Increase in cell-surface *N*-acetylglucosaminide $\beta(1 \longrightarrow 4)$galactosyltransferase activity with retinoic acid-induced differentiation of F_9 embryonal carcinoma cells

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Exposure of F_9 cells to all-*trans*-retinoic acid over a period of 6 days resulted in 4-fold induction of cell surface *N*-acetylglucosaminide $\beta(1 \longrightarrow 4)$galactosyltransferase (GT) activity. The retinoic acid-induced GT activity was further enhanced by treatment of the cells with 8-bromo cyclic AMP. The ability of retinoic acid alone, or retinoic acid in combination with 8-bromo cyclic AMP, to induce GT activity was inhibited by both actinomycin D and cycloheximide. These findings indicate that the induction of galactosyltransferase activity noted with differentiation of F_9 cells involves de novo synthesis of new enzyme protein.

F_9 cell *Retinoic acid* *8-Bromo cyclic AMP* *Galactosyltransferase activity* *Differentiation* *De novo synthesis*

1. INTRODUCTION

F_9 embryonal carcinoma cells, the stem cells of teratocarcinomas, provide an in vitro system for the study of early biochemical events involved in embryonic differentiation and development [1,2]. Exposure of F_9 cells to all-*trans*-retinoic acid induces differentiation to an endodermal cell type [3]. Cyclic AMP has been implicated as a modulator of cellular differentiation in a variety of cell types [4,5] and has been shown to promote the differentiation of F_9 cells treated with retinoic acid [3,6].

Cell surface and extracellular components are required for a number of cell–cell interactions. These components are usually glycoconjugates and their participation in mediating biological responses in a number of cell types has been established (review [7]). One such class of glycoconjugates are glycosyltransferases [8]. These enzymes synthesize a variety of complex carbohydrates by catalyzing the transfer of saccharides from sugar donors to the growing polysaccharide chain. On the cell surface, glycosyltransferases could participate in cellular interactions by binding specific carbohydrate substrates on adjacent cell surfaces [7]. In this manner, these cell surface enzymes have been postulated to modulate such biological processes as cell–cell adhesion [9] and cell migration [10].

Retinoic acid is known to alter cell surface glycoprotein metabolism [11,12] and in some cases this alteration appears to be a specific effect upon the addition of distal sugars [12]. Changes in the activity of glycosyltransferases have been described during viral transformation, altered proliferation, and differentiation of cells [13]. To determine if changes in galactosyltransferase activity might correlate with changes related to the onset of embryonal carcinoma cell differentiation, we have investigated the effect of retinoic acid treatment of F_9 cells on this enzymatic activity.

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2. MATERIALS AND METHODS

2.1. *Materials*

Dulbecco-Vogt's modified Eagle's medium (DMEM) and fetal calf serum (FCS) were from Gibco. All-*trans*-retinoic acid was purchased from Eastman Kodak. Cycloheximide, 8-bromo cyclic AMP, UDP-galactose and *N*-acetylglucosamine were from Sigma; actinomycin D was from Calbiochem. UDP-D-[6-^{3}H]galactose (16.3 Ci/mmol) was purchased from Amersham.

2.2. *Growth of cells*

The F_9 line of embryonal carcinoma cells was kindly provided by Dr Anton Jetten (NIEHS, Research Triangle Park, NC). Stock cultures of F_9 cells were maintained in DMEM with 10% FCS in a 5% CO_2 humidified atmosphere. Cells were passaged every 3 days by mild trypsinization with 0.05% trypsin containing 0.02% EDTA. Cells were counted with a Coulter counter (Coulter Electronics, Hialeah, FL).

2.3. *Differentiation of F_9 cells*

F_9 cells were seeded at 1×10^5 cells per 100 mm dish and allowed to attach and grow for 24 h. The growth medium then was changed to DMEM with 10% FCS containing 0.1 μM all-*trans*-retinoic acid in the presence and absence of 1 mM 8-bromo cyclic AMP. All treatments with retinoic acid were carried out in the dark.

