Role of Expansin in Cell Enlargement of Oat Coleoptiles

Analysis of Developmental Gradients and Photocontrol

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Expansins are wall proteins that mediate a type of acid-induced extension in isolated plant cell walls (S. McQueen-Mason, D.M. Durachko, D.J. Cosgrove [1992] Plant Cell 4: 1425–1433). To assess the role of these proteins in the process of cell enlargement in living tissues, we compared the spatial and temporal growth patterns of oat (Avena sativa L.) coleoptiles with four wall properties related to expansin action. These properties were (a) the ability of isolated walls and living segments to extend in acidic buffer, (b) the ability of heat-inactivated walls to extend upon application of expansins, (c) the amount of immunologically detectable expansin in wall protein extracts, and (d) the extractable expansin activity of walls. Growth rate was maximal in the apical half of dark-grown coleoptiles and negligible in the basal region. This growth pattern correlated with properties a and b; in contrast, the amount and activity of extractable expansin (properties c and d) were reduced only in the most basal region. Upon exposure to white light, coleoptiles abruptly ceased elongation at 8 to 10 h after start of irradiation, and this cessation correlated with reductions in properties a to c. The growth cessation at 8 to 10 h also coincided with the loss of growth response to exogenous auxin and fusicoccin in excised coleoptile segments. These results lend correlative support to the hypothesis that expansin action is important for growth responses of living oat coleoptiles (e.g., responses to acidic buffers, auxin, fusicoccin, aging, and light). Our results suggest that changes in the susceptibility of the wall to expansin action, rather than changes in expansin activity, may be a key determinant of the growth patterns in oat coleoptiles.

Cell enlargement during plant growth depends on biochemical "wall loosening," which gives rise to wall relaxation, water uptake, and wall surface expansion (Cosgrove, 1993). Despite considerable study, the biochemical nature of wall loosening remains uncertain. Many studies have implicated hydrolytic breakdown of matrix polysaccharides as a wall-loosening mechanism (Fry, 1989; Hoson et al., 1991; Inouhe and Nevins, 1991; Carpita and Gibeaut, 1992). Recent interest has also focused on the action of xylolucan endo-transglycosylase, which can cleave xylolucan chains and transfer the nonreducing end to another xylolucan (Farcas et al., 1992; Fry et al., 1992; Nishitani and Tominaga, 1992; de Silva et al., 1993). However, none of these enzymes has been shown to catalyze extension of isolated cell walls, so their role in control of wall extension remains largely conjectural.

Recently, McQueen-Mason et al. (1992) used a reconstitution approach to identify two cucumber wall proteins that can induce extension of isolated walls held in tension in acidic pH. Here we refer to such proteins as "expansins," designating the class of wall-associated proteins that can mediate acid-induced extensions of isolated walls. Li et al. (1993) identified a single, active expansin in wall extracts from oat coleoptiles; its biochemical and antigenic characteristics were very similar to the 29-kD expansin from cucumber hypocotyls. These and related results led us to propose that expansins constitute an evolutionarily conserved family of proteins that mediate a significant part of the acid-extension response common to the walls of many plant species (Li et al., 1993).

Two pertinent and unresolved issues concern the mechanism by which expansins catalyze wall extension and the role that they play in normal cell enlargement. The biochemical activities of expansins remain uncertain, but they do not have detectable exoglycanase, endoglycanase, or xylolucan transglycosylase activities (McQueen-Mason et al., 1992, 1993). Assays of the mechanical strength of paper suggest that expansins may act by disrupting hydrogen bonding between wall glucans (McQueen-Mason and Cosgrove, 1993).

