Calcif Tissue Int (2013) 93:338–347
DOI 10.1007/s00223-013-9723-9

ORIGINAL RESEARCH

Bone Collagen: New Clues to Its Mineralization Mechanism from Recessive Osteogenesis Imperfecta

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Received: 15 November 2012 / Accepted: 1 March 2013 / Published online: 19 March 2013

Abstract Until 2006 the only mutations known to cause osteogenesis imperfecta (OI) were in the two genes coding for type I collagen chains. These dominant mutations affecting the expression or primary sequence of collagen α1(I) and α2(I) chains account for over 90 % of OI cases. Since then a growing list of mutant genes causing the 5–10 % of recessive cases has rapidly emerged. They include *CRTAP*, *LEPRE1*, and *PPIB*, which encode three proteins forming the prolyl 3-hydroxylase complex; *PLOD2* and *FKBP10*, which encode, respectively, lysyl hydroxylase 2 and a foldase required for its activity in forming mature cross-links in bone collagen; *SERPINH1*, which encodes the collagen chaperone HSP47; *SERPINF1*, which encodes pigment epithelium-derived factor required for osteoid mineralization; and *BMP1*, which encodes the type I procollagen C-propeptidase. All cause fragile bone in infancy, which can include overmineralization or undermineralization defects as well as abnormal collagen posttranslational modifications. Consistently both dominant and recessive variants lead to abnormal cross-linking chemistry in bone collagen. These recent discoveries strengthen the potential for a common pathogenic mechanism of misassembled collagen fibrils. Of the new genes identified, eight encode proteins required for collagen posttranslational modification, chaperoning of newly synthesized collagen chains into native molecules, or transport through the endoplasmic reticulum and Golgi for polymerization, cross-linking, and mineralization. In reviewing these findings, we conclude that a common theme is emerging in the pathogenesis of brittle bone disease of mishandled collagen assembly with important insights on posttranslational features of bone collagen that have evolved to optimize it as a biomineral template.

Keywords Collagen · Bone · Cross-linking · Osteogenesis imperfecta · Posttranslational modification

Introduction

Despite intensive study over 50 years, the exact molecular mechanism of mineralization of bone collagen to form an intimate ordered biocomposite remains elusive. In particular, the question is still contested whether bone collagen itself has evolved special features or is essentially generic and passive in a process largely driven by extrafibrillar inhibitors of solid-phase calcium phosphate nucleation and, thus, acts primarily as a structural constraint on nanocrystal growth and not as a nucleator (Fig. 1). Here, we summarize recent genetic discoveries on osteogenesis imperfecta (OI) that we believe reinforce a long-held view that highly evolved posttranslational features of bone collagen itself are intimately involved in regulating the structure of the eventual composite of polymeric collagen embedded with oriented nanocrystal plates of hydroxyapatite [1, 2]. When steps in any of the complex pathways modulating the unique posttranslational chemistry and assembly of collagen in bone go wrong, severe OI can result [3–16].

OI and Bruck Syndrome

Recent advances in OI research were reviewed by Cundy [17]. Most OI cases are caused by dominant mutations in

The authors have stated that they have no conflict of interest.

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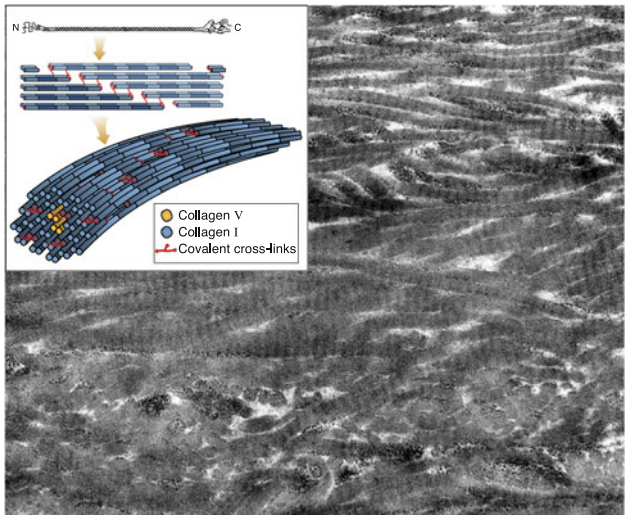


