Molecular Therapy
Nucleic Acids
Commentary

Orchestrating epigenetic readers: Progress in understanding the functions of bromodomain-containing protein 4 complexes

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Cellular differentiation programs involve precise temporal coordination of regulatory gene networks. Epigenetics plays a major role in this process, in part by regulating chromatin access to nucleic acid-modifying enzymes. Although certain DNA modifications are stable and heritable epigenetic regulators, histone post-translational modifications (PTM) play a role in dynamic epigenetic regulation. The spectrum of histone PTMs includes acetylation, methylation, phosphorylation, ubiquitination, and others. Of these, acetylation/methylation switches are the best characterized. H3 Lys (K) acetylation (H3K27Ac) is a modification that relaxes nucleosomal structure, making a permissive environment for gene expression, whereas H3K27 trimethylation (H3K27me3) is a modification that compacts nucleosomes, occluding polymerases causing repression. Understanding how these epigenetic events are orchestrated is central to many fundamental biological questions in immunity, development, and oncogenesis.

Binding of chromatin regulatory proteins by histone code "readers" designates genes for activation or inhibition. Of the readers, bromodomain-containing protein 4 (Brd4) has garnered much recent attention. Through its bromodomain, Brd4 has binding affinity for acetylated histone H4 and H3 residues and is implicated in inducible regulation of cytokine responses in the innate pathway, DNA repair, and maintenance of differentiated cell state. At the molecular level, BRD4 forms complexes with >900 transcription factors, enhancers, cyclin-dependent kinases, RNA splicing, and core RNA polymerase II (Pol II) machinery.[1,2] These proteins enable the Brd4 complex to promote gene expression through regulated transcriptional elongation and formation of transcription factor "hubs" known as "superenhancers."

Two recent studies have added to the understanding of the dynamic and functional activities of the Brd4 reader in cellular differentiation through studies on differentiation of allergic T helper 2 cells (Th2)[3] and vascular smooth muscle proliferation in response to injury, known as intimal hyperplasia (IH[4]). These studies have revealed unexpected complexity because Brd4 forms dynamic protein complexes with the multisubunit Polycomb repressive complex 2 (PRC2), containing H3K27me3 binding and methylase and deactylase activity. Of the members of PRC2, Brd4 complexes with ectoderm development (EED) to produce gene silencing.

Differentiation of naive (pluripotent) T helper cells into Th2 cells mediates antigen-driven immune responses protective of helminth infections. In developed countries, where parasitic infections are rare, Th2 polarization is associated with allergic diatheses, including asthma, allergic rhinitis, atopic dermatitis, and others. Using a Brd4 inhibitor (Brd4i), Zhao et al. demonstrated the importance of Brd4 in Th2 differentiation and surprisingly found that Brd4i treatment upregulated a small population of genes. Comparing RNA-seq with ChIP-seq led to the identification of genes directly bound and silenced by Brd4, including the transcription factor, *Foxp3*, and the ubiquitin ligase, *Fbxw7*. In Th2 differentiation program, inhibition of *Fbxw7* is permissive for full expression of interleukins IL-4, IL-5, and IL-13, all mitogenic cytokines producing a Th2 phenotype. The investigators went on to demonstrate that Brd4i treatment induced ubiquitin-mediated degradation of GATA3 transcription factor by *Fbxw7*; GATA3 is a known central effector of the Th2 phenotype. The investigators applied sequential ChIP-reChIP assays to confirm that Brd4 forms a complex with YY1, GATA3, and that these transcription factors were involved in targeting Brd4 to *IL-4*, *IL-5*, and *IL-13*. This suggested that Brd4 suppresses *Fbxw7*, resulting in GATA3 stability and Th2 differentiation.

Noting that Brd4i reduced H3K27Me3 abundance on *Foxp3/Fbxw7*, the investigators found that the Brd4 separately recruited the PRC2 subunits EED, Suz12, and Ezh2 to the target genes through their interaction with the second Brd4 bromodomain (BD2),[5] and that, of these subunits, the Brd4-EED interaction is the most robust. They mapped the site of interaction, finding that the Lys[19]-acetylated EED residue is recognized by Brd4 BD2. Further studies show that selective Brd4 BD2 inhibitors interfered with Foxp3 silencing and IL-4 activation during Th2 differentiation. The investigators conclude that Brd4 cooperates with Gata3 and YY1 to transcriptionally activate Th2 cytokines as well as recruiting PRC2 to repress *Foxp3* and *Fbxw7*, further adding to the diversity of Brd4 functions, as a gene silencer and activator within the same cellular differentiation program.

