BioTechnologia
vol. 94(3) • pp. 317-335 • 2013



**Plenary Lecture**

**PL2.1**

# New proteases of the chloroplast envelope – what do they do there?

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The repertoire of chloroplast proteases is now expanding to include not only thylakoid and stromal enzymes, but also envelope membrane ones. Homologues of the intra-membrane serine protease rhomboid, designated AtRBL8 and AtRBL9, have been found in the inner envelope membrane. Compared to other envelope proteins, their abundance is very low. Arabidopsis knockouts look mostly normal, but have been reported to demonstrate aberrant flower morphology and reduced fertility. Proteomic analysis of double mutants has shown alterations in the level of only a few proteins. One of these, allene oxide synthase (AOS), is involved in the synthesis of jasmonic acid, which is known to regulate flower morphology. However, how rhomboids regulate the level of AOS is unknown. A second envelope protease is FtsH11, a member of the family of FtsH metalloproteases found in mitochondria and thylakoid membranes. Mutants in this protease are sensitive to elevated temperatures. Complementation of mutant lines with constructs encoding proteolytically inactive enzyme, but containing the ATPase domain, suggests that it is the protease function that is essential, but how this protease is involved in thermotolerance is not clear yet. More specific functions of envelope proteases will be discussed.

**PL2.2**

# The retromer: a multiprotein complex mediating multiple functions

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Mechanisms controlling intracellular trafficking of molecules play a crucial role in cell function as they allow timely and accurate location of the molecules at the right place for completing their specific function. These mechanisms are not only important for cell fate but also for the proper development of whole multicellular organisms by positioning, maintaining or removing the tremendous variety of signaling molecules (hormone receptors, stress-sensing receptors, ion channels, immune related molecules, etc.) to/from their active sites so as to mediate cell communication. Mislocalization of molecules can cause dramatic cellular effects leading for example to severe developmental defects, diseases, or even death. Retromer is a pentameric protein complex conserved from yeast to human that localizes to endosomal membranes and is involved in intracellular protein sorting. In plants, retromer components are required for the targeting of vacuolar storage proteins and phytohormone auxin receptor recycling. We previously showed that retromer loss-of-function mutants display altered cell polarity and organogenesis defects in Arabidopsis. In this presentation, we will illustrate how the loss of a functional retromer complex alters the localization of different proteins and the phenotypic consequences of this mislocalization on seed and seedling development.

**PL2.3**

# Immune signaling and vesicular trafficking – intertwining networks regulated by E3 ubiquitin ligases

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Pathogens are perceived in plants by pattern-recognition receptors (PRRs) which are activated upon binding to pathogen-associated molecular patterns (PAMPs). Ubiquitination and vesicle trafficking are intricately involved in the regulation of immune signaling. We identified Exo70B2, a subunit of the exocyst complex which mediates vesicle tethering during exocytosis, as a target of the plant U-box type ubiquitin ligase (PUB) 22 which acts in concert with related PUBs as negative regulators of PAMP-triggered responses. Exo70B2 is required for both immediate and late responses triggered by diverse PAMPs, suggestive of a role in signaling. Exo70B2 is also required for immune responses against different pathogens. Additional candidate targets of PUB22, as well as of related ligases, are suggestive of a general function in the regulation of vesicle trafficking during immune signaling. This is consistent with the observation of a tripartite localization of PUB22, which includes motile globular compartments and association to the cytoskeleton. The activity of PUB22 is regulated by autocatalytic ubiquitination that results in high turn-over. Activation of immune signaling transiently stabilizes PUB22 protein levels. Together, we propose that PUB22 and related E3 ligases act in concert to reshape vesicle trafficking during immune responses.

**Oral Presentation**

**O2.1**

# Interplay between phosphoinositides and $Ca^{2+}$ in phototropin1- and phototropin2-mediated chloroplast movements in *Arabidopsis*

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Phototropins are plasma membrane localized UVA/blue-light receptors in plants. They control a range of responses (phototropism, stomatal opening, leaf flattening and chloroplast movements) that serve to promote plant growth and optimize photosynthetic efficiency. The model plant *Arabidopsis thaliana* contains two phototropins, phot1 and phot2. In the presence of weak blue light, both phot1 and 2 mediate chloroplast movements towards light (accumulation response). On the other hand, irradiation with strong blue light results in phot2-controlled movement of chloroplasts away from light (avoidance response). The phototropin-induced phosphoinositide metabolism has been shown to be essential for stomatal opening and phototropism. However, the role of phosphoinositides in phototropin-controlled chloroplast relocations remains poorly understood. Our aim was to determine which phosphoinositide species are involved in the control of chloroplast movements in *Arabidopsis* and to elucidate the nature of their involvement. For the above purpose, we inquired in what way the inactivation of phospholipase C (PLC), phosphatidylinositol 3-kinase (PI3K) and phosphatidylinositol 4-kinase (PI4K) affect chloroplast relocations. The inhibition of the phosphatidylinositol 4,5-bisphospahte [PI(4,5)P2] – PLC pathway, using neomycin and U73122, suppressed the phot2-mediated chloroplast accumulation and avoidance responses, without affecting movement responses controlled by phot1. On the other hand, PI3K and PI4K activities are more restricted to phot1- and phot2-induced weak-light responses. The inactivation of PI3K and PI4K by wortmannin and LY294002 severely affected the accumulation

response but had little effect on the avoidance response. The suppressive effect observed with phosphoinositide metabolism inhibitors was, at least partly due to a disturbance in $Ca^{2+}$ signaling. Using the transgenic aequorin system, we have shown that these inhibitors suppress the blue-light-induced transient cytosolic $Ca^{2+}$ rise. Besides $Ca^{2+}$ signaling, phosphoinositides, particularly PI(4,5)P2, also regulate actin-cytoskeleton dynamics. Actin filaments provide tracks for chloroplast movements in the cell. Pretreatment with neomycin showed no obvious effect on the arrangement of actin network visualized with Alexa-fluor phalloidin in mesophyll cells of *Nicotiana tabacum* under dark conditions or after blue light irradiation. A hypothetical model showing differential phosphoinositide control over chloroplast responses and the modulation of cytosolic $Ca^{2+}$ signaling by phosphoinositides has been proposed.

## O2.2

# Asparagine slows down the decomposition of autophagic bodies in sugar starved embryo axes of lupin (*Lupinus* spp)

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Experiments were conducted on embryo axes of yellow lupin (*Lupinus luteus* L.), white lupin (*Lupinus albus* L.), and Andean lupin (*Lupinus mutabilis* Sweet). Embryo axes were isolated from imbibed seeds and cultured *in vitro* for 96 h on liquid mineral medium in six trophy variants: medium with 60 mM sucrose (+S) or without sucrose (−S) and on media additionally supplemented with asparagine (+S+Asn and −S+Asn) or sodium nitrate (+S+$NO_3$ and −S+$NO_3$). Ultrastructural observations were performed in embryo axes root meristematic zone cells. Sugar starvation (−S) in embryo axes of all three investigated species (irrespectively of Asn and $NO_3$ nutrition) caused a significant increase in cell vacuolization. In vacuoles of starved cells (−S) many plasma membrane structure were observed. Simultaneously, a decrease in phosphatidylcholine level was observed. These results pointed on advanced autophagy. In cells of embryo axes fed with asparagine (−S+Asn) the decomposition of autophagic bodies was remarkably lover than in cells of embryo axes non-fed with asparagine (−S). This allowed to more or less precise identification of cell components which were degraded during autophagy. Inside vacuoles of −S+Asn axes the following cell components were visible: cytoplasm, ribosomes, oil bodies, mitochondria, and probably peroxisomes. Ultrastructure of cells of axes fed with nitrate (−S+$NO_3$ and +S+$NO_3$) was very similar to −S and +S, respectively. This work was supported by the National Science Centre (Grant No. N N310 003540).

