A Comparison of Membrane Components of Normal and Transformed BALB/c Cells†

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ABSTRACT: Membrane glycolipids, glycoproteins, and surface proteins of normal and transformed BALB/c cell lines have been compared. Several virally and spontaneously transformed cell lines showed differences in membrane components compared to normal A_{31} cells. These differences consisted of increased amounts of simpler gangliosides, absence of the large external transformation sensitive (LETS) protein, and the appearance of a major new glycoprotein band of about 105 000 molecular weight. In contrast, the spontaneously transformed cell line that caused the fastest growing tumors in vivo and the most rapid animal death (3T12T) did not have these changes. A_{31} and 3T12T glycolipid profiles appear similar as did glycoproteins and cell surface proteins detected by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. When Pronase-generated glycopeptides were analyzed by Sephadex G-50 chromatography, an enrichment in faster-eluting species was seen in two killing tumor lines (c5T and 3T12T) compared to A_{31}. Regressing tumor lines (MSC, c5) did not show this change. Isolated membrane glycoproteins yield glycopeptides of different sizes after Pronase digestion. In addition, several 3T12T glycoproteins yield glycopeptides that are larger than those from the corresponding glycoproteins of A_{31} cells. It appears that glycopeptide alterations associated with transformation occur in several membrane glycoproteins.

In recent years there has been great interest in the role of cell membrane components in the loss of growth regulation characteristic of neoplasia. One of the changes detected in virus transformed cells is the absence of a 240 000 molecular weight glycoprotein (LETS)[1] (Hynes, 1973; Gahmberg and Hakomori, 1973; Hynes and Humphreys, 1974; Hogg, 1974). Efforts to relate the loss of this glycoprotein directly to loss of growth control (Teng and Chen, 1975; Blumberg and Robbins, 1975; Pearlstein et al., 1976) have indicated that LETS may not be directly involved in regulating cell division. The biological role of LETS remains unclear. Another change detected in transformed cell glycoproteins is the appearance of glycopeptides generated by Pronase digestion that elute earlier on Sephadex G-50 than those of normal cells (Meezan et al., 1969; Buck et al., 1970). If the apparently larger transformed cell glycopeptides are treated with neuraminidase, they elute similar to normal cell glycopeptides on Sephadex G-50 (Warren et al., 1972), implying that increased sialic acid is involved in this change. Evidence that the alteration of transformed cell glycopeptides involves more than sialic acid differences was presented by Glick (1974) and Ogata et al. (1976). The latter group showed that the large transformed cell glycopeptides and smaller normal cell glycopeptides demonstrated differential binding to concanavalin A–Sepharose, with or without prior neuraminidase treatment.

Changes in glycolipids associated with transformation have also been detected in a number of different systems. The transformed cells generally show a reduced level of hematoside or higher gangliosides due to the loss of specific glycolipid glycosyltransferases. For example, BHK cells transformed by polyoma virus have lower levels of hematoside (Hakomori and Murakami, 1968) and lower levels of CMP-NAN:lactosylceramide sialyltransferase (Den et al., 1971) than do normal BHK cells. Similar decreases in hematoside or more complex gangliosides have been detected in virus, carcinogen, and spontaneous transformants of mouse, chicken, rat, and human cells (for review, see Hakomori, 1975).

We have previously reported chemical and biological studies of a variety of spontaneously transformed and virally transformed cell lines derived from BALB/c mice. These studies have indicated that carbohydrate changes typical of viral transformation such as increased concanavalin A agglutination (Van Nest and Grimes, 1974), altered glycosyltransferase levels (Patt et al., 1975), and reduced carbohydrate compositions (Grimes and Greegor, 1976) are not always found in spontaneous transformants. Among the various transformants studied, decreased carbohydrate levels seem to be associated with cell lines causing regressing tumors while carbohydrate levels of killing tumor lines are similar to those of normal cells (Grimes and Greegor, 1976). In the work presented here, we have characterized glycolipids, glycoproteins, and surface proteins of these same cell lines.

Materials and Methods

Cell Lines and Cell Culture. All cell lines were derived from BALB/c mice. A_{31} is a cloned line of BALB/c 3T3 fibroblasts and was a gift from Dr. G. Todaro of NIH. c5 is a transformed cell line cloned from A_{31} as previously described (Van Nest and Grimes, 1974). c5T was isolated from a tumor caused by injecting c5 cells into a BALB/c mouse. MSC was derived from a Moloney strain murine sarcoma virus induced tumor in a

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[1] Abbreviations used are: LETS, large external transformation sensitive protein; DMM, Dulbecco's modified minimal essential medium; TEMED, *N,N,N',N'*-tetramethylethylenediamine; PPO, 2,5-diphenyloxazole; G_{M3}, *N*-acetylneuraminylgalactosylglucosylceramide; G_{M2}, *N*-acetylgalactosaminyl[*N*-acetylneuraminyl]galactosylglucosylceramide; G_{M1}, galactosyl-*N*-acetylgalactosaminyl[*N*-acetylneuraminyl]galactosylglucosylceramide; G_{D1a}, *N*-acetylneuraminylgalactosyl-*N*-acetylgalactosaminyl[*N*-acetylneuraminyl]galactosylglucosylceramide; G_{T1}, *N*-acetylneuraminylgalactosyl-*N*-acetylgalactosaminyl[*N*-acetylneuraminyl-*N*-acetylneuraminyl]galactosylglucosylceramide; BHK; baby hamster kidney cells; CMP-NAN, cytosine monophosphate-*N*-acetylneuraminic acid; PBC, primary BALB/c cells; Tris, 2-amino-2-hydroxymethyl-1,3-propanediol.

