Final Scientific Report

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Project Title:

Studies of novel cytoskeletal regulatory proteins that are involved in abiotic stress signaling

Investigators

Institutions

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ARO

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Keywords. Myosin, Golgi, Organelle,

Abbreviations commonly: GFP-green fluorescent protein, RFP-red fluorescent protein

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US: 175,000\$

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Einat Sadot

Signature
Principal Investigator

Signature
Authorizing Official, Principal Institution

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Publication Summary (numbers)

	Joint IS/US authorship	US Authors only	Israeli Authors only	Total
Refereed (published, in press, accepted) BARD support acknowledged			2	2
Submitted, in review, in preparation				
Invited review papers				
Book chapters				
Books				
Master theses				
Ph.D. theses				
Abstracts			5	5
Not refereed (proceedings, reports, etc.)				

Postdoctoral Training: List the names and social security/identity numbers of all postdocs who received more than 50% of their funding by the grant.

1. Dror Avisar 028655181

Cooperation Summary (numbers)

	From US to Israel	From Israel to US	Together, elsewhere	Total
Short Visits & Meetings			2 (GRC meetings)	2
Longer Visits (Sabbaticals)		11 month Abu-Abied		1

Description Cooperation:

The cooperation between the labs involves discussions, exchange of materials that includes seeds and constructs, the training of Mohamad Abu-Abied in Purdue for 11 months and coordinated efforts to express a recombinant active myosin XI-K head. The latter is done in yeast, whereas the constructs are built in Israel the purification is done in the USA.

Patent Summary (numbers)

	Israeli inventor only	US inventor only	Joint IS/US inventors	Total
Submitted				
Issued (allowed)				
Licensed

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Abstract

In the original proposal we planned to focus on two proteins related to the actin cytoskeleton: TCH2, a touch-induced calmodulin-like protein which was found by us to interact with the IQ domain of myosin VIII, ATM1; and ERD10, a dehydrin which was found to associate with actin filaments. As reported previously, no other dehydrins were found to interact with actin filaments. In addition so far we were unsuccessful in confirming the interaction of TCH2 with myosin VIII using other methods. In addition, no other myosin light chain candidates were found in a yeast two hybrid survey. Nevertheless we have made a significant progress in our studies of the role of myosins in plant cells.

Plant myosins have been implicated in various cellular activities, such as cytoplasmic streaming (1, 2), plasmodesmata function (3-5), organelle movement (6-10), cytokinesis (4, 11, 12), endocytosis (4, 5, 13-15) and targeted RNA transport (16). Plant myosins belong to two main groups of unconventional myosins: myosin XI and myosin VIII, both closely related to myosin V (17-19). The *Arabidopsis* myosin family contains 17 members: 13 myosin XI and four myosin VIII (19, 20).

The data obtained from our research of myosins was published in two papers acknowledging BARD funding. To address whether specific myosins are involved with the motility of specific organelles, we cloned the cDNAs from neck to tail of all 17 *Arabidopsis* myosins. These were fused to GFP and used as dominant negative mutants that interact with their cargo but are unable to walk along actin filaments. Therefore arrested organelle movement in the presence of such a construct shows that a particular myosin is involved with the movement of that particular organelle. While no mutually exclusive connections between specific myosins and organelles were found, based on overexpression of dominant negative tail constructs, a group of six myosins (XIC, XIE, XIK, XI-I, MYA1 and MYA2) were found to be more important for the motility of Golgi bodies and mitochondria in *Nicotiana benthamiana* and *Nicotiana tabacum* (8). Further deep and thorough analysis of myosin XIK revealed a potential regulation by head and tail interaction (Avisar et al., 2011). A similar regulatory mechanism has been reported for animal myosin V and VIIa (21, 22). It was shown that myosin V in the inhibited state is in a folded conformation such that the tail domain interacts with the head domain, inhibiting its ATPase and actin-binding activities. Cargo binding, high Ca^{2+} , and/or phosphorylation may reduce the interaction between the head and tail domains, thus restoring its activity (23). Our collaborative work focuses on the characterization of the head tail interaction of myosin XIK. For this purpose the Israeli group built yeast expression vectors encoding the myosin XIK head. In addition, GST fusions of the wild-type tail as well as a tail mutated in the amino acids that mediate head to tail interaction. These were sent to the US group who is working on the isolation of recombinant proteins and performing the in vitro assays. While stress signals involve changes in Ca^{2+} levels in plants cells, the cytoplasmic streaming is sensitive to Ca^{2+} . Therefore plant myosin activity is possibly regulated by stress. This finding is directly related to the goal of the original proposal

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Achievements

The main achievements included the following:

In order to compare the involvement of all the annotated Arabidopsis myosins with Golgi movement, we isolated, sequenced and sub-cloned a fragment containing the IQ, coiled-coil and tail domains of all of them downstream to eGFP, (Fig. 1). The idea was to create dominant negative clones of each myosin family member that lacks the head actin-binding domain but should still be able to bind cargo. This should saturate the binding sites and compete out the function of endogenous wild-type myosin molecules.