## O2.3

# A simple bi-layer model of sunflower (*Helianthus annuus*) epidermis cell wall folding

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The inner layer of the cell wall in tissues that are under tensile stress *in situ*, shows a pattern of more or less regular folds when the tissue is isolated and plasmolysed. Such fold pattern of inner surfaces of the outer periclinal cell walls can be observed by Nomarsky microscopy or AFM (atomic force microscope). The folds are postulated to be caused by buckling of the inner cell wall layer due to the compressive force exerted on this layer by the outer wall layers, when they shrink after the removal of stresses (tissue isolation and plasmolysis). We will present a simple two layer model of the cell wall explaining the fold formation. It shows how different mechanical parameters of the cell wall layers, like Young modulus, initial stress in the cell wall layers that occurs in situ, and anisotropy of cell wall reinforcement, influence the fold pattern. The model is applied to the hypocotyl epidermis of sunflower for which

the fold pattern has been quantitatively analyzed. This work is financially supported by the MAESTRO research grant No 2011/02/A/NZ3/00079 from the National Science Centre, Poland.

**Posters**

**P2.1**

## The influence of auxin transport inhibitors on chloroplast movement and phototropin expression in *Arabidopsis thaliana*

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The role of auxin in plants and the functioning of the plant cytoskeleton have both been widely studied for many years. Recently, more and more links between the cytoskeleton and auxin are emerging. For example, both of these components take part in establishing and maintaining cell polarity. Auxin is also known to play a major role in phototropism, a process controlled by blue light via phototropins. On the other hand, both phototropins and the actin cytoskeleton are key elements in the process of chloroplast movement. These connections between auxin and blue light signaling pathways suggest the existence of a link between auxin and chloroplast movements. In this study auxin transport inhibitors were used to investigate the role of auxin in blue light-induced chloroplast relocations. *Arabidopsis thaliana* plants were cultured in vitro on inhibitor-containing media. Several different auxin influx and efflux inhibitors were tested. In mature plants the expression of phototropin 1 and 2 was evaluated at the mRNA level and chloroplast responses were measured using a photometric method. Chloroplast movement parameters were reduced and phototropin expression levels changed in some of the inhibitor-treated plants. These differences point to a modulatory role of auxin in the signaling pathway controlling chloroplast relocations.

**P2.2**

## Degradation of apoproteins of the major light harvesting complex of photosystem II (LHCII) in response to stresses is mediated by chloroplastic FtsH heterocomplex

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A proteolysis of chloroplast proteins is considered to be a vital factor influencing chloroplast structure, function and maintenance by exerting a control function over protein quality and turnover. Chloroplastic heterocomplex consisting of AtFtsH1, 2, 5 and 8 proteases, integrally bound to thylakoid membrane was shown to play a critical role in degradation of photodamaged PsbA molecules, inherent to photosystem II (PSII) repair cycle. As no other thylakoid-bound apoprotein was identified as target for the heterocomplex-mediated degradation we investigated the role of the chloroplastic FtsH heterocomplex in stress-related degradation of apoproteins of the major light harvesting complex of photosystem II (LHCII) and the modulations in an overall composition of LHCII trimers of PSII enriched membaranes (BBY paricles). We have shown that exposure of *Arabidopsis thaliana* leaves to desiccation, cold and high irradiance led to step-wise disappearance of Lhcb1 and Lhcb2 apoproteins, while Lhcb3 level remained unchanged, except for high irradiance which caused significant Lhcb3 decrease. Furthermore we demonstrated that the stress-related disappearance of Lhcb1-3 is a proteolytic phenomenon for which a metalloprotease is responsible. Using the leaf-variegated *var1-1* mutant lacking AtFtsH5 (and AtFtsH2/8 as well) we demonstrated that chloroplastic AtFtsH heterocomplex is involved in desiccation, cold and high irradiance-dependent degradation of Lhcb1 and Lhcb2 and high irradiance-dependent degradation of Lhcb3. Moreover nondenaturing isoelectrofocusing-based ana-

lyses revealed that changes in abundances of individual LHCII trimers of BBY particles of wild type plants took place following desiccation stress resulting from AtFtsH heterocomplex-dependent differential Lhcb1-3 degradation.

**P2.3**

## Structural organization of photosynthetic apparatus in mesophyll and bundle sheath chloroplasts of C4 plants of NADP-ME subtype

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In C4 plants photosynthesis take place in two different types of cells: mesophyll (M) and bundle sheath (BS). In our experiments we examinated three NADP-ME grasses: *Digitaria sanguinalis, Zea mays* and *Echinochloa crus-galli*. We investigated the organization of photosystem I (PSI) in grasses in which BS chloroplasts differ in granal composition. Using sucrose gradient centrifugation, BN-PAGE, absorption spectroscopy and low temperature fluorescence (77 K) analyses we show the differences in the cell-type specific organization and relative functioning of PSI. M and BS thylakoids from all grasses were isolated mechanically, according to Romanowska et al. 2006. To characterize further, each sucrose density fraction was analyzed by SDS-PAGE and immunoblotting. Three main Chl-containing bands were obtained for both types of thylakoids. The top fraction (LHCs) was enriched in the light-harvesting polypeptides, mainly LHCII, but also LHCI, as shown by immunoblotting. The second fraction contained a mixture of PSII and PSI core subunits. The lowest band contained the PSI core subunits and LHCI polypeptides. Our SDS-PAGE analysis showed that the PSI-enriched fraction isolated from *Digitaria sanguinalis* BS and *Echinochloa crus-galli* M and BS thylakoids contains the LHCII polypeptides, such as Lhcb2. We demonstrate that the three species from the NADP-ME subtype differ in the amount of LHCs polypeptides, especially in BS thylakoids. The amount of light harvesting complexes and content of PSI complexes is higher in *Digitaria sanguinalis* and *Echinochloa crus-galli* than in maize. Room temperature absorption spectroscopy revealed the highest similarity in Chl-protein complexes only in M and BS thylakoids of *Digitaria sanguinalis*. We did not observed the differences in the activity of PSI isolated from the lowest sucrose gradient fraction on the contrary to PSI electron transport activity in isolated M and BS thylakoids of *Echinochloa crus-galli*, *Digitaria sanguinalis* and *Zea mays* plants growing under the same light conditions (200 μmol photons $m^{-2}s^{-1}$). A higher activity of PSI in BS chloroplasts was accompanied with a larger amount of PSI-LHCI. The results show that M and BS thylakoids in C4 species, even of the same subtype, differ in organization and activities under the same light conditions during growth. These studies were financed by the grant from the National Science Centre. We thank to R. Mazur for help with fluorescence measurements.

**P2.4**

## Dynamics of excitation energy transfer in PSII particles of *Arabidopsis thaliana* mutant *clpC1*

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PSII is a dimeric complex composed of two PSII cores (containing reaction centers and internal antennae), up to six LHCII trimers (containing Lhcb1-3 apoproteins) and two copies of minor peripheral antenna complexes CP24, CP26 and CP29 (with Lhcb6, Lhcb5 and Lhcb4 as apoproteins of respective minor antenna complexes). The effect of modified composition of the antenna polypeptides on the overall excitation energy transfer rate was tested by time-resolved fluorescence with ~3,5 picoseconds temporal resolution. The main function of PSII energetic antennae,

both internal and peripheral ones is an efficient transfer of light-induced excitation energy towards P680, the primary electron donor in PSII. Small part of time-resolved fluorescence measurements with ~3.5-ps temporal resolution were performed for PSII-enriched membranes (BBY membranes) prepared from a *clpC1 A. thaliana* mutant, devoid of AtClpC1 chloroplast regulatory protein (chaperone) and from *A. thaliana* wild type plants. The mutant displayed remarkably reduced amount of Lhcb2 apoprotein whereas the level of Lhcb1 and Lhcb3-Lhcb6 apoproteins in the mutant's PSII particles remained unchanged. The results of time-resolved fluorescence studies reported in this communication allowed us to make a significant progress in understanding individual function of Lhcb2 in excitation energy transfer in PSII particles.

