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Human Dermatosparaxis: A Form of Ehlers-Danlos Syndrome That Results from Failure to Remove the Amino-terminal Propeptide of Type I Procollagen

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Summary

Dermatosparaxis is a recessively inherited connective-tissue disorder that results from lack of the activity of type I procollagen N-proteinase, the enzyme that removes the amino-terminal propeptides from type I procollagen. Initially identified in cattle more than 20 years ago, the disorder was subsequently characterized in sheep, cats, and dogs. Affected animals have fragile skin, lax joints, and often die prematurely because of sepsis following avulsion of portions of skin. We recently identified two children with soft, lax, and fragile skin, which, when examined by transmission electron microscopy, contained the twisted, ribbon-like collagen fibrils characteristic of dermatosparaxis. Skin extracts from one child contained collagen precursors with amino-terminal extensions. Cultured fibroblasts from both children failed to cleave the amino-terminal propeptides from the proα1(I) and proα2(I) chains in type I procollagen molecules. Extracts of normal cells cleaved to collagen, the type I procollagen synthesized by cells from both children, demonstrating that the enzyme, not the substrate, was defective. These findings distinguish dermatosparaxis from Ehlers-Danlos syndrome type VII, which results from substrate mutations that prevent proteolytic processing of type I procollagen molecules.

Introduction

Dermatosparaxis ("torn skin") is a recessively inherited connective-tissue disorder that was identified initially in cattle (Hanset 1971) and subsequently has been seen in sheep (Fjölstad and Helle 1973), dogs (Holbrook and Byers 1982), and cats (Counts et al. 1980; Holbrook et al. 1980). The disorder results from a defect in the processing of type I procollagen to collagen, with accumulation of molecules that retain the amino-terminal propeptide in most tissues (Lenaers et al. 1971). Procollagen N-proteinase, the enzyme that normally removes the propeptides, is defective (Lapière et al. 1971). The abnormal collagen molecules form characteristic ribbonlike fibrils (Simar and Betz 1971) that fail to provide normal tensile strength to tissues (Piérard and Lapière 1976). Affected animals have very fragile skin that may be torn during delivery or after minor trauma, and death often results from infection of large areas of avulsed dermis.

We recently identified two children with dermatosparaxis, whose skin contains the abnormal ribbon-like collagen fibrils characteristic of the disorder in animals and whose cultured dermal fibroblasts failed to remove the amino-terminal propeptide of type I procollagen. These two children appear to represent the first identified examples of humans with dermatosparaxis.

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Subjects and Methods

Clinical Summary

When first studied, proband 1 (our cell strain 90–041) was a 10-mo-old white female who was less than

the third percentile for height and weight. She was the first child born to an 18-year-old woman; the father was not identified, and parental consanguinity could not be excluded. She was delivered at 26–28 wk of gestation, after premature rupture of membranes, and her postnatal care was complicated by respiratory distress syndrome, hypoglycemia, left subependymal hemorrhage, hypocalcemia, right-sided pneumothorax, and hypothyroidism. At the time of a detailed physical examination at 10 mo of age, she was noted to have malrotated and low-set ears, prominent eyes with protuberant eyelids, hypoplastic chin, prominent skin folds of the neck and limbs, and a large umbilical hernia. Her skin was soft and had a doughy feel. At 7 mo of age she was noted to bruise easily, and at 8 mo of age she experienced the first in a series of injuries in which the skin was avulsed from the underlying soft tissues. With each episode the skin was easily approximated and healed well in a short period of time. At 20 mo of age a minor fall while walking resulted in a left subdural hematoma that produced right-sided weakness and hand twitching. Evacuation of the hematoma resulted in restoration of motor coordination. Routine laboratory tests were normal. She was thought to be hypothyroid, on the basis of studies done in the perinatal period; circulating levels of T-4 and thyroid-stimulating hormone were 10.8 μg/dl and 2.4 μU/ml, respectively, while she was taking 10 μg of Synthroid daily (for details, see Wertelecki et al., in press).

Proband 2 (cell strain 91-252) was born to nonconsangineous, physically normal parents (our cell strains 91-548 [mother] and 91-549 [father]) by cesarean section at 35 wk of gestation, because of breech presentation and premature rupture of membranes. Apgar scores were 8 and 9 at 1 and 5 min, respectively. At birth he was noted to have bilateral inguinal skin tears, a large umbilical hernia, puffy eyelids with excessive periorbital skin, redundant lax skin of the posterior neck, micrognathia, and large fontanels. Additional evaluation at that time demonstrated a bicuspid aortic valve and dental laminal cysts. He had a normal male karyotype. Both inguinal tears healed with minimal scarring. At 4 mo of age he was noted to have easy bruising, joint hyperextensibility, umbilical hernia, large fontanels, blue sclera, puffy eyelids, and soft, sagging, redundant skin. The child's skin tore easily and healed quickly after suturing. Platelet function and number were normal. At 8 mo of age he was below the third percentile for head circumference, height, and weight, but motor skills were within normal limits, and psychosocial development appeared normal. At 12 mo of age, minor trauma resulted in a jagged laceration of the face, which healed quickly without major scar formation. At 18 mo of age he was not yet walking, although fine motor skills were normal.

