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Mini Review

Integrating Cellular and Organismic Aspects of Vascular Differentiation

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Vascular differentiation can be studied at two levels, and they should complement one another: as an aspect of integrated plant development and as cellular processes. The differentiation of organized strands that connect between organs is induced by polar auxin flow, towards the roots. Anatomy, therefore, can be a complementary method of observing polarity and its changes. As expected for a self-correcting and essential system, vascular patterning mutations are relatively rare and have pleiotropic effects, including modifications of responses to auxin and its transport. Tissue polarity both expresses and depends on auxin transport, a feedback that could account for the determined nature of polarity as well as the gradual canalization of differentiation to vascular strands. This predicts that the molecules responsible for polarity will be localized gradually as differentiation proceeds. Further, a modified location of these molecules can be expected to precede anatomical expressions of a new, regenerated, polarity. Tracheary differentiation is probably the best studied example of cell differentiation. Within the plant, however, this differentiation is coupled to oriented cell growth either along or at right angles to the axis of auxin flow, depending on tissue competence. Differentiation is also coupled to the differentiation of the other components of the vascular system. There are, presumably, early joint stages to these differentiation processes, but what they are remains an intriguing problem.

Key words: Auxin — Cell orientation — Cell patterning — Polar transport — Polarity — Tracheary elements.

The differentiation of vascular tissues can be considered from two points of view. The first is cellular: what are the precise processes that lead to the maturation of these unique cells (Fukuda 1994, 1997a, Stacey et al. 1995)? How are the processes of differentiation dependent on genetic information, and to what extent are differentiation events similar or even overlapping in various cell types? A second point of view emphasizes plant organization. The tracheary elements must be located and oriented as parts of functional vascular strands and these strands must connect to the various plant organs. How, therefore, are various developmental events correlated in neighboring cells and throughout the plant, leading to the formation of a functional whole? The general purpose of this minireview is to consider ways in which these complementary points of view, the cellular and the organismic, could illuminate one another. It will attempt to show that the differentiation of tracheary elements is a unique example in which the gaps between biological studies at different levels are relatively close to being bridged. More specifically, hypotheses based on simple experiments—organ removal, grafts, wounds, hormone applications, and manipulations of tissue cultures—will be considered in relation to the results of molecular studies. How do these hypotheses fare in view of new results and new methods? Could they offer insights about the meaning of new information and suggest new experiments?

Auxin flow and organized development—The differentiation of vascular contacts is prevented, or, in later stages stopped, when a developing organ is removed. The same vascular differentiation can be induced in unusual patterns when organs are grafted or induced to form in adventive locations. It follows that developing organs are the source of signals that induce vascular differentiation. One of these signals is auxin: it is known to be formed in developing shoot organs and its exogeneous local application replaces their effects on vascular differentiation (Fig. 1A, Sachs 1981, 1991a, b).

The vascular tissues, furthermore, are organized as longitudinal strands, running between the various organs of the shoots and the roots. Simple experiments have suggested a general hypothesis that accounts for this major aspect of plant organization. Vascular differentiation occurs along an axial flow of auxin (and possibly other, unknown signals), towards the roots. Auxin flow thus determines the orientation of the vascular strands relative to the plant organs they serve. This suggests that vascular differentiation, and especially xylem formation, can be used as an anatomical expression of auxin flow.

Auxin flow acts back on organ development and survival, not only on vascular differentiation. Apical dominance is a prominent example: the formation of new leaves and other shoot organs is enhanced wherever auxin flow is interrupted by shoot removal or by local wounds. Root initiation is enhanced where auxin accumulates (Sachs 1991a). This suggests that the role of auxin flow is even wider than suggested above: it is a major factor in the in-

tegrated relations between the various organs of the plant. Rather than specifying any one developmental process, the role of auxin is to carry information about the presence and state of the various components of a functional plant (Sachs 1981, 1991a).

Mutant analysis is the present method of choice for the dissection of developmental and other processes. Mutations expressed by changes of vascular patterning have therefore been sought, and such mutations have been found (Berleth and Jürgens 1993, Carland and McHale 1996, Przemeck et al. 1996, Candela et al. 1999, Hobbie et al. 2000). These mutations are pleiotropic (Nelson 1998) and many of the changes are the ones expected from a relation to auxin flow: modified leaf growth (Schneeberger et al. 1998), which is also seen in various garden plants (for example, in *Coleus blumei*), root development, and auxin transport (Przemeck et al. 1996).

