The meprin α subunit, a multidomain metalloproteinase, is synthesized as a type I membrane protein and proteolytically cleaved during biosynthesis in the endoplasmic reticulum (ER), consequently losing its membrane attachment and COOH-terminal domains. The meprin α subunit is secreted as a disulfide-linked dimer that forms higher oligomers. By contrast, the evolutionarily related meprin β subunit retains the COOH-terminal domains during biosynthesis and travels to the plasma membrane as a disulfide-linked integral membrane dimer. Deletion of a unique 56-amino acid inserted domain (the I domain) of meprin α prevents COOH-terminal proteolytic processing and results in the retention of this subunit within the ER. To determine elements responsible for this retention versus transport to the cell surface, mutagenesis experiments were performed. Replacement of the meprin α transmembrane (αT) and cytoplasmic (αC) domains with their β counterparts allowed rapid movement of the α subunit to the cell surface. The meprin αT and αC domains substituted into meprin β delayed movement of this chimera through the secretory pathway. Replacement of glycines in the meprin αT domain GXXG motif with leucine residues, alanine insertions in the meprin αT domain, and mutagenesis of basic residues within the meprin αC domain did not enhance the movement of the α subunit through the secretory pathway. By contrast, a mutant of meprin α (C520AαT) that did not form disulfide-linked dimers or higher order oligomers was transported through the secretory pathway, although more slowly than meprin β. Taken together, the data indicate that the meprin αT and αC domains together contain a weak signal for retention within the ER/cis-Golgi compartments that is strengthened by oligomerization.

Meprins are metalloproteinases of the “astacin family” and “metzincin superfamily” that are oligomeric complexes of α and/or β subunits (1, 2). The multidomain subunits are ~42% identical in amino acid sequence, and both are highly glycosylated and have molecular masses of ~85 kDa (3). Homo- or hetero-oligomeric complexes containing meprin α subunits are referred to as meprin A (EC 3.4.24.19); homo-oligomeric complexes of meprin β subunits are referred to as meprin B (EC 3.4.24.63) (4). Mouse, rat, and human meprin A homo-oligomers consist of disulfide-linked meprin α dimers that self-associate to form high molecular mass multimers of ~1–6 MDa (3, 5, 6). Whereas the high molecular mass forms of meprin A are secreted proteins, meprin B is a disulfide-linked, dimeric type I plasma membrane-bound protein (3, 4). The hetero-oligomeric meprin A complex remains membrane-bound by virtue of the meprin β transmembrane anchor and is a tetramer (3, 4, 7). Meprin B and hetero-oligomeric meprin A are found on the brush-border membranes of the kidney proximal tubules and intestines of mice, rats, and humans, whereas homo-oligomeric meprin A is secreted and found in the urine and intestinal lumina (8, 9). Meprins are also expressed in certain cancer cells and in the leukocytes of the intestinal lamina propria, implying roles in the growth and metastasis of cancer cells and in inflammatory processes (9–11).

The deduced amino acid sequences of meprin α and β subunits (Structure 1) contain a signal sequence (S) at the NH2 terminus, followed by a prosequence (Pro), a catalytic domain (Protease), and the interaction domains MAM (meprin, A-5 protein, receptor protein-tyrosine phosphatase ζ) and TRAF (tumor necrosis factor receptor-associated factor; previously referred to as the MATH (meprin and TRAF homology) and AM (after MATH) domains), which are important for the folding, secretion, activity, and oligomerization of the meprin proteins (12, 13). Both meprin α and β subunits contain epidermal growth factor-like (E),1 transmembrane (T), and COOH-termi- nal cytoplasmic (C) domains. Meprin α contains a unique 56-amino acid sequence (the I domain) inserted between the TRAF and E domains. This subunit is proteolytically cleaved within or near the I domain during subunit maturation in the endoplasmic reticulum (ER) (7, 14). This allows the meprin α subunit to be released from its membrane anchor and to be secreted from cells. Previous studies have demonstrated that the I domain is sufficient and necessary to direct the proteolytic release and secretion of the mature meprin α ectodomains (14). Insertion of the I domain into the meprin β subunit (βαIββ) leads to proteolysis and secretion of this subunit. Deletion of the I domain from meprin α (αIα1) causes the retention of the mutant protein in the secretory pathway prior to the acquisition of complex glycosylation that occurs in the medial-Golgi network (14, 15).

