Retinoic Acid-induced Differentiation of the Mouse Teratocarcinoma Cell Line F9 Is Accompanied by an Increase in the Activity of UDP-galactose:β-D-Galactosyl-α1,3-galactosyltransferase*

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We report here that both the mouse teratocarcinoma F9 cells and F9 cells induced to differentiate by treatment with retinoic acid contain cell surface glycoconjugates with terminal α-linked galactose residues, as shown by agglutination of cells with antisera to blood type B, but not to type A. In addition, both cell types contain high numbers of binding sites for *Griffonia simplicifolia*-1, a lectin which binds to terminal α-linked galactose residues, although differentiated F9 cells contain approximately 50% more binding sites/cell for this lectin. We have also confirmed that differentiation is accompanied by a decrease in the expression of the fucose-containing stage-specific embryonic antigen (SSEA)-1, as evidenced by the fact that F9 cells, but not differentiated F9 cells, are agglutinated by monoclonal antibody to this antigen.

Since these results indicate that surface glycoconjugates contain terminal α-linked galactose residues, we assayed cell extracts for the enzyme UDP-Gal:β-D-Gal-α1,3-galactosyltransferase. We have found that F9 cell extracts contain this activity, and differentiation results in a significant increase in the specific activity of the enzyme, from approximately 2 nmol/mg h in F9 extracts to 7 nmol/mg h in RA/F9 extracts.

It has been suggested that the loss of the SSEA-1 antigen upon differentiation of F9 cells is due to decreased activity of the enzyme GDP-Fuc:β-D-GlcNAc-α1,3-fucosyltransferase. We therefore determined the activities of this fucosyltransferase and several other glycosyltransferases, which included UDP-GlcNAc:β-D-Gal-β1,3-N-acetylgalactosaminyltransferase, UDP-Gal:β-D-GlcNAc-β1,4-galactosyltransferase, and GDP-Fuc:β-D-GlcNAc-α1,6-fucosyltransferase. We have found that extracts from both cell types contain these enzyme activities; differentiation, however, does not result in substantial changes in any of these activities.

The mouse teratocarcinoma cell line F9 contains many surface carbohydrate antigens which are found in early mouse embryos (1-6). F9 cells differentiate upon the addition of retinoic acid into parietal endoderm-like cells (7-10), which begin to synthesize basement membrane proteins, including type IV collagen and laminin. Thus, these cells are a useful model system for events accompanying early embryonic development.

Surface glycoconjugates in the F9 cells have been reported to contain the repeating disaccharide [Galβ1,4GlcNAcβ1] or poly-N-acetyllactosamine sequence (11-13) and the stage-specific embryonic antigen (SSEA)-1, which has the partial structure Galβ1,4(Fucα1,3)GlcNAcβ-R (1, 14). Differentiation results in decreases in the sizes of cell surface-derived glycopeptides and dramatic changes in the antigenicity of surface glycoconjugates and loss of the SSEA-1 antigen (3, 8, 9, 11, 12, 16). It has been proposed that the loss of the SSEA-1 antigen is due to decreased activity of the fucosyltransferase that is involved in synthesizing the antigen (17). However, the detailed structures of the surface glycoconjugates in these cells and the changes in glycosyltransferases accompanying differentiation are not well described.

In our initial studies described here, we examined the reactivity of the F9 cells with a variety of plant lectins and anti-blood type antisera. We have found that both F9 and RA/F9 cells are agglutinated by anti-blood type B, but not by anti-blood type A, sera. In addition, the F9 cells contain high numbers of receptors for the plant lectin *Griffonia simplicifolia*-1, which interacts with high affinity with glycoconjugates containing the terminal sequence Galα1,3Gal-R (18). Such terminal sequences have been found on glycoproteins from many sources and on surface glycoconjugates in many different mouse cells (18-24).

We have also determined that F9 cell extracts contain relatively high amounts of UDP-Gal:β-D-Gal-α1,3-galactosyltransferase activity and that cellular differentiation is accompanied by marked increases in the activity of this enzyme. Interestingly, we detect no significant changes in the activities of a number of other glycosyltransferases upon cellular differentiation.

Our findings demonstrate that a feature of surface glycoconjugates in F9 and differentiated F9 cells is the presence of terminal α-linked galactose residues and suggest that the alterations in the activity of the α-galactosyltransferase may contribute to the reduction in size and altered antigenicity of glycoconjugates in the cells upon differentiation.

**EXPERIMENTAL PROCEDURES**

*Materials—ConA-Sepharose was purchased from Pharmacia LKB Biotechnology Inc. Fibrinogen, fetuin, porcine thyroglobulin, bovine serum albumin, UDP-Gal, UDP-GlcNAc, raffinose, EDTA, green coffee bean α-galactosidase, chitin, Amberlite MB-3, α-methylmannoside.

