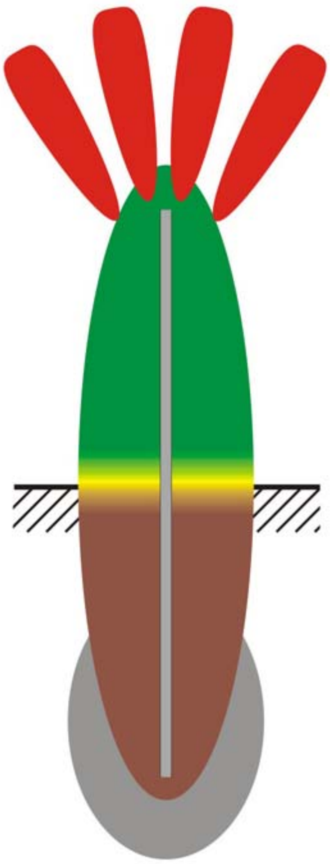
Accademia dei Georgofili

The First Symposium on Plant Neurobiology

May 17-20, 2005
Florence, ITALY



BOOK OF ABSTRACTS

ENTE
CASSA DI RISPARMIO
DI FIRENZE

Programme

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10.30-10.50 **Eric Davies & Bratislav Stankovic:** Electrical signals, the cytoskeleton, and gene expression: current hypotheses on the coherence of the cellular responses to environmental insult

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14.30-14.50 **Michel Thellier:** Memory effects in the response of plants to environmental signals
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16.50-17.10 **Molly Craxton:** Synaptotagmin genes in plants and other eukaryotes
17.10-17.30 **Günther Witzany:** Serial Endosymbiotic Theory: the biosemiotic update 2005
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12.20-12.40 **Gian-Pietro Di Sansebastiano:** Syntaxin 1 as the central element of regulated exocytosis in plants
12.40-13.00 **Viktor Žársky:** Rab GTPases and Exocyst in plants

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15.50-16.10 **Enrico Scarpella:** Leaf vascular patterning in monocots and dicots

16.10-16.30 **Refreshment**

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09.30-09.50 **Tobias Baskin:** A Phytosynapse? The plant's use of glutamate receptors to respond to aluminium
09.50-10.10 **Pia Walch-Liu:** Glutamate signalling and root development in *Arabidopsis*
10.10-10.30 **Hillel Fromm:** Genetic and pharmacological evidence for a role of the GABA shunt in maintaining the levels of reactive oxygen intermediates in plant cells

10.30-10.50 **Refreshment**

10.50-11.10 **Frank Turano:** Glutamate receptors and GABA in plant responses to environmental stress
11.10-11.30 **Frank Ludewig:** *Arabidopsis* knock out mutants of GABA metabolism and their response to different growth conditions
11.30-11.50 **Erwan Le Deunff:** Does GABA act as a long-distance signal in the regulation of nitrate uptake in plants?
11.50-12.10 **Vadim Demidchik:** Is ATP a signaling agent in plants?
12.10-12.40 **Stanley Roux:** Signaling role of ATP in growth control and in wound responses
12.40-13.00 **Benoit Lacombe:** Ion channels in plants: from DNA sequence to integrative biology
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Electrophysiology

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14.50-15.10 **Kazimierz Trebacz:** Action potentials – from mechanisms to functions
15.10-15.30 **Sergey Shabala:** Oscillations in plants
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15.50-16.10 **Refreshment**

16.10-16.30 **Edgar Wagner:** Hydro-electrochemical integration of the higher plant – basis for electrogenic flower induction
16.30-16.50 **Aart von Bel:** Forisomes as sensors and aphids as reporters of Ca^{2+}influx and efflux during depolarization waves along sieve tubes
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10.00-10.20 **Thijs Pons:** Shade avoidance without photoreceptors

10.20-10.40 **Refreshment**

Plant-to-Plant Communication and Ecophysiology

Chairman: **Janet Braam**

10.40-11.00 **Laura Perry:** Root exudation and rhizosphere biology: allelochemicals and cell death
11.00-11.20 **Ragan Callaway:** The transmogrification of plant invaders: biogeographic differences in allelopathic effects and native evolutionary responses
11.20-11.40 **Jürgen Engelberth:** Interplant communication: from induced volatiles to signal transduction pathways
11.40-12.00 **Ninkovic Velemir:** Communication between undamaged plants by volatiles: the role of allelobiosis
12.00-12.20 **Manuela Giovannetti:** Self/non-self recognition in mycorrhizal – root system networks
12.20-12.40 **Wilhelm Boland:** Early chemical and electrical signals in herbivore stressed plants
12.40-13.00 **General Discussion**

13.00-14.00 **Lunch**

14.00-15.00

'Plant Neurobiology': where to go now?

Chairmen: **Stefano Mancuso & František Baluška**

1. Journal

2. Society

3. The next meeting

Abstracts

Charles Darwin and the plant root brain: closing the gap in plant living systems theory

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Charles Darwin was "always pleased to exalt plants in the scale of organised beings" and "felt an especial pleasure in showing how many and what admirably well adapted movements the tip of a root possesses." (1) Indeed, Darwin went so far as to say that the root tip acts like a brain, located within the anterior end (sic) of the plant body, "receiving impressions from the sense organs and directing the root's several movements." (2) What clearly impressed Darwin was the ability of the root to perceive, often simultaneously, multiple vectorial stimuli – touch, moisture, light, gravity – and then make a "decision" about which "final purpose", or bending response, to follow. In connection with these growth responses, Darwin had also said that "plants do not of course possess nerves or a central nervous system." (2)

According to J.G. Miller's "Living Systems Theory" (LST), developed over the last three decades mainly for human organisms and human societies, (3) there are sets of 20 subsystems which, within each level of organisation, from cellular to supranational, are functionally equivalent and govern each level's activity. LST can be adapted, apparently with success, to plant organisms also. (4) About half of the LST subsystems concern the processing of information. Of interest in the present plant-neurobiological context is the subsystem "Channel and Net". (5) In an earlier formulation of plant LST, (4) this subsystem was equated, at the cellular level of organisation, with cytoskeleton and endomembranes, and with the symplasm at the organ level. Now, in the light of recent discoveries from plant cell biology, these designations appear to be confirmed, reinforcing the idea that plants do possess a form of nervous system which, moreover, makes use of molecules and organelles similar to those found in animal systems. As a result, the systems-analytical approach to the constructional hierarchy of plant life converges upon that already recognised for animals, (3) hence providing a truly coherent LST for the two major living systems of Plants and Animals.

Darwin C. 1880. The Power of Movements in Plants. London: John Murray

Darwin C. 1876/1983. Autobiography. In G de Beer, ed; Charles Darwin and TH Huxley, Autobiographies. Oxford: Oxford University Press. p 8-88.

Miller JG. 1978. Living Systems. New York: McGraw-Hill Publishing Co.

Barlow PW. 1999. Living plant systems: How robust are they in the absence of gravity? Adv Space Res 23:1975-1986.

Miller JL, Miller JG. 1995. Greater than the sum of its parts III. Information processing subsytems. Channel and net. Behav Sci 40:238-268.

The green plant as an intelligent organism

Tony Trewavas

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Intelligent behaviour, a complex adaptive phenomenon, is designed to increase fitness in variable environmental circumstances particularly those involving resource foraging and competition. Biologists suggest that intelligence encompasses the characteristics of detailed sensory perception, information processing, learning, memory, choice, efficient optimisation of resource sequestration with minimal outlay, self-recognition, and foresight by predictive modelling. There is good evidence that individual plant species exhibit all of these intelligent behavioural capabilities. Plants should therefore be regarded as prototypical intelligent organisms, a concept that has considerable consequences for evolutionary convergence and investigations of whole plant communication, computation and signal transduction.

Electrical signals, the cytoskeleton, and gene expression: current hypotheses on the coherence of the cellular responses to environmental insult

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When plant tissue is abiotically injured by crushing, cutting, flaming, electrical stimulation or several other means, the injured (perceiving) tissue generates electrical signals, including action potentials and variation potentials. These are transmitted to distant regions (responding tissue) where they evoke apparently disparate responses, including callose formation, closing of plasmodesmata, stoppage of cytoplasmic streaming, inhibition of ribosome movement along mRNA, and ultra-rapid but transient accumulation of several hundred transcripts, which are degraded without being translated. These apparently disparate responses can be reconciled by one fundamental hypothesis that assumes that "the plant does now know what hit it" and thus "expecting the worst" mounts a holistic defense response against its most potent nemesis, a putative viral invasion.

The basis for this response is calcium influx into the cytoplasm via voltage-gated channels (action potential) associated with the microtubules, or via mechano-sensitive channels (variation potential) associated with microfilaments. The calcium interacts with calcium and/or calmodulin-dependent cytoskeleton-associated protein kinases. This causes the phosphorylation of myosin, which stops cytoplasmic streaming, and of elongation factor 2F, which slows elongation and causes ribosomes to pile up on polysomes, thereby decreasing protein synthesis, but protecting pre-existing "host" transcripts from degradation. The phosphorylation signal then passes into the nucleus where it phosphorylates RNA polymerase II, which goes into overdrive, (i.e., does not stop at accuracy check-points), thus causing the synthesis of large amounts of mis-made mRNA. The mRNA is transported into the cytoplasm where it is scanned (checked for accuracy) by ribosomes, and found to be incorrect. This surveillance mechanism stimulates ribonuclease activity which degrades the free (non-polysome-associated), mis-made RNA, but leaves the original, "host" mRNA unscathed since it is protected by ribosomes. The ribonuclease also (and here is the crux of the matter) attack other free mRNAs, including viral mRNAs, so these are disposed before they can be translated. Within minutes this reaction is over, cytoplasmic steaming resumes, translation continues, ribosomes are released and so can be used to translate new (correctly-made) transcripts.

Neurobiological view of plants and their body plan

<u>František Baluška</u>[1,*], Stefano Mancuso[2], Dieter Volkmann[1], and Peter W. Barlow[3]

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In the centuries following the time of Aristotle and his students, who made the first philosophical attempts to understand plants in their complexity, interest in herb plants was limited mainly to their medical usage. This changed in the sixteenth century when the first biological attempts were done to understand the basic principles of structure and function of plants. At first, studies were largely devoted to plant distribution, taxonomy, and morphology. Later, taking the lead from medicine, anatomy and cytology of plants were added to the curriculum of plant sciences, as studied in the early universities. In fact, the cellular nature of living organisms was first elaborated using plants (Hooke 1665). By the end of the 19th century, it was realised that plants were even more similar to animals than had been thought hitherto. Advances in physiology helped confirm this, especially with regard to some of the basic physiological processes, such as respiration, digestion, and cell growth, where plants often provided the material of choice for experimental studies. All principal metabolic biochemical pathways are conserved in both animal and plant cells. As a big surprise, plants have been shown to be identical to animals from several other rather unexpected perspectives. For their reproduction, plants use identical sexual processes based on fusing sperm cells and oocytes. Next, plants attacked by pathogens develop immunity using the corresponding processes and mechanisms operating in animals. Last but not least, both animals and plants use the same molecules and pathways to drive their circadian rhythms.

Currently, plant science has reached another cross-road as the critical mass of new data has been accumulated, culminating in the emergence of plant neurobiology as the most recent area of plant sciences. Plants are intelligent organisms which perform complex information processing (Trewavas 2003, 2004) and which use not only action potentials but also synaptic modes of cell-cell communication(Baluška et al. 2003, 2004, 2005). Thus, the term 'plant neurobiology' appears to be justified. In fact, the word neuron was taken by animal neurobiologists from Greek where the original meaning of this word is vegetal fibre. Moreover, there are several surprises if we apply a 'neurobiological' perspective to how the plant tissues and body are organized. Auxin emerges as a plant-specific neurotransmitter (Baluška et al. 2003). Root apices are specialized not only for the uptake of nutrients but also seem to support neuronal-like activities based on plant synapses (Baluška et al. 2004, 2005). All this suggests that root apices represent the anterior pole of the plant body. In accordance with this perspective, shoot apices act as the posterior pole. They are specialized for sexual reproduction and the excretion of metabolic products via hydatodes, trichomes, and stomata. Next, vascular elements allow the rapid spread of hydraulic signals and action potentials, resembling nerves. As plants are capable of learning and they take decisions about their future activities according to the actual environmental conditions (Trewavas 2003, 2004), it is obvious that they possess a complex apparatus for the storage and processing of information.

Baluška F, Šamaj J, Menzel D (2003) Polar transport of auxin: carrier-mediated flux across the plasma membrane or neurotransmitter-like secretion? Trends Cell Biol 13: 282-285

Baluška F, Mancuso S, Volkmann D, Barlow PW (2004) Root apices as plant command centres: the unique 'brain-like' status of the root apex transition zone. Biologia 59: 7-19

Baluška F, Volkmann D, Menzel D (2005) Plant synapses: actin-based domains for cell-to-cell communication. Trends Plant Sci 10: 106-111

Hooke R (1665) Of the schematisme or texture of cork, and of the cells and pores of some other such frothy bodies. Observation 18. Micrographia, pp 112-116. London

Trewavas A (2003) Aspects of plant intelligence. Ann Bot 92: 1-20 Trewavas A (2004) Aspects of plant intelligence: an answer to Firn. Ann Bot 93: 353-357

Competitive self/non-self discrimination in plants

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Competition usually involves the allocation of limiting resources to non-reproductive functions. Natural selection is expected to favour mechanisms that increase competition with nonself neighbours and limit wasteful competition with self. We compared the growth of plants that were grown in the presence of neighbors that belonged to the same physiological individual, were separated from each other for variable periods, or originated from adjacent or remote tillers on the same clone. The results demonstrate that Pisum sativum, Buchloe dactyloides and Trifolium repens plants are able to differentiate between self and nonself neighbors and develop fewer and shorter roots in the presence of other roots of the same plant. Furthermore, once Buchloe dactyloides cuttings that originate from the very same node are separated they become progressively alienated from each other and eventually relate to each other as genetically alien plants. The results suggest that the observed self/nonself discrimination is mediated by physiological coordination among roots that developed on the same plant rather than allogenetic recognition. The observed physiological coordination is based on, an as yet, unknown mechanism and has important ecological implications for it allows the avoidance of competition with self and the allocation of greater resources to alternative functions.

How can plants choose the most promising organs?

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Almost all plants have numerous apical meristems, each with the potential of forming an entire shoot system. Since resources are limited, a plant must ‘choose’ the best alternatives, based on integrated though incomplete information from various sources and its predictions about future performance. An additional challenge is that the development of any meristem must be correlated with appropriate vascular differentiation and other developmental events, throughout the plant. The presentation will aim to show how, in the absence of a central decision organ, integration of both types can depend on the formation of, and responses to, auxin and other simple molecular signals.
Experiments were carried out on a model system, pea seedlings with two shoots. In most plants only one of these shoots would continue growing. We studied the influence of both local and overall environmental conditions, the removal of leaves of different ages, and the previous developmental rate of individual shoots on the choice of which shoot will continue growing.
The evidence of both experiments and comparative observations supports the following hypothesis. All components of a shoot are sources of auxin and presumably other signals. The synthesis of different amounts of the very same hormones depends on the immediate environment and developmental stage of individual organs. The responses to auxin occur throughout the plant and include the orientation of vascular differentiation towards organs that are its strongest source. This results in competition between alternative organs according to integrated information about their state. This information predicts their future contribution according to the adaptations and evolutionary experience of the species.

The role of root apices in shoot growth regulation

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Long distance communication between roots and shoots of higher plants is essential for regulating whole plant adaptations to environmental changes. For example, decreased availability of essential mineral nutrients or water in the soil is often followed by a seemingly adaptive inhibition of shoot growth. Chemical, hormonal, electrical or hydraulic signals may be used to transmit status information between plant organs. The roots will clearly be the first to generate such signals in response to rhizosphere changes. Whether active neuronal type changes in the root apices or simple passive changes are required in order to generate and transmit such information to the developing shoots, is the subject of this chapter. Several examples of laboratory experiments in which roots appear to respond actively in response to changes in nutrient or water availability are first discussed. However, other examples indicate that shoot responses to rhizosphere changes can occur without the active intervention of root apices. The conclusion is that while neuronal types of communication may occur in plants, they are not always essential for early plant growth responses to environmental changes.