Electron Microscopy

A 2-mm punch biopsy (inner aspect of the upper arm) was obtained from proband 1 at 10 mo of age, was fixed in half-strength Karnovsky fixative, was postfixed in osmium tetroxide, was stained en bloc in saturated aqueous uranyl acetate, and was dehydrated through graded ethanol. An additional sample of skin was obtained at the time of umbilical hernia repair. For transmission electron microscopy (EM), samples were carried through propylene oxide and were embedded in Epon 812. Ultra-thin sections were collected onto copper grids, were stained sequentially with 0.5% phosphotungstic acid, 1% uranyl acetate, and Reynold's lead citrate, and were viewed with a Philips 420 transmission electron microscope. For scanning EM, samples were taken from 100% ethanol into liquid carbon dioxide, critical point dried, mounted, coated with gold-palladium, and viewed with an ETEC scanning electron microscope. The biopsies from proband 2 (obtained at 10 mo of age from the lateral aspect of his lower leg) and from each of his parents were processed as described above, except that, prior to sectioning, they were embedded in Spurr's resin.

Extraction of Collagen from Skin

Skin samples from the first proband, an unaffected newborn control (foreskin), and from a normal adult control were minced, homogenized in 1 M NaCl, 50 mM Tris-HCl pH 7.5, stirred overnight at 4°C, and centrifuged to yield a supernatant (S1) and a pellet (P1). The pellet was suspended in 0.5 M acetic acid, homogenized, stirred overnight at 4°C, and centrifuged to yield supernatant (S2) and a pellet (P2) that was in turn suspended and homogenized in 8 M urea, stirred overnight at 4°C, and centrifuged to yield supernatant (S3) and the residue (P3). Each supernatant was dialyzed into 0.5% acetic acid, lyophilized, and suspended in SDS-PAGE sample buffer, and the collagen chains were identified by SDS-PAGE (Laemmli 1970).

Analysis of Collagen Synthesized by Dermal Fibroblasts

Fibroblasts were cultured from explanted dermal biopsies under routine conditions by using Dulbecco-modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum and penicillin-strepto-

mycin (GIBCO). Confluent fibroblasts were incubated with 2,3,4,5-[^{3}H]proline (100 Ci/mM; Amersham Corp, Arlington Heights, IL) in DMEM supplemented with ascorbic acid (50 μg/ml), and collagenous proteins were harvested according to a method described elsewhere (Bonadio et al. 1985). In some experiments, dextran sulfate was added (final concentration 0.01%; sodium salt M_r 500,000; Pharmacia) to enhance proteolytic processing of procollagens by collagen N- and C-proteinases in the cell layer. After incubation for 20 h, both the proteins in the medium and the cell layers were harvested separately in the presence of protease inhibitors and examined by SDS-PAGE.

Conversion of Procollagen to Collagen

Cells were incubated overnight with 2,3,4,5-[^{3}H]-proline in the presence of ascorbic acid (as above), and the medium containing radioactively labeled collagenous proteins was harvested without protease inhibitors and was supplemented with nonradioactive proline (final concentration 10 mM) to inhibit further incorporation and with dextran sulfate (0.01% final concentration) to facilitate enzymatic processing. The medium samples were then added to 35 mm dishes that contained 250,000 cells that had been plated at the time of initial plating of the cells used for labeling, but that had not been incubated with [^{3}H]proline. After incubation for 20 h, the medium and cell layers were harvested separately, and the collagenous proteins were examined by SDS-PAGE, under reducing conditions.

Results

Dermis Contains Abnormal Collagen Fibrils

The dermis from both children contained abnormal collagen fibrils in ribbon-like sheets (fig. 1), identical to those seen in skin from animals with dermatosparaxis. Collagen fibrils of the sheaths around blood vessels, nerves, adnexa, and the reticular lamina underlying the dermal/epidermal junction were serrated or notched in cross section (fig. 2). Collagen fiber bundles in skin from the affected individual (fig. 3*a*) were more loosely packed than those in skin from controls (fig. 3*b*). Collagen fibrils in skin from both parents of proband 2 were normal (data not shown).

