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Stimulation of radial expansion in arabidopsis roots by inhibitors of actomyosin and vesicle secretion but not by various inhibitors of metabolism

Tobias I. Baskin, Nathan J. Bivens*

Division of Biological Sciences, University of Missouri, Columbia, MO 65211, USA

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Abstract. Plant morphogenesis depends on accurate control over growth anisotropy. To learn to what extent the control of growth anisotropy depends on cellular metabolism, we surveyed the response of growing roots to a range of inhibitors. Seedlings of *Arabidopsis thaliana* L. (Heynh), 7–8 d old, were transplanted onto plates containing an inhibitor, and elongation and radial expansion of roots were measured over the subsequent 2-d period. Fourteen inhibitors of diverse metabolic processes inhibited root elongation but failed to stimulate radial expansion. These inhibitors were aluminum sulfate, aphidicolin (DNA synthesis), caffeine (cell-plate formation), cisplatin (DNA synthesis), cycloheximide (protein synthesis), 3,4-dehydro-L-proline (proline hydroxylation), 6-dimethylaminopurine (protein kinases), dinitrophenol (mitochondrial ATP synthesis), galactose (UDP-glucose formation), Lovastatin, formerly mevinolin (isoprenoid formation), methionine sulfoximine (glutamine synthetase), methotrexate (folate metabolism), XRD-489 (synthesis of branched-chain amino acids), and high or low calcium treatments. These results show that various types of metabolic disruption, although inhibitory to elongation, do not reduce the high degree of anisotropic growth of the root. However, five chemicals did stimulate radial expansion; namely, the detergent, digitonin; two inhibitors of vesicle secretion, monensin and brefeldin A; and two inhibitors of actomyosin, cytochalasin B and butanedione monoxime. The maximum radial expansion induced by these compounds (except butanedione monoxime) was greater than that caused by ethylene, and the morphology of treated roots did not resemble that of roots treated with ethylene. These results indicate that vesicle secretion and actomyosin play a role in controlling anisotropic expansion.

Key words: Actomyosin – *Arabidopsis* (root expansion) – Growth anisotropy – Inhibitor – Root morphology – Vesicle secretion

Introduction

Plant form, whether baroque as an orchid flower or modernist as a horsetail leaf, has long captivated the human imagination. To understand how forms are sculpted by the plant, we need to know how cell expansion is controlled in different directions. Without such control, all plant cells would be spherical, because the driving force for cell expansion, turgor pressure, is equal in all directions, or isotropic. Cells with other than spherical shapes result when expansion rates are not equal in all directions, that is, when expansion is anisotropic. Cells are able to expand anisotropically because the mechanical properties of the cell wall are anisotropic; expanding walls yield to turgor pressure more in one direction than in another. The most prominent anisotropic elements of the cell wall are the cellulose microfibrils, and the ordered deposition of these polymers is generally believed to be essential for the control of plant cell shape (Green 1987).

The control of the directional deposition of cellulose has often been found to depend on the alignment among microtubules in the cortical cytoplasm. Many herbicides inhibit anisotropic growth and hence cause extreme swelling (e.g., "colchicine tumors") by depolymerizing cortical microtubules (Hess 1982). Moreover, instances where the polarity of growth shifts by 90 degrees often involve preceding shifts in microtubule alignment (Shibaoka 1991). However, controlling anisotropic growth means specifying not only the direction of maximum expansion, but also the degree of anisotropy. In other words, for a given rate of expansion along one axis, cells may expand at any

* *Present address*: Department of Plant Biology, University of Illinois, Champaign-Urbana, IL, USA

Abbreviations: BDM = 2,3-butanedione monoxime; DCB = 2,6-dichlorobenzonitrile; DMAP = 6-dimethylaminopurine; XRD-489 = triazolopyrimidine sulfonamide

Correspondence to: T.I. Baskin; FAX: 1 (314) 882 0123;
E-mail: baskin@biosci.mbp.missouri.edu

fraction of that value in the orthogonal axis. Very little is known about the mechanisms used to specify the degree of growth anisotropy, or even whether microtubules and cellulose microfibrils are involved. Yet without specifying the degree of anisotropic expansion of cells, plants could not build specific forms.

