Proteasomal Degradation of Unassembled Mutant
Type I Collagen Pro-α1(I) Chains*

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We have previously shown that type I procollagen pro-α1(I) chains from an osteogenesis imperfecta patient (OI26) with a frameshift mutation resulting in a truncated C-propeptide, have impaired assembly, and are degraded by an endoplasmic reticulum-associated pathway (Lamandé, S. R., Chessler, S. D., Golub, S. B., Byers, P. H., Chan, D., Cole, W. G., Silence, D. O. and Bateman, J. F. (1995) J. Biol. Chem. 270, 8642–8649). To further explore the degradation of procollagen chains with mutant C-propeptides, mouse Mov13 cells, which produce no endogenous pro-α1(I), were stably transfected with a pro-α1(I) expression construct containing a frameshift mutation that predicts the synthesis of a protein 85 residues longer than normal. Despite high levels of mutant mRNA in transfected Mov13 cells, only minute amounts of mutant pro-α1(I) could be detected indicating that the majority of the mutant pro-α1(I) chains synthesized are targeted for rapid intracellular degradation. Degradation was not prevented by brefeldin A, monensin, or NH₄Cl, agents that interfere with intracellular transport or lysosomal function. However, mutant pro-α1(I) chains in both transfected Mov13 cells and OI26 cells were protected from proteolysis by specific proteasome inhibitors. Together these data demonstrate for the first time that procollagen chains containing C-propeptide mutations that impair assembly are degraded by the cytoplasmic proteasome complex, and that the previously identified endoplasmic reticulum-associated degradation of mutant pro-α1(I) in OI26 is mediated by proteasomes.

The major fibrillar collagens (types I, II, and III) are the principal structural components of the extracellular matrix of many tissues, forming characteristic architecturally precise fibrils (1). They are synthesized as precursor molecules with a central triple-helical region containing a Gly-X-Y amino acid repeat motif, flanked by carboxyl- and amino-terminal propeptide globular domains (for review, see Ref. 2). Assembly of three individual pro-α-chains to form a triple helix occurs within the endoplasmic reticulum (ER),¹ and is initiated by interactions between the C-propeptides. Triple helix folding then occurs sequentially from the COOH to the NH₂ terminus, and is essential for efficient secretion of the procollagen molecules (3). Mutations in the pro-α1(I) and pro-α2(I) chains of type I collagen which compromise initial chain association or disturb the folding of the triple helix result in the brittle bone disease osteogenesis imperfecta (OI) (4–7) and one of the important biosynthetic consequences of these mutations is an increase in intracellular collagen degradation (7).

Intracellular degradation is an essential process for regulating the levels of many proteins and an important “quality control” mechanism which minimizes the accumulation within cells and the secretion of mutant or malfolded proteins. Several cellular compartments have been identified as sites for degradation, including the lysosomes which contain acid hydrolases, a post-Golgi non-lysosomal compartment, the ER, and the cytoplasm where the 26 S proteasome, a large catalytic protease complex, is responsible for the degradation (8, 9). While the molecular basis of intracellular collagen degradation has not been fully defined, three of these four cellular compartments have been implicated as the site of collagen proteolysis. In fibroblasts, approximately 15% of normal procollagen is degraded intracellularly by a process that has been termed basal degradation (10, 11), and degradation is significantly increased in cells synthesizing procollagens with structurally abnormal triple helical domains (7, 12, 13). Degradation of both normal and structurally abnormal procollagen molecules can be inhibited by NH₄Cl (12–14), and brefeldin A (15), a drug which causes the cis- and medial-Golgi to fuse with the ER and prevents further intracellular transport. These results suggest that degradation occurs in the distal region of the secretory pathway after the brefeldin A block, in regions that are susceptible to NH₄Cl inhibition such as the trans-Golgi and secretory vesicles or in the lysosomes. Recent studies have demonstrated directly the localization of procollagen I in the lysosome/endosome system (16) identifying this system as a site of collagen degradation, however, normal procollagen degradation in I-cell disease (Mucolipidosis II) fibroblasts, which are deficient in lysosomal hydrolases, suggests that trans-Golgi and secretory vesicles also represent sites of procollagen degradation (17).

