



Society for Experimental Biology Annual Main Meeting 28th June – 1st July 2009, Glasgow, UK

P1 – MEMBRANE TRANSPORT IN BIOTIC AND ABIOTIC STRESS

P1.1

09:25 Sunday 28th June 2009

Viral ion channels teach us lessons on protein sorting

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Viruses have often served as tools to solve basic questions in biochemistry and structure biology. For example many biochemical pathways have been discovered because viruses use the cellular systems for transcription, translation and protein targeting and use these pathways for their own purposes. In this way the analyses of viral pathways have helped to uncover basic cellular mechanisms, which otherwise would have been difficult to study. Likewise, because of their small size, structural studies on virus proteins have often uncovered the basic architectural features of more complex homologous proteins. In this context we have discovered two small, similar viral encoded K⁺ channels that undergo different cellular sorting; one protein (K_{esv}) is targeted to the mitochondria and the other (K_{cv}) to the plasma membrane. By creating protein chimeras and mutants we find that mitochondrial sorting of K_{esv} depends on a hierarchical combination of N- and C-terminal signals. Other than expected from canonical sorting we find that the length of the second transmembrane domain is crucial for mitochondrial targeting; extending its C-terminus by >2 hydrophobic amino acids shifts its location from a mitochondrial membrane to a plasma membrane. Only upon this modification it is possible to record K_{esv} activity on the plasma membrane of cell.

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P1.2

10:40 Sunday 28th June 2009

Extracellular ATP and NADPH oxidase regulate plasma membrane calcium channels

Julia Davies (University of Cambridge)

Extracellular ATP can be involved in regulating plant cell viability, response to pathogens (Chivasa et al., 2005) and adaptation to abiotic stress (Jeter et al., 2004). Despite the apparent absence of equivalents to animal purinergic receptor genes in higher plant genomes, plant cells respond to extracellular ATP with transient increases in cytosolic free calcium as a potential second messenger (Jeter et al., 2004; Demidchik et al., 2003). Influx of calcium across the plasma membrane generates a significant part of such transients (Jeter et al., 2004; Demidchik et al., 2003). A combination of electrophysiology, imaging and luminometry has been used here to delineate a pathway in which activation of a plasma membrane NADPH oxidase lies downstream of extracellular ATP perception in *Arabidopsis* root epidermis. The resultant reactive oxygen species then activate plasma membrane calcium influx channels (Demidchik et al., 2009). This system could be relevant to both abiotic and biotic stress responses. The identity of those channels is now under investigation.

Chivasa et al. (2005) *Plant Cell* 17, 3019–3034

Jeter et al. (2004) *Plant Cell* 16, 2652–2664.

Demidchik et al. (2003) *Plant Physiology* 133, 456–461.

Demidchik et al. (2009) *Plant J.* doi:[10.1111/j.1365-313X.2009.03830x](https://doi.org/10.1111/j.1365-313X.2009.03830x)

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P1.3

11:10 Sunday 28th June 2009

Identification of key transport processes across the nematode induced giant cell plasma membrane

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The root-knot nematode (*Meloidogyne incognita*) is a root pathogen that causes crop losses worldwide. Upon entry into roots the root-knot nematode selects several plant cells, which are induced

to grow into giant cells. The giant cells serve as essential feeding sites for the nematode throughout its lifecycle. Because the giant cell plasma membrane has few connections to surrounding cells, membrane transporters will play an important role in the uptake of specific nutrients required for nematode growth and development.

To identify the key transporters in the giant cell membranes we used the *Arabidopsis* ATH1 Genome Array. We identified many genes encoding transport proteins that were upregulated 1, 2 and 4 weeks after nematode infection (Hammes et al., 2005). We focused on transporters in an amino acid superfamily (Wipf et al., 2002). We characterized the function of specific transporters (Hammes et al., 2006; Yang et al., 2002) and studied the effects of transporter knockout on the ability of nematodes to infect *Arabidopsis* roots. Using *Arabidopsis* mutants lacking the amino acid permeases *AAP3* or *AAP6*, we tested whether disruption of amino acid transport could affect the parasitism of the nematodes. We found decreased parasitism in *Arabidopsis* lines lacking the *AAP3* or *AAP6* amino acid transporters.

Hammes, U. et al., 2005 MPMI 18: 1247–1257

Hammes, U.Z., Nielsen, E., Honaas, L.A., Taylor, C.G., Schachtman, D.P. et al., 2006 Plant J. 48: 414–426

Wipf, D., et al., 2002 Trends Biochem. Sci. 27: 139–147

Yang, Y., et al., 2002 Curr. Biol. 16: 1123–1127

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P1.4

11:40 Sunday 28th June 2009

PINOID counteractively regulates ABCB/PGP- and PIN-mediated auxin transport

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Members of the P-GLYCOPROTEIN (PGP/ABCB) and PIN-FORMED (PIN) family independently and coordinatively mediate the efflux of the auxin, IAA. A regulatory key player is the AGCVIII protein serine-threonine kinase, PINOID (PID) that has recently been demonstrated to determine polar PIN locations. However, the impact of PID-mediated phosphorylation events on the regulation of auxin transport activity remains still unclear. In a large-scale proteomic approach using the tandem-affinity purification (TAP) system, we identified PID as a component of the auxin efflux complex, characterized by the immunophilin-like FKBP42, TWD1, and its regulatory target, ABCB1. PID but not an inactive mutant version, MPID, blocked ABCB1-, ABCB1/PIN1- and ABCB1/PIN2-mediated auxin export in yeast but had no significant effect on PIN-driven export alone. The flavonoid quercetin, a well-known auxin transport and PKC inhibitor, blocked the PID effect on ABCB1. In vitro phosphorylation assays demonstrate that transport inhibition is a result of the inhibitory effect of quercetin on PID autophosphorylation. Although ABCB1 phosphorylation awaits confirmation, our data are inline with a proteomic analysis demonstrating ABCB1 phosphorylation in its regulatory linker domain.

pid protoplasts showed drastically elevated IAA transport capacities, verifying in planta the negative regulatory impact of PID in yeast. Efflux assays using co-transfected tobacco protoplasts resulted in a down-regulation of PIN1-mediated auxin efflux to vector control level. Surprisingly, ABCB1-catalyzed export was significantly enhanced in the presence of PID. In summary, we demonstrate that PID effects

counteractively ABCB- and PIN-mediated auxin transport in an action that is fine-tuned by auxin transport modulators, like flavonoids.

