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VIEWPOINT

Epigenetic and transcriptional control of interferon-β

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The three classes of interferons (IFNs) share the ability to inhibit viral replication, activating cell transcriptional programs that regulate both innate and adaptive responses to viral and intracellular bacterial challenge. Due to their unique potency in regulating viral replication, and their association with numerous autoimmune diseases, the tightly orchestrated transcriptional regulation of IFNs has long been a subject of intense investigation. The protective role of early robust IFN responses in the context of infection with SARS-CoV-2 has further underscored the relevance of these pathways. In this viewpoint, rather than focusing on the downstream effects of IFN signaling (which have been extensively reviewed elsewhere), we will summarize the historical and current understanding of the stepwise assembly and function of factors that regulate IFNβ enhancer activity (the "enhanceosome") and highlight opportunities for deeper understanding of the transcriptional control of the *ifnb* gene.

After pathogen sensing, rapid and robust induction of IFN is often required to mount an effective antimicrobial response. IFNs are so named for their ability to interfere with viral replication and are essential host features in most antiviral immune responses. A tribute to their potency, aberrant expression of IFN is associated with autoimmune disorders (Taft and Bogunovic, 2018). Thus, a better understanding of the transcriptional control of IFN genes may lead to clinically relevant therapeutic advances. Type I IFNs (composed of IFNs α and β, as well as the less well-characterized IFNs τ and ω) are broadly expressed by both immune and nonimmune cell types to initiate immune responses against a wide variety of pathogens, though characteristically after viral infection. Type II IFN (composed solely of IFNγ) is expressed primarily by activated natural killer and T cells, though recent studies have shown it can also be expressed by B cells, dendritic cells, and macrophages (Castro et al., 2018). Type III IFNs and their receptors are expressed by and act predominantly in epithelial tissues as an early antiviral defense. Type I IFNs are required for an antiviral response by acting, in part, to prevent viral replication. Type I IFN is broken up into two main categories, IFNα (composed of >13 different genes) and IFNβ (composed of a single gene). Due to the heterogeneity of the IFNα genes, less is known about their transcriptional regulation. In the case of RNA viruses like H5N1, endosomal TLRs or RIG-I–like receptors bind to virus-associated molecular patterns, such as viral nucleic acids, and initiate an antiviral immune response, acting through a series of adaptor proteins (e.g., MyD88, TRAF, and others). RIG-I–like receptors specifically interact with the mitochondrial antiviral-signaling protein, which promotes activation of the TBK1 complex and activates the IκB kinase, IKKα/β, which in turn phosphorylates the inhibitor of κB kinase (IκB), causing degradation of IκB and release of the transcription factor NF-κB (Ivashkiv and Donlin, 2014). NF-κB then translocates to the nucleus, where it binds to genomic regions containing a Rel-binding site, including motifs at the IFNβ promoter, where NF-κB binding initiates recruitment of chromatin remodelers required for transcription.

Signaling via TRIF adaptor activates TBK1 and IKKε, which phosphorylate IRF3/7, inducing their dimerization, nuclear translocation, binding to ISREs within the IFNβ locus and, cooperatively with NF-κB and AP1, the induction of *ifnb* gene transcription. IFNβ autocrine and paracrine signaling activates the JAK-STAT pathway that results in the assembly of ISGF3, a heterotrimeric (STAT1/STAT2/IRF9) transcription factor that activates a variety of IFN stimulated genes including IRF3 and IRF7, which can amplify expression of type I IFN in a positive feedback loop. Thus, IRF3 and NF-κB are activated by parallel pathways working in concert with AP1 to regulate the antiviral response via IFNβ (Freaney et al., 2013). Apart from activation and DNA binding of transcription factors (TFs), it has long been appreciated that these TFs precipitate highly orchestrated changes in the chromatin "scaffold" at the *ifnb* locus that regulate transcription.

