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REVIEW

A coming-of-age story: activation-induced cytidine deaminase turns 10

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The discovery and characterization of activation-induced cytidine deaminase (AID) 10 years ago provided the basis for a mechanistic understanding of secondary antibody diversification and the subsequent generation and maintenance of cellular memory in B lymphocytes, which signified a major advance in the field of B cell immunology. Here we celebrate and review the triumphs in the mission to understand the mechanisms through which AID influences antibody diversification, as well as the implications of AID function on human physiology. We also take time to point out important ongoing controversies and outstanding questions in the field and highlight key experiments and techniques that hold the potential to elucidate the remaining mysteries surrounding this vital protein.

A fundamental question in biology is that of how an organism, or more simply a population of cells, is able to respond to an almost infinite and unknown array of environmental stimuli given only a limited genome. This problem arises in a variety of systems in biology. Neuronal connections generate a stable network that is able to maintain information but dynamic enough to learn new information; pathogens display an ever-changing pattern of coat proteins on their surface to evade recognition by host immune systems; and finally, the focus of this review, B lymphocytes have evolved mechanisms to produce a repertoire of antibodies diverse enough to respond to the vast number of possible foreign antigens.

Over 50 years ago Frank MacFarlane Burnet, with no experimental evidence, hypothesized the existence of a randomization process that would result in the alteration and variation of immunoglobulin molecules[1]. At that time the only biological precedent for such a process was Lederberg's study on mutation in phage adaptation[2]. The first experimental evidence that such a process does indeed occur came with the demonstration that immunization alters the amino acid sequence of immunoglobulin-λ light chains by introducing single–amino acid changes[3–5]. Half a decade later, after the advent of recombinant DNA technology, it was shown that in addition to mutation, a somatic gene-rearrangement event assembles functional immunoglobulin genes from individual gene segments[6]. Together these two discoveries began the movement to characterize the molecular basis of this process, which corresponded closely with Burnet's original hypothesis of randomization[7].

Today there is a far better understanding of the mechanisms involved in immunoglobulin gene diversification. Recombination of variable (V), diversity (D) and joining (J) gene segments generates the primary repertoire of antibodies in an antigen-independent manner[8–10] (**Fig. 1a**). Later, the encounter of a B cell with its cognate antigen initiates secondary diversification processes at the immunoglobulin loci; these processes include somatic hypermutation (SHM; **Fig. 1b**), immunoglobulin gene conversion (GCV) and class-switch recombination (CSR; **Fig. 1c**). SHM and GCV increase the variability of the antigen-binding domain of the immunoglobulin, and CSR alters immunoglobulin effector function by switching the constant regions of the immunoglobulin heavy chain. As GCV is very similar to SHM in terms of the role of AID (and thus far has only been reported for birds and rabbits), we will mostly focus on SHM; however, almost all findings should be applicable to both processes.

Because SHM and CSR are very different processes—SHM induces the accumulation of point mutations, whereas CSR induces double-strand breaks (DSBs) and genomic recombination—it was astonishing when AID was identified as the key participant in both reactions (**Fig. 1b**,**c**). Like the discovery of the RAG-1–RAG-2 recombinase complex[8,9], the discovery of AID was the seminal finding that gave rise to all subsequent major advances toward understanding the molecular mechanisms involved in secondary immunoglobulin diversification. Although there is still much to learn, molecular immunologists have begun to rapidly uncover the molecular foundation that supports Burnet's theory of immunoglobulin gene randomization. Here we focus on the advances that have been made in AID biology since its discovery 10 years ago. We will focus mainly on the AID protein itself and less on SHM and CSR. The latter have been reviewed elsewhere[11–13].

Discovery and characterization of AID

The discovery of AID and the elucidation of its mechanism were greatly facilitated by the generation of the B lymphocyte cell line CH12F3, which was selected to inducibly undergo CSR at a high frequency. Theorizing that a specific recombinase was responsible for CSR, Muramatsu and Honjo applied a PCR-based 'subtraction method' to screen genes upregulated after stimulation of CH12F3 cells, ultimately identifying AID[14].

