The role of DAAM subfamily formins in muscle development

Summary of the Ph.D. thesis

Imre Molnár

Supervisor: József Mihály Ph.D.

Biological Research Centre of the Hungarian Academy of Sciences,

Institute of Genetics

University of Szeged

Doctoral School of Biological Sciences

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INTRODUCTION

The initial step of myofibril assembly is the formation of a regular array of sarcomeres. These sarcomeres later grow in width and in some cases in length, and eventually align and attach to each other and the sarcolemma. An important question in muscle differentiation is how the striated muscle cell produces these myofibrils with such regular arrays of sarcomeres. Whereas the structural properties of the sarcomeric complexes have been resolved in remarkable details over the past decades, much less is known as to how these cytoskeletal filament systems assemble *in vivo*. In particular, the initial assembly of thin filaments, the regulation of actin dynamics and organization during myofibril formation and maintenance remained poorly understood.

Although the actin nucleation factors are heavily studied in a number of different model systems, the essential nucleation factors in developing muscles have so far not been clearly identified. Besides nucleation, the other important aspects of actin dynamics are filament elongation and length regulation. Because unbranched straight actin filament is the major form in striated muscle cells, it is possible that a formin family protein serves as the key regulator of actin dynamics in myofibrils.

Given the fact, that at the beginning of our work the mechanism of actin assembly in muscle cells was largely unknown, taking in account our previous experimental data, we decided to examine the role of *Drosophila* DAAM in myofibrillogenesis.

AIMS OF THE STUDY

Our major goal was to reveal the function of dDAAM insight the striated muscle's sarcomere. In this purpose we planed to perform the following experiments:

- as first step we wanted to examine in more detail the localization of the dDAAM protein in the Indirect Flight Muscle's sarcomeres of *Drosophila*

- besides to the wild type localization studies we intended to expand the examination to loss of function and protein overexpression mutants

- with the use of loss of function mutants and the RNA silencing constructs we wished to analyze the role of dDAAM in the formation of actin filaments and sarcomere assembly

- additionally, we aimed the investigation of the molecular function of dDAAM and the identification of the potential muscle protein interactors by genetic interaction experiments and biochemical, biophysical studies as well

METHODS

- *Drosophila* genetics:
    - creation of transgenic strains
- Recombinant DNA - techniques:
    - PCR
    - molecular cloning
- Preparation of *Drosophila* embryos:
    - fixing with methanol
    - „slow" fix
- Immunohistochemistry:
    - preparation of *Drosophila* indirect flight muscle, larval body-wall muscle, and heart tube
    - preparation of mouse muscle tissues
- Western-blot
- Fluorescence and confocal microscopy
- Atomic-Force Microscopy
- Electron Microscopy
- Actin filament annealing tests

SUMMARY OF FINDINGS

- the dDAAM protein is enriched in several different types of muscles including the larval body wall muscles, the indirect flight muscle (IFM) and the heart tube
- our protein localization data are consistent with the idea that, although being a barbed end binding protein in non-muscle cells, dDAAM is both enriched in the vicinity of the barbed and pointed ends of the thin filaments in muscle cells
- the lack of dDAAM causes a flightless phenotype, as 16% of adults homozygous for the viable, hypomorphic $dDAAM^{Ex1}$ allele were flightless
- RNAi silencing in a *dDAAM* mutant background (subsequently referred to as $dDAAM^{Ex1}$*, UDT*) caused nearly all males to be flightless
- as we could show by the use of the Western blot analysis, the strength of the flightless phenotypes correlated with the partial reduction of dDAAM protein levels in $dDAAM^{Ex1}$ IFM and its near absence in IFM from the RNAi genotypes
- the flightless phenotype exhibited by $dDAAM^{Ex1}$ mutants could be rescued by muscle-specific expression of the dDAAM protein
- the IFM from the $dDAAM^{Ex1}$*, UDT* mutant combination showed the worst alterations in IFM fiber morphology. The myofibrils were thinner than in wild type and their organization was irregular. The sarcomere length reduction in these myofibrils reached even 38%
- at 48 hours after puparium formation (APF) the pupal IFM of $dDAAM^{Ex1}$*, UDT* flies already showed all the muscle phenotypes observed in adults
- electron microscopy (EM) of the IFM of $dDAAM^{Ex1}$*, UDT* mutants confirmed and extended all the major myofibrillar defects seen in the confocal images
- Atomic Force Microscope (AFM) was used to measure the transverse elasticity of individual myofibrils in rigor conditions. The elasticity (Young's

