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# Intercellular Contact and Cell-Surface Galactosyl Transferase Activity

**(cell culture/mouse/radioautography/contact inhibition/*cis*- and *trans*-galactosylation)**

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**ABSTRACT** **Evidence is presented suggesting the presence of galactosyl transferases and galactosyl acceptors on the outer surfaces of intact Balb/c 3T3 cells. In addition, the data indicate that these transferases may only be capable of transferring galactose from uridine diphosphate galactose to galactosyl acceptors on adjacent cells after intercellular contact is made (*trans*-glycosylation). Intact Balb/c 3T12 cells, by contrast, show no requirement for intercellular contact in order to carry out this reaction suggesting that these cells, which do not exhibit contact inhibition of growth, may be able to transfer galactose to acceptors situated on the same cell as the enzyme (*cis*-glycosylation).**

**Electrophoretic and radioautographic assays were used to detect surface transferase activities in these two cell lines. Results of experiments on cells from sparse and dense cultures, and under conditions where intercellular contact was regulated, are consistent with the above hypothesis.**

Communication between adjacent cells occurs in embryonic, regenerating, and pathological tissue, but understanding these interactions on a molecular level remains one of the central problems of developmental biology. Attempts to isolate specific "informational" molecules whose passage from cell to cell might account for such phenomena as embryonic induction and growth control between normal cells have yielded equivocal results (1, 2). An alternative model for intercellular communication is suggested by the data reported in this paper.

Our results indicate that the established cell lines Balb/c 3T3 and Balb/c 3T12 from mouse embryos possess galactosyl transferases on their cell surfaces, as do at least two other cell types (3, 4). In addition, evidence is presented that suggests that the highly contact-inhibited 3T3 cells catalyze the transfer of galactose from uridine diphosphate galactose to acceptors on adjacent cells ("*trans*"-glycosylation), whereas the highly malignant and relatively non-contact-inhibited 3T12 cells can catalyze this transfer to acceptors located on the same cell ("*cis*"-glycosylation).

The glycosyl transferases transfer single glycose units from glycose donors (nucleotide sugars) to glycose acceptors (nonreducing termini of oligosaccharides). Each enzyme shows a high degree of specificity for a particular donor, as well as for a particular acceptor. The transferases catalyze the following general reaction (5). Nucleotide-X + Y-Z → nucleotide + X-Y-Z, where X, Y, and Z are single glycose units. Except for the sialyltransferases, manganese is a required cofactor for all of these enzymes (5).

Recent work has shown that glycosyl transferases may be present on the outer surface of plasma membranes of cells of the chick-embryo neural retina (3) and human blood platelets (4). In the case of the neural retina (3), evidence has been presented suggesting that the interaction of these surface enzymes with their appropriate substrates on adjacent cells is at least partially responsible for the adhesive recognition demonstrated by these cells. In the case of the blood platelets (4), it has been suggested that these surface enzymes might be responsible for platelet adhesion to collagen oligosaccharides. Since the platelets themselves have no glycose acceptor activity, a purely synthetic function for these enzymes is unlikely.

## MATERIALS AND METHODS

*Cells.* Balb/c 3T3 (3T3) and Balb/c 3T12 (3T12) cells used in these experiments were grown from inocula kindly supplied by Drs. Todaro and Aaronson. They were cultured (6) in Dulbecco's modified Eagle's medium with 10% calf serum. Cells were harvested with 0.1% trypsin (Difco, 1:250).

*Transferase Assays.* Unless stated otherwise, incubation mixtures for detection of galactosyl transferase activity were assayed in 3-ml conical centrifuge tubes containing 100 $\mu$l of intact cells suspended in medium "J" (glucose-, phosphate-, and bicarbonate-free Hanks' basic salts solution with 10 mM $MnCl_2$ and 10 mM $NaN_3$, all buffered to pH 7.2 with 0.01 M Hepes [Calbiochem, Inc.] buffer). Uridine diphosphate[$^{14}$C]galactose (UDP-Gal) was used at 29 $\mu$M and 35 × $10^6$ cpm/$\mu$mol. Incorporation was determined (3) by high-voltage electrophoresis in borate buffer of an aliquot of the incubation mixture. When known galactosyl acceptors were added, the incubations were centrifuged (500 × *g*) for 6 min, and only the supernatants were examined.

