinic/apyrimidinic endonuclease (APE1) cleaves the phosphodiester bond on the 5′ side of a damaged nucleotide at the basic site, resulting in the formation of a 3′-OH group and a 5′ damaged nucleotide, and creates a single-strand break (SSB) in the DNA. Closely spaced, a staggered DSB can be formed by similarly generating SSB on the opposite strand.

In vitro studies have shown that deletion of Ape1 gene may reduce CSR efficiency to 20% of the wildtype level, strongly suggesting that APE1 is essential for CSR process [70]. Moreover, fewer Sμ region DSBs were demonstrated in Ape1 deficient B cells undergoing CSR, providing the evidence that APE1 is required for incisions at AP sites during CSR [71].

The U can also be identified as a U-G mismatch and can be repaired by MMR [72, 73]. The dU:dG mismatch is recognized by MSH2-MSH6 heterodimer which recruits MLH1/PMS2/EXO1 complex. Next, an incision of the mismatch can be made by the endonuclease complex PMS2 and MLH1 (Figure 5). Then, exonuclease EXO1, which has 5′ to 3′ exonuclease activity, creates a single-stranded gap. dU-containing sequences excision on opposite DNA strands thus would create DSBs. Some research demonstrated that inactivating PMS2 or EXO1 results in CSR impairments in human and mice because of defects in DSB formation in S regions [74, 75]. These DSB with a 5′ overhang then can be processed to a blunt DSB by DNA polymerases which is used by NHEJ pathways to complete CSR [76].

Non-homologous end-joining (NHEJ)

The sliding of the protein complex, the Ku70/80 heterodimer, over both ends of the broken DNA molecule is the basic step in the earliest stages of the NHEJ process. Crystallography studies of Ku70/80 proposed that open ring-shaped

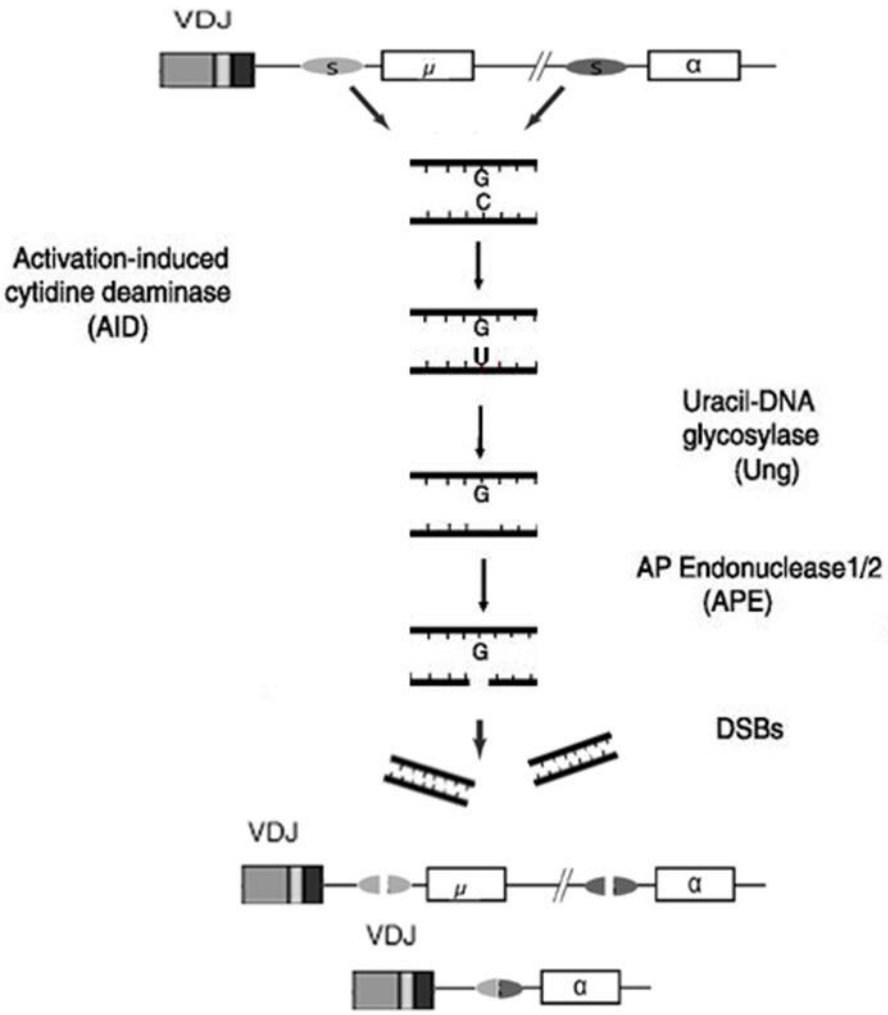


Figure 4. Model for DSB formation by BER in class switch recombination (CSR). AID converts DNA cytosines to uracils in switch (S) region. These uracils are excised by UNG2 enzyme. UNG2-generated abasic sites is converted to single-strand breaks after Ap Endonuclease (APE) enzyme activity. DSB is generated after introducing opposing single-strand breaks. Non-homologous end joining factors then process DSB to complete CSR event [42].

