Golgin-160 is a member of the golgin family of Golgi-localized membrane proteins. The COOH-terminal two-thirds of golgin-160 is predicted to form a coiled-coil, with an NH2-terminal “head” domain. To identify the Golgi targeting information in golgin-160, full-length and deletion constructs tagged with green fluorescent protein were generated. The head domain alone was targeted to the Golgi complex in the absence of assembly with endogenous golgin-160. Further truncations from both ends of the head domain narrowed the Golgi targeting information to 85 amino acids between residues 172 and 257. Surprisingly, certain truncations of the head domain also specifically accumulated in the nucleus. Both a nuclear localization signal (masked in the full-length protein) and information for nuclear retention contributed to the nuclear localization of these truncations. Because the golgin-160 head is cleaved by caspases during apoptosis, we examined the localization of epitope-tagged proteins corresponding to all potential caspase cleavage fragments. Our data suggest that three of these fragments could be targeted to the nucleus, provided that these are released from Golgi membranes after cleavage. The finding that both Golgi and nuclear targeting information is present in the same region of golgin-160 suggests that this protein may have more than one function.

The Golgi complex of higher eukaryotic cells is composed of stacks of cisternal membranes that function in processing and sorting of lipids and proteins en route from the endoplasmic reticulum to the plasma membrane and other destinations (1, 2). The enzymes responsible for the modification of many cellular and secretory proteins reside within the Golgi complex. These enzymes, including glycosyltransferases, glycosidases, and sugar transporters, have unique but overlapping distributions (3). The elaborate structure of the Golgi complex is thought to be important for localization and efficient function of these enzymes. The conservation of Golgi structure throughout the eukaryotic kingdom implies an essential role in Golgi function (4). Understanding the formation and maintenance of Golgi structure as it relates to function is an active area of research.

Identification of Golgi-localized spectrin and ankyrin isoforms (5, 6), and a “detergent-insoluble Golgi matrix,” (7) suggests that the structure of the Golgi complex may be organized by an “exoskeleton” (8). This exoskeleton is proposed to be an extensive meshwork of cytoskeletal and Golgi matrix proteins that sequester and align enzyme-containing membranes, limit diffusion, and provide shape to Golgi cisternae. A family of Golgi proteins, known as golgins, has been implicated as components of this Golgi exoskeleton. Whereas this family was first identified by antibodies from patients with autoimmune diseases, the most characteristic feature of golgins is a large coiled-coil domain similar to that of the myosin family. The golgin family of proteins includes the peripheral membrane proteins GM130 (7), golgin-97 (9), golgin-160 (10, 11), golgin-230/245 (12, 13), and GMAP-210 (14), as well as the integral membrane proteins golgin-67 (15), golgin-84 (16), and giantin (17).

The best studied of the golgin proteins are GM130 and giantin. Initially identified as a component of the detergent-insoluble Golgi matrix, GM130 was later found to bind to GRASP65, a cis-Golgi membrane protein required for stacking of Golgi cisternae in vitro (18). During mitosis, phosphorylation of GM130 is crucial for proper disassembly of the Golgi (19). GM130 also binds to the vesicle docking protein, p115, and to Rab1, a GTPase required for endoplasmic reticulum-to-Golgi transport (20, 21). In addition, giantin binds p115 suggesting the presence of a complex that tethers Golgi cisternae containing GM130 to giantin on vesicles via p115 (22). Disruption of GM130-giantin tethering complexes inhibits transport through the Golgi, and causes the accumulation of transport vesicles (23, 24). Taken together, these observations suggest that golgins couple Golgi structure to Golgi function.

Recently, golgin-160 was shown to be a caspase substrate during programmed cell death (25). Golgin-160 is predicted to form a characteristic coiled-coil structure in the COOH-terminal two-thirds of the protein, with an NH2-terminal non-coiled-coil (head) domain. Although the normal function of golgin-160 is unknown, the cleavage of the head domain during apoptosis is required for efficient apoptotic disassembly of the Golgi (25), suggesting that cleavage of golgin-160 may disrupt protein-protein interactions important for Golgi function.

