Plant Physiol. (1991) 96, 705–712
0032-0889/91/96/0705/08/$01.00/0

Received for publication November 13, 1990
Accepted February 16, 1991

Heterogeneity and Cell Type-Specific Localization of a Cell Wall Glycoprotein from Carrot Suspension Cells[1]

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ABSTRACT

EP1, an extracellular protein from carrot (*Daucus carota*) cell suspensions, has been partially characterized by means of an antiserum and a cDNA clone. In both embryo and suspension cultures different molecular mass EP1 proteins were detected, some of which (31, 32, 52, and 54 kilodaltons) were bound to the cell wall and released into the medium, whereas others (49, 60, and 62 kilodaltons) were more firmly bound to the cell wall and could be extracted with a salt solution. Immunoprecipitation of *in vitro* translation products revealed a single primary translation product of 45 kilodaltons, suggesting that EP1 heterogeneity is due to differential posttranslational modification. In seedlings organ-specific modification of EP1 proteins was observed, a phenomenon which did not persist in suspension cultures initiated from different seedling organs. In culture EP1 proteins were only found to be associated with vacuolated, nonembryogenic cells, and on these cells they were localized in loosely attached, pectin-containing cell wall material. Purified 52/54 kilodaltons EP1 proteins did not alleviate the inhibitory effect of the glycosylation inhibitor tunicamycin on somatic embryogenesis.

Embryogenic carrot (*Daucus carota*) suspension cultures grown at a high cell density in the presence of 2,4-D contain a variety of cells, ranging from different kinds of single cells through small cell clusters to large cell clumps. Among these are meristematic cell clusters, referred to as proembryogenic masses (11), which are able to develop somatic embryos upon dilution in auxin-free medium. Embryo cultures are initiated from a culture fraction enriched for these proembryogenic masses, providing a system of high frequency somatic embryogenesis that is widely used as a model system to study plant embryogenesis (20).

Three observations suggest that proteins secreted into the culture medium play an important role in somatic embryo development. First, a number of embryogenic cell lines all secrete a characteristic set of proteins into the medium, whereas the extracellular protein patterns of nonembryogenic mutant cell lines deviate from this characteristic pattern (2). Second, the acquisition of embryogenic potential in a newly initiated culture has been shown to be accelerated by the addition of high-molecular weight, heat-labile components from an established embryogenic cell line (3). Third, inhibition of somatic embryogenesis with the glycosylation inhibitor tunicamycin can be complemented by the simultaneous addition of extracellular proteins from an uninhibited embryo culture (2).

To obtain more information about the nature of cell suspension extracellular proteins, we screened a carrot λgt11 cDNA expression library with an antiserum raised against total embryo medium proteins. In this report we describe the initial characterization of one of the clones obtained, termed EP1,[4] employing an antiserum raised against the EP1-encoded fusion protein. We show that the EP1 mRNA codes for a small number of secreted glycoproteins that are associated with cell walls of vacuolated cells in culture and that the observed heterogeneity of these proteins appears to arise from different posttranslational modifications.

MATERIALS AND METHODS

Plant Material and Culture Conditions

Embryogenic high-density suspension cultures of carrot (*Daucus carota*, cell line "10" [2]) were initiated from cut seedling roots (3), maintained in liquid B5 medium (10) containing 2,4-D and subcultured with 14-d intervals at an initial cell density of 1.10^6 cells/mL. Seven days after subculturing embryo cultures were initiated by inoculating a culture fraction enriched for proembryogenic masses at a density of 2.10^4 cells/mL in B5 medium. Inhibition/complementation assays with tunicamycin were performed as described previously (2). High-density suspension cultures from fennel (*Foeniculum vulgare*), parsley (*Petroselinum crispum*), and caraway (*Carum carvi*) were initiated from seeds obtained locally and maintained under the same conditions as the carrot 10 cell line. Tomato (*Lycopersicon esculentum*) suspensions were cultured in R3B medium (18). Carrot seedlings were grown from "Flakkese" SG766 Trophy seeds and dissected when the first leaves had fully expanded. Zygotic embryos were ob-

[1] This work was supported in part by a grant awarded to S. C. de V. (NL 0092) in the framework of the Biotechnology Action Program of the European Economic Community.

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[4] Abbreviations: EP1, extracellular protein 1; TBS, Tris-buffered saline; SSC, 0.15 M NaCl/15 mM Na citrate (pH 7.0).

tained by gently squeezing embryos from seeds after imbibing for 2 h. Pericarps and seedcoats were removed from the remaining seeds and the endosperms collected. Tissues were immediately frozen and stored at −80°C.

