

# Genetic reprogramming of host cells by bacterial pathogens

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## Abstract

During the course of infection, pathogens often induce changes in gene expression in host cells and these changes can be long lasting and global or transient and of limited amplitude. Defining how, when, and why bacterial pathogens reprogram host cells represents an exciting challenge that opens up the opportunity to grasp the essence of pathogenesis and its molecular details.

## Introduction and context

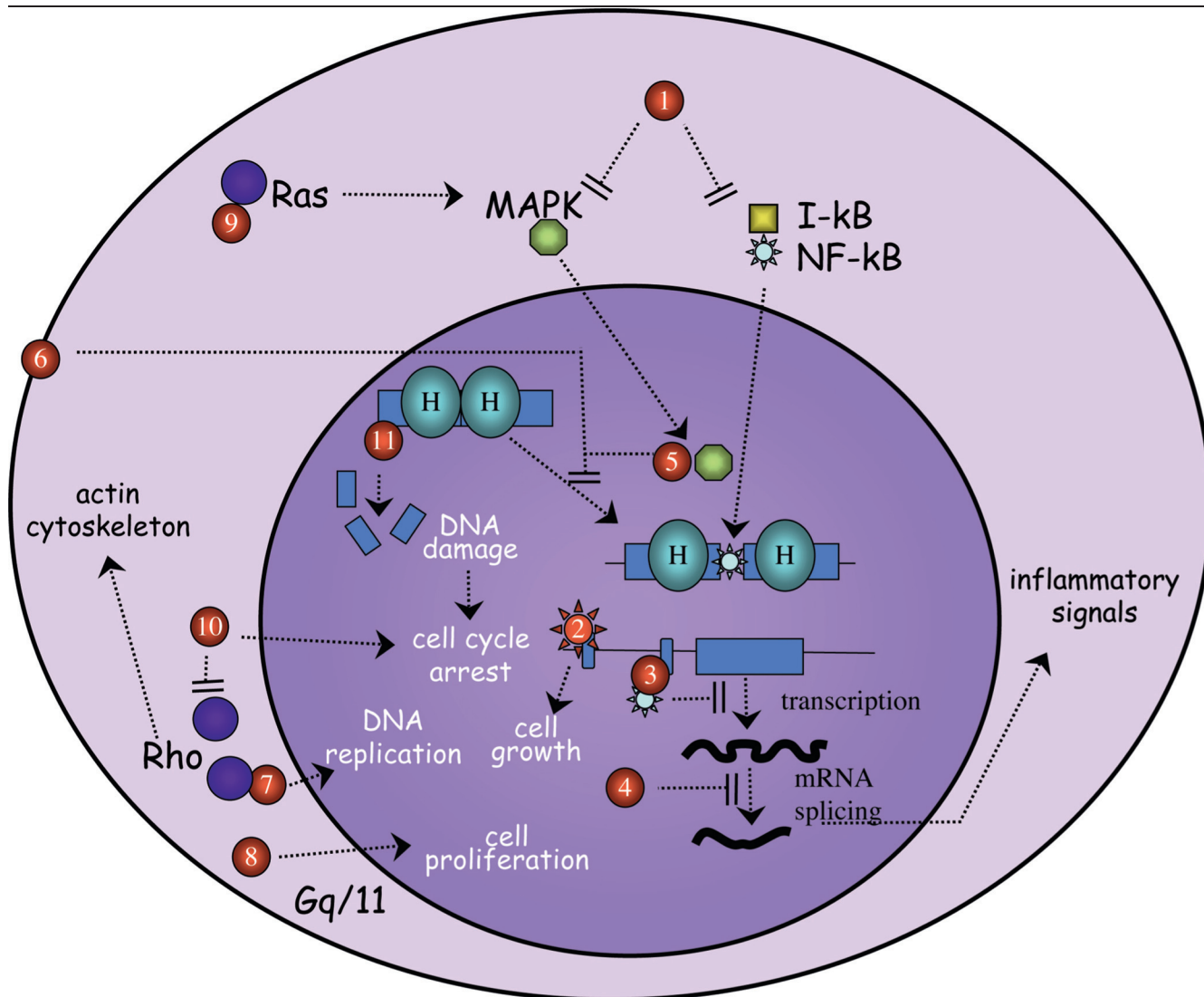
It has been known for decades that various viruses, bacteria, and parasites can manipulate the host cell transcriptome to downregulate the host cell inflammatory response or to promote cell proliferation. With the development of microarray technology and transcriptomic analysis, a plethora of gene expression profiling studies in various pathogen models led to the characterization of Toll-like receptor (TLR)-common and -specific responses to microbial molecular patterns in the early 2000s and of a 'core host response' independent of the cell and pathogen types corresponding to a 'global alarm' to infection [1]. These studies also pointed to the fact that some pathogens affect the host cell genetic program. The large majority of studies report examples of pathogens that downregulate innate immunity. However, many pathogens and virulence factors also affect the cell cycle or induce cell growth.

