Charles University, Prague
Faculty of Science

Department of Plant Physiology

Hormonal regulation of plant cell division and elongation

Ph.D. Thesis

Jan Petrášek **2007**

Hormonal regulation of plant cell division and elongation

A dissertation presented to the Department of Plant Physiology, Faculty of Science, Charles University in partial fulfilment of the requirements for the PhD degree

Jan Petrášek

from Prague

Charles University

Prague

2007

SUPERVISOR

Ass. Prof. Dr. Eva Zažímalová

Institute of Experimental Botany ASCR, Prague, Czech Republic
Department of Plant Physiology, Faculty of Science, Charles University, Prague, Czech Republic

CO-SUPERVISOR

Prof. Dr. Zdeněk Opatrný

Department of Plant Physiology, Faculty of Science, Charles University, Prague, Czech Republic

ACKNOWLEDGEMENTS

I would like to thank to all co-authors of the original articles included in this thesis. Their valuable contributions are specified in detail in the heading of respective chapters.
For their constant support I am very grateful to my family, all my dear colleagues and both supervisors in particular. Thank you all very much!
This work was supported by several local and international grant projects. Their numbers are specified in headings of respective chapters.

This is to certify that this thesis is not a subject of any other defending procedure. It contains set of original results that have been published in the international peer reviewed scientific journals.

Prague 1.2.2007 Jan Petrášek

On behalf of the co-authors of the papers published, I hereby confirm the agreement with inclusion of the papers below into the dissertation thesis of Jan Petrášek. The papers were produced as a team work and the particular contribution of Jan Petrášek is specified at the beginning of relevant chapters of the thesis.

Prague 1.2.2007 Eva Zažímalová

CONTENTS

ABSTRACT

The body of any eukaryotic organism, including plants, consists of many individual cells. The resulting physiological state as well as the morphology of the multicellular organism is determined to a certain level by processes running at the cellular level. Besides basic metabolic processes, both oriented cell division and growth play the key role in the organization of multicellular organisms. In plants, which are formed from cells enwounded with a rigid cell wall, the spatial orientation of these processes is crucial for shaping the plant and for maintaining the developmental plasticity. Auxins and cytokinins are one of the major plant regulatory compounds playing role in the induction and sustaining of cell division and growth. The processes of their biosynthesis and metabolic modifications, as well as the intra- and intercellular transport of these compounds are crucial for setting of the effective concentrations of phytohormones; the model of organogenesis *in vitro* was suggested, based on the concentration ratio between auxins and cytokinins.

This dissertation thesis is focused on hormonal regulation of basic physiological processes taking place in plant cells cultured *in vitro*. It is shown that plant cells can keep balanced levels of physiologically active cytokinins by means of their secretion into the cultivation medium. This process might actually reflect hormonal cell-to-cell communication. Following work is concentrated entirely on the auxin and its transport. It was shown that directional flow of auxin is important for maintaining the polarity of cell division. Fluorescence and *in vivo* localizations proved that inhibitors of auxin transport have very specific effect on putative auxin efflux carrier activity rather than they generally inhibit vesicle-mediated protein traffic to the plasma membrane. The data emphasise the importance of actin filaments and endomembranes in vesicle-mediated trafficking of proteins and in the cycling of the auxin efflux catalysts. The measurement of auxin accumulation in tobacco cells provided one of the evidences for the novel mechanism of auxin action. It is based on the stimulation by auxins of their own transport by means of modulation of auxin transporters vesicle trafficking. Controlled overexpression of PIN proteins in tobacco cells and subsequent quantitative measurements of auxin accumulation suggested a direct involvement of PINs in catalyzing cellular auxin efflux. The overproduction of PINs induced phenotypic changes, which were previously reported to be typical for the response to auxin depletion. These changes include the cessation of cell division, stimulation of cell elongation and amyloplasts formation. The fact that the application of inhibitors of auxin transport as well as higher concentration of exogenous auxin were capable of preventing all the observed "auxin-depletion"-induced changes points to the modification in auxin efflux after overexpression of PINs.

Results summarised in this thesis contributed to the understanding of the molecular processes involved in hormonal control of plant cell development, namely the efflux of auxin at the cellular level and the mechanism of action of auxin transport inhibitors.

ABSTRAKT

Tělo každého organismu, včetně rostlin, je tvořeno mnoha buňkami. Výsledný fyziologický stav a také tvar těla jsou přitom dány povahou procesů, které probíhají na buněčné úrovni. Vedle základních metabolických procesů v širokém slova smyslu jsou to zejména procesy buněčného dělení a růstu, které jsou klíčové v organizaci těla mnohobuněčných organismů. U rostlin, které jsou tvořeny buňkami s poměrně rigidní buněčnou stěnou, navíc nabývá na důležitosti prostorové směrování těchto procesů, dávající výsledný tvar celému rostlinnému tělu a napomáhající udržovat vývojovou plasticitu. Auxiny a cytokininy jsou základní růstové regulační látky rostlin, nezbytné pro navození a udržování procesů dělení a růstu buněk. Účinná koncentrace těchto látek je určena mnoha procesy zahrnujícími nejen jejich biosyntézu a metabolické přeměny, ale také jejich vnitrobuněčný a mezibuněčný transport. Na určitých, přesně vymezených poměrech koncentrací auxinů vůči koncentracím cytokininů je pak založen model organogeneze v tkáňových kulturách rostlinných buněk pěstovaných *in vitro.*

Tato disertační práce je zaměřena na hormonální regulace základních fyziologických procesů probíhajících u rostlinných buněk kultivovaných v podmínkách *in vitro*. Bylo zde prokázáno, že rostlinné buňky jsou schopny exkretovat fyziologicky aktivní cytokininy do svého okolí a že tento proces může představovat prostředek mezibuněčné komunikace. Další práce, zaměřená na auxiny a jejich transport, vedla ke zjištění, že směrovaný tok auxinu je klíčový v udržení polarity buněčného dělení. Výsledky fluorescenčních lokalizací a lokalizací *in vivo* naznačují, že inhibitory transportu auxinu nepůsobí prostřednictvím obecné inhibice vezikulárního transportu, ale že tyto látky velmi specificky inhibují proteiny exportující auxin z buněk. Data navíc ukazují na důležitou roli aktinových filament a endomembrán při transportu membránových váčků i vlastního transportéru auxinu z buňky. Měření akumulací auxinu v buňkách tabáku dále poskytlo jeden z důkazů naznačujících nový mechanismus působení auxinů, který spočívá ve stimulaci jejich vlastního exportu z buněk inhibicí internalizace proteinů PIN. Řízená nadprodukce proteinů PIN v buňkách tabáku a následné měření akumulace auxinu pomohlo podat důkaz, že proteiny rodiny PIN se účastní procesu přenosu auxinu z buněk přímo, velmi pravděpodobně jako vlastní přenašeče. Jejich nadprodukce je spojena s fenotypovými změnami buněk, které byly dříve popsány jako reakce na auxinové hladovění. Jedná se o zastavení buněčného dělení, tvorbu škrobových zrn a stimulaci buněčného prodlužování. Aplikace inhibitorů transportu auxinu, stejně tak jako zvýšené koncentrace externě přidávaných auxinů, odvrátily všechny tyto projevy. To jasně ukazuje, že příčinou těchto fenotypových projevů byly změny v aktivitě přenašeče auxinu ven z buněk.

Výsledky shrnuté v této disertační práci přispěly k pochopení molekulárních procesů podílejících se na hormonální regulaci vývoje rostlinných buněk, především s ohledem na transport auxinu z rostlinných buněk a mechanismus působení inhibitorů transportu auxinu.

GENERAL INTRODUCTION

Cell division and elongation: setting the way of plant morphogenesis by phytohormones

Functional totipotency of plant cells as postulated already by Haberland (1902) reflects sessile life strategy of plants, where every individual cell is ready for switch in its developmental program facilitating many alternative ways of morphogenesis. Plant cells are enwound with cell wall, a structure formed from polysacharides and proteins. Therefore, after mitosis both orientation of freshly formed cell plate at the end of cytokinesis and subsequent cell growth is crucial for maintaining this developmental plasticity. Equal and inequal cell division, cell elongation and isodiametric cell growth - they all allow establishing, forming and maintaining character of tissues, organs and whole bodies during plant development.

Scientific research has always been focused on regulatory compounds, the action of which induces detectable and observable responses. By uncovering the exact mechanisms of their mode of action, many intermediate messengers were found. However, classical concept of “major regulators” survived until these days. Indeed, plant hormones, small organic molecules with wide range of effects, are typical representatives of such compounds, studied already around one hundred years. They are synthesised and utilised in various tissues throughout the whole plant and they could be transported in vasculature to long distances.

The research of recent years contributed remarkably to the understanding of signalling cascades triggered by plant hormones, including auxins and cytokinins. This knowledge greatly helps to understand how these hormones influence morphogenetic processes leading to particular organogenesis.

The aim of this general introduction to six chapters (published papers), which form this dissertation thesis, is to provide brief overview on the knowledge about hormonal regulation of the processes of cell division and growth. It is focused on the role of auxins and cytokinins, phytohormones with possibly the most extensive range of biological effects described, including their activity in *in vitro* cultures. The main attention is paid to the transport of these hormones across membranes, its regulation and how it influences plant cellular morphogenesis.

Auxin action and its regulation

Auxin (indole-3-acetic acid, IAA) as an endogenous plant compound participates on control of cell division, polar elongation and differentiation. In general, processes regulating auxin metabolism coordinate both spatial and temporal aspects of plant growth and development (review by Woodward and Bartel, 2005; Teale et al., 2005; Jenik and Barton, 2005; Teale et al., 2006). According to present knowledge, the mechanism of action of auxin includes its binding to specific binding protein(s) of TIR1 (TRANSPORT INHIBITOR RESPONSE 1) family belonging to F-box proteins, subunits of SCF protein complex (review by Badescu and Napier, 2006; Quint and Gray, 2006) and subsequent activation of nuclear proteasome-mediated specific degradation of auxin-inducible Aux/IAA transcription factors (review by Leyser, 2006). This mechanism represents an elegant and efficient control of gene expression triggered by auxin and is common for several other phytohormones (review by Lechner et al., 2006; Bishopp et al., 2006). However, auxin action is much more complex and, besides its intracellular metabolism, it depends on the cell-to-cell transport of auxin molecules.