## P2.5

# The method to study inner cell wall surface using AFM

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Cellulose microfibrils, embedded in a matrix composed of polysaccharides and proteins, provide the cell wall reinforcement. Anisotropy of microfibril arrays is important for the cell wall mechanics. The aim of the present study was to develop a method of observation of microfibril arrays in the inner (the youngest) cell wall layer using atomic force microscopy (AFM). The inner side of onion (*Allium cepa*) epidermis cell walls was studied. In the course of material preparation the first challenge is to expose the inner cell wall surface. For this purpose cells were torn using double sided adhesive tape attached to periclinal cell walls on both sides of the epidermis. Although it causes apparent shrinking this treatment has to be preceded by tissue drying in cool air. In order to remove proteins (protoplast remnants) the samples were treated with sodium hypochlorite (NaClO); removal of matrix polysaccharides was done using sodium hydroxide (NaOH) (Dinand et al., 1996). Both treatments were performed in ultrasonic cleaner in order to improve the obtained surface quality. Since it is not known how such treatments affect arrangement of cellulose microfibrils, and how much of proteins and polysaccharides are actually removed, samples not treated with NaClO or NaOH (untreated samples) were also examined. For this purpose before the epidermal cells were torn, they had been plasmolyzed with 5% NaCl to provide access to intact inner cell wall surface. These samples were also dried. Arrangement of cellulose microfibrils in cell walls was imaged using AFM. Contact AFM imaging was performed in air. Vertical Deflection Images were used for further analysis. Other studies show that cellulose microfibrils in the dehydrated walls are thicker than those in the hydrated walls due to microfibril aggregation (Thimm et al., 2000). Thus it can be expected that cellulose microfibrils in our studies aggregate in bundles and their diameter exceeds that observed in other studies, in which microfibrils were isolated or observed in water. Therefore, the arrangement of cellulose microfibrils was compared in both treated and untreated cell walls and in the various cell wall areas. The described methods can be used to observe microfibrils but during the image interpretation an influence of specific treatments has to be considered. This work is financially supported by the MAESTRO research grant No 2011/02/A/NZ3/00079 from the National Science Centre, Poland.

## P2.6

# Mechanical properties and ultrastructure of cell walls of scarious bracts surrounding Golden Everlasting capitulum

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The mature scarious involucral bracts of *Helichrysum bracteatum* have the ability to undergo profound hygroscopic movements (Uphof 1924; own observations). These are reversible deformations in response to changes in humidity: the bracts bend outward from the capitulum center in a dry state, or inward – when wet. The bending region

is located approximately in the one third of the bract length. Our preliminary results show that the structure of bending region resembles that of other organs exhibiting hygroscopic movements. Not the entire organ is involved in providing the driving force of the movement, but only its part called the actuating motor (actuating tissue). The actuating tissue is built of an active and resistance parts, and thus resembles a thermally actuated bimetallic strip that curves in response to temperature changes (Reyssat and Mahadevan, 2009). The active part is able to contract or swell and the resistance part stabilizes the developing stress or torsion (Elbaum et al., 2008). During hygroscopic movement of the bract, cells on its abaxial surface in the bending region (active part) undergo profound deformation of shapes and sizes. The deformation in these cells is often as high as 20-40% of the initial cell surface area. It is also strongly anisotropic. The deformation is related to cell wall ultrastructure in this region (cellulose microfibril orientation), and depends on mechanical properties of cell walls. We assess mechanical properties of the actuating tissue of the bract, and try to relate them to the anisotropy of cellulose microfibril arrangement in the cell walls. First we check if there is any specific arrangement of microfibrils in the walls of active and resistance parts. To investigate cellulose microfibril orientation we use polarized light microscopy, scanning electron microscopy, and atomic force microscopy. Next we assess the mechanical properties of actuating tissue in hydrated and dehydrated states. Measurements of stiffness and strength of this tissue is performed with the aid of a tensiometer.

**P2.7**

## Why chloroplastic ferredoxin: $NADP^+$ oxidoreductase binds to lipids – model membranes study?

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Ferredoxin: $NADP^+$ oxidoreductase (FNR) is an enzyme, involved in photosynthetic electron transfer both in all plants and cyanobacteria. FNR interacts with photosystem I in linear electron transfer chain, and with cytochrome $b_6f$ or directly with thylakoid membrane in cyclic electron flow. The isoforms of FNR play a role in non-photosynthetic tissues in biosynthetic pathways. Due to its location and possible substrates, FNR was postulated to directly or indirectly bind to membrane. We have already addressed this problem in model studies and proved that FNR isolated from spinach binds directly to artificial membranes, as evidenced by Langmuir-Blodgett monolayer techniques (LB). Here we present the continuation of that research using FNR enzyme from cyanobacterium, *Synechocystis sp. PCC 6803*, expressed and purified from *E.coli* expression system. FNr from *Synechocystiss* and from spinach share only 5% amino acid sequence homology but the secondary and tertiary structure and the enzymatic characteristics are almost identical. The comparison should help in the identification of binding mechanism and its role in physiological reactions. We again applied LB technique to characterize FNR binding to selected lipids (dipalmitoylphosphatidylcholine-DPPC, monogalactosyldiacylglycerol-MGDG, digalactosyldiacylglicerol-DGDG), in two different pH conditions (pH 5 and pH 8) and for two starting surface pressure conditions (10 and 20 mN/M, respectively). The mixture of MGDG/DGDG and MGDG/DPPC, corresponding to more natural situation, were included in the tests. The MGDG and DGDG were chosen as major lipids of thylakoids of spinach and also of cyanobacterial cells. These two lipids are different in their packing parameter, and forming reversed hexagonal phase or bilayer, respectively. DPPC, which is not present in thylakoids, serves as a control for recognition mechanism – it forms bilayer, but in contrary to DGDG, it has its polar head group charged. Different pH conditions simulate changes observed in chloroplast during illumination. As we shown previously, pH change induces also conformational changes of FNR. To complete the picture, we also compared FNR binding to the membrane in presence of ferredoxin, a small protein with iron-sulfur cluster, natural reaction partner of FNR in linear electron transfer. We found mainly that FNR binds to all tested lipids. However, binding differs in rate and maximum level of surface pressure change. In general, FNR binds to lipids in all tested variant. Binding is faster in pH 5. Change in surface pressure was also more significant in lower pH. Presented work was financed by Iuventus PLUS grants no 0166/IP/2011/71 from Ministry of Science and Higher Education, Poland. Measurements were partially performed in the NanoFun laboratories co-financed by the European Regional Development Fund within the Innovation Economy Operational Programme POIG.02.02.00-00-025/09.

## P2.8

# Gamma-secretase complex in plant cells

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Gamma-secretase is a multisubunit transmembrane protein complex, which is involved in production of Alzheimer disease related β-Amyloid peptides. Gamma-secretase has an activity of intramembrane protease and it is responsible for the final cleavages of β-Amyloid Precursor Protein (APP) and length of the final product β-Amyloid. Gamma-secretase has been widely investigated within mammalian cells. The way of the complex formation, its involvement in intramembrane proteolysis, calcium homeostasis, apoptosis, synapses functioning, and intercellular junctions' formation has been described. Many substrates of γ-secretase have been discovered i.a. Notch receptor, Delta, cadherins, Erb4. Unfortunately foregoing research has not been crowned with obtaining efficient Alzheimer disease medicine. We found that homologous genes of gamma-secretase subunits (i.e. Ps1\2, APH-1, PEN-2, Nct) are present in plant genomes and in the case of *A. thaliana* we showed that these genes are transcriptionally active. Ps1\2, APH-1, PEN-2, Nct proteins localizations in plant cells endomembrane compartments like ER, AG, endosmoes was shown by the usage of WAVE cellular compartments markers. We observed that Ps1\2, APH-1, PEN-2, Nct are very often colocalized with each other within reticular and vesicular structures. Direct interactions between them by FRET-FLIM were also proved. What is more, β-Amyloid Precursor Protein (APP) was overexpressed in Arabidopsis protoplasts, either WT and ps1\ps2 double knock-out line. In result the APP cleavage within TMD has been shown in plant cells, but this process was independent from abundance or lack of presenilins (Ps). Human presenilin 1 (HsPs1) showed similar subcellular localization in plant cells as plants presenilin 1. Moreover colocalization between HsPs1 and other plants APH-1, PEN-2, Nct in protoplast was on the similar level as colocalization between plants Ps1 and APH-1, PEN-2, Nct. Our results are the first concerning Arabidopsis γ-secretase, but still functions and activity of γ-secretase in plant cells are unknown.

