Auxin and the Communication Between Plant Cells

Peter Nick

Abstract Multicellularity allows one to assign different functions to the individual cells. Cell fate could be defined by a stereotypic sequence of cell divisions or it might arise from intercellular communication between cells. Patterning in the totipotent plant cells results mainly from coordinative signals. Auxin is central in this respect, and this chapter ventures to give a survey on the role of auxin as a coordinative signal that regulates patterning of cell differentiation, cell division and cell expansion.

Abbreviations 2,4-D: 2,4-Dichlorophenoxyacetic acid; ARF: ADP-ribosylation factor: ARP; Actin-related protein: BFA; Brefeldin A: GFP; Green fluorescent protein; IAA: Indole-3-acetic acid; NAA: 1-Naphthaleneacetic acid; NPA: Naphthylphthalamic acid; RFP: Red fluorescent protein; TIBA: 2,3,5-Triiodobenzoic acid

1 Introduction

The polar flux of auxin has been used for more than 375 million years to generate and regulate the pattern of vascular differentiation of parenchymatic cells and thus coordinates the organization of the telomes, the building block of cormophytic land plants. In addition to the patterning of vasculature, auxin mediates the coordinative signalling that controls phyllotaxis, the formation of new leaves according to an orderly, species-dependent pattern. The phyllotactic pattern is shaped by competition of young primordia for free auxin, such that the neighbourhood of an existing primordium will be depleted of auxin. Since auxin limits the formation of new primordia, this simple mechanism ensures elegantly that new structures will be laid down at a minimal distance from preexisting primordia.

P. Nick(✉)
Botanisches Institut 1, Kaiserstr. 2, 76128 Karlsruhe, Germany
e-mail: peter.nick@bio.uni-karlsruhe.de

F. Baluska and S. Mancuso (eds.), *Signaling in Plants,*
DOI: 10.1007/978-3-540-89228-1_1, © Springer-Verlag Berlin Heidelberg 2009

Polar auxin transport can regulate the synchrony of cell divisions, with actin organization emerging as a central factor defining the pattern of cell division, probably by polarizing the flow of vesicles that deposit auxin-efflux carriers to the cell pole and thus determining the directionality of auxin efflux. Since the organization of actin, in turn, is regulated by auxin, a feedback loop is established that contains auxin-efflux carriers, intracellular auxin and actin filaments as central elements.

Regulated cell expansion represents the central adaptive response of the sessile plants to environmental challenges and is therefore highly responsive to stimuli, such as light or gravity. These adaptive responses involve a spatiotemporal pattern of cell expansion, which is most evident for tropistic curvature. Actually, auxin was originally identified as a signal that coordinates the pattern of cell expansion. The Cholodny–Went model explains tropism by a signal-induced redistribution of auxin fluxes across the stimulated organ. Although the Cholodny–Went model is repeatedly disputed mainly because of discrepancies between the observed response (a gradient of growth) and the amplitude of the induced gradient of auxin, it is shown that the model is still valid if the redistribution of auxin fluxes is complemented by parallel gradients of auxin responsiveness.

The chapter ends with a speculative consideration of why, during evolution, such a simple molecule as indole-3-acetic acid (IAA) has acquired such a central role for intercellular coordination. This is attributed to the molecular properties of auxin that determine its transport properties (multidirectional influx through an ion-trap mechanism, but unidirectional efflux through the localized activity of auxin-efflux carriers). On the intracellular level, this system is able to establish a clear cell polarity from even minute and noisy directional cues. On the level of tissues, this system is ideally suited to convey lateral inhibition between neighbouring cells. It was sufficient to put the localization of the efflux transporter under the control of auxin itself to reach a perfect reaction-diffusion system *in sensu* Turing (1952). Such systems are able to generate clear outputs from even minute and noisy directional cues and provide a robust mechanism to generate patterns of cell differentiation, cell division and cell expansion under the special constraints of plant development, such as signal-dependent morphogenesis and the lack of specialized and localized sensing organs.

Plant morphogenesis is not based on fixed hierarchies – there is no such a thing as a "Great Chairman" that assigns differential developmental pathways to the individual cells. Plant cells rather "negotiate" on their individual developmental fates in a fairly "democratic" manner with hierarchies being created ad hoc by mutual interactions. It seems that auxin has evolved as a central tool for this "cellular democracy" characteristic for plant development.

2 Plant Development and Cell Communication

"Why do cells exist?" – with this question Philip Lintilhac (1999) starts his thoughtful essay on the conceptual framework of cellularity. Multicellularity initially probably evolved as a strategy to increase in size and thus escape the fate of being eaten.

During growth, the volume of a cell (its "internum" *in sensu* Lintilhac) increases with the third power of the radius; its surface, though, increases only with the second power of the radius. When a cell grows, an increasing gap between consumption (by the "internum") and subsistence (through the boundary with the "externum") has to be bridged that will limit further expansion of the cell. Multicellularity allows an increase of the surface in relation to the volume – for the cell population *as an entity*. This made it possible for the cell to become bigger, again for the cell population *as an entity*. The selective advantage (not to be devoured by predators) paid off for each individual cell. However, the full potential of this achievement emerged only when the individual cells of the newborn organism began to assign different functions to individual members of the population. For the individual cell, differentiation represents a risky investment, because it implies that specific (Lintilhac coined the term "hypercellular") tasks have to be upregulated at the cost of other "hypocellular" functions that are downregulated and therefore have to be compensated by corresponding hypercellular output from neighbouring cells. This culminates in a situation where the individual cells cannot survive outside the organismal context.

Differentiation therefore requires an intensive flow of information between individual cells to maintain the subtle balance between hypercellular and hypocellular functions. Although in some systems the differentiation of individual cells seems to follow a predetermined internal programme, cell–cell communication is important at least in the initial phase, when this programme is defined and triggered. Plant cells with their principal totipotency and their comparatively diffuse differentiation have to be especially communicative. Owing to their developmental flexibility, the balance between hypercellular and hypocellular functions has to be reestablished continuously. It thus seems that cell differentiation in plants resembles more or less the ancestral situation of multicellularity. In addition, plant cells are immobile, such that temporal patterns of differentiation become manifest morphologically and are not obscured by cell migrations.

The primordial form of cell differentiation is developmental dichotomy as characteristically observed during the first formative cell division of zygotes or spores in many algae, mosses and ferns or during the first division of the angiosperm zygote. In the Volvocales, a monophyletic clade of the green algae, it is still possible to follow the evolutionary line from a cell population over cell colonies (consisting of equivalent members that are completely autonomous) to a true organism, where two cell types are coupled by hypocellular and hypercellular interactions. Genetic analysis of differentiation mutants in *Volvox carteri* has uncovered a transcription factor, *regA*, repressing nuclear encoded genes of the chloroplast in mobile, somatic cells such that growth of these cells is suppressed, leading to a delayed cell cycle (Kirk 2003). In contrast, a group of four or five *late gonidia* factors suppress the motile phase in reproductive cells and thus promote their division. The activities of *regA* and *lag* differ as early as from the first division of the mature gonidium. This primary developmental dichotomy is under the control of two or three *gonidialess* factors – mutations in those genes render the first division symmetric such that the resulting daughter organism lacks reproductive cells. In fact, the dichotomy of

the first gonidial division is a cornerstone of August Weismann's concept of inheritance, where he defined the separation of the differentiating, but mortal *soma* from the non-differentiating, but immortal *germ line*as a primordial event of multicellular development (Weismann 1894).

Developmental dichotomy could be based on a gradient of developmental determinants within the progenitor cell that are then differentially partitioned to the daughter cells (formative cell division). According to this mechanism, the ultimate cause for differentiation would reside in cell lineage (Fig. 1a). Alternatively, developmental dichotomy could arise from communication between initially equipotent daughter cells and therefore would be independent of cell lineage (Fig. 1b).

As diverse as these two mechanisms might appear, it can be difficult to discriminate between them in nature since the commitment for a certain developmental pathway and the manifestation of this commitment as differentiation are not always clearly separated in time. However, the principal totipotency of plant cells is easier to reconcile with a model where differentiation is not defined a priori by a formative division (Fig. 1a), but a posteriori by intercellular communication (Fig. 1b).

The impact of intercellular communication on differentiation is heralded in the (prokaryotic, but plant-like) cyanobacteria during the differentiation of heterocysts. Heterocystsexpress (as hypercellular function) a nitrogenase that is able to release the constraints placed on cell division by the limited supply of bioavailable nitrogen.

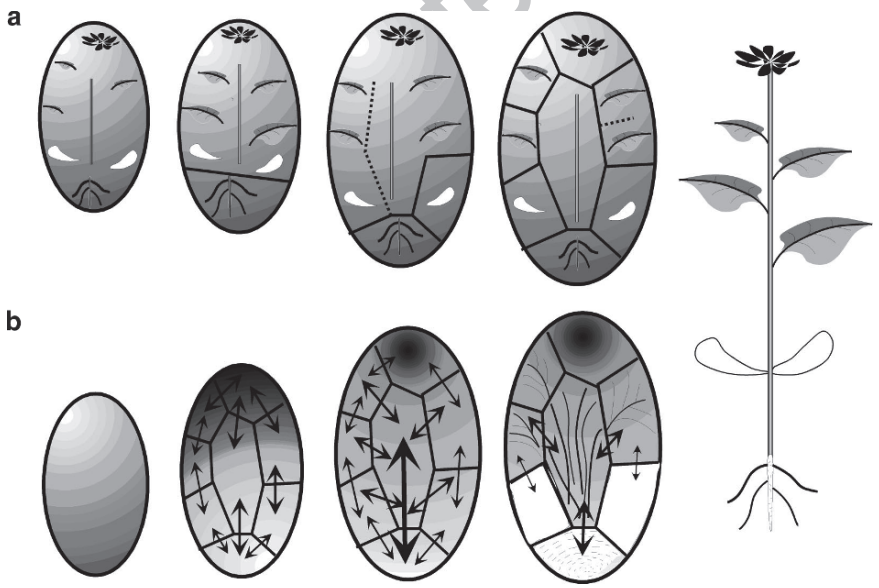

Fig. 1 Mechanisms for the establishment of developmental differentiation. (**a**) Mosaic development, where developmental fate is determined a priori and then assigned to individual daughter cells by a stereotypic sequence of formative cell divisions. (**b**) Regulative development, where developmental fate is not predetermined, but is defined a posteriori by communication between equipotent cells

This nitrogenase dates back to the earliest, anoxic phases of life on this planet and is therefore highly sensitive to oxygen; therefore, to safeguard nitrogenase activity, any photosynthetic activity has to be excluded from heterocysts. These cells are therefore hypocellular with respect to assimilation. The balance between nitrogen export and assimilate import has to be maintained although the total number of cells grows continuously. This balance is kept by iterative patterning, whereby preexisting heterocysts suppress the differentiation of new heterocysts in a range of around ten cells. When, in consequence of cell divisions, the distance between them exceeds this threshold, a new heterocyst will differentiate between them. By the analysis of patterning mutants in *Anabaena* the factor responsible for this lateral inhibition could be identified as the diffusible peptide patS (Yoon and Golden 1998). Differentiation (including the synthesis of patS) will begin in clusters of neighbouring cells; however, one of these cells will advance and then suppress further differentiation in its neighbours (Yoon and Golden 2001). This demonstrates that the differentiation of a heterocyst is not predetermined, but is progressively defined by signalling between neighbouring cells.

