Summary of Ph.D. thesis

GENETIC, CELL BIOLOGICAL AND BIOCHEMICAL ANALYSIS OF THE TISSUE SPECIFIC FUNCTIONS OF A NEW *DROSOPHILA* FORMIN

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Introduction

The cytoskeleton plays a fundamental role in many physiological processes, including organelle transport, cytokinesis, plasticity, and cell movements. Among the main structural elements (macrofilaments, intermediate filaments, and microfilaments/actin cytoskeleton) the actin cytoskeleton is a highly dynamic structure, new filaments born and die in every second, as our cells adopt different size and shape according to the environmental cues. Therefore the organization and the targeting of the new actin filaments has to be tightly regulated.

Formins were identified as members of the family of actin assembly factors. Formins are single polypeptide, multidomain proteins. All formins studied to date are dimeric, due to dimerization of their formin homology 2 (FH2) domain. The FH2 domain is responsible for driving actin nucleation and formin-nucleated filaments are not branched. After nucleation, the FH2 domain remains bound at the barbed end and moves processively as it elongates, promoting elongation by preventing the access of capping proteins. Phylogenetic analysis of the FH2 domain has led to classification of formin family proteins into phylogeny-based groups. Metazoan formins segregate into 7 groups: DIA, FMN, FHOD, delfilin, INF, FRL, and DAAM. The DIA, DAAM, FRL, FHOD and delfilin groups possess strong group-specific similarities outside of the FH2 domain, supporting the FH2-based groupings.

The most extensively studied group to date is the Diaphanous related formins (DRFs), which act as direct effectors of Rho family GTPases. The DRFs include the metazoan DIA, DAAM, and FRL formins, and the yeast Bni1, Bnr1, and SepA. The domain architecture of DRF proteins is preserved and contains two parts: a C-terminal region, which is directly involved in actin assembly, and an N-terminal regulatory region, which mediates an intramolecular interaction with the C terminus to maintain DRFs in an auto-inhibited state. The C-terminal half of DRFs contains three structural and functional elements: the profilin-binding FH1 domain, the actin-binding FH2 domain, and the Diaphanous Auto-regulatory Domain (DAD) motif. The N-terminal part includes a GTPase-

binding domain (GDB), which binds Rho-family GTPases in their activated (GTP bound) state, the Diaphanous Inhibitory Domain (DID), which binds the C-terminal DAD motif, and the dimerization domain (DD).

The DAAM class of formins was originally discovered in a yeast two-hybrid screen for proteins interacting with the PDZ domain of the Wnt signaling protein Dishevelled. The results lead to a model in which Daam1 recruits Rho GEFs to activate Rho and effect cytoskeletal remodeling to polarize the cell. Recent studies examining the mechanism of Daam1 activation have provided insight into how Daam1 and other DRFs such as Dia may be regulated to perform multiple functions. One study has found that auto-inhibition is relieved by Dsh/Dvl interaction, rather than Rho GTPase binding, allowing Daam1 to induce Rho activation in the PCP pathway. On the other hand, different studies have found that Rho can relieve the DID/DAD-mediated inhibition of Daam1 actin filament assembly, suggesting that Rho may indeed be upstream of Daam1.

Objectives

Previous data about the functions of the members of the DAAM family were gained mostly form cell cultures, and *in vitro* biochemical experiments, but our knowledge was very limited about how DAAM proteins work at the level of the whole organism. Our modell organism, the *Drosophila melanogaster* allows to investigate the function of the single *Drosophila* DAAM ortholog (dDAAM) with large scale of genetic, cell biological and biochemical techniques including the true loss of function analysis of the gene and examining its function(s) not only in the whole fly, but even at cellular levels. Therefore we decided to investigate the possible roles of *dDAAM* in the PCP pathway, and based on the expression data of vertebrate DAAM proteins in the developing embryonic nervous system and in other tissues affected by *dDAAM* loss-of function.

