GLYCOLIPID SYNTHESIS IN BABY-HAMSTER-KIDNEY FIBROBLASTS TRANSFORMED BY A THERMOSENSITIVE MUTANT OF POLYOMA VIRUS

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1. Introduction

Transformation of animal cells by oncogenic viruses *in vitro* leads to altered glycosphingolipid composition of the cells [1–8]. This alteration is, in some instances, due to reduced activity of one of the glycosyl transferases required in the biosynthesis of complex glycosphingolipids [9, 10]. Non-transformed cells increase their amounts of complex glycolipids as they reach confluency and this ability is lost by virus transformation [3–5]. As glycolipids are mainly present in the cytoplasmic membrane, it has been suggested that the complex members of these lipids are required for stopping DNA synthesis and cell division in confluent cultures. To test this hypothesis we grew baby-hamster-kidney (BHK) cells transformed by a thermosensitive mutant of polyoma virus in the presence of [1-^{14}C]-palmitic acid and determined the amount of radioactivity in phospholipids and glycolipids. These cells are transformed at 31° and non-transformed at 39° [11, 12]. Normal BHK cells and BHK cells transformed by wild type polyoma virus were used for control experiments. The results showed that the normal cells increased their synthesis of ceramide trihexoside (Gl-3) four times as they became confluent. The wild type transformants and the thermosensitive transformants each incorporated 20-fold less radioactivity in Gl-3 than confluent normal cells. The decrease in Gl-3 synthesis was independent of growth temperature and the cells containing mutant virus exhibited growth control at 39° in spite of the "abnormal" glycolipid pattern.

2. Experimental procedure

2.1. Cell cultures

BHK Cl 13 (a clone of normal BHK cells [13]), BHK Wt Cl 2A (a wild type polyoma-transformed line of strain Cl 13), and BHK ts-3 Cl 7C (obtained from strain Cl 13 by transformation with a thermosensitive mutant of polyoma [11]) were kindly supplied by Dr. W. Eckhart (The Salk Institute, La Jolla, USA). Cultures were initiated by seeding 3.3×10^5, or 3.3×10^6 cells of strain Cl 13 (low and high cell density, respectively), and 8×10^5 or 4×10^6 cells of strains Cl 2A or Cl 7C (for growth at 39° and 31°, respectively) per 100 mm plastic petri dish (Falcon). The conditions for cell growth, the method for DNA analysis and the test for absence of mycoplasma contamination have all been described [14].

2.2. Measurements of Glycolipid Synthesis

[1-^{14}C]Palmitic acid (58 μCi/μmole) dissolved in ethanol (25 μCi/μl) was added to the cell cultures (1.3 μCi/ml). The addition was made after 16 hr of growth (strain Cl 13) or when the amount of DNA per culture corresponded to that of a dense culture (cf. the growth curves in [14]). After 48 hr of labelling, cell sheets were washed with isotonic Tris-HCl, pH 7.4 and removed with a rubber policeman after addition of 60% aq. methanol. Chloroform and methanol were added to give a chloroform–methanol–water ratio of 40:20:3 (v/v). After filtration, 0.2 vol of 2 M KCl was added

and the lower phase evaporated to dryness. The residue was subjected to thin-layer chromatography (TLC) on Silica Gel precoated aluminum sheets, 20 × 20 cm (Merck, N:o 5553) using the two-dimensional system of Gray [15] with modifications [6]. Labeled lipids were localized by radioautography, cut out and counted in a Packard Tri-Carb 3375 instrument. The lipids were identified by comparison of R_f values for reference compounds, chromatographed under identical conditions. Glucosyl ceramide (Gl-1) was further characterized by borate TLC and by gas-liquid chromatography [16].

3. Results

The dpm values for phosphatidyl choline (PC), sphingomyelin (SM), phosphatidyl ethanolamine (PE), glucosyl ceramide (Gl-1), dihexosyl ceramide (Gl-2) and trihexosyl ceramide (Gl-3) were determined and multiplied by factors (≤ 1) which gave PC + SM values = 1000 × 10^3 dpm. The amount of labeling in PC + SM was proportional to the number of cells analyzed.

3.1. BHK Cl 13

Glycolipid patterns were analyzed at 37° in confluent and non-confluent cultures (83 and 28 μg of DNA/dish, cf. [14]). The results are shown in table 1.

