Role of the microtubular cytoskeleton in coleoptile phototropism

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Introduction

Phototropism is based on the ability to regulate cell elongation in response to asymmetric illumination. The classical system to study phototropism has been the Graminean coleoptile, and studies on the phototropism of grass coleoptiles date back to the time of Darwin and Darwin [1]. So far, all work concerning the role of the cytoskeleton during the phototropic response of higher plants has been performed in coleoptiles and this review will therefore focus on coleoptile phototropism. The coleoptile, a leaf-like organ, is formed late during embryogenesis from the base of the scutellum and sheaths the apical meristem. Physiologically it behaves like a leaf although it is not correct to treat it as a homologue of the cotyledons. The coleoptile has the biological function to protect and guide the young leaves during the growth through the soil. As soon as the coleoptile reaches the surface it ceases to grow almost immediately and the primary leaves pierce through the coleoptile tip. Later, crown roots emerge at the node separating the coleoptile from the mesocotyl, and these crown roots replace the primary root that dies soon afterwards.

From the biological function of the coleoptile, the advantage of this organ for plant physiology can be easily derived: (1) Coleoptiles grow fast - the leaves must reach the light, before the resources of the seed become exhausted. (2) Coleoptiles respond sensitively to light and gravity - these are the stimuli that guide the seedlings towards the surface. (3) Coleoptiles grow exclusively by cell expansion - this is the most economic way of growth. (4) Coleoptiles possess a clear apico-basal polarity - the tip must direct the growth of the whole organ.

The first experiments by Darwin and Darwin [1] demonstrated already that the perception of the phototropic stimulus is situated in the very tip of the coleoptile, whereas the bending response moves towards the base of the organ. This implies the transport of signals from the tip to the base of the coleoptiles. These signals transmit two types of information: (A) about the fact that the coleoptile tip has perceived light, and (B) about the direction of the light stimulus. Independently, Cholodny [2] and Went [3] discovered that a growth promoting agens is redistributed during tropistic bending across the coleoptile cross section. The activity of this agens could be measured by a famous bioassay and the agens was eventually identified as the first plant hormone - auxin [4].

There have been attempts by the school of Blaauw [5] to explain phototropism independently of signal transport - if the light causes a localized growth inhibition, then a gradient of light should lead to a gradient of cell growth that does not require the exchange of intercellular signals. The debate between the disciples of the Cholodny-Went theory [2,3] and those of the Blaauw hypothesis [5] has continued ever since. However, the evidence for a displacement of growth [6] and a displacement of auxin [7] towards the shaded flank of the coleoptile are overwhelming, and it is certainly justified to discuss phototropism together with the auxin response.

The growth response to auxin is situated in the epidermis that limits and guides the expansion of the coleoptile [8]. Auxin causes epidermal wall loosening and thus releases the constraint put by the epidermis upon the expansion of the inner tissues. In the framework of the Cholodny-Went theory, it is the control of epidermal elongation by auxin that is the central element of phototropic bending. Cortical microtubules have been considered in this context, because they define the direction of cellulose deposition in the cell wall and thus the

axis of cell expansion [9]. This review will therefore consider the evidence supporting a role of microtubules for phototropism and contrast this with findings where microtubule orientation and growth are discongruent. In a final part, these contradicting observations will be reconciled by introducing the concept of a stable phototropic polarity that can be separated from phototropic bending. It is this phototropic polarity that is intimately linked to a blue-light induced reorientation and fixation of cortical microtubules.

Do microtubules respond to phototropic stimulation? Yes!

The driving force for cell growth is a gradient of water potential with a more negative water potential in cytoplasm and vacuole as compared to the apoplast [8]. The resulting pressure by itself is not directional, and the cell is therefore expected to grow isotropically. In fact, if the cell wall is removed, the cell will assume an isodiametric shape. In intact cells, the direction of growth is actively controlled by the cell wall. From biophysical considerations it is expected that epidermal cells that are approximately cylindrical in shape, should grow preferentially in lateral direction. In other words, they must be endowed with some kind of *reinforcement mechanism* to maintain their original axiality [10]. This reinforcement mechanism seems to reside in the cell wall and was first uncovered for the long internodal cells of the alga *Nitella* [11]. In these elongate cells, the cellulose microfibrils were demonstrated by electron microscopy to be arranged in transverse rings, especially in the newly deposited inner layers of the wall. It is evident that the transverse arrangement of microfibrils can account for the reinforcement mechanism that maintains the longitudinal growth axis in cylindrical cells. In the meanwhile, such a correlation between transverse microfibrils and cell elongation has been demonstrated in numerous cases [reviewed in 9, 12, 13].

Moreover, reorientation of the growth axis is often accompanied by a loss or a reorientation in the anisotropy of cellulose deposition [14-16]. Therefore, the correlation between guided cellulose deposition and cell growth seems to be very tight. The so called terminal complexes responsible for cellulose synthesis are usually organized in rosette-like hexagonal arrays [13]. It is generally believed that these rosettes slide within the membrane leaving behind them bundles of crystallizing cellulose fibers, the microfibrils (**Figure 1A**). Cortical microtubules seem to be responsible for the guided movement of the terminal complexes and thus for the axiality of cell expansion. The evidence for this statement can be summarized as follows:

(1) Cortical microtubules are closely associated with the plasma membrane, and in plasmolyzing cells a direct contact between cortical microtubules and newly formed cellulose microfibrils has been detected by electron microscopy [12].
(2) The prospective sites where secondary wall thickenings will form are already marked by parallel thick bundles of cortical microtubules [17,18]
(3) In those cases, where changes of the preferential axis of cellulose deposition (and, concomitantly, the axis of cell growth) occur, this reorientation is heralded by a reorientation of cortical microtubules (14, for the ethylene response; 19 for the auxin response; 20 for the gibberellin response; 21 for wood formation).
(4) Elimination of cortical microtubules by antimicrotubular drugs results in a gradual loss of growth anisotropy and a block of cell elongation leading, in extreme cases, to lateral swelling (reviewed in 12).

The exact mechanism by which microtubules drive and guide cellulose deposition has been under debate since the discovery of cortical microtubules by Ledbetter and Porter [22], and a manifold of different hypotheses have been proposed (reviewed in 12,13). The principal debate can be summarized into two alternative models:

(1) According to the original model by Heath [23], cortical microtubules are physically linked to the terminal complexes and the linking molecule(s) can be pulled along the microtubules by dynein-like motor proteins. Thus the whole complex will be moved in a direction parallel to the adjacent microtubules (**Figure 1B**). It has been observed in several cases that removal of the cell wall during formation of protoplasts causes a dramatic restructuring of cortical microtubules [24] and makes them susceptible to cold [25]. These observations demonstrate a stabilization of cortical microtubules by the cell wall and indicate a physical link between microtubules and microfibrils across the plasma membrane.

(2) The alternative model is based on the observation that, in some cases, the terminal complexes have been observed in the interspaces outlined by the microtubules rather than being directly attached to them [13]. The guiding of rosette movement, according to this model, is not caused by a physical link of the terminal complexes to microtubule motors. Microtubules are rather supposed to induce membrane channels that impede lateral deviations of rosette movement (**Figure 1C**). The driving force for the movement would be cellulose crystallization itself, propelling the terminal rosette through the microtubule-dependent membrane channels.

At the present stage, it is difficult to decide between both models. Moreover, none of them seems to be complete and able to accomodate all observations. It is necessary to understand, on the molecular level, the interaction between microtubules and the plasma membrane, and the potential role of motor proteins for guided cellulose deposition. In this context microtubule-binding proteins are interesting. Such proteins might mediate the association of microtubules with the plasma membrane and could interact through the membrane with the terminal complexes. The exact mechanism by which cortical microtubules guide the deposition of cellulose and thus define the axis of cellular growth remains to be elucidated. Nevertheless, the close relation between microtubule orientation and the direction of growth suggests that the main function of cortical microtubules has to be sought in the control of cell shape by external and internal signals. This view is supported by the microtubular response to auxin and phototropic stimulation:

In coleoptiles that undergo rapid elongation, cortical microtubules are found to be transverse in both, the cells of the inner tissue and in the epidermis (maize: 19,26; rice: 20). Consistently, cellulose microfibrils are deposited in transverse direction reinforcing the elongation of the cell (maize: 19; rice: 20). Upon excision of the coleoptile tip (the major source of auxin) and incubation of the coleoptile segments in water microtubules change their orientation from transverse to longitudinal and the cellulose-microfibrils are deposited in longitudinal direction [19]. This results in a loss of growth reinforcement and, consequently, in a block of coleoptile elongation. This process can be reversed by addition of exogenous indole-acetic acid [19, 26,27]. Microtubules respond within 10 to 15 min after addition of indole-acetic acid [26,27] and they complete their reorientation within one hour.

