Multiexon Deletions in the Type I Collagen COL1A2 Gene in Osteogenesis Imperfecta Type IB

MOLECULES CONTAINING THE SHORTENED α2(I) CHAINS SHOW DIFFERENTIAL INCORPORATION INTO THE BONE AND SKIN EXTRACELLULAR MATRIX*

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Stefan Mundlos, Danny Chan, Yi Ma Weng, David O. Sillence, William G. Cole, and John F. Bateman

From the Orthopaedic Molecular Biology Research Unit, Department of Paediatrics, University of Melbourne, Royal Children's Hospital, Parkville, Victoria 3052, Australia, and the Department of Clinical Genetics, The New Children's Hospital, Paramatta, New South Wales 2124, Australia

Osteogenesis imperfecta (OI) type IB is a rare subset of the mildest form of OI, clinically characterized by moderate bone fragility, blue sclera, and dentinogenesis imperfecta. Cultured skin fibroblasts from two unrelated individuals (OI-197 and OI-165) with the typical features of OI type IB produced shortened α2(I) chains. Reverse transcription-polymerase chain reaction of the α2(I)-cDNA revealed deletions in the triple helical domain of 5 exons (exons 7–11) in OI-197, and 8 exons (exons 10–17) in OI-165. This exon skipping was caused by genomic deletions in one allele of COL1A2 with the breakpoints located in introns 6 and 11 in OI-197, and introns 9 and 17 in OI-165. The secretion and deposition of the mutant collagen into the matrix was measured in vitro in cultures of skin fibroblasts and bone osteoblasts, grown in the presence of ascorbic acid to induce collagen matrix formation and maturation, as well as in collagen extracts from skin and bone. The secretion of mutant collagen was impaired and long term cultures of fibroblasts showed that the mutant collagen was not incorporated into the mature collagenous matrix produced in vitro by skin fibroblasts from both patients. Likewise, the shortened α2(I) chain was not demonstrable in skin extracts. In contrast, bone extracts from OI-197 showed the presence of the mutant collagen. This incorporation of the abnormal collagen into the mature matrix was also demonstrated in long term cultures of the patient's osteoblasts. The deposition of the mutant collagen by bone osteoblasts but not by skin fibroblasts demonstrates a tissue specificity in the incorporation of mutant collagen into the matrix which may explain the primary involvement of bone and not skin in these patients.

Osteogenesis imperfecta (OI)* is a brittle bone disease that varies in severity from perinatal lethal to mild forms. In spite of the clinical variability, mutations in the genes for the pro-α1(II) chains (COL1A1) and pro-α2(I) chains (COL1A2) of type I collagen have been defined as the basis of the disease in more than 90% of cases studied to date (for reviews see Refs. 1–3). The most common and mildest form of OI, OI type I (OI-I), is characterized by blue sclerae, bone fragility with minimal deformities, and autosomal dominant inheritance (4). Dentinogenesis imperfecta occurs in some patients and this is used to subclassify patients into OI-IA (no dentinogenesis imperfecta) and OI-IB (dentinogenesis imperfecta present) (4, 5).

Biochemically, patients with OI-I commonly show reduced production of structurally normal type I collagen as a result of a COL1AI "null" allele caused by structural mutations that prevent procollagen assembly (6) or more commonly, by mutations that introduce premature stop codons, producing either unstable mRNA or the synthesis of truncated unstable collagen (7). Structural mutations within the triple helical domain of type I collagen usually cause more severe OI phenotypes; however, exon-skipping (7, 8) and glycine substitution mutations (9–16) have been defined in OI-I, but these are clustered toward the amino-terminal end of the triple helix domain, presumably reducing their impact on helix propagation and structure (10). Furthermore, in contrast to the structurally abnormal collagens, which are incorporated into the extracellular matrix in severe forms of OI (17, 18), the normal collagen chains in OI-I may be excluded from matrix formation (12) producing a milder phenotype. Although COL1AI is the predominant disease locus, OI-I can also result from COL1A2 exon-skipping mutations, which also clustered toward the amino-terminal end of the triple helix (19–22), and in a single reported case, from a glycine substitution mutation in COL1A2 (23).

