Uncoupling of Chondroitin Sulfate Glycosaminoglycan Synthesis by Brefeldin A

Robert C. Spiro,* Hudson H. Freeze,† Deepak Sampath,‡ and Joseph A. Garcia*

*Department of Immunology, The Scripps Research Institute, La Jolla, California 92037; and †Glycobiology/Carbohydrate Chemistry Program, The La Jolla Cancer Research Foundation, La Jolla, California 92037

Abstract. Brefeldin A has dramatic, well-documented, effects on the structural and functional organization of the Golgi complex. We have examined the effects of brefeldin A (BFA) on the Golgi-localized synthesis and addition of chondroitin sulfate glycosaminoglycan carbohydrate side chains. BFA caused a dose-dependent inhibition of chondroitin sulfate glycosaminoglycan elongation and sulfation onto the core proteins of the melanoma-associated proteoglycan and the major histocompatibility complex class II-associated invariant chain. In the presence of BFA, the melanoma proteoglycan core protein was retained in the ER but still acquired complex, sialylated, N-linked oligosaccharides, as measured by digestion with endoglycosidase H and neuraminidase. The initiation of glycosaminoglycan synthesis was not affected by BFA, as shown by the incorporation of [6-3H]galactose into a protein-carbohydrate linkage region that was sensitive to β-elimination. The ability of cells to use an exogenous acceptor, p-nitrophenyl-β-D-xyloside, to elongate and sulfate core protein-free glycosaminoglycans, was completely inhibited by BFA. The effects of BFA were completely reversible in the absence of new protein synthesis. These experiments indicate that BFA effectively uncouples chondroitin sulfate glycosaminoglycan synthesis by segregating initiation reactions from elongation and sulfation events. Our findings support the proposal that glycosaminoglycan elongation and sulfation reactions are associated with the trans-Golgi network, a BFA-resistant, Golgi subcompartment.

The biosynthesis of glycosaminoglycan (GAG) carbohydrate side chains involves an extensive series of posttranslational enzymatic reactions that occur during the intracellular transport of core proteins from the ER through the Golgi complex. These reactions use specific initiation, elongation, and sulfation enzymes to modify appropriate core proteins and assemble them into mature proteoglycan molecules. For chondroitin sulfate (CS) GAGs, the carbohydrate–protein linkage region is initiated by a xylosyltransferase that transfers xylose from UDP-xylose to the hydroxyl groups of specific serine residues within a core protein. Initiation is completed by the addition of two galactosyl and one glucuronyl residue through the action of galactosyltransferase I, II, and glucuronyltransferase I, respectively. Once initiated, the repeating disaccharide backbone of glucuronyl and N-acetylgalactosaminyl residues is elongated by specific glucuronyl- and N-acetylgalactosaminyltransferases. Sulfation of the repeating disaccharides by the 4- and/or 6-sulfotransferases completes the synthesis of mono- or disulfated CS (27) (see Fig. 11 for summary).

It is generally accepted that GAG synthesis is a Golgi complex-related event. Early studies using electron microscopy and radioautography showed that the Golgi complex is the major site of GAG chain synthesis (21, 24, 25). Golgi-enriched preparations isolated from some cell types have also been shown to contain GAG synthesizing activity (32). More recent studies examining the kinetics of entry of radiolabeled sugars into GAG chains, as well as the localization of GAG sugar nucleotide and sulfate donor transport systems, have clearly localized GAG synthesis to the Golgi complex (14, 18, 23). The exact spatial organization of the cascade of enzymatic reactions involved in GAG synthesis is not known, due, in part, to the lack of understanding of the dynamics and spatial differentiation of the Golgi complex.

The Golgi complex can be divided into at least four distinct regions, the cis-, medial, and trans-Golgi and the trans-Golgi network (TGN) (2, 5, 12, 13, 15). The specific changes induced by the fungal metabolite, brefeldin A (BFA), have provided new information on how the structural organization of the Golgi complex relates to function. BFA dramatically, yet reversibly, alters Golgi morphology and function by disrupting the budding of nonclathrin-coated transport vesicles responsible for anterograde movement (3, 22). The result is a block in anterograde protein transport out of the ER (20) and a redistribution of cis-, medial, and trans-Golgi–resident enzymes back to the ER through a retrograde, microtubule-dependent pathway (16, 17). In contrast, the compartmen-
talization processes involved in the formation of the TGN, lysosomes, and endocytic vesicles are resistant to the redistribution induced by BFA (2, 20).

