Molecular Cloning, Characterization, and Dynamics of Rat Formiminotransferase Cyclodeaminase, a Golgi-associated 58-kDa Protein*

(Received for publication, July 24, 1998, and in revised form, September 26, 1998)

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The golgi complex plays a key role in the processing and sorting of proteins destined for secretion or delivery to other intracellular compartments (1). It receives newly synthesized cargo from the ER,1 and through the activities of glycosylating, sulfating, and proteolytic enzymes it generates products that are then sorted and segregated into distinct transport intermediates and delivered to distinct post-Golgi compartments (2). In mammalian cells, the Golgi complex consists of a ribbon-like structure composed of stacks of tightly opposed flattened cisternae with extensive vesicular and tubular profiles associated with the rims of the stacks (3). The stacks are functionally and morphologically polarized and contain pleomorphic tubulovesicular structures on the incoming cis-face and the outgoing trans-face (4–6). The tubulovesicular structures near the cis-Golgi have been called the cis-Golgi network, ER-to-Golgi intermediate compartment (ERGIC) or vesicular tubular clusters (VTCs) (reviewed in Refs. 7–10). The exact identity and fate of these structures is still debated, but it appears that VTCs represent transport intermediates ferrying cargo from the ER to the Golgi (11, 12).

In addition to the VTC-mediated ER-to-Golgi movement of material, selective retrograde recycling from the Golgi to the ER has been shown to be involved in the retrieval of specific proteins (e.g. see Refs. 13–15). Recent evidence suggests that active cycling through the ER and Golgi may be common to all Golgi proteins (16–18). The retrograde redistribution of the cycling protein KDEL receptor (KDEL-R) can be seen in untreated cells and seems to involve tubular elements originating from the Golgi region and extending into the periphery of cells (19). Retrograde transport pathways appear to be preferentially enhanced in cells treated with the fungal metabolite brefeldin A (BFA). In BFA-treated cells, resident Golgi proteins (mannosidase II and Gal-T) appear to be mediated by tubules that extend from the Golgi and fuse with the ER (19, 20). The overall structure of retrograde tubules containing KDEL-R in untreated cells, and Gal-T in BFA-treated cells appears similar (19). However, whether KDEL-R is redistributed through tubular structures in BFA-treated cells is less clear. A comparison of mannosidase II and KDEL-R redistribution during BFA treatment shows that KDEL-R redistributes more rapidly than mannosidase II and that it does not utilize tubules during its relocation (21). The evidence suggests that multiple pathways for relocation from the Golgi may exist for distinct types of resident and cycling molecules.

A novel 58-kDa protein (58K) that may be involved in linking Golgi to microtubules was partially sequenced. The obtained sequences show a high level of identity with sequences of porcine formiminotransferase cyclodeaminase (FTCD), suggesting that 58K is rat FTCD. Rat FTCD is structurally similar to porcine FTCD, a metabolic enzyme involved in conversion of histidine to glutamic acid, and exists in dimeric, tetrameric, and octameric complexes resistant to proteolysis. To define parameters of FTCD association with the Golgi, comparison of its behavior with various Golgi and ER-to-Golgi intermediate compartment marker proteins was examined under specific conditions. The results show that extraction parameters of FTCD are similar to those of GM130, a tightly associated Golgi matrix protein. FTCD appears to be a dynamic component of the Golgi, and a proportion of FTCD molecules cycle between the Golgi and earlier compartments of the secretory pathway. FTCD remains associated with Golgi fragments during microtubule disruption and is not released into cytosol during brefeldin A treatment. Instead, FTCD relocates from the Golgi, but the time course of its redistribution is distinct from that of mannosidase II relocation. FTCD is already dispersed into small punctate structures at a time when mannosidase II is still largely localized to Golgi structures. FTCD is not observed in tubules originating from the Golgi and containing mannosidase II. Instead, it appears to redistribute in small vesicles arranged in a linear “pearls on a string” pattern. These results suggest that FTCD relocation is temporally and spatially distinct from mannosidase II relocation and that FTCD provides a novel marker to study Golgi dynamics.

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1 The abbreviations used are: ER, endoplasmic reticulum; ERGIC, ER-to-Golgi intermediate compartment; VTC, vesicular tubular cluster; KDEL-R, KDEL receptor; 58K, 58-kDa protein; BFA, brefeldin A; Gal-T, galactosyl transferase; FTCD, formiminotransferase cyclodeaminase; NRK, normal rat kidney; TBS, Tris-buffered saline; SG, stacked Golgi; PAGE, polyacrylamide gel electrophoresis; mAb, monoclonal antibody; IF, immunofluorescence; PNS, postnuclear supernatant.

