

Auxin levels and auxin binding protein availability in *rolB* transformed *Beta vulgaris* cells

T. XING, D.-Y. ZHANG*, J.F. HALL** and E. BLUMWALD

Department of Botany, University of Toronto,

25 Willcocks Street, Toronto, Ontario M5S 3B2, Canada

Shanghai Institute of Plant Physiology, 300 Fonglin Rd., Shanghai, China 200032*

Department of Biological Sciences, De Montfort University,

Scraptoft, Leicester LE7 9SU, UK**

Abstract

The final biological effect of auxin depends both on free auxin levels and on auxin perception capacity. *RolB* transformed *Beta vulgaris* L. hairy roots provide a system for studying both factors. Highly purified plasma membrane fractions were prepared with aqueous two-phase partitioning. Individual hairy root clones were assessed for the binding activities of plasma membrane-bound auxin binding proteins and for their free intracellular indole-3-acetic acid levels. The presence of a high affinity auxin binding protein with a dissociation constant of 9.07×10^{-7} M was detected in the plasma membrane fractions isolated from non-transformed seedling roots and the six clones of *rolB* transformed hairy roots. However, the levels of specific IAA binding considerably varied among different hairy root clones and between transformed and non-transformed roots. The levels of the detectable polypeptide in immunoblotting with an antibody against maize 22-kD auxin binding protein subunit were in good agreement to the levels that were detected in auxin binding assays. Differences in the indole-3-acetic acid levels were found between transformed and non-transformed roots and also between different transformed hairy root clones. A negative correlation was observed between free intracellular IAA levels and its specific binding to the plasma membrane-bound auxin binding proteins. A latency study indicated that the binding site for auxin may be located on the exterior face of the plasma membrane.

Additional key words: hairy roots, indole-3-acetic acid, red beet.

Received 28 June 1995, accepted 18 December 1995.

Abbreviations: ABP - auxin binding protein; GS-SIM-MS - gas chromatography-selected ion monitoring-mass spectroscopy; IAA - indole-3-acetic acid; PM - plasma membrane.

Acknowledgments: We would like to thank Dr. R. M. Napier (Horticulture Research International, East Malling, Kent, UK) for the generous gifts of antibodies.

Fax: (416) 978-5878, E-mail: txing@botany.utoronto.ca (T. Xing - corresponding author)

Introduction

Auxins are a major class of plant hormones and influence a wide range of developmental processes in plants, including cell division, elongation, differentiation, root development, apical dominance and tropisms (Davies 1991, Palme *et al.* 1991). It has been long suspected that in any of the various cellular responses to auxin, the first step must be the recognition of the hormone by molecules that may be considered operationally as receptors coupling auxin to an appropriate cellular response. It is now evident that auxin levels are not the sole determinant for its biological effect. Both auxin and specific auxin binding proteins (receptors) are crucially involved in the determination of the final effect of auxins on plant growth and development (Hagen 1989, Napier and Venis 1991, Jones and Prasad 1992).

The best studied auxin binding protein is that identified in maize (Palme *et al.* 1991, Jones and Prasad 1992). This protein is a dimer having two approx. 22-kD subunits and shows some of the properties of the expected auxin receptor (Löbner and Klämbt 1985a,b, Shimomura *et al.* 1986, Napier *et al.* 1988). This receptor (ABP1, auxin binding protein 1) was shown to be located in the ER lumen or on the ER membrane (Shimomura *et al.* 1988, Jones *et al.* 1989) and contained a KDEL sequence (Tillman *et al.* 1989, Inohara *et al.* 1989), an ER lumen retention signal (Munro and Pelham 1987). Jones and Herman (1993) found that ABP1 on ER could be transported to PM/cell wall via the secretory system without losing KDEL.

Auxin binding proteins on PM have been more intensively investigated in connection with the primary sites of auxin action. A system was developed, relying on the use of antibodies against ABP and the fact that hyperpolarization of isolated protoplasts is an auxin-specific response (Ephritikhine *et al.* 1987, Shen *et al.* 1988). Monoclonal antibodies to maize ABP blocked this response in tobacco protoplasts and an antiserum to a PM H⁺-ATPase blocked auxin-induced hyperpolarization (Barbier-Brygoo *et al.* 1989). This work suggests that some ABP is present on the outside of the PM, and they are tightly coupled to the activity of the H⁺-ATPase and are essential for this auxin-induced response. Protoplasts from plants transformed with *Agrobacterium rhizogenes* were over 100-fold more sensitive with respect to auxin-induced hyperpolarization than normal protoplasts (Shen *et al.* 1988).

