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Capturing in vivo Dynamics of the Actin Cytoskeleton Stimulated by Auxin or Light

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We present here a transient expression system that allows the response of actin microfilaments to physiological stimuli (changes in auxin content, light) to be observed in single cells in vivo. Etiolated, intact rice seedlings are attached to glass slides, transfected biolistically with talin fused to yellow-fluorescent protein to visualize actin microfilaments, and either treated with auxin or irradiated. The talin marker labels distinct populations of actin that are differentially expressed depending on the physiological state of the coleoptile (active elongation versus ceased elongation). Whereas longitudinal transvacuolar bundles prevail in cells that have ceased to elongate, fine cortical strands are characteristic for elongating cells. The visualized actin structures remain dynamic and responsive to signals. Exogenous auxin triggers a loosening of the bundles and an extension of the cortical strands, whereas irradiation reorientates cortical strands into longitudinal arrays. These responses correspond in quality and timing to the signal responses inferred previously from fixed specimens and biochemical studies. In big advantage over those methods it is now possible to observe them directly at the single cell level. Thus, the rice coleoptile system can be used as a convenient model to study actin dynamics in vivo, in response to physiologically relevant stimuli.

Keywords: Actin — Biolistic transfection — Coleoptile — Rice — Talin.

Abbreviations: FPs, fluorescent proteins; YFP, yellow fluorescent protein; CFP, cyano fluorescent protein.

Introduction

During the control of plant growth, the cytoskeleton mediates a range of crucial functions that depend on the respective mode of growth. Detailed morphological studies on the level of single cells, e.g. root hairs, pollen tubes (Kost et al. 1998, Cai et al. 1997), trichomes (Mathur et al. 1999, Szymanski et al. 1999) and protonemata (Braun and Wasteneys 1998) have provided insights into the characteristic distribution of the cytoskeleton during development. For example, actin is typically found distributed as fine, short arrays in the tip zone of polar growing cells [for review see Geitmann and Emons (2000)]. Within a tissue context cell expansion is not confined to the cell poles, but occurs along the entire cell wall, preferentially along a clear axis. This so-called anisotropy of cell expansion is closely related to cortical microtubules that are aligned in parallel bundles orientated perpendicular to the main axis of expansion [for a recent review see Smith (2003)]. Irrespective of the given growth mode, the cytoskeleton is able to respond to a range of physiologically relevant stimuli and this response represents an essential element for the adaptive response of plant growth to the environment [for reviews see Nick (1998) for microtubules or Staiger et al. (2000) for actin microfilaments]. Although the phenomenon of cytoskeletal response to environmental stimuli has been frequently described, the mechanisms behind it are still far from understood. This is true even for the well-studied field of chloroplast movement, a well-known actin-dependent and precisely regulated photo-response (Takagi 2003).

Although the role of actin in the growth of cells within a tissue is less evident than in tip growth, it clearly exists (Baluška et al. 2003). It is known that actin is organized into longitudinal strands that reflect the preferential axis of tissue expansion (Parthasarathy 1985). Moreover, disruption of the actomyosin system with blockers of actin assembly interferes with anisotropic growth (Baskin and Bivens 1995, Baluška et al. 2001). However, the role of actin in controlling the direction of cell expansion is far from clear. Measurements of the actin tension in soybean cells (Grabski and Schindler 1996) have shown that the rigor of the microfilament system can be regulated by factors that control cell expansion suggesting that microfilaments mechanically limit cell expansion. On the other hand, the inhibition of cell growth with blockers of microfilament assembly (Thimann et al. 1992, Baskin and Bivens 1995, Wang and Nick 1998, Baluška et al. 2001) implies that microfilaments support cell expansion. The increase in information about cytoskeleton-associated proteins, along with the development of new techniques, has allowed more sophisticated questions concerning the signalling mechanisms which remodel the plant cytoskeleton to be posed [for a recent review see Wasteneys and Galway (2003)]. For example, a recent study of overexpression of the actin-binding protein formin in pollen tubes provided new insights into the role of actin in the maintenance of polar cell extension (Cheung and Wu 2003).

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A system to analyze the role of actin in anisotropic tissue growth should meet the following conditions:

1. Growth should be exclusively based on cell expansion, not on cell division, so that it is possible to link growth rate with cellular and biochemical events.
2. It should be possible to control growth rapidly, reversibly and consistently by appropriate signals.
3. The cells undergoing growth should be suitable for cell biological studies.

