L2-1: PIN-dependent auxin gradients as a general mechanism in plant development

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

O2-1: Proteolytic control of PIN2 mediates root gravitropism

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Arabidopsis PIN proteins represent a family of membrane proteins, suggested to act as facilitators of auxin efflux. Specifically, the finding that several PIN proteins exhibit an asymmetric distribution at the plasma membrane provided evidence for their involvement in the regulation of polar auxin transport. The positioning of PINs at the plasma membrane appears to be controlled by a mechanism of continuous intracellular protein cycling, a response that would allow to adjust intracellular PIN localization in response to external and internal stimuli. Another, rather unexplored aspect of post-transcriptional control of PIN proteins, addresses the regulation of their proteolytic turnover. Here we show that the root-specific PIN2/EIR1/AGR protein is subject to asymmetric, cell-specific proteolysis in response to a gravity-stimulus. Moreover, it could be demonstrated that proteolytic turnover of PIN2 depends on its intracellular transport and the activity of the proteasome. We therefore postulate that control of gravity-mediated root bending involves a mechanism that mediates proteolytic turnover of the putative auxin efflux carrier PIN2.

L2-2: MDR/PGP-mediated auxin transport in *Arabidopsis*

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Plant orthologs of human multiple drug resistance/ P-glycoproteins (MDR/PGPs) function in cellular auxin transport, as *pgp* mutants exhibit reduced growth and auxin transport. Heterologous expression of PGP1/19 in mammalian cells resulted in increased auxin efflux, while PGP4 expression resulted in auxin influx. PGP expression also increased transport of synthetic auxins and benzoic acid, but not mammalian MDR substrates. Synergistic increases in auxin efflux resulted when PGP1/19 were coexpressed in HeLa cells. PIN2 expression, which appears to activate mammalian organic acid transporters, antagonized PGP1/19-mediated transport. PGP4-mediated efflux was antagonized by PIN1, but enhanced by PIN2. PGP-PIN coexpression resulted in increased auxin specificity and sensitivity to auxin transport inhibitors. PGP-PIN interactions were confirmed using yeast two-hybrid and co-IP assays. Mislocalization of PIN1 induced in *pgp19* hypocotyls by treatment with Triton X-100 reflects co-localization of PGP19 and PIN1 in detergent resistant “lipid rafts”. PGP19 appears to stabilize PIN1 in these structures, as PIN1 was preferentially detergent solubilized from *pgp19* and not wildtype membranes.

O2-2: ANTIMAX1, auxin transport and shoot branching

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Auxin has long been known to regulate shoot branching, but the mechanism by which it acts has been elusive. The *Arabidopsis MAX* genes may form part of this response, since mutants in these genes have increased branching. Here we report the characterization and cloning of *ANTIMAX1* (*ATM1*), an activation tagging suppressor of the *max1* phenotype.The *antimax1* lines have an overall wild-type appearance, but show novel phenotypes apparently related to an auxin over-response. We show that *ATM1* encodes a phospholipase A2, which acts locally in the stem to modulate auxin transport capacity. Furthermore, we demonstrate that the *max* mutants have increased auxin transport capacity, and that it is this which causes their branching phenotype. Elimination of this increased auxin transport restores branching to wild-type levels. We are thus able to present an integrated model that provides explanations for a mechanism of action of both *ANTIMAX1,* and auxin itself, in the regulation of shoot branching. We are currently testing this model.