Another, though 'negative', result is that remarkably few mutants have been identified (Candela et al. 1999). Yet the search for these mutants has been fairly thorough, at least in *Arabidopsis*. The hypothesis above suggests two explanations for mutants being rare. The first is that vascular patterning is an expression of major controls of organization. Changes in these controls are likely to be expressed even in embryos (Berleth and Jurgens 1993), thus leading to early death rather than modified patterning. A second suggestion is that the development of vascular patterns is robust, or well buffered. Since patterns depend on gradual feedback controls, they are restored following severe wounds (Fig. 1B–D), and this could also occur when they are perturbed by mutations. Rather than following a strict, determined program, development resembles biochemical systems (Normanly and Bartel 1999) in being able to reach a functional mature form by varied, redundant paths (Sachs 1994).

The vascular strands running between plant organs are polar: they always form connections of shoot tissues with roots. There are no direct contacts between shoot organs nor between individual roots. The transport of materials between similar organs is possible, of course, but only indirectly, crossing from one channel to another. Polarity is also expressed by the regeneration of new organs on plant cuttings (Vöchting 1906). Both the location of vascular differentiation and of organ initiation can be accounted for by the polar auxin transport. This, again, is supported by mutant analysis. As mentioned above, genetic changes in vascular patterning have been repeatedly found to be associated with defective responses to auxin and its transport (Carland and McHale 1996, Przemeck et al. 1996, Nelson 1998). Phytotropins, substances that specifically interfere with polar auxin transport, have the expected far-reaching effects on plant organization and vascular differentiation (Matteson et al. 1999).

It was mentioned above that one component of signal flow from the shoot tissues to the root apices is the known hormone auxin. Auxin is placed in a special category by its

In the three figures the original shoot was above the page and the roots below—the axis was thus vertical. The figures are schematic illustrations of changes of this axis as a result of bud growth, auxin treatments and wounds.

Fig. 1 Changes and maintenance of tissue polarity during axis regeneration. Blue lines indicate new vascular tissues, formed after the plants were cut or treated. The actual vessels were much more numerous than the blue lines in the figure. A. The apical parts of the shoot were removed. On the left a bud grew, replacing the missing shoot. Development above this bud ceased, and new vascular tissues were formed, newly oriented so as to connect the growing shoot with the rest of the plant. On the right a local exogeneous source of auxin had the same effect on vascular differentiation. B. The removal of a wedge of tissue interrupted the continuity of plant axis. Vascular regeneration, oriented around this wound, re-established connections between the shoot and the root. C. The axis was cut, as in B, but the cut was oblique, separating a stump pointing towards the roots. Unfunctional differentiation, which did not connect to the roots, continued along the original tissue polarity and into the stump. Some of the new vessels formed closed loops. Limited vascular channels connected the lower parts of the stump with the roots. This differentiation indicated a reversal of the original polarity of the tissues. D. The same wound as in B was cut in meristematic tissues, close to the tip of the shoot. The parts that formed a direct contact with the root increased in girth; there was no reorientation or other development in the interrupted parts of the axis.

Fig. 2 Cellular expressions of polarity changes. Events above a wound, as in Fig. 1B. Cell walls formed after the plants were wounded are in blue. A. Regeneration of a new vessel around a wound. The cells did not divide and yet they re-differentiated: they formed special wall structures and died. Apertures in the cell walls define a new vessel, at right angles to the original tissue polarity. The wall is thickened in bands (only a few shown) that are continuous between neighboring members of the new vessel. These bands were formed according to the original vertical axis, at right angles to their expected, normal orientation relative to the new vessels. B. A similar and adjoining regeneration of a phloem sieve tube (in blue). Unlike the regeneration of the vessels, this re-differentiation is associated with cell divisions. C. Regeneration of the cambium above a wound. The cells divided at right angles to their original axis. As shown by one cell (blue) these divisions were followed by intrusive growth which re-established the elongated form at an angle with the original axis.