To study the effect of actinomycin D and cycloheximide on the induction of galactosyltransferase activity, F_9 cells were treated with retinoic acid for 48 h, followed by continued treatment with retinoic acid for an additional 24 h in the presence of cycloheximide (1 μg/ml medium) or actinomycin D (0.2 μg/ml medium). In experiments with 8-bromo cyclic AMP, F_9 cells were exposed to retinoic acid for 6 days, followed by treatment with 1 mM 8-bromo cyclic AMP for an additional 24 h in the presence and absence of cycloheximide and actinomycin D as indicated.

2.4. *Galactosyltransferase assay*

F_9 cells attached to the culture dish were washed 3 times with phosphate-buffered saline and then twice with Hepes buffer (pH 7.2), containing Na^+ and K^+ (4.76 g/l Hepes, 7.5 g/l NaCl, 0.4 g/l KCl). Cells were detached from the dish by forcing this Hepes buffer over the cells. The detached cells were collected by centrifugation at 200 × *g* for 10 min and the harvested cells washed once with the Na^+- and K^+-containing Hepes buffer. The cell pellet was resuspended in the Hepes buffer system to a final concentration of $1-2 \times 10^7$ cells/ml. The galactosyltransferase activity was linear with increasing cell number up to 10^6 cells/ml.

Galactosyltransferase activity was determined by measuring the transfer of [^{3}H]galactose from UDP-[^{3}H]galactose to *N*-acetylglucosamine to form *N*-acetyllactosamine. The reaction was initiated by the addition of 40 μl of cell suspension (~4×10^4 cells) to a reaction mixture containing 125 μM UDP-[^{3}H]galactose (16.3 Ci/mmol), 6.3 mM $MnCl_2$, 26.6 mM Tris–HCl (pH 7.4), and 20 mM *N*-acetylglucosamine in a total volume of 100 μl. After incubation at 37°C for 30 min, the reaction was terminated by the addition of an equal volume of ice-cold 20 mM EDTA. The terminated reaction mixture then was applied to a column (4 × 0.6 cm) of Dowex AG-1-X8 equilibrated with water. After sample addition, the column was washed twice (0.2 ml/wash) with deionized water. The column then was eluted with 3 0.5-ml washes with deionized water to collect the *N*-acetyl[^{3}H]lactosamine formed, and the 3 elution washes combined. The amount of *N*-acetyl[^{3}H]lactosamine produced was determined by counting for radioactivity in the presence of Aquasol. To eliminate artifacts due to variations in UDP-galactose hydrolase, controls were run in the absence of *N*-acetylglucosamine, and radioactivity determined in these samples (less than 5% of experimental values) were subtracted from the amount of radioactivity noted in the presence of *N*-acetylglucosamine. Enzyme activity is expressed as pmol *N*-acetyllactosamine formed/60 min per μg protein.

2.5. *Protein determination*

Protein was determined as in [14] with bovine serum albumin as the standard.

3. RESULTS AND DISCUSSION

To determine possible changes in cell surface galactosyltransferase activity which might occur during the differentiation of embryonal carcinoma cells, enzyme activity was measured with control F_9

cells and with F_9 cells treated with 0.1 μM retinoic acid for various time periods up to 6 days (fig.1). Little change in galactosyltransferase activity was observed during the first 24 h of retinoid treatment. After 2 and 3 days of retinoic acid treatment, however, there is a doubling in enzyme activity. This would correspond to the results of previous studies which have described a 2–3-day lag between initial treatment of F_9 cells with retinoic acid and the appearance of cells with parietal endoderm-like properties [3,15,16]. The amount of galactosyltransferase activity was further increased in cells exposed to retinoic acid for 4 and 6 days. This would agree with studies which indicate that there is continued conversion to the endoderm cell type with treatment of F_9 cells with retinoic acid up to 10 days [16].

