Pleckstrin domains: from allosteric regulation of protein function towards novel tools for monitoring intracellular reactions (Weiterführung)

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Pleckstrin homology (PH) domains play a major role in lipid-mediated protein translocation and signaling events in proteins. Pleckstrin itself becomes activated upon phosphorylation by protein kinase C (PKC). During the previous funding period a FRET-based sensor (KCP-1) was developed that was able to monitor PKC activity in living cells. However, the structural basis for the conformational alterations linked to the FRET changes still needs to be demonstrated. In order to optimize KCP-1 for NMR-studies and its perfomance in cells, a reduction in size and an improvement of the FRET change to above 100% needs to be achieved. The dual parameter FRET probe KCAP-1 that permits the independent recording of PKC- and PKA-induced phosphorylation in cells opens the way to an entire platform of reporters useful for the intracellular dissection of signaling pathways and for screening. Efforts shall be continued to prepare fluorescently labeled, membran-permeant phosphoinositide derivates to enable monitoring of phospholopid-PH domain interaction by FRET. Furthermore, photoactivatable derivates of phosphoinositide will be added to the toolbox. The interaction of fluorescently labeled phosphoinositide with cytoskeletal PH domains from the Rho-GEF obscurin will be studied by using NMR and FRET.

Projektbeteiligte

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