Studies of chimeric meprin constructs of mouse α/β and human α/β subunits indicated that the COOH-terminal region

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The abbreviations used are: E, epidermal growth factor-like; T, transmembrane; C, cytoplasmic; ER, endoplasmic reticulum; Endo H, endoglycosidase H; PNGase F, peptide N-glycosidase F; PBS, phosphate-buffered saline.
(encompassing the E, T, and C domains) of meprin α is important for retention within the ER (14, 16). However, no systematic study demonstrating which of the COOH-terminal domains or elements therein is essential for the localization and trafficking of the subunits has been conducted. Thus, the studies herein were designed to determine whether any one domain or element in the meprin α or β COOH terminus is responsible for retention within the ER cis–Golgi compartments or movement of the subunits to the cell surface.

The T domain of the meprin α (but not β) subunit contains a repeating glycine motif (GXXGG) that is present in the transmembrane region of proteins such as glycophorin A, β2-adrenergic receptor, N-syndecan, and major histocompatibility complex class II α and β subunits and is essential for dimerization of these proteins and multimerization of the VacA toxin membrane channel of Helicobacter pylori (17–22). The glycines are positioned along one face of the transmembrane α helix, allowing for tight packing with a second transmembrane helix, thereby stabilizing the helix-helix interactions (see, for example, Fig. 4B) (17). Previous studies with glycophorin A and the VacA toxin show that mutation of the glycine residues disrupts dimerization and multimerization (21, 22). In addition, alanine scanning insertion mutagenesis disrupts the alignment of the glycine residues within the transmembrane α helix and thereby disrupts glycophorin A dimerization (23). It seemed possible that the transmembrane structure of meprin α is important for protein-protein interactions (multimerization or hetero-oligomerization) that could lead to retention of the subunit in the ER cis–Golgi compartments and that mutation of the meprin α T domain by site-directed or alanine insertion mutagenesis could disrupt this retention. Therefore, this type of study was conducted with meprin α.

There are two well characterized ER retention motifs found in the cytoplasmic region of ER-retained type I transmembrane proteins, a dilysine motif (KKXX(COOH)) and an RXR motif (24, 25). Basic residues in meprin α C domain (KKRX(COOH)) share similarities with both motifs. For this reason, the basic residues of the meprin α COOH-terminal tail were modified, and the effects on retention or movement of meprin α out of the ER were determined.

**EXPERIMENTAL PROCEDURES**

Reagents and Materials—[35S]Methionine/cysteine was purchased from PerkinElmer Life Sciences. Pansorbin was from Calbiochem. The mammalian expression plasmid pcDNA3.1−, Dulbecco’s modified Eagle’s medium, Opti-MEM, methionine-free Dulbecco’s modified Eagle’s medium, and Dulbecco’s modified Eagle’s medium/Ham’s F-12 medium were from Invitrogen. The QuikChange site-directed mutagenesis kit was from Stratagene. Protein A-Sepharose was from Sigma. Endoglycosidase H (Endo H) and peptide N-glycosidase F (PNGase F) were from New England Biolabs Inc. Complete mini EDTA-free protease inhibitor tablets were from Roche Applied Science. n-Octyl glucoside was from Fisher.

**Plasmid Construction and Mutagenesis—**All cDNAs were cloned into the expression vector pcDNA3.1+. The full-length wild-type mouse meprin α subunit, the αΔI mutant, and the αΔI/βETC mutant were described previously (14). Wild-type meprin α and αΔI were subcloned into pcDNA3.1+ by restriction digestion with HindIII and XbaI. The COOH-terminally His6-tagged wild-type meprin α I mutant was constructed by PCR amplification with an internal 5′-primer and a 3′-primer containing a Gly-Gly-Gly-Ser spacer, a His6 tag sequence, and an Xhol restriction site. This PCR fragment was restriction-digested with BspEI and Xhol and used to replace the corresponding region of the αΔI mutant. The COOH-terminally His6-tagged full-length wild-

