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Restricting activation-induced cytidine deaminase tumorigenic activity in B lymphocytes

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Summary

DNA breaks play an essential role in germinal centre B cells as intermediates to immunoglobulin class switching, a recombination process initiated by activation-induced cytidine deaminase (AID). Immunoglobulin gene hypermutation is likewise catalysed by AID but is believed to occur via single-strand DNA breaks. When improperly repaired, AID-mediated lesions can promote chromosomal translocations (CTs) that juxtapose the immunoglobulin loci to heterologous genomic sites, including oncogenes. Two of the most studied translocations are the t(8;14) and T(12;15), which deregulate cMyc in human Burkitt's lymphomas and mouse plasmacytomas, respectively. While a complete understanding of the aetiology of such translocations is lacking, recent studies using diverse mouse models have shed light on two important issues: (1) the extent to which non-specific or AID-mediated DNA lesions promote CTs, and (2) the safeguard mechanisms that B cells employ to prevent AID tumorigenic activity. Here we review these advances and discuss the usage of pristane-induced mouse plasmacytomas as a tool to investigate the origin of Igh–cMyc translocations and B-cell tumorigenesis.

Keywords: activation-induced cytidine deaminase; chromosomal translocations; class switch recombination; somatic hypermutation; tumorigenesis

Activation-induced cytidine deaminase, a multitask enzyme

Upon antigen encounter, B lymphocytes migrate to primary follicles within peripheral lymphoid organs where they proliferate extensively and establish the germinal centre reaction. Within this specialized microenvironment, activated B cells undergo two unique genetic processes: somatic hypermutation (SHM) and class switch recombination (CSR). Somatic hypermutation is defined as the random introduction of nucleotide substitutions at the variable domains of heavy and light chain genes at a rate of 10^{-3}–10^{-4} per base pair per generation.[1] In conjunction with antigen-mediated selection, SHM promotes affinity maturation of antibody receptors during the humoral immune response. Class switching, on the other hand, replaces the heavy chain constant domain $C\mu$ for one of a set of downstream C_H exons ($C\gamma$, $C\varepsilon$ or $C\alpha$).[2] The different C domains or isotypes impart antibodies with distinct physiological effector functions.

Despite expected mechanistic differences between SHM and CSR, both processes are initiated by activation-induced cytidine deiminase (AID). This B-cell-specific enzyme was first identified by the Durandy and Honjo laboratories as being essential for SHM and CSR both in humans and mice.[3,4] In hens as well, AID promotes immunoglobulin gene conversion,[5,6] and an AID homologue has also been recently implicated in antigen receptor diversification in hagfish.[7]

Precisely how AID mediates such an array of immunoglobulin gene diversification reactions is only beginning to be unravelled, but a large body of evidence clearly demonstrates that upon recruitment to the immunoglobulin loci AID deaminates cytidines into uracils.[8–13] This targeted deamination occurs only in the context of single-strand (ss) DNA exposed by the RNA polII holoenzyme during transcription and stabilized by replication protein A (RPA).[14] However, even within ssDNA, not all cytidines are good deamination substrates, as AID preferentially targets WRCY motifs (where W = A or T, R = A or G, and Y = C or T),[12,15] an intrinsic hotspot sequence that is also evolutionarily conserved from bony fishes to humans. Based on protein sequence conservation between AID and the RNA editing enzyme APOBEC1, AID has

also been proposed to deaminate RNA,[16] although a direct messenger RNA (mRNA) deamination target has not yet been identified.

Somatic hypermutation

Because uracils in genomic DNA can be mutagenic, eukaryotic cells have, not surprisingly, developed several base excision repair (BER) deglycosylases to remove them. During hypermutation, uracils are primarily recognized and removed by uracil-N-glycosylase 1 (UNG), resulting in DNA abasic sites at heavy and light chain V genes.[17] Like spontaneous dC deamination, a significant fraction of AID-mediated deamination events is faithfully reverted to cytidines by BER (Fig. 1). The extent of such error-free repair activity becomes evident in mouse and human B cells that are defective in BER, which accumulate large numbers of C to T (or G to A) transition mutations at immunoglobulin genes as a result of DNA replication of unrepaired uracils.[18,19] In the presence of UNG, however, abasic sites in cycling cells stall the DNA replication fork and engage the activity of REV1 (REV1 in higher organisms is a deoxycytidyl transferase homologue of the *Saccharomyces cerevisiae* REV1 gene) and possibly other translesion synthesis (TLS) DNA polymerases.[20,21] Insertion of dNTPs opposite the non-instructional abasic site results both in transition and transversion mutations at the immunoglobulin loci. Consequently, AID-mediated deamination is either faithfully repaired in G1 or becomes mutagenic if UNG recognizes the uracil at the replication
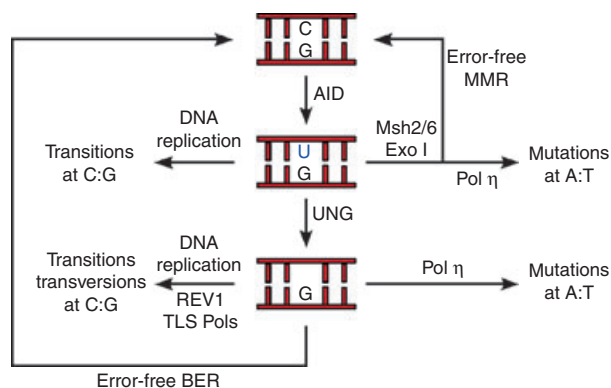

Figure 1. Current model of somatic hypermutation (based on ref. 123). Activation-induced cytidine deaminase (AID)-mediated deamination of cytidines at immunoglobulin genes results in U : G mispairs. DNA replication over uracils will promote transition mutations (C/G to T/A). Uracil can also be repaired in an error-free fashion by either base excision repair (BER) or mismatch repair (MMR). If uracils are recognized by uracil-N-glycosylase 1 (UNG) in the context of DNA replication however, abasic sites would be repaired by REV1 and other translesion polymerases leading to both transition and transversions at C : Gs. Following replication, U : G mismatches or abasic sites would engage the activity of polymerase η (pol η) resulting in mutations primarily at A : T pairs.

fork in the S phase (see refs 22, 23 and Fig. 1). Although this model remains to be fully proven, it predicts that the extent of SHM would be directly proportional to the rate of B-cell division, i.e. how frequently the replication fork encounters unrepaired uracils at the immunoglobulin loci. Considering the extraordinary turnover of germinal centre B lymphocytes, the model would explain how mutations are favoured over error-free repair during the immune response.

Uracils are not only foreign to DNA, but also create U : G mismatches, which are conventionally recognized and removed by the mismatch repair (MMR) pathway. Intriguingly, gene targeting of the MMR factors Msh2, Msh6 or Exo1 results in significant alterations in the mutation spectrum of immunoglobulin genes, indicating that MMR proteins are also directly involved in SHM.[18,24,25] The MMR acts primarily in a post-replicative fashion, correcting errors of nucleotide misincorporation downstream of DNA polymerase α, β or ε.[26] Like BER, MMR is a high-fidelity repair process and so it might appear counterintuitive that it potentiates DNA hypermutation. This conundrum was explained by studies demonstrating that MMR proteins recruit the error-prone translesion DNA polymerase η to the hypermutating immunoglobulin loci.[27,28] This low-fidelity polymerase introduces a significant number of misincorporation errors during patch DNA synthesis, particularly at A/T pairs.[29] Why DNA mismatches other than those induced by AID fail to recruit polymerase η is unclear. In short, SHM can be viewed as the result of DNA replication of unrepaired uracils, REV1/TLS polymerase mutagenic activity at C/G pairs, and nucleotide misincorporation at A/T pairs by polymerase η before or after UNG activity (Fig. 1).

