# SUBCELLULAR COMPARTMENTALIZATION OF SACCHARIDE MOIETIES IN CULTURED NORMAL AND MALIGNANT CELLS

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## ABSTRACT

We studied subcellular localization of saccharide moieties in cultured normal and malignant cells fixed in paraformaldehyde and treated with a nonionic detergent, using lectins specific for various sugar residues as probes in fluorescence microscopy. In normal cells, concanavalin A and Lens culinaris agglutinin, specific for mannose-rich carbohydrate cores in glycoproteins, labeled the endoplasmic reticulum as a wide perinuclear region. Other lectins, on the other hand, stained the Golgi apparatus as a juxtanuclear reticular structure. A similar compartmentalization was also seen in all malignant cells studied, although the Golgi apparatus in these cells was distinctly vesicular in appearance. Our results indicate that saccharide moieties in both normal and malignant cells are similarly compartmentalized, and thus speak in favor of a unidirectional subcellular flow for both membrane and secreted glycoconjugates.

Several recent studies have suggested a common glycosylation pathway for both membrane and secretory glycoproteins in cells (12, 17, 18, 23). According to these studies, the core-region saccharide moieties of asparagine-linked oligosaccharides, mannose and N-acetylglucosamine with some glucose residues, are linked to the newly synthetized peptide moiety during the peptide synthesis in the cisternae of rough endoplasmic reticulum (cf. references 18, 22, and 23). Thereafter, the glycosyl moieties are further processed in the cisternae of the Golgi apparatus, where the terminal saccharides are also included (4, 12, 23). Other glycosylation events, e.g., generation of o-glycosidically linked saccharide moieties of glycoproteins and glycosylation of glycolipids, probably take place exclusively in the Golgi apparatus (4, 12, 13, 14, 22, 23). Mature glycoconjugates remain in intracellular membranes, are delivered to the cell surface, or are secreted (13).

A contrasting view has recently been provided by Dallner and his co-workers (1, 3, 24). In their view, mature glycoproteins could be delivered backward from the Golgi apparatus to the cisternae and to the cytoplasmic surface of the endoplasmic reticulum by a cytoplasmic intermediate (3, 24). In agreement with their findings, it has been claimed that terminal saccharides of glycoproteins also are located in glycoproteins of nuclear and endoplasmic reticulum membranes, at least in malignant cells (21, 31).

The aim of our study was to investigate whether saccharide moieties of cellular endomembranes can be visualized in cultured cells with fluoro-chrome-coupled lectins as probes. With the aid of a method allowing intracellular labeling of lectin-binding sites (10), we show that in both normal and malignant cultured cells saccharide moieties are similarly compartmentalized into different cell organelles, the terminal saccharides of glycoproteins being detectable only in the Golgi apparatus.

## MATERIALS AND METHODS

### Cell Culture

The following cells were studied: normal human embryonal and adult fibroblasts obtained from a local source, canine kidney

epithelial cells (MDCK), human fibrosarcoma (HT 1080) and rhabdomyosarcoma cells, SV40 virus-transformed human fibroblasts Wi38Va13 (Va13 cells), HeLa cells, and rat hepatoma cells (HTC). The malignant cells were obtained from the American Type Culture Collection (Rockville, Md.). The cells were cultured on glass coverslips in plastic petri dishes in either minimal essential medium or Roswell Park Memorial Institute 1630 medium supplemented with 10% fetal calf serum (Gibco Bio-Cult, Glasgow, Scotland) and antibiotics. In some experiments, the cells were incubated in the presence of 2 μg/ml of vinblastine sulphate (Eli Lilly Co., Indianapolis, Ind.) for 3 h and in the presence of 1 μM monensin (courtesy of Dr. R. Hamill, Eli Lilly Co.) for 2 h.

## Fluorescence Microscopy

For fluorescence staining, the cells grown on glass coverslips were fixed in 3.5% paraformaldehyde made in 0.1 M phosphate buffer, pH 7.2, washed in phosphate-buffered saline (PBS), and treated with 0.05% Nonidet NP40 (BDH Chemicals Ltd., Poole, England) for 30 min to make the cells permeable to fluorochrome-coupled lectins (10). Thereafter, the cells were thoroughly washed in PBS.

