Noise Yields Order – Auxin, Actin, and Polar Patterning

P. Nick

Institut of Botany 1, University of Karlsruhe, Kaiserstraße 2, 76128 Karlsruhe, Germany

Abstract: Plant patterns have to integrate environmental cues and to cope with a high level of noise in the sensory outputs of individual cells. In the first part of this review, we demonstrate that local self-amplification linked to lateral inhibition can meet this requirement. In the second part, we describe the search for candidates for such self-amplification loops in the context of auxin-dependent cell growth using Graminean coleoptiles as a model. Auxin-dependent reorganization of actin microfilaments interfered with the auxin sensitivity of growth. Auxin might control the intracellular transport of factors important for auxin sensing via the actomyosin system. By means of a rice mutant with elevated auxin responsiveness, we identified an auxin response factor *(OsARF1),* whose expression is upregulated by auxin as a second candidate for a self-amplification loop. We studied the cross-talk between auxin signalling and environmental cues in the rice mutant *hebiba,* where the photoinhibition of growth is impaired. We found that jasmonate plays a central role in this cross-talk correlated to a downregulation of auxin responsiveness. To obtain an insight into auxin-dependent coordination, we analyzed a tobacco cell line with axial cell divisions. By a combination of modelling and physiological manipulation, we could demonstrate that auxin synchronizes the divisions of adjacent cells on the background of strong heterogeneity of individual cells. We conclude that self-amplification of auxin signalling coupled to mutual competition for available auxin provides a versatile tool to fulfill the special requirements posed by patterning in plants.

Key words: Actin, polarity, pattern, cell communication, auxin.

Pattern Formation and Indeterminate Morphogenesis

Plant morphogenesis is indeterminate: the manifestation of the "Bauplan" in an individual plant depends strongly on the environmental conditions encountered during development. This developmental flexibility includes a flexible response of cell expansion, for instance when cell elongation in the dark is compared to that under irradiation (Lockhard, 1960). These (quite rapid) responses are complemented by (somewhat slower) addition of morphogenetic elements, such as cells, pluricellular structures, or organs. This addition of morphogenetic elements is ordered in both space and time, corresponding to the classical definition of pattern formation (Bünning, 1965). The patterning process is driven by inherent factors as seen, for instance, from the species-specific arrangement of leaves or flower organs. It can, in addition, integrate signals from the environment, for instance, when a meristem becomes committed for flowering in response to lighting conditions and forms flower organs rather than vegetative leaves. Thus, the patterning process must be both highly flexible and robust.

More specifically, the patterning process must meet two requirements: 1) It must be able to cope with signals that can vary over several orders of magnitude for the strength of the control signal (termed in the following as sensory buffering, Fig. **1 A**). 2) New elements have to be added such that the pattern formed by the preexisting elements is perpetuated and/or complemented (termed in the following as iteration, Fig. **1 B**), because the field to be patterned in many cases is formed concomitantly with the patterning process as such.

Why Noise is Not Only Tolerated, But Even Required

The first of these requirements, sensory buffering, has to be seen in the context of plant-specific pecularities. Plant sensing occurs in a rather diffuse manner – there are no such things as eyes, ears, or tongues, there are populations of relatively undifferentiated cells that perform environmental sensing. Nevertheless, plant sensing is surprisingly sensitive – anybody who has studied so-called very low-fluence responses knows how difficult it is to perform such experiments, because the experimental plants are diverted even by minute traces of stray light (Mandoli and Briggs, 1981). 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 during the first steps of the transduction chain. This will inevitably result in all-or-none outputs of individual cells. The efficient amplification of weak stimuli on the one hand, but at the same time the ability to discriminate between very strong stimuli of different amplitude, poses special challenges to plant signalling.
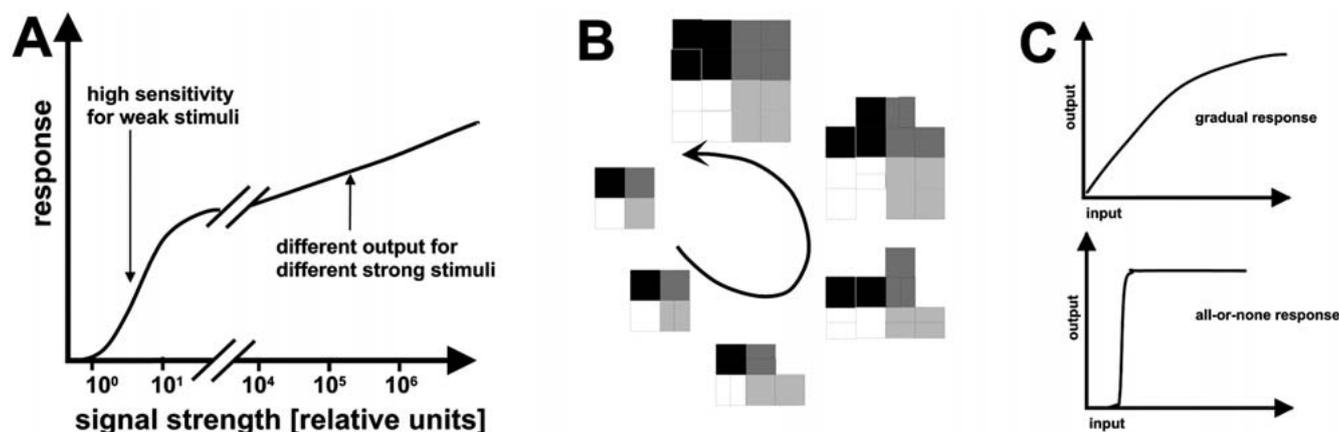
Fig. 1 Illustration of concepts and terms used in this review. (**A**) Sensory buffering: In a system capable of sensory buffering, high sensitivity is combined with the ability to discriminate between strong inputs of different amplitude. For weak stimuli, the output is based on efficient amplification involving sigmoidal input-output functions; for strong stimuli, the output increases progressively with increasing inputs. (**B**) Iterative patterning: In a patterning system that undergoes indeterminate morphogenesis, the formation of additional elements is guided by pre-existing elements in a way that the nature of the pattern is perpetuated. This is illustrated by a pattern of dividing cells that belong to different cell types (symbolized by the different shadings). (**C**) Gradual response: For increasing inputs, the output increases steadily (upper graph), in contrast to an all-or-none response (lower graph), where the output is constant for inputs that exceed a certain threshold.

