

Activation-induced deaminase: light and dark sides

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Activation-induced deaminase (AID) is required for class switch recombination (CSR) and somatic hypermutation (SHM), which are responsible for secondary diversification of antibodies in germinal centers. AID initiates these processes by deamination of cytosines on the immunoglobulin (Ig) locus, a potentially mutagenic activity. AID expression is restricted to germinal-center B cells, but the mechanisms that regulate its target specificity are not completely understood. Here, we review the most recent findings on the regulation of AID targeting and discuss how AID activity on non-Ig genes is relevant to the generation of chromosome translocations and to lymphomagenesis.

Activation-induced deaminase and antibody diversification

The immune system of vertebrates has evolved to mount adaptive responses against pathogens through the generation of a myriad of receptors for antigens of virtually unlimited diversity. During lymphocyte differentiation, T and B cells are programmed to re-arrange somatically the genes that code antigen receptors by the VDJ recombination reaction, thereby giving rise to the primary repertoire of antigen receptors. In contrast to T cells, B cells have an additional chance to diversify their antibody repertoire in germinal centers (see Glossary) after they encounter the antigen in peripheral tissues. The germinal-center reaction enables the generation of higher affinity antibodies with various functional specificities that fine-tune their response against pathogens. The two molecular mechanisms responsible for retuning the antibody repertoire are somatic hypermutation (SHM) and class switch recombination (CSR) (Figure 1). SHM involves the introduction of mutations, usually single-nucleotide substitutions, in the variable antigen-recognition portion of immunoglobulin (Ig) genes. Only those B cells in which SHM has introduced mutations that improve the affinity for their cognate antigen survive and proliferate. CSR is a region-specific recombination reaction that takes place between two switch regions of the heavy-chain Ig locus, so that the C μ primary Ig constant region is substituted by a downstream constant region and the intervening DNA sequence is excised (Figure 1). As a result of this recombination reaction, antibodies with new effector capabilities are generated. Despite temporal and spatial overlap, CSR and SHM are distinct reactions that can take place

independently of one another [1,2]. An increasing number of molecules have been associated with these two reactions, but it was not until the discovery of activation-induced deaminase (AID) by the Honjo laboratory [3] that the pieces of the puzzle started to fit to provide a clearer picture of SHM and CSR.

The identification of AID can in turn add to the understanding of B-cell lymphomagenesis. About 95% of diagnosed lymphomas are of B-cell origin, the majority of which are thought to derive from germinal-center or post-germinal-center B cells. A major hallmark of mature-B-cell lymphomas is the presence of chromosome translocations that involve the Ig locus and a proto-oncogene and that have etiological significance to disease development. The aim of this review is to provide a general view of AID function and regulation in normal physiology, focusing on some of the most recent developments in the field, and its potential contribution to the generation of malignant situations. For a more extensive perspective of AID, the reader should refer to some excellent reviews that have been recently published [4–9].

What is the function of AID?

AID was first identified as a gene selectively expressed in B cells that are activated to undergo CSR [3]. AID sequence has the closest homology to apolipoprotein B mRNA editing enzyme, catalytic polypeptide 1 (APOBEC1), an mRNA cytidine deaminase involved in lipid metabolism. Soon after its identification, AID was shown to be essential for both CSR and SHM in mice [10], and mutations in the AID gene were found to be associated with hyper-IgM syndrome type 2 [11,12]. Moreover, AID, when heterologously expressed in fibroblasts, is sufficient to promote CSR and SHM on transcribed substrates [13,14], implying that AID is the only B-cell-specific factor required to initiate these reactions. The actual mechanism by which AID initiates CSR and SHM has been a matter of intense debate. Because of its homology to APOBEC1, AID was first proposed to be an mRNA deaminase responsible for editing mRNA that codes for a protein, possibly an endonuclease, that would be directly or indirectly involved in SHM and CSR. However, this precursor mRNA has not yet been identified. The alternative hypothesis is that AID would deaminate cytidines directly in the variable or switch regions of the Ig locus to trigger SHM or CSR, respectively (Figure 2). As a consequence of this DNA deamination reaction, a U:G mismatch would be generated in the Ig locus that is likely to be processed through

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Glossary

Abasic site: a sugar phosphate backbone of DNA in which a nitrogenous base has been removed.