Figure 1. A cDNA fragment of all 17 Arabidopsis myosins that contains the IQ, coiled-coil and tail domains was isolated, sequenced and fused to GFP with a bridge of 10X Alanines as depicted in Avisar et al., 2009.

To check which of these constructs is able to arrest the movement of Golgi bodies in Arabidopsis, a transient expression method was applied according to (Marion *et al.*, 2008). Each myosin tail fragment fused to GFP was transiently co-expressed in *Arabidopsis* seedlings, with a RFP marker for Golgi bodies. Several time-lapse movies were acquired and the velocity of a few hundred Golgi bodies in the presence of each myosin tail fragment was calculated using Volocity software. Fig. 2A shows that the same group of myosin tails—MYA1, MYA2, XIC, XIE, XI-I, and XIK—

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previously reported to affect organelle's movement in *Nicotiana benthamiana* (Avisar et al., 2009) were major inhibitors of Golgi body movement in *Arabidopsis* leaves, allowing only ~20% of the Golgi's velocity compared to control. Velocity of Golgi bodies was then checked in plants knocked out for *mya1*, *mya2*, *xic*, *xie*, *xi-i* and *xik* (Peremyslov et al., 2008). When a Golgi marker was transiently expressed in these plants, its velocity was only dramatically reduced (~20% of control) in the *xik* mutant, whereas in the other knockout plants, although significantly different, the Golgi's velocity remained at 70 to 85% of control WT plants (Fig. 2B).

Figure 2. Relative mean velocity of Golgi bodies in *Arabidopsis* epidermal cells in the presence of tail fragments of all *Arabidopsis* myosins and in *Arabidopsis* knockout plants. Time-lapse movies were acquired from at least 10 cotyledon epidermal pavement cells from 3 different plants and Golgi velocity was calculated using Volocity software. The graph represents velocity as percent of control (velocity of Golgi alone in A or Golgi in wild-type plants in B). Columns overlaid with different shapes are statistically different at $P < 0.05$ as tested by Scheffe analysis. Standard error bars are shown (A) GFP fusions of myosin tail fragments were transiently expressed together with a RFP-Golgi marker in *Arabidopsis*

seedlings. (B) RFP-Golgi marker was transiently expressed in *Arabidopsis* seedlings of WT or myosin-knockout lines.

To further elucidate what is unique to the tail of myosin XIK, single or multiple mutations were introduced in the tail of myosin XIK from *N. benthamiana*. Tail fragments containing these mutations were expressed in *N. benthamiana* and their ability to arrest Golgi motility was tested. It was found that a single change of Arg 1386 to Pro or a change of Arg 1386 and Arg 1443 to Ala could abolish myosin XIK's ability to arrest Golgi movement. Sequence alignment with mouse myosin Va and a further literature survey indicated that the corresponding amino acids (Lys 1706 and Lys 1779) from myosin Va are those that mediate the tail and head interaction in this myosin (Li et al., 2008). It has been suggested that tail folding and interaction with

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the head is a regulatory mechanism inhibiting myosin ATPase activity and function of myosin Va (Li *et al.*, 2008). This suggests that myosin XIK can fold and form a head-tail interaction. To test this interaction using recombinant proteins, the tail domain, either WT or mutated R1443A or R1386A were fused to GST in a bacterial expression vector, and the head domain will be expressed in yeast (Figure 3).

Figure 3. Preliminary attempts at purification of myosin XIK head by Ni-NTA affinity chromatography. A *N. benthamiana* myosin XI-K head construct fused with 6X His tag was introduced into *S. cerevisiae* strain W303 and protein expression induced with galactose for 6h at 25C. Affinity chromatography followed standard protocols.

Details of cooperation:

While the work described in Figures 1 and 2 and in the two publications of Avisar *et al.*, (2009, 2011) was done in the Israeli lab, the continuing work described in Figure 3 was carried out in the USA lab. In addition to the collaborative work on myosins, during the second year of funding Dr. Mohamad Abu-Abied from the Israeli lab was trained in the USA lab for 11 months.

List of Publications:

Avisar, D., Abu-Abied, M., Belausov, E., Sadot, E., Hawes C. and Sparkes I.A. (2009) A comparative study on the involvement of 17 Arabidopsis myosin family members on the motility of Golgi and other organelles. *Plant Physiol.* 150(2) 700-709.

Avisar D., Abu-Abied M., Belausov E. and Sadot E. (2011) Myosin XIK is a major player in cytoplasm dynamics and is regulated by two amino acids in its tail. *J. Exp. Bot.* In press

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