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Restricted homology between human α1 type IV and other procollagen chains

(basement membrane/carboxyl propeptide/cDNA clones/collagen/multiple mRNAs)

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ABSTRACT **Screening of a human fibroblast cDNA library with a mouse type IV procollagen clone resulted in one 1.05-kilobase isolate that was used to identify a 1.7-kilobase clone overlapping the former by <150 nucleotides. *Eco*RII digestion revealed that the larger clone exhibited the pattern characteristic of DNA coding for a collagenous sequence. Blot hybridization to RNA from mouse F9 stem cells and from these cells treated with retinoic acid and $N^6,O^{2'}$-dibutyryl-cAMP showed induction of type IV mRNA. DNA sequencing and comparison of the derived amino acids with the reported protein data demonstrated that the clones encode part of the α1 chain of human type IV procollagen. Alignment of α1(IV) with other human procollagens showed minimal but detectable homology. A small cluster of charged residues in the α chain is partially shared by type IV. In close proximity is an interruption in the α1(IV) Gly-Xaa-Yaa region corresponding to the 3′ end of a unique proline-free, and therefore also less rigid, area in other collagen triple helices. Analysis of the carboxyl-terminal α1(IV) peptide showed a repeat symmetry possibly dictated by the six cysteines in each half of the structure. The position of five cysteines in addition to four tyrosine/tryptophan groups allowed a correlation to be drawn between the 3′ noncollagenous type IV region and the larger, highly conserved carboxyl propeptides of other human procollagens. Such similarities in the different chains may define functional domains conserved throughout evolution.**

Basement membranes are complex structures that play an important role in morphogenesis, filtration, support, and separation of epithelia from underlying connective tissue (1). They are composed primarily of the proteins, type IV procollagen, laminin, and entactin and the heparan sulfate proteoglycan (2). One of these components, type IV procollagen, consists of two genetically distinct chains, α1(IV) and α2(IV) with M_rs of 185,000 and 170,000, respectively (3–5). These polypeptides have been isolated from a number of tissues, including glomeruli, lens capsule, placenta, parietal yolk sac, and the EHS sarcoma. Amino acid analysis revealed unique differences from the interstitial procollagens (types I, II, and III), including increased glycosylation, a high content of hydroxylysine and 3- and 4-hydroxyproline, and low levels of arginine and alanine (6). Other distinctive features characteristic of type IV procollagen are the frequent interruptions in the repeating Gly-Xaa-Yaa region, little, if any, processing of the amino and carboxyl noncollagenous peptides, and the formation of these more flexible molecules into a nonfibrillar meshwork, in contrast to the prominent cross-striated fibrils of the interstitial collagens (7–9).

Therefore, determination of the unusual structure of type IV procollagen has been the primary aim of a number of investigators. The protein sequence of the human α1(IV) M_r 95,000 pepsin fragment corresponding to the carboxyl-terminal two-thirds of the collagenous region was reported recently (10). Comparison of this large peptide, containing 12 interruptions, with the partial sequence of the mouse (11) and bovine (12) α1(IV) chains showed about 85% homology (10). Using a mouse cDNA clone (13) representing part of the 3′ untranslated area of the α1(IV) mRNA (unpublished data), we have been able to identify human α1(IV) DNA clones from cDNA libraries. Here we report the nucleotide sequence coding for 182 residues of the collagenous region and the entire 229-residue noncollagenous 3′ peptide. Analysis of these highly divergent structures allowed limited but significant parallels to be drawn between α1(IV) and the conserved α chains and carboxyl propeptides of other human procollagens.

MATERIALS AND METHODS

Materials. Sources of enzymes, nucleic acids, chemicals, and isotopes have been described (14). Cell lines GM3348 and GM2962 were provided by the Human Genetic Mutant Cell Repository (Camden, NJ) and CCL136 was provided by the American Type Culture Collection (Rockville, MD).

Construction and Screening of cDNA Libraries. Construction of the cDNA libraries has been detailed (14). Double-stranded DNA was dC-tailed and annealed to pBR322 that had been cleaved with *Pst* I and dG-tailed (New England Nuclear). Hybrids were used to transform *Escherichia coli* strain MC1061. Replica-plated clones were hybridized under low stringency to the mouse type IV insert or under high stringency to the human type IV insert, and the nitrocellulose filters were washed accordingly (14).