It is unclear whether the OI-IA and OI-IB phenotype results from distinct types of collagen mutations. This is due, in part, to the paucity of detailed clinical information provided in most publications on patients with defined COL1AI or COL1A2 mutations, which does not allow classification of the patients as OI-IA and OI-IB with any degree of certainty. However, two of the probands with COL1AI2 mutations displayed dentinogenesis imperfecta and could be classified as OI-IB (21, 22) and detailed linkage analyses by Sykes et al. (24) further suggest...
that COL1A2 mutations may be a major cause of OI-IB.

While the definition of the spectrum of mutations causing OI remains an important goal, the major research challenge is the definition of the molecular mechanisms by which collagen structural mutations affect the complex organization and homeostasis of the extracellular matrix. In the present report, we describe the molecular defects in two probands and one affected parent with OI type IB. In contrast to the mild clinical phenotype, they had multi-exon deletions of the COL1A2 gene. The effects of these mutations on collagen synthesis, secretion, and matrix deposition were studied using skin, bone, and long term cultures of fibroblasts and osteoblasts. These studies demonstrate that skin and bone cells respond differently to the production of the mutant collagens, displaying differential incorporation of the mutant collagen into the extracellular matrix. These differences in the metabolism of the mutant collagen in bone and skin may account for the presence of bone fragility and the absence of clinical abnormalities of the skin.

**EXPERIMENTAL PROCEDURES**

**Clinical Summary**—A diagnosis of OI type IB was made for both patients based on persistence of gray-blue scleral color in association with dentinogenesis imperfecta and bone fragility (4).

**OI-165**—This 11-year-old boy with OI, whose birth length was on the 10th percentile, was diagnosed at birth because of leg bowing. A skeletal survey showed healing fractures in the leg, arm, and rib region and approximately 40 fractures including malunion of a left femoral fracture at 2 months that resulted in a shortening with an 8.5-cm leg length discrepancy. His sclera were moderately dark blue-gray, grade 4/8 (25). Dentinogenesis imperfecta was present in primary teeth, although his permanent teeth were not noticeably affected. Head circumference was on the 98th percentile and height less than the 3rd percentile. He had marked hypermobility of distal and middle interphalangeal joints and moderate hypermobility of the left knee. Apart from the leg-length discrepancy and mild anterior bowing of both legs, there were no other deformities of the long bones. He had a mild postural scoliosis due to his leg length discrepancy. Skeletal radiographs showed generalized osteopenia but relatively wide cortices at the mid-shaft of long bones (Fig. 1a). Skull x-rays showed multiple Wormian bones. A CT scan of the crano-cervical junction showed basilar impression. While neither parent was affected, heterozygosity for the mutation in the proband indicated that this was a new dominant mutation.

**OI-197**—This family included an affected father and daughter. The proband was a female aged 13 years. Her birth length was on the 3rd percentile. She had her first fracture aged 13 months and subsequently had more than 120 fractures. There were no known fractures of femora or vertebrae. Her sclera were intensely blue-gray, grade 5/8. Dentinogenesis imperfecta was present in both primary and permanent teeth. Head circumference was on the 98th percentile and height less than the 3rd percentile. Her skull showed temporal bulging and ocipital bossing. The right arm had a fixed flexion deformity due to an old fracture. There was no structural deformity in the lower limbs apart from right genu valgum. She had marked hypermobility of proximal and distal interphalangeal joints of the fingers and in metatarso-phalangeal joints. Hearing was normal. Skeletal radiographs showed generalized osteopenia in the long bones (Fig. 1b) and spine with normal height of most vertebrae (Fig. 1c). Skull x-ray showed multiple Wormian bones without evidence of basilar impression. The 51-year-old father had intensely blue-gray sclerae, arcus corneae, height less than the 3rd percentile, head circumference on the 98th percentile, and hearing impairment. His teeth had prematurely worn and were extracted at 15 years of age. He had no fractures but suffered back and knee pain associated with osteoporosis. X-rays of his spine and long bones showed mild osteopenia and widened disc spaces.

