CELL SURFACE GLYCOSYL TRANSFERASES AND ACCEPTORS IN NORMAL AND RNA- AND DNA-VIRUS TRANSFORMED FIBROBLASTS[1]

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Summary Cell surface glycosyl transferases and acceptors were found to be present in normal and oncogenic virus transformed fibroblasts. In cells from confluent cultures, the reaction was elevated 2- to 4-fold in MSV-3T3, RSV-3T3 and PY-3T3 cells compared to normal 3T3 cells, but in cells from sparse cultures, the reaction was essentially equivalent in the normal and transformed cells. Using exogenous acceptors, elevated surface levels of some glycoprotein:glycosyl transferases were demonstrated in the RNA- and DNA-virus transformed cells. The results may be important in phenomena such as cell:-cell adhesion and contact inhibition.

Introduction

One of the foci of cell oncogenic transformation is the plasma membrane. Differences in contact inhibition, antigenic sites, agglutination, and plasma membrane structure occur in normal and neoplastic cells. Although the cell plasma membrane is composed mainly of protein and lipid, interest has centered around cell surface glycoproteins and glycolipids. In particular, glycoprotein:glycosyl and glycolipid:glycosyl transferase activities are either elevated (1,2) or depressed (3-6) and glycosidase and proteolytic activities are elevated (7-9) in oncogenic virus transformed cells. Most glycoprotein:-glycosyl transferases are located in the smooth endoplasmic reticulum (Golgi apparatus) of the cell (10), although plasma membrane localization of the collagen:glycosyl transferases (10,11) has been reported and most glycoprotein:- or glycolipid:glycosyl transferases are thought to be responsible for synthesis of glycoproteins or glycolipids.

Recently, however, Roseman (12) has postulated that glycosyl transferases may function as bridge or combination molecules in cell:cell adhesion phenomena and contact inhibition, the formation of the acceptor:transferase activated

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complex being the chemical entity which determines adhesion. This postulate has been given some credence by the demonstration of glycosyl transferases on the surfaces of chick-embryo neural retina cells (13) and human blood platelets (14,15); in the latter instance it has been suggested that the surface enzymes might be responsible for the adhesion of collagen to platelets and platelet:platelet aggregation (15,16). Recently Roth and White (17) have demonstrated galactosyl transferase and acceptors on Balb/C3T3 cells and Balb/C3T3 cell surfaces and implicated these transferases in cell adhesion and as possibly regulating contact inhibition. In the present work I describe the presence of a large number of glycosyl transferases and acceptors on intact 3T3 cell plasma membrane external surfaces and describe elevated levels of the reaction in 3T3 cells transformed by either RNA or DNA viruses.

Materials and Methods

CMP-[^{14}C]NANA (specific activity 100 Ci/mole), UDP-[^{14}C]glucose (240 Ci/mole), UDP-[^{14}C]galactose (240 Ci/mole), GDP-[^{14}C]mannose (241 Ci/mole), UDP-N-[^{14}C]acetylgalactosamine (40 Ci/mole), UDP-[^{14}C]xylose (100 Ci/mole), UDP-[^{14}C]arabinose (100 Ci/mole), and UDP-N-[^{14}C]acetylglucosamine (40 Ci/mole) were purchased from New England Nuclear Corp. Protein determinations were made by the procedure of Lowry et al. (18). Fetuin (19), guinea pig skin collagen (20,21), and their degraded products were prepared as previously described (22,23). Four lines of fibroblasts were cultured and examined: Balb/3T3, an established cell line of mouse fibroblasts; MSV-3T3, an established line of murine sarcoma virus transformed 3T3 fibroblasts; PY-3T3, an established line of polyoma virus transformed 3T3 fibroblasts; and RSV-3T3, an established line of Rous sarcoma virus transformed 3T3 fibroblasts. Transformed cell lines were from Balb/3T3 cells. Initial cell clones were kindly supplied by Dr. G. J. Todaro, National Institutes of Health. All cells were grown in monolayer culture in the Dulbecco-Vogt modification of Eagles medium supplemented with 10% calf serum.

Results and Discussion

The data in Table 1 demonstrate that when cell cultures were removed by scraping off the monolayer and incubated with appropriate nucleotide diphosphate monosaccharides, Mg^{++}, Mn^{++}, and Dulbecco's medium without serum, transfer of radioactive monosaccharide onto acid, ether-ethanol insoluble material occurred. Transfer of radioactivity indicates levels of both enzyme and acceptor, since either can be rate-limiting to the reaction. In the 3T3 cells high levels of transfer occurred from UDP-glucose, GDP-mannose, CMP-

NANA, and UDP-galactose. For the UDP-galactose reaction in the 3T3 cells, these results confirm the work of Roth and White (17). Moderate amounts of transfer occurred in the UDP-N-acetylgalactosamine and UDP-N-acetylglucosamine systems while very low levels of transfer occurred with the UDP-xylose and UDP-arabinose systems; xylose and arabinose are not present to any great extent in mammalian glycoproteins.

