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Review

Antibody diversification caused by disrupted mismatch repair and promiscuous DNA polymerases

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ABSTRACT

The enzyme activation-induced deaminase (AID) targets the immunoglobulin loci in activated B cells and creates DNA mutations in the antigen-binding variable region and DNA breaks in the switch region through processes known, respectively, as somatic hypermutation and class switch recombination. AID deaminates cytosine to uracil in DNA to create a U:G mismatch. During somatic hypermutation, the MutSα complex binds to the mismatch, and the error-prone DNA polymerase η generates mutations at A and T bases. During class switch recombination, both MutSα and MutLα complexes bind to the mismatch, resulting in double-strand break formation and end-joining. This review is centered on the mechanisms of how the MMR pathway is commandeered by B cells to generate antibody diversity.

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Contents

1. Introduction to AID and canonical DNA repair

Cells have evolved multiple pathways to maintain genomic integrity. These pathways include mismatch repair (MMR) to correct DNA replication errors, base excision repair (BER) and nucleotide excision repair to mend base damage from genotoxic agents, and translesion synthesis to bypass lesions. In most cells, these pathways work to efficiently remove DNA mispairs and damaged bases, and faithfully restore DNA to its original sequence. However, B cells use the MMR and BER pathways to generate DNA mutations as part of the antibody diversification process. Initially, the antibody repertoire is created in pre-B cells by the recombination of immunoglobulin (Ig) V(D)J (variable, diversity, joining) gene segments, and by the pairing of heavy and kappa or lambda light chains [1]. The antibody pool is subsequently expanded in mature B cells upon antigen exposure. These antigen-activated B cells undergo further diversification through somatic hypermutation (SHM) of rearranged variable genes and class switch recombination (CSR) of heavy chain constant genes, both of which are initiated by the enzyme activation-induced deaminase (AID) [2].

AID was discovered by Muramatsu et al. in 1999 [3], and was shown to be a member of the mRNA-editing APOBEC protein family [4]. However, further work revealed that AID acts upon DNA [5–7], where it deaminates cytosine to uracil in single-strand regions of DNA formed during transcription [8]. The protein is

Abbreviations: AID, activation-induced deaminase; APE, apurinic/apyrimidinic endonuclease; BER, base excision repair; bp, base pair; CSR, class switch recombination; D, diversity gene segment; EXO1, exonuclease 1; Ig, immunoglobulin; J, joining gene segment; pol, polymerase; MMR, mismatch repair; NHEJ, non-homologous end joining; SHM, somatic hypermutation; SMUG1, single-strand-selective monofunctional uracil-DNA glycosylase; UNG, uracil DNA glycosylase; V, variable gene segment.

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highly expressed in germinal centers from spleen, lymph nodes, and Peyer's patches [3,9]. AID features an 11 amino acid C-terminal recognition loop, LYFCEDRKAEP, that favors binding to the C in WGCW (where W = A or T) sequence hotspots and deaminates both DNA strands [10–14]. The complete mechanism behind the upregulation and targeting of AID activity exclusively to the Ig loci is currently unknown, although enhancer regions and RNA polymerase II pausing are believed to play major roles [15–18]. AID targeting must be strictly regulated, because deaminations in non-Ig genes can generate translocations that lead to the development of diseases such as B-cell lymphomas [19]. The AID-induced U:G mismatch will mimic T:G, resulting in a C:G to T:A transition following DNA replication of the uracil. Alternatively, the improper uracil either can be repaired via canonical repair pathways, or can employ disrupted repair and translesion polymerases (pol) to generate antibody diversification by SHM and CSR.

As discussed in more detail elsewhere in this issue [20], DNA repair of base damages, including mismatched uracils, relies on the MMR and BER pathways [21a,22]. Canonical MMR uses a heterodimer complex formed by either MutSα, consisting of MSH2 and MSH6, or MutSβ, formed by MSH2 and MSH3, to recognize and bind to mismatches. MutSα targets single nucleotide mismatches, while MutSβ targets loops formed by inserts, deletions, and multi-base mispairs. A MutL heterodimer, containing either MLH1 and PMS2 (MutLα), MLH1 and PMS1 (MutLβ), or MLH1 and MLH3 (MutLγ) is then recruited to the mismatch [21b]. This review will emphasize MutLα, as the other MutL complexes may not be involved in the immune response [23]. MutLα introduces a nearby nick that acts as an exonuclease entry point. Exonuclease 1 (EXO1) removes the mismatch and adjacent bases, creating a single-strand gap. The PCNA sliding clamp recruits a high-fidelity DNA pol, such as pol δ or ε, to accurately resynthesize the gap, followed by DNA ligase I to seal the freshly-repaired strand. Alternatively, BER uses uracil DNA glycosylase (UNG) to remove rogue uracils. This leaves behind an abasic site, which is then cleaved by an apurinic/apyrimidinic endonuclease (APE), producing a single-strand break. Pol β excises the 5′ deoxyribose phosphate group and inserts the correct base, and DNA ligase III closes the nick.

