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STUDIES OF THE PLASMA MEMBRANE OF NORMAL AND VIRUS-TRANSFORMED 3T3 MOUSE CELLS

ROSE SHEININ AND K. ONODERA*

Division of Biological Research, Ontario Cancer Institute and Department of Medical Biophysics, University of Toronto, Toronto, Ontario (Canada)

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SUMMARY

Electrophoretic analysis, using polyacrylamide gels, has been applied to purified plasma membrane preparations derived from 3T3 mouse fibroblasts, 3T3 cells transformed by SV40 and polyoma viruses, as well as to ouabain-resistant 3T3 cells. Differences have been detected at the level of the membrane peptides and the glucosamine-containing macromolecules between normal and virus-transformed cells.

INTRODUCTION

The major physiological properties of neoplastically transformed cells have led many to consider the cell surface as an important site at which are expressed the genetic changes associated with oncogenesis. These properties must derive from biochemical and molecular changes, some of which have now been identified[1-15].

Our own investigations have been concerned with two moieties at the cell exterior; the plasma membrane and the surface component, which is located at the periphery of the cell external to the cell membrane[12]. Differences between glycoproteins of the surface component of normal 3T3 mouse cells and those transformed by polyoma and SV40 viruses have already been reported[11,12]. In addition it was demonstrated that the ganglioside patterns of the purified plasma membranes of such cell types are also divergent (ref. 7; and G. Yogeeswaran, R. Sheinin, J. R. Wherett and R. K. Murray, *J. Biol. Chem.*, (1972) in the press).

The present work has been directed towards a study of the proteins and protein complexes of plasma membrane preparations derived from normal and virus-transformed 3T3 mouse fibroblasts. Differences between the plasma membranes of these cell types have been detected. The data confirm and extend those described in previous investigations[7,16,17].

Abbreviation: GlcN, glucosamine.

* Present address: Kaken Chem. Corp., Ltd, 28-8, 2-chome, Honkomagome, Bunkyo-ku, Tokyo, Japan.

MATERIALS AND METHODS

Cells

The cell lines were derived from a clone of 3T3 mouse fibroblasts* [18]. They were designated as follows: (i) normal 3T3 mouse fibroblasts: 3T3*; (ii) SV40-transformed 3T3 mouse fibroblasts: 3T3-SV-479**,[19], 3T3-SV-CE56*,[20] and 3T3-SV-A26*; (iii) polyoma-transformed 3T3 mouse fibroblasts: 3T3-Py6*,[20]; (iv) ouabain-resistant 3T3 mouse fibroblasts: 3T3-oua^R ***. The cells were maintained and cultivated by procedures already described[11]. Their properties are described elsewhere (G. Yogeeswaran, R. Sheinin, J. R. Wherett and R. K. Murray, *J. Biol. Chem.*, (1972) in the press).

Cultivation of radioactively labelled cells

To obtain glucosamine (GlcN)-labelled cells, cultures were plated at about $1.1 \cdot 10^4$ cells/cm^2 and incubated till the cells covered about $^1/_4$–$^1/_3$ of the growth surface. The medium was decanted and replaced with medium containing 3.3 mg/ml of glucose, 7 % (w/v) of foetal calf serum and either [^{3}H]GlcN·HCl (0.77 mM; 13 Ci/mmole) or [^{14}C]-GlcN·HCl (0.19 mM; 270 mCi/mmole).

To label cells in DNA and RNA, they were grown from low inoculum as noted above in medium containing the nucleosides [*Me*-^{3}H]T (0.036 μM; 18.4 Ci/mmole) and [2-^{14}C]U (0.003 μM; 29.2 Ci/mmole).

The proteins of cells were labelled using [^{14}C]leucine. Cells were grown from low cell number in medium containing $^1/_{10}$ the usual leucine concentration and supplemented with [U-^{14}C]leucine (0.067 μCi/ml; 312 mCi/mmole).

Plasma membrane isolation

The procedure of Brunette and Till[21] was adapted to the isolation of plasma membrane from animal cells which grow on a solid substratum. A major factor which contributed to the success or failure of the technique was the method used to harvest the cells from the growth surface. It proved essential to obtain viable and freely suspended single cells. To achieve this, cells which had been grown to confluence were incubated at room temperature with trypsin for 1 min[11]. The cultures were inverted, drained of excess trypsin, and monitored for the time at which the cells began to round (usually no more than 2 min). The cells were suspended in phosphate-buffered saline[22] *plus* 1 % foetal calf serum (v/v), combined and spun at 800 × *g* for 15 min. The suspension of single cells was processed to obtain purified plasma membrane preparations[7]. These were stored at —20 °C pending analysis.

In experiments in which comparisons were made between different cell types, the cell membrane material was isolated as follows: The cells were grown to confluence in independent culture lots either in non-radioactive medium, or in medium containing a desired radioactive precursor substance. The cells, plated singly at low cell concentration (approx. $1 \cdot 10^4$ cells/cm^2) were observed daily during their growth. The cultures were left for 24 h after the time at which the cells appeared to be totally in contact. The cells, independently harvested from their culture flasks, were combined immediately and processed. Thus the two plasma membrane preparations were isolated as nearly as possible under identical conditions, making the analyses described herein more reliable.

*,**,*** These various cell lines were very kindly given to us by (a) Dr Marguerite Vogt, (b) Dr Michael Oxman and (c) Dr R. M. Baker.