Phototropic stimulation of intact coleoptiles causes a reorientation of cortical microtubules in the lighted, auxin-depleted flank, whereas the microtubules in the shaded, auxin-enriched, flank reinforce their transverse orientation [26,27]. This gradient of microtubule orientation is correlated with a gradient of growth (inhibition of growth in the flank, where microtubules are longitudinal, stimulation of growth in the flank, where microtubules are transverse) resulting in tropistic bending. Microtubule reorientation becomes detectable from 10 min after stimulation and is complete within one hour. Phototropic curvature, in contrast, becomes detectable from 20 to 30 min after stimulation and reaches a maximum at two hours after stimulation [26]. Thus, for phototropic stimulation, the microtubular response clearly precedes the growth response. This is not trivial, because there exist blue-light responses such as the light-inhibition of stem elongation in peas, where microtubule reorientation occurs at a slower rate as compared to the growth response [28].

The analysis of microtubule reorientation in response to auxin and phototropic stimulation lead to a model (**Figure 2**), where phototropic stimulation caused a displacement of auxin across the coleoptile (Cholodny-Went theory). The resulting auxin gradient will then produce a gradient of microtubule orientation with longitudinal microtubules in the auxin-depleted flank, and transverse microtubules in the auxin-enriched flank of the coleoptile. The gradient of microtubule orientation will then be translated into a corresponding gradient of cellulose microfibril deposition and thus a gradient of cell wall extensibility between the lighted and the shaded coleoptile flank. This will eventually culminate in an inhibition of growth in the lighted flank and a stimulation of growth in the shaded flank causing phototropic bending towards the light. This model is supported by the observation that other stimuli that produce lateral auxin transport, such as gravitropism, produce a similar gradient in the orientation of microtubules [26].

Do microtubules control tropistic curvature? No!

This model for the role of the microtubular cytoskeleton mediating between auxin displacement and asymmetric growth seems to be oversimplified, though. Microtubule reorientation and bending can be separated experimentally [29].

(i) Following phototropic stimulation by a pulse of blue light curvature reaches a maximum at two hours after stimulation. Curved coleoptiles experience a gravitropic counterstimulation and therefore they straighten again within a few hours. Microtubules are found to be longitudinal in the lighted side, but remain transverse in the shaded side. Interestingly, this gradient of orientation develops within one hour after the light pulse and is maintained throughout the period of gravitropic straightening (**Fig. 3A**). The original hypothesis (**Fig. 2**) would have predicted an inversion of the orientation gradient prior to the onset of gravitropic straightening, if the two tropistic responses produce asymmetric growth by the same mechanism.

(ii) Upon rotation of maize coleoptiles on a horizontal clinostat in the absence of tropistic stimulation, a nastic curvature develops in the dorsiventral axis of the coleoptile [30]. This curvature is strong, when the coleoptiles are symmetrically irradiated by red light, and it becomes weaker, when the plants are irradiated by blue light. Under red light, the microtubules are transverse on both flanks of the bending organ, and they are longitudinal

under blue light (**Fig. 3B**). However, not any gradient of microtubule orientation across the organ could be detected [29] in contradiction to the original model (**Figure 2**).

(iii) When, two hours after a phototropic stimulus, the coleoptiles are subjected to a phototropic counterstimulation of equal strength, but opposing direction, and the coleoptiles are rotated on a clinostat (to exclude gravitropic counterstimulation), they interrupt their bending towards the first stimulus and start bending towards the counterstimulus [31]. This dominance of the counterstimulus is transient, though: Already one hour later, the coleoptiles „remember" the direction of the first stimulus and return to their original mode of curving, that is subsequently maintained for many hours. The orientation of microtubules is longitudinal in the side that had been hit by the first stimulus. In contrast to bending itself, microtubules do not respond at all to the second, opposing light [29, 32]. The original hypothesis (**Fig. 2**) would have predicted an inversion of the orientation gradient in response to the counterstimulus and a second inversion back to the original gradient.

This analysis leads to the conclusion that a gradient of microtubule orientation is neither necessary nor sufficient for asymmetric growth. In other words: microtubule orientation and phototropic curvature appear not to be causally linked but seem to develop as parallel phenomena.

If microtubules do not control phototropism, why do they respond to phototropic stimulation?

To give an answer to this question, it is important to introduce the concept of phototropic polarity. In contrast to the hypothesis proposed by Blaauw [5], the light gradient is not immediately translated into a gradient of growth. Consistent with the model proposed by Cholodny and Went [2,3], there exists an intermediate step: The gradient of blue light results in a transverse polarization of the tissue. This polarity controls asymmetric growth. It is possible to separate this polarity from tropistic bending and to make it manifest in form of a so called directional memory [31]:

The bending response of maize coleoptiles to blue light is transient, reaching a maximum at two hours after induction and disappearing subsequently, a phenomenon that can be understood in terms of gravitropic straightening [31]. However, when the gravitropic counterstimulation experienced by the curving coleoptile is eliminated by rotation on a horizontal clinostat, a stable bending towards the inducing pulse is observed. When the coleoptiles are transferred to the clinostat after the phototropic bending has already vanished due to gravitropic straightening, nevertheless a stable curvature in direction of the first pulse develops (**Fig. 4**). This demonstrates the existence of a directional memory that had been induced by the stimulus and that persisted even during the time of gravitropic straightening. This memory can thus be separated from bending itself.

A similar directional memory has been repeatedly described for both gravitropism [33,34] and phototropism [35]. In order to detect a directional memory following tropistic stimulation, the expression of the tropistic response was suppressed by cold or auxin depletion [33,34] or by specific ion-channel blockers [35]. If the suppressive treatment was eventually removed, the response to the stimulus developed.

Such experiments indicate that the stimulus can induce a tropistic polarity that can be separated from its expression as curvature and can nevertheless persist over a long time even if it is prevented from becoming manifest. The suppressive condition in case of the phototropic memory of maize coleoptiles is the gravitropic counterstimulation experienced by curved coleoptiles - it is removed by clinostat rotation allowing for expression of the memory as stable bending [31].

When does the spatial memory become stable? This question can be addressed by challenging the memory induced by a stimulus with a counterdirected stimulus after variable time intervals [31]. If the opposing pulse is administered early after the first pulse, it can reverse the memory completely and a strong stable bending response towards the second pulse is observed (**Fig. 5A**). If the opposing pulse is administered later, it fails to reverse the memory. It first does reverse, however, the bending response (**Fig. 5B**). This reversal in the sign of bending remains transient though, and subsequently the original response (directed towards the first pulse) is restored and maintained over a long time [31]. A detailed fluence-response study for the first and the second stimulation [36] demonstrated that, independently of fluence, the spatial memory becomes irreversibly fixed at two hours after the first, inducing pulse. This fixation time is independent of the interaction between the two stimuli and the direction of the spatial memory.

The relevance of microtubules for phototropism seems to be linked to the establishment and fixation of phototropic polarity rather than to phototropic bending *per se*. This conclusion is supported by the following evidence:

(i) The gradient of microtubule orientation [29,32] as well as the spatial memory [31] both persist during gravitropic straightening.
(ii) The gradient of microtubule orientation [29,32] as well as the spatial memory [31,36] do not respond - in contrast to curvature - to a phototropic counterstimulus that is administered late (two hours) after the inducing pulse.
(iii) The gradient of microtubule orientation [32] as well as the spatial memory [31,36] can be reversed by a counterstimulus that is administered early (one hour) after the first pulse.
(iv) The orientation of microtubules is irreversibly fixed at the same time, when the spatial memory becomes irreversibly fixed [32,36].

These observations suggest that the reorientation of microtubules is the cellular expression of phototropic polarity, and that the fixation of microtubule orientation is the cellular correlate to the irreversible fixation of this memory.

