3rd International Symposium on Plant Neurobiology

(14-18 May 2007, Štrbské pleso, SLOVAKIA)

Book of Abstracts

SOCIETY FOR Plant Neurobiology

Slovak Academy of Sciences

Faculty of Natural Sciences
Comenius University

PURO CREATIVE

PROGRAMME

Monday May 14

08:30 – 09:00	**Opening session** Fedor Čiampor: Wellcome address for the Presidium of Slovak Academy of Sciences
	Morning session (Chairman Elizabeth Van Volkenburgh)
09:00 – 09:40	Ladislav Kováč: Information in biology: A time for rethinking the fundamentals
09:40 – 10:10	Virginia Shepherd: From semi-conductors to the rhythms of sensitive plants: The research of J.C. Bose
10:10 – 10:40	Mark Staves: Responses to environmental stimuli by internodal cells of *Chara corallina*
10:40 – 11:00	Coffee break
11:00 – 11:40	František Baluška: Plant neurobiology: paradigm shift in plant sciences
11:40 – 12:10	Fatima Cvrčková: Plant intelligence: why, why not, or where?
12:10 – 12:40	Paco Calvo Garzón: Are eukaryotes truly intelligent?
12:40 – 14:00	Lunch
	Afternoon session (Chairman Dieter Volkmann)
14:00 – 14:40	Frank Telewski: A unified hypothesis of mechanoperception in plants
14:40 – 15:10	Stefano Mancuso: Spatio-temporal dynamics of the electrical network activity in the root apex. A multi-electrode array (MEA) study
15:10 – 15:40	Peter Barlow: The minimum set of cells required to enervate the 'root brains' of plants
15:40 – 16:00	Coffee break
16:00 – 16:40	Paul Galland: Mechanisms of magnetoreception in plants and fungi
16:40 – 17:10	Daniel Robert: Insect hearing and nanoscale mechanoreception
17:10 – 17:30	**General Discussion**
20:00	Welcome party

Tuesday May 15

	Morning session (Chairman Viktor Žárský)
09:00 – 09:40	Akihiko Nakano: Roles of endocytosis regulation in plant physiology and development
09:40 – 10:10	Lukáš Synek: EXO70A1, a putative exocyst subunit, is important for polar growth and plant development
10:10 - 10:30	Jan Martinec: Inositol trisphosphate receptor in plants – is it real?
10:30 – 11:00	Coffee break
11:00 – 11:40	Bruce Veit: Stem cell signaling networks in plants
11:40 – 12:10	Patrick Masson: A novel class of microtubule-binding proteins control root growth behavior and anisotropic cell expansion in Arabidopsis
12:10 – 12:40	Przemyslaw Wojtaszek: Domain-specific cell wall-plasma membrane interface
12:40 – 14:00	Lunch
	Afternoon session (Chairman Bruce Veit)
14:00 - 14:40	Julian Schroeder: Guard cell ion channel signaling
14:40 – 15:10	Nan Yao: Endogenous programmed cell death triggers in plants
15:10 – 15:40	Toshiaki Mitsui: Plastid targeting of glycoproteins in rice cells
15:40 – 16:00	Coffee break
16:00 – 16:40	François Chaumont: Plant aquaporin regulation and cell signaling
16:40 – 17:10	Thomas Paul Jahn: Controlled and facilitated diffusion of H_2O_2 as a potential mechanism involved in signaling and ROS scavenging
17:10 – 17:40	Sakiko Okumoto: The role of glutamate in plants and its potential function as a signaling molecule
17:40 – 18:10	Frank Ludewig: Plant GABA metabolism - approaches to identify genes *in vivo*
18:10 – 18:40	Ian B. Cole: Indoleamines and flavonoids in neuroprotective plant physiology
19:30 – 22:00	Poster session with beer and wine

Wednesday May 16

	Morning session (Chairman Julian Schroeder)
09:00 – 09:40	Jutta Ludwig-Müller: Indole-3-butyric acid as a signal in early events of arbuscular mycorrhizal associations
09:40 – 10:10	Günther Scherer: A role for phospholipase A in auxin gene regulation and auxin responses. The receptor may not be TIR1
10:10 – 10:30	Michal Grunt: Evolutionary history of the domain architecture of plant formins
10:30 – 11:00	Coffee break
11:00 – 11:40	Teun Munnik: Phospholipid-based signaling - 'seeing is believing'
11:40 – 12:10	Susan Murch: The role of human neurotransmitters
12:40 – 12:40	**General Discussion**
12:40 – 14:00	Lunch
	Afternoon session (Chairman Teun Munnik)
14:00 – 14:40	Axel Mithöfer: Jasmonates as inducers of Ca^{2+} signals in the nucleus and the cytosol of plant cells
14:40 – 15:10	Viktor Žárský: Plasma membrane NADPH oxidases (NOXs) in plants – beyond ROS signaling
15:10 – 15:40	Jianping Hu: The role for PEX11 and dynamin-related proteins in Arabidopsis peroxisome proliferation
15:40 – 16:00	Coffee break
16:00 – 16:40	Jinxing Lin: Myosin and actin function in directing mitochondria movement in living pollen tubes of *Picea wilsonii*
16:40 – 17:10	Sonia Philosoph-Hadas: Actomyosin-mediated gravisensing and early transduction events in gravistimulated snapdragon spikes
17:10 - 17:40	Sergio Mugnai: Temporary changes in gravity conditions affect oxygen influx at root level
17:40 – 18:30	**General discussion (especially on the name Plant Neurobiology)**
19:30 – 22:00	Poster session with beer and wine

Thursday May 17

	Morning session (Chairman Mary-Jane Beilby)
09:00 – 09:40	Minoru Ueda: Chemical factors inducing leaf-movement in *Fabaceae* and carnivorous plants
09:40 – 10:10	Arnaldo Schapire: Vesicular trafficking as a mechanism of abiotic stress tolerance in plants
10:10 – 10:40	Amit Levy: A plasmodesmata associated ß-1,3-glucanase in Arabidopsis regulates plasmodesmata function
10:40 – 11:00	Coffee break
11:00 – 11:40	Ralph Hueckelhoven: Cellular polarization for membrane dynamics in interaction of barley with pathogenic *Blumeria graminis*
11:40 – 12:10	Hans Thordahl-Christensen: Syntaxin SYP121 is involved in a number of pathogen defence mechanisms
12:10 – 12:40	Yangdou Wei: Mining iron for host defense and pathogen virulence
12:40 – 14:00	Lunch
	Afternoon session (Chairman Irene Lichtscheidl)
14:00 – 14:40	Mary-Jane Beilby: Action potentials in Charophytes
14:40 – 15:10	Alexander Volkov: Electrophysiology of Venus flytrap (*Dionaea muscipula* Ellis)
15:10 – 15:40	Mary A. Bisson: Effects of acetylcholine on the blue-light response of dark-grown Arabidopsis seedlings
15:40 – 16:00	Coffee break
16:00 – 16:30	Edgar Wagner: Photoperiodic adaptation by systemic control of growth and rates and planes of cell division via systemic electrophysiological communication from the cellular to the organismic level
16:30 – 17:00	Elizabeth Van Volkenburgh: Mesophyll cells are the driving force for light- and acid-induced leaf blade expansion of *Pisum sativum* var. *Argenteum*
17:00 – 17:30	Ed Etxeberria: The linear phase of sucrose uptake concentration curve in sink organs is largely mediated by fluid phase endocytosis
17:30 – 18:00	Miroslav Kaminek: Cytokinin oxidase/dehydrogenase activity in oat xylem sap
18:00 – 18:30	**General discussion**
20:00	Farewell party

Friday May 18

	Morning session (Chairman Wilhelm Boland)
09:00 – 09:40	Mark Mescher: Host-location by parasitic plants
09:40 – 10:10	Renata Bogatek: Allelochemicals as a signaling molecules in the negative plant-plant interaction
10:10 – 10:40	De Oliveira R.F.: Chemical communication between roots and shoots in tomatoes
10:40 – 11:10	Ralf Oelmueller: Molecular analysis of the interaction between *Arabidopsis thaliana* and the growth-promoting fungus *Piriformospora indica*
11:10 – 11:30	Coffee break
11:30 – 12:10	Ton Timmers: Common cellular mechanisms of endosymbiotic root infection
12:10 – 12:40	Charlotte Poschenrieder: Neurotoxicity of aluminium: parallelism between plants and animals (including men)
12:40 – 13:10	Heiko Maischak: Ion channel-forming compounds in caterpillar regurgitate: A way to manipulate the plant plasma membrane potential during herbivory?
13:10 – 13:40	Lukas Schreiber: Cutinized and suberized plant/environment interfaces: structure, biosynthesis and function
13:40 - 14:00	**Closing session**
14:00	Lunch

GENERAL TOPICS

Information in biology: a time for rethinking the fundamentals

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All biological species live in their own species-specific world (Umwelt), delimitated by their sensors. They acquire knowledge in their species-specific manner and construct their own species-specific reality. The human species is no exception. Humans live in a world of medium dimensions (macroworld). The worlds of small dimensions (microworld), of large dimensions (megaworld), and of great complexity (multiworld) are inaccessible to them, lying outside Kant's barriers [1]. Humans are the exceptional species on Earth due to artefacts: artefacts empower humanity to gain knowledge on the world that exists behind the boundary erected by human biological sensors. To describe this unfamiliar world, humans use the concepts of their life world (Lebenswelt), and these concepts function as metaphors [2]. Science is replete with metaphors no less than is art. Biology of the second half of the 20th century has been dominated by the metaphor of information. It has been customary to consider cognition at the exclusive property of humans, with the human mind as an organ of conscious perception, thinking, and memory, busy with "information processing". Cognition has often been analyzed in terms of formal systems, and, accordingly, it has been thought that, in principle, cognition might be embodied in any kind of "hardware", including the human-made computers. Upon new discoveries in biology (restricted number of genes, not much different in flies, plants, and humans; organization of genes, proteins and metabolites as scale-free networks; histone code; the heredity of frames; multiple controlling roles of small RNAs) the paradigm of information in biology may need a revision.

(1) The notion of "information" is vague not only in common life, but in biology itself. It should be used in an unambiguous restricted manner introduced by Claude Shannon. He defined information entropy, or uncertainty, in terms of well defined question Q (in which all possible answers ΣAi are implicated) and an apriori knowledge Ka (assigning probabilities Σpi to the answers) as $S(Q|Ka) = - \text{const} \Sigma\, pi \ln pi$. Information I in a message is difference between two entropies, one associated with apriori knowledge before a message Ka and another associated with aposteriori knowledge Kp after a message: $I = S(Q|Ka) - S(Q|Kp)$ [3]. It is obvious that information is a variable the magnitude of which depends on the nature of well defined question. As the question depends on the receiver, information is a subjective quantity. A different question is being posed by a communication engineer, a human patient addressing a physician, a sperm cell heading toward an ovum, a ribosome translating messenger RNA, a molecular sensor specific for a particular ligand. Information is measured in bits, but it is neither a thing nor a sequence of digits or other units. It can be neither "processed" nor "stored". What can be stored, embodied in sequences of DNA, in texts, in structures, is knowledge, not information. Information need not be compared with "substances" like matter or energy, but rather with "processes" like heat and work. Information is a specific, subject-dependent process of transforming data into knowledge.

(2) Life on Earth, natural (n-) life, is not a formal system, but a chemical system, ruled and constrained by the laws of chemistry. Chemical interactions differ fundamentally from other kinds of interactions, such as mechanical combinations of Lego parts. Chemistry is a science of emergence. Electromagnetic interactions between atoms produce profusion of molecules, with properties qualitatively distinct from those of their constituents. Molecules combine and/or self-assemble into supramolecular structures. Specific conditions of the environment admit specific sets of chemical construction processes, driven thermodynamically or kinetically. But the environment has also another function: it selects, or, to use a more telling metaphor, culls the products of the processes, according to their stability in the environment, their capacity to

"survive" under the particular conditions. This capacity is largely dependent on a degree of isomorphism, functional rather than structural, between the products of the constructions processes and the environment. This isomorphism represents knowledge. Selection for stability introduces into chemical dynamics the second "time arrow", in addition to the first one imposed by the second law of thermodynamics: evolution. Chemical evolution on Earth has produced chemical systems with particularly great stability, which we call, somewhat arbitrarily, living systems. To maintain stability, organisms are unceasingly performing ontic work, assisted by epistemic work. A specific manner of maintaining stability is the reproduction of a system as a whole, or of its "construction plans", or "construction algorithms", present in the form of genes, frames, memes, or other kind of "book-keepers" [4]. They all are storing knowledge, not information. Biological evolution is a progressing process of knowledge acquisition (cognition) and, correspondingly, of growth of complexity. The acquired knowledge is embodied in constructions of organisms. The structural complexity of those constructions which carry embodied knowledge corresponds to their epistemic complexity [1].

(3) There are two kinds of knowledge and of cognition and, correspondingly, two kinds of well posed questions. There is knowledge that reduces uncertainty; the corresponding questions are inquisitory questions, and the process of knowledge acquisition consists in assimilating data by the process of information. Another kind of knowledge serves to reduce ignorance; its corresponding variables are exquisitory questions and exformation (which enables novel inquisitory questions). Because of steady accumulation of knowledge, biological evolution is advancing as a Bayesian ratchet. There is much less "information processing" than it is assumed by the "life-as-information" or "life-as-computation" metaphor [5]. Constructions at all levels, from protein molecules, through cells, tissues, individual organisms, up to social institutions and culture, represent embodied knowledge. Triggering of pre-determined responses (usually as one-bit information) seems to be a more appropriate description of life functioning than information processing.

(4) The information metaphor in biology rendered a valuable service in unravelling processes of protein synthesis and topogenesis and in deciphering nucleic acid and protein sequences. In the postgenomic era, the concept of information may become retardant or misleading. Organisms may be viewed as multihierarchical chemical systems, consisting of loosely bound modules. In their evolution, distinct selections operate at each level of hierarchy. Biological individuality is hierarchically nested, from molecular sensors up to individual organisms, communities, species and terrestrial life as a whole (Gaia), an individual at each level of hierarchy being a distinct cognitive subject engaged in ontic and epistemic work. At the deepest and most elementary level, the loosely bound modules constitute sets of molecular engines. Engine, work, embodied knowledge, and triggering may become four metaphors of a new conceptual armoury.

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From semi-conductors to the rythmns of sensitive plants: the research of J.C. Bose

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Jagadish Chandra Bose (1858-1937) was one of India's first modern scientists, and one of the world's first biophysicists. His work with semi-conductors, radio, and microwave technology, published between 1985 and 1900 in journals including the *Proceedings of the Royal Society*, the "*Philosophical Magazine*" and "*The Electrician*", was well-respected then, and remains so today. In 1900, after winning the admiration of physicists such as Rayleigh and J.J Thompson, Bose crossed the border into plant biophysics. He became a controversial figure in the West. Inventing unique instruments for simultaneously measuring bioelectric potentials and for quantifying plant movements, Bose studied plants that made rapid movements, such as the touch-sensitive *Mimosa pudica* and the Indian Telegraph plant *Desmodium*, as well as "ordinary" plants that did not make obvious rapid movements (e.g. *Nauclea*, the mango and the carrot). Against the tide of the times, Bose concluded that plants and animals have essentially the same fundamental physiological mechanisms. All plants have a well-developed nervous system. All plants co-ordinate their movements and responses to the environment through electrical signalling. All plants are sensitive explorers of their world, responding to it through a fundamental, pulsatile, motif involving coupled oscillations in electric potential, turgor pressure, contractility, and growth. Bose's overall conclusion that plants have an electromechanical pulse, a nervous system, a form of intelligence, and are capable of remembering and learning, was not well received in its time. A century later, some of these concepts have entered the mainstream literature.

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Insect hearing and nanoscale mechanoreception

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In animals, sensory systems can operate at the limits of what is considered physically possible (1). The mechanosensitive neurons of auditory systems are sensitive to extremely low levels of incident stimulus energy. In effect, thresholds of detection can be at energy levels close to thermal noise (kBT), or some 4•10-21 Joules (4zJ). In insects, hearing has been shown to be exquisitely sensitive, relying on mechanically well balanced receivers (2) capturing sound energy associated with a collection of neurones sensitive to mechanical stimuli (3, 4). In mosquitoes and Drosophila, the hearing organs are the antennae, with a mechanosensory organ at their base – Johnston's organ (5). For these animals, as for any other insect and crustacean, hearing relies on the mechanotransduction performed by ciliated neurones embedded in multicellular assemblies called scolopidia. Previous research has shown that active mechanisms are at work in the hearing organs of vertebrates (review in 6), enhancing sensitivity to faint sounds and sharpening frequency selectivity. In *Drosophila* and the mosquito *Toxorhynchites brevipalpes*, hearing has been shown to be an active mechanism and several nonlinear response characteristics have been identified, such as nonlinear dynamic compression and autonomous vibrations (7, 8). Functionally, these active sensory mechanisms contribute to the nanoscale sensitivity and the response dynamics of the auditory organ (9). In insects, the basis of such active mechanisms resides in the mechanical motility of the mechanoreceptive neurons; the first neurons demonstrated to be mechanically motile (8). The molecular machinery subtending motility in insect mechanosensitive neurons is not entirely known, but it is deemed to rely on the function of the axonemal structure of the ciliated scolopidial neurons. Using mutant analysis in *Drosophila*, it was shown that the action of the motor molecule dynein on microtubules pairs was required, and that the transducer channels nompc contributed to the nonlinear, active response (10). Interestingly, the oscillation energy of the entire receiver –eg the antenna- could be shown to fluctuate above thermal noise under the concerted action of the receptor neurons (7-10). This suggests that cellular metabolism alone can modulate or adjust the sensitivity of response coherence of the receptor cell. It also shows that the process can take place at low energy levels. A telltale sign of such process is the presence of autonomous vibrations (7). Remarkably, autonomous vibrations have been observed in yeasts (11), yet their function is still unknown. In the opinion of the author it is likely that nanoscale mechanical vibrations will be discovered in yet other organisms. Whether they are related to the reception of mechanical energy remains to be tested. Enticingly, the increasingly refined knowledge on plant mechanoreception (see contributions by F. Telewski and F. Baluska) offers novel possibilities for comparative research. Because of their respective experimental amenability at the genetic level, tantalizing comparisons could be drawn between mechanoreception in *Drosophila* and that of *Arabidopsis*. The outcome of future research will be an integrated and general understanding of mechanisms of cellular motility and information processing, including the processes of energy dissipation and transduction at the molecular, cellular and systemic levels.

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Plant neurobiology: a paradigm shift in plant sciences

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Sensory plant biology and plant electrophysiology were two lively disciplines up until the 1970s (Bünning 1959, Haupt and Feinleib 1979) but then, for somewhat obscure reasons, they showed no further development. In the last few years, however, there have been numerous advances in plant sciences which necessitate not just a revival of plant sensory biology but also the introduction of plant neurobiology (Baluška et al. 2006). First of all, and contrary to all 'mechanistic' predictions based on the high turgor pressure of plant cells, endocytosis has been found to be an essential process of plant cells which impinges upon almost all aspects of plant life (Šamaj et al. 2005, 2006). Moreover, recent advances in the plant molecular biology have identified, besides classical neurotransmitters, also several proteins typical of animal neuronal systems, such as acetylcholine esterases, glutamate receptors, GABA receptors and endocannabinoid signalling components, as well as indicating signalling roles for ATP, NO and ROS (Baluška et al. 2006). Importantly, plant action potentials have turned out to control processes such as actin-based cytoplasmic streaming, plant organ movements, wound responses, respiration and photosynthesis, as well as flowering (Wagner et al. 2006, Fromm and Lautner 2007). Last, but not least, there have been significant advances in ecological studies on plant-plant and plant-insect communications, in behavioral studies on memory and learning phenomena in plants (Trewavas 2005a,b), as well as the revelation that complex plant behaviour implicates neuronal signal perception, processing, and the integration of ambient signals.