Chromatin state transitions regulate cellular differentiation and responses to extracellular stimuli. These alterations are facilitated by posttranslational modifications of histones, as well as methylation of the 5′-carbon

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of the pyrimidine ring at cytosine nucleotides (5-mC) and their oxidation products (5-hmC, 5-fC, 5-caC). In the context of the immune system, these epigenetic mechanisms, and their interplay with the binding of lineage-defining and stimulation responsive TFs, allow for the development of a diverse array of cellular states, as well as the rapid and robust transcriptional response that occurs after pathogen sensing.

Beyond histone posttranslational modifications and regulation of the stages of the transcription cycle, DNA methylation can play a role in control of IFNβ transcription, as recently described by Gao et al. (2021). 5-mC is associated with repression of regulatory DNA, X-chromosome inactivation, endogenous retro-element repression, and when located within the transcription unit, splicing (Morales-Nebreda et al., 2019). De novo DNA methylation (DNAme) is mediated by DNMT3 a/b, whereas after cell division, DNMT1 is recruited by UHRF1 to hemi-methylated DNA where it methylates the newly synthesized DNA strand. DNAme occurs on the fifth carbon of the cytosine-phosphate-guanine motif (CpG), and processive oxidation is initiated by the ten-eleven translocation (TET) family of enzymes (Liu et al., 2013). In general terms, CpG DNA methylation at promoters inhibits binding of so-called methyl-sensitive TFs and recruits repressive DNAme readers (e.g., MeCP2, SETDB1), thus restricting transcription. In contrast, processive oxidation products of DNAme are generally associated with active chromatin and result in loss of DNAme, either passively, through cell division, or actively, via base excision repair.

In light of these dynamic transitions between DNA methylation and its oxidative products, 5-mC is increasingly appreciated as a potentially dynamic stimulus-responsive epigenetic mark, departing from its historical consideration as a more stable, cell cycle–dependent event. For example, when dendritic cells encounter *Mycobacterium tuberculosis*, hundreds to thousands of genomic loci are de-methylated or oxidized, which allows for the appropriate inflammatory response (Pacis et al., 2015). Similarly, 5-mC is associated with proper immune cell development and regulation of transcriptional programs that instruct immune cell phenotypes; for example, M2 macrophage and T helper cell polarization, cytokine gene expression, and response to stimuli (Morales-Nebreda et al., 2019; Izzo et al., 2020). Unsurprisingly, aberrant DNA methylation has been associated with a variety of autoimmune diseases and pathologies, including rheumatoid arthritis, systemic lupus erythematosus, and numerous cancers (Imgenberg-Kreuz et al., 2018; Zhu et al., 2019).

