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Unilateral reorientation of microtubules at the outer epidermal wall during photo- and gravitropic curvature of maize coleoptiles and sunflower hypocotyls*

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Abstract. Auxin (indole-3-acetic acid) controls the orientation of cortical microtubes (MT) at the outer wall of the outer epidermis of growing maize coleoptiles (Bergfeld, R., Speth, V., Schopfer, P., 1988, Bot. Acta **101**, 57–67). A detailed time course of MT reorientation, determined by labeling MT with fluorescent antibodies, revealed that the auxin-mediated movement of MT from the longitudinal to the transverse direction starts after less than 15 min and is completed after 60 min. This response was used for a critical test of the functional involvement of auxin in tropic curvature. It was found that phototropic (first phototropic curvature) as well as gravitropic bending are correlated with a change of MT orientation from transverse to longitudinal at the slower-growing organ flank whereas the transverse MT orientation is maintained (or even augmented) at the faster-growing organ flank. These directional changes are confined to the MT subjacent to the outer epidermal wall. The same basic results were obtained with sunflower hypocotyls subjected to phototropic or gravitropic stimulation. It is concluded that auxin is, in fact, involved in asymmetric growth leading to tropic curvature. However, our results do not allow us to discriminate between an uneven distribution of endogenous auxin or an even distribution of auxin, the activity of which is modulated by an unevenly distributed inhibitor of auxin action.

Key words: Auxin (unilateral growth) – Gravitropism (coleoptile, hypocotyl) – *Helianthus* (microtubules in tropisms) – Microtubule (cell extension) – Phototropism (coleoptile, hypocotyl) – *Zea* (microtubules in tropisms)

Introduction

The involvement of auxin in the phototropic and gravitropic curvature of axial shoot organs (e.g., coleoptile, hypocotyl) is still a matter of controversial debate. The differential growth on the opposite organ flanks underlying tropic bending was originally explained by the Cholodny-Went hypothesis (Went and Thimann 1937) which states that unequal cell elongation is caused by a lateral movement of auxin from the slower-growing side to the faster-growing side of the organ. Incorporating the fact that organ growth is controlled by the epidermis (or a few peripheral cell layers), a more recent modification of this hypothesis postulates that auxin accumulation in the faster-growing side – and auxin depletion in the slower-growing side – is brought about by a corresponding transport of the hormone between epidermal and inner cell layers (Iwami and Masuda 1976; McDonald and Hart 1987). In a critical review on this subject, Firn and Digby (1980) reached the conclusion that there is no compelling evidence for the causal involvement of auxin in tropic curvature. On the other hand, in a more recent comprehensive article (devoted exclusively to phototropism) Briggs and Baskin (1988) expressed the view "that in a great many cases the Cholodny-Went hypothesis is sufficient to account for the growth changes bringing about curvature".

A possibility of contributing to a resolution of this controversy is offered by the recent finding that auxin-induced growth of maize coleoptile segments is correlated with a concomitant reorientation of microtubules (MT) in the outer epidermis (Bergfeld et al. 1988). This auxin-mediated response is strictly confined to the outer periclinal wall of the epidermis which demonstrates a parallel change in the direction of newly deposited microfibrils at its inner surface. Although definite proof for a direct causal relationship between MT and microfibril orientation is not yet available (see Preston (1988) for a critical assessment of this point), it is quite clear that auxin controls the orientation of both MT and microfibrils specifically at the growth-controlling wall of the coleoptile. Both in terms of tissue specificity as well as temporal expression the effect of auxin is qualitatively different from the effects of other hormones on MT orientation (ethylene: Lang et al. 1982; Roberts et al. 1985; kinetin: Shibaoka 1974; Volfová et al. 1977; gibberellin: Shibaoka 1974; Sawhney and Srivastava 1975; Volfová

* The authors dedicate this paper to Professor Dr. Drs. h.c. Hans Mohr on the occasion of his 60th birthday

Abbreviations: IAA = indole-3-acetic acid; MT = cortical microtubules; OEW = outer epidermal wall (outer epidermis of maize coleoptile)

et al. 1977) and can therefore be used as a diagnostic test for an auxin-mediated growth response. In the present paper we use this cytological feature as a marker for the disputed uneven effectiveness of endogenous auxin in the epidermis of tropically bending maize coleoptiles and sunflower hypocotyls.

Material and methods

Plant materials. For experiments with indole-acetic acid (IAA) subapical coleoptile segments (10 mm long, 3 mm below the tip) were isolated from 4-d-old maize seedlings (*Zea mays* L., cv. Brio, from Asgrow Comp., Buxtehude, FRG) grown in darkness (with a pulse of red light 16 h before harvest) at 25° C as described previously (Kutschera and Schopfer 1985). Segments were incubated in aerated IAA solution (10 $\mu mol \cdot l^{-1}$) in normal laboratory light.