Plants perform neuronal-like computation not just for rapid and effective adaptation to an ever-changing physical environment but also for the sharing of information with other plants of the same species. Plants societies increase their immunity to damage after receiving warnings from attacked neighbours (Engelberth et al. 2004, Ton et al. 2007). Strategies involve, among others, the release of volatiles which then attract the enemies of the attacking herbivores (D'Alessandro et al. 2006). Moreover, there are examples of 'war-like' phenomena whereby invading plants kill other plants via the release of toxic allelochemicals from their root apices (Bais et al. 2006). That this hostility can be caused by root apices of other plants is a new discovery. However, roots are also well known for their ability to avoid dangerous places by actively growing away from hostile soil patches. Also in war-like mode, the root apices of parasitic plants actively recognize the roots of their prey, grow towards them and then, in order to gain control over them, send out root-hair-like processes that later develop into parasitic haustoria (Tomilov et al. 2005). Thus, by using a vast diversity of volatiles, plants are able to attract or repel diverse insects and animals, and thereby are able to shape their biotic niche. The number of volatile compounds released and received by plants for biotic communication is immense, requiring complex signal-release machinery, as well as an unprecedent 'neuronal' decoding apparatus for correct interpretation of received signals. These aspects of plant activity have not yet been much studied.

The plant neurobiological perspective reveals several surprises when the classical plant hormones like auxin, abscisic acid, ethylene, and salicylic acid are considered from this angle. Auxin and abscisic acid elicit immediate electric responses if applied to plant cells from outside (Pickard 1984, Felle et al. 1991, Roelfsema et al. 2004, Pei and Kuchitsu 2005), suggesting that their regulated release within plant tissues may be a part of neurotransmitter-like cell-to-cell communication (for auxin see Schlicht et al. 2006). Abscisic acid signaling pathway is conserved between plants and animals and this signalling molecule both stimulates and is endogenously produced in human granulocytes in a way suggesting that it acts as endogenous proinflammatory cytokine (Bruzzone et al. 2007). Importantly, biologically active abscisic acid was isolated from brains of vertebrates (Le Page-Degivry et al. 1986) indicating possible roles of abscisic acid in

the central nervous system. Salicylic acid activates similar subset of MAPKs as voltage pulses (Link et al. 2002). Ethylene, a classical plant hormonone, is an anaesthetic (Campagna et al. 2003), a fact that plant physiologists have ignored. Interestingly, anaesthetics used on animals including man, induce anaesthetising effects on roots similar to those of ethylene (Powell et al. 1973). Ethylene is released in mechanically stressed plant tissues, and structurally diverse anaesthetics activate mechanosensitive channels (Martinac et al. 1990, Patel and Homore 2001, Patel et al. 2001). As ethylene is released immediately after wounding, it might act to relieve 'pain' in plants. Similarly, ethanol is known to relieve pain (Benedikt et al. 2007), and plants, especially roots, synthesize ethanol under stress conditions such as hypoxia and anoxia. There are numerous other plant-derived substances which manipulate the pain receptors in animals, such as capsaicin, menthol, camphor. Interestingly, the monoterpene volatiles, menthol and camphor induce oxidative stress and inhibit root growth in maize (Zunino and Zygadlo 2004), indicating that they, too, act as plant signalling molecules. Finally, plants express inhibitors that are specific to the neuronal nitric oxide synthases (Lowe et al. 2007, Osawa et al. 2007). Another example of neuronal behavior of plants is the report that prevention of nyctinastic movements of leguminous leaves causes their death while leaves allowed to 'sleep' stayed healthy (Ueda and Nakamura 2006). This resembles the situation in animals (Cirelli et al. 2005). Although melatonin was discovered in plants more than ten years ago (Kolár and Machácková 2005, Arnao and Hernandez-Ruiz 2006, Pandi-Perumal et al. 2006), there no scores for melatonin in the highest ranking plant journals, despite the fact that it is biochemically closely related to auxin. Melatonin mimics auxin in the induction of lateral root primordia from pericycle cells (Arnao and Hernandez-Ruiz 2007).

The Arabidopsis genome encodes ten NADPH oxidases (RbohA-J) of which six are expressed only in root apices (A, B, C, E, G, I) and two (D, F) are expressed in whole seedlings including the root apices (Sagi and Fluhr 2006). Expression of eight of these molecules in root apices makes this one of the most complex signal-mediated ROS-generating organs. It is currently unknown for what developmental and signalling purposes so many different NADPH oxidases in roots are needed. A similar perplexing complexity, unique also for root apices, concerns polar auxin transport. Five types of PIN molecule (PIN1,2,3,4,7) are expressed in root apices (Blilou et al. 2005), whereas only one PIN (PIN1) is sufficient for the morphologically more complex shoot apices (Reinhardt et al. 2003, Reinhardt 2006)! What, then, is so special about root apices? This is a tough question, but answers seem to be emerging in the multitude of recent data, not easily interpretable by the classical plant physiological approach, but comprehensible from the approach of plant neurobiology (Baluška et al. 2005, Brenner et al. 2006). One of them involves the idea that the transition zone of root apices acts as some kind of 'command centre' (Baluška et al. 2004).

Despite a relatively simple body organization, plants need sophisticated sets of coordinative processes. Besides their root-shoot coordination, there is also need for coordination amongst radial tissues, especially within and between the cortex and stele. Action potentials run preferentially in an axial direction and they link root and shoot apices. Despite the modular and apparently decentralized organization of the plant body, there are several critical situations requiring 'centralized' decisions, such as, for instance, the onset of flowering as well as the onset and breakage of dormancy. Although these decisions are based on information retrieved via numerous distant organs, they imply some central 'processor' which would reliably control the whole plant body. Importantly, any wrong decision would have detrimental consequences for the whole plant. Moreover, internal circadian pacemakers of animals are located in their brains. The transition zone of root apices is the only zone in the plant body showing 'brain-like' oscillatory patterns of cellular activities responding also to leaf wounding (Mancuso and Marras 2006). Moreover, cells of this zone are the only ones to express up to five different PIN efflux carriers (Verbelen et al. 2006, Bandyopadhyay et al. 2006). Across the F-actin and myosin VIII-enriched plant synapses (Baluška et al. 2005), PINs drive complex transcellular patterns of polar auxin transport. As this auxin transport is driven via vesicular secretion (Schlicht et al. 2006), auxin elicits electrical responses in adjacent cells (Felle et al. 1991), and it synchronizes cell activities within a cell file (Nick 2006, Maisch and Nick 2007), auxin fulfils the minimum criterion for being a neurotransmiter-like signalling molecule in plants.

Human perception of the outside world relies on a so-called 'neural code' which links sensory signals and neuronal responses. Similarly, in plants, numerous parameters of the physical environment, especially, light, temperature, and gravity, are continuously monitored. Polar auxin transport translates perceived and processed sensory information into adaptive physiological and

motoric responses. New concepts are needed, and new questions must be asked, for advancing our rudimentary understanding of the communicative nature of sensory plants.

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Plant intelligence: why, why not, or where?

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The concept of plant intelligence, as proposed by Anthony Trewavas, has raised considerable discussion that has contributed also to the birth of plant neurobiology (see Trewavas 2002, 2003, 2004, 2005a,b, Firn 2004, Brenner et al. 2006). However, plant intelligence remains loosely defined, and, as a result of attempts to persuade its opponents, it became either practically synonymous to Darwinian fitness ("adaptively variable behaviour" or "ability of an individual to perform in its environment"), or reduced to a mere decorative metaphor. A more strict view can be taken, with emphasis on individual memory and learning. Even this has to be done cautiously, the main problem being the definition of memory itself. To qualify as memories, traces of past events have to be not only stored, but also actively accessed (or at least accessible). We propose a variety of Occam´s razor approach for eliminating false candidates of possible plant intelligence phenomena in this stricter sense: a particular behavior of the plant may be considered "intelligent" only if it cannot be approximated by an algorithmic model that does not require recourse to stored information about past states of the individual or its environment. Re-evaluation of the phenomena previously presented as examples of plant intelligence shows that only some of them pass our test, while others do not.

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Are eukaryotes truly intelligent?

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Although Plant Neurobiology emphasizes the interdisciplinary effort whose ultimate target is the study of the complex patterns of behaviour of plants *qua* information-processing systems, it is not clear what we mean by "information-processing system". Most researchers adopt a computational perspective, according to which information-processing boils down to the manipulation of symbols /subsymbols according to algebraic or statistical rules. We may nevertheless adopt an embodied, embedded perspective (Thelen and Smith, 1994), and interpret information-processing systems in non-computational and/or non-representational terms (Calvo Garzón, in press). According to this view, cognition is to be understood in the continuous interplay of brain, body and environment. In my talk, I propose to study the integration of contemporary scientific knowledge in Cognitive Neuroscience (Gazzaniga et al., 2002) and Plant Neurobiology (Baluška et al., 2006) under this lens in order to assess whether eukaryotes can be interpreted as genuinely intelligent.

Trewavas has defended the integration of scientific knowledge of plants and animals in a number of works (2005; and references therein), arguing that plants do indeed count as intelligent organisms in much the same way as animals do. However, although sympathetic to Trewavas (2005) position, I shall turn his framework upside down. In particular, I shall consider time-estimation in relation to the distinction between online plant behaviour (flower heliotropism) and offline plant behaviour (leaf heliotropism) - specifically, plants' nocturnal reorientation in the absence of solar-tracking (Schwartz and Koller, 1986). This case neatly illustrates why plants and animals' (anticipatory) competencies can indeed be interpreted as two sides of the same coin: Both animals and plants can solve complex problems and react adaptively to environmental contingencies. In my view, nevertheless, once an embodied-embedded picture is granted, all eukaryotic organisms, although subject to an information-processing analysis with individual cells as computational building blocks, should be interpreted in non-computational terms. This view will allow us to reassess Trewavas' insights under a new light. An embracing picture of eukaryotic anticipatory capacities will allow us to place amoebae, plants, and animals (human and non-human) along a continuum. Once we look at the shared cellular and molecular mechanisms of these life forms, we have a reason to unify the knowledge obtained along the spectrum; in non-computational and non-representational terms, however. The ultimate aim is to discuss the sort of novel predictions that such a model may generate in order to test intelligence.

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A unified hypothesis of mechanoperception in plants

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The ability to sense and respond to physical environmental stimuli is of key importance to all living things. Among the common environmental stimuli detected by living organisms are light, temperature, and a variety of chemical signals. A number of environmental stimuli appear to be closely related and can be considered as physical-mechanical stimuli, requiring the perception of a differential mechanical force or pressure gradient by the living cell. These include the perception of gravity, self-loading and internal growth strains, mechanical loading, touch, sound, and the state of hydration within a cell (turgor pressure). Recent advances have lead to the proposal of a plant-specific mechanosensory network within plant cells that is similar to that previously described in animal systems (Jaffe et al. 2002, Baluška et al. 2003). This sensory network is the basis for a unifying hypothesis which may account of the perception of numerous mechanical signals including gravitropic, thigmomorphic, thigmotropic, self-loading, growth strains, turgor pressure (drought and flooding stress), xylem pressure potential, and sound (Telewski 2006). The current state of knowledge of a mechanosensory network in plants is reviewed and considerations given to two different mechanoreceptor models: a plasmodesmata-based cytoskeleton-cell membrane-cell wall (CMCW) network (Baluška et al. 2003) vs. stretch-activated ion channels (Ding and Pickard 1993, Pickard and Fujiki 2005). Post-mechanosensory physiological responses to mechanical stresses are also reviewed along with recommendations for directing future research in the area of mechanoperception and response.

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Spatio-temporal dynamics of the electrical network activity in the root apex. A multi-electrode array (MEA) study.

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Root cells have been a popular research tool for decades because they allow easy access to individual cells for electrophysiological recording and stimulation, pharmacologic manipulations and high resolution microscopic analysis. However, it is technically difficult to record from and stimulate more than three cells using standard intracellular microelectrodes, and those cells usually die within minutes or, barely, hours. Thus any distributed/synchronized electrical activity is missed without a multi-unit approach. Multi-electrode arrays (MEAs) provide a tool to record from and stimulate many cells (up to hundreds) of the same root apex, concurrently and non-invasively. Since the array substrate is made of transparent glass, cell morphology can be easily monitored by the use of an inverted microscope or using fluorescent labels and a confocal. Here, for the first time in plant science, we use a 60-channels MEA to study in thick root apex slices the spatio-temporal characteristics of the electrical network activity. We observed an intense spontaneous electrical activity as well as stimulation-elicited bursts of spikes locally propagating. Our data indicate that synchronous activity of the cells emerges spontaneously throughout the time evolution. The strict similarity of the electrical behaviour recorded with the behaviour showed by neural cell culture may reflect an intrinsic capacity of the root apex to generate functional networks.

The minimum set of cells required to enervate the 'Root Brains' of plants

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When a root system – which includes the collective 'heads' of a plant – burrows down into the soil, all its 'brains', which are continually being created as the root system extends and ramifies, have to be supplied ('enervated') with nervous tissue [1] that connects with the posterior portion of the plant. So, two important questions are: what are the minimal tissue and neuro-physiological systems that enervate a 'root brain', and how are these systems continuously developed?

A minimal 'nervous' tissue can be discerned in so-called 'hair roots' – modified roots which are characteristic of the plant families Ericaceae and Epacridaceae [e.g., 2, 3]. Published papers on hair-root anatomy [e.g., 3] allow analysis of the structure of the plerome (vascular cylinder), a tissue constituting the Channel and Net of the nervous system which supplies the root brain at the root apex. To support this anatomical analysis, a stereotypic cell lineage of the plerome was gleaned from the literature on *Arabidopsis* [4]. Here, the plerome is derived from four apically located stem cells. Each stem cell gives rise, via radial divisions, to one quadrant of plerome whose cell numbers are subsequently amplified by transverse divisions. One pair of self-similar plerome quadrants contains both xylem and phloem cells; the other pair of quadrants contains xylem only; present in all four quadrants are parenchyma and pericycle cells, some of which are probably essential for root nervous function.

On the basis of the above analysis of *Arabidopsis*, we can now see that the anatomy of the hair roots helps to define the minimal plerome tissue composition which is required by any root. Thus the hair-root plerome indicates that hair roots can be of two types: the first contains just one of the xylem-only quadrants; roots of the second type bear one of the xylem-plus-phloem-containing quadrants (which include one sieve tube plus a strand of companion cells, and one file of tracheids). We presume that the single xylem-containing quadrant could not support much root growth, and so roots of this first type are expected to have a limited life. By contrast, one xylem-plus-phloem-containing quadrant can evidently support viable root extension and solute uptake, as well as providing an integrated 'nervous' function for the plant.

Although aspects of our analysis pertaining to hair roots are somewhat conjectural owing to lack of direct evidence from this material, especially from the crucial embryogenic stages of development, we suggest that a root which contains only one of the four plerome quadrants has had three of its plerome stem cells deleted sometime after the establishment of the complete root organ either in the embryo or in a lateral root primordium. The remaining single plerome stem cell can support development of a root if the tissue derived from it includes phloem. However, such a root has a much reduced diameter – i.e. it is a hair root. This putative stem-cell death scenario reminds us of that which attends animal neural tissue development [5].

The continued differentiation and penetration of plerome into the growing hair-root apex, even after much of the usual plerome tissue complement has been deleted, is due to the phenomenon of homeogenetic induction [6, 7]. This process allows the retention of a diminished plerome, but one that possesses a neuronally active xylem-plus-phloem quadrant.

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Mechanisms of magnetoreception in plants and fungi

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The ability to respond to magnetic fields is ubiquitous among the five kindoms of organisms. Apart from the mechanisms that are at work in bacterial magnetotaxis (ferrimagnetism) none of the numerous magnetobiological effects is as yet completely understood in terms of the underlying physical principles. Plants react in many ways to the geomagnetic field and to strong continuous as well as alternating magnetic fields (Galland and Pazur 2005). Because of a lack of model organisms and model reactions the magnetobiology of plants, fungi and microorganisms has remained largely on a phenomenological level. The problem is compounded by the fact that magnetic effects are observed for a huge range of magnetic flux densities that cover more than 10 orders of magnitude. To come to grips with such a huge dynamic range which is similar to that of human vision and numerous photoresponses of plants one would expect the study of dose-response relationships to be of paramount importance. It comes thus as a surprise that such studies are practically nonexistent. They would be particularly needed in view of the fact that responses are elicited by weak magnetic fields, such as the geomagnetic field, whose energy content is several orders of magnitude below the thermal energy content (kT- paradox). As a result most of the studies are characterized by a lack of mechanistic insight even though physics provides several theories that serve as guideposts for biological experimentation and that offer solutions for the kT-paradox.

Beside ferrimagnetism, which is well proven for bacterial magnetotaxis and some cases of animal navigation, three further mechanisms for magnetoreception receive currently major attention: (i) the “radical-pair mechanism” consisting in the modulation of singlet-triplet interconversion rates of a radical pair by weak magnetic fields, and (ii) the “ion cyclotron resonance” mechanism, and (iii) the “coherence” mechanism. Recent studies with Arabidopsis (Ahmad et al. 2007) and Phycomyces show that blue-light reception and magnetoreception are intimately connected, an observation that is best explained in the context of the radical-pair mechanism.

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Responses to environmental stimuli by internodal cells of *Chara corallina*

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The giant (2-10 cm long, 0.5-1.0 mm wide) internodal cells of *Chara corallina* provide a simple, easily manipulated tool for investigating responses to environmental stimuli in single cells. We have identified three responses to environmental stimuli: polarity of cytoplasmic streaming induced in response to gravity or hydrostatic pressure; action potential generation in response to mechanical or electrical stimulation and; tropistic growth in response to light and gravity.

As can be predicted by their large size, *Chara* internodal cells exhibit a rapid (*ca.* 100 $\mu m\ s^{-1}$) rotational cytoplasmic streaming. Streaming proceeds at equal rates basipetally and acropetally in horizontal cells. In contrast, gravity induces a polarity of cytoplasmic streaming in vertically-oriented cells such that the downwardly-directed stream moves *ca.* 10% faster than the upwardly-directed stream – regardless of the morphological identity of the cell ends. This gravity-induced polarity of cytoplasmic streaming can be mimicked by the application of a unilateral hydrostatic pressure to either end of a horizontal cell. Hydrostatic pressure applied to the bottom of a vertically-oriented cell can eliminate or even reverse the gravity-induced response. The induction of a polarity of cytoplasmic streaming by both gravitational pressure and hydrostatic pressure is Ca^{2+}-dependent and requires both ends of the cell to be intact.

Cytoplasmic streaming in *Chara* internodal cells ceases in response to an action potential. The ability of electrical and mechanical stimulations to generate action potentials in *Chara* is Ca^{2+}-dependent and the response to each stimulus may be inhibited in a similar way by Ca^{2+} antagonists. Since ligated cells exhibit cessation of cytoplasmic streaming in response to mechanical and electrical stimulation, intact cell ends are not required for this response.

Chara internodal cells exhibit tropistic growth in response to both light and gravity signals. In the absence of light, *Chara* internodal cells are negatively gravitropic. A light stimulus, opposite to the vector of gravity, will induce phototropic growth which will inhibit (at a flux of ca. 1 $\mu mol\ m^{-2}\ s^{-1}$) or reverse (at a higher flux) the gravity-induced response. Intact cell ends are required for gravitropism but not photropism of internodal cells.

Because of their large size and responsiveness to environmental stimuli, the internodal cells of *Chara* are particularly suited as a model system to elucidate signal transduction pathways in plants. Some progress in revealing insights into interactions between signal transduction pathways in these cells will be discussed.

