Discussion Letter

GLYCOSYLTRANSFERASES: DO THEY EXIST ON THE SURFACE MEMBRANE OF MAMMALIAN CELLS?

T. W. KEENAN and D. James MORRÉ
Departments of Animal Sciences, Biological Sciences and Botany and Plant Pathology, Purdue University West Lafayette, Indiana 47907 USA

Received 27 March 1975

1. Introduction

According to the membrane flow or endomembrane hypothesis [1,2], membrane biogenesis includes the physical transfer of membranes from one subcellular compartment to another. Endoplasmic reticulum membrane is transferred to the Golgi apparatus where it is transformed into plasma membrane-like membranes. This transformed membrane is utilized in formation of secretory vesicles which arise from cisterna of the Golgi apparatus. The secretory vesicle membranes fuse with the plasma membrane and during this process the vesicle contents are discharged into the extracellular space [3,4]. Secretory vesicle membranes can thus contribute to growth or renewal of membranes of the cell surface. Additionally, constituents of surface membranes, especially glycolipids and glycoproteins, have the potential for being synthesized within the context of this export route; their synthesis need not be restricted to the cell surface [5–9]. Yet, recent views and hypotheses assume partial or even exclusive localization of the carbohydrate transferases of glycoprotein and glycolipid synthesis in or on the surface membrane [10–25]. In this report we critically evaluate the evidence relevant to localization of these transferases within eucaryotic cells.

2. Discussion

Glycosyltransferases appear to control the structure and specificity of the carbohydrate portions of complex polysaccharides [11,25]. In mammalian systems these transferase enzymes transfer a carbohydrate from a sugar nucleotide to an acceptor, the latter being usually the incomplete carbohydrate chain of a protein or lipid. Additionally, glycosyltransferases have been suggested to function directly as part of the cell surface receptor complex involved in intercellular adhesion [11–13]. Much of the argument for the localization of glycosyltransferases in plasma membranes is based on indirect evidence (see [21 and 26] for a review of pertinent literature). Further, glycosyltransferase activities have not yet been demonstrated in isolated plasma membrane fractions of defined purity. Nevertheless, several hypotheses advanced to explain tumorigenesis, cellular adhesion and other cellular phenomena are based on presumed surface localization of glycolipid and glycoprotein glycosyltransferases.

Both external and internal membranes of cells are asymmetrically substituted with carbohydrate groups capable of reaction with certain lectins conjugated with ferritin or other markers [27,28]. The carbohydrate groupings of glycoproteins are exposed on the inner face of internal cellular membranes [27,29, 30]. In addition, glycoproteins of microsomal vesicles are labeled by lactoperoxidase-catalyzed iodination only when the vesicles are made permeable by treatment with detergents [31]. At the cell surface, carbohydrate groupings are exposed along the outer or environmental face of the membrane only [27–29]. These observations have been interpreted as resulting from a flow of membranes with retention of configuration from intracellular biosynthetic sites to the cell surface (the intraluminal face of endoplasmic reticulum being equivalent to the external face of the plasma

 North-Holland Publishing Company – Amsterdam

membrane). To achieve this extracellular distribution, glycosyltransferases must otherwise function in the extracellular environment or completed glycolipids and glycoproteins must be asymmetrically inserted into the membrane.

