J. Membrane Biol. 6, 183–194 (1971)

Changes in the Structural Organization of the Surface Membrane in Malignant Cell Transformation

HANNAH BEN-BASSAT, MICHAEL INBAR, and LEO SACHS

Department of Genetics, Weizmann Institute of Science, Rehovot, Israel

Received 18 February 1971

Summary. Malignant transformation of normal cells resulting in agglutinability by the carbohydrate-binding protein Concanavalin A can be explained by three types of changes in the structural organization of sites on the surface membrane. There can be an exposure of cryptic sites, a concentration of exposed sites by a decrease in cell size, and a rearrangement of exposed sites without a decrease in cell size resulting in a clustering of sites.

The carbohydrate-binding protein Concanavalin A (Con. A) (Summer & Howell, 1936) can agglutinate cells transformed by a variety of carcinogenic agents, but it only agglutinates normal cells after they have been treated with trypsin (Inbar & Sachs, 1969*a*). This agglutination was reversed by competition with α-methyl-D-glucopyranoside and other carbohydrates that strongly bind to Con. A (Goldstein, Hollerman & Smith, 1965). Two other agglutinins of plant origin have been reported to agglutinate transformed cells by interacting with specific sites on the surface membrane, but they agglutinate normal cells only after they have been treated with proteolytic enzymes. These agglutinins are a glycoprotein from wheat germ by interacting with N-acetyl-D-glucosamine-like sites (Burger, 1969) and a glycoprotein from soybean by interacting with N-acetyl-D-galactosamine-like sites (Sela, Lis, Sharon & Sachs, 1970; Lis, Sela, Sachs & Sharon, 1970). With soybean agglutinin, transformed cells from hamsters, but not from mice, rats or humans, are agglutinated only after prolonged treatment with pronase (Sela *et al.*, 1970).

Results obtained with these three agglutinins have suggested that the α-methyl-D-glucopyranoside (α-MG)-like sites, the N-acetyl-D-glucosamine-like sites, and the N-acetyl-D-galactosamine-like sites, exposed on the surface membrane of transformed cells, are in a cryptic form in the untransformed parent cells. Direct evidence for this assumption for the α-MG-like sites was obtained by measuring the adsorption of Con. A molecules whose metal

sites required for α-MG binding were labeled with ^{63}Ni. The results indicated that normal and transformed cells have a similar number of sites for Con. A, but that about 85% are in a cryptic form on normal cells (Inbar & Sachs, 1969*b*).

The present experiments were undertaken to determine whether the change in the structure of the surface membrane produced by transformation, as measured by binding sites for Con. A, can be ascribed only to an exposure of cryptic sites or also to changes based on redistribution of already exposed sites on the surface membrane. The cell size of transformed cells can be smaller than that of normal cells (Inbar & Sachs, 1969*b*), and it was found that cell size can be influenced by growth conditions. The present experiments were therefore carried out to obtain evidence for redistribution of the already exposed sites by determining the correlation between cell size, number of sites for Con. A, and Con. A agglutinability in cells grown under different conditions.

Materials and Methods

Cell Cultures

We used normal cells from secondary cultures of golden hamster embryos, lines of golden hamster embryo cells transformed *in vitro* by polyoma virus or after treatment with the chemical carcinogen dimethylnitrosamine (DMNA) (Huberman, Salzberg & Sachs, 1968), a line derived from a simian virus 40 (SV 40)-induced hamster tumor, untransformed cells from the mouse cell line 3T3, and a line of 3T3 cells transformed by SV 40 (Todaro, Green & Goldberg, 1964). Cells were cultured in plastic Petri dishes, in Eagle's medium with a fourfold concentration of amino acids and vitamins with 10% serum. Two batches of adult calf serum, adult 1 and adult 2, and one batch of fetal calf serum were used. Cells were subcultured every 4 days, and the cell lines were grown in each batch of serum for at least 10 subcultures before they were used in the experiments. In all experiments, cells were seeded at 1×10^6 cells per plate in 100 mm Petri dishes. There was no detectable mycoplasma contamination as shown by testing the cultures on mycoplasma agar, according to Chanock, Hayflick, and Barile (1962). Cell volume was measured by centrifuging a known number of cells in a centrifuge tube containing a graduated capillary tube of 1 mm diameter. After 20-min centrifugation at $300 \times g$, the cells had pelleted in the capillary tube, and the surface area of a single cell was calculated from the volume assuming that cells are spherical (Inbar & Sachs, 1969*b*). Triplicate samples were assayed for each point. The results were reproducible with a variation of less than $\pm 5\%$. Total protein per cell was measured by the method of Lowry, Rosenbrough, Farr, and Randall (1951). For both measurements, cell suspensions were prepared as for the agglutination assay.

