Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 891–895, February 1989
Botany

Functional evidence for an auxin receptor at the plasmalemma of tobacco mesophyll protoplasts

(electrical potential difference/evacuolated protoplasts/ATPase)

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Communicated by Jozef Schell, October 19, 1988

ABSTRACT **Tobacco mesophyll protoplasts were previously shown to respond to naphthaleneacetic acid by modifying their transmembrane potential difference. In the present work, evacuolated protoplasts were used to show that this response resides only at the plasmalemma. This electrical response was investigated by using polyclonal antibodies directed against plasma membrane antigens presumably involved in the reception and transduction of the auxin signal. An IgG fraction from an antiserum directed against the membrane auxin-binding protein from maize coleoptile completely inhibited the naphthaleneacetic acid-induced response of tobacco protoplasts. The suppression of the auxin-induced variation in the transmembrane potential difference by an IgG preparation directed against the plasmalemma ATPase from yeast demonstrated the involvement of the ATPase in the electrical response. Variation induced by fusicoccin in the transmembrane potential difference of tobacco protoplasts was unaffected by the anti-auxin-binding protein IgG fraction but was completely suppressed by the anti-ATPase IgG preparation. These results demonstrate the presence of a membrane receptor for auxin at the plasmalemma, the binding of the hormone to this receptor leading to the activation of the proton-pumping ATPase. They also show that at least the primary steps of activation by naphthaleneacetic acid are distinct from those of the fusicoccin-induced response.**

Many studies have described auxin-binding sites in membrane preparations from various tissues and species (for a review, see ref. 1). Membranes from maize coleoptiles have been studied most extensively and several laboratories have characterized an auxin-binding protein (ABP) (2–6). According to its binding properties, this site was referred to as the site I described by Dohrmann *et al.* (7) and was probably located on the endoplasmic reticulum. However, Löbler and Klämbt (8), using a preparation of monospecific IgG directed against the ABP, showed by indirect immunofluorescence that ABP was found primarily at the plasmalemma of epidermal cells. The best evidence that maize ABP functions as a receptor is that anti-ABP IgG blocked the auxin-induced growth response of maize coleoptiles (8). However, Shimomura *et al.* (4) prepared an antibody directed apparently against the same auxin-binding entity that did not affect coleoptile elongation. As discussed by Guern (9), these contradictory data show that auxin receptors are far from being rigorously identified, notably due to the absence of identifiable primary functional responses induced by the binding of the hormone to its receptor.

The effects of auxins on the transmembrane potential difference (Em) of various plant cells—including, for example, pea internode segments (10) and maize (11) and oat (12) coleoptiles—could constitute such a functional response. Such effects on mesophyll protoplasts isolated from tobacco (*Nicotiana tabacum*, cv. Xanthi) have been described (13). On these protoplasts, the Em modifications induced by naphthaleneacetic acid (NAA) varied with the auxin concentration in the external medium as shown by a nonmonotonous dose–response curve. This NAA-induced response at the membrane level (Em variation) was shown to closely correlate with the biological effect of auxins on protoplast-derived cells (stimulation of cell division and cytotoxicity) (13, 14). Furthermore, the rapidity of the membrane response suggests that it resides probably in the early steps of the transduction pathway of the hormonal signal, close to the reception events.

The origin of the Em variation induced by auxin is yet to be elucidated. It has been widely suggested that the observed hyperpolarization of organs involves the stimulation of the H^+-pumping ATPase located at the plasmalemma. However, as stressed by Libbenga *et al.* (1), this has been based only on indirect correlations, because of the absence of direct evidence that auxin activates the proton pump by binding to a membrane receptor at the plasmalemma. Isolated protoplasts, which offer direct access to the plasmalemma, allow us to study membrane proteins by using antibodies directed against antigens on the outer surface of the membrane. The auxin-induced electrical response of tobacco protoplasts was used here as a functional test to investigate the effects of antibodies directed against two membrane proteins (the auxin-binding protein and the H^+-pumping ATPase) located at the plasmalemma and possibly involved in the reception and transduction steps of the auxin signal.