A developmental dichotomy in the complete absence of a predefined gradient has been impressively demonstrated for the somatic embryogenesis of embryogenic carrot cell suspensions. Here a single cell can be induced to produce an entire embryo that is indistinguishable from a sexually produced plant. Similar to zygotic development, the initial event is an asymmetric cell division giving rise to a highly vacuolated basal and a smaller apical cell endowed with a very dense cytoplasm (McCabe et al. 1997). Whereas the vacuolated cell will undergo programmed cell death, this apical cell will undergo embryogenesis. The vacuolated cell expressed a surface marker that was recognized by the monoclonal antibody JIM8 that had originally been raised as a marker of cell fate in root development. By use of ferromagnetic antibody conjugates it was possible to remove cells expressing the JIM8 marker from the suspension. These cultures lost their embryogenic potential. However, a filtrate from a culture containing JIM8-positive cells was able to restore the embryogenic potential of the JIM8-depleted culture. The JIM8 marker, a small soluble arabinogalactan protein secreted by the vacuolated cells, was therefore necessary and sufficient to confer an embryogenic fate to the densely vacuolated apical cells. Thus, the formative division is controlled by intercellular signalling.

Whereas intercellular signalling is relatively evident in these two examples, it might be more widespread. The classic experiment to dissect the role of cell lineage versus intercellular signalling in animal embryology is to transplant tissue to a different site of the embryo and to test whether the explant develops according to position (favouring a signalling mechanism) or according to its origin, as would be expected for differentiation based on cell lineage (Spemann 1936). This experiment has rarely been undertaken in plants, and so intercellular signalling might have been overlooked in many cases. For instance, the highly stereotypic cell lineage in the root meristem of *Arabidopsis thaliana* seemed to indicate that here cell fate is defined by cell lineage that could be traced back to early embryogenesis (Scheres et al. 1994). However, by very elegant laser ablation experiments (Van den Berg et al. 1995) and the analysis of mutants with aberrant definition of tissue layers (Nakajima

et al. 2001) it could be shown that even in this case cell fate was defined by signals (such as the transcription factor shortroot) from adjacent cells.

Generally, the principal totipotency of plant cells is difficult to reconcile with a strong impact of cell lineage. Patterning in cells thus seems to result mainly from coordinative signals. However, as discussed in the next section, the impact of intercellular communication on development seems to reach beyond the realm of individual cells to the coordinative development of entire organs.

3 Auxin as a Pattern Generator in Cell Differentiation I: Vasculature

As consequence of their light dependency, plants increase their surface in an outward direction, which means that they have to cope with a considerable degree of mechanic load. As long as they were aquatic, this was no special challenge, because mechanical strains were counterbalanced by buoyancy, allowing for considerable size even on the basis of a fairly simple architecture. However, the transition towards terrestrial habitats increased the selection pressure towards the development of flexible and simultaneously robust mechanical lattices. Plant evolution responded to this selective pressure by generation of load-bearing elements, the so-called telomes (Zimmermann 1965). These modules are organized around a lignified vascular bundle surrounded by parenchymatic tissue and an epidermis to limit transpiration (Fig. 2a). The telomes were originally dichotomously branched, but by asymmetric branching ("overtopping") hierarchical branching systems emerged that were endowed with main and side axes. By planation and

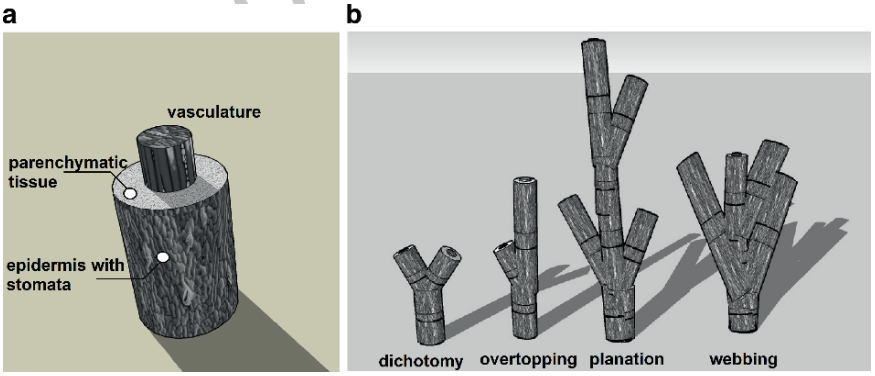

Fig. 2 Modular structure of terrestrial plants. The building block of cormophytes are the telomes (**a**), tubular elements organized around vasculature bundles that are surrounded by parenchymatic tissue and protected by an epidermis with stomata for the regulation of transpiration. By combination of the telomes in combination with simple modulations of their geometry (**b**) progressively complex hierarchical structures have been produced during the evolution of land plants

subsequent fusion of the parenchymatic tissue (so-called webbing) the telomes developed into the first leaves (Fig. 2b). This is still evident in the leaves of certain ferns and the primitive gymnosperm *Ginkgo biloba*, where, interestingly, occasionally atavistic forms are observed that uncover the original dichotomous telome structure. By spherical fusion and reduction of individual telomes globular structures arose that later evolved into sporangiophores and flower organs. Eventually, webbing of non-planar telomes generated the vasculature that has since been used throughout cormophyte evolution. In summary, the whole architecture of land plants derives from the patterned organization of these versatile modules. In other words, if one wants to understand the morphogenesis of land plants, one needs in the first place to understand the patterning of vessels as a core element of these building blocks.

Vessel patterning is central for the success of grafting in horticulture (Priestley and Swingle 1929) and therefore shifted into the focus of botanical research many years ago. As long ago as the eighteenth century regenerative events in grafting were explained by a theory where two morphogenetic factors, a heavy "root sap" and a light "shoot sap", moved towards the respective poles driven by gravity, accumulated there and triggered the formation of roots and shoots, respectively (Du Monceau 1764). In fact, the existence of such morphogenetic factors and their transport in the phloem was elegantly demonstrated by incision experiments (Hanstein 1860). By elaborate cutting and regeneration studies Goebel (1908) arrived at the conclusion that an apicobasal flux of an unknown substance defines the regeneration of new shoot and root elements. If this flux is interrupted or inverted, locally restricted inversion of shoot-root polarity becomes manifest as a gradient in the formation of vasculature and the ability to regenerate adventitious shoots or roots, respectively. The factor that was produced by developing leaves and that was able to induce the differentiation of new vasculature from parenchymatic tissue located basipetally of the leaf was later shown to be the transportable auxin IAA (Camus 1949). This finding opened up the possibility to experimentally manipulate the spatial pattern of vascular bundles, an approach that was exploited by Tsvi Sachs in a series of ingenious experiments. He could demonstrate that "differentiated vascular tissue whose source of auxin has been removed attracts newly induced vascular strands. This attraction is expressed in the joining of the new strands to the pre-existing vascular tissue. Differentiated vascular tissue which is well supplied with auxin inhibits rather than attracts the formation of new vascular strands in its vicinity" (Sachs 1968). This basic experiment and numerous experimental derivatives culminated eventually in a canalization model of auxin-dependent patterning of vasculature: if, within an initially homogeneous distribution of auxin across the parenchymatic tissue, the polar flux of auxin is increased locally (for instance by blocking other drainage paths), the increase leads to accelerated differentiation of vessels at this site. Since those developing vessels can already transport more auxin per unit time, they will deplete the neighbouring areas of auxin. A few vessels will form and mutually compete for auxin. With time, the vessels differentiate progressively and, eventually, the pattern is stabilized by lignification (Sachs 1981).

The cellular basis of this drainage model is the polarity of vascular cells that are aligned with the shoot–root axis. The vasculature of a leaf, however, does not reveal such an obvious polarity and it was therefore not clear whether the auxin canalization theory could be generalized to leaf veins as well. However, when transverse vascular strands were investigated, they were found to include adjacent vessels with opposite polarities that did not mature at the same time (Sachs 1975). Similar vessels without clear polarity could be induced experimentally when the location of the auxin source was changed repeatedly. Thus, the axis of a vessel seems to precede its polarity, and the differentiation of a network without clear directionality (as typical for dicotyledonous leaves) is thought to arise from non-synchronous auxin transport across the leaf blade. Since auxin transport has been observed to oscillate in intensity (Hertel and Flory 1968), the formation of an axial, but non-polar vessel could also originate from auxin movement in opposite directions at different times even through the same cells.

This model predicts that inhibition of polar auxin transport should impair the differentiation of leaf veins. This has been tested experimentally in *Arabidopsis* leaves treated with 1-naphthylphthalamic acid (NPA), an inhibitor of auxin transport (Mattsson et al. 1999). When the concentration of the inhibitor was raised, the vasculature was progressively confined to the leaf margin, indicating that the central regions of the leaf were depleted of auxin. The canalization model is further supported by a series of mathematical models that can explain a variety of common venation pattern (for a recent review see Roeland et al. 2007). The molecular base of canalization is generally seen by alignment of auxin-efflux transporters, [Au1] as the PIN proteins, with the flux of auxin, such that these fluxes are amplified even further. In fact, PIN1 is polarized along the putative direction of auxin flow prior to the formation of vasculature during early leaf development (Scarpella et al. 2006).

A central element of the canalization model is the feedback of auxin flux on cell polarity. This implies that the cell responds to the flow rather than to the local concentration of auxin, which poses sophisticated challenges for modelling. Alternatively, PIN proteins could localize differentially to cell membranes depending on the local auxin concentration in the cell adjacent to this membrane as proposed for phyllotaxis (Jönsson et al. 2006). When this readout of local auxin concentration is combined with an auxin-dependent expression of the channel, it is possible to model channelling patterns that are consistent with those of the classical canalization model (Roeland et al. 2007).