BALB/c mouse, and was the gift of Dr. S. Russell of Scripps Clinic and Research Foundation. 3T12T cells were isolated from a tumor caused by injecting 3T12 cells (a gift from Dr. Todaro) into a BALB/c mouse. PBC cells are early passage BALB/c embryo fibroblasts (Van Nest and Grimes, 1974). Cells were grown in monolayer cultures in Dulbecco's modified minimal essential medium supplemented with 10% fetal calf serum (Grand Island Biological Co., Berkeley, Calif.) and penicillin and streptomycin at concentrations of 50 units and 50 μg/mL, respectively. Cells were routinely passaged with a 5-min treatment of 0.5% trypsin at 37 °C. Routine tests for mycoplasma contamination were performed (Levine, 1972; Peden, 1975). Cultures testing positive were not used for any experiments and were discarded.

Radioactive Labeling. Cell surface proteins were labeled by the [^{125}I]lactoperoxidase method as described by Hynes and Humphreys (1974). Approximately 10^6 cells were plated on 60-mm petri plates and incubated for 24 h in normal growth medium. The monolayers were then washed three times with PBS (0.14 M NaCl, 2.7 mM KCl, 15.3 mM Na_2HPO_4, 1.5 mM KH_2PO_4, 0.9 mM $CaCl_2$, 0.5 mM $MgCl_2$, pH 7.2), and labeled for 10 min at room temperature in 0.5 mL/plate of PBS containing 5 mM glucose, 20 μg/mL lactoperoxidase (Calbiochem, La Jolla, Calif.), 0.1 unit/mL glucose oxidase (Calbiochem), and 250 μCi/mL Na^{125}I (17.0 Ci/mg). The reaction was stopped by adding PBS containing 1.47 mg/mL cold NaI and the monolayers were washed three times with the same solution. The cells were then scraped from the plates into PBS containing 2 mM phenylmethanesulfonyl fluoride and pelleted by centrifugation at 2300 rpm. The labeled pellets were stored at −70 °C. In some experiments the cells were subjected to mild trypsin treatment immediately after labeling. Such cells were washed as above and incubated for 10 min at room temperature with 10 μg/mL trypsin in a buffer consisting of 0.046 M Tris, 0.015 M $CaCl_2$, 0.088 M NaCl, pH 8.1. Control plates were incubated in the buffer alone. Control and trypsinized cells were then harvested and stored as above. Labeling of glycoproteins with [^{14}C]- or [^{3}H]glucosamine was performed by incubating cell monolayers for 24–48 h in DMM containing 10% fetal calf serum and either 5 μCi/mL [^{3}H] glucosamine (10.74 Ci/mmol) or 2 μCi/mL [^{14}C]glucosamine (237.7 mCi/mmol). The labeled cells were washed three times with solution A (calcium- and magnesium-free PBS), and removed from the flask with one round of freeze-thawing in solution A containing 2 mM phenylmethanesulfonyl fluoride. The cells were then homogenized (Dounce) and centrifuged at 1300 rpm for 5 min to remove nuclei and large pieces, and the crude mixture of labeled membrane fragments remaining in suspension was pelleted by centrifugation at 100 000g for 90 min. The pellet was collected and stored at −70 °C.

Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis. Labeled membrane samples were dissolved in a solution containing 20% sucrose, 1% sodium dodecyl sulfate, 0.05% dithiothreitol, 0.027 M H_2SO_4, 0.054 M Tris, pH 6.1. The samples were boiled in the above solution and applied to either slab or tube gels. The complete electrophoresis system was as described by Neville (1971) and consisted of the following. Upper reservoir buffer: 0.04 M boric acid, 0.041 M Tris, 0.1% sodium dodecyl sulfate, pH 8.64; lower reservoir buffer: 0.031 N HCl, 0.424 M Tris, pH 9.19; stacking gel: 3% acrylamide in 0.027 M H_2SO_4, 0.054 M Tris, 5% glycerol; resolving gel: 7.5% acrylamide in lower reservoir buffer containing 10% glycerol. The ratio of acrylamide to *N,N'*-methylenebisacrylamide was 25:1 in all gels. Stacking and resolving gels were polymerized by the addition of 0.08% ammonium persulfate and 0.05% TEMED. Slab gels were dried and radioactivity was detected by autoradiography on Kodak RP/R-14 film. Tube gels were sliced into 1-mm sections and glycoproteins were eluted by shaking in 1% sodium dodecyl sulfate overnight. Radioactivity was determined in a Beckman liquid scintillation counter in a mixture containing 2900 mL of xylene, 960 mL of Triton X-114, and 125 mL of Liquifluor (New England Nuclear, Boston, Mass.).