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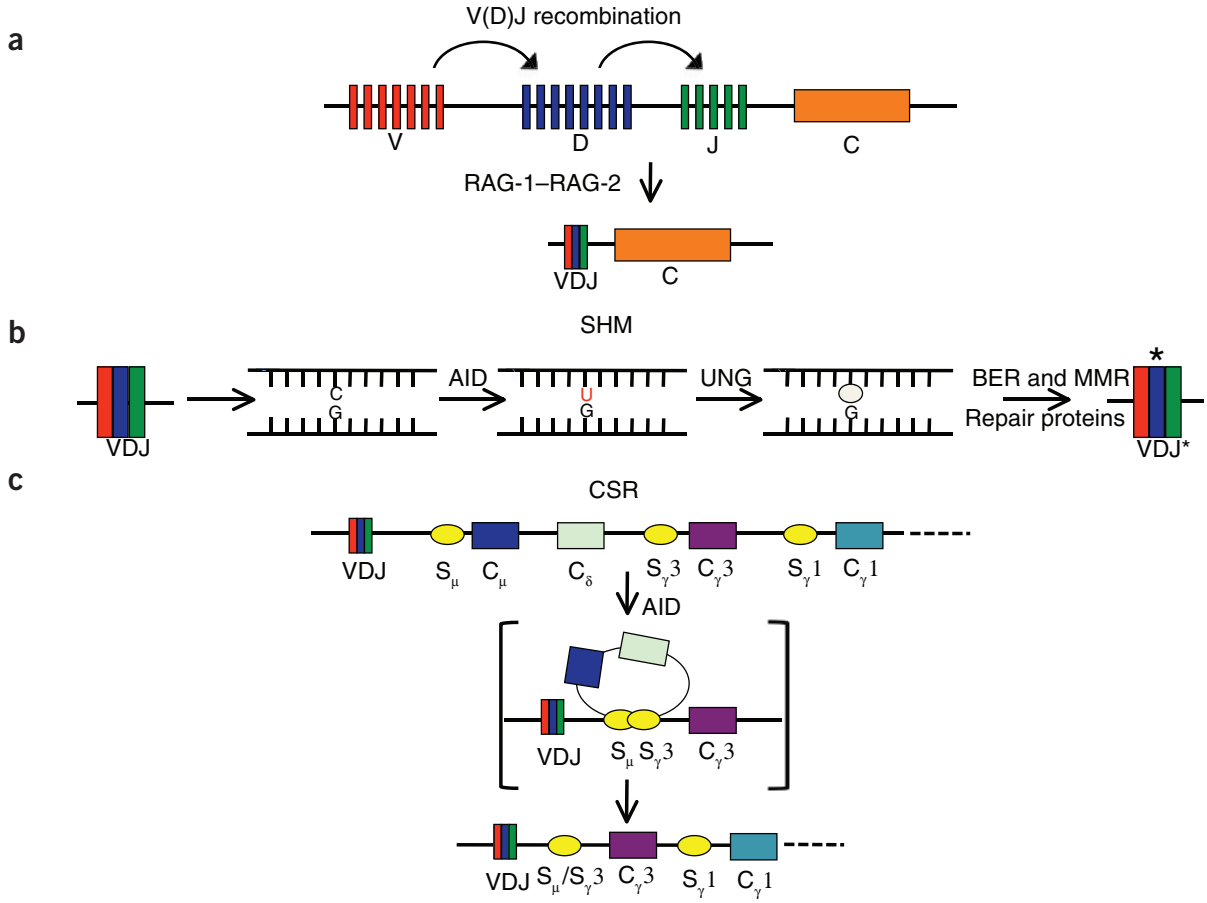

Figure 1 Antibody diversification. (**a**) A deletional recombination event between individual V, D and J segments creates the variable region of the immunoglobulin gene. This process is catalyzed by the RAG-1–RAG-2 recombinase complex and occurs in an antigen-independent way. C, constant. (**b**) SHM, the first of two secondary antibody-diversification processes, results in the accumulation of point mutations in the recombined variable region. AID initiates this process through the deamination of cytidine to uridine, followed by removal of the uracil base by UNG and repair by several base-excision repair (BER) and mismatch-repair (MMR) enzymes. The asterisk indicates the rearranged, mutated variable region. (**c**) CSR completes the secondary antibody diversification and results in the exchange of the default constant region, C_μ (IgM), for one of many downstream regions ($C_\gamma3$ (IgG3) is presented here). AID is thought to initiate this process through deamination of bases in the switch (S) region (yellow circles) upstream of each constant region, resulting in the formation of DSBs and recombination.

