SUBSTRATE-ATTACHED GLYCOPROTEINS MEDIATING ADHESION OF NORMAL AND VIRUS-TRANSFORMED MOUSE FIBROBLASTS

LLOYD A. CULP

From the Department of Microbiology, School of Medicine, Case Western Reserve University, Cleveland, Ohio 44106

ABSTRACT

When BALB/c 3T3, simian virus 40 (SV40)-transformed 3T3 (SVT2), and revertant variants of the transformed cells are removed by EGTA treatment from the substrate on which they were grown, they leave behind a layer of glycoprotein which has been characterized biochemically (Terry, A. H. and L. A. Culp. 1974. *Biochemistry.* **13**:414.)—substrate-attached material (SAM). The influence of SAM from normal and from transformed cells on cellular attachment to the substrate, morphology, movement, and growth has been examined. All three cell types displayed a 30% higher plating efficiency when grown on 3T3 SAM. The morphology of SVT2 colonies and of individual SVT2 cells was dramatically affected by growth on 3T3 SAM—the cells (*a*) were more highly spread on the substrate, (*b*) resisted crawling over neighboring cells, and (*c*) resisted movement away from the edge of colonies; SVT2 SAM was not effective in causing these changes. A cell-to-substrate attachment assay using thymidine-radiolabeled cells and untreated or SAM-coated cover slips was developed. SVT2 cells attached to 3T3 SAM- or SVT2 SAM-coated cover slips with a faster initial rate and to a higher saturation level than to untreated substrate, whereas 3T3 and revertant cells exhibited no preference; there was no species specificity in these cell-substrate attachment phenomena. Trypsin-released cells attached much more slowly than EGTA-released cells. 3T3 SAM, however, was not effective in lowering the saturation density of mass cultures of virus-transformed cells. These experiments suggest that the substrate-attached glycoproteins of normal cells affect the cellular adhesivity, morphology, movement, and perhaps growth patterns of virus-transformed cells—i.e., causing partial reversion of these properties of transformed cells to those found in contact-inhibited fibroblasts. A model for the involvement of substrate-attached glycoproteins in cell-to-substrate adhesion, and possibly cell-to-cell adhesion, has been proposed.

When normal or SV40-transformed mouse fibroblasts are removed from the plastic or glass substrate on which they are grown by the Ca^{++}-specific chelating agent EGTA,[1] they leave a layer of glycoprotein firmly bound to the substrate (1, 2). The morphologically flat, contact-inhibited 3T3

[1] *Abbreviations used in this paper:* con A, concanavalin A; EDTA, (ethylenedinitrilo) tetraacetic acid; EGTA, ethylenebis (oxyethylenenitrilo) tetraacetic acid; MEM × 4, Eagle's minimal essential medium supplemented

cells or reverted variants of the SV40-transformed 3T3 cells, selected with con A (3), appear to deposit much more of this material than the pleomorphic and spindle-shaped SV40-transformed cells (2), based on incorporation of radioactive glucosamine. It has been postulated that substrate-attached material (SAM) plays an important role in adhesion of cells to the substrate, and its study may suggest mechanisms for cell-to-cell adhesion (1). This substrate-attached material may be a component of the cellular "microexudates" reported by others (4–8).

Terry and Culp (1) examined the biochemical composition of this material and found the polysaccharide to homogeneously consist of hyaluronic acid chains of very high molecular weight. The protein portion of the material appeared to be relatively small in size, and preliminary evidence suggests that it is covalently linked to the polysaccharide chains. Qualitatively, SAM from normal cells and SAM from virus-transformed cells appeared to be similar. The quantitative correlation of SAM with cell morphology and adhesiveness (9, 2), the tenacity with which it is bound to the substrate (2), and its highly electronegative properties (1) suggested the involvement of SAM (with Ca^{++} cross-linking) in cell-to-substrate adhesion.

In the present study, I have examined the biological significance of SAM from BALB/c 3T3 cells and their SV40-transformed counterparts by measuring several parameters of growth, morphology, and attachment of cells grown on surfaces covered by natural deposition of SAM, and have compared them with the same parameters of cells grown on untreated surfaces.

MATERIALS AND METHODS

Cells

BALB/c 3T3 cells (clone A31), SV40-transformed 3T3 cells (clone SVT2), and the concanavalin A-selected revertant[2] of SVT2 cells (revertant clone 84) have been described (2, 3). The contact-inhibited hamster cell clone Nil-B1 (10) was kindly provided by Dr. Phillips Robbins; a clone of SV40-transformed Nil-B1 cells was isolated in this laboratory by Timothy Mapstone. Swiss 3T3 cl 1 and a clone of its SV40-transformed counterpart (SV-2-3T3 cl 1) were isolated by Adele Schultz in this laboratory. Cells were used between their 8th and 18th passages after cloning and were grown in Eagle's minimal essential medium (MEM × 4) supplemented with four times the concentration of amino acids and vitamins, 10% fetal calf serum, penicillin (250 U/ml), and streptomycin (0.25 mg/ml). Cells were incubated in an environment of 5% CO_2 in humidified air at 37°C; were routinely passaged in Brockway glass 32-oz tissue culture bottles by using a trypsin (0.05% wt/vol Difco trypsin 1:250)-EDTA (0.5 mM) solution; and were screened for and found to be free of *Mycoplasma* with a radiolabelling assay (2).

For the experiments described in this paper, cells were subcultured by treatment at 37°C for 30 min with 0.5 mM EGTA in PBS to minimize surface membrane glycoprotein damage (2). Occasionally for comparative purposes, cells were subcultured with the trypsin-EDTA solution (treatment for 5 min at 37°C); identical phenomena were found with cells subcultured with TPCK-trypsin (0.005% wt/vol; treatment for 5 min at 37°C). Cells were harvested from cultures in which only 60–70% of the substrate surface was covered with cells to minimize the influence of cell-to-cell interaction, and the cells have been growing for 72 h to minimize the effects of prior subculturing. All experiments were performed in Falcon or Lux plastic tissue culture dishes.

