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Access Guide to Antigen Receptor Genes

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Pillars of Immunology

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Somatic V(D)J recombination randomly assorts gene segments within each AgR locus to generate the potential for B and T lymphocytes to recognize a diverse array of Ags. In a key publication at the turn of this century, McMurry and Krangel (1) provided insight into regulatory mechanisms that guide this process.

V(D)J recombination is initiated by RAG products 1 and 2 that introduce dsDNA breaks at recombination signal sequences (RSSs) associated with rearrangeable gene segments. All seven AgR loci (*IgH*, *Igκ*, and *Igλ*; and *TCRα*, *TCRβ*, *TCRγ*, and *TCRδ*) contain closely related RSSs; however, BCR-encoding genes rearrange to completion only in the B lineage, whereas *TCR* genes fully rearrange only in the T lineage. Because RAG1/2 are essential for recombination in both lineages, the basis for lineage-restricted rearrangement of only appropriate AgR loci posed a major regulatory conundrum. The problem was further accentuated by observations that, even within a lineage, different loci rearranged at distinct developmental stages. For example, *IgH* and *IgL* chain genes rearrange at pro- and pre-B cell stages, respectively, whereas *TCRβ*- and *α*-chain genes rearrange in $CD4^{-}CD8^{-}$ (double-negative [DN]) and $CD4^{+}CD8^{+}$ (double-positive [DP]) stages, respectively. How are RAG1/2 directed to specific loci within, and between, lineages?

The first insights into this question came from a series of studies carried out by Alt and colleagues (2, 3). They demonstrated that rearranging gene segments were transcriptionally active and sensitive to DNase I digestion. These observations led them to propose the accessibility hypothesis, which posits that the recombinase machinery (which was unknown at the time) gains access to only a specific AgR locus at a time determined by cell lineage and developmental stage. The association between V(D)J recombination and transcription also led Alt and colleagues to propose that promoters and enhancers may regulate access of the recombinase. The accessibility hypothesis has guided research into the regulation of V(D)J recombination ever since (4).

The role of promoters and enhancers in this process was soon substantiated using cell culture models and transgenic mice that carried recombination-competent gene segments regulated by enhancers from different AgR gene loci (5, 6). In such experiments, Krangel and colleagues (7) demonstrated that an enhancer located close to the Cδ exons (referred to as the *TCRδ* enhancer, Eδ) activated recombination in DN cells, whereas the *TCRα* enhancer (Eα) activated recombination at a later developmental stage in DP cells. The icing on the cake was provided by deleting enhancers from endogenous loci by gene targeting in embryonic stem cells. Deletion of the *TCRβ* enhancer abolished *TCRβ* recombination in DN cells, whereas Eα deletion substantially reduced *TCRα* rearrangements in DP cells (8, 9). In contrast to locus-wide consequences of enhancer deletions, more localized effects on V(D)J recombination were observed by deleting promoters in each of these loci (10, 11). In all circumstances, transcription and DNase I sensitivity (when it was assayed) correlated with recombination potential, thereby strongly substantiating the accessibility hypothesis. However, the molecular basis for chromatin-level accessibility remained unclear.

Enter the paper highlighted here as a *Pillars of Immunology* (1). In this article, McMurry and Krangel demonstrated that loci permissible for V(D)J recombination were associated with acetylated forms of histone H3, thereby directly linking a form of chromatin modification to recombinase accessibility. In addition, they showed that generation of the permissible state required activity of associated transcription enhancers. Cumulatively, these observations identified a chromatin-based mechanism that connected the hallmarks of the accessibility hypothesis.

Their studies were based on the then-emerging idea that promoters and enhancers could alter the histone modification state by recruiting transcriptional coactivators with histone acetyl transferase activity (12) and that hyperacetylated chromatin domains were sensitive to DNase I digestion (13). Putting these concepts together with their experience with human *TCRδ* minilocus transgenes, they queried histone acetylation of substrates that underwent developmentally appropriate enhancer-dependent recombination. Eα-containing transgenes that recombined fully to the VDJ state were hyperacetylated throughout the minilocus in DP thymocytes, whereas H3 acetylation was substantially reduced within transgenes that lacked an enhancer. (Such transgenes had been shown to rearrange only partially.) Concordant with earlier activation of Eδ compared with Eα, Eδ-containing transgenes were hyperacetylated in DN thymocytes ($RAG2^{-/-}$ × Eδ) that maintained the substrate in unrearranged configuration. Attenuating Eδ functionality by using a mutated enhancer ($RAG2^{-/-}$ × Eδm core) or by promoting differentiation to the DP stage via introduction of a *TCRβ* transgene ($RAG2^{-/-}$ × Eδ × *TCRβ*) reduced histone acetylation. These studies demonstrated that developmental stage-specific activity of *TCRα* and *δ* enhancers drives histone acetylation of these transgenes.

McMurry and Krangel further extended their analyses to the endogenous *TCRα/δ* locus, at which *TCRδ* genes rearrange in DN cells and *TCRα* genes rearrange in DP cells. They found that parts of the *δ* locus, but not of the adjacent *α* locus, were

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Abbreviations used in this article: DN, double-negative; DP, double-positive; Eα, TCRα enhancer; Eδ, TCRδ enhancer; RSS, recombination signal sequence.

