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Signaling-to-chromatin pathways in the immune system

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Abstract

Complex organisms are able to respond to diverse environmental cues by rapidly inducing specific transcriptional programs comprising a few dozen genes among thousands. The highly complex environment within the nucleus—a crowded milieu containing large genomes tightly condensed with histone proteins in the form of chromatin—makes inducible transcription a challenge for the cell, akin to the proverbial needle in a haystack. The different signaling pathways and transcription factors involved in the transmission of information from the cell surface to the nucleus have been readily explored, but not so much the specific mechanisms employed by the cell to ultimately instruct the chromatin changes necessary for a fast and robust transcription activation. Signaling pathways rely on cascades of protein kinases that, in addition to activating transcription factors can also activate the chromatin template by phosphorylating histone proteins, what we refer to as “signaling-to-chromatin.” These pathways appear to be selectively employed and especially critical for driving inducible transcription in macrophages and likely in diverse other immune cell populations. Here, we discuss signaling-to-chromatin pathways with potential relevance in diverse immune cell populations together with chromatin related mechanisms that help to “solve” the needle in a haystack challenge of robust chromatin activation and inducible transcription.

Keywords

chromatin; histone phosphorylation; immune responses; signaling

1 | INTRODUCTION

Survival of all living forms, from simple prokaryotes to complex multicellular organisms, depends on their ability to detect environmental stimuli, integrate the information, and elaborate appropriate highly specialized responses. Implementation of this ability is reflected in the principal functions of diverse immune cells, inflammatory processes, and the cellular response to tissue damage. At the cellular level, the cell’s capacity to generate a response to external cues relies on three general steps: (a) ligand-receptor engagement on the cell surface, endocytic compartments, or cytosol; (b) receptor coupling to cytoplasmic signaling pathways involving the activation of transcription factors (TFs); and (c) signal transduction to chromatin, consisting of DNA and histone proteins, and deployment of

specific transcription programs. The signaling field has devoted considerable effort to the identification of receptors, signaling pathways, signal-induced TFs, and transcription programs targeted by specific stimuli. However, the implications of select gene transcription in the complex and condensed chromatin template have not been subject to such intense study. Early in the field, predominant thinking was that histones were exclusively DNA packaging material. Indeed, basic, positively charged histone proteins enable the compaction of the roughly two-meter genome into an approximately five-micron diameter nucleus. However, we now appreciate that in addition to the packaging function, histones and other chromatin-associated proteins are a highly dynamic and potent regulatory apparatus with extensive function, including defining the fundamental characteristics of accessibility and selective factor recruitment to associated DNA. Related to signaling, histones, and other chromatin factors are direct targets of signaling kinase activity, and in this sense can act as scaffolds that “host” signaling cascades, a characteristic that is well described in the DNA damage response¹ and a conceptual framework that we suggest is well-applied in thinking about transcription through chromatin, especially at stimulation-induced genes. Rapid induction of specific transcription programs involving a small number of genes scattered among thousands² along the condensed two-meter genome, represents an immense “epigenetic challenge.” We propose that features of signaling cascades—including their speed, amplification potential, and the biophysical changes associated with addition of negatively charged phosphates—are efficiently employed and well-adapted to solve this challenge.

The dynamic nature of chromatin, a defining feature that enables a single genome to instruct diverse cell types and environmental responsiveness, is orchestrated by epigenetic regulatory events that prominently include DNA and histone chemical modifications and the incorporation of histone variants. The fundamental unit of chromatin is the nucleosome,^{3,4} with a core particle composed of ~146 bp of DNA wrapped around a histone octamer containing one H3–H4 tetramer and two H2A–H2B dimers.^{5–7} N-terminal tails of histone proteins are flexible disordered regions that protrude from the surface of the nucleosome and can be extensively post-translationally modified (reviewed in 8). A plethora of post-translational modifications (PTMs) have been described so far, among them some of the best-studied include methylation and acetylation of lysines (K), and phosphorylation of serines (S), threonines (T), and tyrosines (Y). These modifications are the result of an equilibrium between the activities of enzymes that catalyze them, such as methyltransferases, acetyltransferases, and kinases (“writers”), and the ones that remove them, for example, demethylases, deacetylases and phosphatases (“erasers”). Downstream effects are mediated by multiple factors endowed with one or more domains for the recognition of specific PTMs (“readers”) and also by the direct impact of the modifications on the chromatin biophysical properties. With regard to transcriptional status, methylation relates both to activation (eg, H3K4, H3K79) and repression (H3K9, H3K27) depending on the modified residue, while acetylation and phosphorylation are typically associated with active chromatin. The latter could be partly due to their ability to directly disrupt DNA-nucleosome interactions, since their addition decreases histone’s positive charge, potentially reducing its electrostatic interactions with DNA, and therefore allowing access to other factors. Different genomic regions and transcriptional states are enriched for specific

PTMs.^{9–13} For instance, active promoters are associated with both H3 and H4 lysine acetylation at different residues and the trimethylation of histone H3 at K4 (H3K4me3); transcribing genes are enriched for H3K36me3 and H3K79me3 at gene bodies; enhancers are marked by H3K4me1, and their activity is associated with the acetylation of H3K27 (H3K27ac). On the other hand, more compacted and repressive chromatin is associated with H3K9me3 and H3K27me3. As discussed below in detail, histone phosphorylation has highly distinctive characteristics with regard to genomic localization and gene specificity, thereby targeting its dedicated function, including crosstalk with other PTMs and amplification of transcription, to stimulation-induced genes.

In this review, we will highlight the challenges of linking cellular signaling to the induction of select genes scattered throughout the 3 billion base-pair genome and the dedicated molecular mechanisms that we and others have been studying that “solve” these challenges to enable rapid cellular responses. The macrophage inflammatory response has been extensively studied in this regard, so it will serve to illustrate many of the mechanisms discussed here, though we propose that the general principles and molecular mechanisms intuitively apply across diverse immune cell subsets (Figure 1).

2 | SENSING EXTERNAL STIMULI—SIGNALING PATHWAYS: FROM CELL SURFACE TO CHROMATIN

Host-pathogen coevolution has been a crucible for selection of numerous pathogen- and stress-sensing receptors across diverse immune cell types. Despite their varied forms and expression patterns, many of these receptors share (a) common signaling machinery; and (b) a biological function that necessitates rapid and high-level induction of target genes. They allow the recognition of distinct environmental cues and are coupled to a number of signaling pathways that often rely on phosphorylation cascades within the cytoplasm and ensure a rapid and transient signaling that activates transcription programs. The speed and scope of these mechanisms are therefore absolutely critical for the appropriate response to infection or damage with the organism’s survival at stake. We suggest that this selective pressure has resulted in dedicated stimulation-dependent chromatin regulatory and transcription mechanisms, typically not employed as part of steady-state transcription. We term these “signaling-to-chromatin” pathways that enable such honed transcription responses in the immune system (and in other contexts where rapid transcription responses are crucial such as neuronal immediate early gene transcription¹⁴).

Signal transduction to the genes in chromatin is, in part, achieved by the phosphorylation and activation of TFs and subsequent recruitment to their cognate DNA binding motifs for the stimulation of a specific subset of genes. Due to the mostly cytoplasmic localization of major signaling kinases, their activity within the nucleus was initially not taken much into consideration. However, later studies showed that signals can be transduced directly to chromatin and that histone proteins can indeed be direct targets of signaling kinase cascades. Induced histone phosphorylation was initially observed in a series of seminal studies during the 1960s-1970s. Administration of glucagon and insulin to rats resulted in a rapid increase in the phosphorylation of histone H1, and it was suggested as a mechanism by which

various hormones could induce transcription.¹⁵ Moreover, lymphocytes stimulated with phytohemagglutinin or with cyclic AMP also resulted in the phosphorylation of different histone proteins and this was coincident with a high transcription rate.^{16–18} Years later, nuclear translocation of protein kinases¹⁹ and phosphorylation of histone proteins started to be explored more in detail. Regarding histone phosphorylation, work in fibroblasts described how extracellular stimuli, such as different growth factors and stressors, triggered the rapid and transient expression of the immediate early genes and was concomitant with the phosphorylation of the core histone H3 and the nucleosome-bound high mobility group protein HMGN1, in a process that was termed the “nucleosomal response”.^{20–22} These works represented early links between signaling pathways, histone phosphorylation, and gene expression, and opened the path to follow up studies and the signaling-to-chromatin field.

