Toward an Understanding of the Gene-Specific and Global Logic of Inducible Gene Transcription

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Virtually all living organisms have evolved mechanisms to adapt to their environment by sensing environmental stresses and inducing the transcription of appropriate sets of response genes in a coordinated fashion. In the vertebrate immune system, the highly selective response to an environmental stimulus, often an invading microorganism, plays an especially important role in regulating the activities of, and interactions among, the many cell types involved in innate and adaptive immunity. It is now widely appreciated that the selective response to a stimulus requires the concerted action of signal transduction pathways, transcription factors, and chromatin structure. Many proteins and pathways that help to regulate a response have been characterized. However, our understanding of the gene-specific and global logic through which a highly selective response is elicited has only recently begun to emerge.

Humans and all other living organisms have acquired the ability to respond to a broad range of environmental stresses. The responses are initiated by the sensing or recognition of the stress, usually leading to the activation of signal transduction pathways that culminate in the altered expression of genes encoding proteins that help the organism to tolerate the stress. Changes in transcription are of central importance for the gene expression changes.

The responses to environmental stresses possess special significance in the context of the vertebrate innate and adaptive immune systems that comprise a large number of cell types that coordinate their response to microbial pathogens and other environmental stresses through the induction of innate effector pathways and/or a wide range of highly specific B- and T-cell responses (Akira et al. 2006). Because of the complexity of the immune system and the diversity of responses that can be elicited, exquisite specificity is needed. As one example, the specific cytokines expressed by cells of the innate immune system in response to microbial infection help to regulate the differentiation of naive T cells into one of the several T-helper-cell types, with different T helper subsets essential for the control of different microbial pathogens.

The exquisite specificity of transcriptional responses is readily observed by measuring mRNA levels following exposure of cells to different stimuli. For example, in response to microbial signaling through most Toll-like receptors (TLRs), macrophages and dendritic cells potently induce transcription of the *Il12b* gene that encodes the p40 subunit of the IL-12 and IL-23 cytokines that play critical roles in Th1 and Th17 differentiation, respectively (Trinchieri 2003). In contrast, in response to tumor necrosis factor (TNF) stimulation through the TNF recep-

tors, the *Il12b* gene remains silent, despite the common induction of hundreds of other genes by TLRs and the TNF receptors. A major goal is to understand how this exquisite selectivity is achieved, not only at the level of an individual gene such as *Il12b*, but also at a broader systems level. That is, what is the gene-specific and global logic through which highly specific responses to diverse stimuli are regulated?

In the simplest scenario, each stimulus induces a different set of signal transduction pathways, each of which activates one or more transcription factors that bind the control regions of the potential target genes; transcriptional induction then occurs at genes bound by an appropriate combination of induced transcription factors (Carey et al. 2009). Although the signal transduction pathways induced by a stimulus play a central role in dictating the transcriptional response, and although combinatorial mechanisms have long been known to be essential for the diverse gene regulation patterns needed in complex organisms (Britten and Davidson 1969; Georgiev 1969; Gierer 1973), it is now widely appreciated that other factors, such as the developmental history of the cells and chromatin features of candidate genes, collaborate with signaling pathways and inducible transcription factors to regulate the response. This collaboration has been studied extensively and has been the topic of several recent reviews (Medzhitov and Horng 2009; Smale 2010; Fowler et al. 2011; Natoli et al. 2011; Nechaev and Adelman 2011; Smale et al. 2014). This article focuses on the experimental strategies used in our laboratory to pursue a better understanding of the logic through which transcription factors and chromatin structure collaborate with signaling pathways to induce a selective response to a microbial stimulus.

BASIC STUDIES OF *II12b* TRANSCRIPTIONAL INDUCTION

Our studies of stimulus-induced transcription began with an emphasis on the molecular mechanisms regulating induction of the *II12b* gene in mouse macrophages in its native chromosomal environment. These studies began soon after the critical role of chromatin in regulated transcription became widely appreciated, due to compelling evidence that histone acetyltransferases and ATP-dependent nucleosome remodeling complexes help to regulate transcription (Côté et al. 1994; Imbalzano et al. 1994; Kwon et al. 1994; Brownell et al. 1996). Until recently, a preferred approach toward understanding gene regulation in a specific biological setting was to examine the regulation of a specific model gene, with the expectation that regulatory principles emerging from studies of the model gene would be broadly relevant. The *II12b* gene was chosen as the model for our studies because of its potent induction in mouse macrophages in response to microbial stimuli and because the critical role of IL-12 (and later IL-23) in bridging innate and adaptive immune responses had recently been described (Trinchieri 2003).

