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Reorganization of microfilaments in protonemal tip cells of the moss *Ceratodon purpureus* during the phototropic response

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Summary. The F-actin distribution in caulonemal tip cells of the moss *Ceratodon purpureus* was examined by rhodamine-phalloidin staining. Gravitropically-growing caulonemal tip cells of the moss possess a distinct alignment of microfilaments (MFs) in their apices. Axially oriented actin bundles run from subapical regions to the apex where they converge towards a central area of the tip, although bundles are absent from the central area itself thus forming a collar-like structure. During a unilateral red light irradiation the actin strands of the apical dome become reoriented towards the irradiated apical flank and still surround an area free of MFs, the point of prospective outgrowth. This process is closely correlated with the morphological effect of bulging and precedes the light-directed outgrowth. The collar structure is essential for the tubular growth form. In darkness, under the influence of antimicrotubule agents the structure is decomposed, the actin strands drift along the cell flanks and finally accumulate in randomly distributed areas where further growth takes place. The microtubules (MTs) are not involved in the phytochrome-mediated reorientation of the microfilaments. Unilateral red light suppresses the distorting effect of antimicrotubule drugs and restores the collar structure with a pronounced light-directed orientation. Instead, the MTs seem to be responsible for restricting the reorientation to the cell tip. This notion is based on the observation that the small area in the apical dome, which is normally the exclusive location of the light-regulated MF rearrangement, extends towards the cell base when MT inhibitors are applied before the unilateral red light irradiation. This in turn leads to a non-tubular expansion of the light-directed cell flank.

Keywords: Actin; Microfilaments; Microtubules; Moss protonema; Phototropism; Phytochrome.

Abbreviations: DIC differential interference contrast; DMSO dimethyl sulfoxide; EGTA ethyleneglycol-bis-(beta-aminoethylether) N,N,N′,N′-tetraacetic acid; MF microfilament; MT microtubule; MTSB microtubule stabilizing buffer; MBS 3-maleimidobenzoic acid N-hydroxysuccimide ester; PIPES piperazine-N,N′-bis(2-ethane-sulfonic acid); RP rhodamine labeled phalloidin.

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Introduction

Moss protonemata consist of two types of tip-growing cells. Caulonemal apical cells, which differentiate from chloronemal cells (Cove and Ashton 1984), show extension growth exclusively located in their apical dome.

Such tip cells exhibit a polar cytoplasmic organization based on their cytoskeleton. The structural alignments of microtubuli and microfilaments can be visualized using indirect immunofluorescence or rhodamine-phalloidin staining and show an abundant distribution even in the apices of the tip cells (Doonan et al. 1985, 1988; Goode et al. 1993; Quader and Schnepf 1989; Schwuchow et al. 1990; Tewinkel et al. 1987). Functional analyses of the cytoskeleton in the establishment of cell polarity have been based largely on inhibitor studies. In general antimicrofilamental drugs slowed down or abolished tip growth (Schmiedel and Schnepf 1980, Doonan et al. 1988), whereas antimicrotubule drugs led to a depolarized growth of the tip cells, resulting in subapical protrusions (Schmiedel and Schnepf 1980, Wacker et al. 1988, Doonan et al. 1988). Isopropyl N-(3-chlorophenyl)-carbamate (CIPC), an inhibitor which perturbs microtubule organizing centres, disrupted the organization of apical foci and caused filament bending (Doonan et al. 1985). From these results it was concluded that the microtubule cytoskeleton is essential in the maintenance of the cell shape whereas the microfilament cytoskeleton establishes the local exo-

cytosis and therefore growth per se (Schnepf 1986, Doonan 1991).

It was argued that the ability of caulonemal tip cells to respond to changes in environmental stimuli such as unidirectional light and gravity by reorientation of the vectorial growth (Cove et al. 1978) should be accompanied by a structural reorganization of the cytoskeleton (Doonan 1991). This assumption was confirmed by studies in gravitropic caulonemal tip cells of the moss *Ceratodon purpureus*, where a significant reorientation of MTs in response to an altered gravity vector was observed (Schwuchow et al. 1990). Studies of cytoskeletal rearrangements during the phototropic response in moss protonema have not been done so far. Kadota and Wada (1992), though, showed that subapically-located circular arrays of microtubules and microfilaments reoriented in the phytochrome-mediated phototropic response of protonemal tip cells of the fern *Adiantum*.

The aim of this work was to gain an insight into the structural features of the MF cytoskeleton and its arrangements during the phytochrome-mediated phototropic response (Hartmann et al. 1983) in protonemal tip cells of the moss *C. purpureus*. We concentrated our work on this part of cytoskeleton because functional analyses performed with antimicrotubule drugs indicated that the microtubule system is less involved in this process (Hartmann 1984). Inhibitor studies directed towards the other cytoskeletal component, the MFs, were equivocal as any interference with MFs led to an inhibition of growth itself, a prerequisite for the phototropic reaction. We show that light mediates a rapid rearrangement of microfilaments associated with phototropic growth; whereas microtubules seem to be involved only indirectly.

