Abstract

This work is a collection of three research articles and one review article focused on flotillins (FLOTs) and hypersensitive induced reaction proteins (HIRs) in *Arabidopsis thaliana*. FLOTs and HIRs are closely related membrane-associated proteins forming two subfamilies both belonging to SPFH domain superfamily. While FLOTs are present in organisms of all evolutionary lineages HIRs are plant specific proteins. The review article sums up the knowledge gained on FLOTs and HIRs from different organisms in terms of cellular localization, interaction with cellular membranes and with other proteins, and physiological functions. The research articles were targeted at three aspects of *At*FLOTs and *At*HIRs: involvement in response to exogenous stimuli; determination of protein interactors; and subcellular localization and dynamics. The first aspect was approached by transcription measurement of *AtFLOT*s and phenotypic screen of single loss-of-function mutants of *AtFLOT*s upon various treatments covering biotic and abiotic stress and phytohormone application. Although we observed changes in transcription none of the treatments provoked a phenotype manifestation in any of *AtFLOT* mutants. In the second article we focused on interactome of *At*FLOT2 and performed co-immunoprecipitation followed by mass spectrometry determination of co-precipitated proteins. Several proteins involved in cellular transport, water stress or plant pathogen interactions were revealed and direct interaction of *At*FLOT2 with some of them was verified using split-ubiquitin system. These interactors point to the possible physiological functions of *At*FLOT2. The manuscript of the third article covers the investigation of localization and dynamics patterns in all isoforms of *At*FLOTs and *At*HIRs. We present general plasma membrane localization for both subfamilies with one exception where the protein is associated exclusively with the tonoplast. However, the presence of a minor tonoplast pool accompanying the predominant plasma membrane localization is shared among some other isoforms as well. At the plasma membrane the signal of both *At*HIRs and *At*FLOTs is clustered in membrane microdomains. These microdomains are very stable over time, especially in *At*FLOTs. Despite the overall immobility revealed by FRAP approach for both subfamilies, a slightly higher dynamics was measured for *At*HIRs. Proteins from the both groups are restricted from linear patterns within the plasma membrane, so called corrals. We found these corrals to align with microtubules, however the disruption of cytoskeleton did not induce any change of *At*FLOT or *At*HIR localization. Finally, we observed an increase in mobility in *At*HIR1 upon pharmacological inhibition of cellulose synthesis and the same effect was also observed under partial enzymatic cell wall digestion in *At*HIR1 and *At*FLOT2. Altogether our findings suggest that plasma membrane microdomain localized FLOTs and HIRs interact with the cell wall which decreases their mobility. This interaction may be important for the communication events at the interface between the cell and its environment. *At*FLOTs may be involved in these events, especially in process like plant-pathogen interaction or water stress, which is suggested by the physiological functions of protein interactors of *At*FLOT2 and transcription responses of *AtFLOT*s to such stimuli.