Lamp-1 does not acquire the large polylectosaminoglycans characteristic of F9 cells

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Although F9 cells labelled with [³H]glucosamine synthesize many glycoproteins that bind to *Datura stramonium* agglutinin-agarose, only a small proportion of these were immunoprecipitated with monoclonal antibodies to lamp-1 and lamp-2 (lamp = lysosomal membrane protein). Differentiation of F9 cells by retinoic acid increased labelling of all *Datura stramonium*-bound glycoproteins, including lamp-1 and lamp-2. Although the large polylectosaminoglycans excluded from Bio-Gel P-6 that are characteristic of F9 cells were obtained from total glycoproteins,

little of these large polylectosaminoglycans was found on lamp-1 and lamp-2. There was no increase in large polylectosaminoglycans of lamp-1 and lamp-2 after retinoic acid treatment, but an increase in the size of small polylectosaminoglycans (included on Bio-Gel P-6) and tri- and tetra-antennary complex oligosaccharides. Therefore, other factors besides the expression of specific glycosyltransferases determine the extent of elongation of polylectosaminoglycans on lysosomal membrane proteins.

INTRODUCTION

Polylectosaminoglycans are large oligosaccharides containing a variable number of repeating *N*-acetyl-lactosamine units (Gal- β 1,4-GlcNAc- β 1,3), which occur both *N*- and *O*-linked to proteins (for review, see Fukuda, 1985). These oligosaccharides carry blood-group antigenic determinants, developmental antigens and tumour-associated antigens. During embryonic development, antigenic determinants such as SSEA1 associated with polylectosaminoglycans (Childs et al., 1983) undergo specific programmed changes which are believed to participate in cellular interactions essential to normal embryogenesis. Aberrant expression of these developmental antigens on some tumour cells has been correlated with their metastatic properties (for reviews, see Feizi, 1985; Feizi and Childs, 1985; Muramatsu, 1988; Hakomori, 1989).

Studies on granulocytes (Fukuda et al., 1981) and related cells, such as chronic myelogenous leukaemia cells, HL60 cells (Carlsson et al., 1988; Lee et al., 1990) and mouse lymphoreticular MDAY-D2 tumour cells (Heffernan et al., 1989), showed that the lysosomal membrane proteins lamp-1 and lamp-2 are the major carriers of polylectosaminoglycans in these cells. Lamp-1 and lamp-2 are related highly glycosylated proteins of variable size around 110 kDa (for review, see Fukuda, 1991). The function of lamp-1 and lamp-2 is not known, but they are primarily located in lysosomal membranes with their carbohydrate moieties oriented toward the lumen of the lysosomes. Some studies indicate that lamp molecules are sometimes found at the cell surface, where they may play a role in intercellular reactions (Fambrough et al., 1988; Laferté and Dennis, 1988). Lamp-1 and lamp-2 glycosylation is affected when cells undergo differentiation, but the effects observed are cell-specific. Differentiation of HL-60 cells to granulocytes induced by dimethyl sulphoxide causes an increase in the number and length of polylectosaminoglycans on both lamp-1 and lamp-2 (Lee et al., 1990). In contrast, spontaneous differentiation of CaCo-2 human colonic adenocarcinoma cells to enterocytes results in a decrease in

polylectosaminoglycans which are primarily associated with lamp-1 and not with lamp-2 (Youakim et al., 1989).

Mouse F9 teratocarcinoma cells, which can be induced to differentiate to endoderm by retinoic acid, synthesize large polylectosaminoglycans that have been well characterized previously (Muramatsu et al., 1979a, b, 1983). Since lamp-1 and lamp-2 are the major carriers of polylectosaminoglycans in other cells, the effects of differentiation induced by retinoic acid on lamp-1 and lamp-2 glycosylation was examined to determine whether glycosylation of these lysosomal glycoproteins changes with differentiation of F9 cells. We report that, although F9 teratocarcinoma cells synthesize the expected large polylectosaminoglycans excluded from Bio-Gel P-6, few of these large oligosaccharides are associated with lamp-1, and only a small proportion is found on lamp-2. Retinoic acid causes an increase in shorter polylectosaminoglycans (included on Bio-Gel P-6) and in tri- and tetra-antennary complex oligosaccharides present on lamp-1 and lamp-2, as well as on other membrane glycoproteins. A preliminary report of this work has been published (Romero and Herscovics, 1990).