Maize seedlings used for photo- and gravitropic experiments were grown at 25° C for 2 d in weak red light (0.3 $W \cdot m^{-2}$) followed by 1 d in darkness. This treatment results in seedlings with repressed mesocotyl growth and straight coleoptiles responding rapidly and uniformly to tropic stimulations (Nick and Schäfer 1988). The seedlings were kept for 1 h under red light (2.3 $W \cdot m^{-2}$) and then subjected either to a phototropic stimulation (unilateral blue-light pulse: 30 s, 450 nm, 1.9 $\mu mol \cdot m^{-2}$, followed by various periods under red light), or a gravitropic stimulation (1 h horizontal orientation under red light). The flat side of the coleoptile opposite to the caryopsis was always oriented towards the blue-light source (phototropism) or at the upper side (gravitropism). After labeling the side oriented towards the stimulus, coleoptile segments of about 6 mm length (3 mm below the tip) were isolated and immediately fixed under red light.

Sunflower (*Helinanthus annuus* L. from Hambrecht, Freiburg, FRG) seedlings were grown at 25° C on damp vermiculite in white light from fluorescent lamps (5 klx) for 6 d. Seedlings were oriented towards the stimulus for 2 h in a random axial position.

Immunocytochemistry. The following procedure is a modified version of the method used by Quader et al. (1986). Incubations were performed in 3-ml plastic vials kept under slow rotating movement. Coleoptile segments were fixed for 1 h at room temperature in microtubule-stabilizing buffer [MSB = 0.1 $mol \cdot l^{-1}$ 1,4-piperazine-di-ethanesulfonic acid (Pipes), 1 $mmol \cdot l^{-1}$ $MgCl_2$, 5 $mmol \cdot l^{-1}$ ethylene glycol-bis (β-aminomethyl ether)-N,N,N′,N′-tetraacetic acid (EGTA); pH 6.8 adjusted with NaOH] containing 50 $g \cdot l^{-1}$ *p*-formaldehyde and 50 $ml \cdot l^{-1}$ dimethylsulfoxide. After three washings (5 min each) in MSB and 15 min of incubation in $NaBH_4$ followed by three washings in MSB, epidermal strips were peeled from the flat sides of the segments and extracted with Nonidet P-40 (Sigma, Deißenhofen, FRG; 20 $ml \, l^{-1}$) in MSB for 30 min. After five washings in MSB, portions of 10–20 epidermal strips were incubated for 1 h at 37° C in 50–100 µl phosphate-buffered saline (PBS = 8 $g \cdot l^{-1}$ NaCl, 0.2 $g \cdot l^{-1}$ KCl, 1.44 $g \cdot l^{-1}$ Na_2HPO_4, 0.2 $g \cdot l^{-1}$ KH_2PO_4, 0.132 $g \cdot l^{-1}$ $CaCl_2 \cdot 6\,H_2O$, 0.1 $g \cdot l^{-1}$ $MgCl_2 \cdot 6\,H_2O$; pH 7.3 adjusted with NaOH) containing rat antibodies directed against tubulin (monoclonal anti-tubulin, clone YL 1/2; Sera-Lab., Cameron, Wiesbaden, FRG; dilution 1:15). After three washings with PBS the strips were incubated for 1 h at 37° C in 50–100 µl PBS containing fluorescein isothiocyanate(FITC)-labeled secondary antibody (anti-rat IgG from Sigma, dilution 1:15). After washing three times with PBS and incubating with PBS containing 1 $ml \cdot l^{-1}$ Triton X-100 overnight the strips were mounted in PBS-glycerol (1:1, v/v) containing 10 $g \cdot l^{-1}$ *p*-phenylenediamine and viewed under a fluorescent microscope (UM 35; Zeiss, Oberkochen, FRG). Photographs were taken on Kodak (Rochester, N.Y., USA) TriX pan (400 ASA) film using a Zeiss Neofluar 40 lens.

Determination of MT orientation. The orientation of MT at the outer face of epidermal cells was generally not homogeneous but changed along the long cell axis. However, within short cell surface sections of a length of two to three times cell width, the MT were virtually parallel and their direction could be described by the angle formed between the array of MT and the longitudinal cell axis. Therefore such cell sections were selected at random in photographs of epidermal strips and the MT angle measured using a protractor. Angular distributions are based on 68–285 measurements obtained from at least 20 different epidermal strips per distribution. All experiments were repeated at least four times. In test experiments with epidermal strips marked at the basal end we always obtained symmetrical MT distributions, irrespective of whether the strips were viewed upside up or upside down, indicating a random distribution of 'left-turns' and 'right-turns' in cell sections with oblique MT directions. Therefore the polar orientation of epidermal cells was disregarded in subsequent experiments.