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noside, N-acetyllactosamine, sodium tetrasborate, GlcNAc, Sephadex G-25, ATP, bovine milk UDP-Gal:β-D-GlcNAc-β1,4-galactosyltransferase, and β-D-galactono-1,2-lactone were obtained from Sigma. Preparation was purchased from Behring Diagnostics and Dowex AG 1-X8 resin was purchased from Bio-Rad. Apiezon A oil was obtained from Bio-Rad. Hyamine 1300 (Pall, PA) was used to continue for 1 h at 37°C and acid was removed by evaporation under reduced pressure. The samples were desalted by chromatography over a column of Sephadex G-25 (5 X 100 cm) equilibrated in 7% 1-propanol. The glycopeptides were desialylated by incubation with 2.0 M acetic acid for 1 h at 10°C, and acid was removed by evaporation under reduced pressure. The samples were then resuspended in 1 ml of buffer and the glycopeptides were isolated by chromatography over a column of Sephadex G-25 (5 X 100 cm) equilibrated in 7% 1-propanol. The glycopeptides were desialylated by incubation with 2.0 M acetic acid for 1 h at 10°C, and acid was removed by evaporation under reduced pressure. The concentration of this desialylated glycopeptide was determined by phenol/sulfuric acid assay of neutral hexose.

Some of the desialylated biantennary glycopeptides from fibroblasts were treated with jack bean β-galactosidase to remove terminal galactose residues. Briefly, 4 μmol of glycopeptide was incubated with 14 milliunits of β-galactosidase in 0.1 ml of 0.1 M citrate buffer, pH 4.6, for 24 h at 37°C in a toluene atmosphere. An additional 14 milliunits of enzyme was then added and the incubation continued for 24 h. The sample was then desalted by passage over Sephadex G-25, as described above, and the glycopeptides were recovered in the void fractions. To test for the efficient removal of galactose residues by the enzyme, one-tenth of the treated glycopeptides was resuspended in PBS and passed over a column (1 X 18 cm) of R. communis agglutinin-I-Sepharose, which binds with high affinity to glycopeptides containing terminal β-linked galactose residues. Greater than 80% of the treated glycopeptides did not bind to the immobilized lectin and were recovered in the run-through fractions of the column, indicating that galactose had been removed from the glycopeptides. Porcine thyroglobulin (1 mg) was dissolved in 10 ml of PBS buffer containing 20 mg of Pronase. Incubation was allowed to proceed at 60°C in a toluene atmosphere for 18 h, at which time additional Pronase (20 mg) was added. The incubation was continued for 2 h and the mixture was then boiled for 5 min. The sample was centrifuged at 10,000 X g for 1 h, and the supernatant was saved. The pellet was resuspended in water and again centrifuged; this step was repeated twice. The water washes were combined with the original supernatant, and the material was dried in a shaker bath evaporator under reduced pressure. The sample was resuspended in water containing 7% 1-propanol and desalted on a 45 X 2.5 cm column of Sephadex G-25-80 in the same solvent. The glycopeptides were detected by phenol/sulfuric acid assay and were recovered in the void volume, pooled, and dried. To isolate the complex-type biantennary Asn-linked oligosaccharides, the porcine thyroglobulin glycopeptides were resuspended in 3 ml of water and applied to a 30-ml column of ConA-Sepharose equilibrated in 10 ml Tris, 1 mm CaCl2, 1 mm MgCl2, 150 mM NaCl, 0.02% NaNO3, pH 8.0 (TBS-NaNO3). Bound glycopeptides were eluted with 10 mM α-methylglucoside in TBS-NaNO3. The bound and eluted glycopeptides were pooled, dried, and resuspended in 5 ml of water. The glycopeptides were desalted and haptoc sorbed by passage over the column (45 X 2.5 cm) of Sephadex G-25-80, as described above. The structure of these glycopeptides has been described by Yamamoto et al. The complex-type biantennary Asn-linked oligosaccharides containing a residue of fucose-located α1,6 to the innermost N-acetyllactosamine of the N,N'-diacetyltrehalobioside (32). As would be predicted, >95% of these glycopeptides were bound by immobilized lentil lectin, which binds with high affinity to biantennary N-linked oligosaccharides containing the core fucose residue. The concentration of glycopeptide was determined by phenol/sulfuric acid assay. The biantennary glycopeptides from porcine thyroglobulin were desialylated as described above for fibroblasts glycopeptides. The radioactivity desaccharide [6-H]GlcNAc was prepared by the following procedure. Five hundred nmol of UDP-Gal in 70% ethanol was dried in a tube with 10,000,000 ppm of UDP-[6-H]Gal was added 10 mg of GlcNAc as art of 500 mg of M25a and 38 μl of UDP-Galβ-D-GlcNAc-β1,4-galactosyltransferase (38 millimolar). The reaction mixture was incubated at 37°C for 2 h. The mixture was passed directly over a column of AG 1 X8 resin equilibrated with 5% borate (34). The radioactive product N-acetyllactosamine was recovered in the haptocyte and used directly in glycosyltransferase assays. HPLC analysis of the material was performed as described by Varzi and Kornfeld (35) on a column of AX-5 resin using a gradient of water in acetonitrile.
trile. This revealed that greater than 98% of the radioactivity was recoverable as N-acetyllactosamine.