Not all mutant collagen is degraded in the distal region of the secretory pathway. Procollagens with mutations in the pro-α1(I) C-propeptide which compromise chain association are degraded by a process which is not prevented by brefeldin A, and was therefore assumed to occur within the ER (4). It has recently been shown that a number of soluble and integral membrane proteins that have been translocated into the ER and were thought to be degraded there, are in fact, degraded by

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§ The abbreviations used are: ER, endoplasmic reticulum; C-propeptide, carboxyl-terminal propeptide; OI, osteogenesis imperfecta; PAGE, polyacrylamide gel electrophoresis; ZL₃al, sulfate carboxybenzyl-leucyl-leucinyl-leucinal; ZL₃VS, carboxybenzyl-leucyl-leucyl-leucine-vinylsulfone; bp, base pairs; BiP, immunoglobulin heavy chain-binding protein.

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the cytoplasmic proteasome complex (18–23). This process requires reverse transport of protein back to the cytoplasm, a process which may be mediated by interaction of the protein with the Sec61 complex, one of the major constituents of the translocation apparatus (22), and could also involve molecular chaperones (24). In this study we explore the role of proteasomes in the ER-associated degradation of assembly impaired mutant type I procollagen. We examined two pro-α(1)(I) C-propeptide OI mutations. The first mutation, an engineered frameshift mutation near the COOH-terminal end of the mouse pro-α(1)(I) chain, was analogous to a mutation defined in a patient with OI type I (25). Fibroblasts from that patient contained both mutant and normal pro-α(1)(I) mRNA, but mutant protein could not be detected in cells suggesting that it was rapidly and completely degraded prior to assembly (25). In addition, we examine fibroblasts from a patient (OI26) in which a heterozygous frameshift mutation impaired, but did not prevent subunit assembly (4). In both cases the use of specific proteasome inhibitors demonstrated a primary role for cytoplasmic proteasomes in the selective degradation of procollagen chains with mutations within the C-propeptide.

**EXPERIMENTAL PROCEDURES**

**Cell Culture**—Dermal fibroblast cultures from controls and a patient with the lethal perinatal form of OI (OI26) were established and maintained in culture as described previously (7). The molecular defect in OI26 is a heterozygous single base insertion in the last exon of COL1A1 (6). The codon reading frameshift generated by the mutation alters the amino acid sequence of the pro-α(I) C-propeptide and results in a chain which is 37 amino acids shorter than normal (Fig. 1B). Mouse Mov13 cells (26) were provided by Dr. R. Jaenisch (Whitehead Institute for Biomedical Research, Cambridge, MA). Mov13 cells synthesize no endogenous pro-α(I) chains since the transcription of both COL1A1 genes is blocked by a retroviral insertion in the first intron (26, 27).

**Production of Stably Transfected Mov13 Cell Lines Expressing an Elongated Pro-α(I) Chain**—The mutant mouse COL1A1 gene construct was a derivative of the previously described control expression construct pWTCl-IA (28). This control construct contains a functionally neutral Met<sup>22</sup> → Ile substitution<sup>2</sup> within the triple helix that allows the cell to be distinguished from wild-type α(I) by its altered CNBr cleavage pattern (29), and a silent Met<sup>1899</sup> → Ala substitution within the C-propeptide. A clone was isolated that contained a 2-bp deletion predicted a pro-α(I) chain extended by 84 amino acids (25). While mutant mRNA was present within the cell layer and medium fractions were treated separately (7, 32). The mutant mouse COL1A1 frameshif mutant construct, pWTCl-IAFs, in Mov13 Cells—Mov13 cells are a unique model system in which to study intracellular collagen degradation. Expression of mutant COL1A1 genes in these cells allows the fate of the resultant mutant pro-α(I) chains to be easily followed without the complications of endogenous pro-α(I) expression (28, 29). The mutant mouse COL1A1 frameshif mutant construct, pWTCl-IAFs (Fig. 1), contains a mutation which is similar to one characterized in a patient with type I OI, where a 2-bp deletion predicted a pro-α(I) chain extended by 84 amino acids (25). While mutant mRNA was present within the OI cells and could be isolated in the translation system, the protein was not detected in cell culture presumably that the aberrant protein was rapidly degraded intracellularly.

