Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 9648–9652, November 1991
Genetics

Reduced amounts of cartilage collagen fibrils and growth plate anomalies in transgenic mice harboring a glycine-to-cysteine mutation in the mouse type II procollagen α_1-chain gene

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Communicated by Elizabeth D. Hay, June 22, 1991

ABSTRACT **We have generated transgenic mice harboring a glycine-to-cysteine mutation in residue 85 of the triple helical domain of mouse type II collagen. The offspring of different founders displayed a phenotype of severe chondrodysplasia characterized by short limbs and trunk, cranio–facial deformities, and cleft palate. The affected pups died of acute respiratory distress caused by an inability to inflate lungs at birth. Staining of the skeleton showed a severe retardation of growth for practically all bones. Light microscopic examination indicated a decrease in cartilage matrix density, a severe disorganization of growth plate architecture, and the presence of streaks of fibrillar material in the cartilage matrix. Electron microscopic analysis showed a pronounced decrease in the number of typical thin cartilage collagen fibrils, distension of the rough endoplasmic reticulum of chondrocytes, and the presence of abnormally large banded collagen fibril bundles. The level of expression of the mutant type II procollagen α_1 chain transgene in cartilage tissues was approximately equal to that of the endogenous gene in two of the strains. We propose that the principal consequence of the mutation is a considerable reduction in density of the typical thin cartilage collagen fibrils and that this phenomenon causes the severe disorganization of the growth plate. We also postulate that the abnormal thick collagen fibrils are probably related to a defect in crosslinking between the collagen molecules. The cartilage anomalies displayed by these transgenic mice are remarkably similar to those of certain human chondrodysplasias.**

The extracellular matrix of cartilages is a multicomponent system in which type II collagen constitutes the major structural protein. After their secretion into the extracellular milieu, the triple-helical type II collagen molecules interact with each other to form highly ordered fibrils that constitute a regular three-dimensional network throughout the cartilage matrix and provide sites for interaction with other extracellular matrix components. In addition to type II collagen, these fibrils also contain type IX and type XI collagen (1, 2).

From studies mainly with mutant human type I, II, and III collagens, we know that mutations that replace glycine residues in the triple-helical domain act as dominant negative mutations presumably because they interfere with collagen assembly and/or fibril function (3, 4).

Given the dominant nature of mutations in fibrillar collagens, the transgenic mice system appeared well-suited to examine the effects of mutations in different domains of type II collagen. Previous work had in fact indicated that transgenic mice bearing a mutant type I procollagen α_1 chain [proα1(I) collagen] transgene with a substitution mutation similar to that which causes perinatal lethal osteogenesis imperfecta in humans presented a phenotype that resembled the human disease (5).

We have generated transgenic mice harboring a Gly-85 → Cys change of the triple-helical domain of mouse type II collagen. The mutation causes striking alterations in the cartilage matrix and the organization of the growth plate cartilages which result in short-limb dwarfism.

MATERIALS AND METHODS

Generation and Identification of Transgenic Mice. The 39-kilobase (kb) insert containing the reconstituted mouse proα1(II) collagen gene (6) (see Fig. 1), 3 kb of 5′ flanking sequences, and 7 kb of 3′ flanking sequences was released from the cosmid vector by *Not* I digestion. DNA was microinjected into pronuclei of one-cell mouse embryos, obtained from C57BL/6 × DBA/2J F_1 (hereafter called B6D2F_1) females mated with B6D2F_1 males, which were implanted into BALB/c × DBA1 (hereafter called CD1) pseudopregnant foster mothers (7). The transgenic founders were identified by Southern hybridization of *Nco* I-digested tail genomic DNA with an *Eco*RI–*Pst* I DNA fragment from intron 6 to intron 8 (see Fig. 1*C*). This probe hybridizes to 540- and 628-base-pair (bp) fragments in the endogenous type II collagen gene and to a 1168-bp fragment in the transgene. Positive founder mice were mated with B6D2F_1 mice. In some instances they were also mated with C57BL/6J mice. The same phenotype was observed in the offspring of both matings.

Tissue Preparation, Histology, and Electron Microscopy (EM). The skeletons of whole mice were stained with alcian blue and alizarin red S as described (8). Samples for both light microscopy and EM were from 19-day-old embryos obtained by Cesarean section. Light microscopy was performed on tissues that were fixed in 4% buffered formaldehyde, embedded in Spurr resin, sectioned, and stained with 1% toluidine blue. For EM analysis the growth plates of long bones were carefully dissected under the microscope and immediately fixed in 1 ml of 1.5% glutaraldehyde/1.5% paraformaldehyde/0.1 M cacodylate buffer with 5 mg of ruthenium hexamine trichloride per ml. The samples were dehydrated, embedded in Spurr resin, sectioned, and stained with 4% uranyl acetate and Reynold's citrate.

