IgH Chain Class Switch Recombination: Mechanism and Regulation

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IgH Chain Class Switch Recombination: Mechanism and Regulation

Janet Stavnezer and Carol E. Schrader

IgH class switching occurs rapidly after activation of mature naive B cells, resulting in a switch from expression of IgM and IgD to expression of IgG, IgE, or IgA; this switch improves the ability of Abs to remove the pathogen that induces the humoral immune response. Class switching occurs by a deletional recombination between two switch regions, each of which is associated with a H chain constant region gene. Class switch recombination (CSR) is instigated by activation-induced cytidine deaminase, which converts cytosines in switch regions to uracils. The uracils are subsequently removed by two DNA-repair pathways, resulting in mutations, single-strand DNA breaks, and the double-strand breaks required for CSR. We discuss several aspects of CSR, including how CSR is induced, CSR in B cell progenitors, the roles of transcription and chromosomal looping in CSR, and the roles of certain DNA-repair enzymes in CSR. The Journal of Immunology, 2014, 193: 5370–5378.

After immunization or infection, activated naive B cells can switch from expressing IgM and IgD on their surface to expressing IgG, IgE, or IgA. This isotype/class switch changes the effector function of the Ab and improves its ability to eliminate the pathogen that induced the response. Isotype switching involves replacement of the μ and δ H chain C (Cμδ) regions of the expressed Ig with γ, ε, or α C regions, and it occurs by a DNA recombination event termed “class switch recombination” (CSR). Fig. 1 is a diagram (not to scale) of the Cμδ genes and CSR in mice; human Cμδ genes are similarly arranged although not identical.

CSR is a deletional DNA recombination occurring between switch (S) regions, which are located upstream of all of the Cμδ genes, with the exception of Cδ6, and are 1–10 kb in length (1). Recombination occurs between dsDNA breaks (DSBs) introduced into the donor μ S (Sμ) region and a downstream/acceptor S region located from ~65–160 kb downstream; occasionally, downstream S regions can subsequently recombine with an S region farther downstream. S regions are G rich and have a high density of WGCW (A/T-G-C-A/T) motifs, the preferred target for activation-induced cytidine deaminase (AID), the enzyme that initiates CSR by deaminating cytosines within S-region DNA, converting deoxycytidine to dU (2, 3). Enzymes of the base excision repair (BER) and mismatch repair (MMR) pathways then convert the dUUs to DNA DSBs, which are required for CSR (4, 5) (Fig. 2). The DSBs are subsequently recombined by an end-joining type of DNA recombination, predominantly by nonhomologous end joining (NHEJ). The use of NHEJ, rather than homologous recombination, is consistent with the facts that S-region DSBs are induced and recombined during the G1 phase (6–9) and that different S regions do not share long stretches of identity (1), which are required for homologous recombination.

CSR occurs very rapidly postinfection or immunization, prior to formation of germinal centers, which generally form 7–10 d after exposure to Ag. For example, using mice expressing a transgenic BCR, both IgMα and IgG2α+ cells were detected in B cell follicles from days 2 to 4 after immunization, but only IgG2α+ cells were detected in germinal centers, indicating that CSR occurred prior to germinal center formation (10). Also, CSR was detected in nontransgenic mice 4 d postinfection with Salmonella (11). However, CSR is also detected in germinal center B cells from human tonsils (12), and IgA CSR occurs in Peyer’s patch germinal centers (13–15), in which B cells are constantly stimulated by the gut microbiota. CSR also occurs during T-independent responses, which do not induce germinal centers (16). Thus, CSR starts prior to somatic hypermutation (SHM) of V region [V(D)J] genes, an AID-dependent process that occurs mainly in germinal centers, and which, after selection, can result in Abs with increased affinity for Ag. The data suggest that CSR may continue as long as B cells are undergoing activation.

Induction of CSR

Many studies of CSR have been performed using cultures of mouse splenic B cells, because these cells can be induced in culture to undergo robust CSR within ~3 d by treatment with the B cell mitogen LPS, acting through the innate receptor

Abbreviations used in this article: AID, activation-induced cytidine deaminase; AP, apurinic/apyrimidinic; APE, AP endonuclease; ATM, ataxia-telangiectasia mutant; BER, base excision repair; 3C, chromosome conformation capture; Ca, μ C region; Cμδ, H chain C; CSR, class switch recombination; DSB, dsDNA break; Eap, Ea intron enhancer; 5′Eco, 5′ Eco enhancer; GLT, germline transcript; HEL, hen egg lysozyme; MMR, mismatch repair; MRE11-NS1-RAD50; NHEJ, nonhomologous end joining; Pol, polymerase; Sμ, μ S; S, switch; SHM, somatic hypermutation; ss, single stranded; SSB, ssDNA break; UNG, uracil DNA glycosylase; WT, wild-type.