Although the cellular details of auxin channelling remain to be elucidated, it is clear that this pattern generator is evolutionarily very ancient. Evidence for polar auxin transport can be found in algae and mosses (for a review see Cooke et al. 2002) and polar auxin transport has been proposed to be responsible for vascular differentiation in early land plants (Stein 1993). In recent conifers or dicotyledonous plants the vasculature follows straight lines, but forms characteristic whirlpools near buds, branches or wounds when the presumed axial flow of auxin is interrupted. Identical circular patterns also occur at the same positions in the secondary wood of the Upper Devonian fossil progymnosperm *Archaeopteris*, thus providing the first clear fossil evidence of polar auxin flow (Rothwell and Lev-Yadun 2005). Thus, already 375

million years ago ancient land plants used polar auxin flux as a tool to establish and maintain a contiguous vascular pattern throughout their telomic modules.

4 Auxin as a Pattern Generator in Cell Differentiation II: Phyllotaxis

In addition to the patterning of vasculature, auxin is a central player in the coordinative signalling that controls phyllotaxis, the formation of new leaves according to an orderly, species-dependent pattern. It has been known for a long time that the position of a prospective leaf primordium in the apical meristem is defined by inhibitory fields from the older primordia proximal to the meristem (Schoute 1913). This was demonstrated by isolation of the youngest primordium by tangential incisions that shifted the position of the subsequent primordia (Snow and Snow 1931). At that time, this shift was interpreted in terms of a first available space model, where the additional space created by the incision would allow the incipient primordia to move to a position where they otherwise were excluded. However, this result is consistent with inhibitory fields emanating from the primordia. There has been a long debate on the nature of these inhibitory signals that were originally thought to be chemical agents, but were later interpreted to be of mechanical nature. Since a growing meristem is subjected to considerable tissue tension, the inhibition could be merely mechanical, because the preexisting primordia would induce stresses upon surrounding potential sites of primordium initiation. The expected stress–strain patterns can perfectly predict the position of prospective primordia (for a review see Green 1980). If the inhibition were mechanical, local release of tissue tension by beads coated with extensin should alter phyllotaxis. In fact, such beads could invert the phyllotactic pattern (Fleming et al. 1997). However, a closer look showed that the extensin-induced structures did not always develop into true leaves, but in some cases resembled mere agglomerations of tissue that did not express leaf markers such as photosynthetic proteins. True leaf development was only initiated when the extensin bead was placed in a site where according to the natural phyllotaxis a primordium would have been laid down. This meant that mere mechanical tension was not sufficient to explain phyllotaxis and this led to a rehabilitation of chemical signals as the cause of the inhibitory field emanating from preexisting primordia. Chemical inhibition was supported by studies in apices that had been freed from primordia by application of auxin-transport inhibitors (Reinhardt et al. 2000), an experimental system that allows study of the de novo generation of a pattern without the influence of preexisting structures. In this system, the coordinative signal was found to be auxin. However, against textbook knowledge, the preexisting primordia did not act as sources, but as sinks for auxin. Within the apical belt that is competent for the initiation of leaf primordia there is mutual competition for auxin as a limiting factor and this competition is biased in favour of certain sites (where, in consequence, a new primordium is initiated) by the preexisting primordia that attract auxin fluxes from the meristem (Reinhardt et al. 2003).

The phyllotactic pattern could be explained by a mechanism where PIN1that continuously cycles between an endocellular compartment and its site of activity at the plasma membrane acts as a sensor for intercellular auxin gradients (Roeland et al. 2007). When the endocytosis of PIN1 becomes suppressed by extracellular auxin (for instance through a membrane-bound or apoplastic auxin receptor; Fig. 3a), auxin will be preferentially pumped upstream by an auxin gradient (Fig. 3b). In fact, the endocytosis of PIN1 has been shown to be suppressed by exogenous auxins (Paciorek et al. 2005) providing the positive amplification loop required for the auxin-dependent inhibitory field.

Phyllotaxis and induction of vasculature are the two classic examples for auxin-dependent pattern formation. What can be generalized from these examples? Both patterns are highly robust against stochastic fluctuations of the input, they rely

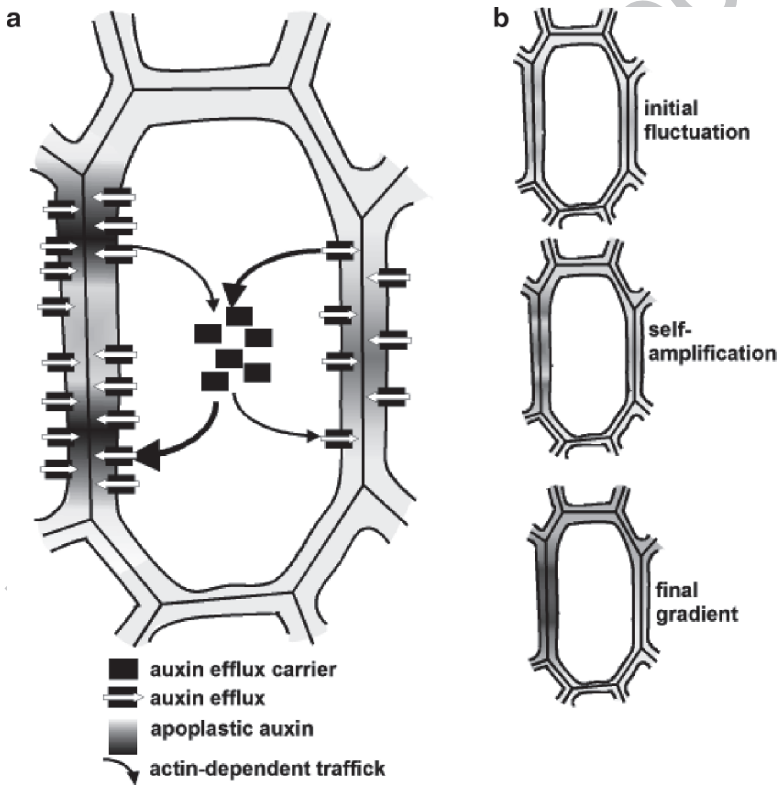

Fig. 3 Model for the self-amplification of transcellular auxin gradients. Auxin-efflux carriers cycle between the plasma membrane (their site of action) and an intracellular pool. Endocytosis of these carriers is locally inhibited by apoplastic auxin and is dependent on actin-mediated vesicle traffic (**a**). The competition between the two flanks of the cell for a limited number of the intracellular carriers in combination with local suppression of carrier endocytosis will amplify initial fluctuations of apoplastic auxin concentration progressively into clear gradients in the concentration of apoplastic auxin (**b**)

on lateral inhibition between the patterned elements, and they culminate in qualitative decisions that are probably brought about by autocatalytic feedback loops. Such mechanisms can be described by the mathematics of reaction-diffusion systems that was adapted to biology (Turing 1952), and has been quite successfully used to model various biological patterns such as foot–head patterning in *Hydra* (Gierer et al. 1972), segmentation in *Drosophila* (Meinhard 1986) and leaf venation (Meinhard 1976). In these reaction-diffusion systems, a locally constrained, self-amplifying feedback loop of an activator is linked to a far-ranging mutual inhibition (Gierer and Meinhard 1972). Auxin-dependent patterning seems to follow this model, but differs in one aspect: rather than employing an actual inhibitor as a positive entity, in auxin-dependent patterning lateral inhibition is brought about by mutual competition for the activator.

5 Auxin as a Pattern Generator in Cell Division

In addition to cell expansion, auxin can induce cell division, a fact that is widely employed for tissue culture and the generation of transgenic plants. Investigation of lateral-root formation in *Arabidopsis* suggested that auxin regulates cell division through a G-protein-dependent pathway (Ullah et al. 2003, for a review see Chen 2001). This was dissected further in tobacco suspension cells, early auxin signalling was dissected further, using the artificial auxins 1-naphthaleneacetic acid (NAA) and 2,4-dichlorophenoxyacetic acid (2,4-D). This study (Campanoni and Nick 2005) demonstrated that these two auxin species affected cell division and cell elongation differentially. NAA stimulated cell elongation at concentrations that were much lower than those required to stimulate cell division. In contrast, 2,4-D promoted cell division but not cell elongation. Pertussis toxin, a blocker of heterotrimeric G-proteins, inhibited the stimulation of cell division by 2,4-D but did not affect cell elongation. Conversely, aluminium tetrafluoride, an activator of the G-proteins, could induce cell division at NAA concentrations that were otherwise not permissive for division and even in the absence of any exogenous auxin. These data suggest that the G-protein-dependent pathway responsible for the auxin response of cell division is triggered by a different receptor than the pathway mediating auxin-induced cell expansion. The two receptors appear to differ in their affinity for different auxin species, with 2,4-D preferentially binding to the auxin receptor responsible for division and NAA preferentially binding to the auxin receptor inducing cell growth.

This bifurcation of auxin signalling (Fig. 4) appears to imply a differential interaction with the cytoskeleton as suggested by a recent detailed study on the effect of auxin on root growth in *Arabidopsis thaliana* (Rahman et al. 2007). When the contributions of cell division and cell elongation were assessed separately, the natural auxin IAAalong with NAA and the auxin-transport inhibitor 2,3,5-triiodobenzoic acid (TIBA)were observed to inhibit cell elongation while leaving filamentous actin basically unaltered. In contrast, 2,4-D and the polar transport inhibitor NP Ainhibited cell division and at the same time eliminated actin filaments.