Fig. 1 Transmission electron micrograph of decalcified bone matrix showing the dense, woven packing arrangement of collagen fibrils. *Inset* individual fibrils are a covalently cross-linked composite of collagen type I polymerized on a template of type V collagen

one of the two genes encoding type I collagen that affect both the amount and structure of the assembled matrix collagen. Though a systemic defect, fragile bone is the most pronounced tissue consequence. For many years no causative genes were known for the 5–10 % of recessive cases, beyond a few cases of parental mosaicism for type I collagen mutations. This changed with the discovery that mutations in *CRTAP* (encodes cartilage-associated protein) caused recessive OI [3]. This protein forms a complex with prolyl 3-hydroxylase 1 (P3H1) and peptidyl prolyl isomerase B (PPIB or cyclophilin B) in the endoplasmic reticulum (ER) [18], which is responsible for 3-hydroxylating single prolines in the collagen α1(I) and α2(I) chains at α1(I) Pro986 and α2(I) Pro707 [19]. In rapid succession, further mutations in *CRTAP* and mutations in *LEPRE1* (encodes P3H1) and *PPIB* (encodes cyclophilin B) were found to cause recessive OI [4–8]. Mutations in the other genes listed in Table 1 were soon identified [9–16, 20–23]. Bruck syndrome, which exhibits the bone fragility of OI and joint contractures, results from defective lysyl hydroxylase 2 activity caused by mutations in either *PLOD2*, which encodes the enzyme [24], or *FKBP10*, which encodes a member of another class of ER resident peptidyl prolyl isomerases [11, 12]. Null mutations in both genes produce close phenocopies of Bruck syndrome both clinically and in terms of an abnormal collagen cross-linking. Only the cross-linking products of telopeptide lysine aldehydes are present in the structural bone collagen of affected patients [14, 25]. The cross-linking of cartilage type II collagen, however, appears not to be affected, whereas ligament type I collagen is.

Mutations in the genes *SERPINH1*, which encodes the well-known ER collagen chaperone heat-shock protein 47 (HSP47), and *SERPINF1*, which encodes pigment epithelium-derived factor (PEDF), were also found to cause recessive OI [10, 20, 21]. The latter protein, PEDF, is produced and secreted by osteoblasts, can bind to collagen, is antiangiogenic, and circulates in the serum and, when missing, osteoid seams fail to mineralize.

More recently, mutations in *BMP1*, which encodes the protease responsible for cleaving the C-propeptides from type I collagen, were found to cause recessive OI cases [15, 16]. In addition to these seven genes, all of which are involved in collagen type I processing or its mineralization, a homozygous mutation in the gene encoding SP7/Osterix

Table 1 Noncollagen genes in which mutations cause osteogenesis imperfecta variants

Gene	Protein	Phenotype	Bone collagen abnormalities
CRTAP	CRTAP	AR, bone fragility, reduced mineral density	α1(I)P986 and α2(I)P707 under prolyl-3-hydroxylation, high HP/LP (*CRTAP*, *LEPRE1*), low HP/LP (*PPIB*)
LEPRE1	P3H1,prolyl 3-hydroxylase (P3H1 complex)		
PPIB	CYPB, cyclophilin B		
FKPB10	FKBP65	AR, Bruck syndrome: bone fragility, joint contractures	Lack of telopeptide hydroxylysines produces skin-like cross-links
PLOD2	LH2, lysyl hydroxylase 2		
SERPINH1	HSP47, heat-shock protein 47	AR, bone fragility (type III OI)	High HP/LP and abnormal arrangement of cross-linking bonds
SERPINF1	PEDF, pigment epithelium-derived factor	AR, bone fragility, low bone mass and wide osteoid seams	Defective mineralization, no other collagen abnormalities detected
BMP1	Procollagen type I C-propeptidase	AR, bone fragility, high mineral density	Defective C-propeptide removal, potential cross-linking defects
IFITM5	Bril, osteoblast-specific small transmembrane protein	AD, bone fragility, hyperplastic callus (type V OI)	None reported

OI osteogenesis imperfecta, *AR* autosomal recessive, *AD* autosomal dominant, *HP* hydroxylysylpyridinoline, *LP* lysylpyridinoline

(a transcription factor required for osteoblast differentiation) causes recessive OI [26]. Mutations in low-density lipoprotein receptor-related protein (LRP5) [5] can cause low bone mass without OI features [27] or osteosclerosis [28], depending on the mutation, through effects on WNT signaling specific to bone. Most recently, the radiographically distinguished dominant variant OI type V was shown to be caused by a heterozygous mutation in a gene for osteoblast-specific transmembrane protein, *IFITM5* [22, 23] (Table 1). The function of this protein in osteoblasts is still unclear.