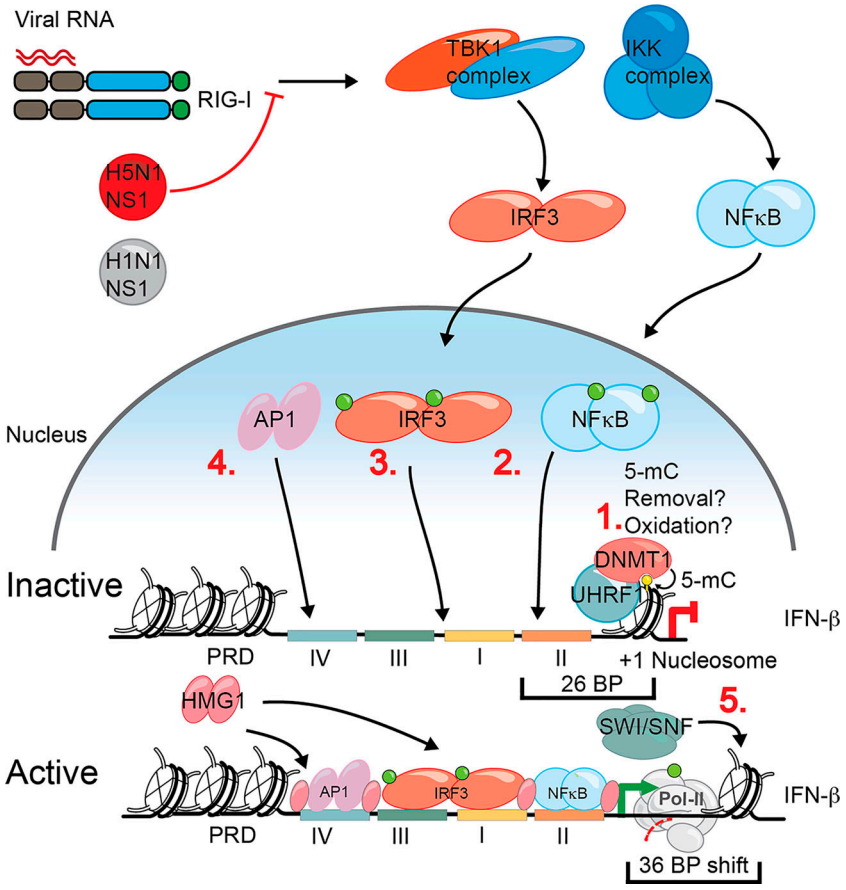

Figure 1. **A link between DNAme and IFNβ enhanceosome regulation.** After viral sensing, maintenance of UHRF1/DNMT1-mediated methylation of the +1 nucleosome is lost (1), and subsequent NF-κB binding and DNAme removal (2) allows for the binding of IRF3 and cJUN/AFT2 enhanceosome recruitment (3/4). SWI/SNF moves the +1 nucleosome 36 bp downstream, opening up access of Pol-II to TATA box and subsequent transcriptional elongation (5).

Recently, Gao et al. (2021) compared the induction of IFNβ during infection with influenza A virus strains, H5N1 and H1N1, and found a substantially dampened type I IFN expression in the highly pathogenic H5N1. Avian H5N1 infections usually occur through a zoonotic route, are characterized by a remarkably high fatality rate (42–82%), and remain a public health concern, especially if a related strain with increased transmissibility became prevalent. In contrast, swine-derived H1N1 has become pandemic, though it has a greatly diminished virulence. Thus, a complete mechanistic understanding of the antiviral IFN response is critical in understanding the differences in the pathogenesis of these diseases and strain-specific therapeutic targets. This study revealed that a major difference between the response to these viruses is the function of a single nucleotide methylation event near the IFNβ promotor and the function of DNAme and H3K9me3 reader UHRF1 (Houliston et al., 2017) that diminishes IRF3 binding and subsequent transcription in H5N1 infection. Due to the unique function of the type I IFN in generating an antiviral response, and its association with autoimmune disorders such as systemic lupus erythematosus, its transcriptional regulation has long been a focus of study; as a result, the IFNβ promoter is one of the most well-characterized and understood in mammalian gene expression. This recent study reveals an additional crucial regulatory step involving regulation of a single DNA methylation site, though the mechanisms that link this event

to upstream TF binding and augmented transcription are still unclear (Thanos and Maniatis, 1995; Agalioti et al., 2000).

IFNβ locus is controlled by four positive regulatory domains (PRD) in a nucleosome-depleted region, and the +1 nucleosome that blocks the TATA box and prevents transcription (Fig. 1). PRD I/III are IFN response factor (IRF)–binding sites, PRD II contains an NF-κB motif, and PRD IV is bound by ATF2/cJUN. The IFNβ enhanceosome is formed by signal-activated TFs: IRFs are bound to their consensus sequence, and high-mobility group protein I recruits NF-κB and cJun/ATF2 (Fig. 1). This fully assembled enhanceosome recruits the histone acetyl transferases PCAF/GCN5, which acetylates histone H4 and H3K9. This acetylation is thought to recruit chromatin remodeler and SWI/SNF family member BRG1, which, in combination with TF II D, slides the +1 nucleosome 36 bp downstream, uncovering the TATA box and the transcription start site and allowing for transcriptional initiation (Fig. 1; Thanos and Maniatis, 1995). This highly concerted sequence of signal-activated TF-binding along an array of regulatory elements has not been previously shown to depend on 5-mC. However, it is still unclear if this regulatory methylation occurs in all IFNβ-expressing cell types, as the authors only examined human peripheral blood mononuclear cells and mouse bone marrow–derived macrophages. Unexpectedly, the single nucleotide 5-mC described by Gao et al. (2021) is located 124 bp downstream of the PRD I IRF-binding site itself, within the +1 nucleosome, just 21 bp from the transcription start site (Fig. 1). This observation opens up several interesting possibilities, potentially revealing an additional layer of control of IFNβ gene expression. 5-mC can be removed through lack of propagation (by Dnmt1) during DNA replication and cell division. Additionally, TET-mediated oxidative products may abolish binding of sensitive methyl-C "readers" or recruit new readers of oxidation intermediates able to alter chromatin and regulate transcription. Alternatively, these oxidation products could be converted back to the unmodified cytosine by thymine DNA glycosylase and the base excision repair pathway. It will be particularly interesting to understand if oxidation intermediates (5-hmC, 5-fC, 5-caC) are present at this +1 nucleosome 5-mC site, as well as potential functional consequences of these states for cofactor recruitment, +1 nucleosome remodeling, and IRF3 binding. Interestingly, stimulation with TNF, a potent activator of NF-κB, is not sufficient for the removal of the +1 nucleosome and *ifnb* transcription. However, if the +1 nucleosome is artificially removed, then TNF alone is sufficient for IFNβ expression (Honda et al., 2006). This observation points toward unexplored interactions between the +1 nucleosome and IRF3, with a potential requirement for removal of 5-mC at the +1 nucleosome for efficient IRF3 binding.

