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Update on Development

The Acid Growth Theory of Auxin-Induced Cell Elongation Is Alive and Well[1]

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Plant cells elongate irreversibly only when load-bearing bonds in the walls are cleaved. Auxin causes the elongation of stem and coleoptile cells by promoting wall loosening via cleavage of these bonds. This process may be coupled with the intercalation of new cell wall polymers (1). Because the primary site of auxin action appears to be the plasma membrane or some intracellular site, and wall loosening is extracellular, there must be communication between the protoplast and the wall. Some "wall-loosening factor" must be exported from auxin-impacted cells, which sets into motion the wall-loosening events (15).

About 20 years ago, it was suggested that the wall-loosening factor is hydrogen ions. This idea and subsequent supporting data gave rise to the Acid Growth Theory (7, 13–15), which states that when exposed to auxin, susceptible cells excrete protons into the wall (apoplast) at an enhanced rate, resulting in a decrease in apoplastic pH. The lowered wall pH then activates wall-loosening processes, the precise nature of which is unknown. Because exogenous acid causes a transient (1–4 h) increase in growth rate, auxin must also mediate events in addition to wall acidification for growth to continue for an extended period of time. These events may include osmoregulation, cell wall synthesis, and maintenance of the capacity of walls to undergo acid-induced wall loosening. At present, we do not know if these phenomena are tightly coupled to wall acidification or if they are the products of multiple independent signal transduction pathways.

EVIDENCE FOR AND AGAINST THE ACID GROWTH THEORY

Four major lines of qualitative evidence (6, 7, 13, 15) support the Acid Growth Theory: (a) auxin-treated stem and coleoptile sections excrete protons in response to auxin, lowering the pH of the apoplast by as much as a full pH unit; (b) treatment of auxin-sensitive tissues with acidic buffers of pH 5.0 can cause cells to elongate at rates comparable to or greater than that induced by auxin; (c) neutral buffers infiltrated into the apoplast can inhibit auxin-induced growth; and (d) the fungal toxin fusicoccin, whose main action is to promote extensive acidification of the apoplast, also causes rapid cell elongation. These results, obtained in many laboratories using a variety of species, provide strong support for the Acid Growth Theory.

Critics of acid growth as applied to auxin action generally do not dispute the qualitative evidence mentioned above, but they have expressed concerns about quantitative considerations. Before discussing these data, it is important to note the principal reason why quantitative information about the Acid Growth Theory has been difficult to obtain. The apoplast of stems and coleoptiles is protected by a waxy cuticle. Thus, protons excreted into the wall cannot easily escape from, and added buffers cannot readily penetrate into, the apoplast unless the cuticular barrier is breached.

There are two ways to circumvent this barrier (Fig. 1), but both create potential problems. The first is abrasion of the cuticle, which creates scattered holes in the cuticle and underlying epidermis. Unless care is taken to ensure that abrasion is extensive, only a minority of surface cells may be exposed to the outside solution. The growth response of incompletely abraded sections will be a composite of the growth of cells protected by the cuticle and those open to the solution. For example, Schopfer (17) estimated that only 30% of the surface of his *Avena* coleoptile sections had been opened to the external solution by abrasion. The most likely reason that neutral buffers, in Schopfer's hands, were unable to completely inhibit auxin-induced growth is that up to 70% of the cells were inaccessible to the outside solution.

The Acid Growth Theory predicts that cells elongating at a maximal rate in response to acidic buffers would not exhibit a further increase in rate when auxin is added. That is, if exogenous acidic buffers and auxin ultimately cause wall loosening by acidification of the apoplast, optimal concentrations of either one, applied alone or together, should produce equivalent initial growth rates. However, Schopfer reported that abraded maize (9) and *Avena* (17) coleoptile sections showed enhanced rates of elongation when auxin was added to pH 4.0 buffers. Inadequate abrasion is, again, the likely explanation for the reports. In this case, only a fraction of the cells would respond to exogenous acid, whereas those that were protected by the cuticle (and therefore having an apoplastic pH much above pH 4.0) would extend in response to auxin-mediated proton extrusion.

The second method to circumvent the cuticular barrier is to peel off the epidermal layers. This effectively removes the cuticle, but has the potential for causing damage to the underlying tissue. In addition, this may greatly alter the ionic composition of the apoplast and/or result in loss of solutes from the exposed cells. Nevertheless, long-term auxin-induced growth has been obtained with *Avena* and *Zea* coleoptiles and pea stems from which the epidermis has been completely removed (2, 16).

