Autonomy versus rhythm – What is needed to build a plant organism?

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Abstract. This essay develops a conceptual framework for a theory of plant organisms. This framework has to consider the specificities of the plant lifestyle: the lack of contiguous borders, a centrifugal architecture based on continuous addition of modular elements, the lack of developmental hierarchy, a strong autonomy of individual cells, the absence of cell-lineage or predefined developmental programmes, and, most importantly, the lack of physical body *contiguity* (defined as continuous interface between internal and external space). The challenges posed by these constraints are met by an organisation that is highly modular. This "LEGO principle" of plant organisation becomes manifest on the level of architecture (as so called telomes, modules comprising vascular tissue accompanied by ground tissue that can differentiate into vasculature), cellular organisation (as innate polarity of individual cells), and genetics (as modular genetic programmes

that can be rearranged in time to yield a great variety of combinations). The assembly of these versatile modules is controlled through robust self-organisation driven by autocatalytic loops linked to lateral inhibition. The origin of this self-organisation can be traced back to photosynthetic prokaryotes as outlined for heterocyst differentiation of cyanobacteria. Lateral inhibition can use actual chemical agents, but in many cases it is based on mutual competition for a limiting patterning signal. This inhibition by competition is demonstrated for phyllotaxis (the pattern by which leaves are laid down in the shoot apex), and vascular differentiation, phenomena that are both regulated by the plant hormone auxin. To address the dynamic formation of plant organisms, we have established cell lines derived from the ground tissue of tobacco as experimental system. These cells produce, upon addition of auxin, pluricellular files with distinct axis and polarity partially recapitulating the developmental programme of their progenitor tissue. The individual cell divisions within a file are synchronised through weak coupling based on a directional flow of auxin and thus constitute a simple minimal organism that can be used to get insight into the process of self-organisation. Using this system we have identified an oscillatory circuit as central element of self organisation. This self-refering circuit connects auxin-dependent remodeling of the actin cytoskeleton with actin-dependent remodeling of auxin flux. We can manipulate this oscillator, and consequently, the temporal pattern of cell divisions, by genetic engineering of actin structure, but also by optical engineering of auxin gradients within a file. The essay concludes with the working hypothesis that the *contiguity* of plant organisms is manifest in time ("rhythm") rather than in space ("body"). Plant organisms are manifest as resonance between highly autonomous oscillators (telomes, cells, genetic programmes) achieved by weak coupling. The resonance proceeds on the background of a strong noise of the individual oscillations. This strong noise represents a system property of plant organisms which can be explained and deduced from the diffuse organisation of plant sensing. A plant „organism" should therefore be understood as process – as entrainment of the initially dissonant individual rhythms. As soon as synchrony between the individual oscillators is established, a plant "organism" will vanish behind the resonating individual cells. This culminates in a paradox: the organismic flow of plant organisms is directed to self-abolition.

1. Why plants challenge our concepts of "organism" and "individuality"

The surface-volume dilemma. Life is usually linked to growth. In a growing body, surface will increase by the second power of the radius, volume, however, by the third power. The material resources required to sustain a body have to pass through the cell surface. As consequence of growth, supply (surface, r^2) and consumption (volume, r^3) will progressively diverge. This gap can be bridged when the interface is increased by invaginations or protrusions, a phenomenon already

manifest in unicellular organisms. Such surface increases confer a selective advantage, because a larger organism acquires buffering against environmental fluctuations, and, most important, is less readily devoured by competitors.

As a consequence of their photosynthetic lifestyle, plants have to increase surface by centrifugal extension, generating a considerable degree of mechanical load (Fig. 1A). As long as plants remained aquatic, this load was at least partially relieved by buoyancy, allowing considerable sizes even for fairly simple architectures. However, when plants began to conquer terrestrial habitats, they had to develop flexible, yet robust, mechanical supports. The invention of vasculature-based modules, so-called *telomes* (Zimmermann, 1965), became a decisive factor for the evolutionary success of land plants (Fig. 1B). Mechanical load shaped plant architecture down to the cellular level and is of tremendous agronomical impact – the reduction of lodging in cereals is considered as pivotal factor for the success of the so called Green Revolution (for the cellular details refer to Nick, 2012). Plant cells are endowed with a rigid cell wall, and this affects cell division and cell expansion, both specifically and fundamentally.

These biophysical constraints have channeled plants towards a sessile lifestyle.

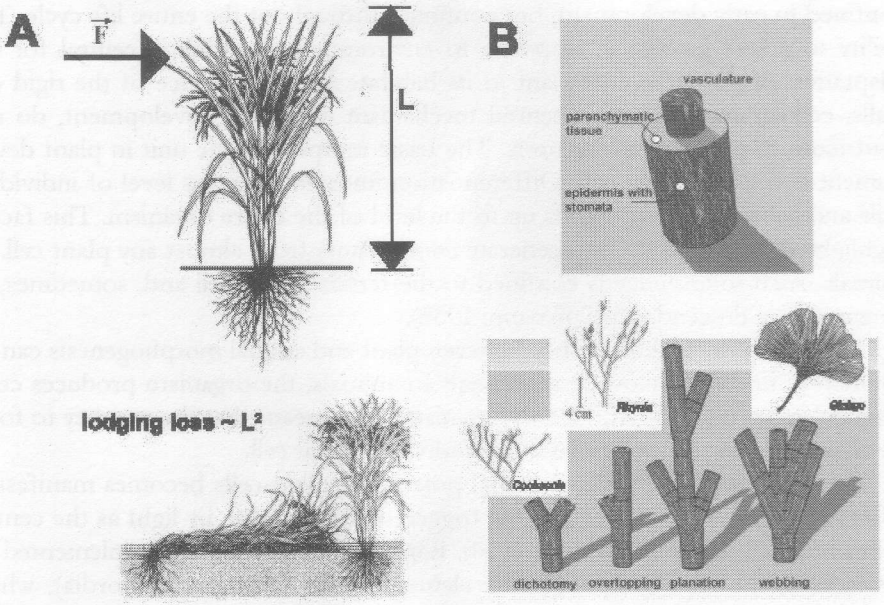

Fig. 1. *Plant architecture is shaped by mechanical load.* ***A*** *Lodging of cereal crops depends on the elongation of the culm. Yield losses by lodging increase with the square of culm length (Oda et al., 1966).* ***B*** *The development of telomes as load-bearing architectural module in the early Devonian was decisive for the evolutionary success of land plants (from Nick, 2009).*

