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MolekularBiologie
der Pflanzen

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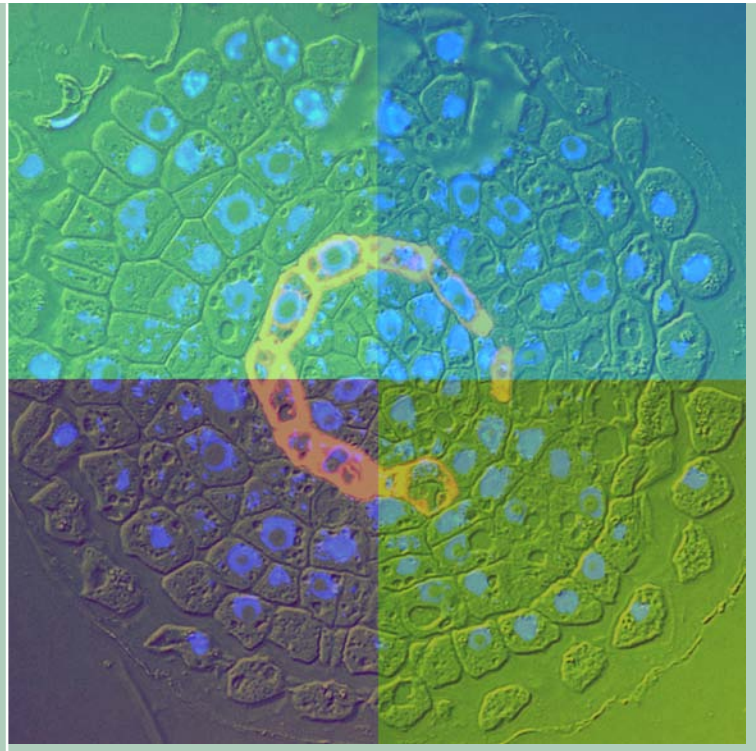

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Research Activities 2006

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Preface

Claudia Oecking

The Center for Plant Molecular Biology (ZMBP) at the University of Tübingen was inaugurated by the Minister for Science and Research of Baden-Württemberg and the Rector of the University in May 1999. After five years of joint funding by the State Government of Baden-Württemberg and the University of Tübingen, the ZMBP emerged as a leading center for plant molecular biology research and was very positively evaluated by a panel of experts appointed by the State Ministry of Science. As a result of this evaluation, the University of Tübingen took over the funding in 2004 by allocating staff positions to the ZMBP.

A key feature of the ZMBP is its departmental structure. Independent research groups supported by central service facilities replaced the former Chairs ("Lehrstühle") of General Genetics, Developmental Genetics and Plant Physiology from the Faculty of Biology. In 2003, the originally projected inter-faculty structure of the ZMBP was implemented by the integration of a new research unit for Plant Biochemistry belonging to the Faculty of Chemistry and Pharmacology. Thorsten Nürnberger was appointed head (C4 Professor) of Plant Biochemistry and Georg Felix (University of Basel) will join the ZMBP in 2006 as a senior group leader (Associate Professor of Plant Biochemistry). The focus of this research unit is in the field of plant innate immunitiy which complements the scientific spectrum covered by the other units at the ZMBP.

In 2003, one of the founding members, Wolf Frommer, moved to the Carnegie Institution of Washington (Stanford, USA). The ZMBP managed to fill the vacant position quickly and could attract Klaus Harter who will strengthen the focus on molecular plant physiology and cell biology.

Two further founding members, Vera Hemleben and Hanns-Ulrich Seitz, retired in 2004. Unfortunately, budget constraints made it impossible to refill their positions.

Research at the ZMBP is regularly evaluated by an International Scientific Advisory Board (ISAB) that also gives advice on the appointment of group leaders.

By several criteria, the ZMBP has established itself as a high-ranking research institute. Its recognition within the international scientific community is mainly due to the professional activities of its members. As an institution, the ZMBP is recognised by the State Research Advisory Board (Landesforschungsbeirat) as a model for research-friendly structures at universities. ZMBP groups also contribute substantially to the DFG-funded Local Research Program 446 on "Mechanisms of Cell Behaviour". The ZMBP has taken the initiative to found a platform called "Plant Molecular Biology - South West" with links to plant research groups at two nearby universities, Ulm and Hohenheim. The idea is to coordinate activities in research and teaching within Baden-Württemberg. Success can also be measured by the career opportunities of the group leaders with temporary contracts. Eight former ZMBP members were appointed professors at universities in Germany or abroad. As a consequence, the ZMBP has seen a substantial turnover of groups.

Recruitment of new group leaders is a multi-layered process: advertising vacant positions in international journals, minisymposia with shortlisted applicants to identify excellent young scientists in research areas of interest to the ZMBP, voting by the group leaders and consultation with the ISAB prior to appointment. In this way, the ZMBP has been strengthened by two new research group leaders in 2004/2005, Volker Lipka from the MPI Köln (plant pathogen interaction) and Rita Gross-Hardt from the University of Zürich (female gametophyte development). Furthermore, Jiri Friml (Developmental Genetics) was awarded a Volkswagen Foundation Junior Group Leader position in October 2002 and Wolfgang Koch (Plant Physiology) has recently been given project leader status in acknowledgement of his scientific contributions.

At present, the research groups of the ZMBP are spread over several buildings on the Sciences Campus, including the SAMOA building opened in 2001. A major requirement for the future is a new building for the entire ZMBP which would be highly beneficial for cooperation, synergism and the efficient use of resources. Unfortunately, the construction of such a new building has been postponed due to financial constraints.

In spite of all the decisive changes the ZMBP has faced in the past years, research activities have been maintained at high level as documented in the progress reports of the individual groups. This success would have been impossible without the excellent contributions of dedicated graduate students and postdocs. In 2005, there were some 50 Ph.D. students enrolled at the ZMBP. Several of the Ph.D. theses submitted in the past years were awarded prizes for their outstanding scientific quality.

Tübingen, November 2005

Thorsten Nürnberger, Klaus Harter, Claudia Oecking, Fritz Schöffl, Gerd Jürgens

Current Research Group Leaders

Georg Felix	Receptor kinases and pathogen recognition
Jiri Friml	Auxin transport and action
Rita Gross-Hardt	Cell specification in the female gametophyte of Arabidopsis
Klaus Harter	Plant signal perception, transduction and integration
Frank Hochholdinger	Genetic dissection of root formation in maize
Gerd Jürgens	Apical-basal patterning in Arabidopsis embryogenesis / Cytokinesis
Volker Lipka	Molecular dissection of non-host resistance in Arabidopsis
Uwe Ludewig	Molecular biology and electrophysiology of nitrogen transport
Thorsten Nürnberger	Plant Innate Immunity
Claudia Oecking	Function of plant 14-3-3 proteins
Fritz Schöffl	Regulation and function of heat stress response
Karin Schumacher	pH-homeostasis in the endomembrane system
Claus Schwechheimer	Regulated plant growth by regulated proteolysis
Ulrike Zentgraf	Leaf senescence of annual plants

Project Leaders

Wolfgang Koch	Uptake and long distance transport of organic nitrogen

Former Research Group Leaders

Wolf B. Frommer (Stanford, USA)	Molecular physiology of metabolite transport and sensing
Vera Hemleben (retired)	Genome organization and molecular evolution in higher plants
Hanns-Ulrich Seitz (retired)	Plant-microbe interactions

Former Project Leaders

Kathrin Schrick	Role of Steroids in plant embryogenesis (Claremont, USA)
Burkhard Schulz	Signal transduction in plant architecture (West Lafayette, USA)

Developmental Genetics and Cell Biology

Overview

Developing organisms are growing populations of cells that exchange information about their relative positions and, in response, adopt specific fates. This is, in essence, the process of pattern formation that transforms the fertilised egg cell into a multicellular organism with its distinct spatial arrangement of cell types, tissues and organs.

Animals establish their body organisation during embryogenesis so that the new-born is a miniature adult. By contrast, plant embryogenesis generates a juvenile form, the seedling, that bears little resemblance to the adult plant. During post-embryonic development, plants produce new structures such as leaves and flowers from self-maintaining stem-cell systems called meristems. Meristems themselves are part of the seedling body organisation that is established during embryogenesis. Recent evidence suggests that meristems are naive cell populations that produce new structures in response to signals from the existing body organisation. Furthermore, several genes involved in pattern formation during embryogenesis also appear to be required for post-embryonic development. These observations suggest that the same patterning mechanisms act from early embryogenesis to flower formation.

Our research focuses on plant developmental mechanisms. *Arabidopsis thaliana* is our model organism whose genome sequence was completed in 2000. *Arabidopsis* also offers experimental advantages. Mutants with specific developmental defects can be isolated with limited labour, and genes identified by mutant phenotype can be cloned on the basis of their position within the genome relative to molecular markers (map-based cloning). Transgenic plants are easily generated for functional studies, and large populations of plants with T-DNA or transposon insertions can be screened for mutations in gene sequences („reverse genetics"). *Arabidopsis* is also amenable to cell-biological studies.

Plants and animals evolved independently from unicellular ancestors. It is therefore anticipated that plant development involves different mechanisms to those found in animal systems. In addition, plant cells have their own distinct organisation. For example, plant cells are encased by rigid cell walls that prevent them from leaving their neighbourhood. As a consequence, temporal and spatial control of cell division rates and planes and oriented cell expansion underlie morphogenetic processes that shape the embryo or individual organs. Another particularity of plants is the occurrence of plasmodesmata that connect neighbouring cells. These cytoplasmic channels can be regulated to facilitate the passage of large molecules including transcription factors and are thus means to exchange positional information during development. In spite of the cell wall, cell signaling also occurs by local ligand-receptor interaction at the plasma membrane. Finally, plants make use of long-distance signaling that serves to integrate developmental processes. The directional transport of the hormone auxin, for example, mediates initiation of root and leaf primordia. Cells can respond to signaling by activating gene expression or by degrading mRNAs or proteins. One surprising finding from the analysis of the *Arabidopsis* genome sequence is that targeted protein degradation appears to play a much greater role in plant development than in animals or yeast. Protein degradation may reflect the organisation of plant cells and also enable plants to respond rapidly to changing environmental conditions.

As molecular cell biology is likely to give insight into developmental mechanisms, the projects in the Developmental Genetics unit are increasingly focused on cell-biological problems in the context of development. The following presentations give more detailed information about the research activities of the individual research groups

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Anne Vieten

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Female gametophyte development

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Christina Kägi
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Cordula Moll

Technical staff
Steffi Nagel

Auxin transport and action

Jiří Friml

Plants have evolved a highly flexible development which has adapted their phenotype to the demands of the environment. Post-embryonic development involving the activity of stable stem cell populations (meristems), *de novo* organ formation and changes in growth direction, provide plants with exceptional flexibility in terms of growth and survival. Recent data has disclosed that the differential distribution of the plant signalling molecule auxin (auxin gradients) (Fig. 1) underlies many of these developmental events (Friml et al., 2002a; Friml et al., 2002b; Benkova et al., 2003).

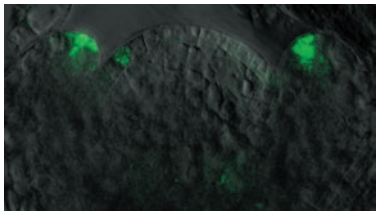

Fig. 1: Local auxin accumulation in *Arabidopsis* shoot apical meristem as monitored by auxin responsive reporter *DR5rev::GFP* (depicted in green). Tips of primordia and presumptive position of new flower initiation are labeled.

Auxin has long been known as a major hormonal regulator of plant development. Unique among currently known plant signalling molecules, auxin is transported in a strictly regulated, polar fashion from cell to cell through plant tissues. According to long-standing theories, auxin transport at cellular level occurs through the action of postulated influx and efflux carriers. The key feature of polar auxin transport – its controlled directionality - was postulated to result from the asymmetric, subcellular localisation of the efflux carrier proteins within auxin-transport competent cells.

Molecular genetic studies in the model plant *Arabidopsis thaliana* have identified putative auxin efflux components encoded by the family of *PIN* gene sequences. Although the function of PIN proteins as efflux carriers has not been biochemically demonstrated, there are many lines of evidence strongly supporting their important role in cellular auxin efflux and polar auxin transport (Friml 2003). The *Arabidopsis PIN* gene family consists of eight members, and homologous genes have been found in other plant species, including maize, rice, soybean and tobacco. Recent analyses have revealed the roles of the different PIN proteins in the establishment of local auxin gradients mediating multiple developmental processes; e.g. PIN1 – apical organogenesis and phyllotaxis (Reinhardt et al., 2003); PIN2, PIN3 – gravitropic and phototropic growth (Müller et al., 1998; Friml et al., 2002b); PIN4 – root meristem patterning (Friml et al., 2002a); and PIN7 – embryonic axis formation (Friml et al., 2003). Remarkably, exactly as predicted, PIN proteins display an asymmetric polar localisation within cells, which correlates with the known directions of auxin flow (Fig. 2). It seems that PIN proteins are key components in a complex network, which mediates auxin fluxes for the establishment of local gradients in multiple developmental processes.

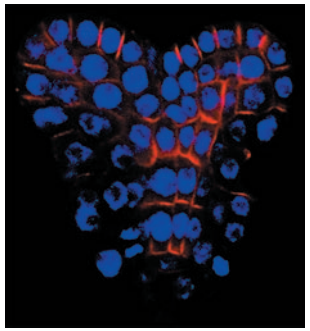

Fig. 2: Polar PIN1 localization (in red) in heart stage *Arabidopsis* embryo. PIN1 shows localization predominantly toward the base of the embryo. DAPI-stained nuclei are stained in blue.

The directional throughput of the auxin distribution network can be modulated by changes in the subcellular polarity of PIN proteins. Cell biological approaches revealed that polar targeting of PIN proteins is related to their permanent subcellular movement between endosomes and the plasma membrane (Geldner et al., 2001) (Fig. 3).

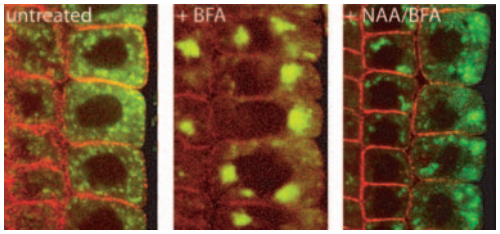

Fig. 3: Constitutive recycling of proteins between the plasma membrane and endosomes. Plasma membrane localized proteins such as PINs or H^+-ATPase (depicted in red) reversibly internalize into aggregated endosomes (labeled by anti-ARF1 antibody in green) in response to Brefeldin A (BFA). Auxins such as NAA inhibit the internalization of plasma membrane proteins but not endosome aggregation.

This constitutive cycling of auxin transport components provides an entry point for internal and external signals (such as gravity) (Fig. 4), which in this manner can rapidly modulate the polarity of PIN proteins, thus redirecting auxin flow and triggering a particular developmental response.

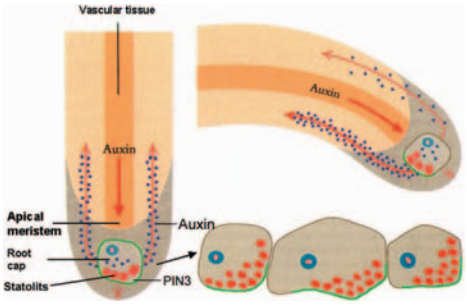

Fig. 4: Model of root gravitropism. Auxin is provided to the root tip through the stele, is laterally distributed symmetrically from the columella and is transported upward through the lateral root cap and epidermis to the elongation zone of the root. After reorientation of the root, statoliths in the columella sediment to the lower side of the cells (inset), PIN3 is relocated and facilitates auxin transport to the lower side of the root. From there auxin is transported to the elongation zone, where it inhibits elongation resulting in downward bending.

The recently characterised protein kinase PINOID is an important factor in mediating the decision about the apical or basal PIN polar targeting (Friml et al., 2004). As cellular levels (and thus the activity of PINOID) are dependent on auxin itself, this provides a possible regulatory connection between auxin and PIN polarity. In addition, auxin can also influence the transcription of PIN proteins and their subcellular distribution (Vieten et al., 2005; Paciorek et al., 2005). Hence, it seems that multiple feed-back regulations operate at different levels in the auxin transport network (Fig. 5) and stabilize changes in local auxin distribution.

