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Transcriptional Regulation in the Innate Immune System

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Abstract

In cells of the innate immune system, the transcriptional response to a microbial stimulus is tailored to both the stimulus and cell type, suggesting the existence of highly sophisticated regulatory mechanisms. Early studies suggested that specificity is dictated by sets of differentially induced transcription factors that synergistically activate target genes containing their binding sites. However, recent studies have revealed additional inter-related regulatory layers, which are the topic of this article. In particular, individual transcription factors may require different post-translational modifications and co-regulatory interactions to regulate different target genes. Furthermore, competence for induction is programmed at an early stage of development by factors involved in lineage commitment, and the architecture and chromatin structure of each promoter play critical roles in transcriptional specificity.

Introduction

Cells of the innate immune system are programmed to elicit rapid and robust transcriptional responses to microbial stimuli or danger signals. A response is often initiated when cell-surface or intracellular pattern recognition receptors (PRRs) encounter pathogen-associated molecular patterns (PAMPs) [1,2]. The PRR-PAMP interaction promotes the activation of several signal transduction pathways, which activate transcription factors and other proteins that are directly involved in the regulation of gene expression [2-4].

The response is best described as a transcriptional cascade. Signaling pathways and transcription factors that are directly activated by the stimulus via post-translational mechanisms first activate a collection of primary response genes [5,6**,7]. Although some primary response genes are activated within minutes, others are activated more slowly, as some signaling pathways appear to proceed more rapidly than others and the events needed for transcription by RNA polymerase II may be completed with different rates at different genes [6**,8]. For example, primary response genes that do not require nucleosome remodeling for transcriptional activation may be activated more rapidly than remodeling-dependent primary response genes. Regardless of their activation kinetics, genes are assigned to the primary response on the basis of their ability to be transcriptionally activated in the presence of protein synthesis inhibitors, such as cycloheximide [5]. Some primary response genes encode cytokines, chemokines, and effector molecules that directly contribute to protection from the microbial challenge, but many others encode transcription factors and signaling molecules that help activate secondary response genes in the

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transcriptional cascade [6**,7,8**]. A subset of the cytokines induced during the primary response can also contribute to the secondary response by engaging their receptors on the plasma membrane and thereby activating additional signaling pathways, transcription factors, and target genes in an autocrine or paracrine manner [9,10**]. The cascade can progress for many hours, with numerous overlapping waves of transcriptional activation and attenuation [10**].

A critical aspect of the transcriptional cascade is that it is tailored to the stimulus and cell type [3,4]. Although responses can diverge dramatically, the differences between stimuli and cell types are often highly specific. For example, lipopolysaccharide (LPS) activates many of the same genes in macrophages and fibroblasts, but a few key genes whose products may be especially important in antigen presenting cells are potently activated only in macrophages. Examples of such genes are those encoding subunits of cytokines like IL-12 and IL-23, which may be selectively activated in antigen presenting cells because of their functions in polarizing T helper cell differentiation.

Because individual genes and subsets of genes are known to be tightly regulated in a stimulus- and cell type-specific manner, an important goal has been to elucidate the mechanisms responsible for transcriptional selectivity. An understanding of the selectivity mechanisms may lead to therapeutic strategies to enhance anti-microbial immune responses. Furthermore, this knowledge may suggest strategies for the selective suppression of key genes that contribute to diseases associated with excessive inflammation and hyperactivation of the immune system, while limiting the impact on the anti-microbial response.

Early models of transcriptional induction

Drawing on studies of inducible transcription in various eukaryotes in response to a broad range of stimuli, early models to explain the transcriptional response to a microbial stimulus were consistent with the following scenario: the microbial stimulus would activate several signal transduction pathways, which would then activate the DNA-binding activities of a number of transcription factors. The transcription factors would bind to recognition elements in the promoters and enhancers of target genes and act in synergy to recruit the general transcription machinery and RNA polymerase II to the core promoter and transcription start site [11,12]. Since factors that participate in transcriptional response, such as NF- κ B, have long been known to activate different target genes in different cell types and in response to different stimuli, mechanisms must exist to limit the response to a subset of the potential target genes. According to early models, specificity would be conferred primarily by cooperative binding and synergy between the various transcription factors that are selectively activated by the stimulus [11,12]. A gene will be induced only in cell types that express appropriate cell type-specific factors, and only in response to stimuli that activate an appropriate set of inducible factors.

