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Highlights

- *L. pneumophila* manipulates the host translational machinery
- *L. pneumophila* manipulates the host transcriptional machinery
- *L. pneumophila* created a novel histone mark to reprogram host gene expression
- *L. pneumophila* uses molecular mimicry of eukaryotic proteins to subvert host functions

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ABSTRACT

Intracellular bacterial pathogens modulate the host response to persist and replicate inside a eukaryotic cell and to cause disease. *Legionella pneumophila,* the causative agent of Legionnaires' disease, is present in fresh water environments and represents one of these pathogens. During co-evolution with protozoan cells, *L. pneumophila* has acquired highly sophisticated and diverse strategies to hijack host cell processes. It secretes hundreds of effectors into the host cell that manipulate many host signaling pathways and key cellular processes. Recently it has been shown that *L. pneumophila* is also able to alter the host's transcriptional and translational machinery and to exploit epigenetic mechanisms in the cells it resides to counteract the host response.

Subversion of the transcriptional and translational machinery by pathogenic bacteria

Pathogens have evolved many different strategies allowing them to persist and replicate in eukaryotic host cells. The regulation of the host's gene expression, at the transcriptional level as well as at the level of mRNA translation is an emerging theme for how intracellular bacterial pathogens may control and alter the host environment for their advantage. They are able to modulate gene expression by interfering with signaling pathways and by directly targeting the transcriptional machinery. During evolution, bacterial abilities were selected to interfere with the pro-inflammatory transcriptional response activated by the cell to recruit phagocytic cells and other components of the immune response to the site of infection. For example *Shigella* spp. secrete type III effectors that modulate nuclear factor κB (NF-κB) activity and mitogen-activated protein kinase (MAPK) activation to reduce inflammation in *Shigella*-infected tissues [1-3]. Other bacteria also target MAPK and NF-κB pathways to manipulate the pro-inflammatory transcriptional response of the host: *Bacillus anthracis* and *Vibrio parahemolyticus*, as well as *Yersinia* spp. block MAPK activation [4-6], whereas *Salmonella typhimurium, Chlamydia trachomatis* or enteropathogenic and enterohemorrhagic *Escherichia coli* target NF-kB pathways [7-9]. Furthermore, pathogenic bacteria not only interfere with signaling pathways regulating transcription factors, but also target the transcriptional machinery directly: *Shigella flexneri* controls gene expression in the nucleus of the host cell by inhibiting the phosphorylation of Ser10 of histone H3 (H3S10) at the promoter of specific genes [10]. Similarly, *Listeria monocytogenes*, *B. anthracis* and *Mycobacterium tuberculosis* induce histone modifications thereby changing chromatin organization at gene promoters [11-14].

More recent studies investigated the role of pathogenic bacteria acting downstream of transcription, by arresting protein synthesis [15]. Host gene translation is a crucial process in the regulation of the innate immune defenses of the host. For example, *Pseudomonas entomophila* globally suppresses protein translation in the gut of its host *Drosophila melanogaster* [16], and exotoxin A (ToxA) from *Pseudomonas aeruginosa* targets elongation factor 2, a component of the host translation elongation machinery [17].

In this review we consider the recent literature that addresses how the intracellular pathogen *Legionella pneumophila,* evades host defenses. We will focus on how *L. pneumophila* hijacks the host's transcriptional and translational machineries to attack and colonize the host cell.

Legionella pneumophila: a paradigm for highly adapted intra-vacuolar pathogens

Legionella pneumophila is an intracellular pathogen and the causative agent of Legionnaires' disease, a severe and atypical pneumonia [18]. *Legionella* are primarily environmental bacteria that replicate intracellularly in aquatic protozoa. Co-evolution with these aquatic hosts led to the acquisition of a pool of virulence traits, which allow *Legionella* to infect lower eukaryotes as well as human cells [19]. *L. pneumophila* disseminates by contaminated aerosols through artificial water systems. The exposure of the lung to water droplets containing bacteria leads to its replication in alveolar macrophages and to the progression of disease.

To proliferate within its hosts, *L. pneumophila* relies primarily on a type IV secretion system (T4SS) known as Dot/Icm system (**Box. 1)** [20,21]. The Dot/Icm T4SS translocates over 300 effector proteins into the eukaryotic host with sophisticated temporal and spatial fine-tuning that allows it to establish a replication-permissive vacuole called *Legionella*-containing vacuole (LCV) (see **Glossary**) [22,23]. Thus, through a complex and well-orchestrated process, the translocated effector proteins permit evasion of the phagosome from the endocytic pathway, recruitment of vesicles from the endoplasmic reticulum (ER) and biogenesis of the LCV [24]. Within the LCV, *L. pneumophila* efficiently replicates until nutrient deficiencies mark the end of the intracellular cycle. This mark may signal *L. pneumophila* to reprogram its gene expression and to synthesize virulence traits that promote host cell lysis, which leads to the scattering of bacteria until they re-establish a replicative niche within a new host [25].