structure of Ku70/80, which is composed of one Ku70 (73 kDa) and one Ku80 (86 kDa) proteins, allows the Ku70/80 dimer to slide over the DNA termini, thus, explaining the high affinity of Ku70/80 for DNA termini [77]. Several studies demonstrated that the binding of a DNA end with the Ku heterodimer gives other NHEJ key enzymes binding capability to DNA ends (Figure 6) [64]. By reaction of the Ku70/80 heterodimer with the broken DNA ends, the Ku-DNA complex generates a scaffold for the association of a 460-kDa serine/threonine kinase:DNA-PKCS [78]. These reactions preserve the DNA termini against degradation and premature ligation. Biochemical experiments have demonstrated that DNA-PKCS molecule holds the DNA ends together prior to ligation [79] and generates a synaptic complex that juxtaposes two DNA termini, two Ku dimers, and two DNA-PKCS molecules. The association of DNA-PKCS with the Ku-DNA complex is essential for the activation of the DNA-PKCS serine/threonine kinase at the carboxy-terminus of the DNA-PKCS molecule. The DNA-PKCS molecule has 16 amino-acid residues that can be autophosphorylated by the DNA-PKCS kinase [80-82]. It is indicated that autophosphorylation process of DNA-PKCS occur when Ku and DNA-PKCS form synapsis with the two DNA ends. The

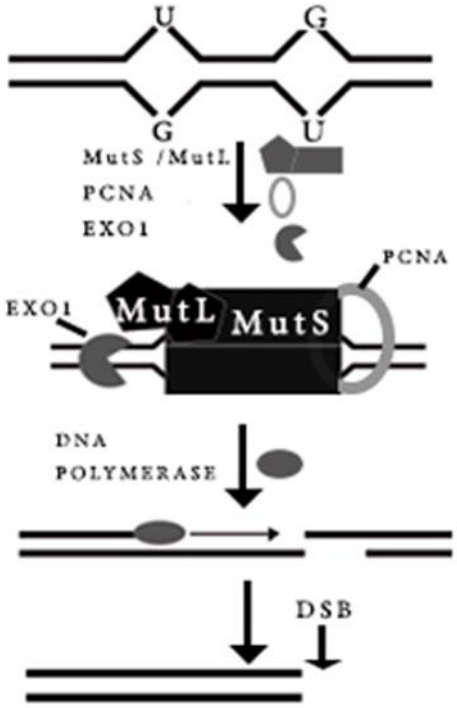


Figure 5. Mismatch repair model for DSB formation during CSR. U:G mismatch is recognized by Mut S-MutL which recruits Exo I protein to 5' side of the U:G mismatch. Exo I introduces single strand breaks at a nicked site. A patch of DNA can be removed by Exo I until it reached the nicked site on the other strand. The staggered end of the DNA is filled by DNA polymerase and form a DSB.

phosphorylation of the DNA-PKC has influence on conformation of the synaptic complex and unphosphorylated form at the DNA termini blocks the assembly of processing enzymes and ligases to the synaptic complex [83-85].

Several studies have demonstrated that the Ku70/80 heterodimer assembles several of the processing enzymes and ligation enzymes to the synaptic repair complex in a manner similar to the recruitment of DNA-PKCS. DNA termini will not be directly ligated because one of the strands will have a 3' or 5' single-strand overhang. A single-strand DNA overhang can be processed either by generating a complementary strand or by resection of the overhang. DNA polymerase μ, DNA polymerase λ, and human terminal deoxynucleotidyl transferase (TdT) are identified as critical enzymes which are capable of synthesizing a complementary strand by adding nucleotides in NHEJ. Pol μ and Pol λ are the members of the Pol X family of polymerases which participate in NHEJ. Both Pol μ and Pol λ can incorporate both dNTPs and rNTPs in a template-dependent or template-independent manner [86, 87]. DNA Pol μ and Pol λ have an N-terminal BRCA1 domain that enables them to interact with Ku [26]. Primary mice cells with genetic knockouts of both Pol μ and Pol λ have shown deficit in DSB repair [88]. Pol X family members also involve terminal deoxynucleotidyl transferase (TdT) which is able to incorporate nucleotides during NHEJ in a template-dependent manner.

Terminal deoxynucleotidyl transferase (TdT) is only expressed in early B- and T-lymphocytes which undergo NHEJ repair that occurs during V(D)J recombination process [89, 92]. The endonuclease Artemis has been demonstrated as an essential enzyme which displays a 5'-3' endonuclease activity for resection of single-stranded overhangs. The Artemis protein itself has a 5'-3' exonuclease activity. After association with the DNA-PKCS molecule, Artemis protein acquires the endonuclease activity that