Abnormal Skin Contains Extended α Chains of Type I Collagen

Skin extracts, available only from proband 1, contained α1(I) and α2(I) chains of type I collagen and,

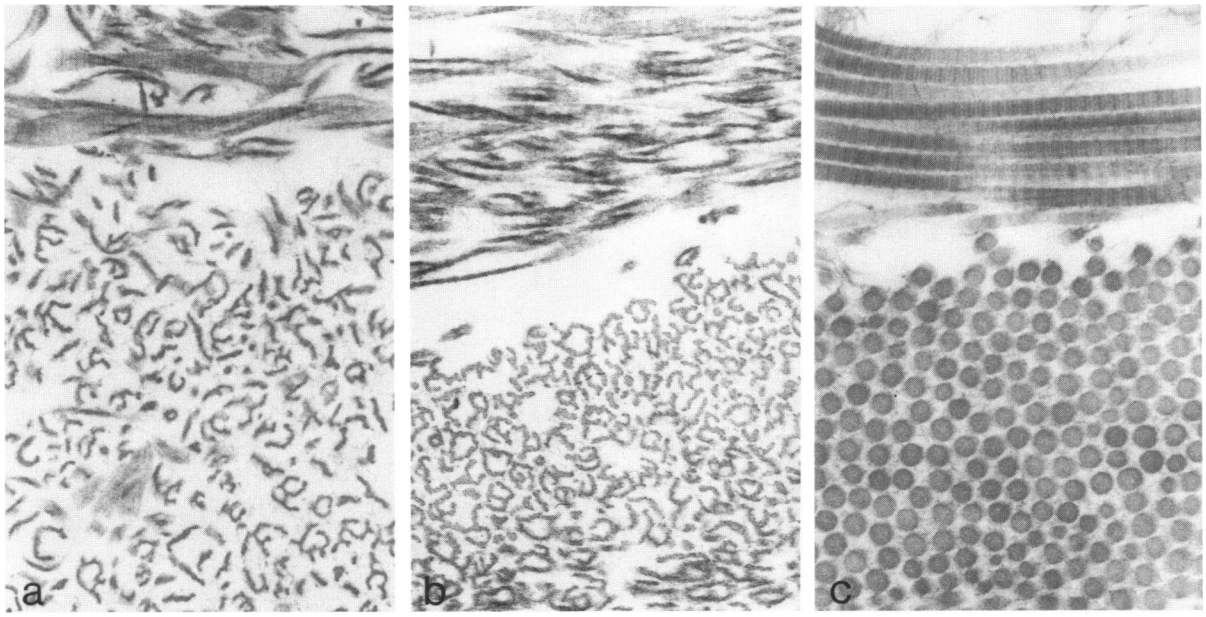

Figure 1 Transmission electron micrographs of reticular dermis from proband 1 (*a*), proband 2 (*b*), and control (*c*). Periodic banding is apparent along the twisted ribbon structures (*a* and *b*) and in normal collagen fibrils (*c*). The hieroglyphic profiles (*a* and *b*) contrast with the round profiles of normal collagen fibrils in cross section (*c*). Magnification is 42,500×.

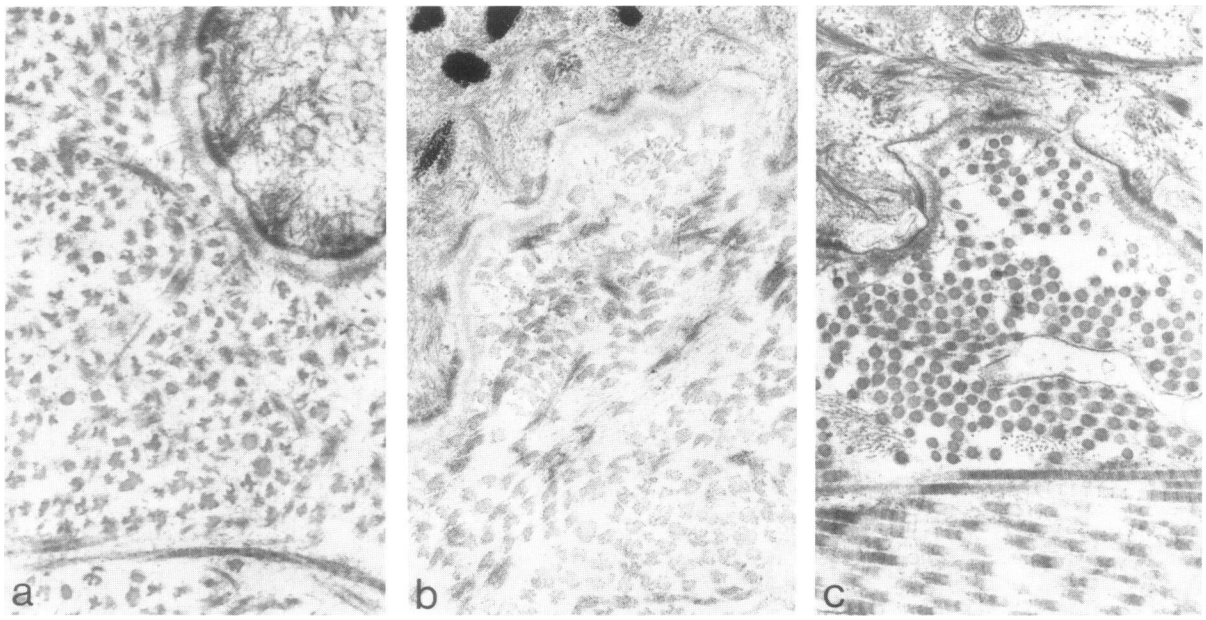

Figure 2 Transmission electron micrographs of the dermal/epidermal junction in the skin of proband 1 (*a*), proband 2 (*b*), and control (*c*). In the connective tissue underlying the basement membrane, the fine collagen fibrils have abnormally serrated borders (*a* and *b*). In normal skin, the collagen fibrils have smooth borders. Magnification is 32,500×.