To investigate sensory buffering on a cellular level, in previous work we had analyzed cellular aspects of photomorphogenesis (Nick et al., 1992, 1993). A cellular analysis of phytochrome-induced anthocyanin patterns in mustard cotyledons, a classical system of a light-dependent plant pattern (Mohr, 1972), revealed that the responses of individual cells are heterogenous (Nick et al., 1993). Even adjacent cells exhibited almost qualitative (all-or-none type, Fig. **1 C**) differences although they had obtained the same fluence of light. However, when the frequency of responsive cells in a given situation was scored and plotted over the strength of the stimulus, a highly ordered function was observed. Thus, the level of individual cells was reigned over by chaos, order emerged only on the level of the whole organ. This heterogeneity was not only observed for the final product, anthocyanin, but also for a precursor activity, the transcripts of chalcone synthase (a key enzyme for the pathway culminating in the synthesis of anthocyanin). The peculiar spatial distribution was, therefore, concluded to originate from the earliest responses to light (Nick et al., 1993). A similar highly stochastic, all-or-none type response of individual cells was found for the blue light-induced reorientation of cortical microtubules in maize coleoptiles, either as 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. Are these responses "noisy" because the flexible physiology of plant cells simply can tolerate this? Or do they represent an innate system property of 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 (termed in the following as gradual response, Fig. **1 C**). A gradual response at the level of the whole organ is necessary because the plant has to respond appropriately to stimuli that vary in intensity over several orders of magnitude. It is the very heterogeneity of individual cells that is important and, thus, heterogeneity is not only tolerated by the plant, it is a clear necessity. It is thus the heterogeneity of individual cells that allows the combination of extreme signal amplification (at the level of individual cells) with a gradual response that depends on the amplitude of the input signal (at the level of the whole organ). Thus, the contradictory requirements posed to plant signalling are met, at least in the cases mentioned above, by spatial separation: high sensitivity is achieved by signal amplification in individual cells, whereas the gradient of the response at the level of the whole organ is secured by interactions among members of a population of heterogeneous individual cells. It remains to be elucidated to what extent intercellular heterogeneity is developed in other signal responses – it is expected to be pronounced in very sensitive responses, less in responses where inputs are stronger and thus a clear output can be achieved at a lower degree of amplification.

Patterning During Indeterminate Morphogenesis Depends on Coordinative Signalling

The indefinite growth that is typical for plants requires that the pattern develops concomitantly with the extension of the field to be patterned. This could be achieved, in principle, by assigning different developmental fates during mitosis. The pattern would then result from an ordered sequence of such asymmetrical cell divisions. Such a mechanism, at first glance, seems to work in the root meristem of *Arabidopsis thaliana* that is patterned in the context of a highly stereotypic cell lineage (Scheres et al., 1994). However, using 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 (transcription factors) from adjacent cells. Generally, the principal totipotency of plant cells is difficult to reconcile with a strong impact of cell lineage. Patterning

in plants thus results from coordinative signals between the already defined (older) regions of the pattern and the newly formed elements of the field that still have to acquire a specific identity.

Coordinative signalling is impressively illustrated by recent advances in understanding the mechanisms underlying phyllotaxis. 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). When, for instance, the youngest primordium is isolated from its environment by tangential incisions, this will shift the position of primordia that are formed later (Snow and Snow, 1931). At that time, this shift was interpreted in terms of the additional space created by the incision that would allow the incipient primordia to move to a position where they otherwise were excluded (first available space model). However, this result is consistent with inhibitory fields emanated from the primordia. The nature of these inhibitory signals has been under debate for a long time – the tissue tension present in a growing meristem would allow efficient inhibition by mechanical stresses originating from buckling from the older primordia upon surrounding potential sites of primordium initiation. In fact, modelling of stress-strain patterns could perfectly predict the position of prospective primordia (for review see Green, 1980). Further support for this idea came from experiments, where local release of tissue tension by beads coated with extensin could cause inversions of the phyllotactic pattern (Fleming et al., 1997). Alternatively, it might be a chemical signal from the preexisting primordia that inhibits the initiation of a new primordium in their proximity. This model 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. These studies led to the (expected) outcome that the coordinative signal is auxin and to the (unexpected) outcome that the preexisting primordia do 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).

A second aspect of phyllotaxis has not attained the same attention, although it is also important: the analysis of genes that are expressed during the commitment of a new primordia shows patches of strongly expressing cells embedded in an environment of cells that express the marker only weakly (for instance figures in Reinhardt et al., 2003), suggesting that there are more or less smooth gradients in terms of primordial gene expression. However, there are other aspects of primordia initiation that appear not to be gradual events, but correspond to a qualitative decision. This is visible, for instance, in the very sharp boundary between the primordium initial and the non-committed neighbours concerning the axis of cell elongation. One of the earliest events of initiation is a reorientation of cortical microtubules that are perpendicular with respect to the microtubules of the non-primordial neighbouring cells (Hardham et al., 1980). Only later, when the primordium already becomes manifest as a small bulge, will this sharp, all-or-none

type difference be smoothed by a transitional zone of cells with oblique microtubules.

A similar mechanism has been elegantly demonstrated for the formation of conductive tissues in wound healing of internodes (Sachs, 2000) or for venation in developing leaves (Mattsson et al., 1999). In this case, parenchymatic cells that are all competent for transdifferentiation into vessels compete for a limited supply of auxin. Some of these cells (and there is a strong stochastic component in the choice of those cells) will transport more auxin than their neighbours and thus deplete them of auxin. The differentiation is accelerated depending on the flux of auxin that has passed through the cell and, conversely, the capacity for polar auxin flux grows with progressive differentiation. This positive feedback, in combination with a lateral inhibition (caused by mutual competition for auxin), will result in an ordered pattern of conductive tissue. This "auxin canalization" model has been extensively studied and modelled mathematically and is capable, for instance, of predicting venation patterns in leaves (for review see Berleth and Sachs, 2001).

