Chapter 29

Actin and signal-controlled cell elongation in coleoptiles

Frank Waller, Qi-Yan Wang, Peter Nick
Institut für Biologie II, Schänzlestr. 1, D-79104 Freiburg, Germany

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Abstract: The role of actin for cell growth has been investigated in great detail for tip-growing cells such as root hairs or pollen tubes. The role of actin for the growth of interphase cells within a tissue context is much less understood and has therefore been analyzed for signal-controlled cell elongation in Graminean coleoptiles using phytochrome and auxin as triggers. Growth inhibition is found to be correlated to actin bundling, whereas a stimulation of cell elongation is accompanied by the formation of a more delicate actin meshwork especially in the region of the cell poles. The response of actin to light is found to be rapid and to precede changes in growth rate. The analysis of the *Yin-Yang* mutant in rice, where actin disassembles in response to auxin indicates the presence of a highly dynamic actin population that is important for cell growth. The experimental findings are discussed in the framework of three alternative models for a role of actin in the elongation of cells within a tissue context: 1. A mechanical limitation of cell expansion by the rigor of the actin meshwork, 2. A control of cell-wall extensibility via an actin-controlled orientation of cortical microtubules, and 3. A control of actin-guided vesicle transport towards sites of wall synthesis. The chapter ends with an outlook on approaches that will distinguish between these alternatives.

Scope of the study

The role of actin microfilaments has been analyzed in great detail for tip-growing cells such as root hairs, pollen tubes, trichomes, protonemata, fungal hyphae or rhizoids (see e.g. chapters 18, 20, 21, 23, 24 of this book) uncovering a polar transport of vesicles along actin microfilaments towards the growing tip of those cells, where the vesicles unload their cargo leading

to intussusception of new cell-wall material into the growing areas that are confined to the very tip of these cells.

In contrast, the function of actin during the expansion of cells that are integrated into a tissue context, is much less understood. It seems that in those cells growth is not confined to a single site, but extends over larger areas. This more diffuse localisation of growth activity is accompanied by a different mode of expansion - rather than increasing wall area via intussusception of new cell-wall material, those cells expand by extending the distance between cellulose microfibrils and by apposing new microfibrils to the inner layers of the cell wall. In this growth mode, the regulation of a growth axis seems to be intimately linked to the orientation of cellulose microfibrils. The synthesis of new microfibrils is guided by underlying microtubules: Transverse orientation of microtubules and, consequently microfibrils, supports cell elongation, whereas a loss of this transverse orientation causes a shift of cell expansion towards lateral thickening (for reviews see Giddings and Staehelin 1991; Williamson 1991; Nick 1998). Interestingly, the orientation of cortical microtubules can change in response to a range of signals such as light, gravity, or various plant hormones. These responses are rapid (Himmelspach et al. 1999), and they are accompanied by corresponding changes in cell expansion (for review see Nick 1998). Whereas cellular morphogenesis of tip-growing cells has been discussed in terms of signal-dependent changes of polar vesicle transport, it thus seems that, in tissues, it is signal-triggered microtubule reorientation that drives morphogenetic responses to the environment.

A closer look uncovers, however, several inconsistencies between microtubule responses and growth (Nick et al. 1991; Nick and Schäfer 1994). Moreover, although in many cases the microtubular response itself is rapid enough to precede the growth response (Nick et al. 1990; Himmelspach et al. 1999), one has to take into account that at least a certain part of the cellulose fibers has to be deposited in the new direction, before a change of growth rate is to be expected. These considerations indicate that there must be rapid mechanisms that are independent of microtubules and that can inhibit growth. These might include events that cause a general decrease of wall-extensibility such as cross-links between cellulose microfibrils or lignification (e.g. Müsel et al. 1997). However, the stimuli that induce growth inhibition, very often also change the proportionality of the cell (Baskin and Bivens 1995), which means that this non-microtubular mechanism must be based on some kind of directionality.

The prime candidate for such a directional mechanism, capable of rapid responses and connected to signaling is very well known from tip-growing cells: it is the actin cytoskeleton. In elongating tissues, actin is usually organized in interconnected, long strands that are aligned with the axis of cell growth. These strand have been found to drive cytoplasmic streaming in cells, where this effect is prominent (Nagai 1979; Shimmen et al. 1995). However, they are also observed in tissues that are not characterized by a dramatic extent of cytoplasmic streaming. The function of these actin strands that are present in a broad range of tissues remains obscure. Thus, the question seems reasonable, whether it is these actin strands that are responsible for microtubule-independent rapid growth responses of cells within a tissue context.

Advantages of the coleoptile system

A system, where these questions can be addressed, should ideally fulfill the following conditions:

1. Growth should be based exclusively on cell expansion, not on cell division to allow correlations between growth rate, cellular and biochemical events.
2. Growth should by accessible to rapid and consistent control by external signals.
3. The cells should be large enough to allow for cell biological studies
4. The system should develop synchronously to provide a homogenous population for biochemical studies.
5. The physiology of growth should be well established.

These requirements are met by the Graminean coleoptile (Fig.29.1). This organ develops as outgrowth of the scutellum (the Graminean homologue of the cotyledon) during late embryogenesis and ensheaths the apical meristem and the embryonic leaves. Following germination it grows rapidly. Growth is carried exclusively by cell elongation. Germination and development can be easily synchronized to high homogeneity by soaking the caryopses in water. The biological role of the coleoptile is to guide the primary leaves through the soil to the surface. As soon as it has reached the surface, it ceases to grow further and is pierced by the primary leaves. It continues to live, however, for several weeks and seems to play a role in the organization of crown-roots, the major rooting system in adult grasses (Nick 1995).

