EM and X-ray Studies of HupR, A Response Regulator from the NtrC Family

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HupR, a member of the NtrC response regulator family, enhances transcription of a membrane-bound hydrogenase in response to environmental change. It is a 53kDa protein composed of 3 domains: An N-terminal receiver domain, a central putative AAA+ ATPase domain, and C-terminal DNA binding domain. Regulation of transcription occurs via a two-component signalling pathway resulting in the phosphorylation of the receiver domain. In most NtrC-like proteins, phosphorylation causes the oligomerisation of the central domain, which activates ATP hydrolysis and promotes interaction with the σ^{54} RNA polymerase (RNAP). Transcription is initiated using the energy released from ATP hydrolysis.

HupR is an unusual member of the NtrC family, as phosphorylation inhibits HupR-dependent transcription. We have calculated a low-resolution structure of the full-length protein using electron crystallography. This model shows HupR crystallised as a dimer. The volume is half that expected suggesting only part of the protein was imaged. We believe this part is the central domain. In addition, 3D crystals of the receiver domain $\pm BeF_3^-$ have been obtained. Preliminary results show the domain has the classic receiver fold with an extended $\alpha 5$. The protein forms a weak dimer which is strengthened by BeF_3^-. The dimer interface involves $\beta 5$ & $\alpha 5$.

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