What can be generalized from these two classical examples of auxin-dependent patterning? Both patterns are highly robust against stochastic fluctuations in the initial situation, they rely on lateral inhibition between the elements within the patterned field, and they contain qualitative decisions that are probably brought about by autocatalytic feedback loops. This type of mechanism can be described by the mathematics of reaction-diffusion systems that was adapted to biology (Turing, 1952) and have been quite successfully used to model various biological patterns (foot-head patterning in *Hydra,* Gierer et al., 1972; segmentation in *Drosophila,* Meinhard, 1986; 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 differs in one aspect from the original model, assuming an actual inhibitor as positive entity. In the two cases described above, lateral inhibition is brought about by mutual competition for the activator (Fig. **2**).

Open Questions and Experimental Systems

Three issues emerge from this survey on iterative patterning in plants:
1. The cellular base of auxin-dependent patterning is linked to the establishment of a directional flow. It is generally believed that it is mainly the polarized activity of an auxin efflux carrier that is responsible for this directionality (for review see Morris, 2000). During recent years, this polar auxin flux has been linked with directional intracellular traffic (for review see Friml, 2003). This intracellular traffic must contain positive feedback loops that lead to efficient self-amplification of initially weak gradients into strong and clear outputs that are qualitatively independent from stochastic fluctuations of the initial situation. What are the players in these feedback loops?
2. These feedback loops must be open to environmental cues (such as light) that regulate the morphogenetic response. How can those cues interfere with a process that, because of its system properties (self-amplification and lateral inhibition), leads to almost qualitative outputs?
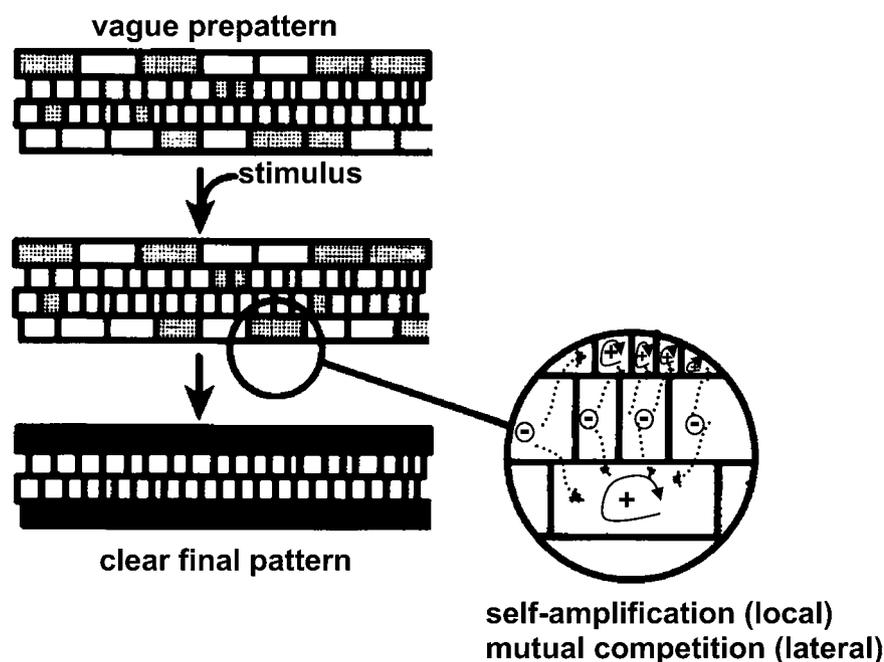
Fig. 2 Formation of signal-dependent pattern in plants. A highly heterogenous cell population (as a consequence of diffuse distribution of signal perception) forms a vague prepattern, whose manifestation can be triggered by environmental stimuli. Local self-amplification loops (indicated by +) in combination with mutual competition of adjacent cells for mobile components that are required for self-amplification (indicated by –) produces a clear final pattern that is almost independent of the initial situation.

3. The heterogeneity of individual cells, which is an innate system property of a non-localized sensing process, must be integrated into ordered patterning. How is this achieved in a field that increases in size concomitantly with the patterning process itself?

We have addressed these questions using two different experimental systems.

(i) The Graminean coleoptile has been *the* classical system to study the action of auxin. The stimulation of coleoptile elongation in the context of environmental signals such as light (Went, 1926) and gravity (Cholodny, 1927) culminated in the identification of indolyl-3-acetic acid as the major natural auxin. Auxin causes a loosening of the epidermal cell wall that limits the expansion of the subtending tissues (Kutschera et al., 1987). Growth is exclusively based on cell expansion, not on cell division, so that it is possible to link growth rate directly with cellular and biochemical events. Coleoptile growth can be controlled rapidly and reversibly by several signals, such as light, gravity, and hormones. This control is intimately linked to signal-triggered cytoskeletal reorganization (for review on microtubules see Nick, 2001, for actin: Waller et al., 2000), and the target tissue, the epidermis, is amenable to both cell biological manipulation and observation in the context of the intact organ, either by microinjection of fluorescent compounds (Himmelspach et al., 1999) or by biolistic transfection (Holweg et al., 2004).

(ii) To study patterning in a system where cell number increases, we had to change the experimental system. In order to avoid the high complexity of plant organs or even tissue explants that contain a great number of different cell types, we searched for a simpler system consisting of only one or a few cell types. Nevertheless, this system should be endowed with axiality, polarity, and a predictable developmental response. This system should be amenable to control by exogenous signals, easy to handle, and be accessible to cell biological analysis. The tobacco cell line VBI-0 was found to meet all of these requirements. This cell line derives from stem pith parenchyma, i.e., the cells that can differentiate into vascular tissue in response to auxin flow. In the same way as its ancestor cells within the tissue, this cell line grows in cell files exhibiting basic characteristics of patterning, such as clear axis and polarity of cell division and growth (Opatrný and Opatrná, 1976; Petrášek et al., 1998). The progression into the culture cycle, the duration of the lag phase, the rate of cell division, and the length of the exponential phase are strongly dependent on auxin in this cell line (Campanoni et al., 2003). Polarity and axiality of VBI-0 cells depend on the transport of auxin (Petrášek et al., 2002). The cell files are formed from singular cells, such that positional information inherited from the mother tissue does not play a role. If there are patterns of competence within a cell file, they must originate *de novo* during the culture cycle.