As adaptation to its biological function, coleoptiles possess an astounding ability to adjust growth with respect to external and internal stimuli:

1. Growth of older coleoptiles is inhibited by red- and far-red light, perceived by the plant photoreceptor phytochrome as well as by blue light, perceived by the plant photoreceptor cryptochrome. This is biologically meaningful, because light indicates that the coleoptile has reached the surface and fulfilled its task. In contrast, the growth of very young coleoptiles is stimulated by phytochrome, but inhibited by blue light. This might be an adaptation to the changed spectral composition of light that penetrates into the soil - whereas blue light is scattered strongly and cannot penetrate, red and especially far-red light can penetrate more deeply. A high red to blue ratio thus indicates that the coleoptile is approaching the surface, but has not yet reached it - an acceleration of growth is therefore a meaningful response (Du Buy and Nuernbergk 1930).
2. In addition to light, the direction of coleoptile growth is guided by gravity. If maize coleoptiles are displaced from a vertical orientation, they respond by gravitropic curvature with a delay time of less than 30 min (Nick and Schäfer 1988; Himmelspach et al. 1999), the response of rice coleoptiles is even faster (Godbolé et al. 2000).
3. The coleoptile responds to asymmetries in the light distribution by phototropic curvature and the formation of a stable transverse polarity that guides the development of the crown roots such that they are preferentially emerging at the shaded side (Nick 1995).
4. These growth responses are carried by changes in the direction and the extent of auxin fluxes from the coleoptile tip (where auxin is produced) towards the base of the coleoptile. The inhibition of growth by red light is triggered by a phytochrome-induced inhibition of auxin transport from the tip into the elongating zone of the coleoptile (Furuya et al. 1969). Gravitropic and phototropic bending are caused by a redistribution of auxin across the coleoptile towards the lower or the shaded flank (Cholodny 1927; Dolk 1936; Went 1928).

The target of these signals seems to be the epidermis that poses a mechanical constraint to the extension of the inner tissues. When the epidermis is removed, the inner tissues extend spontaneously, whereas the epidermis shrinks (Kutschera et al. 1987). By application of auxin, this shrinkage is reduced, whereas the extension of the inner tissue is maintained. This means that, in the intact coleoptile, growth is limited by a reduced extensibility of the epidermis and that auxin can stimulate growth by increasing this extensibility. Electron microscopical studies (Bergfeld et al. 1988) show that it is the outer surface of epidermal cells, where this constraint of growth is located. Thus it is possible to explain the signal response of an entire organ (the coleoptile) in terms of signal-triggered

changes in the extensibility of the outer epidermal cell wall. The coleoptile epidermis consists of well-polarized, relatively large cells that are accessible to a range of cell-biological and histological approaches and is thus an ideal system to study the role of actin for the growth of cells that are integrated into a tissue context.

Light-induced bundling of actin in maize coleoptiles

To obtain insight into the possible role of actin in cell growth, the structure of actin microfilaments was analyzed in epidermal cells of maize coleoptiles under different light conditions. In this system, cell growth is tightly controlled by light perceived by the phytochrome system: When maize seedlings are grown in darkness, the mesocotyl is elongating strongly whereas the coleoptile shows only reduced development. Grown in continuous far-red light, a light quality perceived only by the phytochrome system, mesocotyl growth is suppressed but growth of the coleoptile and primary leaf is promoted. The growth kinetic differs in these tissues: Coleoptile elongation is accelerated by far-red light, leading to a length of about 40 mm within 4 days after germination. In darkness, the final length is identical, but is accomplished only after 6 days. In contrast, the mesocotyl in dark-grown seedlings is more than five times longer compared with that of seedlings grown in far-red light (Waller and Nick 1997).

The light response of the coleoptile is based on changed cell elongation

The analysis of cell length and cell number in epidermal cells of the coleoptile during the growth period from 2 to 6 days after germination reveals a close correlation between coleoptile length and cell length. The growth curve of epidermal cells and of the whole organ closely resemble each other and cell numbers remain constant. This suggests that growth of the coleoptile is accomplished exclusively by cell elongation without any contribution of cell division (Waller and Nick 1997). Epidermal cells in dark-grown mesocotyls also show an elongation during the first days after germination, but from day 5 to day 6 after germination, average cell length declines drastically and pairs of adjacent short cells can be observed (Waller and Nick 1997). Thus, in contrast to the coleoptile, cell elongation cannot account completely for the growth response of the mesocotyl.

The cells that control growth possess specific actin microfilaments

Upon staining with fluorescence-labelled phalloidin and analysis by fluorescence microscopy and laser scanning confocal microscopy a fine meshwork of actin microfilaments can be visualized in many cell types.