The lectins were purchased as fluorescein isothiocyanate (FITC) or tetramethylrhodamine (TRITC) conjugates. Concanavalin A (Con A, Miles Laboratories, Inc., Elkhart, Ind., Vector Laboratories, Burlingame, Calif.), and Lens culinaris agglutinin (LcA, Vector Laboratories) specific for mannose-rich cores in glycoproteins (5, 8, 28), wheat germ agglutinin (WGA, E-Y Laboratories, San Mateo, Calif., Vector Laboratories) reacting both with sialic acids and with N-actylglucosamine moieties of glycoconjugates (5), Ricinus communis agglutinin 120 (RCA 120, Miles Laboratories, E-Y Laboratories), Helix pomatia agglutinin (E-Y Laboratories) reacting with galactose and N-acetylgalactosamine moieties (5), Ulex europaeus agglutinin I (UEAI, E-Y Laboratories), Lotus tetragonolobus agglutinin I (LTA I, Sigma Chemical Co., St. Louis, Mo.) specific for L-fucosyl groups in glycoproteins (5), and Limulus polyphemus agglutinin (LPA, E-Y Laboratories) most specific for sialic acid moieties (5) were used in fluorescence stainings at concentrations of 100–150 μg/ml for 30 min in PBS supplemented with $Ca^{++}$ and $Mg^{++}$. The specimens were thereafter embedded either in sodium-barbital-glycerol, pH 8.4, or in PBS (RCA 120).

Lectin staining in fluorescence microscopy could be completely inhibited by preincubation of the lectin conjugate with the respective haptenic mono- or disaccharide (0.2 M in PBS, for 30 min): Con A and LcA with α-methyl mannoside (Sigma Chemical Co.), WGA with N-acetylglucosamine (Sigma Chemical Co.), RCA 120 with D-galactose (Merck AG, Darmstadt, W. Germany), HPA with N-acetylgalactosamine (Sigma Chemical Co.), UEAI and LTAI with L-fucose (Sigma Chemical Co.), and LPA with N-acetylneuraminic acid lactose (Sigma Chemical Co.).

To locate cellular fibronectin by the indirect immunofluorescence technique, specific rabbit antifibronectin antibodies were used (6; courtesy of Prof. A. Vaheri). FITC- and TRITC-coupled sheep anti-rabbit IgG antibodies were from Statens Bakteriologiska Laboratoriet, Stockholm, Sweden. Thiamine pyrophosphatase activity was shown in paraformaldehyde-fixed cells by the method of Novikoff and Goldfisher (16).

A Zeiss Universal microscope equipped with an epiilluminator (IIIRS) and filters for FITC and TRITC fluorescence was used for fluorescence microscopy. The absence of autofluorescence was confirmed by the use of ultraviolet and green excitation light. Agfapan Professional 400 film was used for photography.

## RESULTS

When cultured, paraformaldehyde-fixed human embryonal or adult fibroblasts were stained with FITC–Con A or FITC-LcA after detergent treatment, a bright perinuclear cytoplasmic staining could be seen (Figs. 1 and 3). Similar cytoplasmic staining was seen also when the cells were stained for indirect immunofluorescence microscopy (IFL) with antifibronectin antibodies (Fig. 2). However, when the cells were stained with any other lectin conjugate, a distinctly different juxtanuclear reticular structure was decorated, as was seen after double-staining of cultured embryonal fibroblasts with FITC–Con A and TRITC-WGA (Figs. 3 and 4) or with TRITC–RCA 120 (Fig. 5) or after staining of MDCK cells with FITC-HPA (Fig. 6).

