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Brd4 is on the move during inflammation

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Abstract

Enhancer landscapes are shaped by the integrated functions of lineage-specific and signal-dependent transcription factors. A new study by Brown et al. suggests that the signal-dependent transcription factor NF-kB can modulate global enhancer activities by altering the occupancy of Brd4, a BET bromodomain coactivator protein, across the genome. This work reveals new principles of enhancer dynamics and insights into the therapeutic modulation of enhancer function with BET bromodomain inhibitors.

Keywords

Brd4; p65; NF-kB; enhancer; super-enhancer; inflammation; JQ1; BET bromodomain

The rate at which a gene is transcribed is determined by an assortment of regulatory DNA elements, which includes promoter proximal regions as well as those that act over large genomic distances, such as insulators and enhancers [1]. Comprised of dense clusters of transcription factor (TF) binding motifs, enhancers generally act in *cis* to increase the transcription rate of specific nearby genes by DNA looping to the target gene promoter. While enhancers are classically defined by their functional effects in reporter assays, tools now exist for annotating enhancers on a genome-wide scale by measuring levels of histone modifications, coactivators, chromatin accessibility, and non-coding RNA transcription. Such approaches have shown that the functional enhancer landscape is remarkably unique in each cell type and defined by lineage-specific TFs [1]. However, emerging evidence also reveals how acute signaling events can lead to reprogramming of enhancer configurations to modulate gene expression.

The inflammatory response represents one example of how rapid fluctuations in gene expression are triggered by extracellular signals, with TFs of the NF-kB family being key

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downstream mediators of this process. Receptor activation (e.g. by TNFα ligands) leads to the entry of NF-kB into the nucleus where it orchestrates a large number of transcriptional changes by associating with various promoter and enhancer regions. The transcriptional output of NF-kB is highly cell type-specific, in accord with NF-kB operating within a pre-existing enhancer landscape unique to each cell type [2]. At such elements, lineage-specific TFs have been shown to 'prime' the enhancer for subsequent NF-kB binding, with enhancer-promoter looping interactions pre-existing prior to stimulation [2, 3]. However, enhancer priming is not a universal property of inflammatory transcriptional induction, as NF-kB can establish active enhancers *de novo* in the absence of pre-existing histone marks or TF occupancy at a subset of its occupied sites [4, 5].

A recent theme in enhancer research has been the classification of regulatory elements based on their functional or structural attributes. Numerous studies have described enhancers that harbor exceptional levels of transcription factors, histone marks, and coactivators, which have been termed super-enhancers, stretch enhancers, or HOT regions (reviewed in [6]). More specifically, super-enhancers have been defined based on their width (>10 kb) and robust enrichment of transcriptional coactivators, like the BET bromodomain protein Brd4 [7]. Super-enhancers tend to exhibit stronger potency in plasmid-based reporter assays than other enhancers; however, their unique functional properties in a chromosomal context remain unclear [7]. It has been proposed that genes regulated by super-enhancers are more sensitive to chemical inhibition of BET proteins than genes regulated by classical enhancers [7].

Earlier work had shown that BET inhibitors potently suppress the inflammatory transcriptional response, which might be related to a direct interaction between Brd4 and the acetylated p65 subunit of NF-kB [8, 9]. A new study by Brown et al. has extended these observations by investigating the genome-wide relationship between p65 and Brd4 occupancy in response to TNFα stimulation in endothelial cells [10]. A key observation was that p65-occupied enhancers displayed significant variability in Brd4 recruitment, with a subset of p65-bound sites meeting the criteria of super-enhancers following TNFα stimulation. Furthermore, the genes located near super-enhancers exhibited an ~1.5-fold greater induction than genes found near typical enhancers following TNFα exposure. This relationship implies that the relative affinity of individual enhancers for the coactivator machinery might tune the biological output of NF-kB signaling. Blocking NF-kB activity using an IkB kinase inhibitor prevented the accumulation of Brd4 at inducible super-enhancers, consistent with a direct or indirect role for p65 in recruiting Brd4 to these sites. In the converse experiment, chemical inhibition of Brd4 with JQ1 had little effect on the immediate binding of p65 to DNA, but suppressed the transcriptional output of inflammatory signaling. Consistent with prior observations, the inhibitory effects of JQ1 tended to be greater for genes located near super-enhancers [7]. Surprisingly, TNFα stimulation also triggered substantial losses of Brd4 occupancy at a subset of pre-existing super-enhancers, suggesting that transcriptional suppression might be an additional consequence of global remodeling of Brd4 occupancy. These relationships were not limited to endothelial cells, but were also found in previously published datasets obtained from stimulated macrophages [9]. Collectively, these experiments indicate that a subset of NF-kB

regulatory functions occur in the context of super-enhancers and might be effectively suppressed through chemical inhibition of BET proteins.

In a mouse model of atherosclerosis, a disease associated with aberrant inflammation of endothelial cells, the authors showed that JQ1 administration significantly attenuated disease progression. Thus, targeting Brd4 exhibits promising specificity for inflammatory responses *in vivo*, consistent with prior observations in animal models of sepsis [9].

This work highlights an impressive plasticity in coactivator recruitment within the enhancer landscape in response to transient extracellular signals. Remarkably, a one-hour pulse of TNFα is sufficient to redistribute Brd4 occupancy across the genome, which has the potential to alter enhancer activities on a global scale. Surprisingly, some of the dynamic gains and losses in Brd4 occupancy were found to occur without proportional changes in histone acetylation, thus calling into question whether Brd4 is in fact acting as a reader of histone marks for its enhancer-relevant function. One possibility is that interactions with transcription factors, instead of chromatin modifications, are the major driving force that localizes Brd4 to enhancers.

It still remains unclear whether super-enhancers are a unique class of *cis*-regulatory elements, particularly since super-enhancers are defined by applying arbitrary enrichment cutoffs to ChIP-seq data. One technical issue in super-enhancer studies has been the method for assigning enhancers to their targets, which is often performed by assuming enhancers regulate the nearest expressed gene. This method can prove problematic for the subset of elements that loop away from neighboring genes to regulate distal targets. Implementation of chromatin interaction maps (e.g. using 3C technologies) might clarify the correlation between enhancer configurations and gene expression in such cases. Ultimately, genetic studies that compare the impact of deleting regulatory DNA elements will be needed to determine whether a true specialization of function exists for super-enhancers *in vivo*.

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