Fig. 3 Possible molecular changes underlying cell polarization during unperturbed and reoriented differentiation. The location and relative concentration of auxin channels is represented by red rectangles. A. The state assumed to prevail in a primary meristem. The tissue is polar—there are more channels on the basal side of the cells—but there are no differences between the cells. B. During continued development some cells, the future vascular channels, become more polar—their channels are more concentrated on their basal side. These cells are the preferred auxin transporters, and this results in their neighbors transporting less auxin and becoming less polar. The elongation of the more polar cells occurs with fewer divisions at right angles to their long axis. C. Reorientation of polarity above a horizontal cut (as in Fig. 1B). The location of the channels changes in response to the movement of auxin along a new axis.

polar transport and ability to determine the orientation of new vascular tissues. Other substances enhance vascular differentiation under appropriate conditions—in treated plants, in culture, or both (Aloni 1987, 1995, Fukuda 1997a). Prominent among these are cytokinins and gibberellins (Aloni 1982), ethylene (Kalev and Aloni 1999), and brassinosteroids (Yamamoto et al. 1997, Clouse and Sasse 1998). None of these replace the effects of leaves in inducing and orienting the differentiation of organized vascular strands; they only enhance differentiation where auxin is already present. The effects of varied substances on differentiation clearly require further study; especially so since there are two ways in which they could be understood. Some substances, such as ethylene and brassinosteriods, might well modify the polarity of cellular responses to auxin, a possibility that is considered below. Others, cytokinins for example, might influence differentiation indirectly, changing the 'well-being' of the cells, the readiness with which they can be induced to differentiate or re-differentiate and become vascular elements.

Canalized flow and integration at the tissue level—It is now necessary to turn from the whole plant to the tissue level. Polarity of organ initiation (Vöchting 1906) and auxin transport (Goldsmith 1977) can be stable or determined. The hypothesis outlined above further suggests that determined polarity could be expressed by vascular differentiation. Vascular anatomy adds dramatic manifestations of a determination which can prevent or greatly delay vascular joining across grafts in which one of the members had been inverted (Sachs 1981), even when tissue joining is

