Identification of the molecular interaction partners of the formin dDAAM

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Background

The formin proteins are an important and evolutionarily well conserved class of actin binding proteins with essential biological functions, including cell division, cell migration and organelle transport. In these processes the best understood molecular role of formins is to promote the nucleation and elongation of unbranched actin filaments, although some formins have also been implicated in the regulation of microtubules. We have previously shown that the single Drosophila DAAM ortholog, dDAAM, is involved in multiple aspects of trachea development and axonal growth regulation, however the molecular mechanisms underlying these morphogenetic functions remain to be uncovered. To gain a better understanding of the molecular functions of dDAAM, we aim to identify the protein interaction partners of dDAAM with biochemical and genetic methods. The biochemical interaction partners is aimed to be identified by affinity chromatography. To this end, we created a dDAAM-Flag fusion protein by tagging the dDAAM gene *in situ* by site specific mutagenesis. To complement the biochemical approach, we also aim to identify interaction partners by a genetic interaction screen using the hypomorphic dDAAM^{EX1} allele exibiting a moderately strong axonal growth phenotype in the adult brain.

Results

1 *In situ* Flag tagging of the *dDAAM* gene with P-element mediated gene conversion

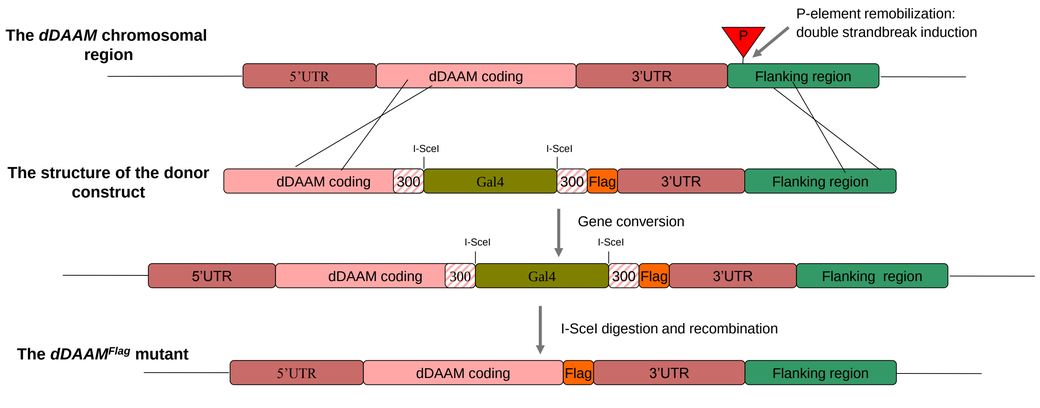


Figure 1. A P-element insertion, that is located in *dDAAM* flanking region, is remobilizied by the Δ2-3 protein. This process induces DNA double stranded breaks, that facilitates the integration of the donor construct into the fly genome in the vicinity of the break by activating the DNA repair mechanisms. The integration can be detected with the help of the Gal-4 marker gene. Subsequently, I-SceI can be used to induce double stranded breaks again to promote the recombination of two homologous (300 nucleotides long) sequences of the donor construct leading to Gal-4 excision and C-term Flag taging of dDAAM.