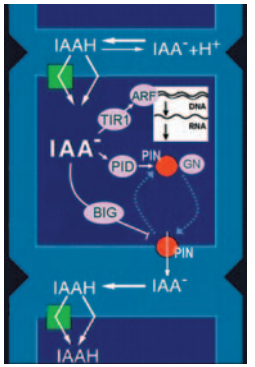

Fig. 5: Model of cellular auxin signaling and transport. Auxin binds the TIR1 receptor and, through auxin response factors (ARFs), activates the expression of auxin-inducible genes (including PID kinase and PIN auxin efflux facilitators). PID contributes to the decision about PIN polarity. Many plasma membrane proteins including PINs cycle constitutively between endosomal compartment(s) and the plasma membrane. In *Arabidopsis*, Brefeldin A inhibits the endosomal ARF GEF GNOM, thus interfering with the exocytosis step of cycling. Auxin, interferes with the endocytosis step of constitutive cycling, via an unknown pathway which includes the BIG protein.

Thus the auxin transport process is a unique model system to study the functional link between basic cellular processes, such as vesicle trafficking or cell polarity establishment, and their developmental outcome at the level of the multicellular plant organism.

Cell specification in the female gametophyte of Arabidopsis

Rita Gross-Hardt

The formation of gametes is a key step in the life cycle of any sexually reproducing organism. In contrast to animal development, plant gametes are formed by a separate haploid generation, the gametophyte. The female gametophyte consists of seven cells that develop from a single haploid spore. These seven cells differentiate into four different cell identities (Fig. 1). Two cells, the egg cell and the central cell, get fertilized and form the seed. These reproductive cells are flanked by accessory cells (synergids and antipodals) that aid in fertilization.

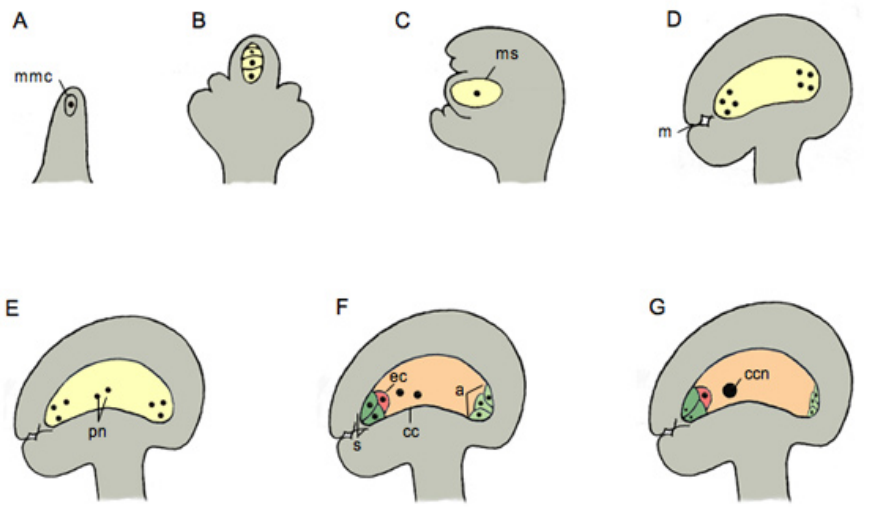

Fig. 1: Development of the female gametophyte.
The female gametophyte (colour) develops within the sporophytic ovule tissue (grey). (A) The megaspore mother cell undergoes meiosis resulting in the formation of four haploid spores (B). Only the functional megaspore survives (C) and undergoes three mitotic divisions to generate an eight nucleate syncytium (D). After the migration of the polar nuclei (E) and cellularisation a seven celled gametophyte is formed (F) that contains two synergids (blue) and one egg cell (red) at the micropylar end, three antipodal cells (green) at the opposite end and a central cell (yellow) containing the two polar nuclei inbetween. Prior to fertilisation, the two polar nuclei fuse to a large central cell nucleus and the antipodal cells degenerate (G). a, antipodal cells; cc, central cell; ccn, central cell nucleus; ec, egg cell; m, mikropyle; mmc, megaspore mother cell; ms, functional megaspore; pn, polar nuclei; s, synergids.

What regulates egg cell identity?

Our research interest is to study the mechanisms that underlie the specification and regulation of the various cell types, with a focus on the regulation of egg cell identity. Our key tool is an *Arabidopsis* transgenic line that expresses two *GUS* reporters in the female gametophyte, one in the egg cell and another in the antipodal cells at the opposite end. This line has been mutagenized and screened for mutants that still express the antipodal marker but show a deviated expression of the egg cell marker, indicating a specific defect in the regulation of egg cell identity.

The mutants isolated fall into two categories (Fig. 2): Mutants of the first category fail to express the *GUS* reporter (Fig. 2b), indicating that the mutant genes could be involved in the activation of egg cell identity. Many of these lines show an impaired fertility (Fig. 3) indicating a functional defect in the specification of egg cell identity.

The lack of a correctly specified egg cell in the mutants allows us to address the central issues of cell specification in the female gametophyte. Our research focus is to:

I. Analyze the role of the egg cell for the specification of neighboring cell types.

II. Characterize cell-cell communication in the female gametophyte.

For this we look for morphological, molecular and functional clues that indicate the identity of the other cell types in the mutant gametophytes.

Mutants of the second category express the egg cell marker ectopically in cells neighboring the egg cell (Fig. 2c). The respective genes could therefore be involved in restricting egg cell identity to just the egg cell.

The characterization of mutants defective in the regulation of egg cell identity and the isolation of the respective genes can generate a valuable resource for further analysis of cell specification processes in the female gametophyte of *Arabidopsis*.

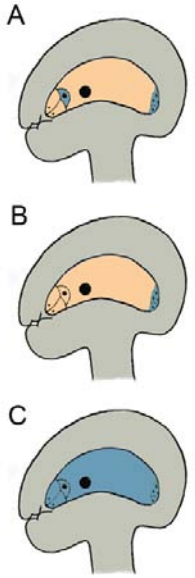

Fig. 2: Schematic representation of wildtype and mutant ovules. (A) wildtype ovule showing *GUS* expression in the egg cell only. (B) category 1 and (C) category 2 mutants which either fail to express the *GUS* gene or express the *GUS* gene ectopically.

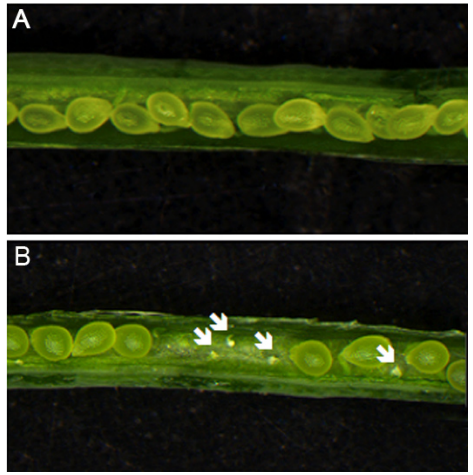

Fig. 3 Silique of wildtype and semisterile plant. (A) Wildtype silique showing full seed set. (B) Semisterile plant showing unfertilized ovules (arrows).

Apical-basal patterning in Arabidopsis embryogenesis / Cytokinesis

Gerd Jürgens

Embryogenesis generates the basic body organisation of the seedling. Along the main axis of polarity, a linear array of structures forms the apical-basal pattern: shoot meristem, cotyledons, hypocotyl, root and root meristem. These structures originate from specific cell groups of the young embryo in a position-dependent manner (Fig. 1). We address two problems: (1) How is the axis of polarity established? (2) How are specific pattern elements generated?

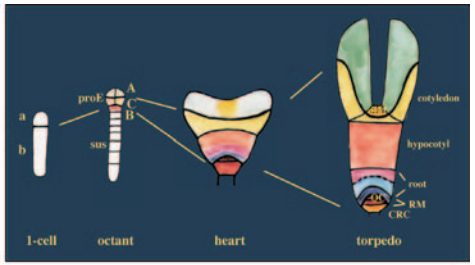

Fig. 1: Development of apical-basal pattern in embryogenesis.
The apical (a) daughter cell of the zygote generates the proembryo (proE) with the apical (A) and central (C) regions. The basal (b) daughter cell generates the suspensor (sus) and the basal (B) region. SM, shoot meristem; RM, root meristem; QC, quiescent centre; CRC, central root cap.

Embryos lacking the GNOM protein - a regulator of vesicle trafficking - fail to establish the coordinated polar localisation of the auxin-efflux regulator PIN1. The fungal toxin brefeldin A (BFA) blocks GNOM activity and disrupts rapid cycling of PIN1 between the basal plasma membrane and endosomal compartments (Geldner et al., 2001). Plants expressing BFA-resistant GNOM are no longer sensitive to BFA in regard to PIN1 cycling and polar auxin transport (Fig. 2; Geldner et al., 2003). These results suggest a role for GNOM in polar vesicle trafficking during axis formation, resulting in polar flow of auxin. Current studies aim to bridge the gap between the cellular function of GNOM and its role in development.

Auxin response in embryogenesis, as visualised by auxin-responsive GFP expression, initially occurs in the apical daughter cell of the zygote and its derivatives - proembryo - and then shifts to the adjacent

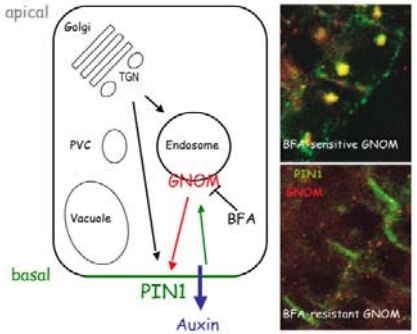

Fig. 2: GNOM-dependent recycling of the auxin-efflux regulator PIN1.
(Left) Model of polarised cell, with site of action of BFA-sensitive GNOM indicated. TGN, trans-Golgi network; PVC, prevacuolar compartment. (Right) PIN1 and GNOM co-localisation caused by BFA treatment in wild-type (top) but not in BFA-resistant GNOM (bottom).

extra-embryonic cell which becomes the hypophysis and initiates root meristem development (Fig.3; Friml et al., 2003). Auxin response and hypophysis specification require the ARF transcription factor MP and its Aux/IAA inhibitor BDL. BDL is degraded when a high level of auxin mediates its interaction with auxin receptor F-box proteins (Dharmasiri et al., 2005). MP and BDL are expressed in the proembryo, suggesting auxin-mediated signaling in root meristem initiation (Fig. 3). Current studies aim to identify the regulatory networks underlying pattern formation in early embryogenesis.

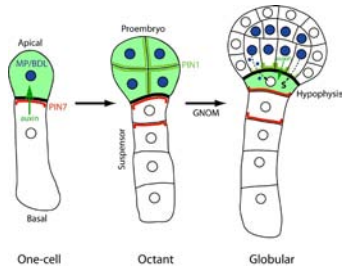

Fig. 3: Model of auxin transport and responses in early embryogenesis (Weijers and Jürgens, 2005).
Auxin accumulation is shown in green, nuclei accumulating MP and BDL in blue. Green arrows indicate auxin transport. PIN1, PIN7, auxin-efflux regulators; s, MP/BDL-dependent signal.

Cytokinesis partitions the cytoplasm of the dividing cell. The two daughter cells may adopt different fates in development, either in response to external signals or due to the asymmetric division of a polarised cell. In plant cytokinesis, the partitioning membrane (cell plate) originates from homotypic fusion of membrane vesicles targeted along a plant-specific cytoskeletal array (phragmoplast) to the centre of the division plane (Fig. 1; Jürgens, 2005). The initially compact phragmoplast is transformed into a widening hollow cylinder, delivering vesicles to the margin of the expanding cell plate which grows towards and eventually fuses with the parental plasma membrane.

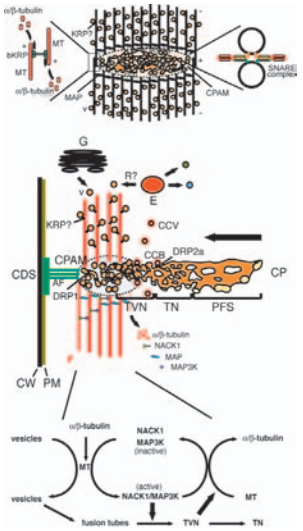

Fig. 4: Model of plant cytokinesis.
(Top) Initial stage of cell plate formation (left) by homotypic vesicle fusion in the centre of the division plane. (Middle) Lateral displacement of the phragmoplast results in centrifugal growth of the cell plate by fusion of newly-arriving vesicles. (Bottom) Model of microtubule dynamics.

Vesicle fusion requires SNARE complexes which are formed between synaptobrevin /VAMP on the vesicle (v-SNARE) and two or three t-SNAREs on the target membrane (syntaxin plus SNAP25 or two t-SNARE light chains). The cycle-regulated cytokinesis-specific syntaxin KNOLLE (KN) is required for vesicle fusion during cell-plate formation and may be activated by the interacting Sec1/Munc18 protein KEULE. A KN-interacting SNAP25 homologue, SNAP33, co-localises with KN during cell plate formation (Heese et al., 2001). Attempts are being made to identify the missing VAMP. To determine syntaxin specificity during cytokinesis, relevant KN domains are analysed by expressing non-cytokinesis syntaxins and chimeric proteins (Müller et al., 2003).

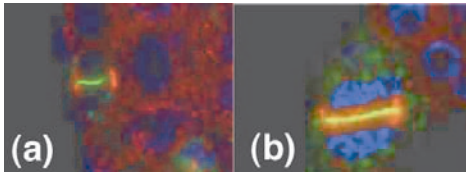

Fig. 5: Prolonged stability of phragmoplast microtubules during cytokinesis in hik cells (b) as compared to wild-type (a). Note overlap of phragmoplast microtubules (red) with KNOLLE-positive cell plate (green) in the centre (yellow, b). Blue, DAPI-stained nuclei.

The dynamic microtubule arrays of the phragmoplast assist in plant cytokinesis, as indicated by cytokinesis-defective (*ruk*, *hik*) and cell division-arrested (*pilz*, *kis*) mutants. The putative Ser/Thr protein kinase RUK associates with mitotic microtubules. The cell cycle-regulated kinesin HIK (NACK1) is required for phragmoplast reorganisation during cell-plate expansion (Fig. 5; Strompen et al., 2002). *PILZ* and *KIS* genes encode tubulin-folding cofactors required for microtubule formation, whereas actin filaments are present in mutant embryos (Fig. 6; Steinborn et al., 2002). Cell growth is not affected, while KN trafficking to the plane of cell division is blocked. These results suggest that microtubules, but not actin filaments, play essential roles in vesicle trafficking during cytokinesis.

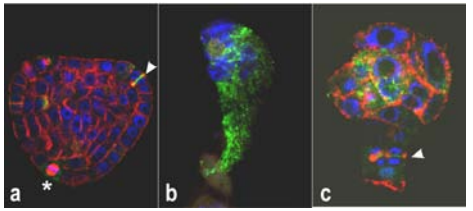

Fig. 6: KNOLLE protein (green) and microtubules (red) in wild-type (a), por (b) and kis (c) mutant embryos. Note absence of microtubules in por mitotic cell (b). Arrowheads, cytokinetic cells; asterisk, metaphase cell; blue, DAPI staining.

Regulated plant growth by regulated proteolysis

Claus Schwechheimer

Proper plant growth is the result of a regulated and coordinated sequence of cell divisions, cell elongations, and cell differentiations. Cell cycle activities and other cellular processes are controlled by phytohormones as well as by external cues and stresses. It is a commonly accepted fact that most of these responses require specific changes at the level of gene expression. It is however becoming increasingly clear that, at least in plants, many response pathways are also controlled by the degradation of regulatory proteins via the ubiquitin-proteasome system.

To contribute to the understanding of protein degradation in plant development, we investigate the COP9 signalosome as a component of the ubiquitin-proteasome system and the signal transduction cascade that leads to the degradation of the unstable repressor proteins RGA and GAI in response to the phytohormone gibberellic acid (GA).

The ubiquitin-proteasome system: The degradation of a protein in the eukaryotic 26S proteasome is preceded by its ubiquitylation through the consecutive activities of an E1 ubiquitin activating enzyme, an E2 ubiquitin conjugating enzyme and an E3 ubiquitin ligase. E3 ligases confer specificity to the process since they recognize the degradation substrates. In plants, E3 ligases form a surprisingly large superfamily with hundreds of representatives and it can be hypothesized that a similarly large number of proteins is targeted for degradation by these E3s. However, the identity of these unstable proteins, as well as the biological role of these E3 ligases, is largely unknown.

In plants as well as in other eukaryotes, cullin-RING E3s form an important E3 family within the E3 superfamily. These protein complexes are composed of a cullin protein subunit (AtCUL1, AtCUL3 or AtCUL4), the RING-finger protein RBX1 as well as one or two specific subunits required for the recognition of the degradation substrate. SCF^{TIR1} and SCF^{SLY1} are two representatives of this E3 family and they have been shown to mediate the degradation of AUX/IAA and DELLA protein growth repressors in response to the phytohormones auxin and gibberellic acid, respectively.

Fig. 1: The COP9 Signalosome is essential for plant development. Loss of function mutants (right panels) have a seedling lethal phenotype.