In summary, most OI cases, whether recessive or dominant, are associated with a defective molecular assembly of bone collagen. The new findings reveal and highlight the importance of understanding the mechanistic intricacies controlling the posttranslational quality of collagen assembly in bone. Can these new genetic insights also aid in understanding whether certain posttranslational properties unique to bone collagen are essential for its characteristic pattern of nanocrystal mineralization?

Fibrillar Architecture and Cross-Linking of Bone Collagen

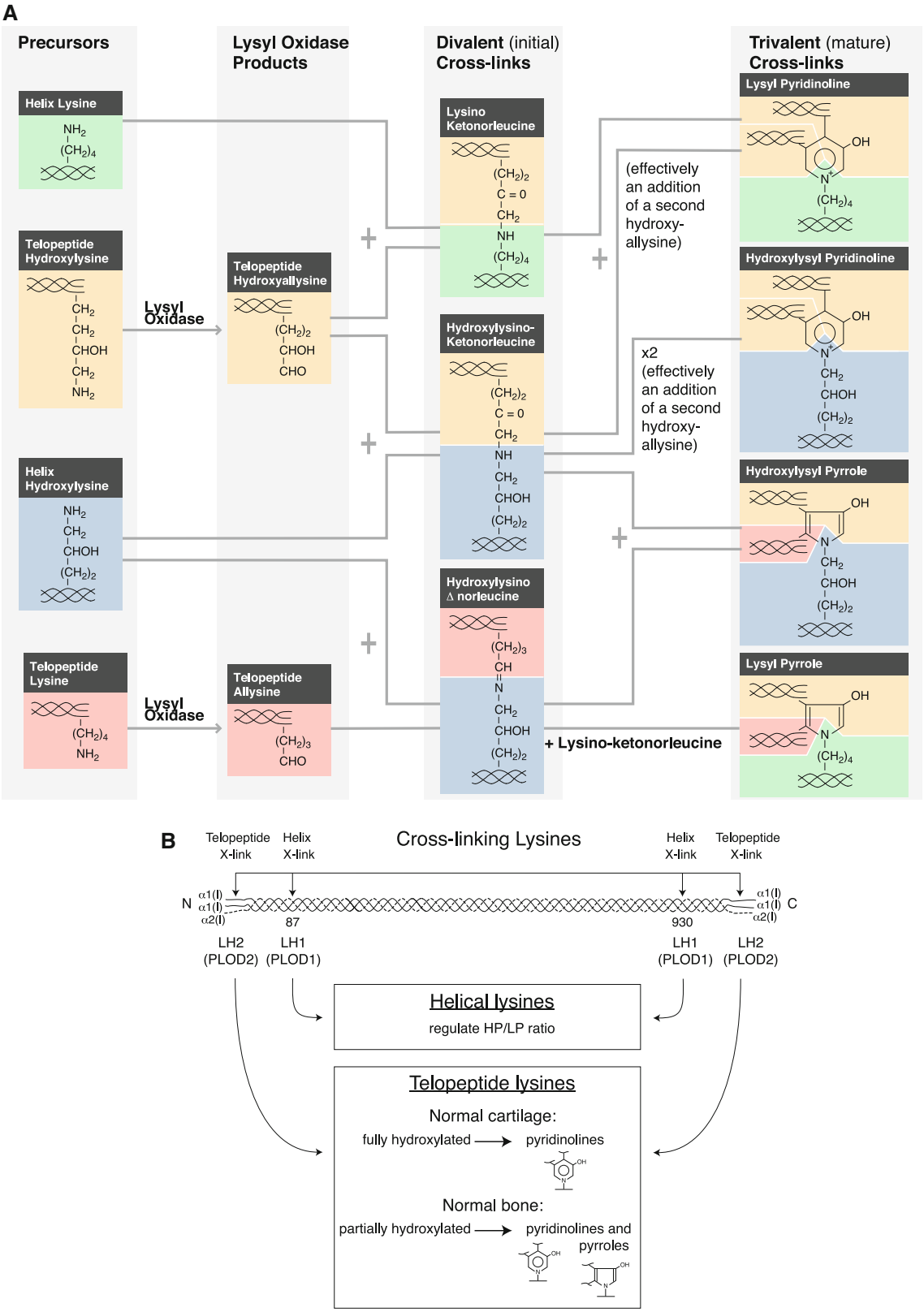
Collagen type I of bone has the same translated primary sequence as that in skin, tendon, and other tissues. The template for the type I collagen polymeric fibril is collagen type V of composition $[(\alpha1(V)]_2$, $\alpha2(V)$ in developing bone with increasing amounts of $\alpha1(XI)$ substituting for $\alpha1(V)$ in mature bone [29]. The functional significance of this isomorphic developmental change in chain usage structurally and biologically is unknown. In mature bone, as in other tissues, the molar ratio of type I/types V and XI collagens is about 30/1 [30].