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**Appendix**

**Experimental Procedures**

**Reagents and Materials—**[35S]Methionine/cysteine was purchased from PerkinElmer Life Sciences. Pansorbin was from Calbiochem. The mammalian expression plasmid pcDNA3.1+, Dulbecco’s modified Eagle’s medium, Opti-MEM, methionine-free Dulbecco’s modified Eagle’s medium, and Dulbecco’s modified Eagle’s medium/Ham’s F-12 medium were from Invitrogen. The QuikChange site-directed mutagenesis kit was from Stratagene. Protein A-Sepharose was from Sigma. Endoglycosidase H (Endo H) and peptide N-glycosidase F (PNGase F) were from New England Biolabs Inc. Complete mini EDTA-free protease inhibitor tablets were from Roche Applied Science. n-Octyl glucoside was from Fisher.

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type meprin β subunit was constructed by Dr. Greg P. Bertenshaw (Pennsylvania State University). Briefly, the entire wild-type meprin β cDNA was PCR-amplified with a 5′-primer containing a KpnI restriction site and a 3′-primer containing a Gly-Gly-Gly-Ser spacer, a His tag sequence, and an XhoI restriction site. This PCR fragment was digested with KpnI and XhoI and cloned into pcDNA3.1 + with the corresponding domains of meprin β with those of meprin α and to replace the C domain of meprin β with that of meprin α, respectively. These mutants were subcloned into pcDNA3.1 + by restriction digestion with HindIII and XhoI. The C320A mutant was constructed by replacing the NH2-terminal fragment containing Cys320 of meprin α with Asp320 of meprin β.

The α/βTC mutant was constructed by fusion PCR in which the wild-type βT and βC domains replaced the corresponding fragments of meprin α. Fusion PCR was also used to construct the βTC/βC mutant, in which the T domain of meprin β was replaced with the corresponding region of meprin α, and the βTC/βC mutant, in which the E T, and C domains of meprin β were replaced with the corresponding domains of meprin α. The α/βTC mutant was constructed by restriction digesting the βTC/βC mutant with PmlI and ApaI and cloning the αTC fragment into αC digesting with the same enzymes. The αLAXAXCOOH mutant was created by site-directed mutagenesis using the QuickChange site-directed mutagenesis kit and mutagenic primers designed to mutate KLRLQCOOH in the meprin αC domain to ALAQQCOOH.

Cell Culture and Transient Transfection—Human embryonic kidney 293 cells (CRL-1573, American Type Culture Collection) were cultured as described previously (12). Cells were transiently transfected with 10 μg of DNA/10-cm2 dish using the HEPES-buffered saline calcium phosphate-mediated transfection method (27). Briefly, 500 μl of 2× HEPES-buffered saline (50 mM HEPES, 1.5 mM Na2HPO4, and 280 mM NaCl, pH 7.05) was added dropwise to 10 μg of DNA in 500 μl of 125 mM CaCl2. After DNA-containing precipitates were allowed to form for 30 min at 25 °C, the reaction mixtures were then added to the 293 cells and incubated overnight at 37 °C. If meprin expression in the culture medium was to be assayed, the culture medium was changed 16 h post-transfection to serum-free Dulbecco’s modified Eagle’s medium/Ham’s F-12 medium or serum-free Opti-MEM. Transfection efficiencies were ~10% for mutant and wild-type proteins. Transfected proteins had similar levels of expression as determined by Western blot analysis of media using similar volumes, antibody dilutions, and film exposure times. The results shown in Fig. 1, 2, and 4–6 are representative of at least three separate experiments.

Preparation of Media and Membrane Fractions and Azocaseinase Activity Assay—Tissue culture media and membrane fractions were prepared 48 h post-transfection and analyzed for the expression of meprins. Media samples were collected; the serine protease inhibitor phenylmethylsulfonyl fluoride (1 mM) was added; and samples were incubated overnight at 37 °C. If meprin expression in the culture medium was to be assayed, the culture medium was changed 16 h post-transfection to serum-free Dulbecco’s modified Eagle’s medium/Ham’s F-12 medium or serum-free Opti-MEM. Transfection efficiencies were ~10% for mutant and wild-type proteins. Transfected proteins had similar levels of expression as determined by Western blot analysis of media using similar volumes, antibody dilutions, and film exposure times. The results shown in Fig. 1, 2, and 4–6 are representative of at least three separate experiments.

