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.

Keywords

V(D)J recombination; RAG-1; RAG-2; lymphocyte development; allelic exclusion; cell cycle; DNA repair; non-homologous end joining; homologous recombination; proteasomal degradation; cyclinA/ Cdk2; Skp2-SCF; epigenetic control; DNA methylation; nucleosome phasing; histone acetylation; histone methylation; plant homeodomain; locus accessibility

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 [1-3], mediates binding to recombination signal sequences (RSSs) [4-6] and makes contacts with the coding flanks [7, 8]. 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 [4-6, 9]. Accordingly,

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mutations that impair recombinase-mediated cleavage and joining have been identified in core RAG-2 [10].

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 [11-16], increases production of hybrid joints [17], impedes endogenous V_H-to-DJ_H joining [12, 18, 19] and promotes aberrant recombination [20]. The mechanisms underlying these effects may be complex, as the non-core region includes multiple functional domains (Fig. 1B).

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 [21-23]. Consequently, the appearance of recombination signal end intermediates [24, 25] and RAG-signal end complexes [26] 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 [21]. Overlapping the RAG-2 degradation domain (Fig. 1B) is a non-canonical nuclear localization sequence that supports binding of importin 5 and nuclear import of RAG-2 [27]. 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 [28].

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 non-homologous 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 [29]. In thymocytes of mice expressing RAG-2 (T490A), aberrant recombinants resembling products of abortive homologous recombination are observed to accumulate [21]. 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.

Locus specificity: general remarks

The V(D)J recombinase is directed toward particular sets of gene segments, depending on lymphoid lineage and developmental stage. Recent work has begun to provide a framework for understanding how this targeting is achieved. At the level of unchromatinized DNA, the V(D)J recombinase is targeted to antigen receptor gene segments by means of specific interactions with flanking RSSs, and this recognition does not require the non-core regions of RAG-1 or RAG-2. Not all RSSs support recombination with the same efficiency, because RSSs exhibit considerable sequence variation. Although sequence variation among RSSs can indeed affect gene segment usage [30], these differences cannot account for the dynamic shifts in locus specificity that accompany commitment to distinct lymphoid lineages and developmental transitions within lineages. Rather, ordered rearrangement of antigen receptor gene segments is associated with the imposition or relief of epigenetic marks. Specific chromatin modifications in the vicinity of RSSs are strongly associated with the presence or absence of ongoing rearrangement. The propensity of a particular locus to undergo rearrangement has

been thought to be determined by accessibility to the RAG complex, a view that ascribes a passive role to the recombinase. Recent findings, however, indicate that the recombinase - through direct binding to modified chromatin - is an active partner in the epigenetic regulation of rearrangement. We discuss below how epigenetic marks interact with the V(D)J recombinase to promote locus-specific rearrangement.

Epigenetic modifications of possible relevance to V(D)J recombination

An alteration in gene function is termed epigenetic if it is maintained through cell division and does not involve a change in the DNA sequence. One extensively studied epigenetic mark is DNA methylation on cytosine, which in mammals occurs at most CpG dinucleotides. A far more complex set of epigenetic marks are associated with the protein components of chromatin. The basic unit of eukaryotic chromatin is the nucleosome. This consists of a histone core - two molecules each of the histones H2A, H2B, H3 and H4 - around which are wrapped about 146 base pairs of DNA. Histones are subject to a variety of posttranslational modifications including acetylation, methylation, phosphorylation, ubiquitylation and sumoylation. Differences in the degree and stereospecificity of modification contribute substantially to the complexity of these marks. Lysine, for example, can be mono-, di- or trimethylated, while arginine can be dimethylated symmetrically or asymmetrically. In addition to chemical modification, the register in which DNA is wrapped around the histone core - termed nucleosome phasing -can have profound effects on the accessibility of specific sequences to interacting factors. Observations relating these modes of epigenetic regulation to the activation or suppression of V(D)J recombination are summarized in turn below.

DNA methylation

Methylation of CpG dinucleotides is normally associated with the suppression of transcription. Consistent with a general correlation of recombination with transcription, CpG methylation over antigen-receptor-gene segments is also associated with suppression of V(D)J recombination [31]. Deletion of PDβ1, a promoter located 5′ to the Dβ1 gene segment or Eβ, an enhancer located 3′ to the TCRβ locus, is accompanied by increased CpG methylation in the Dβ1-Jβ1 region and defects in TCRβ1 rearrangement [32-34]. Conversely, demethylation of DNA has been associated with activation of rearrangement. In developing B cells, for example, the Ig κ allele that is first activated for rearrangement is demethylated over the Jκ - Cκ region, while the opposite allele remains hypermethylated and is recruited to heterochromatin [35, 36].

