Binding of AID to DNA Does Not Correlate with Mutator Activity

Allysia J. Matthews, Solomon Husain and Jayanta Chaudhuri

*J Immunol* 2014; 193:252-257; Prepublished online 30 May 2014;
doi: 10.4049/jimmunol.1400433
http://www.jimmunol.org/content/193/1/252

Supplementary Material

http://www.jimmunol.org/content/suppl/2014/05/29/jimmunol.140043
3.DCSupplemental

References

This article cites 40 articles, 14 of which you can access for free at:
http://www.jimmunol.org/content/193/1/252.full#ref-list-1

Subscription

Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

Permissions

Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts

Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Binding of AID to DNA Does Not Correlate with Mutator Activity

Allysia J. Matthews, Solomon Husain, and Jayanta Chaudhuri

The DNA deaminase activation-induced cytidine deaminase (AID) initiates somatic hypermutation (SHM) and class switch recombination (CSR) by deaminating cytidines to uridines at V region (V) genes and switch (S) regions. The mechanism by which AID is recruited to V genes and S region DNA is poorly understood. In this study, we used the CH12 B lymphoma line to demonstrate that, although S regions can efficiently recruit AID and undergo mutations and deletions, AID neither binds to nor mutates the V gene, thus clearly demonstrating intramunomoglobin locus specificity. Depletion of the RNA-binding protein polypyrimidine tract binding protein-2, previously shown to promote recruitment of AID to S regions, enables stable association of AID with the V gene. Surprisingly, AID binding to the V gene does not induce SHM. These results unmask a striking lack of correlation between AID binding and its mutator activity, providing evidence for the presence of factors required downstream of AID binding to effect SHM. Furthermore, our findings suggest that S regions are preferred targets for AID and, aided by polypyrimidine tract binding protein-2, act as “sinks” to sequester AID activity from other genomic regions. The Journal of Immunology, 2014, 193: 252–257.

A ctivation-induced cytidine deaminase (AID) is essential for somatic hypermutation (SHM) and class switch recombination (CSR) (1, 2). During SHM, AID deaminates deoxycytidines to deoxyuridines at the V region exons (V gene) of the Ig H and L chains (3). Engagement of base excision repair and mismatch repair pathways, along with DNA synthesis by error-prone DNA polymerases at the deoxycytidine:deoxyuridine mismatch, mutates the V genes at a high rate (≈10⁻² –10⁻³ mutations/ bp/generation), leading to selection of B cells with increased Ag affinity (4). CSR exchanges the initially expressed IgH C region Cµ for an alternative set of downstream Cµ exons (or genes), such as Cγ, Cε, or Cα, altering the B cell expression from IgM to a secondary Ab isotype (IgG, IgE, IgA) with distinct effector function (5). CSR is a deletional-recombination reaction that is initiated by AID-mediated deamination of transcribed, repetitive switch (S) region DNA elements that precede each Cγ gene (5). End-joining of double strand breaks (DSBs) between two distinct S regions deletes the intervening DNA as an extrachromosomal circle and juxtaposes a new Cγ gene downstream of the rearranged VDJ segment. Thus, CSR allows for the generation of Ig molecules with the same affinity for Ag but with new effector function.