KDEL-R, KDEL receptor; 58K, 58-kDa protein; BFA, brefeldin A; Gal-T, galactosyl transferase; FTCD, formiminotransferase cyclodeaminase; NRK, normal rat kidney; TBS, Tris-buffered saline; SG, stacked Golgi; PAGE, polyacrylamide gel electrophoresis; mAb, monoclonal antibody; IF, immunofluorescence; PNS, postnuclear supernatant.
of the protein. We cloned and sequenced 58K, and in this report we show that 58K has extensive overall sequence identity with porcine FTCD and is likely to represent rat FTCD. FTCD is a metabolic enzyme involved in the conversion of histidine to glutamic acid (23). Its formiminotransferase activity transfers a formiminoglutamate group from N-formimino-L-glutamic acid to tetrahydrofolate to generate glutamic acid and 5-formiminotetrahydrofolate, and the cyclodeaminase activity then converts the 5-formiminotetrahydrofolate to 5,10-methenyl tetrahydrofolate and ammonia. FTCD is an octameric enzyme with eight identical subunits arranged in a planar ring (23). We examined parameters of rat FTCD association with the Golgi and show that the binding is tight but not dependent on presence of intact microtubules. Interestingly, FTCD appears to be a dynamic component of the Golgi and cycles between the Golgi and earlier compartments of the secretory pathway. FTCD remains associated with membranes in the presence of BFA, but the protein rapidly redistributes from the Golgi, utilizing a temporal and spatial pathway distinct from that used by mannose-dase II. FTCD therefore represents a novel marker with which to explore ER-Golgi dynamics.

MATERIALS AND METHODS

Reagents and Antibodies—Restriction enzymes and molecular reagents were from Promega (Madison, WI) and Qiagen (Chatsworth, CA). The cross-linker 3,3′-dithiodiisouccinimidyl propionate was from Pierce. BFA and nucodazole were from Sigma. BFA was stored as a 5 mg/ml ethanol stock and used at a final concentration of 5 µg/ml in culture medium. Nucodazole was stored as a 10 mg/ml Me2SO stock and used at 20 µg/ml in culture medium. All other chemicals were from Sigma. Protein G-Sepharose was from Amersham Pharmacia Biotech. Protein concentration of samples was determined using Protein Assay Reagent from Bio-Rad with bovine serum albumin as a standard.

The CHO-K1 cell line was a generous gift from Dr. George Bloom (University of Texas Southwestern Medical Center, Dallas, TX). Purified IgG from 58K-9 ascites was used in immunofluorescence studies. Polyclonal rabbit serum was raised against rat FTCD. Secondary antibodies and then with Protein G-Sepharose (1 h, room temperature); supernatants were first incubated (2 h, room temperature) with antibodies and then with Protein G-Sepharose (1 h, room temperature); washed once with 3 M NaCl in TBS and three times with TBS, 0.05% Tween 20; and processed for SDS-PAGE. Immunoprecipitation—SG fraction or NRK PNS was solubilized in TBS containing 2% Triton X-100 and 1 mM phenylmethylsulfonyl fluoride. Samples were centrifuged at 16,000 × g for 5 min, and the supernatants were first incubated (2 h, room temperature) with antibodies and then with Protein G-Sepharose (1 h, room temperature); washed once with 3 M NaCl in TBS and three times with TBS, 0.05% Tween 20; and processed for SDS-PAGE.

SDS-PAGE and Immunoblotting—Samples were analyzed by SDS-PAGE as described previously (26). Following SDS-PAGE, proteins were transferred to nitrocellulose filters. Filters were blocked (2% milk/TBS, 1 h), incubated with primary antibodies (2% milk/TBS, 2 h), washed with PBS/Tween 20, incubated with donkey anti-rabbit or anti-mouse antibodies conjugated to horseradish peroxidase (in 2% milk/TBS, 60 min), washed again, and incubated in ECL detection reagent (Amersham Pharmacia Biotech). All incubations were done at room temperature. Filters were then exposed to x-ray film.

