Rous–Whipple Award

The Rous–Whipple Award was established by the American Society for Investigative Pathology to recognize a career of outstanding scientific contribution.

The 1994 recipient of the Rous–Whipple Award, Dr. Nicholas K. Gonatas, delivered a lecture entitled "Contributions to the Physiology and Pathology of the Golgi Apparatus" after the presentation of the award on Tuesday, April 26, 1994 in Anaheim, California, at the meeting of the Federation of American Societies for Experimental Biology.

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Rous-Whipple Award Lecture

Contributions to the Physiology and Pathology of the Golgi Apparatus

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Ο Βιος βραχύς, η δέ τέχνη μακρή.
"The life is short, the craft is long."
Hippocrates, c. 460–373 B.C.
Aphorisms

The importance of the Golgi apparatus in the transport, processing, and targeting of proteins destined for secretion, plasma membranes, and lysosomes has emerged from numerous studies. In this paper we review studies from our laboratory dealing with 1) the Golgi apparatus during mitosis and the role of microtubules in maintaining the structure of the organelle, 2) the endocytosis of antibodies, exogenous lectins, and toxins into the Golgi apparatus of several cells including neurons* in vivo *and* in vitro*, 3) the traffic of MG-160, a membrane sialoglycoprotein of the medial cisternae of the Golgi apparatus, from the trans-Golgi network to the Golgi cisternae, and 4) the involvement of the Golgi apparatus of motor neurons in the pathogenesis of amyotrophic lateral sclerosis. We conclude with a summary of ongoing work on the primary structure of MG-160 and introduce evidence suggesting that this intrinsic membrane protein of the Golgi apparatus may be involved in the regulation of endogenous, autocrine, basic fibroblast growth factor. We hope that this review will stimulate studies on the Golgi apparatus of neurons, which may lead to the discovery of neuron-specific properties of this important organelle and its involvement in the pathogenesis of neurodegenerative disorders. (Am J Pathol 1994, 145:751–761)

I am honored to receive this award named after Peyton Rous and George Whipple, eminent pathologists and Nobel laureates for their fundamental discoveries on tumor-inducing viruses and vitamins, respectively.

As we are approaching the 100th anniversary of the discovery of the Golgi apparatus by Camillo Golgi (1844–1926), professor of histology and general pathology at the university of Pavia, we should be reminded that for a long time the status of this important organelle was controversial.

Until the late forties, the Golgi apparatus-complex was considered by many cytologists as an artifact of fixation and tissue processing.[1] Over the last 40 years the status of the Golgi apparatus has been upgraded from artifact to "center stage," which is not an exaggerated characterization considering the central roles of the organelle in the transport, processing, and targeting of polypeptides destined for secretion, plasma membranes, or lysosomes.[1] In addition to the above centrifugal functions, the Golgi apparatus, and more precisely GERL or TGN, is involved in the retrograde or centripetal transport of antibodies, lectins and toxins.[2–4]

Many of the studies to be reviewed here were performed by Jacqueline Gonatas and Anna Stieber of our laboratory, who over the last 25 years have devoted their energies, insights, skills, and experience in cellular and molecular studies on the Golgi apparatus. Recent collaborators are Zissimos Mourelatos, Kate Johnston, and Jun Chen, who contributed to studies on the retrograde traffic and cDNA sequencing of MG-160. An important collaborator to our recent studies has been the late Becca Fleischer who shared with us her enormous knowledge and vast experience in the enzymology and cell biology of the Golgi

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apparatus. Her untimely death has deprived the community of cell-molecular biologists of the Golgi apparatus of a highly original scientist who has made classic contributions to the field.

Ongoing research on the Golgi apparatus is voluminous and in "full effervescence." Due to the nature of this presentation only a few relevant contributions from other laboratories will be discussed. Summaries of the results will be presented under Review of Previous Studies, while brief discussions of the significance of the results will be presented under Implications and Future Directions.

Review of Previous Studies

The Golgi Apparatus during Mitosis and the Role of Microtubules in Maintaining the Structure of the Organelle

I was introduced to cell biology by Elliott Robbins. In our first study, published in 1964, we examined by transmission electron microscopy and cytochemical techniques for acid phosphatase and thiamine pyrophosphatase HeLa cells during various stages of mitosis.[5] This paper has been a standard reference to students of mitosis over more than a quarter of a century (ISI, Current Contents, Life Sciences, September 25, 1989, vol. 32, no. 39). In this study, we were impressed by the apparent "disappearance" of the Golgi apparatus during early prophase and its prompt "reappearance" in telophase.[5] Furthermore, in prophase the diffuse network of the normally present cytoplasmic microtubules disappeared, while simultaneously the lysosomes were aggregated in peripherally located clusters. This observation led us to examine the effect of the microtubule-disrupting agent colchicine on interphase HeLa cells.[6] Under the influence of colchicine, the Golgi apparatus of interphase cells fragmented while the lysosomes were distributed in circumferential clusters similar to those seen in mitotic cells.[6] We attributed the "disappearance" of the Golgi apparatus during mitosis and the fragmentation of the organelle in colchicine-treated cells to the same cause, namely to the disruption of microtubules, which in mitotic cells occurs physiologically and in interphase cells after treatment with colchicine.[6]

Perhaps one of the most important findings of these early studies was the realization that axons contained microtubules or "neurotubules" as we called them. In a paper, also published in 1964,[7] we showed that axons and dendrites contained structures "homologous" to spindle tubules, and not elements of the endoplasmic reticulum as it was previously claimed.