The COP9 Signalsome: We are interested in the COP9 Signalosome (CSN). This multiprotein complex was initially identified in plants based on mutants with a constitutive photomorphogenic (cop) phenotype, thus a light-grown phenotype in dark-grown seedlings (Fig. 1). CSN is conserved in all eukaryotes and is strikingly similar in overall organization and subunit composition to the ‚lid' of the 26S proteasome. CSN mediates its function, at least in part, through its interaction with cullin-RING E3s (Fig. 2). This interaction is essential for the degradation of E3 targets and, given the large number of culin RING E3s, it is expected that many if not all of the E3s' degradation targets are not properly degraded in CSN mutants. It thus can be assumed that this is the molecular basis for the pleiotropic and seedling lethal phenotype of CSN mutants (Fig. 1).

The biochemical function of CSN remains to be elucidated. Due to the similarity between CSN and the 26S proteasome, lid', it has been postulated that CSN mediates the degradation of E3 targets as part of an alternative proteasome. Another hypothesis suggests that CSN functions as an assembly platform for cullin-RING E3 complexes. The latter hypothesis is largely based on the finding that CSN, through the activity of its subunit CSN5, can remove the essential NEDD8-modification from the cullin subunit of the E3 complexes (Fig. 2). The role of NEDD8 conjugation and deconjugation for E3 function is not understood. It is clear however that both processes are essential for plant growth and development. We have demonstrated this by showing that loss of the deneddylating CSN5 subunit leads to developmental defects that are as severe as loss of the entire protein complex (Fig. 1). Taken together this finding suggests that deneddylation is the predominant function of the CSN protein complex.

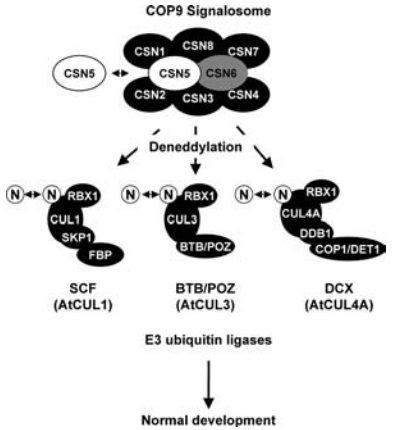

Fig. 2: The COP9 signalosome (CSN) interacts with cullin-RING E3 ligases and this interaction is required for proper protein degradation. CSN exerst its function at least in part by removing the NEDD8 (N) modification from the E3's cullin subunit.

The fact that the interactions between CSN and E3 ligases are essential for E3 function, and the fact that there are hundreds of E3 ligases that mediate the degradation of unstable regulators, suggest that the strong CSN mutant phenotype may be the cumulative effect of the accumulation of these unstable proteins. Alternatively, however, there may also be only a small number of proteins that function as key repressors of plant growth in the CSN mutants. Using genetic interaction studies, we are currently trying to identify such key regulators.

The gibberellic acid signaling pathway: The phytohormone gibberellic acid (GA) regulates important aspects of plant growth and development such as germination, cell elongation, plant height and flowering time. GA responses require the GA-dependent degradation of the so called DELLA repressor proteins, a small family of proteins that includes RGA and GAI in Arabidopsis. A receptor for the GA hormone has recently been identified from plants and it was shown that this receptor can interact in a GA dependent manner with the rice orthologue of RGA and GAI, possibly inducing its GA dependent degradation.

Thus, the signal transduction cascade from GA perception to DELLA protein degradation is very short and seemingly direct. However, GA responses are modulated by a number of other plant hormones including auxin. It is also known that RGA and GAI are phosphoproteins and the role of this phosphorylation for RGA and GAI function is unknown. Using a combination of genetic, biochemical and cell biological studies we are investigating two types of protein kinases that interact physically with RGA and GAI. These studies will help us to understand how GA signal transduction is regulated and possibly also how GA signal transduction cross-talks with other hormone pathways.

Fig. 3: Gibberellic acid signal transduction requires the degradation of the so-called DELLA proteins. Mutants that fail to degrade these growth repressors, such as the barley mutant on the right, are dwarfed.

Overview

Plant life, growth and development, is genetically determined but also greatly influenced by the environment. The rather inefficient exploitation of the genetic potential (on average only about 20-30 % in crop plants) is attributed to environmental stresses. On the other hand plants have the capacity to survive extreme abiotic stress conditions. Owing to their sessile life style, it is of the utmost importance that plants integrate diverse signals from the environment and respond by the induction of protective mechanisms.

The focus in the General Genetics unit is on basic research of functional genetics, investigating the environmental influences on plant growth and development. Model systems such as *Arabidopsis thaliana*, *Nicotiana tabacum,* and *Zea mays* are studied by genetic and reverse genetic approaches. The major areas are: the relation between gene function and phenotype, molecular mechanisms of regulation of gene expression, signal perception and transfer. Part of this work has strategic aspects important for agriculture and plant biotechnology.

The environmental aspects of our research are linked to the heat shock response. Crucial to the perception of stress and the initiation of a response are heat shock transcription factors (HSF) which represent an amazingly complex family in plants. Their functional roles appear to be very diverse and are not yet understood. HSF-dependent gene expression includes not only heat shock proteins but also a number of novel target genes involved in different cellular functions and biochemical pathways. Long-term goals are directed towards the understanding of the molecular mechanisms of common stress tolerance.

The research on oxidative stress is complemented by another environmentally influenced phenomenon – plant senescence. Very little is known about the developmental regulation of cell death in plants and why the protective systems against oxidative stress fail during leaf senescence. The regulatory network governing leaf senescence appears to be very complex. Crucial parameters are the actual leaf age and the developmental stage of the plants. Different cellular and physiological reactions are governed via common signaling components and regulatory proteins. WRKY or NAC factors seem to play a role.

Another unique feature of the plant kingdom is the development of a differentiated root system. Despite their agronomical importance and pivotal function in water and nutrient uptake, little is known about the genetic and molecular basis of root formation in cereals. Defined mutants that affect various aspects of maize root formation are employed as models and reverse genetic approaches are used to study the molecular interactions during root formation in cereals.

The experimental techniques include: (i) microarray gene expression profiling and real time PCR assays, proteome analysis (ii); generation/analysis of transgenic plants and gene knock out mutants; (iii) in gel staining of enzyme activities; (iv); expression of recombinant proteins in E. coli, yeast cells; (v) DNA-protein interaction in vivo/in vitro using laser cross-linking, gel retardation assay; (vi) protein:protein interaction by electrophoresis, cross linking, yeast two-hybrid assays, BiFC; (vii) tissue isolation by laser capture microdissection. For teaching, e-learning modules are accessible under www.uni-tuebingen.de/genetics/lernmodul. An advanced version "VipGen-virtuelles Praktikum Genetik" is provided on CD.

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Genetic dissection of root formation in maize

Frank Hochholdinger

Root systems of terrestrial plants serve many important purposes among which anchorage as well as water and nutrient intake are the most important ones. Cereals including maize (*Zea mays*) account for about 70% of the human calorie uptake worldwide. Despite their agronomic importance a systematic genetic analysis of root formation in cereals has only recently been initiated (Hochholdinger et al., 2004a, b). The root system of maize can be divided into an embryonic root system consisting of a single primary root and a variable number of seminal roots and a post-embryonic root system which is made up of shoot-borne roots formed at consecutive under and aboveground nodes. Lateral roots, which emerge from all major root-types, also belong to the post-embryonic root system.

Mutant analysis of maize root formation

Recently, we have identified a number of monogenic recessive mutants specifically affected in various aspects of shoot-borne and lateral root formation (Hochholdinger et al., 2004a, b).

Shoot-borne roots make up the major backbone of the maize root system and are unique to cereals. Therefore, we are particularly interested in this root-type and were able to identify a mutant (*rtcs*) specifically affected in shoot-borne root initiation. After cloning of the *rtcs* gene, which encodes for a lob domain transcription factor, a functional characterization of this gene and its interactions is under way.

In maize, genetic evidence suggests that at least two distinct pathways control lateral root initiation in embryonic and postembryonic roots (Hochholdinger et al., 2004b). This notion is supported by the identification of mutants that are specifically affected in lateral root formation of the embryonic roots, while lateral root formation in the postembryonic root system is not affected. In addition to several lateral root mutants which we had characterized previously we have recently identified a novel mutant *rum1*, which is specifically affected in the initiation of seminal roots and lateral roots in the primary root (Fig. 1; Woll et al., 2005). The reduced auxin transport and delayed gravitropism of this mutant suggest that the affected gene might be involved in the auxin mediated initiation of the affected roots.

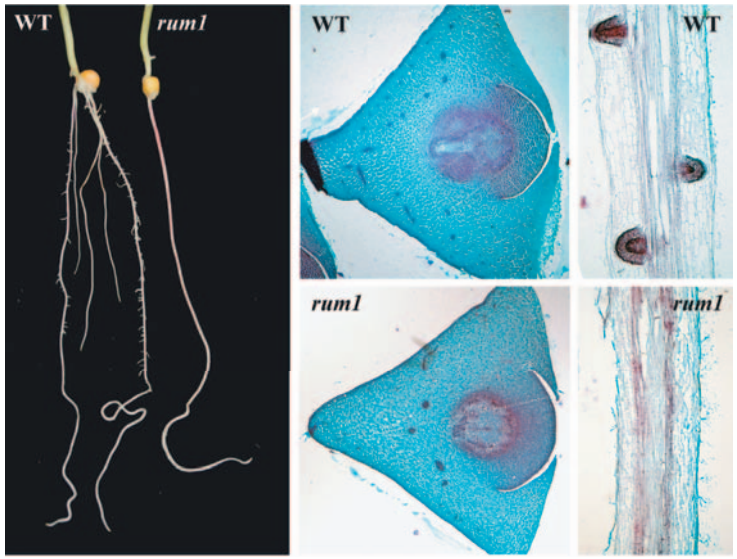


Fig. 1: The novel root mutant *rum1* is affected in lateral and seminal root formation (left) before the initiation of these root types (right).

Our laboratory is also involved in a co-operative project with the goal of functionally characterizing two recently cloned root hair mutants of maize encoding for a *sec3* homolog (*rth1*; Wen et al., 2005) and a phytochelatine synthase (*rth3*).

Reverse genetic analysis of maize root formation

In addition to the characterization of novel root mutants of maize and the cloning of the affected genes we are utilizing these specific mutants for high throughput microarray and proteome analyses (Hochholdinger et al., 2005b). The goal of these studies is a better understanding of the molecular networks that are active during the early stages of maize root formation and identification of the genes and proteins that are differentially expressed between wild-type and mutant genotypes. Such genes might be downstream regulators of the mutated genes and are the starting point of reverse genetic studies, which aim on the knock out of these genes and the subsequent functional characterization of these novel mutants.

Recently, we have extended our microarray analyses to cell-type specific gene expression studies (Schnable et al., 2004) by combining laser capture microdissection (Fig. 2) with microarray hybridizations (Woll et al., 2005).

Proteomics combines the resolution of 2-D gel electrophoresis with the sensitivity of mass spectrometry. On the proteome level we studied various aspects of maize root formation including lateral (Hochholdinger et al., 2004c) and shoot-borne root initiation and the early proteome changes during primary root development (Hochholdinger et al., 2005a).

Manifestation of heterosis in seedling roots

Heterosis is a phenomenon that describes the superior performance of F_1-hybrids over their homozygous parental inbred lines. Typically heterosis is measured in terms of increased yield or vigour. However, heterosis is already manifested during early seedling development and can be measured as increased primary root length, cortical cell length or lateral root density. Therefore, we chose the young maize seedling root system as a model to study the early molecular events leading to heterosis. Currently we are comparing the gene expression patterns of four local maize inbred lines and their reciprocal hybrids in an extensive microarray analysis (Keller et al., 2005). Subsequently, differentially expressed genes will be functionally characterized and their allelic contribution to gene expression will be determined.

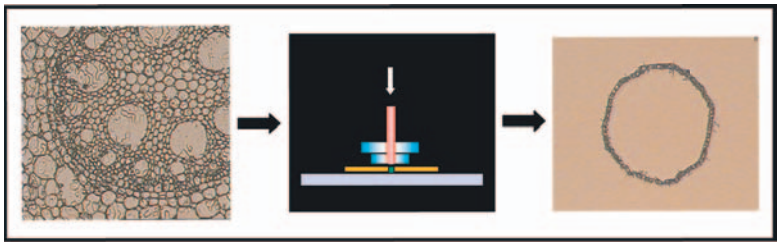


Fig. 2: Isolation of pericycle cells via laser capture microdissection.

Regulation and function of abiotic stress response

Fritz Schöffl

Plants have the capacity to interact with the environment in many different ways and to survive under extreme abiotic and perhaps also biotic stress conditions. The response to heat stress (hs) is highly conserved in organisms but owing to their sessile life style it is of the utmost importance to plants. The hs-response is characterised by (i) a transient alteration of gene expression (synthesis of heat shock proteins: HSP) and (ii) by the acquisition of a higher level of stress tolerance (acclimation). The induction of HSP-expression is not restricted to high temperature stress, HSPs are also linked to a number of other abiotic stresses including cold, freezing, drought, dehydration, heavy metal, and oxidative stresses. HSPs are molecular chaperones, which either prevent complete denaturation (small HSP: sHSP) or support proper folding (other HSP) of enzymes under or after protein denaturing conditions. Manipulation of the hs-response has the potential to improve common stress tolerance that may lead to a more efficient exploitation of the inherent genetic potential of agriculturally important plants. A number of HSPs are expressed in the absence of external stressors, during microspore formation embryogenesis and seed maturation. HSP also play an important role in the normal life of plants.

HSF - central regulators of heat stress and common stress response

HSF are DNA binding proteins which recognise the conserved HSE sequences present in the promoter and upstream regions of hs-genes (encoding HSPs). We have used UV-laser cross-linking of DNA:protein interaction *in vivo* to determine the activation and binding of HSF3 to selected target genes (Zhang et al., 2003, 2004). These and other data support the hypothesis of a feed back loop and a negative role of as yet unknown HSF in the modulation of the hs-response. Currently we are studying HSF-activation, interaction and localization *in vivo*.

In Arabidopsis 21 potential HSF genes have been identified. Single knock out mutations in individual HSF genes show no obvious phenotype, but it was possible to generate a trans-dominant negative mutation of the hs-response by transgenic expression of HSF-repressor constructs (Wunderlich et al., 2003). By the analysis of HSF double-knock-out mutants we are determining the differences in specificity, function and regulation. So far, HSF1 and HSF3 have been identified as response regulators for immediate early expression of target genes upon heat stress

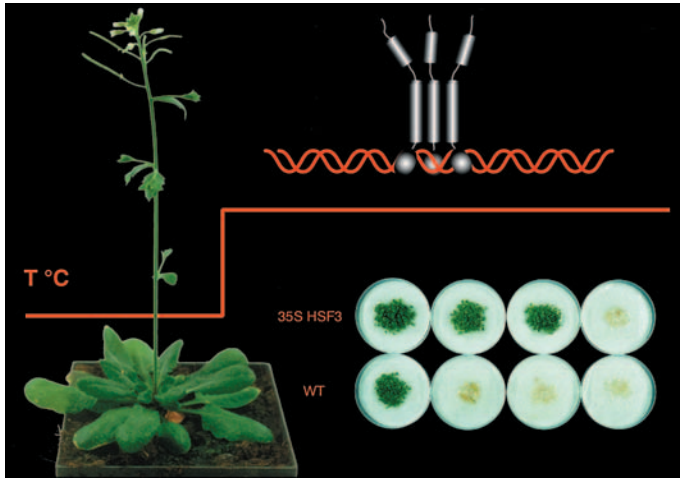


Fig. 1: *Arabidopsis thaliana*, the genetic model for studying and manipulating the heat stress response. Transgenic overexpression of Arabidopsis heat shock transcription factor HSF results in a synthesis of HSPs at normal temperature and significantly increased basal thermotolerance.

(Lohmann et al., 2004). Using microarray expression profiling (Fig. 2), a total of 112 HSF1/3 target genes have been identified, which are involved in different cellular processes, including an almost complete set of genes for the biosynthesis of raffinose family oligosaccharides (Busch et al., 2005).

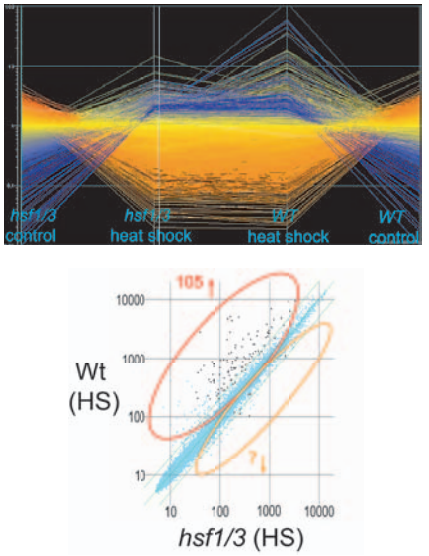


Fig. 2: Expression profiling: Global changes (upper panel) in gene expression in *hsf1/3* double-knockout plants compared to wild type (WT) *Arabidopsis thaliana*. Data plot for HSF1/3-dependent genes (lower panel).