**Assays of Glycosyltransferases**—All enzymes were assayed in a final volume of 15–25 μl using 5 to 50 μg of protein cell extracts in assays for up to 2 h at 37°C in 100 mM cacodylate buffer, pH 7.0. To prepare cell extracts F9, RA/F9, and PYS-2 cells were scraped from dishes in PBS and recentrifuged 15 min at 10000 x g. The cell pellets were solubilized in 100 mM cacodylate buffer, pH 7.0, and protein was determined by the method of Lowry et al. (36), using bovine serum albumin as a standard. The sonicate was adjusted to 1% Triton X-100 and stored on ice for 30 min prior to use to allow complete solubilization.

UDP-Galβ3,4Galβ1,3Galα1,5-galactosyltransferase—UDP-Galβ3,4Galβ1,3Galα1,5-galactosyltransferase was assayed using either N-acetyllactosamine, thymoglobin glycopeptide, or fibrinogen glycopeptide as acceptors. Reactions mixtures routinely contained 20 nm MnCl2, 0.5 mM UDP-[3H]Gal (35,000 cpm/nmol), and acceptor in a final volume of 25 μl. In addition, the reaction mixtures contained 5 mM ATP to inhibit sugar nucleotide hydrolysis and 50 mM D-galactono-1,4-lactone to inhibit hydrolysis of the acceptor and product by endogenous glycosidas.

The reaction mixtures containing glycopeptide acceptors were boiled, diluted to 1 ml with TBS- NaN3, and passed directly over a 1 ml column of ConA-Sepharose. The reaction products were eluted with 100 mM α-methylmannoside directly into scintillation vials. The amount of product could then be determined and compared to the total amount bound by ConA-Sepharose.

**UDP-Fucα3,3Galβ1,4GlcNAcα1,3-fucosyltransferase**—The GDP-Fucα3,3Galβ1,4GlcNAcα1,3-fucosyltransferase was assayed using either asialofetuin or N-acetyllactosamine as acceptors, exactly as described (17, 42). Reactions contained either 1 mM asialofetuin or 10 mM N-acetyllactosamine, 20 mM MnCl2, 5 mM ATP, 15 mM fucose, and 16 μM GDP-[3H]Fuc (45,000 cpm/nmol) in a final volume of 15 μl. Controls assays were done in the absence of added acceptors.

In assays using the asialofetuin as acceptor, the reaction mixture was boiled 5 min, diluted to 1 ml with water, and applied to a column (1 x 50 cm) of Sephadex G-25–80 equilibrated in 0.1 M pyridine/acetic acid, pH 5.0, to separate reactants and products. The fucosylated asialofetuin product was recovered in the void fractions, and portions of the fractions were removed and radioactivity determined by liquid scintillation counting.

When N-acetyllactosamine was used as an acceptor, the reaction mixture was boiled 5 min and extracted three times with chloroform/methanol, as described above. The aqueous extract was resuspended in water and applied to a 3-ml column of Amberlite MB-3 in water. The radiolabeled product was recovered in the wash water of the column, dried in a shaker bath evaporator, and resuspended in 40 μl of water. The material was spotted on Whatman No. 1 paper for descending paper chromatography in ethyl acetate/pyridine/acetic acid/water (5:5:1:3). Lanes were cut in 1-cm sections and radioactivity determined by liquid scintillation counting. The triascharide product Galβ1,4(Fuc)α3Galβ1,3GalNAc was isolated by ascending paper chromatography and eluted with 100 mM phosphate buffer, pH 6.4. Incubations proceeded at 37°C for 24 h in a toluene atmosphere. The samples were then spotted directly for descending paper chromatography in the solvent system ethyl acetate/pyridine/acetic acid/water (5:5:1:3). After chromatography for 24 h, 1 cm strips of the lanes were removed for counting in liquid scintillation mixture (Scintiverse I from Fisher).

**UDP-GlcNAcα2,3Galβ1,3N-acetylgalcosaminyltransferase**—This enzyme transfers N-acetylgalcosamine in β1,3 linkage from UDP-GlcNAc to the terminal galactose residues of complex-type N-linked oligosaccharides (37–40). Our assays employed the asialo derivatives of biantennary acceptor glycopeptides from either fibrinogen or thymoglobin. Enzyme reaction was performed in 25 μl of 50 mM MnCl2, 5 mM ATP, and 0.2–0.8 mM acceptor glycopeptide in a final volume of 25 μl. In addition, the reaction mixtures contained 50 mM 1-N-acetyl-β-D-glucopyranosylamine to inhibit breakdown of products by endogenous β-hexosaminidases. After incubation, the mixture was boiled for 5 min and diluted to 1 ml with water. The mixture was applied directly to a 1 ml column of ConA-Sepharose. The radiolabeled product of the reaction binds to the immobilized lectin and is eluted with 10 mM α-methylglucoside. The radiolabeled product eluted was quantified by removing portions from collected fractions and determining radioactivity by liquid scintillation counting.