Preparation of RNA and Allele-Specific Amplification of cDNAs. Total RNA was isolated by homogenization of mouse

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Abbreviations: proα1(I) and proα1(II) collagen, type I and type II procollagen α_1 chain; RT-PCR, reverse transcription-coupled PCR.

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limbs in 4 M guanidine thiocyanate followed by sedimentation through 5.7 M cesium chloride. cDNA was synthesized from 1 μg of total RNA by extension of random hexamers with 200 units of reverse transcriptase (BRL). Synthesis of the second cDNA strand and 20–30 cycles of cDNA amplification were performed with 2.5 units of *Taq* polymerase (Cetus) in the presence of [α-^{32}P]dATP and [α-^{32}P]dCTP to generate radioactive fragments. Allele-specific PCR was performed either with the A6-wt primer (5′-CTGTGACCCTTGACACC-3′) together with the common A4 primer (5′-AACTTCGCGGCTCAGATGGCTG-3′) for amplification of wild-type endogenous cDNA or with the A6-mut primer (5′-CTGTGACCCTTGACACA-3′) together with the common A4 primer for amplification of mutant-transgene cDNA. For allele-specific amplification, an annealing temperature of 65°C was determined empirically.

Measurement of the Relative Levels of Transgene and Endogenous RNA by the Polymerase Chain Reaction (PCR). During the logarithmic phase of the amplification process, 5-μl aliquots of the allele-specific reactions were removed and digested with *Nco* I. After electrophoretic separation, the radioactivity incorporated in the 99-bp DNA fragment, diagnostic for the endogenous mRNA, and the 108-bp DNA fragment, specific for the transgene RNA, were measured with a Betascan counter. These values were plotted vs. the number of cycles on a semilogarithmic scale. The resulting slope represents the efficiency of amplification of each allele-specific PCR reaction. By extrapolation to zero amplification cycles, the ratio of the relative concentrations of endogenous proα1(II) collagen RNA to transgene proα1(II) collagen RNA can be calculated (9).

RNA Analysis by RNA (Northern) Blot Hybridization. Aliquots (10 μg) of normal and transgenic 19-day embryo limb RNAs were fractionated by electrophoresis on a 1% agarose/formaldehyde gel and blotted onto Hybond N+ nylon membrane (Amersham). The hybridization probes were pMco12a1-1 for proα1(II) collagen mRNA and pMco19a1-1 for α1(IX) collagen mRNA (10). The mouse β-actin probe was a 318-bp cDNA fragment synthesized by reverse transcription-coupled PCR (RT-PCR) and cloned in Bluescript vector (D.T., unpublished data). The filters were hybridized and washed at high stringency (0.015 M NaCl/0.0015 M sodium citrate, pH 7/0.1% SDS at 65°C for 60 min) as recommended by the manufacturers. Hybridization signals were quantified by using a Betascan-counter.

RESULTS

Construction of the Mutated proα1(II) Collagen Gene. Fig. 1 outlines the steps that were used to create both a glycine-to-cysteine mutation and a silent mutation in the mouse proα1(II) collagen gene (*Col2a-1*). A silent mutation, which was needed to distinguish the transgene and its transcript from their endogenous counterparts, was generated by changing the sequence of a *Nco* I restriction site in exon 7 without altering the amino acid sequence (Fig. 1 *A–E*). The GGC codon for Gly-85 in the triple-helical domain was changed to a TGC codon encoding cysteine.

