MS9 Enzyme reactions and dynamics in crystals

Chairs: Gunter Schneider, Arwen Pearson

MS9-P1 CckA regulation by c-di-GMP: how to steer a bacterial histidine kinase into the phosphate mode

Badri N. Dubey[1], Urs Jenal[2], Tilman Schirmer[1]

1. Focal Area of Structural Biology and Biophysics, University of Basel, CH-4056 Basel, Switzerland.
2. Focal Area of Infection Biology, University of Basel, CH-4056 Basel, Switzerland.

email: b.dubey@unibas.ch

Cyclic diguanosine monophosphate (c-di-GMP) is a ubiquitous signaling molecule coor- dinating, amongst others, bacterial development and behavioural programs[1]. Recently, we have shown that histidine kinase CckA from Caulobacter crescents is regulated by c-di-GMP in that it inhibits its kinase and stimulates its phosphatase activity[2]. Thus, the phosphorylation state of CtrA, the ultimate target of the CckA signaling pathway and a master transcription factor controlling cell replication, is controlled indirectly by the cellular c-di-GMP concentration.

In order to unravel the molecular mechanism of c-di-GMP induced reversal of CckA activity, we have biophysically (MALS, ITC) and structurally investigated various con- structs of CckA. Interestingly, in the full-length context CckA binds c-di-GMP with low micro-molar affinity only in presence of ADP and not of ATP, whereas, the isolated cata- lytic domain (CA) binds c-di-GMP with medium affinity irrespective of the mononucleo- tide state. Thus, c-di-GMP binding appears to dependent on the functional state (do- main constellation) of the enzyme and, in this way, stabilize the ADP complexed form.

Crystal structures of the CA domain in complex with c-di-GMP/ATP and of the DHp-CA enzyme core in complex with ADP have been determined. The first structure shows c-di-GMP bound with one of its guanine bases to a specific binding pocket on the CA domain, whereas the second base is not involved in any interactions. In the context of the DHp-CA double domain structure, the second base would come to lie close to helix 1 of the DHp domain suggesting c-di-GMP mediated cross-linking. Taken together, we propose that c-di-GMP binding stabilizes a domain constellation, which allows access and dephosphorylation of the cognate receiver domain and, at the same time, prevents formation of the auto-kinase constellation.

References:

1- Romling U, Galperin MY, Gomelsky M. Cyclic di-GMP: the first 25 years of a universal bacterial second messenger. Microbiol Mol Biol Rev. 2013; 77(1):1-52.

2- Lori, 2- C; Ozaki, S; Steiner, S; Böhm, R; Abel, S; Dubey, B N; Schirmer, T; Hiller, S; Jenal, U (2015). Cyclic di-GMP acts as a cell cycle oscillator to drive chromosome replication. Nature. 2015; 523, 236- 239.

Keywords: Bi-functional enzyme, Histidine kinase, c-di-GMP, signalling, Crystal structure