An intracytoplasmic rather than extracytoplasmic site of addition of carbohydrate prosthetic groups was early inferred from studies with whole cells and intact tissues. For example, Bosmann et al. [32] found parallel rates of radioactive glucosamine incorporation into plasma membrane glycolipids and glycoproteins of HeLa cells as well as evidence for transfer of these constituents from intracellular membranes to the plasma membrane. A central role for the Golgi apparatus in protein and lipid glycosylation was first suggested by autoradiographic studies (cf. [9]] for a recent review). In these studies, attachment of sugars was shown to be a late event of glycoprotein formation and to follow to completion of the polypeptide chain [9,33]. Early cell fractionation studies served to localize glycoprotein glycosyltransferases in microsomal fractions, particularly those microsomal subfractions enriched in smooth membranes [34–36]. Later studies with highly purified and thoroughly characterized cell fractions from rat liver have shown several different glycoprotein [6,7,37–40], and glycolipid [9] glycosyltransferases to be concentrated in Golgi apparatus with lesser amounts being detected in endoplasmic reticulum. With the marker enzyme *N*-acetyl-lactosamine synthetase (a galactosyltransferase whose in vivo function appears to be the galactosylation of glycoproteins), up to 70% of liver homogenate activity van be recovered in highly purified Golgi apparatus fractions [37,39]. Since recovery of Golgi apparatus from homogenates is no more than 70%, it follows that the true percentage of this galactosyltransferase which is associated with Golgi apparatus must be greater than 70%. The several glycosyltransferases involved in conversion of lactosyl ceramide to the disialoganglioside *N*-acetylneuraminic acid-galactose-*N*-acetylgalactosamine-(*N*-acetylneuraminic acid)-galactose-glucose-ceramide (G_{Dla}) are concentrated in Golgi apparatus from rat liver with much lower specific activities being found in endoplasmic reticulum [8]. The total of these activities in endoplasmic reticulum plus Golgi apparatus accounted for more than 85% of the activity of the total homogenate [8]. Similarly, the galactosyltransferase of lactose biosynthesis [41] is concentrated in mammary gland Golgi apparatus fractions [42,43] as are glycosyltransferases of ganglioside synthesis [44]. Glycosyltransferases are also concentrated in Golgi apparatus fractions from a diversity of other animal tissues (for example pancreas [45], thyroid [46], snail mucopolysaccharide secreting gland [47], testis [48] and small intestine [49]).

Based on the above observations, it is clear that Golgi apparatus alone or in combination with endoplasmic reticulum accounts for a large proportion of several glycosyltransferase activities, at least in liver. Thus there is only a small proportion of the total activities available for distribution among other membranes of the cell. Mitochondrial fractions have been reported to contain some glycolipid and glycoprotein glycosyltransferase activities (eg. [50]); however, evidence to rule out an origin from contamination of the mitochondrial fractions by Golgi apparatus has not been presented. Sialic acid [51] and galactose [10] transferase activities have been reported for plasma membrane fractions from rat liver. Here, the procedures used to obtain and characterize there fractions do not satisfactorily account for the possibility of extensive contamination by either Golgi apparatus, smooth endoplasmic reticulum, or both. In contrast, *N*-acetylactosamine synthesis [37,38], UDP-*N*-acetylglucosamine: glycoprotein transferase [37] and transferases of ganglioside synthesis [8] could not be detected in highly purified plasma membrane fractions from liver. These transferases were also not detected in bovine milk fat globule membrane [44,52], a membrane known to be derived directly from apical plasma membrane of mammary secretory cells (eg. [53]). Rabbit erythrocyte membranes were similarly found to be devoid of sialyltransferase activity [54] and lysed chicken erythrocytes had no detectable galactosyl or sialyltransferase activities. (T. W. Keenan and W. W. Franke, unpublished). However, glycosyltransferases were detected in immature chicken erythrocytes induced by phenylhydrazine; these erythrocytes contain at least fragments of intracellular endomembranes [55]. In summary, there has been no unequivocal demonstration of glycosyltransferase actvity in isolated plasma membranes. The opposite conclusion, that there are no such activities in isolated plasma membranes, is favored by currently available experimental evidence.

In recent years an ever increasing number of publications have appeared which purport to demonstrate

membrane glycosyltransferase activities on the surface membranes of cultured mammalian cells [12,13, 15–24,56]. The impetus for these studies appears to be the suggestion of Roseman [11] that glycosyltransferases are involved in intercellular adhesion. In his hypothesis Roseman visualized adhesion as being due, at least in part, to a lock and key fit between glycosyltransferases on a cell surface with their substrate glycoprotein or glycolipid carbohydrate chains on surfaces of other cells. He further predicted that one difference between normal and transformed cells would be a reduced level of glycosyltransferases, and consequently a reduced degree of interaction between surface membranes of the latter cell types. The general experimental design used to test this hypothesis has been to add radioactively labeled sugar nucleotides to suspensions or monolayers of cells in buffer, incubate for various periods, collect particulate matter by precipitation and centrifugation or filtration, and determine radioactivity Any radioactivity incorporated is assumed to be in surface membrane protein or lipid and to have been incorporated into these structures by glycosyltransferases in the surface membrane. Occasionally glycoprotein [19,21,22] or, more recently, immobilized glycolipid [56] acceptors have been added to the incubation mixture. A common observation has been enhanced glycosyltransferase activity in transformed cells in comparison with untransformed controls [17,20–22]. Investigators have been undaunted in interpretation of such results as supporting Roseman's hypothesis, even though just the opposite result, lower levels of transferases on the surface of transformed cells, is predicted by the hypothesis.

