Abstract

Epithelial cell polarization is required for establishment and maintainance of the integrity of tissues and organs. A variety of PDZ domain containing proteins are known to be crucial for the regular distribution of proteins to the basolateral or apical membrane domain. Disruption of these processes may cause diseases such as inflammation or tumor metastasis.

This work presents a first characterization of the FERM and PDZ domain containing protein FRMPD2. It could be shown that the protein is localized to the basolateral membrane of epithelial cells where it co-localizes with proteins of adherens and tight junctions. The FERM domain of FRMDP2 interacts with PtdIns(3,4)P₂ and is required for membrane localization whereas the interaction of the PDZ2 domain with the armadillo protein p0071 mediates basolateral restriction of the protein. Furthermore, recruitment of FRMPD2 to sites of cell/cell contact is E-Cadherin dependent and knockdown of FRMPD2 in Caco-2 cells is associated with an impairment of tight junction formation. In addition, MDCKII cells stably expressing EGFP-FRMPD2 show an increased migration rate in Boyden chamber experiments. According to the results, FRMPD2 may functionally be involved in adherens and tight junction formation as well as cell migration processes.

This work also provided novel insights in the functional role of the protein tyrosine phosphatase PTP-BL whose modular structure resembles closely to the FRMDP2. PTP-BL is suggested to play a role in recycling of the Fas receptor, the regulation of the actin cytoskeleton and during cytokinesis. Here, the interaction of the FERM domain of PTP-BL and SDCCAG3, a protein involved in the recycling of the TNF receptor, was characterized in detail and a role for SDCCAG3 in the regulation of cytokinesis was revealed. A stretch of 25 amino acids within SDCCAG3 was shown to be necessary and sufficient to bind to PTP-BL and further mapping identified several aminoacids within this site to be crucial for interaction. Furthermore, a vesicular distribution of SDCCAG3 was detected in HeLa cells in addition to a strong localization at the midbody during cytokinesis. Overexpression as well as down-regulation of SDCCAG3 led to the formation of multinucleated cells, a phenotype similar to the one detected for overexpression of PTP-BL.

Additionally, the characterization of the interaction of SDCCAG3 and the ArfGAP protein GIT1 revealed two binding sites within GIT1 with the N-terminus of SDCCAG3. First results suggest that GIT1 may also be involved in the regulation of cytokinesis, because knockdown or overexpression of an ArfGAP-deficient mutant led to multinucleated cells, however, the detailed molecular basis remains unclear. In summary, the data from this work provides a
possible regulation of cytokinesis through a complex of the scaffolding protein PTP-BL together with SDCCAG3 and GIT1.

In another part of this work an analysis of the Rab-binding properties of the PtdIns-5-phosphatase OCRL1 was performed in collaboration with the Max-Planck-Institute for Molecular Physiology in Dortmund. An OCRL1 mutation occurring in Lowe syndrome was characterized using a crystal structure of the C-terminal part of OCRL1 in complex with Rab8a and further biochemical methods. The OCRL1 point mutation F668V resulted in decreased affinity to Rab proteins which is likely due to the reduced hydrophobicity of valine compared to phenylalanine according to the crystal structure. Furthermore, compared to the wildtype protein which is localized at the Golgi apparatus and endosomal structures, OCRL1\textsubscript{F668V} showed an increased cytosolic localization in HeLa cells. In contrast to previously studied OCRL1 variations the pointmutation F668V is so far the only Lowe syndrome mutation which selectively affects binding to Rab proteins, while the interaction with the endosomal protein APPL1 remains unchanged. The results indicate that binding to Rab proteins is an important regulatory mechanism for proper function of OCRL1.

In summary, this work presents new insights into the role of different proteins which are involved in cell polarity. FRMPD2, which influences adhesion processes in epithelial cells, as well as PTP-BL and OCRL1, which regulate vesicular trafficking, are involved in asymmetric distribution of proteins and lipids within the cell.