J. Membrane Biol. 3, 267–279 (1970)

Different Locations of Carbohydrate-Containing Sites in the Surface Membrane of Normal and Transformed Mammalian Cells

BEN-AMI SELA, HALINA LIS, NATHAN SHARON, and LEO SACHS

Departments of Genetics and Biophysics, Weizmann Institute of Science, Rehovoth, Israel

Received 1 July 1970

Summary. A soybean agglutinin was found to agglutinate mouse, rat and human cell lines transformed by viral carcinogens, but not hamster cells transformed by viral or non-viral carcinogens. Normal cells from which the transformed cells were derived were not agglutinated by this agglutinin, but they were rendered agglutinable after short incubation with trypsin or pronase. The transformed hamster cells, on the other hand, became agglutinable only after prolonged treatment with pronase. The agglutination was specifically inhibited by N-acetyl-D-galactosamine, indicating that N-acetyl-D-galactosamine-like saccharides are part of the receptor sites for soybean agglutinin on the surface membrane. Such sites exist in a cryptic form in normal cells; they are exposed in transformed mouse, rat and human cells, but become less accessible in transformed hamster cells. The receptor sites for soybean agglutinin differ from the receptors for two other plant agglutinins (wheat germ agglutinin that interacts with N-acetyl-D-glucosamine-like sites and Concanavalin A that interacts with α-D-glucopyranoside-like sites) which become exposed upon transformation of all lines tested. In normal hamster cells, the receptors for all three agglutinins become exposed after incubation with trypsin, but the exposure of N-acetyl-D-galactosamine-like sites requires the longest enzyme treatment. The results indicate a difference in the location of different carbohydrate-containing sites in the surface membrane. The differences in the exposure of carbohydrate-containing sites in the membrane could not be correlated with the levels of carbohydrate-splitting glycosidases in normal and transformed cells.

The change in cellular regulatory mechanism that is produced by the transformation of normal cells by carcinogenic agents can be ascribed to a change in the cell surface membrane (Sachs, 1965). Agglutinins with specific binding sites should be of value in determining the nature of this structural change. Two agglutinins of plant origin have been reported to agglutinate cells by interacting with specific sites on the surface of transformed cells, but not to agglutinate the untransformed parent cells: these are a glycoprotein from wheat germ by interacting with N-acetyl-D-glucosamine-like sites (Burger & Goldberg, 1967), and Concanavalin A, a protein from Jack bean, by interacting with α-D-glucopyranoside-like sites (Inbar &

Sachs, 1969*a*, *b*). Results obtained with these two agglutinins have indicated that both the N-acetyl-D-glucosamine-like sites (Burger, 1969) and the α-D-glucopyranoside-like sites (Inbar & Sachs, 1969*a*, *b*; Inbar, Rabinowitz & Sachs, 1969; Ben-Bassat, Inbar & Sachs, 1970) exposed on the surface membrane of the transformed cells are in a cryptic form in the untransformed parent cells. Recently it has been found that the agglutination of red blood cells by a soybean agglutinin (SBA) was specifically inhibited by N-acetyl-D-galactosamine or by disaccharides in which this sugar occupies the non-reducing end (Lis, Sela, Sachs & Sharon, 1970). It was therefore of interest to use SBA as a marker for another specific terminal carbohydrate in studies on the structural change of the surface membrane that occurs during transformation. In this paper we report results concerning (1) the agglutination of several transformed cell lines by SBA, and its specific inhibition by N-acetyl-D-galactosamine, (2) the lack of agglutinability of transformed hamster cells by SBA, and (3) the different location of various carbohydrate-containing sites in the surface membrane of normal and transformed cells.

Materials and Methods

Cell Cultures

The cell types used in the present experiments are shown in Table 1. Cells were grown in Eagle's medium (EM) with a fourfold concentration of amino acids and vitamins and 10% calf serum in plastic Petri dishes (Falcon Co.). All cells were passaged twice weekly in 0.25% trypsin solution (Difco, 1:300). There was no detectable mycoplasma contamination as shown by testing the cultures according to Chanock, Hayflick and Barile (1962).