MATERIALS AND METHODS

Protoplast Isolation. Mesophyll protoplasts were isolated from tobacco plants (*Nicotiana tabacum*, cv. Xanthi) as described by Caboche (15). The protoplasts were suspended as a stock solution ($3–6 \times 10^6$ protoplasts per ml) in culture medium T_0 (see ref. 15) without NAA and used to measure the Em.

Evacuolation of Mesophyll Protoplasts. The procedure of Griesbach and Sink (16) was used with slight modifications. Washed protoplasts were resuspended in a buffer solution containing 0.5 M sorbitol, 5 mM Mes·imidazol (pH 5.7), 1 mM $CaCl_2$, 0.1% bovine serum albumin, and 0.05% polyvinylpyrrolidone. One milliliter of the suspension ($1–2 \times 10^6$ protoplasts) was then layered on top of a self-forming Percoll gradient containing 0.5 M mannitol, 0.1 M $CaCl_2$, and 20 mM Hepes·imidazol (pH 7.0). The gradient was centrifuged for 45 min at 26,000 rpm (Beckman SW 41 rotor). The green band

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Abbreviations: NAA, naphthaleneacetic acid; FC, fusicoccin; Em, transmembrane potential difference; ABP, auxin-binding protein.
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corresponding to the evacuolated protoplasts was collected from the gradient. This fraction was then washed three times by centrifugation (100 × g, 7 min) in the saline buffer solution used for protoplast washing, resuspended in the culture medium T_0 without NAA, and used for Em measurements.

Antibodies. To obtain antibodies directed against the auxin receptor, the membrane-associated ABP from maize (*Zea mays*) was prepared by the method of Shimomura *et al.* (4) with some simplifications. Maize coleoptiles (300 g) including the primary leaves were homogenized in a solution of 50 mM Tris·citric acid (pH 8.0), 0.25 M sucrose, 1 mM EDTA, and 0.1 mM $MgCl_2$ and filtered through four layers of gauze. The combined filtrates were centrifuged at 5000 × g for 10 min. After adding $CaCl_2$ to a final concentration of 10 mM and stirring for 10 min, the mixture was centrifuged at 10,000 × g for 30 min to obtain a Ca^{2+}-promoted sedimentation of membrane vesicles (17). The vesicles were resuspended in 2.5 mM sodium phosphate, pH 8.0/0.5 mM EDTA (⅓ vol of the homogenized fresh weight), and an equal volume of 1-butanol was added. The mixture was shaken a few times, kept on ice for 20 min, and then centrifuged at 2000 × g for 20 min. The lower phase was withdrawn and dialyzed overnight against TM buffer (10 mM Tris·HCl, pH 7.4/5 mM $MgCl_2$). The dialysate was applied to a DEAE-cellulose column (30 ml). After washing with TM buffer (150 ml), the ABP-containing fraction was eluted with 0.3 M NaCl/TM buffer. This eluate was applied directly to an NAA-linked AH-Sepharose 4B column (50 ml). After washing of the column with 0.5 M NaCl/TM buffer overnight, the column was eluted with 10 mM NAA/0.4 M NaCl/TM buffer. The resulting eluate of 10–12 ml was desalted, freed from NAA, and used as antigen for immunization of rabbits. After repeated booster injections, rabbits were bled and IgG was prepared from antisera by $(NH_4)_2SO_4$ precipitation and DEAE-cellulose column chromatography. Most of the experiments presented in this paper were performed with this total IgG preparation (anti-ABP IgG). To test the specificity of the effects observed using this crude fraction, an aliquot of the polyclonal IgG was further purified by affinity chromatography on an ABP-Sepharose column as described by Löbler and Klämbt (8).

Rabbit antisera raised against the purified native ATPase from *Schizosaccharomyces pombe* were obtained as described by Clément *et al.* (18). This anti-ATPase IgG was previously shown (18) to inhibit the plasma membrane ATPase from another yeast, *Saccharomyces cerevisiae*, and from maize shoots.

The gamma-globulin fractions directed against the maize ABP or the yeast ATPase were obtained from an $(NH_4)_2SO_4$ precipitate. They were dialyzed overnight in phosphate-buffered solution (10 mM sodium phosphate, pH 7.4/0.15 M NaCl). The final protein concentrations were 5.8 mg/ml and 2.4 mg/ml, respectively, for the anti-ABP IgG and the anti-ATPase IgG.