Results

Kinetics of auxin-induced microtubule reorientation. The effect of external application of IAA on the orientation of MT at the outer epidermal wall (OEW) of maize coleoptiles (Bergfeld et al. 1988) was reinvestigated by labeling MT with fluorescent antibodies (Lloyd 1987). Since a rapid response to IAA was expected, based on our previous electron-micrographic analysis, it was necessary to make sure that the fixation procedure used was rapid enough in order to provide a true image of MT position during IAA-induced movement. Figure 1 shows the effect of the fixation medium on IAA-mediated growth of coleoptiles, compared with the effect of an equiosmolal mannitol medium. As expected, a 5-min incubation in osmoticum leads to shrinkage of the segment followed by a rapid resumption of growth upon removal of the osmoticum. Fixation medium of equal osmotic potential produces quite different elongation kinetics. Osmotic shrinkage is stopped within 3 min and the seg-

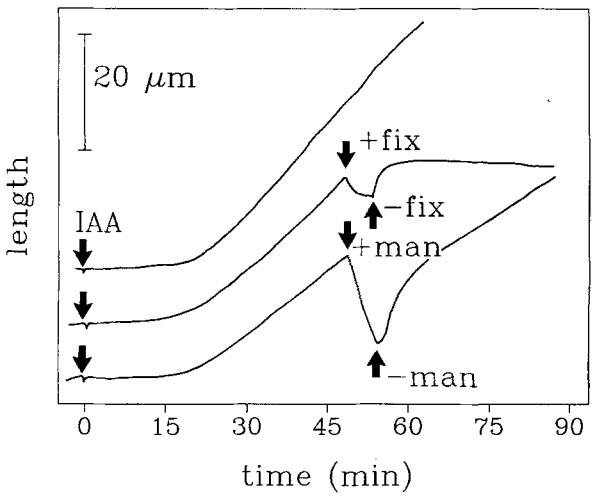

Fig. 1. Effect of fixation medium on IAA-mediated elongation of maize coleoptile segments. Indole-3-acetic acid (10 $\mu mol \cdot l^{-1}$) in distilled water was added to single segments 1 h after cutting and their growth recorded with a displacement transducer (Kutschera and Schopfer 1985). After about 50 min of steady-state growth a segment was transferred to fixation medium + IAA (*+fix*) and after further 5 min back to IAA solution (*−fix*). As an osmotic control, a segment was similarly treated with a mannitol solution (including IAA) of equal osmolality (*+man, −man*)