Protein Isolation and Immunoblot Analysis

Medium protein samples were prepared by passing conditioned medium through a 0.22-μm filter and concentrating by pressure dialysis (2). Cell wall proteins were isolated from living cells by washing once with fresh culture medium followed by incubation in 0.1 M $CaCl_2$ on ice for 20 min. Cells were removed by centrifugation, the solution was passed through a 0.22-μm filter and mixed with two volumes of ethanol. Precipitated proteins were collected by centrifugation, vacuum-dried, and dissolved by boiling in SDS-PAGE sample buffer (14) for 5 min. The viability of $CaCl_2$-extracted cells was determined by Lysamine Green staining and found to be similar to nontreated cells, indicating that contamination of cell wall protein preparations with cytoplasmic proteins was negligible. For the isolation of total cellular protein, frozen plant tissue was ground in SDS-PAGE sample buffer, boiled for 5 min, and centrifuged to remove insoluble material. The anti-EP1 serum was obtained by immunizing a rabbit with the EP1-encoded β-galactosidase fusion protein isolated by preparative SDS-PAGE from *Escherichia coli* Y1089 lysogenized with λgt11-EP1 phage (12). A control serum was raised against β-galactosidase isolated from a wild-type λgt11 lysogen. Immunoblot analysis was carried out as described by Burnette (1), using 12.5% polyacrylamide gels and antisera in a 2000- to 3000-fold dilution.

In Vivo Labeling, *in Vitro* Translation, and Immunoprecipitation

Proteins were pulse-labeled *in vivo* by growing cells for 18 h in the presence of *N*-acetyl-D-[1-^{14}C]glucosamine (Amersham) or L-[^{35}S]methionine (NEN). In one experiment tunicamycin (Calbiochem) was added 1 h before pulse labeling. In pulse-chase experiments, cold L-methionine to a concentration of 10 mM was added after 16 h pulse labeling. Medium samples were taken after 0, 6, or 90 h and immunoprecipitated (see below). For *in vitro* translations 0.5 μg of poly(A$^+$) RNA was incubated for 30 min at 30°C in 10 μL reticulocyte lysate (Green Hectares) with [^{35}S]methionine. Labeled proteins were adjusted to 0.5 × SDS-PAGE sample buffer, boiled for 1 min, and centrifuged (5 min, 14,000*g*). Immunoprecipitation of the supernatant was then carried out as described by Franssen *et al.* (8).

cDNA Library Screening, RNA Isolation, and Northern Blotting

The λgt11 cDNA library was prepared with RNA from an unfractionated embryo culture and was kindly provided by Dr. T. L. Thomas (Texas A&M University, College Station, TX). This library was screened with an antiserum raised against concentrated embryo culture medium according to the protocol described by Huynh *et al.* (12). Alkaline phosphatase-conjugated goat-anti-rabbit IgG was used to detect positive plaques. Total RNA was isolated as described by de Vries *et al.* (4). PolyA(+) RNA was selected by oligo(dT)cellulose chromatography according to standard procedures (16). For RNA gel blot analysis, RNA was denatured with DMSO/glyoxal, electrophoresed on agarose gels, and transferred to Gene Screen membranes (New England Nuclear). Hybridization of the filters was carried out according to the manufacturer's instructions.

Immunofluorescence Labeling

Cells were washed with fresh medium and fixed in 96% ethanol for 2 min., followed by 2% (w/v) paraformaldehyde in 10 mM sodium phosphate for 10 min. Fixed cells were washed once with 10 mM Tris-HCl, 150 mM NaCl (pH 7.5) (TBS), blocked for 1 h with 2% (v/v) calf serum in TBS, and incubated for 2 h in blocking solution containing antiserum (1:500) or JIM5 monoclonal antibody supernatant (1:50 [13]). Subsequently, the cells were washed with TBS, incubated for 30 min in blocking solution with fluorescein isothiocyanate-conjugated second antibody (1:50), washed twice with TBS, and mounted for microscopy.

Purification of Medium EP1 Proteins

Conditioned medium from a 10 d embryo culture was filtered through a 0.2 μm filter. The filtrate was adjusted to pH 8.5 with solid Tris, added to DEAE-sepharose FF (Pharmacia), and incubated for 1 h at 4°C. All buffers used in subsequent steps contained 25 mM Tris-HCl (pH 8.5). Bound proteins were eluted with 0.2 M KCl, diluted, applied to a DEAE-sepharose FF column, and eluted with a gradient from 0 to 250 mM KCl. Final purification was done by Mono Q anion exchange FPLC (Pharmacia) with the same KCl gradient.

RESULTS

Cell Suspensions Contain Multiple, Differentially Modified EP1 Protein Forms

Screening of a λgt11 cDNA library with an antiserum raised against total embryo culture medium proteins yielded several positive cDNA clones. Rabbits were immunized with fusion proteins prepared from lysogens of positive phages to identify the corresponding medium protein. The serum raised against the fusion protein encoded by clone EP1 recognized a doublet with an M_r of 52,000 and 54,000 on a protein gel blot of conditioned medium proteins from both high-density suspension and embryo cultures (Fig. 1A). These proteins correspond to the 52/54 kD proteins that were previously observed in carrot culture media (2, 3). An additional doublet of 31/32 kD was recognized in the medium of cultures with a high cell density, regardless of the presence of 2,4-D (Fig. 1A). The effect of cell density on the appearance of these proteins was confirmed by a serial dilution of proliferating cell suspensions, which showed an increasing amount of 31/32 kD proteins in register with the increase in initial cell density from 1.10^4 to 1.10^7 cells/mL (Fig. 1B).