This basic model has not been dismissed. However, recent studies have revealed additional regulatory layers that must be superimposed on the basic need for synergy between transcriptional activators bound to promoters and enhancers. First, individual inducible transcription factors may possess the capacity to use different co-regulatory proteins to regulate different sets of target genes, raising the possibility that this mechanism makes a major contribution to selectivity of the response to a stimulus. Second, cell type-specificity of the response may depend, not only on which factors are expressed when a differentiated cell encounters a stimulus, but also on events that occur early in development at enhancers for inducible target genes; these events appear to confer competence for transcriptional activation in differentiated cells. Third, transcriptional induction of each target gene is influenced, not only by the transcription factors that are constitutively expressed and

induced in response to a stimulus, but also by the basic properties of the gene's control regions and the chromatin structure assembled at the control regions prior to cell stimulation. These three inter-related topics are discussed below.

Differential regulation of inducible transcription factors

Several transcription factors contribute to inducible transcription in cells of the innate immune system and the basic mechanisms by which some of these factors are activated have been uncovered. For example, NF- κ B, a dominant regulator of inducible transcription in response to many microbial and cytokine stimuli, is activated via the inducible phosphorylation of the I κ B inhibitor proteins [13]. In unstimulated cells, NF- κ B dimers are generally sequestered in the cytoplasm by the I κ Bs. Stimulus-dependent phosphorylation of an I κ B leads to its degradation, thereby releasing the NF- κ B dimer for translocation to the nucleus, where it can bind control regions of target genes and activate transcription [13]. Diverse mechanisms are involved in the activation of other transcription factors implicated in inducible transcription, including phosphorylation-dependent nuclear translocation, phosphorylation-dependent DNA binding, and transcriptional upregulation of the transcription factor gene.

Although it has long been known that a mechanism must exist for the activation of each factor that directly participates in inducible transcription, recent studies of NF- κ B suggest that this factor and perhaps others are subject to several distinct modes of regulation. Furthermore, the diverse regulatory mechanisms may make an important contribution to the selectivity of the response to a stimulus. Specifically, the RelA member of the NF- κ B family appears to be regulated by multiple post-translational modifications, including multiple phosphorylation, acetylation, and methylation events, among others [4,13,14]. Several of these modifications appear to promote interactions with specific co-regulatory or chromatin proteins, which can contribute to transcriptional activation or repression. Importantly, the discovery of diverse regulatory strategies raises the possibility that each post-translational modification and co-regulatory interaction may contribute to the regulation of a distinct set of target genes. It is not yet known whether such a large number of modifications have been reported for NF- κ B because this prominent factor is uniquely susceptible to a diverse range of regulatory mechanisms, or because NF- κ B has simply been studied much more extensively than other factors involved in inducible transcription.

The strongest evidence in support of the hypothesis that different post-translational modifications contribute to selectivity of the response to a stimulus has emerged from studies of RelA serine 276 (S276) phosphorylation by cyclic AMP-dependent protein kinase (PKAc). Analysis of mice in which the S276 phosphoacceptor was genetically altered revealed that S276 phosphorylation – and, therefore, signaling via PKAc or other S276 kinases – is required for the activation of a specific subset of NF- κ B target genes in stimulated macrophages and fibroblasts [15**]. S276 phosphorylation promotes an interaction between NF- κ B and the p300/CBP coactivators, leading to the suggestion that NF- κ B is responsible for the recruitment of these coactivators to only a subset of NF- κ B target genes [16**,17**].

It is not yet known whether target genes that are unaffected by the S276 mutation are activated independently of p300/CBP or whether these common coactivators are recruited to these genes by other mechanisms. It also is not yet known whether S276-independent target genes require one of the other coregulatory proteins that have been found to interact with NF- κ B. Nevertheless, the biological implication of the above finding is that, although NF- κ B is activated by almost all stimuli that promote an innate immune response, it may also play a direct role in tailoring the response via its susceptibility to different inducible post-

translational modifications, each of which may be important for the activation of a distinct set of target genes. This hypothesis needs to be tested more rigorously in the future, in part by asking whether NF- κ B-inducing stimuli can be identified that fail to promote S276 phosphorylation. If so, do these stimuli fail to activate target genes that appear to be dependent on S276 phosphorylation, and is the differential regulation of these genes biologically relevant?

