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A SURVEY OF DIFFERENCES BETWEEN MEMBRANE POLYPEPTIDES OF TRANSFORMED AND NONTRANSFORMED CHICK EMBRYO FIBROBLASTS

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Plasma membrane-associated polypeptides of chick embryo fibroblasts and cells transformed by the Schmidt-Ruppin wild-type strain of Rous sarcoma virus and its temperature-sensitive tsNY68 mutant were compared by two-dimensional gel electrophoresis. Polypeptide and glycoprotein alterations were identified after incubation of cells with [^{35}S]methionine and [^{3}H]mannose and by staining of the gels with ^{125}I-labeled concanavalin A and Coomassie brilliant blue. Polypeptides found to be consistently transformation-sensitive included a group of five polypeptides that were detected only by short-term labeling with methionine, fibronectin, a 180 kDa polypeptide with a p*I* of 5.6, a mannose-containing glycoprotein of 48 kDA and an unusually high p*I* of 8.4, and a 19 kDa polypeptide with a p*I* of approx. 4.5. Several of these polypeptides appear to be particularly interesting for further characterization.

Introduction

Cells transformed by oncogenic viruses show dramatic alterations in growth properties and morphology when compared to their normal counterparts. The cell surface is thought to play an important role in these changes in behavior of transformed cells. Identification of altered polypeptide composition has been an important tool in beginning to determine the differences between these cell types at the molecular level [1–4] (for reviews, see Refs. 5–9).

Only in a few recent reports have powerful two-dimensional electrophoretic separation techniques been applied to this problem [10–13]. Surprisingly, there have been no systematic comparisons of purified plasma membranes by this method using different precursor labeling conditions and oncogenic viruses that permit conditional expression of the transformed phenotype.

This study describes a systematic comparison of plasma membrane polypeptides of chick embryo fibroblasts that were uninfected or transformed with the Schmidt-Ruppin wild-type strain of avian sarcoma virus or with the temperature-sensitive tsNY68 mutant of this strain, using this sensitive two-dimensional protein analysis technique.

Explanted fibroblasts and an RNA tumor virus system were chosen in preference to permanent cell lines in order to mimic in vivo events more closely, and the tsNY68 temperature-conditional mutant system was used to minimize epiphenomena, although the latter choice requires documentation of simple temperature-sensitive changes in labeling. Proteins were quantitated by labeling for 2 or 24 h with [^{35}S]methionine and Coomassie brilliant blue staining, and differences in glycoproteins were examined by [^{3}H]mannose incorpora-

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tion and ^{125}I-labeled concanavalin A binding. These experiments identify new polypeptide changes and further define previously described alterations, with the goal of determining which of the many membrane polypeptides deserve study in more biochemical and immunological detail.

Materials and Methods

Materials

The viral mutant tsNY68 was a kind gift of Drs. S. Kawai and H. Hanafusa. Tissue culture media were obtained from GIBCO Laboratories (Grand Island, NY) or from Flow Laboratories (McLean, VA) and calf serum from Colorado Serum Company (Denver, CO). [^{35}S]Methionine (750 Ci/mmol) was purchased from Amersham (Arlington Heights, IL), whereas all other radiochemicals, [2 $-^{3}$H]mannose (17 Ci/mmol), carrier-free ^{125}I-sodium iodide and ^{125}I-labeled concanavalin A (136 mCi/mg) were from New England Nuclear (Boston, MA). EN^{3}HANCE was also purchased from the same company.

All other chemicals were purchased from Sigma (St. Louis, MO), Bethesda Research Laboratories (Rockville, MD), or BioRad (Richmond, CA), and were reagent grade or purer.

Cell culture

Uninfected, tsNY68- and Schmidt-Ruppin wild-type Rous sarcoma virus-infected chick embryo fibroblasts were cultured in Vogt's PGM medium for 3 days at 36°C [14]. Secondary cultures were maintained for 2 days in GM medium [14] with one change of medium. Cells were passaged to tertiary cultures using 0.25% trypsin, maintained for another 2 days at either 36 or 41°C with one change of medium, then cells were either directly solubilized in 2% SDS with incubation for 3 min in a boiling water bath, or subjected to the plasma membrane purification protocol of Stone et al. [1]. Cells were scraped into Dulbecco's phosphate-buffered saline containing 10% sucrose, centrifuged, and washed with the same buffer. The pellet was incubated in a buffer containing 15 mM iodoacetate, 10 mM Tris-HCl, pH 8.0, and 2% DMSO. After 5 min the cells were homogenized with a Dounce tissue grinder. After centrifugation at $3000 \times g$ for 15 min at 4°C, the pellet was resuspended in the chilled two-phase system described by Brunette and Till [23]. After 10 min centrifugation at $11\,700 \times g$ in a swinging bucket rotor, plasma membranes were recovered from the interface. The two-phase fractionation procedure was repeated and the membranes were washed. These identical membrane preparations have previously been shown to consist of plasma membrane ghosts by phase contrast, scanning, and transmission electron microscopy [1]. The purified plasma membranes were solubilized in boiling 2% SDS. All preparations were immediately frozen in powdered solid CO_2 and stored at -20 or -80°C.