Nucleosome phasing

Together, the core RAG-1 and RAG-2 fragments catalyze RSS-specific nicking and transesterification of DNA substrates in vitro. Efficient cleavage is not observed, however, when chromatinized nuclear substrates are used [37]. RAG-mediated DNA cleavage in vitro is impeded when the target RSS is incorporated into a nucleosome [38-40]; the degree of inhibition has been variously proposed to be dependent [38] or independent [39] of nucleosome phasing relative to the RSS. The resistance of mononucleosomal substrates to cleavage may result from inaccessibility of histone-associated DNA to the RAG complex as well as from helical distortion induced by wrapping of the DNA around the histone core [40, 41]. The impediment to RAG-mediated DNA cleavage observed with mononucleosomal substrates in vitro can be relieved synergistically by histone acetylation and SWI/SNF-dependent remodeling, possibly as a result of alterations in chromatin structure that enhance accessibility of the RSS to the RAG complex [40, 41].

Histone acetylation

Acetylation of histones H3 and H4 is associated with active chromatin. A positive correlation between histone acetylation and active antigen receptor gene rearrangement has been widely documented. Decreased acetylation of H3 and H4 is associated with diminished germline transcription at unrearranged antigen receptor loci and is important for allelic exclusion [36], [42-45]. During B cell development, diminished IL-7 signaling is associated with decreased histone acetylation and reduced accessibility to nucleases over distal V_H segments [43]. A similar relationship is observed over Vβ segments during the transition of intrathymic T cell progenitors from the CD4$^-$CD8$^-$ to the CD4$^+$CD8$^+$ stage [46]. Thus, decreases in histone acetylation are associated with diminished rearrangement. Consistent with this relationship, Ig κ alleles at which recombination is active exhibit increased acetylation of histone H3 [36].

Histone H3 K9 methylation

Dimethylation of histone H3 at lysine 9 (H3K9me2), which is associated with silent chromatin, is positively correlated with inhibition of V(D)J recombination [47-49]. Dimethyl marks at H3K9 are removed over V_H segments at the pro-B to pre-B cell transition, at which stage V_H-to-DJ_H joining occurs; H3K9 demethylation is dependent on expression of the transcription factor Pax5 in pro-B cells [47]. A role for H3K9me2 in the control of V(D)J recombination was suggested in an experiment that targeted G9a, a histone H3K9 methyltransferase, to a TCRβ minilocus. In this setting, directed H3K9 methylation was found to inhibit both germline transcription and V(D)J recombination, overriding the presence of cis-acting accessibility control elements [49]. An interpretation of these findings is complicated, because ablation of the G9a methyltransferase in mice had no significant effects on lymphoid development or stage specificity of V(D)J recombination, despite suppressive effects on λ light chain usage, B cell proliferation and plasma cell differentiation [50].

Histone H3 K4 methylation

Methylation of histone H3 lysine 4 (H3K4) is a phylogenetically conserved modification that has been linked to transcriptional activation in yeast and metazoans [51]. The relationship between histone H3K4 methylation and V(D)J recombination has been the subject of much recent study [36], [48], [52], [53]. Dimethylated histone H3K4 (H3K4me2) [48], [53] and H3K4me3 ([54], [55] exhibit distinct patterns of enhancement within the D-J_H cluster in pro-B cells poised to undergo D-to-J_H rearrangement. Moreover, the recombinationally active Ig κ allele in pre-B cells is marked by hypermethylation of H3K4 [36].

Monoubiquitylation of histone H2B at lysine 123 (ubH2B) promotes histone H3K4 methylation in yeast [56-58]. UbH2B is associated with transcriptionally active chromatin both in yeast [59-63] and in mammalian cells [60], [64]. Patterns of ubH2B deposition have yet to be extensively mapped. As H2B ubiquitylation appears to be a prerequisite for H3K4 hypermethylation, it will be of interest to know whether the density of ubH2B is enhanced at sites of active V(D)J recombination, possibly extending the chain of causation one step upstream.

Direct recognition of modified histone H3 by the V(D)J recombinase

The observations outlined above, while essential to an understanding of epigenetic control, do not in themselves provide mechanistic insight into how histone modification is linked mechanistically to V(D)J recombination. Building on recent progress in the understanding of how histone methylation patterns are read, several studies have combined biochemical, structural and genetic approaches to outline how one such linkage is established.