Rabbit IgG from pooled normal sera (Zymed Laboratories) was used as control nonimmune IgG.

Em Measurements. The Em was measured by the microelectrode technique. Under the microscope, vacuolated and evacuolated protoplasts were individually immobilized in a microholder as described by Rona *et al.* (19), and impaled with a glass micropipette filled with 1 M KCl, connected to an Ag/AgCl electrode. The Em was measured between the microelectrode inserted into the protoplast and a reference Ag/AgCl electrode connected to the bathing medium through a 1% agar/1 M KCl bridge.

For Em measurements, aliquots were taken from the stock protoplast suspensions stored at 4°C and diluted to 10^4 protoplasts per ml just before use. For each experiment, a series of measurements was first obtained in T_0 medium depleted of NAA to obtain a mean reference Em. Another series was then taken on another aliquot of the suspension immediately after NAA was added to the external medium. The auxin-induced Em variation was calculated from mean Em values obtained for each experimental condition from 15 to 50 individual measurements. To test the effect of an IgG fraction on the Em variation, protoplasts were preincubated with the IgG solution for 2.5 hr at room temperature. Measurements were then carried out in the absence or presence of NAA. A similar procedure was used to test the effect of fusicoccin (FC) on Em and the effect of IgG fractions on the FC-induced Em variation. Results were always consistently reproduced in two or three experiments. Data are given either as averages of the various replicated measurements or reported from one representative experiment.

RESULTS

Effects of Auxin and FC on the Em of Vacuolated and Evacuolated Protoplasts from Tobacco Leaves. The protoplasts isolated from tobacco mesophyll cells have been shown to respond to the addition of exogenous auxin analogs by a modification of their Em (13). A concentration of 5 μM NAA was chosen because it induced the maximal hyperpolarization of protoplasts from tobacco with a wild-type genotype (13). In the present study for protoplasts suspended in the culture medium, the Em was made more negative (Em variation of −7 mV) by the addition of 5 μM NAA (Table 1). In the same series of experiments, FC induced hyperpolarization in tobacco protoplasts; the amplitude was dependent upon the FC concentration in the external medium (data not shown). The maximal Em variation reproducibly occurred at 1 μM FC, although its amplitude varied from one protoplast suspension to another (from −4.5 mV to −7.5 mV); maximal hyperpolarization was constant up to 10 μM FC, the highest FC concentration tested.

As emphasized (13), the NAA-induced changes in the overall Em of protoplasts could reflect plasmalemma as well as tonoplast Em variations. To determine which membrane was involved in the observed variations, the effect of NAA on evacuolated protoplasts was investigated. Evacuolated tobacco mesophyll protoplasts appeared as small spherical units, with chloroplasts densely packed together (20), and a mean diameter (17 μm) that was ≈60% of the initial protoplast diameter. The potential difference measured on evacuolated protoplasts appeared slightly more negative than that of the protoplasts from which they originated (Table 1).

Table 1. Effects of NAA and FC on the Em of vacuolated and evacuolated tobacco protoplasts

Protoplast material	Addition to medium	Em, mV	Em variation, mV
Vacuolated	None	−5.5 ± 0.2	—
	NAA (5 μM)	−12.6 ± 0.3	−7.1
	None	−5.6 ± 0.3	—
	FC (1 μM)	−10.7 ± 1.0	−5.1
Evacuolated	None	−11.4 ± 0.6	—
	NAA (5 μM)	−17.9 ± 0.6	−6.5
	FC (1 μM)	−16.1 ± 0.5	−4.7

Em measurements on vacuolated and evacuolated protoplast suspensions were done in the complete culture medium in the absence (control) or presence of NAA or FC. For the vacuolated protoplasts, Em values are expressed as mean ± SEM calculated from independent experiments on different protoplast suspensions over a 6-month period (n = 10 for NAA experiments and n = 5 for FC experiments). In each of these independent experiments, 20 individual protoplast measurements were taken and averaged in each experimental condition. For the evacuolated protoplasts, data are given from one representative experiment where 20 individual measurements were made for each experimental condition.