MATERIALS AND METHODS

Materials

The following materials were obtained from the companies indicated: Dulbecco's modified essential medium, fetal-calf serum, non-essential amino acids, trypsin and EDTA, Grand Island Biological Laboratories, Burlington, Ontario, Canada; penicillin and streptomycin, Flow Laboratories, McLean, VA, U.S.A.; D-[6-³H]glucosamine (sp. radioactivity 27 Ci/mmol), ICN Radiochemicals, Irvine, CA, U.S.A.; Pronase and aprotinin, Boehringer Mannheim Canada, Laval, Quebec, Canada; chitin, Pfanstiehl Laboratories, Waukegan, IL, U.S.A.; Bio-Gel P-6 (200–400 mesh), Bio-Rad Laboratories, Richmond, CA, U.S.A.; *Datura stramonium* agglutinin (DSA)-agarose, E-Y Laboratories, San Mateo, CA, U.S.A.; phenylmethanesulphonyl fluoride, leupeptin, pepstatin, chymostatin and *Lycopersicon*

Abbreviations used: DSA, *Datura stramonium* agglutinin; LEA, *Lycopersicon esculentum* agglutinin; m-lamp, mouse lysosomal membrane protein; NP-40, Nonidet P-40.

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esculentum agglutinin (LEA), Sigma Chemical Co., St. Louis, MO, U.S.A.; *Escherichia freundii* endo- β -galactosidase (sp. activity 3 units/mg), ICN Biomedicals Canada Ltd., Mississauga, Ontario, Canada; Protein A-Sepharose CL-4B, Pharmacia LKB Biotechnology AB, Uppsala, Sweden; Enhance, Dupont, Lachine, Quebec, Canada; all-*trans*-retinoic acid and Kodak X-OMAT AR film, Eastman-Kodak, Rochester, NY, U.S.A. The coupling of the LEA to the Sepharose was done as described by Merkle and Cummings (1987). Rat hybridomas 1D4B and ABL-93, secreting antibodies to m-lamp-1 and m-lamp-2 (IgG2a) respectively, were obtained from Dr. Thomas August, the Development Studies Hybridoma Bank, Johns Hopkins University, Baltimore, MD, U.S.A. An irrelevant monoclonal antibody of the same subtype and rabbit anti-rat antibody was provided by Dr. A. Fuks, McGill Cancer Centre, Montreal, Canada.

Cell culture

F9 cells obtained from Dr. H. Jakob (Institut Pasteur, Paris, France) were cultured in T75 flasks coated with gelatin in 20 ml of Dulbecco's modified essential medium (4.5 g/litre of glucose) containing 15% (v/v) fetal-bovine serum, 50 units/ml penicillin, 50 μ g/ml streptomycin and 4 mM L-glutamine. Cells were seeded at a density of $3\text{--}5 \times 10^5$ per flask and allowed to grow for 20–24 h. They were then incubated for 2–3 days in the same medium with or without 0.3 μ M retinoic acid. Higher concentrations of retinoic acid were toxic. The cells were then labelled for 24 h with 5 μ Ci/ml [3 H]glucosamine in 20 ml of low-glucose (1 g/litre) Dulbecco's modified essential medium with and without retinoic acid. After labelling, the medium was discarded, and the cells were washed with ice-cold PBS (8 mM Na_2HPO_4 , 1.5 mM KH_2PO_4 , 5.4 mM KCl, 137 mM NaCl, pH 7.4), scraped from the flasks, and resuspended in 10 ml of Tris/mannitol buffer (2 mM Tris/HCl, 50 mM mannitol), pH 7.1, containing a mixture of protease inhibitors (1 mM phenylmethanesulphonyl fluoride and 10 μ g/ml each of aprotinin, leupeptin, pepstatin and chymostatin). The cells were then disrupted by using a Dounce homogenizer with approx. 150–200 strokes. The homogenate was centrifuged at 2800 *g* for 10 min at 4 °C. The supernatant was centrifuged at 100 000 *g* for 60 min at 4 °C. The resulting pellet was extracted overnight at 4 °C with 0.5% Nonidet P-40 (NP-40) containing 0.02% NaN_3 , and the NP-40 extract obtained after centrifugation was used for subsequent analysis. The hybridomas were cultured as suggested by the supplier.

