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RAG2 involves the *Igκ* locus demethylation during B cell development

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ABSTRACT

The genes encoding the immunoglobulin κ light chain are assembled during B cell development by V(D)J recombination. For efficient rearrangement, the *Igκ* locus must undergo a series of epigenetic changes. One such epigenetic mark is DNA methylation. The mechanism that the *Igκ* locus is selectively demethylated at the pre-B cell stage has not previously been characterized. Here, we employed bisulfite DNA-modification assays to analyze the methylation status of the *Igκ* locus in primary pre-B cells from RAG-deficient mice with pre-rearranged *Igh* knock-in allele. We observed that the *Igκ* locus was hypermethylated in RAG2-deficient pre-B cells but hypomethylated in RAG1-deficient pre-B cells, indicating that wild-type (WT) RAG2 involves the *Igκ* locus demethylation in a RAG1-independent manner prior to rearrangement. We generated a series of RAG2 mutants between residue 350 and 383. We showed that these mutants mediated the *Igκ* rearrangement but failed to regulate the *Igκ* gene demethylation. We further analyzed that these mutants could increase RAG recombinase activity *in vivo*. We conclude that residues 350–383 region are responsible for endogenous *Igκ* locus demethylation at pre-B cells. We propose that WT RAG2 has an intrinsic function to regulate the *Igκ* locus demethylation.

1. Introduction

During B lymphocyte development, the genes encoding immunoglobulins (Igs) are assembled from discrete variable (V), diversity (D), and joining (J) gene segments through a site-specific recombination reaction named V(D)J recombination (Gellert 2002; Schatz and Swanson 2011). This process is initiated by the proteins encoded by recombination-activating gene 1 (*RAG1*) and *RAG2*, which together cleave DNA at recombination signal sequences (RSSs) that flank the V, D, and J gene segments. RSSs consist of conserved heptamer (5′-CACAGTG) and nonamer (5′-ACAAAAACC) elements separated by a spacer whose length is either 12 or 23 bp (12RSS or 23RSS, respectively). DNA cleavage involves nicking at the junction between the RSS and coding sequence, followed by transesterification to generate a blunt 5′-phosphorylated signal end and a sealed hairpin coding end. Then, the classical non-homologous end joining (c-NHEJ) repair pathway joins the DNA ends in a recombinant configuration, forming a coding joint (CJ) (the rearranged Ig gene) and a reciprocal signal joint (SJ) (McBlane et al., 1995; Rooney et al., 2004; Swanson 2004). Efficient recombination requires a 12RSS and 23RSS, a restriction known as the 12/23 rule (Schatz and Swanson 2011). In this manner, B lymphocytes give rise to the multitude of antigen-recognition specificities that constitute the adaptive immune system.

RAG1 and RAG2 are convergently expressed in developing B and T lymphocytes (Kuo and Schlissel 2009). RAG1 plays crucial roles in both RSS binding and DNA cleavage. It contains 1040 residues, with a core region spanning residues 384–1008 that interacts with the nonamer and heptamer, as well as 3 catalytic residues (D600, D708, and E962) that are responsible for DNA cleavage (Fugmann et al., 2000; Sekiguchi et al., 2001). The functions of RAG2 are poorly understood. RAG2 is composed of 527 amino acids, with a canonical core region (cRAG2) comprising residues 1–383 that cooperates with RAG1 and has no detectable DNA-binding activity by itself, but is indispensable for recombination (Swanson 2004). The C-terminal, non-core region of RAG2 possesses multiple unique features that are indication of regulatory roles. A plant homeodomain finger (PHD; residues 415–487), which binds specifically to tri-methylated histone H3 lysine 4 (H3K4me3), enhances the catalytic activity of the RAG complex and guides RAG2 to regions of active chromatin (Liu et al., 2007; Matthews et al., 2007). Moreover, H3K4me3 acts allosterically at the PHD to relieve auto-inhibition imposed by the domain between the core and noncore regions of RAG2 (Lu et al., 2015). The hinge region (residues 350–410) restricts DNA double-strand breaks (DSBs) induced by RAG in the high fidelity c-NHEJ repair machinery to ensure genomic stability (Coussens et al.,