Immunofluorescence Microscopy—Cells were grown on glass coverslips, fixed in 3% paraformaldehyde (10 min) and washed in PBS. Cells were permeabilized with 0.01% Triton X-100 (7 min); washed in PBS, 0.05% Tween 20; and incubated in PBS, 0.05% Tween 20, 0.4% fish skin gelatin, 0.05% Triton X-100 (20 min); washed with PBS, 0.05% Tween 20; and incubated in ECL detection reagent (Amersham Pharmacia Biotech). All incubations were done at room temperature. Filters were then exposed to x-ray film.

RESULTS

58K Is Tightly Associated with Golgi Membranes—Previous work has shown that 58K codistributes with cytologically oriented, peripheral membrane proteins in isolated Golgi fraction (22), but the relative recovery of 58K during the fractionation was not reported. To more quantitatively examine the distribution of 58K, postnuclear supernatant of rat liver homogenate was fractionated on a discontinuous sucrose density gradient to generate a cytosolic fraction, an SG fraction, an
58K is tightly associated with Golgi membranes. A subcellular fractions enriched in cytosol (cyto), stacked Golgi (SG), ER elements (ER), and a residual pellet (pellet) were isolated from rat liver. An aliquot of each fraction representing the same volume of rat liver postnuclear supernatant was analyzed by SDS-PAGE and immunoblotting with antibodies against 58K and marker proteins for Golgi (GM130), β-COP, VTCs (ERGIC/p58), and ER (calnexin). 58K was predominantly found in a cytosolic and Golgi-enriched fraction, with lower but significant amounts in the ER fraction. B, subcellular membrane fractions were subjected to high speed (150,000 g) centrifugation, and resulting supernatants and pellets were analyzed by SDS-PAGE and immunoblotting with antibodies against 58K and marker proteins. A proportion of 58K is recovered with Golgi and ER membranes. C, aliquots of SG pellet (prepared as in B) were treated with 1 M KCl in the absence or presence of saponin and then subjected to high speed centrifugation, and the resulting supernatants and pellets were analyzed by SDS-PAGE and immunoblotting with antibodies against 58K and marker proteins. 58K is tightly bound to Golgi membranes with extraction properties similar to those of GM130.

ER-enriched fraction, and a residual pellet fraction. The relative homogeneity of each fraction was analyzed by the presence of specific marker proteins. As shown in Fig. 1A, the tightly peripherally associated Golgi matrix protein GM130 (28) is detected predominantly in the SG fraction, with small amounts visible in the cytosolic fraction. The peripherally associated coatamer subunit β-COP (29, 30) is present predominantly in the cytosol but can also be detected in the SG and ER fractions, consistent with its cycling dynamics (12). The ER marker, calnexin (31, 32), is concentrated in the ER fraction but is also detected in the SG fraction, indicating partial contamination of the SG with ER elements. The ERGIC marker, p58 (33) is detected in the SG fraction, indicating partial contamination of the SG fraction with ER elements. The ERGIC marker, p58 (33) is detected in the SG fraction, indicating partial contamination of the SG fraction with ER elements.

58K has been shown previously to be partially extractable from Golgi fraction with 0.5 M KCl (22). To further examine the parameters of 58K association with Golgi membranes, the 150,000 g pellet of the SG fraction was extracted with 1 M KCl, saponin, or saponin and 1 M KCl together, and the distribution of 58K and marker proteins was analyzed. Saponin was used in these studies to perturb the integrity of the SG membrane. As shown in Fig. 1C, in the absence of salt and saponin, 58K was minimally released from the SG pellet (lanes 1 and 2), while treatment with 1 M KCl released approximately half of 58K (lanes 3 and 4). Treatment with saponin alone released low amounts of 58K (lanes 5 and 6), while treatment with saponin and 1 M KCl led to the release of majority of 58K (lanes 7 and 8). The extraction profile of 58K was similar to that of GM130, a protein tightly associated with the Golgi (28). GM130 can be extracted to ~50% by salt alone (lanes 3 and 4) and is not significantly released with saponin alone (lanes 5 and 6) but can be extracted to a higher extent with saponin and salt (lanes 7 and 8). The extraction parameters for 58K and GM130 differ from that of the peripherally associated Golgi protein p115 (34). Almost complete extraction is seen after salt treatment alone (lanes 3 and 4), saponin treatment alone (lanes 5 and 6), or salt and saponin treatment (lanes 7 and 8).

