Alterations of Auxin Perception in rolB-Transformed Tobacco Protoplasts

Time Course of rolB mRNA Expression and Increase in Auxin Sensitivity Reveal Multiple Control by Auxin

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Expression and physiological effects of the root-inducing rolB gene of Agrobacterium rhizogenes T-DNA were studied simultaneously in tobacco (Nicotiana tabacum) mesophyll protoplasts. The kinetic study of the expression of rolB mRNA following exogenous auxin application showed that auxin transiently stimulated rolB expression, with mRNA levels starting to accumulate 6 to 9 h after auxin was supplied and increasing 300-fold after 12 to 18 h. The parallel study of the auxin sensitivity of rolB-transformed protoplasts, as assayed by their electrical response to the hormone, showed that the auxin treatment generated an increase in sensitivity by a factor of up to 100,000, whereas in untransformed protoplasts the same auxin treatment induced an increase in auxin sensitivity that never exceeded 30- to 50-fold. This reflects a strong cooperative effect of auxin and rolB in transformed protoplasts. Surprisingly, the maximal increase in sensitivity was observed several hours before the maximal accumulation of rolB mRNA, suggesting that the dramatic control of auxin sensitivity by auxin in rolB-transformed protoplasts requires only low levels of rolB expression. Antibodies directed against ZmER-abpl, the major auxin-binding protein from maize, differentially altered the auxin sensitivity of the electrical response of rolB-transformed and normal protoplasts. This suggests that alterations of the auxin reception-transduction pathway at the plasma membrane of rolB-transformed protoplasts may account for their increased auxin sensitivity.

Auxin has been implicated in the control of growth and developmental processes at the cellular, tissue, and plant levels (reviewed by Davies, 1987). The molecular mechanisms by which this phytohormone affects so many different responses are not yet known. In the last few years a large number of auxin-binding proteins have been reported, with much discussion as to their possible role as auxin receptors (Venis and Napier, 1992). One of these, maize ZmER-abp1, has been characterized in detail and has been used to explore the perception of auxins at the plasma membrane of different plant cells. Several lines of electrophysiological evidence have shown, for example, that antibodies directed against ZmER-abp1 alter the auxin response of the plasma membrane of tobacco (Nicotiana tabacum L.) mesophyll protoplasts (Barbier-Brygoo et al., 1989, 1991) and of maize coleoptile protoplasts (Rick et al., 1993), and specific fragments of this protein modify the electrical properties of the plasma membrane of Vicia faba guard cells (Thiel et al., 1993).

Further insight into the mechanisms of auxin perception at the plasma membrane can be provided by the characterization of plant systems altered in their responses to auxin. Protoplasts from tobacco plants transformed by Agrobacterium rhizogenes T-DNA, especially by the single rolB gene, exhibit an increased sensitivity of their electrical response to auxin (Maurel et al., 1991b). This finding is in agreement with a number of reports in which an increased sensitivity to auxin was described for excised organs, isolated protoplasts, or membrane vesicles from various plant materials containing the whole pRi T-DNA or some of its subfragments (Shen et al., 1988, 1990; Spano et al., 1988; Barbier-Brygoo et al., 1991; Vansuyt et al., 1992). These findings suggested that morphogenetic effects of pRi T-DNA transformation might involve changes in hormone responsiveness and/or hormone content. Biochemical characterization of the RolB protein allowed Estruch et al. (1991) to show that RolB displays a β-glucosidase activity. However, recent experimental evidence from the same group (Spano et al., 1993) and others (Nilsson et al., 1993; Delbarre et al., 1994) invalidate the early proposal that this activity is involved in the direct release of free auxin from β-glucoside conjugates. Thus, the function of the RolB protein in transformed cells remains unknown.