Little is known of the role that expansins might play in the endogenous control of plant cell enlargement. Previous work found that the acid-extension response of isolated cucumber walls correlated with the endogenous growth of the intact hypocotyl (Cosgrove, 1989). For example, treatments with red light or with a gibberellin biosynthesis inhibitor suppressed both hypocotyl elongation and the acid-induced extension of isolated hypocotyl walls. Cells in the basal part of the cucumber hypocotyl exhibited very little growth and their walls did not extend in response to acidic pH. Similarly, isolated walls from bean leaves lost their acid-extension response as the leaves matured (Van Volkenburgh et al., 1985). These observations indicate that the ability of isolated walls to extend under acidic conditions is developmentally regulated and is correlated with tissue growth. It is not clear, accordingly, how such a process might be controlled by expansins.

Abbreviations: Ex29, 29-kD expansin; PA1, antibody directed against cucumber Ex29; PBST, phosphate-buffered saline with Tween-20.

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however, whether this developmental control is mediated principally by modulation of the amount of expansins, the activity of expansins, the susceptibility of the wall to expansins, or by some other means.

In this study we have pursued this issue in greater detail. We used oat (Avena sativa L.) coleoptiles for this study because they appear to have a single, active expansin in their walls (Li et al., 1993), whereas cucumber hypocotyls have at least two expansins (McQueen-Mason et al., 1992). We studied two conditions in which cell extension varies, namely, as a function of the natural growth pattern along the axis of the oat coleoptile and as a function of light exposure. Our approach was to compare spatial and temporal patterns of coleoptile cell enlargement with corresponding patterns of several properties related to expansin action. Our results pertain not only to the relationship between expansins and the acid-growth behavior of tissues, but also to the broader issue of the role of expansins in endogenous cell growth.

MATERIALS AND METHODS

Plant Material and Growth Conditions

Oat seedlings (Avena sativa L. cv Olge from Carolina Biological Supply) were grown in the dark in moist vermiculite at 27°C. Five-day-old plants with coleoptiles 4 to 5 cm long were used for most experiments. Except as noted below, coleoptiles were harvested under room light without prior exposure to green safelight.

To measure growth distributions, the coleoptiles of 4.5-d-old seedlings (coleoptiles about 3.5 cm long) were marked under dim, green light (Kigel and Cosgrove, 1990) with india ink or oil-based block printing ink at 2.5-mm intervals. Separation of the marks was measured with a ruler after 8 to 10 h.

For light-growth experiments, the intact coleoptiles of 4-d-old, dark-grown seedlings were marked (1 cm from the tip) under dim, green light. The seedlings were then repeatedly to assess growth, whereas other plants from the same flat were harvested at different time points to assess expansin-related properties.

For segment-growth experiments, coleoptiles were cut to the specified length with a double-blade cutter under room lights. The primary leaf was removed with forceps and the coleoptile segments were floated on water until the start of the experiment (total collection time was 5–10 min). For acid-growth experiments, the coleoptiles were abraded with a slurry of carborundum (320 grit, well washed prior to use; Fisher Scientific, Fair Lawn, NJ) and rinsed well prior to cutting to length.

Endogenous Extension Activity of Isolated Walls

Coleoptiles were harvested and frozen at −20°C. The cuticle of the frozen coleoptiles was abraded with a slurry of carborundum as above. Because the frozen coleoptiles were more difficult to handle than fresh coleoptiles, we often abraded the coleoptiles before freezing, with little effect on the results. The abraded coleoptile was cut into four equal sections of about 1.2 cm (designated a to d from the tip to base) and pressed for about 5 min between two glass slides under a constant weight to remove cell fluids and thereby facilitate clamping. Wall specimens were clamped in a constant-force extensometer at 20-g tension, with a distance of 5 mm between the clamps (Cosgrove, 1989). To assay endogenous extension activity, the wall was incubated first in 50 mM sodium phosphate, pH 7.0; in some cases 50 mM sodium Hepes, pH 6.8, was used. After 20 to 30 min, the buffer was replaced with extension assay buffer (50 mM sodium acetate, pH 4.5) and extension was monitored with an electronic position transducer connected to the lower clamp (Cosgrove, 1989). The extension rate at pH 4.5 minus the rate at pH 7.0 was taken as the acid-induced extension.