We have characterized the Golgi targeting of golgin-160 as a first step toward understanding its function. We report that Golgi targeting information resides in an 85-amino acid sequence present in the NH2-terminal head of golgin-160. Surprisingly, we also identified cryptic nuclear targeting information in the same region of the head domain. Our data suggest that specific caspase cleavage fragments of golgin-160 could be translocated from the Golgi complex to the nucleus.

EXPERIMENTAL PROCEDURES
Cells and Transfection—HeLa cells were maintained in Dulbecco’s modified Eagle’s medium (Invitrogen, Carlsbad, CA) containing 10% fetal calf serum and 0.1 mg/ml normocin-O (InvivoGen) at 37 °C in 5% CO2. Thirty-five-mm dishes of HeLa cells (70–80% confluent) were transfected with 8 μg of Lipofectin (Invitrogen) and 2 μg of pEGFP-C1 (CLONTECH) or pcDNA3.1/Myc-His (+), (Invitrogen) encoding the ap-
propriate cDNA. Steady state expression was analyzed 16 h after transfection. For the experiments described in the legend to Fig. 6, HeLa cells were first infected with recombinant vaccinia virus encoding phage T7 RNA polymerase (vTF7-3) (26). vTF7-3 was absorbed at a multiplicity of infection of 5 in monolayers of serum-free Dulbecco’s modified Eagle’s medium (DMEM) in the presence of brefeldin A; NLS, nuclear localization signal.

Sequence was ligated into pEGFP-C1 at the residue at the NH2 terminus) was synthesized, purified, and coupled to previously described (25). A synthetic peptide corresponding to the 14 amino acids (beginning at 394. The coiled-coil mutant, GFP/g160-(393–393), was generated by promoting the c-Myc epitope was generated by introduction of a restriction site, codon, or base pair changes were generated using a PCR-based QuickChange site-directed mutagenesis kit (Stratagene, La Jolla, CA), and mutations were confirmed by dideoxy sequencing. Mutant golgin-160 proteins were named with both the tag type and the amino acids represented cDNAs lacking these 5 base pairs were found. Furthermore, the mouse homolog of golgin-160, Mea-2, contained these 5 base pairs (27). Searching the nucleotide and protein data bases showed that golgin-160 has no overall homology to any known protein with the exception of the mouse Mea-2 protein. Secondary structure analysis predicts that the protein encoded by our cDNA consists of an NLS-terminal non-coiled-coil domain of 393 amino acids followed by a long a-helical domain of 1105 amino acids with a strong coiled-coil prediction (Fig. 1B).

To confirm that the version of golgin-160 represented by our cDNA is expressed, polyclonal anti-peptide antiseras were raised against the predicted NH2- and COOH-terminal sequences. HeLa cells were labeled with [35S]methionine and -cysteine, and subjected to immunoprecipitation with the NH2- and COOH-terminal antibodies. Both antibodies specifically immunoprecipitated an ~170-kDa protein, indicating that a golgin-160 protein with the COOH terminus predicted by our cDNA is expressed endogenously in HeLa cells (Fig. 1C). The observed doublet of golgin-160 is because of phosphorylation (Ref. 11 and data not shown).

The substantial predicted coiled-coil region of golgin-160 suggested it might form a homodimer. To test this hypothesis, we transfected HeLa cells with plasmids encoding GFP-tagged golgin-160 and Myc-tagged golgin-160 separately or together (Fig. 1D). In cells transfected with both plasmids, the presence of Myc-tagged golgin-160 in the anti-GFP immunoprecipitation and vice versa (lanes 7 and 8) indicated that golgin-160 can indeed dimerize, and express Golgi localization.