Preparation of SAM-Coated Substrates

To compare the effects of substrate-attached glycoproteins on cell growth and behavior, SAM-coated plastic tissue culture dishes or glass cover slips (in plastic tissue culture dishes) were prepared as follows. Cells were inoculated into tissue culture dishes containing MEM × 4; the medium was changed 24 h later. After an additional 48 h of growth during which the cells had completely covered the dish and SAM deposition was maximal (1), the cell layer was removed by EGTA treatment and washed well with PBS. Medium was then added to untreated or SAM-coated dishes, which were incubated in 5% CO_2 for at least 1 h to equilibrate the pH of the medium (13) before experimental cells were inoculated. To test for incomplete removal of cells, SAM-coated dishes were occasionally incubated for 3 wk to test for colony formation; no cell regrowth was ever observed. For preparation of SAM-coated glass cover slips[3], cells were grown in 10-cm plastic tissue culture dishes containing twelve 1.1 × 2.2-cm glass cover slips, with care that the cover slips did not overlap during cell growth.

with four times the concentration of vitamins and amino acids; PBS, phosphate-buffered saline; PBS-II, phosphate-buffered saline supplemented with 100 mg/L of $CaCl_2$ and $MgSO_4 \cdot 7H_2O$; SAM, substrate-attached material or substrate-attached glycoproteins (1); SDS, sodium dodecyl sulfate; SV40, simian virus 40; TCA, trichloroacetic acid; TdR, thymidine; TPCK-trypsin, L-(1-tosylamido-2-phenyl) ethyl chloromethylketone-treated trypsin.

[2] Revertant cells are flat, contact-inhibited variants of transformed cells which have retained the partially expressed SV40 genome (3, 11, 12).

[3] Cover slips were cleaned by rinsing in PBS and 100% ethanol.

Attachment Assay

In order to assay for attachment of cells to a particular substrate, cells were radiolabeled in their DNA as follows. Cells (0.5×10^6 3T3, 1.2×10^6 SVT2, 0.5×10^6 revertant, or 0.7×10^6 Nil-B1 cells) were inoculated into 10-cm tissue culture dishes containing MEM × 4 plus 1 μCi/ml of [^{3}H]TdR[4] (sp act: 20 Ci/mmol). After 24 h of growth, nonradioactive medium was substituted for radioactive medium to chase [^{3}H]TdR into DNA during 48 h of continued growth (the cells were approximately 50% confluent); all the cells appeared to be uniformly labeled as determined autoradiographically by silver grain density over the radio-labelled cells, and all the radioactivity in the cells was TCA precipitable. The cells were then removed from the substrate by EGTA, trypsin-EDTA, or TPCK-trypsin treatment as described above, pelleted by centrifugation at 600 *g* for 10 min, and resuspended in MEM × 4 at a density of $2–3 \times 10^6$ cells/ml. It is very important to resuspend the cells with vigorous pipetting to minimize the number of cell aggregates in the suspension. 1-ml aliquots were inoculated into 100-mm tissue culture dishes (15 ml of MEM × 4) containing untreated cover slips or cover slips which were coated with SAM by prior growth of the particular cell type to confluence and removal by EGTA. Duplicate cover slips were removed at the indicated times, rinsed twice in three different batches of PBS-II, drained on a Kimwipe, and placed in scintillation vials for counting in Bray's fluid. Care must be taken while picking up the cover slips to prevent them from (*a*) overlapping and (*b*) dragging across the surface of the tissue culture dish, thereby accumulating a layer of radioactive cells artifactually, which are not easily rinsed off the edge of the cover slip. Time course experiments of 1 h were performed at 22°C, while longer experiments were performed with dishes maintained at 37°C in a CO_2 incubator. No attachment occurred at 4°C.

Materials

Materials were purchased from the following sources: [5-^{3}H]thymidine (sp act: 20 Ci/mmol) from Amersham/Searle Corp., Arlington Heights, Ill.; 32-oz glass culture bottles from Brockway Glass Co. Inc., Brockway, Pa.; 100-, 60-, and 35-mm plastic tissue culture dishes from Falcon Plastic, Div. BioQuest, Oxnard, Calif. or Lux Scientific Corp.; trypsin 1:250 from Difco Laboratories, Detroit, Mich.; TPCK-trypsin from Worthington Biochemical Corp., Freehold, N. J.; EDTA and EGTA from Eastman Organic Chemicals Div., Eastman Kodak Co., Rochester, N. Y.; fetal calf serum from Flow Laboratories Inc., Rockville, Md.; Wright's stain from Fisher Scientific Co., Pittsburgh, Pa.; 1.1 × 2.2-cm glass cover slips (catalog no. 6663-F10) from Arthur H. Thomas Co., Philadelphia, Pa.

[4] This concentration of TdR was not toxic to the growth of the Nil-B1 or SV40-transformed cell lines, but was somewhat toxic to BALB/c 3T3 cells. The same phenomena reported in this study for 3T3 cells were observed with cells grown in 0.05 μCi/ml of [^{3}H]TdR, a concentration not toxic to cell growth.

RESULTS

Plating Efficiency

To test for long-term survival of cells grown on a surface containing substrate-attached glycoproteins, the ability of cells to form colonies on new dishes was compared with their ability to form colonies on 3T3 SAM-coated dishes. The results in Table I indicate that, in general, 30% more colonies grew on SAM-coated dishes, whether the inoculated cells were subcultured with EGTA or trypsin. This was also true for two very different types of cells—the flat, contact-inhibited 3T3 or revertant cell lines and the pleomorphic, non-growth-inhibited SVT2 cells. The exception was trypsinized SVT2 cells, which plated equivalently on new or SAM-coated surfaces. It was also interesting to note that the revertant cells have a low plating efficiency similar to that of 3T3 cells and not comparable to the higher plating efficiency of the SVT2 cells from which they were derived.

Colony and Cell Morphology

Although the size dimensions of colonies formed on new versus SAM-coated dishes were approximately the same, there were several morphological parameters which were considerably different. In Fig. 1, the colony morphologies of 3T3 cells grown on new or 3T3 SAM-coated substrates were approximately the same. SVT2 colonies grown on 3T3 SAM (Fig. 1-B2) demonstrated much less cell movement at the periphery of the colony and much less piling of the cells in the center of colonies. The presence of SAM appeared to reduce the tendency of transformed cells to (*a*) move off the substrate and over nighboring cells and (*b*) move away from neighboring cells at the periphery of colonies. Continued growth of these colonies did, however, result in extensive cell overgrowth in the centers of these colonies. Although revertant cells are contact-inhibited and do not pile, 3T3 SAM was effective in reducing cell movement at the periphery of these colonies as well (Fig. 1-C2).

