ORGANIZATION OF GLYCOLIPIDS AND GLYCOPROTEINS IN SURFACE MEMBRANES: DEPENDENCY ON CELL CYCLE AND ON TRANSFORMATION*

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Summary - Ceramide penta-, tetra-, and trisaccharides of hamster NIL cells were maximally labeled during the G1-phase, and minimally during the S-phase using a surface labeling procedure with galactose oxidase and tritiated borohydride. Since the chemical concentration of ceramide penta- and tetrasaccharides in NIL cells was found nearly constant throughout various phases, the observed change of the label during the cell cycle can be ascribed to the alternation of the exposure rate of glycolipids due to altered organizational assemblage in surface membranes. In polyoma transformed NIL cells, the chemical quantity of higher glycolipids was reduced; nevertheless the rate of exposure was much greater than in NIL cells and was invariant during the cell cycle. There was a unique label in a ceramide tetrasaccharide ("ceramide X") which was not found in non-transformed NIL cells. The label in "galactoprotein a" of NIL cells appears at the G1-phase and increases at later G1-phases. In transformed NIL cells, this label is always absent, throughout the cell cycle.

Proliferation of eukaryotic cells through the mitotic cell cycle may be controlled by modulation of structure and function of the cell surface membrane. Modulation of the surface structure occurring in "contact inhibited" cells has been assessed by the enhanced glycolipid synthesis (1-5) and by the enhanced uptake of *Ricinus* lectin (6) although it is not decided whether these changes occurred as a result of intercellular interaction due to cell contact or as a consequence of arrested cell growth at the G1-phase.

Cell cycle-dependent change of membrane heteroglycans has been suggested by variation of the reactivities with fluorescein-labeled wheat germ lectin (7) or by ^{3}H-labeled Con A (8) during different phases of the cell cycle, by the enhanced incorporation of ^{3}H-fucose into HeLa cell plasma membranes in late S-phase (9)

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Abbreviations: NIL, hamster embryo cells (see 3-5); NILpy, NIL cells transformed by polyoma virus.

and synthesis of a certain glycolipid (10) and a particular sialofucoglycopeptide in M-phase (11). Chatterjee et al. demonstrated that incorporation of ^{14}C galactose into various glycolipids of mouse embryonic fibroblasts increased several fold in the M- and G1-phases as compared to other phases (12), while the H-2 antigen was shown to be expressed more in G1 than other times of cell cycle (13).

Recently, the surface-exposed glycolipids and glycoproteins in mammalian cells have been labeled by galactose oxidase followed by reduction with tritiated sodium borohydride (14). A glycoprotein with an apparent molecular weight of 200,000 ("galactoprotein a") was detected in confluent NIL and BHK cells and was low in growing cells; however, transformed cells show no label of "galactoprotein a" (15,16). The labeling of "galactoprotein a" was deemed, therefore, to be cell-contact dependent as well as cell-cycle dependent. A similar protein was detected using the lactoperoxidase iodination method in confluent normal hamster, mouse, rat, and chick cells (17-19).

Consequently, cell-cycle dependent changes of the surface label of hamster NIL cells and its polyoma-transformants have been studied systematically. This paper reports a striking cell cycle-dependent variation of the glycolipid label which may be due to changing organizational assemblage in surface membranes, and the initiation of the label in "galactoprotein a" in the G1-phase. The magnitude of such variation of the label during the cell cycle is minimal or non-existent in polyoma-transformants.

EXPERIMENTAL METHODS

Condition of the synchronized cell culture: Synchronization of normal NIL cells was obtained by trypsinization of completely "contact inhibited" confluent cells. NIL 2E cells were culture in Eagle's medium containing a doubled concentration of amino acids and vitamins. The confluent cells were kept as such for at least three days to ensure complete confluency; they were trypsinized and cells were seeded in 15 cm Falcon plastic dishes. NIL cells were also synchronized by a double thymidine block in 2.5 mM thymidine (20) and NILpy cells only by this method.

Surface labeling: Cells were treated with 10 units of galactose oxidase (21) on 15 cm Petri dishes for 30 minutes, and then collected by treatment with ethylenediamine tetraacetate and reduced with tritiated sodium borohydride, as described previously (15).

