The Possible Correlation of Growth Rate and Expression of Transformation with Temperature-dependent Modification in High-Molecular-Weight Membrane Glycoproteins in Mammalian Cells Transformed by a Wild-type and by a Thermosensitive Mutant of Avian Sarcoma Virus

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SUMMARY

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis has been used to study plasma membrane fractions from normal rat kidney cells transformed by a temperature-sensitive derivative of Rous sarcoma virus. A comparison of the plasma membrane protein profiles from cells that displayed the transformed phenotype at 33° and the normal phenotype at 37° revealed the disappearance of a high-molecular-weight glycoprotein in preparations from cells grown at 33°.

A similar study of normal rat kidney cells transformed by the wild-type avian virus in which the transformed cells were grown at 37° and 33° revealed no temperature-mediated difference in the morphology and transformed properties of the cells. However, a comparison of plasma membrane preparations from wild-type-transformed cells grown at 37° and 33° did reveal a marked decrease of a high-molecular-weight glycoprotein in cells grown at 37°, concurrent with an increased rate of growth in cells propagated at that temperature.

INTRODUCTION

Most previous comparisons of plasma membrane proteins of normal and transformed cultured mammalian cells have used permanent cell lines and clones derived by transformation with tumor viruses in which transformed cells were grown at 37° and 33° revealed no temperature-mediated difference in the morphology and transformed properties of the cells. However, a comparison of plasma membrane preparations from wild-type-transformed cells grown at 37° and 33° did reveal a marked decrease of a high-molecular-weight glycoprotein in cells grown at 37°, concurrent with an increased rate of growth in cells propagated at that temperature.

MATERIALS AND METHODS

Cell Cultures. The temperature-sensitive cell line used in the present study was a clone (NT3-KR) derived from ts-NT3-KR.2 These cells exhibited the characteristics of transformed cells at 33° and of the normal phenotype at 37° when propagated in Dulbecco's medium (Grand Island Biological Co., Grand Island, N. Y.; Catalog No. H-16) supplemented with 10% fetal calf serum, 10% Tryptose phosphate broth, and 1% dimethyl sulfoxide, as described by Graf and Friis (7). These cells as well as Pr-RSV-NRK were most kindly given to us by Dr. John Wyke (Imperial Cancer Research Fund, London, England).

Plasma Membrane Preparations. These were prepared in every case from exponentially growing cultures that were about two-thirds confluent. Monolayers from cells grown at 37° or 33° were detached by brief exposure to 0.5 mM EDTA in phosphate-buffered saline, pH 7.0, at 37°. Cells were then harvested by low-speed centrifugation, resuspended to 15 times their packed volume in 0.001 M ZnCl2 (16), and homogenized in a tight-fitting Kontes homogenizer with about 30 strokes. Cell rupture and preservation of nuclei was monitored by phase-contrast microscopy, and subsequent isolation of the plasma membrane fraction was carried out by the 2-phase procedure (1). Final preparations showed a morphology and an enrichment of Na+K+-ATPase activity (1) comparable to that reported for plasma membrane preparations by Brunette and Till (1). Parallel experiments revealed less than 4% of the total DPNH diaphorase activity present in the plasma membrane fraction (1).

Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis. This was carried out by the high-resolution method of Laemmli (11) using 8.5-cm running gels with 5% polyacryl-
amide and a 1-cm stacking gel with 3.3% polyacrylamide. In every experiment, 100 μg protein samples of plasma membranes were used for comparative fractionation. Before electrophoresis, samples were dissociated by exposure for 3 min at 90° to a solution of 2% sodium dodecyl sulfate, 0.1 M β-mercaptoethanol, 0.1 M Tris-HCl buffer (pH 6.5), and 0.002 M phenylmethyl sulfonyl fluoride. After electrophoresis to 5 mm of the end of the gel, samples were fixed and stained with Coomassie blue and the Schiff reagent for protein and glycoprotein, respectively, as described by Fairbanks et al. (3). Approximate molecular weights were determined by comparison of the relative migration of plasma membrane proteins with standard proteins as described elsewhere (3).

RESULTS AND DISCUSSION

The ts-NT3-KR cells used in the present study showed not only the transformed morphology at 33° and the untransformed phenotype at 37°, but also a temperature-dependent growth in agar. In essential agreement with Graf and Friis (7), who originated this type of mutant cells, we observed that NT3-KR cells seeded in agar at 33° did form colonies as much as Pr-RSV-NRK at 37° or 33°. However, no such behavior was observed in NT3-KR cells seeded in agar at 37°; essentially no colony formation was observed, a finding similar to that usual in untransformed cells (12) (not shown).

Examination of the plasma membrane fractions from ts-NT3-KR cells grown at 33° and 37° revealed the presence of over 20 protein components in both cases. However, in spite of the expected similarity between the 2 preparations, a selective increase in a protein with an apparent molecular weight of about 48,000 became evident (Chart 1) in preparations of ts-NT3-KR cells, in which the transformed phenotype was expressed at 33°. The possible involvement of glycosylation in the temperature-mediated changes was also tested by staining the plasma membrane proteins with the Schiff-glycoprotein stain (3). Chart 2 shows the definite presence of several glycoproteins in plasma membrane fractions from ts-NT3-KR cells. A most interesting change is that a 250,000 molecular weight glycoprotein component, clearly visible in preparations from cells grown at 37°, is clearly diminished or absent in plasma membranes isolated from cells grown at the lower temperature, which is permissive for the expression of transformation.

