Pathogenic mechanisms of intracellular bacteria

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Purpose of review
We wished to overview recent data on a subset of epigenetic changes elicited by intracellular bacteria in human cells. Reprogramming the gene expression pattern of various host cells may facilitate bacterial growth, survival, and spread.

Recent findings
DNA-(cytosine C5)-methyltransferases of *Mycoplasma hyorhinis* targeting cytosine-phosphate-guanine (CpG) dinucleotides and a *Mycobacterium tuberculosis* methyltransferase targeting non-CpG sites methylated the host cell DNA and altered the pattern of gene expression. Gene silencing by CpG methylation and histone deacetylation, mediated by cellular enzymes, also occurred in *M. tuberculosis*-infected macrophages. *M. tuberculosis* elicited cell type-specific epigenetic changes: it caused increased DNA methylation in macrophages, but induced demethylation, deposition of euchromatic histone marks and activation of immune-related genes in dendritic cells. A secreted transposase of *Acinetobacter baumannii* silenced a cellular gene, whereas *Mycobacterium leprae* altered the epigenotype, phenotype, and fate of infected Schwann cells. The 'keystone pathogen' oral bacterium *Porphyromonas gingivalis* induced local DNA methylation and increased the level of histone acetylation in host cells. These epigenetic changes at the biofilm–gingiva interface may contribute to the development of periodontitis.

Summary
Epigenetic regulators produced by intracellular bacteria alter the epigenotype and gene expression pattern of host cells and play an important role in pathogenesis.

Keywords
bacterial effector, bacterial virulence, DNA methylation, epigenome, histone modification, pathoepigenetics

INTRODUCTION

Extracellular bacteria grow outside the cells of the infected host, whereas obligate intracellular bacteria enter into cells and use host cell resources for their replication [1]. Furthermore, a growing number of facultative intracellular bacteria can multiply both inside and outside host cells [2]. In addition to structural virulence factors, bacterial effector proteins that interact with the host may also facilitate disease initiation and progression [3,4,5▪▪]. The transport of effectors from the bacterial cytosol into the host cells is typically mediated by translocation complexes called type III, type IV, and type VII secretion systems [6▪–9▪,10].

After entering the host cell, bacterial effector proteins may undergo covalent modification by cellular enzymes and their interaction with their targets may affect key processes of cellular physiology [4,5▪▪]. Various bacterial effectors are located to discrete cellular compartments [5▪▪]. A set of effector proteins is translocated to the nucleus and interacts with the chromatin [11,12▪]. Such effectors, some of them called nucleomodulins, may alter the cellular epigenotype and gene expression pattern [13,14,15▪,16▪▪,17].

Although it is well documented that intracellular bacteria, including *Campylobacter rectus*, uropathogenic *Escherichia coli*, and *Helicobacter pylori* silence host genes by the induction of DNA hypermethylation, the exact mechanisms mediating epigenetic dysregulation by these pathogens has not been clarified [18,19]. Recent studies, however, elucidated the mechanism of *de novo* methylation in

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KEY POINTS

- Bacterial DNMTs target the host cell DNA and alter the host cell methylome.
- Intracellular bacteria may possess an array of epigenetic regulators that target the cellular epigenetic machinery.
- The epigenetic alterations elicited by the same bacterial pathogen depend on the host cell phenotype.
- Epigenetic regulators produced by intracellular bacteria may facilitate bacterial growth, survival, and spread.
- The epigenetic changes induced by intracellular bacteria may serve as diagnostic tools and as targets of epigenetic therapy.

cells infected by *Mycoplasma hyorhinis* and *Mycobacterium tuberculosis* [20▪▪,21,22,23▪▪].

Epigenetic reprogramming of host cells by *Mycoplasma hyorhinis* DNA methyltransferases

Although *Mycoplasma* was considered as extracellular bacteria, their reduced genome may reflect a strictly parasitic lifestyle [24▪]. *Mycoplasma* species either adhere to host cells or invade cells and replicate intracellularly for prolonged periods [25▪,26▪]. *Mycoplasma salivarium* was detected in the epithelial cells of oral leukoplakia, a premalignant condition of the oral cavity, whereas *Mycoplasma hominis* infection was associated with prostate cancer [27,28▪].