Although our objective was to understand *II12b* regulation in the context of chromatin, it was first important to identify DNA elements and transcription factors that contribute to *II12b* regulation. In the 1980s and 1990s, the most common strategy toward this goal was to develop a functional assay in which the activity of the promoter could be examined; if proper regulation of the wild-type promoter was observed, mutations could be introduced systematically into promoter sequences, allowing the identification of DNA elements that are important for promoter activity (Carey et al. 2009). Electrophoretic mobility shift assays, DNase I footprinting, and other assays could then be used to identify transcription factors that bind the important elements. Using a functional assay in which macrophage cell lines were transfected with plasmids containing the *II12b* promoter fused to a luciferase or chloramphenicol acetyltransferase gene, followed by lipopolysaccharide (LPS) stimulation of the transfected cells, putative binding sites for NF- κ B, C/EBP, AP-1, and NFAT were identified (Plevy et al. 1997; Zhu et al. 2001, 2003). Subsequent studies led to the identification of an *II12b* enhancer located 10 kb upstream of the transcription start site that also appears to contribute to *II12b* induction (Zhou et al. 2007).

Importantly, none of the transcription-factor-binding sites identified in the *II12b* promoter or enhancer provided an obvious explanation for the selectivity of induction (e.g., its induction by TLRs but not by the TNF receptor). That is, all of the factors identified were known to be induced by a broad range of stimuli. It is important to emphasize, however, the inherent challenges in evaluating data obtained in conventional gene regulation analyses. For example, all of the transcription factors implicated in *II12b* regulation are members of multiprotein families, with different family members often recognizing similar sequences but potentially contributing different functions. Redundancy among family members and other technical

limitations make it difficult to identify the family member that contributes to regulation and rigorously dissect regulatory mechanisms. Furthermore, transfection assays often do not accurately reflect the regulation of an endogenous gene; DNA elements and transcription factors that are critical for regulation of the endogenous gene may play no role in the regulation of a transfected promoter-reporter plasmid; one reason is that transfected plasmids do not assemble into a chromatin structure that resembles the chromatin structure found at the endogenous gene in its native chromosomal location (Smith and Hager 1997; Carey et al. 2009).

NUCLEOSOME REMODELING AT *II12b* REGULATORY REGIONS

To determine whether chromatin contributes to *II12b* regulation, we first used micrococcal nuclease to map the locations of nucleosomes in the vicinity of the *II12b* promoter (Weinmann et al. 1999). The data suggested that, in unstimulated macrophages, a nucleosome encompasses all of the key promoter elements that had been identified. A restriction enzyme accessibility assay was then used to determine whether chromatin structure was altered in response to LPS stimulation (Weinmann et al. 1999). In this assay, nuclei from unstimulated or LPS-stimulated macrophages are incubated with a restriction endonuclease that cleaves in the genomic region of interest. Because the genome remains assembled into chromatin in the nuclei, most genomic regions will be largely resistant to restriction enzyme cleavage. However, the cleavage efficiency is generally higher at regions where nucleosomes have been removed or physically remodeled.

Using the restriction enzyme accessibility assay, the *II12b* promoter region was found to be largely resistant to cleavage in unstimulated cells, but cleavage efficiency was substantially higher in nuclei from LPS-stimulated cells (Fig. 1) (Weinmann et al. 1999; Ramirez-Carrozzi et al. 2006). Similar results were later obtained with the *II12b* enhancer (Fig. 1) (Zhou et al. 2007). These results suggested that nucleosome-remodeling events at the *II12b* promoter and enhancer accompany transcriptional activation. These findings led to speculation that the regulation of nucleosome remodeling may be of central importance for the selective regulation of transcriptional induction. For example, TLR4 and TNF receptors both induce NF- κ B, AP-1, and other common inducible transcription factors. However, according to our hypothesis, most of these factors cannot gain access to their binding sites in the promoter and enhancer unless a prior event promotes nucleosome remodeling. Consistent with this hypothesis, nucleosome remodeling at the *II12b* promoter and enhancer was not observed in TNF-stimulated cells.

