The Plant Cell, Vol. 1, 1003–1009, October 1989

Receptor-Mediated Endocytosis in Plant Cells

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We have employed fluorescein and ^{125}I-labeled elicitors of the defense response in soybeans to monitor the cellular distribution and movement of elicitors following their addition to a soybean cell suspension culture. Our results indicate that the macromolecular elicitors first bind to the cell surface and then internalize in a temperature- and energy-dependent endocytotic process. Within a few hours, virtually all of the elicitor is concentrated in the major vacuole or tonoplast of the cell. Nonspecific (control) proteins neither bound to the cell surface nor internalized in parallel assays.

INTRODUCTION

When extracellular ligands such as polypeptide hormones, lectins, or antibodies bind to their receptors on animal cell surfaces, the receptor ligand complexes are commonly internalized via a process termed receptor-mediated endocytosis (Schlessinger, 1980; Pastan and Willingham, 1985). By this mechanism cells not only remove hormones from their receptors after signals have been transmitted, but they also clear their surfaces of unwanted ligands, regulate receptor numbers in their plasma membranes, and carry both desired and undesired molecules into their cell interiors (Schlessinger, 1980; Pastan and Willingham, 1985).

Remarkably, receptor-mediated endocytosis has not been demonstrated in plants. Because virtually all of the known plant growth regulators are sufficiently small to enter their target cells via facilitated transport or simple diffusion, the need for a mechanism to clear the cell surface of endogenous hormones has not been compelling. Furthermore, theoretical arguments claiming that plants cannot generate sufficient pressure-volume work to form vesicles against the strong turgor pressure within the cell have been offered (Cram, 1980), and these have undoubtedly discouraged exploratory experiments into the occurrence of endocytotic processes in plants. Thus, despite observations that higher plants contain coated vesicles (Mersey et al., 1985), can internalize heavy metals (Wheeler and Hanchey, 1971; Hubner, Depta, and Robinson, 1985), and can even take up larger molecules once their cell walls have been removed (and turgor pressure drops to zero), (Joachim and Robinson, 1984; Tanchak et al., 1984; Hillmer, Depta, and Robinson, 1986), the evidence for receptor-mediated endocytosis has been insufficient to motivate a thorough evaluation of the process.

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We have believed that the inability of researchers to demonstrate receptor-mediated endocytosis in plants has largely been due to an inappropriate choice of ligands. While auxins, gibberillins, cytokinins, etc., may pass through the plasma membrane to receptor sites within the cell, a second class of signal molecules in plants, termed elicitors, are clearly too large and polar to do so. Thus, most well-characterized elicitors of the plant's defense response are either oligo- or polysaccharides, or water-soluble proteins with no ability to permeate lipid membranes (Nothnagel et al., 1983; Sharp, McNeil, and Albersheim, 1984). Instead, these molecules are seen to bind to the cell surface in a saturable manner (Yoshikawa, Keen, and Wang, 1983; Schmidt and Ebel, 1987; Cosio et al., 1988). Because clearance of such polar macromolecules from a plant cell's surface could proceed via an endocytotic pathway, we decided to examine the fate of two distinct elicitor preparations following their interactions with putative receptors at the plant cell surface.

RESULTS

Uptake of Fluorescein-Labeled Macromolecules

Figure 1 displays the sequence of events observed when a fluorescein isothiocyanate (FITC)-labeled elicitor preparation $M_r > 30,000$ from the fungal pathogen *Verticillium dahliae* is incubated with cultured soybean cells. Importantly, this elicitor fraction was found to be fully active in stimulating glyceollin (a phytoalexin defense product) formation in the suspension culture. In Figure 1 each timepoint is illustrated by a phase contrast image of a cell or a cell cluster (a, c, e, g, i, k, m, o, and q), followed by a

