6 Summary

This thesis deals with the characterization of the activation mechanism of the small GTPase Cdc42 by the exchange factor SopE from *Salmonella typhimurium* as well as with the regulation of the p21-activated protein kinase αPAK by Cdc42.

SopE is essential for *Salmonella* invasiveness. It binds directly to Cdc42 and activates it in a Dbl-like fashion. However it has no sequence similarity with these Rho-specific eukaryotic exchange factors.

The catalytic domain of SopE (SopEc, aa 78-240) was isolated and crystallized in a complex with its Rho-target protein Cdc42 (aa 1-178). The crystals diffracted up to 2.3 Å. The x-ray structure of the SopEc-Cdc42 complex could be solved with a combination of molecular replacement and multiple isomorphous replacement including anomalous scattering contribution (MIRAS). The three dimensional structure of Cdc42 shows the classical G-domain, which is found in almost all GTP-binding proteins. Conformational changes are mainly limited to the switch regions.

SopEc is a V-shaped protein formed by two α-helical bundles. It interacts with the switch regions of Cdc42 and stabilizes the nucleotide free state of the GTPase. The conformation of the switch regions is strikingly similar to that of Rac1 in complex with the eukaryotic Dbl-like exchange factor Tiam1. However SopEc has an entirely different architecture than Tiam1 and interacts with its Rho-target protein via different amino acids. Therefore, SopE represents the first example of a non-Dbl-like protein capable of inducing guanine nucleotide exchange in Rho family proteins.

The p21-activated protein kinases (PAKs) are involved in signal transduction processes. They become activated by interaction with GTP-bound Rho-type small GTP-binding proteins Cdc42 and Rac1, thereby relieving the inhibition of the regulatory domain (RD) on the catalytic domain (CD) of PAK.

To investigate the mechanism of activation of αPAK by GTP-binding proteins, fragments of the GST-fusion proteins were proteolytically produced. The proteolytic digestion of αPAK with chymotrypsin produces a heterodimeric RD-CD complex consisting of a regulatory fragment (aa 57-200) and a catalytic fragment (aa 201-491), which is active in the absence of Cdc42. Fluorescence titration demonstrated that Cdc42-GppNHp binds with low affinity ($K_D = 0.6 \mu M$) to the intact kinase, whereas the affinity to the isolated regulatory fragment is much higher ($K_D = 18 nM$). The full-length kinase, the isolated RD, and surprisingly also their complexes with Cdc42 behave as dimers on a gel filtration column. Cdc42-GppNHp interaction with the RD-CD complex is also of low affinity and does not dissociate the RD from the CD. After autophosphorylation of the kinase domain, Cdc42 binds with high (14 nM) affinity and dissociates the RD-CD complex. Assuming
that the RD-CD complex mimics the interaction in native PAK, this indicates that the small G protein does not simply release the RD from the CD domain and activates the latter. It acts in a more subtle allosteric control mechanism to induce autophosphorylation, which in turn induces the release of the RD and thus full activation.