## P2.9

# Mass spectrometry-based identification of transglutaminase protein substrates in senescing chloroplasts of barley leaves

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Transglutaminases (TGases) catalyze the post-translational modification of proteins by establishing ε-(γ-glutamyl) links and by the covalent conjugation of polyamines. In light of the confirmed role of TGases in animal cell apoptosis, we decided to investigate the activity of chloroplast transglutaminases (ChlTGases) and the fate of chloroplast-associated polyamines in barley leaves, where the senescence process was induced by darkness (day 0) and continued until chloroplast degradation (day 12). Once the TGase catalytic activity was detected in senescing chloroplasts and we confirmed the presence of physiological protein substrates for chloroplast TGases (ChlTGases), we aimed to identify the plastid proteins modified by ChlTGase-mediated cross-linking. The autoradiography of the SDS-PAGE gel-separated plastid proteins, which were bound to polyamines such as [$^3$H]PU (putrescine) and [$^3$H]SD (spermidine), revealed 8 and 5 protein bands, respectively. Both polyamines were conjugated to multiple proteins that are involved in the organization and regulation of the photosystem apparatus and were detected in all time points, including in the control. Histone-like protein (12 kDa) was observed from days 0-7, whereas peroxiredoxin and class I heat shock protein (23 and 18 kDa, respectively) were not detected before senescence induction and appeared as TGase substrates, beginning on day 3. IAA-amino acid hydrolase (75 kDa) was observed beginning on day 5 and two proteins, starch synthase (71 kDa) and ent-copalyl diphosphate synthase (37 kDa), were visible only in the late

senescence stages (beginning on day 10). The MS/MS identification of plastid proteins conjugated with exogenous polyamines indicated that the ChlTGases are engaged in the post-translational modification of proteins involved in photosystem organization, stress response and oxidation processes, as well as in the structural reorganization of degrading chloroplasts. In summary, the results presented in this study deepen our knowledge of the elusive physiological role of ChlTGases in leaf senescence. Funding: this work was supported by the National Science Center [research grant No. N N303 418236].

**P2.10**

## Proteoliposomes vs. Thylakoids

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Plants' chloroplasts, the site of the photosynthetic reactions, contain continuous system of thylakoids' membranes. Within these membranes the stromal (unstacked) and granal (stacked) regions can be singled out. It is a common knowledge that varying light, temperature and abiotic stress conditions can change the stromal to granal regions ratio. In our previous study we showed that the chilling sensitive plant – bean has a smaller content of appressed regions (grana) than the chilling tolerant one, pea. Recently we created a 3D model of whole pea and bean chloroplasts. We showed that during magnesium ions induced aggregation pea chloroplasts contain large, distinctly separated, appressed domains, while the appressed regions present in bean thylakoids are less distinguished. So even during forced aggregation we saw distinct differences between species. For the first time we created proteoliposomes consist of galactolipids and incorporated LHCII complexes (light-harvesting complex of photosystem II) as a model for aggregation studies. The aim of this study is to visualize isolated, non-fixed aggregated thylakoids and compare them with aggregated proteoliposomes. We have shown that aggregated pea thylakoids also contain appressed regions and some long, singular membranes. Since the aggregated regions are very similar to the ones contained in intact chloroplasts described previously, a chloroplast as a whole is probably not necessary for the membrane rearrangement process to occur. Proteoliposomes' structure is very similar to the native ones, so we think that this model can be used with success as thylakoids' research simplifier.

**P2.11**

## The subcellular localization of UVR3 and two putative photolyases, PHR2 and At4g25290

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Photolyases are blue light-activated enzymes involved in repair of UVB-induced DNA damage. In *Arabidopsis* genome five genes encoding photolyases were found. The best studied is AtPHR1 having a CPD photolyase activity. The second is AtUVR3, an enzyme with a photolyase activity specific for (6-4PP)s. The third, AtCRY3 acts as a single strand DNA photolyase. Two others, PHR2 and At4g25290 have never been tested yet and their photolyase activities have been proposed only due to *in silico* analysis. Up to date, the subcellular location of only AtPHR1 and AtCRY3 has been confirmed. Whereas AtPHR1 is found in nucleus, AtCRY3 is located in both mitochondria and chloroplasts. This organellar localization of AtCRY3 is induced after UV irradiation. In our studies GFP-tagged proteins were used. Using a transient transformation of *Nicotiana benthamiana* leaves we observed a fluorescence signal under confocal microscope. AtUVR3 localized mainly in nuclei with diffuse signal and one, strongly labeled area. Thus, most probably, this photolyase is located mainly in nucleolus. AtUVR3-GFP was also present in cytoplasm and in chloroplasts. The localization did not change under UVB irradiation. Preliminary experiments have shown AtPHR2 in chloroplasts

only and At4g25290 both in chloroplasts and nuclei. It may suggest that in addition to AtCRY3 also other proteins with photolyase activity are localized in chloroplasts. However, these results need further confirmation. The study was supported by Polish National Science Centre, a grant no. UMO-2011/03/D/NZ3/00210.

## P2.12

# Functional conservation between yeast and plant mitochondrial m-AAA proteases

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AAA proteases (also called FtsH proteases) belong to a conserved family of ATP-dependent metallopeptidases with members found in bacteria, yeast, mammals and plants. FtsH proteases are membrane-bound, bifunctional enzymes in which the ATPase module with chaperon-like properties is fused to the proteolytic domain. Previously we identified and partially characterized two matrix (m)-AAA proteases (AtFtsH3 and AtFtsH10) in mitochondria of *A. thaliana*. Both proteases assemble with prohibitins into high-molecular-weight complexes similarly to their yeast counterparts (Yta10 and Yta12). To confirm that AtFtsH3 and AtFtsH10 are functional homologues of yeast m-AAA proteases, functional complementation assay in yeast was performed. Expression of *Arabidopsis* m-AAA proteases restores the respiratory deficiency of yeast cells lacking Yta10 or/and Yta12. This result indicates that both plant homologues can substitute for the function of the yeast m-AAA proteases. It is thought that respiratory disturbances that are associated with the loss of m-AAA proteases in yeast are mainly caused by a defect in processing of a ribosomal subunit MrpL32. To study if plant m-AAA proteases have a similar function, immunoblotting (using anti-MrpL32 antibodies) with mitochondria isolated from *yta10, yta12* knock-out strains transformed with plant AtFtsH3 or AtFtsH10 was performed. As controls yeast wild-type cells, disruptans *yta10* and/or *yta12* were used. This analysis revealed that yeast m-AAA mutants accumulated only precursor form of MrpL32 whereas mutant cells transformed with plant homologues resembled the wild type pattern, in which MrpL32 was cleaved to a mature form. Thus, we have shown that yeast MrpL32 is a proteolytic substrate for *Arabidopsis* m-AAA. It is possible that plant m-AAA proteases act as processing enzymes mediating maturation of proteins important for biogenesis of plant mitochondria. Further experiments are underway to reveal if the plant m-AAA proteases have a similar processing activity towards *Arabidopsis* putative homologues of yeast MrpL32 (At1g69485; At1g26740).

