Hypothesis

Mechanisms of auxin-induced plant cell elongation

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An alternative to the wall acidification theory of auxin-induced elongation growth is presented. A central effect of auxin is postulated to be cytoplasmic acidification, possibly via an increase in the levels of cytoplasmic Ca^{2+}. Activation of the outwardly directed electrogenic proton pump resulting from acidification would lead to changes in $\Delta\psi$ and transmembrane ion gradients. We argue that these changes, rather than wall acidification, are involved in elongation growth.

Plant growth regulator *Cell wall acidification* *Cytoplasmic Ca^{2+}* *Cytoplasmic pH* *Membrane potential* *Transmembrane ion gradient*

1. INTRODUCTION

Auxins such as indoleacetic acid (IAA) dramatically stimulate cell elongation in seedling stems and coleoptile tissue. It has been proposed that the primary effect of the hormone is stimulation of an outwardly directed proton pump in the plasma membrane of target cells [1,2]. The consequent acidification of the cell wall would loosen it either by direct breakage of acid-labile cell wall bonds or by activation of a wall-loosening enzyme(s) located in the cell wall. As predicted by the theory, cell wall acidification is stimulated by auxin [3–7] and growth may be stimulated in auxin-responsive tissue by acid solutions [8–10]. However, other results (see later) indicate that wall acidification is unlikely to be a factor in long-term responses to auxin, although it may contribute to the initial response [11].

We suggest that the primary effect of auxins is to lower the cytoplasmic pH. Here we examine the ways in which this may be brought about and the consequences which could lead to elongation growth. Experimental results related to these points are briefly discussed and a working hypothesis presented.

2. GENERAL CONSIDERATIONS

2.1. *Mechanisms by which auxin may induce cytoplasmic acidification*

2.1.1. Effects on cellular metabolism

Protons will be generated in the cytoplasm from several sources under aerobic conditions. These include CO_2/H_2CO_3, citric cycle acids and protein synthesis [12]. Hence, auxin could influence these proton sources by as yet unknown mechanisms.

2.1.2. Release of acids from organelles

Auxin might increase the permeability of the tonoplast or of mitochondria to acids and protons.

2.1.3. Increase in cytoplasmic-free Ca^{2+} levels

Binding of auxin to its specific receptors on the plasma membrane or ER could lead to release or uptake, respectively, of Ca^{2+} into the cytoplasm. Mitochondria would take up this Ca^{2+} and eject protons into the cytoplasm.

2.2. *Consequences of cytoplasmic acidification leading to elongation growth*

2.2.1. Stimulation of the electrogenic proton pump

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Provision of additional substrate (protons) will stimulate the outwardly directed proton pump. Possible consequences will be:

(i) An increase in H^+ concentration in the cell wall;
(ii) Hyperpolarization of the plasma membrane;
(iii) Effects on metabolism via a feedback loop.

2.2.2. Effects of protons independent of the pump stimulation

Such as changes in membranes permeability and modulation of metabolism (e.g., via enzyme activities).

3. EXPERIMENTAL

3.1. *Cytoplasmic pH and growth*

1-Naphthyl acetate, which is rapidly taken up by cells and hydrolyzed to 1-naphthol and acetic acid, can be used to generate intracellular acid and will induce growth [13]. Raising the cytoplasmic pH with weak bases (e.g., procaine) inhibits auxin-induced elongation of maize coleoptiles [14]. Using spectroscopic probes generated in situ to measure pH, we found that IAA rapidly lowers the cytoplasmic pH of coleoptile cells (paper in preparation).

3.2. *Mechanisms of cytoplasmic acidification*

Auxin receptors have been reported associated with the plasma membrane, ER and tonoplast [15]. While the plasma membrane receptors may be involved in auxin transport into the cell, auxin-binding to tonoplasts may change the permeability of vacuoles. In animal cells, increasing the cytoplasmic Ca^{2+} concentration provides a mechanism for cytoplasmic acidification since the Ca^{2+} is taken up by mitochondria and protons are released [16–19]. Cytological evidence suggests the ER may act as a calcium sequestering organelle in plants [20]. In oat coleoptiles calcium is also associated with the cell wall in close proximity to the plasma membrane [21]. It is tempting to suggest that by binding to its receptors auxin stimulates uptake of extracellular Ca^{2+} and release of Ca^{2+} from ER. Evidence for Ca^{2+} involvement is still meagre (and contradictory), although we found EGTA and compounds which block Ca^{2+} channels (Co^{2+}, La^{3+}) do inhibit auxin-induced growth [14]. Although impermeant, when EGTA is added to plant cells it leads to a redistribution of intracellular Ca^{2+} [22]. Fluphenazine only inhibits IAA-induced growth after a long lag phase [14], so calmodulin is apparently not directly involved. (Calcium ions appear to inhibit the biochemical wall-loosening process, but only at very high concentrations [23].)