rolB transformed tobacco plants have been used in several studies, trying to elucidate the mechanism of the action of the *rolB* protein. This gene from *Agrobacterium rhizogenes* has been shown to efficiently promote root formation in different host plants (White *et al.* 1985, Cardarelli *et al.* 1987, Spena *et al.* 1987). Phenotypic changes in *rolB* transformed plants were suggested to result from an enhanced level of free intracellular auxin due to a *rolB* catalyzed hydrolysis of auxin conjugates in some studies (Estruch *et al.* 1991), while in others (Nilsson *et al.* 1993, Schmulling *et al.* 1988), *rolB* transformation did not produce a phenotype that resembled that of IAA overproducing plants, nor did it alter the level of free IAA, nor had a significant effect on the rate of IAA metabolism or on the overall rate of IAA biosynthesis. With these inconsistent observations, tobacco protoplasts isolated from plants transformed with *rolB* gene were used to study the possible alteration of auxin sensitivity (Delbarre *et al.* 1994). This work showed that protoplasts expressing *rolB*

had an increased sensitivity to the auxin-induced hyperpolarization of the PM and strongly suggested that the *rolB*-dependent behaviour of transgenic tobacco protoplasts is not a consequence of modifying the intracellular auxin pool but a consequence of changes in the auxin perception pathway. In *rolB* transformed tobacco cells, the auxin binding activity was significantly enhanced in fractions removed from the microsomal membrane preparations by 0.5 M salt, while the binding activity still attached to the membranes after salt washes remained unchanged. The latter was immunologically analogous to maize ABP (Filippini *et al.* 1994).

To further investigate the possible relation of free intracellular IAA levels and auxin perception sensitivity, *rolB* transformed hairy root clones were produced from red beet seedling petioles. A direct ligand binding assay was conducted for the evaluation of the levels of specific auxin binding proteins, which could function as auxin receptors and represent one of the earliest elements that mediate auxin signal perception and transduction. Our results suggest that *rolB* neither consistently increases intracellular free IAA levels, nor consistently increases the availability of specific auxin binding proteins on PM. Instead, a negative correlation of these two factors has been observed.

Materials and methods

Hairy root production: Seeds from red beet (*Beta vulgaris* L.) were surface sterilized and germinated in moist sterile vermiculite. *A. tumefaciens* C58C1 harbouring the hairy root inducing plasmid pRiA4b (Huffman *et al.* 1984) was transformed with pBI121. Transformation was confirmed by plasmid isolation and agarose gel electrophoresis. Petiole sections (2 - 3 cm long) were infected by stabbing with a needle dipped into an overnight culture of *A. tumefaciens* C58C1 harbouring the rhizogenic plasmid pRiA4b, in which *rolA* and *rolC* were removed and the transcription of the *rolB* gene is driven by the cauliflower mosaic virus 35S RNA promoter (35S promoter). Infected petioles were cultured on MS medium containing 3 % (m/v) saccharose and 0.8 % (m/v) agar for 12 d before transfer to a similar medium containing 0.5 g dm⁻³ carbenicillin to remove bacteria. Individual hairy root tips were excised 4 weeks after infection and cultured on MS medium containing 0.5 g dm⁻³ cefotaxime for 3 weeks. They were then transferred to half-strength Gamborg's B5 medium containing 4 % (m/v) saccharose, 0.6 % (m/v) agar, 0.5 g dm⁻³ cefotaxime and 20 mg dm⁻³ kanamycin sulphate. After five passages, the cultures, whose axenic status was confirmed by nutrient broth incubation, were transferred to the same medium but lacking cefotaxime. The expression of *rolB* gene in these roots was confirmed by Northern assay.

Isolation of PM by aqueous two-phase partition: Harvested roots were rinsed twice with ice cold distilled water and homogenized with an equal volume of isolation buffer (50 mM Tris, 1 mM Na₂EDTA, 0.1 mM MgCl₂ and 0.25 M saccharose, pH 8.0) for 5 - 6 min. The homogenate was centrifuged at 4 000 g for 10 min. The pellets were discarded and the supernatant was centrifuged at 50 000 g for 45 min.

The pellet was then resuspended in phase resuspension buffer (5 mM K phosphate buffer, 250 mM saccharose, 1 mM DTT, pH 7.0). Phase partitioning was as described previously (Thomson *et al.* 1993) except replacing sorbitol with saccharose. The upper phase was referred to as the PM. For routine IAA binding assay, PM pellets were resuspended in assay buffer (see below) and used immediately for binding analysis. The whole isolation procedure took place at 4 °C.

Marker enzyme analysis: ATPase activity and K⁺-stimulated pyrophosphatase activity were determined by measuring Pi release according to the method of Ohnishi *et al.* (1975) with slight modifications (Williams *et al.* 1990) except using 0.025 % (m/v) rather than 0.01 % (m/v) Triton X-100, unless indicated otherwise. Antimycin A-insensitive NADH-cytochrome-*c* reductase and cytochrome-*c* oxidase determinations were carried out according to Hodges and Leonard (1974).

