

Review

Induction and Fixation of Polarity — Early Steps in Plant Morphogenesis

(polarity induction/polarity fixation/microtubules/plants/tropism)

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1. Introduction

Specific and complex forms of plants and animals emerge from simple, very often symmetric, origins. This process includes a chain of basic events whereby two poles develop along an originally more or less homogeneous axis. Since the eighteenth century such phenomena are referred to by the term “polarity”. This concept simply designates the specific orientation of activity in space and involves no assumptions whatsoever as to its causes (112). For example, growth gradients (41), the spatial order of cell divisions (28), directed transport of hormones across tissue (91, 103, 104, 105) and structural or physiological differences between two ends of a cell (95) have been described using this concept. The concept of polarity is widely employed not only in theoretical and experimental studies (32), but also in horticulture and agriculture (93). Polarity in animals has been discussed extensively (23, 30, 36, 66, 69, 72, 106, 113, 122, 128). Polarity in plants has been described in several reviews (9, 16, 54, 95, 103, 104, 105, 112).

Despite the accumulation of a considerable amount of descriptive evidence, the molecular and cellular mechanisms responsible for induction and fixation of polarity have remained obscure. In recent years, powerful molecular techniques have been applied with increasing frequency in conjunction with the concepts established by classical developmental biologists. At this stage, a review of the classical concepts of polarity may be useful for the design of future research. If we are to be

able to survey the multitude of phenomena associated with polarity, a formal classification of polarity is a necessary first step: We must ask, how are the poles defined? How is a clear output guaranteed under conditions of high informational noise? To what extent does polarity really evolve *de novo* and what is the role of external information? At what level of complexity is polarity encribed into the organism? To what extent is the formation of polarity reversible? How stable is polarity? These are questions, to which, with our present knowledge, we are still unable to provide a more than a merely phenomenological answer. Nevertheless, such phenomenological answers might help us to identify the best questions to be asked.

2. Brief synopsis of the induction of polarity in animals

To construct a conceptual framework, it is worth beginning with a brief consideration of the induction of polarity in non-plant organisms.

A. Simple or complex polarity?

In cases of polarity in animal systems, one pole might simply lack a property (Fig. 1), that is present at the other pole (**simple polarity**), or both poles could be defined positively by a specific property (**complex polarity**). Transplantation and fusion experiments have shown two antiparallel subpolarities for **animal-vegetative polarity** of sea urchin eggs (11, 47, 118) and **amphibian** embryos



**Simple
Polarity**

**Complex
Polarity**

Fig. 1. Simple versus complex polarity.

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(33, 46, 121), for *prestalk-prespore polarity* in *Dictyostelium* (127), and for head-foot polarity in *Hydra* (10). In *Dictyostelium* (127), as well as in *Hydra* (44, 75, 108), the substances involved in the induction of the subpolarities have been identified. Genetic analyses of *anteroposterior* and *dorsoventral polarity* in *Drosophila* have revealed *complex polarities* (2, 25, 26, 50, 62, 87). However, the ventral and the anterior poles appear to be necessary for the correct establishment of the dorsal and the posterior poles. The dorsal and posterior poles, on the other hand, are not required for the correct establishment of the ventral and the anterior poles.

B. Graded or all-or-none polarity?

The difference between the poles (Fig. 2) could be merely quantitative (*graded polarity*) but it could also be qualitative (*all-or-none polarity*). Gradients are usually found at the early stages of establishment of polarity. Thus, foot and head activators in *Hydra* (10, 108), the morphogens involved in *prestalk-prespore polarity* in *Dictyostelium* (127) and the products of maternal polarity genes in *Drosophila* (2, 26, 62) are distributed in gradients along the axis of polarity. However, all-or-none polarities often prevail in establishment of the final outcome. In *Hydra* (10), *Drosophila* (88), *Dictyostelium* (127) and the sea urchin embryo (22) the final organisms look fairly normal even after artificial imbalance is introduced at early stages. In *Hydra*, for instance, "partial heads" or "partial feet" do not occur. However, the frequency, with which a head or a foot is formed depends on the initial gradients (10). In such cases, so-called *proportion regulation* is observed, i.e., the natural proportions are established even for severely imbalanced initial states. This phenomenon implies a kind of decision-making mechanism that transforms gradients of precursors into all-or-none polarities.