To test whether EP1 proteins are cell wall proteins that become leached from cell walls by the culture medium, we

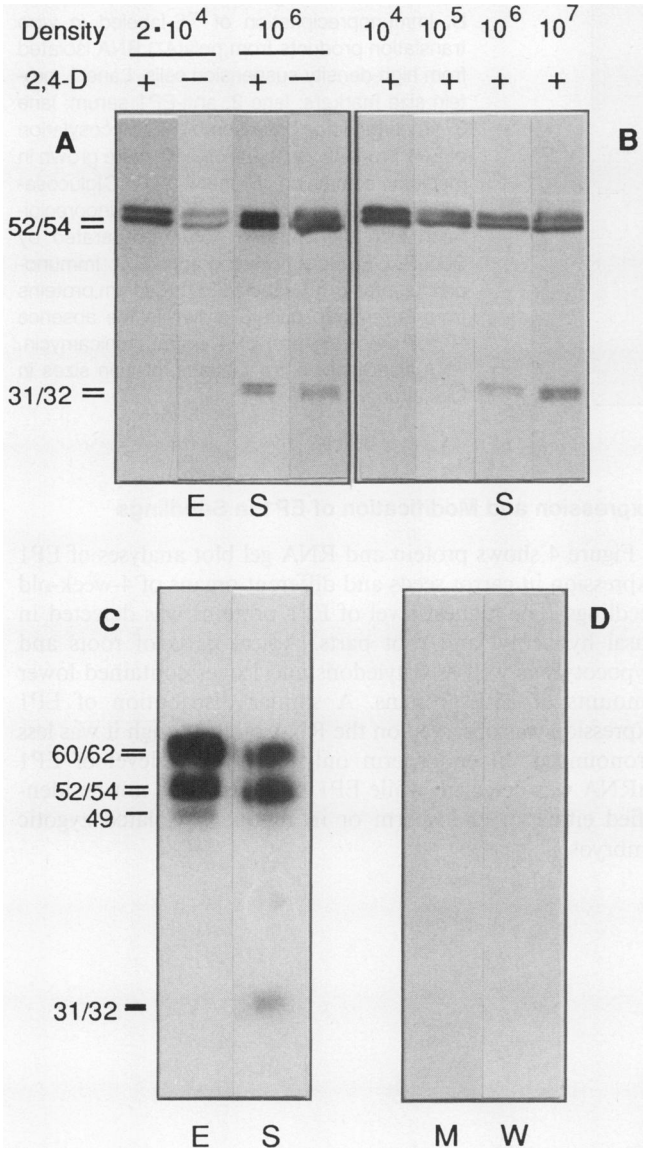

Figure 1. Analysis of EP1 proteins in conditioned media and cell walls. A, B, SDS-PAGE immunoblots of conditioned medium proteins from carrot cell suspensions, 7 d after transfer to fresh culture medium. The blot was developed with the anti-EP1 serum. Initial cell densities (cells/mL) and the presence (+) or absence (−) of 2 μM 2,4-D are listed across the top. Lanes marked E and S below the figure correspond to conditions used for embryo and suspension culture, respectively. Lanes contain equal amounts of protein. C, Occurrence of EP1 proteins in cell walls. Anti-EP1 immunoblots of cell wall proteins extracted with calcium chloride from cells in a 7-d-old suspension (S) or embryo (E) culture. Lanes contain 5 μg of cell wall protein. Sizes are indicated in kilodaltons. D, Immunoblot of medium (M) and cell wall (W) proteins from a high-density suspension culture, developed with anti-β-galactosidase control serum.

extracted living suspension cells with a calcium chloride solution. With this method ionically bound cell wall proteins can be isolated without contaminating cytoplasmic proteins (21). Immunoblotting showed that the medium EP1 proteins of 31/32 and 52/54 kD are indeed present in the cell wall (Fig. 1C). Repeated washing of the cells with fresh culture medium before calcium extraction removed these proteins from the wall (data not shown), indicating that the binding of these proteins to the cell wall is weak. Three additional EP1 polypeptides of 49, 60, and 62 kD that were not found in the medium, were also detected in the cell wall, but could not be rinsed from the wall with culture medium.

Since plant cells are known to secrete β-galactosidase (19), the complex pattern of EP1 proteins could have resulted from antibodies directed against the β-galactosidase part of the EP1 fusion protein. This possibility was ruled out by a control antiserum raised against *E. coli* β-galactosidase, which did not recognize any medium or cell wall proteins (Fig. 1D). Furthermore, it is important to stress that the anti-EP1 serum was raised against a protein that was synthesized in *E. coli* and, thus, does not contain antibodies directed against glycoprotein oligosaccharide side chains.