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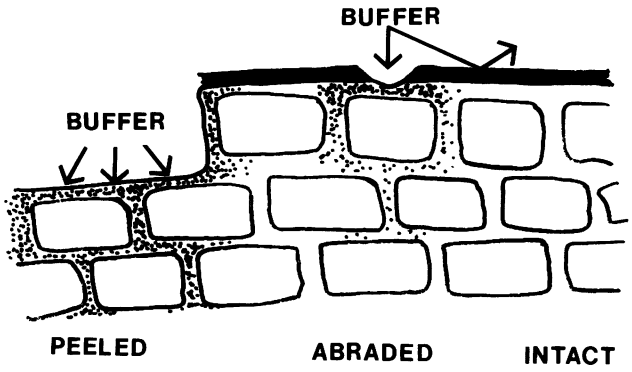

Figure 1. Schematic illustration of how abrasion of the cuticle and peeling (removal of the cuticle and underlying epidermis) impacts access of buffers to the apoplast. Abrasion creates scattered holes. If these holes occur infrequently, buffer penetration will be limited. Peeling maximizes buffer access to the cell walls. Stippled areas indicate the relative amount of buffer penetration into the apoplast. The escape of protons from the apoplast to the outside are similarly affected by the cuticular barrier.

One of the most significant objections to the Acid Growth Theory centered on the pH profile for acid-induced extension. The Acid Growth Theory predicts that solutions with a pH identical to that of the apoplast of auxin-treated tissues should cause nonauxin-treated tissues to elongate at a rate comparable to that induced by auxin. Although direct measurement of the apoplastic pH is not yet possible, the pH of solutions in contact with the apoplast indicate that in the absence of auxin the pH is 5.5 to 6.5, and pH 4.5 to 5.0 in the presence of auxin. It is generally agreed that solutions of pH 6.0 to 7.0 cause little elongation, but the response of tissues to pH 4.5 to 5.0 buffers is a matter of controversy.

Rayle reported that the initial elongation rate of peeled *Avena* coleoptile sections in pH 5.0 solutions was comparable to the rate induced by optimal auxin concentration (13). On the other hand, Kutschera and Schopfer (9) and Schopfer (17) reported that abraded sections of maize and *Avena* coleoptiles only underwent significant acid extensions at pH values lower than 4.5. They concluded that the apoplastic pH of auxin-treated coleoptiles was not low enough to explain the auxin-induced growth rate.

The issue of the pH profile for acid-induced elongation of coleoptile and epicotyl sections has recently been reinvestigated (4, 6). In our opinion, this profile is indeed consistent with the Acid Growth Theory. Reports to the contrary appear to have underestimated the actual rates of potential extension at any particular pH due to a combination of technical problems. One of these problems appears to have been inadequate abrasion of the cuticle (discussed above).

Another technical problem involved preincubation of peeled or abraded sections in distilled water. Acid-induced extension of coleoptiles persists for only 90 to 120 min; a reapplication of fresh solutions at the same pH causes no further extension, although some extension can be induced by solutions of lower pH. Kutschera and Schopfer (9) and Schopfer (17) preincubated sections for 1 or 2 h in distilled water prior to incubation in buffers. During the preincubation period, the sections underwent a burst of extension comparable to that induced by a pH 5.0 buffer. It is hardly surprising, then, that these sections failed to extend in response to a subsequent pH 5.0 buffer application, although they retained a limited ability to extend at lower pH values. On the other hand, when abraded or peeled sections are incubated in a pH 5.0 buffer without any pretreatment, or after pretreatment in a pH 6.5 buffer, the rate of extension is comparable to that induced by optimal auxin (4). We conclude that when technical problems are eliminated, the initial rate of auxin-induced wall loosening can be explained by the acid-mediated wall loosening that occurs at apoplastic pH values known to exist in auxin-treated tissues.

We do not fully understand how distilled water can cause a "pH 5.0-like" response in certain situations. It is possible that in these cases the distilled water itself is acidic due to dissolved CO_2. Another explanation, perhaps more likely, is that it lowers the pH of the Donnan free space, where the wall-loosening enzymes are presumed to reside, because of a marked reduction in available Donnan cations; the normal Donnan cations will diffuse into the distilled water and can only be replaced by H^+ (18). Consistent with this idea is the fact that pretreatment of peeled or abraded coleoptile sections in distilled water whose pH was adjusted to 6.5 with KOH or NaOH caused no "pH 5 growth burst." Such sections subsequently respond to pH 5.0 buffers in a manner similar to sections receiving no pretreatment or a pretreatment with pH 6.5 buffer solution (4).

IS THE EPIDERMIS A UNIQUE TARGET TISSUE FOR AUXIN IN ELONGATION GROWTH?

