COMMENTARIES

Mutations in Genes Encoding Components of a Post-Translational-Modifying Protein Complex Cause Another Collagen Disease

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Osteogenesis imperfecta (OI) defines a group of human disorders that are clinically and genetically heterogeneous and range from mild to lethal in utero or shortly after birth. Most forms of OI are dominantly inherited and are due to mutations in type I collagen genes. In the October 20, 2006 issue of Cell, Morello et al. (1) describe recessive forms of OI caused by mutations in the gene encoding cartilage associated protein (CRTAP), a member of a complex of proteins that function in collagen synthesis and in hydroxylation of a single prolyl residue (986) in type I collagen α1(I) chains to yield peptide-bound 3-hydroxyproline. In addition to CRTAP, the protein complex includes cyclophilin B and prolyl 3-hydroxylase (P3H1). CRTAP is closely related to P3H1 but does not contain the catalytic hydroxylation domain. The mutations in CRTAP result in severe OI with markedly distorted bone structure. Type I collagen synthesized by fibroblasts from affected probands is over-modified (increased lysyl hydroxylation and glycosylation) and has little to no 3-hydroxyproline. Morello et al. (1) also target a null mutation in Crtap in mice that results in a clinical and biochemical phenotype similar to the CRTAP-deficient human disorder. These exciting observations provide a new understanding of how collagen synthesis is controlled and modified in genetic diseases.

Collagens are among the proteins that undergo many post-translational modifications. Some of these take place during elongation of the nascent chains in the endoplasmic reticulum (e.g., prolyl and lysyl hydroxylations) and others after the trimeric molecules are secreted from the cell (e.g., lysyl oxidation). The major skeletal collagens, types I and II, have large, uninterrupted collagen triple helices, comprising three polyproline II-like chains supercoiled around a common axis. The structure contains glycine (Gly) as every third residue, while the high content of proline (Pro) and 4-hydroxyproline (4-Hyp) residues stabilizes the polyproline-II-like helices characteristic of collagens (2). The enzymatic modification of Pro residues to 4-Hyp further stabilizes the collagen helices. Whereas Pro is found in either the -X- or -Y- position of the Gly-X-Y- tripeptide repeat, 4-Hyp is found in mammalian collagens only in the -Y- position. 4-Hyp is an abundant modification in type I and II collagens, with approximately 85-90 residues/1000 amino acids (about 40% of the total Pro + 4-Hyp). Another post-translational modification of collagens, hydroxylation of lysyl residues in nascent chains to form 5-hydroxylysine (5-Hyl), is less frequent, with approximately 4 residues/1000 amino acids (about 12% of the total Lys + 5-Hyl). 5-Hyl functions in
inter- and intramolecular crosslinking of collagen and as a site for O-linked glycosylation.

The enzyme responsible for generation of 4-Hyp in collagen is collagen prolyl 4-hydroxylase (P4H), a \( \alpha_2 \beta_2 \) tetramer located in the endoplasmic reticulum (ER), where the chaperone protein disulfide isomerase (PDI) is the \( \beta \) subunit and the hydroxylase is the \( \alpha \) subunit; there are three isoforms of the \( \alpha \) subunit in humans. Lysyl hydroxylase also has at least three isoforms (LH1-3); it is a dimer of the \( \alpha \) subunit and does not contain PDI. Mutations in two isoforms of the collagen lysyl hydroxylases in humans cause Ehlers-Danlos syndrome type VI (LH-1) and Bruck syndrome (LH-2). Spontaneous mutations of collagen P4H have not been reported, and a targeted null mutation of one isoform in mice is embryonic lethal (2). These collagen hydroxylases all require molecular oxygen, 2-oxoglutarate (\( \alpha \)-ketoglutarate), ferrous iron, and ascorbate, but substrate affinities vary depending upon the enzyme and its isoform. There has been considerable recent interest in the 4-prolyl hydroxylases that are critical in regulating responses to hypoxia by hydroxylating specific Pro residues (not in collagen-like sequences) in the hypoxia-inducible transcription factor, HIF\( \alpha \) (3). The HIF\( \alpha \) hydroxylases have substrate requirements similar to those of the collagen prolyl 4-hydroxylases but do not hydroxylate collagens, have different affinities, and do not have PDI subunits. Prolyl hydroxylation of HIF\( \alpha \) alters its binding to the von Hippel Lindau tumor suppressor protein (pVHL) and regulates its activity (3). The most abundant hydroxylated amino acid in collagen, 4-Hyp, was first isolated in 1902! The least abundant hydroxylated amino acid, 3-hydroxyproline (3-Hyp), was first isolated in 1961-1962 (4). 3-Hyp content in type I collagen is 1 residue/1000 amino acids, approximately 1% that of 4-Hyp, and 3-Hyp is found only in the -X- position of the Gly-X-Y- triplet with the sequence Gly-3-Hyp-4-Hyp- at residue 986 in the human \( \alpha_1(1) \) chain. There was little information, however, about potential biological functions and metabolism of 3-Hyp until a prolyl 3-hydroxylase (P3H) was isolated, cloned, and characterized in chick embryos by Vranka et al. in 2004 (5). (Since other potential members of the P3H family have subsequently been identified, the first member is called P3H1). The structure of chick P3H1 indicates that it is the orthologue of a previously described ER protein named leprecan (6), known also as the growth suppressor, Gros1. Leprecan cloned from the mouse has structural features in common with the other collagen hydroxylases; i.e., it is a member of the family of 2-oxoglutarate- and ferrous iron-dependent dioxygenases (5). Importantly, Vranka et al. (5) demonstrated that P3H1 specifically binds to denatured collagen and to at least two other proteins, cyclophilin B (CYPB) and CRTAP (7;8). CRTAP appears to be a member of the P3H family but lacks the catalytic dioxygenase domain and therefore cannot function as a collagen prolyl 3-hydroxylase. CYPB and CRTAP are also not required for full prolyl 3-hydroxylase activity, since assays performed in their absence showed no additional activity after they were added to the assay mixture (5).