Material and methods

Plant material

Protonemata of *Ceratodon* were transferred to small, square sheets of sterile cellophane on petri dishes containing a modified Knop's agar medium (Hartmann et al. 1983). For dark adaptation these samples were placed vertically in darkness for at least 5 days. Under these conditions the cultures produce negatively-gravitropic caulonemata that adhere to the cellophane and are therefore easy to handle. All subsequent manipulations with such darkadapted material were performed in absolute darkness using infrared goggles (CIR, Rome, Italy) to avoid any influence of stray light.

Irradiation experiments

The dark-grown protonemata were transferred to chamber slides (Nunc Inc., Naperville, Ill., U.S.A.) partially filled with agar. The slides were placed vertically in a holder to maintain the gravity vector and unilaterally irradiated with red light (30 $\mu mol/m^2/s$) for various periods. As light source a projector (Zeutschel, Tübingen, Federal Republic of Germany) was used, equipped with an interference filter of 664 nm (Schott, Mainz, Federal Republic of Germany). The direction of irradiation was marked within the sample by cutting off several protonemata from the light-directed side.

Cytoskeletal inhibitors

Oryzalin was diluted from a stock solution in DMSO and mixed with solidifying growth medium. The final concentration was 1 µM and 0.5% DMSO, respectively. Control plants were grown on agar medium containing 0.5% DMSO. In some cases the microtubule inhibitor colchicine (Sigma, München, Federal Republic of Germany) was used at a final concentration of 1 mM.

Cytochalasin D (Sigma) was added to the agar medium, diluted from a 1 mg/ml stock solution in DMSO to a final concentration of 10 µg/ml inhibitor and 1% DMSO. Control plants were transferred to growth media containing 1% DMSO.