Activation-induced deaminase (AID): the cytidine deaminase that is required for CSR and SHM, which take place in germinal-center B cells.

Chromosome translocations: chromosomal rearrangements in which part of a chromosome is detached by breakage and subsequently joined to a non-homologous chromosome.

Class switch recombination (CSR): a region-specific recombination reaction that takes place between two switch regions of the heavy chain Ig locus so that the C_μ primary Ig constant (C) region is replaced by a downstream C region and the intervening DNA sequence is excised.

c-Myc: a proto-oncogene that is often involved in chromosome translocations found in human B-cell lymphomas.

Cytidine deamination: the process by which the amino group of a cytidine base is removed causing its transformation into a uracil.

Epigenetic modifications: reversible modifications of chromatin that occur without a change in the sequence of the encoding DNA, such as DNA methylation and histone modifications.

Germinal centers: spherical masses in the center of a lymph node that contains actively proliferating B cells in which the SHM and CSR processes take place.

Non-homologous end joining (NHEJ): a DNA-repair mechanism in which two non-homologous DNA ends are repaired by ligation to one another.

P19 and p53: tumor suppressor proteins (i.e. their activity stops the formation of tumors).

Proto-oncogene: a normal cellular gene whose activation is linked to malignant transformation.

Somatic hypermutation (SHM): the process by which mutations, usually single-nucleotide substitutions, are introduced in the variable, antigen recognition portion of immunoglobulin genes.

Switch regions: highly repetitive regions that are present in the introns of the immunoglobulin heavy-chain genes that are targeted in CSR.

Uracil-N-DNA glycosylase (UNG): an enzyme that removes uracils from DNA.

uracil removal by uracil-N-DNA glycosylase (UNG) or by alternative pathways, including base-excision repair and mismatch repair, leading to CSR or SHM. Some prominent evidence supporting the DNA deamination model for

AID function includes the following observations: (i) AID deaminates single-stranded DNA (ssDNA) *in vitro* and it does so with the same sequence preference that is observed in B cells [15–18]; (ii) genetic evidence shows that, in the absence of UNG, mutations at G–C pairs are biased towards transitions [19,20] and CSR is severely impaired [21]. Moreover, the mismatch repair protein MSH2 is involved in both SHM and CSR [6,7], and the combined absence of MSH2 and UNG completely abolishes CSR [22] (Figure 2); and (iii) AID has been reported to interact with switch region DNA in B cells that undergo CSR [23]. Therefore, although the RNA-versus-DNA controversy still exists [24], we consider that the experimental data available to date point to a DNA-deamination activity of AID, which is the accepted view of this review. A comprehensive review of the molecules that are downstream of DNA deamination is beyond the scope of this review [6,7], but some of them are outlined in Table 1 for clarity.

How is AID function regulated?

AID's activity has intrinsic mutagenic potential (Figure 2) and, therefore, a tight regulation is required to restrict this potential to the appropriate cell type, time and loci, and to avoid DNA lesions throughout the genome. Conceptually, two types of regulatory mechanisms can be envisioned to prevent unwanted DNA damage by AID: limiting AID availability to the appropriate cell type (i.e. germinal-center B cells) and restricting the accessibility of DNA sequences to AID function. We will briefly outline some of the evidence for these regulatory mechanisms.

