Lysine Residue at Position 22 of the AID Protein Regulates Its Class Switch Activity

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Abstract

Background: Activation induced deaminase (AID) mediates class switch recombination and somatic hypermutation of immunoglobulin (Ig) genes in germinal centre B cells. In order to regulate its specific activity and as a means to keep off-target mutations low, several mechanisms have evolved, including binding to specific cofactors, phosphorylation and destabilization of nuclear AID protein. Although ubiquitination at lysine residues of AID is recognized as an essential step in initiating degradation of nuclear AID, any functional relevance of lysine modifications has remained elusive.

Methodology/Principal Findings: Here, we report functional implications of lysine modifications of the human AID protein by generating a panel of lysine to arginine mutants of AID and assessment of their catalytic class switch activity. We found that only mutation of Lys22 to Arg resulted in a significant reduction of class switching to IgG1 in transfected primary mouse B cells. This decrease in activity was neither reflected in reduced hypermutation of Ig genes in AID-mutant transfected DT40 B cell lines nor recapitulated in bacterial deamination assays, pointing to involvement of post-translational modification of Lys22 for AID activity in B cells.

Conclusions/Significance: Our results imply that lysine modification may represent a novel level of AID regulation and that Lys22 is important for effective AID activity.

Introduction

Activation induced deaminase (AID) induces class switch recombination (CSR) and somatic hypermutation (SHM) of Ig genes in B cells during the germinal centre reaction [1,2,3]. CSR changes the constant region of the antibody, thereby changing its effector function, whereas SHM increases the affinity of the antibody towards specific antigen by mutating the variable domain followed by selection of high affinity variants. AID initiates both processes by deaminating cytosines within the Ig genomic DNA, thereby generating uracils, which lead to DNA mismatches [4,5,6]. These mismatches are either processed into dsDNA breaks for CSR, or into mutations for SHM [7,8].

Since AID is a DNA-mutating enzyme, its activity has to be tightly controlled to avoid collateral damage. Although AID is primarily targeted to the Ig locus for exerting its mutating function, leakiness of this process is evidenced by AID-mediated off-target mutations in non-Ig genes, including a panel of proto-oncogenes like c-myc, Bcl6 and p53 [9,10]. In addition, as a result of mistargeted CSR, AID also contributes to the translocation of proto-oncogenes into the Ig locus, thereby accelerating malignant transformation and tumorigenesis [11,12]. To ensure that AID-activity is largely restricted to the Ig locus, AID is regulated on different levels including (i) transcription [13,14,15], (ii) phosphorylation of residues T27, S38, Y184 and T140 [16,17,18], (iii) by binding of partner proteins to AID (reviewed in [8], (iv) by protein stability and (v) by subcellular localization [19,20,21].

Though AID acts on genomic DNA, it is largely restricted to the cytoplasm due to a strong C-terminal Exportin/Crm1-dependent nuclear export sequence (NES), which can be blocked by leptomycin B (LMB) [22,23,24]. Deletion or mutation of the NES leads to nuclear accumulation of AID, based on active import, which is mostly dependent on the structural integrity of AID and independent of a defined nuclear localization sequence (NLS) [21]. Interestingly, while deletion of the NES leads to nuclear accumulation and an increased mutational activity at Ig- as well as non-Ig genes, the CSR-activity of AID is strongly impaired [23]. Additionally, nuclear accumulation is accompanied by degradative ubiquitination and decreased protein stability [20,19]. Based on these findings, it is supposed that AID is restricted to the cytoplasm to minimize the level of off-target mutations and to allow the production of stable AID protein, which is necessary for CSR.

Although it could be previously shown that lysine residues within the AID protein are ubiquitinated when nuclear export is blocked, functional implications of lysine modifications have not...
been assessed [19]. In our study, we investigated whether modification of lysine residues within the AID protein is important for CSR and SHM activity. Using a lysine to arginine mutagenesis screen, we found that mutating Lys22 to Arg (termed mutant K3) hampers CSR activity in B cells. However, despite of reduced activity in CSR, mutant K3 was active to wildtype levels in somatic hypermutation and in bacterial deamination assays. Our experiments suggest a role of Lys22 in potentiating CSR activity of AID, providing evidence for a novel level of AID regulation.