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P1.5

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Characterisation of PIN, AUX/LAX, and ABCB/PGP auxin transporters in a *Schizosaccharomyces pombe* heterologous expression system optimized for analysis of plant membrane proteins

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A number of heterologous systems have been used to study the activity of the PIN, ABCB/PGP, and AUX/LAX auxin transporters, but all have limitations resulting in non-functional expression of at least one major auxin transporter. Further, except for *Saccharomyces cerevisiae*, all require specialised expertise. *Schizosaccharomyces pombe* has plant-like, polarized, sterol-rich membrane domains and a more plant-like N-glycosylation mechanism than *S. cerevisiae*. As *S. pombe* has only 11 ABC transporters and single copies of many other transporter genes, we have developed an *S. pombe* expression system that includes knockouts of a number of membrane transporters, Gateway expression vectors, and epitope tags for subsequent subcellular localization of expressed proteins. We expressed *Arabidopsis* ABCB1/PGP1 and ABCB19/PGP19 in ABC knockout lines under an inducible promoter, and ABCB19 exhibited greater ³H-IAA export activity than ABCB1. After knocking out the *auxin effluxer like 1* gene, we expressed PINs and showed that PIN1 exhibits greater activity than PIN7 and PIN2. Expression of AUX1 in a permease-deficient *vat3* mutant resulted in increased net auxin uptake activity. The comparatively higher activities of AUX1, PIN1 and ABCB19 are consistent with their primary auxin transport function in *planta*. Finally, ABCB4 displayed a concentration-dependent reversal of ³H-IAA transport and substrate activation that is consistent with its physiological roles in *planta* and structural models indicating that ABCB4 has three substrate docking sites rather than the two found in ABCB1 and ABCB19. These results suggest that the *S. pombe* system can be employed for comparative analyses and structure/function tests of plant transport proteins.

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P1.6

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Plant nutrition, plant stress, and plant silicon

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The science of plant nutrition deals with the introduction into the terrestrial biosphere of 87.5% of all the chemical elements it requires, and with their transport and functions in plants. Plant nutrition thus is of the utmost importance in the chemical economy of nature. That importance, however, is not at all reflected in the enterprise of plant biological research. That neglect is puzzling because many stresses that plants exposed to the field are plant nutritional, such as mineral deficiencies and excesses. The element silicon, largely disregarded even in research on plants under stress, often plays an important prophylactic or mitigating role under just such conditions. The prevailing inattention to plant nutrition, and of silicon within that

context, is no longer tenable and ought to change. That is important not only for the sake of science per se. We must grow more food on a finite area of land, the best of which is already cropped. Denser planting on such land, or pressing marginal land into production, will put crops under stress – the very condition under which the role of silicon often looms large.

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P1.7

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Cation transport at the plasma membrane and tonoplast

Frans Maathuis (University of York)

K⁺ is the most abundant cation in plants and involved in protein synthesis, enzyme activation, photosynthesis and pH homeostasis. K⁺ is also important in phloem transport, turgor regulation, and stomatal movements. Plants require large amounts of K⁺ and the consequent need for K⁺ fertilisation in agricultural settings, constitutes a major cost for agriculture.

In contrast, Na⁺ is not essential for plants and frequently leads to salinity stress. Growing demand on fresh water supplies, irrigation with low grade water and climate change, mean that soil salinisation will increase significantly in the near future.

Salinity stress, drought stress and K⁺ nutrition are inextricably linked through competitive inhibition of K⁺ uptake in roots by Na⁺, through salinity induced osmotic stress, or through effects of K⁺ on water relations. We started to study these interactions in rice and barley to improve salt resistance, reduce K⁺ fertilisation input and drought stress. Work has concentrated on two gene families involved in Na⁺ and K⁺ transport at respectively the plasma membrane (HKTs) and the tonoplast (TPKs). Expression and patch clamp studies show that TPK isoforms in rice localise to different types of vacuole and thus may have different functions in spite of similar conductance and regulatory properties. KO mutants in OsTPKb, which localises to protein storage vacuoles, show stunted growth and reduced fertility. In contrast, overexpression of barley HvHKT1, which can transport K⁺ and Na⁺, increases salt tolerance and tolerance to K⁺ deficiency. The latter appears to occur through changes in the root shoot partitioning of both ions.

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Potassium uptake into growing barley leaf cells

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In the present study we use a combined molecular and biophysical approach to study the mechanisms through which barley (*Hordeum vulgare* L.) leaf cells accumulate potassium during growth. We clone and analyse the expression pattern of four putative potassium channels (HvAKT1, HvAKT2, HvKCO1, HvKAT2) and one putative potassium transporter (HvHAK4). Functional expression of two

candidate channels (HvAKT1; HvAKT2) in *Xenopus laevis* oocytes confirms that these channels are potassium-selective inward-rectifying channels. Growth-zone specific expression is observed in particular for HvHAK4. Channels which are tested for tissue-site of expression in leaves (HvAKT1, HvAKT2, HvKAT2) are expressed in particular in mesophyll cells. Potassium nutrition causes major and differential changes in potassium concentrations in epidermal and mesophyll cells but hardly any changes in expression of candidate genes; this points to regulation of channels at the level of the protein. Patch-clamp analyses of protoplasts isolated from the leaf elongation and mature zone support a role of HvAKT1 in potassium uptake into growing mesophyll cells. The data further suggest that channel activity is in excess of what is required to sustain growth-associated accumulation of potassium in cells.