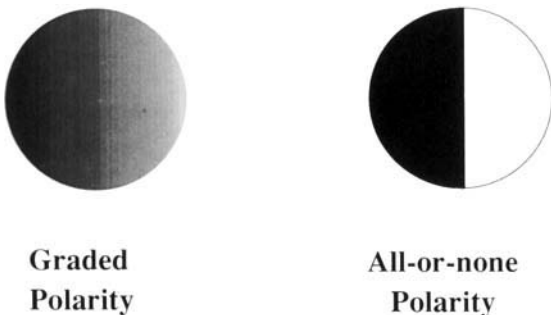


Fig. 2. Graded versus all-or-none polarity.

C. Establishment of polarity: integration, redistribution or inheritance?

Polarity simultaneously implies both unity and difference and there are two principal ways, by which this dichotomy can be produced (34): Either the poles are initially isolated (Fig. 3) and are later linked by long-range signals (*polarity by integration*) or the unity precedes the difference, i.e., polarity appears as a result of the redistribution of a uniformly distributed agent (*polarity by redistribution*). *Polarity by integration* is found in *Hydra*, where locally restricted autocatalytic processes in combination with long-range lateral inhibition bring about *foot-head polarity* (30, 31). The long-range interactions do not necessarily rely on actual inhibitors-simple competition of local sites for limiting resources can fulfil the same function, as seen in the establishment of *prestalk-prespore polarity* in *Dictyostelium* (127). In many cases, however, polarity is not really induced *de novo*, but derives from a maternal polarity (*polarity by inheritance*). The *anteroposterior* and *dorsoventral polarities* of the *Drosophila* embryo depend on maternal mRNAs, which are deposited during oogenesis by the follicle of the mother (1, 2, 67). The polarity of the egg is, therefore, based upon the polarity of the follicle. This conclusion is supported by the

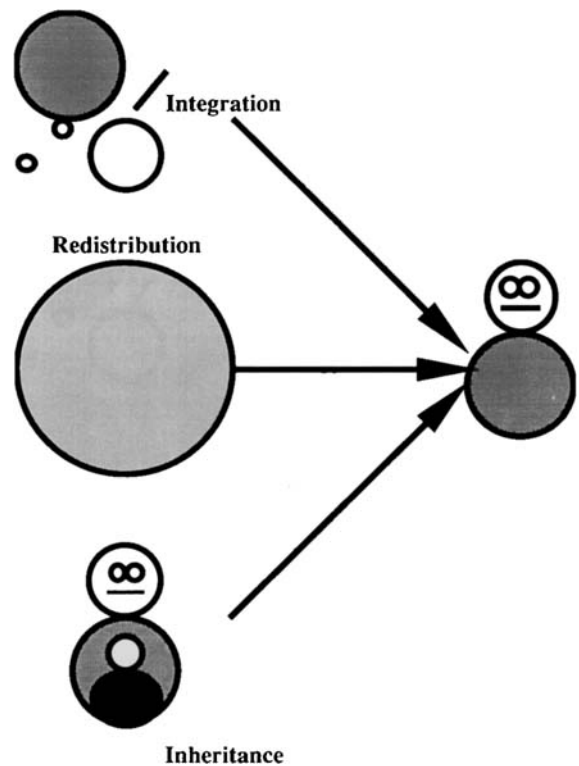


Fig. 3. Principal models for the establishment of polarity.

effects of mutations that influence follicle shape: they also interfere with the dorsoventral polarity of the embryo (2). Impressive examples of **polarity by inheritance** can be found in unicellular organisms, for example the **flagella-stalk polarity** in *Caulobacter crescentus* (17, 110, 111) or the dorsoventral polarity of *Paramecium* (58). In these cases, during each cell cycle, one pole is regenerated in what could be described as the "semiconservative replication" of polarity.

D. Tissue polarity, cell polarity or both?

Polarity can arise from a gradient across the tissue (Fig. 4), even if individual cells are not polar (**tissue polarity**). Alternatively, the cells may be polar but there may be no asymmetry at the level of the whole tissue (**cell polarity**). Eventually, polar cells may become arranged in a polar fashion along the tissue. In multicellular nonplant organisms, **tissue polarity** appears to prevail: the determinants of **anteroposterior polarity** in the *Drosophila* oocyte are deposited in the poles in the form of

mRNA (65) and, after translation, the corresponding proteins, spread in gradients along the long axis of the embryo. Secondary genes then become activated, depending on the levels of constituents of the primary gradients, with a resultant coarse subdivision of the embryo even before cellularization occurs (67, 87, 88). These areas interact sequentially with one another and cause an increasingly precise determination, down to stripes of only one cell-width (51). In the end, the cells themselves become polar, as shown by transplantation experiments with inverse transplants (69, 70). However, **cell polarity** is secondary and derives from tissue polarity in the manner of a **harmonic-equipotential system** (23). Progressive determination of the Bauplan down to the level of individual cells by intercellular interactions seems to be typical of animal development as found in amphibia (47, 113, 116), nematodes (129), *Drosophila* (72, 97), sea urchins (76), *Hydra* (10), and *Dictyostelium* (127).

