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Regulatory events in early and late B-cell differentiation

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Abstract

We are studying transcriptional control of critical developmental decision points in B lymphocytes. Commitment to the B-lymphocyte lineage is dependent on the transcriptional regulator Pax5 and committed B lymphocytes represent the first developmental stage when V_H-to-DJ recombination occurs in the immunoglobulin (Ig) heavy chain locus. We summarize our recent studies showing that methylation of histone H3 lysine 9, a heterochromatic chromatin modification, is present in the Ig V_H region in hematopoietic progenitors and in non-B lineage hematopoietic cells. Pax5 is both necessary and sufficient to remove this heterochromatic mark in B cells.

Using genetically altered mice, we have shown that terminal differentiation of B cells to memory and Ig-secreting plasma cells depends on the transcriptional repressor Blimp-1. Recent studies demonstrating a requirement for Blimp-1 in the formation of pre-plasma memory B cells, Ig-secreting plasma cells as well as preliminary data suggesting a requirement for Blimp-1 in the maintenance of long-lived plasma cells are summarized. We also summarize our recent studies on the regulation of Blimp-1, showing direct repression by Bcl-6 and providing evidence for activation by NF-κB following toll-like receptor signaling.

Keywords: Chromatin; Histone methylation; V(D)J recombination; Plasma cells; Blimp-1

1. Introduction

Transcriptional regulators are well-known to play key roles during development. Our studies have focused on events at the earliest stages of B-cell commitment, when the immunoglobulin (Ig) heavy chain locus is made accessible for rearrangement, and on events at the latest stages of B-cell development when antibody secreting effector cells are formed. Below, we review our recent work and suggest models, which integrate our findings with work from many other laboratories studying these interesting regulatory mechanisms.

1.1. Regulation of immunoglobulin heavy chain V-to-DJ recombination—a critical event in early B-cell development

1.1.1. V(D)J recombination must be tightly regulated

Clonotypic antigen receptors, expressed on both B and T lymphocytes, are critical for the diversity and specificity of humoral and cellular immune responses. It is well-established that DNA recombination of variable (V), diversity (D) and joining (J) gene segments (V(D)J recombination) in three immunoglobulin loci (heavy chain, kappa and lambda) and three T cell receptor (TCR) loci (alpha/delta, beta and gamma) provides the basis for formation and expression of diverse Ig and TCR receptors. Lymphocyte-specific recombination activating gene (RAG) 1 and 2 proteins recognize, bind and cleave conserved DNA sequences called recombination signal sequence (RSS) elements which flank V, D and J gene segments. The broken ends are subsequently joined using ubiquitously expressed DNA double-strand break repair enzymatic machinery (for review see, Bassing et al., 2002).

In both B and T cells, the order of V(D)J recombination of different loci is strictly regulated and successful completion of V(D)J recombination on one allele leads to expression of μ polypeptide that provides a developmental checkpoint and signals further development (Mostoslavsky et al., 2003). In addition, because V(D)J recombination involves the creation

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of double-strand breaks, it must be appropriately restricted to provide genome stability and guard against loss or aberrant rearrangement of DNA. Thus, V(D)J recombination is subject to multiple and stringent controls.

In B cells, V(D)J recombination begins at the immunoglobulin heavy chain locus by the joining of the D_H and J_H segments followed by the joining of V_H-to-DJ (Bassing et al., 2002). It is the second step, joining of V_H-to-DJ that is subject to the most stringent regulation. Although D_HJ_H rearrangement can occur in progenitors not fully committed to the B-cell lineage (Borghesi et al., 2004), V_H-to-DJ rearrangement is limited to committed B cells at the pro-B cell stage. Following functional V_H-to-DJ rearrangement and expression of a transmembrane μ protein, V_H-to-DJ rearrangement is actively repressed in pre-B cells in a process termed allelic exclusion (Bassing et al., 2002; Mostoslavsky et al., 2003). Therefore, this joining step serves as a model for developmental and lineage specific gene control, requiring mechanisms for both activation and repression.

Packaging of DNA into chromatin constrains enzymatic processes, such a recombination, repair and transcription that use DNA as a substrate. Thus, it has long been suspected that alterations in chromatin accessibility play an important role in controlling V(D)J recombination. Indeed, a large body of work now supports the notion that dynamic changes in chromatin structure actively regulate V(D)J recombination (Krangel, 2003).