O2-3: TWISTED DWARF1 modulates auxin transport activities of ABC transporter, AtPGP1, by protein-protein interaction

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The immunophilin-like TWD1 has been demonstrated to interact with ABC transporters PGP1 and PGP19. Analysis suggested a positive regulatory role of TWD1 on PGP1 and PGP19 thought to catalyze the active export of the auxin IAA. Coexpression of TWD1 and PGP1 in yeast showed that TWD1 inhibited auxin transport while IAA export from twd1 protoplasts is even more reduced than in pgp1 pgp19 mutants. Export of the synthetic auxin 1-NAA is reduced in atpgp1 atpgp19 but surprisingly not in twd1 mutants suggesting that TWD1 defines not only transport activities but also specificities. Moreover, polar auxin transport rates are also further decreased in twd1 mutants compared to atpgp1 atpgp19 plants. As a result twd1 mutant roots reveal elevated levels of free IAA that might cause the strong developmental phenotype. While atpgp1 and atpgp19 mutants have been shown to perform hyperphoto- and hypergravitropic responses, roots and shoots of twd1 plants are agravitropic. Additionally, twd1 and pgp1 pgp19 roots reveal reduced sensitivities toward auxin transport inhibitors, like NPA, suggesting that a functional TWD1/PGP1/PGP19 auxin export complex is a target of those. We provide molecular insights into the twd1 syndrome suggesting that TWD1 functions as regulator of auxin transport activities.

O2-5: PINOID, a calcium-regulated protein kinase directing auxin efflux

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Polar auxin transport depends on the polar sub-cellular localisation of the PIN auxin efflux regulators. Previous research has shown that overexpression of the PINOID (PID) protein kinase induces a basal-to-apical shift in PIN localisation, resulting in loss of auxin gradients and strong defects in embryo and seedling roots. Conversely, *pid* loss-of-function induces an apical-to-basal shift in PIN1 polar targeting at the inflorescence apex accompanied by defective organogenesis. Thus, a PINOID-dependent binary switch controls PIN polarity and mediates changes in auxin flow to create local gradients for patterning processes (Friml et al., 2004, Science 306). Our current research focuses on elucidating the PID signaling cascade. Through a two-hybrid screen in yeast we have identified three upstream components of PID. Two of these proteins are calcium binding proteins (Benjamins et al., 2003, Plant Physiol. 132) that interact with PID to regulate its activity in response to changes in cellular calcium levels. The third protein binds PID through its BTB domain, and seems to link PID with cytoskeletal proteins. We are currently developing a generic phospho-peptide-based tool to assist us in our search for phosphorylation targets of PID and the PID-like kinases.

O2-4: Fish bone; a rice auxin transport mutant with pleiotropic phenotypes

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The plant hormone auxin plays many important roles on the plant development, and a number of mutants with defects in polar auxin transport (PAT) have been so far reported in various plant species. Most of these mutants show abnormal phenotypes in either above- or under-ground organs. We have identified a rice mutant, fish bone (fib), which exhibits pleiotropic abnormalities in most of above- and under-ground organs. fib plants show abnormal phyllotaxy, disrupted vascular systems, aberrant floral organs, reduced numbers of crown roots and lateral roots, agravitropism and so on. The wild type plants treated with PAT inhibitor mimicked most of the fib phenotypes, suggesting that fib has defects in PAT. Then, we have measured PAT activities in root and inflorescence stem. The results indicate that PAT activity is impaired in fib plants. In addition, fib plants also show reduced auxin contents and increased auxin sensitivity, but detailed analyses suggest that these are secondary effects caused by PAT defects. Thus, many abnormalities in fib are caused by defects in PAT. In summary, fib is distinct from other PAT mutants in that it shows pleiotropic abnormalities in both above- and under-ground parts. The FIB gene would play important and novel roles in controlling PAT.

O2-6: Expression of AtPINs in tobacco cells increases auxin efflux and induces phenotype changes typical for auxin depletion

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The polar auxin transport plays a key role in the regulation of growth and development in plants. Morphogenic gradients of auxin concentration in various plant tissues are sustained, among others, by the activity of PIN proteins, putative auxin efflux carriers. However, the direct evidence for PINs as real "auxin efflux catalysts" is still not available. We have shown, that the overexpression of *AtPIN6* and *AtPIN7* genes in transformed tobacco BY-2 cells decreased strongly auxin accumulation inside cells suggesting the increase of auxin efflux. Consequently, enormous changes in cell morphology typical for the response to auxin depletion were brought about. These changes included the cessation of cell division, stimulation of cell elongation and amyloplasts formation. Both 1-*N*-naphthylphthalamic acid (NPA), an inhibitor of auxin efflux, and ten-times higher external auxin (2,4-dichlorophenoxyacetic acid) were capable to prevent all observed "auxin-depletion"changes. Altogether, our observations strongly support the idea that PIN proteins are the auxin efflux catalysts, exporting auxin out of the cell. (The work was supported by the Grant Agency of the ASCR, No. A6038303.)