Pronase Digestion. Labeled membrane preparations or sodium dodecyl sulfate gel sections containing labeled glycoproteins were incubated in a solution of 0.1 M Tris, 20 mM $CaCl_2$, pH 7.8, and Pronase (Calbiochem) for 5–8 days at 37 °C. Routinely, 100 μg of Pronase was included on day 0 and 50 μg was added every other day. A few drops of toluene were added to inhibit bacterial growth.

Gel Chromatography. Pronase glycopeptides were analyzed on a Sephadex G-50 column (1.5 × 90 cm). Samples were applied and eluted with 0.05 M potassium phosphate, 0.02% sodium azide, pH 7.2. Two-milliliter fractions were collected, and radioactivity was determined in a Beckman liquid scintillation counter.

Glycolipid Analysis. Cells were labeled with [^{14}C]- or [^{3}H]glucosamine as described for glycoproteins. Labeled membrane fragments were extracted three times with $CHCl_3$–MeOH (2:1) and the extracts were pooled and dried. Labeled glycolipids were then separated using thin-layer chromatography on Silica G-25 plates and developed with CH_3Cl_2–MeOH–0.25% $CaCl_2$ (60:35:8) along with glycolipid standards. Glycolipid standards were detected with iodine vapor. Labeled glycolipids were detected by cutting the developed TLC plate into 0.5-cm strips and counting in a scintillation counter or by autofluorography using Kodak RP/R-14 film after spraying plates with chloroform containing 10% PPO.

Results

The biological properties of these cell lines have been previously described (Van Nest and Grimes, 1974; Russell and Cochrane, 1974). Briefly, A_{31} is a contact inhibited cell line that is nontumorigenic in BALB/c or nude mice. C5 cells cause tumors that always regress in BALB/c mice but which grow progressively, killing nude mice. C5T and 3T12T cause tumors that grow progressively to kill BALB/c mice with 100% frequency. MSC cells can be manipulated to cause either regressing or killing tumors by altering the inoculation cell dose. The 3T12T line can be classified as nonimmunogenic, since injecting irradiated tumor cells or tumor excision does not protect mice from subsequent challenge by 3T12T cells. MSC and C5T are immunogenic, since the same procedures protect animals from rechallenge. Interestingly, mice which have had C5 tumors that regressed are protected from subsequent challenge by C5T cells, indicating shared antigens between C5 and C5T cells.

Glycolipids. Glycolipids were labeled, extracted, and analyzed as described under Materials and Methods. Figure 1 shows the results of thin-layer chromatography of [^{14}C]glucosamine-labeled glycolipids from the cell lines. It can be seen that confluent (panel A) and growing (panel B) A_{31} cells as well as 3T12T cells (panel F) show similar labeling patterns, while MSC, c5, and c5T (panels C,D,E) cells show different patterns. For a more detailed comparison, cells were labeled with [^{3}H]glucosamine, chloroform–methanol soluble material was chromatographed on thin-layer plates, and the amount of radioactivity migrating with known glycolipid standards was quantitated (Table I). In extracts from A_{31} cells and 3T12T

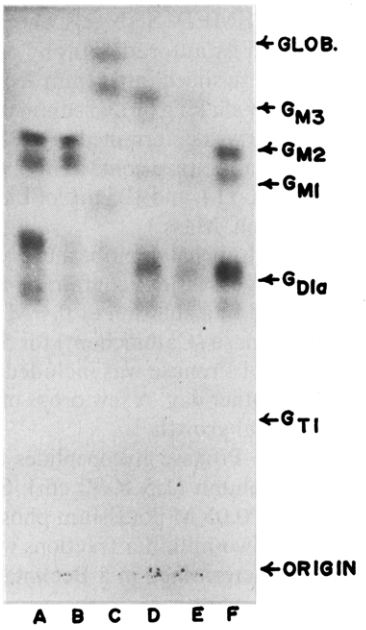

FIGURE 1: Thin-layer chromatography of [^{14}C]glucosamine-labeled glycolipids. Glycolipids were labeled and extracted as under Materials and Methods. Glycolipids were applied to Silica G-25 plates and developed with $CHCl_3$–MeOH–0.25% $CaCl_2$ (60:35:8). After drying, the chromatogram was sprayed with $CHCl_3$ containing 10% PPO, dried, and exposed on x-ray film. Arrows at right mark the migration of glycolipid standards. (A) Confluent A_{31}; (B) growing A_{31}; (C) MSC; (D) c5; (E) c5T; (F) 3T12T.

TABLE I: Thin-Layer Chromatography of [^{3}H]Glucosamine-Labeled Glycolipids.[a]

	% of total $CHCl_3$–MeOH soluble cpm in:					
Cell line	G_{T1}	G_{D1a}	G_{M2}	G_{M3}	Globoside	Total
A_{31} confluent	1	54	33	5	1	94
A_{31} growing	2	47	39	2	1	91
MSC	0	29	11	33	24	97
C5	1	52	19	20	2	94
C5T	0	58	27	12	0	97
3T12T	0	50	38	3	5	96

[a] Glycolipids were labeled with [^{3}H]glucosamine and extracted as outlined under Materials and Methods. Samples were applied to Silica G-25 chromatography plates and developed with chloroform–methanol–0.25% $CaCl_2$ (60:35:8). After drying, the plates were cut into 0.5-cm strips and the strips were placed in scintillation vials and counted in a Beckman liquid scintillation counter. Standard glycolipids were visualized by iodine vapor.

cells almost all the label comigrates with G_{M2} and G_{D1a}. Very little G_{M3} or globoside is detected. MSC cells show increased G_{M3} and globoside and decreased G_{M2} and G_{D1a} labeling compared to A_{31}. c5 and c5T are very similar and show increased G_{M3} and decreased G_{M2} compared to A_{31}. These results are similar to those of Fishman et al. (1976), using sialic acid determination to compare ganglioside levels of normal and transformed 3T3 cells. It should be noted that normal 3T3 cells have been shown to demonstrate glycolipid changes at early stages of cell touch (Yogeeswaran and Hakomori, 1975). The methods used here compare only growing and confluent A_{31} cells and would not detect such changes.

