

Plants, likewise animals, have an ability to generate action potentials, APs, in response to changing environmental factors. Action potentials are short-lasting changes in the cell electrical potential, which when evoked locally, can spread over whole plants or their substantial parts. This enable plant to code and transmit information about changing environment leading to quick responses. The goal of the project is to compare the mechanisms of APs in representatives of fresh water algae – Charophyta, which are the objects of the study of the Lithuanian team, and liverworts, whose bioelectrical properties have been examined for years by the Polish group. An important aspect of this research will be an attempt to answer the question: how did the system of responding to stimuli change upon the passage from water to terrestrial environment. During the evolution, this occurred in the Middle Ordovician, i.e. ca. 470 million years ago. Liverworts were the first plants to settle the land, whereas characean algae are regarded as their nearest ancestors. Special attention will be paid to the role of calcium ions in AP formation. These ions after appearance in the cytoplasm in a substantial amount, lead to physiological responses such as changes in respiration and photosynthesis rates, cessation of cytoplasmic streaming, and closing traps and digesting preys in carnivorous plants. It has not been unequivocally determined whether an increase in the  $\text{Ca}^{2+}$  ion concentration in the vacuole during AP results from an influx of these ions from the external environment or from internal stores, in particular from the vacuole – the largest cell compartment. Ion channels of the SV/TPC type localized in the tonoplast could be responsible for the influx of  $\text{Ca}^{2+}$  ions from the vacuole to the cytoplasm. Both research teams possess experience in examination of ion channel activity in plant vacuoles using the patch-clamp technique (P-C). It enables registration of ion fluxes passing through a single protein molecule – an ion channel. Ervin Neher and Bert Sakmann received a Noble Prize in 1991 for development of this method. It is scheduled to examine, using the P-C technique, vacuolar ion channels of the SV type in the liverwort *Marchantia polymorpha* and in nodal cells of the algae *Nitellopsis obtusa*. Ion channels of the SV type are abundant in plants, animals (in endomembranes), and even in bacteria. So far, their activity has not been recorded in giant Charophyta internodal cells. Probably, this is a result of the way of tonoplast isolation in the form of an envelope of cytoplasmic droplets squeezed from these giant cells. One of the tasks of the project will be an attempt to isolate vacuoles from relatively small nodal cells and examination of ion channel activity using the P-C technique. Mutants of *M. polymorpha* with a knock-out gene encoding the SV/TPC-like channel will be included in the research. It will be examined how cutting off this putative way of  $\text{Ca}^{2+}$  influx to the cytoplasm affects generation and propagation of AP.

A recent study conducted on human cells has revealed that homologues of SV/TPC channels: TPC1 and TPC2 from endolysosomes are responsible, among others, for Ebola virus infection, participate in formation of new veins supplying cancer tumors, and play a role in appearance of  $\text{Ca}^{2+}$  waves in cardiac ischemia. An important task of the project will be to examine the influence of medicines against human TPC1 and TPC2 channels on plant ion channels. Research on human TPC1 and TPC2 channels face substantial technical difficulties due to problems of isolation of clear endolysosome membranes. In the case of positive results of the proposed experiments, a low-cost system will be proposed to test these important medicines in a plant model. Complementary skills and experience of Lithuanian and Polish research teams will be advantageous in reaching these scientific goals.