P2-1: A pharmacological approach to study auxin redistribution

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Active auxin transport is catalysed by two carriers, the influx and efflux carriers, working in opposition at the plasma membrane. Phytotropins such as NPA have been invaluable in demonstrating a role for the auxin efflux carrier in coordinated polar auxin transport; however the function of the influx carrier at the level of the whole plant is still unclear. To help understand the role of these carriers in various aspects of plant development our laboratory is working to identify novel compounds able to alter auxin distribution patterns. To this end, Thirty-five aryl and aryloxyalkylcarboxylic acids were assayed for their ability to perturb the accumulation of 2,4-dichlorophenoxyacetic acid (2,4-D) and naphthalene-1-acetic acid (1-NAA) in suspension cultured tobacco cells (*Nicotiana tabacum* L.). One candidate similar in structure to 2-Naphthylacetic acid, the non steroidal anti-inflammatory drug, (*S*)-(+)-2-(6-methoxy-2-naphthyl)propionic acid (Naproxen) was found to increase 1-NAA accumulation in cells. We show here that, despite its ability to inhibit auxin efflux, in preliminary experiments using hypocotyls sections; Naproxen appears to have little effect on PAT. We also report the effects of Naproxen on auxin transport in root sections and present a detailed characterisation of the phenotypic effects of Naproxen application on plant development.

P2-2: Characterization of OsENT2 encoding an equilibrative nucleoside transporter suggests a function in cytokinin transport

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We identified four genes for potential equilibrative nucleoside transporters (ENTs) from *Oryza sativa* (designated OsENT1 through OsENT4). Growth analysis of budding yeast (*Saccharomyces cerevisiae*) cells expressing OsENTs showed that OsENT2 transported adenosine and uridine with high affinity (adenosine, Km = 3.0 μM; uridine, Km = 0.7 μM). Purine or pyrimidine nucleosides and 2'-deoxynucleosides strongly inhibited adenosine transport via OsENT2, suggesting that OsENT2 possesses broad substrate specificity. In competition experiments with various cytokinins (CKs), adenosine transport by OsENT2 was inhibited by isopentenyladenine riboside (iPR). Direct measurements with radiolabeled CKs demonstrated that OsENT2 mediated uptake of iPR (Km = 32 μM) and trans-zeatin riboside (Km = 660 μM), suggesting that OsENT2 participates in iPR transport in planta. In mature plants, OsENT2 was predominantly expressed in roots. The OsENT2 promoter drove the expression of the beta-glucuronidase reporter gene in the scutellum during germination and in vascular tissues in germinated plants, suggesting a participation of OsENT2 in the retrieval of endosperm-derived nucleosides by the germinating embryo and in the long-distance transport of nucleosides in growing plants, respectively.

P2-3: Does PaLAX1 function as an auxin influx carrier?