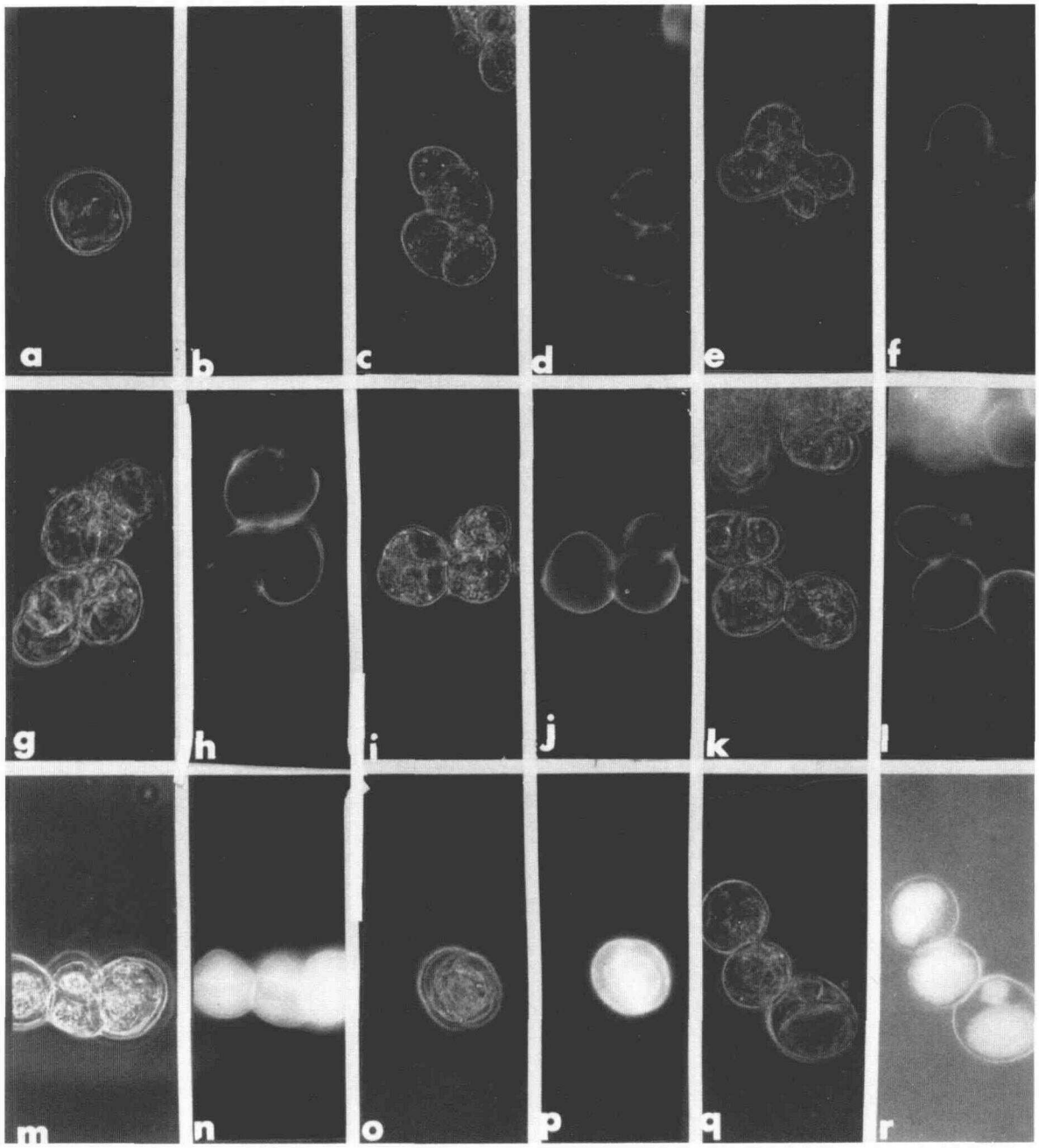

Figure 1. Time Course of Internalization of Fluorescein-Labeled Elicitor from *V. dahliae* into Cultured Soybean Cells.

The micrographs are displayed in pairs with the phase contrast image of a cell or cell cluster on the left and the corresponding fluorescent image of the same cell or cell cluster on the right to allow visualization of the location of the fluorescent elicitor. The micrographs are arranged chronologically from the time of introduction of fluorescein-labeled elicitor into the cell suspension.
(a) and **(b)** 0 min.
(c) and **(d)** 20 min.
(e) and **(f)** 1 hr.
(g) and **(h)** 2 hr.
(i) and **(j)** 3 hr.
(k) and **(l)** 4 hr.
(m) and **(n)** 5 hr.
(o) and **(p)** 6 hr.
(q) and **(r)** 7 hr.

fluorescence image of the same field showing the location of the fluorescent elicitor (b, d, f, h, j, l, n, p, and r). As seen in the micrographs, soon after addition of the elicitor, sufficient amounts of the ligand accumulated at the cell surface to form a fluorescent outline surrounding each cell (e.g., Figures 1c to 1j, 1 hr to 4 hr). Then, over a period of 1 hr to 2 hr, the fluorescent elicitor appears to concentrate in the cell's major vacuole or tonoplast (Figures 1q and 1r), leaving the cytoplasm relatively dark and void of elicitor. By changing the plane of focus of the microscope in Figures 1m to 1r, it could be confirmed that the fluorescence indeed extended across the entire thickness of the cell (not shown). Furthermore, a cursory scan of the entire field of cells revealed that >95% of the soybean cells participated in the uptake process.