Perinuclear staining similar to that seen in normal cells with FITC–Con A and FITC-LcA was also seen in all malignant cells studied (Fig. 7; HT 1080 cells). Analogous to the staining in normal cells, other lectins also gave a bright but distinctly vesicular juxtanuclear staining in malignant cells, as is shown with TRITC-WGA (Fig. 8; HT 1080 cells), with FITC-HPA (Fig. 9; HeLa cells), and with TRITC-UEAI (Fig. 10; Va13 cells). Interestingly, only the malignant cell lines could be stained with lectins specific for fucosyl moieties UEAI and LTAI.

To further characterize the observed lectin-staining patterns, cultured fibroblasts were treated with puromycin (2 μg/ml for 2 h), which is known to deplete cells of their secretory proteins (32). After this treatment, FITC–Con A still gave a perinuclear cytoplasmic staining, although diminished in intensity (Fig. 11), whereas, in indirect IFL with antifibronectin antibodies, only a fibrillar cell-surface staining was seen in the same cells (6, 32; Fig. 12). Also, the juxtanuclear labeling obtained with other lectins resisted this treatment in both normal and malignant cells.

Treatment of cultured embryonal fibroblasts with vinblastine sulphate (10 μg/ml for 3 h) or monensin (1 μM for 2 h) had no influence on the staining pattern obtained with FITC–Con A (Figs. 13 and 15), whereas the juxtanuclear reticular fluorescence seen with other lectins changed to a distinctly vesicular appearance (Figs. 14 and 16), an alteration typical of the Golgi apparatus after these treatments (15, 24–26). In line with this phenomenon, the staining patterns obtained with lectins other than Con A or LcA codistributed with the cytochemical reaction of thiamine pyro-
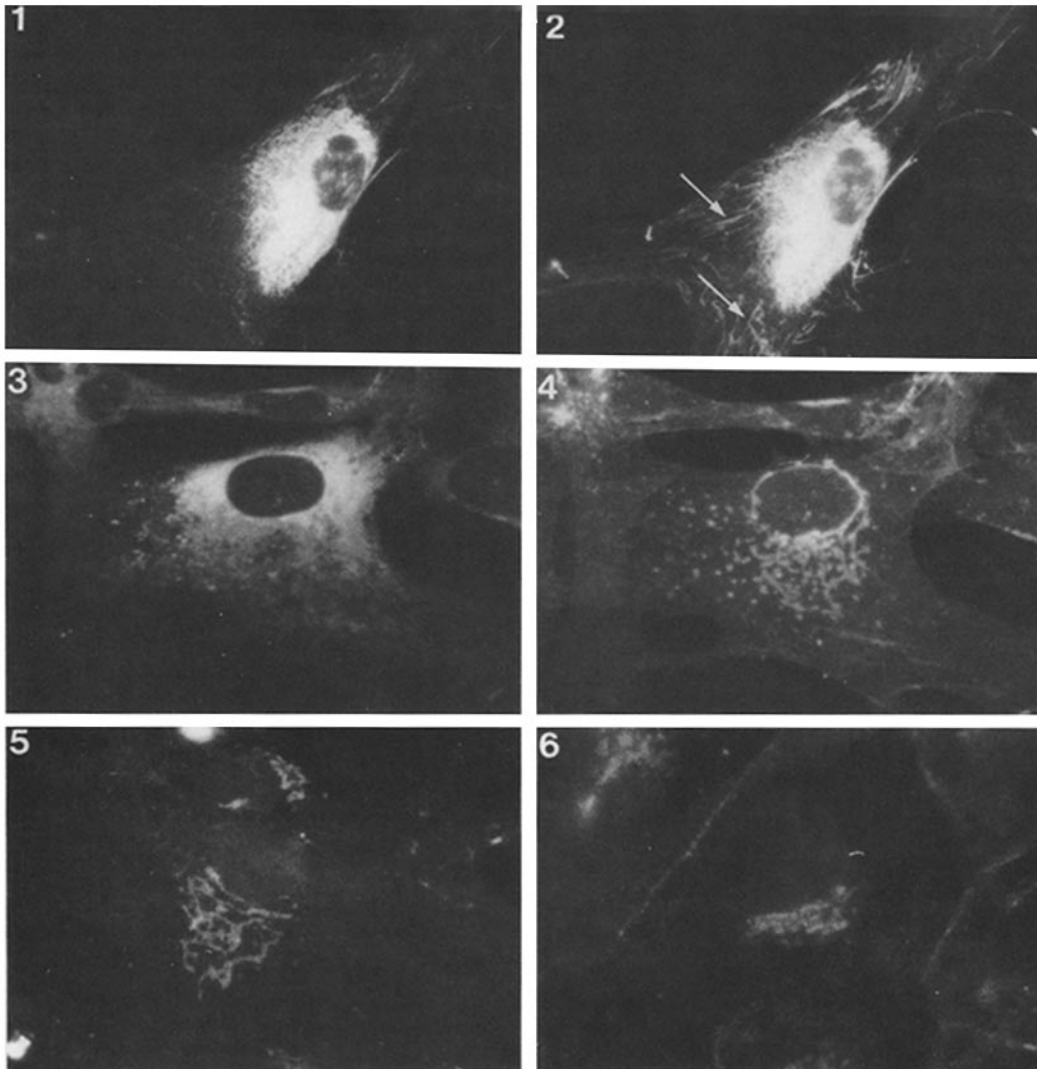
FIGURES 1 and 2    Cultured normal human embryonal fibroblasts after double-staining with FITC–Con A (Fig. 1) and antifibronectin antibodies followed by TRITC–anti-rabbit IgG (Fig. 2). Note the codistribution of the perinuclear fluorescences but the lack of fibrillar staining in Fig. 1 (arrows in Fig. 2).