*Identification of Incorporated Label.* After 3 hr of incubation of whole 3T3 cells with UDP-Gal, the mixture was sonicated and dialyzed exhaustively against phosphate-buffered saline (pH 7.4) and then water. Fractions of the retentate were either acid-hydrolyzed (3.6 N $H_2SO_4$ at 100°C for 4 hr) or subjected to a Folch extraction procedure ($CHCl_3$–MeOH, ref. 7) to determine the amount of label in neutral lipids. The acid hydrolyzate was desalted with ion-exchange resins and the free sugars were compared with standards by electrophoresis in borate buffer and paper chromatography (butanol–pyridine–water 6:4:3). The $^{14}$C extracted by chloroform–methanol 2:1 was hydrolyzed in 0.5 N KOH in 50% methanol at 37°C for 24 hr. The hydrolyzate was fractionated by column chromatography on silicic acid and analyzed by thin-layer chromatography (TLC) with authentic glycolipid standards.

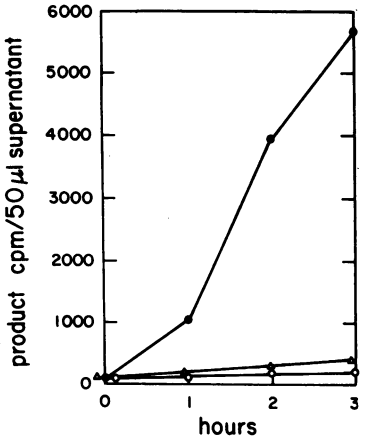


FIG. 1. Increase in galactosylated acceptor formation in reaction-mixture supernatants, as a function of the addition of known galactosyl acceptors. Each reaction mixture contained $1.6 \times 10^7$ 3T3 cells/ml. *Solid circles*, 2.5 mM *N*-acetylglucosamine; *open circles*, 1.7 mg/ml of sialidase-treated ovine submaxillary mucin; *open triangles*, no additions. Reactions were stopped by the addition of 10 μl of 0.3 M EDTA per 100-μl reaction mixture.

*Preincubation of Cells.* Where indicated, cells trypsinized from petri dishes were washed once in culture medium and incubated in 10 ml of this medium in 25-ml DeLong flasks on a gyratory shaker (80 rpm) at 37°C for 30 min. Cell concentrations during this incubation did not exceed $10^6$ cells/ml. Cells were then washed twice in medium "J", diluted to appropriate concentrations, and added to the incubation tubes containing the isotope.

*Radioautography.* Incubations were as described above, except that uridine diphosphate-[$^3$H]galactose (2.6 μM; 1.54 Ci/mmol) was used. After the reaction, the incubation tubes were centrifuged at $500 \times g$ for 2 min and the pellets were fixed with Bouin's fluid. The pellets were then embedded in paraffin and sectioned at 5 μm. The sections were dipped in Kodak NTB-2 Nuclear Track Emulsion, dried, and exposed for 3 weeks at 4°C. For radioautographic analysis of the reaction among cells in monolayer culture, the cells were grown in 35-mm Falcon culture dishes. At the desired cell density, the dishes were washed with Hanks' basic salt solution and 500 μl of medium "J" with [$^3$H]UDP-Gal was added to each dish. The dishes were incubated at 37°C for 3 hr and then washed with Hanks' basic salt solution and fixed with Bouin's fluid. After repeated washes with distilled water, these dishes were treated as were the slides from sectioned pellets.