The aim of this study was to further define the dynamic topology of GAG synthesis using BFA and the human melanoma-associated proteoglycan (MPG) model system. We demonstrate that BFA reversibly uncouples GAG chain initiation from elongation and sulfation events. In the presence of BFA, the MPG core protein is retained in the ER and acquires complex N-linked oligosaccharides but is not converted to a CS proteoglycan form. In addition, BFA-treated cells fail to elongate and sulfate GAG chains onto the exogenous, artificial GAG acceptor, p-nitrophenyl-β-D-xylolside (βDX). Initiation of GAG synthesis in the presence of BFA proceeds through to the addition of the xylosyl residue and the galactosyl residues of the carbohydrate–protein linkage region. These results indicate that GAG chain elongation and sulfation are BFA-resistant events which are associated with the TGN.

Materials and Methods

Materials

BFA was obtained from Sandoz Pharmaceuticals (Basel, Switzerland). Deoxynojirimycin (DMJ) and endoglycosidase H (endo-β-N-acetylglucosaminidase) were obtained from Boehringer Mannheim Diagnostics, Inc. (Indianapolis, IN) and Genzyme Corp. (Boston, MA). Sephardes G-25 PD-10 columns and protein A-Sepharose CL-4B were purchased from Pharmacia Fine Chemicals (Piscataway, NJ). [35S]Methionine (1,259 Ci/mmol), [35S]sulfate (25–40 Ci/mmol), [6-3H]galactose (25.5 Ci/mmol) and Na[3H]I (15.8 mCi/tg) were obtained from Amersham Corp. (Arlington Heights, IL). All reagents used for cell surface iodination procedures (Enzymobeads) and SDS-PAGE were purchased from Bio-Rad Laboratories (Richmond, CA). Neuraminidase (Clostridium perfringens or Vibrio cholera), chondroitinase ABC, chondroitin sulfate A and C carriers, βDX, FITC- and TRITC-conjugated wheat germ agglutinin (WGA) and anti-mouse immunoglobulin antibodies were from Sigma Chemical Co. (St. Louis, MO). Cycloheximide was purchased from Calbiochem-Behring Corp., San Diego, CA). The AX5 HPLC column was purchased from Varian Instruments (Palo Alto, CA). β-Galactosidase (chicken liver) was purchased from Oxford Glycosystems (Rosedale, New York). [35S]Acetate was purchased from Amersham Corp. (Piscataway, NJ). [3H]Galactose-labeled MPG was produced as described previously (10). La Jolla, CA) and was produced as described previously (10). Sera from preimmunized rabbits or fusion partner culture supernatants served as negative controls in all immunoprecipitation experiments.

Radiolabeling and Immunoprecipitation Analysis

Metabolic radiolabeling, cell surface iodination, and immunoprecipitation of specific antigens were performed as described previously (33, 34). 5–8 x 10⁶ cells per sample were used in all experiments. Labeling with [6-³H]galactose was carried out in RPMI 1640 or DME culture media containing 5 mg glucose/ml with 100 µCi/ml of [³H]galactose for 4–6 h at 37°C. The choice of [6-³H]galactose is important because it cannot be converted into [³H]glucuronic acid. Quantitation of SDS-PAGE profiles was performed by densitometric scanning of autoradiograms.

Quantitation of Proteoglycans and β-D-Xyloside-initiated GAGs

The quantitation of [35S]sulfate-labeled macromolecules was carried out as previously described (33, 34).

Isolation of [³H]Galactose-labeled Molecules

Immunoprecipitates isolated with mAb 9.2.27 from [6-³H]galactose-labeled cells were washed extensively in PBS containing 0.5% Tween-20 and 1 mg/ml ovalbumin. [³H]Galactose-labeled O-linked oligosaccharides were then released from the MPG core protein by treatment with 20 µl of 0.05 N NaOH and 0.3 M NaBH₄ overnight at room temperature. The released chains were neutralized with acetic acid and evaporated three times in MeOH/CH₃CN to remove boratesalts. The sample was diluted to 1.5 ml in 2 mM Tris base and passed over a 2-cm column of QAE-Sephadex equilibrated in 2 mM Tris. Neutral species that ran through the column (56% of the starting material) were desalted on a mixed bed ion exchange resin (MB3). Recovery was >90%.

