A Lasting Impression: Epigenetic Memory of Bacterial Infections?

Jorge M Pereira, Mélanie A Hamon, Pascale Cossart

A lasting impression: epigenetic memory of bacterial infections?

Jorge M Pereira [1,2,3,4] , Mélanie A Hamon [4, #] , Pascale Cossart [1,2,3, #]

[1] Institut Pasteur, Unité des interactions Bactéries-Cellules, Paris F-75015, France

[2] INSERM, U604, Paris F-75015, France

[3] INRA, USC 2020, Paris F-75015, France

[4] Institut Pasteur, G5 Chromatine et Infection, Paris F-75015, France

Corresponding authors:

Pascale Cossart

Phone: + 33 1 45688841

Fax: + 33 1 45688706

E-mail: pcossart@pasteur.fr

Mélanie A Hamon

Phone: + 33 1 45688995

Fax: + 33 1 45688706

E-mail: melanie.hamon@pasteur.fr

Abstract: Bacteria can reprogram host gene expression during infection, often through epigenomic mechanisms. However, the lasting impact of such effects remains understudied. This forum discusses examples suggesting that bacterial infection can result in long-lasting memory encoded in epigenomic mechanisms and speculates on the potential of others.

Introduction

Transcriptional regulation in Eukaryotes is a result of the combined effect of transcription factors responding to signaling cascades as well as various modifications of both the DNA and histones, proteins important for packaging DNA into chromatin. The basic subunit of chromatin is the nucleosome, an octamer of histone proteins with which the DNA closely interacts. The state of compaction of nucleosomes plays a major role in gene expression by controlling DNA accessibility to the transcriptional machinery. The chromatin structure is remodeled in a dynamic process by ATP-dependent complexes and covalent modifications of histones.

The different chromatin modifications have variable abilities to be maintained over time and persist through cell division. Therefore different chromatin marks, depending on their stability, will have varying potentials to transmit information over time, through cell division or even cell differentiation (Figure 1). Labile modifications of histones, such as acetylation and phosphorylation, are strongly induced by stimulation with extracellular signals, but are very short-lived and erasure of the mark occurs rapidly after stimulation, in the range of minutes. In contrast, methylation of histones have a longer half-life, from several hours to days, depending on the specific

residue, which is modified and therefore is thought to be the most durable histone mark reported so far. DNA methylation, in contrast, is highly stable, having the ability to be maintained through cell division. Therefore, since DNA methylation is long lasting by sustaining the effects of transient stimulation, it is considered to be the canonical epigenetic mark.

DNA methylation during development is well studied and is required for initiating and maintaining the transcriptional program in differentiated cells. Indeed, during development, the specific conditioning of chromatin will promote specialization of a cellular identity and establish a cell lineage. The epigenetic modifications which maintain the memory of cell fate through cell division can be established at gene coding regions to ensure proper gene silencing or activation, or at distant non-coding regions, termed enhancers, that reinforce transcriptional regulation. Differentiated cells, although committed to a specific cell lineage, can still adapt in a lasting manner to environmental signals making the line between a differentiated or an adapted cell very blurry.

The interaction of bacteria with host cells leads to an alteration of host transcriptional programs, a phenomenon that is well documented over the years and has been shown to involve epigenomic mechanisms (Bierne et al., 2012). Bacterial-induced transcriptional changes can affect the function of host cells either to promote host defense against invading pathogens or to benefit bacterial persistence. Pathogens have evolved several strategies to target host gene expression through regulation of chromatin. Virulence factors modify the epigenomic landscape through targeting of host signaling cascades, or chromatin complexes directly. Additionally, some bacterial factors have intrinsic catalytic activity enabling them to directly modify chromatin. Bacteria-mediated histone marks were reported to map to individual gene

promoters and enhancers, and are correlated with transcriptional reprograming of the host. Since chromatin modifications have the potential to generate a transcriptional memory of the initial stimulus, this facet of bacteria-host interactions raises the interesting possibility that there could be some memory of infection that would be established after pathogen encounter and would last in time even after pathogen clearance. Indeed, similarly to cell differentiation, bacterial encounters have the possibility through chromatin to induce cell adaptations. We believe that these considerations are crucial, as adapted cells would then respond in a distinct manner than naïve cells to subsequent infection or stimuli. Although this forum will focus on bacteria-mediated epigenomic modifications, it should be noted that virus, fungi and parasites also induce similar processes.

