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Does DNA repair occur during somatic hypermutation?

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Abstract

Activation-induced deaminase (AID) initiates a flood of DNA damage in the immunoglobulin loci, leading to abasic sites, single-strand breaks and mismatches. It is compelling that some proteins in the canonical base excision and mismatch repair pathways have been hijacked to increase mutagenesis during somatic hypermutation. Thus, the AID-induced mutagenic pathways involve a mix of DNA repair proteins and low fidelity DNA polymerases to create antibody diversity. In this review, we analyze the roles of base excision repair, mismatch repair, and mutagenesis during somatic hypermutation of rearranged variable genes. The emerging view is that faithful base excision repair occurs simultaneously with mutagenesis, whereas faithful mismatch repair is mostly absent.

Keywords

Activation-induced deaminase; Base excision repair; Mismatch repair; DNA polymerases

1. Introduction

After it was shown that AID can initiate all antibody diversity mechanisms in mature B cells [1, 2], an extensive search was undertaken to identify its mode of action. Initially, AID was thought to be an RNA editing enzyme, due to homology with the RNA deaminase protein APOBEC1 [3], but recently it has been clearly shown that it deaminates DNA in B cells [4]. Thus, AID deaminates cytosine (C) to uracil (U) and thereby introduces an unwanted base at a high frequency into the immunoglobulin (*Ig*) loci, and at a low frequency elsewhere in the genome [5]. Proteins in the base excision repair (BER) and mismatch repair (MMR) pathways then deal with the resulting U:G mismatches. Normally, the uracils would be repaired without error, but this is not the case during antibody diversification. In the *Ig* loci, AID-induced uracils produce DNA lesions and strand breaks to generate somatic hypermutation (SHM), class switch recombination (CSR), and gene conversion (GC) to ensure diversity in antibodies against pathogens. During this flurry of DNA damage, does error-free BER and MMR occur in the *Ig* loci? In this review, we will describe the balance between DNA repair and mutagenesis during the processing of AID-induced damage.

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2. Canonical DNA repair pathways

2.1 BER pathway

BER recognizes small base modifications, abasic sites, and single strand breaks [6]. Repair of uracils occurs by the following steps: (1) excision of uracil by uracil DNA glycosylase (UNG), (2) incision at the resulting abasic site by apurinic/apyrimidinic endonuclease 1 (APE1), (3) replacement of the excised nucleotide by DNA polymerase (pol) β , (4) removal of the 5'-deoxyribose phosphate group by pol β , and (5) sealing of the final nick by DNA ligase 3 (Lig3) (Fig. 1). X-ray cross complementing 1 (XRCC1) is a scaffold protein that coordinates pol β and Lig3 activity [7], and is involved in steps 3–5. As pol β has relatively high fidelity, C is usually inserted opposite template guanine (G) to generate error-free repair of the deaminated cytosine.

2.2 MMR pathway

MMR repairs mismatches and other types of damage made during DNA replication and recombination [8]. Repair of mismatches occurs by the following steps: (1) recognition of the mismatch by the MSH2-MSH6 heterodimer, (2) recruitment of MLH1 and PMS2 to introduce a single strand nick near the mismatch, (3) excision of the mismatch and adjacent bases by exonuclease 1 (Exo1) to generate a gap, (4) synthesis in the gap by pol δ bound to the proliferating cell nuclear antigen (PCNA) clamp, and (5) ligation of the ends by DNA ligase 1 (Fig. 1). Synthesis by the high fidelity pol δ ensures that the correct bases are inserted opposite their complementary bases to produce error-free repair of the mismatch.

3. SHM mutagenesis

During SHM, mutations accumulate in rearranged variable (V), diversity (D), and joining (J) genes on the heavy (H), kappa (κ) and lambda (λ) *Ig* loci. The mutations are mostly single base substitutions, along with occasional tandem double base substitutions, deletions, and insertions. Mutations start 100–200 bp downstream of the transcription initiation site and extend for 1.5–2.0 kb [9]. The frequency of mutation, which is highest in the V(D)J coding exon and the downstream J intron [10–14], occurs at 10^{-2} to 10^{-3} mutations per bp, which is a million times higher than mutation levels in the rest of the genome. As recorded from the nontranscribed strand, C and G nucleotides are mutated equally, implying that AID deaminates C on both DNA strands [15]. However, adenine (A) bases are mutated twice as frequently as the complementary thymine (T) bases, which is likely due to synthesis on the nontranscribed strand by DNA pol η , a low fidelity polymerase that preferentially synthesizes mispairs when copying T bases located on the transcribed strand [16–21]. In terms of the nature of the mutations, transitions are more frequent than transversions, and many mutations occur at C within WGCW (W = A/T), an *in vivo* hot-spot motif for AID [22–25]. Transcription is required for SHM, and the rate of transcription is related to the frequency of mutations [26–28].