HPLC Analysis of Neutral Oligosaccharides

Neutral oligosaccharides were analyzed on an AX5 anion-exchange column using a 40–80% gradient of acetonitrile in 25 mM NaPO₄, pH 6.5, in 30 min at a flow rate of 1 ml/min. Fractions of 0.5 ml were counted. Each run included an internal standard of [¹⁴C]-glucitol and an ovalbumin glucopycoglute (1464).[³H]Acetate was purchased from Amersham Corp. (Piscataway, NJ). β-Galactosidase digestion was performed on 50 µl of 100-mg citrate-P0₄ buffer at pH 4.5 for 16 h at 37°C using 0.05 U of enzyme.

Immunofluorescence Microscopy

M21 cells were grown on 12-mm round glass coverslips placed in 24-well culture dishes and pretreated with BFA for 1 h at 37°C. The coverslips were then washed in ice-cold PBS/0.1% BSA and fixed immediately in a 3% paraformaldehyde solution in 0.1 M sodium phosphate, pH 7.4, for 15 min on ice. The coverslips were then washed in PBS followed by 0.1 M glycerine. Cell surface immunofluorescence was blocked before permeabilization with a human/mouse chimeric form of mAb 9.2.27, containing human heavy and light chain constant regions (11), and with WGA for 30 min at room temperature. The fixed and blocked cells were then permeabilized in 0.15% saponin in PBS/0.1% BSA for 15 min and incubated with primary antibody for 1 h at room temperature. After washing, the coverslips were incubated with fluorescein labeled anti-mouse secondary antibody or WGA for 1 h at room temperature, washed and mounted on glass slides in Fluoromount G (Southern Biotechnology Assoc., Birmingham, AL). Mounted slides were viewed with a 100× oil Neofluor lens on a microscope (both from Carl Zeiss, Inc., Thornwood, NY) equipped with barrier filters to prevent crossover of fluorescein and rhodamine fluorescence.

Results

BFA Inhibits CS GAG Chain Elongation and Sulfation on the MPG Core Protein

The human melanoma MPG antigen recognized by mAb 9.2.27 is synthesized initially as a 240-kD precursor that con-
M21 cells were pretreated with the indicated concentrations of BFA for 1 h before a 4-h label with [35S]methionine at 37°C in the presence of drug. The MPG complex was then isolated from detergent lysates of cells by immunoprecipitation with mAb 9.2.27 and was subjected to SDS-PAGE (5% gel) and autoradiography. A dose-dependent inhibition of the conversion of the 240-kD form to the 250-kD and proteoglycan form (PG) is observed in the presence of BFA. Molecular mass standards are in kilodaltons.

Treatment of M21 human melanoma cells with increasing concentrations of BFA results in a dose-dependent inhibition of the conversion of MPG core protein to its CS proteoglycan form (Fig. 1). Inhibition becomes apparent between 16-32 ng/ml and is complete at higher concentrations. There is no effect on protein synthesis or viability under these labeling conditions up to 50 μg/ml (data not shown). An intermediate form of the core protein accumulates along with the 240-kD species in the presence of BFA. At higher concentrations of BFA all of the core protein is converted to the 240-kD form (Fig. 1). No high molecular mass form or chondroitinase ABC-sensitive form (data not shown) of the MPG is observed in the presence of BFA, suggesting an inhibition of CS GAG chain elongation. The complete inhibition of incorporation of [35S]sulfate into the MPG complex is also consistent with an absence of GAG chain elongation and sulfation (Fig. 2, left panel). Identical results were obtained in three additional human melanoma cell lines (data not shown).

BFA Blocks MPG Core Protein Transport out of the ER

The complete transport and assembly of the MPG complex results in the expression of both the 250-kD and proteoglycan components on the cell surface (33, 34). Truncated forms of both these species are also released into the culture media (Spiro, R., unpublished observation). MPG forms could not be isolated from either 125I cell surface–labeled M21 cells that were trypsinized and pretreated with BFA or from the [35S]methionine–labeled culture media of such cells (Fig. 2, middle and right panels). The absence of MPG cell surface expression and release is consistent with an arrest in intracellular transport of the core protein. To determine the subcellular site of the arrest, the distribution of the MPG core protein in the presence of BFA was examined by immunofluorescence microscopy with mAb 9.2.27.