Con. A

Con. A was prepared (by Miles Yeda) from Jack bean meal (Sigma Chemical Co.) by two crystallizations (Summer & Howell, 1936) and stored as a solution in saturated NaCl. ^{63}Ni-labeled Con. A was prepared as described previously (Inbar & Sachs, 1969*b*) according to the method of Kalb and Levitzki (1968).

Agglutination Assay

Cultured cells were washed twice with phosphate-buffered saline (PBS), and removed from the Petri dish with a solution of 0.02% disodium versenate (Inbar & Sachs, 1969*a*). The suspended cells were washed twice with PBS and then diluted in PBS at a concentration of 1 to 2×10^6 cells per ml. To test for agglutination, 0.5 ml of Con. A diluted in PBS was mixed with 0.5 ml of the cell suspension in a 35 mm Petri dish at a final concentration of 500 μg Con. A per ml. The density and size of aggregates was scored in a scale from − to + + + + after 30-min incubation at room temperature.

Assay of Con. A Binding

Cells were prepared as for the agglutination assay and diluted in PBS at a concentration of 4×10^6 cells per ml. To test for Con. A binding, 0.5 ml of labeled Con. A diluted either in PBS or in PBS containing 10^{-1} M α-MG was mixed with 0.5 ml of cell suspension in a centrifuge tube at a final concentration of 500 μg Con. A per ml. After 30-min incubation at room temperature (Inbar & Sachs, 1969*b*), the cells were washed three times with 5 ml PBS by centrifugation. The 30-min incubation period was chosen so as to saturate all the Con. A sites (Fig. 1). The pellet was dissolved in 0.1 N NaOH, suspended in Bray's solution (Bray 1960), and the radioactivity counted in a Packard Tri-Carb liquid scintillation spectrometer. Triplicate samples were assayed for each point. The results were reproducible with a variation of ±10%. To calculate the amount of labeled Con. A which is bound specifically by its α-MG binding sites, the amount

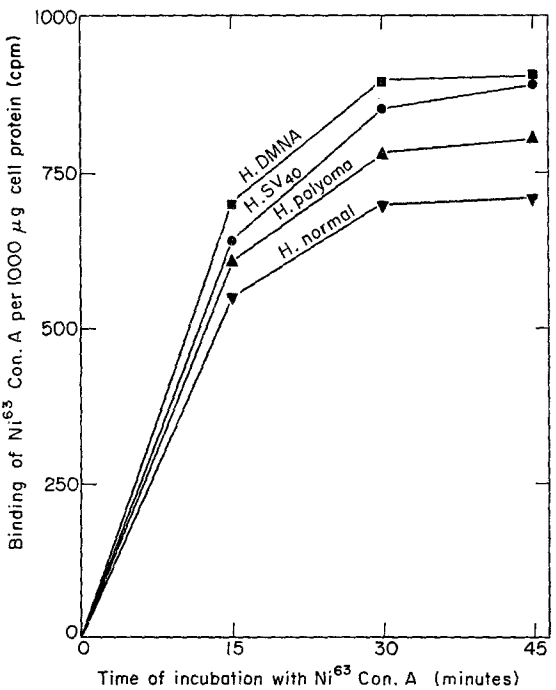

Fig. 1. Binding of Ni^{63} Con. A to normal and transformed hamster cells. The cells were tested 4 days after subculture. They were grown in adult 2 calf serum, and incubated with 500 μg/ml Ni^{63} Con. A at room temperature

bound at saturation in the presence of α-MG was subtracted from the amount bound in the absence of α-MG. This difference is referred to as α-MG specific binding, and the data on Con. A binding given in the present experiments always refer to the α-MG specific binding. The number of Con. A molecules bound was calculated from the observation that 1 mg of labeled Con. A used gave 40,000 cpm and the molecular weight of the protein was taken as 55,000 (Yariv, Kalb & Levitzki, 1968; So & Goldstein, 1968).