The occurrence of a set of seven anti-EP1-reactive proteins of different sizes raised the question whether these proteins are derived from different mRNAs or arise through posttranslational modification. Low-stringency hybridization of RNA from high-density suspension cells with the EP1 cDNA revealed only a single mRNA band of 1.6 kb (Fig. 2A). In addition, when ^{35}S-labeled *in vitro* translation products of poly(A$^+$) RNA from high-density suspension cells were immunoprecipitated with the anti-EP1 serum, only one polypeptide band of 45 kD was detected (Fig. 2B). The anti-β-galactosidase control serum did not recognize any polypeptides (Fig. 2B). Glycosylation of the 49, 52/54, and 62 kD proteins was demonstrated by immunoprecipitation of cell wall proteins from a high-density suspension culture pulse-labeled with *N*-acetyl-D-[1-^{14}C]glucosamine (Fig. 2C). The 31/32 and 60 kD proteins were not labeled and may not have accumulated above the detection level during the 18 h labeling period. After [^{35}S]methionine pulse-labeling the same pattern was observed, which remained unaltered after chase periods of 6 to 90 h (not shown). The addition of tunicamycin, an inhibitor of *N*-glycosylation, resulted in the synthesis of only one medium form of 46 kD (Fig. 2D), suggesting that the size difference between the 52 and 54 kD EP1 proteins is caused by differences in *N*-glycosylation. Deglycosylation of purified 52/54 kD proteins with trifluoromethanesulfonic acid (5) yielded a single broadband that was too diffuse to confirm this observation (not shown). Taken together these results provide strong evidence that the size heterogeneity of EP1 proteins arises from different posttranslational modifications of a single precursor polypeptide.

Conservation of EP1 Epitopes

Total protein extracts from suspension cells of three other members of the *Umbelliferae,* fennel, caraway, and parsley, contained proteins with an M_r between 50,000 and 65,000 that were recognized to different extents by the anti-EP1 serum (Fig. 3). The similarity of the carrot and parsley patterns

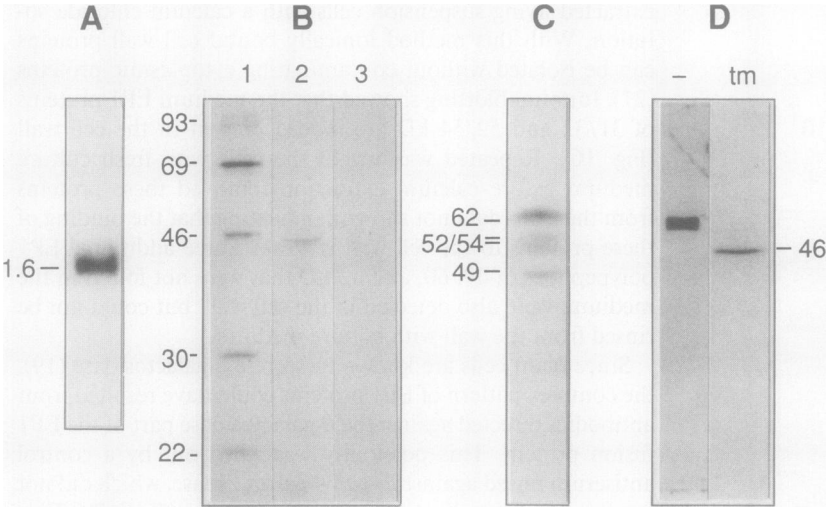

Figure 2. A, RNA gel blot analysis of 10 μg of total RNA from high-density suspension cells, hybridized with the 650 base pair EP1 cDNA insert. Final washing was in 2 × SSC[4] at 50°C. B, Immunoprecipitation of ^{35}S-labeled *in vitro* translation products from poly(A$^+$) RNA isolated from high-density suspension cells. Lane 1, protein size markers; lane 2, anti-EP1 serum; lane 3, anti-β-galactosidase serum. C, Glycosylation of EP1 proteins. Suspension cells were grown in medium containing *N*-acetyl-D-[1-^{14}C]glucosamine. Cell wall proteins were immunoprecipitated with the anti-EP1 serum, separated by SDS-PAGE and autoradiographed. D, Immunoprecipitation of ^{35}S-labeled EP1 medium proteins from an embryo culture grown in the absence (–) or presence (tm) of 4 μg/mL tunicamycin. RNA size is given in kilobases, protein sizes in kilodaltons.

was striking and suggests that between these species not only the recognized epitope but also a major part of the modification of the protein is conserved. Apparently, the EP1-homologous proteins in these species are more strongly bound to the cell wall, because they were not found in the medium (Fig. 3). Homologous proteins were also detected in media from suspension cultures of barley (K Nielsen, Risø, Roskilde, Denmark, personal communication) as well as in *Citrus* embryo cultures, but not in nondifferentiating *Citrus* cultures (R Fluhr, Weizmann Institute, Rehovot, Israel, personal communication). No anti-EP1-reactive proteins were found in a tomato suspension culture (Fig. 3, lanes 1, 6).

Expression and Modification of EP1 in Seedlings

Figure 4 shows protein and RNA gel blot analyses of EP1 expression in carrot seeds and different organs of 4-week-old seedlings. The highest level of EP1 proteins was detected in basal hypocotyl and root parts. Apical parts of roots and hypocotyls as well as cotyledons and leaves contained lower amounts of EP1 proteins. A similar distribution of EP1 expression was observed on the RNA blots, though it was less pronounced. In endosperm only a very low level of EP1 mRNA was detected, while EP1 proteins could not be identified either in endosperm or in manually isolated zygotic embryos.