Isolation of the protein fraction of purified plasma membrane preparations

Sedimented plasma membrane, fresh or in frozen form, was suspended and washed 3 times in ice-cold Tris–HCl (0.1 M) at pH 7.0. The washed membrane pellet was suspended in 10 ml chloroform–methanol (3:1, v/v) solution or in ethanol–ether (3:1, v/v) solution and then shaken overnight. The solvent-insoluble material was spun down at 30000 × *g* for 30 min, and was washed 3 times with the appropriate solvent system. The final pellet was dried at room temperature and was used immediately for electrophoretic analysis.

Polyacrylamide gel electrophoresis in sodium dodecyl sulphate of total membrane material

Fresh or frozen plasma membrane material was dissolved (at an approximate concentration of 1–3 mg protein/ml) in a solution of sodium dodecyl sulphate (2 % w/v) and β-mercaptoethanol (1 %, v/v) in 0.1 M sodium phosphate buffer at pH 7.5 (ref. 23), with or without boiling for 2 min. The dissolved material was applied in a 100–200-μl aliquot (100–400 μg protein) to columns (about 10–15 cm × 0.6 cm) of polyacrylamide gel.

Experiments, in which analysis was dependent upon staining of peptide bands, were carried out by the procedure introduced by Laemmli[24]. 7 % (w/v) polyacrylamide (containing 0.1 % sodium dodecyl sulphate) was used in the lower gel. The material was electrophoresed at pH 8.5 at 5 mA/gel until the tracking dye reached the bottom of a control gel (about 5 h). The peptide material was stained using Coomassie Brilliant Blue[25]. In general duplicate comparative gels were processed together, using a 12-place electrophoresis apparatus (Buchler Instruments). A single membrane preparation gave similar results when processed over a period of several months.

In those experiments in which radioactively labelled materials were examined, analyses were carried out using conditions described by Baenziger *et al.*[26]. The lower gel contained 5 % (w/v) polyacrylamide (*plus* 0.1 % sodium dodecyl sulphate). The material was run at pH 7.5 at 8 mA/gel as above. The gels were cut using a semi-automatic arrangement which gave slices of approximately 1.5–2.0 mm width. The slices were dropped as cut into glass vials. To each was added 1 ml 90 % (v/v) Nuclear Chicago Solubilizer. The slices were swollen overnight at 37 °C. To each vial was added 15 ml scintillation fluid and the radioactivity in each sample was counted. Where material doubly labelled with ^{3}H and ^{14}C was analyzed, the data were processed by a computer program devised by Becker[27]. The program yielded the radioactivity of each isotope, as per cent of the total radioactivity and the percent differenc with respect to radioactivity contributed by ^{14}C and ^{3}H. Automatically produced plots of these parameters have been used in this report.

Following the procedure of Becker[27] the per cent difference area, *i.e.* the total absolute per cent difference, divided by 2, has been used as a measure of sameness or difference of gel patterns. In analyzing comparative membrane preparations the F-test[28] was applied, to assess the significance of difference between the variance of distributions of glucosamine-containing molecules in the polyacrylamide gels.

Polyacrylamide gel electrophoresis in acetic acid–urea of protein extracted from plasma membrane

The protein fraction of isolated plasma membrane, prepared as described above was dissolved in 2 ml of phenol–acetic acid–urea (2:1:1, by vol.). The solubilized

protein was analyzed in gels of polyacrylamide (5 %, w/v); *N,N*-methylenebisacrylamide (0.1 %, w/v); acetic acid (50 %, v/v) and urea (5 M). Sample aliquots of 100 μl were applied to the top of the column in 60 % (w/w) of sucrose. Electrophoresis was carried out for 5–5.5 h in acetic acid (10 %, v/v) *plus* urea (1 M) at 2.5 mA per tube. The gels were stained with Coomassie Brilliant Blue (0.05 %, w/v) in trichloroacetic acid (12.5 %, (w/v) and then destained using standard procedures[25]. Under the staining conditions used, it did not prove possible to obtain complete destaining in the peptide-free regions of the gels. This made photographic reproduction of bands containing small amounts of material very difficult. The gel patterns have therefore been presented in illustrated form.

Surface component, specific labelling with radioactive glucosamine

The procedure for the specific labelling with GlcN of the surface component of 3T3 mouse cells, resident at the extreme periphery of the plasma membrane, has been fully decsribed[11]. Briefly, cells grown to confluence were removed from the glass with trypsin under conditions which left the plasma membrane functionally intact, but removed the surface component. The cells were then plated and exposed 12–13 post-inoculation to [^{3}H]GlcN. In cells so incubated, 85–90 % of the [^{3}H]GlcN incorporated into macromolecules was located in the surface component. In experiments in which proteins were generally labelled with [^{14}C]leucine, it was calculated that the surface component represents less than 0.02 % of the total cellular protein[11].

Enzyme assays

The procedure of Hafkenscheid and Boting[30] was employed to detect the Na^+–K^+-activated Mg^{2+}-ATPase (ATP phosphohydrolase, EC 3.6.1.3) of various cell fractions. This activity was expressed as μmoles of inorganic phosphate (measured by the method of Lowry and Lopez[31]) released per h per mg of membrane protein (measured by the technique of Lowry *et al.*[32]).

NADH diaphorase activity (NADH: (acceptor) oxidoreductase, EC 1.6.99.3) was measured using the procedure described by Wallach and Kamat[33] to give data expressed as μmoles NADH oxidized/min per mg protein.

NADH oxidase activity (NADH–cytochrome *c* reductase, EC 1.6.2.1) in cell fractions was detected using the method of Cooperstein and Lazarow[34].