MOLECULES, SIGNALLING & CELL BIOLOGY

Roles of endocytosis regulation in plant physiology and development

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Crucial roles of endocytosis in various plant functions are emerging recently, but its molecular mechanism and physiological significance still remain largely unknown. Using a model plant, *Arabidopsis thaliana*, we have been studying the molecular mechanism of endocytosis with a special focus on Rab5 GTPases. Three Rab5 members, Ara7, Rha1 and Ara6, are encoded in the *Arabidopsis* genome, which are all involved in endocytosis. Ara7 and Rha1 are orthologs of mammalian Rab5, and Ara6 is a plant-unique type of Rab5 member. Through genetic analysis, we have found that these two subgroups function antagonistically in various developmental stages, although they are all activated by the practically sole GEF, AtVps9a. Moss and spikemoss also have the Ara6-type Rab5, thus this subgroup is well conserved among land plants. These data indicate that land plants have evolved a quite unique mechanism for the regulation of endocytosis, which is essential for the plant life.

EXO70A1, a putative exocyst subunit, is important for polar growth and plant development

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The exocyst is a hetero-oligomeric protein complex involved in exocytosis and has been extensively studied in yeast and animal cells. Current analyses of mutations in genes encoding plant homologs of three subunits (*A. thaliana SEC8*, *EXO70A1*, and maize *sec3*) support the notion that an exocyst complex is also present in plant cells. Our bioinformatic analysis revealed that the Arabidopsis genome contains 23 *EXO70* genes. Based on expression analysis, we identified *EXO70A1* as the main *EXO70* gene in Arabidopsis. We characterized two independent T-DNA insertional mutants in *EXO70A1* gene. Heterozygous *EXO70A1*/*exo70A1* plants appear normal and segregate in the 1:2:1 ratio. However, *exo70A1* homozygotes exhibit multiple phenotypic defects. Polar growth of root hairs and stigmatic papillae is disturbed. Organs are generally smaller, plants show loss of apical dominance and an indeterminate growth where instead of floral meristems new lateral inflorescences are initiated in a reiterative manner. Both *exo70A1* mutants have dramatically reduced fertility. These results suggest that EXO70A1, the putative exocyst subunit, is involved in cell and organ morphogenesis.

Inositol trisphosphate receptor in plants – is it real?

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The receptor for D-*myo*-inositol 1,4,5-trisphosphate ($InsP_3$-R) has been well documented in animal cells. It constitutes an important component of the intracellular calcium signalling system. Today the corresponding genes in many species have been sequenced and the antibodies against some of the $InsP_3$-Rs are available.To the contrary very little is known about its plant counterpart. Only few published works have dealt directly with this topic. We have summarized the available relevant data and figured out some properties of the putative plant receptor(s) including the *in vivo* evidence, its electrophysiology, parameters of the $InsP_3$-induced calcium release and $InsP_3$ binding, its immunological cross-reactivity and its subcellular localization. Phosphatidylinositol-specific phospholipase C is undoubtedly parts of plant signalling pathways. Nevertheless, it is not sure that the $InsP_3$-R is present in plant cells unless any corresponding gene is identified.

Identification of novel abscisic acid signal transduction components and ion channel regulation mechanisms in guard cells

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Guard cells have been developed as a model system for dissecting ion channel functions and regulation mechanisms. Previous studies have shown that two classes of calcium-induced stomatal closing can be separated: rapid Ca2+ reactive and long term Ca2+ programmed stomatal closing (G. Allen et al., 2001, *Nature* 411). However, genetic evidence has been lacking for Ca^{2+} sensor mutants that disrupt Ca^{2+}- and abscisic acid-regulated stomatal movements. In addition, a Ca^{2+}-independent pathway functions in the abscisic acid (ABA) response. We have recently identified two calcium-dependent protein kinases (CDPKs) that function in abscisic acid (ABA) and Ca^{2+} regulation of guard cell ion channels and stomatal closing (I. Mori et al., 2006 *PLoS Biol*.). Furthermore, several independent signal transduction analyses suggest a new model for how plant cells can achieve specificity in calcium signaling through "priming" and "de-priming" of Ca^{2+} sensitive mechanisms (J. Young et al., 2006 *PNAS*). Further evidence that correlates with this "Ca^{2+} sensor priming" hypothesis will be presented. An important target of ABA and cytosolic Ca^{2+} signaling is the activation of S-type anion channels in guard cells. Progress at identifying new genes that are essential for mediating this response will be presented. Evidence for a parallel pathway that functions in the ABA signaling network will also be presented.

Genetic, genomic and signal transduction analyses in several laboratories indicate that genetic redundancies and robustness exist within the abscisic acid signal transduction network. To address this complexity we have pursued gain-of-function genetic screens (e.g. J. Kuhn et al., 2006 *Pl. Physiol*.) and genomic approaches (e.g. N. Leonhardt et al., 2004 *Pl Cell;* Mori et al., 2006 *PLoS Biol*.). More recently we have developed a chemical genetics approach that allows high-throughput screening for molecules and mutants that affect ABA signal transduction. Progress at isolating a small molecule that blocks ABA responses and isolation and characterization of mutants in ABA signaling that are insensitive to this compound will be presented.

A novel class of microtubule-binding proteins control root growth behavior and anisotropic cell expansion in Arabidopsis

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In plants, anisotropic cell expansion is a tightly regulated process that contributes to morphogenesis. In addition to modulating overall growth rates, this process orchestrates directional growth responses to environmental cues, allowing plant organs to grow toward environments that are better suited for their primary functions. A screening for *Arabidopsis thaliana* mutants displaying defective seedling-root growth behavior on hard surfaces allowed us to identify *WVD2*, a gene that regulates both anisotropic cell expansion and the spiral growth of most organs. WVD2 over-expressing plants also display enhanced thigmomorphogenesis and sensitivity to salt and sucrose treatments, suggesting a role in the transduction of these signals. *WVD2* encodes a microtubule (MT)-binding protein that promotes the bundling of MTs in vitro, and affects the organization of cortical MTs in expanding cells of the root (Yuen et al, 2003; Perrin et al, 2006). This protein shares a 95 amino-acid motif with 7 other Arabidopsis proteins, and initial phenotypic analyses of mutants that either over-express or are defective in one or several of these genes suggest distinct, though overlapping, roles for the WDL proteins in the regulation of MT-dependent morphological processes. We hypothesize that WVD2 and related WDL proteins contribute to the control of organs growth behavior in response to environmental and endogenous cues by regulating the organization and/or dynamic properties of cortical MTs in expanding cells, thereby modulating the patterns of anisotropic cell expansion.

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Domain-specific cell wall-plasma membrane interface

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At the end of XX century, Phil Lintilhac, discussing various concepts of cellularity, proposed that the idea of the basic unit of life could be reduced to "event surface" – the boundary separating internum from externum (Lintilhac 1999). Autopetic theory added one additional requirement to this concept: the boundary has to be constructed and maintained by the living system itself (Varela et al. 1974). Here, we will use these metaphors as a useful tool for description of some of the functionalities of the cell wall-plasma membrane-cytoskeleton (WMC) continuum with special attention paid to the plant wall component.

Cell walls are the outermost functional zone of plant cells. Although they surround the individual cells, at the same time they form a part of supracellular structure – the apoplast. In suspension-cultured cells, cell walls are also embedded in the culture medium which can be thought of as a kind of superapoplast. Cell walls have been usually considered as a structural component of the cell and of the plant. Here we would like to draw attention to other wall functions, namely the signalling one and that of physical anchor for other cellular components. To illustrate this, some recent data indicating the possibility of extracellular generation of signals affecting the cell fate will be presented. Moreover, potential other routes for auxin transport will also be discussed. On the other hand, we will also present data showing that the WMC continuum can be treated as a complex sensory medium detecting and transmitting information from the walls to the cytoskeleton for signalling and regulation.

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Stem cell signalling networks in plants

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Although many aspects of multicellularity differ between plants and animals, both feature groups of cells that enable indeterminate patterns of cell division and growth. In an effort to understand how the behaviour of these cells is regulated, we review different types of meristematic tissues in higher plants, with a particular focus on the shoot and root apical meristems (SAM and RAM) (1). We consider whether concepts developed to explain stem cell behaviour in animals may also have relvance to plants, particularly with regard to how such groups of cells are established and maintained. Molecular genetic data is reviewed that suggests that while the establishment and organogenic related functions of the SAM and RAM differ, conserved mechanisms operate that help maintain initial cells in a pluripotent state. Finally we consider how a unique plant specific family of RNA binding proteins, termed MEI2-like, may function as part of larger signalling networks to regulate differentiaton related processes (2,3,4).

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Endogenous programmed cell death triggers in plants

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It has been clear that the precursors and breakdown products of both chlorophyll and heme, such as porphyrins and related molecules, are extremely phototoxic; thus, their synthesis and degradation are highly compartmentalized and regulated. Accumulation of porphyrin compounds is known to cause cell death in both plants and animals. Moreover, ceramides and their related sphingolipid derivatives are bioactive lipids that play important roles as second messengers and as dampening signals for apoptosis in animals (Hannun and Obeid 2002). In an effort to search plant programmed cell death (PCD) triggers, we used Arabidopsis two mutants termed *accelerated cell death 2* (*acd2*) and *acd5*. *ACD2* and *ACD5* encode red chlorophyll catabolite (RCC) reductase (Mach et al., 2001) and ceramide kinase (Liang et al., 2003), respectively. We found that protoporphyrin IX (PPIX, a precursor to heme and chlorophyll) and C2 ceramide trigger an apoptotic-like response in Arabidopsis protoplasts (Liang et al., 2003; Yao et al., 2004). PPIX was enhanced in ACD2-deficient plants and reduced in ACD2-overexpressing plants, indicating that PPIX triggers apoptotic cell death dependent on ACD2. Furthermore, PPIX induced altered ACD2 localization and levels (Yao et al., 2006). We also found that C2 ceramide induced PCD via its effect on mitochondrial permeability transition. The data suggest that a mitochondrial membrane potential loss was commonly induced early during plant PCD and was important for PCD execution, as evidenced by the concomitant reduction of the change in mitochondrial membrane potential and PCD by cyclosporin A. Our data suggest that RCC (and related porphyrin compounds such as PPIX) and ceramides are endogenous cell death triggers.

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Plastid targeting of glycoproteins in rice cells

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Nuclear-encoded plastidial proteins are usually synthesized in the cytosol and posttranslationally imported into the organelle. However, recent our investigations revealed that some starch metabolism-related enzymes are transported into the plastid through unusual pathway. A rice novel ADP-glucose hydrolytic nucleotide pyrophosphatase/phosphodiesterase 1 (NPP1) was shown to be glycosylated, since it contains numerous N-glycosylation sites, binds to Concanavalin A, stains with periodic acid-Schiff reagent and can be digested by Endo-H. Both immunocytochemical analyses and confocal-fluorescence microscopy of rice cells expressing NPP1-GFP revealed that NPP1 occurs in the plastidial compartment. Brefeldin A treatment to NPP1-GFP expressing cells prevented NPP1-GFP accumulation in the chloroplasts (Nanjo et al. 2006). Rice α-amylase I-1 (AmyI-1) is a well-known secretory enzyme bearing typical N-linked oligosaccharide chain. We found that AmyI-1 also occurs in the plastids in living rice cells (Asatsuma et al. 2005). In the targeting of AmyI-1, the effects of the dominant mutants of AtSAR1 and AtARF1 GTPases, which are engaged in the protein traffic from the ER to the Golgi apparatus, were tested in onion epidermal cells. These AtARF1 and AtSAR1 mutants severely arrested the targeting of AmyI-1 into plastids. These experiments provide strong evidence that the plastidial N-glycosylated glycoproteins are transported from the Golgi to the plastid through the secretory pathway in rice cells.

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Plant aquaporin regulation and cell signaling

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Plant growth and development are dependent on the tight regulation of water uptake and transport across cellular membranes and tissues. This water movement can be controlled by the regulation of water channels or aquaporins. These channels are widespread in biological membranes and plant aquaporins are believed to act as "cellular plumbers" allowing plants to rapidly alter the membrane permeability in response to environmental cues (Hachez et al., 2006). In addition, plant aquaporins can also facilitate the transport of other important molecules such as CO_2, ammonia, H_2O_2, boron and silicon.

Recent data indicate that plant aquaporins are regulated by many different mechanisms modifying their subcellular localization and gating (Chaumont et al., 2005; Tornroth-Horsefield et al., 2006). The factors affecting aquaporin trafficking and gating behavior possibly involve phosphorylation, heteromerization, pH, calcium, pressure, solute gradient, temperature. For instance, plasma membrane aquaporins (PIP) are phosphorylated by calcium-dependent protein kinases resulting in the pore opening and an increase of the water channel activity. Interestingly, high osmotic pressure in the apoplast induces a decrease in the phosphorylation status of PIPs, probably preventing water exit from the cells. We also recently showed that non-functional and functional PIPs form heteromers in oocytes and plant cells leading to a relocalization of inactive PIPs from the secretory pathways to the plasma membrane.

Recent progress in the elucidation of plant aquaporin regulation and cell signaling will be discussed.

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Controlled and facilitated diffusion of H_2O_2 as a potential mechanism involved in signaling and ROS scavenging

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The production of reactive oxygen species (ROS) is the natural consequence of aerobic metabolism. ROS are inter-convertible molecules with various degrees of reactivity and the potential to damage cellular components such as lipids, nucleic acids and proteins. Hydrogen peroxide (H2O2) is a rather stable ROS and has recently been shown to be involved in various signaling pathways. To minimize potential damage by ROS and to control a signaling role of H2O2 and other ROS, the concentration of ROS has to be tightly regulated. Such control may involve mechanisms of production and scavenging as well as transport across membranes. Mechanisms for production and scavenging have been studied in great detail. However, the aspect of transport of H2O2 is of particular interest, because a mechanism of transport is part of the definition of signaling molecules.

Until recently it was assumed that H2O2 crosses the membrane by simple diffusion. Contrary to the concept of simple diffusion it was suggested that water channels facilitate the diffusion of H2O2. Henzler and Steudle (2000) showed that mercury, an aquaporin blocker, repressed H2O2 accumulation in internodial cells of the algae Chara corallina. The authors therefore suggested that some aquaporins in Chara served as peroxoporins.

We have used the heterologous expression system yeast to test the hypothesis that specific aquaporins may flux H2O2 across membranes. Yeast is a very useful tool for this type of study, since many mutants are available that differ in the ability to metabolize and detoxify ROS. This allowed us to control the scavenging capacity of the yeast cells while asking the question, if the heterologous expression of aquaporins increased the sensitivity to externally supplied H2O2. In a comprehensive screen testing 24 aquaporin isoforms from plants and mammals we found that expression of plant aquaporins of the TIP1 group and human AQP8 increased the sensitivity of yeast cells towards H2O2 in the medium. Aquaporin-mediated H2O2 transport was further investigated in a fluorescence assay with intact yeast cells using an intracellular ROS-sensitive fluorescent dye. Our data provide molecular genetic evidence that human AQP8 and plant aquaporin AtTIP1 have the potential to facilitate the diffusion of H2O2 across membranes.

The challenge is now to demonstrate a physiological role of hydrogen peroxide transport through aquaporins. We will present current strategies.

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The role of glutamate in plants and its potential function as a signaling molecule

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Glutamate is one of 20 proteogenic amino acids, and serves as an acceptor molecule in primary ammonium assimilation in plants. It is also one of five major translocated amino acids found in phloem and xylem. Metabolism and synthesis of glutamate by enzymes such as glutamine synthetase (GLN1) and asparagine synthetase (ASN1) are regulated by light, carbon and nitrogen, suggesting that the regulation of glutamate level plays a significant role in the control of C/N balance in plants.

In addition to its role as a nutrient and nitrogen transportation form in plants, glutamate might play a role in intracellular signaling. Externally applied glutamate induces membrane depolarization and cytosolic calcium spikes. Arabidopsis genome encodes for 20 putative glutamate receptor homologs, and the disruption of one of these glutamate receptors, GLR3.3, partially abolishes membrane depolarization and cytosolic calcium spikes (Qi et al. 2006). AtGLR1.1 deficient plants (antiAtGLR1.1) exhibit conditional germination phenotype that is sensitive to C:N ratio in the growth media (Kang and Turano 2003).

The role of glutamate as a signaling molecule, however, is unclear. For most plant glutamate receptors the ligand specificity has not been identified. In addition, it is not known whether plants have mechanism that control local glutamate concentration. In order to understand the role of glutamate and glutamate receptors in plants, it is important to know the glutamate level in specific cell types or in subcellular compartments. A method to measure the concentration of glutamate in all cell types would provide valuable information. We have developed a protein-based, Fluorescence Resonance Energy Transfer (FRET) nanosensors for glutamate (Okumoto et al. 2005). Glutamate sensors, when expressed in mammalian cells, were able to detect real-time glutamate concentration change.

Results from Arabidopsis plants expressing glutamate sensors anchored to the outside of the plasma membrane suggest that these sensors can detect glutamate concentration changes in the apoplast. We will introduce a new technique for the analysis of *in vivo* glutamate fluxes in plants and discuss our latest results.

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Plant GABA metabolism - approaches to identify genes involved

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GABA metabolism is compartmentalized. Anabolism takes place in the cytosol and catabolism occurs in mitochondria. The GABA catabolic *succinic semialdehyde dehydrogenase* (*ssadh*) mutant is strongly impaired in growth, most likely due to the accumulation of a toxic compound. The *ssadh* phenotype can be rescued by simultaneously knocking out the *GABA-transaminase* (*gaba-t*) gene, the gene upstream in GABA catabolism. This phenotype suppression can be explained by preventing a toxic intermediate to accumulate in double *knock-out* plants.

Based on this finding and searching for unknown genes that are involved in GABA metabolism, an *ssadh* suppressor screen has been performed, where *ssadh* mutants have been mutagenized using EMS. Under short day conditions, unlike normal growth conditions, *ssadh*/*gaba-t* double mutants display a phenotype that resembles the one of *ssadh* mutants. To explain this phenotype, a second suppressor screen has been performed, mutagenizing *ssadh*/*gaba-t* double *knock-out* plants with EMS. Suppressor mutants of either screen have been collected and are currently analyzed. Ultimately, rescueing EMS mutations will be mapped and cloned to assign functions to the respective genes in GABA metabolism or regulation of the pathway.

To further gather more information on the GABA pathway in plants, a recombinant inbred line (RIL) analysis was performed. RILs were grown in the presence of potentially toxic intermediates of the pathway to determine quantitative trait loci (QTLs) for resistance/sensitivity against/for the respective substances. Overlapping findings with those of suppressor screens are deliberately taken into account.

Indoleamines and flavonoids in neuroprotective plant physiology

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The plant genus *Scutellaria* is a rich source of neurologically active phytochemicals and treatments for a wide range of human diseases including cancers, neurological disease, fevers and immune system dysfunction. Very few of the 350 species in this genus have been extensively studied, but there is traditional and ethnobotanical evidence of efficacy of *Scutellaria* species from around the world. The objective of the current study was to compare the phytochemical diversity in three species of *Scutellaria* from vastly different geographical locations and ecosystems. Metabolomic analysis revealed that the Chinese species, *Scutellaria baicalensis* had 1388 compounds that were not present in extracts of the other species. The North American *Scutellaria lateriflora* had a spectrum of 1261 unique compounds while the Central and South American species *Scutellaria racemosa* had 1733 unique phytochemicals. Equally interesting was the conserved phytochemistry. The neurotransmitters, melatonin and serotonin, were found in all three *Scutellaria* species. The neuroprotectant wogonin was also found in all 3 species of *Scutellaria* along with the flavonoids, baicalin, baicalein, and scutellarin. Wogonin was found at the highest levels in *Scutellaria racemosa*, a plant with traditional indigenous use as a narcotic. Recent studies with animal models indicate neuroprotective capacityin extracts from this species. The presence and conservation of neurologically active phytochemicals across plants from different geographical locations and ecosystems may provide new opportunities for studies of their potential role in plant adaptation and plant development.

