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Increased expression of the gene for the proα1(IV) chain of basement-membrane procollagen in cultured skin fibroblasts from two variants of osteogenesis imperfecta

Taina PIHLAJANIEMI,*,** Jeffrey McKEON,† Steffen GAY,‡ Renate GAY,‡ Wouter J. DE WET,§ Jeanne C. MYERS‖ and Darwin J. PROCKOP¶

*Collagen Research Unit, Biocenter and Department of Medical Biochemistry, University of Oulu, Kajaanintie 52 A, 90220 Oulu, Finland, †Department of Biochemistry, UMDNJ–Rutgers Medical School, Piscataway, NJ 08854, U.S.A., ‡Department of Medicine, University of Alabama, Birmingham, AL 35294, U.S.A., §Department of Biochemistry, University of Potchefstroom, Potchefstroom, South Africa, ‖Departments of Medicine and Human Genetics, and Connective Tissue Research Institute, University of Pennsylvania, Philadelphia, PA 19104, U.S.A., and ¶Jefferson Institute of Molecular Medicine, Jefferson Medical College, Philadelphia, PA, U.S.A.

Fibroblasts from two lethal variants of osteogenesis imperfecta were shown to synthesize increased amounts of type IV procollagen. Previous studies established that one of these variants had a non-functional allele for the proα2 chain of type I procollagen, whereas the other proα2(I) allele contained a mutation leading to synthesis of shortened proα2(I) chains. In the two variants, the relative level of mRNA for proα1(IV) was 31 and 42% of the level of mRNA for proα1(I) chains. A value of less than 2% was found for a third lethal and four non-lethal variants of osteogenesis imperfecta. Immunofluorescent staining of fibroblasts from the two variants synthesizing increased amounts of type IV procollagen indicated that a homogeneous population of cells synthesized both type IV and type I procollagen. The results suggest that mutations in the type I procollagen genes that result in osteogenesis imperfecta can be associated with increased expression of the genes for type IV procollagen.

INTRODUCTION

Collagens are major structural components of the extracellular matrix. In all, 11 genetically distinct types of collagen have been identified in vertebrates, and at least 22 genes code for their constituent α-chains (Cheah, 1985; Miller, 1985; Vuorio, 1986; Burgeson & Morris, 1987). Mutations in the genes for collagen types I and III have been described, but no good association with diseases has been described for the other collagen types. Mutations of type I collagen are best characterized and comprise a heterogeneous group of heritable diseases. A major category of these diseases is osteogenesis imperfecta (OI), characterized by brittleness of bone, osteoporosis and frequently blue sclerae, opalescent teeth, thin skin and deafness (Prockop & Kivirikko, 1984; Cheah, 1985; Byers & Bonadio, 1985; Prockop & Kuivaniemi, 1986; Vuorio, 1986).

Type I collagen consists of two α1(I) chains and one α2(I) chain. These chains are synthesized as precursor proα chains that include a central collagen domain and *N*- and *C*-terminal propeptides that are cleaved after assembly into type I procollagen and secretion from the cell. One moderately severe variant of OI is caused by a 4 bp deletion of sequences coding for the carboxyl-propeptide of the proα2(I) chain. The mutation changes the last 33 amino acids of the protein and prevents the proα2(I) chains from being incorporated into trimers of type I procollagen (Pope & Nicholls, 1981; Deak *et al.*, 1983; Dickson *et al.*, 1984; Pihlajaniemi *et al.*, 1984). A lethal variant of OI was caused by a sporadic mutation in which 643 bp containing three exons in one proα1(I) allele were deleted. The result of the deletion was synthesis of unstable trimers containing shortened proα1(I) chains (Barsh & Byers, 1981; Williams & Prockop, 1983; Barsh *et al.*, 1985; Chu *et al.*, 1985). Several other variants of OI analysed at the protein level or by R-loop mapping of the RNA are caused by deletions in the collagenous region (Byers *et al.*, 1983; de Wet *et al.*, 1983*b*, 1986; Sippola *et al.*, 1984; Byers & Bonadio, 1985). Half-normal levels of either proα1(I) or proα2(I) chains and alterations in the ratios of proα1(I) and proα2(I) mRNAs were observed in several variants of OI (Barsh *et al.*, 1982; de Wet *et al.*, 1983*a*,*b*; Rowe *et al.*, 1985). Three variants of OI are caused by mutations that introduce a cysteine residue into the triple-helical domain of the proα1(I) chain (Nicholls *et al.*, 1984; Steinmann *et al.*, 1984; Cohn *et al.*, 1986; de Vries & de Wet, 1986). In addition to these defects in the primary structure of type I collagen, several other observations in variants of OI have been made. A decrease in the ratio of type I to type III collagen in skin from patients, and a decrease in the ratio of type I to type III procollagen in the medium of cultured fibroblasts (Müller *et al.*, 1975; Penttinen *et al.*, 1975; Sykes *et al.*, 1977; Turakainen *et al.*, 1980; Williams & Prockop, 1983), as well as an increase in the amount of mRNA for type III procollagen in fibroblasts (Chu *et al.*, 1985), have been observed. A very common finding is overmodification of α1(I) and α2(I) chains (Prockop & Kivirikko, 1984; Cheah, 1985; Byers & Bonadio, 1985; Prockop & Kuivaniemi, 1986; Vuorio, 1986). In some patients, an abnormal occurrence