FIGURES 3 and 4    Cultured fibroblasts after double-staining with FITC–Con A (Fig. 3) and TRITC–WGA (Fig. 4). Note the perinuclear staining in Fig. 3 and the distinctly different juxtanuclear reticular staining in Fig. 4.

FIGURES 5 and 6    Cultured normal human fibroblasts stained with FITC–RCA 120 (Fig. 5) and MDCK cells stained with FITC-HPA (Fig. 6) showing a bright reticular juxtanuclear fluorescence.

phosphatase (Fig. 17 and 18), a marker enzyme for the Golgi apparatus (16).

## DISCUSSION

In this study we have shown that saccharide moieties in both cultured normal and malignant cells are similarly compartmentalized into different cell organelles, the terminal saccharides of glycoconjugates being detectable only in a juxtanuclear reticular organelle meeting the criteria for Golgi apparatus.

As we have shown in this and in a previous
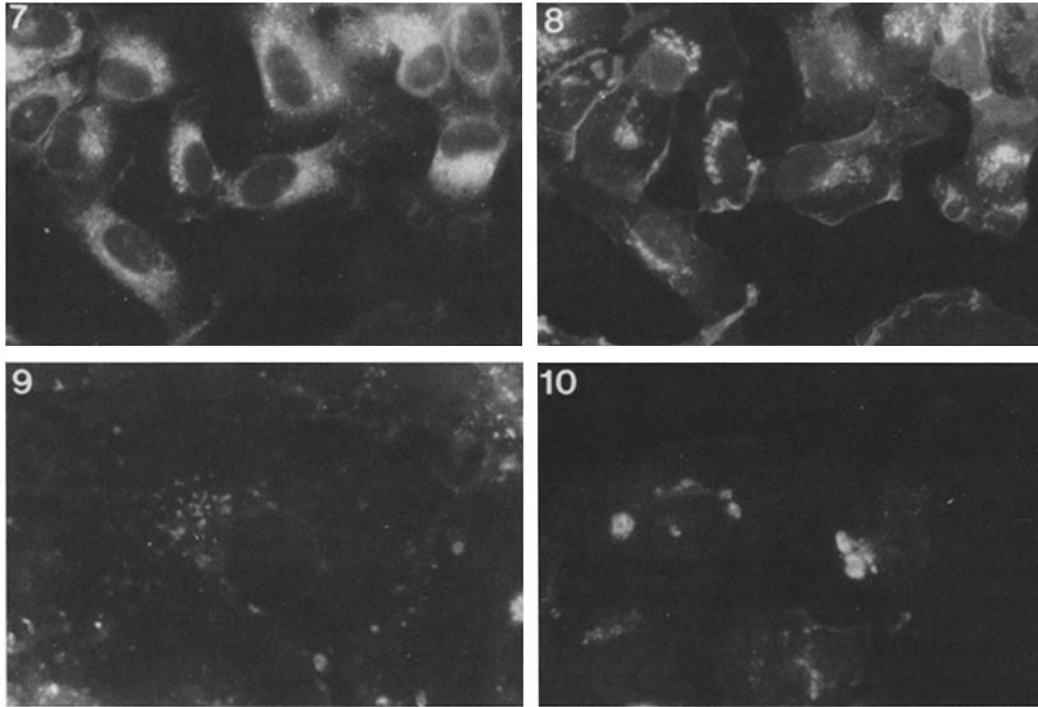
FIGURES 7 and 8   Cultured human fibrosarcoma cells (HT1080) double-stained with FITC–Con A (Fig. 7) and TRITC-WGA (Fig. 8). Note the bright perinuclear staining in Fig. 7 and the vesicular juxtanuclear fluorescence in Fig. 8.