Generation of Transgenic Mice. Four transgenic founder mice were generated by microinjection of the 39-kb reconstituted gene into fertilized oocytes. The silent mutation produces a diagnostic 1168-bp fragment when the DNA of positive pups is digested with *Nco* I (Fig. 2). Quantitative analysis of Southern blots showed that two male founders harbored ≈50 (Gly-85-1) and 25 (Gly-85-3) copies of the transgene, respectively, whereas two female founders (Gly-85-2 and Gly-85-4) had 10 and 2 copies, respectively (Fig. 2). Southern analysis of *Eco*RI-digested genomic DNAs from each of the four transgenic animals, hybridized with the entire mouse proα1(II) collagen gene, demonstrated that the

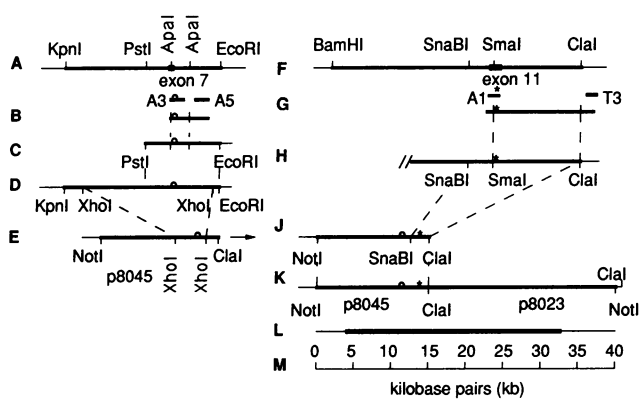

FIG. 1. Schematic description of transgene construction. (*A–E*) Generation of a silent mutation (removal of a *Nco* I site) in exon 7 was performed by a PCR-based site-directed mutagenesis. The sequence of oligonucleotide A3 corresponds to part of the sequence of exon 7 and contains an *Apa* I recognition site and a single-base mutation that changes the *Nco* I site (TGCAGGGCCCTATGGGACCCCGTGG, with the mutation in boldface type). Oligonucleotides A3 and A5 were incorporated into DNA by PCR by using a fragment of the wild-type proα1(II) collagen gene as template (*A* and *B*). The PCR product was digested with *Apa* I and the 150-bp *Apa* I–*Apa* I fragment was first cloned into a *Pst* I–*Eco*RI subclone (*C*), which was then cloned into a 2.2-kb *Kpn* I–*Cla* I genomic subclone (*D*) from which a 1.9-kb *Xho* I–*Xho* I fragment was inserted into a 13-kb clone (p8045). (*F–J*) Site-directed mutagenesis of codon 85 of the triple helical domain in exon 11. Oligonucleotide A1 spans a *Sma* I site and contains a single-base mutation changing a glycine codon to a cysteine codon (GAACCCCGGGTCTCCCCTGTGTCAAGGGTC with the mutation indicated in boldface type). Oligonucleotide A1 and the universal T3 primer were used as primers in a PCR reaction with a 1.3-kb wild-type *Bam*HI–*Cla* I subclone as template (*F* and *G*). The PCR fragment was digested with *Sma* I and *Cla* I and cloned into the original template plasmid substituting the wild-type *Sma* I–*Cla* I fragment (*H*). The 5′ half of the gene was reconstructed by inserting a 600-bp *Sna*BI–*Cla* I fragment containing the glycine mutation into clone p8045 carrying the silent mutation (*J*). Reconstruction of the gene was completed by addition of the 28-kb *Cla* I fragment of plasmid p8023 containing the 3′ half of the gene (*K*). The solid dark rectangle in *L* represents the structural part of the proα1(II) collagen gene.

entire transgene had integrated into the genome of founder animals without major rearrangements.

Chondrodysplasia in the Offspring of Several Transgene Carriers. The four founder animals were phenotypically normal except that founder Gly-85-1 showed a distinctly smaller size and mildly disproportionate growth. However, the offspring of founders Gly-85-1 and Gly-85-3 had a severe form of chondrodysplasia, which included short limbs and trunk, hypoplastic thorax with short ribs, distended abdomen, short snout, protruding tongue, and cleft palate (Fig. 3 *A* and *B*). The offspring of founder Gly-85-3 displayed the most severe phenotype with only rudimentary limbs (Fig. 3*B*). Most of the positive pups of the Gly-85-1 line and all of the positive pups of the Gly-85-3 founder died at birth after a short period of acute respiratory distress. When the pups were delivered by Cesarean section, the positive animals could be easily identified by their appearance; they attempted to breathe but, unable to inflate their lungs, became cyanotic and died shortly after birth. Although a vast majority of the transgenic offspring of founder Gly-85-1 also exhibited a perinatal lethal phenotype, approximately 5–7% of the transgenic pups showed a much milder phenotype and survived the perinatal period, their small size becoming evident later. The positive animals with this phenotype have again transmitted a severe form of the disease to their own offspring. The incomplete penetrance of the lethal phenotype occurred without any change in copy number of the transgene and