Analysis of surface-labeled glycolipids and glycoproteins: The surface-labeled cells were dissolved in sodium dodecyl sulfate and 2-mercaptoethanol and electrophoresed on 5% acrylamide gels; gels were sliced and the radioactivities were

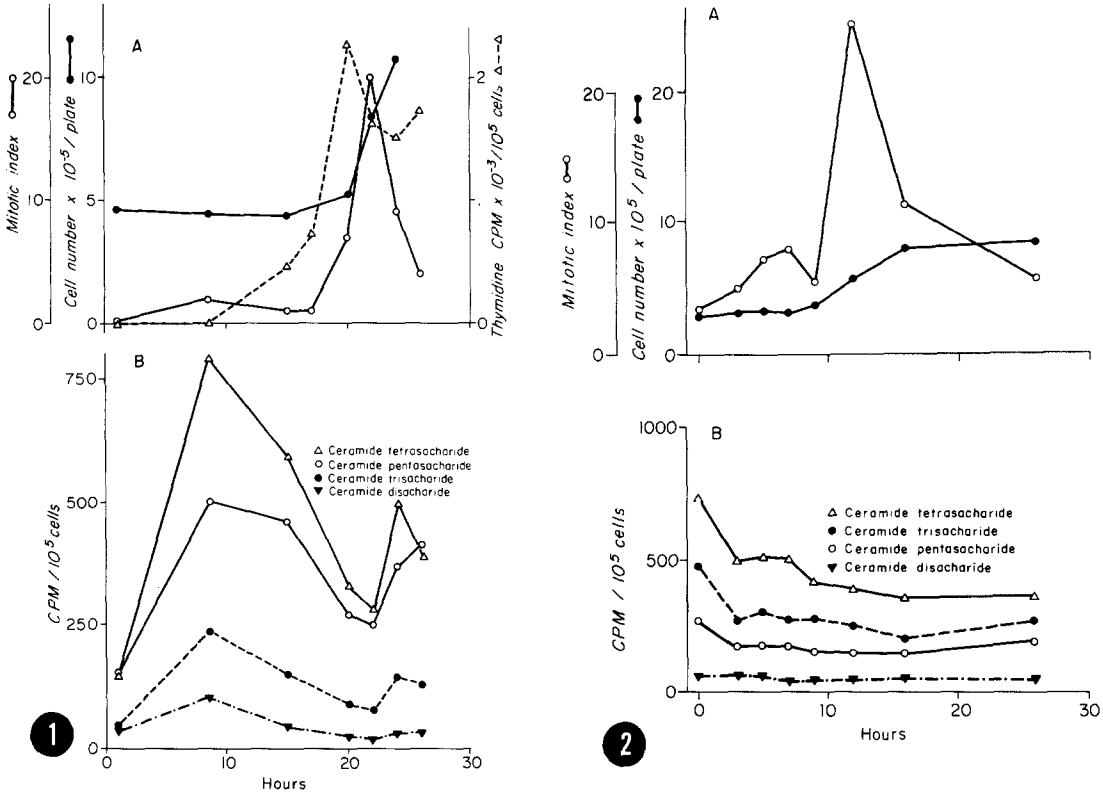

Figure 1.
Variation of the surface-label activities of glycolipids in synchronized hamster NIL cells. Synchronization started from the confluent, trypsinized cells. Upper chart (A) shows the changes of mitotic index, cell number and thymidine incorporation into DNA. Lower chart (B) indicates the label activities of various glycolipids at the corresponding time as shown in Chart A. It is clear that S-phase occurred at 16-20 hours and the mitosis occurred at 22-26 hours after seeding. Note that a pronounced peak of the label occurred at G1-phase, decreased at S-phase, and increased again after mitosis. A similar glycolipid label was obvious in synchronized cells with double thymidine block method.

Figure 2.
Variation of the surface-label activities of glycolipids in synchronized hamster NILpy cells. Synchronization was followed after thymidine block of cell growth at G-S interphase. The upper chart (A) showed the change of mitotic index and cell numbers; the lower chart (B) showed the change of glycolipid label corresponding to the time as in the upper chart (A).

