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AIDing antibody diversity by error-prone mismatch repair

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Abstract

The creation of a highly diverse antibody repertoire requires the synergistic activity of a DNA mutator, known as activation-induced deaminase (AID), coupled with an error-prone repair process that recognizes the DNA mismatch catalyzed by AID. Instead of facilitating the canonical error-free response, which generally occurs throughout the genome, DNA mismatch repair (MMR) participates in an error-prone repair mode that promotes A:T mutagenesis and double-strand breaks at the immunoglobulin (Ig) genes. As such, MMR is capable of compounding the mutation frequency of AID activity as well as broadening the spectrum of base mutations; thereby increasing the efficiency of antibody maturation. We here review the current understanding of this MMR-mediated process and describe how the MMR signaling cascade downstream of AID diverges in a locus dependent manner and even within the Ig locus itself to differentially promote somatic hypermutation (SHM) and class switch recombination (CSR) in B cells.

Keywords

mismatch repair; MMR; class switch recombination; CSR; somatic hypermutation; SHM; AID; DSB; cytosine deamination; epigenetic; antibody diversity

1. Introduction

Organisms at every level of evolutionary development are constantly exposed to genotoxic stress that can damage and alter the structure of their genetic material. To counteract this threat, a large variety of highly conserved repair mechanisms have been generated throughout evolution [1]. It is estimated that 2-4% of the genes in mammalian cells are devoted to the repair of DNA damage and these are tightly integrated through various signal transduction pathways with the regulation of the cell cycle and cell death [2]. Paradoxically, the generation and propagation of “useful” mutations could potentially confer an evolutionary advantage [3]. Furthermore, in complex organisms it is sometimes important to somatically produce increased levels of genetic diversity [4, 5].

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Perhaps the most extreme example of engineering and tightly regulating genomic instability for a selective advantage is the adaptive immune response in higher organisms, where it is essential to spawn an enormous repertoire of antigen binding sites in B and T cell antigen receptors. This is achieved by the combinatorial rearrangement of a small number of variable (V), diversity (D) and joining (J) genes so that the organism can mount a protective immune response against most foreign antigens that it encounters [6]. B cells need to generate antibodies of high affinity to neutralize and inactivate pathogenic agents in the blood stream, in tissues and even in mucosal spaces. To achieve this V(D)J regions that encode low affinity antibodies are somatically hypermutated (SHM) so that they achieve the high affinities required to neutralize toxic foreign agents [7, 8]. In order to distribute these protective antibodies throughout the body and enable them to carry out different effector functions, it is also necessary to mutate the switch (S) regions that are upstream from each of the constant region genes and to convert those mutations into double stranded DNA breaks (DSBs). This allows the heavy chain V(D)J regions encoding the antigen-binding site to be rearranged from the μ constant region to the downstream γ , ϵ , and α constant regions through a process, termed class switch recombination (CSR) [9, 10].

In B cells, a potent mutator known as activation-induced deaminase (AID), initiates SHM of the Ig V(D)J and CSR of the Ig S regions by deaminating Cs and generating U:G DNA mismatches at a very high frequency. This occurs primarily in the activated B cells in the germinal centers of secondary lymphoid organs, such as the lymph nodes, spleen and tonsils where AID is expressed at very high levels. In those germinal center B cells, AID induced mutations recruit base excision (BER) and mismatch repair (MMR) processes that in other cell types and at non-Ig genes repair DNA lesions with high fidelity [11, 12]. However, in B cells, the BER and MMR that are recruited by AID-induced U:G mismatches facilitate an error-prone repair of these mismatches, and MMR is responsible for as many as half of the mutations that arise during SHM and for most of the mutations that occur at A:T bases [13]. While a great deal is known from biochemical and yeast studies about the role of MMR proteins in the normal process of high fidelity MMR, less is known about how these factors are targeted to particular mismatches *in vivo* and to the regulation of the repair process in different lineages of mammalian cells.

In this review we will describe the orchestration of MMR-mediated error-prone repair in both antibody V(D)J and S regions following the enzymatic production of DNA mismatches by AID. We will also point out the many unresolved features of this atypical and potentially dangerous repair process. Through comparison of the complete loss and separation-of-function mutants in the MMR proteins *in vivo* and the detailed examination of the outcome of this process as it is reflected in the sequences of individual antibody V(D)J and S regions, we are learning new aspects of how error-prone MMR generates antibody diversity in B cells. We are also gaining new insights into how high fidelity MMR is regulated in general and how its misregulation can lead to tumorigenesis.