Results

Growth Conditions and Cell Size in Normal and Transformed Cells

Previous results (Inbar & Sachs, 1969*b*) have indicated that the cell size, i.e., the cell surface area, was higher in normal hamster and untransformed 3T3 cells than in transformed cells, when the cells were grown in adult 1 calf serum and tested at 3 to 5 days after subculture. Experiments were then carried out to determine the cell size of normal and transformed hamster cells grown in another batch of adult calf serum, adult 2, or fetal calf serum. The cells were tested at 1 to 4 days after subculture. The results indicate (Fig. 2, left) that the surface area per cell of SV 40-transformed cells decreased by 51% from 1 to 4 days after subculture, when the cells were grown

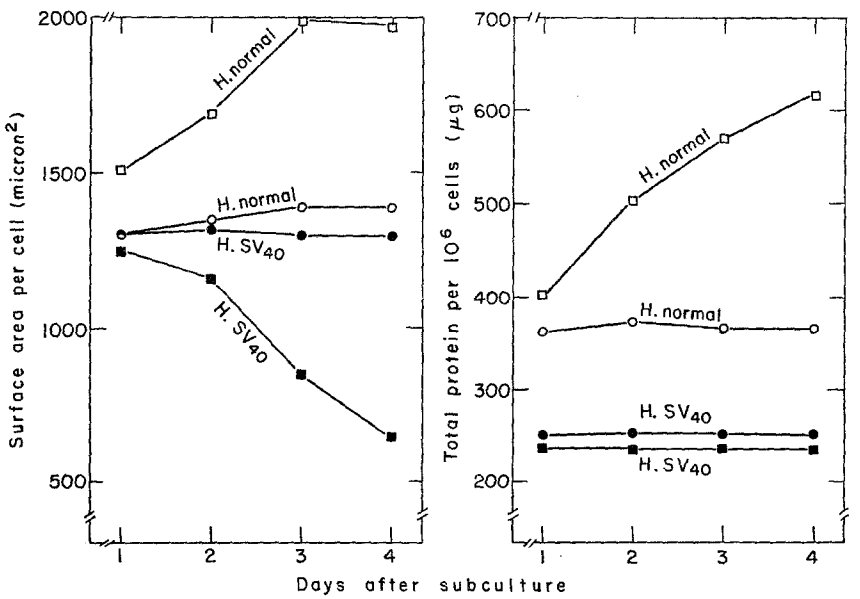

Fig. 2. Cell size and total cell protein of normal and SV 40-transformed hamster cells grown in adult 2 and fetal calf serum. Left = Surface area per cell. Right = Total cell protein. The numbers of cells per plate in adult 2 and fetal serum were: 1.1, 1.1×10^6 (normal), and 1.4, 1.6×10^6 cells (transformed) at 1 day after subculture, and 3.9, 4.0×10^6 (normal), and 19.8, 20.8×10^6 cells (transformed) at 4 days after subculture, respectively. Square symbols = adult 2 serum. Round symbols = fetal serum