How do pathogens alter the host genetic program? The various strategies that have been described so far encompass virtually every possible means of classical gene expression manipulation. Bacteria can alter host gene expression by integration of exogenous bacterial DNA in the host genome, as observed in the case of the soil bacterium *Agrobacterium tumefaciens*, which induces neoplastic growth in plants [2]. Obviously, such irreversible manipulation leading to permanent changes in the host cell physiology suggests an intricate

coevolution between the pathogen and the host that is expressed in the context of a slow-progressing pathology at the frontier with symbiosis. Alternatively, plant pathogens have been reported to activate an antibacterial program in plant cells by inducing the synthesis of microRNA, which interferes with auxin signaling [3].

Bacterial virulence factors can act within the cytosol of host cells by altering signaling pathways regulating the transcription of proinflammatory genes. Among the most studied, the *Yersinia* YopJ/YopP, a bacterial factor delivered into host cells by a type III secretion system belonging to the YopJ/YopP/AvrBsT family, has been associated with multiple functions. YopJ/P is a deubiquitinating enzyme that inhibits the activation of nuclear factor-kappa-B (NF-κB) and was also shown to have an acetyltransferase activity that prevents the phosphorylation and activation of mitogen-activated protein kinase (MAPK) (Figure 1, #1) [4-6]. Bacterial type III effectors that translocate into the host cell nucleus may also alter transcription in various ways. The plant pathogen *Xanthomonas* AvrBs3 family proteins are effectors that mimic a host transcriptional activator, which upregulates Upa20, a master cell regulator controlling cell size (Figure 1, #2) [7]. The *Chlamydia trachomatis* CPAF (*Chlamydia* protease activity factor) protein is secreted into the cytoplasm of host cells and is responsible for the degradation of transcription factors required for major histocompatibility complex (MHC)-protein expression or

Figure 1. How bacterial effectors manipulate host cell genetic programs



The *Yersinia* YopJ type III effector can affect gene expression leading to proinflammatory signals by interfering with mitogen-activated protein kinases (MAPKs) and nuclear factor-kappa-B (NF-κB) signaling pathways. Type III effectors that translocate in the cell nucleus can also affect the transcription of proinflammatory genes in various ways. The *Xanthomonas* AvrBs3 family proteins mimic a host transcriptional activator controlling cell size. Various bacterial effectors regulate the cell cycling or cell proliferation. The bacteria effectors are depicted as solid red circles with numbers referenced in the text: 1, *Yersinia* YopJ; 2, *Xanthomonas* AvrBs3; 3, *Xanthomonas* XopD; 4, *Shigella* IpaH9.8; 5, *Shigella* OspF; 6, *Listeria monocytogenes* lysteriolysin O, *Clostridium perfringens* perfringolysin, and *Streptococcus pneumoniae* pneumolysin; 7, *Escherichia coli* cytotoxic necrotizing factors; 8, *Pasteurella multocida* toxin; 9, *Helicobacter pylori* CagA; 10, *Clostridium botulinum* C3 exoenzyme and *E. coli* cycle inhibiting factors; 11, cytolethal distending toxins. H, nucleosome; I-κB, inhibitor of kappa-B.