However, the variations induced by NAA as well as by FC were exactly the same on vacuolated and evacuolated protoplasts (Table 1), indicating that the plasmalemma alone was involved in the auxin- or FC-induced responses.

Effect of Anti-ABP IgG on the Em Variations Induced by NAA and FC. In a preliminary experiment, the effect of anti-ABP IgG on the NAA-induced Em variation was tested by measuring the Em variation in the presence of various concentrations of anti-ABP IgG. As shown in Table 2, the Em shift induced by NAA was progressively reversed by increasing the anti-ABP IgG concentration in the medium. For the highest concentration tested (0.4 μM anti-ABP IgG), the NAA-induced Em variation was almost nullified. Thus 0.4 μm anti-ABP IgG was used in further investigations of the effect of anti-ABP IgG, either alone or with NAA, on the Em.

Anti-ABP IgG alone induced no significant modification of the Em of tobacco protoplasts (Table 2). The anti-ABP IgG inhibition of the NAA-induced Em variation is illustrated in Fig. 1, where results from three protoplast suspensions isolated from different plants are reported. The distributions of Em values in the control medium in the absence or presence of NAA appeared to be homogeneous, whereas values obtained in the presence of anti-ABP IgG contained a marked subpopulation, ≈10% of values being more negative than −10 mV. A similar subpopulation (5–10%) was sometimes observed in the presence of the IgG alone (data not shown). This phenomenon, which suggests that the IgG fraction itself affects the Em, was not further investigated as it does not impede the detection of IgG effects on the NAA-induced response.

The specificity of the anti-ABP IgG inhibition of the NAA-induced response was tested by using a total IgG preparation purified from normal rabbit serum. These IgG preparations were tested at two concentrations covering the concentration range where anti-ABP IgG was effective in modifying the Em variation (Table 3): they modified significantly neither the reference Em nor the hyperpolarization induced by auxin. The inhibition previously observed with the anti-ABP IgG was thus specific to the serum from the rabbit immunized with the ABP-enriched fraction. However, anti-ABP IgG could modify the NAA-induced membrane response through mechanisms affecting general membrane functions rather than specifically involving the auxin receptor. The effect of anti-ABP IgG was thus tested on the FC-induced Em variation. The results shown in Table 3 indicate that anti-ABP IgG used at the concentration that

Table 2. Effect of anti-ABP IgG on the Em of tobacco protoplasts and on the Em variation induced by NAA

Addition to medium	Em, mV	NAA-induced Em variation, mV
None	−5.3 ± 0.3	—
NAA (5 μM)	−12.7 ± 0.3	−7.4
Anti-ABP IgG (4 nM) + NAA (5 μM)	−11.2 ± 0.4	−5.9
Anti-ABP IgG (40 nM) + NAA (5 μM)	−8.6 ± 0.4	−3.3
Anti-ABP IgG (0.4 μM) + NAA (5 μM)	−6.0 ± 0.4	−0.7
None	−5.6 ± 0.2	—
Anti-ABP IgG (0.4 μM)	−5.8 ± 0.2	—

Em measurements were done in the complete culture medium in the absence of auxin (control) or in the presence of 5 μM NAA. Aliquots from the same protoplast suspension were preincubated with various concentrations of anti-ABP IgG for 2.5 hr at room temperature and NAA was added to 5 μM. In an independent experiment, the effect of anti-ABP IgG at 0.4 μM alone was tested on Em. Incubation conditions were as described above. Em values are presented as mean ± SEM calculated for 17–40 individual measurements in each condition.

