Biochem. J. (1992) 288, 131–135 (Printed in Great Britain)

Characterization of three osteogenesis imperfecta collagen α1(I) glycine to serine mutations demonstrating a position-dependent gradient of phenotypic severity

John F. BATEMAN,* Ingrid MOELLER, Marnie HANNAGAN, Danny CHAN and William G. COLE
Department of Paediatrics, University of Melbourne, Royal Children's Hospital, Parkville, Victoria 3052, Australia

Type I collagen α1(I) glycine to serine substitutions, resulting from G-to-A mutations, were defined in three cases of osteogenesis imperfecta (OI). The Gly substitutions displayed a gradient of phenotypic severity according to the location of the mutation in the collagen triple helix. The most *C*-terminal of these, Gly^{565} to Ser, led to the lethal perinatal (type II) form of OI, whereas the more *N*-terminal mutations, Gly^{415} and Gly^{352} to Ser, led to severe OI (type III/IV) and moderate OI (type IVB) respectively. These data support the notion that glycine substitutions towards the *C*-terminus of the α1(I) or α2(I) chains will be more clinically severe than those towards the *N*-terminus. This results from the more disruptive effect of the mutations at the *C*-terminus on helix initiation and *C*- and *N*-terminal helix directional propagation. This generalization must be modified by considering the nature of the glycine substitution and the surrounding amino acid sequence, since the helix is composed of subdomains of differing stability which will affect the ability of helix re-nucleation and propagation.

INTRODUCTION

Mutations in the genes for the proα(I) chains (*COL1A1*) and proα2(I) chains (*COL1A2*) of type I collagen have been defined as the molecular basis of the heritable connective tissue disorder, osteogenesis imperfecta (OI), in at least 90% of cases studied to date (for reviews see [1,2]). These mutations cover the gamut of possible gene defects, including rearrangements, deletions, insertions and a spectrum of point mutations.

A relatively common type I collagen mutation in OI is the substitution of glycine residues within the helical domain of the α1(I) and α2(I) chains. The occurrence of glycine in the triplet amino acid repeating sequence Gly-Xaa-Xaa is critical for the efficient formation and stability of the triple helix, the functional unit of the extracellular collagenous matrix. The substitution of any of these glycine residues disturbs helix propagation, leading to increased levels of lysine post-translational modification, reduced collagen secretion, increased degradation and reduced helical stability [3–5]. It has been proposed that glycine substitutions towards the *C*-terminus of the α-chains will be more clinically severe than those towards the *N*-terminus, consistent with the model that, since helix formation propagates from the *C*- to *N*-terminus, those mutations towards the *C*-terminus will be more disruptive to helix formation [1]. The best evidence in support of this comes from the α1(I) Gly to Cys mutations [1,2]. However, the correlation of mutation position and clinical severity with other Gly substitutions is less clear, and it is becoming apparent that the nature of the glycine substitution and the surrounding amino acid sequence must be considered [1,6]. Many more mutations spanning the collagen helical domain will need to be characterized before any strict correlation can be made between the biochemical and clinical phenotypes.

In this paper we define point mutations resulting in Gly to Ser substitutions in the proα1(I) chains of three cases of OI. The most *C*-terminal of these, Gly^{565} to Ser, leads to the lethal perinatal (type II) form of OI, whereas the more *N*-terminal mutations, Gly^{415} and Gly^{352} to Ser, lead to severe OI (type III/IV) and moderate OI (type IVB) respectively.

EXPERIMENTAL

Clinical summary

A male baby, O139, died 6 h after birth from perinatal lethal (type II) OI [7]. O158 had a severe form of OI that was classified as type III/IVB. She died unexpectedly at 19 months of age. She had pale blue sclerae, dentinogenesis imperfecta, and severe fragility and bowing of the long bones. O168 has type IVB OI. He is a 7-year-old boy with pale blue sclerae and dentinogenesis imperfecta. He has moderately severe short stature, well-aligned limbs, few fractures and a normal gait. Skin biopsies were obtained from the patients, the parents of O139 and O168, and controls with the approval of the Institutional Human Ethics Committee.