Class switch recombination

Class switch recombination is targeted to specific S recombination regions by 5′ (I) promoters that mediate germline or sterile S-C_H transcription.[30] The 5′ and 3′ heavy chain enhancers appear to promote S-S synapses,[31] which are critical to target recombination from Sμ to the appropriate downstream S domains and to impart accessibility to AID (Fig. 2). In contrast to SHM, which occurs via ssDNA lesions, CSR relies on DNA double-strand break (DSB) intermediates, which presupposes AID activity on both template and non-template DNA strands. At least two unique features of S domains may explain how DNA breaks are generated during CSR. First, S regions are characterized by a G-rich non-template strand, containing large numbers of repetitive, palindromic RGWY motifs.[32] During transcription, these palindromes are displaced as R-DNA loops by the nascent germline transcripts that stably hybridize to the template strand.[33–35] *In vitro* experiments suggest that the AID/RPA complex
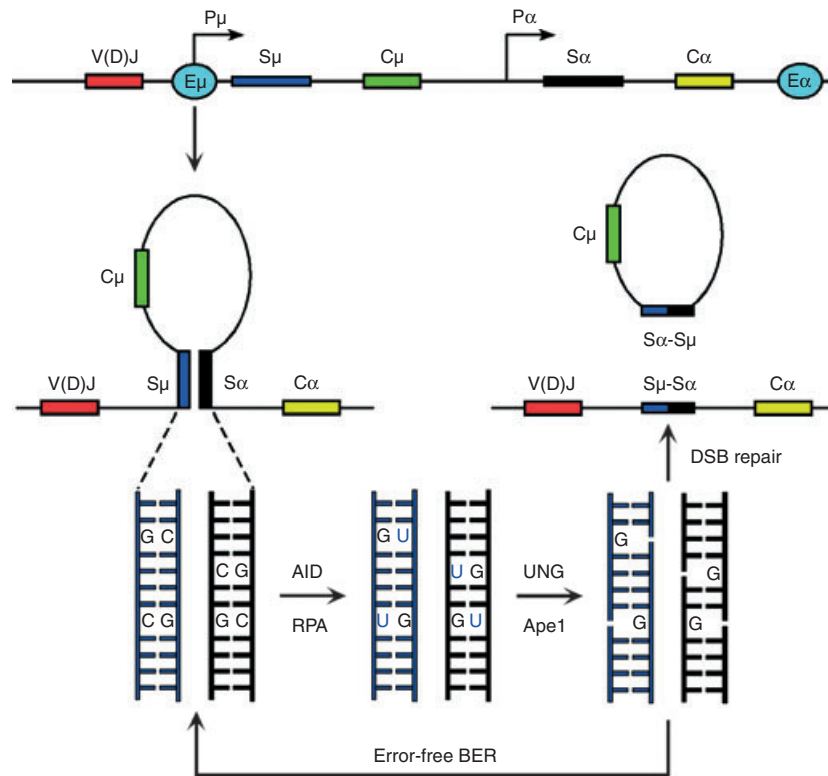
Figure 2. Schematics representing class switch recombination from Sμ to Sα. Recombining switch domains are rendered accessible to activation-induced cytidine deiminase (AID) and replication protein A (RPA) by the concerted action of I-promoters and the μ (5′) and α (3′) enhancers (E). Removal of uracils by uracil-*N*-glycosylase 1 (UNG) and nicking of the DNA backbone by Ape nucleases results in the formation of staggered DNA breaks, which are either repaired in an error-free manner by base excision repair (BER) or serve as substrates for double-strand break (DSB) repair and/or recombination. Although not depicted, mismatch repair activity induces class switch recombination via a similar mechanism.

can directly act on the displaced ssDNA.[11,12,36] Antisense or bidirectional transcription of immunoglobulin genes might explain, on the other hand, how the template strand becomes accessible to deamination.[37,38] The current CSR model proposes that in RGYW-packed S domains, DNA nicks on both DNA strands result in staggered DNA breaks intermediate to recombination (Fig. 2).

Like other DNA DSBs occurring during pre-replicative phases of the cell cycle, AID-mediated breaks engage the non-homologous end-joining repair pathway (NHEJ). The first molecules recognizing DNA DSBs within this pathway are members of the Mre11, Rad50 and Nbs1 (MRN) complex.[39] These molecules recruit and activate the serine/threonine kinase ATM, which in turn phosphorylates numerous repair factors such as MDC1, 53BP1, Nbs1 and H2AX, leading to arrest in cell cycle progression and repair.[40] The importance of this pathway in mediating efficient CSR is evident in ATM−/−, 53BP1−/− or H2AX−/− B cells, where AID-induced breaks at S domains persist unrepaired throughout the cell cycle.[41–43]

In addition to ATM, another member of phosphatidyl-inositol 3-kinase family of proteins, DNA-PKcs, is involved in CSR. The DNA-PKcs acts as the catalytic sub-unit of the DNA–PK complex, composed of the Ku70/Ku80 heterodimer, Artemis, XRCC4, Ligase IV and Cernunnos. In the context of switching, Ku proteins appear to be absolutely required for repair of CSR DNA ends,[44,45] although these experiments were complicated by the proliferation defects of Ku-deficient cells. Yet, DNA-PKcs−/− B cells or cells homozygous for a severe combined immunodeficiency (*scid*) mutation are severely impaired in CSR, with the exception of switching to immunoglobulin G1 (IgG1), which occurs at low but reproducible levels.[46] This residual activity could be ascribed to functional redundancy between DNA-PKcs and other phosphatidylinositol 3-kinases like ATM. Alternatively or in addition, a different repair pathway other than NHEJ could mediate CSR, or at least function as a backup system when NHEJ is crippled. In keeping with this notion, XRCC4 and Ligase IV conditional knock-out B cells retain the capacity to undergo switching to nearly 50% of wild-type levels.[47,48] This underlying backup repair pathway appears to rely on microhomology judging from analyses of the recombination sites at S domains of XRCC4−/− and Ligase IV−/− B cells.[47–49] In conclusion, a

very complex set of repair mechanisms promote and resolve DNA DSBs downstream of AID. It should be pointed out, however, that some of the pathways driving somatic hypermutation also target switch domains during CSR (see for instance ref. 50). How this plethora of enzymatic activities is regulated to ensure mostly hypermutation at V genes and recombination at C genes is under intense investigation.

Chromosomal translocations

In spite of the mechanisms outlined above, DNA lesions intermediate to CSR are occasionally misrepaired, leading to chromosomal single-stranded breaks, DSBs, translocations, deletions and aneuploidy. A well-studied case involves the t(8;14)(q24;q32) chromosomal translocations (CTs) that juxtapose immunoglobulin heavy chain (Igh) to the proto-oncogene cMyc in human Burkitt's lymphomas.[51,52] Reciprocal translocations between these two loci are also pathogenic in mouse plasmacytomas [T(12;15)] and rat immunocytomas [t(6;7)] (reviewed in ref. 53). In all cases, cell transformation is achieved by cMyc transcriptional deregulation at the hands of Eμ and/or Eα immunoglobulin enhancers. That tumours in different mammalian species deregulate cMyc in a similar fashion suggests a common underlying mechanism at the origin of Igh–cMyc translocations.

Peritoneal plasmacytomas (PCTs) in mice can be induced by injection of non-metabolizable materials such as pristane oil. This agent promotes the formation of chronic inflammatory oil granulomas (OGs) rich in interleukin-6 (IL-6) and other cytokines essential for plasma cell differentiation and transformation.[54–56] Plasmacytomagenesis is highly dependent not only on the OG microenvironment but also on the genetic makeup of BALB/cAnPt (B/c) mice. Following three injections of 0·5 ml pristane given at days 0, 60 and 120, $\sim$ 40–60% of B/c mice develop PCTs by day 300.[53] In contrast, most other inbred strains, such as DBA/2, C57BL/6 or 129, are resistant to PCT induction. Development of PCT also requires a conventional environment as tumour incidence in B/c mice raised under specific pathogen-free conditions is in the order of 5%.[57]

In addition to the pristane-treated B/c model, PCTs carrying T(12;15)s spontaneously develop in H-2L^D-IL-6 transgenic mice.[58] Overexpression of IL-6 in these animals initially results in massive hyperplasia of terminally differentiated, non-neoplastic plasma cells involving almost all lymph nodes.[58] This stage is followed by the appearance of atypical plasmablast-like cells in the medullary cords of mesenteric lymph nodes. By 300 days of age, $\sim$60% of IL-6 transgenic mice ultimately develop PCTs.

Both pristane-induced and IL-6-induced tumour incidence can be increased and their latency can be reduced by overexpression of Bcl-2 or Bcl-xL (refs 59–61 and

M.P. unpublished observations). These anti-apoptotic molecules are believed to salvage B cells carrying cMyc translocations from programmed cell death. Consequently, between 70% and 95% of Bcl-2 or Bcl-xL transgenic mice develop a PCT within 100 days. These models lend themselves to establishing chronological and anatomical relationships of CT formation and PCT development. An additional advantage of Bcl-2 and Bcl-xL transgenes is that PCT induction can be carried out in some genetically resistant strains and hybrids.

In conclusion, whether plasmacytomas arise spontaneously in IL-6 transgenic mice or are induced by pristane injections in the B/c background, they critically rely on the IL-6 cytokine and are dramatically accelerated by anti-apoptotic signals.

