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Polarity induction versus phototropism in maize: Auxin cannot replace blue light

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Abstract. In a previous study (Nick and Schäfer 1991, Planta **185**, 415–424), unilateral blue light had been shown, in maize coleoptiles, to induce phototropism and a stable transverse polarity, which became detectable as stable curvature if counteracting gravitropic stimulation was removed by rotation on a horizontal clinostat. This response was accompanied by a reorientation of cortical microtubules in the outer epidermis (Nick et al. 1990, Planta **181**, 162–168). In the present study, this stable transverse polarity is shown to be correlated with stability of microtubule orientation against blue light and changes of auxin content. The role of auxin in this stabilisation was assessed. Although auxin can induce reorientation of microtubules it fails to induce the stabilisation of microtubule orientation induced by blue light. This was even true for gradients of auxin able to induce a bending response similar to that ellicited by phototropic stimulation. Experiments involving partial irradiation demonstrated different perception sites for phototropism and polarity induction. Phototropism starts from the very coleoptile tip and involves transmission of a signal (auxin) towards the subapical elongation zone. In contrast, polarity induction requires local action of blue light in the elongation zone itself. This blue-light response is independent of auxin.

Key words: Auxin – Blue light – Coleoptile – Microtubule – Phototropism – Transverse polarity – *Zea*

Introduction

Plants can adapt their development to changing environmental conditions. This implies the ability to sense and process environmental signals and the ability to tune cellular morphogenesis with this processed information (Mohr 1972). Microtubules are candidates for the link between signal transduction and morphogenesis: they reorient swiftly in response to hormones, light, gravity and endogenous factors (Iwata and Hogetsu 1989; Nick et al. 1990b, 1991b; Sakiyama and Shibaoka 1990; Zandomeni and Schopfer 1993). They appear to guide the directional deposition of cellulose microfibrils, an important mechanism of growth control (Robinson and Quader 1982).

Phototropism of coleoptiles is one of the most sensitive and rapid morphogenetic responses known, with a lag of only 20–30 min (Iino 1988). In maize coleoptiles, reorientation of cortical microtubules in the illuminated coleoptile flank precedes the bending response to phototropic stimulation (Nick et al. 1990b). An early model postulated that the light-induced depletion of auxin in the illuminated coleoptile flank should trigger the observed microtubule orientation from transverse to longitudinal. Deposition of cellulose microfibrils in the longitudinal direction should then produce a stiff cell wall in the illuminated flank and, in consequence, an inhibition of growth. However, a more detailed analysis demonstrated that conspicuous curvatures could arise without or even against gradients of microtubule orientation, and that gradients of microtubule orientation can be induced that are not followed by bending (Nick et al. 1991a). Thus, microtubule reorientation was found to be neither necessary nor sufficient for tropistic bending. The responses are only correlated, not causally linked.

In addition to the tropistic response, blue light can evoke a stable transverse polarity in the direction of stimulation (Nick and Schäfer 1988, 1991). This polarity can withstand opposing gravitropic or phototropic stimuli for many hours and becomes manifest as a stable, long-lasting curvature, if the gravitropic counterstimulation experienced by curved plants is removed by rotation on a horizontal clinostat. This transverse polarity evolves from a labile precursor, which becomes stable 90 min after induction (Nick and Schäfer 1991). Thus, unilateral irradiation by a pulse of blue light triggers a triple response: phototropic curvature (lag 20–30 min, Iino 1988),

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induction of a stable transverse polarity (lag 90 min, Nick and Schäfer 1991), and reorientation of cortical microtubules (lag 10–20 min, Nick et al. 1990b). It has been shown previously (Nick et al. 1991a) that microtubule orientation and phototropic curvature are not always correlated. Thus, the present publication attempts to clarify the connection between microtubule orientation and stable transverse polarity.

Materials and methods

Plants and light conditions. Seedlings of maize (*Zea mays* L. cv. Brio42HT; Asgrow, Bruchsal, Germany) were grown under 0.4 W·m^{-2} red light for 2 d at 25°C and then kept in darkness for one further day. This treatment yielded plants with straight coleoptiles, since mesocotyl elongation and nutations are suppressed by red light (Kunzelmann and Schäfer 1985). All experiments were performed in a symmetrical and saturating red background light (2.5 W·m^{-2}) to level out possible effects of phytochrome gradients induced by phototropic stimulation (Hofmann and Schäfer 1987). For details on growth conditions and selection procedures refer to Nick and Schäfer (1988) and Nick et al. (1992).

