

Transcription-coupled mutagenesis by the DNA deaminase AID

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Published: 27 February 2004

Genome Biology 2004, **5**:211

The electronic version of this article is the complete one and can be found online at <http://genomebiology.com/2004/5/3/211>

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Abstract

Activation-induced deaminase (AID) initiates switch recombination and somatic hypermutation of immunoglobulin genes in activated B cells. Compelling evidence now shows that AID travels with RNA polymerase II to deaminate actively transcribed DNA.

A common mechanism for class-switch recombination and somatic hypermutation

In all cells, high-fidelity pathways repair DNA to maintain the integrity of the genome. A handful of genes exempt themselves from this standard of immutability, however, most notably the loci that encode antigen receptors in B cells and T cells. Early in B- and T-cell development, site-specific cleavage and rejoining of V, D and J gene segments occurs to encode functional antigen receptors. Later, upon B-cell activation, the immunoglobulin loci undergo two additional and distinct genetic alterations (Figure 1). In class-switch recombination, regulated DNA deletion replaces one heavy chain constant region with another, changing the antibody's class but not its antigen specificity and thus optimizing clearance of antigen from the body. In somatic hypermutation, targeted and rampant mutagenesis alters the sequences of the expressed heavy and light chain variable (VDJ) regions. Coupled with selection for B cells expressing high-affinity antigen receptors, hypermutation enhances the efficiency and specificity of the immune response.

The first evidence that switch recombination and somatic hypermutation share any mechanistic components came in 2000, when a pair of papers [1,2] from Honjo, Durandy and collaborators showed that a single polypeptide, activation-induced deaminase (AID), induces both switch recombination and somatic hypermutation in mice [1] and humans [2]. AID is homologous to APOBEC1, a deaminase that edits a specific cytidine in the apolipoprotein B transcript to produce a nonsense codon that results in the expression of a

truncated polypeptide [3]. This evolutionary relationship cast a long shadow over initial scenarios for how AID might function. It was anticipated that a specific RNA target for AID would soon be identified, and would in all likelihood be found to encode a master regulator in the form of a critical nuclease or transcription factor [4]. If such a target does exist, it has yet to be discovered. Instead, overwhelming experimental evidence supports the view that AID deaminates a C to a U in transcribed DNA.

Transcription-coupled mutagenesis

Evidence that AID acts in concert with transcription comes from a lovely recent paper [5]. The process of switch recombination is activated and targeted by the transcription of switch (S) regions, guanine-rich, 2-10 kilobase stretches of DNA located just upstream of those constant regions that participate in switch recombination (Figure 1). Transcription of each S region is driven by a dedicated promoter that is responsive to specific transcription factors, which in turn are regulated by extracellular signals delivered by cytokines. Shimizu and collaborators [5] used chromatin immunoprecipitation to show that AID is present at the transcribed S γ 1 and S ϵ switch regions in murine splenic B cells cultured in conditions that induce switching to γ 1 and ϵ (for immunoglobulins IgG1 and IgE, respectively); and, conversely, that AID could be found only at S γ 1 and not at S ϵ if cells were cultured in conditions that stimulated switching to γ 1 but suppressed switching to ϵ . Furthermore, they obtained evidence for direct association between AID and RNA polymerase II.