Cells versus organisms – why the plant approach is different. In addition to plant architecture, the sessile lifestyle has shaped the mode of morphogenesis. In animals, the *Bauplan* is laid down early in development. In some cases, even maternal factors have been found to complement the DNA of the embryo providing a kind of "morphogenetic inheritance". For instance, the anterior-posterior polarity in the *Drosophila* embryo is determined by a gradient of maternal, untranslated mRNA encoding transcription factors such as BICOID or NANOS (Nüsslein-Volhard, 1995). Even in classical models for epigenetic morphogenesis, such as the amphibian embryo (Spemann, 1936), the dorsiventral polarity of frog eggs is established by autocatalytic feedback of polarising signals (gravity, sperm entrance) to inherited patterns. These inherent pattern not only includes preformed morphogenetic movements, but also transport and translation of maternal mRNA coding for cytoskeletal proteins and polar determinants (Elinson and Rowning, 1988). In these models, the *Bauplan* is laid down during early development, often prior to cellularisation. Here, differentiation initiates at the level of the entire organism and subsequently proceeds down to the level of individual cells.

The situation in plants differs fundamentally: The genetic determination of plant shape is not as stringent as for animal development, but depends strongly on the environment. As central feature of this open morphogenesis, growth is not confined to early development, but continues throughout the entire life cycle. The ability to adjust growth in response to environmental stimuli is central for the adaptation of the individual plant to its habitat. As consequence of the rigid cell walls, cellular movements, a central mechanism in animal development, do not contribute to plant morphogenesis. The basic morphogenetic unit in plant development is the individual cell. Differentiation initiates from the level of individual cells and subsequently proceeds up to the level of the entire organism. This fact is highlighted by the ability to regenerate entire plants from almost any plant cell. In animals, such totipotency is confined to the fertilised egg cell and, sometimes, to its immediate descendants (Spemann, 1936).

Thus, the principal difference between plant and animal morphogenesis can be condensed into the following statement: In animals, the organism produces cells, whereas in plants, cells produce an organism. This means that the potency to form an organism must be enshrined in the individual plant cell.

Open patterning. The developmental potency of plant cells becomes manifest in a flexible response of cell expansion, triggered, for instance, by light as the central environmental factor (Lockhard, 1960). Rapid cell expansion is complemented by a slower addition of morphogenetic elements (cells or organ primordia), which does not occur randomly, but is ordered in space and time. This pattern formation (*in sensu* Bünning, 1965) depends, on one hand, on intrinsic signals that are obviously defined by genetic factors (otherwise there would be no base for classical plant taxonomy!). On the other hand, plant patterning can integrate signals from the environment. Environmental integration is manifest, for instance, when a shoot meristem is committed for flowering controlled by daylength and subse-

quently will form floral instead of vegetative organs. In animal patterning, the elements that are organised during pattern formation are generated prior to being differentiated. In a fruit-fly embryo, for instance, numerous nuclei are produced before they are patterned depending on gradients derived from maternal factors. Plant development follows different rules – here, new elements are continuously added *during* the patterning process perpetuating the pattern in an iterative manner.

This pattern iteration could be achieved, in principle, by assigning different developmental fates to the daughter cells during cell division. The pattern would then result from an ordered sequence of such formative divisions. Such a mechanism had been proposed for the root meristem of the model plant *Arabidopsis thaliana*, which is characterised by a highly stereotypic cell lineage (Scheres *et al.*, 1994). However, elegant laser ablation experiments (Van den Berg *et al.*, 1995), and mutants with aberrant tissue layers (Nakajima *et al.*, 2001) clearly demonstrated that even in this stereotypic system, cell fate was defined by signals (transcription factors) from adjacent cells and not by cellular genealogy.

Generally, the principal totipotency of plant cells is difficult to reconcile with a strong impact of cell lineage. Patterning in plants must result 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.

Procaryotic precursors of coordinative signaling. Plants acquired photosynthesis through a sustainable symbiosis with autotrophic cyanobacteria. Filamentous cyanobacteria already acquired multicellularity and are capable of a simple cell differentiation yielding so called heterocysts that can convert atmospheric nitrogen into ammonium and thus overcome the limitations of bioavailable nitrogen. Since filamentous cyanobacteria combine open patterning with developmental flexibility, coordinative signaling would be expected already in these prokaryotic precursors of the plant lifestyle. The nitrogenase required for the fixation of nitrogen dates back to the earliest, anoxic phases of life on this planet and is therefore highly sensitive to oxygen. To safeguard the functionality of nitrogenase, any photosynthetic activity (releasing oxygen) has to be excluded from heterocysts. Thus, the heterocysts must be supplied with assimilates from their photosynthetic neighbours. Nitrogen export and assimilate import have to be balanced even though the total number of cells grows continuously, which represents a classical problem of open patterning. This balance is regulated by an iterative algorithm, whereby preexisting heterocysts suppress the differentiation of new heterocysts over a range of around ten cells. When, as a consequence of cell division, the distance between the heterocysts exceeds this threshold, a new heterocyst will differentiate between them. Using 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 excel the

others and then immediately start to suppress further differentiation in its neighbourhood (Yoon and Golden, 2001).
Thus, already in photosynthetic pluricellular prokaryotes, cell differentiation is not predetermined, but laid down by signalling between neighbouring cells.