As an approach to understand how plants control the forms of their organs, we have isolated temperature-sensitive mutants in arabidopsis with altered root morphology at the restrictive temperature (Baskin et al. 1992). We have focused on roots because of their high elongation rates, geometrical simplicity, and experimental accessibility. The appearance of these root-morphology mutants is, in general, similar to that of roots treated with either a cellulose-synthesis inhibitor, or with a microtubule inhibitor. For this reason, we suggested these phenotypes result from mutations at loci involved in the synthesis or orientation of either microfibrils or microtubules. However, the path between a mutation and an organismal phenotype can be complex; indeed, several morphology mutants of *Neurospora* are known that result from mutations in the metabolic enzyme, glucose-6-phosphate dehydrogenase (Harold 1990). In plants, might not lesions in cellular processes or structures other than microtubules or microfibrils lead to distorted morphology? Perhaps the radially swollen phenotype of the root-morphology mutants is a non-specific consequence of "metabolic malaise"?

We did the work reported here to see by how much the control of growth anisotropy depends on the metabolic well-being of cells. We inhibited root elongation with a variety of metabolic inhibitors, such as inhibitors of amino-acid synthesis, oxidative phosphorylation, sterol synthesis, folate metabolism, etc., and we quantified root diameter over the concentration range where elongation was inhibited. The diminished root elongation indicates that the tested drug is active, and any concomitant stimulation of radial expansion indicates that the drug reduces the degree of growth anisotropy. By using a wide variety of metabolic and other inhibitors, we can see if inhibited elongation is commonly accompanied by reduced growth anisotropy, and we may identify cellular components that take part in governing growth anisotropy.

Materials and methods

Seeds of *Arabidopsis thaliana* L. (Heynh), ecotype Columbia, were stored at 4 °C, and at day zero were surface-sterilized and plated on agar-solidified, modified Hoagland's solution, with 3% sucrose. The exact composition of the medium is given by Smith et al. (1994). Plates with seeds were placed vertically in a growth chamber, with constant light (80 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and temperature (19 °C). Light from 40-W warm-white fluorescent bulbs and 25-W incandescent bulbs was filtered through a sheet of yellow acrylic (Plexiglas J2208; Cope Plastic, St Louis, Mo., USA). Yellow filters were used to remove ultraviolet and blue wavelengths, which have been shown to drive various deleterious photo-oxidizing reactions in plant growth media (Hangarter and Stasinopoulos 1991).

Seven or eight days after plating, seedlings were transferred to freshly prepared plates containing the inhibitors. To mark the initial position of the root tip, in some experiments, the agar was cut with a scalpel beside each root tip; whereas in others, the back of the plastic plate was scored at the position of the root tip. Plates were returned to the growth chamber for 48 h. Then, plates were photocopied with 150% enlargement. In those experiments where agar had been cut, the cut was made visible by filling it with a few drops of milk. Root elongation was measured with a digitizing tablet as the distance along the root from the tip to the mark. Distance was calibrated by photocopying a ruler at the same enlargement. Root diameter was measured by placing a coverslip over the roots directly on the agar surface, wetted by a few drops of an aqueous 0.01% (w/v) Triton X-100 solution. Mounted roots were viewed through a compound microscope at low magnification, and diameters measured with a calibrated, filiar, ocular micrometer, or in some experiments with a video digitizer (Image 1/AT; Universal Imaging Co., West Chester, Pa., USA). For roots with conspicuous swellings, diameter was measured at its apparent maximum. For control roots, and others without apparent swelling, diameters were measured at the region of root hair initiation. For those treatments that inhibited root hair initiation, root diameter was measured just proximal of the zone of cell elongation.

For each inhibitor tested, three plates were made for each concentration, and ten seedlings were transferred to each plate. Root elongation data are reported as mean $\pm$ SE for the three plates. Diameter data are reported as mean $\pm$ SD for two plates.

The following list of compounds used in this study gives the source (if other than Sigma Chemical Co. St Louis, Mo., USA), the solvent used to make stocks (if other than dimethylsulfoxide, DMSO), the maximum stock concentration, and any other relevant details. For some compounds, stocks were not made; instead, after autoclaving, powder was added to the agar medium followed by 15 min of stirring at 50 °C, with controls stirred similarly. Actinomycin-D, 1 mM stock; aluminum sulfate, added as powder (prior to autoclaving, the medium was adjusted to pH 4.5); aphidicolin, 6 $mg \cdot ml^{-1}$ stock in 95% ethanol; brefeldin A, 10 $mg \cdot ml^{-1}$ stock; 2,3-butanedione monoxime (BDM) added as powder; caffeine, 1 M stock in water; cisplatin, 100 mM stock; cycloheximide, 3.55 mM stock in water; cytochalasin B, 1 $mg \cdot ml^{-1}$ stock; 3,4-dehydro-L-proline, 100 mM stock in water; 2, 6-dichlorobenzonitrile (DCB) (Aldrich Chemical Co.), 10 mM stock; digitonin, 0.1 $g \cdot ml^{-1}$ stock; 6-dimethylaminopurine (DMAP), 100 mM stock; 2,4-dinitrophenol, 10 mM stock; galactose, added as powder; Lovastatin (formerly mevinolin), a gift from Merck Research Laboratories, Rahway N.J., USA, the lactone was opened chemically by a procedure suggested by Merck, 4 $mg \cdot ml^{-1}$ stock in water; L-methionine sulfoximine, 250 mM stock in water; methotrexate, 10 mM stock; monensin, 20 mM in methanol; oryzalin (Chem Service, West Chester, Pa., USA), 10 mM stock; triazolopyrimidine sulfonamide (XRD-489), a gift from DowElanco, Indianapolis, Ind., USA, 10 mM stock. For calcium treatments, either 100 mM $CaCl_2$, or for "control" 100 mM KCl, was added to the medium, or for the "zero" treatment, the usual 5 mM $Ca(NO_3)_2$ stock was replaced by 10 mM KNO_3.

