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Buder revisited: cell and organ polarity during phototropism*

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ABSTRACT

The induction of a radial polarity by environmental stimuli was studied at the cellular and organ levels, with phototropism chosen as a model. The light gradient acting on the whole coleoptile was opposed to the light direction acting upon individual cells in the classical Buder experiment, irradiating from the inside out. Alternatively, the stimulus was administered to the coleoptile tip with a microbeam-irradiation device. Tropistic curvature was assayed as a marker for the response of the whole organ, whereas cell elongation and the orientation of cortical microtubules were taken as markers for the responses of individual cells. Upon tip irradiation, signals much faster than basipetal auxin transport migrate towards the base. The data are discussed in terms of an organ polarity that is the primary result of the asymmetric light signal and affects, in a second step, an endogenous radial polarity of epidermal cells.

Key-words: *Zea mays* L.; Poaceae; maize; microtubules; phototropism.

INTRODUCTION

One of the fastest manifestations of polarity induction in higher plants is the tropistic response. The tropism of coleoptiles, for instance, can be observed as early as 20 min after induction by a brief pulse of asymmetric blue light (Iino 1991). It is interesting to ask whether tropistic polarity is a function of tissue polarity or cell polarity. This question might appear fairly academic, but it addresses a basic problem of plant development.

Cell polarity can be explained in terms of autonomous responses of individual cells not relying upon intercellular communication. In contrast, such intercellular communication is likely to participate in the establishment of true tissue polarity. It is in principle possible, though, to obtain tissue polarity without any interaction between individual cells – when each cell simply responds exclusively to the locally perceived quantity of the inducing stimulus (Nick & Furuya 1992). In this case, the inducing environmental gradient should result in a corresponding gradient of individual cell responses causing tissue polarity.

Such a model has been invoked by Blaauw (1918) to explain phototropism, but later it was shown independently by Cholodny (1927) and Went (1928) that the cells in the two flanks of a phototropically stimulated coleoptile somehow sense the strength of stimulation in the opposing flank. They demonstrated a growth-stimulating factor, auxin, that was transported across the tissue from the lighted to the shaded flank of the coleoptile. The Cholodny-Went theory describes this redistribution of auxin across the tissue and has been supported by a wealth of data from experiments tracing the lateral transport of radioactively labelled auxins (for a review see Pickard 1985). Moreover, phototropic stimulation leads to a redistribution of growth from the lighted to the shaded coleoptile flank (Iino & Briggs 1984), and a redistribution of a factor interfering with the orientation of cortical microtubules (Nick, Schäfer & Furuya 1992).

These data imply some kind of intercellular communication during the establishment of the transverse polarity induced by phototropic stimulation and clearly contradict the cell-autonomous model proposed by Blaauw (1918). However, they do not allow any conclusion to be drawn about the way in which the plant is able to sense the direction of the stimulus. It is still possible that each cell is able to recognize the direction of light individually and responds individually by producing a transverse cell polarity. This cell polarity would then determine the direction of auxin efflux (Pickard 1985; Sachs 1991). The tissue response described by the Cholodny-Went theory would then arise only at that stage by mere summation of individual cell responses. Alternatively, the gradient of light might be recognized by the cell population as a whole, and a true tissue polarity might emerge from intercellular signalling as proposed by Gierer (1981) by locally self-amplifying activation in concert with far-ranging mutual inhibition. This tissue polarity would then induce a parallel cell polarity leading to transverse auxin transport.

A debate between Heilbronn (1917) and Buder (1920) some 70 years ago contributes to this problem. Heilbronn claimed that the plant perceives the direction of the light. Buder, in contrast, insisted on the gradient of light as the signal to be perceived by the coleoptile. This dispute culminated in an ingenious experiment by Buder (1920), in which the gradient of light and light direction were

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**Dedicated to Prof. Dr E. Schäfer on the occasion of his 50th birthday.*

opposed to each other. To achieve this, he irradiated the coleoptile from inside out using a prototype of a light-piping device. He was able to demonstrate bending of the coleoptiles towards the lighted flank, although the direction of the incident light should have induced bending in the opposite direction. This result, in the context of the Cholodny-Went theory, clearly demonstrates that individual cells must communicate with each other in order to sense the asymmetry of the stimulus. This result was confirmed later by perpendicular irradiation of coleoptile tips, whereby one half of the tip was screened. Bending was observed in the plane of the screen towards the lighted side, i.e. perpendicular to the direction of the light (Meyer 1969).

These experiments demonstrated clearly that the direction of the light is sensed as a gradient of light across the tissue. However, they do not exclude the possibility that individual cells, in addition, are able to sense the light direction directly (i.e. intracellular light gradients), because tropistic bending is not an appropriate assay for cell polarity. In the meantime, it has become possible to assay not only the phototropic response at the level of the whole organ, by analysing curvature, but also the polarity of individual cells in the tissue using the cytoskeleton of epidermal cells – where coleoptile growth seems to be controlled (Kutschera, Bergfeld & Schopfer 1987) – as a marker. Cortical microtubules can be visualized by means of immunofluorescence labelling. Following phototropic induction, longitudinal microtubules prevail in the lighted flank of the coleoptile, whereas the microtubules in the shaded side are found to be transverse (Nick & Schäfer 1994). The orientation of cortical microtubules can control the deposition of cellulose microfibrils in the same direction and this determines the axis of cell expansion (Green 1980; Giddings & Staehelin 1991). This gradient in the orientation of microtubules becomes detectable from 10 min after induction and is irreversibly fixed within 2 h. It is correlated with a stable directional memory (Nick & Schäfer 1994). This directional memory can be expressed as asymmetric growth under permissive conditions, but persists even if its expression is prevented by gravitropic counterstimulation (Nick & Schäfer 1988) or low temperature (Nick & Schäfer 1991). The microtubules mark a distinct and probably inherited cell polarity in so far as it seems that only the microtubules adjacent to the outer epidermal cell wall are capable of reorientation, whereas the microtubules at the inner cell wall are reluctant to give up their transverse orientation (Nick *et al.* 1990). The combination of immunofluorescence labelling of cortical microtubules as markers for cell polarity with the set-up of the Buder experiment should yield some insight into the question of how plants establish tissue polarity, and how this tissue polarity possibly interacts with inherited cell polarities. To detect directly the cellular cross-talk inferred from the studies described above, this approach is complemented by direct observation of cell growth in response to microbeam irradiation.