Chemicals and reagents

All chemicals were of analytical grade. The radioactive GlcN was obtained from New England Nuclear Corporation and from Amersham/Searle. Trypsin and foetal calf serum came from Grand Island Biochemicals and from Hyland Laboratories and Reheis Chemical Co. The fluors and phosphors for the scintillation fluid came from Packard, the Nuclear Chicago Solubilizer from Nuclear Chicago.

RESULTS

Properties of isolated plasma membrane preparations

It has already been shown[7] that various 3T3 mouse fibroblast derivatives can be processed to yield large membranous sheets which have the appearance of cell ghosts and which have a length approximately equivalent to the perimeter of intact

cells. Phase-contrast and electron microscopy indicated that the plasma membrane preparations obtained were essentially free of intact nuclei, mitochondria, inner mitochondrial membranes and rough endoplasmic reticulum. This conclusion was supported by enzyme analyses summarized in Table I.

TABLE I

DISTRIBUTION OF ENZYME ACTIVITIES AMONGST FRACTIONS OF 3T3 MOUSE CELLS

Enzyme activity units are given in Materials and Methods. Numbers in parentheses in all tables show number of assays. In the many preliminary experiments no NADH oxidase was ever detected in the membrane fractions, although activity was detected in the homogenate and in the supernatant fractions.

Enzyme	*Cell type*	*Homogenate*	*Supernatant*	*Membrane*
$(Na^+–K^+–Mg^{2+})$-ATPase	3T3 (3)	0.329	0.280	3.08
	3T3-SV-479 (2)	0.204	0.322	4.45
	3T3-Py (1)	Not detectable	0.565	2.86
NADH oxidase	3T3 (3)	Not done	Not done	Not detectable
	3T3-SV-479 (2)	Not done	Not done	Not detectable
	3T3-Py (1)	Not done	Not done	Not detectable
NADH diaphorase	3T3 (3)	6.31	4.56	0.36
	3T3-SV-479 (2)	4.64	2.87	0.30
	3T3-Py (1)	5.07	2.28	0.18

The plasma membrane fraction appeared to be enriched about 10–20 times with respect to the $(Na^+–K^+–Mg^{2+})$-ATPase, now known to be associated exclusively with the plasma membrane of animal cells[35]. Indeed the other cell fractions, including the homogenate exhibited very low, often undetectable ATPase activity. The $(Na^+–K^+–Mg^{2+})$-ATPase activity of the plasma membrane preparations derived from 3T3; 3T3-SV-479; 3T3-SV-CE56; 3T3-SV-A26 and 3T3-Py were, respectively, 2.27 $\pm$ 0.25 (5 samples); 4.40 $\pm$ 1.11 (3 samples); 1.84 $\pm$ 0.40 (3 samples); 2.66 (1 sample) and 2.12 (2 samples) μmoles inorganic phosphate released/mg protein per h. These activities were in the same range as those obtained by others with plasma membranes derived from L-cells[21,36], chick embryo fibroblasts[37] and HeLa cells[38] grown *in vitro*.

As indicated in Table I, the plasma membrane obtained from all cells examined to date was devoid of detectable NADH oxidase activity, normally associated with the inner mitochondrial membrane[39]. This fraction did, however, exhibit some NADH-diaphorase activity usually considered a marker for the endoplasmic reticulum[39,40]. However, the activity in the plasma membrane was only about 2–3 % of that found in the homogenate.

These various observations suggested that the plasma membrane isolates were free of major contamination by all other membrane-containing structures of the cells. No DNA or RNA could be detected using standard chemical methods. Additional analyses were carried out to test for the presence of specific radioactively labelled macromolecules. The data with respect to DNA, RNA and glycoprotein surface component, shown in Table II, indicated once again the purity of the preparations. For of the total cellular [^{3}H]DNA, [^{14}C]RNA and [^{3}H]GlcN-labelled surface component, only about 0.06 %, 0.006 % and 3.4 %, respectively, was recovered in the plasma membrane fraction.

TABLE II

RECOVERY IN PLASMA MEMBRANE PREPARATIONS OF CELLULAR COMPONENTS

Recovery in % ± S.E. of component present in homogenate. Protein was measured directly by the procedure of Lowry *et al.*[32]. Cells were grown through several generations in [^{3}H]GlcN, [^{3}H]T and [^{14}C]U, as indicated in Materials and Methods, to label all GlcN-containing macromolecules, DNA and RNA, respectively. DNA and RNA were distinguished as follows: Labelled cells, washed 3 times with medium containing excess T and U, were treated with 5% (w/v) trichloroacetic acid to precipitate all macromolecules. The 3-times washed precipitate was dissolved in 1 M NaOH and left at room temperature overnight to permit alkaline degradation of RNA. The solution was neutralized and treated with trichloroacetic acid to precipitate the DNA. The filtrate containing solubilized [^{14}C]uridylic acid represented the [^{14}C]RNA and the filtered residue contained the [^{3}H]DNA. Surface component was specifically labelled with [^{3}H]GlcN as noted in Materials and Methods.