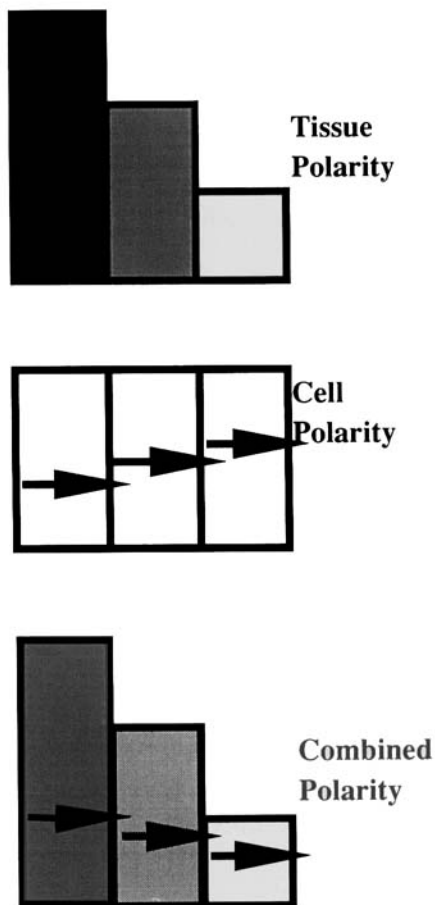


Fig. 4. Classification of polarity with respect to the element that is polar.

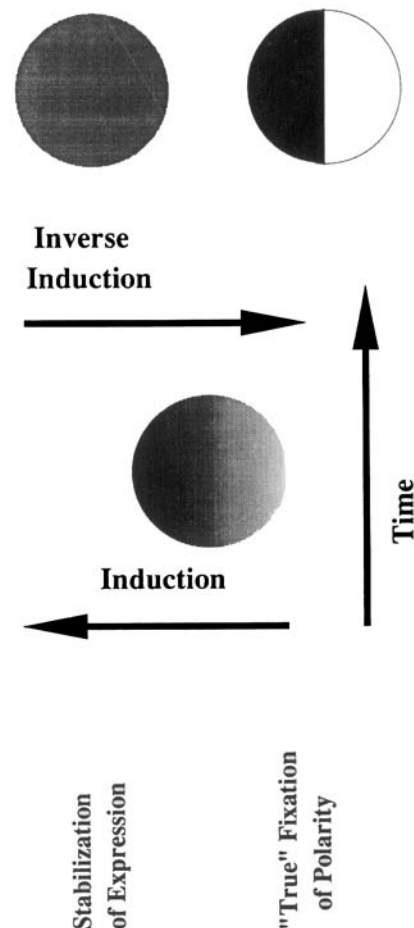


Fig. 5. Stabilisation of the expression of polarity versus the true fixation of polarity.

E. Fixation of polarity

After polarity has been established, it causes developmental changes that can be long-lasting or even irreversible. One might say that the manifestation of polarity has become stable (Fig. 5). However, polarity could be already fixed before it is expressed in a visible manner. In fact, in the *Drosophila* embryo (2) and in amphibian eggs (29) **dorsoventral polarity** becomes stable before it is expressed. If eggs of *Xenopus* are tilted just at the time when **polarity fixation** occurs, embryos with two dorsal sides result. Establishment and fixation of **dorsoventral polarity** appears to rely on microtubules in the cortical plasma (24). A role for the cytoskeleton in **polarity fixation** has also been suggested for **odontoblast polarity** in the mouse embryo (63), which depends on the presence of an actin-binding 169-kDa transmembrane protein.

