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Cell Surface and Golgi Pools of β-1,4-Galactosyltransferase Are Differentially Regulated during Embryonal Carcinoma Cell Differentiation

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β-1,4-Galactosyltransferase (GalTase) has two functionally distinct subcellular distributions. In the Golgi apparatus, GalTase participates in the glycosylation of secretory and membrane-bound glycoproteins, whereas on the cell surface it mediates specific aspects of intercellular adhesion. For this study, a murine GalTase clone was obtained by screening a λgt10 cDNA library made from F9 embryonal carcinoma cells with a heterologous bovine GalTase cDNA probe. The murine GalTase cDNA probe was used in conjunction with assays of GalTase activity to investigate the expression and distribution of GalTase during differentiation of F9 stem cells into secretory endodermal epithelium. During the initial phase of F9 cell differentiation, GalTase mRNA levels remained relatively constant; however, as differentiation progressed, as assayed by expression of the differentiation-specific marker laminin B1, GalTase mRNA levels and enzyme activity rose dramatically. Furthermore, subcellular fractionation of these cells showed that the increased GalTase levels were specifically associated with the Golgi apparatus, whereas GalTase specific activity on the plasma membrane remained constant. These results show that levels of cell surface and Golgi GalTase change relative to one another during F9 cell differentiation and suggest that these functionally distinct pools of GalTase are independently and differentially regulated.

Embryonal carcinoma (EC) cells are the malignant, pluripotential stem cells found in teratocarcinomas (13, 19). EC cells are able to differentiate into derivatives of all three embryonic germ layers in vivo and into specific terminally differentiated cell types in vitro (13, 19). When F9 EC cells are grown in monolayer culture in the presence of retinoic acid (RA), they differentiate into cells analogous to extraembryonic endoderm (13); addition of dibutyryl cyclic AMP (db cAMP) increases the efficiency of RA-induced differentiation (13). F9 cell differentiation is characterized by the transformation of small EC cells with high nucleus-to-cytoplasm ratios into large, secretory epithelial cells with extensive intracellular membranes and an extracellular basal lamina. F9 cell differentiation is accompanied by the loss of some cell surface antigenic markers, e.g., stage-specific embryonic antigen type 1, and increased expression of basal lamina constituents such as laminin. The biochemical and morphological changes induced in F9 cells by retinoids are irreversible and appear to be heritable (28, 29).

Since EC cells are analogous in many respects to cells of the preimplantation embryo, they have been a useful model for identification of cell surface components that mediate intercellular adhesions in the preimplantation embryo. In this regard, uvomorulin (similar to L-CAM, E-cadherin) (14) and β-1,4-galactosyltransferase (GalTase) (2, 27) are two distinct cell adhesion molecules that mediate a variety of cellular interactions, including F9 EC intercellular adhesion. These two cell adhesion molecules appear to operate sequentially during preimplantation development, with uvomorulin mediating the initial phases of morula compaction (14) and GalTase mediating the maintenance of preexisting cell adhesions, leading to blastocyst formation (2).

GalTase is found in two distinct subcellular pools, where it has different biological functions. On the cell surface, GalTase mediates intercellular adhesions by binding to terminal *N*-acetylglucosamine (GlcNAc) residues on multivalent lactosaminoglycan substrates, thereby bridging cells (B. Shur, Biochim. Biophys. Acta, in press). GalTase is also found in the Golgi apparatus, where it functions in the biosynthesis of complex glycoconjugates (30). During differentiation, EC cells undergo transformation from small, nonsecretory stem cells into large epithelial cells that synthesize an extensive extracellular matrix. In this study, we examined the expression of GalTase during EC cell differentiation and determined its subcellular distribution before and during endodermal differentiation. An F9 EC cell cDNA library was prepared, and a 3.1-kilobase (kb) GalTase partial cDNA clone was isolated. With this clone used as a probe, GalTase mRNA levels were shown to rise threefold during differentiation, coincident with increased mRNA levels for the differentiation marker laminin B1. The increase in GalTase mRNA levels paralleled an increase in GalTase enzymatic activity during differentiation. The increased GalTase activity was not, however, uniformly distributed between cell surface and Golgi pools. Subcellular fractionation of these cells showed that Golgi GalTase specific activity increased fivefold during the transformation of EC cells into epithelial cells, whereas the plasma membrane specific activity remained constant. Since a specific increase in Golgi GalTase was not accompanied by a parallel increase in plasma membrane GalTase, these functionally distinct GalTase pools appeared to be independently and differentially regulated.

MATERIALS AND METHODS

Cell culture. F9 EC cells were seeded at a density of 0.7×10^6 cells on gelatinized 100-mm-diameter culture dishes and maintained in Dulbecco modified Eagle medium (GIBCO

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Laboratories, Grand Island, N.Y.) supplemented with 15% heat-inactivated bovine calf serum (Hyclone, Logan, Utah) plus penicillin, streptomycin, and kanamycin (GIBCO). Cultures were maintained at 37°C in a humidified atmosphere of 10% CO_2 and were not allowed to exceed 80% confluency. Cells were dissociated with 2 mM EDTA in Ca^{2+}-Mg^{2+}-free Eagle balanced salt solution containing 2% chicken serum, washed, and collected by centrifugation (200 × *g*, 5 min, 23°C).