Surface Proteins. Figure 2 shows the surface labeling patterns of A_{31} cells (panel A) and PBC cells (panel B) as detected

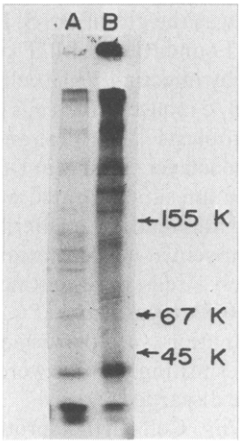

FIGURE 2: Sodium dodecyl sulfate–polyacrylamide gel electrophoresis of ^{125}I-labeled normal cell surface proteins. Approximately 50 000 cpm of each labeled cell material was applied to a 7.5% slab gel and electrophoresed at 150 V for approximately 2 h. The gel was dried and exposed on x-ray film. Molecular weight markers used include: goat IgG, 155 000; bovine serum albumin, 67 000; hen egg albumin, 45 000: (A) A_{31}; (B) PBC.

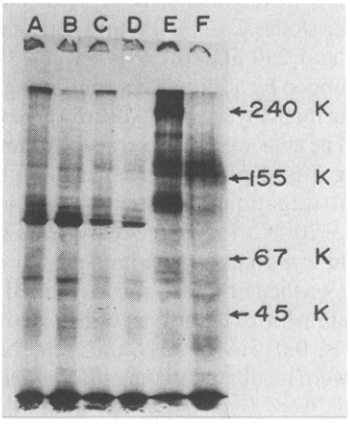

FIGURE 3: Sodium dodecyl sulfate–polyacrylamide gel electrophoresis of ^{125}I-labeled transformed cell surface proteins. Electrophoresis and autoradiography was identical to Figure 2. Molecular weight markers used include: spectrin (large band), 240 000; goat IgG, 155 000; bovine serum albumin, 67 000; hen egg albumin, 45 000; (A) c5; (B) c5 trypsinized; (C) c5T; (D) c5T trypsinized; (E) 3T12T; (F) 3T12T trypsinized.

by the [^{125}I]lactoperoxidase procedure. Both cell lines show labeling of a band analogous to the LETS glycoprotein detected in other systems. Other regions of the gel appear similar between A_{31} and PBC, with a few differences appearing in smaller molecular weight regions. Figure 3 shows ^{125}I profiles for the three spontaneously transformed cell lines before and after mild trypsinization (10 μg/mL for 10 min at room temperature). c5 shows virtually identical patterns before (panel A) and after (panel B) trypsin treatment, as does c5T (panels C and D). Virtually no labeling is seen in the LETS region, while a very heavily labeled band of about 105 000 molecular weight appears in both these cell lines. 3T12T (panel E) is strikingly different from the other two transformed lines, with the heaviest label appearing in a band of 240 000 molecular weight, analogous to the LETS glycoprotein. This band is sensitive to mild trypsinization (panel E), as has been reported for the LETS glycoprotein. 3T12T also shows much greater labeling of several bands of about 165 000 (which are not trypsin sensitive) and about 135 000 molecular weight (which are trypsin sensitive) than do c5 and c5T. There is no labeling of the 105 000 molecular weight band in 3T12T.

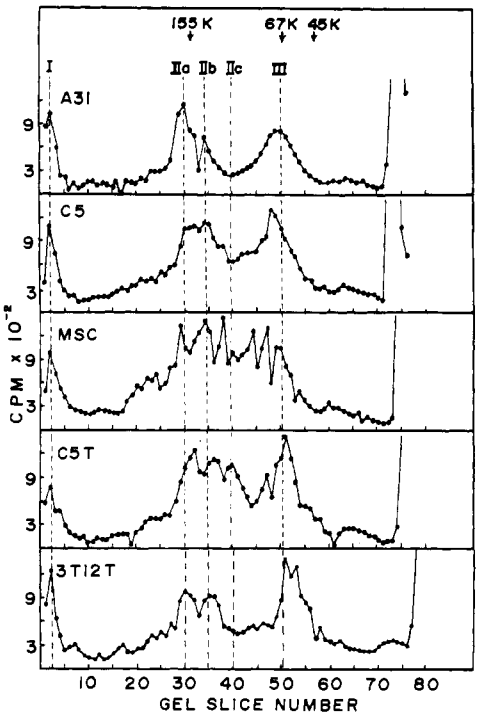

FIGURE 4: Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of [^{3}H]glucosamine-labeled glycoproteins. 60 000 cpm of each labeled membrane preparation was applied to 7.5% gels in 6.0 × 120 mm glass tubes. Gels were electrophoresed at 3 mA/tube for approximately 3 h. Gels were rimmed from the glass tubes, frozen, and cut into 1-mm slices. Slices were shaken overnight in 2 mL of 1% sodium dodecyl sulfate to elute glycoproteins and the eluted material was counted in a Beckman liquid scintillation counter. Molecular weight markers used include: goat IgG, 155 000; bovine serum albumin, 67 000; hen egg albumin, 45 000.