Localization of Golgin-160 Is Distinct from That of GM130 in Brefeldin A-Treated Cells—Brefeldin A (BFA), a fungal metabolite, induces redistribution of Golgi membrane proteins. In BFA-treated cells, Golgi enzymes are redistributed back to the ER, most coat proteins to the cytoplasm, and a growing number of Golgi matrix proteins (such as golgin-160) to Golgi “remnants”
Characterization of a cDNA encoding golgin-160 with a novel COOH terminus: A, the nucleotide sequence and translation of previously identified GCP170 cDNA (accession number D63997), and golgin-160 cDNA (accession number AF48558) are compared in the region where the different 3' ends are generated. The dotted line marks the predicted border between exons 22 and 23. The asterisks mark the position of five additional nucleotides present in the golgin-160 cDNA, which produce a frameshift and create a new termination codon. The position of five additional nucleotides present in the golgin-160 cDNA, which produce a frameshift and create a new termination codon. The nucleotide sequence and translation of Golgi matrix. In BFA-treated cells, GM130 was completely redistributed to distinct puncta spread throughout the cell (Fig. 3). In contrast, golgin-160 appeared mostly cytoplasmic, with a bright spot at or near the centrosome. The centrosomal staining pattern is reminiscent of the trans-Golgi network protein, TGN38, in BFA-treated cells (31). Thus, golgin-160 is clearly not associated with Golgi matrix components as detected by this assay.

The NH2-terminal Domain of Golgin-160 Contains Golgi Targeting Information:—The bulk of golgin-160 is predicted to form an α-helical coiled-coil structure with the exception of the first 393 amino acids, which make up the NH2-terminal head domain (Fig. 1B). To examine the role of the head and coiled-coil domains in Golgi targeting, a full-length fusion protein of golgin-160 was first created by fusing the GFP to the 5' end of the coding sequence (Fig. 3A). Double label immunofluorescence microscopy verified that the full-length fusion protein was targeted to the Golgi complex in transfected HeLa cells. The localization of GM130 is shown in the right panels to mark the Golgi complex. NH2- and COOH-terminal deletion mutants were constructed as illustrated in Fig. 3A, and localization was examined in transfected HeLa cells. Both the NH2-terminal head domain and the COOH-terminal coiled-coil domains were efficiently localized to the Golgi complex (Fig. 3B). Both truncations were expressed at higher levels than the full-length fusion protein. Saturable binding to the Golgi complex may explain the increased cytoplasmic staining present in cells expressing the truncations compared with the full-length fusion protein.

Because golgin-160 forms a homodimer when exogenously expressed (Fig. 1D), we tested the ability of both the NH2-terminal head domain and the COOH-terminal coiled-coil domain to assemble with endogenous full-length golgin-160. Assembly with endogenous golgin-160 would limit any conclusion about the ability of the truncation mutants to specify Golgi targeting on their own. HeLa cells were transfected with GFP vector, GFP-tagged full-length golgin-160, or one of the GFP-tagged deletion mutants. Transfected cells were labeled with [35S]methionine and -cysteine for 30 min, chased for 2 h, and lysed. Expression of each protein was confirmed by immunoprecipitation with anti-GFP antibody (Fig. 4A). Immunoprecipitates of the endogenous protein were analyzed for the presence of the GFP-tagged truncation mutant by sequential immunoprecipitation. To accomplish this, endogenous golgin-160 was first created by fusing the GFP to the 5' end of the coding sequence (Fig. 3A). Double label immunofluorescence microscopy verified that the full-length fusion protein was targeted to the Golgi complex in transfected HeLa cells. The localization of GM130 is shown in the right panels to mark the Golgi complex. NH2- and COOH-terminal deletion mutants were constructed as illustrated in Fig. 3A, and localization was examined in transfected HeLa cells. Both the NH2-terminal head domain and the COOH-terminal coiled-coil domains were efficiently localized to the Golgi complex (Fig. 3B). Both truncations were expressed at higher levels than the full-length fusion protein. Saturable binding to the Golgi complex may explain the increased cytoplasmic staining present in cells expressing the truncations compared with the full-length fusion protein.