Self-Amplification I: A Feedback Loop between Auxin and Actin

Auxin is produced in the coleoptile tip (where environmental signals such as light and gravity are perceived) and moves from there in a basipetal direction towards the proximal elongation zone. When the flux of auxin is linked with directional intracellular traffic (for review see Friml, 2003), this should become manifest in the coleoptile system by an effect of acto-myosin-driven traffic on responses that depend on directional auxin flux. The idea that the signalling role of auxin is related to intracellular traffic is actually quite old. Already 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, Thimann returned to this idea and could show that elimination of actin blocked auxin-dependent growth very efficiently (Thimann et al., 1992; Thimann and Biradivolu 1994).

These findings contrasted with laser tweezer measurements, where the rigor of actin was released by auxin (Grabski and Schindler, 1996). In the framework of the actin-rigor model, the elimination of actin would be expected to stimulate rather than inhibit auxin-dependent growth.

To understand the role of actin in signal-dependent growth, we had analyzed phytochrome-triggered cell elongation in maize coleoptiles (Waller and Nick, 1997). We identified two populations of microfilaments with distinct function. Cells that underwent rapid elongation were endowed with fine strands of actin 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). Auxin can dissociate actin bundles into fine strands in both rice (Wang and Nick, 1998) and maize (Waller et al., 2002 a) coleoptiles, and we could later follow this auxin response in living cells (Holweg et al., 2004).

We succeeded in separating these two actin populations biochemically, based on their different sedimentation rates. The bundled arrays correlated with vesicle-bound actin, whereas the fine strands correlated with a cytosolic fraction of actin (Waller et al., 2002 a). Treatment with Brefeldin A, a compound that interferes with the budding of vesicles at the endoplasmatic reticulum (Orci et al., 1991), shifted actin from the cytosolic into the microsomal fraction and caused rapid bundling of microfilaments despite the presence of auxin, i.e., under conditions where actin otherwise would be cytosolic and microfilaments would be arranged in fine strands. This response was dependent on the concentration of auxin and of Brefeldin A. In parallel, Brefeldin A shifted the dose-response curve of auxin-dependent growth to higher concentrations. In other words, Brefeldin A decreased auxin sensitivity (not to be mixed up with auxin responsiveness!).

This study (Waller et al., 2002 a) suggests that microfilaments might transport vesicles. This transport requires active auxin signalling. When the cells are depleted of auxin or when essential components of auxin signalling become mislocalized upon treatment with Brefeldin A, the transport is blocked and actin is trapped on the endomembrane system and partitioned into the microsomal fraction. The cellular correlate of this trapping would be a bundling of actin strands into dense bundles. The cargo of these vesicles might principally be either components of auxin signalling (such as auxin efflux carriers) or structural components of the cell wall. The observed association of actin with the cell poles, i.e., at a site where cell walls extend slowly, favours the scenario where the cargo consists in regulatory elements related to cell polarity. This is supported by the shift of the dose-response curve of auxin-induced growth, indicating that early components of auxin sensing are affected by Brefeldin A.

When components of auxin signalling are transported along actin, and when this transport depends on auxin, this constitutes a self-amplification loop, as predicted from the survey on auxin-dependent patterning (Fig. **3**). The existence of such a loop is also supported by the work on the PIN proteins, polarity markers that are involved in the regulation of polar auxin flux. For several of these PIN proteins it was demonstrated that they cycle continuously between the plasma membrane (their expected site of action) and intracellular structures. This cycling is affected by treatment with inhibitors of actin assembly (Steinmann et al., 1999) and is dependent on auxin (Paciorek et al., 2005).

We probed this feedback by interference with the presumptive motors that transport cargo along actin microfilaments. In the tobacco cell line VBI-0, where axial cell division is regulated by polar auxin flux (Campanoni et al., 2003; Campanoni and Nick, 2005), treatment with the *bona fide* myosin inhibitor 2,3-butanedione monoxime (BDM) delays the onset of cell division in response to auxin and affects the correct organization of actin microfilaments along with centrifugal vesicle flux (Holweg et al., 2003). Interestingly, it leaves auxin-dependent cell elongation basically unaltered. If BDM acted by inhibiting the response to auxin, it would be expected to affect both cell elongation and cell division to a similar extent. Auxin-dependent cell division and auxin-dependent cell elongation have been demonstrated, in the same cell line, to run through different signalling events (Campanoni et al., 2005). Myosins thus are involved mainly in the signal chain controlling auxin-dependent cell division. Further support for a link between myosins and auxin transport comes from a thale cress insertion mutant, where one of the class XI myosins, *mya2*, is functionally null (Holweg and Nick, 2004). The phenotype of this mutant includes reduced apical dominance, male sterility caused by affected staminal growth, reduced velocity of large vesicles, and reduced polar auxin transport. However, it is still unclear why the loss of *mya2* cannot be compensated for by other myosins of that class.

Although there are still many gaps to be filled, our work demonstrates that events related to the sensing or early transduction of auxins are part of self-amplification loops, and that these loops are linked with actomyosin-driven vesicle transport. This conclusion is supported by findings from other groups that have demonstrated: a) that the polar localization of PIN1 depends on actin (Gälweiler et al., 1998), and b) that the cycling of at least some of the PIN proteins is regulated by auxin (Paciorek et al., 2005).

Self-Amplification II: A Feedback Loop between Auxin and an Auxin Response Factor

To identify molecular components in the regulatory loop between auxin and actin, we used a rice mutant with an elevated auxin response to actin. In this mutant, *Yin-Yang*, the sensitivity of cell elongation to cytochalasin D (an inhibitor of actin assembly) was strongly increased in response to auxin (Wang and Nick, 1998). In this mutant, microfilaments collapsed in response to auxin, leaving a basket-like structure of short actin rods surrounding the nucleus.