The Endocytosis of Antibodies, Exogenous Lectins, and Toxins in the Golgi Apparatus

Two important methodologic innovations made possible these studies. The first was the method for the light and electron microscopic visualization of horseradish peroxidase (HRP), introduced by Graham and Karnovsky.[8] The second were the methods, introduced by Avrameas and Ternynck,[9–11] for the preparation of immunoadsorbents and the covalent conjugation of HRP with antibodies and lectins.

These studies were conducted both *in vitro* using cultured rat neurons, rat pheochromocytoma (PC12) cells, murine neuroblastoma cells, mouse or rat lymphoid cells, and cultured human fibroblasts, and *in vivo*, using injections of ligands into rat brains, eyes, and submandibular glands.

In vitro *studies*

In the first of these studies, we observed that peroxidase-labeled antibodies against IgG were internalized in clusters of tubules resembling the Golgi apparatus in rat and mouse plasma cells. On retrospect, these compartments probably corresponded to GERL (Golgi-endoplasmic reticulum-lysosome) or TGN (the *trans*-Golgi network).[2,12,13] Originally, Novikoff and Novikoff,[12] using the cytochemical techniques for thiamine pyrophosphatase and acid phosphatase, had identified a system of acid phosphatase-positive tubules, vesicles, and cisternae at the mature or *trans* aspect of the Golgi apparatus which they named GERL, for Golgi apparatus-Endoplasmic Reticulum-Lysosome. They postulated that GERL served as a direct conduit of molecules from the rough endoplasmic reticulum (RER) to the lysosomes. A few years later, Griffiths and Simons[13] renamed GERL as TGN for *trans* Golgi network, and proposed that TGN serves as the common site of exit of all proteins processed through the Golgi apparatus.

Conclusive evidence for the existence of a retrograde pathway between cell surface receptors to ligands and the Golgi apparatus was provided by two experiments involving the internalization of peroxidase-labeled ricin and wheat germ agglutinin in cultured neurons.[3,4] In subsequent studies, performed with cultured neurons, neuroblastoma, rat pheochromocytoma (PC12) and lymphoid cells, we confirmed the existence of a retrograde pathway of

endocytosis into GERL-TGN of ligands with affinities to several cell surface receptors.[14–24] The endocytosis was specifically inhibited when the ligands were incubated with soluble receptors, namely, D-galactose with ricin, N-acetylglucosamine with wheat germ agglutinin, and GM_1 ganglioside with cholera toxin.[4,11,15,16] Furthermore, in cells deficient for the receptor to cholera toxin, the GM_1 ganglioside, exogenous ganglioside became associated with plasma membranes and mediated the subsequent endocytosis of the toxin into the Golgi apparatus.[16,17] Because of the high number of cell surface receptors of neuroblastoma cells to ricin (3×10^7 to 8×10^7 per cell), we referred to the process of internalization of lectins and toxins as adsorptive endocytosis in contrast to the receptor-mediated endocytosis, which usually involves a smaller number of cell surface receptors.[18] Comparative studies between fluid phase and adsorptive endocytosis confirmed that the entry of exogenous macromolecules into GERL-TGN is determined by the presence of cell surface receptors to the ligands used, and that, in the cell systems used, native HRP was not internalized into the Golgi apparatus.[19,25]

In lymphocytes, which lack a well developed Golgi apparatus, both antigens and antibodies were internalized into vesicles and tubules adjacent to the Golgi apparatus, which probably represent either late endosomes or GERL-TGN.[23,24]

We then investigated the effect of nerve growth factor-induced "differentiation" of PC12 cells on the adsorptive endocytosis of conjugates of HRP with ricin (ricin-HRP) or wheat germ agglutinin (WGA-HRP).[20,21] While in "differentiated" cells, both ligands were internalized in a clearly outlined GERL-TGN, in "undifferentiated" cells, internalized ricin-HRP or WGA-HRP was noted in several cisternae throughout the Golgi apparatus (Figures 1–3). Furthermore, in NGF "differentiated" PC12 cells there was a 10-fold increase of the endocytosis of WGA-HRP into GERL-TGN.[21] These results led us to conclude that one of the effects of NGF on PC12 cells was the segregation of elements of GERL-TGN from the Golgi apparatus proper and the concomitant increased levels of the adsorptive endocytosis of WGA-HRP into GERL-TGN.[21]

In the last *in vitro* experiment, we examined by a quantitative autoradiographic method the sequential adsorptive endocytosis of ^{3}H-WGA by murine neuroblastoma cells.[22] The results of this study indicated that the Golgi apparatus complex was specifically labeled with [^{3}H]WGA earlier than lysosomes, and that the organelle constituted a quantitatively significant pathway of endocytosis of the ligand.[22]