Binding assay for auxin binding proteins: The membrane suspension (0.5 cm³) was incubated with 4×10^{-8} M ³H-IAA (specific activity 1.11 TBq mmol⁻¹, Amersham) and different amounts of unlabelled IAA from 0 to 10⁻⁴ M in polycarbonate tubes. A final assay volume of 2.5 cm³ was made up with binding assay buffer (50 mM Na₃citrate/citric acid, 5 mM MgCl₂, 0.25 M saccharose, pH 5.5). The samples were incubated with gentle shaking for 30 min at 4 °C and then centrifuged at 75 000 *g* for 30 min. The supernatants were decanted and 1 cm³ ethanol was placed in each tube. The pellets, together with the ethanol, were left at room temperature overnight and then transferred to scintillation vials for counting. Scatchard (1949) analysis was used in the calculation of dissociation constant and the number of binding sites. The bound/free is plotted as a function of bound and the resulting plot is a straight line with slope -K_a (K_d = - 1/K_a), intercepting the abscissa at the number of binding sites (bound/free = 0).

SDS-polyacrylamide electrophoresis and immunoblotting: PM proteins were separated on SDS-PAGE and electro-transferred to nitrocellulose support (Xing *et al.* 1994). Blots were probed with a polyclonal antibody raised against a 22-kD maize ABP (a generous gift from Dr. R. Napier, Horticulture Research International, East Malling, Kent, UK) according to Napier *et al.* (1988).

Quantification of IAA: IAA from hairy roots or seedling roots was quantitatively determined by GC-SIM-MS using [¹³C₆] IAA as internal standards (Sitbon *et al.* 1992, Sundberg 1990).

Protein quantification: Protein content was determined according to Bradford (1976) with BSA as standard.

Results

Isolation of PM: Aqueous two-phase partitioning was used to obtain highly purified PM for auxin binding protein studies. Microsomal preparations from non-

transformed red beet seedling roots and *rolB* transformed hairy roots were applied to phase partitioning. Marker enzyme analysis of the upper phase and the microsomal fraction is shown in Table 1. Vanadate-sensitive ATPase was enriched in the upper

Table 1. Distribution of marker enzymes in phase partitioned-PM of red beet seedling roots. Marker enzyme assays were also applied to phase partitioned-PM of each red beet hairy root clones. Marker enzyme analysis was carried out on five different membrane preparations and the assays were conducted in triplicate. The results shown are from representative experiments using seedling roots. Since the variations in enzyme activities in *rolB* transformed hairy roots were always less than 6 % to those detected in seedling root PM preparations, the data were omitted in this table.

Membrane fraction	Vanadate-sensitive ATPase [% inhibition]	K ⁺ -stimulated PPase [nmol (PPi) mg ⁻¹ s ⁻¹]	NADH-Cyt reductase [nmol mg ⁻¹ s ⁻¹]	Cyt <i>c</i> oxidase [nmol mg ⁻¹ s ⁻¹]
microsomal	60.7	0.94	2.21	1.25
PM	92.1	0	0	0

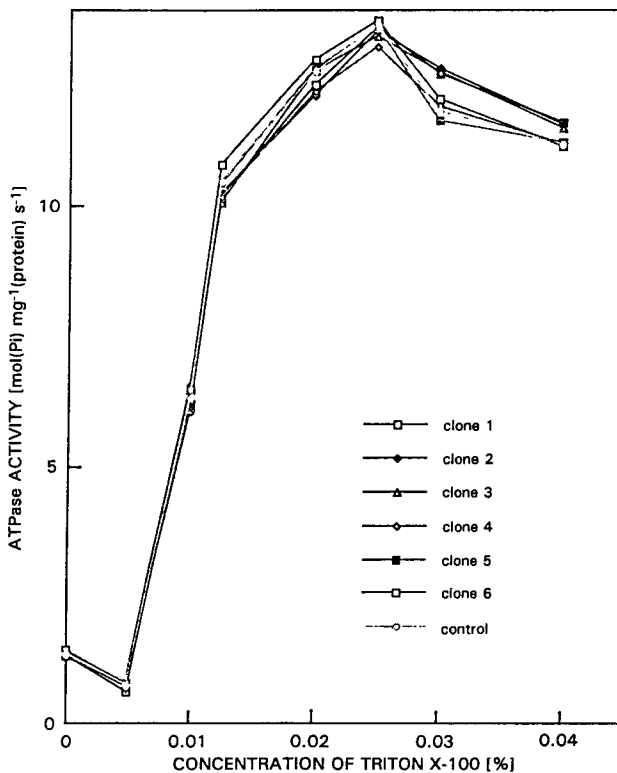


Fig. 1. Latency of ATPase activities of isolated PM vesicles from *rolB* transformed red beet hairy roots (clones 1 - 6) and from non-transformed red beet seedling roots (C). The values represent the mean $\pm$ SD of 4 independent experiments with at least three replicates each.

phase but K^+ -stimulated pyrophosphatase, NADH-dependent cytochrome-*c* reductase and cytochrome-*c* oxidase were low or undetectable. It is thus suggested that the PM preparations were significantly highly purified.