the HIF1α (hypoxia inducible factor 1α)-dependent response to hypoxia [8,9]. CPAF is also responsible for massive morphological changes and nonapoptotic death of host cells [10]. XopD, another *Xanthomonas* type III effector, is a SUMO (small ubiquitin-like modifier) protease that was reported to bind DNA and to repress defense- and senescence-related plant genes in a process involving its EAR (ERF-associated amphiphilic repression)

motifs suggestive of its association with transcription factors (Figure 1, #3) [7,11]. The *Shigella* type III effector IpaH9.8 was also reported to regulate the activity of the U2AF mRNA splicing factor to downregulate proinflammatory processes (Figure 1, #4) [12]. Hypermethylation of promoters during *Helicobacter pylori* infection of gastric epithelial cells has also been involved in the inhibition of transcription and immunosuppression [13].

## Major recent advances

Recently, with the growing awareness of the importance of epigenetic regulation, there has been considerable interest in the regulation of host gene expression through chromatin remodeling by bacterial virulence factors [14]. The *Shigella* type III effector OspF was shown to prevent histone H3 phosphorylation at serine 10 [15]. This property, associated with the OspF ability to inhibit MAPK activation by virtue of its phosphothreonine lyase activity, would prevent the access of the NF- $\kappa$ B transcriptional activator to specific promoters, such as the interleukin-8 promoter, involved in the mounting of the inflammatory response (Figure 1, #5) [16]. The suppression of the interferon response by *Mycobacterium tuberculosis* was also correlated with chromatin remodeling in a process that involves histone deacetylase activity [17]. Bacterial soluble toxins were also shown to modify chromatin, indicating that pathogens can alter gene expression in a paracrine manner in cells that are not directly infected by bacteria. The pore-forming toxins such as the *Listeria monocytogenes* listeriolysin O, the *Clostridium perfringens* perfringolysin, and the *Streptococcus pneumoniae* pneumolysin induce the dephosphorylation of histone H3 combined with the deacetylation of histone H4, which correlate with reduced transcription of immunity genes (Figure 1, #6) [18].

Various pathogenic bacteria such as *H. pylori* or *Bartonella bacilliformis* promote cell proliferation. Bacterial toxins can also induce DNA replication and cell proliferation (reviewed in [19]). In addition to their effects on the host cell cytoskeleton, the *Escherichia coli* cytotoxic necrotizing factor and *Bordetella* dermonecrotic toxin are deamidases that activate Rho family GTPases, leading to Rho-dependent mitogenic signals and to the formation of multinucleated giant cells (Figure 1, #7). The *Pasteurella multocida* toxin induces cell proliferation in a process that depends on the heterotrimeric protein Gq/11, but independently of its effects on RhoA (Figure 1, #8). The stimulatory role of cell proliferation by these toxins during bacterial infection is unclear. In contrast, there is a possible link with cell proliferation induced by the *H. pylori* CagA type IV effector protein that activates Ras signaling, possibly by directly binding to the growth factor receptor-bound protein 2 (Grb2) adaptor (Figure 1, #9) [20,21]. CagA, however, has been implicated in multiple functions and could also alter epithelial cell polarity to favor bacterial replication in the gastric mucosa [22].