Detection and classification of Igh–cMyc chromosomal translocations

Highly sensitive polymerase chain reaction (PCR) methodologies have been developed for detecting CTs in different tumours and tissues. In early publications, Igh–cMyc junctions were amplified by a combination of degenerate primers and two rounds of nested PCR.[62,63] More recently, a long-distance PCR protocol that uses highly specific primers has been the system of choice.[61,64–66] In general, long-distance PCR generates products of considerably great fidelity but is not as sensitive as the consensus or degenerate method. On the other hand, when pushed to the limits of sensitivity, the degenerate method has the potential of yielding artefacts.[67] These probably result from accidental polymerase 'jumping' between templates,[68] or switch region insertion at cMyc.[69] There are several important considerations that must be addressed when using nested PCR methodologies, such as the stringency of the annealing process (65°); appropriate, relatively low amounts of Taq polymerase (1·25 U/50 μl reaction volume); confirmatory re-isolation of the same fragment from the starting DNA; and hybridization of derivative 12 translocations with IgC region probes.

Illegitimate rearrangements between the immunoglobulin loci and cellular oncogenes were first isolated from a few human B-cell malignancies and mouse plasmacytomas.[70–74] Early molecular characterization of translocation breakpoints consistently revealed the involvement of immunoglobulin switch regions, giving credence to the notion that such chromosomal abnormalities were the result of aberrant switch recombination.[75–83] While mostly correct, this hypothesis has been further refined by characterization and classification of a large number of CT breakpoints isolated from mouse plasmacytomas over several years. Based on a study of 103 IL-6 transgenic and 86 pristane-induced PCTs, we classify CTs into three categories (Fig. 3). First, a significant proportion of plasma-
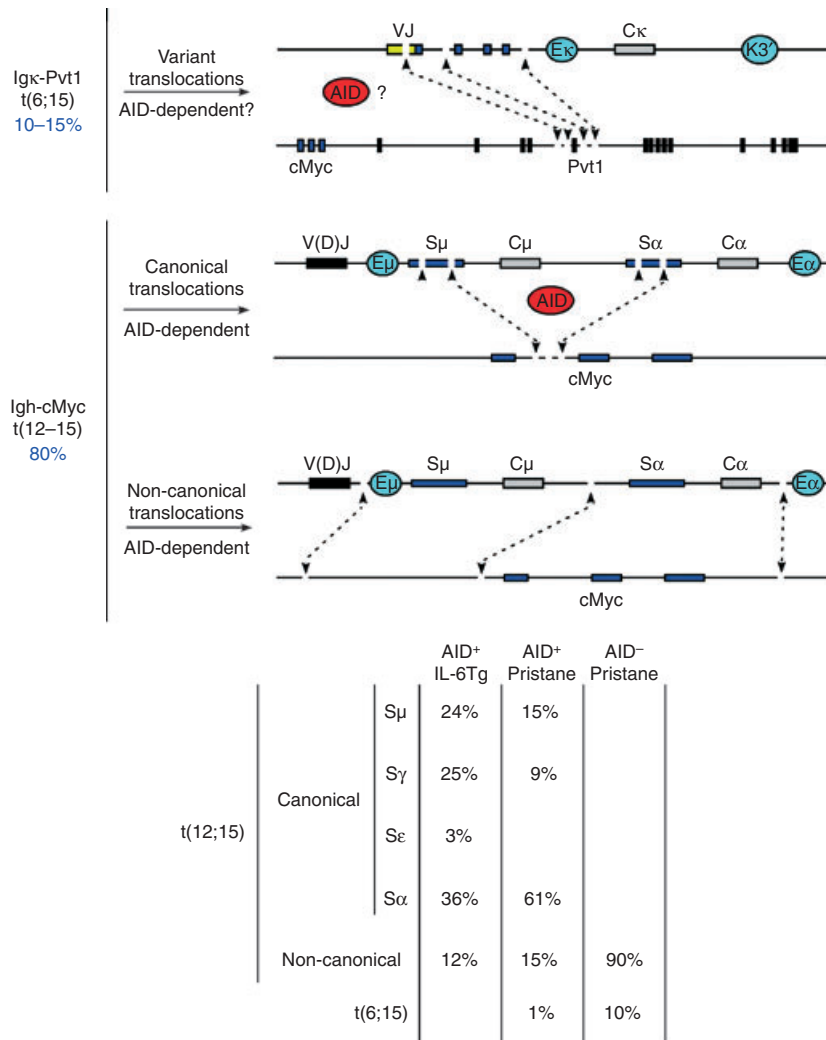
Figure 3. Classification of chromosomal translocations isolated from mouse plasmacytomas. Upper schematic exemplifies variant translocations [t(6;15)] that juxtapose VJk domains to *Pvt1* exon 5. Middle schematic shows activation-induced cytidine deaminase (AID)-mediated deamination during class switch recombination results in the formation of DNA double-strand breaks at S domains and less frequently at cMyc intron 1. Aberrant repair of these lesions can lead to the juxtaposition of cMyc to the immunoglobulin loci. Lower schematic: although at a significantly lesser extent, random, AID-independent DNA breaks can also translocate the cMyc gene to the immunoglobulin loci leading to T(12;15). Translocation breakpoints in these non-canonical translocations often map several kilobases away from S domains or the cMyc gene itself. Right table indicates the percentage of CTs involving diverse S domains in AID$^{+/+}$ interleukin-6 transgenic mice, and pristane-treated AID$^{+/+}$ and AID$^{-/-}$ B/c mice.

cytomas (∼10–15%) deregulate cMyc by means of variant translocations,[84] which juxtapose the Igk locus and the *Pvt1* gene positioned downstream of cMyc in mouse chromosome 15. *Pvt1* mRNA, which does not appear to encode a protein but a cluster of microRNAs,[85] is also translocated to light chain genes in Burkitt's lymphomas.[86] As most variant translocation breakpoints fall within or near *Vκ-Jκ* genes,[84] aberrant repair of hypermutation lesions is expected to play an aetiological role to t(6;15). On the other hand, it is unclear whether *Pvt1* breaks, which fall mostly near exon 5, are AID-mediated. It is interesting to note that in T-cell lymphomas, *Pvt1* exon 5 is a preferred provirus integration target,[87] implying that this genomic site is highly accessible in lymphocytes.

A second group of CTs in mouse plasmacytomas juxtapose the Igh allele to cMyc (∼80%, Fig. 3). Based on breakpoint location, these CTs can be further subclassified into: (1) canonical, when translocations precisely join S domains and cMyc intron 1, and (2) non-canonical, when CT breakpoints are randomly distributed on both alleles (Fig. 3). Canonical translocations clearly result from AID deamination activity both at cMyc and S domains.[88] In IL-6 transgenic or B/c mice treated with pristane, canonical translocations can be isolated from nearly 90% of all plasmacytomas.[61] The preferred cMyc translocation part-

ner in both tumour models is Sα (Fig. 3), suggesting that tumour precursors probably originate in gut lymphoid tissues, where IgA switching is prevalent. Some canonical translocations are complex or atypical in that in addition to S regions, they display unusual inversions or insertions of DNA from other chromosomes into cMyc (see ref. 70 for examples).

Non-canonical translocations, which randomly recombine the Igh and cMyc alleles at sites other than S domains or cMyc intron 1 comprise 10–15% of all T(12;15)s. A role for AID in the origin of these translocations was excluded based on recent studies.[61,65,89] Using AID$^{-/-}$ IL-6 transgenic mice raised under specific pathogen-free conditions, Ramiro et al. failed to amplify T(12;15)s from hyperplastic lymphoid tissues.[65] In B/c pristane-treated mice on the other hand, Igh–cMyc junctions were detected at early time-points using degenerate primers and low stringency annealing temperatures (60°), but were absent from Peyer's patches and gut.[89] A third study investigated both the development of CTs and plasma cell tumours in conventionally raised AID$^{-/-}$BclxL B/c mice.[61] Despite the loss of knock-out mice during the experiment to a wasting syndrome (of possible viral origin) and the slow progression of PCTs, T(12;15)s were nonetheless detected by fluorescence in situ hybridization in the absence of AID. Of the nine examples isolated, there were six T(12;15), one t(6;15) and one chr12 inversion that resulted in the juxtaposition of Igh–nMyc.[61] Interestingly, none of the T(12;15) were mapped to S domains or cMyc intron 1, revealing their non-canonical origin. Non-canonical CTs have also been recently isolated from IL-6 transgenic mice (M.C. Nussenzweig, personal communication).

In summary, these studies demonstrate that whereas AID is the major catalyst for generating Igh–cMyc translocations, other cellular mechanisms can promote illegitimate recombination of these alleles in activated, cycling B lymphocytes.

