*Plant Cell Physiol.* 32(7): 999–1006 (1991)


# Do Microtubules Control Growth in Tropism? Experiments with Maize Coleoptiles

Peter Nick [1, 2, 3], Masaki Furuya [1] and Eberhard Schäfer [2]

[1] *Frontier Research Program, Riken Institute, Hirosawa 2-1, Wako-shi, Saitama, 351-01 Japan*
[2] *Institut für Biologie II, Schänzlestr. 1, 78 Freiburg, Federal Republic of Germany*

A recently proposed hypothesis [Nick et al. (1990) *Planta* 181: 162] suggests that, in maize coleoptiles, tropistic curvature might be caused by a stimulus-induced trans-organ gradient over the orientation of cortical microtubules adjacent to the outer cell wall of the outer epidermis. This gradient, in turn, is controlled by a light-induced redistribution of auxin. The hypothesis was tested by following the behaviour of microtubules for various light stimuli using indirect immunofluorescence in epidermal strips as assay. Analysis of gravitropic straightening, nastic curvature on the horizontal clinostat, effects of tonic irradiation with red and/or blue light, and experiments involving opposing light pulses demonstrate that bending direction and microtubule orientation gradients are not as closely linked as predicted: Considerable bending can be produced without detectable gradients of microtubule orientation, and conspicuous gradients of microtubule orientation are not necessarily expressed as corresponding curvature. Thus, a monocausal relationship between microtubules and tropism is excluded. Furthermore, a comparison of tonic light effects on microtubules to earlier studies into the impact of light upon auxin content indicate that the relationship between auxin and microtubules might be more complex than hitherto assumed. It is concluded that, at least in maize coleoptiles, growth can be regulated by various mechanisms, and that microtubules, although somehow related to tropism, are probably not the cause of the fast tropistic responses.

**Key words:** Blue light — Microtubules — Nastic response — Phototropism — Red light — *Zea* (phototropism).

More than a century after Darwin's first investigation into the mechanism of the phototropic response (Darwin and Darwin 1881), those mechanisms still have remained obscure. Blaauw (1918) proposed that tropism results from the summation of locally restricted light-growth responses. In contrast, the Cholodny-Went theory (Cholodny 1927, Went 1928) suggested that tropistic stimulation evoked a displacement of growth-promoting factors ("auxins") from the lighted towards the shaded side of the organ. "Auxin" increases the extensibility of the outer epidermis by causing a cell-wall relaxation (Masuda 1990). This relieves the constraints put upon cortex extension by the limited extensibility of the epidermis (Kutschera et al. 1987, Masuda 1990). The so called acid-growth theory (Rayle and Cleland 1970, Hager et al. 1971) supposed that auxin achieves this effect by stimulating a proton pump in the plasma membrane, which in turn activates glycanases in the cell wall. However, the predicted auxin-mediated changes of pH could not be detected (Kutschera and Schopfer 1985). Alternative, biomechanical approaches (Green and King 1966, Green 1969) stressed the point that, in a wide range of species, the wall of elongating cells was formed by *transverse* cellulose fibres. The microtubule-microfibril hypothesis claims that microtubules adjacent to the cell membrane serve as leading trails for the movement of cellulose-synthesizing enzyme-complexes residing in the plasma membrane (Robinson and Quader 1982). However, the fine structure of the wall—microfibrils are found to be sometimes interwoven or intertwined (Preston 1988)—is not always consistent with this hypothesis. Nevertheless, in an impressive number of cases, cortical microtubules are found to be parallel to newly deposited microfibrils (Shibaoka 1974, Iwata and Hogetsu 1988, Lang et al. 1982, Bergfeld et al. 1988). Additionally, changes of mi-

Abbreviations: BL, blue light (450 nm); CR, clinostat rotation; RL, red light (660 nm).

[3] To whom correspondence should be addressed.

crofibril orientation are usually preceded by corresponding reorientations of cortical microtubules (Kristen 1985, 1986, Robinson and Quader 1982).

If there is a causal link between light, auxin, microtubules, and growth, one would expect considerable changes of microtubule arrays in response to phototropic stimulation. In fact, a phototropically optimal blue-light pulse could induce a large gradient of microtubule orientation in maize coleoptiles (Nick et al. 1990). Whereas microtubules in the shaded side of the plant maintained their transverse array, microtubules in the lighted flank switched into the longitudinal position. This movement initiated about 10 to 15 min after stimulation and was completed within 60 min, i.e., it preceded tropistic bending becoming visible from 20 to 30 min after stimulation. It should be mentioned that a gravitropic stimulus, too, was able to elicit such a gradient of microtubule orientation. From these data the following working hypothesis emerged: According to the Cholodny-Went hypothesis (Cholodny 1927, Went 1928), photo- or gravitropic stimulation induces a trans-organ displacement of a growth-promoting factor ("auxin") from the lighted (or upper) towards the shaded (or lower) flank of the plant. Depletion of auxin in the one side is followed by reorientation of cortical microtubules from their original transverse into the longitudinal array. In the auxin enriched side, however, they remain transverse. This gradient of microtubule orientation, as outlined by the microtubule-microfibril hypothesis (Robinson and Quader 1982), is translated into a corresponding gradient of microfibril orientation. Thus, the cell-wall extensibility in the auxin-depleted organ flank will diminuish, and cell elongation will cease. Eventually, differential growth will bring about the observed bending according to the stimulus.