3.3. *Inconsistencies of the wall acidification hypothesis*

If auxin increases H^+ concentration in the cytoplasm, the proton pump will be stimulated and cell wall pH fall in accordance with the wall-acidification hypothesis. (There is no evidence that auxin directly activates the pump.) Unfortunately, no straightforward correlation between proton efflux and growth has been found [13,58]. Although acid buffers do stimulate elongation, this is only a short-term effect. Moreover, there is evidence that IAA does not stimulate growth by the same mechanism as acid [24]. Different buffers exhibit different pH optima for growth induction [2]. We suspect they may permeate the cells and acidify the cytoplasm, the different pH optima reflecting different permeabilities. Owing to the polyanionic nature of plant walls the local pH is in any case low (pH 3–5) and is strongly dependent on organic phosphate concentration in the outer bulk phase [25–27].

When coleoptiles are incubated in alkaline buffer containing Na^+, the carboxylic ionophore monensin leads to acidification of the cell wall. Under the same conditions wall acidification by IAA is less, but IAA induces growth whereas monensin does not (paper in preparation). This suggests proton excretion must occur via the pump for growth to occur. In neutral buffer, monensin transports protons into the cytoplasm, stimulates the pump and induces growth [14].

3.4. *Changes in membrane electrical potential and ion gradients induced by activation of the proton pump*

Decreased cytoplasmic pH, by accelerating the pump and thus driving current through the membrane, would be expected to produce membrane hyperpolarization. Hyperpolarization of animal cell plasma membranes may be associated with the primary mode of action of glycoprotein hormones (e.g., thyrotropin), certain toxins and interferon

[28–32]. Effector-receptor binding must either increase the permeability of the membrane to anions or decrease its permeability to cations. Hyperpolarization [33–35], but also depolarization [36], in response to IAA has been reported. The fungal toxin fusicoccin hyperpolarizes plant cells, probably by directly activating the proton pump, and induces growth in many plant tissues [37,38].

Some processes are known to be modulated by $\Delta\psi$ where it apparently exerts an effect independent of any obvious function in which it serves as a driving force for transport or synthetic reactions (see [39]). Alterations in $\Delta\psi$ would affect bilayer fluidity [40] (and hence enzyme activity [41]), polarity and distribution of lipids and conformation and positioning of proteins in the bilayer [39]. A transmembrane $\Delta\psi$ is required for cellulose synthesis in intact *Acetobacter xylinum* cells [39] and by cotton fiber membrane vesicles [42], whereas maintenance of ΔpH is not essential. The rate of cellulose synthesis could be regulated by $\Delta\psi$, possibly by activating the enzyme complex or by its influence on the excretion of newly-synthesized microfibrils through membrane pores [39].

Changes in $\Delta\psi$ can also lead to changes in transmembrane ion gradients which are postulated to serve as an alternative to cAMP as a second messenger in bacterial, fungal and animal cells (reviewed in [43]).

Cytoplasmic pH must be controlled within narrow limits, requiring constant removal of protons. Although the electrogenic proton pump in eukaryotic non-animal cells is believed to be involved, its stimulation alone will have little net effect on cytoplasmic pH because it would simply lead to the return of an increased number of protons (see [44] for reasons). In *Neurospora* this dilemma is overcome by the induction of an increased leak to non-protons (not K^+, but possibly organic anions), allowing the pump to accomplish net H^+ ejection [44]. It would be elegant if auxins made use of such a mechanism so that by lowering the cytoplasmic pH they change the ion permeability of the plasma membrane. Such changes in transmembrane gradients would constitute the second messenger referred to above and could influence processes such as protein synthesis and gene transcription [51–54]. (Although fusicoccin causes a large, rapid stimulation of K^+ uptake in oat coleoptile cells, auxin increases K^+ uptake only slightly after a long lag (see Cleland and Lomax in [50]).)

Induction of plant growth by electric fields is a long-known phenomenon [46,47], and exposure of coleoptiles to short periods of electric current stimulates elongation at a rate equivalent to IAA [14]. Changes in ion fluxes across the cell membrane may be involved as suggested for the stimulus of bone cell growth by weak magnetic fields [48]. The rapidly generated, bidirectionally transmitted signal evoked by wounding in mature plant tissues, which induces polysome formation, probably consists of changes in membrane potential and ion fluxes [49]. Local changes in the cell wall ion concentration may also be important in growth since they can regulate the activity of wall enzymes [25–27].