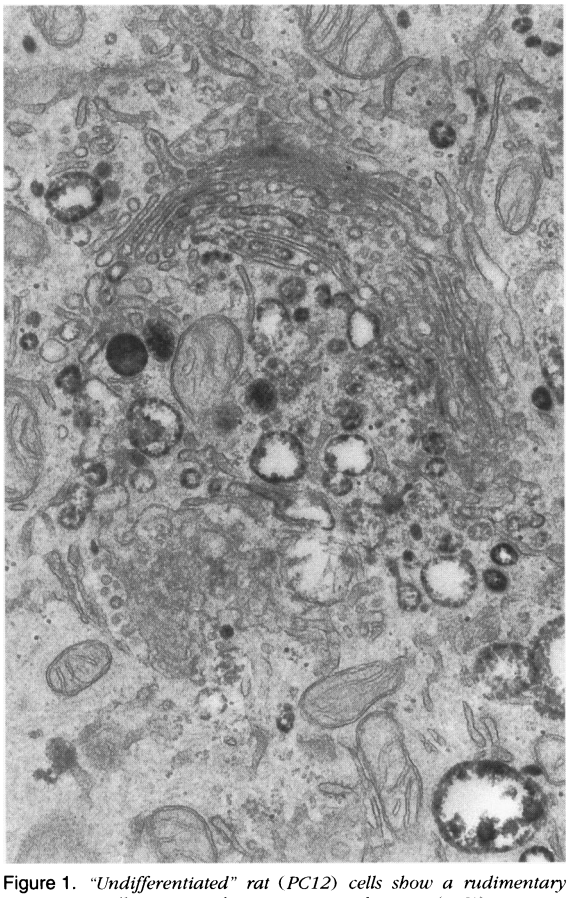

Figure 1. *"Undifferentiated" rat (PC12) cells show a rudimentary TGN. PC12 cells grown without Nerve Growth Factor (NGF), were incubated with 50 μg/ml of WGA-HRP, at 37 C for 90 minutes before fixation with glutaraldehyde and staining for HRP with diaminobenzidine tetrahydrochloride as substrate according to Graham and Karnovsky.[8] Note HRP reaction in dense bodies, probably representing lysosomes and endosomes, and in small vesicles and tubules at the* trans *aspect of unstained cisternae of the Golgi apparatus which label* in vivo *the TGN. Compare TGN with that of PC12 cells exposed to NGF (see Figures 2 and 3). Magnification ×33,000. Permission of the J Cell Sci.*[37]

In vivo *studies*

The purpose of these studies was to investigate whether conclusions derived from experiments with cells in culture were relevant to the *in vivo* condition. Since most of the *in vitro* studies used either normal or neoplastic neurons, we elected to conduct these experiments in rat central or peripheral nervous systems.[26–32] These studies have basically confirmed that the neuronal GERL-TGN is involved in the adsorptive endocytosis of exogenous lectins or toxins and that this pathway of internalization is involved in orthograde, retrograde, and transsynaptic traffics (summarized in ref. 33). The *in vivo* experiments gave two unexpected results, which should be emphasized. First, unlike the early labeling of the Golgi apparatus by [^{3}H]WGA in cultured neuroblastoma cells,

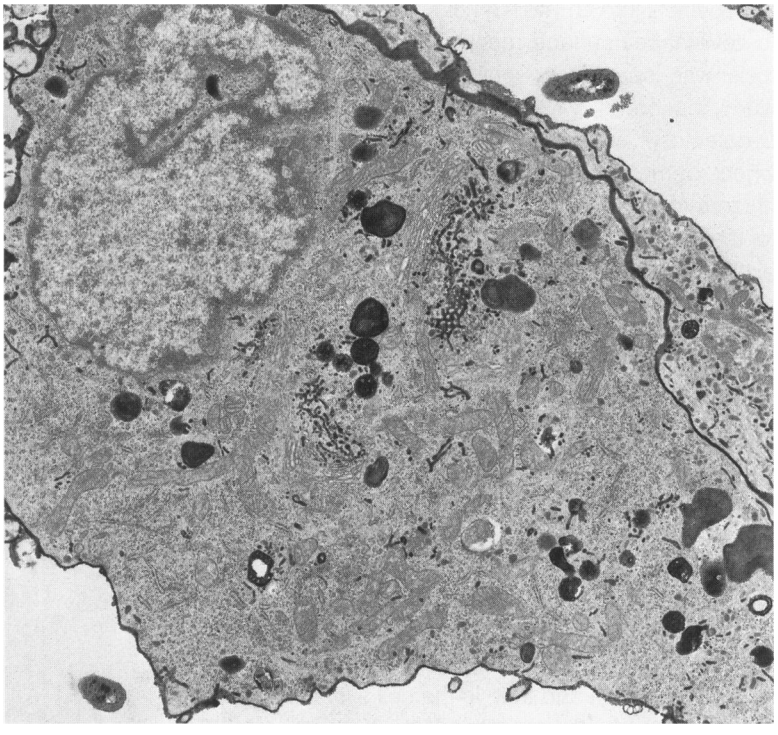

Figure 2. *NGF induces an expansion of TGN. Rat PC12 cells, grown in the presence of 20 ng/ml NGF, were incubated with WGA-HRP and processed as in Figure 1. Reaction for HRP is found on the cell surface within dense bodies probably representing endosomes and lysosomes, and in the TGN represented by an expanded network of anastomosing tubules at the* trans *aspect of unstained cisternae of the Golgi apparatus. Magnification ×12,000.*