2. AID-mediated cytosine deamination instigates a highly mutagenic cascade

Because it is extremely mutagenic, highly expressed in centroblast B cells, and generates mutations characteristic of antibody V and S regions, it was originally thought that AID was a B cell specific deaminase primarily devoted to the generation of antibody diversity [8]. However, subsequent studies revealed that many other genes were mutated in activated B cells some of which were repaired with high fidelity while others were also subjected to error-prone repair [14, 15] (see Saribasak and Gearhart *this issue*). The recent report that there are ~1 million sites occupied by AID in activated mouse B cells is surprising considering its genotoxic potential [16]. This apparent promiscuity of targeting may, to

some extent, be explained by the fact that AID mediated cytosine deamination can also cause the active demethylation of 5-methylcytosines or 5-hydroxymethylcytosines with potential consequences for protein expression and epigenetic inheritance. The finding of AID in germ cells and the demonstration of a role for AID-induced active demethylation in early differentiation and even in neuronal function [17-20] may, to some extent, explain why AID is so widely distributed. However, in B cells AID seems to cause mutations to various extents in most of the genes that it targets [15, 16].

AID selectively deaminates the C in WRCY (W=A/T, R=A/G, Y=C/T) generating a U:G mismatch (WRUY). While this motif is enriched in the parts of the V(D)J regions that form the antigen-binding site and in the S regions, this alone does not explain the specific targeting to the Ig locus since the WRCY motif is widely distributed throughout the genome. The finding that single-stranded DNA (ssDNA) is the substrate for AID in part explains why high rates of transcription, accompanied by the high potential for transcription stalling [21], are required for AID mutagenesis. Indeed, ssDNA accessible regions and transcription bubbles provide an excellent substrate for AID [22-24], but this does not explain why only some but not all highly transcribed genes are targeted by AID in B cells [16]. As will be discussed in other chapters in this issue (Kenter *this issue*), some cis-acting sequences such as E2A binding sites [25, 26] and chromatin modifications provide a partial explanation of how AID mutagenesis might be largely restricted to the V(D)J and S regions of the Ig gene [27-30], but these are also very widely distributed throughout the genome. Therefore, the question remains whether there is any specific genetic or epigenetic signature that licenses AID and error-prone repair to target the V and S regions of the Ig locus more frequently and efficiently than other parts of the Ig locus and non-Ig genes.

Mutations in AID that lead to a loss of SHM and CSR in patients with Hyper-IgM Syndrome type II and subsequent site directed mutagenesis and exon swapping experiments suggested that there are likely to be associated proteins and posttranslational modifications that regulate AID levels and its concentration in the nucleus, and also selectively target AID to parts of the Ig gene. While a number of associated proteins have been discovered (see Orthwein and Di Noia; Larijani and Martin; and Häslér, Rada, and Neuberger *this issue*), some of which like RPA and Spt5 probably maintain the single stranded nature of V(D)J and S regions [21], it is still unclear how much of the rest of the genome is protected from the mutagenic AID activity [15]. These factors also do not explain how AID is regulated to carry out active demethylation as opposed to hypermutation [31]. In fact, it has been suggested that a different set of associated proteins including Gadd45 α and glycosylases like MBD4 and TDG are required for AID to act as a promoter of cytosine demethylation through its deaminase activity [32, 33]. These issues have become even more important as it is now clear that AID is expressed, at least at low levels, in cells of many lineages, and that its mutagenic activity may not only be responsible for B cell malignancies, but also for many other types of cancers [34, 35]. It has thus become very important to understand exactly how AID is recruited to the Ig V(D)J and S regions. It is possible that the factors or DNA and chromatin structures that are responsible for the selective targeting of AID may also cause MMR to be error-prone at the Ig gene and in some of the other genes that are targeted by AID [15].