Although requiring significantly less time, endocytosis of a fully active fluorescein thiosemicarbazide-labeled oligogalacturonic acid elicitor isolated from citrus pectin followed a similar sequence of events (Figure 2). Thus, within 20 min, the oligogalacturonide had accumulated on the cell surface (Figures 2c and 2d), and by 2 hr, most of the elicitor was internalized (Figures 2g and 2h). This similar behavior, despite the probable differences in chemical nature between the two elicitors, suggests that plant cells may remove different elicitor molecules from their cell surface receptors by common endocytotic pathways.

Because extracellular material can fortuitously enter animal cells via a pinocytotic or nonreceptor-mediated endocytotic pathway, it was important to evaluate the relative rate of such receptor-independent uptake to eliminate it as an explanation of the observed internalization events. For this purpose, FITC-labeled bovine serum albumin and FITC-labeled insulin were incubated in parallel experiments with the same cells employed in Figures 1 and 2. As seen in Figure 3, the animal proteins did not bind to the cell surface, and no uptake occurred. Thus, even after 8 hr incubation under the same conditions employed in the elicitor experiments, no measurable fluorescence was detected inside the soybean cells (Figures 3q and 3r).

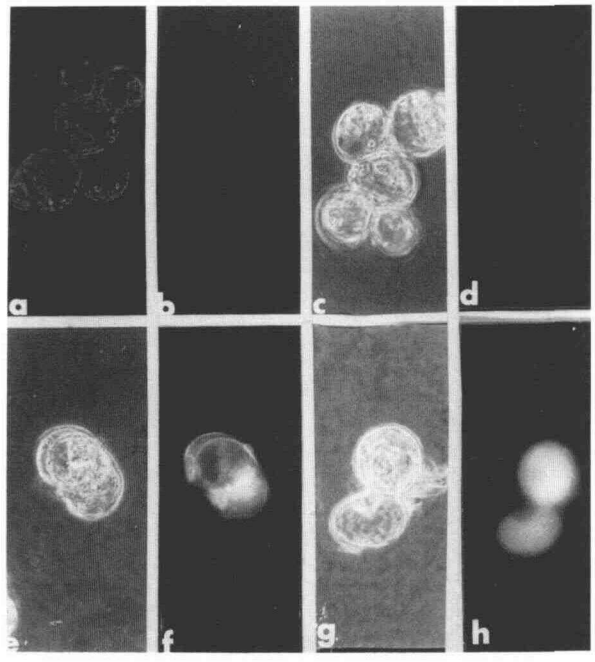

Figure 2. Time Course of Internalization into Cultured Soybean Cells of Fluorescein-Labeled Polygalacturonic Acid Elicitor Isolated from Citrus Pectin.

The micrographs are displayed as in Figure 1 and are arranged chronologically from the time of introduction of labeled elicitor into the cell suspension.
(a) and **(b)** 0 min.
(c) and **(d)** 20 min.
(e) and **(f)** 1 hr.
(g) and **(h)** 2 hr.

Uptake of ^{125}I-Labeled Macromolecules

To establish further that receptor-mediated uptake of elicitor is ligand-specific and much more rapid than any possible nonspecific uptake pathways, the endocytotic process was also monitored using radiolabeled ligands. As shown in Figure 4, uptake of ^{125}I-labeled polygalacturonic acid (closed circles) proceeded at a rate of $\sim 10^6$ molecules cell^{-1} min^{-1}, at least for the first 2 hr when the internalization process was still far from completion. However, when 1 mM KCN was added to the cell suspension to block any energy-dependent processes, no internalization was detected (not shown). Furthermore, when a 10-fold excess of unlabeled polygalacturonic acid was added to compete for specific receptors on the plant cell surface, the rate of uptake of ^{125}I-polygalacturonic acid was reduced at least 10-fold (Figure 4, open squares). Thus, internalization of the polygalacturonic acid elicitor would appear to proceed by an energy-dependent, receptor-mediated process.