Cell type	*Protein*	[^{3}H]*GlcN macromolecules (trichloroacetic acid-precipitable)*	[^{3}H]*DNA*	[^{14}C]*RNA*	^{3}H-*labelled surface component*
3T3	5.14 ± 1.04 (14)	5.86 ± 1.49 (13)	0.061 (1)	0.009 (1)	3.7 (2)
3T3-SV-479	5.18 ± 1.39 (9)	5.11 ± 1.50 (4)	0.057 (2)	0.004 (2)	3.1 (2)
3T3-SV-CE56	6.29 ± 1.63 (5)	6.79 (2)			
3T3-SV-A26	6.27 ± 1.72 (7)	4.04 ± 1.39 (5)			
3T3-Py6	6.26 ± 1.30 (16)				
3T3-oua^R	5.56 ± 0.15 (3)	6.25 (2)			

As isolated, the various plasma membrane preparations are seen to make up approximately 5.54 ± 1.31 % of the cellular protein. The values for each different cell type are given in Table II. Data are also presented which show that the plasma membrane preparations contain about 5.71 ± 1.65 % of the total cellular GlcN-containing macromolecules.

Analysis of plasma membrane preparations by electrophoresis in polyacrylamide gels

It was the purpose of the studies which follow to gather information concerning protein and carbohydrate moieties of plasma membrane preparations derived from various 3T3 fibroblast cell types. Two kinds of membrane preparations were analyzed: (i) fully solubilized membrane; (ii) lipid-free material, solubilized with phenol–acetic acid–urea.

(*i*) *Analysis of fully solubilized plasma membrane.* The data presented in Fig. 1 were acquired in an electrophoretic analysis of plasma membrane derived from 3T3-SV-479 cells generally labelled in protein with [^{14}C]leucine and in carbohydrate with [^{3}H]-GlcN. In four experiments (two each with 3T3 and 3T3-SV-479 cells) approximately 95.6 % of the trichloroacetic acid-insoluble [^{14}C]leucine was recovered within the gel. Under the conditions of running, proteins of molecular weight about 50000–300000 were resolved within the gel (as determined with the marker proteins fibrinogen, bovine serum albumin, liver alcohol dehydrogenase and lysozyme[23,41,44]).

Of interest is the observation that although the ^{14}C-labelled proteins were largely recovered from the gel, only 61.5 % of the [^{3}H]-GlcN-containing macromolecules were resolved within it. This is perhaps not surprising. It is known that sodium dodecyl sulphate dissociates some lipids from proteins[42–44], and so it is pos-

sible that some of the loss is due to the movement of free GlcN-containing lipids with the solvent front. Such lipid movement has been shown to occur in sodium dodecyl sulphate-dissociated plasma membrane material derived from *Micrococcus lysodeikticus*[42] and human erythrocytes[44]. Additional loss may result by leaking of free lipids from the gels during fixation and subsequent processing.

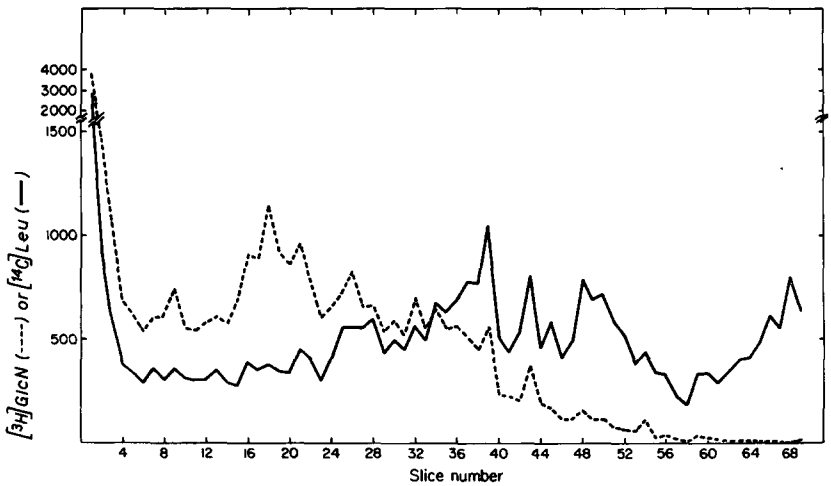


Fig. 1. Electrophoresis of fully solubilized plasma membrane preparations of 3T3-SV-479 cells. Approx. $7.7 \cdot 10^8$ [^{3}H]GlcN-labelled and $3.5 \cdot 10^8$ [^{14}C]Leu-labelled 3T3-SV-479 cells were processed for isolation of plasma membrane, which was solubilized in sodium dodecyl sulphate–mercaptoethanol (with boiling) and electrophoresed at pH 7.5 in sodium dodecyl sulphate–containing polyacrylamide gels (5% w/v). Anode position in this and all other gels was to the right.

Of the GlcN-containing macromolecules which were recovered, a substantial proportion ran in the gel in the position of material with fairly large molecular weight. In view of what is known about the composition of plasma membranes it seemed likely that these molecules are glycoproteins or glycolipoproteins[45].

The distribution of [^{14}C]leucine within sodium dodecyl sulphate–polyacrylamide gels (Fig. 1) indicated that the plasma membrane of the various 3T3 mouse cells under study contained a fairly large number of resolvable peptide moieties. This was confirmed by the analysis illustrated in Fig. 2. When solubilized plasma membrane was subjected to electrophoresis at pH 7.5, in the presence of sodium dodecyl sulphate, some 25–30 peptide bands were detected by staining. Using suitable molecular weight markers, it was established that the peptides resolved under the running conditions used varied from about molecular weight 15000 to 200000.

The peptide patterns obtained with plasma membranes derived from the different cell types examined showed a fair degree of similarity. However, differences were detected, both quantitative and qualitative. Attempts to amplify these differences have been made by examining the lipid-free peptides of the total plasma membrane, as well as those molecules which contain GlcN; this because GlcN-containing macromolecules have been implicated as being of great functional importance in normal and neoplastic differentiation of cells[7,17,45].