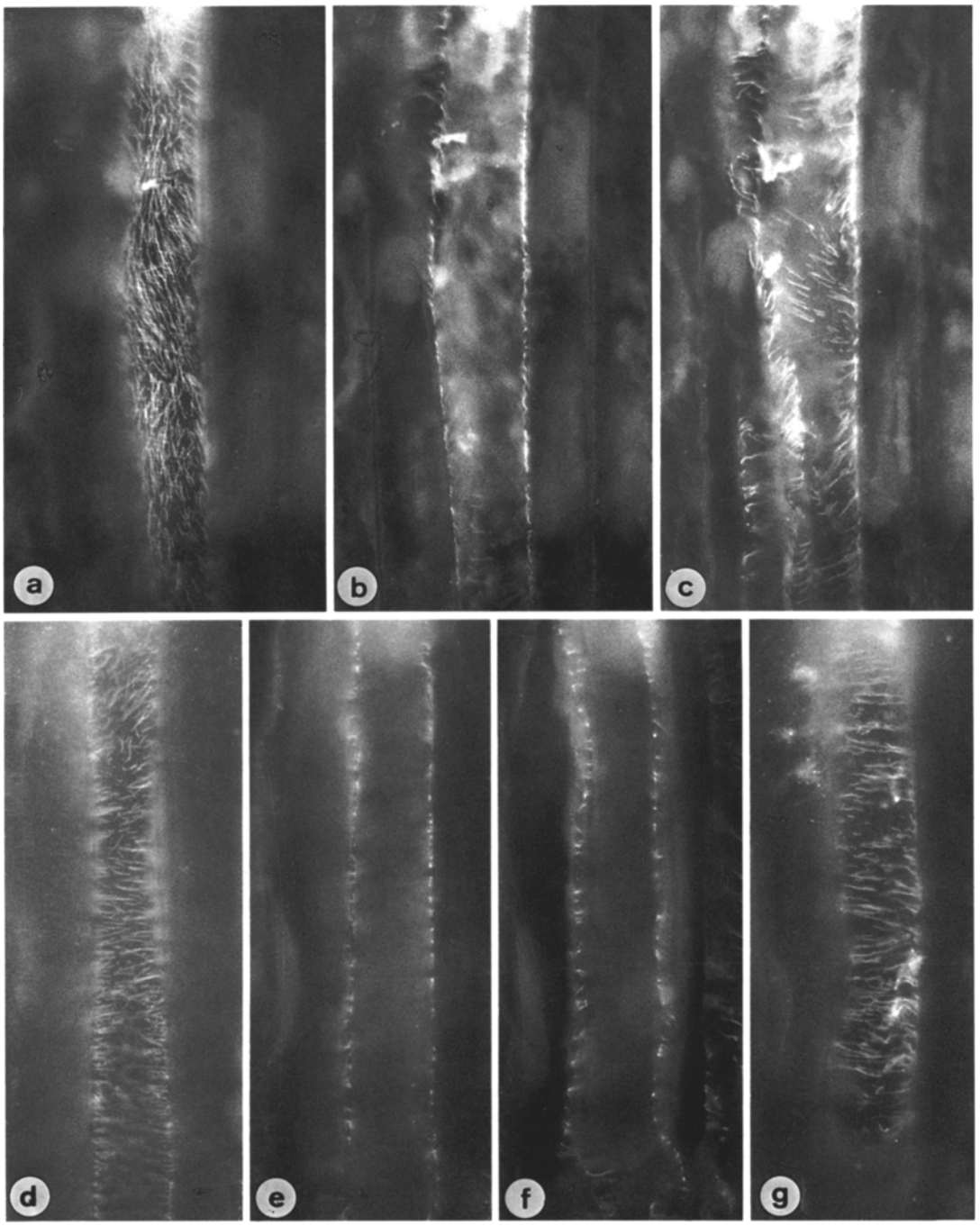

Fig. 2a–g. Orientation of MT in the outer epidermis of maize coleoptile segments in the absence and presence of IAA. Treatment after cutting: incubation for 2 h in water (**a–c**) or 2 h in water followed by 45 min in IAA solution (10 $\mu mol \cdot l^{-1}$, **d–g**). The focal plane was located at the outer surface (OEW), middle region, or inner surface of epidermal cells (from *left* to *right*). The long cell axis represents the long axis of the coleoptile

ment does not continue to elongate after retransferring it to IAA solution without fixative. These data indicate that the *p*-formaldehyde (5%) used for tissue fixation in the following experiments penetrated into the growth-controlling epidermal cells within less than 5 min.

Figure 2 shows the arrangement of MT in the epidermal cells of water- and IAA-treated coleoptile segments as documented by immunofluorescent labeling. By shifting the focal plane of the microscope it is possible to delineate the MT pattern of both the outer and the inner periclinal face of the epidermis. Confirming previous observations with electron-microscopic techniques, the pictures of Fig. 2 demonstrate that the MT of the inner periclinal cell face are transversely (=perpendicular to the long cell axis) oriented both in the absence and in the presence of IAA. In contrast, the MT of the outer cell face show a transverse pattern only after treatment with IAA. The MT arrangement induced by exogenous IAA in water-pretreated (IAA-depleted) segments is similar to the arrangement observed in the intact coleoptile (see Fig. 5). In the absence of IAA the orientation of MT at the outer epidermal face is more or less longitudinal (=parallel to the long cell axis). Thus, the IAA effect on MT orientation is restricted to the population of MT localized at the plasma membrane underneath the OEW. The MT pattern of subepidermal (and other inner) cells was strictly transverse both in the presence and absence of IAA.

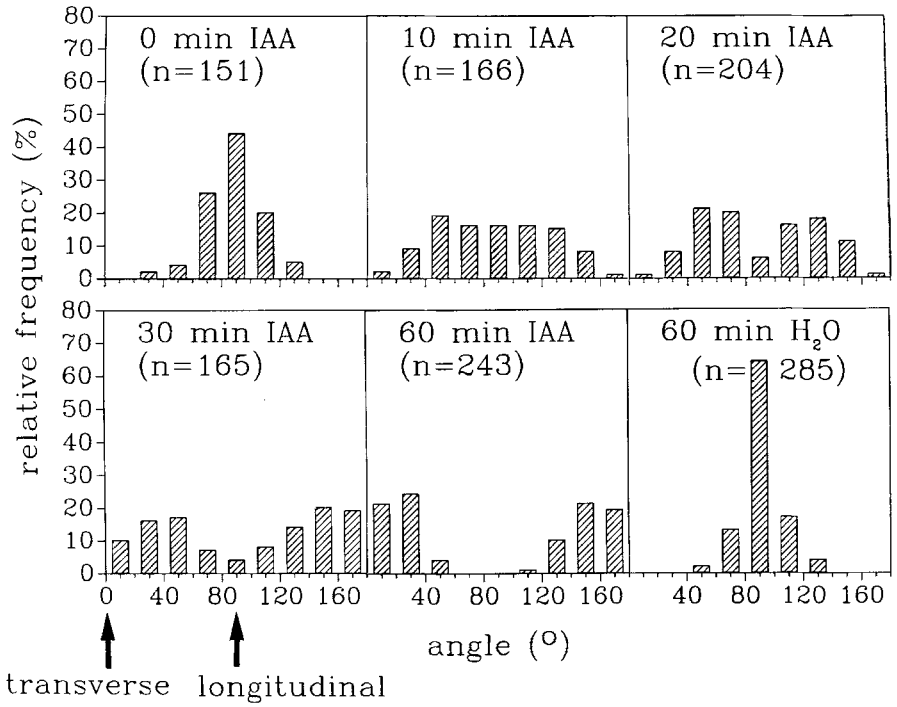

Fig. 3. Frequency distribution of MT orientations at the outer epidermal cell face of maize coleoptile segments during IAA-induced reorientation from the longitudinal to the transverse direction. After cutting, coleoptile segments were incubated for 2 h in water and then transferred to IAA solutions (10 μmol · l^{-1}) for 10, 20, 30, 60 min or further kept on water for 60 min. *n*, number of cell sections measured

The time course of reorientation of MT at the outer epidermal face by IAA was analyzed by determining angular MT distribution patterns from pictures such as those shown in Fig. 2. Figure 3 indicates that IAA produces a significant change towards a transverse MT orientation within 10 min. Taking into account a fixation time of 5 min, this means that the MT respond to the hormone treatment with a lag of less than 15 min. A complete reorientation into the transverse direction was reached after 60 min.