Agglutinins

Purified SBA was isolated from untoasted soybean flour by a procedure previously described (Lis, Sharon & Katchalski, 1966), and was dissolved in Ca^{++}-Mg^{++}-free phosphate-buffered saline (CMB), pH 7.4. Concanavalin A was prepared from Jack bean meal (Sigma Chemical Co.) and purified by two crystallizations (Sumner & Howell, 1936). It was stored in saturated NaCl at room temperature. Purified wheat germ agglutinin was kindly supplied by Dr. M. M. Burger.

Carbohydrates and Enzymes

The disaccharides β-D-GalNAc (1→3)-D-Gal and β-D-GalNAc (1→6)-D-Gal were a gift of Drs. D. Shapiro and A. Acher of this Institute; all other saccharides were products of the highest purity available. Purified Pronase (Lot 2, Seikagaku Fine Biochemicals, Tokyo, Japan) was a gift of Dr. T. Okuyama. Crystallized and lyophilized trypsin and soybean trypsin inhibitor were purchased from Worthington Biochemical Corp.

Assay of Agglutination

Cultured cells, 3 to 4 days after subculturing, were washed with CMB and removed from the Petri dishes with a solution containing 2 g disodium versenate, 8.0 g NaCl,

Table 1. *Origin of cell cultures*

Designation	Description	Origin
Ham normal	Hamster secondary embryo cells	This laboratory
Ham PV1	Polyoma virus (Strain SP_2) transformed hamster embryo cells	This laboratory
Ham PV2	Polyoma virus (strain LP11) transformed hamster embryo cells	This laboratory
Ham SV	SV40 transformed hamster cells	Flow Lab, Rockville, Md.
Ham Adeno 3	Adeno virus 3 transformed hamster cells	Flow Lab, Rockville, Md.
Ham Adeno 12	Adeno virus 12 transformed hamster cells	Flow Lab, Rockville, Md.
Ham Rous	Rous sarcoma virus transformed hamster cells	Flow Lab, Rockville, Md.
Ham DMNA	Hamster embryo cells transformed after treatment with dimethylnitrosamine	This laboratory (Huberman, Salzberg & Sachs, 1968)
Ham BP	Benzo(a)pyrene transformed hamster embryo cells	This laboratory (Berwald & Sachs, 1965)
3T3	Mouse spontaneous cell line	H. Green (Todaro & Green, 1963)
3T3 PV	3T3 polyoma virus transformed cells	H. Green (Todaro, Green & Goldberg, 1964)
3T3 SV	3T3 SV40 transformed cells	H. Green (Todaro, Green & Goldberg, 1964)
3T3 PVSV	3T3 polyoma and SV40 double-transformed cells	H. Green (Todaro, Habel & Green, 1965)
Mouse normal	Mouse SWR secondary embryo cells	This laboratory
Mouse SV	SV40 transformed mouse SWR embryo cells	This laboratory
Rat normal	Rat secondary embryo cells	This laboratory
Rat PV	Polyoma virus transformed rat cells	This laboratory
Human normal	Human tertiary embryo cells	Tel Hashomer Hosp., Israel
Human SV	SV40 transformed human cells	H. Green (Todaro, Green & Swift, 1966)

0.2 g KCl, 1.15 g Na_2HPO_4, and 0.02 g KH_2PO_4 per 1,000 ml distilled water. The cells were washed three times with CMB and suspended at a concentration of 10^6 cells per ml. For the agglutination assay, 0.5 ml of CMB containing varying amounts of the agglutinin was mixed with 0.5 ml of cell suspension in a 35-mm Petri dish at room temperature. The cells were examined with a binocular microscope after different time intervals, up to 30 min, and the degree of agglutination was estimated with a serological scale of (−) to (++++).