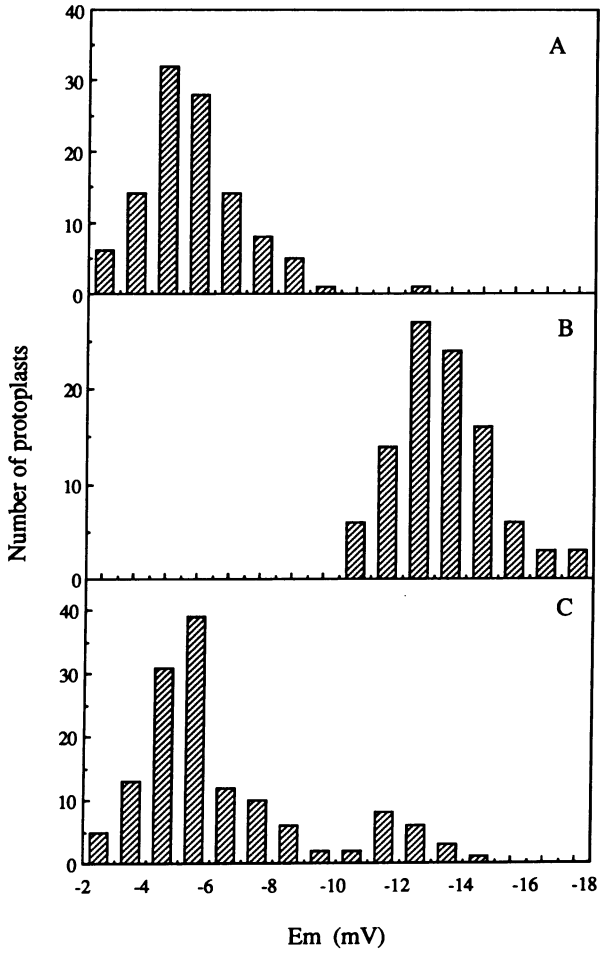

FIG. 1. Effect of anti-ABP IgG on the NAA-induced Em variation of tobacco protoplasts. Em distribution represents the data from three experiments. Protoplasts were in the complete culture medium in the absence (*A*) (control) or in the presence (*B*) of 5 μM NAA. The Em variation induced by auxin calculated from the mean Em values (−5.3 ± 0.2 mV, n = 84, and −13.5 ± 0.2 mV, n = 86, for *A* and *B*, respectively) was −8.2 mV. The protoplasts were then incubated with 4×10^{-7} M anti-ABP IgG for 2.5 hr at room temperature before the addition of NAA to 5 μM (*C*). In this last condition, the Em variation calculated from the mean Em value in *C* (−6.5 ± 0.3 mV, n = 138) and the reference value in *A* was −1.2 mV.

almost totally suppressed the NAA-induced Em variation had no effect on the membrane response induced by FC. Furthermore, Table 3 shows that the monospecific affinity-purified anti-ABP IgG completely inhibited the NAA-induced Em variation when applied at a concentration 100 times lower than the concentration of the crude anti-ABP IgG fraction required to produce the same inhibitory effect (see Table 2).

Effects of the Anti-ATPase IgG on the Em Variations Induced by Auxin and FC. As shown in Table 4, the IgG directed against yeast ATPase, when added to the tobacco protoplast suspension, reduced the Em. Furthermore, anti-ATPase IgG completely inhibited the response induced by NAA as well as that induced by FC (Table 4).

DISCUSSION

Em of Isolated Protoplasts and its Modifications Induced by Auxin and FC. The results presented here show that tobacco protoplasts respond to FC by a membrane hyperpolarization whose amplitude is similar to that observed for the hyperpolarization induced by auxin in the micromolar concentra-

Table 3. Specificity of the effect of anti-ABP IgG on the variation of Em induced by NAA on tobacco protoplasts

Addition to medium	Em, mV	NAA- or FC-induced Em variation, mV
None	−5.6 ± 0.3	—
NAA (5 μM)	−11.4 ± 0.3	−5.8
Nonimmune IgG (0.7 μM)	−5.6 ± 0.2	—
Nonimmune IgG (0.7 μM) + NAA (5 μM)	−11.0 ± 0.2	−5.4
None	−5.6 ± 0.2	—
FC (1 μM)	−10.2 ± 0.3	−4.6
Anti-ABP IgG (0.4 μM) + FC (1 μM)	−10.3 ± 0.3	−4.7
None	−5.5 ± 0.2	—
NAA (5 μM)	−11.2 ± 0.2	−5.7
Monospecific anti-ABP IgG (4 nM)	−5.4 ± 0.2	—
Monospecific anti-ABP IgG (4 nM) + NAA (5 μM)	−5.9 ± 0.2	−0.5