Preparation of Media and Membrane Fractions and Azocaseinase Activity Assay—Tissue culture media and membrane fractions were prepared 48 h post-transfection and analyzed for the expression of meprins. Media samples were collected; the serine protease inhibitor phenylmethylsulfonyl fluoride (1 mM) was added; and samples were subjected to centrifugation at 16,000 × g for 10 min. Supernatant fractions were concentrated to 500 μl with Centrisert 60 concentrators (Millipore Corp.). Cells were washed twice with phosphate-buffered saline (PBS), removed from the plate by scraping using a plate scraper in 2 ml of PBS, and centrifuged at 200 × g for 5 min. Cell sediment was suspended in 500 μl of PBS containing 1× Complete mini EDTA-free protease inhibitor tablet mixture and sonicated for 30 s to disrupt the cells. Total membrane fractions were isolated by centrifugation at 100,000 × g for 30 min. Membranes were washed twice with PBS and solubilized in 200 μl of 1% n-octyl glucoside in 1× PBS and 1× Complete protease inhibitor tablet mixture. Azocaseinase activity was measured as described previously (28).
lized total membrane fractions were subjected to electrophoresis in the presence of β-mercaptoethanol on 7.5% Ready gels (Bio-Rad) in SDS gel electrophoresis buffer and transferred to nitrocellulose membranes. The polyclonal antibody used to detect meprin α subunits was HMC14 (14). Polyclonal antibodies against meprin β were produced in rabbits using purified recombinant rat meprin β protein (PSU56). Horseradish peroxidase-conjugated anti-rabbit secondary antibodies (Amersham Biosciences) were detected by chemiluminescence using the SuperSignal Dura substrate (Pierce).

**Endoglycosidase Treatment**—Proteins were prepared for deglycosylation using Endo H and PNGase F following the manufacturer’s instructions. Briefly, protein samples were denatured by boiling in 1× Complete protease inhibitor tablet mixture were added, and proteins were incubated for 2 h at 37 °C. Deglycosylated proteins were analyzed by SDS-PAGE and immunoblotting.

**Pulse-Chase Experiments**—Pulse-laboradialbeling was performed as described previously (12). Briefly, cells were radiolabeled for 30 min with [35S]methionine/cysteine (100 μCi/ml/dish). The cells were washed once with PBS and lysed. Cell lysates were incubated with Pansorbin for 1 h at 4 °C and centrifuged at 6500 × g for 30 min. Supernatant fractions were incubated with anti-mouse meprin β antibodies (PSU56) for 10 min at 37 °C and then overnight at 4 °C. Immune complexes were immunoprecipitated with 40 μl of a 50% suspension of protein A-Sepharose for 3 h at 4 °C. Beads were washed, boiled in 1× denaturation buffer for deglycosylation for 5 min, and incubated for 2 h at 37 °C in the presence or absence of Endo H. Following deglycosylation, samples were subjected to SDS-PAGE. Gels were dried, and the immunoprecipitated proteins were visualized by fluorography.

**Size Exclusion Chromatography of Membrane-bound Meprins**—Total membrane fractions were prepared by a modification of the method of Booth and Kenny (29). Briefly, stably transfected 293 cell lines grown to confluency on 10-cm² dishes were scraped and washed once with 10 ml of 1× PBS, pH 7.5. Cells were sedimented at 200 × g for 5 min at 4 °C. PBS was removed by aspiration, and the sediment was resuspended in 1 ml of 2 mM Tris and 10 mM mannitol, pH 7.0, and sonicated in an ice bath for 1 min. CaCl₂ was added to a final concentration of 10 mM to precipitate the membranes. Membranes were sedimented at 16,000 × g for 30 min at 4 °C; the supernatant fraction was removed; and precipitates were solubilized overnight in 25 mM HEPES, 150 mM NaCl, and 1% n-octyl glucoside, pH 7.5, on a rotating platform at 4 °C. Unsolubilized membrane components were sedimented at 16,000 × g for 30 min at 4 °C, and the supernatant fraction was subjected to chromatography on a Superose 6 size exclusion column (Amersham Biosciences) equilibrated in 25 mM HEPES, 150 mM NaCl, and 1% n-octyl glucoside, pH 7.5. Fractions (1 ml) were collected, and the presence of meprin protein was detected by Western blot analysis using HMC14 polyclonal antibodies. Western blots were analyzed by densitometry.

