Cell-density-dependent changes in cell-surface glycopeptides and in adhesion of cultured intestinal epithelial cells

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We studied mannose-containing glycopeptides and glycoproteins of subconfluent and confluent intestinal epithelial cells in culture. Cells were labelled with D-[2-³H]mannose for 24 h and treated with Pronase or trypsin to release cell-surface components. The cell-surface and cell-residue fractions were then exhaustively digested with Pronase and the resulting glycopeptides were fractionated on Bio-Gel P-6, before and after treatment with endo- β -N-acetylglucosaminidase H to distinguish between high-mannose and complex oligosaccharides. The cell-surface glycopeptides were enriched in complex oligosaccharides as compared with residue glycopeptides, which contained predominantly high-mannose oligosaccharides. Cell-surface glycopeptides of confluent cells contained a much higher proportion of complex oligosaccharides than did glycopeptides from subconfluent cells. The ability of the cells to bind [³H]concanavalin A decreased linearly with increasing cell density up to 5 days in culture and then remained constant. When growth of the cells was completely inhibited by either retinoic acid or cortisol, no significant difference was observed in the ratio of complex to high-mannose oligosaccharides in the cell-surface glycopeptides of subconfluent cells. Only minor differences were found in total mannose-labelled glycoproteins between subconfluent and confluent cells by two-dimensional gel analysis. The adhesion of the cells to the substratum was measured at different stages of growth and cell density. Subconfluent cells displayed a relatively weak adhesion, which markedly increased with increased cell density up to 6 days in culture. It is suggested that alterations in the structure of the carbohydrates of the cell-surface glycoproteins are dependent on cell density rather than on cell growth. These changes in the glycopeptides are correlated with the changes in adhesion of the cells to the substratum.

In vivo, undifferentiated intestinal epithelial cells divide rapidly and migrate from the crypt to the villus as they differentiate. Differentiated cells lose the ability to divide and are detached at the tip of the villus. This continuous renewal of intestinal epithelial cells is associated with alterations in mannose-containing cell-surface glycopeptides obtained by Pronase treatment of the lateral-basal membranes; the ratio of labelled complex to high-mannose oligosaccharides is greater in crypt cells than in villus cells (Herscovics *et al.*, 1980). In other studies on the relationship between plasma-membrane glycoprotein structure and cell growth, differences in cell-surface glycoproteins between growing and non-growing cells in culture have been demonstrated (Muramatsu *et al.*, 1973; Ceccarini *et al.*,

1975; Muramatsu *et al.*, 1976; Hakimi & Atkinson, 1980). High-mannose oligosaccharides were predominant in glycopeptides derived from fast-growing cells, whereas glycopeptides from non-growing cells were enriched in complex oligosaccharides (Ceccarini *et al.*, 1975; Muramatsu *et al.*, 1976).

In the present study, we have compared cell-surface mannose-labelled glycopeptides obtained from subconfluent and confluent IEC-6 intestinal epithelial cells in culture and have examined the effect of growth inhibition with retinoic acid and cortisol. We found that alterations in cell-surface glycopeptides are not necessarily growth-dependent, but are density-dependent. Furthermore, a significant increase in adhesion of the cells to the substratum has been observed as the cells become confluent.

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Materials and methods

Cell culture

Rat small-intestinal epithelial cells (IEC-6 cells) were cultured in Dulbecco's minimal essential medium (with 4.5 g of glucose/l), containing 5% (v/v) foetal bovine serum, 10 µg of insulin/ml, 50 units of penicillin/ml, 50 µg of streptomycin/ml and 4 mM-L-glutamine, as previously described (Quaroni *et al.*, 1979), except that the medium was changed daily. For 60 mm- and 100 mm-diameter dishes, 4 ml and 10 ml of medium were used respectively. Cells were seeded at a density of about 0.2×10^5 – 0.3×10^5 cells/ml of medium. Cell growth was measured as described previously (Quaroni *et al.*, 1979). Retinoic acid (Sigma Chemical Co., St. Louis, MO, U.S.A.), 0.1 µg/ml in 0.1% ethanol (final concn.), was added to the medium 2 or 3 days after seeding, and ethanol alone was added to control dishes. Dishes were sheltered from light. Cortisol (0.6 µg/ml) was added to the medium 4 days after seeding. After 2 days of treatment with retinoic acid or 4–5 days of treatment with cortisol the medium was changed to low-glucose Dulbecco's medium and the cells were labelled with D-[2-³H]mannose.