This hypothesis averts two causal steps: (1) light controls microtubule orientation via auxin, and, (2) auxin controls growth via microtubule orientation. Support for this view comes from the correlation between the microtubule reorientation evoked by tropistic stimulation and changes of "auxin" content. In addition, microtubule reorientation occurs earlier than visible bending. As to transform those correlations into the causal chain proposed above, one has to investigate, whether microtubule reorientations are necessary and sufficient for explaining the light effect upon growth. This will be undertaken in the following.

## Materials and Methods

*Plants and light conditions*—Maize seedlings (*Zea mays* L. cv. Brio42HT, Asgrow; Bruchsal, F.R.G.; stored in the dark at 3°C) were raised as described in Nick and Schäfer (1988a, b). Immediately after sowing, they were kept for two days in 0.2 W $m^{-2}$ red light, as to suppress mesocotyl growth and nutations (Kunzelmann and Schäfer 1985). Thereafter, they remained in the dark for one day until the onset of experiments. As to circumvent effects of blue-light induced phytochrome-gradients (Hofmann and Schäfer 1987), the experiment was performed in a symmetrical and saturating red background-light (2.5 W $m^{-2}$). Blue light for phototropic induction was isolated as described in Nick and Schäfer (1988a, b). Further details on light sources and measurements are given therein.

*Stimulation treatments*—Three types of experiments were used involving unilateral, symmetrical, and alternative stimulation by blue light. In the *unilateral schedule*, seedlings were subjected to 1.9 $\mu$mol $m^{-2}$ blue light within the shorter coleoptile cross-section, and then maintained in upright position in the red background-illumination for variable time intervals. The *symmetrical schedule* involved a blue-light pulse of 1.9 $\mu$mol $m^{-2}$ from above (this was achieved by means of a mirror) with a subsequent waiting time of 1 h in upright position. Alternatively, plants were irradiated with the same blue-light fluence from above and transferred to a horizontal clinostat (0.5 rpm) for 12 h. It should be emphasized that from stimulation onwards the plants were held in saturating red light if not stated otherwise. As a control the blue light was omitted and plants maintained in upright position for 1 h or rotated for 12 h on the clinostat in the red light used for background illumination. The *alternating stimulation* implied two unilateral light-pulses of equal fluence (1.9 $\mu$mol $m^{-2}$, 30 s), but opposing direction, again parallel to the shorter cross-section of the coleoptile. Hereby, the second pulse was administered 120 min after the first. As to account for the asymmetry of the coleoptile within the short transverse axis (Nick and Schäfer 1989), special care was taken as to keep the spatial setup constant. Therefore, the first pulse was given such that the coleoptile flank adjacent to the caryopsis was facing the light. Then, the plants were put onto the clinostat until the counterstimulation was applied. Immediately afterwards they were returned to the clinostat and rotated under the red light until response evaluation. Special attention was paid as to minimize uncontrolled gravity stimulation. The beakers containing the seedlings were therefore mounted on the already moving clinostat. Moreover, in all experiments, irradiation time for blue light was confined to 30 s. This had the additional advantage to avoid influences of sensory adaptation (Iino 1987) caused by prolonged stimulation.

*Response evaluation*—Phototropic curvature was followed using the method of Nick and Schäfer (1988a, b). At given time points the orientation of cortical microtubules adjacent ot the outer cell wall of the outer epidermis was screened by means of indirect immunofluorescence-staining in epidermal strips utilising monoclonal anti-tubulin antibodies (Sera MAS 077b, Clone YL 1/2, Camon Labor Service; Wiesbaden, F.R.G.). Hereby, the two flanks of the coleoptile, marked by incisions,

were carefully separated. The tissue was fixed by 1% glutaraldehyde and 3% paraformaldehyde in microtubule-stabilizing buffer (100 mM piperazin-1,4-diethansulfonic acid (PIPES), purchased from Merck (Darmstadt, F.R.G.), 1 mM $MgCl_2 \cdot 6H_2O$, 5 mM ethylenglycol-bis-(2-aminomethyl)-tetra acetic acid (EGTA), purchased from Fluka (Buchs, Switzerland), pH 6.8) for one hour and prepared for staining as described in detail in Nick et al. (1990). The orientation of the epidermal strips on the slide glass was kept constant to be sure that the outer face of the epidermis was screened. This is necessary, because microtubules adjacent to the inner cell wall appear to be immobile and constantly maintain a transverse array (Bergfeld et al. 1988, Nick et al. 1990). By means of an epimicroscope (IM 35, Zeiss; Oberkochen, F.R.G.) cells were photographed and microtubule orientation was assessed for patches of one cell width in breadth and two cell widths in length. Within such patches, microtubule orientation was more or less homogeneous, whereas it could change considerably between different parts of a cell. Transverse microtubules were characterized by an angle of 0°, longitudinal microtubules by +/− 90°. Frequency distributions over microtubule orientation were constructed and checked for symmetry using a t-test (Snedecor and Cochran 1972). However, significant asymmetries could not be detected. Distributions comprise the data from 101 to 398 patches (63 to 197 cells) corresponding to 35 to 45 plants. For the unilateral and symmetrical stimulation schedule four replicas were undertaken, three for the experiments involving alternating stimuli.