**UDP-Fucα3,3Galβ1,4GlcNAcα1,3-fucosyltransferase—**UDP-Fucα3,3Galβ1,4GlcNAcα1,3-fucosyltransferase was assayed using fern leaf α-galactosidase and 35 μl of green coffee bean α-galactosidase and 35 μl of 100 mM phosphate buffer, pH 6.4. Incubations proceeded at 37°C for 24 h in a toluene atmosphere. The samples were then spotted directly for descending paper chromatography in the solvent system ethyl acetate/pyridine/acetic acid/water (5:5:1:3). After chromatography for 24 h, 1 cm strips of the lanes were removed for counting in liquid scintillation mixture (Scintiverse I from Fisher).

**UDP-GlcNAcβ3,4GlcNAcβ1,3, N-acetylgalcosaminyltransferase—**This enzyme transfers N-acetylgalcosamine in β1,3 linkage from UDP-GlcNAc to the terminal galactose residues of complex-type N-linked oligosaccharides (37–40). Our assays employed the asialo derivatives of biantennary acceptor glycopeptides from either fibrinogen or thymoglobin. Enzyme reaction was performed in 25 μl of 50 mM MnCl2, 5 mM ATP, and 0.2–0.8 mM acceptor glycopeptide in a final volume of 25 μl. In addition, the reaction mixtures contained 50 mM 1-N-acetyl-β-D-glucopyranosylamine to inhibit breakdown of products by endogenous β-hexosaminidases. After incubation, the mixture was boiled for 5 min and diluted to 1 ml with water. The mixture was applied directly to a 1 ml column of ConA-Sepharose. The radiolabeled product of the reaction binds to the immobilized lectin and is eluted with 10 mM α-methylglucoside. The radiolabeled product eluted was quantified by removing portions from collected fractions and determining radioactivity by liquid scintillation counting.

**UDP-GlcNAcβ3,4GlcNAcβ1,6-fucosyltransferase—**UDP-GlcNAcβ3,4GlcNAcβ1,6-fucosyltransferase was assayed using fern leaf α-galactosidase and 35 μl of green coffee bean α-galactosidase and 35 μl of 100 mM phosphate buffer, pH 6.4. Incubations proceeded at 37°C for 24 h in a toluene atmosphere. The samples were then spotted directly for descending paper chromatography in the solvent system ethyl acetate/pyridine/acetic acid/water (5:5:1:3). After chromatography for 24 h, 1 cm strips of the lanes were removed for counting in liquid scintillation mixture (Scintiverse I from Fisher).

**Methylation Analysis—**Oligosaccharides were methylated and hydrolyzed in strong acid using the method of Hakomori (43). The methylated galactose derivatives were analyzed by thin layer chromatography, using the methylation standards 2,3-dimethylgalactose, 2,3,6-trimethylgalactose, and 2,4,6-trimethylgalactose, as described previously (24).

**RESULTS**

**Agglutination of Cells by Anti-blood Type Antibodies and Binding of Radioiodinated G. simpliplicifolia-1 to F9 and RA/F9 Cells—**We initially examined the surface glycoconjugates of F9 and RA/F9 cells by studying the interactions of these cells with a number of plant lectins and anti-blood type reagents. We found that suspensions of both cell types were not agglutinated by anti-blood type A sera, but they were agglutinated by anti-blood type B sera (Fig. 1, A–D). These results suggest that the cell surface glycoconjugates contain the terminal sequence Galα1,3Gal-R, which has previously been found in surface glycoconjugates of many mouse cells and tissues (18–22).

To assess the efficiency of differentiation of F9 cells in response to 0.1 μM all-trans-retinoic acid, we examined immunofluorescent staining of F9 and RA/F9 cells with antibody to the SSEA-1 antigen. A majority of the F9 cells were brightly positive for the SSEA-1 antigen; however, after a 3-day treatment with retinoic acid, a majority of the cells were not noticeably positive for SSEA-1 (data not shown). In addition, we examined the agglutination of the cells by monoclonal anti-SSEA-1. The F9 cells were agglutinated by both 1:1,000

**α-Galactosyltransferase in F9 Teratocarcinoma Cells**

513
and 1:50,000 dilutions of antibody, whereas there was little, if any, agglutination of the retinoic acid-treated cells (Fig. 1, E and F). These results indicate that most of the F9 cells differentiated in response to retinoic acid.

To provide further evidence for the presence of the terminal α-linked galactose residues on surface glycoconjugates of F9 and RA/F9 cells, we determined the binding parameters of the cells with the plant lectin G. simplificofilia-I. Other investigators have shown that this lectin contains both A4 and B4 isolectins (44, 45). The A4 isolectin specifically binds to blood type A sequences, and binding is inhibited by N-acetyllactosamine; whereas the B4 isolectin specifically binds to blood type B determinants, and binding is inhibited by oligosaccharides containing terminal nonreducing α-linked galactose, such as raffinose (44). As shown in Fig. 2A, F9 cells bound the radioiodinated G. simplificofilia-I in a saturable manner. This binding was inhibited greater than 95% by the inclusion of 50 mM raffinose in the assay (data not shown). Scatchard analysis (Fig. 2B) of the binding data indicate the presence of 17 molecules bound per cell in a 1:1000 dilution of monoclonal anti-SSEA-1 (E and F). After mixing for 3 min at room temperature, the photographs were taken.