Labelling of cells and preparation of glycopeptides

Subconfluent (about 30–40% confluent) and confluent cells grown in 100 mm dishes were labelled with D-[2-³H]mannose (5 µCi/ml; sp. radioactivity 16 Ci/mmol; Amersham, Arlington Heights, IL, U.S.A.) in 10 ml of low-glucose (1 g/l) Dulbecco's medium (Gibco, Grand Island, NY, U.S.A.) supplemented with dialysed 5% foetal calf serum, 50 units of penicillin/ml, 50 µg of streptomycin/ml, 10 µg of insulin/ml and 4 mM-L-glutamine. After 24 h the cells were washed with phosphate-buffered saline, pH 7.9 (0.1 g of CaCl₂/l, 0.2 g of KCl/l, 0.15 g of KH₂PO₄/l, 0.1 g of MgCl₂·6H₂O/l, 8 g of NaCl/l, 1.2 g of Na₂HPO₄/l), and incubated for 5 min at room temperature in 2 ml of phosphate-buffered saline, pH 7.9, containing 100 µg of Pronase CB (Calbiochem, San Diego, CA, U.S.A.)/ml to remove cell-surface material, except that the cells treated with cortisol and the corresponding control cells were treated with 4 ml of phosphate-buffered saline containing 0.1% trypsin (Difco, Detroit, MI, U.S.A.) and 0.05% EDTA for 10 min at room temperature. The extracted cell-surface material was removed with a Pasteur pipette and the cells were washed with 2 ml of phosphate-buffered saline, pH 7.9. Both supernatants were combined, and centrifuged for 5 min at 800 g to remove any detached cells. The resulting supernatant was used as a source of cell-surface glycopeptides. The cells were scraped from the dishes and extracted three times with chloroform/methanol/water (10:10:3, by vol.) to remove lipid-linked oligosaccharides. This material is

referred to as residue. Less than 4% of the radioactivity was extracted from the residue with chloroform/methanol/water. The cell-surface and residue preparations were exhaustively digested with 100 µg of Pronase CB/ml and 10 mM-CaCl₂ in phosphate-buffered saline, pH 7.9, for 3 days at 37°C in the presence of one drop of toluene. Pronase (100 µg/ml) was added every 24 h. Digestion was stopped by boiling for 3 min.

Radioactive sugars present in the glycopeptides after 24 h of labelling with D-[2-³H]mannose were determined as described by Spiro (1966). The glycopeptides were hydrolysed in 0.5 M-H₂SO₄ for 8 h at 100°C. Paper chromatography was performed in ethyl acetate/pyridine/water (8:2:1, by vol.) for 30 h. The migrations of sugars, expressed as *R*_{galactose}, were: glucose, 1.24; mannose, 1.62; fucose, 2.44. About 95% of radioactivity migrated as mannose and the remaining 5% as fucose.

Two-dimensional slab-gel analysis

Equilibrium isoelectric focusing (a gradient of pH 4.3–7.3) was carried out as described by O'Farrell (1975). Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis was carried out as described by Reid & Bielecki (1968) and 7.5% gels were prepared as described by Laemmli (1970). Fluorography was performed by the method of Bonner & Laskey (1974). Membrane preparations were prepared by homogenization of the labelled cells in 0.25 M-sucrose/0.5 mM-EDTA in 5 mM-histidine/imidazole buffer, pH 7.0, in a glass/Teflon Potter homogenizer. The homogenate was centrifuged at 1500 g for 15 min and the membranes were sedimented from the resulting supernatant at 42 000 g in rotor SW 50.1 for 2 h. The membranes were solubilized by boiling for 3 min in 50 mM-Tris/HCl, pH 6.8, containing 2% sodium dodecyl sulphate and 0.5 mM-MgCl₂.