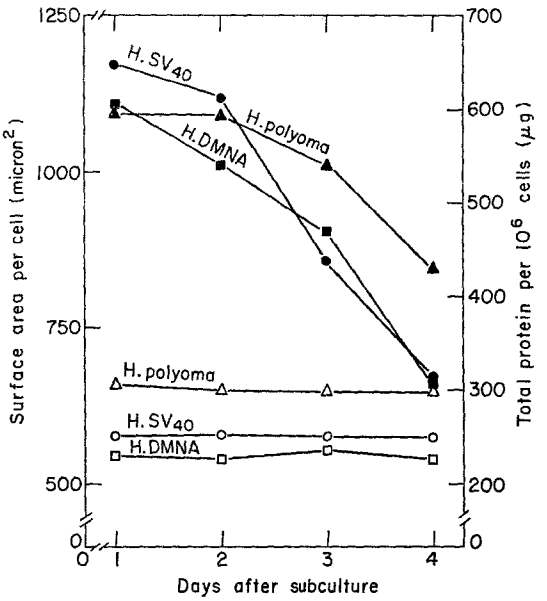

Fig. 3. Cell size and total cell protein of polyoma, SV 40- and dimethylnitrosamine (DMNA)-transformed hamster cells grown in adult 2 serum. The numbers of cells per plate for polyoma, SV 40- and DMNA-transformed cells were: 1.6, 1.4 and 1.1×10^6 cells at 1 day after subculture, and 16.5, 19.8 and 18.0×10^6 cells at 4 days after subculture, respectively. Closed symbols = surface area per cell Open symbols = total cell protein

in adult 2 serum. This decrease in cell size in the transformed cells was not associated with a change in the total protein per cell (Fig. 2, right). The surface area of normal cells in the same serum increased by 26%, and this was associated with an increase in total protein per cell. The surface area and cell protein of normal and transformed cells did not change from 1 to 4 days after subculture when the cells were grown in fetal serum (Fig. 2). Results obtained with two other lines (H. polyoma and H. DMNA) also showed a decrease in cell size, not accompanied by a change in protein per cell, during subculture in adult 2 serum (Fig. 3) and no such decrease in size in fetal serum. Transformed cells did not acquire the growth pattern of normal cells in any of the sera used.

Cell Size, Number of Con. A Molecules Bound, and Agglutinability by Con. A

The next experiments were carried out to determine the relationship between cell size, number of α-MG specific Con. A molecules bound, and agglutinability by Con. A. The results on agglutinability indicate that all the lines of transformed cells were not agglutinated at 1 day but that they

Table 1. *Cell size, number of Con. A molecules bound, and agglutination by Con. A, at 1 and 4 days after subculture of transformed hamster cells in adult 2 and fetal calf serum*

Type of calf serum	Cells	Days after subculture	Surface area per cell (μ^2)	No. of molecules bound		Agglutination by Con. A (500 μg/ml)	Structural changes in membrane sites between 1 and 4 days
				per cell ($\times 10^6$)	per μ^2 surface ($\times 10^3$)		
Adult 2	H. polyoma	1	1,109	40	36	−	Concentration of exposed sites
		4	724	40	56	++	
	H. DMNA	1	1,166	44	38	−	
		4	639	43	68	++++	
	H. SV 40	1	1,279	41	32	−	
		4	651	45	70	+++	
Fetal	H. polyoma	1	1,206	38	32	−	Rearrangement of exposed sites
		4	1,379	39	28	++	
	H. DMNA	1	1,384	49	36	−	
		4	1,411	46	33	++++	
	H. SV 40	1	1,332	41	31	−	
		4	1,181	39	33	+++	

were agglutinated at 4 days after subculture, when grown in adult 1, adult 2, or fetal serum. The data for adult 2 and fetal serum are given in Table 1. The non-agglutinating transformed cells became agglutinable after they had undergone one to two cell replications. When the transformed cells were grown under conditions that enhanced cell replication, such as growth on a feeder layer of 4,000 R X-irradiated rat cells, they were agglutinated even at 1 day after subculture. Normal cells were not agglutinated by Con. A at 1 or 4 days after growth in either the adult or fetal serum.

Experiments on the number of Con. A molecules bound to hamster transformed cells at 1 and 4 days after subculture have shown that, in all the transformed lines, a similar number of molecules were bound per cell at both times in adult 2 and fetal serum. Since there was a decrease in cell size between 1 and 4 days in adult 2 but not in fetal serum, the number of Con. A molecules bound per μ^2 cell surface increased from 1 to 4 days in adult 2 serum but was the same in fetal serum (Table 1). This indicates that the gain of agglutinability at 4 days in adult 2 serum was due to a concentration of exposed sites produced by a decrease in cell size. The gain of agglutinability at 4 days in fetal serum was presumably due to a rearrangement of exposed sites without a decrease in cell size (Table 1).