Work from our group has uncovered a previously unappreciated role of signaling-to-chromatin pathways and histone phosphorylation in the activation of immune transcriptional responses^{23,24} (Figure 1). These and our ongoing studies aim to test the hypothesis that features of speed and scope of transcription at stimulation-induced genes are enabled by dedicated mechanisms involving chromatin control and augmented activity of the general transcription apparatus. These studies have heavily relied on the model system of activation of mouse primary macrophages with Toll-like receptor 4 engagement by bacterial lipopolysaccharide (LPS), in part because we consider that the innate immune cell response to pathogen sensing is a highly adapted process, tuned for robust transcription responses. The histone modifications showing greatest increases after LPS stimulation of macrophages (with negligible levels in resting cells) were the phosphorylation of histone H3 at serine 28 (H3S28ph) and H3.3 at serine 31 (H3.3S31ph) as assessed by mass spectrometry. Chromatin immunoprecipitation and sequencing show that very quickly after treatment they are deposited at inflammatory genes. In agreement with other studies,^{25,26} our data suggest that the two signaling pathways coupled to these two phosphorylation events are the mitogen-activated protein kinase (MAPK) pathway with H3S28 and the nuclear factor- κ B (NF- κ B) pathway with H3.3S31, two central pathways regulating diverse cellular immune responses.^{23,24} Therefore, we hypothesized that these signaling-to-chromatin pathways could be a central feature of inducible transcription in different cell populations of the immune system. Indeed, following our observations in primary macrophages we extended our studies to additional immune cell types responding to their respective signature stimuli. We detected low basal levels and rapid increase of both H3S28ph and H3.3S31ph in bone marrow-derived dendritic cells (BMDCs) upon LPS stimulation, natural killer cells (NK) stimulated with IL-12 and IL-18, naïve B cells stimulated with anti-IgM, anti-CD40, and IL-21 and in CD4⁺ T cells upon activation with phorbol-myristate-acetate and ionomycin (PMA/I; Figure 1). Our findings indicate that H3S28ph and H3.3S31ph could be part of a dedicated histone code for inducible transcription that is exploited by diverse cell types, of the immune system and beyond (eg, in neurons, see Figure 1C in REF. 24), to augment selective transcription responses to external cues.

Although our work is focused on the study of histone phosphorylation on signaling-dependent transcription activation in interphase, it is very important to consider its prominent role on cell cycle regulation, specifically on mitosis.²⁷ Histone phosphorylation

during mitosis is pervasive and in mammalian cells it has been linked to a function in metaphasic chromosome condensation.^{28,29} The activity of various mitotic kinases (ie, Aurora B or haspin) on multiple histone residues such as H3S10, H3S28, or H3T3 have been described so far.^{30–34} It is important to consider this function while studying signaling-dependent phosphorylation in cycling cells because inducible phosphorylation events might be obscured by the abundant mitotic modifications. Indeed, all primary immune cells represented in Figure 1 are experimentally treated in a manner to assure that they are non-cycling; even a modest proportion of mitotic cells would drown out the more selectively deposited stimulation-dependent histone phosphorylation. This technical challenge together with difficulties generating highly specific antibodies discerning histone PTMs (currently subject to significant improvements [chromatinantibodies.com]) have held back progress in the histone phosphorylation field despite early findings and intuitive relevance of histone phosphorylation regulating chromatin dynamics. It is also worth noting that, in addition to direct phosphorylation of histone proteins which is the focus of this review, upstream signaling pathways also instruct epigenetic reprogramming and transcriptional programs through the phosphorylation of histone-modifying enzymes, as in the extensive regulation of histone methyltransferases and demethylases by signaling kinases.³⁵

Here, we briefly review receptors and signaling pathways central to various immune cell types. Rather than a comprehensive discussion of signaling intermediates, we will focus our discussion on their potential to directly phosphorylate histones in chromatin.

Pattern recognition receptors (PRRs) have adapted to recognize specific molecular motifs, pathogen-associated molecular patterns (PAMPs), conserved across different classes of microbes. Toll-like receptors (TLRs) are a type of PRRs with major roles in the initiation of inflammatory and immune responses; therefore, although expressed in multiple cells, they are specially relevant for the functionality of members of the innate immune system such as monocytes, macrophages, or dendritic cells (DCs), the first-line responders.³⁶ The different TLRs precipitate distinct downstream responses that depend on their association with different adapter proteins such as myeloid differentiation primary response 88 (MyD88) or TIR domain-containing adapter-inducing interferon β (TRIF), along with TIR domain-containing adapter protein (TIRAP) and TRIF-related adapter molecule (TRAM) that connect TRIF or MyD88 with specific TLRs.³⁷ Importantly, signaling cascades downstream of *both* TRIF and MyD88 lead to the activation of TGF- β activated kinase 1 (TAK1), a central component of the MAPK and NF- κ B pathways.³⁸ TAK1 activity can result in the activation of the MAPK pathway, a kinase cascade consisting of various serine/threonine kinases that results in activation of downstream members such as extracellular-signal-regulated kinases 1 and 2 (ERK1/2), p38, and c-Jun terminal kinase (JNK).^{37,39} Importantly, within this pathway both ERK1/2 and p38 can phosphorylate and activate the nuclear kinases mitogen- and stress-activated protein kinases 1 and 2 (MSK1/2) that have activity on the histone substrates H3S10 and H3S28²⁵ (Figure 2B and D). Moreover, JNK has also been described to phosphorylate H3S10 in neurons⁴⁰ and H3S28 after UV light exposure.⁴¹ As previously mentioned, TAK1 activity initiates the canonical NF- κ B pathway beginning with the phosphorylation of I κ B kinase β (IKK β) that together with IKK α and IKK γ (NEMO) comprise the IKK complex. IKK phosphorylates I κ B to elicit its ubiquitination and proteasomal degradation, and subsequent release of the TF NF- κ B

for its translocation to the nucleus.³⁷ With the three IKK factors' canonical roles squarely in the cytosolic regulation of I κ B/NF- κ B, an intriguing nuclear function of IKK α is emerging, including its capacity to phosphorylate histone proteins. After LPS and TNF- α stimulations in macrophages and fibroblasts, IKK α is recruited to target genes through the interaction with NF- κ B and presumably additional TFs,⁴² to catalyze the phosphorylation of H3.S31^{24,26} (Figure 2C) and H3S10.^{43,44}

The central role of both the MAPK and the NF- κ B pathways in immune responses is highlighted by the fact that multiple immune receptors are able to engage these pathways. This includes the antigen-specific T-cell receptor (TCR) and B-cell receptor (BCR). In both instances upon antigen encounter, a series of similar membrane-proximal signaling events precipitate the activation of phospholipase C- γ 1 (PLC- γ 1) and PLC- γ 2, respectively. This step precedes the engagement of signaling branches including the activation of the protein kinase C- θ (PKC- θ) and PKC- β downstream of TCR and BCR, respectively, and the activation of rat sarcoma (RAS) that links with the MAPK pathway and activates JNK, p38, and ERK1/2. Interestingly, in a different context PKC- β has been described to phosphorylate histone H3 at threonine 6 to protect H3K4me3 from demethylases and activate androgen-dependent transcription⁴⁵ (Figure 2F). PKC- β was detected occupying androgen response elements (AREs) in resting conditions and was then activated by protein-kinase-C-related kinase 1 (PRK1 -a H3T11 kinase⁴⁶ [Figure 2G]) upon treatment with androgen receptor agonists. In the context of T and B lymphocytes, PKC- θ and PKC- β initiate the NF- κ B pathway through different intermediates that will ultimately lead to the activation of TAK1 and eventual nuclear translocation of NF- κ B.⁴⁷ Similar to TCR and BCR, activating receptors of NK cells signaling through immunoreceptor tyrosine-based activation motifs (ITAMs) can induce the NF- κ B pathway⁴⁷ and the MAPK pathway.⁴⁸

Additionally, several members of the tumor necrosis factor receptor (TNFR) superfamily such as CD40,⁴⁹ TNFR2,⁵⁰ or B-cell activating factor receptor (BAFFR)⁵¹ can activate the non-canonical NF- κ B pathway. This pathway is initiated by the accumulation of NF- κ B-inducing kinase (NIK) and activation of IKK α , which ultimately result in the nuclear translocation of NF- κ B and transcriptional activation.⁵² Additional signaling pathways can be engaged by these receptors; for example, upon CD40 ligand binding, CD40 receptor promotes the recruitment of TNFR-associated factors (TRAFs) that function to activate different pathways including MAPKs ERK1/2, JNK, and p38 pathways and also the canonical NF- κ B pathway.⁵³

The Janus kinase (JAK)-signal transducer and activation of transcription (STAT) pathway is an essential pathway for signaling through multiple cytokine receptors and IFN receptor and has also been implicated in the phosphorylation of histones.⁵⁴ Within this pathway, the protein kinase JAK2 has been described to phosphorylate histone H3 at tyrosine 41 (H3Y41ph) at STAT5-target gene promoters (and potentially other STATs) upon IL-3 treatment^{55,56} (Figure 2D).