In our view, none of the above-cited experiments with intact cells have produced results which conclusively demonstrate the occurrence of glycosyltransferase activities on the surface of cultured cells. Experiments purporting to show such localization can be faulted for one or a combination of the reasons which follow:

2.1. *That hydrolysis of the sugar nucleotide and entry of the free carbohydrate into the cell may account for the findings*

In these experiments it is normally assumed that sugar nucleotides are not transported through the plasma membrane. While this appears to be true, it is also well established that plasma membranes contain enzymes which rapidly degrade sugar nucleotides with generation of free sugars [37,52,57,58]. From in vivo labeling experiments it is apparent that free sugars are accumulated by cells and readily incorporated into glycoproteins and/or glycolipids (eg. [5,9,59]). It was recently found that BHK cells rapidly liberate free galactose from UDP-galactose through the intermediate of galactose-1-phosphate [26]. While most investigators have apparently neglected this possibility,Roth et al. [13] added unlabeled free sugar to their cell suspensions in an effort to block incorporation of liberated radioactive sugar. Unfortunately, recent results demonstrate that the levels of free sugar added by Roth et al. were inadequate to effectively block entry of radioactive galactose liberated from UDP into BHK cells [26]. It was further observed that sodium azide completely abolished incorporation of carbohydrate into exogenous acceptors in intact cells. Since sodium azide had no effect on galactosyltransferase activity in cell homogenates, it was concluded that it blocked entry of the free carbohydrate into the cells [26]. Identical results have been obtained when cytochalasin B, an inhibitor of sugar accumulation by cells, was added to suspensions of mammary carcinoma cells (T. W. Keenan and W. W. Franke, unpublished).

2.2. *That release of glycosyltransferases before or during incubation by cell lysis or secretion of solubilized transferases may be responsible*

While cell intactness is often simply assumed [15–18,21–24], sometimes it is measured by trypan blue exclusion and it is normally reported that 'nearly all' or 'greater than ___%' of the cells were found to exclude the dye [13,19,20]. Nevertheless, the levels of carbohydrate actually incorporated represent a very small mass or proportion of the total sugar nucleotide added, and one wonders if this incorporation is due to the presence of intracellular membranes, freed by cell lysis, in the incubation mixture. This could be, but apparently never has been, tested by removal of intact cells and assay of the resultant supernatant for glycosyltransferase activities. Further, while glycosyltransferases are membrane-bound within the cell, the fact that they are found in soluble form in blood serum [60], amniotic fluid [61] and milk [62] suggests that they can be discharged in free form from at least certain cell types. This discharge could

conceivably occur in buffer suspensions of cells. This possibility, or cell lysis, could readily account for incorporation of carbohydrates into exogenous acceptors which can reasonably be expected not to enter intact cells [13,15–17,19,21,22,56].

2.3. *That glycosyltransferases are associated with mammalian cell surfaces but are of a unique class not involved in membrane protein or lipid glycosylation*

The best evidence for association of glycosyltransferases with cell surfaces comes from plant [63,64] or bacterial [65] cells where such transferases may be involved, not in glycosylation of membrane proteins or lipids (i.e. membrane biogenesis) but rather in the elaboration of surface coats. Clearly, a role for the plasma membrane (in addition to that of the Golgi apparatus) is indicated in cellulose formation [63]. Enzymes of chitin biosynthesis apparently act at or near the surface of the cell [66]. Glycosyltransferases reported to be present on the surface of the alga *Chlamydomonas* [64], for example, might be an expression of a more general tendency of plants to utilize surface transferases in the biosynthesis of walls and surface coats. Similarly, the D-glucosyltransferase involved in attaching the terminal glucosyl residues of collagen is exclusively associated with plasma membranes [67] and belongs to the category of transferases functioning in formation of extracellular coat materials in animal cells.