These requirements are fully met by Graminean coleoptiles (Waller et al. 2000). The growth of this organ responds swiftly to hormones, light or gravitropic stimuli. Following asymmetric stimulation with blue light or gravity the growth response leads to tropistic bending, providing an innate reference system, where the responses of the differentially growing flanks can be directly compared. A crucial role in the control of these growth changes is played by the epidermal cell layer where growth responses to diverse stimuli have been studied in great detail on the level of microtubules as well as actin microfilaments [for review see Nick (1999)]. The epidermis is composed of large, clearly axial cells that are easily accessible to manipulation and observation even in the context of the intact organ. Moreover, dark-grown coleoptiles are devoid of chloroplasts, allowing usage of the full wavelength range for fluorescent markers.

Previous studies of maize coleoptiles (Waller and Nick 1997, Waller et al. 2002) have discriminated between two morphologically and biochemically distinct populations of actin: longitudinal bundles corresponding to a sedimentable fraction of actin predominated in cells with arrested elongation, whereas a finer cortical meshwork correlated with a soluble fraction prevailed during active cell elongation. However, these findings were based on specimens that were mildly fixed and later labelled by fluorescent phalloidin. It is quite possible that even a mild fixation destroys the finer microfilaments or traps them in artificial structures. The same argument holds true for alternative protocols, where actin is cross-linked prior to fixation (Sonobe and Shibaoka 1989). The organization of the fine cortical meshwork thought to be responsible for the promoting effect of actin on cell elongation is therefore particularly difficult to assess accurately under these conditions. Moreover, those methods do not provide the possibility of following the signal responses of individual cells. It is therefore necessary to visualize actin in vivo. Microinjection of fluorescent actin or fluorescent actin-binding proteins has been successfully employed in tip-growing cells (Hussey et al. 1998, Valster et al. 1997) but is extremely cumbersome in the often highly vacuolated and turgescent cells of typical plant tissues. Alternatively, fluorescent fusion proteins have proved to be versatile and convenient tools for observations in vivo (for a recent review see Brandizzi et al. 2002a). Actin-binding proteins such as talin and formin fused to different fluorescent proteins (FPs) have been successfully used to visualize microfilaments in both stable and transient expression systems (Banno and Chua 2000, Kost et al. 1998, Mathur et al. 1999). Stable transformation is difficult and time-consuming in Graminea and may alter development. To gain access to the advantages of in vivo approaches we therefore developed a transient expression system for rice coleoptiles. We introduced a fusion of the actin-binding protein talin (Kost et al. 1998) with the yellow fluorescent protein (YFP) into intact coleoptiles by biolistic transfection. Following expression of the fluorescent tag, we were able to observe the responses of actin microfilaments to auxin and light (the major stimuli that control growth in rice coleoptiles) in the epidermis, i.e. in those cells that actually control the growth response. We found the quality and timing of the response to be consistent with previous data obtained from staining with fluorescent phalloidin and biochemical analysis (Waller and Nick 1997, Wang and Nick 1998, Waller et al. 2002). We show further that double-transfection with different fluorescent tags can be achieved at high frequency, which opens the possibility of studying the function of proteins associated with the signal-dependent response of microfilaments in a well-known and well-controlled physiological context. In summary, the described technique provides a valuable tool to investigate dynamics of the cytoskeleton by live-cell imaging.

Results

The efficiency of the assay determined on the level of expression

For a transient assay involving in-vivo observation of cellular responses, a high number of cells expressing the tag is crucial. To obtain a high number of hits, we attached intact rice seedlings densely on glass slides (Fig. 1a) and bombarded them with gold particles coated with the DNA of interest. The seedlings were cultivated in the dark for an additional 16–30 h – depending on the expression level of the respective protein – and then viewed by confocal laser scanning microscopy. During this period the coleoptiles elongated at a rate of about 5–10 mm day^{-1}, which is the growth rate observed in untreated coleoptiles raised in the dark (Wang and Nick 1998). Preliminary experiments showed that the response to red light (inhibition of elongation) and that to exogenous auxin (stimulation of elongation) was maintained (data not shown).

In order to evaluate the biolistic system as a base for future in vivo studies with FPs in coleoptiles, statistical measurements were made. The coleoptiles were transformed with YFP alone driven by a 35S-promotor (Fig. 1b). The number of expressing cells per coleoptile was plotted against the distance from the scattering centre of emitted gold particles (Fig. 1c). The data show that up to almost 20 cells per coleoptile expressed the tag, but the number of hits decreases rapidly towards the margin which emphasizes the importance of stacking the coleoptiles densely.