MATERIAL AND METHODS

Plants and light conditions

Seedlings of maize (*Zea mays* L. cv. Percival; Asgrow, Bruchsal, Germany) were raised under red light (0·2 W m^{-2}) for 2 d at 25 °C and then kept in darkness for one further day. This treatment produces plants with straight coleoptiles, because elongation and nutations of the mesocotyl are suppressed in red light (Kunzelmann, Iino & Schäfer 1988). All seedlings were kept under a saturating background of red light (2·5 W m^{-2}) from about 5 min prior to phototropic induction to suppress possible effects of phytochrome gradients induced by the phototropic stimulation (Hofmann & Schäfer 1987). Seedlings were selected twice for straightness and length prior to the experiment (Nick & Schäfer 1988).

Stimulation treatment

Straight coleoptiles were carefully excised from the node, such that the primary leaves remained on the node (Fig.1a). The coleoptile was then placed in a strictly upright position on micropipette tips that had been glued to the bottom of a water tank. The tank was filled with water, such that the cut surface of the coleoptiles was imbibed (Fig.1b). Under these conditions, the coleoptiles were capable of normal elongation for up to several hours (Fig.1c). Phototropic induction was administered using a light-piping device (Flexilux 150 HL; Schölly Fiberoptik, Denzlingen, Germany) either from outside (Fig.1d) or from inside (Fig.1e) the coleoptile. After phototropic induction, the coleoptiles were returned to the water tank and remained there for 2 h, until the maximum curvature was reached (Kunzelmann, Iino & Schäfer 1988). Broadband blue light

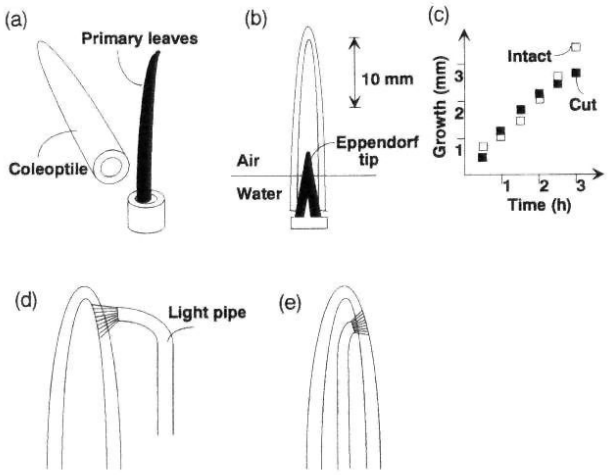

Figure 1. Experimental protocol for the Buder experiment. (a) Preparation of excised coleoptiles. (b) Position of the excised coleoptiles after induction in the incubation tank. (c) Growth of intact (white squares) and excised (black squares) coleoptiles in the incubation tank. (d) Phototropic induction from outside. (e) Phototropic induction from inside (Buder experiment).

(λ_{max} 450 nm, T_{max} 25%, halfband width 25 nm) was isolated through blue plastic (Schott; Mainz, Germany), and measured using a radiophotometer (YSI model 65 A, Yellow Springs Instrument Co.; Yellow Springs, USA). To estimate light scattering across the coleoptile, photographs were taken and the blackening quantified by means of a gel scanner (Shimadzu S65; Tokyo, Japan). Phototropic curvature was determined using a simple xerographic method (Nick & Schäfer 1988).

Microtubule staining

Two hours after phototropic stimulation, the side facing the inducing pulse was marked by an incision, and then subapical segments (2–12 mm below the tip) were excised and fixed for 45 min at room temperature in 3·7% (w/v) paraformaldehyde in microtubule-stabilizing buffer (0·1 kmol m^{-3} 1,4 piperazine-diethanesulfonic acid, 1 mol m^{-3} $MgCl_2$, 5 mol m^{-3} ethylene-bis-(β-aminomethyl-ether)-N,N,N',N' tetraacetic acid, 0·25% v/v Triton X100 and 1% v/v glycerol, pH 6·9). Tangential sections were cut separately for each side of the coleoptile using a Vibratome (Vibroslice VSL, World Precision Instruments, Inc.; Sarasota, USA) at 120 μm thickness. Sections were mounted on coverslips with the outer side of the epidermis facing upwards and then glued in place using a small drop of 1·2% (w/v) agar molten in microtubule-stabilizing buffer without paraformaldehyde. Sections were then incubated for 20 min at room temperature with goat normal serum (Sigma; Neu-Ulm, Germany) diluted 1:20 in Tris-buffered saline (20 mol m^{-3} Tris-HCl, 150 mol m^{-3} NaCl and 0·25% v/v Triton X100) and then treated for 1 h at 37 °C with mouse monoclonal antibodies against α-tubulin and β-tubulin (Amersham; Braunschweig, Germany) diluted 1:1000 in Tris-buffered saline. The sections were washed three times for 5 min in Tris-buffered saline and then incubated for 45 min at 37 °C with a fluorescein-isothiocyanate-labelled secondary antibody (anti-mouse immunoglobulin G from goat, diluted 1:20 in Tris-buffered saline; Sigma, Neu-Ulm, Germany). They were thoroughly washed again with Tris-buffered saline and then mounted in moviol containing 0·1% w/v p-phenylene diamine (Wako Ltd.; Tokyo, Japan). Microtubules were viewed on a confocal laser microscope (DM RBE, Leitz; Bensheim, Germany) using an Argon-Krypton laser at 488 nm excitation, a beam splitter at 510 nm and a 515 nm emission filter. Microtubule orientation at the outer and the inner sides of the cell was recorded with respect to the longer axis of the cell (Nick & Schäfer 1994).

