Plant Physiol. (1986) 81, 931–933
0032-0889/86/81/0931/03/$01.00/0

Short Communication

Auxin-Controlled Glycoprotein Release into the Medium of Embryogenic Carrot Cells

Received for publication December 17, 1985 and in revised form April 11, 1986

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ABSTRACT

Glycoproteins released from carrot cells into culture media were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and visualized by staining with Coomassie brilliant blue or with the periodic-acid Schiff procedure. The appearance or disappearance of two glycoproteins of M_r 65,000 (GP65) and M_r 57,000 (GP57) was closely related to the formation of somatic embroys. GP65 was released specifically from embryogenic cells cultured in a medium without 2,4-dichlorophenoxyacetic acid, in which they can form somatic embryos. GP57 was released from the same embryogenic cells, if they were cultured in a medium with 2,4-dichlorophenoxyacetic acid, in which they cannot form somatic embryos. Nonembryogenic cells which cannot form somatic embryos, released only GP57.

Small carrot cell clusters suspended in auxin-containing medium can form somatic embryos at a high rate, when they are transferred to auxin-free medium (11). As a result of this finding, cultured carrot cells have been used as suitable material to investigate the mechanism of differentiation and biochemical events occurring during embryogenesis (2, 12). In animals, some quantitative and qualitative differences in extracellular glycoproteins have been reported between certain organs and *in vitro* cultured cells. Some of these glycoproteins have been shown to play important roles in cell-to-cell interactions as growth factors or as matrix components, and consequently in the differentiation of cells (6, 10).

In tobacco cells, the appearance of β(1,3)glucanese was shown to be closely related to hormones (5). The synthesis of the extracellular enzyme (14) is induced by auxin and strongly inhibited by cytokinin (4). In carrot cells, some glycoproteins such as peroxidase (1) and acid phosphatase (3) have been shown to be released into the medium.

These findings imply the existence of a correlation between the release of extracellular glycoproteins and the differentiation of cells. We report here variations in extracellular glycoproteins released by carrot cells which were cultured under different conditions, and discuss the implication of this finding in the morphogenesis of carrot cells.

MATERIALS AND METHODS

Plant Materials and Culture Conditions. Cell suspension of *Daucus carota* L. cv US-Harumakigosun was obtained from 1-month-old nodular callus which had been formed by culturing 1-week-old hypocotyl segments on MS[1] agar medium (9) containing 2,4-D (1 mg L^{-1}). Small pieces of the nodular callus were suspended in 100 ml of MS liquid medium containing 1 mg L^{-1} 2,4-D in a 300 ml flask and allowed to grow on a gyratory shaker (100 rpm) at 28°C in darkness. After 2 weeks of culture, the cell suspension was sieved through stainless steel mesh (1 mm in pore size), and the cell clusters remaining on the mesh were transferred to 100 ml of fresh medium containing 2,4-D. This operation was repeated every 2 weeks for 3 to 6 months, and the resulting cells were designated as embryogenic cells. The cell clusters passed through the mesh in the first sieving were also transferred to 100 ml of fresh medium containing 2,4-D. After repeating this operation every 2 weeks for 3 to 6 months, the resulting cells were designated as nonembryogenic cells.

Cell clusters were obtained from both the 2-week-old embryogenic and nonembryogenic cells by successive sieving through meshes. The cell clusters which passed through 63 μm mesh and