DSA-agarose affinity chromatography of labelled glycoproteins

Portions of labelled NP-40 extract (about 5×10^5 d.p.m. of trichloroacetic acid-precipitable material) were adjusted to a volume of 0.5 ml containing a final concentration of 0.5% NP-40 in 6.7 mM KH_2PO_4 /150 mM NaCl, pH 7.4, and 0.02% NaN_3 . The solution was mixed for 24 h at room temperature on a rotating apparatus with 0.1 ml of DSA-agarose, with or without 25 mg of chitin hydrolysate enriched in *NN'*-diacetylchitobiose and *NN'N''*-triacetylchitotriose. After incubation, the supernatant was removed, and the DSA-agarose was washed with 7×1 ml of the same NP-40 solution.

Immunoprecipitation of labelled glycoproteins

Portions [(2–4) $\times 10^5$ d.p.m. of trichloroacetic acid-precipitable material]] were incubated with continuous mixing at 4 °C for 1 h with 20 μ l of hybridoma culture supernatant containing monoclonal antibody to either lamp-1 or lamp-2 in a total volume of

250 μ l containing final concentrations of 0.1% gelatin, 0.5% NP-40, 5 mM EDTA, 150 mM NaCl and 10 mM Tris/HCl, pH 7.5. Then 15 μ l of rabbit anti-rat second antibody was added, and the mixture was incubated as above for 1–4 h. After incubation, 100 μ l of a 20% (v/v) suspension of Protein A-Sepharose equilibrated in the same buffer was added, and the suspension was incubated on a rotating apparatus for 1 h at 4 °C. After centrifugation in an Eppendorf centrifuge, the supernatant was removed and the pellet was washed 2–4 times with 20 mM Tris/HCl, pH 7.6, containing 2.5 mM KCl, 100 mM NaCl, 1 mM EDTA and 0.5% NP-40, and once with 20 mM Tris/HCl, pH 7.6. In some cases, sequential immunoprecipitation was done in which the supernatant obtained after immunoprecipitation with monoclonal antibody to lamp-2 was then treated with monoclonal antibody to lamp-1. Preliminary experiments with different concentrations of antibody established that these conditions were optimal for lamp-1 and lamp-2 immunoprecipitations.

Preparation and fractionation of labelled glycopeptides

Glycoproteins present in the NP-40 extract or isolated by immunoprecipitation were digested at 60 °C for 48 h with 1.25 ml of Pronase (20 mg/ml) in 100 mM Tris/HCl, pH 8, containing 1.5 mM CaCl_2 and 0.04% NaN_3 . Additional Pronase (0.625 ml) was added after 24 h. The Pronase had been preincubated at 37 °C for 2 h before use to inactivate any contaminating glycosidases. After Pronase treatment, the mixture was centrifuged (5000 *g*) and the supernatant was removed. The pellet was washed with 4×0.25 ml of water, and the washes were combined with the supernatant and boiled for 30 min. The labelled glycopeptides were fractionated on a column (1 cm $\times$ 110 cm) of Bio-Gel P-6 as described previously (Youakim et al., 1989); 1 ml fractions were collected and samples were assayed for radioactivity.