The MPG complex shows a localized perinuclear staining pattern in fixed and permeabilized M21 cells when stained with mAb 9.2.27 and a FITC-conjugated secondary antibody (Fig. 3 A). This staining pattern is typical of the Golgi complex as confirmed by the co-localization of the TRITC-
conjugated distal Golgi stack marker, WGA (Fig. 3 B). In contrast, treatment of the cells with BFA before fixation and permeabilization results in a more diffuse, reticular staining pattern that is typical of the ER (Fig. 3 C). The golgi-specific WGA staining also becomes more diffuse, consistent with a BFA-induced morphologic Golgi redistribution (Fig. 3 D) (16). These results suggest that the transport of the MPG core protein is blocked between the ER and the Golgi in the presence of BFA.

The MPG N-linked Oligosaccharides Are Processed in BFA-treated Cells

The BFA-induced effects on the Golgi complex have been previously shown to cause a redistribution of cis-, medial, and trans-Golgi-resident enzymes back to the ER (2, 16, 17). To examine if Golgi enzymes are redistributed in BFA-treated melanoma cells, the processing of the MPG core protein N-linked oligosaccharides was monitored by endo H digestion of MPG immunoprecipitates isolated from M21 cells pretreated, pulse labeled, and chased in the presence of BFA. Immediately after the pulse label, the 240-kD form of the MPG is susceptible to endo H digestion in both control and BFA-treated cells. After 15–30 min, and throughout the remainder of the chase period in control cells, the 240-kD precursor is converted to the 250-kD, endo H–resistant form and the high molecular mass CS proteoglycan (Fig. 4 A) (33, 34). In BFA-treated cells, the 240-kD form gradually acquires endo H resistance during the chase period and becomes completely resistant by 9 h. This acquisition of endo H resistance is accompanied by a slight increase in molecular mass, but complete conversion to the 250-kD or the proteoglycan form is inhibited by BFA (Fig. 4 a). Neuraminidase digestion of the MPG isolated from cells labeled with [35S]methionine for 1 h and chased for 3 h in the presence of BFA results in a shift in mobility of the core protein, indicating the acquisition of sialic acid residues (Fig. 4 b). These results are consistent with the recycling, back to the ER, of the Golgi resident enzymes that trim and terminally process the N-linked oligosaccharides of the ER-retained MPG core protein. The absence of the proteoglycan form of the MPG suggests that the redistribution of CS GAG enzymes to the ER does not occur or that their redistribution results in a loss of functional activity.

BFA Does Not Block Initiation of CS GAG Synthesis on the MPG Core Protein

The extent of CS GAG chain initiation occurring in the presence of BFA was evaluated by metabolically labeling the carbohydrate–protein initiation linkage region (Ser-Xyl-Gal-Gal-GlcUA-) with [6-3H]galactose. Pretreatment of cells with DMJ was used to inhibit the labeling of complex N-linked oligosaccharides, thereby limiting the incorporation of [3H]galactose exclusively to O-linked oligosaccharides. DMJ has previously been shown to block the processing of the MPG core protein N-linked oligosaccharides at the high mannose stage without effecting conversion to the pro-
teoglycan form (34) (Fig. 5, left side). In control M21 cells labeled with \[^{3}H\]galactose, label is incorporated into both the 250-kD and proteoglycan forms of the MPG complex (Fig. 5, right side). In cells pretreated with DMJ, incorporation into the core protein form is blocked by 97%, but is only partially reduced in the proteoglycan form. Cells pretreated with BFA also incorporate \[^{3}H\]galactose into the MPG core protein, even in the presence of DMJ (Fig. 5, right side). This result is consistent with the addition of the xylosyl residue and at least one of the galactosyl residues of the GAG carbohydrate–protein linkage region in the presence of BFA.

To precisely determine the effects of BFA on the synthesis of the carbohydrate–protein linkage region, the \[^{3}H\]galactose-labeled sugar chains were released from the MPG core protein by β-elimination and their size and charge were analyzed by HPLC and QAE-Sephadex ion exchange chromatography, respectively. About 56% of the released chains are neutral, and two peaks are seen in HPLC analysis (Fig. 6).