Several *Mycoplasma* species including *M. hyorhinis,* encode DNA-(cytosine-5)-methyltransferase (DNMT) enzymes that target cytosine-phosphate-guanine (CpG) dinucleotides, similarly to their mammalian counterparts [20▪▪,29,30]. These enzymes establish the methylation patterns of the bacterial genomes. When expressed in human cells, they are also capable to translocate into the cell nucleus and create aberrant methylation patterns of the host cell DNA [20▪▪,21].

Chernov *et al.* [21] demonstrated that expression of *Mhy1*, a *M. hyorhinis* gene coding for a CpG-specific bacterial DNMT caused genome-wide methylation changes of the host DNA in transfected human fibrosarcoma and trophoblast cells. In parallel, the expression of certain cellular genes was upregulated, whereas another gene set showed a decreased level of transcription. All of these changes resulted in the activation of proliferation-specific pathways. A dual-specific DNMT encoded by *Mhy2* was also active in human cells, it methylated both CpG dinucleotides and cytosines in a non-CpG sequence [21]. A subset of CpG dinucleotides was also modified in human cells by *Mhy3*, a *M. hyorhinis* methylase targeting GATC sequences [21]. 'Reprogramming' of the host epigenome by *Mycoplasma* species may contribute to the initiation or progression of malignant tumours [21,31▪,32].

Alteration of the host cell epigenotype by *Mycobacterium tuberculosis*: noncanonical and classical epigenetic mechanisms

M. tuberculosis, the causative agent of tuberculosis, infects humans by the respiratory route. Although the bacterium is engulfed by alveolar macrophages, its survival strategies, such as the inhibition of phagosome–lysosome fusion, allow intracellular persistence and replication [33,34▪,35▪]. Sharma *et al.* [22] observed that Rv2966c, a secreted protein encoded by the genome of *M. tuberculosis* could enter human monocytic cell line derived from an acute monocytic leukemia patient (THP1) cells. Following nuclear transport, Rv2966c methylated the host cell DNA at cytosines located outside CpG dinucleotides, typically at cytosine-phosphate-adenine and cytosine-phosphate-thymine sites [22]. Methylation at non-CpG sites occurs in stem cells and neoplastic cells in mammals, but it is usually absent from adult differentiated cell types, with few exceptions [36].

Sharma *et al.* [22] argued that non-CpG methylation could persist for a prolonged period in the genome of *M. tuberculosis* infected, nondividing macrophages. Non-CpG methylation in the intronic region of the histone variant gene *H2AFY2* and in the *GRK5* gene encoding a G protein-coupled receptor kinase caused transcriptional repression. Rv2966c could bind to specific DNA sequences and interacted with the activating chromatin marks *H3K4me3* and *H3K36me3* (histone H3 trimethylated at lysine 4 and 36, respectively) [22]. In a follow-up study, Sharma *et al.* [23▪▪] observed genome-wide de novo methylation at non-CpG dinucleotides in THP1 macrophages infected with *M. tuberculosis* strain H37Rv. In addition to hypermethylated regions, hypomethylated regions were also detected. Differentially methylated regions, especially hypermethylated sequences, frequently carried a 28 base pair motif with a conserved 'GCCTCC' core sequence [23▪▪]. The H37Rv-induced changes in the host cell epigenome and transcriptome may establish a favourable environment for the intracellular persistence of the bacterium.

Infection of THP-1-derived macrophages with clinical isolates of *M. tuberculosis*-induced hypermethylation at CpG dinucleotides in promoter sequences of several interleukin receptor genes, including the promoter for interleukin 6 receptor [37▪]. This

epigenetic change may downregulate the level of interleukin 6 receptor protein mediating the effects of the pleiotropic regulator interleukin 6.