Abbreviations used: OI, osteogenesis imperfecta; bp, base-pair.

** To whom correspondence and reprint requests should be sent.

of type III and V collagens in bone was found (Müller *et al.*, 1974; Pope *et al.*, 1980; Bateman *et al.*, 1986).

A lethal variant of OI was shown to be a compound heterozygote in that a mutation of one allele for the proα2(I) chain caused synthesis of shortened proα2(I) chains and the other allele was not expressed (de Wet *et al.*, 1983*b*, 1986). We previously observed that fibroblasts from this variant synthesized collagen which had the same mobility on electrophoretic gels as type IV procollagen (de Wet *et al.*, 1983*b*). Here we have explored this observation further and measured the mRNA levels for type I, III and IV procollagen in cultured fibroblasts from this variant and six other variants of OI. We also demonstrate that the same cultured cells are synthesizing both type I and IV procollagen.

MATERIALS AND METHODS

Cell cultures

Normal skin fibroblasts from a fetus (CRL-1106) and a 9-year-old donor (IMR-3348) were obtained from the American Type Culture Collection, Rockville, MD, U.S.A. Fibroblasts from two lethal variants of OI (IMR-2962 and IMR-2328) were obtained from the Institute for Medical Research, Camden, NJ, U.S.A. Fibroblasts from the parents of one of the variants of OI (IMR-2962) were obtained from Dr. Thaddeus E. Kelly, Department of Pediatrics, School of Medicine, University of Charlottesville, Charlottesville, VA, U.S.A. Fibroblasts from a third variant of OI (RMS-401) were obtained from Dr. Joseph Alper, Division of Dermatology, Department of Medicine, School of Medicine, Brown University, Providence, RI, U.S.A. Furthermore, locally established cell lines from four patients with mild OI were studied.

Fibroblasts were grown in 25 cm^2 or 150 cm^2 flasks under standard conditions in Dulbecco's modified Eagle's minimum essential medium containing 10% (v/v) fetal-calf serum. Cultures of the fibroblasts were studied at passages 3–10. Before labelling, the medium was replaced with fresh medium containing 50 μg of ascorbate/ml. In order to detect radioactively labelled procollagens in the medium, the cultures were incubated at near confluency in medium lacking fetal-calf serum and containing ascorbate and 25 μCi of L-[2,3,4,5-^{3}H]proline (100 Ci/mmol; Amersham)/ml.

Analysis of medium proteins

Fibroblasts were grown as described above to near confluency and then labelled for 8 h in medium lacking fetal-calf serum. The proteins in the medium were precipitated by $(NH_4)_2SO_4$ (176 mg/ml) in the presence of proteinase inhibitors (de Wet *et al.*, 1983*b*). Half of the $(NH_4)_2SO_4$ precipitates were prepared for electrophoresis by adding sample buffer to give a final concentration of 2% (w/v) SDS, 10% (v/v) glycerol, 0.001% Bromophenol Blue and 0.125 M-Tris/HCl, pH 6.8, and boiled for 5 min. For examination of proteins in the medium after bacterial collagenase treatment, half of the $(NH_4)_2SO_4$ precipitates were dialysed against 10 mM-$CaCl_2$ in 0.1 M-Tris/HCl, pH 7.5, at 4 °C. Next, bacterial collagenase (0.18 mg/ml; Sigma, type VII) was added and the samples were digested at 37 °C for 4 h. The reactions were stopped by adding sample buffer and were boiled for 5 min. The samples were fractionated by electrophoresis on 5% (w/v) polyacrylamide gels in the presence of SDS (de Wet *et al.*, 1983*b*) and fluorograms were prepared (Laskey & Mills, 1975).