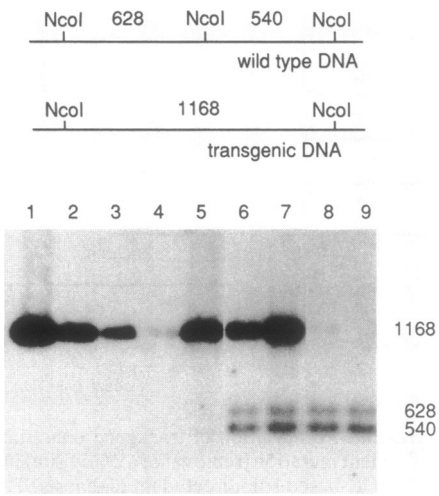

FIG. 2. Identification of transgenic founder mice. Aliquots of the proα1(II) collagen gene cosmid corresponding to 50, 20, 10, and 1 copy per diploid mouse genome (lanes 1–4) and tail DNA samples (lanes 5–8) (10 μg; 5 μg for Gly-85-1) from four founder animals Gly-85-1 to -4 and from a wild-type mouse (lanes 5–9) were digested with *Nco* I, fractionated by electrophoresis on a 1% agarose gel, blotted onto Hybond-N membrane, and hybridized with a ^{32}P-labeled 800-bp *Pst* I–*Eco*RI fragment (Fig. 1*C*). A 1168-bp *Nco* I fragment is diagnostic of the transgene, whereas 628- and 540-bp fragments are diagnostic of the endogenous gene.

could suggest that other genetic or epigenetic factors may play a role in the pathogenesis of the chondrodysplasias in these transgenic mice. Both founders Gly-85-1 and Gly-85-3 are alive probably because they are mosaic for the transgene.

The progeny of founder Gly-85-2 did not show any detectable phenotypic abnormalities, but the mating of heterozygous transgenic mice has generated mice homozygous for the transgene, exhibiting a severe perinatal lethal phenotype with short limbs similar to the phenotype seen in the other two strains. The progeny of Gly-85-4 appeared to be normal and have not been studied in detail.

The abnormal development of the bony and cartilaginous skeleton in the offspring of Gly-85-1 and Gly-85-3 founders was most dramatically demonstrated by staining the skeleton with alcian blue and alizarin red (Fig. 3*C*) (11). The abnormalities included smaller size and delayed mineralization of the whole skeleton. The long bones in the limbs of the transgenic animals also exhibited greatly reduced length/thickness ratios. The total length of long bones in 19-day Gly-85-1 embryos was an average of 0.6 that of the normal littermates. Significant deformities of the head were also seen, with a reduced anterior–posterior axis and short snout.

Structural and Ultrastructural Anomalies of Cartilages. Several different developmental stages of chondrocytes can be visualized in the epiphyseal and growth plate cartilages of long bones: epiphyseal cartilage contains "resting" chondrocytes, whereas the growth plate consists of proliferating and hypertrophic chondrocytes normally arranged in a characteristic columnar pattern (Fig. 4*A*).

The most striking feature in the offspring of founder Gly-85-3 was the complete disappearance of the normal growth plate architecture (Fig. 4*C*). The columnar arrangement was replaced by an irregular transition from epiphyseal cartilage to hypertrophic cartilage. Few proliferative chondrocytes were observed, and the matrix septa that normally define clusters of proliferative and hypertrophic chondro-

