

Cell polarity and tissue patterning in plants

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Summary

Cell polarization is the specialization of developmental events along one orientation or one direction. Such polarization must be an early, essential stage of tissue patterning. The specification of orientation could not occur only at the level of the genetic system and it must express a coordination of events in many cells. There is a positive feedback relation between cell polarization and the transport of the known hormone auxin: polarity determines oriented auxin transport while transport itself induces both new and continued polarization. Since cell polarization increases gradually, this feedback leads to the canalization of transport – and of the associated cell differentiation – along defined strands of specialized cells. Recent work has shown that the same canalized flow can also be an important determinant of cell shape. In primordial, embryonic regions cell growth is oriented

along the flow of auxin from the shoot towards the root. In later developmental stages the cells respond to the same flow by growing in girth, presumably adjusting the capacity of the tissues to the flow of signals. Finally, disrupted flow near wounds results in the development of relatively unorganized callus. Continued callus development appears to require the participation of the cells, as sources and sinks of auxin and other signals. The overall picture to emerge suggests that cell patterning can result from competition between cells acting as preferred channels, sources and sinks for developmental signals.

Key words: auxin, callus, cell growth, cell polarity, cell shape, cellular interactions, pattern formation, polar transport, polar regeneration, regeneration, vessels.

Introduction

A cell is said to be polarized when some of its physiological and developmental processes are special, or at least more pronounced, along one preferred, dominant direction. Unfortunately, the same term, polarity, is also used to refer to a preferential expression of events along a specialized axis, irrespective of direction (Bloch, 1965; Sachs, 1984). Polarity is an essential and early component of the biological pattern formation; in plant embryos and apices it is the first recognizable differentiation of cells. An example of polarized development that is amenable to experimental manipulations is the differentiation of the files of cells forming the vessels that transport water in plants (Fig. 1A): these cells have one specialized axis, along the cell files. But the vessels and other vascular or vein cells are only an extreme example; polarity can also be seen in the growth and divisions, and hence the shape, of the cells associated with the vascular strands (Fig. 1B).

From the point of view of pattern formation a central question raised by polarity is how the orientation and direction of the cells is specified, and specified in ways which integrate the cells of entire tissues. This question of the specification of orientation is different from, and complementary to, that of the intracellular or organelle nature of polarity (Schnepf, 1986; Doonan *et al.* 1988;

Sylvester *et al.* 1989). This question can not be understood and answered only on the basis of commonly held ideas concerning differentiation and tissue patterning. Thus differentiation is commonly considered, or even defined, as a configuration of gene expression. Even though it is likely that the polarization of a cell involves controls of gene expression, gene activity is not known to be directional; thus differences between cells that are polarized along different axes must be due to sub-cellular specialization and not only to the activity of the genetic system. Patterning is also thought to reflect the early establishment of 'pre-patterns' of 'morphogens' (Wolpert, 1971, 1989; Meinhardt, 1982). The local differences in the concentrations of these substances should be precisely interpreted by the cells, resulting in complex patterns of differentiation. But polarity could not be specified only by concentrations; gradients or flows of signals are required, and these must be such as to determine differences within embryonic cells, over distances of less than 10 μm . At the same time polarization requires correlative mechanisms that integrate the activity of neighboring cells and of entire tissues, over relatively large distances. Finally, cell polarity in plants is changed during regenerative processes even in relatively mature tissues, so it could not be specified only by an early pre-pattern or program of development.

The purpose here is first to review, very briefly, the

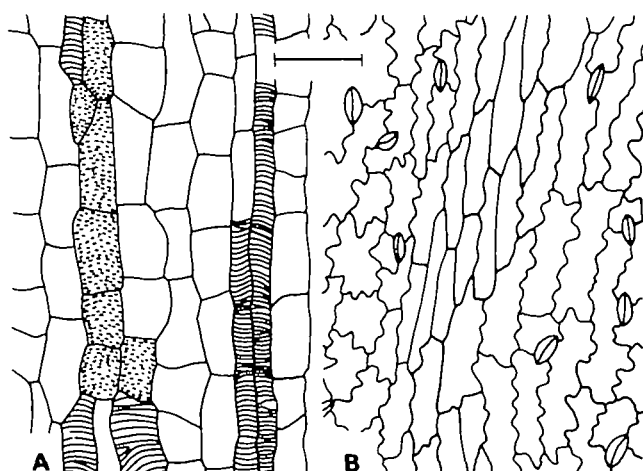


Fig. 1. Two expressions of cell polarization in plants. (A) Vessels in a drawing of a tangential section of a bean stem. Because the stem had been wounded there were both normal and regenerative vessels. These vessels consist of rows of specialized cells with thick walls, which can be recognized by their helical reinforcements or bordered pits (marked by short lines). The individual cells, the vessel members, have a distinct axis which is continuous from one cell to another. (B) The cellular structure of the lower epidermis of a pea leaf. The cells in the center adjoin a minor vein and are elongated – polarized – along the axis of this vein. The oval structures consisting of two short cells are stomata. Bar, 50 μ m.

polar induction of vascular differentiation. This is a highly favorable case, in which polarity and its changes can be seen microscopically and the nature of the chemical inducer of a cellular pattern has been known for a long time. Work on the induction of the vascular system is the basis of the ‘canalization hypothesis’, which accounts for the organization of specialized plant cells in long files on the basis of known cellular traits. Following this brief review, present work will be considered, attempting to increase the scope of the canalization hypothesis to include additional aspects of polarity, those expressed by cell shape. This leads to a discussion of callus tissues, which are characterized not by an absence of cell differentiation but rather by the absence of any consistent polar axes. Finally, the controls of polarization in plants are used as a basis for a discussion of principles of patterning where early pre-patterns and programs of development could not suffice.