These effects are particularly noticeable in the higher magnification photomicrographs of Fig. 2. The morphology of the individual 3T3 cells was unaffected, while SVT2 cells grown on 3T3 SAM possessed a flatter, more fibroblastic morphology. There was considerably less tendency for SVT2 cells to crawl over (or under) each other when

TABLE I

*Plating Efficiency**

Cell type	Treatment method	Number of grown colonies‡		Increased efficiency§ on SAM-covered dishes
		New dishes	3T3 SAM-covered dishes	
				%
3T3	EGTA	70.6 ± 27.1	81.8 ± 21.2	30
	Trypsin	70.9 ± 22.9	96.0 ± 21.7	35
SVT2	EGTA	124.3 ± 12.4	165.6 ± 10.6	32
	Trypsin	125.3 ± 5.6	132.3 ± 11.3	6
Revertant	EGTA	90.0 ± 9.4	116.6 ± 15.4	30
	Trypsin	73.3 ± 3.0	95.0 ± 8.0	30

* Subconfluent cells were removed from cultures with EGTA or Trypsin-EDTA as described in Materials and Methods. 600 cells were inoculated into 60-mm plastic tissue culture dishes, some of which were covered with SAM from 3T3 cells previously grown to confluence and removed with EGTA.

‡ The number of colonies were determined after fixation with methanol and staining with Wright's stain. The error calculations were made from the results of three or four independent experiments, using three or four dishes per experiment.

§ This increased percentage of plating on SAM-covered dishes was determined by: $(Colonies_{SAM} - Colonies_{New})/(Colonies_{New}) \times 100 = \%$.

grown on SAM (Fig. 2-B2) as compared to growth on new dishes (Fig. 2-B1). Approximately 90% of SVT2 colonies grown on 3T3 SAM possessed these unique morphological properties. Revertant cells also appear to be more highly spread when grown on 3T3 SAM (Fig. 2-C2). Similar morphological changes were observed when Swiss SV3T3 cells were grown as colonies on Swiss 3T3 SAM.

The morphological changes observed above were most dramatic when the transformed cells were subcultured with EGTA and less dramatic when they were (*a*) subcultured with trypsin-EDTA or (*b*) plated on SVT2 SAM after EGTA-mediated removal. Although SVT2 SAM was somewhat effective in promoting increased cell spreading and reduced movement at the edge of colonies, 3T3 SAM was much more effective in inducing these changes.

Kinetics of Cell-to-Substrate Attachment

An assay for measuring the rate and saturability of cell attachment to an untreated or SAM-coated substrate was developed (see Materials and Methods) by growing cells in a radioactive precursor of DNA. DNA in cells is highly stable and resistant to ambiguities caused by handling of cells and surface membrane damage. All of the [^{3}H]TdR was incorporated into TCA-precipitable material.

Fig. 3 A indicates that 3T3 cells attach similarly to 3T3 SAM-coated or new glass cover slips. The maximum number of cells had attached within 15–20 min after inoculation. SVT2 cells (Fig. 3 B), on the other hand, attached with a faster initial rate to 3T3 SAM-coated cover slips, and a larger number of cells attached. The flat, contact-inhibited revertant cells (Fig. 3 C) displayed no substrate preference.

The stability of this attachment is displayed in Fig. 4. The plateau level of attached cells was achieved during the first 30 min and dropped only slightly during the first 24 h of culturing these cells. Attachment did not occur at 4°C, indicating a requirement for cell metabolism.

Only a portion of cells in the inoculum attached to a substrate (in our experience approximately one-half) and only a fraction of these remained viable to form colonies (see data in Table I).

The attachment experiments described above were performed with inoculated cells which had been previously removed from their substrate with EGTA. The data in Fig. 5 illustrate the kinetics of attachment of cells removed from the substrate with trypsin-EDTA. Again, there was no preference by 3T3 cells for the type of substrate, although attachment continued throughout the first hour and only became maximal between 1 and 2 h of culturing (unpublished data). SVT2 cells