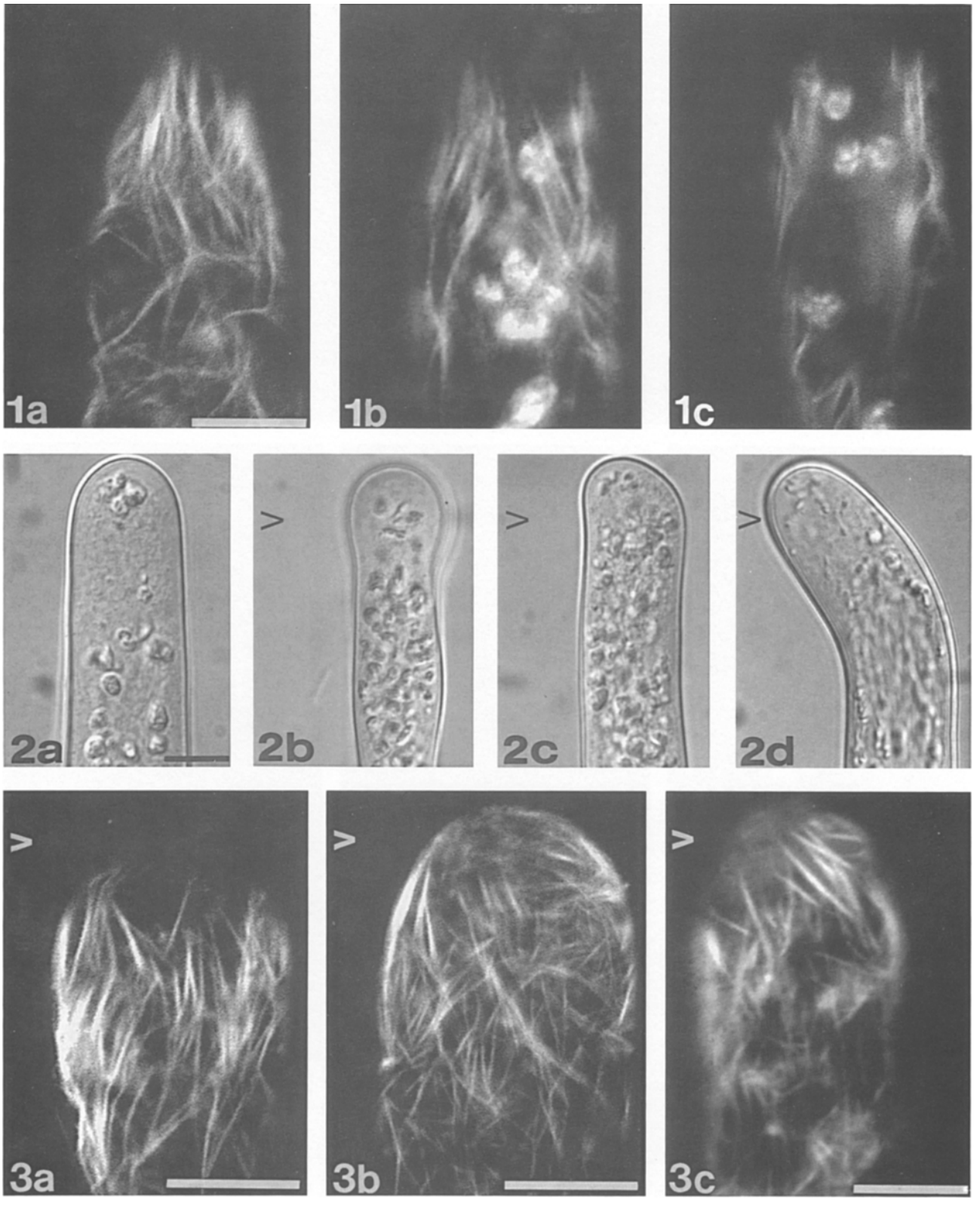
Microfilament staining

For F-actin staining the protonemata were incubated for 20 min in MTSB (0.1 M PIPES pH 6.8, 10 mM EGTA, 5 mM $MgCl_2$), 1% DMSO containing the crosslinker MBS at a final concentration of 100 µM (Sonobe and Shibaoka 1989), derived from a 10 mM stock solution in DMSO. A subsequent fixation with formaldehyde or glutaraldehyde partially decomposed the filamentous structures and was thus avoided. After a three-fold wash with MTSB (5 min each) the moss was immersed for 15 min in MTSB, 5% methanol (Tewinkel et al. 1989), containing 0.1 µM rhodamine phalloidin (Molecular Probes, Eugene, Oreg., U.S.A.). Finally the moss was rinsed three times with MTSB again and mounted on a microscope slide in a drop of the same buffer. Samples were examined with a fluorescence microscope (Axiovert 35; Zeiss, Oberkochen, Federal Republic of Germany) attached to a confocal laserscan system (MRC 600; Bio-

Fig. 1 a–c. Actin filament patterns in tips of gravitropically growing protonema in different planes of focus. Note the parallel axial alignment of the MFs converging to the tip in a zone free of actin bundles. The fluorescence of chloroplasts is prominent in inner sections of the cells. Bar: 10 µm

Fig. 2 a–d. Light micrographs (DIC) showing tip cells in different stages of the photoresponse. Unilateral red light (30 $\mu mol/m^2/s$) was applied from the left indicated by the open arrowhead. **a** Unirradiated cell. **b** 5 min irradiation, note the swelling of the tip (bulging). **c** 20 min irradiation, the bulging is asymmetrical and the new growth point is already established. **d** 60 min irradiation, light-directed growth is well expressed. Bar: 10 µm

Fig. 3 a–c. Structural changes of the MF alignments in the tips of protonema during the photoresponse. Unilateral red light (30 $\mu mol/m^2/s$) was applied from the left side (open arrowhead) for **a** 5, **b** 10, and **c** 20 min. **a** The bundle-free zone is extended, several actin bundles are reoriented whereas most of the MFs retain their predominant axial alignment. **b** Most of the apical MFs are reoriented and are directed to a diffuse stained zone located at the irradiated apical flank. **c** The reoriented actin strands are lengthened. Bars: 10 µm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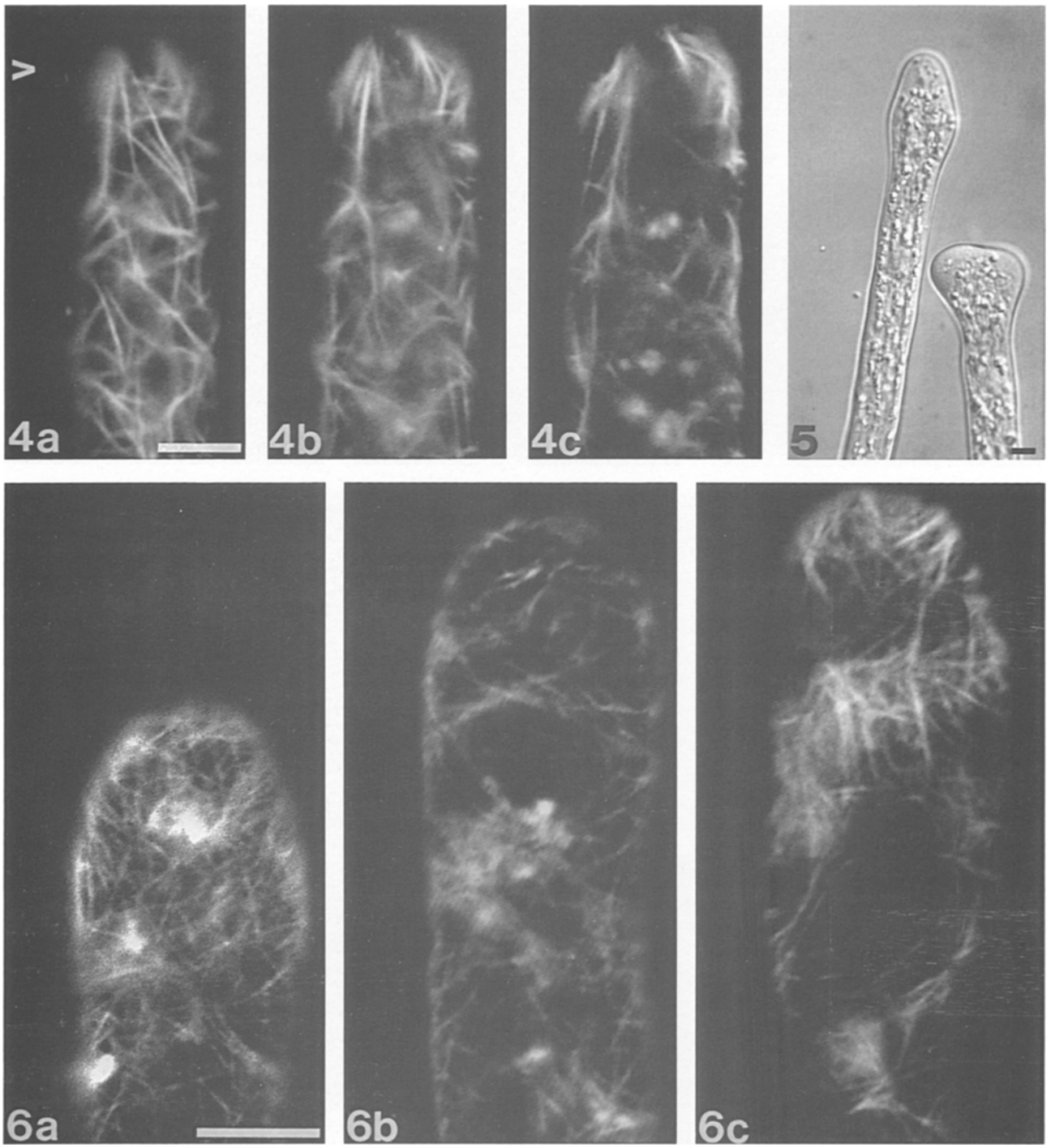