AID is a general mutator and can mutate and induce DSBs at many non-Ig genes (6–11). In fact, aberrant AID activity on oncogenes is a major contributing factor in the ontogeny of a large number of mature B cell lymphomas (12). Despite the ability of AID to target non-Ig genes, the V genes and S region DNA serve as major AID targets, with the efficiency of AID association at the Ig loci being several fold higher than at non-Ig genes (7, 8). In addition to specificity for the Ig loci, there is evidence for intra-Ig locus specificity, because B cells undergoing CSR in culture do not mutate their variable regions (13, 14). Thus, mechanisms must exist to actively recruit AID to V genes and S regions during SHM and CSR, respectively. Several factors, including Spt5, polypyrimidine tract binding protein-2 (Ptbp2), RNA exosome subunits and 14-3-3 adapter proteins, have been implicated in the recruitment of AID to S regions (7, 15–17), although the precise role of these proteins in CSR is yet to be fully elucidated. The mechanism by which AID is specifically recruited to V genes is even more enigmatic. Unlike S regions that are unique in their G:C richness and in their ability to form RNA:DNA hybrid structures (R-loops) upon transcription (18, 19), V genes do not present a recognizable primary or predicted secondary structure that could explain specificity for AID binding. The RGYW (R = A/G, Y = C/T, W = A/T) tetranucleotide does serve as an SHM hot-spot motif, and E2A-transcription factor binding sites promote SHM (6, 20); however, the ubiquitous nature of these sequences at almost all transcribed genes fails to explain AID specificity.

Previously, we identified Ptbp2 as an AID interactor (15). Depletion of Ptbp2 significantly impaired CSR as a result of a defect in the recruitment of AID to S regions. In this study, we used the B lymphoma cell line CH12 to show that when AID recruitment to S regions is impaired through Ptbp2 depletion, association of AID with the expressed V gene is remarkably promoted. Surprisingly, despite the binding of AID to V genes, SHM is not induced. Therefore, AID binding does not correlate with mutation activity, suggesting that SHM requires specific factors and/or subversion of DNA repair pathways that operate downstream of AID binding.

Materials and Methods

Cell culture and protein analysis

CH12 cells (21) were stimulated at a density of 0.25 × 10⁶ cells/ml for 96 h with anti-CD40 Ab (CTT) (1 µg/ml; HM40-3; eBioscience, San Diego, CA), IL-4 (12.5 µg/ml; 404-ML; R&D Systems, Minneapolis, MN), and TGF-β1 (0.1 ng/ml; 240-B; R&D Systems). IgA⁺ cells were generated

from CIT-stimulated CH12 cells by negative selection with anti-IgM MicroBeads (Miltenyi Biotec, San Diego, CA), followed by positive selection using anti-IgA biotin Ab (eBioscience) and Streptavidin MicroBeads (Miltenyi Biotec). The predominantly IgA* cell population was subcloned by serial dilution.

Flow cytometry and Western blotting

Cells were stained with IgM–PE-Cy 7 (R-6–60.2; BD Biosciences, San Jose, CA) and IgA–FITC (C10–3; BD Biosciences) and acquired on an LSR II (BD Biosciences). Data were analyzed using FlowJo software (TreeStar, Ashland, OR). IHC (Invitrogen, Grand Island, NY) was used for exclusion of dead cells. Whole-cell lyses were performed in Nonidet P-40 lysis buffer (20 mM Tris [pH 7.5], 5% [v/v] glycerol, 150 mM NaCl, 5 mM 2-ME, and 0.5% [v/v] Nonidet P-40). The following primary Abs were used: anti-Ptpb2 (ab57619; Abcam, Cambridge, MA), anti-AID (22), and anti-Gapdh (loading control; 6C5; Millipore, Billerica, MA).