Another particular feature of *L. pneumophila* was discovered through genome analyses. The long-lasting co-evolution of *L. pneumophila* with protozoa has shaped the *L. pneumophila* genome significantly, as it encodes a high number of eukaryotic like proteins and protein domains (see **Glossary)** [26]. The functions which these proteins have in eukaryotic cells, suggest that *L. pneumophila* uses molecular mimicry of eukaryotic proteins as a major virulence strategy [26,27]. These genes are predicted to be acquired through horizontal gene transfer (see **Glossary)** from the protozoan hosts thereby helping *Legionella* manipulate host functions [28]. Indeed, during the last decade, intensive investigations confirmed this hypothesis, and led to the discovery of many *Legionella* effectors targeting conserved eukaryotic pathways to delay the cellular response to invasion and promote the biogenesis of the replication niche [29].

L. pneumophila controls its own replication during the infectious cycle by targeting different cellular pathways and by undermining host-cell functions using eukaryotic-like proteins and effectors that exhibit no similarity with host proteins. After phagocytosis, the bacterium rapidly avoids lysosomal digestion to persist in the infected cell by interfering with the trafficking machinery [24]. Then *L. pneumophila* targets several other pathways, like ubiquitination signaling and autophagy to ensure replication and spreading, but also to evade the host immune response [30,31].

Manipulation of the Induction of the Pro-inflammatory Transcriptional Response

To evade the cellular defense machinery and replicate intracellularly, *L. pneumophila* can sabotage host vesicular trafficking, the ubiquitination machinery, and the autophagy pathway. However, recent studies suggest *L. pneumophila* can employ also other strategies such as manipulation of the pro-inflammatory response. Rapidly after invasion, the cellular surveillance system detects, through pattern-recognition receptors like toll-like receptors (TLRs), bacterial structures such as the surface exposed lipopolysaccharide (LPS) or bacterial DNA-containing methylated CpG motifs. The activation of TLRs induces an anti-microbial response through downstream signal-transduction pathways, in particular the activation of NF-κB and MAPK, leading to increased transcription of proinflammatory cytokines [32,33]. Interestingly, *L. pneumophila* evades detection by TLRs by degrading certain microbial structures that would otherwise bind them. The T4SS effector EnhC, a periplasmic protein, interferes with the degradation of peptidoglycan, allowing bacteria to control the production of pattern recognition receptor ligands and to evade immune recognition by suppressing Nod1-dependent NF-kB activation [34]. Although the induction of a proinflammatory transcriptional response is coordinated by TLR signaling early during infection, its induction in later stages is T4SS-dependent [35]. Analyses of the temporal activation of the NF-kB pathways suggest a biphasic regulation: early activation dependent on TLR5 and MyD88 and a second, later activation independent of patter-recognition receptors, but dependent on the Dot/Icm system [36]. Indeed, strains defective in the Dot/Icm system show very low activation of MAPK and NF-kB pathways [35,37]. In addition to the MAPK and NF-kB pathways, this biphasic activation was observed for the induction of IL-6 and downstream TLR activation [38]. Intriguingly, several Dot/Icm translocated proteins may directly interfere and independently contribute to the activation of these pathways. The eukaryotic-like protein LegK1/Lpg1483, encoding a serine/threonine protein kinase, directly phosphorylates the NF-kB inhibitor IkBα leading to robust NF-kB activation, independent of the IKK (Ikβ kinase) complex [39]. LnaB, another T4SS effector without sequence similarity to known proteins, also strongly activates NF-kB [40]. While no effector directly targeting MAPK proteins is currently known, a recent study identified five effectors that, by inhibiting host translation, contribute to a T4SS-dependent MAPK activation [41] (**Figure 1**).

Thus, *L. pneumophila* is able to modify the proinflammatory signal during human infection, probably to recruit more potential host cells to the site of infection to help its spread. Furthermore, the activation of NF-kB signaling promotes host survival by inducing the transcription of anti-apoptotic genes, which promotes bacterial invasion. Accordingly,

