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The Confined Function Model of the Golgi Complex: Center for Ordered Processing of Biosynthetic Products of the Rough Endoplasmic Reticulum

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I. Introduction

The organized and characteristic elements of the Golgi complex (GC)[1] are the stacked smooth-surfaced cisternae which are found in the centrosphere of all eukaryotic cells. These cisternae, in conjunction with other associated smooth-surfaced membranes, are responsible for executing net unidirectional intracellular transport (ICT) from the rough endoplasmic reticulum (RER) toward more distally located structures, e.g., lysosomes and the cell surface (24, 35, 40, 47, 85, 111, 134). The present article is not centered on this apparently most primary of Golgi functions, but rather on the broad range of accessory activities which occur during transport, the family of "posttranslational modifications." These

[1]Abbreviations: endo H, endoglucosaminidase H; Gal, galactose; GalNAc, *N*-acetylgalactosamine; GC, Golgi complex; GDP, guanosine diphosphate; Glc, glucose; GlcN, glucosamine; GlcNAc, *N*-acetyl glucosamine; GlcUA, glucuronic acid; ICT, intracellular transport; IdUA, iduronic acid; Ig, immunoglobulin; Man; mannose; RER, rough endoplasmic reticulum; TPP, thiamine pyrophosphate; UDP, uridine diphosphate; VSV, vesicular stomatitis virus, Xyl, xylose.

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events are, in all likelihood, not essential for the "primary" function of theGC yet they are crucial in allowing the cell to tailor its biosynthetic products for its own needs and the needs of the organism as a whole. In addition to modifying products of the RER, the GC may be involved in processing events as a result of its participation in other routes of vesicular traffic, e.g., centripetal traffic from the cell suface. At present, although the existence of such a return route is widely accepted, the only suggestions of processing are no more than fragmentary (23, 24, 47, 105).

The vast body of structural and enzymological information which describes these processing event has been the subject of recent detailed reviews. In the present article no attempt is made to give a thorough exposition of this literature. Instead, the present article highlights those aspects of processing that may shed light on the detailed route and underlying mechanisms of transport. For example, posttranslational modifications may serve as a set of potential Golgi markers for describing the heterogeneity of the organelle. Furthermore, the results of such modifications (e.g., addition of a particular sugar) may provide circumstantial data indicating the itinerary followed by transported molecules. In this sense, these molecules may be considered a family of probes of the transport machinery. One is led to inquire which products are transported at what rate via which processing sites, or, if the structure of these molecules is altered, e.g., by genetic means, are they transported in the same fashion?

Various nonequivalent criteria have been used to ascribe processing events to the GC—autoradiography, preparative or analytic subcellular fractionation, interruption by ICT inhibitors, delay in the impact of cycloheximide, etc. Each one, alone, provides necessarily incomplete information. The research covered here makes use of each of these approaches. In order to emphasize their nonequivalence and to avoid certain possible confusions, attention is paid to distinguishing between anatomic terms ("proximal," "medial," and "distal" cisternae) and kinetic terms ("early" vs "late"). Although the equivalences proximal = early and distal = late may be correct the needed supporting data for this notion are not yet at hand. Nevertheless, contradictions of this hypothesis are not known.

II. The Confined Function Model of the Golgi Complex

Given the body of anatomic and histochemical data which describes the structural heterogeneity of the stacked cisternae of the GC (24, 35, 134), it is plausible to suppose that selected posttranslational modifications are confined to distinct subcompartments of the GC. Such functional subcompartments might correspond to the relatively more proximal or distal cisternae, although lateral specialization might exist as well—electron microscopic observations suggest that vesicles may bud from the extremities of the cisternae and certain histo-

chemical reaction products are not uniformly distributed along their length (24).

Several conceptually quite different approaches have been used to discriminate among and ascribe function to distinct subcompartments:

1. *The pharmacologic approach* which makes use of monensin to interrupt ICT. As has been discussed elsewhere (134–137), the carboxylic ionophore monensin causes partial equilibration of intra- and extracellular Na^+ and K^+, rapid (within seconds) dilation of all Golgi cisternae, and an impressive slowing of ICT. The agent is highly selective, at least after brief treatment (1 μM monensin, 1 hour, 37°C): ATP levels and intracellular pH are little changed, many biosynthetic events continue [protein synthesis, lipid addition to glycoproteins (54) hyaluronic acid synthesis (84) etc.], and the effects of the ionophore are reversible.[2]

Since in the presence of monensin newly synthesized proteins continue to exit from the rough endoplasmic reticulum (RER), and gain access to but do not traverse the GC, one may inquire whether given posttranslational modifications occur. If they do, they may be assigned to a relatively early subcompartment. If they do not, given appropriate control experiments, they may be assigned to relatively late subcompartments.[3] The first indications that the site of interruption of ICT by monensin lies only part way across the GC came from study of the progress of N-linked oligosaccharide maturation of Ig (139); however, a recent use of monensin should be mentioned since it adds independent support for this idea (37). When BHK cells are infected with Sindbis or Semliki Forest virus, viral budding is normally observed at the cell surface. If monensin is present, transport of the glycoproteins is interrupted in the GC (37, 54, 56) and viral nucleocapsids are found adhering to and budding across the membranes of dilated vacuoles (''ICBMs'') in the Golgi region. These nucleocapsids are presum-

[2]In the context of the later discussion, the study of hyaluronic acid biosynthesis is especially important since it strongly suggests that sugar nucleotide metabolism is little perturbed and that the effects of monensin on sugar incorporation (e.g., into glycoproteins) are directly related to interruption of ICT. Hyaluronic acid, since it lacks a protein core, would be free from such effects.

[3]The appropriate controls vary according to the event being studied. For example, in the case of Ig carbohydrate maturation although monensin blocks [^{3}H]Gal and [^{3}H]Fuc labeling of Ig, the two isotopes continue to be incorporated into other acid-insoluble material (139). In the case of the inhibition of acquisition of endo H resistence by transferrin in VSV-infected hepatoma cells, an internal control is provided by the G protein, which does become endo H resistant (133). Were posttranslation modifications due to enzymes with acid pH optima, inhibition by monensin might be due to alkalinization of the cisternal content resulting from H^+–Na^+ (or K^+) exchange. However, all oligosaccharide processing enzymes studied have roughly neutral pH optima. Furthermore, there are no indications that they are sensitive to relative Na^+/K^+ levels. To date the investigators who use monensin unfortunately do not all conform to the rather short-term protocols first used. Comparison of data is therefore often difficult. The extent to which ICT is perturbed by monensin is thought to depend on the alterations of cytoplasmic ion levels which it provokes. The impact of the ionophore may therefore reflect the activity of the Na–K-ATPase of the cell under study.

ably signaling the presence of and adhering to an overaccumulation of the viral glycoproteins. Since other nearby Golgi-derived elements are histochemically positive for thiamine pyrophosphatase (a histochemical marker of distal cisternae) but ICBMs are negative one can conclude that the viral glycoprotein has only partly traversed the Golgi stack. (The author's interpretation of these data is in fact that the site of monensin arrest is within "medial" or "intermediary" cisternae since the ICBMs bear ricin binding sites.) Both the nucleocapsid-laden ICBMs and the nucleocapsid-free vacuoles can be partially separated from each other (101) (vide infra).

The use of colchicine is more limited than that of monensin, since in a number of cell types ICT proceeds in the presence of the drug. Studies of the liver suggest that the site of arrest of ICT by colchicine may be later than the monensin site (103, 134).

2. The approach of *analytic subcellular fractionation* (20, 34a, 100, 101, 135, 147) in which microsomal fractions or subfractions are spread, for example, on continuous density gradients. The goal of this method is to be all inclusive rather than to enrich for selected markers which might prove nonrepresentative of the whole. At present the cytologic origin(s) of such subfractions cannot be systematically identified.[4]

3. The *in vitro reconstitution* approach (108, 112) in which an attempt is made to simulate selected steps of transport by mixing appropriate subcellular fractions or cell extracts. The read-out from such experiments may involve the monitoring of posttranslational modifications which are considered to be diagnostic of transport. This is a method which may provide proof of the adequacy of subfractionation procedures and lead toward elucidation of the mechanisms of transport.