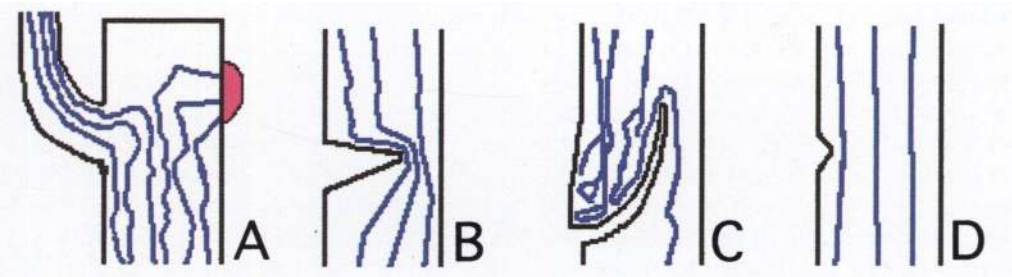

Fig. 1

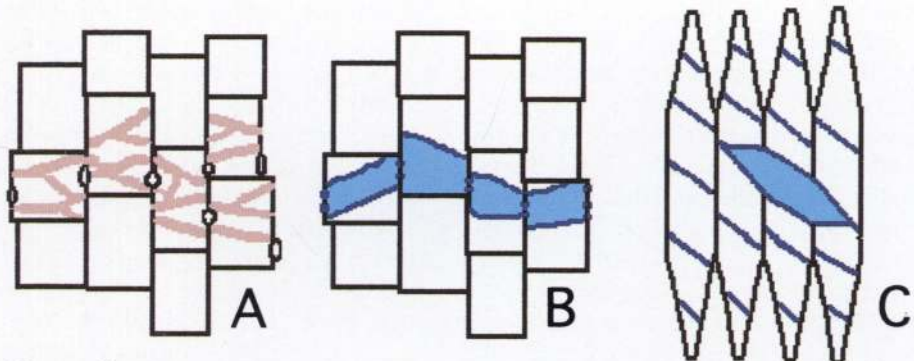

Fig. 2

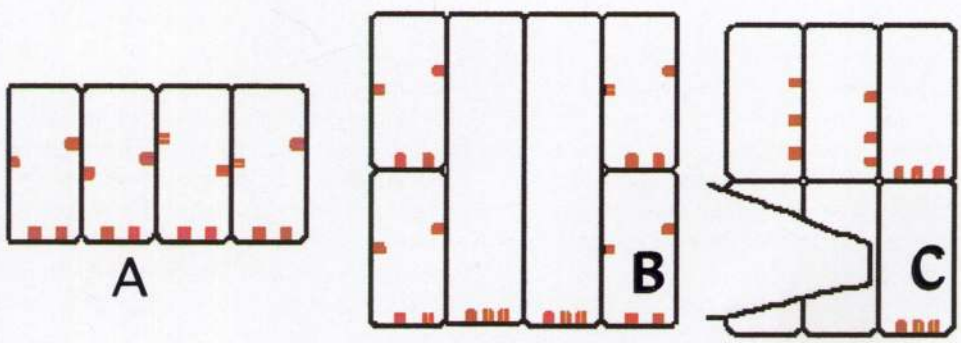

Fig. 3

required for the survival of the grafted plant. In cut plants new vascular strands connect into stumps, where they could have no functional role (Fig. 1C).

Yet the opposite result, vascular differentiation at various angles to original tissue polarity, is also common. It is found next to adventive organs and auxin sources, as well as around wounds (Fig. 1A, B). Measurements of radioactive auxin confirmed the anatomical evidence for a new transport polarity (Gersani and Sachs 1984). The orientation of vascular strands even suggests 180° changes, actual reversals of the original polarity (Fig. 1C). Such revesal has also been seen in culture (Warren Wilson et al. 1994).

These facts about the stability and lability of polarity appear contradictory: when is the original tissue polarity changed rather than maintained? A generalization can be readily suggested. The original polarity prevails wherever possible; near wounds or a new auxin source (either endogeneous or exogeneous) there may be no polar, pre-determined alternative, and then a new auxin polarity appears readily. This generalization suggests that polarity is induced wherever auxin accumulates and diffuses passively through competent tissues (Gersani and Sachs 1984). Put differently: there is a positive feedback between auxin flow and tissue polarity. This positive feedback accounts for polarity being highly determined wherever it can be maintained, and yet readily labile where auxin is forced to follow a new route (Sachs 1981, 1991b).

It follows that the unique response to locally applied auxin, the new orientation of vascular differentiation, is an expression of a new polarity. The new tissues, furthermore, include files of specialized cells, the transporting channels of the xylem and the phloem. Canalization of differentation can be expected to result from a gradual increase in the polar transport capacity of some cell files while auxin is drained from their neighbors (Sachs 1981, 1991a, b). This would mean that when the temporal parameter of the induction of polarity is considered the feedback between auxin flow and tissue polarity accounts for a major differentiation pattern. The evidence for this concept, though strong, is at present only indirect. There is experimental evidence that xylem differentiation is induced gradually, over a period that overlaps much of the time required for the maturation of new vascular tissues. It also is known that the degree of polarity, expressed by the rate of auxin transport, can differ even in neighboring cells (Bourbouloux and Bonnemain 1979). The determination of which files actually continue to differentiate can depend on chance rather than on any predetermined cellular traits (Sachs 1981).

Molecular work provides markers of vascular differentiation whose expression precedes anything that could be seen anatomically (Baima et al. 1995, Igarashi et al. 1998, Hardtke and Berleth 1998, Palme and Gälweiler 1999). A gradual canalization has been seen in the expression of a gene required for vascular differentiation (Hardtke and Berleth 1998). Mutants have been used for the identification of the proteins required for the response to auxin and the polarity of its transport (Bennett et al. 1998, Hardtke and Berleth 1998, Palme and Gälweiler 1999). This has made it possible to visualize polarity by the distribution of membrane proteins (Gälweiler et al. 1998; an earlier attempt at such visualization by another group has not been continued or confirmed). It should thus be possible to see cell polarity at the molecular level, and, as expected from the canalization hypothesis, the asymmetric localization of the auxin transport proteins is gradual (Steinmann et al. 1999). Further detailed temporal information of molecular polarity differences could provide additional tests of the canalization hypothesis. Changes of polarity during regeneration could be visualized at the molecular level (Fig. 3C). Since differences of polarity are expressed by the location rather than presence of molecules, they need not be dependent on changes in gene expression.