## RESULTS

### Reaction properties with intact 3T3 cells

With preincubated 3T3 cells from sparse cultures, the transfer of galactose to intact cells: (*a*) increased linearly with time of incubation for 5.5 hr; (*b*) increased linearly with cell concentrations between $1.8 \times 10^6$ cells/ml and $3.3 \times 10^7$ cells/ml; (*c*) required $Mn^{++}$ for detectable activity, with 10 mM $Mn^{++}$ being optimum; and (*d*) increased with increasing UDP-Gal concentration, although no plateau was reached up to 53 μM UDP-Gal. These results are similar to those obtained with embryonic-chick retinal cells (3).

### Competition

In order to detect entry of UDP-Gal into preincubated 3T3 cells from sparse cultures, and possible intracellular utilization of the glycose donor, assays were done in the presence of a 4-fold molar excess of unlabeled UDP-Glu. No decrease in incorporation was detected. Therefore, labeled UDP-Gal is either not entering the cells or it is not equilibrating with unlabeled UDP-Glu. Unlabeled galactose and galactose-1-$PO_4$ (100 μM) similarly had no effect on galactose incorporation.

### Galactose transfer to added acceptors

Fig. 1 shows the transfer of galactose from UDP-Gal to two known galactosyl acceptors. For these assays, only the incubation supernatants were tested. Preincubated 3T3 cells from sparse cultures were incubated with *N*-acetylglucosamine or desialized ovine submaxillary mucin, and their supernatants were compared with a control with no additions. With intact cells, only *N*-acetylglucosamine served as a detectable acceptor. Identical aliquots of these cells were sonicated and the sonicates were tested against the same two acceptors under the same conditions, but in the presence of 0.1% Triton X-100. Addition of *N*-acetylglucosamine gave 23,000 cpm above the endogenous value (2200 cpm), while addition of the desialized mucin acceptor gave 1400 cpm above the endogeneous value. With the same intact cell suspension, large increases in supernatant radioactivity were obtained with desialized, degalactosized orosomucoid. This is a galactose acceptor of high molecular weight with a terminal, β-linked, *N*-acetylglucosamine residue. Fig. 2 shows that the transfer activity for *N*-acetylglucosamine resides with the cells and is not in the supernatant. In this experiment, one cell suspension with UDP-Gal and *N*-acetylglucosamine was incubated in medium "J". At the indicated times, aliquots were taken, and centrifuged, and only the supernatants were spotted for electrophoresis as in Fig. 1. An identical suspension, but without *N*-acetylglucosamine and UDP-Gal, was incubated for 2 hr; the suspension was then centrifuged and only the supernatant was incubated with UDP-Gal and *N*-acetyl-

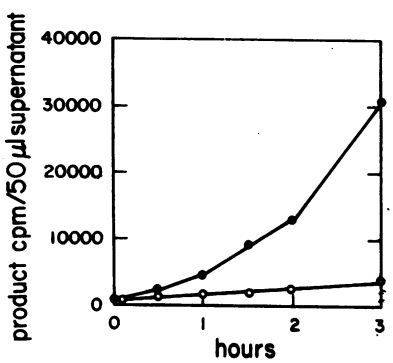


FIG. 2. Different transferase activities toward 2.5 mM *N*-acetylglucosamine in intact 3T3 cells and in a supernatant from a 3T3 cell suspension after incubation without isotope or acceptor for 2 hr. Each incubation tube contained $10^7$ cells/ml, 32 μM UDP-Gal ($70 \times 10^6$ cpm/μmol) and 2.5 mM *N*-acetylglucosamine. *Solid circles*, reactions in the presence of whole cells, as in Fig. 1. At the times indicated, reaction supernatants were spotted for electrophoresis; *open circles*, reactions with the supernatants of 2-hr mock incubations of an identical aliquot of the cells used for whole-cell study.