Results

CSR activity of AID lysine to arginine mutants

As depicted in figure 1A, the human AID protein comprises eight lysine residues. To dissect the involvement of lysine modification in AID activity, we performed a mutational analysis by generating individual lysine to arginine mutants (K1 to K8, Fig. 1A) and tested the resulting mutants in cell switch assays. Therefore, we retrovirally transduced primary AID deficient B cells with the respective AID mutants (K1 to K8) and determined class switching to IgG1 upon stimulation with LPS and IL-4. Interestingly, only mutation of lysine at position 22 (mutant K3) showed an apparent effect on CSR activity (Fig. 1B, C and D), which prompted us to more thoroughly investigate the influence of this residue on CSR in higher sample numbers. We could show that mutating Lys22 significantly affected CSR activity, leading to a decrease from 14.3% for AID, to 10.0% for K3 of IgG1 switched B cells (two-tailed paired t-test, p = 0.0001, n = 11, Fig. 1B and D).

Enzymatic activity of mutant K3 is not perturbed

As mutant K3 showed a decrease in CSR activity, we next asked whether this decrease is reflected in impaired biochemical activity of the mutant per se, irrespective of a putative lysine modification at this position. We therefore tested mutant K3 and wildtype AID for cytidine deaminase activity in ung deficient E.coli cells, determined by reversion of an inactive Rif resistance gene [4]. We found a similar frequency of Rif’ colonies in E.coli cells expressing K3 mutant as opposed to wildtype AID, showing that mutant K3 is catalytically active at a similar level as wildtype AID (Fig. 2). However, lysineless AID mutant in which all eight lysine residues are mutated to arginine (mutant deltaK) has lost enzymatic activity, probably due to structural destabilization (Fig. 2).

Mutating Lys22 does not impair nuclear import

Because Lys22 is located within a region of the AID protein previously found to be responsible for nuclear import [21,23], we assessed whether mutation of Lys22 to Arg would interfere with nuclear/cytoplasmic shuttling of AID, which we speculated to be causative for the observed decrease in CSR activity. We found that in steady-state conditions, GFP-fusions of AID and mutant K3 were strictly localized to the cytoplasm of transfected B mouse B cell line k46 and non-B cells (HEK293T) (Fig. 3). Nuclear accumulation of GFP-tagged AID and K3 could be induced by inhibition of nuclear export using LMB, indicating that nuclear import of AID is not impaired by Lys22Arg mutations (Fig. 3).

Mutant K3 is active in hypermutation

To determine the mutational activity of K3 in eukaryotic cells, we tested its ability to hypermutate an immunoglobulin V region gene in the B cell line DT40. Therefore, we transfected AID+/− ΨV+/− IgM+ DT40 cells with AID and K3 constructs, respectively and determined V region diversification by measuring the number of IgM-loss variants in several independent clones. We found similar activity in SHM from K3 transfected cells compared to those expressing wildtype AID (10.6% vs 8.7% mean IgM-loss; 9.3% vs 5.4% median IgM-loss; p = 0.34, unpaired two-tailed t-test; Fig. 4A). Also, protein levels were similar for wildtype and mutant K3 (Fig. 4C). To more thoroughly determine the mutational activity of K3, we sorted for AID or K3 expressing DT40 variants and analyzed the mutations within the V-region. We found that the amount of mutations within the V-region was similar for AID (3 out of 21 sequences mutated) and K3 (3 out of 24 sequences mutated) expressing DT40 cells, with no apparent difference in hotspot preference being observed (Mutation frequency 4.1 vs 3.9×10−5; p = 0.58, unpaired two-tailed t-test; Fig. 4B and S1). To test off-target activity of AID versus K3, we analysed mutations within the intronic region of Bcl6, which is known to be subjected to SHM in germinal centre B cells and germinal centre derived B cell lymphoma [26,27] and which was shown to accumulate AID dependent mutations also in mice [28]. Therefore, we retrovirally transduced primary AID deficient murine B cells with constructs encoding AID or K3 and sequenced a 600 bp fragment of the intronic Bcl6 locus from GFP+ sorted cells. Strikingly, we found mutations in AID-transduced B cells (2 out of 44 independent sequences contained one mutation), whereas no mutations could be detected in K3 transfused B cells (p = 0.16, unpaired two-tailed t-test; Fig. 4D, S2). From these results we conclude that Lys22 of AID is more important for CSR than for IgV diversification but might also lead to an increase in off-target mutations.