4. The approach of *cytochemistry* (36, 109, 110, 138) making use of antisera which recognize processing enzymes or lectin conjugates. This method cannot indicate at which site processing enzymes are active, but it should locate the enzymes in a cytologic context. The lectin conjugates localize products of sugar transferase activity, with the reservation that it may prove difficult to establish whether a given sugar residue is borne by an N- or O-linked or possibly lipid carrier.

[4]The presence of lipoprotein particles within the Golgi cisternae of the hepatocyte has major implications for liver Golgi subfractionation. Preparative Golgi fractions can be obtained free from smooth ER and plasma membrane because of the density perturbation which results from this buoyant content. For analytic isopycnic fractionation one would anticipate a dispersion of density in proportion to variability in the number of lipoprotein particles contained within Golgi fragments produced by homogenization. Moreover, the relative isopycnic density of different subregions of the GC would reflect the degree of maturation of the lipoproteins. If the lipid content—and as a result the density shift—increases monotonically during ICT then the relative densities of Golgi subfractions might correspond inversely to their position along the time axis of transport; however, there is no proof that such a monotonic relation applies.

5. A possible means of localizing a Golgi component relative to selected processing enzymes is to inquire whether or not it has undergone proteolytic cleavage, terminal sugar addition to N-linked oligosaccharides, initiation or completion of any O-linked oligosaccharides etc. The degree to which it has undergone posttranslational modifications should reflect its ontogeny and site of residence.

6. A kinetic ordering of processing events can be obtained by performing, for example, an amino acid labeling pulse–chase experiment and inquiring as a function of time, when the product in question undergoes one or another maturation step. Distinct kinetics need not, of course, imply distinct compartmentalization. The resolution of this approach is limited by any dispersion of transport rates of the pulse-labeled molecules.

The exploitation of these approaches has by no means been sufficiently extensive to allow assignment of the multitude of processing events to subregions of the GC. Moreover, the present lack of detailed knowledge of the GC is such that it is the analysis of numerous processing events which may actually serve to enumerate the number of subcompartments which exist. Judging from histochemical data (24, 35, 111, 134), at least three classes of cisternae can be distinguished: the most proximal (overstained by OsO_4), medial cisternae (reactive for nicotinamide adenine dinucleotide phosphatase at pH 5 in certain cells) and the most distal (reactive for nucleoside diphosphatase = uridine diphosphatase = thiamine pyrophosphatase). In addition, the structurally somewhat removed tubules and cisternae of "GERL" are histochemically reactive for acid phosphatase. Present studies employing lectins to stain the Golgi cisternae of various cells have also distinguished two or three distinct staining regions along the proximal-to-distal axis (36, 37, 109, 138) (Table I).

The idea of subcompartmentalization of the GC is discussed here in the context of distinct varieties of posttranslational processing several of which are illustrated, in abbreviated form, in Figs. 1–4. To a certain extent, these events might all be found within a single cell; however, since the biosynthetic repertoire (e.g., of exported glycoconjugates) varies among cell types, there is every reason to anticipate significant enzymologic variability of the GC as well. In fact, it is clear that selected sugar transferases are highly enriched only in certain tissues. There also must exist a set of altogether constant Golgi components—those which are responsible for its characteristic structure and endow it with the ability to conduct transport. No such components have yet been identified.

A secondary task in this mapping of Golgi functions is to position the processing paths one relative to the next. For example, is the compartment responsible for proteoglycan sulfation the same as that responsible for galactose addition to asparagine-linked oligosaccharides? The ideal systems to investigate to obtain such information are those in which a single polypeptide undergoes several

TABLE I

SUB-GOLGI LOCALIZATION OF HISTOCHEMICAL MARKERS AND PROCESSING EVENTS

Histochemical marker	Cell type	Localization	Reference
Overosmicated material	Many cells	Proximal cisternae	134
Nicotinamide adenine dinucleotide phosphatase (pH 5)	Ameloblasts, spermatids	Medial cisternae	13a, 124a
Nucleoside diphosphatase (UDPase, TPPase)	Many cell types	Distal cisternae	134
Acid phosphatase	Many cell types	GERL (and lysosomes)	134
Lectin-binding sites			
Concanavalin A	BHK cells	All cisternae (and RER)	36
	Myelomas (Ig^+, Ig^-)	Proximal cisternae (and RER)	138
Ricinus 120	BHK cells	Medial and distal cisternae	36
Wheat germ agglutinin	Myelomas	Distal cisternae	138
Helix lectin	Goblet cells	Proximal cisternae (A^+, A^-) Distal cisternae (A^+)	109

Event	Approach—cell type	Observations	Reference
Proteolysis			
Proalbumin cleavage	Liver SCF,[a] *in vivo* amino acid label	Proalbumin is enriched in "cisternal" subfractions; albumin in "postcisternal" subfractions	92, 102, 103
Proalbumin cleavage	Same, with colchicine	Overaccumulation of albumin and proalbumin, especially in "post-cisternal" fractions	102, 103
Proalbumin cleavage	Primary liver culture, biosynthetic label ∓ taxol	Overaccumulation of albumin	94
Proalbumin cleavage	Same, ∓ monensin (1 hour)	Overaccumulation of proalbumin	93

SFV[b] glycoprotein cleavage	Biosynthetic label of BHK cells ∓ monensin (1.5 hours)	Interruption of cleavage	37
Sindbis glycoprotein cleavage	Biosynthetic label of chick fibroblasts ∓ monensin (3 hours)	Interruption of cleavage	54
Corona virus glycoprotein cleavage	Biosynthetic label of 17Cll cells ∓ monensin (16 hours)	Interruption of cleavage	88
Friend murine leukemia virus glycoprotein cleavage	Biosynthetic label of Eveline cells ∓ monensin (1 hour)	Slowing of cleavage	127a
SFV glycoprotein cleavage	Biosynthetic label of chick embryo fibroblasts ∓ monensin (2 hours)	No effect on cleavage?	98
Proopiomelanocortin cleavage	Biosynthetic label of rat pituitary culture ∓ monensin (2 hours)	Interruption of cleavage	17
Xylose-linked chain elongation			
Repeating disaccharide synthesis	Biosynthetic label of chick chondrocytes ∓ monensin (3 hours)	Inhibition of synthesis	90, 91
	Biosynthetic label of rat chondrosarcoma ∓ monensin (7 hours)	Inhibition of synthesis	84
	Biosynthetic label of human melanoma ∓ monensin (18 hours)	Inhibition of synthesis	12
Sulfation	As above	Inhibition of synthesis	90, 84, 12
Relative timing of sulfation and chain elongation	$^{35}SO_4$ biosynthetic label, sizing of monomer, rat chondrosarcoma	Sulfation follows elongation, 5 minutes before discharge	64
Relative timing of chain elongation and acquisition of endo H resistance by N-linked units	Amino acid label, gel analysis human melanoma	Roughly simultaneous	12

(*continued*)

TABLE I (*continued*)

Event	Approach—cell type	Observations	Reference
Relative timing of chain elongation and GalNAc addition to O-linked units	Labeling with [^{3}H]GlcN of rat chondrosarcoma	Roughly simultaneous	141
Hyaluronic acid synthesis	Biosynthetic labeling ∓ rat chondrosarcoma monensin (6 hours)	No change from controls	84
Asparagine-linked oligosaccharide addition			
Cytologic localization of β(1–4) Gal transferase	Immunocytochemistry of HeLa cells	Restricted to distal (TPPase$^+$) cisternae	110
Relative density of Gal transferase and mannosidase I	SCF of liver	Gal transferase is lower density than MI^c	100
	SCF of CHO	Same	20
	SCF of BHK	Coisopycnic	101
Relative density of terminal sugar transferases	SCF of liver	Coisopycnic	10, 147
	SCF of lymphoma	Slight differences	34a
Relative density of phosphotransferase and phosphodiesterase	SCF of liver	Coisopycnic with MI	100
Relative density of phosphotransferase, diesterase, and Gal transferase	SCF of lymphoma, macrophage	Progressively decreasing	34a
Phosphotransferase action	Biosynthetic label of human fibroblasts ∓ monensin	Similar to controls	100a
Terminal sugar addition to Ig	Biosynthetic label of plasma cells, myeloma ∓ monensin (1 hour)	Interruption of terminal sugar addition	134, 135
Terminal sugar addition to transferrin	Biosynthetic label of hepatoma ∓ monensin (1 hour)	Same	133