Kinetics of microtubule reorientation induced by unilateral blue light. Induction of phototropic bending by a pulse of blue light (first phototropic curvature) is accompanied by a unilateral reorientation of MT at the OEW of maize coleoptiles. Figure 4 shows MT patterns of epidermal cells from the irradiated and the shaded side of the organ 120 min after blue-light treatment. As in the case of IAA-dependent reorientation, the MT of the inner periclinal cell face are unaffected by the phototropic stimulus. The MT of the outer periclinal cell face change from a transverse into a longitudinal orientation, however, only at the irradiated side of the coleoptile. The time course of this directional change during the period of 2 h required for the expression of curvature is documented in Fig. 5. Owing to the action of endogenous IAA the initial orientation of outer-cell-face MT is transverse on both sides of the intact coleoptile. The irradiated side of the coleoptile shows a significant deviation from the transverse direction 20 min after stimulation and a complete reorientation after 60 min while the shaded side remains essentially unchanged (transverse, with a tendency towards increased uniformity within the MT population) during the bending response. Control seedlings irradiated for 1 h with red light from above had a transverse MT orientation on both sides, very similar to the distributions shown for 0 and 10 min after unilateral irradiation in Fig. 5 (data not shown). Thus, blue light induces a reorientation of the MT underneath the OEW from transverse to longitudinal at the irradiated side whereas the corresponding MT at the shaded side maintain (or even reinforce) their transverse orientation. Basically the same result was obtained in similar experiments with the hypocotyl of sunflower seedlings which were irradiated with unilateral blue light for 2 h (data not shown).

Reorientation of microtubules induced by gravitropic stimulation. Reorientation of the MT at the outer epidermal face can also be observed during gravitropic bending. Figure 6 shows that the epidermis of the upper side has a longitudinal MT pattern in contrast to a transverse pattern at the lower side of the gravitropically curved maize coleoptile. Very similar results were obtained in the epidermis of gravitropically curved sunflower hypocotyls (Fig. 7). Thus, in agreement with the phototropic response, differential cell lengthening at the organ surface induced by gravity is accompanied by a differential orientation of MT both in the coleoptile and in the hypocotyl. Reduced lengthening (at the irradiated, or the upper side, respectively) is correlated with a longitudinal MT orientation whereas increased lengthening (at the shade, or the lower side, respectively) is correlated with a transverse MT orientation.

Discussion

Using indirect immunofluorescence labeling of MT in situ we have shown that differential growth of opposite organ flanks leading to phototropic or gravitropic curvature in maize coleoptiles is correlated with changes in the orientation of the MT underlying the outer epidermal wall, whereas the MT of inner epidermal (and parenchymal) cell surfaces of the organ maintain their original