Indole-3-butyric acid as a signal in early events of arbuscular mycorrhizal associations

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Plant hormones are suitable candidates to function as continuous signals between roots and arbuscular mycorrhizal (AM) fungi during the establishment of symbiosis. Auxins might play a role during early events of an arbuscular mycorrhizal association with respect to changes in root morphology and gene expression. As examples several different plant-AM fungus systems will be compared. Inoculation of Zea mays with Glomus intraradices resulted in the significant increase in the percentage of lateral roots during early stages of colonization which coincided with an increase in the levels of the auxin indole-3-butyric acid (IBA). Addition of TFIBA, an inhibitor of IBA-induced root growth and lateral root induction, to roots inoculated with AM-fungi reduced the formation of fine roots and the amount of endogenous free IBA as well as the percentage of colonization. The increase in IBA levels was accompanied by increased enzymatic synthesis of IBA. In the model legume Medicago truncatula IBA was also induced during AM. Transcripts from Medicago truncatula roots differentially induced by IBA and AM were identified by microarray analysis. A small set of genes was simultaneously regulated by both factors. We have validated the expression levels of several transcripts by RT-PCR and found up-regulation of a leghemoglobin by IBA and AM and down-regulation of a high-affinity nitrate transporter by both factors. A model will be presented which summarizes the possible effects of plant hormones, especially auxins, during AM symbiosis.

A role for phospholipase A in auxin gene regulation and auxin responses. The receptor may not be TIR1

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The only plant cytosolic phospholipase A form is the $iPLA_2$ or patatin-related PLA around which our work centers. Auxin increases phospholipase A activity within 2 min (Paul et al. (1998) Plant J. 16: 601-611) and phospholipase A inhibitors, ETYA and HELSS, inhibited PLA activation and elongation growth of etiolated Arabidopsis hypoctyls (Holk et al. (2002) Plant Physiol. 130: 90-101). To identify the mode of action, rapid auxin-regulated gene expression was tested for sensitivity to PLA2 inhibitors using seedlings harbouring the synthetic auxin-responsive reporter *DR5::GUS*. ETYA and HELSS inhibited auxin-induced increases in GUS activity, the steady-state level of the corresponding GUS mRNA and the mRNAs encoded by auxin-activated genes (*IAA1*, *IAA5*, and *ARF19*). Auxin regulation of the steady-state level of Aux/IAA proteins is mediated by the auxin receptor, E3 ubiquitin ligase, TIR1. The velocity of the auxin-induced decrease of an IAA1-luciferase fusion protein was unaltered by ETYA and HELSS during the first 20 minutes when biosynthesis of IAA1-luciferase was prevented by cycloheximide addition. In kinetics of auxin-induced degradation IAA1-luciferase measured without cycloheximide these inhibitors blocked the auxin-induced decrease in steady-state levels. When two phospholipase A genes were over-expressed in the DR5-GUS background the sensitivity of DR5 promoter to auxin was greatly increased. The results here suggest that phospholipase A mediates auxin-regulated gene transcription via a receptor other than TIR1 and may act upstream of TIR1.

Knockout lines for the PLA genes AtPLA I, AtPLA IVA, and AtPLA IVC were isolated and found to be damaged in typical auxin-related functions. The knockout for AtPLA I is expressed in vascular tissue in stems, roots and leaf veins, additionally in pollen and trichomes. The corresponding knockout plants are defect in gravitropism, phototropism, nutation and root tip coiling. The slower response to laterally applied auxin of the AtPLA I knockout suggests a regulatory function of PLA I of auxin transport. The AtPLA IVA is expressed strongly in the root and the knockout for AtPLA IVA is defect in side root formation suggesting a function in auxin-stimulated side root formation. The gene AtPLA IVC is expressed in the gynaecium. With ABA treatment expression of PLA IVC is enhanced in roots and in veins in the shoot. The knockout shows a defect in the response of the root to phosphate starvation. The main root growth is not repressed but side roots are, contrary to the wild type response. Phosphate starvation affects auxin transport which may be disturbed in the pla IVC mutant. The hyocotyl is elongated in these knockouts pointing out a relevance to cell elongation for PLA IVC.

Taken together, the phenotypes of PLA knockout plants point out their function in auxin signalling. PLA is involved in the regulation of early auxin genes. The receptor, however, may not be TIR1.

Evolutionary history of the domain architecture of plant formins

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Formins (FH2 proteins) are ancient actin-binding proteins believed to participate in actin filament nucleation. They are defined by the hallmark FH2 domain, usually preceded by a Pro-rich FH1 region. In addition, metazoan, fungal and *Dictyostelium* formins often contain a N-terminal GTPase-binding domain mediating interaction with Rho GTPases. In angiosperms, two groups of formins can be defined on the basis of FH2 domain sequence; each group also exhibits typical domain architecture. Class I formins are usually transmembrane proteins, while Class II formins often possess a PTEN-related domain that might also mediate membrane association. Thus, formins are good candidates for a link between the actin cytoskeleton and the surface structures of the plant cell. We have performed a thorough analysis of over 100 plant FH2 protein sequences, as well as more than 120 formins encoded by fully sequenced non-plant genomes representing the metazoans, fungi, amoebae, chromalveolates and excavates. The characteristic plant Class I and Class II formins are indeed both universal for the plant kingdom. Moreover, we found a novel domain combination including a RhoGAP domain, present in some algal, moss and lycophyte formins (but absent in angiosperms), suggesting an ancient formin-mediated functional association between Rho GTPases and the actin cytoskeleton.

Phospholipid-based signalling - 'seeing is believing'

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Phospholipids are essential molecules contributing to the structural definition of cell membranes and participating in the regulation of cellular processes as signaling molecules and reservoirs of lipid messengers. While the bigger pools of phospholipids are involved in membrane structure, those involved in cell signalling are usually very small and consequently have been failed to be picked-up for years. Especially polyphosphoinositides (PPI) and phosphatidic acid (PA) have been emerging as signalling molecules.

Over the last few years, we and others have shown that a number of pathways involved in their metabolism, are activated in response to a wide variety of biotic- and abiotic stresses (see reviews). In general, these activations are fast (seconds to minutes) and the lipid responses small and transient, exhibiting rapid turnover. '*How*' these pathways are activated, '*where*' in the cell or plant this takes place, and '*what*' the functional significance of these activations are, is still a big mystery. To start addressing these questions, a number of opportunities are being explored in my laboratory. These include, i) *Arabidopsis* T-DNA insertion lines, ii) GFP-based lipid biosensors and iii) proteomic approaches to identify PA targets. Some recent developments in these areas will be discussed.

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The role of human neurotransmitters in plant cell division

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Each individual plant cell has the potential to become a whole plant through a process of dedifferentiation, redifferentiation and tissue regeneration. It has been hypothesized that this process is induced by alterations in the balance of two classes of plant growth regulating compounds viz. auxin and cytokinin but the exact mechanisms by which these compounds induce plant cell division is not known. In previous research, we have shown that mediation of endogenous neurotransmitter metabolism impaired plant regenerative functions but exogenous application of human neurohormones did not alter plant growth. In this communication, we show for the first time, that exogenous application of the human neurohormone serotonin induced three different developmental pathways in isolated tobacco mesophyll cells: (1) changes in cell structure including formation of pearl-necklace and torpedo-like cells, (2) low-frequency cell division, and (3) high frequency cell division followed by callogenesis when combined with auxin. These data provide the first evidence that serotonin acts as a growth regulator in plant cells and that synthetic auxin induces serotonin-melatonin production, potentially as a precursor to the indoleamine biosynthetic pathway.

Jasmonates as inducers of Ca^{2+} signals in the nucleus and the cytosol of plant cells

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Jasmonates representing a group of oxylipin phytohormones are shown here to differentially induce changes in intracellular Ca^{2+} concentrations in two distinct compartments of plant cells, the cytosol and the nucleus. Based on the Aequorin technique, a structure-activity analysis revealed that jasmonates and related compounds fall into three distinct classes: (1) compounds inducing Ca^{2+} changes in both the cytosol and the nucleus (2) compounds inactive on either compartment or (3) compounds acting selectively on the nucleus.

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Plasma mebrane NADPH oxidases (NOX) in plants – beyond ROS signalling.

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Our recent results show that tip-localised ROS produced by a NOX enzyme are needed to sustain the normal rate of pollen tube growth. As in root hairs and growing root cells the same phenomenon is observed, it is likely that this activity is a general mechanism related to plant cells expansion. The regulation of NOX activity is also in plants related to the Rac/Rop GTPases activity and calcium signalling; and signalling relay involving ROS is well documented in plants. Here we will discuss possible significance of NOX activity in electrogenic processes at the growing plant cell domain.

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The role for PEX11 and dynamin-related proteins in Arabidopsis peroxisome proliferation

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Plant peroxisomes are highly dynamic organelles that play pivotal roles in development and in stress response. To establish a model for peroxisome proliferation in plants, which is largely unknown, we have taken forward and reverse genetic and proteomic approaches using *Arabidopsis thaliana*. Except for Pex11p, which in yeast is involved in peroxisome proliferation with an unknown mechanism, the Arabidopsis genome does not contain obvious sequence homologues to most proteins that operate in yeast to control peroxisome proliferation. The five-member Arabidopsis PEX11 protein family is composed of three subfamilies: PEX11a, PEX11b, and PEX11c to PEX11e, all of which target to peroxisomes, as demonstrated by fluorescence microscopy and immunobiochemical analysis. Overexpression of At *PEX11* genes in *Arabidopsis* increased peroxisome elongation and number, whereas reduction in gene expression lowered peroxisome abundance. PEX11c and PEX11e partially complemented the growth phenotype of the *S. cerevisiae pex11* null mutant on oleic acid. Our results suggested that the *Arabidopsis* PEX11 proteins promote peroxisome proliferation with some functional specificity between subfamilies (Orth et al. 2007 Plant Cell). Using genetic screens and proteomic analysis of the peroxisome membrane, we also identified a subset of the dynamin-related large GTPases that mediates the division of both peroxisomes and mitochondria. These data indicated that despite their distinct evolutionary paths, peroxisomes and mitochondria may use the same set of dynamin-related proteins (DRPs) for division. Additional forward genetic screens and proteomic experiments are conducted in the lab to uncover more plant-specific components of the peroxisome proliferation machinery.

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Myosin and actin function in directing mitochondria movement in living pollen tubes of *Picea wilsonii*

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Mitochondria are dynamic organelles providing subcellular spatial energy as needed and serving as fundamental elements in intracellular signalings in plant cells. However the nature of the mitochondria-cytoskeleton interactions in pollen tubes has not been explored. By using time lapse confocal microscopy, total internal reflection fluorescence microscopy (TIRFM) and spinning-disk confocal microscopy (SDCM), we investigated the effects of cytoskeletal inhibitors on the transportation and positioning of mitochondria in living pollen tubes. It was revealed that the actin filament disrupting drug latrunculin B (LATB), the myosin ATPase inhibitor, 2, 3-butanedione 2-monoxime (BDM) and the actin filament stabilizing drug jasplakinolide (Jas), apparently inhibited mitochondrial motility, while microtubule disrupting drug taxol and oryzalin showed slight effects, demonstrating that intact actin cytoskeleton is required for active mitochondrial movement. Two-dimensional (2-D) trajectory and velocity of individual mitochondrion was obtained to characterize the mitochondrial movement. It was showed that mitochondria of *Picea wilsonii* pollen tubes underwent three classes of linear movements: rapid movement (> 5.0 μm/s instantaneous velocities), slow movement (<5.0 μm/s instantaneous velocities) and mixed movement (ranging from 0.16 to 10.35 μm/s instantaneous velocities). Jas treatment abolished mixed mitochondrial movement and rapid mitochondrial movement, while rapid movement and slow movement were not found in BDM-treated pollen tubes. Taxol treatment increased the frequency of positioning and velocities of mixed mitochondrial movement. Oryzalin treatment caused curve mitochondrial trajectories with similar velocities compared to the control pollen tubes. Taken together, these findings suggested that actin cables provided tracks for mitochondrial slow movement which are powered by myosin, on the other hand, actin cables also served as “conveyor belts” to drive mitochondrial mixed movements via actin polymerization. Therefore, microtubule dynamics regulated mitochondrial positioning, velocities and trajectories via affecting the actin filament dynamics, rigidity and flexibility.

Actomyosin-mediated gravisensing and early transduction events in gravistimulated cut snapdragon spikes

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* Dedicated to the memory of Prof. Abraham H. Halevy, from the Faculty of Agriculture, The Hebrew University of Jerusalem, Rehovot, Israel, who had actively participated in this research.

Horizontal placement of snapdragon (*Antirrhinum majus* L.) flowering shoots initiates an upward gravitropic bending via the chain reaction of gravity perception, signal transduction and growth response (Philosoph-Hadas et al. 1996, Friedman et al. 1998, 2003). Our previous studies (Friedman et al. 2003) have demonstrated that the actin cytoskeleton within the cells of snapdragon (*Antirrhinum majus* L.) spikes is necessary for normal amyloplast displacement upon gravistimulation. Pharmacological disruption of the actin cytoskeleton with cytochalasin D (CD), demonstrated in cortical and endodermal cells, and with latrunculin B (Lat B), demonstrated in cortical cells, delayed the displacement of amyloplasts and resulted in a significant inhibition of the gravitropic bending.

In the present study we have investigated the involvement of myosin in addition to actin in the different phases of the gravitropic response of snapdragon spikes. Using indirect immunofluorescence double-labeling of actin and myosin, we have demonstrated that no organization changes in actin filaments occurred in cortical and endodermal cells of the stem bending zone during gravistimulation. These results suggest that actin depolymerization is not required for amyloplast sedimentation. The amyloplasts in the endodermis were found to be surrounded by actin and myosin, and seem to be attached to the actin filaments via the motor protein, myosin. This suggests the involvement of myosin in amyloplast translocation. This suggestion was supported by the findings showing that pulsing spikes with the myosin inhibitor, 2,3-butanedione-2-monoxime (BDM), inhibited the gravity-induced amyloplast displacement in the endodermis. Indeed, the BDM treatment altered characteristic distribution patterns of myosin-like proteins in the cortex and disrupted the normal organization of the actin network and microtubules. This further indicates that myosin functions in the normal actin network organization. Both BDM and CD inhibited lateral auxin transport and stem bending. It seems therefore, that the inhibitors which affect amyloplast displacement also inhibit the subsequent event of lateral auxin transport leading to inhibition of stem bending. Taken together, our results suggest that the acto-myosin system mediates displacement of amyloplasts, which under normal conditions possibly move along the actin filaments, using myosin as a motor protein, to reorient their position following gravistimulation.

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Temporary changes in gravity conditions affect oxygen influx at root level

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Oxygen influx shows a gravity-regulated asymmetry in the transition zone (TZ) of root apices on ground when root orientation varies from vertical to horizontal. In details, oxygen influx increased only on the upper side of TZ, remaining stable on the lower one, since 18 ± 2 sec after changing the root position. Considering that the tilting procedure took around 15 s, the first O_2 signal can be hypothesized to appear just few seconds after gravistimulation. This rapid change in the oxygen flux into root apices is by far the fastest ever reported plant response to gravity. To analyze this phenomenon in a very low gravity situation, an experiment has been set up on a parabolic flight, which permits a sequence of normal, hyper- and microgravity conditions. During the flight, oxygen flushes in roots of *Zea mays* seedlings have been constantly monitored by selective microelectrodes and a respirometer. A clear and distinct signal in oxygen fluxes has been detected only in the apex zone, starting just 2.0 ± 0.5 s after the imposition of microgravity conditions, while no significant changes have been monitored neither in normal nor in hypergravity conditions. The significance of these results on the nature of the graviperception will be discussed.

Chemical factors inducing leaf-movement in *Leguminosae* and carnivorous plants

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Chemical aspects of two different types of plant leaf movements, the circadian leaf movement known as nyctinasty and trap leaf-closure of Dionaea muscipula, will be discussed.

Nyctinastic leaf movement is induced by the swelling and shrinking of motor cells in the pulvinus, joint-like thickening located at the base of the petiole. A flux of potassium ions across the plasma membrane of the motor cells is followed by massive water flux, which results in swelling or shrinking of these cells. An issue of great interest is the regulation of the opening and closing of the potassium channels involved in nyctinastic leaf movement.

Each of nyctinastic plants of five different genuses so far examined contained a pair of factors, one of which induces leaf closure and another induces leaf opening. The relative contents of the closing and opening factors changed correlating with the nyctinastic leaf movement. Use of fluorescence-labeled and photoaffinity labeled factors revealed that the factors bind to motor cells and that the membrane fraction of the pulvini contained two potential receptor proteins which can bind to the factor.

Venus's flytrap (*Dionaea muscipula*) is known as representative insectivorous plant. This plant traps the insects by its large leaves called trap, and digested them between the traps by digestive enzymes. Interestingly, there observed some "memory" in the leaf-closure of Dionaea. The rapid closure of the traps requires twice stimuli within thirty seconds on their sensory hairs which exist on the internal surface of the trap leaves. Only one stimulus never induces leaf closure. This phenomenon suggested that Dionaea memorizes the first stimuli on the sensory hair. We found that Dionaea has endogeneous chemical factor which induce the closure of traps without stimuli. This plant "memory" can be explained by the stepwise accumulation of secreted chemical factor.

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Vesicular trafficking as a mechanism of abiotic stress tolerance in plants

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Synaptotagmins are a family of transmembrane proteins that function as transducers of Ca^{2+} signaling in membrane fusion events. All members of the synaptotagmin gene family span membranes once, have short lumenal domains and long cytoplasmic regions containing two C2 domains connected by a short linker. There are 16 known vertebrate synaptotagmins. Detailed biochemical and *in vivo* studies of the best characterized isoform, synaptotagmin1 (syt 1), have provided compelling evidence that it functions as a calcium sensor for fast neurotransmitter release at synapses. In *Arabidopsis* there are six synaptotagmin-like genes (sytA-F) with unknown functions. In search for genes that were essential for salt stress tolerance, we screened a T-DNA population at high NaCl concentration. We found that loss-of-function of synaptotagmin A (SytA) in *Arabidopsis* produces hypersensitivity to sodium but only at low Ca^{2+} concentration. We analyzed the phospholipid binding properties of SytA. These studies revealed that only C2A binds phospholipids in a Ca^{2+} dependent manner, while C2B shows phospholipid binding independently of Ca^{2+}. These results, combined with SytA localization in the plasma membrane, suggest that Ca^{2+} dependent membrane trafficking mediated by SytA is important for plant survival under abiotic stress conditions.

A plasmodesmata associated ß-1,3-glucanase in Arabidopsis regulates plasmodesmata function

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Plasmodesmata (Pd), plasma-membrane-lined channels that connect plant cells, are not static organelles, but rather show a high degree of plasticity and can change in a transient manner from 'closed' to 'open' to 'dilated'. The dynamic properties of Pd play an important role in regulating the direct cell-to-cell transport of molecules between cells, in providing a cell-to-cell passageway for plant viruses and in the organization and functioning of symplasmic domains. Two different mechanisms are assumed to produce these focused changes in the tunnels. The first model suggests that conductivity changes due to alterations in plasmodesmal structure motivated by plasmodesmal associated cytoskeleton proteins actin, myosin and centrin. The second model suggests that changes in the wall sheath surrounding the Pd, mediated by callose (ß-1,3-glucan) synthesis and hydrolysis, cause changes to Pd structure that alter its conductivity. Recently we have identified the first ß-1,3-glucanase Arabidopsis enzyme that is associated to the Pd complex, termed AtBG-pap (plasmodesmal associated protein) (Levy et al. 2007). When fused to GFP, this previously identified GPI anchored protein localizes to the ER and the plasma membrane, where it appears in a punctuate pattern that co-localizes with callose present around Pd. In T-DNA insertion mutants that do not transcribe AtBG-pap, GFP cell-to-cell movement between epidermal cells is reduced and callose levels around Pd are elevated.

Many plant ß-1,3-glucanases are "Pathogenesis Related" (PR) proteins, and are induced in response to microbial pathogen infection. Measuring the RNA levels of AtBG-pap following infection with cucumber mosaic virus (CMV) and *Pseudomonas syringae* pv. *tomato* showed no significant increase of AtBG-pap transcription levels, suggesting that AtBG-pap is not a PR protein and is not involved in virus cell-to-cell spread.