2 Immunohistochemical analyses of *dDAAM*^{Flag}

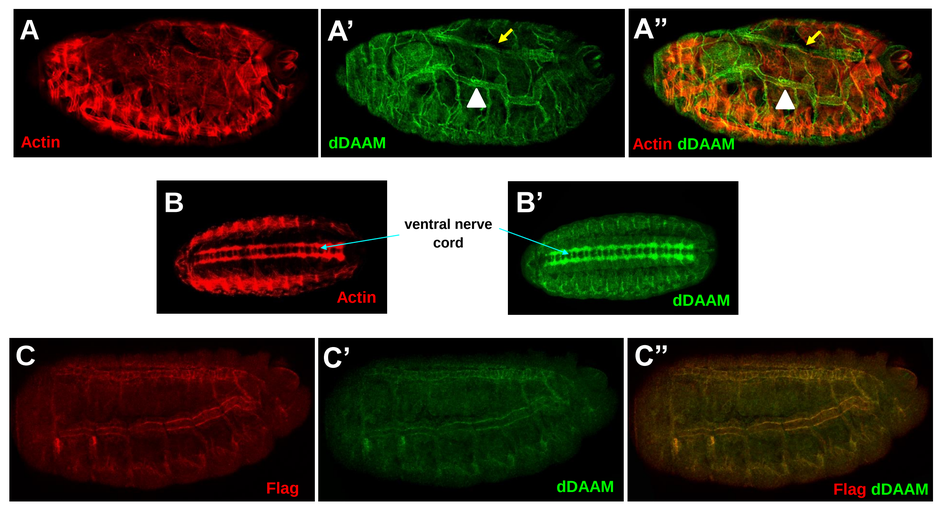


Figure 2. Confocal images of stage 16 *dDAAM*^{Flag} embryos. The dDAAM protein can be detected in the prominent dDAAM expression domains, such as the heart tube (arrow in A' and A"), the dorsal trunk (arrowhead in A' and A") and the ventral nerve cord (B and B') demonstrating that the Flag tagged protein is expressed very similarly to wild type dDAAM. C-C" shows a double staining with anti-Flag (in red) and anti-dDAAM (in green), note that the two stainings overlap almost completely.

3 Biochemical analysis of *dDAAM*^{Flag} strain 8.1

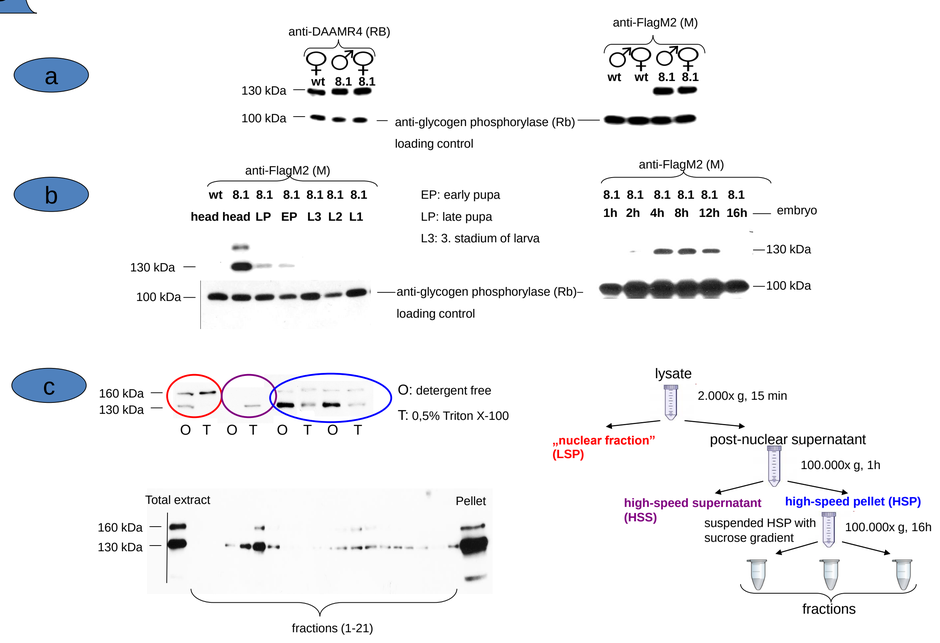
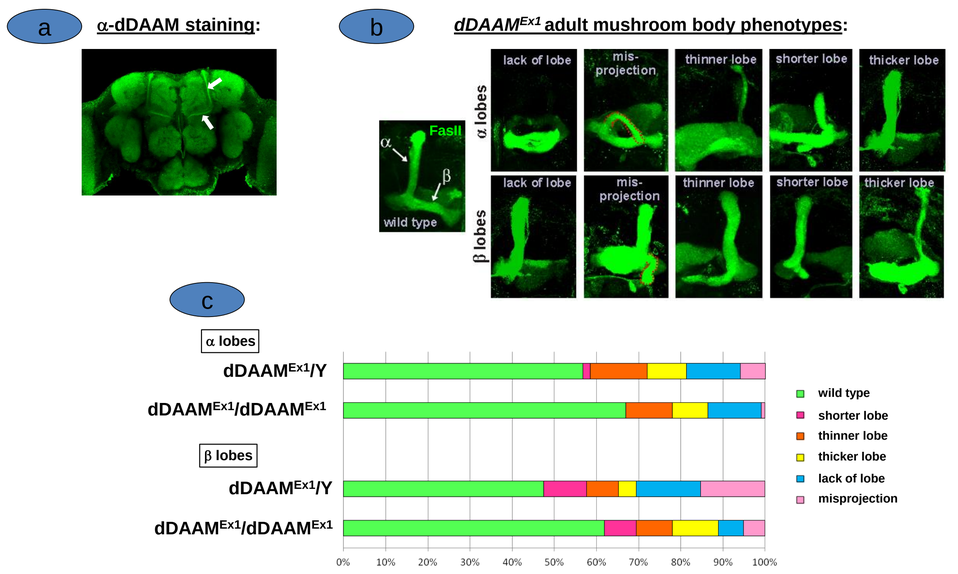