On the basis of the above results and hypothesis, a critical goal is to identify the inducible factor or events that regulate inducible remodeling at the *II12b* promoter and enhancer. Nucleosome remodeling at the promoter and enhancer, like *II12b* transcription, is known to be strongly inhibited by the protein synthesis inhibitor, cy-

Figure 1. Nucleosome remodeling at the *Il12b* promoter and enhancer as monitored by restriction enzyme accessibility. The presence of nucleosomes in the vicinity of the *Il12b* promoter and ~ 10 kb enhancer in unstimulated cells is depicted schematically at the top. Cleavage sites for the restriction enzyme *SpeI* are shown. Restriction enzyme accessibility quantified by genomic Southern blot is shown at the bottom for both the enhancer and promoter in unstimulated and LPS-stimulated bone-marrow-derived macrophages. The band labeled “Uncut” corresponds to a restriction fragment generated by complete cleavage of the purified genomic DNA with a restriction enzyme whose sites flank the critical *SpeI* cleavage site at the enhancer or promoter. The fraction of this fragment cleaved by *SpeI* in the intact nuclei before purification of the genomic DNA is labeled as “Cut.” The absence of the “Cut” band when the cells were stimulated in the presence of cycloheximide (CHX) shows the protein synthesis dependence of the inducible nucleosome remodeling. (Adapted from Ramirez-Carrozzi et al. 2006.)

cloheximide (Weinmann et al. 1999; Zhou et al. 2007). This result suggests that one or more proteins synthesized during the primary response to TLR4 signaling plays a critical role in the inducible remodeling. Unfortunately, the identity of this key inducible factor remains unknown.

NUCLEOSOME REMODELING BY SWI/SNF COMPLEXES

Although the factor responsible for the inducibility of nucleosome remodeling at the *Il12b* promoter and enhancer remains elusive, the enzyme that catalyzes nucleosome remodeling appears to have been identified. Importantly, further analysis of this enzyme’s role in TLR4-induced transcription provided a starting point toward elucidation of the global logic through which transcriptional responses to microbial and environmental stimuli are regulated.

As described above, the increased access to restriction endonuclease cleavage upon macrophage activation was

the most pronounced chromatin change observed at the *Il12b* promoter and enhancer. (Increases in histone modifications have also been observed [Weinmann et al. 1999; Ramirez-Carrozzi et al. 2006, 2009], but the magnitudes of these changes are relatively small.) The protein complexes that have been implicated most frequently in physical changes to nucleosome cleavage are ATP-dependent nucleosome-remodeling complexes that use the energy of ATP hydrolysis to disrupt interactions between the histone octamer and DNA, resulting in nucleosome eviction, sliding, or simply increased accessibility of the nucleosomal DNA (Clapier and Cairns 2009).

To determine whether an ATP-dependent nucleosome remodeling complex might be responsible for the increased accessibility of the *Il12b* promoter and enhancer, different complexes were depleted in a macrophage cell line by retroviral delivery of small hairpin RNAs (shRNAs) directed against the mRNAs encoding the catalytic subunits of the complexes (Ramirez-Carrozzi et al. 2006). Transcriptional induction of the *Il12b* gene was greatly reduced only upon expression of an shRNA target-

ing the two catalytic subunits of the SWI/SNF remodeling complex, Brg1 and Brm. These two proteins act with partial redundancy through their association with a similar set of Brg1-associated factors (Clapier and Cairns 2009). Simultaneous depletion of both catalytic subunits was achieved through the use of an shRNA that targets a homologous region of their mRNAs.