**RESULTS**

The Transmembrane and Cytoplasmic Domains of the Meprin α and β Subunits Are Important for Transport of the Proteins through the Secretory Pathway—Chimeras of meprins...
of perturbations caused by an alanine insertion is shown in the right panel. B, deglycosylation analysis of alanine insertion mutants. Wild-type meprin α (WTα), the ΔI mutant, and the alanine insertion ΔI mutants were analyzed for susceptibility to endoglycosidase treatment. For wild-type meprin α, the medium was collected; for the meprin ΔI mutants, membrane fractions were prepared. Media and membranes were subjected to enzymatic deglycosylation for 2 h at 37 °C in the absence (−) or presence of Endo H (H) or PNGase F (F) as indicated. After deglycosylation treatment, the mutants were subjected to SDS-PAGE and Western blot analysis using anti-meprin α antibodies. The relative positions of the molecular mass markers (in kilodaltons) are shown.

\[ \text{Fig. 5. Alanine scanning insertion mutagenesis of the meprin } \alpha T \text{ domain. A, two-dimensional representation of the mouse meprin } \alpha T \text{ domain and an alanine insertion mutant. The insertion sites of alanine residues within the meprin } \alpha T \text{ domain are indicated by arrows. An example of perturbations caused by an alanine insertion is shown in the right panel. B, deglycosylation analysis of alanine insertion mutants. Wild-type meprin } \alpha \text{ (WTα), the } \Delta I \text{ mutant, and the alanine insertion } \Delta I \text{ mutants were analyzed for susceptibility to endoglycosidase treatment. For wild-type meprin } \alpha \text{, the medium was collected; for the meprin } \Delta I \text{ mutants, membrane fractions were prepared. Media and membranes were subjected to enzymatic deglycosylation for } 2 \text{ h at } 37 \, ^\circ \text{C in the absence (−) or presence of Endo H (H) or PNGase F (F) as indicated. After deglycosylation treatment, the mutants were subjected to SDS-PAGE and Western blot analysis using anti-meprin } \alpha \text{ antibodies. The relative positions of the molecular mass markers (in kilodaltons) are shown.} \]
8 h, indicative of impeded transport, and correlated with their steady-state Endo H profiles (Fig. 2B).

**Mutation of the Glycine Residues in the Meprin αT Domain—**To determine whether the glycine repeat motif within the meprin αT domain is responsible for ER/cis-Golgi retention of the αI mutant, one or more glycine residues were systematically mutated to leucine (Fig. 4A). Fig. 4A shows that the meprin α mutant glycine repeat motif is not present in meprin β and the similarity to the glycoporphin A glycine repeat. Wild-type and mutant mouse meprin α cDNAs were transiently transfected into 293 cells, and the meprin proteins associated with the cells or media were detected by immunoblotting (Fig. 4C). The SDS-PAGE mobility of the subunits after treatment with Endo H and PNGase F was again used to determine whether the subunits contained high-mannose oligosaccharides or were complex glycosylated as an indication of retention prior to or movement beyond the cis-Golgi apparatus. The deglycosylation profiles of the single and multiple Gly-to-Leu mutants of the meprin αI transcripts were very similar to those of the meprin αI mutant, indicating that the repeating glycine motif is not responsible for retention of the subunit in the ER/cis-Golgi compartments.

**Alanine Scanning Insertion Mutagenesis of the Transmembrane Domain Has No Effect on Retention of the αI Subunit in the ER/cis-Golgi Compartments—**Insertion of an alanine residue within the transmembrane domain will cause a 90° shift of the residues NH2-terminal to the insertion and consequently disrupt helical alignments (Fig. 5A) (21). Alanine residues were inserted at various positions in the meprin αT domain within the glycine repeat region and COOH-terminal to it (Fig. 5A). The meprin αI alanine insertion mutants all showed similar deglycosylation patterns compared with the αI mutant, indicating that they did not pass through the medial-Golgi compartment. These results are consistent with those obtained with the glycine mutants, indicating that the structure of the T domain alone is not a major determinant of ER/cis-Golgi retention.