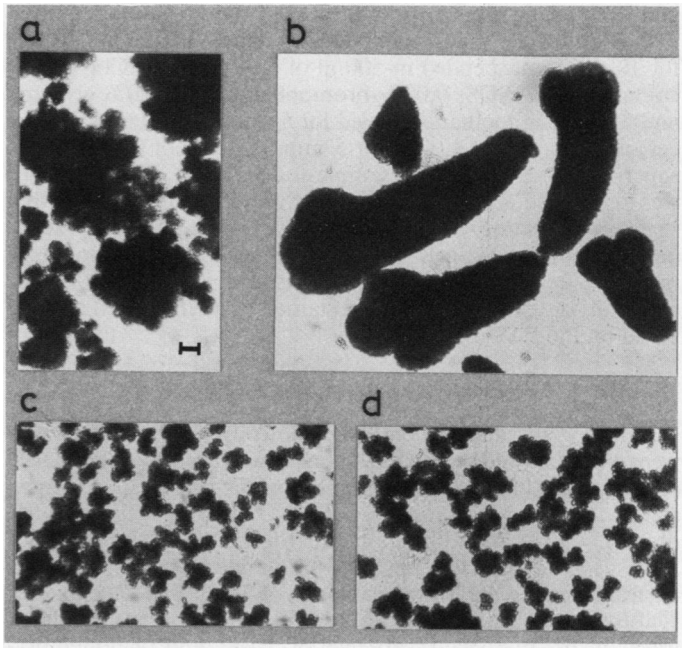

FIG. 1. Morphogenic features of embryogenic or nonembryogenic cells cultured in the presence or absence of 2,4-D. The cell clusters (37–63 μm) of embryogenic (a, b) or nonembryogenic cells (c, d) were cultured for 2 weeks in the presence (a, c) or absence (b, d) of 2,4-D (1 mg L^{-1}) as described in "Materials and Methods." Bar, 100 μm.

[1] Abbreviations: MS, Murashige and Skoog; GP65, M_r 65,000 glycoprotein; GP57, M_r 57,000 glycoprotein; PAS, periodic-acid Schiff.

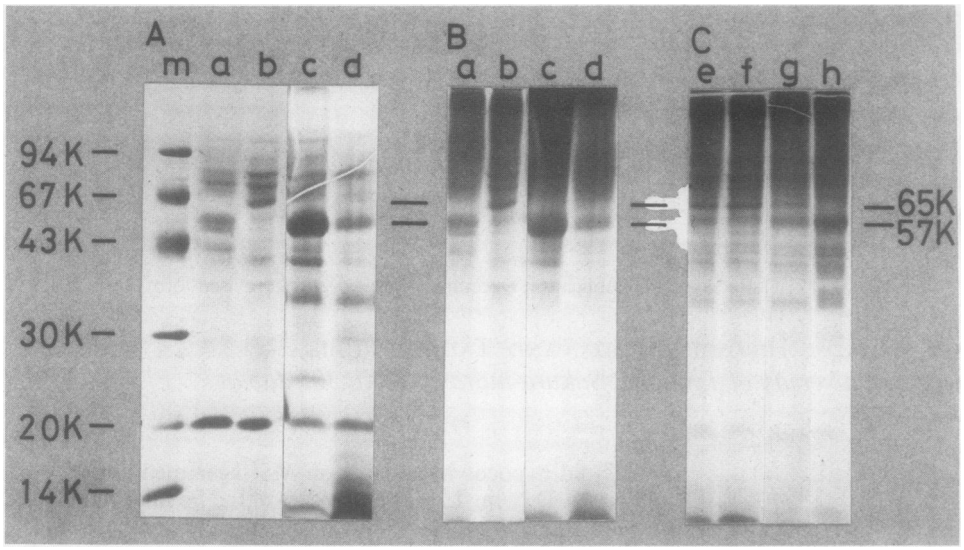

FIG. 2. Analysis by SDS-PAGE of glycoproteins in the medium under different culture conditions. The ethanol-insoluble macromolecules were obtained from the 10 ml media of 2-week-old cultures and subjected to SDS-PAGE. The gels were stained with Coomassie brilliant blue (A) or PAS reagent (B and C). The cells were cultured as described in Figure 1, and a, b, c, and d in the figure correspond to those in Figure 1. Effects of various concentrations of 2,4-D on the appearance of glycoproteins were shown in C. The embryogenic cells (37–63 μm) were cultured for 2 weeks in the absence (lane e) or presence of 10 nM (lane f), 0.1 μM (lane g), and 1 μM (lane h) 2,4-D. m, molecular weight markers: phosphorylase b (mol wt 94,000), BSA (mol wt 67,000), ovalbumin (mol wt 43,000), carbonic anhydrase (mol wt 30,000), trypsin inhibitor (mol wt 20,100), and β-lactalbumin (mol wt 14,400).