Fig. 4 a–c. Three optical sections through a phototropically-stimulated tip cell (25 min red light irradiation). Focal planes are located in the cortex (**a**) at 2 µm (**b**) and 4 µm (**c**) towards the interior of the cell. Note the light-directed shift of the gap. Light direction is marked by the open arrowhead. Bar: 10 µm

Fig. 5. Dark grown tip cells incubated with 1 µM oryzalin for 3 h. Apical and subapical protrusions at both flanks arise. Bar: 10 µm

Fig. 6 a–c. MF patterns of dark grown tip cells incubated for various times (**a** 30, **b** 60, **c** 120 min) with 1 mM colchicine (**a** and **c**) or 1 µM oryzalin (**b**). A distortion of the apical MF alignment proceeds with a prolonged exposure to the inhibitors. **a** The typical MF structure has vanished the actin strands becoming directed to the apical flanks, ×2,000. **b** The MFs are transversely oriented, spanning the breadth of the cell, ×2,000. **c** The actin bundles are arranged in distinct clusters distributed randomly in the tip cell, ×2,000. Bar: 10 µm

rad, Hemel Hempstead, England). The laser light source was a Krypton/Argon-mixed gas laser with an emission line at 568 nm. The filter block in use consisted of an excitor filter 568 DF 10, a dichroic reflector 585 DRLP and an emission filter 585 EFLP. A plan apochromat ×63 oil immersion lens (NA 1.4) was used for observation.

Results

Microfilament arrangements in tip cells

The microfilamental alignments were examined by staining protonemata, following 5 days vertical growth in darkness. MFs formed a cortical network with preferentially axially-oriented actin bundles in the vacuolized cell base. Chloroplasts were attached to this network. In the unvacuolized subapical part of the cell the actin bundles were shorter and more divergent oriented (Fig. 1 a). Connections to chloroplasts could also be observed in that region of the cell. Directed towards the apex, the MFs formed a cortical collar-like structure, consisting of actin bundles with a parallel axial alignment. These MFs converged towards a weakly stained region in the tip of the cell from which bundles were absent (Fig. 1 a–c).

MF reorganization during phytochrome-mediated phototropism

The process of phytochrome-mediated phototropism shows typical stages. The first visible morphological effect of unilateral red light irradiation is an apical swelling of the cell. The intensity of that bulging is variable and is not always distinct. A prolonged irradiation leads to an asymmetrical bulging after 20 min and finally to an outgrowth shifted towards the light direction at an average angle of 30° (Fig. 2 a–d).