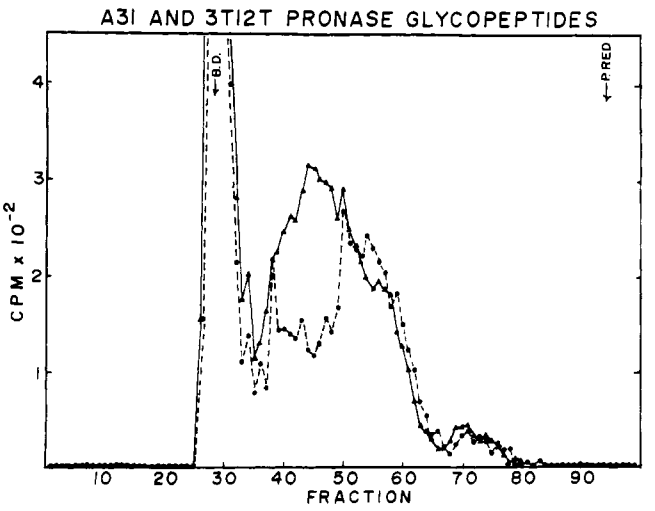

FIGURE 5: Sephadex G-50 chromatography of A_{31} (●) and 3T12T (▲) Pronase glycopeptides. [^{3}H]Glucosamine-labeled A_{31} and [^{14}C]glucosamine-labeled 3T12T membrane preparations containing equal amounts of radioactivity were mixed and digested for 5 days with Pronase. An aliquot of the digested material was then applied to a Sephadex G-50 column (90 × 1.5 cm). B.D., blue dextran 2000; p. red, phenol red.

Membrane Glycoproteins. Crude membrane glycoproteins were labeled with [^{3}H]glucosamine and prepared as described under Materials and Methods. The labeled preparations were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis with the results shown in Figure 4. Three labeled regions were found in all the cell lines tested: region I, about 220 000–250 000; region II, about 165 000–105 000; and region III, appearing about 70 000 molecular weight. There were virtually no differences in region I patterns between any of the cell lines. Region II labeling patterns vary between the cell lines tested. A_{31} and 3T12T cells appear to possess at least two glycoproteins in this region, IIa and IIb. c5, c5T, and MSC show these same glycoproteins as well as an additional glycoprotein, IIc, of about 105 000 molecular weight. Region III again appears similar among all the lines. This consists of a broad peak that actually separates into a doublet on some gels. MSC also shows labeling of a group of bands between regions II and III. In comparing each region it was found that A_{31} always shows relatively lower labeling of region III than do the transformed lines.

Glycopeptides. We next compared glycopeptides from these cell lines. [^{14}C]- and/or [^{3}H]glucosamine labeled membrane fragments were digested exhaustively with Pronase and the resulting glycopeptides were analyzed using Sephadex G-50 chromatography. Figure 5 shows the cochromatogram of [^{3}H]glucosamine-labeled A_{31} and [^{14}C]glucosamine-labeled 3T12T membranes after Pronase digestion. It can be seen that 3T12T is enriched in higher molecular weight glycopeptide species compared to A_{31}, confirming the work of Meezan et al. (1969) and Buck et al. (1970). If the A_{31}-3T12T glycopeptide mixture was treated with neuraminidase prior to Sephadex G-50 chromatography, the elution patterns of glycopeptides from the two cell lines appeared very similar. 3T12T glycopeptides also showed an enrichment in faster-eluting species when compared to PBC glycopeptides. Identical comparisons were made of glycoproteins from A_{31} and the other transformed cell lines as well as from growing and confluent A_{31} cells. As shown in Figure 6, 3T12T, and c5T, the two killing tumor lines, both show an increase of higher molecular weight glycopeptides compared to A_{31}. MSC shows perhaps a small enrichment of the larger glycopeptides, while c5 glycopeptides are identical to those from A_{31} cells. There were no differences detected between glycopeptides of growing and confluent A_{31} cells.

The individual glycoprotein regions were separated from [^{3}H]glucosamine-labeled A_{31} and [^{14}C]glucosamine labeled 3T12T membranes by electrophoresis in a large (1.5 × 15 cm) tube gel. Portions of the gel corresponding to regions I, II, and III were separated, digested exhaustively with Pronase, and analyzed on Sephadex G-50. The results are presented in Figure 7. In both cell lines, the glycopeptides from region I appear to be smallest in size, those from region II are of intermediate size, and those of region III are largest. 3T12T region I and II glycopeptides appear larger than A_{31} region I and II glycopeptides, respectively. Region III glycopeptides from both cell lines appear the same size. There appears to be a multiple cause for the enrichment of large glycopeptides in Pronase digests of crude 3T12T membrane preparations. 3T12T has several glycoproteins (regions I and II) that produce larger glycopeptides than do the corresponding glycoproteins from A_{31}, and 3T12T shows consistently more glucosamine incorporation into glycoproteins that contain the largest glycopeptides from both cell lines (region III). If the separated region I, II, and III glycopeptides are treated with neuraminidase prior to Sephadex G-50 chromatography, the migration of all three regions is shifted to a smaller molecular weight (Figure 8). The size order of region III > region II > region I remains, however, after neuraminidase treatment. In addition, 3T12T region I and II glycopeptides remain larger than A_{31} regions I and II glycopeptides after neuraminidase treatment.