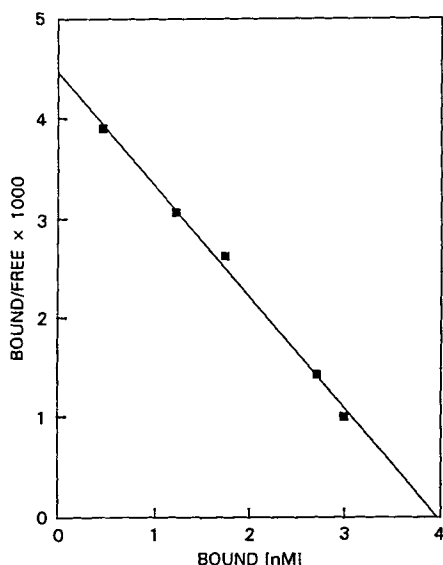


Fig. 2. A representative Scatchard plot of PM-bound auxin binding proteins isolated from non-transformed red beet seedling roots.

Sidedness of PM: Previous studies indicated that auxin could bind to its specific binding sites on the exterior face of the PM (Venis *et al.* 1990). The sidedness of the isolated PM may determine the detectable level of specific auxin binding. Latency studies were applied using Triton X-100 as detergent (Larsson *et al.* 1987). In the absence of Triton X-100 very little ATP hydrolytic activity was present. Addition of Triton X-100 at low concentrations increased the levels ATP hydrolysis. A concentration higher than 0.025 % (m/v) started to show less effect (Fig. 1). This latency work indicates that the isolated PM vesicles are dominantly right-side-out.

IAA binding analysis: The presence of a high affinity auxin binding protein was detected in the PM fractions isolated from non-transformed seedling roots. The dissociation constant to IAA was shown to be $9.07 (\pm 0.16) \times 10^{-7}$ M ($n = 8$) in Scatchard (1949) analysis (Fig. 2). All the six clones of *rolB* transformed hairy roots contained a PM-bound auxin binding protein with the same dissociation constant to IAA in Scatchard analysis (data not shown). The levels of specific IAA binding were various among different hairy root clones and between transformed and non-transformed roots (Fig. 3). The levels of specific IAA binding were also determined in the presence of 0.025 % (m/v) Triton X-100 and the levels were shown to be the same to those detected in the absence of Triton X-100.

A polyclonal antibody raised against maize 22-kD ABP subunit (Napier *et al.* 1988) was used to evaluate the levels of auxin binding proteins on the PM isolated from these hairy roots and seedling roots. The levels of the immuno-detectable 22-kD

polypeptide in different extracts were in good agreement to the levels that were detected in auxin binding assays (Fig. 4).

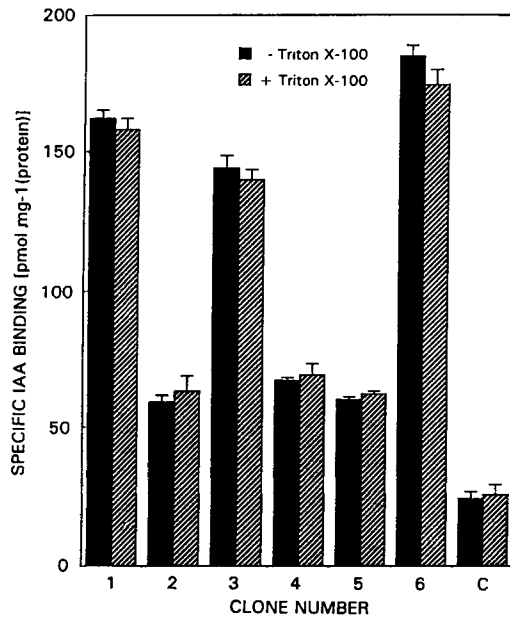


Fig. 3. Specific IAA binding in PM-enriched preparations from *rolB* transformed red beet hairy roots (1 - 6) and from non-transformed red beet seedling roots (C). The levels of specific IAA binding were obtained from Scatchard analysis. *Stippled bars* represent specific IAA binding detected in the absence of Triton X-100. *Hatched bars* represent specific IAA binding detected in the presence of 0.025 % (m/v) Triton X-100. The values represent the mean $\pm$ SD of 6 independent experiments with three replicates each.