168 microarray analyses revealed an upregulation of genes involved in NF-kB signaling as well as
169 genes with anti-apoptotic functions [37,42]. In addition, two T4SS effectors, SidF and SdhA
170 prevented host cell apoptosis in response to infection, clearly supporting a role for T4SS in
171 hijacking the proinflammatory cascade during infection with *L. pneumophila* [43,44].
172
173 ## Manipulation of the host epigenetic machinery elicited upon *L.*
174 ## *pneumophila* infection
175 The finding that bacterial and viral pathogens may gain epigenetic control of host genes
176 to interfere with key cellular processes and to reprogram defense genes is an emerging topic
177 (see **Box.2, Glossary** and for a review [45] and [46]). The first report that *L. pneumophila*
178 infection leads to histone modifications (see **Glossary**) was published in 2008 when a
179 modification of the histone acetylation of infected cells that was partly dependent on the
180 presence of flagellin was observed [47]. However, the exact mechanism leading to this
181 genome-wide change in histone acetylation is not known yet, although it seems that this
182 modification is to some extent dependent on T4SS effectors. Analysis of the *L. peumophila*
183 genome sequence did not give clues to the effector that might be involved in the acetylation
184 of histone H3 and histone H4, but other proteins that could modify host histones were
185 identified. One of those is a protein that encodes a SET-domain (see **Glossary**) [26]. SET
186 domains occur in a large family of evolutionary conserved proteins, first described within
187 *Drosophila Su(var)3-9* and later in the mammalian homologue SUV39H1 [48]. The 130-
188 amino-acid SET domain harbors enzymatic activity allowing histone lysine methylation.
189 Methyltransferases, together with other enzymes, are responsible for post-translational
190 modifications of the NH_2-terminal tails of histone proteins. Indeed, several SET-domain
191 proteins are described to methylate lysine residues of H3 or H4 histone proteins [49]. Thus,
192 by directly targeting chromatin structure and therein modifying the stability and accessibility
193 of DNA for the transcriptional machinery, methyltransferases alter the gene activity of the
194 eukaryotic cell. Interestingly, after LPS stimulation of the p38 pathway, the chromatin
195 structure was affected for a subset of cytokine and chemokine genes, leading to unmasked
196 NF-kB binding sites [50].
197 The presence of a SET-domain encoding protein in a bacterial pathogen like
198 *L. pneumophila* strongly suggested that it could exploit epigenetic mechanisms of the host.
199 Indeed, the *L. pneumophila* strain Paris protein (Lpp1683/RomA) possesses a very specific
200 and strong histone methyltransferase activity [51]. Similarly, LegAS4/Lpg1718, the
201 homologous protein of strain *L. pneumophila* Philadelphia 1 (Lp02), encodes
202 methyltransferase activity [52]. The exciting question was, which lysine is *L. pneumophila*

targeting as this knowledge may allow to learn which host cell functions of the host cell *L. pneumophila* manipulates. By using antibodies targeting several lysines previously shown to be methylated, researchers analyzed the *in vitro* methyltransferase activity of LegSA4 against core histone proteins and measured an increase of histone H3 Lysine 4 di-methylation, leading to the conclusion that LegSA4 methylates H3K4 [52]. However, more precise mass spectrometry analyses revealed that RomA targets only and specifically lysine 14 of histone H3 (H3K14) and tri-methylates it [51]. H3K4 methylation indeed also slightly influences H3K14 methylation suggesting that it could be part of a motif required for RomA binding to its substrate [51]. This H3K14 specific methylation activity is conserved in *L. pneumophila* as shown by the analyses of seven different strains (Paris, Lens, Philadelphia 1 (Lp02), Corby, Lorraine, HL06041035) [51] and **Figure 2**. Furthermore a genetic screen for *lpp1683* encoding RomA in over 100 *L. pneumophila* strains of different origin and sequence types revealed 100% amino acid conservation among them, further underlining its importance during *L. pneumophila* infection (unpublished data). Since H3K14 methylation had never been reported in mammalian cells before, the findings of RomA and its activity prompted many new questions like *(i)* what is the origin of the SET-domain conferring a specificity for a different lysine of histone H3 and *(ii)* what are the gene targets of H3K14 methylation.

In depth phylogenetic analyses of the origin of the SET-domain suggested a eukaryotic origin, but also showed that this protein undergoes an accelerated evolution. Thus, the ability of RomA to impose a seemingly new epigenetic mark on the host cell may have evolved after the horizontal acquisition of a SET-domain from a eukaryotic host protein targeting another histone residue, which had adapted during evolution to a new target, Lysine 14 of histone H3. Alternatively, this histone mark may have been overseen in mammalian cells, and analysis of *L. pneumophila* infection discovered a currently undescribed mechanism of gene regulation in mammalian cells [51]. Thus RomA is *(i)* the first *Legionella* T4SS effector that directly targets the nucleus, and *(ii)* the first bacterial protein that produces a new epigenetic mark on the eukaryotic chromatin landscape (**Figure 1**).

