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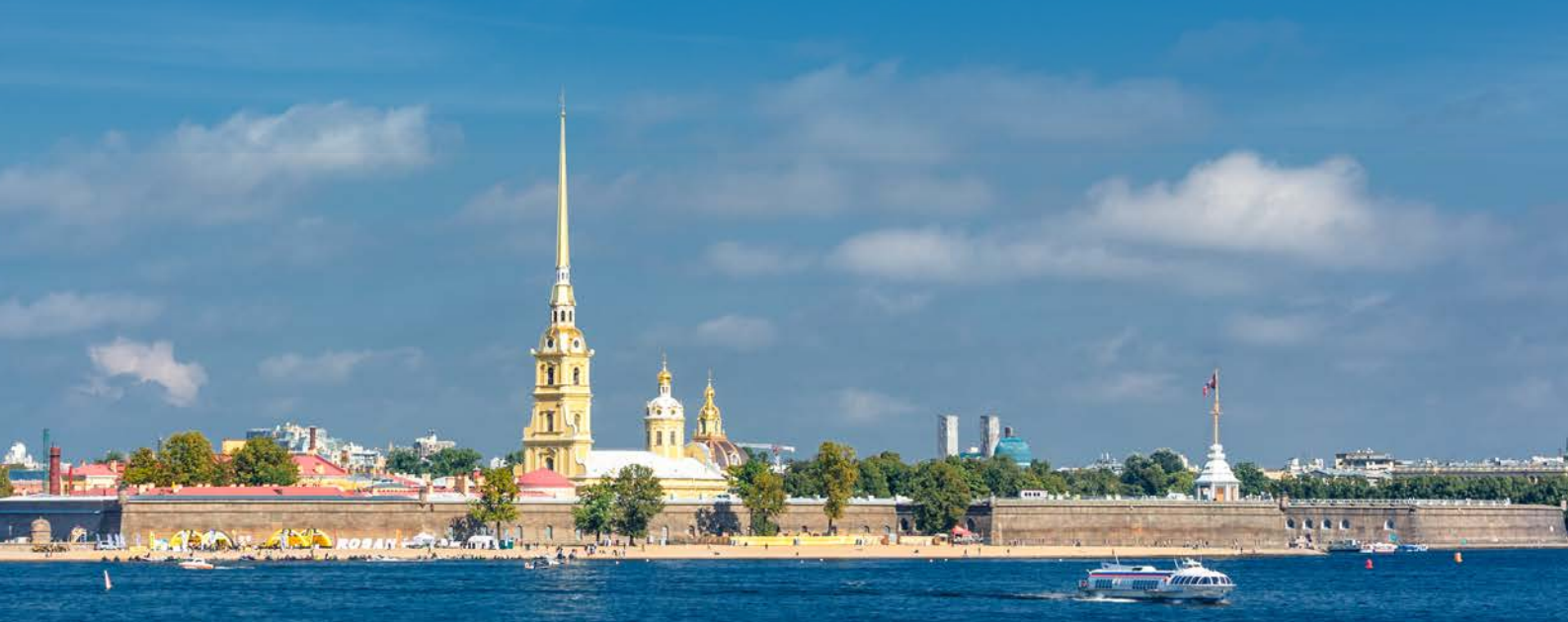

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SESSION 2

Ion Channels, Transporters and Electrophysiology

Modeling *Chara* action potential under salinity stress: similarities to animal Ca^{2+} signaling?

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Thiel and colleagues demonstrated conclusively that the all-or-none nature of *Chara* action potential (AP) is determined by formation of inositol trisphosphate (IP_3), which in turn releases Ca^{2+} from internal stores (1). The Ca^{2+}-activated Cl^- channels are the main agent of the depolarization phase of the AP. Once the Ca^{2+} is re-sequestered by the calcium pumps, the chloride conductance drops and together with depolarization-activated potassium conductance, the membrane potential difference (PD) returns to resting level. In *Chara* cells subjected to 50 mM NaCl medium, the AP duration increases from ~ 3 s to up to 30 s and the APs are often spontaneous. The lack of stimulating pulse and our faster data-logging speeds revealed a sharp positive spike at the beginning of each AP (similar spikes were also observed in APs generated with the cell in normal pond water). We hypothesize that *Chara* plasma membrane may contain transient receptor potential (TRP) channels, which can be activated by diacylglycerol (DAG) formed at the same time as IP_3 by hydrolysis of phospha-tidylinositol biphosphate. While TRP channels seem to be absent from genomes of land plants, they were found in some chlorophyte algae (2) and may be present in Characeae. The second messengers IP_3 and DAG mediate release of Ca^{2+} as well as rapid inflow of either Ca^{2+} or Na^+ from the outside through TRP channels in many animal systems. Cardiomyocytes, for instance, display AP of similar shape to Characeae with initial sharp spike due to opening of the TRP channels (3). Our simulation departs from the Thiel model (1), converting the Ca^{2+} concentration changes into change in membrane PD, to gain understanding into the increase of AP duration under salinity stress. The modeling will reveal if the Ca^{2+} pumps are affected by the rising Na^+ concentration in the cytoplasm. The recently sequenced *Chara* genome will be scanned for TRP channels and the initial spike will be modeled accordingly.