Microbeam irradiation

The system for microbeam illumination was developed in collaboration with Olympus Optical Co. (Tokyo, Japan) and has been described in detail in Schmidt *et al.* (1990). Irradiation took place five cells down the coleoptile tip using a spot size of 2·5 μm, resulting in the illumination of about five cells. Blue light was isolated by means of an interference filter (λ_{max} 450 nm, T_{max} 78%, halfband width 32 nm, no. 8910–1113 13 P77, Olympus; Tokyo, Japan). Fluence was varied by changing the irradiation time and by intercalating various neutral glasses (Olympus; Tokyo, Japan). Coleoptiles were cultivated as described above. Prior to stimulation they were marked with dots of Indian ink to allow identification of individual cells. The intact seedlings were fixed horizontally to the slide by a drop of molten agar (1·2% w/v). Following the irradiation with blue light, growth of individual cells was followed over time at various distances from the coleoptile tip. For this purpose, time-lapse images were recorded using biologically inactive infrared safelight optics (Schmidt *et al.* 1990). It should be emphasized that the seedlings were raised and pretreated in exactly the same way as for the Buder experiment, i.e. they were kept under a saturating background of red light prior to micro-irradiation to suppress possible effects of phytochrome gradients induced by the blue light (Hofmann & Schäfer 1987). In order to counteract the gravitropic stimulation experienced during the experiment, the slides with the coleoptiles were rotated around a horizontal axis (at 0·5 r.p.m.) in the intervals between the image recordings. As a result of this treatment, the gravitropic curvature was negligible throughout the experimental period. Once they had been fixed on the slide, coleoptiles were handled under a dim green safelight that did not induce any phototropic response, even for very prolonged exposures (data not shown).

RESULTS

Fluence–response relations for phototropic stimulation from outside and from inside

The phototropic stimulation was administered by a light-piping device to the very tip of the coleoptile, where the perceptive sites for phototropism are located (Iino 1991), from outside (Fig.1d) or alternatively, in the way described by Buder (1920), from inside (Fig.1e). For these stimulations, fluence–response relations were determined. Since scattering and reflection phenomena in the tissue were expected to obscure the outcome of the experiment (Mandoli & Briggs 1982), the fluence–response curves were determined for various fluence rates.

For stimulation from outside (Fig.1d), a bell-shaped fluence–response curve was obtained with a threshold at around 0·05–0·1 μmol m^{-2} blue light, maximum bending towards the stimulus of about 30° at about 1 μmol m^{-2}, and a decrease to almost 0° for higher fluences (Figs 2a–c). Although the fluence rate was varied by a factor of about 50 (between $8{\cdot}1 \times 10^{-9}$ mol m^{-2} s^{-1} and $4{\cdot}6 \times 10^{-7}$ mol m^{-2} s^{-1}), neither the maximum curvature nor the positions of threshold and peak for this curve displayed any significant change.

In contrast to the data obtained for stimulation from inside (Fig.1e), in the Buder experiment, the shape of the fluence–response curves depended on the fluence rate (Fig.2d–f). For the low fluence rate ($8{\cdot}1 \times 10^{-9}$ mol m^{-2} s^{-1}), the resulting curvature was negative with respect to stimulus direction (Fig.2d). The threshold for this negative

