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The electrical response of maize to auxins

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The electrical response of *Zea mays* coleoptiles and suspension cultured cells to several growth-promoting auxins (IAA, IBA, 2,4-D, 2,4,5-T, 1-NAA) and some of their structural analogues (2,3-D, 2-NAA) has been tested. In coleoptiles two typical electrical responses to IAA are observed: an immediate rapid depolarization, and a hyperpolarization following 7–10 minutes after the first external addition of IAA. Of the other tested compounds only 1-NAA significantly depolarized the cells, whereas all auxins as well as the analogues evoked delayed hyperpolarizations. In contrast, the suspension cells were not hyperpolarized by any of the tested compounds, but were strongly depolarized by IAA, 1-NAA, and to a lesser extent by 2-NAA. In these cells IAA and 1-NAA induced inwardly directed currents of positive charge which both saturated around 12 mA/m^2. The strong pH-dependence together with the half-maximal currents 0.49 μM IAA and 0.76 μM 1-NAA point to a symport of the anions with at least $2H^+$. The delayed plasma membrane hyperpolarization is a different response, and seems to be initiated by the protonated auxin species. In accordance with the current literature, it is interpreted as consequence of a stimulated proton extrusion. The finding that all tested compounds evoked a hyperpolarization, makes this response unspecific. It is concluded that a stimulation of proton extrusion is a necessary, but not sufficient step to induce elongation growth.

Introduction

Externally added IAA induces electrical responses in grass coleoptiles [1–6]. Although some of these effects have been known for some time, their importance for elongation growth is still a matter of considerable debate. Improved techniques and new ideas of auxin action enable us to reconsider older concepts and reevaluate some of the earlier data. In this context the acid growth theory [7,8] plays an important role, although some of its basic statements have been critisized lately [9,10].

Electrophysiological tests of auxin action during the elongation growth of coleoptiles are experimentally demanding, because the expanding tissue often terminates the measurements. In many instances a reimpalement is unsuccessful or leads to less reliable results. Moreover, quantitative measurements of electrical membrane current cannot be carried out on coleoptiles, mainly because of current leakage through the network of plasmodesmata and the possibility of occasional vacuolar impalements. To circumvent such problems, for some tests we have chosen suspension cultured maize cells which, because of their spherical form, allow quantitative measurements of the auxin induced electrical membrane current.

So far, electrophysiological work on auxin effects has mainly focussed on IAA. Using other growth-promoting auxins and some of their structural analogues, in this study we try to bring the electrical responses on a more general basis. We therefore attempt to analyze the carrier-mediated auxin uptake and the auxin-induced stimulation of the plasma membrane H^+-ATPase.

Materials and Methods

Coleoptiles

Zea mays L. (Santos) seeds were soaked for 2 h in tap water. They were then placed on moist vermiculite and grown in the dark for 4–5 days at 24°C.

For electrophysiological experiments the coleoptile segments were excised and abraded with strips of emery cloth (Vitex KK14F No 392; VSM AG Hannover,

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F.R.G.). They were then fixed upright in a Plexiglas chamber which was constantly perfused with the test medium (see below) and which allowed horizontal approach of the microelectrodes from both sides [11]. For these tests standard equipment (S-7000; WPI-Instruments, New Haven, CT, U.S.A.) was used [12–14]. Micropipettes were pulled vertically on a Getra instrument (Weilheim, F.R.G.) from borosilicate glass tubing with solid fiber (Hilgenberg), and were filled with 0.5 M KCl from the rear end by capillary displacement. The incubation time prior to the first impalement was generally 2 h. The tests showed that the segments produced a stable membrane potential about 1.5 h after incubation in the standard test solution.

Cell suspensions

The cultures were grown in Erlenmeyer flasks on a shaker at 60 rpm in the dark in Murashige-Skoog medium which was supplemented with 2,4-D. The cells were harvested by transferring them into the standard test medium which comprised 0.1 mM KCl, 0.1 mM NaCl, 0.1 mM $CaCl_2$ and a mixture of 5 mM Mes + Tris, adjusted to the respective pH.