Bacterial toxins can also induce cell cycle arrest. This particular function could represent a strategy to prevent maturation and exfoliation of epithelial cells

to favor bacterial colonization or to alter the integrity of the epithelial barrier. The *Clostridium botulinum* C3 exoenzyme, an acetyltransferase that inhibits the small GTPase RhoA, induces cell cycle arrest in the G<sub>1</sub> phase (Figure 1, #10). The cytolethal distending toxins (CDTs) first reported in *E. coli*, for which orthologs have been reported in various Gram-negative bacteria (including *Shigella dysenteriae*, *Campylobacter* spp., and *Salmonella typhi*), are tripartite (CdtA, B, and C) holotoxins, with CdtB being the active component responsible for cell blockade [23]. Although CdtB is an endonuclease, it is unclear whether this activity is directly responsible for DNA damage. CdtB induces the recruitment and activation of Mre11, a nuclease of the central sensor of DNA double-strand breaks, DNA damage, and chromatin injury leading to cell cycle arrest [24] (Figure 1, #11). Of note, CdtB also harbors a phosphatidyl 3,4,5 inositol phosphatase activity that may also play a role in cell cycle arrest by a mechanism that remains to be clarified [24]. The Cif (cycle inhibiting factor) family of proteins are type III effectors originally reported in enteropathogenic *E. coli*/enterohemorrhagic *E. coli* strains but also found in *Yersinia pseudotuberculosis* and *Burkholderia pseudomallei*, which induce cell cycle arrest in a process involving the stabilization of the cyclin-dependent kinase inhibitors p21<sup>waf1/cip1</sup> and p27<sup>kip</sup> [25,26]. In addition to its role in the delivery of type III effectors into host cells, the *Shigella* protein was shown to bind to Mad2L2, an inhibitor of the anaphase-promoting complex, and thereby to promote cell cycle arrest [27].

## Future directions

*How?* Many bacterial effectors are proteases/actetyltransferases/deubiquitinases, some of them are endowed with multiple activities, and many of them lack identified targets. The question remains as to what the precise nature and spectrum of these targets are. Addressing these issues could allow us to clarify the links between signaling pathways such as those regulating proteasomal degradation, inflammation, cell proliferation, or cell death.

*When?* Efforts should also aim at addressing the function of effectors in cell types that play a role during infection. For example, it is conceivable that toxins that induce cell cycle arrest in epithelial cells also act as immunosuppressive agents by preventing the clonal expansion of lymphocytes, as reported for the fusobacterial immunosuppressive protein A (FipA) [28]. The development of new technologies to visualize, in real time, virulence factor delivery into host cells should provide us with clues for understanding how various effectors 'orchestrate' the inflammatory response.

*Why?* Besides effects on the global host response, there are, however, examples in which a specific cluster of inflammatory genes is targeted by a given pathogen or by pathogens that specifically alter the host response in a given cell type. Such examples are likely to reflect specific features of the pathogen's physiopathological strategy. Also, in some instances, there are seemingly paradoxical findings for the action of bacterial effectors. For example, a hallmark of bacillary dysentery induced by *Shigella* is acute inflammation. It has been thought that bacteria need to elicit an inflammatory program to colonize the colonic mucosa. As presented above, this view is now being challenged by the discovery that many *Shigella* type III effectors appear to downregulate inflammation. This suggests that we are lacking a model that recapitulates critical steps of the infectious process and that efforts need to be made to develop relevant readouts. Recent years have seen the success of what one could call 'molecular mechanistic' of virulence determinants. Along with the molecular dissection of the mode of action of bacterial virulence determinants, getting the right messages from studying genetic reprogramming of host cells by pathogens will obviously imply efforts to integrate the function of these effectors in the infectious context.

## Abbreviations

CDT, cytolethal distending toxin; Cif, cycle inhibiting factor; CPAF, *Clamydium* protease activity factor; EAR; ethylene response factor (ERF)-associated amphiphilic repression; Grb2, growth factor receptor-bound protein 2; HIF1 $\alpha$ , hypoxia inducible factor 1 alpha; MAPK, mitogen-activated protein kinase; MHC, major histocompatibility complex; NF- $\kappa$ B, nuclear factor-kappa-B; FipA, fusobacterial immunosuppressive protein A; SUMO, small ubiquitin-like modifier; TLR, Toll-like receptor.

## Competing interests

The authors declare that they have no competing interests.

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