Plant patterning and coordinative signaling - phyllotaxis. 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 the susbsequently formed primordia (Snow and Snow, 1931). At the time of this experimental discovery, the positional 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). Alternatively, inhibitory fields emanating from the older primordia have been proposed. 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 the cell-wall loosening protein extensin could cause inversions of the phyllotactic pattern (Fleming *et al.*, 1997). In fact, as one of the earliest events of incipient primordial commitment, membrane-associated microtubules reorient sharply and subsequently align with the stress-strain pattern (Hardham *et al.*, 1980). This phenomenon has been revisited using plants, where microtubules could be followed *in vivo* based on labeling with the green fluorescent protein in developing shoot apices (Hamant *et al.*, 2008) accompanied by mathematical modeling of stress-strain pattern. This approach culminated in a model, where microtubules sense mechanic load, align with the direction of maximal stress, and cause a tilted axis of cell expansion generating the bulging primordium. As alternative to the stress-strain model, chemical signals from the older primordia were proposed to inhibit 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 in the absence of any prepattern.

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. 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).

Plant patterning and coordinative signaling – vasculature. Architecture of all land plants (except the archaic mosses) is shaped by the arrangement of load-bearing modular elements, the telomes. The telomic module consists of conductive woody vasculature embedded in a cylinder of developmentally open ground tissue enclosed by an epidermal layer (**Fig. 1A**). The arrangement of vasculature represents the core process of plant architecture, and therefore has been studied intensively, for instance during the healing of wounds (Sachs, 2000) or for the venation of developing leaves (Mattsson *et al.*, 1999). The cells of the ground tissue are all competent for transdifferentiation into vessels. This differentiation depends on the flow of the plant hormone auxin through the ground tissue. Auxin (indolyl-3-acetic acid) is a weak acid and relatively small. In the acidic environment at the outer face of a plant cells, it will be uncharged and therefore can enter the cells from any direction. In the more or less neutral cytoplasm, auxin is deprotonated and thus acquires a negative charge that will prevent its spontaneous exit. Due to this ion-trap mechanism, it will accumulate in the cell. However, it can exit by means of specific export pumps that are localised asymmetrically, guided by cell polarity. The combination of non-directional influx and directional influx produces a mutual competition of individual cells for free auxin and a directional flow in the direction of cell polarity. A cell with more active or more localised auxin exporters will transport more auxin than its neighbours and therefore cause a drainage of auxin. This mechanism for lateral inhibition of individual elements is now combined by autocatalytic feedback: The differentiation from the ground state into a vascular cell fate is induced by the flux of auxin passing through the respective cell, and the differentiation promotes cell polarity resulting in a stronger gradient of auxin exporters what, in turn, will further stimulate the drainage of auxin.

This positive feedback, in combination with a lateral inhibition (caused by mutual competition for auxin) drives the pattern of conductive tissue, and thus the arrangement of telomes. This "auxin canalisation" 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).

Plant patterning – order without a "Great Chairman". Biological patterns are shapes that become manifest on the level of a population of cells or organs. They are holistic in quality and represent classical system properties that *emerge* when the system is considered as an entity. At first glance, this would call for a strong hierarchy controling the behaviour of the individual elements. To use a metaphor from human societies: collectivism is usually bound to strong (and often autocratic) leader personalities. This approach will not work for plant development, though. As pointed out above, plant cells maintain a high level of autonomy and are not easily subdued to the rule of a "Great Chairman". In addition, plant cells behave in a highly stochastic manner, a property that ultimately can be attributed to the diffuse organisation of environmental sensing (details are given in Nick, 2006): Plants lack specialised sensory organs. In shorthand, each individual cell is able to sense most environmental signals in a monadic way and therefore has to

employ extreme amplification of the sensory input resulting in all-or-none type outputs on the level of individual cells. If all cells of a tissue would respond in a homogenous manner, plant responses would be saturated already at very low input. In case of light, the input from the new moon would produce the same output as full sunlight at noon time. Due to the strong variation of sensory thresholds plants can differentiate between weak and strong stimuli by the frequency of individual cells where sensing is activated. By integration over the population of activated cells (through intercellular signaling) plants can extend their dynamic range of sensing combining high sensitivity with differential responses to different signal input.

What can we generalise from phyllotaxis and vascular 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 brought about by autocatalytic feedback loops. They follow an algorithm described by the mathematics of reaction-diffusion systems that were adapted to biology by Turing (1952), and have been quite successfully used to model various biological patterns such as foot-head patterns in *Hydra* (Gierer *et al.*, 1972; segmentation in *Drosophila* (Meinhard, 1986), and leaf venation (Meinhard, 1976). In 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, where the inhibitor is usally described as a positive entity (such as the patS peptide acting in cyanobacterial patterning). In auxin-dependent patterning, lateral inhibition is brought about by mutual competition for the activator (Fig. 2).