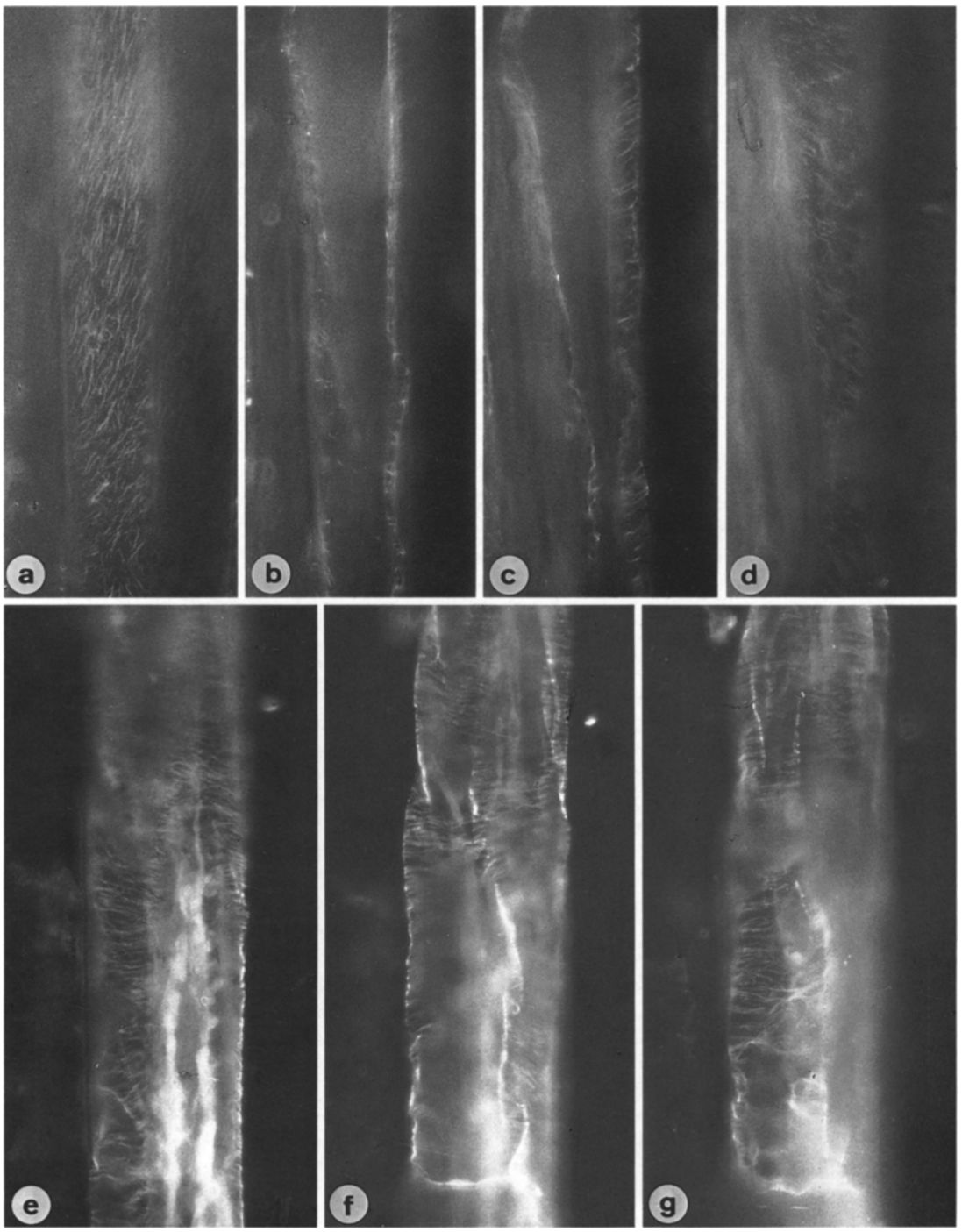

Fig. 4a–g. Orientation of MT in the outer epidermis of coleoptile segments of intact maize seedlings 120 min after unilateral irradiation with a pulse of blue light (30 s, 16.7 $mW \cdot m^{-2}$). **a–d** Irradiated side; **e–g** shaded side. The focal plane was located at the outer surface (OEW), middle region, or inner surface of epidermal cells (from *left* to *right*, compare Fig. 2)

orientation. Similar observations have been made with sunflower hypocotyls, indicating that this phenomenon may be of general importance in plant organs capable of tropic responses. As a rule, transverse MT orientation is associated with a high rate of growth and longitudinal MT orientation is associated with a low rate of growth of the respective epidermal cells. The reorientation of MT during tropic bending is restricted to the cell face covered by the OEW. Moreover, this response demonstrates a time course in close agreement with the time course of the phototropic response (lag-phase approx. 20 min, completion after approx. 2 h). There is a striking similarity between the MT orientation accompanying tropic bending and the reorientation of MT elicited by exogenously applied IAA. In both cases the directional transition is initiated within less than 20 min and involves only the MT subjacent to the OEW. Moreover, a high growth rate is always correleated with transverse MT orientation and a low growth rate with a longitudinal MT orientation, in agreement with many previous investigations (see Bergfeld et al. 1988 for a discussion of the importance of MT and microfibril orientation for cell-wall expansion).

These results bear on several aspects of the mechanism of tropic curvature. Firstly, they are in agreement with the notion that IAA-dependent straight growth