The notion that the epidermis mechanically limits growth and serves as a unique target tissue for auxin action (and presumably H^+ excretion) has become entrenched in the literature (for example, see ref. 8). This model has served to direct thinking regarding the spatial distribution of auxin-binding proteins, auxin up- and down-regulated genes, and biochemical processes. It has also been argued that much of the evidence in support of the Acid Growth Theory is irrelevant because it has been obtained with tissues from which the outer cell layers had been removed.

Recent evidence suggests that we need to reevaluate our thinking about the role of the epidermis. With *Avena* and *Zea* coleoptiles and pea epicotyl sections, it is now quite clear that the epidermis is not a unique target tissue for auxin-induced elongation because peeled sections can be made to undergo long-term auxin-induced growth comparable to that of sections with intact epidermis (2, 16). Previous failures to detect such a large growth response may be due to one of several causes: tissue damage, preincubation conditions, medium composition, or length of incubation (for a more complete discussion of these potential problems, see refs. 2, 4, 12, 16).

Additional recent evidence that the epidermis is not a unique target for auxin action has come from the demonstration by Peters *et al.* (12), which shows that both membrane hyperpolarization and H^+ excretion are induced by auxin in peeled sections. In addition, those auxin up-regulated genes and gene products that appear tightly coupled to elongation are not limited to the epidermis (5). We conclude that auxin

receptors and the signal transduction pathway for auxin action and elongation are likely to exist in all the cells of auxin-responsive stems and coleoptiles. Thus, evidence obtained from peeled sections supporting the Acid Growth Theory is valid.

PROLONGED AUXIN-INDUCED GROWTH

The fact that acid-induced elongation of stem or coleoptile tissues is short-lived as compared with the auxin growth response provides strong evidence that auxin must have an essential role in cell elongation in addition to the promotion of proton excretion. Vanderhoef and Dute (20) suggested that this second role is the promotion of long-term extension by a completely different mechanism than the acid-growth mechanism; that is, the wall-loosening reactions during prolonged growth might be different from those occurring during the early acid-mediated extension response. Alternatively, auxin might simply be regenerating the capacity of the walls to undergo acid-induced wall loosening, either by replacing lost wall-loosening enzymes or by replacing substrate.

It is not yet possible to distinguish between these two alternatives. Cleland has shown, however, that in peeled *Avena* coleoptile sections the pH optimum for auxin-induced growth during the prolonged growth phase is 5.5 to 6.0 rather than about 4.5 to 5.0, which is the optimal pH during the acid-growth phase (3). This suggests that the auxin-mediated rate-limiting step may be different during the prolonged phase relative to the initial phase of growth. For example, the new rate-limiting step might involve regeneration of the capacity for acid-induced wall loosening, with the actual loosening step still having a much more acidic pH optimum. Alternatively, the long-term pH profile could reflect a completely different wall-loosening process, which becomes established after several hours of rapid extension growth. These data caution that the Acid Growth Theory may need to be modified when applied to the prolonged phase of auxin-induced cell elongation.

ROLE OF ACID GROWTH IN OTHER PLANT ORGANS AND IN INTACT PLANTS

Most research on the Acid Growth Theory of auxin action has focused on stem and coleoptile sections, and the evidence from such research is compelling. But what about other organs and intact plants? In these areas, evidence is not as extensive or clear. There is evidence that acid extension may be involved in light-induced leaf expansion of some but not all dicotyledons (19). The correlation between H^+ excretion and the growth of roots suggests that the endogenous growth of this organ might also be acid mediated (10).

Evidence for acid-mediated growth responses during the gravity-induced curvature of intact roots and shoots has also been obtained. For example, neutral buffers prevent gravicurvature of sunflower hypocotyls and *Avena* coleoptiles, and asymmetric application of acidic solutions can cause curvature responses that closely mimic gravitropism. Asymmetric proton excretion has also been shown to occur in gravistimulated roots and stems, and this response appears to be well correlated with and predictive of asymmetric growth (11).

As suggestive as these data may be, it must be pointed out that in most cases the data are qualitative rather than quantitative. Precise pH measurements on the upper and lower surfaces of gravistimulated roots and shoots, coupled with accurate acid dose-response curves, for example, would provide strong evidence for or against the role of acid growth in intact organs responding because endogenous auxin. Such data would be especially useful because they would allow simultaneous comparisons between wall pH during the cessation of elongation (*e.g.* the upper side of a gravistimulated shoot) and the acceleration of growth (*e.g.* the lower side of the same shoot). Further, such measurements could be compared spatially and kinetically with auxin redistribution.