To test whether the number of expressing cells depends on the construct used for transformation, we compared the number of cells expressing the talin–YFP fusion with those expressing the unfused YFP-construct, both driven by a 35S-promotor

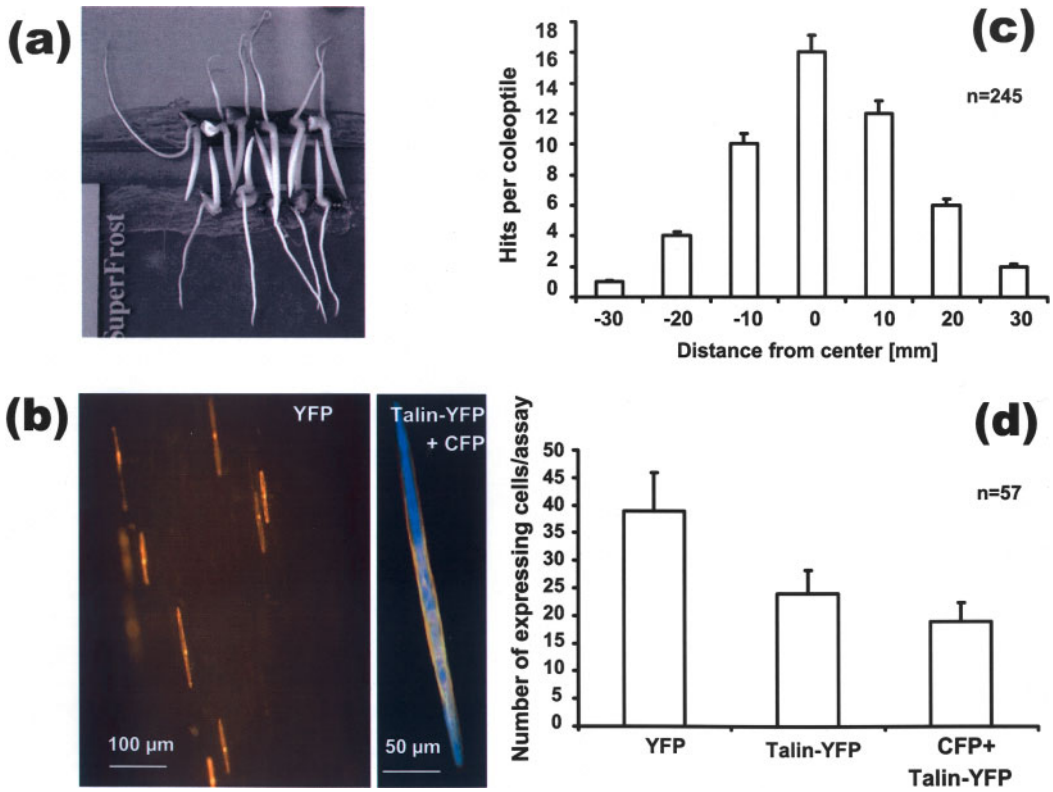

Fig. 1 Arrangement of rice seedlings for particle bombardment, statistical evaluation and determination of success rates of different non-fused and fused FP vectors. (a) Double row of rice seedlings stuck onto glass slides arranged for bombardment with gold particles. After incubation for 16 h in the dark, the frequencies of hit cells in coleoptiles were determined by epifluorescence microscopy. (b) Expression of YFP (left) as single and CFP plus talin–YFP (right) as double expression experiment. (c) Scattering effect of bombardment reflected by frequencies of hits in epidermal cells at various distances from the center. (d) Transformation rate in comparison for expression of an empty YFP-vector, a fused talin–YFP construct and, for double expression of an empty CFP vector together with talin–YFP.

(Fig. 1d). We found that the use of the talin–YFP fusion construct reduced the number of expressing cells by almost 50% as compared to the unfused YFP. This result indicates that the number of expressing cells is indeed affected by the nature of the construct. For a successful transformation event two conditions have to be met: first, the foreign DNA has to be delivered into the cell; second, the cell has to express the introduced gene.

Talin–YFP visualizes microfilament bundles as well as the cortical actin meshwork

Actin is organized in two major arrays in epidermal cells of Graminean coleoptiles (Waller et al. 2000): longitudinal, often transvacuolar bundles and delicate, mostly cortical strands. Depending on the developmental stage of the cell these arrays are manifest to a different degree. We therefore asked if such differences could also be visualized via transient expression of talin_YFP. When the coleoptiles are transfected during the final stages of elongation growth when growth rates are below 0.5 mm day^{-1} (Fig. 2a), long strands of bundled microfilaments are labeled that extend through the entire cell whereas pole regions are often deprived of actin. When the coleoptiles are subjected to transfection at an earlier stage (at the beginning of rapid elongation with growth rates of more than 3 mm day^{-1}), a fine cortical meshwork composed of longitudinal and transverse strands spans through the entire cell cortex and reaches the cell poles (Fig. 2f). Thus, talin_YFP correctly labels both arrays present in these cells, yielding images that are consistent with those obtained by mild fixation followed by visualization with fluorescent phalloidin (Waller et al. 2002).