Intermolecular covalent cross-links between collagen telopeptide and helical domains are essential for fibril strength. Bone collagen presents a unique chemical profile of cross-linking since the telopeptide lysines, which lysyl oxidase converts to reactive aldehydes, are only 50 % hydroxylated compared with 0 % in skin type I collagen and 100 % in type I and type II collagens of cartilages [31]. The mechanism regulating this is unclear. Consequently, the initial divalent and mature trivalent cross-links of bone collagen give a distinctive pattern on peptide analysis with about equal amounts of mature pyrroles and pyridinolines in human bone collagen [32]. Figure 2a summarizes the pathways initiated by the reactive aldehydes generated in telopeptides by lysyl oxidase. Both lysine and hydroxylysine aldehydes react with helical cross-linking site lysine/hydroxylysine residues. Pyridinoline cross-links are found in the collagen of many tissues including bone but are absent from normal skin and cornea. Pyrrole cross-links appear to be restricted to bone and high-load tendons among major connective tissue [32, 33]. The ratio of pyrroles to pyridinolines has been shown to vary between bone types, for example, between osteoporotic and control human bone, with a high pyrrole content associated with a finely meshed trabecular architecture [34]. In Bruck syndrome, the lack of telopeptide hydroxylysines prevents pyridinoline and pyrrole formation and results in a cross-linking profile that resembles that of type I skin collagen [14, 25]. Bruck syndrome is also distinguished clinically by neonatal joint contractures, which it is reasonable to suspect may be a consequence of an altered cross-linking chemistry in tendons and related supporting tissues.

We conclude that pyrrole cross-links contribute unique properties to bone collagen for its role in producing an intimate mineral microcrystal/protein composite. The pyrrole structure predicted from the molecular mass and other properties of isolated cross-linked pyrrole peptides [31], including its likely mechanism of formation by interaction of two divalent cross-links, is a 3-hydroxypyrrole (Fig. 2a). Model *N*-alkylated 3-hydroxypyrroles are highly reactive; for example, they can spontaneously form a dimer or add another aldehyde moiety through their C2 carbon [35]. (Aldehyde addition is the basis of the p-DIMAB colorimetric reaction product used to assay these cross-links in collagen [36].) Pyrroles have the potential therefore to progress to more complex multivalent cross-links as collagen fibrils mature. If telopeptide lysines become more hydroxylated than normal as part of the general overmodification seen in many OI variants as a consequence of delayed triple-helical folding [14], then the pyridinoline to pyrrole ratio will increase in bone in concert with the reported increase in hydroxylysylpyridinoline/lysylpyridinoline (HP/LP) ratio [37]. Conceivably this could produce a more brittle mineralized composite with a lower threshold for microdamage and crack propagation and, hence, increase bone fragility. Posttranslational overmodification of lysine residues in collagen is well recognized in OI cases caused by mutations in *COL1* genes, *CRTAP*, *LEPRE1*, and *PPIB* [3–7]. It should be noted, however, that such collagen overmodifications in OI studies are usually based on the properties of collagens synthesized by skin fibroblasts in culture. When compared with collagen from bone tissue of such patients, the results can be misleading, particularly when 3Hyp levels are being reported (Eyre and Weis unpublished).

