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DISSERTATION SUMMARY

Analysis of a novel CDPK kinase in *Arabidopsis thaliana*

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The *Arabidopsis PRL1* (pleiotrop regulatory locus) gene encodes a regulatory WD protein that plays an important role in carbon metabolism, cytokinin and glucose signaling, cell cycle, photosynthesis, and general stress responses (Németh et al. 1998). The CRK1 protein kinase was isolated as a PRL1 interacting partner in yeast two hybrid system. The CRK1 protein is an atypical member of the Ca^{2+}-dependent protein kinases (CDPKs). CDPKs form a large subfamily of protein kinases in plants that have been implicated in the control of numerous aspects of plant growth and development. The CDPKs have four well characterized conserved motifs: 1) ATP-binding; 2) catalytic domain; 3) an autoinhibitor is predicted in the region immediately following the kinase domain; 4) number of functional calcium-binding EF-hands in a C-terminal regulatory domain. In the CRK subgroup, these EF-hands are degenerated. Until now 28 different CDPKs and 8 CRKs have been identified in *Arabidopsis.*

The function of the CRK1 kinase is not known. We use different molecular and genetical approaches to characterize the CRK1 function in plants. Screening of different cDNA libraries in yeast two hybrid system (whole plants, and cell suspension library) resulted in the identification of further interacting partners of the CRK1 protein. From cell suspension library: dihydroflavonol reductase with epimerase domain, two others CDPK-related protein kinase, ER-type calcium ATP-ase, unknown protein (SEC14 domain); from whole plants library: centromer protein homologue, photosystem I subunit II precursor, unknown protein (bZIP domain).

Biochemical characterization of the CRK1 protein has been initiated. A loss-of function mutation was generated by PCR mutagenesis by eliminating an ATP-binding site in the conserved kinase domain (CRK1m). In order to analyze the kinase activity of the CRK1 protein, His-tagged recombinant CRK1 and CRK1m clones were overexpressed in *E.coli*, and the proteins were purified from bacterial extracts. Activity of the wild type and mutant CRK1 protein was tested by phosphorylation of the myelin basic protein (MBP) as a hypotetical substrate. CRK1 but not the CRK1m protein was able to phosphorylate MBP in a calcium-independent fashion.

In order to test interaction of the CRK1 and other *Arabidopsis* proteins in plant cells, the CRK1 and CRK1m cDNAs were cloned into HiA epitope tagging vector (Ferrando et al. 2000). These constructs we transformed to GV3101/pMP90RK *Agrobacterium tumefaciens* strain. In a transient expression system, *Arabidopsis* cell suspension was infected by the *Agrobacteria.* Transformed cells were collected five days after the *Agrobacterium* inoculation. Immunolocalization studies suggested the nuclear localization of the CRK1 protein. Salutation of CRK1 kinase-protein complex has been initiated in order to identify the putative interacting members of the protein complex.

Biological function of the CRK1 protein is being studied by creating CRK1 and CRK1m overexpressing transgenic *Arabidopsis* plants and identification of a knock-out insertion mutant using reverse genetic approaches. Full length CRK1 and CRK1m cDNA were cloned into pPCV-type plant expression vectors in sense and antisense orientation. Transgenic plants were generated by an *Agrobacterium*-mediated in-planta transformation method. Insertion mutant was identified by screening of pooled *Arabidopsis* DNA templates, representing 60,000 independent insertions. PCR characterization of the transgenic lines and the tagged CRK1 mutant is in progress.

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