The above results clearly correlate with temperature-mediated changes that affect both the ability to grow in agar and the morphology of ts-NT3-KR cells (7, 14). However, in spite of the lack of effects of temperature on the ability of the NRK cells infected with the wild-type avian virus to change their morphology or to grow on agar upon growth at 33° or 37°, a most unexpected observation was made of a selective, reproducible, and significant difference in plasma membrane preparations from Pr-RSV-NRK cells when grown at 37° or 33°. In apparent contrast to the above results with ts-NT3-KR cells, in which the expression of the transformed phenotype at 33° correlates with the disappearance of a glycoprotein detectable in cells expressing the normal phenotype at 37°, we observed that Pr-RSV-NRK cells exhibited the preferential appearance of a glycoprotein that also exhibited an apparent molecular weight of about 250,000 at 33° and its disappearance at 37° (Charts 3 and 4), despite the continued expression of the transformed phenotype in such cells at both 37° and 33°. The fact that the glycoprotein component that is absent at 37° in Pr-RSV-NRK cells (Chart 4) cannot be detected with

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Chart 1. Coomassie blue pattern of an electrophoretogram of plasma membrane proteins from ts-NT3-KR cells. Plasma membrane proteins were prepared and separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis as described in "Materials and Methods." Samples were fixed and subsequently stained with Coomassie blue (3) for detection of plasma membrane protein bands. A, plasma membrane proteins from ts-NT3-KR cells grown at 33°, temperature at which the cells exhibit the transformed phenotype; B, plasma membrane proteins from ts-NT3-KR cells grown at 37°, temperature at which the cells exhibit the untransformed phenotype. Arrow, region of most evident temperature-mediated changes.
Glycoprotein Alterations with Transformation and Rate of Growth

Chart 2. Schiff-glycoprotein pattern of an electrophoretogram of plasma membrane glycoproteins from ts-NT<sub>3</sub>-KR cells. The conditions of preparation and separation of samples were identical to those described in Chart 1 and corresponded to similar samples run in parallel although subsequently processed for glycoprotein staining (3). A, plasma membrane glycoproteins from ts-NT<sub>3</sub>-KR cells grown at 33°; B, plasma membrane glycoproteins from ts-NT<sub>3</sub>-KR cells grown at 37°. Arrow, region of most evident temperature-mediated changes.

the protein stain either (Chart 3) implies that the change is not due just to a decreased glycosylation of an otherwise existing polypeptide as a result of the growth conditions. Nevertheless, the fact that the transformation phenotype is clearly apparent both at 37° and 33° in Pr-RSV-NRK cells implies that the temperature-mediated change in glycoprotein components described above does not correlate merely with the expression of transformation.

Chart 3. Coomassie blue pattern of an electrophoretogram of plasma membrane proteins from wild-type Pr-RSV-NRK cells. Plasma membrane proteins from Pr-RSV-NRK cells were prepared and separated by sodium dodecyl sulfate-polyarylamide gel electrophoresis and stained as indicated in "Materials and Methods" and in Chart 1. A, plasma membrane proteins from cells grown at 33°; B, plasma membrane proteins from cells grown at 37°. Arrow, region of most evident temperature-mediated changes.

Hence, we decided to examine whether such temperature-mediated glycoprotein alterations observed in Pr-RSV-NRK cells could be correlated with an altered rate of growth. It can be seen in Chart 5 that Pr-RSV-NRK cells do indeed show slower growth at 33°, and tend to reach a lower saturation density at 33°, the temperature at which they exhibit the
Chart 5. Relative growth of Pr-RSV-NRK cells at 33° and 37°. Cells were seeded in 5-cm Petri dishes at an initial concentration of about 1.65 x 10^4 cells in Dulbecco's medium containing 10% fetal calf serum, and parallel duplicate cultures were incubated in a 5% CO² atmosphere at either 33° or 37°. The medium was changed every 48 hr, and at the intervals indicated cells were trypsinized and counted. The results presented correspond to duplicate counts, which were found to agree within 7%. O, growth at 33°; •, growth at 37°.

glycoprotein species of an approximate molecular weight of 250,000 disappears during the expression of transformation, it seems apparent from our data that the above-described glycoprotein changes do not correlate only with the expression of transformation but seem to depend also on the growth rate of transformed cells, which appears to be also susceptible to alteration by temperature changes.

Out interpretation of the temperature-mediated alteration in glycoprotein components of transformed cells seems more related to findings in other systems in which glycopeptide alterations have been found to occur not only as a result of transformation with RNA or DNA tumor viruses (2) but also because of the influence of the growth state and stage in the cell cycle (4, 6, 10).

In support of our interpretation of the data presented above, it is particularly relevant to quote a very recent review (10) on a “transformation-sensitive” protein that exhibits an electrophoretic mobility and a molecular weight essentially identical to the glycoprotein species which we find to change as a result of transformation or altered growth rate. The labeling of this 250,000 molecular weight external protein also shows variation with growth state and stage in the cell cycle (4, 10).

ADDENDUM

After the completion of this work, the authors learned that results essentially similar to those presented above, describing alterations in glycoprotein components as a result of virus-mediated transformation, have been recently reported in chick embryo fibroblasts transformed by Rous sarcoma virus (15, 17).

REFERENCES

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