3.1 Initial phase

AID is a master catalyst which regulates SHM, CSR, and GC [1, 2, 29–31]. Early biochemical studies shed light on the catalytic activity of AID (Fig. 1). AID deaminates C to U on single strand DNA, but not on double strand DNA, DNA:RNA hybrids, or RNA in any form [32–35]. To achieve single strand substrates, DNA could be exposed in transcription bubbles or in RNA-DNA loops. A major question is how AID is targeted to *Ig* genes at a higher frequency than to other genes. There are three levels of targeting to consider. (1) Global targeting, to the H, κ , and λ loci. This is the most enigmatic level and may involve *cis* DNA sequences located at the 3' region of the constant genes to recruit AID [36–38]. (2) Regional targeting to V and switch regions. Mutation occurs for 2–4 kb downstream of

promoters located before rearranged V genes and switch regions. It is believed that AID is brought to stalled RNA polymerases, which increases its activity on single strand DNA exposed during transcription [39, 40]. (3) Local targeting to hotspots. *In vitro*, AID has preferential activity at C within the WRC (R = A/G) motif, which is a subset of the WGCW motif defined *in vivo*. We and others have recently shown that a loop of 11 amino acids located in the C-terminal region of AID recognizes the WRC sequence [41–44]. If this loop is replaced by a different loop from the APOBEC3 family of DNA deaminases, there is less CSR and SHM.

3.2 Developing phase

Once AID transforms cytosines into uracils in *Ig* genes, proteins in two distinct repair pathways, BER and MMR, can recognize the uracil. It is still not completely understood why some of the canonical repair proteins in the BER and MMR repair pathways are shared by the mutagenic pathways during the developing phase (Fig. 1) [45].

Shared proteins in the BER/mutagenic pathway include UNG and APE1. UNG excises the uracil from U:G to create an abasic site opposite G. Other uracil glycosylases, such as MBD4 and SMUG, do not significantly contribute to SHM [46, 47]. APE1 then converts the abasic site to a single strand break. There is some evidence that the MRN (MRE11-RAD50-NBS1) complex, normally involved in double strand break repair, can also participate as an endonuclease in this step [48, 49]. Single strand breaks then provide entry for DNA polymerases to synthesize either faithfully or mutagenically, and multiple breaks initiate CSR.

Shared proteins in the MMR/mutagenic pathway involve MSH2, MSH6, and Exo1. The heterodimer MSH2-MSH6 binds to the U:G mismatch [50], and Exo1 creates a gap [51]. Chromatin immunoprecipitation studies also showed that Exo1 is associated with mutating V regions in the BL2 cell line which undergoes SHM [52]. However, a major unanswered question is what causes a DNA nick to allow entry of Exo1 to create a gap? It has been suggested that APE1 might make the nick [53], but the high frequency of A:T mutations in *Ung*^{-/-} mice [54], which would have very reduced abasic sites and nicks, argues for another source to create the single-strand breaks. There is no strong role for participation of the other MMR proteins MSH3, PMS2, or MLH1 in generating mutations [55–60]. Exo1 normally acts after PMS2 and MLH1 involvement during MMR, and it is not known how PMS2 and MLH1 are excluded from the site during mutagenesis (see Chahwan et al. *this issue*).

3.3 Resolution phase

After the uracils are removed by UNG or Exo1, low fidelity DNA polymerases engage with the DNA to produce mutations. In the BER/mutagenic pathway (Fig. 1), the intermediates to be resolved include abasic sites and single strand breaks. Mutations in this pathway occur predominantly at C:G bp. (1) Abasic sites could be bypassed by Rev1, a deoxycytidyl transferase, which generates C:G to G:C transversions in mice and chicken DT40 cells [61, 62]. Moreover, Rev1 activity in DT40 cells is dependent on monoubiquitination of the PCNA clamp [63, 64]. The modified PCNA recruits Rev1 and other error-prone polymerases to perform synthesis at sites of DNA lesions to prevent replication collapse. Abasic sites can also be bypassed by other low fidelity polymerases to produce transitions and transversions. For example, it is not known which polymerase produces C:G to A:T transversions. (2) Single strand breaks produced by APE1 could provide entry points for pol η , which can displace the adjacent DNA strand during synthesis. This patch synthesis by pol η produces mutations of A:T bp in the absence of MSH2 and MSH6 proteins, and is also dependent on PCNA monoubiquitination [65] (see also Chahwan et al. *this issue*).