Lysyl Hydroxylase 2, FKBP65, and Bruck Syndrome

Figure 2b shows the four cross-linking sites in the type I collagen molecule, two telopeptide and two triple-helical, through which intermolecular bonds can form when polymerized in fibrils. Lysyl hydroxylase 2 (LH2) is solely



responsible in osteoblasts for telopeptide lysine hydroxylation. When effectively null due to mutations in *PLOD2*, no hydroxylysine aldehyde cross-links can form and the result is Bruck syndrome 2 [24]. It turns out that mutations in *FKBP10* can produce a very similar pathology (Bruck syndrome 1) through a lack of telopeptide hydroxylase activity

◀ **Fig. 2** **a** As well as a characteristic fibril organization, the pathway of covalent intermolecular cross-linking in bone collagen is unique in using precursor aldehydes produced by lysyl oxidase from both lysines and hydroxylysines in telopeptide domains (shown as *allysine* and *hydroxy-allysine*). The resulting mature cross-links consist of both pyridinolines and pyrroles in about equal amounts. **b** Three lysyl hydroxylases (LH1, LH2, and LH3) encoded by three different genes (*PLOD1, PLOD2*, and *PLOD3*) regulate the chemical nature of the cross-links, which varies between different tissue types. In bone collagen LH2 partially hydroxylates telopeptide lysines at both ends of the type I collagen molecule and LH1 partially hydroxylates the two helical-site lysines in α1(I) and α2(I) chains. In addition, the hydroxylysines at K87 in α1(I) and α2(I) are mostly glycosylated with a single galactose residue with lesser amounts of glucosylgalactose. This pattern is a distinguishing, unique posttranslational feature of bone type I collagen compared with type I collagen of skin, tendon, and other soft tissues

[13, 14, 25]. The most likely mechanism is that the protein it encodes, FKBP65, a peptidyl prolyl isomerase, is required to fold lysyl hydroxylase 2 correctly for it to be active in the ER. Note that lysyl hydroxylase 1 is primarily responsible for hydroxylating the helical cross-linking-site lysines [38], so a delayed collagen triple-helical folding can also result in the increased HP/LP pyridinoline ratio seen in other forms of OI. Conversely, null mutations in *PLOD1* (encodes LH1), which cause Ehlers-Danlos syndrome VIA, result in a very low HP/LP ratio in bone [38]. The bone collagen defects in Ehlers-Danlos syndrome type VIA, both Bruck syndrome variants, and other forms of OI can be detected as abnormal ratios of HP/LP in patients' urine [14, 24, 39].

Collagen Prolyl 3-Hydroxylation

Early in evolution, prolyl hydroxylase activity added functionality to ancestral collagens. Thermal stability of the triple helix was increased by hydrogen bonding through 4-hydroxyproline (4Hyp) residues [40]. Though 3-hydroxyproline (3Hyp) was also present (at about one residue per type I collagen chain and 10 per type IV collagen chain), its function is still essentially unknown. Not until a lack of 3Hyp in type I collagen of *CRTAP* null mice had led to *CRTAP* mutations as a cause of recessive human OI did interest focus on possible functions for 3Hyp.

The finding of several sites of partial 3Hyp occupancy in types I and II collagen molecules spaced D-periodically (234 ± 3 residues) implied a possible role in fibril assembly [19]. Peptide-binding experiments indicated selective affinity between like regions containing a 3Hyp residue [41]. From such evidence and other considerations, including the outward pointing direction from the triple helix of the 3Hyp 3-hydroxyl in a –Gly3Hyp4Hyp– triplet [42], short-range hydrogen bonding between collagen triple helices was considered a possibility [19]. This implied a role in supramolecular assembly.

Figure 3 shows identified sites of 3Hyp in type I collagen molecules (clade A gene products). Only one (A1, Pro986) is fully hydroxylated. Partially occupied A2, A3, and A4 are spaced D-periodically apart. More, unrelated 3Hyp sites are present in the type V/XI collagen α1(V) and α1(XI) chains, which are clade B gene products. Three that are heavily occupied are shown (B1–B3). Multiple other GPP sequences in α1(V) have also revealed low levels of 3Hyp occupancy that vary in occupancy with cellular origin [43]. When packed in fibrils, the A2, A3, and A4 D-periodic sites align in the molecular overlap region, which also contains the A1 site (Fig. 3). The 3Hyp locations are shown placed to scale relative to the uranyl acetate-stained banding pattern of a collagen fibril and to sites where certain small leucine-rich proteoglycans (SLRPs) bind to collagen fibrils. Fibromodulin and lumican bind close to the a and c bands, where telopeptide to helix cross-links also occur [44, 45].