Cytokine receptors engage mainly the JAK-STAT pathway; however, certain receptors can also activate other signaling cascades including the NF- κ B and MAPK pathways. For example, IL-12 and IL-18 can act synergistically for the activation of NK cells and the

production of IFN- γ . Binding of IL-12 to its receptor results in the activation of the JAK2-STAT4 pathway, while IL-18 binding to its cognate receptor leads to the activation of the MAPK, p38, and NF- κ B pathways downstream of MyD88. Engagement of the MAPK/p38-dependent pathway has been described to mediate the stabilization of IFN- γ mRNA.⁵⁷

As described in this section, a multitude of prominent immune cell receptors have the ability to initiate signaling through MAPK, NF- κ B, and JAK-STAT pathways, and activate protein kinases with function at the chromatin level, and ability to phosphorylate histone proteins. This highlights the central role that these signaling-to-chromatin pathways have in regulating diverse cell responses and in development, not only through the well-established role of signaling kinases in the activation of TFs, but also through their direct activity on the chromatin template. Histone phosphorylation has the potential to profoundly affect chromatin biophysical properties and also its ability to interact with other factors. Therefore, in order to improve our understanding of inducible transcription, we should expand the traditional view on signaling pathways and include their link to chromatin and histone phosphorylation.

3 | FINDING A NEEDLE IN A HAYSTACK: TURNING ON SPECIFIC TRANSCRIPTION PROGRAMS

Receptor engagement initializes parallel kinase cascades that result in the phosphorylation and activation of TFs *and* the chromatin template to synergistically activate chromatin and augment transcription at select genes. Stereotypical of this type of response is the inflammatory response in macrophages, where hundreds of genes are expressed within a time frame of minutes.⁵⁸ Achieving such rapid, robust, and selective transcription represents a major “chromatin challenge” given that: (a) humans have around 57,000 protein-coding and non-coding genes²; (b) these genes are distributed along a two-meter genome; and (c) genomes are tightly packaged by histones into chromatin thereby presenting a daunting spatial search and identify quandary. In this section, we will review some chromatin features and mechanisms that facilitate or modulate the search and activation of inducible genes.

3.1 | Transcription factors and cis-acting regulatory elements

To best contextualize signaling-to-chromatin pathways and their activity in augmenting transcription, we will first discuss the concepts and features of transcription and gene control. The number of TFs known to control the human genome is around 1600, and from these, one third are tissue and cell type specific.⁵⁹ Each specific external stimulus is coupled to a limited number of signaling pathways and subsets of TFs. TFs activate transcription (historically, they are also referred to as “activators”) by binding to cis-acting regulatory regions scattered along the non-coding genome (approximately 98% of the genome)⁶⁰ and recruiting appropriate coregulators, so-called coactivators and corepressors depending on the effects they exert on chromatin and transcription. For example, many coactivators directly make chromatin more amenable to transcription through introduction of new histone PTMs that are recognized by the transcription apparatus, like the histone acetyltransferase p300, or through ATP-dependent shifting or removal of nucleosomes (by chromatin remodelers) that may occlude other TF motifs or hinder general transcription machinery access to a gene.

Corepressors work at odds with coactivators and prominently include histone deacetylases (HDACs) that remove these activating histone PTMs or remodelers that occlude activating DNA sequence, though corepressors can also work via more complex mechanisms (see Zmynd11 below).⁶¹ Promoters and enhancers are examples of cis-acting regulatory regions, stretches of DNA of around 200 base pairs (bp) containing clusters of TF-binding sites (TFBSs).⁶² In simplistic terms, the repertoire of TFs and their available binding sites determine the genomic location of regulatory events, while the regulatory factors that TFs recruit, coactivators and corepressors, typically are what mechanistically precipitate next steps, for example, altering histone PTMs, recruiting “downstream” general transcription factors (eg, TATA-box binding protein [TBP] and transcription factor IID [TFIID]) and factors that directly regulate the transcription cycle (eg, positive-transcription elongation factor b [P-TEFb]).

Of the entire repertoire of genomic regulatory elements, only a tiny fraction is accessible for TF binding in any given cell type, and this determines the functional identity of the cell by restricting transcription activation to unique gene regulatory networks.^{60,62} *Cis*-regulatory landscapes are set up by lineage-determining TFs (LDTFs) that generally have pioneer activity.⁶³ Unlike many important stimulation-dependent TFs (SDTFs) such as NF- κ B, that are limited to bind their cognate motifs in nucleosome depleted regions,⁶⁴ many LDTFs have the intrinsic ability to bind and modify nucleosomal structure.^{65,66} For example, early biochemical studies on the pioneer TFs HNF3 α (FoxA1) and GATA-4 demonstrated their ability to bind the albumin enhancer present in H1-compacted nucleosome arrays and to increase its accessibility.⁶⁶ More recent crystal structures of SOX pioneer TFs bound to the nucleosome particle indicate that they are capable of using binding energy to initiate detachment of DNA from the histone octamer and relocate the H4 N-terminal tail, thus potentially disrupting canonical nucleosome-nucleosome contacts and chromatin compaction.⁶⁷ While “pioneer” factor binding occurs on nucleosomal DNA and can contribute to initial “loosening” of DNA-histone contacts, effective displacement of nucleosomes from TFBSs is often only achieved by the subsequent cooperative binding of multiple TFs and with the association of ATP-dependent chromatin remodelers.⁶⁷ Then subsequent, constitutive binding of LDTFs is often necessary to keep regulatory regions nucleosome depleted thereby poising them for future responsiveness.⁶⁸

Examples of pioneering factors in the hematopoietic compartment are TCF-1, that determines the enhancer landscape of T-cells,⁶⁹ EBF-1 in B cell progenitors,⁷⁰ and the well-studied ETS family TF PU.1 (also known as SPI-1), required for the generation of common lymphoid and granulocyte-macrophage progenitors and for the late differentiation of macrophages and B-cells.^{68,71–73} In macrophages, PU.1 in combination with the TFs C/EBP α / β and AP-1 promote chromatin accessibility to macrophage-specific enhancers.⁷² PU.1 binds DNA proximal to the nucleosome entry site (where DNA starts to wrap nucleosomes and is more readily “peeled”) and recruits the coactivator, ATP-dependent switch/sucrose non-fermentable (SWI/SNF) remodeling complex, likely through the interaction with the N-terminal AAD domain of PU.1.⁷⁴ SWI/SNF in turn increases accessibility of PU.1-bound regulatory regions thereby revealing additional TF motifs for additive or subsequent regulatory potential of the enhancer.^{72,75} Furthermore, PU.1 can both directly and indirectly recruit mixed-lineage leukemia (MLL) coactivators that

deposit H3K4me1 around enhancers conferring additional positive regulatory activity.^{10,76,77} Pre-existing levels of H3K4me1 facilitate the recruitment of the histone acetyltransferases (HATs) to acetylate H3K27 for enhancer activation.^{78,79} Therefore, LDTFs, endowed with pioneer activity, determine the cell type specific chromatin-accessible regulatory regions to guide SDTFs and the transcription apparatus to activate cell-specific transcription programs.