A further characterization indicated that the blue-light effect on microtubule reorientation is mediated by auxin [32], whereas the blue-light induced fixation of microtubule orientation is neither mediated by a local depletion of auxin nor by a gradient of auxin. The blue-light induced fixation of microtubules does not require a light gradient and resides in the coleoptile base as shown by partial irradiation using a light pipe. The perception for the blue-light induced reorientation of microtubules, in contrast, resides in the coleoptile tip.

The results of this analysis can be summarized as follows: Tropistic stimulation triggers three chains of events:

(i) Perception in the coleoptile tip causing a displacement of auxin towards the shaded side, resulting in an inhibition of cell elongation in the lighted side and a stimulation of growth in the shaded side. The asymmetric growth causes then the bending towards the light.
(ii) Reorientation of microtubules, caused by a displacement of auxin.
(iii) Perception of blue light in the coleoptile base triggering the production of a factor (that is independent of auxin) that can fix microtubules (irrespective of their actual orientation). This fixed microtubule orientation seems to be the cellular base of a stable transverse polarity that can be rendered manifest by removal of gravitropic counterstimulation.

How is the phototropic polarity established? Intercellular cross-talk versus autistic cells.

Phototropic polarity might be a function of tissue polarity, i.e. it could arise from mutual interactions between the individual cells across the tissue. Alternatively, the phototropic polarity could arise as a cell polarity, i.e. as an autonomous response of the individual cell that does not require intercellular communication. The findings of Cholodny [2] and Went [3] that a growth promoting agens (auxin) is redistributed across the coleoptile suggests that the individual cells interact during the establishment of phototropic polarity. However, the Cholodny-Went theory does not allow any conclusion to be drawn about the way in which the plant is able to sense the direction of the stimulus. It is still possible that each cell is able to recognize the direction of light individually by producing a radial cell polarity. This cell polarity would subsequently determine the direction of auxin efflux. The tissue response described by the Cholodny-Went theory would then arise only at that stage by mere summation of individual cell responses. Alternatively, the gradient of light might be recognized by the cell population as a whole, and a true tissue polarity might emerge from intercellular signalling, for instance by locally self-amplifying activation in concert with far-ranging mutual inhibition [37]. Such a tissue polarity would then induce a parallel cell polarity leading to transverse auxin transport.

A debate between Heilbronn [38] and Buder [39] at the beginning of this century contributes to this problem. Heilbronn claimed that the plant perceives the direction of the light. Buder, in contrast, insisted on the gradient of light as the signal to be perceived by the coleoptile. This dispute stimulated an ingenious experiment by Buder [39], in which the gradient of light and light direction were opposed to each other. To achieve this, the coleoptile was irradiated from inside out using a prototype of a light-piping device. Buder was able to demonstrate bending of the coleoptiles towards the lighted flank, although the direction of the incident light should have induced bending into the opposite direction.

The debate between Blaauw [5] and Cholodny-Went [2,3] and the debate between Heilbronn [38] and Buder [39] both touch the question, by what mechanism the phototropic polarity is established. This process can be divided into two subsequent steps: (i) sensing of the light direction and (ii) induction of a parallel phototropic polarity. The sensing could be based upon the gradient of the light (Buder) or it could be brought about by

actually sensing the direction of the light (Heilbronn). The phototropic polarity could evolve as a cell polarity or it could emerge as a tissue polarity. From these considerations one can design four alternative possibilities, how a phototropic stimulus might result in a phototropic polarity in a multicellular organ such as a Graminean coleoptile (**Figure 6**). In order to distinguish between these four alternatives, it is necessary to find a marker for cell polarity. Tropistic curvature cannot be the appropriate marker, because it reflects merely the integrated response of the individual cells over the entire coleoptile. In contrast, the orientation of cortical microtubules permits the analysis of individual cells and it has been, so far, the closest marker for phototropic polarity [32].

For this reason, the Buder experiment (irradiation from inside out by means of a light pipe) was combined with immunofluorescence analysis of microtubule orientation in the outer epidermis of maize coleoptiles [40]. By measuring fluence-response curves for phototropic curvature the original findings of Buder could be confirmed, i.e. the coleoptiles curved according to the gradient of the light, although this gradient was opposed to the direction of the incident light. Surprisingly, this type of irradiation caused a reversal of cell polarity in most of the epidermal cells: In irradiated cells that were stimulated conventionally (from outside in), only those microtubules that were adjacent to the outer epidermal wall showed a reorientation from transverse to longitudinal (**Figure 7A**). Upon irradiation from inside out, reorientation was more frequent at the inner face of the epidermal cells (**Figure 7B**). In a few cases, transitions between both responses could be observed. These findings might be interpreted such that microtubules can sense the direction of the light. However, the site, where the light is perceived, and the site, where the microtubular response takes place, are separated by several millimeters as shown by microirradiation of individual cells [40]. This means that the information about the light gradient must be transmitted from the tip downwards in form of unknown signals. To detect such signals, the growth response of individual cells was followed over time in response to microirradiation of the coleoptile tip. In fact, a growth-regulating signal could be detected that travels in basal direction with a speed of around 60 $mm \cdot h^{-1}$, i.e. five times faster than auxin. It is possible to imagine that such a signal can embody the information about the tissue polarity - there might be less of this signal in one flank of the coleoptile and more in the other. It is harder to imagine how this signal could convey the information about intracellular light gradients (light direction). But this is exactly implied by the reversal of cell polarity expressed in the inverted microtubular response. To resolve this apparent contradiction it is important to consider endogenous polarities - cells that are organized in a tissue are never symmetric, but usually exhibit distinct endogenous polarization. In case of the epidermis, this endogenous polarity becomes manifest by the different microtubular response to auxin - the reorientation from transverse to longitudinal in response to auxin depletion is confined to those microtubules that are adjacent to the outer wall [19,26]. The reversal of this endogenous polarity upon irradiation from inside out might be brought about in response to superoptimal local levels of the fast growth-regulating signal that is released from the coleoptile tip after phototropic stimulation. Such a mechanism has been invoked to explain the blue-light induce inversion of the gravitropic response during clinostat rotation [41].

How is phototropic polarity established? The combination of the Buder experiment with the analysis of microtubule orientation as indicators of cell polarity can detect the following steps (**Figure 8**): (i) Upon phototropic irradiation of the coleoptile tip, the light gradient is sensed. (ii) This gradient results in the release of signals that migrate towards the coleoptile base much faster (60 $mm \cdot h^{-1}$) than auxin transport (10-12 $mm \cdot h^{-1}$).

(iii) In parallel, a gradient of auxin is established by lateral transport of auxin. This auxin gradient migrates downwards as well, but at a lower speed (10-12 $mm \cdot h^{-1}$). (iv) The fast blue-light induced signal interacts with the endogenous cell polarity of epidermal cells and determines the cellular (microtubular) response to auxin. (v) The depletion of auxin reaches the cells in the lighted flank of the coleoptile base and causes a reorientation of microtubules. This reorientation response to auxin depletion depends on the interaction with the fast signal - there can be reorientation at the outer cell wall, there can be reorientation at the inner wall, there can be no reorientation response (if the phototropic polarity has become fixed). This microtubular response is the cellular base for phototropic polarity (vi) Phototropic curvature develops in parallel to phototropic polarity by a direct response of cell elongation to the local concentration of auxin. Therefore the phototropic curvature is initiated at the tip and migrates downwards with about the same speed as auxin transport itself (i.e. around 10-12 $mm \cdot h^{-1}$). (Vii) Phototropic curvature vanishes within a few hours after the end of stimulation, phototropic polarity instead persists over several days and can induce a stable *photomorphosis* in the coleoptilar node (see below).

Why does the coleoptile need phototropic polarity? Is phototropic curvature not enough?

The coleoptile is usually regarded as an ephemeral organ that has merely the function to guide the primary leaves through the soil towards the surface. It is soon afterwards torn open by the emerging primary leaves. This behaviour poses the question, what the physiological significance of a stable phototropic polarity might be. At a closer look the coleoptile is not as ephemeral as usually written in the text books - it remains alive for several weeks and can even grow actively. However, the axis of cell growth is tilted from elongation to stem thickening. In addition it seems to influence the emergence of the nodal crown roots that will later form the major root system [42]. The dorsiventrality of the coleoptile (it is homologous to a leaf) appears to be imprinted upon the coleoptilar node: the crown roots emerge later at the ventral flank of the coleoptilar node (adjacent to the caryopsis). Interestingly, this gradient can be shifted by phototropic stimulation [42]. The emergence of crown roots is delayed in the lighted flank and promoted in the shaded flank. This effect of phototropic stimulation occurs several days after a stimulation with a light pulse lasting for a few seconds. The phototropic curvature, induced by this pulse, is transient though and disappears within a few hours. The light effect on crown-root formation is not correlated with phototropic curvature and cannot be mimicked by gravitropic stimulation that induces a similar degree of curving [42]. On the other hand, this effect can be reverted by counterstimulation within one hour, it becomes irreversibly fixed at two hours, i.e. the time, when the microtubule-dependent phototropic polarity becomes stable [42]. If microtubules in the basal part of the node are depolymerized by antimicrotubular herbicides, this leads to a suppression of the phototropic shift of crown-root emergence.