In addition to an unusual non-CpG DNA methylase, the genome of *M. tuberculosis* encodes an 'unorthodox' protein arginine methyltransferase, too. Unlike its eukaryotic counterparts that target the long N-terminal tail of histones, the bacterial enzyme Rv1988 dimethylated an arginine residue located to the core region of histone H3 [38]. Rv1988 repressed a series of host defence genes coding for enzymes generating reactive oxygen species and nitric oxide. The expression of tumour necrosis factor receptor-associated factor 3, coding for an ubiquitin ligase of the tumour necrosis factor receptor-associated factor family, was also suppressed [38]. Thus, a bacterial histone arginine methyltransferase may block host defences, possibly promoting thereby pathogen survival.

A classical epigenetic mechanism, promoter silencing by the upregulation of the cellular histone deacetylase 1, was also observed in *M. tuberculosis*-infected macrophages [39]. Silencing of the interleukin-12β promoter was accompanied by the recruitment of *HDAC1* and histone H3 deacetylation. Thus, *M. tuberculosis* may suppress the expression of a key regulator of the T helper 1 response by epigenetic modification [39].

M. tuberculosis may also elicit, however, histone acetylation, because Rv3423.1, a bacterial histone acetyltransferase could be detected in the chromatin of infected macrophages [40▪]. Rv3423.1 generated euchromatic histone marks by acetylating histone H3 at lysine K9 and K14.

In contrast to THP-1-derived macrophages, human monocyte-derived dendritic cells permit only a constrained intracellular survival for *M. tuberculosis* [41]. The cellular epigenome in *M. tuberculosis*-infected dendritic cells was characterized by demethylation at distal enhancer elements and an increased level of activating histone modifications [42]. Transcriptional activation of the immune-related genes *REL*, *CD83*, and *NFKB1* occurred before DNA demethylation [42]. Thus, the epigenetic alterations induced by *M. tuberculosis* are cell type specific, that is, they differ in macrophages and dendritic cells (Table 1).

Identification of epigenetic 'signatures' in granulocytes and monocytes of patients with active tuberculosis and latent *M. tuberculosis* infection may help to monitor the development and progression of tuberculosis [43]. Because epigenetic processes are reversible, microbe-induced epigenetic alterations are potential targets of epigenetic therapy.

Induction of promoter hypermethylation in host cells by an *Acinetobacter baumannii* transposase

The genome of *Acinetobacter baumannii*, a nonoral facultative intracellular bacterium involved in nosocomial infections and periodontitis, encodes a transposase protein that is associated with secreted outer membrane vesicles (OMVs) [44,45]. After delivery via OMVs to host cells, the transposase translocates to the nucleus and acts as a bacterial effector by inducing hypermethylation and silencing of the cellular E-cadherin promoter [45]. Thus, *A. baumannii* may induce epigenetic alterations and downregulation of the tumour suppressor protein E-cadherin in uninfected host cells that take up OMVs. Further studies may reveal the exact mechanism of transposase-mediated CpG methylation.

Epigenetic reprogramming of host cells by *Mycobacterium leprae*, an obligate intracellular bacterium

Intracellular bacteria including *Helicobacter pylori*, *Lactobacillus acidophilus*, and *Mycobacterium leprae* may alter the phenotype and fate of somatic cells

Table 1. *Mycobacterium tuberculosis* induced epigenetic alterations in host cells: recent advances

Effector	Function	Effect	Reference
Rv2966c	DNA-(cytosine-5)-methyltransferase targeting CpA and CpT	Gene silencing in macrophages	[22,23▪▪]
ND	Induction of DNA methylation at CpG dinucleotides	Gene silencing (?) in macrophages	[37▪]
Rv1988	Protein arginine methyltransferase dimethylating histone H3 arginine 42 (H3R42)	Gene silencing in macrophages	[38]
ND	Upregulation of HDAC1 (cellular histone deacetylase)	Gene silencing in macrophages	[39]
Rv3423.1	Bacterial histone acetyltransferase	Gene activation (?) in macrophages	[40▪]
ND	Induction of DNA demethylation and deposition of euchromatic histone marks	Gene activation in dendritic cells	[42]

CpA, cytosine-phosphate-adenine; CpG, cytosine-phosphate-guanine; CpT, cytosine-phosphate-thymine; HDAC1, histone deacetylase; ND, not determined.