To make inhibitor-containing plates, the required amount of inhibitor stock was added to a known volume of previously autoclaved medium. It was not necessary to filter-sterilize the inhibitor stocks. The mixture was vigorously mixed and plates were poured. Control plates were made similarly by adding the maximum amount of pure solvent used for a given set of concentrations. The maximum concentration of solvent in the plates seldom exceeded 0.1%.

Results

To compare the effects of compounds on elongation and radial expansion, we grew arabidopsis seedlings for 7–8 d on control medium and then transplanted them to medium into which a known concentration of drug had been incorporated. We measured the root elongation that occurred over the following 2 d, and the maximum root diameter at the end of this time. Because the maximum root diameter of controls scarcely changes over the 2-d period, we could compare elongation and the diameter at

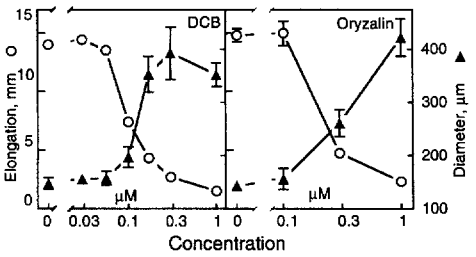

Fig. 1. Growth responses of arabidopsis roots to DCB or oryzalin, compounds known to exert a major effect on growth anisotropy. *Open circles* (left axes) plot elongation increment over 2 d, with SE values (3 replicates) shown when bigger than the symbol; *filled triangles* (right axes) plot maximum root diameter at the end of the 2-d treatment, with SD values (20 seedlings) shown when bigger than the symbol

a final time point to find out whether a given treatment affected the anisotropic expansion of the root.

Inhibitors of microtubule polymerization or cellulose synthesis. Chemicals that inhibit microtubule polymerization, such as oryzalin (Hugdahl and Morejohn 1993), and chemicals that inhibit cellulose synthesis, such as DCB (Hogetsu et al. 1974), are known to cause root swelling (Avers and Goodwin 1956; Hess 1982). In arabidopsis, for both oryzalin and DCB, elongation was inhibited over the same concentration range as radial expansion was stimulated (Fig. 1). The observed concomitant decrease in elongation and increase in radial expansion presumably reflect a single mode of action of each compound to reduce the degree of anisotropic expansion.

Inhibitors of various metabolic pathways. Figure 2 shows the results for 15 inhibitors of various metabolic pathways. Cellular processes targeted by these inhibitors include: protein synthesis (cycloheximide, Lin and Key 1968), synthesis of branched-chain amino acids (XRD-489, Mourad et al. 1993), glutamine synthetase (methionine sulfoximine, Ronzio et al. 1969), isoprenoid formation (Lovastatin, formerly mevinolin, Gray 1987), mitochondrial ATP production (dinitrophenol), DNA synthesis (cisplatin, Rosenberg 1985; and aphidicolin, Sala et al. 1980), cell-plate formation (caffeine, Paul and Goff 1973), folate metabolism (methotrexate, Crosti 1981), mRNA transcription (actinomycin-D, Lin and Key 1968), protein kinases (DMAP, Néant and Guerrier 1988), proline hydroxylation (dehydroproline, Cooper and Varner 1983), UDP-glucose formation (galactose, Inouhe et al. 1986), and calcium homeostasis (high and low calcium treatments). Finally, one compound, aluminum sulfate, has an unknown mode of action (for recent discussion, see Delhaize and Ryan 1995).

