Shigella hacks host immune responses by reprogramming the host epigenome

Hiroshi Ashida¹ & Chihiro Sasakawa¹,²,³

Bacterial pathogens alter host transcriptional programs to promote infection. *Shigella OspF* is an essential virulence protein with a unique phosphothreonine lyase activity. A new study in *The EMBO Journal* (Harouz et al, 2014) reveals a novel function of OspF: targeting of heterochromatin protein 1γ (HP1γ) and downregulation of a subset of immune genes. These results illustrate how bacterial pathogens exploit epigenetic modifications to counteract host immune responses.

See also: H Harouz et al (November 2014)

The intestinal epithelium deploys multiple defense systems to sense bacterial components, transmit danger alarms to the immune system, clear bacteria, and renew injured epithelial lining. In response to various stimuli associated with bacterial infection, host cells trigger inflammatory signaling pathways such as mitogen activated protein kinases (MAPK) and transcription factor nuclear factor κB (NF-κB), leading to expression of inflammatory cytokines and chemokines that are crucial for bacterial elimination. Nevertheless, many bacterial pathogens, including *Shigella*, are able to efficiently colonize the intestinal epithelium by deploying multiple countermeasures against the host immune responses, including reprogramming of the host transcriptional responses.

*Shigella*, a Gram-negative bacterium, has a highly evolved invasive system that enables it to enter host cells, multiply therein, spread from cell to cell, and succeed in colonization within the cells, ultimately leading to severe inflammatory colitis termed bacillary dysentery (shigellosis). *Shigella* delivers a subset of virulence proteins, called effectors, via the type III secretion systems (T3SS), and these effectors enable bacteria to evade host defense systems and promote colonisation. In this issue of *The EMBO Journal*, Harouz et al demonstrate that OspF, one of *Shigella*’s anti-inflammatory effectors, modulates host inflammatory responses by targeting epigenetic modifications.

Gene expression is tightly regulated at the levels of transcription, RNA splicing, and translation and is controlled by a large number of regulatory proteins, including histones. Regulation of transcription requires post-translational histone modifications, such as phosphorylation, acetylation, and methylation, which induce changes in chromatin structure and control recruitment of the transcription machinery to specific promoter regions. For example, phosphorylation of histone H3 at Ser10 by MAPK increases the accessibility of NF-κB and RNA polymerase II (RNAP II) to chromatin on the IL8 promoter region (Arbibe et al, 2007).

Previous reports showed that *Shigella OspF*, which has a unique phosphothreonine lyase activity, translocates into the nuclei of epithelial cells, where it targets the MAPK pathway by irreversibly dephosphorylating MAPKs (Erk and p38) through beta-elimination of the phosphate group (Arbibe et al, 2007; Li et al, 2007). This dephosphorylation inactivates MAPK and inhibits the downstream phosphorylation of histone H3Ser10 at the promoters of a subset of innate immune genes, such as IL8, thus promoting chromatin condensation and inhibiting transcriptional activation by masking NF-κB binding sites. Consistent with this, the *Shigella AospF* mutant induces high levels of IL-8 production and mucosal destruction, leading to increased recruitment of neutrophils in the rabbit ileal-loop infection model (Arbibe et al, 2007).

In addition to this effect on histone modification, Harouz et al now find that OspF also alters the activity of the chromatin reader heterochromatin protein 1 (HP1) and reprograms host gene expression during *Shigella* infection. HP1 family proteins (HP1α, HP1β, and HP1γ in humans) have a conserved chromo-domain that binds methylated histone H3K9, the hallmark modification of heterochromatin (a transcriptionally inactive state), resulting in transcriptional repression (Kwon & Workman, 2011). In addition to the gene-silencing function of HP1, recent studies have shown that HP1 proteins can positively regulate euchromatin (a transcriptionally active state) and activate transcription. HP1γ is recruited to the coding region of active genes and interacts with RNAP II. In this state, phosphorylation of HP1γ at serine 83 determines its localization to sites of transcription elongation (Lomberk et al, 2006). Therefore, HP1 proteins play important roles as both transcriptional repressors and activators in fine-tuning gene expression.

In an *in vitro* guinea pig rectal-infection model, Harouz et al show that infection with the *Shigella DmxiD* (T3SS deficient) mutant dramatically increased phosphorylation of HP1γ at serine 83 in the lamina propria and epithelium, relative to WT *Shigella* infection. Thus, *Shigella* harbors a T3SS-dependent tactic that prevents HP1γ phosphorylation. Because their *in vitro* analysis of *Shigella* infection showed that HP1γS83 phosphorylation is dependent on MAPK activation, the authors hypothesized the involvement of the
T3SS effector OspF, which inactivates Erk and p38 through its phosphothreonine lyase activity, and found that OspF is a key player in dephosphorylation of HP1γ. Consistent with this, the *Shigella ΔospF* mutant increased the level of HP1γS83 phosphorylation relative to that in HeLa cells infected with WT *Shigella*. However, rather than directly dephosphorylating HP1γ, OspF acts by inactivating Erk and consequently reducing the activity of downstream kinase MSK1, a novel kinase for HP1γ at Ser83. In MSK1-null MEF cells infected with the *Shigella ΔospF* mutant, phosphorylation of HP1γS83 was no longer detected, whereas levels of phosphorylated Erk were elevated relative to those in WT infection, strongly indicating that OspF prevents HP1γ phosphorylation through inactivation of Erk/MSK1. As a result of this dephosphorylation, HP1γ dissociates from sites of transcriptional activation at OspF-target genes, such as *IL8*.

The host transcriptional program plays a pivotal role in terminating bacterial infection. *Shigella* overcomes this defense mechanism by delivering a subset of T3SS effector proteins to regulate gene expression and counteract host immune responses (Ashida *et al*, 2011). For example, OspG, OspI, and IpaH target components of the NF-κB pathway and interfere with signaling, thereby inhibiting NF-κB activation. IpaH9.8 binds to U2AF35, a mRNA splicing factor, and inhibits the U2AF35-dependent splicing reaction. Thus, *Shigella* hijacks the transcriptional program at multiple steps, including transcriptional activation, RNA splicing, and post-translational and epigenetic modification, thereby repressing pro-inflammatory cytokine genes.

The work of Harouz *et al* provides novel molecular insights on how bacterial effectors can alter the transcriptional program of the host cell. *Shigella* OspF represses host transcriptional responses by influencing two
epigenetic events: (i) decreasing the level of phosphorylated histone H3 and (ii) altering the activity of HP1 through dephosphorylation of MAPK. As in Shigella, many other bacterial pathogens dramatically alter the host transcriptional program for their own benefit. For example, Listeria monocytogenes hijacks and induces translocation of histone deacetylase SIRT2 from cytoplasm to nucleus, resulting in deacetylation of histone H3 on lysine 18 and regulation of a subset of host genes (Eskandarian et al., 2013). Furthermore, L. monocytogenes manipulates host immune responses by secreting virulence factor LntA, which interacts with the chromatin repressor BAHD1 and impedes heterochromatin formation, thereby promoting the expression of interferon-stimulated genes (Lebreton et al., 2011).

Although accumulating evidence has revealed the importance of epigenetic modifications during bacterial infection, our knowledge of bacterial strategy is still in its infancy. We predict that future studies of the interaction between epigenetic and bacterial pathogens will facilitate development of new drugs and therapeutic approaches for overcoming bacterial infections.

References