At present, the increased auxin sensitivity of the membrane response of transformed tobacco protoplasts remains the only identified cellular effect of the rolB gene. As such, it deserves

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Abbreviations: $E_{m}$, transmembrane electrical potential difference; GUS, β-glucuronidase; NAA, 1-naphthaleneacetic acid; T-DNA, transferred DNA.
to be analyzed in more detail to obtain additional information on rolB action and to allow additional use of rolB-transformed plants in auxin perception studies. In the present work, taking into account the auxin dependence of rolB promoter expression (Maurel et al., 1990; Capone et al., 1991), we investigated the questions of how fast rolB mRNA expression was stimulated in rolB-transformed protoplasts by the application of exogenous auxin, and how this stimulation was associated with alterations in the sensitivity of their membrane response to auxin. We also explored changes at the plasma membrane of rolB-transformed protoplasts that could be associated with the changes in their sensitivity to auxins. For this we used IgGs directed against the maize protein ZmER-abp1 as a probe for immunologically related proteins at the surface of tobacco protoplasts.

MATERIALS AND METHODS

Bacteria and Plasmid Manipulations

Bacterial strains and recombinant DNA techniques were as previously described (Maurel et al., 1990). For construction of pCMB-B:GUS, pBSE15, a BlueScript M13+ vector (Stratagene) carrying the EcoRI 15 fragment of pRI1855 was used to isolate a 1377-bp SalI-BamHI fragment containing the first 4 bp of the rolB coding sequence and 5' flanking, noncoding sequences. This fragment was cloned in the corresponding unique restriction sites of pBl101 (Jefferson et al., 1987). A plant transformation vector carrying the GUS coding sequence with the nopaline synthase polyadenylation elements, giving rise to pBGUS10. A 2593-bp Hpal-Smal fragment encompassing the rolB gene with 1177 bp of the 5' and 646 bp of the 3' noncoding sequences was also isolated from pBSE15 and cloned in the Smal site of Bluescript M13+. The resulting plasmid was linearized by digestion with Nael and cloned in pBGUS10 at the unique Hpal restriction site located 1177 bp upstream of the rolB start codon, thus giving rise to a construct carrying a rolB gene and a rolB:GUS gene fusion in tandem.

Plant Transformation and Growth

DNA constructs were introduced into Nicotiana tabacum cv Xanthi via Agrobacterium tumefaciens transformation in leaf disc inoculations according to Horsch et al. (1985). The transgenic plants selected for further study were grown in a greenhouse (22°C; 9 h of light per d) and corresponded to either in vitro-propagated primary transformants or to their progeny obtained after selfing.

Southern Blot Analysis

Plant DNA was purified from leaves as described (Chilton et al., 1982), digested with the restriction enzymes indicated, subjected to electrophoresis on a 1% agarose gel (10 µg of DNA per lane), and blotted on a GeneScreenPlus membrane (New England Nuclear). DNA blotting, hybridization to DNA probes, and membrane washings were performed according to the manufacturer's manual. DNA fragments of PCR amplification were subjected to electrophoresis on a 2.5% NuSieve agarose gel (FMC, Rockland, ME) and blotted on Hybond N+ membrane (Amersham) in alkaline conditions. 32P-labeled probes (1 × 106 to 2 × 108 dpm/µg DNA) were obtained by random priming using a labeling kit (Amersham or Boehringer Mannheim).

Mesophyll Protoplast Isolation and Culture

Leaf tissues were digested over a 13- to 15-h period in To medium (Caboche, 1980) with 0.1% cellulase R10 (Yakult), 0.02% macerozyme R10 (Yakult), 0.05% driselase (Sigma), 5 × 10⁻⁶ M benzylaminopurine, and no auxin. After digestion, protoplasts were washed twice at 4°C in 0.3 M KCl, 5 mM CaCl₂, 1 mM Mes, pH 5.7. Protoplasts were then resuspended in To medium without auxin, at a density of 5 × 10⁶ protoplasts/mL, and stored at 4°C until use within an 8-h period. In all kinetic experiments, isolated protoplasts were cultured at 21°C for the indicated time in To medium (5 × 10⁶ protoplasts/mL) in the absence or the presence of 10⁻⁵ M NAA; protoplasts were washed twice in the absence of auxin prior to electrophysiological measurements. During the culture period used in these experiments (up to 24 h), rolB-transformed and untransformed protoplasts behaved similarly, and auxin-treated protoplasts did not differ from control protoplasts. As revealed by calcofluor staining, all preparations underwent wall regeneration, starting a few hours after isolation. When protoplast culture was pursued over a few days, cell division started between the 2nd and 3rd d, but only when auxin was present in the culture medium.