All the experiments considered here were performed on plants, but the general conclusions were meant to have a wider scope. There are a number of important advantages in using plants for the study of cell patterning in general and cell polarization in particular. Plants have specialized meristematic regions which continue their embryonic development, forming new organs and organized tissues, as long as the individual plant is alive. This means that vigorous seedlings can be used for experimental purposes, instead of fragile embryos. The cells of plants have thick walls so they are easily observed, and, more important, these cells do not

move relative to one another. Furthermore, plants have remarkable regenerative abilities, which require changes in cell polarity even in mature tissues. These changes allow for experimental perturbations which can be used to test and constrain hypotheses about the controls of cell polarization.

The canalization of polar transport and differentiation

The vascular tissues of plants can often regenerate around a wound (Fig. 2A; Jacobs, 1952; Sachs, 1981). If the orientation of the vascular cells along the transporting files is taken as an expression of polarity, this regeneration must mean that polarity can be re-oriented. A new polarity, as judged by the organization of newly differentiated cells, can also be readily induced by chemical means: the application of the hormone auxin to appropriate tissues leads to the differentiation of new vascular strands at all angles to the original polarity of the tissues (Fig. 2B).

The interpretation of the induction of a new polarity by applied auxin requires consideration of the physiological role of hormones of the auxin group. The hypothesis which led to the discovery of plant hormones was that each developmental process must be controlled by a specific substance. Development was thought to be preceded and determined by the distribution – the pre-pattern – of these determinants or ‘morphogens’. Auxins were thus isolated using a bioassay which was thought to reveal the action of hormones that specifically control cell elongation, but soon after the isolation of the first auxin it was found to have numerous and varied effects on all aspects of plant development. Yet it appears that though the original hypothesis was wrong, auxins do have a special role in the plant (Sachs, 1986, 1988a, 1991). Auxins are major signals of growing shoot tissues, coordinating their growth with varied developmental events throughout the plant. This means that the response to auxins depends on the tissue on which they act and is not specific to the auxin molecules. This role of auxins includes their being natural differentiation-inducing substances, the signals by which shoot tissues *orient* (Fig. 2B) the vascular tissues that connect them with the rest of the plant (Jacobs, 1952; Sachs, 1981, 1986, 1991). The inductive effects are on all aspects of this process, including cell divisions and cell differentiation in apical meristems, in the cambium and in regenerating tissues.

Though the response to auxins is not specific, there is one aspect of auxin physiology that is special if not unique. The transport of auxin through tissues is polar, depending primarily on the developmental history of the tissue and not on concurrent auxin gradients (Goldsmith, 1977; Sachs, 1984; Gersani and Sachs, 1984). This transport is thus an expression of an innate polarity of the tissues, a polarity which is maintained even in the absence of differentiated sources and sinks for auxin. The polar transport of auxin accounts for two

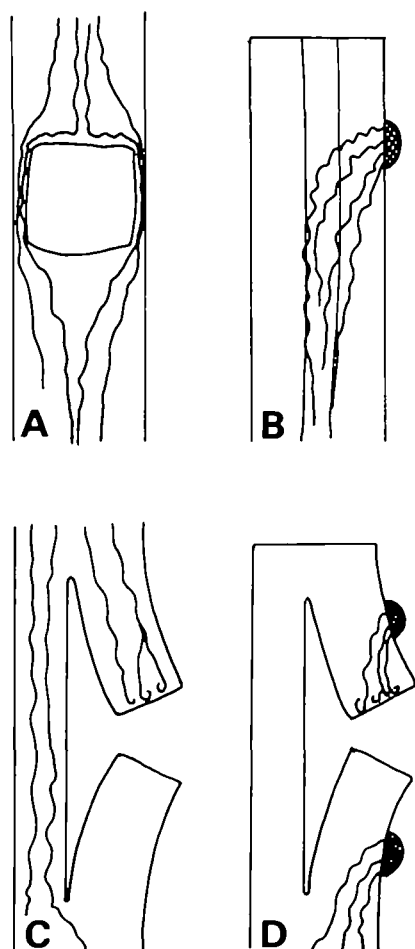


Fig. 2. The induction of new vessel differentiation. The course of the new vessels is not straight and is marked by wavy lines; the original vessels are not shown. (A) Regeneration around a wound in an *Impatiens* stem. Near the wound the new vessels, formed within five days, were at various angles to the original shoot–root polarity of the tissues. (B) Auxin (1% Indoleacetic acid in lanolin) was applied to the left of a decapitated, wounded stem of a pea seedling. The new vessels are organized, multicellular structures and close to the source of auxin they have a new polarity, at right angles to the original polarity of the tissue. (C) Regeneration in cut bean stems showing that new vessels follow the original tissue polarity and do not require contact with the roots. Two cuts, one vertical and one horizontal, separated tissue flaps pointing towards and away from the original direction of the roots. New vessels (and other vascular tissues) formed only in the flap connected with the young leaves pointing in the original direction of the roots. This flap points downwards in the drawing, but the same result is obtained when the entire plants are kept in horizontal, so it could not depend on a response to gravity. (D) As in C, but the shoot above was removed and local sources of auxin (1% Indoleacetic acid in lanolin) were applied to the two flaps of tissue. The absence of the young shoot tissue reduced new vessels' differentiation. The local auxin induced such differentiation in both tissue flaps and the new vessels followed the original polarity of the tissues, in the direction of the roots.

other expressions of the polarity of plant tissues. The first is the regeneration of new organs, especially roots. This regeneration occurs on the root side of cuttings, even where environmental clues could not specify any direction (Vöchting, 1878). This polar regeneration can be understood as a response to the polar accumulation of auxin, which induces the initiation of new roots (Sachs, 1984). A second expression of polarity, and one which can be observed microscopically, is the course and orientation of vascular differentiation, which occurs along the path of polar auxin movement (Sachs, 1969, 1981). Differentiation continues along the polarity of the tissues even if the contact to the roots is severed (Fig. 2C,D; Sachs, 1968, 1981). This is best seen in isolated stem or root storage tissue sections, where there are no root apices: new vascular tissues are formed along the polarity of the tissues, in the original direction of the roots and along the expected course of auxin transport (Gersani, 1987).

