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RAG2's Acidic Hinge Restricts Repair-Pathway Choice and Promotes Genomic Stability

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SUMMARY

V(D)J recombination-associated DNA double-strand breaks (DSBs) are normally repaired by the high-fidelity classical nonhomologous end-joining (cNHEJ) machinery. Previous studies implicated the recombination-activating gene (RAG)/DNA postcleavage complex (PCC) in regulating pathway choice by preventing access to inappropriate repair mechanisms such as homologous recombination (HR) and alternative NHEJ (aNHEJ). Here, we report that RAG2's "acidic hinge," previously of unknown function, is critical for several key steps. Mutations that reduce the hinge's negative charge destabilize the PCC, disrupt pathway choice, permit repair of RAG-mediated DSBs by the translocation-prone aNHEJ machinery, and reduce genomic stability in developing lymphocytes. Structural predictions and experimental results support our hypothesis that reduced flexibility of the hinge underlies these outcomes. Furthermore, sequence variants present in the human population reduce the hinge's negative charge, permit aNHEJ, and diminish genomic integrity.

INTRODUCTION

DNA double-strand breaks (DSBs) must be detected promptly and repaired accurately to ensure genomic stability. While two major DSB repair pathways, classical nonhomologous end-joining (cNHEJ) and homologous recombination (HR), have been extensively studied (Weterings and Chen, 2008), an increasing number of additional error-prone joining mechanisms (e.g., alternative NHEJ [aNHEJ]) are being recognized (Corneo et al., 2007; Helmink and Sleckman, 2012; Kabotyanski et al., 1998; Simsek and Jasin, 2010; Zhu et al., 2002). aNHEJ can cause chromosome translocations (Mladenov and Iliakis, 2011; Simsek et al., 2011; Simsek and Jasin, 2010; Yan et al., 2007; Zhu et al., 2002) and is implicated in oncogenesis (Wang et al., 2008; Zhu et al., 2002). aNHEJ's ability to join chromosomal DSBs (Bogue et al., 1997, 1998; Malynn et al., 1988; Soulas-Sprauel et al., 2007; Yan et al., 2007) suggests that pathway-choice control could be an important safeguard of genomic integrity. Scant evidence, however, links defects in pathway-choice control with genomic instability.

V(D)J recombination employs DSBs to generate antigen receptors (Helmink and Sleckman, 2012), providing a tractable, physiologically relevant model to investigate pathway choice. The recombination-activating gene 1/2 (RAG1/2) complex assembles on a pair of recombination signal sequences (RSSs), forms a synaptic complex (Hiom and Gellert, 1998; Schatz and Swanson, 2011), generates a pair of DSBs, and remains associated with the four broken DNA ends in a post-cleavage complex (PCC) (Agrawal and Schatz, 1997; Jones and Gellert, 2001), which is essential for pathway-choice control (Arnal et al., 2010; Corneo et al., 2007; Lee et al., 2004). One model posits that the PCC forms a scaffold, retaining the broken DNA ends to facilitate proper repair by cNHEJ and discourage repair by HR and aNHEJ (Corneo et al., 2007; Lee et al., 2004; Tsai et al., 2002), both of which could lead to inappropriate and dangerous outcomes.

To investigate the role of pathway-choice control in maintaining genomic integrity during V(D)J recombination, we focused on RAG2, which lacks known DNA binding or catalytic activity but is essential for recombination and has several regulatory roles. Thymocytes from mice bearing a truncated RAG2 mutant ($RAG2^{core/core}$) display genomic instability and, in the absence of p53, rapidly succumb to thymic lymphomas with complex chromosomal aberrations involving the *Igh* and *Tcrα/δ* loci (Deriano et al., 2011). This truncation removes the carboxy (C) terminus and its regulatory domains (Figure 1A), including a plant homeodomain (PHD) finger that interacts with active chromatin (Callebaut and Mornon, 1998; Ji et al., 2010; Jones