These observations indicate that the coleoptile can imprint a polarity upon the node and that this polarity is then expressed as a gradient of crown-root emergence at a time, when the coleoptile itself has already ceased to elongate. The spatial pattern of crown-root emergence is thus defined by events that take place in the coleoptile several days earlier. These early events are polarized by phototropic stimulation, they are dependent on intact microtubules, they can be separated from phototropic bending as such, and they become irreversibly fixed in space two hours after phototropic induction.

What is the physiological significance of microtubule-based stable phototropic polarity? It is not required for the fast, transient bending that is induced by phototropic pulse stimulation. It is intimately linked to a long-lasting transverse polarization of the coleoptile that is induced by phototropic stimulation and that can persist for several days. In the experiment, this transverse polarization can be rendered manifest by removing gravitropic counterstimulation. Under natural conditions, this microtubule-dependent polarity becomes imprinted upon the coleoptilar node and guides the emergence of nodal crown roots several days after the phototropic stimulus. The shift of crown-root emergence towards the shaded side of the coleoptilar node is certainly of adaptive value and helps the seedling to align its axis with respect to direction of the light. The polarizing mechanism seems to be flexible within a temporal window of about two hours after phototropic induction before it becomes fixed irreversibly. This flexibility is ecologically meaningful, because it allows the seedling to distinguish between stochastic changes of light quantity (caused, for instance, by passing clouds) and more stable light gradients (caused, for instance, by neighbouring plants).

Summary and Outlook

The reorientation of cortical microtubules following phototropic induction was initially interpreted in terms of a role for microtubules in the mediation of the phototropic response [26]. This model did not bear closer scrutiny but lead to the discovery of long-lasting effects of phototropic stimulation (stable phototropic polarity). These stable effects develop in parallel to phototropic curvature, but they are not involved in the phototropic response *per se*. The response of cortical microtubules to phototropic stimulation is linked to the establishment and stabilization of this stable phototropic polarity rather than to phototropism itself. The functional significance of the stable phototropic polarity seems to be related to a developmental imprinting of the coleoptilar node by the coleoptile guiding the emergence of crown roots and thus shaping the architecture of the root system. It appears not appropriate and even misleading to discuss these stable effects in the context of phototropism - it would be more correct to designate them as *photomorphosis*. The developmental impact of *photomorphosis* should not be underestimated - it helps the seedling to adjust axis and architecture with respect to the light distribution in the canopy and thus supports and stabilizes the adaptive role of phototropism itself.

To understand *photomorphosis* in molecular terms, it is necessary to understand the mechanism of blue-light induced reorientation of microtubules. The focus will be on those proteins that control the assembly and disassembly of microtubules. The isolation of a microtubule-associated protein from maize that is expressed in response to phytochrome and associates with bundles of cortical microtubules [43] stimulates the search for similar proteins that are formed in response to blue light. The recent finding that blue light causes an increased acetylation of tubulin in maize coleoptiles indicates that the dynamics of assembly and disassembly becomes reduced in response to blue light, what might be the molecular base for the fixation of microtubule orientation and the fixation of the blue-light induced polarity. The isolation and cloning of the factor that is responsible for this reduced dynamics should allow a molecular approach to the problem of *photomorphosis*.

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Figure Legends

Figure 1: Role of microtubules in directional cellulose synthesis. **A** Transmembrane localization of a cellulose-synthetizing complex (terminal complex). **B** Original model by Heath [23], where the terminal complexes are connected to microtubule motors and actively moved along cortical microtubules. **C** Model by Giddings and Staehelin [13], where cortical microtubules induce membrane ridges that confine the movement of the terminal complexes. According to this model, the driving force for the movement originates from the crystallization of cellulose.

Figure 2: Original model about the role of microtubules in the phototropic response according to [19]. The phototropic stimulus causes a lateral displacement of auxin transport and a gradient of auxin with elevated levels of the hormone in the shaded side, whereas the lighted side is depleted from auxin. Microtubule orientation responds to the local level of auxin with transverse microtubules in auxin-enriched areas and longitudinal microtubules in auxin-depleted areas. The gradient of microtubule orientation is translated into a corresponding gradient in the direction of cellulose microfibrils. The longitudinal extensibility of the epidermal wall will decrease in the lighted side causing a reduction of growth. The growth gradient will eventually drive phototropic bending.

Figure 3: Separation of microtubules orientation and bending response. **A** During gravitropic straightening, the direction of bending is inverted, the gradient of microtubule orientation is maintained. **B** During nastic curvature, microtubule orientation is identical on both side of the bending organ.

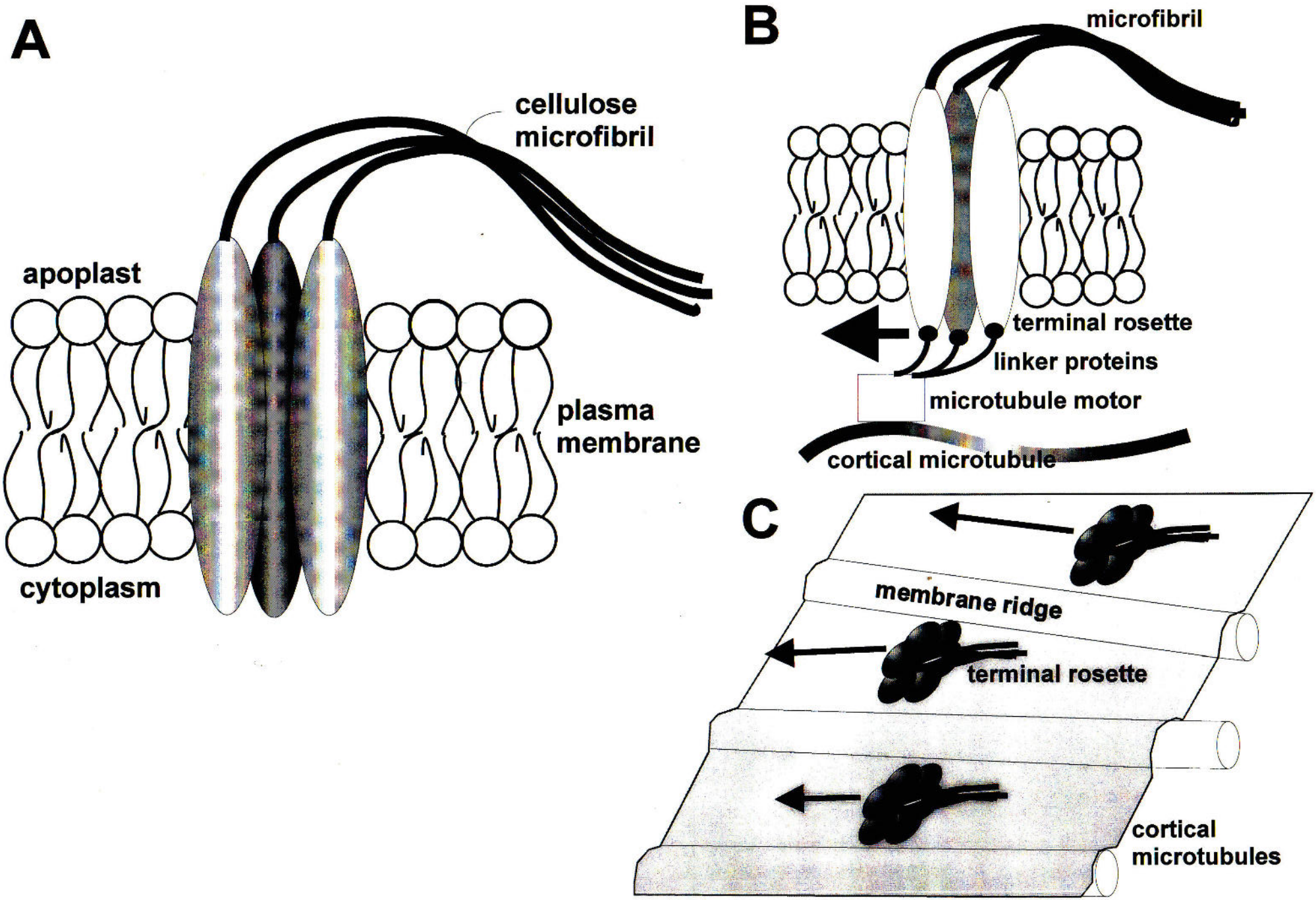
Figure 4: Demonstration of phototropic polarity. The phototropic bending response remains transient, due to gravitropic straightening. When gravitropic counterstimulation is removed (by rotation on a horizontal clinostat), the stable phototropic polarity can be expressed as stable curvature. Despite being suppressed by gravity for many hours, the phototropic polarity has remained stable.