Information processing by stomatal networks

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Stomata must respond to environmental factors such that they open to admit enough CO_2 for photosynthesis, yet close sufficiently to prevent excessive water loss. Much of this regulation occurs at the level of the individual pore, through signal transduction pathways in the guard cells. However there is increasing evidence that stomata interact with each other over short distances and can therefore be said to form a locally-connected network. We present evidence showing that stomatal networks may be processing information in a manner similar to artificial networks that perform distributed emergent computation. This information processing may allow stomata networks to optimize gas exchange for an entire leaf or plant despite the fact that each individual stoma has only local information.

Memory effects in the response of plants to environmental signals

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Plants are sensitive to stimuli from the environment (e.g. wind, rain, contact, pricking, wounding). They usually respond to such stimuli by metabolic or morphogenetic changes. These changes may occur in tissues at a distance from the stimulated region, which implies that the stimulus must be transmitted in some form from the stimulated region to the region where it takes effect (i.e. the stimulus and the response then are spatially separate). Sometimes the information corresponding to a stimulus may be "stored" in the plant where it remains inactive until a second stimulus "recalls" this information and finally allows it to take effect (i.e. the stimulus and the response then are temporally separate). Two experimental systems have proved especially useful in unravelling the main features of these memory-like processes.

In the system based on *Bidens* seedlings, an asymmetrical treatment (pricking, or gently rubbing one of the seedling cotyledons) causes the cotyledonary buds to grow asymmetrically after release of apical dominance by decapitation of the seedlings. This information may be stored within the seedlings, without taking effect, for at least two weeks; then the information may be recalled by subjecting the seedlings to a second, appropriate, treatment that permits transduction of the signal into the final response (differential growth of the buds). Whilst storage is an irreversible, all-or-nothing process, recall is sensitive to a number of factors, including the intensity of these factors, and can readily be enabled or disabled. In consequence, it is possible to recall the stored message several times successively.

In the system based on flax seedlings, a mechanical stimulus (e.g. wind, touching) has no apparent effect. If, however, the plant is subjected at the same time to a 2-day calcium deprivation, numerous meristems form in the stem. When the calcium deprivation treatment is applied a few days after the mechanical treatment, t he time taken for the meristems to appear is increased by a number of days exactly equal to that between the application of the mechanical treatment and the beginning of the calcium deprivation treatment. This means that the information corresponding to the mechanical treatment has been stored in the plants, without any apparent effect, until the calcium deprivation treatment recalls this information to allow it to take effect. The response of flax seedlings to cold shock, to radiation from a GSM telephone and to low intensity electromagnetic radiation at 105 GHz is the same as to mechanical treatment, namely storage of the information and recall after calcium deprivation. The calcium status of the plants before, during and after the calcium deprivation treatment has been studied by SIMS (secondary ion mass spectrometry). Gel electrophoresis has shown that a few protein spots are shifted (compared to controls) in flax seedlings that have stored information corresponding to a cold shock signal but that are not in a state to recall it; a SIMS investigation has revealed that the shift of one of these spots is probably due to protein phosphorylation. Similar modifications of the proteome have been observed in *Arabidopsis* seedlings subjected to the same stimuli.

We suggest that storage/recall processes in plants constitute an elementary form of « memory » and that, given their simplicity relative to the animal brain, these plant processes may be used as experimental systems for testing hypotheses about memory storage and evocation.

Auxin distribution network as a means to integrate and process signals in plant development

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Plant hormone auxin is a prominent intercellular signal in plants. Auxin distributed over long distances largely contributes to the coordination and integration of growth at the whole plant level. On the other hand, directional, active, cell-to-cell transport over short distances mediates local, differential auxin distributions (gradients), required for various patterning processes, including apical-basal axis formation and organogenesis. Also growth responses to environmental cues such as light or gravity utilize a similar mechanism involving auxin gradients. Differentially expressed auxin transport facilitators of the PIN family, each with specific polar, subcellular localization form a network for auxin distribution and formation of these local gradients. The activity of PIN proteins can be regulated at the single cell level by changes in their vesicle trafficking-dependent polar targeting in response to developmental and environmental cues. Thus, this auxin distribution network, whose directional throughput is modulated by both endogenous and exogenous signals, provides, by means of mediating auxin fluxes and creating local gradients, a common mechanism for the plasticity and adaptability of plant development.

Active export of auxin by MDR-type ATP-binding cassette transporters

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The plant hormone auxin regulates virtually all plant developmental processes. On the cellular level, a protein complex characterized by the PIN proteins is thought to mediate auxin efflux, however, PIN-mediated transport has not been conclusively demonstrated. Recent findings suggest that multidrug resistance (MDR)-like p-glycoproteins (PGPs) function in auxin transport, but the molecular basis remains elusive. Here we report decreased auxin efflux in *atpgp* mutant protoplasts as well as the export of IAA and synthetic auxins by AtPGP1 and AtPGP19 heterologously expressed in yeast and HeLa cell systems. In contrast to the gradient-driven model proposed for PIN-mediated transport, biochemical and pharmacological analysis demonstrates an energy-dependent, primary active transport mechanism for AtPGP-mediated auxin transport. Further, the auxin transport inhibitors NPA and quercetin that bind AtPGP1 and AtPGP19 were shown to inhibit AtPGP function. Basipetal auxin transport in *atpgp1* roots is reduced, which correlates well with AtPGP1 basal plasma membrane localization in cortical root cells. Our data suggest the presence of a primary energized auxin transport system involved in polar auxin transport.

The brain of plants: neurotransmitters, neuroregulators and neurotoxins

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The existence of humans in our world is dependent on plants and human culture is profoundly influenced by those plants which affect the function of our brains. Some plants are chosen and cultivated for their capacity to change our moods while other plants contain toxins that impair our cognitive functions. One of the more fascinating questions in plant biology is the role of these compounds in the growth and development of higher plants. It has been hypothesized that neurologically active secondary metabolites in plants function as anti-herbivory compounds or attractants. Additionally, recent evidence suggests that the compounds may play regulatory roles in plants similar to those observed in other species. The discovery of the mammalian neurohormone melatonin in growing plants and the correlation of this compound with photoregulated processes is one example of a human hormone that mediates plant growth. Further studies have uncovered a range of neurologically-active plant-based compounds with functions and activities in organisms throughout the ecosystem. As new techniques are developed to study these compounds and their activity in plant and animal systems, there is enormous potential to develop a greater understanding of plant life, human health and human diseases.

Synaptotagmins and cell-to-cell communication at neuronal synapse

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Intracellular membrane traffic is governed by a conserved set of proteins including SNARE fusion proteins and synaptotagmins (Syts). The mammalian Syt family consist of 16 isoforms. Syts are membrane proteins that possess tandem C2 domains (C2AB) implicated in calcium-dependent phospholipid binding. We performed a pair-wise amino acid sequence comparison together with functional studies of rat Syt C2ABs to examine common and divergent properties within the mammalian family. Sequence analysis indicates three different C2AB classes, the members of which share a high degree of sequence similarity. All the other C2ABs are highly divergent in sequence. Interestingly, nearly half of the Syt family does not exhibit calcium-dependent phospholipid binding. Syts do however possess a more conserved function, namely constitutive binding to target SNARE heterodimers. All tested isoforms bound the target SNARE dimer composed of syntaxin1 and SNAP-25. Our study suggests that both calcium-sensitive and insensitive Syt isoforms function in membrane traffic to interact with the target SNARE heterodimer on the pathway to membrane fusion.

Synaptotagmin genes in plants and other eukaryotes

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Synaptotagmin genes are not just present in animals with nervous systems, but also occur in plants. Sequence analysis indicates the presence of synaptotagmin genes in all animals and all land plants, but there is no evidence of synaptotagmin genes in unicellular organisms or those with simple forms of multicellularity. Animals posess diverse synaptotagmin genes, the number of which varies with organism complexity. There have been different patterns of synaptotagmin gene acquisition and loss in different animal lineages. All animals posess a Syt1 homologue which provides the function of fast, synchronous, calcium regulated synaptic vesicle exocytosis, essential for efficient neurotransmission. The diverse other synaptotagmin homologues appear to be involved in other types of specific vesicle trafficking within and beyond the nervous system.
There appear to be three different types of synaptotagmin genes in plants, and varying numbers of each type in different species. Sequence analysis shows that these genes are related to groups of genes present in a wide variety of eukaryotes including animals, plants, fungi and protozoa. These relatives all have C2 domains, but none have the N-terminal transmembrane, linker, C2A, C2B arrangement which defines synaptotagmins. Unfortunately, these genes are all poorly functionally characterized. Although plants lack Syt1 homologues, and their synaptotagmin genes are clearly divergent from those of animals, it still remains to establish whether the functional roles of the plant and animal genes overlap.
Functional studies of the synaptotagmin genes of the primitive moss *Physcomitrella*, and of *Arabidopsis* and other higher plants are now in progress.

Serial Endosymbiotic Theory (SET): the biosemiotic update 2005

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The serial endosymbiotic theory (SET) explains the origin of nucleated organisms through merging between archae-bacterial and eubacterial cells. The paradigmatic change to former evolutionary theories is that the driving force of evolution is not ramification but merging.
Lynn Margulis describes the symbiogenetic processes in the language of the classical biology in termslike "merging", "fusion", etc. within natural laws. *Biosemiotics* has proved all cell-cell-interactions are *rule-governed sign-mediated* interac-tions (*rsi's*). *Rsi's* between and within cells and cell societies can be described better in terms of a *biology as an understanding social science*, than in terms of the classical biology. The decisive difference between natural laws and semiotic rules is that every living being is underlying natural laws in a strict sense, as opposed to semiotic rules may be followed or not, may be changed or not, may be generated or not; so living beings have a relationship to *rsi's* but not to natural laws.
The change from the classical biology to a *biology as understanding social science* may be also a change from the 3 rd person perspective (external observer) to the 1 st/2 nd person perspective (*performative participant*). It leaves behind the subject-object-dichotomy and integrates the Umwelt –concept (J.v.Uexkuell) into a Mitwelt- concept, in which *all* living beings are participants of a universal community of communicating life.

Intellectual property rights in smarty plants

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Advances in plant biology are being affected by the continuous strengthening of intellectual property protection for biological inventions. The acquisition of rights in intellectual property controls the research and commercial use of protected techniques, materials, genes, seeds, vectors, cell lines, etc. Efforts are underway to harmonize intellectual property right regimes in the international arena.
The recognition of the existence of plant neurobiological phenomena creates new opportunities for the scientists that study smarty plants. This presentation will highlight the above issues and will educate scientists so that they can make informed decisions regarding their research practices and the licensing of their discoveries. Patented neurobiological inventions relating to human and animal model systems will be used as reference points. Parallels will be drawn, and the potential for obtaining plant-specific intellectual property rights will be presented.

Functional characterisation of *Arabidopsis* glutamate-like receptors

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There are twenty members of the *Arabidopsis* glutamate-like receptor family (AtGLR) that have been so named as they share significant similarity with the animal ionotropic glutamate receptors (iGLuR). Animal iGLuR are non-selective cation channels that have been demonstrated to function predominately as Ca^{2+} and Na^{+} influx pathways, particularly at synaptic junctions. Although it is predicted that AtGLR have the 3+1 channel-like structure of iGLuR, and they also share a ligand-binding domain of significant similarity to the animal iGluR, the amino acid sequence within the putative pore of AtGLR is unlike any other known channel. This makes predictions of AtGLR selectivity, if they are indeed ion channels, impossible. In an attempt to determine AtGLR function, the expression of several members of the family has been attempted in *Xenopus* oocytes, a popular expression system for iGLuR.
Preliminary results indicating successful heterologous expression and characterisation of AtGLR as Ca^{2+}, K^{+} and Na^{+} permeable ion channels have been reported at several conferences over the last five years. So far, however, these reports have not been successfully published in peer reviewed journals. iGLuR form functional channels by combining subunits into hetero- or homo-tetramers therefore, as well as the expression of single members of the AtGLR family we are undertaking co-expression studies to screen for AtGLR function. The utility and difficulty of using Xenopus oocytes as an heterologous expression system for plant ion channels and transport proteins will therefore be discussed with reference to our own attempts to characterise AtGLRs.
In the light of difficulties in the definitive functional characterisation of the AtGLRs in heterologous systems, we are also turning our attention to the plant resource and conducting electrophysiological studies *in planta* in an attempt to elucidate AtGLR function.

Keeping in touch with plant glutamate receptors

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The *Arabidopsis* genome encodes for 20 members of putative ligand-gated channels, termed glutamate receptors (GLR). Despite the fact that initial studies suggested a role of GLRs in various aspects of photomorphogenesis, calcium homeostasis or aluminium toxicity, their functional properties and physiological role in plants remain elusive. Here we focussed on *AtGLR3.4*, which is ubiquitously expressed in *Arabidopsis* including roots, vascular bundles, mesophyll cells and guard cells. *AtGLR3.4* encodes a glutamate-, touch-, and cold-sensitive member of this gene family. Abiotic stress stimuli such as touch, osmotic stress or cold stimulated *AtGLR3.4* expression in an abscisic acid independent, but calcium dependent manner. In plants expressing the Ca^{2+}-reporter apoaequorin glutamate as well as cold elicited cytosolic calcium elevations. Upon glutamate treatment of mesophyll cells, the plasma membrane depolarised by about 120 mV. Both glutamate responses were transient in nature, sensitive to glutamate receptor antagonists, and were subject to desensitisation. One hour after eliciting the first calcium signal, a 50 % recovery from desensitisation was observed, reflecting the stimulus-induced fast activation of *AtGLR3.4* transcription. We thus conclude that AtGLR3.4 in particular and GLRs in general could play an important role in the Ca^{2+}-based, fast transmission of environmental stress.

Pharmacological approaches to understand plant glutamate receptors

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Plants produce a variety of compounds which affect the human nervous system. Although their effects on humans have been well studied, their role in plants is poorly understood. One such compound, BMAA [S(+)-beta-Methyl-alpha, beta-diaminopropionic acid], is common to all species of the most ancient, living seed plants, the cycads. BMAA in cycads has been incriminated as the etiological source of Guam's dimentia, a disease in the South Pacific--manifested often years after exposure, that results in an Alzheimer's and Parkinson's disease. In the animal nervous system, BMAA acts upon ionotropic and metabotropic glutamate receptors (iGluRs), which are ligand-gated ion channels that transmit synaptic signals necessary for a variety of functions including vision and memory. It is not known if BMAA has a physiological role in plants; however, genes with high sequence similarity to animal iGluRs have been identified in a number of plant species. We have taken a pharmacological approach to uncover the role of plant glutamate receptor (AtGLR) genes, by examining the effects of BMAA, a cycad-derived iGluR agonist, on Arabidopsis morphogenesis. When grown in the presence of BMAA, Arabidopsis seedlings show a two to three fold increase in hypocotyl length and a significant inhibition of cotyledon opening. The effect of BMAA on hypocotyl elongation is light-specific and can be reversed by the simultaneous application of glutamate, the native iGluR agonist in animals. A genetic screen was devised to isolate Arabidopsis mutants with a BMAA insensitive morphology (*bim)*. When grown in the light on BMAA, *bim* mutants have shorter hypocotyls then wildtype. Analysis of the *bim* mutants, as well as the role of BMAA, will be discussed.