[³H]Concanavalin A binding

Cells were grown on 60 mm-diameter dishes. The medium was removed and the cells were washed with phosphate-buffered saline, pH 7.1 (as described above, except that 0.2 g of KH₂PO₄/l and 1.14 g of Na₂HPO₄/l were present). Then 1 ml of phosphate-buffered saline, pH 7.1, and 10 µl (1 µCi) of [³H]concanavalin A (sp. radioactivity 27.8 Ci/mmol; New England Nuclear, Boston, MA, U.S.A.) were added. Cells were incubated with or without 20 mM-α-methyl D-mannopyranoside for 30 min at room temperature. The cells were then washed with phosphate-buffered saline and treated with 2 × 4 ml of cold 5% (w/v) trichloroacetic acid for 5 min and then with 2 × 4 ml of water. Next 1 ml of 0.1 M-NaOH was added for 10 min and the resulting

hydrolysate was used for protein and radioactivity determinations.

Cell-adhesion assay

Cells grown on 60 mm-diameter dishes obtained at different stages of growth were used. The medium was removed and the cells were washed with phosphate-buffered saline, pH 7.1. The cells were then incubated on a rotary shaker at 140 rev./min for 15 min in 1 ml of phosphate-buffered saline, pH 7.1, containing 100 μ g of trypsin [treated with 1-chloro-4-phenyl-3-L-tosylamidobutan-2-one ('TPCK'); Worthington, Freehold, NJ, U.S.A.]/ml which had been further purified by gel filtration (Codington *et al.*, 1970).

The detached cells were removed with a Pasteur pipette. The attached cells were treated with 1 ml of phosphate-buffered saline containing 100 μ g of 'TPCK'-treated trypsin/ml and the cells were scraped with a rubber policeman. The number of detached and attached cells was determined by using an electronic Coulter Counter.

Glycosidase treatment

Digestion with endo- β -N-acetylglucosaminidase H was carried out in 50 mM-citrate/phosphate buffer, pH 5.0, with 5 munits (1 enzyme unit represents 1 μ mol/min) of endo- β -N-acetylglucosaminidase H (Seikagaku Kogyo Co., Tokyo, Japan) for 16 h at 37°C. For this and subsequent incubations one drop of toluene was added.

Digestion with jack-bean α -mannosidase was carried out in 50 mM-sodium acetate buffer, pH 5.0, containing 0.2 mM-ZnSO₄, 0.1 M-NaCl and 0.1% bovine serum albumin. α -Mannosidase (Boehringer, Mannheim, W. Germany) (1.5 units) was added at 0, 5 and 19 h of incubation. Incubation was continued for a total of 25 h at 37°C. The enzyme preparation was contaminated with N-acetylglucosaminidase. The extent of contamination was determined by the manufacturer to be less than 1%.

Glycosidase treatments were terminated by boiling for 3 min and the products were analysed by gel filtration.

Results

Effect of retinoic acid on cell growth

A typical growth curve of IEC-6 cells is shown in Fig. 1. The cells grew exponentially starting 1 day after seeding and continuing up to 5–6 days in culture. After this time the growth rate gradually decreased and the cells reached confluency after 9–10 days in culture. Retinoic acid at a concentration of 0.1 μ g/ml completely inhibited cell growth after 48 h of treatment (Fig. 1). The effect of retinoic acid was concentration-dependent between 0.01 and 10 μ g/ml, and was not cytotoxic as

Fig. 1. *Effect of retinoic acid on cell growth*
Cells were seeded at a density of about 10⁵ cells/dish (60 mm diameter). After 3 days in culture 0.1 μ g of retinoic acid/ml in 0.1% ethanol (final concns.) was added. To control dishes only 0.1% ethanol was added. The arrow indicates the time when retinoic acid was added. The determinations were done on duplicate dishes. —, Control cells; ····, retinoic acid-treated cells.

determined by ⁵¹Cr release (May *et al.*, 1981). The treatment with 0.1 μ g of retinoic acid/ml also caused about 90% inhibition of [³H]thymidine uptake after 48 h.