3. MMR mediates the resection of ssDNA patches and the introduction of A:T mutations

The current model (Figure 1) suggests, the U:G mismatch that is generated when AID mutates an Ig V(D)J region is either replicated over to produce a U->T mutation, recognized by UNG to initiate a sometimes error-prone BER (see also Saribasak and Gearhart *this issue*), or recognized by the MutSa MMR heterodimer composed of the MSH2 and MSH6

proteins (Figure 1). This MMR-mediated sensing of the U:G lesion initiates a series of processes that are responsible for the mutations at surrounding A:T bases. When the U:G mismatch is recognized by MutS α , ATP-mediated conformational changes [36-38] allow it to recruit PCNA and 5'-3' exonucleases, such as EXO1 [39]. The mismatch is subsequently excised to create a single stranded patch. The excised strand is then replaced by a new strand of DNA that might acquire additional mutations at A:T bases. This error-prone resynthesis is mediated, at least in part, by the polymerase eta (Pol η) (Figure 1), a translesional polymerase that is most error-prone when it copies A and T bases [40-44]. As illustrated in Figure 1, during normal DNA replication the resynthesis of the excised strand is carried out by high fidelity polymerases δ and ϵ , but at the V – and possibly S regions – Pol η is recruited by mono-ubiquitylated PCNA [45, 46]. The length of this patch has been estimated to be 20-30 bp [14, 15, 47]. It is unclear what restricts the patch to this size since in biochemical studies much longer strands of DNA can be excised by EXO1 [48, 49]. Presumably nicks that are made 3' and/or 5' to the site of recruitment of MSH2 and MSH6 determine where EXO1 or some as yet unknown nuclease(s) initiates the excision process. While recent studies in cell free systems and in mice suggest that a latent endonuclease activity in PMS2 could generate nicks 5' or 3' to the mismatch [50-52], several studies have shown that the MLH1-PMS2 heterodimer (MutL α) is not required to excise the strand of DNA containing the AID-generated U:G mismatch during SHM (reviewed in [53]). This is especially peculiar since MutL α is involved in MMR as it occurs in most other circumstances *in vivo* [38, 54], suggesting that it is important to prevent MLH1 and PMS2 from being recruited to the V regions. The mechanism and factors responsible for this restriction are not known.

3.1 MutS α is the sensor that initiates A:T mutagenesis during SHM

The evidence that supports the model described above and illustrated in Figure 1 comes from studies in which various MMR proteins have been genetically deleted or replaced by proteins with point mutations (reviewed in [53]). For example, the early studies in DNA repair deficient mice suggested that the A:T mutations observed in the V region were introduced during a second phase of SHM, where error-prone MMR and long-patch BER are responsible for the mutations surrounding the initially targeted C [13, 39, 55-58]. The absence of the BER protein UNG does not have a significant impact in the accumulation of A:T mutations [55], suggesting that MMR pathway is predominant during the phase 2 of SHM. In fact, MSH2-, MSH6-, and EXO1-deficient B cells lack 80-90% of all A:T mutations [39, 56, 58]. Interestingly, in the absence of proper MMR, a basal 10-20% of A:T mutations can be introduced in a UNG-dependent manner as revealed by *Msh2/Ung* [13] and *Msh6/Ung* [59] double-deficient mice. Therefore, an alternative long-patch version of BER, which exposes a bigger gap than the traditional one-base short-patch BER, may be responsible for some of the A:T mutations of SHM. What is not fully understood yet is if both MMR and BER mechanisms collaborate or compete in resolving AID-generated U:G mispairs. As discussed by Saribasak and Gearhart (*this issue*), it is possible that the relative roles of MMR and BER may vary during different phases of the cell cycle, perhaps because of different levels of expression of the relevant proteins. Recently, it has been suggested not only that both pathways are noncompetitive, but that they might even collaborate [60, 61]. This collaboration will create situations where MMR-mediated resynthesis of a ssDNA gap can encounter a BER-generated abasic site on the complementary strand, promoting mutations at G:C sites by untemplating the polymerase. These observations suggest that BER/MMR cooperation might be responsible for some of the G:C mutations, even though MMR tends to be error-free over U:G mismatches and is perverted to be error-prone at A:T neighboring sites [62].

