Regulation of the AAA+ protein ClpC by adaptor proteins

Dissertation

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1. Gutachter: Prof. Dr. K. Turgay
2. Gutachter: Prof. Dr. R. Hengge

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Parts of this study have been published in the following journals

Original articles

1. **Kirstein J**, Dougan DA, Gerth U, Hecker M & Turgay K
   The tyrosine kinase McsB is a regulated adaptor protein for ClpCP
   EMBO J. 2007 Apr 18;26(8):2061-2070.

2. **Kirstein J**, Schlothauer T, Dougan DA, Lilie H, Tischendorf G, Mogk A, Bukau B, Turgay K.
   Adaptor protein controlled oligomerization activates the AAA+ protein ClpC.
   EMBO J. 2006 Apr 5;25(7):1481-91.

3. Andersson FI, Blakytny R, **Kirstein J**, Turgay K, Bukau B, Mogk A, Clarke AK.
   Cyanobacterial ClpC/HSP100 protein displays intrinsic chaperone activity.
   J Biol Chem. 2006 Mar 3;281(9):5468-75.

4. **Kirstein J**, Zuhlke D, Gerth U, Turgay K, Hecker M.
   A tyrosine kinase and its activator control the activity of the CtsR heat shock repressor in *B. subtilis*.
   EMBO J. 2005 Oct 5;24(19):3435-45.

Review article

Kirstein J, Turgay K.
A new tyrosine phosphorylation mechanism involved in signal transduction in *Bacillus subtilis*.
J Mol Microbiol Biotechnol. 2005;9(3-4):182-8. Review.

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Summary

The main objective of my PhD thesis was the analysis of the functional relationship of the HSP100/Clp protein ClpC with its adaptor proteins. ClpC is not only involved in the removal of misfolded and aggregated proteins, but also controls, through regulated proteolysis, key steps of several developmental processes in the Gram-positive bacterium *Bacillus subtilis*. ClpC differs from other members of the HSP100 family in that it requires an adaptor protein for virtually all its activities.

A variety of molecular biological, biochemical and biophysical methods were employed to address the mechanistic and functional interplay of ClpC and its adaptor protein network.

(1) It could be demonstrated that the activation of ClpC is based on the adaptor mediated assembly of the ClpC hexamer and that the oligomerized complex constitutes the functional and substrate binding species of this chaperone complex.

(2) It could be shown that the formation of the whole proteolytic complex, ClpCP, depends on the preceding adaptor mediated assembly of the ClpC oligomer. Once assembled, hexameric ClpC facilitates the oligomerization and thereby activation of the otherwise monomeric proteolytic component, ClpP. Thus, ClpCP mediated proteolysis appears to be regulated in a hierarchic mode governed by an adaptor protein.

(3) A functional characterization of a ClpC homolog from a photobiontic organism revealed that the adaptor dependent activation is not a common feature among the ClpC homologs. Thus, ClpC of *B. subtilis* exhibits a unique characteristic even within the ClpC family.

Besides the activation of ClpC, adaptor proteins also fulfill a substrate recognition role enabling the specific degradation of a huge variety of substrate proteins by ClpCP. The substrate spectrum of ClpCP includes key regulators such as ComK, ComS, SpoIIAB, CtsR and MurAA. The wide range of ClpC substrates argues for a substantial number of ClpC adaptors to allow a specifically controlled degradation. The cognate adaptor protein for one of these regulatory proteins, CtsR, could be identified and thus (4), adding McsB to the adaptor protein network of ClpC. McsB could be characterized as tyrosine kinase, exhibiting adaptor properties only in its kinase-on state. The kinase activity is crucial for the regulation of the class III heat