remained on 37 μm mesh, were rinsed 3 times with MS medium without 2,4-D, inoculated at a density of 0.2 to 0.5 μl packed cell volume (100*g*) per ml medium in a 300 ml flask containing 100 ml of liquid MS medium with or without various phytohormones and cultured on a gyratory shaker (100 rpm) at 28°C under dim light. After 2 weeks of culture, the culture media were separated from cells by filtration through a glass filter (Whatman GF/F) and used for further experiments. Morphogenesis was observed under a binocular microscope.

Preparation and Analysis of Glycoproteins in Culture Media. To the medium obtained, 2.5 volumes of ethanol were added and allowed to stand overnight at 4°C. The resulting ethanol-insoluble residue was precipitated by centrifugation at 15,000*g* for 10 min, resuspended in 500 μl of 60 mM Tris-HCl (pH 6.8) containing 2% SDS, 0.02% bromophenol blue, 10% glycerol, and 5% mercaptoethanol, boiled for 5 min in a water bath, then centrifuged again at 1,000*g* for 5 min. An aliquot (50 μl) of the supernatant was used as a sample for SDS-PAGE. SDS-PAGE was performed according to the method of Laemmli (8) with a slight modification. A running gel was made with 12% acrylamide. Proteins on gels were stained with 0.25% Coomassie brilliant blue R-250 dissolved in 50% isopropanol and 10% acetic acid. Carbohydrates on gels were stained by an improved method based on the PAS reaction (7).

RESULTS AND DISCUSSION

Embryogenic and nonembryogenic cell clusters (37–63 μm) were cultured for 2 weeks in the medium with or without 2,4-D (1 mg L^{-1}). Almost all of the embryogenic cell clusters formed somatic embryos in the absence of 2,4-D, but not in the presence of 2,4-D (Fig. 1, a and b). Nonembryogenic cell clusters did not form somatic embryos but just proliferated as small clumps, regardless of the presence or absence of 2,4-D (Fig. 1, c and d). The fresh weight of embryogenic cells (100 ml) Cultured for 2 weeks in the presence or absence of 2,4-D, and of nonembryogenic cells cultured for the same period in the presence or absence of 2,4-D was 2.2, 3.5, 2.9, and 2.0 g, respectively.

The ethanol-insoluble materials from each 10 ml medium were subjected to SDS-PAGE and the gels were stained for protein and carbohydrate. A polypeptide with M_r 65,000 and referred to as GP65 was stained by Coomassie brilliant blue and PAS reagent, and specifically detected in the culture medium of embryo forming cells (embryogenic cells cultured without 2,4-D) (Fig. 2, b). A polypeptide with M_r 57,000 and referred to as GP57 also stained by Coomassie brilliant blue and PAS reagent, and was detected in the culture medium of nonembryo-forming cells (embryogenic cells cultured with 2,4-D and nonembryogenic cells) (Fig. 2, a, c, and d). GP57 was much more abundant in the medium of nonembryogenic cells in the presence of 2,4-D than in the absence of 2,4-D (Fig. 2, c and d).

In the next experiment, the effects of the concentration of 2,4-D on the appearance of both proteins in the medium of embryogenic cells (37–63 μm) were examined by using PAS-reagent. The formation of somatic embryos was strongly and moderately inhibited by the addition of 1 μM and 0.1 μM 2,4-D, respectively, but not by 10 nM 2,4-D. Embryo formation was correlated with the abundance of GP65 and GP57. GP57 decreased in abundance as 2,4-D concentration decreased, while GP65 greatly increased in abundance as 2,4-D concentration decreased (Fig. 2, C).

Our results indicate that somatic embryogenesis is accompanied by the release of a specific glycoprotein into the medium. It is likely that protein is synthesized and secreted by the cells. It is not clear whether these events are the consequence of embryogenesis or whether this glycoprotein has a regulatory role.

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