**Sequences of Rat 58K**

**Are Highly Homologous to Porcine FTCD Sequences**—To initiate the molecular characterization of 58K, we used mAb 58K-9 to screen a λ ZAP II rat liver cDNA expression library. 6 × 10^5 plaques were screened, and 15 positive clones were isolated. Restriction mapping showed eight distinct groups of clones, containing inserts ranging in...
size from ~1.2 to ~2.5 kilobase pairs. Six distinct clones were partially sequenced, and obtained sequences were compared with sequences present in the GenBank/EMBL databases.

A high level of identity (black boxes) or conservation (gray boxes) is observed between the sequences. A segment spanning amino acids 160–175 is underlined and represents a putative tetrahydrofolate binding site in porcine FTCD. B, aliquots of SG were subjected to proteinase K digestion in the presence (lanes 5–8) or absence (lanes 1–4) of urea as a denaturing agent. Samples were analyzed by SDS-PAGE and immunoblotting with mAb 58K-9 and polyclonal anti-polymeric IgA receptor (pIgA-R) antibodies. FTCD is largely resistant to proteolysis in the absence of urea but is readily degraded when denatured. C, aliquots of rat liver cytosolic fraction were untreated (lanes 1 and 2) or cross-linked with 3,3'-dithiobis(sulfosuccinimidyl propionate) (lanes 3 and 4) and analyzed by SDS-PAGE in the absence (lanes 2 and 4) or presence (lanes 1 and 3) of reducing agent and immunoblotted with mAb 58K-9 antibodies. FTCD is recovered as higher order dimers, tetramers, and octamers. Additional bands (marked by asterisks) are visible and may represent FTCD complexed with an additional protein. The arrow denotes the interface of stacking and separating gel. D, NRK lysate was immunoprecipitated with mAb 58K-9, and SG fraction was immunoprecipitated with mAb 58K-9 and monoclonal anti-FLAG antibody. The immunoprecipitates were analyzed by SDS-PAGE and immunoblotting with mAb 58K-9. A band migrating at 58 kDa is evident in mAb 58K-9 immunoprecipitates from both sources but not in the control immunoprecipitate. IgG heavy and light chains of mAb 58K-9 and control IgG are also detected.

Fig. 2. 58K has molecular and structural similarities to porcine FTCD. A, obtained nucleotide sequences of 58K were used to obtain corresponding open reading frames, and those are aligned with relevant sequences in porcine FTCD. A high level of identity (black boxes) or conservation (gray boxes) is observed between the sequences. A segment spanning amino acids 160–175 is underlined and represents a putative tetrahydrofolate binding site in porcine FTCD. B, aliquots of SG were subjected to proteinase K digestion in the presence (lanes 5–8) or absence (lanes 1–4) of urea as a denaturing agent. Samples were analyzed by SDS-PAGE and immunoblotting with mAb 58K-9 and polyclonal anti-polymeric IgA receptor (pIgA-R) antibodies. FTCD is largely resistant to proteolysis in the absence of urea but is readily degraded when denatured. C, aliquots of rat liver cytosolic fraction were untreated (lanes 1 and 2) or cross-linked with 3,3'-dithiobis(sulfosuccinimidyl propionate) (lanes 3 and 4) and analyzed by SDS-PAGE in the absence (lanes 2 and 4) or presence (lanes 1 and 3) of reducing agent and immunoblotted with mAb 58K-9 antibodies. FTCD is recovered as higher order dimers, tetramers, and octamers. Additional bands (marked by asterisks) are visible and may represent FTCD complexed with an additional protein. The arrow denotes the interface of stacking and separating gel. D, NRK lysate was immunoprecipitated with mAb 58K-9, and SG fraction was immunoprecipitated with mAb 58K-9 and monoclonal anti-FLAG antibody. The immunoprecipitates were analyzed by SDS-PAGE and immunoblotting with mAb 58K-9. A band migrating at 58 kDa is evident in mAb 58K-9 immunoprecipitates from both sources but not in the control immunoprecipitate. IgG heavy and light chains of mAb 58K-9 and control IgG are also detected.
resistant to proteolysis in the absence of urea (lanes 1–4) but is readily degraded when protease K and urea are used (lanes 5–8). This behavior was observed when digestion was performed in the presence of 1 mM KCl and 2% TX-100 (data not shown). Proteolysis of the type I transmembrane protein, the polymeric IgA receptor (Fig. 2, pIgA-R) is shown as control (26). The cytoplasmic ~20-kDa tails of the 116- and 120-kDa forms of the polymeric IgA receptor are efficiently cleaved by protease K in the absence (lanes 1–4) or presence (lanes 5–8) of urea, resulting in ~96- and ~100-kDa intraluminal and proteolysis-protected fragments.