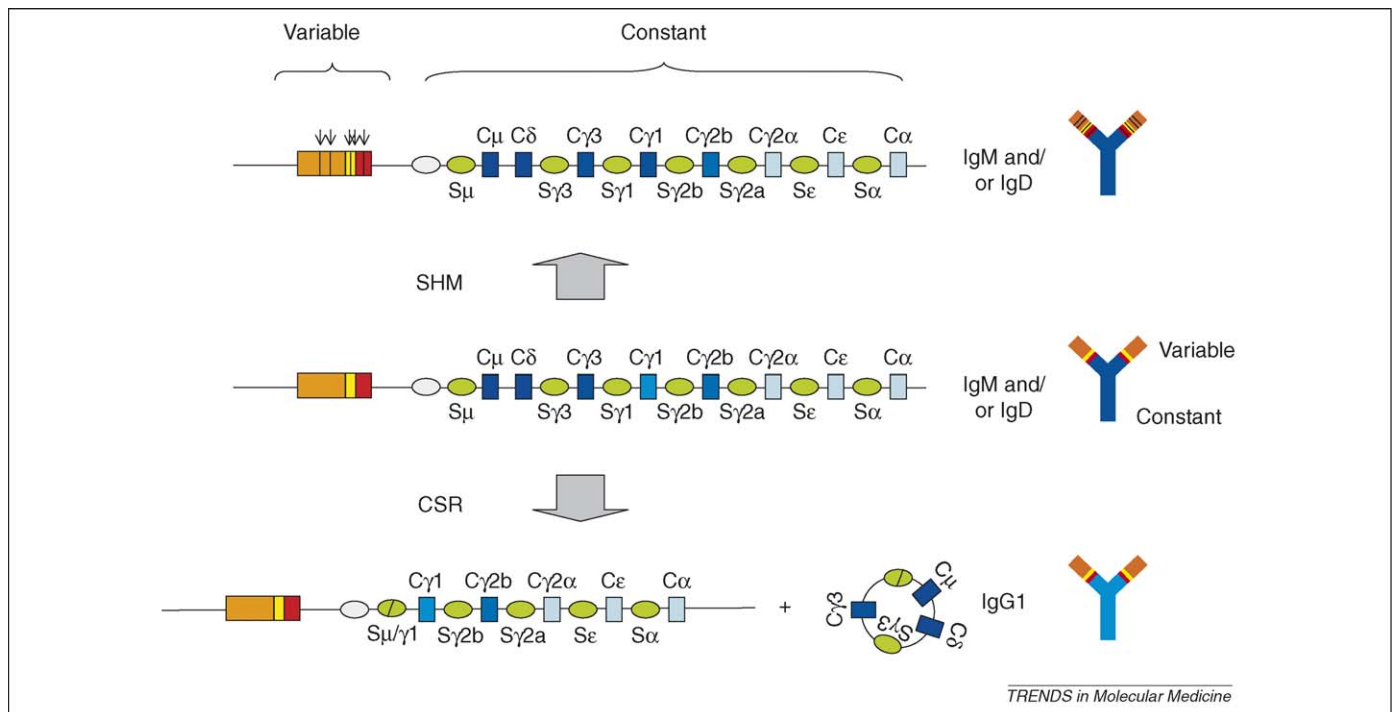


Figure 1. Generation of the secondary antibody repertoire by SHM and CSR. Two molecular mechanisms are responsible for the secondary diversification of antibodies in germinal centers. SHM alters the antigen-binding region of the antibody by introducing nucleotide substitutions (depicted as arrows) in the rearranged variable region of the Ig genes, thereby enabling the generation of antibodies with higher affinity for antigen. CSR is a region-specific recombination reaction that exchanges the primary C_μ constant region with a downstream constant region (blue boxes), giving rise to antibodies with new effector functions. The recombination takes place between the switch regions (green ovals) preceding the corresponding constant regions and results in the excision of the intervening DNA sequence. The figure illustrates recombination from S_μ to S_{γ1}. Molecules on the right side of the figure represent IG proteins encoded by the Ig genes shown on the left.