[46]. In an experimental model of human leprosy, *M. leprae* infection of mouse primary Schwann cells caused downregulation of the master regulator Sry-related HMG (high mobility group) box 10 in host cell nuclei, possibly because of bacterium-induced, methylation-mediated silencing of the *Sox10* gene [47]. In parallel, the transcription of the *Sox10*-regulated myelin genes decreased, whereas a series of genes involved in epithelial–mesenchymal transition (EMT) were demethylated and activated. Thus, the *M. leprae*-infected Schwann cells acquired the phenotype of progenitor/stem-like cells. Accordingly, they could differentiate into myofibers and smooth muscles both *in vitro* and *in vivo*, a phenomenon that may facilitate the dissemination of bacteria [47,48].

The exact mechanism of *M. leprae*-induced reprogramming remains to be elucidated. We suggest that EMT was initiated by the virulence factor phenolic glycolipid (PGL) which is loosely linked to the outer cell wall layer of the bacterium. PGL can modulate macrophage function by reducing the levels of inflammatory mediators (tumor necrosis factor-α, interleukin 6, and interleukin-1β) released from the activated cells [49▪]. Other candidates for the induction of EMT are the putative ligands binding to Toll-like receptor 4 (TLR4) [50▪]. These molecules comprise lipooligosacharides, proline–glutamate/proline–proline–glutamate proteins that are abundant in pathogenic mycobacteria, and putative *M. leprae* orthologs of TLR4-binding proteins characterized in *M. tuberculosis* [50▪]. The function of a number of proteins encoded by the *M. leprae* genome is unknown at present [51]. Their characterization may reveal further important aspects of *M. leprae*–host cell interactions.

Epigenetic alterations induced by oral bacteria

Polymicrobial communities of oral bacteria regularly form biofilms on surfaces within the mouth [52▪▪]. Dental plaques, the biofilms located to the tooth surface play a crucial role in the development of such highly prevalent oral diseases as caries, gingivitis, and periodontitis [53]. Oral bacteria were implicated in the pathogenesis of oral carcinoma, too [54▪,55▪]. Moreover, it was suggested that *Porphyromonas gingivalis*, the 'keystone pathogen' of periodontitis and *Fusobacterium nucleatum* promote colorectal carcinogenesis [56▪].

P. gingivalis, a low-abundance biofilm species is capable to trigger an inflammatory response and impair, in parallel, various host defence mechanisms [53,57▪,58▪]. These changes allow enhanced replication of other bacteria located to the dental biofilm. The relative abundance of individual members of the bacterial community is also altered, resulting in periodontitis, a dysbiotic disease. Periodontitis is characterized by complement activation, tissue destruction, increased efflux of gingival crevicular fluid, periodontal pocket formation, and alveolar bone loss [59,60▪]. *P. gingivalis* excerts a disproportionately large effect on the structure of the microbial community resulting in the transition of a 'healthy' plaque into a 'pathological' plaque [53,59]. This keystone pathogen coaggregates with other bacteria and alters the gene expression pattern of the microbial community [61,62,63▪,64▪].

In addition to interacting with bacteria located to the dental plaque, *P. gingivalis* also adheres to gingival epithelial cells (GECs) and persists and multiplies intracellularly [65,66▪]. After internalization into early phagosomes the bacterium is protected from immune mechanisms, and by the activation of cellular autophagy, it gets access to host proteins that are utilized as sources of carbon and nitrogen. *P. gingivalis* is able to exit the host cell and reenter into neighbouring cells [66▪]. A recent study demonstrated that a prolonged interaction with *P. gingivalis* increased the methylation of the *TLR2* promoter in GECs [67]. Using a reporter construct, the activity of the methylated *TLR2* promoter was reduced compared with the unmethylated one. Because TLR2 is involved in the recognition of the pathogen and it is indispensable for the upregulation of cytokine and antimicrobial peptide production following bacterial stimulation, methylation-mediated inhibition of *TLR2* expression may result in innate immune dysbiosis manifested as hyporesponsibility of the GECs to *P. gingivalis* [67].