Stimulation treatments. The protocol for alternating stimulation involved two unilateral light pulses of identical fluence (1.9 µmol·m^{-2}, 30 s) but opposing direction, parallel to the shorter diameter of the coleoptile. The second, counteracting, pulse was administered either 1 or 2 h after the first, inducing, light pulse. In one set of experiments, the first pulse was applied not unilaterally, but from above by means of a mirror. Except for during the irradiation treatments, the plants were kept rotating on a horizontal clinostat at 0.5 rpm until response evaluation as described in detail in Nick and Schäfer (1991). In a variation of this procedure the counteracting light pulse was replaced by decapitation and subsequent incubation in water or a solution of 0.1 mM indole-3-acetic acid (IAA) according to Nick et al. (1992). Energy fluxes were determined as described previously (Nick and Schäfer 1988).

Localized illumination. The procedure described above was varied by giving the first light pulse only to specific regions of the intact coleoptile (see Fig. 3). This was achieved by using a light-piping device (Flexilux 150 HL; Schölly Fiberoptik, Denzlingen, Germany). The inducing light pulse was given either to the very tip (treatment 2 in Fig. 3) or 20 mm below the tip (treatment 3 in Fig. 3). The stability of the resulting curvature was then tested by a counter-directed light pulse at variable time intervals after induction. This counterpulse was applied to the entire length of the coleoptile. The fluence of the inducing light spot was reduced to 0.8 µmol·m^{-2} blue light with a spot diameter of 0.5 mm to minimize light-piping effects within the tissue (Mandoli and Briggs 1982). The counterpulse was equal in fluence to the inducing pulse. A control experiment recorded the response to 0.85 µmol·m^{-2} blue light, where both, inducing and opposing light pulses were given over the entire length of the coleoptile (see Fig. 3, treatment 1). Both light pulses were of maximally 30 s duration. A second control assayed the tropistic responses to tip and base illumination for omission of the counterstimulation. Each datum point in Fig. 3 represents the average of 12 individual seedlings.

Response evaluation. Phototropic curvature was determined using a simple xerographic method (Nick and Schäfer 1988). Cortical microtubules were stained by means of immunofluorescence. Coleoptile segments (length 20 mm, 2 mm below the tip) were excised, the primary leaf was discarded, and the side facing the inducing pulse was marked by an incision. After prefixation for 45 min at room temperature in 3.2% (w/v) paraformaldehyde in microtubule-stabilizing buffer (0.1 M 1,4 piperazine-diethanesulfonic acid, 1 mM $MgCl_2$, 5 mM ethylene glycol-bis-(β-aminomethyl-ether)-N,N,N',N'-tetraacetic acid, 0.2% Triton X100, pH 6.8), tangential sections were cut under a drop of microtubule-stabilizing buffer from the flat sides of the coleoptile and collected separately with respect to coleoptile flank. Then fixation in the same solution was continued for a further 40 min. After three washings in the same buffer without paraformaldehyde the sections were incubated for 20 min at room temperature with goat normal serum (Nordic Immunology, Tilburg, The Netherlands; diluted 1:20 in phosphate-buffered saline, PBS) and then treated for 1 h at 37°C with a mouse monoclonal antibody raised against β-tubulin (Amersham, UK) diluted 1:1000 in PBS. The sections were washed with PBS and incubated for 50 min at 37°C with a fluorescein-isothiocyanate-labeled secondary antibody (anti-mouse immunoglobulin G from sheep, 1:20 diluted in PBS, Amersham), washed again and then mounted in an antifading agent (Citifluor, Amersham, UK) with the outer face of the epidermis facing upwards. They were viewed under a fluorescence microscope (Orthopan, Leitz, Wetzlar, Germany) and photographed on Kodak TriX Pan 400 ASA film (Kodak, Rochester, New York, USA). Since there seems to be no preferential handedness to the obliqueness of microtubules within the tissue (Nick et al. 1990b), their orientation was scored according to four classes with 0° designating transverse microtubules, 30° slightly oblique microtubules, 60° steeply oblique microtubules and 90° longitudinal microtubules. Frequency distributions were constructed from the data from 20–45 plants corresponding to at least two independent sets of experiments and mean orientation calculated from these distributions (Tables 1–5).