Physiological measurements of 20 days old AtBG_pap mutant plants suggest that growth of these mutants is inhibited. Germination of the mutant seeds is severely delayed, and totally inhibited in ~50% of the seeds. AtBG-pap RNA levels were shown to be induced during germination just prior to testa and endosperm rupture. These results suggest that callose degradation by AtBG-pap at Pd is required for the regulation of germination, possibly by the release of seed dormancy and the activation of symplasmic connection between cells.

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Cellular polarization for membrane dynamics in interaction of barley with pathogenic *Blumeria graminis*

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When parasitic *Blumeria graminis*, the grass powdery mildew fungus, attempts to penetrate into barley epidermal cells, the plant reacts by cellular reorganization. The cytoplasm polarizes, the filamentous actin cytoskeleton focuses, and the nucleus migrates to the site of attack. Consequently dynamic reorganisation of the endomembrane system takes place, and endocytotic multivesicular bodies (MVBs) form. MVBs either target the vacuole in a lysosomal-like pathway or they are redirected to fuse with the plasma membrane and to release their vesicular cargo into the apolast. MVB-based secretion is involved in formation of cell wall appositions in which *B. graminis* is restricted. However, both actin reorganization and MVB formation are also involved in compatibility leading to accommodation of fungal feeding structures, haustoria, in intact cells. This might indicate corruption of cellular defence mechanisms for generation of the haustorial complex. Accordingly, barley RHO-like proteins, which is involved in actin organization, and presumably defensive NADPH oxidase are required for successful penetration of *B. graminis* into epidermal cells of barley.

Syntaxin SYP121 is involved in a number of pathogen defence mechanisms

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Plant disease resistance is the result of the collective activity of separate defence mechanisms. We have previously discovered that the syntaxin gene *SYP121* (*PEN1*) in *Arabidopsis* is required for penetration resistance[1,2]. SYP121 is necessary for vesicle trafficking leading to formation of papillae, which are local cell wall appositions functioning as barriers against fungal penetration. Based on the use of a functional GFP-SYP121 fusion, we demonstrate that SYP121 in addition to its plasma membrane localization, also is found in endosomal compartments involved in polarized secretion. Pharmacological analyses have demonstrated the involvement of endocytosis and endosomal secretion in the SYP121-dependent penetration resistance.

The most closely related syntaxin gene, *SYP122*, is not required for penetration resistance. Meanwhile, the phenotype of the *syp121 syp122* syntaxin double mutant have shown that these genes act as negative regulators of several signalling pathways leading to pathogen defence-related programme cell death. The *syp121 syp122* plant exhibits a lesion mimic phenotype, which we by introducing knock-out mutations have shown partly to be due to an active SA signalling pathway[3]. Introducing knock-out mutations in a number of other well-known defence pathways have unravelled that several of these also contribute to the lesion mimic phenotype. The fact that several defence pathways are activated in the syntaxin double mutant *syp121 syp122* has allowed us to study genetically to what extend these pathways are parallel.

Re-mutagenesis of *syp121 syp122* has led to isolation of a large number of triple mutants, which at varying degree rescues the lesion mimic phenotype. The third mutations have occurred in *SUPPRESSOR OF SYNTAXIN-RELATED DEATH* (*SSD*) genes. While a number of these have been positionally cloned, many of them have been placed in signalling pathways using high through-put genetics based on examining hundreds of F_2 populations of crosses between triple mutants. Often combination of two *ssd* mutations lead to an enhanced suppression of the lesion mimic phenotype, indicating that the *SSD* genes control parallel signalling pathways, each contributing to the lesion mimic phenotype of the *syp121 syp122* double mutant. In summary, our observations have helped us to draw a map of signalling pathways that are active in *syp121 syp122*.

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Mining iron for host defense and pathogen virulence

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Iron (Fe) is a ubiquitous redox-active element essential to both pathogenic microorganisms and their hosts. In the plant immune response, the formation of localized cell wall appositions, the oxidative burst and the production of pathogenesis-related proteins are hallmarks of plant defense reactions. Using a wheat-powdery mildew pathosystem, we have shown that Fe is a central mediator linking these three phenomena. Upon powdery mildew attack, Fe in leaf epidermis redistributes to the apoplast, which leads to Fe deficiency in the cytosol of attacked cells. The accumulated apoplastic Fe mediates the oxidative burst, which further stimulates Fe efflux and intracellular Fe deficiency. H_2O_2 and Fe deficiency induce expression of defense-related genes while suppressing the expression of Fe storage-related genes. Our work identifies Fe as an underlying factor regulating cereal defenses, and establishes links between disease-related Fe homeostasis in plants and animals.

Fungal pathogens have evolved at least two pathways for Fe uptake from plant hosts regulated by siderophore-assisted Fe mobilization and reductive Fe assimilation systems. To examine the relative contribution of the reductive and siderophore pathways of Fe uptake, we created mutants disrupted at the ferroxidase gene *Fet3* (*Δfet3*) or the siderophore biosynthetic gene *SidA* (*ΔsidA*) from the head blight pathogen *Fusarium graminearum*. Targeted disruption of the *Fet3* gene has no effect on virulence, whereas the *SidA* gene is an essential virulence attribute in the wheat-*F. graminearum* pathosystem. Together, these data show how pathogenic fungi compete with the host for Fe and how the host uses Fe to counteract this threat, providing insights on developing novel strategies for plant disease control.

Plant Physiology & Electrophysiology

Action potential in charophytes

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The plant action potential (AP) has been studied for more than half a century. The experimental system was initially provided by the large charophyte cells, which allowed insertion of multiple electrodes and manipulation of cell compartments. The early experiments were modelled on the Hodgkin and Huxley (1952) (HH) voltage clamp technique developed for the squid axon. The HH analysis identified sodium ion inflow and potassium ion outflow as the as the depolarising and repolarising phases, respectively, of the nerve AP. The plant AP was also modelled in terms of voltage-gated opposing ion flows. The return to the resting potential difference (PD) was, indeed, due to the outflow of potassium ions. However, the depolarising agent was found to be the outflow of chloride ions with involvement of the calcium ions in activation of the chloride channels (Hope and Findlay, 1964). Later the patch clamp technique characterised the chloride ion channels, but the source of the calcium increase in the cytoplasmic space remained unclear (Thiel et al., 1993). Further, using tonoplast-free cells, Japanese researchers obtained APs without chloride ions in the perfusion medium in the charophyte cells, suggesting that calcium could be the depolarising ion under some circumstances (Shimmen and Tazawa, 1980).

At the turn of the century, the paradigm of the charophyte AP shifted to include several chemical reactions, second messenger activated channel and calcium ion liberation from internal stores. The threshold voltage pulse mobilizes the second messenger inositol-1,4,5,-triphosphate (IP3) from its membrane-bound precursor phosphatidyl inositol 4,5-biphosphate (PIP2). IP3 has to reach critical concentration to stimulate calcium concentration rise in the cytoplasm (Biskup et all, 1999; Wacke et al. 2003). Many aspects of this new model await further clarification.

The role of AP in plant movements is well documented in higher plants. The charophytes, on the other hand, are a good system to study the involvement of the AP in wound signalling (Shimmen, 2002) and turgor regulation (Beilby and Shepherd, 1996; 2006), which will be discussed in more detail.

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Electrophysiology of Venus flytrap (*Dionaea muscipula* Ellis)

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Electrical signaling and rapid closure of the carnivorous plant *Dionaea muscipula* Ellis (Venus flytrap) have been attracting the attention of biophysicist and electrophysiologists since the nineteenth century [1,2]. When an insect touches the trigger hairs of the Venus flytrap, mechanosensors on these trigger hairs generate an electrical signal that acts as an action potential which activate the motor cells. Six trigger hairs protruding from the upper leaf epidermis act as mechanosensors, with three of the trigger hairs located in the center of each half of the lamina. The exact mechanism of Venus flytrap closure is still unknown. Moreover, a traditionally used slow data acquisition systems cannot capture plant electrical signals with frequencies higher than half of the sampling frequency. Using an ultra-fast data acquisition system with measurements in real time, we found that action potentials in the Venus flytrap have an average speed of 10 m/s with a duration time of about 1.5 ms and are fast enough to induce the closure of the leaves by the motor cells. A few minutes after closing of the Venus flytrap, electrical signaling was also detected in the lower part of the leaf of the Venus flytrap in the form of graded potentials with amplitudes of 20 mV or less. In terms of electrophysiology, Venus flytrap responses can be represented as the following sequence: stimulus perception, signal transmission, and induction of response. We discovered that the electrical impulse between a midrib and a lobe allows the Venus flytrap leaf to close by activating motor cells without mechanical stimulation of trigger hairs. The average closing time of Venus flytraps by electrical stimulation of motor cells is 0.3 s, which is the same as mechanically induced closing by a small piece of a gelatin or cotton thread. Our results demonstrate that electrical stimulation can be used to study mechanisms of fast activity in motor cells of the plant kingdom.

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Effects of acetylcholine on the blue-light response of dark-grown Arabidopsis seedlings

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An early response of etiolated (dark-grown) *Arabidopsis* to light is a transient depolarization. It has been proposed (Lewis & Spalding, 1998) that this depolarization is due to activation of a Cl^- channel in a ligand-gated channel family, and blocking channel function prevents the inhibition of growth that is part of the greening response. Based on previous experiments with *Chara* that showed a chloride channel activated by acetylcholine (ACh) (Gong & Bisson, 2002), we explored the possibility that the light-stimulated *Arabidopsis* channel is similarly activated by ACh. Since the *Chara* experiments indicated that the binding site for ACh is cytoplasmic, we microinjected ACh into the cytoplasm of hypocotyl cells of etiolated plants, and determined the effect on the light-induced depolarization. We found, instead of activation, that microinjection of ACh inhibited the depolarization due to light. Because nicotine potentiated the effect of ACh in *Chara*, we microinjected nicotine with ACh in *Arabidopsis*. Instead of potentiating the effect, nicotine restored the depolarization, although it significantly delayed it. To test the physiological significance of these effects, we treated etiolated *Arabidopsis* plants with ACh with and without nicotine. Treatment decreased growth in the dark somewhat, but eliminated the inhibitory effect of light. In fact, plants treated with ACh consistently grew longer after brief exposure to light than those in constant darkness. We explore models for the action of ACh on the greening process.

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Photoperiodic adaptation by systemic control of growth and rates and planes of cell division via systemic electrophysiological communication from the cellular to the organismic level

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Vegetative growth and the transition to reproductive growth involve continuous communication between all plant organ systems and their response to the networking of internal and external signals. Time-lapse photography is clear evidence of such adaptative behaviour to environmental signals resulting in a precise pattern of rhythmic phenomena. Rhythmic integration of the whole plant involves modulation of turgor pressure via stretch-activated ion channels and concomitant changes in membrane potentials, potentially leading to action and/or variation potentials.

As evident on the cellular level, induction of polarity, the basis for changes in rates and planes of cell division, involves latent changes in patterning of plasmamembrane electrochemistry, which eventually becomes stabilised by structural polarity involving cytoskeleton elements. It is proposed that the dynamic electrochemical activity is continuously integrating internal and external signalling for developmental adaptations in a changing environment.

Mesophyll cells are the driving force for light- and acid- induced leaf blade expansion of *Pisum sativum* var. *argenteum*

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Dicot leaves have a laminate structure of four cell layers: the two epidermal layers surrounding the palisade and spongy mesophyll tissues. There has been persisting uncertainty as to which of these layers is driving the expansion of leaf blades. To solve this problem we made use of the Argenteum mutant of pea, where viable epidermal layers can be easily removed from the leaflets. Removal of the main vein or just one epidermis did not alter the growth rate of excised leaflet strips, but removal of the second epidermis caused a rapid increase in the growth rate. Long-term experiments confirmed that the light-response of isolated mesophyll strips excedes that of the complete-leaflet strips by 50 %, while isolated epidermis strips expand only when pulled by an external force. Both isolated mesophyll and epidermal tissues undergo rapid elongation in response to a change in solution pH from 6.0 to 4.5. Previous experiments comparing the ability of isolated epidermis and mesophyll layers to pump protons in response to light found the response of the mesophyll to be larger (Stahlberg & VanVolkenburgh 1999). These results support the idea that the mesophyll layers drive and control the rate of leaf expansion.

The linear phase of sucrose uptake concentration curve in sink organs is largely mediated by fluid phase endocytosis

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Biochemical studies have demonstrated that sucrose uptake kinetics into sink cells consist of multiple components collectively characterized by a bi-phasic curve. Whereas the hyperbolic phase at low external sugar concentration is believed to represent a high-affinity, membrane-bound, carrier-mediated component, the linear non-saturable phase at higher concentrations denotes facilitated diffusion presumably mediated by a sucrose binding protein. Previous observations that FPE in celery parenchyma was only induced at high external mannitol prompted us to re-examine the possible role of FPE and membrane-carrier transport within both phases of the characteristic concentration uptake curve. At low external concentration (5 mM), sucrose uptake into turnip (*Brassica campestris*) hypocotyl discs was inhibited by the sucrose carrier inhibitor phloridzin (2 mM) but unaffected by the endocytic inhibitor latrunculin-B (10 µM). When sucrose concentration was increased to 100 mM, transport was significantly reduced by both phloridzin and latrunculin-B. Uptake of the endocytic marker Alexa-488 was strongly inhibited by latrunculin-B at both external sucrose concentrations with no effect noted in the presence of phloridzin. Analyses of the data and of Alexa-488/sucrose 'specific activity' revealed that the characteristic linear phase of sucrose uptake concentration curves is largely mediated by fluid phase endocytosis. Time-lapse photography using confocal laser scanning microscopy captured the rapid fusion of a ~5 µm vesicle with the vacuole supporting earlier suggestions that most of the endocytic uptake occurs through a clathrin-independent micropinosome system. In other photographs, the inclusion of multivesicular bodies within the vacuole was evident. The presence of multivesicular bodies (containing fluorescent vesicles) strongly suggest the existence of a retrograde vesicle transport system from the vacuole capable of reconciling vacuolar volume and constituting the energy-dependent phase that concentrate solutes within.

Cytokinin oxidase/dehydrogenase activity in oat xylem sap

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Cytokinins affect plant growth and development by stimulating cell division and differentiation. Modulation of flux of cytokinins from roots to shoots via xylem flow in response to environmental signals has been repeatedly reported. The concentration of cytokinins in apoplast can be potentially modulated by extracellular cytokinin metabolism. We found the cytokinin oxidase/dehydrogenase (CKX) activity in xylem sap of oat (*Avena sativa* L.) plants. The enzyme exhibited pH optimum at 8.5 and its activity was associated with glycosylated protein. Since the pH of root-sourced xylem sap is much lower (6.1) the activity of the CKX leaving the roots is suppressed protecting the co-transported cytokinins from degradation. The potential role of CKX in control of the cytokinin concentration in xylem sap in response to environmental signals was tested by the exposure of 12 d old plants for 48 h to nutrient solutions differing in NO_3^- concentration (16-1000 μM). The flux of the root-sourced CKX activity was increased with the increasing NO_3^- supply up to 7-fold correlating well with the increasing flux of cytokinins [trans-zeatin riboside, *trans*-zeatin and N^6-(2-isopentenyl)adenine]. The flux of *cis*-isomers of zeatin was, with exception of *cis*-zeatin *O*-glucoside, not affected by the exogenous NO_3^- indicating different regulation of biosynthesis of *trans*- and *cis*-zeatins by nitrate

ECOLOGY

The role of volatiles in plant-to-plant communication

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Plant volatiles have long been known to mediate many important interactions between plants and insects, but their importance in interactions among plants has been much debated. We discuss two recent projects dealing with volatile mediated plant-to-plant communication. The first demonstrates that seedlings of the parasitic dodder plant *Cuscuta pentagona* use volatile cues to locate host plants and to distinguish between more and less preferred hosts. Several individual compounds present in volatile blend of the preferred host tomato (*Lycopersicon esculentum*) are shown to be attractive to *C. pentagona* seedlings, while one compound present in the non-host wheat (*Triticum aestivum*) is shown to be repellent. The second project shows that volatiles released by herbivore-wounded leaves of hybrid poplar (*Populus deltoides x nigra*) prime defenses in adjacent leaves on the same plant that have little or no vascular connection to the wounded leaves. Undamaged leaves exposed to volatiles from wounded leaves on the same stem had elevated defensive responses to feeding by gypsy moth larvae (*Lymantria dispar*) compared to leaves that did not receive volatiles. While previous research has focused on signaling between plants, self-signaling via volatiles is consistent with the short distances over which plant response to airborne cues has been observed to occur, suggesting that within-plant signaling may greater ecological significance than previously realized.

Allelochemicals as a signaling molecules in the negative plant-plant interaction

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Allelopathy phenomenon is defined as the influence of one plant on another through chemicals (allelochemicals) released into the environment. The action of allelochemicals in target plant is diverse and affects a large number of biochemical reactions resulting in the modifications of variety physiological processes. Sunflower (*Helianthus annuus* L.) actively influences the growth of surrounding plants due to its strong allelopathic potential. We investigated mode of action of sunflower allelochemicals during germination of mustard (*Sinapis alba* L.) seeds. Inhibition of germination was associated with alterations in reserve (lipids, proteins) mobilization and energy (ATP) generation in the catabolic phase of germination (Kupidlowska et al. 2006). Additionally, sunflower allelopathic compounds induced oxidative stress manifested as enlarged production and accumulation of reactive oxygen species (ROS) (Oracz et al. 2007). It correlated well with loss of membrane integrity (Bogatek et al. 2006). Therefore we suggested that in allelopathy stress ROS (H_2O_2) may act as signaling molecules leading to disturbances in the balance of phytohormones crucial for seed germination. ABA concentration in seeds increased after exposition to sunflower allelochemicals, in the contrast, ethylene emission was strongly repressed (Gniazdowska et al. 2007). Low ethylene concentration, resulting from inhibition of key enzymes activities of ethylene biosynthesis may enhance seed sensitivity to ABA. The alteration in phytohormones level leads to decreasing metabolic activity of the embryo and blocking seed germination as well as growth of young seedlings. The putative relationship between ROS and phytohormones in allelopathy interaction will be discussed.

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Chemical communication between roots and shoots in tomatoes

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Abscisic acid (ABA) is not only synthesized in leaves, but also in roots and it is conventionally accepted that root-sourced ABA plays a key role upon water deficit, triggering stomatal closure in the leaves. Here, we used the ABA-deficient mutant *notabilis* (*not*) in *Lycopersicon esculentum*, its isogenic cultivar Lukullus (Luk) and a naturally desiccation-resistant wild relative *L. pennellii* (*pen*) to study the relative importance of leaf and root-derived ABA on stomatal closure. We conducted a series of graftings with these genotypes in all possible shoot/rootstock combinations and then imposed water stress on the plants. Measurements of stomatal conductance, transpiration and water potential were performed. Thesuccess of grafts was minimal when *not* was the scion or *pen* was the rootstock. In graftings involving a *not* shoot, stomatal conductance and transpiration were reduced during water stress and the recovery period if *pen* or Luk was used as rootstock rather than *not* itself. Conversely, low stomatal conductance was also observed in *pen* even when the rootstock was *not*. The *not*/*not* graftings attained the permanent wilt point in 5 days whereas *not*/*pen* survived without irrigation for 21 days. These results suggest that the genotype of the shoot determines stomatal activity under normal irrigation and that under dehydration and the subsequentrecovery the control is given by a root-derived substance, which appears to be in a higher dose in *L. pennellii*. This opens interesting perspectives for the basic and applied aspects of water stress resistance in plants.

