

Transcriptional regulators which participate in the regulation of *Legionella pneumophila* pathogenesis determinants

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Abstract

Legionella pneumophila is a Gram-negative bacterium and the causative agent of the Legionnaires' disease, a serious respiratory infection. As an intracellular pathogen, it invades and replicates within alveolar macrophages and freshwater protozoa using its primary virulence machinery, the Icm/Dot type IVB secretion system. This system facilitates infection by translocating over 300 effector proteins into host cells, where they modulate various cellular processes to support bacterial survival and replication. The effector-encoding genes (EEGs) are regulated by a complex regulatory network that detects environmental cues and ensures the effectors timely expression. Several global regulatory systems directly control the expression of roughly 120 EEGs in *L. pneumophila*. The main regulators include the PmrAB and CpxRA two-component systems, the LetAS-RsmYZ-CsrA regulatory cascade, and the Fis1 and Fis3 repressors. Additionally, the global regulator Fur contributes to the regulation of a single effector gene. Beyond these global regulatory systems, certain EEGs are regulated by local regulators. These include the copper-responsive LciRS two-component system, which controls one adjacent EEG located within the same genomic island; RegK3, a LuxR-family regulator responsible for the expression of two neighboring EEGs; and LelA, a LysR-type transcriptional regulator (LTTR) that regulates three EEGs, one adjacent and two located elsewhere in the genome. These local regulators are found in only a subset of *Legionella* species, are often acquired through horizontal gene transfer, and typically control one or two adjacent EEGs, although in some cases they also regulate a small number of distantly located targets.

The first chapter of this study describes two novel LysR-type transcriptional regulators, LelB and LelC, which function as local regulators of EEGs in *L. pneumophila*. Bioinformatic analysis, expression assays, site-directed mutagenesis, and in vitro binding experiments led to the discovery that in *L. pneumophila*, LelB controls the expression of three EEGs, and LelC controls the expression of a single EEG. Moreover, we discovered that LelB is a versatile regulator that can serve either as a global or local regulator of EEGs. The LelB orthologs in the *Legionella* genus were divided into three clades, but only in two of them, LelB is located adjacent to its target genes and functions as a local regulator. In the third clade, which contained five *Legionella* species, the validated LelB regulatory element was identified in multiple genes. Examination of this collection of genes uncovered that most of them harbor common effector domains and other known effector features. Furthermore,

examination of some of these genes from one of these species, *Legionella longbeachae*, indicated that LelB regulates their expression. Lastly, the expression of both LTTRs was shown to be positively regulated by the RpoS sigma factor, while LelB expression was also found to be repressed by the Fis1 and Fis3, thus connecting them to the extended effector regulatory network. In summary, the results reveal that LelB functions as a local regulator of EEGs in several *Legionella* species and as a global regulator of EEGs in other *Legionella* species.

In the second chapter, we identify and characterize a LuxR-type regulator, LexR1, that was found to control the expression of EEGs in *L. pneumophila* and other *Legionella* species. The *L. pneumophila* LexR1 ortholog was shown to directly activate the expression of four EEGs by binding to a conserved inverted-repeat element located upstream of these genes. Activation of these target genes was found to depend not only on the levels of LexR1 but also on the presence of casamino acids, indicating that LexR1 is activated in response to a nutrient-derived ligand found in casamino acids. Consistently, several LexR1 orthologs in other *Legionella* species exhibited the same casamino acid-dependent activation of their respective target EEGs. Comparative genomic analysis revealed nearly 30 genes across seven *Legionella* species that contain the LexR1 regulatory element, most of which encode validated effectors or proteins containing common effector domains. Notably, some of these genes were shown to overlap among the seven species and appeared to be co-transferred with their regulatory sequences through horizontal gene transfer. Finally, LexR1 expression was shown to be positively regulated by the RpoS sigma factor and repressed by Fis repressors, integrating it into the broader effectors regulatory network. Overall, these findings establish LexR1 as an EEGs local regulator that links nutrient sensing to effector gene expression across the *Legionella* genus.

Collectively, the two chapters of this study describe three novel local regulators of EEGs, thus doubling the number of known members of this group. This expansion enabled a comprehensive comparison, allowing us to define both the shared characteristics and the distinct features of all six local regulators that control EEGs expression in *L. pneumophila* and other *Legionella* species. The increasing number of identified local transcriptional regulators of EEGs, along with their integration into the broader effectors regulatory network, highlights the complexity and interconnectivity of the pathogenesis regulatory network in *L. pneumophila*.

