Epitope mapping reveals conserved regions of an auxin-binding protein

Richard M. NAPIER and Michael A. VENIS

Horticulture Research International, East Malling, Maidstone, Kent ME19 6BJ, U.K.

There is now good evidence that maize (*Zea mays*) auxin-binding protein (ABP) functions as a receptor. We have synthesized sequential overlapping hexapeptides to map the epitopes recognized by a number of antisera to ABP. Only a few regions of the protein are recognized, and these are shown to be exposed on the surface. Three epitopes predominate, and these are clustered around, but do not include, the glycosylation site. A comparison is made between these maps of sera against purified ABP, maps of sera raised against recombinant maize ABP expressed in *Escherichia coli* and computer antigenicity predictions. Our anti-(maize ABP) serum recognizes ABP counterparts in other plant species. We have used immunoblotting to affinity-purify the immunoglobulins which cross-react from the antiserum. Epitope mapping of these immunoglobulins suggests that two of the three predominant epitopes may be conserved in both monocotyledonous and dicotyledonous plants. The possible functional significance of these conserved epitopes is discussed.

INTRODUCTION

Auxins are a major class of plant hormones that elicit a wide range of growth and developmental responses, including cell elongation and differentiation. A putative receptor for auxin has been identified and purified [1–3] from maize (*Zea mays* L.) shoots and, subsequently, the cDNA for this protein was sequenced [4–6]. The putative receptor binds the synthetic auxin 1-naphthaleneacetic acid with high affinity ($K_D = 5.7 \times 10^{-8}$ M) and shows a specificity for auxins and anti-auxins which broadly matches the known structure–activity relationships of these compounds *in vivo* [7,8]. Until recently it was characterized primarily by its ability to bind auxins and is, consequently, referred to as ‘auxin-binding protein’ (ABP) [1–6]. However, recent studies with tobacco (*Nicotiana tabacum*) mesophyll protoplasts have suggested that ABP mediates a characteristic auxin-induced protoplast hyperpolarization [9] and is, therefore, functioning as a receptor. The key observation was that auxin-induced hyperpolarization could be blocked by preincubation of tobacco protoplasts with antibodies raised to maize ABP [9]. Additionally, maize ABP added to tobacco protoplasts can complement the endogenous auxin-perception mechanism and increase auxin sensitivity [10].

The ability of antibodies raised against maize ABP to inhibit auxin-induced hyperpolarization in tobacco cells suggests not only that ABP is a receptor, but also that it is highly conserved. Maize ABP is a dimer of two (probably identical) 22 kDa subunits *N*-glycosylated at a single site [3,5]. Immunoblotting experiments have shown that anti-(maize ABP) serum cross-reacts with ABP counterparts in a number of species [13]. Knowledge of the epitopes to which antibodies bind might help us to identify which parts of ABP are highly conserved. By implication these conserved regions are likely to be functionally important.

We have used a commercially available epitope-mapping kit, based on the peptide-synthesis protocols described by Geysen and co-workers [14,15] (see the Materials and methods section), to map a number of maize ABP antisera and to compare the maps with computer predictions of antigenicity. In addition, immunoblotting has been used as an affinity-purification step for

cross-reacting immunoglobulins in order to identify epitopes that may be conserved in other plant species.

MATERIALS AND METHODS

Antibodies were raised against maize ABP as previously described [3]. Serum IgGs were purified by $(\text{NH}_4)_2\text{SO}_4$ precipitation and, after extensive dialysis, passage through a column (4 cm $\times$ 1 cm) of DEAE-Bio-Gel A (Bio-Rad, Hemel Hempstead, Herts., U.K.) (for 1 ml of serum) in half-strength phosphate-buffered saline (PBS). The unretained protein was collected.