For immunostaining of electrophoretically separated type I and IV procollagens, fibroblasts were grown as described above to near confluency and then labelled for 24 h. The proteins in the medium were precipitated with $(NH_4)_2SO_4$ and fractionated by polyacrylamide gel-electrophoresis as described above. The fractionated proteins were blotted on to a nitrocellulose filter (Schleicher und Schuell) as described by Towbin *et al.* (1979) and Burnette (1981) and treated for two-step immunostaining. The filter was pretreated at 37 °C for 1 h with 3% (w/v) bovine serum albumin in 0.15 M-NaCl and 0.1 M-Tris/HCl, pH 7.4 (Tris/saline buffer). The filter was washed with the Tris/saline buffer and then incubated for 1 h at 37 °C with a mouse monoclonal IgG (1:500 dilution) specific for the proα1(I) chain of type I human procollagen provided by Dr. Harold Foellmer, Department of Pathology, School of Medicine, Yale University, New Haven, CT, U.S.A. The filter was then incubated for 1 h at room temperature with anti-mouse IgG linked to peroxidase (U.S. Biochemicals) and stained with diaminobenzamide and peroxide. The same filter was then treated under the same conditions first with rabbit antiserum to mouse type IV procollagen (1:50 dilution) and then with anti-rabbit IgG linked to peroxidase (U.S. Biochemicals). The antiserum to mouse type IV procollagen was kindly supplied by Dr. Jean-Michael Foidart, Department of Medicine, State University of Liège, Liège, Belgium.

Assays of mRNAs

Poly(A)-enriched RNA was extracted from cultured fibroblasts at near confluency by using an oligo(dT)–cellulose affinity column (Pihlajaniemi & Myers, 1987). For Northern-blot analysis the RNAs were electrophoresed in a 0.7%-agarose gel containing 2 M-formaldehyde and transferred to a nitrocellulose filter (Thomas, 1980). Filter-bound RNAs were hybridized to ^{32}P-nick-translated cDNA clones Hf32 and HT-21, coding for human proα2(I) collagen (Myers *et al.*, 1981) and human proα1(IV) collagen chains (Pihlajaniemi *et al.*, 1985) respectively. To assay the steady-state levels of mRNAs, 2.5–30 ng of poly(A)-enriched RNA was dissolved in 70 μl of water and combined with 5 μl of yeast tRNA (20 mg/ml; Boehringer-Mannheim), and 75 μl of a solution containing 3 parts of 20 × SSC (1 × SSC = 0.15 M-NaCl/0.015 M-sodium citrate, pH 6.8) and 2 parts of 12 M-formaldehyde were added. The samples were heated at 68 °C for 15 min and applied with a low vacuum to the nitrocellulose filter using a slot-blot apparatus (Schleicher und Schuell). The filters were washed three times with 10 × SSC and baked at 80 °C for 2 h. Duplicate filters were incubated with cloned human cDNAs α12, E6 and HT-21 specific for proα1(I) and proα1(III) (Loidl *et al.*, 1984), and proα1(IV) (Pihlajaniemi *et al.*, 1985) chains respectively. The cDNA clones containing 2400–2500 bp inserts were labelled with ^{32}P by nick-translation to the same specific radioactivity. The filters were incubated with the labelled cDNA clone (10 ng/ml), 50% (v/v) formamide, 5 × SSC, denatured salmon sperm DNA (0.1 mg/ml), 0.1% SDS, 0.2% polyvinylpyrrolidone, 0.2% Ficoll and 0.2% bovine serum albumin for 24 h at 43 °C. The filters were washed in a series of decreasing concentrations of SSC beginning with 2 × SSC and ending with 0.2 × SSC

and 0.1% SDS at 65 °C. The filters were exposed to X-ray film with an intensifying screen and the films were scanned with a Gilford spectrophotometer.