Assay of Glycosidase Activity

Enzymes were extracted and assayed as described elsewhere (Bosmann, 1969). Cells were seeded at 10^6 per 100-mm Petri dish, and were harvested after 2 days in the subconfluent phase. The cells were homogenized at 0 °C with 30 volumes of 0.1% Triton X-100 by 12 strikes in a Kontes glass homogenizer. The homogenate was extracted for 16 hr at 4 °C with stirring; the mixture was centrifuged at 30,000 × *g* for 1 hr; and the supernatant was tested for glycosidase activity. The assay mixture contained cell extract (0.1 ml), the p-nitrophenol glycoside of the appropriate sugar (0.25 μmoles in 0.05 ml H_2O), and 0.1 M citric buffer (0.05 ml), pH 4.3. Incubation was carried out for 1 hr at 37 °C; the reaction was terminated by the addition of 1 ml of 0.02 M glycine:NaOH buffer, pH 10.5; and the optical density of the released p-nitrophenol was measured at 420 nm. To assay for extracellular activity of glycosidases, the culture media of the tested cells were collected, lyophilized and made up to the same volume as that used for the homogenization and extraction of the cells. Protein was determined by the method of Lowry, Rosenbrough, Farr and Randall (1951), using crystalline bovine plasma albumin as standard.

Results

Agglutination of Normal and Transformed Cells by SBA

Hamster, human mouse and rat cells were used for the agglutination studies with SBA. It was found that with the exception of transformed hamster cells, SBA at a concentration of 50 to 100 μg/ml agglutinated all transformed cells tested (Table 2). Agglutination usually occured within

Table 2. *Agglutination of transformed cell lines with SBA*

Cell line	Degree of agglutination[a]			
	Concn. of SBA (μg/ml)			
	10	50	100	250
3T3 PV	++	+++	++++	++++
3T3 SV	±	++	+++	++++
3T3 PVSV	++	+++	++++	++++
Mouse SV	++	+++	+++	++++
Rat PV	++	++	+++	++++
Human SV	+++	++++	++++	++++
Ham PV1	−	−	−	−
Ham PV2	−	−	−	−
Ham SV	−	−	−	−
Ham Adeno 3	−	−	−	−
Ham Adeno 12	−	−	−	−
Ham Rous	−	−	−	−
Ham DMNA	−	−	−	−
Ham BP	−	−	−	−

[a] The degree of agglutination was scored after 30-min incubation.