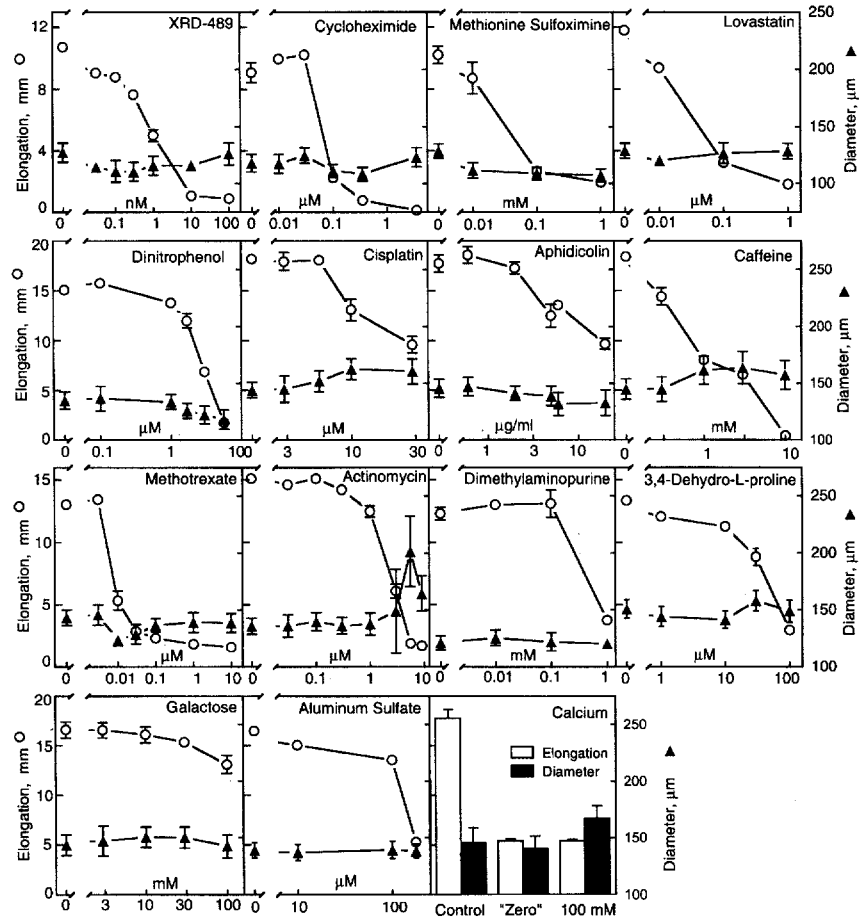

Fig. 2. Growth responses of arabidopsis roots to various metabolic inhibitors. Details of the plots are the same as for Fig. 1, except that the y-axes for diameter are plotted at twice the scale. Note that there are only minor effects on root diameter despite major inhibition of elongation

Over the concentration range where these compounds inhibited root elongation, notable radial swelling was stimulated by only one, actinomycin-D (Fig. 2; note that the diameter scale is double that of Fig. 1). However, the radial swelling induced by this compound was unusual, affecting only some of the roots (note the size of the standard deviations for the actinomycin diameter data), and making a short bulge, about as long as wide, located far away from the tip (more than 1 mm at 3 μM). This indicates a highly restricted response, in which only a few cells swell. By contrast, the inhibition of elongation by actinomycin-D was uniform. This transient swelling might relate to reported interactions between RNA synthesis and microtubule organization (Utrilla and de la Torre 1991; Kaneta et al. 1993).

As shown in Fig. 2, some chemicals caused roots slightly to thin (e.g., methionine sulfoximine) or thicken (e.g., cisplatin). These changes amount to 10% of root diameter at most, far less than the 100–200% changes seen in root-morphology mutants (Baskin et al. 1992). We have not tested the reproducibility of these small changes or explored their physiological meaning. We conclude that a variety of metabolic processes may be inhibited without greatly perturbing the ability of the root to grow anisotropically.

Inhibitors that stimulated radial expansion. Certain compounds were found to inhibit elongation and concomitantly to stimulate radial expansion (Fig. 3). Radial expansion in roots is well known to be caused by the plant hormone, ethylene, and results for ethylene treatment on arabidopsis roots are included for comparison (replotted from Baskin et al. 1995). Radial swelling was caused by the detergent, digitonin, below concentrations that in onion roots have been shown to disrupt mitosis (Olah and Hanzely 1973). Swelling was also stimulated by two inhibitors of vesicle secretion, monensin and brefeldin A, at or below concentrations used to demonstrate ultrastructural or biochemical effects on plant secretion (Driouich et al. 1994; Satiat-Jeunemaitre and Hawes 1994). Finally, swelling also occurred with an inhibitor of actin polymerization, cytochalasin B (Cooper 1987), and of myosin ATPase, BDM (Herrimann et al. 1992). Effects of BDM on plant material have to our knowledge not been previously reported. Cytochalasin B is typically active against cytoplasmic streaming at or above the effective range shown in Fig. 3 (Williamson 1993). The radial swelling induced by these compounds decreased at the highest concentrations, presumably because the overall rate of expansion becomes limiting. The maximum swelling caused by any drug in this group was less than the extreme swelling caused by either oryzalin or DCB (Fig. 1), but was nevertheless highly significant.