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Molecular physiology of nitrate transport in *Arabidopsis thaliana*

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Nitrate, an essential element for plant growth and development, is taken up from soil solution by active transport through the plasma membrane of root cells. It is then translocated in the whole plant by different channels or transporters. To cope with large variations in nitrate concentrations in soils, two uptake systems coexist within plants, a low-affinity nitrate transport system (LATS) and a high affinity transport system (HATS). The NRT1 and NRT2 gene families are thought to be involved more specifically in either the LATS or the HATS system, although the ATNRT1.1 gene was shown recently to be a dual affinity nitrate transporter. In *Arabidopsis thaliana*, the NRT2 family contains 7 genes, distributed on three chromosomes, and differentially regulated at the level of organ specificity or in response to environmental conditions. This raised the question of their functional redundancy or specificity. We recently demonstrated that in addition to NRT2.1, which acts as the main component of nitrate uptake through root plasma membranes, an *Arabidopsis* NAR2 gene, homologous to *Chlamydomonas reinhardtii* genes, is also involved in this process and is essential for the presence of an active NRT2.1 protein in the cells. On the other hand, we showed that the NRT2.7 gene is predominantly expressed during seed maturation and controls seed nitrate content. Work is in progress to elucidate the role of other members of the family, NRT2.4, NRT2.5 and NRT2.6.

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P1.10

16:20 Sunday 28th June 2009

Interactive effects of potassium and nitrogen on root system architecture and expression of transporters

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Using EZ-Rhizo software for easy and quantitative analysis of root system architecture (RSA, Armengaud et al., 2009) we currently monitor the effects of different combinations of mineral nutrients on root development of *Arabidopsis thaliana*. In agreement with a strong effect of potassium (K) on nitrate uptake and assimilation in plants (Armengaud et al., in press), RSA shows a complex pattern depending on the relative amounts of K and nitrogen (N) as well as the relative amounts of N sources (e.g. nitrate, ammonium, glutamine). The observed RSA phenotypes partly mirror the dependence of transcript levels of K- and nitrate-transporters on relative K/nitrate/ammonium levels, and are therefore likely to be related to the internal nutrient status of the plant.

Armengaud, P. et al. (2009) Plant J 57: 945–956

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P1.11

10:30 Monday 29th June 2009

Handling heavy metals: How plants deal with the essentials and the toxins

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Transition metals are essential for life, where they play roles in catalysis and in stabilising protein structure. Plants often absorb from soils these essential metals that are present at very low activities. In many cases, though, transition metals are present at soil concentrations that are potentially toxic. The evolutionary strategies that enable plants to cope with these dual problems will be explored. The particular roles of the Cation Diffusion Facilitator (CDF) family of membrane proteins will be explored. CDF proteins play a special role in transition metal homeostasis, and newly-discovered features that determine their metal specificities will be presented. Prospects for improving human dietary intake of essential metals will also be discussed in the context of expression of CDF family members in plants.

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Arsenic transport in plants

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Arsenic (As) is ubiquitous in the environment and its accumulation by plants may cause phytotoxicity or transfer of As to the food chain. Arsenic poisoning affects millions of people worldwide through As exposure from drinking water and from consumption of foods. Rice is particularly efficient in accumulation of As compared with other staple food crops. Our recent studies have shown that arsenite is mobilised rapidly under the reducing conditions in flooded paddy soils, and that arsenite is taken up by rice roots mainly through the silicon (Si) pathway. Two Si transporters, a Si influx transporter (Lsi1) belonging to Nodulin26-like Intrinsic Protein (NIP) and a Si

efflux carrier Lsi2, are able to transport arsenite, which is present predominantly as uncharged neutral molecule in the normal pH range found in soils and plant cells. A number of NIP proteins are able to transport arsenite bidirectionally, dependent on the concentration gradient. Evidence will also be presented for a role of Lsi1 in the uptake of methylated As species in rice roots. In aerobic environments, arsenate is the main form of As taken up by plant roots through the phosphate transporters. Arsenate is reduced to arsenite rapidly in roots, which is then effluxed to the external medium, complexed by thiol compounds or transported to shoots. Recent progress in the understanding of these processes will be discussed.

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C³: Chloride channels, cadmium, and calcium

Frank Lanfermijer (University of Groningen), Frank Moradi (University of Groningen), Theo Elzenga (University of Groningen)

In higher plants chloride channels function in the control of the electrochemical gradients across membranes and play a role in the maintenance of ion-fluxes and signal transduction cascades. Seven *chloride channel (ClC)* genes are identified in the *Arabidopsis* genome.

To assess the function of these ClC proteins in *Arabidopsis*, we obtained three knock out mutant plants with T-DNAs inserted in the *AtClCa*, *b*, and *d* genes. Using these single mutants we made the three double and the one triple mutant plants. The single, double and triple mutant plants were exposed to various growth conditions, like low pH, high osmotics and heavy metals. We monitored the effects of the treatments on plant growth, primary root growth and morphology of roots. We also studied ion fluxes in and out of the root using the MIFE vibrating probe system.

The mutants show differences in their nitrate fluxes at the root. In all mutant plants root growth is inhibited by cadmium like in wild type. Calcium is able to alleviate this inhibition, except in one of the double mutants and the triple mutant. The roles of the different ClCs in plant and root growth are discussed.

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An assessment of ionic changes in *Chlamydomonas reinhardtii* during phosphorus deficiency and cadmium stress

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Phosphorus is an essential nutrient and its deficiency is a significant nutritional problem. The effects of altered P levels on the accumulation of essential and toxic metals such as cadmium are not fully clear. The unicellular alga *Chlamydomonas* is an excellent system in which to study the impacts of nutrient stress at the cellular level. We have used *Chlamydomonas* to assess the impact of altered P regimes and Cd stress on the cell's elemental composition. Following acute, high dosage Cd exposure, the rate of Cd uptake was highest in the early log phase of growth and this was coincident with expression of the tonoplast Cd transporter CrMRP2. The effects of longer-term

exposure to various Cd concentrations and the interaction of Cd uptake with P nutrition on trace metal accumulation were studied. High P availability (1 mM) stimulated high cell growth and consequently greater total uptake of Cd from the growth medium, however, the Cd cell load (atoms/cell) was significantly higher when cultures were P limited (10 μ M). Significant changes in cell metal content were observed when P availability was limited, including a large increase in Zn content. Moreover, Cd treatments appeared to alter the accumulation of some metals. Further investigations using a *Chlamydomonas* mutant (*psr1*) which has an impaired P starvation response, will reveal whether these changes in metal uptake are linked to known mechanisms of P scavenging. Additionally, experiments are assessing gene expression changes in response to these stresses to determine the pathways responsible for the metal changes.