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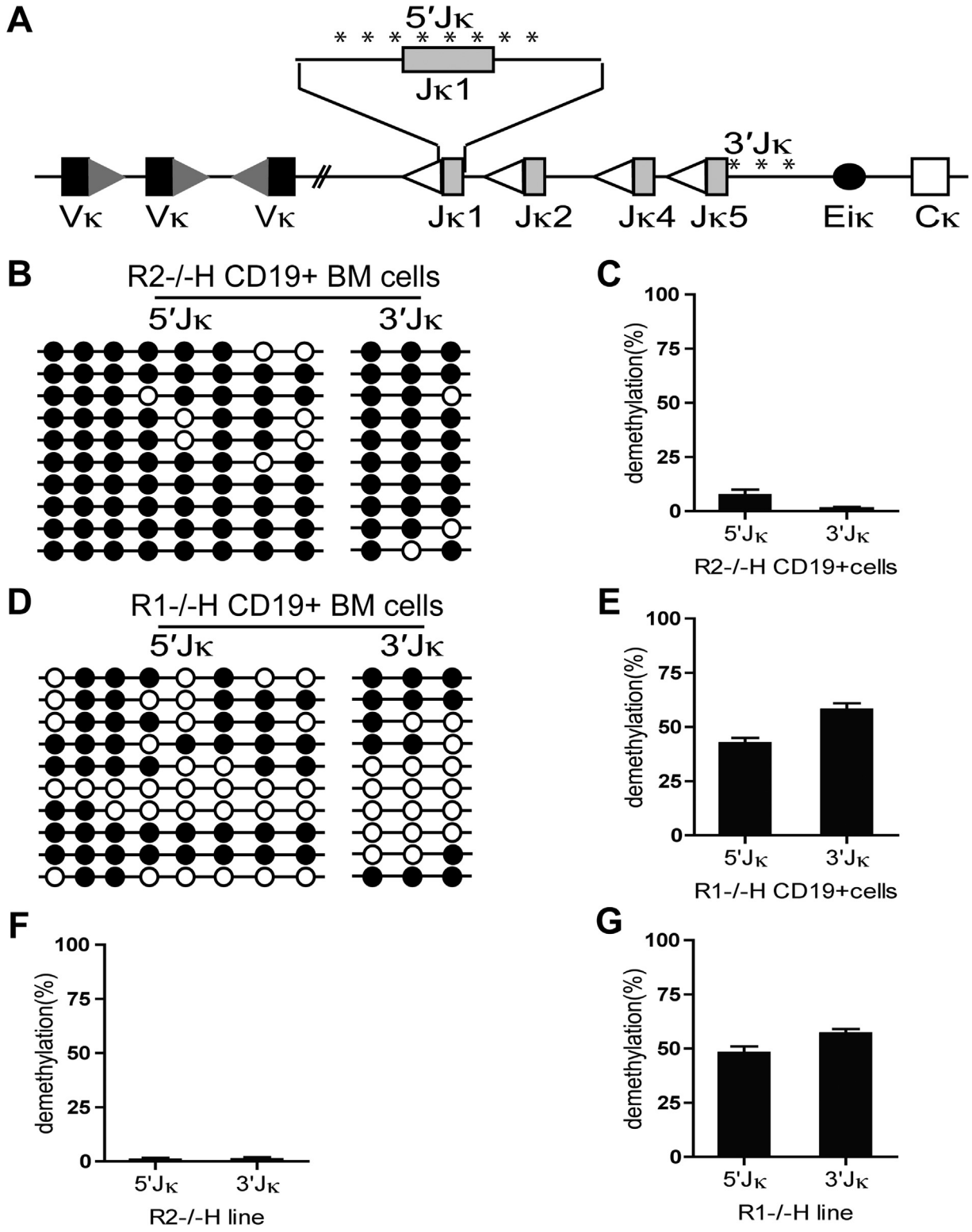

Fig. 1. DNA methylation status of the *Igκ* locus in RAG-deficient mice. (A) Schematic of the murine germline *Igκ* locus (not to scale). Partial Vκ and the 4 individual functional Jκ gene segments are represented with black and gray rectangles. The 12RSSs and 23RSSs elements as represented with gray and white triangles. The intronic enhancer (Eiκ) is represented with a black oval, and the constant region exon (Cκ) is represented with a white rectangle. Each CpG site is indicated with an asterisk. The 5′Jκ contains 8 CpG sites and 3′Jκ includes 3 CpG sites. (B) Genomic DNA was extracted from CD19+ bone marrow cells from R2-/-H mice and subjected to bisulfite-modification assays. Black circles signify methylated CpGs, and white circles signify demethylated CpGs. The methylation status of 5′Jκ and 3′Jκ were analyzed. (C) Graphical representation of the average demethylation levels in (B). (D) The methylation status of 5′Jκ and 3′Jκ in CD19+ bone marrow cells from R1-/-H mice. (E) Graphical representation of the average demethylation levels in (D). (F) Graphical representation of the average demethylation levels of 5′Jκ and 3′Jκ in v-abl transformed R2-/-H pre-B cell line. (G) Graphical representation of the average demethylation levels of 5′Jκ and 3′Jκ in v-abl transformed R1-/-H pre-B cell line. Methylation was measured in DNA from 2 independent preparations.