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hyperacetylated in DN cells from RAG2$^{-/-}$ mice. Transition of thymocytes to the DP stage in (RAG2$^{-/-}$ × *TCRβ*) mice was accompanied by robust hyperacetylation of *TCRα* sequences. Thus, locus hyperacetylation corresponded closely with the developmental stage at which these genes rearranged. Importantly, Eα-deleted alleles were hypoacetylated in DP cells, indicating that this state was largely determined by Eα. Three important observations resulted from McMurry and Krangel's clever combination of model transgenes, developmentally staged, yet homogeneous, cell populations, and enhancer-deleted endogenous alleles. First, histone H3 hyperacetylation correlated with recombination potential in DN and DP cells. Second, histone modification status preceded recombination, suggesting a causative connection between histone acetylation and recombination. Third, enhancers, such as Eα and Eδ (which had been shown to control recombination), also affected histone acetylation. McMurry and Krangel proposed "a model for V(D)J recombination in which *cis*-regulatory elements direct access to RAG proteins in vivo by inducing the region- and developmental stage–specific hyperacetylation of histone H3."

The observed correlation between histone hyperacetylation and recombination was soon extended to include other AgR loci. Since then, additional modes of epigenetic regulation of recombination have been identified. The demonstration that a plant homeodomain in RAG2 binds trimethylated lysine 4 on histone H3 (14, 15) established a direct link between chromatin and the recombinase machinery. In addition, the concept that the epigenetic information present in the three-dimensional state of AgRs regulates recombination has since gained prominence (16). Interestingly, both of these epigenetic features are controlled by transcriptional enhancers, placing these sequences at the hub of regulated recombination.

By providing the first insights into the role of epigenetics in functionally distinguishing between genetically similar RSSs at different AgR loci, McMurry and Krangel established a paradigm that led to an increasingly sophisticated understanding of chromatin dynamics associated with V(D)J recombination.

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Disclosures

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References

1. McMurry, M. T., and M. S. Krangel. 2000. A role for histone acetylation in the developmental regulation of V(D)J recombination. *Science* 287: 495–498.
2. Yancopoulos, G. D., T. K. Blackwell, H. Suh, L. Hood, and F. W. Alt. 1986. Introduced T cell receptor variable region gene segments recombine in pre-B cells: evidence that B and T cells use a common recombinase. *Cell* 44: 251–259.
3. Blackwell, T. K., M. W. Moore, G. D. Yancopoulos, H. Suh, S. Lutzker, E. Selsing, and F. W. Alt. 1986. Recombination between immunoglobulin variable region gene segments is enhanced by transcription. *Nature* 324: 585–589.
4. Stanhope-Baker, P., K. M. Hudson, A. L. Shaffer, A. Constantinescu, and M. S. Schlissel. 1996. Cell type-specific chromatin structure determines the targeting of V(D)J recombinase activity in vitro. *Cell* 85: 887–897.
5. Capone, M., F. Watrin, C. Fernex, B. Horvat, B. Krippl, L. Wu, R. Scollay, and P. Ferrier. 1993. TCR beta and TCR alpha gene enhancers confer tissue- and stage-specificity on V(D)J recombination events. *EMBO J.* 12: 4335–4346.
6. Oltz, E. M., F. W. Alt, W. C. Lin, J. Chen, G. Taccioli, S. Desiderio, and G. Rathbun. 1993. A V(D)J recombinase-inducible B-cell line: role of transcriptional enhancer elements in directing V(D)J recombination. *Mol. Cell. Biol.* 13: 6223–6230.
7. McMurry, M. T., C. Hernandez-Munain, P. Lauzurica, and M. S. Krangel. 1997. Enhancer control of local accessibility to V(D)J recombinase. *Mol. Cell. Biol.* 17: 4553–4561.
8. Bouvier, G., F. Watrin, M. Naspetti, C. Verthuy, P. Naquet, and P. Ferrier. 1996. Deletion of the mouse T-cell receptor beta gene enhancer blocks alphabeta T-cell development. *Proc. Natl. Acad. Sci. USA* 93: 7877–7881.
9. Sleckman, B. P., C. G. Bardon, R. Ferrini, L. Davidson, and F. W. Alt. 1997. Function of the TCR alpha enhancer in alphabeta and gammadelta T cells. *Immunity* 7: 505–515.
10. Whitehurst, C. E., S. Chattopadhyay, and J. Chen. 1999. Control of V(D)J recombinational accessibility of the D beta 1 gene segment at the TCR beta locus by a germline promoter. *Immunity* 10: 313–322.
11. Hawwari, A., C. Bock, and M. S. Krangel. 2005. Regulation of T cell receptor alpha gene assembly by a complex hierarchy of germline Jalpha promoters. *Nat. Immunol.* 6: 481–489.
12. Kuo, M. H., J. E. Brownell, R. E. Sobel, T. A. Ranalli, R. G. Cook, D. G. Edmondson, S. Y. Roth, and C. D. Allis. 1996. Transcription-linked acetylation by Gcn5p of histones H3 and H4 at specific lysines. *Nature* 383: 269–272.
13. Hebbes, T. R., A. L. Clayton, A. W. Thorne, and C. Crane-Robinson. 1994. Core histone hyperacetylation co-maps with generalized DNase I sensitivity in the chicken beta-globin chromosomal domain. *EMBO J.* 13: 1823–1830.
14. Matthews, A. G., A. J. Kuo, S. Ramón-Maiques, S. Han, K. S. Champagne, D. Ivanov, M. Gallardo, D. Carney, P. Cheung, D. N. Ciccone, et al. 2007. RAG2 PHD finger couples histone H3 lysine 4 trimethylation with V(D)J recombination. *Nature* 450: 1106–1110.
15. Liu, Y., R. Subrahmanyam, T. Chakraborty, R. Sen, and S. Desiderio. 2007. A plant homeodomain in RAG-2 that binds hypermethylated lysine 4 of histone H3 is necessary for efficient antigen-receptor-gene rearrangement. *Immunity* 27: 561–571.
16. Bossen, C., R. Mansson, and C. Murre. 2012. Chromatin topology and the regulation of antigen receptor assembly. *Annu. Rev. Immunol.* 30: 337–356.