Touch-responsive gene expression

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Plants can perceive environmental stimuli with exquisite sensitivity. Indeed, even the seemingly innocuous stimulus of touch can elicit elaborate and unexpected responses in plants (reviewed in (1)). Plants use the sense of touch to capture prey, enhance the likelihood of crosspollination and climb other objects to reach heights resplendent in sunshine. Even nonspecialized plants respond to mechanical perturbations, such as wind and gravity, through morphogenetic alterations. Darwin noted that plant roots turn and grow away from points of contact (2). Jaffe coined the term thigmomorphogenesis to describe the touch-induced decreased elongation and enhanced radial expansion of plant shoots (3).
Single cells, in addition to organisms, are likely to perceive and respond to mechanical stimuli. To maintain turgor homeostasis, individual cells must sense turgor pressure and wall integrity. In addition, subcellular organelles can translocate in response to mechanical perturbations.
Signaling molecules and hormones, including intracellular calcium, reactive oxygen species, octadecanoids and ethylene have been implicated in touch responses.
Touch stimulation can also rapidly alter gene expression regulation. The *Arabidopsis thaliana TCH* genes were discovered as genes whose expression is upregulated in plants perturbed by touch or wind (4). The *TCH* genes encode proteins predicted to function in calcium signal transduction and cell wall modification.
Recently, we investigated the prevalence of touch inducibility among plant genes (5) . Touch-induced gene expression is surprisingly widespread; more than 2.5% of *Arabidopsis* genes are rapidly upregulated in expression in touch-stimulated plants. In addition to encoding calcium-binding and cell wall modifying proteins, the induced genes include many encoding proteins predicted to function in defense, as transcription factors and as protein kinases. With these genes as tools, we are employing molecular genetic methods to begin to elucidate mechanisms of touch perception, signal transduction and response regulation.

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Ca^{2+}-permeable channels in the plant pathogen response

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Production of reactive oxygen species (ROS) and hypersensitive cell death are characteristic features of the induced pathogen resistance in plants. Ca^{2+}-signals preceding the pathogen-induced cell death have been shown to involve Ca^{2+} entry via the plasma membrane. We therefore analyzed Ca^{2+}-signals and Ca^{2+}-channels involved in the responses to pathogen attack and ROS. For this purpose, we used the Ca^{2+}-reporter protein aequorin as well as the combined single-cell fluorescence imaging and patch clamp technique. Moreover, we compared Ca^{2+}-channel activities in *Arabidopsis* wild type and the pathogen-resistant mutant, *dnd1* (Yu et al., 1998) . Cellular responses to ROS were significantly reduced in *dnd1*. Since *DND1* was shown to encode the Ca^{2+}-permeable and <u>c</u>yclic <u>n</u>ucleotide-<u>g</u>ated ion <u>c</u>hannel CNGC2 (Clough et al., 2000) , this channel represents a possible candidate for H_2O_2-dependent Ca^{2+} entry via the plasma membrane. We thoroughly examined this hypothesis and will present recent results on how CNGC2 transmits cellular responses to reactive oxygen species, a step essential for the control of hypersensitive cell death.

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14-3-3 brain proteins in plants: master regulators that couple signaling to ion transport

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14-3-3 proteins were originally identified during a systematic classification of brain proteins. The brain does contain high levels of 14-3-3 protein and it was thought initially that they were specific for neuronal tissue. Not surprisingly, diseases like Alzheimer, Parkinson and SCA1 have been linked to 14-3-3 function. It is now clear that 14-3-3 protein are ubiquitously expressed in almost all eukaryotic cells and that they have a wide range of functions due to the phosphorylation dependent interaction with numerous (>300) partner proteins (1). During evolution the basic structure of 14-3-3 proteins has been conserved and in contrast to the initial picture it looks like there is also functional conservation. Thus, it becomes clear that both in plant and in animals 14-3-3 proteins are essential for the activity of certain ion transporters, like pumps and channels (2). Here we report on the function of plant 14-3-3 proteins in coupling chemical (ABA) and light (blue/red) signals, to electrical signals and gene expression. Whether functional conservation goes as far as the demonstrated role of brain 14-3-3s in learning and memory formation remains to be seen (3).

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MAP kinases in neurons and plants

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Mitogen activated-protein kinases (MAPKs) such as ERK and p38 play a crucial role in synaptic plasticity since they are involved both in neuritogenesis and in neuronal polarity, differentiation and survival (Howe et al. 2001, Krapivinski et al. 2003, Nakata et al. 2005). These MAPKs are polarly localized in tip-growing neuronal axons. ERK, as an essential component, participates on signalling induced by neuronal growth factor (NGF) during retrograde transport of NGF receptor from synapses to the cell body, as well as on the signalling of glutamate receptors of the NMDA subtype underlying memory processes (Howe et al. 2001, Krapivinski et al. 2003). Recently, a novel p38 MAPK pathway was shown to play a role in pre-synaptic development (Nakata et al.2005). Interestingly, scaffolded molecular members of ERK pathway including NGF recepror, TRK, RAS, RAF, MEK and ERK itself are associated with clathrin-coated vesicles and with signalling endosomes upon NGF activation (Howe et al. 2001). On the other hand, hydrogen peroxide induces p38 and accelerates endocytosis via stimulation of GDI:Rab5 complex (Cavalli et al. 2001). In plants, nothing is known about the function of MAPK membrane scaffolding. Previously, we revealed that plant MAPK, polarly localized to vesicular structures, is associated with tip-growth of root hairs (Šamaj et al. 2002). Our recent data suggest that MAPKs are enriched at transversal cross walls of root cell files (representing putative plant synapses), and some MAPKs are subcelularly targeted to clathrin-coated vesicles and endosomal membranes, a phenomenon which is enhanced by oxidative and other abiotic stresses.

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SNARE molecules indicate the complexity of the post-Golgi traffic in plant cells

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Although the endomembrane system is well conserved among all eukaryotic cells, the plant endomembrane system also has unique features. In mammals and yeast, each cell has only one type of vacuole, whereas plant cells possess two-types of functionally different vacuoles, a lytic vacuole and a protein storage vacuole. The lytic vacuole is comparable to the mammalian lysosome and the yeast vacuole with an acidic pH of inside of the compartment, while protein storage vacuole is the specialized vacuoles having the capacity to store proteins in seed or vegetative cells. Therefore, protein sorting in plant cells is likely to be more complicated than other eukaryotes because two separate pathways diverge from the Golgi apparatus or plasma membrane to two different vacuole destinations.

In all eucaryotic cells, specific vesicle fusion during vesicular transport is mediated by membrane-associated proteins called SNAREs (soluble N-ethyl-maleimide sensitive factor attachment protein receptors). In the *Arabidopsis* genome, 54 SNARE and 57 Rab GTPase genes have been identified. These numbers are greater than those of yeast and mammalians, indicating the complexity of the plant endomembrane system. SNAREs and Rab GTPase are necessary for plant-unique higher order physiological function, suggesting that plants have adopted the membrane trafficking system to plant specific phenomenon.

A series of transient expression assays using green fluorescent protein (GFP) fused proteins revealed that most of SNARE proteins were located on specific intracellular compartments: 6 in the endoplasmic reticulum, 9 in the Golgi apparatus, 4 in the *trans*-Golgi network (TGN), 2 in endosomes, 17 on the plasma membrane, 7 in both the prevacuolar compartment (PVC) and vacuoles, 2 in TGN/PVC/vacuoles, and 1 in TGN/PVC/plasma membrane. S ome SNARE proteins showed multiple localization patterns in two or more different organelles, suggesting that these SNAREs shuttle between the organelles. Furthermore, the SYP41/SYP61-residing compartment, which we define as the TGN, was not always located along with the Golgi apparatus, suggesting that this compartment is an independent organelle distinct from the Golgi apparatus. We will discuss possible combinations of SNARE proteins on all subcellular compartments, and suggest the complexity of the post-Golgi membrane traffic in higher plant cells.

Syntaxin 1 as the central element of regulated exocytosis in plants

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The best-known secretory models in plants appear more closely allied to the idea of constitutive secretion**.** In contrast with the events of neurotransmitter release, in each of these cases secretion is prolonged or continuous, rather than transient; the secretory event is not preceded by a large accumulation of vesicles beneath the target membrane; and the exocytotic fusion does not appear tightly coupled to endocytosis and vesicle recycling. Nevertheless the secretion in plants is frequently targeted to specific regions of the cell or triggered by external stimuli, and now there is growing evidence pointing to vesicle pools that are released on stimulation.

Specific interactions between SNAREs, regulated by specific GTPases (Rab proteins), represent a central event of vesicular traffic and modulate secretion. SNAREs study, carried out essentially with biochemical approaches, presents great difficulties because the specificity found in function and localization does not correspond to an equivalent differentiation of chemical and physical characteristics. All SNAREs share, to a certain extent, unspecific affinity for each other.

Ten different syntaxins seem to contribute to the last steps of exocytosis and endocytosys. Their role is far from being fully characterised.

Nicotiana tabacum Syntaxin 1 (Nt-Syr1 or SYP121) is a SNARE protein required for ABA control of ion channels and it seems to be involved in exocytosis. In fact the expression of a dominant negative fragment of Nt-Syr1 indicated that this syntaxin mediates the traffic between the Golgi complex and the plasma membrane.

Using the approach of dominant negative mutants, we proved that this syntaxin is involved in a secretory process independent from the constitutive secretion of cell wall polysaccharides.

By analogy, we expect this process to be essentially the same known in the synaptic transmission but used by plants for rapid variations of the plasma membrane surface (stomata movements and turgor). To study the sorting and recycling of this protein, we generated a collection of GFP-tagged variants. Even if GFP tagged SYP121 appeared to be localised essentially on the plasma membrane, other variants suggested that this protein was anchored on internal membranes and then sorted to the plasma membrane. These GFP-tagged variants may allow to visualise post-Golgi intermediate compartments of post-transcriptionally regulated secretory events, in this case ABA dependent. The pool of secretory vesicles ready to answer ABA stimulus and controlled by the tSNARE SYP121, could also assemble in structures similar to the secretory granules described in the animal systems but never observed in plants.

Rab GTPases and Exocyst in plants

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Oriented cell expansion in plant cells is regulated by members of Ras-related superfamily of GTPases as in other eukaryots. Functional cycle of Rab family of protens is dependent on C-terminal double-cysteine motif modification by geranylgeranyl hydrophobic moiety. We found plant-specific features of Rab-geranylgeranyltransferase (RabGGTase) subunits and using molecular phylogenetic analysis we proposed a new scenario of the GDI/REP superfamily of neurobiological proteins evolution. Analysis of a mutant in one of the RabGGTase subunits points to the feedback regulation of photomorphogenesis by cell expansion. We are studying exocyst complex in plants as a putative effector of small GTPases including Rab proteins. Our biochemical as well as genetic evidence points to the exocyst involvement in plant cell polarity regulation.

Receptor-like kinases: how plants sense their environment and can tell us what they "see"

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Because plants cannot physically change environments, they must be able to sense and respond dynamically to numerous environmental cues. This probably explains why an exceptionally large fraction of the *Arabidopsis* genome comprises genes encoding receptor proteins, particularly receptor-like kinases (RLKs), which initiate often complex local and systemic signaling networks. We have found that several RLKs are expressed in response to insect attack and may participate in "sensitizing" the plant for subsequent responses. We are attempting to discern the function of some of the approximately 600 RLKs in *Arabidopsis* by creating chimeric proteins having various extracellular (sensor) domains linked to a single intracellular (kinase) domain which produces a consistent visible report. We report the creation of the first such chimeric receptors expressed in planta, and discuss the value of the "RLK Kits" we will be producing to investigators in many disciplines.

Nitric oxide functions in plant disease resistance

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A widespread feature of plant disease r esistance is the hypersensitive response (HR), which is characterized by the formation of necrotic lesions at the infection site and by the restriction of pathogen growth and spread. Following this local resistance response, tissue distal to the infection site develops a systemic acquired resistance (SAR) to secondary infection by the same or by different pathogens.

Similarly to what is observed in the macrophage action during the immune response, one of the earliest events in the HR is the rapid accumulation of reactive oxygen species (ROS) and nitric oxide (NO) through the activation of enzyme systems similar to neutrophil NADPH oxidase and nitric oxide synthase. Both NO and ROS are necessary to trigger host cell death, and several lines of evidence suggest that this cell death results from the activation of suicide processes. In fact, the HR is thought to be a form of programmed cell death, a genetically programmed process well known in animals, which is characterized by a distinct set of morphological and biochemical features.NO and ROS are also components of a highly amplified and integrated defense system that triggers the local expression of resistance genes. NO also functions independently of ROS in the induction of various defence genes including pathogenesis-related proteins and enzymes of phenylpropanoid metabolism involved in the production of lignin, antibiotics and the secondary signal salicylic acid. NO signaling functions depend on its reactivity and ROS are key modulators of NO in triggering cell death, although through mechanisms different from those commonly observed in animals.

The establishment of SAR, an inducible plant defense response, involves the existence of a systemic signal that migrates from infected to systemic, non infected leaves and requires SA. Compelling evidence indicates that SA, although necessary both for local resistance and for SAR induction, is not the long-distance signal molecule that triggers systemic resistance. In mammals, NO circulates in the blood as S-nitroso adducts of proteins, or as low molecular weight S-nitroso thiols, such as nitroso glutathione (GSNO). This molecule, believed to act as both an intra- and intercellular NO carrier, is a powerful inducer of plant defense genes. Since glutathione is a major metabolite in the phloem, where the SAR signal is transmitted, it can be hypothesized that excess NO produced during the HR binds to glutathione and that in this form it may act as a long distance SAR signal.

In summary, although several hypotheses still await experimental demonstration it is now clear that NO and ROS play a key role in disease resistance responses, and further studies of their regulation and mechanisms of action will offer the possibility to exploit new ways for improving plant resistance to a number of biotic and abiotic stresses.

Nitric oxide and auxin interactions in root formation

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Plants have developed a series of mechanisms for perception and transduction of environmental or endogenous stimuli and for integration and synchronization of physiological responses. Signal transduction, the way cells construct responses to a stimulus, is a recently defined focus in plant biology. Moreover, in addition to the accumulation of data generated by structural and functional genomic analysis, a revival of pharmacological, surgical and physiological experimental approaches assessed in the past years have led to a clear advancement in the comprehension of molecular mechanisms involved in plant physiological responses. As well as it was demonstrated in animal systems that nitric oxide (NO), cGMP and Ca^{2+} were interconnected to play a central role in vascular cell physiology, the same results are forthcoming from plant systems. Currently, the molecular basis that are responsible of transducing plant hormone messages into physiological responses are being deciphered. The auxin indole acetic acid (IAA) is the main hormone involved in determining the root architecture. Recently, we have demonstrated that NO is required in both the IAA-induced adventitious root formation (ARF) [Plant Physiol. (2002) 129: 954; (2003) 132: 1241; (2004) 135: 279] and lateral root development (LRD) [Planta (2004) 218: 900]. During ARF, the NO action is accomplished by at least two parallel mechanisms: i) the increase in cGMP level and ii) the activation of a MAPK pathway. The induction of LRD by NO is accomplished through a NO-mediated induction of cell cycle regulatory genes (cyclins and cyclin-dependent kinases) that leads to the activation of pericycle cells and their progress through G1-S phase. These and other recent findings concerning NO-related mechanisms that control root development will be presented.

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Oxygen/Auxin influx and NO efflux: neural-like features of the transtion zone of the root apex

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Root apices are composed of three distinct regions: an apical meristem where all cell divisions are taking place, a basal region of rapid cell elongation where cells extend parallel to the apical-basal axis of the root, and an interpolated transition zone (TZ). In contrast, shoot apices do not show such a clear zonation since cell divisions and rapid cell elongation occur side-by-side. In the root apex, the transition zone cells maintain an active cytoarchitecture, having central nuclei from which radiate both microtubules and actin filaments towards the peripheral plasma membrane. This specific cytoarchitecture is proposed to be optimally suited for the effective signal transmission between the plasma membrane and nucleus (Baluška et al. 2003a). Cells of the distal part of the transition zone, occupying approximately the region of 1,0 – 1,5 mm from the root tip, but not those of the meristem (approx. 0,0 – 1,0 mm from the root tip) and elongation region (approx. 2,0 – 7,0 mm from the tip), are characterized by the maximal degree of oxygen and IAA influx (Mancuso et al. 2005) into the root apex. Moreover, cells of the TZ are also specifically involved in the production of NO in response to environmental stimuli such as hypoxia. Immediately after the onset of hypoxia, NO is emitted specifically from the TZ of the root apex with a mean peak efflux of 925 ± 102 fmol NO $cm^{-2}s^{-1}$. Approximately 230 pmol NO cm^{-2} are produced at the TZ. Interestingly, dramatic NO efflux was recorded only in the TZ of the root apex. Outside of the TZ, only small effluxes of NO (1/10 lower than those produced in the TZ) were detectable.
To further support the idea of the TZ as a kind of sensory zone, w e will present, simultaneous measurements of tissue oxygenation and single-cell electrical activity in the TZ, showing that action potentials were accompanied by immediate decreases in tissue oxygenation. This behaviour surprisingly resembles results recently published on the neural activity of cats (Thompson et al. 2003). Obviously, cells of the transition zone are accomplishing processes requiring very large amounts of oxygen. All these advancements converge towards a concept proposing that the transition zone of the root apex represents a highly specialized sensory and information processing region which encompasses neuronal features (Baluška et al. 2003b). An attractive scenario would be that this root apex region acts as some kind of 'brain-like' command centre in higher plants (Baluška et al. 2004). Future studies should answer this important question of plant biology.