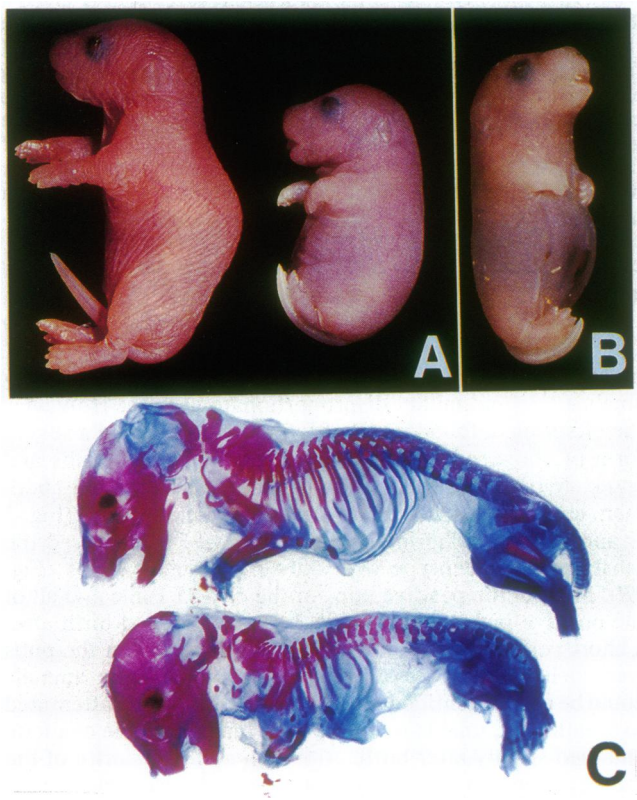

FIG. 3. Major phenotypic abnormalities of transgenic mice. (*A*) Gly-85-1 F_1 transgenic 19-day embryo (*Right*) and normal nontransgenic littermate (*Left*). (*B*) Gly-85-3 transgenic newborn F_1 mouse. (*C*) Lateral view of skeletons of normal (*Upper*) and Gly-85-1 F_1 transgenic (*Lower*) 19-day embryos stained with alizarin red S and alcian blue dyes for bone and cartilage, respectively.

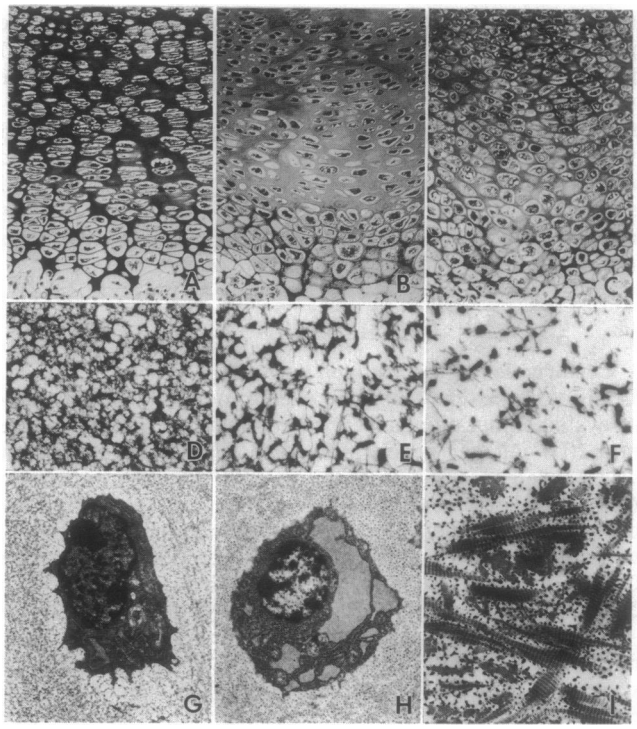

FIG. 4. Structure and ultrastructure of cartilage of transgenic mice. (*A–C*) Light microscopy of growth plate cartilage of humerus from normal (*A*), Gly-85-1 (*B*), and Gly-85-3 (*C*) 19-day embryos, stained with 1% toluidine blue. (×90.) (*D–I*) Territorial compartment (*D*, *E*, and *F*), typical chondrocytes (*G* and *H*), and interterritorial compartment (*I*) of the epiphyseal cartilage matrix. Samples are from wild-type (*D* and *G*), Gly-85-1 (*E*), and Gly-85-3 (*F* and *I*) 19-day embryos. (*D–F* and *I*, ×5400; *G* and *H*, ×1980.)

cytes were absent. Furthermore, the cartilage-bone interface appeared very uneven. A pronounced reduction in extracellular matrix was seen in both epiphyseal and growth plate cartilages. Randomly distributed streaks of darkly staining fibrous-like matrix were observed throughout the cartilage (Fig. 4*C*). The histological findings in Gly-85-1 and homozygous Gly-85-2 mutant mice were generally similar to those in the offspring of Gly-85-3 (Fig. 4*B* and data not shown).

EM examination of epiphyseal and growth plate cartilages showed that the principal anomaly in the Gly-85-1 and Gly-85-3 offspring was a reduction in the number of characteristic thin collagen fibrils throughout the extracellular matrix (ECM) (Fig. 4 *E* and *F*), more pronounced in the Gly-85-3 than in the Gly-85-1 mutants. A similar severe reduction in the density of typical cartilage fibrils was seen in the ECM of the trachea. In many areas of the Gly-85-1 and Gly-85-3 matrix, large fibrils of banded collagen frequently arranged in thick bundles were found in locations corresponding to the streaks of fibrous material seen by light microscopy (Fig. 4*I*). The general morphology of epiphyseal chondrocytes in the transgenic animals resembled that of wild-type chondrocytes, but mild distension of the rough endoplasmic reticulum was observed in many Gly-85-1 cells; in Gly-85-3 cartilages this phenomenon was more pronounced (Fig. 4 *G* and *H*).