Fibroblast collagen biosynthetic labelling

After culture for 3 days in growth medium containing 0.25 mM-sodium ascorbate (Sigma), confluent fibroblast cultures were labelled with 10 μCi of L-[5-^{3}H]proline/ml (30 Ci/mmol; Amersham Corp.) for 18 h in medium containing 10% (v/v) dialysed fetal calf serum (Flow Laboratories), 0.25 mM-sodium ascorbate and 0.1 mM-β-aminopropionitrile fumarate (Sigma) [4,5]. The cell layer and medium fractions were separated for analysis and the procollagens, precipitated with 25%-saturated $(NH_4)_2SO_4$, were subjected to limited proteolysis with pepsin [4,5].

SDS/polyacrylamide gel electrophoresis

Collagen α-chains were resolved on 5% (w/v) polyacrylamide separating gels containing 2 M-urea [4,5]. The α-chains were detected by fluorography [8].

Thermal stability of collagen

The freeze-dried [^{3}H]proline-labelled collagens were dissolved in 0.4 M-NaCl/0.1 M-Tris/HCl buffer, pH 7.4, at 4 °C. The samples were warmed stepwise (1 °C/min) from 34 °C to 43 °C, and at 0.5 °C intervals samples were taken and digested with a mixture of trypsin and chymotrypsin [9]. The proportion of the collagen that was resistant to proteolysis at each temperature

Abbreviations used: OI, osteogenesis imperfecta; T_m, thermal denaturation temperature.

* To whom correspondence should be addressed.

interval was determined by scintillation counting of collagen α-chain bands that were resolved by electrophoresis and identified by fluorography [10]. The thermal denaturation temperature, T_m, was defined as the temperature at which half of the collagen was degraded.

Formation and chemical cleavage of mRNA:cDNA heteroduplexes

Total RNA was isolated from confluent fibroblast cultures [11,12]. mRNA:cDNA heteroduplexes were formed in a volume of 50 μl containing approx. 5 ng (50000–100000 d.p.m.) of labelled cDNA probe, 5–10 μg of total RNA, 80% (v/v) formamide, 40 mM-Pipes, pH 6.4, 1 mM-EDTA and 0.4 M-NaCl. The mixture was denatured at 80 °C for 5 min, incubated at 60 °C for 2 h and ethanol-precipitated. Chemical modification of mismatched nucleotides with hydroxylamine or osmium tetroxide, cleavage with piperidine and electrophoresis of the products on denaturing 7 M-urea/5% acrylamide gels have been described [13–16].

The apparent site of the mutation in the collagen chains, based on the localization of regional protein overhydroxylation, was used as a guide to the selection of suitable control cDNAs for heteroduplex formation. A control α1(I) cDNA probe, chosen to span the abnormal region, was purified and end-labelled with [α-^{32}P]dCTP by the fill-in reaction using the Klenow fragment of DNA polymerase I [17].

Amplification and sequencing of cDNA

First-strand cDNA was synthesized from total RNA using a cDNA synthesis kit (Amersham Corp.) primed with specific oligonucleotide primers which were also used for PCR amplification. Approx. 50 ng of cDNA was amplified by the PCR [18] through 30 cycles using Taq polymerase (Perkin Elmer–Cetus) [19]. Each cycle consisted of denaturation at 92 °C for 30 s, annealing of primers at 64 °C for 1 min and primer extension at 72 °C for 3 min [15,16]. In samples from OI58 and OI68 a 466 bp fragment corresponding to bases 1505–1970 [numbered from the start of transcription of the proα(I) mRNA] of the α1(I) cDNA [20] was amplified with the primers 5′-TGCTGGAGAGGAAG-GAAA-3′ and 5′-TCCAGCCTCTCCATCTTT-3′. Sample O139 was amplified with the primers 5′-AAGAGGCGAGAGAG-GTTT-3′ and 5′-GACCACTTTCACCCTTGT-3′ to give a 332 bp fragment covering bases 2144–2475 of the α1(I) cDNA sequence.