Neurotoxicity of aluminium: parallelism between plants and animals (including men)

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Although epidemiological studies in men are still inconclusive, it is well established that aluminium (Al), in available form, is extremely toxic to all organisms. The neurotoxic effects in animals and men are well documented. High tissue Al concentrations have been found in patients with dialytic encephalopathy, amyotrophic lateral sclerosis or Alzheimer disease. In plants, Al mainly affects root growth and development. A stunted root system with reduced capacity to explore the soil for water and nutrients is the main visible symptom of the Al toxicity syndrome. Besides these obvious differences in the outcome of Al toxicity, there are striking similarities in the mechanisms of Al toxicity in both plant and animals, including men. This presentation will give a comprehensive overview on the basic mechanisms by which Al may cause neurotoxicity in both animals and plants. At the present stage of knowledge, it is getting clear that specific cells are the primary targets of Al toxicity in both humans and plants: astrocytes in the animal or human brain and transition zone cells in roots, the "brain-like cells" (Baluška et al. 2004, Illeš et al. 2006) of plants. Special attention will be paid to Al interactions with the plasma membrane, to Al-induced oxidative stress, and to the glutamate metabolism at these primary sites of toxicity. Perception and transmission of the Al signal and its consequences for adaptative root growth in plants will be discussed.

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Common cellular mechanisms of endosymbiotic root infection

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The mutual beneficial relationships between plants and arbuscular mycorrhizal (AM) fungi and nitrogen-fixing bacteria known as rhizobia, are highly important both from an agricultural and ecological point of view. Plants exchange photosynthate products for phosphate in the first and nitrate in the latter, and provide a safe niche for their microbial partner. The AM association is wide-spread, while the rhizobial symbiosis is limited to leguminous plants. During both interactions the microbes invade internal root tissues developing specific intracellular symbiotic structures called arbuscules in the AM association and symbiosomes in nodulation. In legumes, the entry of both AM and rhizobial symbionts appears to be controlled by a common signal transduction pathway concerning a small number of plant genes (*DMI1*, *-2*, *-3* and their homologues). The initial entry into root tissue is intracellular involving the formation of a host membrane/cell wall interface which physically separates the microbe from the host cell cytoplasm. During nodulation, invasion takes place through curled root hairs and a subsequently formed plant-derived structure, the so-called infection thread. For the passage through root cortical cells the infection thread makes use of a predefined way consisting of cytoplasmic bridges named pre-infection threads. AM fungi penetrate directly root epidermal cell surfaces and pass through a recently identified structure named the pre-penetration apparatus (Genre et al. 2005). Thus, in both symbioses the plant appears to be the principal partner in control of initial infection and infection progression. Comparative ongoing studies on both endosymbiotic associations in our laboratory will be presented in relation to the strategies developed by plants to control beneficial microbe entry into host tissues.

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Piriformospora indica: A cultivable symbiotic fungus with multiple biotechnological applications: Molecular analysis of its interaction with *Arabidopsis thaliana*

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Piriformospora indica is a wide-host root-colonizing fungus which allows the plants to grow under extreme physical and nutrient-limiting conditions. The fungus promotes growth and seed production and confers resistance against biotic and abiotic stresses (1). We study the molecular basis of the interaction between *P. indica* and the model plant *Arabidopsis thaliana* (cf. 2, 3) Based on mutant screens, expression profiling, proteomics as well as biochemical techniques, we could identify plant components, which are required for this beneficial plant/microbe interaction. Components involved in recognition, early signalling events and maintenance of the symbiotic interaction will be discussed. In a second screen, we identified *Arabidopsis* mutants in which growth and development is inhibited rather than promoted by the fungus. Apparently, only a few components in plants need to be manipulated to convert a beneficial into a pathogenic interaction.

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Ion channel-forming compounds in caterpillar regurgitate: A way to manipulate the plant plasma membrane potential during herbivory?

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When insects feed on plants, they introduce oral secretions (OS) into the plant tissue. These OS contain several molecules that are known to be involved in the induction of plant defence reactions and subsequent processes. OS was analyzed with regard to their membrane activities using the black lipid membrane (BLM) technique. Transmembrane ion fluxes were generated by OS of eight different lepidopteran larvae, which all displayed comparable ion channel-forming properties in artificial membranes. These currents were characterized by long lasting opening times and high conductivities. The OS from *Spodoptera exigua* exhibited channels with a preference of cations over anions. OS also induced a transient increase of the cytosolic calcium concentration in soybean cells, which was determined by the aequorin technique.
Other compounds of the OS, fatty acid- amino acid conjugates (FACs), also interfere with BLMs. But unlike OS, they do not form long lasting channels.
Since ion fluxes and depolarization are early responses upon insect feeding, OS-derived components may directly be involved and interact with the plant membranes.
The focus of ongoing work lies on the purification and subsequent identification of the substance(s) responsible for the channel-formation.

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Cutinized and suberized plant/environment interfaces: structure, biosynthesis and function

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As a prerequisite for colonisation of the mainland, plants developed lipophilic biopolymers forming the interface between the plant and the surrounding air and soil environment. Leaf surfaces are covered by cuticles and waxes, stem and root interfaces are formed by suberized cell walls. As main function lipophilic interfaces form efficient transport barriers protecting land-living plants from uncontrolled water loss and at the same time they protect living plant tissue from infection by pathogens. Various aspects of our ongoing research related to the structure, biosynthesis and function of cutinized and suberized plant/environment interfaces will be presented and discussed.

POSTERS

Endocytic uptake and traffic of sucrose linked to both starch and cellulose biosynthesis are processes specifically triggered by sucrose that require the synthesis *de novo* of proteins

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We have recently established that an important pool of sucrose linked to starch biosynthesis in heterotrophic cells is taken up by endocytosis (Etxeberria et al. 2005 Plant Cell Physiol. 46, 474-481; Baroja-Fernandez et al. 2006 Plant Cell Physiol. 47, 447-456). Whether this mechanism is also involved in the sucrose-cellulose conversion process was investigated by comparing the rates of cellulose accumulation in sycamore cells cultured in the presence or absence of the endocytic inhibitors wortmannin-A, 2-4(4-morpholynyl-)8-phenyl-4H-1 benzopyran-4-1 (LY294002) and latrunculin B. These analyses revealed that sucrose-cellulose conversion involves two phases, the second of which being 35% sensitive to the effect of endocytic inhibitors. Whether endocytic uptake, traffic and metabolism of sucrose requires the *de novo* synthesis of proteins was investigated by comparing the rates of accumulation of the endocytic marker lucifer yellow, sucrose and starch in sycamore cells in the presence or absence of the transcriptional inhibitor cordycepine and the translational elongation inhibitor cycloheximide. These analyses revealed that the two compounds exerted a strong inhibitory effect on the accumulation of lucifer yellow, sucrose and starch. The stimulatory effect of sucrose in the endocytic uptake of external solutes could not be replaced by the non-metabolizable sucrose analogues palatinose and turanose. The overall results (a) provide a first indication that the endocytic uptake of sucrose linked to both starch and cellulose biosynthesis requires *de novo* synthesis of proteins and (b) further strengthen that the endocytic uptake of external solutes is very specifically triggered by sucrose.

Multiple isoforms of phospholipase D are involved in the regulation of plant cell morphogenesis

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Phospholipase D (PLD) cleaves structural phospholipids, namely phosphatidylcholine, producing second messenger phosphatidic acid (PA). PLD and PA play crucial role in many signal transduction pathways across eukaryotic kingdom. In animal and yeast cells, PLD was implicated in the regulation of vesicular trafficking and dynamics of actin cytoskeleton. In plants, PLD research is mainly focused on its role in responses to various stresses and involvement of PLD/PA in the mechanisms controlling cell polarity is only partially characterised. Here we present data showing that multiple PLDs are required for polar growth of tobacco pollen tubes. We cloned five partial PLD cDNAs from tobacco pollen tubes and BY2 cells covering all major PLD subfamilies. RT-PCR analysis suggested differential expression of studied cDNAs. In order to functionally characterize distinct PLD isoforms, we used gene specific knock-down mediated by antisense oligonucleotides. The suppression of NtPLDbeta1 and NtPLDdelta lead to lower growth rates, whereas exogenously applied PA restored normal growth, thus confirming the importance of PLD signaling for polar growth of pollen tube and raising the question of downstream targets of PA. Visualization of actin cytoskeleton indicated the involvement of PLD/PA signalling in cytoskeletal dynamics.

Plant γ-tubulin is essential for noncentrosomal microtubule nucleation from microtubules and membranes associated dispersed sites

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Microtubules (MTs) nucleated independent of defined microtubule organizing centres such as centrosomes or spindle pole bodies have been only recently shown to play an important role in designing cytoskeleton architecture. γ-Tubulin is required for MT nucleation at defined microtubule organizing centres but its role in nucleation of noncentrosomal MTs is much less understood. In higher plants where all somatic and gametic cells are acentrosomal, there are several microtubular arrays organized during cell cycle progression from undefined dispersed sites. Well characterized γ-tubulin ring complexes that are essential for centrosomal MT nucleation in animal cells have not yet been identified in plants. Rather we found the presence of heterogeneous protein complexes of γ-tubulin in cytoplasm, in association with membranes and MTs. Large γ-tubulin complexes were active in microtubule nucleation. To further analyze the role of γ-tubulin, we conditionally downregulated γ-tubulin by inducible expression of RNAi constructs in *Arabidopsis thaliana*. After induction of RNAi γ-tubulin was gradually depleted from all known cellular locations including the microsomal and the microtubular fraction. We found that γ-tubulin as a component of cortical nucleation templates guides cortical MTs. The regrowth of MTs from perinuclear membrane rich region after drug depolymerization was delayed in cells with reduced γ-tubulin levels. Similarly, immunodepletion of γ-tubulin from *A. thaliana* extracts strongly compromised the *in vitro* polymerization of MTs. Almost complete RNAi depletion of γ-tubulin led to the absence of microtubules. In summary, we showed that γ-tubulinu is essential for MTs nucleation from dispersed sites in acentrosomal plant cells. Further characterization of γ-tubulin forms and their protein interactions is under progress.

Glutamate and ethanol deplete F-actin and inhibit vesicle recycling at "plant synapses" in root apices

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L-Glutamate is a well-known neurotransmitter in brain and it has also dramatic impacts on plant root apices (Filleur et al. 2005, Walch-Liu et al. 2006). Specifically, the primary root apex is sensitive to L-Glutamate, which does not affect apices of young lateral root primordia (Walch-Liu et al. 2006). Plants also express glutamate-like receptor family proteins (GLRs) gated by glutamate and glycine (Dubos et al. 2003, Gilliham et al. 2006). Glutamate gated GLRs emerge to act in plants, similarly like in animals, as calcium channels (Demidchik et al. 2004, Kang et al. 2006, Qi et al. 2006) involved in the response of plants to sensoric stimuli and to stress from the environment (Kim et al. 2001, Sivaguru et al. 2003, Kang et al. 2004, Meyerhoff et al. 2005). Genetic evidence suggest, that GLR are essential for the organization and functioning of primary root apices (Li et al. 2006). Here we have analyzed effects of glutamate on the actin cytoskeleton and vesicle trafficking in primary root apices of Arabidopsis and maize. Our data reveal, that the most sensitive subcellular domains are the cell end-poles, which represent what we have defined as plant synapses (Baluška et al. 2005). Especially in the transition zone (Verbelen et al. 2006), F-actin gets depleted and vesicle trafficking inhibited at plant synapses in primary root apices. Surprisingly, similar effects have been scored also with ethanol at concentrations even lower as those (Offenhäuser et al. 2006) which has been recently reported to affect F-actin at mouse brain synapses (Offenhäuser et al. 2006, Sordella and Van Aelst 2006). In the future, we will study the behavior and performance of roots challenged with exogenous glutamate and ethanol.

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γ-Tubulin and its role in *Arabidopsis* development

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We found that inducible RNAi depletion of γ-tubulin led to serious distortions of development in *A. thaliana* seedlings. Cells with decreased levels of γ-tubulin could progress through mitosis, but late mitotic events and cytokinesis were strongly affected. Particularly, we observed that polar distribution of γ-tubulin during late mitosis was disturbed and the phragmoplast formation failed. In contrast to the control cells where anaphase spindles were rearranged into the phragmoplast, long anaphase spindles persisted between separated nuclei in RNAi cells. The cell plate formation sites were often misaligned. These discrepancies in late mitosis and cytokinesis often resulted in bi- or multi-nuclear cells and disruption of regular cell files and some morphogenic changes were observed. Strict developmental pattern of stomata was disrupted and clusters of two to four stomata were observed in RNAi expressing plants with reduced γ-tubulin levels. In addition to the stomata clustering, the cytokinetic defects of guard cells were found when γ-tubulin was severely depleted. Dorsoventral polarity during leaf development was disturbed in seedlings with reduced γ-tubulin levels. Ectopic root hairs formation was observed in cells with randomized microtubules, anisotropic growth of root hairs was disturbed, formation of two growth axes was often observed. We suggest that some functions of γ-tubulin that are important for cytokinesis, cell specification and polar growth might be microtubule independent and require further analysis.

Physiological basis for altered responsiveness to auxin and light in modern corn hybrids – role of auxin-binding proteins

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Modern varieties of corn (*Zea mays* L.) developing erect leaves have been selected for their ability to maintain production in dense planting. We showed earlier that on the whole plant level, and at the cellular and molecular levels, the modern hybrid 3394 is less sensitive to exogenous auxin than two older hybrids 307 and 3306. Others confirmed our results since there is a decline in response to auxin over the decades of varieties release. We also showed that the levels of endogenous free IAA in 307 and 3394 were similar.

The modern hybrid 3394 growing in the dark was also less sensitive to exogenous auxin than other two older hybrids 3366 and 317. Also, excised mesocotyl segments of 3394 were less responsive to NAA than segments of the older varieties. An additional modern hybrid, Benecia developing erect leaves, showed less sensitivity to exogenous auxin than older hybrids PR39A37 and PR39G83 with less erect leaves for leaf angle development and expression of *ABP4* (auxin-binding protein 4). Interestingly, the three hybrids did not differ in the level of endogenous free IAA in etiolated mesocotyls.

We published recently that growth of 3394 seedlings is less inhibited than growth of older hybrids by red (R) and far-red light (FR). Here we found that 3394 mesocotyl is also less responsive than all the tested older hybrids to the inhibitory effect of blue light (BL). In contrast to R or FR, BL in our experimental conditions promoted elongation of coleoptile, and the stimulatory effect was much stronger in 3394 than in the older varieties. Interestingly, under BL, coleoptile elongation of the modern variety 3394 was inhibited by NAA significantly more than growth of coleoptile in 307.

To understand more the role of maize ABPs in growth and development, we analyzed maize single mutants *abp1* and *abp4*, and the double mutant *abp1abp4*. Mutations in *ABP1* and *ABP4* genes caused changes in development of leaf angle. In comparison with the corresponding wild-type (WT), *abp1* and *abp4* developed more and less erect leaves, respectively. Interestingly, etiolated WT, *abp1*, and *abp4* seedlings exhibited similar responses to exogenous auxin for coleptile, mesocotyl, and root growth. However, mesocotyls of double mutant *abp1abp4* were distinctly less sensitive to the inhibitory effect of exogenous auxin. Analysis of endogenous auxin in etiolated mesocotyls revealed that all the *abp* mutants contain significantly greater levels of free IAA.

Our results support the existence of interaction between auxin and light in regulation of growth and development of young corn seedlings. The results further indicate that ABP1 and ABP4 are involved in mesocotyl growth and leaf angle development, but also suggest ABP redundancy in maize. Finally, our data support the hypothesis that modern corn hybrids developing erect leaves are less responsive to exogenous auxin. Specific function of blue light in development of maize seedlings is discussed.

Possible interaction between blue light and anion and water channels during plant responses to abiotic stresses

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We previously reported that spontaneous mutant *7B-1* in tomato is resistant to osmotic and salt stress in seed germination specifically under blue light (BL). We showed that in tomato wild-type (WT), BL strongly inhibits seed germination, but essentially less than in *7B-1*. Relative to the WT, *7B-1* seedlings develop longer hypocotyl in WL and BL. The long-term objective of our work is to determine whether *7B-1* gene is involved in blue light signaling, and what is the role of *7B-1* product in plant tolerance to stresses. Here we report that NPPB, an anion-channel blocker, can intensify the inhibitory effect of BL on germination in tomato, when applied on WT seeds cultured under BL. Interestingly, germination of *7B-1* seeds in BL is almost completely resistant to NPPB. Aquaporins could represent a prerequisite for strategies against osmotic stress. Like NPPB, $HgCl_2$, an inhibitor of aquaporin, under BL powerfully inhibits seed germination in WT, but not in *7B-1*. Differently from *7B-1*, mutations *cry1-1* and *cry1-2* do not result in the resistance of seed germination to mannitol, and do not alter sensitivity of seed germination to the inhibitory effect of NPPB or $HgCl_2$. On the other hand, defects in two *Arabidopsis* genes coding for anion channels result in increased sensitivity of seed germination to mannitol, specifically under BL. Our results suggest that functional anion-channels and/or aquaporins may be involved in ability of seeds to tolerate osmotic stress. Data also indicate that BL is involved in the process. *7B-1* mutant seems to have some traits of plants less responsive to biotic stress. We found that in the dark and BL hypocotyls of the *7B-1* mutant are less sensitive than WT to the inhibitory effect of *Pseudomonas syringae* phytotoxin coronatine added to the culture medium. Interestingly, the resistance was associated with the fact that in contrast to the WT plants, level of endogenous salicylic acid (SA) and jasmonic acid (JA) in *7B-1* hypocotyls could not be altered by BL. Our results suggest that BL plays a role in plant tolerance to abiotic stresses, and that anion channels and/or aquaporins may be involved in the process. Pleiotropic effect of the mutation suggests that *7B-1* gene product can function as an upstream element in light signaling pathway(s). We hypothesize that specifically in BL, *7B-1* mutation enhances activity of anion and/or water channels, which can help the mutant seeds to tolerate osmotic stress. Analysis of recently obtained cDNA microarray data is in progress, and cloning of *7B-1* gene will follow.

Acetylcholine signalling targets "plant synapses"

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The classical neurotransmitter acetylcholine (ACh) is well-known for propagating action potentials across neuronal synapses (Phillis 2005). At the neuromuscular synapse, the entire process of signal transmission, including vesicular release ofACh, its diffusion across the synaptic cleft, reversible binding with nicotinic ACh receptor, and finally the enzymatic hydrolysis of ACh by acetylcholinesterase (AChE) takes only a few milliseconds. In plant tissues, ACh is an abundant molecule, which increases its endogenous concentrations under stress situation (Tretyn 1991). Plants express AChE, which is inhibited by neostigmine bromide, a specific inhibitor of the animal AChE (Sagane et al. 2005). Older studies already revealed that exogenous ACh stimulates plant cell elongation (Evans 1972). Moreover, endogenous ACh levels are sensitive to light and oscillate (Tretyn and Tretyn 1990). It has also been shown, that ACh-hydrolyzing activity in maize is essential for the root graviresponse (Momonoki 1997, Momonoki et al. 2000). Here we report, that experimental manipulations of ACh levels exerts specific actions on root apices of Arabidopsis and maize. The F-actin-enriched cross-walls in the root transition zone, which we have previously defined as "plant synapses" (Baluška et al. 2005), emerge as the most sensitive intracellular domains. Excess of ACh, induced either by addition of exogenous ACh or by inhibition of the AChE, has a dramatic impact on spatial control of cell division planes, F-actin assembly, endocytosis and vesicle recycling activities, as well as on the overall architecture of the plant synapse.

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Involvement of ABA in the generation and propagation of electrical signal upon local burning demonstrated on wild-type and ABA-deficient tomato mutant plants

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An important role of electrical signals in short-term long-distance (systemic) plant responses to local wounding has been recently established (Koziolek et al. 2004, Hlaváčková et al. 2006). Simultaneously, a rapid action of plant hormones (abscisic and jasmonic acids) in systemic tobacco responses upon local stress has been shown (Hlaváčová et al. 2006, Hlaváčková and Nauš 2007). However, little is known about the interactions of these signals (electrical and chemical) and about the mechanisms whereby they mediate the systemic responses.

