Abstract

Vesicle tethering complex exocyst is one of the key regulators of the cell polarity and morphogenesis in eukaryotes. The complex interacts with the secretory vesicle, as well as plasma membrane, and facilitates formation of cis SNARE complex leading into fusion of the vesicle with target destination. Two of the eight exocyst subunits, the SEC3 and EXO70 are known to bind plasma membrane via protein and lipid interactors in Opisthokont model organisms. Genomes of angiosperm plants encode a surprisingly wide repertoire of EXO70 isoforms with over 20 present in both Arabidopsis and diploid tobacco genome. It has been proposed that different EXO70 isoforms would form parts of functionally distinct subtypes of the plant exocyst complex driving membrane trafficking to various membrane domains. Specific interactions of peripheral membrane proteins with particular membrane phospholipids largely contribute to targeting of cellular components to subcellular compartments and membrane domains. This thesis focuses on role of protein-lipid interactions in regulation of plant cell polarity and contributes to functional analysis of the plant EXO70 family diversity. We introduce the topic with the theoretical reviews summarizing role of protein-lipid interactions in establishing plant cell membrane domains at various spatio-temporal scales and role of the exocyst complex at the interface of cytoskeleton and membrane trafficking in eukaryotes. Analyzing the roles of diverse EXO70 isoform within single cell is the core of the first study that systematically compares subcellular localization of the whole EXO70 repertoire using the growing tobacco pollen tube, a well established model system for addressing regulation of plant cell polarity. Transferring the pollen tubes with constructs encoding the tagged native tobacco EXO70 isoforms, we uncovered targeting of the proteins to various compartments, including vesicle-enriched inverted cone, nucleus and two spatially distinct plasma membrane domains overlapping to a different degree with spatial maxima of anionic phospholipids phosphatidylinositol-4,5-bisphosphate and phosphatidic acid. This is followed by the detailed analysis of EXO70A1, the EXO70 isoform expressed across many plant tissues and known to drive polar exocytosis in different cell types. We combined live cell imaging of Arabidopsis root cells and tobacco pollen tubes with in vitro protein-lipid binding assays and molecular dynamics simulations to demonstrate importance of

EXO70A1 direct interaction with anionic phospholipids in membrane targeting of the plant exocyst complex. The thesis further includes functional studies that address the roles of Arabidopsis EXO70A1, EXO70B1 and EXO70B2 isoforms in targeting trafficking to various membrane domains and subcellular compartments, and to which I contributed. In the form of comprehensive review, we discuss our data and the so far published studies addressing the relation between the subcellular localization and function of plant EXO70 isoforms.