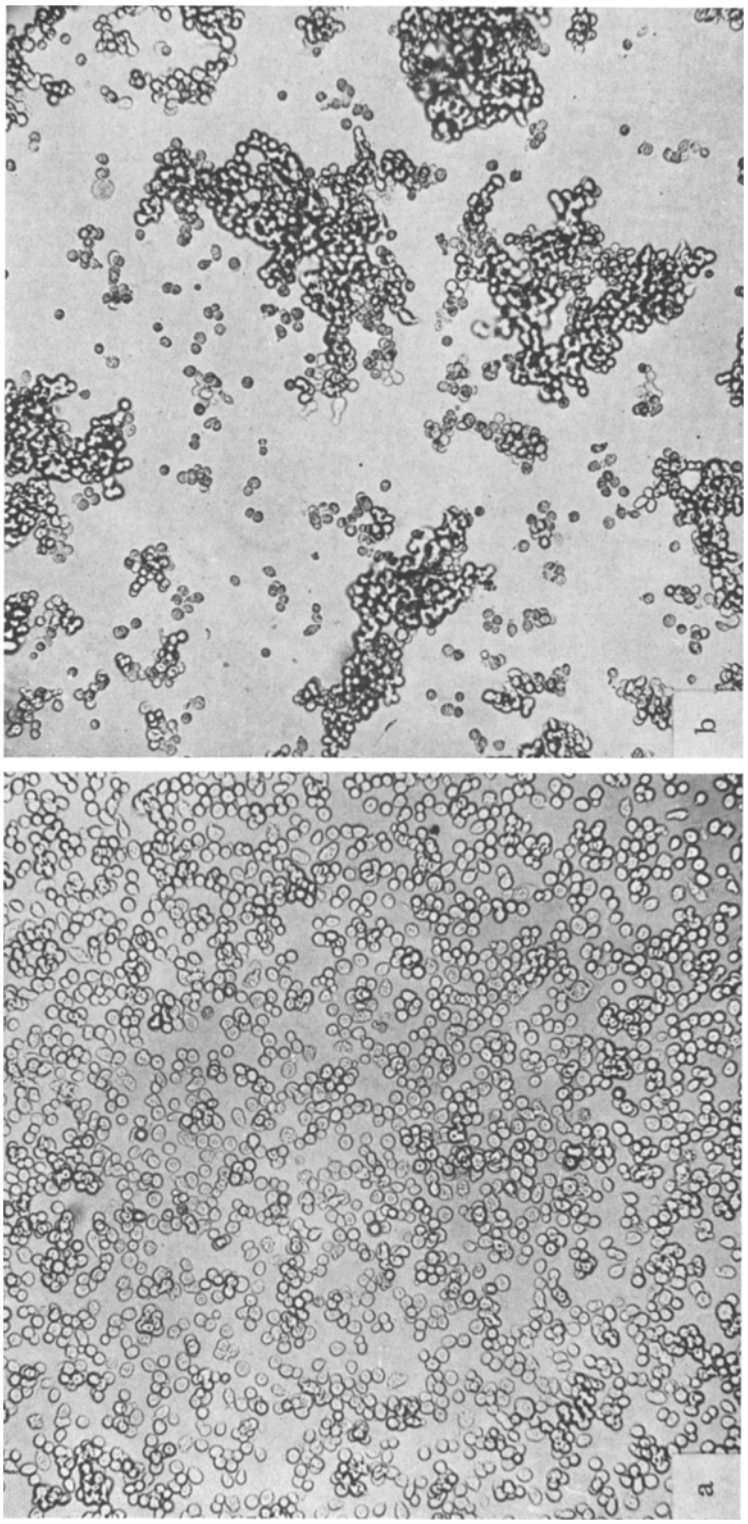

Fig. 1. (a) Normal rat cell and (b) polyoma virus-transformed rat cells after 30-min incubation with 100 μg/ml soybean agglutinin (X 100)

2 to 3 min after the addition of SBA, and the size of aggregates increase with time to give large agglutinates after 30-min incubation (Fig. 1). N agglutination was observed even after 30 min with 500 μg/ml with any c the cultured normal cells tested (Table 1) or with the untransformed 3T cells, or with cells taken directly from hamsters, such as red blood cell and bone marrow cells, prepared as described (Paran & Sachs, 1968) There was no agglutination in control experiments in which cells wer incubated in CMB only.

*Inhibition of Agglutination by N-Acetyl-*D*-galactosamine*

Various sugars, some of which are known to appear as constituents c cell surfaces, were tested for their inhibitory effect on the agglutinatior and for their ability to disperse cell aggregates produced by SBA. In hapte inhibition experiments, the sugar was incubated with the agglutinin i CMB for 5 min at room temperature; the mixture was added to an equa volume of a suspension of agglutinable cells; and agglutination was score after 30 min. The concentration of agglutinin used in these experiment was 100 μg/ml, which was the minimal concentration necessary to give th highest degree of agglutination of most cell lines. It was found (Fig. 2)