Extraction of Cell Wall Proteins

About 60 to 100 excised coleoptile segments (variable between experiments), corresponding to the regions used in the extension assays described above, were homogenized in 2 mL of buffer A (15 mM sodium phosphate, pH 6.0). The homogenate was centrifuged in an Eppendorf centrifuge for 5 min to pellet the walls and the supernatant was discarded. The pellet was washed four times by resuspending and centrifuging in buffer A. The final wall pellet was resuspended in 0.3 to 0.5 mL of buffer B (50 mM Hepes, pH 7.0, 1 M NaCl, 2 mM EDTA, and 2 mM sodium metabisulfite) for at least 1 h. After centrifugation, the supernatant was desalted and concentrated with a Centricon-10 Microcentrator (Amicon, Beverly, MA) for reconstitution assays. If the protein concentration was high enough, the NaCl extract was diluted directly with extension assay buffer, provided the final concentration of NaCl was less than 150 mM. Proteins were quantified colorimetrically with Coomassie protein assay reagent (Pierce, Rockford, IL).

For determination of cell wall dry weight, the salt-extracted walls were incubated for 20 h at 37°C in 50 mM sodium phosphate, pH 7.0, containing 2 mg/mL of protease (from Streptomyces griseus; Sigma). The enzyme-digested walls were washed twice with water, twice with 70% ethanol, dried in an oven at 85°C for 24 h, and weighed.

Reconstitution Assays

For tests of the susceptibility of oat walls to expansin activity, coleoptile walls were assayed in a reconstitution assay in which the walls were challenged with a fixed level of exogenous expansin activity (Li and Cosgrove, 1993; McQueen-Mason et al., 1993). Briefly, 1-cm segments were cut from designated regions of the coleoptile, frozen at −20°C, abraded with carborundum, and dipped for 15 s in boiling water to inactivate the endogenous expansin activity. Each wall specimen was mounted in the extensometer as described above and incubated in extension assay buffer. After 20 to 30 min in extension assay buffer, the buffer was replaced with 0.4 mL of the same solution containing about 30 μg of cucumber wall proteins with expansin activity. The
difference in the extension rate before and immediately after addition of proteins (i.e. the first 10-15 min) was taken as the reconstitution activity. Controls, in which the buffer was exchanged for fresh buffer with or without inactive proteins (BSA or boiled expansins), failed to induce wall extensions (data not shown). Cucumber proteins for these assays were extracted from cucumber hypocotyl walls with 1 M NaCl and precipitated with ammonium sulfate, as described previously (McQueen-Mason et al., 1992). In the assay using light-treated coleoptile walls, we used about 75 μg of proteins partially purified by HPLC on a C₅ column (McQueen-Mason et al., 1992).

Oat expansin activity was assayed in a similar reconstitution assay. Walls from etiolated hypocotyls of 4- to 5-day-old cucumber seedlings (Cucumis sativus L. cv Burpee Pickler, from A.W. Burpee, Westminster, PA) were used as substrate for these assays because they broke less often than oat coleoptiles and they were more responsive to added proteins than were oat walls (Li et al., 1993). Walls from the apical 1 cm of the cucumber hypocotyls were collected, frozen, abraded, boiled, and clamped in the extensometer, exactly as described above. After about 30 min in extension assay buffer the solution was replaced with 0.4 mL of extension assay buffer containing proteins extracted from designated regions of the oat coleoptile (usually 20–30 μg of crude wall protein, extracted as described above).