For immunological investigations, the protoplasts were prepared with NAA present during digestion.

mRNA Isolation and Amplification of rolB cDNA

Total RNA was prepared from about 1.5 × 10⁷ protoplasts as described by Han et al. (1987). Poly(A)⁺ RNA was selected by chromatography on oligo(dT)-cellulose (Pharmacia) according to the standard method (Aviv and Leder, 1972).

For cDNA first-strand synthesis, 1 µg of poly(A)⁺ RNA was used with 0.5 µg of oligo(dT)₁₂₋₁₈ as a primer and Moloney murine leukemia virus reverse transcriptase (Gibco BRL). After an incubation period of 90 min at 42°C, one-third of the reaction volume was used to carry out the amplification procedure. A pair of 24-mer primers corresponding to rolB sequences 5'-TGGCGCAACAGTACCAGC-CAATAC-3' and 5'-GTCGCCGCAAGCTAACATACATAG-3' was used for PCR amplification of a 255-bp fragment from rolB cDNA. After a first cycle with a heating denaturation of 2 min, a PCR of 35 cycles (each with 95°C, 50 s; 53°C, 1 min; 72°C, 1 min) was performed in 50 µL of the reaction mixture containing 200 µM each of 2'-deoxynucleoside 5'-triphosphates, 2 µM each of the primers, and 1 unit of Taq polymerase (Promega) in the corresponding buffer.

Electrical Response of Mesophyll Protoplasts to Auxin

The Eₘ of protoplasts was measured under the microscope by the microelectrode technique as described previously (Ephritikhine et al., 1987; Barbier-Brygoo et al., 1991). For each NAA concentration tested, 15 to 20 individual measurements were performed at room temperature on an aliquot of
the protoplast stock solution diluted to $5 \times 10^4$ protoplasts/mL in To medium with the appropriate NAA dose, and a mean $E_m$ value was calculated. $\Delta E_m$ represents $E_m$ variations from the mean $E_m$ value measured in the absence of auxin. For each tobacco clone and for each protocol of protoplast preparation, the dose-response curve to auxin of the electrical response of protoplasts was established in at least two independent experiments.

### Inactivation of the Electrical Response to Auxin by Anti-ZmER-abpl Antibodies

The polyclonal IgG raised against ZmER-abpl, an auxin-binding protein from maize coleoptiles (Hesse et al., 1989) expressed in *Escherichia coli*, was affinity purified with homogenous ZmER-abpl coupled to a BrCN-Sepharose column (K. Palme, unpublished data) and were kindly provided by Dr. K. Palme (Köln, Germany). The cross-reactivity to tobacco proteins of this anti-ZmER-abpl antibody was assayed on leaf microsomal proteins that were partially purified, subjected to SDS-PAGE, and immunoblotted as described by Venis et al. (1992).

Protoplasts were incubated in To medium at a density of $10^6$ protoplasts/mL for 5 min at 4°C in the presence or the absence of the indicated IgG dose. Auxin was added and $E_m$ measurements were performed over the next 20 min as described above. The effects of one IgG concentration on the auxin response of a protoplast preparation were studied as follows: two $E_m$ dose-response curves to auxin were simultaneously determined in the presence and in the absence of IgG. For the control IgG, we used rabbit IgG from pooled sera of unimmunized animals (Zymed Laboratories, San Francisco, CA).