Regulating AID physiological and pathological activity

Mounting evidence demonstrates that B lymphocytes minimize the potential tumour-inducing ability of AID through a variety of mechanisms. First, expression of the AID gene is largely restricted to germinal centre or T-cell-independent activated B lymphocytes.[90,91] This spatiotemporal regulation is fine-tuned by the counterbalanced activities of transcriptional activators and repressors such as nuclear factor-κB, Pax5, E47, Blimp1, Id2, Id3, Irf8 and HoxC4,[92–97] which are recruited to the AID gene (Aicda) basal promoter or intronic enhancer. In B cells undergoing CSR, chromatin at these two genomic sites is extensively remodelled by acetylation and methylation (Fig. 4 and ref. 91). Chromatin remodelling activity is further recruited to

conserved-non-coding-sequence X (CNSX, Fig. 4). This phylogenetically conserved element is required for AID-gene expression under physiological conditions,[91] but does not appear to function as a conventional enhancer based on its inability to induce luciferase transcription in reporter assays (our unpublished observations). There is also evidence of a fourth conserved element 5′ of AID (CNSVIII), which is H3K4 methylated, H4 acetylated, but surprisingly lacks H3 acetylation (Fig. 4 and ref. 91). Deciphering how all these elements confine AID transcription to germinal centre B cells awaits further biochemical and genetic characterization.

A link between AID transcription levels and the extent of AID pathological activity was recently uncovered by studies using AID heterozygous mice, which display reduced AID mRNA and protein compared with wild-type littermates.[66,98,99] In the context of isotype switching, AID$^{+/-}$ lymphocytes show impaired inter- and intra-switch recombination and a substantial decrease in the frequency of S mutations and chromosomal breaks. Similarly, during the humoral immune response AID$^{+/-}$ mice display increased numbers of germinal centre cells,[99] but fewer B-cell clones with high-affinity antibodies.[66] On the autoimmune MRL/lpr background, these defects result in lower levels of specific anti-dsDNA antibodies in the serum, with a concomitant delay in kidney damage relative to controls.[98] Haploinsufficiency for AID is also evident by the reduced ability of AID$^{+/-}$ B cells to undergo intrachromosomal Igh–cMyc translocations and ultimately B-cell tumour development.[66] It is important to note that the extent of off-target translocations depends on AID expression levels to a much larger extent than do on-target hypermutation or CSR. This feature, which holds true both under conditions of AID overexpression[43,88] and underexpression,[66] suggests that AID enzymatic activity is constrained to varying degrees depending on the genomic location[100] and/or cell type.[101]

AID transcript amounts, and consequently AID-mediated deamination, are also indirectly regulated by at least two microRNAs. First, the lymphocyte-specific miR-155 decreases the half-life of AID mRNA by associating to its 3′ untranslated region.[102,103] This regulation is physiologically relevant as demonstrated in gene targeted (Aicda^{155}) or BAC AID^{GFP} transgenic mice lacking an AID miR-155 binding site, which displays enhanced CSR and SHM relative to controls.[102,103] Likewise, Igh–cMyc translocations are significantly upregulated in miR-155$^{-/-}$ or Aicda^{155} B cells.[102] The microRNA miR-181b has also been shown to associate with the AID 3′ untranslated region.[104] Accordingly, overexpression of miR-181b in lipopolysaccharide + IL-4-stimulated B cells suppresses AID and CSR levels.[104] Conditional inactivation of miR-181b in the B-cell compartment might help to determine whether it also influences the incidence of Igh–cMyc translocations and lymphomagenesis.
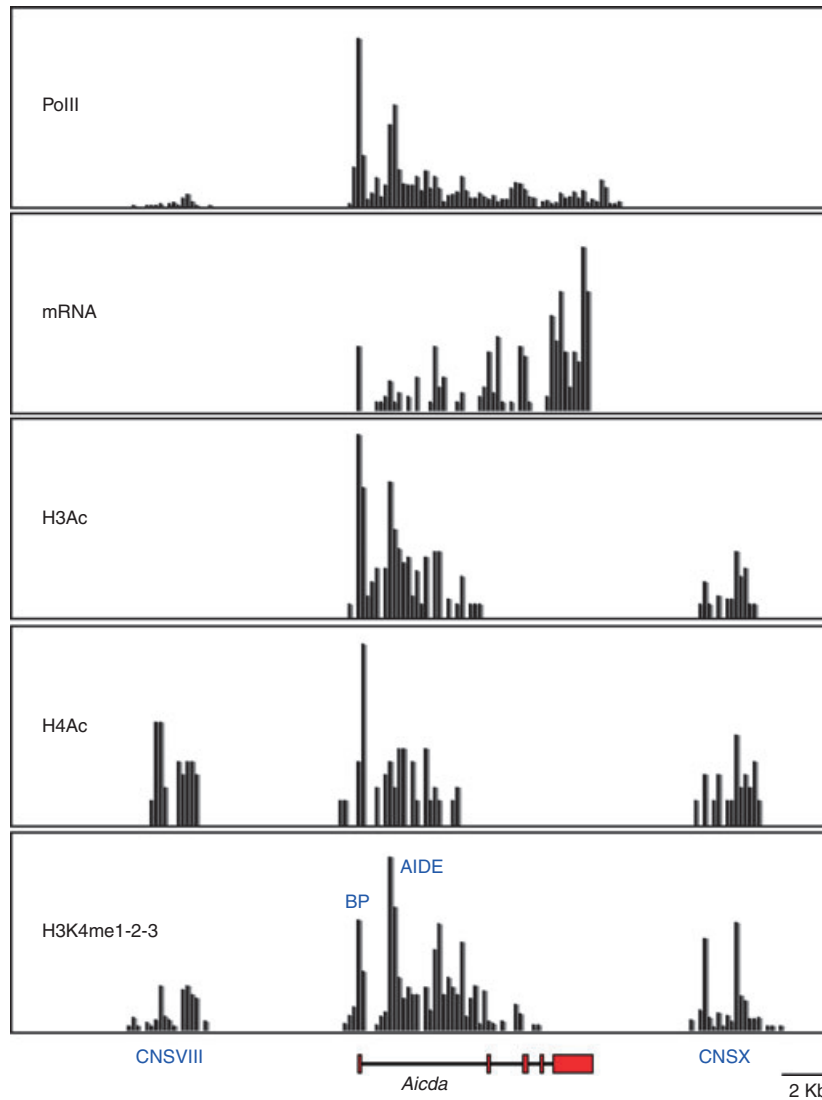
Figure 4. Partial epigenetic map of the mouse *Aicda* locus (35, 400 bps) in lipopolysaccharide + interleukin-4 activated B cells depicting polymerase II binding, messenger RNA (mRNA) production, histone H3 and H4 acetylation, and histone H3 lysine 4 mono-, di-, or tri-methylation (H3K4me1-2-3). Phylogenetically conserved elements CNSVIII, activation-induced cytidine deiminase (AID) basal promoter (BP), AID intronic enhancer (AIDE), and CNSX are also highlighted. Mapping (200 base pair resolution) was produced by Solexa DNA microsequencing of immunoprecipitated chromatin (PolII, H3Ac, H4Ac, H3K4me1-2-3) or polyA[+] isolated mRNA (Yamane and Casellas, manuscript in preparation).

One puzzling feature of AID is that although it directly deaminates immunoglobulin DNA, its protein is mostly confined to the cytoplasm (refs 105,106 and Fig. 5). AID nuclear exclusion appears to be achieved by a combination of selective nuclear degradation and a CRM1-dependent export mechanism.[101,107–109] Consistent with results obtained with cells overexpressing AID, increasing AID nuclear concentration enhances off-target hypermutation but has little effect on the overall mutation rate of immunoglobulin genes.[101] Based on this observation it is tempting to speculate that the counterintuitive AID compartmentalization might have evolved primarily to minimize AID pathological activity during the immune response.

Post-translational phosphorylation constitutes another level of AID regulation. Mass spectrometry and biochemical analyses have identified at least two phosphorylation target sites in AID: serine 38 and threonine 140.[99,110–113] Phosphorylation of S38, carried out either by protein kinase A and/or protein kinase C,[99,111] is thought to facilitate AID interaction with RPA, which imparts accessibility to transcribed DNA.[14] Mice carrying an S38A mutation show 20–35% of wild-type CSR and SHM levels.[99] The origin of T140 phosphorylation is less clear, but T140A mutant mice show preferential defects in hypermutation levels.[99] Considering the effect of AID expression levels on AID physiological and pathological activity, it is reasonable to expect that AID hypo- or
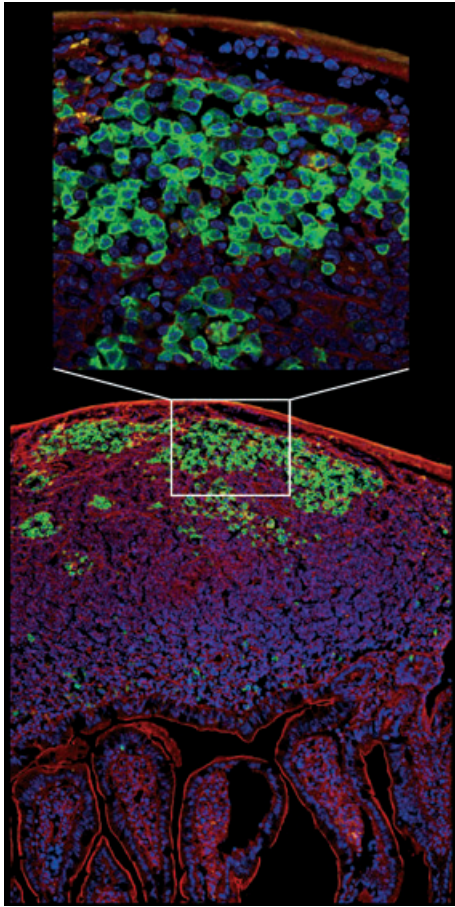
Figure 5. Micrograph showing a longitudinal section of intestinal villi and Peyer's patches from the jejunum of activation-induced cytidine deiminase-green fluorescent protein (AID-GFP) mice. A close-up view of the germinal centre reveals AID localization is mostly cytoplasmic. Cells were stained with DAPI (blue) and F-actin was labelled with phalloidin (red). The image was prepared by Susan Lim and collected by Kristien Zaal using a Leica SP5 confocal microscope.