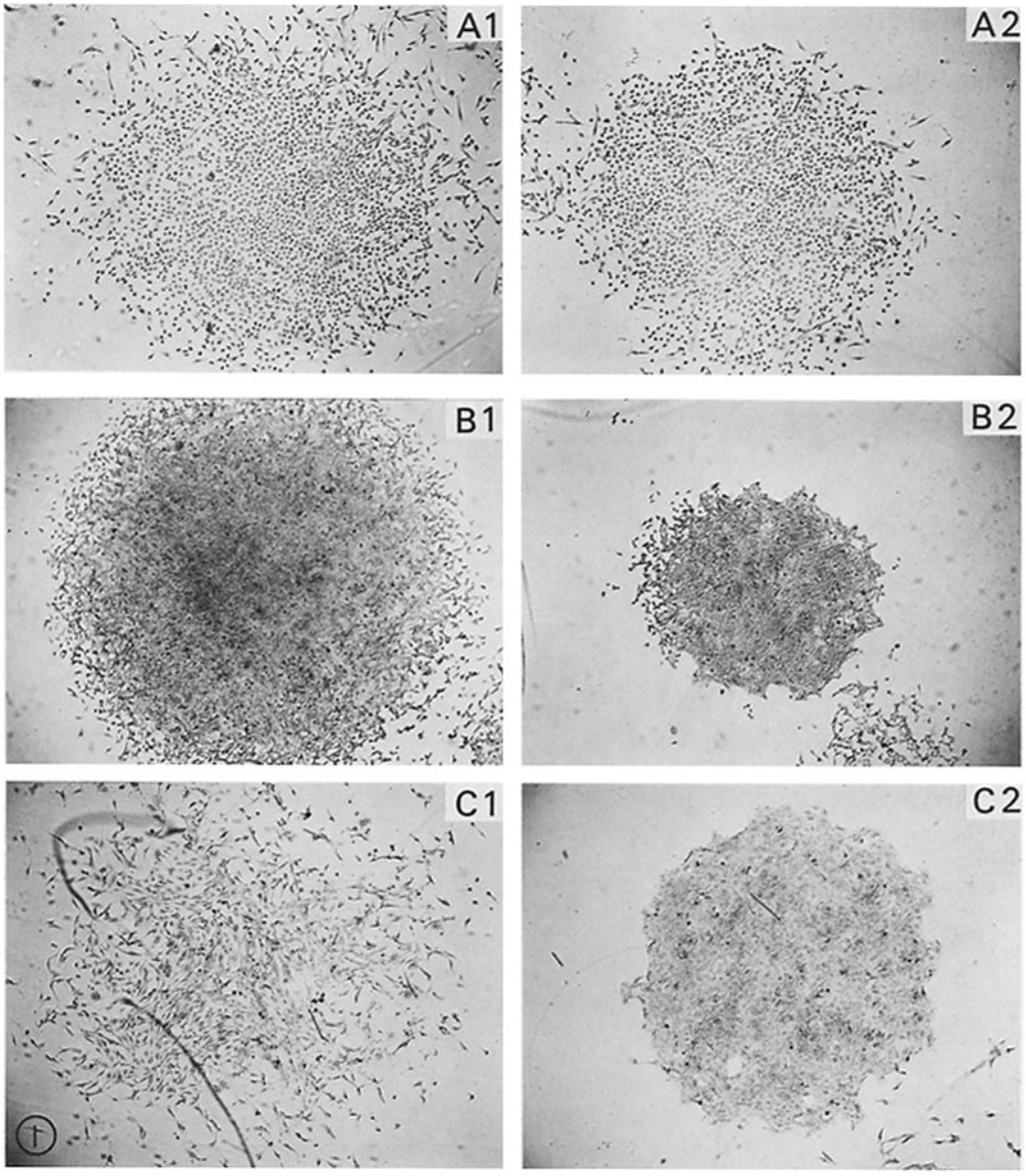

FIGURE 1 Effects of substrate-attached glycoproteins on colony morphology. Cells were plated sparsely in new plastic tissue culture dishes (series 1) and in 3T3 SAM-coated dishes (series 2). Colonies developed during cell growth over a 2-wk period, were then fixed with methanol, and were stained with Wright's stain. All cells were subcultured using EGTA. (A) BALB/c 3T3 cells; (B) BALB/c SVT2 cells; and (C) con A revertant cells. × 45.

(Fig. 5 B) still attached faster and to a higher maximal level to SAM-coated cover slips after trypsin treatment, although attachment did not become maximal until 2 or 3 h after inoculation.

Fig. 6 illustrates the kinetics of attachment of normal and transformed cells to SVT2 SAM. 3T3 cells demonstrated a preference for SAM-coated cover slips (Fig. 6 A), although the SVT2 cells again showed a greater preference (in terms of both initial rate and saturability) for the SAM-coated surface (Fig. 6 B).

Is there species specificity for the nature of

SAM? In Fig. 7, the contact-inhibited hamster cell line Nil-B1 was tested for its attachability to mouse 3T3 SAM. The untransformed Nil cells showed no preference or aversion to 3T3 SAM and behaved like 3T3 cells in this regard. SV40-transformed Nil-B1 cells attached to the SAM-coated surfaces with a faster initial rate and to a higher saturating level. Thus the attachment of homologous or heterologous transformed cells was more efficient when compared to that of their normal