Not all the regulatory regions become available during differentiation, some gain accessibility upon stimulation.^{80–82} For example, a subset of enhancers that are not marked by H3K4me1 in resting macrophages gain de novo accessibility upon stimulation.^{81,82} In this case, increased accessibility to these latent enhancers and subsequent methylation of H3K4 are achieved by the cooperative binding of LDTFs and SDTFs, and most likely by the activity of chromatin remodelers. Interestingly, while these enhancers lose accessibility, PU.1 and SDTF occupancy and H3K27Ac upon stimuli withdrawal, 30% of the de novo enhancers keep H3K4me1 for days, suggesting a poised chromatin configuration after resolution of the initial stimulus. Indeed, H3K4me1-mediated enhancer priming resulted in a faster or more robust response after a second challenge.⁸¹

Several studies indicate that SDTFs can interact with nuclear kinases and direct them to phosphorylate the chromatin template (histones and other chromatin-associated proteins) at specific target regions, linking cell stimulation events to signaling kinase cascades and chromatin regulation. Signaling kinases with known catalytic activity on histones can bind and activate TFs; for instance, the kinase MSK1 interacts and activates Creb⁸³; p65⁸⁴ and the AP-1 member ATF1.⁸⁵ Additionally, IKK α can interact with CREB binding protein (CBP) and form a complex with p65 for the phosphorylation of H3S10 and induction of NF- κ B targets in fibroblasts.⁴⁴ Interestingly, IKK α nuclear functions have also been related to transcription regulation mediated by TFs other than NF- κ B.⁴² For instance, it can interact with SMAD3 to activate its target genes downstream of the TGF β pathway⁸⁶, with E2F-1 in response to estrogen treatment or with Notch for the activation of target genes in colorectal tumors.⁴² Another example is the protein kinase JAK2, which is brought to the chromatin template through its interaction with STAT5 (and possibly other STATs), where it can phosphorylate H3Y41.^{55,56} Further, MYC forms a complex with PIM1 to phosphorylate H3S10 and activate MYC-target genes⁸⁷ and co-occupancy of ERK2 and estrogen receptor α (ER α) has been observed at their target genes.⁸⁸ These examples serve to illustrate that a general feature of signaling-to-chromatin in the context of inducible transcription is the shepherding of central signaling kinases to specific target regions and genes by interacting SDTFs partner factors.

3.2 | Chromatin architecture and regulatory hubs

The development of chromatin conformation capture technologies, such as genome-wide chromatin conformation capture (Hi-C) assay, and the improvement of microscopy-based techniques⁸⁹ have facilitated a new understanding of the organization of the genome. We can now appreciate that the chromatin fiber is packaged into the nucleus in a non-stochastic manner; it is organized into different domains that are thought to represent functional genomic structures. Indeed, enhancers and their cognate promoters, that, in vertebrates, are located an average of ~20–50 kb apart, are brought into close proximity in 3D space within

single chromatin domains, and such physical proximity is required for transcription.^{90,91} At supra-megabase scale, chromatin consists of compartments characterized by the segregation of transcriptionally active euchromatic regions, characterized by a high accessibility and the presence of activating histone modifications, and inactive, compacted heterochromatic regions, enriched for repressive histone modifications and generally transcriptionally silenced.^{92,93} Other sub-megabase structures are dependent on the presence of architectural proteins such as the insulator CCCTC-binding factor (CTCF) and the cohesin complex. Within this category, the biggest domains are called topologically associated domains (TADs)^{94–96} and the ones formed by shorter genomic regions have been called loop domains,⁹³ insulated neighborhoods⁹⁷ or sub-TADs.⁹⁸ These topological features might serve to increase interaction frequency between cognate regulatory regions situated far apart in the linear genome and also might preclude inappropriate interactions with adjacent domains and thus aberrant gene expression.⁹⁹ Actually, spatial confinement appears to be the major determinant regulating chromatin interaction probabilities between distal V_H and D_{HJH} regions comprising immunoglobulin genes.¹⁰⁰ This suggests that the same could be applied to interactions between regulatory DNA elements, with smaller confinement volumes increasing interaction frequencies. Biophysical characteristics of the chromatin fiber are central in this type of broad domain-level chromatin structure and organization. Signaling events, and prominently MAPK and NF-κB activity, underlie chromatin state transitions and cell activation phenotypes and cell fate decisions.^{23,24} Histone kinases and the phosphorylation of histones have the potential to (a) disrupt the interactions between acidic DNA and basic histone proteins, (b) eject repressive or chromatin compaction factors, and (c) provide scaffolding or binding sites for positive regulatory factors that activate chromatin (Figure 2). We propose that these features of histone phosphorylation critically influence other chromatin architecture and regulatory events to direct cell responses to stimuli.

The most accepted mechanism to achieve chromatin looping and TAD formation is the loop extrusion model involving the cohesin complex and CTCF.^{101–104} Cohesin is a ring-shaped ATPase complex formed by the subunits SMC1, SMC3, and RAD21. It can entrap chromatin into the ring, extrude a loop and slide the fiber until it encounters CTCF “barriers” that are two molecules of CTCF in a convergent orientation at the base of the loop structure.¹⁰¹ This model also has been proposed to be the mechanism by which enhancers find their promoters, although perhaps in a CTCF-independent manner, since CTCF is rarely localized at the base of loops connecting promoters and enhancers.⁹³ Therefore, the localization of cognate regulatory regions within the same CTCF-dependent domain could serve to restrict interactions to the domain, thus decreasing the search time of promoters and enhancers through the chromatin-sliding activity of cohesin to enable prompt transcription. Indeed, although loss of CTCF or cohesin do not have a great repercussion on general steady-state transcription,¹⁰⁵ both are critical for the proper transcriptional response to pro-inflammatory signals in macrophages.^{106,107} Thus, cohesin and CTCF, and chromatin architecture dynamics in general, may be crucial for dynamic cell responses and the chromatin state changes that underlie and direct cell fate decisions, and we suggest that signaling-to-chromatin and histone phosphorylation may play an important upstream role in these processes.

As mentioned earlier, enhancer-promoter proximity is required for gene expression, and however, proximity does not necessarily mean an automatic induction of transcription. Instead, in some instances, it might keep genes in a poised configuration to be rapidly induced upon arrival of a specific signal. As an example, promoters of the genes encoding interleukins 4, 5, and 13, become proximal to the T helper type 2 (T_H2) locus control region (LCR) during specification of different T-cell lineages, forming a poised structural configuration that may be responsible for the rapid and coordinated expression of the interleukin genes after subsequent activation.¹⁰⁸ This primed structural organization is “hardwired” in the chromatin 3D chromatin conformation during differentiation. The initial cluster of cytokine proximal promoters is present in T-cells, B-cells, and NK cells. The reorganization event that brings the T_H2 LCR near the initial promoter cluster is a T-cell-specific event. Indeed, rewiring of the regulatory “interactome” is pervasive during cell-lineage specification and results in cell type-specific chromatin topologies.^{109,110} Interestingly, developmental lineage inter-relationships of the hematopoietic tree can be recapitulated by clustering cell type-specific promoter interactomes of primary human hematopoietic cells.¹¹¹ In general, regarding inducible transcription, we can find examples where promoter-enhancer proximity are (a) instructive, or rate-limiting to drive de novo transcription, and also (b) permissive, setting up highly poised states awaiting subsequent signals for rapid transcription.¹¹² However, it is intuitive to think that in scenarios where it would be beneficial for the transcription reaction to occur rapidly, such as at inflammatory genes in macrophages, pre-existing permissive chromatin conformations around rapid response genes would be more prevalent. In this context, different events such as the phosphorylation and activation of TFs and histone proteins could be the necessary steps to trigger transcriptional activation. Indeed, we propose that histone phosphorylation could be effectively utilized to integrate instructive upstream signaling components, directed by SDTFs, DNA sequence and adapted regulatory element circuitry to alter: (a) probabilities of gene induction within a population of cells, for example, by changing biophysical properties of chromatin across large architectural domains to alter probabilities of regulatory intra-domain interactions; (b) kinetics (speed) of de novo transcription, for example, by augmenting the transcription cycle or rapidly releasing poised transcription; and (c) the level (output) of transcription. Such effects (and others) could be mediated through biophysical, “in *cis*” effects on chromatin, and also through the ejection of corepressors and stimulation or recruitment of coactivators (Figure 2).