Although canonical DNA repair is desirable under most circumstances, a significantly altered process ensues in B cells during antibody development. In a mechanism initially proposed by Rada et al. [24], adjustments to the MMR pathway can introduce mutations at A and T bases, while a modified BER pathway is responsible for generating mutations at C and G bases. MMR and BER proteins also participate in switching between constant genes during CSR. This review focuses on how the MMR pathway is manipulated by B cells to generate antibody diversity.

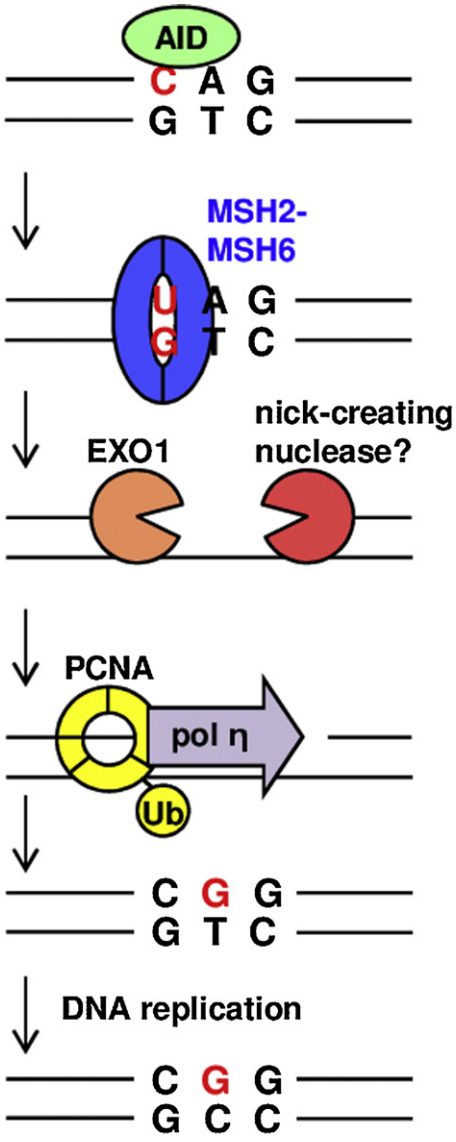

Fig. 1. MMR proteins create mutations at A:T bp during SHM. AID deaminates cytosine to uracil in immunoglobulin variable region DNA and generates a U:G mismatch that is recognized by the MSH2–MSH6 heterodimer. A single-strand DNA gap is produced at the mismatch by EXO1 and an unknown nick instigator. Monoubiquitinated PCNA homotrimer recruits the error-prone DNA pol η to fill in the gap, copying the original T with a G instead of an A. DNA replication results in the G:T mismatch being permanently affixed in one of the two daughter cells as a mutation to G:C.

2. MutSα complex generates A:T mutations during SHM

2.1. Pathways responsible for creating Ig variable region diversity

Antibodies contain both a rearranged V(D)J gene, which regulates antigen binding, and a constant gene, which determines isotype. Upon antigen exposure, B cells in germinal centers undergo successive rounds of SHM [25]. Mutations occur in two regions of DNA: (1) the variable region containing the rearranged VDJ or VJ gene, and (2) the switch region preceding each constant gene. Mutations start just downstream of transcription start sites in the promoter (variable region) and intronic enhancer (switch region), indicating that transcription is necessary for AID activity [26,27]. These mutations typically occur as single base substitutions and form at an elevated frequency of 10^{-2} mutations/bp, compared to spontaneous mutation in other loci, which occurs at a frequency of 10^{-8} mutations/bp [28]. The result of SHM in variable regions is increased affinity of the antibody for antigen, and the result of SHM in the switch region is increased double-strand breaks for CSR from IgM to IgG, IgA, and IgE. The absence of AID in humans leads to type II hyper-IgM syndrome [29], where individuals are at increased risk of disease because they can only produce low affinity antibodies of the IgM isotype. Contrary to intuition, deficiencies in MMR proteins actually lead to decreased mutagenesis in variable and switch regions [30,31]. This occurs because SHM relies on a hijacked version of the MMR pathway to create mutations at A and T residues [32] (Fig. 1).

The MutSα heterodimer binds to an AID-induced U:G mismatch and recruits a nick-creating nuclease. The identity of the nuclease is unknown, but it is unlikely to be the MutLα complex employed in canonical repair; possible suspects are examined in more detail in Section 2.2. This nuclease acts along with EXO1 to remove the mismatch and adjacent bases. Monoubiquitinated PCNA then encircles the gap and binds error-prone DNA pol η, which favors synthesis