Lectin affinity chromatography and endo- β -galactosidase treatment of labelled glycopeptides

Glycopeptide fractions obtained from Bio-Gel P-6 were first fractionated on a small column (2 ml) of Concanavalin A-Sepharose as described previously (Romero and Herscovics, 1986). The labelled glycopeptides that were not retained on Concanavalin A-Sepharose were desalted on a column of Bio-Gel P-2 (1 cm $\times$ 45 cm), and then incubated with or without 20 m-units of *E. freundii* endo- β -galactosidase in 0.1 ml of 0.1 M sodium acetate buffer, pH 5.5, containing 0.02% NaN_3 . The samples were boiled for 5 min and were chromatographed on a 1 ml column of DSA-agarose or LEA-Sepharose, essentially as described previously (Youakim et al., 1989). The samples were applied in 0.5 ml of PBS (6.7 mM KH_2PO_4 /150 mM NaCl, pH 7.4) containing 0.02% NaN_3 . The columns were first eluted with the buffer (10 ml). The DSA column was then eluted with the same buffer containing 8 mg/ml chitin hydrolysate enriched in *NN'*-diacetylchitobiose and *NN'N''*-triacetylchitotriose and the LEA column was eluted with the buffer containing 20 mg/ml chitin hydrolysate enriched in *NN'N''*-triacetylchitotriose and *NN'N''N'''*-tetra-acetylchitotetraose (6–8 ml); 1 ml fractions were collected and assayed for radioactivity. The recovery from the affinity columns was greater than 90%.

SDS/PAGE analysis

The glycoproteins present in the immunoprecipitates or bound to DSA-agarose were solubilized by boiling in sample buffer, and a

portion was fractionated by SDS/PAGE on a 7%-polyacrylamide gel as described by Laemmli (1970). The gels were fixed for 1 h in methanol/acetic acid/water (3:1:6, by vol.), and were processed for fluorography with Enhance, dried, and exposed to Kodak X-OMAT AR film at -70°C .

RESULTS

Isolation of DSA-agarose-bound glycoproteins

To identify polylactosaminoglycan-containing glycoproteins, F9 teratocarcinoma cells were incubated with [^3H]glucosamine for 24 h, and labelled membrane-bound glycoproteins were extracted with NP-40. The incorporation of radioactivity into trichloroacetic acid-precipitable material was similar in control and in retinoic acid-treated cells. The NP-40 extracts were fractionated on DSA-agarose, which binds polylactosaminoglycans in addition to tri- and tetra-antennary complex oligosaccharides which contain lactosamine β -1,6- and β -1,2-linked to mannose (Cummings and Kornfeld, 1984; Yamashita et al., 1987). About 5% of the radioactivity of the NP-40 extract of control cells was bound to DSA-agarose, compared with about 13% of the radioactivity from retinoic acid-treated cells. Binding to DSA-agarose was greatly inhibited in the presence of chitin hydrolysate, indicating that the interaction with the lectin was carbohydrate-specific. The bound glycoproteins were then fractionated by SDS/PAGE. Several diffuse labelled protein bands were observed, ranging in size from about 70 kDa to above 200 kDa. The labelling of these glycoprotein bands was much more intense after retinoic acid treatment (Figure 1a). One of these bands migrated with an apparent molecular mass of about 110 kDa.