To compare data from all of the inhibitors, we plotted the percent increase in root diameter against the percent inhibition of root elongation (Fig. 4). Curves for DCB and oryzalin (filled circles) have slopes of about one, indicating the reciprocal relationship between decreases in elongation and increases in radial expansion. The slope of one is consistent with the idea that these two compounds affect radial and axial expansion by a single mechanism. Further, curves for the large group of metabolic inhibitors

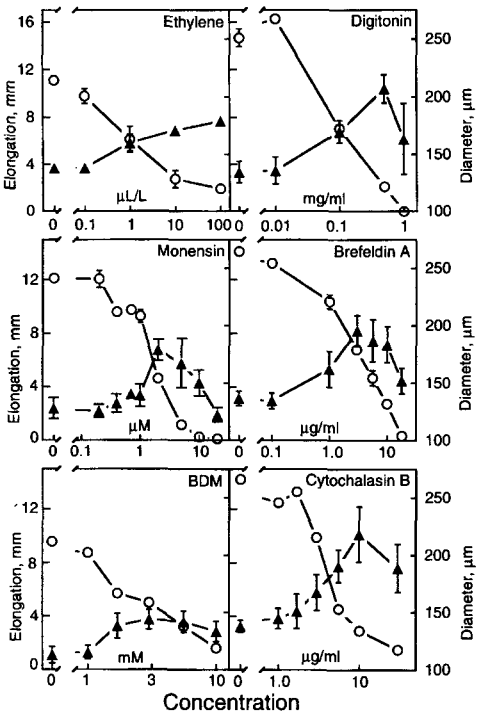

Fig. 3. Growth responses of arabidopsis roots to certain inhibitors that caused significant radial swelling. Details of the plots are the same as for Fig. 1, except that the y-axes for diameter are plotted at twice the scale. Data for ethylene are replotted from Baskin et al. (1995). Note the significant stimulation of radial expansion by this group of compounds

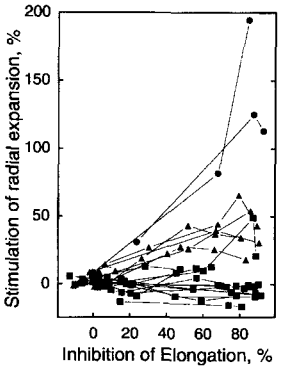

Fig. 4. Comparison of the extent of radial expansion as a function of inhibition of elongation for all of the compounds used. *Circles* replot the data from Fig. 1; *squares* from Fig. 2, and *triangles* from Fig. 3. Data points with more than 96% elongation inhibition have been omitted for clarity

(filled squares) have slopes near zero, showing they inhibited elongation without much affecting radial expansion. Finally, curves for the group of chemicals causing intermediate radial expansion (filled triangles) have slopes between zero and one with y-intercepts near the origin, indicating that this group had a proportionally greater effect on axial than on radial expansion. This would happen if these compounds not only reduced the degree of anisotropic expansion but also inhibited expansion itself.

Data presented thus far report the maximum diameter induced by a given treatment, but the appearance of the swollen roots is also informative (Fig. 5). Because the mean diameter of control roots varied on different days, to compare roots treated with different compounds, we used drug concentrations that caused maximum radial swelling in a single experiment. Measurements of root elongation and radial expansion in this repeat experiment agreed with the previous results, confirming the reproducibility of these effects. Root swelling induced by each of these compounds was distinct morphologically from that caused by ethylene (Fig. 5), suggesting that radial expansion induced by these compounds was not elicited through a response to ethylene. This suggestion is supported by our preliminary results with an ethylene-insensitive mutant of arabidopsis, *etr1*, whose roots, in response to these compounds, swelled just as much as did those of the wild type.