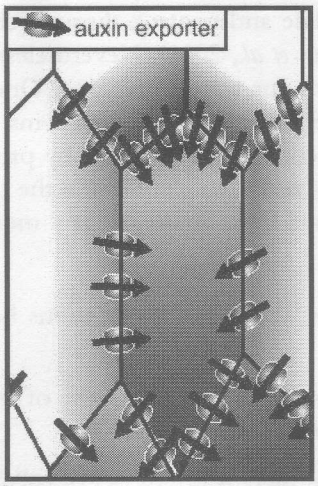

Fig. 2. *Auxin based self-organisation in plants. The supply with auxin is limited, as is the number of auxin exporters. The localisation of exporters is dynamically linked with local concentration of auxin (represented by the shading). Since auxin can permeate into and accumulate in the cell from any direction by an ion-trap mechanism, even a minor asymmetry in the localisation or activity of auxin exporters (represented by the density of arrows) will cause lateral inhibition upon the neighbouring cells. This auxin drainage represents the core element of auxin canalisation.*

Self activation combined to mutual competition also provides proportional harmony, which means that it can set up a new holistic organisation that is independent of size, when the original system is either divided or fused. This astounding ability becomes manifest as the *lack of physical body individuality*, which is so typical for plants (for further comparison refer to the contribution by Bereiter-Hahn in the same issue): the plant body can be subdivided and the parts will readily organise a new independent plantlet that in shape and architecture resembles its progenitor organism. By the way, the lack of physical body individuality has been a core element of the neolithic revolution, because it allowed humans to copy interesting plants for there use in agriculture. Especially the domestication of the so called 'first fruit crops' (grapevine, dates, olives) around eight millenia ago would not have been possible without this lack of physical body individuality.

2. Interlude: The LEGO principle

The plant version of 'organism' and 'identity' is modular. For obvious psychological reasons, the conventional concept of 'organism' is fairly anthropocentric and linked to the model of a 'body' with clear borders delineated by a more or less smooth and contiguous surface. There is some self-deception in this simplistic concept, illustrated, for instance, by the recent discovery that the microorganisms living in

our guts can manipulate and control, through chemical communication, the way how we behave (Hejtz *et al.*, 2011), Nevertheless, the "organism=body concept" continues to dominate our way of thinking. One reason for the perseverance of this concept might be that it seemingly redeems us to think too deeply about the much more difficult concept of 'identity' by providing a seemingly easy answer: 'identity' is defined as realm residing inside the border of the 'body'. This is certainly wrong and plants force us to adopt a more volatile concept of 'organism' and 'identity':

> In plant organisms, there is no contiguous border due to their centrifugal architecture.
>
> In plant organisms, there is no hierarchy of the 'body' over its parts due to strong cell autonomy.
>
> In plant organisms, there is no impact of cellular genealogy on the set-up of the *Bauplan*.
>
> In plant organisms, there is no physical body individuality.
>
> In plant organisms, there is no predefined developmental programme.

Nevertheless, plants are clearly organisms:
They can complete their lifecycle and propagate very efficiently safeguarding their lifecycle against a broad range of environmental fluctuations. Their buffering capacity even excels that of animals by orders of magnitude. Despite strong variations in the details of individual development (which is tuned with the respective environmental conditions), the characteristics of each plant species become manifest as a specific way to develop, respond and propagate. These characteristics form the base of classical plant taxonomy.
The seemingly paradox combination of flexible and species-characteristic development proceeds on the base of a pronounced modularity. To use a metaphor: plant development resembles a play of LEGO bricks. Each brick is simple in shape and robust enough to survive most if not all challenges posed by a young architect. The assembly of these bricks is extremely flexible, though, and allows for almost any conceivable variation of architecture.

What are these "LEGO bricks" of plant development? There are principally three types of bricks: architectural, cellular, and genetic (Fig. 3).

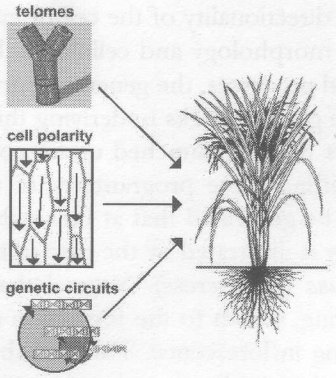

Fig. 3. Modular organisation of plant development. Plant architecture is based on morphological modules (telomes) that are combined in a flexible manner depending on environmental conditions. Individual plant cells are endowed with innate directionality (cell polarity) that is dynamically aligned by signal flow through the morphological modules. Self-referring robust genetic circuits guide the differentiation of the cellular and the morphological modules and are recombined in temporal patterns.

Architectural modules. The architectural bricks are the telomic modules arranged in a flexible way integrating mechanical load with a panel of environmental signals (with light as central component). The arrangement of telomic modules is under control of auxin flux from the aerial organs towards the roots driving the differentiation of ground tissue into vasculature (as core element of the developing telome). This architectural principle is simple, robust, and flexible. Cellular details of recently discovered fossile finds of the progymnosperm *Archaeopteris* (Rothwell and Lev-Yadun, 2005) suggest that the arrangement of telomes was controlled by auxin flow already in the Upper Devonian 375 million years ago. The secret of the land plant success story seems to reside in this modular morphology.

Cellular modules. The cellular bricks forming the base for the telomic principle correspond to the polarity of vascular cells that is aligned with the shoot–root axis. The directional transport of auxin is brought about by the combination of non-directional influx (due to the ion-trap mechanism described above) and directional efflux (due to the polar localisation of auxin-efflux carriers). The polar localisation of auxin-efflux carriers is a continuous process rather than a fixed structure: These carriers cycle continuously and rapidly (the lifetime of the carriers at the membrane are in the range of a few minutes!) between an endocytic compartment and the site of their activity at the plasma membrane. The intensity of cycling depends on the presence of auxin (Paciorek *et al.*, 2005) providing the positive amplification loop required for the the auxin canalisation mechanism driving vascular patterning. This loop is continuously "questioned" by sensory input on the auxin distribution between the cellular neighbours and subsequently either

reinforces the existing directionality of the cell or evokes the events culminating in a new polarity. When morphology and cellular architecture are brought about by modular, self-organised processes, the genetic control might be relatively simple.