Table 2. *Cell size, number of Con. A molecules bound, and agglutination by Con. A, at 4 days after subculture of normal and transformed hamster cells, in adult 1, 2, and fetal calf serum*

Type of calf serum	Cells	Surface area per cell (μ^2)	No. of molecules bound		Agglutination by Con. A (500 μg/ml)	Structural changes in membrane sites in transformation
			per cell ($\times 10^6$)	per μ^2 surface ($\times 10^3$)		
Adult 1	H. normal	1,130	9	8	−	Exposure of cryptic sites and concentration of exposed sites
	H. polyoma	810	39	48	++	
	H. DMNA	950	55	58	++++	
Adult 2	H. normal	1,962	65	33	−	Concentration of exposed sites
	H. polyoma	724	41	56	++	
	H. DMNA	639	44	68	++++	
	H. SV 40	651	47	73	+++	
Fetal	H. normal	1,306	38	29	−	Rearrangement of exposed sites
	H. polyoma	1,379	39	28	++	
	H. DMNA	1,285	42	33	++++	
	H. SV 40	1,301	40	31	+++	

The comparison of normal and transformed hamster cells at 4 days after subculture indicates that the structural changes in the membrane sites for Con. A that result in agglutinability in cell transformation can be due to three types of changes depending on the serum used (Table 2). When the cells were grown in adult 1 serum, the surface area of the normal cells was somewhat larger than that of the transformed cells (about 1,100 μ^2 compared to about 900 μ^2), but the number of Con. A molecules bound per cell and per μ^2 were both about sixfold higher in the transformed than in the normal cells. The increase in the number of molecules bound per transformed cell indicates that transformation resulted in an exposure of cryptic sites, and the slight decrease in size can also have produced some concentration of exposed sites (Table 2).

When the cells were grown in adult 2 serum, the surface area of normal cells was about 1,800 μ^2 compared to about 700 μ^2 for transformed cells. The difference in the surface area between normal and transformed cells was thus higher in adult 2 than in adult 1 serum. In adult 2 serum, the number of molecules bound per cell was even lower in transformed than in normal cells, but, owing to the decrease in cell size, the number of molecules bound per μ^2 was about twofold higher in transformed than in normal

cells. In this serum, there was no evidence for exposure of cryptic sites, and the increase in the number of molecules bound per μ^2 was by a concentration of exposed sites due to a decrease in cell size (Table 2).

In fetal serum, the surface area, number of molecules bound per cell, and number of molecules bound per μ^2 were similar in the normal and transformed cells. The agglutinability of the transformed cells compared to the lack of agglutinability of the normal cells in fetal serum, which was not associated with a difference in cell size, was thus presumably due to a rearrangement of exposed sites without a decrease in cell size (Table 2). It was also found that in fetal serum the number of molecules bound to normal cells was similar or even lower after the cells were treated with trypsin than without trypsin treatment. The gain of agglutinability of normal cells after trypsin treatment was thus presumably also due to the same mechanism.

It has previously been shown that infection of normal cells with polyoma virus and infection of 3T3 cells with SV 40 result in a gain of agglutinability by abortively transformed cells (Inbar & Sachs, 1969*a*; Ben-Bassat, Inbar & Sachs, 1970). Experiments were therefore undertaken to relate the agglutinability of abortively transformed cells to cell size, and the number of Con. A molecules bound per cell and per μ^2 cell surface, and to compare abortively transformed cells with 3T3 cells hereditarily transformed by SV 40. The cells were grown in adult 2 calf serum. The results indicate (Table 3) that

Table 3. *Cell size, number of Con. A molecules bound, and agglutination by Con. A of 3T3 cells abortively and hereditarily transformed by SV 40*

Cells	Type of transformation	Days after infection[a] or subculture[b]	Surface area per cell (μ^2)	No. of molecules bound		Agglutination by Con. A (500 µg/ml)	Structural changes in membrane sites in transformation
				per cell ($\times 10^6$)	per μ^2 surface ($\times 10^3$)		
3T3	—	1[a]	1,900	25	13	—	
		4[a]	2,197	26	12	—	
SV 40-infected 3T3	Abortive	1[a]	2,011	24	12	—	Concentration of exposed sites
		4[a]	1,575	29	19	+ + +	
SV 40-transformed 3T3	Hereditary	1[b]	1,109	31	28	—	Concentration of exposed sites and exposure of cryptic sites
		4[b]	744	36	48	+ + + +	

[a] Growing cultures (Ben-Bassat, Inbar & Sachs, 1970) of 3T3 cells were infected with 10 plaque-forming units of SV 40 per cell at 1 day after subculture.