Gel Electrophoresis and Immunoblot Analysis

Crude wall proteins were concentrated by filtration in a Centricon-10 (above) or by precipitation with ammonium sulfate (0.4 g added per mL). They were separated by one-dimensional SDS-PAGE (Laemmli, 1970) or by two-dimensional gel electrophoresis (O’Farrell et al., 1977) using a Mini-PROTEAN II system (Bio-Rad), 15% acrylamide gels, and Sigma Pharmalyte 2D ampholytes (pH 3–10). Proteins in gels were visualized by silver staining (Oakley et al., 1980). Proteins were electrophoretically transferred to nitrocellulose membrane (Schleicher and Schuell PH 79) in a solution of 192 mM Gly, 25 mM Tris, 20% methanol (v/v) at 10 V/cm for 3 h. The membrane was blocked for at least 1 h with 3% BSA in PBST, then incubated for 2 h in PBST containing antiserum (dilution of 1:1000 to 1:5000). The membrane was washed three times with PBST and then incubated for 2 h with goat anti-rabbit immunoglobulin-G-conjugated alkaline phosphatase (Sigma; dilution of 1:4000) in PBST containing 0.5% BSA. The immunoblot was developed with bromochloroindolyl phosphate/nitroblue tetrazolium and the reaction was stopped with 10 mM EDTA (Harlow and Lane, 1988).

The antiserum, designated PA1, was obtained from a female New Zealand White rabbit injected with purified Ex29 from cucumber hypocotyls (Z.-C. Li and D.J. Cosgrove, unpublished data). In brief, Ex29 was extracted from cucumber hypocotyl walls and purified by sequential ammonium sulfate precipitation, HPLC fractionation, and SDS-PAGE (McQueen-Mason et al., 1992). The band containing Ex29 was cut from the gel, dried, pulverized, and homogenized with Freund’s adjuvants prior to immunization of the rabbit (Harlow and Lane, 1988). Preimmune serum and nonimmune sera at the same dilution did not detect any specific proteins in immunoblots.

RESULTS

Developmental Gradients along the Coleoptile Axis

Figure 1A shows the growth rate (elongation rate) as a function of position along the dark-grown oat coleoptile. The

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**Figure 1.** Comparison of the growth pattern of etiolated oat coleoptiles (A) with the pattern of acid-induced growth of excised segments (B), acid-induced extension of isolated walls (C), and susceptibility of isolated walls to extend in response to exogenous expansins (D). Figure 1A illustrates the top four coleoptile regions used for the experiments in this paper; the corresponding growth rate distribution is shown below. Shaded regions designate the tissues used in the wall-extension assays and the segment-growth assays. The growth of the marked coleoptiles was measured after 8 to 10 h and relative growth rates were calculated as \( \frac{\ln(\text{final size}) - \ln(\text{initial size})}{\text{time interval}} \). The growth rates are plotted against the logarithmic mean position of the midpoint between marks (= exp(\ln(initial position) + ln(final position))/2). Data represent the means of 20 coleoptiles. This experiment was carried out three times with similar results. B, Acid-growth response of different coleoptile regions. Abraded segments (initial length = 5 mm) were incubated for 2 h with shaking at 27°C in acid or neutral buffers (10 mM potassium citrate/phosphate at pH 4.5 or 7.0, respectively). The response was calculated as mean change in length in acid buffer minus the mean change in length in neutral buffer. This experiment was carried out three times with similar results. C, Representative traces of acid-induced extensions from isolated oat coleoptile walls. The letters A to D designate the various regions of the coleoptile and the numbers in the inset represent the initial rate of extension upon addition of extension assay buffer (mean ± se, n = 4). D, Extension of walls from different coleoptile regions in response to addition of exogenous expansin. The extension rate of heat-inactivated coleoptile walls in extension assay buffer was measured for about 30 min, then the buffer was exchanged for the same buffer containing approximately 30 μg in 0.4 mL of crude cucumber expansin. The response was quantified as the increase in extension rate in the first 15 min after addition of expansin. Means ± se, n = 6.
growth rate was highest in the apical 12 mm. This region corresponds to the region we designated a. There was little or no growth in the basal region d and intermediate growth rates in the middle regions b and c. If this spatial distribution of growth were dependent on expansin-mediated wall extension, then one might expect a similar pattern of acid-growth response along the coleoptile (this assumes that expansins mediate acid-growth responses; see McQueen-Mason et al., 1992; Li et al., 1993). Therefore, we assayed the four regions of the oat coleoptile for their ability to undergo two types of acid-induced extension.