Galactinolsynthase 1 was characterized as a key enzyme for heat-induced synthesis of galactinol and raffinose in Arabidopsis leaves (Panikulangara et al., 2004); its biological roles, e.g. in stress tolerance and stress-dependent phloem transport of raffinose is currently being investigated. Another focus of our research is the functional role of HSF, which are expressed after heat shock in a HSF-dependent fashion. Such HSF must be important for later stages of the heat shock response or during recovery. There is evidence that members of this group are involved in the regulation of pathogen defence in Arabidopsis.

Oxidative Stress – Heat stress response

Heat stress and oxidative stress show overlapping effects on target gene expression. In our present analysis we investigate the generation of H_2O2 upon heat stress and the effects of heat stress and oxidative/antioxidative compounds on the expression of selected target genes. There is evidence that - at normal temperature - H_2O_2 is an efficient inducer of sHSP expression, which can be counteracted by inhibitors or peroxide scavengers. The aim of this work is to elucidate the role of oxidative stress in HSF regulation and signalling.

Developmental expression of sHSP

The biological role of developmentally induced HSP, occurring at certain stages of the plant life cycle, e.g. during seed maturation and microspore embryogenesis, is unclear. The heat induction pattern of sHSP in pollen is, compared to leaf tissue, incomplete. However, there is a developmental expression of different subsets of sHSP at different stages of microspore formation (Volkov et al., 2005). The developmental induction may be related to desiccation tolerance and dormancy of seeds. A future goal for investigating sHSP function is the identification of preferential substrates *in vivo*.

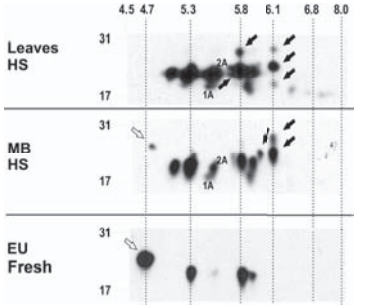


Fig. 3: Developmental expression of class I sHsp in pollen and in leaf tissue of tobacco. 2D-patterns exemplify mid-bicellular (MB) or early-unicellular (EU) pollen, without (fresh) of after heat stress (HS) compared to HS of leaf tissue.

Leaf senescence of annual plants

Ulrike Zentgraf

Natural or harvest-induced senescence is a major determinant factor causing agricultural crop losses, both in the field and during transport from the producer to the consumer. The timing of senescence affects many different agriculturally important traits like timing of seed set, number and quality of seeds, fruit ripening etc. Increased longevity can have an important impact on human health as fungi and other microorganisms thrive on senescing tissue. In addition, climatic extremes can trigger senescence; as a result of global climate changes, we will be forced to develop crop plants that cope better with the changed environmental conditions. Despite the importance of the senescence processes, our knowledge of the regulation of leaf senescence is still poor. Senescence is not a chaotic breakdown but an orderly loss of normal cell functions under genetic control of the nucleus. We are interested in understanding the mechanisms involved in the regulation of leaf senescence in annual plants using *Arabidopsis thaliana* as a model system. In general, leaf senescence is characterised by the breakdown of macromolecules and the mobilisation of nutrients out of the senescing tissues.

The rapid loss of chlorophyll, the lowering of protein and RNA levels or the leakiness of the cell membranes are typical senescence markers. The selective activation of gene expression, but also the shutoffs of certain RNA production and/or protein synthesis, are likely to initiate and regulate this process.

1) Senescence associated gene expression

For detailed gene expression analyses during leaf senescence we have designed a hybridization matrix for gene profiling using the Affymetrix high density genome arrays representing approximately 8.200 genes. If we analyze gene expression according to the age of the individual leaves of one plant in comparison to leaves harvested from plants with different ages we get very different expression profiles. These data indicate that gene expression is governed by two parameters, leaf age and plant age. The differential expression of genes according to the age of the leaves within one rosette was also analyzed using suppression subtractive hybridization (SSH). A transcription factor of the WRKY family, WRKY53, was isolated in this screen. This transcription factor exhibits a very interesting expression pattern: there is a switch from a leaf age dependent expression in 6- week-old plants to a strong systemic plant age dependent expression in 7-week-old plants (Fig.1).

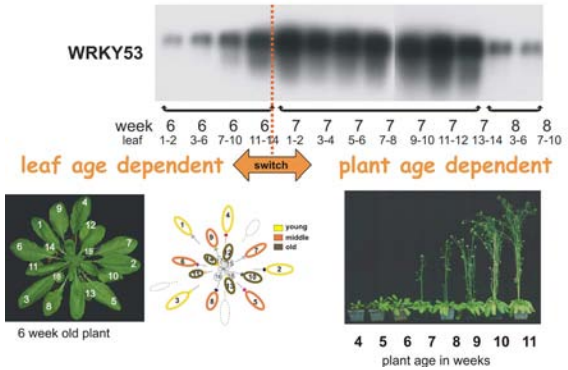


Fig. 1: Characterization of *WRKY53* expression of 6-week-old and 7-week-old plants of *Arabidopsis thaliana* by Northern blot analyses using single leaves marked with a color code.

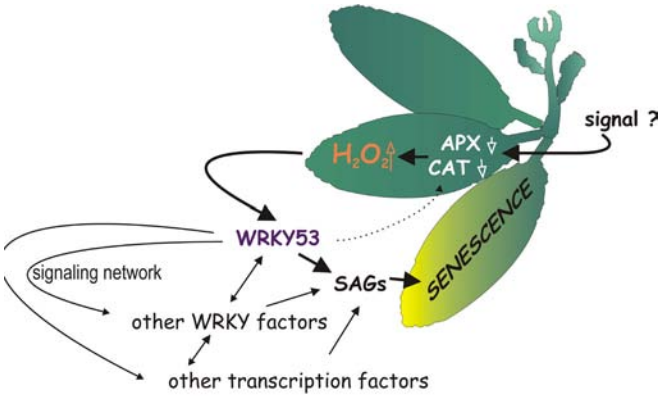


Fig. 2: Model for a signal transduction mechanism leading to induction of leaf senescence.

To understand the regulatory role of the senescence related *WRKY53* factor, we identified targets of this transcription factor by a pull down assay using genomic DNA and recombinant WRKY53 protein. We isolated a number of candidate target genes including other transcription factors, also of the WRKY family, stress- and defence related genes, and senescence-associated genes (SAGs). WRKY53 protein could bind to these different promoters *in vitro* and *in vivo* and it could act either as a transcriptional activator or a transcriptional repressor depending on the sequences surrounding the W-boxes. *WRKY53* expression can be induced by H_2O_2 and can regulate its own expression in a negative feed-back loop. Our results suggest that *WRKY53* acts in a complex transcription factor signalling network regulating senescence specific gene expression and that hydrogen peroxide might be involved in signal transduction. Currently, we are investigating the upstream regulatory factors involved in the transcriptional regulation of WRKY53.

2) Oxygen free radicals and antioxidative capacity (A5, SFB 446)

Oxygen free radicals are thought to play an essential role in senescence.

Therefore, the activities of the different isoforms of the H_2O_2 scavenging enzyme catalase and ascorbate peroxidase and the expression of the different genes were analyzed during senescence of *Arabidopsis thaliana*. If plants of different developmental stages were compared, CAT2 and APX1 activities decreased with the time of bolting even before the loss of chlorophyll could be measured. At the same time point, the H_2O_2 content increased. Subsequently, the stress inducible CAT3 isoform was activated and APX1 activity was recovered leading again to a decline of the H_2O_2 content. Treatment of cell cultures with either H_2O_2 or paraquat led to an inactivation of APX activity, indicating that the decrease of APX-activity during the time of bolting might be a secondary effect. Expression of *WRKY53* can also be induced by H_2O_2 and H_2O_2 concentration increases exactly at the time point when *WRKY53* expression changed from leaf age to plant age dependence. Therefore, we think that H_2O_2 might serve as a signal to induce leaf senescence (Fig. 2). Currently, we are investigating the regulatory factors involved in the transcriptional regulation of the catalases, especially CAT2. Besides WRKY53, three different proteins have been isolated which interact with the CAT2 promoter.

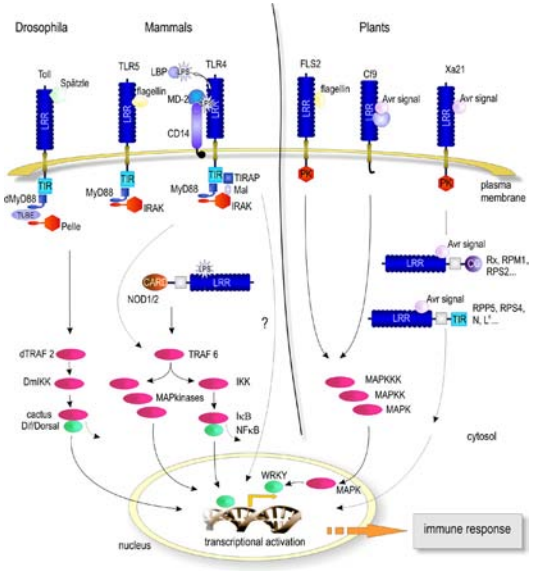
Plant Biochemistry

Overview

Immunity of multicellular organisms to microbial invasion is based upon the host's ability to discriminate between "self" and potentially dangerous "non-self" structures. Like animals, plants have evolved perception systems for multiple, highly invariant pathogen-associated molecular patterns (PAMP) that trigger basal or non cultivar-specific defense responses in plants. Non-self recognition is brought about by specific plant receptors that are structurally similar to TOLL-like receptors mediating PAMP perception and activation of innate immune responses in animals. Beside inducible plant immune responses, constitutive physical or chemical barriers contribute to the defensive arsenal of a plant. In addition to PAMP-mediated plant innate immunity, disease resistance programs are also often initiated through plant cultivar-specific recognition of microbial race-specific virulence factors, a recognition specificity that is not known in animals. Plant species and plant cultivar-specific resistance represent evolutionarily linked types of immunity that are collectively referred to as the plant innate immune system.

Research activities in the Plant Biochemistry unit aim at unravelling the molecular basis of non-cultivar-specific plant innate immunity. More specifically, we focus on the identification and functional characterization of plant elements that provide constitutive protection against attempted microbial invasion as well as of components that are implicated in the activation of inducible immune responses, such as PAMP perception systems and PAMP-induced signal transduction cascades. The majority of our research can be classified as basic research carried out on the model plant, *Arabidopsis thaliana*. To meet our research goals we use a combination of forward and reverse genetics screens, protein-protein interaction studies, cell biological assays and genome/proteome-based approaches. In the long term, knowledge gained from this work is expected to contribute to molecular breeding of genetically durable disease resistance in crop plants.

Thorsten Nürnberger



Conservation of signaling pathways mediating the activation of innate immunity in insects, mammals and plants.

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Receptor kinases and pathogen recognition

Georg Felix

Plants and animals can sense microbial attack by detection systems for "pathogen-associated molecular patterns" (PAMPs). These molecular patterns are conserved among whole groups and classes of microorganisms and can serve as hall-marks for the detection of 'non-self' by the hosts. So far, a series of PAMPs originating from fungi, oomycetes and bacteria have been described that act as potent elicitors of defense responses in plants. However, the corresponding receptors of the plants remain unknown for most of them. By concentrating on detection mechanisms for bacteria we have characterized the two bacterial proteins flagellin (Felix et al., 1999) and the elongation factor Tu (EF-Tu, Kunze et al., 2004) as PAMPs active in the model plant *Arabidopsis*. Perception in both cases is specific for particular domains of the proteins and synthetic peptides representing these epitopes, termed flg22 and elf18, fully mimic the elicitor activity of the intact proteins (Fig. 1). Using genetic and reverse-genetic approaches the flagellin receptor FLS2 (Gómez-Gómez and Boller, 2000) and the EF-Tu receptor EFR (Zipfel et al. unpublished) of *Arabidopsis* have been identified. Both receptor proteins have an ectodomain thought to function as the binding site for the respective ligand, a single-pass transmembrane domain and a cytoplasmic kinase domain. In plants, receptor kinases form a large family of proteins with >600 members in *Arabidopsis*. Based on genetic evidence it is clear that members of this family play crucial roles in aspects of the plant's life ranging from growth and development, to fertilization, reproduction and interaction with symbionts and pathogens. However, most of these putative receptors remain poorly characterized with respect to the signals they perceive. Similarly, little is known about the molecular mechanism by which this important class of plant receptors converts extracellular signal inputs into intracellular signal outputs.

Current and future work will base on the well characterized ligand-receptor pairs flg22/FLS2 and elf18/EFR as experimentally well suited models for studying the mechanism of receptor activation and transmembrane signaling (Fig. 2). Based on results with related receptors from animals one can anticipate that processes of homo- and/or hetero-dimerization with as yet unknown co-receptor elements relay extracellular signals to cytoplasmic signal output. To study these protein-protein interactions we will make use of site-specific alterations and tagging of the receptors, affinity crosslinking, screens for interacting proteins and approaches with chimeric forms of FLS2 and EFR. These latter approaches will also help to identify the domains of the receptors responsible for interaction with the PAMPs.

Plants have perception systems for bacterial PAMPs in addition to flagellin and EF-Tu. For example, lipopolysaccharide (LPS), a classical PAMP for the human innate immune system, has been reported to be detected by plants as well. Using the rapid and convenient cell-culture based bioassays that were successful in identifying flagellin and EF-Tu, we will try to identify further bacterial structures for which plants have evolved perception systems. Knowing more about the repertoire of the PAMPs recognized will allow studies on the interplay of the different perception systems in the defense response. Flagellin and EF-Tu activate a common signaling pathway and a congruent set of defense responses, but without a clear additive or synergistic effect. Thus, the apparent redundancy of perception systems might serve to ensure and enhance the detection for microbes that evolved to camouflage one or the other of the PAMPs. PAMPs usually represent highly conserved structures which carry essential functions and, consequently, are difficult to change or mutate. Nevertheless, some of the bacterial species pathogenic to plants have conspicuous changes in flagellin and EF-Tu which renders them 'invisible' to the detection systems of the plants. To test whether these changes in the PAMPs are due to selective pressure imposed by the defense system of the plant hosts, it will be important to study the virulence of these pathogens when these camouflaged forms of the PAMPs are exchanged with forms that can be detected by the plant defense system.

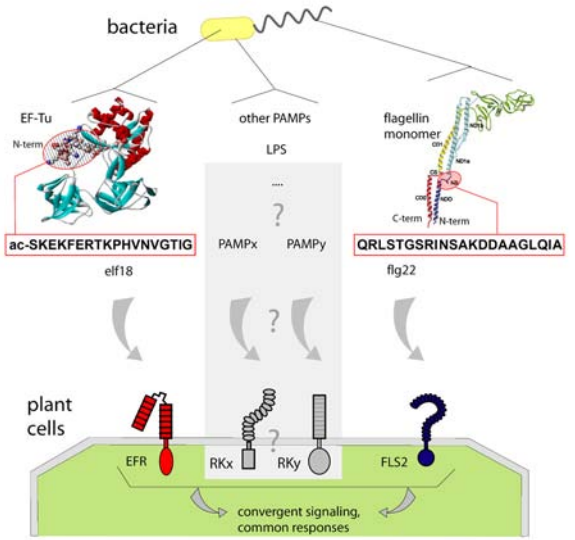

Fig.1: Schematic view of EF-Tu and flagellin, two bacterial-derived PAMPs detected by *Arabidopsis* cells. The N-terminus of EF-Tu (elf18), recognized by the receptor termed EFR, and the epitope flg22 of flagellin, recognized by the flagellin receptor FLS2, are highlighted in red. Apart from flagellin and EF-Tu, plant cells can detect further bacterial PAMPs, including lipopolysacharides (LPS) and other, as yet unidentified, molecular patterns. Similarly, the receptors for these additional PAMPs are currently unknown (shaded, question marks). Based on the common set of responses induced by all PAMPs one can speculate that these receptors might resemble the receptor kinases EFR and FLS2.