Proteins playing role in cell-to-cell transport of auxin

Auxin is the only plant hormone that undergoes polarized transport in the cell-to-cell manner, a complex process resulting from both passive diffusion of auxins into cells and the activities of auxin-influx and efflux carriers. Directional transport of auxin coordinates many processes of plant development (reviews by Friml and Wisniewska, 2005; Tanaka et al., 2006a), it is inherent part of auxin signalling (reviews by Paciorek and Friml, 2006; Berleth et al., 2005; Vogler and

Kuhlemeier, 2003) and it is realized by coordinated action of carrier proteins and their regulators (reviews by Fleming, 2006; Blakeslee et al., 2005a; Benjamins et al., 2005).

The explanation of polarity of cell-to-cell auxin transport was simultaneously proposed by Rubery and Sheldrake (1974) and Raven (1975) and it is known as chemiosmotic polar diffusion model (Goldsmith, 1977). According to this model, an undissociated, lipophilic form of native auxin molecule (IAA) can easily enter the cell cytoplasm from slightly acidic extracellular environment (pH 5.5) by passive diffusion. Since the pH of cytoplasm is more alkaline (pH 7), IAA molecules dissociate and resulting hydrophilic auxin anions (IAA^-) are trapped in the cytosol. The exit or uptake of IAA^- were proposed to be assisted by the auxin efflux and influx proteins, respectively, and the polarity of auxin transport was explained by their asymmetric distribution on the plasma membrane of the cell (see Chapter 6 of this thesis, Fig. 1).

During past decade, several promising candidates for auxin-transporting proteins and relevant regulatory mechanisms were suggested and tested. Because of the lack of direct evidence of auxin-transporting role of some of these candidates, they are very often named as "facilitators".

Characterization of *Arabidopsis thaliana auxin resistant* (*aux1*) agravitropic mutants showing resistance to IAA, led to the identification of AUXIN-RESISTANT1/LIKE AUX1 (AUX/LAX) transmembrane proteins sharing similarity with plant amino acid permeases, group of proton-gradient-driven transporters (Bennett et al., 1996; Parry et al., 2001; Swarup et al., 2004). AUX1 protein regulates root gravitropism by facilitating auxin uptake (Marchant et al., 1999) and its asymmetrical localization in the plasma membrane of root apical and epidermal cells may contribute to the loading of auxin into the root tip from the phloem (Swarup et al., 2001). It was shown recently that heterologously expressed AUX1 is capable of transporting IAA in *Xenopus* oocytes (Yang et al., 2006) providing quite clear biochemical evidence for the role of AUX1 as auxin-influx carrier. It should be noted that in addition to AUX1, members of a group of aromatic and neutral transporters (ANTs) of amino acid transporters were shown to transport auxin inside cells (Chen et al., 2001) in *Arabidopsis thaliana*.

The investigation of several other mutants of *Arabidopsis thaliana* resulted in the identification of candidates for auxin efflux carriers. These were root gravitropic mutants *agravitropic* (*agr*) and *wavy6* (*wav6*) (Bell and Maher, 1990; Okada and Shimura, 1990), root-specific ethylene-insensitive mutant *ethylene insensitive root1* (*eir1*) (Roman et al., 1995) and one of the floral mutant *pin-formed* (*pin*) (Kappert 1959; Goto et al., 1987). The mutant phenotype of *pin1* with disturbed organ initiation and phyllotaxy resulting in the absence of flower buds was shown to be phenocopied by auxin efflux inhibitors. These mutants had drastically decreased transport of IAA (Okada et al., 1991). Corresponding *PIN1* gene encodes the protein with two transmembrane domains separated by a hydrophilic region sharing sequence similarity with bacterial membrane transporters (Gälweiler et al., 1998). Subsequently, other genes corresponding to all above mentioned mutations were identified and shown to be allelic with other member of *PIN* family, *PIN2*. These were *AGR1/WAV6/EIR1/PIN2* (Chen et al., 1998; Utsuno et al., 1998; Luschnig et al., 1998; Müller et al., 1998). Because of its sequence similarity with bacterial membrane transporters of small molecules and the ability to export toxic analog of IAA, 5-fluoro-indole, when heterologously expressed in yeast, EIR1 has been suggested to transport auxin (Luschnig et al., 1998). To these days, eight members of AGR/WAV/EIR/PIN protein family were identified in *Arabidopsis thaliana* and commonly referred as PIN1-8 (reviewed by Paponov et al., 2005; Zažímalová et al., 2007). In addition, there are seven other, yet uncharacterized, *PIN*-like genes in *Arabidopsis thaliana* (Chen and Masson, 2005). Particular PIN proteins, namely PIN1, PIN2, PIN3, PIN4, PIN6 and PIN7 have been shown to be involved in controlling various morphogenetic processes such as embryogenesis (Friml et al., 2003), root development (Friml et al., 2002a; Blilou et al., 2005), organ formation (Benková et al., 2003), vascular differentiation (Mattsson et al., 2003; Scarpella et al., 2006), tropisms (Friml et al. 2002b; Blakeslee et al., 2004) and phyllotaxis (Reinhardt et al. 2003). In

these processes, PIN proteins form functionally redundant network and their asymmetrical intracellular distribution seems to reflect main streams of directed auxin flow; the basipetal transport of auxin in shoots (towards stem basis) through cells of stele continuing in the acropetal transport towards shoot apex and an opposite basipetal transport (towards basis of root) through epidermal and cortical cells (for review see Chen and Masson, 2005; Tanaka et al., 2006a). The role of PIN5 and PIN8 proteins, lacking central hydrophilic loop, is not clear yet. Similar way as with AUX1 protein, heterologous expression systems were used to provide biochemical evidence for the role of PIN proteins as auxin efflux carriers. Yeast cells expressing *AGR* gene released preloaded IAA more rapidly than control cells (Chen et al, 1998). Chapter 5 of this thesis brings the evidence that heterologously expressed *PIN2* and *PIN7* genes in mammalian and yeast cells export auxin out of the cell. Moreover, it is shown there that *Arabidopsis* PIN1, PIN4, PIN6 and PIN7 play rate-limiting function in auxin efflux from *Arabidopsis* and tobacco cells.

Another proteins playing role in auxin influx and efflux are plant orthologs of mammalian ABC (ATP-binding cassette)-type transporters of multidrug resistance/p-glycoprotein (MDR/PGP) protein family (Noh et al., 2001; reviewed by Geisler and Murphy, 2006). In the screen for proteins having affinity to phytotropin 1-naphthylphthalamic acid (NPA) (Murphy et al., 2002; Terasaka et al., 2005) potential candidates for auxin efflux (AtPGP1, AtPGP4 and AtPGP19) and influx proteins (AtPGP4) have been identified. Although the phenotype of some *pgp* mutants indicates disturbed polar auxin transport, it is, in contrast to *pin* mutants, usually not mimicked by treatment with auxin efflux inhibitors. Biochemical evidence for auxin transporting role of PGPs have been provided by heterologous expression of *AtPGP1* and *AtPGP4* in HeLa and yeast cells resulting in their ability to export and import auxin and fluoroindoles (Geisler et al., 2005; Terasaka et al., 2005; Santelia et al., 2005). Consistently with these results, in chapter 5 of this thesis, increased efflux of auxin from tobacco cells overexpressing *AtPGP19* is shown. AtPGP1 and AtPGP4 are asymmetrically distributed in endodermal and cortex cells of root elongation zone, however, their intracellular distribution in the root apex is uniform (Geisler et al., 2005; Terasaka et al., 2005). How this localization reflects directional auxin flow remains to be answered.

There is one more candidate for auxin-transporting protein, transmembrane protein TM20 (Jahrmann et al., 2005). Heterologous expression of this protein in *Xenopus* oocytes was able to modify transport of IAA across membrane. The asymmetric distribution of TM20 was observed in more differentiated tissues of maize embryo and it has no sequence similarity with any protein encoded in *Arabidopsis thaliana* genome. Therefore, it may represent a novel component in auxin transport across membrane.

Regulation of auxin transport

One of the most fruitful approaches in studying the regulation of auxin transport machinery is the application of various inhibitors. The most widely used inhibitor of auxin efflux is NPA, which belongs to a group of inhibitors known as phytotropins (Rubery 1990). The application of NPA to plant tissues results typically in the increase of auxin accumulation, presumably due to the inhibition of auxin efflux activity (for review see Morris et al. 2004). Detailed knowledge about the mechanism, by which NPA and other phytotropins inhibit auxin efflux, is still lacking. NPA probably binds to a specific high affinity NPA-binding protein (NBP) located on the cytoplasmic face of the PM (Sussman and Gardner 1980), where it is associated with actin cytoskeleton (Cox and Muday 1994; Dixon et al. 1996; Butler et al. 1998). In addition, more general, inhibitory effect of phytotropins on endocytotic processes was reported (Geldner et al. 2001).

Another set of results that helped the understanding of auxin transport machinery comes from the studies using fungal toxin brefeldin A (BFA), the inhibitor of Golgi-mediated anterograde vesicle trafficking and endosomal recycling. BFA inhibits auxin efflux activity in zucchini hypocotyls (Morris and Robinson 1998) and blocks auxin transport through the tissue

(Robinson et al. 1999). Since BFA treatment effectively changes the proportion of proteins localized at the plasma membrane and in the endosomal space (review by Geldner 2004), it is an ideal tool for studying constitutive cycling of both putative auxin efflux and influx carriers.

It is very probable that the function of all yet characterized auxin transport-facilitating proteins is coordinated. Indeed, as shown recently by Blakeslee et al. (2007), *Arabidopsis thaliana* PGP1 and PGP19 colocalize with PIN1 in the shoot apex and with PIN1 and PIN2 in root epidermal cells. Yeast two-hybrid assay as well as coimmunoprecipitations suggested interaction between PINs and PGPs in contrast to PGPs and AUX/LAX proteins. Moreover, co-expression of PINs and PGPs in heterologous systems enhances auxin transport, suggesting that both transport systems act independently, but might interact in a tissue-specific manner. The authors proposed a model, where PINs, PGPs and AUX/LAX proteins define independent transport mechanisms that are coordinated at whole-plant level. PINs are primary determinants of a directional auxin movement required in developmental processes and PGPs may export auxin from tissues with high auxin production, i.e. from apical tissues.