Purification of ABP from barnyard grass (*Echinochloa crus-galli*) and mung bean (*Vigna radiata*; ‘*Phaseolus aureus*’)

Shoots were homogenized in borate grinding buffer (50 mM-disodium tetraborate/0.25 M-sucrose/0.1 mM-MgCl₂/1 mM-EDTA/5 mM-6-amino-n-hexanoic acid/0.25 mM-phenylmethanesulphonyl fluoride, pH 8.0). The brei was filtered through nylon gauze, centrifuged at 6000 *g* for 20 min and the supernatant centrifuged again at 50000 *g* for 45 min. The final supernatant was collected and protein was precipitated with 75% -satd. $(\text{NH}_4)_2\text{SO}_4$. Proteins were redissolved in citrate buffer (10 mM-sodium citrate/5 mM-MgSO₄, pH 6.0) and loaded on to an affinity column (5 cm $\times$ 1.5 cm) consisting of 4-hydroxyphenylacetic acid coupled to epoxy-Sepharose (Pharmacia, Milton Keynes, U.K.) (D. Klämbt & G. Viola, personal communication) and tumbled to mix for 15 min. The column was then allowed to repack, washed thoroughly with citrate buffer and eluted with 50 mM-Tris/HCl/5 mM-MgSO₄, pH 9.0. Eluted proteins were precipitated with trichloroacetic acid (10%, w/v) and then subjected to preparative SDS/12% PAGE. Proteins were transferred to nitrocellulose (0.45 μ m pore size; Hybond-C; Amersham International, Aylesbury, Bucks., U.K.) using a Bio-Rad Transblot cell.

From other species, ABP was purified by using anti-(maize ABP) immunoglobulins coupled to Reactigel (Pierce and Warriner, Chester, U.K.). Protein was prepared as described above, but solubilized in $\frac{1}{5}$ -strength PBS and incubated overnight at 4 °C with the affinity matrix. After thorough washing in $\frac{1}{5}$ -strength PBS the protein was eluted with 0.1 M-glycine/HCl, pH 2.5.

Abbreviations used: ABP, auxin-binding protein; PBS, phosphate-buffered saline (10 mM-potassium phosphate/150 mM-NaCl, pH 7.4); PBST, PBS plus 0.1% Tween 20.

Immunoaffinity purification of immunoglobulins from barnyard-grass and mung-bean ABP counterparts

Test strips from the edges of blotted membranes were stained with anti-(maize ABP) serum and preimmune serum to identify immunoreactive bands. Strips were then cut from the remainder of the membrane to excise the relevant protein bands. Control strips were also taken. The strips were incubated in blocking solution (PBS+0.1% BSA+0.1% Tween 20) for 30 min. Immunoglobulins from the anti-(maize ABP) serum were diluted in blocking solution (5 µg of IgG/ml) and incubated with the strips for 1 h at room temperature. After extensive washing in PBS+0.1% Tween 20 (PBST) the IgGs were eluted from the membrane in 0.1 M-glycine/HCl, pH 2.5 (2 ml/100 mm × 10 mm strip). The eluate was neutralized with 0.1 ml of 1.5 M-Tris/HCl, pH 8.8, and the IgGs were then dialysed extensively against PBST.

Epitope mapping kit

The kit was purchased from Cambridge Research Biochemicals (Cambridge, U.K.). Every sequential overlapping hexapeptide from the amino acid sequence of ABP [6] (but without the signal peptide) was synthesized on activated pegs according to the directions supplied. Before each assay the pegs were blocked with blocking solution (containing 1% BSA; 300 µl/well) for 1 h at room temperature. The test antibody was diluted in blocking solution and incubated with the peptides overnight at 4 °C (200 µl/well). All subsequent steps were at room temperature. The pegs were washed in PBST and developed by using either biotinylated sheep anti-rabbit IgG (Sigma, Poole, Dorset, U.K.) or biotinylated sheep anti-rat IgG (Amersham), each diluted 1:2000 in PBST, followed by streptavidin-biotinylated horseradish peroxidase (Amersham), also diluted 1:2000. The data from the e.l.i.s.a. plate reader (model 3550; Bio-Rad) were analysed by using the software supplied with the kit.