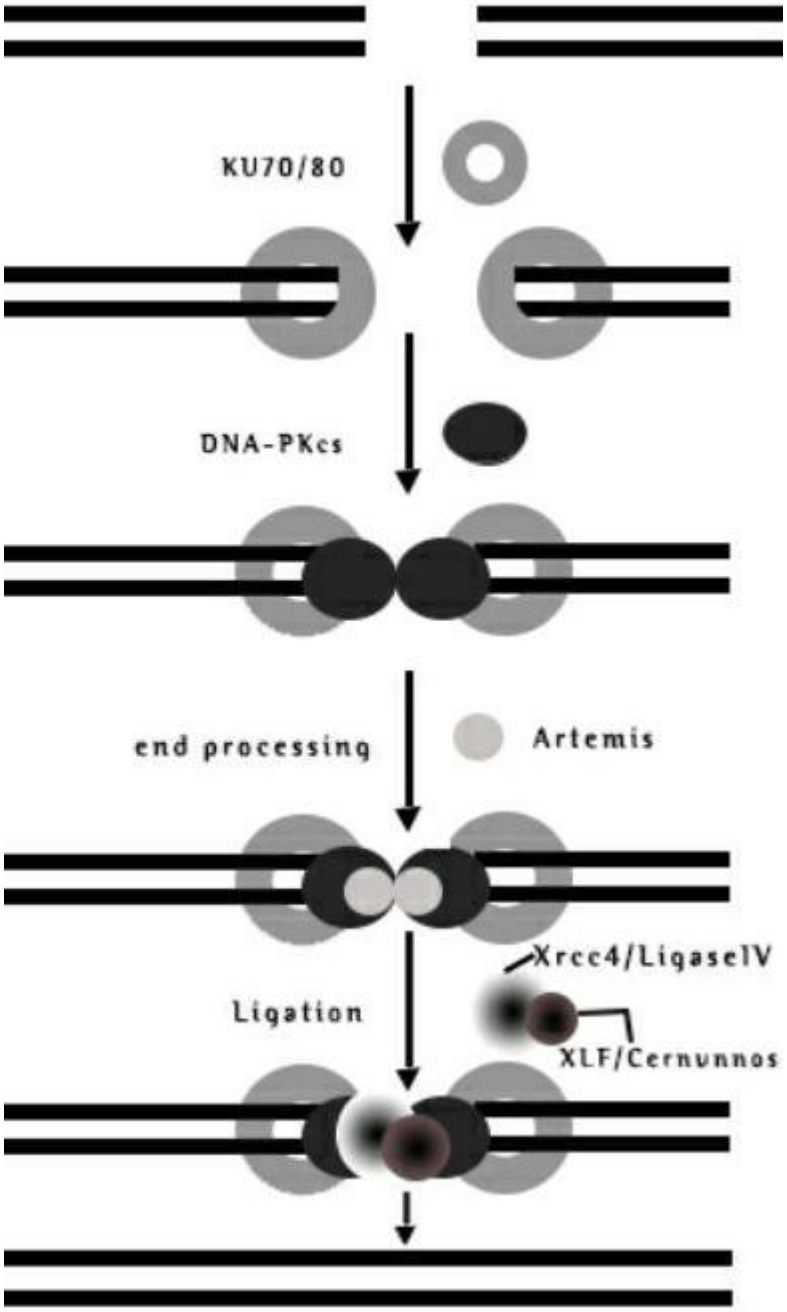


Figure 6. Model for NHEJ. The Ku70/80 heterodimer binds to broken DNA ends. Ku molecules recruit DNA-PKCS. The DNA-PKCS molecules on both DNA ends form a synaptic complex which tethers the DNA ends. Trans DNA-PKCS autophosphorylation generates accessible DNA termini for other NHEJ enzymes. DNA ends are processed before ligation by Artemis nuclease. Finally, the ligase complex IV/XRCC4 mediates rejoining of DNA double-strand breaks [114].

processes hairpin opening during V(D)J recombination [93, 94].

Processing of DNA ends also can be mediated by mammalian polynucleotide kinase (PNK). PNK can add 5′ phosphate groups to DNA termini which are essential for the ligation reaction [95]. The PNK, the apurinic/apurinic endonuclease (APE1), the tyrosyl-DNA phosphodiesterase (TDP1), and the endonuclease Artemis are

identified as critical enzymes for removing 3' phosphoglycolates in certain case before ligation reaction [96].

Finally, the ligase complex IV/XRCC4 stimulates binding of processed DNA ends. One *in vitro* study has indicated that, after interaction with Ku70/80, the ligation activity of ligase IV/XRCC4 increases 20-fold, suggesting that Ku is critical to activate ligase IV/XRCC4 [97]. Ligase IV is demonstrated to be composed of the N-terminus, which includes a DNA binding domain, an adenylation domain, an oligo-binding domain, and the C-terminus containing two BRCA1 C-terminal (BRCT) domains [98]. BRCT motifs mediate protein–protein and protein–DNA interactions [99–101]. XRCC4 has a globular amino-terminal head and a long carboxyterminal stalk [102]. The amino-terminal head interacts with DNA and the carboxy-terminal region between amino acids 173 and 195 enhances the association of XRCC4 with ligase IV [103]. The BRCT domains associate with Ku [104] and the homodimer of XRCC4 binds to the region between the two BRCT domains. The association of XRCC4 with DNA ligase IV form bridging between the two DNA ends [105, 106]. Together, XRCC4 and Ligase IV create a stable complex, and XRCC4 absence results in degradation of Ligase IV. Consequently, XRCC4 is identified as essential enzyme to both stabilize and stimulate ligase IV/XRCC4 ligation activity [107, 108]. This ligation reaction may be enhanced by the existence of what has been recently discovered which is called XLF/Cernunnos protein [109]. Cernunnos-XLF is phosphorylated by DNA-PKCS in human cells in response to DSBs [110]. XLF/Cernunnos factor interacts with the ligase IV/XRCC4 complex and modulates the ligation efficiency of ligase IV/XRCC4 [111]. Recent studies proposed that XLF interacts with XRCC4 and creates a helical filament of alternating Cernunnos-XLF/XRCC4 dimers which, thereby, stabilizes DNA end synapsis [112]. Ligase IV is needed to stabilize both the association of Cernunnos-XLF/XRCC4 and its recruitment to the sites of DNA breaks. Cernunnos-XLF deficiency results in a defect in DSB repair including both signal and coding joins in V(D)J recombination [113]. Taken together, NHEJ is critical for the efficient repair of DNA double-strand breaks (DSBs) during V(D)J recombination and CSR and point mutations in the core NHEJ factors may result in genomic instability and carcinogenesis.

