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Keywords

Listeria, *Mycobacterium*, interferon-lambda, BAHD1, epigenetics, chromatin complex

Abstract

Bacterial infections, like their viral counterparts, trigger the onset of innate immune defense mechanisms through the release of cytokines, including interferons (IFNs). While type I and II IFN responses to bacteria have long been explored, type III remains poorly addressed. We have recently reported that the pathogen *Listeria monocytogenes* triggers the expression of type I and III IFN genes in epithelial cells, and is able to fine-tune downstream signaling at the chromatin level. This bacterium can negatively or positively modulate the expression of interferon-stimulated genes (ISGs) by manipulating the function of BAHD1, a component of a host chromatin-silencing complex. To this end, *L. monocytogenes* tightly controls the secretion of a BAHD1 inhibitory factor, LntA. Here, we further document the current knowledge about chromatin mechanisms modulating interferon responses during host-bacteria interplay, and discuss their physiological consequences.

When intracellular bacteria infect eukaryotic cells, a number of signaling events lead to a drastic reprogramming of the host cell transcriptional landscape. A growing body of evidence indicates that these local or genome-wide changes can proceed from bacterial-induced chromatin modifications[1,2]. Most of the reported mechanisms of chromatin subversion by bacteria result in the silencing of key host defense genes. For instance, the secreted protein phosphatase OspF from *Shigella flexneri* prevents the phosphorylation of histone H3 on serine 10 (H3S10) at the promoters of a set of innate immune genes including IL-8, and consequently down-regulates these genes[3]. Exposure of HeLa cells to bacterial pore-formin toxins, such as *Listeria monocytogenes* listeriolysin O (LLO), also results in the rapid dephosphorylation of H3S10 at the promoters of various host genes, including the innate immune-related genes *CXCL2* and *IFIT3*, and correlates with their down-regulation[4]. However, gene silencing does not restrict to histone modifications; this multi-step process requires the coordinated action of DNA–, RNA– and histone–binding, –modifying and –remodeling factors. These components assemble into large complexes, establishing a condensed chromatin state referred to as heterochromatin, and leading to repression of transcription. We focus here on the subversion by bacteria of some of these chromatin regulators, in the context of interferon responses.

Two bacteria can manipulate chromatin to regulate interferon responses

Interferons are major cytokines produced in response to both viral and bacterial infection[5]. After recognition of pathogen determinants, type II IFNs (IFN-γ) are produced exclusively by immune cells, while types I and III (IFN-I and IFN-III) can be produced by a wide range of cell types. Upon binding to their cognate surface receptor, they activate JAK/STAT (Janus Kinase/Signal Transducer and Activator of Transcription)-signaling pathways. Typically, IFN-γ will trigger the translocation of phosphorylated STAT1/STAT1 homodimers to the nucleus, and induce the transcription of a subset of genes characterized by the presence of a GAS (gamma-interferon activated site) box in their promoter sequence. In contrast, IFN-I and IFN-III stimulations predominantly induce the formation of a phosphorylated STAT1/STAT2/IRF9 heterotrimer, also named ISGF3 (ISG Factor 3). After entering the nucleus, this complex binds to promoters containing Interferon-Sensitive Response Elements (ISREs) and drives the transcription of downstream genes.

Mycobacterium tuberculosis and *avium* were the first bacteria reported to control interferon-induced pathways at the chromatin level[6-8]. During infection of macrophages stimulated with IFN-γ, mycobacteria repress some IFN-γ-responsive genes, in particular genes encoding major histocompatibility complex class II (MHC-II) molecules and their transactivator CIITA (Fig. 1). The recognition of a mycobacterial cell wall lipoprotein, LpqH, by TLR2, and subsequent signaling through the MAPK pathways, result in the binding of the transcription factor C/EBP at the promoter of *CIITA*. This prevents the recruitment of the SWI/SNF chromatin remodeler at this site and leads to histone deacetylation and *CIITA* repression[7]. CIITA-regulated genes, such as *HLA-DR,* are (*i*) down-regulated by the decrease in CIITA amounts, and (*ii*) further repressed by the recruitment at their promoter of a chromatin complex containing histone deacetylases (HDACs) and the co-repressor mSin3A, in response to TLR2 activation[8].