Less is known about the transcriptional regulation of the various IFNα genes, in part because of the large number of genes (15) with overlapping functions. Though most *Ifna* genes do contain PRD-like domains, they do not undergo the same remodeling of the +1 nucleosome and do not require AP1 family members for transcriptional activation (Lin et al., 2000). Thus far, the exact role of these epigenetic differences in IFNα and IFNβ regulation in type I IFN responses are unexplored.

Although the classical understanding of the function of UHRF1 is to propagate hemimethylated DNA through DNMT1 recruitment, the findings of Gao et al. (2021) invoke a novel function of UHRF1 in repressing IFNβ in the absence of activating signals. One possibility is that UHRF1 binds the 5-mC (and perhaps H3K9me3, if present at the *ifnb* promoter) and locks the +1 nucleosome in position, requiring TET activity, demethylation, or possibly the presence of 5-mC oxidation products, to eject UHRF1, to facilitate chromatin activity amenable to IRF3 binding (by unknown mechanisms). Another possibility is the potential for early-acting NF-κB–associated coactivators to antagonize UHRF1 association with the +1 nucleosome, to enable stepwise *ifnb* activation. For example, the NF-κB–associated histone kinase IKKα could phosphorylate H3S10 to eject UHRF1 from H3K9me3 binding (Armache et al., 2020; Martínez de Paz and Josefowicz, 2021; Yamamoto et al., 2003). Since the IFNβ response is typically tuned down after ∼24 h, it will be interesting to examine the role of this 5-mC, UHRF1, and the +1 nucleosome in negative feedback of IFNβ transcription. Further, exploring the presence of this methylation event and UHRF1 association in other cell types will be important. Additionally, future studies should explore the mechanism by which H5N1 modulates UHRF1 activity, though it is tempting to speculate that the viral protein NS1's known inhibition of RIG-I signaling may be involved, via an indirect mechanism. As compared with H1N1, H5N1 NS1 is more abundant in the cytosol, which could allow for greater ability to inhibit RIG-I (Killip et al., 2017; Dankar et al., 2013). Reduced RIG-I activity could then result in diminished NF-κB activity, including putative NF-κB–dependent mechanisms of UHRF1 ejection.

The diverse mechanisms that viruses employ to inhibit type I IFN production highlight its critical role in the antiviral response. Various viruses contain virulence factors that inhibit TBK1, IKKε, or directly dephosphorylate IRF3/7. Many picornaviruses and flaviviruses encode proteases that directly cleave IRF7 or induce proteosome-dependent degradation of IRFs. Kaposi's sarcoma–associated herpes virus encodes an IRF-like protein that associates with IRF3/7 and inhibits their DNA binding. Thus, a better understanding of type I IFN transcriptional regulation and viral evasion mechanisms is critical for developing more advanced antiviral therapeutic strategies (Chiang and Liu, 2019).

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