For electrophysiological tests the cells were transferred into a flow-through chamber. Single cells or small cell clusters were sucked in a glass holding pipette, and were impaled from the opposite side. Prior to the impalement the cells remained for 3 h in this medium which was constantly stirred and aerated. The electrical membrane current was measured applying the one-electrode method by balancing the series resistance (including the microelectrode) to zero using a bridge circuit. Since the method relies on the precondition that the electrode resistance should be negligable towards the total membrane resistance of the cell measured on ($R_{el}/R_m = 1/1000$), this condition has been checked carefully after the tests and the withdrawal of the electrodes. Current-voltage curves were obtained by feeding trains of rectangular current pulses into the spherical cells, and recording the pertinent changes in membrane potential. The auxin-induced current was extrapolated by graphical subtraction of the curve measured after auxin addition from the control *I-V* curve measured before the auxin treatment.

Cytosolic pH-tests were carried out with pH-selective microelectrodes, built as described by Felle and Bertl [15].

Intact vs. excised coleoptiles

In order to deplete intracellular IAA it is custom to remove the coleoptile tip several hours prior to the respective experiments. We did not compare IAA levels of intact and decapitated coleoptiles [16–19], but found that the ability of the coleoptiles to react to externally-added auxins was in no way affected by the removal of the coleoptile tip. The data to these tests are not shown.

Results

Maize coleoptile cells react to externaly added IAA with two typical electrical responses: an immediate fast depolarization, and a delayed hyperpolarization which is observed 7–10 min after the IAA addition [20].

The fast reaction

Cells of maize coleoptiles as well as of suspension cultures respond to addition of external IAA with an immediate depolarization. For maize suspension cells this is shown in Fig. 1 together with the strong concentration and pH dependence. As demonstrated in Fig. 2, this response is completely different from the reaction to weak acids. For maize coleoptiles this depolarization is restricted to IAA and 1-NAA, whereas cells of maize suspension cultures are also depolarized by 2-NAA, although less substantial (data not shown). Also, the response to IAA is faster in suspension culture cells than in coleoptiles. The reason for these differences could be due to different affinities and specificities of the auxin carriers, but very likely also arise from the better accessibility of the tested compounds to single suspension cells. For both cell types the maximal auxin induced depolarization never exeeded 30 to 35 mV.

On the single spherical suspension cells the IAA-induced inwardly directed electrical current of positive charge has been measured. Two current-voltage (*I-V*) curves are shown in Fig. 3A. The control curve was measured before addition of IAA, the second one (+IAA) was taken in the presence of IAA and in the moment of maximal membrane depolarization (E'_m). At E'_m, both curves have the same slope (= membrane conductance) which indicates that the observed depolarizations were not caused by an increase in membrane conductance. Graphical subtraction of both curves yields the IAA induced membrane current (ΔI_{IAA}). This curve (dashed) is virtually voltage independent and as such is similar to *I-V* curves obtained in earlier work for hexoses and amino acids [12]. The same procedure carried out for the other IAA concentrations each, yields the saturation curve of Fig. 3B with a maximal

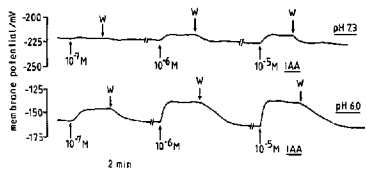

Fig. 1. Depolarization of *Zea mays* suspension cells after the addition of the indicated IAA concentrations and at different external pH. W, return to control solution.

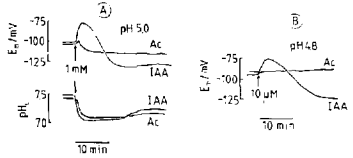

Fig. 2. Comparison of the effects of acetic acid (Ac) and IAA at the indicated concentrations and external pH on membrane potential (E_m) and on cytosolic pH (pH_c) of abraded *Zea mays* coleoptile segments.