2 Amino acids are numbered from the start of the triple helix.

Briefly, after disruption of the cell layer by sonication, procollagens and collagens were precipitated from the cell and medium fractions with ammonium sulfate at 25% saturation. The precipitate was redissolved in 2 ml of 50 mM Tris-HCl, pH 7.5, containing 0.15 M NaCl and the protease inhibitors 5 mM EDTA, 10 mM N-ethylmaleimide, and 1 mM phenylmethylsulfonil fluoride. Aliquots of procollagens were precipitated with 75% ethanol and either analyzed directly or subjected to limited pigeon digestion (100 μg/ml pepsin in 0.5 M acetic acid, 4°C, 16 h) to determine if the collagens had formed pepsin-resistant triple helices.

For the pulse-chase analysis of procollagen degradation, skin fibroblasts or transfected Mov13 cells in 6-well plates were grown to confluence and treated with 0.25 mM sodium ascorbate overnight. The cells were preincubated in 1 ml of Dulbecco’s modified Eagle’s medium without l-methionine and l-cysteine (Life Technologies Inc.) for 1 h then pulse-labeled for 1 h with 300 μCi of L-[35S]methionine (Trans<sup>35</sup>S)-label, 1032 Ci/mmol, ICN Pharmaceuticals Inc.). Cells were treated with the proteasome inhibitors: carboxybenzyl-leucinyl-leucinyl-leucinal (ZL, ala) (Sigma), carboxybenzyl-leucinyl-leucinyl-vinylsulfone (ZL, VSY) (kindly provided by H. Ploegh, MIT, Boston, MA (33)), clasto-lactacytin β-lactone (Calbiochem), 1 μg/ml brefeldin A (Roche Molecular Biochemicals), 10 μg/ml monensin (Sigma) or 50 mM NH<sub>4</sub>Cl throughout the preincubation, pulse labeling and chase periods. Cells were washed once with ice-cold phosphate-buffered saline, scraped into 1 ml of 0.25 M sucrose, and centrifuged briefly in a clinical centrifuge. Cells were lysed in 0.5 ml of lysis buffer (150 mM NaCl, 50 mM Tris-HCl, pH 7.5, containing 5 mM EDTA, 20 mM N-ethylmaleimide, 1 mM 4-(2-aminoethyl)-benzenesulfonyl fluoride, 2 mM iodoacetamide, 1% (w/v) Nonidet P-40) on ice for 30 min. Lysed cells were centrifuged for 5 min at 10,000 × g to remove insoluble material and the supernatant precipitated with 100 μl of 20% Protein A-Sepharose (Amersham Pharmacia Biotech) at 4°C for 2 h. Type I procollagen prochains in the supernatant were immunoprecipitated with LF-67 (1/1000 dilution), a rabbit polyclonal antibody which recognizes the pro-α(I) carboxyterminal telopeptide of both human and mouse collagens (34) (kindly provided by Dr. Larry Fisher, National Institute of Dental Research, Bethesda, MD), and 100 μl of 20% Protein A-Sepharose at 4°C overnight. Immunoprecipitated complexes were washed three times each with 50% (v/v) lysis buffer, 50% (v/v) NET buffer (50 mM Tris-HCl, pH 7.5, containing 1 mM EDTA, 150 mM NaCl, and 0.1% Nonidet P-40), twice with NET buffer, then once with 10 mM Tris-HCl, pH 7.5, containing 0.1% Nonidet P-40. Immunoprecipitated chains were eluted into electrophoresis loading buffer at 65°C for 10 min.