Conclusion

In this review, we presented the roles of DSBs in V(D)J recombination and CSR during B cell development. V(D)J recombination process divided into cleavage and joining stages. RAG1 and RAG2 recombinase enzymes are the essential factors which modulate V(D)J recombination process by generating DSB. These DSBs are processed by the non-homologous end joining (NHEJ) proteins yielding IgM B cell. After immunization, IgM B cells undergo CSR and SHM which are initiated by AID enzyme activity. AID mediates cytosine deamination reaction which would yield U:G mismatches that stimulate either the MMR or the BER pathways. Both MMR and BER pathways are sufficient in processing U:G mismatch into DSB which are rejoined by NHEJ pathway to complete CSR events. Aberrant repair of such DSBs can lead to chromosomal breaks and translocations, which are critical factor in B-cell lymphomagenesis.

References

1. Schatz DG. 2004. V(D)J recombination. Immunol Rev. 200:5–11.
2. Brandt VL, Roth DB. 2004. V(D)J recombination: how to tame a transposase. Immunol Rev. 200:249–260.
3. Sohail A, Klapacz J, Samaranayake M, Ullah A, Bhagwat AS. 2003. Human activation-induced cytidine deaminase causes transcription-dependent, strand-biased C to U deaminations. Nucleic Acids Res. 31:2990–2994.
4. Pham P, Bransteitter R, Petruska J, Goodman MF. 2003. Processive AID-catalysed cytosine deamination on single-stranded DNA simulates somatic hypermutation. Nature. 424(6944):103-107.
5. Ramiro AR, Stavropoulos P, Jankovic M, Nussenzweig MC. 2003. Transcription enhances AID-mediated cytidine deamination by exposing single-stranded DNA on the nontemplate strand. Nat Immunol. 4(5):452–456.

