DEFECTIVE TYPE I PROCOLLAGEN MOLECULES ARE DEGRADED BY THE CYTOSOLIC PROTEASOME.

James M. Pace and Peter H. Byers. University of Washington, Seattle, Washington, USA.

Synthesis, assembly and secretion of type I procollagen are stringently regulated processes. Generally, procollagen molecules are shuttled through the secretory pathway and secreted into the extracellular spaces only when they have acquired a fully folded and assembled conformation. Conversely, defective procollagen molecules from individuals with osteogenesis imperfecta (OI) are often sorted from normal molecules, retained within the rough endoplasmic reticulum (RER) and degraded by a quality control system, thereby promoting the structural integrity of the extracellular matrix. Little is known about the mechanisms and proteolytic systems that mediate RER degradation of abnormal pro alpha chains. Abnormal proteins in the cytosol and a small number of ER lumenal (alpha1-antitrypsin) or membrane (CFTR) proteins are marked for degradation by conjugation to ubiquitin and then hydrolyzed by the 26S proteasome. The present study investigated whether the proteasome participates in the degradation of abnormal type I procollagen molecules.

Cultured dermal fibroblasts from two OI patients with mutations in the C-terminal propeptide coding region of COL1A1 were used. The first cell strain had a complex deletion/insertion mutation that predicted synthesis of truncated proalpha1(I) chains, 45 amino acid residues shorter than normal molecules. The second harbored a frameshift mutation that resulted in the synthesis of short-lived, elongated proalpha1(I) chains that extended 84 amino acids beyond normal termination. Cells were incubated with specific inhibitors of the 26S proteasome (lactacystin and MG-132), pulse labeled with [35S]methionine, and chased with non-radiolabeled methionine for up to 240 minutes. Labeled proalpha1(I) chains were immunoprecipitated and separated by SDS-PAGE.

Proteasome inhibitors markedly retarded the disappearance of abnormal proalpha1(I) chains. In the first cell strain, absence of lactacystin and MG-132 permitted progressive decay of proalpha1(I) chains, but in the presence of inhibitor, proalpha1(I) chains disappeared only minimally over a 4-hour chase period. In the second cell strain, abnormal product was degraded so rapidly that it was not detectable by SDS-PAGE. However, in the presence of inhibitors, the elongated chain persisted for over 160 minutes. If incubation with inhibitors was extended to 18 hours before labeling, degradation was not delayed.

Slowed disappearance of defective proalpha1(I) chains in the presence of inhibitors suggests that the cytosolic proteasome degrades abnormal proalpha1(I) chains. Although degradation was slowed in proteasome-inhibited cells, some loss did occur. Furthermore, cells treated with inhibitors for long periods of time adapted and resumed degradation of abberant molecules, suggesting that another proteolytic system also contributes to type I procollagen quality control.

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