The specification of oriented cell differentiation—The overall levels of auxin have also been increased (Klee et al. 1987) and decreased (Romano et al. 1991) by the methods of genetic engineering. As expected, these changes led to quantitative modifications of vascular tissues (as well as other expected changes, such as modified branching). From the point of view of the present discussion, however, the important point might be what did not happen. Though the quantities of critical hormones were changed, the overall course of the vascular tissues was maintained. The contrast with an older case of genetic engineering might be significant: Agrobacteria cause tumors, disrupting all aspects of normal organization, by manipulating hormone synthesis. Here the modification is local, not in the plant as a whole, and it prevents the occurrence of an organizing flow through the developing tissues. This suggests that present techniques of turning genes on and off locally could lead to the formation of new forms, perhaps even ones as complex as the organized galls induced by insects. A novel, organized structure has been induced by exogeneous auxin—and this required repeated local applications, presumably inducing a long term auxin flow (Sachs 1993).

New methods have also been used to measure auxin concentrations in minute amounts of tissue. Careful work by Uggla et al. (1998) has demonstrated sharp differences in the auxin content of neighboring cells in the cambial region. These differences have been taken to mean that auxin can be a morphogen, a substance whose precise concentration specifies different cell fates and, therefore, specifies patterned differentiation. The hypothesis that underlies this suggestion is quite different from the one outlined above. It does not consider the axial and polar, and thus vectorial, properties of vascular differentiation (Sachs 1981, Kramer 1999).

This raises the question of the parameters of the presence of auxin to which the cells actually respond. Auxin concentration in the cells might be critical for differentiation, as is commonly assumed. Concentration alone, however, could not suffice; it could not specify cell orientation. The alternatives are either the gradient of auxin across the cells or its actual flow through the cells. Intuitively a gradient appears likely, yet experiments in which local auxin sources were added proved that the information about the presence of auxin is required over a long period, of two days (Sachs 1981, 1991a). For responses along large distances—many meters in trees—this would require gradients that are vanishingly small. Further, auxin can induce vessels in the form of closed loops (Fig. 1C, Sachs and Cohen 1982): long term circular gradients are impossible, while there could be a circular flow that follows a determined polarity. The differentiation of individual cultured cells might be an exception—but here, too, auxin transport inhibitors reduce differentiation and an auxin flux might be generated locally (Burgess and Linstead 1984). It is unknown, of course, how cells could measure flow, but mechanisms involving intracellular, rather than intercellular, gradients are certainly possible. In any case the required mechanisms are a far cry from the intuitive assumptions, often taken for granted, of all-or-none switches of cell differentiation in response to critical auxin concentrations at a precise time.

The oriented, vectorial properties of vascular differentiation raise another problem. A prediction from the induction of vascular differentiation by polar, canalized auxin flow is that all strands should be polar—they should have one side connecting, however indirectly, to shoot tissues and another to the roots. As mentioned above, all major vascular strands meet this expectation. The vascular tissues of Angiosperm leaves, however, form a complex network, and within this network there are regions to which no polarity can be assigned (Sachs 1975). A modified hypothesis, suggested by vascular connections at the base of some leaves, can resolve this apparent conflict between actual differentiation and a dependence on auxin flow. The strands that have no defined polarity are, in fact, anatomical expressions of continued auxin flow, but this is a flow whose direction changed a number of times during the course of their early differentiation. There is limited experimental evidence for this possibility and it is in accordance with the limitation of complex, a-polar networks to Angiosperm leaves in which development is not synchronous (Sachs 1975, 1989, 1991b). The modified hypothesis suggest an important principle: the first stage in the determination of cell orientation could be the choice of a preferred axis rather than a preferred polarity or direction; there is an 'axiality' that precedes polarity. Flow continues preferentially along the induced axis of such cells even where its direction changes repeatedly. The molecular basis of such axiality could be an important future challenge.