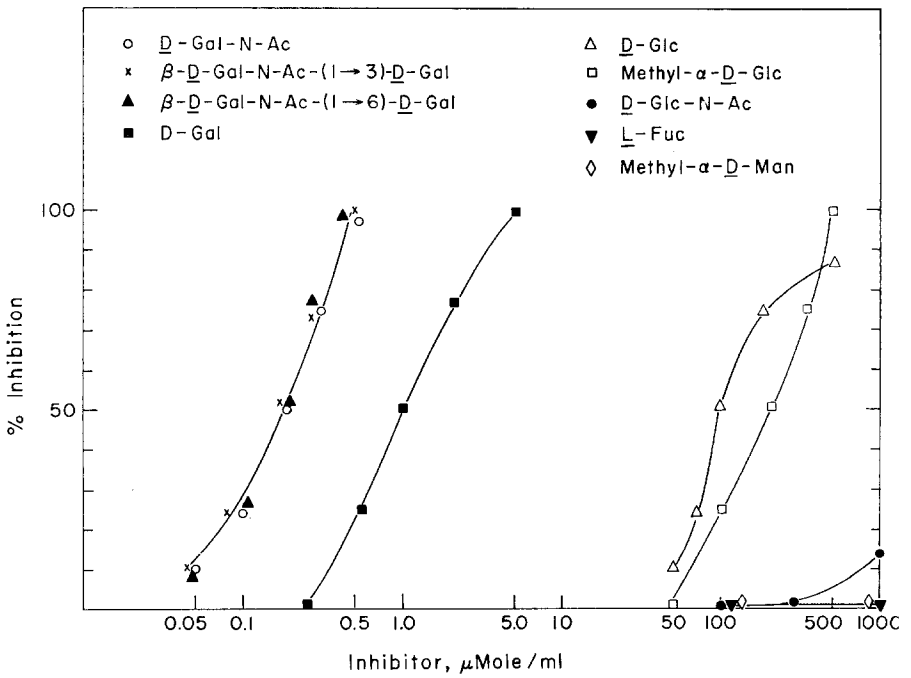

Fig 2. Inhibition by various saccharides of the agglutination of agglutinable transforme cells by soybean agglutinin

that N-acetyl-D-galactosmine and its disaccharides are strong specific inhibitors of agglutination by SBA. At a concentration of 0.15 μmole/ml, these sugars gave 50% inhibition of the agglutination (from ++++ to +-+), whereas 1 μmole/ml of D-galactose was needed to inhibit cell aggregation to the same extent. D-glucose, and its α-methyl glycoside which was found to be a specific inhibitor for the interaction of transformed cells with Concanavalin A (Inbar & Sachs, 1969*a*), gave 50% inhibition at a concentration of 150 μmole/ml, 1,000-fold higher than N-acetyl-D-galactosamine. All other sugars tested, including N-acetyl-D-glucosamine, L-fucose, and methyl-α-D-mannoside, were found to be very poor inhibitors, if at all, even at a concentration of 1 mmole/ml. No significant differences were observed in the concentration of N-acetyl-D-galactosamine needed to inhibit the agglutination of cells from all the agglutinable lines shown in Table 2. Further evidence for the specificity of SBA for N-acetyl-D-galactosamine was obtained in experiments in which the effect of various saccharides on aggregates was tested. The aggregates of transformed cells, formed after incubation with 100 μg/ml of SBA for 30 min, were washed three times with CMB and suspended in CMB containing varying amounts of different saccharides. N-Acetyl-D-galactosamine at a concentration of 0.2 μmole/ml caused complete dispersion of the aggregates within 20 min; D-galactose caused dispersion at the concentration of 4 μmole/ml; N-acetyl-D-glucosamine, L-fucose and D-mannose were without effect even at a concentration of 1 mmole/ml. The dispersed cells were collected by centrifugation and washed three times in CMB. Incubation of these cells with SBA (100 μg/ml) caused agglutination to the same degree as that obtained with fresh cells, and the aggregates could again be dispersed by the addition of N-acetyl-D-galactosamine. These results demonstrated that agglutination by SBA is reversible.

Effect of Trypsin and Pronase on Cell Agglutinability

Normal, untransformed 3T3 and transformed cells were tested for agglutination by SBA after treatment with trypsin and pronase. The cells (20×10^6) in 20 ml of CMB containing 10 μg/ml of the enzymes were incubated for different periods at 37 °C. The cells were washed three times with CMB, and treated with SBA (100 μg/10^6 cells/ml). The results (Table 3) show that, subsequent to incubation with trypsin or pronase for 5 min, all normal cells tested and the untransformed 3T3 cells became agglutinable. Longer incubation with proteolytic enzymes did not significantly affect the degree of agglutination of 3T3, rat and human transformed cells. Transformed hamster cells, on the other hand, became agglutinable only after