The experimental design of these studies was as follows: 3T3 cells and the derivative 3T3 cells under test were grown and differentially-labelled with [^{3}H]- and

Fig. 2. Electrophoretograms of plasma membrane preparations of various 3T3 mouse cell types, analyzed in sodium dodecyl sulphate-containing polyacrylamide gels. Plasma membrane preparations were obtained from the following cell suspensions: (a) $1.11 \cdot 10^9$ 3T3-SV-A26 cells; (b) $6.6 \cdot 10^9$ 3T3-SV-CE56 cells; (c) $3.7 \cdot 10^9$ 3T3-SV-479 cells; (d) $6.7 \cdot 10^9$ 3T3-Py cells; (e) $5.2 \cdot 10^9$ 3T3 cells. The membrane preparations were dissolved as described in Materials and Methods. Material in 10-μl volumes containing, respectively, 368, 432, 443, 327 and 250 μg of protein was subjected to electrophoresis for 5 h at 8 mA/gel, as described earlier. Intensity of staining is designated as follows: most intense blue, solid black; decreasing density of stain by the decreasing degree of stipling.

[^{14}C]GlcN. The cells were mixed and processed for isolation of plasma membrane, which was solubilized and examined by polyacrylamide gel electrophoresis. To assess the feasibility of this approach, studies were first carried out with differentially labelled plasma membrane material derived from the same kind of cells. Representative data derived with 3T3-SV-479 cells have been plotted in Fig. 3. As expected, the ^{14}C and ^{3}H labelled plasma membrane from like cells yielded very similar patterns of electrophoresis of GlcN containing molecules. Analogous results were obtained when cells were marked in the reverse differential labelling pattern.

Experiments were then carried out to assess the similarity between the plasma membrane of 3T3 mouse fibroblasts and that of each of a number of derivative 3T3 cells. Studies were made first using one differential labelling situation, and then using the reverse labelling condition. Comparable results were always obtained.

Representative data, shown in Fig. 4, revealed considerable differences between the plasma membrane preparations of the parental 3T3 cells and that of virus transformed cells. In contrast the electropherograms obtained with preparations derived from mixtures of 3T3 and 3T3-oua^{R} cells were remarkably similar.

The quantitative data relevant to these analytical experiments have been summarized in Table III. Gel distribution patterns obtained with like cells labelled with [^{14}C]- and [^{3}H]GlcN differed one from the other to the extent of about 4.35 %. The comparative electropherograms of GlcN-containing molecules in plasma membrane of 3T3 and virus-transformed 3T3 cells differed to the extent of 8.71–27.1 %. Calculations of variance of the gel distribution patterns obtained with normal and virus-transformed cells revealed that these were significantly different. In contrast the electrophoretic mobilities of GlcN-containing molecules in plasma membrane of

3T3-oua^R cells were found not to be significantly different from analogous substances in 3T3 mouse fibroblasts.

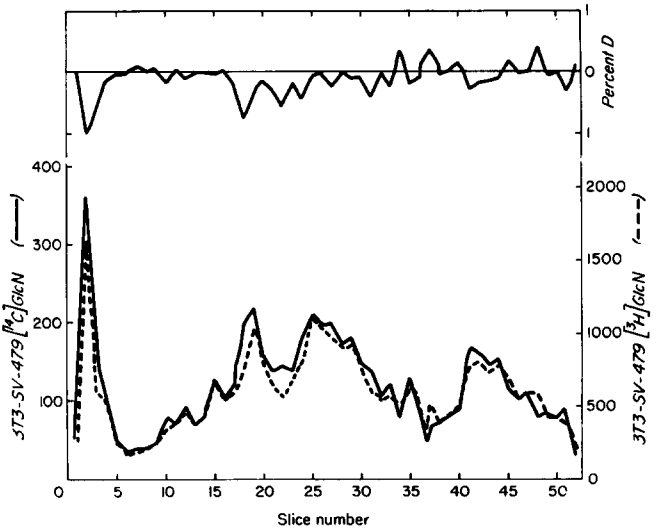
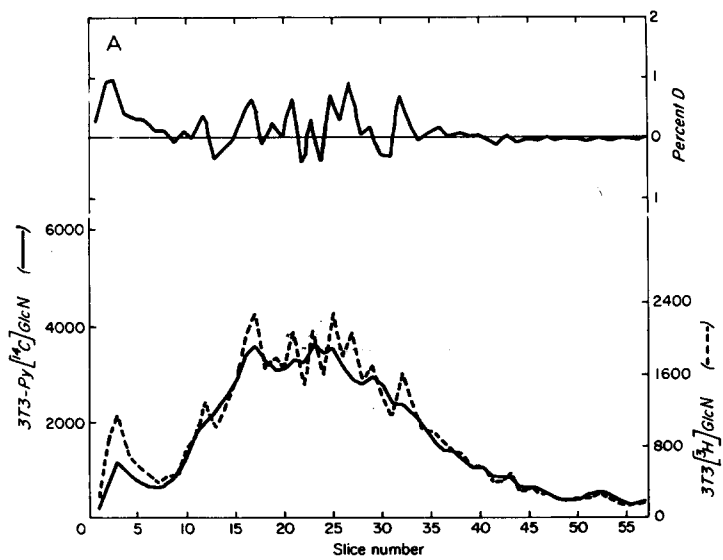
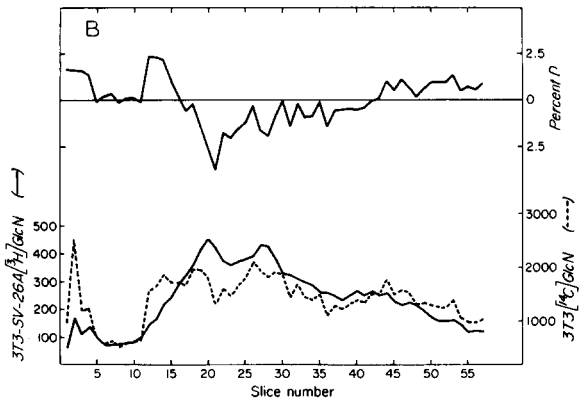