The peptide-linked pegs were washed free of bound antibodies in an ultrasonic bath (Dawe type 6444 AF; Philip Harris, London W.3, U.K.), containing 1% SDS and 0.1% 2-mercaptoethanol in 0.1 M-sodium phosphate, pH 7.2, at 55 °C, for 30–45 min. The pegs were rinsed in water at 55 °C, then washed for 2 min in boiling methanol before air-drying. They could then be re-used or stored dry. We have been able to re-use the peptides up to 50 times without any noticeable deterioration, although some antibodies were found to be particularly difficult to wash off. On these occasions the washing procedure was repeated until a satisfactory low background was obtained.

Assay for surface epitopes

The pegs were prepared and incubated with antibody as above. After thorough washing, antibodies were eluted from the pegs with 200 µl of 0.1 M-glycine/HCl, pH 2.5, for 5 min with agitation. After neutralization with 15 µl of 1.5 M-Tris/HCl, pH 8.8, 100 µl was taken and added to plates previously coated with purified non-denatured ABP [3]. After incubation the plates were washed in PBST and developed with biotin anti-rabbit IgG (Sigma) diluted 1:1000 in PBST and then with avidin-peroxidase (Sigma) diluted 1:2000 in PBST.

RESULTS

Epitope mapping of polyclonal immunoglobulins

We have synthesized all the linear hexapeptides within the mature maize ABP (i.e. not including the signal peptide) on activated peg supports. Using these peptides in an e.l.i.s.a. has allowed us to identify which peptides are recognized by our antiserum (Fig. 1*a*). Pre-immune serum is shown in Fig. 1(*b*).

Only a small proportion of the peptides is recognized (less than 15%), and these are confined to a few discrete regions of the ABP sequence. Antisera from a number of different sources (but all to ABP purified from maize) all show reaction to two sets of peptides between peptides 85–90 and 96–100 (Figs. 1*a* and 1*c–1e*), which may, therefore, represent immunodominant regions of ABP. Each of the maps shows that, in each serum, there are also antibodies to a few other discrete regions of ABP.

Comparison of the epitope maps shown in Fig. 1, and data described below, suggests that the peptides within the immunodominant regions can be subdivided into three groups (Fig. 1*a*). For convenience we have named them group I (peptides 85–87), group II (peptides 88–90) and group III (peptides 96–100). Antibodies in some sera recognize either group I (e.g. Fig. 1*d*) or group II (e.g. Fig. 1*c*) or both (Figs. 1*a* and 1*e*). All the antisera recognize group III.

We have compared the epitope maps (Figs. 1*a–1e*) with a computer analysis of the ABP sequence (Fig. 1*f*). This program (described in [16]) collates predictions for structural flexibility [17], antigenicity [18], β -turn potential [19] and hydrophilicity [20] for each peptide. Higher-than-average scores [16] in three or four of these indices suggest that the peptide is likely to be exposed on the surface of ABP and thus be an antigenic site [21]. These scores are plotted in Fig. 1*f*.

To test whether the epitopes identified by our antiserum are exposed on ABP, we repeated the mapping experiment, but eluted the antibodies from the immobilized peptides and challenged them with native ABP in an e.l.i.s.a. (Fig. 2*a*). Comparison of Fig. 2(*a*) with Fig. 1(*a*) shows the two maps to be

Fig. 1. Epitope maps of anti-(maize ABP) sera

The e.l.i.s.a. absorbance (*A*, in arbitrary units) is plotted for each hexapeptide. Peptide 1 represents residues 1–6 of the mature ABP (signal peptide cleaved), peptide 2 residues 2–7 etc., and so the epitope map can be read as if it represented a linearized protein with the *N*-terminus on the left. (*a* and *b*) Immune and preimmune serum respectively [3]. (*c* and *e*) Antisera from D. Klämbt and co-workers. (*d*) Antiserum from K. Palme. All these antisera were raised to ABP purified from maize. (*f*) Map of peptides predicted to be most highly antigenic [16] (see the text). The predominating epitopes discussed in the text, namely I, II and III, are identified in (*a*).

