# GANGLIOSIDE BIOSYNTHESIS IN MOUSE CELLS: GLYCOSYLTRANSFERASE ACTIVITIES IN NORMAL AND VIRALLY-TRANSFORMED LINES

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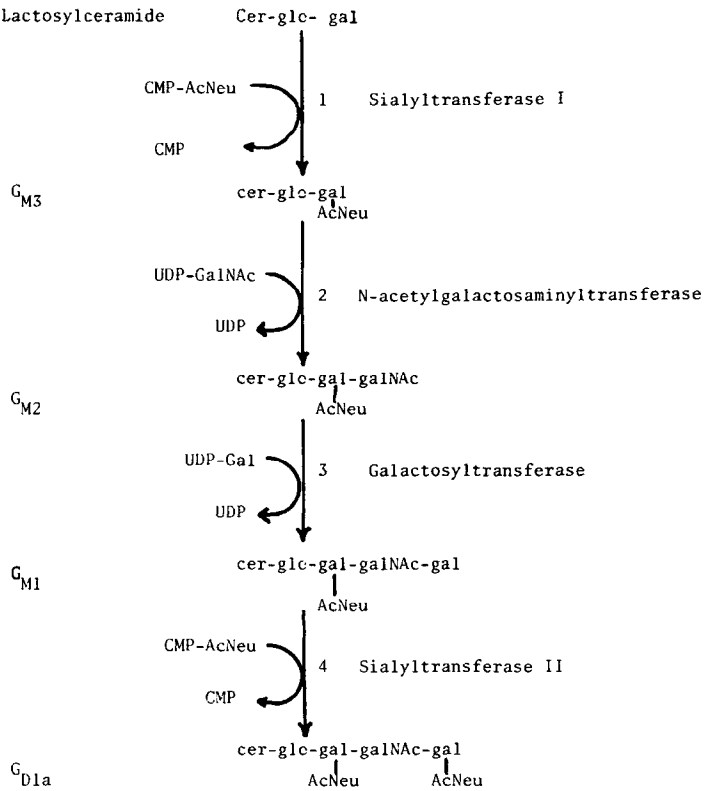
Summary - The activities of four glycosyltransferases involved in ganglioside biosynthesis were measured in two different established mouse cell lines and in the SV40 and polyoma transformed variants of these lines. The only consistent change observed was the very reduced activity of UDP-GalNAc: hematoside N-acetyl-galactosaminyltransferase in all of the transformed cell lines (15-20% of normal cells). There was no significant differences in any of the glycosyltransferases with increased cell density and cell-to-cell contact.

Cells in culture transformed by oncogenic DNA viruses have altered growth properties (1) loss of contact inhibition (2) and cell surface changes (3-5). Several laboratories have reported differences in glycolipid and glycoprotein compositions in such transformants (6-9). Studies with established mouse cells indicate a less complex ganglioside composition in SV40 and polyoma transformed lines. Whereas the major gangliosides are $G_{M3}$[1], $G_{M2}$, $G_{M1}$, and $G_{D1a}$, generally the virally-transformed cells have mainly $G_{M3}$ (6,10-12). The absence of these higher gangliosides appears to be due to a decrease in the enzyme UDP-N-Acetylgalactosamine: hematoside N-acetylgalactosaminyltransferase (13,14).

Since the biosynthesis of gangliosides appears to proceed by the sequential addition of monosaccharide units from sugar nucleotide

[1] The abbreviations used are: AcNeu, N-acetyl-neuraminic acid; cer, ceramide (N-acylsphingosine). For glycosphingolipids containing sialic acid the symbols proposed by Svennerholm (17) are used. $G_{M3}$NAc, N-acetylneuraminylgalactosylglucosylceramide (hematoside); $G_{M2}$, N-acetylgalactosaminyl-[N-acetylneuraminyl]-galactosylglucosylceramide; $G_{M1}$, galactosyl-N-acetylgalactosaminyl-[N-acetylneuraminyl]-galactosylglucosylceramide; $G_{D1a}$ N-acetylneuraminyl-galactosyl-N-acetylgalactosaminyl-[N-acetylneuraminyl]-galactosylglucosylceramide.

FIGURE 1. BIOSYNTHETIC PATHWAY FOR GANGLIOSIDES




donors to the elongating glycolipid acceptor (Fig. 1), (15), and the biosynthetic enzymes are presumed to exist as a multi-enzyme complex (15,16), it was important to determine if viral transformation results in a decrease in one specific glycosyltransferase or whether the entire biosynthetic pathway is altered. The present communication compares the levels of these enzymes in normal and virally-transformed cells.