β-galactosidase digestion eliminates both peaks and generates a monosaccharide peak, presumably free \[^{3}H\]galactose, which elutes slightly later than the \[^{14}C\]fucitol standard, as expected (7). The major peak is probably the linkage region, XylH β1→4-Gal β1→3-Gal, and the minor peak is probably XylH β1→4-Gal. Chromatography on QAE-Sephadex showed that 43% of the labeled material carries one to three negative charges, based on comparison with appropriate standards (data not shown). The characterization and identity of this material will be reported elsewhere. These results suggest that the BFA-induced block in the MPG GAG chain elongation and sulfation is not due to a complete block in the initiation events.

**BFA-treated Cells Fail to Synthesize GAG Chains onto Xyloside Acceptors**

To evaluate the activity of the GAG chain enzyme machinery
in the presence of BFA, independent of the MPG core protein, treated cells were tested for their ability to elongate and sulfate GAG chains onto the exogenous acceptor, βDX. As an artificial GAG acceptor, βDX diffuses freely into cells to the sites of GAG synthesis where it substitutes for the xylose residue of the initiation linkage region and allows polymerization and sulfation of GAGs onto itself. The smaller βDX-bound GAGs are then efficiently exocytosed into the culture media (30). Since initiation and elongation are prerequisites for sulfation, the integrity of the GAG enzyme machinery can be evaluated, independent of core protein structures, by monitoring the increase in incorporation of [35S]sulfate into GAG chains in the presence of βDX.

In a representative experiment, incubation of M21 cells with 1 mM βDX results in a 10-fold increase in the mean incorporation of [35S]sulfate into [35S]-macromolecules (33, 34) (Fig. 7). The proportion of [35S]-macromolecules released into the culture media increases from 40 to 79% in the presence of βDX, representative of the exocytosis of the free, βDX-initiated, GAG chains. In contrast, pretreatment of the cells with BFA results in a dose-dependent inhibition of incorporation of sulfate into βDX-initiated macromolecules. The observed inhibition is not due to a block in the uptake of βDX since identical results are obtained in cells that are preloaded before BFA treatment (data not shown). At 20 ng/ml of BFA, incorporation of [35S]sulfate is only 59% of control cells, and at higher concentrations (0.2 and 2 μg/ml) the inhibition is virtually complete with incorporation diminishing to 1% of control cells (Fig. 7). Similar experiments performed with [6-3H]galactose demonstrated the accumulation of [H]galactose intermediates linked to βDX (data not shown). The dose response range demonstrated here correlates well with that observed in Fig. 1 for inhibition of conversion of the MPG core protein to the proteoglycan form. These results indicate that BFA completely disrupts the ability of cells to use an exogenous, artificial acceptor for the elongation and sulfation of GAG chains. To determine if BFA permanently damages the GAG enzyme machinery, the reversibility of its effects was tested in the absence and presence of new protein synthesis.

**Reversibility of BFA Effects**

The effects of BFA on the synthesis of the MPG proteoglycan form are completely reversible. In M21 cells pretreated with BFA for 30 min, pulse-labeled for 10 min, and then washed and chased in the absence of BFA, conversion of the MPG core protein to the proteoglycan form begins between 1 and 2 h of release from BFA (Fig. 8). After an overnight release, both the 250-kD and CS proteoglycan forms can be isolated from the BFA-treated cells, as demonstrated by chondroitinase ABC digestion of the immunoprecipitates before SDS-PAGE analysis (in Fig. 8, 24 hour time point). Interestingly, after recovery from BFA treatment, a higher proportion of the MPG is expressed as the 250-kD rather than the proteoglycan form. This indicates that not all of the core protein that accumulates in the presence of BFA can serve as substrate for chondroitin sulfate synthesis after removal of the drug. The reversibility of the BFA-induced effects does not require new protein synthesis. Identical results are obtained in cells treated with BFA and released under conditions where protein synthesis is inhibited >90% by cycloheximide (data not shown).