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counted as previously described (14). Glycolipids were extracted with chloroform-methanol and the total glycolipid fraction was separated from neutral lipids and phospholipids by the acetylation procedure (22). The total glycolipids obtained as acetylated derivatives were deacetylated and further separated by thin-layer chromatography on Silica gel G (Analtech, precoated plate); silica gel zones containing different glycolipids were scraped and solubilized with "NCS solubilizer" (Amersham/Searle) and counted in a scintillation counter.

Chemical quantity of glycolipids: The glycolipids fraction was prepared by acetylation procedure (22) and the chemical quantity of glycolipids present in the extract determined by gas chromatography after methanolysis, N-acetylation, and O-trimethylsilylation (23). Inositol was used as an internal standard. *Cell number* was determined by Coulter counter; *mitotic index* was determined as the percentage of mitotic cells against the total cell number present in several arbitrary microscopic sights. *Thymidine uptake* was determined by incubation with ^{3}H thymidine for 30 minutes followed by precipitation of cells with ice-cold trichloroacetic acid. The insoluble precipitate was filtered through a "Millipore filter" and the radioactivity was determined.

RESULTS

Change of surface-exposed glycolipid label during cell cycles of NIL and NILpy cells: As seen in Figure 1, the surface-exposed label of glycolipids in NIL cells increased severalfold at the G1-phase, and decreased significantly at the S-phase and increased again after mitosis. Such a striking variation of the label depending on cell cycle was observed in synchronized cells with different techniques; i.e., the synchronized cells started from confluent culture and from double thymidine block (not shown here).

The chemical quantity of ceramide pentasaccharide[1] and ceramide tetrasaccharide[1] was found nearly constant irrespective of different phases of cell cycle although ceramide tri- and dihexoside increased significantly at G1 and mitotic phases (Table I). Since a large variation of the label occurred in ceramide penta- and tetrasaccharide, the observed change of the surface label should depend on the rate of exposure of these glycolipids.

The label of glycolipid in NILpy cells did not vary during phases of cell cycle; i.e., no maximum label was found at the G1-phase (see Figure 2). The chemical quantity of ceramide penta- and tetrasaccharide in NILpy cells was much lower than that of NIL cells in agreement with the previous reports (2-5, 24). Nevertheless, the surface label of NILpy cells in these glycolipids was not very much reduced. This finding indicates that the labeling efficiency of glycolipids in NILpy cells is generally higher than in NIL cells.

The presence of a label in a specific glycolipid of NILpy cells ("ceramide

[1] As previously reported (24), the majority of ceramide pentasaccharide is a Forssman glycolipid (αGalNAcl→3βGalNAcl→3αGall→4βGall→4Glc→ceramide) and that of ceramide tetrasaccharide is globoside (βGalNAcl→3αGall→4βGall→4βGlc→ceramide) in NIL cells. Each glycolipid fraction, however, contains other glycolipids with similar structures.