Table 3. *Effect of proteolytic enzymes on the agglutination of normal, untransformed* **3T3**, *and transformed cells with SBA*

Cells[a]	Degree of agglutination[b]								
	Time of incubation with pronase (min)					Time of incubation with trypsin (min)			
	0	5	30	60	90	5	30	60	90
3T3	−	++++	++++	+++	+++	++++	++++	+++	+++
3T3 SV	++++	++++	++++	+++	+++	++++	++++	+++	+++
Rat normal	−	++++	++++	++++	+++	++++	++++	++++	+++
Rat PV	++++	++++	++++	+++	+++	++++	++++	+++	+++
Human normal	−	++++	++++	++++	+++	++++	++++	++++	+++
Human SV	+++	++++	++++	++++	+++	+++	++++	++++	+++
Ham normal	−	++++	++++	++++	+++	++++	++++	++++	+++
Ham PV1	−	+	++	++++	++++	−	−	+	+++
Ham PV2	−	−	−	+	+++	−	−	−	−
Ham SV	−	−	−	++	++++	−	−	−	−
Ham DMNA	−	−	−	−	++	−	−	−	−

[a] The cells were treated with 0.001% trypsin or pronase. [b] Agglutination was tested with 100 μg SBA/ml.

Table 4. *Agglutination of trypsinized normal hamster cells by different agglutinins*

Agglutinin[a]	Degree of agglutination					
	Time of incubation with trypsin (min)					
	1	2	3	4	5	6
Wheat germ agglutinin	±	++	++++	++++	++++	++++
Concanavalin A	−	−	++	++++	++++	++++
SBA	−	−	−	±	++	++++

[a] Concanavalin A and SBA were added at 100 μg/ml, and wheat germ agglutinin at 25 μg/ml. The trypsin was used at a concentration of 1 μg/ml. Digestion was stopped by addition of 5 μg/ml soybean trypsin inhibitor.

prolonged incubation with pronase (30 to 90 min depending on the cell line; *see* Table 3). Treatment with trypsin resulted in the agglutinability only of Ham PV1, whereas two other lines did not agglutinate even after 90 min of trypsin incubation. Agglutination by SBA after protease treatment was inhibited by N-acetyl-D-galactosamine.

In previous studies it was found that the transformed hamster cells used are agglutinated by Concanavalin A (Inbar & Sachs, 1969*a*). They are also agglutinated by wheat germ agglutinin. It was therefore of interest to examine if there are any differences in the accessibility of the receptor sites for the three agglutinins in membranes of normal hamster cells. For this purpose, normal hamster cells were treated with trypsin (1 μg/ml) for 1 to 6 min, and the reaction was stopped by the addition of an excess (5 μg/ml) of soybean trypsin inhibitor. Cells were suspended in CMB and then tested in parallel for agglutination with wheat germ agglutinin, Concanavalin A and SBA. The cells were strongly agglutinated by all three agglutinins after 6-min incubation with trypsin, but there was a small difference in the time required for the exposure of the receptor sites for the different agglutinins; namely, agglutination with SBA was preceded by that with wheat germ agglutinin or Concanavalin A (Table 4). We have found similar results with hamster bone marrow cells, where enzyme treatment required to expose the binding sites for Concanavalin A is shorter than the treatment required to expose the SBA binding sites.

Glycosidase Activity in Normal and Transformed Cells

To examine if the absence of agglutination of untrypsinized normal cells by all three agglutinins and of transformed hamster cells could be caused by increased levels of glycosidases, the activity in the cells and the media

Table 5. *Glycosidase activity in extracts of normal, untransformed 3T3, and transformed cells*

Enzyme	Glycosidase activity[a]					
	Cells					
	Ham normal	Ham PV2	Ham DMNA	3T3	3T3 SV	3T3 PVSV
N-acetyl-β-D-glucosaminidase	114±12	92±9	106±18	94±6	310±21	218±32
β-D-galactosidase	109±9	95±16	90±12	58±7	166±21	94±15
N-acetyl-β-D-galactosaminidase	97±12	82±6	94±19	61±2	185±24	141±15