Results

Orientation of microtubules is stable 2 h after irradiation with blue light. Following sequential stimulation with two opposing blue-light pulses of equal strength (1.9 µmol·m^{-2}, 30 s), curvature developed under conditions of symmetric gravity during rotation on an horizontal clinostat (Fig. 1). When the opposing pulse was given 60 min after the inducing stimulus, final curvature was dominated by this opposing stimulation (Fig. 1, left panel). Microtubules, which had been transverse on both flanks of the coleoptile at the time of induction, were found to be longitudinal in the illuminated side and transverse in the shaded side 60 min later (Fig. 1, left panel, Fig. 2). This gradient of microtubule orientation was reversed briefly after the application of the counterpulse, following the inversion of curvature. Eventually, microtubules became longitudinal in the shaded side (facing the second pulse), where they had been transverse, whereas in the flank facing the first stimulus, they turned back from longitudinal to transverse (Fig. 1, left panel, Fig. 2). Thus, neither the phototropic response nor the gradient of microtubule orientation induced by the first pulse showed any stability against an opposing stimulation. In fact, a comparison with a control, where the first pulse had been omitted, gives the impression that the counterpulse had erased all traces of the original stimulation.

A fundamental difference was observed when the counterpulse was administered 90 min after the inducing stimulus (Fig. 1, right panel). Although the second pulse could control bending for the first hour after counterstimulation, bending eventually was reversed and plants curved towards the first light pulse. The final result was indistinguishable from control experiments in which the counterpulse had been omitted. The gradient of micro-

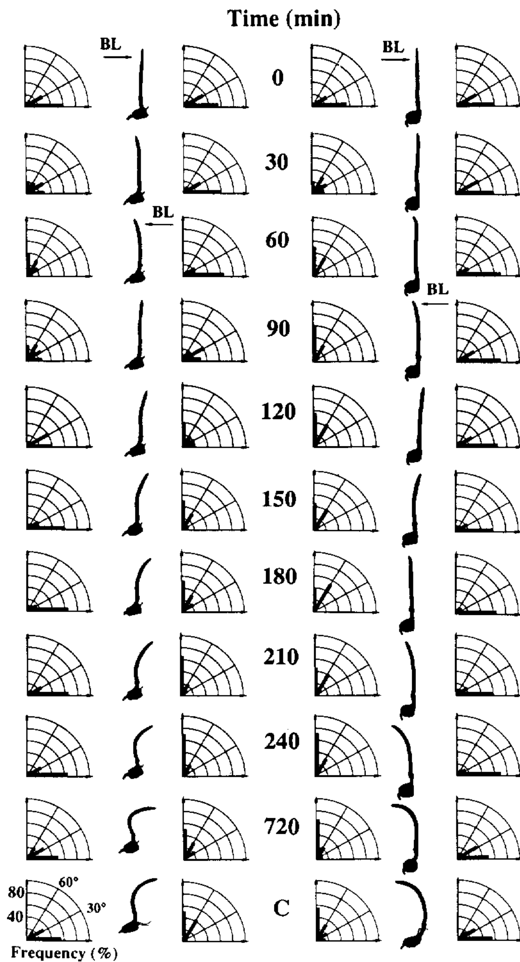

Fig. 1. Stabilisation of transverse polarity and microtubule orientation by unilateral blue light in *Zea* coleoptiles. *Left-hand panel*: Labile polarity for counterstimulation 60 min after phototropic induction. *Right-hand panel*: Stable polarity for counterstimulation 90 min after phototropic induction. The shadowgraphs show the bending response of a typical seedling. Frequency distributions represent the orientation of cortical microtubules in the epidermis in the corresponding coleoptile flank, respectively with 0°, transverse, and 90° longitudinal microtubules

tubule orientation induced by the first light pulse did not reveal any effects of the counterstimulus. Microtubules remained longitudinal in the side facing the inducing stimulus and transverse in the opposite coleoptile flank throughout the experiment, i.e. even during the short period when the seedlings transiently bent towards the counterpulse (Fig. 1, right panel). It thus appeared that, 90 min after induction, both the transverse polarity in the direction of the light (Nick and Schäfer 1988, 1991) and the gradient of microtubule orientation had attained stability against the opposing stimulation.