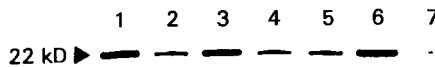


Fig. 4. Immunoblotting of PM proteins. PM (40 mg of protein) was subject to SDS-PAGE (12.5 %) and immunoblot analysis using polyclonal antibody raised against maize ABP1. Lanes 1 - 6 represent *rolB* transformed red beet hairy root clones 1 to 6, respectively. Lane 7 represents non-transformed red beet seedling roots. The *arrow* indicates the molecular mass of the cross-reacted polypeptide band.

Free intracellular IAA levels: Intracellular IAA levels were determined in extracts from six *rolB* transformed hairy root clones and from the non-transformed red beet seedling roots. Considerable variation in IAA levels was observed among hairy root clones and between transformed and non-transformed roots (Fig. 5). When compared to the levels of the detected specific IAA binding proteins, a negative correlation exists between the IAA levels and the specific IAA binding levels.

Discussion

Estruch *et al.* (1991) reported that the *rolB* gene product could *in vitro* hydrolyze indoxyl- β -glucoside. It was thus postulated to interfere with hormone metabolism in transformed plants. However, this cannot be simply explained by a direct release of IAA from its conjugates as *rolB* is unable to hydrolyze ester conjugates of IAA

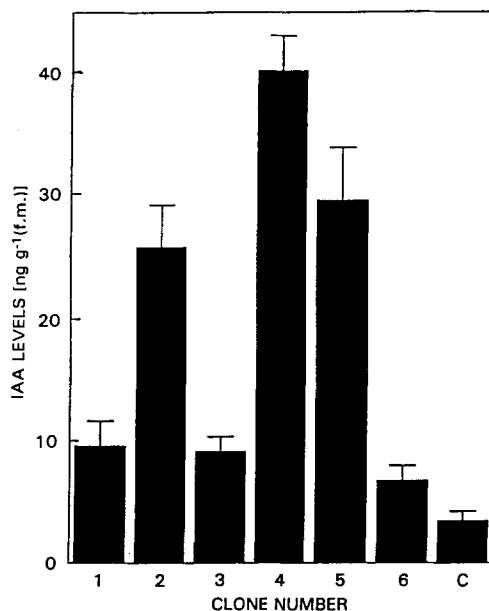


Fig. 5. Free intracellular IAA levels in *rolB* transformed red beet hairy roots (clones 1 - 6) and from non-transformed red beet seedling roots (C). The values represent the mean $\pm$ SD of 6 independent experiments with at least three replicates each.

(Spena *et al.* 1993). Furthermore, no gross alteration could be detected in IAA concentrations in transgenic tobacco tissues overexpressing *rolB* (Schmülling *et al.* 1993), and neither the size of the free IAA pool nor the rate of IAA metabolism were affected in these tissues (Nilsson *et al.* 1993). One of the hypotheses was that the *rolB*-enhanced protein increases the sensitivity of plant cells to auxin (Shen *et al.* 1988, Maurel *et al.* 1991, Spano *et al.* 1988). Delbarre *et al.* (1994) tested both auxin sensitivity and intracellular auxin levels in *rolB* transformed tobacco protoplasts. It was shown that *rolB* raised the auxin sensitivity of the electrical response of *rolB* transformed protoplasts, but had no effect on auxin levels and metabolism. The *rolB*-dependent behaviour of these transgenic protoplasts was not a consequence of modifying the intracellular IAA concentration but likely results from its perception system. Here, we investigated the possible changes of both PM-bound auxin binding proteins and intracellular IAA levels due to *rolB* transformation.

Highly purified PM fractions were prepared from *rolB* transformed hairy roots and from non-transformed seedling roots using aqueous two-phase partitioning. The

specific auxin binding activities detected in these preparations were believed to represent PM-bound auxin binding proteins. A specific auxin binding protein was detected in all the *rolB* transformed hairy roots and in the non-transformed seedling roots, with a K_d of $ca. 9.07 \times 10^{-7}$ M (Fig. 2). The amount of this protein in each hairy root clone was evaluated with Scatchard (1949) analysis. Two binding assay conditions were used, *i.e.* in the absence or in the presence of Triton X-100. To each of the root clones, the same specific binding activity was detected under both conditions (Fig. 3). This supports the view that the binding site of this protein is located on the exterior face of the PM (Barbier-Brygoo *et al.* 1989, 1991, Venis *et al.* 1990). Differences in specific IAA binding were found between transformed clones and non-transformed materials and also between these transformed clones (Fig. 3). The different expression levels of this protein in different root clones were reinforced by immuno-blotting (Fig. 4). A comparison of intracellular IAA levels with auxin binding levels shows a tendency for clones with higher specific binding to have lower free IAA levels and *vice versa* (Fig. 3 and 5). It seems that transformed hairy roots somehow gained the ability, due to the introduction of *rolB* gene, to build up a new balance of net auxin accumulation and its perception sensitivity. While consistent with the work showing that *rolB* transformation changes the auxin perception sensitivity (Shen *et al.* 1988, Delbarre *et al.* 1994, Maurel *et al.* 1991, Spano *et al.* 1988), our results are not in agreement with the work showing that hairy roots from bittersweet nightshade (Gartland *et al.* 1991) and pea (Schaerer and Pilet 1993), and shoots from T_L -DNA-transformed rape seed (Julliard *et al.* 1992) displayed normal levels of endogenous IAA. More specifically, our results did not agree with the work showing that the expression of *rolB* gene in tobacco plants had no significant effect on the endogenous levels of IAA (Schmülling *et al.* 1988; Nilsson *et al.* 1993). It is possible that in these previous work the examined *rolB* transformed materials obtained significantly high auxin sensitivity and an increase of IAA levels became unnecessary.