IAA induced membrane current of 12.5 mA/m^2; the half-maximal current is induced by 0.49 µM IAA. For 1-NAA a maximal current of 12.1 mA/m^2 has been extrapolated. This points to a carrier jointly used by both auxins, however, with a lower affinity for 1-NAA, according to the half-maximal current at 0.76 µM 1-NAA.

The delayed reaction

(a) Growth promoting auxins. A remarkable response of coleoptiles to IAA is the delayed hyperpolarization [3,21–24]. It occurs usually 7–10 min after the external addition of IAA and has been interpreted as an indication for the stimulation of the plasma membrane H^+-ATPase. This idea is well supported by measurements of proton extrusion through coleoptiles [7,26], and it is assumed that this stimulation of the H^+-ATPase is of central importance for elongation growth. To our knowledge, so far only IAA but neither other growth promoting auxins, nor their growth-inactive structural analogues have been tested as to their effectivity to elicit this delayed electrical response. For this reason we tested 1-NAA, 2,4-D, 2,4,5-T and IBA, and found that all evoked responses similar to IAA (Fig. 4), i.e. a delayed hyperpolarization which usually turned into an oscillation [13,24].

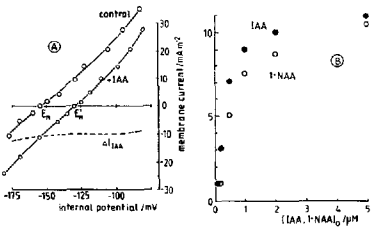

Fig. 3. Analysis of the IAA^- induced electrical current in *Zea mays* suspension cells. (A) Current-voltage curves, measured before (control) and after the addition of 10 µM IAA. The dashed curve represents the IAA-induced positively charged, inwardly directed electrical current (ΔI_{IAA}) which was obtained by graphical subtraction of the IAA-curve from the control (see text). E_m resting potential before adding the IAA, E'_m membrane potential in the presence of IAA at maximal depolarization. (B) Difference currents, as obtained by the method shown in (A), plotted against the external IAA (●) and 1-NAA (○) concentrations, as indicated. Each point represents the current measured at maximal depolarization in the presence of the respective auxin concentration.

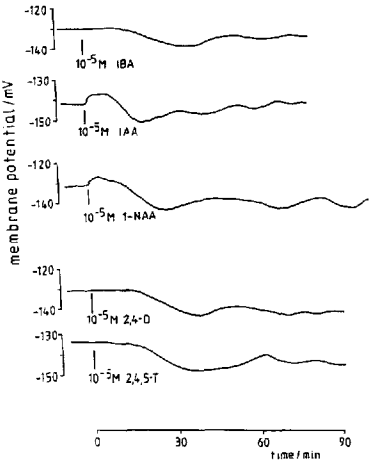

Fig. 4. Comparison of the effects of different growth promoting auxins on the membrane potential of abraded *Zea mays* coleoptile segments. IBA, indolylbutyric acid; 1-NAA, 1-naphthylacetic acid; 2,4-D, 2,4-dichlorophenoxy acid; 2,4,5-T, 2,4,5-trichlorophenoxy acid.

(b) Structural analogues. To our surprise, the analogues 2-NAA and 2,3-D of the strong auxins 1-NAA and 2,4-D, respectively, also caused clear-cut hyperpolarizations (Fig. 5) and, as shown elsewhere, also acidified the external medium [26]. However, the hyperpolarizations were even more delayed (about 12 min), and the concentrations needed to evoke quantitatively comparable effects, were higher. Consequently, when 1-NAA or 2,4-D were added after 2,3-D and 2-NAA had already hyperpolarized the cells, the effect was enhanced. In Figs. 5A–D the effects of some auxin combinations are given: 5 µM 1-NAA + 5 µM 2-NAA, added after 10 µM 2-NAA, first depolarized and then further hyperpolarized the membrane; addition of 10 µM IAA then had only a minor effect (Fig. 5A). 10 µM 2-NAA exchanged for 10 µM 1-NAA, caused an immediate hyperpolarization, showing the typical proton pump overshoot usually observed after taking the de-