Remarkably, the H3K14 tri-methylation by this bacterial effector strongly decreased the acetylation of the same residue, a well-known marker of transcriptional activation [53,54]. Acetylation of lysine residues of the NH_2-terminal tail of histone proteins is the result of a fine-tuned equilibrium between histone acetyl-transferase (HDAC) and histone deacetylase (HAT) activities. They control the acetylation status at the promoter level to regulate the transcription of defined genes [55]. Thus, stable and covalent modification of this residue by a specific bacterial effector would result in a winner strategy to hijack the HDAC/HAT

equilibrium permanently and stably downregulate target gene expression. Indeed, ChIP-seq analyses revealed that RomA activity leads to a genome wide epigenetic modification of the methylation status of H3K14, targeting over 4000 genes [51]. Furthermore, it was reported that the homologous protein encoded by strain Philadelphia 1 (Lp02) specifically targets the host nucleolus and shows specificity for rDNA promoters through a direct binding to HP1α/γ, a major constituent of heterochromatin [52]. This results is however at odds with previously published work showing that HP1α/γ binding is likely linked to methylation at H3K9 (H3K9Me) [56,57]. With these different results many questions remain, but they also open the way for a new field in *Legionella* research: analyses of T4SS effectors that directly target the nucleus of the infected host and modify the chromatin landscape to control the host transcriptional response directly.

Manipulating the host translational machinery

The role of inhibiting the host translation machinery during infection by pathogens has become a new field of research in the past few years [58]. Indeed, *L. pneumophila* is also able to directly inhibit the translational activity of the host cell it infects. To date, five *L. pneumophila* effectors have been characterized that inhibit the host translation machinery (Lgt1, Lgt2, Lgt3, SidI and SidL/Ceg14). Lgt1, Lgt2 and Lgt3 are glycosyltransferases that modify the mammalian elongation factor eEF1A and block host translation both *in vitro* and *in vivo* [59,60]. The fourth effector, SidI, while not a glycosyltransferase, also targets the elongation factor eEF1A, as well eEF1Bγ, leading to the inhibition of protein synthesis in *L. pneumophila* infected cells and the induction of the host stress response [61]. The fifth effector, SidL/Ceg14 shows protein translation inhibition *in vitro* [62,63]. Analysis of the global effects of Lgt1, Lgt2, Lgt3, SidI and SidL/Ceg14 in correlation with the transcriptional response of the cell during *L. pneumophila* infection revealed that the inhibition of the host protein synthesis is critical for the induction of the innate immune response [62]. This global decrease in host translation prevents the synthesis of the NF-kB inhibitor IkB and shapes the transcriptional profile of the innate immune response through MAPK activation in the host [41] (**Figure 1**). Furthermore, it has recently been suggested that SidL/Ceg14 modulates the host cytoskeleton [63]. As targeting the host cytoskeleton induces the immune response [64,65], SidL/Ceg14 may further contribute in a specific manner to the immune induction upon *L. pneumophila* infection.

A recent study, however, found that the observed translational suppression was triggered by an effector protein–independent pathogen-detection pathway [66]. Ubiquitination of the Akt protein resulted in the down-regulation of mTOR activity. Given that the mTOR

pathway also regulates translation by inactivating a translation negative regulator, 4E-BP1 [67], a decrease in its activity during *L. pneumophila* infection resulted in a reduced translation activity, which in turn promoted cytokine biasing. By using a Δ5less mutant, which has a chromosomal deletion of all five effectors previously identified, the authors convincingly demonstrated that translational suppression was indeed triggered by an effector protein–independent pathogen-detection pathway [66]. This work provides new insight in the emerging concept that host gene translation is a crucial cellular process targeted during bacterial infections, while also being an important regulator of the innate immune defense of the host. However, the presence of an effector-independent effect suggests that protein translation inhibition is an unspecific mechanism as mammalian cells are not the natural host of *L. pneumophila* The immune response observed upon *Legionella* infection may thus rather be a cell response than a direct targeting by the bacteria and *Legionella* is a wonderful tool to reveal the mechanisms of these recognitions.

Moreover, these findings add to the understanding of the complex and tightly regulated control that bacteria impose on the cell. *L. pneumophila* is not only capable of controlling the transcriptional response of the host cell at the nuclear level by modulating signaling pathways and epigenetic modifications, but also at the translational level by directly regulating the abundance of mRNA produced by the cell in response to the bacterial invasion.