Microfilaments remain dynamic after visualization with talin–YFP

To check whether the association with talin–YFP leads to a stabilization of actin microfilaments that would impair their dynamic organization, individual cells were followed over time in intact coleoptiles. Comparison of subsequent frames captured in intervals of 10 min (Fig. 3a, b) reveals that the overall organization of the microfilaments is maintained over long time periods. It is therefore possible to distinguish between bundles of microfilaments and to locate them through several frames (white arrows). A closer look reveals, however, that orientation, thickness, and fine structure of individual strands differ between frames. New strands appear, others disappear, neigh-

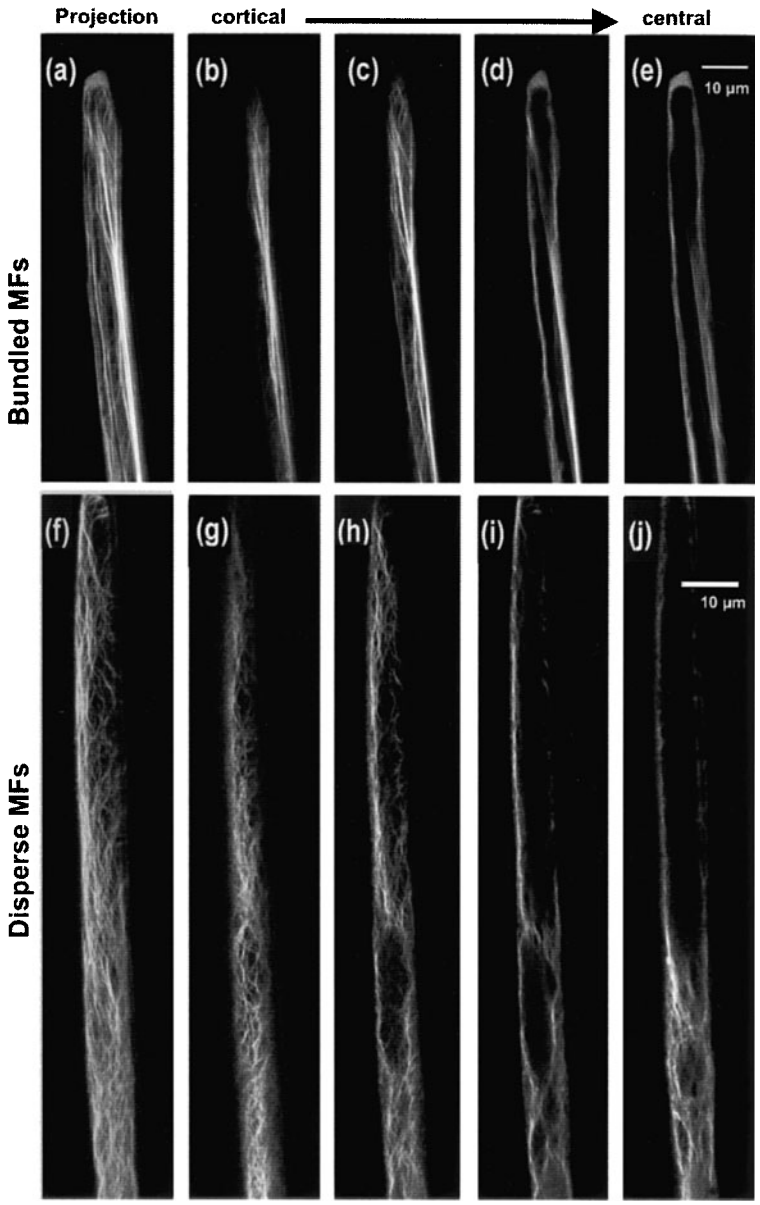

Fig. 2 Talin–YFP visualizes both microfilament bundles as well as the cortical meshwork. (a, b) Cells were scanned in four confocal sections from the cell cortex to the centre (B–E, G–J) and shown as projection in A and F, respectively. Actin in (a) is represented by predominantly axially aligned thick bundles of microfilaments (MFs) whereas in (b) a cell with a fine and dispersed mesh of MFs is shown (b).

bouring strands merge or seem to deviate (see white arrows). This indicates a considerable degree of fluctuation on the level of individual microfilament strands, despite an apparently stable organization of the array as an entity.

To test whether talin-labeled actin filaments remain sensitive to actin depolymerising drugs, cells were treated with latrunculin B and cytochalasin B. The actin meshwork of a cell treated with 1 µM latrunculin B disappeared within 30 min (Fig. 3c). Stable longitudinal bundles of actin typical for a cell with arrested elongation (Fig. 3d) virtually vanished after 45 min of treatment with 10 µM of cytochalasin B, indicating that talin–YFP does not affect actin dynamics. Interestingly, a cloudy structure appearing as a "basket" of short actinfilaments around one center were observed after treatment with this inhibitor. By bright-field microscopy (data not shown) this center could be identified as the site where the gold particle had entered.