Although 3Hyp is found throughout animal phyla from the most primitive sea sponges [46], the Pro986 substrate did not appear until early chordates (Eyre and Weis unpublished findings). This coincides with the appearance of the *CRTAP* gene and just before vertebrates and bone emerged [47]. The Pro986 3Hyp site therefore appears to be a newly recognized substrate when CRTAP and the P3H1/CRTAP/PPIB complex emerged [19]. To speculate, this acquired Pro986 modification may have helped equip collagen molecules for a polymeric architecture that was better organized to enable ordered hydroxyapatite nanocrystal growth within fibrils. An effect on cross-linking and molecular packing is one possibility we are exploring.

Alternatively, the P3H1/CRTAP/PPIB complex may function primarily as a chaperone that aids in native collagen molecule assembly [17]. The consequences of the defective complex include ER distress, the unfolded protein response (UPR) and disrupted cell level regulation of bone formation and turnover. This could be the primary cause or at least a significant contributor to OI pathobiology. The two concepts are not mutually exclusive, and lack of Pro986 3Hyp and ER distress probably both contribute.

The Collagen-Specific Chaperone HSP47 and *SERPINH1* Mutations

The exact mechanism by which *SERPINH1* mutations result in brittle bones is unclear, but clearly it involves collagen misassembly. The mutations reported in human OI, L78P, and in dog OI, L326P, both result in sequence changes at the active site through which HSP47 binds to the collagen triple helix as shown from the crystal structure of the complex [48].

Since HSP47 binds strongly only to a native triple helix at specific sites containing arginine, it acts as a quality control