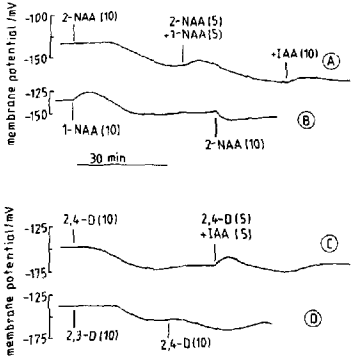

Fig. 5. Comparison of the effects of some growth promoting auxins, their inactive structural analogues (2-NAA, 2,3-D), and combinations thereof on membrane potential of abraded *Zea mays* coleoptile segments. 2-NAA, 2-naphthylacetic acid; 2,3-D, 2,3-dichlorophenoxy acid. Numbers in brackets denote concentration in μM. See text.

polarizing substrate (here 1-NAA) out (Fig. 5B). 5 μM IAA + 5 μM 2,4-D, added after 10 μM 2,4-D, depolarized the cell, but caused only weak further hyperpolarization (Fig. 5C). Finally, 2,4-D, exchanged for 2,3-D, clearly further hyperpolarized the cells (Fig. 5D).

(c) The effect of external pH: IAAH or IAA⁻? The acid growth theory emphasizes the importance of the external pH. In our experiments 10 μM IAA neither depolarized nor hyperpolarized the maize cells at pH 9. But already at pH 7.95 a 5 mV hyperpolarization was observed and at pH 6.1 the hyperpolarization was almost maximal with 28 to 30 mV. This raises the question for the effective IAA species, IAAH or IAA^-?

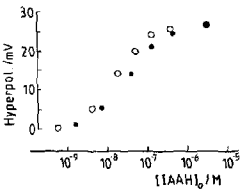

Fig. 6. Dose-response relationships of the effect of the protonated IAA (IAAH) on abraded *Zea mays* coleoptile segments, measured as the maximal hyperpolarization. (○) Data points represent measurements obtained in the presence of 10 μM IAA, but at differ.nt external pH. (●) All points obtained at constant pH 6.0 in the presence of various IAA concentrations.

Since from pH 7.95 to 6.1 the IAA^- concentration just drops from 9.99 μM to 9.5 μM, whereas the IAAH concentration increases from 7 nM to about 70-fold, the binding site evidently recognizes IAAH. This is supported by corresponding tests carried out with different IAA concentrations but at constant pH 6. The data from both approaches only differ marginally and are shown as dose-response curves in Fig. 6.

Discussion

The depolarization, a weak acid effect?

Clearly, the results given in Figs. 1 to 3 cannot be interpreted as weak acid effects on the plasma membrane [27]. In Fig. 2 the action of acetic acid and IAA on maize coleoptiles are directly compared. At an external pH 5, 1 mM acetic acid and IAA both induced similar shifts in cytosolic pH, which is evidently the result of the protonated weak acids entering the cells and releasing protons according to the higher cytosolic pH [15,28]; electrically however, IAA and acetic acid cause different responses: whereas IAA at first strongly depolarizes the cells, acetic acid causes an immediate hyperpolarization. At pH 4.8 the different mode of action of acetic acid and IAA is even more evident. Whereas 10 μM IAA immediately depolarizes the cells followed by the hyperpolarization typical for coleoptiles, the same amount of acetic acid has no measurable effect. It should be emphasized, however, that weak acids do have effects on membranes and can indeed lead to depolarizations. Extensive work on the action of fatty acids carried out by Frachisse et al. [28] have revealed that weak acids induce time-variant changes in membrane conductance, which however were detectable only at much higher concentrations than those of the auxins used here.

