

State of the art:

Molecular membranes in all living organisms serve to protect cells from the environment and to separate parts of the cell dedicated to particular functions such as for example storage of certain compounds, protection of genetic material or synthesis of proteins. All biological membranes are composed of lipids (among them large, stiff molecules of sterols) and proteins. Most of the membranes in a plant cell are not isolated identities, but rather form a continuous network. Communication of intracellular compartments, demarcated by membranes, as well as perceiving environmental signals (for example hormones) and responding to them, is achieved mainly by the means of vesicular transport. Vesicles bud from one membrane, travel along cytoskeleton cables and fuse to another membrane. This movement is highly regulated by many proteins embedded or interacting with the membranes, among them Rab proteins.

Rab proteins are modified by two lipid moieties, geranylgeranyl groups, to enable their stable binding to vesicles. Decrease in this modification level in the *rgtb1* mutant (in an enzyme called Rab Geranylgeranyl Transferase- RGT) leads to slowing down vesicular transport and disturbance in growth and development of our model plant, *Arabidopsis thaliana*. All mentioned processes are common to land plants.

Aims of the project:

- 1) In the project we want to see whether the problems with plant growth in the *rgtb1* mutant result from faulty hormone (brassinosteroid) perception on the outer membrane of the cell. We plan to directly observe if the receptor of this hormone (named BRI1) is mislocalized in *rgtb1* plant cells. If this is true, transcription of numerous genes governed by cell response to brassinosteroid will be changed, and we will measure it. Among these are Rab proteins and RGT coding genes as well as sterol biosynthesis genes.
- 2) We speculate that lack of BRI1-containing vesicle recycling, and in consequence lack of brassinosteroid dependent transcription, will cause imbalance in sterol biosynthesis and in turn, abnormal sterol composition of the cell. This is known to be dangerous for organism survival, as all transport and cell integrity relies on proper protein and lipid equilibrium in the membranes. We will measure the sterol content in the *rgtb1* mutant and a mutant with nonfunctional BRI1 (named *bri1*).
- 3) Alternatively, slowing down of vesicular transport in the the *rgtb1* mutant may cause a situation, when sterol biosynthesis is normal, but sterol molecules cannot escape from their place of synthesis, because vesicular transport is out of work. By isolation of different cellular compartments from the *rgtb1* mutant we will be able to answer the question, if sterols are trapped where they are synthesized.
- 4) Finally we want to ask a question opposite to the second one, how induced sterol imbalance, as in the selected sterol biosynthesis mutant named *smt2/3*, influences the structure of intracellular compartments, Rab protein localization, Rab geranylgeranylation and transport efficiency. In the *smt2/3* mutant the proportion of two main plant sterols sitosterol and campesterol, normally constituting 75% and 20% of Arabidopsis membranes, is reversed, leading to the situation that campesterol is prevalent. We expect to see by the microscopic and biochemical methods that in such a situation Rab containing vesicles do not behave normally (for example they fuse, cluster etc.).

Expected impact of the project:

Summarizing, the results of this investigation will complement the knowledge concerning the basic cellular processes. In particular it will broaden our understanding of reciprocal regulation of major vesicular transport players such as Rab proteins and sterol lipids. In the project we will answer the question whether brassinosteroids are the crucial link. We will also find feedback loops in sterol-brassinosteroid-Rab protein dependent processes. This knowledge may become useful in the future applied agricultural studies, as brassinosteroids, the growth hormones, are new candidates for "green revolution", boosting the crops for starving areas of our planet. Rab proteins are already under study to improve edible plants properties and studying plant sterols is important for discovery of new potent pharmaceuticals.

Results of this project will be discussed with the specialists in field during scientific conferences and become available in public domain as works published in professional journals. We plan to prepare a comprehensive popular article in Polish on Rab proteins function in plants.