CHAPTER 13

Temporal and Spatial Regulation of V(D)J Recombination: Interactions of Extrinsic Factors with the RAG Complex

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Abstract

In the course of lymphoid development, V(D)J recombination is subject to stringent locus-specific and temporal regulation. These constraints are ultimately responsible for several features peculiar to lymphoid development, including the lineage specificity of antigen receptor assembly, allelic exclusion and receptor editing. In addition, cell cycle phase-dependent regulation of V(D)J recombinase activity ensures that DNA rearrangement is completed by the appropriate mechanism of DNA repair. Regulation of V(D)J recombination involves interactions between the V(D)J recombinase—a heteromeric complex consisting of RAG-1 and RAG-2 subunits—and macromolecular assemblies extrinsic to the recombinase. This chapter will focus on those features of the recombinase itself—and in particular the RAG-2 subunit—that interact with extrinsic factors to establish patterns of temporal control and locus specificity in developing lymphocytes.

Functional Organization of RAG-1 and RAG-2

RAG-1 and RAG-2 are 1040 and 527 amino acid residues long, respectively. Residues 384 through 1008 of RAG-1 constitute the core fragment, which contains the catalytic site for DNA cleavage, mediates binding to recombination signal sequences (RSSs) and makes contacts with the coding flanks. The core RAG-2 fragment (Fig. 1), consisting of residues 1 through 387, extends interactions of RAG-1 with the RSS and is essential for helical distortion near the scissile bond, a possible prerequisite for transesterification. Accordingly, mutations that impair recombinase-mediated cleavage and joining have been identified in core RAG-2.

Residues 387 through 527 of RAG-2 comprise the non-core region (Fig. 1) and are dispensable for DNA cleavage by the RAG proteins in vitro. Nonetheless, removal of this region reduces the efficiency of extrachromosomal recombination, increases production of hybrid joints, impedes endogenous V_H-to-D_J_H joining and promotes aberrant recombination. The mechanisms underlying these effects may be complex, as the non-core region includes multiple functional domains (Fig. 1B).

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Temporal Regulation of V(D)J Recombination through Interactions with the RAG-2 Non-Core Region

The non-core region of RAG-2 supports the periodic destruction of RAG-2 protein. RAG-2 accumulates in quiescent cells and in dividing cells during the G1 phase; rapid degradation of RAG-2 begins at the G1-to-S transition and continues until the following entry into G1. Consequently, the appearance of recombination signal end intermediates and RAG-signal end complexes is restricted to G0/G1. Destruction of RAG-2 is triggered by phosphorylation of threonine 490, which lies within a phylogenetically conserved cyclin-dependent kinase (CdK) target site and is also dependent on a lysine-rich interval spanning amino acid residues 499-508. Overlapping the RAG-2 degradation domain (Fig. 1B) is a noncanonical nuclear localization sequence that supports binding of importin α and nuclear import of RAG-2. At the G1-to-S transition, phosphorylation of RAG-2 by cyclinA/Cdk2 permits association of RAG-2 with the Skp2-SCF ubiquitin ligase. This phosphorylation-dependent interaction is mediated by the F-box protein Skp-2 and its associated protein Cks1. Upon polyubiquitylation of RAG-2 by Skp2-SCF, RAG-2 is subjected to proteasomal degradation.

The cell cycle dependence of V(D)J recombination may play a role in the coupling of DNA cleavage by the RAG complex to DNA repair. V(D)J recombination is normally completed by a form of DNA repair termed nonhomologous end joining (NHEJ). NHEJ is active throughout the cell cycle, but an alternative mechanism for double-strand DNA repair, homologous recombination (HR), is nearly inactive during G1. In thymocytes of mice expressing RAG-2(T490A), aberrant recombinants resembling products of abortive homologous recombination are observed to accumulate. These observations suggest that restriction of RAG-2 accumulation to the G0 and G1 cell cycle phases promotes the correct repair of V(D)J recombination intermediates by NHEJ, perhaps by temporal sequestration of RAG activity from HR.

Figure 1. Regulatory domains of RAG-2. A) Schematic representation of mouse RAG-2. Core and non-core regions are designated; amino acid residues are numbered below. KL, Kelch-like propeller domains; L, linker domain; PHD, plant homeodomain finger; D, domain governing programmed degradation and nuclear import of RAG-2. B) Detailed representation of the non-core region. Amino acid residues at domain boundaries are numbered above. L (black rectangle), PHD (gray rectangle) and D (hatched rectangle) as defined in (A). The hatched interval denotes the extent of the domain governing cell cycle-dependent degradation of RAG-2; the shaded region within this interval marks the nuclear import signal that resides within the degradation domain. Shaded arrowheads, sites of mutations in the linker domain that impair V(D)J recombination. Open arrowheads, targets of mutations in the PHD domain that abolish H3K4me3 binding and impair V(D)J recombination. Black arrowhead, cyclinA/CDK2 phosphorylation site, essential for programmed degradation of RAG-2 at the G1-S transition. Shaded diamond, target of mutation that selectively impairs nuclear import of RAG-2.