3. Induction of polarity in plants

Transplantation experiments have provided a useful approach to studies of the polarity in animals. However, they are difficult to carry out in plants because the cell walls are rigid and the vacuoles are large (3). Why is it then worth investigating plant polarity at all? There are two main answers to this question: (i) plant cells are often omnipotent and able to regenerate a whole organism (114), and it is certainly worth asking what happens to cell polarity during this process; and (ii) plants have to tune their morphogenesis to conform to their environment, - thus, induction of polarity must be linked to transduction of external stimuli.

The induction of polarity in plants has been studied extensively in the following two systems: (A) **Thallus-rhizoid polarity** in phaeophycean zygotes and pteridophycean spores (89, 95, 99, 100, 101), and (B) **Shoot-root polarity** in higher plants (32, 102, 104, 120).

A. Induction of cell polarity: *Thallus-rhizoid polarity*

In the zygote of *Fucus*, a colourless rhizoid appears at one pole and the plastids migrate into the opposite thallus pole. The direction of this polarity can be influenced by a range of environmental factors, such as light, temperature, an electric field or a chemical gradient (101, 124). The effects of light are due to as yet unidentified blue-light receptors (7, 40). However, in certain species of ferns and mosses, induction of polarity

can be triggered by a membrane-bound form of the biliprotein phytochrome, which absorbs in the red region of the visible spectrum (56). The environmental gradients that induce polarity can be very small and the system is fairly resistant to noise, suggesting extensive signal-amplification mechanisms (30, 39). Sensitivity can become so high that, even in a putatively symmetric environment, stochastic fluctuations can act as polarizing stimuli (8). As demonstrated with the spores of *Equisetum*, a variation in the strength of the inducing gradient does not affect the expression of polarity in the individual cell. However, it affects the frequency, within the population, with which polarity is aligned correctly (39). This phenomenon resembles the situation in *Hydra* and is consistent with the idea of an **all-or-none polarity**. Irradiation with strong polarized light can cause doubling of the rhizoid pole (53), which suggests that polarity is induced *de novo* and is not inherited. The mechanism of induction of polarity has been partially clarified for the *Pelvetia* zygote (99, 100). Polarity develops parallel to gradients of calcium, sodium and potassium ions, which were measured by a very elegant technique (57): the cells were sucked onto a fine nickel mesh and, thus, served as insulators of the chambers above and below the mesh. The ionic composition of the chambers could be changed and currents measured with a vibration electrode. The future rhizoid pole exhibited an increased influx of calcium ions into the cell, whereas the opposite pole was characterized by a decreased influx of calcium ions. For stimulation by an external gradient of calcium ions, these data imply redistribution of calcium pumps towards the site at which the external concentration of calcium ions is minimal (i.e., the future rhizoid pole). Similar autocatalytic "self-electrophoresis" (55) was found in green algae and mosses (124) and it appears to be a general requirement for induction of polarity by various environmental stimuli (98). Thus, **thallus-rhizoid polarity** evolves by redistribution. How it is transduced into the final form and whether and how it is fixed, remain questions that must be answered. Polar transport of ribosomes (94), polar activation of calmodulin and, as a consequence, the polar phosphorylation of proteins (13) and directed changes in the cytoskeleton (60) are some of the phenomena that have been discussed in this context.

B. Induction of polarity in multicellular plants: Shoot-root polarity

Because of the horticultural importance of polarity in grafting (93), polarity was already the focus of scientific interest many years ago. Polarity was detected as a basoapical gradient in the capacity of root stocks to regenerate adventitious shoots. In addition, there is an antiparallel gradient in the capacity of the scion to regenerate adventitious roots (32). The earliest theory on **shoot-root polarity** was formulated by Marquise Duhamel du Monceau in the eighteenth century. He postulated the existence of two morphogenetic factors, a heavy "root sap" and a light "shoot sap", which were directed by gravity towards the respective poles, accumulated there and triggered the formation of roots and shoots, respectively (74). In fact, the existence of such morphogenetic factors and their transport in the phloem was demonstrated in elegant incision experiments (35). The question then arose as to whether this polarity could be oriented by gravity. Early experiments with inverted sunflower seedlings by gravity. Early experiments with inverted sunflower seedlings yielded positive results (113), but it was not possible to reverse polarity in adult plants. In a famous experiment, an inverted segment of a *Salix* branch regenerated roots and shoots according to the original polarity, and not according to its actual orientation (120). By contrast, in rhizome cuttings of *Cordyline*, incubated horizontally, a new polarity was inducible perpendicular to the original polarity, although the latter still persisted and, as a result, a quatripolar situation developed (32). In the siphonal alga *Caulerpa*, polarity could be inverted easily even by light (86). Depending on their experimental material, researchers either favoured the hypothesis of Sachs (102) that polarity is induced *de novo* or the opposing view, formulated by Vöchting (120), that polarity is inherited and stable. A synthesis was attempted by Goebel (32), who showed, in elaborate cutting and regeneration studies, that an apicobasal flux of an unknown substance defines **shoot-root polarity**. If this flux is interrupted or inverted, locally restricted inversion of polarity can occur. This flux, in recent years, has been shown to be that of auxin (103, 104, 105). Vessel regeneration in *Coleus* stems, as directed by **shoot-root polarity**, was manipulated in a predictable way by a series of ingenious experiments in which incision was combined with local application of auxin (104, 105). The mechanism of induction, again, involves autocatalytic cycles: if within an initially homogeneous