This apparent stability of microtubule arrays against counterstimulation could be caused by a loss of responsiveness to blue light 90 min after irradiation. Alternatively, microtubule orientation itself might become stable at this time. With the intention of deciding between these possibilities, the second light pulse was replaced by a different treatment: changes in the content of endogenous auxin or exogenous IAA, respectively. Although 1 h after a unilateral light pulse a clear gradient of microtubule orientation could be detected, this gradient (longitudinal microtubules in the illuminated side, transverse microtubules in the shaded side) could be eliminated by changing the content of auxin/IAA (Table 1). Depletion of endogenous auxin for 1 h yielded longitudinal microtubules in both sides, incubation with saturating concentrations of IAA yielded transverse microtubules in both sides. Identical results were obtained for unstimulated coleoptiles (Table 1). Two hours after induction, the light-induced gradient of microtubule orientation had become stable – this time against changes in the content of auxin/IAA (Table 1). It should be emphasized that this stabilisation of a gradient extended to microtubule orientation in the shaded side. Those microtubules maintained their transverse orientation against depletion of endogenous auxin, although they had not experienced a light-induced reorientation response. This means that a loss of responsiveness to blue light can be ruled out as an explanation and that microtubule orientation per se has become stable.

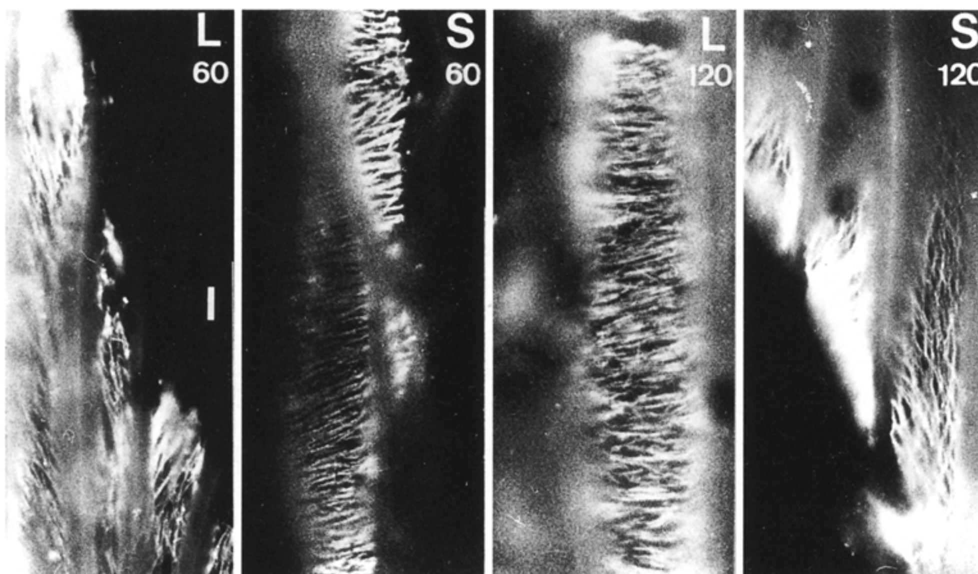

Fig. 2. Inversion of the gradient in the orientation of cortical microtubules of *Zea* coleoptiles by phototropic counterstimulation applied 60 min after induction. Cortical microtubules stained by immunofluorescence in the lit (*L*) and shaded (*S*) flanks of a coleoptile 60 min after phototropic stimulation (*left*). A counteracting phototropic stimulation of equal strength was administered at this time on the shaded side of the coleoptile. The gradient in the orientation of microtubules between lit and shaded sides had been reversed by 120 min after the original light pulse (*right*), corresponding to the left column in Fig. 1. Bar = 10 μm; ×480