As mentioned above, the localization of auxin-transporting proteins in the plasma membrane is crucial for directional flow of auxin. It must be noted that auxin efflux carriers seem to be parts of plasma membrane-localized complex, consisting of auxin transporting molecule(s) itself and other regulatory proteins. Therefore, the regulation of the activity as well as targeting of auxin-transporting proteins into the plasma membrane has great impact on all auxin-triggered processes. In this respect, the most studied are the vesicle-cycling machinery (including cytoskeleton) and the phosphorylation status of its components.

There is a growing amount of evidence that AUX1, PINs and PGPs cycle between plasma membrane and endosomes by processes of constitutive cycling of proteins. Using vesicular trafficking inhibitor BFA, it was shown that PINs might undergo constitutive cycling between plasma membrane and an endosomal space (Steinmann et al., 1999; Geldner et al., 2001), as already indirectly suggested by Robinson et al. (1999). Similarly, BFA interfered with the localization of AUX1 (Grebe et al., 2002) and PGPs were shown to be associated with a number of secretory proteins suggesting common mechanism of their trafficking (Murphy et al., 2002; reviewed by Blakeslee et al., 2005b). The whole concept of trafficking and proper localization as well as function of components of the auxin efflux carrier complex was proposed, based among other things on the similarities with the trafficking of mammalian glucose transporter GLUT4 (Muday and Murphy, 2002; Muday et al., 2003). Trafficking processes of candidates for auxin efflux carriers and the immunolocalization of IAA in endosomes (Schlicht et al., 2006) suggest another attractive similarity between auxin and neurotransmitter release from neuronal cells (for review see Brenner et al., 2006). However, mechanisms underlying the constitutive cycling of proteins in plants are still very poorly understood (reviewed by Murphy et al., 2005; Geldner and Jürgens, 2006).

It was shown that constitutive cycling of PIN1 is regulated by GNOM, one of the ADP-ribosylation factors (ARF)-GDP-GTP (Steinmann et al., 1999; Geldner et al., 2003) and that other ARFs might contribute to the trafficking of PINs (Xu and Scheres, 2005). Since there are differences in the sensitivity of various PINs to BFA, there might exist several distinct paths for trafficking of various PINs (Geldner et al., 2003). In case of trafficking of PIN2, pathway containing *Arabidopsis thaliana* SORTING NEXIN 1 (AtSNX1), a protein implicated to play a role in mammalian endosomal sorting, has been described recently (Jaillais et al., 2006). Moreover, for both PIN1 and PIN2, clathrin-dependent endocytosis has been shown to play a role in their endosomal trafficking (Dhonukshe et al., 2007). *Arabidopsis* protophloem cells of root tip were used to show novel GNOM-independent and BFA-insensitive endosomal pathways for trafficking of AUX1 (Kleine-Vehn et al., 2006), the asymmetrical distribution of which seems to be assisted by endoplasmic reticulum accessory protein AXR4 playing the role probably in posttranslational modifications of AUX1 (Dharmasiri et al., 2006). Independent regulation of trafficking of putative auxin influx and efflux carriers might be crucial in physiological processes

such as root gravitropism where AUX1 in concert with PIN2 mediates basipetal auxin transport (Swarup et al 2005). In case of PGPs, the activity of AtPGP1 and AtPGP19 seems to be regulated by their interaction partner, plasma membrane glycosylphosphatidylinositol (GPI)-anchored protein TWISTED DWARF1 (TWD1) and FASCICLIN-LIKE ARABINOGALATAN PROTEIN2 (FAGP2)/FASCICLIN2 (FAS2) (Geisler et al., 2003; Bouchard et al., 2006). TWD1 has been proposed to stabilize auxin efflux complex at the plasma membrane, to play a role in endocytic trafficking of PGPs (Geisler et al., 2003) and to modulate auxin efflux activities in *Arabidopsis thaliana* (Bouchard et al., 2006). Since mammalian GPI-anchored proteins and PGPs are known to stabilize sterol-rich microdomains in the plasma membrane, plant PGPs might have similar role in the stabilization of auxin efflux complexes containing PINs shown previously by Noh et al. (2003). Indeed, sterol composition of membranes reflecting the activity of STEROL METHYL TRANFERASE1 (SMT1) enzyme was shown to be crucial for the positioning of PINs in the plasma membrane as was suggested by Willemsen et al. (2003).

Interestingly, the endocytosis-dependent cycling of proteins in plant cells was shown to be regulated by auxin efflux inhibitors (Geldner et al., 2001) as well as by auxin itself (Chapter 4 of this thesis). By inhibition of endocytosis of PINs, auxin increases abundance and thus capacity of efflux transporters at the plasma membrane resulting in increased efflux of auxin from the cell (Chapter 4). The feedback regulation was postulated by Sachs (1991) and it is known as canalization hypothesis. According to this hypothesis, auxin has a feedback effect on both its own transport and directionality of its flow.

The role of phosphorylation in the regulation of auxin transport was studied in *Arabidopsis thaliana* mutants with phenotypes typical for altered auxin transport *root curl in NPA 1* (*rcn1*) and *pinoid* (*pid*). *RCN1* gene encodes a regulatory subunit of protein phosphatase-2A (PP2A), a heterotrimeric serine/threonine protein phosphatase (Garbers et al., 1996; Deruere et al., 1999). Treatment with inhibitors of protein phosphatases were shown to mimic *rcn1* phenotype (Rashotte et al, 2001) and the activity of these phosphatases sensitive to cantharidin and okadaic acid (Shin et al., 2005) regulated PIN2-mediated gravitropism in roots of *Arabidopsis thaliana*. *Arabidopsis* mutant *pid* has phenotype similar to *pin1* and corresponding *PID* gene encodes auxin-inducible serine/threonine protein kinase, playing a role during embryogenesis and organ development (Bennett et al., 1995; Christensen et al., 2000; Benjamins et al., 2001; Furutani et al., 2004). It was demonstrated that the activity of PID controls the polarity of PINs targeting (Friml et al., 2004) and enhances auxin efflux (Lee and Cho, 2006). Moreover, *Arabidopsis thaliana* 3-phosphoinositide-dependent protein kinase 1 (PDK1) has been shown recently to stimulate the activity of PID kinase, providing the evidence that upstream phospholipid signalling might play a role in the regulation of auxin transport (Zegzouti et al., 2006). Since the amino acid sequence of PINs contains phosphorylation motifs (Zažímalová et al., 2007) it is possible that PINs themselves might be one of the possible targets of PID activity, directly or through other kinases. Altogether, auxin-inducible PID protein might be under feedback regulation of auxin (see above) thus controlling by specific phosphorylation the polar targeting of PINs at the plasma membrane.

At least for auxin efflux, the correct positioning of plasma membrane auxin-transporting complex seems to be assisted by the components of cytoskeleton. The evidence came from the experiments with the application of actin drugs that resulted in the reduced polar auxin transport in maize coleoptiles (Cande et al., 1973) and in zucchini hypocotyls (Butler et al. 1998). The process of endosomal cycling of PINs is actin-dependent (Geldner et al., 2001; reviewed by Muday and Murphy, 2002). The application of myosin inhibitor 2,3-butanedione monoxime (BDM) was shown to impair auxin-induced cell division (Holweg et al. 2003) and the mutation in *Arabidopsis* myosin VI led to the inhibition of basipetal auxin transport (Holweg and Nick 2004). However, as shown in Chapter 3 of this thesis, the application of some phytotropins was not affecting the structure of cytoskeleton itself. Together, these observations strongly suggested that actin filaments are involved in both intracellular trafficking of PINs and in their correct

positioning at the plasma membrane. Based on the results with anti-tubulin drugs, it seems that also microtubules might provide PINs with certain positional information (Boutté et al., 2006), although their role is not clear yet.

Cytokinin action and its regulation

Cytokinins, often defined as substances promoting cell division in plant tissue cultures with optimal concentration of auxins (Horgan, 1984), are adenine derivatives with pleiotropic physiological effects (review by Sakakibara, 2006). Their mechanism of action is based on their binding to receptor histidine kinase and subsequent autophosphorylation of receptor molecule and phosphotransfer protein followed by phosphorylation of response regulator that initiates transcription of cytokinin response genes (review by Bishopp et al., 2006; Ferreira and Kieber, 2005). So, in contrast to auxin, cytokinins do not act *via* specific degradation but their response regulators are positive or negative regulators of a specific expression. Although there are some evidences about the existence of a small purine permease, activity of which is competitively inhibited by cytokinins (Gilliseen et al., 2000; Burkle et al., 2003), a possible directional cell-to-cell transport of cytokinins has not been demonstrated. It seems that the translocation machinery of cytokinins is probably shared with purines and nucleosides.

Cell division and growth and their regulation by auxins and cytokinins

In principle there are two major ways by which plants increase in their size. They are somatic cell multiplication (division) and cell expansion (growth). Cell multiplication is realized by nuclear division (mitosis) followed by the partitioning of the cytoplasm (cytokinesis). Regulation of cytokinesis requires a series of links between the nuclear cycle, the cell cortex, the Golgi apparatus, and the membrane trafficking apparatus (reviewed recently by Jürgens, 2005a,b). The polarity and quality of cytokinesis is set by the sequence of events securing correct positioning of newly formed cell wall arising from the specialized membrane structure, cell plate. These events are realized by directional deposition of material along actin and microtubular cytoskeleton to the site of newly formed cell plate. Here, vesiculo-tubular network is formed with help of dynamins, ubiquitous eukaryotic GTPases (review by Konopka et al., 2006). The cytokinesis could be understood as a specialized form of secretion (Assaad, 2001) with targeted deposition of material to the cell plate. In plants, cell division is restricted to meristems containing repeatedly dividing cells in their centre. Cells that are gradually released from meristems may undergo various developmental programs, depending on their position and function in the tissue. This so-called cell fate is largely influenced by the processes of directional cell expansion leading to the establishment and maintenance of cell axiality and polarity. In general, there are two main types of plant cell growth: diffuse and tip growth. Diffuse type of growth runs along the whole surface of the cell (cells released from root, shoot or leaf meristems) and it is the main type of cell growth shaping plant body. In contrast, tip growth is restricted to the tip of the cell (pollen tubes, root hairs) and represents the most dynamic growth of plant cells running by targeted secretion of cell wall components.