Additional differentiation responses to auxin flow—The vascular system of land plants consist of three tissues, the xylem, the phloem, and, except in Monocotyledons and present day ferns, a meristematic cambium. The individual tissues, in turn, include diverse cell types, including heterogeneous parenchyma (Sachs 1981, Fahn 1990). Yet local auxin applications, with no additional substances, induce the differentiation and determine the orientation of entire vascular systems. The induction of phloem sieve tubes by auxin (Aloni 1995) is if anything more rapid and prevalent than the induction of tracheary elements; it is ignored only because it is hard to follow. These facts emphasize, again, that auxin is not a specific inducer of any one type of cell differentiation. This raises two related questions: what aspects of the varied differentiation processes are overlapping or identical and how their locations could be specified (Sachs 1981). In both cases mutants could be useful, but the problem, of course, is that such mutants should be specially hard to detect: screening anatomical structures, though possible, requires much effort; further, it damages the very plants that must be nurtured once their special traits are noticed. Reports of a mutant that specifically affects fiber differentiation (Zhong et al. 1997) and another that changes the spatial relations between the component vascular tissues (Zhong et al. 1999) could therefore be quite important.

It is reasonable to assume, at least as a working hypothesis, that the specification of early differentiation stages of the various cell types could be similar or identical. The study of gene expression on intact tissues, however, has barely started (Allona et al. 1998). The emphasis has been on xylem differentiation in culture (Warren Wilson et al. 1994), where it readily occurs from specialized parenchyma and not only from meristematic cells. It even occurs, in a well defined experimental system, by a re-differentiation of cells with no cell divisions (Fukuda 1994, 1997a). Here the expression of genes in tracheary differentiation has been followed in some detail (Demura and Fukuda 1994, Igarashi et al. 1998), especially of genes related to the programmed cell death of the tracheary elements (Fukuda 1997b, Fukuda et al. 1998). These isolated cells are, in fact, the best studied cellular differentiation in plants. The system has allowed the characterization of three stages in the process of single cell re-differentiation forming tracheary elements: the loss of the parenchyma differentiation, the re-structuring of the cells, and, finally, the formation of the specialized cell walls and cell autolysis (Fukuda 1997a). Of these stages the most relevant to the differentiation of other vascular cell types is likely to be the second, the processes that could be expected to include the determination of cell orientation. Comparisons of the changes in gene expression in the various component cells and tissues of the vascular system, however, are not yet

available. Their study is likely to be a fruitful approach in the future.

It is now necessary to turn from differentiation processes to the spatial relations between the various cells and tissues of the vascular system. Information is available from following intact and perturbed development in various plants. Though this is scattered evidence, it suggests the following spatial mechanisms, all or part of which could contribute to a general answer:

[a] The induction of the different cells and tissues could require auxin to act together with other substances. Thus sugar was found to enhance phloem differentiation (Warren Wilson 1978). This, however, may only be an enhancement of the formation of the callose used to detect the sieve tubes (Aloni 1980). Primary phloem fiber differentiation is induced and oriented by signals originating in growing leaves (Sachs 1981), and in this case they can not be replaced by a local source of auxin. Fiber formation in secondary xylem can be induced by combinations of auxin and gibberellins (Hess and Sachs 1972, Aloni 1987). Combinations of auxin and other hormones, presented in defined temporal orders, induce the formation of fibers even in vitro (Aloni 1982).

[b] There are exceptions to the generality of the effects of auxin on the formation of entire vascular systems, indicating a need for additional inductive factors. For example, auxin does not induce the differentiation of the procambium within shoot apices (Young 1954, Sachs 1981). The problem could be technical: diffusion at the dimensions of apices must be rapid, preventing exogeneous auxin application from having a local, orienting effect.

[c] Xylem vessels are often accompanied by parenchyma (Braun 1983, Fahn 1990). These cells could be the ones that remain 'partially induced' when the cells that later differentiate as dead vessel elements become, as long as they are alive, the preferred channels of auxin transport (Sachs 1981). The varied, species specific relations between the parenchyma and the vessels could depend on the parameters of the canalization of auxin flow.

[d] Vessel diameters vary greatly. This variation stands out in comparisons of different species, and even in the wood formed in different conditions and seasons in an individual plant. The different vessel diameters could be a function of the concentrations of the inductive auxin: when the concentration is high differentiation can be expected to be rapid, allowing only limited time for cell growth and resulting, therefore, in narrow vessels (Aloni 1987, 1995).