Chromatin immunoprecipitation analysis

Knockdown in CH12 cells was described previously (15). Chromatin immunoprecipitation (ChIP) assays were performed as described previously (15, 23). PCR amplification and detection were carried out on a Bio-Rad CFX96 system, and threshold cycle (Ct) values were calculated using CFX Manager software by setting the threshold within the linear phase using a log-amplification plot. For detection of specific DNA sequences in ChIP. Averaged, IQ SYBR Green Supermix (Bio-Rad, Hercules, CA) was used for Sα and p53 amplifications, whereas FastStart Universal Probe Mix was used for VDJ amplification along with FAM-labeled Universal Probe #45 (both from Roche, Indianapolis, IN). Melt-curve analysis was used for SYBR reactions to verify the presence of a single amplicon of the correct size. Chim “relative units” were calculated by first normalizing the Ct value of each Ab sample by the Ct value of the input sample (CtAb/CtInput). The inverse of this normalized Ab Ct value was then taken (1/NormCtAb). The resulting value for the nonspecific IgG Ab sample was corrected using the geometric mean of the Ct values obtained for unstimulated IgA+ cells, suggesting that SHM was not induced (Fig. 1B). As in CH12 cells, AID did not associate with IgH regions in unstimulated IgA+ cells or with the Trp53 (p53; non-Ig control) genomic sequence under any condition (Supplemental Fig. 1B, 1C, 1F). However, contrary to our predictions, AID binding to the IgH V gene segment was not detected in CIT-stimulated IgA+ cells (Fig. 1C, Supplemental Fig. 1F). Instead, AID was found associated with the recombinated Sμ–Sα DNA (Fig. 1C, Supplemental Fig. 1F). Thus, AID exhibits intra-IgH locus specificity with a bias for binding to S regions, not only in bulk CH12 cells undergoing CSR, but also in IgA+ cells in which the IgH V gene might be expected to have a better chance of competing favorably against the remaining S region for AID binding.

Activated CH12 cells do not undergo SHM

To test the possibility that the failure to detect AID at the V gene is not due to the transient nature of the interaction, we isolated genomic DNA from IgA+ cells and sequenced the expressed IgH V gene using sequence-specific primers (Supplemental Fig. 1A, Supplemental Table II). The rate of mutation of the IgH V gene (VDJ) in CIT-stimulated IgA+ cells was similar to that observed for unstimulated IgA+ cells, suggesting that SHM was not induced (Fig. 2A, Supplemental Table I). In contrast, the recombinated Sμ–Sα sequence in CIT-stimulated IgA+ cells had significant levels of mutations (Fig. 2B, Supplemental Table IB). Further, the original recombinated Sμ–Sα junction present in the unstimulated IgA+ cell line underwent deletions to create novel S junctions (Fig. 2B, 2C). Thus, although AID in IgA+ cells bound to and mutated the remaining S region, it neither associated with nor induced SHM at the IgH V gene.

Localization of AID at the IgH locus is altered upon Ptpb2 depletion

The RNA-binding protein Ptpb2 interacts with AID and facilitates the recruitment of AID to S regions (15). We investigated the possibility that, in Ptpb2-depleted cells, AID that fails to bind to S regions can be “retargeted” to the V gene segment. Using a short hairpin RNA (shRNA) directed against the 3’ untranslated region of Ptpb2 mRNA, we knocked down Ptpb2 expression in CH12 cells, with “scrambled” shRNA serving as a control (Fig. 3A). As expected (15), AID binding to S regions was significantly reduced (~2-fold, p = 0.003), and CSR was impaired (Fig. 3B, 3C, Supplemental Fig. 1G). Strikingly, the reduction in AID at S regions was accompanied by a significant increase (>6-fold, p = 0.01) in AID binding specifically to the IgH V gene of stimulated...
Ptbp2-depleted cells (Fig. 3C, Supplemental Fig. 1D, 1G). AID was not substantially enriched at other regions of the Igh locus, including 1 kb upstream and downstream of the rearranged VDJ (5’Vh1-53 and 3’Jh2, respectively), Cμ, and the Iμ promoter (Fig. 3D). Surprisingly, AID was not detected at the expressed VJκ L chain locus (Fig. 3D). This could be due to occlusion of the Ab-binding epitope while AID is bound to the L chain locus or, more interestingly, a suggestion that the level of Ptbp2 preferentially effects AID relocalization within the Igh locus during SHM; thus, only the binding of AID to the S region and Igh V gene exons is altered upon Ptbp2 depletion. Finally, AID was not found to be associated with control genomic sequences, such as Trp53 (p53) or other non-Ig genes that were shown to be upregulated in B cells undergoing CSR (23) (Fig. 3E). Thus, when the association of AID with S regions is impaired, its interaction with the expressed Igh V gene segment is specifically and significantly promoted.

