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Preparing Targets for V(D)J Recombinase: Transcription Paves the Way

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The adaptive immune system has evolved a brute force strategy to combat the enormous numbers of pathogens we encounter on a daily basis. Billions of distinct Ag receptors (AgRs) are generated during our lifetime, each with a unique spectrum of binding specificities, and each is clonally distributed so that every mature lymphocyte expresses a signature TCR or BCR. In the 1970s, after several decades of intense study, the work of the Tonegawa, Leder, and Hood laboratories (1–3) finally solved the mystery of how this vast AgR repertoire is produced during lymphocyte development. Their work demonstrated that B and T lineage cells, unlike any other somatic cell type, manipulate their genomes, performing targeted recombination at loci encoding the Ag-binding portions of the TCR and BCR proteins. This process, called V(D)J recombination, is a DNA cut-and-paste reaction that creates unique variable region coding exons via random selection of V, D, and J gene segments in each precursor lymphocyte.

Over the next 15 years, the field focused on the enzymes responsible for this process [called V(D)J recombinase], until Schatz, Oettinger, and Baltimore (4, 5) discovered its critical components, which they dubbed RAG-1 and RAG-2. However, during that decade and a half, important molecular aspects of V(D)J recombination and its regulation were revealed through studies of primary lymphoid tissue and transformed cell models, especially pre-B cells transformed by the Abelson murine leukemia virus (Abl pre-B cells). It became clear that assembly of Ig and Tcr loci is controlled at the levels of tissue specificity (Tcr genes only rearrange in thymocytes and Ig genes only rearrange completely in B cell precursors) and allelic exclusion (lymphocytes functionally rearrange only one of two available alleles for each receptor component) (6). Using Abl pre-B cells, Alt, Baltimore, and colleagues (7) showed that Ig loci are also assembled in a specific order, with D to JH recombination preceding V to DJH at IgH, followed by rearrangement of the Ig L chain loci.

Several explanations for how V(D)J recombination is regulated during lymphocyte development were put forth, including the possibility that the recombinase exists in multiple tissue- or stage-specific forms, each of which targets a given AgR locus. However, no evidence to support this or other models existed at the time (the mid-1980s). This month’s Pillars of Immunology article by Yancopoulos and Alt (8) provided the first breakthrough on how such regulatory mechanisms may work. Through a series of experiments in both cell lines and lymphoid tissues, they showed that transcription of unarranged VH gene segments is coincident with their targeting for active recombination. Prior to this article, it was thought that VH gene segments are transcriptionally silent until V to DJH recombination brings their upstream promoters into the proximity of a strong enhancer (Eμ) located near the JH cluster (>100 kb away from the VH) (9). Transcription of germline Igk gene segments had been reported by the Perry laboratory (10), but these Jk segments are situated close to active enhancers, and Jk transcription could not be correlated with their active recombination.

An important aspect of the Yancopoulos and Alt article (8) was how the authors synthesized their findings with prior observations in the field to generate a paradigm-shifting model for recombinase control. Two of the more important observations were: 1) the DNA sequences of recombinase targets (termed recombination signals), which flank all Ig and Tcr gene segments, are essentially indistinguishable; and 2) the IgH locus undergoes partial assembly in thymocytes (D to JH rearrangement) (11, 12). The authors speculated (correctly so) that, collectively, the available data supported the existence of a single recombinase, which was targeted to specific regions within AgR loci during appropriate developmental windows but was excluded from these regions at all other stages.

Thus, Yancopoulos and Alt (8) upended several aspects of existing dogma, showing that VH expression could be Eμ independent and, more importantly, provided the first correlation between so-called germline transcription (glt) of gene segments and their recombination. To accomplish this, the authors used VH probes in a series of Northern blotting studies to identify novel transcripts in Abl pre-B cells. These transcripts also hybridized to probes prepared from 3′ VH sequences, which would be deleted upon V to DJH recombination, suggesting the transcripts were derived from unarranged VH segments. The proof for this important point came from the cloning and sequencing of germline VH transcripts (VH-glt). Importantly, expression of VH-glt was specific to precursor B cells, reflecting both the tissue and stage specificity of IgH recombination. In transformed cells, VH-glt predominated in fetal liver-derived Abl pre-B lines, most of which undergo V to DJH recombination. In contrast, VH-glt was...
detected only sporadically in bone marrow-derived lines, many of which had completed VDJH assembly on at least one IgH allele.