Concluding remarks

The pathogenesis of infection is a constantly evolving battle between the host and the pathogen. *L. pneumophila* is a paradigm for intracellular pathogens teaching us many lessons. This bacterium needs to replicate inside the host cell to be transmitted to another one and therefore it manipulates the host by establishing a fine balance between the immune response and infection [68]. *L. pneumophila* has adopted many global and parallel strategies to intercept and modulate the immune response by hijacking key cellular processes. How does *L. pneumophila* achieve this? A particular feature of *L. pneumophila* is that it encodes a very high number of proteins in its genome that exhibit structural and functional mimicry of eukaryotic proteins that reflect the diversity of eukaryotic pathways that are exploited by this bacterium during infection of phylogenetically diverse eukaryotic hosts [26,27]. Indeed, many of these proteins encode activities of eukaryotic cells and *L. pneumophila* secretes them to manipulate host pathways for its own advantage. Partly based on the identification of these eukaryotic like proteins, much progress has been made in characterizing novel mechanisms by which this pathogen regulates the transcriptional and translational machineries. Thus,

L. pneumophila targets key cellular processes allowing it to gain control over the host response. Undoubtedly, more processes will be uncovered over time.

Analyzing one of these eukaryotic like proteins showed recently that *L. pneumophila* directly targets the host cell nucleus where it modifies its epigenetic landscape to impact the regulation of host cell transcription [51,52]. These findings are the beginning of nuclear microbiology, a new field of research in *Legionella* that will answer many remaining questions of how *L. pneumophila* manipulates its hosts as diverse as protozoa and human macrophages. Future studies will focus on whether *Legionella* sets other epigenetic marks in the host epigenome through specific eukaryotic enzymes acquired during evolution. The analyses of the epigenetic profile of the infected cell will give new insight into the extent of epigenetic modifications induced by *L. pneumophila*. Furthermore, other bacterial effectors might be identified that play an important role in the transport of these enzymes to the cell nucleus. One promising candidate was recently identified, the T4SS effector LegG1/*lpg1976*, a RanGTPase activator that was shown to play a role in nucleo-cytoplasmic transport [69,70]. Thus, LegG1 may, by affecting cellular processes regulated by Ran, regulate the nuclear transport of *Legionella* nucleomodulins like RomA. With the rapid development of new techniques and an increase in their sensitivity, comprehensive analysis of diverse genome-wide modifications combined with transcriptional profiling, and analyses of translational regulation induced at different stages of infection will allow important insight into how *Legionella* attacks a host cell as well as the host's response. Importantly, these crucial cellular processes seem to be tightly associated with the host's innate immune defenses.

L. pneumophila does not only possess very diverse effectors for controlling the host response, but it also possesses an incredibly high number of over 300 effectors that interestingly show important redundancy. Thus deleting single or even multiple effectors often has no or little impact on its capacity to multiply in eukaryotic hosts [71]. To overcome this phenomenon, a method called insertional mutagenesis and depletion (iMAD) was recently developed to define sets of *Legionella* proteins that employ redundant virulence mechanisms [72]. This phenomenon may be due to the presence of highly related effectors with similar functions, probably a result of gene duplications that occurred by divergent evolution, or due to the presence of effectors that target similar cellular processes but use different enzymatic activities. Furthermore, multiple variants of one effector may have evolved in different host environments. However, effector redundancy may also exist at the level of two different effector proteins mediating similar processes through completely independent pathways. Many pathogens multiply strategies to target a single event in the disease process [73]. Thus it will be essential to gain a better understanding of the single

contribution of each bacterial effector to the disruption of a cellular pathway and to learn how they work in concert with respect to their spatial-temporal regulation. These endeavors will allow us to understand how *L. pneumophila* is able to exert a quasi global control over the cellular responses of the eukaryotic host cell.

Figure legends:

Figure 1: Modulation of transcriptional and translational processes by *L. pneumophila*. A) After entry and avoidance of the endocytic pathway, *L. pneumophila* establishes a safe replicative niche, the *Legionella*-containing vacuole (LCV). **B)** *L. pneumophila* targets the transcriptional host machinery both in a T4SS-dependent and -independent manner. Independent of T4SS, TLR is activated, leading to a MyD88 dependent activation of NF-kB and MAPK pathways, inducing a specific regulation of immune defense genes in the nucleus of infected cells. In parallel, in a T4SS-dependent manner, the secreted effector RomA targets the host chromatin to methylate Lysine 14 of histone H3 to down-regulate gene transcription genome-wide **C)** *L. pneumophila* targets the translational host machinery both in a T4SS-dependent and -independent manner. Through inhibition of the Akt/mTORC1 signaling pathway, *L. pneumophila* decreases host translation initiation. In parallel, specific T4SS secreted effectors inhibit translation elongation.