### RESULTS

#### Introduction into Tobacco of B-B:CUS, a Construction with Both the rolB Gene and a rolB:GUS Fusion

We constructed pCMB-B:GUS, a bacterial plasmid suitable for gene transfer to plants via *A. tumefaciens* and carrying a tandem gene construct named B-B:GUS. Figure 1 shows that the B-B:GUS construction carries a chimeric gene with a rolB promoter fragment up to $-1177$ bp, the coding sequence of the GUS reporter gene (Jefferson et al., 1987), and the nopaline synthase terminator. The construct also carries a functional rolB gene with identical upstream regulatory elements and its own polyadenylation sequences.

B-B:GUS was introduced into tobacco by *A. tumefaciens*-mediated transformation of leaf discs. Forty to 50% of the kanamycin-resistant plants displayed GUS activity in histochemical assays. Some of them displayed slight developmental alterations, including reduced size, lanceolate leaves, and early flowering. A primary transformant (clone BBGUS6) was chosen for further characterization. T-DNA structures were investigated by Southern analysis using the rolB promoter as a probe. For three restriction enzymes tested (EcoRI, HindIII, BamHI), bands were detected at the expected positions for internal T-DNA fragments, showing that at least some of the T-DNA inserts were colinear with the construction (data not shown).

#### Time Course of Auxin Effects on rolB Gene Expression in rolB-Transformed Protoplasts

The effects of exogenous auxin on rolB promoter expression were first investigated by monitoring GUS enzymatic activity on protoplasts isolated from BBGUS6 plants prepared in the absence of auxin and then cultured in the absence or in the presence of a range of auxin concentrations. Auxin stimulated GUS expression in a dose-dependent manner, with a maximal stimulation at $10^{-5}$ M NAA (data not shown).

In additional experiments, BBGUS6 protoplasts were thus cultured in the absence or presence of $10^{-5}$ M NAA, and poly(A)$^+$ RNA was purified after various times of culture. A PCR procedure, via cDNA synthesis, was performed to allow the amplification of a 255-bp fragment from rolB sequences present in protoplast mRNAs. This fragment was detected by hybridization of PCR products with a radiolabeled rolB probe. The autoradiograms in Figure 2, A and B, show that rolB mRNA was barely detectable in freshly isolated protoplasts. In protoplasts cultured in the absence of auxin (Fig. 2A), the RNAs accumulated as early as 15 min after the beginning of the protoplast culture, reached a peak accumulation after 1 h, and then decreased to the initial level. The quantification of hybridization signals in three independent experiments confirmed that rolB mRNA levels were transiently increased by 50- to 100-fold after 0.5 to 2 h of culture (Fig. 2C). In protoplasts cultured in the presence of auxin, a similar transient accumulation of rolB mRNA occurred in the early hours of the culture, followed by a decrease of rolB mRNA to the initial level after 6 h of culture (Fig. 2B). However, a second increase in rolB mRNA levels was triggered specifically by the presence of auxin. rolB mRNA levels were maximal after 12 to 18 h of culture, with an accumulation of 300- to 500-fold over the initial level (Fig. 2D).

#### Time Course of Auxin Effects on Auxin Sensitivity of rolB-Transformed Protoplasts

The auxin-induced hyperpolarization was used to characterize the auxin sensitivity of BBGUS6 protoplasts under experimental conditions corresponding to those used for the mRNA expression studies.

We characterized the auxin-induced $E_m$ variations of BBGUS6 protoplasts cultured in the absence or in the pres-
Incubation time (hours) 0 0.25 0.5 1 2 3 6 9 12 18 25

5 10 15 20

Figure 2. Time course of rolB mRNA levels in tobacco mesophyll protoplasts cultured in the presence or in the absence of 10^{-5} M NAA. A and B, PCR products obtained after amplification of rolB cDNA from BBGUS6 protoplasts cultured for the indicated times in the absence (A) or in the presence (B) of 10^{-5} M NAA were hybridized with a radiolabeled full-length rolB probe. The Southern blot is from a representative experiment. No hybridization signal was detected when the same procedure was performed using untransformed protoplasts. C and D, Quantitative analysis of rolB expression. Slot-blot hybridization signals corresponding to serial dilutions of PCR products from three independent kinetic experiments were quantified by a Bio Image Analyzer System (Millipore). The intensity of each hybridization spot is represented in arbitrary units for the various incubation times in the absence (C) or in the presence (D) of auxin. For each independent experiment, the highest value for protoplasts cultured in the absence or in the presence of auxin was taken as the internal reference of 300 arbitrary units.