Table 1. Stabilisation of microtubule orientation against auxin or IAA after phototropic induction. *Zea* coleoptile segments were excised at variable time intervals after phototropic induction and incubated for 1 h either in water (causing depletion of exogenous auxin) or in 10 µM IAA. The gradient in the orientation of cortical microtubules became stable 2 h after induction. Values represent mean ± SE of frequency distributions constructed for microtubule orientation, with 90° indicating longitudinal orientation and 0° transverse orientation

Time after induction (Δt)	Control: intact coleoptiles			Excision at Δt and incubation in water for 1 h Microtubule orientation (°)		Excision at Δt and incubation in IAA for 1 h Microtubule orientation (°)	
	Tropistic curvature (°)	Microtubule orientation (°)					
		Lit side	Shaded side	Lit side	Shaded side	Lighted side	Shaded side
Unstimulated	2.3±1.5	15±7	20± 6	85±12	83± 9	13± 6	15± 8
Controls							
1 h	12.4±2.4	84±7	16±10	80±9	89±12	12±16	15±10
2 h	25.3±1.8	85±3	19±12	81±15	13±10	82± 9	17± 8

Table 2. Failure to induce stable microtubule arrays by auxin. Maize coleoptile segments were incubated at time 0 h in water and the resulting longitudinal microtubule array assayed for stability against 10 µM IAA for 1 h (causing transverse orientations). For definition of values refer to Table 1

Time after decapitation (Δt)	Incubation in water Microtubule orientation (°)	Incubation in water, followed by incubation in IAA Microtubule orientation (°)
0 h	18±16	19±13
1 h	86± 6	12±16
2 h	80±21	16± 9
3 h	78±19	22± 6

Table 3. Failure to induce stable microtubule arrays by gradients of auxin in maize coleoptiles. Half of the coleoptile tip was removed to mimick the auxin depletion produced by phototropic stimulation. Segments were excised at variable time intervals and the stability of the resulting microtubule arrays (longitudinal underneath the removed half of the tip and transverse underneath the remaining half of the tip) was assayed by incubation in 10 µM IAA or water, respectively. For definition of the values refer to Table 1

Time after decapitation (Δt)	Control: coleoptiles where half of the tip was removed			Excision at Δt and incubation in water Microtubule orientation (°)		Excision at Δt and incubation in IAA	
	Induced curvature (°)	Microtubule orientation (°)					
		Concave side	Convex side	Concave side	Convex side	Concave side	Convex side
0 h	1.9±2.3	10±17	12±16	81±15	82±19	12±16	25±18
1 h	10.7±1.4	81±14	19±13	79±19	86±10	22± 8	16±14
2 h	21.8±4.8	86±13	20±19	85±12	83±16	12±19	23±18
3 h	27.9±3.2	80±15	29±15	75±11	81±21	19±10	16±22

The role of blue light and auxin in the induction of stable microtubule arrays. It might be that the action of blue light upon stabilisation of microtubule arrays is transduced by the depletion of auxin induced by the irradiation. If this were true, decapitation and subsequent depletion of endogenous auxin for 2 h should elicit stable microtubule arrays. One hour after decapitation microtubules exhibited a longitudinal orientation (Table 2), but they readily returned to the transverse position after addition of indole-acetic acid, even as late as 3 h after decapitation (Table 2).

It was considered that this experiment, using symmetric changes of auxin content, was only roughly mimicking the effects of unilateral blue light. A transverse gradient of auxin should be closer to the situation after phototropic stimulation. To produce such a gradient, half of the coleoptile tip was removed and the otherwise intact seedlings kept up to 3 h under red light. From 1 h after decapitation, microtubules were found to be longitudinal in the cells subtending the excised tip-half, they were transverse or slightly oblique in the opposite coleoptile flank (Table 3). However, this gradient of microtubule orientation was erased if coleoptile segments were incubated in water or in 0.1 mM IAA, even when this incubation was delayed for 3 h (Table 3). Thus, all attempts to induce stable microtubule arrays without blue light failed.