Significant changes in the structural alignment of the apical MFs accompanied that red light-modulated process (Fig. 3 a–c). After 5 min of unilateral irradiation the arrangement of the MFs became loose, associated with the expansion of the tip during the first period of bulging. The bundles remained predominantly tip oriented while the apical bundle-free zone became slightly extended (Fig. 3 a). During a prolonged irradiation the actin bundles of the apical dome gradually altered their orientation (Fig. 3 b). This reorientation was directed towards the irradiated apical flank and was apparently based on a vectorial MF synthesis. Some observations make it likely that pre-existing actin bundles served as initial sites for this directed polymerization. The grade of the microfilamental reorientation depended on the actual location of the precursor MF and was generally more pronounced in MFs most distant from the stimulus (Figs. 3 b, c and 4 b, c). The new actin bundles converged in a small area where formerly located actin strands had disappeared. This array was marked by a diffuse staining pattern (Fig. 3 b, c). During further unilateral irradiation the reoriented bundles lengthened but did not congregate to form a closed cortical envelope. These MFs still formed a hem around an area in which bundles remained absent (Fig. 3 c). The process of microfilamental rearrangement always preceded that of light-oriented growth and was strictly light-directed. The result of the whole process is a directionally-translocated gap, the presumptive new centre for outgrowth (Fig. 4 a–c).

For a statistical evaluation, protonemata were irradiated for 30 min to assure that the microfilament reorientation was well established and easy to interpret. A further advantage of the prolonged irradiation was that the growth direction was already morphologically manifested in an asymmetrical bulging, so that the cytoskeletal arrangement could be compared with growth at the same time. The MF orientation always accorded to the growth direction. Thus the MF imaging data reflected results obtained by an ordinary irradiation experiment of the same irradiation time, in which about 75% of the tip cells show a positive phototropical response. The only exception was the slightly increased proportion of cells with a light-averted MF arrangement; this might be due to some cells having become twisted during the staining procedure (Table 1).

Effects of oryzalin and colchicine on protonemal growth

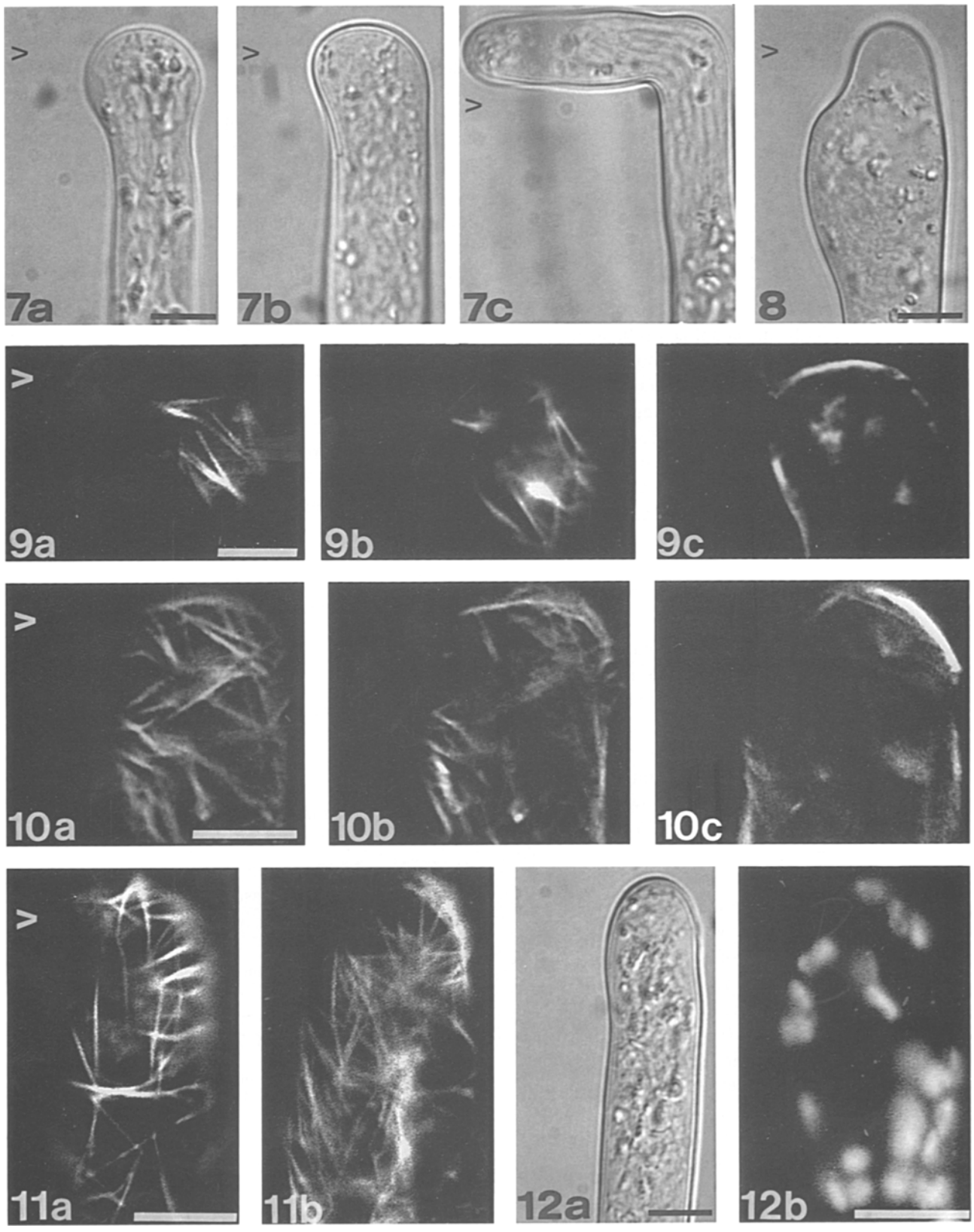
Dark-adapted protonemata were incubated with 1 µM oryzalin or in some cases with 1 mM colchicine, inhibitor concentrations which were found to be