ence of auxin (10^{-5} M NAA) over time periods up to 12 h. In each case, a dose-response curve to auxin was established and auxin sensitivity was deduced from the auxin concentration inducing the maximal hyperpolarization. Examples of dose-response curves corresponding to different time periods of culture in the absence or presence of auxin are shown in Figure 3. BBGUS6 protoplasts cultured in the absence of auxin exhibited a fairly constant auxin sensitivity over the time of culture, with maximal hyperpolarization induced by 10^{-6} to 10^{-7} M NAA, close to the sensitivity observed on freshly isolated protoplasts (Fig. 3C). In the presence of auxin during protoplast culture, the maximal hyperpolarization was successively obtained for 3 \times 10^{-9} M (Fig. 3A), 3 \times 10^{-10} M (Fig. 3B), and 10^{-11} M NAA (Fig. 3C) after 1, 2, and 5 h of auxin treatment, respectively; beyond this period, the auxin sensitivity of the response remained stable (Fig. 3D).

Data from these experiments and others are collected in Figure 4, which shows the auxin sensitivity of the membrane response as a function of the incubation time of protoplasts in the absence or presence of auxin. In contrast to what was observed in the absence of auxin, the auxin sensitivity of BBGUS6 protoplasts was raised sharply over the first hours of culture in the presence of auxin. The maximal sensitivity level was reached after 5 to 6 h of auxin treatment and corresponded to a 10,000-fold increase over the initial level. The same increase in sensitivity was observed when BBGUS6 protoplasts were prepared by overnight digestion of leaf tissues in the presence of 10^{-5} M NAA (data not shown).

We also performed similar experiments using untransformed protoplasts. Freshly isolated protoplasts or protoplasts cultured in the absence of auxin exhibited a maximal hyperpolarization at a concentration of 10^{-4} M NAA. After 1 h of incubation in the presence of auxin in the culture medium,
the maximal hyperpolarization of untransformed protoplasts was observed at $3 \times 10^{-6}$ M NAA, which corresponded to an increase in auxin sensitivity by a factor of 10- to 30-fold (data not shown). For longer incubation times, no further change in the sensitivity of untransformed protoplasts to auxin was observed. These results show that auxin was able to induce a large amplitude shift in auxin sensitivity only in transformed protoplasts.

### Responsiveness of rolB-Transformed Protoplasts to IgGs Directed against ZmER-abp1

IgGs raised against the maize protein ZmER-abp1 produced in E. coli were assayed for their ability to recognize abp1 homologs in tobacco leaves. In proteins partially purified from microsomal fractions, anti-ZmER-abp1 IgGs specifically detected an abp1 homolog of 22 kD (Fig. 5A). The effects of these IgGs on the dose-response curve of NAA-induced $E_m$ variations in untransformed and rolB-expressing BBGUS6 protoplasts were evaluated on freshly isolated protoplasts prepared by digestion of leaves in the presence of $2 \times 10^{-6}$ M NAA. Figure 5B shows the auxin sensitivity of the response as a function of an anti-ZmER-abp1 IgG concentration. In all the experiments, anti-ZmER-abp1 IgG from unimmunized animals used at the highest concentration tested for anti-ZmER-abp1 IgG ($10^{-7}$ M) had no effect on the electrical response of either type of protoplast to auxin.