## Results

*Behaviour of cortical microtubules during gravitropic straightening*—Since cortical microtubules can reorient in the response to phototropic as well as to gravitropic stimulation (Nick et al. 1990), their behaviour during gravitropic straightening was analysed. Following unilateral stimulation with a blue-light pulse of 1.9 $\mu$mol $m^{-2}$ for 30 s, curvature reached a maximum of more than 30° towards the light within two hours (Fig. 1a). Then it gradually decreased again. At about 10 h after the stimulus plants grew

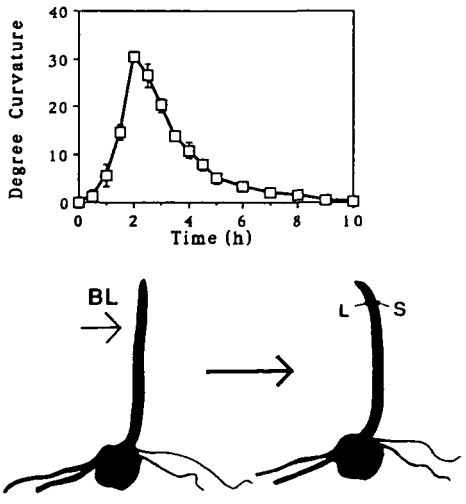


Fig. 1a

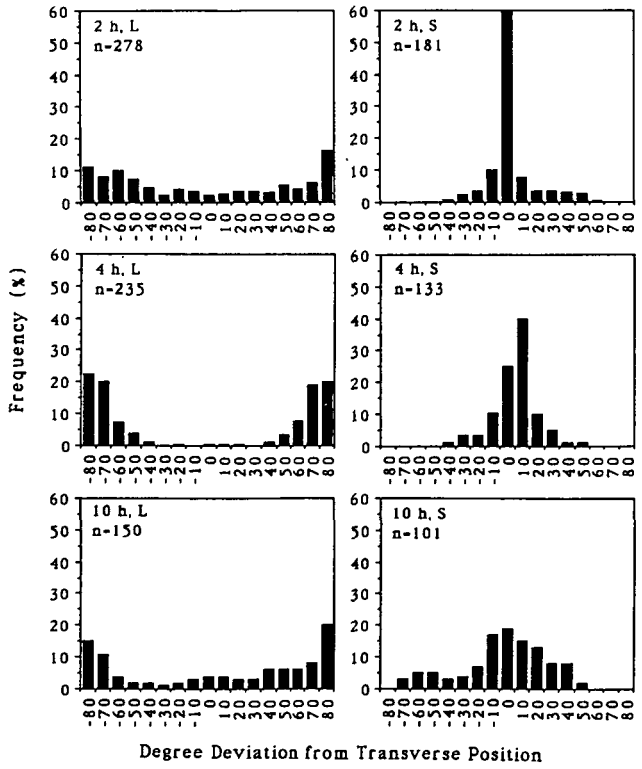


Fig. 1b

**Fig. 1** Orientation of cortical microtubules during gravitropic straightening. a) Time course of phototropic curvature following a phototropically optimal stimulation with a unilateral blue-light pulse of 1.9 $\mu$mol $m^{-2}$ for 30 s and curvature development under saturating red-light (2.5 W $m^{-2}$). Microtubule orientation was followed over time at the lighted (L) and shaded (S) side of the coleoptile. b) Microtubule orientation over time in the lighted and shaded side for the situation described in a) with 0° indicating transverse +/− 90° longitudinal microtubules.

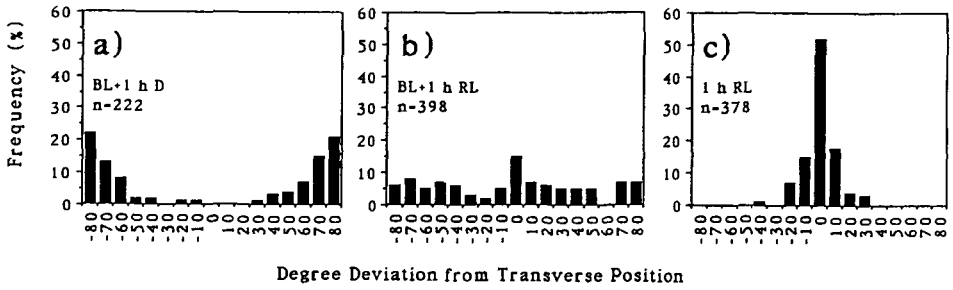


**Fig. 2** Orientation of cortical microtubules for symmetric irradiation. a) A blue-light pulse of 1.9 $\mu$mol $m^{-2}$ from above for 30 s, followed by 1 h incubation in the dark. b) As in a), but incubation under saturating red-light (2.5 W $m^{-2}$). c) As in b), but omitting the blue-light. Data represent one experiment out of four. The variation between experiments was typically in the range of 4 to 8 % per class.

