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Identification of the Diaphanous-Related Formin FHOD1 as Regulator of Nuclear Migration and Post-Mitotic Cell Spreading

Promotionsfach: Infektiologie
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Diaphanous-related formins are effectors of Rho-GTPases, which are required for actin nucleation as well as elongation and capping of actin filaments. Besides remodeling of the actin cytoskeleton, several DRFs have a dual function in microtubule based cytoskeletal processes, by stabilization, orientation or co-alignment of MTs with F-actin. Important cellular processes which require formin activity are cell motility, polarity, division and regulation of gene expression. DRFs are characterized by the GTPase binding domain (GBD) and the formin homology (FH) domains FH1 and FH2, which mediate actin binding and nucleation, respectively. The activity of DRFs is controlled by intramolecular interaction between the N-terminal Diaphanous inhibitory domain (DID) and the C-terminal Diaphanous regulatory domain, which is released upon Rho-GTPase binding and additional triggers.
FH1/FH2 domain-containing protein 1 (FHOD1) is a DRF family member which differs from the other 14 DRF family members in structural and functional aspects. First, the N-terminal GBD and DID domains are not well defined in FHOD1. As the FHOD1 N-term contains an extended linker loop and a ubiquitin superfold of unknow function. Second, despite missing intrinsic actin nucleation activity of its FH2 domain, FHOD1 forms thick actin filaments by bundling of preformed actin filaments via lateral side binding to preformed actin filaments. All knowledge about FHOD1 functions derives from experiments with over-expressed FHOD1 or by biochemical assays with purified FHOD1. Relevant cellular functions of endogenous FHOD1 had not been reported at the starting point of this thesis. The general goal of this thesis was to define FHOD1 functions by characterization of its interaction with protein ligands.
The first part of this thesis aimed to investigate the functional interplay between FHOD1 and Aurora B in post-mitotic cell spreading in co-operation with the Lindon lab. FHOD1 was shown to be phosphorylated by Aurora B at multiple serine and threonine residues by *in-vitro* kinase assay and mass spectrometry. Phosphorylation was most abundant in a small cluster of

five serine and threonine residues within the coiled coil domain of FHOD1. *In-vitro* kinase assay with FHOD1 after mutating the serine threonine cluster to alanine (FHOD1-5A) revealed that these five residues are most relevant for FHOD1 *in-vitro* phosphorylation by Aurora B. Rescue assays with over-expressed FHOD1-5A after depletion of endogenous FHOD1 resulted in perturbed post-mitotic cell spreading. IN contrast a phosphomimetic FHOD1-5D mutant efficiently rescued cell spreading, which correlated with the requirement for active Aurora B in this context. These observations indicate that FHOD1 is required for daughter cell spreading by organizing and mediating crosstalk between MFs and MTs at the cell cortex, under control of Aurora B.

In the second project, a yeast two hybrid screen for novel FHOD1 interacting proteins identified an N-terminal fragment of the outer nuclear envelope protein nesprin-2G (Giant). Nesprin-2G is the largest isoform of nesprin-2 which contains an N-terminal CH domains which binds to actin filaments. In addition to the N-terminal CH domain that binds to actin filaments. In addition, nesprin-2G contains an N-terminal KASH domain which links nesprin-2G to the nuclear membrane. The long rod-shaped, flexible part of nesprin-2G between CH domains and KASH domain is unstructured and contains many spectrin repeats (SRs).

Nesprin-2G containing filaments linked to dorsal actin cables, referred to as transmembrane actin-associated nuclear (TAN) lines attach the nucleus to retrogradely moving actin cables to promote rearward nuclear movement. Rearward nuclear movement together with the process of centrosome centration leads to centrosome re-orientation with the centrosome being localized between the nucleus and the leading edge of the cell.

In co-operation with Gregg Gundersen`s lab (Columbia University, NYC, USA) the interaction of FHOD1 with nesprin-2G was reconfirmed and mapped to the N-term of FHOD1 (aa1-339) and the N-terminal spectrin repeats SRs 11-13 of nesprin-2G. FHOD1 was shown to be required for nuclear migration by knockdown with siRNA. In line with missing intrinsic actin nucleation, FHOD1 was neither involved in dorsal actin cable formation nor in establishment of retrograde actin flow. Immune fluorescence analysis of polarizing FHOD1 over-expressing cells revealed that FHOD1 co-localized with nesprin-2G at TAN-lines, and depletion of FHOD1 resulted in decreased TAN line formation. Thus, FHOD1 was identified as a TAN line component. The molecular determinants in FHOD1 which were required for TAN line formation were the N-terminal nesprin-2G interacting domain and the adjacent additional actin binding site (ABS), which is unique to FHOD1. Together these results suggest that FHOD1 acts as an adaptor between dorsal actin cables and nesprin-2G, to provide mechanical stability to TAN lines during transportation of the nucleus.

The third project was to identify additional FHOD1 interacting partners by tandem affinity purification (TAP) of over-expressed FHOD1 from mammalian cells. TAP allowed to express FHOD1 in a physiological more relevant environment in which FHOD1 interacts with its endogenous binding partners. The background of an additional search for interacting proteins was to gain further insight into the mechanism and regulation of the FHOD1 dependent processes of post-mitotic cell spreading and TAN line formation. Several promising candidate proteins with MF-, MT- and mitotic functions were found.