Early studies demonstrated that hallmarks of accessible chromatin, such as germ-line transcription and DNase I sensitivity, increased at V, D and J loci prior to their recombination (Krangel, 2003). Subsequent work has shown that nucleosomes, the basic element of chromatin, inhibit recombination in vitro at the level of both RAG binding and cutting (Golding et al., 1999; Kwon et al., 1998; McBlane and Boyes, 2000), indicating that chromatin can act as a barrier to recombination in certain situations. In these in vitro studies, histone modifications, which occur on histone tails, were implicated as a mechanism by which nucleosome inhibition can be overcome because tailless histones partially relieved nucleosome repression (Golding et al., 1999; Kwon et al., 2000), as did histone acetylation (Kwon et al., 2000). Recently, a specific histone modification associated with gene repression, methylation of lysine 9 on histone H3 (H3K9), was shown to alter the ability of a gene to recombine when this modification is directed to the proximal promoter (Osipovich et al., 2004). It seems that both activating and repressive modifications can influence V(D)J recombination.

1.1.2. Covalent histone modifications in the V_H region change during early B-cell development

While in vitro data and artificial recombination substrates have provided important information for how chromatin can regulate V(D)J recombination, it is also important to provide evidence for the presence of these modifications at endogenous antigen receptor loci. We have focused on the V_H heavy chain locus, which spans ∼2 MB on murine chromosome 12 (Chevillard et al., 2002). The locus is comprised of over 100 functional genes that can be grouped into families based on homology, each gene having its own promoter and RSS element. Due to the size of the locus, investigation of associated histone modifications enables us to ask questions about both local and global regulation. Additionally as the locus must be both activated and repressed in a lineage specific and developmental manner, we can assay for the presence of modifications that both increase and decrease DNA accessibility. These data can help us to unravel the complex mechanisms by which chromatin structure protects the genome from unwanted rearrangement while also directing developmental and lineage specific antigen receptor rearrangement.

The Chromatin Immunoprecipitation assay (ChIP) allows one to determine if a particular modification is associated with a specific DNA sequence in vivo. Using primers, which amplify ∼300 bp fragments of the V_H locus (including individual V_H gene promoters, RSS elements and intergenic DNA at least 1 kb from a V_H gene segment) (Johnson et al., 2003), we were able to assay for the presence or absence of specific modifications at the endogenous V_H locus.

Due to the fact that H3K9 methylation is associated with inactive chromatin in many species (Sims et al., 2003) and can inhibit V(D)J recombination of an engineered substrate (Osipovich et al., 2004), we considered the possibility that this modification was involved in the stringent lineage inaccessibility of the V_H locus in non-B cells. We found that a significant portion of V_H locus is associated with H3K9 methylation in non-B cells (Johnson et al., 2004). Interestingly this association was not restricted to V_H gene segments or their location within the locus and was found in multiple cell types including T cells, macrophages and fibroblasts. Importantly, there was no significant H3K9 methylation found associated with the same DNA regions in B cells. The single exception in B cells was the promoter of the S107 V_H gene family, V11. This gene has been found to rearrange at much lower frequency then other family members despite the high degree of homology in both the promoter and RSS element (Love et al., 2000). Morshead et al. (2003) also studied H3K9 methylation in the IgH locus and did not find this pattern. The difference between their results and ours is likely to be due to the fact that they analyzed cell lines and we analyzed primary cells. Since local methylation was shown to be able to inhibit V(D)J recombination (Osipovich et al., 2004), we believe the presence of H3K9 methylation at this promoter explains the low level of rearrangement of gene. These data place H3K9 methylation at the endogenous V_H region, implicating this modification in the lineage specific control of V_H-to-DJ recombination.

To understand whether commitment to non-B cell lineages involves active histone methylation at the V_H locus or whether commitment to the B cell lineage involves removal of this heterochromatic modification, we determined when H3K9 methylation at the V_H locus appeared during hematopoiesis by analyzing *Pax5*$^{-/-}$ bone marrow cells. Pax5 is a B-cell specific transcription factor whose expression