To confirm further that the above elicitor endocytosis was ligand-specific and not a consequence of random pinocytosis of growth medium components, two additional control experiments were conducted. First, the uptake of ^{125}I-ovomucoid, a third small animal protein presumed to have no affinity for plant cell surface receptors, was also analyzed. As shown in Figure 4 (open circles), little internalization was detected over the entire 5-hr time course of the experiment. Thus, random uptake of extracellular molecules appears to proceed at a rate many times slower than elicitor endocytosis by the same cells. Second, to assure that the intact polygalacturonic acid molecule was, in fact, internalized and not simply digested to its monomeric units and then transported into the cell, the endocytosed polygalacturonide was subsequently extracted

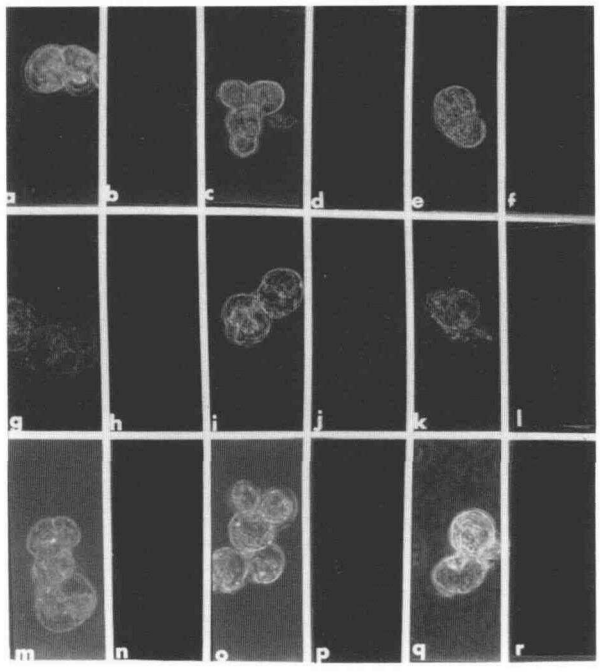

Figure 3. Time Course of Treatment of Cultured Soybean Cells with Fluorescein-Labeled Insulin.

The micrographs are displayed as in Figure 1 and are arranged chronologically from the time of introduction of labeled protein into the cell suspension.
(a) and **(b)** 0 min.
(c) and **(d)** 20 min.
(e) and **(f)** 1 hr.
(g) and **(h)** 2 hr.
(i) and **(j)** 3 hr.
(k) and **(l)** 4 hr.
(m) and **(n)** 5 hr.
(o) and **(p)** 6 hr.
(q) and **(r)** 7 hr.
These results are identical to those of soybean suspension cultures treated with fluorescein-labeled bovine serum albumin.

from the soybean cells and sized on a gel-filtration column. After allowing the internalization process to proceed for 40 min, the isolated elicitor eluted in the same column fraction as unmodified elicitor and significantly ahead of the monomer elution peak (data not shown). Thus, taken together with the fluorescence microscopy data, these results demonstrate that rapid elicitor uptake is an energy-dependent process that requires molecular recognition at the membrane and is not mediated by random "drinking" of molecules from the extracellular medium.

Inhibition of Elicitor Uptake at 4°C

Because one of the hallmarks of receptor-mediated endocytosis in animal cells is the interruption of internalization (but not surface binding) upon cooling to 4°C, we determined whether the same property might characterize the internalization of elicitors by plant cells. For this purpose, soybean cells were cooled to 4°C and incubated with either the fluorescein-labeled elicitors, the aforementioned fluorescent animal proteins, or ^{125}I-labeled polygalacturonic acid, and then washed at various time intervals to remove unbound ligand. As seen in Figure 4, the ^{125}I-labeled polygalacturonic acid uptake was inhibited ~88% at 4°C relative to the rate at 23°C. As seen further in Figure 5, elicitor binding apparently proceeded at the lower temperature, except that it occurred at a much slower rate. However, unlike the process at 23°C, the fluorescent ligands at 4°C never entered the cells, remaining at their periphery for the entire 8 hr of incubation. (Compare the 7-hr and 8-hr timepoints of Figures 2 and 5, which differ only in the temperatures of incubation.) Control experiments with the nonspecific animal proteins revealed the expected absence of binding seen in Figure 5 (not shown). Furthermore, as with endocytosis in animal cells, internalization of the elicitor by the soybean cells resumed once the cells were returned to 23°C (Figure 6). Thus, endocytosis in plants would appear to exhibit the same temperature dependence as endocytosis in animals.