We examined short-term (up to 1 h) local and systemic electrical responses of wild-type (WT) and abscisic acid (ABA)-deficient tomato mutant (sitiens) plants to local burning (12s) of an upper leaf. A lower endogenous concentration of ABA (about one third of WT) in leaves located below the burned one was detected in untreated and also burned sitiens plants compared to the WT tomato. The electrical recordings obtained from the wounded leaf and three others situated below the burned one (in basipetal direction) revealed significant differences between both variants. Firstly, the amplitude of electrical signal of WT (50-60 mV) plants was twice as high as that of sitiens (20-35 mV) plants. Secondly, the way of electrical signal propagation seemed to be influenced by ABA. While in the WT plants the amplitude and propagating velocity of electrical signal decreased with increasing distance from the site of burning (indicating the variation potential, VP), it did not hold for sitiens plants. Although the 5th leaf in the sitiens plants was closer (27 cm) to the burned site (6th leaf), the VP wave was delayed as compared to that of the more distant 4th (29 cm) and 3rd (31 cm) leaves. Taken into account the angle position of measured leaves on sitiens tomato plants, the VP wave propagated faster along the leaf trace of burned leaf (vascular bundles coming from the burned leaf) down the stem in tomato sitiens plants.

Comparing amplitudes and propagation velocities of electrical potential changes in WT and sitiens mutant tomato plants, our results suggest a participation of ABA in the electrical signal generation and propagation in tomato plants after local wounding. Thus, an interaction of both, electrical and chemical signals, in rapid systemic plant stress responses is plausible.

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Photoelectrical reaction of pumpkin plants and its interaction with action potential induced by electrical and thermal stimuli

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Many studies have shown that plants possess numerous mechanisms which enable them to perceive, transduce and respond to a variety of environmental signals. Light signals are amongst the most important environmental factors that regulate plant growth and development. The gol of this study was to determine the interaction of light-induced changes in electlical potential of pumpkin plants with the action potentials evoked by electrical and thermal stimuli. The experiments were carried out with 14-16 day-old pumpkin plants (*Cucurbita pepo* L.) grown in Hoagland`s medium under incandescent and luminescent light. The measurements of electrical potential difference were done with a non-invasive, surface-contact electrodes. The electrical reactions of pumpkin were induced by electrical (square current pulses), thermal (burning) and light (400 W m^{-2}) stimuli. The action potential (AP) generated by electrical pulses in the lower part of the hypocotyl was transmitted and propagated with decrement to cotyledons and leaves. These voltage transient changes had a shape of single peak and fulfilled all-or-none law. The action potential triggered by thermal stimulus (local burning of cotyledons) differed in terms of amplitude and duration as compared to electrically induced AP. The irradiation of the plants with white light caused an electrical response of a specific nature which did not resemble the APs induced by electrical and thermal stimuli. The light-induced response comprised two phases: a relatively fast hyperpolarization followed by a slower depolarization. The potential difference measured with surface electrodes changed with an identical time course but opposite polarity as compared to intracellular recordings. The amplitude and time course of depolarization phase depended on the duration of irradiation. When pumpkin plants were stimulated simultaneously with both light and electrical or thermal stimuli an additive effect was observed. The results are discussed taking into account the ionic basis of both light-induced electrical response and action potential triggered by electrical or thermal stimuli.

Functional adaptation of suspension-cultured tobacco BY-2 cells to the osmotic stress

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Plant cells are continuously exposed to changing osmotic conditions, dependent on the versatile water accessibility. Long-term osmotic stress evokes activation of adaptive mechanisms, leading to changes in e.g. cellular metabolism. As a consequence, the cell is able to survive the stress. However, biochemical adaptations are not the only ones. Another group constitute mechanical adaptations, and the continuum between cell wall, plasma membrane and actin cytoskeleton is the major player here. The wall-protoplast interactions are particularly important for mechanical stabilization of the cells subjected to changing environmental conditions. They prevent either bursting of the protoplast under iso- and hypotonic conditions, or collapsing of the plasmolysing protoplast in hypertonic environment. The cytoskeleton plays an important role at the protoplast side in controlling cell shape and mediating intracellular signalling. Both microtubules and actin filaments might be anchored at the plasma membrane and further in the surrounding cell walls. This anchoring could be crucial for the proper functioning of cytoskeletal networks.

The very special case of the cell's response to stress is the adaptation to such conditions that are lethal to non-adapted cells. We have adapted the suspension-cultured tobacco BY-2 cells to extreme osmotic stress conditions evoked by high levels of ionic (NaCl, KCl) and nonionic (mannitol, sorbitol, polyethylene glycol) agents. The concentrations of these agents were chosen in such a way as to cause similar changes of the water potential. Nonionic and ionic osmotica act in different manner and result in specific responses of adapted cells. Ionic agents increase adhesive properties of the cells, and formation of cell aggregates. On the other hand, nonionic agents stimulate strictly positioned cell divisions and thus induce formation of cell files. Surprisingly, analyses of actin and tubulin cytoskeletons in adapted cells and non-adapted, unstressed, cells reveal no significant changes. However, tobacco BY-2 suspension cells exposed to short-term osmotic stress could cope with it in a cytoskeleton-dependent manner. Such cells reveal disruption of fine networks of cortical microfilaments and microtubules, and, most probably, formation of thicker cables. Changes in the actin cytoskeleton occur at membrane zones detached from cell walls – protoplast's regions especially subjected to mechanical stress. It seems that upon prolonged exposure to osmotic stress conditions adaptive, alterations in cell wall composition will occur. This will probably change anchoring of the cytoskeleton in the walls and further modify functioning of the whole cell wall-plasma membrane-cytoskeleton continuum. In that way, cell's mechanical balance restoration will be ensured and, in consequence, cell will be able to resist osmotic pressure and divide in severe stress conditions.

Signal transduction from elicitation with N-acetylchitooligosaccharide to biophoton generation

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Biophotons are ultraweak light emissions from biochemical reactions in a living body. Elicitor-responsive photon emissions (ERPE) increase in suspension-cultured rice (*Oryza sativa* L.) cells when elicited by *N*-acetylchitooligosaccharide. Biochemical analyses were undertaken to clarify the emission mechanism of ERPE. Exogenously applied phosphatidic acid (PA), the second messenger leading to the reactive oxygen species (ROS) generation in the signal transduction of disease response, raised photon emissions in rice cells. Comparisons of photon emissions from PA and ERPE regarding time courses, spectral compositions, and the inhibition ratios of several inhibitors, as well as a loss- and gain-of-function assay using the protein synthesis inhibitor cycloheximide and PA, showed the possibility that ERPE were generated through PA, an intermediate of phospholipid signaling. The effects of protein phosphorylation (K252a) and the Ca^{2+} signaling inhibitors (EGTA and $LaCl_3$), caused ERPE to decrease. It is clear that ERPE are regulated by Ca^{2+} signaling and protein phosphorylation. ERPE were suppressed when cells were pretreated with ROS-generating inhibitors: pyrocatechol-3,5-disulfonic acid disodium salt (Tiron); diphenylene iodonium (DPI); and salicylhydroxamic acid (SHAM). Conversely, exogenously applied ROS (superoxide and hydrogen peroxide) was able to induce photon emissions. ERPE are closely associated with the ROS-generating system. In addition, we found that the pattern of ERPE is almost identical to that of hydrogen peroxide generation. ERPE were inhibited with the pretreatment of NO scavenger, cPTIO. Interestingly, exogenously applied NO did not induce biophotons, but suppressed ERPE dose-dependently when applied together with *N*-acetylchito-oligosaccharide. It appears that NO plays a role of controlling ERPE through interacting with the ROS-generating system.

Alterations of actin cytoskeleton - the signalisation highway - in root hairs of GFP-FABD2 *Arabidopsis thaliana* treated with Pb^{2+}

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We are grateful to Prof. Diedrik Menzel (University of Bonn, Germany) for generous gift of the GFP-FABD2 *Arabidopsis thaliana* line.

Our earlier studies show that in response to lead tip growing *Funaria hygrometrica* protonemata formed cell wall thickenings (CWT) localized at the apex. The thickenings were built mainly from pectins able to bind Pb^{2+} and callose. In fact, they accumulated large amounts of this metal (Krzeslowska et al. in press). It seemed to be probable that such reaction was the result of alterations in actin cytoskeleton caused by lead. We supposed moreover that it might be a typical one for tip growing cells. This hypothesis was verified in root hairs of GFP- FABD2 *Arabidopsis thaliana*.

GFP- FABD2 *A. thaliana* incubated 10 days *in vitro* on MS medium, were treated with 16 µM Pb by 24h, applied as an aqueous solution of $PbCl_2$. Control material was incubated for the same amount of time on distilled water. Afterwards, *in vivo* studies of the actin filaments (MFs) and cell wall (CW) structure and composition in the control and in lead treated root hairs, were carried out. Callose was detected by aniline blue and pectins by ruthenium red. All observations were carried out in fluorescence microscope Axiovert 200M and laser scanning confocal microscope LSM 510 (Carl Zeiss, Jena, Germany).

Treated with lead root hairs often formed cell wall thickenings (CWT), localized mainly at the tip of the cell. Preliminary studies of their composition showed that they contained first of all pectins and callose. Microfilament bundles in growing control root hair run parallel to the onger axis of the cell and they do not reach the tip. Opposite to this, in lead treated material MFs bundles were much more thick than in control and reached the tip of the root hair which often was swollen. In this region MFs array was not parallel to the longer axis of the cell and were running in various directions. In root hairs where CWT appeared the number of MF bundles was lower. Some of them were running just under the CWT, often parallel to its edges. Other MF bundles were arrayed in various directions. If the CWT occurred in subapical or lateral cell walls, one or a few MF bundles, running more or less parallel to the longer axis of the cell in this region, curved and run directly to CWT.

Tip growing cells showed similar response to lead: swollen tips and formation of CWT. CW formation during cell elongation requires both proper transport of vesicles from GA to growing tip and recycling of cell wall compounds via endocytotic pathway. Both processes are strongly dependent on actin cytoskeleton (Ovecka et al. 2005). The results of our studies shown, however, that in root hairs of *A. thaliana* treated with lead the array of MFs is markedly altered, especially in the tip region of the cell. Thus we conclude that its disturbation was probably one of the main reason of CWT formation in lead treated plant cells. Furthermore, disturbations of cell wall – cytoskeleton continuum strongly suggests some alteration(s) in signalisation within stressed plant cells.

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Exocyst subunit Sec8 of *A.thaliana* is essential for proper seed coat mucilage development

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Exocyst, a large protein complex of 8 subunits, has been shown to be required for proper post-Golgi vesicle targeting at the plasma membrane. Its orthologs can be found in most of eukaryotes. In Arabidopsis, defects of exocyst subunits Sec8 and Exo70 show a drastic phenotype, most visible at pollen-tubes and root hair defects. Post-Golgi secretion is required for both polarized growth and secretion. Here we show a new phenotype of *sec8* mutants affecting seed coat mucilage. Seed coat is missing in plants lacking Sec8 protein and is significantly smaller with truncated Sec8. These data support an idea, that seed coat mucilage can be used as a marker of exocytosis in *Arabidopsis thaliana*.

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Structural and functional modification of WMC proteins caused by nitric oxide

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In recent years, nitric oxide was reported to be involved in many physiological processes in plants - in fact it is regarded as an important signaling molecule in the plant world (1). NO can modulate the functioning of proteins, either through binding to transition metal ions (such as Fe^{+2} in heme groups in guanylate cyclase (2)) or *via* modification of amino acid residues. Among the latter modifications, the key roles play S-nitrosylation of cysteine thiols and nitration of thyrosine residues. Both modifications can affect the structure and the activity of proteins.As a consequence, S-nitrosylation can be treated as an nitric oxide-dependent signaling modification, involved in the mechanism for redox-based regulation of signal transduction pathway (3). There were two aims of this work. First, to identify proteins of the cell wall-plasma membrane - cytoskeleton continuum (WMC) potentially modified by NO. Second, to investigate the effects exerted by various NO donors and modulators of NO activity on the functioning of WMC continuum as a whole and the actin cytoskeleton as part of it. Nitric oxide was localized in *Arabidopsis thaliana* (in suspension-cultured cells and seedlings) of utilizing DAF-2-FM diacetate (4). For the analyses of protein modifications, MALDI TOF mass spectrometry was used. The proteins were identified using specific antibodies and western blot analysis. These studies were complemented with the microscopic studies of the organization of the actin cytoskeleton in roots of *Arabidopsis thaliana* seedlings and *in vivo* immunolocalization of S-nitrosotiols (SNO) and nitrotyrosines in cell suspension culture.

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Abnormal cell wall formation as a reaction to abiotic stress

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In growing plant cells, cell wall deposition is a highly organized process that is completed when cell expansion comes to an end. The formation of additional callose is assumed to be a general defense reaction which can be elicited either by physical stress, e.g. mechanical perturbations (Foissner *et al*. 1996), or by organic and anorganic chemical agents. It requires the plasma membrane bound enzyme 1,3-ß-glucan synthase which polymerizes the callose from glucose within the cytoplasm, wherefore normally the participation of organelles is ruled out. And actually there are only few cases where the involvement of the Golgi apparatus is discussed as well (reviewed by Kauss 1996).

We induced abnormal callose synthesis in differentiated onion inner epidermal cells by mechanical stress (puncturing of the cell by a micro-needle) and by incubating onion cells in solutions of copper sulphate (Kartusch 2003), and we stimulated aberrant cell wall thickening and branching in growing root hairs of *Triticum aestivum*. We describe changes of the cytoskeleton and of the motility of the organelles and the nucleus.

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Crawling roots: animal-like behaviour in plants

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Exploratory root movements closely resemble behaviour of lower animals (Darwin 1880) due to their co-ordinated bendings in two different zones: the transition and elongation region (Wolverton et al. 2000). This allows invasively growing root apices to be highly flexible in avoiding obstacles as well as dangerous soil patches due to limited amount of water or increased amounts of toxic metals. Roots of parasitic plants actively detect and grow towards root apices of their host plants and to colonize them using haustorial hairs which penetrate into the transition zone of pray roots (Tomilov et al. 2005). Moreover, roots growing down along gravity vector and hitting mechanical obstacle start "to probe the shape" of this mechanical obstacle and use the first possibility to grow down the gravity vector (Massa and Gilroy 2003). Very similar behavior can be documented by growing roots up of a slope when ethylene signalling proved to be essential to accomplish worm-like crowling of roots searching for weak sites in the substratum (Hahn et al. 2006). In order to understand this complex animal-like behavior of roots, we have performed a series of experiments using both intact and decapped maize (*Zea mays*, cv. Careca) roots. Scored behaviour of roots implicate gravity sensing in decapped roots (Mancuso et al. 2006) and document that root bendings in the transition zone and elongation region are highly coordinated to perform the worm-like crawling movements. Root cap removing is perturbing this coordinated behavior of two bending domains, suggesting that the intact root apex is essential for this coordinated root behavior. Removing of the root cap even promotes the root growth (see also Mancuso et al. 2006) but these roots grow straight and are impaired in their abilities to grow down the gravity vector which is an inherent part of their crawling movement, allowing them to analyze the substrate properties as well as to avoid dangerous environments. In future, we will use this new experimental system to challenge growing roots, both of wild-type as well as relevant mutant lines, treated with drugs and neurobiologically active substances to analyze their roles in animal-like behaviour of roots.

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Detecting electrical network activity in root apex by multielectrode arrays (MEAs)

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All processes of living organisms examined with suitable and sufficiently sensitive measuring techniques generate electric fields that must be regarded as one of the most universal properties of living organisms. Many studies have demonstrated that bioelectrochemical signals exist in plants at all levels of evolution (action potentials or excitation waves). AP are possible mechanisms for intercellular and intracellular communication in the presence of environment changes. Plants respond to environmental stimuli and excitation can be dispersed throughout the entire plant, travelling from the top of the stem to the root and from the root to the top of the stem. Though excitation waves appear strongly after stimulation, a basic electrical activity can be found in the whole plant. The theoretical description of the electrical activity and the propagating model through single cells is still not understood. Simultaneous multisite recording is a prerequisite to understand the nature of electrical phenomena. For extracellular recording from electrogenic cells pursuing these goals, substrate integrated, planar microelectrode arrays (MEAs) have been developed to monitor spikes and local field potentials. A typical setup for MEA recording is based on metal microelectrodes fabricated on a planar chip, discrete-element preamplifiers located close to the MEA device and a multi-wire cable that conducts the pre-amplified analog signals to a data acquisition card. Here we report for the first time recordings of single-unit spike activity with MEAs in acute slice of *Zea mays* L. root apex. Field potentials were recorded simultaneously from 60 electrodes (30 µm diameter) with high spatial and temporal resolution. This new technique allowed us to map functionally discrete regions of the root and to observe the space-time relationships and the spontaneous, synchronous electrical activity of the root apex. The nature of spike shapes has been studied on each MEA electrodes (200 µm interelectrode spacing). We conclude that extracellular recording of independent single-unit spike activity with MEAs is indeed suitable to monitor electrical network activity in root apex, making MEAs an exceptionally useful tool for the assessment of fast network dynamics in plants.

Bioorganic study of nyctinasty on genus *Albizzia* using molecular probes

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Most leguminous plants close their leaves in the evening, as if to sleep, and open them in the morning according to the circadian rhythm controlled by a biological clock. Nyctinastic plants have a pair of endogenous bioactive chemical factors that control leaf movement. Potassium ß-D-glucopyranosyl-12- hydroxyjasmonate and *cis-p*-coumaroylagmatine were isolated as leaf-closing factor (LCF) and leaf-opening factor (LOF) of leguminous plants bolonging to genus *Albizzia*, respectively. Our studies focus on the mechanism of leaf movement using these chemical factors as molecular probes.

We developed molecular probes consisting of modified leaf-movement factors of *Albizzia* plants in order to identify their target cells. We conducted a double fluorescence-labeling study using FITC-labeled LCF and rhodamine-labeled LOF. Interestingly, both of the probes bound to the same motor cells called extensor cells in the pulvini. Therefore, the motor cell with a set of receptors for leaf-movement factors is located on the extensor side of pulvini. Since extensor cells are difined as cells that increase their turgor during opening, and decrease their turgor during closing, the leaf-movement factor must facilitate a decrease or increase in the turgor of extensor cells. In *Albizzia* plants, the trigger for leaf-movement might be related to the change in turgor of extrensor cells.

We also synthesized a pair of enantiomers of FITC-labeled LCF, and used them for fluorescence studies. Comparing the results, FITC-labeled LCF of natural stereochemistry bound to the extensor cells of *Albizzia* plants, whereas its enantiomer could not bind to it. The results demonstrated the involvement of a receptor in the extensor cell, which recognizes the stereochemistry of jasmonate-type LOF.

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Use of an extracellular oxygen vibrating microelectrode system to detect rapid changes in oxygen fluxes in electrotropically-stimulated maize roots

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It is well known that the direction of growth of certain plant cells or organs can be modified by an applied electric field. This phenomenon, known as electrotropism has been reported in fungi (McGillavray and Gow, 1986) and algae (Brower and Giddings, 1980) as well as in the pollen tubes (Marsh and Beams, 1945), roots (Ishikawa and Evans 1990; Wolverton et al. 2000), and shoots (Schrank 1959) of higher plants.

The correlation between electrical changes and gravitropic curvature suggests the possibility that the curvature of roots in an electric field results from electrical changes within the root that mimic those caused by gravistimulation. This possibility is strengthened by reports that root electrotropism is suppressed by inhibitors of auxin transport (Moore et al. 1987). We examined the effects of electrotropism in solutions of low electrolyte concentration using primary roots of maize (*Zea mays* L., variety Merit). When submerged in oxygenated solution across which an electric field was applied, the roots curved rapidly and strongly toward the positive electrode (anode). The strength of the electrotropic response increased and the latent period decreased with increasing field strength. At a field strength of 1.5 volts per centimetre the latent period was few minutes and we were able to analyse changes in oxygen fluxes outside the root thanks to the vibrating probe. The experimental measurement of ion or gaseous molecules fluxes in roots is fundamental when discriminating normal physiological function from abnormal or stressed states. We took measures in three basic anatomical parts of root apex: meristematic zone, transition zone, and elongation zone (Verbelen et al. 2006).