Immunofluorescent staining of cultured fibroblasts

Fibroblasts were grown in 25 cm^2 flasks as described above. Medium was removed and the cells were washed several times with phosphate-buffered saline (0.015 M-phosphate/0.135 M-NaCl, pH 7.2) and air-dried. The cells were stained first with a monoclonal antibody to human type IV collagen (IgG_1, κ) and then with a second fluorescein isothiocyanate-conjugated goat anti-mouse IgG (Calbiochem; 1:20 dilution). Cells from a duplicate flask were stained with polyclonal antibodies against human type I procollagen as described by Gay & Rhodes (1986) and Gay & Fine (1987) and a second fluorescein isothiocyanate-conjugated goat anti-rabbit IgG (Calbiochem; 1:10 dilution).

RESULTS

Analysis of culture medium from normal and OI fibroblasts

Proteins from the medium of cultured fibroblasts of one OI variant (IMR-2962) fractionated by DEAE-cellulose chromatography were previously shown to contain two prominent collagenous protein bands which co-migrated with a type IV procollagen standard (de Wet *et al.*, 1983*b*). To identify conclusively the procollagen chains, the same fibroblasts and fibroblasts from another variant of OI (IMR-2328) were incubated with [^{3}H]proline, the media were separated by SDS/polyacrylamide-gel electrophoresis and the gel was examined by both fluorography and immunostaining (Fig. 1). Protein bands corresponding to proα1(I) and proα2(I) chains of type I procollagen were detected in the fluorogram prepared from the polyacrylamide gel of the medium proteins. A high-molecular-mass bacterial-collagenase-sensitive band was identified at the position where type IV procollagen migrates in both OI variants (Fig. 1*a*). In the control cells the same band was very faint. The band was identified as type IV proα chains by immunoblotting of polyacrylamide-gel-fractionated medium proteins with an antibody to type IV procollagen. In these experiments the proα1(IV) and proα2(IV) chains migrated as a single band. As shown in Fig. 1(*b*), medium from both lines of normal fibroblasts showed a band of proα(IV) chains. The amount of proα(IV) chains was much greater, however, in medium from two variants of OI (Fig. 1). For comparison, the same nitrocellulose filter was also immunoblotted with a monoclonal antibody for the proα1 chain of type I procollagen. Because this antibody is directed against the *C*-terminal propeptides, it detected both unprocessed and partially processed proα1(I) chains.

Northern-blot analysis of mRNAs

To examine the synthesis of type IV procollagen further, Northern-blot analysis was performed. A portion (300 ng) of poly(A)-enriched RNA from normal fibroblasts and fibroblasts from two variants of OI were electrophoresed under denaturing conditions and hybridized with ^{32}P-labelled cloned cDNAs for proα2(I) chain of type I procollagen and the proα1(IV) chain of type IV procollagen. As indicated in Fig. 2, the expected bands (Myers *et al.*, 1981) of differently sized mRNAs for the proα2(I) chain were readily detected. On the same filter, a band corresponding to the mRNA for the