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TLR4, or by signaling through CD40, the most important receptor for T cell help. LPS alone induces AID expression, but a cytokine, such as IL-4, must be added to induce AID when Ab to CD40 (anti-CD40) is used. These ligands also induce cell proliferation, another requirement for CSR (17, 18). Surprisingly, splenic B cells do not require a signal provided by the BCR to switch in culture, because neither LPS nor anti-CD40 triggers signaling via the BCR. This might be explained by the large amounts of LPS (usually 10–50 μg/ml) used in these cultures, which would not normally be provided to B cells in vivo. Also, much more continuous CD40 signaling is provided in these cultures than normally would be available in vivo. In vivo, CD40 signaling would be delivered to Ag-specific B cells during contacts with Ag-specific Th cells. CD40 signaling is very important for CSR in vivo, as demonstrated by the lack of B cell proliferation and CSR in response to T-dependent Ags in mice and humans deficient in CD40 signaling (19, 20). Also, TLR signaling is important for in vivo immune responses and CSR in response to viruses (21).

Although Ag is not necessary to obtain CSR in culture, addition of low levels (1–100 ng/ml) of soluble hen egg lysozyme (HEL) Ag to LPS- or anti-CD40–stimulated mouse splenic B cells expressing a HEL-specific BCR increases CSR (22). Increasing the amount of Ag to 1 μg/ml inhibited CSR. Also, low levels (nanogram amounts) of anti-IgD dextran added to LPS-activated cultures of splenic B cells increases CSR (23–25). The increase in CSR is not due to increased cell proliferation (24, 26). In contrast, addition of large concentrations (10 μg/ml) of anti-IgM to LPS cultures or anti-CD40 cultures reduces CSR, although it does not inhibit cell proliferation (27, 28). The inhibitory-signaling pathway has been studied, and it appears to be a feedback response to extensive BCR cross-linking.

BCR signaling appears to be important for CSR in culture under conditions when signals provided by accessory/secondary signals are limiting. Ligands for the innate receptors TLR1/2, TLR7, and TLR9 were shown to induce little or no CSR in culture. When anti-IgD dextran is added to these cultures, CSR to several isotypes is increased synergistically (24). Likewise, when HEL is added to HEL-specific B cells activated with TLR7 or TLR9, CSR to IgG1 is also greatly increased (22). Unlike mouse B cells, human peripheral blood or tonsillar B cells switch poorly in culture to either CD40 or TLR signaling (29); perhaps activation through BCR signaling would help.

In conclusion, it is likely that CSR in vivo depends upon activation of B cells via their BCR, in addition to secondary signals from CD40 and TLR signaling. It is unclear whether the BCR signaling is required for inducing CSR or is only required for initial activation of the B cells, and the T cell signals are responsible for inducing CSR. In addition, it appears likely that TLR signaling is important for both T-independent and T-dependent responses in vivo. It is possible that primary and multiple secondary signals given together increase the robustness of the CSR response.

Regulation of isotype specificity by transcription of unrearranged C_{H} genes

Naive B cells have the potential to switch to any isotype. Isotype specificity is directed by induction of transcription across S regions, because AID-induced deaminations and CSR are restricted to S regions that are undergoing transcription (5, 30–33). Located upstream of each acceptor S region are transcription promoters, which are activated by cytokines that induce CSR to that specific isotype. Transcription from these promoters produces germ line transcripts (GLTs), so-called because they are transcribed from unrearranged genes. GLTs are spliced, as diagrammed in Fig. 1 for γ2b GLT. Because of their unusually high G content, S-region transcripts form R-loops (RNA–DNA hybrids) with the bottom strand DNA, rendering the nontranscribed (top strand) single stranded (ss) (34–36). The substrate for AID is ssDNA; thus, R-loops cause the top strand to become an extensive AID substrate. However, it is known that AID attacks both the top and bottom strands at S regions nearly equally (37). Thus, the R-loop must be removed during CSR to expose the bottom strand to AID.

