Abstract

Exocyst is a protein complex composed of eight subunits, evolutionarily conserved in yeasts, animals, and plants. The main function of exocyst is to mediate the tethering of secretory vesicles to the plasma membrane. However, the involvement of exocyst in some other processes, especially in autophagy, has been recently discovered. Plant exocyst is specific because most of its subunits have multiple paralogs. The most diversified subunit is EXO70, which is encoded by 23 paralogous genes in *Arabidopsis thaliana*. In this thesis, I dealt with subunit AtEXO70E2 (AT5G61010), which has been localized to double-membrane compartments considerably reminiscent of autophagosomes. These compartments were named EXPOs (for exocyst-positive organelles) and described as a component of unconventional protein secretion pathways. There are also hints that EXO70E2 could play a role in autophagic processes. However, details of this relationship remained unexplored. For my experiments, I used stably transformed lines of *A. thaliana* and transiently transformed leaves of *Nicotiana benthamiana*. I performed numerous colocalization experiments, applied various pharmacological treatments to the studied lines, and analyzed a mutant line in the *EXO70E2* gene.

According to my observations, protein EXO70E2 is expressed especially in root tips, while in leaves I observed a very weak expression of *EXO70E2*. I noticed a partial colocalization of EXO70E2 with important autophagy markers, ATG8 proteins. Although EXO70E2 possesses ATG8-interacting motives (AIMs), its interaction with ATG8E and ATG8F is likely indirect. On the other hand, I did not observe colocalization of EXO70E2 with selected endomembrane compartment markers (*trans*-Golgi network, multivesicular bodies). Selected pharmacological treatments did not affect the intensity or localization of the fluorescent signal of EXO70E2-GFP, the only exception was wortmannin. This autophagy inhibitor caused an increase in the cytoplasmic fluorescent signal of EXO70E2-GFP, enlargement of EXPOs, and also increase in their number. Expression of EXO70E2-GFP in *atg5* and *atg9* mutants provided interesting results. In these lines, I observed a strong accumulation of EXO70E2 and at the same time an almost complete absence of EXPOs, both in root tips and leaves.

Phenotypic analysis of *exo70E2* mutant suggested that protein EXO70E2 might operate in specialized autophagic pathways important for coping with the lack of energy (carbon starvation). On the other hand, this protein may not play an essential role in the regulation of autophagic pathways induced by nitrogen starvation. However, these results may have been affected by a second mutation (in LAZ1 HOMOLOG1 promoter) in the *exo70E2* mutant line I used. I observed partial colocalization of EXO70E2 with chitinase (AT2G43570), even in the area outside the plasma membrane. The results of this thesis suggest that subunit EXO70E2 may play a regulatory role in specific autophagic pathways connected with unconventional (or also conventional) protein secretion pathways. Thus, EXPOs could be a part of so-called secretory autophagy pathways. It seems that autophagy is important for the degradation of EXO70E2 at the same time.

Keywords: *Arabidopsis thaliana, Nicotiana benthamiana*, exocyst, EXO70E2, autophagy, ATG proteins, carbon and nitrogen starvation, secretion, chitinase