Em measurements were done in a complete culture medium in the absence of auxin (control). This medium was then supplemented with NAA or/and IgG purified from a nonimmune rabbit. Protoplasts were preincubated with IgG for 2.5 hr at room temperature before the addition of NAA. The same procedure was used to test the effect of the IgG fraction containing anti-ABP IgG on the response induced by FC and the effect of the monospecific anti-ABP IgG on the NAA-induced response. Em values are presented as mean ± SEM calculated from 15 to 40 individual measurements for each condition. Each experiment was repeated two (monospecific anti-ABP IgG) or three (nonimmune IgG and anti-ABP IgG) times; data presented are from a representative experiment.

tion range (Table 1). The removal of the vacuole from these protoplasts slightly modified the absolute value of the Em but did not affect the response induced by either NAA or FC (Table 1). This constitutes direct evidence that the observed responses reside at the plasmalemma alone.

The involvement of the plasma membrane ATPase in the Em of isolated tobacco protoplasts was revealed by using the yeast anti-ATPase IgG that decreased the Em (Table 4). These results indicate the existence of a strong cross-immunoreactivity between yeast and tobacco ATPases and correlate well with those of Clément *et al.* (18). The electrogenic component of Em revealed by the blockage of the ATPase appeared to have a small amplitude, which could be accounted for by the absence of a complete inhibition of the

Table 4. Effect of anti-ATPase IgG on the variation of potential difference induced on tobacco protoplasts by auxin or FC

Addition to medium	Em, mV	NAA- or FC-induced Em variation, mV
None	−6.1 ± 0.3	—
Anti-ATPase IgG	+0.8 ± 0.6	—
NAA (5 μM)	−12.5 ± 0.4	−6.4
Anti-ATPase IgG + NAA (5 μM)	−0.2 ± 0.5	−1.0
FC (1 μM)	−10.8 ± 0.4	−4.7
Anti-ATPase IgG + FC (1 μM)	+0.9 ± 0.6	+0.1

Em measurements were done in the complete culture medium in the absence (control) or presence of the effectors (NAA and FC). The medium was then supplemented with the anti-ATPase IgG (0.4 μM) and protoplasts were preincubated for 2.5 hr at room temperature before measurements. Anti-ATPase IgG was tested either alone or in combination with NAA or FC. Em values are presented as mean ± SEM calculated from 20 individual measurements for each experimental condition (data from one representative experiment is shown).

ATPase, as observed by Clément *et al.* (18) on maize. However, the Em measured with microelectrodes on vacuolated and evacuolated protoplasts, as well as the amplitude of the auxin- or FC-induced Em variations, were very low compared to values from intact cells. The hypothesis of a strong increase in the relative permeability of the plasmalemma to anions on isolated protoplasts would be compatible with the results obtained here and with the decrease in plasma membrane resistance observed by Cornel *et al.* (21).

The description of a stretch-activated anion channel in the plasma membrane of protoplasts from stem-derived tobacco cell suspensions (22) could provide insights into this problem. The characteristics of this channel, activation by suction, selectivity for anions over cations (P_{Cl}/P_K = 9.5), large conductance (100 pS), and long open time, suggest that it could mediate osmoregulation. Channels exhibiting similar properties have been described also on the plasma membrane of *Escherichia coli* spheroplasts (23). Preliminary results, obtained by the patch-clamp technique, indicate that pressure-induced channels, the selectivity of which has still to be determined, are present also on the plasmalemma of tobacco protoplasts (R. Hedrich and H.B.-B., unpublished results). It is tempting to suggest that such anion channels would be opened on protoplasts during Em measurements with microelectrodes, their opening being possibly induced by the slight negative pressure applied inside the microholder to maintain the protoplasts during impalement. This hypothesis could account for a depolarized state of protoplast plasmalemma as well as for the correlated decreases in the membrane resistance and in the electrogenic contribution of the ATPase.