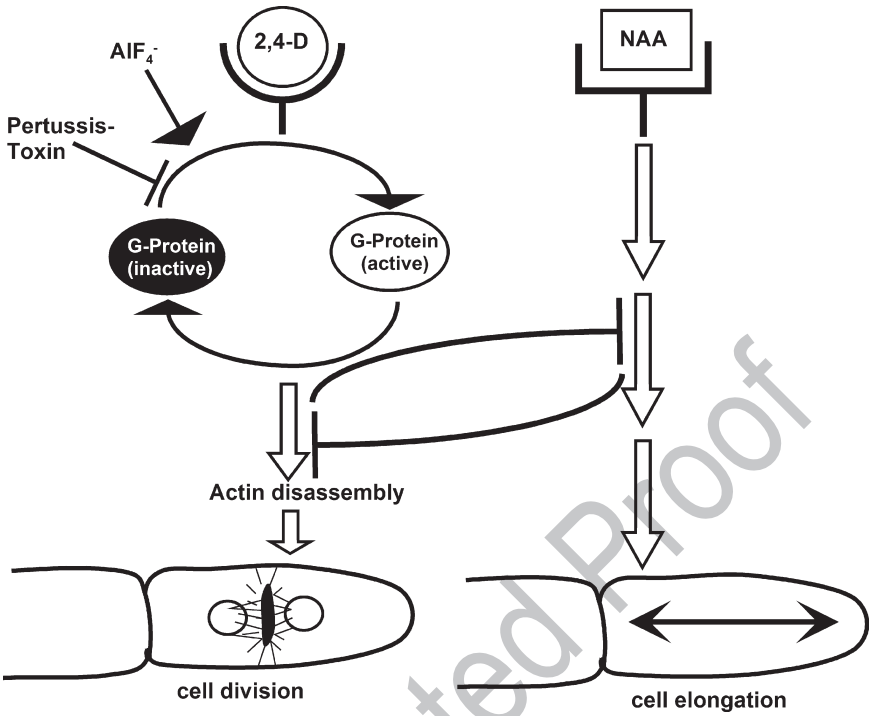

Fig. 4 Model for the bifurcation of auxin signalling in the regulation of cell division and cell elongation in tobacco cells according to Campanoni and Nick (2005) modified according to Rahman et al. (2007). The auxin receptor with high affinity for 1-naphthaleneacetic acid regulates cell elongation and is independent of G-protein activity and does not cause a disassembly of actin, whereas the auxin receptor with high affinity for 2,4-dichlorophenoxyacetic acid triggers a signal chain that involves the activity of a G-protein and triggers the disassembly of actin filaments. This signal chain is inhibited by pertussis toxin and is activated by aluminium tetrafluoride. Both pathways are mutually inhibitory

The root represents a very complex system consisting of different tissue layers that differ with respect to molecular machinery, auxin sensitivity and cytoskeletal organization. Moreover, the frequency of cycling cells, even in a rapidly growing root, is relatively modest, which makes it difficult to study the control exerted by intercellular auxin signalling on cell division on a quantitative level. However, a clear pattern of cell divisions is evident, with the cells of the quiescent centre acting as stem cells for the generation of proliferative tissues. As pointed out already, in the primary root of *Arabidopsis*, where this phenomenon has been dissected most intensively, this pattern can be traced back to early embryogenesis, whereas it seems to be more flexible in meristems of the Graminea. Nevertheless, the pattern of cell division is already established when the root meristem becomes accessible to cell-biological inspection and it is very difficult, if not impossible, to manipulate these patterns in a fundamental manner. Thus, root meristems represent a beautiful

system to study pattern perpetuation, but for the analysis of pattern induction simpler systems that are less determined might be more appropriate. Suspension lines of tobacco are such models to study the primordial stages of division patterning and, in general, cellular aspects of cell division. These lines usually proceed from unicellular stages through a series of axial cell divisions towards cell files that are endowed with a clear axis and, in most cases, with a clear polarity. As will be explored in more detail below, these cell files are not a mere aggregation of autonomous, independent, cells, but display holistic properties such as an overall directionality and a pattern of cell division. In other words, these files are nothing other than a very reduced, but entire version of a multicellular "organism". Owing to this extreme reduction in the level of complexity, it may be easier to study the intercellular negotiations of hypercellular and hypocellular functions rather than in a highly complex and differentiated meristem. Two tobacco cell lines have been studied in more detail with respect to cell–cell communication:

- The cell line VBI-0 (Opatrný and Opatrná 1976; Petrášek et al. 1998) derives from stem pith parenchyma, i.e. the cells that can differentiate into vascular tissue in response to auxin flow. These cells have preserved the ability to generate the structured cell-wall thickenings characteristic for xylogenesis (Nick et al. 2000). In the same way as its parenchymatic ancestor cells, this cell line grows in files where fundamental characteristics of patterning, such as clear axis and polarity of cell division and growth, are manifest. The progression into the culture cycle, the duration of the lag phase, the rate of cell division and the length of the exponential phase (Campanoni et al. 2003), but also cell polarity and axiality (Petrášek et al. 2002), can be controlled by auxin. The cell files are formed from singular cells, such that positional information inherited from the mother tissue probably does not play a role. If there are patterns of competence within a cell file, they must originate de novo during the culture cycle.
- The widely used cell line BY-2 (Nagata et al. 1992) has generated a wealth of data on the role of phytohormones during the plant cell cycle. Compared with VBI-0, the temporal separation between cell division and cell expansion phases is less pronounced (probably as a consequence of the extremely high mitotic activity and short culture cycle). Moreover, the subsequent differentiation of these cells cannot be observed because they very rapidly lose viability if they are not subcultured directly after the logarithmic phase. However, BY-2 is transformed much more easily than VBI-0, such that a broad panel of different transgenic lines expressing fluorescently tagged marker proteins has become available. Moreover, although not as clearly manifest as in VBI-0, the basic features of patterning as well as file axis and polarity can be observed as well in this line.

During the work with these two cell lines, the cell divisions within the file were found to be partially synchronized, leading to a much higher frequency of cell files with even cell numbers than cell files with uneven cell numbers (Campanoni et al. 2003; Maisch and Nick 2007). The experimental data could be simulated using a mathematical model derived from non-linear dynamics, where elementary oscillators (cycling cells) were weakly coupled, and where the number of these oscillators was not conserved, but increased over time. The model predicted several non-intuitive

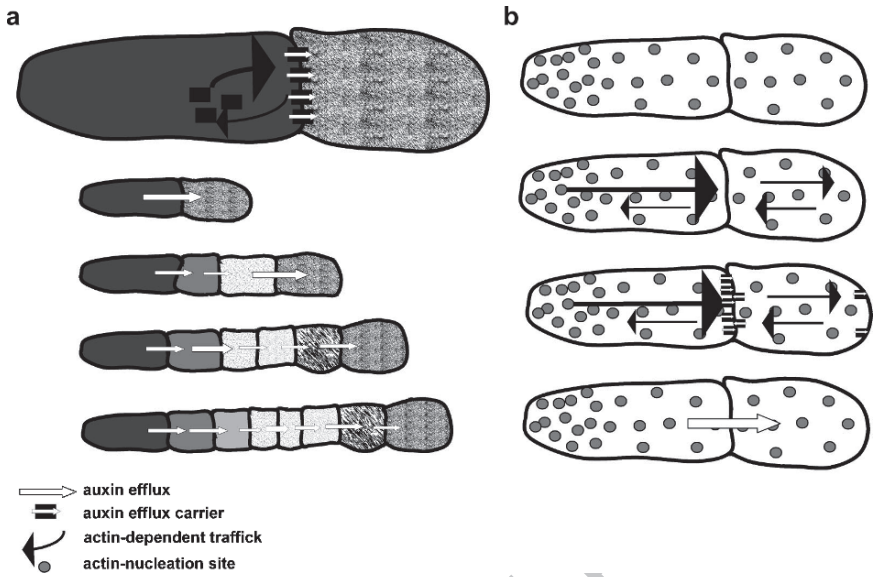

Fig. 5 Model for the regulation of cell division patterns in tobacco cell cultures by polar auxin transport. **a** Actin-dependent cycling of auxin-efflux carriers results in a polar distribution of the carrier and a polar flow of auxin through the cell file. Divisions of neighbouring cells are synchronized by this flow such that even cell numbers become more frequent than uneven cell numbers. **b** Actin-related protein 3 as marker for actin-nucleation sites is distributed in a gradient in the polarized tip cells, but not in the other cells of the file. The gradient of actin nucleation should result in a gradient of actin-dependent traffic that in turn will generate a graded distribution of auxin-efflux carriers such that auxin flow is polarized along the file axis

properties of the experimental system, among them that this coupling is unidirectional, i.e. that the coordinating signal was transported in a polar fashion. The coupling corresponds to a phase shift in the cell cycle, i.e. a dividing cell will cause its downstream neighbour to accelerate its cell cycle such that it will also initiate mitosis. The synchrony of cell divisions could be inhibited by low concentrations of the auxin-efflux inhibitor NPA. Although it has been known for a while that auxin is necessary for the progress of the cell cycle, and thus can be used to synchronize the cell cycle in plant cell cultures (for a review see Stals and Inzé 2001), this was the first time that auxin was shown to coordinate the divisions of adjacent cells. The modelling and the time courses of cell division showed that the noise in this system was considerable, with high variation in the cycling period over the cell population. Nevertheless, the division of adjacent cells was synchronized to such a degree that files with uneven cell numbers were rare compared with files with even numbers (Fig. 5a). Frequency distributions over the cell number per file thus exhibited oscillatory behaviour with characteristic peaks at the even numbers.

Since auxin efflux carriers cycle between the plasma membrane and an endocytotic compartment, auxin signalling has been linked to the organization of actin (for a review see Xu and Scheres 2005). However, this presumed link has recently been

questioned by experiments, where PIN1 and PIN2 maintained their polar localization, although actin filaments had been eliminated by 2,4-D or NPA (Rahman et al. 2007). For the phytotropins TIBAand 2-(1-pyrenoyl) benzoic acid, it was shown very recently that they induce actin bundling not only in plants, but also in mammalian and yeast cells, i.e. in cells that are not to be expected to utilize auxin as a signalling compound (Dhonukshe et al. 2008). This has been interpreted as supportive evidence for a role of actin filaments in polar auxin transport. However, it was mentioned in the same work that NPA failed to cause actin bundling in non-plant cells, suggesting that its mode of action must be different.

Irrespective of the suggested direct effect of TIBA and 2-(1-pyrenoyl) benzoic acid on microfilaments, actinorganization has been found to be highly responsive to changes in the cellular content of auxins (which would explain the NPA effect, for instance). This finding is actually quite old. During the classical period of auxin research, Sweeney and Thimann (1937) proposed that auxin might induce coleoptile growth by stimulating cytoplasmic streaming that is indeed very prominent in the coleoptile epidermis. In a series of publications, the late Kenneth Thimann returned to this idea and showed that elimination of actin very efficiently blocked auxin-dependent growth and argued that microfilaments are necessary for cell growth (Thimann et al. 1992; Thimann and Biradivolu 1994). These findings contrasted with laser tweezer measurements, where the rigour of actin limiting cell expansion was shown to be released by auxin (Grabski and Schindler 1996). In the framework of this actin-rigour model, the elimination of actin would be expected to stimulate rather than inhibit auxin-dependent growth. On the other hand, at that time there was no alternative model that could explain how actin filaments would support cell growth.