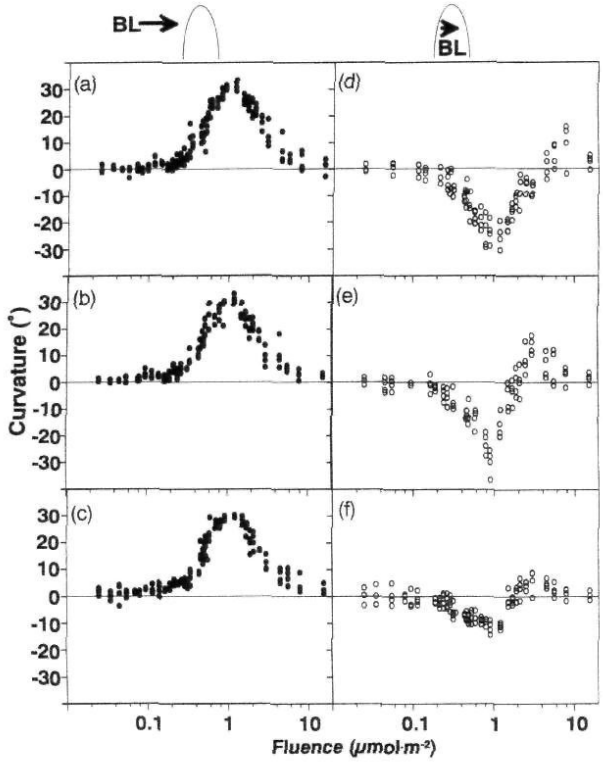

Figure 2. Fluence–response relations for phototropic induction from outside (a–c) and from inside (d–f) the coleoptile. Positive curvatures indicate bending towards the light source. Curvature was measured 2 h after induction. The low fluence rate (a,d) was $8{\cdot}1 \times 10^{-9}$ mol m^{-2} s^{-1}, the intermediate fluence rate (b,e) was $5{\cdot}7 \times 10^{-8}$ mol m^{-2} s^{-1}, and the high fluence rate (c,f) was $4{\cdot}6 \times 10^{-7}$ mol m^{-2} s^{-1}.

curvature was around 0·1 μmol m^{-2}, a maximum of about −20° to −25° was reached at 1 μmol m^{-2}, and curvature rapidly returned to 0° if the fluence was raised to 3 μmol m^{-2}. Surprisingly, for fluences between 3 and 15 μmol m^{-2}, a positive curvature was observed reaching maximum values of more than 10° at 8 μmol m^{-2}.

If the same experiment was repeated using an intermediate fluence rate ($5{\cdot}7 \times 10^{-8}$ mol m^{-2} s^{-1}), the resulting fluence–response curve exhibited some significant changes (Fig.2e). Again, from about 0·1 μmol m^{-2} increasing fluences produced curvatures that were negative with respect to stimulus direction, and again, the peak of this negative bending occurred at about the same fluence as found for the low fluence rate. However, the curvature increased back to 0° at a lower fluence. Moreover, the positive curvature at high fluences reached a maximal value not at 8 μmol m^{-2} but at 3 μmol m^{-2} blue light.

For an even higher fluence rate ($4{\cdot}6 \times 10^{-7}$ mol m^{-2} s^{-1}), the negative peak was markedly reduced to less than −15°, although the position of this peak appeared to be unaltered. The fluence at which the curve crossed the abscissa was lowered further, and the positive peak was rather small.

Orientation of cortical microtubules after stimulation from inside and outside

The orientation of cortical microtubules in epidermal cells can change in response to auxin and phototropic stimulation, and this reorientation is confined to the microtubules adjacent to the outer cell wall (Nick *et al.* 1990). This difference between the two sides of an epidermal cell might be caused by the intracellular gradient of the incident light, or, alternatively, it might be caused by a pre-existing endogenous transverse polarity of this cell. To discriminate between the two alternatives, the behaviour of microtubules has to be assessed for a situation where an intracellular light gradient is opposed to the putative endogenous transverse cell polarity. There are two occasions where such an opposition occurs. For phototropic stimulation from outside, it occurs at the shaded side of the coleoptile (Fig.3b). For phototropic stimulation from inside, it occurs at the illuminated side of the coleoptile (Fig.3d). Therefore, the orientation of cortical microtubules was recorded for the inner and the outer sides of epidermal cells 2 h after phototropic induction for both types of stimulation (Fig. 3). In order to maximize the light gradient across the coleoptile, the fluence rate was kept small ($8{\cdot}1 \times 10^{-9}$ mol m^{-2} s^{-1}). The experiments were performed at a fluence of 1 μmol m^{-2}, yielding large curvatures for both types of irradiation (Figs 2a&d).

For phototropic stimulation from outside, microtubules at the lighted flank exhibited a longitudinal array at the outer side of the epidermal cell, but remained transverse at the inner side (Fig.3a). In the shaded flank, they were found to be transverse on both the outer and the inner sides of the cell (Fig.3b).

For phototropic stimulation from inside, the non-illuminated coleoptile flank exhibited transverse microtubules on both the outer and the inner sides of the cell (Fig.3c). In the lighted coleoptile flank, a complex pattern was observed (Fig.3d). In some cells, the microtubules were longitudinal at the outer and transverse at the inner side of the cell, similar to the situation in the lighted coleoptile flank after phototropic stimulation from outside (Nick *et al.* 1990 and Figs 4A&B, broad arrowheads). However, other cells behaved in an unusual way, with transverse microtubules at the outer and longitudinal microtubules at the inner side of the cell (Figs 4A&B, narrow arrowheads). In some cells, microtubules were found to be steeply oblique at both the outer and the inner sides of the cell, giving the impression of a spiral around the cell periphery (Fig.4C). The pitch of this spiral was sometimes in the same sense in the outer and the inner walls, and sometimes in a different sense. The two situations even coexisted in various regions of an individual cell (Fig.4C). Such regions were joined by an area in which the usual strict parallelity of microtubules was lost (Fig.4C, arrowhead). All these cell types occurred in a mosaic pattern, such that neither at the inner nor at the outer cell face could a clear preferential direction be attributed to the microtubules of the whole cell population (Fig.3d, right-hand panel). However, if the fluence was raised by a factor of 10 (resulting in positive curvatures;