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It has been suggested that the product of *AUX1* gene from *Arabidopsis thaliana*, as well as other *AUX1*-type genes identified in various plant species, can act as an auxin influx carrier. We have isolated the cDNA of the gene Pa*LAX1* from *Prunus avium*. The gene and its product are sequentially highly identical to both the cDNA and the corresponding protein sequences of *AUX1*-type genes. We report the overexpression of *AUX1*-type gene in transgenic plants. We have characterized transformed *Nicotiana tabacum* and Arabidopsis lines, which carry gene Pa*LAX1* controlled by constitutive promoters. In both plant species, the uptake of auxin, 2,4-dichlorophenoxyacetic acid, was four times higher in transgenic lines when compared to the wild-type lines. Moreover, the internal concentration of indole-3-acetic acid, the major endogenous auxin, was also four times higher in transgenic tobacco plants than in the wild-type control plants. The phenotypical changes caused by the overexpression of Pa*LAX1* gene in transformed plants corresponded closely to the increase in endogenous levels of auxin. Therefore, we conclude that the product of the gene Pa*LAX1* promotes the uptake of auxin and, as an auxin influx carrier, affects the overall content of endogenous auxins in transgenic plants.

P2-4: The mechanism of accumulation of cytokinins in suspension-cultured tobacco cells

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Cytokinins are small non-polar molecules thought to be transported across cell membranes by simple diffusion, even if a purinpermease was reported in Arabidopsis to transport zeatin. We have studied kinetics of cytokinin transmembrane transport by direct measurement of labelled cytokinin accumulation in the cells of tobacco line BY-2 (*Nicotiana tabacum* L., cv. Bright Yellow-2). Isopentenyladenine, isopentenyladenosine, zeatin and zeatin riboside were accumulated rapidly in the cells; the process was linearly time-dependent for concentrations from 2 nM to 200 nM. The accumulation of isopentenyladenine was saturated at concentration 10^{-7} M, accumulation of isopentenyladenosine seemed to be more effective. Benzyladenine and dihydrozeatin inhibited the influx of isopentenyladenine, while kinetin and adenine did not. Isopentenyladenosine accumulation was inhibited by cytokinin ribosides, but not by adenosine itself. Isopentenyladenine, but not isopentenyladenosine, was metabolised promptly after uptake. Together, our findings suggest that, in tobacco cells, isopentenyladenine is accumulated *via* simple diffusion, whereas iPR accumulation seems to be partly dependent on active carrier-driven transport. (The work was supported by the Grant Agency of the ASCR, No. A6038303.)

P2-5: Characterisation of tobacco cell lines transformed with the PaLAX1 gene

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Auxin is the only plant hormone which is transported through plant body by a special polar auxin transport machinery that includes a balanced system of passive diffusion as well as influx and efflux carriers. We report transformation of the BY-2 tobacco cells with the gene Pa*LAX1* from *Prunus avium*, which is a gene sequentially highly homologous to *AUX1* (coding for a putative auxin influx carrier). We have obtained 15 transgenic lines with various phenotypes differing from controls. Two transgenic lines with remarkably contrasting phenotypes, one with elongated cells and the other one with spherical cells, were chosen for further studies. The treatments with 1- and 2-naphthoxyacetic acids (inhibitors of auxin influx) resulted in a significant delay of the commencement of cell divison in both these transgenic lines while the control cells, under the same treatment, did not divide at all. The measurements of accumulation of radioactively labelled 2,4- dichlorophenoxyacetic acid, and the determination of internal content of native auxin, indole-3-acetic acid, suggest that the gene product, PaLAX1, plays an important role in the uptake of auxin into cells. (The work was supported by the Grant Agency of the ASCR, No. A6038303 and B6038203.)

P2-7: *Arabidopsis* and tobacco cell suspensions as tools to study the mechanism of auxin transport

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A substantial amount of molecular and cytological data about the polar auxin transport machinery comes from studies at the tissue level on *Arabidopsis thaliana* and its mutants. However, the diversity of cell types within even the tiny roots of *Arabidopsis* makes it almost impossible to investigate biochemical aspects of auxin transport at the cell level in a meaningful way. Thus there is a need for some alternative system that would enable detailed quantitative studies of auxin transport. Cell suspension cultures provide good model systems for these studies at the level of a single cell and its compartments. In comparison with the well-known tobacco cell line BY-2, all *Arabidopsis* cell suspension cultures reported so far were not convenient for direct measurements of auxin accumulation because they tended to form cell clusters. We show here that cell cluster formation in *Arabidopsis* cell culture could be partly diminished by manipulation with auxin concentration in cultivation medium. Such *Arabidopsis* cell suspension allowed us to study quantitative aspects of accumulation of radioactively labelled auxins and to compare them with the auxin accumulation kinetics in tobacco BY-2 and *PIN*-transformed cells. (The work was supported by the Grant Agency of the ASCR, project No. A6038303.)