Figure 5: Assay for fixation of the phototropic polarity. The phototropic polarity induced by a stimulus is questioned by a counterstimulus of equal strength administered at various time intervals after the first irradiation. **A** If the time interval is short, the phototropic polarity (and the gradient of microtubule orientation) can still be inverted. **B** If the time interval is long, the phototropic polarity (and the gradient of microtubule orientation) is irreversibly fixed and resists a counterstimulation.

Figure 6: Different models about the establihsment of phototropic polarity. The four models can be understood as combinations from two parameters: (1) Do the cells act autonomously or do they communicate? (2) Does the plant sense the gradient of light over the tissue or the direction of the light (i.e. the intracellular light gradient)?

Figure 7: Inversion of cell polarity in the Buder experiment. **A** For phototropic stimulation from outside in, only the microtubules at the outer cell wall in the lighted coleoptile flank respond by reorientation. **B** For phototropic stimulation from inside out the microtubules reorient at the inner cell wall of the lighted coleoptile flank (inversion of epidermal cell polarity).

Figure 8: Model about the role of microtubules in the phototropic response. The gradient of blue light induces, in parallel, two events: (1) Release of a fast signal that migrates basipetally with a speed of 60 $mm \cdot h^{-1}$. This signal interacts with the endogenous radial polarity of epidermal cells and defines their microtubular response to auxin or auxin depletion. (2) Lateral auxin transport towards the shaded coleoptile flank. The auxin gradient migrates basipetally with a speed of 10-12 $mm \cdot h^{-1}$. The local auxin depletion in the lighted side induces a reorientation of cortical microtubules from transverse to longitudinal. The microtubule orientation is fixed after two hours, in response to a blue-light induced signal that is not auxin. The orientation of microtubules defines a phototropic polarity that remains stable over days and participates in developmental imprinting of the node by the coleoptile that is relevant for crown-root formation.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B
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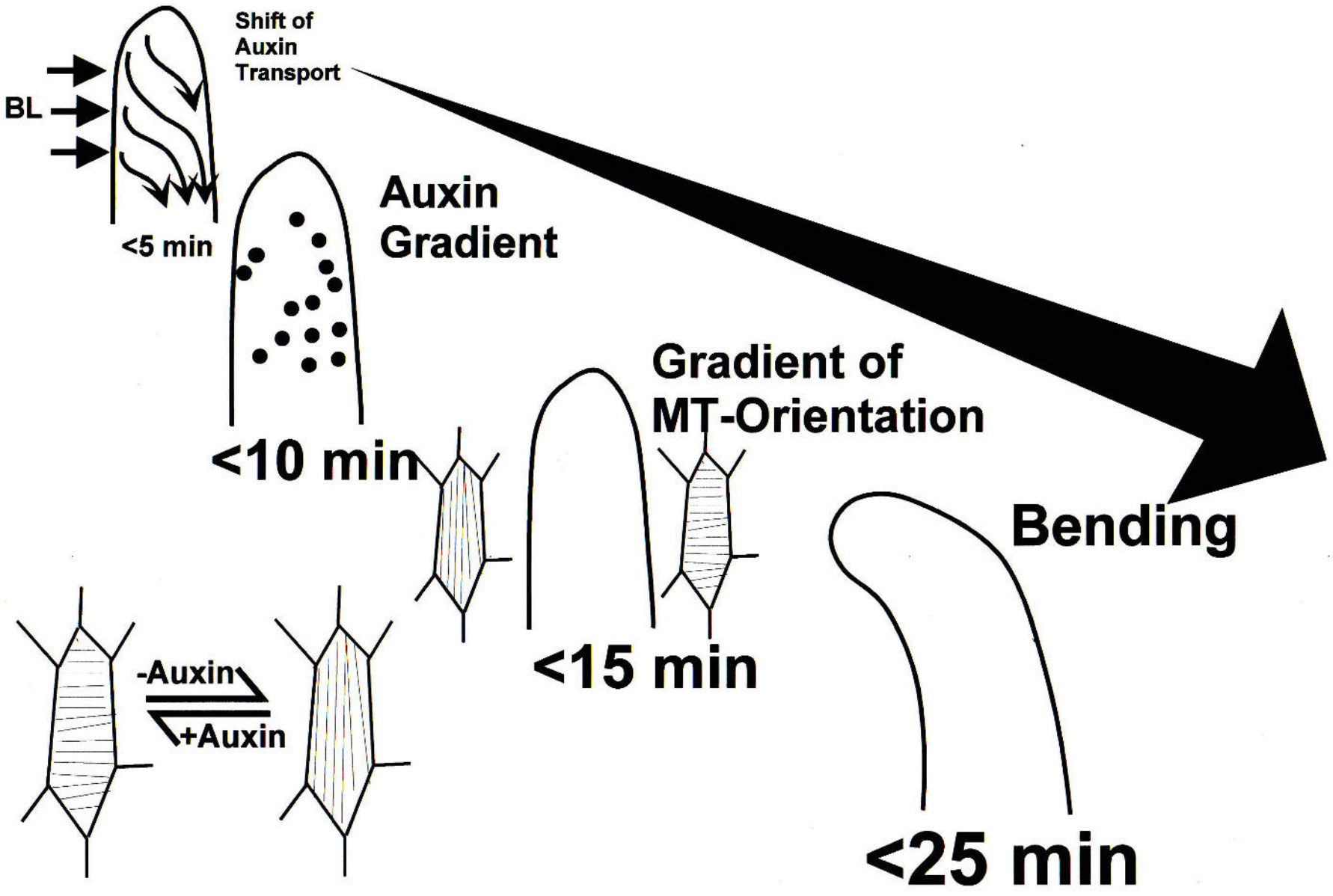
cortical microtubule
C
membrane ridge
terminal rosette
cortical microtubules