All-trans RA (Sigma Chemical Co., St. Louis, Mo.) was added to cell cultures (final concentration of 0.1 μM from a 1 mM stock prepared in ethanol and stored at −20°C) 24 h after seeding. Addition of RA was considered time zero. Cells were maintained in medium containing RA in the presence or absence of 1 mM db cAMP (Sigma). Medium was changed every 48 h, and cultures were not allowed to exceed 80% confluency. At 24-h intervals, cells were collected from 22 100-mm-diameter plates as described above, approximately 10% of the cells were assayed for total GalTase activity, and RNA was isolated from the remainder. Cells from 50 parallel cultures were dissociated for subcellular fractionation.

Maintenance of PYS cells was similar to that of F9 cells except that PSY cells were grown on untreated tissue culture dishes rather than on gelatin-coated surfaces.

Preparation of RNA. Total RNA was extracted from F9 EC and PSY cells (10, 16). Where indicated, poly(A)$^+$ RNA was isolated from total RNA by affinity chromatography on oligo(dT)-cellulose (Collaborative Research, Inc., Belford, Mass.) as described elsewhere (1).

Construction and screening of an F9 EC cell λgt10 cDNA library. DNA complementary to F9 EC cell poly(A)$^+$ RNA was prepared essentially as described by Gubler and Hoffman (12), using a commercial cDNA synthesis system (Amersham Corp., Arlington Heights, Ill.). After cDNA synthesis and electrophoresis on an agarose gel, double-stranded cDNA (>2.0 kb) was isolated by migration onto DE81 DEAE-cellulose. The eluted cDNA was treated with *Eco*RI methylase (New England BioLabs, Inc., Beverly, Mass.), and *Eco*RI linkers (Collaborative Research) were added. Excess linkers were removed by *Eco*RI digestion and gel filtration chromatography on a Sepharose CL-4B-200 (Sigma) column. The cDNA was ligated into the *Eco*RI restriction site of λgt10 and packaged by using Gigapack Plus packaging extracts (Stratagene, La Jolla, Calif.). The resulting bacteriophage library was plated on *Escherichia coli* C600hfl$^-$ cells and screened essentially as described previously (17) by hybridization with the 0.9-kb *Pst*I-*Kpn*I fragment of clone pLbGT-1, encoding bovine GalTase (provided by P. K. Qasba [23]). Restriction enzymes were purchased from Bethesda Research Laboratories, Inc. (Gaithersburg, Md.) or International Biotechnologies Inc. (New Haven, Conn.), T4 DNA ligase and DNA oligolabeling kit were purchased from Pharmacia Fine Chemicals (Piscataway, N.J.), and radioactive nucleotides were obtained from Dupont, NEN Research Products (Boston, Mass.).

Nucleotide sequence analysis. Selected restriction fragments were subcloned into the bacteriophage vector M13, and the DNA sequence was determined by the dideoxy-chain termination method as described elsewhere (20, 24). Reagents for subcloning and sequencing were purchased from International Biotechnologies.

RNA blot analysis. RNA samples and marker RNA were denatured by heating at 60°C for 15 min in a solution containing 50% (vol/vol) formamide, 6% (vol/vol) formaldehyde, 20 mM morpholinepropanesulfonic acid (MOPS; pH 7.0), and 5 mM EDTA and fractionated by electrophoresis on 1% agarose–6% formaldehyde gels. To confirm RNA concentrations and determine integrity, RNA was stained with ethidium bromide for UV visualization. For Northern (RNA) blot analysis, RNA was transferred to nitrocellulose (Schleicher & Schuell, Inc., Keene, N.H.) (17). Alternatively, total RNA bound to nitrocellulose by the slot blot method of Gasser et al (11) was used to quantitate relative levels of GalTase, laminin B1, and *ras* mRNAs. RNA bound to nitrocellulose was hybridized with ^{32}P-labeled cDNA inserts of 2.0 kb from the F9 EC cell GalTase cDNA clone described in this study, 1.1 kb from c-Ki-*ras*2 (Oncor), and 1.1 kb from the laminin B1 clone pPE47 (kindly provided by Brigid Hogan). Depending on the experiment, the RNA blots were hybridized separately with the individual probes or with all three cDNA probes pooled.

Hybridizations were done overnight at 42°C in a solution containing 10% (wt/vol) dextran sulfate, 4× SSC (SSC is 0.15 M NaCl plus 0.015 sodium citrate), 50% (vol/vol) formamide, 10 μg of denatured salmon sperm DNA per ml, 1× Denhardt solution (0.02% [wt/vol] Ficoll [Pharmacia], polyvinylpyrrolidone, bovine serum albumin), and 50 mM sodium phosphate (pH 7.0). After hybridization, the filters were washed once in 2× SSC containing 1% sodium dodecyl sulfate at room temperature and twice in 0.5× SSC containing 0.1% sodium dodecyl sulfate at 45°C. Hybridization was visualized by autoradiography, using Kodak XAR-5 film (Eastman Kodak Co., Rochester, N.Y.). To quantitate GalTase, B1 laminin, and *ras* mRNAs on the slot blots, the appropriate regions of the nitrocellulose paper were cut out, and the bound radioactivity was counted by liquid scintillation spectroscopy.

