Research News

Auxin and phyllotaxis

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Our understanding of phyllotaxis is still largely based on surgical and pharmacological experiments carried out before 1970. Recent experiments implicate the plant hormone auxin in the regulation of phyllotaxis. A recent paper shows how the polar auxin transport mutant, *pin1-1*, which fails to make flowers, affects the expression of well known meristem genes. This work opens the door for the genetic analysis of phyllotaxis.

If *Trends in Plant Science* had existed 200 years ago, which trends in plant science would have been featured? Phyllotaxis might have been high on the list because the scientists of those times appreciated the aesthetic side of biology. They could get truly exalted when writing about a structure as pleasing to the eye as the spiral arrangement of florets in a sunflower. More prosaically, after 200 years of research, the mechanism by which these patterns arise is still a mystery. But things are moving.

Phyllotactic patterns are highly regular and predictable and hardly influenced by environmental factors[1–3]. Most prevalent are spiral arrangements, in which successive organs are displaced by the Fibonacci angle of ~140 degrees. Spirals are easily visible in a sunflower head, but with a little effort one can also see them in the arrangement of the leaves of a tomato plant or the flowers of *Arabidopsis*.

The phyllotactic patterns arise at the shoot apical meristem. Along the apical–basal axis, the meristem consists of three distinct subpopulations of cells (Fig. 1a). At the apex is the central zone, which serves as a source of cells for the two meristematic zones below it, the rib zone and the peripheral zone. The rib zone forms the tissues in the central part of the stem, and the peripheral zone gives rise to the lateral organs and the outer stem tissues. Although the cells in rib and peripheral zones have obviously different fates, both zones are involved in cell differentiation. This is in contrast with the cells of the central zone, which can be considered as 'stem cells'. Therefore, it might be useful to group rib and peripheral zones together. In such a view, the meristem consists of two apical–basal patterning elements: an apical element equivalent to the central or 'stem cell' zone and a basal element consisting of rib and peripheral zone. The rib and peripheral zone are involved in cell differentiation.

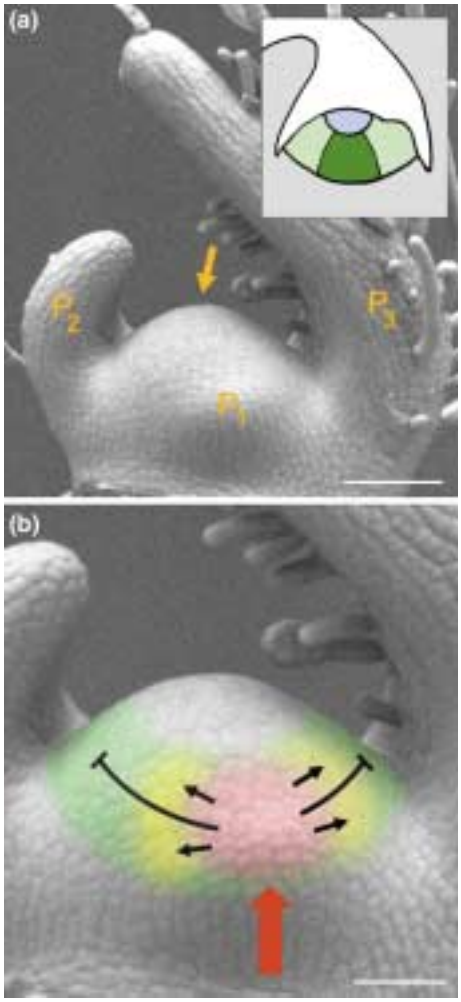

Fig. 1. Lateral views of the tomato shoot apical meristem. (a) P_3, P_2 and P_1 are the leaf primordia, the tip of the apical meristem is indicated by an arrow. Scale bar = 100 μm. Inset: diagram of the apical–basal organization of the meristem. The most apical element, the central zone is shown in blue. The two basal elements, the peripheral and rib zones are shown in light- and dark-green, respectively. (b) Modified from Ref. 13. PIN1-dependent auxin transport from differentiated tissues (red arrow) causes localized expression of organ identity and organ outgrowth genes, such as *aintegumenta* and *leafy* (pink area). Lateral signaling leads to boundaries (*cuc2* expression, yellow area) and inhibition of organogenesis in the remaining part of the peripheral zone (green area). Scale bar = 50 μm.

Genetics of phyllotaxis has been surprisingly difficult

Genes specifying the apical–basal organization of the meristem have been the subject of intense study in recent years, and we are beginning to understand how the central and peripheral zones are set up[4–7]. We also know some of the genes that initiate organ formation. However, all this elegant work has not solved the central problem in phyllotaxis. This problem relates to the radial organization of the meristem as distinct from apical–basal (note that 'radial' can have two different meanings, we use it here as in 'radial symmetry', not as in epidermis versus internal tissues). At any time only a few dozen cells, at a characteristic angle relative to the previous primordium, engage in organ formation. Why are these cells and not their neighbors selected from among the cells within the peripheral zone?

Different approaches have been used to address this question, for example, surgical and pharmacological experiments and, to a limited extent, genetic manipulation. Although genetic approaches have yielded numerous mutants with irregular phyllotaxis, in most cases these mutants display highly pleiotropic defects. It would be extremely helpful to have 'homeotic' phyllotaxis mutants, that is, mutants in which the only phenotypic alteration is a transformation of one type of phyllotaxis into another. Only one putative 'homeotic' mutant has been described, the *abphyl1* mutant of maize, which puts two opposite leaves into one node instead of the normal alternate phyllotaxis[8]. The molecular characterization of *abphyl1* is eagerly awaited.