Shift of
Auxin
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BL
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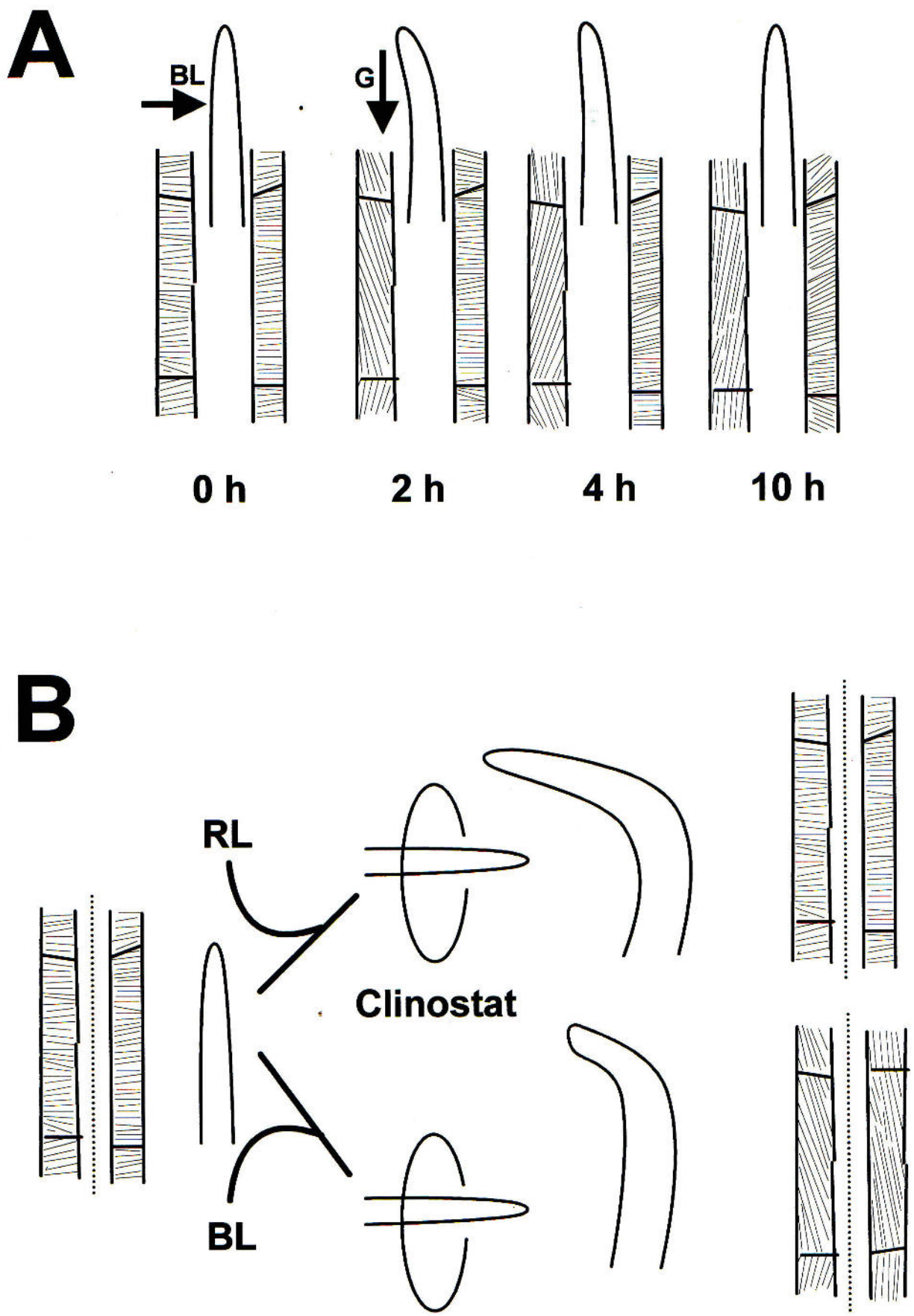
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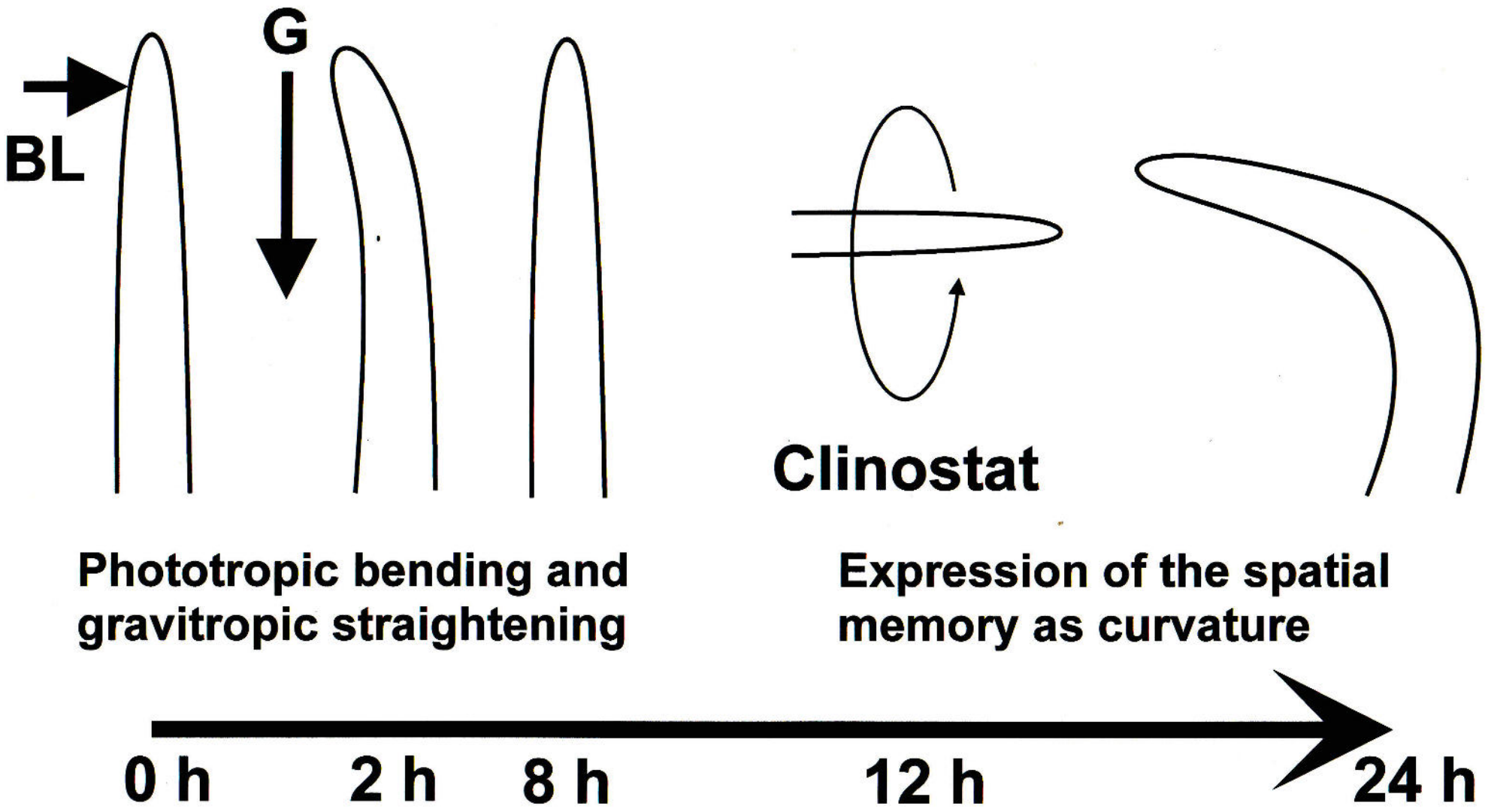
G
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Phototropic bending and
gravitropic straightening
Expression of the spatial
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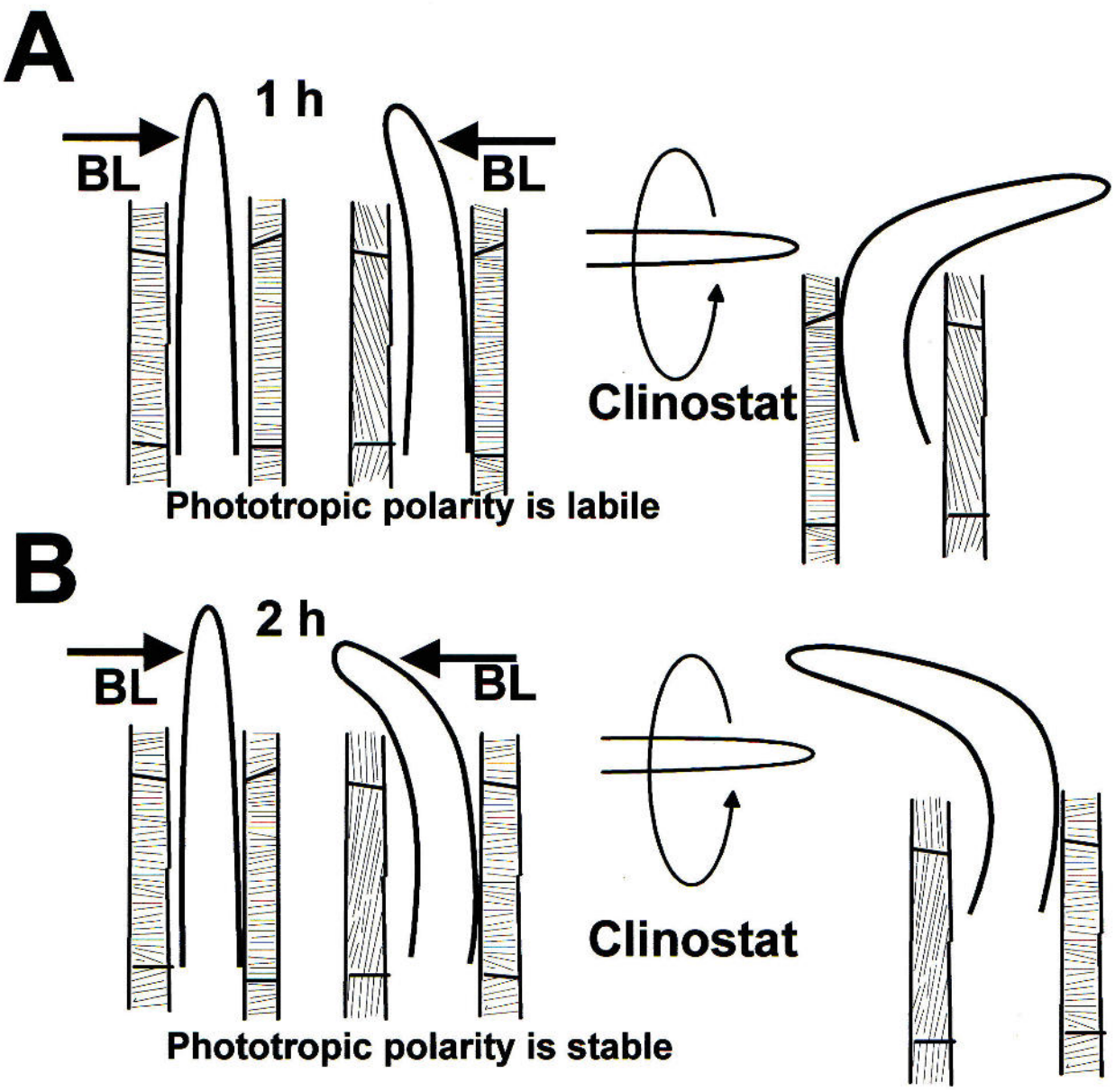