This pattern is observed in epidermal cells of primary leafs, in primary leaf companion cells and in subepidermal cells of coleoptiles. In contrast to the cells of the inner tissues, epidermal cells of the coleoptile (and of the mesocotyl) are characterized by bundles of actin filaments spanning the longitudinal axis of the cell, forming an array of finer filaments at the poles of the cell only. This difference is especially striking, if epidermal cells are compared to the immediately neighboured subepidermal cells: Thick longitudinal bundles of actin filaments are only observed in epidermal cells whereas neighbouring subepidermal cells do not exhibit this pattern (Waller and Nick 1997). This difference is significant if one takes into account that it is the epidermis that limits and regulates coleoptile growth (Kutschera et al. 1987), whereas the inner tissues play a more passive role in the regulation of growth.

Condensed microfilament arrays correlate with reduced cell elongation

When the behaviour of actin filaments in the coleoptile epidermis is followed over time in dark- and light-grown seedlings, a significant change in the state of longitudinal bundles is observed: In young seedlings (2.5 to 3 days after germination) that had been raised under far-red light, the longitudinal arrays of actin filaments consist of a number of fine strands spanning the longitudinal axis of the cells. In addition, a fan of fine filaments can be observed near the cell poles. When growth slows down after day 4, these filaments become increasingly bundled and the fine polar strands disappear. In this stage most of the longitudinal actin filaments are concentrated in two to four thick longitudinal actin bundles.

In dark-grown seedlings, longitudinal filaments are comparatively bundled up to day 3 after germination along with a slow growth of the coleoptile. They loosen into arrays with more and finer longitudinal filaments and fine strands near the poles between days 4 and 5 when dark-grown coleoptiles undergo rapid elongation. Eventually, the microfilament recondense into thick bundles from 6 days after germination, accompanied by a rapid decline in growth rate.

These observations can be summarized as follows (Waller and Nick 1997): During periods of fast elongation (2 to 3 days after germination for far-red treated seedlings; 4 to 5 days after germination for dark-grown seedlings) of the coleoptile longitudinal actin filaments in the epidermal cell layer are organized into a number of fine strands, whereas prior or subsequent to the phase of strong elongation these filaments are observed to form few thick bundles.

The light response of actin microfilaments is fast

In epidermal cells of the mesocotyl, a similar correlation between cell elongation and the organization of longitudinal actin filaments has been observed. These actin filaments respond rapidly to changes in growth rate: When dark grown maize seedlings are transferred to far-red light, mesocotyl growth slows down by about 70 % within one hour after onset of irradiation. This growth inhibition is accompanied by a rapid and dramatic response of actin microfilaments: The originally fine strands are replaced by dense bundles that have, in addition, approached the lateral cell walls.

The main findings of this study on the relation between actin microfilaments and phytochrome-controlled cell elongation can be summarized as follows:

1. The microfilament structure of epidermal cells, where the control of growth is located, differs significantly from the underlying tissue.
2. Epidermal microfilaments occur in two configurations: massive longitudinal bundles or a loose array consisting of fine longitudinal strands.
3. The bundled configuration is observed in cells, where elongation is blocked, the loose array is observed in cells, where elongation is elevated.
4. The transition from the loose array to the bundled array in response to light is rapid and parallel to an inhibition of cell elongation.

Auxin-induced dynamics of actin in rice coleoptiles

The response of Graminean coleoptiles to phytochrome seems to be controlled by changes in auxin transport (Furuya et al. 1969). This plant hormone that is produced by a very limited region in the very tip of the coleoptile, is transported in basipetal direction and this transport can be regulated by a number of signals such as red light (Furuya et al. 1969), blue light (Went 1928; Nick et al. 1992), and gravity (Godbolé et al. 2000). By limiting the flow of auxin into a given region of the coleoptile, cell growth in this region can be controlled allowing, in case of auxin asymmetries, for curving responses such as photo- and gravitropism.

Screen for cytoskeletal rice mutants

On this background, mutants were selected in rice, where the response of the cytoskeleton to auxin as a key signal was disturbed (Nick et al. 1994). Such mutations should become manifest as alterations of coleoptile growth, when the cytoskeleton is differentially eliminated depending on its dynamics. This is possible for drugs that inhibit the addition of monomers to cytoskeletal polymers. Cytoskeletal elements with a high level of dynamic equilibrium between assembly and disassembly should be more susceptible to such compounds as compared to more inert structures with low rates of monomer addition and polymer disassembly. In other words: it is possible to screen for mutants with reduced cytoskeletal dynamics by searching for seedlings, where coleoptile elongation has acquired resistance to cytoskeletal assembly inhibitors such as the aryl carbamates, the dinitroaniline herbicides (in case of microtubules) or the cytochalasins (in case of microfilaments). Since these compounds cause a loss of growth axiality, the growth of susceptible plants is dramatically blocked, accompanied by lateral swelling, whereas resistance becomes manifest as unimpaired coleoptile elongation.

Yin-Yang, a mutant with elevated resistance to aryl carbamates

Among mutants that were found to be resistant to aryl carbamates such as phenyl urethane or propham, *Yin-Yang* was isolated as a recessive mutant due to a moderately increased resistance of seedling growth. It was found to be cross-resistant to drugs with a different site of action such as colchicine, indicating that the mutation was not based on alterations of the drug-binding site of tubulins (Wang and Nick 1998). A physiological analysis revealed that cell number was increased in the epidermis of mutant coleoptiles, which was compensated by a reduced cell length. Microtubules were found to be oriented strictly transverse at a time, when they had already assumed a longitudinal orientation in the wild type, accompanied by a prolonged period of coleoptile elongation.