The carrier-mediated auxin uptake: an electrophoretic IAA / proton symport?

The data from Figs. 1 and 3 clearly point to a saturable electrophoretic auxin uptake mediated by a carrier [29–32]. In parallel, IAA pH-dependently also enters the cell in its protonated form. Applying radioisotope techniques, it has always been difficult to sharply separate this unspecific intrusion of IAAH from the carrier mediated transport. In measuring the IAA-induced depolarization and the corresponding electrical membrane current we detect the carrier-mediated IAA uptake only; the half-maximal IAA-induced current of 0.49 μM IAA (Fig. 3B) almost corresponds with the K_m of 0.3 μM given by Hertel [32] for *Cucurbita*. Three observations point to a proton/IAA^- symport: (1) IAA^- induced positive charge moves into the cell; (2) this current is saturable; (3) the electrical responses are strongly pH-dependent. But, which species of IAA (IAAH or IAA^-) is symported with how many H^+? If

we decide on an IAAH/nH$^+$ symport, basically 1 H$^+$ would be sufficient to induce a membrane depolarization and an inwardly directed positive current. In the tests carried out at pH 7.3 and 6.0 (Fig. 1), respectively, large differences of depolarizations (and membrane currents) are observed. For example, at pH 7.3, 1 μM IAA depolarizes the plasmalemma by 5 mV, but by 22 mV at pH 6. Calculating the concentrations of both species within this pH range, we find that $[IAA^-]_o$ does not change significantly, whereas $[IAAH]_o$ does so by a factor of 20; this would point to IAAH as the symported species. But at pH 7.3 only 3 nM IAA are protonated. This would not only require an unusually high affinity of the carrier for IAAH, but is also not warranted by the measured half-maximal current of 0.49 μM IAA (Fig. 3B). This means that the pH sensitivity is not due to an increase in the transported substrate, but rather to more H$^+$ at lower pH. We therefore favour IAA^- as transport substrate and suggest that the auxin influx carrier cotransports IAA^- with at least 2 H$^+$.

The specificity of the uptake carrier

Apparently, not all growth promoting auxins induce an electrical current. For both *Zea mays* coleoptiles and suspension cells we found that IAA and 1-NAA were the only compounds to induce a significant electrical current, whereas the other tested auxins, even at concentrations of 10 μM, had no effect in coleoptiles and at the most minor effects in suspension cells. This is in an interesting contrast to the results obtained by Sabater and Sabater [33], and Hertel [32] who found in binding studies in *Lupinus* and *Cucurbita*, respectively, that the uptake carrier had a much higher affinity for IAA and 2,4-D (and 2-NAA) than for 1-NAA. Rubery [34] found in crown gall cells, that beside IAA also 2,4-D had a saturable uptake component. We are not excluding the possibility that the nondepolarizing compounds are also bound by this uptake carrier, but we are certain that in maize they are not transported electrophoretically. It is conceivable, however, that the auxin uptake carriers of monocots and dicots posess different specificities.

The stimulation of the plasma membrane H$^+$-ATPase

(a) Coleoptiles. It is known for some time that externally added IAA hyperpolarizes the plasma membrane of maize coleoptiles with a lag time of 7–10 min [3,7,23,24,27]. Our observation that all the other tested growth promoting auxins similarly hyperpolarize the coleoptiles (Fig. 4) and also stimulate proton extrusion [35,36] is new and seems to confirm the idea that the plasma membrane H$^+$-ATPase is activated by auxins in general. The IAA-induced stimulation of proton extrusion has always been a pillar of the acid growth theory. Thus, we found it surprising that the growth-inactive structural analogues 2-NAA and 2,3-D also caused similar reactions of the coleoptiles, viz. a delayed hyperpolarization (Fig. 5), as well as proton extrusion [36]. Since we found in auxiliary experiments that these compounds did not stimulate the elongation growth of maize coleoptiles at concentrations 10 μM or lower (data not shown), our observations indicate that beside the auxin-induced acidification of the cell wall space other processes must take place as well to induce growth. Clearly, to evoke responses which were quantitatively comparable to the responses to IAA, higher concentrations of 2-NAA and 2,3-D had to be added which, however, were still below the level needed to cause trivial weak acid effects.