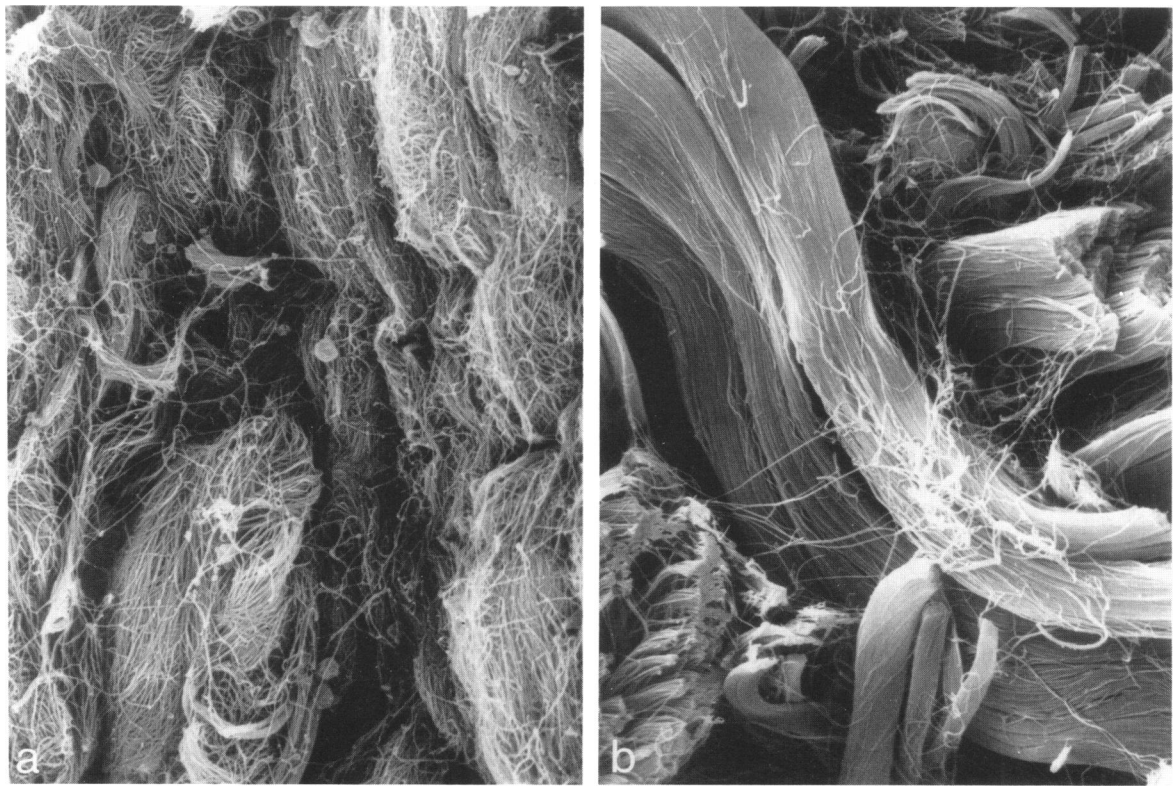

Figure 3 Scanning electron micrograph of dermal collagen fibers from proband 1 (*a*) and a control (*b*). In proband 1, the surfaces of collagen fibers have tangled collagen fibrils (*a*) that contrast with the organized parallel alignment of fibrils in normal dermis of the control (*b*). Magnification is 2,000×.

in addition, chains that migrated in the position of those that retained the amino-terminal propeptides (pNα chains) (fig. 4). Control skin samples contained only the α chains and the normal cross-linked chains (β-components). The skin from the proband contained multiple dimers, indicating that at least some of the pNα chains were involved in interchain cross-links.

Cultured Cells Fail To Cleave the Amino-terminal Propeptides from the Chains of Type I Procollagen

After cells from both probands and from the parents of proband 2 were incubated with [^{3}H]proline for 16 h, and after the medium and cell-layer collagenous proteins were examined by SDS-PAGE, the efficiency of conversion of type I procollagen to collagen was limited (fig. 5*A*), with accumulation of pNα chains. Control cells generally process procollagens with limited efficiency (for comparison, see fig. 5*A*), but, among cell strains, there is marked variation in the extent of conversion. As a consequence, in these studies it was difficult to distinguish the proband's cell strains from others, including parental cells. To augment the efficiency of enzymatic conversion by partial proteolysis, cells were incubated with [^{3}H]proline, in the presence of dextran sulfate (Bateman and Golub 1990). Under these conditions, control cells and cells from the parents of proband 2 retained little procollagen in the medium and converted to collagen virtually all type I procollagen that was found in the cell-associated matrix (fig. 5*B*). In contrast, cells from both probands removed the carboxyl-terminal propeptides efficiently but failed to remove the amino-terminal propeptides and, as a result, accumulated pNα chains in the pericellular matrix (fig. 5*B*).