Fig. 3. Electrophoresis of fully solubilized plasma membrane preparations of 3T3-SV-479 cells, labelled with [^{3}H]- and [^{14}C]GlcN. [^{3}H]GlcN-labelled ($2.8 \cdot 10^9$) and [^{14}C]GlcN-labelled ($3.5 \cdot 10^9$) 3T3-SV-479 cells were processed for the isolation of plasma membrane. 0.1 ml of solubilized membrane material (containing 259 μg of protein) was electrophoresed at pH 7.5 in 5% polyacrylamide; the gel was sliced and the slice content of [^{3}H]- and [^{14}C]GlcN was measured. Percent D, a measure of the % sameness or difference between the distribution of [^{3}H]GlcN and [^{14}C]GlcN, was calculated as described under Materials and Methods. Recovery of [^{3}H]GlcN = 50.7%; of [^{14}C]GlcN = 49.8%.




B
Percent D
3T3-SV-264[³H]GlcN
3T3[¹⁴C]GlcN
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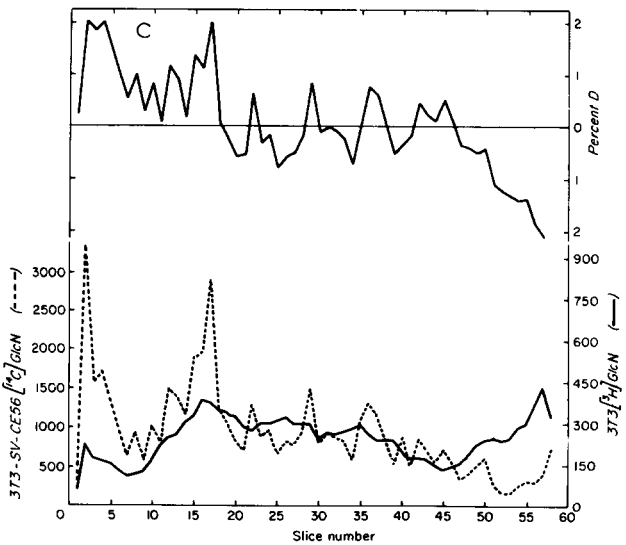
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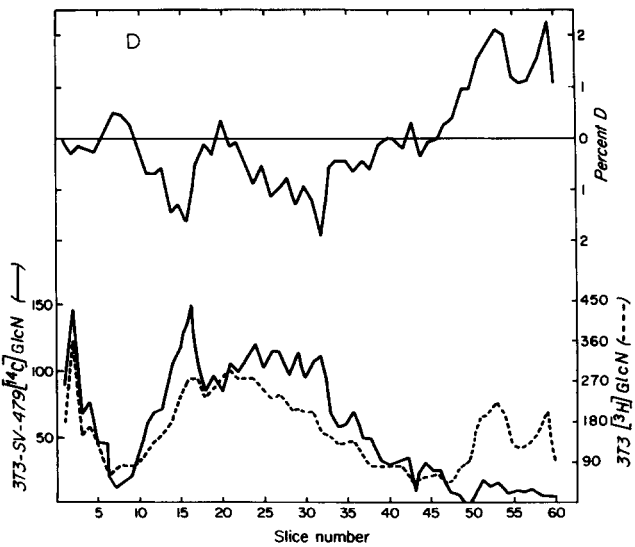
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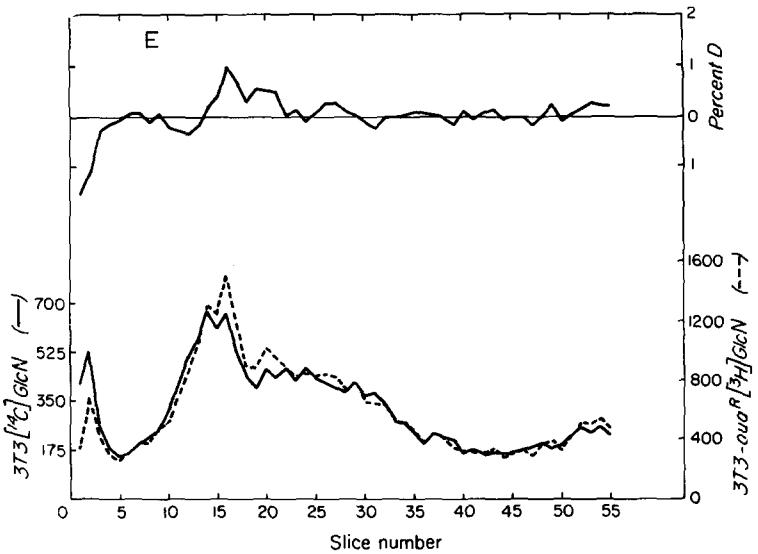