The R-loop is thought to cause RNA polymerase (Pol) II to stall during transcription of S regions, resulting in accumu-
RNA from the 3′ substrates for the RNA exosome, a complex that degrades paused Pol II, causing an unknown ubiquitin ligase to poly-ubiquitinate Pol II, leading to degradation of Pol II (42). Pol II degradation leads to transcription termination, creating substrates for the exosome. Interestingly, AID is found associated with polyubiquitinated Pol II, and Nedd4 ubiquitination activity was shown to promote interaction of AID with Spt5 and with the RNA exosome, as well as to promote binding of the RNA exosome to transcribed S regions. Nedd4 also reduces the level of γ1 GLTs, presumably due to degradation by the exosome. Thus, Nedd4 activity appears to increase the amount of ss bottom strand DNA and to help recruit AID to S regions. This model has been supported by results indicating that Pol II pausing leads to transcription termination and increased SHM in IgH V genes, dependent on the RNA exosome (43, 44).

GLTs must be spliced to support CSR, although the reason for this is unknown (33, 45–49). Because splicing is cotranscriptional, it is possible that splicing factors are involved in recruiting AID to S regions (50) and/or that splicing is required for R-loops to form, perhaps because the RNA must thread back into the DNA to form an R-loop (36). The splicing regulator PTBP2, a protein that binds AID and S-region transcripts, is important for efficient CSR. Knockdown of this protein reduces the association of AID with S regions (51, 52). PTBP2 is known to regulate alternative splicing and many aspects of RNA metabolism (53), although its specific role(s) in CSR is not defined. Also, CTNNBL1, a component of the spliceosome, interacts with AID and is important for both CSR and SHM (50). These interesting studies suggest avenues that might lead to an understanding of why splicing of GLTs is important for CSR, although at this time the role of splicing is not understood.

**Regulation of CSR by chromosome looping**

Sμ and the acceptor S regions must be in contact to recombine. Chromosome conformation capture (3C) experiments showed that, in mature naive splenic B cells prior to CSR, the Eμ intron enhancer (Eμ) and the 3′ Cα enhancer (3′Eα)/regulatory region, located ∼220 kb apart in the genome, are positioned sufficiently near each other in the nucleus to be cross-linked by formaldehyde treatment via proteins bound to them (54–57). Gene targeting experiments showed that segments of the 3′Eα are essential for CSR to all isotypes (58). This Eμ–3′Eα interaction causes Sμ and the downstream S regions to be located within the same chromosomal loop (Fig. 3A). In cells activated to switch, but that have not yet switched to IgG1 by treatment with LPS plus IL-4, the Eμ–Sμ–Cμ (μ C region) and Sy1-Cy1 loci are found in 3C experiments to be positioned near each other and the 3′Eα segment (Fig. 3B). In cells treated with LPS alone, which induces CSR to IgG3, the Sy3-Cy3 locus is associated with Eμ–Sμ–Cμ and with 3′Eα instead of with Sy1-Cy1 (54, 55). Because LPS plus IL-4 induces γ1 GLTs, these results suggest that factors that induce transcription from the GLT promoter and/or the GLTs themselves are involved in recruiting the Sy1-Cy1 locus to the positions of the Eμ-Sμ-Cμ locus and 3′Eα. Also, it is possible that looping allows transcriptional activators that bind the enhancers to gain access to GLT promoters; thus, association of a S-Cα1 acceptor locus with the Eμ-Sμ-Cμ locus and with 3′Eα might contribute to transcription or even be required for germline transcription.
It is important to identify the proteins regulating loop formation between the Eμ and 3′Eα enhancers and Cé genes in B cells before and during CSR. YY-1, a protein that binds both the 3′Eα and Eμ enhancers is a candidate (59, 60). Better evidence is available for PTIP, which was shown to interact with the B cell–specific protein Pax5 and to be important for the interaction between the 3′Eα and the γ1 GLT promoter (61). Mice deficient in PTIP have reduced γ3, γ2b, and γ1 GLTs and reduced CSR to these isotypes (62), but it is unknown how much of this effect is due to reduced interaction between the Cé loci and the 3′Eα and how much is due to the fact that PTIP is important for recruiting the histone methyl transferase MLL, which produces the activating histone modification H3K4me3. It is possible that MLL and/or H3K4me3 are important for the looping, CTCF and cohesin, two proteins involved in contraction/looping of the Vé and Vk gene loci (63), do not appear to bind within the 180-kb region encoding the Cé genes nor to the 3′Eα. However, cohesin was shown to be required for optimum CSR, but the mechanism of its contribution is unknown (64, 65).