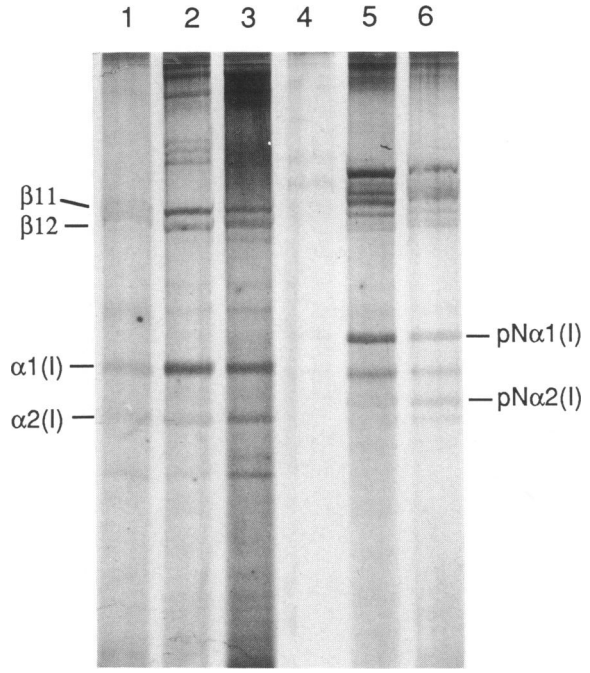

Figure 4 SDS-PAGE of skin extracts from a control (lanes 1–3) and from proband 1 (lanes 4–6). Collagen was extracted from skin by means of 0.5 M acetic acid (lanes 1 and 4) or 8 M urea (lanes 2 and 5), and the residual pellet (lanes 3 and 6) was dissolved in buffer. In the control lanes, α1(I) and α2(I) chains of type I collagen and the β11 and β12 dimers are the major collagen components (lanes 1–3). In extracts from the skin of proband 1, two novel chains that migrate in the expected positions for pNα1(I) and pNα2(I) are present (lanes 4–6), and there are additional cross-link components in the region of the β dimers.

Complementation of the Defect in Proteolytic Processing by Normal Cells

When procollagens synthesized by control cells were added, along with dextran sulfate to augment proteolytic processing to control cells, conversion to collagen was complete, and the molecules were deposited in the cell-associated matrix (fig. 6). When the procollagen molecules synthesized by cells from either proband were added back to cells from each proband, proteolytic removal of the carboxyl-terminal propeptides was complete, but virtually no molecules had the amino-terminal propeptides removed. As a result, these cells accumulated pNα chains in the cell-associated matrix (fig. 6). In contrast, procollagen molecules synthesized by cells from each proband were converted to collagens by control cells, and only α chains could be identified. Procollagens synthesized by control cells and then incubated with cells from the affected infants were partially converted to collagen, but more than half remained as pNα chains (fig. 6); partial conversion probably reflects the presence of residual enzyme in the procollagen preparation from the normal cells. These results indicate that there is a defect in the activity of the N-proteinase in cells from both probands and that the defects cannot be complemented by enzymes from the other. These findings are compatible with deficient activity of the type I procollagen N-proteinase rather than with a substrate mutation that prevents proteolytic processing.

Discussion

The two children described here have dermatosparaxis, a disorder previously recognized only in animals.