Table 1. Comparison of growth direction and MF orientation in separate samples

Number of tip cells	% tip cells		
	light-directed	unaffected	light-averted
128 (MF orientation)[a]	71.9	20.3	7.8
193 (growth direction)[b]	76.2	22.8	1

[a] Seven samples were irradiated with unilateral red light (30 min, 30 µmoles/m²/s) and the MFs were stained as described. Well stained tips were analysed with conventional fluorescence microscopy

[b] Two samples were irradiated with unilateral red light (30 min, 30 µmoles/m²/s) and the protonemal growth direction was analysed with light microscopy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7b
7c
8
9a
9b
9c
10a
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10c
11a
11b
12a
12b

effective in microtubule disassembly of *Ceratodon* tip cells (Weber 1989, Schwuchow et al. 1990).

The MT inhibitors had pronounced effects on the cell morphogenesis of protonemata growing in darkness. The centre of outgrowth shifted from the apex to the flanks of the tip cell, the regular tubular habit being replaced by a disproportionate form of growth with subapical protrusions sometimes at one but often at both cell flanks (Fig. 5). This effect was enhanced by a prolonged exposure to the inhibitor. The chloroplasts sedimented to the base of the cell or to the basal part of protrusions. In most cases the former apex of such tip cells became strongly vacuolized (data not shown). Both inhibitors had similar effects on the microfilamental alignments of the tip cells. The ordered arrangement in the tip with its axially-oriented MFs in the subapical part was disrupted (Fig. 6 a). The microfilaments oriented divergently a dislocation enhanced by prolonged exposure to the inhibitors. In some cases transversely-running MFs spanned the breadth of the cell (Fig. 6 b), whereas in others an accumulation of MFs in various parts of the cell occurred. Within these aggregations the actin bundles had a more or less pronounced preferential orientation towards presumptive growth areas (Fig. 6 c).

The antimicrotubule drugs did not inhibit the phototropical response of *Ceratodon* tip cells, but the shape of growth was affected, depending on the starting point of the unilateral irradiation during the inhibitor incubation. If light was applied simultaneously with MT inhibitors the phototropic growth started inside the apical dome (or slightly subapically), shifted exactly towards the light direction. The form of the cell remained tubular (Fig. 7 a–c). The MFs in these samples showed a pronounced light-directed reorientation, in some cases this rearrangement occurred in subapical areas but the filaments were placed closely together, so that its collar-like structure was maintained (Fig. 9 a–c). A different form of growth could be observed in samples in which protonemata were preincubated with antimicrotubule drugs 0.5–1 h prior to the red light irradiation. An extended area of the light-directed cell flank expanded directionally towards the light source (Fig. 8). The MFs in such samples were preferentially oriented towards this expanding part of the cell flank (Fig. 10 a–c). A prolonged preincubation resulted in a further extension of the area in which light-directed MF reorientation took place (Fig. 11 a, b).

Effects of cytochalasin D on protonemal growth

The presence of 10 µg/ml cytochalasin D in the agar medium led to a growth inhibition within 15 min and was accompanied by a disturbance of the chloroplast distribution. *Ceratodon* caulonemata possess a characteristic zonation in apical region of the tip cell (Fig. 4). Chloroplasts of the apical dome are separated from distal chloroplasts by a plastid-free zone (Walker and Sack 1990). Under the influence of cytochalasin this plasid-free zone vanished (Fig. 12 a). MFs were completely absent from the apices of cytochalasin-treated cells; only the chloroplasts were visualized as a result of their autofluorescence (Fig. 12 b). Microfilament staining of control samples showed

Fig. 7 a–c. Sequence of light micrographs (DIC) showing tip cells in different stages of the photoresponse under the influence of 1 µM oryzalin. Unilateral red light (30 µmol/m^2/s) was applied from the left (open arrowhead). **a** 5 min irradiation. **b** 20 min irradiation, note that the centre of growth is shifted nearly in a right angle. **c** 2 h irradiation, the shape of growth is still tubular. Bar: 10 µm

Fig. 8. Light micrograph (DIC) of a tip cell submitted to 1 µM oryzalin for 1 h in darkness and subsequently for 2 h with unilateral red light. Note that the light growth has switched from tubular to a nontubular flank growth. Bar: 10 µm