Fig. 2. Epitopes on the surface of ABP

(a) Antibodies were eluted from the epitope-mapping pegs and used in an e.l.i.s.a. presenting native ABP (see the Materials and methods section). Only eluted antibodies recognizing native ABP would register in the e.l.i.s.a. The results are presented as in Fig. 1, the serum used for this experiment being the one mapped in Fig. 1(a). (b) Immunoglobulins ($2.5 \mu\text{g} \cdot \text{ml}^{-1}$) were mixed with purified ABP ($1.5 \mu\text{g} \cdot \text{ml}^{-1}$) in PBST+1% BSA and incubated at 37°C for 2 h before mapping (blocked; ii). Control immunoglobulins were incubated without ABP (i).

the same, suggesting that all the antibodies detected by the epitope-mapping kit are directed towards the surface of the intact purified protein. As a further test for surface-exposed epitopes, we preincubated the immunoglobulins with purified soluble ABP (Fig. 2b). Binding to peptides was blocked for all epitopes: completely for epitope group I, extensively for group II and by approx. 70% for group III. Antibodies mapping to C-terminal epitopes seem to be under-represented in the maps in Fig. 2(b) (and Fig. 1) if comparison is made with Fig. 2(a). This is likely to be due to a much reduced affinity of the immunoglobulins for C-terminal peptides, because the peptides are attached to the plastic support pins through their C-termini. A free and mobile C-terminus can be presented by a protein but not by the peptides. Consequently, antibodies mapping to C-terminal epitopes are easily washed off the peptides during the standard prolonged washing and development procedure (see the Materials and methods section) (Fig. 2b), but if eluted early after initial washes they can bind strongly to immobilized ABP (Fig. 2a).

Antisera have been raised to recombinant maize ABP expressed in *E. coli* (K. Palme, unpublished work). Epitope maps of these (Fig. 3) show broadly the same characteristics as maps for antisera to purified maize ABP (Fig. 1), the major differences being the absence of reaction to group III peptides, but the presence of antibodies binding to peptides between groups II and III, namely to peptides 91–95 (Fig. 2b).

Conserved epitopes

Our anti-(maize ABP) serum (Fig. 1a) recognizes on immunoblots counterpart ABPs from other plant species, both monocotyledonous and dicotyledonous (Fig. 4; [13]). In both mung bean and barnyard grass the principal counterpart has a molecular mass close to 24 kDa. In maize and a number of other species the molecular mass of ABP is 22 kDa in our hands (Fig. 4, lanes C–E). In addition to the 24 kDa counterpart of ABP in mung bean, a second protein, of 40 kDa (Fig. 4, lane B), is recognized by our anti-(maize ABP) serum. A protein of similar molecular mass can also be detected in barnyard grass and other species (lanes A and C), but in lower abundance. One or two other bands are stained relatively weakly, and these may be

Fig. 3. Epitope maps of antisera raised to recombinant maize ABP expressed in *E. coli*

(a, b) Two antisera obtained from K. Palme.

Fig. 4. ABP counterparts in other plant species

Partially purified ABP preparations were separated by SDS/PAGE, blotted on to nitrocellulose, and probed with anti-(maize ABP) serum (see Fig. 1a). Lane A, barnyard grass; lane B, mung bean; lane C, rape (*Brassica napus*); lane D, pea (*Pisum sativum* var. Alaska); lane E, maize (*Zea mays*) for comparison.

breakdown products of the more abundant proteins discussed above or isoforms of ABP [5]. None of these proteins was stained by preimmune serum (results not shown).

Having shown that antibodies in the serum map to a spectrum of epitopes, we decided to try to identify which epitopes were conserved in other species. Cross-reacting antibodies were affinity-purified by immunoblotting and subsequent elution from the nitrocellulose-blotting membrane (see the Materials and methods section). In order to purify sufficient antibody, four preparative SDS-containing gels were used for each species, and each blot was used repeatedly.

The two immunoreactive bands from mung bean were treated separately. Antibodies affinity-purified from the 24 kDa band were mapped to a single conserved epitope, epitope III (Fig. 5a). Antibodies affinity-purified from the mung-bean 40 kDa band also map to a single epitope, but to epitope I (Fig. 5b). Interestingly, antibodies affinity-purified from the barnyard-grass 24 kDa ABP (Fig. 4) map to both epitopes I and III (Fig. 5c). Antibodies binding to epitope-II peptides were not purified in any of these experiments, even though, in the full serum, the mapping response was as strong to these peptides as to groups I and III (Fig. 1a).