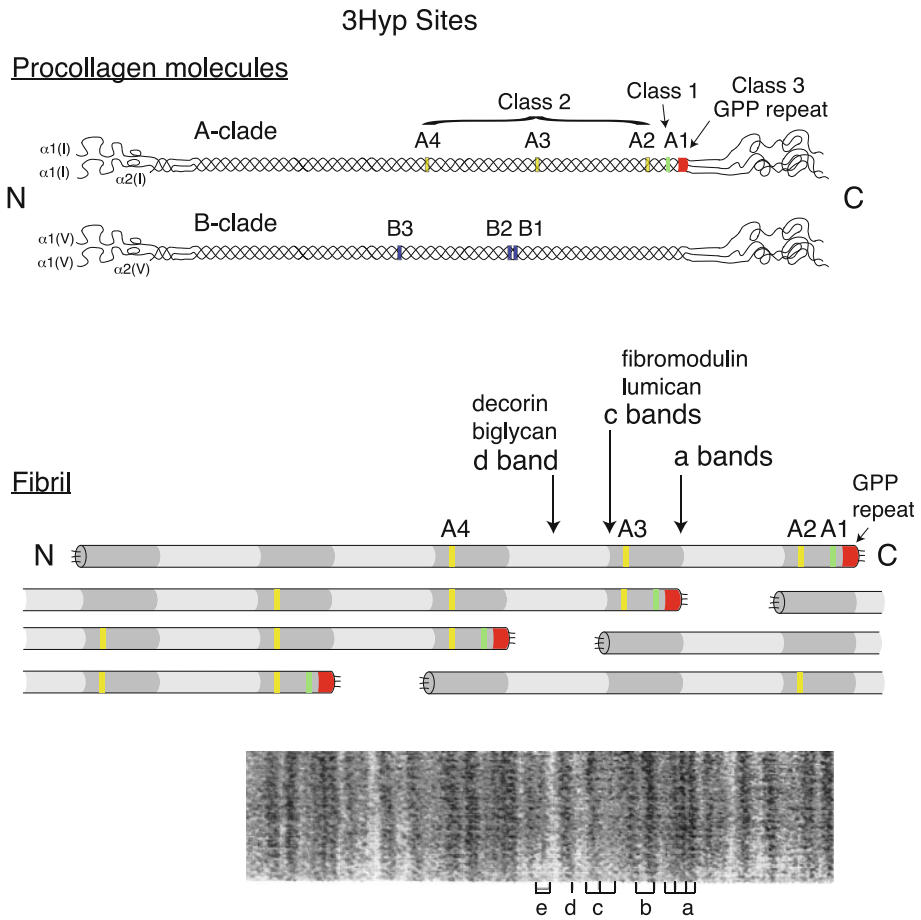


Fig. 3 Location of 3Hyp residues in type I and type V collagen molecules (*top*) with their resulting aligned positions in a fibril relative to the D-periodic stagger and band pattern of fibrils as seen by transmission EM (*bottom*). A single fully 3-hydroxylated proline occurs at Pro986 (A1 site) in α1(I) and at Pro707 (A3 site) in α2(I). All four sites (A1–A4) are fully or partially 3-hydroxylated in α2(V), and the spacing between A2, A3, and A4 is D-periodic (234 ± 3 residues). Multiple sites of 3Hyp occur in the α1(V) chain [19, 41] with a high occupancy and D-periodic spacing between B1 and B3 [19]. It is notable that fibril surface binding sites for one class of small leucine-rich proteoglycans (e.g., fibromodulin, lumican) are close to 3Hyp sites

for folding and prevents premature aggregation of native procollagen molecules until after they are transported from the ER to the Golgi and released at low pH [48].

The cross-linking of bone collagen from the OI dachshund [9] shows abnormalities consistent with a defective molecular assembly and posttranslational chemistry (Eyre and Weis unpublished). The mechanism and significance of this continue to be explored.

SERPINF1/PEDF, OI Type VI, and Osteoid Mineralization

OI type VI is characterized by a unique bone histomorphometry, with large seams of unmineralized osteoid and an abnormal lamellar pattern [49]. Collagen biochemistry seems normal as far as it has been investigated. The causative defect (mutations in *SERPINF1*) disrupts PEDF, a secreted protein apparently required for mineralization of osteoid [20, 21]. A mechanistic role for PEDF as a chaperone in the osteoblast ER also remains a possibility, so posttranslational effects on collagen in bone cannot yet be ruled out.

However, it seems more likely that the defect is downstream from matrix collagen assembly and involves a regulatory step of mineral deposition extracellularly. Understanding the mechanism may reveal another pathway and signaling defects through which mutations can cause OI.

BMP1 and Collagen C-Propeptide Processing Defects

Autosomal dominant collagen type I mutations (COLA1 and COLA2) that alter the C-propeptide cleavage site sequence result in relatively mild OI with an abnormally high mineral density in patients on DXA scans [50]. Retention of propeptide sequences conceivably could result in more space for mineral within and between fibrils, which may explain the high bone density. Altered collagen cross-linking remains a possibility. Recessive mutations in *BMP1*, which encodes the collagen type I C-propeptidase, also cause OI with high bone density, presumably through a similar mechanism of delayed propeptide removal and disrupted fibril assembly and mineralization [13, 14]. Note that BMP1 processes other matrix molecules including lysyl oxidase (*LOX*) activation by removal of its N-terminal propeptide domain [51]. Thus, more than one mechanism may be operating.

IFITM5/Bril and OI Type V

A single autosomal dominant new mutation in another gene *IFITM5* (interferon-induced transmembrane protein 5) was most recently shown to cause OI type V in multiple individuals and families [22, 23, 52]. This heterozygous mutation in the 5′-UTR adds five amino acids to the N terminus of the signal peptide, which is predicted to have gain-of-function effects. The gene encodes a small transmembrane protein highly restricted to mineralized tissue and known to be expressed in osteoblasts at the point in bone formation when mineral is deposited [53, 54]. The findings strongly suggest a specific role in the control of osteoblast expression and the mineralization pathway of bone and cartilage. Curiously, the protein product of *IFITM5* (Bril, bone-restricted ifitm-like protein) is associated with yet another peptidyl prolyl isomerase in the 15-member human FKBP gene family, FKBP19 (encoded by *FKBP11*) [54].