**Cell Culture**—Samples of skin and bone from patients and age-matched controls were obtained during routine surgery with informed consent and approval of the Ethics Committee of this hospital. Skin fibroblasts were established from biopsies and grown as described previously (26, 27). Osteoblasts cultures (OI-197) were established from biopsies and grown as described previously (26, 27). Osteoblasts cultures (OI-197) were established from biopsies and grown as described previously (26, 27).

**In Vitro Matrix Analysis**—Matrix deposition was induced by ascorbate in long term culture of fibroblasts and osteoblasts (18, 28). From confluence, the osteoblasts were grown for another 14 days, and fibroblasts for another 21 days, in DMEM medium containing 10% (v/v) fetal calf serum and 0.25 mM ascorbic acid. The medium was then replaced with 10 ml of DMEM medium containing 10% (v/v) dialyzed fetal calf serum, 0.25 mM sodium ascorbic acid, and 10 μCi/ml L-[2,3-3H]proline (44.5 Ci/mmol, DuPont NEN) for 18 h in DMEM containing 10% (v/v) dialyzed fetal calf serum (26, 27). The cell and medium fractions were harvested separately, and procollagen were precipitated with (NH₄)₂SO₄ at 25% saturation and converted to collagen by limited pepsin digestion before electrophoresis (26, 27). In some experiments the 0.25 mM sodium ascorbate in the labeling medium was replaced with 0.1 mM α-dipyrpyridyl to prevent the post-translational hydroxylation of the procollagens. The cell fraction was collected, and the unhydroxylated procollagen was analyzed by electrophoresis after precipitation with (NH₄)₂SO₄ at 25% saturation.

**Collagen Biosynthetic Labeling**—After culture for 3 days in DMEM containing 10% (v/v) fetal calf serum and 0.25 mM ascorbate (Sigma), confluent cells were labeled with 10 μCi/ml L-[2,3-3H]proline (44.5 Ci/ml, DuPont NEN) for 18 h in DMEM containing 10% (v/v) dialyzed fetal calf serum (26, 27). The cell and medium fractions were harvested separately, and procollagen were precipitated with (NH₄)₂SO₄ at 25% saturation. The phenotype of the osteoblasts was verified by measurement of alkaline phosphatase activity.

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diodeactive gels are described elsewhere (26, 27). Coomassie-stained collagen bands were quantified by densitometry (Bio-Rad GS-670 densitometer) by comparison to standard collagen samples loaded on to each gel. The radioactivity in each collagen band was determined by excision and scintillation counting (29).

RT-PCR and cDNA Sequencing—Total cytoplasmic RNA was purified from skin fibroblasts cultures (30), and first-strand cDNA was synthesized using a RT-PCR kit (Perkin-Elmer) using an α2(I) specific primer COL1 (Table I). cDNA corresponding to exons 6–30 of the COL1A2 gene was amplified with COL23 and COL1 primers (Table I).

The PCR products were purified by electrophoresis on a 0.8% (w/v) agarose gel and recovered by electroelution, phosphorylated with T4 polynucleotide kinase and cloned into the SmaI site of M13mp18 vector (Amersham Corp.). Single-stranded DNA preparations from the individual clones were sequenced using a Sequenase kit (U. S. Biochemical Corp.) (31).

Southern Blot Analysis—Genomic DNA was prepared from confluent skin fibroblasts (32) established from OI-165 and OI-197, and from unrelated healthy individuals as controls. Whole blood genomic DNA was also prepared from the father of OI-197 (33). A 1341-bp PstI/Ncol fragment encompassing exons 9–30 of the COL1A2 gene was isolated from a cDNA clone (34) and used as a probe for Southern blot analysis. Approximately 30 ng of the probe was labeled with [32P]dCTP (3000 Ci/mmol, Amersham Corp.) (31).