In the MMR/mutagenic pathway (Fig. 1), the intermediate to be resolved is a single-strand gap produced by Exo1. Mutations in this pathway occur mostly at A:T bp. In *Msh2*^{-/-}, *Msh6*^{-/-}, *Exo1*^{-/-}, and *Polη*^{-/-} mice, the frequency of mutations at A:T bp decreased by about 75% [20, 21, 23, 51, 56, 66–69], suggesting that these proteins participate in the same pathway. To address this hypothesis, we showed that MSH2-MSH6 binds to pol η *in vitro*, and stimulates its catalytic activity of nucleotide incorporation [50], which may be one reason why pol η is recruited to the gap. The A:T error signature in *Polη*^{-/-} mice correlates to the biochemical activity of pol η *in vitro*, which is known to be more mutagenic when copying A and T bases [70]. Recruitment of pol η also occurs through the modified PCNA clamp, as it was shown that mice with a K164R mutation, which prevents monoubiquitination of PCNA, had a 93% decrease in mutations at A:T bases [71, 72]. To determine if the residual A:T mutations depended on pol η synthesis using another DNA clamp, Jacobs and colleagues examined mice with a defective RAD9-RAD1-HUS1 (9-1-1) clamp, but no change was found [73]. Thus, PCNA monoubiquitination at K164 and pol η are required for A:T mutations, since *Polη*^{-/-} PCNA^{K164R} mice had virtually no A:T mutations [74]. However, the 9-1-1 clamp did appear to play a role in GC in DT40 cells, suggesting it was involved in homologous recombination [75].

In the absence of pol η, some of the residual A:T mutations are contributed by pol κ [76], which is presumably recruited by MSH2-MSH6 and the monoubiquitinated PCNA clamp. However, to see if pol η is the only polymerase functioning in the absence of MSH2, Reynaud and colleagues produced *Msh2*^{-/-} *Polη*^{-/-} double knockout mice [25]. To their surprise, they discovered that all A:T mutations were abolished when compared to the single mutants, indicating that the residual A:T mutagenesis in MSH2-deficient (and MSH6-deficient) mice is contributed by pol η, which has now been recruited by UNG during strand displacement in the BER/mutation pathway. In fact, there is complete loss of all A:T mutations in mice doubly deficient for UNG-MSH2 or UNG-MSH6 [77, 78]. Thus, pol η is the sole contributor of A:T mutations in the normal physiological context [79]. Other polymerases, such as pol θ and pol ι, were analyzed for roles in SHM, but there is no clear evidence for their involvement [80–84].

4. Balance between repair and mutagenesis

It has been estimated that AID generates a minimum of 800-fold more uracils in the *Ig* loci than are found elsewhere during cellular metabolism [4]. Whether these rogue bases are repaired or undergo mutagenesis depends on several factors. (1) G1 vs. S phase. Although AID is expressed throughout the cell cycle [85], cytosine deamination likely takes place in G1 when transcription occurs. AID is linked to proteins in the transcription complex [40, 86, 87] and single strand DNA, the target of AID, is exposed in the transcription bubble. If the resulting U:G mismatch is recognized by MSH2-MSH6 in G1, the resulting gap can be either faithfully repaired by pol δ, or mutagenically processed by pol η to produce mutations at A:T bp. BER also occurs in G1, since UNG and APE1 are active on double strand DNA, and pol β can perform faithful repair. However, UNG is up-regulated during S phase [88], and if the uracil from deamination persists into the replication fork, it will be rapidly removed by UNG, leaving an abasic site. Abasic lesions in single strand DNA are either poor substrates for APE1 or are protected by replication protein A binding, preventing APE1 cleavage [89]. Thus, these persistent abasic sites would likely be replicated by the low fidelity polymerases Rev1 and others, leading to mutations of C:G in S phase [47, 90, 91]. (2) UNG and MSH2. The UNG and MSH2 mutagenic pathways may work together, with APE1 initiating a nick at the UNG-generated abasic site to attract Exo1 and pol η [53, 92]. Clearly, both mutagenic pathways are operating in wild type mice, and could be recruited to the AID-generated uracils to work in concert during G1. (3) DNA repair vs. SHM. To what extent does DNA repair occur in the hyper-actively mutating *Ig* loci? The high frequency of