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Delayed leaf senescence induces extreme drought tolerance in crop plants

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We investigated the effects of the expression of *IPT* (isopentenyl-transferase) and cytokinin production on several aspects of photosynthesis in transgenic tobacco plants grown under optimal or restricted (30% of the optimal) watering regimes. There were no significant differences in stomatal conductance between leaves from wild-type and transgenic plants expressing the *IPT* gene under the control of the *SARK* promoter grown under optimal or restricted watering. On the other hand, there was a significant reduction in the maximum rate of electron transport as well as the use of triose phosphates only in the wild-type plants during growth under restricted watering, indicating a biochemical control of photosynthesis during the growth under water deficit. The transgenic plants displayed an increase in catalase inside peroxisomes, a physical association between chloroplasts, peroxisomes and mitochondria and an increase in the CO₂-compensation point, indicating the cytokinin-mediated occurrence of photorespiration in the transgenic plants. The contribution of photorespiration to the tolerance of the transgenic plants to water deficit was also supported by the increase in transcripts coding for enzymes involved in the conversion of glycolate to RuBP. Moreover, the increase in transcripts was further enhanced in the transgenic plants grown under restricted watering conditions, indicating a cytokinin-induced increase in photorespiration and the contribution of photorespiration to protecting photosynthetic processes and playing a beneficial role during water stress. Our results indicate the possibility of generating transgenic plants with increased water use efficiency and increased tolerance to water deficit.

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Differential regulation of the genes encoding the high-affinity K⁺ transporters HAK5 of *Thellungiella halophila* and *Arabidopsis thaliana* in response to salinity

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One of the biggest challenges for a plant growing under high salinity is to ensure the uptake of essential mineral nutrients such as K⁺ while restricting the accumulation of potentially toxic ions such as Na⁺. When in addition K⁺ is a limiting factor, high-affinity K⁺ uptake systems with a high discrimination between K⁺ and Na⁺ are crucial to maintain K⁺ supply and K⁺/Na⁺ homeostasis.

Group I HAK K⁺ transporters are major contributors to high-affinity K⁺ uptake. The regulation of these genes is complex and may be mediated by responses to the tissue K⁺ concentrations, hormones, reactive oxygen species or the plasma membrane potential. These genes are highly induced in K⁺-starved roots. However in tomato plants, salinity represses *LeHAK5* expression. To characterize the regulation of this type of genes under salinity in plants differing in their salt tolerance *Arabidopsis thaliana* and its halophyte relative *Thellungiella halophila* have been employed. The results show that, as in glycophytes, *ThHAK5* is a major contributor to high-affinity K⁺ uptake in *T. halophila*. However, whereas *ThHAK5* transcripts are detected in K⁺-starved plants grown with NaCl, transcripts of the *A. thaliana* homologue *AtHAK5* are absent. In parallel, high-affinity K⁺ uptake is reduced to a lesser extent in *T. halophila* than in *A. thaliana* when plants are grown with NaCl. The results presented here indicate that plants which differ in salt tolerance show differential regulation of the expression of genes encoding K⁺ transporters.

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Systems analysis of membrane transport and homeostasis in stomatal guard cells

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Guard cells play a vital role in regulating photosynthetic CO₂ uptake and transpirational water loss from plants. The mechanisms that drive stomatal movement have been intensively studied at the level of the guard cells in epidermal peels and in intact leaves, protoplasts and membrane patches. However, our knowledge of the internal mechanisms that control the dynamic continuum of stomatal apertures is very poor. Using systems analysis, we are bridging this gap in understanding of the dynamic range of stomatal apertures and its regulation by plasma membrane and tonoplast transport. An important focus for us is to understand how transport integrates with homeostasis in response to environmental stress and how such integration determines the 'set point' for stomatal aperture. To this end, a software platform is under development for quantitative mathematical modelling of guard cell membrane transport and homeostasis involving ion and solute channels, pumps, carriers and co-transporters. Individual models of the key transporters are being programmed in the C++ language, based on the detailed kinetic information of guard cell transporters. Concurrently, we are using

imaging, electrophysiology and related techniques to examine the oscillatory behaviour of guard cells. These studies will be used to test and validate our model's predictions.

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P1.18

16:00 Monday 29th June 2009

Role of aquaporins in the regulation of membrane osmotic water permeability in maize cultured cells in normal and stress conditions

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Plant growth and development rely crucially on a fine regulation of the water transport through cellular membranes. This control can be mediated by water channels named aquaporins. We are studying the function and regulation of plasma membrane aquaporins (ZmPIPs) in maize "Black Mexican Sweet" (BMS) suspension cells. Using protoplast swelling assays and cell pressure probe experiments, we observed that the membrane osmotic water permeability coefficient (P_f) of BMS cells increases according to the culture stages (Moshelion et al., submitted). Interestingly, several ZmPIPs show higher RNA and protein levels at the end of the exponential and stationary phases. We are investigating the contribution of these ZmPIPs in the control of cell membrane permeability using isoform specific RNA interference approach. Silencing vectors were prepared and introduced in BMS cells by biolistic, and silenced cell lines were selected. When submitted to a hypo-osmotic shock, silenced protoplasts swelled at a slower rate compared to wild-type cells. The P_f of the silenced lines was therefore significantly shifted to lower values in comparison with wild-type ones, indicating that ZmPIPs play an essential role in regulating the water permeability of maize suspension cells. We are currently studying the impact of different stresses (osmotic stress, chemical compounds, temperature shock...) on the growth of the silenced lines to highlight the physiological role of ZmPIPs in maize cells exposed to changing environment.