Fig. 4. Electrophoresis of solubilized plasma membrane preparations from mixtures of two different kinds of cells differentially labelled with [^{3}H]- and [^{14}C]GlcN. Cell lots were grown up as noted below in medium containing radioactive GlcN. Differentially labelled plasma membrane preparations were made and analyzed as discussed under Fig. 1: (A) [^{14}C]GlcN 3T3-Py6 ($7.7 \cdot 10^9$) cells *plus* [^{3}H]GlcN 3T3 ($2.8 \cdot 10^9$) cells. Recovery of [^{14}C]- and [^{3}H]GlcN was 74.5% and 69.8%, respectively. (B) [^{14}C]GlcN 3T3 ($3.4 \cdot 10^9$) cells *plus* [^{3}H]GlcN 3T3-SV-A26 ($5.8 \cdot 10^9$) cells. Recovery of [^{14}C]- and [^{3}H]GlcN was 54.7% and 52.4%, respectively. (C) [^{14}C]GlcN 3T3-SV-CE56 ($4.3 \cdot 10^9$) cells *plus* [^{3}H]GlcN 3T3 ($7.5 \cdot 10^8$) cells. Recovery of [^{14}C]- and [^{3}H]GlcN was 73.3% and 77.7%, respectively. (D) [^{14}C]GlcN 3T3-SV-479 ($4.1 \cdot 10^9$) cells *plus* [^{3}H]GlcN 3T3 ($2.2 \cdot 10^9$) cells. Recovery of [^{14}C]- and [^{3}H]GlcN was 79.9% and 73.8%, respectively. (E) [^{3}H]GlcN 3T3-oua^R ($5.6 \cdot 10^8$) cells *plus* [^{14}C]GlcN 3T3 ($5.1 \cdot 10^8$) cells. Recovery of [^{14}C]- and [^{3}H]GlcN was 61.3% and 67.8%, respectively.

TABLE III

COMPARISON OF ELECTROPHORETIC ANALYSES OF GlcN-LABELLED MACROMOLECULES IN PLASMA MEMBRANE PREPARATIONS DERIVED FROM MIXED LOTS OF 3T3 MOUSE FIBROBLASTS

% difference area is calculated according to Becker[27] from the % distribution of [^{3}H]- and [^{14}C]GlcN throughout the gels (see Materials and Methods). It is expressed as [(Total % difference)/2] ± S.E. Average variance values significantly different at the 1% level are denoted by an asterisk.

Cells analyzed		*% Difference area*	*Variance*
[^{3}H]3T3	*plus* [^{14}C]3T3	4.83 ± 0.50 (4)	0.050
[^{3}H]3T3-SV-479	*plus* [^{14}C]3T3-SV-479	3.86 ± 0.20 (3)	0.031
[^{3}H]3T3	*plus* [^{14}C]3T3-Py6	8.82 ± 1.33 (3)	0.329*
[^{14}C]3T3	*plus* [^{3}H]3T3-Py6	8.61 ± 0.23 (4)	0.318*
[^{3}H]3T3	*plus* [^{14}C]3T3-SV-A26	14.35 ± 1.17 (2)	0.341*
[^{14}C]3T3	*plus* [^{3}H]3T3-SV-A26	15.49 ± 1.63 (5)	0.341*
[^{3}H]3T3	*plus* [^{14}C]3T3-SV-CE56	21.45 ± 1.08 (2)	0.825*
[^{14}C]3T3	*plus* [^{3}H]3T3-SV-CE56	23.13 ± 1.66 (3)	0.669*
[^{3}H]3T3	*plus* [^{14}C]3T3-SV-479	26.48 ± 1.65 (6)	0.694*
[^{14}C]3T3	*plus* [^{3}H]3T3-SV-479	27.72 ± 2.55 (2)	0.465*
[^{3}H]3T3	*plus* [^{14}C]3T3-oua^R	5.47 ± 1.12 (2)	0.082
[^{14}C]3T3	*plus* [^{3}H]3T3-oua^R	4.77 ± 0.45 (2)	0.083

(ii) Electrophoretic analysis of the protein moiety of plasma membrane preparations

The foregoing experiments have permitted gross analyses of GlcN-containing molecules of the plasma membrane. To examine the proteins directly, the plasma membrane fractions of the various cells of interest were first freed of lipid and the residual insoluble protein fraction was dissolved in phenol–acetic acid-urea solution. This material was analyzed by electrophoresis in polyacrylamide gels to yield the data represented in Fig. 5.