Terminal sugar addition to viral glycoproteins	Biosynthetic label of Eveline cells infected with MuLV ∓ monensin (2 hours)	Same	127a
	Biosynthetic label of BHK cells infected with Lacross virus ∓ monensin (2.5 hours)	Partial block	79a
	Biosynthesis label of chick fibroblasts infected with SFV ∓ monensin (2 hours)	Partial block in oligosaccharide maturation	98
	Biosynthetic label of chick fibroblasts infected with VSV ∓ monensin (6 hours)	G becomes resistant to endo H	54
	Biosynthetic label of hepatoma infected with VSV ∓ monensin (2 hours)	G becomes resistant to endo H	133
Terminal sugar addition to proopiomelanocortin	Biosynthetic label of pituitary culture ∓ monensin (2 hours)	Proopiomelanocortin becomes resistant to endo H	17
Relative timing of lipid addition and acquisition of endo H resistance	Biosynthetic label of Vero cells infected with VSV	Palmitate addition precedes endo H resistance	119
Relative density of site of lipid addition and Mannosidase I	SCF of pulse [^{3}H]palmitate-labeled VSV-infected CHO cells	Site of G acylation is coisopycnic with mannosidase	20
	SCF of pulse [^{3}H]palmitate-labeled SFV infected BHK ∓ monensin (4 hours)	As above, unless monensin has increased mannosidase density	101
Lipid addition	[^{3}H]Palmitate label of Sindbis- or VSV-infected chick fibroblasts ∓ monensin (3 hours)	Little or no inhibition	54

(*continued*)

TABLE I (*continued*)

Event	Approach—cell type	Observations	Reference
GalNAc-linked chain elongation			
Site of mucin Gal addition	Autoradiography of mucin-secreting cells of stomach (2 minute) [^{3}H]Gal pulse)	Incorporation is over distal cisternae and vesicles	72
Relative timing of internal GalNAc and N-linked peripheral GlcNAc addition	Biosynthetic label of chorionic gonadotropin-secreting cells [^{3}H]GlcN label	Similar rates of labeling in secreted hCG	41
Oligosaccharide elongation	Biosynthetic label ∓ monensin (16 hours) 17C11 cells infected with coronavirus	Interruption of sugar addition	88
	Biosynthetic label ∓ monensin (6–9 hours) HEp-2 cells infected with Herpes virus	Inhibition of shift in glycoprotein gel mobility	54a
Glycolipid elongation			
Conversion of glucosyl ceramide to complex glycosphingolipids	Biosynthetic label of glioma [^{3}H]Gal ∓ monensin	Monensin blockes conversion	26a
Transport of gangliosides to cell surface	[^{3}H]Gal pulse–chase, evaluation of surface exposure ∓ monensin	No change from controls	26a

[a]SCF, Subcellular fractionation.
[b]SFV, Semliki Forest virus.
[c]MI, Mannosidase I.

modifications. Possibly those hybrid structures which do not exist in nature might be constructed by appropriate gene splicing.

III. Covalent Modifications

A. Proteolysis

Numerous secretory and membrane proteins are selectively cleaved during their passage through the GC to the cell surface. The best studied examples are proalbumin, the spike glycoproteins of many enveloped virus, and the family of precursors of polypeptide hormones [pro-insulin, -glucagon, -parathyroid hormone, -gastrin, -enkephalin (14), -opiomelanocortin (48), vasopressin-neurophysin precursor (74)]. Many of the data implicating the GC are in fact only indirect. The approaches used involved study of the effect of agents which block secretory protein exit from the RER (e.g., uncouplers) (130), correlation between the kinetics of ICT and cleavage, or, best of all, analysis of smooth microsomal or Golgi-enriched subcellular fractions [e.g., of hepatocytes (92, 102) or virally infected cells (68)].

In the case of Sindbis and Semliki Forest virus it has been suggested that cleavage occurs at the moment of arrival at the cell surface since extracellular antisera directed against their glycoproteins can block cleavage and since precursor species are not detected by surface-labeling procedures (8,151). However, the cytologic site of cleavage may depend on both the virus and the host cell—for several paramyxovirus, influenza virus and certain RNA tumor virus cleavage has been reported to occur intracellularly (68). For Rous sarcoma virus the suggested site of cleavage ranges from the GC to the virion itself (7a). Moreover, certain host cells do not cleave the viral glycoproteins and therefore may yield noninfectious virus.

The covalent site of proteolysis is strikingly uniform (18, 32). In all the cases mentioned above, cleavage occurs adjacent to one to four basic amino acids and results in the release of lysine or arginine. Such amino acid excision and the generation, for example, of insulin from proinsulin, have been ascribed to the concerted action of both trypsin-like and carboxypeptidase B-like activities (18), however, none of the claims of attribution of these activities to identifiable enzymes is unequivocally accepted. Such protease activities have yet to be used as Golgi markers.

An outstanding example of differential cleavage is the case of proopiomelanocortin. The same precursor is cleaved (always adjacent to basic amino acids) to yield different products by cells of the anterior or intermediary lobe of the pituitary (46, 48). Moreover, in other endocrine cells minor amounts of unorthodox or incomplete cleavage products may be detectable (18). The result is a

variety of microheterogeneity reminiscent of the microheterogeneity of processed carbohydrate structures of glycoproteins.

It has been suggested that the proteolysis of proinsulin to insulin may occur both within the GC and in secretion granules (18, 130). The experiments on which this idea is based involve study of isolated granule fractions derived from biosynthetically labeled tissue. These data suggest that protease(s) of the GC may be transported along with the secretory product. In the case of proopiomelanocortin processing, successive cleavage events may occur within different compartments—β-lipotropin and ACTH are generated during transport, while further conversion to β-endorphin occurs in granules (32a, 38).

There are no indications that cleavage is a prerequisite for transport. In the case of proalbumin, proinsulin, and the precursors of viral proteins, altered polypeptides which resist cleavage (mutant species or the products of incorporation of amino acid analogs) have been shown to arrive at the cell surface (18, 68).

Amino acid labeling pulse–chase experiments show that monensin interrupts the cleavage of viral surface glycoproteins, proalbumin, and proopiomelanocortin. (Table I). Therefore, one can ascribe cleavage to a late subcompartment. This conclusion is consistent with the liver Golgi subfractionation studies which show that proalbumin–albumin conversion occurs upon entry into "postcisternal" Golgi elements—those which carry the greatest load of lipoprotein (92, 103). Experiments on the influence of colchicine on N-linked oligosaccharide maturation of secretory glycoproteins show that it interrupts ICT at a very late site (102, 103). It is therefore not surprising that it causes an accumulation of postcisternal elements containing an excess of both albumin and proalbumin (102, 103). When taxol is used to stabilize microtubules cleavage to albumin proceeds, and discharge is much reduced (94).

In the case of glycoproteins which undergo proteolytic cleavage prior to arrival at the cell surface there are insufficient data to generalize with respect to the relative order of the events of carbohydrate maturation and proteolysis. Nevertheless, pulse–chase studies of proopiomelanocortin biosynthesis employing monensin suggest that the acquisition of mature carbohydrate units precedes proteolysis (17). In this study the inhibition by monensin may reflect neutralization of intragranular pH since the granule protease has a pH optimum at pH 5 (69).

B. Initiation and Elongation of Xylose-Linked Oligosaccharides

Chondroitin sulfate, dermatan sulfate, heparin, and heraran sulfate all contain the same internal tetrasaccharide structure (Xyl-Gal-Gal-GlcUA) linked to multiple serine residues of a protein core.

The four corresponding monosaccharide transferases have been characterized and substantially purified, and a limited amount of information is available concerning the polypeptide recognized by the initiator xylosyl transferase (15).

