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SURFACE GLYCOSYLTRANSFERASES ON CULTURED MOUSE FIBROBLASTS

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Previous work has suggested the presence of galactosyltransferases on the outer surface of the plasma membrane of a malignant and a nonmalignant cell line. This paper summarizes data indicating that three other classes of glycosyltransferases are similarly located on cell surfaces. In addition to the original two cell lines examined, BALB/c 3T3 and BALB/c 3T12, two other lines of BALB/c origin have been investigated. These are the SV40–transformed 3T3 line and one of the revertants of the virally infected cells that is no longer malignant but retains a viral genome.

INTRODUCTION

The glycosyltransferases synthesize oligosaccharide chains by catalyzing the addition of a single sugar from its nucleotide derivative to the nonreducing end of a specific monosaccharide or a polysaccharide (1). These sugar acceptors may be free or attached to proteins or lipids. For example, the reaction for the transfer of N-acetylglucosamine to any acceptor may be written as UDP–NAcGm + acceptor $\xrightarrow[\text{transferase}]{\text{N–acetylglucosaminyl–}}$ acceptor←NAcGm + UDP where N–acetylglucosamine (NAcGm) is transferred to the acceptor from its nucleotide form, uridine diphosphate N–acetylglucosamine (UDP–NAcGm). The reaction products are the newly glycosylated acceptor plus free UDP. Most transferase reactions examined require a divalent cation for optimum activity.

The cellular transferases are usually associated with membranes and most have been localized in the Golgi apparatus (2). Soluble transferases have been found in extracellular fluids such as colostrum (3), human sera (4), and in the fluid surrounding the embryonic chick brain (5).

Recently, a number of reports have suggested that some of the cellular transferases may be associated with the external surface of the plasma membrane. These data come from experiments using chick embryo neural retina cells (6), human blood platelets (7, 8), and cultured mouse fibroblasts (9, 10). The evidence for a surface localization of these enzymes may be summarized as follows:

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1. Intact cells (6, 9, 10) catalyze the transfer of sugars from sugar nucleotides to endogenous acceptors under conditions where there is no evidence for uptake of the sugar nucleotide.

2. Radioautography shows that the glycosylated endogenous acceptor is located at the cell periphery (6, 9).

3. There is no evidence for uptake and subsequent internal utilization of labeled breakdown products of the sugar nucleotides (6, 9).

4. In the presence of intact cells and sugar nucleotide, transfer occurs not only to endogenous acceptors but also to exogenous acceptors of varying molecular weights (6–10). The glycoslylated acceptors remain in the supernatant of the cell suspension while the enzyme activity is associated with the intact cells.

Using normal and malignant cells in culture, we have reported (9) that endogenous galactosyltransferase activity is low in confluent normal cells compared to normal cells from sparse cultures or malignant cells from either type of culture. Galactosyltransferase activity toward two exogenous acceptors was high and approximately equal in both of these cell types and did not vary according to the density of the cultures from which the cells were harvested. In addition, we observed (9) that transfer of galactose from UDP–galactose to endogenous acceptors on intact normal cells could be decreased if the degree of cell contact during the incubation was decreased. The degree of cell contact had no effect on the endogenous reaction when the intact malignant cells were similarly assayed. From these and other observations we made two suggestions. First, normal cells may glycosylate one another when they make contact in culture so that glycosyl acceptors are relatively saturated on normal cells from confluent cultures. Second, it is possible that, on normal cells, enzymes and their substrates are sufficiently separated so that glycosylation depends on cell contact. When contact is made, enzymes on one cell can utilize substrates on the adjacent cell. Since the malignant cells did not require cell contact for endogenous galactosyltransferase activity, it was suggested that these enzymes and their substrates could interact optimally on a single cell surface.

In this paper we will summarize similar data obtained with three additional classes of glycosyltransferases and another pair of cultured cells. The data will appear in a later, more complete, publication. In general, our observations on surface galactosyltransferase activity in Balb/c 3T3 and 3T12 cells can be generalized to surface sialyl – and N+acetylglucosaminyltransferase activities on these cells as well as SV3T3 and SV3T3 FR cells. Surface N–acetylgalactosaminyltransferase activity, however, seems not to behave as do the other three enzyme systems.

