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Domain Arrangement of Der, a Switch Protein Containing Two GTPase Domains

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Beamline(s): X4A

Introduction: The EngA subfamily of essential bacterial GTPases has a unique domain structure consisting of two adjacent GTPase domains (GD1 and GD2) and a C-terminal domain. *E. coli* der is an essential gene required for cell viability (for review see ref. 1).

Methods and Materials: The structure of *T. maritima* Der was solved at 1.9 angstrom resolution by single wavelength anomalous diffraction (SAD) using selenium. Initial phasing attempts relying on two ordered endogenous methionine residues, proved unsuccessful until 5 additional methionine substitutions were engineered into the protein at positions predicted to be on the internal faces of amphipathic helices. These mutations greatly improved the size, mosaicity and diffraction limits of the subsequently grown selenomethionine crystals.

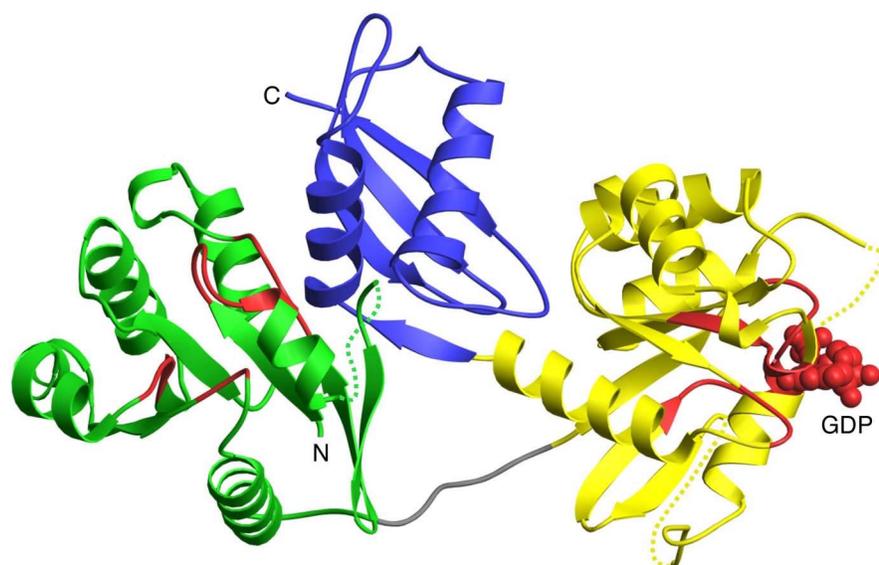
Results: The structure of *Thermotoga maritima* Der bound to GDP was determined at 1.9 angstrom resolution. (2) The structure revealed a novel domain arrangement in which the GTPase domains pack at either side of the C-terminal domain, arranged in a cloverleaf shape. Unexpectedly, the C-terminal domain resembles a KH domain, missing the distinctive RNA recognition elements. Conserved motifs of the nucleotide-binding site of GD1 are integral parts of the GD1-KH domain interface, suggesting the interactions between these two domains are directly influenced by the GTP/GDP cycling of the protein. In contrast, the GD2-KH domain interface is distal to the GDP-binding site of GD2.

Conclusions: This structure provides the first description of a GTPase containing multiple G domains and establishes a scaffold for ongoing studies aimed at characterizing both intra and intermolecular regulation of this essential bacterial protein.

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References:

1. J. Hwang and M. Inouye, M. 2001 "An Essential GTPase, Der, Containing Double GTP-Binding Domains from *Escherichia coli* and *Thermotoga maritima*," J. Biol. Chem. **276**, 31415-31421.
2. V. Robinson, J. Hwang, E. Fox, M. Inouye and A. Stock "Domain Arrangement of Der, a Switch Protein Containing Two GTPase Domains," Structure In Press.



Ribbon diagram of *T. maritima* Der. Loops that form the guanine nucleotide binding sites in the two G domains are highlighted in red. A GDP molecule bound in the second G domain is shown in red as a space-filling model.