Using tobacco cells, Filippini *et al.* (1994) found that *rolB* transformation led to a several fold increase in auxin binding activity in fractions removable from the membranes by 0.5 M NaCl, while the binding activity still attached to the membranes after salt washes remained unchanged. However, the binding activity in the washed-off fractions was not depressed by antibodies against maize ABP, while the membrane-attached binding activity was. In contrast, antibodies against the *rolB* protein completely prevented auxin binding activity in the washed-off fractions but not that in the remained membrane fractions. They suggested that there existed an auxin binding activity associated with a protein immunologically related to *rolB* and its activity was significantly enhanced in *rolB* transformed cells while the auxin binding protein immunologically analogous to maize ABP did not change by *rolB* transformation. It should be noted that microsomal membrane fractions but not partially purified PM or ER were used in their study. In maize, over 95 % of auxin binding activities were recovered in ER (Napier and Venis 1991). It could be difficult to relate the *rolB*-induced changes in their work to primary auxin perception. Although the level of auxin binding proteins detected by antibodies against maize ABP was not shown to be changed in the *rolB* transformed tobacco cells, the

possible *rolB*-induced changes in PM could be easily masked. In our study, a highly purified PM fraction was used for direct ligand binding analysis and immunoblotting. The purified PM is expected to provide some more unequivocal evidence on *rolB* related alterations of auxin perception on PM. We have clearly showed the changes in auxin binding activities on PM in *rolB* transformed *Beta vulgaris* cells.

Our present work indicated that auxin binding proteins could be involved in the regulation of auxin economy in *rolB* transformed hairy roots and that specific auxin binding proteins may co-operate sophisticatedly with auxin during its function. Since the levels of both PM-bound specific auxin binding protein and intracellular IAA were increased in all the six hairy root clones due to *rolB* transformation, it could be expected that the overall auxin perception, *i.e.* the total level of "auxin-auxin binding protein" complex may well be increased. The negative correlation of PM-bound auxin binding protein availability and intracellular IAA levels may indicate a balance of these two factors at a higher level in terms of total signal perception. The possible auxin binding activity of *rolB*-related proteins (Filippini *et al.* 1994) and the possible effect of the *rolB* gene product on the structure of auxin binding proteins are also worth considering. The manipulation of the expression of auxin binding protein genes will provide us with a more precise and less one-sided strategy to regulate auxin effect on plant growth and development.

References

- Barbier-Brygoo, H., Ephritikhine, G., Klamt, D., Ghislan, M., Guern, J.: Functional evidence for an auxin receptor at the plasmalemma of tobacco mesophyll protoplasts. - *Proc. nat. Acad. Sci. USA* **86**: 891-895, 1989.
- Barbier-Brygoo, H., Ephritikhine, G., Klamt, D., Maurel, C., Palme, K., Schell, J., Guern, J.: Perception of the auxin signal at the plasma membrane of tobacco mesophyll protoplasts. - *Plant J.* **1**: 83-93, 1991.
- Bradford, M.M.: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. - *Anal. Biochem.* **72**: 248-254, 1976.
- Cardarelli, M., Mariotti, D., Pomponi, M., Spano, L., Capone, I., Costantino, P.: *Agrobacterium rhizogenes* T-DNA genes capable of inducing hairy root phenotype. - *Mol. gen. Genet.* **209**: 475-480, 1987.
- Davies, P.J.: *Plant Hormones and their Role in Plant Growth and Development*. - Martinus Nijhoff Publishers, Dordrecht 1991.
- Delbarre, A., Muller, P., Imhoff, V., Barbier-Brygoo, H., Maurel, C., Leblanc, N., Perrot-Rechenmann, C., Guern, J.: The *rolB* gene of *Agrobacterium rhizogenes* does not increase the auxin sensitivity of tobacco protoplasts by modifying the intracellular auxin concentration. - *Plant Physiol.* **105**: 563-569, 1994.
- Ephritikhine, G., Barbier-Brygoo, H., Muller, J.F., Guern, J.: Auxin effect on the transmembrane potential difference of wild-type and mutant tobacco protoplasts exhibiting a differential sensitivity to auxin. - *Plant Physiol.* **83**: 801-804, 1987.
- Estruch, J.J., Schell, J., Spena, A.: The protein encoded by the *rolB* plant oncogene hydrolyses indole glucosides. - *EMBO J.* **10**: 3125-3128, 1991.
- Filippini, F., Schiavo, F.L., Terzi, M., Costantino, F., Trovato, M.: The plant oncogene *rolB* alters binding of auxin to plant cell membranes. - *Plant Cell Physiol.* **35**: 767-771, 1994.
- Gartland, K.M.A., McInnes, E., Hall, J.F., Mulligan, B.J., Morgan, A.J., Elliott, M.C., Davey, M.R.: Effects of *Ri* plasmid *rol* gene expression on the IAA content of transformed roots of *Solanum*