**SDS-PAGE and Immunoblotting**—Type I procollagen and collagen chains were analyzed by SDS-PAGE on 5% (w/v) polyacrylamide gels. Where indicated, samples were reduced before electrophoresis by the addition of dithiothreitol to a final concentration of 10 mM. Procollagen chains were also analyzed by two-dimensional gel electrophoresis (35) which resolves the chains on the basis of both charge and size. Radioactively labeled proteins were detected by fluorography. For immunoblotting, procollagen chains resolved by SDS-PAGE were electro- phoretically transferred to nitrocellulose filters. Blots were incubated with LF-67 at a dilution of 1/10,000 and bound antibody detected using horseradish peroxidase-conjugated Protein A (Bio-Rad) and an enhanced chemiluminescence kit (ECL, Amersham Pharmacia Biotech).

**RESULTS AND DISCUSSION**

**Stable Expression of the COL1A1 Frameshift Mutant Construct, pWTCl-IAFs, in Mov13 Cells**—Mov13 cells are a unique model system in which to study intracellular collagen degradation. Expression of mutant COL1A1 genes in these cells allows the fate of the resultant mutant pro-α(I) chains to be easily followed without the complications of endogenous pro-α(I) expression (28, 29). The mutant mouse COL1A1 frameshif mutant construct, pWTCl-IAFs (Fig. 1), contains a mutation which is similar to one characterized in a patient with type I OI, where a 2-bp deletion predicted a pro-α(I) chain extended by 84 amino acids (25). While mutant mRNA was present within the OI cells and could be isolated in the translation system, the protein was not detected in cell culture presumably that the aberrant protein was rapidly degraded intracellularly.

The predicted human and mouse proteins show extensive sequence homology, including a highly positively charged COOH terminus, and might be expected to share similar metabolic fates. To examine the biochemical consequences of the mouse
frameshift mutation and address the question of procollagen subunit stability and the targeting of abnormal chains for intracellular degradation raised by the human type I OI mutation, Mov13 cells were transfected with the mutant construct, pWTCI-IAfs. Individual, stably transfected clones were selected in medium containing G418 then screened for expression of pro-
\( \alpha_1(I) \) mRNA by Northern blot analysis. In contrast to the untransfected Mov13 cells which produced no pro-
\( \alpha_1(I) \) mRNA (Fig. 2, lane 1), high levels of pro-
\( \alpha_1(I) \) mRNA of the correct size were apparent in two transfected cell lines, Mov13-IAfs4 and Mov13-IAfs10 (Fig. 2, lanes 5 and 6), and a third cell line, Mov13-IAfs2, contained low levels of pro-
\( \alpha_1(I) \) mRNA (Fig. 2, lane 4).

**Mutant Frameshift Pro-\( \alpha_1(I) \) Are Degraded Intracellularly in Transfected Mov13 Cells**—To examine the ability of the frameshift mutant pro-
\( \alpha_1(I) \) mRNA to be translated and the mutant pro-
\( \alpha_1(I) \) chains to assemble into functional collagen molecules, stably transfected cells were biosynthetically labeled with \( ^{3}H \) proline for 18 h and cell and medium fractions analyzed by SDS-PAGE after digestion with pepsin. Pepsin removes the NH\(_2\)- and COOH-terminal globular domains but leaves the triple helical domain intact. Thus the presence of pepsin-resistant collagen indicates that stable collagen trimeric assembly has occurred. In untransfected Mov13 cells no pepsin-resistant collagen was present (Fig. 3, lanes 1 and 2) and in cells transfected with the wild-type collagen gene, high levels of pepsin-resistant collagen was present (Fig. 3, lanes 3 and 4).
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**FIG. 2.** Expression of mutant pro-\( \alpha_1(I) \) mRNA in transfected Mov13 cells. Approximately 3 \( \mu \)g of total RNA was fractionated on a 0.8% agarose gel and transferred to nitrocellulose. The filter was hybridized simultaneously to \( ^{32}P \)UTP-labeled mouse \( \alpha_1(I) \) and rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNAs and exposed to x-ray film. The migration positions of the two major pro-\( \alpha_1(I) \) mRNAs and the glyceraldehyde-3-phosphate dehydrogenase mRNA are indicated. Lane 1, parental untransfected Mov13 cells (Mov); Lane 2, Mov13 cells transfected with a wild-type COL1A1 gene (WT); Lanes 3–6, Mov13 cells transfected with the frameshift mutant construct pWTC1-IAfs, Mov13-IAfs8, -IAfs2, -IAfs4, and -IAfs10.