Numerous data in the literature correlate the hyperpolarizing effects of auxins and FC on the Em to the stimulation of the plasma membrane H^+-ATPase and to the corresponding enhancement of H^+ excretion (1, 24). The reconstitution experiments of Thompson *et al.* (25) indicated more directly that the ATPase is involved in the auxin-induced Em change. On tobacco protoplasts (Table 4), both NAA- and FC-induced membrane hyperpolarization were suppressed by anti-ATPase IgG. This provides direct evidence for the involvement of the plasma membrane ATPase in the observed responses and indicates that ATPase activation is a common step in the transduction events induced by both effectors.

A Membrane Receptor for Auxins at the Plasmalemma. In tobacco protoplasts the electrical response induced by NAA and other auxin analogs was shown (13, 14) to closely correlate with the biological effects of NAA and auxins—stimulation of the proliferation of protoplast-derived cells and induction of cytotoxicity. In the present study, antibodies directed against a putative auxin receptor from maize were shown to inhibit the auxin-induced Em variation of tobacco protoplasts (Fig. 1). These results provide further evidence that ABP functions as a receptor and demonstrate a strong cross-immunoreactivity between maize and tobacco ABP. Furthermore, they show that two responses induced by auxins, cell elongation in maize coleoptile and cell proliferation of tobacco protoplast-derived cells, are apparently mediated by the same receptor protein.

The receptor in the protoplasts is probably located in the plasma membrane, because the IgG cannot diffuse into the cell and thus acts at the outer face of the plasma membrane. However, IgG could be internalized by endocytosis, without any specific plasmalemma binding step, and subsequently reach an intracellular target. Low temperature is the most effective inhibitor of endocytosis (26). The fact that anti-ABP IgG exhibited the same blocking effect on the NAA-induced response when the 2.5-hr incubation was at room temperature (19°C) or at 0°C (data not shown) argued against such internalization of IgG and confirmed the localization of the receptor at the external surface of plasmalemma.

As the IgG was obtained by immunization with partially purified ABP fraction, some of the IgGs used were directed against non-ABP proteins. However, the results obtained with the monospecific affinity-purified IgG preparation favor the idea that the effects observed in the present study with the crude total IgG fraction are due to IgG directed specifically against the auxin receptor at the plasmalemma. The specificity of anti-ABP IgG was reinforced by the absence of any inhibitory effect on the Em variation induced by FC (Table 3). This correlates well with the fact that the auxin receptor and the FC receptor, although both located at the plasmalemma, appear to be different proteins (see ref. 1).

In other respects, our results show that the reception of the auxin signal and the expression of its effect involve two proteins of the same membrane. This raises the question of the location of transduction steps between the binding of NAA to its receptor and the activation of the ATPase, either totally within the plasmalemma [as suggested by Thompson *et al.* (25)] or in the cytoplasm, at least partially [as proposed by Felle *et al.* (27)].

CONCLUSION

The present work, combining the use of a functional test for auxin activity and antibodies directed specifically against plasmalemma antigens, confirms the presence of an auxin receptor at the plasmalemma and brings direct evidence for the involvement of the ATPase in the auxin-induced membrane response. Measuring the auxin-induced Em variation of isolated protoplasts presently appears to be the best way to study the short-term functional activity of plasma membrane receptors. Furthermore, the dose–response curves of auxin-induced Em modifications have revealed that this electrical response can be used to characterize the sensitivity of plant cells to auxins. This has been shown by the comparison between the wild-type tobacco genotype and the NAA-tolerant mutant (28, 29), which is ≈10-fold more resistant to exogenous auxins, and by *Lotus corniculatus* roots transformed with *Agrobacterium rhizogenes* that were shown to be more sensitive to auxins than normal roots by a factor of 500–1000 (30), as revealed by auxin-induced responses, such as root elongation, H^+ excretion from root tips, or Em variation on root protoplasts. As noted by Guern (9), these phenomena of resistance and hypersensitivity to auxins, characterized by shifts of the dose–response curve, could result from modifications in the number of active receptors as well as in the efficiency of the transduction pathway.

The results obtained with the anti-ABP IgG fraction show that it is possible to titrate the electrical response with increasing concentrations of IgG (Table 2) and to estimate the number of active receptors on the outer surface of the plasmalemma.

We are grateful to Drs. A. Goffeau and S. C. Brown for critical reading of the manuscript.

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