distribution of auxin across the (still parenchymatic) tissue, the auxin flux is increased locally (for instance, by blocking other drainage paths), the increase leads to accelerated differentiation of vessels at this site. Since those developing vessels can already transport more auxin per unit time, they will deplete the neighbouring areas of auxin. A few vessels will form and mutually compete for auxin. With time, the vessels differentiate progressively, cortical microtubules are arranged in transverse rings and, eventually, lignification leads to some kind of stabilisation (104, 105). Strictly speaking, it is the expression of polarity that becomes stable, not the polarity itself (103). Separation of the two phenomena was emphasized by Vöchting and illustrated by an experiment with dandelion roots (Fig. 6): a root segment was buried upside-down after the shoot pole had been sealed with resin. Under these circumstances, adventitious shoots formed at the (now upper) root pole. This formation of shoots continued for several months, and the original polarity seemed to be reversed because shoots emerged preferentially at the former root pole. However, when a segment was cut from this inverted plant and buried without sealing of the poles, the original polarity was clearly expressed and adventitious shoots emerged exclusively at the original shoot pole. Thus, the original polarity persisted unaltered even though its manifestation had remained latent for several months.

A precursor of **shoot-root polarity** can be detected already in the zygote: The zygote displays a distinct **cell polarity** with a vacuolar pole at the micropylar site (the future suspensor or root pole) and an opposed cytoplasmic pole (114). It appears that the cytoplasmic pole exerts an inhibitory effect upon the suspensor pole, which can be removed by microsurgery or loss-of-function mutations (71). Differentiation is initiated from the cytoplasmic, embryonic pole (73, 96, 109, 114, 123). It is unknown, whether or not the maternal tissue imprints its polarity upon the embryo sack. Maternal mRNAs, as in case of the *Drosophila* embryo, appear, however, to play a minor role (71). The role of auxin fluxes in the early establishment of **shoot-root polarity** remains to be defined. It is clear, however, that the apical meristem does not have the same impact on embryonic polarity as it has during postembryonic development (114). In the vegetative shoot, the polarity, although maintained by auxin fluxes, originates from continuous and unequal cell divisions in the meristem initials (114). This process can be disturbed by simple

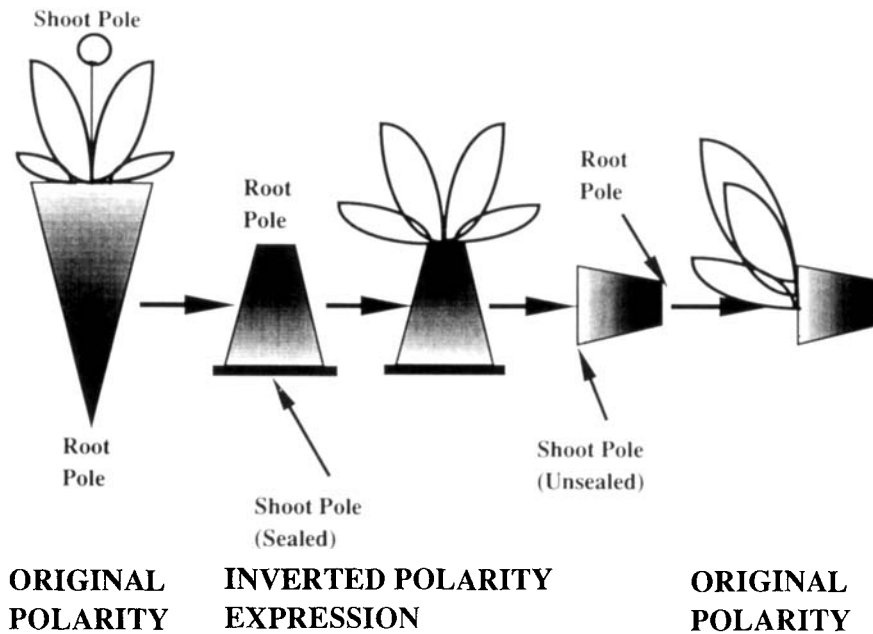


Fig. 6. Schematic representation of Vöchting's experiment with *Taraxacum*.