Table 1
Incorporation of [1-^{14}C]palmitic acid into glycolipids and phospholipids of BHK Cl 13.

Lipid	37°, low density	37°, high density	37°, high density / 37°, low density
	Radioactivity (× 10^{-3} dpm)		
PC + SM	1000	1000	1.0
PE	107	190	1.8
Gl-1	4.3	5.6	1.3
Gl-2	1.1	1.4	1.3
Gl-3	1.4	5.9	4.2

Labeling was carried out for 48 hr. After extraction, thin-layer chromatography and radioautography, the radioautograph pattern was used as a template for removing spots for liquid scintillation counting. PC = phosphatidyl choline, SM = sphingomyelin, PE = phosphatidyl ethanolamine, Gl-1 = glucosyl ceramide, Gl-2 = dihexosyl ceramide and Gl-3 = trihexosyl ceramide.

The amounts of labelling of Gl-1 and Gl-2 were not much affected by cell density. On the other hand the amount of radioactivity in Gl-3 increased four times in confluent cells. This cell-density dependent increase of Gl-3 synthesis was also found for cultures grown at 31° or 39°. For comparison, NIL 2 cells at 37° increased their synthesis of Gl-3, tetrahexosyl ceramide and pentahexosyl ceramide as they reached confluency [4, 5].

3.2. BHK Wt Cl 2A

Glycolipid synthesis was measured at high cell density after growth at 31° or 39° (185 and 163 μg of DNA/dish at 31°, and 39°, respectively). The results are shown in table 2. The Gl-3 synthesis was reduced 20 times compared to high density BHK Cl 13 cultures, and there was some accumulation of radioactivity in the precursors Gl-2 and Gl-1. It seems possible that polyoma transformation represses synthesis of the glycosyl transferase required to attach the terminal galactose of Gl-3 to dihexosyl ceramide, but experimental evidence for this is lacking.

3.3. BHK ts-3 Cl 7C

These cells contain a mutant polyoma virus genome. Fig. 1 illustrates the growth characteristics observed at 31° and 39°. At 39° (●—●—●) the amount of DNA per plate never exceeded 100 μg, whereas at 31° (▼—▼—▼) 175–225 μg of DNA per plate were easily obtained. Similarily, the percentage of labelled nuclei, as judged by radioautography after 60 min pulses with [^{3}H]thymidine [14], decreased rapidly with time at 39° (● - - ● - - ●) and much slower at 31° (▼- - ▼- - ▼). Table 3 shows the results of glycolipid analyses with

Table 2
Incorporation of [1-^{14}C]palmitic acid into glycolipids and phospholipids of BHK Wt Cl 2A.

Lipid	39°, high density	31°, high density
	Radioactivity (× 10^{-3} dpm)	
PC + SM	1000	1000
PE	91	217
Gl-1	8.2	3.0
Gl-2	6.9	1.5
Gl-3	0.2	0.3

For details, see legend to table 1.

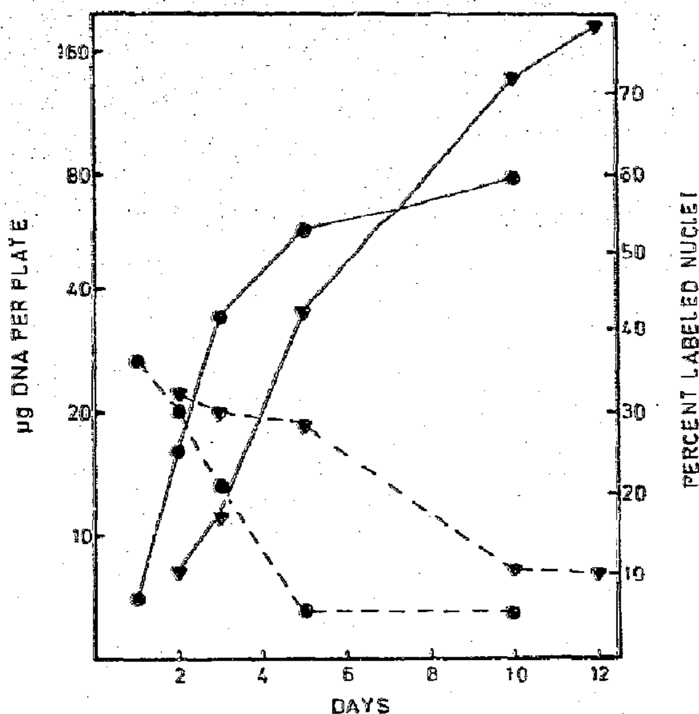

Fig. 1. DNA synthesis in BHK ts-3 Cl 7C at either 31° (▾) or 39° (●). Parallel cultures to those used for analyses of glycolipid synthesis were analyzed for content of DNA (solid lines) and percentage of labeled nuclei (broken lines) at different times after seeding.