Figure 2. Lysine 14 methylation of Histone H3 (H3K14) upon infection of alveolar epithelial cells is conserved in different *L. pneumophila* strains. The A549 cell line was infected with seven *L. pneumophila (L.p.)* and one *L. longbeachae* strain and their H3K14 methylation activity was analyzed. Each *L. pneumophila* strain encodes a homologous SET-domain containing protein and each strain methylates H3K14 in the host cell. *L. longbeachae* does not encode a SET-domain protein and accordingly no methylation of H3K14 was observed. After 8 hours of infection, cells were fixed in 4% paraformaldehyde, pemeabilized with PBS-triton 0.1% and stained with 4-6-diamidino-2-phenylindole (DAPI) (Invitrogen; cyan blue), anti-LPS (red), anti-H3K14me (Euromedex, H3-2B10; green) and Alexa Fluor633-phalloidin (Invitrogen). Scale bar, 10μm. Strains tested are: Paris (accession number: NC_006368), Philadelphia (NC_002942), Philadelphia 1 (Lp02) (Study accession number: SRP020472 [74]), Lorraine (NC_018139), (NC_013861), Corby (NC_009494), Lens (NC_006369), HL06041035 (NC_018140) and *Legionella longbeachae* strain NSW150 (NC_013861).

Glossary

***Legionella*-containing vacuole (LCV):** specialized compartment derived from the endoplasmic reticulum (ER) that avoids fusion with the lysosome and contains replicating bacteria.

Eukaryotic-like proteins: bacterial proteins containing domains preferentially found in eukaryotes or proteins having a higher similarity to eukaryotic proteins than to bacterial ones.

Horizontal Gene Transfer (HGT): transmission of DNA between chromosomes of different bacterial strains, species or even between organisms belonging to different domains of life.

Epigenetic: literally, *epi-* (Greek: *επί-* over, outside of, above) and genetics. It refers to changes and controls of gene activity without alterations of the DNA sequence, often altering the physical structure of DNA.

Histones: proteins constituting the basic structural unit of chromatin, the nucleosome. Two dimers of histones H3, H4 H2A and H2A form octamers around which DNA is wrapped to form the nucleosome.

SET domain: (suppressor of variegation, enhancer of zest and thiotorax) protein domain encoding lysine methyltransferase activity.

Box 1. *L. peumophila*'s type IV secretion system

Legionella transfers bacterial proteins into eukaryotic host cells through a type IV secretion system (T4SS), a membrane-associated multiprotein secretion apparatus encoded by the *dot/icm* genes related to type IV secretion systems present in other bacteria [20,21]. T4SSs, restricted to Gram-negative bacteria, are specialized machineries that span the bacterial outer membrane and the plasma membrane of the cell into which the secreted proteins or the DNA are delivered [75-77].

L. pneumophila utilizes T4SS to translocate a large number of substrates, which are predicted to manipulate host cell processes to promote intracellular replication, into the host cell. Over the last about 10 years, more than 300 Dot/Icm-translocated proteins have been identified by using multiple approaches: *(i)* genome analyses and genetic screens [71]; *(ii)* yeast two-hybrid screens using *icm* genes as bait [78]; *(iii)* machine learning methods with considerably high prediction accuracy [79] and *(iv)* searching for genes essential for specific host cellular activities [80,81]. Moreover, chemical genetics revealed that effector translocation involves host cell factors to initiate a close contact event for the translocation of pre-synthesized effector molecules [82].

An intriguing aspect of the multi-step organization of the secretion system is the fine-tuning of timely secretion and specific localization of each effector during cell invasion. Indeed, once delivered into the eukaryotic cell, effector localization is predicted to be important in regulating its activity. Some are associated to the LCV like SidC and its paralogue SdcA, both of which have been shown to bind to phosphatidylinositol 4-phosphate present on the LCV membrane [83]. Other effectors are targeted to different regions of the cell directly according to the host functions they modulate. For instance RomA, a SET-domain encoding methyltransferase, is directed to the host cell nucleus, where it specifically targets histone proteins [51]. In addition to effector spatial regulation, their expression is tightly regulated. Genes encoding several effectors have been shown to be up-regulated at the transition of *L. pneumophila* growth from the exponential phase to the stationary phase, revealing that their expression and protein production is tightly regulated allowing the bacteria to quickly invade the host cell [84]. Moreover, it may be possible that host responses also influence reprogramming of the T4SS function during infection.

Box 2. Epigenetic targeting by bacterial pathogens

Understanding how pathogens induce chromatin modifications to affect the host is an emerging topic. For years, researchers focused on interfering with cellular pathways to understand the mechanisms used to modify host gene expression; however, it has become increasingly evident that some pathogens translocate epigenetic modulating factors, recently called “nucleomodulins” [85].