The subsequent generation of AID-deficient mice, which exhibit a striking inability to undergo CSR and SHM, confirmed that AID is a key participant in both processes[15]. At the same time, AID was identified as the molecule responsible for a subset of human hyper–immunoglobulin M (IgM) syndrome, which results in a complete loss of CSR (and thus an overproduction of the IgM isotype) and SHM[16].

Initial investigations revealed that AID has a cytidine-deaminase domain with homology to a known RNA deaminase, APOBEC1 (apolipoprotein B mRNA–editing catalytic polypeptide 1)[14]. Thus, it was initially hypothesized that AID edits mRNA, a model now referred to as the 'RNA-editing hypothesis'. To account for the involvement of AID in both SHM and CSR, it was imagined that AID targets either distinct mRNAs (a DNA mutator in the context of SHM and a DNA recombinase in the context of CSR) or a single mRNA that functions in both SHM and CSR, with the likely involvement of task-specific cofactors.

At the core of the alternative 'DNA-editing hypothesis' is the concept that AID mutates DNA directly. This model provides substance to earlier theories proposed by Peters and Storb[17], Scharff and Edelmann[18] and Neuberger[19], who all postulated the existence of a mutating factor that is directly targeted to the immunoglobulin locus. A number of later studies demonstrated that ectopic expression of AID is able to induce mutations in the genome of a number of mammalian cell types (plasma cells, HEK293T cells and NIH3T3 cells)[20,21], yeast[22,23] and even bacteria[24]. As it is unlikely that AID would edit the same mRNA in prokaryotic and eukaryotic cells to generate a novel DNA mutator, the simplest interpretation of these data is that AID is a true DNA mutator.

Although it is straightforward, that interpretation is complicated by the fact that expression of APOBEC1, the prototypical mRNA editor, also gives rise to DNA mutations in bacteria[25]; by analogy, AID may act on a specific mRNA as well. Analysis of the enzymatic properties of purified AID has shown deaminase activity on DNA but not on RNA substrates[26–30]. However, AID can bind both DNA and RNA[28,31] and, like APOBEC1, may require a (unknown) specific mRNA substrate. Overall, given that *in vitro*, AID 'prefers' to deaminate WRC motifs (where 'W' is adenosine or thymidine; 'R' is purine; and 'C' is cytidine)[32,33], a 'preference' that coincides well with SHM 'hot spots' observed *in vivo*[34], and that AID seems to be bound to immunoglobulin loci *in vivo*[35,36], the present evidence is in favor of the DNA-editing model.

Robust experimental support for the DNA-editing hypothesis also emerged from studies focused on an important intermediate of the SHM reaction in this model: U-G mismatches. Mice deficient in uracil DNA glycosylase (UNG), the main glycosylase that removes uracil from DNA

in the context of base-excision repair, mutate their immunoglobulin loci at rates identical to those of their wild-type littermates, but the spectra of mutations that accumulate are very different[37]. Mutations at G and C are strongly biased toward C-to-T and G-to-A mutations as a result of direct replication of such mismatches; however, mutations at A and T bases are similar in both *Ung*$^{-/-}$ and wild-type mice. In addition, CSR is substantially lower in the absence of UNG[37–39].

More recently, variants of AID and UNG shown to be catalytically inactive *in vitro* have been found to display a surprising activity *in vivo*, restoring CSR in AID- and UNG-deficient activated B cells, respectively[40,41]. Although these data are intriguing, concerns have arisen regarding whether the mutants determined to be catalytically inactive *in vitro* were in fact inactive *in vivo*. Overall, although a number of reports question the DNA-editing hypothesis, there is still no direct evidence in support of the RNA-editing model, as no AID-edited mRNA transcript has been reported.