A
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BL
Clinostat
Phototropic polarity is labile
B
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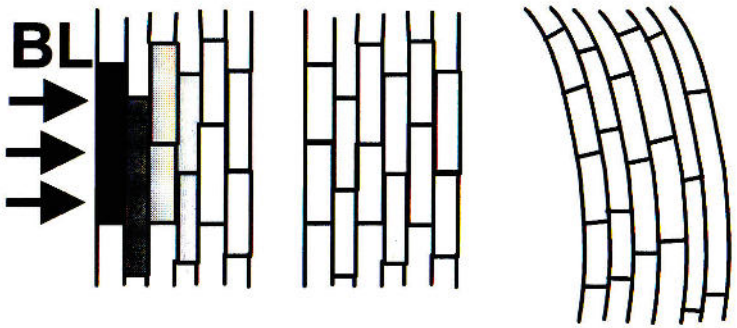
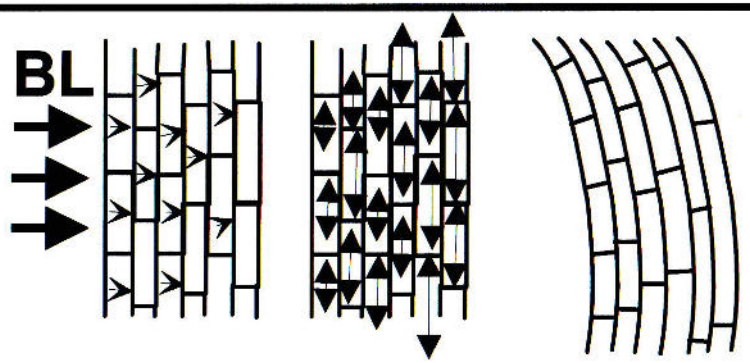
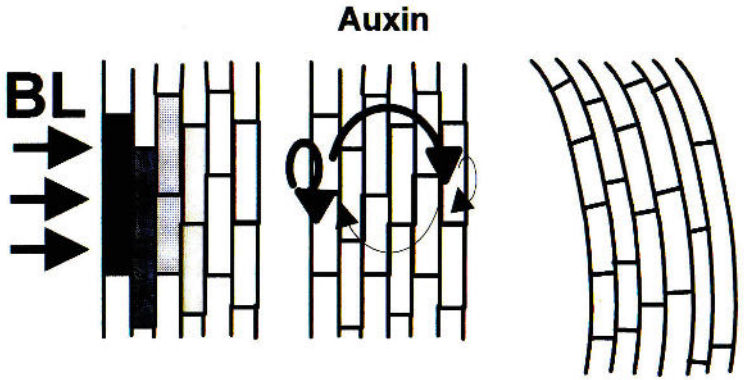
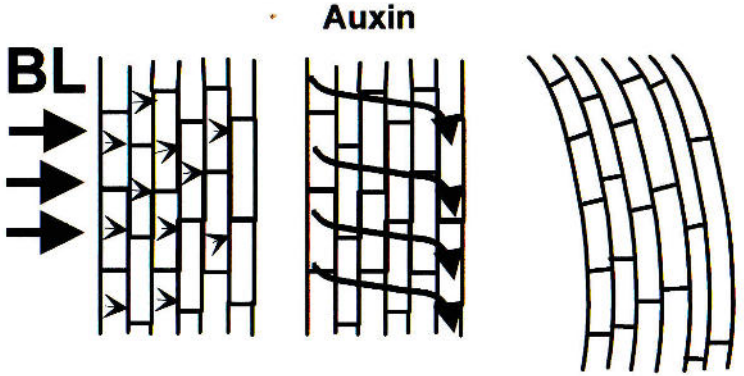
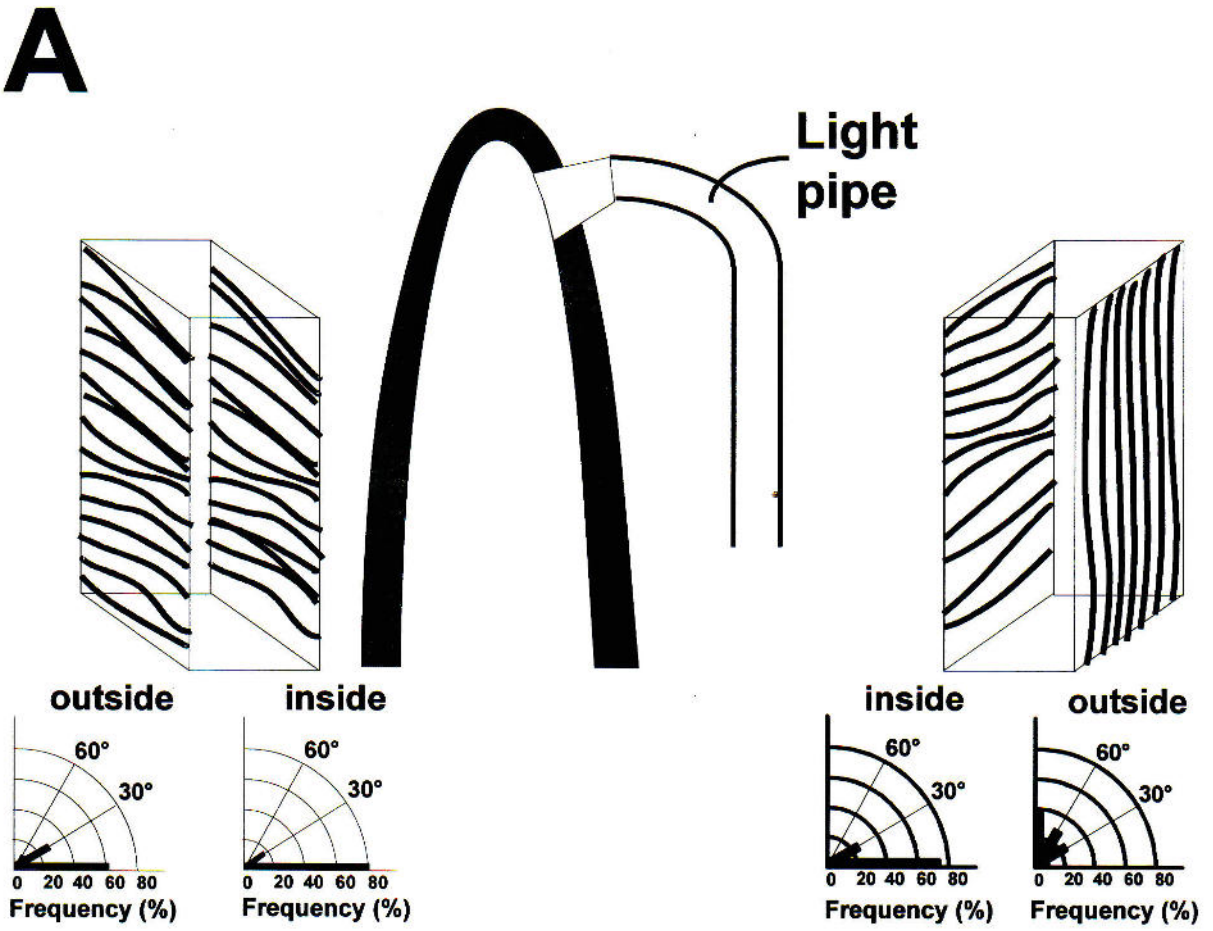
BL
BL
Clinostat
Phototropic polarity is stable