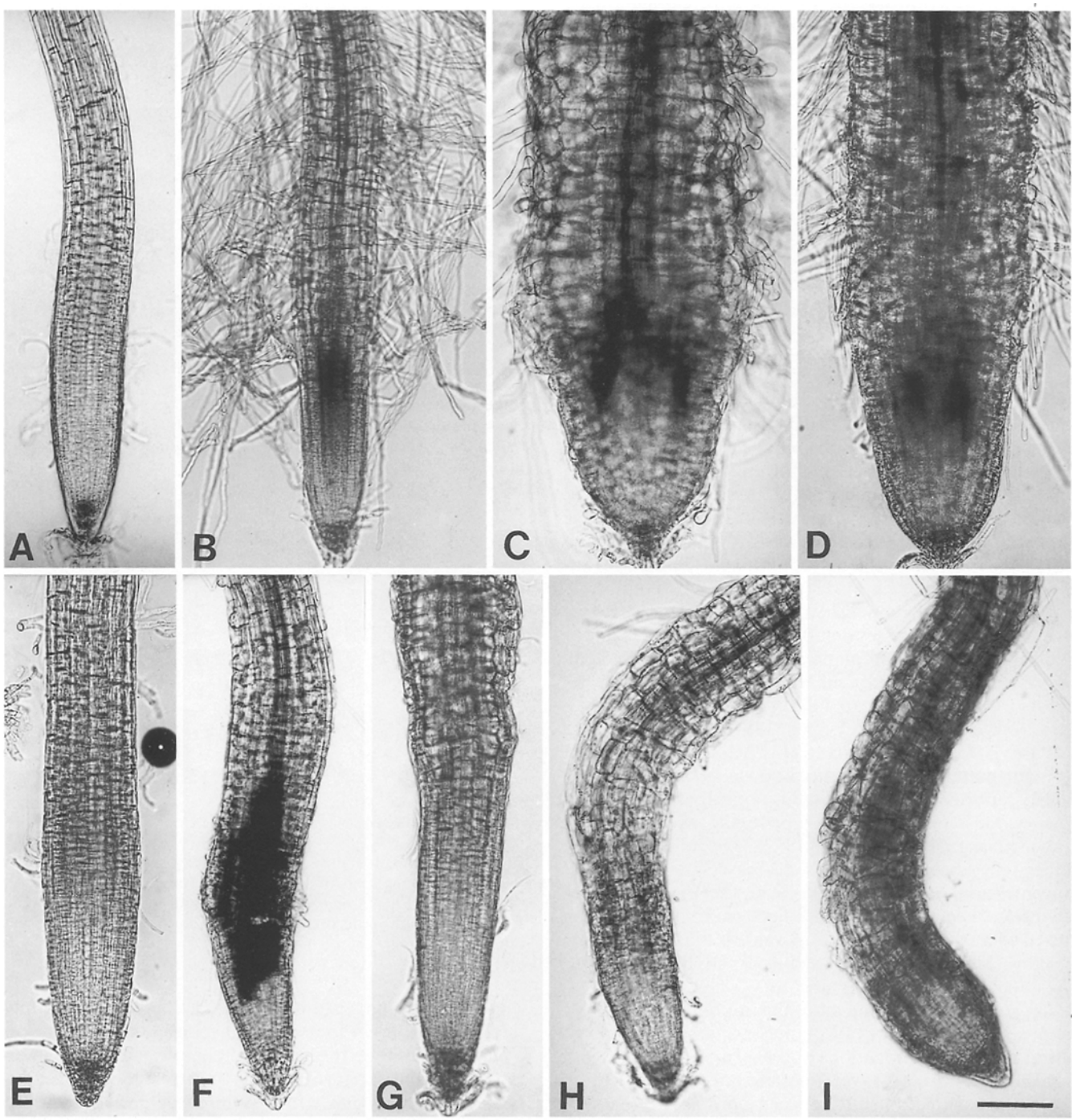

Fig. 5A–I. Micrographs of arabidopsis roots treated with inhibitors that cause swelling. **A**, control; **B**, 100 $\mu l \cdot l^{-1}$ ethylene; **C** 1 μM oryzalin; **D** 0.3 μM DCB; **E** 3 mM BDM; **F** 3 μM monensin; **G** 3 $\mu g \cdot ml^{-1}$ brefeldin A; **H** 10 $\mu g \cdot ml^{-1}$ cytochalasin B; **I** 0.06 $mg \cdot ml^{-1}$ digitonin. Ethylene figure is from Baskin and Williamson (1992). $\times 82$; bar = 0.15 mm

Oryzalin and DCB caused extensive swelling in all growing regions of the root (with the possible exception of the root cap). Similarly, digitonin also appears to have caused swelling throughout growing regions. However, monensin, brefeldin, cytochalasin B and BDM appear to have affected different regions preferentially. Whereas BDM seems to have caused swelling only in the region of cell division, monensin, brefeldin and cytochalasin appear to have caused swelling only in the zone of pure elongation, proximal to the zone of cell division. This visual impression was confirmed for cytochalasin B by measuring root diameter as a function of distance from the tip (not shown): the diameter of treated roots was the same as that of control roots in the zone of cell division (i.e., in the apical 300 μm of the root tip).