**FIG. 3.** Expression of \( \alpha_1(I) \) chains in transfected Mov13 cells. SDS-polyacrylamide gel electrophoresis of \([^{3}H]\)proline-labeled pepsin-resistant collagen from the cell layer (C) and medium (M) fractions of Mov13 cell cultures. Lanes 1 and 2, untransfected Mov13 cells (Mov); lanes 3 and 4, Mov13 cells transfected with a wild-type COL1A1 gene (WT); lanes 5–12, Mov13 cells transfected with the frameshift construct, pWTC1-IAfs, Mov13-IAfs8, -IAfs2, -IAfs4, and -IAfs10. The protein loadings are equivalent in all lanes and the gels were exposed for the same time. The migration positions of type I collagen \( \alpha_1(I) \) and \( \alpha_2(I) \) chains and type V collagen \( \alpha_1(V) \) and \( \alpha_2(V) \) chains are indicated.

Proteasomes are essential for the degradation of misfolded proteins. They are composed of a core particle and a regulatory complex, and their activity is regulated by proteasome inhibitors such as bortezomib. The results from the experiments with Mov13 cells transfected with the frameshift construct pWTC1-IAfs confirm that the frameshift mutant \( \alpha_1(I) \) chains, when expressed in these cells, are targeted for degradation by the proteasome.

Despite the accumulation of small amounts of type I collagen in these cultures over the 18-h labeling period, intracellular precursor \( \alpha_1(I) \) chains could not be detected by proline radiolabeling and fluorography (data not shown). This failure to detect intracellular degradation of \( \alpha_1(I) \) chains is consistent with the results obtained in other cell lines transfected with frameshift mutants. The degradation of the mutant \( \alpha_1(I) \) chains was compared with that of control COL1A1 chains using the polyclonal antibody LF-67. Proteins were transferred to nitrocellulose and the filter probed with antibody LF-67. Mov13-IAfs10 cells synthesized a pro-\( \alpha_1(I) \) subunit that migrated more slowly than control pro-\( \alpha_1(I) \) (Fig. 4, lane 3). This was consistent with the prediction that the mutant protein would be 85 amino acids larger than normal and may be substituted with an additional \( N \)-linked oligosaccharide group. The mutant \( \alpha_1(I) \) subunits migrated as monomers when analyzed without reduction (Fig. 4, lane 5), indicating that interchain disulfide bonds had not formed and suggesting that trimer assembly is severely impaired.

**Proteasome Inhibitors Protect the Frameshift Mutant Pro-\( \alpha_1(I) \) Chains from Intracellular Degradation.** To determine the site of intracellular degradation, Mov13-IAfs10 cells were treated with several protease and vesicular traffic inhibitors and the relative levels of mutant protein compared. Cells were pretreated for 1 h with inhibitors, metabolically labeled with \([^{35}S]\)methionine for 2 h, chased for 30 min in the presence of inhibitor, and the intracellular pro-\( \alpha_1(I) \) chains immunoprecipitated with LF-67. In these untreated cells, mutant pro-\( \alpha_1(I) \) was not detected, indicating that complete degradation of the mutant protein produced during the 2-h pulse had occurred within the 30-min chase period (Fig. 5A, lane 1). The mutant subunit was not detected in cells treated with brefeldin A, which blocks protein transport into the Golgi and results in redistribution of most of the Golgi into the ER (36), monensin, a carboxylic ionophore which blocks intracellular traffic within the trans-Golgi, or NH\(_4\)Cl, which raises the pH of acidic compartments such as the trans-Golgi and lysosomes (Fig. 5A, lanes 2–4). Since these agents inhibit intracellular vesicular traffic, but inhibit lysosomal function, degradation of the mutant frameshift pro-\( \alpha_1(I) \) does not occur in the distal regions of the secretory pathway. This suggests that, as in lethal OI patients with C-propeptide mutations (4), the degradation pathway may be “ER-mediated.”