Genetic modules. The genetic bricks underlying the *Bauplan* of land plants are robust regulatory circuits that are launched under control of fairly permissive temporal patterns. By shifting these programmes in time (so called heterochrony), new architectures can be generated that at first sight can be very spectacular. The power of heterochrony is illustrated by the case of the 'Skye' ecotype of the model plant *Arabidopsis thaliana* (thale cress). Normal thale cress plants produce a leaf rosette but, upon bolting, switch to the formation of small, single leaves protruding from the elongating inflorescence. This switch is impaired in the 'Skye' ecotype resulting in a fundamentally altered architecture with aerial rosettes formed from the axillary meristems of the bolting inflorescence (Grbić and Bleeker, 1996). It could be shown that this spectacular change of the *Bauplan* was caused by mutations in two relatively unspectacular genes that modulate, among numerous other factors, the timing of developmental processes. The mutations simply delay the inactivation of the vegetative programming. The ongoing vegetative development at simultaneous launch of a floral programme accounted for a fundamentally different morphology that at first sight seemed to result from 'macroevolution'. A comparative approach on plant development rapidly reveals that many evolutionary adaptations of plant architecture can be deduced from heterochronic shifts between fairly simply morphogenetic processes (for review see Li and Johnston, 2000).

The secret of plant morphogenesis - robust modules, flexible assembly. In summary, plant organisms assemble morphological, cellular, and genetic modules to integrate signals from the environment with the innate necessities of physiology. These modules stem from fairly robust self-organisation providing a mechanism to maintain the specific quality of the respective plant. The assembly of these modules is rather flexible and can be tuned with the exogenous necessity of environment. Since the modules are relatively robust and autonomous, the signals that regulate modular assembly may be very simple. Complexity is provided by the receiving modular process, not by the signal triggering this process. For instance, indole acetic acid, the natural auxin, is astonishingly small and simple. However, it combines three molecular properties (none of which is spectacular): Auxin is a small organic acid and therefore easily moves through the acidic environment of the apoplast. Auxin carries a lipophilic indole ring and therefore can permeate the cell membrane from any direction, which allows a cell to *explore* the auxin levels in its neighbourhood. Auxin is a weak acid, and thus readily trapped in the neutral cytoplasm and has to be actively exported by carriers, which allows to create a directionality of auxin efflux. It was sufficient to shift the localisation of the efflux transporter under the control of auxin itself to reach a perfect reaction-diffusion system *in sensu* Turing (1952).

The plant version of 'organism' and 'identity' is volatile. When the secret of plant development is enshrined in the flexible assembly of morphogenetic, cellular, and genetic modules, "organism" in plants cannot mean the "body" of a plant, but turns into something more volatile. The plant version of "organism" resides at the interphase *between* these modules and apparently depends on signals that due to their simple nature cannot encode complex information. It is evident that any attempt to address the essence of plant organisms by conventional molecular approaches (even including the fashionable high-throughput "-omics" strategies) will lead to nothing. Even a simple plant organ is too complex to get access to a phenomenon that works on the meta-level of self-organisation processes and clearly transcends the molecular nature of the conveying signals. In other words, any deeper approach to the essence of plant organisms poses conspicuous challenges to scientific reduction.

3. Chemical resonance and minimal organisms

"Leaves in the test tube" – experimental reduction of plant self-organisation. Plants grow during their whole lifetime by adding new cells to the tip of roots and shoots. Cell differentiation in the mitotically active zone, termed meristem, is controlled by three-dimensional intercellular signaling (for the root meristem see, as example, van den Berg *et al.*, 1995). However, differentiation is already channeled, when the meristem becomes accessible to cell-biological inspection. At this stage, it is very difficult, if not impossible, to manipulate the pattern in a fundamental manner. Thus, 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. Several years ago, we have introduced cell lines derived from the ground tissue of tobacco shoots as experimental system to study the primordial stages of division patterning (Campanoni *et al.*, 2003). These cell lines can be readily cultivated in suspensions maintained under continuous rotation. These suspension cell lines are generally considered as dedifferentiated and have even been designated as 'HeLa-cells of plant biology' (Nagata *et al.*, 1992). However, they have preserved certain features from their ancestry, such as the ability to generate the structured cell-wall thickenings characteristic for vascular cells (Nick *et al.*, 2000), the ability to produce, through a series of axial cell divisions, cell files with a clear axis and polarity, and the responsiveness to auxin as controling signal. Since these files are formed from singular cells, 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.

Weak coupling of autonomous oscillators. During the work with these tobacco cell files, we observed that files consisting of even numbers of cells were dominating over files with uneven cell numbers (Campanoni *et al.*, 2003, Maisch and Nick, 2007).

At first sight, frequency peaks of even numbered files might occur, when the cell cycle proceeds with a precise timing (Fig. 4). This should generate files homogenously consisting of

$$f(n) = 1, 2, 4, 8, \ldots 2^n$$

individual cells (with n representing the number of cell cycles). However, the length of individual cell cycles varies over a broad range, and there is, in addition to the expected peaks at 2^n, a curious frequency peak for files composed of 6 cells (in some cases accompanied by a smaller peak of 10 cells). The experimental data could be simulated using a mathematical model derived from non-linear dynamics, where elementary oscillators (cycling cells) with a high level of noise (variation in the length of individual cell cycles) were weakly coupled, and where the number of these oscillators was not conserved, but increased over time (Campanoni *et al.*, 2003). In contrast to concurrent models, the weak-coupling algorithm was able to predict the observed frequency peak of hexacellular files. Moreover, it predicted several non-intuitive properties of the experimental system, among them that coupling was 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. Unidirectional signaling is a diagnostic feature of auxin transport. In fact, the predominance of even-numbered cell files could be eliminated by low concentrations of 1-*N*-naphthylphthalamic acid, a specific inhibitor of auxin exporters (and thus of directional auxin transport). Although the noise in this system was considerable, with high variation in the cycling period over the cell population, the division of adjacent cells was synchronised to such a degree that files with uneven cell numbers were rare compared to files with even numbers. Frequency distributions over the cell number per file thus exhibited oscillatory behaviour with characteristic peaks at even cell numbers (Fig. 4).