Characterization of D-[2-³H]mannose-labelled glycopeptides

Subconfluent and confluent cultures were incubated with D-[2-³H]mannose for 24 h, and very mild Pronase treatment was employed to separate cell-surface from cell-residue mannose-labelled glycopeptides. Under these conditions very few cells were detached from the plastic surface and between 24 and 27% of the radioactivity in the glycopeptides was released from the cells. The total incorporation of label into subconfluent cells was about twice the incorporation into confluent cells, expressed per μ g of protein or per number of cells.

Fig. 2. Comparison between cell-surface and residue glycopeptides

Chromatography on Bio-Gel P-6 (200–400 mesh) of D-[2- 3 H]mannose-labelled glycopeptides from the cell surface (—) and from the residue (.....) of (a) subconfluent and (b) confluent cells. The glycopeptides were eluted from the column (1 cm $\times$ 116 cm) with 0.1 M-pyridine acetate, pH 5.0, containing 0.5 mM- NaN_3 . Flow rate was 4 ml/h and 0.72 ml fractions were collected. Bovine serum albumin was used to determine V_0 (void volume).

After extensive Pronase digestion the glycopeptides from the cell surface and from the residue of subconfluent and confluent cells were analysed by chromatography on Bio-Gel P-6 (Fig. 2). The cell-surface fractions contained a higher proportion of larger-sized glycopeptides (fractions 40–56) than did the residue fractions. This difference was particularly evident in confluent cells (Fig. 2b).

The comparison between cell-surface glycopeptides from subconfluent and confluent cells is shown in Fig. 3(a). The material from confluent cells contained a much greater proportion of the higher-molecular-weight glycopeptides than did the material obtained from subconfluent cells. Treatment with endo- β -N-acetylglucosaminidase H did not affect the elution of the larger glycopeptides (fractions 40–56, Fig. 3b), but it caused a shift of the elution of the lower-molecular-weight glycopeptides (fractions 57–68, Fig. 3a) to smaller molecular weight (fractions 64–80, Fig. 3b). These results indicate that the smaller glycopeptides obtained from both subconfluent and confluent cells contained high-mannose oligosaccharides. About 61% of the glycopeptides from the cell surface of subconfluent cells and about 32% of the glycopeptides from the cell surface of confluent cells were susceptible to endo- β -N-acetyl-

Fig. 3. Comparison between confluent and subconfluent cells

Bio-Gel P-6 column chromatography of D-[2- 3 H]-mannose-labelled glycopeptides from the cell surface of (—) subconfluent and (.....) confluent cells; (a) intact glycopeptides; (b) glycopeptides after endo- β -N-acetylglucosaminidase H treatment. Gel filtration was done as described in Fig. 2.

glucosaminidase H treatment. About 69% and 66% of the labelled glycopeptides obtained from the residue of subconfluent and confluent cells, respectively, was susceptible to this enzyme (results not shown). Treatment of the cell-surface glycopeptides with α -mannosidase followed by Bio-Gel P-6 chromatography showed the release of labelled mannose and the disappearance of glycopeptides containing high-mannose oligosaccharides. The higher-molecular-weight glycopeptides were not affected by that treatment. About 49% of the labelled mannose was released from the cell-surface glycopeptides of subconfluent cells, but only about 22% was released from the cell-surface glycopeptides of confluent cells. These data also show that the glycopeptides of higher molecular weight contain complex oligosaccharides, since they were not affected by endo- β -N-acetylglucosaminidase H and α -mannosidase. Furthermore, these glycopeptides could be metabolically labelled with [3 H]fucose. The fucose-labelled material was resistant to mild alkaline treatment (50 mM-KOH/1 M- NaBH_4 , 24 h, 45°C), indicating the absence of O-glycosidically bound oligosaccharides (results not shown).