CSR in B cell progenitors

Pre-B cells purified from mouse bone marrow express low amounts of AID and were shown to have ongoing H chain switch recombination (μ > γ2b), despite the fact that they do not express L chains and IgM (66). This was determined by two molecular assays for switch recombination events at the DNA level, detection of RNA transcripts from the excised DNA circles (circle transcripts), and postswitch transcripts produced by transcription of the recombined genes. In a different study, pro-B cells from Rag1-deficient mice could be induced in culture to switch at the DNA level from μ > γ2b and from μ > ε, although these cells do not express either μ-chains or L chains (67). The physiological role of this H chain switching is unknown, although AID activity in pro- and pre-B cells might contribute to autoimmunity (68).

Interestingly, and unlike CSR regulation in mature B cells, μ > γ2b, but not μ > γ3, switching occurs in pro-B cells activated with LPS plus CD40L, and μ > ε, but not μ > γ1, switching occurs in cells treated with LPS plus CD40L plus IL-4 (67). GLT expression correlates with CSR (i.e., γ2b and ε GLTs, but not γ3 or γ1 GLTs, are detected in pro-B cells, activated without or with IL-4, respectively). The explanation for this restricted choice of isotypes appears to be that pro-B cells have a chromosomal loop between the Cy3 and Cy1 loci that sequesters these genes away from the Eμ and 3′Eα enhancers (67). This loop was not detected in mature splenic B cells. Likewise, ex vivo bone marrow pre-B cells from C57BL/6 mice switch to γ2b but not to γ3; surprisingly, however, BALB/c pre-B cells switch to γ2b and γ3 (66). The pre-B cells in both mouse strains also switched to α, consistent with results indicating that mice that cannot express IgM can undergo CSR to IgA (69). Two mature mouse B cell lines (CH12F3 and I.29μ) can be induced to switch in culture, but the switching is restricted to IgM, or rarely, to I.29μ, to IgE or IgG2a. Perhaps these cell lines have a chromosomal loop that sequesters the other Cé loci from the enhancers. Much more research is needed to understand how specific chromosomal loops are regulated and the roles of chromosomal loops in regulating CSR.

Introduction of DSBs in S regions by AID involves the BER and MMR pathways

Although AID is rapidly induced after B cell activation, the enzymes and proteins that convert the AID-induced dUs to DSBs are constitutively expressed, because the lesions that these proteins repair occur in all types of cells. Uracil, whether as a result of AID activity or caused by spontaneous hydrolysis of cytosine, can be excised by the BER enzyme uracil DNA glycosylase (UNG), which leaves an abasic (apurinic/apyrimidinic [AP]) site (Fig. 2A). Although cells express four uracil DNA glycosylases (UNG, Smug1, MBD4, and TDG), deficiency in UNG alone results in a 95–99% reduction in CSR in mice and humans (4, 70, 71) and reduces S-region DSBs detected during CSR in cultured splenic B cells nearly to levels observed in air−/− B cells (72). Recently, it was found that, in ung−/− mice, Smug1 can partially substitute (i.e., in smug1−/− ung−/− splenic B cells, CSR is reduced another 5-fold) (71). Smug1 deficiency by itself has no effect on CSR, probably as a result of its low abundance (73) and low activity on dU in ssDNA, whereas UNG is abundant and more active on ssDNA than on dsDNA (74). Because AID only has activity on ssDNA, it is possible that UNG excises most dUs prior to reformation of the DNA duplex.