FIGURES 9 and 10   HeLa cells stained with FITC-HPA (Fig. 9) and Val3 cells stained with TRITC-UEA (Fig. 10). A bright vesicular juxtanuclear staining is seen in both types of cells.

study (10), paraformaldehyde fixation of cultured cells followed by treatment in nonionic detergent permits fluorescent staining with good preservation of cytoplasmic morphology. In cultured cells, Con A and LcA, which are most specific for mannosyl and glucosyl moieties (3), seemed to label mainly the endoplasmic reticulum, as indicated by the codistribution of Con A labeling (see also reference 10) with that obtained in double-staining with antifibronectin antibodies, a major secreted protein in cultured fibroblasts (32). This also agrees with our previous electron microscope findings (29) and those of others (7, 20). The distinct staining obtained with Con A even after treatment of the cells with puromycin, which is known to deplete cells of their secretory proteins after termination of protein synthesis (32), suggests that the staining is due to labeling of saccharide moieties of both secretory and membrane glycosyl moieties.

The juxtanuclear staining with lectins other than

Con A or LcA seen in normal cells apparently represents labeling of the Golgi apparatus, which is compatible with the known morphology of the Golgi apparatus (14) and with the behavior of this cell organelle after treatment with microtubule-disrupting drugs, such as vinblastine sulphate (15, 27), or with monovalent ionophores, such as monensin (25, 26). Further confirmation of the localization in the Golgi apparatus was obtained by the codistribution of the reticular juxtanuclear fluorescence with the reaction product of thiamine pyrophosphatase, an enzyme that localizes in the Golgi apparatus (14, 16). Interestingly, in all the malignant cells studied, the Golgi apparatus was distinctly vesicular in appearance, differing from the decoration seen in normal cells. Thus far, an altered morphology of the Golgi apparatus in malignant cells has been noted in only a few reports (see, e.g., references 2 and 30).