We applied a fluorescent differential display (FDD) approach (Ito et al., 1994; Kuno et al., 2000) and searched for genes that were: (i) differentially regulated between wild type and *Yin-Yang*, and (ii) rapidly induced by auxin (Waller et al., 2002 b). Using this approach, we succeeded in isolating the first auxin response factor from rice, *OsARF1*.

Auxin response factors (ARFs) are transcription factors that bind to a conserved element (auxin-responsive element, AuxRE) in promotors of auxin-responsive genes that have been
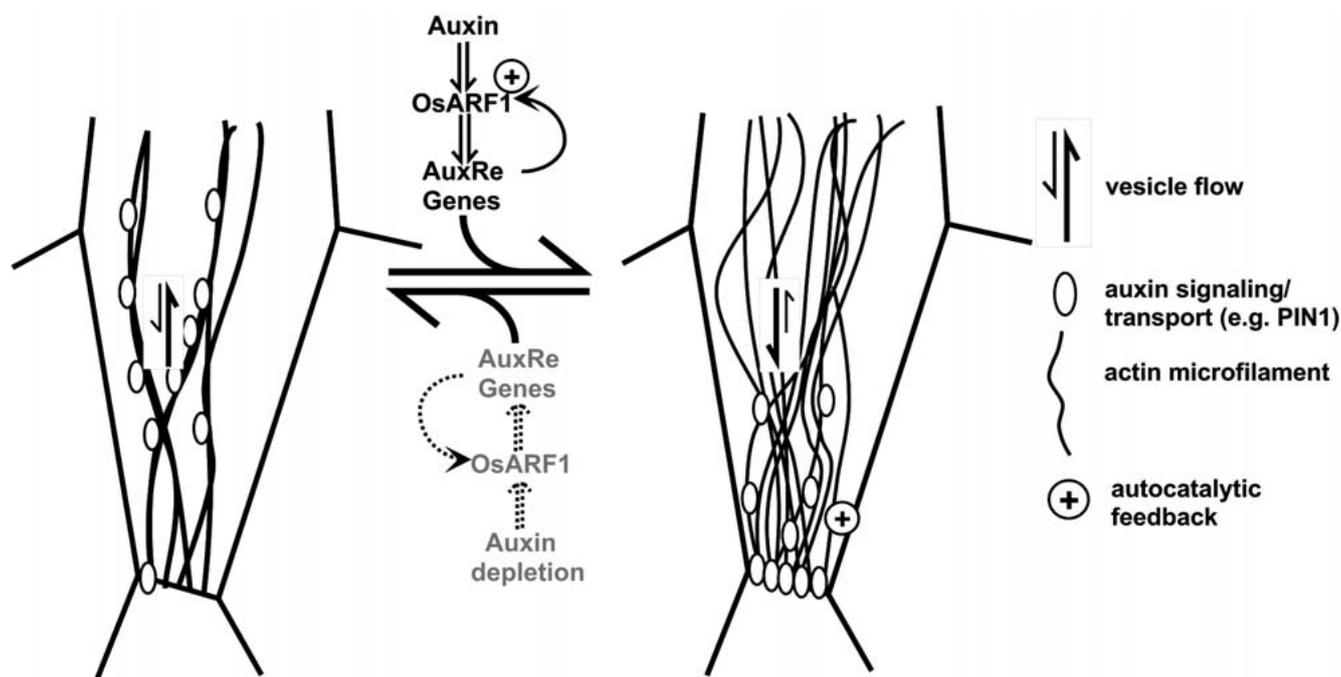
Fig. 3   Model for the role of autocatalytic feedback loops in the Graminean actin response to auxin. In the absence of auxin, actin microfilaments are bundled, preventing the delivery of vesicle cargo essential for auxin signalling and/or transport (such as PIN1) to the cell pole. This configuration of actin is correlated with a preferentially endocytic flow of vesicles. Upon addition of auxin, actin bundles are dissociated into finer strands that allow delivery of the cargo to the cell pole accompanied by reduced endocytotic and/or increased exocytotic activity. Auxin induces the activity of the early auxin response gene *OsARF1* that will trigger expression of auxin-responsive genes (*AuxRe* genes), which, in turn, leads to transcriptional activation of *OsARF1* establishing an additional positive feedback loop.

identified in *Arabidopsis thaliana* (Ulmasov et al., 1997). The ARF gene family consists of 23 members in this species that are able to form homo- and heterodimers with other members of this family and with some of the Aux/IAA genes (Ulmasov et al., 1999 a), and some of these ARFs have been shown to be able to repress or to activate expression of reporter genes with an AuxRE promoter element (Ulmasov et al., 1999 b).

The expression of *OsARF1* correlates with auxin-induced cell growth. *OsARF1* was upregulated in the mutant, but also in the faster-growing flank of gravitropically-stimulated rice coleoptiles. Surprisingly, we observed that *OsARF1* is upregulated by auxin – the first reported case for an auxin response factor. Furthermore, *OsARF1* could be shown to be a primary auxin-responsive gene, a class of genes that is auxin-induced even in the absence of *de novo* protein synthesis and likely to play an important role in mediating the growth-stimulating effect of auxin.

The promotor of *OsARF1* contains a canonical AuxRE element (Waller et al., 2002 b). As ARFs are responsible for regulating early auxin-induced genes via this element, OsARF1 might regulate its own transcription, which could provide the molecular basis of a further feedback mechanism in auxin signalling. It should be mentioned that some of the ARFs, such as *ARF3* and *ARF5,* have been shown to be involved in auxin-dependent patterning processes (Sessions et al., 1997; Hardtke and Berleth, 1998). The inferred positive feedback of *OsARF1* on its own synthesis would be an ideal candidate for the self-amplification loop central to auxin-driven patterning (Fig. **3**).