In order to assess the importance of a gradient of blue light for the stabilisation of microtubules, an inducing pulse was given symmetrically from above. One or two hours after induction, putative stability effects were tested either by a unilateral "counterpulse" (Table 4) or

Table 4. Stabilisation of microtubule orientation against asymmetric blue light after symmetric irradiation of maize coleoptiles. Plants were irradiated from above by a pulse of blue light (1.9 $\mu mol \cdot m^{-2}$) and then phototropically induced by a second, unilateral, light pulse of equal strength either 1 h (left panel) or 2 h (right panel) after the symmetric irradiation. Negative curvatures indicate tropistic bending towards the second light pulse; lit and shaded side are defined with respect to the unilateral light pulse. For definition of orientation values refer to Table 1

Time after induction (Δt)	Second light pulse after 1 h			Second light pulse after 2 h		
	Induced curvature (°)	Microtubule orientation (°)		Induced curvature (°)	Microtubule orientation (°)	
		Lit side	Shaded side		Lit side	Shaded side
0 h	+ 3.3 ± 1.7	12 ± 7	18 ± 13	− 0.9 ± 1.5	12 ± 19	22 ± 8
1 h	+ 2.9 ± 0.4	79 ± 12	80 ± 23	+ 2.2 ± 0.9	76 ± 16	82 ± 18
2 h	− 12.8 ± 2.8	83 ± 23	18 ± 12	+ 0.5 ± 1.7	85 ± 16	82 ± 15
3 h	− 37.8 ± 4.9	79 ± 18	23 ± 19	− 24.3 ± 1.9	82 ± 17	80 ± 20
12 h	− 98.4 ± 19.3	82 ± 14	18 ± 18	+ 5.7 ± 4.9	79 ± 13	86 ± 22

Table 5. Stabilisation of microtubule orientation against auxin after symmetric irradiation. Maize coleoptiles were irradiated from above as in Table 4 and the stability of the resulting longitudinal microtubule array questioned by incubation in 10 μM IAA for 1 h (inducing transverse arrays). For definition of values refer to Table 1

Time after irradiation (Δt)	Control: intact plants Microtubule orientation (°)	Incubation in IAA Microtubule orientation (°)
0 h	18 ± 11	10 ± 23
1 h	78 ± 16	17 ± 14
2 h	82 ± 13	76 ± 19

by decapitation and subsequent incubation in IAA (Table 5). One hour after vertical induction, microtubules were longitudinal in both coleoptile flanks (Tables 4, 5). After unilateral irradiation by the "counterpulse" they returned to the transverse array on the side opposed to this "counterpulse" (Table 4, left). This was accompanied by a strong curvature towards the unilateral light pulse. A reorientation of microtubules from longitudinal to transverse could also be achieved by incubation in IAA (Table 5, left). Two hours after a vertical light pulse, the longitudinal microtubule orientation had become stable against unilateral light pulses (Table 4, right) and incubations with IAA (Table 5, right). For these conditions the unilateral "counterpulse" could evoke only a slight, ephemeral bending response (Table 4, right).

Induction of transverse polarity by partial illumination. If coleoptiles were unilaterally stimulated over their entire length with a pulse of 0.85 $\mu mol \cdot m^{-2}$ blue light (treatment 1 in Fig. 3), a strong curvature of about 100° towards the light pulse could be observed 1 d later (control 1 in Fig. 3). A counterpulse of equal strength could reverse this response, if it was applied up to 1 h after induction (curve 1 in Fig. 3). After that time the effects induced by the first pulse had become stable and were expressed as a stable curvature in the direction of the inducing pulse. When the same inducing fluence was not distributed over the entire length of the coleoptile, but confined to a small spot of 0.5 mm diameter on the very tip of the coleoptile (treatment 2 and curve 2 in Fig. 3), no stabilisation against the counterpulse could be observed, even if the counterpulse was applied as late as 3 h after induction. However, the phototropic response, elicited by such a tip illumination (control 2 in Fig. 3) was only slightly reduced as compared to the response produced by stimulation of the entire coleoptile (control 1 in Fig. 3). When the inducing pulse was directed to the base of the coleoptile, 20 mm below the tip (treatment 3 in Fig. 3), only a significantly smaller phototropic response was observed (control 3 in Fig. 3). Surprisingly, this response escaped reversibility as early as 1 h after induction, as fast as for irradiation of the entire coleoptile (curve 3 in Fig. 3). Thus, with respect to induction of transverse polarity, partial stimulation in the coleoptile base was found to be as effective as irradiation of the entire coleoptile.