### DISCUSSION

The rolB gene from Agrobacterium rhizogenes has been recognized for almost 10 years to play a pivotal role in the development of the hairy root disease (White et al., 1985). However, thus far no one has been able to detect the presence of the RolB protein in transformed plants, although anti-RolB antibodies have been described (Trovato et al., 1990). The only report on the presence of the rolB mRNA in plants has shown that when rolB expression was directed by its own promoter, massive amounts of mRNA were needed, in the most favorable case of tobacco stem tissues, to detect hybridization signals in northern blots (Schmülling et al., 1988). The present work provides data concerning the expression of a full rolB gene in tobacco mesophyll protoplasts. This expression could be detected with the use of a high-sensitivity detection method via PCR amplification. We characterized here the levels of rolB mRNA in freshly isolated rolB-transformed protoplasts and their change upon protoplast culture in the absence or in the presence of auxin. The transient increase in rolB mRNA levels during the early hours of the protoplast culture was independent of the presence of auxin and may result from either protoplast preparation or handling prior to culture, suggesting that stimuli other than exogenous auxin are able to induce the expression of rolB.

On the other hand, auxin elicited a specific and massive increase in auxin sensitivity of untransformed protoplasts cultured in the presence (○) or in the absence (□) of $10^{-5}$ M NAA. Protoplasts were isolated by overnight digestion of BBGUS6 leaf tissues in the absence of auxin and subsequently cultured in To medium in the presence or absence of $10^{-5}$ M NAA for the indicated times. Auxin sensitivity was studied using the electrical response of protoplasts to the hormone and estimated by the NAA concentration inducing the maximal hyperpolarization ($-\log$ optimal [NAA] in molar concentration). The gray area corresponds to the maximal range of sensitivities observed in freshly isolated protoplasts.

![Figure 4](image-url) Evolution with time of the auxin sensitivity of BBGUS6 protoplasts cultured in the presence (○) or in the absence (□) of $10^{-5}$ M NAA. Protoplasts were isolated by overnight digestion of BBGUS6 leaf tissues in the absence of auxin and subsequently cultured in To medium in the presence or absence of $10^{-5}$ M NAA for the indicated times. Auxin sensitivity was studied using the electrical response of protoplasts to the hormone and estimated by the NAA concentration inducing the maximal hyperpolarization ($-\log$ optimal [NAA] in molar concentration). The gray area corresponds to the maximal range of sensitivities observed in freshly isolated protoplasts.

![Figure 5](image-url) Activity of anti-ZmER-abp1 IgG in tobacco. A, Blot of partially purified proteins from leaf microsomal fraction (wild-type genotype) probed with anti-ZmER-abp1 antibodies, showing a tobacco abp1 homolog of 22 kD. B, Effects of anti-ZmER-abp1 IgG on the electrical response of rolB (○) and BBGUS6 (□) protoplasts to auxin. Protoplasts were prepared by overnight digestion of leaf tissues in the presence of $2 \times 10^{-6}$ M NAA. Aliquots of the protoplast stock suspension were either pretreated or not pretreated with various concentrations of anti-ZmER-abp1 IgG for 5 min at 0°C, and dose-response curves of $E_m$ to auxin were established at room temperature as described in "Materials and Methods" and in the legend to Figure 2. For each curve, sensitivity was estimated as the NAA concentration inducing the maximal hyperpolarization ($-\log$ optimal [NAA] in molar concentration) and plotted as a function of IgG concentration. In all the experiments, anti-ZmER-abp1 IgG had no significant effect on the electrical response of either type of protoplast to auxin.
rolB mRNA accumulation starting 6 to 9 h after auxin was supplied to the protoplasts. The time course of this effect indicates that rolB is not an early auxin-regulated gene, and is consistent with the time course of GUS accumulation in rolB;GUS protoplasts that could be detected after 6 h of auxin treatment (Maurel et al., 1990). However, rolB mRNA levels declined after 18 h of culture in the presence of auxin (Fig. 2, B and D), whereas GUS activity was stable up to 24 h (Maurel et al., 1990). This may reflect regulation processes of rolB mRNA synthesis and/or stability that could not be detected using the stable GUS reporter enzyme.