During experiments on the gravitropic response of rice coleoptiles, a striking 'misbehaviour' was observed for the *Yin-Yang* mutant (Fig. 29.2): The gravitropic lag phase (around 30 min for the wild type) was drastically reduced, the gravitropic response became detectable virtually immediately after the onset of stimulation. On the other hand, the rate of curving was reduced as compared to the wild type resulting in a response that initiated earlier, but required a longer time until the coleoptile was completely vertically again. Moreover, the curving response did not stop when the

coleoptile had recovered its originally vertical orientation, but continued leading to a significant overresponse in the mutant. These alterations of gravitropism were accompanied by a changed microtubular response in the bundle-sheath cells of the coleoptile, where the gravitropic stimulus is perceived: In the wild type, microtubules are found to be strictly transverse in these cells, but reorient rapidly in response to gravitropic stimulation by a reorientation into longitudinal arrays (Godbolé et al. 2000). In the mutant, microtubules are found to be longitudinal before the onset of gravitropic orientation (Nick et al. 1997), which has been correlated with a redistribution of auxin fluxes from basipetal transport to lateral transport towards the lower flank of the rice coleoptile (Godbolé et al. 2000).

Interestingly, all these aspects of the altered gravitropic response in *Yin-Yang* mutants could be mimicked in the wild type by pretreatment with the actin-polymerization blocker cytochalasin D: Microtubules in the gravisensing cells assumed a longitudinal orientation in the absence of gravity (Nick et al. 1997), the gravitropic lag phase was drastically reduced, and the rate of bending was reduced accompanied by significant overbending (Wang and Nick 1998). This raised the possibility that the mutation was only indirectly related to changes in microtubular dynamics, and that the phenotype might be caused by alterations of the actin microfilament system.

To test this assumption more directly, the *Yin-Yang* coleoptiles were analyzed with respect to the response of cell elongation to auxin (Wang and Nick 1998). Coleoptile segments were incubated in the presence of 5 μM auxin, a concentration that represents the optimum of elongation growth, and increasing concentrations of the actin-assembly blocker cytochalasin D were added into the assay to obtain a dose-response relation. The resulting curve revealed that auxin-induced elongation became inhibited in wild-type coleoptiles with concentrations exceeding 0.2 μM of cytochalasin D. In the Yin-Yang mutant, the threshold of inhibition was lowered by a factor of 10. Interestingly, if the experiment was repeated at lower auxin concentrations that were suboptimal for cell elongation, the elevated cytochalasin-D sensitivity in the mutant disappeared. In order to observe this increased sensitivity, a minimal concentration of 200 nM of auxin had to be added into the assay. This type of study was repeated for different temperatures to obtain different growth rates and produced three major results:

1. The *Yin-Yang* mutant is more sensitive to cytochalasin D.
2. This elevated sensitivity is induced by auxin.
3. This elevated sensitivity does not depend on growth rate.

Stimulated by these physiological studies, the *Yin-Yang* mutant was further analyzed with respect to the structure of actin microfilaments in the coleoptile epidermis. In coleoptile segments, where the endogenous auxin had been depleted by incubation in water, the microfilaments of the wild type were organized in dense bundles as was expected from these cells that showed a low rate of elongation (see above). Upon addition of auxin, these bundles became loosened and dispersed into finer strands that were parallel to each other. Again, this was to be expected from the observations in maize, where such a loose array was characteristic for cells with stimulated elongation. The situation in the *Yin-Yang* mutant differed conspicuously: upon auxin depletion, the microfilaments still maintained a loose configuration similar to that observed in the wild type after addition of auxin. After addition of auxin, these fine actin strands disappeared rapidly and were replaced by basket-like networks of short filaments that formed around the nucleus. The formation of such nuclear baskets could be mimicked in the wild type by treatment with cytochalasin D. As observed already for growth, the *Yin-Yang* mutant exhibited an elevated sensitivity of microfilament integrity with respect to cytochalasin D. In the rice mesocotyl that is under control of gibberellic acid rather than auxin (Toyomasu et al. 1994), the formation of nuclear baskets could be induced by gibberellic acid in the *Yin-Yang* mutant, but not in the wild type.

Since cytochalasin D inhibits the addition of actin monomers to growing microfilaments, the sensitivity of a given microfilament to this drug depends on its rate of turnover (Cooper 1987): Dynamic microfilaments with a high dynamic equilibrium between assembly and disassembly are eliminated by low concentrations of cytochalasin D, whereas inert microfilaments with slow turnover are expected to be relatively resistant. The elevated sensitivity of the *Yin-Yang* mutant to cytochalasin D could thus be a consequence of an elevated dynamics of microfilaments.

Auxin (and gibberellin in case of the mesocotyl) seems to stimulate the dynamics of actin microfilaments, because the sensitivity of cell elongation (and of microfilament integrity) to cytochalasin D is elevated in the presence of auxin. This effect of auxin is very dramatic in the *Yin-Yang* mutant, but can be observed in a less pronounced fashion in the wild type as well (Wang and Nick 1998). This stimulation of actin dynamics by auxin must occur in the wild type in a tight balance between elevated disassembly and elevated assembly, because the microfilaments would disappear otherwise. In the mutant, this balance seems to be disturbed in the mutant with disassembly being stimulated by auxin, whereas the assembly seems to lag behind, leading to an increased pool of monomeric G-actin that is then

repartitioned into the nuclear meshwork characteristic for auxin-treated *Yin-Yang* cells. This situation can be mimicked in the wild type by treatment with cytochalasin D. Again, the resulting elevated level of G-actin is organized into the nuclear basket. The observation that, in *Yin-Yang*, the actin microfilaments are found in the loose configuration even upon depletion of endogenous auxin suggests that the mutation might partially decouple actin dynamics from auxin signalling. Auxin perception and early signalling seems to be intact, because auxin sensitivity (as assessed from the dose-response relationship) is not affected in the mutant. The target of the mutation must be located further downstream, either in late steps of auxin signalling or in factors that regulate the dynamics of the actin system.