First, the acid-growth responses of living coleoptile cylinders excised from regions a to d were measured. As shown in Figure 1B, the apical region (a) extended the greatest amount in acid buffer (pH 4.5), whereas the basal region (d) hardly grew at all, and regions b and c were intermediate in acid-growth response. It should be noted that the segments used in this assay were taken from the middle 50% of regions a through d (shown as the shaded regions in Fig. 1A). Thus, the acid-growth pattern paralleled the pattern of endogenous coleoptile growth, except that region a was somewhat more responsive to acid treatment than expected from the growth distribution.

Second, we measured the acid-induced extensions of isolated coleoptile walls from regions a to d (Fig. 1C). The initial rates of wall extension were highest for region a and were lower for the more basal regions. Region d lacked a substantial acid-induced wall extension under our assay conditions. The final rates of extension (e.g. 2 h after addition of acidic buffer; see inset in Fig. 1C) were highest in regions a and b and lower in the more basal regions. These results show that acid-induced wall extension correlates with endogenous coleoptile growth.

We further dissected the acid-induced wall extension into two parts: the extractable expansin activity and the susceptibility of the walls to expansins. This dissection assumes that the acid-induced extension of coleoptile walls is principally due to the activity of expansins and that we can extract this activity in a quantitatively reliable fashion (see "Discussion"). Wall susceptibility and expansin activity were tested by a reconstitution assay: a crude expansin fraction was added to walls that were inactivated with a brief heat treatment. The final rates of extension (e.g. 2 h after addition of acidic buffer; see inset in Fig. 1C) were highest in regions a and b and lower in the more basal regions. These results show that acid-induced wall extension correlates with endogenous coleoptile growth.

In immunoblot analyses of the extracted wall proteins, PA1 detected a protein that was most abundant in regions a and c and less abundant in region d (data not shown). In immunoblots of two-dimensional gels, this antibody gave a strong signal to a single protein of minor abundance in the protein extract from oat walls (Fig. 3). We also confirmed by two-dimensional immunoblots that the antibody detected the same, single protein in wall extracts from regions a and d (data not shown). Previous results showed that the protein detected by this antibody co-purified in HPLC separations with the oat expansin activity (Li et al., 1993). Our results indicate that only the basal region d of the coleoptile was diminished in extractable expansins because they were much easier to obtain in quantity than were coleoptile expansins in this reconstitution assay.
Role of Expansin in Oat Coleoptile Growth

Figure 3. Two-dimensional immunoblot (A) and silver-stained gel (B) of crude (ammonium sulfate-precipitated) wall proteins from the apical 2.5 cm of the oat coleoptile. The arrows mark the location of the single protein detected by PA1 (at 1:5000 dilution).

The wall to expansin action may be an important determinant of this growth pattern.

Light Inhibition of Oat Coleoptile Growth

We further analyzed the relationship between growth and expansin action by examining the response of coleoptiles to white light. Oat seedlings showed two kinetic phases in their light-growth responses. In the first 8 h of light treatment, the coleoptiles grew at a rate of 60 to 90% of the dark controls (variable between experiments) and then abruptly ceased growth at 8 to 10 h after start of irradiation (Fig. 4).

The acid-induced extension of native coleoptile walls was assayed at various times after the start of irradiation. Walls taken 4 h after start of the white-light treatment were equally responsive as the dark controls, but walls lost most of their acid-extension response at the 8-h point and were completely unresponsive at 24 h (Fig. 5). Likewise at the 8- to 10-h time point oat coleoptiles lost much of their growth response to exogenous auxin (Fig. 6A) and to fusicoxcin (Fig. 6B). These results show that the growth inhibition that initiated 8 h after onset of light was correlated with a reduction of the acid-extension response of isolated coleoptile walls and with the responsiveness of coleoptile segments to auxin and fusicoxcin.