DISCUSSION

As a first step toward dissecting the steps associated with elicitor signal transduction, we have employed two meth-

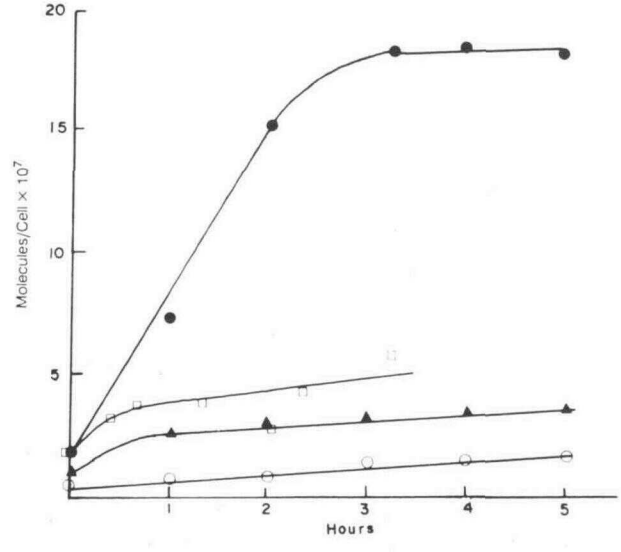

Figure 4. Time Course of Internalization.

Time course of internalization of ^{125}I-labeled polygalacturonic acid (●, ▲) and ^{125}I-labeled green pea fowl ovomucoid (○) at 23°C (●, ○) and 4°C (▲), or in the presence of a 10-fold molar excess of unlabeled polygalacturonic acid elicitor at 23°C (□).

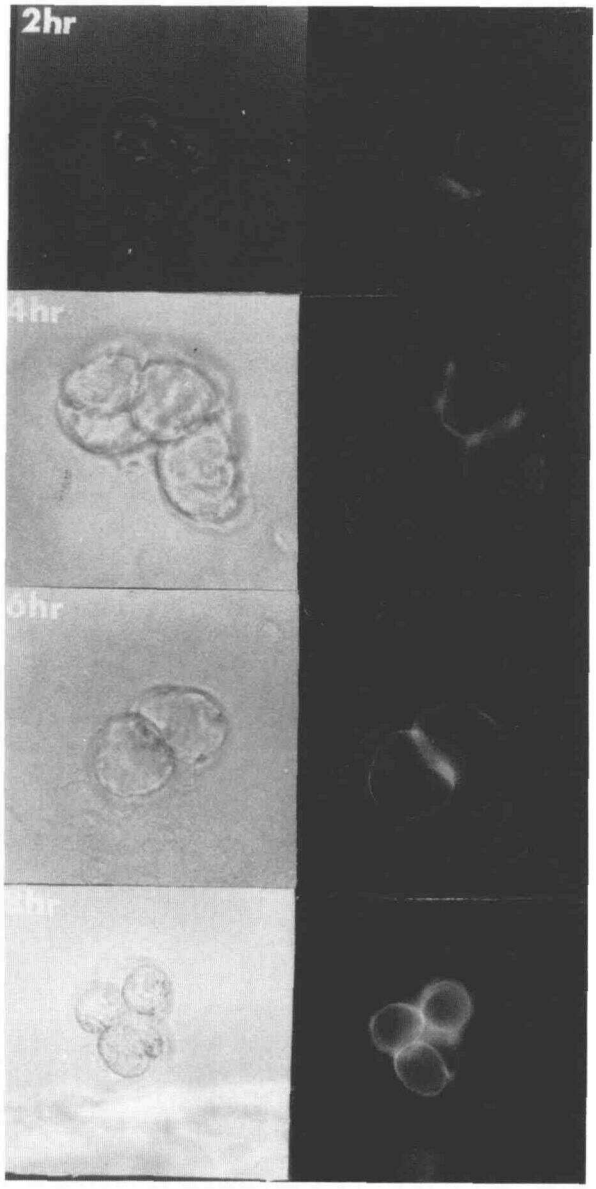

Figure 5. Inhibition of Internalization of Fluorescein-Labeled *V. dahliae* Elicitor into Cultured Soybean Cells upon Chilling to 4°C.

The micrographs are arranged as in Figure 1 with the times of incubation shown.

ods to determine the fate of the elicitor molecules responsible for triggering the defense response. With ^{125}I-labeled polygalacturonic acid, we have measured the rate of uptake of elicitor ($\sim 10^6$ molecules cell^{-1} min^{-1}) and have shown that its endocytosis, as anticipated, is highly temperature-sensitive and competitively inhibited by unlabeled elicitor. We have also demonstrated that the elicitor is internalized in its undigested form, and that its degradation, if it occurs at all, must be delayed for several hours after elicitor entry into the cell.