The most striking evidence that VH-glt and recombination are linked came from in vivo studies using mouse fetal liver tissue corresponding to days 15–17 of embryonic development. At these time points, the fetal liver contains populations of precursor B cells that undergo sequential and nearly synchronous waves of IgH recombination. Strikingly, Yancopoulos and Alt (8) found the highest levels of VH-glt in the developmental window immediately preceding V to DJH recombination, with levels waning at later times, when most of the pre-B cells have completed IgH rearrangement. As the authors pointed out, these findings strongly suggested that transcription is a component of the regulatory mechanisms that prepare gene segments for recombination, perhaps by establishing an open chromatin environment, a concept now known as the accessibility model.

As with any groundbreaking work, the Yancopoulos and Alt article (8) triggered a flurry of research activity. Within a year of this article’s publication, work from the Alt laboratory supported two basic tenets of the accessibility model. First, they showed that Igα and Igαγ loci were likely targeted by a common recombinase because Igαγ-derived gene segments rearrange efficiently when introduced as plasmid substrates into pre-B cell lines (13). Second, using recombination substrates that were stably integrated into the chromatin of Abl pre-B cells, Blackwell et al. (14) found that transcription of a neighboring gene enhances substrate recombination efficiency. Over the next decade, correlations between glt and recombination were broadened to encompass nearly all categories of AgR gene segments (6, 15). This now even includes antisense transcription detected throughout large clusters of unarranged gene segments, which may be a pioneering process that opens broad domains of chromatin (16). In this regard, many features of open chromatin, including RAG-nuclease sensitivity, DNA methylation status, and histone modifications, were connected to glt and accompanying recombination (17–21). Ferrier et al. (22) also provided the first genetic evidence for the accessibility model, showing that transcriptional enhancer elements are required for efficient recombination of transgenic substrates, a connection that has been solidified by the recombinatorially inert phenotype of AgR loci after targeted deletion of promoters and enhancers that drive their glt (6, 23, 24).

Definitive proof of the accessibility model awaited the identification of RAG-1/2 as V(D)J recombinase in the late 1980s. Schatz and colleagues (15) showed that RAG-1/2 expression in fibroblasts is sufficient to recombine accessory extrachromosomal substrates, but endogenous AgR loci remain unarranged, presumably because they are packaged in recombinase-inaccessible chromatin. More recently, the connection between glt, chromatin, and recombination has been forged at a biochemical level by the Oettinger and Desiderio laboratories (25, 26), who found that a domain of RAG-2 recognizes a specific modification highly enriched on nucleosomes near active promoters. These data strongly suggest that glt marks chromatin associated with AgR gene segments to facilitate tethering of the RAG complex.

The concept of AgR accessibility control originating from the Yancopoulos and Alt article (8) remains strong today. Numerous laboratories still investigate questions that arose from this work because regulatory mechanisms employed by developing lymphocytes to coordinate AgR gene assembly are more broadly applicable to transcriptional control in other cell types. Indeed, in the Pillars article, Yancopoulos and Alt (8) foreshadowed a major issue that remains unresolved today: the identity of cis elements that drive VH-glt and presumably recombination of the complex VH cluster (8). Progress has been slow, but a recent report that certain mouse VH clusters have PAX5 target elements harboring intrinsic enhancer activity may provide a much-needed boost (27). Beyond chromatin accessibility, it is now clear that the three-dimensional structure of AgR loci plays an important role in facilitating long-range recombination, including V to DJH (28, 29).

However, the cause–effect relationships, if any, among chromatin accessibility, transcription, and changes in locus topology remain key questions for the future. Finally, in a more global sense, the Yancopoulos and Alt article (8) set the stage for our current appreciation that transcription is not only required to generate mRNA templates for protein translation, but the process itself also contributes to important regulatory functions when cells reset gene expression programs during development. How these "sterile" transcripts induce localized, regional, and even long-range changes in chromatin remain important objectives of the field.

Disclosures
The authors have no financial conflicts of interest.

References