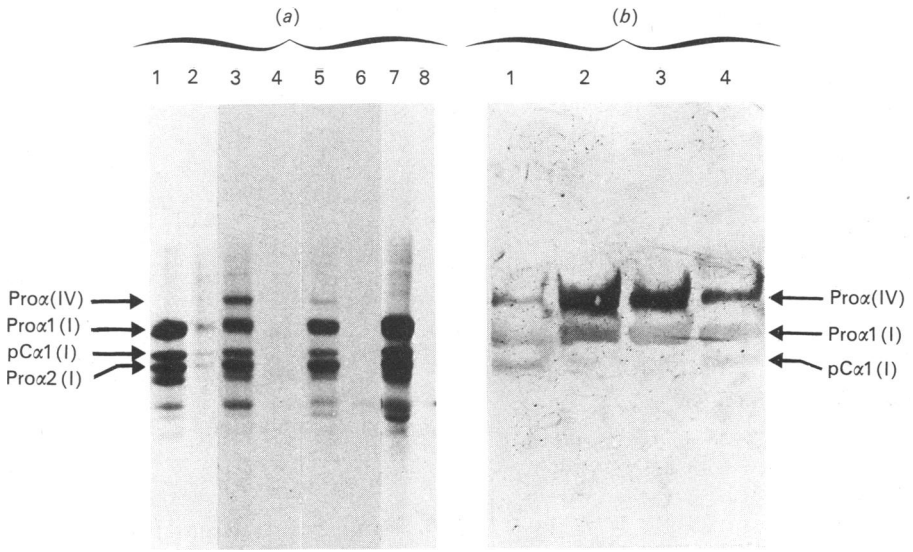

Fig. 1. Proα1(IV) chains in culture medium from normal and OI fibroblasts

(*a*) Fluorogram prepared after separation of [^{3}H]proline-labelled medium proteins by SDS/polyacrylamide-gel electrophoresis. Lane 1, medium from control fibroblasts from a 9-year-old donor (IMR-3348); lane 3, medium from one lethal OI variant (IMR-2962); lane 5, medium from a second lethal OI variant (IMR-2328); lane 7, medium from a normal fetus (IMR-1106); lanes 2, 4, 6 and 8, bacterial-collagenase digestion of the media proteins. (*b*) Immunoblot prepared from gel-electrophoretic fractionation of medium proteins. Lane 1, medium from control fibroblasts (IMR-3348); lane 2, medium from OI fibroblasts (IMR-2962); lane 3, medium from OI fibroblasts (IMR-2328); lane 4, medium from control fibroblasts (IMR-1106). The filter was first immunostained with a monoclonal antibody against the *C*-terminal propeptide of the proα1 chain of the type I procollagen and then with antiserum to type IV procollagen. Proα1(I) chain lacking the *N*-terminal propeptide is indicated by 'pCα1(I)'.

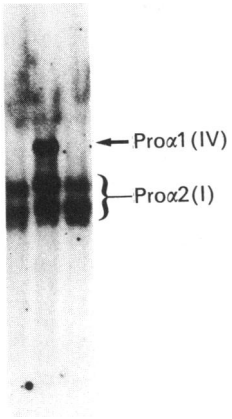

Fig. 2. Northern-blot analysis of poly(A)-enriched RNAs

The blot was hybridized first with a ^{32}P-nick-translated cDNA for proα2(I) and then a cDNA for proα1(IV). Bands corresponding to proα2(I) and proα1(IV) mRNAs are indicated. Left-hand lane, control (IMR-3348); middle lane, lethal OI variant (IMR-2962); right-hand lane, lethal OI variant (RMS-401).

Table 1. Steady-state levels of mRNAs for proα1(IV) and proα1(III) chains compared with the steady-state level of mRNA for proα1(I) chains

Results are means ± S.D. for the numbers (n) of analyses shown.

Cell line	$100 \times \frac{\text{Proα1(III)}}{\text{Proα1(I)}}$	$100 \times \frac{\text{Proα1(IV)}}{\text{Proα1(I)}}$
Normal 9-year-old (CRL-3348)	46.6 ± 1.5 (n = 4)	< 2 (n = 4)
Normal fetus (CRL-1106)	29.9 ± 2.9 (n = 5)	8.4 ± 3.8 (n = 7)
Lethal OI (IMR-2328)	38.6 ± 5.5 (n = 4)	31.0 ± 8.0 (n = 5)
Lethal OI (IMR-2962)	36.6 ± 4.3 (n = 6)	42.1 ± 10.0 (n = 8)
Father of IMR-2962	35.1 ± 2.7 (n = 4)	5.8 ± 3.3 (n = 4)
Mother of IMR-2962	39.2 ± 3.6 (n = 4)	< 2 (n = 4)

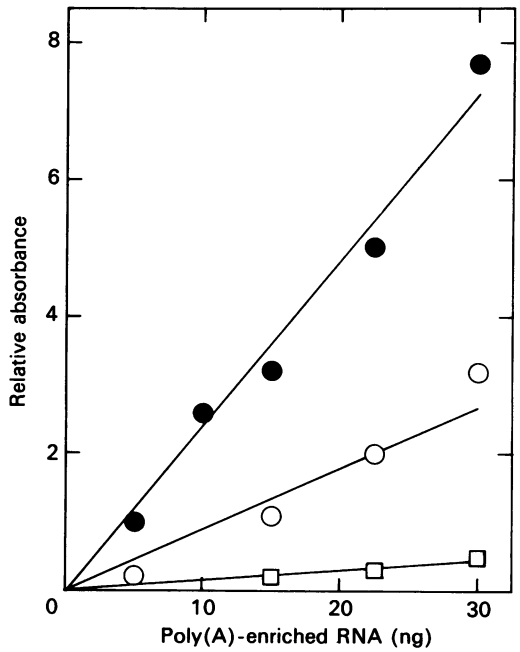

Fig. 3. Assays of poly(A)-enriched RNAs from control fibroblasts (IMR-1106) by slot-blot hybridization

Poly(A)-enriched RNA (2.5–30 ng) was dotted on to nitrocellulose filters and hybridized with cDNA probes. The RNA–[^{32}P]DNA hybrid bands were detected by autoradiography and quantified by densitometry. ●, Hybridization with a ^{32}P-nick-translated cDNA for proα1(I); ○, hybridization with a ^{32}P-nick-translated cDNA for proα1(III); □, hybridization with a ^{32}P-nick-translated cDNA for proα1(IV).