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Polar signals in plant vascular tissue development

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Polar signals have long been implicated in vascular tissue pattern formation (summarized in 1). To better understand the molecular cues directing the patterned differentiation of vascular tissues we have adopted a number of strategies for the identification and functional characterization of genes preferentially expressed at early stages of vascular development.
Several of these approaches are based on the observations that auxin signaling through the Auxin Response Factor (ARF) MP is critical for vascular differentiation in *Arabidopsis* and that vascular patterning depends on proper auxin transport at critical stages of organ development (1, 2). At least one other ARF acts redundantly in auxin signaling in vascular development and at least one Aux/IAA co-regulator functions as an *in planta* antagonist in this process (3). Auxin response maxima foreshadow sites of procambial differentiation (4) and other early markers indicate a polar mode of preprocambial cell fate acquisition (5). We report experiments addressing the acquisition of cell polarity during early stages of procambium formation and strategies towards the identification of signal transduction genes expressed at these early stages . Finally, further early vascular genes are identified in a collection of indirect enhancer-trap lines, which also served as genetic backgrounds in the search for mutants with abnormal preprocambial patterns.

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Leaf vascular patterning in monocots and dicots

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In leaves, vascular strands of distinct hierarchical orders are organized in closed (i.e. connected) networks. Vascular networks are generated de novo during the development of each leaf primordium. Procambial cells, the precursors of all vascular cells, differentiate from preprocambial cells, which represent a molecularly defined subset of ground meristem cells in the leaf (1). Mechanisms integrating aligned cell differentiation during vascular strand formation were probably not reinvented in the evolution of leaves, but recruited and revised by leaf-specific controls. In addition, species-specific cues are likely to be involved in leaf vascular patterning, as shown by the successful use of species-specific leaf vascular patterns as taxonomic diagnostic features (2). Whereas most dicot leaves show a ramified pattern of progressively branched veins, most monocot leaves have striate venation patterns in which major veins lie parallel along the proximodistal axis of the leaf and are connected transversely by minor transverse veins (3).
Auxin signals have a profound impact on vascular patterns of dicot leaves. Local auxin application induces the formation of a new vascular strand (4). Further, both defective auxin signal transduction and impaired auxin transport alter leaf vascular patterns in characteristic ways (5-8), and positions of leaf procambium formation are foreshadowed by sites of elevated auxin response (9).
The reduced auxin sensitivity of monocot leaves (10), and their highly reproducible distribution and arrangement of veins (3) suggest that vascular patterns in monocot leaves may be rigidly specified. Recent pharmacological and genetic evidence suggest, however, a role for auxin transport and signal transduction in vascular patterning of monocot leaves (11,12).

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Signals and targets triggered by self-incompatibility: recognition of "self" can be deadly!

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Self incompatibility (SI) is one of the most important mechanisms for promoting outbreeding in flowering plants. It prevents self-fertilization through a genetically controlled cell-cell recognition system [1,2]. My lab studies SI in Papaver rhoeas (the field poppy), where SI is mediated by an interaction between the pistil S-gene product when it meets “self” (incompatible) pollen. This triggers a Ca^{2+}-dependent signalling cascade in incompatible pollen, resulting in inhibition of pollen tube growth.
The SI-specific signals involve activation of several protein kinases, including a MAP kinase, p56 [3]. Another phosphoprotein, p26, which is hyper-phosphorylated rapidly in response to SI, has been identified as a soluble inorganic pyrophosphatase [4], which we postulate may play an important role in inhibition of incompatible pollen. Another target of the SI response is the pollen actin cytoskeleton, which undergoes rapid reorganization and depolymerization [5,6]. We have recently demonstrated that programmed cell death (PCD) is triggered by the SI response and that a caspase-like/DEVDase activity is activated by SI in incompatible pollen [7]. Compelling evidence suggests that the caspase-like activity is triggered very early in the signalling cascade, and also that it plays an active role in processes leading to inhibition.
SI-induced PCD appears to involve a number of signalling cascades and targets which recent data suggest may be involved in cross-talk. I will discuss progress on identifying the signals that mediate SI, the involvement of the actin cytoskeleton and PCD.

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Signal perception and transduction in plant innate immunity

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Immunity of an entire plant species to microbial infection (non-host resistance) is determined by intertwined layers of defense including both constitutive barriers and inducible reactions. Activation of non plant cultivar-specific inducible responses is likely based upon recognition of pathogen-associated molecular patterns, which bind to plant receptors. We have identified a cell wall transglutaminase (TGase) from phytopathogenic *Phytophthora* spp. that triggers defense responses in parsley and potato. A surface-exposed fragment within this TGase (Pep-13) was shown to be indispensable for both elicitor and TGase activity of the protein, suggesting a crucial role of this domain for protein stability and/or enzymatic activity. Ligand-induced receptor activation gives rise to elevated levels of cytoplasmic calcium, subsequent posttranslational activation of MAPK, production of reactive oxygen species and antimicrobial phytoalexins. NPP1, another *Phytophthora*-associated surface structure was shown to trigger a similar cascade of events through a receptor system distinct of the Pep-13 receptor. Currently, conditional expression of NPP1-induced cell death in *Arabidopsis thaliana* is employed to isolate mutants impaired in NPP1 perception. In addition, two molecular patterns associated with phytopathogenic pseudomonads, lipopolysaccharide and the typeIII effector, HrpZ, were shown to synergistically activate plant defense in parsley. Moreover, LPS alone as well as type III secretion-deficient pseudomonads were shown to trigger systemic acquired resistance (SAR) in Arabidopsis, suggesting that the plant defense-inducing capacity of microbial surface structures contributes to physiologically relevant resistance responses, such as SAR. Microarray experiments using the Affymetrix ATH1 chip and RNA prepared from pathogen-infected as well as elicitor-infiltrated *A. thaliana* plants were performed to identify genes whose expression pattern and predicted molecular function implied a role in signal perception and transduction. Plants carrying homozygous T-DNA insertions in a gene encoding a leucine-rich repeat receptor kinase were identified that showed an altered disease resistance. A molecular characterization of the protein will be presented.

TMV as a model for the analysis of RNA transport via plasmodesmata

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Studies in higher plants have revealed the existence of proteins and RNA species that travel cell-to-cell and through the vasculature to serve as signaling molecules in plant development and gene silencing, thus confirming the role of plasmodesmata (Pd) in the mediation and control of intercellular and systemic communication via macromolecules. Compelling evidence for macromolecular trafficking through Pd comes from RNA viruses, which encode movement proteins (MP) to interact with Pd and other components of the RNA transport machinery in order to spread their genomes from cell-to-cell. The MP of *Tobacco mosaic virus* (TMV) is believed to form a ribonucleoprotein complex (RNP) with viral RNA (vRNA) and to represent the core of the infectious particle that spreads between cells. This hypothesis is supported by the ability of MP to bind single-stranded nucleic acids *in vitro*, by its localization to plasmodesmata (Pd), its interaction with cell wall proteins, as well as by its ability to modify the size exclusion limit (SEL) of Pd. Our research is aimed at elucidating whether the MP indeed forms an RNP *in vivo*, and also at understanding the cellular mechanism that targets the viral RNA genome (e.g. the RNP) and potentially other RNA molecules to Pd.

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Hydrotropism: root growth responses to water

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Survival of terrestrial plants depends upon the capacity of roots to obtain water and nutrients from the soil. Directed growth of roots in relation to a gradient in moisture is called hydrotropism and begins in the root cap (RC) with the sensing of the moisture gradient. Even though the lack of sufficient water remains the single-most important factor affecting world agriculture, there are surprisingly few studies on hydrotropism. Using a screening system with a water potential gradient, we isolated a no hydrotropic response (*nhr1*) semi-dominant mutant of *Arabidopsis* that continued to grow downwardly into the medium with the lowest water potential contrary to the positive hydrotropic and negative gravitropic response seen in wild type (wt) roots. The lack of hydrotropic response of nhr1 roots was confirmed in a system with a gradient in air moisture. The hydrotropic response of wt roots in the screening system occurred with a concurrent drop in both starch content in columella cells and in gravitropic downward growth response. However, hydrotropicaly stimulated *nhr1* roots in the screening system contained unusually large amyloplasts that persisted throughout the 8-day treatment. Since nhr1 roots maintained their large amyloplasts during hydrostimulation, their perception and gravitropic response in the screening system was not affected as in wt roots. Furthermore, *nhr1* roots had abnormal RC morphogenesis and displayed decreases in auxin maximum. The genetic analysis of hydrotropism has provided new insights about the mechanisms that the RC uses to perceive and respond simultaneously to moisture and gravity signals. This knowledge might allow us to understand how RC processes environmental signals that are capable of regulating whole plant growth.

Cell wall integrity signalling in *Arabidopsis thaliana*

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Signalling mechanisms coordinating interaction between the cell wall and the plant cell are crucial in several different biological contexts such as (a)biotic stress response, cell wall polysaccharide biosynthesis and cell morphogenesis. Over the last years evidence has surfaced that hints at the existence of a mechanism monitoring cell wall integrity. Both ethylene and jasmonic acid signalling mechanisms have been implicated while lignin and pectin biosynthetic processes are apparently downstream targets. We have performed expression profiling experiments monitoring the transcriptional response to cell wall stress caused by inhibition of cellulose biosynthesis through the herbicide isoxaben. One of the genes responding very strongly on the transcriptional level encodes a putative glutamate receptor protein (*GLR*). Homozygous mutant seedlings for this *GLR* exhibit cell wall defects and reduced lignification upon treatment with isoxaben. We will present our results regarding the biological function of this receptor.

N-Acylethanolamines: emerging Lipid Mediators of Seedling Development

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N-Acylethanolamines (NAEs) are lipid mediators derived from the hydrolysis of the membrane phospholipid, *N*-acylphosphatidylethanolamine (NAPE). In animal systems, this reaction constitutes part of the endocannabinoid signaling pathway, which regulates a variety of physiological processes, including cell proliferation, immune cell signaling, neurotransmission, and embryo development. NAEs have been identified and quantified in a variety of plant tissues, and reports of biological activities of these lipids in plants are rapidly emerging. For example, NAEs have been implicated in the signal-mediated activation of gene expression, the inhibition of phospholipase D (PLD) alpha activity, and the regulation of ABA-induced stomatal closure. NAEs are prevalent in desiccated seeds and are metabolized rapidly during imbibition. We have shown previously that sustained levels of NAE12:0 have profound effects on Arabidopsis seedling growth and development, which includes a reduction in primary root and cotyledon expansion, increased radial swelling of root tips, and an inhibition of root hair initiation. Light and electron microscopic analysis of Arabidopsis roots reveal that the effects of NAE12:0 also are apparent at the cellular level as defects in cell division, cytoskeletal organization, membrane dynamics, and cell wall organization. We have developed a battery of tools that we expect will help in extending our understanding of NAE function in seedling development in *Arabidopsis thaliana*. These tools will be supported by sensitive quantitative procedures (gas chromatography/mass spectrometry) for determining endogenous levels of NAEs in plant extracts. Characterizing the effects of NAEs on seedling growth through these interdisciplinary approaches should provide new insights into the regulation of plant development by this endogenous group of bioactive lipids.

A Phytosynapse? The plant's use of glutamate receptors to respond to aluminium

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The synapse is a hallmark of the animal nervous system, sited literally at the crossroads between electrical and chemical information. Plants don't have synapses, so it provokes surprise to discover that plants do have proteins that are homologues of ionotropic glutamate receptors. In animals, these receptors are synaptic cation channels, whose opening is gated by the neurotransmitter glutamate. In plants, we have found that glutamate receptors appear to be key players in signal transduction. Our data suggest that in the presence of aluminum, plant roots secrete glutamate, which binds to its receptor, allowing a calcium influx. The calcium transient leads to plasma-membrane depolarization as well as depolymerization of the cortical microtubules, both actions that will spread the message throughout the cell. Our original work was done with *Arabidopsis* roots and we are now finding a similar response network in wheat. This work suggests that in plants the concentrated synapse and brain of animals are replaced by a delocalized intelligence.

Glutamate signalling and root development in Arabidopsis

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Like the rest of the plant, the root system has no pre-defined body plan, rather its development is continuously modified by interactions with environmental factors, including nutrients. Plants are known to forage for localised supplies of nitrate by proliferating their lateral roots within nitrate-rich patches. Recent evidence suggests that roots are also capable of sensing and responding in a highly specific manner to the presence of organic N in the soil. *Arabidopsis* root growth is very sensitive to the presence of external glutamate, but not to most other amino acids. The ability to sense glutamate appears to reside in the root tip itself and is common to both primary and lateral roots. Different ecotypes of *Arabidopsis* differ markedly in their glutamate sensitivity and we have used recombinant inbred lines to map a major QTL for glutamate sensitivity to chromosome 5. We will discuss our current understanding of the genetic and physiological basis of this phenomenon and its possible relationship to the existence of a family of glutamate receptor genes in plants.

Genetic and pharmacological evidence for a role of the GABA shunt in maintaining the levels of reactive oxygen intermediates in plant cells

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Disruption of the *Arabidopsis* gene encoding the last enzyme of the GABA shunt (SSADH) results in high levels of reactive oxygen intermediates (ROIs), cell death, and dwarfism, associated with hypersensitivity to stresses such as UV and heat. The *ssadh* mutants also contain high levels of gamma-hydroxybutyrate (GHB) a known neurotransmitters in mammals, which is derived from the GABA shunt. Currently we are undertaking genetic approaches aimed at isolating suppressor mutants of GABAshunt deficient mutants (*ssadh* in particular) with the hope of identifying novel components involved in GABA metabolism and signaling. A few suppressor mutants, which resemble wt plants in spite of the fact that they are homozygous for the disrupted *SSADH* gene, have been isolated and the genes with the suppressor mutations are being cloned. By taking a pharmacological approach, we found that vigabatrin (VGB), a specific inhibitor of mammalian GABA-transaminase, reduces the levels of GHB in *ssadh* mutants, similar to the effect of the drug in mammals deficient in SSADH. Moreover, VGB substantially reduces the levels of ROI, and improves plant growth. Thus both genetic and pharmacological evidence suggest the involvement of the GABA shunt in maintaining the levels of ROI in plant cells. Moreover, our studies reveal novel similarities between plants and animals in relation to two neurotransmitters: GABA and GHB.