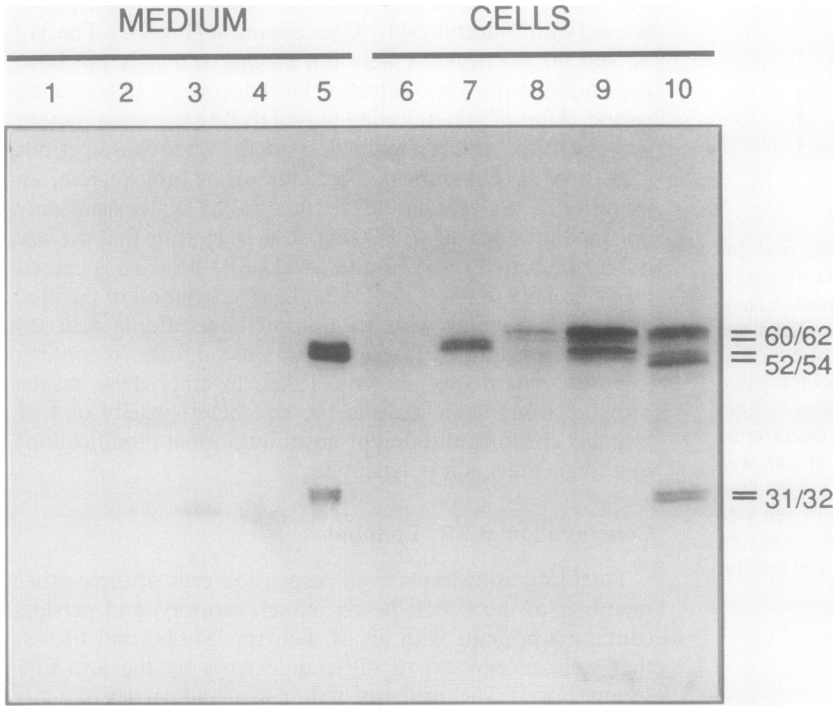

Figure 3. Conservation of EP1 epitopes. SDS-PAGE immunoblot of medium (lanes 1–5) or cellular (lanes 6–10) proteins isolated from high density suspension cultures of tomato (lane 1, 6), fennel (lane 2, 7), caraway (lane 3, 8), parsley (lane 4, 9), or carrot (lane 5, 10), developed with the anti-EP1 serum. Protein sizes are given in kilodaltons.

Striking differences in EP1 protein patterns were observed in the different seedling organs (Fig. 4A). Roots contained 52/54 and 60/62 kD doublets, but not the 31/32 and 49 kD proteins present in suspension cultures. The latter proteins were not found in any other parts of the seedlings. In hypocotyls and cotyledons only the 60 and 62 kD proteins coexisted with a low amount of a 34 kD polypeptide which itself was neither encountered in roots nor in cultured cells. Leaves did not contain any of the EP1 proteins observed in suspension cultures, but only produced the 34 kD polypeptide, irrespective of the degree of leaf expansion.

Pattern of EP1 Proteins in Cell Suspensions Is Not Related to the Source of the Explant

The similarity of the EP1 protein pattern in roots and root-derived suspension cultures suggests that the EP1-producing cells of the explant persist in tissue culture and maintain, at least in part, the original modification pattern. Since in leaves none of the EP1 forms characteristic of cell suspensions are present, a suspension culture was initiated from primary leaf to see whether the subsequent suspension culture had the EP1 modification pattern of the explant. Except for a relatively low level of 31/32 kD proteins in the cell wall, the pattern of EP1 medium and cell wall proteins in the leaf-derived embryogenic culture was indistinguishable from that seen in a root-derived culture. This indicates that the observed modification of EP1 proteins in carrot cell suspensions is not inherited from the source of the explant but appears to be characteristic of suspension culture growth.

52/54 kD EP1 Proteins Do Not Complement Tunicamycin Inhibition of Somatic Embryogenesis

Previously, we have postulated that the 52/54 kD medium proteins may be responsible for the complementation with extracellular glycoproteins of tunicamycin-inhibited somatic embryogenesis (2). Therefore, the 52/54 kD EP1 proteins were purified and tested at different concentrations in tunicamycin-inhibited cultures (Table I). No significant tunicamycin-complementing activity was observed with purified 52/54 kD proteins, indicating that, by themselves, these proteins are not sufficient to prevent proembryogenic mass disruption as observed with total unfractionated medium proteins.

Table I. *Effect of Purified 52/54 kD EP1 Medium Proteins on Inhibition of Somatic Embryogenesis by Tunicamycin*

Additions	Somatic Embryos[a]
None	100 ± 5
tm[b]	7 ± 3
tm + total medium concentrate	64 ± 7
tm + purified 52/54 kD EP1 proteins	11 ± 3[c]

[a] Mean of three assays ± SE. The number of somatic embryos in control cultures was set at 100%. [b] tm, 0.75 μg/mL tunicamycin. [c] Highest value of four different concentrations.

EP1 Proteins are Associated with Cell Walls of Nonembryogenic Vacuolated Cells in Culture

The finding that EP1 proteins are not present in zygotic embryos (Fig. 4A) prompted us to investigate the distribution of EP1 proteins between different cell types in embryo and suspension cultures. Both in suspension and in embryo cultures (Fig. 5A) EP1 cell wall proteins were associated only with the vacuolated single cell population and not with somatic embryos, proembryogenic masses, or large callus clumps. Also, the EP1 mRNA was only present in single cells of an embryo culture (Fig. 5B).