## P2.13

# Stabilization of actin filaments with phalloidin has no influence on chloroplast movement in *Arabidopsis* mesophyll cells

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Actin cytoskeleton is the basis of chloroplast-orientation movements. These movements are directed by blue light in the leaves of terrestrial angiosperms. In strong blue light chloroplasts perform avoidance responses, and in weak blue light they perform accumulation responses. In the present work, we studied chloroplast movements in *Arabidopsis thaliana* mesophyll cells after treatment with Alexa Fluor® 488-conjugated phalloidin. Phalloidin is an actin stabilization agent from *Amanita phalloides*. Each phalloidin molecule binds to an actin subunit and two neighboring actin subunits through hydrogen bonds and van der Waals interactions. Fluorescently labeled phalloidin provides evidence that the inhibitor enters the plant cells. In our experiments, phalloidin conjugated with Alexa 488 stained the actin cytoskeleton without fixation of the tissue. Phalloidin did not influence chloroplast movements assessed by photometric method. This suggests that F-actin alone cannot be responsible for chloroplast movements and puts in question the concept of cp-actin being the motor for chloroplast relocations.

**P2.14**

# Application of TSA technique in localization of snRNP-rich cytoplasmic bodies

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snRNPs are key components of the spliceosome involved in pre-mRNA maturation in eukaryotic cells. Biogenesis of these biomolecules includes two nuclear phases separated by cytoplasmic stage. Despite rich molecular knowledge on snRNPs formation, little is known about the spatial organization of this process on cellular level. In patricular, there is no significant data on structures responsible for UsnRNA hypermethylation and assembly of UsnRNPs. Previous *in situ* investigation on larch microsporocytes revealed cyclic occurence of cytoplasmic structures enriched in snRNPs components like U1 and U2 snRNA, Sm proteins and m3G cap snRNA. Additionally, during first stages of meiosis in these cells, assembly of cytoplasmic bodies precedes *de novo* formation of Cajal bodies in the nucleus (Smoliński et al., 2011). Here we present an application of Tyramide Signal Ampification (TSA) method in localization of snRNP-rich cytoplasmic bodies in larch microsporocytes during first meiotic prophase. This technique is an enzyme-mediated detection method that utilizes the catalytic activity of horseradish peroxidase (HRP) to generate high-sensitivity labeling of a target protein or nucleic acid sequence *in situ*. Amplification of signal with this technique enabled us to investigate molecular composition of cytoplasmic bodies in more detail, including localization of other spliceosomal UsnRNA. TSA is a useful tool in detection of weak signals, hard or impossible to localize in light microscope using standard hybridization, as well as immunofluorescence methods.

**P2.15**

# Cooperation of motor proteins with microtubules in generations of lipotubuloid's motion in *Ornithogalum umbellatum* ovary epidermis

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In plant cells, the cytoskeleton consists of two major networks of protein polymers, actin filaments and microtubules. Intracellular transport along actin filaments and microtubules depends on the associated motor proteins, myosins connected with actin filaments and kinesins and dyneins connected with microtubules. Lipotubuloids of *O. umbellatum* ovary epidermis are structures do not have their own membrane, but are surrounded by a tonoplast. Lipotubuloids are agglomerations of half-unit-membrane-surrounded lipid bodies connected with microtubules, containing ER, ribosomes and a scarce number of mitochondria, dictyosomes, microbodies and autolytic vacuoles. This structures exhibit progressive-rotary motion. The aim of the current work was to check with the use single and double immunogold method presence and co-localization of motor proteins with cytoskeleton could make a motion of lipotubuloids. Studies showed that microtubules and actin filaments of lipotubuloids might be connected with one another by myosin and kinesin. Moreover, myosin was also detected in Golgi bodies in lipotubuloid. In lipotubuloids immunosignals were also detected after the use of an antibody to dynein light chains but spectroscopy mass analysis showed that in *O. umbellatum* epidermis lacked dynein heavy chains. The presence of dynein light chains, which was revealed in lipotubuloids with the immunogold technique, is another interesting issue. However, it is not connected with a cell and lipotubuloid movement. Lack of dynein heavy chains observed in Western blot indicates that in *O. umbellatum* lipotubuloids dynein light chains did not form dynein. The activity of kinesin and myosin together with two cytoskeletal elements, microtubules and actin filaments, seems to be the driving force generating specific, variable, very dynamic and complex, autonomic rotation of lipotubuloids.

**P2.16**

# The effect of abscicsic acid and dimethyl sulfoxide and different temperatures on the cryopreservation process of *Abies nordmanniana* (Steven) Spach embryogenic callus

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Cryopreservation, as a method of plant material storage at liquid nitrogen temperature (LN – 196 °C), is a relatively safe treatment for the viability of tissues and organs. This technique is also important due to the possibility of generating a large amount of seedlings from somatic embryos formed on embryogenic callus for unlimited time in the process of somatic embryogenesis. Six *in vitro* cultured lines of Nordmann fir (*Abies nordmanniana* (Steven) Spach) embryogenic callus were used as a plant material for cryopreservation. The cryopreservation experiments were divided into two parts, i.e. the one with the application of slow freezing and thawing protocol (temperature change by 1 °C/minute) and rapid freezing and thawing (abrupt temperature changes with LN). Each of the plant tissue freezing rates was divided into two variants that differed from one another by the type of cryoprotectant used: abscisic acid (ABA) or dimethyl sulfoxide (DMSO). Pretreatment of the callus lines with low temperature (7 ±1 °C) for 8 weeks with three concentrations of ABA gave unsatisfactory results in the first part of the experiment, i.e. with the application of slow freezing and thawing procedure. Application of these conditions resulted in far-reaching dehydration of the callus cells, as ice crystals mechanically damaging the cell structure were generated. Much better results were obtained with the same cryoprotectant using the abrupt temperature changes with LN. The most optimal concentration of ABA applied during pretreatment of the callus cell lines was 25 μM/l. The callus cells showed the best recovery rate and formed somatic embryos. In the remaining concentrations of ABA (10 and 50 μM/l), the callus did not resume cell divisions and died after 8 weeks. Callus treated with DMSO as the cryoprotectant resumed cell divisions after the cryopreservation procedure, but the callus cells did not differentiate in the further stages of somatic embryogenesis, which means that somatic embryos did not develop. In contrast, the control experiments showed that regardless of the temperature combination after the cryoreservation process, all callus lines dehydrated, turned brown and did not resume cell divisions, and eventually died. The research on the Nordmann fir's callus cryopreservation showed that comparing the two tested cryoprotectants, the pretreatment-phase-introduced abscisic acid (ABA), the protectant that plays an important role in the water management regulation and adaptation to extreme environments (including low temperatures) proved to be more effective than dimethyl sulfoxide (DMSO), which is applied on a larger scale, but mutagenic cryoprotectant.