Expression of the Mutant Transgene. The proα1(II) collagen transgene RNA is identical to the endogenous mouse RNA except for the glycine substitution mutation and the silent mutation that alters a *Nco* I-cleavage sequence. To distinguish between these two mRNAs, we used RT-PCR with allele-specific oligonucleotide primers bracketing the silent mutation that differed from each other by a single nucleotide located at the 3′ end of the primers at the site of the glycine mutation (A6-wt and A6-mut) (Fig. 5*A*). Under proper annealing temperature, these specific primers directed amplification only of their corresponding allelic cDNAs (Fig. 5*B*). With RNA from wild-type mice, only the wild-type primer generated a DNA product; no DNA was generated with the mutant oligonucleotide primer. With RNA from transgenic mice, the wild-type oligonucleotide primer directed synthesis of wild-type DNA, identified by the 99-bp *Nco* I fragment, whereas the mutant primer directed synthesis only of transgene DNA, identified by the 108-bp fragment. This allele-specific PCR was then used to compare the relative levels of wild-type and mutant RNAs in limbs of transgenic animals. Measurements of the RNA levels, calculated by extrapolation of the levels of amplified cDNA at various steps, indicated that the ratio of type II collagen transgene RNA to endogenous wild-type Col2a-1 RNA was approximately 1:1 in limbs of Gly-85-3 mutants (Fig. 5*C*). A similar level of expression of the mutant transgene was found for limb RNA from the Gly-85-1 offspring.

Northern hybridizations were performed to further compare the levels of the 5-kb proα1(II) collagen mRNA in limb RNA from Gly-85-1 offspring and in limb RNA from wild-type mice (Fig. 5*D*). To verify that the same amount of chondrocyte-specific RNA was present in the two RNA preparations, filters were hybridized with a cDNA probe for mouse α1(IX) collagen, another chondrocyte-specific marker. The results indicated that after normalization with the values obtained for α1(IX) collagen RNA, the total levels of the 5-kb α1(II) collagen RNA were twice as high in the limbs of Gly-85-1 as in limbs of wild-type animals, confirming the values obtained by allele-specific PCR measurements. This experiment also strongly suggested that the size of the proα1(II) collagen transgene mRNA is the same as that of the corresponding endogenous mRNA.

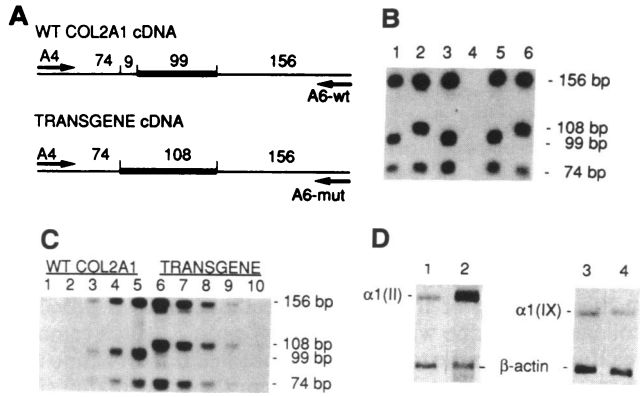

FIG. 5. Expression of the transgene. (*A*) Schematic presentation of the *Nco* I restriction map of wild-type and mutant proα1(II) collagen cDNA. A4, A6-wt, and A6-mut represent PCR primers. A6-wt and A6-mut differ by a single base at their 3′ end. (*B*) Allele-specific amplification of wild-type and transgenic limb cDNAs from normal (lanes 3 and 4), Gly-85-1 (lanes 1 and 2), and Gly-85-3 (lanes 5 and 6) 19-day embryos. Each cDNA was amplified with either A4 and A6-wt primers (lanes 1, 3, and 5) or with A4 and A6-mut primers (lanes 2, 4, and 6). After digestion of the PCR products with *Nco* I, the DNAs were fractionated by electrophoresis on a 15% acrylamide gel. (*C*) Kinetics of amplification of the endogenous (lanes 1–5) and transgenic (lanes 6–10) cDNA by allele-specific RT-PCR. Samples were obtained after 22 (lanes 1 and 10), 24 (lanes 2 and 9), 26 (lanes 3 and 8), 28 (lanes 4 and 7), and 30 (lanes 5 and 6) cycles, digested with *Nco* I, and fractionated as in *B*. (*D*) Northern analysis of RNAs. Total RNA (10 μg) prepared from limbs of wild-type (lanes 1 and 3) and Gly-85-1 (lanes 2 and 4) transgenic 19-day-old embryos were fractionated by electrophoresis. The mouse β-actin probe was used to normalize amounts of RNA on filters, and the α1(IX) collagen probe was used to verify that similar amounts of chondrocyte-specific RNAs were present in the RNA preparations. Individual hybridization signals were counted by betascanner-counter. The ratios of proα1(II) collagen to β-actin RNA were 0.97 for wild-type RNA and 1.96 for Gly-85-1 RNA; the corresponding ratios of proα1(IX) collagen to β-actin RNA were 0.51 and 0.54. Duplicate experiments showed the same ratios.