in rat retinal neurons, labeling of the Golgi apparatus by the ligand is a relatively late phenomenon suggesting that the bulk of [^{3}H]WGA destined for lysosomes does not pass through the Golgi apparatus.[22,31] The second observation clarifies the specificity of the transsynaptic transport of WGA in the rat visual system. Following intraocular injection in adult rats of [^{125}I]WGA and examination by ultrastructural autoradiography of the superior colliculus and lateral geniculate, label was found not only over postsynaptic neuronal elements but also over adjacent glia.[32] This finding indicates that [^{125}I]WGA, transported by orthograde axoplasmic flow to presynaptic terminals, is released by exocytosis at the synaptic cleft; however, subsequently [^{125}I]WGA diffuses along extracellular pathways and is internalized not only by the tightly coupled postsynaptic elements but also by the adjacent glia.[32]

An unexpected outcome of these studies was the realization that conjugates of WGA-HRP were much more sensitive than native HRP for anatomical studies of neuronal connections.[26] These conjugates were subsequently used in numerous neuroanatomic studies for the tracing of axonal transports (ISI, Current Contents, Clinical Medicine, September 9, 1991, vol. 19, no. 36).

Biochemical Studies on MG-160, a Membrane Sialoglycoprotein Residing in the Medial Cisternae of the Golgi Apparatus, Are Consistent with the Conclusion That a Physiologic Retrograde Transit Pathway Exists in the Distal Golgi Apparatus

Two methodologic innovations contributed to the development of molecular approaches in our studies of the Golgi apparatus of neurons. The first was a method for the preparation of enriched fractions of the Golgi apparatus from rat brain neurons.[34] The second innovation was the method of Louvard and colleagues (quoted in ref. 35) for the preparation of organelle specific antibodies. It should be stated here that our original goal to identify strictly neuron-specific membrane proteins of the Golgi apparatus is still elusive.

We have elected to concentrate our efforts in studies of MG-160, an intrinsic membrane protein residing in the medial cisternae of the Golgi apparatus of rat brain neurons, pituitary, PC12, and other cells for the following reasons: 1) the protein contains complex carbohydrates including sialic acid, as indicated its from resistance to digestion with the enzyme Endo-H,

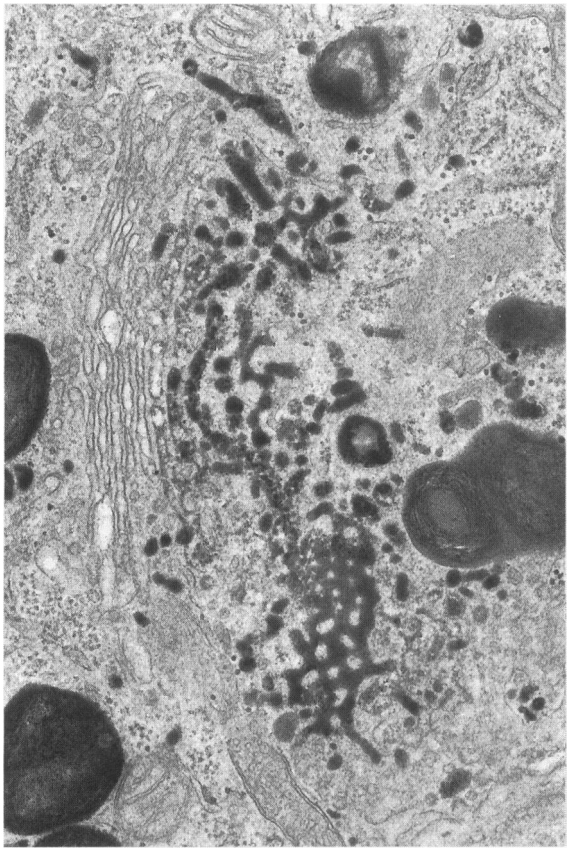

Figure 3. *Higher magnification from Figure 2 showing at the* trans *aspect of unstained cisternae of the Golgi apparatus the peroxidase-positive system of anastomosing tubules characteristic of TGN. Magnification ×35,000.*