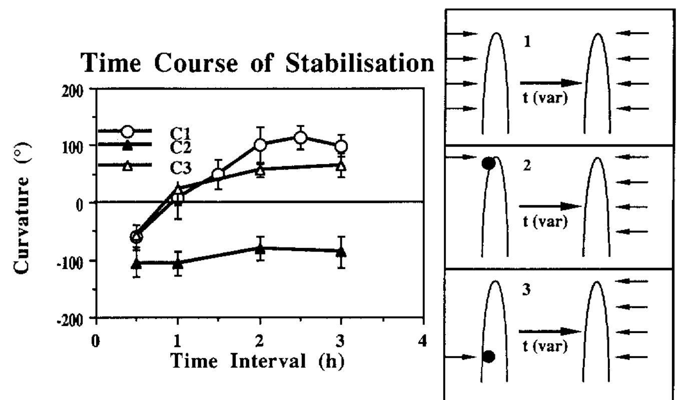

Fig. 3. Stabilization of directional memory induced by localized stimulation. The stability of the response to the inducing light pulse was assayed by counterdirectional stimulation over the whole length of the coleoptile. The inducing pulse was either distributed over the entire length of the coleoptile (treatment 1), or in the very tip of the coleoptile (treatment 2), or in the base, 20 mm below the tip (treatment 3). Positive curvatures indicate bending towards the inducing pulse, negative curvatures inversion of the response by the counterstimulus. Controls C1 to C3 show the response for the respective induction for omission of the counterpulse

Discussion

Stable arrays of microtubules and transverse polarity. Phototropic stimulation can confer a stable transverse polarity which controls long-term changes in growth (Nick and Schäfer 1988, 1991). It evolves from stabilizing a labile precursor, which becomes detectable from 20 min after irradiation. This labile precursor is based upon a gradient across the coleoptile and can be reoriented by opposing light pulses (Nick and Schäfer 1991). However, 2 h after irradiation, transverse polarity attains resistance to counterstimulation.

A gradient across the coleoptile can be detected for the orientation of cortical microtubules from 10 to 20 min after phototropic stimulation, with longitudinal microtubules in the illuminated side and transverse microtubules in the shaded side of the coleoptile (Nick et al. 1990b). It is labile and can be reoriented by opposing light pulses (Figs. 1 and 2). However, 2 h after irradiation, this gradient of microtubule orientation has acquired stability against opposing light pulses (Fig. 1).

Phototropic curvature and this gradient of microtubule orientation are correlated with respect to time course, direction, fluence-dependence and relation with auxin (Nick et al. 1990b, 1992). Nevertheless, they were shown to be parallel phenomena, not causally linked to each other (Nick et al. 1991a). One important difference is the stability of microtubule orientation beginning from 2 h after tropistic stimulation (Nick et al. 1991a, Fig. 1). In contrast, tropistic curvature can be transiently reversed by opposing gravitropic or phototropic stimuli (Nick and Schäfer 1988; Nick et al. 1991a,b).

On the other hand, the gradient of microtubule orientation and the stable spatial memory did correlate in all cases tested so far (Fig. 1 and Nick et al. 1991a). This includes temporal (Nick et al. 1990; Nick and Schäfer 1991) as well as spatial aspects. One might argue that the stability of microtubule arrays from 2 h after irradiation is only apparent, due to long-term sensory adaptation or habituation (Galland 1989) inactivating the transduction chain responsible for the blue-light action upon microtubule orientation. However, the observation that microtubule orientation is not only resistant to opposing blue-light pulses, but also to changes in the content of auxin or IAA indole (Table 1), favours of a true stability of microtubule arrays. If habituation were involved, it would be expected in a very late event of transduction and should affect a step necessary for the cellular response to auxin rather than signal transduction in sensu stricto.

It thus appears justified to assume that microtubules are stabilized 2 h after irradiation with blue light. The gradient of microtubule orientation across the coleoptile might embody the information on the direction of light-induced spatial memory. The stability of microtubule arrays might be the cause for the stability of this spatial memory. In other words: in maize coleoptiles, light-induced stable arrays of microtubules might be the cellular marker for the light-induced stable transverse polarity.