Figure 1 SDS/PAGE of [^3H]glucosamine-labelled glycoproteins isolated by affinity chromatography on DSA-agarose and by immunoprecipitation with antibodies to lamp-1 and lamp-2

(a) F9 cells were preincubated for 3 days with (lane 2) or without (lane 1) $0.3\ \mu\text{M}$ retinoic acid. The cells were then labelled for 24 h with $5\ \mu\text{Ci}/\text{ml}$ [^3H]glucosamine in low-glucose medium with or without retinoic acid, and fractionated as described in the Materials and methods section. A sample of NP-40 extract containing 5×10^5 d.p.m. of trichloroacetic acid-precipitable material was subjected to SDS/PAGE and fluorography. (b) F9 cells were preincubated for 2 days with (lanes 2 and 4) or without (lanes 1 and 3) $0.3\ \mu\text{M}$ retinoic acid. The cells were fractionated as described in the Materials and methods section, and a sample of NP-40 extract containing 4×10^5 d.p.m. of trichloroacetic acid-precipitable material was immunoprecipitated with antibodies to either lamp-1 (lanes 1 and 2) or lamp-2 (lanes 3 and 4). About one-half of the immunoprecipitated material was subjected to SDS/PAGE and fluorography. Exposure to film was 12–14 days. The position of standard molecular-mass markers (kDa) is indicated.

Immunoprecipitation with antibodies to lysosomal membrane proteins

To determine whether the labelled glycoprotein band of about 110 kDa contains lysosomal membrane proteins, the NP-40 extract was subjected to immunoprecipitation with monoclonal antibodies to m-lamp-1 and m-lamp-2. In control cells about 2.8% and 0.4% of the radioactivity in the NP-40 extract was associated with lamp-1 and lamp-2, respectively. After retinoic acid treatment the proportion of labelled immunoprecipitated glycoproteins increased to 7.1% and 0.9% in lamp-1 and lamp-2, respectively (Figure 1b). Similar results were obtained in two different experiments.

Characterization of glycopeptides obtained from glycoproteins extracted with NP-40

To demonstrate the presence of polylactosaminoglycans in F9 cells, the labelled glycoproteins in the NP-40 extract were exhaustively digested with Pronase, and the resulting glycopeptides were analysed sequentially by gel-filtration chromatography, and by DSA-agarose affinity chromatography before and after treatment with endo- β -galactosidase, which selectively cleaves lactosaminoglycans containing at least two lactosamine units (Fukuda and Matsumura, 1976). Labelled glycopeptides that bind to DSA-agarose and are sensitive to endo- β -galactosidase contain polylactosaminoglycans (Merkle and Cummings, 1987; Carlsson and Fukuda, 1990). The glycopeptides were first fractionated on a column of Bio-Gel P-6 (Figures 2c and 2f). About 45% of the labelled glycopeptides were excluded from the gel (Fraction I) in both control and retinoic acid-treated cells. Retinoic acid treatment caused an increase in size of the included glycopeptides of Fraction II compared with control cells. When the labelled glycopeptides of Fraction I from control cells were subjected to DSA-agarose affinity chromatography, about 36% of the radioactivity bound to the lectin. This was decreased to about 19% by treatment with endo- β -galactosidase (Table 1). After retinoic acid treatment, the proportion of radioactivity that bound to DSA-agarose was increased to 67%, and a much larger proportion of these oligosaccharides was sensitive to endo- β -galactosidase (Table 1). These results, together with previous detailed structural studies (Muramatsu et al., 1979a, b, 1983), confirm that the F9 cells being used in this study synthesize large polylactosaminoglycans (fraction I).

Characterization of glycopeptides obtained from lysosomal membrane glycoproteins

To determine whether the increased [^3H]glucosamine labelling of lamp-1 and lamp-2 observed after retinoic acid treatment is caused by a change in glycosylation of these glycoproteins after differentiation, the immunoprecipitated glycoproteins were digested with Pronase, and the glycopeptides were fractionated on Bio-Gel P-6. Surprisingly, most of the labelled glycopeptides obtained from lamp-1 were included in the gel, and only about 5% of the radioactivity was found in the excluded Fraction I in both control and retinoic acid-treated cells (Figures 2a and 2d). These results indicate that lamp-1 contains little of the large polylactosaminoglycans characteristic of F9 cells found in Fraction I of the NP-40 extract (Figures 2c and 2f).