Previous analysis has indicated that porcine FTCD exists as a dimer organized into higher order structures, tetramers and octamers (37). To determine whether rat FTCD shows similar structural characteristics, rat liver cytosolic fraction was either untreated or subjected to cross-linking, analyzed by SDS-PAGE in the presence or absence of reducing agent, and, after transfer to nitrocellulose filters, immunoblotted with mAb 58K-9. As shown in Fig. 2C, only a 58-kDa band is detected when untreated cytosol is analyzed (lanes 1 and 2). When cytosol was cross-linked but reduced prior to analysis, the 58-kDa band was detected, and an additional minor species migrating at a position expected for an FTCD dimer (~115 kDa) was apparent (lane 3). The dimer band probably results from incomplete reduction of disulfate bonds by β-mercaptoethanol. When an analogous sample was analyzed without reduction, a more complex pattern was evident (lane 4). In addition to the 58-kDa band, bands with the expected mobilities of FTCD dimers, tetramers, and octamers could be detected. Interestingly, three additional bands were seen (marked by asterisks) that may represent dimers, tetramers, and octamers complexed in a stoichiometric ratio with an additional protein. The observed changes in mobilities are most consistent with a single ~35-kDa protein cross-linking to each FTCD dimer, but we cannot currently exclude the possibility that a complex of proteins rather than a single ~35-kDa molecules is cross-linked. It is unlikely that the cross-linking of the ~35-kDa moiety is artifactual, since it is associated in stoichiometric amounts (one per FTCD dimer) and is not cross-linked to FTCD monomers. Additional proteins have not been previously observed in FTCD preparations purified from porcine liver (23), but in our experiments crude rat liver cytosolic fraction was cross-linked, and this may preserve unstable associations that do not persist during extensive purification. The identity of the cross-linked moiety is unknown, but it is not required for FTCD enzymatic activity, since purified and recombinant FTCD generated without it are active (35, 37). It is possible that the cross-linked moiety is irrelevant to the enzymatic activity of FTCD but plays a role in the association of the protein with the Golgi membrane.

**FTCD Cycles between the Golgi and Earlier Compartments of the Secretory Pathway**—The initial report characterizing 58K showed it localized to the Golgi complex in rat (Faza) and human (HepG2) cell lines (22). Since we detected FTCD in a SG fraction and in an ER fraction (Fig. 1A), we examined whether FTCD is a cycling protein. FTCD distribution was analyzed by immunofluorescence (IF) in cells incubated at 37 °C or at a reduced temperature of 15 °C and in cells incubated at a reduced temperature and allowed to recover at 37 °C for 18 min prior to analysis. Incubation of cells at 15 °C inhibits the anterograde transport of VTCs from the periphery to the Golgi region without significantly influencing the retrograde Golgi to ER transport. This results in partial entrapment of cycling molecules in peripheral VTCs, and the subsequent shift of such cells to 37 °C for a short time amplifies the accumulation of cycling proteins in the peripheral structures (21, 38, 39).

Prior to the IF experiments, NRK lysates were examined by immunoprecipitation and immunoblotting with mAb 58K-9 to ensure that the antibodies reacted with FTCD in this cell type, since FTCD has been reported to be liver-specific (35). As shown in Fig. 2D, a band of the same mobility (58 kDa) is immunoprecipitated and immunoblotted from NRK lysate and from rat liver SG, indicating that mAb 58K-9 can be used to localize FTCD in NRK cells.

NRK cells were incubated at 37 °C or at 15 °C for 2 h and then either analyzed directly or shifted to 37 °C for 18 min prior to IF analysis. The distribution of FTCD was compared with the distribution of the Golgi protein GM130 and two cycling proteins p58 and p115. In cells cultured at 37 °C, FTCD is present in a characteristic perinuclear Golgi pattern (Fig. 3A) and co-localizes with GM130 (Fig. 3B). In cells cultured at 15 °C, FTCD is present in Golgi structures that appear slightly fragmented (Fig. 3C), and co-localizes in those structures with GM130 (Fig. 3D). In addition, FTCD can also be detected in small punctate structures scattered throughout the cells (arrowheads in C) that do not contain GM130. A similar situation is seen in cells shifted from 15 °C to 37 °C, with FTCD (Fig. 3E) and GM130 (Fig. 3F) co-localizing in morphologically normal Golgi but FTCD also present in small scattered puncta (arrowheads in Fig. 3E) that do not contain GM130.