DNA Sequencing and Preparation of Oligonucleotide Primers. The following restriction fragments of the α1(IV) cDNA clone, KK4 (Fig. 1), were cloned into appropriately cleaved M13 mp18 and mp19 vectors: *Pst* I, *Pst* I/*Sph* I, *Pst* I/*Bam*HI, and *Bam*HI/*Hin*dIII. Conditions for ligation, transformation, and Sanger dideoxy-sequencing reactions were essentially as described (15). The universal primer of 17 nucleotides (Collaborative Research, Waltham, MA) was used for the sequencing as well as six additional 15-nucleotide primers that were synthesized by using an apparatus from Sequemat (Watertown, MA) and purified by high-pressure liquid chromatography (16). Specific type IV primer sequences, noted in Fig. 1 by triangles, were derived from those coding for the collagenous domain 5′ → 3′, carboxyl terminus 5′ → 3′ (two primers) and 3′ → 5′ (two primers), and untranslated region 3′ → 5′.

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Abbreviation: kb, kilobase(s).

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RESULTS

Isolation of Human Procollagen cDNA Clones. The construction of two human cDNA libraries prepared from 28–35S normal fibroblast (GM3348) and rhabdomyosarcoma (CCL136) poly(A)$^+$ RNA has been described (14). Previous screening of 12,000 colonies yielded procollagen α1(III) and α2(V) clones from both libraries (14, 17) and α1(I) and α2(I) from the GM3348 library (ref. 14 and unpublished data). The possibility that type IV procollagen sequences might also be represented in these libraries was explored, since type IV is synthesized by the CCL136 cell line (18) as well as by a fibroblast culture (GM2962) from an osteogenesis imperfecta patient (19).

First, CCL136, GM2962, and GM3348 poly(A)$^+$ RNAs were blotted (20) and hybridized under low stringency to the mouse type IV cDNA clone pcI5 (13). One major and several minor species were seen with the fibroblast RNAs and a diffuse area of hybridization was seen with the CCL136 RNA (not shown). Control filters hybridized to α1(I) and α1(III) cDNA clones showed that the type IV region was just above the larger α1(I) transcript. Therefore, screening of the GM3348 library with the mouse type IV DNA under low stringency was initiated. One positive clone, NB3, containing a 1.05-kilobase (kb) insert, was characterized by restriction mapping (Fig. 1) and Southern blot hybridization to pcI5 cleaved with different restriction enzymes (not shown). NB3 was then rehybridized to the GM3348 clones under highly stringent conditions and also to clones from the CCL136 library. Two additional positive clones were identified, and their restriction maps were compared with the first human isolate, NB3 (Fig. 1). Whereas the CCL136 clone RR6, with a 1.15-kb insert, was almost entirely contained within NB3, the 1.7-kb insert of the GM3348 clone KK4 overlapped NB3 by <150 nucleotides and extended another 1.55 kb.

Characterization by *Eco*RII Digestion and Southern Blot Hybridization. If the total 2.6 kb of cloned DNA coded for part of a procollagen chain, some sequences representing the Gly-X-Y domain might be present. One reproducible method for screening the plasmids has been digestion by the enzyme *Eco*RII, which recognizes the site CC$\frac{A}{T}$GG (Pro-Gly) found multiple times in DNA coding for the Gly-Xaa-Yaa collagenous region. Only KK4 showed the characteristic pattern of small DNA fragments (Fig. 2) similar to the α2(I) procollagen clone Hf32 that covers part of the α chain and carboxyl propeptide (21). The human clones NB3 and RR6 (Fig. 2) and the mouse clone pcI5 (not shown) were negative by this assay.