Microfilament responses to auxin and light visualized by talin–YFP

Since talin–YFP was found to truly label both microfilament arrays characteristic for these cells and to preserve their innate dynamics, we asked whether it was possible to follow the signal-dependent responses of microfilaments in this system. The stimuli of major biological relevance in these coleoptiles are auxin and light. Whereas auxin triggers a stimulation of growth, light causes growth cessation.

To follow the response to auxin, transformed cells with thick bundles of actin were followed over 2 h prior (Fig. 4a,

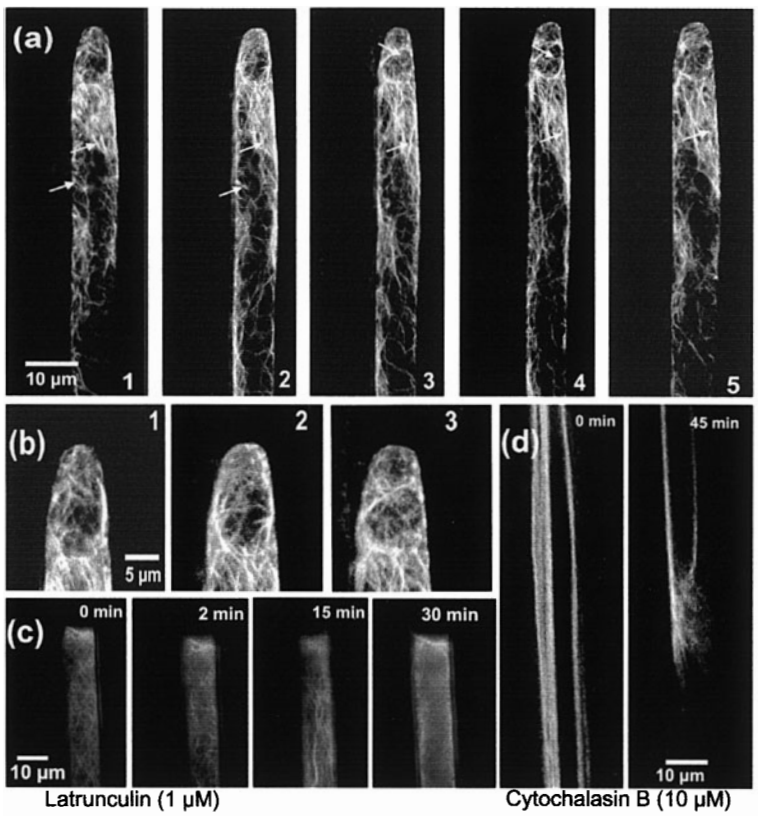

Fig. 3 New formation and disappearance of talin–YFP-labeled actin microfilaments. (a) Time series of a cell at intervals of 10 min showing fluctuations of actin filaments in the poles of epidermal cells. New strands are formed which appear, disappear or apparently maintain their original position (indicated by arrows). (b) Magnified pole regions of (a). (c, d) Disappearance of the actin meshwork after application of actin drugs (c) Time series (0, 2, 15 and 30 min) of a cell treated with 1 µM latrunculin B. (d) Disrupted actin filaments after treatment with 10 µM cytochalasin for 45 min. At the same time, a cloudy structure, appearing as a "basket" of actin arrays, is visible close to the cell surface where the gold particle had entered.

frames 1–4) and more than 2 h subsequent (Fig. 4a, frames 5–8) to addition of auxin. The long, dense and apparently stable bundles remained more or less unchanged before auxin was added (Fig. 4a, frames 1–4). In addition to these bundles, only a few, mostly longitudinal cortical strands were visible that only rarely reached into the cell pole. After addition of auxin, the bundles were rapidly replaced by finer strands and the cortical fine microfilaments became more prominent with an increased tendency to deviate more from the long axis of the cell (Fig. 4a, frames 5–8). Moreover, the newly formed alignment appeared to be much more dynamic with microfilaments changing their orientation rapidly between subsequent frames (Fig. 4a, frames 5–8). This observation is also true for strands appearing at cell poles (after addition of auxin) that continuously changed their position, created new interconnections within the cell pole or simply disappeared.

When the fine cortical microfilaments characteristic for rapidly elongating coleoptiles are followed during exposure to white light, they display a reorientation from initially transverse to oblique into a clearly longitudinal direction (Fig. 4b, c). Fig. 4c demonstrates that the preferred axial arrangement of actin filaments still present after 45 min of light exposure is kept during the entire following period of 8 h after the onset of light. Interestingly, these microfilaments did not form bundles comparable to those seen in auxin-depleted cells (compare Fig. 4b, frame 2 with Fig. 4a, frame 1) but remained dissociated in individual strands. Additionally, microfilaments were still observed to reach into the cell pole. Thus, although both auxin depletion and light cause a cessation of cell elongation, the microfilament organization observed under these conditions differs considerably.