Binding of Radioiodinated Tomato Lectin to F9 and RA/F9 Cells—We have found recently that tomato lectin binds with high affinity to glycoconjugates containing the polycl-α-lactosamine sequence [3Galβ1,4GlcNAcβ1] (46). Because other investigators have suggested that differentiation of F9 cells is accompanied by decreases in the amount of poly-N-acetyllactosamine chains, we performed binding studies on the F9 and RA/F9 cells with radiiodinated tomato lectin. The binding of lectin to both cell types was saturable (Fig. 3A). The number of binding sites, as determined by Scatchard analysis, for F9 cells was 2.2 × 10^6 sites/cell with an apparent K_a = 4.6 × 10^6 M⁻¹. Similar binding experiments were performed with RA/F9 cells. There were approximately 50% more binding sites for the lectin in the RA/F9 cells (25 × 10^6 sites/cell; K_a = 3.6 × 10^6 M⁻¹) (Fig. 2A and B). Because the G. simplificofilia-I preparation contains both A4 and B4 isolectins, we performed similar binding experiments with purified G. simplificofilia-I-B4. We obtained similar numbers of binding sites for G. simplificofilia-I-B4, as that found for G. simplificofilia-I for both F9 and RA/F9 cells (data not shown). These data indicate that many surface glycoconjugates on both F9 and RA/F9 cells contain terminal α-linked galactose residues.

Assay of UDP-Galβ-d-Gal-α1,3-galactosyltransferase in F9 and RA/F9 Cell Extracts—The above results suggest that both the F9 and RA/F9 cells contain the enzyme UDP-Galβ-d-Gal-α1,3-galactosyltransferase, which adds terminal α-linked galactose residues to terminal β-linked galactose residues. This enzyme has recently been purified from calf thymus (47) and Ehrlich ascites tumor cells (48). These investigators demonstrated that the purified enzyme utilizes N-acetyllactosamine as an acceptor to synthesize the trisaccharide Galα1,3Galβ1,4GlcNAc. In addition, the enzyme utilizes a variety of other glycoconjugates containing terminal β-linked galactose residues.

We therefore chose to use N-acetyllactosamine as the acceptor in one method of assaying for the presence of the α-
galactosyltransferase. As an alternative method, we developed a rapid and sensitive assay for the enzyme using as an acceptor a biantennary complex-type N-linked oligosaccharide isolated from human fibrinogen. The fibrinogen glycopeptide was de-nacetylated to expose terminal β-linked galactose residues. After transfer of radioactive α-linked galactose from UDP-[3H]Gal to the glycopeptide substrate by α-galactosyltransferase, the radiolabeled product could be isolated quantitatively on a column of ConA-Sepharose and eluted from the column into vials by 500 mM α-methylglucoside (Fig. 4).

The activity of the enzyme in both F9 and RA/F9 extracts toward the glycopeptide acceptor was nearly saturated with approximately 0.8 mM glycopeptide acceptor (Fig. 5B) and protein concentration (up to 1 mg/ml) (Fig. 5C). The activity was entirely dependent on UDP-Gal. Greater than 75% of the UDP-Gal was recovered intact from both incubation mixtures after a 2-h assay (data not shown).

To examine the possibility that cell extracts contained soluble inhibitors or activators of the α-galactosyltransferase, we assayed the activity of the α-galactosyltransferase in mixtures of F9 and RA/F9 extracts. The amount of product formed in mixed extracts of RA/F9 and F9 cells close to that expected, based on the additive activities in the two preparations (Table I). Determination of the Structure of the Oligosaccharide Product of the UDP-Gal:β-D-Gal α1,3-galactosyltransferase—To confirm the structure of the product of the enzyme, we prepared [3H]galactose-labeled disaccharide acceptor. The acceptor was prepared by incubating UDP-Gal:β-D-GlcNAc-β1,4-galactosyltransferase with UDP-[3H]Gal and N-acetylglucosamine, and the disaccharide product was purified by high performance liquid chromatography as described under "Experimental Procedures."

Approximately 100,000 cpm of the radiolabeled acceptor disaccharide [3H]Galβ1,4GlcNAc was incubated with 30 μg of...
a-Galactosyltransferase in F9 Teratocarcinoma Cells

![Graph A](image1.png)

**Fig. 5.** UDP-Gal:β-D-Gal-α1,3-galactosyltransferase in F9 and RA/F9 cells as a function of glycopeptide acceptor concentration, time of incubation, and protein concentration. A, approximately 50 μg of protein extract from both F9 and RA/F9 cells in 1% Triton X-100 was incubated for 2 h at 37 °C in a complete reaction mix of 25 μl containing the indicated concentration of glycopeptide acceptor. The product was isolated as described under "Experimental Procedures" and as shown in Fig. 4. B, extracts of F9 and RA/F9 cells were incubated under standard assay conditions, as described in A for the indicated times, using 0.8 mM acceptor glycopeptide. C, the activity of the α-galactosyltransferase was determined as a function of protein concentration in extracts of F9 and RA/F9 cells using 0.8 mM acceptor glycopeptide in 2-h assays, as otherwise described in A. ■—■, F9; ○—○, RA/F9.