[b] Days after subculture of hereditarily transformed cells.

4 days after infection, when about 50% of the cells had become agglutinable, the infected cells were smaller than the uninfected cells. Although the uninfected and infected cells bound a similar number of Con. A molecules per cell, there was about a 50% increase in the infected cells in the number of molecules bound per μ^2. This indicates that in the abortively transformed cells the gain of agglutinability was associated with a concentration of exposed sites due to a decrease in cell size.

The 3T3 cells hereditarily transformed by SV 40 were about 30% of the size of the untransformed 3T3 cells at 4 days after subculture. The hereditarily transformed cells also showed at this time about 50% increase in number of molecules bound per cell, and 400% increase in the number of molecules bound per μ^2 cell surface (Table 3). Similar results had previously been obtained with cells grown in adult 1 calf serum (Inbar & Sachs, 1969*b*). The gain of agglutinability in the hereditarily transformed 3T3 cells was thus associated with a concentration of exposed sites and an exposure of cryptic sites.

Discussion

The present and previous data (Inbar & Sachs, 1969*b*) on the binding sites for Con. A on the surface membrane of normal and transformed cells can explain the mechanism of the changes in the structure of the surface membrane in cell transformation (Fig. 4). The results indicate that there

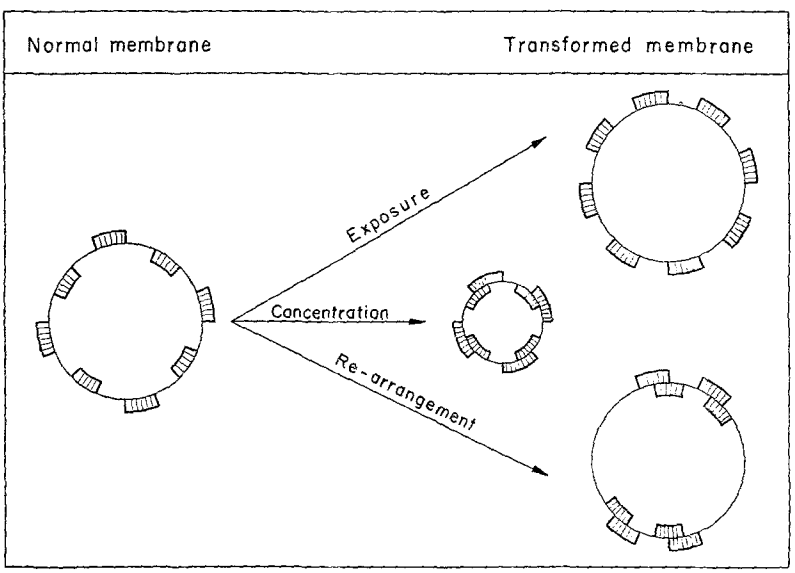

Fig. 4. Model of the three types of changes in the structural organization of surface membrane sites for Con. A in cell transformation

can be three types of changes in the structural organization of sites on the surface membrane in cell transformation that result in agglutinability by Con. A: (1) an exposure of sites that are cryptic in normal cells; (2) a concentration of exposed sites by a decrease in cell size; and (3) a rearrangement of exposed sites without a decrease in cell size. The results have shown that the type of change produced can be influenced by growth conditions, which were changed in the present experiments by using different types of serum in the culture medium.