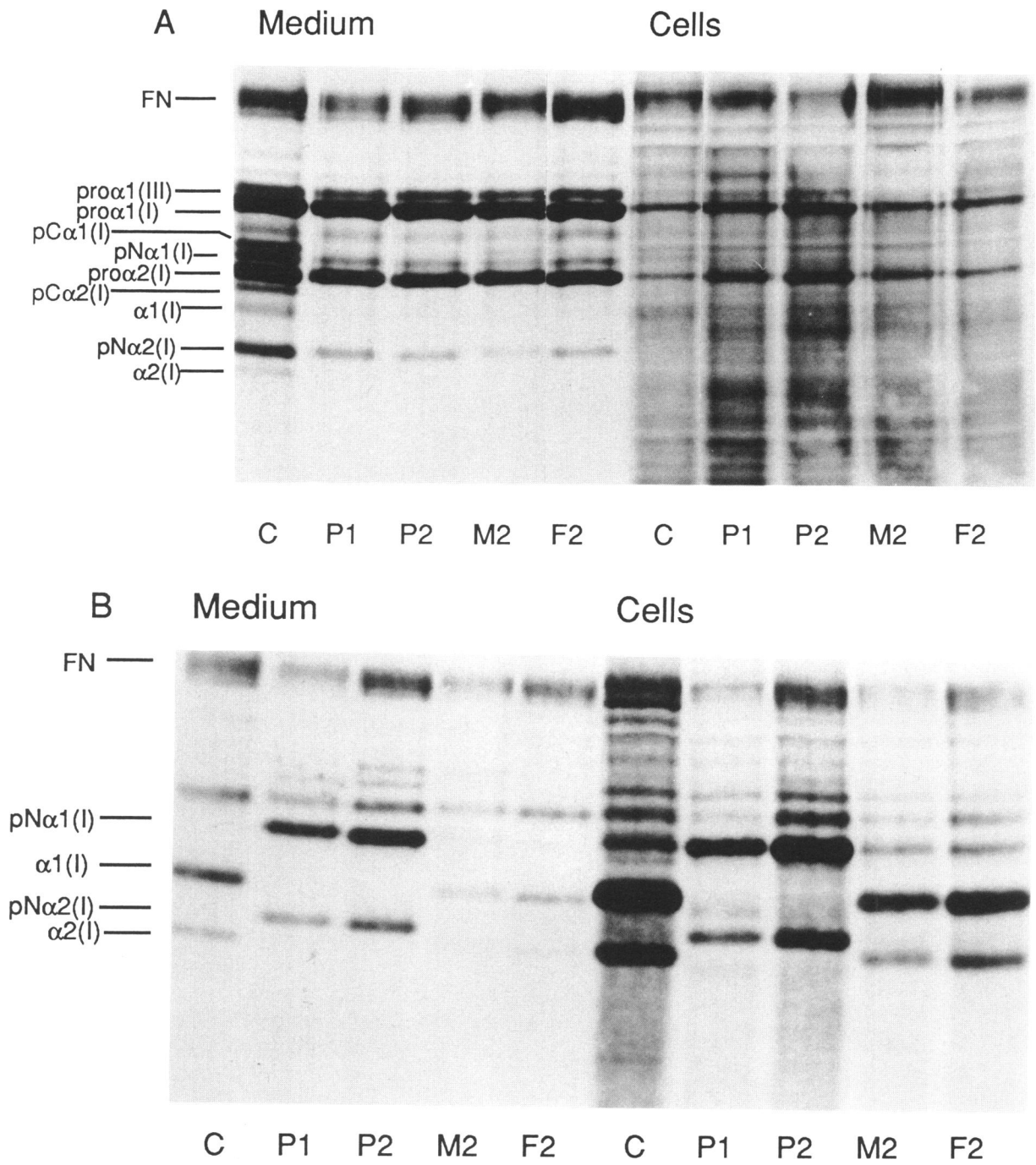

Figure 5 Collagens synthesized and processed by cultured dermal fibroblasts. *A*, Procollagen synthesized by skin fibroblasts cultured from a control (lane C), proband 1 (lane P1), proband 2 (lane P2), and the mother (lane M2), and the father (lane F2) of proband 2. Cells were incubated overnight in the presence of ascorbic acid and [^{3}H]proline. Proteins in medium and cell layer were harvested separately and then were analyzed by SDS-PAGE under reducing conditions. Under these conditions, the control cells can cleave the amino-terminal propeptide, the carboxyl-terminal propeptide, or both, from the type I procollagen molecules in the medium. The conversion of procollagen to collagen is incomplete. Cells from both probands and from the parental cells of proband 2 appear to be able to cleave the carboxyl-terminal propeptide more efficiently than they cleave the amino-terminal propeptide, thus accumulating pNα chains. The pCα chains contain the carboxyl-terminal propeptide; pNα chains contain the amino-terminal propeptides. FN = fibronectin. *B*, Procollagen processing in the presence of dextran sulfate by cells from a control (lanes C), both probands (lanes P1 and lanes P2), and from both parents (lanes M2 and