Amplification products of the predicted size were purified, treated with T4 polynucleotide kinase [17] and cloned into a *Sma*I-cut dephosphorylated M13mp8 vector (Amersham). Multiple clones of OI39 and OI58 DNA from independent amplification reactions were sequenced using a Sequenase kit (United States Biochemical Corp.) and the M13 universal primer. The amplification products of OI39 and OI68 were also directly sequenced [21] using the primers 5′-GACCACTTTC-ACCCTTGT-3′(O139) and 5′-TGCTGGAGAGGAAGGAAA-3′(O168).

RESULTS

Collagen protein analysis

Three cases of OI showed abnormal, slowly migrating type I collagen α1(I) and α2(I) chains when compared with age-matched control fibroblast collagen α-chains (Fig. 1). The slow migration in OI39 was directly demonstrated to be due to excessive levels of post-translational hydroxylation of lysine [5]. Analysis of the CNBr peptides of the α1(I) chains showed that the over-modification was restricted to the α1(I) CB8 and CB3 peptides, suggesting that the underlying mutation was in the central region of the helix (results not shown). Fibroblast type I collagen secretion was impaired and bone and dermal tissue showed a reduction in type I collagen to approximately half control values [5]. In the less severe cases, OI58 and OI68, fibroblast collagen production was not reduced relative to age-matched control fibroblasts and collagen secretion was not noticeably impaired [22].

Electrophoretic analysis of the type I collagen produced by fibroblast cultures from the parents of OI39 and OI68 showed only normally migrating α1(I) and α2(I) chains (results not shown). The parents of OI58 were unavailable for study.

Detection and sequencing of abnormal mRNA sequences

Heteroduplexes were formed using a control α1(I) cDNA 2278 bp *Xho*I-*Xho*I fragment which spanned the central portion of the collagen helix, and mRNA extracted from cultured OI and control dermal fibroblasts. Hydroxylamine/piperidine treatment resulted in cDNA cleavage in all three cases, demonstrating the presence of mismatched C nucleotides producing labelled cleavage fragments of control cDNA of 176 bp, 365 bp and 815 bp in OI68, OI58 and OI39 respectively (Figs. 2*a* and 2*b*). Treatment with osmium tetroxide/piperidine did not reveal any T mismatches.

Hydroxylamine/piperidine treatment of heteroduplexes formed with mRNA from fibroblast cultures of the parents of OI39 and OI68 indicated that the mutations were not present.

To define the mutations, short lengths of first-strand α1(I) cDNA were amplified by the PCR using unique oligonucleotides chosen to span the regions containing the mismatches. For OI58, sequencing of M13 subclones of the amplified products identified the mutation as a single base substitution at base pair 1896 which converted the codon for glycine (GGC) to AGC (serine) at amino acid position 415 (numbered by the standard convention in which the first glycine of the triple helical domain of the α-chain is numbered one) of the helix (Fig. 3). The abnormal sequence was confirmed by sequencing of multiple clones from two separate PCR reactions. Clones containing the normal sequence (GGC) were also identified, indicating that the patient was heterozygous for the α1(I) mutation.