MECHANISM OF AUXIN-INDUCED WALL ACIDIFICATION

A variety of mechanisms could, in theory, lead to auxin-induced wall acidification. However, the accumulated indirect and/or circumstantial evidence has made it likely that auxin-induced proton excretion is mediated by the plasma membrane H^+-ATPase. This hypothesis has been greatly strengthened by an elegant paper by Hager and coworkers (6). Using an antibody against one isoform of the plasma membrane H^+-ATPase and a chemiluminescence immunoblotting system, Hager *et al.* (6) have shown that auxin treatment of *Zea* coleoptiles increases the amount of immunologically detectable H^+-ATPase in the plasma membrane by approximately 80%. This response has kinetics that closely match those for auxin-induced growth; both phenomena are detectable about 10 min after the addition of auxin and reach steady-state levels in about 30 min. The translation inhibitor cycloheximide, a rapid and potent inhibitor of auxin-induced growth, quickly diminishes the auxin-enhanced level of the H^+-ATPase (apparent half-life of about 12 min). In addition, cordycepin, a transcription inhibitor, has a similar inhibitory effect on both growth and antibody-detectable H^+-ATPase in the plasma membrane (Fig. 2). Interestingly, growth induced by fusicoccin does not increase levels of the H^+-ATPase. This indicates that the observed increase in ATPase levels is a response to auxin and not a response to growth *per se*. The molecular mechanism of the auxin-induced increase in antibody-detectable H^+-ATPase levels and the enhanced transport of secretory vesicles that accompany this phenomenon are unknown. Further, it has not yet been directly shown that increases in immunologically detectable levels of the H^+-ATPase reflect and/or are responsible for enhanced proton pumping.

Alternative ways that auxin might stimulate H^+-ATPase activity have been proposed. One method is by causing a reduction in cytoplasmic pH. Because the pH optimum for the enzyme is 6.5, whereas the cytoplasmic pH is normally 7.2 to 7.5, a reduction in cytoplasmic pH should cause a large increase in ATPase activity. Such a decline in cytoplasmic pH in response to auxin has been recorded both with pH microelectrodes and with fluorescent pH dyes and confocal microscopy. Another possibility is that auxin activates the ATPase via phosphorylation by a protein kinase. The effect of auxin would be indirect, possibly either via enhancement of the inositol trisphosphate cycle or by an increase in cyto-

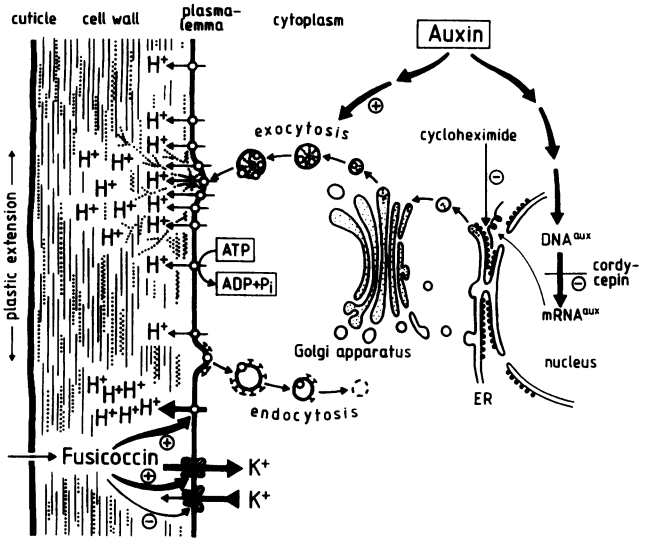

Figure 2. Schematic representation of one possible mechanism for auxin-induced cell wall acidification (Reproduced with permission from Ref. 6, Figure 11). In this model, auxin-enhanced excytotic vesicle transport and insertion of a rapidly turning-over H^+-ATPase into the plasma membrane are envisioned to stimulate hydrogen ion excretion into the apoplast and initiate wall loosening. In this model, fusicoccin stimulates protein excretion via a separate independent mechanism. DNA^{aux} and RNA^{aux} = auxin-activated specific nucleic acid sequences.

plasmic Ca^{2+}. A problem with this concept is that it has not been shown that phosphorylation of the ATPase causes activation and it has not been shown that auxin can activate protein kinases. Finally, a direct effect of auxin on the V_{max} of ATP-driven proton pumping has been reported to occur in tobacco leaf plasma membrane vesicles. As yet, this has not been demonstrated for any other tissue.