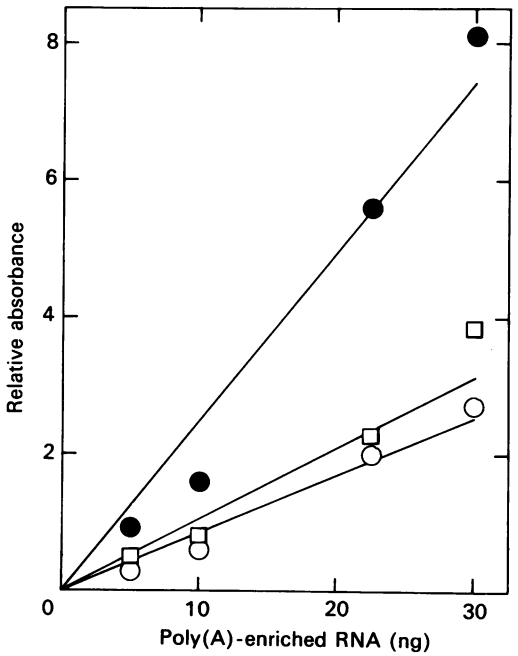

Fig. 4. Assays of poly(A)-enriched RNAs from a lethal OI variant (IMR-2962) by slot-blot hybridization

Symbols are as for Fig. 3.

proα1(IV) chain of type IV procollagen was also detected in one of the OI variants (IMR-2962). Proα1(IV) mRNA was also seen with poly(A)-enriched RNA from a second variant of OI (IMR-2328) (results not shown). However, no mRNA for the proα1(IV) chain was seen in another variant with a lethal form of OI (RMS-401) and in control fibroblasts (Fig. 2).

Slot-blot analysis of steady-state levels of mRNAs

To obtain a quantitative estimate of the amounts of mRNA for type IV procollagen and other procollagens, poly(A)-enriched RNA from fibroblasts was examined by a slot-blot procedure. With samples from normal fetal fibroblasts (IMR-1106), low levels of mRNA for proα1(IV) chains were detected. Parallel filters were hybridized with a proα1(I) cDNA probe of the same size and specific radioactivity as the type IV procollagen