To analyze the cells containing the EP1 proteins in more detail, intact suspension cells were subjected to anti-EP1

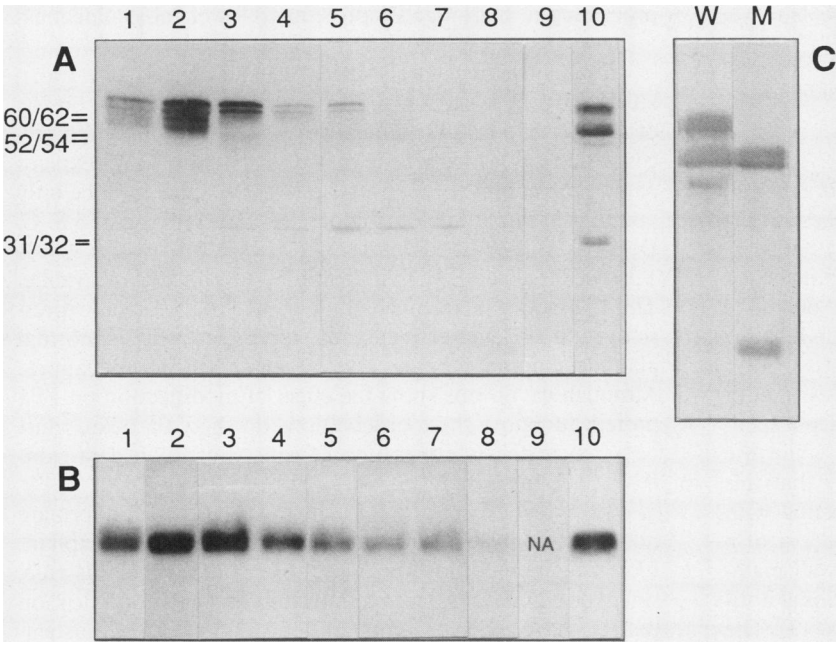

Figure 4. Expression and modification of EP1 in seedlings and suspension cultures. A, Protein gel blot of 25 μg of total protein isolated from seedling root tips (lane 1), basal part of roots (lane 2), hypocotyls (lane 3), shoot apices (lane 4), cotyledons (lane 5), young leaves (lane 6), expanded leaves (lane 7), endosperm (lane 8), seed embryos (lane 9). For comparison suspension cells are included in lane 10. The blot was developed with the anti-EP1 serum. Protein sizes are in kilodalton. B, RNA gel blot of 10 μg of total RNA from different seedling tissues and suspension cultures as indicated in (A), hybridized with the EP1 cDNA insert. Final washing was in 0.5 × SSC at 50°C. NA = not analyzed. C, Modification of EP1 proteins in a leaf-derived suspension culture. Anti-EP1 immunoblot of proteins from a suspension culture initiated from seedling primary leaf. W, cell wall proteins; M, medium proteins.

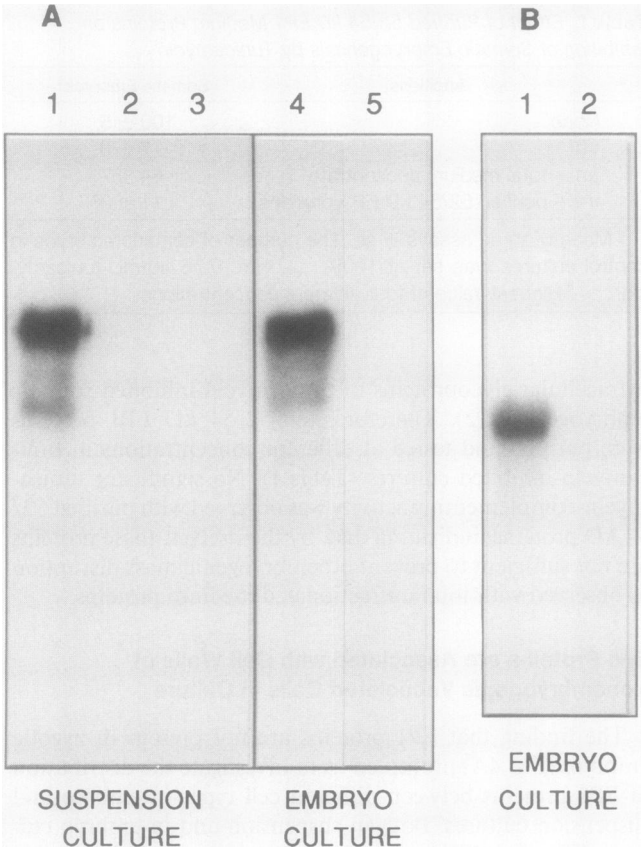

Figure 5. Distribution of EP1 expression in cell suspensions. A, Protein gel blot. Seven day suspension (lanes 1–3) or embryo (lanes 4 and 5) cultures were fractionated by sieving and Percoll gradient centrifugation or manual selection. Total protein was isolated from different fractions, subjected to SDS-PAGE, and immunoblotted with the anti-EP1 serum. Lane 1: <50 μm, <20% Percoll, vacuolated single cells. Lane 2: 50 to 125 μm, dense cell clusters were picked up manually. Lane 3: >170 μm, >20% Percoll, large dense cell clumps. Lane 4: <125 μm, <20% Percoll, vacuolated single cells. Lane 5: >125 μm, 30–40% Percoll, globular to torpedo-stage embryos. B, RNA gel blot of total RNA isolated from embryo culture single cells (<50 μm, lane 1) and somatic embryos (lane 2), hybridized with EP1 cDNA insert. Final washing was in 0.5 × SSC at 50°C.