Total cell lysate. RA-treated or untreated F9 cells were dissociated as described above and washed twice in medium B (140 mM NaCl, 4 mM KCl, 20 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid [HEPES], pH 7.2) containing a cocktail of protease inhibitors (PIC; 2 μg of antipain per ml, 0.1% aprotinin, 10 μg of benzamidine per ml, 1 μg of chymostatin per ml, 1 μg of leupeptin per ml, 1 μg of pepstatin per ml). A total of 10^6 cells were resuspended in 2 ml of medium B-PIC and briefly homogenized in a Polytron PT 10-35 (setting 2; Brinkmann Instruments, Inc., Westbury, N.Y.). Broken-cell preparations were diluted with *n*-octylglucoside (final concentration, 30 mM; Sigma) to a concentration of 2.5 × 10^6 cells per ml. Cells were extracted for 60 min on ice, with frequent trituration. A 40-μl (approximately 4 μg of protein) amount of the solubilized lysate was used for each GalTase assay (see below).

Subcellular fractionation. A total of 50 × 10^6 RA-treated or untreated F9 cells were washed, suspended in 2 ml of 0.25 M sucrose–1 mM triethanolamine containing PIC, and homogenized in an ice slurry, using a Polytron PT 10-35 at setting 10 for 10 s, followed by 30 s at setting 6. Large debris and unbroken cells were removed by centrifugation (500 × *g*, 10 min). Total cellular membranes were collected from the postnuclear supernatant by centrifugation (33,000 × *g*, 60 min), and the pellet was suspended in 1 ml of 55% sucrose in 1 mM Tris hydrochloride, pH 8.0. Plasma membranes were separated from other cellular components on a continuous sucrose gradient as described by Wilcox and Olson (33). Briefly, the suspended pellet was layered over 0.5 ml of saturated sucrose and overlaid by a continuous sucrose gradient ranging from 25 to 45%. The gradient was centrifuged overnight at 4°C in an SW40 rotor at 75,000 × *g* and collected in 1-ml fractions, which were subsequently diluted with 8 ml of medium B-PIC and centrifuged (100,000 × *g*, 60

min, 4°C). Pellets were washed twice more in medium B-PIC and centrifuged at 33,000 × *g* for 30 min. Each membrane pellet was resuspended and divided into five equal portions for enzyme assays and protein determination.

Enzyme assays. Detergent-solubilized cell lysates or fractionated subcellular membranes were assayed for GalTase activity as described by Shur (26). Membrane samples containing 1 to 13 μg of protein were incubated at 37°C in a total volume of 50 μl of medium B-PIC containing 100 μM UDP-[^{3}H]Gal (574 dpm/pmol; Dupont, NEN), 3 mM 5′-AMP (to competitively inhibit UDP-Gal degradation), 10 mM $MnCl_2$, and 30 mM GlcNAc at 37°C. Except for the 1-h time courses, the reaction was terminated after 30 min by addition of 10 μl of ice-cold 0.2 M EDTA–Tris hydrochloride, pH 7.2. A 50-μl sample of the reaction mixture was subjected to high-voltage borate electrophoresis to separate the ^{3}H-labeled galactosylated product from unused UDP-[^{3}H]Gal and its breakdown products. The radiolabeled product remaining at the origin was quantitated by liquid scintillation spectroscopy. β-1,3-Galactosyltransferase activity was determined by using 0.25 mg of asialo bovine submaxillary mucin rather than GlcNAc as the substrate (18). Asialo bovine submaxillary mucin was prepared by the method of Carlson et al. (6). GalTase activity toward endogenous substrates (i.e., in the absence of added GlcNAc or asialo bovine submaxillary mucin) was subtracted from activity toward exogenous substrates. Alkaline phosphatase activity was determined spectrophotometrically, using 3.5 mM *p*-nitrophenyl phosphate as the substrate (4). Protein content was measured by the method of Bradford, using bovine serum albumin as the standard (3).

RESULTS

Isolation and characterization of an F9 EC cell GalTase cDNA clone. Our preliminary experiments (data not shown) and previous reports (23) indicated that the bovine GalTase cDNA clone hybridized strongly with a murine mRNA of approximately 4.4 kb and to a lesser extent with a 2.2-kb mRNA. A 0.9-kb *Pst*I-*Kpn*I fragment of the bovine clone containing the GalTase-coding sequence was used to screen 10^6 recombinants from an F9 EC cell λgt10 library for clones encoding murine GalTase.

One positive clone (GTcDNA-1) identified by hybridization with the bovine sequences was shown to contain three *Eco*RI fragments of 1.9, 0.85, and 0.35 kb, for a total insert size of approximately 3.1 kb. These fragments were individually subcloned into the *Eco*RI site of pUC19 for further characterization. When they were used individually as probes for hybridization to Northern blots of F9 EC cell poly(A)$^+$ RNA, each fragment hybridized strongly with the 4.4-kb message expected to encode murine GalTase (Fig. 1). Only the 1.9-kb fragment showed even a slight hybridization with the 2.2-kb mRNA species. Subsequent isolation of several clones that cross-hybridized with the untranslated region of clone GTcDNA-1 and encoded a 2.2-kb mRNA whose sequence was unrelated to that of GalTase (data not shown) suggested that this hybridization was due to repetitive sequences found in portions of the 3′ untranslated region of GalTase mRNA (23, 25).