treatments that lead to symmetric divisions of meristem cells (5). Nevertheless, the basis of polarity in meristem cells (which is the ultimate cause of *shoot-root polarity*) remains obscure. From this initial cell polarity, tissue polarity emerges as a secondary phenomenon, as can be concluded from the capacity of apical fragments for conspicuous *proportion regulation* (4, 92, 115). In these respect, their behaviour resembles that of split amphibian embryos (113). Interactions between different apical regions eventually determine the plant pattern, as shown by microsurgery (64, 92, 114) and the analysis of pattern mutants (59, 68, 71). Thus, determination proceeds from the cellular to the tissue level, which, in a sense, is the mirror image of the *harmonic-equipotential system* in animals (22).

4. Polarity and tropism

Polarity in the unicellular zygote of *Fucus* can be manipulated easily by environmental factors. *Shoot-root polarity* of higher plants is not as readily accessible. However, it does occur in multicellular organisms in which intercellular interactions can be studied. Combining the advantages of both types of systems is warranted. Although they have not been on the mainstream of research on plant morphogenesis, photo- and gravitropism provide appropriate systems with which to examine such a combination. In fact, tropism and the

induction of polarity share many characteristic traits: autocatalytic signal amplification (42), efficient elimination of stochastic noise (30, 42), induction by as yet unidentified blue-light receptors (7, 21, 40) and the importance of auxin (91, 103, 104, 105). Thus, it is not astonishing that polarity has been described as a tropistic signal chain which is extended by a fixation step (42). Moreover, tropism, as well as the induction of polarity, cannot be explained in terms of responses of isolated cells, but they imply interactions between cells. This "holistic" nature of tropism has been demonstrated repeatedly, for example, in balancing experiments with the sporangiophore of *Phycomyces* (27), by the observation that auxin and growth are redistributed across photo- or gravitropically stimulated grass coleoptiles (14, 19, 48, 49, 90, 91, 125, 126), by the failure to induce a red-light tropism in coleoptiles, even though a gradient of red light is detected by the plant (45), and by the finding that the coleoptile recognizes the direction of light by measuring the gradient of light across the tissue (15, 48, 61). Phototropism appears to induce a *tissue polarity* (15), which is then expressed as the transverse transport of auxin (19, 91, 125, 126). This phenomenon is remarkable because gravitropism must derive from some sort of *cell polarity* (77). The gradient of gravitational acceleration across the coleoptile is certainly too small to be sensed. As for *shoot-root polarity* (32, 120) tropistic polarity can be separated from

its expression as curvature: if curvature is suppressed by cold treatment (20), decapitation (12) or by specific inhibitors of plasma membrane ion-pumps (37, 38), a long-lasting memory of the experienced tropistic stimulation is, nevertheless, retained. This tropistic "mneme" (12, 20) or "memory" (37, 38) persists for many hours and is expressed readily as curvature if the suppression is removed. The similarity to the experiment with segments of dandelion roots (120) is evident (Fig. 6).