In addition to the reduced *I12b* transcription observed following knockdown of Brg1 and Brm, LPS-induced restriction enzyme accessibility at both the *I12b* promoter and enhancer was reduced. Inducible Brg1 recruitment to the *I12b* promoter and enhancer, which was observed by chromatin immunoprecipitation (ChIP), was also reduced following Brg1 knockdown (Ramirez-Carrozzi et al. 2006). Together, these results strongly suggest that *I12b* induction requires nucleosome remodeling at the promoter and enhancers by the SWI/SNF-remodeling complexes following their specific recruitment by a recruitment mechanism that remains unknown. Interesting, a study by Chi et al. has suggested that, following its recruitment, SWI/SNF activity requires an additional calmodulin-dependent signaling event (Lai et al. 2009).

VARIABLE ROLE OF SWI/SNF COMPLEXES AT INDUCIBLE GENES

The evidence that inducible nucleosome remodeling is needed for transcriptional induction of *I12b* was initially difficult to reconcile with results emerging from the study of other inducible genes. Specifically, classic studies of heat-shock genes in *Drosophila* had revealed that their promoters are assembled into constitutively accessible chromatin (Tsukiyama et al. 1994; Shopland et al. 1995; Nechaev and Adelman 2011). Not only are their promoters accessible, but RNA polymerase II appears to have initiated transcription, although it remains paused slightly downstream from the transcription start site. Heat-shock induction then promotes elongation of the paused polymerase, with its release serving as a major regulator of transcriptional induction (Nechaev and Adelman 2011). Initiation of transcription and pausing before cell stimulation was also reported at the inducible human immunodeficiency virus long-terminal repeat promoter (Verdin et al. 1993). In contrast, similar to the inducible nucleosome remodeling observed at the *I12b* promoter, inducible nucleosome remodeling at the promoter appeared to be important for induction of the yeast *PHO5* gene, the mouse mammary tumor virus long-terminal repeat, and the human *IFN-β* gene (Almer et al. 1986; Fryer and Archer 1998; Agalioti et al. 2000).

These findings raised the question of why different chromatin properties are present at different inducible genes before their induction. One possibility is that the variability simply reflects the evolution of diverse mechanisms to regulate inducible transcription, with no biological significance to the variability. A more interesting possibility is that the variability is an important feature of the global logic through which selective responses to diverse stimuli are elicited. A closer examination of the

impact of Brg1/Brm knockdown in macrophages provided an opportunity to explore this important question.

Specifically, an examination of the mRNA levels for several LPS-induced genes in mouse macrophages revealed that these genes, upon their induction in a single cell type by a single stimulus, could be grouped into different categories on the basis of their sensitivity to Brg1/Brm knockdown (Ramirez-Carrozzi et al. 2006, 2009). Many genes, including a substantial fraction of primary response genes (genes induced in the presence of cycloheximide), were induced in a Brg1/Brm-independent manner. In contrast, the remaining primary response genes and most secondary response genes were induced at greatly reduced levels following Brg1/Brm knockdown.

MECHANISTIC AND BIOLOGICAL INSIGHTS INTO VARIABLE NUCLEOSOME REMODELING REQUIREMENTS

To examine the significance of the variable SWI/SNF dependence of inducible genes in LPS-stimulated macrophages, the impact of Brg1/Brm knockdown on the expression of 67 inducible genes was examined by quantitative reverse transcription–polymerase chain reaction (RT-PCR) (Fig. 2) (Ramirez-Carrozzi et al. 2009). An evaluation of the chromatin properties and biological properties of the SWI/SNF-dependent and SWI/SNF-independent genes suggested models to explain the variability at both the mechanistic and biological levels.

Mechanistically, the promoters of genes activated in a SWI/SNF-independent manner were generally assembled into a chromatin structure in unstimulated macrophages resembling that found at transcriptionally active genes. Specifically, the promoters were accessible to restriction enzymes in unstimulated cells, with little change upon induction (Fig. 2, *Tnf*). ChIP experiments further revealed that the promoters of most of these genes contained high levels of histone H3 lysine 4 trimethylation (H3K4me3) in unstimulated macrophages, with relatively little increase following LPS stimulation (Ramirez-Carrozzi et al. 2009). In addition, RNA polymerase II was associated with the promoters of many SWI/SNF-independent genes in the unstimulated cells; initiation and pausing appears to occur at some of these genes before stimulation, as observed at the *Drosophila* heat-shock genes (Adelman et al. 2009; Hargreaves et al. 2009). Together, these results suggest that inducible remodeling by SWI/SNF complexes is not needed at these genes because their promoters are pre-assembled into an active chromatin state in unstimulated cells.