MATERIALS AND METHODS

Cells

The four cell lines used in these experiments are of Balb/c mouse origin. The characteristics of each line are summarized in Table I. One of the two malignant cell types was virally transformed and the other is a "spontaneous" transformant selected for its ability to grow at high cell densities. The "flat revertants" originated from SV40–transformed Balb/c 3T3 cells which were selected for their inability to grow at high densities (11). All cells were grown as described previously (9) in Dulbecco's modification of Eagle's medium plus 10% calf serum in the absence of antibiotics.

TABLE I. Characteristics of the Cell Types Used

	Balb/c 3T3 (3T3)	Balb/c 3T12 (3T12)	SV40 Balb/c 3T3 (SV 3T3)	SV40 Balb/c 3T3 FR (SV FR)
Shows contact inhibition of growth	yes	no	no	yes
Malignant	no	yes	yes	no

Transferase Assays

Nucleotide sugars labeled with ^{14}C in the sugar moiety were purchased from New England Nuclear Corporation and used in the assay incubations at subsaturating concentrations. Cells to be assayed were harvested with 0.1% crude trypsin as described previously (9). Before use, the cells were incubated in the serum-containing culture medium for one hour. This generally resulted in higher transferase activities than if the intact cells were assayed immediately after trypsinization. After this one hour incubation, the cells were washed twice in Ca^{++}–, Mg^{++}–, glucose-free Hanks saline (HBS) and buffered to pH 7.2–7.4 with 0.01 M HEPES and suspended in HBS to the appropriate concentrations. Fifty μl of the cell suspensions were added to small incubation tubes. These tubes contained the appropriate amounts of sugar nucleotide and cation as well as the exogenous acceptor when such an acceptor was to be used. These components of the assay mixture were previously added to the tubes in solution and evaporated to dryness in a rotary evaporator. The reactions were stopped by the addition of 10 μl of 0.2 M EDTA. Glycosylated product was measured by subjecting 50 μl of the stopped reaction mixture to high voltage electrophoresis on 1% borate-impregnated paper as previously described (9). When assaying transfer of the acetylated hexosamines, the electrophoretogram origins were subjected to ascending chromatography in 80% ethanol to remove any remaining free hexosamine. The origins were then counted in a Nuclear Chicago Scintillation Spectrometer.

When disrupted cells were to be assayed, an aliquot of the cell suspension was sonicated before addition to the incubation tubes. In addition to sugar nucleotide and cation, these tubes contained 10 μl of 0.1% Triton X–100 which also was evaporated to dryness before addition of the sonicated cell suspension.

RESULTS

Table II shows the optimum divalent cation conditions for transfer of four different sugars to endogenous acceptors on intact 3T3 cells. Two points should be noted. First, the cation requirement varies according to the nucelotide sugar being assayed. All of the transferase classes require Mn^{++} for highest activities although the necessary concentrations vary.

The second point is that in two of the three cases examined, the cation requirement varies qualitatively when the endogenous reaction on intact cells is compared to that on disrupted cells in the presence of detergent. With UDP–NAcGm, intact cells require 0.8 mM Mg^{++} although sonicates of the identical cell suspension require 10 mM Mn^{++}. Not only is manganese not required for the reaction with whole cells, it is inhibitory.

TABLE II. Cation Requirements for Optimal Endogenous Transferase Activities with 3T3 Cells

Sugar donor	Intact cells	Disrupted cells
UDP–N–acetylgalactosamine (UDPGalNAc)	5 mM Mn^{++}	n.d.[a]
UDP–galactose (UDPGal)	10 mM Mn^{++}	10 mM Mn^{++}
UDP–N–acetylglucosamine (UDPNAcGm)	5 mM Mn^{++}	10 mM Mn^{++}
CMP–N–acetylneuraminic acid (CMPNAN)	10 mM Mn^{++}	none[b]

[a]Not determined.

[b]Cations tested were: Ca^{++}, Cd^{++}, Co^{++}, Cu^{++}, Fe^{++}, Mg^{++}, Mn^{++}, Ni^{++}, Zn^{++}.

Table III shows the numbers of monosaccharides transferred to the cell types assayed. These data were obtained by incubating each of the cell types with each of the indicated nucleotide sugars under conditions which have been determined as optimal for the 3T3 cells. In order to calculate the maximum number of sugars transferred to the cells, each reaction was carried out until the amount of product formed no longer increased with time. No distinct quantitative or qualitative correlations exist between endogenous activity and malignancy.