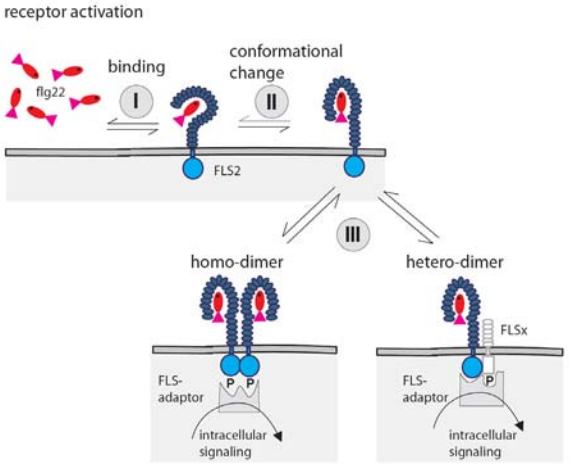

Fig.2: Model for activation of the flagellin receptor FLS2. FLS2 is a receptor kinase with an ectodomain composed of leucine rich repeats (LRR). LRR-domains are involved also in PAMP detection by the Toll-like receptors of animal innate immunity and have been shown to adopt a horse-shoe type of configuration. Interaction of flg22 with FLS2 is a two-step process that leads to non-reversible binding of the ligand (steps I and II). Connected to this process one can imagine a structural change in the ectodomain that then allows an oligomerisation process (either homo- or hetero-dimerization, III) that leads to a corresponding change on the cytoplasmic side and activation of intracellular signaling. FLSx and FLS-adaptor are hypothetical elements of the flagellin receptor.

Molecular dissection of non-host resistance in Arabidopsis

Volker Lipka

"Non-host resistance" describes the phenomenon that an entire plant species is normally immune to all genetic variants of a particular parasite. Although non-host resistance represents the most common form of plant resistance in nature, it has so far been poorly understood at the molecular level, due to the lack of tractable genetic systems. To take remedial measures we developed a system for the genetic dissection of non-host resistance in *Arabidopsis*, which allowed the isolation of *Arabidopsis* mutants (*pen1* and *pen2*) with compromised pathogen entry control against the biotrophic barley powdery mildew *Blumeria graminis* f. sp. *hordei* [*Bgh*] (Collins *et al.*, 2003).

PEN1 and *PEN2* were identified by map-based cloning and encode a syntaxin and a family 1 ß-glycosyl hydrolase, respectively (Fig. 1). Systematic gene interaction analyses have provided evidence of a two-layer concept of operationally distinct mechanisms mediating non-host resistance in *Arabidopsis* (Lipka *et al.*, 2005).

Syntaxins belong to a super-family of proteins known as SNAREs (soluble *N*-ethylmaleimide-sensitive fusion protein attachment protein receptors), which mediate secretory vesicle fusion events at target membranes by SNARE-complex formation with additional SNARE proteins (SNAPs) and specific vesicle anchored v-SNAREs. Expression of GFP-PEN1 fusions revealed that PEN1 localizes to the plasma membrane. Interestingly, GFP-tagged PEN1 accumulates at fungal penetration sites suggesting that PEN1 is required for timely and spatially controlled membrane traffic to the cell periphery, preventing pathogen entry into epidermal cells. To gain insight into the molecular mechanics of this phenomenon we generated transgenic lines expressing fluorescent versions of *Arabidopsis* v-SNAREs. These lines were used to trace tagged vesicle dynamics in pathogen challenge experiments in order to identify potential vesicle-localized v-SNARE complex partners of PEN1 (shown in the fluorescence microscopy insert of Fig.1). We currently are using magnetic activated organelle sorting (MAOS) for isolation of fluorescence tagged vesicle populations and their subsequent biochemical characterization.

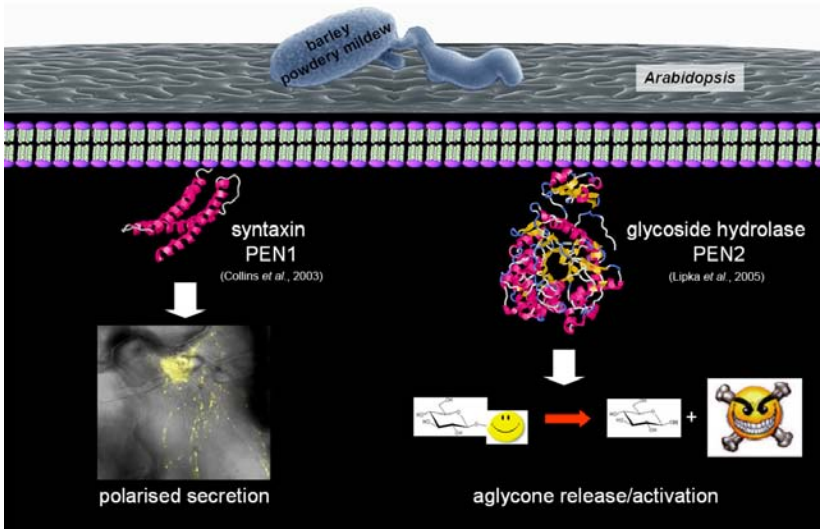

Fig. 1: Pathogen entry control at the cell periphery is mediated by the proteins PEN1 and PEN2. Our current working model suggests a function of the syntaxin PEN1 in polarized secretion processes. The glycoside hydrolase PEN2 is probably involved in the enzymatic release of a biologically active aglycone from an inactive precursor metabolite.

In plants, ß-glycosyl hydrolase activity is involved in processes such as compartmentalization of phytohormones, floral development and pigmentation, defense mechanisms, lignification and cell wall decomposition. Transgenic complementation analyses with wt *PEN2*-cDNA as well as catalytically inactive variants recently revealed that catalytic activity is required for PEN2 function in non-host resistance. Similarly, the unique C-terminal extension of the PEN2 protein appears to be crucial for *in planta* function. Functional GFP fusions of the PEN2 protein associate with peroxisomes which also focally accumulate at pathogen interaction sites, reinforcing the importance of induced cell polarization processes for pathogen entry control (Lipka *et al.*, 2005). Comparative metabolic profiling and substrate trap technology aiming at the identification of the *in planta* substrate(s) of PEN2 are currently applied in collaboration with research groups at the Max-Planck-Institute in Cologne (Paul Schulze-Lefert) and the IPB in Halle (Dierk Scheel, Stephan Clemens).

The distinct entry control mechanisms represented by PEN1 and PEN2 are characterized by a variable and restricted efficiency in host range demarcation against different pathogens. Indeed, only *pen2* mutations affect basal and non-host penetration resistance to multiple pathogens, including the inappropriate hemibiotrophic cucurbit pathogen *Colletotrichum lagenarium*. This finding prompted us to develop a non-host interaction model system employing a collection of evolutionarily more and less distantly related non-adapted *Colletotrichum* species. This *Arabidopsis-Colletotrichum* model system has the significant advantage that both interacting organisms can be genetically manipulated by *Agrobacterium*-mediated T-DNA transfer. In addition, challenge inoculations of a chemically induced *Arabidopsis* mutant population provided novel mutant candidates with aberrant interaction phenotypes.

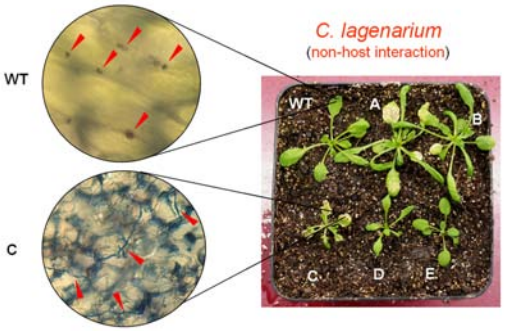

Fig. 2: Novel mutant candidates (A, B, C) exhibiting aberrant non-host interaction phenotypes compared to WT plants upon inoculation with the non-adapted cucurbit pathogen *C. lagenarium*. Microscopic analysis reveals papilla-associated penetration resistance on WT and enhanced colonisation success on mutant plants (i.e. hyphal growth in mutant C).

Characterization of these mutants and isolation of the mutated genes will allow a comparison of the *Arabidopsis-Bgh* and *Arabidopsis-Colletotrichum* non-host model systems and will reveal mechanistic differences, commonalities and particularities. Additionally, the genetically tractable inappropriate and compatible *Colletotrichum* species employed promise the identification of pathogen-derived effector molecules, which are either responsible for betrayal of the intruder to the plant's surveillance system or sabotage of the latter and establishment of compatibility. Collaborative projects with research groups in Japan (Yoshitaka Takano) and at the Max-Planck-Institute in Cologne (Richard O'Connell) address these issues.

Thorsten Nürnberger

Most plant species are resistant against complete species of potential microbial invaders, a phenomenon that is termed non-host, species or non-cultivar-specific resistance or immunity. Non-host resistance relies on multiple protective mechanisms that comprise both constitutive barriers and inducible reactions. Multiple pathogen-associated molecular patterns (PAMPs) have been shown to trigger receptor-mediated, inducible pathogen defense reactions in non-host plants.

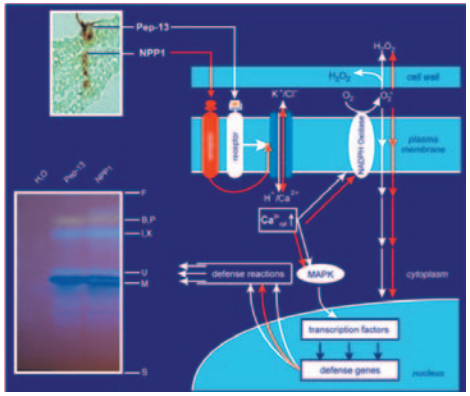

Fig. 1: Hypothetical model of PAMP-induced signal transduction in parsley.

Recognition of such signals is very likely to activate immune responses in natural plant-microbe encounters and thus to contribute to disease resistance. A major research topic in our group is the identification of new pathogen-associated structures that exhibit plant immunity-stimulating activities. Moreover, we analyze the molecular architecture of PAMP perception complexes as well as of PAMP-induced signal transduction cascades in various plant systems.

We have identified a cell wall transglutaminase (TGase) from phytopathogenic *Phytophthora* species that triggers pathogen defense responses in parsley and potato. A surface-exposed 13-mer fragment within this TGase (Pep-13) was shown to be necessary and sufficient to activate non-cultivar-specific immune responses in these plants. Binding of Pep-13 to its receptor evokes a Ca^{2+}-inward current that results in sustained elevated levels of cytoplasmic Ca^{2+}, subsequent posttranslational activation of MAPK cascades, production of reactive oxygen species and, eventually, the production of antimicrobial phytoalexins (Fig. 1). Recently, biochemical purification of the Pep-13-receptor was completed and sequence information of the protein was obtained by MS-based protein sequencing.

NPP1, another *Phytophthora*-associated surface structure was shown to trigger a similar cascade of events through a perception system distinct from the Pep-13 receptor (Fig. 1). In contrast to Pep-13, NPP1 stimulates plant immune responses, such as a hypersensitive cell death response, not only in parsley, but in virtually all dicotyledonous plants, including *Arabidopsis thaliana*. NPP1 is among the founding members of an emerging class of proteins that are present in various plant pathogenic oomycetes, fungi, or bacteria. These proteins may be identified by the presence of a common domain, called the NPP1 (necrosis-inducing *Phytophthora* protein) domain. Currently, conditional execution of NPP1-mediated cell death in *Arabidopsis* is employed to isolate mutants impaired in NPP1 perception or NPP1-induced plant cell death (Fig. 2).

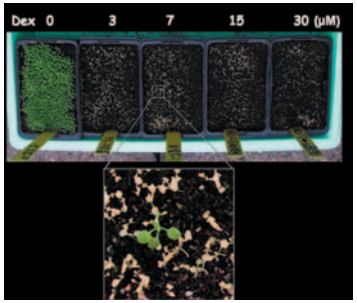

Fig. 2: Dexamethasone-based conditional lethal screen for NPP1-insensitive *Arabidopsis* mutants.

In addition, pathogen-associated molecular patterns from phytopathogenic bacteria, such as lipopolysaccharides (LPS), the virulence-associated protein, HrpZ, or peptidoglycans, are analyzed with respect to their proposed plant immune response-stimulating potential.

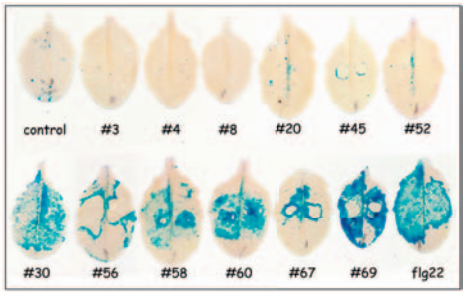

Fig. 3: PAMP-induced reporter gene activity (GUS) in *Arabidopsis*.

Moreover, various natural compound libraries comprising bacteria-derived carbohydrate, lipid and protein structures are screened for their ability to drive the expression of a GUS-reporter gene controlled by a PAMP/pathogen-responsive promoter (Fig. 3). This approach will help answer the question whether plants have evolved PAMP perception systems that recognize a structural variety of PAMPs similar to that recognized by their animal counterparts.

Both, PAMP-mediated non-cultivar-specific and AVR-mediated plant cultivar-specific immunity make use of receptor-like kinases consisting of an extracellular LRR-domain, a transmembrane region and a cytoplasmic kinase domain (LRR-RLK). A concerted approach of various laboratories to build up a gene expression atlas of *Arabidopsis* by means of microarray analyses (*ATGenExpress-Project)* enabled us to study global alterations of the transcriptome upon pathogen or PAMP treatment. Transcript levels of 49 out of 235 LRR-RLK genes increased upon either pathogen infection and/or elicitation (Fig. 3).

One of these genes was previously described to encode the brassinosteroid receptor BRI1-Associated Kinase 1 (BAK1) that is implicated in regular plant growth and development. T-DNA insertions in the BAK1 gene resulted in enhanced susceptibility to infection with necrotrophic fungi (*Alternaria brassicicola, Botrytis cinerea*), but not to infection with biotrophic bacteria or oomycetes. Interestingly, numerous *Arabidopsis* mutants impaired in brassinosteroid sensitivity or biosynthesis did not vary in their resistance against any of the pathogens tested. Moreover, exogenously applied brassinosteroids rescued the moderate dwarf growth phenotype of BAK1 mutants, but they neither rescued plant disease resistance to necrotrophic fungi in these lines nor enhanced disease resistance in wild type plants. Our data suggest that BAK1 is a crucial component of plant disease resistance to necrotrophic fungal infection and may exert a dual function in brassinosteroid-independent plant immunity and brassinosteroid-dependent plant development.

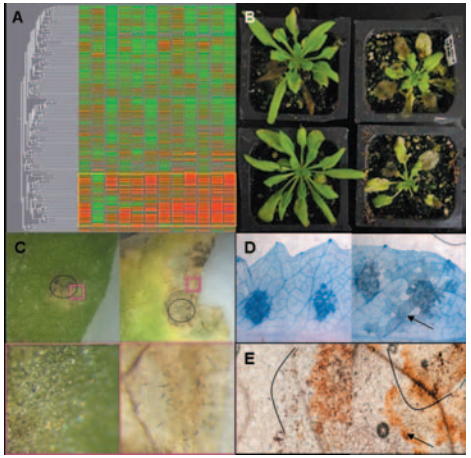

Fig. 4: Cluster analysis of LRR-RLK gene expression in pathogen-infected Arabidopsis plants **(A)**. Enhanced disease susceptibility of *bak1* mutants to infection with *Botrytis cinerea* **(B)** or *Alternaria brassicicola* **(C)**. Runaway cell death **(D)** and accelerated ROS production **(E)** in *bak1* mutants infected with *Alternaria brassicicola*.

Overview

Physiology studies the function of an organism, encompassing the dynamic processes of growth, metabolism, reproduction and interaction with the biotic and abiotic environment. Modern physiology combines a wide spectrum of molecular biological, biochemical, biophysical, cell biological and genetic techniques to obtain insight into how an organism functions.

Plants as sessile organisms face the problem that they are confined to their habitat and have to endure and adapt to a broad array of environmental conditions (Fig. 1). Furthermore, plants have to convert the “diffuse” energy source “light” into biochemical energy and to enrich mineral nutrients from the soil. To optimize growth and development in a highly competitive environment, plants depend on a multitude of molecular signal perception, processing and integration systems. Although several specific and more-or-less linear signalling pathways have been described, far less is known about the components, composition and molecular mechanisms underlying signal processing and integration in higher plants.

The focus of our research - conducted by five independent research groups - is on the specific features of plant signal perception, signal transduction and information integration. Furthermore, we are interested in intracellular protein trafficking as well as nutrient acquisition.

The majority of the research in our department can be classified as basic research. However, the transfer of results and technological knowledge could lead to potential biotechnological application in the field of crop improvement.

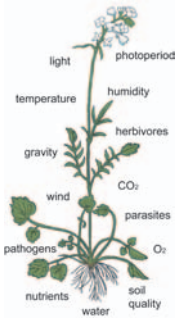

Fig. 1: Environmental signals to be processed by plants.