Antibodies affinity-purified from mung-bean blots, and used for mapping conserved epitopes, did recognize maize ABP on

Fig. 5. Conserved epitopes

Immunoglobulins from anti-(maize ABP) serum were affinity-purified on blots of mung bean (a and b) or barnyard grass (c) proteins (see the Materials and methods section), then epitope-mapped. Maps of immunoglobulins eluted from control parts of the blots are presented underneath in each case. (a) Elutions from the 24 kDa band of mung bean (see Fig. 4b). (b) Elutions from the 40 kDa band of mung bean. (c) Elutions from barnyard grass (24 kDa).

blots (results not shown). We have also repeated the affinity-purification and mapping experiment with deglycosylated barnyard-grass ABP, obtaining epitope maps that are the same as those found with the parent glycosylated protein (Fig. 5c), i.e. with the same two conserved epitopes (results not shown).

DISCUSSION

Epitope mapping by using sequential hexapeptides synthesized on activated pegs has proved to be a convenient method for establishing regions of ABP that are immunoreactive. Strictly, the peptides to which the antibodies bind can be described only as partial epitopes [21], and the maps we have presented are not complete pictures of the range of epitopes recognized by our antisera. However, it has been suggested that, within the three-dimensional structure of an epitope [21], only five or six residues, possibly continuous, contribute most of the binding energy [22], in which case mapping with peptides should give a reliable description of the major antigenic determinants. We might have missed antibodies recognizing conformationally dependent epitopes, or antibodies for which the oligosaccharide side group is an important determinant. Nevertheless, the epitope-mapping kit can provide a great deal of valuable information about antibodies and their antigens.

The hexapeptides we have named groups I–III appear to represent immunodominant epitopes of maize ABP. Antibodies recognizing these peptides were found to be present in all the sera mapped (Fig. 1). Group III is separated from groups I and II by the site of *N*-glycosylation, namely the asparagine residue at position 95, which is included between peptide 90 (at the C-terminus) and peptide 95 (at the N-terminus). Peptides 92–95 are not recognized by antibodies from any of the sera (Fig. 1). Antibodies to these peptides are found in sera raised to recombinant ABP expressed in *E. coli* (and which are, therefore, not glycosylated) and, at the same time, antibodies to group-III peptides are lost (Fig. 2). It appears that the oligosaccharide group may influence antigenicity and/or protein folding in this part of ABP. It has been shown that oligosaccharides can decrease the conformational mobility of the amino acid backbone and side groups around glycosylation sites [23]. Maps of sera to

recombinant ABP show (Fig. 3) that regions I and II are immunodominant, even without the adjacent oligosaccharide.

According to Laver *et al.* [21], the weight of evidence suggests that most, perhaps all, of the surface of protein antigens is antigenic. The ABP preparations used to raise the sera mapped in Fig. 1 were of non-denatured protein [3,5,6], and all the antibodies eluted from the mapping peptides recognized non-denatured ABP (Fig. 2a). However, it cannot be assumed that ABP adsorbed to e.i.s.a. plates retains its native conformation, and so we used ABP in solution to block antibodies to surface epitopes before mapping. Blocking was extensive for all epitopes, the lowest level of blocking being 70% for group-III peptides (Fig. 2b). Both these sets of data suggest that the epitopes picked up by the mapping peptides lie exposed on the surface of ABP. Antibodies recognizing C-terminal epitopes seem to be under-represented on peptide maps (Fig. 2b), probably due to attachment of the mapping peptides through their C-terminus. We have monoclonal antibodies which recognize the C-terminus of ABP (and do not map on these peptides) and which precipitate ABP from solution [8]. In conjunction with the data of Fig. 2(a), this suggests that the C-terminus of ABP is also exposed, which would allow interaction between the KDEL terminus and KDEL receptors [24] to retain ABP in the endoplasmic reticulum [11].