A variety of protein domains are capable of binding the N-terminal region of histone H3 when this is hypermethylated at lysine 4. These include the chromodomains of CHD1 [65, 66], the double tudor domain of JMJD2A [67] and the plant homeodomain (PHD) fingers of ING2 [68-71], BPTF [68, 71] and Yng1 [72]. Crystallographic analysis reveals that the PHD fingers of ING2 [69], BPTF [68] and Yng1 [72] all contain an aromatic cage that mediates binding to methyl-lysine, a feature shared by other methyl-lysine-binding domains [73]. The structural basis of H3K4me2 or H3K4me3 binding by the PHD finger is of particularly broad significance, because this recognition domain is present in many chromatin-associated proteins that carry out histone modification [74, 75].

The ability of the PHD finger to mediate binding to H3K4me2 and H3K4me3 led several groups to examine the function of a similar domain that earlier had been identified within residues 419 through 481 of the non-core region of RAG-2 [76]. This non-canonical PHD finger (Fig. 1B) was shown to mediate direct binding of RAG-2 to histone H3 di- or trimethylated at K4, with a preference for H3K4me3 [54, 55]. Mutations that abolish binding of the RAG-2 PHD finger to H3K4me3 (Fig. 1B) were found to impair V(D)J recombination both within extrachomosomal substrates and at endogenous loci [54, 55]. Moreover, the association of the RAG-2 PHD finger with chromatin across the immunoglobulin heavy chain locus is positively correlated with the density of H3K4me3 [54]. Mutations that disrupt H3K4me3 binding or Zn^{++} coordination by the RAG-2 PHD finger had been associated earlier with combined hereditary immunodeficiencies in humans [77-81], underscoring the physiologic importance of these interactions.

The crystal structures of complexes between the RAG-2 PHD finger and modified H3 peptides have shown that this domain, while functionally related to its canonical cousins, exhibits the unusual ability to integrate epigenetic marks [82]. In the complex with a peptide bearing K4me3, the trimethyl ammonium group of K4 is buried in an "aromatic cage" similar to that of other methyl-lysine-binding domains. An important difference between the PHD finger of RAG-2 and other H3K4me3-binding domains, however, was observed: an enhanced affinity for a doubly modified histone – namely, H3 bearing both K4Me3 and a symmetrically dimethylated arginine at position 2 (R2Me2s). This is possible because the RAG-2 PHD finger lacks a side chain carboxylate that in homologous domains forms salt bridges with unmodified R2. In RAG-2 this is replaced by tyrosine, which mediates interactions with H3R2me2s [82]. An important consequence is that binding of RAG-2 to an H3 peptide bearing K4me3 is enhanced by the presence of R2Me2s [82]. While the differential affinities of RAG-2 for singly and doubly modified histone H3 could in principle contribute to locus discrimination by the V(D)J recombinase, the physiological relevance of this property remains unclear, because symmetric methylation of histone H3 R2 has as yet not been detected in vivo.

Evidence for allosteric regulation of V(D)J recombinase activity by histone H3 trimethylated at lysine 4

The engagement of histone H3K4me3 by the RAG-2 PHD finger provides a bridge between one chemical mark of active chromatin and the V(D)J recombinase machinery. Paradoxically, while V(D)J recombination is profoundly impaired by a point mutation that abolishes H3K4me3 binding by the RAG-2 PHD finger, complete removal of the non-core region, including the entire PHD finger, has only a modest debilitating effect [54, 55]. To reconcile these observations it has been proposed that an inhibitory domain resides within the non-core region of RAG-2, and that suppression of recombinase activity by this domain is relieved upon engagement of the PHD finger by H3K4me3 (Fig. 2). Consistent with this proposal is a crystal structure in which the RAG-2 PHD finger - in the absence of an H3K4me3 ligand - is occupied by an amino-terminal peptide encoded by the expression construct [82]. It may be that hypermethylated H3K4 does not simply act as a docking site for the recombinase but rather plays a more active role as an allosteric trigger of RAG catalysis.

Future directions: deposition and integration of epigenetic signals controlling V(D)J recombination

The link between transcriptional activation and locus-specificity of V(D)J recombination has long suggested that transcription and V(D)J recombination are controlled by shared epigenetic mechanisms. Progress in understanding these mechanisms has awaited the chemical characterization of epigenetic marks and the development of methods by which the genomic distribution of these marks could be mapped. These approaches have begun to provide a detailed view of epigenetic change at antigen receptor genes as a function of development. Several important questions will continue to dominate the field.