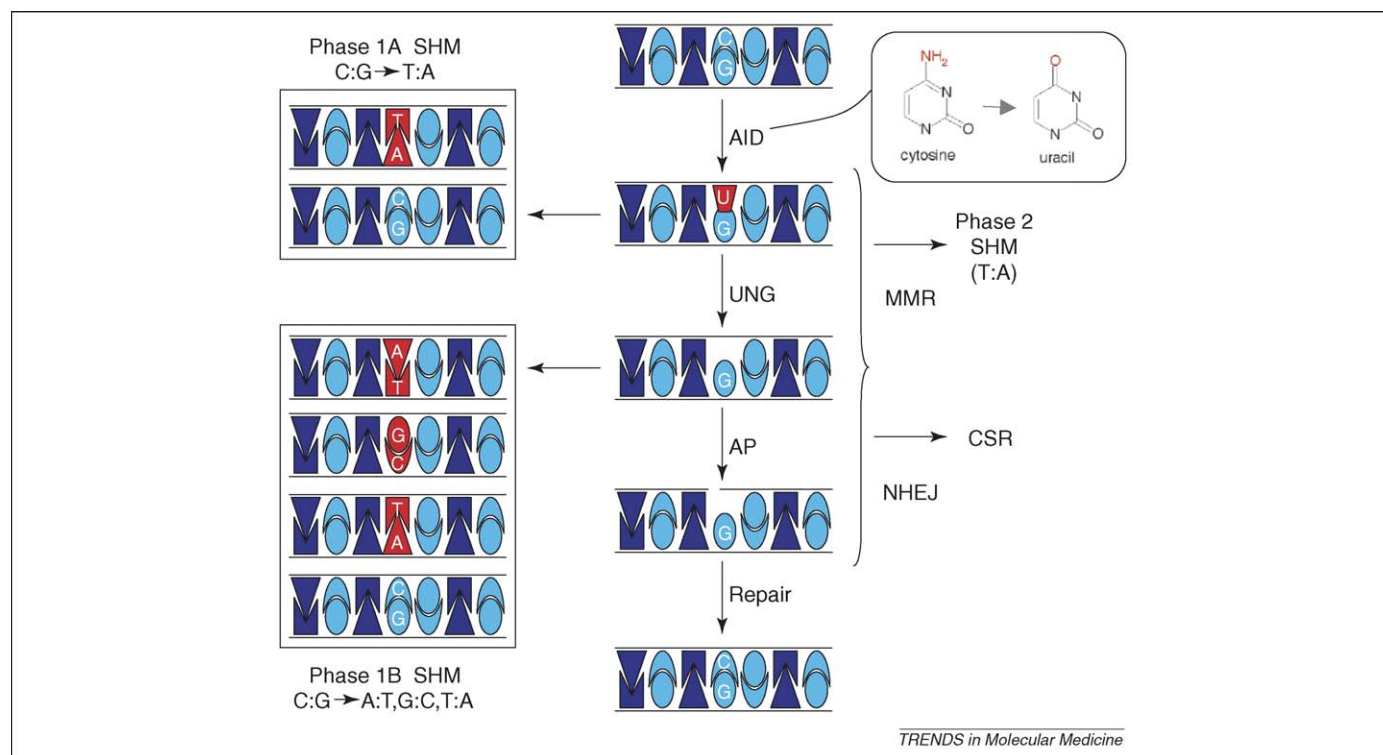


Figure 2. DNA deamination model for AID function in SHM and CSR. AID deaminates dC residues on variable or switch regions of the Ig genes to generate a dG:dU mismatch. This initial dU:dG mispairing can have three different outcomes that lead to SHM: (i) it can be replicated over, leading to transition mutations at dC:dG pairs (phase 1A SHM); (ii) it can be subject to uracil excision by UNG generating an abasic site, which can lead to both transitions and transversions at C–G pairs upon replication (phase 1B SHM); or (iii) it can be recognized by the mismatch-repair (MMR) machinery, which would account for the majority of A–T focused mutations (phase 2 SHM). The abasic site that is generated by UNG-mediated excision of a dU residue can also be substrate for an apyrimidinic endonuclease (AP), leading to a nick at the DNA strand that contains the abasic site. The DNA nick might then be repaired by the conventional base-excision repair pathway, which would eliminate the lesion in the DNA caused initially by AID or, alternatively, it can be processed by non-homologous end joining (NHEJ) and lead to CSR. The figure is adapted from the model originally proposed by Petersen-Mahrt *et al.* [19].