Listeria monocytogenes is the second example of a bacterium controling the expression of ISGs by acting on the chromatin remodeling machinery[9]. In contrast to *Mycobacterium*, *Listeria* chromatin modulation targets IFN-I and IFN-III pathways in epithelial cells (Fig. 1). Our recent work suggests that infection by *Listeria* promotes the repressive function of a chromatin-silencing complex at the promoter of a set of ISRE-dependent ISGs. We established that two components of this complex, the heterochromatinization factor BAHD1[10] and the co-repressor KAP1, participate in the down-regulation of ISGs. Further studies will be needed to investigate the signaling pathways triggering this bacterial-mediated repression of ISGs, as well as the role of BAHD1/KAP1 partners (Fig. 2).

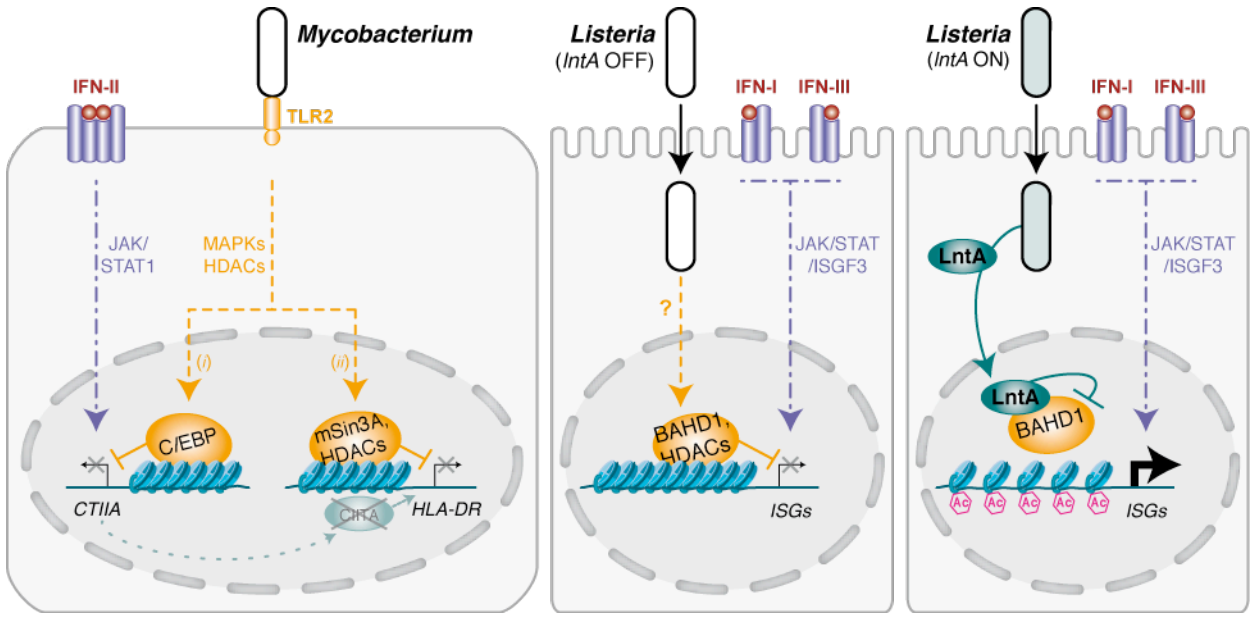

Figure 1. Bacterial modulation of interferon responses by chromatin-related mechanisms. In macrophages, the activation of TLR2 by *Mycobacterium* counteracts IFN-II-dependent signaling. Activation of MAPKs triggers the activation of histone deacetylases and (*i*) the silencing of the *CIITA* gene, (*ii*) the repression of CIITA-dependent gene expression. In epithelial cells, infection by *Listeria* leads to the expression of IFN-I and IFN-III genes (not represented). Subsequently, these cytokines activate the JAK/STAT pathway. However, by an unknown pathway, infection also drives the BAHD1-associated chromatin complex to repress ISGs. When *Listeria* secretes LntA, this factor enters the nucleus where it interacts with BAHD1, destabilizes the silencing complex, restores H3K9 acetylation (Ac) and enhances the expression of ISGs.