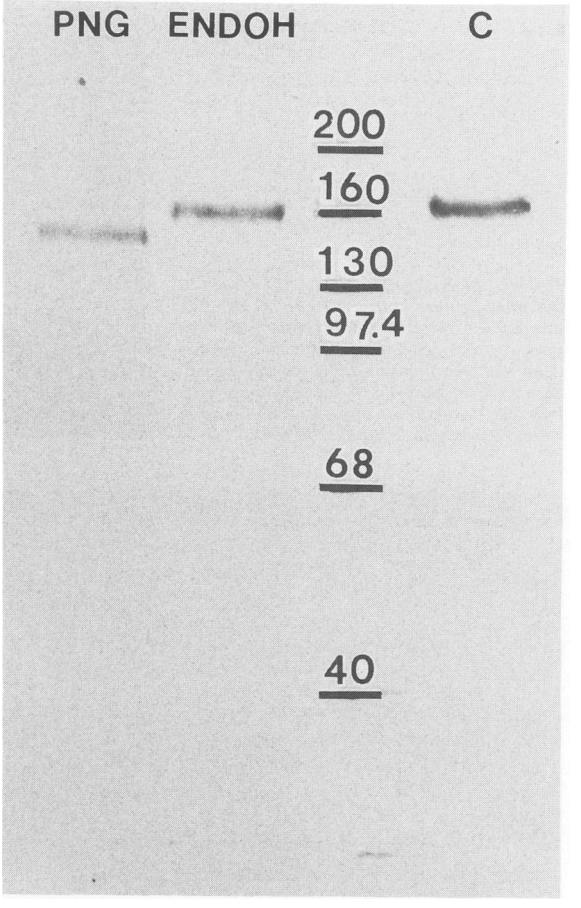

Figure 4. *MG-160, the sialoglycoprotein of the medial cisternae of the Golgi apparatus, is resistant to endo H and susceptible to PNGase digestions. Neuronal golgi fractions, 20 ng, from rat brain were incubated with peptide N-glycosidase F (PNGase), endo H, and buffer alone, C. The shift of fractions treated with PNGase indicates that the protein contains asparagine-linked carbohydrates. The resistance to endo H digestion indicates the presence of complex carbohydrates. Permission of J Biol Chem.*[36]

staining with limax flavus lectin, and by its susceptibility to neuraminidase digestion; 2) in the presence of reducing agents, MG-160 migrates in polyadrylamide gels as a single 160-kd band, and at 130 kd under nonreducing conditions, consistent with the presence of intrachain disulfide bonds; 3) digestions of intact Golgi vesicles from rat brain neurons with trypsin revealed that MG-160 has long intralumenal and/or intramembrane domains and a short cytoplasmic tail; 4) MG-160 is a significant component of the brain Golgi apparatus with a yield/g of rat brain of 0.9 μg, representing approximately 3% of the Golgi proteins (Figures 4–6).[36]

The biochemical characterization of MG-160 raised the question whether under physiologic conditions there is a retrograde flow through the Golgi apparatus, since the sialoglycoprotein MG-160 has been identified in the medial cisternae of the organelle while sialyltransferases have been localized in *trans* cisternae of the organelle or in TGN in most but not all cells.[36] In order to further probe the possibility for a physiologic retrograde transport through the Golgi apparatus, we investigated in PC12 cells the effect of the secretion blocker Brefeldin A (BFA) on the morphology of the Golgi apparatus and the sialylation of MG-160. In BFA-treated cells, MG-160 dispersed throughout the RER while TGN collapsed around the centriole. The physical separation of the MG-160-containing compartment from TGN, the putative sialyltransferase-containing compartment, was associated with biochemical changes of MG-160, which acquired resistance to Endo-H, but failed to be sialylated (Figures 7 and 8). Promptly upon removal of BFA, the Golgi apparatus was reassembled, and MG-160 became sialylated.[37] These results strongly suggest that under physiologic conditions there is a retrograde transit pathway between TGN and cisternae of the Golgi apparatus (Figure 9).

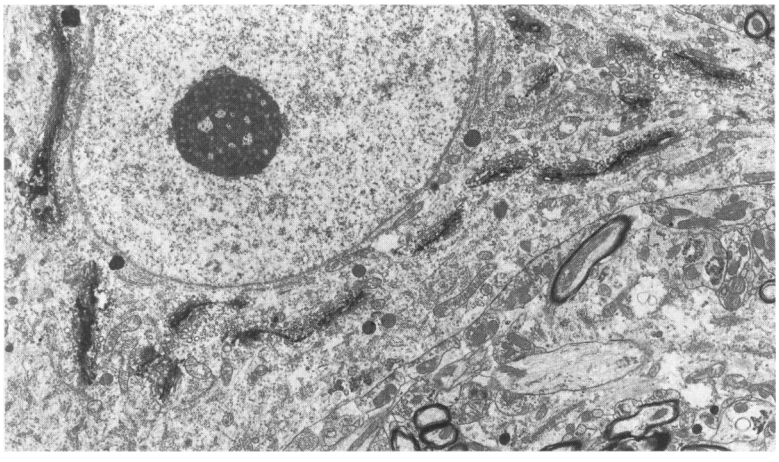

Figure 5. *Monoclonal antibody 10 A8, used in the identification and isolation of MG-160, stains the Golgi apparatus of rat brain neurons. In this immunocytochemical reaction done with the pre-embedding immunoperoxidase method, only the Golgi apparatus of a Purkinje neuron is stained. Magnification ×6000. Permission of J Biol Chem.*[36]