The effects of BFA on the ability of M21 cells to use the exogenous βDX acceptor are also completely reversible. Cells pretreated with BFA and then released in drug-free media for 2 h incorporate [35S]sulfate into βDX-initiated GAG chains at levels equal to control cells. Identical results are obtained in cells released in the presence of cycloheximide (Fig. 9). The reversibility in the absence of new protein synthesis indicates that the GAG elongation and sulfation enzymes are present, but inactive, in BFA-treated cells.

**BFA Inhibits the Conversion of the Class II–associated Invariant Chain to a Proteoglycan Form**

To address the generality of the BFA-induced inhibition of GAG chain synthesis, the effects on the conversion of the ma-
Figure 7. BFA inhibits β-d-xyloside-initiated GAG synthesis. M21 cells were pretreated for 1 h with or without BFA (1 μg/ml) and p-nitrophenyl-β-d-xyloside (1 mM) for 15 min before a 1 h label with [35S]sulfate in the presence or absence of BFA. Cell-associated (hatched region) or exocytosed (nonhatched region) 35S-labeled macromolecules were quantitated after their exclusion from Sephadex G-25 under dissociative conditions as described (33, 34). The height of the bar indicates total [35S]sulfate incorporation. The percent of total [35S]sulfate incorporation into control cells is indicated at the top of each bar. A dose-dependent inhibition of the ability of cells to elongate and sulfate GAGs onto an exogenous acceptor is observed in the presence of BFA. Data are expressed as the mean of duplicate cultures with the range of values indicated.

Figure 8. The effects of BFA on assembly of the MPG are reversible. M21 cells were pretreated for 1 h with or without BFA (1 μg/ml) before a 10-min pulse with [35S]methionine at 37°C. The cells were then washed and chased in unlabeled media in the absence of BFA. At the indicated time points, an aliquot of cells was harvested and MPG immunoprecipitates were isolated from detergent cell lysates with mAb 9.2.27. Digestion or mock digestion of immunoprecipitates with either endo H (Endo H) or chondroitinase ABC (Case ABC) was performed for 1 h at 37°C before being subjected to SDS-PAGE (5% gel) and autoradiography. Conversion of the MPG core protein to the proteoglycan form (PG) begins to appear after 1–2 h of release from BFA treatment. Molecular mass standards are in kilodaltons.
The results presented in this study demonstrate that the Golgi-specific changes induced by BFA have a profound effect on CS GAG synthesis. BFA caused a dose-dependent, yet completely reversible inhibition of CS GAG elongation and sulfation onto the MPG and invariant chain core proteins, as well as onto the exogenous, artificial GAG acceptor, βDX. BFA did not affect the initiation of CS GAG synthesis, including addition of the xylosyl residue and the galactosyl residues of the initiation linkage region. The simplest explanation for these findings is that CS GAG chain elongation and sulfation enzymes are resistant or insensitive to the redistribution induced by BFA, while the initiation enzymes are not (Fig. 11). This results in the complete uncoupling of CS GAG chain initiation from the elongation and sulfation events in a manner completely analogous to the BFA-induced uncoupling of glycosphingolipid synthesis (39, 40).

The strongest line of evidence supporting the idea that BFA uncouples CS GAG synthesis comes from the experiment that demonstrates GAG initiation, in the absence of elongation and sulfation, by analyzing the incorporation of [14C]galactose into the carbohydrate–protein linkage region. Under conditions where incorporation was restricted to O-linked oligosaccharides, by treatment with DMJ, label was incorporated into the MPG core protein that accumulated in the presence of BFA (Fig. 5). Since GAG chains represent the majority of, if not the only, O-linked oligosaccharides of the MPG complex (9, 28), the incorporation of [14C]galactose into the BFA-treated core protein is consistent with the addition of the xylosyl residue and the galactosyl residues of the initiation linkage region. Results from the fine structure analysis indicated that the [14C]galactose is predominately (56%) incorporated into neutral species that are sensitive to β-galactosidase digestion and probably represent the linkage region (Fig. 6). A portion (43%) of the material is anionic and its identity will be reported elsewhere.