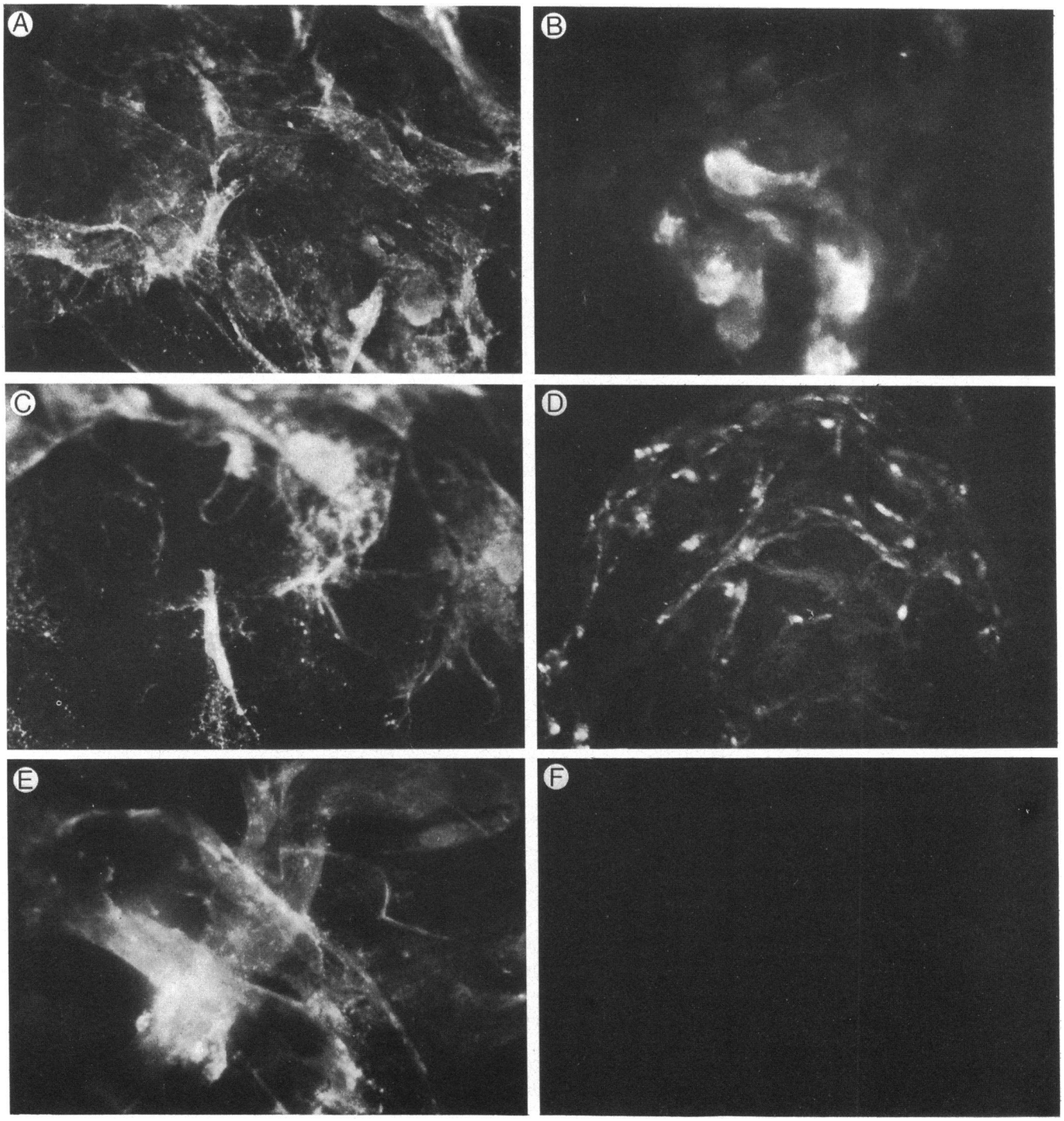

Fig. 5. Immunofluorescence staining of fibroblasts from two lethal OI variants and a control cell line

Panels A and B, lethal OI variant IMR-2962; panels C and D, lethal OI variant IMR-2328; panels E and F, control fibroblasts IMR-3348. In panels A, C and E the cells were stained with anti-(type I procollagen) antibodies. In panels B, D and F the cells were stained with anti-(type IV procollagen) antibodies. The magnification is 320×.

cDNA clone. The results suggested that, in the normal fetal fibroblasts, the level of proα1(IV) mRNA was about 8% of the proα1(I) mRNA levels (Fig. 3; Table 1). No measurable amounts of mRNA for proα1(IV) chains was found in fibroblasts from a 9-year-old normal donor (IMR-3348) and four non-lethal variants of OI (not shown). Under the assay conditions, we estimated that we could have detected mRNA from proα1(IV) chains that was about 2% of that of proα1(I) chains.

In contrast, the levels of mRNA for proα1(IV) were elevated in fibroblasts from two lethal variants of OI (IMR-2962 and IMR-2328). As shown in Fig. 4, the level of mRNA for proα1(IV) chains in one of the variants (IMR-2962) was about 42% of the level of mRNA for proα1(I) chains. The level of proα1(IV) mRNA in the second variant (IMR-2328) was about 31% of mRNA for proα1(I) (Table 1). There was no change in the relative amounts of these two RNAs when cells were

examined at passages 5–10. Fibroblasts from the parents of one of the variants (IMR-2962) with high levels of proα1(IV) mRNA were also examined. The level of proα1(IV) mRNA in fibroblasts from the father, who had a non-functioning proα1(I) allele (de Wet *et al.*, 1983*b*), was 5.8% of the level of mRNA for proα1(I). The level for proα1(IV) mRNA in the mother's fibroblasts was less than 2% of the level of mRNA for proα1(I). The mother had neither of the defects detected in the patient's proα2(I) alleles (de Wet *et al.*, 1983*b*).

Parallel filters of poly(A)-enriched RNA were also hybridized with a ^{32}P-labelled cDNA for proα1(III) chains. As indicated in Figs. 3 and 4 and Table 1, the ratio of mRNAs for the proα1(III) chains compared with proα1(I) chains ranged from 29.9 to 46.6% in both the control and OI fibroblasts.