Cytokinesis and cell growth are under complex mutual regulation that forms the architecture of plant tissues (review by Traas and Bohn-Corseau, 2005; Ramirez-Parra et al., 2005; Gutierrez, 2005). Auxins and cytokinins are involved in this regulatory network at several levels ranging from the regulation of the expression and activity of specific protein kinases and cyclins at the G1/S and G2/M checkpoints (Richard et al., 2002; review by Swarup et al., 2002) to the complicated mutual cross-talk (review by Swarup et al., 2002). Nice example of the hormone-triggered regulation of signalling pathway is the process of lateral root initiation in cells of pericycle, which seems to be dependent on the interplay between auxin, cell cycle progression, cell fate and the developmental status of the pericycle (Vanneste et al., 2005; De Smet et al., 2006). The crosstalk between auxins and cytokinins play an important role in the regulation of apical dominance. Auxin, perhaps by repressing the expression of isopentenyltransferase, a key

enzyme in the cytokinin biosynthesis, is able to prevent biosynthesis of cytokinins in the nodal stem and thus to inhibit outgrowth of axilary buds (Tanaka et al., 2006b; Nordström et al., 2004).

Hormonal regulation of plant cell division and growth in simplified models

When studying the hormonal regulation of cell division and growth, various cultures of *in vitro* propagated organ, tissue or cell cultures are often used. These models allow investigation of morphogenetic processes leading to particular organogenesis without the "background" of the rest of plant body. Auxins and cytokinins are among the most intensively studied plant hormones and their balanced levels in tissue culture are determining pattern of development. Morphoregulatory effect of auxin-cytokinin ratio was described in tobacco tissue culture, where more cytokinins induced shooting, more auxins rooting and balanced levels of both regulators induced callus formation (Skoog and Miller, 1957). Today, this concept is still valid and balanced levels of exogenously applied auxins and cytokinins are often used as the only essential hormonal factors maintaining repeated cell division in cell cultures.

With respect to processes of cell division, growth and elongation, cell lines cultured in liquid medium could serve as fruitful models. Highly friable cell lines of *Nicotiana tabacum*, L., cv. Bright Yellow (BY-2; Nagata et al., 1992; Geelen and Inzé, 2001) and cv. Virginia Bright Italia VBI-0 (Opatrný and Opatrná, 1976) are among only very few models, if not the only ones, which simulate in their growth phases roughly the behaviour of meristematic, elongating and even root cap cells (Sakai et al., 2004). In the life cycle of such axially dividing cell cultures, timely separated cell division and cell growth (elongation) could be understood as the simplified example of patterning process.

However, there exist cell cultures with various levels of habituation, acquired, mitotically transmissible ability to proliferate without exogenous supply of phytohormones. Transcriptome analysis of cytokinin-habituated *Arabidopsis thaliana* cell culture provided important data on changes in the gene expression in cytokinin habituated lines (Pischke et al., 2006). These authors reported changed expression of genes playing role in cytokinin signalling as well as their reception. Therefore, it seems that changed levels of intracellular concentration of cytokinins as the only reaction to external hormone withdrawal is not the only way to overcome the absence of exogenous cytokinins. Although in a very simplified systems of protoplast cultures there is no initiation of cell division without exogenous supply of auxin (Schell et al., 1999) and cells derived from tobacco mesophyll divide extensively when both auxin and cytokinin is supplied (Stickens et al., 1996; Tao and Verbelen, 1996), at the level of cell suspension culture both hormone-independent growth and cell division occur very often. Auxin-autotrophic lines have been derived from the individual VBI-0 cells by selection on hormone-free medium (Petrášek, unpublished). In one of these lines, VBI-I1, cells are spherical and the polarity of cell files is less expressed as compared to VBI-0. If standard concentrations of synthetic auxins, naphthalene-1-acetic acid (NAA) and 2,4-dichlorophenoxyacetic acid (2,4-D), are added the polarity of cell files is re-established and even enhanced (Campanoni et al., 2001). Interestingly, the selection of hormone-autonomous cell line 2B-13 from BY-2 cell line (Shimizu et al., 2006) and subsequent analysis of a substance that is inducing cell division when added to auxin-starved cells (Shimizu et al., 2006) led to the identification of a protein structurally related to P-glycoprotein from *Gossypioides kirkii*, which belongs to ATP-binding cassette (ABC)-transporters. Indeed, transcripts of ABC transporters are among the most frequent ones in reaction to auxin starvation in BY-2 cells (Gallis et al., 2004; Matsuoka et al., 2004).

Since it is difficult to study the biochemical aspects of auxin transport by measurements at the whole-plant and organ level (see above), plant cells cultured in liquid medium represent a valuable alternative. In Chapter 6 of this thesis results obtained using BY-2 cell line as well as other tobacco cell lines in the study of auxin transport mechanisms are summarized. In contrast to cell suspensions of *Arabidopsis* (which tend to form big cell clusters), the major advantage of

intensively dividing cell cultures of tobacco is that the effects on cell morphology of various inhibitors as well as of auxin itself can be observed in parallel to the measurements of auxin accumulation. Since tobacco cell lines of a good quality are completely friable, it is possible to express all measured data as an equivalent per cell number. During the period of cell division, such high-quality tobacco cell lines usually form polar cell files instead of cell clusters. Based on the studies using tobacco cell line VBI-0, the inhibition of auxin transport by NPA and the consequent rise in the internal auxin level delayed the onset of cell division and disrupted its polarity (Chapter 2 of this thesis). Disturbed polarity resulting from NPA treatment was was observed later also in BY-2 cells by Dhonukshe et al. (2005). Mathematical modelling suggested that NPA possibly disturbs the gradient in auxin concentration along cell files of VBI-0 cells (Campanoni and Nick 2003). This effect might be mediated by the actin cytoskeleton as shown by Holweg et al. (2003) using inhibitors of myosin action. Based on these results, it can be proposed that auxin actually synchronizes pattern of cell division (review by Nick, 2006).

VBI-0 line is routinely maintained on both NAA and 2,4-D. These two auxins may control cell division and cell elongation by different pathways (Campanoni and Nick 2005). Therefore, in this respect BY-2 cell line is a better model, because it is only 2,4-D-dependent and 2,4-D is not so good substrate for its auxin efflux carriers as it is in VBI-0 cell line (Chapter 5 of this thesis).

Objectives and outlines of this thesis

The main objective of the work presented in this thesis was to contribute to the understanding of hormonal regulation of plant cell division and growth. For this purpose, simplified models of tobacco cell lines VBI-0 and BY-2 were chosen. In these cytokinin-autotrophic cell lines, cell division intensity as well as its polarity can be manipulated by changing the exogenous supply of auxin. Effective actual concentrations of auxins and cytokinins inside cells result from many processes. These include changes of exogenous sources of phytohormones, biosynthesis, conjugation and degradation of internal phytohormones and their intracellular and intercellular transport. Changes of both exogenous and endogenous auxin and cytokinin levels were studied with respect to processes of cell division and growth. Major questions that have been raised in this thesis are connected with the mechanism of auxin transport, because, as stated above, it is critical for maintenance of balanced auxin levels and thus for keeping proper cell and organ polarity. However, at the beginning of the work, cytokinins as necessary partners of auxins were addressed, and their secretion into the cultivation medium in relation to cell development was studied.

Chapter 1 of this thesis is the continuation of the work started in our laboratory by my supervisor, Dr. Eva Zažímalová. During the studies of regulation of internal levels of hormones it was observed that decreasing auxin levels in the cultivation medium resulted in the substantial increase of levels of biologically active cytokinins during exponential phase of growth of VBI-0 cells (Zažímalová et al., 1996). At the time, when the work started, there were not many evidences about possible release of endogenous cytokinins into the cultivation medium as well as about the proportion of internal and released cytokinins. In this Chapter, we describe how cells of the cytokinin-autonomous tobacco cell line VBI-0 keep balanced levels of physiologically active cytokinins. Cytokinins were excreted from the cells during the whole subcultivation period, and their concentrations in the cultivation medium were found to be roughly proportional to their momentary levels inside the cells. The excretion might thus represent one of the mechanisms controlling internal cytokinin concentrations and maintaining hormonal homeostasis. As shown in our following work (Motyka et al., 2003), inducible expression of isopentenyl-transferase gene (encoding the key enzyme of cytokinin biosynthesis) in tobacco suspension cells (*Nicotiana tabacum*, L., cv. Wisconsin 38) led to the increase of cytokinin excretion into the cultivation medium. Moreover, overproduction of cytokinins resulted in increased cell division activity resulting in higher number of smaller cells. These results strongly suggest the existence of regulatory mechanisms controlling transport of cytokinins across plasma membrane.

Similar to cytokinins, the ratio of external to internal concentration of auxins is crucial for the regulation of plant cell division and growth. In **Chapter 2**, a description of relationship between auxin accumulation (reflecting actual auxin flow through the cell) and cell division in tobacco VBI-0 cell line is presented. It was shown earlier (Zažímalová et al., 1995) that decreased levels of auxin in the cultivation medium are compensated by increased production of endogenous auxin IAA. In this work we have described (for the first time at cellular level) the effect of NPA, an inhibitor of polar auxin transport on the level of auxin efflux, on cell division polarity. The application of NPA to tobacco VBI-0 cells results in increased accumulation of labelled synthetic auxin [^{3}H]NAA in cells and temporary and reversible inhibition of cell division activity. Partial loss of cell polarity and disturbed orientation of cell division were observed when cell division activity resumed. The data suggest that an NPA-binding regulatory protein is involved in directing of a proper localisation of auxin efflux carrier to specific regions of the plasma membrane. The protocol for the measurement of auxin transport characteristics based on the method by Delbarre et al (1996) was adapted for VBI-0 cells and described in detail in Zažímalová and Petrášek (2000).

Chapter 3 is the contribution to the research on the mechanism of action of phytotropins, inhibitors of auxin transport, and how they regulate cell division polarity. We have compared the effect of phytotropin NPA and vesicle trafficking inhibitor BFA on both auxin transport and cellular structures (cytoskeleton, endoplasmic reticulum) in tobacco BY-2 cells. Both these compounds strongly decreased auxin efflux, but treatment with BFA, in contrast to NPA, had substantial impact on structures of actin cytoskeleton and membranes of endoplasmic reticulum. This observation together with dose-response relationships suggest that NPA, and perhaps other phytotropins as well, have very specific effect on putative auxin efflux carrier activity rather than they generally inhibit vesicle-mediated protein traffic to the plasma membrane (Geldner et al., 2001). Furthermore, the data emphasise the importance of actin filaments and possibly endomembranes in vesicle-mediated trafficking of proteins and in the cycling of the auxin efflux catalyst itself.