mutation could either be due to a lack of DNA repair or the plethora of AID-induced damage could overwhelm repair. This question can be addressed by removing proteins in the BER and MMR pathways and examining the effect on SHM frequency and spectra. In general, one would expect to see an increase in the frequency of mutation after an essential gene is eliminated if DNA repair occurs at the same time as SHM. The spectra of mutations would change depending on alterations in the BER pathway affecting C:G bp or in the MMR pathway affecting A:T bp. Table 1 summarizes the changes that occurred in the absence of certain DNA repair genes, and these are discussed below.

4.1 Deficiency of BER genes

UNG-deficient mice were first analyzed in 2002 by Neuberger and colleagues [54]. These results, in combination with their previous papers on the role of UNG in bacteria [93] and DT40 cells [94], nailed the mechanism of AID to DNA deamination. The overall frequency of mutation in the J_H4 intron was unchanged in *Ung*^{-/-} mice, although there was a drastic drop in C:G transversions (Table 1). However, when the absolute frequencies were measured, there was a significant rise in C:G transitions [65, 95]. There was also a seven-fold increase in the frequency of C:G transitions in VJ genes from *Ung*^{-/-} DT40 cells [14]. This indicates that UNG participates in error-free repair, and in its absence, the mutation frequency increases. Thus, UNG is involved in both generating C:G transversions at uracils and removing uracils causing C:G transitions during canonical BER.

Polβ^{-/-} mice were examined for SHM in the S_μ region by Stavnezer and colleagues, and there was an increased frequency, particularly of A:T mutations [96]. These results suggest that pol β is involved in error-free synthesis during BER, and in its absence, pol η may be recruited to generate mutations at A:T bp. Another study showed that BL2 subclones that had spontaneously down-regulated pol β contained a high frequency of SHM in the VDJ gene [97]. Most mutations in the BL2 cell line are at C:G bp, so it is unknown if other polymerases could substitute in the absence of polβ.

Xrcc1^{+/-} mice were tested in our lab for SHM in the J_H4 region, and they had a significant two-fold increase compared to wild type littermates [98]. These results were striking considering that the haploinsufficient B cells still retained 50% of the XRCC1 protein. The spectra of mutations was unchanged, indicating that the increased mutations were generated by both the UNG and MSH2 mutagenic pathways to affect C:G and A:T substitutions, respectively.

In summary, canonical BER is active during SHM, since in the absence of UNG, polβ, and XRCC1 proteins, the frequency of mutations increased. This suggests that the plethora of AID-induced lesions, including abasic sites and single strand breaks, allows some of them to escape faithful BER and become substrates for the mutagenic pathways.

4.2 Deficiency of MMR genes

MSH2-deficient mice were analyzed by several groups and found to have a decreased frequency of SHM, and most notably, a decrease in mutations of A:T bp (Table 1). The concomitant decrease in frequency and A:T mutations in B cells from mice deficient for MSH6 and Exo1, as well as DNA polη, led to a straight-forward model of MSH2-MSH6 recognition of U:G mismatches, followed by gap formation by Exo1, and fill-in by pol η. In the absence of each of these three MMR proteins, the overall frequency was expected to decrease because there were fewer A:T mutations. However, the results from mice doubly-deficient for MSH2 and MSH6 led to another conclusion, when Scharff and colleagues [99] showed that the SHM frequency was unchanged. Although A:T mutations decreased, C:G transitions actually increased, leading to the same overall SHM frequency found in wild type

mice. Increased C:G transitions have been reported in single-deficient mice by others [23, 56, 65–69], but this study documented a larger increase in the absence of both MSH2 and MSH6. They proposed that in wild type mice, MSH2-MSH6 recruits Exo1 to create a gap, and pol η replicates over C and G bases correctly, but copies A and T bases incorrectly. Thus, in the absence of MSH2-MSH6, and MSH2-pol η [25], uracils could remain in the DNA and are replicated as C:G transitions. It is also possible that MSH2-MSH6 stimulates UNG activity, and in their absence, uracils persist to generate C:G transitions.