The complete inhibition of βDX-dependent CS GAG chain
polymerization and sulfation induced by BFA is also consistent with an uncoupling of GAG synthesis. As a xylose analogue, βDX diffuses freely into the cell to substitute for the normal carbohydrate–protein linkage residue (30). The anterograde intracellular transport of βDX molecules through the Golgi complex results in the formation of core protein free GAG chains that are efficiently exocytosed from the cell. In the presence of BFA, the βDX molecules that diffuse into the ER become properly initiated due to the redistribution of enzymes from BFA-sensitive compartments. The initiated molecules do not, however, gain access to the elongation and sulfation enzymes in BFA-resistant compartments because of the BFA-induced block in anterograde vesicular transport. Similarly, the βDX molecules that initially diffuse to the BFA-resistant compartments do not become properly initiated to serve as substrates for elongation and sulfation. This results in the complete inhibition of βDX-dependent CS GAG synthesis. Upon removal of BFA, transport between BFA-sensitive and resistant compartments is reestablished and βDX-initiated CS GAG synthesis quickly recovers within 1 h. The complete recovery in the absence of new protein synthesis is further proof that the elongation and sulfation enzymes are present but segregated from the initiation enzymes in BFA-treated cells.

There are other possible explanations for the inhibition of CS GAG synthesis induced by BFA, including the direct inhibition of the elongation and sulfation enzymes or the absence of redistribution of the proper nucleotide sugar and sulfate donor transport systems. Either mechanism would result in the observed inhibition of CS GAG synthesis, regardless of the location of the elongation and sulfation enzymes after the BFA-induced redistribution. However, results from previous studies argue against these possibilities. BFA does not inhibit the in vitro activity of several glycosyltransferases and does not affect the uptake of UDP-sugars (20, 36). In addition, the sialylation of the MPG core protein in the presence of BFA demonstrates that the Golgi-resident CMP–sialic acid transport system (14, 23) and some sialyltransferase can be redistributed to the ER. The recent data demonstrating that BFA causes the disruption of a Golgi-specific structural protein suggests that its mode of action is directed towards the structural integrity of the Golgi rather than the resident enzymes (3, 22). For these reasons it is more likely that the BFA-induced inhibition of CS GAG synthesis is due to the segregation of initiation and elongation–sulfation enzymes into BFA-sensitive and -resistant compartments, respectively (Fig. 11).

The results presented here strongly support the proposal that CS GAG chain elongation and sulfation are TGN-associated events. This follows directly from the results of Chege and Pfeffer (2) who showed that the TGN is a BFA-resistant, Golgi sub-compartment. The localization of CS elongation and sulfation events to the TGN extends the list of TGN-resident enzymes to include the specific glycosyl- and sulfotransferases involved in these reactions. It has been previously shown that some classes of sialytransferases and the GA2/GM2/GD2 synthase are localized in a BFA-resistant compartment (2, 31, 38–40). Further structural studies on the specific enzymes involved in these reactions will help to determine the mechanism responsible for their specific targeting to the TGN.

The mapping of CS GAG elongation and sulfation to the TGN is completely consistent with previous kinetic studies of GAG synthesis (18, 19, 35). While the exact site of the initial xylosylation reaction has been debated, it is generally agreed that elongation and sulfation are later Golgi events. The results presented here do not distinguish between an ER or early Golgi site for GAG initiation, but clearly segregate it from GAG elongation and sulfation. It will be important to determine whether a similar topology exists for the synthesis of other classes of GAGs.

The TGN differs from the cis–medial, and trans–Golgi in that it functions to sort proteins into transport vesicles that are bound for distinct organelles or domains of the cell surface (2, 4, 8, 26, 37). It is interesting to speculate that the addition of a GAG side chain might influence the packaging of core proteins into distinct transport vesicles bound for different regions of the cell. The expression of core proteins,
such as the MPG and invariant chain, in both glycoprotein and proteoglycan forms, may ultimately reflect distinct subcellular localization and/or function.

We are grateful to Sandoz Pharmaceuticals (Basel, Switzerland) for the gift of brefeldin A.

This work was supported by National Institutes of Health grants CA49243 (to R. C. Spiro) and CA38701 (to H. H. Freeze). H. H. Freeze is an Established Investigator of the American Heart Association. This is Scripps publication number 6765-IMM.

This work was supported by National Institutes of Health grants CA49243 (to R. C. Spiro) and CA38701 (to H. H. Freeze). H. H. Freeze is an Established Investigator of the American Heart Association. This is Scripps publication number 6765-IMM.

Received for publication 11 March 1991 and in revised form 12 August 1991.

References