Electrophoresis of collagens produced from skin fibroblasts in culture. a, fibroblasts were labeled with [3H]proline in the presence of ascorbic acid (see Experimental Procedures for details). The collagens in the cell layer (lanes 1–3) and secreted into the medium (lanes 4–6) were digested with pepsin and analyzed on a 5% (w/v) polyacrylamide gels. Unhydroxylated procollagens were also produced from fibroblast cultures in the presence of α,α-dipyridyl, and the procollagens in the cell layer were analyzed after reduction with 10 mM dithiothreitol (lanes 7–9). Shortened forms of the type I collagen chains were designated as α1(I)*α2(I) for OI-197 and α1(I)**α2(I)** for OI-165. b, pepsin-digested collagens from the medium fractions were also subjected to fibroblast collagenase digestion, and the resultant TCA and TcB fragments were analyzed on a 7.5% (w/v) polyacrylamide gels.

**Fig. 2.** Electrophoresis of collagens produced from skin fibroblasts in culture. a, fibroblasts were labeled with [3H]proline in the presence of ascorbic acid (see Experimental Procedures for details). The collagens in the cell layer (lanes 1–3) and secreted into the medium (lanes 4–6) were digested with pepsin and analyzed on a 5% (w/v) polyacrylamide gels. Unhydroxylated procollagens were also produced from fibroblast cultures in the presence of α,α-dipyridyl, and the procollagens in the cell layer were analyzed after reduction with 10 mM dithiothreitol (lanes 7–9). Shortened forms of the type I collagen chains were designated as α1(I)*α2(I) for OI-197 and α1(I)**α2(I)** for OI-165. b, pepsin-digested collagens from the medium fractions were also subjected to fibroblast collagenase digestion, and the resultant TCA and TcB fragments were analyzed on a 7.5% (w/v) polyacrylamide gels.
fragments from controls. Within the 14-kb fragment, there is a polymorphic EcoRI site (35) resulting in cleavage of this fragment into 10.5- and 3.5-kb fragments. The proband and the affected father of OI-197 were (−/−) for the EcoRI polymorphism. An additional fragment of approximately 11 kb was evident in OI-197 and her affected father, whereas an additional 8-kb band was observed in OI-165 (Fig. 5). These findings indicated genomic deletions of approximately 3 and 6 kb within the 14-kb EcoRI fragment of OI-197 and OI-165, respectively. OI-165 was (del/−) for the EcoRI polymorphism since the deletion encompasses the region of the polymorphic EcoRI site within the mutant allele. While the affected father of OI-197 showed a milder phenotype, he is unlikely to be a mosaic for the mutation since the mutant and normal bands are of equal intensity in both the father and the proband (Fig. 5).

Genomic Deletion Breakpoints—The deletion breakpoints were identified using genomic PCR with primers positioned in adjacent exons (Table I). The primer sets selected for genomic PCR would not have amplified any fragments from controls as the expected products were too large for the PCR protocol used. However, a 2.7-kb product was obtained using primer set COL2/3 COL31 from OI-197 and her affected father (Fig. 6b) and a 0.27-kb product was obtained using primer set COL32/3 COL39 from OI-165 (Fig. 6a). Sequencing of the 0.27-kb fragment from OI-165 showed the deletion breakpoints to be in introns 9 and 17 (Fig. 6a). Sequencing from both the upstream and downstream ends of the 2.7-kb product from OI-197 showed sequences from intron 6 and intron 11, respectively (36). The deletion breakpoint in intron 6 was approximately 2250 bp from its upstream end and approximately 1150 bp from...

**Fig. 3.** Electrophoresis of RT-PCR products amplified from fibroblast mRNA. CDNA was amplified for 35 cycles of the PCR using primers COL1 and COL23 as described under “Experimental Procedures.” The location of the primers relative to the pro-α2(I) chain and the CNBr peptides is shown diagrammatically. All of the fragments and products were determined in retrospect from the published sequence (35) and are indicated. Lane 1, HaeIII-digested αX174 molecular weight markers; lane 2, control; lane 3, OI-197; lane 4, OI-165.

**Fig. 4.** Amino acid sequence deduced from cDNA sequencing of the RT-PCR products. The in-frame multi-exon deletion of the COL1A gene from the probands are shown in relation to the exon number and the amino acid sequence of the pro-α2(I) chain.