As shown in Table 1, the MSV-3T3 cell line had elevated levels of surface glycosyl transferases and/or surface acceptors; this was especially true for the GDP-mannose and UDP-galactose systems, which had more than 3 times the activity of the comparable 3T3 systems. RSV-3T3 cells also had increased levels of glycosyl transferases and/or acceptors, but the increases were not as great as those for the MSV-3T3 cells (Table 1). The PY-3T3 cell line had elevated levels of surface transferases and/or acceptors in all instances except for GDP-mannose system, which was lower in the PY-3T3 than in the 3T3 cells.

The following series of experiments were performed to determine whether the transferases were indeed surface located. First, although it is unlikely that the nucleotide diphosphate monosaccharides penetrate the cell plasma membrane to any great extent during a 30-minute incubation, the following experiments were performed. The experiments of Table 1 were repeated exactly except that instead of termination of the experiments with phosphotungstic acid and trichloroacetic acid precipitation, the cells were merely centrifuged at 2000 x g for 5 min and washed twice with saline, and the radioactivity in the cells was determined. In every instance the amount of radioactivity was no greater than 10% higher than that given in Table 1. This demonstrates that large amounts of radioactivity were not present in intracellular pools as might be expected if the nucleotide diphosphate monosaccharides were entering the cell and synthesis of glycoprotein was occurring within the plasma membrane rather than on its external surface. Secondly, the experiments of Table 1 were repeated exactly, except that after the assay incubation but before acid precipitation the cells were incubated with 1 ml of 0.25% trypsin in Tris buffered saline at 37°C, pH 7.0, for 15 minutes. This treatment, which releases peptides from the cell surface but does not lyse the cells, caused in every instance 95-100% of the radioactivity present in the cells, as shown in Table 1, to be released, and no longer associated with the cell pellet. Thus the radioactivity transferred was indeed associated with the external surface of the cell plasma membranes. Thirdly, before procedures such as those in Table 1 were repeated, cells were treated with 1 ml of 0.25% trypsin in Tris buffered saline at 37°C, pH 7.0, for 15 min. The cells were centrifuged out

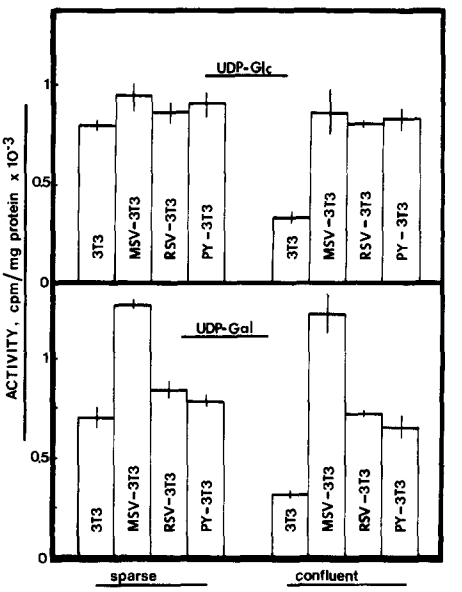

Figure 1. Activity of UDP-galactose and UDP-glucose reactions in normal and oncogenic virus transformed cells from sparse and confluent cultures. Experiments were performed as given in Materials and Methods and the legend to Table 1. Means ± 1 S.D.

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of this solution and washed twice with saline, and the experiments of Table 1 were repeated exactly with these trypsin treated cells. In every instance between 0 and 15% of the activity of Table 1 was found; that is, between 85 and 100% inhibition of transfer of monosaccharide activity occurred when the cells were treated with trypsin. Lastly, the activities were demonstrated to follow requirements for enzyme activity. Boiling the cells abolished the activity; activity was linear with respect to time of incubation, amount of substrate and amount of cells; and activities were dependent on temperature of incubation, presence of divalent cations, and pH of incubation.