Long-lasting epigenomic modifications

We will start by discussing the examples that show most stable epigenomic changes in response to bacterial infection and therefore have the potential to generate a memory of response.

Cell de-differentiation

Infection with *Mycobacterium leprae*, the agent responsible for human leprosy, was shown to dramatically change the identity of the host cells by modulating the strict transcriptional program imposed after cell differentiation. This pathogenic bacterium reprograms fully differentiated and functional Schwann cells, glial cells from the peripheral nervous system, to a progenitor/stem-like cell (Masaki et al.,

2013). Interestingly cellular reprogramming correlates with changes in DNA methylation, leading to derepression of key mesoderm genes and silencing of the master regulator of Schwann cell lineage (Masaki et al., 2013). These events are important for *M. leprae* infection since de-differentiated cells re-acquire initial migratory properties exploited by the bacteria to spread throughout the host.

Carcinogenesis

An abnormal cellular reprogramming may promote carcinogenesis. Several types of cellular stress, such as pathogenic infections, have been identified as risk factors for cancer, and *Helicobacter pylori* infection is a major causal risk for gastric cancer development. The underlying molecular mechanisms are still unclear but data suggest that epigenetic reprogramming might be involved. Indeed, *H. pylori* induces aberrant DNA methylation in human gastric mucosa at the promoter of genes found to be methylated in gastric cancer cells (Maekita et al., 2006). Interestingly, although eradication of *H. pylori* leads to a reduction in promoter methylation, a basal level still remains.

Tolerance

A lasting epigenomic marking was observed during the tolerance response induced by the anthrax lethal toxin. Macrophages exposed to sublethal doses of the toxin exhibited deacetylation of histone H3 on lysine 27 and became refractory to subsequent cytolytic doses of toxin. Interestingly, this toxin-induced tolerant state was shown to last up to 6 weeks in a subpopulation of cells (Ha et al., 2014).

Endotoxin tolerance was coined to name the response to sustained stimulation by lipopolysacharide (LPS), the major constituent of the cell wall of gram-negative bacteria. It was considered to be a hyporesponsive state, which allowed for the tight control of inflammation over time. A closer examination of gene transcription revealed that the response is more complex as not all genes are tolerized, while others are highly induced. At the chromatin level, transcriptional repression is facilitated by remodeling from an active to a silent state at specific gene promoters, an effect that lasts long after removal of LPS (Biswas and Lopez-collazo, 2009). Recently, an interesting effect of LPS on enhancers has been demonstrated and will be discussed in the section below.

"Trained Immunity"

The concept that innate immunity exhibits memory is another illustration of lasting epigenomic mechanisms (Hamon and Quintin, 2016; Netea et al., 2011). The term "trained immunity" has been proposed, and although still controversial, it explains well the concept that innate immune cells can become adapted to a certain stimulus and then respond in a stronger manner to a second exposure to the same or other stimulus. This effect was initially observed upon vaccination with Calmette-Guérin bacilli (BCG), which is designed to prevent *Mycobacterium tuberculosis* infection but was also shown to provide long-term protection to other unrelated bacterial, viral and fungal pathogens. Through subsequent investigations with other microbial stimuli, it was determined that this innate immune memory might be conferred through epigenomic mechanisms. Stimulation of monocytes with the fungal

cell wall component, β-glucan, induced functional reprogramming of monocytes, leading to enhanced cytokine production, associated with stable changes in histone H3 trimethylation. More recently, the effect of LPS on enhancers also supports epigenomic marks involving innate immune memory. Indeed, new distant enhancers become marked upon LPS stimulation by monomethylation at lysine 4 on histone H3, a modification which is maintained even when LPS is no longer present (Ostuni et al., 2013). Thus LPS is able to modify the pre-established gene regulatory landscape of the cell by generating and maintaining this new class of enhancers, making cells exposed to LPS transiently different from naïve cells.