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Cation channels are sensors of ROS and oxidative stress in plants

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ROS are critically important for plants' life. They are generated by intracellular and extracellular mechanisms and accumulate in apoplastic space, the compartment with low antioxidant activities. Moderate generation of ROS is necessary for normal physiology, but their overproduction results in oxidative stress associated with damage and dysfunction of cell components (Demidchik, 2015, Environ Exp Bot). The question of sensing ROS is still open. It is proposed here that cation channels are one of prime targets of ROS in plants. They catalyse initial and very rapid sensing of ROS.

In the plasma membranes of lower and higher plants, ROS instantaneously activate two major classes of ion channels: Ca^{2+}-permeable nonselective cation channels (NSCCs) and K^+ outwardly-rectifying channels (KORs encoded by GORK). Activation of cation channels by ROS leads to dramatic influx of

Ca^{2+} for signalling and nutritional needs and K^+ loss (electrolyte leakage) inducing cell shrinkage, programmed cell death and autophagy. Ca^{2+} entry also rearranges actin cytoskeleton and modifies vesicular transport. ROS-activated ion channels reveal complex nature of activation, depending on the developmental stage and oxidative capacity of tested ROS. The transition metal binding centres have recently been identified in some members of cyclic nucleotide-gated channels, a subclass of NSCCs (Demidchik *et al*. 2014, JXB). These centres potentially produce hydroxyl radicals from H_2O_2 directly in the channel's macromolecule. Mutation in ROS-sensitive moieties in K^+ efflux GORK channel leads to decrease of ROS-sensing capacity, suggesting that distinct molecular groups are responsible for ROS sensing by ion channels. These moieties probably confer physiological properties related to ROS, such as programmed cell death and autophagy.
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Tip localized hyperpolarization activated Ca^{2+}-channels mediate pollen tube growth control via kinase-dependent anion channel regulation

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Fertilization as well as plant sexual reproduction in general and crop yield in particular rely on the successful transport of immobile sperm cells by the pollen tube (PT) to the female gametophyte. A tip focused cytosolic Ca^{2+}-gradient represents the major determinant for growth control and navigation of PTs. By simultaneously performing microelectrode based voltage-clamp measurements with live-cell Ca^{2+}- and anion imaging, we identified hyperpolarization-activated Ca^{2+}-channels (HACCs) localized at the PT apex. Activation of HACCs by hyperpolarization pulses was accompanied by (i) an increase in the apical cytosolic Ca^{2+}-concentration ($[Ca^{2+}]_{cyt}$), (ii) high anion channel activity and (iii) a decrease in the apical cytosolic anion concentration. HACC inhibition eliminated the $[Ca^{2+}]_{cyt}$ increase and abolished anion channel activity suggesting a Ca^{2+}-dependent protein kinase (CPK)-mediated mechanism of anion channel activation. Molecular and cell biology data together with electrophysiological analyses of anion channel- and CPK-loss of function mutants revealed a CPK2/20/6-dependent activation of SLAH3, ALMT12, ALMT13 and ALMT14 anion channels in growing Arabidopsis PTs. Interaction of anion channels and CPKs was demonstrated via anion current measurements and bimolecular fluorescence complementation in *Xenopus* oocytes. The observed growth retardation phenotypes in single and multiple loss-of function anion channel- and CPK mutants together corroborate the physiological significance of a kinase dependent Ca^{2+}-decoding pathway in controlling PT growth performance via anion channel activation.

The vacuolar channel TPK1 forms a complex with a regulatory kinase involved in ABA induced stomatal closure

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In response to drought, the hormone ABA causes lowered stomatal conductance. Stomata also close in response to elevated CO_2. In both cases, the vacuolar K channel TPK1 is involved, contributing to salt release from the guard cell vacuole. In spite of considerable detailed knowledge about trans plasma membrane fluxes, virtually nothing is known about the mechanism that couples stimuli such as ABA to