Radioactive labeling

Cells were incubated with [^{35}S]methionine (Amersham, 750 Ci/mmol) for 2 h at 45 μCi/ml or for 24 h at 15 μCi/ml. The medium for the 2-h incubations was depleted of unlabeled methionine by the use of methionine-free medium and dialyzed calf serum. Incubations with [2-^{3}H]mannose (New England Nuclear, 17 Ci/mmol) were performed at 15 μCi/ml for 24 h in regular GM medium. Cell surface labeling with ^{125}I was performed by the lactoperoxidase method as described by Hynes [18] using carrier-free ^{125}I-sodium iodide (New England Nuclear).

Two-dimensional gel electrophoresis

Isoelectric focusing in the first dimension was performed according to Cabral and Schatz [19]. The gels were loaded with 150 μg of protein in a final concentration of 1% SDS. The electrophoretic current was maintained at 0.3 mA/gel until the voltage reached 260 V. The total isoelectric focusing time was 24 h. The pH gradient of reference gels loaded with an equal aliquot of SDS without protein was determined by slicing the gels, adding 1 ml of H_2O, and measuring the pH of the pieces after trituration by a glass rod. After incubation of the sample gels in a buffer containing 0.0625 M Tris-HCl, pH 6.8, 10% glycerol, 0.1 M dithiothreitol, 2.5% SDS, and 0.01% Bromphenol blue, the gels were transferred to slab gels containing resolving gel acrylamide concentrations of either 7.5% or 12%. Electrophoresis in the second dimension was performed as described previously [20,21]. After fixing and staining with Coomassie brilliant blue R250, the gels were treated with

EN^{3}HANCE (New England Nuclear), dried, and autoradiographed using Kodak X-Omat film. The average exposure time was 3 days. Concanavalin A-binding proteins were identified in gels according to the gel overlay method of Burridge [22] using ^{125}I-labeled concanavalin A (New England Nuclear, 36 mCi/mg).

The proteins used as molecular weight standards were: myosin with a molecular weight of 200000, RNA polymerase with 4 bands: 160000, 150000, 90000 and 39000, phosphorylase *b* (94000), bovine serum albumin (68000), ovalbumin (43000), α-chymotrypsinogen (25700) and β-lactoglobulin (18400).

Protein assays

$(Na^+ + K^+)$-ATPase activity was determined as described by Moore and Pastan [15]. NADH-ferricyanide reductase activity was assayed according to Wallach and Kamat [16]. Protein determinations were performed as described by Lowry et al. [17] with bovine serum albumin standards.

Results

Characteristics of membrane preparations

The protein recovered from the interface of the two-phase system constituted 5–9.5% of total protein in original cell homogenates. The recovery was similar to that reported by Brunette and Till [23] and Glick and Warren [24] for L cell membranes using two-phase systems.

After labeling of the cell surface with ^{125}I by the lactoperoxidase method and isolating the membrane fraction, the specific activity was 7-times higher than in the supernatant. This fraction was previously shown by electron microscopy to consist of purified plasma membrane ghosts [1]. The activity of the membrane $(Na^+ + K^+)$-ATPase was found to be too low to be a reliable marker for the purity of the membrane fraction, in agreement with Stone et al. [1] using the same purification method.

NADH-ferricyanide reductase is an aspecific marker for electron transport activity. We found only 3–4% of the total activity in the plasma membrane fraction. This is in agreement with the findings of Brunette and Till [23] and Branton and Landry-Magnan [25] and indicates a low level of contamination with endoplasmic reticulum. Fig. 1 shows the differences in protein composition between the purified plasma membrane fraction and cytosol or total cell homogenate. The polypeptide composition was clearly different, consistent with purification relative to other cellular proteins.

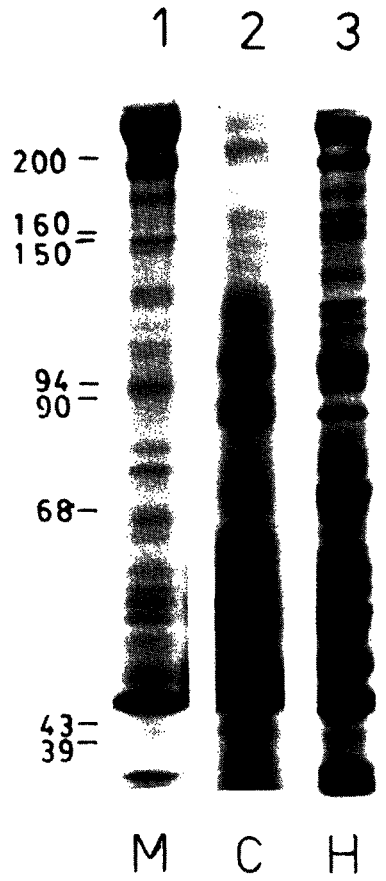

Fig. 1. Comparison of the protein composition of a membrane fraction (M) of chick embryo fibroblasts obtained as described in Methods (lane 1), a cytoplasmic fraction (C) obtained by centrifugation of the lysed cells at 100000 × *g* for 60 min (lane 2), and the original total cell homogenate (H, lane 3).