Abasic (AP) sites are subsequently cut by AP endonuclease (APE)1 and/or APE2, creating an ssDNA break (SSB) (Fig. 2A). If SSBs are sufficiently near each other on both DNA strands, DSBs are produced. APE1 is essential for viability; although apex1−/− mice have not been produced, apex1−/− mice have DNA repair defects. Both APE1 and APE2 contribute to CSR in splenic B cells induced to switch in culture (75). S-region DSBs are greatly reduced in apex1−/− apex2−/− splenic B cells but are only marginally reduced in apex1−/− or apex2−/− cells, indicating that these enzymes are partially redundant. However, deletion of these enzymes in CH12F3 B lymphoma cells gave different results. APE1-deficient cells have an 80% reduction in CSR, whereas APE2 deficiency has no effect on CSR (76). The different results in splenic B cells and CH12F3 cells remain a puzzle.
APE2 is a very inefficient endonuclease, about 1000-fold less active than APE1. APE2 has stronger exonuclease activity than APE1; thus, one could envision APE1 producing the SSB and APE2 creating a gap at the SSB, which could increase the probability that an SSB might become a DSB. However, evidence suggesting that APE2 acts as an endonuclease at AID-induced AP sites in splenic B cells was obtained by determining the locations of S-region DSBs in APE-deficient mice compared with wild-type (WT) mice. APE2 deficiency reduced their preference for G:C bp, so that the proportion of breaks at G:C bp were not significantly different from their proportion in the sequence itself, whereas DSBs in apex1+/- cells were similar to WT cells (75). It is not known why APE2 is important for CSR, especially because APE1 is a much more efficient endonuclease.

A major question in the field is why AID-induced lesions are not accurately repaired, despite the fact that BER is a highly active and error-free repair pathway. In most cells, DNA Pol β accurately replaces the excised nucleotide, ligase III–XRCC1 seals the phosphodiester backbone, and DSBs are not generated (Fig. 2A). In fact, DNA Pol β modestly inhibits S-region DSBs and CSR, suggesting that it competes with DSB formation but is overwhelmed by the numbers of AID-induced SSBs (77). Another study suggested an additional possibility. In this study, SHM of Ab V region genes was examined in germinal center B cells from Peyer’s patches (78). These cells are undergoing constant stimulation by gut microbes and undergo robust SHM. The results suggest that the use of APE2, instead of APE1, in repair of AID-induced lesions in germinal centers converts BER to an error-prone repair pathway (78). Germinal center B cells express very low levels of APE1 protein and mRNA compared with APE2. APE2 was found to be important for mutations at A:T bp, whereas apex1+/- B cells have unperturbed SHM (78). Because an SSB is required as an entry point for the error-prone translocation polymerase DNA Pol η to introduce mutations at A:T bp, this suggests that APE2 is indeed acting as an endonuclease during SHM, creating SSBs. It is possible that the use of APE2 might inhibit error-free repair of AID-induced lesions. Interestingly, APE2, but not APE1, interacts with PCNA, a protein that recruits DNA Pol η to DNA (79).

When the AID-UNG-APE pathway induces SSBs that are too far apart on opposite DNA strands to produce DSBs, MMR can convert the SSBs to a DSB (5) (Fig. 2B). The Msh2-Msh6 heterodimer binds to U:G mismatches in duplex DNA, as well as recruits Mlh1-Pms2 and exonuclease 1, which initiates resection from a SSB located 5’ to the mismatch (80, 81). Pms2 also has endonuclease activity and can create additional SSBs on the previously nicked strand, providing additional entry sites for exonuclease 1 or other nucleases (82, 83). As diagrammed in Fig. 2B, this resection should result in creation of a DSB with a long ss tail, which, when filled in by DNA Pol, creates a blunt or nearly blunt DSB appropriate for NHEJ (5). Several types of data support this model, including the facts that S-region DSBs and CSR are reduced by 2–5-fold in MMR-deficient B cells (7, 84, 85) and that, in the absence of Sp or tandem repeats, with their numerous AID target hotspots, CSR is absolutely dependent upon Msh2 (86).

Recombination of S-region DSBs by NHEJ

S–S recombination occurs by NHEJ, which involves binding of the abundant toroidal heterodimer KU70/80 to each DSB, forming a platform for nucleases Artemis and PALF, as well as for DNA polymerases, and greatly stimulating the ligation activity of DNA ligase IV-XRCC4-XLF (reviewed in Refs. 87–89). The Mre11-Nbs1-Rad50 (MRN) complex also rapidly binds DSBs, but it is unclear whether KU and MRN compete or cooperate at S-region DSBs. Rad50 has a long coiled-coil domain with a hook at the end by which MRN complexes bound at different DSBs can interact and tether the DSBs to each other (90). MRN recruits additional factors, including the kinase ataxia-telangiectasia mutant (ATM), which activates and coordinates the cellular response to DSBs. KU and MRN were both shown to bind S-region DSBs (6, 91–93) and contribute to CSR (94–98).