[a] Results are given as nmoles/hr/mg protein. Each assay was performed twice on different cell samples, and results are given as means ± 1 SE.

of eight of these enzymes was measured. In these experiments, normal hamster cells, 3T3 cells and their transformed cells were used. Three enzymes, N-acetyl-β-D-glucosaminidase, N-acetyl-β-D-galactosaminidase, and β-D-galactosidase, have shown the highest activity in extracts of these cells (Table 5). Whereas the level of different enzymes was about the same or lower in transformed than in normal hamster cells, there was an increase of glycosidase activity in transformed 3T3 cells, which ranged from 1.6 to 3.3-fold over the untransformed 3T3 cells. Similar results have also been reported previously (Bosmann, 1969). The other five enzymes tested, α-D-mannosidase, L-fucosidase, α-D-glucosidase, β-D-glucocidace and α-D-galactosidase, showed much lower activity than that measured with the three enzymes listed in Table 5. The activity of these five enzymes in normal or in transformed cells ranged from 3 to 12 nmoles/hr/mg protein. When assayed for extracellular glycosidases, only N-acetyl-β-D-glucosaminidase had measurable activity, which was of the same value in media collected from normal and transformed hamster and 3T3 cells. None of the other seven enzymes could be detected in the extracellular growth medium. The results indicate that the lack of availability of receptor sites could not be accounted for by an increase in the level of glycosidases in cells where the sites were not exposed.

Discussion

In this paper we present data showing that an agglutinin obtained from soybean oil meal agglutinates transformed cell lines from three different species, whereas it does not agglutinate the untransformed cells from which

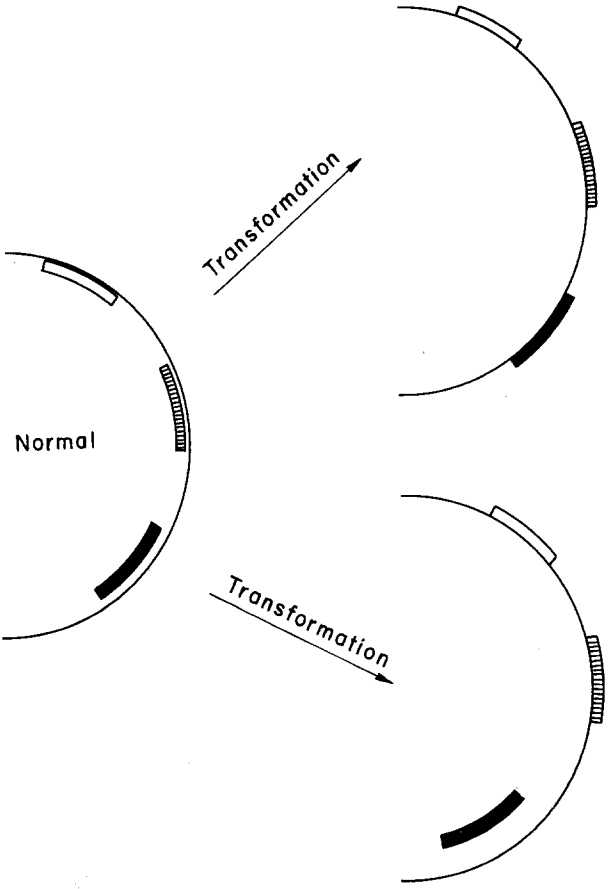

Fig. 3. Model of the location of three carbohydrate-containing sites in normal and transformed cells. ▭ N-acetyl-D-glucosamine-like site; ▥ α-D-glucopyranoside-like site; ■ N-acetyl-D-galactosamine-like site

they were derived. The normal cells become agglutinable upon mild treatment with a proteolytic enzyme. Hapten inhibition studies indicate that the agglutinin binds to N-acetyl-D-galactosamine-like sites on the cell surfaces. It seems therefore that N-acetyl-D-galactosamine-like binding sites, as well as N-acetyl-D-glucosamine-like sites (Burger, 1969), α-D-glucopyranoside-like sites (Inbar & Sachs, 1969*a*, *b*), and "hematoside" sites (Hakomori, Teather & Andrews, 1968), are present in a cryptic form in normal cells and become accessible in some transformed cells.