Short-term interaction of lipopolysacharide (LPS) derived from *P. gingivalis* with human oral keratinocytes also induced epigenetic alterations. Martins *et al.* [68] found that the level of acetylated histone H3, a euchromatic mark associated with active promoters, increased quickly following LPS exposure.

Coinfection by *P. gingivalis* and *Filifactor alocis*, a Gram-positive anaerobic rod associated with periodontitis, downregulated the level of histone H1 family members in epithelial cells [69,70]. This may alter the structure of nucleosome and the host cell transcriptome.

In addition to *P. gingivalis*-derived LPS, short-chain fatty acids (SCFAs) secreted by the bacterium may also increase histone acetylation and activate promoters in host cells. Furthermore, SCFAs inhibit histone deacetylase enzymes that remove acetyl moieties from histone tails, and suppress histone N-lysine methyltransferases that create heterochromatic marks at inactive promoters. SCFAs produced by *P. gingivalis* and *Fusobacterium nucleatum* reactivated latent, epigenetically modified herpesvirus genomes:

they switched on the lytic (productive) replication cycle of Kaposi's sarcoma-associated herpesvirus and Epstein–Barr virus (EBV) [71,72]. In HIV- infected individuals activation of Kaposi's sarcoma-associated herpesvirus replication may contribute to the development of oral Kaposi's sarcoma, whereas EBV infection was associated with chronic periodontitis and with the presence of *P. gingivalis* in Japanese patients [73]. In pregnant women, coinfection of EBV and *P. gingivalis* may also facilitate the development of chronic periodontitis [74].

Thus, bacterium–host cell interactions may contribute to the epigenetic reprogramming of host cells at the biofilm–gingiva interface [71]. The altered host cell epigenotype and gene expression pattern as well as viral coinfections amplified by epigenetic regulators of bacterial origin may play a role in the progression of periodontitis [71,72].

Mobilization of chromosomally integrated human herpesvirus 6 genome by *Chlamydia trachomatis*

The termini of the linear viral genomes of human herpesvirus (HHV) 6 and HHV7 consist of repetitive sequences, similarly to the human telomeric hexanucleotide TTAGGG. Both HHV6 and, HHV7 can integrate to subtelomeric chromosomal regions and have the ability to enter the human germline [75▪]. About 0.5% of the population carries silent, chromosomally integrated HHV6 genomes that are transmitted vertically in a Mendelian fashion. Under specific conditions, such as pregnancy or immune suppression, chromosomally integrated HHV6 genomes may be reactivated and become infectious again, causing disease [76,77].

Chlamydia trachomatis, an obligate intracellular Gram-negative bacterium, causes inflammatory diseases. It uses the ephrin receptor tyrosine kinase A2 as a receptor for cellular entry [78]. Strangely enough, this receptor is also used by hepatitis C virus and HHV8 [79,80]. A nuclear effector protein secreted by *C. trachomatis* has histone methyl-transferase activity targeting histones H2B, H3, and H4 (reviewed in [81]). Because *C. trachomatis* is sexually transmitted and is able to interfere with DNA damage response and telomeric damage response signalling, it was implicated in ovarian carcinogenesis [82]. *In vitro*, *C. trachomatis* superinfection is able to mobilize silenced chromosomally integrated HHV6 genomes from their subtelomeric locations via telomeric circle formation [83]. The unexpected interplay between *C. trachomatis* and HHV6 may be of great importance and warrants further experiments.

CONCLUSION

A diverse set of intracellular bacteria causes epigenetic dysregulation in host cells that contributes to disease initiation and progression.

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Conflicts of interest

There are no conflicts of interest.

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