Remarkably, *Listeria* has evolved a strategy to counterbalance ISG repression in specific conditions, by directly targeting this chromatin complex. A secreted virulence factor, LntA enters the nucleus of infected cells, where it interacts with BAHD1, impedes its function, and thereby promotes the expression of ISGs. We observed a decrease in BAHD1 occupancy of the promoters of *IFITM1* and *IFIT3* in cells infected with LntA-producing bacteria. Consistent with the presence of HDAC1/2 in the BAHD1-associated complex, the level of acetylation of lysine 9 on histone H3, which is a mark of active chromatin, increased at the promoters of ISGs in the presence of LntA. How LntA targets BAHD1 specifically at ISGs remains an open question. Indeed, LntA had no effect on either BAHD1 occupancy or histone acetylation status at the promoter of *IGF2*, one of the genes that BAHD1 represses constitutively[10]. The specificity of LntA recruitment to ISRE-containing promoters might be driven through an interaction with transcription factors activated only in infected cells, such as ISGF3. LntA would thus displace BAHD1 only from a subset infection-dependent targets.

Chromatin complexes regulate interferon response

The above-described studies highlighted that mechanisms controlling interferon responses in infected cells highly depend on chromatin states, and allowed the identification of new chromatin regulators, in particular BAHD1[10]. Remarkably, some of the components that we identified in the chromatin complex associated to BAHD1 (Fig. 2) are general modulators of ISGs in interferon-stimulated cells, and not only during bacterial infections. For example, the histone deacetylase HDAC1 has been shown to directly bind STAT1 and 2, and modulate IFN-α-induced transcription[11]. The heterochromatin proteins HP1 can repress the transcription of ISGs, through the interaction of their chromoshadow-domain with H3, antagonizing the nucleosome-remodeling activity of the SWI/SNF complex[12]. The pleiotropic chromatin regulator KAP1 is able to bind STAT1, 3, 4 and 6 transcription factors[13], and represses type-I interferon-dependent transcription in

HeLa cells[14]. In addition, BAHD1 interacts with H3K9 methyltransferases (HMTs) and the methylated-DNA binding protein MBD1[10]; these factors might promote heterochromatin formation at the promoters of ISGs in response to IFN-I and IFN-III signaling, as was shown for MBD1 in response to IFN-γ[15].

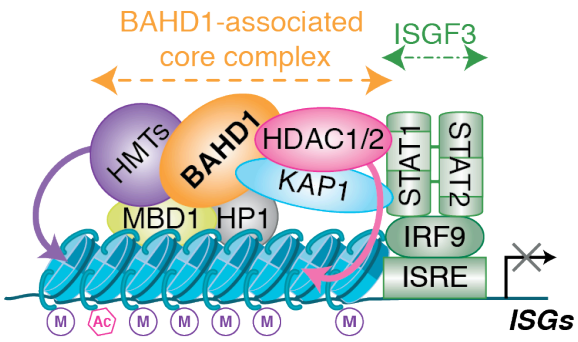

Figure 2. Model of the BAHD1-associated repressive core complex deposited at the promoters of ISGs. The BAHD1-associated chromatin complex contains several heterochromatin components, such as KAP1, HP1 and MBD1, and modifying enzymes, such as histone methyltransferases (HMTs) and deacetylases (HDACs). Since KAP1 and HDAC1 can interact with STAT proteins, we hypothesize that they could bridge the silencing complex with ISGF3, deposited on ISRE sequences. HP1 was also described as a regulator of ISGs. HMTs can methylate histones (M), while HDACs can erase acetylations (Ac), resulting in a closed chromatin state at targeted promoters.

Beside chromatin-silencing factors, proteins that open the chromatin structure, such as the chromatin-remodeling complex of the SWI/SNF family PBAF, can also regulate the expression of ISGs. In response to IFN-α or viral challenge, the BAF47 subunit of the PBAF complex is required for maintaining an open chromatin state at ISG promoters, thus allowing their induction[16], while the BAF200 subunit confers gene selectivity in regulating ISGs[17].

We hypothesize that all these proteins can form distinct regulatory sub-complexes, depending on cell types and stimuli they are submitted to. Specific assortment of factors and targeting of different promoters could thus translate interferon signals into a wide range of cellular responses.

Are interferon responses a blessing or a threat?

