

A fucosyltransferase in teratocarcinoma stem cells

Decreased activity accompanying differentiation to parietal endoderm cells

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Teratocarcinoma stem cell F9 expressed a potent fucosyltransferase activity acting on asialofetuin. A majority of the product was susceptible to α -L-fucosidase I from almond emulsin, indicating that the linkage formed was mainly $\text{Fu}\alpha 1 \rightarrow 3\text{GlcNAc}$. The specific activity of the transferase decreased when the stem cells were induced to differentiate into parietal endoderm cells by retinoic acid and dibutyryl cyclic AMP. Furthermore, PYS-2 cell, a parietal endoderm cell line virtually lacked the transferase. The change in the fucosyltransferase activity could be correlated with cell surface changes occurring during differentiation.

<i>Fucosyltransferase</i>	<i>Glycosyltransferase</i>	<i>Teratocarcinoma stem cell</i>	<i>Cell surface</i>
	<i>Differentiation</i>	<i>Parietal endoderm cell</i>	

1. INTRODUCTION

Cell surface carbohydrates are known to be altered in highly ordered manners during early stages of mammalian embryogenesis [1–8]. The phenomenon probably reflects the importance of protein–carbohydrate interactions to accomplish the embryogenesis. Being interested in the mechanism of the programmed alterations, we have initiated studies on glycosyltransferases responsible for the cell surface changes. Because of the limited availability of early embryos, we employed a model system, namely the teratocarcinoma system, in which the stem cell resembles multipotential cells of early embryos and can be induced to differentiate in vitro [9].

2. MATERIALS AND METHODS

F9 cells [10] were cultured and induced to dif-

ferentiate as in [7]. PYS-2 cells [11] were cultured as in [1]. Asialofetuin was prepared by mild acid hydrolysis (0.1 M H_2SO_4 , 80°C, 30 min) of fetuin (Sigma). Embryoglycan, the glycoprotein-bound large carbohydrates of early embryonic cells [1,12], was prepared from F9 cells as in [12].

For the measurement of fucosyltransferase activity, cells from 2–5 dishes (10 cm, Falcon) were homogenized at 4°C with 0.7–1.0 ml of 50 mM Tris–maleate buffer (pH 7.0) containing 0.5% Triton X-100 and 25 mM MnCl_2 . After ultracentrifugation at $78000 \times g$ for 1 h, 50 μl of the supernatant (or that diluted with the extraction medium) was mixed with 10 μl of aqueous solution of asialofetuin (50 mg/ml) and 10 μl of aqueous solution of $\text{GDP-[}^{14}\text{C]fucose}$ (1.1×10^5 dpm, 0.260 nmol, New England Nuclear). The reaction was continued at 37°C for 30 min, and stopped with 10 μl of 7.5% sodium tetraborate containing 0.2 M EDTA. The reaction mixture was analyzed by gel filtration on a column of Sephadex G-50 (1.4 $\times$ 33 cm), which was equilibrated and eluted with 0.05 M ammonium acetate buffer, pH 6.0. Radioactivity eluted in the excluded volume was

Abbreviations: Diff-F9, F9 cells differentiated by retinoic acid and dibutyryl cyclic AMP; FBP, fucose-binding proteins of *Lotus tetragonolobus*

taken as radioactive fucose transferred to the acceptor, after subtracting the value obtained in the control run in which asialofetuin was omitted from the reaction mixture. The values of the control run were less than 100 dpm in all cases. The activity was roughly proportional to the amount of enzyme (fig.1) and reaction time (up to 30 min) provided that the amount of the transferred fucose was less than 10 pmol.

For the measurement of nucleotide pyrophosphatase, GDP-[^{14}C]fucose was incubated with the cell extract under the condition described above except that asialofetuin was omitted, and the products were analyzed by paper chromatography in ethylacetate/pyridine/acetic acid/ H_2O (5:5:1:3, by vol.) [13]. Digestion with α -L-fucosidase I from almond emulsin [14] was carried out as in [2].