The major findings of the mutant analysis can be summarized into the following statements:

1. Several aspects of the *Yin-Yang* mutant as different as gravitropic response, cell elongation, microtubule orientation in the gravity-sensing cells of the bundle sheath, or formation of nuclear actin baskets can be mimicked in the wild type by treatment with cytochalasin D.
2. The sensitivity to cytochalasin D is elevated in the *Yin-Yang* mutant with respect to cell elongation.
3. This sensitivity is induced by auxin (or by gibberellin in the mesocotyl).
4. Auxin induces a higher dynamic equilibrium of microfilament assembly and disassembly.
5. The pool of G-actin is tightly controlled in the wild type.
6. This tight control is maintained in the mutant for low levels of microfilament dynamics. It is increasingly lost, when the dynamics is increased by auxin or gibberellin.
7. The early events of auxin signalling are not affected in the mutant.
8. Upon depletion of auxin the microfilaments in the *Yin-Yang* mutant behave as if auxin is still present.

Ideas about the role of actin in cell growth

The work described in this chapter suggests a role of actin microfilaments for the control of cell elongation by signals such as light and auxin. The light stimulus activates the phytochrome system which causes changes of auxin transport and, in consequence, changes of intracellular auxin content. Microfilaments respond to these signals (light or auxin, respectively) by passing between two configurations: a loose configuration composed of fine longitudinal strands that seem to consist of highly

dynamic microfilaments, and a bundled configuration of lower dynamics. The loose configuration is closely correlated with elevated cell elongation, whereas the bundled configuration is found in cells that elongate slowly. The microfilament response is confined to the epidermis, the tissue that limits coleoptile elongation.

The results obtained with the *Yin-Yang* mutant demonstrate that specific alterations of microfilament organization (i.e. induction of nuclear baskets by auxin) are accompanied by corresponding changes in the behaviour of cell elongation (reduced cell elongation). Moreover, when these alterations in the microfilament system are mimicked in the wild type by treatment with cytochalasin D, this results in the same effect, namely, an inhibition of cell elongation (Wang and Nick 1998). The signal response of the epidermal actin array is fast – at least as fast as the response of cell elongation (Waller and Nick 1997).

Thus, actin microfilaments are necessary to maintain cell elongation in epidermal cells of Graminean coleoptiles. They can fulfill this function, when they are organized in loose arrays made up of fine strands, they cannot fulfill this function, when they are bundled in response to auxin depletion.

However, this conclusion does not explain, how actin microfilaments control cell elongation. One can imagine three ideas to answer this question:

Idea 1: The rigor of the microfilament system could mechanically constrain cell elongation (Fig. 29.3A). This idea is supported by the following evidence:

A. Actin forms fan-like arrays near the poles of epidermal cells (Waller and Nick 1997).
B. The microfilament system of adjacent cells is coupled through the plasmodesmata (White et al. 1994).
C. Mild treatment with cytochalasin D that still preserves actin microfilaments as such, detaches the actin cytoskeleton from the epidermal poles in such a way that it collapses, very much like a spring that had been allowed to contract (Wang and Nick 1998).
D. In soy-bean cells, the rigor of the actin system had been estimated by means of a laser forceps (Grabski and Schindler 1996) and found to be tightly correlated with cell growth. Auxin makes the actin cytoskeleton relax and stimulates elongation, whereas cytokinin or aluminum that increase the rigor of microfilaments, inhibit cell elongation (Grabski and Schindler 1995, 1996).

E. Bundling of microfilaments is confined to the epidermis, and coleoptile growth is limited by the epidermis (Waller and Nick 1997).
F. Bundled microfilaments are correlated with inhibited cell elongation, both in maize and in rice coleoptiles (Waller and Nick 1997; Wang and Nick 1998).

If microfilaments impede growth due to their rigor, the elimination of microfilaments by cytochalasin D should remove this growth constraint, and elongation should be stimulated. However, the opposite has been observed (Thimann et al. 1992; Wang and Nick 1997).

Idea 2: Actin could control cell elongation by determining microtubule orientation (Fig. 29.3B).