Because of the weak hybridization signal observed when KK4 was detected and the presence of a common *Hin*dIII site only with RR6, we wanted to obtain further proof that KK4 was contiguous with NB3. Therefore NB3, RR6, and KK4 were hybridized to genomic DNA cleaved with *Eco*RI. A unique 11-kb fragment seen with NB3 and RR6 was the same size as one hybridizing to KK4 (Fig. 3). An additional 4.7-kb fragment was also identified with KK4. These two sets of experiments verified that KK4 is orientated 5′ to NB3 and RR6 (Fig. 1) and also showed that the 3′ end of this procollagen gene consists mostly of intervening sequences.

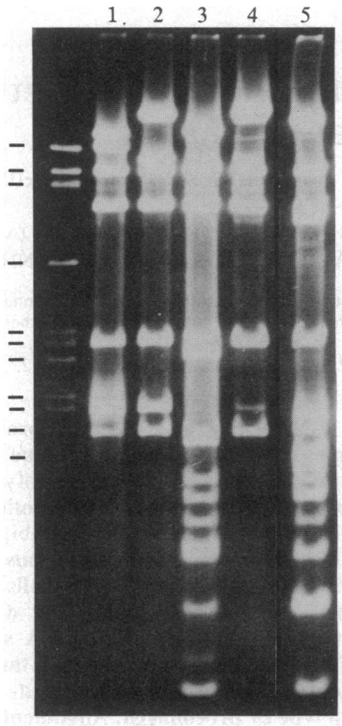

FIG. 2. *Eco*RII digestion of procollagen clones and pBR322. Three micrograms of DNA was digested with *Eco*RII (recognition site CC$\frac{A}{T}$GG) and electrophoresed in a 12% polyacrylamide gel. Lane 1, the α1(IV) clone NB3; lane 2, the α1(IV) clone RR6; lane 3, the α1(IV) clone KK4; lane 4, the pBR322 plasmid; and lane 5, the α2(I) clone Hf32 (21). *Hae* III-cleaved ϕX174 DNA markers shown on the left are 1353, 1078, 872, 603, 310, 281, 271, 234, 194, 118, and 72 base pairs.

RNA Transfer Blot Hybridization to Mouse and Human RNAs. Synthesis of type IV procollagen is induced in F9 mouse teratocarcinoma stem cells after treatment with retinoic acid and N^6,$O^{2'}$-dibutyryl-cAMP (22). Subsequent hybridization of mouse type IV clones to RNA isolated from these cells at different time points after retinoic acid addition showed a concomitant increase in type IV mRNA (13, 23). Thus, additional evidence for the human clones coding for

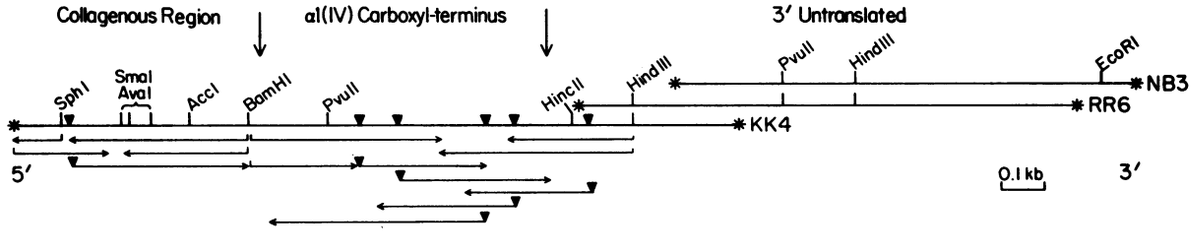

FIG. 1. Characterization of the α1(IV) procollagen cDNA clones by restriction analysis and DNA sequencing. The three clones shown were analyzed independently for the presence of endonuclease restriction sites. Digestion using *Kpn* I, *Cla* I, *Sac* I, *Sal* I, *Xba* I, *Xho* I, *Eco*RV, *Bgl* II, and *Pst* I was negative in the inserts that were cloned into the *Pst* I site of pBR322 (asterisks). The fragments of KK4 cloned into M13 mp18 and mp19 for DNA sequencing are described in *Materials and Methods*. Thin lines show the regions of KK4 sequenced by the Sanger dideoxy procedure (15) multiple times to obtain the reported nucleotides and derived amino acids. The universal primer was used for sequencing from the *Pst* I, *Sph* I, *Bam*HI, and *Hin*dIII sites. Triangles show the location of six 15-nucleotide primers synthesized by using sequences from KK4. Arrows designate the junctions of the three regions indicated.

