S3-4

Identification of genes determining intestinal intermediate filament organization in Caenorhabditis elegans

Hüsken K.[1], Wiesenfahrt T.[2], Überbach D.[2], Davis M.W.[3], Jorgensen E.M.[3], Windoffer R.[1], Bossinger O.[2], Leube R.E.[4]
[1]Department of Anatomy and Cell Biology, Johannes Gutenberg-University Mainz, Mainz, Germany, [2]Institute for Genetics, Heinrich-Heine-University Düsseldorf, Düsseldorf, Germany, [3]HHMI and Department of Biology, University of Utah, Salt Lake City, United States of America, [4]Department of Molecular and Cellular Anatomy, RWTH Aachen, Aachen, Germany

Intermediate filament proteins (IFs) constitute one of the three major cytoskeletal filament systems. They mainly function in maintaining mechanical integrity of cells and are highly abundant in epithelia. Yet, mechanisms of IF formation and organisation in living cells and organisms are still poorly understood. We therefore constructed C. elegans strains expressing fluorescent intestinal IFs. These fluorescent polypeptides label the subapical terminal web region of the rigid intestinal endotube. An optical screen was performed after EMS mutagenesis to search for alterations of the characteristic periluminal fluorescence pattern. In this way two types of non-allelic mutants were identified presenting either multiple bubble-shaped distortions of the intestinal lumen or a rearranged IF cytoskeleton with cytoplasmic granules and prominent junctional localization. We found structurally and functionally intact apical junctional complexes in both types of mutants indicating that the altered distribution patterns are not a consequence of disturbed epithelial polarity. Efforts are under way to identify the mutated gene loci by SNP-mapping. Taken together, our results show that C. elegans IF-reporter strains are valuable tools for the identification of gene products determining IF organization.

katrin.huesken@uni-mainz.de

S3-5

Identification, localization and expression of rice OsKCH1, a novel plant-specific kinesin

Frey N.[1], Nick P.[1]
[1]University of Karlsruhe, Institute of Botany I, Karlsruhe, Germany

Kinesins are ATP-driven microtubule motor proteins that play important roles in intracellular transport and control of microtubule dynamics. Animal kinesins are well characterized. Molecular properties of plant kinesins, however, are only poorly understood so far. We have identified a novel plant-specific kinesin, OsKCH1, from rice. OsKCH1 harbours a C-terminally located motor core containing a microtubule binding domain. The presence of a typical signature neck peptide upstream of the motor core indicates OsKCH1 to be a minus-end directed kinesin. Due to a unique calponine homology (CH) domain at its N-terminus, OsKCH1 can be classified into the KCH-group of CH-domain containing kinesins, a distinct branch of the minus-end directed Kinesin-14 subfamily. KCHs have to date only been found in higher plants and have been associated with microtubule and actin binding. OsKCH1 was found to be expressed in roots, leaves and shoots of 6 days old rice seedlings. Localization studies with transiently expressed YFP-fusions of both the N-terminal region of OsKCH1 including the CH-domain, and the putative motor core in tobacco BY-2 cells and rice seedlings show that the motor domain decorates microtubules. Additionally, by recombinant expression of the corresponding His-fusions in E.coli and affinity purification, this non-canonical kinesin has become accessible for biochemical analysis.

nicole.frey@bio.uni-karlsruhe.de

S3-6

The influence of infections with alpha-cardiac actin mutants on the cytoskeleton of neonatal rat cardiomyocytes by adenoviral vectors.

Mazur A.J.[1], Littwitz C.[2], Mannherz H.G.[1]
[1]Ruhr-University Bochum, Department of Anatomy and Embryology, Bochum, Germany, [2]Ruhr-University Bochum, Department of Physiology, Bochum, Germany

We tested the effect of the alpha-cardiac actin mutants Tyr166Cys and Met305Leu on the cytoskeleton of isolated neonatal rat cardiomyocytes (NRCs) by transfection. For this purpose we prepared adenoviral vectors containing the cDNAs of wild-type and mutant actins containing a N-terminal HA-tag (hemagglutinin). 72h after infection of NRCs with the adenoviral constructs the cells were fixed and stained with either TRITC-phalloidin or several antibodies directed against proteins building focal adhesions such as integrin beta 1, paxilin, talin, vinculin and alpha-actinin and with IgGs recognizing components of intercalated discs like alpha-catenin, plakoglobin, and desmoplakin. In contrast to data obtained from transfections of non-cardiomyocytes, we observed no differences between the WT actin and the Tyr166Cys mutant after transfection into NRCs. Instead, we found that the infected NRCs were able to incorporate the exogenous actins into existing myofibrils and sarcomers. Data will be also presented on the effect of adenoviral infections on focal adhesions.

antonina.mazur@rub.de