**FIGURE 1.** AID expressed in IgA+ cells does not bind the expressed Igh V gene. (A) Flow cytometry analysis of IgM (left panel) or IgA (right panel) expression on unstimulated (U) or CIT-stimulated (S) CH12 or IgA+ cells. (B) Western blot analysis of whole-cell extracts using AID and Gapdh (loading control) Abs. (C) ChIP analysis to detect binding of AID or histone H3 to Sμ and Igh V gene (VDJ) in stimulated CH12 or IgA+ cells. Two independent experiments are shown. “ChIP (Relative Units)” is defined as the reciprocal of the quotient of the crossing threshold (Ct) value of specific Ab immunoprecipitation signal and the Ct value of input signal, with nonspecific IgG immunoprecipitation signal (background) subtracted from this value.

**FIGURE 2.** AID does not induce SHM in CH12 cells. (A) Mutation frequency of the Igh V region (VDJ). Unstimulated (U) IgA+ cells accumulated one mutation in 66,297 bases, and stimulated (S) IgA+ cells had one mutation in 68,593 bases. (B) Mutation frequency of the Sμ–Sα junction. Unstimulated (U) IgA+ cells accumulated 2 mutations in 65,515 bases and 1 sequence with a deletion. Data were obtained from two independent samples. The \( p \) values were calculated using a \( z \)-test. (C) The generation of deletions at the Sμ–Sα junction in CIT-stimulated IgA+ cells. DSBs generated in Sμ and Sα upon initial stimulation of an IgM+ CH12 cell are synapsed to form an initial Sμ–Sα junction. Stimulation of an IgA+ cell generates a new set of DSBs and results in the formation of a new Sμ–Sα junction, deleting the intervening switch sequence.

AID bound to the Igh V gene in PTBP2-depleted cells is phosphorylated

AID is phosphorylated at serine 38 (S38), and mutation of S38 to alanine impairs the ability of AID to mediate SHM and CSR (23, 29–33). To determine whether AID bound to the V gene was phosphorylated at S38 (pS38-AID), we carried out ChIP experiments using pS38-AID–specific Ab (23). In accordance with AID bound to Sμ, the amount of pS38-AID localized to Sμ was reduced in Ptbp2-depleted CH12 cells (Fig. 4A). This reduction was accompanied by a significant increase (~7-fold, \( p = 0.05 \)) in pS38-AID levels specifically associated with the Igh V gene (Fig. 4A). Localization of pS38-AID to the Trp53 (p53; non-Ig control) genomic sequence was not amplified from pS38-AID samples (Supplemental Fig. 1E). Thus, AID is not only specifically targeted to the V gene segment in Ptbp2-depleted CH12 cells, it is phosphorylated at S38.

AID phosphorylated at S38 interacts with the ssDNA-binding protein replication protein A (RPA) (29, 30). To test whether RPA localization also is altered in Ptbp2-depleted cells, we carried out ChIP analyses using an Ab specific for the 32-kDa subunit of RPA. RPA levels at Sμ were significantly reduced (~2.5-fold, \( p = 0.03 \)) in Ptbp2-depleted CH12 cells (Fig. 4A). In contrast, there was a significant increase (~3-fold, \( p = 0.01 \)) in RPA localization specifically to the Igh V gene segment (Fig. 4A, Supplemental Fig. 1E).

Phosphorylation of AID at S38 is mediated by protein kinase A (PKA) (23, 30, 31, 34), and it is believed that AID is phosphorylated by S region–bound PKA to activate the CSR cascade (23, 35). In keeping with AID-independent recruitment of PKA to S regions (23), the catalytic subunit of PKA (PKA-Cα) was detected at Sμ in CIT-stimulated Ptbp2-depleted cells (Fig. 4B). However, PKA-Cα was not detected at the Igh V gene segment (Fig. 4B), even though phosphorylated AID was readily detectable (Fig. 4A). Thus, not all proteins known to bind S regions are re-targeted to the V gene in the absence of Ptbp2, thereby ruling out the possibility that the binding of AID to the Igh V gene is due to general deregulation of protein–DNA associations in Ptbp2-depleted CH12 cells.