	Sensing of light gradient (Buder)	Sensing of light direction (Heilbronn)
Cell-autonomy (Blaauw)		
Cell interaction (Cholodny-Went)		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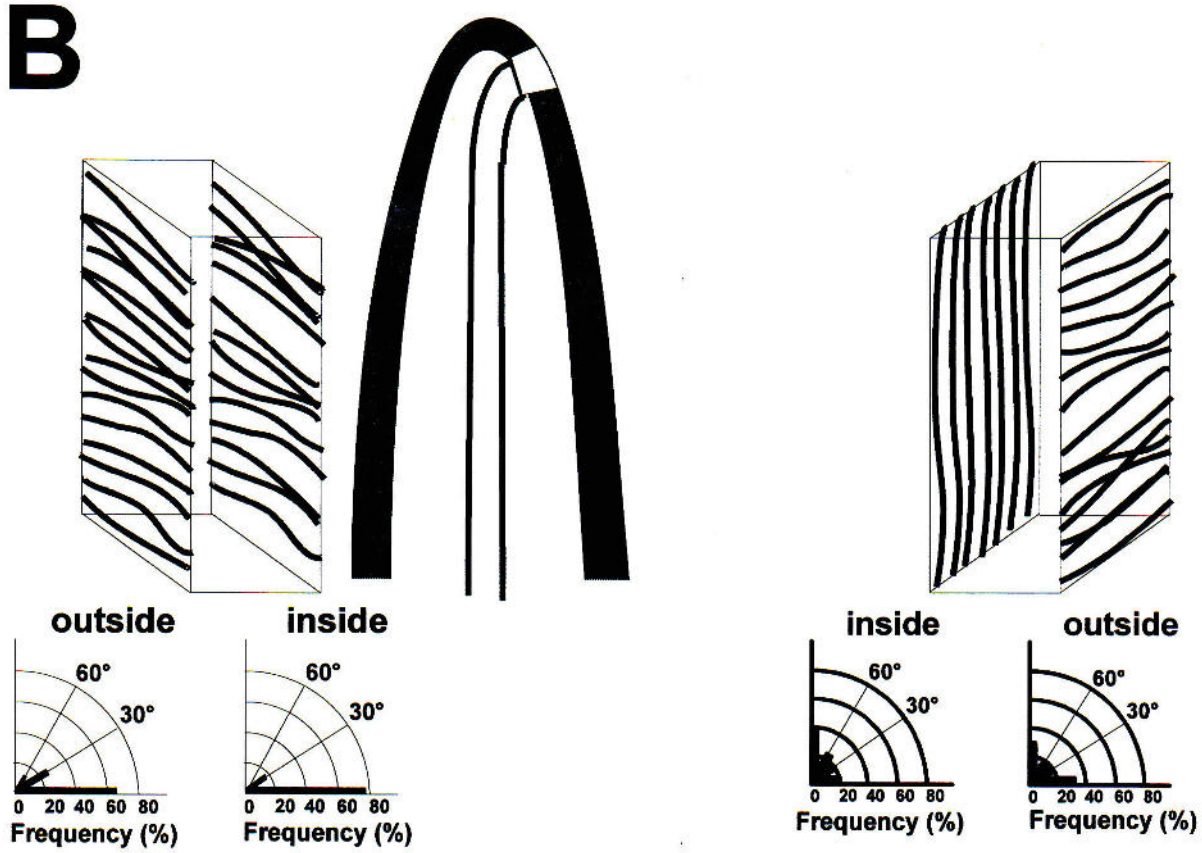
Frequency (%)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30°
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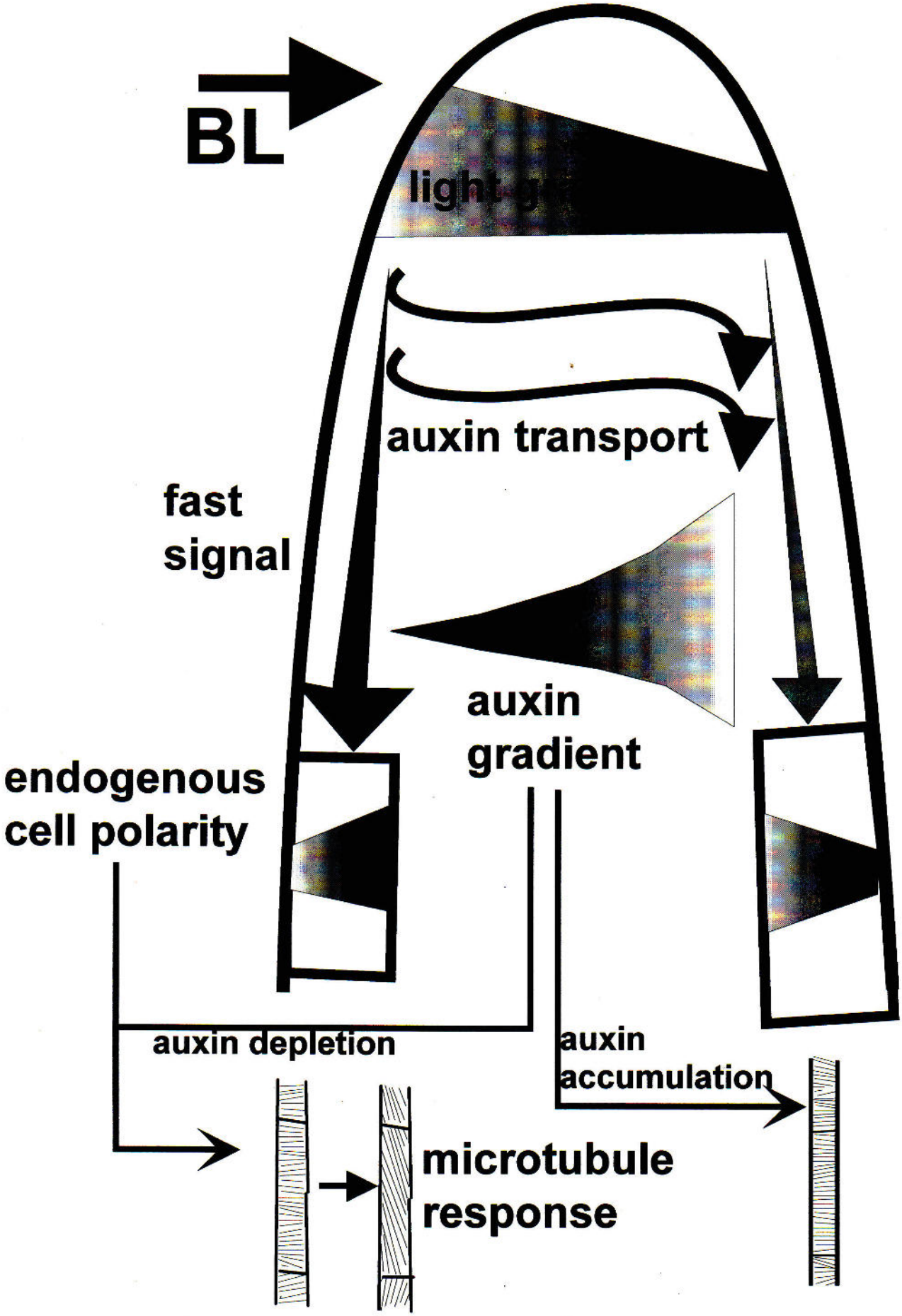
Frequency (%)


BL
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fast
signal
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cell polarity
auxin depletion
auxin
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microtubule
response