Discussion

Radial expansion and inhibitor studies. Metabolic inhibitors have been applied in countless assays of mitosis or plant growth, and comparative information on elongation and radial expansion for many inhibitors might be found in the literature. However, in reading reports on the effects of inhibitors, we rarely found data on radial expansion. The absence of data on diameter in a paper cannot be taken to mean the absence of an effect, because although swelling of the magnitude caused by oryzalin or DCB is unmistakable to the eye, intermediate swelling as reported in figure 3, significant when measured, is easily overlooked. With our system, had we only been assaying elongation, we would almost certainly have missed this intermediate swelling. Therefore, the explicit comparison made here between the sensitivities of elongation and radial expansion to various inhibitors represents a generally novel addition to our understanding of the mode of action of each inhibitor and of how plants grow anisotropically.

Control of growth anisotropy from cell to organ. Our results on the control of growth anisotropy are based on measuring the length and width of the root, and thus they do not pertain directly to anisotropic rates of cellular expansion. In fact, the magnitude of cellular growth anisotropy is a complicated function of time and position in the root (Avers and Goodwin, 1956). Nevertheless, based on our measurements of overall organ form, we can make valid inferences about cellular anisotropic expansion. In arabidopsis, root diameter is set by the radial expansion of cells in the apical portion of the zone of cell division, and by the absence of radial expansion of cells in the zone of rapid elongation (Baskin et al. 1992, 1995). Thus, any treatment that causes the zone of rapid elongation to swell radially must reduce the degree of anisotropic expansion of cells in that zone. In response to most of the compounds we tested, roots did not swell detectably despite the inhibition of elongation; hence, we may conclude these compounds did not affect the anisotropic expansion of rapidly elongating cells. Conversely, for a small group of compounds including secretion and actomyosin inhibitors (except BDM, discussed below), the swollen form of roots almost certainly required the cells in the zone of rapid elongation to have non-zero radial expansion. Hence, we may conclude that these compounds did, in general, reduce the degree of anisotropic expansion of rapidly elongating cells.

Compared with rapidly elongating cells, the anisotropic expansion of the apical dividing cells cannot be as readily inferred from the shape of the root. Apical dividing cells expand slowly in both axial and radial directions. Without direct measurements of the anisotropic expansion at the cellular level, we cannot even say whether the direction of maximum expansion is axial or radial. Nevertheless, based on changes in the organ's shape, we may still make valid statements about changes in average cellular anisotropic expansion. Many compounds that did not reduce the growth anisotropy of rapidly elongating cells caused a slight, dose-dependent thinning of the root (black squares in Fig. 4). Slight root thinning would result from inhibited rates of radial expansion for the apical dividing cells, but need not reflect any change in their cellular anisotropic expansion, provided that radial and axial expansion rates were inhibited proportionally. Finally, the swelling caused by BDM is small enough and in the right position to be caused by stimulated radial expansion specifically of apical dividing cells. But only if expansion of these cells were stimulated proportionally in axial and radial directions, despite the clear inhibition of expansion in rapidly elongating cells, would we have been wrong to infer from our data on organ form that BDM affected anisotropic rates of cellular expansion.

Growth anisotropy and metabolism. We have used a variety of metabolic inhibitors and shown that elongation can be largely inhibited with only slight if any changes in radial expansion. We have scarcely exhausted possible ways to disrupt cellular metabolism; but, the compounds chosen do cover a diversity of metabolic pathways. Although each inhibitor may not have acted according to the cited expectation, there is no reason to think that all, or even most, of the drugs shown in Fig. 2 acted through the same mechanism. From the lack of stimulation of radial expansion by the chosen metabolic inhibitors, we conclude that the perturbation of normal metabolism does not, as a rule, reduce the cell's control of growth anisotropy. Conversely, compounds that do cause swelling can no longer be considered likely to do so by virtue of some type of generalized cell stress.

Two of the inhibitors that caused negligible changes in radial expansion have been reported to interfere with organization or stability of plant cortical microtubules, and for this reason they might be expected to affect radial expansion. Cycloheximide, after application to root tip cells of onion for 1–2 h, has been reported to disturb the organization of cortical microtubules (Mineyuki et al. 1994). Further work will be needed to say whether this alteration in the cortical microtubules is either specific to onion, short-lived, or not able to change the degree of anisotropic expansion. Similarly, kinase inhibitors, including DMAP, have been shown to affect cortical microtubules, for example, in bean stems, preventing a reorganization of microtubules mediated by exogenous gibberellic acid (Mizuno 1994). But for arabidopsis roots, neither DMAP nor another kinase inhibitor, K252a (not shown), affected growth anisotropy, which suggests that

kinases susceptible to inhibition by these compounds are not involved in controlling radial expansion. It is plausible that other protein kinases, unaffected by these inhibitors, play a role in controlling root morphology. Consistent with this expectation, we have recently shown for arabidopsis roots that two inhibitors of protein phosphatases do cause significant radial swelling (Smith et al. 1994).