In O139, subcloning and sequencing identified a G to A mutation at base 2346 of the proα1(I) sequence, resulting in a

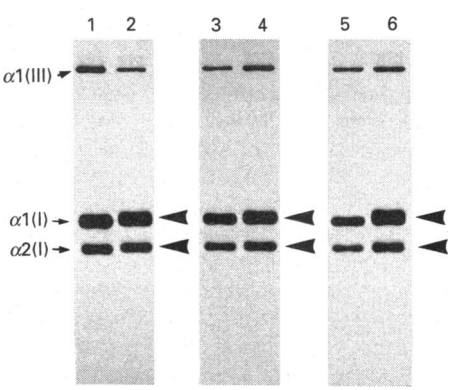

Fig. 1. Electrophoresis of pepsin-digested fibroblast collagens

Fibroblast cultures were labelled for 18 h with [^{3}H]proline and the collagens secreted in the medium were pepsin-digested and analysed without reduction on SDS/polyacrylamide gels (5%) (see the Experimental section for details). Lanes 1, 3 and 5, control fibroblasts; lane 2, OI39; lane 4, OI158; lane 6, OI68 fibroblast collagen. The migration positions of type I collagen α1(I) and α2(I) chains and type III collagen [α1(III)] are shown. The slow migration of the OI fibroblast α-chains due to increased lysine post-translational modification is indicated by arrowheads.

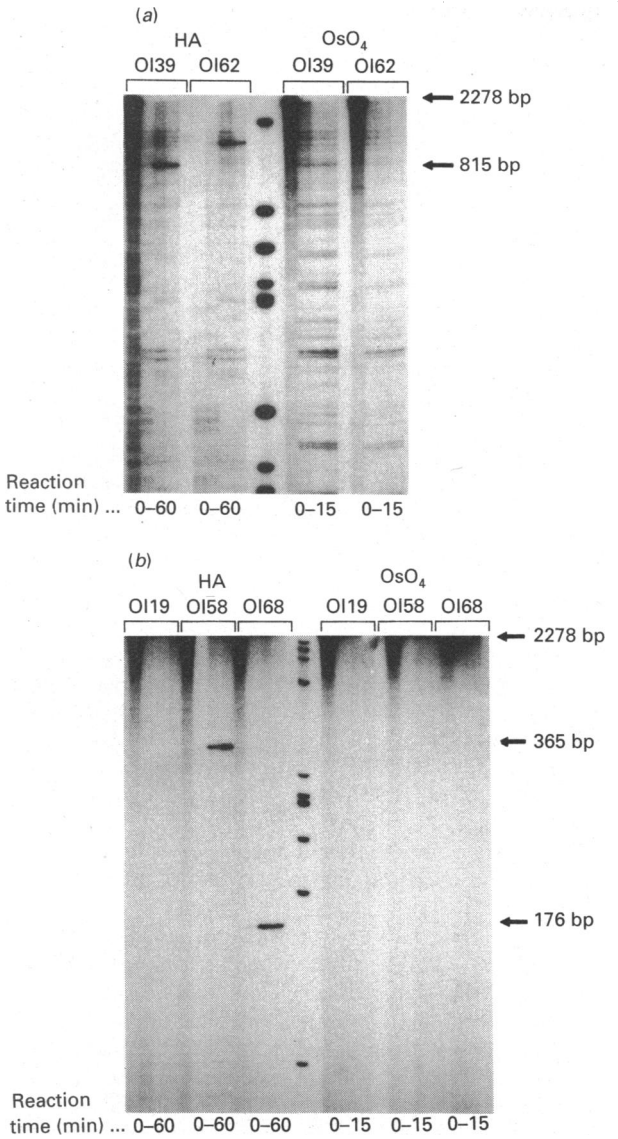

Fig. 2. Detection of the α1(I) mRNA mutations by chemical modification and cleavage