[e] Finally, two aspects of vascular structure are evidence for transverse polar interactions, between the xylem and the phloem and along the radius of the plant axis. The first is the vascular rays, multicellular structures with a radial rather than vertical orientation (Fahn 1990, Lev-Yadun and Aloni 1995). Their presence is just about invariable in vascular systems with continuous cambial activity, and their regular distances are maintained in a dynamic pattern whose details change constantly, even during thousands of years of continued development (Sachs 1981). Ray formation continues, and is even enhanced, where the shoot and root of trees are connected only by narrow cambial bridges (Carmi et al. 1972). This is significant: rays can only be formed at the expense of the cross section area of the xylem and phloem, and in narrow bridges this cross section of the contacts between the shoot and the root must be limiting. It follows that rays must have some essential developmental role. There is, however, no simple relation between the rate of cambial activity and ray density (Lev-Yadun and Aloni 1995), and little that is concrete can be said about the possible role or roles of rays in vascular differentiation.

A second structural expression of transverse polar interactions are the relative locations and inter-dependence of the differentiation of the xylem and the phloem. The relations between the two tissues vary, but they are predictable and stable for any given genotype. In intact or damaged plants xylem does not form without neighboring phloem, and only limited phloem forms with no xylem. In contrast to axial polarity (Fig. 1C), the radial relation is strictly maintained during regeneration, and grafts of tissues with opposite radial polarities do not fuse (Warren Wilson and Warren Wilson 1984). Explanations of this polarity in terms of gradients of sugar and auxin (Warren Wilson and Warren Wilson 1984) don't quite account for its stability, nor for the differentiation of other expression of radial polarity, that of the rays. The same transverse polarity may also be a critical component of leaf structure. Here the phloem is found on the abaxial side, which is, again, the morphologically outer one. Mutants of leaf differentiation have been considered as mutants of this polarity (Eshed et al. 1999). They can also be understood, however, as changes in competence of a specific tissue to respond correctly to its location, not of the any radial or polar transverse interactions that specify cell fate.

Growth in response to polar auxin—The entire plant axis, not only the vascular system, is an oriented, shoot-root system. This suggests that growth could resemble cell differentiation in the vascular system in being a response to auxin flow. It is generally assumed, or at least implied, that auxin concentration can enhance growth along the predetermined polarity of the treated tissue. This was, of course, the bioassay that led to the identification of auxin. Yet even in maturing tissues there is varied evidence that local auxin sources specify not only growth itself but its orientation as well, thus determining cell form (Sachs 1991b). Local auxin applications to maturing parenchyma result in growth along the new axis of auxin flow (Czaja 1935). This growth can be at right angles to the original tissue polarity. As mentioned above, repeated, local auxin applications can even result in the development of an or-

ganized gall (Sachs 1993). Substances that interfere with auxin transport, such as ethylene, also change the axis of cell growth. Where auxin flow is prevented, growth has no defined axis. This is presumably what happens in callus, near wounds (Kirschner et al. 1971), and where auxin is applied in high concentrations, which can be expected to swamp the transport system.

The determination of plant form, however, occurs in meristematic rather than in perturbed, relatively mature tissues. Here changes in the orientation of growth and cell divisions are the of basis leaf initiation (Lyndon 1982, 1998). A dramatic reorientation of growth in response to signal flow occurs in the meristematic cambium. Most of the cambial cells—the fusiform initials and their immediate derivatives—are oriented along the axis of the plant organ. Where vascular reorientation occurs, as where a lateral branch grows or where the axis is cut, the initials divide at various angles to their long axis (Fig. 2C, Kirschner et al. 1971). The elongated cell form is restored by oriented intrusive growth of individual cells, penetrating between their neighbors, rather than tby any growth of the tissue as a whole. Similar oriented cell growth occurs in the cambium in response to local auxin applications.