Effect of retinoic acid and cortisol on cell-surface glycopeptides

Since retinoic acid completely inhibited growth of subconfluent cells after 2 days of treatment, and cortisol has also been shown to inhibit growth of these cells (Quaroni & May, 1980), their effects on the cell-surface glycopeptides were examined to

Fig. 4. Effect of retinoic acid and cortisol. Bio-Gel P-6 column chromatography of D-[2-³H]-mannose-labelled glycopeptides from the cell surface of (a) retinoic acid-treated cells and (b) cortisol-treated cells the growth of which had been inhibited for at least 2 days before labelling. The glycopeptides from control cells (—) and retinoic acid- or cortisol-treated cells (....) were subjected to the endo- β -N-acetylglucosaminidase H treatment. Gel filtration was done as described in Fig. 2, except that 1 ml fractions were collected in (b).

determine whether differences between subconfluent and confluent cells were growth-dependent. The elution pattern from Bio-Gel P-6 columns of mannose-labelled products obtained from the cell surface of control, retinoic acid- and cortisol-treated cells is shown in Fig. 4 after treatment with endo- β -N-acetylglucosaminidase H. No significant difference in the ratio of high-mannose to complex oligosaccharides was found in glycopeptides derived from either control or treated cells (Fig. 4).

Two-dimensional slab-gel analysis of D-[2-³H]-mannose-labelled glycoproteins

To determine whether the differences in carbohydrate structure observed between subconfluent and confluent cells were caused by glycosylation of different proteins as the cells became confluent, the total membrane glycoproteins were studied after labelling the cells with D-[2-³H]mannose. One-dimensional sodium dodecyl sulphate/polyacrylamide-gel electrophoresis showed about 12–14 radioactive bands, with four major labelled glycoproteins which had apparent molecular weights of 180 000, 105 000, 88 000 and 47 000. To improve

Fig. 5. Glycoproteins from confluent and subconfluent cells

Autoradiograms of two-dimensional slab-gel analysis of D-[2-³H]mannose-labelled glycoproteins from (a) subconfluent and (b) confluent cells. Isoelectric focusing (IF) was performed with pH 4.3–7.3 Ampholines and sodium dodecyl sulphate/polyacrylamide-gel electrophoresis (SDS) was carried out in 7.5% slab gel from the top (–) to the bottom (+) of the Figure. About 80 μ g and 250 μ g of protein from subconfluent and confluent cells, respectively was applied to the gels. Time of the exposure for autoradiographs was 1 month.

resolution, two-dimensional isoelectric focusing–sodium dodecyl sulphate/polyacrylamide-slab-gel analysis was used. The comparison between mannose-labelled glycoproteins from subconfluent and confluent cells is shown in Fig. 5. Two-dimensional gel analysis showed at least 40 radioactive spots, with at least 15 major components. Only minor differences, pointed out by the arrows, between the mannose-labelled glycoproteins from subconfluent and confluent cells were observed.

Concanavalin A binding

Since a difference in the composition of mannose-labelled glycopeptides between subconfluent

and confluent cells was evident, and since this difference was concentrated mainly in the cell-surface fraction, subconfluent and confluent cells should display a differential concanavalin-A-binding ability. The binding of [^3H]concanavalin A by IEC-6 cells attached to the dishes is shown at different stages of growth in Fig. 6. The ability to bind concanavalin A decreased with increasing cell density between 2 and 5 days in culture. Cells obtained after 1 day in culture bound about 3.5 times as much concanavalin A as did cells obtained after 5 days in culture. Although the cells after 5 days in culture were still growing, they bound relatively little concanavalin A. No significant difference was observed between growing cells after 5 days in culture and confluent non-growing cells after 12 days in culture.

Similar data were obtained when cells were scraped from the dishes and incubated as a suspension with [^3H]concanavalin A. A 4-fold decrease in concanavalin A binding was observed between days 3 and 5 of culture. However, cells in suspension bound about 12 times as much concanavalin A as did cells attached to the plastic dishes. Almost identical data were obtained when binding was expressed per number of cells. Binding of [^3H]concanavalin A to the cells was inhibited by 85–90% in the presence of 20 mM- α -methyl D-mannopyranoside.