After retinoic acid treatment, there was an increase in size of the included glycopeptides of Fraction II. Most of these labelled glycopeptides (75–80%) from both control and treated cells were not retained on Concanavalin A-Sepharose. They were fractionated on DSA-agarose, or on LEA-Sepharose, which binds

Figure 2 Bio-Gel P-6 chromatography of [^3H]glucosamine-labelled glycopeptides

F9 cells were preincubated for 3 days with (d, e, f) or without (a, b, c) 0.3 μM retinoic acid. The cells were then labelled for 24 h with 5 $\mu\text{Ci/ml}$ [^3H]glucosamine in low-glucose medium with or without retinoic acid. The cells were fractionated as described in the Materials and methods section, and different amounts of NP-40 extract were immunoprecipitated first with antibodies to lamp-2 (b, e), and then with antibodies to lamp-1 (a, d). The immunoprecipitates and untreated NP-40 extracts (c, f) were then digested with Pronase, and fractionated on Bio-Gel P-6. Fractions I and fractions II were pooled, as indicated. The left and right arrows indicate the elution positions of BSA and mannose respectively.

Table 1 DSA-agarose and LEA-Sepharose chromatography of [^3H]glucosamine-labelled glycopeptides

Lamp-1 glycopeptides (fractions II from Figures 2a and 2d) and fraction I glycopeptides obtained from the NP-40 extract (Figures 2c and 2f) that were not retained on Concanavalin A-Sepharose were fractionated on DSA-agarose or on LEA-Sepharose before and after treatment with endo- β -galactosidase (Endo- β -gal), as described in the Materials and methods section. When indicated, samples were hydrolysed with 2 M acetic acid at 100 $^{\circ}\text{C}$ for 1 h. Abbreviations: RA, retinoic acid; N.D., not determined.

Glycopeptide fraction	RA	Acid treatment	Endo- β -gal	Bound radioactivity (%)	
				DSA	LEA
Lamp-1 fraction II	—	—	—	2	N.D.
	—	+	—	2	N.D.
	+	—	—	19	5
	+	—	+	12	2
	+	+	—	21	7
	+	+	+	14	3
NP-40 extract fraction I	—	—	—	36	
	—	—	+	19	
	+	—	—	67	
	+	—	+	12	

glycopeptides containing at least three linear lactosamine units (Merkle and Cummings, 1987). In control cells, only 2% of the radioactivity from lamp-1 was bound to DSA-agarose. This amount increased to 19% after retinoic acid treatment. When lamp-1 glycopeptides from retinoic acid-treated cells were chromatographed on LEA-Sepharose, only about 5% of the radioactivity was bound to the lectin column. Mild acid hydrolysis under conditions that remove sialic acid (Merkle and Cumming, 1987) had little effect on the binding to either lectin column. Treatment with endo- β -galactosidase decreased binding to the

lectin columns (Table 1). These results indicate that lamp-1 contain polylectosaminoglycans with a small number of repeats, since they were included on Bio-Gel P-6, and that differentiation induced by retinoic acid causes an increase in the proportion of these short polylectosaminoglycans and of tri- and tetra-antennary complex oligosaccharides.

When the labelled glycopeptides obtained from lamp-2 were fractionated on Bio-Gel P-6, 33–37% of the radioactivity was found in large glycopeptides excluded from the gel (Fraction I), with little difference between control and retinoic acid-treated cells (Figures 2b and 2e). Retinoic acid treatment caused an increase in size of the included glycopeptides found in Fraction II (Figures 2b and 2e). Since incorporation of [^3H]glucosamine into lamp-2 was much lower than into lamp-1, there was insufficient radioactivity for further fractionation on DSA-agarose.