In secretion Golgi apparatus and other internal membranes appear to carry out two important functions. One is the transformation and terminal glycosylation of membrane constituents. The other is the elaboration, packaging and glycosylation of secretory products including wall components, and mucins and mucopolysaccharides of cell walls and surface coats [68]. Secretory vesicles of Golgi apparatus from both plant [69,70] and animal [71] cells exhibit high glycosyltransferase activities. These are presumed to function in the terminal glycosylations of secretory products as the vesicles migrate to the cell surface [69–71]. Additionally, such transferases have been suggested to continue to function after the vesicle membrane fuses with the plasma membrane, especially in formation of primary cell walls of plants [69,70]. As suggested by Pat and Grimes [21], glycosyltransferases entering plasma membranes by such a route might be able to incorporate carbohydrate moieties of exogenous nucleotide sugars into products. However, such transferases are functionally involved in elaboration of surface coats and not with glycosylation of membrane components. Such a distinction is often overlooked and tested only by establishing acceptor specificity. As far as is known, glycosyltransferases of polysaccharide or mucin formation differ in specificity from those of glycoprotein or glycolipid glycosylation; i.e., they are specific for very different acceptors. Functions and functional implications also differ. The products of one class of transferases are clearly implicated in tumorigenesis, cell recognition and other aspects of regulation of cellular activities. The other class results in the formation of protective walls and surface coats where a regulatory function for glycosylated products is less clear. Additionally, alternative pathways not directly involving sugar nucleotide derivatives might be involved. This could include participation of lipid intermediates analogous to the oligosaccharide-polyisoprenol of bacterial polymers [72].

Until the possibilities enumerated above are tested, there appears to be no valid basis for the conclusion that glycosyltransferases of membrane biogenesis are localized on the surfaces of mammalian cells. It is certainly apparent that assignment of specific roles to glycosyltransferases of the cell surface in cellular processes such, as recognition and tumorigenesis, are premature.

Acknowledgements

Our work is supported by grants from the National Science Foundation and the National Institutes of Health. T.W.K. is supported by a research career development award from the National Institutes of General Medical Science. We thank Professor Dr. W. W. Franke, Deutsches Krebsforschungszentrum, Heidelberg, for stimulating discussions. Purdue University AES Journal Paper No. 5797.

References

[1] Franke, W. W., Morré, D. J., Deumling, B., Cheetham, R. D., Kartenbeck, J., Jarasch, E. and Zentgraf, H. (1971) Z. Naturforsch. 26b, 1031–1039.