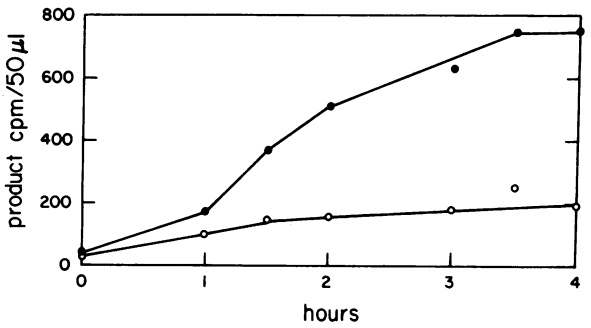


FIG. 3. Incorporation of galactose onto intact 3T3 cells from sparse and confluent cultures. Each reaction tube contained $5 \times 10^6$ cells/ml. *Solid circles*, incorporation onto cells from sparse cultures; *open circles*, incorporation onto cells from confluent cultures.

glucosamine under the same conditions as was the cell suspension. At appropriate times, aliquots were taken and spotted directly for electrophoresis.

This same transfer of galactose from UDP-Gal to *N*-acetylglucosamine can be demonstrated in intact 3T12 cells, but these cells show a definite activity with desialized ovine submaxillary mucin as well.

**Culture conditions**

Fig. 3 shows the effect of the original culture density on galactose incorporation in 3T3 cells. Sparse cultures contained less than $10^4$ cells/cm$^2$ for at least 2 days before harvesting. Confluent cultures contained more than $5 \times 10^4$ cells/cm$^2$. Incorporation into endogenous acceptors was greater in cells from sparse cultures. However, Table 1 shows that cells from confluent cultures showed greater transferase activity, at least towards the added acceptor *N*-acetylglucosamine, whether whole cells or sonicates (in the presence of Triton X-100) were tested.

Incorporation into 3T12 cells was not dependent upon culture conditions. However, preincubation of 3T12 cells before assay resulted in a marked decrease in incorporation. In contrast, 3T3 cells showed a slight increase in incorporation as a result of preincubation in growth medium.

TABLE 1. UDP-Gal: *N-Acetylglucosamine galactosyl transferase activity in whole cells and in sonicates of whole cells*

| Cells | *N*AcGlu 0.5 mM | cpm/50 µl incubation* Cells from sparse cultures | Cells from confluent cultures |
|---|---|---|---|
| Intact | − | 2211 | 699 |
| Intact | + | 4782 | 5555 |
| After sonication† | − | 677 | 939 |
| After sonication | + | 5662 | 8792 |

* Reactions were performed with 28.5 µM UDP-Gal ($35 \times 10^6$ cpm/µmol) with $5.3 \times 10^6$ cells/ml for both sparse and confluent cultures. Time of incubation was 3 hr, and the reaction was stopped as in Fig. 1.

† All incubations with cell sonicates contained 0.1% Triton X-100, to maximize activity of the membrane-bound enzymes.

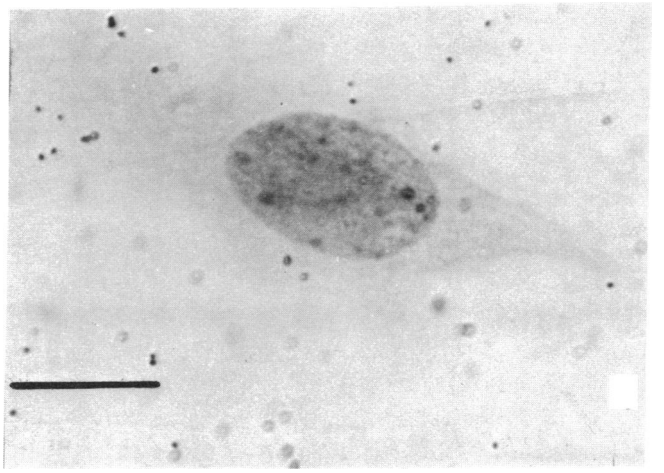

FIG. 4. Radioautograph from a sparse 3T3 culture incubated for 3 hr at 38°C with UDP-[$^3$H]galactose and treated as described in text. *Bar* represents 10 micrometers in this and subsequent figures.