The first is to define precisely the structural features that confer locus specificity to the V(D) J recombinase. While recognition of histone H3K4me3 by RAG-2 provides a link between active chromatin and V(D)J recombination, it is obvious that H3K4me3 – a general mark of transcriptionally active chromatin – is too broadly distributed to act alone in directing the recombinase to specific sites of action. Clearly other modes of regulation must contribute to locus specificity of recombinase activity. While it seems likely that this will involve a combinatorial summation of chromatin modifications and DNA sequence elements, the answer is far from clear. A related question concerns the direct role of modified chromatin in regulating RAG activity. The proposal that the recombinase is allosterically activated upon binding of the RAG-2 PHD finger to modified chromatin will need to be tested, and the relative contributions of modifications at H3K4, H3R2 and elsewhere will need to be defined. Regions of the RAG-2 other than the PHD finger may also mediate functional interactions with chromatin. The RAG-2 linker region, which lies at the amino-terminal side of the PHD finger (Fig. 1B), has been reported to bind core histones and mutations within this region were found to impair V_H-to-DJ_H joining [83]; the basis for this apparently selective effect is unclear. A third question concerns how developmental signals, such as those that emanate from the pre-BCR, govern deposition and removal of epigenetic marks at antigen receptor loci. A resolution of these outstanding issues will provide a starting point from which to address the larger problem of allelic exclusion.