Table 3
Incorporation of [1-^{14}C]palmitic acid into glycolipids and phospholipids of BHK ts-3 Cl 7C.

Lipid	39°, high density	31°, high density
	Radioactivity (× 10^{-3} dpm)	
PC + SM	1000	1000
PE	75	156
Gl-1	6.5	6.6
Gl-2	3.2	11.1
Gl-3	0.3	0.2

For details, see legend to table 1.

high density cultures at 31° (188 μg/plate) and 39° (100 μg/plate). The amounts of labelling of Gl-3 were identical to those observed for the cells transformed by wild type polyoma at both 31° and 39° and thus 20 times lower than those of confluent BHK Cl 13 cells.

4. Discussion

Previously, the glycolipid composition of virus transformed cells has been compared to that of the corresponding non-transformed cells. Here, we have used a cell line transformed by a mutant of polyoma virus. These cells exhibit growth control and normal agglutinability at the non-permissive temperature but uncontrolled growth and increased agglutinability at the premissive temperature [11, 12]. It was thus possible to study both controlled and uncontrolled growth in the *same* cell line and not as previously in different cell lines. Our results showed that polyoma transformation of BHK cells impairs the synthesis of ceramide trihexoside. Also, the synthesis of trihexosyl ceramide in the non-transformed BHK cells was cell density dependent. The BHK cells containing thermosensitive polyoma virus showed impaired synthesis of trihexosyl ceramide at both permissive and non-permissive temperatures. It may thus be concluded that "normal" glycosphingolipid synthesis is no requirement for the growth control exhibited by this cell line. This conclusion is at variance with previous suggestions concerning a correlation between the glycolipid pattern and growth control [1–7, 9].

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References

[1] S.-I. Hakomori and W.T. Murakami, Proc. Natl. Acad. Sci. U.S. 59 (1968) 254.
[2] P.T. Mora, R.O. Brady, R.M. Bradley and V.W. McFarland, Proc. Natl. Acad. Sci. U.S. 63 (1969) 1290.
[3] S.-I. Hakomori, Proc. Natl. Acad. Sci. U.S. 67 (1970) 1741.
[4] P.W. Robbins and I.A. Macpherson, Nature 229 (1971) 569.
[5] P.W. Robbins and I.A. Macpherson, Proc. Roy. Soc. London B 177 (1971) 49.
[6] H. Sakiyama, S.K. Gross and P.W. Robbins, Proc. Natl. Acad. Sci. U.S. 69 (1972) 872.
[7] S.-I. Hakomori, T. Saito and P.K. Vogt, Virology 44 (1971) 609.

[8] L. Warren, D. Critchley and I.A. Macpherson, Nature 235 (1972) 275.
[9] F.A. Cumar, R.O. Brady, E.H. Kolodny, V.W. McFarland and P.T. Mora, Proc. Natl. Acad. Sci. U.S. 67 (1970) 757.
[10] H. Den, A.M. Schultz, M. Basu and S. Roseman, J. Biol. Chem. 246 (1971) 2721.
[11] R. Dulbecco and W. Eckhart, Proc. Natl. Acad. Sci. U.S. 67 (1970) 1775.
[12] W. Eckhart, R. Dulbecco and M.M. Burger, Proc. Natl. Acad. Sci. U.S. 68 (1971) 283.
[13] I.A. Macpherson and M. Stoker, Virology 16 (1962) 147.
[14] K.G. Bjursell, P.A. Reichard and K.L. Skoog, European J. Biochem. 29 (1972) 348.
[15] G.M. Gray, Biochim. Biophys. Acta 144 (1967) 511.
[16] S. Hammarström, and B. Samuelsson, J. Biol. Chem. 247 (1972) 1001.