DISCUSSION

The Gly-85 → Cys mutation in the proα1(II) collagen transgene leads to several clear anomalies. We believe that the major anomaly that is responsible for the abnormal phenotype and is strikingly demonstrated by EM analysis is the considerable reduction in the number of typical type II collagen fibrils in cartilages. This pronounced decrease in type II collagen fibrils must result from anomalies in procollagen assembly and intracellular degradation or from a more rapid turnover of mutant collagen molecules in the extracellular milieu (maybe as a result of defective fibril formation) or from sequestration of collagen molecules in abnormally large collagen fibrils. In fact, all three mechanisms could contribute to the abnormal reduction of typical thin collagen fibrils in cartilage. The distension of the rough endoplasmic reticulum (rER) of chondrocytes suggests anomalies in procollagen assembly, as is seen in fibroblasts of osteogenesis imperfecta patients in whom the distension of the rER correlates with a retention of abnormal type I procollagen molecules in the cells (12).

We propose that the profound derangement of the normal architecture of the growth plate, showing a disappearance of the regular columnar arrangement of the proliferating and hypertrophic chondrocytes, is a direct consequence of the decreased number of typical cartilage collagen fibrils. The postmitotic chondrocytes in the proliferative zone of the growth plate appear to be unable to produce sufficient matrix to separate themselves from each other to form distinct clusters typical of this site. It is very likely that this major growth plate alteration is responsible for the much slower growth of long bones.

We speculate that the large fibril bundles in epiphyseal cartilages are the result of defective crosslinking. Indeed one

of the four crosslinking sites in the collagen polypeptide is located at residue 87. The Gly-85 → Cys mutation almost certainly alters the local three-dimensional structure of the collagen molecule and probably will create an abnormal substrate for the lysyloxidase enzyme acting on Lys-87. These large-banded fibrils were, indeed, very abundant in the cartilage matrix of two different transgenic mice strains harboring a lysine-to-arginine mutation either in the crosslinking site in residue 87 of the triple helical domain or in the amino-telopeptide of α1(II) collagen (unpublished data). In contrast, these thick fibrils were completely absent in the offspring of transgenic mice that harbor a proα1(II) collagen transgene with a deletion of exon 7, which removes residues 4–18 in the triple helix, hence deleting an amino acid segment that is clearly removed from the crosslinking sites. These mice display a very similar phenotype of pronounced chondrodysplasia as the severely affected Gly-85 mice (unpublished data). It should also be noted that the broad fibrils were not seen in transgenic mice harboring a proα1(II) collagen wild type transgene that contained only the silent mutation. These mice in which the levels of transgene mRNA were ≈50% of those of the endogenous Col2a-1 mRNA displayed no abnormal phenotype (data not shown). Finally, it is difficult to attribute the severity of the chondrodysplasia to the presence of the thick-banded collagen fibrils. Indeed, these thick fibrils were abundant in transgenic mice harboring mutations in either of two cross-linking sites that show only minimal alterations of the growth plate and no significant reduction in the density of the normal thin cartilage fibrils.

Type I collagen is not a constituent of these thick fibrils. Indeed, *in situ* hybridization of long-bone cartilage of Gly-85-3 showed a complete absence of type I collagen RNA in chondrocytes, although this RNA could be detected, as expected, in the perichondrium (data not shown). In addition, immunofluorescent studies of long-bone cartilage of Gly-85-3 mutants using three different antibodies that are highly specific for epitopes in the type II collagen polypeptide indicate that type II collagen was present in the large fibrils (data not shown).