Actin microfilaments and microtubules are intimately linked. The transverse microtubule arrays characteristic of elongating cells are spontaneously lost in response to cytochalasin D (Nick et al. 1997; Wang, unpublished results). The reorientation of microtubules in response to a signal has been shown to be based on disassembly of discordant microtubules and reassembly in a new direction (Himmelspach et al. 1999). Disassembly and reassembly are biochemical processes that do not convey directional information *per se*, but require some kind of either lattice or field that provides directional cues that are amplified into a direction-dependent stability. These directional cues would then organize microtubule nucleation and microtubule elongation in space. This lattice or field has to embody some kind of axis and it must respond to signals. Actin microfilaments would meet both conditions. If microtubule-organizing centers (MTOC) would be attached to actin microfilaments they could by dynamically reshuffeled between a disperse distribution (maintained by the loose microfilament configuration), and an aggregated distribution (maintained by the bundled microfilament configuration). By modulating the degree of actin bundling, the distances between the points of the MTOC-lattice could be tuned in such a way that the minimal distance between two MTOC would be different in a direction-dependent manner. As long as the microfilaments remain in the loose configuration, the minimal distance would be smaller in transverse direction, larger in longitudinal direction. This would result in a higher stability of transverse microtubules as compared to longitudinal microtubules. Bundling of microfilaments would increase the minimal distance between neighbouring MTOC in transverse direction, such that the stability of longitudinal microtubules would exceed that of transverse microtubules. All the experimental data reported above are consistent with this actin-MTOC-lattice model, moreover this model would explain, why

the microtubules in the gravity-sensitive inner cells of *Yin-Yang* spontaneously assume a longitudinal array (Nick et al. 1997), and why the *Yin-Yang* mutant is cross-resistant to antimicrotubular drugs with different binding sites (Wang and Nick 1998).

Idea 3: Actin could guide vesicle transport to those areas, where cell wall is produced in a way similar to that found in tip-growing cells (Fig. 29.3C).

The loose microfilament configuration would support this function, the bundled configuration would impede the movement of vesicles along actin resulting in growth inhibition. The advantage of the vesicle model over the actin-rigor model (Fig. 29.3a) consists in the explanation, why cytochalasin D is expected to block cell elongation. If the tracks for vesicle transport are affected, this should result in impaired transport and impaired cell elongation. Further support for this model comes from pharmacological studies of root expansion growth, where a loss of growth axis was observed in response to drugs that affect the actin-myosin system or vesicle secretion (Baskin and Bivens 1995). The cargo of these vesicles might be components of the cell wall or of the plasma membrane.

At this point, it is necessary to emphasize a principal difference with the mechanism that drives growth in tip-growing cells: The growth of cells in a tissue does not occur by intussusception of new cell-wall material into the cell wall, but by apposition of new layers to the inner face of the preexisting cell wall. Cell expansion occurs by changing the distance between preexisting cellulose microfibrils, and by apposition of new microfibrils that allow for further expansion. As pointed out in the beginning of this chapter, this growth mode is intimately linked to the orientation of cortical microtubules. A guided transport of vesicles loaded with cell-wall material might be needed to maintain the synthesis of cellulose, an inhibition of this transport would gradually cause a depletion of monomer supply and thus cellulose synthesis. From this, an inhibition of growth might be expected. However, auxin-induced coleoptile growth can be maintained under conditions, where cellulose synthesis has been severely inhibited (Edelmann and Fry 1992). This indicates that microfibril apposition is not an important target for actin-guided vesicle trafficking.

If growth of cells in a tissue were exclusively driven by extension of lateral walls supported by a mechanism dependent on microtubules and microfibrils, one would expect more or less cylindrical cells that are long in case of rapid elongation and short in case of growth inhibition. What is observed under the microscope, is something else: epidermal cells are more

appropriately seen as extended rhomboids that are separated by a more or less cylindrical zone in the equator (Fig. 29.4). In case of elongated cells the equatorial zone is large in relation to the rhomboid polar regions, such that the cell as a whole resembles a cylinder. When growth is suppressed and the cells remain short, their true shape can be seen more clearly, because the equatorial region is short in relation to the rhomboid poles. This is especially striking in the cells of the coleoptile tip prior to expansion. This shape suggests that the polar region of the cells grow by a different mechanism than the equatorial zone. The prominence of rhomboid cells in the coleoptile apex suggests that growth in the polar regions precedes that in the equator. When these cells elongate by a factor of 20 or more, this means that there is a difference in terms of maturation between cell wall and plasma membrane in the very equator as compared to the polar regions: The nearer they are located to the equator, the more mature they are expected to be

This leads to a model, where in the polar region components are inserted into the young cell wall and into the young plasma membrane that are necessary to maintain intercalar growth once the expanding equatorial zone has reached the respective region of the cell wall (Fig. 29.4). Such components might be either elements of the cellulose-synthetizing complexes residing in the plasma membrane (Giddings and Staehelin 1991), or membrane proteins that link the cortical microtubules to the cell wall or just basic components of primary wall and plasma membrane.

In such a model, elongating cells within a tissue would grow based on a two-phase mechanism: In the early phase of cell-wall formation in the polar regions of the cell, actin microfilaments would act to guide vesicles to the cell poles, whereas microtubules would control the subsequent phase, when the matured cell wall extends and grows by apposition. According to the actin-vesicle model of growth control, the loose microfilament configuration would be necessary to maintain growth because it provides the tracks (and, via myosins, the motile force) to support the immense flow of membrane and wall material necessary for the rapid expansion of epidermal cells. The extent of plasma-membrane flow in these cells is astonishingly high – the complete plasma membrane has been reported to turn over within three hours (Steer 1988). This means that a block of vesicle-trafficking caused by the bundling of actin microfilaments should impair growth within minutes, consistent with the fast response of both, epidermal filaments and cell elongation that has been observed after induction of the phytochrome system (Waller and Nick 1997).