FG Harter: Function of plant two-component signalling systems; target gene identification, post-translational regulation and functional analysis of bZIP transcription factors, identification of regulatory gene networks using microarray approaches and bioinformatics.
PG Koch: Uptake and long distance transport of amino acids; proteins involved in amino acid transport, identifying importers and exporters, analyzing the impact of reduced amino acid transport on plant growth and seed development.
FG Ludewig: Molecular biology and electrophysiology of nitrogen transport; ammonium, amino acid and nitrate transporters and their function in storage and re-mobilization of nitrogen; transport mechanisms; imaging of nitrate and amino acid dynamics.
FG Oecking: Function of regulatory 14-3-3 proteins in plants; regulation of the plasma membrane H^+-ATPase, identification and characterization of novel 14-3-3 target polypeptides, elucidation of the physiological role of 14-3-3 proteins.
FG Schumacher: pH-Homeostasis in the endomembrane system; molecular and physiological analysis of V-ATPase function and regulation, identification of interaction partners, imaging of ion dynamics and V-ATPase assembly.

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Plant signal perception, transduction and integration

Klaus Harter

The research activities of our group focus on plant perception of environmental and endogenous stimuli and the molecular mechanisms of signal processing inside the plant cell.

a) Two-component signal transduction

During evolution higher plants adapted and further developed a unique molecular signal processing mechanism which appears to be perfectly suited for signal perception, transduction and integration: the two-component system (TCS, Fig. 1).

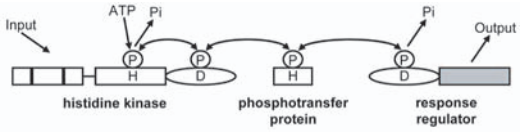

Fig. 1: Basic features of the multistep two-component system (TCS) in higher plants.

In the model system *Arabidopsis thaliana* and other plant species, TCSs are mostly composed of hybrid histidine kinases, histidine phosphotransfer proteins and response regulators. The hybrid histidine kinases function as receptors for specific endogenous or environmental signals and – after autophosphorylation – transfer a phosphoryl residue in a strict one-to-one manner *via* a histidine phosphotransfer protein to a response regulator (Fig. 2). Phosphorylation of response regulators induces their interaction with other proteins or their binding to specific promoter regions of their target genes. In addition to this linear mode of action, the promiscuous interaction capability of TCS elements enables cross-talk between different signalling pathways.

In higher plants, we and other groups have shown that TCSs and TCS-like elements contribute to the perception and transduction of phytohormones and environmental cues, to the regulation of phyllotaxis and the circadian clock, to the promotion of flowering and to female gametophyte development.

On account of their multitude of elements, their molecular features and their way of action, TCSs combine the capacity for signal transduction with unique molecular properties enabling fine-tuning and cross-talk between different signalling pathways and, therefore, contribute to the generation of overlapping physiological and developmental responses.

b) bZIP transcription factors

Transcription factors play crucial roles in almost all biological processes and are classified by their DNA-binding domain. The basic region/leucine zipper (bZIP) transcription factors have a basic region that binds DNA and a leucine zipper for homo- and heterodimerization (Fig. 2).

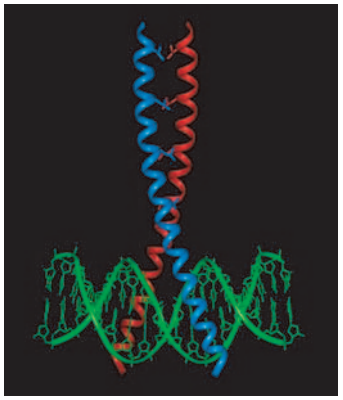

Fig. 2: Schematic representation of a bZIP transcription factor binding to double-stranded DNA (green).

Studies of a few plant bZIP transcription factors have revealed that they participate in the regulation of diverse biological processes. In the course of our research we address the mechanisms of the molecular regulation of four bZIP transcription factors from *Arabidopsis.* These factors form a small but distinct subfamily within the 75 members of the *Arabidopsis* bZIP proteins. Our data indicate that these factors are regulated by post-translational mechanisms, including intracellular distribution, dimerization and phosphorylation.

We are currently studying the functional role of these post-translational mechanisms in regulating the activity of bZIP transcriptional regulators. In addition, we are interested in the *in vivo* target genes of group C bZIP factors. We have, therefore, initiated a combination of different approaches which are aimed at identifying such target genes. Supported by results derived from the physiological analysis of bZIP factor mutants and bZIP factor overexpressors, we expect to obtain insight into the function of these bZIP factors in growth and development and the responses to environmental and endogenous stimuli in *Arabidopsis*.

c) Bioinformatics

It has long been considered that the evolution of regulatory interaction is a major driving force for the generation of evolutionary novelties. As more and more genomic sequences and expression data become available, we are now starting to resolve general issues such as the dynamics of regulatory sequence evolution.

We are also interested in the comprehensive characterisation of *cis*-regulatory elements in the non-coding regions of genes (Fig. 3). These *cis*-regulatory elements are recognized by transcription factors which actually trigger the activity of target genes. The more we understand how the position, orientation and combination of different *cis*-regulatory elements affect transcription, the more we will be able to predict gene expression patterns.

For the broad range determination of gene expression profiles we use microarray data from *Arabidopsis*. As a contributing laboratory of the multinational AtGenExpress project, we have access to a multitude of expression profiles. Combining our present knowledge of physiological processes and signal transduction pathways, we aim to integrate these expression data into our current models of genome-wide gene expression (Fig. 3).

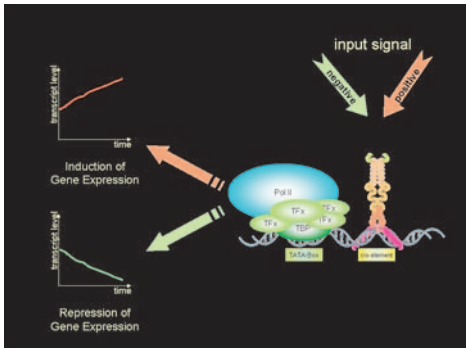

Fig. 3: Regulatory principles and components involved in eukaryotic gene expression.

The regulation of gene expression is not restricted to the binding of transcription factors to *cis*-elements but also depends on the structural properties of the DNA neighbourhood. Thus, another focus of our bioinformatics approach is the analysis of chromatin remodelling and epigenetics. For several reasons plants provide detailed information about chromatin-mediated processes impossible to attain in other eukaryotic system.

d) Technoloy Aspects of our Research

At present our research is predominantly based on the model plant *Arabidopsis thaliana* but is extended to other plant species (e.g. tobacco, tomato, rice). The majority of our methods are non-invasive or minimally-invasive, such as *in planta* protein localisation using fluorescent protein markers, *in planta* protein-protein interaction and *in planta* biochemical studies as well as the characterization of mutants created by both forward and reverse genetics. Comprehensive physiological analyses are accompanied by transcriptome and proteome studies. The non-invasive/minimally-invasive cell biological techniques are supported by *in vitro* biochemical methods and *in vivo* approaches performed in non-plant systems.

Uptake and long distance transport of organic nitrogen

Wolfgang Koch

Amino acids represent the major transport form of organic nitrogen in most plant species. Inorganic nitrogen is assimilated into amino acids in an energy-requiring process. The assimilation of ammonium usually occurs in roots, whereas nitrate can be assimilated in roots or leaves. Likewise, free amino acids can also arise from breakdown of storage proteins in the vegetative or reproductive storage organs to supply the developing plant. From their site of synthesis, excess amino acids can be exported via the vascular tissue to apices, newly developing tissues, and particularly, reproductive organs. Amino acids are transported in both the xylem and phloem, but concentrations in the phloem are about 10 fold higher. The composition of the phloem and xylem sap and of the cytosol of mesophyll cells are highly similar, indicating that non-specific loading processes are involved. At least two transport steps across the plasma membrane are required for the uptake of amino acids into the phloem, one releasing amino acids into the apoplasm and one for uptake into the phloem. In contrast, xylem loading requires only an export step from the xylem parenchyma. Import into embryos requires at least two steps, one for release from the seed coat and one for uptake into endosperm and embryo. Since amino acids are imported into seeds mainly via the phloem, and the composition of the phloem sap changes along the path, additional transport steps are required for xylem-to-phloem transfer along the translocation path.

As may have been expected from the multiplicity of substrates, tissues and, thus, transport events involved, several amino acid transporter genes could be identified in *Arabidopsis,* potato, tomato, pea and other plants. In Arabidopsis 53 putative amino acid transporters were clustered in three different superfamilies with related transporters from yeast and animal. Some of these transporters are localized at the plasma membrane; some members from yeast and animal are known to be involved in the import and export of amino acids at the membrane of intracellular vesicles and vacuoles. In plants, most compartments like plastids, mitochondria, peroxisomes and the cytosol are involved in biosynthesis and degradation of amino acids. Thus, transporters are required to move amino acids between compartments. Furthermore, many transport proteins are targeted and removed from the plasma membrane upon stimulation of the transported substrate, e.g. Glut4 in animals or GAP1 in yeast. In plants little is known about such regulatory influences of substrates on the transporter density at the plasma membrane.

The complexity of N-allocation and the number of genes involved in amino acid transport, combined with biosynthesis and environmental influences, make it necessary to combine the analysis of individual genes with genomic approaches.

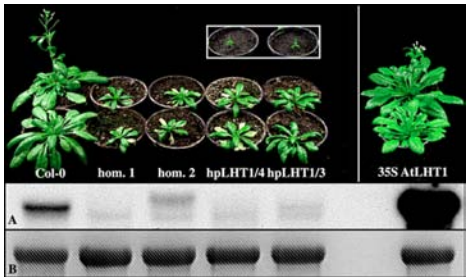

Fig. 1: Growth phenotype of an amino acid transporter mutant. The phenotype was reproduced by RNAi-constructs, whereas overexpression showed no growth effect. A) reduced RNA levels in the mutants B) loading control.

Major research goals of our work are:

- Tissue specific expression of all amino acid transporters: GUS-fusions have been used to identify the tissue specific distribution of the functional AAPs. With this method we could show an individual expression pattern for seven transporters demonstrating an individual function for each of the proteins. In our work, we focus on the transporters potentially involved in long distance transport, i.e. localizing in phloem and xylem of sink and source leaves, seeds and roots (e.g. AAP6 in xylem parenchyma).

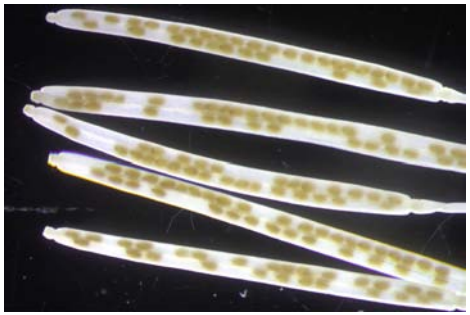

Fig. 2: Seed phenotype of T-DNA insertion lines of AtAAP8, Number of seeds is reduced to ~ 55 %.

- Cellular and subcellular localization using GFP and epitope tags: With this method we could demonstrate that AAP3 is localized at the plasma membrane and in Golgi vesicles. This tagged protein can now be used to study regulation of transporter density upon external signals. A possible targeting and recycling of the proteins could be an efficient way of adapting to environmental conditions.

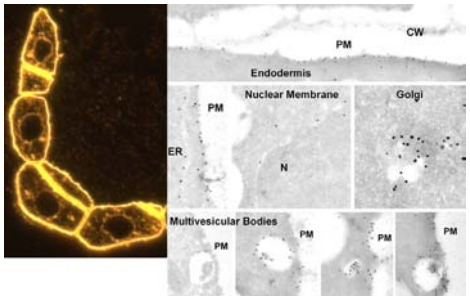

Fig. 3: Localization of c-myc tagged AAP3 in the root endodermis, plasma membrane and at the golgi apparatus.

- Antisense and RNAi approaches: In potato we could show that antisense inhibition of a transporter expressed in source leaves led to reduced amino acid content in tubers (Koch et al. Plant Journal 2003). This showed that it is possible to manipulate transport of amino acids to sink organs despite the number of putative transporters. In Arabidopsis, we use an RNAi approach to reduce the expression of multiple transporters expressed in the same tissue.

- T-DNA-insertion lines: We have identified T-DNA insertion lines for many transporters. Some of these lines show drastic phenotypes such as reduced growth and early senescence. These phenotypes could be reproduced in RNAi plants, other mutants are under analysis

- Biochemical methods: Uptake studies with radiolabelled amino acids and HPLC/GC-MS-analyses are used to follow the impact of T-DNA insertions and gene silencing**.** In RNAi lines, a reduced uptake of amino acids was determined. An altered C/N ratio determined in StAAP1-antisense tubers point to a co-regulation of carbohydrate and amino acid delivery to sink organs.

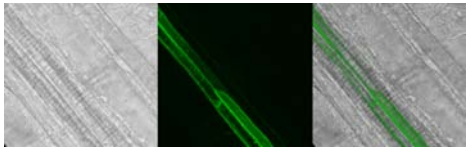

Fig. 4: Root phloem localization of a putative amino acid exporter. At the root level, amino acids are unloaded of the phloem and can enter the xylem to cycle within in the plant or supply sink tissues.

Since the genes examined so far show a distinct expression and localization pattern, revealing the complexity of nitrogen distribution is challenging. Our goal is to learn how a fine tuned network of collaborating genes responds to various conditions to adjust the delivery of organic nitrogen. This analysis will help us to understand processes such as xylem to phloem transfer, differential translocation of amino acids and cycling. Beside this basic research of understanding N-allocation in plants, applied biotechnological aspects are part of our work. Successful manipulation of amino acid transport could lead to enrichment of proteins in seeds or improve processing and quality of agricultural products.

Molecular biology and electrophysiology of nitrogen transport

Uwe Ludewig

Our research activities focus on the molecular physiology of nutrient uptake and metabolite transport in plants. *Arabidopsis thaliana* is the model species used, but certain aspects are also studied in crop plants such as tomato or maize. A few additional studies are performed on another model plant, the moss *Physcomitrella patens*.

Plants are sessile organisms that have to ensure adequate nutrient supply to every single cell during development and growth. Although a certain amount of each nutrient is necessary for optimal development, many nutrients are toxic in excess. Plants have evolved strategies to cope with different environments and regulate optimal uptake from suboptimal soils and nutrients are stored or excluded upon demand. Nitrogen is the most important nutrient acquired from the soil and is limiting in many ecosystems and in agricultural practice. Different nitrogen forms regulate root architecture and plant morphology.

Ammonium transport

Nitrogen is in part taken up as ammonium. Ammonium transport involves NH_4^+ transporters of the AMT/Rh-type. Homologs of plant AMT-ammonium transporters have also been identified in microorganisms and even humans, but we found that the human transporters function distinctly from the plant transporters. We are studying the physiology, regulation and structure of plant ammonium transporters using a wide spectrum of techniques. Reporter plants are used to identify the tissue and subcellular localization of individual AMT-members. Biochemical assays are used to study their function. Two heterologous expression systems are utilized for in-depth biophysical analysis: yeast and *Xenopus* oocytes.

Biophysical analysis, including electrophysiological measurements on oocytes, identified the NH_4^+ transport mechanism in AMTs, their structural assembly as heterooligomers, and the amino acid residues involved in substrate recognition and selective transport.

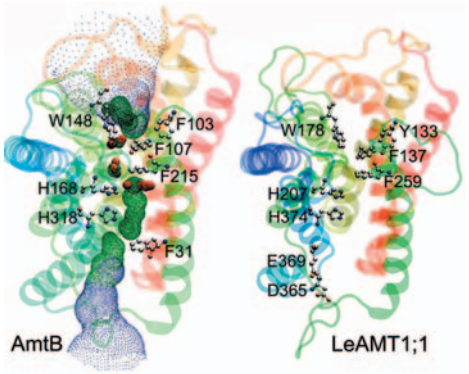

Fig. 2: Structural comparison between bacterial (left) and plant ammonium transporter monomers (right). Functionally important residues are highlighted.

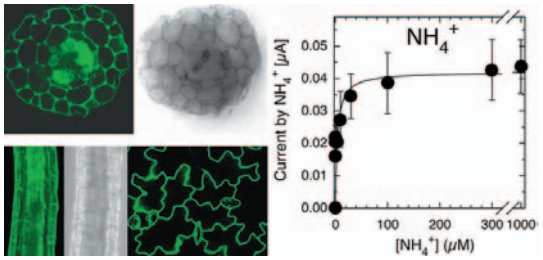

Fig. 1: AtAMT1;1 expression in epidermis and vasculature of root and shoot (left) and saturation kinetics of NH_4^+ currents by AtAMT1;1 (right).