3. RESULTS AND DISCUSSION

F9 cell is a clonal line of teratocarcinoma stem

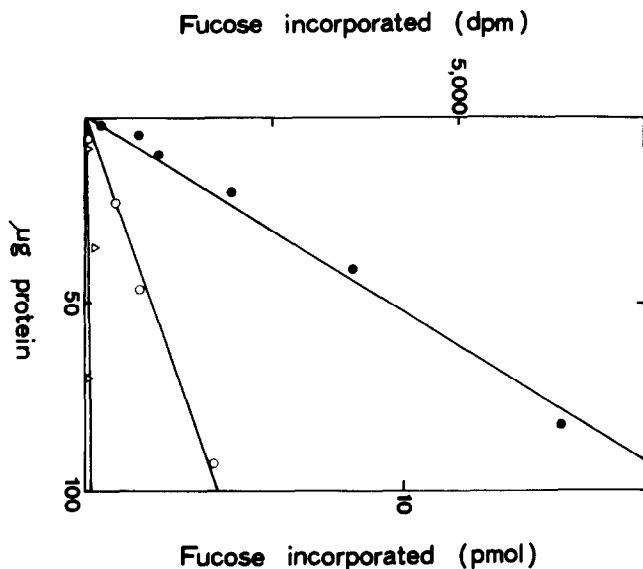


Fig.1. Activities of the fucosyltransferase from F9 cells (●), the cell induced to differentiate into parietal endoderm cells (○, Diff-F9 cells) and PYS-2 cells (Δ). The cell extract was prepared and used for the enzyme assay as described in section 2. The volume of extraction medium used, the number of 10 cm culture dishes used to propagate the cells and the protein concentration in the extracts were as follows: F9 cells, 0.8 ml, 2 dishes, 3.30 mg/ml; Diff-F9 cells, 0.7 ml, 5 dishes, 1.86 mg/ml; PYS-2 cells, 0.7 ml, 2 dishes, 2.80 mg/ml.

cell. A potent fucosyltransferase activity was detected in Triton extract of the stem cells, using asialofetuin as an acceptor (fig.1). When F9 cells were induced to differentiate into parietal endoderm cells (Diff-F9 cells) by treating with retinoic acid and dibutyryl cyclic AMP [15] for 5 days, the specific activity of the fucosyltransferase decreased to 22% of the original one (fig.1). In another experiment performed independently, the specific activity decreased to 20%. Considering that around 10% of the cells remained morphologically undifferentiated at the stage examined, it is apparent that differentiation to parietal endoderm cells was accompanied by marked reduction in the fucosyltransferase activity. Furthermore, PYS-2 cell, a clonal line of parietal endoderm cell, was found to virtually lack the fucosyltransferase (fig.1). It should be noted that the F9 cells and PYS-2 cells were derived from the same teratocarcinoma line, namely teratocarcinoma OTT6050.

The decreased activity of the fucosyltransferase can be explained not only by the decreased amount of the enzyme but also by increased amount of nucleotide pyrophosphate or fucosidase. However, the transferase activity of F9 cells was not inhibited by the extract from PYS-2 cells (table 1) or Diff-F9 cells (not shown). Furthermore, the level of nucleotide pyrophosphate acting on GDP-fucose was higher in the extract from F9 cells as compared to that from PYS-2 cells (table 1) or from Diff-F9

Table 1

Evidence showing that PYS-2 extract did not have substances inhibitory for the assay of the fucosyltransferase

Enzyme preparations ^a	Fucose transferred (dpm) ^b	GDP-fucose decomposed (%) ^c
F9 cells	3714	60
PYS-2 cells	100	3.6
F9 cells + PYS-2 cells	3883	—

^a Protein concentration of the extract from F9 cells was 2.5 mg/ml and that from PYS-2 cells was 2.6 mg/ml

^b The extract (25 μl) was mixed with the extraction medium (25 μl) or with the extract from the other cell (25 μl) and was used for the assay

^c Fifty μl of the extract was used

cells (26% decomposition by 93 μ g protein). These results indicate that enhanced destruction of substrates or products is not the reason for decreased fucosyltransferase activity in parietal endoderm cells.