Back-hybridization of these *Eco*RI fragments to Southern blots of specific restriction endonuclease digestions of the bovine clone showed that the 1.9-kb fragment contained sequences that hybridized with the translated region of bovine GalTase. DNA sequence analysis of this fragment in the region indicated on Fig. 2 showed that the sequence was

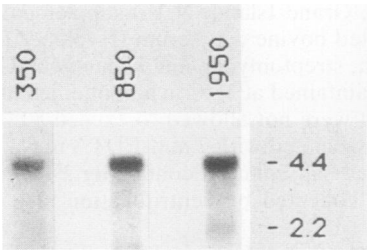

FIG. 1. Hybridization of ^{32}P-labeled *Eco*RI fragments from GalTase cDNA clone GTcDNA-1 to Northern blots of F9 EC cell poly(A)$^+$ RNA. Poly(A)$^+$ RNA (10 μg) from F9 EC cells, separated by electrophoresis on a 1% agarose–6% formaldehyde gel, was transferred onto a nitrocellulose membrane. The blots were hybridized separately with the ^{32}P-labeled 0.35-, 0.85-, and 1.9-kb *Eco*RI fragments of GTcDNA-1, washed, and exposed for autoradiography as described in Materials and Methods. All three fragments hybridized with the RNA indicated at 4.4 kb; only the 1.9-kb fragment hybridized (faintly) with the 2.2-kb RNA. Size markers were murine 28S and 18S rRNAs.

87% homologous to bovine GalTase at both the nucleotide and deduced amino acid sequence levels (data not shown).

Comparison of the restriction endonuclease map and the indicated partial nucleotide sequence of clone GTcDNA-1 with that of GalTase cloned from a mouse mammary cell line (25) showed these clones to be identical in all indicated regions and confirmed that our clone contained 3.1 kb of the 3′ sequence of the 4.4-kb mRNA expected for F9 EC cell GalTase mRNA (Fig. 2). We have since cloned and are completing sequence analysis of the remaining 5′ sequence of GalTase mRNA from a mouse mammary gland cDNA library (L. Lopez and B. Shur, manuscript in preparation).

Changes in GalTase mRNA and activity levels during F9 EC cell differentiation. The F9 GTcDNA-1 clone was used to determine GalTase mRNA levels in F9 cells that had been induced to differentiate by the addition of RA or RA plus db cAMP. RNA prepared from untreated or treated F9 cells was analyzed for specific mRNA expression by Northern blot analysis and quantitated by slot blot hybridization. The concentration and integrity of the RNA were analyzed by ethidium bromide staining and UV visualization (RNA from day 5 RA-treated cells degraded during RNA isolation and was eliminated from subsequent calculations). The levels of mRNA for the laminin B1 chain were used as a positive indicator for differentiation (29). The levels of mRNA for c-Ki-*ras* were determined as well, since upon differentiation, *ras* expression in F9 cells is reported to remain relatively unchanged or to decrease only slightly (5).

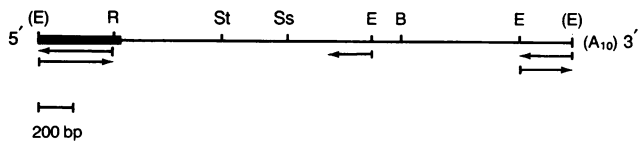

FIG. 2. Restriction map of the 3.1-kb F9 EC cell clone GTcDNA-1 and regions subjected to DNA sequence analysis. The box at the 5′ end represents the protein-coding region, and the thin line represents the entire 3′ untranslated region, terminating with a poly(A) tract. Arrows indicate the direction and extent of sequence determinations. Abbreviations for restriction endonuclease cleavage sites: B, *Bam*HI; E, *Eco*RI; (E), synthetic linker-derived *Eco*RI site; R, *Rsa*I; Ss, *Sst*I; St, *Stu*I. Not all of the *Rsa*I sites are shown. Other than *Rsa*I, the only sites shown are those used to compare the restriction map of GTcDNA-1 with that of another reported murine GalTase clone (25). bp, Base pairs.