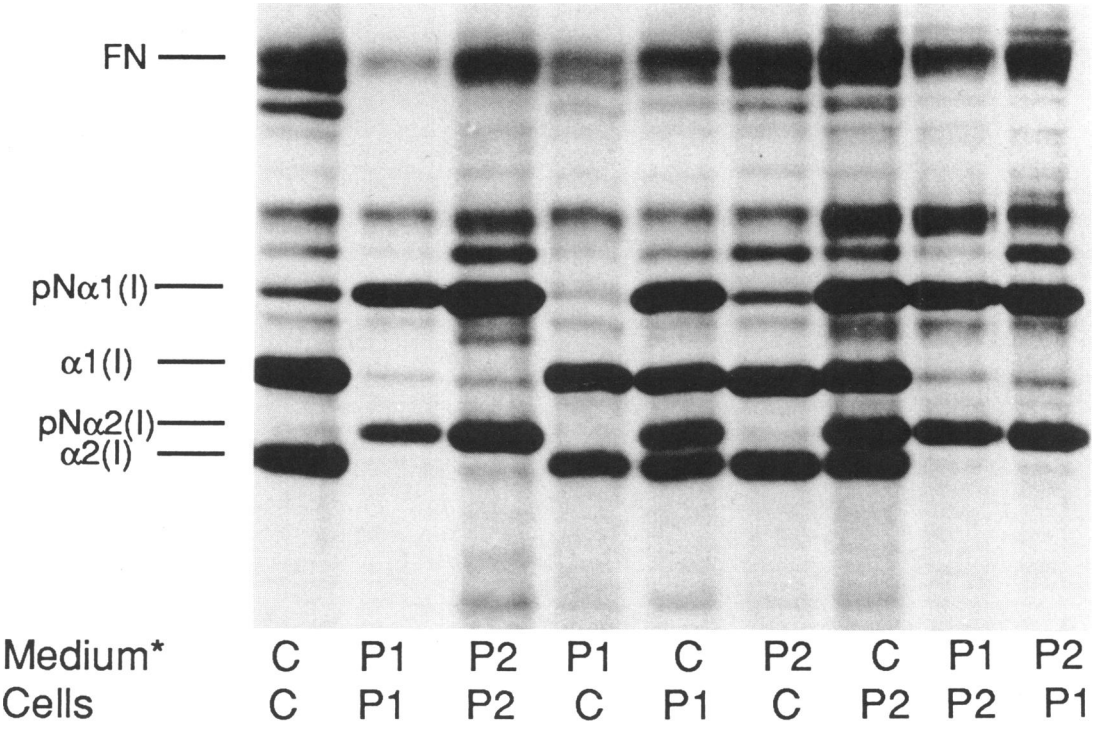

Figure 6 Complementaion of the defect in proteolytic processing of type I procollagen by normal cells. Cells were incubated with [^{3}H]proline, in the presence of ascorbic acid, for 16 h. The medium containing the [^{3}H]proline-labeled proteins was then supplemented with 10 mM proline (to inhibit further incorporation of the radioactive amino acid into protein) and dextran sulfate, 0.01% final concentration (to induce conversion of procollagen to collagen) and was added to cells that had not been exposed to the label. After incubation for an additional 16 h, the cell layers of each dish were harvested, and the proteins were examined by SDS-PAGE under reducing conditions. In these studies, cells from a control (C) were used to synthesize proteins that were then placed on cells from a control, proband 1 (P1), or proband 2 (P2); similarly, cells from probands 1 and 2 were used to synthesize proteins that were then placed on cells from a control, proband 1, and proband 2. Medium* = source of the labeled proteins; Cells = unlabeled cells to which the previously labeled proteins were applied. Control cells convert, to α chains, the type I procollagen synthesized by control cells and by cells from both probands. Cells from each proband process, to pNα chains only, their own molecules and those synthesized by the other proband. Cells from each proband process, to α chains, some molecules made by normal cells but accumulate substantial amounts of pNα chains. The completed processing probably reflects the presence of N-proteinase from the control cells.

The phenotype results from the accumulation of collagen precursors in multiple tissues, as a consequence of a lack of activity of type I procollagen N-proteinase, the enzyme that removes the amino-terminal propeptides from the chains of type I procollagen. Skin from these children contained characteristic ribbon-like collagen fibrils, made up, in part, by molecules that contained extended collagen chains. In animals, the disorder is inherited in an autosomal recessive fashion. Although an enzymatic defect has been demonstrated in cells from both probands, there is no evidence of consanguinity in either family to further support the hypothesis of autosomal recessive inheritance.

Cultured dermal fibroblasts from both probands do not remove the amino-terminal propeptides from type I procollagen molecules. In skin from the first pro-

lanes F2) of proband 2. Cells were incubated with [^{3}H]proline, in the presence of ascorbic acid and dextran sulfate, for 16 h, and the proteins in the medium and in the cell layer were harvested separately and were analyzed by SDS-PAGE under reducing conditions. The majority of type I collagen proteins in the control and parental cell cultures are found in the cell layer, but in both compartments, conversion of procollagen to collagen is complete and only α chains are apparent. In cultured cells from both probands, although most of the type I collagen proteins are in the cell layer, a substantial proportion remain in the medium. Cells from both probands accumulate pNα1(I) and pNα2(I) chains in both compartments; virtually no fully processed molecules are apparent.

band, however, there is an abundance of chains that comigrate with α1(I) and α2(I) chains of type I collagen. We have not determined that the amino-terminal sequence from those chains confirms that the correct cleavage occurred and excludes the possibility of cleavage by an alternative mechanism. The presence of multiple cross-linked components suggests that some processing occurs that leaves cross-link residues in the amino-terminal non-triple-helical region accessible.