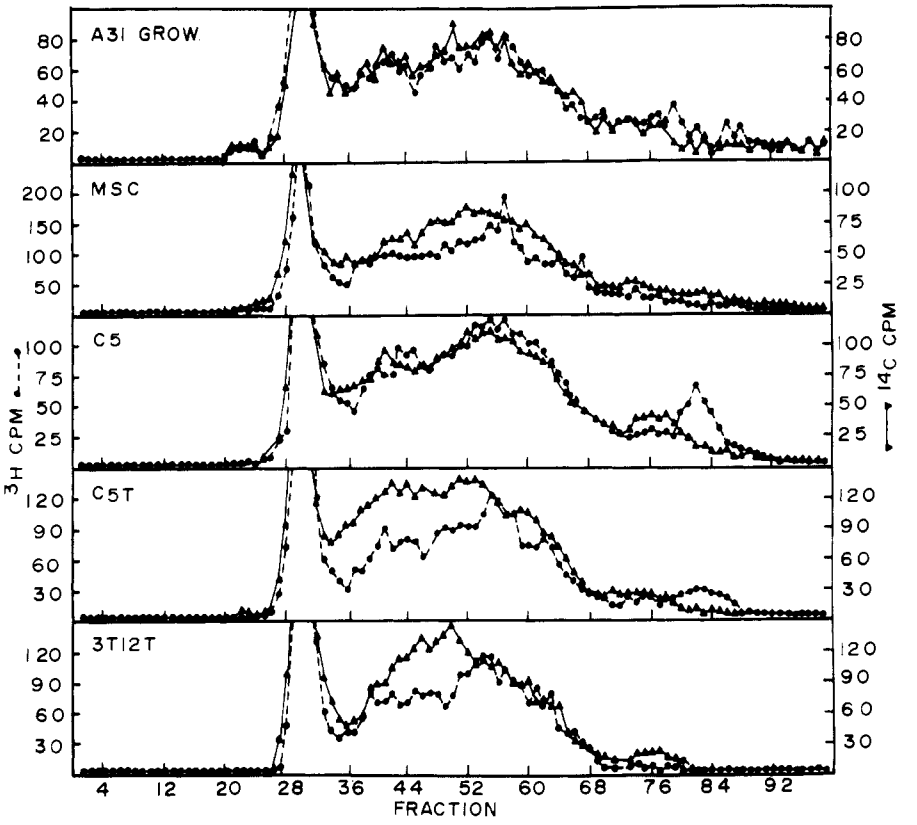

FIGURE 6: Comparison of confluent A_{31} Pronase glycopeptides to growing A_{31} and transformed cell glycopeptides. [^{3}H]Glucosamine-labeled confluent A_{31} membrane preparations (●) were mixed with either [^{14}C]glucosamine-labeled growing A_{31} membrane preparations or [^{14}C]glucosamine-labeled transformed cell membrane preparations (▲) and digested 6 days with Pronase. The Pronase digests were applied to a Sephadex G-50 column (90 × 1.5 cm).