Unless specified otherwise, all of the experiments with 3T3 cells used preincubated cells from sparse cultures.

**Partial product characterization**

After acid hydrolysis of 3T3 and 3T12 retentates, all radioactivity (90% recovery of both) coelectrophoresed with authentic galactose and migrated with this sugar on descending paper chromatography. Of the unhydrolyzed 3T3 product, 21% sedimented at $100{,}000 \times g \times 60$ min; all of the radioactivity in the pellet was extractable with chloroform–methanol 2:1 and could be identified as ceramide lactose. None of the $100{,}000 \times g$ supernatant radioactivity was soluble in chloroform–methanol. The result of an identical centrifugation with the unhydrolyzed product of 3T12 cells left 35% of the total counts in the pellet fraction. Of this pellet fraction, 75% was identified as ceramide lactose and 25% as ceramidetrihexoside.

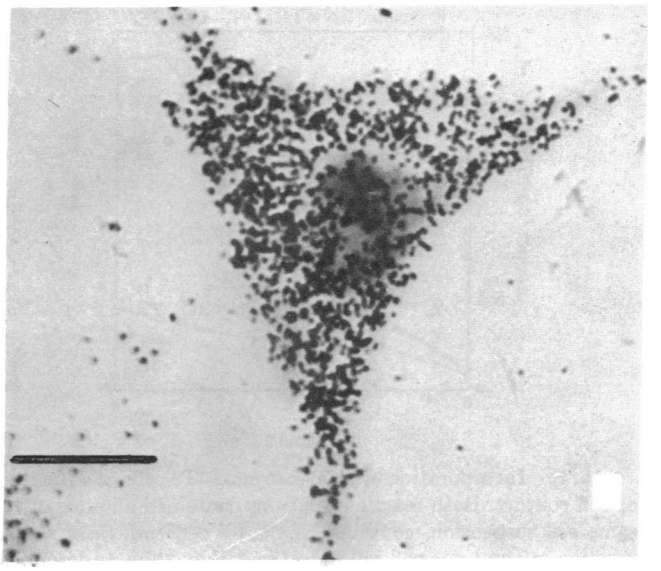

FIG. 5. Radioautograph from a sparse 3T12 culture obtained exactly as in Fig. 4.

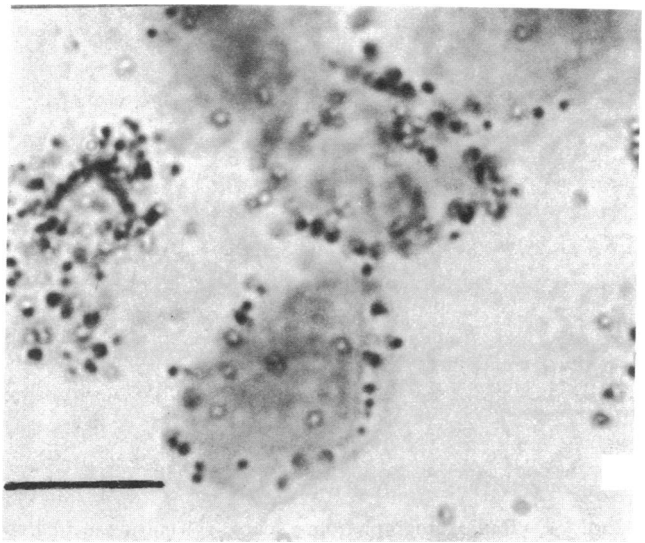

FIG. 6. Radioautograph of a sectioned 3T3 cell pellet incubated for 3 hr at 37°C with UDP-[$^3$H]galactose and treated as described in text.