P2-6: Computational modelling of long-distance auxin transport in a study of the induction of axillary bud outgrowth in pea

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One of the first and most enduring roles identified for the plant hormone auxin is the mediation of apical dominance. We have tested whether indole-3-acetic acid (IAA) inhibits the initial stage of bud release in garden pea (*Pisum sativum* L.), or subsequent stages, by providing a rigorous examination of the dynamics of auxin level, auxin transport and axillary bud growth. We have developed a simple computational model of long-distance auxin transport based on endogenous auxin levels and on a substantial data set of radiolabelled auxin transport in the intact polar auxin transport system over a range of auxin concentrations. Our studies indicate that auxin transport rates in the mature stem are probably not concentration dependent at physiologically relevant auxin concentrations. After decapitation, *initial* bud growth occurs prior to changes in IAA level or transport in surrounding stem tissue and is not prevented by an acropetal supply of exogenous auxin nor induced soon after depletion in auxin content caused by auxin transport inhibitors. The data supporting these findings are underpinned by our "continuous" model of long-distance auxin transport. We propose that auxin mediates apical dominance via inhibition of buds that are already in transition towards sustained growth.

P2-8: Characterisation of tobacco cell lines transformed with the *AtPIN1, 4, 6, 7* genes from *Arabidopsis*

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Since the chemiosmotic theory was formulated in 1974, predicted auxin efflux carriers become an important part of our understanding how uneven and yet coordinative auxin distribution throughout the plant is achieved. In this study we have transformed cells of BY-2 (*Nicotiana tabacum* L., cv. Bright Yellow-2) line with genes from *PIN* family coding for putative auxin efflux carriers in *Arabidopsis*. We have placed At*PIN4, 6, 7* under the control of glucocorticoid-inducible system and after transformation we gained sets of lines for each of investigated genes. We have also constitutively transformed BY-2 cells with *PIN1::PIN1:GFP* gene construct to study the localization of PIN *in vivo*. Independent of the identity of the particular gene or type of overexpression (constitutive or inducible), we have observed characteristic changes in cell morphology typical for the response to auxin depletion. These changes included cessation of cell division, stimulation of cell elongation and amyloplast formation. By measurements of accumulation of radiolabelled naphthyl-1-acetic acid we have shown that the overexpression of At*PIN4, 6, 7* genes decreased auxin accumulation inside cells; these findings are consistent with predicted function of PINs as auxin efflux carriers. (The work was supported by the Grant Agency of the ASCR, no. A6038303.)

P2-9: Auxin-mediated cross-regulation of PIN expression

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The phytohormone auxin (indole-3-acetic acid) has multiple and complex effects on plant development. Important facilitors of auxin efflux, and thus of auxin transport, are the members of the PIN protein family. Although PIN-dependent local auxin distribution is required for the correct apical-basal patterning, organogenesis and tropistic growth, the developmental defects in most of the *pin* single mutants are only mild. Interestingly, these defects become much more pronounced in *pin* multiple mutants revealing a functional redundancy between the different PIN proteins. However, PIN proteins have only partially overlapping domains of expression. How functional replacement is possible became clear by analyzing *pin* k.o. mutants, in which specific PINs were ectopically expressed in the domain of the missing PIN. Moreover, this ectopic PIN expression can be phenocopied by treating plants with auxin or the auxin transport inhibitor NPA. This demonstrates that the cross-regulation of PIN expression is directly or indirectly mediated by auxin. A detailed analysis of the auxin-mediated PIN expression and its role for PIN functional redundancy will be presented.