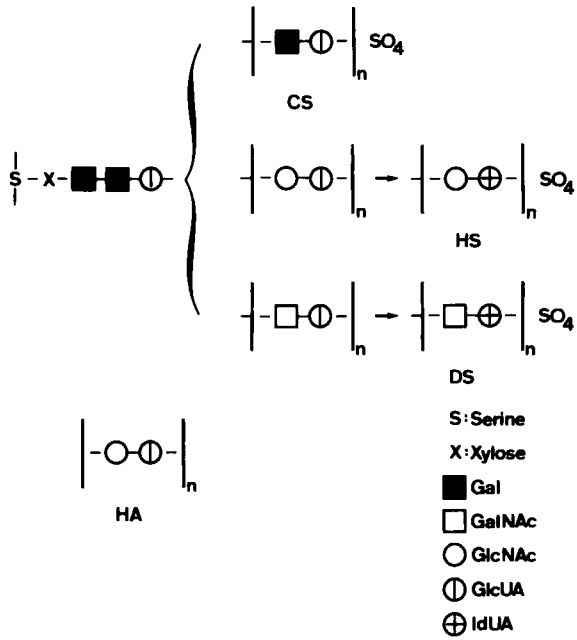

FIG. 1. Structural and biosynthetic relations among xylose-linked proteoglycans and haluronic acid (which lacks a protein core). Serine residues acquire a common tetrasaccharide by sequential monosaccharide addition and are subsequently elongated by addition of repeating disaccharides—again from monosaccharide donors. For heparin, heparan sulfate, and dermatan sulfate a further epimerization of GlcUA to IdUA occurs at many sites. A single core protein may bear both chondroitin sulfate and keratan sulfate (cf. Figs. 2 and 3) as well as O-GalNAc and N-GlcNAc-linked chains. For HS and DS some sulfation precedes epimerization. CS, Chondroitin sulfate; HS, heparan sulfate; DS, dermatan sulfate; HA, hyaluronic acid.

There are provisional indications that at least the xylosyl and first galactosyl transferases might be physically associated with each other—they are both recovered in immunoprecipitates prepared with monospecific anti-xylosyl transferase antisera (121).

The literature is equivocal with respect to the cytologic site(s) of addition of these internal sugar residues. Attempts at subcellular fractionation of embryonic cartilage suggest that the four corresponding enzyme activities belong to both rough and smooth microsomal fractions; however, the enzyme assays employed are in several respects not ideal (51).

Part of the fascination with proteoglycan biosynthesis comes from recognition of their immense size [beautifully visualized by electron microscopy (62)] relative to the dimensions of the machinery of ICT. A monomer of cartilage proteoglycan, for example, consists of a core protein (~ 300 nm long when spread)

which bears on the order of 80 lateral sulfated chains (internal tetrasaccharide and repeating sulfated GalNAc, GlcUA disaccharide) each of which is about 100 residues long.

Recent experiments making use of antisera directed against protein determinants of the proteoglycan monomer indicate that there is a substantial intracellular pool of core protein lacking the repeating disaccharides (12, 65). In amino acid pulse–chase experiments, this pool, which is presumably in the RER, can be chased into mature proteoglycan with a half-time of ~ 80 minutes, i.e., roughly equal to the half-time for the inhibition of proteoglycan sulfation by cycloheximide (64, 83).

Autoradiographic data and experiments employing puromycin (33, 87, 140, 149) are clear in implicating the GC as the site of addition of the repeating disaccharides and their sulfation. Moreover, cell-free synthetic studies employing Golgi-rich fractions of a mastocytoma show (by analogy with other studies of disaccharide growth) that chain elongation is the result of alternate addition of the two component sugars from UDP-GalNAc and UDP-GlcUA (123). No information is available concerning determination of the length of the oligosaccharide chains, which are, in fact, not of altogether uniform length (25). Sulfation appears to occur after disaccharide elongation since 2-minute $^{35}SO_4$ pulse-labeled intracellular chains have already acquired their mature hydrodynamic size (64). The half-time for discharge of $^{35}SO_4$ pulse-labeled chains is only ~ 5 minutes (63). Hence sulfation is a very late event.

Several noncovalent assembly steps result in the ultimate extracellular product: monomeric units of derivatized core protein firmly associated with the hydrophobic "link" glycoprotein and hyaluronic acid. A set of ingenious competition experiments has shown that hyaluronic acid—which is synthesized by the same cells which synthesize the monomer—is bound only after discharge (63). Although intracellular monomer can bind the link protein after solubilization, it is not known whether this event or the association with collagen (121, 143) normally occurs prior to discharge.

A word should be said about the biosynthesis of hyaluronic acid. It is at no time covalently bound to protein and cycloheximide does not interrupt its biosynthesis (80). Its repeating disaccharide (GlcNAc, GlcUA) can be assembled *in vitro* by incubating cell extracts with UDP-activated substrates (2). Growth is at the reducing end of the chain, which is transiently membrane bound, possibly via UDP (100b). Autoradiographic study of synovial cells proves that hyaluronic acid synthesis does indeed occur within the GC (5). The demonstration that its synthesis is not influenced by doses of monensin which block sulfation of chondroitin sulfate by the same cells is consistant with its not having a protein core—sugar addition can occur *in situ* without ongoing ICT (84). In order to assign its synthesis to a subcompartment it will be important to learn whether monensin blocks its secretion.

The most complex of the proteoglycan biosynthetic pathways is that of heparin, which has been in large part elucidated with the help of mast cell tumors. Following the internal tetrasaccharide addition and elongation of the repeating (GlcNAc, GlcUA) disaccharide units, five further enzymes intervene (76, 77): (1) a deacylase which removes most acetyl groups from GlcNAc (this activity is low in cells synthesizing heparan sulfate and is stimulated by N-sulfation at other residues), (2) an *N*-sulfotransferase which acts on most of the sites exposed by (1), (3) the epimerase which converts 70–90% of glucuronic acid residues of the polymer to iduronic acid, (4) an *O*-sulfotransferase which acts at C-2 of many iduronic acid residues, and (5) an *O*-sulfotransferase which acts at C-6 of glucosamine. There are no data indicating whether some or all of these activities are expressed in the same cytologic subcompartment in which disaccharide elongation occurs. Recent studies have indicated the existence of two classes of heparan sulfate. One is released from the cell (e.g., hepatocyte); the other is hydrophobic and membrane-associated. It is thought to have a hydophobic core protein which serves as anchor (66).

Study of the biosynthesis of chondroitin sulfate is potentially of unique interest for defining the subcompartmentalization of the GC since the same core protein which bears 80 xylose-linked repeating disaccharides also bears ~ 12 N-linked complex oligosaccharides, ~ 120 short O-linked GalNAc-containing oligosaccharides, ~ 60 single GalNAc residues, and (in many cases) keratan sulfate chains (44, 78, 79). Such multiple derivatization of a single polypeptide provides ideal material for establishing the relative sub-Golgi sites and rates of processing of such divergent structures. The presently available kinetic data shown that the acquisition of endoglycosaminidase H (endo H) resistance by the N-linked oligosaccharides of chondroitin sulfate is roughly coincident with the addition of the repeating disaccharides (12). Study of the incorporation of labeled glucosamine into the repeating disaccharides and the internal residues of the short O-linked units suggest that these two events occur simultaneously (141).

Other data bearing on the sub-Golgi localization of proteoglycan synthesis come from use of monensin. Both the addition of repeating disaccharides and sulfation of chondroitin sulfate are markedly reduced in the presence of monensin though the pools of ATP and phosphoadenosine phosphosulfate are unchanged (12, 57, 90, 91). One can provisionally conclude that these events take place relatively late during transit through the GC. The limited amount of chondroitin sulfate which is secreted in the presence of monensin is undersulfated and may serve as a model for certain achondroplasias.