### Radioautography

Figs. 4 and 5 show the results of radioautographs made on 3T3 and 3T12 cells in monolayer culture labeled with [$^3$H]-UDP-Gal. Whether in sparse or confluent cultures, 3T3 cells were labeled much less than were 3T12 cells, which were strongly labeled under both conditions. However, radioautographs of cell pellets made by incubation of cell suspensions with [$^3$H]UDP-Gal in small, conical centrifuge tubes showed no detectable differences in labeling. Virtually all of the cells in the 3T3 (sparse and preincubated) and 3T12 (confluent and not preincubated) pellets were labeled heavily and peripherally. Fig. 6 shows a radioautograph of a sectioned 3T3 cell pellet.

### Contact dependence

In order to test more directly the requirement for contact in the transferase reaction between 3T3 cells, two identical

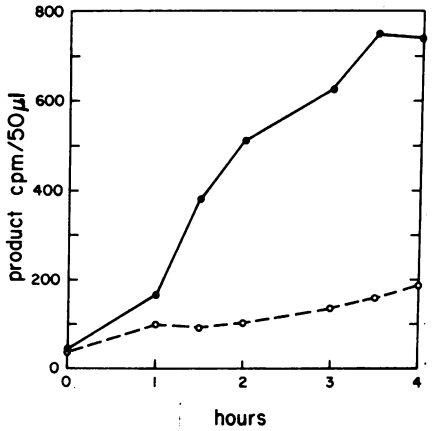


FIG. 7. Incorporation of galactose onto 3T3 cells as a function of cell contact. Both reaction mixtures contained aliquots of the same cell suspension, containing 5 × $10^6$ cells/ml. *Solid circles*, reaction in a stationary 3-ml conical centrifuge tube, as described; *open circles*, reaction in a stirred (200 rpm) shell vial, a treatment that maintains the cells in suspension.

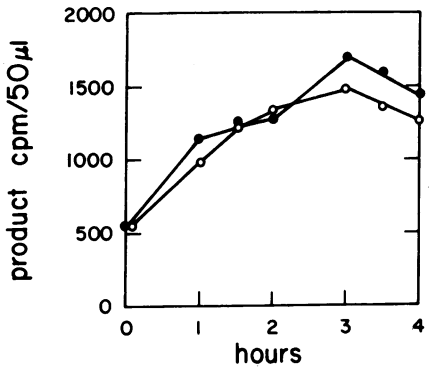


FIG. 8. Incorporation of galactose onto 3T12 cells as a function of cell contact. Both reaction mixtures contained aliquots of the same cell suspension, containing 8 × $10^6$ cells/ml. *Solid circles*, stirred reactions; *open circles*, stationary reactions, as described in the text and Fig. 7. Reactions stopped by rapid chilling to 0°C.

suspensions were made with 5 × $10^6$ cells/ml. One suspension was incubated, as usual, in a stationary, 3-ml conical centrifuge tube. Under these conditions most cells quickly sediment in a loose pellet. The second suspension was placed in a 2-ml flat-bottomed, shell vial with a stirring rod attached to the bottom on the external surface. This vial was stirred at a speed of about 200 rpm during incubation, thereby decreasing cell contact as compared to the stationary tube. Fig. 7 shows that incorporation in the stirred tube was less than in the stationary tube. When the identical experiment was done with 3T12 cells (Fig. 8) no difference in rate or extent of incorporation was seen.

In both of these experiments, stirred and stationary incubation tubes showed a 10–15% loss of cells during the duration of the experiment, as determined by hemocytometer counts. However, the number of cells in the stationary and stirred tubes did not differ significantly from one another.

## DISCUSSION

The data show that whole cells from the established Balb/c 3T3 line are capable of catalyzing the transfer of galactose from UDP-Gal to either cellular or added acceptors. The evidence presented suggesting that galactosyl transferases and acceptors are on the outer surface of the plasma membrane is indirect and consists of the following three points:

(*a*) There is no inhibition of the endogenous reaction by unlabeled UDP-Glu, galactose, or galactose-1-$PO_4$, indicating that entry of UDP-Gal or one of its labeled breakdown products for intracellular utilization is unlikely.