- dulcamara* L. - Plant Growth Regul. 10: 235-241, 1991.
- Hagen, G.: Molecular approaches to understanding auxin action. - New Biol. 1: 19-23, 1989.
- Hodges, T.K., Leonard, R.T.: Purification of a plasma-membrane bound adenosine triphosphatase from plant roots. - Methods Enzymol. 32: 392-406, 1974.
- Huffman, G.A., White, F.W., Gordon, M.P., Nester, E.W.: Hairy-root-inducing plasmid: physical map and homology to tumor-inducing plasmids. - J. Bacteriol. 157: 269-276, 1984.
- Inohara, N., Shimomura, S., Fukui, T., Futai, M.: Auxin-binding protein located in the endoplasmic reticulum of maize shoots: molecular cloning and complete primary structure. - Proc. nat. Acad. Sci. USA 86: 3564-3568, 1989.
- Jones, A.M., Prasad, P.V.: Auxin-binding proteins and their possible roles in auxin-mediated plant cell growth. - BioAssays 14: 43-48, 1992.
- Jones, A.M., Herman, E.M.: KDEL-containing auxin-binding protein is secreted to the plasma membrane and cell wall. - Plant Physiol. 101: 595-606, 1993.
- Jones, A.M., Lamerson, P.L., Venis, M.A.: Comparison of site I auxin binding and a 22-kilodalton protein in maize. - Planta 179: 409-413, 1989.
- Julliard, J., Sotta, B., Pelletier, G., Miginiac, E.: Enhancement of naphthaleneacetic acid-induced rhizogenesis in *T_L*-DNA-transformed *Brassica napus* without significant modification of auxin levels and auxin sensitivity. - Plant Physiol. 100: 1277-1282, 1992.
- Larsson, C., Widell, S., Kjellbom, P.: Preparation of high-purity plasma membranes. - Methods Enzymol. 148: 558-568, 1987.
- Löbler, M., Klämbt, D.: Auxin-binding protein from coleoptile membranes of corn. I. Purification by immnological methods. - J. biol. Chem. 260: 9848-9853, 1985a.
- Löbler, M., Klämbt, D.: Auxin-binding protein form coleoptile membranes of corn. II. Localization of a putative auxin receptor. - J. biol. Chem. 260: 9854-9859, 1985b.
- Maurel, C., Barbier-Brygoo, H., Spena, A., Tempe, J., Guern, J.: Single *rol* genes from the *Agrobacterium rhizogenes* *T_L*-DNA alter some of the cellular responses to auxin in *Nicotiana tabacum*. - Plant Physiol. 97: 212-216, 1991.
- Munro, S., Pelham, H.R.B.: A C-terminal signal prevents secretion of luminal ER proteins. - Cell 48: 899-907, 1987.
- Napier, R.M., Venis, M.: From auxin-binding protein to plant hormone receptor. - Trends Biochem. Sci. 16: 72-75, 1991.
- Napier, R.M., Venis, M.A., Bolton, M.A., Richardson, L.I., Butcher, G.W.: Preparation and characterization of monoclonal and polyclonal antibodies to maize membrane auxin-binding protein. - Planta 176: 519-525, 1988.
- Nilsson, O., Crozier, A., Schmülling, T., Sandberg, G., Olsson, O.: Indole-3-acetic acid homeostasis in transgenic tobacco plants expressing the *Agrobacterium rhizogenes rolB* gene. - Plant J. 3: 681-689, 1993.
- Ohnishi, T., Gall, R.S., Mayer, M.L.: An improved assay of inorganic phosphate in the presence of extra-labile phosphate compounds: application to the ATPase assay in the presence of phosphocreatine. - Anal. Biochem. 69: 261-267, 1975.
- Palme, K., Hesse, T., Moore, I., Campos, N., Fildwisch, J., Garbers, C., Hesse, F., Schell, J.: Hormonal modulation of plant growth: the role of auxin perception. - Mechanism Develop. 33: 97-106, 1991.
- Scatchard, G.: The attraction of proteins for small molecules and ions. - Ann. N.Y. Acad. Sci. 57: 660-672, 1949.
- Schaerer, S., Pilet, P.E.: Quantification of indole-3-acetic acid in untransformed and *Agrobacterium rhizogenes*-transformed pea roots using gas chromatography mass spectroscopy. - Planta 189: 55-59, 1993.
- Schmülling, T., Schell, J., Spena, A.: Single genes from *Agrobacterium rhizogenes* influence plant development. - EMBO J. 7: 2621-2629, 1988.
- Schmülling, T., Fladung, M., Grossmann, K., Schell, J.: Hormonal content and sensitivity of transgenic tobacco and potato plants expressing single *rol* genes of *Agrobacterium rhizogenes*. -