Transcription of AID

AID was originally described as a B cell–specific factor unique to activated germinal center B cells. In this setting, AID expression is induced by signals that induce CSR in naive B cells (lipopolysaccharide, interleukin 4, CD40 ligation, transforming growth factor-β and so on) and SHM in human lymphoma lines (IgM-CD19-CD21 crosslinking)[42]. Using large-scale noncoding sequence-homology predictions[43] combined with histone H3–acetylation patterns[44] and limited 'promoter-bashing' experiments[45], a number of labs have identified four regions of the AID locus (*Aicda*; called '*AID*' here) associated with transcriptional regulation. First, there is a 1.6-kilobase *AID* promoter[43,45], which lacks a TATA box and includes sites for the transcription factors NF-κB, STAT6, HoxC4, Sp1 and Pax5 (refs. 43,45–47). In addition, there exists a putative regulatory region in the first intron that encompasses E-box motifs[48], a 3′ enhancer element[43,44] and a set of uncharacterized elements upstream of the locus[43]. How these *cis*-acting elements (and the *trans*-acting factors that bind them) operate remains to be elucidated by additional *in vivo* experiments.

Recently, AID expression has been reported to be sensitive to estrogen signaling, both in B cells and in estrogen-responsive tissues such as breast and ovary[49]. Because only mRNA was quantified, it remains to be seen whether AID protein is produced in response to estrogen. Furthermore, AID expression has been observed in a number of tissues under a variety of stimulation conditions associated with cellular transformation[49,50]. Even though in all of these cases AID expression is lower than it is in germinal center B cells, expression of AID is mutagenic and thus represents a threat to genomic stability.

Post-transcriptional regulation of AID expression

The microRNA miR-155, previously shown to have a role in the proper activation of B lymphocytes, directly regulates AID protein quantities in response to activating stimuli[51,52]. After being activated, B cells in animals with a disruption of the miR-155 target site in the 3′ untranslated region of *AID* express substantially more AID protein, which results in more CSR *in vitro* and *in vivo* and in temporally deregulated AID expression. Together these results suggest that miR-155 has a role in switching off AID expression in post–germinal center B cells[51]. Additionally, in the absence of miR-155-mediated control, excess AID protein leads to mutation on non-target genes, including the antiapoptotic gene *Bcl6* (ref. 51); importantly, the occurrence of *Myc-Igh* translocations, a known side effect of aberrant AID activity, is 15-fold higher in the absence of miR-155 (ref. 52). Finally, miR-181 family members have also been reported to be differently modulated during CSR[51,53] and to have an effect on AID abundance[53]. However, the *in vivo* importance of miR181 family members in AID expression remains to be determined. As the 3′ untranslated region of AID mRNA contains several AU-rich elements (S.D.F., unpublished data), mRNA-binding proteins that recognize such elements might also be important in the regulation of AID expression by influencing either the stability or translation of AID mRNA.

Post-translational modification of AID

Post-translational modifications of proteins are commonly involved in the regulation of protein activity and also act to provide diversity to protein function[54]. Thus far, much of the focus on AID regulation by post-translational modification has been in the realm of phosphorylation, specifically phosphorylation of serine 38 (Ser38), which is thought to be carried out by the cAMP-dependent kinase PKA (protein kinase A)[55]. 'Knock-in' mice expressing AID with an S38A substitution have 70% less CSR and SHM[56,57]. Evidence suggests that the specific localization of PKA to the immunoglobulin locus results in the phosphorylation of Ser38 of AID at the switch regions during CSR, a modification shown to be essential for the recruitment of the AID cofactor RPA (replication protein A)[36]. The formation of the complex of PKA, phosphorylated AID and RPA at the immunoglobulin locus has a positive effect on CSR, as inactivation of PKA hinders CSR[34]. However, the Ser38-phosphorylation event has also been observed after expression of AID in unstimulated 3T3 fibroblasts and 293T cells[56,58], which raises the question of how specific this mode of regulation is for CSR and SHM in B cells.