The statements made above concerning the dependence of tissue polarity on previous conditions may appear contradictory. A new polarity can be induced in cut stems and by local sources of auxin (Fig. 2A,B). Furthermore, in at least one case regeneration along a new polarity has been shown to be preceded by a new polarity of auxin transport (Gersani and Sachs, 1984), so that changes indicated by microscopy correspond to those measured by physiological means. On the other hand, polarity appears to be a determined cellular trait, as it is maintained even in cut tissues, devoid of sources and sinks for auxin. In fact, differentiation follows polarity even when it is not to the advantage of the plant, forming no functional contacts between the shoots and the roots (Fig. 2C,D). An extreme expression of the determined nature of tissue polarity is seen in grafts where one member has been inverted: new differentiation follows polarity even at the expense of the formation of new contacts between the shoots and the roots (Fig. 3A,B; Vöchting, 1892; Sachs, 1968).

A survey of the conditions in which polarity is or is not maintained suggests a simple generalization (Sachs, 1981, 1984). Auxin flow and the resulting vascular differentiation follow tissue polarity wherever possible (Fig. 3C,D). When the tissue is cut so that there is no polar alternative, auxin accumulates. This auxin may induce the formation of roots, but if it passes through the tissue at some angle to the original polarity it induces a new polarity, expressed both by new auxin transport and by vascular differentiation. What this means is that auxin flow both depends on and induces tissue polarity – a positive feedback relation which could be the basis for the stability of polarity and, at the same time, could account for the ready changes of this polarity when the original feedback relations are completely disrupted.

The interdependence of auxin transport, tissue polarity and vascular differentiation leads to a mechanism which could generate an important cellular pattern (Sachs, 1969, 1981, 1991). If the process of tissue polarization is gradual, the transport of auxin, and the resulting differentiation, should occur first in a wide

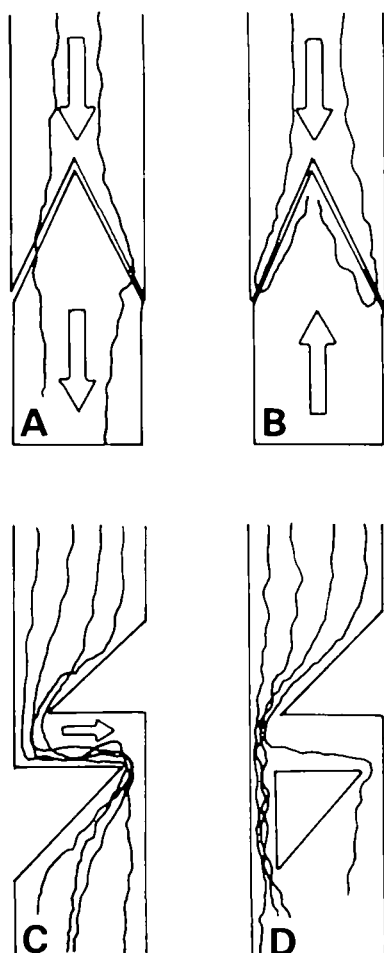


Fig. 3. Varied relations of new vessel differentiation to tissue polarity. (A) Grafts in which the original orientation towards the roots, shown by the arrows, was maintained. The new vessels crossed the graft regions, indicating the functional integration of the two graft members. (B) Grafts similar to A, but the lower member was inverted before the tissues were joined. New vessels did cross the grafted region, but they were directed along the original, determined polarity of the tissues, indicated by the arrows, and had no conceivable function. (C) A stem in which the removal of two tissue sections left only a horizontal bridge connecting the shoot and the root. New vessels differentiated readily along this bridge, at right angles (arrow) to the original polarity of the tissues. (D) As in C, but an additional bridge was left, so the new differentiation could occur either along or at right angles to the original polarity. Most of the new vessels always followed the original polarity of the tissues.

region, through many cell files (Fig. 4). As the cells specialize, as they become 'more polarized' or better transporters, narrower files of cells would suffice to carry the same auxin flow. Thus transport would be gradually canalized to cells whose polarization and differentiation continue. These specialized cells would induce the continued differentiation of the cells above them by draining away the auxin and they would induce the cells below by supplying an increasing flow of the very same auxin. At the same time these specialized cells would inhibit their lateral neighbors, from which

auxin flow had been diverted, preventing their further differentiation as vascular elements (Sachs, 1986). Thus this mechanism of polarity reinforcing polarity would canalize differentiation to defined strands whose cross-section would be determined by the auxin available for transport.

This 'canalization hypothesis' is supported by indirect evidence of various types: it accounts for facts concerning the transport of auxin, the structure of the vascular strands and the contacts made between developing strands (Sachs, 1969, 1981, 1991). It has also been tested by mathematical modelling, showing that the intuitive predictions are reasonable when the quantitative parameters are taken into account (Mitchison, 1980, 1981). This evidence, which is not new, will not be reviewed here.

Induced polarity expressed by cell shape

An advantage of the 'canalization hypothesis' is that it suggests various experimental approaches to cellular patterning (Sachs, 1981) and the one to be followed here is to search for additional cellular expressions of the same canalized controls of polarization. The 'canalization hypothesis' considered above was based only on observations of vessels, whose component cells have thick walls containing lignin, cells which can be seen readily even in three-dimensional, cleared preparations. But the canalization of auxin flow suggests that there should be cells that are 'partially induced': cells which took part in the early polarization processes but were prevented from continued differentiation by the specialization of their neighbors, the ones which became the preferred channels of auxin flow (Fig. 4F). And, as expected, the cells next to the veins are elongated, and in this sense polarized; these cells, furthermore, are the ones that are most readily induced to differentiate as tracheary elements by the application of a source of auxin (Sachs, 1981), again favoring their being viewed as 'partially induced' by their participation in early auxin flow. The working hypothesis to be pursued here, therefore, is that the polar flow of auxin (and presumably other signals) is important in determining the orientation and degree of cell elongation.