immunofluorescence. The anti-EP1 serum labeled cell wall material which was present mostly as diffuse patches on the surface of vacuolated single cells (Fig. 6, A and B). Strongly reactive patches of this material that were completely detached from the cells were also frequently observed (Fig. 6C). The appearance of this material was not a fixation or labeling artefact, since identical structures were observed attached to (Fig. 6D) or detached from cells before fixation. The amount of EP1-positive cell surface material in a high density suspension culture decreased with age of the culture, while the amount of free wall material increased, suggesting that this material detaches from the cells. Nonspecific labeling with the anti-β-galactosidase control serum was negligible (Fig. 6E). To investigate the nature of the EP1-reactive cell wall material, cultures were immunolabeled with the monoclonal antibody JIM5, which is specific for unesterified pectins (13). The JIM5 antibody was found to label structures similar to those labeled by the EP1 serum (Fig. 6, *cf.* F and C), leading us to conclude that EP1 proteins are bound to pectinaceous cell wall material which detaches from cells growing in liquid culture.

DISCUSSION

In this paper we describe the initial characterization of EP1, a set of cell wall glycoproteins occurring in carrot cell suspensions as well as in seedlings. In high-density suspension cells seven EP1 proteins of different sizes occur, while the cells in these cultures contain mRNA encoding a single-size *in vitro* translation product. These observations imply that EP1 size heterogeneity is introduced posttranslationally by differential modification.

N-Glycosylation is likely to be one mechanism responsible for EP1 heterogeneity, since a single 46 kD medium protein instead of the normal 52/54 kD doublet occurs in the medium in the presence of tunicamycin, an inhibitor that prevents the addition of glycans to asparagin residues (6). The wall-bound forms may be glycosylated differently from the 52/54 kD glycoproteins, which could be the basis of their enhanced wall-binding (7). The sizes of the high-M_r EP1 glycoproteins are probably overestimations, since it has been found that glycosylated proteins migrate more slowly in SDS-PAGE than unglycosylated proteins of the same mass (15). Thus, only a small number of oligosaccharide side chains could be sufficient to increase the apparent M_r to 52, 54, 60, or 62 kD. If we assume signal peptide cleavage from the 45 kD primary translation product to a polypeptide of 41 to 43 kD, the 46 kD protein produced in the presence of tunicamycin may contain modifications other than *N*-glycosylation. We have not identified the nature of these modifications yet. Because of the size of the 31/32 kD doublet, about 10 kD smaller than the putative 41 to 43 kD precursor, we assume that proteolytic processing is responsible for the formation of these proteins. However, [^{35}S]methionine pulse-chase labeling did not reveal processing of high-M_r EP1 proteins to lower molecular mass forms.

Although we detect only one EP1 band after *in vitro* translation, we cannot exclude the possibility that this band consists of allelic isoforms that differ in their ability to undergo glycosylation or proteolytic cleavage. This could only explain part of the complex EP1 pattern, as there appears to be only one EP1 gene per haploid genome (F. van Engelen and S. C. De Vries, unpublished data).

In seedlings the EP1 modification pattern is organ specific. Thus, in addition to organ- and tissue-specific gene expression, another level of regulation appears to exist in normal plant development that involves organ-specific modifications. Although we do not know the effect of modification on EP1 protein function, these observations do show that care must be taken in the interpretation of gene expression data from RNA gel blots only.

With a few exceptions (17), organ-specific phenotypes are not inherited by cell cultures initiated from an organ explant. EP1 protein modification appears to conform to this rule, since roots and leaves, which show different EP1 modification patterns, give rise to the same modification pattern in suspen-