Transient epigenomic modifications

Besides the above examples, bacteria have been reported to induce a plethora of other histone modifications. Such modifications imposed by infection were clearly shown to play a role in regulation of host transcription; however, whether they affect cellular identity and/or can persist after the stimulus is cleared remains to be determined.

Transcriptional changes via histone modifications

During infection, bacteria can hijack host signaling pathways to impose histone modifications, with histone acetylation and phosphorylation being the two main modifications reported so far. Examples include *Listeria monocytogenes*-induced deacetylation on histone H3 on lysine 18, a process mediated by a host

deacetylase, Sirtuin 2, and correlating with gene repression during infection (Eskandarian et al., 2013). Interestingly, as for *Listeria*, other bacteria were shown to induce removal of histone marks, such as dephosphorylation and deacetylation (Bierne et al., 2012). In these cases the return to the initial epigenomic status has not been evaluated yet.

Bacteria can also impose other histone modifications through targeting bacterial effectors with enzymatic activity to the nucleus. For example, *Chlamydia trachomatis* and *Legionella pneumophila* are able to methylate histones using bacterial factors with methyltranferase activity, NUE and RomA, respectively (Pennini et al., 2010; Rolando et al., 2013). RomA-dependent methylation was correlated with host transcriptional changes (Rolando et al., 2013), but the role of NUE-induced methylation remains to be determined.

Although classically viewed as chemically unstable, these infection-induced histone marks could persist, or serve as recruiting platforms for the generation of more stable modifications. Future studies will be necessary to elucidate whether the above modifications are able to maintain the reported transcriptional changes after infection is cleared.

Transcriptional changes via chromatin remodeling

Bacteria are also able to target chromatin binding complexes through bacterial factors such as LntA of *L. monocytogenes*. When secreted, LntA inhibits the chromatin repressor BAHD1 in the nucleus of the host cell and induces the activation of interferon-stimulated genes (Lebreton et al., 2011). Here again, whether these changes are long-lasting is unknown and deserves investigation.

Regulatory RNAs

Diverse classes of RNA, such as microRNAs and long noncoding RNAs (lncRNAs), have emerged as important regulators of chromatin as they can act as sequence dependent scaffolds for chromatin modifying complexes.

During bacterial infection the role of regulatory RNAs has increasingly been appreciated. microRNA expression is clearly modified upon infection, although the consequences for the host are still unclear (Maudet et al., 2014) . The role of lncRNAs in the control of gene expression in immune cells has recently been demonstrated in a collection of studies. For example lncRNA-Cox2, which is expressed upon recognition of various bacterial ligands was shown to regulate inflamatory gene expression in macrophages (Carpenter and Fitzgerald, 2015). Another lncRNA, NeST, was shown to control susceptibility to *Salmonella* infection through epigenomic changes involving histone 3 lysine 4 trimethylation on the interferon-gamma gene locus (Carpenter and Fitzgerald, 2015). Whether these noncoding RNA regulate host chromatin and whether their effect could last beyond bacterial clearance remain to be addressed.