**P2.17**

# Sucrose uptake and transport are inhibited by okadaic acid during regeneration of the sugar-starved *Vicia faba* root meristem cells

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Sucrose is the main transport form of photosynthetically assimilated carbohydrate that is partitioned between different sink tissues in higher plants. Cell divisions in root meristems strongly rely on a constant supply of sucrose. Limitation of nutrient supply, by isolating the *Vicia faba* roots from cotyledons and cultivation on the medium without sucrose, results in expression of two principal control points (PCP) in meristems and blockade of the cell cycle at PCP1 in G1 and PCP2 in G2 phase. The sucrose induced resumption of replication and mitotic activity requires effi-

cient coordination of sucrose transport and metabolism as well as mechanisms that control the cell cycle progression. This resumption occurs after twelve-hour metabolic regeneration comprising increased activity of sucrose synthase (SuSy) and hexokinase (HK) as well as starch grain and cell wall matrix polysaccharide biosynthesis. Signaling network in which sucrose participates has not been precisely identified yet. It is known that the processes of protein phosphorylation and dephosphorylation, catalyzed by protein kinases and phosphatases respectively, are its essential elements. Okadaic acid (OA), the specific protein phosphatase 1/2A inhibitor, supplied at the beginning of the recovery period (0-3 h) blocks completely the above listed processes thus making the cell cycle resumption impossible. On the other hand, when added at the end (9-12 h), OA has a weak inhibitory effect. Therefore the aim of these studies was: firstly – establishing how sucrose is transported into cells; secondly – checking whether intensity of sucrose uptake by cells is the same at the beginning of the metabolic regeneration period, when metabolic processes are increased, and at the end of the metabolic regeneration period, when the reactions connected with the activation of replication and mitosis are more intensive; thirdly – finding out whether OA, blocking reactions connected with sucrose metabolism, also interferes with the process of sucrose uptake from the medium; fourthly – establishing what the distribution of sucrose taken into the cell looks like and whether OA interferes with the sucrose movement between cell compartments. The level of [3H]sucrose uptake was measured by liquid scintillation counting while sugar distribution was analyzed using microautoradiography and electron microscopy. The results showed that sucrose entered the meristematic cells along symplastic or apoplastic pathways and to a small extent through endocytosis. Not only cytoplasmic compartments (endoplasmic reticulum, vacuoles, plastids) but also nucleus was [3H]sucrose-labeled. The intensity of [3H]sucrose uptake was nearly 2-fold lower during the initial than during the final period of metabolic regeneration. OA inhibited apoplastic pathway of radioactive molecule uptake and its distribution between cell compartments implicating PP1/2A involvement in the regulation of this transport.

## P2.18

# Electron tomography technique – useful tool for plasmodesmata ultrastructure analysis in maize leaves

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Plasmodesmata are cytoplasmic microchannels (diameter of about 30 nm) linking individual plants cells and providing transport between them. In general, plasmodesmata are constructed of protein-lipid membranes and proteins, where, central rod, called the desmotubule, is the connection of ER adjacent cells. However, there are differences in the construction of various types of plasmodesmata – in the some types additional elements were found (Evert et al., 1977; Robinson-Beers and Evert, 1991). Electron tomography analysis provides three-dimensional (3D) images of subcellular objects with high resolution. The sections (usually of a thickness in the range 200-500 nm) are located in tilt-rotate holder, where electron beam passes through the sample at the following degrees rotation, usually from $+60^\circ$ to $-60^\circ$. 3D images obtained in that way can provide more information about substructure of plasmodesmata than traditional transmission electron microscopy and can be very helpful not only for ultrastructural studies but also for a better understanding of plasmodesmata functioning in plant organisms. Three dimensional shape of plasmodesmata was designed using IMOD software (http://bio3d.colorado.edu/) on the basis on electron tomography micrographs, performed by JEM 1400 (Jeol Co., Japan) equipped with a tilt-rotate tomographic holder and a high resolution digital camera (CCD MORADA; Olympus Soft Imaging Solutions, Germany) in Laboratory of Electron Microscopy of Nencki Institute of Experimental Biology, Polish Academy of Sciences, Poland. This work was supported by grant: Iuventus Plus 0036/IP1/2011/71 from the Ministry of Science and Higher Education, Poland.

**P2.19**

# DNA replication stress-induced biphasic nuclear structures in *Allium cepa* root meristem cells

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Cell division cycles are mediated through the timely activation of cyclin-dependent kinases (CDKs). In plants, the transition from G2 to mitosis is controlled by CDKB proteins peaking in late G2 and mid-M phase for the B1-type and B2-type CDKs, respectively. Prolonged treatment of *Allium cepa* root meristems with changing concentrations of hydroxyurea (HU; an inhibitor of ribonucleotide reductase) results in either premature chromosome condensation (PCC), or an abnormal course of nuclear division, characterized by the biphasic nuclear structures having condensed and decondensed parts of the nucleoplasm. The aim of the current study was to depict conditions that compromise cell cycle checkpoints and convert DNA replication stress into an abnormal course of mitosis. Continuous treatment of onion seedlings with a low concentration of HU results in shorter root meristems, enhanced production of $H_2O_2$ and accumulation of cyclin B-like (CBL) proteins. The induction of DNA double-strand breaks by HU-mediated replication arrest results in gamma-phosphorylation of H2AX histones (H2A variant), resolved as fluorescent foci comprising megabase chromatin domains nearby the sites of incurred DNA damage. Furthermore, HU-induced stress gives rise to cell nuclei resuming S phase with gradients of chromatin states characteristic for various periods of DNA replication (shown in experiments using BrdU labeling), together with axially elongated cells with interphase/mitotic structures (IM-cells). Concurrently, prolonged HU treatment extends both the relative time span and the spatial scale of H3S10 phosphorylation known in plants. The obtained data suggest that a critical cell length and a threshold level of accumulated CBL proteins are both determining factors by which the nucleus attains commitment to induce an asynchronous course of chromosome condensation. Replication stress-induced alterations in an orderly route of cell cycle events reflect a considerable reprogramming of metabolic functions combined with gradients of morphological changes of chromatin spread along the nucleus. Supported by the Ministry of Science and Higher Education (N N303 503038).

**P2.20**

# A study of pigments associated with cell walls of calli of some *Cactaceae* species

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As reported earlier [1], the appearance of bright reddish pigment(s) associated with cell walls of callus cells of *Mammillaria multiceps* (*Cactaceae*) has been observed. The pigmentation has arisen as a response to a stress; the difficulties in extraction of the pigment(s) have been noted. This study founded the similar pigmentation of cell walls of callus cells in stress conditions is not restricted to *M. multiceps* species and is inherent for at least three other cacti species of different genus – *Echinocereus reichenbachii*, *Notocactus floricomus*, *Uebelmannia pectinifera*. Several ways of pigments isolation have been worked out. Freshly isolated cell walls are preferable to use. Complete and fast washing-out of the pigments from the wet cell wall preparations has been achieved by dimethyl sulphoxide, and then followed by an isolation of the substances from the water-diluted DMSO extract by n-butanol. Partial extraction has been achieved by ethanol and chloroform treatment. The presence of three coloured compounds in cell wall preparations of *M.multiceps* callus was detected by HPLC-MS. Hydrophobic and presumably cationic nature of these substances has been noted. Similar molecular weights (m/z 283, 299 and 313), retention times and absorption spectra of the pigments may suggest their related chemical structures. Although the pigmentation appeared in stress

conditions of different types, the clearest and most regular response of calli was caused by an invasion of microorganisms, particularly soil bacteria *Bacillus subtilis* and *Agrobacterium rhizogenes*. The pigments did not show an appreciable antimicrobial activity against these bacterial strains. It has been found, however, that the substances were able to scavenge hydroxyl radicals, which may indicate their possible role in a defense system of the callus cells. We are grateful to Germplasm bank of world flora of Institute of Cell Biology and Genetic Engineering NASU for providing the initial long-term cultivated callus cultures of *Cactaceae*.