Cyclic AMP has been shown to promote retinoic acid-induced differentiation of F_9 cells to endoderm [6,15,16]. In agreement with these studies, in F_9 cells treated for 3 and 6 days with retinoic acid and for an additional 24 h with retinoic acid in the presence and absence of 8-bromo cyclic AMP, cyclic AMP elicits a further 2-fold increase in galactosyltransferase activity (fig.2). This change in enzyme activity is not observed when F_9 cells are treated with 8-bromo cyclic AMP alone (unpublished). Previously, it was reported that exposure of F_9 cells to retinoic acid causes a progressive increase in cyclic AMP-dependent protein kinase activity and provokes a rapid, preferential increase in the amount of the R_{II} regulatory subunit of this kinase system associated with the plasma membrane [17]. On the basis of these findings, it was suggested that an increase in cyclic AMP-dependent protein kinase activity may be an early event of retinoic acid action to mediate other changes which take place during the differentiation process. The present results which show that cyclic AMP further enhances retinoid-induced changes in

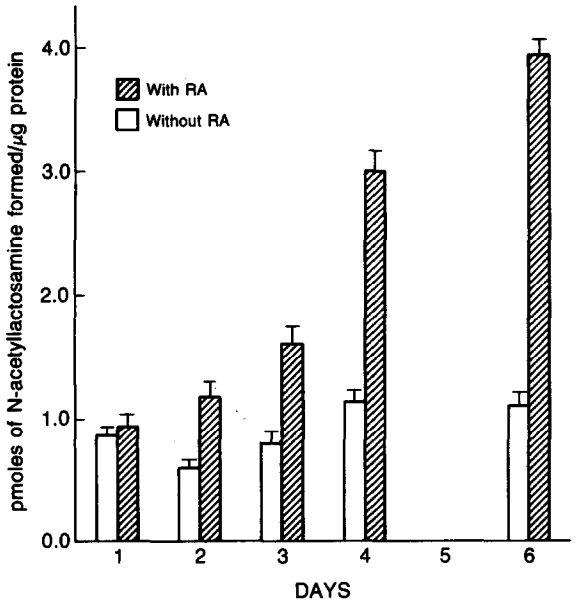

Fig.1. Effect of retinoic acid treatment of F_9 cells on cell surface galactosyltransferase activity. F_9 cells were grown in the presence or absence of 0.1 μM retinoic acid for the times indicated, and then galactosyltransferase activity was determined as described in section 2.

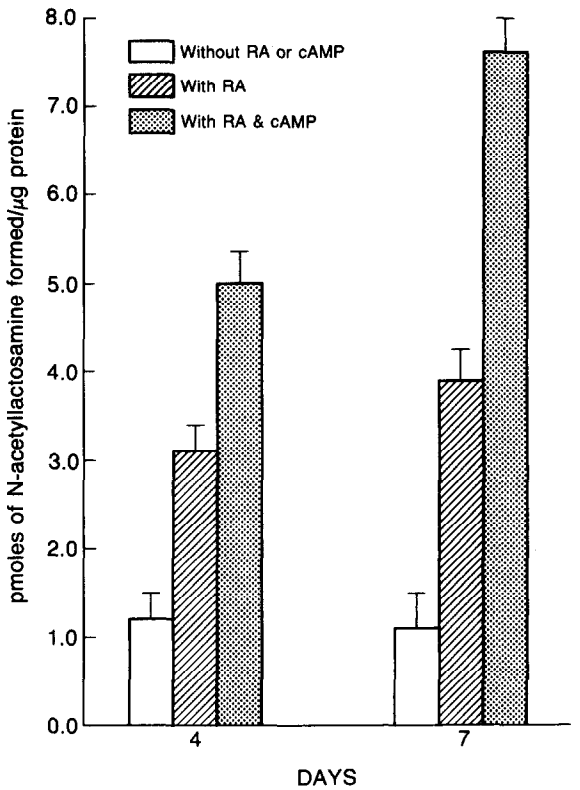

Fig.2. Effect of treatment of F_9 cells with retinoic acid and with a combination of retinoic acid and 8-bromo cyclic AMP on cell surface galactosyltransferase activity. F_9 cells were treated with 0.1 μM retinoic acid for 3 days, followed by an additional 24 h treatment with 0.1 μM retinoic acid with or without 1 μM 8-bromo cyclic AMP. Results are expressed as mean ± SD.

galactosyltransferase activity are consistent with this suggestion.