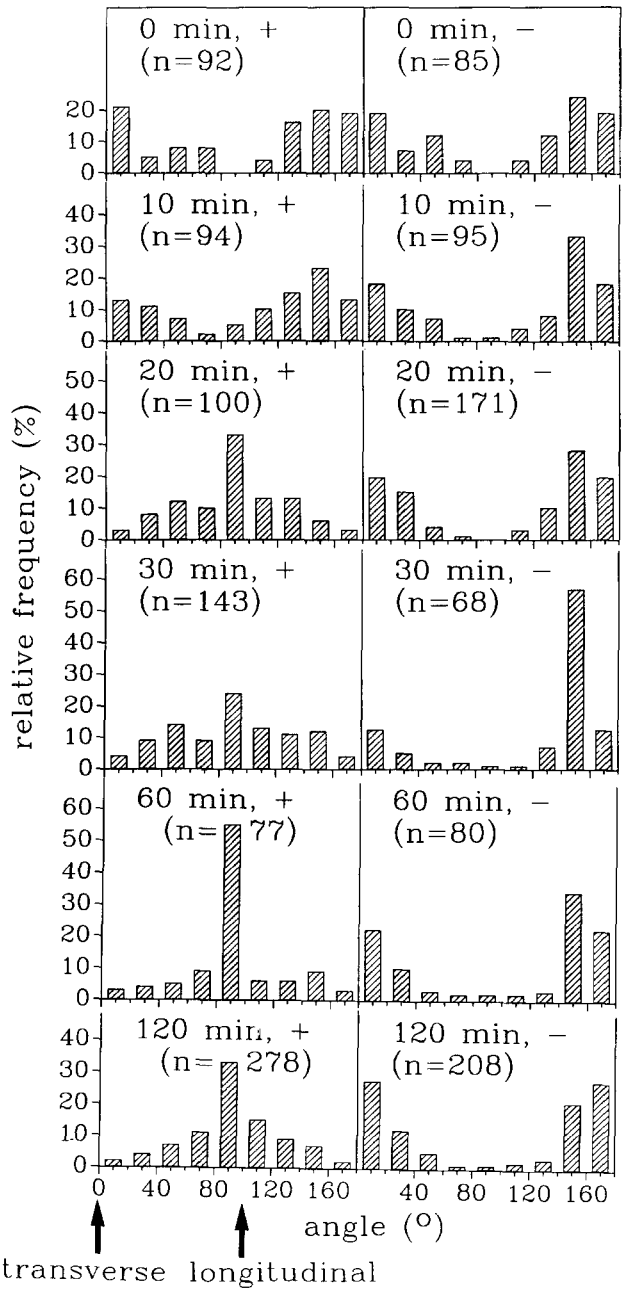

Fig. 5. Frequency distribution of MT orientations at the outer epidermal cell face of maize coleoptiles during phototropic bending. The coleoptile of intact seedlings was exposed to a unilateral blue-light pulse. Segments of the bending zone were isolated 10, 20, 30, 60, 120 min afterwards. After fixation, epidermal peels were prepared from the irradiated (+) and the shaded (−) organ sides. *n*, number of cell sections measured

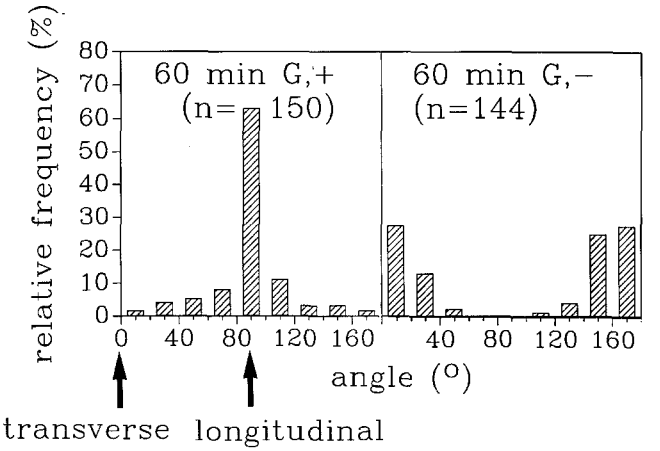

Fig. 6. Frequency distribution of MT orientations at the outer epidermal cell face of maize coleoptiles during gravitropic bending. The coleoptile of intact seedlings was oriented horizontally for 60 min under red light. Subsequently, segments of the bending zone were isolated and processed as in Fig. 5. (+, upper side; −, lower side of the coleoptile)

(Kutschera 1987) as well as tropic bending (Firn and Digby 1977) of coleoptiles and hypocotyls is controlled by the epidermis (more specifically, by changes in the physical properties of the rigid outer epidermal wall). Differential changes in the extensibility of the epidermal wall could be brought about by large differences in epidermal auxin levels which are, however, not detectable by analyzing the auxin distribution in bulk tissue (Iwami and Masuda 1976; McDonald and Hart 1988). In this case, the small differences in auxin levels between organ halves typically found in previous attempts (Pickard 1985) to demonstrate auxin gradients in tropically bending coleoptiles are no valid argument against the involvement of auxin in this response. Secondly, the striking agreement between the changes in MT orientation observed at the OEW during tropic bending and during induction of straight growth by external auxin strongly indicates that auxin is, in fact, functionally involved in mediating asymmetric growth leading to organ curvature. This conclusion is strictly based on the spatial and temporal correlation between the effects of tropic stimuli and the effect of applied auxin on the orientation of epidermal MT and is therefore independent of the problem of whether or not MT orientation and cell-wall expansion are mechanistically related. There is no evidence that short-term growth of epidermal cells is controlled through the orientation of microfibrills (Bergfeld et al. 1988). Also the data of the present paper do not prove a causal relationship between auxin action on MT orientation and tropic curvature. However, our results do show that MT reorientation is a specific auxin-mediated response which can be used as a diagnostic test for an asymmetric distribution of the hormone, correlated with asymmetric organ growth.

Based on a quite different approach, a very similar conclusion has recently been reached by McClure and Guilfoyle (1989). Using a tissue-print technique, these authors showed that gravitropic bending of soybean hypocotyls is correlated with an asymmetric distribution of a family of mRNAs, produced by an asymmetric expression of auxin-controlled genes. These as well as our data do not prove an uneven distribution of auxin molecules at the two flanks of a tropically bending organ. Evidently, this type of result is equally compatible with the assumption that the amounts of endogenous auxin on both organ flanks are equal and that differential growth is produced by the unequal distribution of an inhibitor interfering with the action of auxin (Feyerabend and Weiler 1988; Hasegawa et al. 1989). However,