L. monocytogenes uses sophisticated strategies to fine-tune interferon responses, in epithelial cells as discussed here, and in immune cells[5, 18]. Does this dual capacity to repress or activate innate immune genes translate into a benefit for the pathogen? Most notably, why would a virulence factor be dedicated to the up-regulation of ISGs? Here, we emphasize that, while the function of interferons in anti-viral defense mechanisms is widely acknowledged, their role in the context of intracellular bacterial infections is more ambiguous[5, 19]. Considering the induction of interferon responses only as a line of anti-bacterial defense at the systemic level would constitute a simplistic view.

The production of IFN-γ by macrophages and other immune cells, promotes bacterial clearance and is thus critical in controlling primary *L. monocytogenes* infections[20]. In contrast, several independent studies have reported that stimulation of IFN-I production could increase the virulence of intracellular bacterial pathogens, including *Listeria*, as well as *Mycobacterium, Chlamydia* and *Tropheryma*, through various effector mechanisms[5, 21, 22]. In our study, we found that both IFN-I and IFN-III were induced in response to infection of epithelial cells by *L. monocytogenes*. In the context of viral infections, type III IFNs can substitute for type I in specific tissues, such as epithelia[23]. Taken together, it is tempting to speculate that IFN-III might, like IFN-I, have injurious consequences for the host during listeriosis – and perhaps other bacterial infections, although this has not yet been explored in animal models. If so, LntA, by exacerbating the downstream response, would be beneficial to the pathogen, which would explain why Δ*lntA* mutants are less virulent than wild type strains. In the future, more detailed *in vivo* studies will be required to fully understand the contribution of LntA and of its cellular partners on the outcome of *Listeria* infections. The stimulation of interferon responses might support localized pro-bacterial conditions, such as the

recruitment of vehicle immune cells to infected epithelia, or the facilitation of bacterial shedding at the intestinal or placental barriers, through the destruction of infected tissue or abortion. Otherwise, as was proposed for IFN-I response, the stimulation of ISGs by LntA might inhibit IFN-γ-mediated immunity[24] or promote host T-cell apoptosis[25].

Nevertheless, the pro-bacterial effect of interferon responses is probably limited to a narrow spatiotemporal frame. Indeed, the constitutive expression of *lntA* promotes bacterial clearance. Consistent with this, *BAHD1*$^{+/-}$ mice are more resistant to listeriosis than their wild type littermates. A systemic induction of ISGs during infection thus seems detrimental to bacteria. As a corollary, the expression of *lntA* appears to be tightly controlled by bacteria. The positive function of LntA, paralleling its production, is thus likely restricted to a specific time and place. We propose that an early and/or acute type I/III interferon response could activate the host innate immune defenses and help fight against *Listeria*, as it was reported for other bacteria[5, 19].

ISGs are expressed in distinct combinations depending on the cell type and stimulatory pathways, and they cover a very wide range of cellular or extra-cellular functions. Because physiological effects of interferons are generally studied at the systemic level, functional knowledge concerning individual ISGs remains elusive. An important issue for forthcoming research will thus be to understand more precisely the roles of the ISGs modulated by BAHD1 and LntA. Most of the characterization efforts have so far focused on anti-viral effectors such as the double-stranded RNA-activated serine/threonine protein kinase (PKR) or the 2'-5'-oligo-adenylate synthetase (OAS) gene family, which seem less pertinent to bacterial infections. Functional insights into other ISGs are emerging; for instance, IFI6 (G1P3) and IFI27 (ISG12) are mitochondrial proteins, reported to have respectively anti- and pro-apoptitic effects[26]; the ISG15 deconjugating enzyme USP18 and GBP-1 also display anti-apoptotic properties[27, 28], while IFIT2 (ISG54) is pro-apoptotic[29]. Tuning the balance between various effectors might constitute a way for the bacteria, on the one hand to maintain cell survival during intracellular proliferation, on the other hand to trigger cell death, allowing escape from immune response or dissemination.'

In conclusion, our recent work has highlighted that intracellular bacteria are able to carefully regulate the response of their host cells to type I and III interferons, by targeting directly the cellular chromatin silencing outfit. During infection, this bacteria/host interplay results in modifications of the chromatin status and transcriptional levels of ISGs. Whether these changes will, in the long run, translate into stable epigenetic marks deserves further investigation.

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