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160 was immunoprecipitated in the first round with anti-golgin-160 antibodies whose epitope was not present in the GFP-tagged truncation mutant expressed in the sample (see Fig. 4A). The immunoprecipitates were eluted with SDS, diluted, and re-immunoprecipitated with anti-GFP to assess whether the expressed truncations were present in the original immunoprecipitate via interaction with endogenous golgin-160 (Fig. 4B). The COOH-terminal coiled-coil domain assembled with endogenous golgin-160 (lane 4), while neither GFP alone (lane 1) nor the NH₂-terminal head domain (lane 3) was able to form complexes with endogenous golgin-160. Endogenous golgin-160 was observed in these immunoprecipitates only after a long exposure, because the short label used here results in inefficient labeling because of the long half-life of golgin-160. The Golgi targeting of the coiled-coil domain observed in Fig. 3B could thus be because of assembly with endogenous golgin-160. However, the NH₂-terminal domain of golgin-160 clearly contains Golgi targeting information, demonstrated by its ability to localize GFP to the Golgi in the absence of assembly with endogenous golgin-160. Although we could not rule out the possibility that the coiled-coil domain of golgin-160 contains a Golgi targeting signal, we focused on dissecting the targeting signal in the head domain.

Identification of an 85-Amino Acid Sequence within the NH₂-terminal Head Domain Containing Both Golgi and Nuclear Targeting Information—To identify the minimal sequence within the NH₂ terminus of golgin-160 responsible for localization of golgin-160 to the Golgi complex, progressively smaller truncations from both ends of the 393-amino acid head domain were engineered as GFP fusion proteins (Fig. 5A). Expression of these GFP-tagged mutants in transfected HeLa cells resulted in production of appropriately sized proteins, detected by [35S]methionine and -cysteine labeling followed by immunoprecipitation with anti-GFP antibody (Fig. 5B). The intracellular localization of these truncation proteins was examined by indirect immunofluorescence microscopy (Fig. 5C). Mutant proteins containing either the first 139 amino acids or the last 136 amino acids of the NH₂-terminal head domain (GFP/g160-(1–139) and -(257–393)) were distributed diffusely throughout the cell, indicating that these regions lack any specific Golgi targeting information. In contrast, GFP/g160-(140–257) demonstrated localization of GFP-tagged golgin-160 proteins. A, schematic representation of full-length GFP-tagged golgin-160 and mutants. Full-length golgin-160 contains an extensive coiled-coil domain (shaded boxes) and non-coiled-coil NH₂-terminal head domain. B, HeLa cells were transfected with the indicated cDNA, fixed, and the GFP signal was enhanced by staining with rabbit anti-GFP and fluorescein isothiocyanate-conjugated goat anti-rabbit IgG (left panels). The cells were co-stained for endogenous GM130 with mouse anti-GM130 in combination with Texas Red-conjugated anti-mouse IgG antibody (right panels). Both the NH₂-terminal head domain and the COOH-terminal coiled-coil domain were efficiently targeted to the Golgi complex. Bar, 10 μm.

**Fig. 3.** Localization of GFP-tagged golgin-160 proteins. A, schematic representation of full-length GFP-tagged golgin-160 and mutants. Full-length golgin-160 contains an extensive coiled-coil domain (shaded boxes) and non-coiled-coil NH₂-terminal head domain. B, HeLa cells were transfected with the indicated cDNA, fixed, and the GFP signal was enhanced by staining with rabbit anti-GFP and fluorescein isothiocyanate-conjugated goat anti-rabbit IgG (left panels). The cells were co-stained for endogenous GM130 with mouse anti-GM130 in combination with Texas Red-conjugated anti-mouse IgG antibody (right panels). Both the NH₂-terminal head domain and the COOH-terminal coiled-coil domain were efficiently targeted to the Golgi complex. Bar, 10 μm.

**Fig. 4.** The COOH-terminal coiled-coil domain of golgin-160 assembles with endogenous golgin-160 in vivo. HeLa cells were transfected with plasmids encoding GFP alone, GFP/g160-(1–1498), GFP/g160-(1–393), or GFP/g160-(393–1498). Transfected cells were labeled with [35S]methionine and -cysteine for 30 min, chased for 2 h, and lysed. The lysate was divided into aliquots and immunoprecipitated. Immunoprecipitates were analyzed by SDS-PAGE (8% gels) and fluorography. A, the specificity of the antibodies is shown by a single round of immunoprecipitation with either anti-GFP or anti-golgin-160. A background band migrates slightly higher than the GFP-tagged full-length golgin-160. Note that the endogenous golgin-160 is not well labeled after 30 min, and is difficult to detect. B, sequential immunoprecipitations with anti-golgin-160 followed by anti-GFP were performed as described under “Experimental Procedures.” The GFP-tagged coiled-coil domain was detected in a complex with endogenous golgin-160 (lane 4), whereas the GFP-tagged head domain was not (lane 3).
strated specific Golgi targeting. Interestingly, this protein also showed prominent nuclear localization. All three of the truncation proteins were small enough to diffuse into the nucleus, but only GFP/g160-(140–257) accumulated there, suggesting that this localization is specific.