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Molecular characterisation of protein motifs responsible for PIP aquaporin trafficking in response to abiotic stresses

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Recent studies have shown that changes in the plasma membrane (PM) water permeability of plant cells in response to diverse physiological factors like salt or oxidative stress are accompanied by a cycling of certain aquaporins between the PM and subcellular membranes. Our laboratory has shown that isoforms of the two sequence-related plant aquaporin subfamilies, PIP1 and PIP2, physically interact to regulate their trafficking. When expressed alone in maize protoplasts, ZmPIP1s are retained in the ER and ZmPIP2s are found in the PM. However, when co-expressed, hetero-oligomerization of both PIP isoforms results in ZmPIP1 re-localisation from the ER to the PM. These

data suggest that ZmPIP1s carry ER retention signals which are inefficient upon hetero-oligomer formation. To identify putative ER retention motifs we transiently (co-) expressed a vast set of mutated and chimeric proteins in maize protoplasts and the effect on trafficking was examined by confocal microscopy. Our data indicate that the loop A of PIP1;2 may contain an ER retention signal as its replacement with ZmPIP2,5 loop A leads to some extent to ZmPIP1;2 trafficking to the PM, but only if an additional ER export motif (N-terminal diacidic acid motif from ZmPIP2;5) is present. These results point towards a more complex export mechanism that is naturally governed through hetero-oligomer formation. Using the crystal structure of SoPIP2;1 as a template, amino acids potentially important for the hetero-oligomer formation were targeted and mutated, and the effect on hetero-oligomer formation investigated by monitoring the trafficking in maize protoplasts in response to diverse stresses.

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P1.20

Poster Session – Monday 29th June 2009

Osmoregulation in lily pollen involves the plasma membrane H⁺ ATPase

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Upon landing on a stigma, pollen grains start to generate a pollen tube that penetrates the stigma tissue and grows towards the ovule where fertilization takes place. The tip growth mechanism of pollen tubes is a highly regulated network of various cellular processes involving the cytoskeleton, exocytosis, cytosolic gradients of pH and Ca²⁺, ion transporter activity, signal transduction pathways and water uptake. Although the fastest growing pollen tubes will reach the ovule first, a balance between growth speed, pollen tube cell wall stability, turgor pressure and water uptake has to be established to prevent the tubes from bursting. During the way from the stigma surface to the ovule the pollen tube will be challenged by different osmotic and/or ionic conditions and therefore needs to sense and to regulate its water and ion uptake. By measuring the turgor pressure and the membrane potential simultaneously as well as the H⁺ transport activity of the plasma membrane (PM) ATPase in intact pollen grains during osmotic challenges, we observed that a hyper-osmotic shock reduces the turgor pressure, hyperpolarises the PM and acidifies the external medium via a vanadate-sensitive ATPase. On the other hand, a hypo-osmotic shock increases the turgor pressure with a simultaneous depolarisation of the PM and an alkalisation of the medium. Thus, modulation of the PM H⁺ ATPase activity upon hyper- and hypo-osmotic shock enables the pollen to modify its ion and water uptake to re-adjust the turgor pressure during tube growth.

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P1.21

Poster Session – Monday 29th June 2009

Bioaccumulation of selenium compounds and thioredoxin reductase activity in the green alga *Scenedesmus quadricauda*

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Selenium is a trace element performing important biological functions. It usually affects organisms in a strictly dosage-dependent manner being essential at low and toxic at higher concentrations. The impact of selenium on mammalian and land plant cells has been quite extensively studied. Information about algal cells is rare.

We studied the impact of selenium compounds on the green unicellular chlorococcal alga *Scenedesmus quadricauda*. Both the dose and chemical forms of Se were critical factors in the cellular response. We selected three strains of *S. quadricauda* specifically resistant to high concentrations of inorganic selenium added as selenite – strain SeIV, selenate – strain SeVI or both – strain SeIV+VI.

The total amount of Se and selenomethionine in biomass increased with increasing concentration of Se in the culturing media. The selenomethionine made up 30–40% of the total Se in biomass. The amount of selenium and SeMet in algal biomass was dependent on both the type of compound and its dose.

In both the wild type and Se-resistant strains, the activity of an enzyme thioredoxin reductase, increased rapidly in the presence of the form of selenium for which the given algal strain was not resistant. The activity of thioredoxin reductase was affected by selenium treatment in dose-dependent and toxic-dependent manner. The findings implied that the increase in TR activity in algal cells was a stress response to selenium cytotoxicity.

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P1.22

Poster Session – Monday 29th June 2009

NHX-isoforms in barley under salt stress: Expression and immunolocalization

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Na⁺/H⁺ exchangers found in plant vacuolar membranes extrude Na⁺ from the cytosol into the vacuole to maintain low cytosolic Na⁺ concentration and play crucial role in K⁺ homeostasis and vesicle trafficking. *HvNHX1-3* encodes the proteins, which are predicted to have the typical transporter topology of nine-to-twelve membrane-spanning domains with a hydrophilic C-terminal tail. The *HvNHX1-3* amino acid sequences display 70% total identity, the highest sequence homology among NHXs occurs in the N-terminal part that forms the membrane pore, whereas C-terminal domains are more dissimilar. We used barley seedlings of a salt-tolerant cultivar (Elo) as a material for our experiments. Under salt stress conditions we observed different regulation of expression both on mRNA and protein levels for all three Na⁺/H⁺ antiporter isoforms. The *HvNHX3* protein showed cluster-staining structures in control plants, though salt-treated plants showed granular distribution. We hypothesize that this transporter might be activated by changing its cluster organization. Immunolocalization of the *HvNHX1-3* in roots showed that each isoform was ubiquitously present in all root tissues. These data together with the variation in the C-terminal sequences of these antiporters probably suggest multiple regulation pathways in a cell. Finally, our results make it possible to conclude that the *HvNHX1-3* expression and activity are differently regulated by various protective mechanisms induced in barley under salt stress.