![Graph B](image2.png)

![Graph C](image3.png)

**Fig. 6.** UDP-Gal:β-D-Gal-α1,3-galactosyltransferase activity as a function of UDP-Gal concentration. Enzyme activity was assayed for 2 h in extracts of both F9 and RA/F9 cells using 0.2 mM acceptor glycopeptide and different concentrations of UDP-Gal as indicated. Upper line, RA/F9 extracts; lower line, F9 extracts.

![Graph D](image4.png)

**Fig. 7.** HPLC separation of the radiolabeled acceptor disaccharide N-acetylactosamine and the trisaccharide product of the UDP-Gal:β-D-Gal-α1,3-galactosyltransferase in F9 and RA/F9 extracts. The radiolabeled acceptor disaccharide [3H] Galβ1,4GlcNAc was incubated with extracts of either F9 or RA/F9 cells in the presence of UDP-Gal, and the product trisaccharide and acceptor disaccharide were separated by HPLC as described under "Results" and under "Experimental Procedures." The elution positions are shown for the standards 1, the disaccharide Galβ1,4GlcNAc; and 2, the trisaccharide Galβ1,4GlcNAcβ1,3Gal are shown.

Table I

<table>
<thead>
<tr>
<th>Enzyme source</th>
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<tr>
<td>F9 extract</td>
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</tr>
<tr>
<td>RA/F9 extract</td>
<td>6.94 ± 0.24</td>
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<tr>
<td>F9 extract + RA/F9 extract</td>
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(Expected 3.70)

![Table](image5.png)

**Table I**

UDP-Gal:β-D-Gal-α1,3-galactosyltransferase activity in mixed extracts of F9 and RA/F9 cells

Each assay contained 0.6 mM acceptor glycopeptide and either 0.053 mg of F9 extract or 0.036 mg of RA/F9 extract or a mixture of both extracts. Assays were conducted for 2 h at 37 °C and product was determined as described under "Experimental Procedures." The expected specific activity in mixtures in nmol/mg h was calculated from the separately determined activities in each extract. Each specific activity reported is the mean ± S.E. of two determinations.

![Graph E](image6.png)

**Fig. 7.** HPLC separation of the radiolabeled acceptor disaccharide N-acetylactosamine and the trisaccharide product of the UDP-Gal:β-D-Gal-α1,3-galactosyltransferase in F9 and RA/F9 extracts. The radiolabeled acceptor disaccharide [3H] Galβ1,4GlcNAc was incubated with extracts of either F9 or RA/F9 cells in the presence of UDP-Gal, and the product trisaccharide and acceptor disaccharide were separated by HPLC as described under "Results" and under "Experimental Procedures." The elution positions are shown for the standards 1, the disaccharide Galβ1,4GlcNAc; and 2, the trisaccharide Galβ1,4GlcNAcβ1,3Gal are shown.

RA/F9 incubation and approximately 600 cpm of product was obtained from the F9 incubation. The major peak eluting in the position of a disaccharide was the radiolabeled acceptor disaccharide.

The trisaccharide products from the F9 and RA/F9 cell extract incubations were isolated after HPLC, and its structure was analyzed by methylation analysis to confirm the linkage position of the terminal α-galactose residue. Methylation of the radioactive acceptor disaccharide and subsequent hydrolysis resulted in the recovery of all radioactivity as 2,3,4,6-tetramethylgalactose, as expected (Fig. 8A). Methylation of the trisaccharide product from F9 and RA/F9 cell extracts resulted in production of radioactive 2,4,6-trimethylgalactose (Fig. 8, B and C), which indicates that the trisaccharide product has the structure Galβ1,3[3H]Galβ1,4GlcNAc.

To determine whether the added galactose residue was in α-linkage, we treated both the disaccharide acceptor and the
**Fig. 8. Thin layer chromatography of methylated radioactive galactose residues.** The radiolabeled acceptor disaccharide $[^{3}H]Gal\beta1,4GlcNAc$ (A) and the trisaccharide product recovered after incubation of F9 cell extracts (B) and RA/F9 cell extract (C) with UDP-Gal and the acceptor disaccharide were methylated, and the methylated galactose residues were separated by thin layer chromatography. The lanes were divided into 0.5-cm sections and the distribution of radioactivity determined by liquid scintillation counting. The standards 2,4,6-trimethylgalactose and 2,3,4,6-tetramethylgalactose migrated 5.5 and 8.0 cm, respectively.

**Fig. 9. Descending paper chromatography of radiolabeled disaccharide acceptor and trisaccharide product before and after treatment with exoglycosidases.** A, the radioactive disaccharide acceptor treated with $\alpha$-galactosidase; B, the radioactive trisaccharide product not treated with enzyme; and C, the radioactive trisaccharide product treated with enzyme. The trisaccharide standard Gal$\beta1,4GlcNAc$Gal$\beta1,3Gal$ and the disaccharide Gal$\beta1,3GlcNAc$ migrated 23 and 30 cm, respectively.