We have compared the range of epitope maps with antigenicity predictions from a number of computer analyses (Fig. 1). By combining predictions of structural flexibility, antigenicity, β -turn potential and hydrophilicity, a more catholic index can be obtained for the probability that a peptide might be on the surface of the protein and, thereby, an effective antigen. The predictions are based on analysis of short linear peptide sequences (six residues), and hexapeptides were used in our mapping experiments. Nevertheless, only moderate agreement was found between predictions and maps. Antibodies in the various sera are found to recognize peptides from many of the regions predicted to be most antigenic. However, there are two regions predicted to be strongly antigenic against which no antibody maps, namely between peptides 51 and 59 and between peptides 103 and 118. Also striking is that, in the immunodominant regions, peptide groups I and II are correctly predicted to be strongly antigenic yet group III is missed. Group III is not immunodominant in non-glycosylated recombinant ABP (Fig. 3), and we have discussed the influence of glycosylation on antigenicity. Computer analyses can only give predictions based on known protein structures, and we do not want to imply that the programs have failed. For the hexapeptide sequences where antigenicity is predicted, but corresponding epitopes not found, it is possible that antibodies were generated, but to discontinuous epitopes not detected by our mapping strategy. However, it is also possible that the discrepancies highlight structural features of ABP, such as in the case of the observation made above that glycosylation influences the antigenicity of adjacent amino acid residues.

Immunoblotting with our anti-(maize ABP) serum has shown that counterparts of ABP are found in a range of plant species (Fig. 4, [13]). Preparative SDS/PAGE of partially purified proteins and transfer to nitrocellulose membranes enabled us to use these blots as affinity matrices. Only the immunoglobulins in the serum which specifically cross-react with conserved epitopes are purified. All plants contain much less ABP than maize; barnyard grass (7–10-fold less) and mung bean (20–50-fold less) are the best alternative sources from the range of species we have tested, and even here repetitive elutions from each blot were necessary.

We have observed that a second, 40 kDa, protein in mung bean and possibly other species (Fig. 4) shares some immunological identity with maize ABP. Unlike maize ABP and the 24 kDa mung-bean protein, however, the 40 kDa mung-

bean protein does not seem to be glycosylated (results not shown), and we do not know the relationship of this protein to ABP.

Two of the immunodominant regions of ABP appear to be highly conserved (Fig. 5). These regions are represented by peptide groups I and III. Both epitopes are conserved in the monocotyledonous plants maize and barnyard grass and in the dicotyledonous plant mung bean (Fig. 5). They are likely, therefore, to represent the source of immunological recognition in other species as well (Fig. 4).

This method of detecting conserved sequences can never replace nucleic acid or amino acid sequencing data for completeness. It is clear that antibodies to a protein antigen will only recognize a proportion of the total polypeptide (Figs. 1 and 2), and there are other limitations, as discussed above. Nevertheless, this approach gives a straightforward method for identifying conserved epitopes, without the necessities of either protein purification or cDNA library construction from unknown tissues. The observation that certain epitopes are conserved well enough for the method to work suggests that these regions of the ABP are functionally or structurally important. That they also appear to be immunodominant may suggest that they are sites of protein-protein interaction. We have not yet been able to repeat these experiments for tobacco ABP, but our results and sequence data from dicotyledonous plants (S. Shimomura & C. Lazarus, personal communications) would suggest that these two epitopes are also conserved in tobacco and that it is when anti-(maize ABP) antibodies bind to these epitopes that tobacco protoplast ABP is inhibited from transducing the auxin signal [9,10]. It is too early to suggest that this is anything other than a possibility. However, with the knowledge that parts of the ABP sequence are conserved and antigenic, we can develop more specific antisera. Combining knowledge of antibody epitopes with functional assays [9,10] may enable us to ascribe functional detail to particular domains of ABP.

We thank Professor D. Klämbt and co-workers (Botanisches Institut, D-5300-Bonn, Germany), and Dr. K. Palme (Max-Planck-Institut für Züchtungsforschung, D-5000-Köln 30, Germany), for their gifts of antisera, Dr. M. Partis for running the maize ABP sequence through the analysis program PREDICT, Dr. C. Lazarus and Dr. S. Shimomura for communicating ABP sequence data, Dr. M. Clark for help in interfacing the epitope-mapping programs and Ms. Sue Tolhurst for expert technical assistance. This work was partly supported by the BRIDGE Programme of the European Economic Community.