To get insight into the role of actin in the control of cell growth, the phytochrome-triggered cell elongation of maize coleoptiles was studied in more detail (Waller and Nick 1997), leading to a physiological definition of two microfilament populations that were functionally different. In cells that underwent rapid elongation, actin was organized into fine strands that became bundled in response to conditions that inhibited growth. This transition was rapid and preceded the changes in growth rate. Moreover, this response was confined to the epidermis, i.e. to the target tissue for the signal control of growth (Kutschera et al. 1987). Later, these two actin populations could be separated biochemically owing to differences in sedimentability (Waller et al. 2002). The fine actin filaments correlated with a cytosolic fraction of actin, whereas actin became trapped on the endomembrane system and was partitioned into the microsomal fraction in conditions that induced bundling. The transition between the two states of actin could be induced, in a dose-dependent manner, by light (perceived by phytochrome), by fluctuations of auxin content, or by the secretion inhibitor brefeldin A (BFA). The bundling of actin was accompanied by a shift of the dose-response of auxin-dependent cell elongation towards higher concentrations and thus to a reduced auxin sensitivity *in sensu strictu*. This led to a model whereby auxin signalling caused a dissociation of actin bundles into finer filaments that were more efficient with respect to the polar transport of auxin-signalling/transport components. Thus, any modulation of cellular auxin content (such as that induced by phytotropins) is expected to repartition the ratio between bundled and detached actin filaments.

This short excursion makes clear that although the organization of actin seems to play a role in the polarity of auxin fluxes, there is also a clear effect of auxin on the organization of actin filaments. This bidirectionality in the relation between actin and auxin has to be considered to avoid flaws in the interpretation of inhibitor effects. The feedback circuit between auxin and actinwas addressed using patterning of cell division as a sensitive trait to monitor changes of polar auxin fluxes (Maisch and Nick 2007). If actin were part of an auxin-driven feedback loop, it should be possible to manipulate auxin-dependent patterning through manipulation of actin (Fig. 5a). This prediction was tested using a transgenic BY-2 cell line stably expressing a fusion between the yellow fluorescent protein and the actin-binding domain of mouse talin (Ketelaar et al. 2004). In this cell line, the microfilaments were constitutively bundled, and the synchrony of cell division was impaired in such a way that the characteristic oscillations described above disappeared. When transportable auxin was added (auxin per se was not sufficient), both a normal organization of actin and the synchrony of cell division could be restored. This demonstrated that actin is not only responsive to changes in the cellular content of auxin, but that it also actively participates in the establishment of the polarity that drives auxin transport.

When actin organization is relevant for the synchrony of cell division (mediated by a polar transport of auxin), the factors that regulate the organization and polarity of actin filaments are highly relevant for patterning. A central player might be the actin-related protein (ARP) 2/3 complex, a modulator of the actin cytoskeleton shown by immunofluorescence to mark sites of actin nucleation in tobacco BY-2 cells (Fišerová et al. 2006). ARP2/3 caps the pointed end such that the actin filament grows in the direction of the barbed end. Tobacco Arp3 was cloned and fused to red [Au2] fluorescent protein (RFP) as a marker for bona fide sites of actin nucleation (Maisch J, Fišerová J, Fischer L, Nick P, submitted). By biolistic transient transformation of [Au3] tobacco cells it was possible, for the first time, to visualize ARP3 in living plant cells. With use of dual-fluorescence visualization of actin [by a green fluorescent protein (GFP) fusion of the actin-binding site of fimbrin] the RFP–ARP3 could be shown to decorate actin filaments in vivo. When actin filaments were transiently eliminated (either by treatment with cytochalasin D or by cold treatment) and then allowed to recover, RFP–ARP3 marked the sites from which the new filaments emanated.

With use of this marker, the behaviour of actin-nucleation sites could be followed through patterned cell division in comparison with AtPIN1::GFP-PIN1as a marker for cell polarity. This uncovered a qualitative difference between the terminal (polarized) cells of a file and the (isodiametric) cells in the centre of a file (Fig. 5b). The density of ARP3 was increased in the apex of terminal cells in a gradient opposed to the polarity monitored by PIN1 (which was concentrated at the opposite, proximal cross wall). Upon disintegration of the file into single cells, the graded distribution of ARP3 persisted, whereas PIN1 was redistributed uniformly over the plasma membrane of these cells. In contrast, the isodiametric cells in the file centre did not exhibit a graded distribution of the ARP3 signal, and the accumulation of PIN1 at the cross wall was much fainter than at the terminal cells, indicating that they are caused by residual amounts of PIN1 laid down by the (polar) progenitors of these cells.

The relationship between actin, vesicle flow and polar auxin transport appears to be interwoven by a bifurcated signal chain: vesicle trafficking mediated by ADP-ribosylation factors (ARFs) is required for the polar localization of Rho-related GTPases in plants which control regulators of the ARP2/3 complex (Frank et al. 2004). On the other hand, ARF-mediated vesicle trafficking also controls the localization of PIN proteins which is known to rely on the activity of the serine-threonine kinase PINOID (Friml et al. 2004) and on the function of P-glycoproteins/multiple drug resistance proteins (Noh et al. 2001). When the function of these ARFs is impaired, in consequence of either treatment with the fungal toxin BFAor a mutation in one of the guanine nucleotide exchange factors that activate the ARFs, PIN1 becomes mislocalized and is trapped in intracellular compartments (Geldner et al. 2001). This cellular effect accounts for the phenotype of the corresponding *Arabidopsis* mutant, *gnom*, that suffers from a drastic loss of cell and organ polarity and, in consequence, is not able to establish an organized *Bauplan*. Thus, ARF-dependent vesicle flow controls actin nucleation (through the activity of the ARP2/3 complex) and, in parallel, the localization of PIN proteins. However, the initial cue that controls the spatial pattern of ARF activity remains unknown. ARP3 maintained an intracellular gradient in the polar terminal cells of BY-2, whereas PIN1 was redistributed (Maisch J, Fišerová J, Fischer L, Nick P, submitted), indicating that actin nucleation might be upstream of the events that culminate in a polar distribution of PIN1. However, owing to the split signalling of the ARFs on the Rho-related GTPases and on the ARP2/3 complex, ARP3 and PIN1 might as well be parallel downstream targets of unknown factors that are expressed in response to cell polarity.

[Au4]

Irrespective of these uncertainties in the molecular details, actin filaments have emerged as central players for the directional vesicle flow by which the polar localization of auxin-efflux carriers is established and perpetuated. The cycling of PIN1 is suppressed by exogenous auxin such that PIN1 remains longer in the plasma membrane (Paciorek et al. 2005) and is therefore able to pump auxin more efficiently into the apoplast. On the other hand, the localization of PIN1 depends on the activity of actomyosin and the organization of the actin tracks is in turn under the control of auxin. These interactions will therefore establish a feedback loop with auxin-efflux carriers, intracellular auxin and actin filaments as central elements (Fig. 3a). This feedback loop is nothing other than a reaction-diffusion system *in sensu* Turing and might represent the cellular pacemaker of auxin-mediated pattern formation.

6 Auxin as a Pattern Generator in Cell Expansion

Once a plant cell has been born by cell division, it undergoes rapid expansion by uptake of water. This expansion is impressive: plant cells can increase in size by up to 4 orders of magnitude (Cosgrove 1987). Regulated cell expansion represents the central adaptive response of the sessile plants to environmental challenges and is therefore highly responsive to stimuli, such as light or gravity, and internal factors, including developmental signals and plant hormones. Whereas the mechanisms

driving and regulating cellular expansion have been investigated in great detail over several decades, relatively little attention has been paid to the coordinative aspects of cell expansion. However, historically it was exactly this coordination of cell expansion that led to the discovery of auxin. In their famous *The Power of Movement in Plants*, the Darwins demonstrated for the phototropism of graminean seedlings that the direction of light is perceived in the very tip of the coleoptile, whereas the growth response to this directional stimulus occurs at the coleoptile base (Darwin and Darwin 1880). The signal transported from the tip to the base of the coleoptile must transmit not only information about the fact that the coleoptile tip has perceived light, but also information about the direction of the light stimulus. [Au5]

Simultaneously, but independently, Cholodny (1927), for gravitropism, and Went (1926), for phototropism, discovered that this transmitted signal must be a hormone. By means of the famous *Avena* biotest this hormone was later found to be IAA (Kögl et al. 1934; Thimann 1935). The Cholodny–Went model explains tropistic curvature by an alignment of auxin transport with the stimulation vector. The resulting gradient of auxin between the two flanks of the stimulated organ will then induce a growth differential that drives bending in the direction of the inductive stimulus.

Since its beginnings, the Cholodny–Wentmodel has been challenged by attempts to explain tropism independently of cell communication by mere summation of cell-autonomous responses (Fig. 6a). For instance, when light causes an inhibition of growth, a gradient of light should produce a gradient of growth that would not require the exchange of intercellular signals (Blaauw 1915). Alternatively, each cell could perceive the direction of the stimulus and produce a directionality on its own – without interaction with the other cells – and the individual cell polarities would then add up to the polarity of the entire organ (Heilbronn 1917). This debate stimulated an ingenious experiment by Johannes Buder, where the gradient of light across the tissue and the direction of light were opposite (Fig. 6b). He irradiated the coleoptile from inside-out using a prototype of a light-pipe (Buder 1920). Under these conditions, the coleoptiles bent towards the lighted flank, i.e. according to the gradient of light and opposite to its direction. The outcome of this experiment demonstrated clearly that the direction of light is sensed in the coleoptile tip owing to extensive communication between the perceptive cells and strongly argues against cell-autonomous models of tropistic perception (Nick and Furuya 1996).

The transverse polarity built up in response to phototropic or gravitropic stimulation in the perceptive tissue subsequently redistributes the basipetal flow of auxin and thus transmits the directional information into the responsive tissue at the coleoptile base. This gradient of auxin flow is well established, starting from bioassays (for instance Dolk 1936) and ending up with tracer experiments using radioactively labelled auxin (Goldsmith and Wilkins 1964; Parker and Briggs 1990; Iino 1991; Godbolé et al. 2000) or direct measurements of free auxin across tropistically stimulated coleoptiles (Philippar et al. 1999; Gutjahr et al. 2005).