Coupling of Auxin Signalling to Environmental Stimuli – Jasmonate as Key Player

Self-amplifying feedback loops such as auxin-dependent intracellular transport of auxin-signalling components or auxin-triggered induction of auxin response factors are expected to result in almost qualitative outputs of the cellular response. Nevertheless, these outputs must be flexible enough to integrate the environmental cues that are so important for plant morphogenesis. Light and gravity are certainly the most central of these cues. Classically, they were thought to modulate auxin responses by changes in auxin transport and thus the spatial distribution of auxin. For instance, auxin is diverted to the lower flank of gravitropically stimulated coleoptiles (e.g., Dolk, 1936; Philippar et al., 1999; Godbolé et al., 2000; Gutjahr et al., 2005) and the resulting gradient in the concentration of auxin has been proposed as a cause of gravitropic bending (Cholodny, 1927). In rice seedlings, where coleoptile elongation is exclusively controlled by phytochrome (Pjon and Furuya, 1967; Takano et al., 2001), the inhibition of cell elongation by light had been concluded from classical studies (Furuya et al., 1969) to be caused by inhibition of basipetal auxin transport.

Although the importance of basipetal auxin transport has been confirmed by a wealth of data collected over several decades, the role of auxin in signal-dependent growth appears to be complex. Synthesis, conjugation/deconjugation, catabolism (for review, see Normanly, 1997), and gradients between adjacent tissues can influence growth. For instance, light-induced growth inhibition in pea seedlings was found to correlate with

a specific decrease in the level of auxin in the epidermis (Behringer and Davies, 1992). In addition, sensitivity/responsiveness of the target tissue to auxin have to be considered (Salisbury et al., 1988; Rorabaugh and Salisbury, 1989; for review see Trewavas, 1981). This already indicates that the reduction in the cross-talk between auxin signalling and environmental signals to a mere diversion of auxin fluxes would certainly be too simplistic.

We used two rice mutants with altered growth responses to gravity and light to identify factors in the cross-talk between auxin and environmental signals and to obtain insight into the mode of their interaction. 1) The mutant *Yin-Yang* (Wang and Nick, 1998), with its elevated auxin-responsiveness, was also affected in the response to light and gravity. 2) The mutant *hebiba* (Riemann et al., 2003) had been originally isolated due to a dramatically impaired photoinhibition of coleoptile growth. It was later found to be endowed with a drastically increased responsiveness to auxin. Using fluorescent differential display (Kuno et al., 2000), we screened for phytochrome-induced genes, whose expression was altered in the two mutants as compared to the wild type (Waller et al., 2002 b; Chaban et al., 2003; Riemann et al., submitted).

We identified, in this approach, a new member of the cytochrome P450 (CYP) superfamily that was induced by auxin in the WT, but not in the *Ying-Yang* mutant (Chaban et al., 2003). This gene, *CYP87A3,* was light-responsive and seemed to be a negative regulator of the auxin response. *CYP87A3* was shown to be a primary auxin response gene, that is up-regulated by auxin as well as by light (Chaban et al., 2003). Interestingly, this upregulation was only transient (in contrast, for instance, to the stable upregulation of *OsARF1* that had been isolated from the same mutant). The induction of this gene by auxin was dependent on irradiation. It was strong in etiolated coleoptiles, but strongly reduced after irradiation.

The substrate of *CYP87A3* is yet to be elucidated. The regulation patterns of CYP proteins are strictly correlated to their biological function (e.g., Bak et al., 2001). We therefore think that *CYP87A3* must be involved in the cross-talk between phytochrome and auxin signalling. The expression profile of this gene demonstrates that light acts by reducing the responsiveness to auxin.

The phenotype of the second mutant, *hebiba,* is very drastic: in the wild type, coleoptile elongation is maximal in the dark and efficiently inhibited upon irradiation. In the mutant, elongation is strongly reduced in the dark, and accelerated by light (Riemann et al., 2003). The presence of a dark phenotype argued against a photoreceptor mutation, and we therefore searched for light-induced rapid changes in the content of phytohormones using highly sensitive and reliable multiplex analytics in a gas chromatographic approach combined with two-dimensional mass spectrometry (Müller et al., 2002). We observed that jasmonate and its precursor OPDA were strongly, rapidly, and transiently increased in response to irradiation, but only in the wild type. In the mutant, neither jasmonate nor its precursor could be detected (Riemann et al., 2003). To challenge this observation, we induced a strong production of jasmonate and OPDA by wounding in the wild type, but again failed to detect any jasmonate or OPDA in the mutant. In the next step, we tested whether the observed mutant phenotype

(that also included male sterility) could be rescued by exogenous jasmonate. This was the case, demonstrating that the mutant phenotype was caused by the absence of jasmonate. This means that the jasmonate pathway plays a role in the response of cell elongation to light. Although the classical role for jasmonates is seen in plant defence, a cross-talk with light signalling had already been inferred from the phenotype of the *Arabidopsis* mutant *psi2* that was found to be hypersensitive to red light and at the same time shows activation of a "hypochondrical" pathogen response, i.e., without actually being challenged by a pathogen (Genoud et al., 1998; Genoud and Métraux, 1999). Jasmonates could cause photoinhibition simply by direct downregulation of factors that are necessary for growth *per se*. The complete absence of jasmonate in the mutant (in contrast to a certain basic level in the wild type) should then result in a larger amplitude for the auxin response of growth (as is actually observed in *hebiba*).