hyperphosphorylation would have a more dramatic impact in the incidence of Igh breaks and chromosomal translocations. In this regard, it has been recently shown that SHM species lacking CSR (teleost) harbour a 'constitutively phosphorylated' form of AID, while those capable of inducing CSR (tetrapods) require S38 phosphorylation to fully activate AID.[114] This post-translational control mechanism might thus have evolved to minimize AID-mediated lesions and translocations.[114]

One of the most remarkable aspects of SHM and CSR is that they rely on DNA repair factors from the BER and MMR pathways that commonly correct DNA deamination. To explain this apparent contradiction it has been hypothesized that AID simply overwhelms error-free repair or subverts it into an error-prone mechanism in B lymphocytes. As discussed previously, the engagement of low-fidelity DNA polymerases during hyper-

mutation and DSB repair enzymes during CSR supports the latter scenario. On the other hand, there is clear evidence that AID overcomes UNG/Msh2 error-free repair at non-immunoglobulin genes.[100] This is also the case at the immunoglobulin loci because absence of UNG and/or Msh2 significantly increases the mutation frequency at S domains during isotype switching.[18,115] Based on analyses of polymerase $\beta^{-/-}$ B cells activated under suboptimal conditions it has also been proposed that DNA lesions intermediate to CSR (and by extrapolation to chromosomal translocations) are decreased by error-free BER.[116] The underlying assumption is that faithful correction of dC deamination at S domains by polymerase β, and possibly translesion synthesis polymerases normally prevent DNA break formation. At the same time, it should be remembered that staggered DNA breaks at switch regions would also result from canonical, high-fidelity repair on both DNA strands (Fig. 2). A role for high-fidelity repair in DNA break formation is clear in AID$^{+/-}$ mice, which display CSR and chromosomal translocations at $\sim$ 50% of wild-type levels despite the fact that virtually all S mutations are repaired in an error-free fashion.[66] Furthermore, cMyc and other oncogenes are frequently translocated to the immunoglobulin loci in an unmutated state.[88,100,117,118] These observations have profound implications for B-cell tumorigenesis because they raise the intriguing possibility that while protecting the B-cell genome from mistargeted hypermutation, DNA repair pathways recruited downstream of AID might in fact promote chromosomal translocations.

Concluding remarks

We have summarized various cellular mechanisms that help restrain AID deamination activity (Fig. 6). How efficiently these processes prevent tumorigenesis in the B-cell compartment is clear from studies with transgenic mice expressing AID ectopically.[119] Surprisingly, these animals do not display significant abnormalities in B cells but instead develop spontaneous T-cell lymphomas carrying extensive hypermutation at T-cell receptor, cMyc, Pim1 and other genes. In addition, the mice often develop multiple lung microadenomas and less frequently melanomas, hepatocellular carcinomas, and sarcomas.[120] It is tempting to speculate that some of the mechanisms outlined above might be B-cell-specific, and therefore unable to protect other somatic cells from tumorigenesis when AID is aberrantly expressed. In this context, there is evidence that signalling pathways, including nuclear factor-κB, can upregulate AID in non-B cells leading to neoplastic progression.[121] In the B-cell compartment, transforming viruses can also sustain AID expression for long periods of time,[122] a feature that could promote malignancy in susceptible individuals. It will be interest-
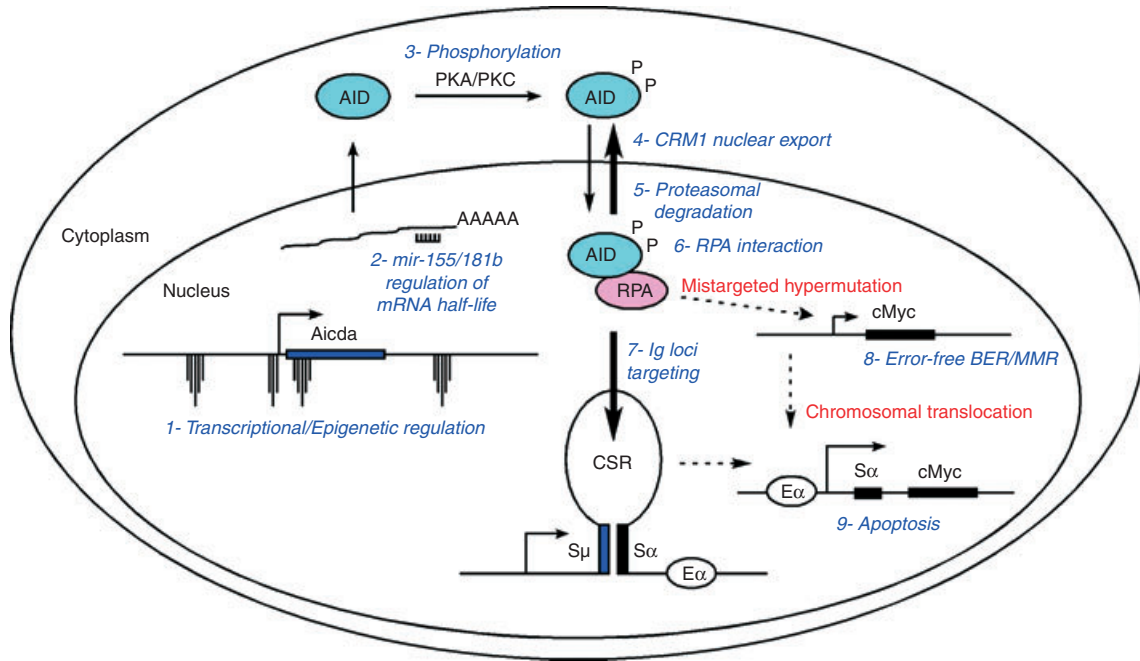
Figure 6. Summary of activation-induced cytidine deiminase (AID) regulatory mechanisms: 1- Spatiotemporal targeting of AID gene transcription to activated and germinal centre B cells. This is achieved by the concerted action of transcription factors and the *Aicda* epigenetic landscape. 2- microRNA regulation of AID messenger RNA half-life. 3- Protein kinase A (PKA) and/or protein kinase C (PKC) -mediated AID phosphorylation. 4- AID nuclear export mediated by CRM1. 5- Nuclear proteasomal degradation. 5- AID interaction with single-stranded (ss) DNA binding protein replication protein A (RPA), which allows AID to deaminate transcribed ssDNA. 7- Preferential recruitment of the AID–RPA complex to immunoglobulin genes. 8- Mistargeted hypermutation to non-immunoglobulin genes like cMyc counteracted by error-free base excision repair (BER) and mismatch repair. 9- Immunoglobulin-oncogene translocations eliminated by apoptosis via P53 activation. This safeguard mechanism might also get rid of B lymphocytes carrying extensive DNA damage.

ing to determine whether tumour susceptibility is in fact related to failure in AID regulatory mechanisms.

Acknowledgements

References

1 Neuberger MS. Antibody diversification by somatic mutation: from Burnet onwards. *Immunol Cell Biol* 2008; **86**:124–32.

2 Honjo T, Kinoshita K, Muramatsu M. Molecular mechanism of class switch recombination: linkage with somatic hypermutation. *Annu Rev Immunol* 2002; **20**:165–96.

3 Revy P, Muto T, Levy Y *et al*. Activation-induced cytidine deaminase (AID) deficiency causes the autosomal recessive form of the Hyper-IgM syndrome (HIGM2). [see comments]. *Cell* 2000; **102**:565–75.

4 Muramatsu M, Kinoshita K, Fagarasan S, Yamada S, Shinkai Y, Honjo T. Class switch recombination and hypermutation require activation-induced cytidine deaminase (AID), a potential RNA editing enzyme. [see comments]. *Cell* 2000; **102**:553–63.

5 Harris RS, Sale JE, Petersen-Mahrt SK, Neuberger MS. AID is essential for immunoglobulin V gene conversion in a cultured B cell line. *Curr Biol* 2002; **12**:435–8.

