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J_H6 downstream intronic sequence is dispensable for RNA polymerase II accumulation and somatic hypermutation of the variable gene in Ramos cells

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ABSTRACT

Activation-induced deaminase (AID) introduces nucleotide substitutions within the variable region of immunoglobulin genes to promote antibody diversity. This activity, which is limited to 1.5 kb downstream of the variable gene promoter, mutates both the coding exon and downstream intronic sequences. We recently reported that RNA polymerase II accumulates in these regions during transcription in mice. This build-up directly correlates with the area that is accessible to AID, and manipulation of RNA polymerase II levels alters the mutation frequency. To address whether the intronic DNA sequence by itself can regulate RNA polymerase II accumulation and promote mutagenesis, we deleted 613 bp of DNA downstream of the J_H6 intron in the human Ramos B cell line. The loss of this sequence did not alter polymerase abundance or mutagenesis in the variable gene, suggesting that most of the intronic sequence is dispensable for somatic hypermutation.

1. Introduction

During B cell development, variable (V), diversity (D), and joining (J) gene segments recombine to create a functional V(D)J exon. This initial diversity is further expanded after antigen encounter, when B cells mutate their V(D)J genes to increase antibody affinity. The process is initiated by activation-induced deaminase (AID) (Muramatsu et al., 2000; Revy et al., 2000), an enzyme which converts cytosine to uracil in single-strand DNA (Di Noia and Neuberger et al., 2002; Maul et al., 2011; Petersen-Mahrt et al., 2002). The presence of uracil in DNA initiates a mutagenic cascade, resulting in the introduction of point mutations within the V region during somatic hypermutation (SHM), and DNA double-strand breaks within switch (S) regions preceding constant (C) genes during class switch recombination. The ability of AID to access single-strand DNA is intimately associated with the process of transcription by RNA polymerase II (Pol II) (Bransteitter et al., 2003; Chaudhuri et al., 2003; Dickerson et al., 2003; Pham et al., 2003; Ramiro et al., 2003; Sohail et al., 2003). It has recently been proposed that AID physically interacts with components of the stalled transcription complex to bring AID to the immunoglobulin locus (Pavri et al., 2010; Willmann et al., 2012). In S regions, Pol II stalling is caused by the generation of R-loop structures (Rajagopal et al., 2009; Yu et al., 2003). These structures occur when the newly transcribed RNA remains annealed to the transcribed DNA strand and occludes the non-transcribed strand from reannealing. However, in V regions, there are no R-loop structures that arise during transcription. Nonetheless, we recently described the accumulation of Pol II which correlates with AID activity (Maul et al., 2014), indicating that other DNA sequences may be involved in pausing Pol II.

During SHM, mutagenesis is limited to a 1.5 kb region downstream of the Ig promoter. This mutation window spans both the leader (L) and V(D)J exons and downstream intronic sequences on both heavy and light chain loci. Interestingly, the distance is independent of which V(D)J gene is used, suggesting that any conserved AID targeting element would not be found within the exon itself. Consistent with this hypothesis, replacement of the V(D)J exon with non-immunoglobulin sequences produced normal frequencies of mutagenesis (Yeap et al., 2015; Yelamos et al., 1995). Thus, any *cis* targeting component might be located within other DNA sequences in the mutation window. The intron sequences downstream of rearranged J gene segments are attractive candidates because they are proximal to V(D)J genes and are shared in every rearrangement. In mice, intronic sequences have frequencies of mutation that are comparable to those in adjacent V(D)J exons (Gearhart and Bogenhagen, 1983; Kim et al., 1981; Lebecque and

Abbreviations: AID, activation-induced deaminase; Pol II, RNA polymerase II; SHM, somatic hypermutation

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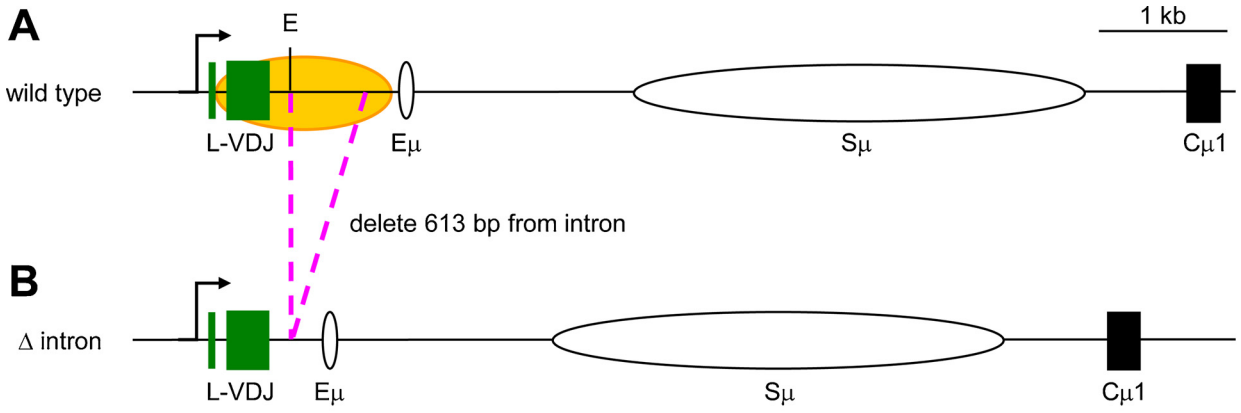