Our findings that PKA-Cα is associated with S regions, but not with the Igh V gene, regardless of Ptbp2 expression, lends credence...
to the proposal (23) that AID phosphorylation has a different role in SHM than in CSR. It is generally believed that transcribed S regions form R-loops, allowing AID to access S regions independent of its phosphorylation status (36). Phosphorylation of AID at S38 is still required to promote formation of DSBs at S regions through interaction with APE1 (35), as well as for the repair of DSBs through recruitment of RPA to S regions (8, 23). In contrast, transcribed V genes do not readily reveal ssDNA in the context of R-loops, and it is likely that AID only binds V genes in the context of a pS38–AID–RPA complex, because of the ability of RPA to bind and stabilize ssDNA within transcription bubbles (29). Our findings that pS38–AID and RPA, but not PKA-Cα, are detected at the Igh V gene support the notion that AID binding to V genes requires prior phosphorylation. However, we cannot exclude the possibilities that PKA-Cα was not detected at the V gene segment as a result of the transient nature of the interaction or that other proteins bound to the V gene segment mask the Ab binding site of V gene–bound PKA-Cα. Additionally, we cannot rule out the possibility that AID bound to the V region exons is not phosphorylated by PKA but is instead modified by an unidentified kinase.

**AID localization to the V gene does not induce SHM**

To determine whether AID binding induced SHM, we sequenced the Igh V gene (VDJ). Surprisingly, the mutation frequency in Ptbp2-depleted cells stimulated for 96 h was similar to that in scrambled cells (Fig. 5A, Supplemental Table IC). Moreover, Ptbp2-depleted cells stimulated continuously for 3 wk did not accumulate additional mutations (Supplemental Table IC). Although the mutation frequency was higher than in unstimulated cells (Supplemental Table IC), it was still considerably lower than typical SHM (32, 33, 37). The absence of SHM activity in Ptbp2-depleted cells cannot be attributed to a deficiency in Igh V gene (VDJ) transcription, because transcription through this region was not markedly altered (<2-fold change) upon Ptbp2 depletion (Supplemental Fig. 2A). Most importantly, there was no correlation...
between the levels of AID bound to the V gene and SHM frequency (Figs. 3C, 5A). In contrast, the region 5′ of the Sμ-repetitive region (34) was highly mutated (Fig. 5B, Supplemental Table ID). A majority of the mutations in this region were transition mutations, and all mutations occurred at C:G bp (Fig. 5C). This spectrum suggests that mutations in CH12 cells arise mainly from replication across deaminated residues, with a small portion being mutated at the same frequency as the RGYW motifs in the V region. Percentage nucleotide substitutions from residue “y” (down left side) to residue “y” (along top), with total percentage substitution for each residue listed on the right. Percentage transitions or transversions are indicated below the figures. The p values were calculated using the z-test.

FIGURE 5. AID binding to the Igh V gene does not induce SHM. (A) Mutation analysis of Igh V gene (VDJ) segment in CIT-stimulated CH12 cells expressing either scrambled or Ptbp2 shRNA constructs. Scrambled cells accumulated three mutations in 65,436 bases sequenced, and Ptbp2 cells had two mutations in 65,436 bases sequenced. (B) Mutation analysis of 5′ Sμ region. Scrambled cells accumulated 14 mutations in 62,160 bases sequenced, and Ptbp2 cells had 9 mutations in 62,720 bases sequenced. Data represent four independent samples. (C) Characterization of mutations found in 5′ Sμ region. Percentage nucleotide substitutions from residue “y” (down left side) to residue “y’” (along top), with total percentage substitution for each residue listed on the right. Percentage transitions or transversions are indicated below the figures. The p values were calculated using the z-test.