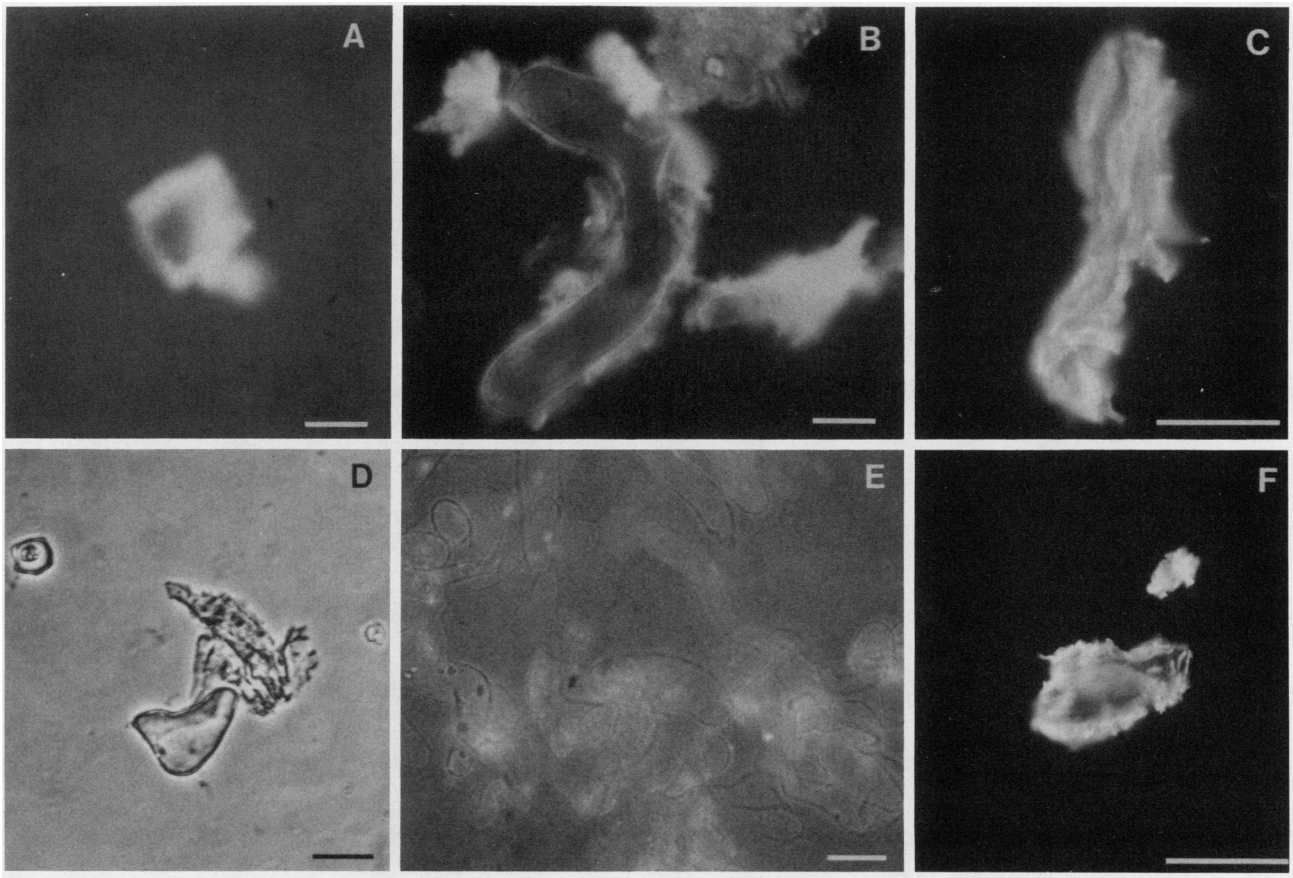

Figure 6. Immunolocalization of EP1 proteins and pectins in suspension cells. Fixed intact cells were treated with anti-EP1 serum (A–C), anti-β-galactosidase control serum (E) or pectin-specific JIM5 monoclonal antibody (F). A and B show EP1-reactive material on the cell surface and in C this material is detached from the cell. Free wall material also labels with JIM5 antibody (F). D is a phase contrast image of a cell that has not undergone any fixation or antibody treatment.

sion culture. As suggested above, the formation of the low-M_r EP1 proteins probably depends on extracellular protease activity. This activity may be high in leaves, but is substantially diluted in culture medium. Thus, the observed changes in modification pattern may not reflect changes in gene expression, but represent a cell suspension artefact.

Satoh and Fujii (22) have described the purification of GP57, a 57 kD extracellular protein from carrot suspensions which is glycosylated and exists in two forms which are secreted by nonembryogenic cells, suggesting homology or identity with EP1. This was not the case, as the anti-EP1 antiserum did not react with purified GP57 and a comparison of EP1 and GP57 sequence data did not reveal homology between these proteins (S Satoh, personal communication).

We have only few clues to the biochemical function of the EP1 proteins. Its conservation in both dicots and monocots suggests that it is a biologically important molecule. The partial EP1 amino acid sequence deduced from the cDNA sequence has not revealed homology with known proteins (F. van Engelen and S. C. De Vries, unpublished data). The EP1 proteins are abundantly present in cell walls of vacuolated cells in culture and in basal parts of seedling roots and hypocotyls, but their level is reduced in both apices of seedlings. This would favor a role in cell elongation, which is thought to be regulated by formation and cleavage of bonds between cell wall polymers (9). Obviously, cell expansion is restricted in proembryogenic masses and early stages of developing embryos, which could explain the absence of cell wall EP1 proteins from these structures. Nevertheless, they are immersed in medium containing the 52/54 (and 31/32) kD proteins, which apparently does not interfere with embryo development. Are these medium EP1 proteins inactivated by modification or is a proper substrate required for EP1 protein function? Such questions can only be resolved after identification of the biochemical function of these proteins.

ACKNOWLEDGMENTS

We thank Paul Knox (John Innes Institute, Norwich, U.K.) for a generous gift of JIM5 monoclonal antibody, Peter van Druten for photography, and Anke de Jong, Roger Pennell, and Ben Scheres for stimulating discussions and useful comments on the manuscript.

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