6. Bak ST, Sakellariou D, Pena-Diaz J. 2014. The dual nature of mismatch repair as antimutator and mutator: for better or for worse. Front Genet. 5:287.
7. Bardwell PD, Martin A, Wong E, Li Z, Edelmann W, Scharff MD. 2003. Cutting edge: the G-U mismatch glycosylase methyl-CpG binding domain 4 is dispensable for somatic hypermutation and class switch recombination. J Immunol. 170:1620–1624.
8. Berek C and Milstein C. 1987. Mutation drift and repertoire shift in the maturation of the immune response. Immunol Rev. 96:2341.
9. Casellas R, Nussenzweig A, Wuerffel R, Pelanda R, Reichlin A, Suh H, *et al*. 1998. Ku80 is required for immunoglobulin isotype switching. EMBO J. 17:2404–2411.
10. Manis JP, Gu YS, Lansford R, Sonoda E, Ferrini R, Davidson L, *et al*. 1998. Ku70 is required for late B cell development and immunoglobulin heavy chain class switching. J Exp Med. 187(12):2081–2089.
11. Hwang JK, Alt FW, Yeap LS. 2015. Related mechanisms of antibody somatic hypermutation and class switch recombination. Microbiol Spectr. 3:MDNA3-0037-2014.
12. Xu Z, Zan H, Pone EJ, Mai T, Casali P. 2012. Immunoglobulin class-switch DNA recombination: induction, targeting and beyond. Nat Rev Immunol. 12:517–531.
13. Carmona LM, Schatz DG. 2017. New insights into the evolutionary origins of the recombination-activating gene proteins and V(D)J recombination. FEBS J. 284:1590605.
14. Mostoslavsky R, Alt FW, Bassing CH. 2003. Chromatin dynamics and locus accessibility in the immune system. Nat Immunol. 4(7):603-606.
15. Chang HH, Lieber MR. 2016. Structure-Specific nuclease activities of Artemis and the Artemis: DNA-PKcs complex. Nucleic Acids Res. 44(11):4991-4997.
16. Swanson PC. 2002. Fine structure and activity of discrete RAG-HMG complexes on V(D)J recombination signals. Mol Cell Biol. 22:1340–1351.
17. Ru H, Mi W, Zhang P, Alt FW, Schatz DG, Liao M, *et al*. 2018. DNA melting initiates the RAG catalytic pathway. Nat Struct Mol Biol. 25:732-742.
18. Kim DR, Dai Y, Mundy CL, Yang W, Oettinger MA. 1999. Mutations of acidic residues in RAG1 define the active site of the V(D)J recombinase. Genes Dev. 13:3070–3080.
19. Landree MA, Wibbenmeyer JA, Roth DB. 1999. Mutational analysis of RAG1 and RAG2 identifies three catalytic amino acids in RAG1 critical for both cleavage steps of V(D)J recombination. Genes Dev. 13:3059–3069.
20. Fugmann SD, Villey IJ, Ptaszek LM, Schatz DG. 2000. Identification of two catalytic residues in RAG1 that define a single active site within the RAG1/RAG2 protein complex. Mol Cell. 5:97–107.
21. McBlane JF, van Gent DC, Ramsden DA, Romeo C, Cuomo CA, Gellert M, *et al*. 1995. Cleavage at a V(D)J recombination signal requires only RAG1 and RAG2 proteins and occurs in two steps. Cell. 83:387–395.
22. Gellert M. 2002. V(D)J recombination: RAG proteins, repair factors, and regulation. Annu Rev Biochem. 71:101–132.
23. Helmink BA, Sleckman BP. 2012. The response to and repair of RAG-mediated DNA double-strand breaks. Annu Rev Immunol. 30:175–202.
24. Gu Y, Seidl KJ, Rathbun GA, Zhu C, Manis JP, van der Stoep N, *et al*. 1997. Growth retardation and leaky SCID phenotype of Ku70deficient mice. Immunity. 7(5):653–665.
25. Zhu C, Bogue MA, Lim DS, Hasty P, Roth DB. 1996. Ku86-deficient mice exhibit severe combined immunodeficiency and defective processing of V(D)J recombination intermediates. Cell. 86(3):379–389.
26. Nussenzweig A, Chen C, da Costa Soares V, Sanchez M, Sokol K, Nussenzweig MC, *et al*. 1996. Requirement for Ku80 in growth and immunoglobulin V(D)J recombination. Nature. 382(6591):551–555.
27. Gao Y, Chaudhuri J, Zhu C, Davidson L, Weaver DT, Alt FW. 1998. A targeted DNA-PKcs-null mutation reveals DNA-PK-independent functions for KU in V(D)J recombination. Immunity. 9(3):367–376.
28. Gell D, Jackson SP. 1999. Mapping of protein-protein interactions within the DNA-dependent protein kinase complex. Nucleic Acids Res. 27(17):3494–3502.
29. Calsou P, Delteil C, Frit P, Drouet J, Salles B. 2003. Coordinated assembly of Ku and p460 subunits of the DNA-dependent protein kinase on DNA ends is necessary for XRCC4-ligase IV recruitment. J Mol Biol. 326(1):93–103.
30. Hsu HL, Yannone SM, Chen DJ. 2002. Defining interactions between DNA-PK and ligase IV/XRCC4. DNA Repair (Amst). 1(3):225–235.
31. Ma Y, Pannicke U, Schwarz K, Lieber MR. 2002. Hairpin opening and overhang procesing by an Artemis/DNA-dependent protein kinase complex in nonhomologous end joining and V(D)J recombination. Cell. 108:781–794.
32. Dong J, Zhang T, Ren Y, Wang Z, Ling CC, He F, *et al*. 2017. Inhibiting DNA-PKcs in a non-homologous end-joining pathway in response to DNA double-strand breaks. Oncotarget. 8(14):22662-22673.
33. Roth DB, Menetski JP, Nakajima PB, Bosma MJ, Gellert M. 1992. V(D)J recombination: broken DNA molecules with covalently sealed (hairpin) coding ends in scid mouse thymocytes. Cell. 70(6):983–991.
34. Ma Y, Pannicke U, Schwarz K, Lieber MR. 2002. Hairpin opening and overhang processing by an Artemis/DNA-dependent protein kinase complex in nonhomologous end joining and V(D)J recombination. Cell. 108:781–794.
35. Gu J, Li S, Zhang X, Wang LC, Niewolik D, Schwarz K, *et al*. 2010. DNA-PKcs regulates a single-stranded DNA endonuclease activity of Artemis. DNA Repair. 9(4):429–437.
36. Rooney S, Sekiguchi J, Zhu C, Cheng HL, Manis J, Whitlow S, *et al*. 2002. Leaky Scid phenotype associated with defective V(D)J coding end processing in Artemis-deficient mice. Mol Cell. 10(6):1379–1390.
37. Grawunder U, Zimmer D, Fugmann S, Schwarz K, Lieber MR. 1998. DNA ligase IV is essential for V(D)J recombination and DNA double-strand break repair in human precursor lymphocytes. Mol Cell. 2(4):477–484.
38. Dahm K. 2008. Role and regulation of human XRCC4-like factor/ cernunnos. J Cell Biochem. 104(5):1534–1540.