C

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                10        20        30        40        50        60        70        80        90        100
                |....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
WT-A            GGCGAGTCCTGGCACAGACGGGAGGTACGGTATGGACGTCTGGGGCCAAGGGACCACGGTCACCGTCTCCTCAGGTAAGAATGGCCACTCTAGGGCCTTT
Δ Intron        GGCGAGTCCTGGCACAGACGGGAGGTACGGTATGGACGTCTGGGGCCAAGGGACCACGGTCACCGTCTCCTCAGGTAAGAATGGCCACTCTAGGGCCTTT
                                                                  JH6
                110       120       130       140       150       160       170       180       190       200
                |....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
                GTTTTCTGCTACTGCCTGTGGGGTTTCCTGAGCATTGCAGGTTGGTCCTCGGGGCATGTTCCGAGGGGACCTGGGCGGACTGGCCAGGAGGGGACGGGCA
                GTTTTCTGCTACTGCCTGTGGGGTTTCCTGAGCATTGCAGGTTGGTCCTCGGGGCATGTTCCGAGGGGACCTGGGCGGACTGGCCAGGAGGGGACGGGCA

                210       220       230       240       250       260       270       280       290       300
                |....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
                CTGGGGTGCCTTGAGGATCTGGGAGCCGAATTCATATGCGTACGTCTGTGGATTTTCCGATGCCTTTGGAAAATGGGACTCAGGTTGGGTGCGTCTGATG
                CTGGGGTGCCTTGAGGATCTGGGAGCCGAATTC----------------------------------------------------------------
                                          EcoRI
                310       320       330       340       350       360       370       380       390       400
                |....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
                GAGTAACTGAGCCTGGGGGCTTGGGGAGCCACATTTGGACGAGATGCCTGAACAAACCAGGGGTCTTAGTGATGGCTGAGGAATGTGTCTCAGGAGCGGT
                ----------------------------------------------------------------------------------------------

                410       420       430       440       450       460       470       480       490       500
                |....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
                GTCTGTAGGACTGCAAGATCGCTGCACAGCAGCGAATCGTGAAATATTTTCTTTAGAATTATGAGGTGCGCTGTGTGTCAACCTGCATCTTAAATTCTTT
                ----------------------------------------------------------------------------------------------

                510       520       530       540       550       560       570       580       590       600
                |....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
                ATTGGCTGGAAAGAGAACTGTCGGAGTGGGTGAATCCAGCCAGGAGGGACGCGTAGCCCCGGTCTTGATGAGAGCAGGGTTGGGGGCAGGGGTAGCCCAG
                ----------------------------------------------------------------------------------------------

                610       620       630       640       650       660       670       680       690       700
                |....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
                AAACGGTGGCTGCCGTCCTGACAGGGGCTTAGGGAGGCTCCAGGACCTCAGTGCCTTGAAGCTGGTTTCCAAGAGAAAAGGATTGTTTATCTTAGGAGGC
                ----------------------------------------------------------------------------------------------

                710       720       730       740       750       760       770       780       790       800
                |....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
                ATGCTTACTGTTAAAAGACAGGATATGTTTGAAGTGGCTTCTGAGAAAAATGGTTAAGAAAATTATGACTTAAAAATGTGAGAGATTTTCAAGTATATTA
                ----------------------------------------------------------------------------------------------

                810       820       830       840       850       860       870       880       890       900
                |....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
                ATTTTTTTAACTGTCCAAGTATTTGAAATTCTTATCATTTGATTAACACCCATGAGTGATATGTGTCTGGAATTGAGGCCAAAGCAAGCTCAGCTAAGAA
                ----------------------------------------CACCCATGAGTGATATGTGTCTGGAATTGAGGCCAAAGCAAGCTCAGCTAAGAA

                910       920       930       940       950       960       970       980       990       1000
                |....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
                ATACTAGCACAGTGCTGTCGGCCCCGATGCGGGACTGCGTTTTGACCATCATAAATCAAGTTTATTTTTTTAATTAATTGAGCGAAGCTGGAAGCAGATG
                ATACTAGCACAGTGCTGTCGGCCCCGATGCGGGACTGCGTTTTGACCATCATAAATCAAGTTTATTTTTTTAATTAATTGAGCGAAGCTGGAAGCAGATG
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Fig. 1. Somatic hypermutation in the IgH locus of Ramos cells. **A)** Wild type, and **B)** Δ intron. Arrow marks the transcription start site. Green boxes represent L and VDJ exons; yellow oval depicts the area targeted for SHM (Qian et al., 2014); white ovals signify the Eμ enhancer and Sμ region; and black box represents the Cμ1 exon. Pink dotted lines show the deletion. E, EcoRI. **C)** Nucleotide sequence (613 bp) that was deleted downstream of the EcoR1 site (underlined) is marked by red dashes. The rearranged J_H6 gene segment is underlined for reference.

Gearhart, 1990; Pech et al., 1981). The frequency then diminishes after 500 bp, suggesting that AID activity declines over distance. The universal nature of SHM in these unselected introns is confirmed by noting that the 3′ flanking sequences on three different loci in mice undergo SHM: *IgH* (Both et al., 1990; Jolly et al., 1997), *Igκ* (Hackett et al., 1990), and *Igλ* (Gonzalez-Fernandez et al., 1994). Furthermore,