Shortly after dermatosparaxis was identified in cattle (Lenaers et al. 1971), Lichtenstein et al. (1973) identified three girls with a form of Ehlers-Danlos syndrome (EDS) type VII, whose skin contained extended α1(I) chains of type I collagen. They hypothesized that the defect was similar to that in cattle. Clinical examination showed that these children did not have fragile skin but, instead, had bilateral congenital hip dislocation and recurrent dislocations of several other joints. Subsequent studies of one of those children (Steinmann et al. 1980; Weil et al. 1989) and then of additional patients with the same phenotype (Eyre et al. 1985; Cole et al. 1986; Wirtz et al. 1987, 1990; Weil et al. 1988, 1989*a*, 1989*b*, 1990; Vasan et al. 1991) demonstrated that EDS type VII results from heterozygosity for mutations that induce skipping of the sequences contained in exon 6 in either the COL1A1 (EDS type VIIA) gene or the COL1A2 (EDS type VIIB) gene (which encode the proα1(I) and proα2(I) chains, respectively, of type I procollagen). In both genes, this exon encodes the domain that contains the type I procollagen N-proteinase cleavage site (D'Alessio et al. 1988). The phenotypic result of mutations in COL1A1 (Cole et al. 1986) appears more severe than the result of mutations in COL1A2, probably because more molecules would be defective with random chain assortment (type I procollagen molecules contain two proα1(I) chains and a single proα2(I) chain). The phenotype of dermatosparaxis can be classified as EDS type VIIC.

The difference between the clinical presentation of mutations that affect the processing enzyme and that of mutations that affect the type I procollagen substrate is striking. The major features of dermatosparaxis include (on the basis of limited clinical experience) lax and fragile skin that tears easily, inguinal hernia, blue sclerae, a small chin, and growth retardation. In contrast, the major features of EDS type VII are congenital hip dislocation, which may be refractory to attempts at repair, and dislocation of multiple other joints.

These differences probably reflect the nature of the abnormal type I collagen molecules that accumulate in tissues. In dermatosparaxis, the enzyme fails to cleave the amino-terminal propeptide, leaving molecules that have extensions on all chains. Other enzymes in the matrix probably produce some cleavage (e.g., see fig. 4), but the majority of molecules involved in fibril formation are those with extended aminotermini. In EDS type VII, when the mutation is in the COL1A2 gene, only half of the molecules contain the abnormal chain, and many of those molecules can be further processed (Watson et al., in press). The cleavage apparently allows the propeptide extension to assume a different conformation that permits fibrillogenesis to occur more normally. When the mutation is in the COL1A1 gene, three-quarters of the molecules formed have abnormal amino-terminal extensions. The mutation in the substrate interferes with cleavage, but the enzyme can, apparently, cleave the proα2(I) and a normal proα1(I) chain, even in molecules that contain an abnormal chain (Wirtz et al. 1990).

The molecular basis of the deficiency of type I procollagen N-proteinase has not been determined for any form of dermatosparaxis. The enzyme functions only on a trimeric substrate (Tuderman and Prockop 1982), is sensitive to the conformation of the substrate molecules, and cleaves the proα1(I) chain prior to cleaving the remaining chains. Mutations that alter the conformation of type I procollagen can decrease the efficiency of cleavage (Minor et al. 1986; Vogel et al. 1988; Bateman and Golub 1990; Wirtz et al. 1990). Type I procollagen N-proteinase has been isolated from chick tendons and has been partially characterized (Hojima et al. 1989). The active enzyme has a high molecular weight and contains multiple subunits, with the activity residing in a polypeptide chain of approximately 200–300 kD. The enzyme from dermatosparaxic animals has not been characterized, so it is not known which subunit harbors mutations. It is possible that mutations in one of several genes could reduce activity and lead to the clinical features of dermatosparaxis.

The three disorders—dermatosparaxis and the two kinds of EDS type VII (A and B, which result from mutations in COL1A1 and COL1A2, respectively)—represent one of the rare examples in human genetic disease in which mutations in the proteolytic enzyme and in the protein product are known to produce recognizable phenotypes. In some of the other disorders, mostly known in the clotting cascade, the phenotypic

endpoint—a bleeding diathesis—is similar for all mutations. Because of defects in procollagen processing, the phenotypes of the enzymatic defect and of the defect in substrate are readily distinguishable, presumably because the nature of the accumulated molecules in tissues dictates the extent of and distribution of the effects on tissues.

The prevalence of "dermatosparaxis" in humans is unknown. On the basis both of the striking clinical findings and of the recognition of the disorder in several animal species, it is surprising that the condition has not been recognized previously in humans. The failure previously to identify affected individuals may reflect the methods used for diagnosis. The efficiency of enzymatic cleavage of type I procollagen by cultured cells varies from time to time within the same cell strain and among normal cell strains. The addition of dextran sulfate to culture medium facilitates cleavage but is not used routinely. In contrast, the transmission electron microscopy findings are dramatic and characteristic of the disorder. It is possible that other children and infants with this disorder have not been identified because appropriate studies have not been performed. Alternatively, the mutations may be exceedingly rare in the human population.

Note added in proof.—A more detailed clinical description of the second proband has been submitted for publication (Petty et al., submitted). A third child with dermatosparaxis was recently identified, and a detailed clinical and biochemical study has been accepted for publication (Nusgens et al., in press).

Acknowledgments

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