Discussion

AID mediates CSR and SHM by deaminating cytidine residues within the genomic DNA of the Ig locus. To keep off-target mutations low and to avoid genomic instability, AID has to be tightly targeted to the Ig locus and its activity regulated by several mechanisms, including restricted expression, nuclear/cytoplasmic shuttling, interaction with binding proteins and phosphorylation at Ser/Thr residues. Phosphorylation at Ser38 was shown to induce interaction with Replication Protein A, a DNA binding protein that is thought to mediate access of AID to transcribed DNA targets [29,17]. Phosphorylation at Thr140 also regulates CSR and SHM as inhibition of phosphorylation at this residue decreases the biological activity of AID [18].

In this study, we aimed at assessing the role of lysine modification on AID activity. In previously published work it could be shown that nuclear AID is ubiquitinated at any of its eight lysine residues to initiate degradation. This was interpreted as means to keep nuclear AID levels low to avoid genomic instability [19]. However, conjugation of ubiquitin to cellular proteins not only initiates their destabilization but alternatively can modulate their specific function or their cellular trafficking [30,31]. In this respect, we investigated the functional implication of single lysine to arginine mutants of the human AID protein and found that Lys22 is important in potentiating the specific activity of AID. While mutating Lys22 to Arg had no significant effect on AID activity in E.coli (Fig. 2) and on SHM in DT40 B cells (Fig. 4A, B, C), the implication on CSR activity was more pronounced, reflected in a significant reduction of IgG1 switched primary B cells (Fig. 1). Although Lys22 had no substantial effect on IgV diversification in DT40, off target Bcl6 mutations were reduced in B cells expressing K3 compared to wt AID (Fig. 4D), implying that increased collateral damage might accompany a more efficient CSR activity.

As lysine-ubiquitination of AID was recently demonstrated to be involved in regulating AID stability/degradation [19,32], we
Figure 1. Effect of lysine to arginine mutations in the AID protein on CSR activity. (A) Schematic representation of AID highlighting the position of the lysine (K) residues. For CSR assays, untagged constructs were expressed bicistronically together with GFP separated by an internal ribosome entry site (IRES). (B) Representative FACS profile for CSR activity of individual Lys to Arg mutants. B cells from AID deficient mice were retrovirally transfected with the respective lysine AID mutant (Mx: empty vector) and CSR to IgG1 was determined upon stimulation of cells with LPS and IL-4. GFP expression of infected cells was driven from an IRES element located 3’ of the stop codon of the cloned AID mutant. The percentage in the upper right quadrants indicate the percentage of IgG1+ cells among the GFP+ (i.e. retrovirally infected) population. (C) Results from three independent experiments of a screen of lysine mutants showing the percentage of IgG1+ cells among the GFP+ population. (D) Results from 11 independent experiments comparing the CSR activity of AID and K3. (E) Western Blot on lysates from B cells retrovirally transfected with the respective constructs. Black arrowheads indicate specific band. White arrowheads indicate nonspecific band. (IB: immune blot).

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speculated whether a functional ubiquitin conjugation onto Lys22 could account for increased CSR activity of wildtype AID compared to mutant K3. In line with a recent publication, we found that Lys22 is a potential target of ubiquitin conjugation, however, ubiquitination is not restricted to a specific lysine residue as also mutant K3 was found to be ubiquitinated in transfected B cell lines (data not shown and [19]). In addition, lysine-independent N-terminal ubiquitination of AID as noticed especially upon impaired nuclear export [19] makes a clear-cut analysis of residue-specific ubiquitination difficult. Therefore, it is difficult to assess whether ubiquitination of Lys22 is actually responsible for the observed efficacy of wt AID compared to mutant K3 or whether other explanations account for the decreased effectiveness of mutant K3. In this regard it is conceivable that Lys22 is modified by other functional groups such as sumo, acetyl or methyl groups, or otherwise, that Lys22 has an intrinsic effect on CSR activity of AID. In either case, Lys22 could either affect targeting of AID to S-regions or the assembly of AID into a putative multiprotein-complex which executes CSR. In this regard it will be interesting not only to test for the presence of post-translational Lys22 modifications but also to analyze whether Lys22 mutation affects interaction with recently identified AID-interacting proteins such as CTNNBL1 [33], PKA [29], Sp5 [34], GANP [35], PTBP2 [36] and 14-3-3 adaptor proteins [37] which were found to recruit AID to its DNA target sites.