FTCD (Fig. 3G) co-localizes poorly with p58 (Fig. 3H) in cells cultured at 37 °C, and the majority of structures containing p58 do not label for FTCD (arrowheads in Fig. 3I). Similarly, in cells cultured at 15 °C, FTCD (Fig. 3I) is predominantly present in morphologically identifiable Golgi, while the majority of p58 (Fig. 3J) in present in peripheral VTCs (arrowheads in Fig. 3J). In cells shifted from 15 to 37 °C, the majority of FTCD (Fig. 3K) is present within the Golgi. The majority of p58 is present in peripheral VTCs (arrowheads in Fig. 3L).

Recent results indicate that p115 is a cycling protein found associated with the cis-Golgi cisternae and with VTCs (24). Consistent with this itinerary, p115 is detected in the Golgi region and in punctate structures in the periphery of cells cultured at 37 °C (Fig. 3N), 15 °C (Fig. 3P), or shifted from 15 to 37 °C (Fig. 3R). The accumulation of p115 in peripheral VTCs is most pronounced in cells incubated at 15 °C (arrowheads in Fig. 3P) or shifted from 15 °C to 37 °C (arrowheads in Fig. 3R). FTCD (Fig. 3, M, O, and Q) co-localizes with p115 in morphologically identifiable Golgi structures in all cells. In addition, FTCD can also be detected in some peripheral structures containing p115 (arrowheads in O and Q). However, the relative proportion of FTCD in peripheral VTCs seems significantly lower than that of p115. It appears, therefore, that although FTCD localizes predominantly to the Golgi complex under steady state, the protein is dynamic, and a proportion cycles between the Golgi and earlier compartments of the secretory pathway. FTCD appears to cycle more extensively than GM130 but less than p58 and p115.

**Membrane Association of FTCD Is Not BFA-responsive and Does Not Depend on Intact Microtubules**—Since FTCD is a soluble enzyme, its association with the Golgi complex is likely to be mediated by protein-protein interactions. To initiate an inquiry into the nature of this association, we first examined its behavior during BFA treatment. Responses of Golgi proteins to BFA treatment vary, and the effect on FTCD behavior was compared with that of mannoseidase II, which redistributes to the ER; p115, which relocates to peripheral VTCs; and β-COP, which is released into the cytosol.

After a 30-min BFA treatment, FTCD (Fig. 4A) is present in a diffuse cellular pattern and in small punctate structures clustered in the perinuclear region (arrowheads). Mannosidase
II (Fig. 4B) is present in a diffuse reticular pattern shown to be the ER, in agreement with previous results (40). p115 (Fig. 4C) localizes in punctate structures that contain p58 (data not shown) and are likely to represent VTCs. β-COP (Fig. 4D) is present in a diffuse cytosolic pattern, in agreement with previous results (40). The BFA-induced redistribution of each protein was completely reversible when BFA was removed (data not shown).

To determine whether the diffuse localization of FTCD represents the redistribution of the protein throughout the ER or its release into the cytosol, NRK cells were first treated with nocodazole to disrupt microtubules and then with BFA. The disruption of microtubules prevents the extension of retrograde tubules and prevents the redistribution of proteins from the Golgi to the ER but does not prevent BFA-induced release of molecules like β-COP from the membrane. FTCD remained associated with Golgi fragments in the presence of nocodazole and BFA (Fig. 4E), as did mannosidase II (Fig. 4F) and p115 (Fig. 4G). That the nocodazole/BFA treatment was sufficient to dissociate a BFA-responsive protein was shown by β-COP release into the cytosol and its lack of association with the Golgi fragments (Fig. 4H). These results show that FTCD association with Golgi membranes is not affected by BFA and suggest that the diffuse FTCD localization pattern seen after BFA treatment most likely represents its relocation. The results also show that FTCD binding to Golgi membranes is independent of the microtubule cytoskeleton.

FTCD Rapidly Relocates from the Golgi in Response to BFA—To examine the dynamics of FTCD redistribution, we analyzed its localization at distinct time points during BFA treatment. In these experiments, β-COP was dispersed into the cytosol after 50 s of BFA treatment (the earliest time point examined) and remained there throughout the length of the experiment (data not shown), in agreement with previous results (40). At each time point, analogous coverslips were used to localize FTCD or mannosidase II.