Three additional phosphorylation sites have been documented on AID isolated from splenic B cells: Thr27, Thr140 and Tyr184. Thus far, the role of these phosphorylation sites is unclear. A T140A 'knock-in' mouse exhibits 30% less CSR and 50% less SHM[56], but the mechanism by which Thr140 phosphorylation promotes CSR and SHM is unknown. It is possible that the phosphorylation of different residues, or of a different combination of residues, plays a part in distinguishing between AID activity during SHM and CSR. This will prove to be an interesting focus for future pursuits.

The study of post-translational modification of AID has expanded to ubiquitination. Nuclear AID has been shown to be polyubiquitinated after treatment of cells with a proteasome inhibitor[59], which suggests that proteasomal degradation of nuclear AID limits its half-life in the nucleus. However, the pattern of ubiquitination observed in these studies is also consistent with multiple monoubiquitination events[59]. The description of a newly identified E3 ubiquitin ligase that can monoubiquitinate AID[60] is interesting in this context. It is possible that both monoubiquitination and polyubiquitination can occur, as is the case with the tumor suppressor p53 and its regulator MDM2 (ref. 61), and it will be interesting to elucidate the effect of ubiquitination on AID activity and cellular function. The interdependence of phosphorylation events themselves, as well as phosphorylation events in combination with other post-translational modifications, will probably prove to be insightful.

AID cellular localization

There is an increasing body of evidence that suggests that the subcellular localization of AID is tightly controlled to limit the amount of enzyme in the nucleus. AID, although mainly cytosolic, has been shown to shuttle between the nucleus and cytoplasm. The mechanism by which AID is exported from the nucleus is fairly well characterized; export is sensitive to the export receptor CRM1 inhibitor leptomycin B[62,63] and requires the final ten amino acids of AID (residues 188–198)[62–64]. Interestingly, substitution of the AID carboxy-terminal nuclear-export sequence also results in less CSR but maintains AID mutational activity[62,63], a

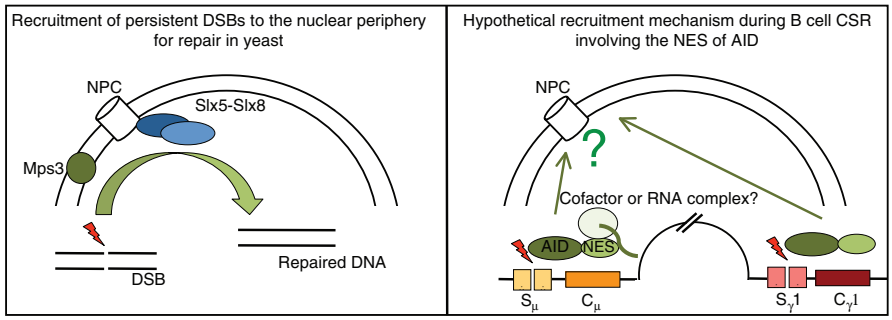

Figure 2 Specific localization to the nuclear periphery may be important for DSB repair. Left, persistent DSBs in *S. cerevisiae* are tethered to the nuclear pore complex (NPC) and are sequestered from the rest of the genome. This localization may require the integral inner nuclear membrane protein Mps3 and is dependent on the kinases Mec1 and Tel1 (ATM and ATR in humans). These interactions are thought to then shuttle the DSBs to a complex containing the pore protein Nup84 and the SUMO ligase Slx5-Slx8 (RNF4 in humans). In yeast, these interactions are thought to facilitate an alternative pathway of repair that enhances gene conversion through template-switch recombination. Right, evidence regarding the importance of the AID carboxy-terminal nuclear-export signal (NES) in CSR suggests that similar sequestration to the nuclear pore may occur and should be studied. It is possible that this is promoted by binding of a cofactor to AID or even by the formation of an RNA-containing complex. This model is completely speculative but emphasizes the importance of a foray into high-resolution microscopy to understand the nuclear localization of AID and the immunoglobulin locus. An understanding of both could provide insight into the mechanism of CSR and also provide an assay for the identification of other proteins or cellular factors necessary for CSR.

phenotype that can also be seen with common carboxy-terminal deletions found in human patients[65]. The potential function of the AID carboxyl terminus in CSR is discussed below.