Notably, Lys22 is highly conserved as it can be found in AID from mammals and birds whereas AID from frog, bony- and pufferfish share an Arg residue at this position (Fig. S3). While SHM is present already in cartilaginous fish and is likely to occur in all jawed vertebrates, CSR appeared late in evolution and can be found only in land vertebrates, starting with amphibians [38]. Together with our observation that mutant K3 has more dramatic effects on CSR than SHM, this raises the possibility that Lys22 had co-evolved with the mechanism of CSR.

AID is regulated at several levels, including transcription, nuclear/cytoplasmic shuttling and phosphorylation. Our notion that Lys22 is important for proper AID function introduces lysine modification as a novel level of AID regulation. Future work will be required to unravel the role of Lys22 in mediating interactions with AID-binding proteins and to identify the presence of any functional lysine modifications at this position, such as sumoylation, methylation or acetylation.

Materials and Methods

Ethics statement

No ethics statement is required: the research described in this manuscript does not involve human participants. Also, no experimental procedures were performed on live animals. Mice used for this research were maintained at the animal facility of the University of Salzburg according to the national guidelines for animal welfare. Mice were killed by CO2 suffocation and spleens were used for further experiments.
Class Switch assay

Class switching was analysed as previously described [20]. Briefly, B cells were purified from AID−/−mice [3] and cultured in the presence of LPS and IL-4 for one day prior to infection with human AID (or variants) encoding retroviruses. CSR to IgG1 was analysed by flow cytometry three days after retroviral infection. For retrovirus production, Plat-E [39] cells were transfected with pMx-Ig-derived plasmids using Genejuice (Novagen). Lysine mutants of human AID were cloned into pMx-Ig plasmids using BamHI/XhoI sites and site-directed mutagenesis.

Somatic Mutation

AID-induced IgV gene somatic mutation was monitored by measuring the frequency of IgM-loss variants in AID−/−IgM−/−IgM+DT40 cells [40] transfected with hAID-encoding vectors based on pExpressPuro2 (gift from J-M Buerstedde, Munich, Germany). Independent stable transfectants were expanded for 4 weeks and analysed by flow cytometry for the percentage of cells retaining surface IgM expression. For analysis of mutations in the IgV, genomic DNA was prepared from GFP+ sorted cells and DNA was amplified using specific primers (V-fwd: 5′-GGT GCA GGC AGC GCT GAC TCA GCC GGC CTC-3′; V-rev: 5′-CCA AAT CAC CAA AAA TCG ACA AAA TGT CAC-3′), and cloned using TOPO-cloning (Invitrogen) followed by sequencing of independent clones.

Off-target mutations within the Bcl6 intronic region were determined from primary retrovirally transfected mouse B cells. Briefly, primary resting B cells from AID deficient mice were retrovirally transfected with AID or K3 encoding constructs as described for CSR-assays. Transfected cells were sorted 3 days after transfection using FACS-sorting based on GFP expression and genomic DNA was isolated. PCR amplification of intronic Bcl6 region was achieved using specific primers as described by Muto et al [28] (5′-CTT TCT TGG TTG GAG TCG AGG C-3′; 5′-CGG GCT TGA GGT CAT TTC TC-3′) followed by TOPO-cloning of purified PCR fragments and sequencing of several independent clones.

AID-induced mutations in E.coli

Bacterial assays were performed essentially as described in Petersen-Mahrt et al. [4]. In brief, AID-induced mutations were monitored by measuring the frequency of Rifampicin (Rif) resistant clones that are generated by mutation of a Rif-sensitive allele in an Uracil-DNA Glycosylase (UNG)-deficient BW310 E.coli strain. Individual clones of BW310 cells transfected with empty vector or AID or K3 (AIDLys22Arg) expressing pTrc99 [4] based constructs were grown overnight in 3 ml IPTG-containing medium at 30°C. Cultures were then spread on Rif and Ampicillin plates and the appearance of Rif-resistant clones was calculated (Mutation frequency).