Glutamate receptors and GABA in plant responses to environmental stress

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The non-protein amino acid γ-aminobutyric acid (GABA) has been shown to rapidly increase in plants upon exposure to a variety of environmental stresses. However, little is known about the physiological role of GABA in higher plants or how alterations in the balance between Glu and GABA affect normal plant growth and development. Both molecules have been proposed to be signaling molecules in plants, we have taken two approaches to address this possibility. One approach focuses on the biosynthetic relationship between the putative ligands, Glu and GABA, while the other approach focuses on the putative receptors in the signaling pathway(s).
GABA is synthesized from glutamic acid by the enzyme glutamic acid decarboxylase (GAD). The completion of the Arabidopsis genome has revealed that there are five *AtGAD* genes. *AtGAD1* and *AtGAD2* are expressed in roots and throughout the plant, respectively. However, the expression of the other three genes (*AtGAD3-5*) has not been reported. In order to gain a better understanding of the temporal and spatial expression of all the members of the *AtGAD* gene family, we made promoter and *uidA* gene fusion (*AtGAD*::GUS) constructs and determined the expression of each gene throughout development and upon exposure to distinct stresses. Our findings suggest that the *AtGADs* have non-redundant roles.
Results from parsimony and incongruence length difference analyses demonstrate that regions of the N-termini of nearly a third of the twenty members of the putative glutamate receptors in *Arabidospis thaliana* (*AtGLRs*) are related to animal GABA B receptors. Immunoblot analyses and immunolocalization of one of these *AtGLR* gene products, AtGLR3.2, show that it is an integral membrane protein that accumulates in rapidly dividing cells and vascular tissue. These findings suggest that AtGLR3.2 may be associated with calcium distribution and allocation.
We used an antisense approach to develop Arabidopsis lines deficient in *AtGLR1.1* (*antiAtGLR1.1*). Our results suggest that AtGLR1.1 has functional characteristics similar to animal ionotropic glutamate receptors (iGLRs). Using a combination of targeted- proteomics and - metabolomics, genome-wide microarray and physiological analyses, we show that there is a strong interaction between carbon (C), nitrogen (N), sulfur (S) and abscisic acid (ABA) metabolic pathways, ABA sensing, and/or H_2O utilization.
In conclusion, our results show that the *AtGLRs* (i) regulate distinct pathways associated with N and S acquisition, distribution and metabolism, (ii) alter the expression of distinct carbon (C) metabolic pathways, (iii) alter the synthesis of the phytohormone ABA and sensitivity of the plant to ABA, and (iv) ultimately coordinate components of the C/N/S and ABA signaling pathways which change stomatal aperture to affect plant responses to H_2O-related stresses. These findings suggest that the AtGLRs may coordinate the control of distinct components of C, N and S metabolism as well as ABA biosynthesis and sensing.

Arabidopsis knock out mutants of GABA metabolism and their response to different growth conditions

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Arabidopsis mutants defective in genes of the g-aminobutyric acid (GABA) pathway were isolated and analysed regarding their growth behaviour on agar plates containing different nitrogen sources. Several carbonic and amino acids including GABA were monitored under these conditions using GC/MS. GABA-transaminase knock out plants were hypersensitive against GABA when grown on agar plates.In contrast to WT plants, mutants did not grow on plates containing high concentrations of GABA as sole nitrogen source. Strikingly, growth of both, WT and mutant plants was much less inhibited without any nitrogen in the medium indicating that GABA sensing differs between *Arabidopsis* WT and mutant plants, i.e. a situation where GABA either can or cannot be used.

Does GABA act as a long-distance signal in the regulation of nitrate uptake in plants?

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Nitrate uptake is the most limiting step of nitrate assimilation and consequently of higher plants growth (1). Nitrate uptake processes involve the functioning of several transporters with low-affinity (NRT1) and high-affinity (NRT2) to nitrate. These transporters are now well characterized at molecular level in many species and are regulated by complex local and long-distance signalling pathways (2). Until now, long-distance signals identified (such as glutamine) were supposed to mediate a feedback repression of nitrate uptake according to the modifications of internal N status varying with N demand of plants for growth.
In order to identify others transported amino acids implicated in long-distance regulation of nitrate uptake, we have analysed in *Brassica napus* plants, composition of phloem exudates during N-deprivation (short-term experiment) and over a growth cycle (long-term experiment) in relation with variations of nitrate influx and *BnNRT2* nitrate transporter gene expression.
Our results show a positive correlation between GABA in phloem exudates and nitrate uptake in short and long-term experiments. The hypothesis that GABA could act as long-distance signal in up-regulation of nitrate uptake was tested by providing an exogenous GABA supply to the roots. By contrast to Glutamine treatment which inhibits both nitrate uptake and *BnNRT2* gene expression, GABA treatment induces a significant increase of *BnNRT2* mRNA expression but has less effect on nitrate influx. Despite the fact that this study provides the first evidence that GABA may act as putative long-distance inter-organ signal in plants, our results raise question of whether GABA could mediate a root-specific response in mineral acquisition via the modifications of root absorbing surfaces or increase conduction of ions transport (3).
We are now investigating the role of GABA in root development, by analysing the effects of GABA treatments on exploratory root system: primary and lateral roots and absorbing root system: root hair development.

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Is ATP a signalling agent in plants?

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Very low concentrations of extracellular purines (ATP, ADP and AMP) can function as neurotransmitters in animal cells (1). At the cell surface they interact with specialised receptors ("purinoceptors") which form transmembrane cation-selective channels (P2X receptors) or activate G-proteins (P2Y-receptors). Low concentrations of extracellular ATP (eATP) have recently been demonstrated to modify auxin distribution, gene expression and gravitropic responses in *Arabidopsis* roots (2). Since these effects have not been related to phosphorylation, a possible explanation is that eATP can act in plants in a neurotransmitter-like mode (2). Here, we test the hypothesis that ATP is a signalling agent in plants that functions in a similar way to animal neurotransmitters (activating ion fluxes at very low concentrations). We have found that [eATP] as low as 300 nM induced transient elevations of root ([Ca^{+2}] cyt) in plants constitutively expressing cytosolic aequorin (3). ADP and non-hydrolysable analogues of ATP also caused [Ca^{+2}] cyt increases, showing that the effect was not induced by phosphorylation. UTP was far less potent than all tested purines. Purine-induced [Ca^{+2}] cyt increases were inhibited by classic purinoceptor blockers (suramin and PPADS) and by Gd^{+3} (3). These results have now been confirmed by Jeter *et al.* (2004) (4). Using the MIFE ® technique we have measured the effect of extracellular purines on net Ca^{+2} and K + fluxes in intact *Arabidopsis* root epidermis. ATP, ADP and the non-hydrolysable ATP analogue *ab* meATP (up to 100 *m* M) simultaneously caused transient Ca^{+2} influx and K^+ efflux. Purine-induced fluxes were far larger in the elongation zone than in the mature epidermis. Using patch-clamp we have identified p urine- induced whole-cell cation conductances and single cation channels in root epidermis-derived protoplasts. Activity resembled animal ionotropic purinoceptors. We propose that extracellular ATP and ADP are important signalling agents in plants acting via plasma membrane cation channels.

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Signaling role of extracellular ATP in growth control and in wound responses

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Externally applied ATP (xATP) can induce increased [Ca2+] cyt in plant cells (Demidchik et al., 2003; Jeter et al., 2004), just as it does in animal cells. To evaluate the physiological significance of this signaling response, we have tested the effects of xATP on growth and development in Arabidopsis. Lower µM levels of xATP enhance and higher µM levels inhibit the hypocotyl growth of seedlings. Sub-µM xATP can induce in leaves an enhanced production of superoxide and downstream gene expression changes associated with wounding. Neither the growth nor superoxide responses can be induced by equivalent concentrations of phosphate or AMP. The inhibitory growth effects of xATP can be blocked by ethylene inhibitors, and the ability of xATP to induce superoxide production can be blocked by antagonists of animal purinoceptors and by inhibitors of calcium uptake into cells.
Ectoapyrases (ecto-NTPDases) are ATP-hydrolyzing enzymes that play a key role in controlling the [xATP]. Two highly similar apyrases in Arabidopsis, Atapy1 and Atapy2, have putative signal peptides and have been postulated to function as ectoapyrases. They are important for pollen germination and other aspects of plant cell growth. Pollen from plants not expressing Atapy1 and Atapy2 (= double knock-out (DKO) plants) cannot germinate. DKO pollen from plants that are complemented with a wild-type gene under the control of a steroid-inducible promoter can be induced to germinate by dexamethasone. Fertilization by this pollen yields seeds that germinate normally, but in the absence of further dexamethasone treatment, the resulting DKO plants are stunted, typically < 10 cm tall at maturity. As evaluated by promoter:GUS constructs and other methods, the highest mRNA level for both apyrases was found in tissue poised for rapid growth (mature pollen), or tissue actually undergoing rapid growth (elongation zone of the root, hypocotyls of etiolated seedlings), consistent with the postulate that apyrases play a key role in growth control.

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Ion channels in plants: from DNA sequence to integrative biology

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Processes involved in plant growth, development and adaptation to changing environmental conditions are frequently accompanied by mass flow of ions or even electrical signals. Therefore signal perception, transduction or processing in higher plants significantly relies on the concerted action of receptors, ion channels and pumps.
The functional properties of most ion channels cloned in *Arabidopsis* (and other plants) remain speculative and rely essentially upon sequence homologies with animal channels. The plant Shaker-like K^+ channel family is a noticeable exception as this ion channel family has been well characterized at the molecular and physiological level.
As their animals homologues, the so-called Shaker-like plant K^+ channels are made of four a - subunits. Nine genes in *Arabidopsis* encode such polypeptides, which display the typical structural scheme with 6 transmembrane segments and one P-domain. In heterologous expression systems (*Xenopus* oocytes, *Sf*9 insect cells, COS cells, yeast) these genes yield K^+ selective channels featuring both absence of inactivation and voltage-gating and are therefore capable of contributing as well to sustained K^+ transport as to excitability. These channels can be sorted in 4 functional groups: (i) inwardly-rectifying (KAT1, KAT2, AKT1, SPIK), (ii) outwardly-rectifying (SKOR and GORK), (iii) weakly inwardly-rectifying channels (AKT2), and (iv) regulatory subunit (AtKC1).
An overview of the current knowledge of these different subunits will be presented. A special emphasis will be given on the approaches that have allowed to decipher their role *in planta*: cloning, heterologous expression, expression pattern, regulation at the transcriptional/post transcriptional level and characterisation of knock-out mutants. Finally, the role of other proteins in the macromolecular assembly and regulation of plant potassium channels will be discussed.

Developmental effects of electric current in thermo- and photoperiodic plants

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The effect of electric current (EC) was tested on flowering induced by vernalization and by photoperiod in winter wheat, stem cuttings of spring and winter rape grown in vitro and to rape grafted plants (non-vernalized scion of the apical part grafted on vernalized stock of winter rape). Different time/voltage combinations were tested as well as both polarities. EC of positive polarity (anode close to apex, cathode in the medium) increased significantly percentage of flowering plants in partially vernalized winter wheat and of non-vernalized scions grafted on vernalized winter rape stocks, both under long and under short days. Transition to flowering of isolated apical stem segments of spring and winter rape was also stimulated by EC of positive polarity. EC of negative polarity inhibited flowering of the grafted scions in winter rape under long days. Thus, negative polarity seems to suppress inductive signals, while the positive polarity either mimics them or restrains the effects of non-inductive conditions.

Action potentials - from mechanism to function

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Action potentials (APs) can be evoked in plants by damaging and non damaging stimuli. After application of damaging stimuli, such as wounding or burning either series of APs or APs superimposed with variation potentials are registered. Non damaging stimuli, like illumination, cooling or direct current lead usually to generation of single AP. An important feature of APs is their ability to spread from the site of stimulation throughout the whole plant, specialized tissue or plant organ. Local circuits consisting of ion fluxes play important role in AP transmission and synchronization of plant response to remote stimuli. It is thus important to determine the ion mechanism of APs.
It was first elaborated for giant characean cells. Later on, basic aspects of the mechanism were confirmed and modified using another model plant - the liverwort *Conocephalum conicum*. *C. conicum* belongs to phylogenically oldest terrestrial plants. Its thallus forms nearly homogenous network of cells connected with plasmodesmata. All cells of the thallus (including rhizoids) are excitable. Basing on experiments with application of ion-selective microelectrodes, and ion channel inhibitors one can conclude that the ion mechanism of APs in *Conocephalum* consists of the following steps. AP is initiated by Ca^{2+} influx into the cytosol. Increasing $[Ca^{2+}]$ cyt activates anion channels, and Cl - efflux leads to cell depolarization. Repolarization occurs as a result of K^+ efflux and enhanced H^+ extrusion through the electrogenic proton pump. Simultaneous application of anion- (A-9-C) and potassium channel inhibitors (TEA) allowed to separate a calcium phase of AP, called VT, and address the question concerning the source of calcium ions entering the cytosol. Substances responsible for Ca^{2+} liberation from internal stores (Sr^{2+}) caused an increase, whereas chemicals blocking internal Ca^{2+} channels (neomycin) suppressed the calcium phase of AP. Partial inhibition of VT was also observed after application of impermeable Ca^{2+} channel inhibitors affecting Ca^{2+} influx through the plasmalemma. It was concluded that APs in *Conocephalum* are initiated by Ca^{2+} influx through the plasma membrane which then causes liberation of additional portion of Ca^{2+} ions according to the process known as calcium induced calcium release.
The extent of $[Ca^{2+}]$ cyt increase determines the physiological response. It was demonstrated that in *Conocephalum* the rate of respiration increases up to twice after passing of AP evoked by non damaging electrical stimuli. Severe stimuli, like cutting of the thallus edge, evoked series of APs followed by shifted in phase oscillations in the rate of respiration. After blocking of AP with ion channel inhibitors, no significant increase in the respiration rate was observed irrespectively of the kind of stimulus.
Significant increase in peroxidase activity is another consequence of excitation in *C. conicum*. It was registered only if the threshold of excitation was exceeded. Here again Ca^{2+} ions seem to play a role of coupling factors between AP and the physiological response. Different physiological responses evoked as a consequence of plant excitation are consistent with the concept of "calcium signature".

Oscillations in plants

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Rhythmical behaviour is a quintessential pattern of life itself. Biological oscillations kept many generations of scientists fascinated, from such diverse areas of knowledge as physiology, biochemistry, biophysics, mathematics, and biological cybernetics. An avalanche-like increase in publication number led to foundation of several specialised journals on biological rhythms. However, plants have always been treated as "Cinderellas" in studies on biological rhythms. In contrast to quite obvious circadian rhythms (with 24 h period), ultradian oscillations (with periods of minutes) are not as widespread in plants as they are in animal organisms, at least at first glance. With a possible exception of plant movements (such as leaves or plant axial organs) and oscillations in stomatal aperture, many plant physiologists treat oscillations as some unwanted "noise". More recently, a breakthrough in understanding of the signalling role of Ca^{2+} in cell metabolism caused a vivid interest in calcium oscillations in stomatal guard cells, as reflected by a large number of excellent reviews [1-3]. The physiological role of ultradian oscillations in other plant tissues and organs is still underestimated. In this review, an attempt is made to summarise the recent progress in this area and highlight the paramount role of oscillatory processes in plant life. First, advantages and principles of oscillatory control are considered in the context of plant physiology, with a major emphasis on feedback control and self-sustained oscillations, as well as on determenistic chaos and "strange" behaviour in plants. Next, a possible role for ultradian rhythms in timekeeping and the link between ultradian and circadian oscillators is discussed. Several models of circadian oscillators are analysed, and various *pros* - and *contras* - for each of them are discussed. The major emphasis is made on possible role of cellular membranes as an important component of the feedback loop in circadian clock mechanism. The importance of membrane- related oscillations is further illustrated by their crucial role as a part of the encoding mechanism, mediating plant-environmental interaction. Practical examples include cell differentiation and morphogenesis, growth, development and adaptive responses to various abiotic and biotic stresses.

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Slow wave potentials – higher plants' very own propagating electrical signals

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Unlike action potentials (APs) slow wave potentials (SWPs, sometimes called variation potentials) are a particular creation of higher plants with no parallel occurrence in animals. The generation of SWPs occurs under different conditions than that of action potentials (APs). APs are generated and can be studied in excised stems and leaves. SWPs depend on the pressure difference between the atmosphere and an intact plant interior. Excision of leaves, anti-transpirants, air of high humidity any other condition that compromises the tension of the plant interior will severely reduce the range of SWP signals or suppress them altogether. SWPs and APs both involve transient depolarizations of plant cells that spread from their place of generation to distant parts of the plants. However, when compared in the same plant the depolarization of APs is always shorter than that of an SWP. The available data suggest that this feature reflects different ionic mechanisms. While opening anion channels mediate most of the depolarization in APs, the depolarization of SWPs probably involves both opening of calcium channels and a transient shutdown of stretch-sensitive H^+-ATPases at the plasma membrane. A third defining particularity of SWPs consists in their mode of propagation. SWPs appear to involve a sequence of local depolarizations that arise in the wake of a rapid wave of positive pressure spreading through the xylem and finally resulting in rapid water uptake and turgor increase by peripheral cells. Since poisoned or killed stem sections do not interrupt SWP propagation, it follows a hydraulic rather than electric mechanism. Studying *Mimosa* responses, Haberlandt suggested as early as 1890 (when only APs were known) to look for a hydraulically propagating signal. It took a long time to realize that such a signal exists in the form of SWPs.