Approaching the problem from a different angle, Zhang et al. examined the role of Brd4-EED in intimal IH. IH is a pathological response to arterial injury associated with restenosis after cardiovascular stenting procedures. Here, Zhang et al. observed that a small-molecule EED inhibitor blocked the injury-upregulated H3K27me3 mark and

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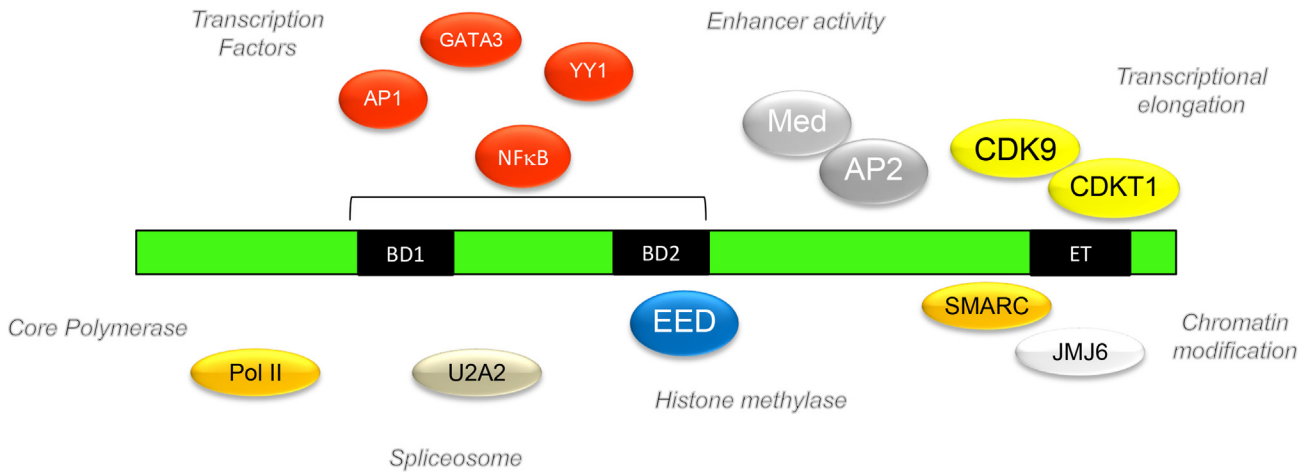

Figure 1. Brd4 protein interactions in gene activation and gene repression

Selected Brd4 protein-protein interactions are diagrammed. The positive transcription factor elongation factor-b (PTEFb)-CDK complex binds the Brd4 COOH terminal extra terminal domain (BET). Recruitment of Brd4-PTEFb complex mediates transcriptional activation of immediate-early genes through regulated transcriptional elongation. Enhancer binding complexes, Med and AP2, interact with Brd4, enabling coupling of enhancers to proximal promoters for gene activation. Acetylated transcription factors interact with Brd4 through the bromodomain (BD); NF-κB/RELA requires interactions with both BDs. Transcription factor binding enables Brd4 repositioning to inducible promoters and superenhancers.

inhibited PDGF-stimulated proliferation. The authors discovered that EED associated with Brd4 and that this complex bound to the *Ccnd1* and *P57* genes. Confirming the above study, Zhang et al. found that JQ1 disrupted Brd4-EED interactions, indicating dependence on acetylated lysine interactions. The transcription factor STAT3 is a tyrosine phosphorylated protein that mediates downstream EED. The authors provide evidence that the NH_2 terminus of EED mediates phospho-Tyr^{705} STAT3 interaction activating *Ccnd1*. Interestingly, inhibition of EED reduces $pTyr^{705}$ abundance; the mechanism was not described.

These two exciting studies have extended the complexity of Brd4-mediated gene regulation to implicate several themes, which are elaborated below and shown schematically (Figure 1).

Brd4 is a functionally pleiotropic enzyme, forming complex with histones, transcription factors, Mediator/AP2 complex, transcriptional elongation factors, and components of the core polymerase machinery (Figure 1).[1,2] Acetylated proteins interact with Brd4 through BD interaction, enabling dynamic formation and dissolution of functionally distinct Brd4 complexes. Although Brd4 interacts with the DNA-associated acetylated histones, Brd4 binding to acetylated transcription factors enables the complex to be dynamically repositioned to specific sequences. Earlier studies have shown that activated NF-κB binds and repositions Brd4 to activate immediate-early genes,[6] reorganizing superenhancers.[7] Through its endogenous RNA Pol II kinase activity and RNA Pol II/CDK9 binding, Brd4 activates immediate-early cytokine genes through a process of transcriptional elongation. BRD4 endogenous histone acetyltransferase on Lys^{122} destabilizes nucleosomal tails to promote transcriptional elongation by evicting nucleosomes from chromatin.[8] The focus studies of this commentary expand the universe of Brd4-binding transcription factors to include YY1 and GATA3. In this context, YY1/GATA3 complexes with Brd4 to activate cytokine genes controlling Th2 differentiation. The requirement of YY1/GATA3 acetylation for Brd4 binding and the effect of this complex on regulated transcriptional elongation is likely, but was not demonstrated.