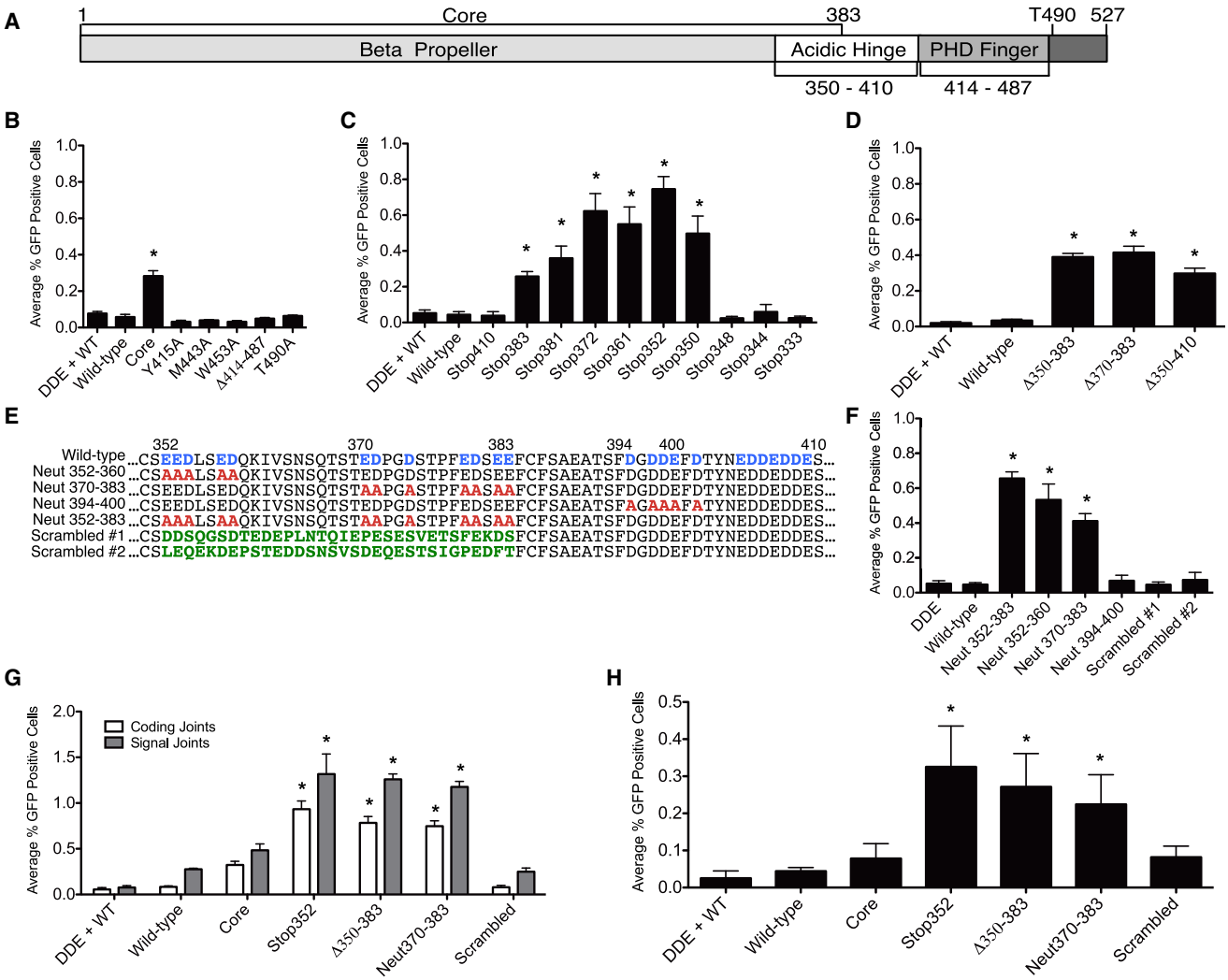

Figure 1. Acidic Residues in the Hinge Region of RAG2 Regulate Repair-Pathway Choice

(A) RAG2 schematic.

(B–F) Quantification of fluorescence-activated cell sorting (FACS) data from three separate experiments for aNHEJ in CHO-K1 cells measuring mutations to known regulatory regions (B), truncation mutations (C), internal deletions (D), and neutralizing mutations (E and F).

(G) XRCC4-deficient CHO cells were tested for their ability to bypass cNHEJ defects using CJGFP and SJGFP.

(H) Recombination on the chromosomal substrate pMX-INV in SCID-MEFs with the indicated RAG2 mutations. Values significantly different from wild-type RAG1 and RAG2 are marked with an asterisk (Student's t test, unpaired, two-tailed, equal variance).

Means from three independent experiments are plotted; error bars reflect the SEM. See also Figures S1 and S2.

and Simkus, 2009; Matthews et al., 2007), and a CDK phosphorylation site (T490) that targets RAG2 for destruction at the G1-to-S transition (Figure 1A; Li et al., 1996; Zhang et al., 2011). Mutating this residue alone (T490A) stabilizes RAG2 protein levels throughout the cell cycle, and T490A knockin lymphocytes contain elevated levels of DSBs (Zhang et al., 2011). These persistent breaks are pathogenic: in a $p53^{-/-}$ background, these T490A mice develop thymic lymphomas with antigen receptor translocations. $Rag2^{core/core}$ $p53^{-/-}$ mice develop lymphomas much more rapidly and with substantially higher penetrance than $Rag2^{T490A/T490A}$ mice, suggesting that additional, unidentified regulatory elements in RAG2's C terminus safeguard the genome.

The C terminus also contains a conserved hinge region (Jones and Simkus, 2009) that lies after a predicted β-propeller (Callebaut and Mornon, 1998) and has a high density of acidic amino acids (Oettinger et al., 1990). A small region of the hinge (residues 402–407) interacts with core histones (West et al., 2005), but the significance of this interaction remains unclear. Nothing further has been reported as to the hinge's function (Jones and Simkus, 2009).

Through a systematic analysis of RAG2's C terminus, we show that the hinge is critical for PCC stability, pathway-choice control, and maintenance of genomic integrity. Based on our data and structural predictions, we hypothesize that the acidic hinge is an intrinsically disordered domain (IDD) that regulates joining