Mechanisms controlling transcription require physical proximity of a gene, DNA regulatory regions and regulatory factors (e.g. coactivators). However, the current understanding extends beyond the classic concept of pairwise stable promoter-enhancer interactions. For instance, Hi-C studies demonstrate broad chromatin interactivity across transcriptionally active domains, but surprisingly few stable promoter-enhancer loops.⁹³ Instead, the current view also revolves over the idea of chromatin topologies forming hubs of multiple regulatory regions interacting simultaneously to regulate transcription.^{90,113–116} As an example, the characterization of the regulome of LPS-activated B-cells using Hi-C has uncovered numerous cases of multiple enhancers controlling a gene (multi-enhancer genes) and different promoters controlled by the same enhancer (multi-gene enhancers), suggesting that these configurations could be more a rule than an exception.¹¹⁷ Although caution must

be taken while interpreting interactomes resulting from experimental approaches based on averaged-populations (where multiple pairwise contacts might be mistaken by multi-way interactions), the utilization of improved technologies to study chromatin structure, such as genome architecture mapping (GAM) or split-pool recognition of interactions by tag extension (SPRITE), has allowed the direct detection of simultaneous interactions genome wide.⁸⁹

Along the same lines, during the last few years, enhancer function has been considered within the context of biomolecular condensates.^{118–121} Biomolecular condensates are membraneless organelles or spatially organized biochemical units that serve to concentrate and compartmentalize functionally related molecules (reviewed in 122). Numerous cellular organelles have been classified within this category: the nucleolus, nuclear speckles, polycomb bodies, and others.¹²³ Some condensates are formed by liquid-liquid phase separation upon reaching a threshold concentration of components that participate in weak multivalent interactions.¹²³ This type of interaction is favored between factors enriched for intrinsically disordered regions (IDRs) and modular domains. Interestingly, in this regard, multiple proteins participating in transcription activation are able to engage in highly dynamic multivalent interactions and form condensates, including the carboxy-terminal domain (CTD) of Pol-II, transactivating domains of TFs, coactivators such as bromodomain-containing protein 4 (BRD4), p300 or mediator, and even DNA and RNA.¹²² Although still speculative (largely because of a lack of studies of these properties in the physiologic context of chromatin), biomolecular condensate concepts highlight how pre-existing chromatin topologies could bring into close proximity and concentrate several regulatory DNA elements and TFBSs. LDTFs and SDTFs cooperatively binding to these regions would crowd a small nuclear volume with coactivators and other factors until reaching a threshold for condensate formation that could drive efficient transcription activation.

Chromatin and histone PTMs are also thought to contribute to condensate formation,^{124–126} and this is intuitive given its features: high valency, polymer form, intrinsically disordered histone tails, abundant histone binding cofactors, interactions with RNA and DNA, and PTM “switches” between functional states. In vitro, nucleosomal arrays are able to create condensates at physiological cation concentrations, and different chromatin attributes, such as nucleosome spacing or histone PTMs can modulate chromatin ability to condensate. For instance, binding of the heterochromatin protein α (HP1 α) to H3K9me3 has been suggested to drive heterochromatin condensate formation^{124,125}; likewise, acetylated chromatin is thought to form condensates upon BRD4 binding.¹²⁶ Essentially, concentrating key components into close proximity could result in formation of condensates in short time scales compatible with fast inducible transcription responses.¹²³ In this context, one could speculate that important determinants might be signaling-dependent TFs and histone PTMs (notably phosphorylation) with their combined ability to interact with a plethora of other factors such as protein kinases, chromatin remodelers, protein scaffolds, and the transcription apparatus itself.

All together, the aforementioned observations, including the finding of chromatin hubs and regulatory condensates, bring into the same framework previous concepts that were

well accepted, such as the existence of superenhancers^{127–129} and transcription factories.¹³⁰ Additionally, they help to reconcile the classic view of static pairwise enhancer-promoter interactions with recent observations of transcription regulation including the clustering of regulatory regions,¹¹⁷ the low number of stable CTCF-loops directly connecting enhancers and promoters⁹³; the short residence time of Polymerase II (Pol-II) and TFs on chromatin,^{131,132} or the decreased enhancer-promoter proximity after transcription activation.¹³³

3.3 | Promoter structure

Certain structural features of promoters controlling inducible genes can influence their expression kinetics.^{134,135} In general, there are two main groups of Pol-II promoters, the ones bearing TATA elements and the ones containing CpG islands (CGIs), which approximately comprise two-thirds of mammalian gene promoters. CGIs are characterized by a high density of unmethylated CpG dinucleotides that contrasts with the heavily methylated CpGs found outside CGIs.¹³⁶ In general, intermediate levels of guanine-cytosine content tend to favor nucleosome assembly, but the high density found in CGIs makes DNA winding around the histone octamer inefficient and tends to create nucleosome depleted regions, largely accessible to the basal transcription machinery.¹³⁷ Furthermore, binding of proteins containing ZF-CXXC domains also contributes to the establishment of a transcriptionally favorable structure.¹³⁶ In this regard, CGI binding of the histone methyltransferase CFP1 facilitates the nucleation of histone H3K4 trimethylation (H3K4me3), a modification associated with transcription activation.¹³⁶ Additionally, CGI binding of histone demethylase KDM2A can deplete the promoter of H3K36 dimethylation (H3K36me2), a modification linked to transcription repression.¹³⁶ Moreover, even in the absence of productive transcriptional activity, CGI promoters tend to be enriched for Pol-II binding,¹³⁸ which would also prevent nucleosome assembly at those promoters and make them prone to rapid transcriptional induction.

The transcriptional response of macrophages to LPS stimulation has been extensively studied with regard to the influence of promoter structure on transcription kinetics.^{80,134,135,138,139} Initial studies suggested the CGI promoters were generally associated with primary response genes (PRGs), that are rapidly transcribed upon stimulation and do not require SWI/SNF-mediated chromatin remodeling.¹³⁴ In contrast, secondary response genes (SRGs) are not enriched for CGI promoters. These genes are dependent on de novo protein synthesis (of their activators) both for nucleosome remodeling of their promoters and for transcription activation, which explains the longer times required for their expression.^{134,139} A subsequent genome-wide analysis confirmed that a large percentage of genes that are rapidly induced contain CGI promoters, however, the authors found that genes activated at later time points can also be driven by CGI promoters.¹³⁵ CGI promoters controlling fast and slow genes are structurally similar in that they have a transcriptionally poised conformation. One interesting difference, however, is that promoters with a low density of CpG dinucleotides are highly prevalent among genes that are potently stimulated (by more than 100-fold in cases such as *IL6* and *IL12b*). Conversely, CGI promoters are enriched among weakly and transiently induced genes.¹³⁵

As mentioned before, a fraction of slower genes (SRG) is controlled by promoters that need to undergo remodeling upon stimulation. Using micrococcal nuclease (MNase) DNA digestion to map genome-wide nucleosome positioning, a study found that such promoters correspond with nucleosomes that are not strongly positioned. In the MNase sequencing data from a population of macrophages, weakly positioned nucleosomes were detected as fuzzy peaks, in contrast with the sharp peaks observed for well-positioned nucleosomes.⁸⁰ These “fuzzy” nucleosomes could possibly contain both H2A.Z and H3.3 histone variants that are found in less stable nucleosome core particles at regulatory regions,¹⁴⁰ likely to facilitate remodeling prior to transcription. The fuzzy central nucleosomes are usually flanked by strongly positioned nucleosomes that are therefore non-amenable for remodeling, perhaps to protect the cell-specific regulatory landscape from disruptions that could perturb cell identity. These varying degrees for the steady-state accessibility of inflammatory response gene promoters clearly define their targeting by SRTFs, and therefore, may similarly define their targeting by TF associated histone kinases, in terms of timing and functional consequences of histone phosphorylation. For example, highly poised and accessible target genes may feature augmented transcription cycles via histone phosphorylation pathways, while histone phosphorylation at target genes with less open chromatin may result in derepression (Figure 2). Lack of histone kinase-associated SRTF motifs and/or lack of accessibility may exclude histone phosphorylation from other genes, even those that are stimulation induced in other contexts or targeted by different signaling pathways.