39. Wei YF, Robins P, Carter K, Caldecott K, Pappin DJ, Yu GL, *et al*. 1995. Molecular cloning and expression of human cDNAs encoding a novel DNA ligase IV and DNA ligase III, an enzyme active in DNA repair and recombination. Mol Cell Biol. 15(6):3206–3216.
40. Di Noia JM, Neuberger MS. 2007. Molecular mechanisms of antibody somatic hypermutation. Annu Rev Biochem. 76:1–22.
41. Manis JP, Tian M, Alt FW. 2002. Mechanism and control of class-switch recombination. Trends Immunol. 23:31-39.
42. Chaudhuri J, Alt F. 2004. Class-switch recombination: interplay of transcription, DNA deamination and DNA repair. Nat Rev Immunol. 4:541–552.
43. Shapiro GS, Aviszus K, Murphy J, Wysocki LJ. 2002. Evolution of Ig DNA sequence to target specific base positions within codons for somatic hypermutation. J Immunol. 168:2302–2306.
44. Hackney JA, Misaghi S, Senger K, Garris C, Sun Y, Lorenzo MN, *et al*. 2009. DNA targets of AID evolutionary link between antibody somatic hypermutation and class switch recombination. Adv Immunol. 101:163-189.
45. Wang M, Rada C, Neuberger MS. 2010. Altering the spectrum of immunoglobulin V gene somatic hypermutation by modifying the active site of AID. J Exp Med. 207(1):141–153.
46. Muramatsu M, Sankaranand VS, Anant S, Sugai M, Kinoshita K, Davidson NO, *et al*. 1991. 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(26):18470-18476.
47. Muramatsu M, Kinoshita K, Fagarasan S, Yamada S, Shinkai Y, Honjo T. 2000. Class switch recombination and hypermutation require activation-induced cytidine deaminase (AID), a potential RNA editing enzyme. Cell. 102(5):553-563.
48. Revy P, Muto T, Levy Y, Geissmann F, Plebani A, Sanal O. *et al*. 2000. Activation-induced cytidine deaminase (AID) deficiency causes the autosomal recessive form of the Hyper-IgM syndrome (HIGM2). Cell. 102(5):565-575.
49. Xu Z, Zan H, Pone EJ, Mai T, Casali P. 2012. Immunoglobulin class-switch DNA recombination: induction, targeting and beyond. Nat Rev Immunol. 12(7):517-531.
50. Kim RJ, Kim HA, Park JB, Park SR, Jeon SH, Seo GY, *et al*. 2007. IL-4-induced AID expression and its relevance to IgA class switch recombination. Biochem Biophys Res Commun. 361:398- 403.
51. Kim HA, Seo GY, Kim PH. 2011. Macrophage-derived BAFF induces AID expression through the 236 p38MAPK/CREB and JNK/AP-1 pathways. J Leukoc Biol. 89:393-398.
52. Lee H, Trott JS, Haque S, McCormick S, Chiorazzi N, Mongini PK. 2010. Acyclooxygenase-2/prostaglandin E2 pathway augments activation-induced cytosine deaminase expression within replicating human B cells. J Immunol. 185:5300-5314.
53. Lee CH, Melchers M, Wang H, Torrey TA, Slota R, Qi CF, *et al*. 2006. Regulation of the germinal center gene program by interferon (IFN) regulatory factor 8/IFN consensus sequence-binding protein. J Exp Med. 203(1):63-72.
54. Gonda H, Sugai M, Nambu Y, Katakai T, Agata Y, Mori KJ, *et al*. 2003. The balance between Pax5 and Id2 activities is the key to AID gene expression. J Exp Med. 198:1427-1437.
55. Yadav A, Olaru A, Saltis M, Setren A, Cerny J, Livák F. 2006. Identification of a ubiquitously active promoter of the murine activation-induced cytidine deaminase (AICDA) gene. Mol Immunol. 43(6):529-541.
56. Sayegh CE, Quong MW, Agata Y, Murre C. 2003. E-proteins directly regulate expression of activation-induced deaminase in mature B cells. Nat Immunol. 4:586-593.
57. Matthews AJ, Zheng S, DiMenna LJ, Chaudhuri J. 2014. Regulation of immunoglobulin class-switch recombination: choreography of noncoding transcription, targeted DNA deamination, and long-range DNA repair. Adv Immunol. 122:1–57.
58. Feldman S, Wuerffel R, Achour I, Wang L, Carpenter PB, Kenter AL. 2017. 53BP1 contributes to Igh locus chromatin topology during class switch recombination. J Immunol. 198(6):2434-2444.
59. Kim A, Han L, Santiago GE, Verdun RE, Yu K. 2016. Class-switch recombination in the absence of the IgH 30 regulatory region. J Immunol. 197:2930–2935.
60. Basu U, Chaudhuri J, Alpert C, Dutt S, Ranganath S, Li G, *et al*. 2005. The AID antibody diversification enzyme is regulated by protein kinase A phosphorylation. Nature. 438:508–511.
61. Rada C, Di Noia JM, Neuberger MS. 2004. Mismatch recognition and uracil excision provide complementary paths to both Ig switching and the A/T-focused phase of somatic mutation. Mol Cell. 16(2):163–171.
62. Ghosh R, Das D, Franco S. 2018. The role for the DSB response pathway in regulating chromosome translocations. Adv Exp Med Biol. 1044:65–87.
63. Khair L, Guikema JE, Linehan EK, Ucher AJ, Leus NG, Ogilvie C, *et al*. 2014. ATM increases activation-induced cytidine deaminase activity at downstream S regions during class switch recombination. J Immunol. 192:4887–4896.
64. Fell VL, Schild-Poulter C. 2015. The Ku heterodimer: function in DNA repair and beyond. Mutat Res Rev Mutat Res. 763:15-29.
65. Lindahl T. 1979. DNA glycosylases endonucleases for apurinic/apyrimidinic sites and base excision-repair. Progr Nucleic Acid Res Mol Biol. 22:135–192.
66. Krokan HE, Bjoras M. 2013. Base excision repair. Cold Spring Harb Perspect Biol. 5:a012583.
67. Nilsen H, Otterlei M, Haug T, Solum K, Nagelhus TA, Skorpen F, *et al*. 1997. Nuclear and mitochondrial uracil-DNA glycosylases are generated by alternative splicing and transcription from different positions in the UNG gene. Nucleic Acids Res. 25:750–755.
68. Imai K, Slupphaug G, Lee WI, Revy P, Nonoyama S, Catalan N, *et al*. 2003. Human uracil-DNA glycosylase deficiency associated with profoundly impaired immunoglobulin class-switch recombination. Nat Immunol. 4(10):1023-1028.
69. Nilsen H, Stamp G, Andersen S, Hrivnak G, Krokan HE, Lindahl T, *et al*. 2003.Gene-targeted mice lacking the Ung uracil-DNA glycosylase develop B-cell lymphomas. Oncogene. 22:5381–5386.