Hydro-electrochemical integration of the higher plant - basis for electrogenic flower initiation

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The integration of activity of *Chenopodium* plants on hydraulic-electrochemical levels is expressed by a diurnal rhythm in resting membrane potential measured with contact electrodes. Membrane state could be gated by the energy state of cells. From earlier studies we compiled evidence in favour of a circadian rhythm in overall energy transduction producing a circadian rhythm in energy charge and redox state (NADP/NADPH). The ratio of metabolic coupling nucleotides would be relatively temperature independent and thus could fulfill the requirements for precise temperature-compensated time keeping. The photoreceptor phytochrome, involved in photoperiodic control of development, could via changes in pyridine nucleotide pool sizes and changes in nucleotide ratios regulate transcription via redox controlled transcription factors.
Spontaneous action potentials (APs) have been shown to correlate with turgor controlled growth movements. The accumulation of spontaneous APs at specific times during daily light-dark spans were recorded giving specific electrophysiograms (EPGs).
There was a switch in predominant propagation direction of APs along the stem axis (acropetal-basipetal) in the transition from vegetative to reproductive growth; opposite in long- and short-day *Chenopodium* plants. The information from EPGs showing the frequency distribution and polarity changes of spontaneous APs in response to flower inducing and non inducing photoperiods have been used to induce flowering in non inductive photoperiods by specific timing and polarity of direct current pulses via contact electrodes. It is anticipated that hydraulic changes at the apex leading to flower initiation are mediated by a specific hydro-electrochemical communication between leaves and the shoot ape

Forisomes as sensors and aphids as reporters of Ca^{2+}-influx and efflux during depolarization waves along sieve tubes

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Experiments on electrical propagation are often lacking of a precise definition of the cell types and the ion species involved. We developed an *in vivo* system that unequivocally shows calcium involvement in electrical long-distance signalling via the sieve tubes in intact plants. Shallow windows paradermally cut in the cortex of major leaf veins of *Vicia faba* allowed observation of events in sieve tubes and the insertion of microcapillaries into sieve elements. Exclusively in sieve elements of Fabaceae, protein bodies (forisomes) occur which reversibly disperse and contract in dependence of calcium. Forisome dispersion enabled to determine the *in vivo* Ca^{2+} concentration in the sieve tubes. Burning of the leaf tip triggered a depolarization wave along the sieve tubes which concurred with an increase of intracellular calcium in the sieve elements as shown by Oregon Green. Upon arrival of the depolarization wave, forisomes often readily dispersed. The forisomes re-contracted several minutes after passage of the wave. The behaviour of forisomes and the depolarization profiles indicate the involvement of calcium channels and calcium pumps in electrical propagation along the sieve elements.

As forisomes temporarily plug sieve plates, the reaction of aphids to burning-induced plugging of the sieve plates - and thus food deprivation - was investigated. Behaviour of aphids and the position of the stylet insertion can be monitored by the so-called EPG-technique. Microelectrodes attached onto the aphid's abdomen record electrical patterns produced by the aphid during foraging. Each specific wave pattern is related to foraging of a defined cell type. After puncturing of a sieve tube, aphids produce watery saliva which is reflected by a E1-wave pattern. As soon as the aphids are settled, an E2-wave pattern establishes which can hold for many hours.

The hypothesis that the aphids would react to sudden food deprivation or turgor drop was tested in the plant/aphid combinations *Vicia faba/Megoura viciae* and *Hordeum vulgare/Schizaphis graminae* by burning the leaf tips. It appeared that plugging of sieve plates causes an immediate transition from the E2- back to the E1-wave pattern. Aphids were then used to mimick a row of microelectrodes along the phloem. The behaviour of aphids at various distances from the site of burning enabled us to record speed and magnitude of the depolarization wave. The degree of the aphid's reaction was supposed to be related to the degree of sieve plate plugging which in turn is related to the degree of local depolarization and Ca^{2+} influx. The EPG-data seem to indicate that the propagation speed slows down with the distance and that the depolarization wave becomes extinct after 10 to 20 cm.

Heat-induced electrical signals change photosynthesis in poplar

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Plants respond to various abiotic stimuli by generation and propagation of electrical signals. To get more information on the response of photosystem II (PSII) in higher plants, we investigated heat-induced long and short-distance signalling in poplar trees (*Populus trichocarpa*) by means of chlorophyll fluorescence imaging. In both types of signalling, PSII quantum yield of electron transport is reduced significantly (from c. 0.55 to 0.35). Two-dimensional imaging analysis of the fluorescence signal manifests the yield reduction which spreads via the veins through the leaves. The different types of signal propagation were shown by microelectrode measurements in leaf-vein phloem via the aphid technique; basipetal signal transduction leads to rapid membrane hyperpolarisation within the same leaf, whereas acropetal long-distance signalling causes depolarisation of the membrane potential in leaf phloem. Moreover, gas exchange measurements revealed that the depolarising signals travel distances across the stem to neighbouring leaves where the net CO_2 uptake rate is temporarily depressed towards compensation. Controls show that after cooling of the stem to +4°C electrical signal transmission via the phloem is disrupted so that leaf gas exchange stays unchanged. By measuring calcium-deficient poplar plants, a much lower amplitude of the electrical signal was detected, and no significant response in gas exchange was observed after heat-induced wounding of leaves. We therefore conclude that electrical signals significantly affect the photosynthetic performance of poplar trees.

Electrophysiology and Phototropism

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Plants generate different types of electrical events in connection to environmental stress. Cells, tissues, and organs transmit electrochemical signals over short and long distances. Action potentials in higher plants may be the information carriers in intercellular and intracellular communication in response to environmental changes. Here, we show the generation of bioelectrochemical responses induced by blue photosensory system in soybean plants. A phototropic response is a sequence of the four following processes: reception of the directional light signal, signal transduction, transformation of the signal to a physiological response, and the production of directional growth response. The irradiation of soybean plants at 450 ± 50 nm induces action potentials with duration times of about 0.3 ms and amplitudes about 60 mV. Action potentials play an active role in the expedient character of the response reactions of plants as a reply to external stimuli. Blockers of ionic channels inhibit phototropism in soybean plants. The role of the electrified interface of the plasma membrane in signal transduction is discussed.

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Signals and signalling pathways in plant wound responses

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A widely studied system is the wound induction of proteinase inhibitors (PIs), and other proteins, that are produced locally by the wounded organ, and systemically in organs distant from the wound site. PIs are inhibitors of a variety of proteases, including those from pathogens and herbivorous insects, and are important components of plant resistance to herbivores. There are several possibilities for the signal(s) that link a local wound to the systemic responses, including various chemical elicitors, a pressure wave caused by release of tension in the xylem, and an electrical signal that could be propagated in the phloem. In tomato seedlings, following a severe wound, evidence favours the hydraulic dispersal of chemical elicitors by reversal of flow in the xylem, a mechanism proposed by Malone and co-workers, whereas minor wounds seem to be associated with the transport of chemical elicitors in the phloem. These various possibilities for signals and signalling pathways will be discussed.

Effects of high frequency electromagnetic stimulation on plants

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Studies on the effects of high frequency electromagnetic stimulation on living beings have grown in number over the last few years, particularly in relation to the exponential increase in use of cell phones among the general population. This telecommunication device uses high frequency, non thermic electromagnetic waves (900–1800 MHz) coupled with a lower frequency carrier (230 Hz). Shortly after the advent of cell phones, the question arose as to whether this kind of radiation could constitute an environmental threat for living organisms, particularly in relation to possible health effects. We chose to use tomato plants as our model biological system along with a Mode Stirring Reverberation Chamber (MSRC) as the stimulating device. Plants offer numerous advantages compared with animals (ease of growth, lack of mobility, absence of psychological side-effects), while the MSRC allows us to generate a highly controlled and reproducible isotropic, homogeneous electromagnetic field. Our investigation was focused at the molecular level, where we measured rapid changes in mRNA accumulation (using qRT-PCR) of stress-related genes as molecular markers. We found that stress-related mRNAs such as calmodulin, bZIP transcription factor and protein kinase strongly accumulate within minutes after a short (10 min), low amplitude (5 V/m), 900 MHz EMF stimulation. This accumulation does not occur if the plants are placed into an EMF-proof container. Futhermore, exposure of a single leaf (while masking the rest of the plant) shows that a traumatic signal rapidly moves from the stimulated leaf to the rest of the plant and elicits accumulation of these same stress-related transcripts. In conclusion, we demonstrated that low amplitude EMF stimulation constitutes an environmental stimulus able to activate stress-related genes in tomato plants.

Shade avoidance without photoreceptors

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One of the best-known examples of adaptive plasticity in plants is the shade avoidance response of plants to canopy density perceived by phytochrome. However, it is now evident that proximity detection by means of this family of photoreceptors is not the only signalling pathway available to plants for this critical information for survival in a competitive situation. Other photoreceptors are involved as well, and ethylene is important also. We found that the response of plants to a mechanical stimulus works in a manner that is consistent with shade avoidance. The examples mentioned so far are concerned with avoidance of shading of leaves, which thus maximizes photosynthetic carbon gain. Another form of shade avoidance is the reallocation of photosynthetic capacity from lower shaded leaves to upper leaves in more favourable light conditions, which has a similar function. Photoreceptors do not play an important role in this process. The distribution of the transpiration stream parallel to the light gradient appeared to be involved in an alternative signalling pathway.

Root Exudation and Rhizosphere Biology: Allelochemicals and Cell Death

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Plant roots release an enormous variety of secondary metabolites into the soil. These root exudates can play important roles in mediating positive and antagonistic root-root and root-microbe interactions. We will focus our discussion on the role of root exudates in negative and positive communication between roots of *Centaurea maculosa* and competing plant species.
Centaurea maculosa , a native species in western Europe, is an invasive weed in North America . Our work on *C. maculosa* root exudates indicates that *C. maculosa* invasions in North America are partly mediated by root-secretion of a potent phytotoxin, (±)-catechin. Roots of susceptible plants such as *Arabidopsis thaliana* exposed to (±)-catechin exhibit waves of ROS and Ca 2+ signals that begin at the meristematic and elongation zones, and lead to genome-wide changes in expression and cell death. We investigated the genes involved in early signal transduction triggered by (±)-catechin using T-DNA mutants. Our studies have shown that some T-DNA mutants exhibit resistance to (±)-catechin phytotoxicity and at least one gene mutation increases (±)-catechin-sensitivity in Arabidopsis. These results indicate a complex network of genes working simultaneously to cause cell death and to counteract cell death, possibly by affecting levels of ROS.
Soil (±)-catechin concentrations in *C. maculosa* populations can be very high (mean = 1.55 mg g -1), and tend to be higher in North American populations than in European populations. Further, North American grassland species tend to be more sensitive to (±)-catechin than the European species with which *C. maculosa* naturally coexists, suggesting that (1) European species may have co-evolved (±)-catechin-resistance and (2) the (±)-catechin-naïvité of North American species may account for *C. maculosa* invasiveness in North America. Our research also suggests that high concentrations of (±)-catechin are autotoxic to *C. maculosa* seedlings and inhibit *C. maculosa* recruitment in well-established stands, indicating chemical control of population density. In addition, we have found that low catechin concentrations can induce growth and defense responses in (±)-catechin-susceptible species, indicating the potential for chemical facilitation by *C. maculosa* under some conditions. Finally, our work on mechanisms of (±)-catechin-resistance has shown that at least two North American species (*Lupinus sericeus* and *Gaillardia grandiflora*) also rely on root exudates to resist (±)-catechin toxicity. *Lupinus* and *Gaillardia* root-secrete oxalic acid in response to exposure to (±)-catechin. Oxalic acid, by working as an antioxidant, protects the resistant species and their (±)-catechin-susceptible neighbors from (±)-catechin toxicity under *in vitro* conditions and in the field. The presence of the resistant species or exogenous oxalic acid reduces *C. maculosa* (±)-catechin production, revealing the potential for chemical cross-talk between allelopathic and allelochemical-resistant species, and the coordination of defense responses between plants.

The transmogrification of plant invaders: biogeographic differences in allelopathic effects and native evolutionary responses

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Many exotic plant species undergo astounding increases in dominance when introduced to new communities by humans. This is primarily attributed to escape from specialist consumers. However, strong allelopathic effects (a form of plant-plant communication) by some powerful invaders and much stronger allelopathic effects in invaded than in native ranges suggest a new theory for invasive success – the novel weapons hypothesis. We discuss the evidence for allelopathic effects of *Centaurea maculosa* and *C. diffusa*, evidence for belowground cross-talk, and propose that some invaders transmogrify because they possess novel biochemical weapons that function as unusually powerful allelopathic agents or as mediators of new plant-soil microbial interactions. Novel biochemical weapons possessed by some plant invaders may provide an advantage based on differences in historical coevolutionary trajectories. Furthermore, exotic plant invasions often cause high mortality in native populations and therefore have the potential to be a selective force on natives. We found that individuals of some North American species that survived *Centaurea maculosa* invasion have higher tolerances to the European invader than individuals from communities that had not experienced invasion. These results provide initial evidence that native plants species may evolve to tolerate the effects of an exotic invader, and in particular an invader's novel allelochemistry.

Interplant communication: From induced volatiles to signal transduction pathways

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Plants under insect herbivore attack have evolved various mechanisms to counteract this threat. Among the measures plants undertake to survive with the least damage are the recognition of insect-derived elicitors, production of proteins that block digestion or disrupt intestinal tissue, and the production of defense-related secondary metabolites, which directly or indirectly affect the herbivore performance. Volatile organic compounds (VOC), a mixture of volatile secondary metabolites from various pathways, serve as signals not only to attract predators and parasites of attacking herbivores, but also can be recognized by neighboring plants resulting in defense-related gene expression. Jasmonic acid (JA) and other lipid-derived compounds (oxylipins), which are activated by wounding and insect elicitors, represent important signals in this process. In corn (Zea mays), insect-derived elicitors not only increase the production of oxylipins locally, but also induce JA distal from the application site within 5-10 min. Green leafy volatiles (GLV), which are rapidly emitted during herbivory, serve as potent volatile signals for neighboring receiver plants. By inducing specific sections of the octadecanoid signaling pathway GLV can act as priming signals preparing those plants against impending herbivory. Structure/function analysis of natural GLV as well as synthetic analogs clearly showed certain structural requirements, but excluded α,β-unsaturated carbonyls as active centers. A comparison of gene expression after wounding, wounding with application of crude regurgitant elicitors (CRE), and exposure to Z-3-hexenyl acetate (Z-3-HAC) further demonstrated the specificity of the GLV signal in plant-plant communication through selective activation of genes involved in JA biosynthesis. In conclusion, inter-plant communication via GLV results in an enhanced preparedness specifically directed against insect herbivore attack mediated by specific activation of distinct parts of the octadecanoid signaling pathway.