3.4 | Paused polymerase

Historically, the recruitment of Pol-II and general TFs forming the pre-initiation complex (PIC) was thought to be the main rate-limiting step for transcription activation. However, this idea was challenged by studies describing premature transcription termination, indicating that Pol-II initiation does not inevitably lead to the transcription of full-length products and additional steps might be highly regulated and rate-limiting.^{141,142} The confirmation of the existence of additional regulatory “roadblocks” after transcription initiation came from studies by the Lis laboratory and others during the 80s and the beginning of the 90s.^{143–146} Using as a model *Drosophila melanogaster* and focusing on the inducible heat shock genes (Hsp), the Lis laboratory showed that transcriptionally competent Pol-II could not start productive transcription until the induction of heat shock. Pol-II was stalled once it had transcribed a short RNA strand of 20–60 nucleotides, an event they termed “paused” Pol-II.

After many years of study and technical improvements, we can now appreciate the complexities of transcription regulation beyond the recruitment of Pol-II, with promoter-pausing, pause-release and initial elongation being heavily regulated steps. After PIC assembly, the TFIIH kinase subunit CDK7 phosphorylates the CTD domain of Pol-II to facilitate the transition to the elongation phase.¹⁴⁷ Here, the initial short RNA and the DNA tilt impairs the subsequent addition of nucleotides, which prevent further elongation. Two factors are required to stabilize paused Pol-II, DRB-sensitivity inducing factor (DSIF), and the negative elongation factor (NELF) complex.^{148,149} The strength and duration of the Pol-II promoter-proximal pausing depend on different factors such as features inherent to

the core promoter,¹⁵⁰ TF binding,¹⁵¹ chromatin-remodeling complexes, and the transcription machinery.¹⁵² Ultimately, release of Pol-II into the gene body requires the phosphorylation of its CTD at position Ser 2 (S2ph) by P-TEFb. This important rate-limiting step in transcription is regulated by a highly controlled stepwise process of (a) TF-recruited histone acetyltransferase coactivators and histone acetylation, (b) binding of BRD4 (and other coactivators) to histone acetylation via bromodomains, and (c) these factors' scaffold activity and recruitment of P-TEFb for the phosphorylation of Pol-II and elongation control. This phosphorylation step by P-TEFb is a general feature of the active transcription cycle at expressed genes, since its inhibition blocks transcription almost entirely.^{153,154} However, some genes appear to be stalled or poised, with the transcription cycle blocked at this step. Among other mechanisms, Pol-II pausing is thought to be important for the rapid and/or simultaneous expression of gene networks upon receiving external cues. Indeed, different studies in resting macrophages have described paused Pol-II preloaded at transcription start sites of inflammatory genes such as tumor necrosis factor alpha (TNF- α).^{138,155,156} In resting macrophages paused Pol-II colocalizes with NELF, which gets rapidly ejected upon LPS stimulation.¹⁵⁷ More than 60% of the inflammatory genes are regulated at this pause-release step, thus it represents a general feature of poised transcription and macrophage readiness.^{157,158}

4 | SIGNALING-TO-CHROMATIN

4.1 | Initial observations

The study of cytoplasmic signaling pathways and signal-induced activation of TFs has received most of the attention in the signaling field, while the direct effect of these pathways on chromatin has been largely overlooked. The first studies of specific histone targets of signaling focused on association with the expression of the immediate early genes *c-fos* and *c-jun* in fibroblasts.^{20–22} Rapid expression of these genes shared strikingly similar transient kinetics with phosphorylation of histone H3 at serine 10 (H3S10ph) suggesting a direct role of histone phosphorylation in transcription. Subsequently, it was shown that the MAPK pathway was required for this activity through the activation of either ERKs after mitogenic stimuli or p38 after stress stimuli.^{22,159} Downstream kinases were later identified and provided clear links between signaling pathways and histone phosphorylation. Namely, ribosomal S6 kinase 2 (RSK2) was found downstream of ERKs,¹⁶⁰ and MSK1/MSK2 downstream of both ERKs and p38.²⁵ Despite a long history of discoveries in the signaling-to-chromatin field, it is still far from clear (a) how kinases are targeted to specific genes and the factors controlling this selectivity, including TF cofactors, chromatin states, and architecture, and (b) what specific downstream regulatory events are precipitated by substrate phosphorylation to alter chromatin and augment transcription. The presence of histone phosphorylation events provided intriguing regulatory possibilities, especially with a diverse collection of other modifications already known to populate the histone tails, and the possibility of crosstalk between them as an exciting idea proposed as a central part of the histone code hypothesis.¹⁶¹ Crosstalk of histone phosphorylation with other histone PTMs is especially appealing as a model for chromatin regulatory changes at genes selectively targeted by histone kinases. Thus began exploration of the potential crosstalk between histone phosphorylation and acetylation given their association with

transcription activation. Initially, it was shown that H3S10ph in stimulated fibroblasts was accompanied by acetylation of lysine 14 at histone H3 (H3K14ac) in the *c-fos* promoter, and this association was abolished upon perturbation of MAPK signaling.¹⁶² Moreover, phosphorylated histones had enhanced ability to recruit GCN5 (KAT2B), the acetyltransferase for H3K9/K14. Subsequent studies suggested a model with H3S10ph precipitating a cascade of pro-transcription events: (a) H3S10ph recruits 14-3-3 adapter proteins, that interact with phosphorylated serine and threonine residues¹⁶³; (b) 14-3-3 recruits large multiprotein complexes containing GCN5 and the SWI/SNF remodeler complex to H3S10ph chromatin¹⁶⁴; (c) 14-3-3 also recruits the acetyl transferase KAT8 (MOF) that catalyzes the acetylation of lysine 16 of histone H4 (H4K16ac)¹⁶⁵; (d) acetylated histones facilitate the binding of BRD4 that in turn recruits and activates P-TEFb; and (e) P-TEFb, namely the subunit cyclin-dependent kinase 9 (CDK9), then phosphorylates the CTD of Pol-II at serine 2 to start transcription elongation¹⁶⁵ (Figure 2E). These studies highlighted the potential of histone phosphorylation to stimulate or recruit coactivators to activate transcription. On the other hand, another set of early studies have highlighted the potential for histone phosphorylation to eject corepressors. Two features of histone phosphorylation make this corepressor ejection function intuitive: (a) the large, negatively charged nature of the modification can disrupt contacts with repressive histone “readers”; and (b) phosphorylated residues are strategically positioned within the histone tail immediately adjacent to or nearby lysine residues that are methylated by corepressors. For example, both S10 and S28 lie within “ARKS” motifs [alanine (A), arginine (R), lysine (K), serine (S)] next to K9 and K27, and S31 is nearby and in between K27 and K36 with potential to affect readers of both residues.²⁴ Indeed, phosphorylation of H3S10 results in ejection of the corepressor HP1 from H3K9me2/3 binding^{30,166} and phosphorylation of H3S28 ejects PRC2.^{167,168}

These initial studies provided some general concepts of how histone phosphorylation could potentially regulate transcription through stimulation of coactivators and ejection of corepressors (Figure 2), among other potential mechanisms (more details below). They also highlighted links between upstream signaling pathways and kinases and chromatin targets. After these initial studies, numerous histone residues have been identified as direct targets of signaling kinases and with the ability to influence transcriptional activation (Table 1).

