Allosteric mutants show that PrfA activation is dispensable for vacuole escape but required for efficient spread and Listeria survival in vivo.

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The transcriptional regulator PrfA controls key virulence determinants of the facultative intracellular pathogen Listeria monocytogenes. PrfA-dependent gene expression is strongly induced within host cells. While the basis of this activation is unknown, the structural homology of PrfA with the cAMP receptor protein (Crp) and the finding of constitutively activated PrfA* mutants suggests it may involve ligand-induced allostery. Here, we report the identification of a solvent-accessible cavity within the PrfA N-terminal domain that may accommodate an activating ligand. The pocket occupies a similar position to the cAMP binding site in Crp but lacks the cyclic nucleotide-anchoring motif and has its entrance on the opposite side of the β-barrel. Site-directed mutations in this pocket impaired intracellular PrfA-dependent gene activation without causing extensive structural/functional alterations to PrfA. Two substitutions, L48F and Y63W, almost completely abolished intracellular virulence gene induction and thus displayed the expected phenotype for allosteric activation-deficient PrfA mutations. Neither PrfA(allo) substitution affected vacuole escape and initial intracellular growth of L. monocytogenes in epithelial cells and macrophages but caused defective cell-to-cell spread and strong attenuation in mice. Our data support the hypothesis that PrfA is allosterically activated during intracellular infection and identify the probable binding site for the effector ligand. They also indicate that PrfA allosteric activation is not required for early intracellular survival but is essential for full Listeria virulence and colonization of host tissues.
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