In summary, canonical MMR seems to be suppressed during SHM, since the absence of PMS2 and MLH1 proteins had little effect on the frequency or spectra of mutations.

5. Concluding remarks

In the absence of both UNG and MSH2 [77], or UNG and MSH6 [78], the frequency of SHM was unchanged, perhaps because these proteins have dual roles in generating and repairing mutations, which may explain why the frequency is not increased in their absence (Table 1). However, the spectrum in the double-deficient mice was dramatically altered, in that there were no A:T mutations and C:G transversions. Thus, these mice display the footprint of AID deamination in the complete absence of repair, which produces only C:G transitions. The large number of mutations indicates that AID introduces abundant deamination events. The increase in mutation frequency in the absence of several BER proteins implies that many of the AID-induced lesions are repaired faithfully by BER. Although there is robust support for BER activity during SHM, there is little evidence for faithful MMR activity, since in the absence of MMR proteins, there was either no change or a decrease in the frequency.

There are still many questions to be answered. For example, why is the MMR pathway not completing faithful repair of the U:G mismatch? What generates the nick that allows entry for Exo1 and pol η ? What other polymerases cause C:G to A:T transversions, and the residual A:T mutations in *Pol η ^{-/-} Pol κ ^{-/-}* mice? Do B cells activated through the Ig receptor *vs.* the toll-like receptors express different levels of UNG, which could produce DNA repair rather than SHM/CSR? Does DNA repair decrease with age, so that B cells from old animals have more translocations than cells from young animals? In conclusion, the simultaneous interactions between DNA repair proteins and low fidelity polymerases for AID-induced damage make the *Ig* loci a war zone for genomic stability, and it will be fascinating to see what circumstances determine who wins.

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Highlights

Activation-induced deaminase generates mutagenic uracils in variable genes

Uracils are processed by either DNA repair or mutagenic pathways

UNG and APE1 proteins participate in both base excision repair and mutagenesis

MSH2-MSH6 and EXO proteins participate in both mismatch repair and mutagenesis

Base excision repair, but not mismatch repair, occurs during somatic hypermutation

Fig. 1.

AID-induced uracils are processed through either DNA repair or mutagenesis. In the initial phase, AID deaminates cytosine to uracil. In the developing phase, the rogue uracils are recognized by two sets of proteins: UNG or MSH2-MSH6. The DNA undergoes incision by APE1 or excision by EXO1, with stars representing the deleted bases. In the resolution phase, the UNG processed substrates can either be faithfully repaired by components in BER pathway, or mutagenically managed by low fidelity DNA polymerases to produce SHM. Similarly, the MSH2-MSH6 processed substrates can either be faithfully repaired by proteins in the MMR pathway, or mutagenically handled by low fidelity polymerases. Polymerases are recruited to the breaks by monoubiquitinated (red circle) PCNA to generate SHM.

Table 1

DNA repair genes affecting SHM.

DNA repair pathway	Gene	Region	Frequency	Spectra	Reference
BER	<i>Ung</i> ^{-/-a}	J _H 4 intron	No change	No C:G tv ^d	54
	<i>Ung</i> ^{-/-b}	VJ	Increase	No C:G tv	14
	<i>Polβ</i> ^{-/-}	Sμ	Increase	Increased A:T	96
	<i>Polβ</i> ^{downc}	VDJ	Increase	No change	97
	<i>Xrcc1</i> ^{+/-}	J _H 4 intron	Increase	No change	98
MMR	<i>Msh2</i> ^{-/-}	VJ, J _H 4 intron	Decrease	Decreased A:T	56, 66, 67
	<i>Msh6</i> ^{-/-}	VDJ, J _H 4 intron	Decrease	Decreased A:T	23, 68
	<i>Exo1</i> ^{-/-}	VDJ, J _H introns	Decrease	Decreased A:T	51
	<i>Msh2</i> ^{-/-} <i>Msh6</i> ^{-/-}	VDJ, J _H introns	No change	Decreased A:T; increased C:G ts ^d	99
BER & MMR	<i>Ung</i> ^{-/-} <i>Msh2</i> ^{-/-}	J _H 4 intron	No change	No A:T; no C:G tv	77
	<i>Ung</i> ^{-/-} <i>Msh6</i> ^{-/-}	J _H 4 intron	No change	No A:T; no C:G tv	78

^aUnless specified, all of the studies were done in mice
^bChicken DT40 cells
^cBL2 cell line
^dtv, transversions; ts, transitions