70. Masani S, Han L, Yu K. 2013. Apurinic/apyrimidinic endonuclease 1 is the essential nuclease during immunoglobulin class switch recombination. Mol Cell Biol. 33:1468–1473.
71. Guikema JE, Linehan EK, Tsuchimoto D, Nakabeppu Y, Strauss PR, Stavnezer J, *et al*. 2007. APE1- and APE2-dependent DNA breaks in immunoglobulin class switch recombination. J Exp Med. 204(12):3017-3026.
72. Wilson TM, Vaisman A, Martomo SA, Sullivan P, Lan L, Hanaoka F, *et al*. 2005. MSH2-MSH6 stimulates DNA polymerase eta, suggesting a role for A:T mutations in antibody genes. J Exp Med. 201:637–6345.
73. Pena-Diaz J, Bregenhorn S, Ghodgaonkar M, Follonier C, Artola-Boran M, Castor D, *et al*. 2012. Noncanonical mismatch repair as a source of genomic instability in human cells. Mol Cell. 47:669–680.
74. Bardwell PD, Woo CJ, Wei K, Li Z, Martin A, Sack SZ. *et al*. 2004. Altered somatic hypermutation and reduced class-switch recombination in exonuclease 1-mutant mice. Nat Immunol. 5(2):224-229.
75. Péron S, Metin A, Gardès P, Alyanakian MA, Sheridan E, Kratz CP, *et al*. 2008. Human PMS2 deficiency is associated with impaired immunoglobulin class switch recombination. J Exp Med. 205(11):2465-2472.
76. Stavnezer J, Guikema JE, Schrader CE. 2008. Mechanism and regulation of class switch recombination. Annu Rev Immunol. 26:261–292.
77. Walker JR, Corpina RA, Goldberg J. 2001. Structure of the Ku heterodimer bound to DNA and its implications for double-strand break repair. Nature. 412:607–614.
78. Smith GC, Jackson SP. 1999. The DNA-dependent protein kinase. Genes Dev. 13(8):916–934.
79. DeFazio LG, Stansel RM, Griffith JD, Chu G. 2002. Synapsis of DNA ends by DNA-dependent protein kinase. EMBO J. 21(12):3192–200.
80. Ding Q, Reddy YV, Wang W, Woods T, Douglas P, Ramsden DA, *et al*. 2003. Autophosphorylation of the catalytic subunit of the DNA-dependent protein kinase is required for efficient end processing during DNA double-strand break repair. Mol Cell Biol. 23(16):5836-5848.
81. Cui X, Yu Y, Gupta S, Cho YM, Lees-Miller SP, Meek K. 2005. Autophosphorylation of DNA-dependent protein kinase regulates DNA end processing and may also alter double-strand break repair pathway choice. Mol Cell Biol. 25(24):10842-10852.
82. Douglas P, Cui X, Block WD, Yu Y, Gupta S, Ding Q, *et al*. 2007. The DNA-dependent protein kinase catalytic subunit is phosphorylated in vivo on threonine 3950, a highly conserved amino acid in the protein kinase domain. Mol Cell Biol. 27(5):1581-1591.
83. Calsou P, Frit P, Humbert O, Muller C, Chen DJ, Salles B. 1999. The DNA-dependent protein kinase catalytic activity regulates DNA end processing by means of Ku entry into DNA. J Biol Chem. 274:7848-7856.
84. Weterings E, Verkaik NS, Bruggenwirth HT, Hoeijmakers JH, van Gent DC. 2003. The role of DNA dependent protein kinase in synapsis of DNA ends. Nucleic Acids Res. 31:7238-7246.
85. Block WD, Yu Y, Merkle D, Gifford JL, Ding Q, Meek K, *et al*. 2004. Autophosphorylation-dependent remodeling of the DNA-dependent protein kinase catalytic subunit regulates ligation of DNA ends. Nucleic Acids Res. 32(14):4351-4357.
86. Bebenek K, Pedersen LC, Kunkel TA. 2014. Structure–function studies of DNA polymerase lambda. Biochemistry. 53:2781–2792.
87. Moon AF, Pryor JM, Ramsden DA, Kunkel TA, Bebenek K, Pedersen LC. 2014. Sustained active site rigidity during synthesis by human DNA polymerase μ. Nat Struct Mol Biol. 21(3):253–260.
88. Bertocci B, De Smet A, Weill JC, Reynaud CA. 2006. Nonoverlapping functions of DNA polymerases mu, lambda, and terminal deoxynucleotidyltransferase during immunoglobulin V(D)J recombination *in vivo*. Immunity. 25(1):31-41.
89. Lieber MR. 2006. The polymerases for V(D)J recombination. Immunity. 25(1):7-9.
90. Daley JM, Laan RL, Suresh A, Wilson TE. 2005. DNA joint dependence of pol X family polymerase action in nonhomologous end joining. J Biol Chem. 280(32):29030-29037.
91. Daley JM, Wilson TE. 2005. Rejoining of DNA double-strand breaks as a function of overhang length. Mol Cell Biol. 25(3):896-906.
92. Daley JM, Wilson TE. 2008. Evidence that base stacking potential in annealed 3' overhangs determines polymerase utilization in yeast nonhomologous end joining. DNA Repair (Amst). 7(1):67-76.
93. Povirk LF, Zhou T, Zhou R, Cowan MJ, Yannone SM. 2007. Processing of 3'-phosphoglycolate-terminated DNA double strand breaks by Artemis nuclease. J Biol Chem. 282(6):3547-3558.
94. Ma Y, Pannicke U, Schwarz K, Lieber MR. 2002. Hairpin opening and overhang processing by an Artemis/DNA-dependent protein kinase complex in nonhomologous end joining and V(D)J recombination. Cell. 108(6):781-794.
95. Chappell C, Hanakahi LA, Karimi-Busheri F, Weinfeld M, West SC. 2002. Involvement of human polynucleotide kinase in double-strand break repair by non-homologous end joining. EMBO J. 21(11):2827-2832.
96. Valerie K, Povirk LF. 2003. Regulation and mechanisms of mammalian double-strand break repair. Oncogene. 22(37):5792-5812.
97. Nick Mc, Elhinny SA, Snowden CM, McCarville J, Ramsden DA. 2000. Ku recruits the XRCC4-ligase IV complex to DNA ends. Mol Cell Biol. 20(9):2996-3003.
98. Ellenberger T, Tomkinson AE. 2008. Eukaryotic DNA ligases: structural and functional insights. Annu Rev Biochem. 77:313–338.
99. Leung CC, Glover JN. 2011. BRCT domains: easy as one, two, three. Cell Cycle. 10(15):2461–2470.
100. Yu X, Chini CC, He M, Mer G, Chen J. 2003. The BRCT domain is a phospho-protein binding domain. Science. 302(5645): 639–642.