3.2 PCNA^{K164} mono-ubiquitylation mediates error-prone polymerase recruitment

The question remains how MMR, which is supposed to be a high fidelity repair mechanism, is diverted into an error-prone mode that is accountable for 80-90% of A:T mutations. We, and others, have demonstrated a critical role for site-specific mono-ubiquitylation of proliferating cell nuclear antigen (PCNA) at lysine 164 in SHM [45, 46, 60, 63] (Figure 1). The lack of A:T mutations in B cells of PCNA^{K164R} mutant mice that cannot modify PCNA at lysine 164 indicated a role for posttranslational modifications of PCNA in processing uracils downstream of AID. This is thought to be the result of the failure to recruit and activate the error-prone polymerase η and to some degree polymerase κ to generate mutations at A:T sites [41-44]. Interestingly, further K63-linked poly-ubiquitylation of PCNA activates a proteasome-independent error-free branch of repair that is not completely understood and that might suppress A:T hypermutation [64-66]. This process requires the E2 ubiquitin conjugases Ubc13/Mms2, which can physically interact with the E3 ligases HLTF and SHPRH. Although HLTF and SHPRH can poly-ubiquitylate PCNA [67, 68], they are not essential for SHM, suggesting the existence of an alternative E3 ligase yet to be discovered [66]. It also remains unclear whether PCNA is mono-ubiquitylated as part of a complex and then recruited to the V region, or if the mono-ubiquitylation occurs locally once PCNA has been recruited to the V region.

3.3 The cellular context of MMR-mediated mutagenesis

The use of reporter genes in mice suggest that the cellular environment of germinal center B cells, compared to other cell types, enables an increased susceptibility to mutations at A:T bases but the frequency of these mutations is still low compared to those seen in the Ig V and S regions [69, 70]. This seems to confirm that the MMR pathway is highly mutagenic only at AID-targeted loci since it is much less mutagenic at a *lacI* transgene that is not targeted by AID [14]. Additional studies of the protein complexes and signaling cascades that accompany AID activity and of the genetic and epigenetic peculiarities of the Ig genes could reveal how MMR mediated mutagenesis is largely restricted to the Ig genes. What is clear is that MMR factors themselves possess high levels of plasticity in B cells to orchestrate and regulate the ultimate outcome of the repair processes triggered by AID-generated U:G mismatches. Post-translational modifications and ATPase-dependent conformational changes seem to modulate the scaffolding and the enzymatic properties of MMR factors, as we discuss in more detail below.

4. MMR promotes DSB formation to facilitate class switching

The mechanisms by which the signaling cascade downstream of AID diverges so as to independently mediate SHM or CSR remains puzzling. One clear distinction is that DSBs are required to initiate CSR [10, 71] (Figure 2), yet exactly how the DSB is initiated continues to be a matter of debate. Much like SHM, ssDNAs are often created as an intermediary step following AID-mediated U:G mismatches [72]. But instead of creating single point mutations, directly or through the recruitment of MMR, PCNA and error-prone polymerases, many of these ssDNA gaps lead to DSB formation during CSR. In that context, AID activity is dependent on the germline expression of the different S regions, which are controlled by transcription factors [73], including BATF [74] and Ikaros [75], and exhibit physical interactions with the IgH 3' regulatory region [76-79]. A more direct mechanism for DSB development is either the fortuitous overlap of two single stranded nicks on opposite strands of the DNA or the short ssDNA end resection in opposite direction of two adjacent DNA nicks [73]. The predominance of DSB may be, in part, due to the difference in sequence characteristics between the V and S regions. Whilst the former encodes an open-reading frame with a distribution of AID-hotspots, the latter is intronic with a number of overlapping AID-hotspot repeats and a propensity for R-loop formation

due to the high G:C content of the transcribed strand of the switch regions [80-83]. Such sequence features have been shown to favor DSB formation even when transplanted into V regions, presumably by increasing the chance of creating nicks in both strands of the DNA [84-87]. Intriguingly, the frequency of mutations at S regions in either MutS α or MutL α mutants inversely correlates with that at the V region in those same mutants. At S regions the overall base mutation frequency increases rather than decreases [88, 89]. This may be because the MMR process is more biased towards error-free repair at S regions, much like the rest of the genome. It is also possible that the MutL α -dependent MMR mechanism leads to the formation of DSBs at S regions, limiting the recruitment or activity of error-prone polymerases and favoring the mobilization of the resecting or recombining factors involved in DSB formation.