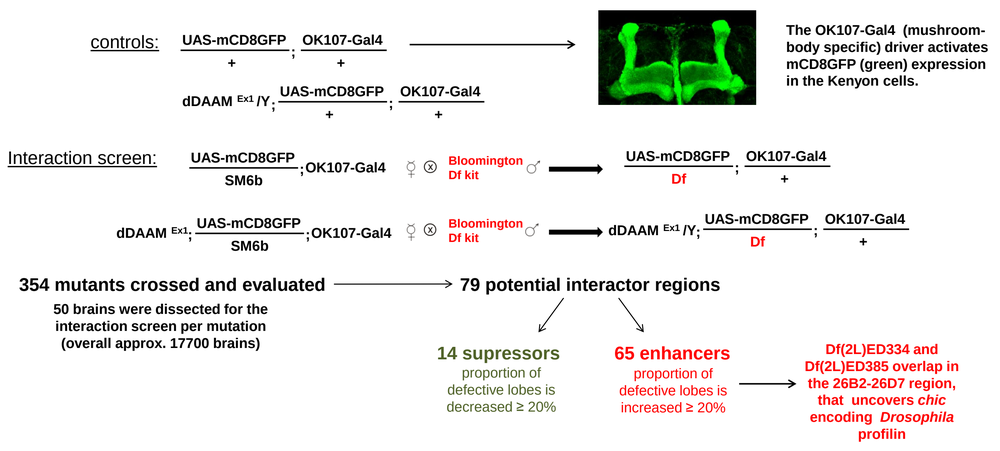
Figure 3. Immunoblot experiments of dDAAM^{Flag} (a) We compared the dDAAM protein level in wild type and dDAAM^{Flag} 8.1 mutant *Drosophila* brains. We revealed no difference in the expression level as compared to wild type type. (b) Comparison of dDAAM^{Flag} expression in different tissues and developmental stages. The most abundant protein level is found in head and 4, 8, 12 hours embryos. (c) Subcellular fractionation with sucrose gradient of the plasmamembrane (HSP), nuclear (LSP) and cytoplasmic (HSS) cpmpartments by centrifugation from protein extracts prepared from *Drosophila* heads. The highest level of dDAAM protein is found in the HSP fraction. These results suggest that most part of the dDAAM protein is membrane associated.

4 Identification of dDAAM interaction partners by a genetic approach



The dDAAM protein is highly enriched in the neuropile region of the adult brain, including the mushroom body (arrows on *a*). We found that the hypomorphic *dDAAM*^{Ex1} mutant allele displays axonal projection defects in the mushroom body (b). The moderately strong penetrance (c) of these defects made it an ideal tool for a dominant genetic interaction assay that aims to identify the suppressor and enhancer mutations of *dDAAM*^{Ex1}.

5 Genetic interaction screen with the Bloomington Deficiency kit



For the genetic screen we used a mushroom body specific driver (OK107-Gal4) to express the mCD8GFP protein allowing the analysis of the mutant brains without antibody staining. We identified 79 potential interactor deficiencies (14 supressors and 65 enhancers). Two overlapping enhancer deficiencies (Df(2L)ED334 and (Df(2L)ED385) uncover the *chic* gene that is a known dDAAM interacting partner, and therefore this observation nicely validates our screen. The mapping of the other interacting regions is still in progress.

Conclusions

1. We generated a *Drosophila* strain (*dDAAM*^{Flag}) in which we inserted a Flag tag at the C-terminus of the protein in situ.

2. Localization of the dDAAM-Flag protein is identical to that of the wild type protein because it is strongly expressed in the trachea, heart tube and ventral nerve cord.

3. Western-blot experiments demonstrate that the dDAAM-Flag protein is expressed at wild type levels.

4. The largest amount of dDAAM-Flag can be found in the head, and it apperas to be membrane associated. We will aim to purify the dDAAM containing protein complex from head extraxts in near future.

5. We established a genetic interaction assay suitable for the identification of dDAAM interaction partners by using the adult *Drosophila* brain.

6. In this screen we identified 79 potential interactor deficiencies whose mapping is in progress

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