## EXPERIMENTAL PROCEDURE

Cells and cell culture: The various established mouse cell lines and conditions used in these experiments have been described previously (6,14). The normal (N-AL/N) and transformed (SVS-AL/N and PY-AL/N) are non-clonal cell lines while the three Swiss 3T3 cell lines (normal Swiss 3T3 and

transformed SV101 and PY11) are clonal derivatives. Cells were harvested just prior to confluency unless otherwise noted.

Preparation of cell homogenates: The cells were harvested as previously described (14). The washed packed cells were suspended in nine volumes of 0.25 M sucrose containing 0.1% 2-mercaptoethanol and disrupted by freeze-thawing four times in a dry ice-ethanol bath. Enzymatic activities obtained with this procedure were comparable to those obtained with the previous technique of rupturing cells by sudden decompression (13)[2].

Glycolipid acceptors and sugar nucleotide donors: $G_{M3}NAc$ was prepared from dog erythrocytes and further purified and analyzed (13). $G_{M2}$ was prepared from the brain of a patient with Tay-Sachs disease (18) and was a generous gift from Dr. Andrew Gal, NINDS. $G_{M1}$ was prepared from bovine brain gangliosides (19). Lactosylceramide was obtained from Miles Laboratories. Sugar nucleotides were purchased from the following sources: UDP-N-Acetyl-1-$^{14}$C-D-galactosamine (14 mCi per mmole) from International Chemical and Nuclear; UDP-galactose-1-$^{14}$C (254 mCi per mmole) and CMP-N-acetylneuraminic acid-[sialic-4,5,6,7,8,9-$^{14}$C] (223 mCi per mmole) from New England Nuclear; UDP-galactose from Sigma (used to dilute UDP-galactose-$^{14}$C to 4 mCi per mmole).

Enzyme Assays: The reaction mixtures and incubation conditions for the various glycosyltransferase assays are described in Table 1. After incubation, the reactions were terminated by adding 20 volumes of chloroform-methanol (2:1 v/v) (CM) and 40 volumes of chloroform-methanol-water (60:30:4.5 v/v/v) (CMW). These mixtures were passed through 1 x 2.5 cm colums of Sephadex G-25 superfine (1g) previously equilibrated with CMW. The columns were then washed with 5 ml of CMW (4 ml for N-acetylgalactosaminyl-transferase assays). This procedure effectively separates the radioactive glycolipid products from the radioactive sugar nucleotide precursors (13). The eluants were dried and the radioactivity determined

[2] Unpublished observations of P. H. Fishman and R. W. Smith.

TABLE I

Reaction Mixtures and Incubation Conditions for Glycosyltransferase Assays

| Component | Glycosyltransferase | | | |
|---|---|---|---|---|
| | Sialyltransferase I[a] | N-acetylgalactosaminyltransferase[b] | Galactosyltransferase[c] | Sialyltransferase II[d] |
| Glycolipid acceptor | 50 nmoles cer-glc-gal | 50 nmoles $G_{M3}NAc$ | 10 nmoles $G_{M2}$ | 50 nmoles $G_{M1}$ |
| Sugar donor ($^{14}C$) | $10^5$ cpm CMP-AcNeu | $10^5$ cpm UDP-GalNAc | $2x10^5$ cpm UDP-Gal | $10^5$ cpm CMP-AcNeu |
| Na Cacodylate buffer | 5 µmoles pH 6.35 | 2.5 µmoles pH 7.2 | 5 µmoles pH 7.2 | 5 µmoles pH 6.5 |
| Detergents | 750 µg cutscum | 200 µg Triton X-100 | 100 µg Triton CF-54 50 µg Tween 80 | 100 µg Triton CF-54 50 µg Tween 80 |
| Cardiolipin | 100 µg | - - - | - - - | - - - |
| Cell homogenate | 20 µl | 10 µl | 20 µl | 20 µl |
| Final volume | 50 µl | 50 µl | 50 µl | 50 µl |

Glycolipid acceptors were added to the reaction tubes in CM and taken to dryness. The remaining components were then added and the reactions initiated by addition of the cell homogenates (4-6 mg protein per ml) followed by vigorous swirling. After incubating at 37°C for 2 hrs. the reactions were terminated by adding 1 ml CM and 2 ml CMW. The radioactive ganglioside products there then isolated as described under "EXPERIMENTAL PROCEDURE."

a) Assay conditions same as ref. 20; b) Assay conditions similar to ref. 13; c) Assay conditions same as ref. 16; d) Assay conditions same as ref. 21.

by liquid scintillation spectrometry. Each determination of enzyme activity represents net incorporation of radioactive sugar onto exogenous acceptor and was calculated by subtracting endogenous incorporation from incorporation with added acceptor.