Fig. 6. [^3H]Concanavalin A binding to IEC-6 cells. The cells attached to the dishes were washed with phosphate-buffered saline, pH 7.1, and then incubated with [^3H]concanavalin A for 30 min at room temperature. Each point represents an average of two determinations from duplicate dishes. Variations between separate determinations were in the range 2–9%.

Adhesion of cells to substratum

Since some cell-surface glycoproteins are thought to be involved in adhesion of cells to the substratum, it was decided to find out whether the observed changes in cell-surface glycopeptides between subconfluent and confluent cells are associated with changes in adhesion of IEC-6 cells. We found that a 30 min incubation with EDTA (0.2 g/l) in phosphate-buffered saline could remove only about 6% of the cells at day 2, and 3% of the cells at day 4 of culture. For that reason purified trypsin was used in the adhesion assay. As shown in Fig. 7, the adhesion of the cells to the substratum increased with increasing cell density. At day 2 of culture over 80% of the cells could be detached during 15 min of incubation, whereas at day 6 of culture only about 10% of the cells could be detached under identical conditions. Cell detachment was dependent on the trypsin concentration and the length of the trypsin treatment. At day 6 of culture, when no decrease in the growth rate was observed (Fig. 1), there was a dramatic increase in the adhesion of cells to the substratum. After 12 days in culture, confluent cells were only slightly more adherent than subconfluent cells after 6 days in culture and only about 4% of the cells could be detached in the standard adhesion assay.

Fig. 7. Adhesion of IEC-6 cells to the substratum. The cells were washed with phosphate-buffered saline and then incubated with shaking at 140 rev./min on a rotary shaker for 15 min in phosphate-buffered saline containing 100 μg of trypsin/ml. Both detached and attached cells were counted and the number of detached cells is expressed as a percentage of the total number of the cells. Each point represents an average of two determinations from duplicate dishes. Variations between separate determinations were in the range 6–15%.

Discussion

We have described differences in mannose-containing glycopeptides derived from the cell surface of confluent and subconfluent intestinal epithelial cells in culture. High-mannose oligosaccharides were predominant in glycopeptides obtained from the cell surface of subconfluent fast-growing cells, whereas glycopeptides from confluent non-growing cells were greatly enriched in complex oligosaccharides. These changes were observed mainly at the cell surface, since residue-derived glycopeptides contained approximately the same ratio of high-mannose to complex oligosaccharides. Although the types of oligosaccharides changed, only minor differences were found in the pattern of total mannose-labelled glycoproteins obtained from subconfluent and confluent cells. These results suggest the possibility that the cell-surface changes were mainly due to differences in the biosynthesis of the carbohydrate units, and not to the formation of different glycoproteins.

The evidence obtained also indicates that alterations at the cell surface of intestinal epithelial cells depends on cell density and not on the growth rate of the cells. When growth of subconfluent cells was completely inhibited with a non-toxic concentration of either retinoic acid or cortisol (Quaroni & May, 1980), the non-growing subconfluent cells still retained the same elution pattern of cell-surface glycopeptides as the growing subconfluent cells. Furthermore, concanavalin A binding was not related to growth rate, since there was a linear decrease in binding only during part of the exponential growth period of the cells (between 2 and 5 days of culture), and there was no significant difference in binding between growing subconfluent cells obtained after 5 days in culture and non-growing confluent cells obtained after 12 days in culture.

The present results also demonstrate that intestinal epithelial cells in culture undergo a marked change in adhesion, as measured by the ability of trypsin to detach the cells from the substratum. A very large increase in adhesion was observed with increased cell density. This change in cell adhesion can be correlated with the ability to bind concanavalin A and with the increased proportion of complex oligosaccharides at the cell surface.