Fig. 9 a–c. MF pattern of the apical dome of a cell treated with 1 µM oryzalin during a simultaneous unilateral red light irradiation (30 µmol/m^2/s) for 30 min. Three optical sections were taken in 2 µm steps. The actin strands converge into a small MF-free area at the irradiated apical flank. Bar: 10 µm

Fig. 10 a–c. Three optical sections in 2 µm steps through a tip stained for MFs. Cell treated with 1 µM oryzalin for 1 h, where the cell was kept 30 min in darkness prior to a 30 min irradiation with unilateral red light (30 µmol/m^2/s). The actin strands converge into an extended MF-free area at the irradiated flank spanning from the apex to subapical parts of the cell. Bar: 10 µm

Fig. 11 a, b. Two optical sections in a distance of 2 µm taken from a tip cell stained with RP. The cell was submitted to 1 µM oryzalin for 1 h in darkness and subsequently irradiated 30 min with unilateral red light (30 µmol/m^2/s) still exposed to the inhibitor. The actin strands direct over a large area towards the irradiated flank. Bar: 10 µm

Fig. 12. a Light micrograph (DIC) of a tip cell treated with 10 µg/ml cytochalasin D for 15 min. Note the disturbed chloroplast distribution in the tip of the cell. **b** RP-staining of a tip cell treated with 10 µg/ml cytochalasin D for 30 min. The focal plane is within the cortex, where the most actin strands are expected. The tip of the cell is lacking completely of F-actin, the chloroplasts are visible cause of their autofluorescence. Bars: 10 µm

that the solvent DMSO had no effect on the cellular MF structures (data not shown).

Discussion

The apical MF-cytoskeleton

The architecture of the apical microfilament cytoskeleton in caulonemal tip cells of *Ceratodon* is definite: Axially oriented MF-bundles are exclusively located in the peripheral cytoplasma. The central cytoplasma is free of RP-stainable F-actin. The cortical actin strands form a closely packed structure, but are absent in the vesicle enriched dome.

The arrangement of the MFs might explain their functional role in the regulation of tip growth. It is assumed that actin bundles are essential for a polarized vesicle transport (Picton and Steer 1982, Brawley and Robinson 1985) and therefore cause a directional exocytosis resulting in vectorial growth. The alignment of the MFs observed in the apical part of *Ceratodon* tip cells might relate to an analogous function. If the tip-located actin strands consist of uniformly polarized MFs, their axial orientation could provide a vectorial vesicle transport to the growing point at the very tip of the cell. Cortical MF-bundles did not decorate the plasma membrane at the exclusion site. This might facilitate the fusion of vesicles with the membrane in this part of the tip. The significance of this structural feature is emphasized by the observation that the MF-bundles in the area of prospective outgrowth disappear during the translocation of the growing point. A similar heterogeneity in the apical MF-composition was reported for other tip growing organisms. In the apical dome of protonema tip cells of *Adiantum* the cytoskeletal elements are rather scarce (Wada and Murata 1991). The MFs in the tip region of pollen tubes of *Nicotiana alata* and *Lilium longiflorum* are few and fine, consisting of small bundles or single elements (Lancelle et al. 1987, Lancelle and Hepler 1992). An apical cleft within the cytoplasmic F-actin was observed in live cells of *Saprolegnia ferax* stained with RP. The position of the cleft predicted the direction of tip growth (Jackson and Heath 1993). The authors suggest that this cleft is either established by vesicle exclusion or is a portion of the in vivo unstained peripheral network.

An additional growth-regulating function besides the vesicle transport is assigned to apical MF-structures of other tip growing cells. In these cases the apical MFs serve directly or indirectly as a regulator for turgor-induced cell expansion: In pollen tubes (Pierson et al. 1986, Heslop-Harrison et al. 1986) and fungal hyphae (Heath 1987, Jackson and Heath 1990) apical actin meshworks regulate the turgor-induced expansion by modulating the cytoplasmic rigidity (Picton and Steer 1982, 1983; Jackson and Heath 1990). In protonema tip cells of *Adiantum* subapically located circular arrays of cortical MTs and cortical MFs (Murata et al. 1987, Kadota and Wada 1989) determine the orientation of the microfibrils at the innermost layer of the cell wall parallel with the cytoskeletal bands (Murata and Wada 1989, Wada et al. 1990). In these cells the regulation of the turgor-induced protrusion might be based on the plasticity and elasticity of the cell wall (Wada and Sugai 1994). The apical MF-structure in *Ceratodon* tip cells seems not to be related to a function like this, as (*i*) cytochalasin neither induced an acceleration of growth, nor forced the swelling of the tip; (*ii*) the apical bundles offered no resistance to the turgor-induced lateral extension of the tip during bulging; (*iii*) a theoretical coalignment of apical MFs and microfibrils would result in axially-oriented microfibrils at the innermost layer of the cell wall. Such a texture in return would be unable to resist lateral expansion-forces within the tip.