Our concepts of phyllotaxis are still based largely on surgical and pharmacological experiments carried out before 1970. It can rightfully be argued that wound effects compromise surgical approaches and that many inhibitors are of questionable specificity. However, now

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Fig. 2. *Arabidopsis pin1* mutant. The mutation in a putative auxin efflux carrier leads to a meristem that is normal with respect to meristem maintenance and stem tissue production but fails to make lateral organs. Scale bar = 2 cm. Inset: meristem at the apex of a *pin1* plant. Local application of auxin (red) to a *pin1* meristem causes induction of a lateral organ with floral identity[12]. Scale bar = 200 μm.

that the concept of linear signaling pathways is being abandoned[9], it seems obvious that genetic interference in crosstalking networks should cause side effects just as well. A clear advantage of inhibitors and other chemical effectors is that their application can be carefully restricted in space and time. It is interesting to note the revival of pharmacological and surgical techniques in plant developmental biology[10–12].

Auxin as a regulator of phyllotaxis

Most theories of phyllotaxis postulate that signals emanate from young primordia and affect organ positioning in the meristem. No signal molecules were chemically identified, although, inevitably, most plant hormones have been implicated. Now two publications, one using a pharmacological approach[12], the other using molecular genetics[13], put auxin firmly at the center of phyllotaxis research. Didier Reinhardt and colleagues[12] applied specific inhibitors of auxin transport to *in vitro* cultivated tomato shoot apices. Leaf production was completely suppressed, but the meristems were otherwise normal. Local application of auxin microdroplets restored leaf formation but only at positions within the peripheral zone. Similar results were obtained with the *Arabidopsis* auxin transport mutant, *pin1*, except that, in this case, auxin induced flower primordia (Fig. 2). The experiments indicate the operation of two independent patterning systems. At one level there is the genetically well defined apical–basal organization of central and peripheral zones, specified by genes such as *wus*, *clv1-3* and *stm1* (Refs 4–7). At another level there is an auxin-dependent system, which specifies the radial pattern within the peripheral zone.

The report by Teva Vernoux and colleagues[13] confirms these results and beautifully integrates them into a genetic framework. The starting point is again the famous *pin1* mutant of *Arabidopsis*, which has reduced polar auxin transport because of a defect in a putative auxin efflux carrier[14] (Fig. 2). A *pin1* plant makes a few misshapen leaves and then produces a bare floral stem with a normal-looking meristem at its apex. It correctly expresses important meristem markers such as *stm* and *wuschel*, and the *clv3* gene function is required for restricting meristem size, all as expected of a well behaved meristem. Thus, two of the three functions of the meristem – meristem maintenance and production of stem tissues – are perfectly normal, whereas the third function, the production of flowers, is totally inhibited in the *pin1* mutant.

If auxin is required for organ initiation as well as radial positioning, what impact does this have on the expression of markers for organ identity? Vernoux *et al.*[13] investigated three such marker genes: *leafy* (Ref. 15) and *aintegumenta* (Ref. 16), which are markers of organ identity, and *cup-shaped cotyledon 2* (Ref. 17), which specifies the boundaries between organs. All three of these genes are expressed in the *pin1* apex. At first sight, this is disappointing because it suggests that *PIN1* affects a late step in organogenesis. However, the situation is somewhat more complicated. The three genes are no longer expressed in distinct phyllotactic patterns but instead are expressed in a ring-shaped domain in or just below the peripheral zone. Thus, the two consequences of the defect in polar auxin transport are that (1) organ formation is blocked, and (2) all the cells of the peripheral zone take on a confused identity with organ as well as boundary character.

Vernoux *et al.* present a plausible diagram of the promotive and inhibitory relationships between the genes (Fig. 1b). More importantly, they integrate auxin and firmly link this elusive plant hormone to a set of well known regulatory genes. So, what is the role of auxin in this scheme of phyllotaxis? Some apparently simple questions still remain unanswered. Where is auxin synthesized and how is it distributed? The shoot tip is far too small to measure auxin distribution by direct physical means and indirect methods do not work well in the shoot tip. So we can only guess. The rescue of the *pin1*-phenotype by micro-application of auxin can be explained most easily by assuming that inhibition of auxin transport depletes the meristem of auxin[12]. Although other scenarios cannot be ruled out, this suggests that the meristem proper is not a site of auxin production. If this turns out to be true, tissues below the meristem must be the sources of auxin. Vernoux *et al.*[13] also showed that the *Pin1* mRNA itself is unequally distributed and, conceivably, the PIN1 protein could channel auxin from the site of production to defined positions within the meristem. A locally high auxin concentration would be the signal for organ outgrowth, presumably by directly or indirectly regulating organ initiation and organ boundary genes. Are genes such as *lfy* and *ant* auxin-responsive? How is the boundary gene *cuc2* regulated? How do other mutations in auxin-related pathways, such as *pinoid*, *monopteros* and *axr1* (Refs 18,19) affect phyllotaxis at the molecular level? And how is the differential expression of *Pin1* itself regulated? Such questions can now be answered. Phyllotaxis is still as beautiful as it was 200 years ago, but it is becoming a little bit less mysterious.

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