An auxin-binding protein (ABP) has been identified by Löbler and Klämbt in maize [37] which, after binding IAA, supposedly activates a signal transduction chain, finally leading to elongation growth. Our data indicate that this cascade is forked. Since both growth-active auxins and growth-inactive structural analogues seemingly stimulate the H$^+$-ATPase (hyperpolarization, proton extrusion), we must assume that they all bind first to the same structure (possibly the described ABP), a process which appears too unspecific to induce growth. On the other hand, when the H$^+$-ATPase is deactivated, no elongation growth takes place. This indicates that the auxin-induced H$^+$-ATPase stimulation and subsequent external acidification is a necessary, but not a sufficient condition to stimulate growth. We therefore suggest that the auxin-induced stimulation of the plasma membrane H$^+$-ATPase (proton extrusion) is a prerequisite for the growth induction, e.g., by stimulating transport of cell wall constituents or precursers thereof. Since many transport processes across the plasma membrane of plant cells are proton-driven, interruption of this proton turnover necessarily leads to severe inhibition of such membrane transport [38].

(b) Suspension culture cells. None of the tested auxins hyperpolarized these cells, nor was proton extrusion stimulated. The observation that FC rapidly stimulated proton extrusion (data not shown) proved that the H$^+$-ATPase was indeed active. It appears that apart from the uptake carrier there is no structure to recognize auxins in connection with a H$^+$-ATPase stimulation, as observed in coleoptiles. One explanation is that by origin the suspension culture cells are mesophyll cells and as such do not respond to IAA with elongation growth, but react otherwise to it (i.e. cell division). These cells may posess the same signal transduction chain(s) as the coleoptiles, but cannot be activated because the receptor (APB) is either missing or not expressed.

Concluding remarks

Since all tested auxins as well as their structural analogues apparently stimulate the H$^+$-ATPase in cole-

optiles, it appears that the activating binding site is rather unspecific. It may well be that we shall find many more non-growth promoting compounds with the ability to induce a delayed stimulation of the H^+-ATPase, and it should be interesting to find out why they activate only part of the signal chain but do not stimulate growth. We must assume that one or more internal binding sites [39,40] are more specific and filter out the growth promoting compounds. Also, the function of the highly specific influx carrier remains unclear yet. IAAH (and 1-NAAH) enter the cells pH-dependently due to their weak acid character, and at the physiological external pH of 4.8 to 5 could easily be accumulated by a factor of 200 to 300. Since our data are in favour of an electrophoretic IAA uptake, the membrane potential of about -90 mV (at pH 4.8, the natural pH of the apoplast; [26]) adds to the proton gradient across the plasma membrane of roughly 2.5 units (≈ -150 mV) to form an electrochemical proton gradient of -240 mV, and hence a thermodynamic driving force of -385 mV for IAA^-! It is possible that the high affinity for IAA^- together with the increased driving force give the cell a possibility to keep external IAA concentration low in order to maintain high sensitivity for IAA.