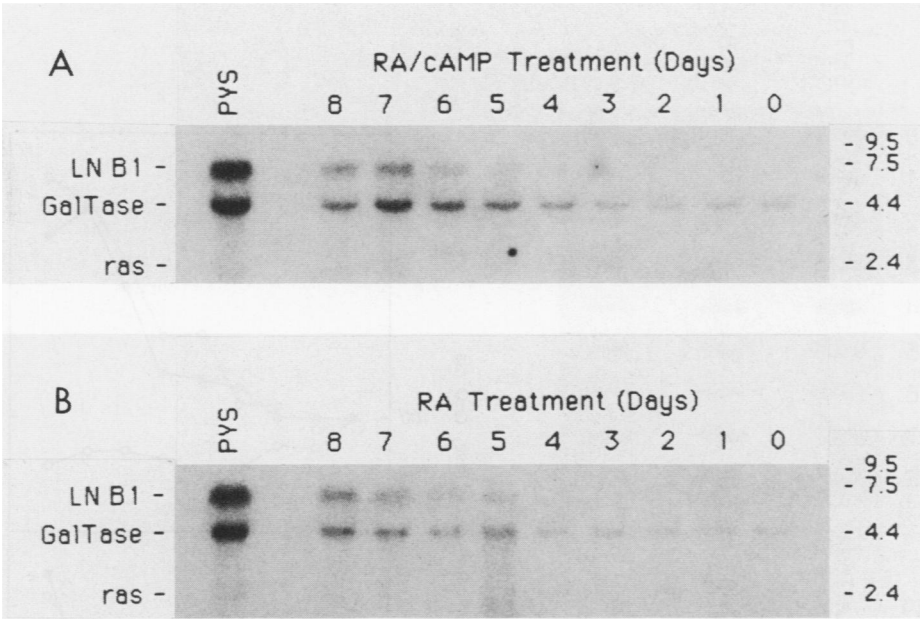

FIG. 3. Northern blot analysis of GalTase, laminin B1, and *ras* mRNAs during F9 EC cell differentiation. RNA was extracted at 1-day intervals from F9 EC cells that had been induced to differentiate for 0 (no treatment) to 8 days by addition of RA-db cAMP (A) or RA only (B). For comparative purposes, RNA from the terminally differentiated endodermal cell line PYS is included as well. Total RNA (10 μg) from each time point, separated by electrophoresis on a 1% agarose–6% formaldehyde gel, was transferred onto a nitrocellulose membrane. The blots were hybridized with the pooled ^{32}P-labeled GTcDNA-1, laminin B1, and *ras* cDNA probes, washed, and exposed for autoradiography as described in Materials and Methods. The expected migration positions of mRNAs for the laminin B1 subunit (6.0 kb), GalTase (4.4 kb), and *ras* (2.0 kb) are indicated on the left. Size markers (in kilobases) are from a commercially available RNA ladder.

Northern blot analysis of RNA from the untreated F9 cells showed that before RA or RA-db cAMP treatment, the 4.4-kb GalTase mRNA was clearly present, whereas the 6.0-kb laminin B1 mRNA was undetectable (Fig. 3). The 2.0-kb *ras* mRNA was not visible at this autoradiographic exposure but was detectable after longer exposures (not shown). Expression of these specific mRNAs throughout the 8 days of differentiation is shown in Fig. 3; for comparative purposes, results for the terminally differentiated endodermal cell line PYS are shown as well. When slot blot analysis was used to quantitate the levels of GalTase, laminin B1, and *ras* mRNAs, both RA and RA-db cAMP treatments induced the expected differentiation of F9 EC cells, as evidenced by the increased laminin B1 mRNA levels after approximately 4 days of differentiation (Fig. 4). Temporally, the increase in GalTase mRNA levels paralleled the results for laminin B1 in that the levels remained fairly constant during the first 3 days of treatment and then began to increase. Conversely, *ras* mRNA levels declined slightly during the first 2 to 3 days of differentiation, after which they remained constant. Similar results were obtained whether differentiation was induced with RA or RA-db cAMP.

The relationship between GalTase mRNA levels and GalTase enzymatic activity during differentiation was determined by assaying for GalTase activity in total cell lysates. GalTase specific activity in RA-treated F9 cells decreased to approximately one-third of control values during the first 3 days of differentiation, without a corresponding decline in GalTase mRNA levels (Fig. 5). The decrease in GalTase specific activity was not due to an increase in total cellular protein, since protein contents per cell were similar in uninduced and 3-day RA-treated cells (data not shown). On day 4 of treatment, GalTase activity began to rise in parallel to the increase in GalTase mRNA levels, as determined by Northern and slot blot analyses. Similar results were found for both RA- and RA-db cAMP-treated cells (RA-db cAMP data not shown).

GalTase subcellular distribution. Since GalTase is found both on the plasma membrane and in the Golgi apparatus, subcellular fractionation was used to determine the relative distribution of cell surface and Golgi GalTase during F9 cell differentiation. For these studies, differentiation was induced with RA only, since RA and RA-db cAMP treatments produced similar effects on GalTase mRNA and activity levels.

Golgi and plasma membranes were isolated by sucrose gradient centrifugation (33), and the identity and purity of these membrane populations were determined by enzyme markers. Alkaline phosphatase and β-1,3-galactosyltransferase have been reported to be plasma membrane and Golgi membrane markers, respectively, consistent with results of this study. These two membrane markers segregated cleanly from one another during fractionation (Fig. 6), occupying relative positions characteristic for plasma membranes and Golgi membranes, respectively (7, 9, 21, 31, 32). Furthermore, the utility of alkaline phosphatase as a surface marker for uninduced and differentiated cells was demonstrated by a coincident distribution of ^{125}I and alkaline phosphatase after Iodobead-catalyzed surface iodination (data not shown). GalTase specific activity in the plasma membranes (normalized per microgram of protein in each fraction) was calculated from fractions enriched for alkaline phosphatase and free of contaminating β-1,3-galactosyltransferase activity (i.e., fraction 5). Conversely, the Golgi GalTase activity was calculated from fractions enriched for the Golgi marker β-1,3-galactosyltransferase (i.e., fraction 11). The fractionation procedures used rely on the flotation of plasma membranes and Golgi membranes out of the total cellular mem-