Less is understood about how AID localizes to the nucleus for SHM and CSR. Ta *et al.* posited the existence of a weak nuclear-localization sequence encompassing the first 20 amino acids of AID[66]; however, others have observed that deletion of this sequence does not result in nuclear exclusion after expression of AID in 293T cells[64]. A subsequent report has suggested that nuclear import is active and is governed by a conformational nuclear-localization sequence dependent on the proper formation of multimers of AID[67], which emphasizes the need for more work focused on understanding the structure and formation of oligomers of AID.

Finally, it is important to note that all investigations into the localization of AID have used cell lines with ectopic expression of AID. It is formally possible that the localization patterns observed thus far are partly due to overexpression of the protein in a heterologous system. Visualization of physiological expression of AID in activated B cells might yield a different picture.

Targeting AID to the immunoglobulin locus

One of the most interesting unanswered questions in the field of AID regulation is the question of how AID-mediated mutation is targeted specifically to the immunoglobulin locus. Despite its ability to deaminate any transcribed substrate *in vitro*[27,28,32,68,69] and to act on selected highly transcribed genes when overexpressed[20,70,71], AID targets the immunoglobulin loci almost exclusively in its physiological context. Non-immunoglobulin gene-mutation events are found only after numerous rounds of mutation in B cells from Peyer's patches *in vivo*[72].

In addition to being targeted to immunoglobulin loci, AID shows a distinct local distribution in the immunoglobulin locus. During SHM, AID acts solely at the variable region (composed of the VDJ or VJ exon), but during CSR its activity is restricted to the switch regions. Although this local distribution of mutation at immunoglobulin loci has been studied for decades, mechanistic insight is still lacking. Below, we discuss the prevailing hypotheses and their supporting evidence.

Local positioning

Even before the discovery of AID, mutations that occur during SHM were found to have a distinct pattern, with a very strong 5′ boundary at the promoter and a less abrupt 3′ boundary about 1 kilobase downstream of the promoter[73]. Similarly, CSR-associated mutations start to appear just downstream of the sterile intron-region (I_μ) promoter, as well as the downstream switch-region promoters[11]. This suggested that the mutation is associated with the onset of transcription at the immunoglobulin locus. Confirming that theory, experiments have shown that promoter deletion results in less mutation[74]; movement of the promoter upstream of its original position results in an identical shift in the distribution of the mutations[75]; duplication of the promoter involving the placement of a copy 5′ of the constant region 'recruits' mutation to this part of the locus[17]; and insertion of exogenous DNA 3′ of the promoter and 5′ of the remaining gene acts as a buffer that protects the gene from mutation[76]. These observations led to the hypothesis that the mutating factor (AID) is loaded on the transcription-initiation complex, follows RNA polymerase II as elongation proceeds, and dissociates stochastically to create the decaying pattern of mutation observed[17,77]. In fact, AID is able to bind RNA polymerase II (ref. 35) and has been shown to be intimately associated with the transcription complex[29]. In addition, observations of B cells activated to undergo CSR *in vitro* have shown that the density of RNA polymerase II complexes correlates with the frequency of mutation in the surrounding switch regions[78].

Cis-acting targeting elements

Although the promoter is necessary for transcription and hence is required for SHM and CSR, the importance of distinct promoter elements for the recruitment of AID is not well understood. Early studies in mice suggested that any promoter is sufficient[74,79], but subsequent studies in the DT40 chicken B cell line have found that replacement of the endogenous *Igl* promoter with the promoter of the human gene encoding elongation factor 1-α supports high transcription but results in less *Igl* mutation[80]. Therefore, it is likely that the nature of the promoter region is important to provide both a platform for loading AID onto the transcription complex and binding sites for AID-targeting factors. In addition, the promoter may provide cell cycle specificity, which is known to be important—for reasons that are not yet clear—for CSR[81]. Little is known about the role of cell cycle in SHM.