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P1.23

Poster Session – Monday 29th June 2009

A tripartite SNARE-K⁺ channel complex involved in *Arabidopsis* potassium nutrition

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In addition to their roles in vesicle delivery and fusion, a few membrane trafficking (SNARE) proteins interact with ion channels, notably mammalian Syntaxin1A which binds to specific Ca²⁺ and K⁺ channels in nerves and neuroendocrine tissues to modulate their gating properties. Such interactions have been thought to be restricted to mammalian tissues in which they serve highly specialized roles to facilitate signaling and its coupling to membrane traffic. We discovered that the SNARE protein SYP121 of the model plant *Arabidopsis* binds directly and selectively to the regulatory ('silent') K⁺ channel subunit KC1, which assembles with different inward-rectifying Shaker K⁺ channels to affect their activities. The Shaker subunits AKT1 and KC1 form heterotetramers that are involved in potassium uptake at the root hair and epidermis. We found that SYP121 promotes gating of the inward-rectifying K⁺ channel AKT1 when heterologously co-expressed with KC1, and that the SYP121–KC1 complex is essential *in vivo* for AKT1-associated K⁺ current, channel-mediated K⁺ uptake at the root epidermis and for growth. These results demonstrate a role for a SNARE as part of protein complex facilitating plant mineral nutrition and they implicate additional roles for SNARE binding to control the activity of other ion channels through the common KC1 subunit.

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P1.24

Poster Session – Monday 29th June 2009

Transcriptional and hormonal adaptation to ammonia stress in *Arabidopsis*

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Ammonium is a major nitrogen source in many plant ecosystems that is primarily taken up into roots by regulated ammonium transporters (AMTs). In the absence of nitrate, however, excess ammonium inhibits the growth of roots and shoots. Using a set of *Arabidopsis* mutants, the importance of various factors at high ammonium was tested. The alteration of the plasma membrane permeability to NH₄⁺ and NH₃ by ectopic expression of ammonium transporters was of minor importance for plant growth. To identify critical factors, the transcriptional response and adaptation of plants to exclusive ammonium, nitrate or mixed nitrogen sources was assayed using full genome microarrays. Minor transcriptomic differences were observed between mixed nitrogen and nitrate, while significant differences occurred with sole ammonium nutrition. These were largely in accordance with the physiological and metabolic adjustments that have been previously reported for

Arabidopsis and many other plants. These include transcriptional regulation of primary ammonium assimilation, down-regulation of photosynthesis, up-regulation of mitochondrial enzymes and some stress-related genes. Furthermore, novel links to cell wall biosynthesis and to hormonal control of growth by auxin, but not cytokinin, were identified. Indeed, low auxin levels were detected in ammonium grown root tips, which were deformed and swollen. The importance of auxin was highlighted by mutants that reversed the reduced root growth, indicating a primary role of hormonal control in the adaptation to ammonium, in addition to metabolic adjustments.

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P1.25

Poster Session – Monday 29th June 2009

Arabidopsis acyl-CoA-binding protein 2 enhances tolerance to cadmium-induced oxidative stress in transgenic plants

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Arabidopsis acyl-CoA-binding proteins ACBP1 to ACBP6 are conserved at the acyl-CoA-binding domain which confers binding to long-chain acyl-CoA esters. Plasma membrane-localized ACBP2 also contains C-terminal ankyrin repeats. Northern blot analysis has shown that the expression of *ACBP2* is induced by lead (Pb) but not cadmium (Cd). However, ACBP2 was observed to interact, in yeast two-hybrid analysis and co-immunoprecipitation assays, with a Cd-inducible heavy metal-binding protein AtFP6. *ACBP2*- and *AtFP6*-overexpressing transgenic *Arabidopsis* were generated by *Agrobacterium*-mediated transformation and confirmed by PCR and northern blot analysis. Both *ACBP2*-overexpressors and *AtFP6*-overexpressors were more tolerant than wild type to Cd in the growth media. *ACBP2*-overexpressors also demonstrated enhanced tolerance to hydrogen peroxide. *In vitro* assays showed that recombinant (His)₆-ACBP2 binds [¹⁴C]linoleoyl-CoA and [¹⁴C]linolenoyl-CoA, the precursors for phospholipid repair following lipid peroxidation from Cd-induced oxidative stress. These results suggest that ACBP2 is likely involved in the repair of peroxidized phospholipids at the plasma membrane.

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P1.26

Poster Session – Monday 29th June 2009

Root aquaporins – Do they matter to whole-plant water flow in barley?

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Root aquaporins have been studied extensively in the context of limiting water uptake by, and flow through plants. The implicit assumption has been that the hydraulic properties of roots actually limit whole-plant water flow. In the present study we test this

assumption on hydroponically-grown barley plants which are 14–18 days old at the time of analyses. A range of techniques is used (cell pressure probe, root pressure probe, vacuum pump, gravimetric analyses) to measure the hydraulic properties of the main components along the transport path of water through plants and to assess the significance of transcellular water flow (involving aquaporins) in roots. The capacity of roots to take up and transport water is in excess of two orders of magnitude of what is required to sustain transpirational water loss. Since aquaporins are unlikely to contribute more than 90% to radial water uptake by roots, the conclusion is that root aquaporins do not limit whole-plant water flow in hydroponically-grown barley. What are these aquaporins actually used for?

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P1.27

Poster Session – Monday 29th June 2009

Regulation of the gating mode of the *Arabidopsis* K⁺ channel AKT2 is important for adaptation to abiotic stress

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The potassium channel AKT2 is unique among the Kv channels from the model plant *Arabidopsis thaliana*. In contrast to inward-rectifying (K_{in}) and outward-rectifying (K_{out}) channels, AKT2 is able to mediate both K⁺ uptake and K⁺ release. When expressed in *Xenopus* oocytes or COS cells, AKT2 mediates K⁺ currents characterized by a leak-like component and a time-dependent hyperpolarization-activated component. Earlier studies showed that these components are carried by AKT2 channels co-existing in two different gating modes. In mode#1 AKT2 behaves as an inward-rectifying channel, whereas in mode#2 it is open in the entire physiological voltage interval. Setting the gating mode depends on post-translational modifications like e.g. phosphorylation. Now, we created transgenic plants that express only mutated AKT2 channels which are impaired in the regulation of the gating mode. These plants allow studying the physiological importance of the post-translational modifications of AKT2. Based upon detailed phenotypical analysis we can conclude that the leak-like gating mode#2 is important for proper plant growth in certain abiotic stress conditions. Plants lacking gating mode#2 are characterized by the delay in development and reduced number of leaves; features characteristic for akt2-1 knockout plants. The presented data provide definite evidence for the existence of leak-like potassium channels *in planta*. Mathematical modeling and computational simulations of transport processes in which AKT2 is involved further allow pinpointing the special role of AKT2 in certain stress conditions.