**Fig. 5.** Southern blot analysis. EcoRI-digested genomic DNA were resolved on a 0.8% (w/v) agarose gel, transferred onto a nylon Hybond N+ membrane, and hybridized to an α2(I) CDNA probe (see “Experimental Procedures” for details). wt (+/+) represents the wild type DNA homogenous for an EcoRI polymorphic site within the 14-kb fragment. wt (−/−) represents the homozygous wild type DNA, which lacks the EcoRI polymorphic site. The position of the EcoRI fragments, the polymorphic EcoRI site (arrow E) and the genomic deletions in the probands relative to the exon/intron organization of the COL1A gene are shown. The COL1A exons that hybridize to the labeled CDNA probe are indicated.
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Fig. 6. Genomic breakpoints. Genomic DNA fragments from both probands were amplified using primers described under “Experimental Procedures.” The resultant PCR products were cloned and sequenced. The exact nucleotide breakpoints for OI-165 (a) and OI-197 (b) are shown relative the exon/intron organization of the COL1A2 gene. The numbers above the vertical bars (exons) represent the exon numbers. The arrows and the corresponding numbers represent the relative location and orientation of the primers used for genomic PCR of both normal and mutant sequences. All the primers are within the exons indicated with the exception of COL45, which is a primer in intron 6.

Fig. 7. Electrophoretic analysis of in vitro and in vivo collagenous matrix from skin and bone. An in vitro matrix was induced by using long term culture of osteoblasts (a) and skin fibroblasts (b), with ascorbate. The cultures were labeled for 18 h with [3H]proline, and the radiolabeled collagens deposited into the in vitro matrices were sequentially extracted with 50 mM Tris/HCl, pH 7.5, containing 150 mM NaCl, 5 mM EDTA, 5 mM phenylmethylsulfonyl fluoride, and 10 mM N-ethylmaleimide (NS), 0.5 M acetic acid (HAC), and limited pepsin-digestion (pepsin) as described under “Experimental Procedures.” These extracts, together with the medium fraction (med), were quantitatively loaded and analyzed on 5% (v/v) polyacrylamide gels. The collagens extracted from the bone matrix (OI-197), and skin matrix (OI-165 and OI-197) were also analyzed following limited pepsin digestion. These samples were not loaded quantitatively, but instead loading volumes were adjusted to obtain similar collagen concentrations. The shortened forms of the α1(I)∗ and the α2(I)∗ (OI-197) are indicated by small and large arrows, respectively, and α1(I)** and the α2(I)** (OI-165) are indicated. The samples were analyzed under non-reducing conditions. The migrations of α1(I), α2(I) chains and cross-linked α-chain dimers (β-components), type I collagen trimers (α1(II)∗), and type V collagen α1(V) and α2(V) chains are shown.

greater proportion of mutant chains in the neutral salt extract, which contained mainly intracellular collagen, than in the medium. However, a significant proportion of the mutant collagen was deposited into the osteoblast-produced extracellular matrix in vitro (Fig. 7a), demonstrated by the presence of the mutant α2(I)∗ chain in the collagen extracted with 0.5 M acetic acid and, to a lesser extent, in the pepsin extract. This result was also consistent with analysis of OI-197 bone, which demonstrated the presence of mutant molecules in pepsin extracts of the mature bone matrix (Fig. 7a).

In contrast, mutant α2(I)∗ chains were not detected in the matrix of long term (21 days) skin fibroblast cultures from OI-197 and OI-165 (Fig. 7b). This finding was consistent in three separate experiments. The mutant α2(I)∗ chains were produced by the fibroblasts but were largely restricted to the neutral salt extracts. In OI-165 fibroblasts, the labeled mutant chains were poorly secreted with only a trace amounts of the mutant molecules in the medium fraction, while more significant proportion of the mutant molecules were secreted from OI-197 fibroblasts (Fig. 7b). These findings were again consistent with the in vivo analysis of the collagen extracted from skin for OI-165 and OI-197, which showed a normal collagen composition with no detectable mutant α2(I)∗ (OI-197) or α2(I)∗ (OI-165) chains. As expected, the fibroblasts from both patients produced a matrix in vitro with a reduced collagen content (estimated from three separate experiments to be approximately 45% for OI-197 and 25% for OI-165 of that produced by control cells) consistent with the reduced collagen content of skin extracts.

