Published in final edited form as:
Semin Immunol. 2010 December ; 22(6): 311–312. doi:10.1016/j.smim.2010.09.002.

Epigenetic regulation of V(D)J recombination

Ann Feeney
The Scripps Research Institute, Department of Immunology and Microbial Science, IMM-22, 10550 North Torrey Pines Rd., La Jolla, CA 92037, phone: (858) 784-2979, fax: (858) 784-8917

Ann Feeney: feeney@scripps.edu

It has been known for over 25 years from the ground-breaking work of Alt and co-workers that the process of V(D)J recombination is under tight regulation, with each of the V(D)J rearrangement steps creating the heterodimeric antigen receptors occurring in a precise order. In addition, although the same RAG recombinase rearranges TCR and Ig genes, there is strict lineage specificity, other than some IgD_H to IgJ_H rearrangement in thymocytes. After making these observations of lineage- and developmental stage-specificity, Alt and coworkers proposed the "Accessibility Hypothesis", which states that only certain parts of certain loci are "accessible" to the recombinase at each given stage of differentiation. This clearly is the case, but what that means in molecular terms is still being worked out. In this issue, several groups of investigators summarize their efforts in unraveling these complex control mechanisms.

The earliest indications that a locus or part of it, was accessible, was the observation that transcription of unrearranged gene segments occurred prior to rearrangement of the genes in that region. For over two decades, it has been unclear if this so called "germline transcription" plays an essential role in making the region accessible, or if it is merely a byproduct of the fact that the locus has been made accessible, and hence is accessible to transcription factors and to RNA polymerase. In addition to this germline transcription in the sense direction, Anne Corcoran and her group uncovered the fact that antisense transcription was also occurring just prior to gene rearrangement within the large V_H gene locus. Interestingly, only the antisense transcription was intergenic in the V_H locus, while the sense transcription used the V_H gene promoters and predominantly ran only through the coding region. Corcoran and colleagues proposed that this antisense transcription remodels the large V_H part of the IgH locus, making the V_H genes accessible for rearrangement. Subsequently, her group and that of Ranjan Sen both described antisense transcription throughout the D_H locus. Although both groups are in full agreement on the data, they differ in their interpretations of the consequences of antisense transcription, with the Corcoran group predicting that the antisense transcription opens the D_H locus, while the Sen group hypothesizes that it represses the D_H locus via a Dicer-dependent repeat-induced silencing mechanism. Both groups discuss their data and hypotheses in this issue.

Research over the past decade has demonstrated that regions of the antigen receptor loci that are accessible for recombination display various histone post-translational modifications (acetylation, methylation) that are characteristic of active genes. Acetylation of histones H3 and H4 were the first modifications analyzed, but since that time, a more complete epigenetic profile of the antigen receptor loci in T and B cell development has been provided by many labs, and is discussed in several articles in this issue including those of Leisso-Degner and

Feeney, Subrahmanyam and Sen, Desiderio, and Osipovich and Oltz. Recent work from two groups, including that of Desiderio, has shown that one histone modification, methylation of lysine 4 on histone 3 (H3K4me3) has a profound direct consequence for ordered V(D)J rearrangement, as is discussed in the article by Desiderio. RAG2 has a PHD domain in its non-core region, and RAG2 binds specifically to H3K4me3 throughout the genome. This histone modification is found almost exclusively on J genes and on closely linked D genes at all receptor loci, but is not present on V genes even when the V locus is accessible. Furthermore, the histone methyltransferase that adds this modification often travels with the RNA Pol II complex, suggesting that the role of germline transcription, which is most prominent over the J genes, may be to add this histone mark. By having this epigenetic flag on the J genes, RAG1/RAG2 would preferentially be recruited to RSS of J genes, thus resulting in the ordered rearrangement of D to J before V to DJ. Desiderio discusses data suggesting that the binding of this non-core region of RAG2 to H3K4me3 acts as an allosteric trigger to increase V(D)J activity. RAG1 also has extra functions in its non-core region: a RING finger is present in the N-terminal region that has E3 ubiquitin ligase activity. Recent data suggest that this non-core region of RAG1 preferentially interacts with and promotes monoubiquitylation of histone H3. Thus, both RAG1 and RAG2 not only catalyze the V(D)J recombination reaction, but they play an important role in regulation of accessibility by interactions with chromatin with domains outside of the catalytic domains. The non-core portion of RAG2 is also critical for the cell cycle regulation V)D)J recombination, and Desiderio describes his recent work demonstrating that the degradation of RAG2 as cells enter S phase is critical for preventing RAG-mediated DNA breaks leading to translocations.