A first question should be answered by descriptive methods: when and how do some cells become longer than their neighbors? Both development and mature structures are variable at the cellular level, so it is not possible to reconstruct all aspects of development from stages found in different leaves and observed by destructive methods. For example, it is not possible to determine with any certainty the relative role of cell elongation and oriented cell divisions in the formation of cells that are elongated relative to their neighbors. However, cell elongation or polarization can be followed in living tissues on the surface of the plant, in the epidermis next to leaf veins. Specific regions of the epidermis have been observed and drawn periodically by epi-illumination microscopy (Sachs, 1978a) but this method is laborious and the information now available is quite limited.

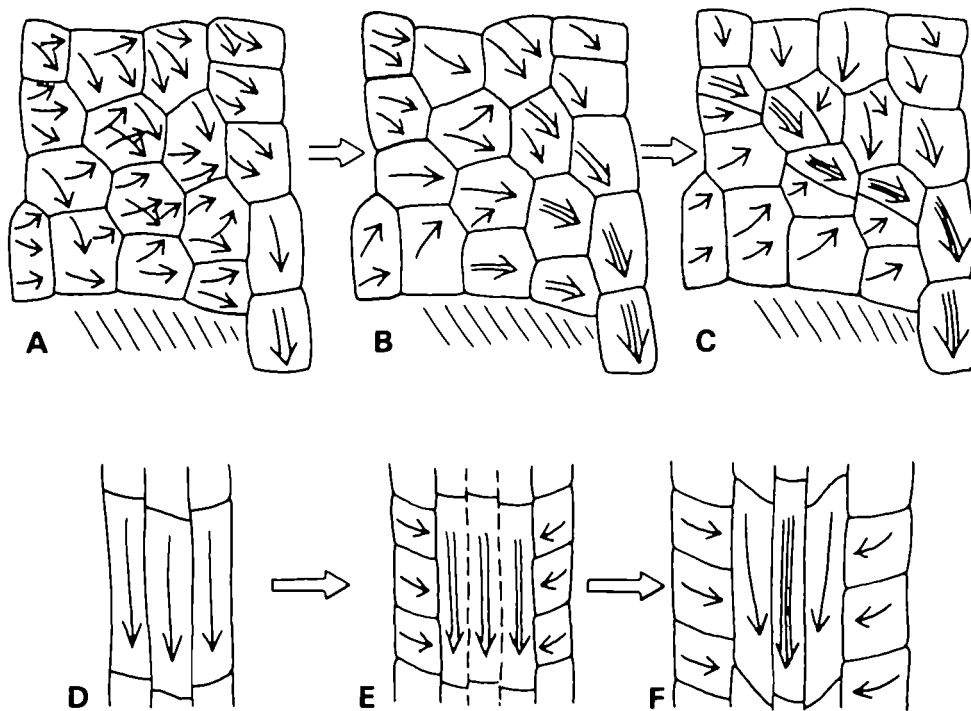


Fig. 4. The canalization hypothesis and some of its consequences. (A–C) Presumed stages in the determination of new vessels in a horizontal bridge above a wound (diagonal lines), such as the one shown in Fig. 3C. The arrows indicate expected auxin movement; arrows with double and triple lines indicate increasing transport and increasing cell polarization. In A diffusion causes movement in varied, even changing, directions, from the source of auxin (ultimately the shoot tissues) on the left towards the ‘sink’, the tissues that transport along their original polarity and were not interrupted by the wound, on the right. In B the cells have started to differentiate a new polarity in response to auxin flow and polar transport appears; this occurs first in varied strands and close to the ‘sink’ on the right, which orients and hastens auxin movement. In C continued differentiation has led to the specialization of one central strand. The transport capacity of this strand is large enough to drain the auxin away from neighboring cells, towards itself. The differentiation of the vascular strands is associated with cell divisions, but its meandering course is evidence for its origin involving random events. (D–E). Differentiation of more than one cell type as the result of a canalization of auxin transport. In D transport occurs equally through three neighboring cells. In E the central cell specializes as a polar transporter and divides into three parallel cells; the other, outlying cells become oriented towards this central channel. Finally, in F the most central file of cells has differentiated to maximize transport. This diverts transport away from neighboring cells, which remain ‘partially differentiated’. The outlying cells, through which some transverse transport has occurred, grow along their new orientation.

An easier method, allowing the development of larger regions to be followed, employs the repeated copying of the surface of a developing epidermis with vinyl polysiloxane impression material, which does not harm living tissues (Williams and Green, 1988). A result of such copying, starting when the cells were more or less isodiametric, appears in Fig. 5. Contrary to expectations, elongation was due only to preferential cell growth along the direction of the vein, and not to oriented cell divisions. When neighboring cells were compared, elongation was associated with a reduced contribution to growth in other directions (Fig. 5C). Thus the polarization of the cells next to the veins was analogous to the re-orientation of polarity observed in the differentiation vascular tissues of wounded plants (Sachs, 1981): a capacity for growth in all directions was re-directed during polarization so it became concentrated along one axis.

Simple experiments demonstrate the possibility of modifying local cell elongation. The formation of new leaves is due to re-oriented cell growth (Lyndon, 1982).

Young leaves orient cell growth towards themselves so that the epidermis is elongated along the axis connecting these leaves with the roots – when these leaves are removed at an early stage the oriented elongation ceases. This is what would be expected if elongation were induced together with vascular differentiation. As explained above, the establishment of a new polarity in disrupted, cut stems was important for understanding the polarity of vascular differentiation, but cell shape does not regenerate and reorient in the same way as do the vascular tissues. The difference between the two expressions of polarity may be quantitative rather than qualitative: cell shape may be determined early, in tissues that are too small for wounding experiments. The difficulty related to size is not only technical, since the wounds themselves have local influences that are size dependent and could mask the possible effects on the flow of signals.