6 Arakawa H, Hauschild J, Buerstedde JM. Requirement of the activation-induced deaminase (AID) gene for immunoglobulin gene conversion. *Science* 2002; **295**:1301–6.

7 Rogozin IB, Iyer LM, Liang L, Glazko GV, Liston VG, Pavlov YI, Aravind L, Pancer Z. Evolution and diversification of lamprey antigen receptors: evidence for involvement of an AID-APOBEC family cytosine deaminase. *Nat Immunol* 2007; **8**:647–56.

8 Sohail A, Klapacz J, Samaranayake M, Ullah A, Bhagwat AS. Human activation-induced cytidine deaminase causes transcription-dependent, strand-biased C to U deaminations. *Nucleic Acids Res* 2003; **31**:2990–4.

9 Ramiro AR, Stavropoulos P, Jankovic M, Nussenzweig MC. Transcription enhances AID-mediated cytidine deamination by exposing single-stranded DNA on the nontemplate strand. *Nat Immunol* 2003; **4**:452–6.

10 Dickerson SK, Market E, Besmer E, Papavasiliou FN. AID mediates hypermutation by deaminating single stranded DNA. *J Exp Med* 2003; **197**:1291–6.

11 Chaudhuri J, Tian M, Khuong C, Chua K, Pinaud E, Alt FW. Transcription-targeted DNA deamination by the AID antibody diversification enzyme. *Nature* 2003; **422**:726–30.

12 Pham P, Bransteitter R, Petruska J, Goodman MF. Processive AID-catalysed cytosine deamination on single-stranded DNA simulates somatic hypermutation. *Nature* 2003; **424**:103–7.

13 Petersen-Mahrt SK, Harris RS, Neuberger MS. AID mutates *E. coli* suggesting a DNA deamination mechanism for antibody diversification. *Nature* 2002; **418**:99–103.

14 Chaudhuri J, Khuong C, Alt FW. Replication protein A interacts with AID to promote deamination of somatic hypermutation targets. *Nature* 2004; **430**:992–8.

15 Huang FT, Yu K, Hsieh CL, Lieber MR. Downstream boundary of chromosomal R-loops at murine switch regions: implications for the mechanism of class switch recombination. *Proc Natl Acad Sci USA* 2006; **103**:5030–5.

16 Honjo T, Muramatsu M, Fagarasan S. AID: how does it aid antibody diversity? *Immunity* 2004; **20**:659–68.

17 Di Noia J, Neuberger MS. Altering the pathway of immunoglobulin hypermutation by inhibiting uracil-DNA glycosylase. *Nature* 2002; **419**:43–8.

18 Rada C, Di Noia JM, Neuberger MS. Mismatch recognition and uracil excision provide complementary paths to both Ig switching and the A/T-focused phase of somatic mutation. *Mol Cell* 2004; **16**:163–71.

19 Kavli B, Otterlei M, Slupphaug G, Krokan HE. Uracil in DNA – general mutagen, but normal intermediate in acquired immunity. *DNA Repair (Amst)* 2007; **6**:505–16.

20 Simpson LJ, Sale JE. Rev1 is essential for DNA damage tolerance and non-templated immunoglobulin gene mutation in a vertebrate cell line. *EMBO J* 2003; **22**:1654–64.

21 Jansen JG, Langerak P, Tsaalbi-Shtylik A, van den Berk P, Jacobs H, de Wind N. Strand-biased defect in C/G transversions in hypermutating immunoglobulin genes in Rev1-deficient mice. *J Exp Med* 2006; **203**:319–23.

22 Peled JU, Kuang FL, Iglesias-Ussel MD, Roa S, Kalis SL, Goodman MF, Scharff MD. The biochemistry of somatic hypermutation. *Annu Rev Immunol* 2008; **26**:481–511.

23 Di Noia JM, Rada C, Neuberger MS. SMUG1 is able to excise uracil from immunoglobulin genes: insight into mutation versus repair. *EMBO J* 2006; **25**:585–95.

24 Martomo SA, Yang WW, Gearhart PJ. A role for Msh6 but not Msh3 in somatic hypermutation and class switch recombination. *J Exp Med* 2004; **200**:61–8.

25 Wiesendanger M, Kneitz B, Edelmann W, Scharff MD. Somatic hypermutation in MutS homologue (MSH)3-, MSH6-, and MSH3/MSH6-deficient mice reveals a role for the MSH2–MSH6 heterodimer in modulating the base substitution pattern. *J Exp Med* 2000; **191**:579–84.

26 Jiricny J. The multifaceted mismatch-repair system. *Nat Rev Mol Cell Biol* 2006; **7**:335–46.

27 Zeng X, Winter DB, Kasmer C, Kraemer KH, Lehmann AR, Gearhart PJ. DNA polymerase eta is an A–T mutator in somatic hypermutation of immunoglobulin variable genes. *Nat Immunol* 2001; **2**:537–41.

28 Delbos F, Aoufouchi S, Faili A, Weill JC, Reynaud CA. DNA polymerase eta is the sole contributor of A/T modifications during immunoglobulin gene hypermutation in the mouse. *J Exp Med* 2007; **204**:17–23.

29 Pavlov YI, Rogozin IB, Galkin AP, Aksenova AY, Hanaoka F, Rada C, Kunkel TA. Correlation of somatic hypermutation specificity and A–T base pair substitution errors by DNA polymerase eta during copying of a mouse immunoglobulin kappa light chain transgene. *Proc Natl Acad Sci USA* 2002; **99**:9954–9.

30 Stavnezer J, Guikema JE, Schrader CE. Mechanism and regulation of class switch recombination. *Annu Rev Immunol* 2008; **26**:261–92.

31 Wuerffel R, Wang L, Grigera F *et al.* S–S synapsis during class switch recombination is promoted by distantly located transcriptional elements and activation-induced deaminase. *Immunity* 2007; **27**:711–22.

32 Tashiro J, Kinoshita K, Honjo T. Palindromic but not G-rich sequences are targets of class switch recombination. *Int Immunol* 2001; **13**:495–505.

33 Daniels GA, Lieber MR. Transcription targets recombination at immunoglobulin switch sequences in a strand-specific manner. *Curr Top Microbiol Immunol* 1996; **217**:171–89.

34 Reaban ME, Griffin JA. Induction of RNA-stabilized DNA conformers by transcription of an immunoglobulin switch region. *Nature* 1990; **348**:342–4.

35 Shinkura R, Tian M, Smith M, Chua K, Fujiwara Y, Alt FW. The influence of transcriptional orientation on endogenous switch region function. *Nat Immunol* 2003; **4**:435–41.

36 Barreto V, Reina-San-Martin B, Ramiro AR, McBride KM, Nussenzweig MC. C-terminal deletion of AID uncouples class switch recombination from somatic hypermutation and gene conversion. *Mol Cell* 2003; **12**:501–8.

37 Ronai D, Iglesias-Ussel MD, Fan M, Li Z, Martin A, Scharff MD. Detection of chromatin-associated single-stranded DNA in regions targeted for somatic hypermutation. *J Exp Med* 2007; **204**:181–90.

38 Chaudhuri J, Basu U, Zarrin A *et al.* Evolution of the immunoglobulin heavy chain class switch recombination mechanism. *Adv Immunol* 2007; **94**:157–214.

39 Petrini JH. The mammalian Mre11-Rad50-nbs1 protein complex: integration of functions in the cellular DNA-damage response. *Am J Hum Genet* 1999; **64**:1264–9.

40 Shiloh Y. ATM and related protein kinases: safeguarding genome integrity. *Nat Rev* 2003; **3**:155–68.

41 Petersen S, Casellas R, Reina-San-Martin B *et al.* AID is required to initiate Nbs1/gamma-H2AX focus formation and mutations at sites of class switching. *Nature* 2001; **414**:660–5.

42 Franco S, Gostissa M, Zha S *et al.* H2AX prevents DNA breaks from progressing to chromosome breaks and translocations. *Mol Cell* 2006; **21**:201–14.

43 Ramiro AR, Jankovic M, Callen E *et al.* Role of genomic instability and p53 in AID-induced c-myc–Igh translocations. *Nature* 2006; **440**:105–9.

44 Manis JP, Gu Y, Lansford R, Sonoda E, Ferrini R, Davidson L, Rajewsky K, Alt FW. Ku70 is required for late B cell development and immunoglobulin heavy chain class switching. *J Exp Med* 1998; **187**:2081–9.

45 Casellas R, Nussenzweig A, Wuerffel R *et al.* Ku80 is required for immunoglobulin isotype switching. *EMBO J* 1998; **17**:2404–11.

46 Manis JP, Dudley D, Kaylor L, Alt FW. IgH class switch recombination to IgG1 in DNA-PKcs-deficient B cells. *Immunity* 2002; **16**:607–17.

