Masters Theses 1896 - February 2014

ANALYSIS OF AN ACTIN BINDING GUANINE EXCHANGE FACTOR, GEF8, AND ACTIN DEPOLYMERIZING FACTOR IN ARABIDOPSIS THALIANA.

Aleksey Chudnovskiy

Follow

Document Type
Open Access

Degree Program
Molecular & Cellular Biology

Degree Type
Master of Science (M.S.)

Year Degree Awarded
2010

Month Degree Awarded
May

Abstract
ABSTRACT ANALYSIS OF AN ACTIN BINDING GUANINE EXCHANGE FACTOR, GEF8, AND ACTIN DEPOLYMERIZING FACTOR IN ARABIDOPSIS THALIANA. April, 2010 Aleksey Chudnovskiy, B.S., UNIVERSITY OF MASSACHUSETTS AMHERST M.S., UNIVERSITY OF MASSACHUSETTS AMHERST Directed by: Professor Alice Y. Cheung Polarized cell growth is a fundamental biological process that is tightly regulated spatially and temporally. In plants the key systems to study polar cell growth are pollen tubes and root hairs. In recent years a lot of work has focused on elucidating the mechanisms that mediate this process. The actin cytoskeleton plays a key role in polarized cell growth. Different studies in plant and animal models show that signaling mediated through small GTP-binding proteins is a common theme in actin signaling. In recent years many groups have shown that small GTP binding proteins regulate actin dynamics through the activity of Actin Binding Proteins (ABP). In this study I explored the function of two ABPs from Arabidopsis Thaliana: Actin depolymerizing factor (ADF) and a novel actin-binding guanine exchange factor (GEF). I used Arabidopsis

protoplasts as a system to study the function of these proteins. We showed through over-expression of the GFP labeled GEF8 under the constitutively active 35 S promoter, that GEF8 labels the prominent cable like structures inside the cell. Using actin and tubulin binding drugs such as Latrunculin and Oryzalin we showed that GEF8 labels actin cables. Using the Yeast 2 Hybrid system we determined that GEF8 binds actin filaments directly. We established that GEF8 interacts with actin through the unique N terminus of the protein. Finally, using the Basic Local Alignment Search tool we showed that the N terminus of GEF8 is homologous to the Actin Binding Protein 140, a well-established protein marker in Yeast. ADF is an established key regulator of the actin cytoskeleton. Much is known about ADF regulation in animal systems. In plants it has been shown that the small Rho type GTP binding proteins, called RAC/ROPS, regulate ADF activity and that overexpression of RAC/ROPs causes the inactivation of ADF through the phosphoryaltion on Serine 6. However, little is known about the proteins that transduce the signal from small GTP binding proteins to the ADF. Here we show some evidence that upon overexpression of Ric 4 (a RAC/ROP effector known to play a role in actin polymerization), ADF gets displaced from the filament. Moreover, ADF is known to be inactivated by phosphorylation at Ser6; the kinase responsible for this phosphorylation has not been identified in plant. We observed that over-expression of Calcium Dependent Protein Kinase 16 (CDPK16) in protoplasts also induced dissociation of ADF from actin cables. These results suggest that both of RIC4 and CDPK16 may play a role in the pathways that regulate ADF activity.

Additional Files

Alex master thesis figures .pdf (9448 kB)
Master thesis figures

Advisor(s) or Committee Chair

Cheung, Alice Y

Wu, Hen-ming