Outlook

To distinguish between the three models discussed above, it is necessary to find experimental answers to the following questions:

1. Where do epidermal cells expand? Is growth completely diffuse over the entire cell, or are there distinct regions, where membrane formation takes place preferentially?
2. What is the role of actin-driven motors (i.e. myosins) in auxin-induced cell elongation?
3. How is auxin-signalling coupled to actin-bundling?
4. How is the polarity of vesicle transport (from the cell center towards the cell periphery) controlled?

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Fig. 29.1: Typical morphology of a Graminean seedling. The coleoptile is separated from the mesocotyl by the crown roots that form the main rooting system in the adult plant. In the Graminea, the primary root does not persist, but dies a few weeks after germination. The apical meristem forming the primary leaves is hidden in the coleoptile and is located slightly above the the node. The primary leaves pierce the coleoptiles a few days after germination depending on the lighting conditions.

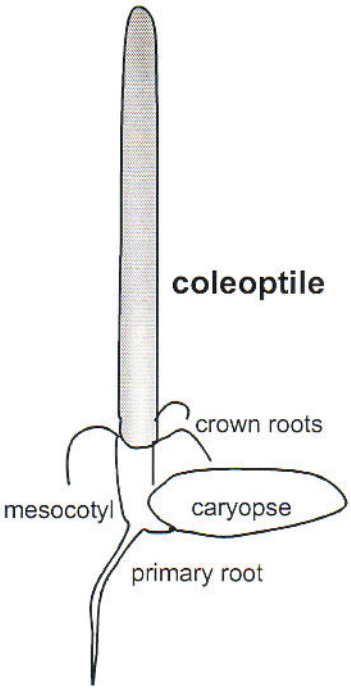

Fig. 29.2: Gravitropic response of the *Yin-Yang* mutant. Rice seedlings were placed horizontally and the gravitropic curvature of the coleoptiles was followed over time in untreated coleoptiles versus plants that had been pretreated for 30 min with 2 µM of cytochalasin D. The response of the wild type is shown in the upper, the response of the *Yin-Yang* mutant in the lower panel. The inset shows a blow-up of the early time course to show the elimination of the gravitropic lag-phase in the mutant (lower panel) or by treatment with cytochalasin D in the wild type (upper panel). The dashed line in the upper panel shows the final curvature reached by wild-type coleoptiles after treatment with cytochalasin D, and the dotted line in the lower panel the final curvature reached by untreated *Yin-Yang* coleoptiles. In both cases, a significant overbending is observed.