(*b*) Whole cells catalyze the transfer of galactose to at least some known galactose acceptors of high and low molecular weights. This activity resides with the cells, and is not present in a 500 × *g* cell supernatant.

(*c*) Radioautographs of cells in pellets after incorporation of [$^3$H]galactose show peripheral labeling patterns.

The results also show that 3T3 cells originating from sparse cultures incorporate galactose to a greater extent than do 3T3 cells from confluent cultures. The enzyme activities in both cell types, at least with regard to the added galactose acceptor, *N*-acetylglucosamine, cannot account for this difference. Therefore, the increased incorporation in sparse 3T3 cells may be due to the presence of greater amounts of galactosyl acceptors. Incubation of 3T3 cells for 30 min in

growth medium after trypsinization has a slightly stimulatory effect on subsequent incorporation of galactose by 3T3 cells.

With the 3T12 cells, on the other hand, cells from sparse and confluent cultures show the same rate of incorporation and this rate is similar to that for sparse, preincubated 3T3 cells. Preincubation has a marked inhibitory effect on incorporation of galactose by 3T12 cells.

In addition, radioautographic analyses of cells labeled in monolayers or in pellets with [$^3$H]UDP-Gal show that 3T3 cells in monolayer, sparse or confluent, show much less incorporation than do 3T12 cells. In radioautographs of sectioned pellets, however, 3T12 cells and 3T3 cells from sparse cultures showed heavy incorporation and were indistinguishable.

These results all suggest that 3T3 cells might only be able to transfer galactose to endogenous acceptors on adjacent cells, making this catalysis dependent upon contact.

It would follow that confluent 3T3 cells, having experienced extensive cell contact in culture, would have a greater number of already galactosylated sites. Sparse cultures would yield cells with a greater number of available sites which, under the conditions of the assay, would become galactosylated when the cells sediment in the conical centrifuge tubes. Preincubation of these cells under conditions where contact is not favored would do little to affect the results except, perhaps, to allow the cells to repair damage done to their transferases by trypsin. Radioautographs of sparse 3T3 cultures would show low incorporation because of a low extent of intercellular contact. Confluent cultures would show low incorporation because the sites had been previously transgalactosylated on contact. In order to test the requirement for contact more directly, incorporation in 3T3 cells was compared between stationary and stirred suspensions. In the stirred suspension, where cell contact is diminished, the incorporation was four-times less than in the stationary suspension, where cells settle and contact is enhanced. The fact that incorporation in 3T12 cells is not affected by identical stirring indicates that these cells do not require contact with adjacent cells to catalyze the transfer reaction.

The assays reported here are complicated by the fact that they measure the activities of an undetermined number of galactosyl transferases, and that they are performed at concentrations of UDP-Gal shown not to be optimal. Nevertheless, the best interpretation of the data is that 3T3 and 3T12 cells possess on their surfaces galactosyl transferases and acceptors. On the malignant 3T12 cell, these enzymes and substrates might be close enough so that *cis*-glycosylation could occur. On the nonmalignant 3T3 cells, the enzymes and substrates might be spatially separated, so that only *trans*-glycosylation could occur when two cells make sufficient contact.

If these conclusions are valid, one of the defects in the malignant cell type, 3T12, could be an architectural one on the plasma membrane. This could explain the minimal differences in quality and quantity of surface glycoproteins and glycolipids between normal and malignant cells (8).

A mechanism of this type could also explain the increased amount of complex glycolipids in confluent cultures of nonmalignant cells, as compared to sparse cultures (9–11). On contact, the cells would simply add glycose units to the oligosaccharide chains of their neighbors.

Whether this contact-dependent reaction of the cell surface is actually an example of how normal cells might alter each other on contact and begin the metabolic events leading to growth cessation remains to be determined.

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