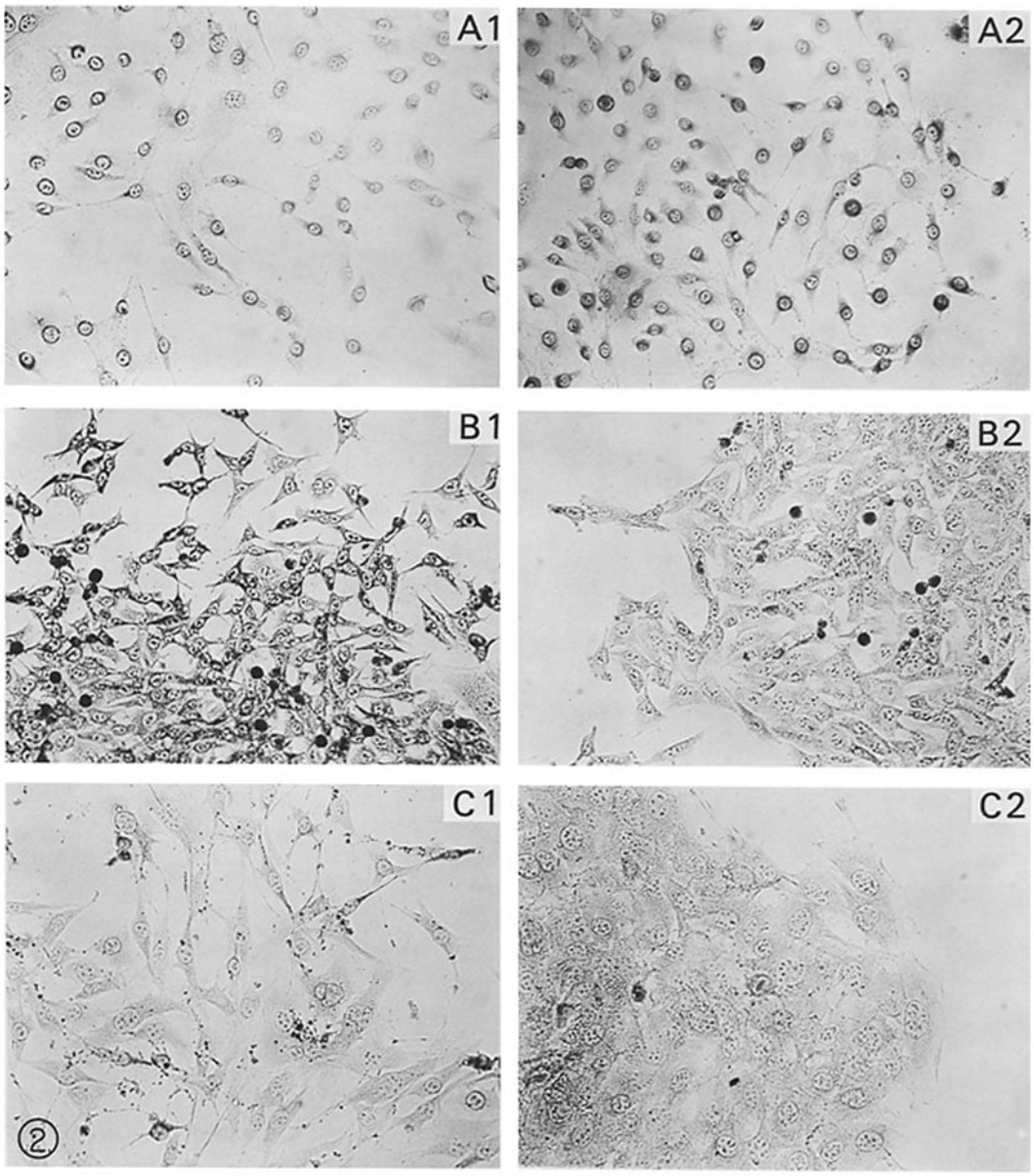

FIGURE 2 Effects of substrate-attached glycoproteins on cell morphology. Cells were treated as described in Fig. 1 by growth in new plastic tissue culture dishes (series 1) and in 3T3 SAM-coated dishes (series 2). All cells were subcultured using EGTA. (A) BALB/c 3T3 cells; (B) BALB/c SVT2 cells; and (C) con A revertant cells. × 200.

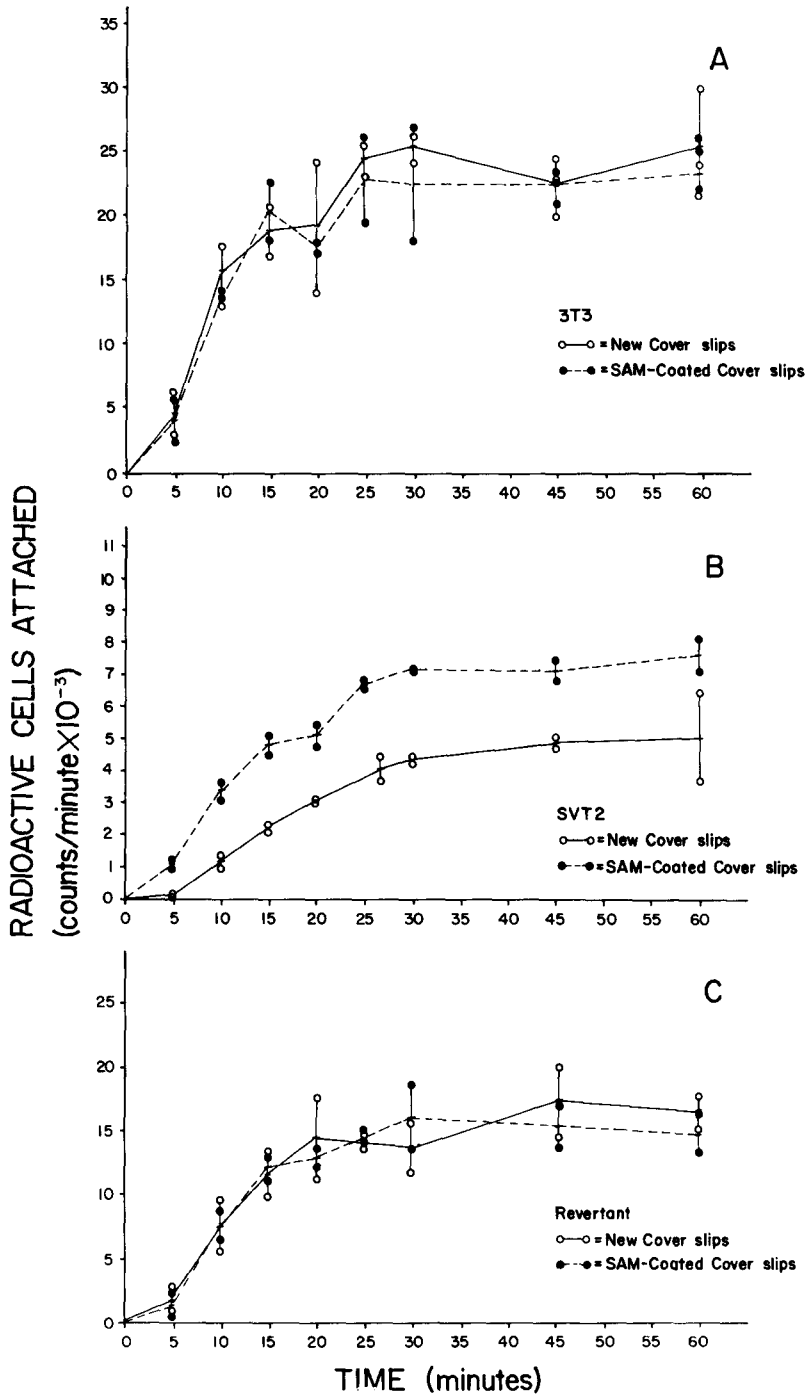

FIGURE 3 Short-term effects of 3T3 substrate-attached glycoproteins on cell-to-substrate attachment. The methodology of measuring attachment of DNA-radiolabeled cells to new glass cover slips (O—O) and to 3T3 SAM-coated cover slips (●- - -●) at 22°C has been described in Materials and Methods. Cells were removed before attachment by EGTA treatment.