Since the young leaves are sources of auxin, it should be asked whether local sources of auxin also induce and orient cell elongation. It is, of course, well known that

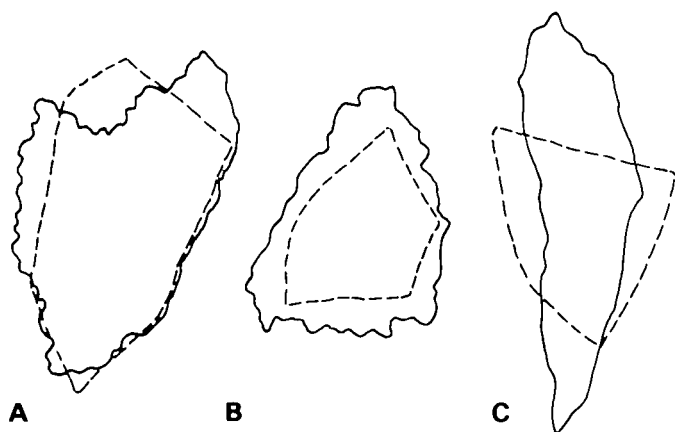


Fig. 5. Shape changes during the development of three epidermal cells on a pea leaf. Individual cells were followed from an embryonic stage to maturity by copying the surface of the leaf repeatedly, using a non-destructive method (Williams and Green, 1988). So as to highlight only changes of cell shape, the comparison ignores cell divisions and subtracts growth by enlarging the embryonic stage (broken lines) 13 times more than the mature stage (continuous lines). The three cells shown were no more than one cell apart; yet their growth differed greatly. The large increase in length at the expense of the contribution to growth in other directions, seen in (C), was characteristic of cells in the vicinity of the veins (Fig. 1B). Elongation is thus a polarization of growth which would otherwise occur in other directions.

auxin increases elongation – this was the basis of the bioassay used for the isolation of auxin, and it is still considered to be the most ‘typical’ effect of auxin, whatever that may mean. But physiological work has dealt with determined tissues, in which the cells were committed to elongate along a given axis and it was only the degree of this elongation, not its direction, that was experimentally varied. However, it was also noticed, long ago, that locally applied auxin does have a directional effect on cell growth (Czaja, 1935; Ruge, 1937). Furthermore, cambial cells are re-oriented along new shoot–root axes, and the directional effect of the shoot can be replaced by a local source of auxin (Balatinecz and Farrar, 1966; Kirschner *et al.* 1971).

So as to confirm these observations on the orienting effect of auxin on cell shape, and extend them to the epidermis, small amounts of auxin (less than 0.1 mg of 1% Indoleacetic acid in lanolin) were applied to the surfaces of embryonic organs of pea seedlings, in the minute apical regions that have not been commonly used for physiological experiments. An elongation of epidermal cells was found along the expected axis of auxin flow, from its point of application and along the polarity of the tissues, towards the roots (Figs 6C, 7C). The response to repeated applications of auxin to the same location included the formation of unique cellular bulges, expressing a novel, reoriented growth. Elongation, however, was not characteristic of all epidermal cells. The competence of the cells to respond by elongation depended on the age of the tissue: cells of stem and leaf tissues that were young and still dividing

elongated most readily, while hardly any response was elicited from mature tissues. Even in young stem regions auxin re-oriented cell growth most readily when the young leaves were removed – when there was no additional stream of auxin maintaining the original polarity of the tissue (Sachs, 1969, 1981). Finally, cells close to a wound reoriented most readily. In all these variables – tissue age, dependence on other polar effects and the influence of wounds – epidermal re-orientation was correlated with the competence of non-epidermal cells to differentiate as new vascular elements in response to auxin treatments (Sachs, 1981). The re-orientation of cellular growth was noticed within a day after treatment, before any new vascular differentiation could be detected. This is as expected, since vascular differentiation is a longer, more complex process.

Elongation along a new axis, parallel to the new flow of auxin, was not the only oriented response to auxin application. When auxin moved along the original polarity of tissues, rather than at some angle to this polarity, it caused a thickening of the stems, a growth in girth (Fig. 6D). The increase in thickness was only in the tissues below the source of auxin, the ones that connected this source of auxin with the direction of the roots (Fig. 6C,D). This cell growth is thus at right angles to the expected polar flow of the auxin.

The influence of applied auxin on growth in girth appears to reflect a normal process which occurs in intact plants. Stem thickening is induced by young leaves: stems which grow with no leaves above them elongate, but they remain considerably thinner than stems of intact shoots (Fig. 6B). Auxin, applied in a lanolin paste, on the top rather than the side of these stems, replaces the effect of the young leaves in inducing stem thickening. This effect of auxin is concentration-dependent; young stems to which a 5% lanolin paste was applied hardly elongated and assumed the shape of small barrels (Fig. 6E). The growth in girth was not accompanied by changes in the width of the epidermal cells: their mature size was about the same (Fig. 7A, B) regardless of whether the stems expanded, to twice or more their diameter, in either intact plants or in stems to which exogenous auxin was applied. This must mean that there were more cells in the wide stems, and that growth was accompanied by cell divisions.

The epidermal cells thus respond to auxin by growth and division in ways that resemble those of the cambium, whose ‘activity’, or growth in girth, is induced by the flow of auxin (Sachs, 1981). The difference is, of course, that the epidermis does not maintain the embryonic capacity to grow and divide for an unlimited period. In both the epidermis and the cambium the response might be understood as an adjustment of the cross-section of the tissues, and presumably their capacity to transport auxin, to the flow of available auxin.