Table IV shows that malignant cells do differ from nonmalignant cells with regard to a requirement for intercellular contact for maximal endogenous activities. With three of the four sugar nucleotides assayed, malignant cell types show no dependence on contact for transferase activity. However, N–acetylgalactosaminyltransferase activity can be decreased in malignant cells by maintaining the cells in suspension during the assay. Conversely, normal cells do show a definite contact dependence for the sugar nucleotides assayed except UDP–Gal NAc.

DISCUSSION

The data summarized in this preliminary report suggest that a wide variety of glycosyltransferases may exist on the outer surface of Balb/c fibroblast plasma membranes. This conclusion is consistent with that published previously by Bosmann (10) with regard to similar experiments on different cell lines.

The work presented here allows four essential points to be made.

1. When assaying a variety of transferase families, it is at least necessary to optimize the conditions for each family. Ideally, these enzyme assays should be carried out in the presence of excess glycosyl acceptor so that the only limiting factor is the amount of enzyme. In practice, this is often impossible since only a relatively small number of exogenous acceptors are available. Even worse, it is not known how valid comparisons are between reactions of membrane associated enzymes and soluble exogenous acceptors and these same enzymes and their insoluble natural acceptors, even if the oligosaccharide portion of the two acceptors is identical. Nevertheless, for these cell types and these endogenous reactions no single incubation condition would yield accurate data concerning the rate or extent of the various transferase reactions with intact viable cells.

2. With respect to the extent of the endogenous reaction, no consistent correlation can be found between the amount of any of the sugars transferred and the degree of malignancy of the cell being assayed.

3. For most transferase activities, optimum endogenous reaction rates depend on intercellular contact if nonmalignant intact cells are assayed. When malignant cells are

TABLE III. Number of Sugar Molecules Transferred to Endogenous Acceptors on Intact Cells Under Optimum Conditions

	Cell Types							
	3T3		3T12		SV3T3		SVFR	
Sugar Donor	per cell ($\times 10^{-6}$)	per μ^2 surface ($\times 10^{-3}$)	per cell ($\times 10^{-6}$)	per μ^2 surface ($\times 10^{-3}$)	per cell ($\times 10^{-6}$)	per μ^2 surface ($\times 10^{-3}$)	per cell ($\times 10^{-6}$)	per μ^2 surface ($\times 10^{-3}$)
UDPGalNAc	11.0	10.0	22.0	20.0	n.d.		n.d.	
UDPGal	5.0	4.5	4.4	3.8	6.4	26.0	5.3	4.8
UDPNAcGm	3.3	2.9	1.4	1.3	4.4	18.0	2.6	2.3
CMPNAN	0.6	0.5	1.0	0.9	0.9	3.8	n.d	

TABLE IV. Requirement for Intercellular Contact[a] for Optimum Endogenous Glycosyltransferase Activity on Intact Cells

	3T3	3T12	SV3T3
Endogenous reaction requires cell contact	UDPGal UDPNAcGm CMPNAN	UDPGalNAC	
Endogenous reaction does not require cell contact	UDPGalNAC	UDPGal UDPNAcGm CMPNAN	UDPGal UDPNAcGm CMPNAN

[a]The criterion for contact dependence was a significant decrease in endogenous activity when intact cells were maintained in suspension during the assay compared with their activity when allowed to form a loose pellet (9).

assayed this dependence is no longer observed. One of the four transferase classes assayed, the N–acetylgalactosaminyltransferases, shows a complete reversal in this regard. At the present time, no transferase has been seen to behave similarly on both the malignant and nonmalignant cells described.

4. The fact that optimum endogenous activity with intact cells often requires different levels or species of cation compared to disrupted cell activities could indicate that internal and external enzymes are regulated separately according to their different requirements.

It should be noted that not all of the criteria for surface transferases outlined in the introduction have been satisfied for all the cell types and all the transferase classes mentioned here. It is possible that some of these data, therefore, will be more applicable to internal enzymes. This possibility is, perhaps, minimized by the observation that at least one cell type shows a dependence on cellular contact for the maximum utilization of each sugar donor. It would be difficult to understand how internally located enzymes would require cell contact for activity, assuming the sugar nucleotides were available.

The roles of these putative surface reactions in the markedly different degrees of growth control shown by some of these cells in culture are currently being examined. Preliminary results indicate that the forced glycosylation of 3T12 cell surfaces has an inhibitory effect on the growth rate of the cells for the next 24 hours.

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