Conclusions and Perspectives

The examples discussed here support the possibility of an epigenetic memory of infection. Whether bacteria change the differentiation status of the cell or lead to a lasting adaptation state, will depend on the stimulus, i.e on the pathogen. Importantly, epigenomic changes are not the only possible marks contributing to epigenetic memory. As proposed by Monticelli and Natoli, every inducible change that is not rapidly reversed, such as protein activation by phosphorylation or relocalization, has the potential to maintain a lasting effect which could contribute to a short term memory of the response (Monticelli and Natoli, 2013). However, up to now, in most cases the true lasting potential of bacteria-mediated epigenomic or other changes has not been evaluated. Indeed, even in the more striking examples of cell de-differentiation, the long-term impact for the host and the lasting potential of the epigenomic signature has not been explored, and the impact of previous encounters and the context of a cell should not be discarded when studying the response to a given stimulus.

Importantly, the lasting potential of chromatin marks not only depends on the kinetics of the epigenome, but also on the stimulus itself. For example, in contrast to LPS, which is rapidly cleared from the organism, BCG and the anthrax toxin may persist in the host organism. Therefore, the lasting epigenomic effect would not be due to memory, but continuous stimulation by persistent pathogens or persistent components. Thus, it will be important in future studies to evaluate the kinetics of stimulation in order to truly describe epigenomic memory.

So far most studies in this field have been performed *in vitro* with fully terminally differentiated cells such as epithelial cells. Since in such cell types cell fate is already established and a short lifespan often occurs *in vivo* this raises the question of whether such memory would be relevant for these cells. The same can be applied to differentiated innate immune cells which also have a short lifetime. Looking at the response of undifferentiated cells such as stem cells appears much more appropriate to further explore the concept of innate immune memory. Research in the coming years in the field will aid in elucidating novel epigenetic mechanisms during infection, which could offer the opportunity to modulate innate immune memory to facilitate the treatment of pathogenic infections. Finally, targeting of chromatin components by pharmaceutical drugs is an exploding field of study for the treatment of diseases such as cancer. The studies that we have detailed here would suggest that a similar strategy could be used to treat bacterial disease.

Acknowledgments

We apologize to colleagues whose work was not cited here due to reference restrictions.

Research in P.C. laboratory receives financial support from the Pasteur Institute, INSERM, INRA, ANR (ERANET Infect-ERA PROANTILIS), European Research Council (ERC) (BacCellEpi #670823), the Balzan Foundation and the Fondation le Roch Les Mousquetaires. P.C. is a Howard Hughes Medical Institute Senior International Research Scholar.

References

Bierne, H., Hamon, M.A., Cossart, P., 2012. Epigenetics and Bacterial Infections. Cold Spring Harb Perpect Med. doi:10.1101/cshperspect.a010272.

Biswas, S.K., Lopez-collazo, E., 2009. Endotoxin tolerance : new mechanisms , molecules and clinical significance 475–487. doi:10.1016/j.it.2009.07.009

Carpenter, S., Fitzgerald, K.A., 2015. Transcription of inflammatory genes: long noncoding RNA and beyond. J. Interferon Cytokine Res. 35, 79–88. doi:10.1089/jir.2014.0120

Eskandarian, H.A., Impens, F., Nahori, M.-A., Soubigou, G., Coppée, J.-Y., Cossart, P., Hamon, M.A., 2013. A role for SIRT2-dependent histone H3K18 deacetylation in bacterial infection. Science 341, 1238858. doi:10.1126/science.1238858

Ha, S.-D., Han, C.Y., Reid, C., Kim, S.O., 2014. HDAC8-Mediated Epigenetic Reprogramming Plays a Key Role in Resistance to Anthrax Lethal Toxin-Induced Pyroptosis in Macrophages. J. Immunol. 193, 1333–43. doi:10.4049/jimmunol.1400420

Hamon, M., Quintin, J., 2016. Innate immune memory in mammals. Semin. Immunol.

Lebreton, A., Lakisic, G., Job, V., Fritsch, L., Tham, T.N., Camejo, A., Matteï, P., Regnault, B., Nahori, M., Cabanes, D., Gautreau, A., Ait-si-ali, S., Dessen, A., 2011. A Bacterial Protein Targets the BAHD1 Type III Interferon Response 2, 1319–1321.