Polarity and callus formation

Auxin applications often influence cell shape even when

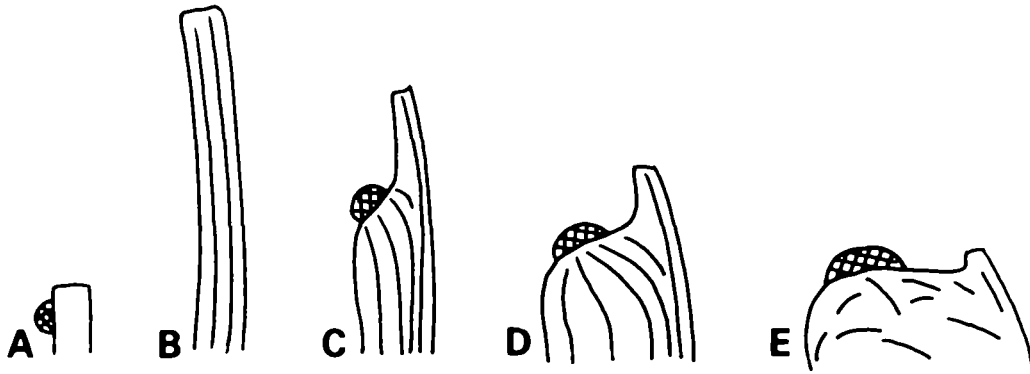
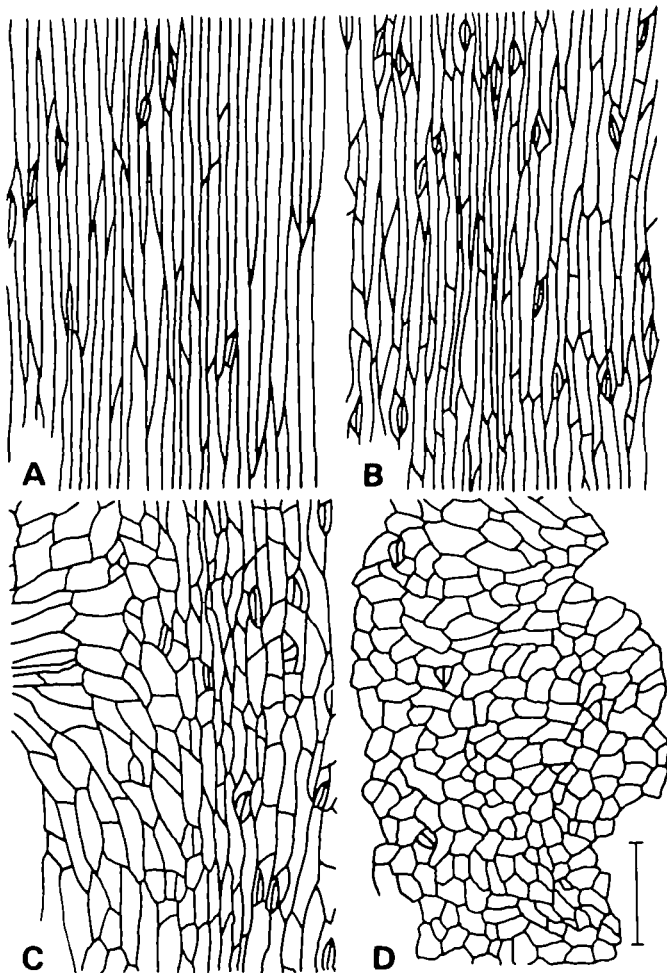


Fig. 6. The reorientation of epidermal cell growth by local auxin applications. The lines mark the long axis of the cells (details shown in Fig. 7) in pea stem internodes treated in various ways when they were about 0.7 mm long and allowed to grow to maturity on the otherwise intact plant. (A) The stem of a pea bud at the time the experiment was started; it was connected to the rest of the plant at its base. The apex of the bud was removed and lanolin, with or without auxin, was applied on one side. This stem is shown in a much larger scale than the mature stems in the other drawings of this figure. (B) A stem to which lanolin with no auxin was applied. On plants from which all shoot apices were removed, such stems elongated to a length of 1 cm or more. (C) Auxin (1% Indoleacetic acid) applied in the lanolin. Close to the point of auxin application there was new, re-oriented growth in the direction of the auxin source. Below the point of application, in the polar direction towards the roots, this auxin caused considerable growth in girth. (D) As in C, but increased response due to increased auxin treatments (higher concentrations, larger amounts of lanolin preparations or repeated applications). (E) When the amount of auxin was increased even further the growth of the tissues lost all obvious polarity. The absence of polarity was also evident in microscopic sections and in the structure of the epidermis (Fig. 7D).



they do not reach the tissues from any one, defined direction. Such responses were seen where auxin was applied at a relatively high concentration and to a relatively wide region: there was growth in response to these treatments, but this growth had no defined, coherent axis (Fig. 6E; 7D). This means a disruption of organization as it is expressed by cell polarity. Such disruption can be understood as a response to inductive auxin flows that occur in varied directions and that may even change with time (Sachs, 1975, 1989). Perhaps the most extreme expression of this disruption is the appearance of large cells that are not elongated along any one axis: and this is the response to high auxin concentrations, especially to synthetic auxins, such as 2,4D, whose concentration is not readily regulated by metabolism and whose polar transport is not as efficient as that of natural auxins (McCready, 1963). Cell growth

Fig. 7. Epidermal structure of stems treated as shown in Fig. 6. Drawing of nail varnish copies of surfaces as seen with a projection microscope. (A) Intact bud. The cells have a coherent long axis which conforms with the axis of the stem. The structures consisting of two short cells are stomata. (B) A stem that developed without the leaves above it, as in Fig. 6B. Such stems were considerably thinner than the intact controls, but this difference was not reflected in the size of their mature epidermal cells. (C) As in Fig. 6C, with auxin applied laterally and renewed one day later. In the region of auxin application there was a reorientation of growth, resulting in a change of polarity of cell shape. Many cells also grew in girth, and were considerably wider than the cells in the controls shown in A and B. (D) An example of the epidermis in stems treated with large amounts of auxin, as in Fig. 6E. The tissue does not have any one consistent axis of elongation, and thus no clear polarity can be defined by cell shape. Bar, 200 μ m, refers to all drawings.