A closer look at the sequence of the head domain of golgin-160 revealed a putative NLS, PREKKTSK, located between amino acids 232 and 239. To test the contribution of the potential NLS to the steady state localization of golgin-160 truncation proteins, we mutated the three lysine residues within this sequence to arginines. The lysine residues in other monopartite NLS-containing proteins have been shown to be essential for nuclear import (32). Mutation of the NLS in GFP/g160-(140–257) resulted in an increase in cytoplasmic staining and a reduction in nuclear, but not Golgi targeting (fig. 5NLS, Fig. 5C).

Subcellular fractionation was performed with the goal of quantifying the nuclear versus Golgi-localized populations of these truncation proteins. However, upon homogenization using several different methods, all of the truncation proteins were found in the cytosolic fraction (data not shown). These data suggest that the interactions responsible for nuclear retention may not be stable enough to withstand the fractionation procedure, allowing leakage of these small proteins into the cytosol through nuclear pores.

To more directly assess the contribution of the NLS to nuclear localization of golgin-160 truncation proteins, we used a vaccinia virus-mediated T7 expression system to allow detection of the proteins early after transfection. HeLa cells were

![Diagram of golgin-160 truncations](image)

**FIG. 5.** Identification of the minimal sequence within the head domain of golgin-160 sufficient for Golgi localization. **A,** a schematic representation of GFP-tagged truncations. A putative nuclear localization signal (gray box) was mutated in the constructs labeled −NLS. **B,** transfected HeLa cells were labeled with [35S]methionine and cysteine for 30 min and lysed. Lysates were immunoprecipitated with anti-GFP, separated by SDS-PAGE (15% gel), and the labeled proteins were visualized by fluorography. **C,** transfected HeLa cells expressing GFP-tagged truncation mutants of golgin-160 were fixed, permeabilized, and the GFP signal was enhanced by staining with rabbit anti-GFP and fluorescein isothiocyanate-conjugated goat anti-rabbit IgG (left panels). Cells were co-stained for endogenous GM130 with mouse anti-GM130 in combination with Texas Red conjugated anti-mouse IgG (right panels). An 85-amino acid sequence within the NH$_2$-terminal non-coiled-coil domain of golgin-160 (residues 172–257) is sufficient to target GFP to the Golgi. Bar, 10 μm.

![Diagram of golgin-160 truncations](image)

**FIG. 6.** A nuclear localization sequence in the head of golgin-160 mediates active import of golgin-160 truncations into the nucleus. HeLa cells were infected with vaccina virus encoding T7 RNA polymerase (VT7-3) and transfected with plasmids encoding GFP/g160-(139–257) or GFP/g160-(139–257)-NLS behind the T7 promoter. At 2 or 4 h post-transfection, cells expressing GFP-tagged truncation mutants of golgin-160 were fixed, permeabilized, and the GFP signal was enhanced by staining with rabbit anti-GFP and fluorescein isothiocyanate-conjugated goat anti-rabbit IgG. The golgin-160 truncation containing the NLS localizes to the nucleus more rapidly and efficiently than the same truncation with a mutated NLS.
FIG. 7. Specific caspase cleavage fragments of the NH2-terminal head of golgin-160 have the potential to be targeted to the nucleus. A, schematic representation of Myc-tagged constructs of all possible caspase cleavage fragments of the head domain of golgin-160. Aspartic acid residues at the caspase cleavage sites within the NH2-terminus of golgin-160 are marked with a star. B, potential Myc-tagged caspase cleavage fragments of golgin-160 were expressed in HeLa cells by transient transfection. Transfected cells were fixed, permeabilized, and stained with mouse anti-Myc antibody and Texas Red-conjugated secondary antibody. Bar, 10 μm.