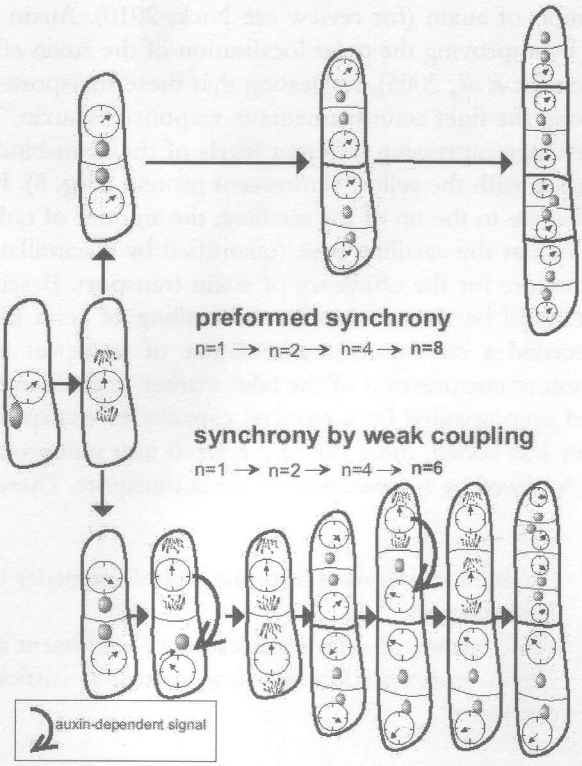

Fig. 4. Synchrony of division patterns in cell lines derived from ground tissue of tobacco. A model of preformed synchrony with precise timing of cell cycles (symbolised by identical timing of the clocks) should lead to a sequence of f(n) = 1, 2, 4, 8,... 2^n. A model, where individual cell cycles are not very precise (symbolised by variable timing of the clocks), but synchronised by weak coupling each time cell initiates mitosis (symbolised by the 12 o'clock position), will produce a sequence of 1, 2, 4, 6,... 2n instead, when the coupling signal is conveyed unidirectionally. The coupling signal was shown to depend on auxin transport.

Sensitive muscles: the actin-auxin oscillator. The polar localization of the auxin efflux carriers is not static, but subjected to a dynamic equilibrium between endocytotic uptake into endosomal compartments and exocytotic targeting to the plasma membrane. This was concluded from experiments with the fungal toxin Brefeldin A (BFA) repartitioning the carriers into intracellular compartments (Geldner *et al.*, 2001). Carrier repartitioning was suppressed by inhibitors of actin assembly. On the other hand, auxin controls the conformation of actin, whereby the massive bundles prevalent in the absence of auxin are rapidly detached into finer filaments

after addition of auxin (for review see Nick, 2010). Auxin can stimulate its own transport by improving the polar localisation of the auxin efflux carriers at the cell poles (Paciorek *et al.*, 2005), suggesting that these transporters are more efficiently moved along the finer actin filaments in response to auxin. This model was tested in rice seedlings expressing different levels of the actin-binding domain of mouse talin in fusion with the yellow fluorescent protein (**Fig. 5**). By feeding radioactively labelled auxin to the tip of the seedling, the amount of radioactivity recovered in an agar block at the seedling base (quantified by a scintillation counter) could be used as measure for the efficiency of auxin transport. Based on this experimental system, it could be shown that the debundling of actin filaments by exogenous auxin preceded a concomitant stimulation of transport efficiency (Nick *et al.*, 2009). Upon overexpression of the talin marker, actin filaments were constitutively bundled accompanied by a reduced capacity to transport auxin. When exogenous auxin was added, these bundles relaxed into numerous fine strands of actin filaments followed by a promotion of auxin transport. These findings demonstrate that

(i) actin reorganisation into fine strands precedes the stimulation of auxin transport,
(ii) fine strands of actin are necessary for efficient auxin transport, and
(iii) actin reorganisation into fine strands is sufficient to promote auxin transport.

Thus, manipulation of actin can be used as tool to manipulate auxin transport. We therefore transferred this strategy to further dissect the role of auxin transport for division synchrony in the tobacco cell model. If actin is part of an auxin-driven feedback loop, it should be possible to manipulate auxin-dependent patterning through manipulation of actin. We tested this prediction, using again the genetic approach, where we expressed the actin-binding domain of mouse talin in fusion with the yellow fluorescent protein. Mouse talin competes with endogenous actin-depolymerisation factors for binding sites on actin such that the actin filaments are progressively trapped in a bundled configuration (Keetelar *et al.*, 2004). In fact, overexpression of the construct in tobacco cells produced constitutively bundled fluorescent actin filaments. As predicted, the synchrony of cell division was impaired in this line, but could be restored by addition of auxins along with a normal organization of actin.