47 Yan CT, Boboila C, Souza EK *et al.* IgH class switching and translocations use a robust non-classical end-joining pathway. *Nature* 2007; **449**:478–82.

48 Soulas-Sprauel P, Le Guyader G, Rivera-Munoz P, Abramowski V, Olivier-Martin C, Goujet-Zalc C, Charneau P, de Villartay JP. Role for DNA repair factor XRCC4 in immunoglobulin class switch recombination. *J Exp Med* 2007; **204**:1717–27.

49 Pan-Hammarstrom Q, Jones AM, Lahdesmaki A, Zhou W, Gatti RA, Hammarstrom L, Gennery AR, Ehrenstein MR. Impact of DNA ligase IV on nonhomologous end joining pathways during class switch recombination in human cells. *J Exp Med* 2005; **201**:189–94.

50 Faili A, Aoufouchi S, Weller S, Vuillier F, Stary A, Sarasin A, Reynaud CA, Weill JC. DNA polymerase eta is involved in hypermutation occurring during immunoglobulin class switch recombination. *J Exp Med* 2004; **199**:265–70.

51 Kuppers R. Mechanisms of B-cell lymphoma pathogenesis. *Nat Rev* 2005; **5**:251–62.

52 Klein U, Dalla-Favera R. Germinal centres: role in B-cell physiology and malignancy. *Nat Rev Immunol* 2008; **8**:22–33.

53 Potter M. Neoplastic development in plasma cells. *Immunol Rev* 2003; **194**:177–95.

54 Nordan RP, Neckers LM, Rudikoff S, Potter M. A growth factor required by plasmacytoma cells *in vitro*. *Curr Top Microbiol Immunol* 1986; **132**:114–20.

55 Suematsu S, Matsusaka T, Matsuda T, Ohno S, Miyazaki J, Yamamura K, Hirano T, Kishimoto T. Generation of plasmacytomas with the chromosomal translocation t(12;15) in interleukin 6 transgenic mice. *Proc Natl Acad Sci USA* 1992; **89**:232–5.

56 Kovalchuk AL, Kim JS, Park SS *et al.* IL-6 transgenic mouse model for extraosseous plasmacytoma. *Proc Natl Acad Sci USA* 2002; **99**:1509–14.

57 Byrd LG, McDonald AH, Gold LG, Potter M. Specific pathogen-free BALB/cAn mice are refractory to plasmacytoma induction by pristane. *J Immunol* 1991; **147**:3632–7.

58 Suematsu S, Matsuda T, Aozasa K *et al.* IgG1 plasmacytosis in interleukin 6 transgenic mice. *Proc Natl Acad Sci USA* 1989; **86**:7547–51.

59 Cheung WC, Kim JS, Linden M, Peng L, Van Ness B, Polakiewicz RD, Janz S. Novel targeted deregulation of c-Myc cooperates with Bcl-X(L) to cause plasma cell neoplasms in mice. *J Clin Invest* 2004; **113**:1763–73.

60 Silva S, Kovalchuk AL, Kim JS, Klein G, Janz S. BCL2 accelerates inflammation-induced BALB/c plasmacytomas and promotes novel tumors with coexisting T(12;15) and T(6;15) translocations. *Cancer Res* 2003; **63**:8656–63.

61 Kovalchuk AL, duBois W, Mushinski E *et al.* AID-deficient Bcl-xL transgenic mice develop delayed atypical plasma cell tumors with unusual Ig/Myc chromosomal rearrangements. *J Exp Med* 2007; **204**:2989–3001.

62 Janz S, Muller J, Shaughnessy J, Potter M. Detection of recombinations between c-myc and immunoglobulin switch alpha in murine plasma cell tumors and preneoplastic lesions by polymerase chain reaction. *Proc Natl Acad Sci USA* 1993; **90**:7361–5.

63 Muller JR, Jones GM, Potter M, Janz S. Detection of immunoglobulin/c-myc recombinations in mice that are resistant to plasmacytoma induction. *Cancer Res* 1996; **56**:419–23.

64 Kovalchuk AL, Potter M, Janz S. DNA sequence analysis of the genetic recombination between Igh6 and Myc in an uncommon BALB/c plasmacytoma, TEPC 1194. *Immunogenetics* 1996; **44**:151–6.

65 Ramiro AR, Jankovic M, Eisenreich T *et al.* AID is required for c-myc/IgH chromosome translocations *in vivo*. *Cell* 2004; **118**:431–8.

66 Takizawa M, Tolarova H, Li Z *et al.* AID expression levels determine the extent of cMyc oncogenic translocations and the incidence of B cell tumor development. *J Exp Med* 2008; **205**:1949–57.

67 Ramiro AR, Jankovic M, Nussenzweig MC. Amplifying Igh translocations. *Nat Immunol* 2005; **6**:117; author reply 8.

68 Paabo S, Irwin DM, Wilson AC. DNA damage promotes jumping between templates during enzymatic amplification. *J Biol Chem* 1990; **265**:4718–21.

69 Kovalchuk AL, Kim JS, Janz S. E mu/S mu transposition into Myc is sometimes a precursor for T(12;15) translocation in mouse B cells. *Oncogene* 2003; **22**:2842–50.

70 Potter M, Wiener F. Plasmacytomagenesis in mice: model of neoplastic development dependent upon chromosomal translocations. *Carcinogenesis* 1992; **13**:1681–97.

71 Gauwerky CE, Croce CM. Chromosomal translocations in leukaemia. *Semin Cancer Biol* 1993; **4**:333–40.

72 Kuppers R, Dalla-Favera R. Mechanisms of chromosomal translocations in B cell lymphomas. *Oncogene* 2001; **20**:5580–94.

73 Leder P, Battey J, Lenoir G, Moulding C, Murphy W, Potter H, Stewart T, Taub R. Translocations among antibody genes in human cancer. *Science* 1983; **222**:765–71.

74 Rabbitts TH. Chromosomal translocations in human cancer. *Nature* 1994; **372**:143–9.

75 Taub R, Kirsch I, Morton C, Lenoir G, Swan D, Tronick S, Aaronson S, Leder P. Translocation of the c-myc gene into the immunoglobulin heavy chain locus in human Burkitt lymphoma and murine plasmacytoma cells. *Proc Natl Acad Sci USA* 1982; **79**:7837–41.

76 Stanton LW, Watt R, Marcu KB. Translocation, breakage and truncated transcripts of c-myc oncogene in murine plasmacytomas. *Nature* 1983; **303**:401–6.

77 Marcu KB, Harris LJ, Stanton LW, Erikson J, Watt R, Croce CM. Transcriptionally active c-myc oncogene is contained within NIARD, a DNA sequence associated with chromosome translocations in B-cell neoplasia. *Proc Natl Acad Sci USA* 1983; **80**:519–23.

78 Hamlyn PH, Rabbitts TH. Translocation joins c-myc and immunoglobulin gamma 1 genes in a Burkitt lymphoma revealing a third exon in the c-myc oncogene. *Nature* 1983; **304**:135–9.

79 Erikson J, ar-Rushdi A, Drwinga HL, Nowell PC, Croce CM. Transcriptional activation of the translocated c-myc oncogene in Burkitt lymphoma. *Proc Natl Acad Sci USA* 1983; **80**:820–4.

80 Dalla-Favera R, Martinotti S, Gallo RC, Erikson J, Croce CM. Translocation and rearrangements of the c-myc oncogene locus in human undifferentiated B-cell lymphomas. *Science* 1983; **219**:963–7.

81 Crews S, Barth R, Hood L, Prehn J, Calame K. Mouse c-myc oncogene is located on chromosome 15 and translocated to chromosome 12 in plasmacytomas. *Science* 1982; **218**:1319–21.

82 Adams JM, Gerondakis S, Webb E, Mitchell J, Bernard O, Cory S. Transcriptionally active DNA region that rearranges frequently in murine lymphoid tumors. *Proc Natl Acad Sci USA* 1982; **79**:6966–70.

83 Adams JM, Gerondakis S, Webb E, Corcoran LM, Cory S. Cellular myc oncogene is altered by chromosome translocation to an immunoglobulin locus in murine plasmacytomas and is

rearranged similarly in human Burkitt lymphomas. *Proc Natl Acad Sci USA* 1983; **80**:1982–6.

84 Siwarski D, Muller U, Andersson J, Notario V, Melchers F, Rolink A, Huppi K. Structure and expression of the c-Myc/Pvt 1 megagene locus. *Curr Top Microbiol Immunol* 1997; **224**:67–72.

85 Beck-Engeser GB, Lum AM, Huppi K, Caplen NJ, Wang BB, Wabl M. Pvt1-encoded microRNAs in oncogenesis. *Retrovirology* 2008; **5**:4.