Discussion

The goal of these experiments was to assess the potential of a transient transfection system to observe in vivo responses of the actin cytoskeleton in Graminean coleoptiles. The Graminean coleoptile was chosen as experimental plant material because of several advantages. Firstly, in contrast to other systems commonly used for transient assays, such as onion epidermis, the epidermal cell layer of coleoptiles has a central function in active growth and exhibits fast growth responses to various stimuli such as gravity, light and auxin. Furthermore, as growth is based exclusively on cell expansion, not on cell division, this allows correlations between growth rate and cellular events such as reorientations of microtubules and actin filaments. Finally, the coleoptile as intact organ is preserved in its entirety during all experimental procedures including treatments and microscopic observation.

Several preceding studies using immunolabeling or phalloidin staining have demonstrated altered cytoskeletal arrangements after the onset of diverse stimuli [for review see Nick (1999)]. However, since these techniques are highly invasive and in their final consequence lethal, information about the

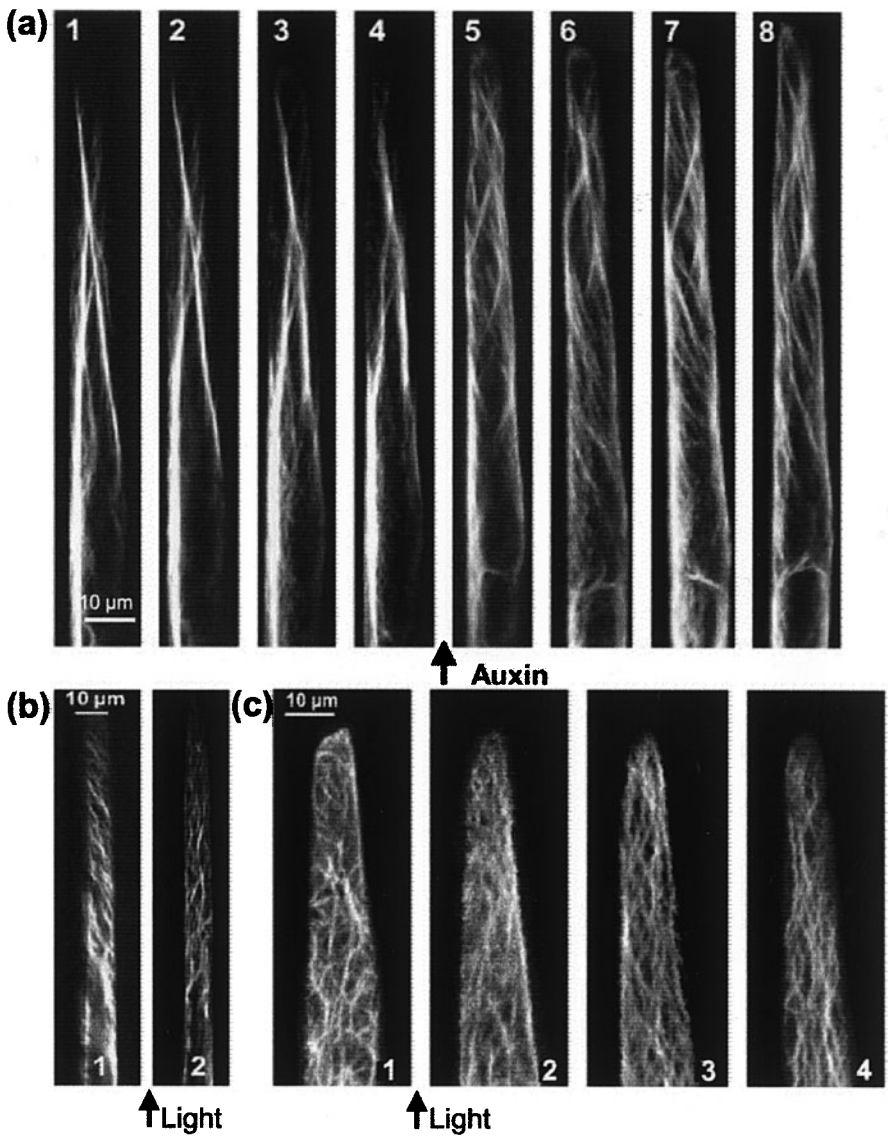

Fig. 4 Reorganization of actin filaments in response to auxin (a) and light (b,c) shown by whole cell scans. (a) Response to auxin. Actin arrays consisting of thick longitudinal bundles remain nearly unchanged during an observation period of 2 h (1–4; intervals of 30 min). Upon addition of auxin (10 µM NAA) bundles disperse and dissociate within 30 min into randomly oriented fine strands (5). Reorientation is subsequently accompanied by continuous realignments of microfilaments for the following period of 2 h (5–8; intervals of 30 min). (b, c) Response to light. (b) Complete reorientation of transversally aligned actin filaments (1) in axial direction (2). (c) Reorientation and withdrawal of microfilaments from the cell pole during exposure to white light. Exposure times 0 min (1), 45 min (2), 4 h (3), and 8 h (4).