It is only in a fairly narrow competence window that auxin applications cause parenchyma growth along the axis of auxin flow. Cambial activity is an obvious example of meristematic cell growth that depends on auxin flow. Yet the axis of most cambial growth is at right angles to auxin flow (Sachs 1981). This appears to be a generalization that holds for primary as well as secondary meristems. As in the cambium, growth within apical meristems accommodates the radius of the axis to the mass of organs that are supported and supplied. Responses to local auxin applications, as well as regeneration following transverse wounds and organ removal, suggest that here, too, auxin flow is a determining control (Sachs 1993).

The response of meristematic tissues to the orientation of auxin movement displays no pre-determined direction or polarity. In contrast to the events in a downwards pointing stump of relatively mature tissue (Fig. 1C), close to the shoot apex such stumps are by-passed, being replaced by additional growth in the intact part of the axis (Fig. 1D, Sachs 1993). This is, presumably, the same 'axiality', rather than polarity, that was mentioned above as necessary to account for the differentiation of complex vascular networks, especially in leaves (Sachs 1975, 1989).

Growth and vascular differentiation are readily observed, but there are, presumably, earlier oriented events of subcellular structures. These could bridge the gaps between overt cell form and differentiation and molecular changes. Such structures have been sought in the cytoplasmic strands that transverse the vacuoles of growing cells, close to the meristematic tips. There is limited evidence that orientation of these strands changes in response to wounds and local auxin (Kirschner and Sachs 1978). Further, following the strands in living cells shows that their connections change constantly, over periods of 20 min or so, as though the cells are 'searching' for the best possible configuration (Sachs 1991a). This temporal element of cell and tissue organization has not been studied. At finer levels, microtubules become arranged along axes that predict the future polarity of differentiation and the pattern of cell wall bands (Warren Wilson et al. 1994, Chaffey et al. 1997). Actin filaments also reorganize in differentiating vascular cells (Kobayashi et at. 1987). Subcellular structures, however, also respond to other sources of vectorial information, such as mechanical stimulation and light (Fischer and Schopfer 1997), and their relations with the information supplied by auxin could be important problem for future study.

Conclusions: unexpected developmental controls and research opportunities—A common paradigm, often implied rather than clearly stated, underlies many if not most studies of cell differentiation. Such differentiation is considered as the result of defined programs that are activated by specific signal configurations at critical times. Overt differentiation is also thought to be preceded by an all or none determination of cell fate, and this determination is an expression of events at the level of genes and their expression. How well does this paradigm fit the facts and hypotheses outlined above?

In contrast with intuitive concepts about simple relations between substances and processes, auxin flow appears both to coordinate the relations between organs and their vascular supplies and to determine cellular patterns within vascular strands. Auxin, therefore, is not an inducer of any specific developmental event. This makes functional sense: the control of varied processes by one substance results in events at the organ and cell levels being parts of an integrated whole. There still is a unique aspect to the processes determined by auxin: the specification of the orientation of growth and differentiation. This very same orienting influence could also be essential for the relations between the various organs, tissues and cells of the organized plant (Sachs et al. 1993).

What, then, replaces a detailed specification by precise configurations of substances? One answer is that the cells respond to information over an extended time, and the temporal parameters greatly enrich the information the cells receive. The differentiating cells, furthermore, interact with the vectorial information: their early changes influence include oriented transport, and thus change the distribution of the signals that determine continued differentiation. Determination is gradual and quantitative, depending on interactions between cells. Early determination can be expressed by vectorial transport, and could therefore depend on the location of molecules and subcellular structures, not necessarily the occurrence of gene expres-

sion.

These differences between the requirements for vascular differentiation and common paradigms are important. They do not provide for any developmental program, at least as 'program' is commonly understood. But they do provide for a selection, according to local conditions and previous events, between many alternative possibilities (Sachs 1991a). The selection does not result in precise, repeatable structural details, but the overall resulting structures are free of functional mistakes. The self-correcting aspects of this development mean that it is robust (Sachs 1991a), suggesting that many mutants could be masked. Further, this revised paradigm raises important, general problems that have not been tackled to date. Ones that stand out in the discussion above as challenges for future research include axial preferences that have no determined orientation, the polarity along the radius of the plant axis and the relations between varied types of growth and differentiation. Mutants of these phenomena are rare or absent—presumably because they have not been sought, and some of them would be specially difficult to detect in the commonly used minute model plant, *Arabidopsis*.

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