Amino acid transport

Besides ammonium transport, amino acid transport is important for plant nutrition and development. More than 60 amino acid transporter genes have been identified in a single plant genome, indicating the complexity of plant amino acid transport. We are studying a subfamily of APC-related amino acid transporters and have identified novel vacuolar amino acid transporters. The role of these in the regulation of vacuolar amino acid storage and release is analyzed by using T-DNA insertion lines, RNAi plants and over-expressers. The transgenic plants are being studied to identify the function of individual transporters in plant life and to indicate their critical role during germination and senescence. Transgenic plants are also studied with selective fluorescent amino acid probes. In addition to their role as metabolites, certain amino acids have been suggested to serve as metabolic signals that regulate carbon and nitrogen assimilation. Fluorescent metabolite sensors have been developed that are specific for, e.g. glutamine. Similar sensors for other nutrients and metabolites are currently being developed and provide a tool for the non-invasive online monitoring of dynamic metabolite changes within individual plant compartments. The fluorescent sensors are used in order to understand how metabolite levels in plants vary with nutrient deficiencies, and biotic and abiotic stresses.

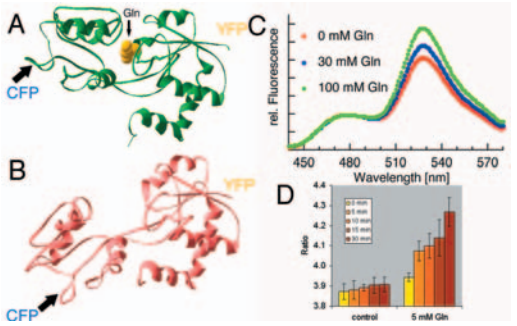

Fig. 3: Design and development of fluorescent nanosensors for glutamine (Gln) A, Closed conformation of a bacterial Gln-binding protein in complex with Gln. B, Open conformation without substrate. Note that the right half of the Gln-binding protein was fixed in the same position. CFP and YFP were attached to the most distant moving parts of the Gln-binding protein C, Fluorescence changes with glutamine D, *In vivo* observation of Gln uptake in yeast.

Nitrate storage

Nitrate, the most important nitrogen source for most plants, is taken up by the roots, transferred to the shoots and transiently stored in leaf vacuoles. The anion transporters involved have been identified and are being analyzed using similar techniques and approaches. Fluorescent nitrate sensors are being applied to measure the nitrate concentrations in subcellular compartments. The regulation of transient nitrogen storage is also being studied with respect to chloride storage, which is important during salt stress. The selectivity of the vacuolar transporters is being studied using electrophysiological methods, such as patch clamp analysis.

Fig. 4: Transformed plant protoplasts expressing GFP-tagged plasma membrane (lower inset) or tonoplast channels/ transporters (upper inset) are identified by their fluorescence. Transport in individual protoplasts or vacuoles is then analyzed by patch clamp.

Biophysical analysis of various other transporters

In several collaborations with other groups, additional transporters have been characterized biophysically using *Xenopus* oocytes. These include metabolite transporters for amino acids, nucleotides, urea, metal chelates, boron, etc. An experimental and computational approach (involving computer simulations and molecular dynamics) has been set up to understand the molecular details of selectivity of certain aquaporins (water channels). Several aquaporins were found to conduct far more substrates than water. A conducted substrate of special interest in some aquaporins is ammonia. The physiological role of the transport of additional substrates is being analyzed by various assays, including the use of transgenic plants.

Although the work focuses on basic research, we aim to identify biotechnologically relevant applications for future optimization of crop nitrogen use efficiency.

Function of plant 14-3-3 proteins

Claudia Oecking

Reversible phosphorylation of proteins is the most widespread signaling mechanism and regulates almost all aspects of cellular life. 14-3-3 proteins were the first molecules to be identified as discrete phosphoserine/threonine binding modules and are highly conserved in both the animal and plant kingdoms. Coordination of a client protein within the amphipathic groove of a 14-3-3 dimer can have a range of context-dependent effects such as conformational change, re-localization and bridging of two molecules. Accordingly, 14-3-3 homologs seem to complete signal-induced and phosphorylation-dependent transitions in protein activity and have emerged as key modulators of signal transduction events.

Research in my laboratory aims to understand the biological functions of 14-3-3 proteins in higher plants. In addition to protein crystallization and structure determination, an array of biochemical, molecular and cell biological approaches is applied.

We have identified 14-3-3 proteins as positive regulators of the plant plasma membrane H^+-ATPase that provides the driving force for nutrient uptake and maintenance of cell turgor. The enzyme is kept at a low activity level by its C-terminal domain, the autoinhibitory action of which is released upon binding of 14-3-3 proteins. According to our data the enzyme's C-terminal domain harbors two nonclassical sites for regulatory 14-3-3 proteins: the phosphorylated extreme C-terminal end (YpTV-COOH) and an unphosphorylated motif located further upstream. The established complex represents the target for the wilt-inducing phytotoxin fusicoccin (FC), a well known activator of the pump *in vivo*. The molecular action of this diterpenglucosid was uncovered by X-ray analysis (Fig. 1). In brief, FC closes a gap that remains in the binding groove after coordination of the atypical phosphorylated binding motif of the H^+-ATPase leading to mutual stabilization as well as increase in binding affinity of both ligands. As a consequence of permanent activation of the H^+-ATPase, stomatal pores are irreversibly opened followed by wilting of plants.

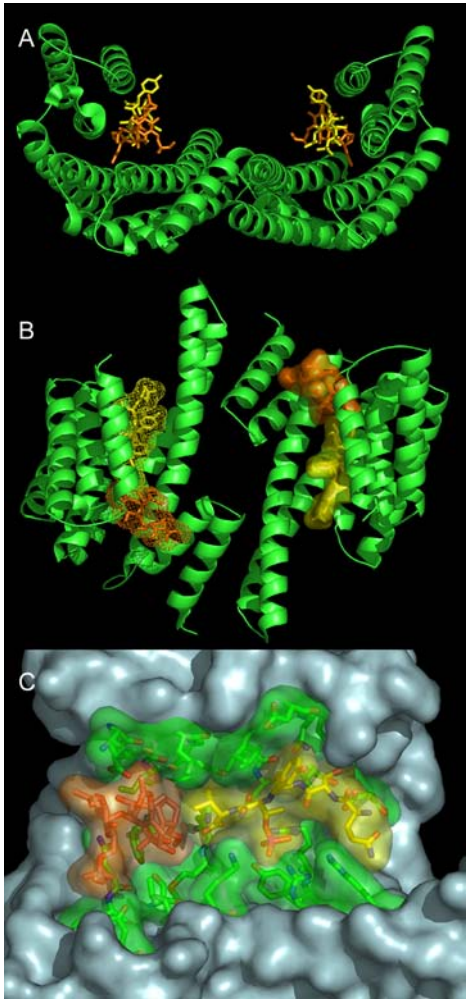

Fig. 1: The ternary 14-3-3/phosphopeptide/FC complex. Ribbon plot (A, B) of the phytotoxin FC (orange) and a phosphorylated pentapeptide representing the plant H^+-ATPase's extreme C-terminal end (QSYpTV-COOH; yellow) coordinated within a 14-3-3 dimer (green). (B) shows the view rotated by 90° about the horizontal axis. (C) Van der Waals surface representation of one amphipathic 14-3-3 binding cleft showing the close contact of both ligands.

Current results show that a 14-3-3 dimer is simultaneously occupied by two entire binding motifs of the H^+-pump – each consisting of two nonclassical sites - in an antiparallel fashion and an unusual conformation. Furthermore, the structure suggests an – up to now – unidentified mode of 14-3-3 action: association seems to induce the assembly of an active H^+-ATPase oligomer. Future experiments aim at elucidating the relevance of 14-3-3 proteins with respect to oligomerization. In addition, we have established an appropriate method for the identification of the protein kinase(s) mediating this important regulatory interaction.

In order to address the question of 14-3-3 function at the level of the whole organism, we have started to analyze gene expression patterns of individual 14-3-3 isoforms as well as their subcellular localization. For this purpose genomic GFP fusions of all thirteen *Arabidopsis* isoforms will be studied for expression in transgenic plants. In addition we have established a proteomic approach to identify novel target polypeptides whose interaction with 14-3-3 proteins will be studied at the molecular and cellular level. In this regard, interaction with the glycolytic enzyme enolase as one identified target was verified both *in vitro* (immunoprecipitation, affinity chromatography) and *in vivo* (bimolecular fluorescence complementation, BiFC). BiFC represents a novel method for direct visualization of protein interactions in living cells and is based on complementation between two nonfluorescent fragments of the yellow fluorescent protein (YFP). Interestingly, functional reconstitution of YFP was exclusively observed in the cytoplasm (Fig. 2 C, D) while homodimerization of the corresponding polypeptides (14-3-3: Fig. 2A, enolase: Fig. 2 B) could be additionally detected in the nucleus. Glycolytic enzymes are often considered rather dull enzymes. However, recent findings have shown that the enolase is bi-functional in that it additionally functions as a transcription factor required for expression of cold-responsive genes. Taken together, 14-3-3 binding possibly sequesters enolase in the cytoplasm and the release of 14-3-3 then allows the enzyme to relocate to the nucleus.

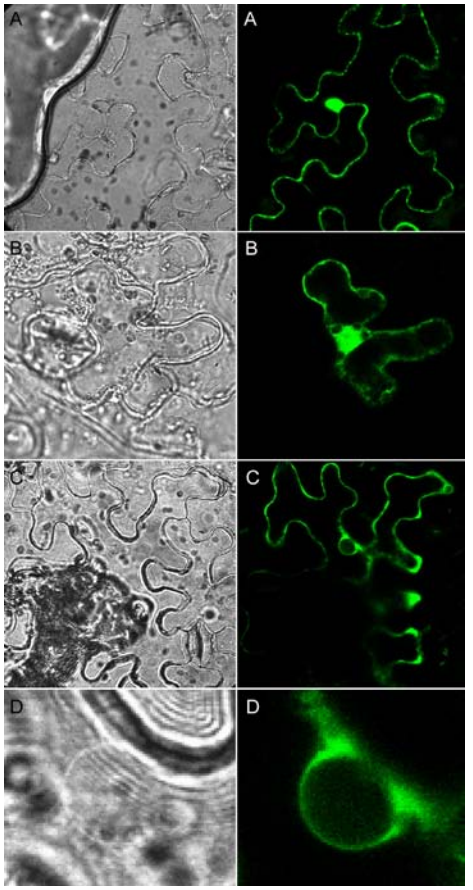

Fig. 2: BiFC visualization of protein-protein interactions in *Agrobacterium*-infiltrated *Nicotiana benthamiana* leaves. Confocal (right panel) and bright field (left panel) images of epidermal leaf cells are shown. (A) Homodimerization of 14-3-3, (B) homodimerization of enolase, (C) interaction between enolase and 14-3-3, (D) enlarged view of the nucleus shown in (C) demonstrating that association is restricted to the cytoplasm.

Last but not least, reverse genetics as well as manipulation of the expression of 14-3-3 homologs aims at elucidating their physiological roles and isoform-specific functions.

pH-homeostasis in the endomembrane system

Karin Schumacher

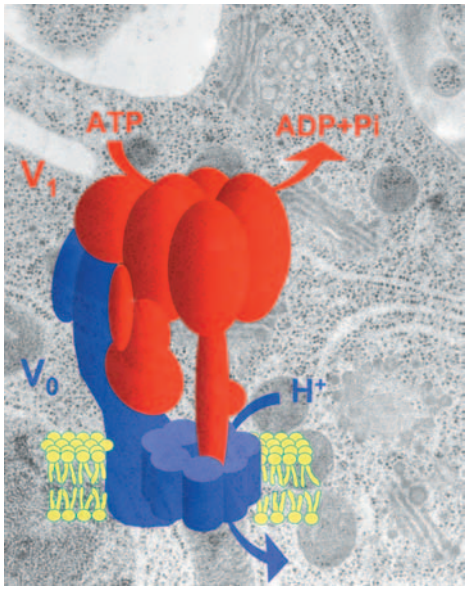

Fig. 1: Structural model of the V-ATPase, a proton pump found in the endomembrane system of all eukaryotes.

All organelles of the highly dynamic endomembrane system have characteristic luminal pH values suited to their biochemical functions. The maintenance and regulation of these acidic microenvironments is necessary, e.g. for membrane transport, protein trafficking and vesicular transport and is achieved by the activity of V-ATPases. V-ATPases are a class of primary electrogenic multi-subunit proton pumps found in all eukaryotes and consist of the cytosolic ATP hydrolysing V_1 subcomplex and the membrane bound proton translocating V_0 subcomplex. Despite their essential primary function, the cellular processes that depend on V-ATPase activity and the mechanisms that regulate V-ATPase activity and integrate it into the cellular signaling networks are largely uncharacterized.

Research in my laboratory aims to identify the biological functions and regulatory mechanisms of the plant V-ATPase. We have identified a number of mutant alleles for genes encoding Arabidopsis V-ATPase genes (VHA) and could show that null-alleles cause severe defects in Golgi morphology leading to lethality of either the male gametophyte (*vha-A*) or the embryo (*vha-E1,* collaboration with Ulrike Mayer). A conditional weak allele (*vha-C-1*) as well as inducible RNAi-lines allows manipulation of V-ATPase activity and studies on the physiological responses immediately after V-ATPase inhibition. For example, we could show that V-ATPase inhibition, leading to reduced cell expansion in the hypocotyl of etiolated seedlings, is accompanied by drastic changes in the transcription profile, including the induction of genes regulated by the phytohormone jasmonic acid. Interestingly, inhibitors of secretory trafficking and cell wall biosynthesis cause similar changes in gene expression.

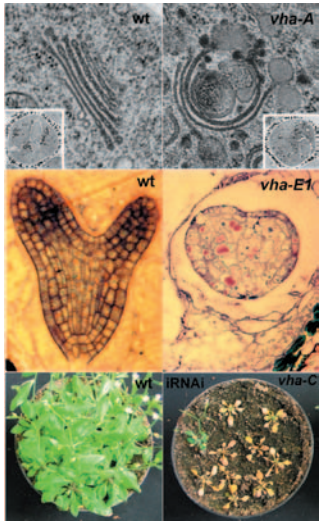

Fig. 2: Loss of V-ATPase activity disturbs Golgi morphology and leads to lethality of the male gametophyte (*vha-A*) or the embryo (*vha-E1*). Plants carrying an inducible VHA-C RNAi construct undergo cell death upon induction.

Furthermore, the mutant alleles serve as genetic backgrounds to study the *in vivo* significance of regulatory mechanisms (e.g. Redox-Regulation, ADP-binding, H^+/ATP coupling rate) that have so far only been analyzed *in vitro*.

In the Arabidopsis genome many of the V-ATPase subunits are encoded by small gene families, thus offering a large potential to adapt the V-ATPase to the requirements of different cell types or organelles.

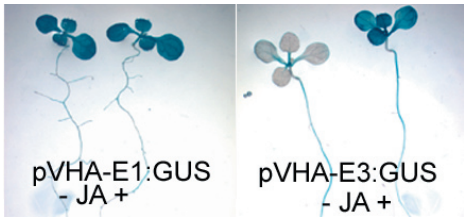

Fig. 3: Transcriptional regulation of V-ATPase isoforms. In contrast to the ubiquitous isoform VHA-E1, VHA-E3 is only expressed in certain tissues and is strongly induced by jasmonic acid.

Whereas most isoforms differ in their expression pattern and transcriptional regulation (Fig.3 A+B), the isoforms of VHA-a are ubiquitously expressed but differentially localized. In contrast to VHA-a2 and VHA-a3 that are found at the tonoplast, VHA-a1 resides in the trans-golgi network (TGN) (Fig4.). Co-localization experiments as well as uptake-studies with the endocytic tracer FM4-64 have shown that the TGN acts as an early endosomal compartment. Concanamycin A, a specific V-ATPase inhibitor causes characteristic changes in Golgi/TGN morphology and interferes with both secretory and endocytic trafficking leading us to postulate that both pathways merge in the TGN.

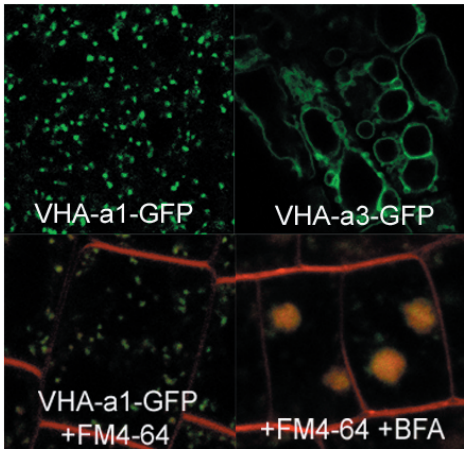

Fig. 4: Subcellular localization of V-ATPase isoforms. VHA-a3:GFP labels the tonoplast, whereas VHA-a1:GFP shows a punctate staining pattern that colocalizes rapidly with the endocytic tracer FM4-64 and accumulates in BFA-compartments.