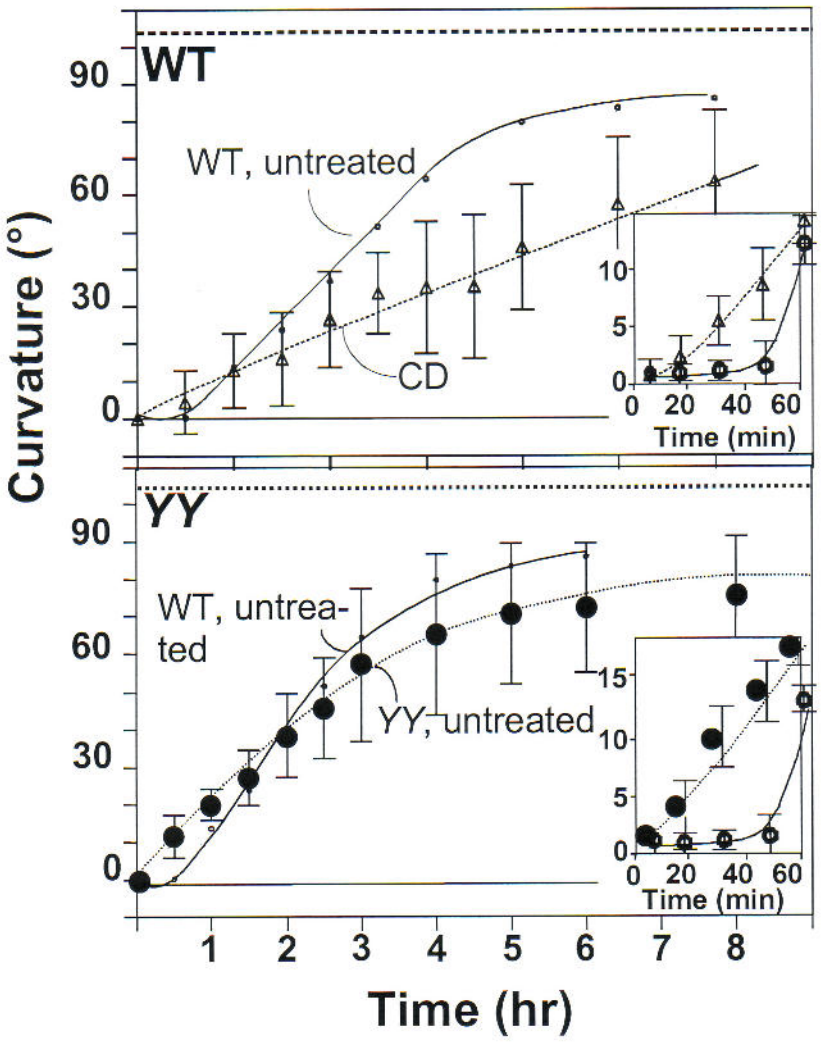

Fig. 29.3: Models for the potential role of actin microfilaments in the control of auxin-dependent elongation growth. **A** Bundling of actin filaments in response to auxin depletion increases the rigor of the actin cytoskeleton and reduces cellular extensibility in longitudinal direction (double-headed arrow). **B** Bundling of the actin lattice causes a repatterning of microtubule-organizing centers (MTOC) increasing the minimal distance between neighboured MTOC in transverse direction over that in longitudinal direction. In consequence, longitudinal microtubules become more stable as compared to transverse microtubules and new microtubules reassemble more frequently in longitudinal direction causing a net reorientation of microtubules into a longitudinal array that causes a longitudinal deposition of cellulose and thus a loss of cell elongation. **C** Bundling of the actin lattice inhibits transport of vesicles to the polar regions of the cell impairing the supply of membrane components that are necessary to maintain cell elongation.

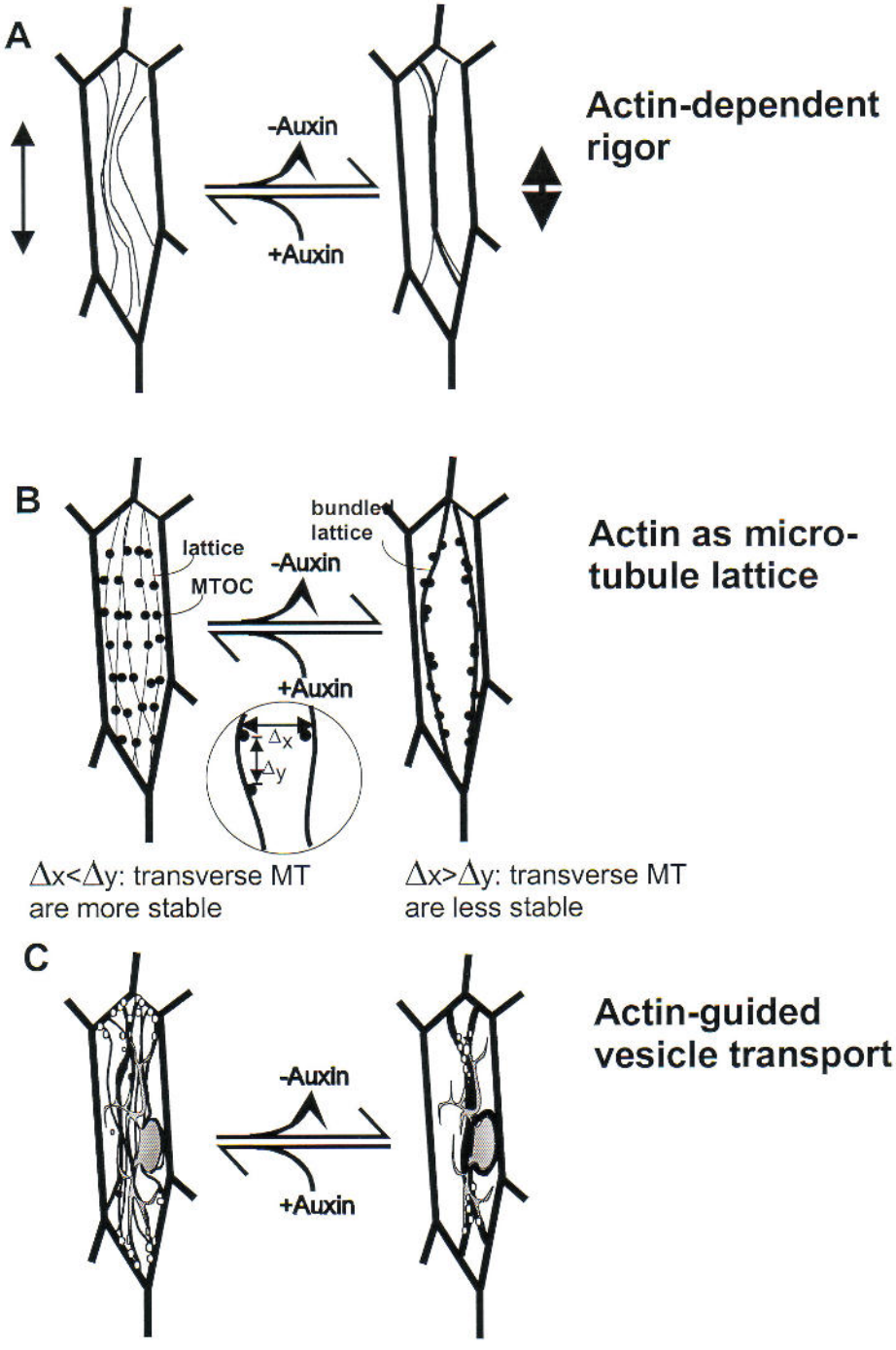

Fig. 29.4: Elongation of an epidermal cell. Elongation is mainly brought about by extension of the central equatorial zone (gray) towards the two poles. Cell-wall components that are necessary to maintain the growth of the equatorial zone (circles) could be transported to the polar zone and would shift to the equatorial zone, when it migrates over the respective position of the plasma membrane. Thus membrane components of the equatorial zone are expected to be have experienced a longer history (symbolized by the gray and black colour of the circles) as compared to the newly inserted membrane components in the polar regions (symbolized by the white colour of the circles).