Besides the above-mentioned role of phytotropins, the regulation of auxin efflux carrier activity could be, in principle, under regulatory control of auxin molecule itself. Modulation of subcellular protein translocation is actually one of the possible mechanisms, how signalling molecules can control cell behaviour. In animal and human cells this mode of regulation is often based on constitutive cycling, which consists of repeated internalisation of proteins from and recycling them back to the plasma membrane. In spite of the fact that several plant proteins, including PINs, exhibit constitutive cycling, no such mechanism of hormone action has been shown in plants yet. In **Chapter 4** a novel mode of plant hormone action is implied. The evidence came, among others, from our experiments with VBI-0 and BY-2 cells. Pre-treatment with 2,4-D, a weak substrate for auxin efflux carrier(s) in BY-2 cells, significantly increased the efflux of NAA (a good substrate for auxin efflux carriers there) and abolished the inhibitory effect of BFA, suggesting that auxin stimulates its own efflux by a vesicle-trafficking-dependent mechanism. Therefore, by modulation of vesicle trafficking of PIN proteins, auxin regulates their incidence and activity at the cell surface, providing a mechanism for the feedback regulation of auxin transport from cells.

Since plant tissues are hardly accessible for direct measurements of auxin accumulation in individual cells, until recently the evidence that PINs are real "auxin efflux catalysts" has been missing. In **Chapter 5** the rate-limiting function of PIN proteins in cellular auxin efflux is described in detail in tobacco BY-2 cells. Moreover, we show here that PINs mediate auxin efflux from mammalian and yeast cells without needing additional plant-specific factors. Conditional gain-of-function alleles and quantitative measurements of auxin accumulation in *Arabidopsis* and tobacco cultured cells revealed that the action of PINs in auxin efflux is distinct from PGP, rate-limiting, specific to auxins, and sensitive to auxin transport inhibitors. This suggests a direct involvement of PINs in catalyzing cellular auxin efflux, and implies the

existence in plant cells of two distinct auxin efflux pathways: PIN-dependent and PGP-dependent. **Chapter 6** has been published as an invited paper in a book devoted to tobacco BY-2 cell line. It summarizes and discusses recent progression in the field of auxin transport that has been achieved using tobacco cultured cells. Their advantages in studies of processes such as cell division, elongation and establishment of cell polarity are highlighted. In addition to that, summary of our recent results is presented in Fig. 3 of this Chapter. It describes phenotypic changes in reaction to the overproduction of PIN proteins, which were previously reported to be typical for the response to auxin depletion. These changes include the cessation of cell division, stimulation of cell elongation and amyloplasts formation. The fact that NPA as well as higher concentration of exogenous auxin were capable of preventing all observed "auxin-depletion"-induced changes points out the modification in auxin efflux after overexpression of PINs.

CONCLUSIONS AND PROSPECTS

The main objective of the work presented in this thesis was to contribute to the understanding of hormonal regulation of plant cell division and growth. For this purpose, simplified models of tobacco cell lines VBI-0 and BY-2 were chosen. In these auxin-dependent and cytokinin-autotrophic cell lines, cell division intensity as well as its polarity can be manipulated by changing the external supply of auxin. Changes of both exogenous and endogenous auxin and cytokinin levels were studied with respect to processes of cell division and growth. Major questions that have been raised in this thesis are connected with the mechanism of auxin transport across plasma membrane, because it is critical for maintenance of balanced auxin levels and thus for promoting the standard growth cycle and keeping proper cell file polarity.

It has been shown in this thesis that cytokinins were excreted from the cells during the whole subcultivation period, and their concentrations in the cultivation medium were found to be proportional to their momentary levels inside the cells. The excretion might thus represent one of the mechanisms controlling concentrations of internal endogenous cytokinins and, generally, the results suggested the existence of possible regulatory mechanisms controlling transport of cytokinins across plasma membrane. Since the cell division and growth of many tobacco cell lines is dependent on the supply of auxin from external source, the ratio of external to internal concentration of auxins is crucial for the regulation of cell division and growth there. It has been observed that inhibition of auxin efflux across membrane resulted in increased accumulation of auxin in cells and in temporary and reversible inhibition of cell division activity accompanied by the change of its polarity. Parallel comparison of the effects auxin efflux inhibitor 1-naphthylphthalamic acid (NPA) and vesicle trafficking inhibitor brefeldin A (BFA) on both auxin transport and the state of cellular structures (cytoskeleton, endoplasmic reticulum) in BY-2 cells revealed their differential effects on cellular structures. Both these compounds strongly decreased auxin efflux, but treatment with BFA, in contrast to NPA, had substantial impact on structures of actin cytoskeleton and membranes of endoplasmic reticulum. This observation together with dose-response relationships suggest that NPA, and perhaps other phytotropins as well, has a very specific effect on putative auxin efflux carrier activity rather than it generally inhibits the vesicle-mediated protein traffic to the plasma membrane. Furthermore, these data emphasise the importance of actin filaments and possibly endomembranes in vesicle-mediated trafficking of proteins and in the constitutive cycling of the auxin efflux catalyst(s). Following search for upstream factors that would modulate auxin transport machinery in tobacco cells implied a novel mode of the plant hormone action: By modulation of vesicle trafficking of auxin-efflux-carrier proteins of the PIN-type, auxin regulates their incidence and thus their capacity at the cell surface, providing a mechanism for the feedback regulation of auxin transport from cells. Such feedback regulation could be under the regulation of proteasome-mediated degradation of auxin-inducible transcription factors. However, this theory is not proved yet. Until recently, the evidence that PINs are real "auxin efflux catalysts" has been missing. In this thesis the rate-limiting function of PIN proteins in cellular auxin efflux is described in detail in tobacco BY-2 cells. Together with other results presented it can be concluded that the action of PINs in auxin efflux is rate-limiting, specific to auxins, sensitive to auxin transport inhibitors and distinct from the action of ABC-like transporters of PGP-type. PINs overexpression in tobacco cells caused phenotypic changes, which were previously reported to be typical for the response of cells to auxin depletion. These changes include the cessation of cell division, stimulation of cell elongation and amyloplasts formation. NPA as well as higher concentration of exogenous auxin were both capable of preventing the observed "auxin-depletion"-induced changes. All these results suggest a direct involvement of PINs in catalyzing cellular auxin efflux, and imply the existence of two distinct auxin efflux pathways in plant cells: PIN-dependent and PGP-dependent.

Suspension-cultured cell lines of tobacco and *Arabidopsis* can serve as an invaluable alternative experimental tool for auxin transport studies, which is complementary to *Arabidopsis* plants. In our laboratory, the collection of various cell lines transformed with components of both auxin influx and efflux machinery was established and it is maintained and continuously extended. This set of experimental materials will be useful for further detailed studies of the composition of the auxin transport machinery and quantitative aspects of its action, as well as for the characterization of the intracellular distribution and dynamics of proteins playing a role in the transport of auxin *in vivo* using fluorescent tags. It may also contribute to uncovering the role of both auxin influx and efflux in the establishment of proper intracellular auxin levels and their relation to the regulation of cell division, cell elongation and establishment and/or maintenance of cell polarity.

REFERENCES

Assaad, F.F. Plant cytokinesis. Exploring the links. Plant Physiol 126: 509-16, 2001.

Badescu, G.O., Napier, R.M. Receptors for auxin: will it all end in TIRs? Trends Plant Sci 11: 217-223, 2006.

Bell, C.J., Maher, P.E. Mutants of *Arabidopsis thaliana* with abnormal gravitropic responses. Mol Gen Genet 220, 289-293, 1990.

Benjamins, R., Quint, A., Weijers, D., Hooykaas, P., Offringa, R. The PINOID protein kinase regulates organ development in Arabidopsis by enhancing polar auxin transport. Development 128: 4057-4067, 2001.

Benjamins, R., Malenica, N., Luschnig, C. Regulating the regulator: the control of auxin transport. Bioessays 27: 1246-1255, 2005.

Benková, E., Michniewicz, M., Sauer, M., Teichmann, M., Seifertová, D., Jürgens, G., Friml, J. Local, efflux-dependent auxin gradients as a common module for plant organ formation. Cell 115: 591-602, 2003.

Bennett, M.J, Marchant, A., Green, H.G., May, S.T., Ward, S.P., Millner, P.A., Walker, A.R., Schulz, B., Feldmann, K.A. Arabidopsis AUX1 gene: a permease-like regulator of root gravitropism. Science 273: 948-950, 1996.

Bennett, S.R.M., Alvarez, J., Bossinger, G., Smyth, D.R. Morphogenesis in *pinoid* mutants of Arabidopsis thaliana. Plant J 8: 505-520, 1995.

Berleth, T., Krogan, N.T., Scarpella, E. Auxin signals - turning genes on and turning cells around. Curr Op Plant Biol 7: 553-563, 2004.

Bishopp, A., Mähönen, AP., Helariutta, Y. Signs of change: hormone receptors that regulate plant development. Development 133: 1857-1869, 2006.

Blilou, I., Xu, J., Wildwater, M., Willemsen, V., Paponov, I., Friml, J., Heidstra, R., Aida, M., Palme, K., Scheres, B. The PIN auxin efflux facilitator network controls growth and patterning in *Arabidopsis* roots. Nature 433: 39-44, 2005.

Blakeslee, J.J., Bandyopadhyay, A., Peer, W.A., Makam, S.N., Murphy, A.S. Relocalization of the PIN1 auxin efflux facilitator plays a role in phototropic responses. Plant Physiol 134: 28-31, 2004.

Blakeslee, J.J., Peer, W.A., Murphy, A.S. Auxin transport. Curr Opin Plant Biol 8: 494-500, 2005a.

Blakeslee, J.J., Peer, W.A., Murphy, A.S. MDR/PGP Auxin transport proteins and endocytic cycling. In: Šamaj J, Baluška F, Menzel, D, editors. Springer Berlin/Heidelberg, Plant Endocytosis. Plant Cell Monographs 1: 159-176, 2005b.

