Summary

Chapter 1: DUF 926 containing proteins: from *Dictyostelium* to mammals

DUF 926 containing proteins are highly conserved from lower to higher eukaryotes. In mammals two proteins contain DUF 926, NKAP (NF kappa beta activating protein) and NKAP-L (NF kappa beta activating protein-Like). Unlike mammals, the *Dictyostelium discoideum* genome carries only one DUF 926 domain containing protein. To identify nuclear interacting partners of SUN1, an inner nuclear envelope protein, we performed immunocoprecipitation studies and found DdNKAP as its interacting partner. N-terminal domain of DdNKAP contains RS repeats with highly basic amino acids; the DUF 926 domain is located at the C-terminus. In mammals, NKAP is a transcriptional repressor and is required for T cell development, maturation and acquisition of functional competency. Moreover, it is important in maintenance and survival of adult hematopoietic stem cells. However, the molecular mechanism underlying its function is poorly understood. In a detailed analysis of DdNKAP we found that during interphase DdNKAP is localised in the nucleus in a punctuated pattern while during mitosis it redistributes to the cytosol. Upon ectopic expression of DdNKAP the cells show a severe growth defect, a delay in stream formation, aggregation and chemotaxis. Overexpression of the N-terminus only also led to a growth defect, however streaming and aggregation were enhanced when compared to wildtype cells. This was paralleled by enhanced chemotactic speed and increased motility. To better understand the function of DdNKAP, we performed gene expression profiling using microarray studies, which revealed up-regulation of genes involved in stress response whereas genes involved in translation were down-regulated. In conclusion *Dictyostelium* DdNKAP is involved in gene regulation, growth and aggregation.

Like DdNKAP, NKAP is localised in the nucleus during interphase and redistributed to the cytosol during mitosis. Specifically, NKAP localizes to the nuclear speckles. Moreover, upon actinomycin D treatment NKAP positive structures rounded up and the size of the speckles was increased. We further report that NKAP is not expressed during G0 phase. Co-immunoprecipitation studies revealed that NKAP interacts with several RNA binding proteins including FUS, a protein important in RNA biogenesis. Additionaly, we also show that NKAP interacts with pre-mRNA as well as spliced

mRNA. Taken together, NKAP has an important role in cell cycle progression and RNA biogenesis.

Chapter 2: Regulation of the Actin Cytoskeleton by an Interaction of IQGAP Related Protein GAPA with Filamin and Cortexillin

Filamin and Cortexillin are F-actin crosslinking proteins in Dictyostelium discoideum allowing actin filaments to form threedimensional networks. GAPA, an IQGAP related protein, is required for cytokinesis and localizes to the cleavage furrow during cytokinesis. Here we describe a novel interaction with Filamin which is required for cytokinesis and regulation of the F-actin content. The interaction occurs through the actin binding domain of Filamin and the GRD domain of GAPA. A similar interaction takes place with Cortexillin I. We further report that Filamin associates with Rac1a implying that filamin might act as a scaffold for small GTPases. Filamin and activated Rac associate with GAPA to regulate actin remodelling. Overexpression of filamin and GAPA in the various strains suggests that GAPA regulates the actin cytoskeleton through interaction with Filamin and that it controls cytokinesis through association with Filamin and Cortexillin.