Comparison of FTCD localization in cells treated with BFA for 50 s (Fig. 5A) with its localization in untreated cells (see Fig. 3, A, G, and M) indicates that FTCD is already partially redistributed. Redistribution was more pronounced after 2.5-min BFA treatment, with the majority of FTCD no longer localized...
in identifiable Golgi structures but dispersed in small punctate structures present throughout the cell (arrowheads in Fig. 5B). FTCD dispersion was almost complete after 6-min BFA treatment, with minor amounts present within recognizable Golgi structures (Fig. 5C). The kinetics of FTCD relocation differ significantly from those of mannosidase II. Mannosidase II was present in morphologically normal Golgi structures after 50-s (Fig. 5E), 2.5-min (Fig. 5F), or 6-min (Fig. 5G) BFA treatment. Mannosidase II was present in the ER at the next time point analyzed, after 20-min BFA treatment (data not shown) and after 30-min BFA treatment (see Fig. 4B). After 2.5- and 6-min BFA treatment, tubular projections emanating from the Golgi region and containing mannosidase II were seen (arrowheads in Fig. 5H). The tubules were similar in size and morphology to structures previously reported to mediate Golgi to ER retrograde transport in BFA-treated cells (19, 40) or in untreated cells (19, 41). Significantly, FTCD was not detected in such tubules (Fig. 5D). Instead, FTCD redistribution appears to be mediated by small punctate structures. Interestingly, the small puncta were often arranged in a “bead on a string” pattern (arrowheads in D), which may indicate that they are associated with microtubules. These results suggest that FTCD relocation is temporally separate from mannosidase II relocation and that it may involve a pathway distinct from that utilized by mannosidase II.

**DISCUSSION**

*58K Identity—*To molecularly characterize 58K, a peripherally associated Golgi protein of unknown function (22), we used monoclonal anti-58K antibodies to screen a rat liver cDNA expression library. Positive clones were identified, characterized, and partially sequenced. Based on the high level of identity between our novel sequences and the sequence of porcine FTCD (35), we conclude that 58K represents rat FTCD. Obtained sequences were also highly homologous to partial sequences of uncharacterized mouse open reading frames that might represent mouse FTCD. FTCD is part of an enzymatic cascade involved in the conversion of histidine to glutamic acid (23). The functional formiminotransferase activity unit of FTCD is a dimer with binding sites for glutamate and folate.
A sequence GPSAFVPSWG (amino acids 163–172 in porcine FTCD) may play a role in tetrahydrofolate recognition (35, 36), and complete conservation of pig, rat, and mouse sequences was seen in this region (Fig. 2A).

In addition to amino acid sequence homology, rat FTCD is structurally similar to porcine FTCD and exists in dimeric, tetrameric, and octameric complexes that are resistant to proteolysis. Surprisingly, we found that such complexes can be cross-linked to an additional protein/proteins (Fig. 2C). Whether such component(s) might represent a linker involved in FTCD localization and/or cycling remains to be determined.

FTCD lacks a typical sequence corresponding to a microtubule binding domain but has been shown to bind to polymerized microtubules (22). This apparent inconsistency can be explained based on the fact that FTCD contains binding sites for polyglutamate (37) and is also likely to bind to glutamate residues. Consequently, its ability to associate with microtubules may be due to the high content of tandem glutamic acids present in β-tubulin (43, 44). Whether FTCD interacts with tubulin in vivo in a physiologically significant manner and what the consequences of such interactions may be are currently unknown.

**FTCD Association with the Golgi**—One of the interesting questions posed by this study is why FTCD, a metabolic enzyme involved in amino acid metabolism is associated with the Golgi, since both the substrates and the products of FTCD are freely soluble. An attractive hypothesis would be that upstream and downstream enzymes involved in generating histidine, its conversion to glutamic acid, and glutamic acid utilization are also associated with Golgi membranes and that such localization might bring together an assembly line of distinct activities to perform sequential modifications of generated products/substrates. This would parallel the arrangement of enzymes within the lumen of the Golgi, with distinct sugar transferases acting in a sequential manner on substrates as they pass through the cisterna. There are currently no data to support such a hypothesis. It is also possible that FTCD is tethered to the Golgi to allow specialized regulation of its enzymatic activity, perhaps in response to changes in physiological status of the cell, or that FTCD performs additional nonenzymatic functions in the Golgi.