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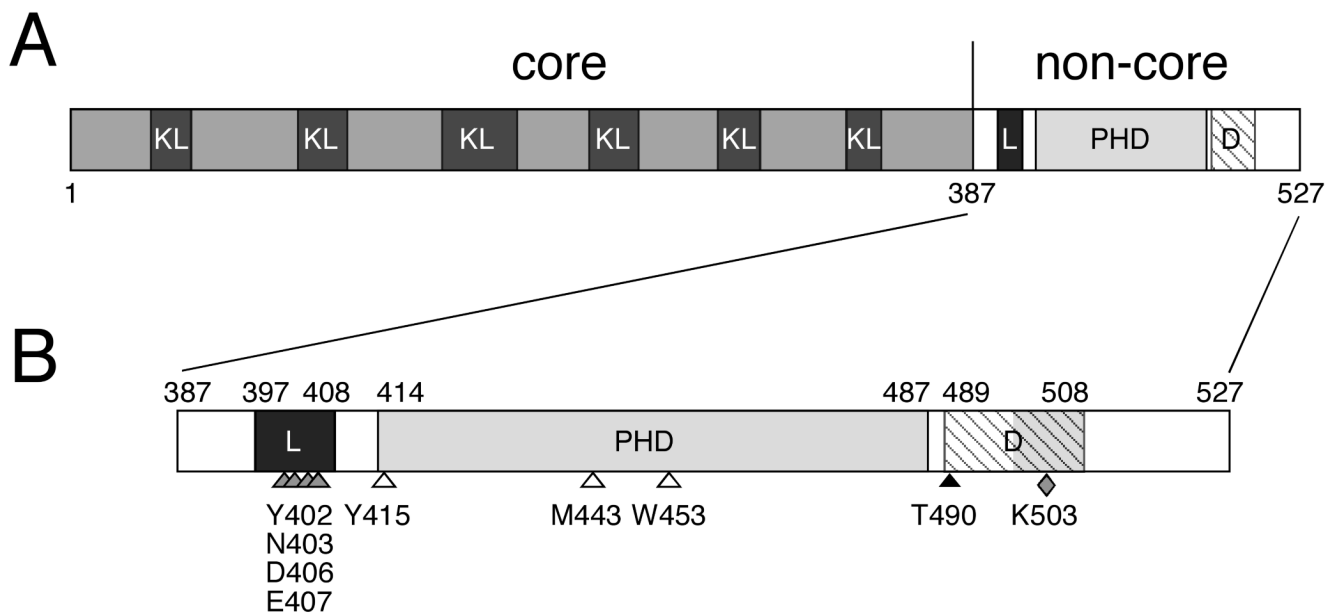
Fig. 1.
Regulatory domains of RAG-2. A, schematic representation of RAG-2. Core and non-core regions are designated; amino acid residues are numbered below. KL, Kelch-like propeller domains; L, linker domain; PHD, plant homeodomain finger; D, domain governing programmed degradation and nuclear import 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.
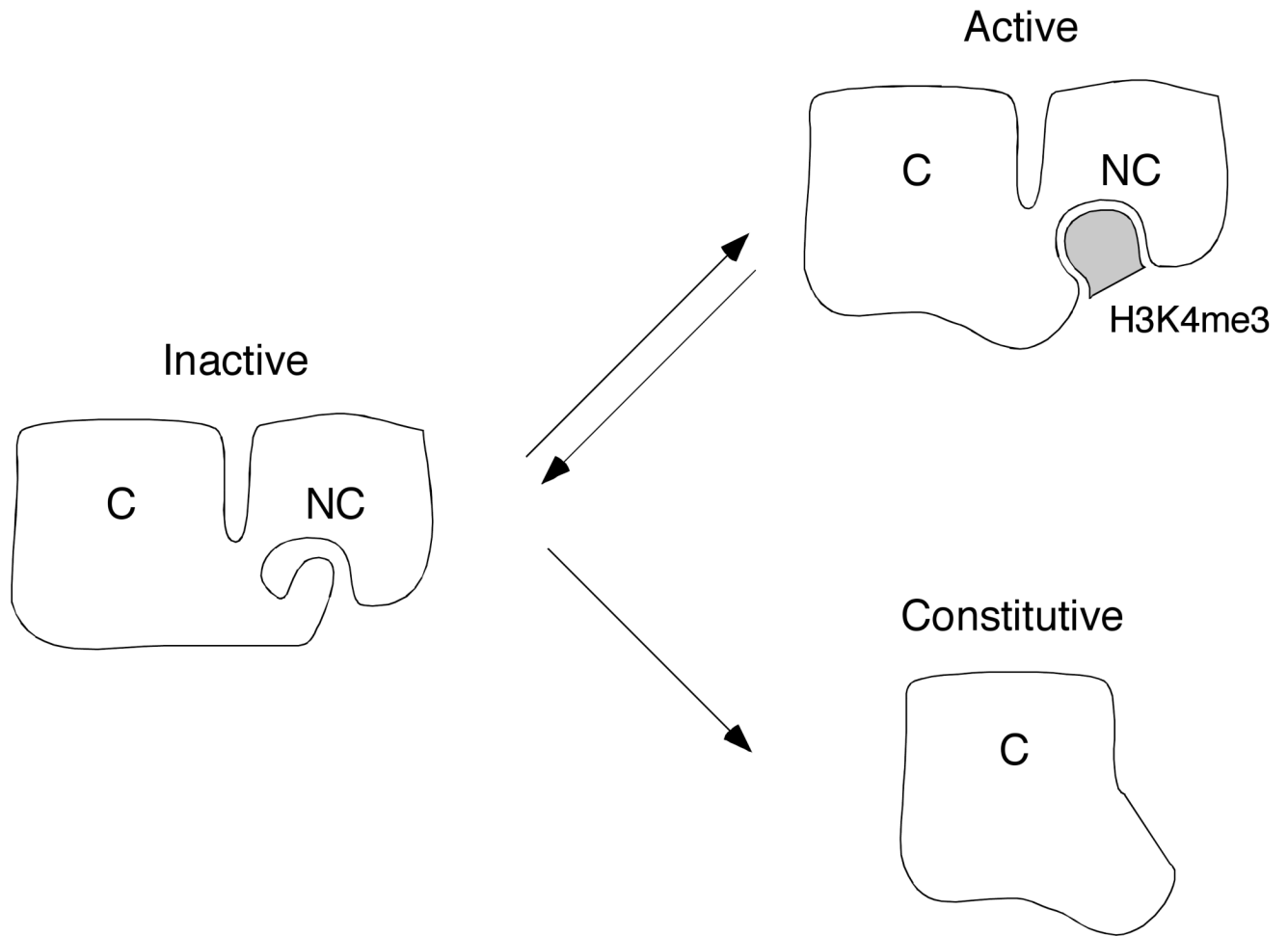
Fig. 2.
A model for allosteric activation of the RAG complex by modified histone H3. White figures
represent RAG-2; C and NC denote core and non-core regions. respectively. Shaded object
represents trimethylated lysine 4 of histone N3 (H3K4me3). In a hypothetical inactive
conformation (left), the aromatic channel of the RAG-2 PHD finger is occupied by an inhibitory
domain residing elsewhere in the non-core region. In the hypothetical active conformation
(upper right), the PHD finger is bound by histone H3K4me3 and the putative inhibitory domain
is released. The RAG-2 core fragment (lower right) lacks both the PHD finger and the putative
inhibitory domain. In this configuration RAG-2 is proposed to assume an active configuration
constitutively. For further discussion, see text.