straight again. This can be attributed to counteracting gravitropic stimulation, experienced by the seedlings after they have curved towards the light (Nick and Schäfer 1988a). The orientation of cortical microtubules in both flanks of the coleoptile was followed over the different stages of gravitropic straightening (Fig. 1b). If no phototropic stimulation is applied, microtubules are transverse in both flanks of the coleoptile (Nick et al. 1990). Two hours after stimulation, when straightening initiates, microtubules are clearly not transverse any more at the lighted, but they are clearly transverse at the shaded side of the organ. This is to be expected from previous work (Nick et al. 1990). Four hours after stimulation, when straightening was very fast, nevertheless, microtubules were longitudinal in the lighted, and transverse in the shaded side. It should be kept in mind that, at this time, the lighted side grew *faster* than the shaded side. This gradient of microtubule orientation persisted to at least 10 h after the stimulus (Fig. 1b), when straightening had been completed. However, the transverse orientation of microtubules at the shaded side gradually faded somewhat. It should be considered that at this time the growth of coleoptiles stops and in some cases the primary leaf already breaks through, such that the supply with endogenous auxin might be limited. This might explain, why many cells showed oblique microtubules causing a broadening of the distribution (Fig. 1b). But, still, microtubules at the shaded side were significantly more transverse than those at the lighted side.

*Response of cortical microtubules to symmetric irradiation*—If the same blue-light pulse (1.9 $\mu$mol $m^{-2}$, 30 s) was given from above, followed by incubation in the dark for 1 h, microtubules were found to be longitudinal (Fig. 2a). However, if the incubation was performed in a saturating, red background-light (2.5 W $m^{-2}$), the situation was not as unequivocal (Fig. 2b): in addition to those cells having longitudinal microtubules, a conspicuous proportion of cells exhibited transverse or oblique microtubules. If the plants were irradiated for 1 h with red light only (Fig. 2c), the situation became simple again. Now, a clear transverse orientation prevailed.

*Clinostat experiments*—During rotation on a horizontal clinostat maize coleoptiles show a very strong nastic bending towards the caryopsis (Nick and Schäfer 1989). Under saturating red light (2.5 W $m^{-2}$), the resulting curvature amounted to about 80° towards the caryopsis (Fig. 3a, upper row). The microtubules were transverse (Figs. 3b, upper row) without any significant difference between the slower and the faster growing side of the organ. If the plants were subjected to a blue-light pulse (1.9 $\mu$mol $m^{-2}$, 30 s) from above prior to the clinostat rotation (Fig. 3a, lower row), the resulting curvature was smaller, but with about 50° still considerable. Here, microtubules were longitudinal on both sides of the organ (Fig. 3b, lower row, Fig. 3c). Again, no significant difference between the slower and the faster growing side could be detected.

*Counterstimulation experiments*—Experiments involving two opposing blue-light pulses revealed long-term effects of phototropic stimulation ("spatial memory"), which could be separated from curvature itself (Nick and Schäfer 1988b). A similar set-up was chosen, as to test, whether, similar to spatial memory, microtubule reorientation can be separated from curvature itself. Herefore, a light pulse (1.9 $\mu$mol $m^{-2}$, 30 s), hitting the flank of the coleoptile, which was adjacent to the caryopsis (Fig. 4a), was followed 2 h later by a pulse of equal strength but opposing direction. Following the counterpulse, curvature first decreased considerably—the plants moved towards the counterpulse. About 1 h after the counterstimulation, plants were almost straight again. But then a new bending towards the first stimulation initiated yielding final curvatures of more than 100°. These changes in the direction of curvature, however, were not accompanied by corresponding changes of microtubule orientation (Figs. 4b): Microtubules were found to be longitudinal on the side facing the first light pulse, and transverse on the opposite side throughout the experiment. Especially striking is this pat-