One-dimensional SDS gels comparing uninfected with wild-type and tsNY68 virus-infected cells generally confirmed previously published data (Refs. 1–3, results not shown). The only unusual result was a major decrease in short-term labeling at 41°C of a band corresponding to the monomer of the membrane-associated glycoprotein fibronectin (apparent molecular weight 235000) and of a polypeptide with an apparent molecular weight of 150000.

TABLE I

SUMMARY OF ALTERATIONS IN PLASMA MEMBRANE POLYPEPTIDES OF CHICK EMBRYO FIBROBLASTS IN-

CB, Coomassie brilliant blue staining; A, incubation at 36°C; B, incubation at 41°C. +, present; −, absent; ±, barely detectable; ↓,

Protein	Mol. wt. ($\times 10^{-3}$)	p*I*	Transformation-specific alterations	Methionine incubation					
				Non-infected					
				2 h		24 h		CB	
				A	B	A	B	A	B
1	235	6.1	↓	+	+/↓	+/↓	+/↓	+	+
2	180	5.6	↓	+	+	+	+	+	+
3	155	8 [b]	↓ [a]	+	+	−	−	−	−
4A	96	7.5–8		−	−	−	−	−	−
4B	96	5.4–5.9		−	−	−	−	−	−
5	94	5.0		+	+	−	−	+	+
6	90	6.5	↓ [a]	+	+	−	−	−	−
7	72	5.8		+	+	+	+	+	+
8	61	6.2	↓ [a]	+	+	−	−	±	±
9	59	6.5		±	±	±	+	+	+
10	48	8 [b]	↓	+	+	+	+	+	+
11	46	4.5 [b]	↑	+	+	+	+	±	±
12	45	5.4	↓	+	+	+	+	+	+
13	31	7.4		+	+	−	−	±	±
14	31	7.2		+	+	−	−	±	±
15	24	5.7	↓ [a]	+	+	−	−	−	−
16	24	5.5	↓ [a]	+	+	−	−	−	−
17	23	7.3		−	−	−	−	−	−
18	19	4.5 [b]	↓	+	+	+	+	±	±
19	155	6.1							

[a] Polypeptide of apparent high turnover rate.

[b] p*I* approximate: Because the gels in the first dimension were loaded with proteins dissolved in a buffer containing SDS, the gradient was pH 4–8 instead of the pH 3.5–9.5 of the ampholines used. The p*I* values listed for the proteins at the end of this gradient are

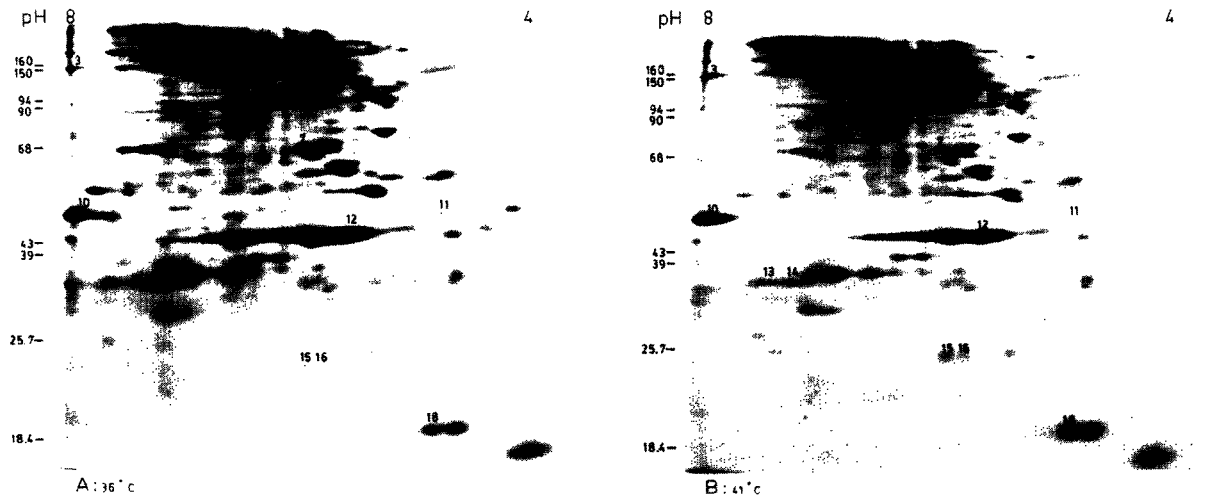

Fig. 2. Polypeptide analysis of the membrane fraction of uninfected chick embryo fibroblasts by two-dimensional electrophoresis after a 2-h incubation with [^{35}S]methionine. Acrylamide concentration of the second-dimension gel was 12%. A: 36°C; B: 41°C. The number refer to the nomenclature of Table I in this and all subsequent figures.