4.2 | Inducible histone phosphorylation in immune cells

As briefly mentioned above, LPS stimulation of bone marrow-derived macrophages is directly linked to the phosphorylation of two histone residues, H3S28 and H3.3S31, with levels becoming detectable by western blot within minutes after addition of LPS to the media.^{23,24} Interestingly, despite similar kinetics, the genomic distribution of these two modifications contrasts strikingly. H3S28ph is found over extended domains surrounding inflammatory genes, and it is largely excluded from transcribed gene bodies, which instead are precisely delineated by H3.3S31ph. These observations suggest that the two events could have non-overlapping, perhaps synergistic effects on transcription activation. H3S28ph appears to be an important step for initial activation of chromatin regions containing stimulation responsive genes, creating phosphorylated neighborhoods primed for transcription activation, while H3.3S31ph is linked to the transcription cycle

itself. Similar to the initial studies of S10 in fibroblasts,²⁵ we have observed that in this context, phosphorylation of S28 is triggered by MAPK pathways and catalyzed by MSK1/2. In the case of H3.3S31ph, we and others have uncovered a role of the NF- κ B pathway and the kinase IKK α .^{24,26} Indeed, IKK α had been previously reported to carry out the phosphorylation of H3S10 after TNF- α stimulation of mouse embryo fibroblasts (MEFs).^{43,44} Interestingly, they found that this role of IKK α is independent of its function in phosphorylating I κ B α , triggering its degradation. After TNF- α treatment, IKK α was found to translocate to the nucleus and bind to promoter regions of the NF- κ B-dependent genes *I κ B α* , *IL-8*, and *IL-6*.^{43,44} At those promoters, IKK α catalyzes the phosphorylation of H3S10 and favors transcription. Promoter *and* gene body activity of IKK α are not mutually exclusive, and one model could represent the extension of the other. Bringing together the findings described in all four works on IKK α and previous models, one could envision the following series of events. First, upon NF- κ B-dependent activation, IKK α is shuttled into the nucleus and recruited to target gene promoters, likely by NF- κ B, but also potentially by other TFs,⁴² where it initially phosphorylates H3S10. Next, as described in the previous section, this first phosphorylation event could recruit factors that promote transcription elongation. IKK α is then “handed off” to the elongating Pol-II (Pol-II S2ph) through its interaction with heterochromatin protein 1 gamma (HP1 γ) and phosphorylates H3.3S31 on genic nucleosomes in a co-transcriptional fashion.^{24,26} A possible shift of IKK α kinase activity from S10 to S31 is plausible since in vitro experiments suggest that HP1 γ interaction with IKK α favors its activity on H3.3S31 over H3S10.²⁶ Also, consistent with this model are the differences in location, with H3S10ph mainly at promoter and +1 nucleosome regions, while H3.3S31ph is extended to gene bodies and over the 3' regions. Moreover, blocking Pol-II elongation by pretreatment with CDK9 (p-TEFb) or topoisomerase 1 (TOP1) inhibitors result in the abrogation of H3.3S31ph, which demonstrate the co-transcriptional character of this phosphorylation.²⁴

Another notable example of a central signaling pathway that has been directly linked to histone phosphorylation is the JAK-STAT pathway. Histone H3 can be phosphorylated at tyrosine 41 (H3Y41) by JAK2, a kinase with prominent roles in hematopoiesis downstream of several cytokine receptors. Dawson and colleagues identified a JAK2 nuclear pool in various hematopoietic cell lines.⁵⁵ Using different stimuli coupled to JAK2 signaling, like interleukin 3 (IL-3), they observed activation of JAK2 concomitant to the increase of H3Y41ph, demonstrating the inducible nature of the phosphorylation event. Moreover, inhibition of JAK2 decreased the phosphorylation and the expression of the *Lmo2* gene. In a follow-up study by the same group, they performed chromatin immunoprecipitation and sequencing (ChIP-seq) and found association of the mark with STAT5-binding regions and actively transcribed genes.⁵⁶ Additionally, after one hour of stimulation with IL-3 they observed increased levels of H3Y41ph at 2 loci known to be targets of JAK2-STAT5 signaling, *Pim1* and *Cish*. Interestingly, JAK2 also phosphorylates H3Y41 at genes that are not STAT5 targets, suggesting a potential coupling of JAK2 with other STATs or different TFs that might guide the kinase to different gene subsets.

Metabolic-state sensing pathways have also been implicated in direct targeting of the chromatin template. Transcription programs linked to metabolic adaptations in stress conditions are controlled by AMP-activated protein kinase (AMPK) and its main upstream

kinase liver kinase B1 (LKB1).¹⁶⁹ Activation of AMPK in fibroblasts through the use of different metabolic stressors, led to AMPK binding to chromatin and direct phosphorylation of histone H2B at serine 36 (H2BS36).¹⁷⁰ AMPK colocalizes with H2BS36ph at promoters and transcribed regions of the AMPK-dependent genes *Cpt1c* and *p21*. The presence of a phospho-mutant form of H2BS36ph (H2BS36A) decreased the expression of the induced genes. In addition to these direct links to signaling-to-chromatin pathways, cellular metabolism has broad potential to influence epigenetic events through regulating the levels of key epigenetic cofactors including Acetyl-CoA, α -ketoglutarate and S-adenosylmethionine.¹⁷¹ A fascinating topic for future investigation includes the integration of, and crosstalk between, concurrent (a) signaling-to-chromatin events; and (b) rapid changes in cellular metabolism that occur during cellular stimulation.

4.3 | Downstream effects of histone phosphorylation

Signal-dependent histone phosphorylation can elicit different downstream effects that in general favor transcription activation. Histone phosphorylation can be necessary for the recruitment of specific cofactors, ejection of transcription repressors, and in some instances, phosphorylation can also modulate enzymatic activity of other factors. Another possibility is a direct effect of the phosphate group itself; the addition of this bulky negative modification could result in the disruption of electrostatic interactions between the negatively charged DNA template and the basic histone proteins. The dynamic wrapping/unwrapping of DNA around the histone octamer can transiently alter its accessibility to other factors, such as those of the transcription machinery or other chromatin-based processes. Along these lines, phosphorylation of the nucleosome entry site (H3Y41 and H3T45) can promote accessibility by unwinding DNA.¹⁷²

Interestingly, the N-terminal tail of H3 encompasses two identical motifs, the “ARKS” motifs mentioned previously. The first one contains K9 and S10, and the second one contains K27 and S28. In both motifs, lysine can either be acetylated or methylated to activate or inhibit transcription, respectively. The antagonistic effects of lysine methylation and acetylation on the transcriptional output together with the proximity to the serine residues suggest that inducible phosphorylation could act by precipitating a switch from the repressive to activated state, what has been termed “methyl switch” or “methyl-acetyl switch.” Indeed, different observations from studies on H3S10ph and H3S28ph support these models. For instance, induction of H3S28ph can disrupt H3K27 methylation.^{167,168} Polycomb repressor complex 2 (PRC2) is necessary to catalyze H3K27me3 marks, and together with other Polycomb group (PcG) proteins maintain this modification to keep a repressive chromatin state critical for differentiation and fate transitions. In response to stress signals, mitogens and differentiation cues, PcG-target genes display temporary accumulation of H3S28ph and H3K27me3S28ph marks. This coincides with the displacement of subunits of the PRC2 complex, decreased H3K27me3 at promoter regions, and transcriptional activation.¹⁶⁷ Consistent with these observations, MSK1 targeting to the polycomb-repressed α -globin promoter is sufficient to promote H3S28ph-mediated displacement of PRC2.¹⁶⁸ Moreover, they also described the corresponding increase of the dual modification H3K27acS28ph and transcriptional activation, which agrees with the

causal effect of H3S28ph on histone acetylation and gene expression found upon targeting deactivated Cas9 fused to a hyper-active variant of MSK1 to gene promoters.¹⁸¹

We have also identified a similar activity in LPS stimulated macrophages.²³ In resting macrophages inducible genes have pre-existent levels of H3K27Ac; once cells are activated the acetylation levels are greatly increased and this effect is abrogated by the use of MSK inhibitors. H3S28ph augments p300 catalytic activity and transcription on chromatinized templates. Therefore, these observations reinforce the idea of an inducible methyl-acetyl switch mediated by phosphorylation of H3S28 (Figure 2B). Such a switch could be important during cell differentiation, where H3K27me3 and the PcG proteins are critical to maintain a repressive state at their developmentally regulated gene sets, and differentiation cues could instruct chromatin activation and histone acetylation to induce their expression and promote differentiation.¹⁷³