Total RNA from control and OI fibroblasts was hybridized to a 2278 bp *Xho*I–*Xho*I ^{32}P-labelled fragment of the α1(I) cDNA and reacted with hydroxylamine or osmium tetroxide for 0–60 min as indicated, then treated with piperidine and the resultant fragments resolved on a 5% (w/v) polyacrylamide gel (see the Experimental section for details). (*a*) In OI39, cleavage at mismatched and thus reactive Cs by hydroxylamine (HA) produced a 815 bp labelled fragment. OI62 has an undefined mutation resulting in different cleavage fragment and is included as a chemical cleavage control for the mismatch in OI39. Treatment with osmium tetroxide (OsO_4), which detects mismatched Ts, did not produce a cDNA cleavage. ^{32}P-labelled molecular size markers of 255, 300, 397, 404, 427, 492, 598 and 1027 bp were produced by *Msp*I digestion of the 21-hydroxylase B gene. (*b*) In OI58 or OI68, cleavage at mismatched Cs by hydroxylamine produced labelled fragments of 365 bp and 176 bp respectively. No mismatched Ts were detected by OsO_4 treatment. The chemical cleavage control was OI19, which contains no mutations in this region of the mRNA. ^{32}P-labelled *Hae*III fragments of ΦX174 DNA molecular size standards were used to calculate the sizes of the cleavage fragments.

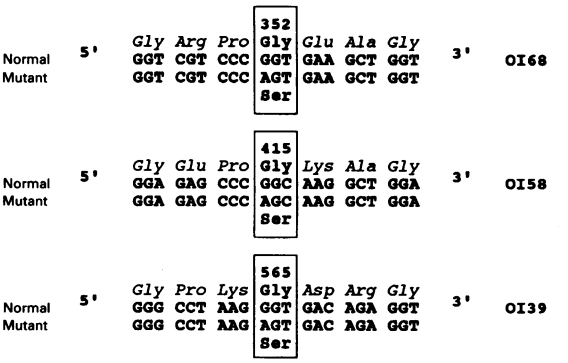

Fig. 3. DNA sequence of the OI mutations

The mRNA regions containing the mismatched bases were PCR-amplified and sequenced (see the Experimental section for details). The coding sequence of the normal and mutant alleles is shown, with Gly to Ser substitutions at positions 352, 415 and 565 of the α1(I) helix indicated.

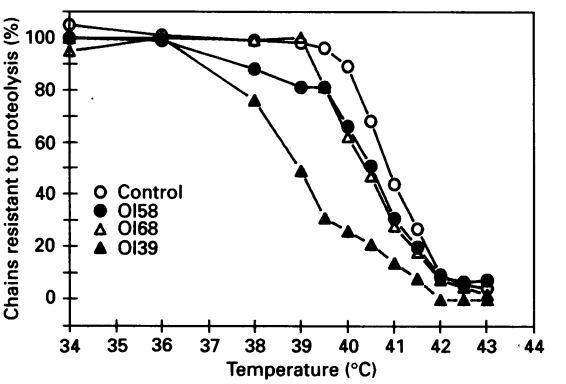

Fig. 4. Thermal denaturation of type I collagen

[^{3}H]Proline-labelled collagens were prepared from the medium of OI45 and control fibroblasts. They were treated with pepsin, and after inactivation of the pepsin the collagens were warmed slowly from 34 °C to 43 °C. Serial samples were taken and digested with trypsin/chymotrypsin, and the resistant chains were resolved by electrophoresis. The melting temperature of the helix was determined by the relative proportion of the α-chains that were resistant to proteolysis (see the Experimental section for details).

heterozygous Gly^{565} to Ser substitution (Fig. 3). This mutation was confirmed by direct sequencing of the PCR-amplified product in which two sequences were apparent (G and A) at base pair 2346, representing the sequence of the mutant and normal alleles (results not shown). In direct sequencing of the OI68 PCR products, two alternatives (G and A) were also identified at base pair 1707, demonstrating that this patient was also heterozygous for a G to A transversion, resulting in the substitution of Gly^{352} by Ser in the product of the mutant allele.

Collagen melting temperature

Estimation of the thermal denaturation temperature (T_m) of type I collagen synthesized by OI and control fibroblasts was made by measuring its resistance to proteinase digestion after heating to different temperatures (Fig. 4). In all cases the T_m was reduced relative to that of control type I collagen, but the magnitude of this reduction was greatest in the clinically most severe case, OI39. The T_m of OI39 type I collagen was approx. 39.0 °C compared with 40.9 °C for control type I collagen. In OI58 and OI68 the T_m was 40.5 °C and 40.4 °C respectively, representing a decrease compared with controls of 0.4 °C and 0.5 °C respectively. Since the OI cases were heterozygous, this reduction in T_m probably represents an underestimate of the

reduction in the stability of the mutant type I molecules. The T_m of OI type III collagen was the same as in the control (results not shown).