Maekita, T., Nakazawa, K., Mihara, M., Nakajima, T., Yanaoka, K., Iguchi, M., Arii, K., Kaneda, A., Tsukamoto, T., Tatematsu, M., Tamura, G., Saito, D., Sugimura, T., Ichinose, M., Ushijima, T., 2006. High levels of aberrant DNA methylation in Helicobacter pylori-infected gastric mucosae and its possible association with gastric cancer risk. Clin. Cancer Res. 12, 989–995. doi:10.1158/1078-0432.CCR-05-2096

Masaki, T., Qu, J., Cholewa-Waclaw, J., Burr, K., Raaum, R., Rambukkana, A., 2013. Reprogramming adult Schwann cells to stem cell-like cells by leprosy bacilli promotes dissemination of infection. Cell 152, 51–67. doi:10.1016/j.cell.2012.12.014

Maudet, C., Mano, M., Eulalio, A., 2014. MicroRNAs in the interaction between host and bacterial pathogens. FEBS Lett. 588, 4140–4147. doi:10.1016/j.febslet.2014.08.002

Monticelli, S., Natoli, G., 2013. Short-term memory of danger signals and environmental stimuli in immune cells. Nat. Immunol. 14, 777–84.

doi:10.1038/ni.2636
Netea, M.G., Quintin, J., Van Der Meer, J.W.M., 2011. Trained immunity: A memory for innate host defense. Cell Host Microbe 9, 355–361. doi:10.1016/j.chom.2011.04.006
Ostuni, R., Piccolo, V., Barozzi, I., Polletti, S., Termanini, A., Bonifacio, S., Curina, A., Prosperini, E., Ghisletti, S., Natoli, G., 2013. Latent enhancers activated by stimulation in differentiated cells. Cell 152, 157–171. doi:10.1016/j.cell.2012.12.018
Pennini, M.E., Perrinet, S., Dautry-varsat, A., Subtil, A., 2010. Histone Methylation by NUE , a Novel Nuclear Effector of the Intracellular Pathogen Chlamydia trachomatis 6. doi:10.1371/journal.ppat.1000995
Rolando, M., Sanulli, S., Rusniok, C., Gomez-Valero, L., Bertholet, C., Sahr, T., Margueron, R., Buchrieser, C., 2013. Legionella pneumophila effector RomA uniquely modifies host chromatin to repress gene expression and promote intracellular bacterial replication. Cell Host Microbe 13, 395–405. doi:10.1016/j.chom.2013.03.004

Figure Legend

Figure 1. Cellular responses to environmental stimuli.

When exposed to extracellular stimuli, such as bacterial factors, host eukaryotic cells mount appropriate responses by adapting their transcriptional programs through short-lived or long-lasting molecular mediators. (**A**) When these mediators are labile, such as protein post-translational modifications (PTM), transient histone modifications, or noncoding RNAs (ncRNAs), there is no memory of stimulation since marks are rapidly removed after resolution of the response. However, when transcriptional mediators are stable, a memory of the initial stimulus is maintained in time. (**B**) For example, LPS stimulation induces phosphorylation of histone H3 on serine 10 (H3S10P) at the IL-1β promoter, which correlates with gene activation (green arrow). Upon restimulation with LPS, tolerance is induced trough deposition of methylation of histone H3 on lysine 9 (H3K9m), correlating with a block of transcription (red arrow). (**C**) Examples of "Trained Immunity". Upon restimulation with β-glucans, a more robust cytokine expression is induced through deposition and maintenance of H3K4Me3 at inflammatory gene promoters. Stimulation with LPS leads to marking of latent enhancers with methylation of histone H3 on lysine 4 (H3K4Me1). Upon restimulation reacetylation of histone H3 on lysine 27 (H3K27ac) and rerecruitment of Pu.1, the macrophage master regulator occur faster than in naïve cells.