Similar changes in the ratio of high-mannose to complex oligosaccharides in cell-surface glycopeptides have been described in human diploid fibroblasts (Ceccarini *et al.*, 1975; Muramatsu *et al.*, 1976). The glycopeptides obtained from Sindbis virus grown in rapidly dividing chick-embryo fibroblasts also displayed a greater proportion of larger-sized high-mannose oligosaccharides than did glycopeptides obtained from the virus grown in confluent non-growing cells (Hakimi & Atkinson, 1980). The above authors stressed the importance of

cell growth on the synthesis and/or metabolism of high-mannose oligosaccharides in the cell-surface glycoproteins.

In other studies, no difference was found in the mannose-labelled glycopeptides of a murine leukaemia virus glycoprotein (gp70) grown in subconfluent or confluent mouse embryo fibroblasts (Rosner *et al.*, 1980) and in the glucosamine-labelled glycopeptides obtained from Sindbis-virus glycoproteins grown in baby-hamster kidney (BHK) cells and chick-embryo fibroblasts at low and high cell density (Keegstra *et al.*, 1975).

Little is known about the factors which determine the ratio of complex to high-mannose oligosaccharides. The major pathway for the formation of both types of oligosaccharides involves the transfer of a common Glc₃Man₉GlcNAc₂ oligosaccharide precursor from dolichyl pyrophosphate to protein. After this transfer, processing of the oligosaccharide occurs to yield specific structures [for reviews see Parodi & Leloir (1979) and Hubbard & Ivatt (1981)]. However, under certain conditions shorter oligosaccharide-lipids can be formed, resulting in endo- β -N-acetylglucosaminidase-H-resistant oligosaccharides in the glycoproteins (Chapman *et al.*, 1979a,b; Kornfeld *et al.*, 1979; Turco, 1980; Rearick *et al.*, 1981). It remains to be determined, therefore, whether the increased complex oligosaccharides at the cell surface observed with increased cell density are caused by differences in the glycosylation mechanism. If this were the case, it could be due to a change in the oligosaccharide transferred to protein, and/or to differences in processing of the same oligosaccharide precursor in confluent and subconfluent cells. To this effect, it is noteworthy that a cell-density-dependent α -mannosidase has been reported (Robbins, 1979), but it is not known whether this enzyme is involved in processing.

Some cell-surface glycoproteins have been shown to play a role in the adhesion of a number of cell lines (Yamada *et al.*, 1976; Hahn & Yamada, 1979; Terranova *et al.*, 1980). A cell-surface glycoprotein which specifically binds high-mannose oligosaccharides has been implicated in cell-to-cell adhesion of teratocarcinoma stem cells (Grabel *et al.*, 1979). Mouse fibroblasts (3T3-12 cells) treated with retinoic acid display a change in mannose-labelled cell-surface glycopeptides along with a dramatic increase in adhesion to the substratum. The more adherent retinoic acid-treated cells express a larger proportion of complex oligosaccharides compared with untreated cells (Sasak *et al.*, 1980; Adamo *et al.*, 1979). In the intestine *in vivo*, undifferentiated epithelial crypt cells express more complex oligosaccharides in their lateral-basal membranes than do differentiated villus cells, and display strong adhesion to underlying basement membranes

(Herscovics *et al.*, 1980). Although the mechanism of adhesion is still poorly understood, it has been shown that a deficiency in complex oligosaccharides at the cell surface of ricin-resistant fibroblasts is associated with decreased adhesion to fibronectin and collagen (Pena & Hughes, 1978; Hughes, 1979).

In vivo, the carbohydrate groups on the cell surface of epithelial cells play an important role in binding of *Escherichia coli* by a mannose-specific lectin (Eshdat *et al.*, 1978). It is also possible that the oligosaccharides are important in the interactions between the cells and the basement membrane during the migration of the intestinal cells from the crypt to the villus.