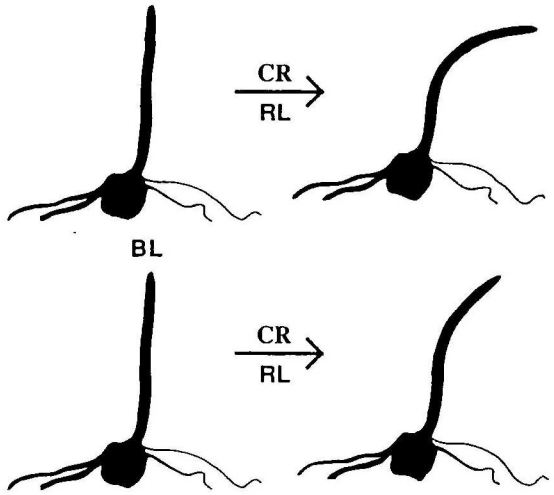


Fig. 3a

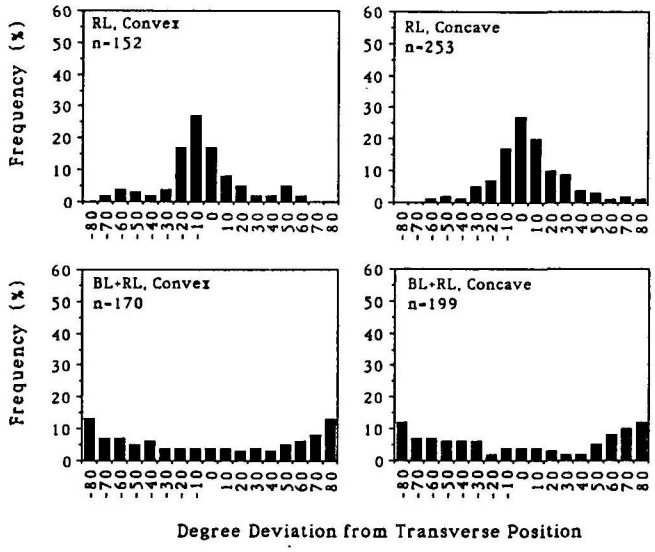


Fig. 3b

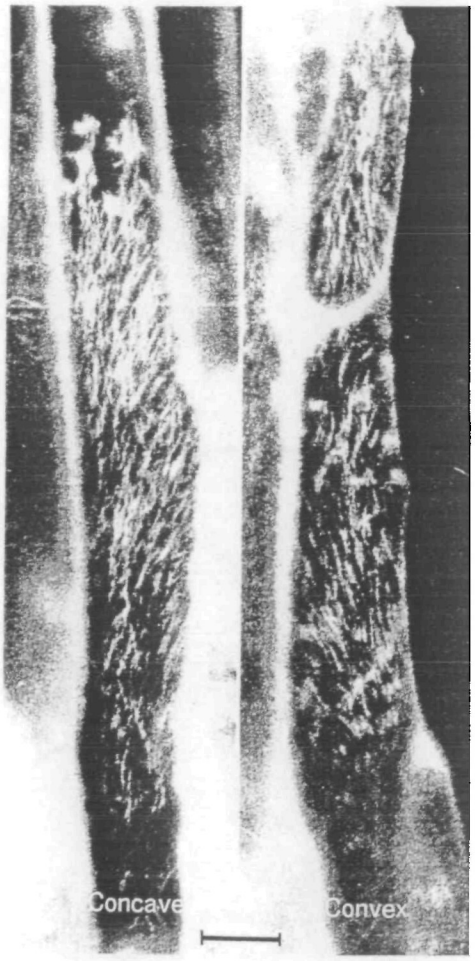


Fig. 3c

**Fig. 3** Orientation of cortical microtubules during nastic curvature. a) Induction of nastic curvature: plants were rotated on a horizontal clinostat with 0.5 rpm under saturating red light 2.5 W $m^{-2}$ for 12 h (upper row). Under these conditions, plants curved by 80° towards the caryopsis. If plants were irradiated with 1.9 $\mu$mol $m^{-2}$ blue light for 30 s from above prior to clinostat rotation (lower row) the resulting curvature was about 50°. b) Microtubule orientation after 12 h of clinostat rotation for the situations described in a) at the convex and concave coleoptile flank. One experiment out of four is shown. Variation between experiments was typically in the range of 2 to 7% per class. c) Immunofluorescence images for the concave and the convex side for 12 h of clinostat rotation after preceding irradiation with blue light. The bar represents 10 $\mu$m.

tern during the transiently negative bending (within the first hour after the counterpulse), because here microtubules were longitudinal at the *faster* growing side, but transverse at the *slower* growing side.

## Discussion

*Is microtubule reorientation a necessary precondition for bending?*—The original hypothesis, proposed by Nick et al. (1990), suggested a causal chain connecting blue-light induced auxin shift (Cholodny 1927, Went 1928) via auxin dependent reorientation of cortical microtubules in the lighted organ flank to differential growth. If this held true, one could predict the following: (1) There is no bending without gradients of microtubule orientation across the coleoptile cross-section. (2) There is no auxin-mediated