Methods used in the study

-P-element remobilization

-complementation analysis

-dominant genetic interactions

-epistasis analysis

-PCR

-Southern-blot

-Western-blot

-Immunoprecipitaiton

-Cell cultures: S2 and P19 (immunohistochemistry)

-Immunohistochemistry on embryos

    -„slow” fix

    -fixing with methanol

-Immunohistochemistry on larval trachea

-RNA *in situ* hibridization

Results

-we generated loss-of-function alleles of the *dDAAM* gene

-we examined the expression of *dDAAM* as well, and found that the mRNA and protein is expressed in the developing embryonic tracheal system, in the developing embryonic central nervous system (CNS), and in cardiac myoblasts

-we found, that in early, transcriptionally inactive embryos *dDAAM* mRNA is highly enriched suggesting that *dDAAM* has a considerable maternal contribution

-the loss-of-function analysis and the epistasis data argue that the *Drosophila* DAAM ortholog is either not required for PCP signaling or plays a redundant role during PCP establishment

-our analysis revealed that in wild-type tracheal cells apically localized actin is arranged into parallel bundles running perpendicular to the axis of the tubes where it is required for the proper assignment of the cuticle secretion in the embryonic trachea, and the function of the dDAAM is to organize these filaments into parallel cables

-our data indicate that dDAAM activity minimally requires the FH1 and FH2 domains, the presence of these domains are necessary and sufficient for the in vivo catalytic activity of dDAAM protein

-our genetic interaction and epistatis data indicate, that *RhoA* works upstream of *dDAAM*, while *Src42* and *Tec29* non-receptor tyrosine kinases can be placed downstream of *dDAAM* in tracheal cells

-*dDAAM*$^{mat/zyg}$ mutant embryos (in which maternal and zygotic *dDAAM* functions are both impaired) showed strong CNS phenotypes, tests with neuron-specific markers did not reveal any obvious alterations in neuron numbers in *dDAAM*$^{mat/zyg}$ mutant CNSs, suggesting that the CNS phenotypes are caused by defects in neurite growth rather than aberrant lineage formation

-based on primary neuronal cell culture data our loss-of-function experiments show that dDAAM regulates filopodium formation in growth cones, which is likely to be the subcellular cause for the axonal growth phenotypes observed *in vivo*

-expression of C-DAAM (a constitutively active dDAAM form) in the embryonic CNS resulted in embryonic lethality and severe fasciculation defects, especially commissures and nerve roots appeared thicker

-in growth cones of cultured primary neurons, the activated dDAAM protein exhibited a very similar localization pattern as the wild type protein, and induced a 35% increase in axonal filopodia number

-in immunoprecipitation experiments some of the activated dDAAM was found to be associated with Ena and Profilin, thus, taken it together with our genetic interaction and localization data, our results indicate that dDAAM operates in close association with Ena and Profilin *in vivo*

-we have shown that dDAAM promotes the formation of neurite-like protrusions when expressed in mouse P19 cells, whereas murine Daam1 can functionally replace dDAAM in *Drosophila*

-our data suggest that the regulation of actin assembly during axon growth is very likely to signify an evolutionary conserved DAAM function

Publications

List of publications directly related to the subject of the thesis

Matusek T., Djiane A., Jankovics F., Brunner D., Mlodzik M., Mihály J.: The Drosophila formin DAAM regulates the tracheal cuticle pattern through organizing the actin cytoskeleton, Development 2006 Mar; 133(5):957-66. **IF=7.603**

Matusek T., Gombos R., Szécsényi A., Sánchez-Soriano N., Czibula Á., Pataki Cs., Gedai A., Prokop A., Raskó I., Mihály J.: Formin proteins of the DAAM subfamily play a role during axon growth, J Neurosci. 2008 Dec; 28(49):13310-9. **IF=7.506**

List of publications not related to the subject of the thesis

Mihály J., Matusek T., Pataki Cs.: Diego and friends play again: old planar cell polarity players in new positions, FEBS J. 2005 Jul; 272(13):3241-52. **IF=3.579**