Immunofluorescent staining of the cultured fibroblasts

One further question asked here was whether the synthesis of type IV procollagen occurred in the same cultured fibroblasts as those which were synthesizing type I procollagen. Cells from two of the OI variants (IMR-2962 and IMR-2328) and a control cell line (CRL-3348) were stained with either anti-(type IV procollagen) antibodies or anti-(type I procollagen) antibodies. The results showed that all three cell lines stained with anti-(type I procollagen) antibodies, but only the OI cells showed staining with anti-(type IV procollagen) antibodies (Fig. 5). Most of the OI fibroblasts synthesized both proteins.

DISCUSSION

Type IV collagen is the major structural component of basement membranes. It can be distinguished from fibre-forming collagens (types I, II and III) by more extensive post-translational modifications, greater sensitivity to proteinases, lack of substantial extracellular processing and formation of network-like structures instead of cross-striated fibres (see Martin *et al.*, 1985; Burgeson & Morris, 1987). The type IV procollagen molecule contains two genetically distinct polypeptide chains, a proα1(IV) chain of approx. 185 kDa and a proα2(IV) chain of about 170 kDA (Crouch & Bornstein, 1979; Tryggvason *et al.*, 1980).

Two of the variants of OI studied here (IMR-2962 and IMR-2328) synthesized increased amounts of proα1(IV) chains compared with control fibroblasts. They also contained considerably higher levels of mRNA for proα1(IV) chains, as shown by Northern- and slot-blot analysis. In these two OI variants the proα1(IV) mRNA levels were 31 and 42% of the proα1(I) mRNA levels, and therefore were about the same as the levels of proα1(III) mRNAs. A value of less than 2% was found for a third lethal OI variant and for four non-lethal variants of OI (results not shown). The fibroblasts of variant IMR-2962 synthesized no normal type I procollagen, since each of the two alleles for proα2(I) chains were defective. One of the alleles contained a sporadic mutation which lead to synthesis of shortened proα2(I) chains, whereas the other proα2(I) allele was non-functional (de Wet *et al.*, 1983*b*). The proband inherited the non-functioning proα2(I) allele from the father (de Wet *et al.*, 1983*b*). As shown here, the father's fibroblasts contained a slightly increased amount of mRNA for proα1(IV) chains.

All specific mutations characterized so far in different variants of OI have been traced to either the proα1 or proα2 chain of type I procollagen (Prockop & Kivirikko, 1984; Cheah, 1985; Byers & Bonadio, 1986; Prockop & Kuivaniemi, 1986; Vuorio, 1986). Furthermore, linkage studies using restriction-fragment-length polymorphism strongly suggest that OI segregates with either the proα1(I) or proα2(I) loci in families with OI (Tsipouras *et al.*, 1984; Sykes *et al.*, 1986). Therefore it is likely that the OI variant IMR-2328 also contains a mutation in one of the two structural genes for type I procollagen. The results obtained here with the two variants of OI suggest that expression of genes for type IV procollagen occurs in some, but certainly not all, variants of this disorder. Whether there is a direct causal relationship between the increased expression of the type IV procollagen and the mutation in type I procollagen in these variants is not known. However, it seems that type IV procollagen cannot replace type I procollagen in tissues because of the large chemical and physical differences between these two collagen types.

Previously, increased synthesis of collagen types III and V have been described in variants of OI. In some cases these observations probably reflect either a decreased rate of synthesis of type I procollagen or synthesis of structurally abnormal type I procollagen that is rapidly degraded. The occurrence of type III collagen in bone (Müller *et al.*, 1974, 1975; Pope *et al.*, 1980; Bateman *et al.*, 1986), a tissue normally devoid of this collagen, and an increase in the amount of type V collagen in bone (Pope *et al.*, 1980; Bateman *et al.*, 1986) possibly represent an attempt to compensate for diminished type I collagen production in some patients. As shown here, increased synthesis of type IV procollagen by two lethal variants of OI reflected an increase in the levels of mRNA for the proα1(IV) chain, whereas the mRNA levels for type I and III procollagens were comparable with the control. The proα1(IV) mRNA levels were elevated without concomitant change in the corresponding gene copy number as indicated by Southern-blot analysis of genomic DNAs from the OI variants and control (results not shown). Therefore the elevated proα1(IV) mRNA levels reflected either increased transcriptional activity of the corresponding gene or increased stability of the mRNA transcripts.

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