Regulation of AID expression and activity

AID expression is restricted to germinal-center B cells, although the insights of this regulation have not been deciphered completely. Interleukin (IL)4 and anti-CD40 signals act synergistically to activate AID expression, possibly through binding of signal transducer and activator of transcription 6 (STAT6) and nuclear factor- κ B (NF- κ B) to the proximal promoter [25,26]. A highly conserved intronic E-box element in the *AID* gene has been reported to contribute to positive and negative regulation of *AID* transcription through the binding of the helix–loop–helix (HLH) transcription factor E47 and the inhibitor of DNA binding HLH protein ID3, respectively [27]. A similar counter-balanced regulation was also suggested for paired box gene 5 (PAX5) and inhibitor of DNA binding 2 (ID2) factors in the *AID* promoter region [28]. Recently, binding sites for ubiquitous Sp transcription factors, a family of four zinc-finger transcription factors that regulate many different promoters by binding to GC box or GT motifs, have been described in the *AID* minimal promoter [29]. Therefore, the specificity and, possibly, levels of *AID* transcription seem to be the result of a complex combination of transcriptional activators – both ubiquitous and tissue-specific – and repressors, whose contribution *in vivo* still awaits experimental assessment. Furthermore, a nuclear-export signal located in the C-terminal end of AID ensures that the vast majority of AID protein stays in the cell cytoplasm [30,31]. Although the mechanisms controlling

AID shuttling within the cell are not yet fully understood [5], this seems an additional regulatory level that limits the concentration of nuclear AID that is available to access its DNA targets. Moreover, it has recently been shown that AID is subject to post-translational modifications, in particular to phosphorylation by protein kinase A (PKA), which seemingly activates AID function in CSR and SHM through mechanisms that might involve subcellular localization, recruitment to DNA or sequence targeting [32–35]. Finally, it has been recently reported that transgenic mice that express high levels of AID in B cells display inefficient CSR and SHM [36]; therefore, AID function might be subject to additional regulatory mechanisms that still need to be unveiled.

Target specificity: primary sequence

Even when it is ensured that AID is specifically expressed and transported in limited amounts to the nucleus of germinal-center B cells, the question still remains as to how its deamination activity is restricted to the Ig loci and spares other genes in the genome. SHM is not random throughout the Ig variable region; rather, it focuses on so called mutational hotspots, defined by the sequence WRC (where W = A or T and R = A or G) [8]. The same sequence preference has been shown in deamination assays *in vitro*, which indicates that it is part of the specificity of AID activity itself [18]. Targeting of WRC hotspots might be further enhanced by AID interaction with replication

Table 1. Proteins involved in SHM, CSR and chromosomal translocations^a

Protein	SHM ^b	CSR ^b	c-MYC-IgH translocations ^c	Refs
B-cell specific				
AID	Required	Required	Required	[10,64,65]
Base-excision repair				
UNG	Required for transversions at dC:dG	Required	Required	[21,65]
Mismatch repair				
MSH2	Involved in A–T mutations	Involved	N/A	[22,69,70]
MSH6	Involved in A–T mutations	Involved	N/A	[71–73]
NHEJ				
DNA-PKs	Dispensable	Involved	N/A	[74–76]
Ku70	N/A	Required	N/A	[77]
Ku80	N/A	Required	Dispensable	[78,65]
Other DNA-repair proteins				
H2AX	Dispensable	Involved	Dispensable	[65,79]
53BP1	Dispensable	Required	Dispensable	[65,80,81]
ATM	Dispensable	Involved	Protective	[65,82,83]
Nbs1	N/A	Involved	Dispensable	[65,84,85]
Tumor suppressor genes				
p53	N/A	Dispensable	Protective	[65]
p19	N/A	Dispensable	Protective	[65]

^aAbbreviations: 53BP1, p53 binding protein 1; ATM, ataxia telangiectasia mutated; DNA-PKs, catalytic subunit of DNA-dependent protein kinase; H2AX, histone 2A family, member X; MSH2, mutS homologue 2; MSH6, mutS homologue 6; N/A, not analyzed; NBS1, Nijmegen Breakage Syndrome 1; NHEJ, non-homologous end joining.

^bProtein involvement in SHM and CSR as assessed in mutant mice. Required means severe blockade (>90%). Involved means mild effect.