AID Localization

Constructs encoding AID or AID-variants fused C-terminally to GFP were generated by PCR-based mutagenesis and cloned into pEGFP-N1 (Clontech). The respective constructs were transiently

Figure 4. Somatic Hypermutation of IgV genes by wt AID and mutant K3. (A) Somatic hypermutation reflected as loss of IgM expression in AID−/−IgV−/−IgM+DT40 cells. AID−/−IgV−/−IgM+DT40 cells were stably transfected with plasmids encoding AID or K3 coupled to IRES-GFP expression. The percentage of IgM-loss variants of GFP+ cells is expressed as the median ± SE of multiple (n) independent clonal transfectants determined three weeks after transfection (untransfected control, n = 12; AID, n = 36; K3, n = 44). (B) Proportion of sequences carrying mutations in IgV genes of DT40 cells. IgV regions were cloned from cDNA of AID and K3 expressing DT40 transfectants. Segment sizes in the pie charts are proportional to the number of sequences carrying the number of mutations indicated in the periphery of each chart. The frequency of mutations per bp sequenced and the total number of independent sequences analyzed is indicated underneath and in the center of each chart, respectively. (C) The abundance of AID and K3 in DT40 transfectants was determined by Western blotting from cell extracts. Blots were reprobed for GFP expression and actin levels as loading control. (D) Proportion of sequences carrying Bcl6 mutations in AID deficient B cells retrovirally transfected with AID or K3 constructs, respectively. Bcl6 was cloned from genomic DNA of sorted mouse B cells, retrovirally transfected to express AID or K3, respectively. Pie charts were generated as described in (B).

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transfected into HEK293T [41] cells in a six-well format using Genetique and replated onto glass slides 24 h after transfection. Alternatively, stable transfectants of the mouse B cell line k46 [42], expressing pEGFP-N1-based AID-GFP fusion constructs were used. Nuclear export of AID was blocked by incubation of cells with Leptomycin B at a final concentration of 10 ng/ml for 5 h. Cells were fixed in 4% paraformaldehyde, permeabilized with 0.5% Triton X-100 and cell nuclei were stained using 1 μg/ml DAPI and covered in mounting medium (Vectashield).

Western Blotting

Approximately 106 retrovirally transfected B cells or 10e5 DT40 cells were boiled in 50 μl of reducing SDS-sample buffer and subjected to SDS/PAGE. Samples were blotted onto PVDF membranes (Millipore) and detected by Western blot analysis using polyclonal rabbit serum specific for human AID (Abcam), GFP (Cell Signaling), or actin (Cell Signaling) and HRP-conjugated secondary antibodies specific for rabbit IgG (Dako).

Supporting Information

Figure S1 Mutations identified within the IgV’ gene from DT40 cells transfected with AID or K3, respectively. The depicted sequence of the IgV’ gene was PCR amplified from transfected DT40 cells. Primer binding sites are underlined. Independent mutations are shown in uppercase letters above the bcl6 intronic sequence. No mutations were found for K3 (n = 49). (Accession No: NT_039624.7) (DOC)

Figure S2 Mutations identified within the bcl6 gene from primary mouse B cells retrovirally transfected with AID or K3, respectively. The depicted sequence of the bcl6 intronic region was PCR amplified from retrovirally transfected and sorted mouse B cells. Independent mutations for AID (n = 44) are shown in uppercase letters above the bcl6 intronic sequence. No mutations were found for K3 (n = 49). (Accession No: NT_039624.7) (DOC)

Figure S3 Lys22 is conserved in higher vertebrates and birds. Alignment of AID protein sequences from various species showing that Lys22 is conserved in birds and mammals. Species competent for switching are marked in bold. Sequences derived from M. Chatterji et al. J. Immunol. 2007;179:5274–5280. (DOC)

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Author Contributions

Conceived and designed the experiments: R. Geisberger. Performed the experiments: R. Geisberger MH FJG NZ. Analyzed the data: R. Geisberger MH FJG NZ. Contributed reagents/materials/analysis tools: AE. R. Greil. Wrote the paper: R. Geisberger. Interpreted results and critically discussed the manuscript: R. Geisberger MH FJG NZ AE R. Greil.

References

1. Reys P, Muto T, Levy Y, Geissmann F, Pfeil A, et al. (2000) Activation-induced cytidine deaminase (AID) deficiency causes the autosomal recessive form of the hyper-IgM syndrome (HIGM2). Cell 102: 565–575.

2. Muramatsu M, Sankaranand VS, Anant S, Sugai M, Kinoshita K, et al. (1999) Specific expression of activation-induced cytidine deaminase (AID), a novel member of the RNAediting deaminase family in germinal center B cells. Journal of Biological Chemistry 274: 18470–18476.

3. Muramatsu M, Kinoshita K, Fagarasan S, Yamada S, Shinkai Y, et al. (2000) Class switch recombination and hypermutation require activation-induced cytidine deaminase (AID), a potential RNA editing enzyme. Cell 102: 553–563.

