MOLECULAR GENETICS OF MYOSIN MOTORS IN ARABIDOPSIS

Final Report

for Period July 1, 1992 - June 30, 1996

John Schiefelbein

University of Michigan
Ann Arbor, Michigan 48109

February 1997

Prepared for

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THE U.S. DEPARTMENT OF ENERGY
AWARD NO. DE-FG02-92ER20068

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DOE/ER/20068--T1

FINAL REPORT

DOE AWARD #: DE-FG02-92ER20068

PROJECT TITLE: "Molecular Genetics of Myosin Motors in Arabidopsis"

PRINCIPAL INVESTIGATOR: John Schiefelbein

PERIOD COVERED BY REPORT: 7/01/92-6/30/96

NAME/ADDRESS OF RECIPIENT ORGANIZATION: The University of Michigan
3003 South State Street
1060 Wolverine Tower
Ann Arbor, MI 48109

AMOUNT OF UNEXPENDED FUNDS: None

SUMMARY OF INVESTIGATION:

The normal growth and development of plant cells depends on the precise organization and distribution of the cellular contents. The basic goal of this investigation was to define a group of the molecules that are involved in organizing and transporting plant cell components. Based largely on studies of animal and fungal cells, one of the molecules thought to be involved in intracellular trafficking in plants is the actin-based motor protein myosin. Therefore, the major aim of this study was to isolate and analyze plant genes encoding myosin proteins. The plant of choice for these experiments was *Arabidopsis thaliana*, which offers numerous advantages for molecular genetics research.

Our research findings during the grant period can be organized into three main areas:

1. Molecular Cloning of a Class V Myosin Gene from Arabidopsis. The polymerase chain reaction technique was used to clone myosin-like gene fragments from Arabidopsis genomic DNA. These fragments were used as hybridization probes to purify a full-length cDNA and genomic DNA clones of a myosin gene, named MYA1. The predicted product of the MYA1 gene is 173 kD and it is structurally similar to the class V myosins isolated from animal and fungal species. The MYA1 is composed of a highly-conserved N-terminal "head" domain, a putative calmodulin-binding "neck" domain, an alpha-helical coiled-coil domain, and a C-terminal domain. Northern blot analysis shows that the Arabidopsis MYA1 gene is expressed in all the major plant tissues (including flower, leaf, root, and stem). These results imply that MYA1 may be involved in mediating transport of vesicles and/or organelles in all plant cells. This work has been published in the article "A myosin from a higher plant has structural similarities to class V myosins" by Mark Kinkema and John Schiefelbein, in the *Journal of Molecular Biology* (1994) 239: 591-597.

2. Characterization of the Myosin Gene Family from Arabidopsis. In many species, multiple myosin isoforms exist, and these are encoded by different genes. In order to define the size and complexity of the myosins in a higher plant, we analyzed the myosin gene family from Arabidopsis. Using a cross-hybridization strategy, several different

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myosin genes were detected from genomic and cDNA clones. A total of six myosin-like genes were identified from three polymerase chain reaction products (named PCR1, PCR11, and PCR43) and from three cDNA clones (named ATM2, MYA2, and MYA3). Sequence comparisons of the deduced head domains suggest that these myosins are members of two major classes. Analysis of the overall structure of the ATM2 and MYA2 myosins shows that they are similar to the previously identified ATM1 and MYA1 myosins, respectively. The MYA3 appears to possess a novel tail domain, with five IQ repeats, a six-member imperfect repeat, and a segment of unique sequence. Northern blot analyses indicated that some of the Arabidopsis myosin genes are preferentially expressed in different plant organs. Combined with previous studies, these results show that the Arabidopsis genome contains at least eight myosin-like gene representing two distinct structural classes. These results have been reported in the article: "Molecular analysis of the myosin gene family in Arabidopsis thaliana" in the journal *Plant Molecular Biology* (1994) 26: 1139-1153.

3. Functional Analysis of the MYA1 Gene Product. In order to define the role of myosin in plants, we have used molecular and genetic methods to examine the function of the MYA1 myosin. Using in situ RNA hybridization, we have analyzed the patterns of expression of the MYA1 gene. The MYA1 gene is expressed in a developmentally regulated manner in specific cells of floral organs (including the sepals and anther filaments), the leaves (including the mature trichomes), and the cotyledons (at the seedling stage). Transgenic plants were produced with constructs designed to either repress or overexpress/ectopically express the Arabidopsis MYA1 myosin. Two different MYA1 cDNA fragments were used to generate antisense RNA under the control of the CaMV35S promoter. Transgenic plants were found to express high levels of antisense RNA for each of the two constructs, but no reduction in the level of endogenous MYA1 mRNA or protein was detected. The phenotype of these antisense lines was indistinguishable from the wild type. The CaMV35S promoter was also used to drive the expression of the entire MYA1 cDNA. Transgenic plants harboring the MYA1-cDNA construct were phenotypically wild type and had levels of MYA1 mRNA and protein that were indistinguishable from those of untransformed plants. These studies suggest that the expression level of the MYA1 gene is tightly regulated and/or that changes in MYA1 expression are detrimental to plant growth and development. These results are currently being organized into a manuscript that will be submitted for publication.

In summary, this investigation has led to new insights into the structure and function of the myosin molecules in plants. This work opens up new possibilities for the manipulation of intracellular trafficking in plant cells. The manuscripts from our published work on this project are appended.