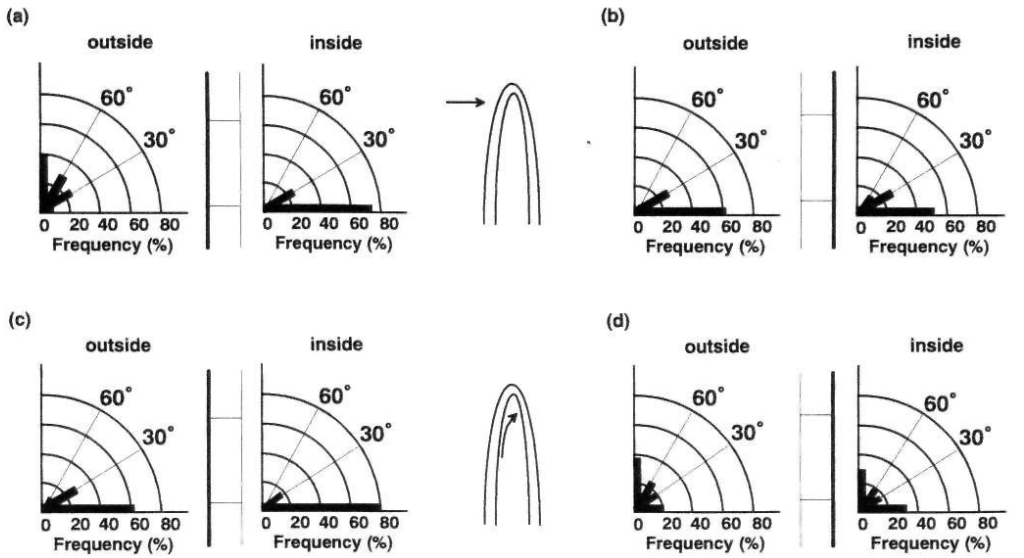

Figure 3. Frequency distribution of microtubule orientation after irradiation from outside and inside. (a) Irradiation from outside, lighted flank; (b) irradiation from outside, shaded flank; (c) irradiation from inside, shaded flank; (d) irradiation from inside, lighted flank. The angles are defined with respect to the short axis of the cell with transverse bars representing transverse microtubules, and longitudinal bars representing longitudinal microtubules. The distributions are based on data from 100 to 200 individual cells. The coleoptiles were induced by 1 μmol m^{-2} blue light.

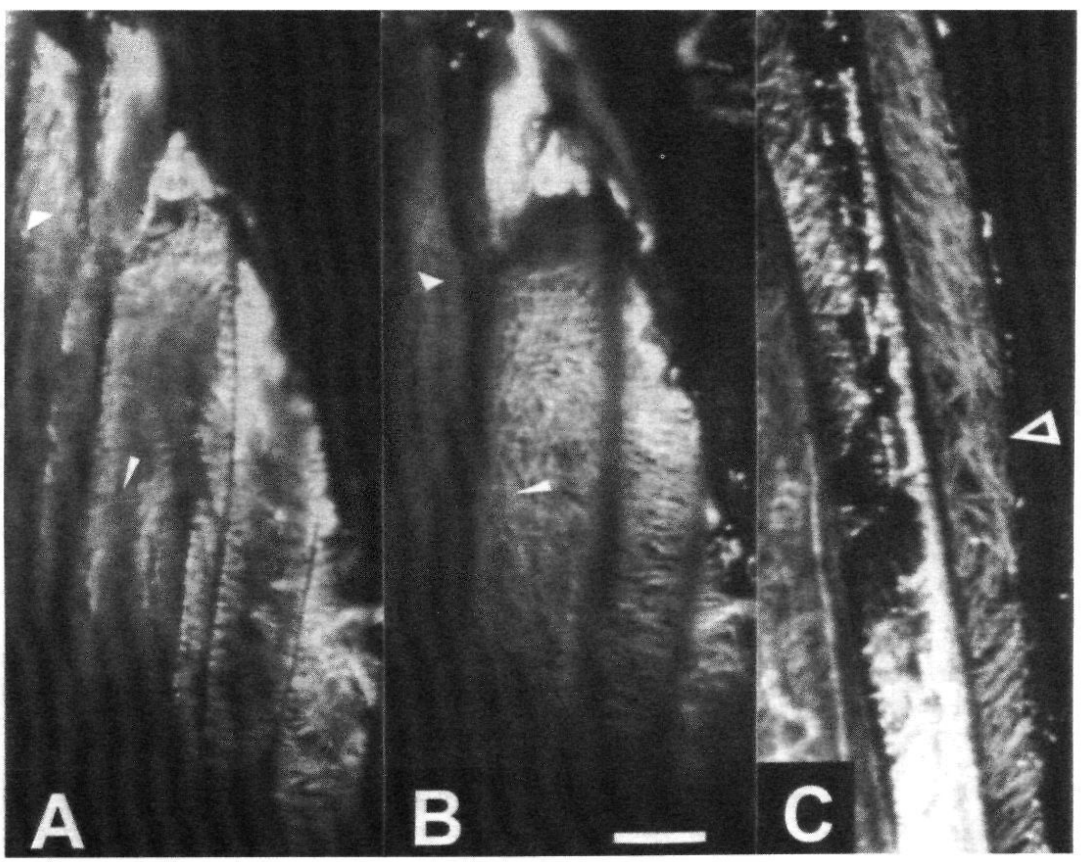

Figure 4. Microtubules at the lighted side of coleoptiles that have been induced by 1 μmol m^{-2} blue light from inside (Buder experiment). (A) microtubules adjacent to the outer and (B) to the inner cell wall of epidermal cells. Broad arrowheads indicate a cell with the usual array (longitudinal at the outer and transverse at the inner side of the cell), while narrow arrowheads indicate a cell in which the usual array has been reversed. (C) Cell with steeply oblique microtubules at both sides with a change of pitch between the lower and the upper halves of the cell (arrowhead). The white bar corresponds to 25 μm.