dynamics of the cytoskeleton could only be inferred by analyzing large population of fixed cells. To get a more detailed view of the dynamics in a single cell it is necessary to observe reorientations of microtubules and actin filaments in the very same cell before and after stimulation. We achieved this by combining the advantages of the coleoptile system with biolistic delivery of fluorescent fusion proteins labeling the cytoskeleton. The use of an in vivo marker for actin allows following the stimuli-induced rearrangements of actin microfilaments continuously in the same cell at a high temporal and spatial resolution. Even very discrete positional changes of individual actin filaments can be observed providing additional indications on functions of the actin cytoskeleton.

From the technical point of view the number of hit cells in biolistic transfection systems is generally low. As the morphology of the actin cytoskeleton can vary considerably among the different cells of a tissue, the question of efficiency is very important. Statistical studies were therefore conducted to estimate success rates with either the non-fused or the fused FP vectors or with double transfections. As seen from the results for talin–YFP, single transformation was found to be highly efficient. Interestingly, double transformation of talin–YFP with an additional cyano fluorescent protein (CFP) vector also resulted in high transfection rates.

With respect to the ability of our in vivo system to reflect the actin cytoskeleton in its natural structure and behaviour, several observations can be made. Firstly, fine transversally orientated actin strands at the cell cortex can be distinguished from thick longitudinal bundles of actin microfilaments. In addition to the fact that fine bundles of actin microfilaments are well preserved throughout the whole cell cortex this is also true for their functionality and responsiveness during exposure to certain stimuli. Exogenous auxin induces a loosening of thick longitudinal bundles and a formation of a fine polar meshwork

which can be now in advantage to previous studies continuously and directly observed.

Following light treatment the actin cytoskeleton showed a dramatic realignment from transverse to loose longitudinal arrays. This was quite surprising considering the cytoskeletal responses known from other studies of growth under light conditions: coleoptile growth in rice, similar to other Graminean seedlings, is controlled by the phytochrome system (Pjon and Furuya 1967). Whereas elongation of individual cells is promoted in the dark, elongation ceases rapidly in response to light, allowing the primary leaf to pierce through the coleoptile tip. In addition to light, elongation in coleoptiles is regulated by auxin as has been shown in detail in dose-response experiments [for rice see Wang and Nick (1998)]. While the application of exogenous auxin promotes cell growth and induces loose arrays of actin microfilaments, growth halts in response to auxin depletion, actin filaments are bundled and the polar mesh disappears (Waller et al. 2002). Assuming that light acts by lowering auxin levels, it would be expected that this crosstalk would be also mirrored by a corresponding organization of actin. This would mean a bundling response in the case of auxin deficiency or light and a loose array formation in the case of auxin promoted growth in dark. However, we observed actin, in response to light, to realign into fine and dispersed longitudinal strands not exhibiting any bundling effect (Fig. 4b, c). The fact that the actin response to light is totally different from that to auxin depletion might indicate signal-specific and independent mechanisms. This could mean that light controls actin via components that are either independent of auxin or act upstream of those events that modulate the actin cytoskeleton in response to auxin depletion.

On the other hand, the longitudinal reorientation of actin induced by light is reminiscent of the characteristic behaviour of microtubules under light conditions. Cortical microtubules are found to be transverse in cells where elongation is promoted. In contrast, they rapidly reorientate longitudinally under light conditions which inhibit growth (Nick 1999, Waller et al. 2000). Thus, the light-induced reorientation of microfilaments is in parallel to that of microtubules and is therefore consistent with a mutual control of both cytoskeletal components (Collings and Allen 2000, Tominaga et al. 1997). Parallel rearrangements were also recently observed in embryogenic cells of maize where redistributions of microtubules and actin filaments to the cell cortex occurred during the developmental switch from unpolarized to polarized cells in response to auxin deprivation (Samaj et al. 2003).

In addition to the realignment of actin filaments another observation was made in respect of the cell poles. The amount of actin filaments appears to be elevated at pole regions of cells when transverse microfilaments predominate along the cell axis (Fig. 4a frame 4; frame 1 in 4b and 4c) but disappears from the furthest pole regions when actin filaments are longitudinally aligned along the cell. Enrichment or deprivation of actin in end-poles of cells might therefore be linked to a certain developmental stage of the cells within tissues like the epidermal layer of higher plants. In elongating cells of maize root apices actin has been observed to be enriched at end-poles which was suggested to be important for the maintenance of polarity (reviewed by Baluška et al. 2003). From the present observation one might therefore conclude that actin extensions at outermost regions of cell-poles in parallel to a transversal orientation of actin filaments at lateral domains does not only reflect a stage where cell-growth is promoted but also represents a manifestation of cell polarity.