DISCUSSION

The three α1(I) point mutations in these cases of OI were heterozygous for G to A transitions in glycine codons at base pairs 1707, 1896 and 2346, resulting in Gly to Ser substitutions at amino acids 352, 415 and 565. The mutations were away from exon–intron boundaries and thus must represent *COL1A1* point mutations. In OI68 (Gly^{352} to Ser) and OI39 (Gly^{565} to Ser), protein analysis demonstrated normal parental collagen. Furthermore, in OI39, analysis of amniotic cell collagen from a subsequent pregnancy showed normal fetal collagen in twin babies (J. F. Bateman & T. Mascara, unpublished work). These data suggest that, as in the majority of other OI cases [1,2], these cases represented sporadic new mutations. Parental mosaicism has been described in OI [23,24] and was not formally discounted in these cases.

These cases demonstrate a gradient of clinical severity from the more *C*-terminal glycine-to-serine substitution (OI39, Gly^{565}) leading to lethal perinatal OI, while mutations at Gly^{415} (OI58) and Gly^{352} (OI68) lead to the progressively less severe forms of OI, type III/IV and type IVB respectively. The α-chains in all three cases displayed high levels of lysine post-translational modification due to disruption of helix propagation by the glycine substitution, a common finding in OI [3–5]. This retardation of helix propagation often results in decreased collagen secretion and increased breakdown of the structurally abnormal molecule [4,5,22]. Decreased secretion was evident in the most severe case, OI39, but in OI58 and OI68 secretion was not significantly impaired. Since correct helix formation is critical for normal secretion, these data suggest that the mutations at Gly^{415} and Gly^{352} may not lead to as severe a structural abnormality of the triple helix.

The differential effect of these mutations on helix structure was also reflected in the decreased melting temperature of the type I collagen. OI39 (Gly^{565} to Ser) had a 1.4 °C reduction in T_m, while the less severe cases (Gly^{415} and Gly^{352} to Ser) had only a small reduction in T_m of 0.4–0.5 °C. However, the overall correlation of the extent of decrease of T_m with clinical severity has been poor, with some clinically mild cases showing a more significant reduction in helix stability than some lethal cases [2]. Of particular interest in this regard is the lethal case Gly^{631} to Ser [25], where the intact helix had a normal T_m but mammalian collagenase subfragments show a reduced stability relative to controls.

There have been four other cases of α1(I) helix Gly to Ser mutations characterized to date. These occur at Gly^{844} [26], Gly^{832} [27], Gly^{631} and Gly^{598} [25]. Preliminary reports have been made of a further five, at Gly^{1009}, Gly^{1003} [28], Gly^{964} [1], Gly^{913} [28] and Gly^{460} [29]. These, in general, fit the pattern that the more *C*-terminal the mutation, the more severe the clinical consequences. There are, however, two cases of OI with Gly to Ser mutations which do not fit this simple model and argue that a more comprehensive model must account for the effect of specific regional sequences. Gly^{832} [27] and Gly^{844} [26] to Ser mutations produce the milder type IV and type III phenotypes, although mutations on the *C*- and *N*-terminal sides of these produce the lethal phenotype.

These data further indicate that the model which considers the collagen helix as a single functional unit in relation to helix formation and stabilization is inappropriate, and a more detailed model must consider the helix as being composed of subdomains of differing stability. One such model considers these domains as co-operative folding blocks [30] which result in non-linear helix formation and stabilization. The position of glycine mutations within these sequence subdomains will thus have a critical role in the effect of helix propagation on destabilization.