REFERENCES

1. Löbner, M. & Klämbt, D. (1985) *J. Biol. Chem.* **260**, 9848–9853
2. Shimomura, S., Sotobayashi, T., Futai, M. & Fukui, T. (1986) *J. Biochem. (Tokyo)* **99**, 1513–1524
3. Napier, R. M., Venis, M. A., Bolton, M. A., Butcher, G. W. & Richardson, L. I. (1988) *Planta* **176**, 519–526
4. Inohara, N., Shimomura, S., Fukui, T. & Futai, M. (1989) *Proc. Natl. Acad. Sci. U.S.A.* **86**, 3564–3568
5. Hesse, T., Feldwisch, J., Balshuseman, D., Bauw, G., Puype, M., Vanderkerckhove, J., Löbner, M., Klämbt, D., Schell, J. & Palme, K. (1989) *EMBO J.* **8**, 2453–2461
6. Tillmann, U., Viola, G., Kayser, B., Seimeister, G., Hesse, T., Palme, K., Löbner, M. & Klämbt, D. (1989) *EMBO J.* **8**, 2463–2467
7. Ray, P. M., Dohrmann, U. & Hertel, R. (1977) *Plant Physiol.* **60**, 585–591
8. Napier, R. M. & Venis, M. A. (1990) *Planta* **182**, 313–318
9. Barbier-Brygoo, H., Ephritikhine, G., Klämbt, D., Ghislain, M. & Guern, J. (1989) *Proc. Natl. Acad. Sci. U.S.A.* **86**, 891–895
10. Barbier-Brygoo, H., Ephritikhine, G., Shen, W. H., Delbarre, A., Klämbt, D. & Guern, J. (1990) in *Transducing Pathways: Activation and Desensitization* (Konijn, T. M., Houslay, M. D. & van Haastert, P. J. M., eds.), pp. 231–244, Springer-Verlag, New York
11. Ray, P. M. (1977) *Plant Physiol.* **59**, 594–599
12. Shimomura, S., Inohara, N., Fukui, T. & Futai, M. (1988) *Planta* **175**, 558–566
13. Venis, M. A. & Napier, R. M. (1990) in *Hormone Perception and Signal Transduction in Animals and Plants* (Roberts, J., Kirk, C. & Venis, M., eds.), pp. 55–65, The Company of Biologists, Cambridge
14. Geyson, H. M., Meloen, R. H. & Barteling, S. J. (1984) *Proc. Natl. Acad. Sci. U.S.A.* **81**, 3998–4002
15. Geyson, H. M., Rodda, S. J., Mason, T. J., Tribbick, G. & Schoofs, P. G. (1987) *J. Immunol. Methods* **102**, 259–274
16. Partis, M. D. & Grimm, R. (1990) *Z. Naturforsch.* **45c**, 987–998
17. Karplus, P. A. & Schulz, G. E. (1985) *Naturwissenschaften* **72**, 212–213
18. Welling-Wester, G. W., Weijer, W. J., van der Zel, R. & Welling-Wester, S. (1985) *FEBS Lett.* **188**, 215–218
19. Chou, P. Y. & Fasman, G. D. (1978) *Annu. Rev. Biochem.* **47**, 251–276
20. Hopp, T. P. & Woods, K. R. (1981) *Proc. Natl. Acad. Sci. U.S.A.* **78**, 3824–3828
21. Laver, W. G., Air, G. M., Webster, R. G. & Smith-Gill, S. J. (1990) *Cell (Cambridge, Mass.)* **61**, 553–556
22. Novotny, J., Bruccoleri, R. E. & Saul, F. A. (1989) *Biochemistry* **28**, 4735–4749
23. Wormald, M. R., Wooten, E. W., Bazzo, R., Edge, C. J., Feinstein, A., Rademacher, T. W. & Dwek, R. A. (1991) *Eur. J. Biochem.* **198**, 131–139
24. Armstrong, J. & Warren, G. (1990) *Nature (London)* **344**, 383–385

Received 10 September 1991/3 December 1991; accepted 10 December 1991