Since UNG plays a minor role in generating V region mutations at A:T sites but a major role in mediating CSR, it is likely that BER is playing an important role in promoting DSBs at S regions (see also Saribasak and Gearhart *this issue*). However, MMR is also important since the deletion of either MutS α or MutL α (Figure 2) results in a decrease of 50% or more in CSR [53]. This switching defect is usually accompanied, especially in the absence of the scaffolding functions of MutL α , by a destabilization of the canonical non-homologous end joining (NHEJ) mechanisms and an increase in the use of longer microhomologies at the S-S junctions [6, 73, 90-92]. Ultimately, if the created DSBs are blunt or nearly blunt on both ends (0-3 bp), repair progresses towards ligation by the canonical NHEJ (cNHEJ) pathway. However, if the DNA ends are staggered (>4 bp), repair could require further processing of ends primarily by the Mre11-RAD50-NBS1 complex [93] and CtIP [94]. This would be followed by ligation through an alternative NHEJ (aNHEJ) pathway that depends on overlapping S-S microhomologies created by the staggered DNA ends which appear to require the MutL α protein for their adequate processing [6, 53, 73].

4.1 MutL α relays MutS α signals to orchestrates DSB formation

Among the inducers and repair proteins involved in antibody diversification, the factors that show the most disparity in phenotype between SHM and CSR are Pol η (Figure 1) and the MutL α complex (Figure 2). MutL α is clearly required to trigger the emergence of a DSB at S regions and to orchestrate the CSR pathway in a manner distinct from that observed in SHM [73]. The MLH1/PMS2 heterodimer is an established MMR adaptor molecule or “match-maker” that coordinates the recognition of the mismatch and its excision via recruitment of downstream factors and introduction of a single stranded nick 5' or 3' to the mismatch [50, 51]. Consequently, MutL α seems to relay the MMR signaling cascade downstream of the MutS α sensor complex to favor the formation of DSBs rather than ssDNA breaks that are common intermediates in classical MMR (Figure 2) [8, 95-97]. Though the exact mechanism is not entirely elucidated yet, there have been many recent developments. PMS2 has been shown to encode an endonucleolytic activity required for its role in MMR [50]. A point mutation of a critical endonuclease residue, PMS2^{E702K}, was sufficient to significantly affect CSR frequency but not the S-S microhomology [52]. This suggests that the endonuclease activity of MutL α is needed to trigger the formation of DSBs in CSR but is not essential for subsequent DNA end processing. This is in contrast to MLH1-null [89, 96, 97] and MLH1-ATPase deficient mice (in press), which show a defect in CSR frequency and a propensity for increased microhomologies. Despite the redundancies in ATPase activities among the MutS α and MutL α complexes, we have shown that the MLH1 ATPase activity is required to elicit the adaptor and effector functions of the MutL α complex during CSR (unpublished data).

One possibility is that MLH1 could independently affect the processivity of downstream exonuclease(s), such as EXO1 or even unknown nucleases, thereby increasing the emergence of DSBs. Although *in vitro* studies have been inconclusive in that regard [48,

49], it is possible that MutL α could affect EXO1 processivity *in vivo*, depending on auxiliary factors that might be missing from the purified proteins in biochemical studies [48, 49]. Alternatively MutL α could bias the system in other ways. For example, the MutL α interactome has identified a number of known and uncharacterized proteins [98]. Among the hits is the interaction of both MLH1 and PMS2 (but not PMS1) with DNAPK-cs, which is considered an orchestrator of the NHEJ repair pathway. Such recruitment could predispose the system towards NHEJ repair thereby curtailing the chance of DNA end resection and development of microhomologies. Furthermore MSH6 was recently shown to associate with Ku70 to regulate double strand break repair [99]. It is therefore possible that at S regions the MutL α complex could actively orchestrate the interplay between MMR and NHEJ. This would be counter-productive in the V region since DSBs would lead to deletions and insertions with frame shifts that could cause the loss of V regions and lead to B cells without surface Ig. This does not explain how MLH1 and PMS2 are being excluded from the V region, and therefore the molecular signature that makes MMR activity distinctive during SHM or CSR remains unknown.