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decreased relative to non-infected or nonpermissive controls; ↑, increased relative to non-infected or nonpermissive controls.

tsNY68 infected						Schmidt-Ruppin wild type infected						Con A staining		Mannose incubation		Protein
2 h		24 h		CB		2 h		24 h		CB		tsNY68		tsNY68		
A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	
±/↓	+	+/↓	+	+/↓	+/↓	±/↓	±/↓	−/↓	−/↓	+/↓	+/↓	+/↓	+	+/↓	+	1
−/↓	+	−/↓	+	−/↓	+	−/↓	−/↓	−/↓	−/↓	−/↓	−/↓					2
−/↓	+	−	−	−	−	−/↓	−/↓	−	−	−	−	+/↓	+	+/↓	+	3
+	+	−	−	−	−	−	−	−	−	−	−	+	+	+	+	4A
−	−	−	−	−	−	+	+	−	−	−	−					4B
−/↓	+	−	−	+	+	−/↓	−/↓	−	−	+	+					5
−/↓	+	−	−	−	−	−/↓	−/↓	−	−	−	−					6
−/↓	+	−/↓	+	+	+	+/↓	+	−/↓	+/↓	+	+					7
+/↓	+	−	−	−	±	±/↓	±/↓	−	−	−	−					8
±	±	+/↑	+	+	+	±	±	+/↑	+/↑	+	+					9
+/↓	+	+/↓	+	+/↓	+	+/↓	+/↓	+/↓	+/↓	+/↓	+/↓	+/↓	+	+/↓	+	10
+/↑	+	+/↑	+	±	±	±/↑	+	+/↑	+	±	±					11
+/↓	+	+/↓	+	+/↓	+	+/↓	+/↓	+/↓	+/↓	+/↓	+/↓					12
−/↓	+	−	−	±	±	−/↓	−/↓	−	−	±	±					13
−/↓	+	−	−	±	±	−/↓	−/↓	−	−	±	±					14
+/↓	+	−	−	−	−	+/↓	+/↓	−	−	−	−					15
+/↓	+	−	−	−	−	+/↓	+/↓	−	−	−	−					16
+/↓	+	−	−	−	−	+	+	−	−	−	−					17
+/↓	+	+/↓	+	±	±	+/↓	+/↓	±/↓	±/↓	±	±					18
												±/↓	+			19

therefore not accurate; e.g., in a pH 7–9 gradient, the p*I* of the 48000 protein is actually approx. 8.4 (results not shown). For this reason, in a few cases this protein and presumably others did not enter gels when the pH gradient did not reach that p*I*.

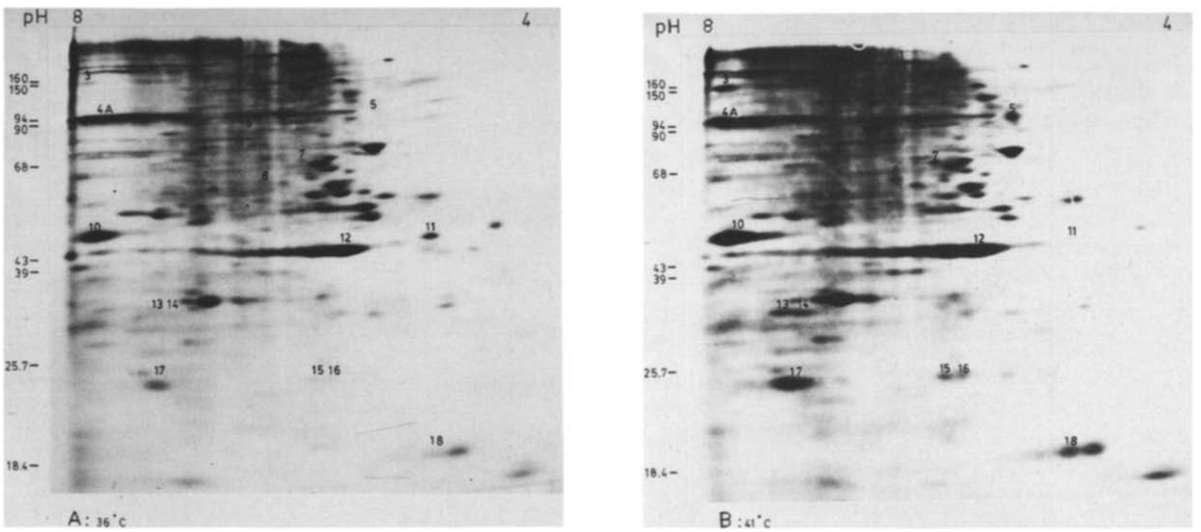

Fig. 3. Two-dimensional autoradiogram of the membrane fraction of tsNY68-infected chick embryo fibroblasts after 2 h incubation with [^{35}S]methionine. Acrylamide concentration of the second-dimension gel was 12%. A: 36°C; B: 41°C.

Comparisons of polypeptide compositions by two-dimensional gel electrophoresis after short-term labeling with [^{35}S]methionine

The experiments were repeated using the two-dimensional electrophoresis system of Cabral and Schatz [19]. Comparisons of the membrane fractions of uninfected cells at 36 or 41°C revealed only a change in labeling of the 235000 polypeptide (Table I, Fig. 2A and B, spot 1). As in one-dimensional gels, this change in short-term labeling was not reflected in the Coomassie blue protein pattern (gels not shown).