The unexpected finding that F9 cells contain an $\alpha$-galactosyltransferase and that differentiation results in an increase in this activity suggested the possibility that many other glycosyltransferases might also be elevated. We therefore assayed the activities of a number of other enzymes. The choices of specific enzymes to assay were based on numerous observations by our own and other laboratories concerning the types of structures present in F9 cell glycoconjugates. The enzymes and their specific activities are reported in Table II. All reactions were linear with time of incubation up to 2 h and protein concentration up to 1 mg/ml extract in the assay. Recovery of radiolabeled sugar nucleotide donor was greater than 65% in all assays.

We chose to assay UDP-Gal$\beta$-d-GlcNAc-$\beta1,4$-galactosyltransferase because this activity is commonly found in most
The presence of the enzyme in the cells is correlated with the antecedent and competitive inhibition of the newly synthesized glycoconjugates. This enzyme is most likely involved in the elongation of poly-N-acetyllactosamine chains containing the type 2 backbone of the repeating disaccharide unit (11-13), which is consistent with our finding that F9 cells contain numerous surface binding sites for tomato lectin. Previous studies have suggested that the apparent size reduction of F9 glycopeptides might be due to altered biosynthesis of the poly-N-acetyllactosamine chains. Because we observed a decreased number of binding sites for tomato lectin in the RA/F9 cells compared to F9 cells, we assayed cell extracts for the enzymatic activity of the enzyme in the latter two tissues (47, 48). These results demonstrate that F9 cells contain relatively high levels of the enzyme UDP-Gal:β-D-Gal-α,1,3-galactosyltransferase and that differentiation of the F9 cells is accompanied by an increase in the apparent activity of the enzyme.

**TABLE II**

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Cell type</th>
<th>Specific activity (nmol/mg h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UDP-Gal:β-D-GlcNAc-β1,4-galactosyltransferase^a^</td>
<td>F9</td>
<td>20.2</td>
</tr>
<tr>
<td></td>
<td>RA/F9</td>
<td>18.6</td>
</tr>
<tr>
<td>UDP-Gal:GlcNAc:β-D-Gal-β3,1-N-acetylglucosaminyltransferase^a^</td>
<td>F9</td>
<td>0.111</td>
</tr>
<tr>
<td></td>
<td>RA/F9</td>
<td>0.130</td>
</tr>
<tr>
<td>GDP-Fuc:β-D-GlcNAc-α1,6-fucosyltransferase^a^</td>
<td>F9</td>
<td>0.098</td>
</tr>
<tr>
<td></td>
<td>RA/F9</td>
<td>0.096</td>
</tr>
<tr>
<td>GDP-Fuc:β-D-GlcNAc-α1,3-fucosyltransferase^a^</td>
<td>F9</td>
<td>0.171^4 0.207^6</td>
</tr>
<tr>
<td></td>
<td>RA/F9</td>
<td>0.216^6 0.273^6</td>
</tr>
<tr>
<td></td>
<td>PYS-2</td>
<td>&lt;0.020^6 &lt;0.020^6</td>
</tr>
</tbody>
</table>

^a Assayed using 20 mM GlcNAc acceptor.

^b Assayed using 0.6 mM glycopeptide acceptor (see Fig. 4).

^c Assayed using 0.6 mM glycopeptide acceptor (see Fig. 4).

^d Assayed using 1 mM asialofetuin acceptor.

^e Assayed using 10 mM N-acetyllactosamine acceptor.

These results demonstrate that the enzyme is present in F9 cells and has been reported in F9 cells (49). The activity of the enzyme in extracts of both F9 and RA/F9 cells was high in the range reported in other cell types by other investigators (50). In over a dozen experiments of this type we failed to find any significant differences in the activities of this enzyme in extracts from F9 and RA/F9 cells (Table II).

Several investigators have found that some F9 cell glycoconjugates contain the repeating disaccharide sequence [3Galβ1,4-GlcNAcβ1] in a poly-N-acetyllactosamine-type chain (11-13), which is consistent with our finding that F9 cells contain numerous surface binding sites for tomato lectin. Previous studies have suggested that the apparent size reduction of F9 glycopeptides might be due to altered biosynthesis of the poly-N-acetyllactosamine chains. Because we observed a decreased number of binding sites for tomato lectin in the RA/F9 cells compared to F9 cells, we assayed cell extracts for the enzymatic activity of the enzyme. UDP-GlcNAc:β-D-Gal-β3,1-N-acetylglucosaminyltransferase. This enzyme is most likely involved in the elongation of poly-N-acetyllactosamine chains containing the type 2 backbone of the repeating disaccharide chain [4GlcNAcβ1,3Galβ1]. The activity of the enzyme was determined by the assay shown in Fig. 4. The activity was detectable but not remarkably different in extracts from both cell types (Table II).