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P1.28

Poster Session – Monday 29th June 2009

Membrane transporters involved in arsenic movement in *Oryza sativa* and *Arabidopsis thaliana*

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Arsenic (As), a metalloid occurring ubiquitously in nature in organic and inorganic forms, is classified as a potent carcinogen. Rice

is the major source of dietary As intake and is often grown in areas with aquifers containing high amounts of As which are prevalent in south east Asian countries.

Currently there is a great need to understand how plants deal with As, both from the perspective as potential sources of dietary As but also as potential mechanisms for phytoremediation. We are therefore using various approaches to identify and characterise plant membrane proteins involved in transport of As.

Data from our lab showed that the *Arabidopsis thaliana* aquaglyceroporin AtNIP7;1 is a pathway for AsIII uptake in this species (Isayenkov and Maathuis, 2008). We are using *nip7;1* and other NIP loss of function mutants (*nip4;1*, *nip5;1* and *nip6;1*) to investigate their putative role in As uptake but also in As efflux. Preliminary results showed that some of the NIP isoforms may participate in As efflux and as such contribute to plant As tolerance.

The *Saccharomyces cerevisiae* (baker's yeast) gene *ACR3* is involved in AsIII efflux from the cytosol (Ghosh et al., 1999). We have transformed *ScACR3* into *Arabidopsis* and rice to assess if this can improve plant.

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P1.29

Poster Session – Monday 29th June 2009

Functional analysis of rice “two-pore” potassium channels (TPKs)

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Potassium is a major nutrient for plant growth and development. It is involved in many important physiological processes and over 50 enzymes are activated by K^+ . In addition, K^+ is important for phloem and xylem transport, turgor regulation and gas exchange through stomatal movements and general water homeostasis.

We are currently studying the role of vacuolar TPK channels in rice, globally one of the most important crops. The *Oriza sativa* genome contains 3 genes that encode two pore K^+ (TPK) channels. In order to evaluate the role of this gene family, manipulation of expression levels was established via stable plant transformation, and analysis of loss of function mutants.

Transgenic rice overexpressing OsTPKa and OsTPKb has been generated and is being analysed. Loss of function mutant lines for OsTPKb show severely retarded growth and compromised seed production.

Transient expression in protoplasts was performed to study localisation of OsTPKs channels. This demonstrated a difference in cellular localisation between OsTPKa and OsTPKb channels with OsTPKa located in the large lytic vacuole whereas OsTPKb is predominantly localised in small (protein) storage vacuoles. To gain insight into localisation features of both channels, several chimeras were created with different domains from OsTPKa and OsTPKb. To study OsTPK trafficking, experiments with the Golgi disrupting compound brefeldin were performed which demonstrated little impact on TPKb localisation but redirection of TPKa to storage vacuoles. This suggests the presence of a Golgi independent trafficking pathway to storage vacuoles.

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P1.30

Poster Session – Monday 29th June 2009

Ion-coupled cation exchangers from *Chlamydomonas reinhardtii* with roles in nutrient stress homeostasis

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Cation exchanger-type transporters play an important role in ion homeostasis in many organisms. CAX genes have been studied most extensively in higher plants and yeast. They are located predominantly at the tonoplast where they mediate the high-capacity transport of divalent cations, usually including Ca^{2+} , into the vacuole. To gain insight into the evolution and conservation of the functional and regulatory characteristics of these transporters, CAX genes have been identified and characterised from the unicellular model alga *Chlamydomonas reinhardtii*. CrCAX1 and CrCAX2 encoding putative Ca^{2+}/H^+ exchangers were cloned. Both genes were more closely related to fungal CAX genes than those from higher plants but have structural characteristics similar to plant Ca^{2+}/H^+ exchangers. When CrCAX1-GFP was expressed in yeast it localized at the vacuole. Both CrCAX1 and CrCAX2 could efficiently suppress the Ca^{2+} hypersensitive phenotype of a yeast mutant following truncation of the N-terminal tail, suggesting the existence of an N-terminal auto-regulatory mechanism. The gain of Ca^{2+} tolerance was due to proton gradient-dependent Ca^{2+}/H^+ exchange activity at the tonoplast. CrCAX1 could also provide tolerance to Na^+ stress when expressed in yeast or *Arabidopsis* due to Na^+/H^+ exchange activity, indicating that CrCAX1 can transport both monovalent and divalent cations into the vacuole. CrCAX genes were transcriptionally regulated in *Chlamydomonas* cells grown under various ionic stress conditions including Ca, Na and P stress. Furthermore, *Chlamydomonas* appears to possess genes similar to mammalian Na^+/Ca^{2+} exchangers, which are absent in higher plants. Experiments are examining whether these putative NCX transporters can mediate Na^+ -coupled Ca^{2+} transport.

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P1.31

Poster Session – Monday 29th June 2009

Effect of maize plasma membrane aquaporin phosphorylation on the channel activity, trafficking and hetero-oligomerisation

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Aquaporins are channel proteins that play an important role in regulating transmembrane water flow. We are studying the function and regulation of maize plasma membrane (PM) intrinsic proteins (ZmPIPs).

We demonstrated by immuno-precipitation and mass-spectrometry experiments that ZmPIPs were phosphorylated *in vitro* and *in vivo* and we identified phosphorylated serine residues. The role of ZmPIP2;1 serine phosphorylation on the channel activity, localization and interaction with ZmPIP1;2 was investigated in *Xenopus laevis* oocytes. We demonstrated that S126 and S203 of ZmPIP2;1 were important for protein activity and its PM localization (Van Wilder et al. (2008) *PCP*, 49, 1364). However phosphorylation was not involved in hetero-oligomerization with ZmPIP1;2. WT and mutated ZmPIP2;1 and ZmPIP1;2 fused to fluorescent tags were transiently expressed in maize mesophyll protoplasts. Protein localization was determined using a confocal microscope. WT CFP::ZmPIP2;1 and

YFP::ZmPIP1;2 were localized to the PM and ER, respectively. We showed that mutation of the phosphorylated serine residues in CFP::ZmPIP2;1 did not modify its trafficking to the plasma membrane. On the other hand, preliminary results showed a lower water channel activity for these mutants. Interestingly, YFP::ZmPIP1;2 mutated isoforms were re-located from the ER to the PM. We are currently testing polyclonal phospho-specific antibodies raised against phosphorylated PIPs. These antibodies could constitute a powerful tool to measure the phosphorylation status in different stress conditions.