## P2.21

# Lead transport and localisation in *Lupinus angustifolius* L. root cells

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Plants exposed to lead commonly accumulate it in their tissues. The uptake and the deposition of the lead in the root tissues of *Lupinus angustifolius* were investigated using histochemical and electron microscopy techniques. Sodium rhodizonate, which forms brown-red precipitate with metal, was used to detect the lead ions. After 7 days of treatment with lead nitrate at the concentration of 100 mM the staining reaction was observed only in the root tips, while after incubation with 1000 mM concentration of the same compound the whole roots were stained. With the time of (2-48 h) with concentration 100 mM of lead, the color gradually appeared further and further from the root tip. The presence of the lead in the lupine roots has been already determined after 2 h of the incubation of the metal solution but only in the root tips. Starting from the 24 h the metal was detected along the whole length of the roots while the hypocotyls remained unstained. The most intense staining in the roots was observed after 48 h of incubation implying the presence of the huge amount of the tested metal. On the cross sections of the stained roots brown-red precipitate was observed especially in the cell walls, the protoplasts of rhizodermal and outer layer cortex cells. After elongated treatment some proto- and metaxylem cells were also stained. Localization of the lead on the ultra-structural level was determined in root meristem cells treated with 100 mM metal solution. Observations in transmission electron microscope revealed a few electron-dense deposits in cells and cellular organelles. These deposits had been already seen in the cell walls, in the intracellular spaces and in the vacuoles after short time of incubation (2-6 h). The amount and the size of the lead deposits in these cell compartments increased with the time of the metal treatment. It seems the apoplast play important role in blocking the lead entry to the protoplast, however further exposition (longer than 24 h) on lead, caused the presence of the metal deposits also in cytoplasm, endoplasmic reticulum and Golgi apparatus. The accumulation of lead deposits depends both on the metal concentration and on the time of exposition.

## P2.22

# The ultrastructure of zinc treated *Triticum aestivum* L. root meristematic cells

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The effect of $Zn^{2+}$ (0.8 mM, 4.8 mM) on the ultrastructure of 7-day-old *Triticum aestivum* root meristematic cells was estimated after 1, 3 and 7 days of treatment. The roots of seedlings kept in the standard Hoagland solution were the control. The root meristems were prepared for analysis in TEM according to the routine procedure. The meristematic cells of the control wheat roots on the $1^{st}$, $3^{rd}$ and $7^{th}$ day of experiment were characterized by typical ultrastructure. Plastids (P) had the electron-dense stroma and rare tylakoids; small starch grains were seen sporadically and no plastoglobuli were visible. Mitochondria (M) displayed electron-transparent matrix and usually narrow cristae. Single endoplasmic reticulum cisternae (ER) were running in different direction. Golgi apparatus (GA) was com-

posed of 5-6 cisternae and a few vesicles. The level of cell vacuolization was low and small vacuoles (V) were electron-transparent. Cell wall (CW) was thin and smooth. $Zn^{2+}$ at the concentration of 0.8 mM slightly enhanced cell vacuolization just after one-day-treatment. Vacuoles contained dispersed small electron-dense granules. 3-day zinc exposure led to further vacuolization and accumulation of numerous electron-dense granules that sometimes aggregated into big complexes. There were many typical M and GA cisternae with numerous vesicles around them in the cytoplasm. CW was thicker and sometimes slightly wavy. The root meristematic cells treated with 0.8 mM $Zn^{2+}$ for 7 days were significantly vacuolized. Vacuoles were filled with countless small granules and frequently large electron-dense deposits occurred. Mitochondria often turned into condensed form. Very small electron-dense granules were noticed in slightly thicker CW. Sporadically ring-shaped nucleoli occurred. $Zn^{2+}$ at the concentration of 4.8 mM triggered far more extensive vacuolization than at the lower dose. This process intensified with time of exposure. After 7 days of treatment large V were packed with numerous huge electron-dense deposits. Small granules were also seen in irregularly thickened CW. The long root exposure to high $Zn^{2+}$ concentration significantly affected cell ultrastructure. The majority of mitochondria had condensed matrix and swollen cristae. Plastids contained plastoglobuli. The cytoplasm was thinned down and lipid droplets were seen in it. Sometimes swollen ER cisternae and quite frequent ring-shaped nucleoli occurred. Concluding, 0.8 mM $Zn^{2+}$ triggered adaptative ultrastructural changes of wheat root meristematic cells leading to compartmentalization of the taken up metal mainly in vacuoles. The higher zinc dose was toxic as symptoms of cell degradation appeared.

**P2.23**

## Unraveling protein-protein interactions using fluorescence imaging techniques

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In living cells most proteins are involved in protein-protein interactions. They create complex network of reciprocal relations, enabling cells to keep their physiological processes going. Scientists have developed number of methods to study these phenomena. Most popular to date are biochemical approaches such as co-immunoprecipitation, pull-down assay or blue native-PAGE. These methods require laborious sample preparations that rely on homogenization of whole tissues and cells. In such severe conditions, most of weak or transient protein-protein interactions are lost from the result during experimental procedure. Different approach to study protein interactions rely on using live cells as an experimental environment, instead of electrophoresis buffer. Utilization of microscope, together with fluorescent proteins as markers of proteins of interest within living cells, enables to use them also in studying interactions. Most popular method is FRET microscopy which consists in excitation of fluorescent protein by a second fluorescent protein. Combining FRET with fluorescence lifetime measurement it is possible to resolve protein-protein interaction not only spatially within live cell but also qualitative and quantitative, even with capability of resolving protein-protein distances. We apply live cell imaging, FRET and its variants to unravel interactions between selected subunits of exocyst complex and proteins involved in signaling in plant cells.

**P2.24**

## Dedifferentiation of *Arabidopsis thaliana* cells is accompanied by large decrease of RNA polymerase II transcription

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After degradation of cell wall plant cells acquire totipotency. Common source of protoplasts are differentiated leaf mesophyll cells. The switching between differentiated cells and undifferentiated totipotent protoplasts is accom-

panied with dissociation of chromocenters which mainly consist of pericentric heterochromatin. It is exceptional that this dissociation of chromocenters leads to decondensation of heterochromatin but transcriptional activity of silent genomic regions, like centromeric 180 bp tandem repeat, doesn't increase (Tessadori et al., 2007). However, transcription level of other genes in isolated protoplasts remains unknown. In this step occurs transition from $G_0$ to $G_1$ phase of cell cycle and after that redifferentiation begins (Zhao et al., 2001). In the next day of protoplasts culture chromatin becomes recondensed, protoplasts passes through the next phases of cell cycle and about 3-4 days after isolation, first divisions are visible. In this study, we wanted to analyze the general level of mRNA transcription in terms of dedifferentiation and redifferentiation of plant cells. For this purpose we used *Arabidopsis thaliana* Col-0 leaf mesophyll protoplasts, in which we measured the amount of RNA polymerase II elongation form (POL II EF) by immunostaining, using monoclonal antibody specific for the phosphoserine-2 in CTD heptapeptide repeat (Chromotek). We measured the amount of examined antigen by quantitative fluorescence signal evaluation in single nuclei using confocal microscope. We have analyzed fluorescence signal from isolated and cultured 1 to 4 days protoplasts and isolated nuclei from leaf mesophyll cells as a reference. We have observed highly significant decrease in amount of POL II EF in isolated protoplasts as compared to leaf nuclei. In next days of protoplasts culture, the POL II EF amount increased gradually, reaching the level similar to leaf nuclei in 3$^{rd}$ day. In 4th day of protoplasts culture, significant decrease of POL II EF level occurs, but probably it is related to division of examined antigen amount, to two daughter nuclei during mitosis. Our results suggest that decondensation of chromatin in isolated protoplasts is not associated with transcription activation, but on the contrary we observed large decrease of mRNA synthesis level. That observed inhibition of genes transcription is related to protoplasts dedifferentiation, and probably is essential for redifferentiation into new cell type. The increase of POL II EF transcription in cultured protoplasts is related to redifferentiation and is relevant for further re-enter the cell cycle and later divisions.