Our results show that the "outward movement" of saccharide determinants and their appearance on the surface of transformed cells cannot be considered as a general trait of all cell lines. An exception to this was found with transformed hamster cells, where N-acetyl-D-galactosamine-like

residues could not be detected by SBA on the cell surface. These receptor appeared on the surface of the transformed hamster cells only after pro longed pronase treatment. This is in contrast to the behavior of the receptor for Concanavalin A and wheat germ agglutinin, which became exposed in all cell lines tested upon transformation. It appears therefore that some carbohydrate determinants are revealed on the surface of transformed cells whereas others are either located deeper than in normal cells (Fig. 3) or have a "cover" that is more resistant to proteolytic enzymes. It is noteworthy that slight differences were observed in the accessibility of the three carbohydrates in normal hamster cells, where exposure of N-acetyl-D-galactosamine-like receptors required the longest enzyme treatment; this difference, however, becomes much more obvious in the transformed cells. Another point of interest is that within the various transformed hamster lines tested, the degree of inaccessibility of N-acetyl-D-galactosamine-like sites differs markedly, indicating different rearrangements of saccharide profiles in the membranes of the different cell lines. It remains to be determined whether the different carbohydrate receptors are located on the same or different carrier molecules. It is also not yet clear whether the proteolytic treatment, which exposes the receptors, splits a single peptide bond adjacent to the cryptic site, thus rendering the site available for interaction with the agglutinin, or whether a whole proteinous cover has to be removed. The differences in the presence of carbohydrate receptors on cell surfaces of normal and transformed cells could not be ascribed to differences in the level of carbohydrate-splitting enzymes. The cellular and extracellular activity of several glycosidases was tested, and there was no increase in cells where the receptor sites were not exposed.

This study was supported by Contract No. 69-2014 within the Special Virus Cancer Program of the National Cancer Institute, N.I.H., P.H.S., and by Grant FG-Is-247 from the U.S. Department of Agriculture.

References

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

Berwald, Y., Sachs, L. 1965. *In vitro* transformation of normal cells to tumor cells by carcinogenic hydrocarbons. *J. Nat. Cancer. Inst.* **35**:641.

Bosmann, H. B. 1969. Glycoprotein degradation: Glycosidases in fibroblasts transformed by oncogenic viruses. *Expl. Cell Res.* **54**:217.

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.

– Goldberg, A. R. 1967. Identification of a tumor-specific determinant on neoplastic cell surfaces. *Proc. Nat. Acad. Sci.* **57**:359.

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

Hakomori, S., Teather, C., Andrews, H. 1968. Organizational difference of cell surface "hematoside" in normal and virally transformed cells. *Biochem. Biophys. Res. Commun.* **33**:563.

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., 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.

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.

— Sharon, N., Katchalski, E. 1966. Soybean hemagglutinin, a plant glycoprotein. I. Isolation of a glycopeptide. *J. Biol. Chem.* **241**:684.

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

Paran, M., Sachs, L. 1968. The continued requirement for inducer for the development of macrophage and granulocyte colonies. *J. Cell. Physiol.* **72**:247.

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

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

Todaro, G. J., Green, H. 1963. Quantitative studies of the growth of mouse embryo cells in culture and their development into established lines. *J. Cell Biol.* **17**:299.

— — Goldberg, B. D. 1964. Transformation of properties of an established cell line by SV40 and polyoma virus. *Proc. Nat. Acad. Sci.* **51**:66.

— — Swift, M. R. 1966. Susceptibility of human diploid fibroblast strains to transformation by SV40 virus. *Science* **153**:1252.

— Habel, K., Green, H. 1965. Antigenic and cultural properties of cells doubly transformed by polyoma and SV40. *Virology* **27**:179.