Establishing transcriptional competence during development

It has been well-established that the molecular events leading to cell-type-specific transcription can begin early in development, long before a gene is transcribed. One example is the *Alb1* (albumin) gene, in which key transcription factors, FoxA1 and GATA4, are known to associate with its liver-specific enhancer in the endoderm [18**,19,20]. The binding of these factors is thought to induce chromatin changes that facilitate the binding of additional transcription factors later in development, culminating in transcriptional activation in hepatocytes.

Recently, this paradigm has been extended to inducible genes involved in innate immunity. Specifically Ghisletti et al. and Heinz et al. have shown that enhancers for inducible genes in macrophages become associated with transcription factors involved in lineage commitment at an early stage of development [21**,22**] (Figure 1). The Ghisletti et al. studies began with a genome-wide chromatin immunoprecipitation (ChIP-Seq) analysis of histone H3 lysine 4 monomethylation (H3K4me1) and the p300 coactivator protein in unstimulated and LPS-stimulated macrophages [21**]. The goal was to identify transcriptional enhancers genome-wide that support inducible transcription. The p300 ChIP-Seq results revealed thousands of relatively sharp ChIP-Seq peaks that were observed only the stimulated cells. Since many of these peaks were in the vicinity of LPS-induced genes, but did not coincide with transcription start sites, they were suggested to correspond to inducible enhancers. Histone H3K4me1, a mark of transcriptional enhancers [23], was broadly distributed over most of the p300 peaks, providing further evidence that the peaks correspond to enhancers. However, the H3K4me1 marks were observed in both unstimulated and stimulated cells, suggesting that the mark is deposited during development.

Importantly, bioinformatics analyses to identify DNA motifs that are enriched at the genomic regions exhibiting inducible p300 binding identified a substantial enrichment for binding sites for the PU.1 transcription factor, which plays an important role in macrophage development [21**, 24, 25]. Additional studies suggested that PU.1, presumably in combination with other critical regulators of macrophage development, binds the enhancers at an early stage of macrophage development. Upon enhancer binding, these factors were proposed to induce H3K4me1 deposition and other changes in chromatin structure that may confer susceptibility to transcriptional induction in the differentiated cells (Figure 1).

The study of Heinz et al. led to the same conclusions, but began with ChIP-Seq analyses of PU.1 in B cells and macrophages, two cell types that rely on this factor for their development [22**]. PU.1 was found to bind thousands of genomic sites in the two cell types, but many of the binding sites differed. In each cell type, the binding sites were often found to be in close proximity to expressed cell type-specific genes, but binding was distant from the promoter, again consistent with the hypothesis that regions identified correspond to enhancers. Additional studies implicated other key regulators of B-cell and macrophage development in the marking of these enhancers at an early stage of development.

These results strongly suggest that the cell type-specificity of genes induced in response to a stimulus is determined at an early stage of development when enhancers for the inducible genes become associated with factors that are involved in development and lineage

commitment and early stages of development. Although these studies provide a major advance toward an understanding of the cell type-specificity of the response to a stimulus, a number of questions remain to be answered. In particular, is there a specific reason why enhancers seemed to acquire chromatin marks at an early stage of development? Chromatin structure has been found to be less compact in stem cells than in differentiated cells [19,26-28]. One possibility is that chromatin changes must take place early in development to prevent genes from assembling into repressive chromatin structures that would be resistant to activation in mature cells. A second key question is how the marking of enhancers for inducible genes early in development influences the chromatin structure and protein-DNA interactions at promoters. The studies discussed above did not carefully analyze promoters. However, studies of individual inducible model genes in macrophages have shown that at least some promoters acquire unmethylated CpG dinucleotides during macrophage development, yet nucleosome remodeling and the binding of inducible transcription factors are still required for transcriptional induction [29]. Additional studies are needed to determine the relationship between events occurring at enhancers and promoters during early development and in response to a stimulus. Direct communication via looping has been well-documented, but the mechanism by which this communication orchestrates transcriptional induction remains to be elucidated.

Influences of promoter and chromatin structure on transcriptional activation

A third recent advance concerns the impact of promoter architecture and chromatin structure on the specificity of a transcriptional response to a stimulus. According to early models, activation of a set of inducible transcription factors would allow these factors to bind and activate genes containing an appropriate collection of recognition sites for the factors in their promoters or enhancers [12]. However, the chromatin structure at the promoters of inducible genes appears to be quite diverse and can strongly influence susceptibility to gene activation and selectivity of a response.