101. Manke IA, Lowery DM, Nguyen A, Yaffe MB. 2003. BRCT repeats as phosphopeptide-binding modules involved in protein targeting. Science. 302(5645):636–639.
102. Junop MS, Modesti M, Guarné A, Ghirlando R, Gellert M, Yang W. 2000. Crystal structure of the Xrcc4 DNA repair protein and implications for end joining. EMBO J. 19(22):5962-5970.
103. Sibanda BL, Critchlow SE, Begun J, Pei XY, Jackson SP, Blundell TL, *et al*. 2001. Crystal structure of an Xrcc4-DNA ligase IV complex. Nat Struct Biol. 8(12):1015-1019.
104. Costantini S, Woodbine L, Andreoli L, Jeggo PA, Vindigni A. 2007. Interaction of the Ku heterodimer with the DNA ligase IV/Xrcc4 complex and its regulation by DNA-PK. DNA Repair (Amst). 6(6):712-722.
105. Grawunder U, Zimmer D, Kulesza P, Lieber MR. 1998. Requirement for an interaction of XRCC4 with DNA ligase IV for wild-type V(D)J recombination and DNA double-strand break repair in vivo. J Biol Chem. 273:24708–24714.
106. Grawunder U, Zimmer D, Lieber MR. 1998. DNA ligase IV binds to XRCC4 via a motif located between rather than within its BRCT domains. Curr Biol. 8:873–876.
107. Grawunder U, Wilm M, Wu X, Kulesza P, Wilson TE, Mann M, et al. 1997. Activity of DNA ligase IV stimulated by complex formation with XRCC4 protein in mammalian cells. Nature. 388(6641):492-495.
108. Bryans M, Valenzano MC, Stamato TD. 1999. Absence of DNA ligase IV protein in XR-1 cells: evidence for stabilization by XRCC4. Mutat Res. 433(1):53-58.
109. Tsai CJ, Kim SA, Chu G. 2007. Cernunnos/XLF promotes the ligation of mismatched and noncohesive DNA ends. Proc Natl Acad Sci USA. 104:7851-7856.
110. Drouet J, Delteil C, Lefrançois J, Concannon P, Salles B, Calsou P. 2005. DNA-dependent protein kinase and XRCC4-DNA ligase IV mobilization in the cell in response to DNA double strand breaks. J Biol Chem. 280(8):7060-7069.
111. Ahnesorg P, Smith P, Jackson SP. 2006. XLF interacts with the XRCC4-DNA ligase IV complex to promote DNA nonhomologous end-joining. Cell. 124(2):301-313.
112. Andres SN, Modesti M, Tsai CJ, Chu G, Junop MS. 2007. Crystal structure of human XLF: a twist in nonhomologous DNA end-joining. Mol Cell. 28(6):1093-1101.
113. Buck D, Malivert L, de Chasseval R, Barraud A, Fondanèche MC, Sanal O, et al. 2006. Cernunnos, a novel nonhomologous end-joining factor, is mutated in human immunodeficiency with microcephaly. Cell. 124(2):287-299.
114. van Gent D, van der Burg M. 2007. Non-homologous end-joining, a sticky affair. Oncogene. 26:7731–7740.