Fig.2d), longitudinal microtubules became more dominant at the inner side of the cell (65% compared to 30% at 1 μmol m^{-2}).

Cell growth after microbeam illumination of the coleoptile tip

Curvature is based on differential growth between the two flanks of the tropistically stimulated coleoptile. This implies inhibition of growth in the illuminated coleoptile flank (Iino & Briggs 1984). In an attempt to detect the putative signals that coordinate the growth responses of individual cells, intact coleoptiles were irradiated from outside with a microbeam of various fluences at a location five cells from the very tip of the coleoptile. For details of the irradiation procedure, refer to Nick *et al.* (1993). The irradiated spot was confined to five cells. The growth of individual cells was then followed over time at variable distances from the tip by means of time-lapse photography in the infrared.

The growth increment over 2 h was plotted against the distance from the tip. The resulting curves (Fig. 5) revealed a complex pattern of cell growth with a stimulation of growth in the apical part of the coleoptile and an inhibition of growth in its basal parts. With increasing fluence the transition between stimulation and inhibition is shifted towards the tip of the coleoptile. Additionally, the stimulation in the apical part becomes weaker, whereas the inhibition in the basal part becomes more pronounced.

Following the time course of cell growth after microbeam irradiation of the tip, it was observed that the time interval between stimulation and growth response increased with the distance of the responding cell from the tip, as shown for two cells in Fig.6a. A putative signal thus appeared to travel from the tip towards the base. If this lag time between irradiation and onset of growth was plotted against the distance from the tip, a more or less straight line emerged (Fig.6b). The slope of this line gives an estimate of the speed at which this putative signal migrates from tip to base. The calculated speed (60 mm h^{-1}) reveals a signal that moves relatively fast.

DISCUSSION

Buder was right: phototropism depends on the light gradient, not on light direction

The issue of debate between Heilbronn (1917) and Buder (1920) was the question of whether the plant is able to sense the direction of light (Heilbronn 1917) or whether it is the gradient of light across the coleoptile that is perceived (Buder 1920). The data presented here (Fig. 2) are consistent with those obtained by Meyer (1969) and confirm that Buder was right.

The Cholodny-Went theory (Cholodny 1927; Went 1928) has in the meantime been well established (Briggs 1963; Pickard 1985; Iino & Briggs 1984; Nick, Schäfer & Furuya 1992). It demonstrates unequivocally the 'holistic' nature of tropism, i.e. the response of the cells at one flank of the coleoptile depends not only on the locally perceived stimulus, but also on the stimulus perceived at the opposite flank of the organ. This is usually interpreted in terms of a displacement of auxin from the lighted to the shaded coleoptile flank.

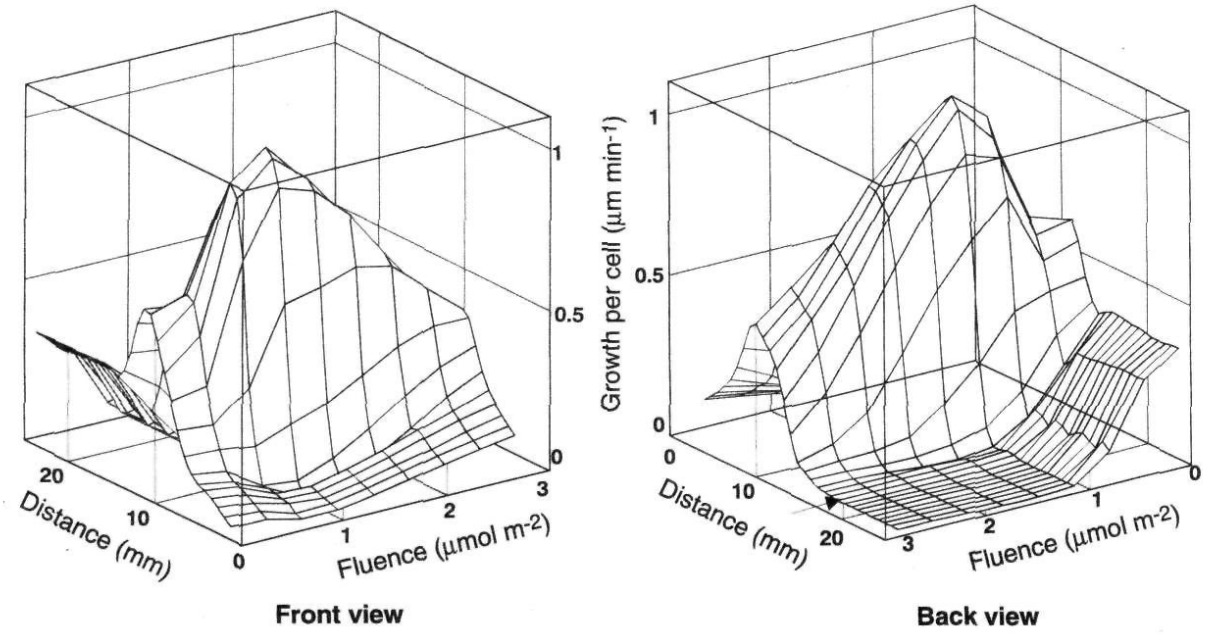

Figure 5. Growth of epidermal cells after microbeam irradiation of the coleoptile tip by various fluences of blue light. Note the stimulation of growth in the apical part and the inhibition of growth in the basal part of the coleoptile. Arrow: growth response obtained for stimulation with 3 μmol m^{-2} at the coleoptile base (15 mm from the tip).