Two fundamental classes of promoters have long been known to exist [30]. The first class, referred to as CpG-island promoters, contains CpG dinucleotides at the abundance that would be expected if all four nucleotides were randomly distributed throughout the genome. The second class, which we refer to as low CpG promoters, contains CpG dinucleotides at the low abundance that is observed through most of the genome in mammals. CpG dinucleotides are underrepresented because 5-methylcytosine can undergo deamination to form thymine; this process is thought to have resulted in the loss of CpG dinucleotides during the evolution of species in which high levels of 5-methylcytosine are observed [30]. Approximately 70% of mammalian promoters contain CpG-islands and the other 30% contain a low CpG content, with only a small number between the two extremes [31, 32]. The precise reason for the evolution and maintenance of these two promoter classes in mammalian genomes remains unknown. However, the properties of the two classes generally are quite distinct among inducible genes.

CpG-island promoters are highly prevalent among primary response genes that are rapidly induced by LPS and other Toll-like receptor ligands [6**,33**] (Figure 2). These genes are usually induced in the absence of nucleosome remodeling by SWI/SNF complexes and, in unstimulated cells, their promoters contain features of active genes, including high histone H3K4me3 and high levels of RNA polymerase association [6**,33**]. In contrast, low CpG promoters are much more prevalent among secondary response genes. These genes require SWI/SNF complexes for their transcriptional induction and their promoters exhibit low H3K4me3 and low polymerase association [6**,33**].

From a biological perspective, CpG-island promoters seem to be enriched among genes that are known to be induced by a broad range of stimuli, such as *Fos*, *Jun*, *Egr1*, *Nfkb1a*, and *Nfkbiz* (Figure 2). In contrast, genes containing low CpG promoters tend to require tight regulation and encode key cytokines and effective molecules, such as *Il12b*, *Il6*, and *Nos2*. The dramatic differences in promoter and chromatin properties may play a major role in selectivity [4,6**,33**]. Common factors like NF-κB and AP-1 are induced by many stimuli and, upon their induction, these factors may be competent for the activation of genes that are assembled into active chromatin in unstimulated cells. However, the nucleosome remodeling requirement observed at many low CpG promoters may confer a requirement for additional transcription factors that are capable of promoting nucleosome remodeling by SWI/SNF complexes. The promoters for these genes often contain binding sites for factors like NF-κB and AP-1. If the nucleosome barrier were not present, these factors may be sufficient for transcriptional activation and the desired selectivity of expression may be lost. Importantly, the distribution of two classes of promoters varies with the stimulus. For example, low CpG promoters are most prevalent during the primary response to Type 1 interferon signaling [6**], perhaps because most genes induced by Type 1 interferons require tight regulation and the transcription factors that are induced – STATs and IRFs – can readily promote nucleosome remodeling [34-36].

Additional support for the hypothesis that chromatin differences can contribute to selectivity of a transcriptional response in cells of the innate immune system has emerged from an effort to identify and characterize small molecule inhibitors of chromatin proteins. The most relevant results have emerged from studies of inhibitors of a class of chromatin-associated adaptor proteins, known as BRD proteins [37**]. BRD proteins contain bromodomains that bind acetylated histones. Upon binding to acetylated histones, BRD proteins recruit P-TEFb, which contributes to transcriptional activation by promoting transcription elongation and RNA processing [33**,38-40]. The proteins therefore serve as a bridge between chromatin and transcription. An important role for BRD proteins in cells of the innate immune system was supported by the finding that LPS stimulation of macrophages enhanced acetylation of histone H4 lysine residues that are known to be responsible for BRD recruitment [33**].

Strikingly, highly selective chemical inhibitors of BRD proteins have been developed and have been found to inhibit a select subset of LPS-induced genes [37**]. The BRD inhibitors appeared to preferentially inhibit genes containing low CpG promoters, suggesting that the activation of many genes in this class may depend on BRD recruitment. Conversely, inducible genes containing CpG island promoters either do not require BRD proteins for their activation, or the BRD proteins are recruited to these promoters via a mechanism that is not disrupted by the small molecule inhibitors. In support of the latter possibility, RNA interference experiments suggested that BRD proteins are required for the activation of all inducible genes [37**]. The chemical inhibitors prevent BRD proteins from binding acetylated histones, suggesting that BRD proteins may be recruited to some promoters by acetylated histones and to other promoters by interactions with transcription factors [41]. Most importantly, these results highlight the diversity of mechanisms by which chromatin and chromatin-associated proteins can contribute to a transcriptional response. The results highlight the great potential for the selective modulation of specific subsets of inducible genes in a therapeutic setting by targeting chromatin-associated proteins.