The data of Figure 1 demonstrate that the reactions were density dependent. In sparse cultures levels of the reaction with either UDP-[^{14}C]glucose or UDP-[^{14}C]galactose as precursor were essentially equivalent with either the normal or the transformed cells (Figure 1); similar results were obtained with the other nucleotide diphosphate monosaccharides as precursors.[9]

The data in Table 2 demonstrate that the surface transferases functioned with exogenous degraded glycoproteins as acceptors; it is clear that these high molecular weight glycoproteins do not enter the intact cells. The data for the transfer of NANA to fetuin minus NANA, galactose, N-acetylglucosamine

TABLE 1. Glycosyl Transferase Activity and Acceptor Presence on the Surface of 3T3 and Virally Transformed 3T3 Cells

^{14}C-Labeled Precursor	Cell Line			
	3T3	MSV-3T3	RSV-3T3	PY-3T3
UDP-glucose	340 ± 21	865 ± 76	806 ± 16	822 ± 61
GDP-mannose	938 ± 32	3685 ± 71	989 ± 84	737 ± 36
CMP-NANA	336 ± 22	823 ± 62	444 ± 31	1268 ± 172
UDP-galactose	338 ± 12	1211 ± 142	739 ± 6	657 ± 81
UDP-xylose	2 ± 4	3 ± 2	3 ± 1	3 ± 1
UDP-N-acetylgalactosamine	126 ± 7	317 ± 38	351 ± 35	203 ± 13
UDP-N-acetylglucosamine	96 ± 6	193 ± 24	255 ± 29	153 ± 11
UDP-arabinose	2 ± 2	4 ± 1	3 ± 3	3 ± 1

Experiments were performed by harvesting cells at confluency by scraping, washing the cells once with 0.1 M Tris, pH 7.6, saline, suspending 0.1 to 0.3 mg as protein of the cells in 0.1 ml Dulbecco's medium without serum, adding 10 µl of 0.1 M $MgCl_2$, 10 µl of 0.1 M $MnCl_2$ and 10 µl of a 3.3 µCi/ml solution of ^{14}C-labeled precursor to a final volume of 0.130 ml. The mixtures were incubated for 30 min at 37°C, after which 3 volumes of 1% phosphotungstic acid in 0.5 N HCl were added and the precipitate was centrifuged out of solution. The precipitate was washed twice with 10% trichloroacetic acid, once with ethanol:diethyl ether (2:1, v/v), dissolved in 1N NaOH and radioactivity determined in a liquid scintillation counter. Data are cpm per mg protein and means ± 1 S.D. from 5 independent observations.

TABLE 2. Activity of Surface Glycosyl Transferases with Exogenous Acceptors

^{14}C-Labeled Precursor	Exogenous Acceptor	Cell Line			
		3T3	MSV-3T3	RSV-3T3	PY-3T3
CMP-NANA	Fetuin minus NANA	370 ± 16	663 ± 76	483 ± 61	492 ± 17
UDP-N-acetyl-glucosamine	Fetuin minus NANA, galactose, N-acetyl-glucosamine	87 ± 7	178 ± 14	240 ± 17	143 ± 10
UDP-glucose	Collagen minus glucose	360 ± 9	141 ± 6	191 ± 29	224 ± 15
UDP-galactose	Collagen minus glucose, galactose	554 ± 72	1320 ± 21	1621 ± 172	531 ± 27

Experiments were performed exactly by the procedure given in Table 1 except that 400 µg of acceptor were added to a volume of 0.160 ml. Data are means ± 1 S.D. for 5 or more observations. Data have endogenous activity subtracted.

indicate that these surface enzymes in the RNA- and DNA-virus transformed cells are present in elevated amounts compared to the 3T3 cells. The collagen:-glucosyl transferase utilizing collagen minus glucose as acceptor is depressed in the transformed cells, consistent with the observation that these transformed cells synthesize less collagen than the untransformed parent cell lines (6). The collagen:galactosyl surface transferase had elevated levels in the RSV-3T3 and MSV-3T3 cell lines and comparable levels of activity in the PY-3T3 cells compared to the 3T3 cells. Experiments in which the cells were centrifuged from the assay mixture and the supernatant precipitated with phosphotungstic acid and washed with trichloroacetic acid and ether-ethanol indicated that all of the exogenous activity was associated with the supernatant and not the cell pellet.

The work clearly demonstrates the existence of glycoprotein:glycosyl transferases and acceptors on the external surfaces of normal and RNA and DNA oncogenic virus transformed cells. It should be noted that the results are consistent with such transferases and acceptors functioning in the phenomena of cell:cell interaction (12). It is important that these transferases and acceptors are present at very low levels compared to the usual Golgi apparatus transferases studied by detergent solubilization (1), and for this reason their function may be other than glycoprotein biosynthesis. The DNA and RNA transformed cells are characterized by higher activities in general than their normal counterparts; such higher activity may be due in part to higher levels of surface transferases or to the higher levels of acceptors in the transformed cells which may be able to carry out cis- (on the same cell) as well as trans- (on adjacent cell) glycosylation, as suggested by Roth and White (17), whereas the normal cell may only function for transglycosylation.

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