FTCD association with the Golgi is not perturbed during
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microtubule disruption, indicating that the protein binds to bona fide Golgi sites. Similar behavior is exhibited by other microtubule/organellar binding molecules such as CLIP170 (45) and kinesin (46). Parameters of FTCD association with the Golgi were compared with the behavior of various Golgi and VTC marker proteins. The results show that a relatively small fraction of FTCD is recovered with Golgi membranes, the major- ity of the protein being present in a cytosolic fraction (Fig. 1A). This distribution may reflect the true cellular localization of the enzyme or may result from the release of FTCD from Golgi membranes during cell disruption. This is a relevant concern in light of our results showing that the peripherally associated p115 is recovered predominantly in the cytosol following cell disruption but is not present in a cytosolic pool in intact cells (47). Based on obtained IF images showing predomin- ant Golgi signal without diffuse cytoplasmic staining, we tend to favor the hypothesis that in vivo FTCD is associated with Golgi membranes, but this association is not maintained during cell disruption.

The extraction properties of FTCD recovered with Golgi membranes are not similar to those of another peripherally associated Golgi protein, p115 (Fig. 1C). p115 is readily ex- tracted with salt and saponin, while FTCD behavior is more similar to that of the tightly membrane-associated Golgi protein GM130. GM130 is recovered in a detergent- and salt-resistant Golgi matrix (28), and it is possible that FTCD might also be a component of such a structure. However, unlike FTCD, GM130 is not recovered in a cytosolic fraction in disrupted cells but remains associated with Golgi elements. GM130 binding to the Golgi is mediated by the N-myristoylated Golgi protein GRASP65 (48), while the Golgi partner for FTCD has not yet been identified. The differential release of FTCD from Golgi membranes may reflect inherent regulation of FTCD binding to its cognate receptor.

FTCD does not contain an obvious lipid binding domain, and its association with the Golgi is likely to be mediated by a protein. Whether it binds directly to a transmembrane Golgi protein or is linked through a peripheral bridging molecule is unknown. However, FTCD association with the Golgi is control- led by a mechanisms not directly regulated by the ADP- ribosylation factor, since it does not dissociate from the mem- branes in response to BFA in the manner shown previously for β-COP (49) and p200 (50).

FTCD Dynamics—Unlike the actively cycling proteins p58 (51) and p115 (24), FTCD cycling between the Golgi and earlier compartments of the secretory pathway seems modest. A small amount of FTCD is already dispersed into small punctate structures 50 s after the BFA addition, at a time when mannosidase II relocation is much faster than mannosidase II and p28 (21, 52). Dispersion of FTCD does not appear to be through formation of tubules, nor is FTCD observed in tubules originating from the Golgi and containing mannosidase II (Fig. 5, D and H). Instead, FTCD appears to redistribute by being incorporated into small punctate structures (perhaps vesicles) arranged in linear fashion like pearls on a string. It is possible that D-KDEL-R utilizes a pathway identical or similar to that used by FTCD, since it is also not found in mannosidase II-containing tubules in BFA-treated cells (21).

In conclusion, FTCD appears to be a novel Golgi protein that may be used as a marker to probe the dynamics of ER-Golgi pathways. Our results showing that FTCD relocation is tem- poral and spatially separate from mannosidase II relocation may define as yet uncharacterized parameters of transport between these two compartments. The finding that FTCD is a dynamic component of the Golgi is intriguing, since other pro- teins exhibiting cycling behavior have been implicated directly or indirectly in membrane transport functions. Whether FTCD is involved in membrane traffic or whether its cycling is not physiologically relevant and reflects cycling of its receptor re- mains to be investigated.

Acknowledgments—We are most grateful to Dr. George Bloom for providing the hybridoma and for sharing unpublished infor- mation. We wish to thank Dr. Robert MacKenzie for antibodies to porcine FTCD and recombinant porcine FTCD. We thank Dr. James Collawn for critical evaluation of the manuscript.

Addendum—While this work was under review, Bashour and Bloom (53) and Hennig et al. (54) reported the identification and characterization of the 58-kDa protein from rat and a p60 protein from chicken liver as FTCD.

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Ya-sheng Gao, Cecilia Alvarez, David S. Nelson and Elizabeth Sztul

J. Biol. Chem. 1998, 273:33825-33834.
doi: 10.1074/jbc.273.50.33825

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