Discussion

Our results clearly demonstrate that S regions are preferred targets for AID binding. Even in cells that have undergone CSR to the last Cμ gene, AID is efficiently recruited to the remaining S region in lieu of the transcribed Igh V gene. This strong preference for S regions might represent a physiological mechanism for AID regulation, wherein S regions act as a “sink” to prevent AID from interacting with non-Ig genomic sequences. Being noncoding, S regions could sustain mutations and deletions without having any deleterious effect on cell viability. This is evident from the abundant mutations and deletions observed at Sμ–Sα junctions upon re-expression of AID in IgA+ cells. Thus, active recruitment of AID to S regions probably functions as a default pathway to sequester AID activity away from other genomic targets.

If AID expressed in a B cell is indeed actively recruited to the S regions, the question that follows is how AID is targeted to the V genes to initiate SHM. Our studies clearly demonstrate that reduction in Ptbp2 levels promotes binding of AID to Igh V gene segments. Thus, in a B cell undergoing SHM, modulating Ptbp2 expression or perturbing the interaction between AID and Ptbp2 through posttranslational modifications of AID and/or Ptbp2 could increase the amount of non-S region–bound AID (Fig. 6). The relative expression of Ptbp2 in germinal center B cells undergoing SHM versus CSR will shed further light onto the differential role of this protein during an immune response.

The most striking finding is that, despite binding to the Igh V gene, AID cannot induce SHM. Over the past few years, several genome-wide association analyses of AID reported that it has the potential to bind to other genomic regions (7, 11, 39). Our findings clearly enforce the notion that binding of AID is not synonymous with mutations. For SHM to take place, at least in the context of CIT-stimulated CH12 cells, additional steps need to occur beyond AID binding. It is feasible that CH12 cells lack a factor(s) required for SHM and/or that CIT induction is not sufficient to induce the expression of all required proteins. Another likely possibility is that AID targeted to the Igh V gene in Ptbp2-depleted cells is indeed actively deaminating, but high-fidelity repair (6), as of detectable SHM in Ptbp2-depleted CH12 cells is not due to a deficiency in the amount of available hot-spot motifs in this region; rather, it is likely due to the absence in expression of required repair mediators or uncharacterized SHM-specific factors in CH12 cells (Fig. 6). In conclusion, although known mediators of SHM, namely AID and RPA, were abundant at the Igh V gene, and the bound AID was phosphorylated at S38, SHM was not induced.
to opposed to error-prone required for SHM, “fixes” the lesions in a nonmutagentic fashion. Under physiological conditions, SHM is associated with germinal center structures found in secondary lymphoid organs, where the interaction of B cells with CD4+ T cells (40) could induce SHM-promoting factors or class switch reversion of high-fidelity repair pathways that are absent from in vitro culture system described in this study.

In conclusion, the data presented in this article intriguingly suggest that targeting of AID to S regions is the default mechanism in a CSR-sufficient B cell line and may indicate a role for S regions as an AID “sink,” sequestering AID away from the rest of the genome. Our findings identify Ptbp2 as a crucial mediator between S region and genome. AID deamination activity or the localization does not determine AID deamination activity or the induction of mutation. Additionally, we describe a cell line–based model system that will be invaluable in further elucidating factors/conditions required for SHM versus CSR.

Acknowledgments
We thank Tasaka Honjo (Kyoto University, Kyoto, Japan) for providing the CH12 cell line and David Schatz (Yale University, New Haven, CT) and members of the Chaudhuri laboratory for helpful discussions and suggestions.

Disclosures
The authors have no financial conflicts of interest.