The enzyme GDP-Fuc:β-D-GlcNAc-α1,3-fucosyltransferase has recently been purified from F9 cells (42). In a previous study Muramatsu and Muramatsu (17) observed that retinoic acid induction of F9 cells resulted in a greater than 80% reduction in the activity of this enzyme. As a control for their assays, these authors demonstrated that extracts from the mouse parietal yolk sac carcinoma cell line 21-23. In support of our present findings, Ozawa et al. (56) reported that the antigenicity of some surface antigens in F9 cells is lost upon treatment with β-galactosidase. Thus, it is likely that the α-galactosyltransferase is present in many cells and tissues. A significant finding in our study is that the activity of the α-galactosyltransferase increases upon cellular differentiation, whereas the activities of many other glycosyltransferases are largely unaffected. These observations may have important implications for understanding the factors involved in regulating glycoprotein biosynthesis in the mouse embryonal carcinoma cells during differentiation. It is possible that competitive reactions between the α-galactosyltransferase and other enzymes may largely influence the structures of newly synthesized glycoconjugates.

There is precedent for the possibility that such competitive enzyme reactions occur and influence the structures of newly synthesized glycoconjugates (50). Enzyme competition has been shown to be important in the biosynthesis of mucin oligosaccharides in porcine liver transfers fucose to biantennary complex-type N-linked oligosaccharides lacking outer terminal galactose residues and containing terminal N-acetylgalcosamine residues. We therefore prepared this acceptor by removing the galactose residues from desialylated biantennary glycopeptides from fibrinogen by treatment with jack bean β-galactosidase, as described under "Experimental Procedures." The activity of the α1,6-fucosyltransferase with this desialylated and degalactosylated acceptor was determined using the identical assay shown in Fig. 4. The activity of the enzyme in F9 and RA/F9 extracts with this acceptor was 0.106 nmol/mg h and 0.994 nmol/mg h, respectively, which is comparable to the activity determined with the galactosylated acceptor shown in Table II.

**DISCUSSION**

These results demonstrate that F9 cells contain relatively high levels of the enzyme UDP-Gal:β-D-Gal-α,1,3-galactosyltransferase and that differentiation of the F9 cells is accompanied by an increase in the apparent activity of the enzyme. The presence of the enzyme in the cells is correlated with the expression of surface glycoconjugates containing terminal α-linked galactose residues, since the cells are agglutinated by anti-blood type B and are bound by G. simplicifolia-I.

Surface glycoconjugates containing terminal α-linked galactose residues have been found in many mouse cell lines (21, 53, 54), and terminal α-linked galactose residues are found on the oligosaccharide side chains of several soluble glycoproteins (21-23). In support of our present findings, Ozawa et al. (56) reported that the antigenicity of some surface antigens in F9 cells is lost upon treatment with β-galactosidase. Thus, it is likely that the α-galactosyltransferase is present in many cells and tissues. α-Galactosyltransferase activity has been found in murine peritoneal macrophages (19), calf thymus (47), and Ehrlich ascites tumor cells (48) and has recently been purified from the latter two tissues (47, 48). These enzymes appear to be similar to the α-galactosyltransferase in F9 cells, in that they transfer α-linked galactose to the terminal β-linked galactose of N-acetylgalactosamine. In this respect, these a-galactosyltransferases are different from the α-galactosyltransferase in human cells involved in synthesizing blood-type B determinants. The human-derived enzyme requires that the terminal β-linked galactose of the acceptor be substituted at the 2-position by α-linked fucose (50).

A significant finding in our study is that the activity of the α-galactosyltransferase increases upon cellular differentiation, whereas the activities of many other glycosyltransferases are largely unaffected. These observations may have important implications for understanding the factors involved in regulating glycoprotein biosynthesis in the mouse embryonal carcinoma cells during differentiation. It is possible that competitive reactions between the α-galactosyltransferase and other enzymes may largely influence the structures of newly synthesized glycoconjugates.

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case in α1,3-linkage (11, 42). This probably accounts for the high level of anti-SSEA-1-reactive material in F9 cells. As shown in the present study, there is a decrease in the expression of the SSEA-1 antigen upon retinoic acid-induced differentiation, which is in agreement with observations by others (8, 9). In contrast to the findings of Muramatsu and Muramatsu (17), however, we could not detect a decrease in the activity of the α1,3-fucosyltransferase upon differentiation. We do not presently have an explanation for these contradictory results. Nevertheless, our studies suggest that factors other than a decrease in the activity of the α1,3-fucosyltransferase activity may be involved in the observed decreased expression of the SSEA-1 antigen. Preliminary studies in our laboratory have shown that a high proportion of all complex-type Asn-linked oligosaccharides from both F9 and RA/F9 cells are bound by immobilized G. simplicifolia-I, which is consistent with the presence of high amounts of terminal α-linked galactose residues. Further detailed characterization of the glycoconjugates synthesized by the teratocarcinoma cell is in progress to allow a more comprehensive understanding of the biochemical regulation of glycosylation in the F9 and RA/F9 cells.

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REFERENCES