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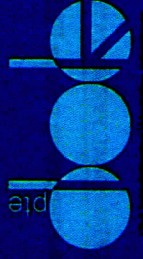
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Carla M. de



Interaction of Myosin 16 Tail Domain

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Myo16 is a recently discovered unconventional motor protein, which has a prominent role in the regulation of cell cycle especially in developing and adult level of Myo16 was described in schizophrenia [1] and the role in autism [2]. Myo16 contains a motor domain, an ankyrin-repeat domain, called the ankyrin domain (My16Ank) and in Myo16b isoform a proline-rich tail domain (My16Tail). The My16Tail contains a proline-rich domain that binds profilin and through this interaction it may regulate the actin-myosin complex. Furthermore, phosphorylation of the My16Tail activates the myosin, which suggests a crucial role for My16Tail. Analysis of purified proteins or whole cells have been critical to deciphering their cellular function. The aim of this project is to study the biophysical and biochemical properties of My16Tail *in vitro*.

Methods: The recombinant My16Tail domain was cloned and expressed in baculovirus/Sf9 system and purified under denaturing conditions. After renaturation, fluorescence spectroscopic assays were performed to measure kinetics and interactions of My16Tail with profilin-1, actin and My16Ank.

Results: However an unspecified interaction between Myo16 and profilin was described by coimmunoprecipitation earlier [4], we found no direct interaction between My16Tail and fluorescent profilin-1 using anisotropy measurements. The profilin-1 regulated actin polymerization was also not influenced by My16Tail. Meanwhile, we also tested the interaction between My16Tail and My16Ank domains, which showed a moderate affinity (KD ~ 5 µM).

Conclusion: Our results showed, that My16Tail does not influence the polymerization of actin through the binding of profilin-1, and we suppose, that the neuronal-specific profilin-2 isoform might be the possible binding partner. On the other hand, it seems My16Tail can bind My16Ank assuming a regulatory function by backfolding of the tail to the N-terminal of the myosin 16.

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References:

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The CT region of DAAM has a supporting role in FH2-mediated actin dynamics regulation

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DAAM is a DRF formin protein essential for the regulation of actin cytoskeleton dynamics in diverse biological processes. The conserved FH1-FH2 domains of DAAM catalyse actin nucleation and processively mediate filament elongation. These activities of FH2 are autoregulated by the N- and C-terminal regions. The C-terminal DAD-CT of formins was suggested recently to directly regulate actin dynamics, as well. We analysed the role of DAD-CT region of *Drosophila* DAAM in its interaction with actin by *in vitro* biochemical and *in vivo* genetic approaches. We found that DAD-CT can bind actin and enhance the nucleating activity of FH2, through the CT region. Despite of being able to bind actin, DAD-CT does not significantly influence actin assembly in the absence of the FH2 domain. Analysis of the 1732A loss-of-function mutation in FH2 and FH2-DAD-CT revealed that DAD-CT can compensate for the compromised actin-binding of the FH2 domain. However, the actin nucleation, elongation and barbed end capping activity of the FH2 domain cannot be restored by the presence of DAD-CT. Consistently, *in vivo* data show that the CT region is not essential, yet contributes to DAAM-mediated filopodia formation in primary neurons. In conclusion, DAAM DAD-CT possesses an FH2-dependent function in actin dynamics. Our data suggest that the FH2 region is the core actin-interacting element of DAAM and DAD-CT has only a supporting role in actin nucleation, presumably through stabilisation of nucleation intermediates.

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