Communication between undamaged plants by volatiles: the role of allelobiosis

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Plant/plant communication via volatiles, allelopathy, may have profound effects on development of exposed plant individuals (1). Hypothetically these responses affect insects adapted to living on and/or around these plants. To identify effects of plant-plant communication via volatiles on different trophical levels we have used a model system consisting of different barley cultivars and two common weeds, an aphid pest, *Rhopalosiphum padi* (L.), and an aphid predator, ladybird, *Coccinella septempunctata* (L.).
Plant-plant communication mediated by volatile messenger substances significantly affect plant leaf temperature (2) and biomass allocation (3) but not relative growth rate and total biomass. Significant changes in leaf temperature and biomass allocation of exposed plants to allelobiotic volatilise show that the allelopathic effect was systemic. Barley plants exposed to volatiles from neighbouring plants were less readily accepted by aphids when specific cultivars of barley were combined (*intra-species*) (2; 4) and between cereals and a number of aggressive weeds (*inter-species*) (5). Exposure to volatiles from creeping thistle, *Cirsium arvense* (L.), also causes barley plants to become more attractive to ladybird (L.) (6).
For this tritrophic effect of plant/plant communication we suggest the term allelobiosis defined as the effects of chemical interactions between plants across trophic leaves (7).
Our results support the hypothesise that plant responses to allelobiosis is an adaptation for coexistence with other which is a basis for follow up effects in higher trophic levels. The positive effect on polyphagous predators may increase the ecological success of listening" plant stand and, also have negative effects on herbivore plant acceptance.

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Self/non-self recognition in mycorrhizal networks

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Most terrestrial plant species establish mutualistic symbioses with arbuscular mycorrhizal (AM) fungi, which develop extensive, belowground extraradical hyphae fundamental for the uptake of nutrients from soil and their transfer to the host plant (1) . Since AM fungi have a wide host range, they are able to colonize and interconnect plants of different species, genera and families, by means of hyphae extending from one root system to another . Such mycorrhizal networks, visualized and quantified in vivo by using a two-dimensional experimental system, extend at a mean growth rate ranging from 738 to 1067 mm per day, depending on the host plant, and reach hyphal densities of 10-40 mm per mm of root length. Moreover, AM extraradical networks are highly interconnected due to the widespread occurrence of hyphal fusions (anastomoses) (2).
Successful anastomoses , first described during the pre-symbiotic growth of AM fungi, occur between hyphae belonging to the same individual and to different individuals of the same strain. They are characterized by self compatibility, consisting in complete fusion of hyphal walls, cytoplasmic flow and migration of nuclei through hyphal bridges, as revealed by time-lapse, video-enhanced and epifluorescence microscopy (3) . By contrast, hyphae of individuals belonging to different genera and species, and even to geographic isolates of the same species, do not fuse, and show rejection responses, either before or after anastomosis , revealing AM hyphal ability to discriminate self from non-self. Incompatibility responses consist of protoplasm retraction from hyphal tips and septum formation in approaching hyphae, even before physical contact, suggesting that specific recognition signals are involved in hyphal fusion (4).
Extraradical mycorrhizal networks maintain the capacity of self recognition, evidenced by the high frequency of anastomosis between hyphae originating from the same root system (2). Recent results show that the root systems of plants belonging to different species, genera and families become interconnected by means of anastomosis formation between mycorrhizal mycelia, which can potentially create indefinitely large continuous fungal network linking together plants in a community (5). The emerging picture of mycorrhizal networks is one of previously unimagined dynamism and provides further support to the view that AM fungal symbionts play a fundamental role in the distribution of resources by the establishment of functional guilds in plant communities.

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Poster Presentations

Charzewska A, Zawadzki T, Krupa M, Stolarz M: The free-running rhythm of circumnutation activity in sunflower (*Helianthus annuus* L.)

Tamás Visnovitz, Ildikó Világi, Zoltán Kristóf: Mechanoreceptor cells on the tercier pulvinus of *Mimosa pudica* L.

Elzbieta Krol, Halina Dziubinska, Kazimierz Trębacz, Maria Stolarz, Maria Filek: Different Responses to cold recorded in spring - and winter - varieties of rape (*Brassica napus* L. var. *oleifera*)

Maria Stolarz, Halina Dziubińska, Elżbieta Król, Maciej Krupa, Agnieszka Charzewska, Tadeusz Zawadzki, Kazimierz Trębacz: Light-induced changes in stem growth and circumnutations in *Helianthus annuus* L.

Viktor E. Tsyganov, Alexander I. Zhernakov, Anna V. Khodorenko, Zlata B. Pavlova, Pavel Y. Kisutin, Andrei A. Belimov, Vera I. Safronova, Tatyana S. Naumkina, Alexey Y. Borisov, Peter Lindblad, Karl-Josef Dietz, Igor A. Tikhonovich: Genetic approach to study pea (*Pisum sativum* L.) adaptations to mechanical and cadmium stresses during development its symbioses with *Rhizobium* and arbuscular mycorrhizal fungi

Kiril N. Demchenko, N.P. Demchenko, Katharina Pawlowski: Myosin VIII, actin and tubulin in the development of symbiotic contacts by actinorhizal and rhizobial root nodules

Miroslav Ovecka, Irene Lichtscheidl, František Baluška: Plant synapses in plant root apex are enriched with lipid rafts

Boris Voigt, Molly Craxton, Bazbek Davletov, Patrick J. Hussey, Diedrik Menzel, František Baluška: Expression and localization of *Arabidopsis* synaptotagmins

Tomohiro Uemura, Takashi Ueda, Akihiko Nakano, Masa H. Sato: Plant SNARE molecules involved in endocytosis and exocytosis

Pilar Gil Montenegro: Root–to–leaf electrical signaling in Avocado (Persea americana Mill.)

Markus Schlicht, Boris Voigt, Alina Schick, Miroslav Strnad, Klaus Palme, Dieter Volkmann, Diedrik Menzel, František Baluška: End-Poles of Root Cells as Auxin Transporting Plant Synapses

Susanna M. Messinger, Keith Mott, David Peak: Noise Enhanced Distributed Emergent Computation in Plants

Maja Kovač, Darja Milovanovič Jarh, Axel Műller, Anita Purnat, Špela Baebler, Hana Krečič, Mojca Milavec, Maruša Pompe-Novak, Kristina Gruden, Maja Ravnikar: Jasmonic acid, salicylic acid and gene expression in early response of potato plants to virus infection

Jolana Albrechtova, Edgar Wagner: Hydraulic signals and plant development

Ying-Lang Wan, Halina Gabrys, František Baluška, Diedrik Menzel: Blue Light-Regulated Vesicular Recycling of Phototropin1 in the Root Transition Zone

Jevin West, Keith Mott, David Peak: Why decentralized computation in plants?

Weronika Krzeszowiec, Halina Gabrys: What do myosins do in blue light?

Vladimíra Hlaváčková, Pavel Krchňák, Petr Ilík, Jan Nauš, Ondřej Novák, Radek Kaňa, Martina Špundová: The first moving signal inducing systemic changes in photosynthesis after local burning of tobacco plants

Wasser K, Müller J, Preuss ML, Holstein S, Böhm N, Nielsen E, Hirt H, Menzel D, Šamaj J: Signalling endosomes in plants: activated MAPKs associate with CCVs and endosomes

Jens Müller, Nils Böhm, Heribert Hirt, Diedrik Menzel, Jozef Šamaj: Dynamic behaviour of SIMKK, a plant stress-induced mitogen-activated protein kinase kinase and its downstream target SIMK

The free-running rhythm of circumnutation activity in sunflower (*Helianthus annuus* L.)

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Introduction: Circumnutation is a revolving movement of elongating cylindrical plant organs such as stems, hypocotyls, tendrils and roots. It is the consequence of helical growth and reversible volume variations occurring in the cells of the moving part of the organ (the bending zone below the apex). The bending is caused by the difference in the water content and cell volume between the convex and concave sides of the bending zone, associated with turgor and ion concentration differences between opposite sides of the stem. It is proposed that turgor changes are generated by endogenous, spontaneous oscillations. As a consequence oscillatory growth and movement are generated. We aimed to compare the rhythmicity of circumnutation in sunflower with regard to the trajectory length, period, shape and surface area of individual nutations in different photoperiod conditions.

Material and Methods: Measurements lasting about 7 weeks were executing using a picture analysis system. Variable photoperiods – a 24h photoperiod (LD 16:8 followed by LL), a 30h photoperiod (LD 20:10 followed by LL and again by LD 20:10) and a 16h photoperiod (LD 8:8 followed by LL) were applied. In order to determine whether a periodicity of circumnutation parameters existed or not, the data were processed by the Fourier spectral analysis and the wavelet analysis.

Results and conclusions: We found that all parameters revealed a beautiful daily modulation in each LD condition applied. After LD-LL transition, the parameters were gradually loosing their modulation. In the transient phase (about 7 days after LD-LL transition) the rhythms of the circumnutation parameters turned out to be close to 24h in the 24h photoperiod as well as in the 30h and 16h photoperiod. These findings suggest the possibility for circumnutation of being circadian regulated. After the LL-LD transition the rhythms regained their daily modulation after one night. These findings strongly support the view that circumnutation in sunflower, widely known as an ultradian rhythm, also possesses daily modulations of its intensity and it can tune in a wide range to the frequency of environment.

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Mechanoreceptor cells on the tercier pulvinus of *Mimosa pudica* L.

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Mimosa pudica is a famous plant, grown for its curious movement; the leaves close up and drop when touched, and re-opening within a few minutes. It has become a subject of many studies (even Charles Darwin dealt with the seizmonastic movement of *Mimosa*), "but the receptor cells have not yet been identified" (Shimen, 2001).
We described a special cell type on the adaxial part of the tercier pulvini. Stimulating these cells, the leaf that contains the pulvinus closed. A very gentle mechanical stimulation, -carried out with a micromanipulator needle - was effective in these cells, but ineffective for any other cells of the tercier pulvinus.
Light and electron microscopy were carried out to reveal the connections between cells and the motor cells. The receptor cells and the motor organ have connections through plasmodesmata. Samples were taken from different stages of developing leaves to study the differentiation of the receptor cell. The sensitivity of the receptor cells for mechanical stimulation requires a special developmental stage.
The electrophysiological studies supported our receptor cell theory. We measured electrochemical potential in these cells. After mechanical stimulation (about 0,3 sec) hyperpolarisation and then a depolarisation peak was recorded. Repolarisation developed about 1 sec later. Leaves in refracter stadium could not produce those potential changes.
We make efforts to understand the function of that cells also at molecular level.

Different responses to cold recorded in spring and winter varieties of rape (*Brassica napus* L. var. *oleifera*)

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In our study we used intracellular microelectorde technique to demonstrate that the spring and winter varieties of *Brassica napus* L. var. *oleifera* respond to cold in different ways. The transmembrane potentials were measured in the mesophyll cells of leaves in both varieties. Cold stimulation was obtained by local application of 0.5 ml of cold solution (1°C) to the leaf surface and a local drop of temperature was approx. to 8° – 10°C. The transmembrane potentials and the durations of cold-induced potential changes depended on a kind of a leaf. The mesophyll cells of a spring variety displayed low transmembrane difference (-100 mV) and its cold-induced potential changes lasted on average 8 s (measured in a half of amplitude). The cells from a winter variety, which had been vernalized, had almost two fold larger transmembrane difference (-185 mV) and their cold-induced potential changes lasted significantly longer – approx. 18 s . The results are consistent with the general assumption that plants have a kind of memory remembering previous environmental conditions.

Light-induced changes in stem growth and circumnutations in *Helianthus annuus* L.

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Many studies including applications of external stimuli like different gravitational forces, touch, high and low temperature and chemicals have been carried out to understand the mechanisms underling circumnutations. Circumnutations are defined as helical movements of a growing plant organ. We investigated the effects of light/dark 16/8h period on stem growth and circumnutations in three-week-old sunflowers. The growth kinetics was being monitored by an angular position-sensing transducer for a three days and for circumnutation characteristic the time-lapse images from top view camera were registered and analyzed. Under 24 h light/dark cycle both the stem growth and circumnutation parameters displayed diurnal rhythm . Besides long-lasting fluctuation in a range of many hours, the light- induced short sunflower stem response in a range of a few minutes were observed. After switching on the light a rapid decrease of the growth rate and even a stem contraction was observed. The kinetics of stem reaction had a characteristic wave-like shape and all in all persisted about 60 minutes. These drastic changes in stem length were accompanied by phase dependent circumnutation disturbances. These results suggest that after non-injured light stimuli hydro-electrochemical signals which might interfere with circumnutation mechanism appeared in sunflower stem.

Genetic approach to study pea (*Pisum sativum* L.) adaptations to mechanical and cadmium stresses during development its symbioses with *Rhizobium* and arbuscular mycorrhizal fungi

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Legume endosymbioses with nodule bacteria and arbuscular mycorrhizal fungi is sensitive to stresses and, at the same time, is able to decrease the stress effects on plants. Using of genetic approach allows to identify plant genes involved in the control over adaptations of legume symbioses to stresses. In this study, two mutants with changed reactions to stresses have been analysed by their ability to interact with *Rhizobium* and mycorhhizal fungi: SGEcrt (*crt*) with hypersensitivity of roots to touch stimuli [1] and SGECd t (*cdt*) with increased tolerance to cadmium (Cd) [2]. It was demonstrated that mutant SGEcrt (*crt*) has decreased nodulation ability which can be restored by addition of ethylene action inhibitors. In contrast, the mutant SGEcrt (*crt*) is characterized with accelerated mycorrhizal colonization. The mutant SGECd t (*cdt*) was characterized with increased tolerance of nodulation to toxic Cd concentration in comparison with wild type.

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Myosin VIII, actin and tubulin in the development of symbiotic contacts by actinorhizal and rhizobial root nodules

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Two groups of plants can enter root nodule symbioses with nitrogen-fixing soil bacteria. Gram-negative, unicellular rhizobia induce nodules on legume roots, while Gram-positive mycelial actinomycetes of the genus *Frankia* induce nodules on the roots of plants from eight angiosperm families, mostly woody shrubs, collectively called actinorhizal plants. Based on molecular phylogenetic studies, both types of root nodule symbioses are supposed to go back to a common ancestor. We compared infection thread growth in actinorhizal and legume nodules by immunolocalization of components of the cytoskeleton. We used myosin VIII-specific antibodies and antibodies against actin and tubulin to analyse the intracellular accommodation of microsymbionts in legume (*Medicago truncatula*, pea) and actinorhizal symbioses (*Casuarina glauca*, *Datisca glomerata*) on light microscopy and TEM level. Myosin VIII accumulated around infection threads in actinorhizal systems, but not around legume infection threads.

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Plant synapses in plant root apex are enriched with lipid rafts

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Structural sterols are integral components of the plasma membrane. They are enriched in spatially differentiated microdomains, "lipid rafts". We visualized structural sterols in root apex of *Arabidopsis thaliana* by fluorescence microscopy. In our study, we compared the distribution of structural sterols in the plasma membrane in two types of root cells with distinct mode of elongation: diffusely expanding meristematic cells arranged in cell files and tip-growing tubular root hairs. Our results show that structural sterols were abundant at the plasma membrane of root hair apices and they were internalized into endosomal compartments. In root meristematic cells they were present in the plasma membrane and endosomal compartments, but in certain developmental zones of the root apex they were enriched in non-growing cross cell poles representing plant synapses. Both actin-enriched root hair apices and non-growing end poles of the cell surface enriched in actin and myosin VIII are cell periphery domains known to be active sites of intense endocytosis and vesicular trafficking. Enrichment of structural sterols in these domains indicates that they can be involved in the modulation of the physiological properties of the plasma membrane responsible for the maintenance of actin-dependent rapid membrane trafficking and recycling.

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Expression and localization of Arabidopsis synaptotagmins

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Synaptotagmins are known Ca^{2+} dependent triggers of exocytosis and endocytosis in animals with nervous system. 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. Synaptotagmins have a common structure, a N-terminal transmembrane sequence is followed by a linker and two distinct C2 domains, C2A and C2B. These two domains are known Ca^{2+} binding domains, but there is nothing more known about the function of the proteins in plants. To get more information about the 6 members of the Arabidopsis synaptotagmin gene family, we are investigating expression patterns by promotor-GUS fusions and cellular localizations by GFP-fusions as well as antibody labelings. Preliminary data suggest that the members of the synaptotagmin gene family are differently expressed, but show similar cellular localization.