[2] Morré, D. J., Keenan, T. W. and Huang, C. M. (1974) in: Advances in Cytopharmacology (Ceccarelli, B., Clementi, F. and Meldolesi, J., eds.), Vol. 2, pp. 107–126, Raven Press, New York.
[3] Beams, H. W. and Kessel, R. G. (1968) Int. Rev. Cytol. 23, 209–276.
[4] Dauwalder, M., Whaley, W. G. and Kephart, J. E. (1972) Subcell Biochem. 1, 225–275.
[5] Haddad, A., Smith, M. A., Herscovics, A., Nadler, N. J. and LeBlond, C. P. (1971) J. Cell Biol. 49, 856–882.
[6] Schachter, H., Jabbal, I., Hudgin, R. L., Pinteric, L., McGuire, E. J. and Roseman, S. (1970) J. Biol. Chem. 245, 1090–1100.
[7] Wagner, R. R. and Cynkin, M. A. (1971) J. Biol. Chem. 246, 143–151.
[8] Keenan, T. W., Morré, D. J. and Basu, S. (1974) J. Biol. Chem. 249, 310–315.
[9] Bennett, G., LeBlond, C. P. and Haddad, A. (1974) J. Cell Biol. 60, 258–284.
[10] Aronson, N. N., Tan, L. Y. and Peters, B. P. (1973) Biochem. Biophys. Res. Comm. 53, 112–118.
[11] Roseman, S. (1970) Chem. Phys. Lipids, 5, 270–297.
[12] Roth, S. (1973) Quart, Rev. Biol. 48, 541–563.
[13] Roth, S., McGuire, E. J. and Roseman, S. (1971) J. Cell Biol. 51, 536–547.
[14] Emmelot, P. (1973) Europ. J. Cancer 9, 319–333.
[15] Roth, S. and White, D. (1972) Proc. Natl. Acad. Sci. U.S. 69, 485–489.
[16] Bosmann, H. B. (1971) Biochem. Biophys. Res. Comm. 43, 1118–1124.
[17] Bosmann, H. B. (1972) Biochem. Biophys. Res. Comm. 48, 523–529.
[18] Bosmann, H. B., Bieber, G. F., Brown, A. E., Case, K. R., Gersten, D. M., Kimmerer, T. W. and Lione, A. (1973) Nature 246, 487–489.
[19] Bosmann, H. B. (1974) Biochim. Biophys. Acta 339, 438–441.
[20] Bosmann, H. B., Case, K. R. and Morgan, H. R. (1974) Exp. Cell Res. 83, 15–24.
[21] Patt, L. M. and Grimes, W. J. (1974) J. Biol. Chem. 249, 4157–4165.
[22] Lamont, J. T., Perrotto, J. L., Weiser, M. M. and Isselbacher, K. J. (1974) Proc. Natl. Acad. Sci. U.S. 71, 3726–3730.
[23] Weiser, M. M. (1973) J. Biol. Chem. 248, 2536–2541.
[24] Arnold, D., Hommel, E. and Risse, H. J. (1973) Biochem. Biophys. Res. Comm. 54, 100–107.
[25] Cook, G. M. W. and Stoddart, R. W. (1973) Surface Carbohydrates of the Eukaryotic Cell Academic Press, New York.
[26] Deppert, W., Werchau, H. and Walter, G. (1974) Proc. Natl. Acad. Sci. U.S. 71, 3068–3072.
[27] Hirano, H., Parkhouse, B., Nicholson, G. L., Lennox, E. S. and Singer, S. J. (1972) Proc. Natl. Acad. Sci. U.S. 69, 2945–2949.
[28] Nicholson, G. L. and Singer, S. J. (1974) J. Cell Biol. 60, 236–248.
[29] Keenan, T. W., Franke, W. W. and Kartenbeck, J. (1974) FEBS Lett. 44, 274–278.
[30] Winqvist, L. Eriksson, L. C. and Dallner, G. (1974) FEBS Lett. 42, 27–31.
[31] Kreibich, G., Hubbard, A. L. and Sabatini, D. D. (1974) J. Cell Biol. 60, 616–627.
[32] Bosmann, H. B., Hagopian, A. and Eylar, E. H. (1969) Arch. Biochem. Biophys. 130, 573–583.
[33] Cook, G. M. W., Laiso, M. T. and Eylar, E. H. (1965) Proc. Nat. Acad. Sci. U.S. 54, 247–252.
[34] Eylar, E. H. and Cook, G. M. W. (1965) Proc. Nat. Acad. Sci. U.S. 54, 1678–1685.
[35] Hagopian, A., Bosmann, H. B. and Eylar, E. H. (1968) Arch. Biochem. Biophys. 128, 387–396.
[36] Wagner, R. R. and Cynkin, M. A. (1969) Arch. Biochem. Biophys. 129, 242–247.
[37] Morré, D. J., Merlin, L. M. and Keenan, T. W. (1969) Biochem. Biophys. Res. Comm. 37, 813–819.
[38] Fleischer, B. and Fleischer, S. (1970) Biochim. Biophys. Acta 219, 301–319.
[39] Bergeron, J. J. M., Ehrenreich, J. H., Siekevitz, P. and Palade, G. (1973) J. Cell Biol. 59, 73–88.
[40] Bizzi, A. and Marsh, J. B. (1973) Proc. Soc. Exp. Biol. Med. 144, 762–765.
[41] Brodbeck, U. and Ebner, K. (1966) J. Biol. Chem. 241, 762–764.
[42] Keenan, T. W., Morré, D. J. and Cheetham, R. D. (1970) Nature 228, 1105–1106.
[43] Keenan, T. W., Huang, C. M. and Morre, D. J. (1972) J. Dairy Sci. 55. 1577–1585.
[44] Keenan, T. W. (1974) J. Dairy Sci. 57, 187–192.
[45] Ronzio, R. A. (1973) Arch. Biochem. Biophys. 159, 777–784.
[46] Chabaud, O., Bouchilloux, S., Ronin, C. and Ferrand, M. (1974) Biochimie 56, 119–130.
[47] Ovtracht, L., Morré, D. J. and Merlin, L. M. (1969) J. de Microscopie 8, 989–1002.
[48] Cunningham, W. P., Mollenhauer, H. H. and Nyquist, S. E. (1971) J. Cell Biol. 51, 273–285.
[49] Mahley, R. W., Bennett, B. D., Morré, D. J., Gray, M. E., Thistlewaithe, W. and LeQuire, V. S. (1971) Lab. Invest. 25, 435–444.
[50] Myers, M. W. and Bosmann, H. B. (1974) Eur. J. Biochem. 47, 173–177.
[51] Procer, W. E. and Ashwell, G. (1971) J. Biol. Chem. 246, 4825–4833.
[52] Keenan, T. W. and Huang, C. M. (1972) J. Dairy Sci. 55, 1013–1015.
[53] Keenan, T. W., Morré, D. J., Olson, D. E., Yunghans, W. N. and Patton, S. (1970) J. Cell Biol. 44, 80–93.
[54] Jancik, J. and Schauer, R. (1974) Z. Physiol. Chem. Hoppe Seyler's 355, 395–400.
[55] Brecher, G. and Stohlman, F. (1962) in: Erythropoiesis (Jacobson, L. O. and Doyle, M., eds.) pp. 216–257. Grune and Stratton, New York.
[56] Yogeeswaren, G., Laine, R. A. and Hakomori, S. (1974) Biochem. Biophys. Res. Comm. 59, 591–599.