There is a great deal of mechanistic flexibility in NHEJ, which is necessary because DSBs can differ greatly, having 3’ or 5’ ss tails of different lengths, and possibly even having hairpins, in addition to being blunt. Generally, the junctions formed between two S regions have 0 or 1 bp of microhomology between Sp and the acceptor S region (i.e., one cannot discern whether the 1 bp came from Sp or the acceptor S region, although this microhomology can increase up to 6 bp or more). In cells deficient in an NHEJ protein (e.g., KU or ligase IV) or deficient in Mlh1 or Pms2, S–S junctions show increased lengths of microhomology, especially if Sp–Sp junctions are analyzed, because these S regions have the most homology with each other (99). To explain the increased lengths of junctional microhomology, investigators have thought that an alternate end-joining pathway, or microhomology-mediated end joining, substitutes for NHEJ (100–102). However, it is not clear whether microhomology-mediated end joining is really a defined pathway or, instead, that NHEJ has partially redundant components. For example, in the absence of ligase IV, ligase I and ligase III can perform end joining, but not as efficiently, and the other components of NHEJ can still participate (88, 103). Deficiencies in an NHEJ component might expose the DSBs to end-resection activities; also, the less efficient recombination might allow time for increased end resection prior to ligation and result in increased use of microhomology, which can help to stabilize the junctions. In situations in which fewer S-region DSBs are induced [e.g., in human patients heterozygous for AID with a C-terminal deletion (104) or in mblh1-/- or pms2-/- mouse and human B cells (7, 99, 105)], junctional microhomology is also increased. Perhaps this occurs because DSBs are limiting, thus delaying recombination. Note that although Msh2-deficient cells have reduced DSBs (7), they do not have increased junctional microhomology, indicating that reduced DSB frequency does not always result in increased use of microhomology. The explanation for this difference from Mlh1- and Pms2-deficient cells is unknown.

The AID C terminus is essential for CSR but not for SHM

The fact that AID lacking the C terminal 8–17 aa (ΔAID) cannot support CSR but appears to support normal SHM of V(D)J segments has been known for several years, but the explanation is still unclear (106, 107). Recruitment of UNG and Msh2-Msh6 to Sp is impaired in cells expressing ΔAID, likely decreasing the introduction of DSBs. However, the numbers of Sp and Sy DSBs detected are normal, suggesting that DSBs accumulate due to defective repair and recombination (93, 108, 109). In agreement, there is reduced recruitment of NHEJ for }.
proteins to Sµ regions and increased lengths of S–S junctional microhomology in cells expressing ΔAID (93, 104, 110, 111). However, how the C terminus recruits NHEJ proteins, and whether this is important for the greatly reduced CSR, is still unknown.

**The role of ATM during CSR and phosphorylation of AID**

ATM kinase is a major regulator of the DNA damage response. ATM is rapidly activated by the binding of MRN to DSBs; in turn, ATM phosphorylates MRN and several downstream effectors of DSB repair (112). Humans with ATM mutations have numerous problems due to poor DNA repair functions. Mouse splenic B cells lacking ATM have reduced CSR (30% of WT B cells) (113, 114) and impaired V(DJ) recombination (115). In response to S-region DSBs, ATM induces phosphorylation of AID at S38 by an undefined pathway, and this increases the ability of AID to induce S-region DSBs and to bind to APE1 via an unknown protein (116). APE2 has not been tested. Thus, AID is activated by a feed-forward mechanism involving ATM. Phosphorylation of AID at S38 is not necessary for its deaminase activity in cell-free experiments, although activated cultured splenic B cells expressing AIDS38A have greatly reduced Sµ DSBs and CSR (116–118). It is hypothesized that this feed-forward mechanism increases AID activity at localized regions that have DSBs, such as at the IgH S regions, helping to explain why AID is so much more active at S regions than at off-target sites (91, 119, 120).

