

## DOCTORAL THESIS

### Characterization of the biological function of AtEXO70E2

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## Abstract

Exocyst positive organelle (EXPO) is a newly discovered double membrane organelle involved in exocytosis and likely other vesicle trafficking processes. EXPO is likely generated from the ER, fused with plasma membrane and released a single membrane vesicle to cell exterior. The *Arabidopsis* protein Exo70E2 was found to be associated with EXPO and therefore is considered as a marker of EXPO and might play a role in EXPO-mediated vesicle trafficking. Understanding the biological function of AtExo70E2 (abbreviated as E2 in this thesis) will be very helpful in unraveling the function of EXPO. The aim of this work was to use various molecular, genetic and physiological approaches to determine the possible role of *Arabidopsis* Exo70E2 in biological pathways.

By using the Exo70E2pro: GUS line, the expression pattern of Exo70E2 was determined. Exo70E2 was expressed mainly in roots, especially in root tips and epidermal cells in the division and elongation zones of roots. Its expression level was induced when the seedlings were treated with Flg22, a peptide derived from bacterial flagillin protein that induces the plant defense response.

The tissue subcellular localization of Exo70E2 was also studied using the 35S:Exo70E2-eYFP and Exo70E2pro:Exo70E2-GFP reporter lines. The GFP fusion protein was found primarily in the epidermal cells of roots even in the 35S:Exo70E2-eYFP lines.

For phenotypic analysis resulting from mutations of the Exo70E2 gene, I obtained three T-DNA insertion mutant lines and generated its overexpression lines. The two mutant alleles, *e2-2* and *e2-3* are in the Columbia ecotype background and further characterized. *e2-2* which has a T-DNA insertion in an exon is likely a knock out line as Exo70E2 gene transcript could not be detected. *e2-3*, which

carries a T-DNA insertion in its promoter region, was found to accumulate a higher level of the transcript, suggesting that the insertion causes its enhanced expression of Exo70E2. There was no obvious difference between wild type and *e2-2* in their phenotypes under different conditions tested in this study. However, *e2-3* had a retarded growth phenotype when grown in soil or on MS medium. The seedlings of *e2-3* on MS medium also had a yellowish color although such a phenotype was not obvious when they were grown in soil. When supplementing the MS medium with sucrose, glucose or mannitol, the growth of *e2-3* was more reduced compared to wild type under these conditions. However, on the medium with NaCl or under phosphate deficiency, the yellowish phenotype of *e2-3* was rescued and the mutant seedlings became relatively healthier than the seedlings under the regular MS medium.

A proteomics approach was taken to compare protein secreted from the seedlings of wild type and the mutants. Proteins secreted by seedlings to the liquid medium were collected, concentrated and subjected to MS analysis. Comparison of the profiles of secreted proteins between the wild type and the mutants led to identification of candidate proteins whose secretion might be affected by the mutation.

My study indicates that Exo70E2 and EXPO are involved in transporting proteins (likely also metabolites) to the exterior of cells and the rhizosphere and might play an important role in stress responses.

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