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References

- Adamo, S., DeLuca, L. M., Akalovsky, I. & Bhat, P. V. (1979) *J. Natl. Cancer Inst.* **62**, 1473–1477
- Bonner, W. N. & Laskey, R. A. (1974) *Eur. J. Biochem.* **46**, 83–88
- Ceccarini, C., Muramatsu, T., Tsang, J. & Atkinson, P. H. (1975) *Proc. Natl. Acad. Sci. U.S.A.* **72**, 3139–3143
- Chapman, A., Trowbridge, I., Hyman, R. & Kornfeld, S. (1979a) *Cell* **17**, 509–515
- Chapman, A., Li, E. & Kornfeld, S. (1979b) *J. Biol. Chem.* **254**, 10243–10249
- Codington, J. F., Sanford, B. H. & Jeanloz, R. W. (1970) *J. Natl. Cancer Inst.* **45**, 637–647
- Eshdat, Y., Ofek, I., Yashour-Gan, Y., Sharon, N. & Mirelman, D. (1978) *Biochem. Biophys. Res. Commun.* **85**, 1551–1559
- Gabel, L. B., Rosen, S. D. & Martin, G. R. (1979) *Cell* **17**, 477–484
- Hahn, L.-H. E. & Yamada, K. (1979) *Proc. Natl. Acad. Sci. U.S.A.* **76**, 1160–1163
- Hakimi, J. & Atkinson, P. H. (1980) *Biochemistry* **19**, 5619–5624
- Herscovics, A., Bugge, B., Quaroni, A. & Kirsch, K. (1980) *Biochem. J.* **192**, 145–153
- Hubbard, C. S. & Ivatt, R. J. (1981) *Annu. Rev. Biochem.* **50**, 555–583
- Hughes, R. C. (1979) in *Glycoconjugates* (Schauer, R., Boer, P., Buddecke, E., Kramer, M. F., Vliegthart, J. F. G. & Wiegandt, H., eds.), pp. 414–415, Georg Thieme, Stuttgart
- Keegstra, K., Sefton, B. & Burke, D. (1975) *J. Virol.* **16**, 613–620
- Kornfeld, S., Gregory, W. & Chapman, A. (1979) *J. Biol. Chem.* **254**, 11649–11654
- Laemmli, U. K. (1970) *Nature (London)* **227**, 680–684
- May, R. J., Quaroni, A., Kirsch, K. & Isselbacher, K. J. (1981) *Am. J. Physiol.* in the press
- Muramatsu, T., Atkinson, P. H., Nathenson, S. G. & Ceccarini, C. (1973) *J. Mol. Biol.* **80**, 781–799
- Muramatsu, T., Koide, N., Ceccarini, C. & Atkinson, P. H. (1976) *J. Biol. Chem.* **251**, 4673–4679
- O'Farrell, P. H. (1975) *J. Biol. Chem.* **250**, 4007–4021
- Parodi, A. J. & Leloir, L. F. (1979) *Biochim. Biophys. Acta* **559**, 1–37
- Pena, S. D. J. & Hughes, R. C. (1978) *Nature (London)* **276**, 80–83
- Quaroni, A. & May, R. J. (1980) *Methods Cell Biol.* **21B**, 403–427
- Quaroni, A., Wands, J., Trelstad, R. L. & Isselbacher, K. J. (1979) *J. Cell Biol.* **80**, 248–265
- Rearick, J. I., Chapman, A. & Kornfeld, S. (1981) *J. Biol. Chem.* **256**, 6255–6261
- Reid, M. S. & Bielecki, R. L. (1968) *Anal. Biochem.* **22**, 374–381
- Robbins, A. R. (1979) *Proc. Natl. Acad. Sci. U.S.A.* **76**, 1911–1915
- Rosner, M. R., Grinna, L. S. & Robbins, P. W. (1980) *Proc. Natl. Acad. Sci. U.S.A.* **77**, 67–71
- Sasak, W., DeLuca, M., Dion, L. D. & Silverman-Jones, C. S. (1980) *Cancer Res* **40**, 1944–1949
- Spiro, R. G. (1966) *Methods Enzymol.* **8**, 5–7
- Terranova, V. P., Rohrbach, D. H. & Martin, G. R. (1980) *Cell* **22**, 719–726
- Turco, S. J. (1980) *Arch. Biochem. Biophys.* **205**, 330–339
- Yamada, K., Yamada, S. & Pastan, I. (1976) *Proc. Natl. Acad. Sci. U.S.A.* **73**, 1217–1221