Figure 5. Acid-induced extensions of native oat coleoptile walls at various times after the start of white-light irradiation. Each curve is representative of eight assays of the acid-extension responses of frozen/thawed coleoptiles in 50 mM potassium acetate, pH 4.5.

Figure 6. Loss of coleoptile growth response to auxin (A) or fusicoxcin (B) following onset of constant white light. Data are the mean increases in the length of initial 6-mm segments incubated on 10 mL of 10 μM indole acetic acid or 1 μM fusicoxcin for 2 h. Fifteen to 20 segments per group. The values are expressed as percent of dark controls, which grew approximately 0.9 mm for auxin treatment and 1.3 mm in fusicoxcin. This experiment was carried out three times with similar results.
We dissected the acid-extension response into the activity of extractable expansins and the susceptibility of coleoptile walls to exogenous expansins. This dissection makes the same assumptions mentioned above. Light treatment strongly reduced the susceptibility of coleoptile walls to added expansins, whereas it had only a small, statistically insignificant effect on the extractable expansin activity (Table II). Light-grown coleoptiles contained more wall material (measured as wall dry weight), probably as a result of continued wall deposition without cell expansion, but the total protein extracted was less. Thus, if expansin activity were expressed on the basis of wall dry weight, the specific activity would be reduced by about half in the light-grown coleoptiles, but in terms of protein-specific activity, there was little effect of light. Immuno blot analyses indicated that light treatment reduced the amount of expansin detected by the PA1 antibody (Fig. 7). The results suggest that changes in wall susceptibility to expansin action are of major importance for the loss of growth at 8 h after onset of light.

**DISCUSSION**

Our experiments were based on the premise that if expansin-mediated wall enlargement were important for coleoptile cell expansion then the spatial and temporal patterns of growth in the coleoptile should correlate with one or more properties related to expansin action. We found that endogenous growth correlated quite well with certain properties associated with the action of expansins, specifically with (a) the acid-induced growth of excised segments, (b) the acid-extension responses of isolated walls, and (c) the susceptibility of isolated walls to extend upon addition of expansins. The amount and activity of extractable expansin was reduced in the nongrowing cells (region d), but otherwise did not correlate with the growth pattern along the coleoptile axis. Similar results were obtained in our studies of the inhibition of coleoptile growth by light. These results offer correlative support for the hypothesis that expansin-mediated wall extension is important for cell growth in oat coleoptiles. However, modulation of the amount or activity of expansin seems to be of secondary importance for the growth patterns we studied. The results suggest that modification of the wall, so as to make it insensitive to expansin action, may be more important as a mechanism for growth control in oat coleoptiles.

**Table II. Effect of light on wall susceptibility and extractable expansin activity**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Wall Proteins*</th>
<th>Wall Dry Weightb</th>
<th>Extractable Activityc</th>
<th>Wall Susceptibilityd</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dark</td>
<td>2.7 ± 0.2</td>
<td>0.33 ± 0.02</td>
<td>2.58 ± 0.32</td>
<td>1.99 ± 0.18</td>
</tr>
<tr>
<td>Light</td>
<td>1.7 ± 0.3</td>
<td>0.57 ± 0.05</td>
<td>2.10 ± 0.27</td>
<td>0.0 ± 0.03</td>
</tr>
</tbody>
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*a n = 3.  b n = 4.  c n = 15.  d n = 6 (dark) or 7 (light).*

Previous studies compared the distributions of growth and of wall-modifying enzyme activities along growing axes. Specific examples include (a) enzymes hypothesized to possess wall-loosening functions, such as glycanases and glycosidases (Nevins, 1970; Goldberg, 1980; Wong and MacAlahan, 1980; Pierrot et al., 1982); (b) enzymes hypothesized to enhance the activity of wall-loosening enzymes, such as pectin methylesterase (Goldberg, 1984); (c) enzymes with transglycosylase activity (Fry et al., 1992); and (d) putative wall-stiffening enzymes, such as peroxidase (Goldberg et al., 1987; Macadam et al., 1992). The spatial distribution of these enzymes is often (although not always) found to be consistent with their putative functions in growth. However, the exact role of these enzymes in the wall-extension process remains largely conjectural since none of them has been shown by direct assays to induce or modify wall-extension processes (Cosgrove, 1993). In contrast, expansins possess a well-defined ability to catalyze extension of isolated walls in acidic pH. This property, in combination with our current results showing a correlation between growth and expansin-mediated wall extension, makes expansins an attractive candidate for the "wall-loosening enzymes" long hypothesized to cause plant cell enlargement (McQueen-Mason et al., 1992).