To establish if the induction of cell surface galactosyltransferase activity with retinoic acid was due either to stimulation of pre-existing enzyme or increased biosynthesis of new enzyme protein, studies were carried out with actinomycin D (an mRNA synthesis inhibitor) and cycloheximide (a protein synthesis inhibitor). In these studies, F_9 cells were treated with retinoic acid for 48 h with exposure to retinoic acid for an additional 24 h in the presence and absence of the inhibitors. The results presented in fig.3 show that both actinomycin D and cycloheximide prevent the retinoic acid-mediated induction of galactosyltransferase activity. Similar results were obtained when actinomycin D and cycloheximide were added to F_9 cells during treatment with retinoic acid in combination with cyclic AMP (fig.4). These findings indicate that the induction of galactosyltransferase activity in response to retinoic acid and cyclic AMP involves de novo synthesis of new enzyme protein.

Increased amounts of cell surface galactosyltransferase during retinoid-induced differentiation of F_9 cells may be required to add carbohydrate moieties to existing or newly synthesized

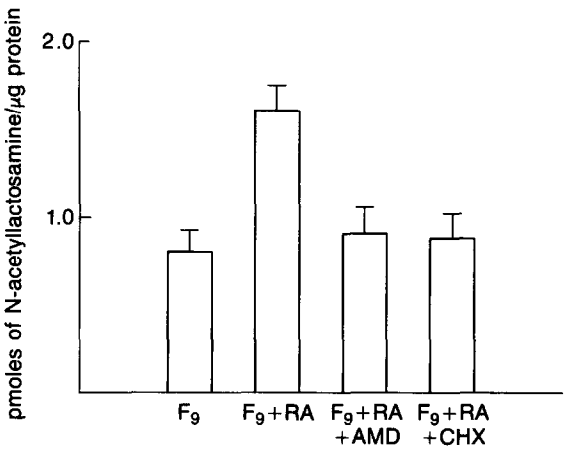

Fig.3. Effect of actinomycin D and cycloheximide on the retinoic acid-mediated induction of galactosyltransferase activity. F_9 cells were treated with 0.1 μM retinoic acid for 2 days, followed by treatment with 0.1 μM retinoic acid for an additional 24 h in the presence and absence of cycloheximide (1 μg/ml growth medium) or actinomycin D (0.2 μg/ml growth medium).

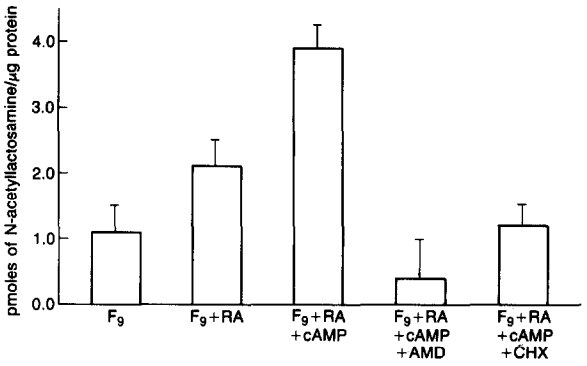

Fig.4. Effect of actinomycin D and cycloheximide on the retinoic acid plus 8-bromo cyclic AMP-mediated induction of galactosyltransferase activity. F_9 cells were treated with 0.1 μM retinoic acid for 6 days, followed by treatment with 0.1 μM plus 1 mM 8-bromo cyclic AMP for an additional 24 h in the presence and absence of cycloheximide (1 μg/ml growth medium) and actinomycin D (0.2 μg/ml growth medium) as indicated.

glycoconjugates. Others have shown alterations in cell surface structure with differentiation [18]. A similar induction of galactosyltransferase activity has been described when human promyelocytic (HL60) cells were treated with retinoic acid [19]. Of interest also is a recent study which reports that a different glycosyltransferase, a fucosyltransferase specific for the Fucα(1$\longrightarrow$3)GlcNAc linkage, is decreased in F_9 cells following treatment with retinoic acid and cyclic AMP [20]. This raises the possibility that reciprocal changes in cell surface glycosyltransferase activities (such as a decrease in fucosyltransferase accompanied by an increase in galactosyltransferase) could conceivably serve to modulate cell–cell interactions and thus to regulate growth and differentiation during embryonic development.

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