Blakeslee, J.J., Bandyopadhyay, A., Lee, O.R., Mravec, J., Titapiwatanakun, B., Sauer, M., Makam, S:N:, Cheng, Y., Bouchard, B., Adamec, J., Geisler, M., Nagashima, A., Sakai, T., Martinoia, E., Friml, J., Peer, W.A., Murphy, A.S. Interactions among PIN-FORMED and P-Glycoprotein Auxin Transporters in *Arabidopsis* Plant Cell First published on January 19, 2007; 10.1105/tpc.106.040782

Bouchard, R., Bailly, A., Blakeslee, J.J., Oehring, S.C., Vincenzetti, V., Lee, O.R., Paponov, I., Palme, K., Mancuso, S., Murphy, A.S., Schulz, B., Geisler, M. Immunophilin-like TWISTED DWARF1 modulates auxin efflux activities of Arabidopsis P-glycoproteins. J Biol Chem 281: 30603-30612, 2006.

Boutté, Y., Crosnier, M.T., Carraro, N., Traas, J., Satiat-Jeunemaitre, B. The plasma membrane recycling pathway and cell polarity in plants: studies on PIN proteins. J Cell Sci 119: 1255-1265, 2006.

Brenner, E.D., Stahlberg, R., Mancuso, S., Vivanco, J., Baluska, F., Van Volkenburgh E. Plant neurobiology: an integrated view of plant signaling. Trends Plant Sci 11: 413-419, 2006.

Burkle, L., Cedzich, A., Dopke, C., Stransky, H., Okumoto, S., Gillissen, B., Kuhn, C., Frommer, W.B. Transport of cytokinins mediated by purine transporters of the PUP family expressed in phloem, hydathodes, and pollen of Arabidopsis. Plant J 34: 13-26, 2003.

Butler, J.H., Hu, S.Q., Brady, S.R., Dixon, M.W., Muday, G.K. *In vitro* and *in vivo* evidence for actin association of the naphthylphthalamic acid-binding protein from zucchini hypocotyls. Plant J 13: 291-301, 1998.

Campanoni, P., Blasius, B., Nick, P. Auxin transport synchronizes the pattern of cell division in a tobacco cell line. Plant Physiol 133: 1251-1260, 2003.

Campanoni, P., Nick, P. Auxin-dependent cell division and cell elongation. 1-naphthaleneacetic acid and 2,4-dichlorophenoxyacetic acid activate different pathways. Plant Physiol 137: 939-948, 2005.

Campanoni, P., Petrášek, J., Opatrný, Z., Nick, P. Manipulation of cell axis, polarity and the exit from the cell cycle via the level and balance of different auxins. 17th IPGSA conference, 1-6 July, Brno, Czech Republic, p. 95, 2001.

Cande, W.Z., Goldsmith, M.H.M., Ray, P.M. Polar auxin transport and auxin-induced elongation in the absence of cytoplasmic streaming. Planta 111: 279-296, 1973.

Chen, L., Ortiz-Lopez, A., Jung, A., Bush, D.R. *ANT1*, an aromatic and neutral amino acid transporter in Arabidopsis. Plant Physiol 125: 1813-1820, 2001.

Chen, R., Hilson, P., Sedbrook, J., Rosen, E., Caspar, T., Masson, P.H. The *Arabidopsis thaliana AGRAVITROPIC 1* gene encodes a component of the polar-auxin-transport efflux carrier. Proc Natl Acad Sci USA 95: 15112-

15117, 1998.
Chen, R., Masson, P.H. Auxin Transport and Recycling of PIN Proteins in Plants In: Šamaj J, Baluška F, Menzel, D, editors. Springer Berlin/Heidelberg, Plant Endocytosis. Plant Cell Monographs 1: 139-157, 2005.
Christensen, S.K., Dagenais, N., Chory, J., Weigel, D. Regulation of auxin response by the protein kinase PINOID. Cell 100: 469-478, 2000.
Cox, D.N., Muday, G.K. NPA binding activity is peripheral to the plasma membrane and is associated with the cytoskeleton. Plant Cell 6: 1941-1953, 1994.
Delbarre, A., Muller, P., Imhoff, V., Guern, J. Comparison of mechanisms controlling uptake and accumulation of 2,4-dichlorophenoxy acetic acid, naphtalene-1-acetic acid, and indole-3-acetic acid in suspension-cultured tobacco cells. Planta 195: 531-541, 1996.
Deruere, J., Jackson, K., Garbers, C., Soll, D., DeLong, A. The RCN1-encoded A subunit of protein phosphatase 2A increases phosphatase activity in vivo. Plant J: 20, 389-399, 1999.
De Smet, I., Vanneste, S., Inzé, D., Beeckman, T. Lateral root initiation or the birth of a new meristem. Plant Mol Biol 60: 871-887, 2006.
Dharmasiri, S., Swarup, R., Mockaitis, K., Dharmasiri, N., Singh, S.K., Kowalchyk, M., Marchant, A., Mills, S., Sandberg, G., Bennett, M.J., Estelle, M. AXR4 is required for localization of the auxin influx facilitator AUX1. Science 312: 1218-1220, 2006.
Dhonukshe, P., Kleine-Vehn, J., Friml, J. Cell polarity, auxin transport, and cytoskeleton-mediated division planes: Who comes first? Protoplasma 226, 67-73, 2005.
Dhonukshe, P., Aniento, F., Hwang, I,, Robinson, D.G., Mravec, J., Stierhof, Y.D., Friml, J. Clathrin-Mediated Constitutive Endocytosis of PIN Auxin Efflux Carriers in Arabidopsis. Curr Biol 2007 Feb 14; Epub ahead of print, 2007.
Dixon, M.W., Jacobson, J.A., Cady, C.T., Muday, G.K. Cytoplasmic orientation of the naphthylphthalamic acid-binding protein in zucchini plasma membrane vesicles. Plant Physiol 112: 421-432, 1996.
Fleming, A.J. Plant signalling: the inexorable rise of auxin. Trends Cell Biol 16: 397-402, 2006.
Ferreira, F.J., Kieber, J.J. Cytokinin signaling. Curr Opin Plant Biol 8: 518-525, 2005.
Friml, J., Vieten, A., Sauer, M., Weijers, D., Schwarz, H., Hamann, T., Offringa, R., Jürgens, G. Efflux-dependent auxin gradients establish the apical-basal axis of *Arabidopsis*. Nature 426: 147-153, 2003.
Friml, J., Benková, E., Blilou, I., Wisniewska, J., Hamann, T., Ljung, K., Woody, S., Sandberg, G., Scheres, B., Jürgens, G., Palme, K. AtPIN4 mediates sink-driven auxin gradients and root patterning in *Arabidopsis*. Cell 108: 661-673, 2002a.
Friml, J., Wisniewska, J., Benková, E., Mendgen, K., Palme, K. Lateral relocation of auxin efflux regulator PIN3 mediates tropism in *Arabidopsis*. Nature 415: 806-809, 2002b.
Friml, J., Yang, X., Michniewicz, M., Weijers, D., Quint, A., Tietz, O., Benjamins, R., Ouwerkerk, P.B.F., Ljung, K., Sandberg, G., Hooykaas, P.J.J., Palme, K., Offringa, R. A PINOID-dependent binary switch in apical-basal PIN polar targeting directs auxin efflux. Science 306: 862-865, 2004.
Friml, J., Wisniewska, J. Auxin as an intercellular signal. In: Fleming, A., editor. Annual Plant Reviews 16, Intercellular Communication in Plants, Blackwell Publishing, Oxford, 1-26, 2005.
Furutani, M. et al. PIN-FORMED1 and PINOID regulate boundary formation and cotyledon development in Arabidopsis embryogenesis. Development 131: 5021-5030, 2004.
Galis, I., Fukuda, H., Matsuoka K. Microarray analysis of gene expression in methyl jasmonate-treated tobacco BY-2 cells. International meeting Cell and Molecular Biology of tobacco BY-2 cells, p. 21, 2004
Garbers, C., DeLong, A., Deruère, J., Bernasconi, P., Söll, D. A mutation in protein phosphatase 2A regulatory subunit affects auxin transport in Arabidopsis. EMBO J. 15: 2115-2124, 1996.
Geldner, N., Friml, J., Stierhof, Y.D., Jürgens, G., Palme, K. Auxin transport inhibitors block PIN 1 cycling and vesicle traficking. Nature 413: 425-428, 2001.
Geldner, N., Anders, N., Wolters, H., Keicher, J., Kornberger, W., Muller, P., Delbarre, A., Ueda, T., Nakano, A., Jürgens, G. The *Arabidopsis* GNOM ARF-GEF mediates endosomal recycling, auxin transport, and auxin-dependent plant growth. Cell 112: 219-230, 2003.
Geldner, N. The plant endosomal system-its structure and role in signal transduction and plant development. Planta 219: 547-560, 2004.
Geldner, N., Jürgens, G. Endocytosis in signalling and development. Curr Opin Plant Biol 9: 589-594, 2006.
Gälweiler, L., Guan, C., Müller, A., Wisman, E., Mendgen, K., Yephremov, A., Palme, K. Regulation of polar auxin transport by AtPIN1 in Arabidopsis vascular tissue. Science 282: 2226-2230, 1998.
Geelen, D.N.V., Inzé, D.G. A bright future for the bright yellow-2 cell culture. Plant Physiol 127: 1375-1379, 2001.
Geisler, M., Kolukisaoglu, H.U., Billion, K., Berger, J., Saal, B., Bouchard, R., Frangne, N., Koncz-Kalman, Z., Koncz, C., Dudler, R., Blakeslee, J., Murphy, A.S., Martinoia, E., Schulz, B. TWISTED DWARF1, a unique plasma membrane-anchored immunophilin-like protein, interacts with *Arabidopsis* multidrug resistance-like transporters AtPGP1 and AtPGP19. Mol Biol Cell 14: 4238-4249, 2003.
Geisler, M., Blakeslee, J.J., Bouchard, R., Lee, O.R., Vincenzetti, V., Bandyopadhyay, A.,

Titapiwatanakun, B., Peer, W.A., Bailly, A., Richards, E.L., Ejendal, K.F.K., Smith, A.P., Baroux, C., Grossniklaus, U., Müller, A., Hrycyna, C.A., Dudler, R., Murphy, A.S., Martinoia E. Cellular efflux of auxin catalyzed by the Arabidopsis MDR/PGP transporter AtPGP1. Plant J. 44, 179-194, 2005.

Geisler, M., Murphy, A.S. The ABC of auxin transport: The role of p-glycoproteins in plant development. FEBS Lett 580: 1094-1102, 2006.