^cProtein involvement in c-MYC-IgH translocations as assessed by PCR analysis in mutant or plasmacytoma bearing mice.

protein A (RPA) [32], a ssDNA-binding protein involved in replication, recombination and repair. AID–RPA interaction seems dependent on AID phosphorylation by PKA at residues S38 and, possibly, T27, a modification that might take place preferentially in B cells and that has been reported to be necessary for CSR [33,34]. However, because WRC preference is also achieved by AID that is ectopically expressed in non-B cells, the primary function of PKA-mediated AID phosphorylation and RPA interaction might be related to accessibility to transcribed substrates or to as yet unknown regulatory mechanisms. In addition, it has recently been shown that AID hotspot preference can be modulated *in vivo* by mutS homolog 6 (MSH6), suggesting that the function of mismatch-repair proteins during SHM would not be limited to a post-mutational stage but might also contribute to targeting [37]. In any case, the degenerate nature of WRC motifs implies that hotspot preference alone cannot be responsible for conferring specificity to Ig genes. Furthermore, variable region sequences and switch repeats can be replaced by heterologous sequences and still enable SHM and CSR to take place [2,9].

Target specificity: transcription

If not primary DNA sequence, what makes Ig genes particularly good substrates for AID activity? It has long been known that both SHM and CSR are associated with transcription [1,2,4,9]. The features of this association can be summarized as follows: (i) transcription of the variable and switch regions is required for SHM and CSR, respectively, and removal of Ig promoters dramatically decreases SHM frequency; (ii) the rate of transcription of variable and switch regions correlate with the rate of SHM and CSR, respectively [2,9]; (iii) mutations start to accumulate 100 bp downstream of the promoter, peak at 200 bp and decrease to undetectable levels over the next 1.5–2 Kb; this mutation profile has been shown to be due to lack of AID accessibility beyond those boundaries [38]; and (iv) AID can deaminate ssDNA but not double-stranded DNA

(dsDNA) *in vitro* [15–17]; however, transcribed dsDNA is subject to AID-mediated deamination both *in vitro* and in *Escherichia coli* and the non-template strand is preferentially mutated [16,18,39,40]. These latter results indicate that transcription has a primarily mechanistic role during SHM and CSR by giving AID access to ssDNA, which is exposed either in the form of transcription bubbles [39,40] or R-loops [16]. If that is the case, what is the specificity of transcription-driven AID targeting? It has been shown that Ig-specific promoter and enhancer sequences can be replaced by heterologous *cis* sequences and still support SHM [2], suggesting that hypermutability of Ig genes might not be conferred by specific regulatory sequences, but, rather, that the main function of *cis* elements might be to enhance transcription of the target genes. Evidence against this view comes from a recent report where an E-box site that is present in all Ig genes was found to enhance SHM frequency without increasing the rate of transcription [41]. However, the mutability increase provided by this E-box motif can hardly account for the actual SHM frequency observed in Ig genes. Moreover, E boxes are relatively widespread in the genome and SHM is not usually associated with them. Therefore, the role of *cis* sequences in determining SHM targeting remains an open question.

Target specificity: epigenetic modifications

Despite the data discussed earlier in the review, transcription alone is not sufficient to recruit AID activity because not all transcribed genes support SHM. Notably, within the Ig locus the variable but not the constant region accumulates mutations during SHM, although they are both being transcribed presumably at similar rates. Local changes in chromatin structure – namely DNA methylation and histone modifications – are known to promote or modulate many DNA-associated processes such as transcription, replication, recombination and repair. Several recent works have evaluated histone modifications of the Ig locus during SHM and CSR in search for

Box 1. Outstanding questions

- How is AID targeted to the Ig loci? How do *cis* sequences and epigenetic modifications participate in AID targeting?
- What is the role of post-transcriptional modifications in AID regulation and function?
- What is the identity of AID partners, and what role do they have in AID regulation?
- Can SHM-associated DNA lesions lead to chromosome translocations?
- What is the contribution of AID to lymphomagenic development?

additional regulatory mechanisms to account for AID accessibility. Histone 3 (H3) hyperacetylation of switch regions correlated with induction of CSR and germline transcription, indicating that this modification might precede and mark the IgH locus for AID to initiate the recombination reaction [23,42,43]. Whether the same modification operates during SHM deserves further investigation because correlation was found in an activated B-cell line [44], but not in B8–1 Ig transgenic mice [45]. Interestingly, hyperacetylation of histone 4 in the switch region correlated with CSR in an AID-dependent manner [43], suggesting that this modification might be downstream of AID function and have a role in recruitment of repair factors to double-strand breaks (DSBs) rather than of AID itself. Although it still remains to be determined to what extent epigenetic modifications have a role in AID targeting independently of transcriptional activation, this is a promising field that will increase understanding of AID specificity.