The epigenetic modification, strictly speaking, involves a heritable status, maintained by a positive feedback loop during cell division (see **Glossary**). It is thus possible that a bacterial infection generates heritable marks in host cell behavior, becoming associated with malignancy. A well-known example is *Helicobacter pylori* infection, resulting in chronic gastritis and cancer [86]. *H. pylori* induces aberrant DNA methylation of promoter CpG islands, leaving epigenetic imprints in infected cells and permanent changes in gene expression [87,88]. Even though some microbes that cause persistent infections and are associated with malignancy often benefit from heritable epigenetic changes, most of them induce epigenetic modifications in a “broader” sense as an inheritable, additional information superimposed on the DNA sequence. *Listeria monocytogenes*, *Shigella flexneri*, *Bacillus anthracis*, *Mycobacterium tuberculosis*, *Porphyromonas gingivalis*, as well as *Helicobacter pylori, induce* signaling pathways leading to histone modifications, mostly to elude the immune response and, in general, the host surveillance processes [46]. In particular, modulation of histone acetylation seems to be a common feature of bacterial infections, especially on host defense gene promoters [11,89].

Some pathogens encode enzymes that directly modify the epigenetic landscape: *(i) Clamydia pneumonia* encodes a SET domain protein that methylates murine histone H3 *in vitro* [90]; *(ii) Clamydia trachomatis* SET-domain containing protein called NUE targets the mammalian cell nucleus and methylates histone H2B, H3 and H4 *in vitro* [91] and *(iii)* RomA of *Legionella pneumophila* tri-methylates histone H3 on Lysine 14 *in vitro* and *in vivo* to decrease its acetylation status therewith reducing transcriptional activity [51].

Moreover, genome sequence analysis has revealed that a significant number of bacteria have SET domain encoding genes [92]. Future studies will help to characterize their enzymatic activity; however, it is also possible that they methylate non-histone substrates [93]. In addition, new enzymatic domains, derived from eukaryotic histone modifying enzymes, might be identified in the genomes of bacterial pathogens.