86 Graham M, Adams JM. Chromosome 8 breakpoint far 3′ of the c-myc oncogene in a Burkitt's lymphoma 2;8 variant translocation is equivalent to the murine pvt-1 locus. *EMBO J* 1986; **5**:2845–51.

87 Tsichlis PN, Lee JS, Bear SE *et al.* Activation of multiple genes by provirus integration in the Mlvi-4 locus in T-cell lymphomas induced by moloney murine leukemia virus. *J Virol* 1990; **64**:2236–44.

88 Robbiani DF, Bothmer A, Callen E *et al.* Activation induced deaminase is required for the chromosomal translocations in c-myc that lead to c-myc/IgH translocations. *Cell* 2008; **135**:1028–38.

89 Unniraman S, Zhou S, Schatz DG. Identification of an AID-independent pathway for chromosomal translocations between the Igh switch region and Myc. *Nat Immunol* 2004; **5**:1117–23.

90 Muramatsu M, Sankaranand VS, Anant S, Sugai M, Kinoshita K, Davidson NO, Honjo T. Specific expression of activation-induced cytidine deaminase (AID), a novel member of the RNA-editing deaminase family in germinal center B cells. *J Biol Chem* 1999; **274**:18470–6.

91 Crouch EE, Li Z, Takizawa M *et al.* Regulation of AID expression in the immune response. *J Exp Med* 2007; **204**:1145–56.

92 Gonda H, Sugai M, Nambu Y, Katakai T, Agata Y, Mori KJ, Yokota Y, Shimizu A. The balance between Pax5 and Id2 activities is the key to AID gene expression. *J Exp Med* 2003; **198**:1427–37.

93 Sayegh CE, Quong MW, Agata Y, Murre C. E-proteins directly regulate expression of activation-induced deaminase in mature B cells. *Nat Immunol* 2003; **4**:586–93.

94 Shaffer AL, Lin KI, Kuo TC *et al.* Blimp-1 orchestrates plasma cell differentiation by extinguishing the mature B cell gene expression program. *Immunity* 2002; **17**:51–62.

95 Lin KI, Angelin-Duclos C, Kuo TC, Calame K. Blimp-1-dependent repression of Pax-5 is required for differentiation of B cells to immunoglobulin M-secreting plasma cells. *Mol Cell Biol* 2002; **22**:4771–80.

96 Sciammas R, Shaffer AL, Schatz JH, Zhao H, Staudt LM, Singh H. Graded expression of interferon regulatory factor-4 coordinates isotype switching with plasma cell differentiation. *Immunity* 2006; **25**:225–36.

97 Dedeoglu F, Horwitz B, Chaudhuri J, Alt FW, Geha RS. Induction of activation-induced cytidine deaminase gene expression by IL-4 and CD40 ligation is dependent on STAT6 and NFkappaB. *Int Immunol* 2004; **16**:395–404.

98 Jiang C, Zhao ML, Diaz M. Activation-induced deaminase heterozygous MRL/lpr mice are delayed in the production of high-affinity pathogenic antibodies and in the development of lupus nephritis. *Immunology* 2008; **126**:102–13.

99 McBride KM, Gazumyan A, Woo EM, Schwickert TA, Chait BT, Nussenzweig MC. Regulation of class switch recombination and somatic mutation by AID phosphorylation. *J Exp Med* 2008; **205**:2585–94.

100 Liu M, Duke JL, Richter DJ, Vinuesa CG, Goodnow CC, Kleinstein SH, Schatz DG. Two levels of protection for the B cell genome during somatic hypermutation. *Nature* 2008; **451**:841–5.

101 McBride KM, Barreto V, Ramiro AR, Stavropoulos P, Nussenzweig MC. Somatic hypermutation is limited by CRM1-dependent nuclear export of activation-induced deaminase. *J Exp Med* 2004; **199**:1235–44.

102 Dorsett Y, McBride KM, Jankovic M *et al.* MicroRNA-155 suppresses activation-induced cytidine deaminase-mediated Myc–Igh translocation. *Immunity* 2008; **28**:1–9.

103 Teng G, Hakimpour P, Landgraf P, Rice A, Tuschl T, Casellas R, Papavasiliou FN. MicroRNA-155 is a negative regulator of activation-induced cytidine deaminase. *Immunity* 2008; **28**:621–9.

104 de Yebenes VG, Belver L, Pisano DG, Gonzalez S, Villasante A, Croce C, He L, Ramiro AR. miR-181b negatively regulates activation-induced cytidine deaminase in B cells. *J Exp Med* 2008; **205**:2199–206.

105 Rada C, Jarvis JM, Milstein C. AID-GFP chimeric protein increases hypermutation of Ig genes with no evidence of nuclear localization. *Proc Natl Acad Sci USA* 2002; **99**:7003–8.

106 Cattoretti G, Buettner M, Shaknovich R, Kremmer E, Alobeid B, Niedobitek G. Nuclear and cytoplasmic AID in extrafollicular and germinal center B cells. *Blood* 2006; **107**:3967–75.

107 Ito S, Nagaoka H, Shinkura R, Begum N, Muramatsu M, Nakata M, Honjo T. Activation-induced cytidine deaminase shuttles between nucleus and cytoplasm like apolipoprotein B mRNA editing catalytic polypeptide 1. *Proc Natl Acad Sci USA* 2004; **101**:1975–80.

108 Brar SS, Watson M, Diaz M. Activation-induced cytosine deaminase (AID) is actively exported out of the nucleus but retained by the induction of DNA breaks. *J Biol Chem* 2004; **279**:26395–401.

109 Aoufouchi S, Faili A, Zober C, D'Orlando O, Weller S, Weill JC, Reynaud CA. Proteasomal degradation restricts the nuclear lifespan of AID. *J Exp Med* 2008; **205**:1357–68.

110 Pasqualucci L, Kitaura Y, Gu H, Dalla-Favera R. PKA-mediated phosphorylation regulates the function of activation-induced deaminase (AID) in B cells. *Proc Natl Acad Sci USA* 2006; **103**:395–400.

111 Basu U, Chaudhuri J, Alpert C *et al.* The AID antibody diversification enzyme is regulated by protein kinase A phosphorylation. *Nature* 2005; **438**:508–11.

112 McBride KM, Gazumyan A, Woo EM, Barreto VM, Robbiani DF, Chait BT, Nussenzweig MC. Regulation of hypermutation by activation-induced cytidine deaminase phosphorylation. *Proc Natl Acad Sci USA* 2006; **103**:8798–803.

113 Pham P, Smolka MB, Calabrese P, Landolph A, Zhang K, Zhou H, Goodman MF. Impact of phosphorylation and phosphorylation-null mutants on the activity and deamination specificity of activation-induced cytidine deaminase. *J Biol Chem* 2008; **283**:17428–39.

114 Basu U, Wang Y, Alt FW. Evolution of phosphorylation-dependent regulation of activation-induced cytidine deaminase. *Mol Cell* 2008; **32**:285–91.

115 Xue K, Rada C, Neuberger MS. The *in vivo* pattern of AID targeting to immunoglobulin switch regions deduced from mutation spectra in msh2$^{-/-}$ ung$^{-/-}$ mice. *J Exp Med* 2006; **203**:2085–94.

116 Wu X, Stavnezer J. DNA polymerase beta is able to repair breaks in switch regions and plays an inhibitory role during immuno-

globulin class switch recombination. *J Exp Med* 2007; **204**:1677–89.

117 Pasqualucci L, Neumeister P, Goossens T, Nanjangud G, Chaganti RS, Kuppers R, Dalla-Favera R. Hypermutation of multiple proto-oncogenes in B-cell diffuse large-cell lymphomas. *Nature* 2001; **412**:341–6.

118 Shen HM, Peters A, Baron B, Zhu X, Storb U. Mutation of BCL-6 gene in normal B cells by the process of somatic hypermutation of Ig genes. *Science* 1998; **280**:1750–2.

119 Okazaki IM, Hiai H, Kakazu N, Yamada S, Muramatsu M, Kinoshita K, Honjo T. Constitutive expression of AID leads to tumorigenesis. *J Exp Med* 2003; **197**:1173–81.

120 Okazaki IM, Kotani A, Honjo T. Role of AID in tumorigenesis. *Adv Immunol* 2007; **94**:245–73.

121 Matsumoto Y, Marusawa H, Kinoshita K *et al. Helicobacter pylori* infection triggers aberrant expression of activation-induced cytidine deaminase in gastric epithelium. *Nat Med* 2007; **13**:470–6.

122 Gourzi P, Leonova T, Papavasiliou FN. A role for activation-induced cytidine deaminase in the host response against a transforming retrovirus. *Immunity* 2006; **24**:779–86.

123 Neuberger MS, Rada C. Somatic hypermutation: activation-induced deaminase for C/G followed by polymerase eta for A/T. *J Exp Med* 2007; **204**:7–10.