## RESULTS AND DISCUSSION

Factors influencing enzyme activities: Each enzyme assay was run under conditions of linearity in terms of time and protein content. Glycolipid acceptor concentrations were saturating for all assays. No significant activation or inhibition was observed when homogenates from normal and transformed lines were assayed together. Sialyltransferase I in all of the cell lines was stimulated 3-7 fold by cardiolipin. Similar findings had been reported for this same enzyme activity in baby hamster kidney (BHK) cells (20). Cardiolipin at 100 ug per incubation mixture produced maximum activity.

Effect of Cell Density on Glycosyltransferase Activities: Since it has been reported that quantity of certain glycolipids increase upon cell-to-cell contact in some contact-inhibited cell lines (22, 23), the influence of cell density on the ganglioside biosynthesizing enzymes was investigated. Swiss 3T3 cells were plated in inocula of $5 \times 10^4$, $10^5$, $5 \times 10^5$ and $10^6$ cells per plate. The cells were harvested after 3 days by gentle trypsinization in order to obtain suspensions for cell counting. The trypsinization procedure had no effect on the transferase activities[2]. Both sialytransferases exhibited a slight decrease in specific activity as cell density increased whereas the other two transferases fluctuated slightly (Fig. 2). For comparative purposes, cells were routinely harvested at preconfluency while still in a logarithmic phase of growth. Similar experiments with clonal derivatives of Balb 3T3 cells indicated that there were no substantial changes in N-acetylgalactosominyltransferase activity with cell density (24). These cell lines were: OA31, a normal Balb 3T3; 50-IIA41 and 11A8, SV40 transformed Balb 3T3; and 10 A 7, a "flat revertant" SV40 transformed Balb

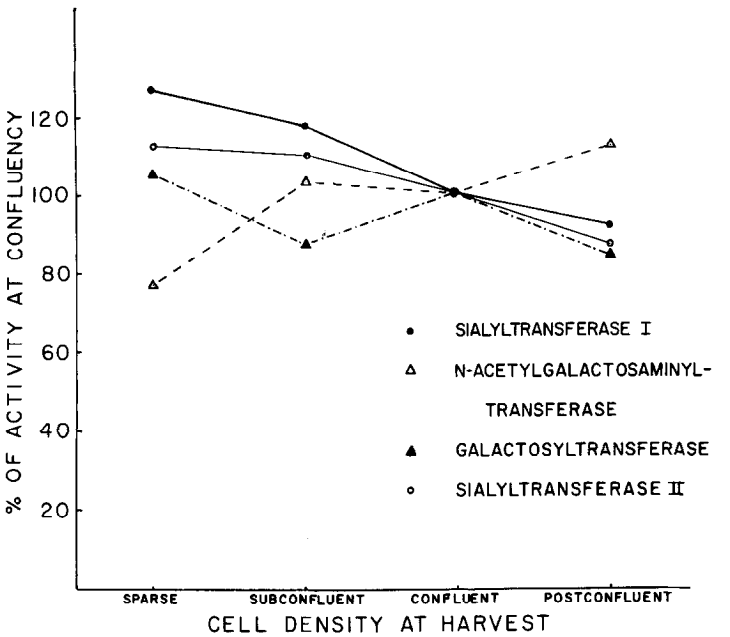



FIGURE 2

Effect of cell density on glycosyltransferases. Swiss 3T3 cells were plated in inocula of $5x10^4$, $10^5$, $5x10^5$, and $10^6$ cells per plate. The cells were harvested after three days and the number of cells were determined. Cell densities (cells per $cm^2$) were: sparse, $1x10^4$; subconfluent, $3x10^4$, confluent, $1x10^5$; post-confluent, $1.5x10^5$. Glycosyltransferase activities were determined as described under "EXPERIMENTAL PROCEDURE." The activities of each transferase have been normalized and are given as the percent activity in confluent cells.

