The c-AMP Receptor-like Protein CLP is a Novel c-di-GMP Receptor Linking Cell-cell Signaling to Virulence Gene Expression in *Xanthomonas campestris*

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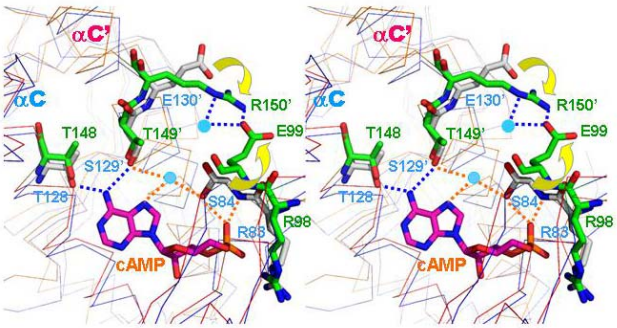
C-di-GMP controls a wide range of functions in eubacteria, yet little is known about the underlying regulatory mechanisms. In the plant pathogen *Xanthomonas campestris*, expression of sub-set of virulence genes is regulated by c-di-GMP and also by the CAP-like protein *Xc*CLP, a global regulator in the CRP/FNR superfamily. Here, we report structural and functional insights into the interplay between *Xc*CLP and c-di-GMP in regulation of gene expression. *Xc*CLP bound target promoter DNA with sub-μM affinity in the absence of any ligand. This DNA-binding capability was abrogated by c-di-GMP, which bound to *Xc*CLP with μM affinity. The crystal structure of *Xc*CLP showed that the protein adopted an intrinsically active conformation for DNA binding. Alteration of residues of *Xc*CLP implicated in c-di-GMP binding through modeling studies caused a substantial reduction in binding affinity for the nucleotide and rendered DNA binding by these variant proteins insensitive to inhibition by c-di-GMP. Taken together, the current study reveals the structural mechanism behind a novel class of c-di-GMP effector protein in the CRP/FNR superfamily and indicates that *Xc*CLP regulates bacterial virulence gene expression in a manner negatively controlled by the c-di-GMP concentrations

C-di-GMP is an important novel secondary messenger involved in modulating a variety of biological activities in eubacteria. In the plant pathogen *Xanthomonas campestris* pv. *campestris* (*Xcc*), c-di-GMP is implicated as a second messenger controlling expression of pathogenicity genes in response to the extracellular diffusible signal factor DSF. DSF signal transduction involves the hybrid sensor kinase RpfC and the response regulator RpfG.. DSF perception by RpfC is believed to lead to its autophosphorylation and subsequent phosphorelay to RpfG. RpfG is unique in that it contains no DNA-binding domain, but an HD-GYP domain that exhibits phosphodiesterase activity capable of degrading c-di-GMP to GMP. Phosphorylation is thought to activate RpfG for c-di-GMP degradation. In this way RpfC/RpfG link perception of the cell-cell signal DSF to alteration in the cellular level of c-di-GMP. What is not clear however is how alteration in c-di-GMP levels is coupled to the downstream pathogenicity gene expression that is under DSF control.

Recently, a number of studies have strongly suggested that *Xc*CLP plays a key role in the DSF-mediated regulatory pathways in *Xcc*; knockout of this protein in *Xcc* significantly alters the expression profile of a number of pathogenicity factors such as exopolysaccharide, extracellular cellulase, and polygalacturonate lyase. The mechanistic link between *Xc*RpfG and *Xc*CLP in pathogenicity gene regulation has, however, remained obscure.

In this manuscript, we report the crystal structure of *Xc*CLP determined by X-ray crystallography and show that it belongs to the CRP/FNR superfamily. We also show that *Xc*CLP is unique in this superfamily because it is intrinsically adapted for DNA binding without the need for any ligand. Furthermore, *Xc*CLP is found to be released from DNA binding when incubated with c-di-GMP in competition EMSA and SPR assay, indicating that c-di-GMP acts as a negative regulator for downstream pathogenic gene expression via changing *Xc*CLP conformation. *Xc*CLP was also shown to directly bind c-di-GMP with a K_D of approximate 3.5 μM by an ITC experiment. *Xc*CLP is thus likely to be one of the long sought-after c-di-GMP receptor proteins in the DSF-mediated pathways.

Figure below shows the close-up of important nucleotide binding sites of *Xc*CLP compared to those in *Ec*CRP/cAMP.



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