

Probing Rice Gravitropism With Cytoskeletal Drugs and Cytoskeletal Mutants

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In multicellular organs, phototropic sensing is based upon gradients in stimulus strength across the entire tissue. The differences in the strength of the gravitational field between the two flanks of a plant are certainly too small to be sensed. This implies that each cell, individually, must align its polarity with the direction of gravity. As the first step in gravity sensing, the gravitational field must be translated into a mechanical force that can then be perceived in the second step. It is generally believed that statoliths of some kind are the targets of the gravitational field. In plants, sedimentable amyloplasts have been conceived of, since the turn of the century, as potential statoliths (Haberlandt, 1900; Nemec, 1900).

In principle statolith sedimentation might be transduced into a new alignment of cell polarity in two ways. Displacement of the statolith could interfere with diffusible molecules, causing a concentration gradient that can then direct the new cell polarity (proximity mechanism). Alternatively, the statoliths could be pressed into a structure that is sensitive to mechanical stress, triggering a signal cascade that would cause a reorientation of cell polarity (pressure mechanism). The observation that the gravitational response is pressure dependent, even for accelerations where amyloplast sedimentation already seems to be saturated, favors a pressure mechanism at least for higher plants (Rawitscher, 1932; Johnsson, 1965; Friedrich and Hertel, 1973). The pressure-perceiv-

ing structure must embody some kind of directionality, if it is to interact with a transducing mechanism and alter cell axis and polarity. Cytoskeletal elements, actin microfilaments and microtubules, are good candidates for the pressure-perceiving system (Table I): they have a distinct axis and polarity, and microtubules have repeatedly been found to be highly sensitive to mechanical stress. Polymerization and depolymerization of tubulin into microtubules is one of the few biochemical processes that have been reported to respond directly to the direction of gravity (Tabony and Job, 1992).

With these considerations as background, we tried to assess the role of microtubules and microfilaments in the gravitropic response of rice coleoptiles. The rice coleoptile is highly sensitive to gravity, and the abundance of amyloplasts can be controlled by submerged growth. The cuticle of rice is very thin, so the cells are more accessible to drugs than are coleoptiles from other species. The coleoptile grows exclusively by cell elongation, and there is a wealth of old physiological data about this process. Accessibility of molecular techniques and a very small genome also make rice an excellent system for molecular studies.

Several steps of the gravitropic response chain were probed with cytoskeletal drugs (Table I). Ethyl-*N*-phenylcarbamate (EPC) and oryzalin block tubulin polymerization and rapidly eliminate microtubules. Taxol blocks depolymerization of tubulin, yielding an increased number of microtubules, and cytochalasin D rapidly eliminates actin microfilaments. The drugs were found to be effective within 15–30 min after application to the gravity-sensing tissue of the Japanese rice cultivar Nihonmasari.

Amyloplast sedimentation, the putative first step of the response chain (Sack, 1991), was investigated in vi-

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Table I

Elements of the plant cytoskeleton and interfering drugs used in this study

Cytoskeletal element	Component	Drug
Microtubules	Tubulin	Eliminating:
		Ethyl- <i>N</i> -phenylcarbamate (EPC)
		Oryzalin
		Colchicin
Microfilaments	Actin	Stabilizing:
		Taxol
		Eliminating: Cytochalasin D (CD)

bratome sections after starch was stained with Lugol solution. Sedimentation was not affected by cytochalasin D, only slightly promoted by microtubule elimination, and strongly inhibited by taxol. In this system, therefore, microtubules, but not microfilaments, are in physical contact with the statoliths. The gravity-induced reorientation of microtubules in the gravity-sensing tissue was analyzed, after paraformaldehyde fixation, in vibratome sections by immunofluorescence and confocal laser microscopy. The usual reorientation from transverse to longitudinal was inhibited by taxol. Cytochalasin D promoted this reorientation, even in the absence of gravitropic stimulation. The gravitropically induced lateral transport of auxin was followed by measuring the asymmetry of radioactively labeled auxin fed to the coleoptile tip. Lateral transport was inhibited by suppression of both tubulin polymerization and depolymerization. This inhibition could be separated from effects on longitudinal transport. Cytochalasin D inhibited auxin transport *per se*, yet we failed to observe a specific inhibition of lateral transport. Gravitropic bending was delayed by the microtubule drugs (irrespective of whether they inhibit polymerization or depolymerization of tubulin), whereas cytochalasin D caused a precocious onset of bending at a slower rate. It should be emphasized that gravitropism occurs, although at low efficiency, in the absence of microtubules and microfilaments. Furthermore, the gravitropic response could be inhibited at concentrations that would permit phototropism (as internal control for differential growth) to proceed.

These data favor the idea that microtubules interact with the gravity-perceiving system in the rice coleoptile. It is not enough that microtubules are present to fulfill this task, they must also be dynamic. Reorientation of microtubules from transverse to longitudinal is a prerequisite for the establishment of a new (radial) cell polarity resulting in a lateral transport of auxin. This would ex-

plain why cytochalasin D, promoting such a reorientation, causes a precocious gravitropic response.

To test this hypothesis further, the gravitropic response was analyzed in two cytoskeletal rice mutants. In the mutant *ER31*, the reorientation response of microtubules to the auxin signal is interrupted, although auxin *per se* is perceived normally, and microtubules are able to respond to other signals (Nick *et al.*, 1994). In this mutant, gravity can reorient microtubules, and gravitropism functions with about the same bending rate as in the wild type, but the bending is delayed by several hours. In the mutant *Yin-Yang*, the auxin-induced stimulation of actin polymerization is disturbed, resulting in the partial decay of actin microfilaments and the formation of nuclear actin baskets in response to auxin (Wang and Nick, unpubl. data). In this mutant, gravitropism begins earlier than in the wild type, but develops at a slower rate. The gravitropic behavior of the mutant *ER31* can be mimicked by treatment with antimicrotubular drugs; the gravitropic behavior of the mutant *Yin-Yang* is similar to that of the wild type after application of cytochalasin D.

The data can be discussed in terms of a physical contact between amyloplasts and microtubules during gravity perception. Microtubules serve as amplifiers for the mechanical stress exerted by the amyloplasts. This stress is then perceived by mechanosensitive channels that are probably located in the endoplasmic reticulum and causes the establishment of a new cell polarity. For this polarity shift, reorientation of microtubules seems to be a time-limiting step. The shifted polarity then causes a shift of auxin transport in the transverse direction and, eventually, gravitropic bending. Parallel to this pathway, a second, slower gravitropic pathway that is independent of microtubules seems to exist. This pathway might function independently of auxin. The observation that rice can respond gravitropically under submerged conditions (when amyloplasts are small) opens up the possibility that two completely different gravitropic pathways exist. The extremely high gravitropic sensitivity of the rice coleoptile might have evolved in response to a situation in which amyloplasts, due to submergence, are small. This high sensitivity might be the result of two mutually enhancing signal chains. We can ask whether the two chains can be separated under conditions of microgravity, when only the more sensitive of the two chains contributes to the gravitropic response.

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