The proton pump is intimately involved in a variety of metabolic processes and there is evidence of a feedback loop between metabolism and the pump in *Nitella* [55]. Similarly, inhibition of the pump by vanadate did not allow H^+ producing processes to lower the cytoplasmic pH in *Neurospora*, possibly because a signal generated by the pump turns off H^+ production at the level of oxidative phosphorylation [56]. (The signal may be related to the fact that the pump is a major consumer of ATP.) Hence, by activating the pump, auxins and fusicoccin may have yet unpredictable effects on metabolism. Alkalinisation of the cytoplasm, for example, may result in increased malic acid formation from CO_2 (Haschke and Lüttge in [50]). Activation of the proton pump may also lead to increased water uptake via electroosmosis and can increase cell turgor (see Fensom, Zimmerman and Steudle in [50]).

Finally, is pump stimulation involved in growth at all or does it simply reflect the cytoplasmic acidification? It is unclear, for example, whether the changes in *Neurospora* membrane permeability are a direct result of decreased intracellular pH or related to the increased activity of the pump [45]. Auxin-induced growth is inhibited by vanadate [57], but since the pump is integrated in metabolism this result is difficult to interpret.

4. WORKING HYPOTHESIS

The central effect of auxins in inducing extension growth in plant cells is to lower the

cytoplasmic pH. This may occur via effects on metabolism or the release of acids/protons from the vacuole and endoplasmic reticulum. However, we postulate that auxins bind to their receptors on the endoplasmic reticulum and plasma membrane and induce an increase in the cytoplasmic levels of free Ca^{2+}. This Ca^{2+} is taken up by mitochondria which secrete protons into the cytoplasm. The externally directed proton pump is then stimulated by the lowered cytoplasmic pH. Although some wall acidification will result and may be involved in the initial growth response, we propose that continuous elongation growth depends on changes in $\Delta\psi$ and transmembrane ion gradients. The former would lead to changes in membrane structure affecting such parameters as permeability and enzyme activity; the latter would act as a second messenger and influence processes such as secretion, protein synthesis and gene transcription.

Other auxin effects, for example on cell division and differentiation, may involve additional mechanisms which might also participate in long-term elongation growth. The interaction between different hormones makes such mechanisms difficult to disentangle, although regulation of cytoplasmic Ca^{2+} levels by auxin could be involved.

REFERENCES

[1] Cleland, R.E. (1971) Annu. Rev. Plant Physiol. 22, 197–222.
[2] Hager, A., Menzel, H. and Krauss, A. (1971) Planta 100, 47–75.
[3] Cleland, R.E. (1973) Proc. Natl. Acad. Sci. USA 70, 3092–3093.
[4] Rayle, D.L. (1973) Planta 114, 63–73.
[5] Marré, E., Lado, P., Rasi Caldogno, F. and Colombo, R. (1973) Plant Sci. Lett. 1, 179–184.
[6] Cleland, R.E. (1976) Plant Physiol. 58, 210–213.
[7] Jacobs, M. and Ray, P.M. (1976) Plant Physiol. 58, 203–209.
[8] Rayle, D.L. and Cleland, R.E. (1970) Plant Physiol. 46, 250–253.
[9] Jacobs, M. and Ray, P.M. (1975) Plant Physiol. 56, 373–375.
[10] Perley, J.E., Penny, D. and Penny, P. (1975) Plant Sci. Lett. 4, 133–136.
[11] Vanderhoef, L.N., Lu, T.-Y. and Williams, C.A. (1977) Plant Physiol. 59, 1004–1007.
[12] Raven, J.A. and Smith, F.A. (1976) Curr. Adv. Plant Sci. 8, 649–660.
[13] Vesper, M.J. and Evans, M.L. (1979) Proc. Natl. Acad. Sci. USA 76, 6366–6370.
[14] Brummer, B. (1982) Dissertation, Universität Zürich.
[15] Rubery, P.H. (1981) Annu. Rev. Plant Physiol. 32, 569–596.
[16] Rasmussen, H. and Goodman, D.B.P. (1977) Physiol. Rev. 57, 421–509.
[17] Scarpa, A. (1978) in: Membrane Transport in Biology, pp.263–347, Springer, Berlin, New York.
[18] Rose, B. and Rick, R. (1978) J. Membrane Biol. 44, 377–415.
[19] Arruda, J.A.L., Dytko, G., Lubansky, H., Mola, R., Kleps, R. and Burt, C.T. (1981) Biochem. Biophys. Res. Commun. 102, 891–896.
[20] Hepler, P.K. (1977) in: Mechanism and Control of Cell Division (Rost, T.L. and Gifford, E.M. eds) pp.212–232, Hutchinson and Ross, Strondsburg, Oowden.
[21] Slocum, R.D. and Roux, S.J. (1982) J. Histochem. Cytochem. 30, 617–629.
[22] Wolniak, S.M., Hepler, P.K. and Jackson, W.T. (1980) J. Cell Biol. 87, 23–32.
[23] Cleland, R.E. and Rayle, D.L. (1977) Plant Physiol. 60, 709–711.
[24] Pope, D.G. (1977) Ann. Bot. 41, 1069–1071.
[25] Noat, G., Crasnier, M. and Ricard, J. (1980) Plant, Cell and Environment 3, 225–229.
[26] Ricard, J., Noat, G., Crasnier, M. and Job, D. (1981) Biochem. J. 195, 357–367.
[27] Crasnier, M., Ricard, J. and Noat, G. (1982) FEBS Lett. 144, 309–312.
[28] Grollman, E.F., Lee, G., Ambesi-Impiombata, F.S., Meldolesi, M.F., Aloj, S.M., Coon, H.G., Kabach, H.R. and Kohn, L.D. (1977) Proc. Natl. Acad. Sci. USA 74, 2352–2356.
[29] Ramos, S., Grollman, E.F., Laxo, P.S., Dyer, S.A., Habig, W.H., Hardegree, M.C., Kaback, H.R. and Kohn, L.D. (1979) Proc. Natl. Acad. Sci. USA 76, 1099–1103.
[30] Friedmann, N. and Dambach, G. (1980) Biochim. Biophys. Acta 596, 180–185.
[31] Friedhoff, L.T., Kim, E., Priddle, M. and Sonenberg, M. (1981) Biochem. Biophys. Res. Commun. 102, 832–837.
[32] Tsuchiya, W., Okada, Y., Yano, J., Murai, A., Miyahara, T. and Tanaka, T. (1981) Exp. Cell Res. 136, 271–278.
[33] Etherton, B. (1970) Plant Physiol. 45, 527–528.
[34] Cleland, R.E., Prins, H.B.A., Harper, T.R. and Higinbotham, N. (1977) Plant Physiol. 59, 395–397.
[35] Nelles, A. (1977) Planta 137, 293–298.
[36] James, R.B., Pierce, W.S. and Higinbotham, N. (1976) in: Rouen Workshop, Proceedings of the Centre National de la Recherche Scientifique.