## P2.25

# Photosynthetic activity of acidophilic red alga *Cyanidioschyzon merolae* under various light intensity and quality during growth

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*Cyanidioschyzon merolae* is unicellular, ultrasmall (1.5-2.0 μm in diameter) eukaryotic and autotrophic red alga lives in acidic sulfate-rich hot springs habitats, optimally at pH 2-3 and 45 °C. It contains only one: chloroplast, mitochondrion, microbody (peroxisome), nucleus and does not have cell wall. Photosystem I (PSI) of *C. merolae* is similar to that present in higher plants but can use phycobilisomes as antennae, while photosystem II (PSII) resembles that exists in *cyanobacteria*, since it contains only phycobilisomes as light-harvesting antennae. In this study, *C. merolae* were grown in artificial medium (pH 2.5, 42 °C) at low (atmospheric) and at high (5%) $CO_2$ concentration and various light conditions. The photosynthesis rate of cells and activity of photosystems (PSI and PSII) in isolated thylakoids were examined. Photosynthetic performance was also determined as a function of oxygen concentration for cells grown in white light supplemented with either red (670 nm) or far-red (720 nm) light. The cells cultivated at low $CO_2$ showed much higher rate of $O_2$ evolution than cells grown at high $CO_2$ during the illumination period. This difference and inhibitory effect of oxygen on photosynthesis were seen only for freshly harvested cells and disappeared when cells were kept for 2-3 h at atmospheric concentration of $CO_2$. Red light caused marked decrease in photosynthesis rate, compared to far-red and white light. Ethoxozolamide (EZ), an inhibitor of carbonic anhydrase (CA), had no effect on the rate of photosynthetic $O_2$ evolution for both low and high $CO_2$ grown cells. The above results clearly show that the $CO_2$-concentrating mechanism (CCM) in *C. merolae* is absent. The PSII activity was higher at low light intensity during growth in comparison with high light, whereas PSI activity did not change. We have also examined the content of PSI and PSII using HPLC, as well as the composition of chloroplast thylakoid protein complexes using BN-PAGE, but results are preliminary.

## P2.26

# Functional characterization of NRT1.2-like proteins in cucumber

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The molecular features of nitrate transport within plants and the regulation of this process have been thoroughly studied mostly in the model plant A. thaliana. It is known that active nitrate transport across plant cell membranes has been attributed to three families of membrane transporters NRT1, NRT2 and CLC. NRT1 family in plants has received particular attention during the last few years. Arabidopsis NRT1.1 (CHL1) and NRT1.2 transporters were shown to be involved in nitrate uptake from the soil solution intro root cells, but until now the characterization of nitrate transporters from other species, especially those important for agriculture is still lacking. In this work we present a comparative functional analysis of the tree NRT1.2- like protein from cucumber. We have identified in cucumber genome 3 differentially expressed homologues of Arabidopsis NRT1.2 genes. The heterologous expression of CsNRT1.2A and CsNRT1.2C in yeast *Hansenula polymorpha* lacking the endogenous nitrate transporter YNT1 restored growth of the mutant in the presence of nitrate in solid and liquid growth media. In addition, the nitrate reductase activity was clearly determined in mutants expressing both cucumber NRT1.2-like genes. The more detailed study of nitrate transport kinetics in yeast cells confirmed that CsNRT1.2A and CsNRT1.2C encode the putative nitrate transporters involved in nitrate uptake into the cells.

## P2.27

# Transcriptional activity of *Petunia* calreticulin gene (*PhCRT*) involved in pistil transmitting tract maturation, progamic phase and double fertilization

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In double fertilization, a reproductive system unique to flowering plants, two immotile sperm cells are delivered to the female gametophyte (the embryo sac) by a pollen tube. The male gametes are released into the embryo sac, usually within one of the two synergid cells commonly known as receptive synergid. One sperm cell fuses with the egg cell to generate a zygote/embryo, whereas the other fuses with the central cell to form the nutritive endosperm. $Ca^{2+}$ plays essential signaling, physiological, and regulatory roles during this multi-step process, which comprises three successive phases: pollination, progamic phase, and gamete fusion. A good candidate protein to facilitate this $Ca^{2+}$ homeostasis is calreticulin (CRT). CRT is a highly conserved and ubiquitously expressed $Ca^{2+}$-binding/buffering protein in multicellular eukaryotes. As an endoplasmic reticulum (ER) – resident protein, CRT plays a key role in many cellular processes including $Ca^{2+}$ storage and release, protein synthesis, and molecular chaperoning in both animals and plants. CRT has long been suggested to play a role in plant sexual reproduction. To address this possibility, fluorescent in situ hybridization (FISH) was used to assess *PhCRT* gene expression in different parts of the pistil before pollination, during different stages of the progamic phase, and at fertilization. The highest level of *PhCRT* mRNA was detected in the stigma of the unpollinated pistil one day before anthesis and during the early stage of the progamic phase, when pollen is germinated and tubes outgrow on the stigma. In the ovary, *PhCRT* mRNA was most abundant after pollination and reached maximum at the late stage of the progamic phase, when pollen tubes grow into the ovules and fertilization occurs. *PhCRT* mRNA transcripts were seen to accumulate predominantly in transmitting tract cells of maturing and receptive stigma, in germinated pollen and growing pollen tubes, and at the micropylar region of the ovule, where the female gametophyte is located. From these results, we

suggest that *PhCRT* gene expression correlates with secretory activity of the pistil transmitting tract cells, pollen germination, and outgrowth of the tubes, and then with gamete fusion and early embryogenesis. This project was supported by the Ministry of Science and Higher Education in Poland, grant N303 023 32/1034 (to ML) and founds provided by Nicolaus Copernicus University for the research program of the Laboratory of Developmental Biology.

## P2.28

# Architecture of the exocyst complex in plant cells

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Exocytosis is a fundamental process in all eukaryotic cells and is indispensible for cell growth and development that require delivery of components to the plasma membrane or to the extracellular environment. Exocytosis is localized process that occurs preferentially at speci c domains. Exocytotic vesicles are tethered at the plasma membrane by the octameric exocyst complex. All proteins in the complex have been conserved during evolution and exocyst complex is functional in plants. Here we show localization of exocyst subunits in living plant cells and unravel some of protein-protein interactions between exocyst subunits using microscopy approaches. Using FLIM-FRET assay we show the potential spatial orientation of the subunits within the complex. This research is supported by the Ministry of Science and Higher Education grant N N303 561539 to AK.

## P2.29

# Morphological and physiological characterization of the Δ*psaL* mutant of cyanobacterium Synechocystis PCC6803

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Cyanobacteria are prokaryotic organisms that conduct photosynthesis with the use of two photosystems (PSI and PSII). Together with other proteins and small molecular weight compounds, PSs are organized in photosynthetic electron transport chain embedded in thylakoid membrane. Respiratory electron transport chain is localized in the same membrane system in cyanobacterial cells and there are interconnections between the two electron transfer pathways on plastoquinone, plastocyanin, and cytochrom *c* levels. In contrast to plant chloroplast, where PSI was found exclusively in monomeric state, in cyanobacterial cells photosystem I and exists as both monomer and trimer. Monomer/trimer ratio is affected by various biotic and abiotic stresses. PsaL subunit is responsible for majority of the interactions between PSI monomers in oligomer. We have created deletion mutant of Synechocystis PCC6803 strain that does not produce native PsaL protein. Morphological and physiological differences between resulting Δ*psaL* strain and wild type were studied. Both strains were cultivated at 30 °C and illuminated with 50 μmol photons $m^{-2}s^{-1}$ white light. Deletion of *psaL* gene leads to a mutant with fully monomerized, photosynthetically active photosystem I complexes. No difference in cells shape or size was found on confocal microscope images. Electron microscope images however, show changes in thylakoid membrane structure due to the accumulation of granules expressing low electron density in Δ*psaL* cells. Analysis of total fatty acid composition of isolated thylakoid membranes reveals minor changes in mono- and polyunsaturated fatty acids ratio in mutant, when compared to the wild type. Inactivation of the psaL gene was accompanied by an enhanced accumulation of carotenoids in Δ*psaL* mutant cells, in comparison to WT. Neither chlorophyll accumulation nor relative PS I/PS II ratio were significantly affected by mutation. Δ*psaL* mutant exhibit slightly smaller functional photosynthetic antenna size and lower excitation level of P700 for given light intensity, than wild type. Additionally significant differences in P700 oxidation upon illumination, and re-reduction in darkness were found.