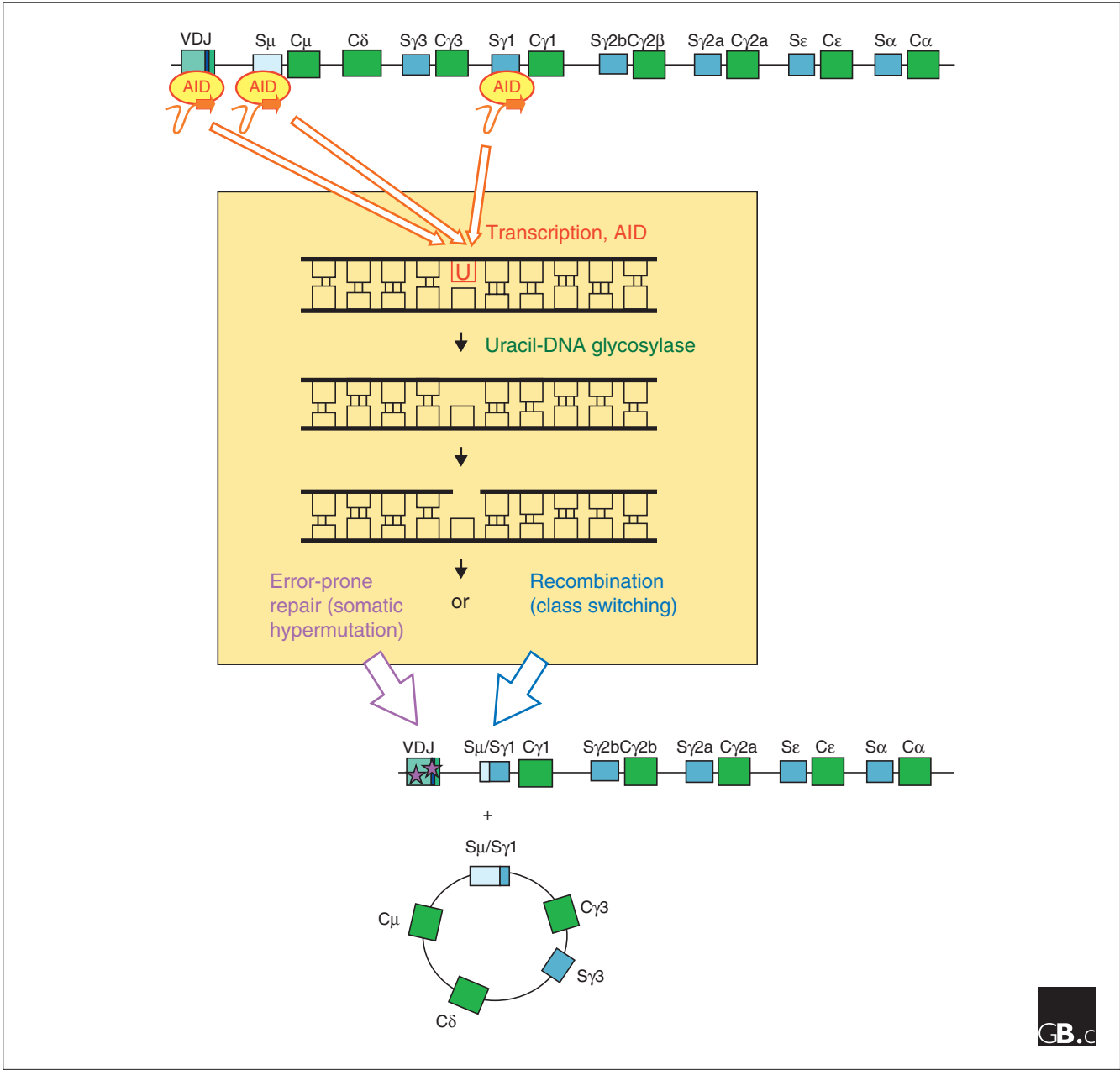


Figure 1
Transcription-coupled mutagenesis initiates class-switch recombination and somatic hypermutation. The murine immunoglobulin heavy chain locus is shown (top line) with a rearranged variable (VDJ) region juxtaposed to the C μ constant region. AID is depicted traveling with the transcription apparatus (tailed arrows) at the expressed variable region and the activated S μ and S γ 1 switch regions. The shaded box illustrates how AID first deaminates C to U, and then uracil-DNA glycosylase removes U, leaving an abasic site. Subsequent steps generate single-strand breaks [17], which become substrates for mutagenic repair or recombination. Somatic hypermutation alters variable region sequence, and switch recombination joins a new constant region (C γ 1) to the expressed variable region, producing an extrachromosomal DNA circle (bottom), which contains the deleted region. The final result is a heavy chain locus containing a mutated variable region (mutations are indicated by stars) and a chromosomal S μ /S γ 1 junction (bottom).