[37] Marré, E. (1979) Annu. Rev. Plant Physiol. 30, 273–288.
[38] Felle, H. (1982) Plant Sci. Lett. 25, 219–225.
[39] Delmer, D.P., Benziman, M. and Padan, E. (1982) Proc. Natl. Acad. Sci. USA 79, 5282–5286.
[40] Lelkes, P.I. (1979) Biochem. Biophys. Res. Commun. 90, 656–662.
[31] Kimelberg, H.K. (1977) Cell Surface Rev. 3, 205–293.
[42] Komor, E., Weber, H. and Tanner, W. (1979) Proc. Natl. Acad. Sci. USA 76, 1814–1818.
[43] Bacic, A. and Delmer, D.P. (1981) Planta 152, 346–351.
[44] Lazo, P.S., Barros, F., De La Pena, P. and Ramos, S. (1981) Trends Biochem. Sci. March, 83–86.
[45] Sanders, D., Hansen, U.-P. and Slayman, C.L. (1981) Proc. Natl. Acad. Sci. USA 78, 5903–5907.
[46] Blackman, V.H., Legg, A.T. and Gregory, F.G. (1924) Proc. Royal Soc. Lond. Ser. B Vol. XCV, 214–228.
[47] Webster, W.W. and Schrank, A.R. (1953) Arch. Biochem. Biophys. 47, 107–118.
[48] Luben, R.A., Cain, C.D., Chen, M.C.-Y., Rosen, D.M. and Adey, W.R. (1982) Proc. Natl. Acad. Sci. USA 79, 4180–4184.
[49] Davies, E. and Schuster, A. (1981) Proc. Natl. Acad. Sci. USA 78, 2422–2426.
[50] Regulation of Cell Membrane Activities in Plants (1977) (Marre, E. and Ciferni, O. eds) Elsevier, Amsterdam, New York.
[51] Zurfluh, L.L. and Guilfoyle, T.J. (1980) Proc. Natl. Acad. Sci. USA 77, 357–361.
[52] Theologis, A. and Ray, P.M. (1982) Proc. Natl. Acad. Sci. USA 79, 418–421.
[53] Zurfluh, L.L. and Guilfoyle, T.J. (1982) Planta 156, 525–527.
[54] Walker, I.C. and Key, J.L. (1982) Proc. Natl. Acad. Sci. USA 79, 7185–7189.
[55] Hansen, U.-P. (1980) in: Plant Membrane Transport: Current Conceptual Issues (Spanswick, R.M. et al. eds) pp.587–588, Elsevier, Amsterdam, New York.
[56] Sanders, D. and Slayman, C.L. (1982) J. Gen. Physiol. 80, 377–402.
[57] Jacobs, M. and Taiz, L. (1980) Proc. Natl. Acad. Sci. USA 77, 7242–7246.
[58] Dahse, I., Keller, E.R.J. and Müller, E. (1983) Plant Sci. Lett. 28, 327–335.