Can AID function entail pathological situations?

As discussed above, the mechanisms that determine AID locus specificity remain unclear (Box 1). Can AID access DNA sequences other than Ig genes? If so, what pathological situations can arise from its activity?

Mutations in non-Ig genes

Heterologous expression of AID induces mutations in non-Ig genes in various experimental systems. First, AID can promote SHM on a green fluorescent protein (GFP) transgene when overexpressed in a B-cell line [46]. Likewise, both SHM and CSR can be induced on transcriptionally active substrates when AID is overexpressed in fibroblasts. The rate of AID activity was roughly proportional to the transcription rate of target substrates [13,47]. Bacterial and yeast genes are also susceptible to AID mutagenic activity when they are being transcribed [19,39,40,48]. Finally, constitutive and ubiquitous expression of AID in transgenic mice caused T-cell lymphomas, whose cells presented extensive mutations in T-cell receptor and *Myc* genes [49]. In all the cases, mutations occurred preferentially at hypermutation hotspots, but extremely biased towards G–C pairs, suggesting that the primary AID sequence preference is maintained in these experimental systems but the resolution of the initial mutation into A–T-focused mutations (second phase of SHM. See Figure 2 and [7]) is either not operative or overwhelmed by AID overexpression. The accessibility of highly transcribed non-Ig genes to AID mutagenesis under overexpression conditions is generally attributed to loose target specificity,

maybe due to deregulation of AID subcellular localization, although this issue has not been addressed directly.

However, non-Ig genes have also been reported to accumulate SHM-like mutations in peripheral blood B cells from normal donors and, therefore, under presumably physiological conditions: notably, B-cell lymphoma 6 (*BCL-6*) [50,51] and *FAS* [52] proto-oncogenes, but also non-oncogenic *B29* (Ig β) and *MB1* (Ig α) [53]. Several oncogenes from different B-cell neoplasias have also been probed for the presence of mutations. *BCL-6*, a proto-oncogene known to be translocated in 35% of diffuse large B-cell lymphoma (DLBCL) cases, is mutated in 73% and 47% of DLBCL and follicular lymphoma cases, respectively, but not in other B-cell malignancies [54]. Similarly, other translocation-prone proto-oncogenes such as *PIM1*, *MYC*, ras homolog gene family, member H (*RHOH*) and *PAX5* are often mutated in DLBCL [55]. Although the dependence of these mutations on AID deamination has not been formally proved, in all cases they occurred preferentially at WRC hotspots and clustered downstream of active promoters. Oncogene mutations in DLBCL have been interpreted as the result of an aberrant hypermutation mechanism that can underlie the origin of DNA DSBs and the generation of chromosomal translocations. Intriguingly, no mutations were found in the *AID* gene to account for a deregulated function [55,56], and levels of AID expression were variable among different DLBCL cases and did not correlate with the levels of SHM detected [56,57]. This inconsistency between AID expression and mutation frequency can be explained considering that expression of AID might have taken place earlier in the disease and that mutations at oncogenes might be positively selected during the progression of the malignancy. If this kind of selection is operative at early stages of the lymphoma, generation of mutations at oncogenes might be due not to aberrant hypermutation but to a bystander function of AID in genes other than Ig. This latter scenario would be consistent with the finding of various genes being mutated in normal B cells and would imply that AID-induced oncogene mutations might contribute to the development of the disease.

Chromosome translocations

The most prominent hallmark of B-cell lymphomas is the presence of chromosome translocations that juxtapose loci encoding the Ig genes and a proto-oncogene. Some of these translocations are associated with a specific type of B-cell lymphomas, such as of *BCL-1*–Ig translocation in mantle-zone lymphoma, *BCL-2*–Ig translocations in follicular lymphoma or *c-MYC*–Ig translocation in Burkitt lymphoma. As a result of chromosome fusion, the oncogene partner comes under control of Ig *cis* sequences, leading to its deregulated, constitutive expression [58,59]. The importance of Ig chromosome translocations in lymphomagenesis can be inferred from the role of the involved proto-oncogenes in cell cycle and apoptosis, and in some cases was proved in transgenic mouse models [60]. Besides Ig-related chromosome translocations, other transforming events might also be important in lymphoma pathogenesis, notably mutations at tumor suppressor genes, such as *p53*, retinoblastoma (*RB*), *p19* or ataxia telangiectasia mutated

(ATM), are common and characteristic of particular B-cell malignancies [59].