The evidence for site exposure in cell transformation was obtained from the observation that transformed hamster cells bound a higher number of Con. A molecules per cell than normal hamster cells when grown in adult 1 serum. The binding of a higher number of Con. A molecules per cell was also found in the comparison of 3T3 and hereditarily transformed 3T3 cells grown in adult 1 and adult 2 serum. The existence of cryptic sites in normal cells has been shown (Inbar & Sachs, 1969*b*) by the experiment that when normal hamster cells, grown in adult 1 serum, were treated with trypsin, they became agglutinable, and the number of Con. A molecules bound per cell increased by about 600% without a change in cell size. The number of exposed sites per μ^2 surface on the trypsin-treated normal cells was similar to that found per μ^2 on transformed cells (Inbar & Sachs, 1969*b*).

When normal and transformed hamster cells were grown in another batch of calf serum, i.e., adult 2, the normal and transformed cells bound a similar number of Con. A molecules per cell and still only the transformed cells were agglutinated. Since the size of transformed cells in this serum was about 30% of the normal cells, the number of Con. A molecules bound per μ^2 surface was increased, so that there was a concentration of exposed sites in the transformed cells. Evidence for a concentration of sites was also obtained in abortively transformed cells.

When cells were grown in fetal calf serum, the cell size and the number of sites were about the same in the normal and transformed cells, and only the transformed cells were agglutinated by Con. A. This suggests that there was a rearrangement of exposed sites on the surface membrane in transformed cells, presumably by movement of sites resulting in the formation of clusters. The difference in agglutinability of transformed cells at 1 and 4 days after subculture in fetal serum and of normal cells treated with trypsin, without a difference in cell size or number of molecules bound, also suggests the same explanation. Assuming that the radius of one Con. A molecule is 20 Å, it can be calculated that only about 14% of the cell surface area of transformed cells is occupied by Con. A molecules at saturation. The results, therefore, do not exclude the possibility that other factors on the surface

membrane, in addition to the distribution of binding sites, are involved in the ability of cells to be agglutinated by Con. A. It has also been shown that a specific metabolic activity on the cell surface is required for agglutination by Con. A (Inbar, Ben-Bassat & Sachs, 1971*a*).

It has been shown that the formation of cell variants with a reversion of properties characteristic of transformation is associated with a reversion of the surface structure of transformed cells resulting in a loss of agglutinability by Con. A (Inbar, Rabinowitz & Sachs, 1969; Rabinowitz, Sela & Sachs, 1971). The reverted cells have a different chromosome constitution than the transformed cells (Rabinowitz & Sachs, 1970; Hitotsumachi, Rabinowitz & Sachs, 1971). Revertants with a higher chromosome number are larger than transformed cells, and there was thus presumably also in these revertants a change in the distribution of binding sites for Con. A due to the change in cell size. It has been shown that the difference in the structure of the surface membrane between normal and transformed cells is reflected by a difference in the location of amino acid and carbohydrate transport sites (Inbar, Ben-Bassat & Sachs, 1971*b*). There is also a differential toxic effect of Con. A on normal and transformed cells *in vitro*, and local treatment of transplanted tumors with this protein resulted in an inhibition of tumor development *in vivo* (Shoham, Inbar & Sachs, 1970). It will be of interest to determine if this differential effect on normal and transformed cells, resulting in an inhibition of cell multiplication and cell killing, can occur with the three types of changes in the surface membrane.

The change in the cellular regulatory mechanism that is produced by malignant cell transformation can be ascribed to a change in the cell surface (Sachs, 1965). According to the proposed model (Fig. 4), the structural organization of the surface membrane resulting in an exposure, a concentration, and/or a rearrangement of sites on the cell membrane may produce the change in cellular regulatory mechanism that is associated with cell transformation.

This work was supported by Contract No. 69-2014 with the Special Virus Cancer Program of the National Cancer Institute (U.S. Public Health Service). We are indebted to Miss Nurit Lapidot for skillful technical assistance.

References

Ben-Bassat, H., Inbar, M., Sachs, L. 1970. Requirement for cell replication after SV 40 infection for a structural change of the cell surface membrane. *Virology* **40**:854.

Bray, C. A. 1960. A simple efficient liquid scintillator for counting aqueous solutions in a liquid scintillation counter. *Anal. Biochem.* **1**:279.

Burger, M. M. 1969. A difference in the architecture of the surface membrane of normal and virally transformed cells. *Proc. Nat. Acad. Sci.* **62**:994.