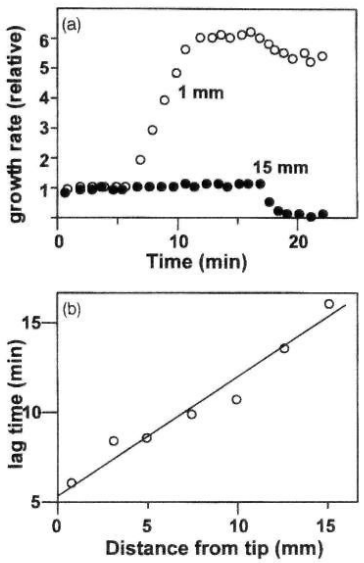

Figure 6. Time course of cell growth after microbeam irradiation in the coleoptile tip (a) and dependence on the lag time of the growth response on the distance from the tip (b). The typical growth responses of an apical cell (open circles) and that of a basal cell (closed circles) are shown in (a) after induction by 1 μmol m^{-2} blue light. The straight line in (b) gives the estimated linear regression describing the increasing lag time of the growth response with increasing distance from the irradiated spot. The slope of this line gives an estimate for the speed of the signal moving from tip to base (about 60 mm h^{-1}).

However, it is worth combining the Buder experiment with the finding of Cholodny and Went: the interaction between individual cells across the coleoptile precedes the transverse shift of auxin. After blue light has been perceived by the individual cells the gradient of blue light across the coleoptile must be somehow sensed. This requires signals that travel from cell to cell and establish some kind of tissue polarity. This tissue polarity subsequently orients a parallel cell polarity that drives the transverse auxin transport.

The combination of the Cholodny-Went theory with the outcome of the Buder experiment leads to the conclusion that individual cell polarities can be aligned with the tissue polarity imposed upon the coleoptile by the gradient of blue light. This implies that the individual cell can sense and respond to an organ polarity (Nick & Furuya 1992).

The cellular light–growth response depends qualitatively on cell position

Tropism, as pointed out above, results from complex interactions between individual cells. To detect the signals interchanged between the individual cells, the microbeam experiment was launched (Figs 5&6). The stimulus was administered to the very tip, where phototropic perception resides (Iino 1991). Two results of this experiment should be emphasized.

First, a signal moves from tip to base (Figs 6a&b). It triggers the cellular growth response to the light that was perceived in the tip. Auxin has been shown to travel at about 10–12 mm h^{-1} in maize coleoptiles (Goldsmith 1967). After phototropic stimulation of the apical 1 mm of the coleoptile, a wave of growth redistribution moves in the basal direction at about the same speed (Iino & Briggs 1984). The signal detected here moves about 6 times faster. It can be hypothesized that similar signals establish the organ polarity driving the tropistic auxin displacement.

The wave of growth redistribution observed after irradiation of the entire tip (Iino & Briggs 1984) becomes detectable from 20 to 30 min after phototropic induction and is probably the manifestation of auxin redistribution. The changes in growth (stimulation in the tip, inhibition in the base) observed after microbeam irradiation of a few tip cells start sooner. It remains an open question why they are not subsequently overlaid (and thus rendered indetectable) by the effects of auxin displacement. The answer might be that a certain minimal number of irradiated cells have to cooperate to bring about a stable auxin displacement. Similar thresholds of cooperation have been repeatedly observed in micro-irradiation experiments (Bischoff 1995; Gressel & Galun 1967).

Secondly, the response to this signal is qualitatively different depending on the physiological state of the target cell (Fig. 5). The physiologically immature cells of the apical region responded by a stimulation of growth, whereas the cells of the elongation zone approaching maturity responded by an inhibition of growth. The transition between stimulation and inhibition depends on the fluence of the stimulus. These qualitative differences in the individual cell responses could explain the complex fluence–response relationships obtained during the Buder experiment (Figs 2d–f).

It may appear astonishing that the response of a cell depends qualitatively on its position along the coleoptile axis. There are two principal ways to interpret this result.

(1) The irradiation could block the apicobasal transport of a factor that is necessary for growth. It would accumulate in the tip and stimulate growth there, whereas it would be depleted in the more basal cells which would cease to grow. With increasing fluence the block would become stronger, explaining the more pronounced inhibition in the basal part. However, if the fluence is increased, the stimulation of growth in the tip should also be more pronounced, as a result of an even stronger accumulation of this putative factor in the tip cells. This was not observed – the growth stimulation in the tip becomes weaker if the fluence increases (Fig. 5). Moreover, microbeam irradiation of the base causes severe inhibition of growth without eliciting such a growth stimulation in the tip (Fig. 5, arrow).

(2) This finding supports the second possible interpretation: it might be that the cells of the tip and base are qualitatively different in their responses to the blue-light-triggered signal. The coleoptile exhibits a steady gradient in cell maturity with elongated, nearly mature cells at the base and almost isodiametric, immature cells at the tip. It might be that the blue-light-triggered signal is interpreted by each

cell according to its position along the coleoptile axis. In other words, the cellular light response depends on the physiological state of the cell.