Fibril-Associated Small Leucine-Rich Proteoglycans in Bone

SLRPs are a widely distributed and diverse group of 18 matrix proteins evolved from a common ancestor that share collagen-binding domains [54]. Decorin, biglycan, lumican, fibromodulin, keratocan, and osteoadherin are among the SLRPs widely distributed and found in bone matrix, where they are bound to collagen fibril surfaces. From studies on mice null for either or both decorin and biglycan, these molecules are important regulators of fibril growth and size limits in many tissues [55]. Osteoadherin is limited to bone and dentin and is deposited just before mineralization occurs [56]. Osteoadherin-null mice have not been described, so the significance of this molecule in mineralization and bone function is unclear. Work on fibromodulin, a related class II SLRP, indicates a role in tendon in regulating fibril size and lysyl oxidase-mediated cross-linking chemistry [45]. Though mutations in SLRPs have not been found in OI, their role in shaping the fibrillar collagen architecture and material properties of extracellular matrices is clear.

Implications for Bone Fragility

In this review we summarize the defective steps in collagen assembly that can result in a childhood brittle bone phenotype. With the growing knowledge a repeated theme is emerging of abnormal cross-linking when bone collagen is examined. We end with a hypothesis that disturbed cross-linking from the normal equal mix of lysine aldehyde and hydroxylysine aldehyde structures is a fundamental feature of the bone pathology in OI. Clearly, collagen strength rests heavily on its cross-linking, but other properties of bone including ductility and resistance to microdamage and crack propagation may depend on the unique pattern of lysyl oxidase-mediated cross-links that characterize normal bone collagen of higher vertebrates. The placement of cross-links and chemical lability of a significant fraction of them (those formed from lysine aldehydes) may be required to produce a malleable framework in which mineral nanocrystals can grow optimally within the fibrils. This concept is in accord with conclusions drawn from ultrastructural and material property observations on fragile bone from the oim mouse, which lacks an α2(I) chain and deposits α1(I) trimeric type I collagen [57].

Clearly, too, other mechanisms produce low bone mass and risk of fragility fractures through signaling-pathway defects as evidenced by the SP7/Osterix [26] and WNT pathway [27, 28] mutations. Thus, it is also possible that defects in collagen assembly have downstream effects on cell matrix interactive signaling as well as inherent structural defects in the collagen matrix itself.

Implications for Mineral Deposition

An association between the presence of 3-hydroxyproline residues in collagen with mineralization can be traced back to silica deposition by Porifera, the most primitive class of multicellular animals [46]. Indeed, it has been proposed that hydrogen bonding between orthosilicic acid and the vicinal two hydroxyls in –Gly-3Hyp-4Hyp– sequences in the meters-long silica spicules of cloud sponges can act as a collagen-based nucleation template for silica crystal growth [46].

It is possible that this same unique peptide sequence has continued to act as a surface template for orienting mineral nanocrystal deposition throughout collagen evolution. It seems more likely, however, that 3Hyp evolved to add structural stability and/or order to collagen assembly, which in turn influenced the packing arrangement and cross-linking of molecules in fibrils to provide an orderly and malleable microenvironment for accommodating mineral crystallite growth. A potential role in orienting solid-phase mineral growth nevertheless deserves some attention.

Future Challenges

- One challenge is to define how tissue specificity in the chemistry and placement of cross-linking bonds is regulated in bone collagen and whether cross-link irregularities are a common mechanism underlying bone fragility in OI. Are pyrrole cross-links important

for the unique material properties of bone, and is their loss a determining factor in the bone fragility of OI?

- Another is to establish the physicochemical and biological advantages that 3Hyp residues have added to the collagen triple helix both ancestrally and through evolution.
- Also, the mechanisms by which fibril-associated SLRPs regulate the lateral and longitudinal growth of collagen fibrils in general and in bone specifically remain to be defined. Do such proteins influence collagen cross-linking and fibril mineralization?
- The biggest challenge will be proving cause-and-effect relationships between the altered posttranslational features of collagen in OI and material properties as a basis for predicting increased bone fragility. The clues we have suggest that abnormal cross-linking may be a common consequence. But is this a critical change that affects material strength, a marker of altered molecular packing that in turn affects mineral organization, or a side show? Multidisciplinary collaborations and genetic approaches will continue to be vital for progress in this rapidly advancing field.

Acknowledgement Research reported in this publication from the authors' laboratory was supported by the National Institute of Arthritis and Musculoskeletal and Skin Diseases of the National Institutes of Health under awards AR36794 and AR37318. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

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