With the fluorescein-labeled *Verticillium* and polygalacturonic acid elicitors, we have further elucidated at low resolution the intracellular fate of elicitors after their introduction into the plant cell suspension. Thus, both elicitors were found to first associate at the cell surface and then to gradually accumulate in the major vacuole. That this gradual sequestration is not likely involved in signal transduction is evidenced by the fact that some defense mechanisms are already operative before significant sequestration has occurred. For example, we have recently demonstrated that toxic H_2O_2 is released by the plant cells within 5 min of elicitor addition, and that this agent itself is responsible for triggering the subsequent production of phytoalexins (Apostol, Heinstein, and Low, 1989). It is, therefore, conceivable that the elicitor is delivered to the vacuole predominantly for the purpose of elicitor disposal and not for any active protective function. In this respect, it is interesting that β-glucanases, which can digest glucan elicitors, have been found to be specifically concentrated in the plant's vacuole (Van den Bulcke et al., 1989).

Comparison of the time course of endocytosis of fluorescein- and ^{125}I-labeled polygalacturonic acid (Figures 2 and 4) reveals that both processes reach completion around 2 hr after elicitor addition. In contrast, the *Verticillium* elicitor, which appears to be a protein (M.A. Horn, P.F. Heinstein, and P.S. Low, unpublished data), was nearly quantitatively delivered to the vacuole only after 5 hr to 7 hr. This difference in processing time must clearly reflect distinct, but perhaps parallel, processing pathways following elicitor binding because the rates of elicitor recognition, i.e., the times of appearance of the H_2O_2 burst after elicitor addition, are within 1 min to 5 min in both

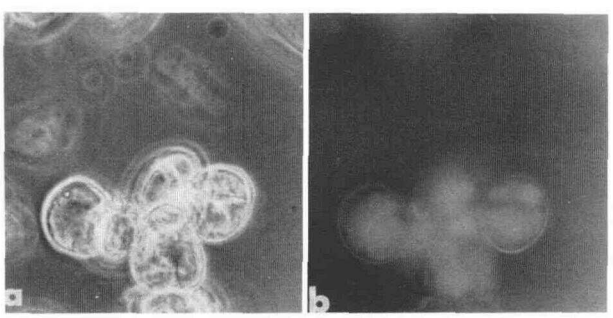

Figure 6. Resumption of Endocytosis of Fluorescein-Labeled *V. dahliae* Elicitor after Raising the Incubation Temperature from 4°C to 23°C.

(a) Cells incubated for 8 hr at 4°C revealing no internalization of elicitor (see Figure 5) were returned to 23°C and examined after 3 hr incubation by phase contrast.

(b) Cells incubated for 8 hr at 4°C revealing no internalization of elicitor (see Figure 5) were returned to 23°C and examined after 3 hr incubation by fluorescence microscopy.

cases (Low and Heinstein, 1986; Apostol et al., 1987; Apostol, Heinstein, and Low, 1989).

The rapid endocytosis of two unrelated elicitor molecules, the competitive inhibition of labeled elicitor uptake by unlabeled elicitors, and the absence of significant internalization of unrelated animal proteins all attest to the requirement for a specific recognition event in the endocytotic process. Although no elicitor receptor was isolated, it is still difficult to conceive of a system displaying the recognition and internalization properties reported here that lacks a specific protein receptor. Saturable elicitor binding to cultured plant cells has, in fact, been reported by several laboratories (Yoshikawa, Keen, and Wang, 1983; Schmidt and Ebel, 1987; Cosio et al., 1988), and an auxin receptor has even been identified recently in tomato plasma membranes (Hicks, Rayle, and Lomax, 1989). However, as yet no elicitor receptor has been isolated and characterized from any plant species.

In conclusion, most molecular stimuli are designed to be transient events, i.e., capable of being switched off after the signal has been transduced, and, therefore, mechanisms must exist for inactivating or removing a regulatory ligand after its message has been delivered. Plant cells that have detected the presence of a pathogen and successfully resisted the attack must also be able to eliminate the stimulus that has diverted their metabolic energies toward defense. In this study, we have shown that one mechanism by which such stimuli might be removed is via receptor-mediated endocytosis.

METHODS

Plant Material

Soybean (*Glycine max* Merr cv Kent) cell suspension cultures were maintained in W-38 medium and subcultured every 7 days to 10 days, as described previously (Low and Heinstein, 1986; Apostol et al., 1987).

Elicitor Preparation

The oligogalacturonide fraction (degree of polymerization = 12) used as an elicitor was prepared from citrus pectin as previously described (Nothnagel et al., 1983). A typical preparation contained 500 μg of galacturonic acid equivalents per milliliter.

The *Verticillium dahliae 277* elicitor was prepared as previously described (Low and Heinstein, 1986; Apostol et al., 1987). A typical elicitor preparation contained 70 μg of protein and 134 μg of glucose equivalents per milliliter.