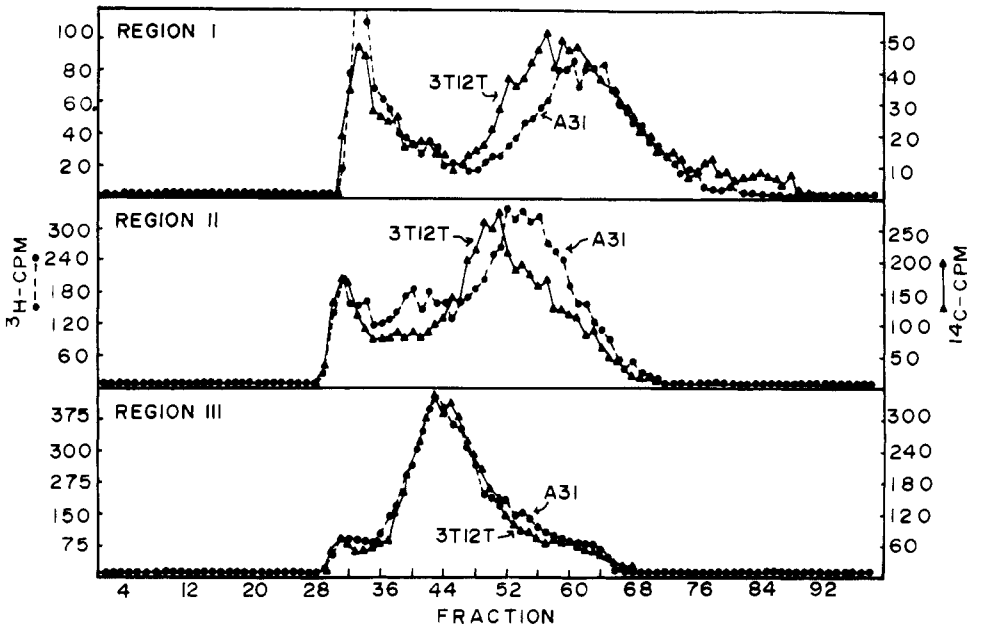

FIGURE 7: Sephadex G-50 chromatography of A_{31} (●) and 3T12T (▲) Pronase glycopeptides of isolated glycoprotein regions. [^{3}H]Glucosamine-labeled A_{31} and [^{14}C]glucosamine-labeled 3T12T membrane preparations were mixed and coelectrophoresed on a 7.5% tube gel (1.5 × 15 cm). Part of the gel was sliced and counted, and portions of the remaining gel corresponding to glycoprotein regions I, II, and III (see text) were excised and incubated for 8 days with Pronase. The digested material from each region was then eluted from the gels, lyophilized, and applied to a Sephadex G-50 column (90 × 1.5 cm).

Discussion

A number of alterations of membrane components have been detected in transformed cells. These include loss of the LETS protein (Hynes, 1973; Gahmberg and Hakomori, 1973), the appearance of large Pronase glycopeptides (Meezan et al., 1969; Buck et al., 1970), simplification of glycolipid patterns (Hakomori, 1975), and altered carbohydrate compositions (Wu et al., 1969; Grimes, 1970; Grimes and Greegor, 1976).

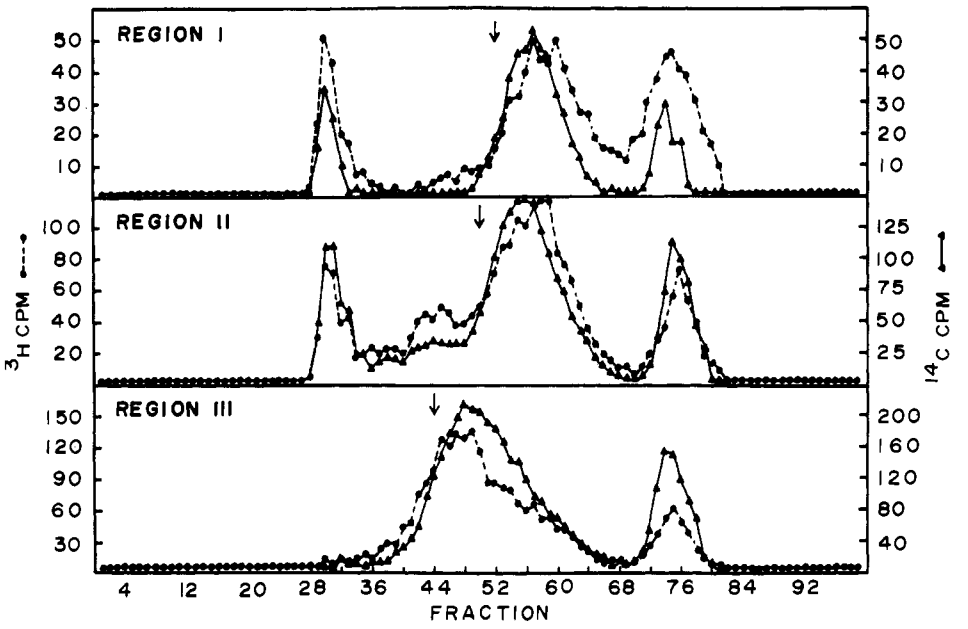

FIGURE 8: Sephadex G-50 chromatography of neuraminidase treated A_{31} (●) and 3T12T (▲) Pronase glycopeptides of isolated glycoprotein regions. [^{3}H]Glucosamine-labeled A_{31} and [^{14}C]glucosamine-labeled 3T12T region glycopeptides were prepared as in Figure 7. The glycopeptides were boiled for 5 min to destroy Pronase activity and 20 mg/mL of bovine serum albumin was added to bind excess sodium dodecyl sulfate. The mixture was then adjusted to pH 5.0 and incubated for 2 h with 10 IU of *Vibrio cholerae* neuraminidase (Calbiochem) at 37 °C. The neuraminidase-treated material was then analyzed by Sephadex G-50 chromatography. Arrows mark the major elution peak of [^{14}C]3T12T glycopeptides before neuraminidase treatment.

Most of these alterations have been detected in cells transformed by tumor viruses. In contrast, there are several examples of spontaneously transformed cells which do not have altered glycolipids or carbohydrate compositions (Sakiyama et al., 1972; Grimes and Greegor, 1976).