The search for *cis*-acting regulatory sequences in and near the immunoglobulin locus began with the use of mouse 'passenger' transgenes and, later, knockout animals, with a focus on known transcriptional control elements[82–86]. The consensus that emerged from these studies was that the known transcriptional enhancers are not essential for the recruitment of AID but are necessary for CSR and SHM, as they provide a high rate of transcription and promote efficient long-range genomic interactions[87]. Subsequently, two labs have identified additional

locus-specific elements that seem to be both necessary and sufficient for AID recruitment. First, a systematic deletion study of the *Igl* locus in the DT40 chicken B cell line has identified a previously unknown regulatory element downstream of the known *Igl* enhancer (the 3′ regulatory region)[88]. The element contains a transcriptional enhancer, as its deletion abolished *Igl* expression; however, the addition of an SV40 enhancer was able to restore transcription but not SHM and GCV. Thus, it seems that this region also contains an element that 'recruits' AID-mediated mutation and whose function can be separated from a role in transcription. Similar observations were generated by the use of a constitutive promoter to overcome the effects of transcription in deletion of noncoding regions from the *Igl* locus in DT40 cells[89]. These experiments identified a targeting element identical to the 3′ regulatory region (the 'diversification activator' DIVAC), which was shown to promote SHM when integrated into a reporter transgene encoding green fluorescent protein independently of its genomic location.

Work still needs to be done to determine the minimal sequence responsible for the 'recruitment' of AID activity and the mechanism by which this recruitment works. For example, is AID directly recruited to this sequence or does it require another recruitment factor? How does the 3′ regulatory region act to support the loading of AID onto a promoter that is more than 6 kilobases upstream? Can this work in a chicken B cell line 'translate' into mammalian cells? As there is no clear sequence homology between the 3′ regulatory region and any mammalian immunoglobulin locus[88], it is possible that the structure of this region is more important than its nucleotide sequence.

AID targeting factors

It has been hypothesized that AID is targeted to the immunoglobulin locus by a 'targeting cofactor' that acts as the bridge between transcription and AID-mediated mutation[17]. This cofactor complex must therefore be able to distinguish between the highly transcribed immunoglobulin locus and other highly transcribed regions, probably by three-dimensional interactions with immunoglobulin-specific *cis*-acting targeting elements. Despite great efforts, no cofactor able to target AID to the immunoglobulin locus has been identified thus far. There is, however, evidence that the presence of the basic helix-loop-helix transcription factor E2A, or similar factors that recognize E-box motifs, is correlated with AID-mediated mutation. Mouse transgenes containing multiple consensus E-box motifs have more SHM[90], and non-immunoglobulin gene targets of AID frequently contain such motifs[72]. Similarly, inactivation of the gene encoding E2A in DT40 cells results in less SHM and GCV[91]. However, direct proof that E2A (or a complex containing E2A) recruits AID to immunoglobulin loci *in vivo* is lacking. Finally, it is important to remember that locus-specific elements may function not in recruiting AID itself but instead in targeting the immunoglobulin locus for error-prone repair[72]. Evidence suggests that AID induces widespread genomic breakage during CSR, and different repair mechanisms, rather than AID targeting, provide genomic specificity to the CSR reaction (unpublished data).

AID cofactors

Although not much is known about the factors that target AID to the locus, recent work has focused on a general search for AID-interacting proteins and their possible functions in SHM and CSR. The AID-interacting partner most studied, RPA, a ubiquitous general single-stranded DNA–binding protein, was originally thought to bind single-stranded DNA near SHM 'hot spots' in the transcription 'bubble', thereby facilitating the access of AID to double-stranded DNA[92,93]. On the basis of that hypothesis, RPA would be dispensable for CSR, as RNA-DNA hybrids (R loops) in the switch regions create a stable single-stranded DNA substrate for AID. Recently, however, experiments have shown that RPA is recruited to switch regions during CSR, which raises the question of what the function of RPA really is[36]. Because it has been observed that an AID mutant (S38A) unable to bind RPA is still recruited to switch regions during CSR, it does not seem that RPA promotes the binding of AID to DNA[36]. Thus, it is possible that RPA has a role in enhancing the activity of AID or is involved in events 'downstream' of the initial AID-mediated deamination event (such as the recruitment of repair factors). For example, in yeast, RPA accumulates at DSBs to recruit telomerase and form new telomeres when the necessary TG repeats are lacking[94]. It is also formally possible that the accumulation of RPA at the immunoglobulin locus facilitates relocalization of the immunoglobulin locus in the nucleus. At this point, we cannot rule out the possibility that RPA has different functions during SHM and CSR.