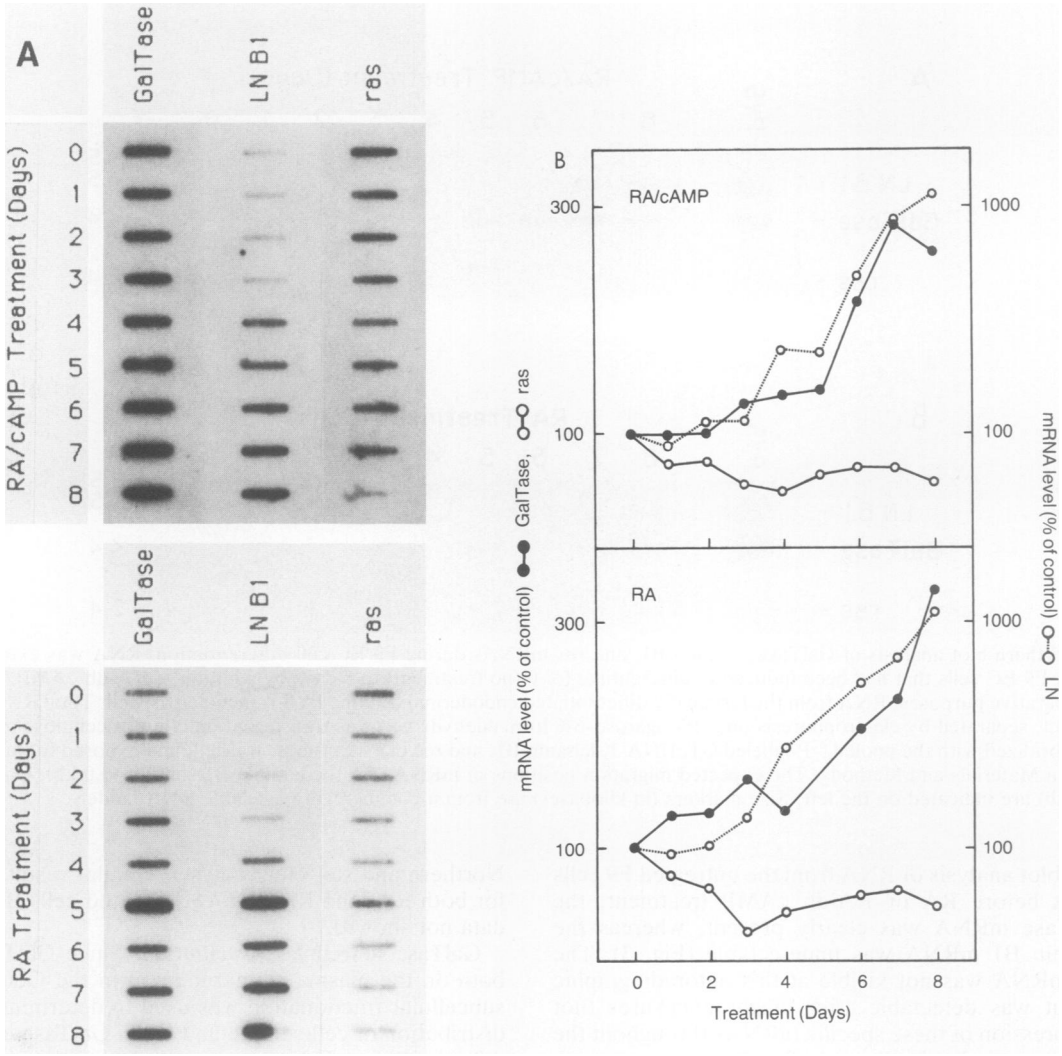

FIG. 4. Quantitation by slot blot analysis of GalTase, laminin B1, and *ras* mRNAs during F9 EC cell differentiation. RNA was extracted at 1-day intervals from F9 EC cells that had been induced to differentiate for 0 (no treatment) to 8 days by addition of RA-db cAMP or RA only. (A) Total RNA (10 μg) from each time point, which was applied to nitrocellulose filters by the slot blot method (in triplicate), hybridized individually with the ^{32}P-labeled GTcDNA-1, laminin B1, or *ras* cDNA probe, washed, and exposed for autoradiography. After a brief exposure, the appropriate regions of the nitrocellulose membrane were cut out, and the bound radioactivity was counted by liquid scintillation spectroscopy. (B) Expression of GalTase, *ras*, and laminin B1 (LN) mRNAs during F9 EC cell differentiation, expressed as a percentage of the control value (day 0, no treatment). Each point is the average of two experiments; panel A shows an autoradiograph of a representative experiment. Descriptions of probes and experimental conditions are given in Materials and Methods. Because of degradation of the RNA sample, results for day 5 of the RA-treated cells are not included on the graph.

brane pellet (i.e., fraction 12). However, the slower-moving, denser Golgi membranes were occasionally contaminated with small amounts of plasma membrane, as shown by the presence of alkaline phosphatase activity. This was particularly evident during fractionation of fully differentiated cells such as 8-day RA-treated F9 cells and PYS cells. However, since the GalTase specific activity of the plasma membrane was found to be insignificant relative to the Golgi specific activity in differentiated cells (Fig. 7), the small amount of plasma membrane contamination did not significantly affect the calculated Golgi specific activity.