- Plant J. 3: 371-382, 1993.
- Shen, W.H., Petit, A., Guern, J., Tempe, J.: Hairy roots are more sensitive to auxin than normal roots. - Proc. nat. Acad. Sci. USA 85: 3417-3421, 1988.
- Shimomura, S., Sotobayashi, T., Futai, M., Fukui, T.: Purification and properties of an auxin-binding protein from maize shoot membranes. - J. Biochem. 99: 1513-1524, 1986.
- Shimomura, S., Inohara, N., Fukui, T., Futai, M.: Different properties of two types of auxin-binding sites in membranes from maize coleoptiles. - Planta 175: 558-566, 1988.
- Sitbon, F., Hennion, S., Sundberg, B., Little, C.H.A., Olsson, O., Sandberg, G.: Transgenic tobacco plants co-expressing the *Agrobacterium tumefaciens iaaM* and *iaaH* genes display altered growth and indoleacetic acid metabolism. - Plant Physiol. 99: 1062-1069, 1992.
- Spano, L., Mariotti, D., Cardarelli, M., Branca, C., Costantino, P.: Morphogenesis and auxin sensitivity of transgenic tobacco with different complements of Ri T-DNA. - Plant Physiol. 87: 479-483, 1988.
- Spena, A., Schmülling, T., Koncz, C., Schell, J.: Independent and synergistic activity of *rolA*, *B* and *C* loci in stimulating abnormal growth in plants. - EMBO J. 6: 3891-3899, 1987.
- Spena, A., Estruch J.J., Hansen, G., Langenkemper, K., Berger, S., Schell, J.: The rhizogenes tale: modification of plant growth and physiology by an enzymatic system of hydrolysis of phytohormone conjugates. - In: Nester, E.W., Verma D.P.S. (ed.): Advances in Molecular Genetics of Plant Microbe Interactions. Pp. 109-124. Kluwer Academic Publishers, Dordrecht 1993.
- Sundberg, B.: Influence of extraction solvent (buffer, methanol, acetone) and time on the quantification of indole-3-acetic acid in plants. - Physiol. Plant. 78: 293-297, 1990.
- Thomson, L.J., Xing, T., Hall, J.L., Williams, L.E.: Investigation of the calcium-transporting ATPases at the endoplasmic reticulum and plasma membrane of red beet (*Beta vulgaris*). - Plant Physiol. 102: 553-564, 1993.
- Tillman, U., Viola, G., Kayser, B., Siemeister, G., Hesse, T., Palme, K., Löbner, M., Klambt, D.: cDNA clones of the auxin-binding protein from corn coleoptiles (*Zea mays* L.): isolation and characterization by immunological methods. - EMBO J. 8: 2463-2467, 1989.
- Venis, M.A., Thomas, E.W., Barbier-Brygoo, H., Ephritikhine, G., Guern, J.: Impermeant auxin analogues have auxin activity. - Planta 182: 232-235, 1990.
- White, F.F., Taylor, B.H., Huffman, G.A., Gordon, M.P., Nester, E.W.: Molecular and genetic analysis of the transferred DNA regions of the root-inducing plasmid of *Agrobacterium rhizogenes*. - J. Bacteriol. 164: 33-44, 1985.
- Williams, L.E., Schueler, S.B., Briskin, D.P.: Further characterization of the red beet plasma membrane Ca^{2+} -ATPase using GTP as an alternative substrate. - Plant Physiol. 92: 747-754, 1990.
- Xing, T., Williams, L.E., Nelson, S.J., East, J.M., Hall, J.L.: Immunological detection and localization of a calsequestrin-like protein in red beet and cucumber cells. - Protoplasma 179: 158-165, 1994.