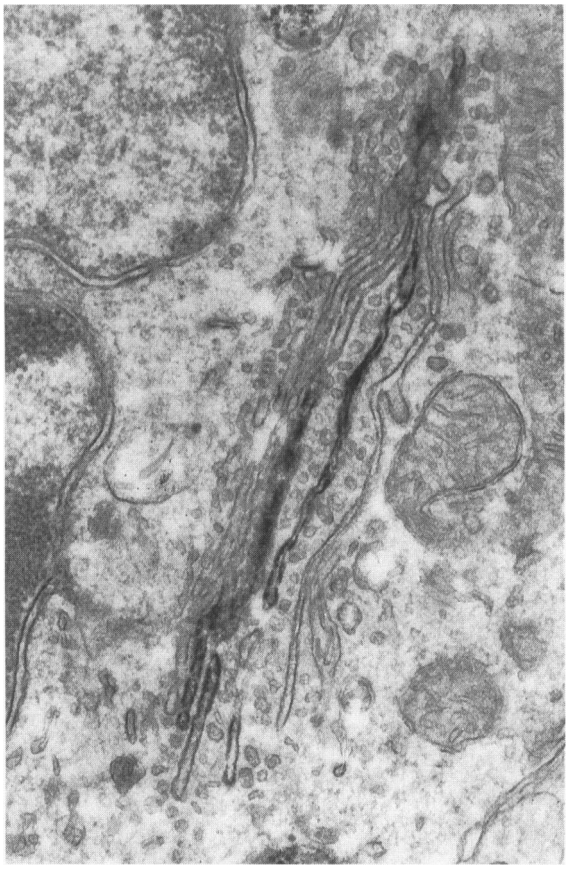

Figure 6. *Higher magnification from Figure 5 shows staining of the medial cisternae of the Golgi apparatus. Magnification ×36,000. Permission of J Biol Chem.*[36]

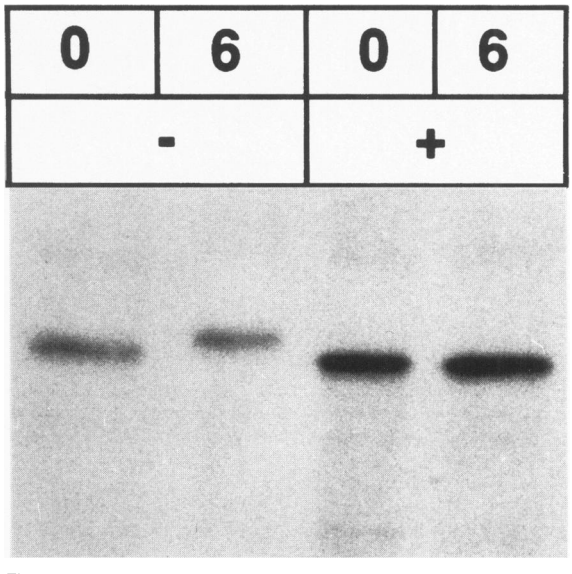

Figure 7. *Brefeldin A (BFA) inhibits the conversion of a 150-kd precursor of MG-160 to the mature 160-kd form. In this experiment PC12 cells were labeled with Tran*^{35}*S-label for 30 minutes, lysed immediately (0), or chased for 6 hours (6), before cell lysis and immunoprecipitation with monoclonal antibody 10A8. BFA-treated cells (+) were preincubated with BFA (30 minutes, 5 μg/ml), pulse-labeled, and chased in the presence of BFA. Precipitates were analyzed by SDS-PAGE. MG-160 matures to its 160 kDa form during 6 hours of chase, but in BFA-treated cells, the maturation of MG-160 is inhibited. Permission of J Cell Sci.*[37]

The Involvement of the Golgi Apparatus of Motor Neurons in the Pathogenesis of Amyotrophic Lateral Sclerosis (ALS)

These studies were made possible with the development of an organelle-specific antiserum against MG-160, which was immunolocalized specifically in the Golgi apparatus of cells of several species including human.[38] An initial experimental study of the Golgi apparatus, immunostained with the rat and organelle-specific monoclonal antibody 10A8, was conducted in the neurons of the hypoglossal nucleus following transection of the hypoglossal nerve at the level of the bifurcation of the carotid artery.[39] This experiment revealed the classic dispersion and reaggregation of the Golgi apparatus in chromatolytic neurons during the onset of the reaction and recovery, respectively.[39] Thus, having validated the usefulness of an organelle-specific antibody in the study of a reproducible neuronal injury, we decided to examine the Golgi apparatus of spinal cord motor neurons from

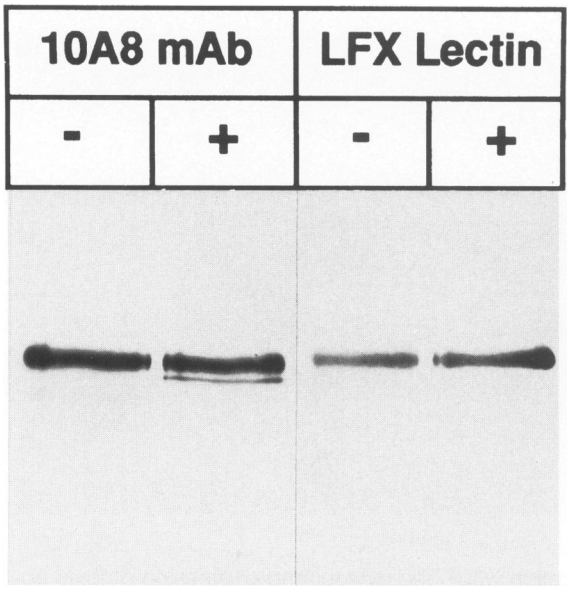

Figure 8. *In the presence of BFA, MG-160 fails to be sialylated. MG-160 was purified from control (−) and BFA-treated cells (+) by immunoaffinity chromatography, with monoclonal antibody 10A8 as the ligand according to a previously described method.*[36] *Protein was analyzed by Western blot stained with either monoclonal antibody 10A8 (10A8 mAb), or with the sialic acid specific lectin* Limax flavus *(LFX lectin). In lane 10A8 mAb BFA*$^+$*, note in the form of a doublet the accumulation of a 150-kd precursor which cross-reacts with the mAb 10A8 but not with the sialic-acid specific lectin. Permission of J Cell Sci.*[37]