Examination of the promoter sequences of the SWI/SNF-independent genes revealed a high prevalence of CpG-island promoters (Hargreaves et al. 2009; Ramirez-Carrozzi et al. 2009). CpG islands are found at ~70% of promoters in mammalian cells, but their functional significance is not fully understood (Deaton and Bird 2011). Early biochemical studies revealed that DNA sequences with an extremely high content of G/C base pairs assemble into unstable nucleosomes (Drew and Travers 1985),

Figure 2. Variable sensitivities of LPS-induced genes to Brg1/Brm knockdown in mouse macrophages. At left, the effect of Brg1/Brm knockdown on mRNA levels for 67 LPS-induced genes (55 primary response genes and 12 secondary response genes) in mouse bone-marrow-derived macrophages is shown. mRNA levels are presented as a percentage of the levels observed in control cells stimulated with LPS. Genes that are induced at nearly normal or enhanced levels following Brg1/Brm knockdown are colored red, whereas genes whose expression is reduced to the greatest extent are colored green. Genes colored brown or yellow showed intermediate effects. At the right, restriction enzyme accessibility results are shown for the promoters of three representative genes: *Tnf*, representative of a Brg1/Brm-independent primary response gene; *Ccl5*, representative of a Brg1/Brm-dependent primary response gene; and *Il6*, representative of a Brg1/Brm-dependent secondary response gene. (Adapted from Ramirez-Carrozzi et al. 2006, 2009.)

raising the possibility that the absence of a remodeling requirement at CpG-island promoters may be due, at least in part, to an intrinsic instability of nucleosomes assembled at these promoters. Consistent with this possibility, intrinsic instability was observed at many CpG-island promoters in nucleosome assembly experiments performed in vitro (Ramirez-Carrozzi et al. 2009). The relevance of this intrinsic instability for SWI/SNF-independent transcription awaits further analysis.

In contrast to the properties of SWI/SNF-independent genes, SWI/SNF-dependent genes were usually found to contain low CpG promoters with low histone H3K4me3 in unstimulated cells (Ramirez-Carrozzi et al. 2009). The

promoters of representative genes in this class showed low accessibility to restriction enzyme cleavage in unstimulated cells, with increased accessibility following stimulation (Fig. 2, *Ccl5*, and *Il6*). Thus, mechanistically, the variable dependence of inducible genes on SWI/SNF complexes correlated closely with the variable chromatin properties observed at their promoters in unstimulated cells.

At a biological level, SWI/SNF-independent induction was generally observed at genes that are known to be induced by a broad range of stimuli, such as the *Fos*, *Jun*, *Egr1*, *Egr2*, *Egr3*, and *Nfkb* genes. This finding suggests a hypothesis in which genes induced by a broad

range of stimuli have little need for a nucleosome barrier to activation. In contrast, SWI/SNF dependence was observed at cytokine and chemokine genes that are known to be selectively regulated, such as *Il12b*, *Il6*, and *Ifnb*. These findings are consistent with the model described above, in which nucleosome remodeling provides a critical barrier to activation that contributes to selectivity of the response to a stimulus.

It is important to emphasize that, although the results described above provide an initial framework, a number of findings during the course of these studies have revealed additional complexity that will need to be examined further to fully understand how chromatin contributes to transcriptional induction and the selectivity of a transcriptional response. For example, although most SWI/SNF-independent genes contain CpG-island promoters that are assembled into constitutively active chromatin, a subset of the SWI/SNF-independent genes were found to contain low CpG promoters assembled into inaccessible chromatin in unstimulated cells (Ramirez-Carrozzi et al. 2009). One possibility is that nucleosomes at these promoters require remodeling by an ATP-dependent nucleosome remodeling complex other than the SWI/SNF complexes. In addition, the *Il6* gene, which is an SWI/SNF-dependent secondary response gene in macrophages, was found to be an SWI/SNF-independent primary response gene with a constitutively accessible promoter in fibroblasts (Ramirez-Carrozzi et al. 2009). Presumably, a factor expressed during fibroblast development helps to catalyze the assembly of a constitutively accessible chromatin structure at the low CpG *Il6* promoter, thereby allowing rapid activation in the absence of nucleosome remodeling. Although the significance of this finding is unknown, one possibility is that it reflects the different biological functions of the IL-6 cytokine in the two cell types. In macrophages, IL-6 is thought to be a major regulator of T-helper-cell differentiation, a function that may require highly selective regulation by different stimuli. In contrast, IL-6 appears to function as a broad regulator of inflammation when expressed by fibroblasts, perhaps suggesting a lesser requirement for tight regulation.