Preparation of FITC-Labeled Proteins

To 1 mL of a 1 mg/mL solution of the protein to be labeled was added 0.5 mL of a 1 mg/mL solution of FITC (Sigma Chemical Co.) in dimethylformamide. The reaction was allowed to proceed for 4 hr in the dark at room temperature. After 4 hr any unreacted FITC was "quenched" with 10 μL of ethanolamine. The quenched reaction mixture was dialyzed against distilled H_2O until the dialysate was free of fluorescence.

Preparation of Fluorescein Thiosemicarbazide-Labeled Polygalacturonic Acid

To 1 mL of the polygalacturonic acid elicitor was added 0.2 mL of a 1 mg/mL solution of fluorescein thiosemicarbazide (Molecular Probes Inc.) in dimethylformamide. This mixture was allowed to react for 4 hr in the dark at room temperature and then dialyzed against distilled H_2O until fluorescence no longer appeared in the dialysate.

Assay for the Uptake of Fluorescein-labeled Macromolecules

A 7-day-old suspension culture of *G. max* Merr cv Kent was gravity-filtered using a fine nylon mesh. One cm^3 of loosely packed cells was transferred to 20 mL of fresh W-38 medium and allowed to grow for 24 hr to 36 hr, which resulted in a cell population in the early exponential growth phase. To this flask was added 20 μg of the desired derivatized molecule, after which the suspension was incubated at the desired temperature. At different time intervals, 1 mL of the cell suspension was removed, vacuum filtered, and washed with 75 mL of fresh W-38 medium. The washed cell pellet was then resuspended in 20 mL of fresh W-38 medium and examined under the fluorescence microscope. All suspensions were brightly fluorescent before washing away the external fluorescent macromolecules, but only the cells that had participated in endocytosis remained fluorescent after the washing procedure.

Preparation of ^{125}I-Labeled Molecules

To radioiodinate polygalacturonic acid it was necessary first to derivatize it with a tyrosine residue. Therefore, to 11 mL of a 1 mg/mL solution of polygalacturonic acid elicitor in H_2O was added 11 mg of tyrosine hydrazine. The reaction was allowed to proceed under inert (N_2) atmosphere for 24 hr. The resulting hydrazone was isolated via gel filtration on a PD-10 column (Pharmacia LKB Biotechnology Inc.) Spectroscopic and chromatographic analysis indicated that the expected polygalacturonic acid tyrosine hydrazone was harvested from this reaction. This derivative was found to retain its full activity as an elicitor in the pyranine oxidation assay (Low and Heinstein, 1986; Apostol, Heinstein, and Low, 1989).

To 1 mL of a 300 mM phosphate buffer, pH 7.0, containing 5 iodobeads (Pierce Chemical Co.) was added 0.8 mCi of ^{125}I-NaI (carrier-free in 1 N NaOH, Amersham), and the mixture was allowed to incubate for 5 min to liberate the active iodine species, according to the supplier's instructions. After activation, 2 mg of polygalacturonic acid tyrosine hydrazone in 0.5 mL of iodination buffer was added. The iodination was allowed to proceed with stirring for 20 min. After the iodination was completed, the product was isolated via gel filtration on a PD-10 column. Typical iodinations were approximately 70% efficient, resulting in products

emitting 1×10^5 cpm/μg. ^{125}I-Labeled green pea fowl ovomucoid was prepared in a similar fashion, except that the iodination was 50% efficient, and resulted in products emitting 1.2×10^5 cpm/μg.

Assay for the Uptake of ^{125}I-Labeled Macromolecules

Soybean suspension culture cells in the early exponential growth phase were obtained as previously described. To each culture was added sufficient ^{125}I-labeled macromolecule to achieve a final concentration of 10 μg/mL, and the suspension was incubated at either 23°C or 4°C for the desired time. After incubation, 0.5 mL of cells was removed, filtered, washed with 200 volumes of W-38 growth medium, and placed in counting vials for determination of ^{125}I-ligand content.

Isolation of Internalized ^{125}I-Labeled Polygalacturonic Acid Tyrosine Hydrazone from Plant Cells via Gel Filtration Chromatography

Soybean cell suspension cultures that had been incubated for 40 min with ^{125}I-labeled polygalacturonic acid were washed with growth medium, homogenized in 10% sodium dodecyl sulfate-containing buffer to break intermolecular interactions, centrifuged (3000*g* for 5 min) to remove cell wall fragments and cell debris, and chromatographed on a Bio-Gel P-6 column (1 × 15 cm, Bio-Rad). The samples were eluted with 0.125 M imidazole-HCl, pH 7.0, containing 300 mM NaCl. The retention time of the radioactive species in the plant cell extract was compared with uninternalized ^{125}I-polygalacturonic acid eluted in an identical fashion.