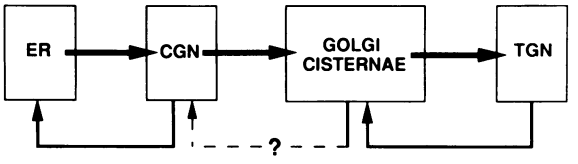

Figure 9. *Proposed model for the bidirectional flow of proteins and membrane proteins through the Golgi apparatus modified from Rothman and Orci.*[56] *In addition to the anterograde vesicular traffic of proteins from the endoplasmic reticulum (ER) to the TGN, we indicate the retrograde transport pathways that recycle proteins from the* cis *Golgi network (CGN) to the ER and from TGN to the Golgi cisternae. Because the retrograde transports of proteins from the Golgi cisternae to CGN and ER has been identified only in BFA-treated cells and in the retrograde transported* Shiga *toxin, we attach a question mark to this pathway which may reflect nonphysiologic conditions.*[51] *Permission of J Cell Sci.*[37]

patients with ALS. Previous neuropathologic studies had shown dispersion of the Nissl substance, or chromatolysis, of neurons in ALS and following axonotomy (quoted in ref. 40). Therefore, we were surprised to find out that the Golgi apparatus of spinal cord motor neurons in ALS was reduced to numerous small round granules, which filled the perikaryon and proximal dendrites in a fashion very similar to the fragmentation of the organelle induced experimentally by agents depolymarizing the microtubules (Figure 10).[40] In subsequent studies we confirmed that in contrast to age-matched normal and neurologic controls, which showed fragmentation of the Golgi apparatus in a low percentage of spinal cord motor neurons, in ALS and related motor neuronopathies a high percentage of motor neurons showed fragmentation of the organelle.[41–43] Furthermore, there was a highly significant correlation between neurons containing a fragmented Golgi apparatus and ubiquitin-positive skein-like or granular inclusions, which are presumably pathognomonic for ALS.[43]

Primary structure of MG-160

In a study soon to be concluded, we describe the primary structure of rat MG-160 derived from the sequencing of cDNAs. In brief, the cDNA encodes a polypeptide of 1171 amino acids with an M_r of 133,403 d. MG-160 displays a short carboxy terminal cytoplasmic tail, a single transmembrane domain, 16 cysteine-rich intralumenal repeats, and a cleavable signal peptide at the amino terminus. The protein contains five potential asparagine-x-threonine glycosylation sites. These findings are quite consistent with previous biochemical studies on MG-160, which disclosed that the protein contains Asn-linked carbohydrates and intrachain disulfide bonds.[36] Antibodies against a 17-amino acid-long synthetic peptide, derived from the cDNA sequence, react by Western blots of rat brain membranes with a 160-kd band, and by immunocytochemistry with the Golgi apparatus of rat Purkinje cells. MG-160 was found to be autologous with a receptor to fibroblast growth factor.[44] Furthermore, MG-160 isolated by immunoaffinity chromatography from rat brain binds basic fibroblast growth factor. These findings are consistent with the hypothesis that MG-160 is involved in the traffic and/or processing of either endogenous (autocrine) or exogenous fibroblast growth factors or both.

Implications and Future Directions

The Golgi Apparatus during Mitosis and the Role of Microtubules in Maintaining the Structure of the Organelle

Mitosis

Using antibodies against Golgi enzymes, and the histochemical marker for the *trans* Golgi cisternae thiamine pyrophosphatase, Warren and colleagues[45,46] have identified a mitotic form of the Golgi apparatus consisting of clusters of vesicles. Metaphase cells contained 10 to 300 Golgi clusters; the distribution of these mitotic elements of the Golgi apparatus between the two daughter cells, presumably, occurs randomly.[45,46] The detailed morphogenesis of

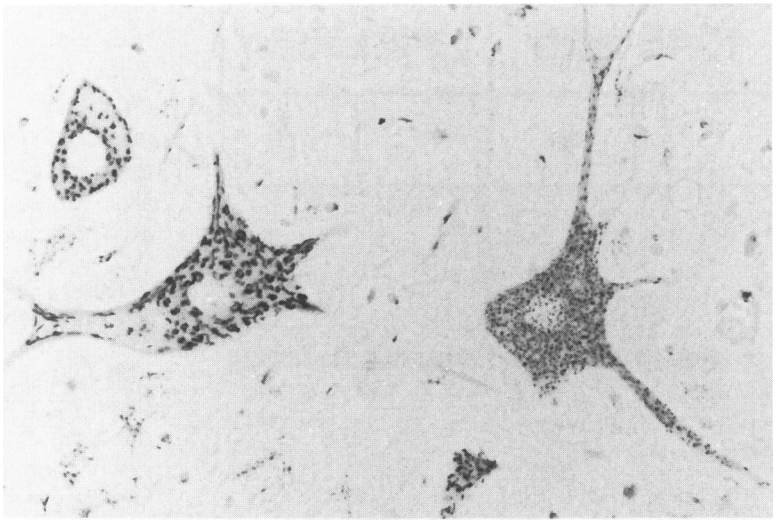

Figure 10. *Fragmentation of the Golgi apparatus in ALS. Section of the spinal cord from a patient with ALS immunostained with a polyclonal antiserum against MG-160. To the left normal motor neuron containing in the perikaryon numerous irregular profiles of the Golgi apparatus. To the right, a motor neuron with numerous round and small elements of the Golgi apparatus resembling the fragmentation of the organelle induced by microtubule depolymerizing agents. Magnification ×960. Permission of Am J Path.*[43]