Plant SNARE molecules involved in endocytosis and exocytosis

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In all eucaryotic cells, specific vesicle fusion during vesicular transport is mediated by membrane-associated proteins called SNAREs (soluble N-ethyl-maleimide sensitive factor attachment protein receptors). Sequence analysis identified a total of 54 SNARE genes (18 Qa-SNAREs/Syntaxins, 11 Qb-SNAREs, 8 Qc-SNAREs, 14 R-SNAREs/VAMPs and 3 SNAP-25) in the *Arabidopsis* genome. Almost all of them were ubiquitously expressed through all tissues examined. A series of transient expression assays using green fluorescent protein (GFP) fused proteins revealed that most of SNARE proteins were located on specific intracellular compartments. As the results , we identified 18 SNARE molecules on the plasma membrane; 9 Qa-SNAREs (SYP111, SYP112, SYP121, SYP122, SYP123, SYP124, SYP125, SYP131 and SYP132), 1 Qb-SNAREs (AtVTI12), 3 Qc-SNAREs (N`PSN11, NPSN12 and NPSN13) and 5 R-SNAREs (AtVAMP721, AtVAMP722, AtVAMP724, AtVAMP725 and AtVAMP726). These SNARE proteins also localized endosomal compartment, suggesting that these SNAREs shuttle between the plasma and endosome. We propose possible combinations of SNARE proteins on plasma membrane, and discuss the SNARE proteins involved in endocytosis or exocytosis

Root–to–leaf electrical signaling in Avocado (*Persea americana* Mill.)

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Our work using phytomonitoring techniques for Avocado (*Persea americana* Mill) tree irrigation indicate that plant response to changed soil water availability is a very fast process. Root–to–leaf ABA transport or hydraulic processes cannot fully explain the almost immediate stomata physiological response (less than 15 minutes) to either water application and/or sudden ETp increments or reductions.

Some studies in Avocado physiology report that root–to–leaf ABA transport is a transpirative mass-flow process. In other tree species a decline in stomatal conductance (gs) was detected when volumetric soil water content declined below 0.12, but the decline in gs, could not have been mediated by increasing [ABA(xyl)] because stomatal closure appeared to precede any increase in [ABA(xyl)]. Sap flow velocity in the range of 30 to 35 cm/h has been reported in avocado, indicating that hydraulic forces cannot either fully explain the fast stomatal response to soil water availability.

This work is aimed to study the eventual existence of an electrical signaling process regulating stomata behavior. Two year old avocado trees were subjected to several drying and re-watering cycles, as well as to modifications on some ETp parameters, as incident radiation and air flux conditions. Extracellular electrical potential was continuously recorded between trunk and leaf petiole; leaf stomata conductance was also registered.

Drying the root system with a continuous air flow at room temperature generated the arrival of an electrical signal to the leaf petiole; the same signal was detected when the root system was re-watered. Our results indicate that a sudden change in soil water availability creates a significant electrical signal, which reaches leaf petiole in 10 to 50 min. Other measurement were made on girdled plants, in several cycles, re-watering plants after 4 drying days, indicating that the electrical signal detected is possibly conducted by the xylem, and not by the phloem tissue.

The eventual existence of root–to–leaf electric information exchange mechanisms opens interesting possibilities to artificially modify plant response to environmental or agronomic management strategies, aimed to increment water use efficiency.

End-Poles of Root Cells as Auxin Transporting Plant Synapses

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Putative auxin influx and efflux carriers (PIN/AUX proteins) show polar localizations in root cells which is in a good agreement with predictions of the chemiosmotic theory as related of the polar auxin transport (PAT). In this classical model, the role of these carriers have been interpreted to act as plasma membrane transporters. However several published data indicate that the exocytosis inhibitor Brefeldin A stops the PAT within few minutes, while the putative transport proteins of the PIN and AUX families are still polarly localised at the plasma membrane of the end-poles. Furthermore, it became clear that PAT inhibitors like TIBA and NPA are, in fact, general inhibitors of the endocytosis in plant cells (1). These data support an alternative model proposing that auxin is secreted in a neurotransmitter-like mode via BFA-sensitive and F-actin dependent vesicular exocytosis (2). This new view of the PAT is in a good accordance with the preferential localization of polarized secretion machinery at the end-poles of maize root cells transporting auxin (3). These end-poles have several other properties allowing to define them as plant synapses (4). Besides abundant F-actin, myosin VIII, dynamins, Rho-GTPases (5); the auxin secreting end-poles are equipped also with plant homologues of mammalian neuronal molecules synaptotagmins. Taken together, all these data enable us to propose that the end-poles of root cells, especially those of the root transition zone which are particularly active in the PAT, resemble neuronal synapses.

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Noise Enhanced Distributed Emergent Computation in Plants

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The idea of biological organisms as natural computers has been tantalizing researchers for decades, but substantial, quantitative proof of computation is elusive and difficult to obtain because of the inherent complexity of biological systems. A recent study has, however, presented evidence supporting the existence of distributed emergent computation in plants (*Proceedings of the National Academy of Science* 101(4), 918-922). Plants daily solve a constrained optimization problem, maximizing CO_2 uptake while minimizing water loss, through the dynamic adjustment of stomatal conductance. Stomata form a locally connected network that collectively processes environmental stimuli to perform the optimization task. Like all biological systems, plants are subject to noise, i.e., random variability in both space and time. Biological noise has traditionally been viewed as a nuisance that organisms cope with by developing sometimes elaborate noise-attenuating mechanisms. Although this view is substantiated by biological processes like DNA replication and some cellular signaling cascades, recent research has revealed that some biological processes are actually improved by, or even driven by, the presence of noise. Motivated by the possibility that noise enhances some biological processes, this study examined the possible effects of noise on distributed emergent computation in plants by introducing similar noise into a standard artificial cellular computing system that also performs distributed emergent computation. We found that small amounts of spatial and temporal noise added to the system independently improve computational efficiency. Added together, spatial and temporal noise improves the computational ability of the system more than either independently. Thus, it may be that biology not only coexists with noise, but actually uses it to enhance adaptive, information processing ability.

Jasmonic acid, salicylic acid and gene expression in early response of potato plants to virus infection

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Jasmonic acid (JA) and salicylic acid (SA) are central players in mediating responses to pathogens and wounding in plants. These molecules induce expression of several defence-related genes. The involvement of JA and SA in early response of plants to virus infection was investigated in potato plants (*Solanum tuberosum* L.) infected with potato virus Y NTN. Various potato cultivars are differently sensitive to the virus. In susceptible variety the symptoms of infection are ring shaped necrosis of the tubers, chlorosis and curling of leaves, accelerated senescence, and sever reduction in crop yield. In our study, two potato cultivars were chosen: sensitive cv. Désirée and resistant cv. Santé.
JA and SA were measured 1 and 3 hours after inoculation using a multiplex GC-MS/MS and HPLC, respectively. The study showed the involvement of JA in very early response of potato to PVY NTN infection, as significant increase of JA, was detected in inoculated leaves 1 hour post-inoculation. The increase was most pronounced in resistant variety indicating the correlation of JA metabolism with the expression of the resistance. The role of SA in very early defence response is less clear.
Gene expression profile of virus-potato plant interaction was studied by cDNA microarrays. Most prominent changes were observed in genes associated with defence response.

Hydraulic signals and plant development

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Systemic responses by definition involve a whole plant body, though they are usually evoked by local signal(s). The signals spread through the plant body via chemical, electrical, hydraulic and molecular pathways. Hydraulic signals are especially important in drought stress and in wound reactions. However, turgor changes also drive rhythmic growth, leaf movements as well as circumnutations and therefore seem to co-ordinate plant development. Hydraulic signals are probably perceived as pressure changes both by mechano-transductive ion channels and as local deformations in cytoskeleton and nucleoskeleton, with the possibility of influencing gene expression.
Physical strain at the surface of the apical meristem was previously suggested to play a key role in the patterning of organogenesis. We have studied the influence of local water transport and turgor changes on organogenesis at the apical meristem of Chenopodium plants. Specific changes in shape and size of the apical meristem were found to precede reorganisation of organogenesis under photoperiodic flower induction. Optical properties of cell walls at the surface of the apical meristem were changing during flower induction. Expression of the aquaporin *CrAQP* increased at the apex during an early phase of flower induction and the application of an inhibitor of aquaporin activity partially inhibited flowering. Changes in ion balance and carbohydrate levels in the cells seem also to be involved in the process. Altogether, the results support a hypothesis about the involvement of hydraulic signals in organogenesis at the apical meristem. It is anticipated that hydraulic changes at the apex leading to flower initiation are mediated by a specific hydro-electrochemical communication between (roots), leaves and the shoot apex.

Blue light-regulated vesicular recycling of phototropin1 in the root transition zone

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Phototropin is essential for phototropism of plants. Phototropin 1 is a 120kD ser/thr kinase, which can receive photons through the LOV domain at the N-terminus. The changes in conformation of the LOV domain activate the C-terminal kinase domain, leading to the autophosphorylation of PHOT1. But how the signal is transduced into the cell and leads to the bending response is still unknown. Our studies use Arabidopsis seedlings transformed by a GFP-PHOT1 construct expressed under the control of endogenous promoter. We have found that PHOT1 recycles between the plasma membrane at cross-poles and endosomes in cells of the root apex transition zone. The recycling pathway is active at a basal level even in darkness and under low light-condition, but can be stimulated by blue light. Stimulation is dependent on the light intensity, and therefore could be the mechanism for sensing the light gradients when the plants are illuminated unilaterally. PHOT1 recycling requires an intact actin cytoskeleton and is sensitive to Brefeldin A (BFA), an exocytosis blocker. BFA induces formation of PHOT1-enriched endocytic compartments. The amount of PHOT1 within these BFA-induced compartments is enhanced by blue light in correlation of the light intensity. This recycling pathway can be affected by ROS, suggesting that PHOT1 could participate in stress response pathways.

Why decentralized computation in plants?

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The *what* part of information processing in leaves has been addressed by the plant physiology community. Plants on a daily basis must solve a constrained optimization problem: how should stomatal aperture should vary for the plant to take up the most amount of CO_2 for a given amount of H_2O loss as environmental conditions change throughout the day? The *how part* of information processing remains an open question. Traditionally, stomata were assumed to operate independently, with each stoma processing signals from the environment and the rest of plant in isolation. However, recent studies have shown that the plant may be solving this constrained optimization task using emergent distributed computation. The purpose of this study is to address the *why* part of the story. Why do plants resort to decentralized computation when there clearly are disadvantages to this type of information processing (e.g., redundancy, pathological deadlocks and increased processing time and resource costs)? To address this question, a theoretical analysis has been done addressing the topics of robustness, metabolic investment costs and evolutionary happenstance.

What do myosins do in blue light?

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Myosins are members of an actin-activated family of ATPases. The Arabidopsis genome contains 17 myosin-like genes that belong to classes VIII and XI. Myosins transport various cargoes along microfilaments and are essential in many physiological processes. They are also components of motor systems responsible for chloroplast photo-orientation in the cell. It has been established that actin cytoskeleton responds to light conditions (e.g. in *Vallisneria*, *Mougeotia*, *Ceratodon*). Photoreceptors mediating chloroplast movements in *Arabidopsis thaliana* are two phototropins, PHOT1 and PHOT2, members of the blue light photoreceptor family. They are involved in phototropism, chloroplast movements and stomatal opening. So far, no evidence has been presented that myosins might be controlled by light absorbed by a photoreceptor. Myosins of both classes are present on chloroplast surface [1,2] and are believed to participate in chloroplast translocations in *Arabidopsis*. The aim of this work was to obtain evidence that the surface-associated myosins are involved in the mechanism of chloroplast movements, by establishing whether they undergo changes under the influence of light. Blue light intensities were applied that induce saturated avoidance and accumulation responses of chloroplasts. The experiments were carried out on mature lives of *Arabidopsis thaliana* wild type, first irradiated and then fixed with paraformaldehyde solution. Myosins were visualized with animal-antibodies: anti-myosin (smooth & skeletal) and secondary FITC-labeled antibodies. The fluorescence was observed in a confocal microscope. Localization of myosins was different in the cells irradiated with strong and weak blue light. Myosins were found on the chloroplast surface in almost all cells irradiated with weak blue light. Strong blue light displaced them from that surface. The effect was blue light-specific and did not occur in strong red light. We suggest that the light-induced reorganization of myosins is essential in the mechanism of chloroplast movements and that it is the final step in the phototropin signal transduction.

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The first moving signal inducing systemic changes in photosynthesis after local burning of tobacco plants

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Plant species developed some systemic self-protecting mechanisms inducible by local stress. Some questions about the nature of moving signals and their connection to systemic changes in the energetic processes including photosynthesis are still unresolved.
A short-term systemic response of tobacco plants (*Nicotiana tabacum* cv. Samsun) to local burning was investigated. A basipetal spreading of electrical signal along the plant, evoked by the local burning of the upper leaf, was detected by changes in the extracellular electrical potential differences (EEPD) between Ag/AgCl electrodes placed on three untreated leaves and a reference electrode placed in the root nutrient solution. Simultaneously, the photosynthetic parameters (gas exchange and chlorophyll fluorescence) were measured on the selected leaves. Concentrations of potential chemical signal compounds - jasmonic (JA) and abscisic acid (ABA) - in measured leaves were measured by the LC-MS method.
Changes in EEPD were observed in several seconds after burning depending on the distance from the site of burning. The extent of EEPD changes was mostly within 10 - 70 mV. Main changes lasted several minutes and a steady state value was achieved in about 1 hour. In most cases the amplitude of changes decreased with increasing distance of the measured leaf from the burned one. The propagation velocity was about 1 cm s^{-1}. The EEPD changes were followed in several minutes by a pronounced decrease in the rate of CO_2 assimilation, transpiration and stomatal conductance. Nearly no change was detectable in the sub-stomatal concentration of CO_2. Similarly, negligible short-term changes in chlorophyll fluorescence induction and photochemical quenching were detectable. In the measured leaves, also changes in JA and ABA concentrations were observed.
We suggest that the short-term changes in photosynthesis evoked by local burning are stimulated (directly or indirectly) by the moving electrical signal. Interestingly, these photosynthesis changes do not include changes of electron transport in PSII.

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Signalling endosomes in plants: activated MAPKs associate with clathrin-coated vesicles and endosomes

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Mitogen-activated protein kinase (MAPK) pathways including ERK and p38 associate with signalling endosomes upon activation of growth factor receptors in mammalian cells. It is believed that this scaffolding and compartmentalization of MAPK pathways is essential for achieving signal specificity and more efficient transduction. In plants, almost nothing is known about targeting of MAPKs to the endomembranes and to the cytoskeleton. We provide biochemical and cell biological evidence that two *Arabidopsis* MAPKs, MPK4 and MPK6 are weakly associated with endosomes (later one also with CCVs) in control cells. Additionally, three MAPKs, MPK3, 4 and 6 are associated in their activated state with endosomes following diverse abiotic stresses. Active MPK4 and MPK6, but not MPK3, associate also with isolated clathrin-coated vesicles (CCVs) upon biotic stress induced by elicitor (flagellin) treatment. These data indicate a constitutive signalling from the endocytic compartment in plants.

Dynamic behaviour of SIMKK, a plant stress-induced mitogen-activated protein kinase kinase and its downstream target SIMK

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SIMKK, a stress-induced mitogen-activated protein kinase kinase (MAPKK) is a specific activator of SIMK, but nothing is known about the dynamic behaviour of these two signalling enzymes in plants. Therefore, we aimed to study dynamic localization changes of SIMKK and SIMK upon diverse abiotic stresses. Both, transiently or stably expressed CFP/YFP-tagged SIMKK and SIMK have shown predominantly nuclear localization under unstressed conditions. By contrast, both kinases partially relocated to the cytoplasm and co-localized on spot-like vesicular structures upon salt and oxidative stress. Other abiotic stresses, such as heat and wounding, but not the exposure to elicitors (flagellin, chitin, β-glucan and ergosterol) also caused relocation of SIMKK to cytoplasmic vesicular compartments. Detailed time-lapse observations revealed that SIMKK was actually recruited to motile endomembraneous organelles (most likely representing endosomes) upon stress.

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