Another inhibitor of root elongation that does not affect radial expansion, dehydroproline (Fig. 2), has recently been shown to disturb the morphology of cells regenerating from protoplasts (Cooper et al. 1994). Treatment with 50 μM dehydroproline prevented normal wall regeneration and cell division; and caused many cells slowly to become extremely swollen. This analog apparently substitutes for proline in most capacities, except as a substrate for prolyl hydroxylase, which it permanently inactivates. Therefore, dehydroproline is a reasonably specific inhibitor of the synthesis of hydroxyproline-rich cell wall proteins (Cooper and Varner 1983; Schmidt et al. 1991). On the basis of the protoplasts regenerating with aberrant shapes, Cooper et al. (1994) argue for a fundamental role for these proteins in controlling morphology. However in our study, dehydroproline inhibited the elongation of arabidopsis roots, but did not significantly disrupt their morphology (Fig. 2). Similarly, dehydroproline inhibited the expansion of tissue culture cells without affecting their morphology (Schmidt et al. 1991). We suggest that the difference between the results for protoplasts versus tissue culture cells and roots can be explained by the absence or presence of a cell wall. Hydroxyproline-rich proteins may be required for wall regeneration de novo; but, these proteins may not have to be secreted continuously for some features of wall organization to persist when a cell wall is present. Nevertheless, our results do point to the importance for rapid expansion of the continued production of hydroxyproline-rich proteins.

Growth anisotropy, vesicle secretion, and actomyosin. Many studies on plants with monensin, brefeldin A or cytochalasin report that these compounds inhibit elongation of organs, including roots, at concentrations within the ranges shown here (e.g., Brummell and Hall 1985; Hoson and Masuda 1992; Thimann et al. 1992; Schindler et al. 1994). However, we could find only a single report where effects on radial expansion were assayed: Thomas et al. (1973) found for onion roots that cytochalasin B at 30 $\mu g \cdot ml^{-1}$ (the maximum shown in Fig. 3) inhibited elongation and also caused radial swelling. Therefore, it remains to be determined whether the inhibition of anisotropic growth caused by these five compounds applies widely to higher plants.

The reduction in growth anisotropy wrought by these five compounds may or may not occur via interactions with cellulose synthesis or cortical microtubules. The action of monensin and brefeldin might result from a decreased delivery of cellulose synthetase to the plasma membrane (Brummell and Hall 1985; Hoson and Masuda 1992); or instead, a diminished delivery of certain wall matrix components may change the environment around the microfibrils and so decrease resistance of the wall to radial deformation. The action of BDM and cytochalasin might result from decreased rates of actomyosin-dependent vesicle secretion (Williamson 1993); hence, similarly to the secretion inhibitors, limiting supplies of cellulose synthetase or of some other relevant wall component. Instead, the actomyosin inhibitors may influence organ form because there is a functional interaction between actin microfilaments and cortical microtubules (Eleftheriou and Palevitz 1992). Furthermore, although BDM has been used successfully in a screen for actomyosin mutants in fission yeast (J. Hyams, University of London, personal communication), there are reports for animal cells of effects of BDM that are not myosin dependent (e.g., Huang and McArdle 1992). Finally, digitonin also may disrupt cellulose synthesis, as suggested by the recent use of this detergent as an effective solublizing agent for cellulose synthetase enzymatic activity (Kudlicka et al. 1995); or instead, digitonin may disorganize cortical microtubules, as suggested by its reported ability in onion roots to abolish mitotic spindles and preprophase bands (Olah and Hanzely 1973). Experiments to test the mechanistic basis for the swelling induced by these compounds are underway.

Conclusion. We tested inhibitors of a variety of cellular processes and most of them inhibited root elongation without stimulating radial expansion. Therefore, mechanisms that control growth anisotropy are in general not linked tightly to cell metabolism. However, we found a small group of chemicals that did stimulate radial expansion. These compounds are a detergent, two inhibitors of vesicle secretion and two inhibitors of actomyosin. We conclude that vesicle secretion and the actin cytoskeleton participate in the control of anisotropic growth.

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