these mitotic forms of the Golgi apparatus, their metamorphosis to Golgi cisternae during telophase, and the control and orchestration of these pleiomorphic changes from interphase cisternae to metaphase vesicles and then back to telophase cisternae are not clear. Presumably, all these changes are somehow related to the disappearance of the interphase microtubules and to their reformation at telophase. The molecular parameters of these complex membrane-microtubule interactions remain to be clarified.[47]

Microtubules and the Golgi Apparatus

The dependence of the structural integrity of the Golgi apparatus on microtubules has been established beyond any reasonable doubt, and some information has been introduced on the molecular mechanisms of these Golgi membrane-microtubule interactions.[48–50] Most likely, the interaction of microtubules with membranes of the Golgi apparatus depends on N-ethylmaleimide-sensitive cytosolic factors and membrane-associated receptors, which have not yet been identified.[49] Apparently, neither intermediate filaments nor microfilaments are involved in the reclustering of scattered Golgi elements along microtubules during the recovery phase of nocodazole induced depolymerization of microtubules.[47]

The Endocytosis of Antibodies, Exogenous Lectins, and Toxins in the Golgi Apparatus

Ricin and certain other protein toxins act on cytosolic targets. Available evidence supports the notion that the internalization of these toxins into an intact Golgi apparatus is prerequisite for their eventual translocation within the cytosol and expression of toxic actions. There is correlation between the disorganization of the Golgi apparatus induced by BFA and protection against ricin, or inactivation of cholera toxin.[50,51] Shiga toxin is internalized not only into TGN, but also into the rest of the Golgi cisternae and the rough endoplasmic reticulum.[51,52] However, the precise molecular events which make possible the translocation of these toxins from the lumen of TGN or other membrane-bound compartments into their cytosolic targets are not known. This retrograde pathway involves not only the transport of toxins but also of physiologic molecules, like TGN38/41, an intrinsic membrane protein predominantly localized to TGN, and the mannose-6-phosphate receptor, which recycle between the cell surface and TGN.[53–55]

Biochemical Studies on MG-160, a Membrane Sialoglycoprotein Residing in the Medial Cisternae of the Golgi Apparatus, Are Consistent with the Conclusion that a Physiologic Retrograde Pathway Exists within the Distal Golgi Apparatus

Investigations of mechanisms involved in intracellular traffics have been enriched from *in vitro* molecular studies, and genetic approaches in yeast.[56] Rothman and others have been able to reproduce in a soluble system an authentic form of the secretory pathway and thus identify and study proteins involved in the budding and fusion of transporter vesicles. Parallel molecular genetic studies in yeast have revealed the conservation of the basic mechanisms of protein transport from low to the high eukaryotes.[56,57] These

studies have assumed that transport through the Golgi apparatus occurs in a unidirectional fashion. However, experiments with the secretion blocker BFA have raised the question whether a physiologic bidirectional transport of proteins occurs within the Golgi apparatus.[57] Experiments on the maturation of MG-160 in cells treated with BFA, summarized earlier in this paper and reported elsewhere, are consistent with the conclusion that a physiologic retrograde pathway exists within the Golgi apparatus (Figures 7, 8).[37] Obviously many questions remain unanswered on the existence and purpose of a physiologic retrograde pathway within the Golgi apparatus. Machamer[58] has advanced an hypothesis based on the notion that retention signals of intrinsic membrane proteins of the Golgi apparatus are not efficient and that additional retrieval mechanisms operate through a retrograde transport of the polypeptide. According to this hypothesis, the steady-state localization in proximal domains of the Golgi apparatus of the sialoglycoprotein MG-160 may require its continuous retrieval from distal domains where it becomes sialylated.[58,59]

The Involvement of the Golgi Apparatus of Motor Neurons in the Pathogenesis of ALS

A variety of human diseases are associated with defects of intracellular protein processing and trafficking, which most likely involve the Golgi apparatus.[54,60,61] In addition, molecular genetic studies have disclosed in certain cases of familial ALS defects of the gene encoding the cytosolic enzyme Cu/Zn superoxide dismutase (SOD).[62–64] The presumed increased levels of free radicals resulting from defects of the SOD enzyme, may initiate neuronal atrophy and death. The Golgi apparatus may be a target of free radicals, since they are toxic to DNA and membranes.[62] An alternative hypothesis for the etiology of ALS, based on results obtained from transgenic mice, proposes that increased expression of subunits of intermediate filaments leads to lesions resembling human motor neuron disease.[64–66] If the transgenic animal models of ALS represent authentic forms of the disease, one might expect to identify in the spinal cords of these animals motor neurons with the characteristic fragmentation of the Golgi apparatus, as it has been encountered in the human.[43]

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