ACKNOWLEDGMENTS

We are grateful to Drs. Michael Laskowski, Jr. and Izydor Apostol for the green pea fowl ovomucoid. This work was supported by National Science Foundation grant DCB-8811465.

Received July 17, 1989; revised August 28, 1989.

REFERENCES

Apostol, I., Heinstein, P.F., and Low, P.S. (1989). Rapid stimulation of an oxidative burst during elicitation of cultured plant cells: Role in defense and signal transduction. Plant Physiol. **90,** 109–116.

Apostol, I., Low, P.S., Heinstein, P.F., Stipanovic, D., and Altman, D.W. (1987). Inhibition of elicitor-induced phytoalexin formation in cotton and soybean cells by citrate. Plant Physiol. **84,** 1276–1280.

Cosio, E.G., Popperyl, H., Schmidt, W.E., and Ebel, J. (1988). High affinity binding of fungal β-glucan fragments to soybean (*Glycine max* L.) microsomal fractions and protoplasts. Eur. J. Biochem. **175,** 309–315.

Cram, W.J. (1980). Pinocytosis in plants. New Phytol. **84,** 1–17.

Hicks, G.R., Rayle, D.L., and Lomax, T.L. (1989). The *Diagetropica* mutant of tomato lacks high specific activity auxin binding sites. Science **245,** 52–54.

Hillmer, S., Depta, H., and Robinson, D.G. (1986). Confirmation of endocytosis in higher plant protoplasts using lectin-gold conjugates. Eur. J. Cell Biol. **41,** 142–149.

Hübner, R., Depta, H., and Robinson, D.G. (1985). Endocytosis in maize root cap cells: Evidence obtained using heavy metal salt solutions. Protoplasma **129,** 214–222.

Joachim, S., and Robinson, D.G. (1984). Endocytosis of cationic ferritin by bean leaf protoplasts. Eur. J. Cell Biol. **34,** 212–216.

Low, P.S., and Heinstein, P.F. (1986). Elicitor stimulation of the defense response in cultured plant cells monitored by fluorescent dyes. Arch. Biochem. Biophys. **249,** 472–479.

Mersey, B.G., Griffing, L.R., Rennie, P.J., and Fowke, L.C. (1985). The isolation of coated vesicles from protoplasts of soybean. Plant **163,** 317–327.

Nothnagel, E.A., McNeil, M., Albersheim, P., and Dell, A. (1983). Host pathogen interactions: XXII. A galacturonic acid oligosaccharide from plant cell wall elicits phytoalexins. Plant Physiol. **71,** 916–926.

Pastan, I., and Willingham, M.C. (1985). Endocytosis. (New York: Plenum Press).

Schlessinger, J. (1980). The mechanism and role of hormone-induced clustering of membrane receptors. Trends Biochem. Sci. **5,** 210–214.

Schmidt, W.E., and Ebel, J. (1987). Specific binding of a fungal glucan phytoalexin elicitor to membrane fraction from soybean *Glycine max*. Proc. Natl. Acad. Sci. USA **84,** 4117–4121.

Sharp, J.K., McNeil, M., and Albersheim, P. (1984). The primary structures of one elicitor-active and seven elicitor-inactive hexa-(β-D-glucopyranosyl)-D-glucitols isolated from the mycelial cell walls of *Phytophthera megasperma* f. sp. glycinea. J. Biol. Chem. **259,** 11321–11336.

Tanchak, M.A., Griffing, L.R., Mersey, B.G., and Fowke, L.C. (1984). Endocytosis of cationized ferritin by coated vesicles of soybean protoplasts. Planta **162,** 481–486.

Van den Bulcke, M., Bauw, G., Castresana, C., Van Montagu, M., and Vandekerckhove, J. (1989). Characterization of vacuolar and extracellular β-(1,3)-glucanases of tobacco: Evidence for a strictly compartmentalized plant defense system. Proc. Natl. Acad. Sci. USA **86,** 2673–2677.

Wheeler, H., and Hanchey, P. (1971). Pinocytosis and membrane dilation in uranyl-treated plant roots. Science **171,** 68–71.

Yoshikawa, M., Keen, N.T., and Wang, M.C. (1983). A receptor on soybean membranes for a fungal elicitor of phytoalexin accumulation. Plant Physiol. **73,** 497–506.