Chanock, R. M., Hayflick, L., Barile, M. F. 1962. Growth on artificial medium of an agent associated with atypical pnuemonia and its identification as a PPLO. *Proc. Nat. Acad. Sci.* **48**:41.

Goldstein, I. J., Hollerman, C. F., Smith, E. E. 1965. Protein-carbohydrate interaction. II. Inhibition studies on the interaction of concanavalin A with polysaccharides. *Biochemistry* **4**:876.

Hitotsumachi, S., Rabinowitz, Z., Sachs, L. 1971. Chromosomal control of reversion in transformed cells. *Nature* **231**:511.

Huberman, E., Salzberg, S., Sachs, L. 1968. The *in vitro* induction of an increase in cell multiplication and cellular life-span by the water soluble carcinogen dimethylnitrosamine. *Proc. Nat. Acad. Sci.* **59**:77.

Inbar, M., Ben-Bassat, H., Sachs, L. 1971*a*. A specific metabolic activity on the surface membrane in malignant cell transformation. *Proc. Nat. Acad. Sci. In press.*

— — — 1971*b*. Location of amino acid and carbohydrate transport sites in the surface membrane of normal and transformed mammalian cells. *J. Membrane Biol.* **6**:195.

— Rabinowitz, Z., Sachs, L. 1969. The formation of variants with a reversion of properties of transformed cells. III. Reversion of the structure of the cell surface membrane. *Int. J. Cancer* **4**:690.

— Sachs, L. 1969*a*. Interaction of the carbohydrate-binding protein concanavalin A with normal and transformed cells. *Proc. Nat. Acad. Sci.* **63**:1418.

— — 1969*b*. Structural difference in sites on the surface membrane of normal and transformed cells. *Nature* **223**:710.

Kalb, A. J., Levitzki, A. 1968. Metal binding sites of concanavalin A and their role in the binding of α-methyl-D-glucopyranoside. *Biochem. J.* **109**:669.

Lis, H., Sela, B., Sachs, L., Sharon, N. 1970. Specific inhibition by N-acetyl-D-galactosamine of the interaction between soybean agglutinin and animal cell surfaces. *Biochim. Biophys. Acta* **211**:582.

Lowry, O. H., Rosenbrough, N. J., Farr, A. L., Randall, R. J. 1951. Protein measurements with the folin phenol reagent. *J. Biol. Chem.* **193**:265.

Rabinowitz, Z., Sachs, L. 1970. Control of the reversion of properties in transformed cells. *Nature* **225**:136.

— Sela, B., Sachs, L. 1971. Changes in the location of carbohydrate containing sites in the surface membrane in relation to growth control in mammalian cells. *In preparation.*

Sachs, L. 1965. A theory on the mechanism of carcinogenesis by small deoxyribonucleic acid tumor viruses. *Nature* **207**:1272.

Sela, B., Lis, H., Sharon, N., Sachs, L. 1970. Different locations of carbohydrate-containing sites in the surface membrane of normal and transformed mammalian cells. *J. Membrane Biol.* **3**:267.

Shoham, J., Inbar, M., Sachs, L. 1970. Differential toxicity on normal and transformed cells *in vitro* and inhibition of tumor development *in vivo* by concanavalin A. *Nature* **227**:1244.

So, L. L., Goldstein, I. J. 1968. Protein—carbohydrate interaction. XX. On the number of combining sites on concanavalin A, the phytohemagglutinin of the Jack bean. *Biochem. Biophys. Acta* **165**:398.

Sumner, J. B., Howell, S. F. 1936. The identification of the hemagglutinin of the Jack bean with concanavalin A. *J. Bacteriol.* **32**:227.

Todaro, G. J., Green, H., Goldberg, B. D. 1964. Transformation of properties of an established cell line by SV 40 and polyoma virus. *Proc. Nat. Acad. Sci.* **51**:66.

Yariv, J., Kalb, A. J., Levitzki, A. 1968. The interaction of concanavalin A with methyl α-D-glucopyranoside. *Biochem. Biophys. Acta* **165**:303.