The Cholodny–Went model has been under continuous debate (see also Trewavas 1992), mainly because there is a discrepancy in amplitude between the gradient of the growth rate and the gradient of auxin concentration. The difference in auxin content between the two flanks of a tropistically stimulated coleoptile is in the [Au6]

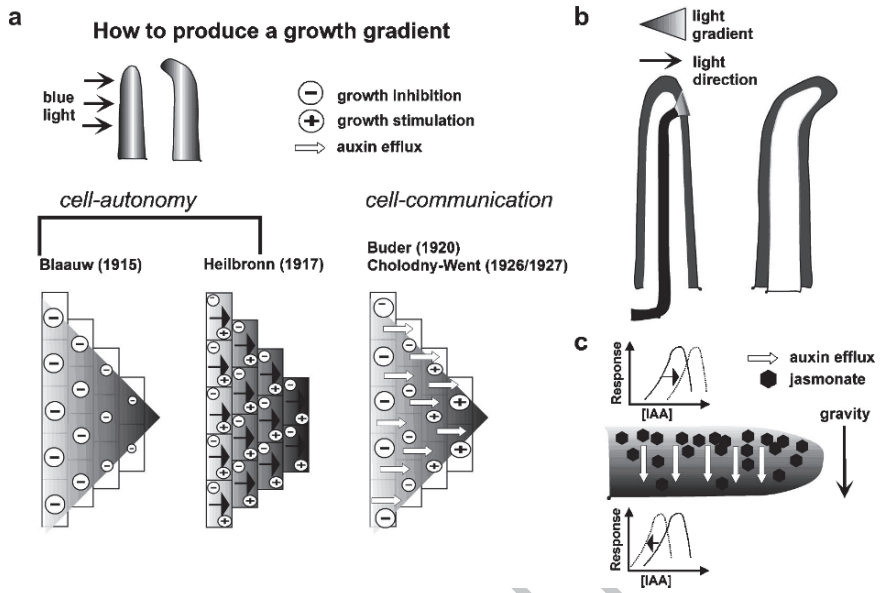

Fig. 6 Patterns of cell expansion during tropistic curvature of graminean coleoptiles. **a** Models for the formation of a growth gradient. According to Blaauw (1915), phototropic curvature emerges from a summation of growth inhibitions in response to the local intensity of light without interaction between cells. According to Heilbronn (1917), individual cells perceive the direction of light and respond by an intracellular gradient of growth. In contrast to these models that are based on complete cell autonomy, Buder (1920) explains curvature by interactions between individual cells across the coleoptile, and Cholodny (1927) and Went (1926) imply a lateral transport of a growth substance ("auxin"). **b** The experiment of Buder (1920), where the light direction and the light gradient across the organ are opposed. The bending is determined by the gradient, not by the direction of the light, contradicting the model postulated by Heilbronn (1917). **c** Extended Cholodny-Went model of gravitropism (according to Gutjahr et al. 2005). The lateral transport of auxin across the stimulated coleoptile is accompanied by a counterdirected gradient of jasmonate abundance and a gradient of auxin responsiveness across the tissue, with elevated responsiveness in the lower flank and reduced responsiveness in the upper flank

range of about 1:2 (Goldsmith and Wilkins 1964; Parker and Briggs 1990; Gutjahr et al. 2005), whereas growth is completely shifted from one flank to the other, i.e. the decrease in growth rate in one flank corresponds to the increase in growth rate in the other flank (Digby and Firn 1976; Iino and Briggs 1984; Himmelspach and Nick 2001). Elongation growth in coleoptiles increases more or less proportionally to the logarithm of auxin concentration (Wang and Nick 1998), such that the observed doubling of auxin concentration in the one flank would not succeed in causing the observed changes in growth rate. Moreover, when gravitropically stimulated hypocotyls (Rorabaugh and Salisbury 1989) or coleoptiles (Edelmann 2001; Gutjahr et al. 2005) were submersed in high concentrations of auxin, they showed positive gravitropism, i.e. they behaved as if they were roots. This is difficult to reconcile with a gradient of auxin as a unique cause for tropistic curvature.

With use of a classic biotest for auxin, the split-pea assay, in gravitropically stimulated rice coleoptiles, it could be demonstrated that, in parallel to the redistribution of auxin itself, a gradient of auxin responsiveness develops (Gutjahr et al. 2005) with elevated responsiveness at the lower flank and reduced responsiveness at the upper flank (Fig. 6c). This gradient of responsiveness can account for the strong redistribution of growth even for relatively modest gradients of auxin. It can even explain the peculiar sign reversal of bending for incubation with high concentrations of auxin beyond the optimum – for such superoptimal concentrations, the elevated auxin responsiveness at the lower flank should result in an inhibition of growth that is less pronounced in the upper flank, where the responsiveness is lower. In parallel to the gradient of auxin, a counterdirected gradient of jasmonate developed with higher concentrations at the upper flank as compared with the lower flank. Jasmonate acts as a negative regulator for auxin responsiveness, because both signal pathways compete for signalling factors such as AXR1 (Schwechheimer et al. 2001). Thus, the observed jasmonate gradient might well account for the observed gradient in auxin responsiveness across a gravitropically stimulated coleoptile. To test this assumption, the jasmonate gradient was either equalized by flooding the coleoptiles with exogenous methyl jasmonate or eliminated in consequence of a mutation that blocks jasmonate synthesis (Gutjahr et al. 2005). In both cases, the gravitropic response was delayed by about 1 h, but was eventually initiated and proceeded normally. This indicates that the jasmonate gradient, although not necessary for gravitropism, acts as a positive modulator. When auxin transport was inhibited by NPA, the jasmonate gradient nevertheless developed, suggesting that it is induced by gravitropic stimulation in parallel to and not in consequence of lateral auxin transport.

In summary, the Cholodny–Went model has to be extended by signal-triggered, modulative gradients of auxin responsiveness, but remains valid in its central statements. This means that tropistic responses, representing nothing other than a patterned distribution of cell expansion over the cross-section of the stimulated organ, can be explained in terms of auxin-dependent cell communication.

The analysis of auxin-dependent cell communication in cell division has identified a feedback loop between actin and auxin. This loop represents also a central element of patterned cell expansion. Actually, it was cell elongation in coleoptiles where the regulation of actin organization by auxin was discovered first (Sweeney and Thimann 1937; Thimann et al. 1992; Thimann and Biradivolu 1994; Waller and Nick 1997; Wang and Nick 1998; Holweg et al. 2004). Treatment with BFA, a fungal inhibitor of vesicle budding, caused, despite the presence of auxin, a rapid bundling of microfilaments and shifted actin from the cytosolic fraction into the microsomal fraction (Waller et al. 2002) depending on the dose of auxin and of BFA. In parallel, BFA shifted the dose-response curve of auxin-dependent growth to higher concentrations. In other words, BFA decreased auxin sensitivity *in sensu strictu*, consistent with an actin-dependent transport of auxin-signalling components such as auxin-efflux carriers. Again, a self-amplification loop emerges, consisting of auxin-dependent organization of actin filaments and actin-dependent transport of auxin-signalling components.

A prediction from this model for the actin-auxin feedback loop would be that a bundling of actin should be followed by a reduction in the activity of polar auxin transport. This prediction is supported by the suppression of division synchrony in tobacco cell lines that overexpress mouse talin (Maisch and Nick 2007). However, to measure auxin transport directly, it would be necessary to administer radioactively labelled auxin to one pole of the cell file and to quantify the radioactivity recovered in the opposite pole of the file. This is not possible in a cell culture that has to be cultivated as suspension in a liquid medium. This approach would be feasible, however, in the classical graminean coleoptile system, where auxin transport can be easily measured by following the distribution of radioactively labelled IAA fed to the coleoptile apex. Transgenic rice lines were generated that expressed variable levels of the actin-binding protein talin (Nick P, Han MJ, An G, submitted). In those lines, as a consequence of talin overexpression, actin filaments were bundled to variable extent, and this bundling of actin filaments was accompanied by a corresponding reduction in the polar transport of auxin and gravitropic curvature (as a physiological marker that relies on the activity of auxin transport). When a normal configuration of actin was restored by addition of exogenous auxin, this restored auxin transport as well. This rescue was mediated by transportable auxin species, but not by 2,4-D, which lacks polar transport. With use of this approach, the causal relationship between actin configuration and polar auxin transport could be shown directly.

A further prediction of the actin–auxin feedback model is oscillations in transport activity, because auxin will, through the reorganization of actin, stimulate its own efflux such that the intracellular level of auxin will drop, which in turn will result in a bundled configuration of actin, such that auxin-efflux carriers will be sequestered, culminating in a reduced efflux such that auxin received from the adjacent cells will accumulate and trigger a new cycle. The frequency of these oscillations should depend on the dynamics of actin reorganization (around 20 min; Nick P, Han MJ, An G, submitted), and the speed of PIN cycling (in the range of 5–10 min; Paciorek et al. 2005) and is therefore expected to be in the range of 25–30 min. In fact, classic experiments on basipetal auxin transport in coleoptiles report such oscillations with a period of 25 min (Hertel and Flory 1968).

We therefore arrive at a model of a self-referring regulatory circuit between polar auxin transport and actin organization, where auxin promotes its own transport by shaping actin filaments. Thus, similar to the patterning of cell division, the actin-auxin oscillator seems also to be the pacemaker for the patterning of cell expansion.

7 Why Auxin - or Order Without a “Great Chairman”

Already in multicellular algae, a polar transport of auxin can be detected (Dibb-Fuller and Morris 1992; Cooke et al. 2002) and seems to play a role in the establishment of polarity (Basu et al. 2002), indicating that the central role of auxin in cell communication is evolutionarily quite ancient and had already been developed prior

to the colonization of terrestrial habitats. Why has evolution selected such a simple molecule for such a central role in intercellular coordination?

Although we are far from providing a full answer to this question, it seems that the answer is related to plant-specific features in the organization of signalling and development: In animal development, cell differentiation is typically controlled by precise and defined regulatory networks that are structured by predetermined hierarchies. In contrast, plant cells are endowed with a pronounced developmental flexibility that is maintained basically throughout the entire life span of a plant cell. Moreover, there are hardly any fixed hierarchies – plant development does not know of such a thing as a "Great Chairman" that assigns differential developmental pathways to the individual cells. Plant cells rather "negotiate" their individual developmental fates in a fairly "democratic" manner with hierarchies being created ad hoc by mutual interactions. It seems that auxin is a central tool in these "negotiations", because it represents a versatile tool to establish ad hoc hierarchies on the background of the high degree of "cellular anarchy" and noise that is characteristic for plant development.

Why is plant development so "noisy"? The strong developmental noise seems to be the tribute paid to indeterminate morphogenesis. The manifestation of the *Bauplan* in an individual plant depends strongly on the environmental conditions encountered during development. This developmental flexibility includes a rapid response of cell expansion, complemented by a somewhat slower addition of morphogenetic elements, such as cells, pluricellular structures and organs. This patterning process can integrate signals from the environment, and must therefore be both highly flexible and robust. More specifically, the patterning process has to cope with signals that can vary over several orders of magnitude for the strength of the control signal, and new elements have to be added such that the pattern formed by the preexisting elements is perpetuated and/or complemented.