modulus) of *dDAAM*^{Ex1} and *dDAAM*^{Ex1}, *UDT* mutant myofibrils was significantly lower than that of wild type

- the subsarcomeric localization of mDaam1 appears similar to that of Drosophila DAAM with regard to accumulation at the Z-disc and alongside the M-line, and this suggests an evolutionary conserved function

- our experiments done on mice cell cultures showed, that mDaam1 is recruited to sarcomeric complexes as early as the actin crosslinker α-actinin protein. Therefore this formin is likely to be an early determinant of myofibrillogenesis

- measurements of the ventral longitudinal 3 (VL3) muscle in the *dDAAM*^{Ex68} null mutants showed a 53% length reduction and 38% width reduction compared to the wild type

- larval heart tube size was also reduced in 100 hour old *dDAAM* ^{Ex68} mutants (40% reduction in diameter)

- the strong dominant genetic interaction between *dDAAM* and the IFM-specific *Act88F* and *Tm2* alleles, and the complete lack of interaction with the non-muscle cell specific isoforms, suggests that the major function of dDAAM during muscle development is linked to the regulation of sarcomeric actin filament formation

- we investigated genetic interactions of *dDAAM* with mutations affecting the pointed end regulator proteins SALS and Tmod. The presence of *sals*^{f07849}/+ in a *dDAAM*^{Ex1} mutant background had no obvious phenotypic effect. In contrast, the *tmod*^{00848} mutation entirely suppressed the weak flightless phenotype of *dDAAM*^{Ex1}

- we also showed, that the reduced dDAAM levels suppress the ‘‘over elongation’’ of the thin filaments seen in the IFM of tmodRNAi flies

- we carried out an *in vitro* F-actin annealing assay with the barbed end binding FH1–FH2 domains of dDAAM. We found that the presence of the FH1–FH2 fragment (100 nM) allowed the end-to end annealing of actin filaments.

Whereas Tropomyosin (TM) enhanced the end-to-end annealing of actin filaments, and the combined effect of TM and dDAAM was even slightly higher than the one of TM alone

- the dominant genetic interactions between *mhc* and *dDAAM* with respect to viability and muscle structure support the possibility that dDAAM acts in concert with Mhc during muscle development

LIST OF PUBLICATIONS

Publication directly related to the subject of the thesis:

Molnár I, Migh E, Szikora S, Kalmár T, Végh AG, Deák F, Barkó S, Bugyi B, Orfanos Z, Kovács J, Juhász G, Váró G, Nyitrai M, Sparrow J, Mihály J.
DAAM is required for thin filament formation and sarcomerogenesis during muscle development in Drosophila.
PLoS Genetics 2014 Feb 27;10(2):e1004166. IF: 8.10

Other publications:

Nelson KS, Khan Z, Molnár I, Mihály J, Kaschube M, Beitel GJ.
Drosophila Src regulates anisotropic apical surface growth to control epithelial tube size.
Nature Cell Biology 14:(5) *pp. 518-U150.* (2012) IF: 19.488

Prokop A, Sanchez-Soriano N, Goncalves-Pimentel C, Molnár I, Kalmár T, Mihály J.
DAAM family members leading a novel path into formin research.
Communicative & Integrative Biology 4:(5) *pp. 538-542.* (2011)