first infected with a recombinant vaccinia virus encoding T7 RNA polymerase, and subsequently transfected with plasmids encoding GFP/g160-(140–257) or GFP/g160-(140–257)-NLS behind a T7 promoter. The localization of these proteins was analyzed by indirect immunofluorescence at 2 and 4 h post-transfection (Fig. 6). At 2 h post-transfection, there was significantly more nuclear staining when the NLS was intact, and this was even more dramatic at 4 h post-transfection. Thus, the NLS seems to increase the rate of nuclear translocation.

Some nuclear accumulation was observed for GFP/g160-(140–257) even when the NLS was mutated (Figs. 5 and 6). We hypothesize that the small size of this protein allows diffusion into the nucleus where interaction with a nuclear component results in nuclear retention. Nuclear retention of GFP/g160-(140–257) seems likely because neither GFP/g160-(1–139) nor GFP/g160-(257–393) accumulate in the nucleus (Fig. 5), even though all three of these proteins are small enough to diffuse into the nucleus. We conclude that a small portion of the golgin-160 head (residues 140–257) contains three distinct targeting signals: information for Golgi localization, for active nuclear import, and for nuclear retention (see summary in Fig. 8).

To further narrow the minimal Golgi targeting signal, we created smaller truncations in the minus NLS background. GFP/g160-(172–257)-NLS was targeted to the Golgi as efficiently as the larger truncations, narrowing the Golgi targeting signal to 85 amino acids (Fig. 5C). This sequence contains no significant homology to other proteins.

Specific Caspase Cleavage Fragments of the Head Domain of Golgin-160 Have the Potential to be Targeted to the Nucleus—Full-length golgin-160 was never observed in the nucleus, raising the possibility that the NLS and putative nuclear retention signal are not physiologically relevant. However, the NH2-terminal head domain of golgin-160 is cleaved into smaller pieces by caspases during programmed cell death (25). The caspase cleavage sites are shown in Fig. 7A. Depending on the order of caspase activation, a number of different fragments of golgin-160 could potentially be released from Golgi membranes. To assess the localization of potential caspase cleavage fragments, we constructed Myc-tagged mutants corresponding to all possible fragments resulting from various combinations of caspase cleavage of golgin-160 (Fig. 7A). In these experiments, we used a small epitope tag to more closely mimic the actual fragments produced by caspase cleavage. HeLa cells were transfected with the indicated plasmids and localization was determined by indirect immunofluorescence microscopy (Fig. 7B). Only those constructs that contained amino acids 140–311 were specifically localized to the nucleus, whereas the remaining constructs were distributed throughout the entire cell. The Myc-tagged constructs containing the 85-amino acid stretch demonstrated to be important for Golgi targeting also showed Golgi localization. The apparent increase in nuclear localization of these fragments compared with those shown in Fig. 5C may indicate that the NLS is more accessible, or that nuclear retention is more efficient. The specific nuclear targeting of g160-(1–311)/Myc, g160-(60–311)/Myc, and g160-(139–311)/Myc suggests that if these fragments are released from the Golgi during apoptosis, they are translocated to the nucleus. A direct demonstration of this will require antibodies that recognize the central portion of the golgin-160 head, which are currently in production.

DISCUSSION

Golgins were originally identified as autoantigens in certain autoimmune diseases, but the definition of the golgin family has been extended to include any Golgi-localized membrane protein that contains an extensive coiled-coil domain. Although some golgins have been implicated in vesicle tethering and/or cisternal stacking, it is likely that different functions for other members of this broadly defined family exist. We previously showed that the NH2-terminal head domain of golgin-160 is cleaved by caspases during programmed cell death (25). Cleavage of golgin-160 during apoptosis may disrupt protein-protein interactions that are critical for Golgi structure and function, contributing to the apoptotic disassembly of the Golgi.

