Shigella hacks host immune responses by reprogramming the host epigenome

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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 1 γ (HP1 γ) 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 κ B (NF- κ B), 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 colonization. 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- κ B 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- κ B 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 (HP1 α , HP1 β , and HP1 γ 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. HP1 γ is recruited to the coding region of active genes and interacts with RNAP II. In this state, phosphorylation of HP1 γ at serine 83 determines its localization to sites of transcription elongation (Lomber *et al*, 2006). Therefore, HP1 proteins play important roles as both transcriptional repressors and activators in fine-tuning gene expression.

In an *in vivo* guinea pig rectal-infection model, Harouz *et al* show that infection with the *Shigella AmsxD* (T3SS deficient) mutant dramatically increased phosphorylation of HP1 γ at serine 83 in the lamina propria and epithelium, relative to WT *Shigella* infection. Thus, *Shigella* harbors a T3SS-dependent tactic that prevents HP1 γ phosphorylation. Because their *in vitro* analysis of *Shigella* infection showed that HP1 γ S83 phosphorylation is dependent on MAPK activation, the authors hypothesized the involvement of the

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DOI 10.15252/emboj.201489934 | Published online 22 September 2014

Figure 1. *Shigella* reprograms host transcriptional response by injecting the T3SS effector OspF.

(left panel) *Shigella* WT delivers OspF, which translocates into the nucleus and dephosphorylates MAPK, thereby blocking phosphorylation of histone H3 at Ser10 and HP1 γ at Ser83. Dephosphorylated histone H3 and HP1 γ repress transcription of specific immunity-related genes such as *IL-8*. (right panel) *Shigella* $\Delta ospF$ infection triggers MAPK activation, which induces phosphorylation of histone H3 at Ser10 and HP1 γ at Ser83. Phosphorylated histone H3 and HP1 γ promote transcriptional activation, resulting in production of IL-8, which in turn recruits neutrophils to limit and clear *Shigella* infection.

T3SS effector OspF, which inactivates Erk and p38 through its phosphothreonine lyase activity, and found that OspF is a key player in dephosphorylation of HP1 γ . Consistent with this, the *Shigella* $\Delta ospF$ mutant increased the level of HP1 γ S83 phosphorylation relative to that in HeLa cells infected with WT *Shigella*. However, rather than directly dephosphorylating HP1 γ , OspF acts by inactivating Erk and consequently reducing the activity of downstream kinase MSK1, a novel kinase for HP1 γ at Ser83. In MSK1-null MEF cells infected with the *Shigella* $\Delta ospF$ mutant, phosphorylation of HP1 γ S83 was no longer detected, whereas levels of

phosphorylated Erk were elevated relative to those in WT infection, strongly indicating that OspF prevents HP1 γ phosphorylation through inactivation of Erk/MSK1. As a result of this dephosphorylation, HP1 γ dissociates from sites of transcriptional activation at OspF-target genes, such as *IL-8*.

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- κ B

pathway and interfere with signaling, thereby inhibiting NF- κ B 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.

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