C. Tailoring of Asparagine-Linked Oligosaccharides

1. *Secretory and Membrane Glycoproteins (7, 71, 113–115, 125, 127)*

With the realization that preassembled dolichol-linked oligosaccharides are cotranslationally added to selected asparagine residues, the further processing of

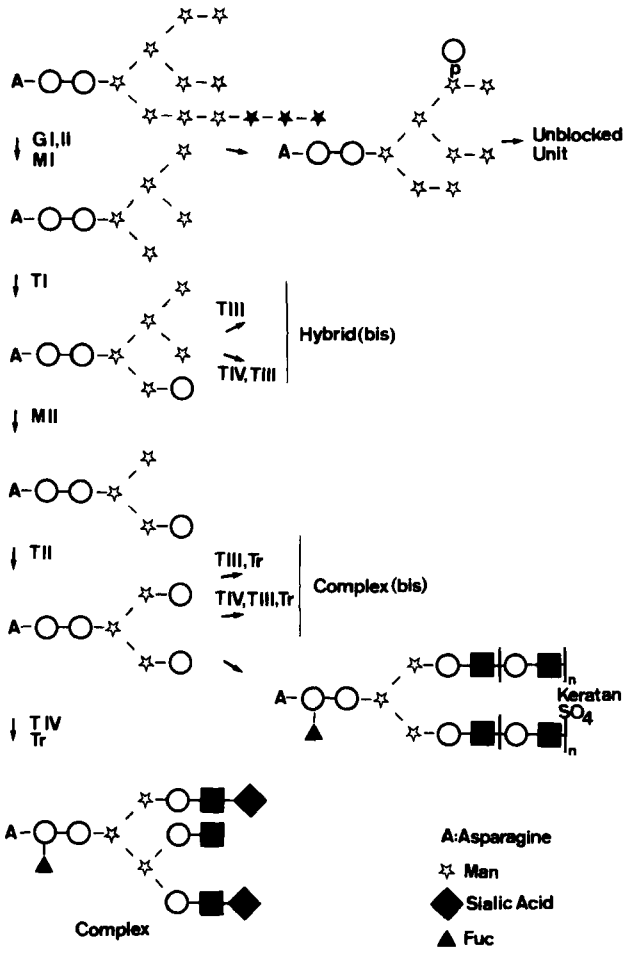

FIG. 2. Structural and biosynthetic relations among asparagine-linked saccharides. The common precursor at the top left can be trimmed and further processed toward complex or hybrid structures, structures characteristic of lysosomal enzymes ("unblocked unit"), or keratan sulfate. As is extensively discussed elsewhere (71,113) along the "complex pathway" several options and control points exist. The activities of GlcNAc transferases (TI–TIV) are of key importance. TIII, for example, generates a "bisected" (bis) structure by addition of GlcNAc to the most internal (β-linked) mannose and as a result blocks the activity of TII, TIV, fucosyl transferase, and mannosidase II. TI action is a prerequisite for action of several further transferases. The structure bearing GlcNAc–phosphate, which can give rise to an unblocked unit, indicates only one of several possible phosphorylated mannose residues. The actual degree of concomitant mannose trimming is progressive but not uniform. Tr, Terminal sugar (fucose, Gal, sialyl) transferases; G, glucosidase; M, mannosidase.

such moieties has been rapidly elucidated. The recent reviews of these investigations are too thorough to warrant an exposition of this now well-known sequence of steps which involves both sugar trimming and terminal sugar addition. The processing to a triantennary complex unit, e.g., of the G protein of vesicular stomatitis viruses (VSV) in infected CHO cells, involves nine enzymes and of these only the first two glucosidases and possibly the first mannosidase act at the level of the RER (71, 127, 135). The rest have been localized to preparative Golgi fractions. The β(1-4) galactosyltransferase responsible for subterminal Gal addition is, at present, the best characterized Golgi enzyme. Not only have its enzymological properties been investigated in several tissues, but in addition first studies of its trans-membrane orientation (30) and biosynthesis (132) have been reported. In HeLa cells the transferase bears both O-linked and endo H-resistant N-linked oligosaccharides (G. Strous, personal communication).

Several laboratories have monitored attempts at sucrose gradient Golgi subfractionation with the use of this set of enzyme activities. The observations to date are fragmentary and the separations only partial. The first mannosidase has been reported to have an isopycnic density in sucrose gradients which is higher than that of galactosyltransferase [CHO cells (20) and liver (100)]. Such a difference is not observed in BHK cells even when they are infected with Semliki Forest virus and treated with monensin so as to increase the density of medial Golgi elements (101). In the case of CHO cells the density differences are small but intriguing since the mannosidase activity is coisopycnic with those vesicles in which the G protein acquires covalently bound lipid (vide infra). Attempts to separate subfractions differentially enriched in the terminal sugar transferases (GlcNAc, Gal, Sialyl) by sucrose gradient analysis have failed (10, 147) or provided only slight differential enrichment (34a). Both affinity methods and countercurrent distribution studies do discriminate between liver Golgi subfractions differentially enriched in galactosyltransferase vs "ER-like" enzyme activities (49, 53).

An outstanding only partly resolved question is what governs the particular path and degree of processing at a given glycosylation site. Among the key considerations is the repertoire of processing activities of a given tissue, possible competition between transferases of overlapping specicificity (7, 113), and the steric environment of the oligosaccharides. In the case of thyroglobulin (127), certain IgM myeloma proteins (1) and certain viral envelope glycoproteins the situation is particularly intriguing since different sites along a single polypeptide ungergo radically different degrees of processing. In this last-mentioned case, it has been observed that when Sindbis virus infects different host cells different sites are processed to different extents (125).

Additional variables in terminal sugar addition concern (1) the degree to which the transferases may be present at the cell surface as well as in the GC, and (2)

the apparent intracellular pool size of secretory glycoproteins which have already accepted their terminal sugars. Both enzymologic (122) and, less equivocally, immunocytochemical studies (99) show that in selected cells galactosyltransferase may be exposed at the (apical) cell membrane. Presumably this is a reflection of some structural change in the enzyme or other determinative Golgi elements which abrogates the normal affinity of the enzyme for the GC. With respect to (2), comparative studies of immunoglobulin synthesis by several mouse myelomas indicate that the intracellular pool of Ig which can be labeled with terminal sugars may be either undetectably small or quite substantial (81, 97). These variations may parallel the degree of differentiation of the myeloma in question. A variable location of the terminal sugar transferases might explain such observations, i.e., in some cases they might act later along the path of ICT than in others.

Several cytologic studies pertain to this pathway. An immunocytochemical study has localized β(1-4) galactosyltransferase to the distal (thiamine pyrophosphatase-positive) cisternae of the GC of HeLa cells (110). The presumed products of terminal sugar transferases have also been localized. Concanavalin A–gold conjugates (which interact primarily with immature asparagine-linked oligosaccharides) stain the cisternal space of the RER and all Golgi cisternae, whereas ricin 120 (which interacts with nonreducing galactosyl residues) stains only the more distal cisternae of BHK cells (36, 37). In a methodologically quite different study of IgM-secreting myeloma cells and an Ig-negative mutant, concanavalin A–peroxidase has been shown to stain the cisternal space of both the RER and proximal cisternae while a wheat germ agglutinin–peroxidase conjugate (specific for clustered sialic acid) stains the cisternal space of more distal cisternae and associated vesicles (138).

Any such studies must be interpreted in light of what is known of the principle glycoproteins and glycolipids of the cells studied. Especially in the case of the IgM myeloma (and to a lesser extent with the BHK cells when they are infected with Semliki Forest virus) one has reason to consider much of the staining to be due to a single glycoprotein. Nevertheless, the study of the myeloma mutant suggests that the oligosaccharides of underlying Golgi components distribute in roughly the same way as the Ig oligosaccharides. Taken as a whole, the cytologic observations argue strongly that the direction of transport is indeed proximal-to-distal and that the terminal sugars are acquired during passage across the stack of Golgi cisternae.

These conclusions are also compatible with the available monensin data. In short-term experiments with IgM-secreting plasma cells and myeloma cells, monensin interrupts ICT within the GC at a site where the Ig heavy chains are still sensitive to endo H and have acquired neither galactose, fucose, nor sialic acid (134, 135). Terminal sugar addition to other glycoproteins continues and Ig

labeled with [^{3}H]galactose or [^{3}H]fucose is rapidly secreted (139). Similar results have been reported for the effect of monensin on maturation of the oligosaccharides of transferrin (133) and of the surface glycoproteins of Semliki Forest virus (37). In other cells and employing somewhat different protocols the major intra-Golgi site of accumulation of membrane proteins in the presence of monensin may be such as to have allowed somewhat further oligosaccharide maturation (Table I). Nevertheless, transport to the cell surface is not observed.