The differences in polypeptide composition between the plasma membrane fractions of the tsNY68-infected cells cultured at permissive and nonpermissive temperatures are shown in Fig. 3, using 2 h of labeling with [^{35}S]methionine and analysis on 12% acrylamide second-dimension gels. For better separation of the higher molecular weight polypeptides 7.5% acrylamide gels were also used (not shown). The major differences are summarized in Table I.

Transformation-sensitive alterations in labeling appeared to affect only approximately 10–15% of all polypeptide spots (Fig. 3).

There were at least 16 major membrane-associated changes in polypeptide labeling, all but one of which was a decrease (Table I). In some cases, e.g., spots 5 and 6, there was virtually complete loss of labeled peptides. In comparison, the well-known decrease in membrane-associated fibronectin (spot 1) was partial.

These alterations in labeling with a 2-h pulse of methionine appeared to be transformation-specific. They were also found in comparisons of tsNY68-infected cells at the permissive temperature with uninfected cells at that temperature, as well as in wild-type virus-transformed cells compared to uninfected cells at either cell culture temperature (Table I, Fig. 2). There was only one exception (spot 7), which was partially restored to more normal levels by incubation of cells transformed by the wild-type virus at 41°C.

Several viral polypeptides could also be detected in plasma membrane fractions after pulse labeling. A polypeptide presumably corresponding to p28 [26] had an apparent molecular weight of 23000 (spot 17) in this electrophoretic system, and was present in membrane fractions of wild-type virus-infected cells and of cells infected with its tsNY68 mutant at either temperature. An apparent viral precursor glycoprotein with a molecular weight of 96000 similar to that described by Hayman [27] was also noted (spot 4). Similar results were also obtained in cells infected by the RAV-7 virus (unpublished data). Many of the polypeptides listed in Table I could not be detected by Coomassie brilliant blue staining, indi-

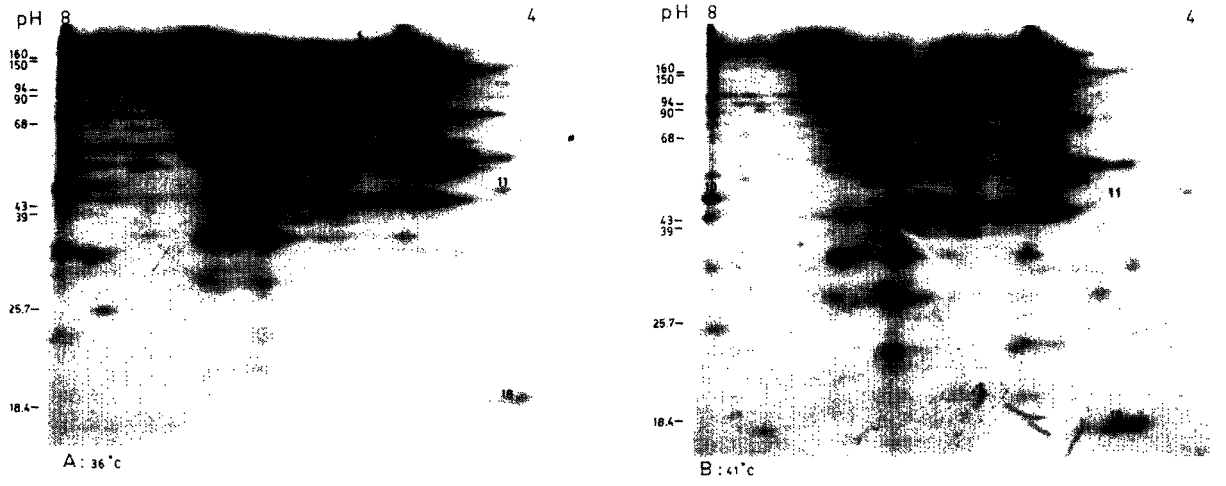

Fig. 4. Two-dimensional autoradiogram of the membrane fraction of tsNY68 virus-infected cells after 24 h incubation with [^{35}S]methionine. 12% acrylamide in the second dimenson. A: 36°C; B: 41°C.