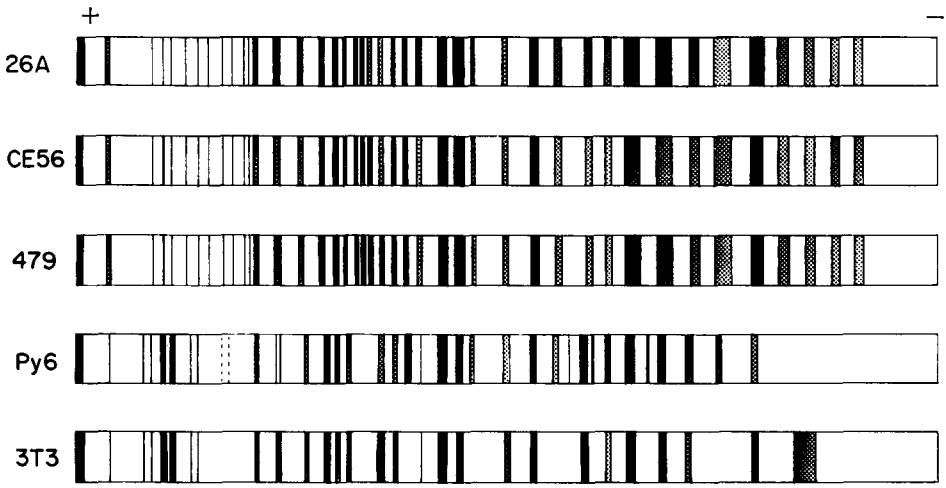

Fig. 5. Electrophoretograms of lipid-extracted plasma membrane preparations of various 3T3 mouse fibroblasts. Plasma membrane preparations of the various 3T3 mouse fibroblasts noted below were obtained and then extracted with chloroform–ethanol as described in Materials and Methods. The residual insoluble material was dissolved in phenol–acetic acid–urea and was analyzed on polyacrylamide gels containing acetic acid and urea. (a) 3T3-SV-A26; (b) 3T3-SV-CE56; (c) 3T3-SV-479; (d) 3T3-Py6; (e) 3T3, containing, respectively, 40.0, 57.6, 56.8, 41.2 and 62.8 μg protein.

Of the order of 20–30 peptide bands were detected in the lipid-free fraction of the plasma membrane of the various 3T3 cell preparations examined. The protein composition of the plasma membrane fractions isolated from normal 3T3 cells differed quite markedly from that of the analogous preparations derived from 3T3 cells transformed by the polyoma and SV40 viruses. Of particular interest was the finding that the peptide patterns obtained with the three SV40-transformed cell lines (479, CE56 and A26) were very similar and differed considerably from the pattern obtained with the polyoma-transformed 3T3 cells. These findings were in accord with those reported earlier[7].

DISCUSSION

Plasma membrane preparations have been obtained from normal 3T3 mouse fibroblasts, from 3T3 cells transformed by SV40 and polyoma viruses and from 3T3 cells resistant to ouabain. The analyses presented here, and the electron microscopic evidence shown elsewhere[16], indicated that these plasma membrane isolates are free of other cellular constituents. They showed that the plasma membrane material con-

tains about 5.5 % of the total cellular protein and 5.7 % of the macromolecular glucosamine. The plasma membranes exhibited (Na^+–K^+–Mg^{2+})-ATPase activity commensurate with that detected in other cells grown *in vitro*[21,36,38].

It has been possible to show that 94 % of the total membrane protein can be resolved by electrophoresis in sodium dodecyl sulphate-containing polyacrylamide gels, which revealed the presence of at least 20–30 peptide-containing moieties in all preparations examined. Analysis of the lipid-free fraction of plasma membrane (in sodium dodecyl sulphate-free gels) also showed the presence of some 20–30 peptide fractions. Using [^{3}H]GlcN labelled material, many GlcN-containing moieties were detected in fully solubilized plasma membrane preparations electrophoresed in sodium dodecyl sulphate gels. On the basis of the co-electrophoresis of components labelled with [^{3}H]GlcN and [^{14}C]Leu it was tentatively concluded that these were glycoprotein or glycolipoprotein. These results are in accord with those of others who have detected marked heterogeneity amongst the proteins of mammalian cell membranes[44,46–49].

Comparative studies carried out with normal 3T3 cells and various virus-transformed derivatives, have indicated that the plasma membrane isolates from these do differ. The differences are several, as revealed by the variable patterns of electrophoresis of the peptides and GlcN-containing molecules of the fully dissolved plasma membrane, and of the peptides of the previously delipidated material.

These various studies reflect extensive differences between the cell surfaces of normal and virus-transformed 3T3 mouse fibroblasts. The very profound extent of these variations is emphasized by the finding that the GlcN containing molecules of the plasma membrane preparations of 3T3 and 3T3 oua^R cells were not detectably unalike. There is evidence (R. Sheinin, unpublished), that the latter drug-resistant cells which differ from normal 3T3 cells with respect to certain parameters of the cell membrane bound (Na^+–K^+–Mg^{2+})-ATPase (M. A. Cescon, R. M. Baker and R. Sheinin, unpublished), carry a single, specific lesion on the cell surface.

Of special interest was the finding that the variation *vis à vis* GlcN-containing molecules, *i.e.* glycoproteins and perhaps glycolipoproteins, was clonally distributed. Thus the three different 3T3-SV cells each gave a distinct pattern of electrophoresis of such membrane molecules; which in turn deviated from that of normal cells. This result was in contrast to those made with respect to the peptide moieties. For these were very similar in the case of three clones of 3T3-SV cells, whether examined in fully solubilized or lipid-free preparations. It seems likely that the observed clonal variation in GlcN-containing molecules bears some relevance to that recently observed in the case of the abnormal ganglioside composition of the plasma membranes of virus-transformed cells[7,17,50].

There is much evidence that the surface of mammalian and avian cells undergoes extensive functional[51–54] and chemical (*cf.* refs 7 and 17) modulation in association with neoplastic transformation. The problem becomes one of understanding how these many variations can issue from the very small amount of genetic information which is added to the cell in the form of integrated virus genome[20]. This puzzle would be resolved if neoplastic transformation is associated with disorientation of the processes which normally regulate the formation and turnover of surface macromolecules, in particular the complex heteroglycans. This postulate, which already has supportive evidence[56–57], would explain not only the many chemical changes

observed; but also the extensive modulation of functional molecules, many of which may be glycoprotein or glycolipid in nature[45]; the apparent derepression of synthesis of embryonic surface antigens[51–54]; as well as the stable expression of Forsmann antigen at the surface of transformed derivatives of normal cells, in which such expression is dependent upon conditions of growth and development[58–61].

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