We have studied the targeting of golgin-160 as a first step toward addressing the contribution of this protein to Golgi structure and function. Our golgin-160 cDNA clone encodes a protein of 1498 amino acids, which differs from the previously reported GCP170 sequence because of a frameshift resulting in a novel COOH terminus (Fig. 1A). Based on the new sequence, golgin-160 is predicted to have a non-coiled-coil NH2-terminal head domain followed by an extensive COOH-terminal coiled-coil tail (Fig. 1B). A number of expressed sequence tags present in the database suggest that the protein represented by our
golgin-160 cDNA is widely expressed. Anti-peptide antisera raised against the COOH terminus of our predicted golgin-160 protein confirmed that it is indeed endogenously expressed in HeLa cells (Fig. 1C). It is likely that golgin-160 exists as a dimer that is mediated by its the coiled-coil domain (Figs. 1D and 4B).

Several golgins are present in a detergent-resistant Golgi matrix, and redistribute to distinct cytoplasmic puncta when cells are treated with BFA (7,33). Golgin-160 does not appear to be a component of the Golgi matrix, because it was not redistributed to GM130-containing puncta in BFA-treated cells (Fig. 2).

Several golgins, including GM130, giantin, golgin-97, and golgin-84, contain Golgi targeting signals in their COOH-terminal regions (16,34–38). Here, we report multiple distinct targeting signals in the NH2-terminal domain of golgin-160 (Fig. 8). We identified an 85-amino acid stretch in the NH2-terminal domain of golgin-160 capable of targeting GFP to the Golgi complex (Fig. 6). This 85-amino acid sequence contains several predicted motifs, such as a “helix-loop-helix” dimerization pattern (amino acids 199–214) that could mediate protein–protein interactions, but no significant homology to other proteins. The efficiency of Golgi localization was increased by the presence of an additional flanking sequence, suggesting that the conformation of the 85-residue domain may be critical for correct targeting. Together, these results indicate that Golgi localization of golgin-160 reflects an intrinsic property of the NH2-terminal head domain in the context of the full-length protein. A further understanding of the Golgi targeting signal will require determining the proteins with which the signal interacts.

Interestingly, some truncations of the golgin-160 head domain showed prominent nuclear localization, suggesting the presence of cryptic nuclear targeting information (Fig. 5C). A putative NLS was identified between residues 232 and 239. When this sequence was mutated, steady state nuclear localization was reduced but not eliminated (−NLS, Fig. 5C). Analysis at early times post-transfection demonstrated that the NLS accelerated the rate of nuclear accumulation of GFP/g160 (140–257) (Fig. 6). The nuclear accumulation of truncation proteins lacking the NLS may be because of passive diffusion into the nucleus and interaction with a nuclear component, resulting in nuclear retention. However, it is important to note that full-length golgin-160 (endogenous or exogenously expressed) was never observed in the nucleus. Thus, the nuclear localization information present in the NH2-terminal head domain does not appear to function in the context of full-length golgin-160. There are a number NLS examples that are masked in the cytoplasm by protein–protein interactions, phosphorylation, or other post-translational modifications. We speculate that the NLS in the golgin-160 head is masked in a regulated manner.

We have previously shown that the NH2-terminal domain of golgin-160 is cleaved by caspases during apoptosis (25). To determine whether caspase cleavage fragments could be targeted to the nucleus, Myc-tagged constructs corresponding to all possible cleavage fragments were generated and localized by indirect immunofluorescence microscopy (Fig. 7B). Three of the six possible cleavage fragments showed specific nuclear localization. Although all six of the fragments are small enough to diffuse into the nucleus, only those containing the region between residues 140 and 311 accumulated there. The three fragments containing residues 172–257 localized predominantly to the nucleus, even though they contain the Golgi targeting region described above. It is possible that the NLS and/or nuclear retention domain is completely unmasked in these fragments, which mimic the proteolytic fragments that would be generated in vivo. Thus, if these caspase-derived fragments are released from Golgi membranes during programmed cell death, they are likely to be translocated to the nucleus. It is tempting to speculate that apoptotic fragments of golgin-160 have a specific function in the nucleus. Studies to address this issue are presently underway.

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