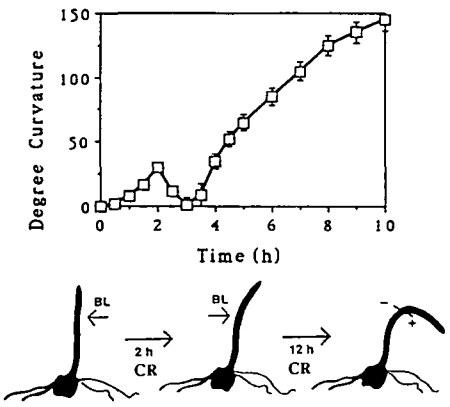


Fig. 4a

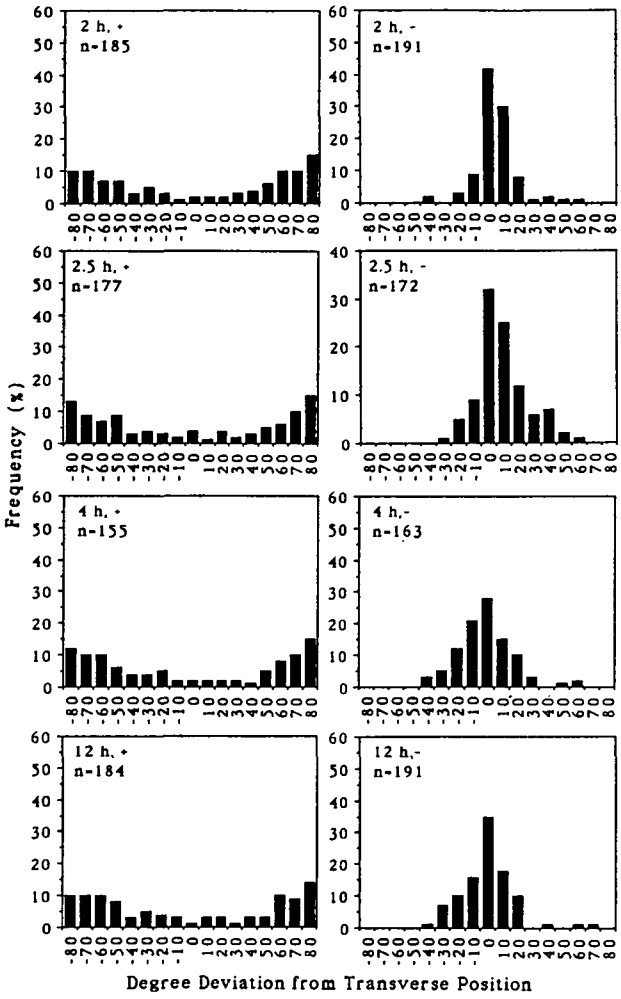


Fig. 4b

**Fig. 4** Orientation of cortical microtubules for opposing stimulation. a) Time course of curvature for phototropically optimal pulse stimulation with blue light (1.9 $\mu$mol $m^{-2}$, 30 s) from the coleoptile flank facing the caryopsis, and opposing blue-light irradiation with the same fluence 2 h later. Between and after the stimulations plants were kept on a horizontal clinostat under saturating red light (2.5 W $m^{-2}$). b) Microtubule orientation over time for the situation described in a) at the side facing the first pulse (+) and the opposite (−) side. One experiment from a set of three replicas is shown. Variation per class amounted to 1 to 8%, except for the 4 h and 12 h distributions, where it was somewhat larger (1 to 13% per class).

elongation growth without cortical microtubules. Fig. 3 shows that the first prediction is wrong: By clinostat rotation conspicuous curvatures can be produced without any gradient of microtubule orientation and this for transverse (Fig. 3a, b, upper row) as well as for longitudinal (Fig. 3a, b lower row) microtubules on both sides of the organ.

The second prediction is disproven by colchicin experiments (Edelmann 1989). That author was able to show that auxin-mediated elongation growth of coleoptile segments could take place in the presence of colchicine for up to 24 h after the onset of drug application. Immunofluorescence staining of cortical microtubules in epidermal strips demonstrates that colchicin eliminates microtubules within 2 h (Nick 1990). After that time no intact microtubules were detectable. Recent experiments (Nick et al. 1991) with the microtubule-eliminating drugs ethyl-*N*-phenylcarbamate and propyzamide revealed that phototropism could proceed, although cortical microtubules in the epidermis had been removed by the drugs (gravitropism, on the other hand, was inhibited). The conclusion is that microtubule reorientation is *not* a necessary precondition for curvature. Auxin can mediate growth by an additional, microtubule-independent, mechanism. The clinostat-elicited curvature is either using this pathway or it does not rely on auxin at all.

*Is microtubule reorientation a sufficient precondition*

*for bending?*—A third prediction made by the initial hypothesis (Nick et al. 1990) claims that, if there is a gradient of microtubule orientation across the organ, there should always be differential growth. Hereby, the direction of bending should be defined by the direction of the gradient in the orientation of microtubules. Plants should curve towards that side, where cortical microtubules are *longitudinal*. Again, this prediction is not consistent with the observation. During gravitropic straightening (Fig. 1a, b), the plants curve towards the side, where microtubules are *transverse*, which is most prominent at 4 h after stimulation. Moreover, 10 h after the light pulse plants grow straight, although there is still a large gradient of microtubule orientation. The hypothesis further predicts that the initial gradient of orientation (longitudinal in the lighted side, transverse in the shaded side) should become inverse just *before* the onset of gravitropic straightening, i.e., somewhat earlier than 2 h after stimulation. This is not the case.

A similar situation was observed in the counterstimulation experiments (Fig. 4a, b). Here, immediately after the counterpulse, plants curved towards the side, where microtubules were transverse, i.e., *opposite* to the gradient of microtubule orientation. However, it should be underlined that the stable, final bending on the clinostat was again parallel to the gradient of microtubule orientation.