No staining reaction with any lectin was seen in the nucleus of the cultured cells studied, although
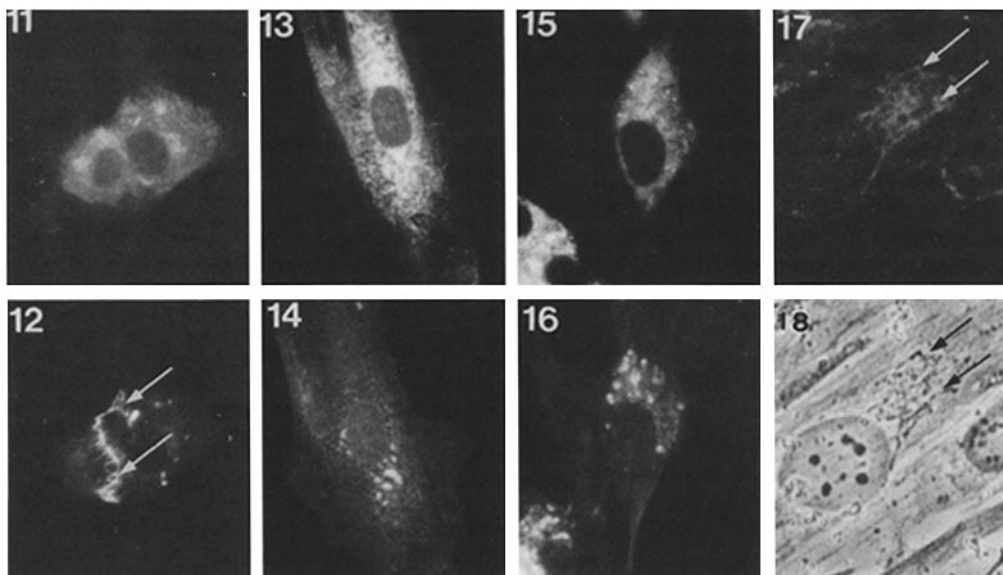
FIGURES 11 and 12   Cultured human embryonal fibroblasts treated with 2 µg/ml of puromycin for 2 h and double-stained thereafter with FITC–Con A (Fig. 11) and antifibronectin antibodies followed by TRITC–anti-rabbit IgG (Fig. 12). Note the bright perinuclear staining with FITC–Con A in Fig. 11. In the same cells, fibronectin antibodies give only a fibrillar, cell-surface type of labeling (arrows in Fig. 12).

FIGURES 13–16   Cultured human embryonal fibroblasts treated with vinblastine sulphate (10 µg/ml for 3 h; Figs. 13 and 14) or monensin (1 µM for 2 h; Figs. 15 and 16). Note the typical perinuclear fluorescence in cells treated with FITC–Con A and the bright juxtanuclear vesicular fluorescence seen in cells after both treatments with TRITC-WGA.

FIGURES 17 and 18   Cultured human embryonal fibroblasts after thiamine pyrophosphatase reaction (Fig. 17) and the same cells stained with FITC-WGA (Fig. 18). Note the codistribution of the enzymatic reaction product and the fluorescence staining (arrows).

the fixation method used in our study allows penetration of macromolecular probes into the nuclear interior (10). This result is in contrast to a number of recent reports suggesting the presence of lectin-binding glycoproteins among the nonhistone chromosomal proteins in the nucleus (9, 11, 19).

The current view of the glycosylation stages in the maturation of an asparagine-linked glycoprotein is that N-acetylglucosamine, mannose, and glucose residues are initially added to the growing polypeptide at the site of its synthesis in the cisternae of rough endoplasmic reticulum (12, 18, 22, 23). Thereafter, the core region is processed in the Golgi apparatus: glucose and some mannose residues are removed, and all the terminal sugars are added to the glycopeptide, namely, N-acetylglucosamine, galactose, fucose, and sialic acids (cf. references 18, 22, and 23). Other glycosyl moieties of glycoproteins and those of glycolipids, on the other hand, seem to be added exclusively in the

Golgi apparatus (4, 13, 14, 23). In the present study we apparently visualize these processes, and our findings contradict the reports (1, 3, 24) proposing cytoplasmic transfer of fully glycosylated glycoproteins from the Golgi apparatus to membranes of the endoplasmic reticulum and nuclei. The data presented here, however, are in agreement with our previous electron microscope findings with ferritin-coupled lectins (29) and are also in agreement with the findings of others (7, 20), suggesting a unidirectional subcellular flow for maturation of both membrane and secretory glycoconjugates (for a recent review, see reference 13).

The results of the present study also suggest that, apart from an increased staining with fucose-specific lectins, cultured malignant and transformed cells have a compartmentalization of saccharide moieties in their cytoplasmic membranes similar to that of normal cells. This is at variance

with several recent reports (21, 31). Our results also demonstrate the suitability of a simple method for fluorescence visualization of cellular endomembrane organelles, especially the Golgi apparatus. This method should prove valuable for studies of the function of this cell organelle.

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