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P1.32

Poster Session – Monday 29th June 2009

Characterization of plant membrane transporters involved in salt tolerance and potassium homeostasis in cereals

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Salinity tolerance in plants is a multigenic trait, with genes encoding stress responsive proteins involved in compatible solute/osmolyte synthesis, polyamine synthesis, antioxidant defence mechanisms, ion transport and compartmentalization of ions. Our research is focussed on the functional characterisation of various Na⁺ and K⁺ transporters which are believed to be relevant in plant salt tolerance. We are studying their exact physiological role in both rice and barley, particularly with regards to salt-tolerance, by employing overexpression and of loss of function approaches. To study the role of rice *OstPKs*, *OsSKOR*, *OsAKT1* and barley *HvHKT1*, a series of constructs for overexpression was made using a binary vector system, pGreen/pSoup, and transgenic overexpressing lines are currently being analysed. Rice plants overexpressing TPKa showed improved growth at low K⁺ levels when compared to wild type, suggesting that *OstPKa* plays important roles in K⁺ nutrition. Barley overexpressing the *HvHKT1* gene was also found to have improved growth at low K⁺. *HvHKT1* appears to be involved in promoting K⁺ translocation from root to shoot and the latter may explain the increased salt tolerance which was also observed in the transgenics. In addition, loss of function mutants for rice *OsAKT1* and *OstPKb* have been isolated and are also being tested to see how they respond to different salt and potassium regimes.

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P1.33

Poster Session – Monday 29th June 2009

The role of membrane and ion channel trafficking in stomatal stress responses

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SNARE proteins constitute the intracellular transport machinery that mediates membrane fusion. They are also linked to a variety of physiological responses. In particular, the plasma membrane SNARE Syp121 has been attributed a role in immune and stomatal responses (Lipka et al., 2007; Sutter et al., 2006a). *Nicotiana tabacum* NtSyp121 was shown to be linked to ABA-mediated ion channel regulation (Leyman et al., 1999) which is central to adjusting cell turgor and thus stomatal aperture. Signals such as ABA evoke rapid changes in channel

activity which represents a short-term stomatal response. By contrast, longer-term stomatal responses might be dependent on ion channel trafficking as recent data suggest. ABA-evoked internalisation of the *Arabidopsis* K_{in}⁺-channel KAT1 was followed by KAT1 recycling to the plasma membrane over a seven-hour time period (Sutter et al., 2007). Delivery and localisation of KAT1 has also been shown to be affected by both NtSyp121 and its *Arabidopsis* homolog AtSyp121 (Sutter et al., 2006b). In a project to explore the role of SNARE proteins and ion channel trafficking in adaptive stress response we are currently investigating stomatal regulation and transpiration in plasma membrane SNARE mutants of *Arabidopsis* as well as quantifying the trafficking of fluorescently tagged ion channels in these and other stress signalling mutants. Elements of this work will be presented and discussed.

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P1.34

Poster Session – Monday 29th June 2009

Na⁺/K⁺ permeability at different salt growth regimes and Na⁺ and K⁺ uptake kinetics in the halophyte *Halimione portulacoides* (L.) Aellen

Lourdes Rubio (University of Málaga), Karima Aithamou (University of Málaga), Beatriz Elena (University of Málaga), María J. García-Sánchez (University of Málaga), José A. Fernández (University of Málaga)

Halimione portulacoides (L.) Aellen, (*Atriplex portulacoides* L.) is a halophyte plant which is usually found in well-drained and upper marshes, growing in soils that can become markedly hypersaline, as those from the shallow and well-mixed Palmones River Estuary (Southern of Spain) (Rubio et al., 2003). As some other halophytes, this species accumulates high salt concentrations in leaves, being Na⁺ accumulation proportional to the external salinity (Redondo et al., 2007). In order to study the salt tolerance mechanisms of this plant, we have analysed Na⁺ transport across the plasma membrane of epidermal root cells. Electrophysiological techniques were used to estimate Na⁺/K⁺ ratio and relative plasma membrane permeability (Fernández et al., 1999) in root cells from plants grown in Hoagland nutrient solution containing different NaCl concentrations (0–75 mM). On the other hand, seeds were germinated and grown in the absence of Na⁺ and K⁺ to analyse the uptake kinetics of both ions. The results indicate that Na⁺/K⁺ relative membrane permeability decreases at high salinity and that this species shows saturation kinetics in the micromolar range for both Na⁺ and K⁺.

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P1.35

Poster Session – Monday 29th June 2009

Salt stress signalling involves ATP release and *Arabidopsis* annexin 1

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of Cambridge)

Plant salt stress signalling pathways sense increases of cytosolic free calcium (1). Under salt stress, plant cells release ATP non-lytically (2). Extracellular ATP has been found to stimulate net calcium influx at the *Arabidopsis* root epidermis and increase cytosolic free calcium in protoplasts from these cells (3). This suggests that extracellular ATP could be part of the salinity signalling network. Here we show that salt-induced release of ATP is impaired in roots of the *Atann1* mutant which lacks annexin 1 (ANN1). Annexins are small, amphipathic proteins capable of binding and inserting into membranes. Maize annexins can stimulate exocytosis (4) and form calcium-permeable channels in vitro (5). Salinity-induced increase in cytosolic free calcium was also impaired in the *Atann1* mutant. This suggests that

the lesion in signalling could be at the level of annexin-mediated exocytotic ATP release or annexin-mediated calcium flux.

Keywords: Salt stress "; Annexin; ATP signalling "; Cytosolic calcium.

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