The goals of the research described in this poster are (a) to determine the changes of oxygen fluxes in different zones of the root related to the electrotropic curvature in maize roots, investigating the early phase of the electrotropic response, from the apply of the current to the visible bending, and (b) to determine the role and the effects of pharmacological manipulation in the electrotropic response and their changes on oxygen fluxes.

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Investigating nanoparticle penetration and transport pathways in plant tissues as new systems for treatment delivery: techniques for *in situ* visualization.

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The great potential of nanoparticles as delivery systems to be directed to specific targets in living beings has been first explored for medical uses. In agriculture, nanotechnology applications can also have a broad range of uses in particular to tackle infections with nanosystems tagged to pesticides or other substances for efficient and local treatments, thus reducing the dose of chemicals released to the environment.

In order to explore the benefits of nanotechnology applications in agriculture, the first level is to achieve the penetration, movement and targeting of the nanoparticles through the plant at specific sites. In this context, the precise localization of the particles in the plant tissues and in the different subcellular compartments is pivotal. We have performed preliminary assays with carbon coated magnetic nanoparticles in plants, the magnetic core allowing allocation of the nanoparticles in the site of interest (affected tissues) using small magnets. In this work, a number of tools for the detection and analysis of magnetic nanoparticles introduced into plants have been evaluated, by using different techniques and levels of observation, ranging from conventional light microscopy to confocal and electron microscopy.

We have inoculated *in vitro* growing plants with a ferrofluid composed of carbon-coated magnetic nanoparticles. Tissue samples were then collected, fixed, cut and observed with different processing techniques to detect the presence of nanoparticles at the above-mentioned microscopy levels. These techniques include conventional light microscopy, fluorescence microscopy, confocal scanning laser microscopy and electron microscopy, combined with different fixation and/or embedding processes. The results showed that the nanoparticles can be visualised by reflection on a confocal microscope; inferred as dark areas in an autofluorescent background (either natural or induced), and as a punctuate pattern on the light microscope, further identified as clusters of nanoparticles on the electron microscope due to their iron core. Our first data showed the presence of nanoparticles both in the extracellular space and within some cells. Further work is needed to evaluate how the nanoparticles penetrate and are transported within the plants, and the mechanism(s) of intracellular internalisation to explore the potential of nanoparticles as smart treatment delivery systems in plants..

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Subtilisin-like proteases of *Arabidopsis thaliana* involved in the regulation of plant development

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Subtilisin-like proteases are serine endopeptidases with catalytic triad of aspartate, histidine and serine. Eucaryotic subtilases belong to two families of subtilisin-like proteases: kexins and pyrolysins, based on amino acid sequence similarity. Kexins, called proprotein convertases, are well known in mammals to play pivotal role in generation of bioactive molecules: polypeptide hormones, growth and neurotrophic factors, receptors, adhesion molecules and other proteases through highly specific proteolytic cleavage. In recent years, a growing body of evidence indicates that regulation of many aspects of plants growth and development depends not only on classical phytohormones but also on peptide signaling. Although many of these peptides and their precursor are being identified, there is still no direct evidence for generating bioactive peptide by plant protease. To gain knowledge about possible roles of subtilases in the regulation of plant growth and development, we have chosen two subtilase genes At5g19660 and At5g59810 from among 56 in *Arabidopsis thaliana* genome. For functional analysis of At5g59810, we decided to utilize the insertion mutants as well as transgenic plants overexpressing this gene under control of CaMV promoter, and a line with *promoter*::GUS construct. Phenotypical changes, immunolocalization and GUS expression analysis will be presented. On the basis of these evidences, we suggest that At5g59810 could be an enzyme involved in the generation of signal peptides regulating plant development. Second subtilisin At5g19660 is an ortholog of S1P/SKI-1, a conservative animal type of subtilases. These are transmembrane proteins located in endoplasmic reticulum/Golgi apparatus. In animals, they catalyze proteolysis of transmembrane precursors of transcription factors, and enable release of active molecules from the endomembrane system. In this communication, bioinformatic data of At5g19660 protein and their potential substrates will be presented. Additionally, we will show evidence for the developmental role of this protease based on the analysis of *Arabidopsis thaliana* insertion mutants.

Cell-type specific disruption and recovery of the cytoskeleton in Arabidopsis epidermal root cells upon heat shock stress

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The cytoskeleton in plant cells plays an important role in controlling cell shape and mediating intracellular signalling. However, almost nothing is known about the reactions of cytoskeletal elements to heat stress, which represents one of the major environmental challenges for plants. Here we show that living epidermal root cells of *Arabidopsis thaliana* could cope with short-term heat shock stress showing disruption and subsequent recovery of microtubules and actin microfilaments in a time-dependent manner. Time-lapse imaging revealed a very dynamic behaviour of both cytoskeletal elements including transient depolymerization/disassembly upon heat shock (40-41°C) followed by full recovery at room temperature (20°C) within 1-3 hours. Reaction of microtubules, but not actin filaments, to heat shock was dependent on cell type and developmental stage. On the other hand, recovery of actin filaments but not microtubules from heat shock stress was dependent on the same parameters. The relevance of this adaptive cytoskeletal behaviour to intracellular signalling is discussed.

Interactions between auxin and retinoid-like signaling in plants

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The C_{18}- apocarotenoid D'orenone, a precursor of the trisporic acid signalling molecules acting as pheromones in soil fungi (zygomycetes, Schachtschabel et al. 2007), exerts extremely rapid effects on roots of higher plants, ranging from the monocot *Zea mays* up to the dicot *Arabidopsis thaliana*. Most sensitive are tip-growing root hairs, which stop their tip growth within a few minutes of exposure to D'orenone. The actin cytoskeleton is rapidly remodelled, involving F-actin depolymerization in root hairs. We have shown this *in vivo* using the transgenic GFP-ABD2 actin-reporter line of Arabidopsis (Voigt et al. 2005) and *in situ* using a polyclonal maize actin antibody on Steedman's wax sections taken from maize root apices (Baluška et al. 1997). D'orenone rapidly depolymerizes F-actin and disintegrates the vesicle-rich 'clear zone' at the very tip of growing roots hairs. Vacuoles protrude up to the very tips of the root hairs as growth is ceasing. Labelling of Arabidopsis roots with the cell permeable Ca^{2+} dye Fluo3-AM and the $O_2^{\cdot-}$-sensitive dye NBT revealed, that a few minutes after the D'orenone treatment the tip-focused ROS and cytoplasmic Ca^{2+}-gradient disappear.

Intriguingly, D'orenone exposed roots display an activation of the auxin response reporter $DR5_{rev}$::GFP specifically in the root tip. A similar phenomenon was monitored for the phosphatidylinositol-3-OH kinase inhibitor wortmannin (Jaillais et al. 2006). Like wortmannin, D'orenone treatment affects both PIN2 abundance and subcellular location. Nearly the complete PIN2–GFP signal, which is normally observed in epidermal cells and cells of the lateral root cap in root tips, vanished and the PIN2–GFP signal started to be expressed strongly in the transition zone cells. PIN2 polarity was still maintained at the plasma membrane, but PIN2–GFP also accumulated within vesicular compartments and at the tonoplast of vacuoles. Double treatment with D'orenone and the general secretion inhibitor brefeldin A (BFA) revealed that PIN2-GFP-positive BFA-induced compartments start to appear only after long treatment periods of more than 2 h instead of usual 30 minutes.

Importantly, external addition of auxin rescues all aspects of the D´orenone induced phenotype, i.e. on the levels of root hair formation, root growth, and root graviresponse. External auxin also makes the roots more resistant to additionally applied D'orenone. All this implicates that D'orenone is a very active biological molecule possibly affecting either the PIN2-dependent auxin efflux or auxin signalling; relevant for both the auxin dependent root hair tip-growth (Lee and Cho 2006) and root growth (Blilou et al. 2005). D'orenone might also function as an important component of the myxomycete – plant communication. Finally, the most attractive scenario would be that endogenous D'orenone-like substances exist in plants and act as a new, hitherto unknown, class of plant hormones.

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History of polar auxin transport - the evolutionary aspects

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Polar auxin transport (PAT) is one of the fundamental processes in the life of higher plants. The cell-to-cell active transport of auxin molecules underlies their uneven and complex spatio-temporal distribution within plant body. The proper function of PAT is crucial for many, often very diverse plant developmental processes and/or situations, and it delimits basic processes such as embryogenesis, polarity maintenance and growth responses to environment. PAT in higher plants is the process with very complex regulation. When studying PAT, plant models representing individual stages in the course of plant phylogeny may help to assess early development of individual traits in auxin transport. The understanding of the evolution of PAT may contribute to decipher various strategies in its regulation in higher plants and - in general - it would give a better insight into the basis of the whole process.

From physical-chemical reasons, the efflux of auxin from cells is the crucial step in PAT. PIN proteins from *Arabidopsis thaliana* were shown to play a rate-limiting role in catalyzing the auxin efflux from cells and their asymmetrical/polar cellular localization determines the direction of cell-to-cell auxin flow. Therefore, we have collected known sequences coding for the homologues of auxin efflux carriers of PIN family and resulting data were related to available information on the distribution of PAT, apical and polarized growth and other PAT-related characteristics during plant evolution. We have also outlined the next possible steps in data-mining strategies related to studies of co-evolution of PINs together with various forms of auxin transport, and some possible implications of PAT for land plant evolution.

Glutamate-induced changes in electrical potential, circumnutations and stem growth in *Helianthus annuus* L.

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In sensitive sejsmonastic and carnivorous plants, action potential is an essential factor that evokes rapid movement of plant organ e.g. leaf or flay-trap. In ordinary plants, APs can also participate in the regulation of plant pollination, fertilization, respiration, photosynthesis, growth and gene expression. Recently it was shown that in plants the glutamate receptor is involved in electrical response and light and growth signalling. Here, the effect of glutamate on membrane potential, stem movement and growth was studied in three-week-old sunflowers. Extracellular and intracellular electrical potential measurements were carried out. Time-lapse photography from a top and side view camera was used for stem movement and growth study. Two drops of millimolar glutamate solution were injected into the lowest part of the sunflower stem. Injection of glutamate solution resulted in a series of APs lasting several dozens of minutes. The evoked APs propagated from the injection site along the stem and were able to enter the petiole. Some were initiated in the upper part of the stem and propagated downwards. The AP series were often accompanied by variation potential. Local application of glutamate resulted in a decreased rate of circumnutation, which reached its minimum in the third hour after the glutamate application. The preliminary experiments with application of the time-lapse photography technique did not show any significant growth inhibition following glutamate injection. The overall results provide a first indication that in higher plants the injection of glutamate solution evokes propagating series of APs and inhibits the endogenous stem movement.

Defects in light receptors affect boron-regulated growth in Arabidopsis seedlings*

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Boron (B) is essential microelement in all vascular plants. Among others, it plays an important role in cell wall synthesis. The physiological role of B in plants is depicted as that of a transducer in several processes initiated by light, gravity, and some plant hormones. Information concerning the role of boron in plant growth and development during photomorphogenesis is very poor. It has been previously observed (Rölfe et al. unpublished results)that under red (RL), but not in blue light (BL) boron can stimulate hypocotyl growth, and that the element effect also depends on light intensity. Here, we studied effects of elevated boron concentrations on *Arabidopsis* growth and development in *in vitro* conditions with respect to light quality signal. Analysis of mutants with defects in light perception could suggest interaction between boron and light signaling pathways during plant growth and development. In this genetic approach we also investigated boron and light effects on growth of mutants with defects in genes involved in synthesis of plant cell wall components, especially cellulose. For the analyses, we used photomorphogenic mutants *cry1 (hy4)*, *cry2*, and *hy2*, and cell wall mutants *rsw1-1* and *rsw1-10*. We found that hypocotyl elongation in all *Arabidopsis* ecotypes tested was stimulated by boron at concentrations from 2 to 3 mM H_3BO_3, but inhibited at higher boron concentrations. We revealed that hypocotyl of *cry1* mutant was not essentially stimulated by boron in BL or RL, and even not in dark. The data suggest that functional photoreceptor CRY1 is positively involved in boron-induced stimulation of hypocotyl growth. In contrast, *cry2* plants grown in the dark, or under BL or RL showed WT responses to boron supplemented in the culture medium. Under normal conditions, etiolated *rsw1-10* seedlings develop very short hypocotyl. We found that in *rsw1-10* mutant H_3BO_3 highly stimulates hypocotyl elongation even at the concentration (10mM) extremely toxic for control plants. The stimulation was associated with strong reduction of *BOR1* expression in mutant hypocotyl. The positive effect of boron on hypocotyl growth was most intensive in dark, but it was also essential in RL and BL, i.e. light quality only reduces growth amplitude. In contrast to *rsw1-10*, mutation *rsw1-1* did not affect hypocotyl responsiveness to high boron concentrations. Results of our experiments led to several important conclusions. First, we revealed that boron at relatively high concentrations could stimulate hypocotyl elongation not only in red light, but also in blue light and in the dark. Other results suggest that in RL and BL, functional photoreceptors in *Arabidopsis* can maintain high capacity of boron to stimulate hypocotyl elongation. Analyses of mutants with defects in cell wall synthesis revealed that mutation *rsw1-10* results in reduction in primary root and hypocotyl sensitivity to toxic effects of high boron concentrations. Differential expression of *BOR1* in the mutant and wild-type plants supports the existence of mechanism by which plants can tolerate toxic effects of high boron concentrations on plant growth

Programmed cell death as an integrated plant cell response to stress treatments which induce changes of developmental programmes

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The immature pollen grain, at the stage of vacuolate microspore can be switched, upon stress, from their normal pollen development programme to the embryogenesis pathway. Pollen embryogenesis is of much interest for basic studies and for applied research, being the best and more used tool to obtain double haploids. This process occurs by the reprogramming of microspores upon an abiotic stress treatment, followed by embryogenesis. The effectiveness of pollen cultures varies among species and essays. On occasions, many of the cells do not progress after the stress treatment. This could be due to a different response after stress, cell death events or different signal transduction pathways. The programmed cell death (PCD) pathways are not well defined in plants. In barley (*Hordeum vulgare* L.), an agronomically interesting species, pollen embryogenesis is induced by a starvation treatment in isolated microspore cultures. Different stages of pollen embryogenesis cultures were analyzed: during and after the stress treatment. Lines of in vitro suspension cells in barley were also developed as a convenient/simpler model system to evaluate the cell response to the inductive treatment and the occurrence of PCD events. The same conditions of the microspore cultures were reproduced on the suspension cells and various PCD markers were evaluated. A study on the characterization of PCD has been undertaken. Ultrastructural, cytochemical and inmunocytochemical analysis showed structural changes during stress treatment in the cultures similar to those established in animal cell apoptosis. After 24 hour of stress treatment different cytoplasmic and nuclear changes were found in the microspores. Results showed an increase of the number of vacuoles during this starvation treatment and a segregation of the cytoplasm after longer treatments. DAPI (fluorocrome specific of DNA) staining of the cultures showed disorganized nuclei with small fluorescent inclusions, similar to the apoptotics bodies in animal cells. The apoptotic features found in the stress-treated in vitro systems were compared with another stress-induced PCD system of plant cycling cells (1, 2) and with the developmental PCD process of tapetal cells, a male germ-derived cell line. In these systems, cytoplasmic release of the cytochrome C, DNA fragmentation, chromatin condensation, RNP segregation and nuclear lobulation were observed. Active cleaved-caspase 3 antigen was detected by Western blot, immunofluorescence and immunogold labelling in the cytoplasm of the treated cells and at specific developmental stages in the tapetum. Enzimatic activity of caspases was detected. Cleaved-caspase 3-like protein has been also localized by inmunofluorescence and immunogold labelling during stress treatment in pollen embryogenesis. All of these changes were not found when microspores and cell suspensions were put in nutrient medium. These results during pollen embryogenesis pathway are indicating a defined PCD assopciated with this developmental process. The knowledge of this process would allow us to influence it with drug treatments to increase the survival of the cultures in the early stages and the efficiency of the system in double-haploid production.

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Arabidopsis synaptotagmin A is enriched at cortical ER domains and is implicated in salt stress tolerance

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Sequence analysis of various animal and plant genomes revealed the presence of synaptotagmin genes in all animals and land plants, but there is no evidence of synaptotagmin genes in unicellular organisms or those with simple forms of multicellularity. In the Arabidopsis genome we find six members, SytA to SytF, which belong to this protein family. They show the same domain pattern like their animal counterparts. They possess a N-terminal transmembrane sequence, which is followed by a linker of different length and two distinct C2 domains, C2A and C2B. Here, we show the ubiquitous expression of SytA in Arabidopsis and its localization via transient transformation of tobacco leaves and stable transformation of Arabidopsis seedlings in distinct cortical ER domains localized at plasma membrane – cell wall adhesion sites. Furthermore, a T-DNA SytA loss-of-function mutant shows response to salt stress through inhibited root growth and aberrant growth of root hairs. These findings suggest possible role(s) of SytA in vesicle- and calcium-mediated salt stress tolerance.

Darkness and blue light illuminations control endocytosis and vesicle recycling at the plasma membrane of plant cells

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The blue light signal is one of the most important environmental signals for plants. It causes phototropism of plant organs, stomatal opening, and chloroplast movements. In the last decades, several members of blue light receptors have been discovered in plants. Phototropin1, the essential photoreceptor of the blue light mediated phototropism, has been considered as an important factor in almost all kinds of blue light responses. In our previous studies (in preparation), we showed that the blue light initiates endocytotic translocation of PHOT1. The level (speed and rate) of the internalization of PHOT1 can reflect the intensity of blue light signals. In this study, using the PIN2::GFP artificial protein, dynamic natures of both styryl dye FM4-64 and PIN2 have been studied under darkness and controlled blue light illuminations. Blue light signals increased the rate of FM4-64 internalization into cytoplasm, changed the localization of PIN2::GFP from vacuole to the plasma membrane, and increased the trapping of both components within Brefeldin A-induced endosomal compartments. We conclude that the blue light signals controls the localization of putative auxin exporter PIN2, and affects homeostasis of the plasma membrane via endocytosis and vesicle recycling. Endocytosis and the endocytic network of plant cells may play roles in the blue light signal transduction.

The subcellular localization and blue-light-induced movement of Phototropin 1-GFP in etiolated seedlings of *Arabidopsis thaliana*

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Phototropin 1 (phot1) is a photoreceptor for phototropism, chloroplast movement, stomatal opening, leaf expansion, and likely solar tracking in response to blue light. Following earlier work with *PHOT1::GFP* (Sakamoto and Briggs 2002), we investigated the pattern of cellular and subcellular localization of phot1 in etiolated seedlings of *Arabidopsis thalinana*. The Phot1::GFP fusion protein is expressed strongly in the abaxial tissues of the cotyledons and in the elongating regions of the hypocotyl. It is moderately expressed in the shoot/root transition zone and the root near the apex. The plasma membranes of mesophyll cells near the colyledon margin appear labeled uniformly except for strongly labeled cell plate-like structures. The pattern of labeling of individual cell types varies with cell type and developmental stage. Label is undetectable in the root epidermis, root cap, and root apical meristem. Blue-light treatment causes PHOT1::GFP, initially relatively evenly distributed at the plasma membrane, to become reorganized into a distinct mosaic with strongly labeled punctate areas and other areas completely devoid of label, a phenomenon best observed in cortical cells in the hypocotyl elongation region. Concomitant with or following this reorganization, PHOT1::GFP moves into the cytoplasm in all cell types investigated. It disappears from the cytoplasm after several hours in darkness. Neither its appearance in the cytoplasm nor its eventual disappearance in darkness is prevented by the translation inhibitor cycloheximide, although the latter process is retarded. We hypothesize that this relocalization modulates blue light-activated signal transduction.

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