**Motifs in the Meprin αC Domain Resembling Known Cytoplasmic ER Retention Motifs Are Not Involved in the Inability of the Meprin α Subunit to Move through the medial-Golgi Compartment—**The meprin αC domain contains only six amino acids (Fig. 6A), in contrast to meprin β, which has 26 amino acids. There are two basic residues in the meprin αC domain (XXXRQCOOH), and although they do not exactly conform to the known ER retention motifs (e.g. KDEL or a dilysine motif at positions −3 and −4 or positions −3 and −5 prior to the COOH terminus) (30), the basic residues were replaced with Ala to test whether the basic residues in the meprin αC domain affect ER retention. A mutant that extended the COOH terminus to 16 amino acids (αI-His6) was also constructed (Fig. 6A). Both mutants had a similar Endo H profile compared with the αI mutant (Fig. 6B), indicating that the basic residues present in the meprin αC domain and the length of the tail do not affect subunit retention.

**The Oligomeric State Affects the Intracellular Retention of the Meprin αI Mutant—**To determine the effects of the oligomeric state of meprin α on ER/cis-Golgi retention of the αI mutant, a mutant lacking the ability to form disulfide-linked dimers was constructed. Previous studies have demonstrated that Cys220 is responsible for the formation of intersubunit disulfide-bonded dimers in wild-type meprin α; mutation of this residue (C320A) abrogates the ability to form higher order oligomeric species (24). Therefore, Cys220 was mutated to Ala in

![Fig. 6. Endo H sensitivity of the meprin αC domain mutants. A, diagrammatic representation of the meprin αC domain and its mutants. Addition of a Gly-Gly-Gly-Ser spacer and a His6 tag to the αI mutant (αI-H−His6) is indicated. TM, transmembrane domain. B, deglycosylation of the meprin αI C domain mutants. The meprin αI C domain mutants were analyzed for susceptibility to endoglycosidase treatment. Solubilized membranes were subjected to enzymatic deglycosylation for 2 h at 37 °C in the absence (−) or presence of Endo H (H) or PNGase F (F) as indicated. After deglycosylation treatment, the mutants were subjected to SDS-PAGE and Western blot analysis using anti-meprin antibodies. The relative positions of the molecular mass markers (in kilodaltons) are shown.

A

- Meprin αI
- αI-H−His6
- αI-XAXAXCOOH

B

- H F - H F - H F

- αI
- αI-H−His6
- αI-XAXAXCOOH
the $\alpha$1 mutant (C320A$\alpha$1), and the oligomeric size and glycosylation state of this mutant were determined.

The $\alpha$1 and C320A$\alpha$1 mutants were solubilized from transiently transfected 293 cells. Membrane fractions were analyzed by size exclusion chromatography on a Superose 6 column. The elution profiles were determined by densitometry of Western-blotted Superose 6 fractions and with the elution profiles of standard proteins (Fig. 7A). The $\alpha$1 mutant eluted with a peak in fraction 12, corresponding to a molecular mass of $\sim$620 kDa, whereas the C320A$\alpha$1 mutant eluted with a peak in fraction 15 corresponding to a molecular mass of $\sim$270 kDa (Fig. 7A). It is likely that the 270-kDa protein is a dimeric form of the C320A$\alpha$1 protein because no evidence could be found for another protein interacting with the C320A$\alpha$1 mutant after immunoprecipitation, two-dimensional gel electrophoresis, NH$_4$-terminal sequencing, and mass spectrophotometry analyses (data not shown).

To determine the effect of the different oligomeric states of the $\alpha$1 and C320A$\alpha$1 mutants on movement through the secretory pathway, the deglycosylation patterns of these mutants were compared. Approximately half of the C320A$\alpha$1 mutant contained Endo H-resistant oligosaccharides, indicating movement out of the ER/cis-Golgi compartments, whereas the $\alpha$1 mutant was primarily Endo H-sensitive, indicating residence within a pre-medial-Golgi compartment (Fig. 7B). Wild-type meprin $\alpha$ protein secreted into the culture medium was completely Endo H-resistant, as expected of a protein that has traversed the secretory pathway. From this result, we concluded that the oligomeric state of the meprin $\alpha$ subunit is a determinant in the ability of this protein to move into the medial-Golgi compartment. In addition, the $\alpha$1 mutant, like the mature wild-type protein, was resistant to extensive degradation by trypsin, indicating that it is properly folded (data not shown). The activated $\alpha$1 mutant was able to hydrolyze the protein substrate azocasein and had a similar specific activity for this substrate ($1083 \pm 164$ units/mg) compared with wild-type meprin $\alpha$ ($977 \pm 268$ units/mg). Thus, although the $\alpha$1 mutant is retained in the ER/cis-Golgi compartments, it has similar enzymatic and oligomerization properties compared with the secreted wild-type protein.