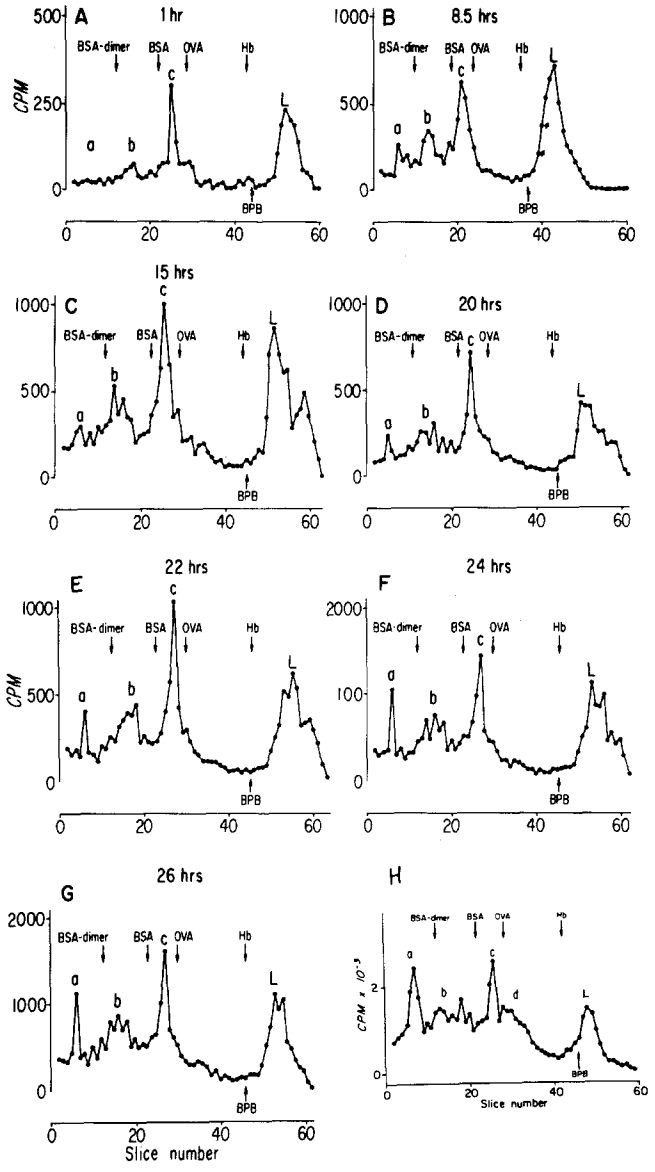

Figure 3.
Variation of cell surface label in glycoprotein during the different phases of NIL cell cycle. BSA-dimer (dimeric bovine serum albumin), BSA (bovine serum albumin), OVA (ovalbumin), and Hb (hemoglobin) indicates the location of the internal references labeled with ^{14}C-formaldehyde; L represents the lipid label. A to G showed the labeling pattern of cells obtained 1 hour to 24 hours after synchronization started from confluent, trypsinized cells. Hours of sampling indicated in each pattern, H showed the labeling pattern of confluent cells. Note that the label for "galactoprotein a" (as marked as peak "a") appeared at 8.5 hours (corresponding to G1 phase) after seeding, and greatly increased on cell contact. The peak c and a part of peak L are due to non-specific label by tritiated sodium borohydride alone.

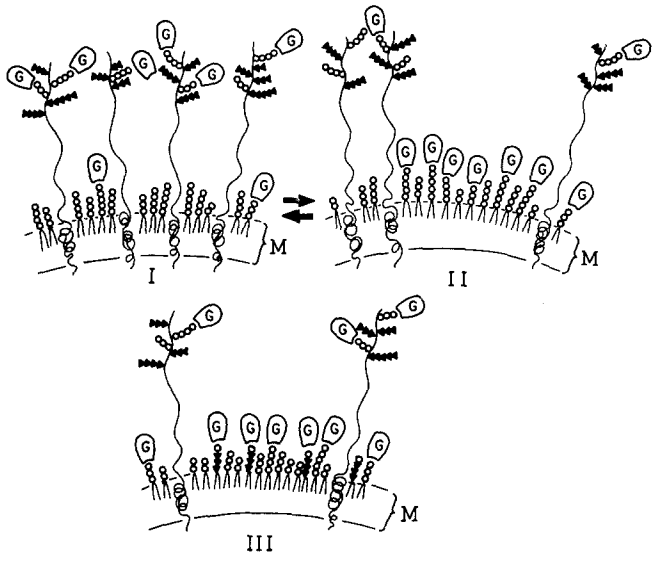

Figure 4.
Idealized version of the assemblage of glycolipids and glycoproteins in surface membranes. Circles represent sugars with known structures; two, three, four, and five circles with two tails represent, respectively, ceramide di-, tri-, tetrasaccharide (globoside), and pentasaccharide (Forssman antigen). *Coiled or stretched lines* represent peptide, *solid triangles* represent sugar unit with unknown structure; they are present mostly on glycoproteins, some in glycolipids of transformed cells ("ceramide X").

NIL cells have a number of glycolipids and glycoproteins with galactosyl and galactosaminyl terminal residues which are easily labelled with galactose oxidase (represented by the G).