4.2 The potential roles of PCNA mono-ubiquitylation and chromatin epigenetics in DSB formation

We have described earlier how PCNA mono-ubiquitylated at residue K164 could bind and activate error-prone polymerases, such as Pol η to mediate SHM. However, PCNA^{K164R} mutation also causes an – as yet – unexplainable CSR defect, even though Pol η -deficient mice do not manifest any detectable CSR defect [42, 100]. This suggests that mono-ubiquitylated PCNA might have additional roles in CSR (Figure 2). Of course this function could be downstream of the DNA DSB, dealing with the adequate progress of the DSB repair process; but it might also be upstream recruiting and/or regulating DSB instigators. A very intriguing possibility is that PCNA could provide the balance between MMR and chromatin assembly [101] at the DNA mismatch site within the Ig locus. It has been shown that disassembly of nucleosomes is associated with MMR catalytic activities [102] and that MMR proteins bind a mismatch within a nucleosome with much lower efficiency than a naked mismatch [103]. Yet histone modifications have the potential to demarcate DNA stretches for AID induced alterations and/or to indirectly tether AID – as well as other DNA repair factors – preferentially to S regions [27, 104-106] (see Kenter *this issue*). For example, H3K9me3 and H3K9ac histone marks have been shown to be associated with CSR [27]. Subsequent studies described how these epigenetic marks can facilitate the recruitment of KAP1 and HP1 proteins to S donor regions tethering with them AID protein to mediate the generation of DSBs [104]. PTIP, a member of the MLL3-MLL4 histone methyltransferase complex has been shown to be associated with increased H3K4me3 mark, ensuring proper targeting, stability, and repair of S regions [105]. Interestingly, PTIP has also been shown to be required for PCNA mono-ubiquitylation [107] along with RNF8, an E3 ubiquitin ligase important for efficient CSR [108-110]. These data suggest that PCNA and its modified forms could be important during the interplay between MMR repair and DSB formation. Since MSH6 possesses a chromatin-binding motif, called PWWP, believed to bind the chromatin assembly factor CAF-1 [101] it would be interesting to study the effects of a PWWP mutation on CSR and SHM.

4.3 Controlling widespread genomic breaks generated by the AID/MMR axis

It is also conceivable that V regions undergo DSB formation [111, 112] via MMR, but since these mutations occur in the open-reading frame of the Ig genes, the likelihood that the DSB creates a frameshift is high, hence such B cells would get selected against because they cannot express an Ig on their surface. Off-target DSBs induced by AID constitute a high risk for the B cell integrity and survival, which has been shown to be prevented by homologous-recombination mechanisms involving XRCC2 [113]. What is yet ill understood is whether

MMR factors are indeed hijacked along with AID to cooperatively promote off-target genomic breaks in B cells leading to genomic translocations and eventually tumorigenesis. At the Ig genes, the last 10 C-terminal amino-acid residues of AID have been demonstrated to be important for cooperative binding to MSH2/MSH6 and UNG, as well as for the function of the protein complex downstream of the DSBs, probably directing the S region DSBs towards NHEJ [114]. Indeed, MSH6 can directly influence AID activity *in vivo* [37]. The C terminus of AID has also been found to interact with the 14-3-3 adaptor protein complex, which specifically binds to the AGCT motifs that are enriched in S regions [115], providing another factor that could contribute to the difference in the repair of DNA damage at the V and S regions.

It is worth noting that the MMR pathway was also suggested to engage in what is termed “futile repair cycles” during the cytotoxic DNA damage response. It is believed that during such a process, MMR does not fully repair the persistently created mismatch, but eventually processes it into an alternative DNA damage intermediate mended by other DNA repair machineries such as ATR-ATRIP [116, 117]. The nature of this MMR activity could favor the generation of DSB by generating futile cycles of DNA resection without it being coupled to canonical mismatch repair. It would have been interesting if the futile cycles of MMR also work during SHM and CSR, respectively. Yet, to date, this does not seem to be the case since all evidence so far indicates that the canonical MMR pathway is the predominant route.