Gillissen, B., Burkle, L., Andre, B., Kuhn, C., Rentsch, D., Brandl, B., Frommer, W.B. A new family of high-affinity transporters for adenine, cytosine, and purine derivatives in Arabidopsis. Plant Cell 12: 291-300, 2000.

Goldsmith, M.H.M. The polar transport of auxin. Ann Rev Plant Physiol 28: 439-478, 1977.

Goto, N., Starke, M., Kranz, A.R. Effect of gibberellins on flower development of the pin-formed mutant of Arabidopsis thaliana. Arabidopsis Information Service 23, 66-71, 1987.

Grebe, M., Friml, J., Swarup, R., Ljung, K., Sandberg, G., Terlou, M., Palme, K., Bennett, M.J., Scheres, B. Cell polarity signaling in *Arabidopsis* involves a BFA-sensitive auxin influx pathway. Curr Biol 12: 329-334, 2002.

Gutierrez, C. Coupling cell proliferation and development in plants. Nature Cell Biol 7: 535-541, 2005.

Haberlandt, G. Kulturversuche mit isolierten Pflanzenzellen. Sitzungsber. Mat Nat Kl Kais Akad Wiss Wien 111: 69-92, 1902.

Holweg, C., Honsel, A., Nick, P. A myosin inhibitor impairs auxin-induced cell division. Protoplasma 222: 193-204, 2003.

Holweg, C., Nick, P. Arabidopsis myosin XI mutant is defective in organelle movement and polar auxin transport. Proc Natl Acad Sci USA 101: 10488-10493, 2004.

Horgan, R. Cytokinins. In: Wilkins MB (ed): *Advanced Plant Physiology*, pp. 53-75, Pitman Publishing Limited, London, 1984.

Jahrmann, T., Bastida, M., Pineda, M., Gasol, E., Ludevid, M., Palacin, M., Puigdomenech, P. Studies on the function of TM20, a transmembrane protein present in cereal embryos. Planta 222: 80-90, 2005.

Jaillais, Y., Fobis-Loisy, I., Miege, C., Rollin, C., Gaude, T. AtSNX1 defines an endosome for auxin-carrier trafficking in *Arabidopsis*. Nature 443, 106-109, 2006.

Jenik, P.D., Barton, M.K. Surge and destroy: the role of auxin in plant embryogenesis. Development 132: 3577-3585, 2005.

Jurgens, G. Cytokinesis in higher plants. Ann Rev Plant Biol 56: 281-299, 2005a.

Jurgens, G. Plant cytokinesis: fission by fusion. Trends Cell Biol 15: 277-283, 2005b.

Kappert, H. Beschreibung der Mutante 24ak, unpublished, 1959.

Kleine-Vehn, J., Dhonukshe, P., Swarup, R., Bennett, M., Friml, J. Subcellular trafficking of the Arabidopsis auxin influx carrier AUX1 uses a novel pathway distinct from PIN1. Plant Cell. 2006 Nov;18: 3171-3181, 2006.

Konopka, C.A., Schleede, J.B., Skop, A.R., Bednarek, S.Y. Dynamin and cytokinesis. Traffic 7: 239-247, 2006

Lechner, E., Achard, P., Vansiri, A., Potuschak, T., Genschik, P. F-box proteins everywhere. Current Opinion in Plant Biology 9: 631-638, 2006.

Leyser, O. Dynamic Integration of Auxin Transport and Signalling. Curr Biol 16: 424-433, 2006.

Marchant, A., Kargul, J., May, S.T., Muller, P., Delbarre, A., Perrot-Rechenmann, C., Bennett, M.J. AUX1 regulates root gravitropism in Arabidopsis by facilitating auxin uptake within root apical tissues. Embo Journal 18: 2066-2073, 1999.

Matsuoka, K., Demura, T., Galis, I., Horiguchi, T., Sasaki, M., Tashiro, G., Fukuda, H. comprehensive gene expression analysis toward the understanding of growth and differentiation of tobacco BY-2 cells. Plant and Cell Physiology 45: 1280-1289, 2004.

Müller, A., Guan, C., Gälweiler, L., Tänzler, P., Huijser, P., Marchant, A., Parry, G., Bennett, M., Wisman, E. Palme, K. *AtPIN2* defines a locus of *Arabidopsis* for root gravitropism control. EMBO J 17, 6903-6911, 1998.

Mattsson, J., Ckurshumova, W., Berleth, T. Auxin signaling in *Arabidopsis* leaf vascular development. Plant Physiol 131:1327-1339, 2003.

Lee, S.H., Cho, H.-T. PINOID positively regulates auxin efflux in *arabidopsis* root hair cells and tobacco cells. Plant Cell 18: 1604-1616, 2006.

Luschnig, C., Gaxiola, R., Grisafi, P., Fink, G. EIR1, a root specific protein involved in auxin transport, is required for gravitropism in *Arabidopsis thaliana*. Genes Dev 12, 2175-2187, 1998.

Morris, D.A., Robinson, J.S. Targeting of auxin carriers to the plasma membrane: differential effects of brefeldin A on the traffic of auxin uptake and efflux carriers. Planta 205: 606-612, 1998.

Morris, D.A., Friml, J., Zažímalová, E. The Transport of Auxins. In: Davies PJ (ed) Plant Hormones: Biosynthesis, Signal Transduction, Action! Kluwer Acad Publ, Dordrecht, pp 437-470, 2004.

Motyka, V., Vaňková, R., Čapková, V., Petrášek, J., Kamínek, M., Schmülling, T.: Cytokinin-induced upregulation of cytokinin oxidase activity in tobacco includes changes in enzyme glycosylation and secretion. Physiol Plant 117, 11-21, 2003.

Muday, G.K., Murphy, A.S. An emerging model of auxin transport regulation. Plant Cell 14: 293-299, 2002.

Muday, G.K., Peer, W.A., Murphy, A.S. Vesicular cycling mechanisms that control auxin transport polarity. Trends Plant Sci 8: 301-304, 2003.

Murphy, A.S., Hoogner, K.R., Peer, W.A., Taiz, L. Identification, purification and molecular cloning

of N-1-naphthylphthalamic acid-binding plasma membrane-associated aminopeptidases from *Arabidopsis*. Plant Physiol 128: 935-950, 2002.

Murphy, A.S., Bandyopadhyay, A., Holstein, S.E., Peer, W.A. Endocytotic cycling of PM proteins. Annu Rev Plant Biol. 56: 221-251, 2005.

Nagata, T., Nemoto, Y., Hasezava, S. Tobacco BY-2 cell line as the "Hela" cell in the cell biology of higher plants. Int Rev Cytol 132, 1-30, 1992.

Nick, P. Noise yields order - Auxin, actin, and polar patterning. Plant Biol 8: 360-370, 2006.

Noh, B., Murphy, A.S., Spalding, E.P. Multidrug resistance-like genes of Arabidopsis required for auxin transport and auxin-mediated development. Plant Cell 13: 2441-2454, 2001.

Noh, B., Bandyopadhyay, A., Peer, W.A., Spalding, E.P., Murphy, A.S. Enhanced gravi- and phototropism in plant mdr mutants mislocalizing the auxin efflux proteini PIN1. Nature 423: 999-1002, 2003.

Nordstrom, A., Tarkowski, P., Tarkowska, D., Norbaek, R., Astot, C., Dolezal, K., Sandberg, G. Auxin regulation of cytokinin biosynthesis in Arabidopsis thaliana: A factor of potential importance for auxin-cytokinin-regulated development. Proc Natl Acad Sci USA 101: 8039-8044, 2004.

Okada, K., Ueda, J., Komaki, M.K., Bell, C.J., Shimura, Y. Requirement of the auxin polar transport system in the early stages of Arabidopsis floral bud formation. Plant Cell 3: 677-684, 1991.

Okada, K., Shimura, Y. Reversible root tip rotation in *Arabidopsis* seedlings induced by obstacle-touching stimulus. Science 250: 274-276, 1990.

Opatrný, Z., Opatrná, J. The specificity of the effect of 2,4-D and NAA on the growth, micromorphology, and the occurrence of starch in long-term *Nicotiana tabacum* cell strains. Biol Plant 18: 381-400, 1976.

Paciorek, T., Friml, J. Auxin signaling. J Cell Sci 119: 1199-1202, 2006.

Paponov, I.A., Teale, W.D., Trebar, M., Blilou, I., Palme, K. The PIN auxin efflux facilitators: evolutionary and functional perspectives. Trends Plant Sci 10: 170-177, 2005.

Parry, G., Marchant, A., May, S., Swarup, R., Swarup, K., James, N., Graham, N., Allen, T., Martucci, T., Yemm, A., Napier, R., Manning, K., King, G., Bennett, M. Quick on the uptake: Characterization of a family of plant auxin influx carriers. Journal of Plant Growth Regulation 20: 217-225, 2001.

Pischke, M.S., Huttlin, E.L., Hegeman, A.D., Sussman, M.R. A transcriptome-based characterization of habituation in plant tissue culture. Plant Physiology 140: 1255-1278, 2006.

Quint, M., Gray, W.M. Auxin signaling. Current Opinion in Plant Biology 9: 448-453, 2006.

Ramirez-Parra, E., Desvoyes, B., Gutierrez, C. Balance between cell division and differentiation during plant development. International Journal of Developmental Biology 49: 467-477, 2005.

Raven, J.A. Transport of indoleacetic acid in plant cells in relation to pH and electrical potential gradients, and its significance for polar IAA transport. New Phytol 74: 163-174, 1975.

Rashotte, A.M., DeLong, A., Muday, G.K. Genetic and chemical reductions in protein phosphatase activity alter auxin transport, gravity response, and lateral root growth. Plant Cell 13: 1683-1697, 2001.

Reinhardt, D., Pesce, E.R., Stieger, P., Mandel, T., Baltensperger, K., Bennett, M., Traas, J., Friml, J., Kuhlemeier, C. Regulation of phyllotaxis by polar auxin transport. Nature 426: 255-260, 2003.

Richard, C., Lescot, M., Inzé, D., De Veylder, L. Effect of auxin, cytokinin, and sucrose on cell cycle gene expression in Arabidopsis thaliana cell suspension cultures. Plant Cell Tiss. Org. 69: 167-176, 2002.

Robinson, J.S., Albert, A.C., Morris, D.A. Differential effects of brefeldin A and cycloheximide on the activity of auxin efflux carriers in *Cucurbita pepo* L. J Plant Physiol 155, 678-684, 1999.

