Structural Basis for the Activity and Allosteric Control of Diguanylate Cyclase

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Recent studies suggest that a novel second messenger, cyclic d-GMP (c-diGMP), is extensively used by bacteria to control multicellular behaviour. This cyclic dinucleotide is synthesised by the diguanylate cyclase (DGC) domain in a reaction that converts two GTP into one c-diGMP and two pyrophosphates. The DGC domain contains a highly conserved GG(D/E)EF sequence motif, and occurs in various combinations with sensory / regulatory domains in bacteria.

We have identified the response regulator, PleD, from *Caulobacter crescentus* as a diguanylate cyclase [1] and have solved its crystal structure in complex with c-diGMP to 2.7 Å [2]. PleD consists of a receiver domain D1 with a phosphorylation site, a receiver-like domain D2, and an effector domain DGC. In the structure, PleD forms a homodimer mediated by D1-D2 interactions. The DGC domain has a similar fold as the catalytic domain of adenylate cyclase but has an active site that reveals different nucleotide binding. The guanine base of c-diGMP is hydrogen bonded to Asn335 and Asp344, while the ribosyl and α-phosphate groups extend over the β2-β3 hairpin that carries the sequence motif. Interestingly, the c-diGMP molecule crosslinks two symmetrically arranged DGC domains from adjacent dimers. We propose that activation of PleD through phosphorylation leads to dimerisation, which allows the two DGC domains of a dimer to align symmetrically for c-diGMP synthesis.

Two intercalated c-diGMP molecules are bound to the domain interface between D2 and DGC. This allosteric binding site explains the observed non-competitive product inhibition. We propose that PleD inhibition is effected by DGC domain immmobilisation to the D1-D2 stem.

[1] Paul R., Weiser S., Amiot N., Chan C., Schirmer T., Giese B., Jenal U., *Genes Dev.,* 2004 **18**, 715. [2] Chan C., Paul R., Samoray D., Amiot N.C., Giese B., Jenal U., Schirmer T., *Proc. Natl. Acad. Sci.*, 2004, **101**, 17084.

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