Recently, it has become apparent that a number of mutant proteins which have been targeted to the ER and were thought to be degraded within that compartment are, in fact, degraded in the cytoplasm by the ubiquitin-proteasome pathway (18–
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FIG. 5. Effect of protease and vesicular transport inhibitors on degradation of mutant [fs]pro-α1(I) collagen. A, Mov13-1Afs10 cells were labeled for 2 h with [35S]methionine then chased for 30 min and lysed. Pro-α1(I) chains were immunoprecipitated with LF-67 and then separated on SDS-polyacrylamide gels. Cells were labeled without treatment (−) (lane 1) or were treated for 1 h prior to and during pulse-chase with the following agents: 1 μg/ml brefeldin A (BFA) (lane 2), 10 μg/ml monensin (mon) (lane 3), 50 mM NH4Cl (lane 4), and 20 μM ZL3al (lane 5). B, identification of pro-α1(I) chains protected by the proteasome inhibitor ZL3al. Mov13-1Afs10 cells either without treatment (−) (lane 1) or treated with 20 μM ZL3al for 2 h were lysed and electrophoresed on an SDS-polyacrylamide gel. Pro-α1(I) chains were identified by immunoblotting with LF-67. The migration positions of [fs]pro-α1(I) chains, and a smaller species, [fs]pro-α1(I)*, are indicated.

To further examine the role of proteases in degradation of unassembled mutant procollagen, the effect of two other specific proteasome proteolytic activity inhibitors ZL3al, labeled with [35S]methionine for 1 h, chased for 30 min and the intracellular pro-α1(I) chains immunoprecipitated with a pro-α1(I)-specific antibody, LF-67 (Fig. 5, lane 5). Incubation with this proteasome inhibitor prevented degradation of the frameshift mutant pro-α1(I) chain. The identity of the protected protein was confirmed by immunoblotting (Fig. 5B, lane 2).

To determine if this pathway was also responsible for the degradation of the frameshift pro-α1(I) chain, Mov13-1Afs10 cells were treated for 1 h with the specific proteasome inhibitor, ZL3al, labeled with [35S]methionine for 1 h, chased for 30 min and the intracellular pro-α1(I) chains immunoprecipitated with a pro-α1(I)-specific antibody, LF-67 (Fig. 6A, lane 5). The larger minor band migrating at the same position as the heavy chain of major histocompatibility complex class I molecules (22) and ribophorin I (38). There are two N-linked oligosaccharides addition sites within the mutant pro-α1(I) C-propeptide (Fig. 1B) which offer possible targets for deglycosylation. However, treatment of the cells during the preincubation and labeling period with tunicamycin did not significantly alter the mobility of either band (data not shown) indicating that the deglycosylation cannot account for the difference in mobility of the two bands. In a pulse-chase experiment in the presence of clasto-lactacycin β-lactone the upper band representing the full-length frameshift mutant protein was converted to the smaller species almost completely after a 1-h chase period (Fig. 6B). These data suggest that the smaller form of the pro-α1(I) is derived from the upper band by the action of a non-proteasomal ER or cytoplasmic protease, and it is the smaller form that is degraded by proteasomes since it is protected from degradation by proteasome inhibitors. Importantly, pro-α2(I) chains did not immunoprecipitate with LF-67 even when the mutant pro-α1(I) chains were protected from degradation (Fig. 6A, lanes 6–8). This suggested either that the mutant C-propeptide sequence rendered the chains completely incompetent for assembly, or that the mutant pro-α1(I) chains were no longer within the ER but had been transported to the cytoplasm by reverse translocation and were therefore unable to interact with pro-α2(I). Together these data demonstrate that the vast majority of the frameshift mutant pro-α1(I) chains have an impaired ability to assemble into trimers and are rapidly degraded in transfected Mov13 cells by proteasomes.