Finally, it has to be noted that, despite a considerable accumulation of the actin-binding protein talin through overexpression, neither the dynamics nor the responsiveness of the actin cytoskeleton seem to be affected. It can also be seen that actin retains its natural dynamics in the response to latrunculin B or cytochalasin B. The preserved actin "basket", which was occasionally observed at the wounded site, can be explained either by a direct action of the drug, since cytochalasin is known to form short rods of actin filament bundles (Collings et al. 1995), or, more speculatively, by a scenario of altered actin dynamics as an element of a wounding response. In fact, changes of actin distribution have been observed in cells after fungal penetration in the way that actin filament bundles became aligned towards the penetration site (Kobayashi et al. 1991, Schmelzer et al. 1995).

The technique presented above employing live-cell imaging of cells of Graminean coleoptiles allows the observation of several signal-dependent responses of the actin cytoskeleton. The method labels fine actin filaments specifically and in a non-destructive manner. The most striking benefit is that fine bundles of actin microfilaments can be continuously observed during reorientation in response to stimuli. Formation of bundles, disassembly and assembly, as well as positional changes of microfilaments can be followed at a high temporal resolution. This advantage is exemplified by the discovery of new and unexpected rearrangements of actin in response to diverse stimuli. With these advantages, the presented in vivo technique opens up a new field for the investigation of cytoskeletal dynamics. As interesting perspective, the mutual control of actin- and microtubular dynamics could be followed through the simultaneous expression of differentially labelled actin and microtubule-binding proteins.

Materials and Methods

Biolistic transformation

For biolistic transformation, gold particles (1.5–3.0 μm, Sigma-Aldrich) were coated with different fusion constructs according to a modified manual of BIO-RAD (PDS-1000/He Particle Delivery System Manual). An amount of 1 μg DNA was used for each transfection, for double transfections DNA combinations were prepared at 1 : 1 (w/v) mixtures. DNA-coated gold particles were placed on macrocarriers (BIO-RAD) and kept at room temperature for total evaporation of ethanol (45 min). Rice (*Oryza sativa* L. *ssp. Japonica cv.* Nihonmasari) seedlings were grown at 25°C for 4 d in the dark on floating meshes as

described by Nick et al. (1994) Under green safe light, 5–6 seedlings were attached close to each other by gluing caryopses trimmed closely to the seedling along the edge of a glass slide (Fig. 1a). Two of these slides were placed opposite to each other horizontally in a particle gun which was constructed according to Finer et al. (1992) and DNA-coated gold particles were delivered to epidermal cells of coleoptiles by three shots at a pressure of 1.5 bar in the vacuum chamber at –0.8 bar. Cells were viewed after growth for additional 12–20 h in the dark.

Vectors for fusion constructs

The *p35S-YFP-talin* construct within a binary vector (pVKH18En6) was a kind gift of Federica Brandizzi. The CFP vector *p35S-CFP-C1* was a kind gift of Thomas Kretsch. It was constructed by fusing the CFP-C1 fragment from pECFP-C1 (Clontech) into a p35S-transient expression vector.

Auxin, light and inhibitor treatments

Microscopic images were obtained before and after the respective treatments. For the application of auxins and cytochalasin B, the coleoptiles were placed in small Petri dishes (3.5 cm in diameter) where the central part of the bottom was replaced by a coverslip such that the coleoptile could be viewed either under upright or inverse microscopes. Auxin was applied as 10 μM α-naphthaleneacetic acid for a total time interval of 2 h. Cytochalasin B was added at 10 μM for 45 min. For light treatments, individual cells were first viewed under the microscope, and then the coleoptile kept in glass cuvettes filled with water. After exposure to white light for 2 h they were again returned to the microscope and the cells that had been viewed before were again relocated and recorded.

Microscopy

For statistical evaluations, an epifluorescence microscope (Axioskop, Zeiss, Oberkochen, Germany) with a digital image acquisition system (Axiovision and Axiocam, Zeiss, Oberkochen) equipped with filter sets for CFP and YFP (Nr. 05 and Nr. 46, Zeiss, Oberkochen). Microfilaments were analyzed with a confocal laser microscope (LSM510, Zeiss, Oberkochen, Germany) with an argon laser. YFP-talin was observed at an excitation wavelength of 514 nm using a beam splitter at 490 nm and a band pass filter between 535 and 590 nm. Images were taken at a resolution of 1,024×1,024 pixels with a mean of four. For whole-cell scans a series of 20 optical sections was scanned at 1 μm *z*-slice distance covering the diameter of an epidermal cell (10–20 μM). 3D images were obtained by projections of these sections.

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