DISCUSSION

The two OI type IB patients were shown to be heterozygous for large multi-exon deletions in the COL1A2 gene for the α2(I)∗
chain of type I collagen. In one patient (OI-197) the genomic deletion of 3 kb encompassed exons 7–11, and in the other patient (OI-165) the 6-kb deletion removed exons 10–17. Both deletions yielded intron junctions that did not alter the splice donor and acceptor sites. The 5′ and 3′ exons were spliced normally, maintaining the translational reading frame and the repetitive Gly-X-Y triplet sequence. The deletions removed amino acids 145-296 and 94–200 from the triple helical domain of OI-165 and OI-195, respectively.

Large deletions of the type I collagen genes are infrequent in OI, and the majority of mutations are point mutations, which result in helix-destabilizing glycine substitutions, single exon-skipping mutations, premature stop codons; and frameshift mutations, which produced shortened or elongated dysfunctional collagens (1, 3, 14). There are two previously described large deletions, both of which lead to OI type II (perinatal lethal). In one of them, a deletion in COL1A1 extends from exon 23 to exon 25 (84 amino acids) (37–39); in the other, the deletion in COL1A2 extends from exon 34 to exon 40 (40). In these cases it was suggested that the deletions were produced by nonhomologous recombination. In the two patients described here, analysis of the sequences around the intron deletion breakpoints did not demonstrate the present of inverted sequences or direct repeats and there was no significant homology between the 5′ and 3′ deletion junctions. Furthermore, it was unlikely that Alu repetitive elements were involved in the deletion, since the Alu repeat elements of COL1A2 (41) are located well away from either of the deletion junctions. Thus, it is most likely that the deletions also arose through nonhomologous intron-mediated recombination.

The deletions produced pro-α2(I) chains with large internal helical deletions, and the type I collagen molecules containing these shortened chains were secreted poorly. It should be noted that the calculation of secretion was based on the distribution of pepsin-stable collagens, and therefore did not take into account intracellular mutant collagen degradation or any altered pepsin stability of mutant α2(I) chain-containing collagen trimers. However, the presence of shortened α1(I) chains following pepsin digestion, and the relatively constant ratio of total α1(I):α2(I) in combined cell and medium pools (1.98 for OI-165; 2.12 for OI-197; 2.12 for controls) suggests that the collagen triple helix carboxyl-terminal to the mutation is largely pepsin resistant. Increased pepsin sensitivity, if present, would probably be most evident in intracellular mutant collagens undergoing assembly and helix folding, and result in dramatically reduced intracellular collagen levels after pepsin digestion. Both this and intracellular degradation of mutant-containing collagen molecules would result in an artificial overestimate of mutant collagen secretion efficiency. These arguments suggest that the reduced collagen secretion measured in our experiments is probably an underestimate of the extent of the mutant collagen secretion defect.

The retarded secretion of structurally abnormal type I collagen is a common finding in OI and represents an important intracellular “quality control” mechanism (26, 42). The retention of the mutant-containing collagen trimers within the cells results in increased intracellular breakdown, via endoplasmic reticulum-mediated and lysosomal degradative pathways (43), resulting in reduced collagen in the extracellular matrix. This deficiency was reflected in the marked reduction in the collagenous matrix deposited by dermal fibroblasts (OI-165 and OI-197) and osteoblasts (OI-197) grown in long term cultures in the presence of ascorbic acid. This collagen deficiency was confirmed in the skin and bone from OI-197 and skin from OI-165.

In OI-165, serial extraction of the in vitro extracellular collagen matrix formed by dermal fibroblasts with acetic acid and then pepsin to extract the progressively more cross-linked collagen, demonstrated that the mutant collagen was excluded from the mature in vitro matrix. Pepsin extracts of skin tissue samples confirmed the absence of the mutant collagen. Exclusion of collagen containing mutant pro-α1(I) chains from a dermal fibroblast matrix formed in vitro in the presence of dextran sulfate has been previously reported (12). Unfortunately bone samples or osteoblast cultures were not available from OI-165, and we were unable to determine whether this mutation was included or excluded from the bone matrix.