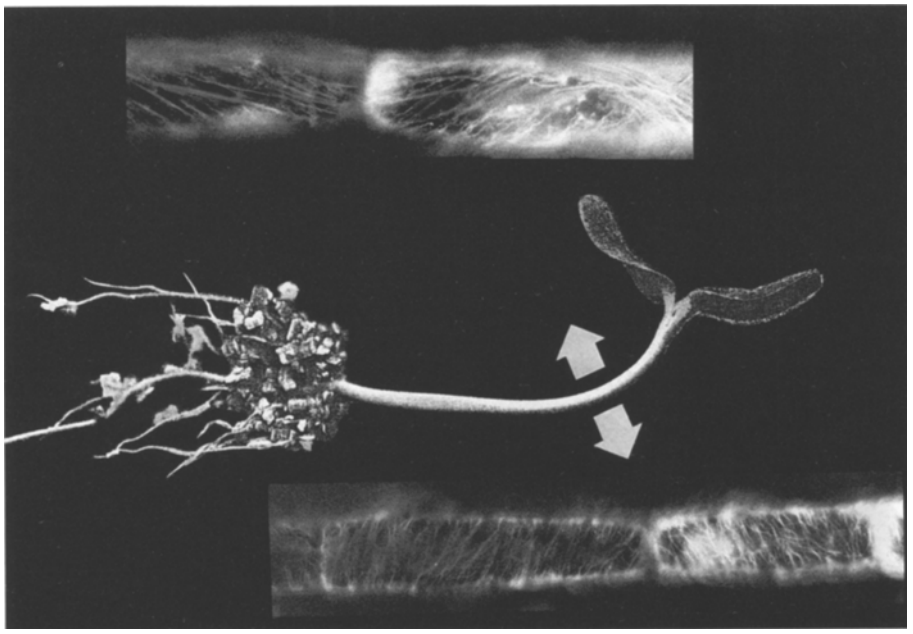

Fig. 7. Orientation of MT at the outer face of epidermal cells of a sunflower seedling. The hypocotyl of a 6-d-old white-light-grown seedling was transferred from the vertical to the horizontal orientation. After 2 h in darkness 10-mm segments from the bending zone were isolated and processed as in Fig. 5. The micrographs demonstrate MT patterns at the outer face of epidermal cell files from the upper and lower hypocotyl sides

it should be borne in mind that the Cholodny-Went hypothesis was based on a bioassay of diffusible auxin and should therefore be evaluated in terms of auxin *activity* rather than extractable auxin *content*. In this limited sense, our results, as well as those of McClure and Guilfoyle (1989), provide strong circumstantial evidence that tropic organ curvature depends on asymmetric distribution of auxin as predicted by the Cholodny-Went hypothesis. Whether this asymmetric auxin distribution is brought about by a lateral transport of the hormone from the slower growing side to the faster growing side of the organ (Cholodny-Went), or by other means, remains an open question.

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References

Bergfeld, R., Speth, V., 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

Briggs, W.R., Baskin, T.J. (1988) Phototropism in higher plants – controversies and caveats. Bot. Acta **101**, 133–138

Feyerabend, M., Weiler, E.W. (1988) Immunological estimation of growth regulator distribution in phototropically reacting sunflower seedlings. Physiol. Plant. **74**, 185–193

Firn, R.D., Digby, J. (1980) The establishment of tropic curvatures in plants. Annu. Rev. Plant Physiol. **31**, 131–148

Hasegawa, K., Sakoda, M., Bruinsma, J. (1989) Revision of the theory of phototropism in plants: a new interpretation of a classical experiment. Planta **178**, 540–544

Iwami, S., Masuda, Y. (1976) Distribution of labeled auxin in geotropically stimulated stems of cucumber and pea. Plant Cell Physiol. **17**, 227–237

Kutschera, U. (1987) Cooperation between outer and inner tissues in auxin-mediated plant organ growth. In: Physiology of cell expansion during plant growth, pp. 215–226, Cosgrove, D.J., Knievel, D.P., eds. American Society of Plant Physiologists, Rockville, Md., USA

Lang, J.M., Eisinger, W.R., 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

Lloyd, C.W. (1987) The plant cytoskeleton: the impact of fluorescence microscopy. Annu. Rev. Plant Physiol. **38**, 119–139

McClure, B.A., Guilfoyle, T. (1989) Rapid redistribution of auxin-regulated RNAs during gravitropism. Science **243**, 91–93

McDonald, J.R., Hart, J.W. (1987) New light on the Cholodny-Went theory. Plant Physiol. **84**, 568–570

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

Pickard, B.G. (1985) Roles of hormones in phototropism. In: Encyclopedia of plant physiology, N.S., vol. 11: Hormonal regulation of development III, pp. 365–417, Pharis, R.P., Reid, D.M., eds. Springer, Berlin Heidelberg New York

Preston, R.D. (1988) Cellulose-microfibril-orienting mechanisms in plant cell walls. Planta **174**, 67–74

Quader, H., Deichgräber, G., Schnepf, E. (1986) The cytoskeleton of *Cobaea* seed hairs. Planta **168**, 1–10

Roberts, I.N., Lloyd, C.W., Roberts, K. (1985) Ethylene-induced microtubule reorientations: Mediation by helical arrays. Planta **164**, 439–447

Sawhney, V.K., Srivastava, L.M. (1975) Wall fibrils and microtubules in normal and gibberellic-acid-induced growth of lettuce hypocotyl cells. Can. J. Bot. **53**, 824–835

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

Volfová, A., Chvojka, L., Haňkovská, J. (1972) The orientation of cell wall microtubules in wheat coleoptile segments subjected to phytohormone treatment. Biol. Plant. (Praha) **19**, 421–425

Went, F.W.., Thimann, K.V. (1937) Phytohormones. MacMillan, New York

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