PROJECT PROPOSAL
for applicants for ITC fellowships (2016/17)

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project title: CYTOSKELETON REGULATION DURING DROSOPHILA NMJ FORMATION

PROJECT SUMMARY
Elucidating the molecular mechanisms of synapse development and function is important for understanding biological processes such as learning and memory. The development and plasticity of these highly specialized cellular junctions requires the appropriate modulation of the underlying neuronal cytoskeleton. The goal of our research is to gain deeper insights into the coordinated regulation of actin and microtubule dynamics during the formation of neuromuscular junctions in Drosophila. We plan to use the combination of genetic, biochemical and cell biological approaches to study a formin type of cytoskeleton regulator that plays a pivotal role in synaptogenesis.

BACKGROUND
Proper functioning of the central nervous system (CNS) relies on information processing within neuronal circuits that requires synaptic connections between neighboring cells. Once formed, synapses must maintain their ability to undergo morphological and functional modifications in response to altered activity and environment. It is well established that many neurological disorders, including autism, epilepsy, schizophrenia and neurodegeneration, are associated with synaptic defects, yet the molecular mechanisms governing synaptic development and plasticity remain to be fully elucidated. In particular, although many pieces of evidence suggest that the neuronal cytoskeleton plays a crucial role in synapse development and function, the mechanisms of actin and microtubule regulation during synaptic growth and plasticity remained poorly understood.

The Drosophila larval neuromuscular junction (NMJ) is a powerful system for dissecting synaptic development, as many of the known regulators are conserved between flies and vertebrates. Our major goal is to use this model system to better understand the coordinated regulation of actin and microtubule dynamics during synapse formation.

CURRENT RESEARCH
The Drosophila NMJs are glutamatergic synapses that display a beads-on-a-stringlike structure formed at the axon terminus and are composed of synaptic boutons (Fig. 1A), which contain active zones for neurotransmitter release. During growth, the NMJ is subject to remodeling to build additional synapses on the growing muscle, which is achieved by the formation of new boutons as well as by budding off from the existing boutons. These processes require cytoskeletal rearrangements. While studying the Drosophila orthologue of the highly conserved formin type of actin assembly factor, DAAM (dDAAM), we noticed that the loss of dDAAM severely impairs NMJ development (Fig. 1B). We have shown that dDAAM is required both presynaptically and postsynaptically, that is consistent with its accumulation at both of these synaptic regions. In addition, we found that similar to its wild type
counterpart, an actin polymerization incompetent dDAAM isoform is still able to partly rescue the lack of dDAAM. Interestingly, recent studies suggested that, besides their role in actin assembly, some formins are also able to bind microtubules. This possibility was confirmed for dDAAM with in vitro assays, moreover, we revealed that the dDAAM mutant NMJs display a reduced level of MAP1B (called Futsch in Drosophila), a major microtubule associated protein with key role in microtubule stabilization. All together, these data suggest that dDAAM is a good candidate to link actin polymerization and microtubule regulation during NMJ formation.

METHODS TO BE LEARNED / APPLIED

- Basic Drosophila genetics to generate the appropriate fly stocks to be used during the course of these studies
- NMJ preparation for immunohistochemical protocols followed by confocal microscopy
- Molecular cloning, in vitro mutagenesis, in vitro formin/microtubule interaction assays by cosedimentation tests
- The creation of transgenic Drosophila stocks, rescue experiments with the mutant transgenes affecting microtubule binding

SUGGESTED READINGS


Representative recent research grants
“Investigating the mechanisms of thin filament assembly during myofibrillogenesis” (Hungarian Scientific Research Foundation, 2013-2017)

Some of the latest students in the laboratory
Bánhidi E, B.Sc. and M.Sc., 2012-present “Identification of the genetic interaction partners of the formin dDAAM required for axonal growth”
Migh E, Ph.D., 2011-present, “Identification of the molecular interaction partners of dDAAM”
Molnár I, Ph.D., 2008-2014, “The role of the DAAM formin subfamily during miofibrillogenesis”