**DISCUSSION**

This work establishes that the meprin $\alpha$T domain in combination with the meprin $\alpha$C domain is responsible for the retention of the subunit in the ER/cis-Golgi compartments and that the E domain does not contribute to the retention process. In addition, the meprin $\beta$T and $\beta$C domains together promote the movement of meprin proteins through the secretory pathway (Fig. 8). No one motif or domain in the meprin $\alpha$ COOH terminus was found to be responsible for the retention; however, the formation of disulfide-linked dimers and higher oligomerization of meprin $\alpha$ subunits enhanced the retention of the complex. One interpretation of these results is that the meprin $\alpha$T and $\alpha$C domains together contain a weak retention signal and that oligomerization serves to cluster multiple signals and to increase the strength for retention. The retention signal is not likely to involve the GXXGX motif of the T domain or the basic residues of the six-member cytoplasmic tail, however, because mutations of these residues did not enhance movement out of the ER/cis-Golgi compartments. But higher oligomerization of subunits containing these motifs may increase interactions with resident ER proteins or disrupt interactions with proteins that are instrumental in moving the proteins out of the ER. This would explain why the dimeric C320A$\alpha$1 mutant and the $\beta\alpha$TC chimera are only partially retained within the early secretory pathway compartments.

This work also demonstrates that higher order oligomeric complexes of meprin $\alpha$ form in the ER. There are several other known protein complexes that assemble in the ER; for example, measles virus envelope glycoproteins, the T cell receptor, and IgE all form oligomers in the ER (20, 31, 32). In these examples, however, proper oligomerization in the ER leads to exit from the ER rather than retention; multimerization of these proteins apparently masks ER retention motifs (32). By contrast, multimerization of meprin $\alpha$ leads to unmasking or strengthening retention signals and retaining the protein unless the luminal protein domains are proteolytically cleaved from the membrane. Thus, the purpose of meprin $\alpha$ retention appears to be to allow the complex to form properly for the COOH-terminal hydrolysis rather than for membrane-associated transport through the secretory pathway. Previous studies have established that multimerization of meprin $\alpha$ is essential for stability and enzymatic activity against substrates; thus, this process has functional significance (26). It is also known from previous studies that meprins must contain the MAM domain for proper folding (12). Mutants lacking the MAM domain, either by truncation after the protease domain or by deletion of
FIG. 8. Diagrammatic representation of the intracellular trafficking of wild-type meprin α and β subunits and mutants. From top to bottom: wild-type meprin α is secreted into the culture medium; wild-type meprin β is cell surface-expressed; αΔI is retained in the ER/cis-Golgi compartments; αΔI/βTC is rapidly transported in the secretory pathway; and C320AΔI and β/αTC are slowly transported out of the ER/cis-Golgi compartments.
this domain from the rest of the protein, are degraded by the ubiquitin/proteasome pathway (12, 33). Thus, the quality control system of the ER monitors for proper folding of the meprin α protein and for proteolytic release of the protein from the membrane.

The meprin αTC and βTC domains have little in common, in contrast to the other domains of the subunits, for which amino acid identity ranges from 35 to 55% (34). This indicates that these COOH-terminal domains have evolved for different functions. The meprin αTC and αC domains appear to have evolved for retention of the subunit in the ER/cis-Golgi compartments for proper proteolytic processing and for formation of a large secreted protease. By contrast, the meprin βTC domains are endowed with features that maintain transmembrane association and that enable interactions with cytosolic proteins for transport and possibly for signal transduction. For example, the 26-amino acid meprin βC domain (YCTRKYRRKRAN-

Movement of Meprin Subunits to the Plasma Membrane

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