The parallel study of the auxin sensitivity of rolB-transformed protoplasts, as assayed by their electrical response to the hormone, showed first that the auxin treatment generates an increase in auxin sensitivity of large amplitude long before the massive auxin-induced increase in rolB mRNA levels (Figs. 2 and 4). Our results indicate that in protoplasts there is no simple relationship between the levels of rolB mRNA expression and auxin sensitivity because similar levels of rolB expression can be associated with different levels of auxin sensitivity. In the absence of auxin, the transient 50- to 100-fold increase in rolB mRNA levels induced no significant change in the sensitivity of the membrane response, whereas in the presence of auxin a similar mRNA increase was associated with a rise in sensitivity by a factor of 1,000 to 10,000 within 3 h (Fig. 4). These results reflect the critical role of auxin in controlling the auxin sensitivity of transformed protoplasts. In wild-type protoplasts, auxin treatment also induced an increase in auxin sensitivity. However, this increase never exceeded 30- to 50-fold. From these control experiments we conclude that auxin and rolB strongly cooperate in increasing the hormonal sensitivity of transformed protoplasts.

In the present work, we used tobacco protoplasts to demonstrate a multiple control by auxin of rolB gene expression on one hand and auxin sensitivity on the other hand. Such control may underlie more integrated processes such as auxin-controlled rhizogenesis in various transformed materials (Cardarelli et al., 1987; Capone et al., 1989; Maurel et al., 1991a). In transformed protoplasts, the levels of rolB mRNA seem to be nonlimiting for enhancement of auxin effects. In transformed plant tissues, prolonged induction of rolB by auxin may be necessary to mediate morphogenetic effects of rolB, possibly involving cellular effects other than the modulation of auxin sensitivity.

Three independent laboratories have now shown that the RoB protein is not acting through a release of auxin from auxin Glc esters (Nilsson et al., 1993; Spena et al., 1993; Delbarre et al., 1994). Interestingly, Delbarre et al. (1994), working on the same biological material that was used in this study, namely transformed protoplasts from the BBGUS6 clone, have shown that normal and transformed protoplasts do not differ in their ability to accumulate and metabolize exogenous auxins. Under the conditions used to measure auxin sensitivity, no difference could be observed either in the intracellular or in the extracellular auxin concentrations between the two types of protoplast. These data support the idea that the increased auxin sensitivity of rolB-transformed protoplasts could follow from alterations in the receptor-transduction of the auxin signal.

Here we have shown that protoplasts expressing the increased sensitivity to auxin quantitatively differ from normal protoplasts in their responsiveness to IgGs raised to ZmER-abp1, a maize auxin-binding protein. We demonstrated previously that these IgGs are able to block the electrical response of tobacco protoplasts, indicating that proteins involved in auxin recognition (tARPs) and sharing common epitopes with ZmER-abp1 are present at the surface of the protoplasts. We have also shown that about 100- to 1000-fold more IgGs were needed to reduce by 30-fold the sensitivity of rolB-transformed protoplasts compared to normal ones. This large difference in the immunoreactivity of the surface of rolB protoplasts could indicate that the perception of the auxin signal or its transduction has been altered (for instance, the number of tARPs may be increased or their efficiency modified), with such changes somehow participating in the shift in auxin sensitivity. The fact that rolB protoplasts show the same increase in sensitivity to the agonist antibody D16 directed against a peptide from ZmER-abp1 and to auxin (Venis et al., 1992) further supports the idea that these protoplasts are altered in the perception step of the signal at the outer face of the plasma membrane. The mechanisms by which the RoB protein could alter or interact with the auxin perception chain at the plasma membrane are still entirely unknown. Nevertheless, the present work demonstrates that tobacco protoplasts transformed by rolB provide a relevant and unique cellular system in which to study the mechanisms of auxin perception.

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LITERATURE CITED

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Han JH, Estruch JJ, Schell J, Spena A (1991a) The protein encoded by the rolB plant oncogene hydrolyses indole glucosides. EMBO J 10: 3125-3128