IN A NUTSHELL

The Acid Growth Theory has a long, complex, and controversial history. First proposed approximately 20 years ago, it states that auxin induces stem and coleoptile cells to excrete protons and that the resulting apoplastic acidification causes the cleavage of load-bearing cell wall bonds and, thus, cell elongation. When the theory was proposed, it was acknowledged that other auxin-dependent processes such as export of wall-loosening enzymes or wall precursors were also required for sustained growth. As time passed, substantial support for the Acid Growth Theory accumulated, but criticism also increased. These criticisms focused primarily on perceived discrepancies between the magnitude of acidification induced by auxin and growth responses generated by equivalent pH values. Concurrently, another notion developed that served to exacerbate the confusion. This concept stated that the epidermis was a unique target tissue for auxin action. Because much of the evidence supporting the Acid Growth Theory was obtained using peeled segments (*i.e.* segments lacking an epidermis), the target-tissue concept seemed to invalidate or at least seriously weaken much of the original supporting data.

These issues have recently been resolved and it is now clear that: (a) the acidification induced by auxin is sufficient to account for at least the first few hours of auxin-induced growth, and (b) the epidermis of coleoptiles and pea epicotyls is not a unique target tissue for auxin action. Reports supporting contrary views appear to have been technically flawed. Thus, the Acid Growth Theory is alive and well in 1992.

LITERATURE CITED

1. **Cleland RE** (1971) Cell wall extension. Annu Rev Plant Physiol **22:** 197–222
2. **Cleland RE** (1991) The outer epidermis of *Avena* and maize coleoptiles is not a unique target for auxin in elongation growth. Planta **186:** 75–80
3. **Cleland RE** (1992) Auxin-induced growth of *Avena* coleoptiles involves two mechanisms with different pH optima. Plant Physiol (in press)
4. **Cleland RE, Buckley G, Nowbar S, Lew N, Stinemetz C, Evans M, Rayle D** (1991) The pH profile for acid-induced elongation of coleoptile and epicotyl section is consistent with the acid-growth theory Planta **186:** 70–74
5. **Dietz A, Kutschera U, Ray PM** (1990) Auxin enhancement of mRNAs in epidermis and internal tissues of pea stem and its significance for the control of elongation. Plant Physiol **93:** 432–438
6. **Hager A, Debus G, Edel H-G, Stransky H, Serrano R** (1991) Auxin induces exocytosis and the rapid synthesis of a high turnover pool of plasma-membrane H^+-ATPase. Planta **185:** 527–537
7. **Hager A, Menzle H, Krauss A** (1971) Versuche und Hypothese zur Primärwirkung des Auxins beim Streckungswachstum. Planta **100:** 47–75
8. **Kutschera U, Bergfeld R, Schopfer P** (1987) Cooperation of epidermis and inner tissues in auxin-mediated growth of maize coleoptiles. Planta **170:** 168–180
9. **Kutschera U, Schopfer P** (1985) Evidence against the acid growth theory of auxin action. Planta **163:** 483–493
10. **Moloney MM, Elliott MC, Cleland RE** (1981) Acid growth effects in maize roots: evidence for a link between auxin-economy and proton extrusion in the control of root growth. Planta **152:** 285–291
11. **Mulkey TJ, Evans ML** (1981) Geotropism in corn roots: evidence for its mediation by differential acid efflux. Science **212:** 70–71
12. **Peters W, Richter U, Felle H** (1992) Auxin-induced H^+-pump stimulation does not depend on the presence of epidermal cells in corn coleoptiles. Planta **186:** 313–316
13. **Rayle DL** (1973) Auxin-induced hydrogen-ion excretion in *Avena* coleoptiles and its implications. Planta **114:** 63–73
14. **Rayle D, Cleland RE** (1970) Enhancement of wall loosening and elongation by acid solutions. Plant Physiol **46:** 250–253
15. **Rayle DL, Cleland RE** (1977) Control of plant cell enlargement by hydrogen ions. Curr Top Dev Biol **11:** 187–214
16. **Rayle D, Nowbar S, Cleland R** (1991) The epidermis of pea is not a unique target tissue for auxin-induced growth. Plant Physiol **97:** 449–451
17. **Schopfer P** (1989) pH-dependence of extension growth in *Avena* coleoptiles and its implications for the mechanism of auxin action. Plant Physiol **90:** 202–207
18. **Sentenac H, Grignon C** (1981) A model for predicting ionic equilibrium concentration in cell walls. Plant Physiol **68:** 415–419
19. **Taylor G, Davies WJ** (1985) The control of leaf growth of *Betula* and *Acer* by photo-environments. New Phytol **101:** 259–268
20. **Vanderhoef LN, Dute RR** (1981) Auxin-regulated wall loosening and sustained growth in elongation. Plant Physiol **67:** 146–149