DISCUSSION

We have shown by DSA affinity chromatography of [^3H]glucosamine-labelled membrane glycoproteins that F9 cells synthesize many glycoproteins containing polylectosaminoglycans, ranging in size from about 70 kDa to over 200 kDa. Similar results were obtained by LEA affinity chromatography (P. A. Romero and A. Herscovics, unpublished work). Retinoic acid, which induces differentiation of F9 cells, causes an increase in the amount of labelled glycoproteins bound to DSA-agarose. Analysis of the labelled glycopeptides obtained from total membrane glycoproteins by gel filtration and DSA-agarose chromatography before and after endo- β -galactosidase treatment showed that large glycopeptides containing polylectosaminoglycans excluded from Bio-Gel P-6 are synthesized by the F9 cells used in the present study, as originally described by Muramatsu et al. (1979b). However, little of these large polylectosaminoglycans are found associated with the lysosomal membrane proteins, lamp-1 and lamp-2. After immunoprecipitation, only a small proportion of the labelled glycopeptides

derived from lamp-1 (5%) was recovered in the large polylectosaminoglycan fraction (Fraction I excluded from Bio-Gel P-6). Although a larger proportion of the labelled glycopeptides derived from lamp-2 (35%) was excluded from Bio-Gel P-6, the amount of labelled lamp-2 immunoprecipitated from these cells is very low, representing less than 1% of the total membrane-bound labelled glycopeptides of F9 cells. These results were unexpected, since, as indicated previously, the lysosomal membrane glycoproteins are major carriers of polylectosaminoglycans in other cells (Fukuda et al., 1981; Carlsson et al., 1988; Heffernan et al., 1989; Lee et al., 1990). These observations indicate that the elongation of oligosaccharides to form large polylectosaminoglycans on lysosomal membrane proteins is controlled by other factors besides the expression of the specific glycosyltransferases required for their synthesis, a conclusion similar to that reached in previous studies of CaCo-2 cells. In these cells, large polylectosaminoglycans excluded from Bio-Gel P-6 are present primarily on lamp-1. These were shown to decrease with differentiation (Youakim et al., 1989), but this decrease was not correlated with the activity of glycosyltransferases directly involved in elongation to polylectosaminoglycans (Brockhausen et al., 1991). It may be that the accessibility of the lysosomal membrane proteins to the specific glycosyltransferases involved in polylectosaminoglycan synthesis varies in different cells, due to differences in membrane localization of the enzymes and/or in the rate of transport of the lysosomal membrane proteins through the Golgi on their way to the lysosomes. To this effect, it has been demonstrated that decreasing the rate of transport through the Golgi by lowering the temperature causes an increase in both the number and the length of polylectosaminoglycans on lysosomal membrane proteins of HL60 cells (Wang et al., 1991).

In the present work, it is shown that retinoic acid has little effect on the small amount of large polylectosaminoglycans associated with lamp-1 and lamp-2, but causes an increase in the proportion of lamp-1 polylectosaminoglycans containing a relatively small number of repeating *N*-acetyl-lactosamine units (Fraction II endo- β -galactosidase-sensitive glycopeptides included on Bio-Gel P-6 that bind to DSA-agarose and LEA-Sepharose). The gel-filtration profile of labelled glycopeptides obtained from lamp-2 suggest that retinoic acid has a similar effect, but insufficient material was available for further characterization. A similar increase in polylectosaminoglycans associated with lamp-1 after retinoic acid treatment has been reported previously (Amos and Lotan, 1990; Heffernan et al., 1993), but the methods used in these studies do not separate polylectosaminoglycans according to size. They observed a somewhat greater effect of differentiation on polylectosaminoglycans associated with the lysosomal membrane proteins. These differences

may be due to the source of F9 cells or to the fact that the cells were not grown on gelatin-coated dishes. Furthermore, Heffernan et al. (1993) induced the cells to differentiate with a combination of retinoic acid and dibutyryl cyclic AMP. These conditions lead to differentiation of F9 cells to parietal endoderm rather than to primitive endoderm, as in the present work with retinoic acid alone (Strickland, 1981).

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