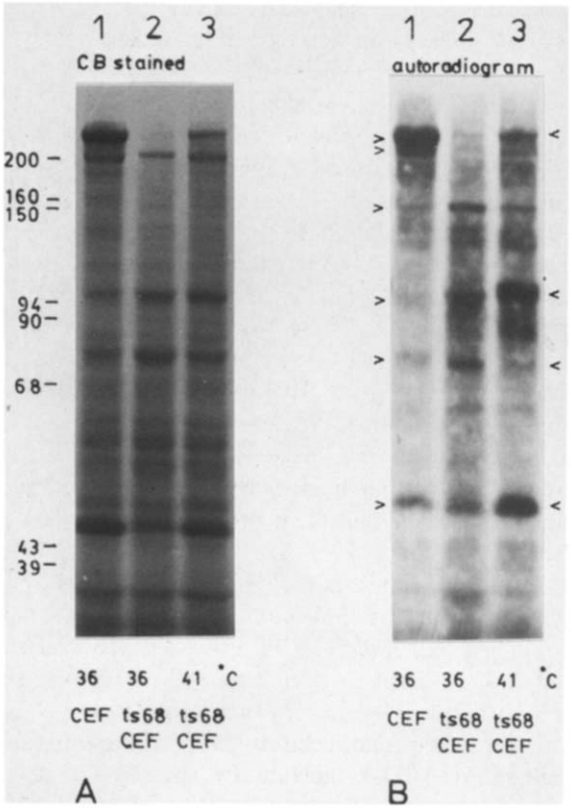

Fig. 5. Coomassie brilliant blue staining pattern (A) and autoradiogram (B) after 24 h incubation with [2−^{3}H]mannose. Acrylamide concentration was 7.5%. Lane 1, membrane fraction of uninfected cells (36°C); lane 2, membrane fraction of tsNY68-infected cells at the permissive temperature (36°C); lane 3, membrane fraction of tsNY68-infected cells at the nonpermissive temperature (41°C). Arrowheads mark proteins discussed in the text.

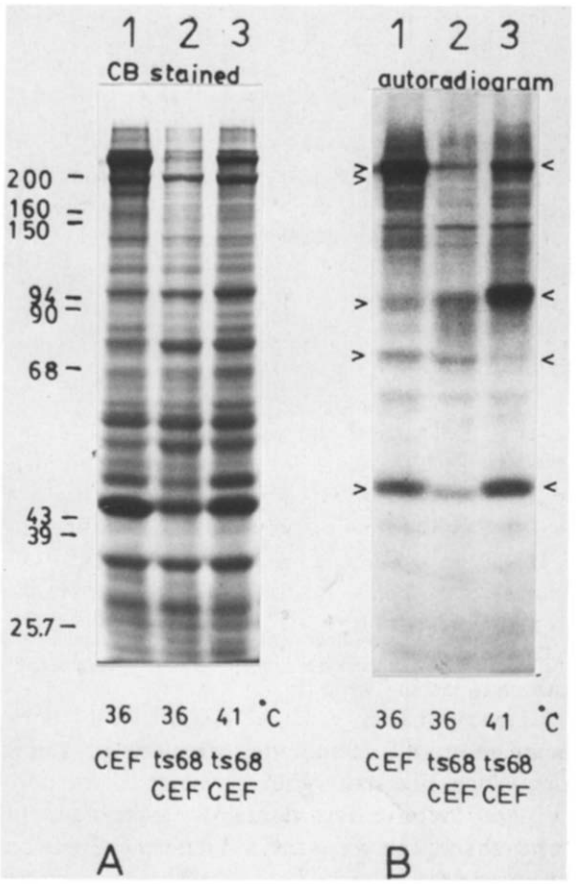

Fig. 6. A. Coomassie brilliant blue staining pattern of the membrane fractions of uninfected cells (lane 1), tsNY68-infected cells at 36°C (lane 2) and at 41°C (lane 3). B. Autoradiogram of the same gel after incubation with [^{125}I]concanavalin A. Arrowheads mark proteins of interest.

cating that they are considerably less abundant than the major structural proteins.

Comparisons of polypeptide compositions after long-term labeling with [^{35}S]methionine

The polypeptide patterns of both uninfected and infected cells incubated for 24 h with [^{35}S]methionine differed substantially from the patterns obtained after only 2 h of incubation when examined by two-dimensional gels (Table I; compare Figs. 2, 3 with 4). Comparisons of the plasma membrane fractions of uninfected cells labeled for these two time periods revealed at least 9 differences in labeling (Table I). A number of spots that were present at 2 h were poorly or no longer labeled at 24 h (e.g., spots 3 and 6). A few spots (e.g., 9) were more clearly visible at 24 h (see also Table I). The spots that were no longer visible with long-term labeling are presumably those with more rapid turnover rates on the plasma membrane.

Fig. 4 shows the differences in incorporation of methionine between the cell membrane fractions of tsNY68-infected cells maintained at temperatures permissive or nonpermissive for transformation. In contrast to the results with a 2-h incubation, significantly fewer polypeptide differences were detected between transformed and nontransformed cells. The differences are summarized in Table I, and they include approximately eight major, transformation-specific changes.