RNA-SEQUENCING ANALYSES OF INDUCIBLE TRANSCRIPTION

A limitation of the studies described above is that they involved an analysis of only 67 inducible genes, with the expression of each gene monitored by quantitative RT-PCR in all experiments. In other words, a true genome-scale analysis was not performed. Efforts to extend the analysis using microarrays were unsuccessful due to the limited quantitation and accuracy of microarray platforms. However, the emergence of RNA-sequencing (RNA-seq) as a more accurate and quantitative alternative to microarrays has provided an opportunity to perform truly genome-scale analyses of inducible gene expression cascades.

An additional advantage of RNA-seq is that it has been used with considerable success to examine RNA popula-

tions other than mature mRNA. Of particular relevance to the goal of dissecting transcriptional cascades, RNA-seq can be used to monitor nascent transcripts for genes that are being actively transcribed. A major benefit of analyzing nascent transcripts when studying transcriptional cascades is that changes in nascent transcript levels following cell stimulation provide a more accurate measure of the kinetics of transcriptional activation and inactivation. After transcription of a gene is maximally induced, its mRNA will continue to accumulate until it reaches its steady state, which is dictated by both its rate of synthesis and degradation. Thus, two genes that are transcriptionally induced at the same time after stimulation will show different mRNA kinetics if their stabilities differ. Similarly, transcriptional inactivation is not accurately measured by mRNA analyses because a stable mRNA can persist for several hours after transcription of the gene is turned off. To understand the global logic through which transcriptional cascades are regulated, it is important to identify groups of genes that are turned on and off with similar kinetics in response to a stimulus; nascent transcript analyses provide a much more accurate measure of transcriptional kinetics than do mRNA analyses.

Several RNA-seq methods have been developed to monitor nascent transcripts, including GRO-seq (genome-wide nuclear runon) (Core et al. 2008), NET-Seq (analysis of RNA polymerase/DNA/RNA ternary complexes) (Churchman and Weissman 2011), and metabolic labeling (Friedel and Dölken 2009; Rabani et al. 2011). Our laboratory has relied extensively on the fourth method, in which chromatin is first separated from the cytoplasm and nucleoplasm of a cell population by biochemical fractionation (Bhatt et al. 2012). RNAs that purify with the chromatin fraction include recently transcribed RNAs and RNAs being actively transcribed. Notably, the chromatin fraction also includes noncoding RNAs that may function in association with the chromatin.

An analysis of chromatin-associated RNAs in LPS-stimulated macrophages allowed groups of genes to be identified that are expressed with similar kinetics (Bhatt et al. 2012). To determine whether coregulated genes might be regulated by similar mechanisms, computational methods were used to determine whether binding sites for specific transcription factors are enriched in the promoters of each coregulated gene cluster. This analysis revealed strong overrepresentation of known regulators of LPS-induced transcription, such as NF- κ B, AP-1, serum response factor, interferon response factor 3 in a limited number of clusters. Binding sites for signal transduction and transcription (STAT) proteins were also found to be overrepresented in specific kinetic clusters, presumably due to the autocrine activation of interferon (IFN) receptors following the earlier induction of IFN- β .

The strong enrichment of transcription-factor-binding sites in genes within specific kinetic clusters provides strong evidence that the global analysis of nascent transcripts by RNA-seq of chromatin-associated transcripts provides an important step toward an understanding of the global logic through which a transcriptional cascade is regulated. However, it is important to emphasize that

much remains to be learned. In particular, although binding sites for transcription factors, such as NF- κ B, were found in several promoters within specific coregulated clusters, several other promoters in these same clusters lacked the overrepresented binding site. This observation, combined with the fact that the genes within each cluster are not perfectly coregulated, suggests that the clusters are not homogenous with respect to regulatory mechanisms. In fact, it seems unlikely that any coregulated cluster presented in published reports is homogenous.