The abnormalities that we have generated in transgenic mice bearing a single glycine-to-cysteine substitution in a proα1(II) collagen transgene strongly resemble the features found in the spondyloepiphyseal dysplasia (SED) group of human chondrodysplasias, which in order of decreasing severity include achondrogenesis type II (Langer–Saldino), hypochondrogenesis, SED congenita, and mild SED with predisposition to osteoarthritis (11). Type II collagen mutations have recently been identified in some cases of the latter three disorders (13–16). The phenotypes of the Gly-85 transgenic mice have also strong homologies with those of several monogenic mutations in mouse for which the mutated genes have not yet been identified (17).

The severity of the chondrodysplastic phenotype appeared to be dependent on the level of expression of the transgene. Indeed the offspring of Gly-85-2, which are heterozygous for the transgene, do not show overt signs of abnormal bone growth and express the mutant proα1(II) collagen transgene at about one-third the level of expression of the endogenous gene. Gly-85-2 mutant mice that are homozygous for the transgene died at birth and showed symptoms of severe growth retardation that were similar to those seen in the offspring of Gly-85-1 and Gly-85-3 founders.

The transgenic mice that we have generated should help to dissect genetically the role of extracellular matrix proteins in cartilages of intact animals and more specifically their role in the function of the growth plate. We believe that the primary lesion in these mice is a severe decrease in functional collagen fibrils in cartilage matrices. This defect results in a considerable disorganization of growth plate cartilages, a phenomenon which, at this time, can only be examined in intact animals.

We are very grateful to Dr. Richard Behringer (The University of Texas M. D. Anderson Cancer Center) for many valuable discussions. We gratefully acknowledge Lee Ann Garrett, Chad Smith, Glenn Decker, Mirta Machado, and Zhao Xia Ren for their help throughout these experiments. We thank Janie Finch for editorial assistance. S.G. is the recipient of an Arthritis Foundation fellowship. This work was supported by National Institutes of Health Grants R01 AR40335 (to B.d.C.) and R01 HD 20691 (to W.H.) and by the Finnish Academy (to E.V.).

1. Mayne, R. (1990) *Ann. N.Y. Acad. Sci.* **599**, 39–44.
2. Vuorio, E. & de Crombrugghe, B. (1990) *Annu. Rev. Biochem.* **59**, 837–872.
3. Byers, P. H. (1990) *Trends Genet.* **6**, 293–300.
4. Prockop, D. J., Baldwin, C. T. & Constantinou, C. D. (1990) *Adv. Hum. Genet.* **19**, 105–132.
5. Stacey, A., Bateman, J., Choi, T., Mascara, T., Cole, W. & Jaenisch, R. (1988) *Nature (London)* **332**, 131–136.
6. Metsaranta, M., Toman, D., de Crombrugghe, B. & Vuorio, E. (1991) *J. Biol. Chem.*, in press.
7. Hogan, B., Costantini, F. & Lacy, E. (1986) *Manipulating the Mouse Embryo: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, N.Y.).
8. McLeod, M. J. (1980) *Teratology* **22**, 299–301.
9. Chelly, J., Kaplan, J.-C., Maire, P., Gautron, S. & Kalm, A. (1988) *Nature (London)* **333**, 858–860.
10. Metsaranta, M., Toman, D., de Crombrugghe, B. & Vuorio, E. (1991) *Biochim. Biophys. Acta* **1089**, 241–243.
11. Spranger, J. & Maroteaux, P. (1990) *Adv. Hum. Genet.* **19**, 1–103.
12. Steinmann, B., Rao, V. H., Vogel, A., Bruckner, P., Giltzelmann, R. & Beyers, P. (1984) *J. Biol. Chem.* **259**, 11129–11138.
13. Lee, B., Vissing, H., Ramirez, F., Rogers, D. & Rimoin, D. (1989) *Science* **244**, 978–980.
14. Vissing, H., D'Alessio, M., Lee, B., Ramirez, F., Godfrey, M. & Hollister, D. W. (1989) *J. Biol. Chem.* **264**, 18265–18267.
15. Tiller, G. E., Rimoin, D. L., Murray, L. W. & Cohn, D. H. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 3889–3893.
16. Ala-Kokko, L., Baldwin, C. T., Moskowitz, R. W. & Prockop, D. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 6565–6568.
17. Johnson, D. R. (1986) *The Genetics of the Skeleton: Animal Models for Skeletal Development* (Oxford Univ. Press, London).