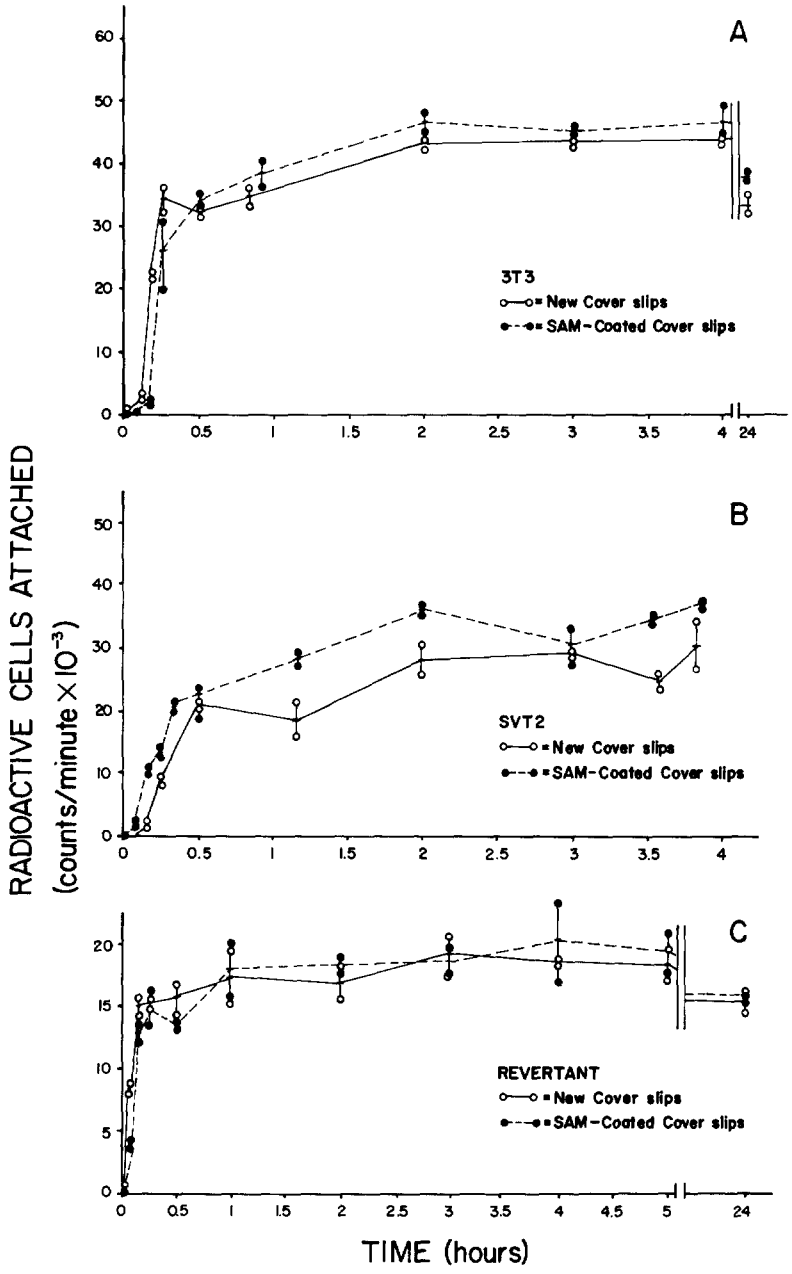

FIGURE 4 Long term effects of 3T3 substrate-attached glycoproteins on cell-to-substrate attachment. The assay has been described in Materials and Methods and the legend to Fig. 3. Attachment to new cover slips (O—O) at 37°C and attachment to 3T3 SAM-coated cover slips (●- - -●) at 37°C was measured. Cells were removed before attachment by EGTA treatment.

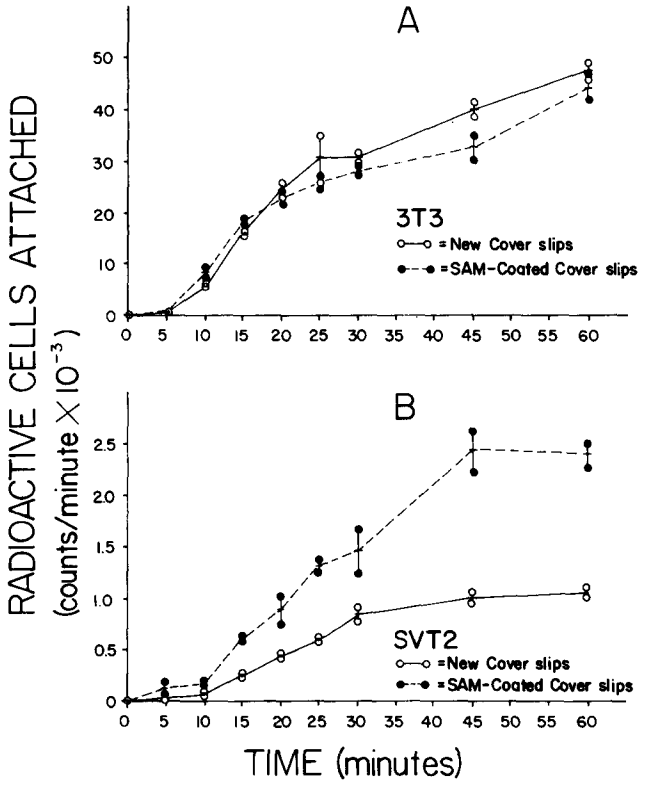

FIGURE 5 Attachment after trypsinization. The assay has been described in Materials and Methods and the legend to Fig. 3. Cells were removed before attachment by trypsin-EDTA treatment. Attachment to new cover slips (O—O) at 22°C and to 3T3 SAM-coated cover slips (●- - -●) at 22°C was measured.

cell counterpart or to that of reverted variants of the transformed cells.

Effects on Cell Growth

The morphological and social behavior changes observed with SVT2 cells plated sparsely on 3T3 SAM-coated dishes (see Figs. 1 and 2) suggested that SAM may be able to induce contact inhibition of growth with SVT2 cells, similar to the lowered saturation density of transformed cells treated with trypsinized Con A (14). SV40-transformed 3T3 cells (BALB/c or Swiss) were inoculated into new and 3T3 SAM-coated tissue culture dishes, and the cell number was followed as a function of time. Fig. 8 indicates that the presence of 3T3 SAM did not lower the saturation density of either BALB/c SVT2 (Fig. 8 A) or Swiss SV3T3 (Fig. 8 B) cells. This indicated that the behavior of transformed cells growing in a small number of colonies on SAM was not reflected by phenotypic induction of contact inhibition of growth in mass cultures of transformed cells growing on 3T3 SAM.

DISCUSSION

These experiments suggest that substrate-attached glycoproteins may play an important role in determining the fibroblastic properties of morphology, attachment to the substrate, movement, and perhaps growth as summarized in Table II. These properties no doubt depend upon the nature of cell-to-substrate adhesion (9, 7, 8, 15).

SAM from 3T3 cells affects (*a*) the spreadability of transformed cells on the substrate, (*b*) movement of cells on the substrate, and (*c*) reassociation kinetics of cell attachment to the substrate (Table II). These data are consistent with the data found previously, namely, (*a*) 3T3 cells and reverted variants (3, 11) of SV40-transformed cells deposit more of this material on the substrate, as determined by accumulation of glucosamine-radiola-

beled material, than their transformed counterpart (2), and (*b*) SAM from normal cells and SAM from transformed cells appear to be qualitatively very similar (1). SAM does not reduce the saturation density of mass cultures of transformed cells, suggesting that either a large number of cells modifies SAM such that it is no longer effective in altering cell movement, spreadability, and overlap (or underlap) as observed in colony growth experiments, or perhaps SAM plays a role in cell-to-substrate adhesion without affecting cell-to-cell adhesion.