The viral polypeptides could not be detected in

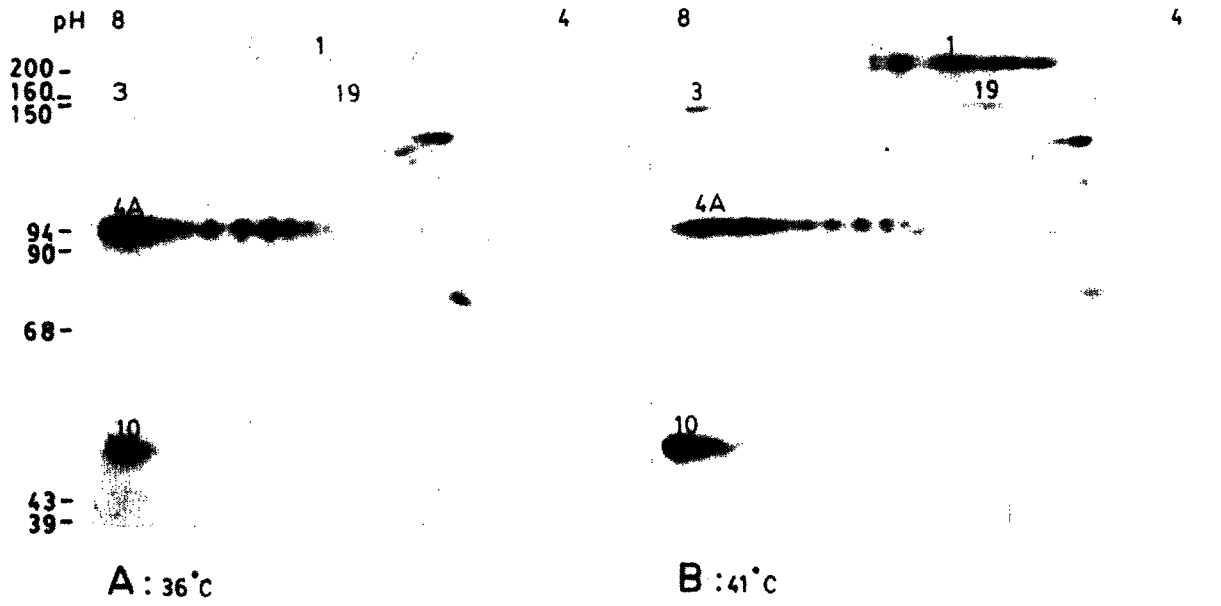

Fig. 7. Staining of two-dimensional gels with [^{125}I]concanavalin A obtained from the membrane fraction of tsNY68-infected cells at the permissive (A) and at the nonpermissive temperature (B). Acrylamide concentration in the second dimension is 7.5%.

association with membranes after this prolonged incubation time, as would be expected for polypeptides that are only transiently associated with membranes. There was general agreement between changes detected in Coomassie blue-stained gels (not shown) and the results of the long-term labeling experiments (Table I), as would be expected for major proteins with average or long half-lives.

Comparisons of transformed and nontransformed cell membrane fractions by other labeling methods

The results of a 24-h incubation with [^{3}H]mannose are shown in Fig. 5. In the membrane fractions of uninfected cells, the two major mannose-labeled polypeptides were the fibronectin subunit (235 000) and a polypeptide with an apparent molecular weight of 48 000. By two-dimensional gel analysis, both polypeptides co-migrated with the analogous methionine-labeled polypeptides listed in Table I (spots 1 and 10) and were thus presumably the same polypeptides. Minor mannose-labeled polypeptides had molecular weights of approximately 200 000, 155 000, 96 000, and 75 000. Comparing the electrophoretograms of tsNY68-infected cell membrane fractions at 36 and 41°C, it is clear that the labeling of fibronectin was less pronounced at 36°C, the permissive temperature. The labeling of the 48 000 polypeptide was also decreased. The Coomassie blue protein staining pattern in Fig. 9B confirms these transformation-releated decreases at the temperature permissive for transformation. Polypeptides with molecular weights of approx. 75 000 and 96 000 also displayed altered labeling. Only the 75 000 polypeptide was more heavily labeled at 36°C. It is noteworthy that the labeling of over half of the more heavily labeled bands containing mannose was altered after transformation, whereas a much lower percentage of polypeptides appeared altered (Fig. 5).

Staining of gels with [^{125}I]concanavalin A according to Burridge [22] is shown in Figs. 6 and 7. The one-dimensional gel autoradiogram of concanavalin A-binding polypeptides in Fig. 6 was strikingly similar to the mannose labeling pattern in Fig. 5; nearly all of the same polypeptides were labeled. In addition, the same alterations in labeling were found between membrane fractions of tsNY68-infected cells at the nonpermissive and permissive temperatures: in the transformed cells there was less labeling of fibronectin, a 96 000 polypeptide, and a 48 000 polypeptide, and there

was heavier labeling of a polypeptide with an apparent molecular weight of 75 000. Fig. 7 shows the two-dimensional analysis of [^{125}I]concanavalin A-binding proteins of tsNY68 virus-infected cell membrane fractions at 36 and 41°C. Some alterations can be detected both by concanavalin A binding and by methionine labeling (cf. Figs. 3, 4, and 7), as indicated in Table I. Spot 4A again is presumably one of the viral polypeptides of tsNY68. A spot with an approximate molecular weight of 155 000 and p*I* of 6.1 (spot 3) appears to bind more concanavalin A in the membrane fractions of transformed cells.

Discussion

The objective of this study was to determine which of many membrane-associated proteins of cells deserve particular attention for future isolation and monoclonal antibody analyses. The identification of distinctive properties such as unusual isoelectric points and the determination of quantities and stability should help focus future efforts on certain polypeptides that are more likely to play a role in transformation.