In contrast, PRC2 is a multisubunit H3K 27me3 reader associated with gene silencing through H3K27me3 formation, deacetylation, and chromatin condensation. Both studies here provide evidence that Brd4 interacts with the PRC2 EED subunit, and, in particular, Zhao et al. demonstrate that EED acetylation is required for interacting with the Brd4 BD2 domain. In pluripotent cells, PRC2 complexes bind to CpG-rich islands characterized by metastable "bivalent" histone marks.[9] The mechanism for Brd4-EED complex binding to differentiation-silenced genes was not demonstrated, but could be mediated by Brd4's affinity for acetyl lysine to bivalent chromatin marks (Figure 2). Studies of inducible PRC2-mediated silencing in models of cell-type transition have shown that bivalent/metastable regulatory domains contain both activating and repressive histone marks.[10] Through its methylase and histone deacetylase activity, Brd4-EED recruitment further shifts the distribution to fully repressive H3K27me3 marks resulting in repression, recruitment of DNA methylases, and long-term silencing.

In summary, these studies identify Brd4 as an activator and inhibitor of gene expression mediated by distinct protein complexes. The diverse functions of Brd4-associated proteins suggest that many more activities await discovery. More work will be required to fully understand how Brd4 complexes coordinate and orchestrate complex cellular

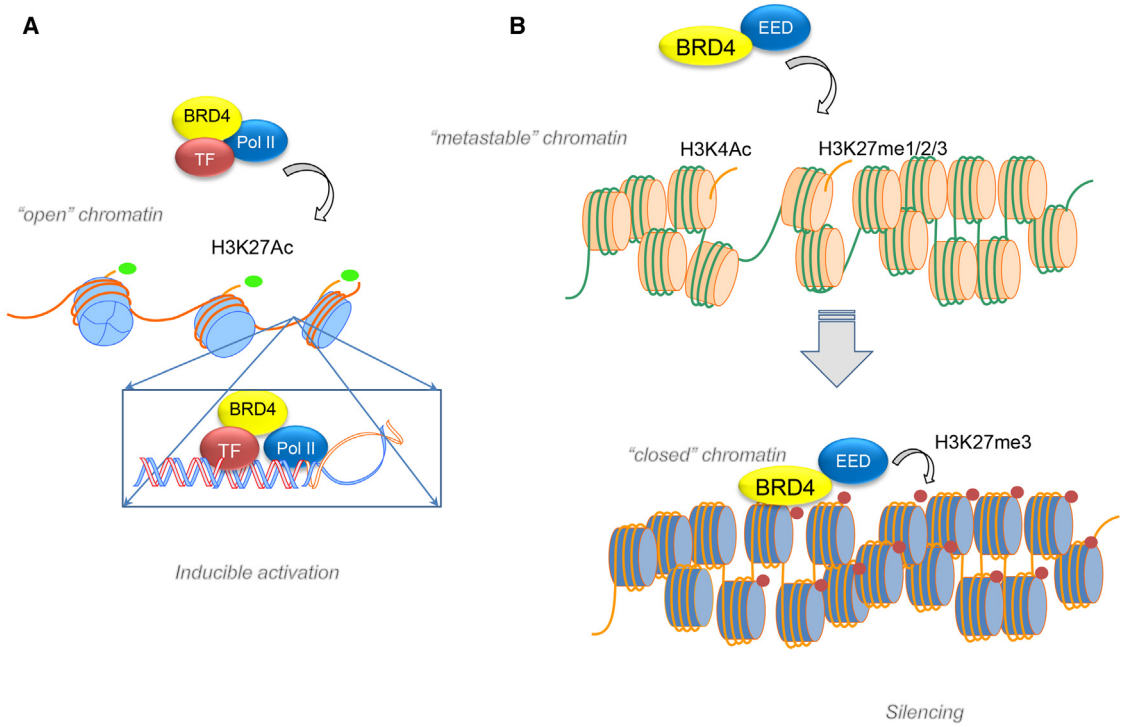

Figure 2. Model of metastable chromatin interactions. Unresolved mechanisms for PRC2-mediated repression

(A) Brd4-PTEFb recruitment to open chromatin domains in enriched in H3K27Ac marks. Enzymatic activities of Brd4 include phosphorylation of the RNA Pol II domain and H3K122 acetylation, disrupting nucleosomes. (B) PRC2-sensitive regulatory domains are enriched in CpG islands. One mechanism is that these regions are in a metastable condition with components of both activating and repressive histone marks.[10] Through Brd4 affinity for histone H3 acetylation, the EED PRC2 recruitment further shifts the distribution to fully repressive H3K27me3 marks resulting in silencing and occlusion to activating signals.

differentiation programs. This understanding that will be essential to therapeutic targets in the myriad disorders facilitated by Brd4.

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DECLARATION OF INTERESTS

The authors declare no competing interests.

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