Numerous examples in the literature describe the qualitative dependence of light responses on the developmental cell state: the induction of anthocyanin synthesis in white mustard depends on the competence of the cell (Steinitz, Drumm & Mohr 1976), for example maturity and state of vacuolization (Steinitz & Bergfeld 1977; Nick *et al.* 1993). In rice, depending on the nature of the organ, a signal triggered by phytochrome is transduced by alterations in auxin transport (Furuya *et al.* 1969) or by changes in gibberellin sensitivity (Toyomasu *et al.* 1994) to produce an inhibition of cell elongation. The causal link of one of the three photoreceptor systems (phytochrome, blue-light receptor and UV receptor) to the transcriptional activity of the chalcone synthase promotor depends on the preceding light regime (Frohnmeyer *et al.* 1992). It is obvious that any interpretation of signal-transduction chains will remain incomplete if the physiological state of the cells is ignored.

Microtubules can sense organ polarity

In the maize coleoptile, epidermal cells exhibit a pronounced transverse polarity: microtubules adjacent to the outer cell wall respond by reorientation to auxin depletion and blue light (Nick *et al.* 1990). Microtubules adjacent to the inner cell wall, in contrast, maintain a transverse orientation after stimulation from outside. This transverse cell polarity might be inherited. For instance, a factor that is required for microtubule reorientation could be absent at the inner side of the cell. Alternatively, it might be the direction of the incident light that enhances reorientation at the outer over that at the inner side of the cell. The Buder experiment provides two important contributions to this problem:

(1) The microtubules adjacent to the inner cell wall can indeed assume a strongly oblique or even longitudinal orientation, if the stimulus is administered contrary to the natural cell polarity (Figs 3d&4). This implies that the molecular components necessary for the reorientation process are present at both the inner and the outer cell walls.
(2) It seems to be the direction of light that can override the natural tendency of the inner side of the cell to maintain transverse microtubules. In some cases this results in cells with a reversal of the usual cell polarity with longitudinal microtubules at the inner, and transverse microtubules at the outer, side of the cell (Figs 4A&B). In some cases, microtubules assume a steeply oblique array on both sides of the cell (Fig. 4C). The two responses can coexist in neighbouring cells or even within individual cells in a kind of chaotic mosaic. The corresponding frequency distributions do not reveal a preferential direction (Fig. 3d), emphasizing the stochastic behaviour of individual cells or even cell domains.

This stochastic outcome indicates that the microtubules at the two sides compete for a limiting factor necessary for the reorientation from transverse to longitudinal in response to auxin depletion. This factor seems to be redistributed depending on intercellular gradients. In the absence of blue light, these gradients favour the accumulation of this factor at the outer cell wall and this may be reinforced at the lighted flank of coleoptiles that have been irradiated from outside (Fig. 3a). At the shaded side the transport of this factor should be impeded. However, the fluence there is low, due to the attenuation of the light along the optical path through the tissue. Without blue light, microtubules are found to be transverse on both sides (Nick *et al.* 1990). If the fluence were increased, such that this attenuation was compensated, in some cells the microtubules at the inner side of the cell would be expected to reorient. This is not observed – microtubules are found to be mainly transverse on both sides of the cell (Nick, Schäfer & Furuya 1992). However, if fluence is increased to 10 μmol m^{-2} and is administered from inside out, the formation of this putative factor seems to be stimulated, resulting in a high frequency (65% instead of 30% at 1 μmol m^{-2}) of cells with inverted radial polarity (i.e. microtubules longitudinal at the inner and transverse at the outer cell face). It is interesting to ask whether this increased frequency of polarity reversal is related to the reversal of phototropic curvature observed for high fluences in the Buder experiment (Fig. 2d).

What, then, is the signal that is sensed by the microtubules themselves? At first sight it seems to be the intracellular light gradient, since the Buder set-up can impede the endogenous transverse polarity of the cell. There is, however, a minor but crucial problem: the site where the light is perceived and the site where microtubules respond are spatially separated by several millimetres in the longitudinal direction. This requires a basipetal signal that somehow transmits information about the direction of the light. It is difficult to imagine how information about a transverse cell polarity could be carried over such a distance.

However, a signal might be released in the tip whose quantity and spatial distribution across the tissue depend on changes in cell polarity. Whether this signal is auxin or the fast factor detected in the microbeam experiments (Figs 5 & 6) cannot be determined at this stage. The target cells might respond to the gradient of this signal across the tissue (i.e. to the organ polarity induced by the blue light). Alternatively, the endogenous cell polarity might tilt in response to superoptimal local levels of this signal. Such a mechanism has been invoked to explain the blue-light-induced inversion of the gravitropic response during clinostat rotation (Sailer, Nick & Schäfer 1990).

The nature of the transmitted signal is not known. However, it must interfere with the elements necessary for microtubule reorientation. Microinjection experiments using fluorescence-labelled tubulin in pea epidermis have demonstrated that reorientation of microtubules is based upon directional control of tubulin polymerization rather than upon movement of polymerized microtubules (Yuan *et al.* 1994). The transition is characterized by patches of microtubules in the new direction, whereas neighbouring patches are still oriented in the old direction. This resembles the stochastic mosaic observed in a situation where light direction and endogenous cell polarity oppose each other (Fig. 4).

The signal travelling from tip to base and carrying information about the light gradient is therefore predicted to control tubulin polymerization and thereby cell polarity. Possible targets for this signal are the microtubule-associated proteins (MAPs) that regulate tubulin dynamics. Little is known about these MAPs in plants, but the recent isolation of two putative MAPs from maize coleoptiles (Nick, Lambert & Vantard 1995) might be a first step in the molecular analysis of cell polarity.

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