4. Petersen-Mahrt SK, Harris RS, Neuberger MS (2002) AID mutates E-coli with accuracy. Nature 419: 99–103.

5. Chaudhuri J, Tian M, Khosong C, Chua K, Pinaud E, et al. (2003) Transcription-targeted DNA deamination by the AID antibody diversification enzyme. Nature 422: 736–730.

6. Di Noia JM, Neuberger MS (2002) Altering the pathway of immunoglobulin hypermutation by inhibiting uracil-DNA glycosylase. Nature 419: 43–48.

7. Neuberger MS (2008) Antibody diversification by somatic mutation: from error-prone DNA synthesis to gene duplication. Annual Review of Biochemistry 76: 1–22.

8. Liu M, Duke JL, Richter DJ, Vinuesa CG, Goodnow CC, et al. (2008) Two levels of protection for the B cell genome during somatic hypermutation. Nature 451: 814–U11.

9. Yoshikawa K, Okazaki EM, Ito T, Kinoshita K, Muramatsu M, et al. (2002) AID enzyme-induced hypermutation in an actively transcribed gene in fibroblasts. Science 296: 2033–2036.

10. Ramiro AR, Jankovic M, Callen E, Dillipantonio S, Chen HT, et al. (2006) Role of genomic instability and p53 in AID-induced c-myc/IgH translocations. Nature 445: 105–109.

11. Ramiro AR, Jankovic M, Eisenrich T, Dillipantonio S, Chen-Kiang S, et al. (2004) AID is required for c-myc/IgH chromosome translocations in vivo. Cell 118: 431–439.

12. Dedegla F, Horvitz B, Chaudhuri J, Alt FW, Geha RS (2004) Induction of activation-induced cytidine deaminase gene expression by IL-4 and CD40 ligation is dependent on STAT6 and NF kappa B. International Immunology 16: 395–404.

13. Crouch EE, Li ZY, Takizawa M, Fichtner-Feigl S, Gourni P, et al. (2007) Regulation of AID expression in the immune response. Journal of Experimental Medicine 204: 1145–1156.

14. Tran TH, Nakata M, Suzuki K, Begum NA, Shinkura R, et al. (2010) B cell-specific and stimulation-responsive enhancers derepress Aida by overcoming the effects of silencers. Nature Immunology 11: 148–157.

15. Basu U, Chaudhuri J, Alpert C, Dutt S, Ranganath S, et al. (2005) The AID antibody diversification enzyme is regulated by protein kinase A phosphorylation. Nature 438: 508–511.

16. McBride KM, Gazumyan A, Woo EM, Barreto VM, Robbiani DF, et al. (2006) Regulation of hypermutation by activation-induced cytidine deaminase phosphorylation. Proceedings of the National Academy of Sciences of the United States of America 103: 8780–8785.

17. McBride KM, Gazumyan A, Woo EM, Schwickert TA, Chait BT, et al. (2008) Regulation of class switch recombination and somatic mutation by AID phosphorylation. Journal of Experimental Medicine 205: 2585–2594.

18. Aoufouchi S, Faiì A, Zober C, DY Orlando O, Weller S, et al. (2008) Proteasomal degradation restricts the nuclear lifespan of AID. Journal of Experimental Medicine 205: 1357–1368.

19. Geisberger R, Rada G, Neuberger MS (2009) The stability of AID and its function in class-switching are critically sensitive to the identity of its nuclear-export sequence. Proceedings of the National Academy of Sciences of the United States of America 106: 6736–6741.

20. Patenaude AM, Orthwein A, Hu Y, Campo VA, Kavith B, et al. (2009) Active nuclear import and cytoplasmic retention of activation-induced deaminase. Nature Structural & Molecular Biology 16: 517–527.

21. Brat SS, Watson M, Diaz M (2004) Activation-induced cytidine deaminase (AID) is actively exported out of the nucleus but retained by the induction of DNA breaks. Journal of Biological Chemistry 279: 26393–26401.

22. Ito S, Nagaska H, Shinkura R, Begum N, Muramatsu M, et al. (2004) Activation-induced cytidine deaminase shuttles between nucleus and cytoplasm like apolipoprotein B mRNA editing catalytic polypeptide 1. Proceedings of the National Academy of Sciences of the United States of America 101: 1973–1980.