This straightforward, but admittedly naive, view of jasmonate-auxin interaction was scattered when we reinvestigated the role of auxin transport and auxin responsiveness in the gravitropic response of rice coleoptiles (Gutjahr et al., 2005). Auxin can pass freely through the cuticle of rice coleoptiles (Godbolé et al., 2000) and the coleoptiles exhibit very efficient gravitropism even in solution (in contrast to most Graminea). This experimental system is therefore ideally suited to analyze different parameters of auxin signalling in the context of gravitropism. We could demonstrate, again using GC-MS-MS analysis of phytohormones in different regions of gravistimulated coleoptiles (Gutjahr et al., 2005), that: 1) efficient gravitropic bending is possible under conditions where the internal gradient of auxin is outlevelled by excessive exogenous auxin; 2) gravitropic bending shows a sign reversal for very high concentrations of auxin that are superoptimal with respect to growth (i.e., the coleoptile then behaves as if it were a root); 3) a qualitative gradient of auxin responsiveness develops within 15–30 min of gravitropic stimulation with a low responsiveness in the upper and a high responsiveness in the lower flank; 4) a gradient of jasmonate develops opposed to the gradient of auxin, i.e., there is more jasmonate in the upper flank as compared to the lower flank; 5) this gradient also develops when auxin transport is blocked by inhibitors, i.e., it is independent of the gravitropic auxin transport; and 6) when this gradient is eliminated either by flooding with exogenous jasmonate or by using the *hebiba* mutant, where jasmonate is absent, gravitropic bending is delayed. These results suggest that the jasmonate gradient is related to the observed gradient of auxin responsiveness. In other words, the interaction of jasmonate with auxin-dependent cell elongation is not a mere additive interaction at the level of growth. This interaction must take place earlier, at the level of auxin signalling.

Is there a mechanism that could explain a reduced responsiveness to auxin after activation of the jasmonate pathway? Recent findings about ubiquitin-related processes in phytohormonal signal transduction (for review, see Frugis and Chua, 2002) suggest that both signalling pathways are working via the 26S proteasome: auxin responses are mediated by interactions of the COP9 signalosome with the E3 ubiquitin ligase SCF^{TIR1} (Schwechheimer et al., 2002), and a similar system acts in jasmonate signalling with the F-box protein COI1 acting as the corresponding E3-type ubiquitin ligase (Devoto et al., 2002). Both ubiquitin ligase-related pathways interact through
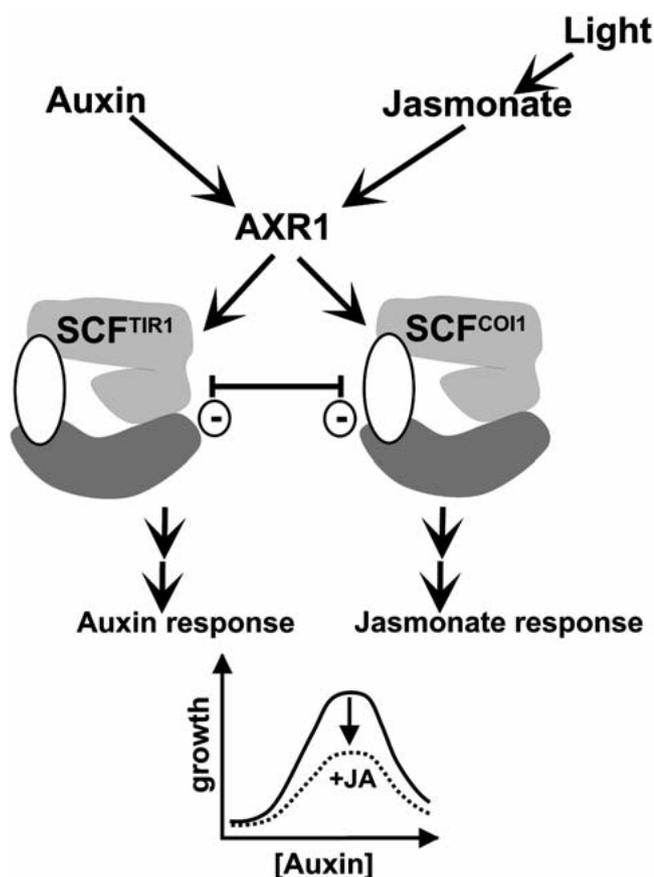
Fig. 4 Cross-talk between auxin and jasmonate signalling in the growth response of rice coleoptiles. Auxin triggers the activation of AXR1 that interacts with the E3 ubiquitin ligase SCF^{TIR1} culminating in the activation of auxin-responsive genes by proteolytic degradation of repressor proteins. Jasmonate signalling competes with auxin signalling for AXR1, which is also required for activation of the E3 ubiquitin ligase SCF^{COI1} (indicated by –). Thus, the formation of jasmonate in response to light will result in a reduced auxin responsiveness of growth and a reduced amplitude of the bell-shaped dose-response curve for auxin-dependent elongation growth.

a process called neddylation that activates several E3 ubiquitin ligases of the SCF type. AXR1, a subunit of the complex responsible for this neddylation, is required for multiple E3-mediated processes (Schwechheimer et al., 2002), and the *axr1* mutant in *Arabidopsis,* which originally was isolated as an auxin response mutant, has been shown to also be defective in jasmonate response (Tyryaki and Staswick, 2002).

A system property of this mechanism is the competition of both pathways for a common factor, namely, AXR1. Activation of the jasmonate pathway therefore has the consequence that auxin signalling becomes depleted from this key player leading to reduced auxin responsiveness (Fig. 4). Conversely, a strong activation of auxin signalling should suppress the responsiveness to jasmonates. To obtain robust outputs, this should be complemented by self-amplification. Such feedback loops have already been discussed above for auxin signalling (for instance, the auxin-induced induction of an auxin response factor). They are known also for jasmonate signalling – at least several of the enzymes involved in jasmonate synthesis are upregulated in response to jasmonate, and the same

seems to hold true for the signalling component COI1 (Riemann et al., unpublished). Thus, what is seen at the level of auxin-dependent patterning, i.e., self-amplification combined with coordination by competition for a common resource whose availability is limited, is also observed at the level of co-ordination between signalling chains. This combination of auxin and jasmonate signalling is exactly analogous to the reaction-diffusion systems described and modelled by Turing (1952). A system property of such Turing systems is the capability of self-organization and the generation of clear robust outputs even with the background of highly heterogeneous inputs that are biased by stochastic fluctuations.