Plant sensing occurs in a rather diffuse manner – there are no such things as eyes, ears or tongues; there are, instead, populations of relatively undifferentiated cells that sense environmental cues and signals. Nevertheless, plant sensing is surprisingly sensitive. When this high sensitivity of signalling is reached without specialized sensory organs, the individual cells must already be endowed with very efficient mechanisms for signal amplification that are active already during the first steps of the transduction chain. This strong signal amplification will inevitably result in all-or-none outputs of individual cells. The efficient amplification of weak stimuli on the one hand, with the simultaneous necessity to discriminate between very strong stimuli of different amplitude, poses special challenges for plant signalling. If all cells of a given organ were absolutely identical and homogeneous, even an extremely weak stimulation would yield a maximal response of the whole organ. It is clear that such a system would not have survived natural selection – the amplitude of the output must vary according to variable amplitudes of the input signal, because the plant has to respond appropriately to stimuli that vary in intensity, even if these stimuli are strong. One way to reconcile the requirement for high sensitivity with the requirement for a graded, variable output would be to assign the two antagonistic tasks to different levels of organization: the high sensitivity to the individual cells that perceive the signal; the graded, variable output to the population

of cells (i.e. the tissue or organ) by an integration over the individual cell responses. But this works only when the sensory thresholds of individual cells differ over the population; in other words, when the individual cells are highly heterogeneous with respect to signal sensitivity and thresholds.

This heterogeneity was actually observed when photomorphogenesis was investigated on a cellular level for phytochrome-induced anthocyanin patterns in mustard cotyledons, a classic system of light-dependent plant patterning (Mohr 1972; Nick et al. 1993) or for microtubule reorientation in coleoptiles triggered by blue light or auxin depletion (Nick 1992). Even adjacent cells exhibited almost qualitative differences although they had received the same dose of the signal. However, when the frequency of responsive cells in a given situation was scored and plotted against the strength of the stimulus, a highly ordered function emerged. Thus, the realm of individual cells was reigned over by chaos; order emerged only on the level of the whole organ. This highly stochastic, all-or-none type response of individual cells becomes especially manifest for an early response to a saturating stimulus or as the final result of weak induction (Nick et al. 1992). It thus appears that early signalling events are highly stochastic, when assayed at the level of individual cells. These responses are not merely "noisy" because the flexible physiology of plant cells can tolerate this. These "noisy" responses rather represent an innate system property of plant signalling. However, this "noisy" inputs poses especial challenges for any ordering principle.

[Au11]

It might be these challenges that have rendered IAA a central integrator and synchronizer of plant development. A molecule that is easily transported through the acidic environment of the apoplast, but that is readily trapped in the cytoplasm and then has to be actively exported is ideally suited to convey lateral inhibition between neighbouring cells. It was sufficient to put the localization of the efflux transporter (whatever its molecular nature may be) under the control of auxin itself to reach a perfect reaction-diffusion system *in sensu* Turing (1952). On the intracellular level, this system is able to establish a clear cell polarity from even minute and noisy directional cues. On the level of tissues, this cell polarity will generate patterns in a manner that meets the special constraints of plant development, i.e. noisy inputs as a consequence diffuse sensing and progressive addition of new elements to the pattern. Since the natural auxin IAA can enter the cell from any direction (because it can enter the cell even independently of import carriers such as AUX1), but will exit in a defined direction defined by the localized activity of the efflux carrier, it can collect the input from several neighbours and focus this input into a clear directional output. It is this property that makes auxin a versatile and robust integrator for cell–cell communication.

References

Basu S, Sun H, Brian L, Quatrano RL, Muday GK (2002) Early embryo development in *Fucus distichus* is auxin sensitive. Plant Physiol 130:292–302

Blaauw AH (1915) Licht und Wachstum II. Bot Zentralbl 7:465–532

Buder J (1920) Neue phototropische Fundamentalversuche. Ber Dtsch Bot Ges 28:10–19

820 Campanoni P, Nick P (2005) Auxin-dependent cell division and cell elongation: NAA and 2,4-D
821 activate different pathways. Plant Physiol 137:939–948
822 Campanoni P, Blasius B, Nick P (2003) Auxin transport synchronizes the pattern of cell division
823 in a tobacco cell line. Plant Physiol 133:1251–1260
824 Camus G (1949) Recherche sur le role des bourgeons dans les phenomenes de morphogenèse. Rev
825 Cytol Biol Veg 11:1–199
826 Chen JG (2001) Dual auxin signalling pathways control cell elongation and division. J Plant
827 Growth Regul 20:255–264
828 Cholodny N (1927) Wuchshormone und Tropismen bei den Pflanzen. Biol Zentralbl 47:604–626
829 Cooke TJ, Poli DB, Sztein AE, Cohen JD (2002) Evolutionary patterns in auxin action. Plant Mol
830 Biol 49:319–338
831 Cosgrove D (1987) Assembly and enlargement of the primary cell wall in plants. Annu Rev Cell
832 Dev Biol 13:171–201
833 Darwin C, Darwin F (1880) Sensitiveness of plants to light: it's transmitted effect. In: The power
834 of movement in plants. Murray, London, pp 574–592
835 Dibb-Fuller JB, Morris DA (1992) Studies on the evolution of auxin carriers and phytotropin
836 receptors: transmembrane auxin transport in unicellular and multicellular Chlorophyta. Planta
837 186:219–226
838 Digby J, Firn RD (1976) A critical assessment of the Cholodny–Went theory of shoot gravitro-
839 pism. Curr Adv Plant Sci 8:953–960
840 Dhonukshe P, Grigoriev I, Fischer R, Tominaga M, Robinson DG, Hašek J, Paciorek T, Petrašek J,
841 Seifertová D, Tejos R, Meisel LA, Zažímalová E, Gadella TWJ, Stierhof YD, Ueda T, Oiwa K,
842 Akhmanova A, Brocke R, Spang A, Friml J (2008) Auxin transport inhibitors impair vesicle motility
843 and actin cytoskeleton dynamics in diverse eukaryotes. Proc Natl Acad Sci U S A 105:4489–4494
844 Dolk HE (1936) Geotropism and the growth substance. Recl Trav Bot Neerl 33:509–585
845 Du Monceau D (1764) La physique des arbres. Winterschmidt, Nuremberg, pp 87–93
846 Edelmann H (2001) Lateral redistribution of auxin is not the means for gravitropic differential
847 growth of coleoptiles: a new model. Physiol Plant 112:119–126
848 Fišerová J, Schwarzerová K, Petrášek J, Opartný S (2006) ARP2 and ARP3 are localized to sites
849 of actin filament nucleation in tobacco BY-2 cells. Protoplasma 227:119–128
850 Fleming AJ, McQueen-Mason S, Mandel T, Kuhlemeier C (1997) Induction of leaf primordia by
851 the cell wall protein expansin. Science 276:1415–1418
852 Frank M, Egile C, Dyachok J, Djakovic S, Nolasco M, Li R, Smith LG (2004) Activation of
853 Arp2/3 complex-dependent actin polymerization by plant proteins distantly related to Scar/
854 WAVE. Proc Natl Acad Sci U S A 101:16379–16384
855 Friml J, Yang X, Michniewicz M, Weijers D, Quint A, Tietz O, Benjamins R, Ouwerk PB, Ljung K,
856 Sandberg G, Hooykaas PJ, Palme K, Offringa R (2004) A PINOID-dependent binary switch
857 in apical-basal PIN polar targeting directs auxin efflux. Science 306:862–865
858 Geldner N, Friml J, Stierhof YD, Jürgens G, Palme K (2001) Auxin transport inhibitors block
859 PIN1 cycling and vesicle trafficking. Nature 413:425–428
860 Gierer A, Meinhard H (1972) A theory of biological pattern formation. Kybernetik 12:30–39
861 Gierer A, Berking S, Bode H, David CN, Flick K, Hansmann G, Schaller H, Trenkner E (1972)
862 Regeneration of hydra from reaggregated cells. Nature 239:98–101
863 Godbolé R, Michalke W, Nick P, Hertel R (2000) Cytoskeletal drugs and gravity-induced lateral
864 auxin transport in rice coleoptiles. Plant Biol 2:176–181
865 Goebel K (1908) Einleitung in die experimentelle Morphologie der Pflanzen. Teubner, Leipzig,
866 pp 218–251
867 Goldsmith MHM, Wilkins MB (1964) Movement of auxin in coleoptiles of *Zea mays* L. during
868 geotropic stimulation. Plant Physiol 39:151–162
869 Grabski S, Schindler M (1996) Auxins and cytokinins as antipodal modulators of elasticity within
870 the actin network of plant cells. Plant Physiol 110:965–970
871 Green PB (1980) Organogenesis – a biophysical view. Annu Rev Plant Physiol 31:51–82
872 Gutjahr C, Riemann M, Müller A, Weiler EW, Nick P (2005) Cholodny–Went revisited – a role
873 for jasmonate in gravitropism of rice coleoptiles. Planta 222:575–585