From the perspective of chromatin structure, the global analysis of chromatin-associated transcripts by RNA-seq confirmed some of our previous findings from analysis of a limited set of genes, but other early models required revision. Specifically, as observed in the earlier studies (Ramirez-Carrozzi et al. 2009), a high percentage of rapidly induced primary response genes was found to contain CpG-island promoters assembled into constitutively active chromatin (Bhatt et al. 2012). Furthermore, the most potently induced late genes contained low CpG promoters that lacked active chromatin in unstimulated cells. Also consistent with our earlier studies, a smaller group of genes containing low CpG promoters and lacking active chromatin was observed among the most rapidly induced genes.

However, in the RNA-seq analysis, the presence of a CpG island correlated most closely with genes that are not only rapidly activated but also rapidly inactivated after their rapid activation (Bhatt et al. 2012). Almost every transiently induced gene was found to contain a CpG-island promoter, whereas a much larger number of genes that show rapid but sustained induction contain low CpG promoters. This finding suggests that a CpG-island promoter may be particularly important for the rapid inactivation of transcription, perhaps because inactivation of these classes of promoters, in which chromatin may play a lesser role, is easier to achieve.

Another new finding from the RNA-seq analysis was the presence of a large number of CpG-island promoters among genes that are activated with relatively slow kinetics during the transcriptional cascade (Bhatt et al. 2012). This contrasts with the finding from the analysis of 67 individual genes that the vast majority of genes induced with slow kinetics contain low CpG promoters (Ramirez-Carrozzi et al. 2009). An examination of possible reasons for this difference revealed that it is due to the inclusion of a broader range of induction magnitudes in the RNA-seq studies. The CpG-island-containing genes induced with slow kinetics are mostly genes induced by only 10- to 20-fold; as with other CpG-islands, the CpG islands at these promoters are assembled into constitutively active chromatin. In contrast, the genes that are most potently induced with slow kinetics contain low CpG promoters assembled into inactive chromatin in unstimulated cells.

These findings suggest that CpG-island promoters are suitable at genes that require transcriptional fluctuations of relatively modest magnitudes, whereas most genes requiring potent induction have evolved low CpG promoters. One possible explanation for this difference was that genes in the latter group are transcribed at a lower basal

level in unstimulated cells, leading to larger fold-induction magnitudes. However, basal transcription levels were not consistently lower in the low CpG genes (Bhatt et al. 2012). We therefore favor a slightly different model, in which the nucleosome barrier present at low CpG promoters helps to reduce the basal transcription levels of genes that have evolved to support extremely high levels of transcription, thereby contributing to the large dynamic range of these genes (not necessarily by conferring a low basal transcription level in comparison to other genes but by conferring a lower basal level than might otherwise be found at a gene capable of supporting extremely high levels of transcription).

CONCLUSIONS

Since the discovery of the first inducible transcription factors (Sen and Baltimore 1986; Bohmann et al. 1987), much has been learned about the mechanisms used to regulate inducible transcription at both a gene-specific and global level. A large number of transcription factors that contribute to inducible transcription have been discovered, signaling pathways that lead to the activation of many of these factors have been characterized, and initial insights into the role of chromatin structure in inducible transcription have been described. However, it can be argued that the field remains in its infancy. Growing evidence suggests that many and possibly all transcription factors will be subject to multiple layers of regulation, with our current knowledge limited to the most basic regulatory layers. Furthermore, our limited understanding of the *in vivo* functions of the dozens of histone modifications that have been described, combined with our limited knowledge of the chromatin structure into which eukaryotic genes are assembled in their native chromosomal environment, have presented formidable barriers to efforts to develop a clear understanding of how chromatin contributes to the regulation of inducible transcription and the selectivity of a transcriptional response to a stimulus. Fortunately, the development of increasingly sophisticated experimental methods for the analysis of transcriptional regulation in cell populations and in single cells continue to suggest novel strategies toward the study of inducible transcription, paving the way for continuing progress toward an understanding of the logic underlying the selective responses to microbial and environmental stimuli.

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