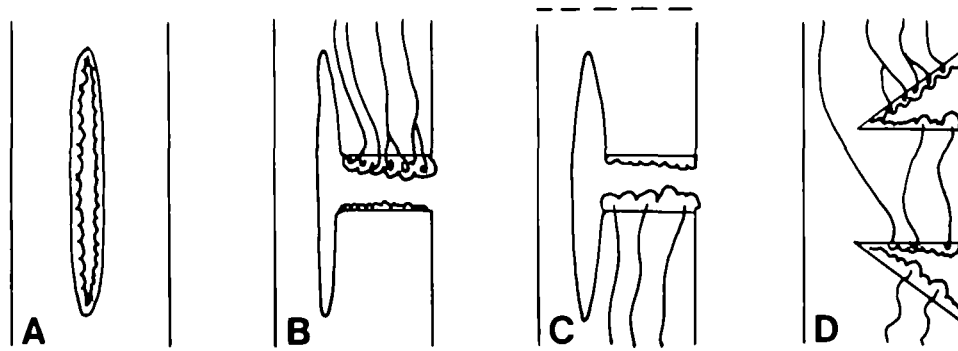


Fig. 8. Polarity and vascular contacts of callus on cut bean stems. Results showing that callus formation is related to the interruption of the polar shoot–root contacts and that in terms of these contacts callus can be of two complementary types. (A) A longitudinal wound, one that does not cut many vascular contacts, leads to the formation of limited amounts of callus. This callus is generally not associated with the formation of new vascular tissues. (B) The same longitudinal wound was accompanied by two additional cuts, leaving tissue flaps pointing towards and away from the roots (as in Fig. 2C). Callus developed primarily on the surface pointing towards the roots. This callus formation was accompanied by the differentiation of new vascular contacts (wavy lines) which connected with the shoots but not the roots. (C) Stem wounded as in B, but the young shoot tissues were removed some distance above the wounded region. The removal of the shoot above reduced the amount of callus formed on the surface which would have connected with this shoot. Callus development was increased, however, on the opposite surface. This callus was also associated with the formation of vascular contacts, but these contacts were always with roots, not the shoots. (D) Two cuts separated a region so that it was not in direct contact with either the shoots or the roots. Limited callus was formed on such isolated, central regions. This callus was associated with weak vascular differentiation, and the strands sometimes ran between the callus on the two complementary surfaces, the ones pointing towards and away from the roots.

with no definable polarity is also the response to ethylene, and ethylene is known to disrupt polar transport (Morgan and Gausman, 1966).

Disrupted organization, indicating the absence of coherent polar flow, is the outstanding characteristic of callus tissues and is thus by no means a rare phenomenon. Experiments performed on the hypocotyls of bean seedlings kept in non-sterile, humid conditions repeat observations that have been made on many plants and show the relation of callus development to the expected flow of polar signals (Fig. 8; Sachs, 1981, 1991). Callus forms on wounded plants, but not all wounds have the same effect. The most pronounced callus growth is in response to wounds that disrupt the polar stream of auxin, and presumably other signals, towards the roots. Furthermore, the most common type of callus formation is enhanced by the presence of young leaves above wounds (Fig. 8), and this effect of leaves could be replaced by an exogenous source of auxin. The importance of polarity is also expressed by the dependence of callus growth on the age of the tissues: wounds made in very young tissues, at the time the cells have a distinct axis but no determined direction (Sachs, 1975, 1989, 1991) do not result in any pronounced callus growth (unless the wound remains unhealed until the tissue becomes polar).

But there is evidence that callus is not only a response to the disruption of the flow of polar signals. Callus, though its influence is weak unless it is tumorous, resembles growing apices in being associated with the polar induction of vascular differentiation (Kirschner *et al.* 1971). The polarity of the contacts of these new vascular tissues are of two types, towards the roots and

towards the shoots (Fig. 8). This suggests that callus forming in contact with shoots but not roots (Fig. 8B) acts as a disorganized root center, or as a sink for the polar flow of auxin. Loss of polarity occurs when cells grow and differentiate towards many local, ill-defined centers which may differentiate as vascular nodules (Fig. 9B). Callus development is less common above wounds, where it is in contact with roots but not shoots (Fig. 8C). The cellular structure of this callus (Fig. 9A) is quite different from its counterpart on the opposite side of the wound: the axes of the cells are more or less parallel with one another and there are no vascular nodules. It is possible that this callus consists of aborted, partially initiated shoot apices and thus of new polarizing centers right below the surface of the callus.

This approach to callus stresses its participation in interactions with the plant as well as its differentiation and partial organization. This view contrasts with the common descriptions of callus as 'undifferentiated', descriptions which are contradicted by microscopic observations and are also inconsistent with differentiation being related to gene expression: callus cells certainly have some and not all genes active and these cells also display a determination, or stability, of various traits associated with gene expression (Meins, 1986). Using this reasoning, callus growing in culture, where a polar flow of auxin or other inductive signals is not expected, may be a complex mixture of different types of cells, whose proportions depend on the constituents of the culture medium.