Glycolipids were directly imbedded in lipid bilayer matrix (M) through ceramide among "bushes of ektoprotein and glycoprotein" (14). Their reactivity with galactose oxidase ("G") may be determined by the spacing of ektoprotein (glycoprotein) bushes. State I shows that glycolipids are "cryptic" among bushes of protein and glycoprotein, representing the S-phase. State II shows that glycolipids are exposed by the change of spacing of the ektoproteins and glycoproteins, representing the G1-phase. Note that more label in glycolipid occurred at the G1-phase due to the larger interspace between ektoproteins. State III shows the surface assemblage of transformed cells where a) most of the glycolipid could be exposed due to fewer ektoproteins and glycoproteins and larger interspace between them b) reduced ceramide penta- and tetrasaccharide, increased ceramide dihexoside, and c) a new label in an unknown glycolipid ("ceramide X").

X"): The major label found in ceramide tetrasaccharide of NILpy cells was not on globoside; it was due to a glycolipid with a galactosyl residue at the non-reducing end containing glucosamine instead of galactosamine, but is as yet not fully characterized (ceramide X). This glycolipid was not labeled, nor chemically detectable in normal NIL cells and is considered to be a specific product of NILpy cells. Ceramide tetrasaccharide of NIL cells was essentially a globoside.

TABLE I

Chemical Concentration of Glycolipids of NIL Cells at Different Phases of Cell Cycle* (Determined by Gas Chromatography)

	The amount of glycolipid found: expressed as galactose μg per 10^7 cells		
	1 hr.	*8.5 hr. (G1-phase)*	*24 hr. (Mitotic phase)*
Ceramide pentasaccharide[1]	1.58	1.28	1.33
Ceramide tetrasaccharide[2]	1.29	1.46	0.85
Ceramide trisaccharide	0.49	0.99	0.95
Ceramide dihexoside	0.07	0.22	0.26

* Synchronized cells started from trypsinized confluent cell culture.
1 Forssman glycolipid and other glycolipid with a similar structure.
2 Globoside and other similar glycolipids containing GalNAc and Gal.

Change of glycoprotein label of NIL cells at various phases of cell cycle: As seen in Figure 3, only "galactoprotein a," with an apparent molecular weight of 200,000, changed significantly during the cell cycle. The "galactoprotein a" label can be removed by trypsinization and appeared at the G1-phase, the intensity of the label remained nearly constant during the S through M phases and significantly increased in the next G1-phase.

As has been shown previously, the label in "galactoprotein a" was lost in transformed NIL and BHK cells (15, 16).

DISCUSSION

The present study clearly indicates a dramatic change in the organization of glycolipids in membranes during the cell cycle of non-transformed NIL cells and the absence of such changes of organization in NILpy cells. Since chemical concentration of ceramide pentasaccharide and tetrasaccharide was not significantly changed through various phases of the cell cycle, the enhanced label in those glycolipids at G1-phase can be explained by the altered organization of glycolipids in membrane; it may be possible that the exposure of glycolipids could result

from enlarged spacing and allocation of the "bushes of ektoproteins" (14) among which glycolipids are seated (see Figure 4 and legend).

The present study also indicates that glycolipids of NILpy cells are exposed to the outer environment at a much higher rate than that of normal cells. The glycolipids on the lipid bilayer can be exposed by either lateral dislocation or by deletion of ektoprotein "bushes" ("State II" and "State III" in Fig. 4). Thus, the idea of "cryptic site" becoming "exposed" during cell cycle (7) and on transformation (25, 26) can be true with regard to glycolipid organization in membranes. It is also possible that such a transition of organization of membrane glycolipids can be closely associated with ektoprotein mobility in the lipid bilayer (27) which can be coupled by the activity of cytoplasmic filaments sensitive to colchicine or cytochalasin B (see e.g., 28, 29).

The elevation of chemical concentrations of some glycolipid in confluent cell culture as previously reported (2-5) must be largely ascribed to cell contact, rather than arrested cell growth at the G1-phase as the chemical concentration of globoside and Forssman glycolipids did not increase at G1-phase.

The presence of an unique, high molecule "galactoprotein a" (mol. wt., app. 200,000) in confluent cell sheets of BHK and NIL cells and its complete absence in virally-transformed cells has been noted previously (15, 16). It is entirely plausible that the spacing and allocation of this ektoprotein in membrane may determine the degree of exposure of glycolipids. As a result of the missing "galactoprotein a," glycolipids of NILpy cells could be highly exposed to the external environment, irrespective of cell cycle phase.

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