Although our results implicate expansin action in the growth patterns of oat coleoptiles, the activity of extractable expansins did not correlate closely with the endogenous growth of the coleoptile or with the other acid-extension properties (Tables I and II). This result suggests that coleoptile growth is not controlled by modulation of expansin activity. We regard this conclusion as tentative, however, because our assay of extractable activity may not correctly represent the expansin activity in situ. At this time we do not know the efficiency with which expansins are extracted from the walls by 1 M NaCl. If our treatments (developmental age and light) influenced the efficiency of wall protein extraction (e.g. by altering expansin binding to the wall), then our reconstitution and immunoblot assays might give misleading results. Likewise, if cofactors or other modulators of expansin activity in situ were lost upon extraction and reconstitution, our activity results could be misleading. Relatively crude protein prepa-
rations were used to assay expansin activity, so it is possible that other proteins in the mixture might have interfered with the extension assay (e.g. proteases, peroxidases). Although Li et al. (1993) found only a single protein with expansin activity in oat coleoptile walls, it is still possible that the crude mixture of proteins used in our assays might contain proteins with synergistic activities; however, attempts to identify such synergisms by combining HPLC fractions failed to reveal such activities (our unpublished results). We also note that exogenous expansins can restore only a fraction of the acid-extension response of oat coleoptiles (Li et al., 1993), unlike cucumber walls that may be fully restored in their extension responses (McQueen-Mason et al., 1992). Thus, some caution is warranted in interpreting these assays of extractable activity from oat coleoptiles. The comparison of the activity results (Tables I and II) with the western blots (Figs. 2 and 6) does suggest that the specific activity of extracted expansins is not constant, but we do not know the basis for this apparent variation in specific activity.

We note that the susceptibility of walls to expansin action correlated well with coleoptile growth in our experiments. The biochemical mechanism for this alteration of wall properties is uncertain, but several possibilities suggest themselves. For instance, the wall might become cross-linked in a light-dependent and age-dependent fashion, e.g. by peroxidative cross-linking (Goldberg et al., 1987; Kim et al., 1989; Kamisaka et al., 1992; Macadam et al., 1992). Such cross-linking might form a load-bearing network that is resistant to the biochemical action of expansins. Alternatively, the wall components on which expansins act might become modified or lost so that the proteins no longer act on them. A third possibility is that the physical porosity or binding properties of the wall become altered so that the expansin protein cannot gain access to its site of action. It may be possible in future work to analyze the change in wall susceptibility by in vitro chemical modification of insusceptible walls followed by reconstitution assays. It is also possible that the same modification that makes the wall resistant to expansins also makes it resistant to other wall-loosening mechanisms; however, other hypothetical mechanisms for wall loosening have not yet been shown to cause wall extension in vitro.

Previous studies concluded that expansins catalyze at least part of the acid-extension responses of isolated walls (McQueen-Mason et al., 1992; Li et al., 1993). Our current results extend this work with evidence that suggests that expansins play an important role in the enlargement of living cells (i.e. correlations between expansin-related properties and coleoptile growth responses to acidic buffers, auxin, fusococcin, aging, and light). Most notably, our results suggest that modifications of the wall that alter its susceptibility to expansins may be of key importance for oat coleoptile growth. We anticipate that cloning of the expansin genes and genetic manipulation of their expression will yield further insights into the developmental significance and mechanism of these novel wall proteins.

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For the full reference list, please see the original publication.


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