There is a line of experimentation indicating the existence of two sequential subcompartments traversed by the G protein of VSV. The ultimate interpretation of the data remains to be determined, but is thought to pertain to the ability of the membranes of Golgi subfractions to undergo fusion with the membrane of a relatively late subcompartment. In the protocols employed (20, 30a, 111) VSV-infected mutant CHO cells (lacking GlcNAc transferase I) are pulse-labeled with [^{35}S]methionine and chased for increasing intervals. At each time point a cell homogenate is mixed with a wild-type homogenate, briefly incubated, and assayed for the proportion of ^{35}S-labeled G (initially sensitive to endo H and perpetually so in the mutant CHO cell) which has acquired resistance to endo H. The *in vitro* maturation is manifest only after a brief chase interval, suggesting that at later times G has gained access to a subcompartment which will no longer participate in such membrane–membrane fusion.

Numerous yeast glycoproteins, for example, secretory invertase and acid phosphatase, bear both asparagine-linked and O-linked polymannosyl oligosaccharides. The asparagine-linked units are donated from the same dolichol-linked intermediates as in mammalian cells, trimmed to a structure containing 2 GlcNAc and 8 Man, and elongated by massive further addition of mannose and phosphate (3). Studies of yeast temperature-sensitive ICT mutants which overaccumulate RER, Golgi-like cisternae, or vesicles suggest that the polymannosyl elongation of the N-linked oligosaccharides of invertase occurs within the GC (21). The phosphorylation of the secretory protein also occurs within this compartment (131).

2. *Lysosomal Enzymes (71, 86, 124)*

In cultured fibroblasts, the proper intracellular targeting of lysosomal enzymes depends on the presence of mannose 6-phosphate on high mannose asparagine-linked oligosaccharides. The phosphorylated structure is the result of the post-translational addition of one or several GlcNAc-1-phosphate units to several oligosaccharides per polypeptide, followed by removal of much of the GlcNAc. Biosynthetic labeling studies show that the "unblocked" phosphate structure is achieved within 1 hour after a [^{3}H]mannose pulse and that phosphoryl groups are ultimately lost, presumably upon entry into lysosomes (31). The enzymes responsible both for addition of the blocked phosphate and subsequent removal of

the GlcNAc are both enriched in preparative liver Golgi fractions. In attempts at liver Golgi subfractionation, the two activities have been reported to be coisopycnic with mannosidase I, i.e., denser than galactosyltransferase (100). Sucrose gradient subfractionation of a mouse lymphoma and macrophage cell line has partially resolved the phosphotransferase, phosphodiesterase, and Gal transferase (34a). There is also a further suggestion that mannosidase I and the phosphotransferase are localized to the same Golgi subcompartment—in ^{3}H-Man pulse-chase studies the degree of trimming to a Man_7 structure correlates with the progressive addition of blocked phosphates (34).

Since lysosomal enzymes also bear complex oligosaccharides (34, 45, 124), they must pass via the terminal sugar transferases (i.e., anatomically distal cisternae), and indeed they can be visualized within all Golgi cisternae by histochemical methods. This route of passage is acutely manifest in the I-cell syndrome in which GlcNAc-1-phosphotransferase activity is missing and many lysosomal enzymes are released from cells. Such molecules contain more sialic acid and are bound to insolubilized ricin to a greater extent than in the wild type (124, 134a). A somewhat analogous situation is also observed for a CHO cell mutant in which lysosomal enzymes bear only complex oligosaccharides (71a). Also consistent with this line of reasoning is a report that the mannose-phosphate receptor—which is thought to mediate the ICT of newly synthesized lysosomal enzymes—bears sialic acid; however, the receptor molecules isolated may largely originate from the cell surface rather than from the GC (129).

How does the cell determine whether or not to add the blocked phosphate to a given oligosaccharide? Two points should be made. (1) The signals have not been identified, but when the GlcNAc-phosphotransferase is presented with any of several high mannose secretory proteins or lysosomal enzymes, transfer is overwhelmingly more active toward the lysosomal enzymes (106, 144), and (2) the studies on processing of lysosomal enzymes once again indicate the existence of microheterogeneity: the degree of addition of blocked phosphate, of unblocking, and of addition of terminal sugars is variable.

In yeast, where lysosomal enzyme oligosaccharides are not essential for targeting to the lysosome (120), lysosomal carboxypeptidase Y is phosphorylated while in the RER (131). Its carbohydrate is processed in two stages while tranversing the GC–temperature-sensitive ICT mutants blocked at the level of the GC accumulate one of two endoglucosamindiase H-sensitive species (131).

Data on the influence of monensin on lysosomal enzyme transport are suggestive. In I-cell fibroblasts monensin reduces hexosaminidase secretion (143b). In normal cells it may actually stimulate their release (100a). It is striking that chloroquine, which also raises intralysosomal pH, also causes release of newly synthesized lysosomal enzymes (124). This effect can be attributed to neutralization of lysosomal content by the ionophore and a consequent tying up of the

mannose–phosphate receptor: however, the situation has not yet been fully analyzed. The secreted lysosomal enzymes are phosphorylated (100a).

3. *Lipid Addition to Membrane Proteins (117, 118)*

The presence of covalently bound lipid is not restricted to proteins which traverse the secretory pathway; however, a number of plasma membrane glycoproteins have been shown to acquire fatty acids posttranslationally. The best studied cases are the envelope glycoproteins of Vesicular Stomatitis virus, Semliki Forest virus, and Sindbis virus, the proteolipid of myelin, the transferrin receptor, HLA antigens, and T200, a lymphocyte surface antigen. The covalent sites of fatty acid linkage are suspected to be cysteine or serine residues. The corresponding enzymes and activated fatty acid precursors have not been identified.

Four lines of experimentation suggest that an early Golgi subcompartment is the site of palmitate addition in virally infected cells. (1) Pulse–chase studies employing labeled palmitic acid show that the half-time required by cycloheximide to shut off acylation of viral proteins is 15 minutes. Hence acylation is certainly not a cotranslational event (119). (2) Within only 3 to 6 minutes after acylation these proteins become resistent to endo H (VSV) or undergo proteolytic cleavage (Sindbis), whereas when amino acids are used to label the same glycoproteins, the half-time for acquisition of endo H resistance is 20–30 minutes (119). (3) Subcellular fractionation of VSV-infected CHO cells pulse-labeled with [^{3}H]palmitate shows that acylated G is coisopycnic with mannosidase I activity (20). In a closely related system, however, this isopycnic coincidence is eliminated when the density of "medial" Golgi elements containing the acylated viral proteins is increased by infecting the cells with Semliki Forest virus and then treating with monensin (101). In this situation mannosidose I is unperturbed and remains coisopycnic with galactosyl transferase. (4) The acylation of these glycoproteins is not blocked by monensin (54).

It is curious that in yeast ICT mutants acylation has been assigned to the RER (M. Schlesinger, personal communication). Since the phosphorylation of yeast lysosomal enzymes also occurs in such RER-blocked mutants (131), but has been localized to preparative liver Golgi fractions (71), one wonders whether the conventional anatomic boundary between RER and GC corresponds to the mutant phenotypes. In higher organisms a partial enzymologic overlap has been noticed between the RER membranes and the GC (49, 53, 111) and electron microscopic lectin-binding studies (36, 135, 138) suggest that it is the anatomically proximal Golgi cisternae that resemble the RER. Moreover, as already mentioned, monensin experiments have assigned both acylation and lysosomal enzyme phosphorylation to an early subcompartment. Perhaps in the yeast ICT

mutants in question the accumulated unit includes what is conventionally considered to be the proximal face of the GC as well as the RER.

4. *Keratan Sulfate I*

An additional modification of asparagine-linked oligosaccharides occurs in the cornea where they may be processed to corneal keratan sulfate, a rather small (70 kilodalton) proteoglycan characterized by a repeating sulfated (GlcNAc, Gal) disaccharide. Since keratan sulfate I biosynthesis can be blocked by tunicamycin (43), these units appear to be the result of an alternate derivatization of the same trimmed oligosaccharide which can be processed to a sialic acid-containing complex oligosaccharide. Indeed, the linkage region is identical to that of the complex sites—only three mannose residues remain and these bear GlcNAc and Gal followed by the repeating disaccharide (89).