The possible effect of the local sequence in determining the effect of the mutation on helical structure by providing domains of relatively low or high local helix stability has been explored by Bachinger & Davis [6]. Mutations in regions of low local stability would be less deleterious to helical stability. Further, according to this model, if sequences *N*-terminal to the mutation are of high stability, re-nucleation of helix formation could occur readily with minimal interruption to helix propagation. The non-lethal mutations at Gly^{832} and Gly^{844} are in such a region of lower stability, with a region of higher stability immediately *N*-terminal [6]. Thus disturbance of the helix folding caused by the mutations may be rescued by a relatively easy re-nucleation of triple helix folding after the mutations. In contrast, the mutations *N*-terminal to this region causing lethal OI (Gly^{565}, Gly^{598} and Gly^{631}) lack such *N*-terminal re-nucleation sites ([6]; H.P. Bachinger, personal communication).

Correlation of the nature of the molecular defect with the clinical severity of OI remains an important goal. The devastating effect of the glycine mutations on the collagenous matrix results, at least in part, from the reduced secretion and increased degradation induced by the defect. The degradation of molecules containing a mutant α1(I) consumes normal α1(I) chains in the same molecule [31] resulting, in the case of α1(I) mutations, in the destruction of as much as 75% of the total collagen. In transgenic mice expression of as little as 10% of a Gly to Cys mutant α1(I) gene resulted in a severe clinical phenotype [32], suggesting that this destruction of mutant-containing type I collagen molecules may not entirely account for the severity of the clinical consequences.

The explanation may lie in the highly organized molecular packing of the collagen fibril, where a small structural abnormality in a relatively few secreted mutant collagen molecules is amplified through the fibrillar architecture resulting in a functionally abnormal matrix. An example of one such structural abnormality was demonstrated by Vogel *et al.* [33]. In this case of OI a Gly^{784} to Cys mutation produced a flexible kink in the triple helix which on incorporation into fibrils *in vitro* delayed assembly and altered fibril morphology [34]. The over-glycosylation of lysine induced by the mutation may also alter the ability of the collagen to pack into the unit cell of fibril structure. X-Ray diffraction studies on non-enzymic glycosylation of rat-tail tendon collagen fibrils has demonstrated a resulting distortion of the unit cell [35]. The relevance of this model to the relatively lower amount of overglycosylation that occurs in OI collagen is still uncertain.

The amplification of small structural distortions within the collagenous fibrillar matrix may exert a profound influence on the functional properties of the matrix. In particular, the molecular effect of these distortions on bone cell expression and mineralization are important areas for further research.

This work was supported by grants from the National Health and Medical Research Council of Australia, the Royal Children's Hospital Research Foundation and the Osteogenesis Imperfecta Foundation.

REFERENCES

1. Byers, P. H. (1990) Trends Genet. **6**, 293–300
2. Kuivaniemi, H., Tromp, G. & Prockop, D. J. (1991) FASEB J. **5**, 2052–2060
3. Bonadio, J. & Byers, P. H. (1985) Nature (London) **316**, 363–366
4. Bateman, J. F., Mascara, T., Chan, D. & Cole, W. G. (1984) Biochem. J. **217**, 103–115
5. Bateman, J. F., Chan, D., Mascara, T., Rogers, J. G. & Cole, W. G. (1986) Biochem. J. **240**, 699–708