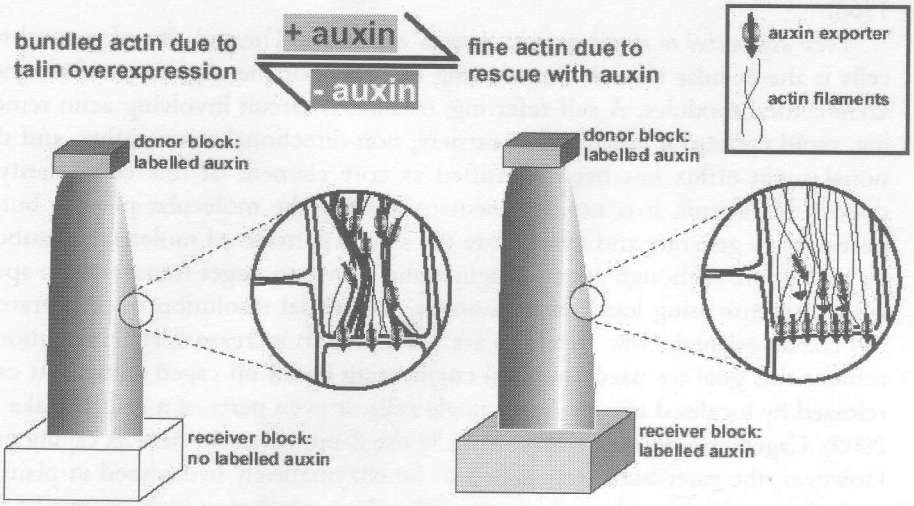

Fig. 5. Auxin controls its own transport by shaping actin filaments. By overexpression of the actin-bundling domain of talin in rice it is possible to cause constitutive actin bundles. This leads to an inhibition of auxin transport (which can be measured by feeding radioactively labelled auxin to the tip of the seedling and measuring the radioactivity arriving at the base of the seedling. By addition of auxin, a normal configuration of actin (consisting of fine strands) can be recovered. This results in a rescue of auxin transport (Nick et al., 2009).

We therefore arrive at a model of a self-referring regulatory circuit between polar auxin transport and actin organisation, where auxin promotes its own transport by shaping actin filaments. This circuit seems to contribute to the self-amplification of auxin transport, a central element in current models of auxin-dependent patterning.The implications of this model are to be explored, but already at this stage it can be used to derive characteristic properties of basipetal auxin transport. For instance, the model predicts that the transport of IAA should oscillate. Auxin will induce fine actin strands that will partition auxin-efflux carriers more efficiently to the plasma membrane, such that the intracellular auxin concentration will decrease. This decrease will cause bundling of actin filaments and, as a consequence, efflux carriers will be sequestered in intracellular compartments, 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 reorganisation (around 20 min), and the speed of carrier cycling which is in the range of 5–10 min (Paciorek *et al.*, 2005). From these parameters, auxin transport is predicted to oscillate with a period of abourt 25–30 min. In fact, such oscillations with a period of 25 min had been observed during classical ex-

periments on basipetal auxin transport in maize coleoptiles (Hertel and Flory, 1968).

New approaches to morphogenesis: chemical engineering. The polarity of ground tissue cells is the cellular module constituting the base for the alignment of telomes as architectural modules. A self-referring, oscillatory circuit involving actin remodeling, rapid cycling of auxin-efflux carriers, non-directional auxin influx, and directional auxin efflux has been identified as core element of this cell polarity. To dissect this circuit, it is not sufficient to identify the molecular players, but it is necessary to generate and manipulate the spatial patterns of molecules at subcellular resolution. Although genetic engineering allows to target transgenes to specific compartments using localisation motives, the spatial resolution of this strategy is too coarse-grained. New strategies are warranted to increase spatial resolution. To achieve this goal we used chemical engineering based on caged auxin that can be released by localised irradiation in single cells or even parts of a cell (Kusaka *et al.*, 2009). Caged compounds conventionally use 2-nitrobenzyl-esters as caging group. However, the ester-bond was found to be enzymatically hydrolysed in plant cells such that auxin was released prior to photolysis producing high unspecific background activities. By molecular modelling of the active centers of these enzymes, an esterase-resistant caging group, (2,5-dimethoxyphenyl)(2-nitrobenzyl) ester, could be designed and employed successfully. We administered this tool to the actin-auxin oscillator to demonstrate in a proof-of-principle experiment that a biological response can be controlled by light at cellular resolution. By using an auxin-inducible promotor (DR5) driving a GFP reporter, we were able to confirm that auxin was released only in the irradiated cell. Subsequently, we used the cell line overexpressing talin in fusion with the yellow fluorescent protein. In this cell line, actin is constitutively bundled, but can be rescued by addition of exogenous auxin (Maisch and Nick, 2007). By feeding caged auxin to this cell line and irradiating individual cells of a file, we could trigger a specific reorganisation of actin filaments that was confined to the irradiated cell (Kusaka *et al.*, 2009). Thus, chemical engineering using light-switchable triggers can now be exploited to steer auxin gradients during self-organisation of the tobacco cell model. At present, we are completing a study, where auxin is released in different cells of a file during specific stages of the culture cycle accompanied by specific changes in division patterns.