Along come meticulous clinical observations and human and mouse genetics to shed light on these issues. OI is clinically very heterogeneous, usually dominantly inherited, and caused by mutations in genes encoding type I collagen (\( \text{COL1A1} \) and \( \text{COL1A2} \)) (2;9). One of the forms of OI, OI type VII, however, has a recessive inheritance and does not map to \( \text{COL1A1} \) or \( \text{COL1A2} \) (10). A major breakthrough in the "P3H1/CRTAP dilemma" thus came with the mapping of a genetic modification in a kindred with OI type VII to a locus on chromosome 3p22.3, although there was no known candidate gene at the time (10). It was subsequently postulated by Morello et al. (1) that CRTAP, included in this region, could be a cause of OI type VII in view of its binding to type I collagen and its potential roles in prolyl 3-hydroxylation (5). Morello et al. (1) then show that there is such a mutation at a splice site that could lead to unstable CRTAP mRNA and a decreased amount of the protein. Furthermore, Morello et al. examine an additional family with
clinically normal parents who had four children, all affected with severe OI clinically considered to be type II OI. Analysis of CRTAP sequences revealed a homozygous single base pair deletion that caused a frameshift mutation in exon 4; both asymptomatic parents were carriers of the mutation. Mass spectroscopic analysis of collagen in medium conditioned by fibroblasts from affected individuals in both kindreds showed no 3-Hyp in residue 986 in type I collagen α1(I) chains, even though CRTAP itself has no P3H activity. Engineering a Crtap-null mutation in mice reproduced many features of the human mutation, including severe osteoporosis and decreased prolyl 3-hydroxylation.

Further observations from Joan Marini's laboratory (BEMB, NICHD/NIH) were reported at the November 1-4, 2006 meeting of the American Society for Matrix Biology, and these have enormously helped to clarify several issues (11;12). Dr. Marini’s group identified 10 patients with severe OI, previously classified as OI type II or III, which is usually lethal in the first year of life but which did not map to COL1A1 or COL1A2. Three of these patients (11) had mutations in CRTAP, with absent CRTAP on Western blots and 3-Hyp levels (α1(I) chain residue 986) at 0-20% of the control levels, consistent with the observations of Morello et al (1). Most importantly for this discussion, the Marini group demonstrated null mutations in both alleles of the P3H1/leprecan gene (P3H1/LEPRE1), with little or no P3H1 protein made by fibroblasts and marked decreases in α1(I) chain 3-Hyp content in the remaining seven probands (12). These patients also had severe OI with short limbs, markedly distorted bone structure, but white sclerae. Bone densitometry showed very low Z scores (some approximately -5 to -7)! Both α1(I) and α2(I) chains synthesized by cultured fibroblasts from affected individuals with mutations in CRTAP or P3H1/LEPRE1 had increased lysyl hydroxylation and glycosylation, indicating longer retention of nascent chains during elongation in the ER. Does this mean that prolyl 3-hydroxylation is critical for collagen helix formation or stabilization? There is no direct evidence for this possibility. Indeed, based on studies of model synthetic polyproline-II-like helices, 3-Hyp destabilizes the triple helical structure (13), in striking contrast to the stabilizing effects of the more abundant 4-Hyp residues present exclusively in the -Y- positions of the collagen triple helical repeat (2;14).

A possible explanation for the type I collagen defects in CRTAP or P3H1/LEPRE1 deficiency can also be found in the paper by Vranka et al. (5). They propose that the "complex of proteins, P3H1, cyclophilin B, CRTAP, and possibly other larger complexes, interact with unfolded procollagen chains in vivo to achieve a fully folded and assembled collagen molecule within the cell." CYPB-deficiency (not yet reported) therefore might also have the phenotype of severe OI with decreased collagen prolyl 3-hydroxylation. Deficiency in collagen 3-Hyp could just be a marker for a dysfunctional complex. In addition, 3-Hyp is more abundant in other collagens, such as type IV collagens; the latter are major components of basement membranes. Is the structure and prolyl 3-hydroxylation of these collagens also disturbed if the P3H1/CYPB/CRTAP complex is abnormal? Do members of the P3H/LEPRE family other than P3H1/LEPRE1 have the function of prolyl 3-hydroxylation of substrates such as type IV collagens? Introduction of a null mutation into P3h1 in mice would confirm the elegant observations from the Marini laboratory in humans and enable further biochemical studies, but would probably not be definitive. On the other hand, a "knock in" of a P3h1 with the catalytic dioxygenase domain deleted would be very informative, particularly if the mouse homozygous for the mutation were to have no 3-Hyp in type I collagen yet no OI phenotype! These are all interesting questions raised by these exciting new views into the genetics, cell biology and biochemistry of extracellular matrix proteins.

Conflict of Interest: The author reports that no conflict of interest exists.
References


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