In maize coleoptiles, such a spatial "memory" can be induced even by a unilateral pulse of blue light and be rendered visible even ten hours later as strong and stable curvature in the direction of the light pulse by transferring the plants to a horizontal clinostat (such that gravity acts symmetrically). This light-induced stable transverse polarity derives from a labile precursor and is fixed about two hours after induction (78, 85). It is expressed in an all-or-none fashion. Incomplete polarity could not be produced even by varying the strength of the applied stimuli (85). By analogy to *head-foot polarity* in *Hydra* (10) and *thallus-rhizoid polarity* in *Fucus* (37) variations in the strength of the inducing gradients are mirrored by the frequency of plants where stable polarity is manifest. Although *transverse polarity* is of the all-or-none type, its early precursors can be shown to be distributed in a gradient (85). It should be mentioned that a similar polarity can be induced by gravity (78) and is expressed by an all-or-none pattern as well (82). However, the respective transduction chain is clearly separate from the blue-light-induced *transverse polarity* (43, 82, 119). By analogy to the double rhizoids in *Fucus* (53), a stable symmetry could be induced if the opposing light pulse was given exactly at the time at which *polarity fixation* takes place (78, 85). This observation indicates the *de novo* induction of blue-light-induced *transverse polarity*, consistent with the old theory of Sachs (102). It should be mentioned, however, that *inheritance of polarity* according to Vöchting (120) exists in the case of the dorsoventral polarity that causes nastic curvature (79), and it interacts with transverse polarity in an all-or-none fashion. Although triggered by blue light, *transverse polarity* relies on a signal chain, which is clearly separate from events that mediate phototropism, as shown by time-course and fluence-response studies (85). The separation occurs before the evolution of the phototropic *tissue polarity* (82, 85).

Cortical microtubules in the outer epidermis respond to light and gravity with a resultant orienta-

tion gradient across the coleoptile (81). Although correlated in terms of timing and direction with tropistic bending, the phenomena can be distinguished by the means of microtubule-eliminating drugs (83) and rotation on a clinostat (84). However, microtubules exhibit a much closer involvement in the light-induced transverse polarity (80), and a fixation of the orientation of microtubules occurs about two hours after a unilateral blue-light pulse, at the same time as the fixation of *transverse polarity*. This fixation is brought about by a loss of microtubule motility and extends to both poles. Thus, both poles of transverse polarity are positively defined (*complex polarity*). Intact actin microfilaments appear to be essential for *polarity fixation* (80). A conspicuous cell polarity can be observed in the case of reorientation of microtubules, since only microtubules adjacent to the outer epidermal wall appear to be responsive (52, 81). The significance of a *cell polarity* is evident from the fact that both gravity- and blue-light-induced transverse polarities can be reversed by symmetric irradiation with blue light (85, 107).

5. Summary and prospects

Our knowledge of the induction of polarity in plants, although still fragmentary, already allows us to make certain generalizations:

1. There is a significant body of evidence for extensive signal amplification that leads to all-or-none decisions. Where the underlying mechanism could be uncovered, locally restricted autocatalytic processes, in combination with long-ranging inhibitory interactions, have been found to be important. In contrast to the situation in animals, this long-ranging inhibition is produced by mutual competition for limiting resources rather than by actual inhibitors.
2. Whereas polarity in animals seems to be complex for the most part, the situation in plants is less unequivocal: *thallus-rhizoid polarity* in *Fucus* appears to be simple; light-induced *transverse polarity* in maize coleoptiles is complex. However, the graminean coleoptile might be a special case because its lifespan is determined and cell divisions do not occur during later phases. Usually, plants are characterized by "open" organization that involves a high degree of flexibility. This requirement might be more easily met by a *simple polarity*, which is less difficult to reorient.
3. *Polarity fixation* appears to occur concomitantly with (*shoot-root polarity*) or later (*light-induced transverse polarity*) than the expression of polarity.

It is possible, however, to distinguish between expression and fixation by preventing the expression of polarity and restoring it after some time with an examination of whether or not the underlying polarity had remained stable. A role for the cytoskeleton, and for microtubules in particular, in the expression of polarity and its fixation has been assumed for both *thallus-rhizoid polarity* and *shoot-root polarity*. Such a role could be demonstrated in the light-induced *transverse polarity* of maize coleoptiles. Thus, stimulus-induced reorientations of microtubules and their membrane interactions play an important role.

Although some formal characterization of the induction of polarity and its fixation in plants appears to be possible, most of the mechanisms involved remain obscure. It is not known how some of the inducing stimuli are perceived, nor are the signals known by which the individual cells communicate in order to bring about an ordered response. The cytoskeleton is very likely to be a mediator for polarity. At least it can serve as a marker of cell polarity. However, it is still unclear by what mechanisms the cytoskeleton can be restructured, how such structural changes are directed, and how the cytoskeleton interacts with the plasma membrane. Many of these questions were, in fact, already posed a long time ago, when they could not be answered, because the necessary tools were not available. Some of these tools are now provided as a result of recent achievements in molecular biology and biochemistry. A successful combination of old questions and new tools must take into account the fact that polarity is based on spatial order. Thus, assays *in situ*, capable of distinguishing the responses of individual cells are warranted.

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