Enhancers and promoters throughout the receptor loci play a critical role in controlling the accessibility of various portions of the different receptor loci. Deletion of the enhancers at several loci showed similar results of greatly impaired rearrangement of D and J genes, and decreased histone acetylation over a large region encompassing D and J gene segments. The role of enhancers at the TCRβ and IgH loci are described in the articles by Spicuglia et al., Osipovich and Oltz, and Subrahmanyam and Sen. Osipovich and Oltz also describe the interactions between promoters and enhancers that regulate locus accessibility, and the downstream consequences of these interactions, such as the recruitment of chromatin remodeling complexes such as SWI/SNF, which results in decreased nucleosome occupancy, and thus more accessibility of the RSS. Transcription factors, by binding to promoters and enhancers, also play an important role in both large-scale locus accessibility as well as localized V gene accessibility, as discussed by Leisso-Degner and Feeney, and Osipovich and Oltz.

Allelic exclusion refers to the fact that most lymphocytes only express one heterodimeric antigen receptor. There are two steps to this control mechanism. The first step is the process by which only one allele undergoes complete V(D)J recombination at a time. Bergman and Cedar, and Spicuglia et al., summarize their data and that of others demonstrating that there are monoallelic differences that may dictate which allele will undergo rearrangement. Some of these mechanisms include monoallelic DNA demethylation, recruitment to heterochromatin or to the nuclear lamina, or packaging one allele only with acetylated histones. They discuss the arguments for and against stochastic mechanisms regulating which of the two alleles is demethylated, associated with acetlylated histones, and not associated with heterochromatin and thus accessible for recombination. In the kappa locus, the data from the Bergman lab is strong that the early replicating allele is favored for recombination, but at the TCRβ locus, existing data suggest that the process may be more stochastic. In the second step of the enforcement of allelic exclusion, once a cell has successfully rearranged a receptor gene on one allele, the accessibility of that locus must be shut off to prevent the generation of a second successful rearrangement event at that locus. These mechanisms are still not fully understood.

Nuclear movement and 3-dimensional organization of the receptor loci is also critical for V(D)J recombination. First, the receptor loci move from the nuclear periphery to the center of the nucleus before recombinational accessibility is established. Then, all of the large receptor loci have been shown by 3D-FISH to undergo locus compaction at the specific time at which that locus is poised to undergo V(D)J recombination. This will bring all of the V genes into proximity to the DJ or J regions to allow relatively equal access of V genes in distal as well as the proximal parts of the locus to undergo V(D)J recombination. This is essential for lymphocytes to produce a diverse repertoire of receptors. Elegant studies from the Murre lab have demonstrated that the IgH locus forms multiple loops, creating a rosette-type structure that compacts the 2.5 Mb V_H region. Leisso-Degner and Feeney describe their studies demonstrating that there are multiple binding sites for CTCF and its cofactor cohesin throughout all of the large receptor loci, making the CTCF/cohesin complex an attractive candidate for creating the loops that compact the loci during V(D)J rearrangement.

The process of V(D)J recombination, essential for creating a diverse receptor repertoire, also has the potential to lead to translocations if not carefully regulated. The articles in this issue describe our current state of knowledge of the tight regulation of V(D)J recombination by transcription factors, enhancers and promoters, and by epigenetic mechanisms. Together, these mechanisms result in precisely controlled accessibility of gene segments to RAG-mediated cleavage and recombination only at the appropriate time for V(D)J recombination to occur. Although great progress has been made in our understanding of this regulation of accessibility, there are still many unanswered questions that will keep this exciting field very active in the future.