Roman, G., Lubarsky, G.B., Kieber, J.J., Rotheneberg, M., Ecker J.R. Genetic analysis of ethylene signal transduction in *Arabidopsis thaliana* : Five novel mutant loci integrated into a stress-response pathway. Genetics 139, 1393-1409, 1995.

Rubery, P.H. Phytotropins: receptors and endogenous ligands. Symp Soc Exp Biol 44: 119-146, 1990.

Rubery, P.H., Sheldrake, A.R. Carrier-mediated auxin transport. Planta 118: 101-121, 1974.

Sachs, T. Cell polarity and tissue patterning in plants. Development 1(Suppl): 83–93, 1991.

Sakai, A., Miyazawa, Y., Kuroiwa, T. Studies on dynamic changes of organelles using tobacco BY-2 as the model plant cell line. In: Nagata T, Hasezawa S, Inzé D (eds) Biotechnology in agriculture and forestry Vol. 53 Tobacco BY-2 cells, Springer, Berlin Heidelberg, pp 192-216, 2004.

Sakakibara, H. Cytokinins: Activity, biosynthesis, and translocation. Ann Rev Plant Biol 57: 431-449, 2006.

Santelia, D., Vincenzetti, V., Azzarello, E., Bovet, L., Fukao, Y., Duchtig, P., Mancuso, S., Martinoia, E., Geisler, M. MDR-like ABC transporter AtPGP4 is involved in auxin-mediated lateral root and root hair development. Febs Letters 579: 5399-5406, 2005.

Scarpella, E., Marcos, D., Friml, J., Berleth, T. Control of leaf vascular patterning by polar auxin transport. Genes Dev 20: 1015-27, 2006.

Schell, J., Bisseling, T., Dulz, M., Franssen, H., Fritze, K., John, M., Kleinow, T., Lessnick, A., Miklashevichs, E., Pawlowski, K., Rohrig, H., van de Sande, K., Schmidt, J., Steinbiss, H.H., Stoll, M. Re-evaluation of phytohormone-independent division of tobacco protoplast-derived cells. Plant J 17: 461-466, 1999.

Schlicht, M., Strnad, M., Scanlon, M.J., Mancuso, S., Hochholdinger, F., Palme, K., Volkmann, D., Menzel, D., Baluška, F. Auxin immunolocalization implicates vesicular neurotransmitter-like mode of polar auxin transport in root apices. Plant Sign Behav 1: 122-133, 2006.

Shimizu, T., Eguchi, K., Nishida, I., Laukens, K., Witters, E., Van Onckelen, H., Nagata, T. A novel cell division factor from tobacco 2B-13 cells that induced cell division in auxin-starved tobacco BY-2 cells. Naturwissenschaften. 93: 278-85, 2006.

Shin, H., Shin, H.S., Guo, Z.B., Blancaflor, E.B., Masson, P.H., Chen, R.J. Complex regulation of Arabidopsis AGR1/PIN2-mediated root gravitropic response and basipetal auxin transport by cantharidin-sensitive protein phosphatases. Plant J 42: 188-200, 2005.

Skoog, F., Miller, C. Chemical regulation of growth and organ formation in plant tissues cultured in vitro. Symposia of the Society for Experimental Biology, No. XI, The Biological Action of Growth Substances: 118-140, 1957.

Steinmann, T., Geldner, N., Grebe, M., Mangold, S., Jackson, C.L., Paris, S., Galweiler, L., Palme, K., Jurgens, G. Coordinated polar localization of auxin efflux carrier PIN1 by GNOM ARF GEF. Science 286: 316-318, 1999.

Stickens, D., Tao, W., Verbelen, J.P. A single cell model system to study hormone signal transduction. Plant Growth Regul 18: 149-154, 1996.

Sussman, M.R., Gardner, G. Solubilization of the receptor for *N*-1-naphthylphthalamic acid. Plant Physiol 66: 1074-1078, 1980.

Swarup, R., Friml, J., Marchant, A., Ljung, K., Sandberg, G., Palme, K., Bennett, M. Localization of the auxin permease AUX1 suggests two functionally distinct hormone transport pathways operate in the Arabidopsis root apex. Genes & Dev 15: 2648-2653, 2001.

Swarup, R., Kargul, J., Marchant, A., Zadik, D., Rahman, A., Mills, R., Yemm, A., May, S., Williams, L., Millner, P., Tsurumi, S., Moore, I., Napier, R., Kerr, I.D., Bennett, M.J. Structure-function analysis of the presumptive Arabidopsis auxin permease AUX1. Plant Cell 16: 3069-3083, 2004.

Swarup, R., Kramer, E.M., Perry, P., Knox, K., Leyser, H.M.O., Haseloff, J., Beemster, G.T.S., Bhalerao, R., Bennett, M.J. Root gravitropism requires lateral root cap and epidermal cells for transport and response to a mobile auxin signal. Nature Cell Biol 7: 1057-1065, 2005.

Tanaka, H., Dhonukshe, P., Brewer, P.B., Friml, J. Spatiotemporal asymmetric auxin distribution: a means to coordinate plant development. Cell Mol Life Sci 63: 2738-2754, 2006a.

Tanaka, M., Takei, K., Kojima, M., Sakakibara, H., Mori, H. Auxin controls local cytokinin biosynthesis in the nodal stem in apical dominance. Plant J 45: 1028-1036, 2006b.

Tao, W.H., Verbelen, J.P. Switching on and off cell division and cell expansion in cultured mesophyll protoplasts of tobacco. Plant Sci 116: 107-115, 1996.

Teale, W.D., Paponov, I.A., Ditengou, F., Palme, K. Auxin and the developing root of Arabidopsis thaliana. Physiol Plant 123: 130-138, 2005.

Teale, W.D., Paponov, I., Palme. K. Auxin in action: signalling, transport and the control of plant growth and development. Nat Rev Mol Cell Biol 7: 847-859, 2006.

Terasaka, K., Blakeslee, J.J., Titapiwatanakun, B., Peer, W.A., Bandyopadhyay, A., Makam, S.N., Lee, O.R., Richards, E.L., Murphy, A.S., Sato, F., Yazaki, K. PGP4, an ATP binding cassette P-glycoprotein, catalyzes auxin transport in Arabidopsis thaliana roots. Plant Cell 17: 2922-2939, 2005.

Traas, J., Bohn-Courseau, I. Cell proliferation patterns at the shoot apical meristem. Curr Opin Plant Biol 8: 587-592, 2005.

Utsuno, K., Shikanai, T., Yamada, Y., Hashimoto, T. *AGR*, an agravitropic locus of *Arabidopsis thaliana*, encodes a novel membrane-protein family member. Plant Cell Physiol 39: 1111-1118, 1998.

Vanneste, S., Maes, L., De Smet, I., Himanen, K., Naudts, M., Inzé, D., Beeckman, T. Auxin regulation of cell cycle and its role during lateral root initiation. Physiol Plant 123: 139-146, 2005.

Vogler, H., Kuhlemeier, C. Simple hormones but complex signalling. Curr Opin Plant Biol 6: 51-56, 2003.

Willemsen, V., Friml, J., Grebe, M., Van den Toorn, A., Palme, K., Scheres, B. Cell polarity and PIN protein positioning in Arabidopsis require *STEROL METHYLTRANSFERASE1* function. Plant Cell 15: 612-625, 2003.

Woodward, A.W., Bartel, B. Auxin: Regulation, action, and interaction. Ann Bot 95: 707-735, 2005.

Xu, J., Scheres, B. Dissection of *Arabidopsis* ADP-RIBOSYLATION FACTOR 1 function in epidermal cell polarity. Plant Cell 17: 525-536, 2005.

Yang, Y., Hammes, U. Z., Taylor, C. G., Schachtman, D. P. and Nielsen, E. High-affinity auxin transport by the AUX1 influx carrier protein. Curr Biol 16: 1123-1127, 2006.

Zažímalová, E., Březinová, A., Holík, J., Opatrný, Z. Partial auxin deprivation affects endogenous cytokinins in an auxin-dependent, cytokinin-independent tobacco cell strain. Plant Cell Rep. 16: 76-79, 1996.

Zažímalová, E., Opatrný, Z., Březinová, A., Eder, J. The effect of auxin starvation on the growth of auxin-dependent tobacco cell culture: dynamics of auxin reception and endogenous free IAA content. J Exp Bot 46: 1205-1213, 1995.

Zažímalová, E., Křeček, P., Skůpa, P., Hoyerová, K., Petrášek, J. Polar transport of plant hormone auxin - the role of PIN-FORMED (PIN) proteins.

Cell Mol Life Sci (Invited paper, accepted after revision), 2007.

Zažímalová, E., Petrášek, J. Estimation of activity of auxin uptake and efflux carriers in the cells of VBI-0 tobacco strain. Biol. listy 65: 253-257, 2000.

Zegzouti, H., Anthony, R.G., Jahchan, N., Bogre, L. , Christensen, S.K. Phosphorylation and activation of PINOID by the phospholipid signaling kinase 3-phosphoinositide-dependent protein kinase 1 (PDK1) in Arabidopsis. Proc Natl Acad Sci USA 103: 6404-6409, 2006.

CURRICULUM VITAE

NAME	Jan Petrášek
SEX	Male
HOME ADDRESS	Wichterlova 2310/2, 182 00 Praha 82, Czech Republic
E-MAIL	petrasek@ueb.cas.cz
DATE OF BIRTH	August 1, 1972
NATIONALITY	Czech
MARITAL STATUS	Married

EDUCATION

1986-1990, Jan Neruda Grammar school in Prague
1990-1995, Charles University, Faculty of Science, Prague, Czech Republic (Mgr., equivalent of MSc.), diploma thesis "Phytohormones in regulation of cell cycle in plants: characterization of the cell division and growth in relation to the structure and function of the cytoskeleton in tobacco cell line VBI-0"
2003, RNDr. degree in plant physiology and anatomy
1995-2006, postgraduate studies, Faculty of Science, Prague, Czech Republic

PROFESSIONAL EXPERIENCE

Since 1998, research assistant, Charles University, Faculty of Science, Department of Plant Physiology, Prague, Czech Republic
Since 1998, research assistant, Institute of Experimental Botany, Laboratory of Hormonal Regulations in Plants, Prague, Czech Republic