Hanstein J (1860) Versuche über die Leitung des Saftes durch die Rinde. Jahrb Wiss Bot 2:392
Heilbronn A (1917) Lichtabfall oder Lichtrichtung als Ursache der heliotropischen Reizung? Ber Dtsch Bot Ges 35:641–642
Hertel R, Flory R (1968) Auxin movement in corn coleoptiles. Planta 82:123–144
Himmelspach R, Nick P (2001) Gravitropic microtubule orientation can be uncoupled from growth. Planta 212:184–189
Holweg C, Süßlin C, Nick P (2004) Capturing *in-vivo* dynamics of the actin cytoskeleton. Plant Cell Physiol 45:855–863
Iino M (1991) Mediation of tropisms by lateral translocation of endogenous indole-3-acetic acid in maize coleoptiles. Plant Cell Environ 14:279–286
Iino M, Briggs WR (1984) Growth distribution during first positive phototropic curvature of maize coleoptiles. Plant Cell Environ 7:97–104
Jönsson H, Heisler MG, Shapiro BE, Meyerowitz EM, Mjolsness E (2006) An auxin-driven polarized transport model for phyllotaxis. Proc Natl Acad Sci U S A 103:1633–1638
Ketelaar T, Anthony RG, Hussey PJ (2004) Green fluorescent protein-mTalin causes defects in actin organization and cell expansion in *Arabidopsis* and inhibits actin depolymerizing factor's actin depolymerizing activity in vitro. Plant Physiol 136:3990–3998
Kirk DL (2003) Seeking the ultimate and proximate causes of *Volvox* multicellularity and cellular differentiation. Integr Comp Biol 43:247–253
Kögl F, Hagen-Smit J, Erxleben H (1934) über ein neues Auxin ("Hetero-Auxin") aus Harn. Hoppe-Seylers Z Physiol Chem 228:104–112
Kutschera U, Bergfeld R, Schopfer P (1987) Cooperation of epidermal and inner tissues in auxin-mediated growth of maize coleoptiles. Planta 170:168–180
Lintilhac PM (1999) Towards a theory of cellularity – speculations on the nature of the living cell. Bioscience 49:60–68
Maisch J, Nick P (2007) Actin is involved in auxin-dependent patterning. Plant Physiol 143:1695–1704
Mattsson J, Sung ZR, Berleth T (1999) Responses of plant vascular systems to auxin transport inhibition. Development 126:2979–2991
McCabe PF, Valentine TA, Forsberg S, Pennella R (1997) Soluble signals from cells identified at the cell wall establish a developmental pathway in carrot. Plant Cell 9:2225–2241
Meinhard H (1976) Morphogenesis of lines and nets. Differentiation 6:117–123
Meinhard H (1986) The threefold subdivision of segments and the initiation of legs and wings in insects. Trends Genet 3:36–41
Mohr H (1972) Lectures on photomorphogenesis. Springer, Heidelberg
Nagata T, Nemoto Y, Hasezava S (1992) Tobacco BY-2 cell line as the "Hela" cell in the cell biology of higher plants. Int Rev Cytol 132:1–30
Nakajima K, Sena G, Nawy T, Benfey PN (2001) Intercellular movement of the putative transcription factor SHR in root patterning. Nature 413:307–311
Nick P, Furuya M (1996) Buder revisited – cell and organ polarity during phototropism. Plant Cell Environ 19:1179–1187
Nick P, Schäfer E, Furuya M (1992) Auxin redistribution during first positive phototropism in corn coleoptiles: microtubule reorientation and the Cholodny–Went theory. Plant Physiol 99:1302–1308
Nick P, Ehmann B, Schäfer E (1993) Cell communication, stochastic cell responses, and anthocyanin pattern in mustard cotyledons. Plant Cell 5:541–552
Nick P, Heuing A, Ehmann B (2000) Plant chaperonins: a role in microtubule-dependent wall-formation? Protoplasma 211:234–244
Noh B, Murphy AS, Spalding EP (2001) Multidrug resistance-like genes of *Arabidopsis* required for auxin transport and auxin-mediated development. Plant Cell 13:2441–2454
Opatrný Z, Opatrná J (1976) The specificity of the effect of 2,4-D and NAA on the growth, micromorphology, and occurrence of starch in long-term *Nicotiana tabacum* L. cell strains. Biol Plant 18:359–365
Paciorek T, Zažimalová E, Ruthardt N, Petrášek J, Stierhof YD, Kleine-Vehn J, Morris DA, Emans N, Jürgens G, Geldner N, Friml J (2005) Auxin inhibits endocytosis and promotes its own efflux from cells. Nature 435:1251–1256

928 Parker KE, Briggs WR (1990) Transport of indole-3-acetic acid during gravitopism of intact
929 maize coleoptiles. Plant Physiol 94:1763–1768
930 Petrášek J, Freudenreich A, Heuing A, Opatrný Z, Nick P (1998) Heat shock protein 90 is associ-
931 ated with microtubules in tobacco cells. Protoplasma 202:161–174
932 Petrášek J, Elčkner M, Morris DA, Zažímalová E (2002) Auxin efflux carrier activity and auxin
933 accumulation regulate cell division and polarity in tobacco cells. Planta 216:302–308
934 Philippar K, Fuchs I, Lüthen H, Hoth S, Bauer CS, Haga K, Thiel G, Ljung K, Sandberg G, Böttger M,
935 Becker D, Hedrich R (1999) Auxin-induced K^+ channel expression respresents an essential step
936 in coleoptile growth and gravitropism. Proc Natl Acad Sci U S A 96:12186–12191
937 Priestley JH, Swingle CF (1929) Vegetative propagation from the standpoint of plant anatomy.
938 USDA Tech Bull 151:1–98
939 Rahman A, Bannigan A, Sulaman W, Pechter P, Blancaflor EB, Baskin TI (2007) Auxin, actin and
940 growth of the *Arabidopsis thaliana* primary root. Plant J 50:514–528
941 Reinhardt D, Mandel T, Kuhlemeier C (2000) Auxin regulates the initiation and radial position of
942 plant lateral organs. Plant Cell 12:507–518
943 Reinhardt D, Pesce ER, Stieger P, Mandel T, Baltensperger K, Bennett M, Traas J, Friml J,
944 Kuhlemeier C (2003) Regulation of phyllotaxis by polar auxin transport. Nature 426:255–260
945 Roeland MH, Merks RMH, Van de Peer Y, Inze D, Beemster GTS (2007) Canalization without
946 flux sensors: a traveling-wave hypothesis. Trends Plant Sci 12:384–390
947 Rorabaugh PA, Salisbury FB (1989) Gravitropism in higher plant shoots VI. Changing sensitivity
948 to auxin in gravistimulated soybean hypocotyls. Plant Physiol 91:1329–1338
949 Rothwell GW, Lev-Yadun S (2005) Evidence of polar auxin flow in 375 million-year-old fossil
950 wood. Am J Bot 92:903–906
951 Sachs T (1968) On the determination of the pattern of vascular tissue in peas. Ann Bot 33:781–790
952 Sachs T (1975) The control of the differentiation of vascular networks. Ann Bot 39:197–204
953 Sachs T (1981) The control of the patterned differentiation of vacular tissues. Adv Bot Res
954 9:152–262
955 Scarpella E, Marcos D, Friml D, Berleth T (2006) Control of leaf vascular patterning by polar
956 auxin transport. Genes Dev 20:1015–1027
957 Scheres B, Wolkenfelt H, Willemsen V, Terlouw M, Lawson E, Dean C, Weisbeek P (1994) Embryonic
958 origin of the *Arabidopsis* root and root meristem initials. Development 120:2475–2487
959 Schoute JC (1913) Beiträge zur Blattstellungslehre. I. Die Theorie. Rec Trav Bot Neerl 10:153–325
960 Schwechheimer C, Serino G, Callis J, Crosby WL, Lyapina S, Deshaies RJ, Gray WM, Estelle M,
961 Deng XW (2001) Interactions of the COP9 signalosome with the E3 ubiquitin ligase SCF^{TIR1}
962 in mediating auxin response. Science 292:1379–1382
963 Snow M, Snow R (1931) Experiments on phyllotaxis. I. The effect of isolating a primordium.
964 Philos Trans R Soc Lond Ser B 221:1–43
965 Spemann H (1936) Experimentelle Beitrage zu einer Theorie der Entwicklung. Springer, Berlin
966 Stals H, Inzé D (2001) When plant cells decide to divide. Trends Plant Sci 6:359–364
967 Stein W (1993) Modeling the evolution of stelar architecture in vascular plants. Int J Plant Sci
968 154:229–263
969 Sweeney BM, Thimann KV (1937) The effect of auxin on cytoplasmic streaming. J Gen Physiol
970 21:123–135
971 Thimann KV (1935) On the plant growth hormone produced by *Rhizopus suinus*. J Biol Chem
972 109:279–291
973 Thimann KV, Biradivolu R (1994) Actin and the elongation of plant cells II: the role of divalent
974 ions. Protoplasma 183:5–9
975 Thimann KV, Reese K, Nachmikas VT (1992) Actin and the elongation of plant cells. Protoplasma
976 171:151–166
977 Turing AM (1952) The chemical basis of morphogenesis. Philos Trans R Soc Lond Ser B Biol
978 237:37–72
979 Ullah H, Chen JG, Temple B, Boyes DC, Alonso JM, Keith RD, Ecker JR, Jones AM (2003) The
980 beta-subunit of the *Arabidopsis* G protein negatively regulates auxin-induced cell division and
981 affects multiple developmental processes. Plant Cell 15:393–409

Van den Berg C, Willensen V, Hage W, Weisbeek P, Scheres B (1995) Cell fate in the *Arabidopsis* root meristem is determined by directional signalling. Nature 378:62–65

Waller F, Nick P (1997) Response of actin microfilaments during phytochrome-controlled growth of maize seedlings. Protoplasma 200:154–162

Waller F, Riemann M, Nick P (2002) A role for actin-driven secretion in auxin-induced growth. Protoplasma 219:72–81

Wang QY, Nick P (1998) The auxin response of actin is altered in the rice mutant *Yin-Yang*. Protoplasma 204:22–33

Weismann A (1894) Aufsätze über Vererbung und verwandte Fragen. Fischer, Jena

Went F (1926) On growth accelerating substances in the coleoptile of *Avena sativa*. Proc Kon Ned Akad Weten 30:10–19

Xu J, Scheres B (2005) Cell polarity: ROPing the ends together. Curr Opin Plant Biol 8:613–618

Yoon HS, Golden JW (1998) Heterocyst pattern formation controlled by a diffusible peptide. Science 282:935–938

Yoon HS, Golden JW (2001) PatS and products of nitrogen fixation control heterocyst pattern. J Bacteriol 183:2605–2613

Zimmermann W (1965) Die Telomtheorie. Fischer, Stuttgart

Author Queries

Chapter No.: 158344_Baluska_01

Queries	Details Required	Author's Response
[AU1]	Please advise if "basis" is meant.	
[AU2]	Please advise if this should be "ARP".	
[AU3]	Kindly note that "Maisch J, Fischer L, Nick P, Submitted" is not found in the reference list. Please add this reference to the list or delete from the text.	
[AU4]	Kindly note that "Maisch J, Fišerová Fischer L, Nick P, Submitted" is not found in the reference list. Please add this reference to the list or delete from the text.	
[AU5]	Kindly note that year in the reference citation Drawin and Drawin (1888) has been changed to (1880) according to the reference list. Please check.	
[AU6]	Kindly note that Trewavas 1992 is not found in the reference list. Please add to the list or delete from the text.	
[AU7]	Please advise if "various" is meant. "Variable" means "changing".	
[AU8]	Kindly note that "Nick P, Han MJ, AnG, Submitted" is not found in the reference list. Please add this reference to the list or delete from the text.	
[AU9]	Please advise if "different" is meant. "Variable" means "changing".	
[AU10]	Kindly note that "Nick P, Han MJ, AnG, Submitted" is not found in the reference list. Please add this reference to the list or delete from the text.	
[AU11]	Please explain exactly what is meant here by "physiology". In Spriger publications"-ology" should only be used for "the study of…"	