The GalTase specific activities for plasma membrane and Golgi membranes isolated from untreated, 24-h RA-treated, and fully differentiated (i.e., 8-day RA-treated) F9 cells are shown in Fig. 7. For comparative purposes, results for the terminally differentiated endodermal cell line PYS are shown as well. GalTase specific activity rose dramatically in the Golgi apparatus coincident with differentiation, consistent with what would be expected of an actively secretory epithelial cell. However, despite the increase in Golgi GalTase, GalTase specific activity on the plasma membrane remained constant. (Specific activities in Fig. 5 are much lower than those in Fig. 7, since total cell lysates were assayed in the former and purified membranes were assayed in the latter.)

Subcellular fractionation of the membrane preparations resulted in a high degree of enrichment and segregation of membrane-specific markers (Fig. 6); consequently, the relative GalTase activities in specific intracellular compartments could be determined by normalization of plasma membrane

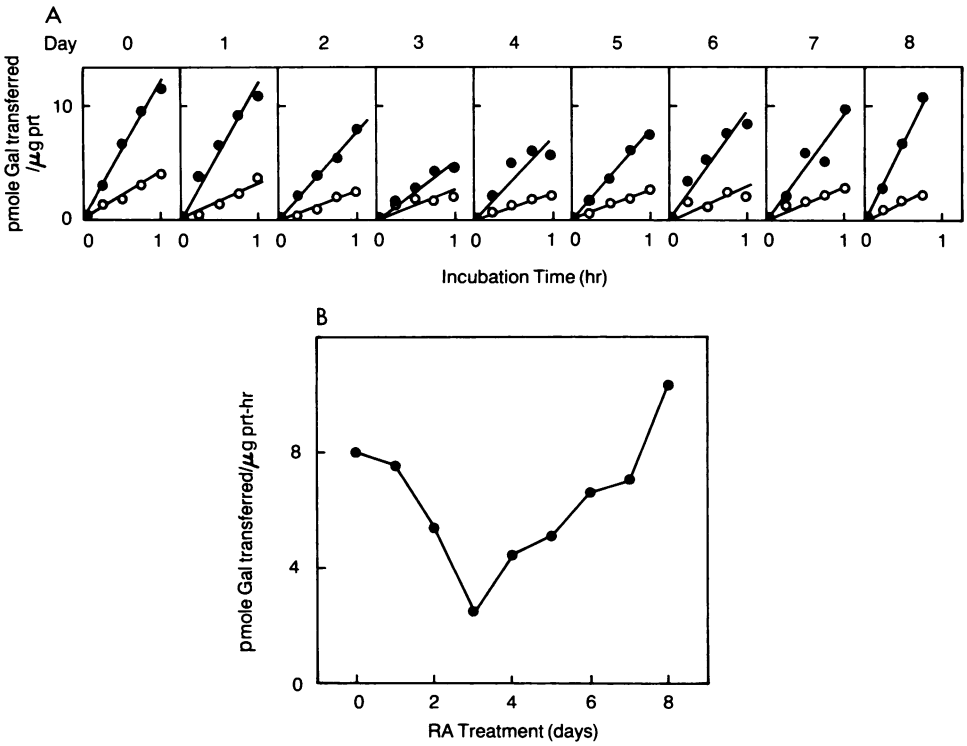

FIG. 5. GalTase specific activity during RA-induced differentiation of F9 cells. (A) GalTase activity measured in cell lysates in the presence (●) or absence (○) of added substrate (30 mM GlcNAc). Length of RA treatment is given above each panel. Each time course was conducted two to three times, with similar results. (B) Enzyme activity toward exogenous GlcNAc substrates during RA-induced differentiation of F9 cells, as calculated from the difference between slopes of the lines in panel A. Similar results were obtained when cells were induced with RA-db cAMP. prt, Protein.

GalTase activity to the plasma membrane marker alkaline phosphatase. This, in turn, permitted the percentage of total GalTase activity (as opposed to specific activity) in the plasma membrane to be calculated; by inference, GalTase partitioning between Golgi and plasma membranes could then be determined. During differentiation, there was a relative decrease in GalTase activity in the plasma membrane compared with the Golgi; in contrast to the 24% of total cellular GalTase associated with the plasma membrane of untreated-F9 EC stem cells, within 24 h of RA treatment less than 10% of GalTase activity was surface associated. This altered GalTase distribution was maintained in the fully differentiated state. After 8 days of induction, 8% of GalTase activity was found in the plasma membrane.

DISCUSSION

In this study, we examined the expression and subcellular distribution of GalTase during differentiation of F9 EC stem cells into secretory epithelial cells. To determined GalTase mRNA levels during differentiation, a GalTase cDNA clone was isolated from an F9 EC cell cDNA library, characterized, and used as a probe. On the basis of restriction endonuclease mapping and DNA sequence analysis, this clone was found to be identical to previously reported murine GalTases cloned from a mammary epithelial cell line and from F9 EC cells (22, 25).

GalTase mRNA levels remained stable during the initial phases of F9 cell differentiation, after which GalTase mRNA levels increased approximately threefold, concomitant with increased levels of laminin B1 mRNA. Although the increase in laminin B1 mRNA levels upon RA induction is thought to be associated with an elevated rate of transcription (15), we did not examine the rates of GalTase mRNA transcription or turnover in this study. That total GalTase enzymatic activity decreased during the initial phases of EC cell differentiation, although mRNA levels remained constant, suggests that either the translational expression of GalTase mRNA was down-regulated or turnover of the enzyme was enhanced. As differentiation progressed, however, enzyme activity increased in parallel with the level of GalTase mRNA. Subcellular fractionation studies indicated that the increased level of GalTase enzyme activity was due to a selective increase in GalTase associated with the Golgi apparatus; plasma membrane GalTase specific activity remained constant.

