Plant Signaling & Behavior

4th International Symposium

Proceedings

St. Petersburg, RUSSIA

19–24 June 2016

Measurements of NaCl-, heavy metal- and hydroxyl radical-induced programmed cell death in *Arabidopsis thaliana* roots

Mackievic V.[1], Chychko A.[1], Voitsekhovskaja O.V.[2], Tyutereva E.V.[2], Samohina V.[1], Sokolik A.[1], Demidchik V.[1,2]
[1] Belarusian State University, Minsk, Belarus
[2] Komarov Botanical Institute RAS, Saint Petersburg, Russia
dzemidchyk@bsu.by

Introduction. Programmed cell death (PCD) is a crucial process in all eukaryotes which plays an important role in developmental pathways and survival of organisms. PCD is also a defense mechanism against stresses. However, it is not always beneficial to plants and can lead to severe loss in crop yield. Understanding of PCD mechanisms is essential not only for development of fundamental concepts on ontogenetic processes in plants, but also for creating means of control and stimulation of plant productivity and stress resistance.
The aim of this study was to determine the role of systems located in the plasma membrane of plant root cells in the development of stress-induced PCD.
Materials and Methods. The following lines of *Arabidopsis thaliana* L. were used: WS-0 (Wild Type: WT), *gork1-1* (lacking root K^+ efflux channel GORK) and *rhd2* (lacking root ROS-producing enzyme NADPH oxidase C). Morphological symptoms of PCD were studied in root atrichoblasts and trichoblasts (root hairs). Viability tests were conducted using Evans Blue (Sigma, USA), Fluorescein diacetate (Sigma, USA) and Nikon epifluorescent microscopy. Cell death protease activity was measured using CaspACE™ FITC-VAD-FMK *In Situ* Marker (Promega, USA).
Results. We have designed a set of robust cell morphology tests to examine stress-induced PCD. This was based on detection of protoplasm condensation, appearance of condensed dark areas in place of nuclei, plasma membrane damage, etc.. We have also adapted very sensitive test of caspase-like protease activity (FITC-VAD-fmk). These two tests showed a great sensitivity in early detection of PCD symptoms induced by oxidative stress (Cu^{2+}/L-ascorbic acid), Ni^{2+} or NaCl. The viability tests with Evans Blue and fluorescein diacetate probes displayed high sensitivity for detection of PCD induced by oxidative stress and NaCl, but these probes did not report cell death caused by Ni^{2+}. Designed techniques allowed high throughout detection of cells with PCD symptoms in different plant lines. Using these techniques, we have found that *gork1-1* and GORK with modified ROS sensing center as well as *rhd2* have delayed PCD development in response to a number of stresses. This also allowed to examine effects of pharmacological agents, such as thiourea, Gd^+, tetraethylammonium and others.
This study was supported by Russian Science Foundation grant #15-14-30008 to VD.

Redox regulation of Autophagy in plants

Minibayeva F.V.[1, 2], Ryabovol V.V.[1], Dmitrieva S.A.[1], Ponomareva A.A.[1]
[1] Kazan Institute of Biochemistry and Biophysics, Russian Academy of Sciences, 420111, Lobachevsky str. 2/31, Kazan, Russia
[2] Kazan (Volga Region) Federal University, 420000, Kremlevskaya str. 18, Kazan, Russia
minibayeva@kibb.knc.ru

Autophagy is a strictly regulated catabolic process that targets damaged or toxic components for vacuolar or lysosomal degradation. In plants, autophagy is involved in development, the response to stress, and programmed cell death. Here we review the intricate link between autophagy and reactive oxygen species (ROS). First, oxidative stress following ROS accumulation is a typical inducer of autophagy. Second, sites of ROS production and signaling are among the primary targets of autophagy. Third, intracellular redox changes can control the formation of autophagosomes by regulating the activity of autophagic (ATG) proteins. Plants use autophagy to survive oxidative stress by degrading and recycling the oxidized proteins and damaged intracellular components, including organelles. For example, our work has shown that in *Triticum aestivim* seedlings oxidative stress induced by the exogenous application of prooxidants such as paraquat and salicylic acid results in macroautophagy. Electron microscopy analysis shows that elimination of mitochondria in autophagolysosomes occurs in paraquat treated wheat roots. Disruption of the mitochondrial electron transport chain by antimycin A, an inhibitor of complex III, causes accumulation of ROS and induces autophagy. The formation of autophagosomes is controlled by the activity of numerous ATG proteins. ATG8, a multifunctional protein from the ubiquitin superfamily, is used as a molecular marker of macroautophagy. The structure of *Ta*ATG8g was found to contain W- and L-sites, necessary for the interactions of ATG8 with various ligands, including ATG4. ATG4 is a cysteine protease and considered to be a direct target for oxidation by H_2O_2. *Ta*ATG4 interacts with *Ta*ATG8 *via* the so-called AIM-motif (ATG8-interacting motif). Modification of ATG8 by the ATG4-mediated cleavage of the C-terminus, a prerequisite for the