For OI-197 skin fibroblasts, bone cells and tissue samples were available, enabling us to compare the behavior of the mutant collagen in these tissues. The results confirm that the mutant pro-α2(I) chains are expressed by both fibroblast and osteoblast cultures (44). Studies comparing matrix formation in vitro in long term fibroblast and osteoblast cultures provided some interesting findings. Collagen deposition into the matrix was dramatically reduced in both fibroblast and osteoblast matrices, consistent with the tissue collagen deficiencies and with previous in vitro experiments demonstrating the reduced collagen deposition by OI fibroblasts (18). A reduction in collagen production has also been demonstrated for OI osteoblasts (45), but in these studies matrix deposition was not assessed. However, while serial extraction demonstrated that the mutant collagen was excluded from the mature in vitro and in vivo skin matrix, when bone cell culture and bone tissues were examined a totally different pattern emerged. In extracts of the osteoblast in vitro matrix, the mutant collagen was incorporated into the mature cross-linked collagenous matrix. The presence of the mutant collagen in pepsin extracts of mature bone tissue directly demonstrated that not only is the mutant incorporated into the matrix, but the mutant collagen is not degraded and remains a stable component of the mature matrix.

The mechanism of how bone and skin matrices discriminate and differentially incorporate the OI-197 mutant collagen is not known. It is becoming increasingly clear that collagen fibrillogenesis and maturation is a complex multistep process involving heterotypic collagen associations and interactions with other matrix components such as decorin and fibromodulin (46, 47). While the predominant collagen of both skin and bone is type I collagen, there are many differences in the composition of other matrix components between these tissues, including the ability to mineralize, which may influence the pattern of collagen deposition and maturation. Several detailed structural studies have determined that the packing of collagen into fibrils, and the resulting molecular arrangement of adjacent collagen molecules, is different in bone to that in soft tissues such as skin and tendon (48, 49). This alternate packing is most noticeably reflected in the different patterns of intermolecular cross-links in soft and hard tissues (50, 51). The inclusion of the mutant collagen in the bone, but not skin matrix, may reflect this difference in packing, suggesting that the molecular arrangement in bone may be more permissive for the incorporation of the mutant molecules.

Previous studies have demonstrated that some collagen helical mutations have a long range effect on procollagen structure at the N-propeptide cleavage site (52). The resulting altered conformation of this cleavage site reduces procollagen processing and results in an accumulation of pN-collagen. The retention of the type I procollagen N-propeptide on a proportion of the mutant collagen molecules in vivo would present a steric barrier to correct fibrillogenesis analogous to that seen in

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2 Amino acids are numbered from the NH2-terminal end of the collagen triple helix, and nucleotides numbered from the transcription start site (35).
Ehlers-Danlos syndrome type VII (53, 54). In these Ehlers-Danlos syndrome patients, deavage site mutations or N-proteinase deficiency result in the processing defect, which manifests clinically in skin and joint laxity, although there are reports of wormian bones and blue sclera (55) suggesting that the defects are not totally confined to the soft tissues. In both the OI type IB patients, the mutant collagen is excluded from the skin matrix, and thus there is no opportunity for the expression of the Ehlers-Danlos syndrome type VII clinical phenotype. In OI-197 bone the mutant collagen, which is shortened by a multi-exon deletion in the helical domain, may also have reduced N-propeptide processing, and the inclusion of this structurally abnormal collagen pN-collagen into the bone matrix may contribute to the pathological effect of the mutations on the bone matrix.

These studies also highlight important issues in the extrapolation from biochemical data obtained in fibroblast culture to the definition of the molecular pathology of the mutant protein in bone, the primary affected tissue. Likewise, these studies also demonstrate the differential matrix incorporation of collagen with different mutations, since our previous studies have shown that collagen with a helical glycine substitution mutation on the incorporation of collagen into a functional matrix may contribute to the pathological effect of the mutations on the bone matrix.

In an attempt to address these questions, further experiments are under way involving the stable transfection of fibroblast and bone cells with mutant α(I) collagen genes and the comparison of the biochemical phenotypes that result.

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