In the present study, the membrane proteins, glycoproteins, and glycolipids of normal cells, a viral transformant, and several spontaneous transformants were compared. MSC, c5, and c5T had increased amounts of the more simple gangliosides. In addition, there was no 240 000 molecular weight surface protein labeled by [^{125}I]lactoperoxidase. These three lines also demonstrated the presence of a new major glycoprotein band not present on normal A_{31} cells. In contrast, 3T12T, the spontaneous transformant that produced the most rapidly growing tumors in vivo, did not have these alterations. The glucosamine-labeled glycolipid patterns of A_{31} and 3T12T appeared similar. The methods used in this study, however, do not exclude possible differences in fucolipids or slow-migrating minority glycolipids (Itaya et al., 1976) between A_{31} and 3T12T cells. 3T12T had normal levels of a ^{125}I-labeled, trypsin-sensitive 240 000 molecular weight surface protein. The glycoprotein patterns of A_{31} and 3T12T as detected by sodium dodecyl sulfate-polyacrylamide gel electrophoresis were also very similar, with no new major bands appearing. Thus, many of the typical transformation specific changes detected in membrane proteins, glycoproteins, and glycolipids of virally transformed cells appear to be secondary in nature and not necessary for expression of the transformed phenotype. It is interesting that the lack of substantial surface alterations in 3T12T parallels the lack of immunogenicity (Grimes and Van Nest, submitted). It appears that a tumor cell that has very similar surface features to normal cells can be more successful in overcoming defenses of the host.

The only consistent difference detected between 3T12T and A_{31} cells was the presence of glycopeptides of higher molecular weight in the transformed cells. These results confirm earlier work using viral transformants (Meezan et al., 1969; Buck et al., 1970). Several isolated glycoprotein regions have glycopeptides of different molecular weight. The smaller glycoproteins tend to have higher molecular weight glycopeptides. These differences are retained even after neuraminidase treatment. These results confirm those of Sakiyama and Burge (1972), indicating that a number of different kinds of carbohydrate structures are likely to be found on cellular glycoproteins. Glycopeptides prepared from regions I and II of 3T12T glycoproteins are both of higher molecular weight than the corresponding regions from A_{31} cells. It is of interest that the 240 000 molecular weight glycoprotein while present in both the normal A_{31} and the spontaneously transformed 3T12T yields glycopeptides which are characteristically altered in the transformed cells. The presumed alteration is therefore occurring on more than one kind of carbohydrate structure. These differences remain after neuraminidase treatment, showing that the change probably involves carbohydrates other than sialic acid, confirming the work of Glick (1974). We are now purifying cell membrane glycoproteins in sufficient amounts to allow detailed chemical study.

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Lipid Activation of Undecaprenyl Pyrophosphate Synthetase from *Lactobacillus plantarum*†

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ABSTRACT: *Lactobacillus plantarum* undecaprenyl pyrophosphate synthetase is a soluble enzyme which has an in vitro requirement for detergent or phospholipid for activity. It is activated by the anionic detergents deoxycholate, dodecyl and cetyl sulfate, as well as Triton X series detergents. Brij 35, 56, and 96 and cetyltrimethylammonium bromide were ineffective in activating the enzyme. *L. plantarum*, *Escherichia coli*, and bovine cardiolipin, egg phosphatidic acid, and oleate are all good activators of the enzyme in the absence of detergent. *L. plantarum* phosphatidylglycerol and lysylphosphatidylglycerol, several lecithins, dipalmitylphosphatidic acid, phosphatidylserine, and the C_6–C_{18} saturated fatty acids (except C_{16}) are all ineffective over a wide concentration range. However, in the presence of 0.1% Triton X-100, dipalmitylphosphatidic acid, phosphatidylserine, and C_6–C_{18} saturated fatty acids exhibit a concentration-dependent stimulatory effect, with the C_{12} and C_{14} fatty acids being most effective. Mixtures of C_{10} or C_{14} fatty acids with Brij 35 demonstrated enzyme activation similar to the fatty acids–Triton X-100 mixtures. Using mixtures of Brij 35 and C_{14} fatty acid, it was observed that a fixed molar ratio of fatty acid to detergent (2:1) was required for optimal activity. Temperature dependence of egg phosphatidic acid activation of the enzyme showed a marked increase in enzyme activity from 30 to 40 °C, while both cardiolipin and Triton X-100 exhibit a decrease in stimulatory activity in this temperature range. These results suggest that an optimal surface charge and fluid lipid bilayer are required for enzyme activity.

Long chain polyprenyl phosphates have been shown to play a central role as glycosyl carriers in the biosynthesis of bacterial cell wall polysaccharides (Hemming, 1974) and have been strongly implicated in a similar role in the biosynthesis of oligosaccharide precursors of glycoproteins (Lennarz, 1975). All the enzymes reported to date which metabolize these polyprenyl phosphates have been described as being membrane associated and activated by a variety of lipids and detergents. Prenyl transferases which synthesize undecaprenyl pyrophosphate, a glycosyl carrier in bacterial systems, have been described in *S. newington* (Christenson et al., 1969), *M. lysodeikticus* (Kurokawa et al., 1971), and *L. plantarum* (Keenan and Allen, 1974a). The enzyme from *L. plantarum* has been partially purified and demonstrated to be stimulated by several detergents and cardiolipin (Keenan and Allen, 1974a,b; Allen et al., 1976). We report here a study of the effectiveness of a variety of natural and synthetic phospholipids, fatty acids, and detergents to stimulate undecaprenyl pyrophosphate synthetase.

Experimental Procedure

Materials

L. plantarum (ATCC 8014) was obtained from the American Type Culture Collection. Sorbents for chromatography were silica gel G (E. Merck), silicic acid (Bio-Sil HA, Bio-Rad Laboratories), and silica gel G without gypsum, on plastic sheets from Brinkmann Instruments. Amberlite XAD-2 was obtained from Mallinckrodt Chemical Works. Farnesyl py-

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