6. Bachinger, H.-P. & Davis, J. M. (1991) Int. J. Biol. Macromol. **13**, 152–156
7. Sillence, D. O., Senn, A. & Danks, D. M. (1979) J. Med. Genet. **16**, 101–116
8. Bonner, W. M. & Laskey, R. A. (1974) Eur. J. Biochem. **46**, 83–88
9. Bruckner, P. & Prockop, D. J. (1981) Anal. Biochem. **110**, 360–368
10. Bateman, J. F., Harley, V., Chan, D. & Cole, W. G. (1988) Anal. Biochem. **168**, 171–176
11. Lui, C. P., Slate, O. L., Gravel, R. & Ruddle, F. H. (1979) Proc. Natl. Acad. Sci. U.S.A. **76**, 4503–4506
12. Wake, S. A. & Mercer, J. F. B. (1985) Biochem. J. **228**, 425–432
13. Cotton, R. G. H., Rodrigues, N. R. & Campbell, R. D. (1988) Proc. Natl. Acad. Sci. U.S.A. **85**, 4397–4401
14. Dahl, H.-H. M., Lamande, S. R., Cotton, R. G. H. & Bateman, J. F. (1989) Anal. Biochem. **183**, 263–268
15. Lamande, S. R., Dahl, H.-H. M., Cole, W. G. & Bateman, J. F. (1989) J. Biol. Chem. **264**, 15809–15812
16. Bateman, J. F., Lamande, S. R., Dahl, H.-H., Chan, D., Mascara, T. & Cole, W. G. (1989) J. Biol. Chem. **264**, 10960–10964
17. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd edn., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
18. Saiki, R. K., Gelfand, D. H., Stoffel, S., Scharf, S. J., Higuchi, R., Horn, G. T., Mullis, K. B. & Erlich, H. A. (1988) Science **239**, 487–491
19. Kogan, S. C., Doherty, M. & Gitschier, J. (1987) N. Engl. J. Med. **317**, 985–990
20. Tromp, G., Kuivaniemi, H., Stacey, A., Shikata, H., Baldwin, C. T., Jaenisch, R. & Prockop, D. J. (1988) Biochem. J. **253**, 919–922
21. Bachmann, B., Luke, W. & Hunsmann, G. (1990) Nucleic Acids Res. **18**, 1309
22. Bateman, J. F., Chan, D., Lamande, S., Mascara, T. & Cole, W. G. (1988) Ann. N.Y. Acad. Sci. **543**, 95–105
23. Wallis, G. A., Starman, B. J., Zinn, A. B. & Byers, P. H. (1990) Am. J. Hum. Genet. **46**, 1034–1040
24. Cohn, D. H., Starman, B. J., Blumberg, B. & Byers, P. H. (1990) Am. J. Hum. Genet. **46**, 1034–1040
25. Westerhausen, A., Kishi, J. & Prockop, D. J. (1990) J. Biol. Chem. **265**, 13995–14000
26. Pack, M., Constantinou, C. D., Kalia, K., Nielsen, K. B. & Prockop, D. J. (1989) J. Biol. Chem. **264**, 19694–19699
27. Marini, J. C., Grange, D. K., Gottesman, G. S., Lewis, M. B. & Koeplin, D. A. (1989) J. Biol. Chem. **264**, 11893–11900
28. Cohn, D. H., Wallis, G., Zhang, X. & Byers, P. H. (1990) Matrix **10**, 236
29. Byers, P. H., Wallis, G. A. & Willing, M. C. (1991) J. Med. Genet. **28**, 433–442
30. Privalov, P. L. (1982) Adv. Protein Chem. **35**, 1–104
31. Prockop, D. J. & Kivirikko, K. I. (1984) N. Engl. J. Med. **311**, 376–386
32. Stacey, A., Bateman, J. F., Choi, T., Mascara, T., Cole, W. G. & Jaenisch, R. (1988) Nature (London) **332**, 131–136
33. Vogel, B. E., Doelz, R., Kadler, K. E., Hojima, Y., Engel, J. & Prockop, D. J. (1988) J. Biol. Chem. **263**, 19249–19255
34. Kadler, K. E., Torre-Blanco, A., Adachi, E., Vogel, B. E., Hojima, Y. & Prockop, D. J. (1991) Biochemistry **30**, 5081–5088
35. Tanaka, S., Avigad, G., Brodsky, B. & Eikenberry, E. F. (1988) J. Mol. Biol. **203**, 495–505

Received 10 March 1992/18 May 1992; accepted 29 May 1992