In fibroblasts isolated from a patient with type I OI, the pro-α1(I) chain contains a COOH-terminal frameshift mutation (25), which is very similar to the engineered pro-α1(I) mutant presented here. mRNA levels for the mutant chain were normal but mutant procollagen chains were not detectable suggesting that the mutant protein was rapidly degraded (25). The similarity of these OI mutant procollagen chains to the engineered pro-α1(I) mutation expressed in Mov13 cells, suggested that degradation of the OI mutant may also be proteasome-mediated.

O126 Mutant Pro-α1(I) Is Also Protected by Proteasome Inhibitors—To determine if proteasomal degradation of procollagen chains with C-propeptide mutations is a general mechanism or is specific to the engineered mutation, we examined the degradation of mutant pro-α1(I) chains in O126 cells, derived from a patient with lethal OI. In O126 a heterozygous frameshift mutation within the C-propeptide results in the synthesis of a truncated pro-α1(I) containing an altered C-propeptide sequence (Fig. 1B) (4, 6). This mutation slowed, but did not
completely prevent, assembly of the mutant chains into triple helical molecules. Mutant unassembled pro-α1(I) chains were selectively degraded in OI26 cells and this degradation was not prevented by brefeldin A, suggesting an ER-associated degradation pathway (4). Because OI mutations are heterozygous it is normally not possible to discriminate the fates of normal and mutant allele products. However, the frameshift mutation in OI26 results in the synthesis of a more basic pro-α1(I) chain, and the normal and mutant chains can be resolved by two-dimensional gel electrophoresis, which separates the proteins on the basis of both charge and size (4, 6). We were able to take advantage of this unique feature of the mutant protein to further explore its intracellular degradation. OI26 fibroblasts were incubated with the proteasome inhibitor ZLαl, labeled with [35S]methionine, and the pro-α1(I) chains immunoprecipitated with LF-67 and resolved by two-dimensional gel electrophoresis. In untreated cells only normal pro-α1(I) chains were seen, however, mutant and normal pro-α1(I) chains were present in comparable amounts in samples treated with the proteasome inhibitor (Fig. 7). These data demonstrate that, as in transfected Mov13 cells, pro-α2(I) chains with a mutant C-propeptide are degraded by proteasomes in OI26 fibroblasts.

Many plasma membrane and secretory proteins that fail to fold correctly within the ER are selectively degraded by a quality control process known as ER-associated degradation. The 26 S proteasome has been shown to be responsible for the ER-associated degradation of transmembrane proteins such as the cystic fibrosis transmembrane conductance regulator (21), connexin-43 (18), major histocompatibility complex class I (19), as well as the secreted proteins connexin-43 (18), major histocompatibility complex class I (19), the cystic fibrosis transmembrane conductance regulator (21), and apolipoprotein B (39). The experiments here demonstrate that BiP associates with type I procollagen in three lines containing C-propeptide mutations that impaired chain association but not with the remaining 17 lines that contained helix mutations and assembled normally (43). Furthermore, we have previously shown that BiP associates with mutant procollagen in OI26 cells and in a second OI cell line that contains a C-propeptide Trp to Cys amino acid substitution (4). In both these lines normal procollagen folding is disturbed and the formation of disulfide-linked trimers is retarded (4, 6), suggesting that BiP specifically associates with procollagen chains that contain C-propeptide mutations and has an impaired ability to assemble but not with chains with mutations in the triple helix which can assemble normally but have abnormal triple helices (43). Procollagen chains with abnormal triple helices are directed to lysosomes for degradation (12, 13, 16), and our results demonstrate that procollagens with an impaired ability to assemble as a consequence of C-propeptide mutations are degraded by proteasomes. BiP binding may thus play a critical role in recognizing and directing mutant procollagen to proteasomes for degradation. BiP may bind to the misfolded procollagen chains and then either escort them to the protein translocation pore for reverse translocation into the cytoplasm, or transfer them to other proteins that chaperone the abnormal procollagen to the ER membrane.

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