D. Initiation and Processing of *N*-Acetylgalactosamine-Linked Oligosaccharides

Present data are largely in favor of the notion that the addition of the most internal GalNAc to serine or threonine takes place in the GC, long after polypeptide chain termination. The data derive from the distribution of GalNAc transferase activity in subcellular fractions of intestinal mucosa (61), and oviduct (42), from study of the impact of cycloheximide on GalNAc addition to mammary glycoproteins (146), and from the comparative evaluation of the kinetics of GalNAc and GlcNAc addition to O-linked and N-linked units, respectively (41). The result of glycosylation is most impressive for mucins—hundreds of oligosaccharides are added and the product may be greater than 80% carbohydrate (11). The enzymes responsible for chain elongation have been studied in sufficient detail as to rationalize the set of structures observed. It has been reported that neuraminic acid of mucins can be acetylated and that acetyl groups can be replaced by glycolyl groups after their addition to mucin (116).

Two approaches localize mucin-specific transferase activities to Golgi subcompartments: (1) an autoradiographic study of [^{3}H]Gal pulse-labeled mucus secreting cells which indicates that incorporation takes place over distal cisternae and associated vesicles (72), and (2) a cytochemical study of thin sections of goblet cells stained with lectin–gold conjugates to detect nonreducing terminal GalNAc (109). Proximal cisternae are stained and medial cisternae negative. Only in blood group A^+ material (where terminal GalNAc is present) are the distal cisternae and secretion granules also stained. Both studies are, therefore, consistent with the idea of progressive sugar addition during proximal-to-distal transport across the Golgi stack.

A single investigation indicates that the subcellular site of incorporation of the

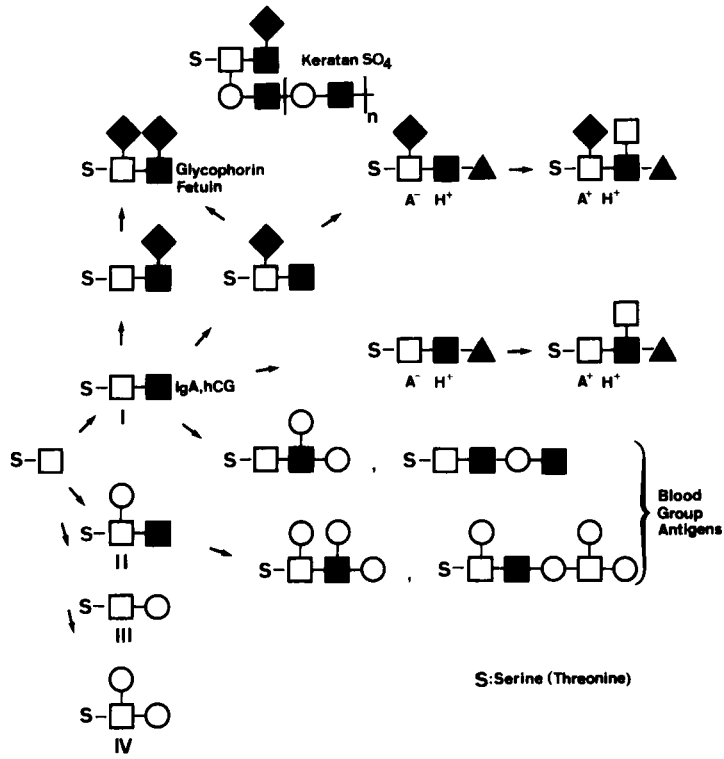

FIG. 3. Structural and biosynthetic relations among common GalNAc-linked oligosaccharides. The four basic core structures (I–IV) [cf. (115)] are derivatized to generate the units found on membrane glycoproteins, hormones, mucins, and keratan sulfate. The blood group H determinant is Fucα(1-2)Gal-, the A$^+$ determinant is GalNAc(1-3)[Fucα(1-2)]Galβ-. The four structures indicated by the bracket "blood group antigens" are precursors of human antigens. Competition of transferases of overlapping specificities explains certain choices among the alternate biosynthetic paths. IgA, Immunoglobulin A; hCG, human chorionic gonadotropin.

internal and peripheral O-linked GalNAc is (kinetically) near the site of addition of peripheral GlcNAc to N-linked oligosaccharides (41). Tissue culture cells synthesizing the beta subunit of human chorionic gonadotropin (which bears both O- and N-linked oligosaccharides) are labeled with [^{3}H]GlcN for periods up to 3 hours. Successive samples of secretion are collected and analyzed for O-linked [^{3}H]GalNAc, and N-linked peripheral and internal [^{3}H]GlcNAc. The kinetics of labeling of the first two are rapid and similar. The internal GlcNAc is labeled only after a lag which is considered a measure of the time required for exit from the RER to the cell surface.

The best characterized surface glycoprotein of the human red blood cell, glycophorin, bears 15 O-linked tetrasaccharides and one N-linked oligosac-

charide. The O-linked units, which begin with GalNAc, should serve as a model for step-by-step analysis of chain elongation, as should the O-linked oligosaccharides of the envelope glycoproteins of murine corona virus. The incomplete biosynthetic data suggest, paradoxically, that O-linked oligosaccharides are added when the glycophorin message is translated in the presence of dog pancrease microsomes (55). The only published data on the corona virus show that very long-term monensin treatment blocks the addition of O-linked sugars (88). A similar observation has been made in the study of the influence of monensin on the biosynthesis of the O-linked oligosaccharides of Herpes virus glycoproteins (54a).

Skeletal keratan sulfate (keratan sulfate II) is produced by chrondrocytes of mature cartilage and contains the same repeating disaccharide as the N-linked variety (keratan sulfate I). In this case, the saccharide is linked via GalNAc to serine or threonine residues of the core protein. By analogy with data discussed above, one would expect both chain initiation and elongation to occur in the GC. Since the same protein core contains short mucin-like O-linked oligosaccharides and since the distribution of these units is not random there must be signals indicating which serine or threonine residues are to receive the repeating disaccharide chains (44, 79).

E. Glycolipid Chain Elongation

The abundant and diverse classes of glycolipids have received little attention in the context of ICT, although glycolipids are enriched in plasma membrane and Golgi-rich fractions. Nevertheless, a unique elegant biosynthetic study of cultured neuroblastoma cells shows that several gangliosides are transported from an intracellular site (presumably the GC) to the cell surface with a half-time of 20 minutes (82).

Following the addition of the most internal glucose or galactose to ceramide, a broad range of further processing pathways may be available, according to the tissue in question. The sugar transferases involved are thought to be largely distinct from the transferases involved in glycoprotein synthesis.

Glycolipid sugar transferases responsible for synthesis of GM3, GM2, GM1, and GD1 have been found enriched in preparative Golgi fractions of rat liver, kidney, and bovine thyroid (22, 58, 96); however, it is striking that the activity responsible for the conversion of GM3 to GD3 is not especially enriched in the thyroid fractions (Fig. 4). Recent [^{3}H]Gal pulse–chase studies of rat glioma cells show a precursor–product relation between glucosylceramide and more complex glycosphingolipids. This conversion is effectively blocked by monensin, suggesting that it involves transport from the early part of the GC (or RER) to a later region. In strict analogy with studies of secretion of [^{3}H]Gal-labeled lg (139), [^{3}H]Gal-labeled gangliosides are rapidly transported to the cell surface in the