AID has also been shown to interact with the spliceosome-associated factor CTNNBL1 (ref. 95), which provided the first mechanistic connection between AID and the 10-year-old observation that splicing of the immunoglobulin transcript is necessary for CSR[96]. Disruption of the CTNNBL1-AID interaction by a specific mutation in *AID* results in less GCV in DT40 cells as well as less CSR in primary B cells stimulated *in vitro*. Furthermore, CTNNBL1-deficient DT40 cells show much less SHM and GCV; however, a specific role for CTNNBL1 *in vivo* awaits the generation of a B cell–specific knockout model. Emphasizing the role of splicing in AID-mediated reactions is recent work showing that depletion of the THO-TREX complex, in the context of AID expression in the yeast *Saccharomyces cerevisiae*, boosts mutation to near the rates in germinal center B cells[97]. THO-TREX functions at the interface among transcription, splicing and mRNA export in yeast[97] and vertebrate cells[98]. Overall, these experiments hint at a central role for the coincident processes of transcription, splicing and mRNA export in CSR.

AID function in the context of the nucleus

AID is generally thought to be targeted to the immunoglobulin locus, but the reverse is equally valid and potentially revealing. It is possible that the immunoglobulin locus is targeted to a mutational and/or recombinational 'factory' that includes AID, error-prone polymerases and other necessary enzymes. Reports indicate that nuclear architecture and subcompartment organization are important regulators of transcription[99]; moreover, observations in *S. cerevisiae* suggest that nuclear architecture is just as important for the repair of damaged DNA. Here, DSBs are sequestered to specific locations in the nuclear periphery and this movement is instrumental in preventing aberrant DNA repair.

In yeast, DSBs and telomeres localize to the nuclear periphery by a mechanism that is dependent on the inner nuclear membrane protein Mps3 (refs. 100–102). This mechanism confines 'hard-to-repair' breaks—which could potentially yield harmful translocations—to a restricted area, which allows alternative repair pathways to become active. The discovery of a Sumo-dependent ubiquitin ligase heterodimer of Slx5-Slx8 that localizes to nuclear pores and binds DSBs may help provide insight into the mechanism by which repair pathways are selected to repair these breaks[103] (**Fig. 2**). A 'hub' can be envisioned in the nuclear periphery where the telomere-proximal, broken *Igh* locus, the transcription, splicing and mRNA-export machinery, and AID in complex with its interacting partners may be temporarily sequestered. Alternative repair pathways active in such a hub could contribute to the error-prone repair of AID-created uracil residues, with error-free repair occurring at different sites in the nucleus. There are hints that the AID carboxy-terminal nuclear-export sequence may be the signal for this sequestration, as the respective mutants are unable to undergo

CSR. Furthermore, a heterologous nuclear-export sequence, although able to restore cellular trafficking of AID, is not sufficient to restore CSR[104]. Finally, elements of the locus (for example, the 3′ enhancers) are probably important for nuclear positioning and proper repair during CSR. In bacterial artificial chromosome–transgenic B cells, CSR occurs almost exclusively at the transgenic locus as long as the enhancers are present; however, in the absence of these elements, repair is distinctly aberrant[105]. At present this is a completely speculative model. However, high-resolution, high-sensitivity microscopy studies of the subnuclear, three-dimensional localization of AID and key regions of the immunoglobulin locus should help provide insight into the mechanisms that facilitate targeted mutation and recombination.

Conclusions

It has been a decade since the discovery of AID and already the field has made great strides toward understanding its role in secondary antibody diversification. However, as in all young fields, there are pieces missing from the puzzle. Which signaling pathways are involved in controlling AID expression, and how these signals are integrated by the *AID* promoter and enhancers, are still not understood. Exposure of the layer of post-transcriptional regulation of AID is only just beginning, and better understanding of the influence of post-translational modification on AID function is needed. Finally, additional work is needed to understand the influence of dynamic relocalization of AID in the cytoplasm and nucleus. Over the next decade, progress should be made in all of these areas to further the understanding of the role of AID in antibody diversification.

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