5. Summary and future perspectives

While an enormous amount has been learned in the last decade about the biochemical, molecular and genetic mechanisms responsible for the generation of antibody diversity, there is still much to be uncovered. One issue is that all of the molecular mechanisms of affinity maturation, have in fact been hijacked and in various ways perverted from normal mechanisms that repair or modify DNA. AID may have evolved as a cytosine deaminase whose role was to modify the epigenetic landscape by triggering active demethylation of CpGs to regulate expression of certain genes, [17, 18, 32]. However at Ig genes in B cells, its major role seems to be skewed towards creating genetic mutations. MMR evolved to protect organisms from post replicative errors and DNA damage that lead to genomic instability, but in B cells, MMR is highly error-prone at least at the Ig gene and probably at few other sites [15, 16]. It seems reasonable to assume that there are associated protein co-factors, DNA structures and increased accessibility through changes in chromatin structures in the V and S regions that could preferentially target AID and distort the usual high fidelity of MMR [8, 118]. However, it is unclear why many other genes that are highly expressed in B cells and are also targeted by AID but repaired instead with high fidelity [15]. It is possible that cis-acting sequences recruit different DNA binding proteins to those sites that are most highly mutated by AID and these could also play a role in modulating the function of MMR [26, 119]. Furthermore, while error-prone MMR is targeted to both V and S regions, the details of how MMR functions at these two regions are quite different. In particular, MutL α is not involved in SHM at V regions even though it is a major orchestrator of MMR repair excision and resynthesis, but it plays an important role in CSR at S regions. In addition, Pol η which is recruited through mono-ubiquitylated PCNA^{K164} is required for mutations at A:T residues, but is not required for CSR even though mono-ubiquitylated PCNA is involved in both processes. Some of the factors involved are probably part of the MMR interactome, which contains many proteins beyond the classical MMR complex and differs in different cell types [98]. It is also possible that posttranslational modifications like phosphorylation and ubiquitylation of MMR proteins determine their exact roles by excluding MutL α from the V region or allowing the recruitment of different co-factors. Some MMR and MMR-associated proteins are expressed

primarily in S phase and it is not yet clear whether AID induced mutations or error-prone repair occur preferentially during replication, although both CSR and SHM appear to occur during G1 and S phases [120, 121]. Since a recent study in yeast suggests that there are separate MMR foci in the nucleus for MutS α and MutL α [122], it is possible that SHM and CSR are occurring in separate foci, and therefore in temporally and/or spatially distinct MMR factories.

These findings suggest that it will be useful to examine the MMR interactome in B cells that are undergoing either SHM or CSR; preferentially at different stages of the cell cycle. It would also be informative to dissect out individual aspects of the MMR process by examining additional separation-of-function mutant mice in the different MMR proteins as well as in other factors that participate in the MMR process.

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Highlights

- The DNA mutator AID catalyzes U:G mismatches recognized by mismatch repair (MMR).
- MMR generally fixes DNA mismatches faithfully, except at the immunoglobulin genes.
- MMR differentially promotes A:T mutagenesis at V regions and DSB at S regions.
- The MMR sensor complex MutS α (MSH2/MSH6) promotes both SHM and CSR.
- The MMR adaptor complex MutL α (MLH1/PMS2) has no role in SHM but promotes CSR.

Figure 1. MMR cascade during A:T mutagenesis at ssDNA patches in Ig genes

MMR pathway plays the major role introducing A:T mutations during SHM in B-cells. The MutSα heterodimer composed of MSH2 and MSH6 recognizes the AID-generated U:G mismatch. This initiates a series of processes to create a ssDNA gap around the original AID-induced lesion, by recruiting scaffolding proteins (e.g. PCNA), nucleases (e.g. EXO1), and yet unknown factors and activities such as the instigation of nick-directed mismatch repair. Finally, the ssDNA patch is resynthesized through a complex cycle of DNA polymerization, which involves the recruitment of low-fidelity error-prone polymerases, like Polη and Polκ, as opposed to the use of canonical high-fidelity polymerases like Polδ and Polε. Post-translational modifications of PCNA, mainly through ubiquitylation, have been shown to play a role in regulating the usage of these polymerases and in orchestrating the decision between error-free and error-prone repair.

Figure 2. MMR cascade orchestrates DSB-repair at Ig genes

DSBs are required to initiate CSR at S regions, and the MMR pathway plays a significant role promoting DSB formation. Similar to SHM, the MutS_α heterodimer senses the AID-generated U:G mismatch, but here, the coordination of EXO1 nuclease activities and the MutL_α heterodimer composed of MLH1 and PMS2, relays the MMR signaling cascade to favor the formation of DSBs. While Polη does not play a major role during CSR, PCNA ubiquitylation has been shown to be important for efficient CSR, therefore suggesting additional role(s). Once the break is produced, canonical DSB sensors like 53BP1, the ubiquitin ligases RNF8/RNF168 and the phosphorylated form of H2AX (γH2AX) ensure the initiation of recombination and ligation of the S junctions through non-homologous end joining (NHEJ) mechanisms. At this stage, MMR scaffolding functions and yet unknown regulating signals fine tune processing of the S-S junctions, balancing the use of two seemingly distinct versions of NHEJ: the canonical pathway and the alternative pathway.