Discussion

A general picture emerges: a flow of signals, which

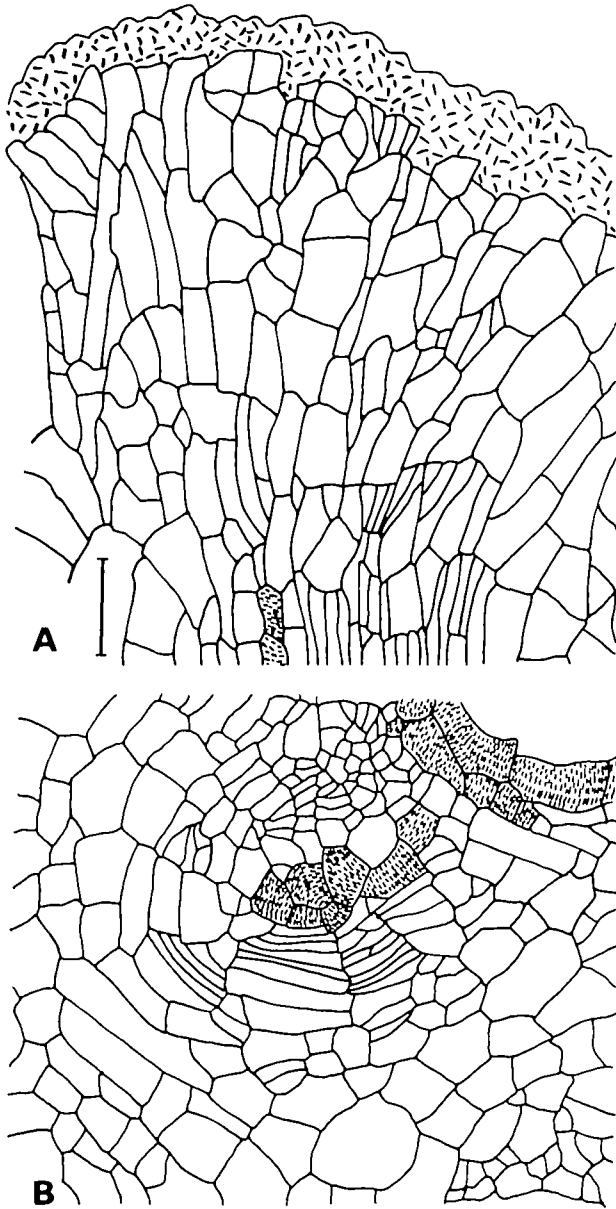


Fig. 9. Cellular details of two types of callus structure found on cut bean stems (as in Fig. 8), indicating that callus can be either a source or a sink of inductive polar signals. Projection microscope drawings of hand cut sections in which the three-dimensional structure could be studied by changing the focus of the microscope. Only two cellular characteristics can be seen, cell shape and the presence of vessel members, which are marked by the short lines representing the bordered pits in their thick walls. (A) Callus on flaps pointing towards the shoots, as in Fig. 8C. This callus has a coherent axis. It includes vascular strands which are relatively straight, and they connect with the direction of the roots, not the shoots. (B) A comparable section through callus on flaps pointing towards the roots, as in Fig. 8B. This callus is distinguished by the absence of a coherent polar structure. The vascular tissues appear in sections as complex nodules. Bar, 100 μ m, refers to both drawings.

include the hormone auxin, moves from the young tissues of the shoots towards the roots. The prominent response to this control system is the differentiation of vascular tissues, but it has additional roles and unexpected traits. It is an important if not major determinant of the shape of cells, and interruptions of the flow of signals are a cause of the formation of callus, or partially organized tissues. The complexity of the reactions to simple signals is due to the gradual differentiation which depends on cell competence, as well as to different cellular responses related to changes of synthesis, transport and use of the same signals. The polar flow thus acts as a major control of various aspects of polarization and patterning, at both the cellular and the tissue level. The signals presumably act on the polar distribution of cellular organelles, especially the microtubules (Schnepf, 1986; Doonan *et al.* 1988; Sylvester *et al.* 1989), but this intracellular aspect of polarization is outside the scope of the present discussion.

This polar control system has a number of characteristics that are unexpected in view of commonly held views concerning cellular interactions and pattern formation. These may be summarized as follows:

(1) The controls suggested here put great stress on the specification of orientation, not only of different types of cell differentiation. The importance of orientation was suggested in early stages of the work on auxin (Czaja, 1935; Ruge, 1937) but has been largely ignored since. Orientation could not be specified only by the concentrations of critical substances; evidence, not reviewed here, strongly indicates that the cells respond to the actual flow of signals, not to their gradients (Sachs, 1981; Sachs and Cohen, 1982).

(2) The determination of cellular polarity is not dependent only on the stability of gene expression. Instead, the stable state of the tissue is due to a positive feedback: signal flow is oriented by polarity and a new polarity can be induced only by changes in this very same signal flow. This involves the same principles, though not the same signals and cellular traits, as the gradual polarization of zygotes by ion channel localization and the electrical fields this localization elicits (Jaffe and Nuccitelli, 1977).

(3) The sensitivity of the cells is not limited to early, critical stages of development. Instead, the cells are subject to continuous control, and vascular differentiation along new axes can even be induced in mature cells, cells which would not have changed in undisturbed tissues.

(4) Varied differentiation processes do not require separate signals. In the examples encountered here, cells which are induced for a short period become cortical parenchyma; longer, continued induction leads to the differentiation of mature vascular elements. Furthermore, cells through which auxin flows differentiate as vascular tissues, while where auxin accumulates new root meristems are formed.

How do cellular patterns arise where they are not a realization of chemical or other pre-patterns? The discussion above does lead to a general suggestion: the cells are active participants in patterning, forming,

transporting and consuming the very signals that control differentiation. These activities of the cells both continue and change throughout most if not the entire time the cells differentiate. The participation of the cells is important because it means that the parameters of patterning need not depend on direct chemical interactions and on the limited distances in which diffusion is effective (Crick, 1970; Wolpert, 1971; Meinhardt, 1982). This participation of the cells could be a cause of cellular patterning in two complementary ways. The first is that patterning could be 'differentiation dependent' (Sachs, 1978b, 1991): it is the activities of the differentiating cells which pattern the distribution of the signals, which in turn determine further differentiation. Thus, in the examples considered here, it is the differentiation of the cells as polarized transporters of auxin which, by a positive feedback between polarized transport and the continued cell polarization, canalizes the auxin flow on which this very same differentiation depends.

A second way by which cell participation could be a source of cellular patterns is 'epigenetic selection' (Sachs, 1988b, 1991): the 'correct' or patterned cells can be selected for continued differentiation from among a larger number of cells which initiate differentiation. This selection could result from the competition between the cells for signals that determine further differentiation, a competition which would increase as the cells differentiate and become better transporters or sinks for limiting signals. Thus, in the example considered above, the cells compete as transporters of the signals from the shoots to the roots. The best transporters – the ones that are along the shortest path, the first to differentiate, etc. – divert the signals away from their neighbors and differentiate as vascular channels. This cell competition, and the resulting selection, is not directly related to evolution; it is an example of 'epigenetic selection' (Sachs, 1988b, 1991), a way of achieving organization in complex developmental systems, where the correction of inevitable mistakes rules out patterning solely by early, strict programs or pre-patterns.

How general are these suggestions? Are they relevant to other cells and to other organisms? Polar transport is known only for auxins in plants. But the discussion above does not depend in any way on transport having a fixed direction. Indeed, even in plants, the details of leaf vein networks indicate that the earliest process is the determination of the axis, but not the direction, of signal transport (Sachs, 1975, 1989). And in general terms what is being suggested is that the cellular properties which require study include the dynamic and directional response of the cells to developmental signals. These responses are likely to be critical in many developmental systems, at least in those that are complex and where the product of development, the mature structure, shows signs of variability rather than a strict, programmed development.

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