References

In conclusion, the data presented in this article intriguingly suggest that targeting of AID to S regions is the default mechanism in a CSR-sufficient B cell line and may indicate a role for S regions as an AID “sink,” sequestering AID away from the rest of the genome. Our findings identify Ptbp2 as a crucial mediator between S region and genome. AID deamination activity or the localization does not determine AID deamination activity or the induction of mutation. Additionally, we describe a cell line–based model system that will be invaluable in further elucidating factors/conditions required for SHM versus CSR.

Acknowledgments
We thank Tasaka Honjo (Kyoto University, Kyoto, Japan) for providing the CH12 cell line and David Schatz (Yale University, New Haven, CT) and members of the Chaudhuri laboratory for helpful discussions and suggestions.

Disclosures
The authors have no financial conflicts of interest.

References

In conclusion, the data presented in this article intriguingly suggest that targeting of AID to S regions is the default mechanism in a CSR-sufficient B cell line and may indicate a role for S regions as an AID “sink,” sequestering AID away from the rest of the genome. Our findings identify Ptbp2 as a crucial mediator between S region and genome. AID deamination activity or the localization does not determine AID deamination activity or the induction of mutation. Additionally, we describe a cell line–based model system that will be invaluable in further elucidating factors/conditions required for SHM versus CSR.

Acknowledgments
We thank Tasaka Honjo (Kyoto University, Kyoto, Japan) for providing the CH12 cell line and David Schatz (Yale University, New Haven, CT) and members of the Chaudhuri laboratory for helpful discussions and suggestions.

Disclosures
The authors have no financial conflicts of interest.

References

In conclusion, the data presented in this article intriguingly suggest that targeting of AID to S regions is the default mechanism in a CSR-sufficient B cell line and may indicate a role for S regions as an AID “sink,” sequestering AID away from the rest of the genome. Our findings identify Ptbp2 as a crucial mediator between S region and genome. AID deamination activity or the localization does not determine AID deamination activity or the induction of mutation. Additionally, we describe a cell line–based model system that will be invaluable in further elucidating factors/conditions required for SHM versus CSR.

Acknowledgments
We thank Tasaka Honjo (Kyoto University, Kyoto, Japan) for providing the CH12 cell line and David Schatz (Yale University, New Haven, CT) and members of the Chaudhuri laboratory for helpful discussions and suggestions.

Disclosures
The authors have no financial conflicts of interest.

References

In conclusion, the data presented in this article intriguingly suggest that targeting of AID to S regions is the default mechanism in a CSR-sufficient B cell line and may indicate a role for S regions as an AID “sink,” sequestering AID away from the rest of the genome. Our findings identify Ptbp2 as a crucial mediator between S region and genome. AID deamination activity or the localization does not determine AID deamination activity or the induction of mutation. Additionally, we describe a cell line–based model system that will be invaluable in further elucidating factors/conditions required for SHM versus CSR.

Acknowledgments
We thank Tasaka Honjo (Kyoto University, Kyoto, Japan) for providing the CH12 cell line and David Schatz (Yale University, New Haven, CT) and members of the Chaudhuri laboratory for helpful discussions and suggestions.

Disclosures
The authors have no financial conflicts of interest.

References

In conclusion, the data presented in this article intriguingly suggest that targeting of AID to S regions is the default mechanism in a CSR-sufficient B cell line and may indicate a role for S regions as an AID “sink,” sequestering AID away from the rest of the genome. Our findings identify Ptbp2 as a crucial mediator between S region and genome. AID deamination activity or the localization does not determine AID deamination activity or the induction of mutation. Additionally, we describe a cell line–based model system that will be invaluable in further elucidating factors/conditions required for SHM versus CSR.

Acknowledgments
We thank Tasaka Honjo (Kyoto University, Kyoto, Japan) for providing the CH12 cell line and David Schatz (Yale University, New Haven, CT) and members of the Chaudhuri laboratory for helpful discussions and suggestions.

Disclosures
The authors have no financial conflicts of interest.

References