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3T3 (25). The results are in contrast to the cell density-dependant changes in glycolipid concentrations reported to occur in hamster cells in culture (22, 23, 26). Although ganglioside concentrations were not determined in sparse and confluent mouse cells, it recently has been reported that cell density dependent changes in glycosyltransferases correlated with changes in glycolipids in hamster cells (27). Whether these different observations

are due to differences in cell type, or in clones (cf. ref. 26, 28), or in class of glycolipid has yet to be determined. However, cell density-dependent changes in glycolipid concentrations does not appear to be a general phenomenon.

Enzyme levels in normal and transformed cells: Levels of the ganglioside glycosyltransferases in normal and virally-transformed clonal derivatives of Swiss 3T3 cells are given in Table II. The results of similar experiments with the various non-clonal AL/N cell lines are presented in Table III. The only consistant change in enzyme levels after viral transformation in both Swiss 3T3 and AL/N cell lines was the marked decrease in N-acetylgalactosaminyltransferase. The altered ganglioside composition of both uncloned and cloned virally-transformed cell lines investigated in this laboratory is in agreement with this observation (6, 10, 11). Sialtransferase I is reduced in a polyoma transformed BHK clonal cell line (20) and was reported to be lower in SV40 transformed Swiss 3T3 (29). This enzyme activity was elevated in SV101 and PY AL/N, unaffected in PY11 and moderately diminished in SVS AL/N. Sialytransferase II was reported to be decreased in SV40 transformed Swiss 3T3 cells (29); lower activity than normal was found by us in the SV101 cells. However, this enzyme activity was higher in both PY AL/N and SVS AL/N than in the control N AL/N cell line.

The results suggest that the alteration in ganglioside biosynthesis after transformation of established mouse cells by oncogenic DNA viruses are complex when considering the change in activities of the various glycosyltransferases. SV40 and polyoma appear to induce somewhat different effects and the host cell may influence any virally mediated changes. The results, however, continue to support a common biochemical change in SV40 and polyoma virus-transformed mouse cells; reduced activity of a specific N-acetylgalactosaminyltransferase. Whether this consistantly observed

TABLE II

GLYCOSYLTRANSFERASE ACTIVITIES IN NORMAL AND VIRALLY-TRANSFORMED SWISS 3T3 CELLS

| GLYCOSYLTRANSFERASE | CELL LINE | | |
|---|---|---|---|
| | SWISS 3T3 | SV 101 | PY 11 |
| Sialyltransferase I[a] | 16, 800 ± 770 | 17,600 ± 6,100 | 18,500 ± 3,300 |
| N-acetylgalactosaminyltransferase[b] | 0.95 ± 0.26 | 0.12 ± 0.14 | 0.16 ± 0.04 |
| Galactosyltransferase[b] | 0.22 ± 0.02 | 1.44 ± 0.04 | 0.37 ± 0.06 |
| Sialytransferase II[a] | 51,000 ± 2000 | 18,500 ± 6,400 | 41,800 ± 12,700 |

[a] Activity is cpm per mg protein per 2 hours.

[b] Activity is nanomoles per mg protein per 2 hours.

The values given are the average of three separate experiments and represent synthesis of gangliosides using exogenous acceptors as determined under "EXPERIMENTAL PROCEDURES."

TABLE III

GLYCOSYLTRANSFERASE ACTIVITIES IN NORMAL AND VIRALLY-TRANSFORMED AL/N MOUSE CELLS

| GLYCOSYLTRANSFERASE | CELL LINE | | |
|---|---|---|---|
| | N-AL/N | SVS-AL/N | PY-AL/N |
| Sialytransferase I[a] | 6560 ± 4950 | 3680 ± 60 | 9370 ± 1540 |
| N-acetylgalactosaminyltransferase[b] | 1.52 ± 0.29 | 0.25 ± 0.05 | 0.22 ± 0.04 |
| Galactosyltransferase[b] | 1.11 ± 0.35 | 0.07 ± 0.06 | 0.12 ± 0.14 |
| Sialyltransferase II[a] | 5740 ± 650 | 10,560 ± 110 | 11,030 ± 630 |

[a] Activity is cpm per mg protein per 2 hours.

[b] Activity is nanomoles per mg protein per 2 hours.

The values given are the average of two separate experiments and represent synthesis of gangliosides using exogenous acceptors as determined under "EXPERIMENTAL PROCEDURES".

change is directly related to viral transformation or a secondary consequence of selection during the transformation process remains to be determined and cloning experiments are underway to explore these alternatives.

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