Essential elements in the establishment of stable microtubule arrays. Depletion of auxin can make microtubules reorient in a fashion similar to blue light (Nick et al. 1990b, 1992). Thus, similar to blue light, it might endow the microtubule arrays with stability. However, even 3 h of auxin depletion were not able to produce stable microtubule arrays (Table 2). A gradient of auxin, although able to induce a gradient in the orientation of microtubules, was equally ineffective in confering stability of this orientation (Table 3). Thus, although blue light causes gradients of auxin across the coleoptile, artificially induced auxin gradients could not mimick all aspects of phototropic stimulation. Although auxin is able to trigger reorientation of microtubules, stabilisation of microtubule arrays requires a blue-light-induced factor, which is not auxin. A similar conclusion has been drawn from a detailed analyses of microtubule reorientation induced by light of different spectral qualities (Zandomeni and Schopfer 1993). It might even be that stabilisation of microtubule arrays does not rely upon auxin at all, but utilizes a different signal-transduction pathway. A similar conclusion was drawn for the fixation of the physiologically defined spatial memory (Nick and Schäfer 1991). Thus, it appears that blue light is essential for the stabilisation of microtubule arrays. The question arises whether it has to be a gradient of blue light or whether symmetrical blue light has the same effect. The answer seems to be the latter: symmetrical blue light can suppress the responses to subsequent stimuli, if its action is allowed to develop for 2 h (Table 4). This can be seen on the physiological level: only transient curvature is observed, similar to the transient bending towards the counterpulse in Fig. 1. On the cellular level, this becomes manifest as a stable longitudinal microtubule array on both flanks of the coleoptile (Tables 4, 5). This experiment directly demonstrates that the two aspects of transverse polarity – direction and stability – can be separated on the whole-organ as well as on the cellular level. This appears to be a general feature of polarity in plants (Jaffe 1958; Nick and Furuya 1992). Extensive fluence-response studies on spatial memory lead to the conclusion that the signal-transduction chains mediating the stabilisation of transverse polarity and the phototropic asymmetry leading to tropistic bending separate before phototropic asymmetry is formed (Nick and Schäfer 1991). A similar conclusion was drawn for blue-light-mediated reorientation of microtubules (Nick et al. 1992). The findings presented here suggest that the same is true for the stabilisation of microtubule arrays (Table 5). This parallelism of the three phenomena further strengthens the view that microtubule reorientation and the stabilisation of microtubule arrays are the cellular correlates of the physiologically defined blue-light-induced transverse polarity.

Stable transverse polarity is a localized response. To analyze the role of longitudinal signal migration in the induction of stable transverse polarity, the stabilisation of the memory of the direction of an inducing pulse was followed for localized irradiation in the tip and base of the coleoptile, respectively (Fig. 3). This experiment yielded two important results: (i) The tropistic response to blue light can be induced best by stimulation of the very tip of the coleoptile, as reported previously (Iino 1988). The

tropistic response to stimulation in the base of the coleoptile is comparatively weak (compare controls 1, 2 and 3 in Fig. 3). (ii) In contrast, even as late as 3 h after induction, no stable transverse polarity could be detected for unilateral tip irradiation (curve 2 in Fig. 3). However, with respect to induction of stable transverse polarity, stimulation at the base of the coleoptile was as effective as stimulation over the entire coleoptile length (compare curves 1 and 3 in Fig. 3). The failure to induce a stable polarity by tip illumination suggests that basal cells have to see the light themselves to bring about this response. In other words: no apicobasal signal can replace the direct action of blue light. This is also valid for blue-light-induced changes in the content of auxin. Thus, in contrast to phototropic curvature, stable transverse polarity is cell-autonomous. It depends upon a signal induced by blue light, a signal which cannot be accounted for by auxin. This is consistent with previous experiments (Nick et al. 1990a) demonstrating that gravitropic stimulation requires blue light to induce a stable transverse polarity (Nick et al. 1990a). The spatial separation of phototropic induction (initiated in the tip of the coleoptile) and the induction of stable transverse polarity (taking place in the base of the coleoptile) provides direct evidence for the view that the two phenomena are not causally linked.

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