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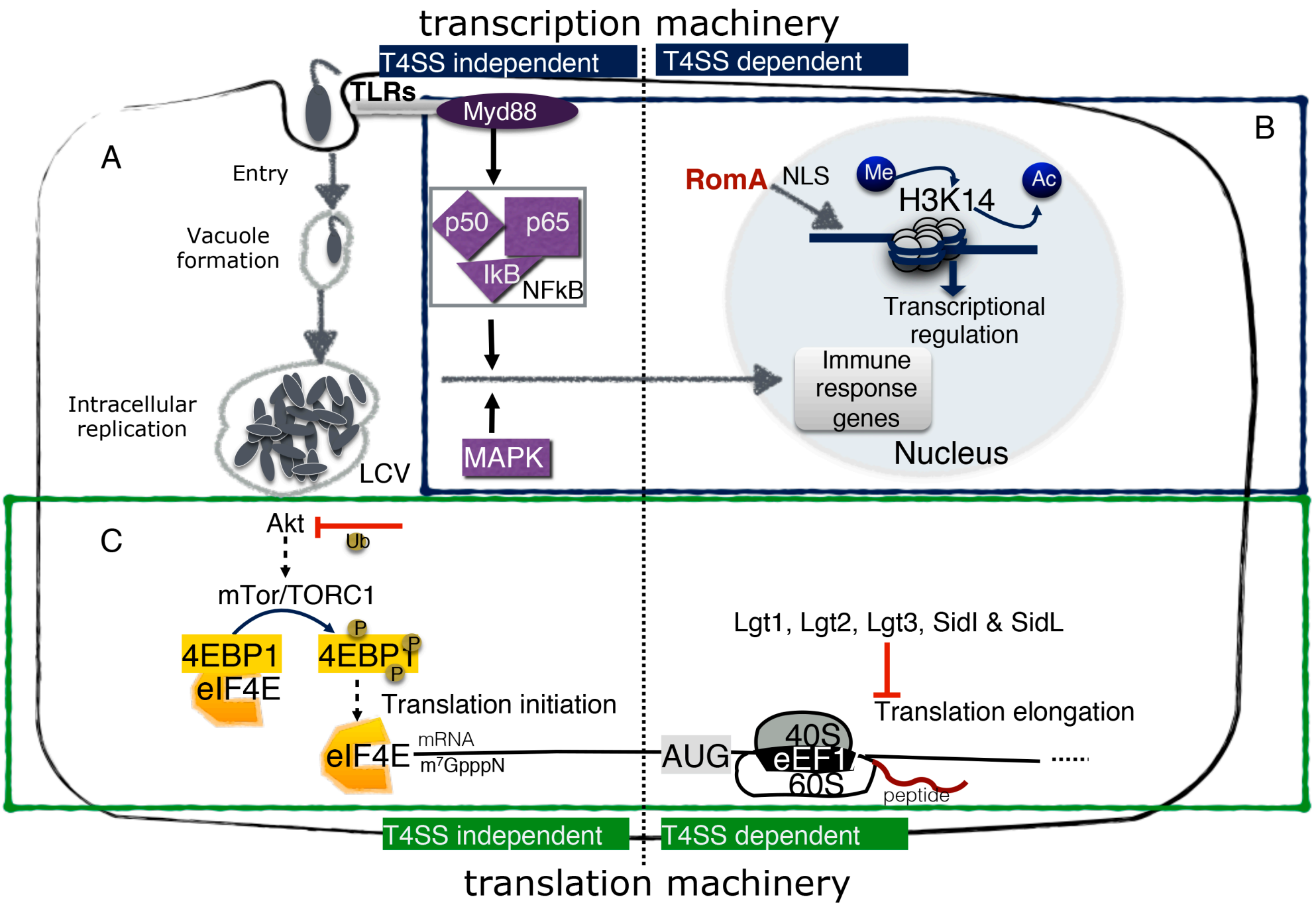
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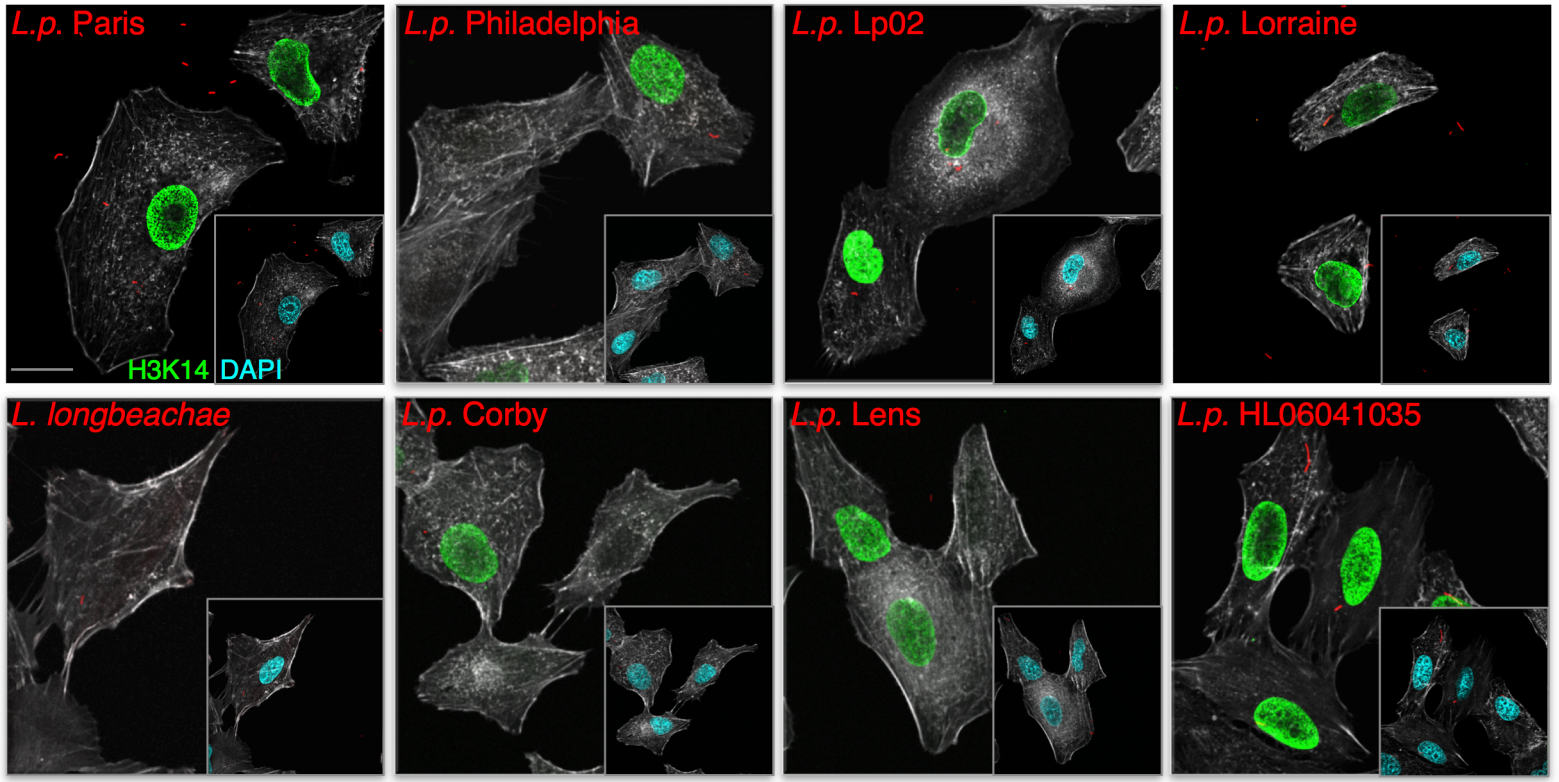
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