3T3 cell morphology and attachment were not affected by the presence of 3T3 SAM, although the plating efficiency was higher. The spreadibility of 3T3 cells on the substrate may be maximal because of limiting intracellular components such as microtubules, a limiting number of sites on the cell surface available for anchorage to SAM on the substrate, or modification of SAM such that it is no longer biologically effective. It is not apparent why 3T3 cells attach more efficiently to SVT2 SAM, even though SAM from normal cells and SAM from transformed cells appear to be highly similar qualitatively (1). Perhaps serum components bind to different types of SAM and this affects the cellular attachment processes; serum components have recently been identified on the surface of the substrate (5). Nothing is known about the topographical distribution of these materials on the substrate and whether this distribution varies between normal and transformed cells. The SAM of transformed cells possesses a higher proportion of the higher molecular weight class of materials (1), and perhaps 3T3 cells interact more favorably with this class of material. Much more biochemical evidence on the nature of SAM may be required before these questions can be answered.

The increased attachability of transformed cells to 3T3 SAM may reflect their slower processes of synthesizing and secreting SAM as compared to

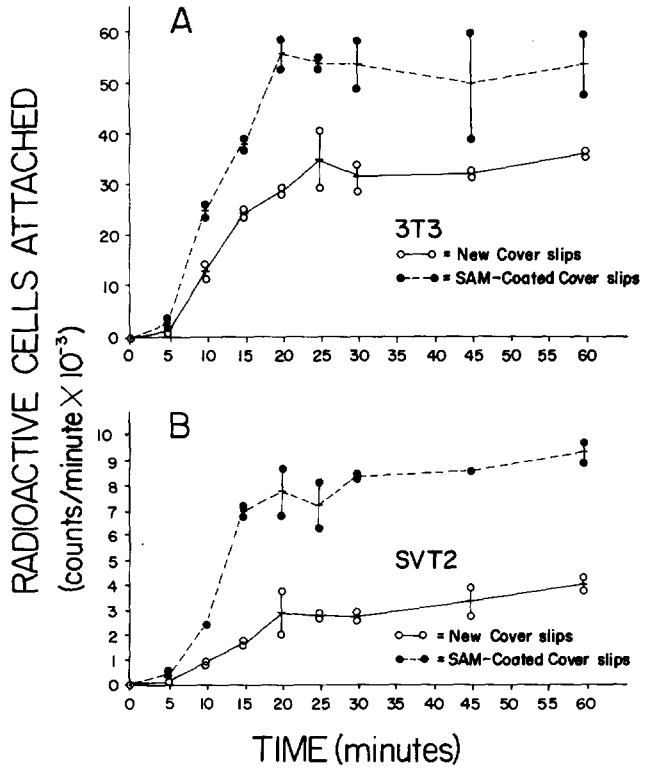

FIGURE 6 Attachment to SVT2 SAM. The assay has been described in Materials and Methods and the legend to Fig. 3. Cells were removed before attachment by EGTA treatment. Attachment to new cover slips (○—○) at 22°C and to SVT2 SAM-coated cover slips (●- - -●) at 22°C was measured.

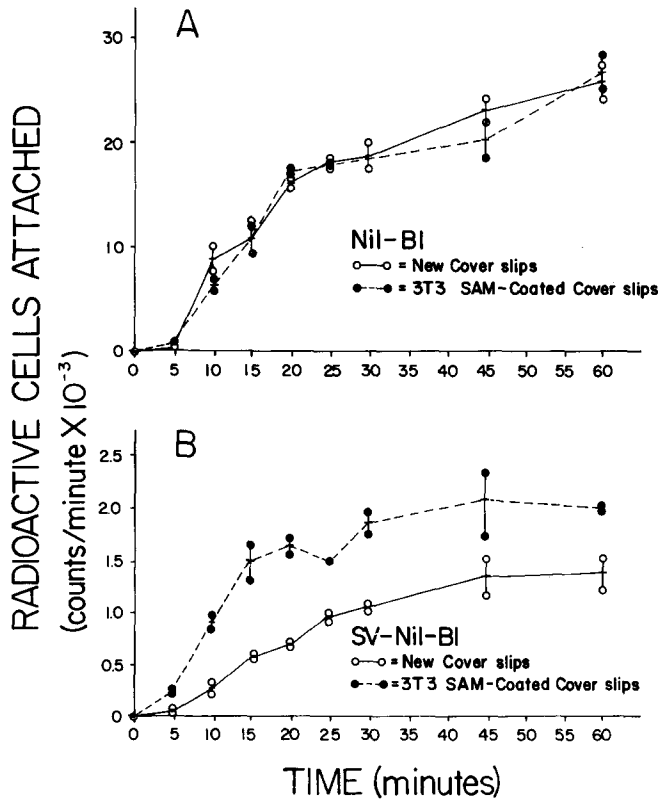

FIGURE 7 Attachment of normal and SV40-transformed Nil-B1 cells to 3T3 SAM. The assay has been described in Materials and Methods and the legend to Fig. 3. Cells were removed before attachment by EGTA treatment. Attachment to new cover slips (O—O) at 22°C or to 3T3 SAM-coated cover slips (●- - -●) at 22°C was measured.

normal or revertant cells after subculturing treatments. This ability may then be initially compensated for by a SAM coating on the substrate to which cells can readily adhere. The much slower kinetics of reattachment of trypsinized cells as compared to EGTA-treated cells presumably reflects the more extensive surface damage caused by trypsin and longer periods of incubation in medium in order to repair this damage.