[57] Fleischer, B. and Fleischer, S. (1969) Biochim. Biophys. Acta 183, 265–275.
[58] Evans, W. H. (1974) Nature 250, 391–394.
[59] Riordan, J. R., Mitranic, M., Slavik, M. and Moscarello, M. A. (1974) FEBS Lett. 47, 248–251.
[60] Mookerjea, S., Chow, A. and Hudgin, R. L. (1971) Can. J. Biochem. 49, 297–299.
[61] Nelson, J. D., Jato-Rodriguez, J. J. and Mookerjea, S. (1974) Can J. Biochem. 52, 42–50.
[62] Magee, S. C., Mawal, R. and Ebner, K. E. (1974) Biochemistry 13, 99–102.
[63] VanDerWoude, W. J., Lembi, C. A., Morré, D. J. Kindinger, J. I. and Ordin, L. (1974) Plant Physiol. 54, 333–340.
[64] McLean, R. J. and Bosmann, H. B. (1974) J. Cell Biol. 63, 218a.
[65] Schuer, M. G., Lennarz, W. J. and Sweeley, C. C. (1968) Proc. Natl. Acad. Sci. U.S. 59, 1313–1320.
[66] Ruiz-Herrera, J. and Bartnicki-Garcia, S. (1974) Science 186, 357–359.
[67] Hagopian, A., Bosmann, H. B. and Eylar, E. H. (1968) Arch. Biochem. Biophys. 128, 387–396.
[68] Morré, D. J., Keenan, T. W. and Mollenhauer, H. H. (1971) in: Advances in Cytopharmacology (Clementi, F. and Ceccarelli, B., eds.) Vol. 1, pp. 159-182. Raven Press, New York.
[69] VanDerWoude, W. J., Morré, D. J. and Bracker, C. E. (1971) J. Cell Sci. 8, 331–351.
[70] Morre, D. J. and VanDerWoude, W. J. (1974) in: Macromolecules Regulating Growth and Development (Hay, E. D., King, T. J. and Papaconstantionou, J., eds.) pp. 81–111. Academic Press, New York.
[71] Merritt, W. D. and Morré, D. J. (1973) Biochim. Biophys. Acta 304, 397–407.
[72] Waechter, C. J., Lucas, J. J. and Lennarz, W. J. (1973) J. Biol. Chem. 248, 7570–7579.