MF-reorganization during phototropical response

Light is a mediator operating on the microfilamental alignments in the apical dome and can shift the former centrally-located gap towards the illuminated apical flank before growth reorientation. This process is marked by an enhanced F-actin turnover in the tip, in which a local degradation of bundles goes hand in hand with the synthesis of reoriented actin strands. The temporary extension of the bundle-free zone and the diffuse staining pattern observed in distinct parts of the tip could be interpreted as signs for the decompositional effect. An elevation of the G-actin concentration could serve in return for a rapid synthesis of the reoriented actin strands, which immediately followed the partial destruction of actin bundles. This new formation has its origin in pre-existing bundles which might help in the rebuilding of the collar-like structure.

The question of course remains regarding the transduction mechanism of the phytochrome-regulated MF-orientation. It is known that tip-growing protonema of *Ceratodon* exhibit a steep tip-to-base gradient of chlortetracycline (CTC)-stainable calcium and that a red light-induced shift of this calcium gradient takes

place, moving along with the translocation of the growth centre (Hartmann and Weber 1988). But care must be taken in the interpretation of these results. As CTC accumulates and fluoresces in compartments in which a high concentration of free calcium exists next to hydrophobic sites, particularly membranes (Tsien 1989), the staining patterns might not reflect the actual distribution of cytosolic free calcium. Assuming that the CTC-calcium gradient ist concomitant with the distribution of cytosolic free calcium in the tip, which has yet to be demonstrated, it is likely that the polymerization of actin is kinetically modulated within such a gradient. Depolymerization is favoured at high calcium concentrations (Kohno and Shimmen 1988), in this case at the apical dome which is therefore free of actin bundles. On the other hand polymerization and subsequent bundling takes place at lower calcium levels in basal parts of the tip. Not only the kinetic but also the direction of polymerization could be determined in such a way. That cytosolic free calcium plays a role in the transduction chain of some phytochrome-regulated processes has been demonstrated (Shacklock et al. 1992, Chae et al. 1990, Tretyn et al. 1991, Neuhaus et al. 1993) and might also be relevant in this context. Causalities between a light-induced Pfr gradient (Kraml 1994), calcium gradient, and microfilament rearrangement remain to be demonstrated, however. It has also to be taken into consideration that actin-associated proteins like fragmin (Hasegawa et al. 1980) or profilin (Valenta et al. 1993, Staiger et al. 1994) might be involved in this process, regulating the formation of actin filaments in a calcium-dependent or -independent manner.

Effects of antimicrotubule drugs on MFs

An intact MT-system is essential to maintain the microfilamental collar structure in the apex of gravitropically growing cells. This assumption is based on the observation that antimicrotubule drugs forced the translocation of the MFs out of the tip, leading to a random distribution of the actin bundles in the subapical part of the cell. As a consequence, the growth in such cells is no longer restricted to the apex and subapical protrusions arise.

The MT-system is not involved in the phytochrome-induced MF rearrangement. It rather seems to be responsible for keeping this process located in the apical dome. In cases where unilateral red light was applied simultaneously with antimicrotubule drugs, the compact arrangement of the MFs was restored within the apex and the extent of their light-induced reorientation was pronounced. Both effects in combination might explain why cells treated in this way grow at right angles to the gravitropic vector, directly towards the light direction, while remaining tubular. In cases where the irradiation was performed after a preincubation with antimicrotubule drugs the reorientation proceeded in an extended area and therefore could not restore the collar-like structure, possibly explaining the delocalized but nevertheless light-oriented outgrowth of these cells. From these results we conclude that the MTs anchor the sites in the central apex that regulate the polarity of the tip and thereby the arrangement of the apical MFs. The restricted distribution of light-regulated sites to a small area might explain why the phototropic response itself and the capacity for altering the orientation of actin strands is limited to an acute angle, less than 90°. A disruption of the MTs results in a translocation of these sites out of the tip leading to a gradual reorientation of the MFs; light-directed bundle reorientation now could take place over a much wider area. Our data are in accordance with both these predictions.

Reports dealing with stimuli-induced cytoskeletal rearrangements in other tip-growing organisms also reveal a complex interaction between the MT and MF cytoskeleton. In protonemal tip cells of *Adiantum* subapically located circular arrays of cortical MTs and cortical MFs rearrange in a phytochrome-mediated response. The reorientation of MFs seems to be primarily light-triggered and the MTs are thought to follow the MFs in their organization. These assumptions are based on the observations that the reorganization of MFs precedes that of MTs and that the MF inhibitor cytochalasin affects both systems, whereas MT-specific drugs exclusively inhibit the MT rearrangement (Kadota and Wada 1992). In *Chara* rhizoids the MT system is essential for the functionally-polar organization of the actin cytoskeleton, although in contrast to the MF it is not involved in the primary events of gravitropism (Braun and Sievers 1994).

We conclude that the microfilament reorientation is an early step in the phytochrome-mediated phototropic response of *Ceratodon* tip cells and establishes the vectorial light growth. Although the microtubule cytoskeleton is not directly involved in this process, it defines the apical dome as the exclusive location of MF-mediated phototropic growth.

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