Fig. 9 is a pictorial model for the possible involvement of substrate-attached glycoproteins in cell-to-substrate adhesion, and perhaps cell-to-cell adhesion. Ca^{++} ions may be the cross-linking mechanism between negatively charged SAM (1), substances which are deposited on the substrate and firmly anchored by some unknown mechanism, and the negatively charged glycocalyx of the cell surface. This model is consistent with (*a*) the efficient release of cells with the Ca^{++}-specific chelating agent EGTA, (*b*) the minimal solubilization of glycoproteins from the cell or substrate during EGTA-mediated removal (2), and (*c*) the positive effects of SAM on cell attachment and morphology and the negative effect on cell movement observed in these studies. It may also be speculated that SAM may be deposited at the cell-cell interface during contact and that normal cells may deposit much more of this "gluelike" material at cellular interfaces, allowing these cells to "cement" themselves together. Considerable efforts are being made to identify SAM-like components at the cell-cell interface.

These substrate-attached glycoproteins are no doubt a portion of the cellular "microexudates" reported by others (4–8) in a wide variety of cell systems. It will be interesting to determine if many different cell types produce the same type of substrate-attached glycoproteins. Hamster Nil-B1

cells used in our studies attached normally in the presence of mouse SAM, whereas SV40-transformed Nil-B1 cells attached preferentially to mouse SAM. There does not appear, therefore, to be species specificity in terms of cell-SAM interaction, and this is consistent with the lack of species specificity for contact inhibition of growth observed by Eagle et al. (16). Perhaps most or all cell types produce common types of intercellular and cell-substrate matrix materials which mediate several social phenomena such as cell morphology, movement, and density-dependent inhibition of growth.

Walther et al. (17) have recently reported an assay like the one described in our studies for measuring cell-to-cell adhesion, using high concentrations of trypsin for subculturing the assay cells. They reported no difference in the attachment kinetics of BHK or polyoma-transformed BHK

TABLE II

*Effects of Substrate-Attached Glycoproteins on Cell Behavior**

Behavior change	Cell Type		
	3T3	SVT2	Revertant
(1) Increased plating efficiency	+	+	+
(2) Decreased motility from colony edge	–	+	+
(3) Increased cell spreading	–	+	–
(4) Decreased overlapping of transformed cells	–	+	–
(5) Prevents growth in interior of colonies	+‡	+	+‡
(6) Decreased saturation density	N.D.§	–	N.D.§
(7) Increased rate and saturation level of cell attachment to substrate	–	+	–

* These are changes affected by cells inoculated onto 3T3 SAM.
‡ These cells are normally contact inhibited.
§ Not done.

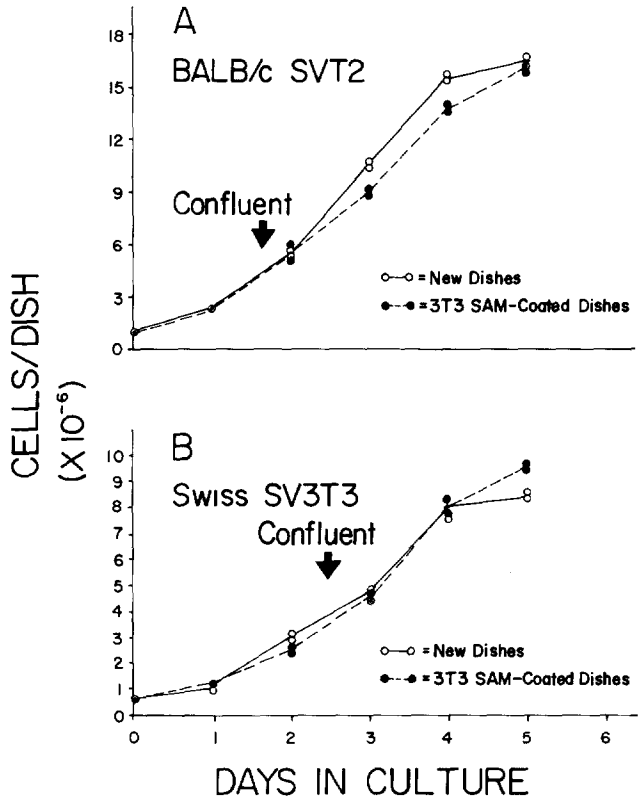

FIGURE 8 Effect on growth of transformed cells by SAM from BALB/c or Swiss 3T3 cells. BALB/c SVT2 cells (A) or Swiss SV3T3 cells (B) were grown on new 60-mm plastic tissue culture dishes or dishes covered with SAM by prior growth to confluence of BALB/c 3T3 cells (A) or Swiss 3T3 cells (B). The saturation density for the normal cell counterparts of these transformed cells was approximately 1.5–2.0 × 10^6 cells/dish for either BALB/c or Swiss 3T3 cells.

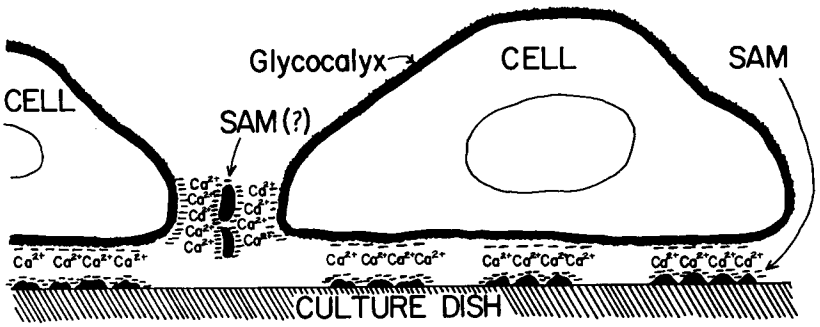

FIGURE 9 Model for SAM involvement in cell-to-substrate and possibly cell-to-cell adhesion.

cells to homologous or heterologous monolayers of cells.

Henkart et al. (18) and Cauldwell et al. (19) have recently reported the purification of sponge cell aggregation factors which are highly species specific. These molecules are very large ($\sim 10^7$ mol wt), are highly negatively charged because of a high proportion of glutamic acid, aspartic acid, and uronic acid, and have a relatively high carbohydrate/protein ratio. Substrate-attached glycoproteins from mouse fibroblasts (1) share these same properties, and perhaps these properties will be common for "supramolecules" which are involved in intercellular adhesive processes.

Much more information on the biochemistry and metabolic properties of substrate-attached glycoproteins will be required before these materials can be definitively assigned a role in cell-to-substrate or cell-to-cell adhesion. Differences in these properties between normal and virus-transformed cells may be critical in determining the phenotypes of these cells.

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