The plasma membranes were obtained by the two-phase system of Stone et al. [1]. As in other frequently used two-phase protocols (Brunette and Till [23]) the membrane fraction contains a low level of contamination by endoplasmic reticulum.

The expression of several polypeptides present in the plasma membrane fraction was found to be sensitive even to temperature alone. For example, the labeling of the membrane-associated and matrix glycoprotein fibronectin in nontransformed cells was unexpectedly decreased after a 5 K elevation in temperature.

A second general point is that the incorporation of methionine was clearly different in several polypeptides at 2 h versus 24 h. For example, many polypeptides were much less intensely labeled at 24 h that at 2 h when compared to average labeling. In fact, many polypeptides that were readily detected after a 2 h incubation with [^{35}S]methionine could not be visualized with Coomassie brilliant blue, strongly suggesting that they have rapid turnover rates. In contrast, after 24 h of incubation, the differences between the autoradiograms and protein staining patterns were much less.

Transformation-sensitive polypeptides

The transformation-specific polypeptide alterations identified in this study are summarized in Table I (third column). Overall, surprisingly few polypeptides (10–15%) were consistently different between the nontransformed cell membrane fractions (chick embryo fibroblasts at 36 and 41°C and tsNY68 virus-infected fibroblasts at 41°C) and the transformed cell membrane fractions (wild-type virus-infected fibroblasts at 36 and 41°C and tsNY68 at 36°C) throughout all the different experiments; these results contrast with those obtained with a permanent cell line [10]. Transformation, therefore, appears to have selective effects on plasma membrane polypeptides.

The use of a two-dimensional gel system and of short-term pulse labeling appears to have increased the ability to identify transformation-sensitive polypeptides. For example, spots 3 and 19 would not be resolved in a standard one-dimensional gel, and only one out of the at least five proteins with an apparent molecular weight of 48 000 were transformation-sensitive.

Perhaps of greatest potential importance are the multiple alterations detected in the 2 h incubations with methionine, which have not been reported previously to our knowledge. These polypeptides appear to be present in minor amounts that are not detectable by Coomassie brilliant blue or 24 h labeling, and their synthesis and turnover rates are probably high. Further studies seem appropriate to attempt to determine their role in the mechanisms of transformation of cells, since some may be regulatory proteins with short half-lives. Their alteration may be of greater importance than the previously described alterations in major structural proteins [1–3].

The well-known change in the extracellular glycoprotein fibronection (spot 1) was readily seen. Another major polypeptide that was consistently decreased in transformed cells was a 180 000 polypeptide with an apparent p*I* of 5.6 (spot number 2; Table I). This polypeptide was visible by protein staining, yet was not labeled by [^{3}H]mannose nor by staining with concanavalin A, which suggests that it does not have mannose-containing oligosaccharides. It is not clear whether this polypeptide is the ω-protein of Robbins et al. [31] (mol.wt. 206 000) or the 200 000 polypeptide of Isaka et al. [3].

A polypeptide with a molecular weight of 48 000 was also consistently decreased. This polypeptide was found to have an unusual p*I* of about 8.4 (spot number 10, Table I). It incorporated mannose and was readily labeled with concanavalin A; in fact, this polypeptide (gp48) and fibronectin were the major concanavalin A-binding polypeptides in this cell type. It is presumably the δ-protein of Robbins et al. [30]. A further unusual feature of this polypeptide is that it cannot be extracted with 0.5% Triton X-100 at pH 7. It remains in the $100\,000 \times g$ pellet, and can, therefore, be operationally considered to be part of the detergent-insoluble cytoskeleton. Furthermore, this polypeptide appears to be present in a variety of fibroblastic cells (chick embryo fibroblasts, mouse L929 cells, BHK cells) and epithelial cell lines such as HeLa and MDCK cells. It is not present in liver cell membranes and in lymphocyte S49 cell membranes (Dubbelman and Yamada, unpublished results). The unusual solubility properties and isoelectric point identified for this polypeptide may prove valuable in the future isolation of this glycoprotein.

Membrane-associated actin is also decreased after transformation [31]. However, there are also other previously undetected polypeptides with the same molecular weight as actin, but at different p*I* values, that are also transformation-specific (Figs. 3 and 4). Another polypeptide that is consistently decreased after transformation has a molecular weight of about 19 000 and a low p*I* of approx. 4.5 (spot 18, Table I). This polypeptide could not be detected by the Coomassie brilliant blue stain for protein.

We could not detect the previously reported transformation-specific changes in the so-called glucose-regulated proteins or GRP's [1–3,32,33], presumably because of our frequent changes of medium to avoid nutritional deprivation. Zala et al. [34] describe a GRP with a molecular weight of 47 000, but because our medium is not deprived of glucose, it is unlikely that this is the same protein as gp48.

The results of this study suggest that it will be useful to focus on the isolation and further characterization of certain membrane-associated polypeptides. Specific candidates include gp48, a glycoprotein that is present in substantial quantities and has an unusual p*I* to facilitate purification, as well as the polypeptides of apparently rapid turnover rates, some of which may be important regulatory proteins.

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