So far two fucosyltransferases, namely $\text{Fuc}\alpha 1 \rightarrow 2\text{Gal}$ transferase and $\text{Fuc}\alpha 1 \rightarrow 3\text{GlcNAc}$ transferase, are known to act on asialofetuin, which have $\text{Gal}\beta 1 \rightarrow 4\text{GlcNAc}$ termini [16]; 77% of the fucose transferred to asialofetuin by the extract from F9 cells was released by α -L-fucosidase I from almond emulsin, which acts on $\text{Fuc}\alpha 1 \rightarrow 3,4\text{GlcNAc}$ linkages but not on $\text{Fuc}\alpha 1 \rightarrow 2\text{Gal}$ linkage, $\text{Fuc}\alpha 1 \rightarrow 6\text{GlcNAc}$ linkage [14] nor on $\text{Fuc}\alpha 1 \rightarrow 3\text{Gal}$ linkage (unpublished). Thus, we propose that the newly formed fucosyl linkage was mainly $\text{Fuc}\alpha 1 \rightarrow 3\text{GlcNAc}$.

The predominant carrier of fucose in plasma membrane of F9 cells is embryoglycan, the glycoprotein-bound large carbohydrates [1,12]. The extract from F9 cells could transfer fucose not only to asialofetuin, but also to embryoglycan, which also has unfucosylated $\text{Gal}\beta 1 \rightarrow 4\text{GlcNAc}$ termini [12] (fig.2). Thus, the transferase under in-

vestigation is involved in formation of cell surface structures of F9 cells.

Differentiation to parietal endoderm cells accompanies drastic alteration in cell surface. Notably, receptors for fucose binding proteins of *Lotus tetragonolobus* (FBP) and SSEA-1 defined by a monoclonal antibody are present in the stem cells but are absent in parietal endoderm cells [3,17]. SSEA-1 is a carbohydrate antigen determined by $\text{Fuc}\alpha 1 \rightarrow 3\text{GlcNAc}$ linkage [15]. Evidence accumulating in our laboratory [2,8,12] suggests that in the stem cells, the major binding site for FBP is also in the form of the $\text{Fuc}\alpha 1 \rightarrow 3\text{GlcNAc}$ linkage. Thus, the decreased activity of the fucosyltransferase can be correlated with the disappearance of the cell surface marker(s) from the differentiated cells. In other words, the differentiation-dependent alterations of cell surface [3,17] appear to be caused not only by the suppression of synthesis of embryoglycan [7], an endogenous acceptor for the fucosyltransferase, but also by suppression of the fucosyltransferase itself.

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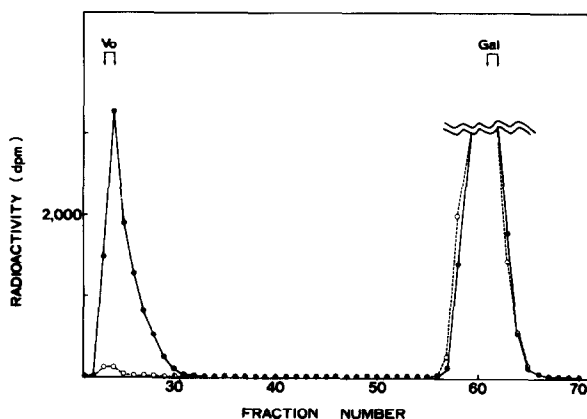


Fig.2. Enzymatic transfer of fucose from GDP-[^{14}C]fucose to embryoglycan. The reaction was carried out as described in section 2 using 160 μ g of the enzyme protein and 50 μ g embryoglycan as the acceptor. The product was analyzed on a column of Sephadex G-50 (1.8 $\times$ 91 cm) equilibrated and eluted with 0.05 M ammonium acetate buffer, pH 6.0. Fractions, 3 ml were collected. Vo and Gal represent the area where blue dextran or galactose was eluted. (●—●) Experimental run; (○---○) control run in which embryoglycan was not added to the reaction mixture.

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