4. Conclusions

Plant organisms – temporal rather than spatial contiguity. As explored in this essay, plant organisms emerge from self-organisation of highly autonomous cells that are not hierarchically ordered. There is neither a preset developmental programme, nor a physical body individuality, and cell lineage (the genealogy of a given cell) is not relevant either. Using tobacco cell lines as models for a minimal organism, we could identify an oscillatory circuit as core element of this non-hierarchical self-

organisation. This circuit utilises auxin-dependent actin remodelling and actin-dependent asymmetric transport of auxin-efflux transporters as central elements and is synchronised between neighbouring cells by directional signals (that might be auxin transport itself or a factor tightly linked with auxin transport). By chemical engineering with light-switchable caged auxin, we can control the temporal pattern of cell divisions during self-organisation. Moreover, we have in the meantime established a method to follow cell division patterns in individual cell files over several days using a perfusion-chamber system with a continuous flow of auxin-containing medium. This allowed to observe synchrony of cell divisions *in flagranti* and revealed that the synchronising signal is not only moving within a given cell file, but can be transmitted between neighbouring files. This observation provides a further example demonstrating that cell lineage is dispensable for plant patterning and that the emerging "organism" transcends the limitations of physical *contiguity* (defined as continuous interface between internal and external space). In this situation, the "organism" becomes manifest in form of resonating chemical oscillations. What transforms an assembly of autonomous, "egoistic" cells into an entity, where cells cooperate and give up their autonomy for the sake of synergistic cooperation? The answer might be: a common (chemical) rhythm plant organisms emerges not by spatial *contiguity*, but by temporal continuity.

"Rule by rhythm" as general principle of biological self-organisation. Temporal continuity might arise from very simple causes: all living beings are non-linear in quality and combine self-amplification loops with lateral (or mutual) inhibition. The lateral (or mutual) inhibition does not necessarily require chemical or physical agents with inhibitory activity – since resources are limited, any upregulation of a process will inevitable consume resources required in a different site, which allows for inhibitory cross-talk. As the examples of phyllotaxis and vascular patterning demonstrates, in plants, lateral inhibition is brought about by mutual competition for a limited supply of auxin. Systems that chain self-amplification to mutual inhibition will inevitably begin to oscillate. Oscillators tend to resonate due to weak coupling (which may be based on various factors that even do not need to be very specific). Rhythmic coordination by resonance is used throughout evolution illustrated by a very long list of examples ranging from the swarming behaviour of starving Myxobacteria (Igoshin *et al.*, 2004), over self-organisation of individual amoeba into a differentiating slug in *Dictyostelium discoideum* (Pálsson et al., 1997), the neural coordination during ring-muscle contraction of jellyfishes (Satterlie, 1979), till the rhythmic clapping of applauding concert visitors or the strict rhythms of monastic or military communities. It seems that "rule by rhythm" is also employed as important (probably as central) strategy to organise a plant organism from autonomous and even anarchic modules.

"Freedom in the small" – why plant cells have to be noisy. "Rule by rhythm" as organisation principle allows the individual elements to keep most of their autonomy and therefore represents the mildest possible form of hierarchy. Since these cells maintain their individual oscillations, but are simply coordinated by resonance

(which is a very subtle and hardly perceptible phenomenon that emerges only on the level of the entity), the individual cell is left with a high degree of freedom. This "freedom in the small" is especially pronounced in plant cells. The modular organisation of plants with robust self-organisation is certainly predestined to cope with a high degree of noise. However, noise may not be just tolerated, but even required for a plant organism (for review see Nick, 2006): Plant sensing occurs in a rather diffuse manner – there are no such things as eyes, ears or tongues; there are, instead, populations of relatively non-specialised cells that sense environmental cues and signals. Nevertheless, plant sensing is surprisingly sensitive. To achieve high sensitivity without specialised sensory organs requires very efficient signal amplification of the individual cells already during the first steps of signaling. Strong signal amplification is expected to produce all-or-none outputs. On the other hand, the organism has to discriminate between very strong stimuli of different amplitude. For instance, a germinating seedling must rely on minute traces of stray light penetrating into the soil to sense, based on the colour of the light, whether it will be shaded by competitors. On the other hand, once it has reached the sunlight, it must be able to position its leaves depending on gradients between "strong" and "even stronger" light. The combination of extreme sensitivity and the need to discriminate strong stimuli in a gradual manner poses special challenges to 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. One way to solve this dilemma of plant sensing is to assign the antagonistic tasks to different levels of organisation: the high sensitivity to the individual cells that perceive the signal; the graded, variable output to the population of cells by integration over the individual cell responses (i.e. by "counting active cells"). Such a mechanism 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 *et al.*, 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.

Organismic identity versus cellular harmony. Let us presume a system composed of perfectly matching elements that function in a precise manner. In such a system of perfect cellular harmony, the individual element (the cells) would behave in an identical way, whether its fellow elements would be present or not. This means,

individual cells would not require interactions between cells, and thus there would be no need for any supercellular regulation either. It is evident that such a system could work only in a situation, where external fluctuations are extremely buffered, either because the organisms lives in an extremely constant environment or because certain parts of the organism can compensate environmental fluctuations. Neither is valid for plant organisms. Here, the behaviour of individual cells has to be constantly negotiated as visible from the entrainment of individual oscillations by intercellular signals.

These considerations lead to a surprising outcome: the plant organism is not manifest in those (rare) situations, when the oscillations of individual cells resonate in harmony. It rather *emerges* in the (common) situations, when the oscillations of individual cells diverge and have to be entrained into a common rhythm. Organismic identity and cellular harmony act as antagonists.

What is a plant organism? It is a temporal pattern ("rhythm") rather than a spatial entity ("body") and thus clearly a floating process and not a tangible thing. The organismic process is directed towards a state of resonating harmony between (oscillating) individual cells. This state is never reached, though: Plants have to face an everchanging environment. However, due to their open development, they continuously create their own new and oscillating environment. If the state of resonance between individual cells were achieved, the "organism" would instantly evaporate. It is during the cumbersome period, when individual oscillations diverge, when the "organism" becomes manifest as entrainment of the dissonant individual rhythms. Thus, it is cellular dissonance rather than cellular harmony that renders "organisms" visible.

The paradox of the organismic flow: it is targeted towards its own abolition.

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