The results of this study are noteworthy for two reasons. First, the increased expression of GalTase mRNA and Golgi-associated enzyme activity provides biochemical and molecular evidence that EC cell differentiation into a secretory endodermlike epithelium involves the elaboration of an extensive Golgi network not present in EC stem cells. More significant, these studies provide evidence that the plasma membrane and Golgi pools of GalTase are independently regulated. Although RA-induced differentiation resulted in a fivefold increase in Golgi-associated GalTase, plasma membrane GalTase remained at constant specific activity. Clearly, the level of surface GalTase is not a reflection of the level of total intracellular GalTase activity. This finding argues against surface GalTase being a product of bulk nonspecific vesicular transport to the plasma membrane; if the level of cell surface GalTase were regulated by a simple partitioning mechanism, then increased GalTase activity in

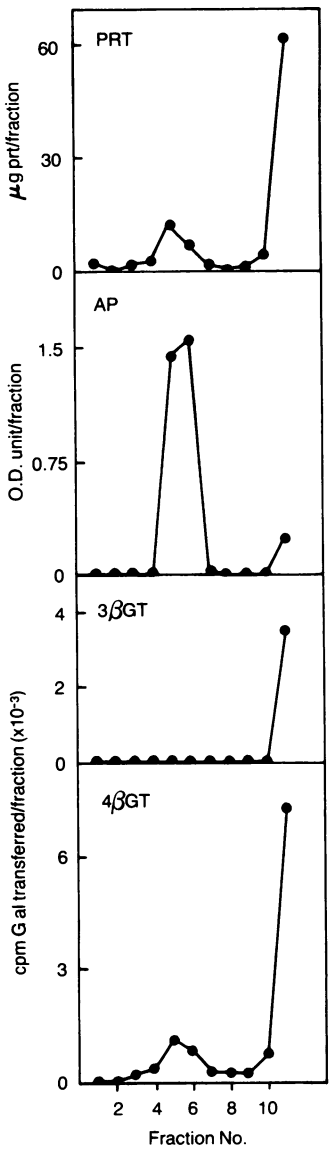

FIG. 6. Characterization of a continuous sucrose gradient for separation of membrane populations. Protein and enzyme activities in membanes from each fraction were determined as described in Materials and Methods. Depicted are protein (PRT) values, alkaline phosphatase (AP) activity (a plasma membrane marker), β-1,3-galactosyltransferase (3βGT) activity (a Golgi marker), and GalTase (4βGT) activity. Data shown are from a representative gradient of uninduced F9 cells. Increasing fraction number is indicative of increasing sucrose density. The bottom fraction of each gradient (i.e., fraction 12) contained residual, unfractionated, total cell membranes and is not included in the figure. O.D., Optical density.

the Golgi would result in a parallel increase in cell surface GalTase. Instead, our observations indicate that GalTase activity in the plasma membrane and in the Golgi are differentially regulated.

Two independent observations are consistent with this concept. First, it has been reported that isoproterenol treatment of the parotid gland induces a 40-fold increase in surface GalTase activity, whereas intracellular GalTase activity remains unaffected (18). Second, recent findings show that laminin, but not fibronectin, induces the recruitment of GalTase from intracellular pools onto cell surface lamellipodia, where it functions during cell migration on laminin (8). Collectively, these observations indicate that cell surface and Golgi pools of GalTase are under separate controls and suggest that the two forms of GalTase differ transcriptionally or posttranslationally or are regulated by other, as yet unidentified gene products that may differentially influence surface GalTase transport, stability, or turnover. In light of the recent identification of two related GalTase mRNAs that differ only in their 5′ extensions, it is possible that the surface and Golgi GalTases are encoded by separate mRNAs. These possibilities are now being investigated.

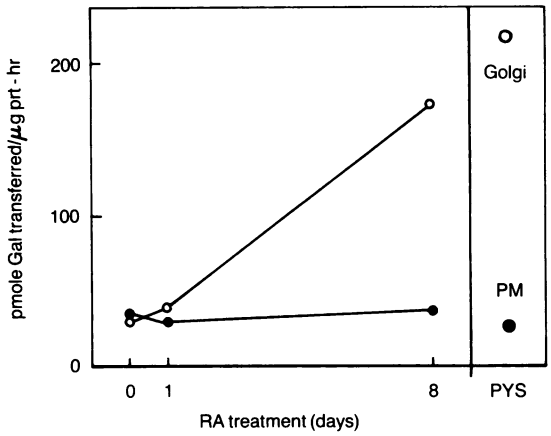

FIG. 7. GalTase specific activity in membrane subpopulations of induced and uninduced F9 and PYS cells. GalTase specific activity was determined in Golgi and plasma membrane (PM) populations for uninduced F9 cells (day 0), cells induced to differentiate by addition of RA for either 1 or 8 days, and for PYS cells. Data shown are from a representative experiment; at least four gradient fractionations were performed for each cell type. prt, Protein.

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