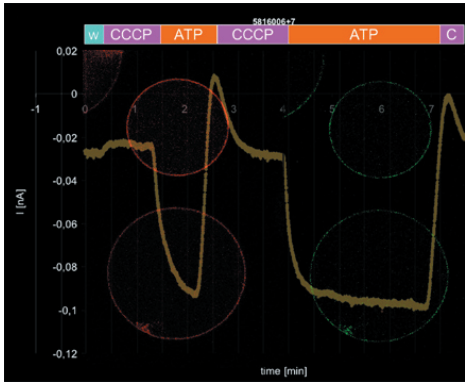

Fig. 5: Patch-clamp analysis and in vivo imaging of V-ATpase assembly are used to monitor V-ATPase activity. Vacuoles are labeled by VHA-A:RFP and VHA-a3:GFP.

Knowledge of the specific isoforms combined with the VHA-mutant alleles as genetic backgrounds enables us to address the functional differences individual isoforms confer to the V-ATPase complex. Biochemical and electrophysiological measurements are used to determine V-ATPase kinetics; moreover, we have established transgenic lines that allow *in vivo* imaging of V-ATPase assembly.

Last but not least, we have used the yeast two-hybrid system to identify proteins that interact with the V-ATPase. Both *in vitro* and *in vivo* (bimolecular fluorescence complementation, BiFC) methods are used to confirm these interactions. Physiological analysis of the respective mutants will show if these proteins are indeed involved in V-ATPase regulation.

The staff members provide the ZMBP with various services. These services include administration, plant transformation, plant cultivation, analytical facility, DNA-sequencing, microscopy and documentation, information technology and the workshop and engineering unit.

ZMBP Administration

Responsibilities

- Efficient and appropriate use of the resources available to the ZMBP
- Implementation of the decisions of the research group leaders with regard to the financial resources of the center
- Opening and management of budget accounts for the research groups of the center
- Financial planning to fulfil the central tasks of the ZMBP
- Management of payment transactions in cooperation with the university financial department

Staff

Dr. Harald Stransky (Head of Central Facilities)

Silvia Röcker (Business Assistant and Public Relations)

Plant Transformation Unit

The unit offers the service of Agrobacterium-mediated transformation of:

- potato (*Solanum tuberosum* L. cv. Désireé)
- tobacco (*Nicotiana tabacum* cv. SNN)
- tomato (*Lycopersicon esculetum* cv. Moneymaker)
- transformation of protoplasts

The main goal of the service unit is

- Generation of transgenic plants for the research groups
- Standardization of existing protocols for transformation and regeneration of plants
- Development of new transformation procedures (e.g. biolistic transformation) for further species

The service includes

- Collection and maintenance of different lines and genotypes
- Cultivation of sterile plant material
- Preparation of media
- Transformation of plant tissue and protoplasts
- Regeneration and cultivation of transformants

Staff

Dr. Wolfgang Koch (Head)

Caterina Brancato (Transformation Technician)

Anja Hoffmann (Transformation Technician)

Plant Growth Unit

These facilities consist of growth chambers (96 m^2), temperature controlled trial areas (100 m^2), glasshouse (400 m^2), six standard greenhouses (270 m^2), field greenhouse (126 m^2), and a two hectare field trial area.

The computer controlled glasshouse is composed of six compartments. Environmental conditions (temperature, humidity and light) in each cabinet are controlled individually.

The greenhouses provide a semi-controlled environment for growth and multiplication of transgenic plants.

The field trial area provides optimum conditions for testing plants in a controlled agricultural environment.

Staff

Tanja Sikler (Coordinator and Head Glasshouses)

Ernst Schwärzli (Coordinator and Head Field Trial)

Muhammad Golkary

Johanna Gray

Sofia Riegger

Ingrid Rudolph

Analytics Unit

The unit focuses on the qualitative and quantitative analysis of inorganic ions (NH_4^+, NO_3^-), primary plant metabolites (organic acids, amino acids, and sugars), lipids, plant pigments, and plant hormones.

The unit develops methods for metabolomic analysis using mass spectrometry coupled with gas chromatography (GC/MS). Due to the large diversity of chemical and physical properties of metabolites, additional methods must be combined to determine plant metabolomes. Therefore, liquid chromatography using micro-HPLC coupled with a quadrupole ion trap mass spectrometer (LC/MS) is still being developed.

Metabolomic analysis using GC/MS and LC/MS will lead us to a comprehensive and quantitative analysis of the metabolites of a biological system.

The service includes

- **Inorganic cations and inorganic anions**
  HPLC separation using size exclusion and conductivity detection
- **Organic acids, sugars and related compounds**HPLC separation using ion moderated partition chromatography (IMP) and multimode detection by conductivity, absorbance, and refractive index
- **Sugars**
  HPLC separation using IMP and multimode detection by conductivity, absorbance, and refractive index.
  HPLC separation using high pH anion exchange (HPAEC) and pulsed amperometric detection
- **Amino acids**
  HPLC separation using a multimodal process (mainly ion exchange, ion exclusion, and partition) on sulfonated crosslinked polystyrene, post column reaction with ninhydrin followed by photometric dual wavelength detection
- **Plant pigments**
  HPLC separation using reversed phase silica gel and diode array photometric detection at multiple wavelengths
- **Plant hormones**
  HPLC separation using reversed phase silica gel columns and fluorescence detection coupled with diode array photometric detection.
- **Metabolomic analysis**
  We use an Agilent 6890 GC and 5973 MSD (quadrupole mass spectrometer with electron impact ionisation). Chromatographic separation is achieved using a 30 m x 0.25 mm Supelco SPB50 column (polysiloxane column coated with 50% methyl and 50% phenyl groups).

Staff

Dr. Harald Stransky, Head Scientist

Bettina Stadelhofer, Technician

Karl Wurster, Chemical Engineer

DNA Sequencing Unit

The unit provides three fully automatic DNA sequencers enabling a rapid and flexible determination of DNA sequences for plant genes.

Staff

Dr. Wolfgang Koch

Markus Wunderlich

Microscopy Unit / Scientific Documentation

The Microscopy Unit supports research in using transmission and scanning electron microscopy as well as conventional and confocal light microscopy on biological samples ranging from molecules to complex tissues.

The Light- and Electron Microscopy Unit provides preparative techniques for morphological studies as well as for immunolocalization of gene products. The focus of our methodological research is on cryoimmobilisation-based specimen preparation procedures for both improved preservation of structural integrity and antigenicity and on methods for detection of low copy number antigens. This allows a detailed analysis of cellular processes on the molecular level in the context of complex biological systems.

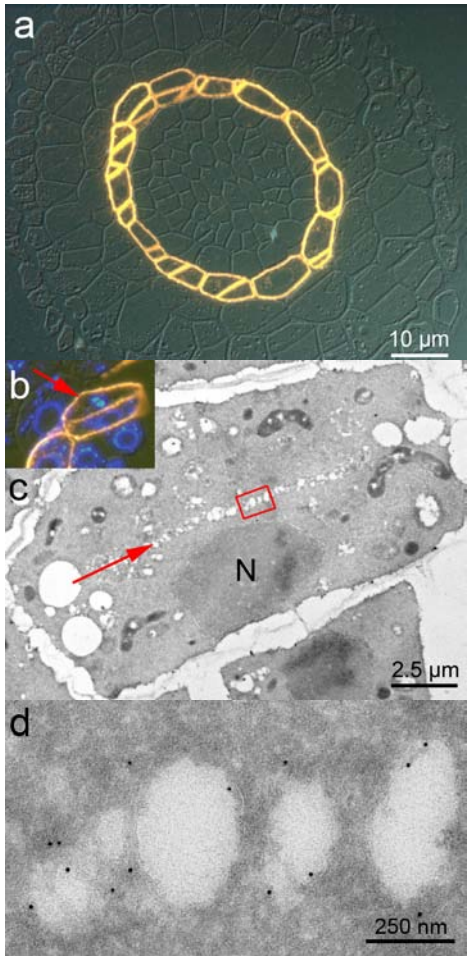

Fig 1: Immunolocalization of the plasma membrane amino acid proton co-transporter AAP3.
(a) Immunofluorescence localization of AAP3 (orange fluorescence) in endodermal cells of a cross-sectioned A. thaliana root tip. A thin cryosection is shown with additional weak differential interference contrast.
(b-d) Correlative LM-EM microscopy: (b) Immunofluorescence labelled dividing cell (blue: nuclei). (c) Immunogold labelling of the identical cell (arrow in (b)) in the corres¬ponding ultrathin TEM cryosection (overview). Arrow points to the cell plate plane. (d) Higher magnification of boxed area in (c) showing electron dense black gold markers on cell plate membranes. N, nucleus. (Collaboration with S. Okumoto & W. Koch).

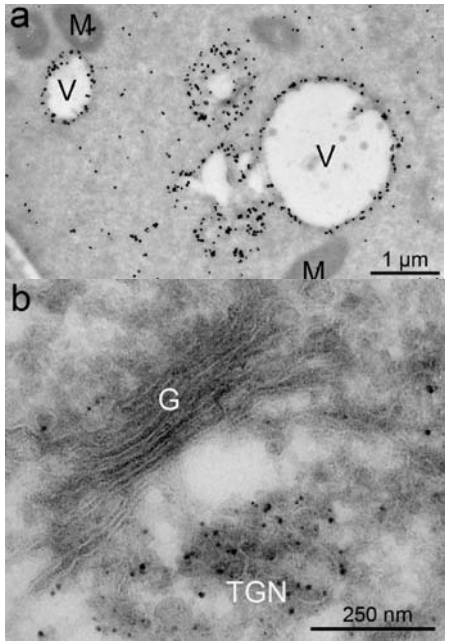

Fig 2: Immunogold localization of Vacuolar H^+-ATPase a (VHA-a) isoforms.
Immunogold localization of VHA-a isoforms fused to GFP on ultrathin cryosections of *A. thaliana* root tip cells using anti-GFP antibodies and silver-enhanced 1 nm gold markers (a,b). Subunit a3 targets the V-ATPase to vacuolar membranes (V) (a), whereas subunit a1 localizes to the trans-Golgi network (TGN) (b) (Collaboration with J. Dettmer & K. Schumacher)..

Techniques provided include

Negative staining, glycerol spraying, metal shadowing, freeze-drying, critical-point drying, cryoimmobilisation (high pressure freezing), freeze-substitution, (low temperature) embedding, ultrathin resin sectioning, ultrathin cryosectioning, immunogold labelling.

Our service includes

- Performing technically demanding and time consuming projects
- Help with designing experiments
- Assistance in preparation of biological samples
- Instruction in use of equipment
- Teaching

Staff

Dr. York-Dieter Stierhof	Head
Dagmar Ripper	Technician
Dr. Ulrike Mayer	Senior Scientist (Confocal microscopy)
Barbara Lahlou	Photographics

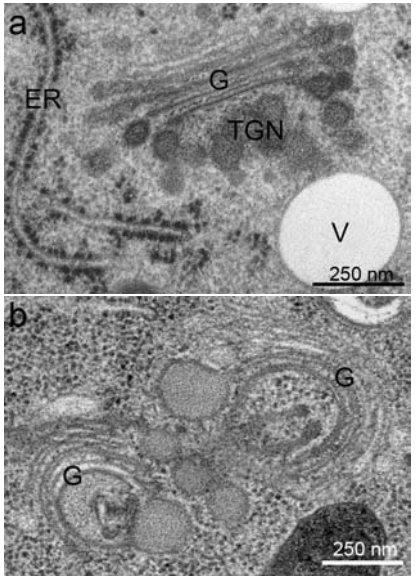

Fig 3: The V-ATPase inhibitor Concanamycin A induces changes in Golgi and TGN structure.
The TEM micrographs in (a) and (b) show resin sections of cryofixed root tips with Golgi stack (G), trans-Golgi network (TGN), endoplasmic reticulum (ER) and vacuoles (V). After treatment with the V-ATPase specific inhibitor Concanamycin A Golgi stacks and TGN are altered (d). (Collaboration with J. Dettmer & K. Schumacher).

Lab Ware Preparation and Autoclave Service

Ghabrab Cuflom

Susanne Dieterich

Rosa Hahn

Brigitte Schneck

Information Technology

This unit maintains the computer network and all communication systems. It is responsible for network security and backup data security. An additional task is the installation of software and support for all standard office computer applications.

The service includes

- Coordination computersystems/Network (CIP/WAP).
- Administration network problems (Windows 2003 Server).
- Computer problems (WindowsNT/2000/XP, Apple OS X).
- Helpdesk hard- & software.
- Computer and software training.
- Administration accounting software (ConAktiv).
- Administration ZMBP websites.

Staff

Dieter Steinmetz, Information Technology

Workshop and Engineering Unit

The ZMBP workshop is responsible for designing and engineering new equipment for experimental set up and for maintenance of scientific equipment.

Staff

Edgar Raidt, Head of Technical Support Staff

Karl Michalik, Head of Electronics Department

Werner Schneck, Master Technician

Anastasios Mourlidis, Workshop Technician

Gerhard Schneck, Technical Support

Martin Ammer, Apprentice

Marco Neumann, Apprentice

Developmental Genetics and Cell Biology

RG Friml

Auxin transport and action

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RG Gross-Hardt

Cell specification

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RG Jürgens

Pattern formation in *Arabidopsis* embryogenesis

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RG Schwechheimer

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General Genetics

RG Hochholdinger

Genetic dissection of root formation in maize

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RG Schöffl

Regulation and function of heat stress response

Zhang, L., Eggers-Schumacher, G., Schöffl, F., Prändl, R. (2001). Analysis of heat-schock transcription factor-DNA binding in *Arabidopsis* cultures by UV laser crosslinking. *Plant J* **28**, 217-223.

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RG Zentgraf

Leaf senesence of annual plants

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Plant Biochemistry

RG Felix

Receptor kinases and pathogen recognition

Chinchilla,D., Bauer,Z., Regenass,M., Boller,T., and Felix,G. (2006). The *Arabidopsis* receptor kinase FLS2 binds flg22 and determines specificity of flagellin perception. *Plant Cell*, *in press*.

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RG Lipka

Molecular dissection of non-host resistance in Arabidopsis

Stein, M., Dittgen, J., Sanchez-Rodriguez, C., Hou, B.-H., Molina, A., Schulze-Lefert, P., Lipka, V., Somerville, S. (2006) The plasma membrane resident ABC transporter PDR8 contributes to non-host resistance in Arabidopsis. *Plant Cell* in press.

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RG Nürnberger

Plant Innate Immunity

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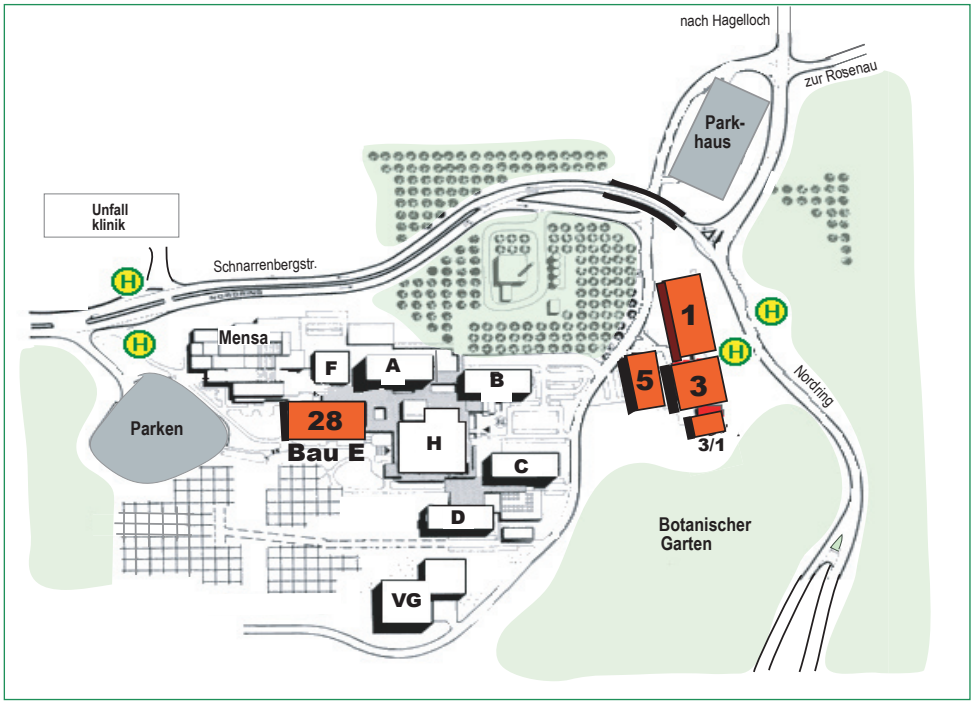

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72072 Tübingen