Thus, AID associates with the transcription apparatus to attack the transcribed S-region DNA (Figure 1).

Other data support and complement this conclusion. In experiments that draw on our understanding of how uracil in DNA is normally repaired, genetic analysis has produced compelling evidence that AID deaminates DNA. C to U deamination is common (100 C to U deamination events occur each day in each mammalian cell). The highly conserved pathways that repair uracil in DNA rely on uracil-DNA

glycosylases to remove the uracil base, and apurinic endonucleases to nick the abasic site so that new DNA synthesis can recreate the duplex [6]. Reasoning that if AID deaminates DNA, repair of AID-induced lesions would depend upon uracil-DNA glycosylase activities, Neuberger and collaborators [7] showed that expression of AID in *Escherichia coli* stimulates mutation at G•C base pairs, and that mutation levels are amplified in a strain deficient in uracil-DNA glycosylase. Climbing the evolutionary ladder, this same group showed that inhibition of uracil-DNA glycosylase alters the mutation spectrum in a hypermutating chicken bursal lymphoma cell line [8], and that switch recombination is impaired and the spectrum of hypermutation altered in mice deficient in uracil-DNA glycosylase [9]. Quite recently, Durandy and collaborators [10] reported a similar phenotype in humans deficient in uracil-DNA glycosylase.

Biochemical analysis of the substrate specificity of the AID deaminase provided a breakthrough on another front. Most tellingly, the Goodman and Alt laboratories [11,12] showed that, while AID does not attack duplex DNA, it will deaminate cytidines in single-stranded DNA within the exposed single-stranded region of an artificial transcription bubble or a transcribed substrate. This provides a mechanistic explanation for the dependence of both class-switch recombination and somatic hypermutation on transcription, and for the correlation between the level of hypermutation and the level of transcription. Moreover, AID was shown to be processive *in vitro* and to preferentially alter sequences at specific hotspot motifs [13]. Thus, enzymatic activity recapitulates two features evident in the sequences of literally thousands of hypermutated variable regions, namely that mutation focuses at hotspots, and that if a variable region is mutated at all, it displays multiple mutations.

When AID was first discovered, the RNA-editing enzyme APOBEC1 [3] was its only characterized relative, and the hypothesis that AID acted on RNA was grounded in this evolutionary precedent. We now know of at least nine members of the APOBEC gene family in humans, including seven genes (or pseudogenes) tandemly linked in the APOBEC3 locus at 22q13 [14]. One of these, APOBEC3G, protects against retroviral infection by specifically deaminating the first-strand cDNA synthesized by reverse transcriptase (reviewed in [15]). The human immunodeficiency virus HIV-1 evades this protective mechanism by expressing a protein, Vif, that binds APOBEC3G and targets it for proteolytic degradation [16]. Thus the evolutionary rationale for believing that AID must have an RNA target is no longer valid.

Knowing that AID induces transcription-coupled mutagenesis by deaminating C to U in DNA, and that uracil-DNA glycosylase removes U to produce an abasic site (see shaded box in Figure 1), we can now look forward to a molecular understanding of the downstream steps in the switching and hypermutation pathways. Many questions remain to be

answered. How is the abasic site processed to create the single-strand breaks characteristic of hypermutating V regions [17]? How is the normally faithful uracil-DNA repair pathway diverted to have a recombinogenic or mutagenic outcome? How are the S regions juxtaposed for recombination? How do other highly expressed genes avoid mutation? We can look forward to progress in these and related areas in the near future.

Acknowledgements

We gratefully acknowledge support from the US National Institutes of Health, grant numbers R01 GM39799 and R01 GM41712 (N.M.) and F32 GM67482 (E.D.L.).

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