Cell Division Patterns: Auxin as Weak Coupler of Noisy Oscillators

To search for auxin-dependent regulatory circuits in the context of environmental signals, we had chosen Graminean coleoptiles as a model. Despite numerous advantages, this is an organ where cell number remains constant. In order to investigate patterning in a field where the number of elements increases during patterning, we had to change the system. Using the above-described tobacco cell line VBI-0 as a model system, we demonstrated that cell division within the file is partially synchronized, leading to a much higher frequency of cell files with even cell numbers as compared to files with uneven cell numbers (Campanoni et al., 2003). The experimental data could be simulated using a mathematical model derived from nonlinear dynamics, where elementary cell division oscillators are coupled weakly, and where the number of oscillators is not conserved.

The model predicted several non-intuitive properties of this experimental system, among them that this coupling is unidirectional, i.e., a polar transport of the coordinating signal (Fig. 5).

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 1-*N*-naphthylphthalamic acid (NPA), a well-known inhibitor of polar auxin transport (for review, see Morris, 2000). 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 review see Stals and Inzé, 2001), this was the first time that auxin was shown to coordinate the divisions of adjacent cells.

The modelling, as well as time courses of cell division, showed that the noise in this system was considerable. The length of the cell cycle was quite variable over the population of cells. Nevertheless, the division of adjacent cells was synchronized to such a degree that files with uneven cell numbers were only rarely observed. This study (Campanoni et al., 2003) demonstrated that patterning in an indefinite field is intimately linked to cell polarity.

Synopsis and Outlook

Auxin certainly represents one of the most universal signals in plant life, and the number of processes where auxin is involved is astounding. Why has evolution selected such a simple mol-
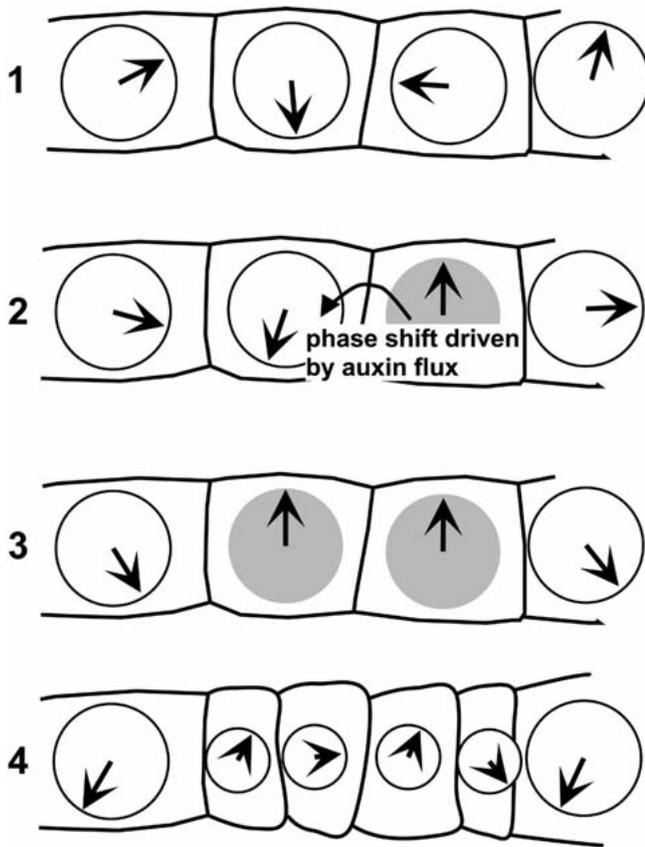
Fig. 5 Synchronization of cell division by weak coupling. Initially, asynchronous cells (1) of a cell file differ strongly with respect to phase and period of the cell cycle (the phase within the cell cycle is symbolized by the direction of the arrows). When a given cell becomes committed for mitoses (symbolized by the arrow in twelve-o'clock direction), it releases a coupling signal (bold arrow) to the adjacent cell (2). However, this signal is released only in one direction with respect to the file polarity. This coupling signal is dependent on auxin efflux and can be inhibited by NPA. *Notabene*: This signal could either be a flux of auxin from the cell committed for mitosis towards its downstream neighbour, but could also be a depletion of auxin from the downstream neighbour towards the cell that is committed for mitosis. As result of this signal, the phase of the downstream neighbour is advanced to reach commitment for mitosis (3). Both cells divide more or less simultaneously. The daughter cells lose synchrony rapidly and advance at different phases (4).

ecule for such a central role in intercellular coordination? Although this is far from being understood, we think that the evolutionary driving force might have been the versatility of auxin as a tool to set up polarity. A molecule that is easily transported through the acidic environment of the apoplast, but is readily trapped in the cytoplasm and has then 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 tissue level, it can generate patterns in a manner that meets the special constraints typical for plants, i.e., noisy inputs as a conse-

quence of diffuse sensing and progressive addition of new elements to the pattern.

There seem to be at least two self-amplification loops in auxin signalling: 1) components of auxin signalling are transported along actomyosin, actomyosin in turn is maintained in a transport-competent state by auxin. These components could be related to elements participating in the regulation of auxin efflux, such as the PIN proteins (Gälweiler et al., 1998). 2) A regulator of auxin-induced genes, auxin response factor 1, is induced by auxin.

Auxin signalling must integrate environmental cues, such as light or gravity. We have identified elements for the interaction with light, and we could show that these elements are related to a reduced responsiveness of cell growth to auxin. Jasmonate was identified as a key element for the interaction between auxin signalling and light in rice coleoptiles. Both signalling pathways compete for AXR1 as limiting factor, both signalling pathways contain self-amplification loops and thus are endowed with the systemic properties analogous to a reaction-diffusion pattern.

Using a system where patterning occurs concomitantly with cell division, we could show that polar auxin flux coordinates the divisions of neighbouring cells that are otherwise highly heterogenous in terms of cell cycle. Future work will focus on the link between actin polarity and auxin-regulated cell division. Whereas one pole can be inherited from the mother cell, there must be *de novo* generation of a new cell pole at the site where a new cross wall is laid down. This is expected to imply some kind of sign reversal. How are microfilaments that are elements of auxin-triggered regulatory circuits behaving during this sign reversal?

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P. Nick

Institut of Botany 1
University of Karlsruhe
Kaiserstraße 2
76128 Karlsruhe
Germany
E-mail: peter.nick@bio.uni-karlsruhe.de

Guest Editor: R. Reski