Acknowledgements

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References

1 Brauner, L. and Diemer, R. (1967) Planta 77, 1–31.
2 Etherton, B. (1970) Plant Physiol. 45, 527–528.
3 Nelles, A. (1977) Planta 137, 293–298.
4 Cleland, R.E., Prins, H.B.A., Harper, J.R. and Higinbotham, N. (1977) Plant Physiol. 59, 395–397.
5 Göring, H., Polevoy, V.V., Stahlberg, R. and Stumpe, G. (1979) Plant Cell Physiol. 20, 649–656.
6 Brummel, D.A. and Hall, J.L. (1987) Plant Cell Environ. 10, 523–543.
7 Cleland, R.E. (1980) in Plant Growth Substances 1979 (Skoog, F., ed.), pp. 71–78, Springer, Berlin.
8 Hager, A., Menzel, H. and Krauss, A. (1971) Planta 100, 47–75.
9 Kutschera, U. and Schopfer, P. (1985) Planta 163, 485–493.
10 Vesper, M.J. (1985) Planta 166, 96–104.
11 Brummer, B., Bertl, A., Potrykus, I., Felle, H. and Parish W.R. (1985) FEBS Lett. 189, 109–114.
12 Felle, H. (1983) Biochim. Biophys. Acta 730, 342–350.
13 Felle, H. (1988) Planta 174, 495–499.
14 Felle, H. (1988) Planta 176, 248–255.
15 Felle, H. and Bertl, A. (1986) J. Exp. Bot. 37, 1416–1428.
16 Sheldrake, A.R. (1973) New Phytol. 72, 433–447.
17 Parsons, A., Firn, R.D. and Digby, J. (1988) J. Exp. Bot. 39, 1331–1341.
18 Parsons, A., Firn, R.D. and Digby, J. (1988) J. Exp. Bot. 39 1343–1354.
19 Weiler, E.W., Jourdan, P.S. and Conrad, W. (1988) Planta 72, 433–447.
20 Evans, M.L. (1974) Annu. Rev. Plant Physiol. 25, 195–233.
21 Brummer, B., Potrykus, I. and Parish, R.W. (1984) Planta 162, 345–352.
22 Göring, H. and Bleiss, W. (1982) in Plasmalemma and Tonoplast: Their Function in the Plant Cell (Marmé, D., et al., eds.) pp. 263–269, Elsevier, Amsterdam, New York.
23 Brummer, B., Felle, H. and Parish, R.W. (1984) FEBS Lett. 174, 223–227.
24 Felle, H., Brummer, B., Bertl, A. and Parish, R.W. (1986) Proc. Natl. Acad. Sci. U.S.A. 83, 8992–8995.
25 Marrè, E., Lado, P., Ferroni, A. and Ballarin-Denti, A. (1974) Plant Sci. Lett. 2, 257–265.
26 Peters, W. and Felle, H. (1991) J. Plant Physiol., in press.
27 Bates, G.W. and Goldsmith, M.H.M. (198)) Planta 159, 231–237.
28 Frachisse, J.M., Johannes, E. and Felle, H. (1988) Biochim. Biophys. Acta 938, 199–210.
29 Hertel, R. (1983) J. Physiol. 112, 53–67.
30 Sabater, M. and Rubery, P.H. (1987a) Planta 171, 501–506.
31 Sabater, M. and Rubery, P.H. (1987b) Planta 171, 507–513.
32 Hertel, R. (1987) in Plant Hormone Receptors (Klämbt, D., ed.), pp. 81–92, Springer, Berlin.
33 Sabater, M. and Sabater, F. (1986) Planta 167, 76–80.
34 Rubery, P.H. (1977) Planta 135, 275–283.
35 Peters, W. and Felle, H. (1991) J. Plant Physiol., in press.
36 Felle, H., and Peters, W. (1989) in Plant Membrane Transport (Dainty, J., DeMichelis, M.I., Marrè, E. and Rasi-Caldogno, F., eds.), pp. 361–366, Elsevier, Amsterdam.
37 Löbler, M. and Klämbt, D. (1985) J. Biol. Chem. 260, 9854–9859.
38 Felle, H. and Johannes, E. (1990) J. Exp. Bot. 41, 587–592.
39 Dohrmann, U., Hertel, R. and Kowalik, H. (1978) Planta 14, 97–106.
40 Jacobs, M. and Hertel, R. (1978) Auxin binding to subcellular fractions from *Cucurbita* hypocotyls: in vitro evidence for an auxin transport carrier.