Nevertheless, the experiments represented by Figs. 1a, b and 4a, b demonstrate that symmetric growth can occur, although there is a gradient in the orientation of microtubules across the coleoptile cross-section (Fig. 1b, situation 10 h after the stimulus). Moreover, the gradient of growth and the gradient of microtubule orientation are sometimes opposing each other (Fig. 1b, situation 4 h after the stimulus, and Fig. 4a, b, situation in the first hour after counterstimulation). The conclusion is that microtubule reorientation is *not* a sufficient precondition for bending.

*Is the light effect upon microtubule reorientation mediated by auxin?*—It has been shown by several researchers that irradiation with red light lowers the auxin content in the subapical part of graminean coleoptiles considerably (Briggs 1963, Furuya et al. 1969, Iino 1981). In maize coleoptiles, a saturating red-light pulse reduces auxin by about 50% (Iino 1981). One would therefore expect that irradiation with red light should be correlated to longitudinal microtubules. Again, the data cannot be reconciled with the prediction made by the hypothesis. Not only microtubules are found to be transverse in red-light treated plants (Fig. 2c), but they even *turn* from longitudinal to transverse in response to red light (Figs. 2a, b). Whereas the longitudinal orientation of microtubules induced by symmetric blue light (Fig. 2a) could be brought about by a light-dependent auxin depletion (Thornton and Thimann 1967, Pickard 1985), this is not possible for red light. Thus, at least red light (presumably mediated by phytochrome) can act via a pathway not relying on changes of *overall* auxin content. Preliminary experiments show that gibberellic acid can reorient microtubules from longitudinal to transverse under conditions of auxin depletion.

*Conclusion: Microtubule reorientation is not the cause for phototropism*—Concluding, one has to state that microtubule reorientations are neither necessary nor sufficient for differential growth. This contradicts a monocausal relationship between light, auxin, microtubules, and growth. The initial hypothesis proposed by Nick et al. (1990) has therefore to be replaced by a more sophisticated view: (1) Light can act upon microtubule orientation via light-dependent changes in the level of auxin (Pickard 1985). In addition, red light turns microtubules into the transverse position, which is not expected from its effects upon overall auxin content. This might be brought about by a different mediator, for instance gibberellic acid. (2) Auxin *can* stimulate growth without using microtubules, for instance after they have been eliminated by colchicin (Edelmann 1989). Moreover, differential growth *can* occur without any gradients of microtubule orientation (Fig. 3a–c). On the other hand, straight growth *can* occur on the background of a gradient in microtubule orientation across the organ (Fig. 1a, b). It is even observed that the flank with longitudinal microtubules *can* grow faster than the flank with transverse microtubules (Fig. 1a, b, 4 h after induction, Fig. 4a, b, first hour after the counterpulse). This means that auxin can regulate growth by several means, only one of which is based upon microtubules.

Thus, the light-induced gradient of microtubule orientation with certainty is *not* the cause for tropism. The question remains, what the function of such a gradient might be. The experiment of Fig. 4a, b gives a first hint. Although visible bending on the clinostat changes its direction in response to the counterpulse (Fig. 4a), the gradient of microtubule orientation remains stable throughout the experiment (Fig. 4b). It is congruent with the stable, final movement on the clinostat, not with the short-term, transient curving towards the counterpulse. This resembles the phototropically induced spatial memory, which is established within 2 h after a blue-light pulse (Nick and Schäfer 1988b). It appears, as if microtubules were related to long-term effects of tropism rather than the fast tropistic bending itself.

This work was supported by the Deutsche Forschungsgemeinschaft, a grant of the Studienstiftung des Deutschen Volkes and a grant of the Science and Technology Agency to P.N.

## References

Bergfeld, R., Speth, V. and Schopfer, P. (1988) Reorientation of microfibrils and microtubules at the outer epidermal wall of

maize coleoptiles during auxin-mediated growth. *Bot. Acta* 101: 57–67.

Blaauw, A. H. (1918) Licht und Wachstum III. *Med. Landbouwhogeschool* 15: 89–204.

Briggs, W. R. (1963) Red light, auxin relationships, and the phototropic responses of corn and oat coleoptiles. *Amer. J. Bot.* 50: 196–207.

Cholodny, N. (1927) Wuchshormone und Tropismen bei Pflanzen. *Biol. Zentralblatt* 47: 604–626.

Darwin, C. and Darwin, F. (1881) *Das Bewegungsvermoegen der Pflanzen*. J. v. Carus, Stuttgart.

Edelmann, H. (1989) Untersuchungen zur Zellwandsynthese während des Auxin-induzierten Zellstreckungswachstums von Maiskoleoptilen (*Zea mays* L.). Inaugural-Dissertation, Albert-Ludwigs-University Freiburg i. Br., F.R.G.

Furuya, M., Pjon, D. J., Fujii, T. and Ito, M. (1969) Phytochrome action in *Oryza sativa* L. III: The separation of photoreceptor site and growing zone in coleoptiles and auxin transport as effector system. *Develop. Growth Differ.* 11: 62–76.