Most B-cell lymphomas are thought to arise from germinal-center or post-germinal-center B cells and harbor translocation breakpoints that cluster either at variable regions or at switch regions of Ig loci. These observations suggested from early on that the generation of Ig-associated translocations was connected with the germinal-center reactions, namely SHM and CSR. The identification of AID as the common initiating mechanism of these two processes finally provided a test of this hypothesis. Mouse models where Ig-associated translocations occur spontaneously are scarce. One of them is the extra-osseous plasmacytoma generated in IL6 transgenic mice [61], where translocations that involve the IgH switch region and the *c-MYC* proto-oncogene, resembling those found in sporadic Burkitt lymphoma, occur at a high frequency. These translocations were absent from hyperplastic lymph nodes of *AID*^{-/-}IL6tg mice, indicating that AID is indeed required for c-MYC-IgH translocations [62]. Interestingly, this observation has been challenged by the suggestion that in pristane-induced plasmacytomas [63], AID might be dispensable for the generation of c-MYC-IgH translocations but necessary for the outgrowth of translocation harboring cells, possibly by virtue of its mutagenic properties [64]. However, AID overexpression by itself can induce the generation of c-MYC-IgH translocations in B cells cultured for short time periods by a mechanism that involves both deaminase and UNG activity [65]. Therefore, although a secondary mutagenic role of AID during tumor progression cannot be ruled out, c-MYC-IgH translocations are initiated by a mechanism common to CSR that is dependent on AID activity.

At physiological levels of AID expression, c-MYC-IgH translocations are extremely rare, both *in vivo* [66] and *in vitro* [65], suggesting that in normal circumstances cellular surveillance mechanisms might prevent the generation of chromosome translocations or the progression of translocation-harboring cells. Indeed, evidence has been provided by two independent groups that B cells from mice that lack DNA-damage response proteins, such as ATM, hisone 2A family, member X (H2AX) or 53 binding protein 1 (53BP1), are more prone to accumulate chromosome breaks and aberrations associated with the IgH locus [65,67] and that these events, at least in the case of H2AX [67] and 53BP1 [65], depend on the presence of AID. In addition, B cells that lack p53 tumor suppressor display a higher AID-dependent c-MYC-IgH translocation frequency [65]. p53 is activated both by the DNA-damage sensor ATM and by oncogenic stress response through p19. Chromosome translocations are also more frequent in *ATM*^{-/-} and *p19*^{-/-} B cells, suggesting that two independent pathways converge in p53, which can protect against the occurrence or spreading of this kind of lesion [65] (Table 1). Interestingly, 53BP1- or H2AX-deficient cells did not show an increase in c-MYC-IgH translocations [65], which suggests that these lesions might be proportional not only to the number of unresolved breaks but also to the degree of impairment in p53 signaling. These findings are interesting in view of the high rate of mutant *ATM*, *p53* and *p19* alleles present in B-cell lymphomas,

such as Burkitt lymphoma and DLBCL [59], and also of the reduced levels of p53 in germinal-center B cells [68]. Therefore, mutant or low levels of p53 combined with AID expression in germinal centers might contribute early in the pathogenesis of lymphoma by facilitating AID-induced translocations.

Concluding remarks

SHM and CSR are the molecular reactions responsible for the secondary diversification of antibodies in germinal centers. The identification of AID as the initiating activity of both processes has been a milestone in the development of this field. AID triggers CSR and SHM through a cytidine deamination reaction with obvious mutagenic consequences. Indeed, AID is involved in the generation of chromosome translocations and, presumably, proto-oncogene mutations commonly found in mature B-cell lymphomas. Unlike the recombinant activating gene (RAG) proteins, which are involved in the V(D)J recombination reaction during lymphocyte development, the regulation and specificity of AID activity are far from being understood and might actually entail a looser targeting spectrum (Box 1). This might in turn relate to the seemingly higher propensity of mature B cells to undergo malignant transformation.

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