Conclusions

Three emerging strategies that appear to make major contributions to the selective regulation of a transcriptional response have been highlighted in this article. However, it is important to conclude by emphasizing that the precise contribution of each of these mechanisms to transcriptional selectivity in specific physiological settings will require further investigation.

It is also important to emphasize that the three mechanisms are closely related to each other. For example, the molecular events occurring during lineage commitment and development that appear to provide competence for transcriptional induction have been closely linked to changes in chromatin structure, which may therefore be responsible for transcriptional competence. Furthermore, a large fraction of the proteins that have been reported to interact with inducible transcription factors like NF- κ B are chromatin proteins. NF- κ B's requirement for different post-translational modifications and co-regulatory interactions at different target genes may therefore be closely connected to the chromatin structure that is established at the control regions of each gene during development. Finally, different promoter architectures (e.g. CpG-island versus low CpG) are closely correlated with differences in chromatin structure, especially in unstimulated cells. Therefore, a full understanding of the mechanisms by which each of these emerging strategies contributes to selectivity of a transcriptional response will require studies that emphasize the interplay between the strategies rather than studies of each mechanism in isolation.

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Highlights

Transcriptional selectivity can be influenced by diverse co-regulatory interactions of individual transcription factors.

Cell-type specificity of induction is directly influenced by key regulators of lineage commitment.

Promoter architecture and chromatin structure make major contributions to transcriptional selectivity.

Figure 1.

Acquisition of competence for transcriptional induction during development. The cell-type specificity of inducible genes appears to be strongly influenced by molecular events that occur during lineage commitment and/or early development. During early development, enhancers for inducible genes become associated with transcription factors that have long been implicated in lineage commitment and early development, including PU.1 [21**,22**]. Binding of PU.1 and other developmental regulators induces changes in chromatin structure and the acquisition of a histone H3K4me1 mark. Enhancers for some inducible genes are associated with transcription factors in pluripotent cells [29]. The molecular events that occur in pluripotent cells and during development may be necessary for transcriptional competence in stimulated mature cells. Upon stimulation, inducible transcription factors carry out additional interactions with the enhancers and promoters of inducible genes and promote additional changes in chromatin structure, culminating in transcriptional activation. The chromatin events required for transcriptional induction vary from gene to gene and are likely to contribute to selectivity of the transcriptional response to a stimulus.

- CpG island promoters are assembled into open, active chromatin prior to induction.
- Readily activated by factors that cannot promote nucleosome remodeling.
- Promiscuously activated genes, including several transcription factor genes (*Fos*, *Jun*, *Egr*).
- Often resistant to BRD inhibitors.

- Low CpG promoters usually lack features of active chromatin in unstimulated cells.
- Nucleosome remodeling by SWI/SNF complexes often required.
- Remodeling facilitated by stimulus-specific factors, such as IRF3 and primary response gene products.
- Sensitive to BRD Inhibitors

Figure 2.

Two distinct classes of promoters associated with inducible genes. Many inducible genes contain CpG-island promoters, which usually possess features of active chromatin in unstimulated cells. These features include high levels of histone H3K4me3, high levels of pre-associated RNA polymerase II, accessibility to restriction endonuclease cleavage, and low nucleosome density. The low nucleosome density may be due in part to the intrinsic instability of nucleosome assembled on CpG-island sequences. These features of active chromatin allow transcriptional induction in the absence of SWI/SNF-dependent nucleosome remodeling. CpG-island promoters are often associated with genes induced by a broad range of stimuli with little selectivity. In contrast, genes with low CpG promoters contain stable nucleosomes at their promoters, lack features of active chromatin in unstimulated cells, and often require nucleosome remodeling for their activation. The requirement for nucleosome remodeling provides an important barrier to transcriptional activation and confers a requirement for transcription factors that can recruit nucleosome remodeling complexes. IRF3 promotes nucleosome remodeling at several LPS-induced, remodeling-dependent primary response genes. Primary response genes that remain to be identified appear to be required for nucleosome remodeling at secondary response genes, as remodeling at these genes is dependent on new protein synthesis.