Interestingly, atm−/− B cells induced to switch showed decreased DSBs at Sµ regions but have increased DSBs at Sµ (9). These data have been interpreted to suggest that the feed-forward activation of AID by ATM is very important for AID activity at acceptor S regions and that, when DSBs are poorly induced in the acceptor S region, Sµ DSBs accumulate due to lack of a downstream partner. These data also suggest that acceptor S-region DSBs are limiting for CSR. It was found previously that AID is more active at Sµ regions than at Sy regions (37, 121), suggesting that AID attacks the Sµ region first (121). Taken together, these data lead to the hypothesis that ATM is activated by Sµ DSBs, resulting in phosphorylation of AID, which increases its activity at the downstream S region that is colocalized due to chromosome looping. The studies of chromosomal looping described above suggest that Sµ and acceptor S regions are located near each other prior to induction of AID and DSBs, because the looping might coincide with expression of GLTs. Thus, when AID is activated by phosphorylation, the acceptor S region might be localized sufficiently near to be attacked. Close localization of the two S regions also decreases the likelihood of aberrant recombination events. Surprisingly, ATM deficiency has no effect on cell cycle regulation of S-region DSBs, because they are restricted to the G1 phase in atm−/− cells, just as in WT cells, indicating that Sµ DSBs only accumulate during the G1 phase and are repaired before the S phase, even if the DSBs do not undergo Sµ–Sx recombination (9).

**Function of 53BP1 in CSR**

53BP1 is one of the DNA repair proteins that binds DSBs in response to the phosphorylation activities of ATM. CSR is reduced by 90% in cells deficient in 53BP1, but its roles in CSR are still not clear. ATM phosphorylates H2AX, converting it to γH2AX, which, in turn, interacts with 53BP1 (122), although additional kinases, other enzymes, and histone modifications also recruit and activate 53BP1 (123, 124). Resection at S-region DSBs during the G1 phase normally is inhibited by 53BP1 and its effector protein Rif1, two proteins very important for CSR that function epistatically and whose activities favor recombination by NHEJ (120, 123, 125–131). Surprisingly, S–S junctions in 53bp1−/− cells do not have increased microhomology (126). Another activity of 53BP1 that is essential for CSR is its ability to oligomerize (127, 132). 53BP1 likely binds DSBs in both Sµ and downstream S regions, and, most likely, the oligomerization of 53BP1 helps to hold two different S regions together in the proper conformation. This might explain why inverted S-region sequences and a segment from the IgH mu enhancer have been detected between the recombining S-region segments in 53bp1−/− cells (126). In addition, in 53bp1−/− cells induced to undergo CSR there are numerous deletions within Sµ, suggesting that DSBs within Sµ failed to find an acceptor S-region recombination partner. Thus, 53BP1 is essential for CSR, and it appears to have multiple roles, although they are still not entirely understood.

**Questions remaining**

There are many more interesting issues to discuss about CSR than can even be discussed briefly in this article because of space limitations. We recommend several recent reviews of CSR that include additional topics (33, 49, 103, 133–135).

Although much is now known about CSR, there are still many questions left unanswered, some of which have been mentioned in this review. Although AID deaminates off-target genomic sites, as well as induces DSBs at off-target sites, most of its activity is directed toward Ig loci. How AID is targeted to S regions is mostly unknown. We know that transcription, and probably RNA stalling and RNA splicing, are important; of course, there are numerous transcribed genes with spliced transcripts and stalled Pol II. What causes the stalling of Pol II at S regions? S regions and S-region transcripts are likely to bind specific proteins, such as 14-3-3 (136) and PTBP2, that help to recruit AID, but are there others? How is the looping of the Cµ gene loci and enhancers regulated? What causes AID-induced S-region DSBs to be restricted to the G1 phase? Is AID activity restricted to the G1 phase, as is UNG activity? What signaling mechanism causes the S-region DSBs to be repaired prior to the S phase, even in the absence of ATM or p53 (9)? What is the role of APE2 in S-region DSBs, and why isn’t APE1 sufficient? Although APE1 is expressed as well as APE2 in splenic B cells induced to switch in culture, it is poorly expressed in germinal center B cells. Does the low expression of APE1 reduce CSR in germinal center cells? Is APE1 well expressed during CSR in vivo in nongerminal center cells? How does phosphorylation of AID at S38 cause increased S-region DSBs? Is this specific for S-region DSBs? What does the C terminus of AID do during CSR? Although it might be involved in recruitment of NHEJ proteins, its deletion has a much greater effect on CSR than loss of NHEJ functions. What is the importance and what are the roles of histone modifications of IgH genes for CSR? What are the functions of AID expression in pro- and pre-B cells? Is this advantageous or only deleterious?
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Disclosures
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References


BRIEF REVIEWS: Ab CLASS SWITCH RECOMBINATION