The H3S28ph methyl switch is similar to what has been observed for H3S10ph. As already mentioned, H3S10ph is abundant in mitosis. It is interesting to note that histone phosphorylation can have distinct functions in interphase and in mitosis, though some of the mechanisms are conserved. For instance, H3S10ph disrupts HP1 proteins binding to chromatin^{30,166} (Figure 2D), and different kinases regulate this function in mitosis or interphase. Phosphorylation by the mitotic kinase Aurora B triggers the ejection of all three HP1 isoforms, however, this does not cause a decrease in histone methylation levels. In this case, phosphorylation is a transient and strictly mitotic event, upon mitosis termination phosphorylation levels decrease allowing HP1 to bind chromatin again.³⁰ In interphase, the *Hdac1* promoter in resting HeLa cells is decorated with H3K9me2 and bound by HP1 γ . Upon engagement of the MAPK p38 pathway the increase of S10 phosphorylation disrupts HP1 γ binding and allows *Hdac1* transcription activation. Similar to the mechanism defined in mitosis, histone methylation levels are maintained and loss of phosphorylation enables re-engagement of HP1 γ .¹⁶⁶ As another example, JAK2-dependent phosphorylation of H3Y41 seems to function through the ejection of HP1 α bound to H3K9me2/3 as well (Figure 2D). In support of this possibility, phosphorylated peptides abrogated HP1 α binding. In addition, JAK2 activity correlated with histone phosphorylation, loss of HP1 α , and transcription of the *Lmo2* gene, whereas the use of JAK2 inhibitors abolished these effects.⁵⁵

As pointed out above in detail, a recurring characteristic of inducible genes is that they tend to display characteristics of transcriptionally active chromatin before they are fully expressed, including prelooped architecture, accessible chromatin, and paused Pol-II. In agreement with this notion, our data in macrophages indicate that inflammatory genes also display levels of H3K27ac and H3.3K36me3 activating marks in resting conditions, while H3S28 and H3.3S31 phosphorylations are strictly stimulation-dependent modifications. This pre-activated state of chromatin seems to be very relevant for the complex regulation of inflammatory gene induction. For instance, H3.3K36me3 is the substrate for the binding of zinc finger MYND-type containing 11 (ZMYND11),^{174,175} a transcriptional repressor that we found bound to many inflammatory genes. H3.3S31 phosphorylation upon LPS treatment ejects ZMYND11 to promote transcription (Figure 2C). Indeed, ZMYND11 knockdown increases transcriptional activation. Another effect of H3.3S31ph in transcription

is the stabilization of the methyltransferase SETD2, that catalyzes H3.3K36me3; H3.3S31ph stimulates SETD2 activity and increase the levels of H3.3K36me3 (Figure 2C).

It is worth considering that histone modifications might have different effects depending on the context, and H3.3S31ph provides a good example of this. In postmitotic immune cells, we observed selective deposition of H3.3S31ph within the gene body and 3'-end of stimulation-induced genes, while in differentiating stem cells this phosphorylation event has been detected at enhancers and promoters of developmental genes.¹⁷⁶ In this context, it promotes transcriptional activation through the induction of p300 activity, which leads to the increase of H3K27Ac.¹⁷⁶ An important function for H3.3S31ph was also observed during *Xenopus laevis* gastrulation and corresponded to increased H3K27ac and decreased H3K27me3.¹⁷⁷ It is intriguing to speculate that in the context of rapid cell division (eg, ESC or gastrulation) widespread mitotic histone phosphorylation, including at H3.3S31, could derepress H3K27me3 chromatin, especially at sites of H3.3 deposition, and thereby link cell division to poising of developmentally regulated enhancer landscapes.

5 | SUMMARY AND FUTURE DIRECTIONS

In this review, we discussed how rapid regulation of gene expression in response to cellular stimulation evokes numerous challenges, including rapidly turning on a small subset of select genes among thousands on chromatin fibers folded into a micron-scale nucleus. “Signaling-to-chromatin” mechanisms involving histone phosphorylation can augment dynamic chromatin regulation, including activation of transcription, and are engaged across a range of stimulation conditions such as pathogen sensing and cytokine responses, and in diverse immune cell types (Figure 1). We reviewed how histone phosphorylation can regulate transcription via numerous mechanisms, with ejection of corepressors and stimulation of coactivators as a recurring paradigm (Figure 2A). It is worth noting that, as selectively engaged chromatin features of stimulation-induced genes, with relevance to inflammation and other disease states, signaling-to-chromatin pathways may represent more selective therapeutic targets than the general transcription machinery.

There is exciting potential for future studies in the signaling-to-chromatin field that would contribute significantly to the understanding of diverse biological processes, including focus in the interplay and crosstalk between rapid changes in cellular metabolism, availability/consumption of metabolites functioning as epigenetic cofactors, and signaling-to-chromatin pathways in stimulated cells. Additionally, high priority should be placed on unraveling how signaling events and histone phosphorylation alter the biophysical features of chromatin and interact with chromatin as a signaling scaffold to alter chromatin states (eg, histone PTMs, 3D architecture, biomolecular condensate characteristics) and transcription. There is also intriguing potential for signaling-to-chromatin pathways to trigger cascades of epigenetic events that result in durable transcriptional memory, as in trained immunity phenotypes. The function of signaling-to-chromatin events, and specific mechanisms of histone reader, writer, and eraser regulation by histone phosphorylation crosstalk and “switches” are likely to potently regulate diverse cell responses and disease states, especially in the context of the immune system. Thus, understanding these processes will reveal important fundamental features that enable rapid chromatin state changes that instruct inflammatory

gene transcription and cell fate choices, and also point to novel features of disease states and therapeutic targets.

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DATA AVAILABILITY STATEMENT

All data (western blots) represented here are available for sharing in original form.

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FIGURE 1.

Signaling-to-chromatin in the immune system. A, Western blot showing levels of histone H3 phosphorylation at residues S28 and S31 (H3.3) upon stimulation of different immune cell types with relevant stimuli for the indicated times. B, Different immune cell receptors are able to engage MAPK and NF- κ B pathways to increase chromatin phosphorylation and stimulate transcription of target genes

FIGURE 2.

Histone phosphorylation-mediated binary switches for inducible transcription. A, General model describing histone phosphorylation activity through ejection of corepressors (left) and stimulation of coactivators (right). B, General model of H3S28ph and H3.3S31ph ability to eject the transcriptional corepressor PRC2 and increase binding and activity of p300. C, Co-transcriptional phosphorylation of H3.3S31 by IKK α ejects ZMYND11 and stimulates the activity of SETD2. D, Common mechanism of action for H3S10ph and H3Y41ph in the ejection of HP1. E, Events linking H3S10 phosphorylation and transcription activation through the stepwise recruitment of coactivators (MOF, GCN5, BRD4, and P-TEFb) and Pol-II CTD phosphorylation. F, Kinase activity of PKC β on H3T6 prevents demethylation

of H3K4 by ejecting corepressors JARID1B and LSD1. G, Phosphorylation of H3T11 by PRK1 recruit coactivator JMJD2C to demethylate H3K9 and activate transcription

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TABLE 1

Histone kinases involved in transcriptional regulation

Kinase	Histone residue	Mechanism		References
		Eject/block corepressor	Recruit/stimulate coactivators	
MSK1/2	H3S10	HP1	GCN5/SWI-SNF	25,162,164,166
MSK1/2	H3S28	PRC2	p300/SWI-SNF	23,164,167,168
IKK α	H3.3S31	ZMYND11	SETD2	24,26
IKK α	H3S10	Unknown	Histone acetylation	26,43,44
PIM1	H3S10	Unknown	MOF	87,165
CHK1	H3.3S31	Unknown	p300	176
CHK1	H3T11	Unknown	Histone acetylation	178
PRK1	H3T11	Unknown	JMJD2C	46
PKC β	H3T6	LSD1/JARID1b	Unknown	45
JAK2	H3Y41	HP1	Unknown	55
ACK1	H4Y88	Unknown	WDR5/MLL2	179
AMPK	H2BS36	Unknowns	Unknown	170
JNK	H3S10	Unknown	Unknown	40
RSK2	H2BS32	Unknown	Unknown	180
RSK2	H3S10	Unknown	Unknown	160