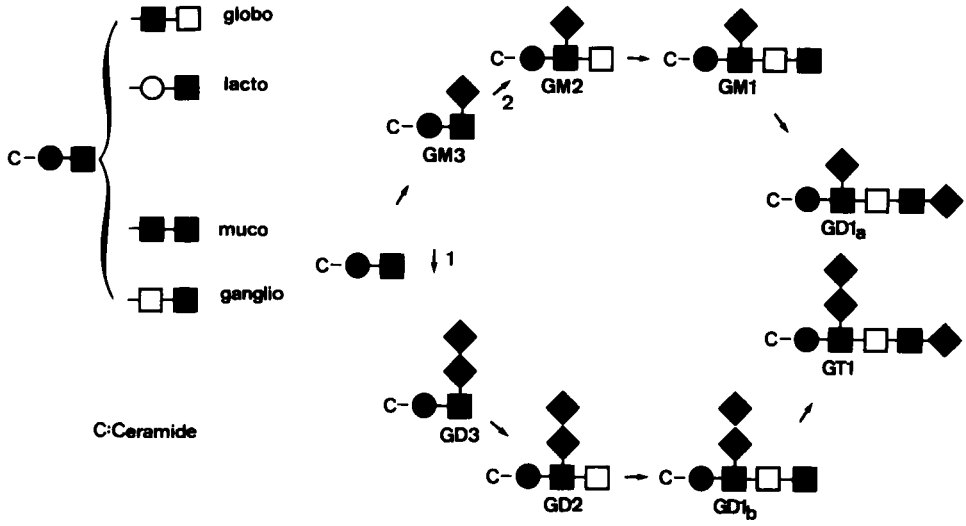

FIG. 4. Structural and biosynthetic relations among glycolipids. After glucose addition to ceramide several paths can be followed which result in the globo, muco, lacto, and ganglio series. Only some of the simplest structures are indicated. The ganglioside biosynthetic pathway is further detailed. It is surprising that enzyme (1) is not highly enriched in Golgi fractions. The conversion of GM3 to GM2 (enzyme 2) occurs with high efficiency in neural tissue. GM3 is the major glycosphingolipid of nonneural tissues.

presence of monensin (P. Fishman and H. Miller-Podraza, personal communication).

Studies of the distribution of sulfotransferases involved in sulfatide synthesis have also localized these enzymes to preparative Golgi fractions of testes and kidney (27–29, 70). A single study demonstrates transport of sulfatides from GC to the cell surface (28).

Since the methods for detailed analysis of glycolipids are now relatively streamlined and since the biological importance of glycolipids is increasingly apparent, further data on their ICT should be forthcoming.

IV. Noncovalent Modifications

In the hepatocyte secretory lipoprotein particles are visible within the smooth endoplasmic reticulum and become conspicuous within the GC, especially after ethanol intoxication (128). The secreted particles contain noncovalently bound triglycerides, phospholipids, and free and esterified cholesterol. Recent studies of isolated Golgi fractions suggest that the phospholipids of the contained lipoproteins are in rapid equilibrium with the phospholipids of the membranes of

Golgi cisternae (52). It therefore appears likely that it is within the GC that they acquire most of their lipids. Nevertheless, since lipoprotein particles recovered from rat and chicken liver Golgi fractions do not have the same lipid composition as in serum, further intra- or extracellular maturation events must be involved (4, 52). In murine macrophages the binding of apolipoprotein E to cholesterol occurs only after discharge from the cell (6).

Secretory cells may be divided into two classes: the nonregulated class (e.g., plasma cells, fibroblasts) and the regulated class (e.g., pancreatic and pituitary cell types). Only members of the regulated class store their secretory products in granules. This content is released by exocytosis upon physiologic stimulation. The genesis of such secretory granules and lysosomes takes place either within distal Golgi cisternae or in vacuoles (e.g., "condensing vacuoles") in their immediate vicinity, according to cell type and physiologic conditions (23, 24). Such impressive condensation of content is in many cases thought to reflect the electrostatic interaction between secretory products, for example, between secretory proteins and proteoglycans (24, 77, 142). Presumably the site of visible condensation corresponds to the region of the GC within which the proteoglycan is synthesized (e.g., sulfated) or to a later region. As already mentioned, experiments employing monensin indicate that sulfation of chondroitin sulfate is a rather late Golgi event. The proteoglycans of secretory granules have been reported to be heparin, heparan, dermatan, and chondroitin sulfate (60, 104, 142, 150).

A fundamental question pertaining to the organization of the GC should also be considered. A wealth of data demonstrates that the terminal sugar transferases, for example, are restricted to a subregion of the GC. Why do these proteins, synthesized in the RER, exit only as far as this portion of the GC and migrate, if at all, only much more slowly to the cell surface? Several explanations might be invoked: (1) the ionic content of the GC is unlike that the the RER and as a consequence a conformational change is induced in such membrane proteins which reduces their mobility—the possibility that the Golgi content may have unique ionic properties has recently been discussed (136), or (2) the protein encounters, within the membrane of Golgi cisternae, certain stable residents (proteins?) for which it has a high affinity, or (3) a posttranslational covalent modification executed within the GC is responsible. To date, no experimental means have been identified which cause mislocalization of such Golgi markers and no information is available to indicate whether one of the three proposed explanations might be correct.

V. Consequences of Processing for the Golgi Complex and the Cell as a Whole

The point of departure for this article was the supposition that the primary function of the GC is to accomplish net unidirectional ICT. It is presumably

through evolution that the GC has acquired an impressive array of other responsibilities. These activities are the subject of the preceding pages and are summarized in Table I. What is the impact of housing these processing events on the GC, and what are some of the dividends for the cell?

1. The GC has become such an exceedingly active center for sugar addition that the content of the cisternae must be a seething bath of activated sugar nucleotides. These species, to the extent that they have been investigated, gain access to the cisternal space by facilitated transport through specific sites (13, 16, 30, 126). This set of transporters may be considered as additional set of Golgi markers.

2. The GC must deal with the side products of these reactions—the mono- and dinucleosides which are produced by the transferases—especially since UDP inhibits GalNAc and Gal transferase hyaluronate synthetase (59, 95, 100b). Both the UDP and GDP and to a lesser extent CMP are broken down within the cisternal space by nucleoside diphosphatase (9, 95). Furthermore, the end-products of hydrolysis must exit from the cisternae in order to function in repetitive cycles. Only a single report pertains to this second class of carriers (30). In the same vein, the ability of the GC to synthesize lactose (in the mammary gland) and the presence of both mannosidase and phosphodiesterase activities suggests that Golgi membranes are impermeable to disaccharides yet permeable to monosaccharides. Experimental observations on the mammary gland and myeloma cells suggest that this is the case (135, 145).

3. The ability to assemble proteoglycans may be considered a prerequisite for the existence of the regulated secretory cell phenotype.

4. The addition of both neutral and negatively charged sugars to intrisic glycoproteins and glycolipids of the GC obviously modifies the environment in which other Golgi activities occur. For example, the local membrane potential may be perturbed at sites where sialic acid has been added, and the presence of ceramide derivatives may result in membrane rigidification (148).

5. The assembly of membrane-bound oligosaccharides clearly allows the cell to endow its surface with negative charge and an array of specific structural sites beyond those simply encoded in its polypeptides.

6. The generation of mannose phosphate groups, in many cells, is essential for targeting of lysosomal enzymes.

Such considerations indicate that the existence of posttranslational processing events is an integral part of the cells repertoire of biosynthetic equipment. Given this realization, it would not be altogether suprising to learn that nucleoside diphosphatase, for example, had subsumed certain functions at the level or overall Golgi structrue and organization. Other proteins involved in more specialized processing events, e.g., select glycolipid sugar transferases, found only in the GC of certain cells, might not be expected to have acquired such dual responsibilities.

Other common features of processing deserve comment, for example, the microheterogeneity which is commonplace among glycoproteins and may also be characteristic of proteolytic cleavage. No experimental studies have attempted to manipulate the degree of microheterogeneity, e.g., by reducing the temperature so as to slow transport through the GC. Nevertheless, in addition to the enzymologic considerations mentioned above (overlapping transferase specificities, etc.), one wonders whether dispersion in the rates of entry and exit and duration of residence within a given subcompartment may not be responsible. In this sense, the length of an oligosaccharide chain, e.g., of chondroitin sulfate, might be considered an indirect measure of the kinetics of transport.

For the moment, knowledge of the sequences of posttranslational processing events can explain the final structures of many macromolecules which pass through the GC. This article has summarized the incomplete data now available which points to subcompartmentalization of these events. It is to be anticipated that as the assignment of specific steps to subcompartments is made with greater certainty, this information will lead to an increased appreciation and understanding of the dynamics of membrane–membrane interactions which underly the operation of the organelle as a whole.

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