23. McBride KM, Barreto V, Ramiro AR, Stavropoulos P, Nussenzveig MC (2004) Somatic hypermutation is limited by CRM1-dependent nuclear export of activation-induced deaminase. Journal of Experimental Medicine 199: 1235–1244.

24. Ta VT, Nagaska H, Catalan N, Durandy A, Fischer A, et al. (2003) AID mutant analyses indicate requirement for class-switch-specific cofactors. Nature Immunology 4: 843–848.
26. Pasqualucci L, Migliazza A, Fracchiolla N, William C, Neri A, et al. (1998) BCL-6 mutations in normal germinal center B cells: Evidence of somatic hypermutation acting outside Ig loci. Proceedings of the National Academy of Sciences of the United States of America 95: 11816–11821.

27. Pasqualucci L, Neumeister P, Geoursens T, Nanjundg S, Chaganti RSK, et al. (2001) Hypermutation of multiple proto-oncogenes in B-cell diffuse large-cell lymphomas. Nature 412: 341–346.

28. Muto T, Okazaki IM, Yamada S, Tanaka Y, Kinoshita K, et al. (2006) Negative regulation of activation-induced cytidine deaminase in B cells. Proceedings of the National Academy of Sciences of the United States of America 103: 2752–2757.

29. Chaudhuri J, Khuong C, Alt FW (2004) Replication protein A interacts with AID to promote deamination of somatic hypermutation targets. Nature 430: 962–968.

30. Sun J, Chen Z (2004) The novel functions of ubiquitination in signaling. Current Opinion in Cell Biology 16: 119–126.

31. Hicke L, Dunn K (2003) Regulation of membrane protein transport by ubiquitin and ubiquitin-binding proteins. Annual Review of Cell and Developmental Biology 19: 141–172.

32. Orthwein A, Paternade AM, Affar E, Lamarre A, Young JC, et al. (2010) Regulation of activation-induced deaminase stability and antibody gene diversification by H9p90. Journal of Experimental Medicine 207: 2751–2765.

33. Conticello SG, Ganesh K, Xue K, Lu M, Rada C, et al. (2008) Interaction between anti body-diversification enzyme AID and spliceosome-associated factor CTNNBL1. Molecular Cell 31: 474–484.

34. Pavri R, Gazumyan A, Jankovic M, Di Virgilio M, Klein I, et al. (2010) Activation-Induced Cytidine Deaminase Targets DNA at Sites of RNA Polymerase II Stalling by Interaction with Sp5. Cell 143: 122–133.

35. Maeda K, Singh SK, Eda K, Kitabatake M, Pham P, et al. (2010) GANP-mediated Recruitment of Activation-induced Cytidine Deaminase to Cell Nuclei and to Immunoglobulin Variable Region DNA. Journal of Biological Chemistry 285: 23945–23953.

36. Nowak U, Matthews AJ, Zheng S, Chaudhuri J (2011) The splicing regulator PTBP2 interacts with the cytidine deaminase AID and promotes binding of AID to switch-region DNA. Nature Immunology 12: 160–166.

37. Xu ZM, Falop Z, Wu GK, Pone EJ, Zhang JS, et al. (2010) 14-3-3-3 adaptor proteins recruit AID to 5'-AGCT-3'-rich switch regions for class switch recombination. Nature Structural & Molecular Biology 17: 1124–1133.

38. Cannon JP, Haire RN, Rast JP, Litman GW (2004) The phylogenetic origins of the antigen-binding receptors and somatic diversification mechanisms. Immunological Reviews 200: 12–22.

39. Morita S, Kojima T, Kitamura T (2008) Plat-E: an efficient and stable system for transient packaging of retroviruses. Gene Therapy 7: 1063–1066.

40. Arakawa H, Sarbasak H, Buerstedde JM (2004) Activation-induced cytidine deaminase initiates immunoglobulin gene conversion and hypermutation by a common intermediate. Plos Biology 2: 967–974.

41. Sena-Esteves M, Saeki Y, Camp SM, Chiocca EA, Breakefield XO (1999) Single-step conversion of cells to retrovirus vector producers with herpes simplex virus-Epstein-Barr virus hybrid amplicons. Journal of Virology 73: 10426–10439.

42. Kim KM, Alber G, Wraber P, Roth M (1993) Differential Signaling Through the Ig-Alpha and Ig-Beta Components of the B-Cell Antigen Receptor. European Journal of Immunology 23: 911–916.