Green, P. B. (1969) Cell morphogenesis. *Annu. Rev. Plant Physiol.* 20: 365–394.

Green, P. B. and King, A. (1966) A Mechanism for the origin of specifically oriented textures with special reference to *Nitella* wall texture. *Aust. J. Biol. Sci.* 19: 421–437.

Hager, A., Menzel, H. and Krauss, A. (1971) Versuche und Hypothesen zur Primaerwirkung des Auxins beim Streckungswachstum. *Planta* 100: 47–75.

Hofmann, E. and Schäfer, E. (1987) Red-light induced shift of the fluence-response curve for first positive curvature of maize coleoptiles. *Plant Cell Physiol.* 28: 37–45.

Iino, M. (1981) Control of maize seedling growth: Light and auxin. Ph. D. Thesis, Australian Natl. University.

Iino, M. (1987) Kinetic modelling of phototropism in maize coleoptiles. *Planta* 171: 110–126.

Iwata, K. and Hogetsu, T. (1989) Arrangement of cortical microtubules in *Avena* coleoptiles and mesocotyls and *Pisum* epicotyls. *Plant Cell Physiol.* 29: 807–815.

Kristen, U. (1985) The cell wall. *Progr. Bot.* 47: 1–18.

Kristen, U. (1986) The cytoskeleton: Microtubules. *Progr. Bot.* 48: 1–22.

Kunzelmann, P. and Schäfer, E. (1985) Phytochrome-mediated phototropism in maize mesocotyls. Relation between light and $P_{fr}$ gradients, light growth response and phototropism. *Planta* 165: 424–429.

Kutschera, U., Bergfeld, R. and Schopfer, P. (1987) Cooperation of epidermal and inner tissues in auxin-mediated growth of maize coleoptiles. *Planta* 170: 168–180.

Kutschera, U. and Schopfer, P. (1985) Evidences against the acid-growth theory of auxin action. *Planta* 163: 483–493.

Lang, J. M., Eisinger, W. R. and Green, P. B. (1982) Effects of ethylene on the orientation of microtubules and cellulose microfibrils of pea epicotyl cells with polylamellate cell walls. *Protoplasma* 110: 5–14.

Masuda, Y. (1990) Auxin-induced cell elongatin and cell wall changes. *Bot. Mag. Tokyo* 103: 345–370.

Nick, P. (1990) Versuch über Tropismus, Querpolarität und Mikrotubuli. Inaugural-Dissertation, Albert-Ludwigs-Universität, Freiburg i. Br., F.R.G.

Nick, P., Bergfeld, R., Schäfer, E. and Schopfer, P. (1990) Unilateral reorientation of microtubules at the outer epidermal wall during photo- and gravitropic curvature of maize coleoptiles and sunflower hypocotyls. *Planta* 181: 162–168.

Nick, P. and Schäfer, E. (1988a) Interaction of gravi- and phototropic stimulation in the response of maize (*Zea mays* L.) coleoptiles. *Planta* 173: 213–220.

Nick, P. and Schäfer, E. (1988b) Spatial memory during the tropism of maize (*Zea mays* L.) coleoptiles. *Planta* 175: 380–388.

Nick, P. and Schäfer, E. (1989) Nastic response of maize (*Zea mays* L.) coleoptiles during clinostat rotation. *Planta* 179: 123–131.

Nick, P., Schäfer, E., Hertel, R. and Furuya, M. (1991) On the putative role of microtubules in gravitropism of maize coleoptiles. *Plant Cell Phisiol.* 32: 873–880.

Pickard, B. G. (1985) Role of hormones in phototropism. *In* Encyclopedia of Plant Physiology, N. S., vol. 11, Hormonal Regulation of Development III. Edited by Pharis, R. P. and Reid, D. M. pp. 365–417. Springer, Berlin.

Preston, R. D. (1988) Cellulose-microfibril-orienting mechanisms in plant cell walls. *Planta* 174: 64–74.

Rayle, D. C. and Cleland, R. E. (1970) Enhancement of wall loosening and elongation by acid solutions. *Plant Physiol.* 46: 250–253.

Robinson, D. G. and Quader, H. (1982) The microtubule-microfibril syndrome. *In* The Cytoskeleton in Plant Growth and Development. Edited by Lloyd, C. W. pp. 109–126. Academic Press, London.

Shibaoka, H. (1974) Involvement of wall microtubules in gibberellin promotion and kinetin inhibition of stem elongation. *Plant Cell Physiol.* 15: 255–263.

Snedecor, G. W. and Cochran, W. G. (1972) *Statistical Methods*. Iowa State University Press, Ames.

Thornton, R. M. and Thimann, K. V. (1967) Transient effects of light on auxin transport in the *Avena* coleoptile. *Plant Physiol.* 42: 247–257.

Went, R. W. (1928) Wuchsstoff und Wachstum. *Rec. Trav. Bot. Neerl.* 25: 1–116.

(Received April 26, 1991; Accepted July 17, 1991)