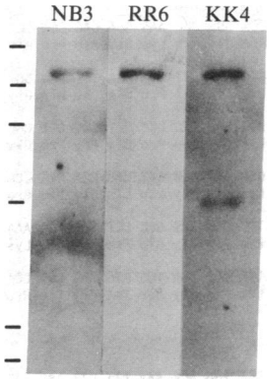

FIG. 3. Southern blot hybridization of α1(IV) clones to genomic DNA. Fifteen micrograms of genomic DNA was digested with *Eco*RI, electrophoresed in 1% agarose gels, and transferred to nitrocellulose filters (20). Plasmid DNAs were ^{32}P-labeled and nick-translated to a specific activity of 6–8 × 10^8 cpm/μg and hybridized in 50% formamide/0.9 M NaCl/0.09 M sodium citrate, pH 6.8, for 20 hr at 38°C. Final washing of the filters was at 68°C in 30 mM NaCl/3 mM sodium citrate, pH 6.8/0.1% $NaDodSO_4$. Autoradiography times were from 48 to 72 hr. *Hin*dIII-cleaved λ DNA markers indicated on the left are 23, 9.4, 6.5, 4.3, 2.3, and 2.0 kb.

type IV procollagen would be generated by the same hybridization pattern. By using NB3 and KK4 as probes, both the increase in intensity and size of the mRNAs were comparable to the profile observed with the homologous mouse type IV clone pcI5 (Fig. 4).

Hybridization of KK4 to human fibroblast mRNA (GM3348) showed the recurring pattern of multiple procollagen transcripts (Fig. 5). With the human α1(I) and α1(III) cDNA clones, two species of about equal intensity are seen (refs. 14 and 24 and Fig. 5). In contrast, the higher molecular weight type IV mRNA (6500 nucleotides) vastly predominates over the two smaller ones (5900 and 5300 nucleotides). We and other investigators have characterized human (24)

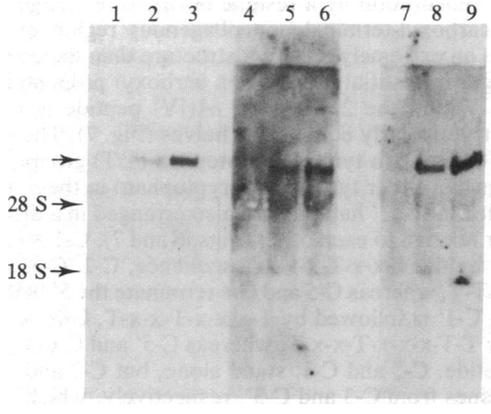

FIG. 4. Analysis of F9 cell mRNAs homologous to pcI5, KK4, and NB3. F9 teratocarcinoma cells were treated with 0.5 μM retinoic acid, 500 μM N^6,$O^{2'}$-dibutyryl-cAMP, and 500 μM theophylline. Five micrograms of total RNA was transferred to nitrocellulose filters from a 1.0% agarose/formaldehyde gel (20) and hybridized to the indicated ^{32}P-labeled, nick-translated plasmids. Lanes 1–3, the mouse clone pcI5 (insert only); lanes 4–6, the human clone KK4; lanes 7–9, the human clone NB3. RNA was from F9 stem cells (lanes 1, 4, and 7) or was from cells treated with retinoic acid/N^6,$O^{2'}$-dibutyryl-cAMP/theophylline for 2 (lanes 2, 5, and 8) or 4 days (lanes 3, 6, and 9). Hybridization and wash conditions were pcI5 insert, 50% formamide at 42°C and 15 mM NaCl/1.5 mM sodium citrate, pH 6.8, at 50°C; NB3 and KK4, 35% formamide at 35°C and 0.15 M NaCl/0.015 M sodium citrate at 50°C. NB3 and KK4 specific activities were 2 × 10^9 cpm/μg. Exposure times were 24 hr for lanes 1–3, 96 hr for lanes 4–6, and 24 hr for lanes 7–9.

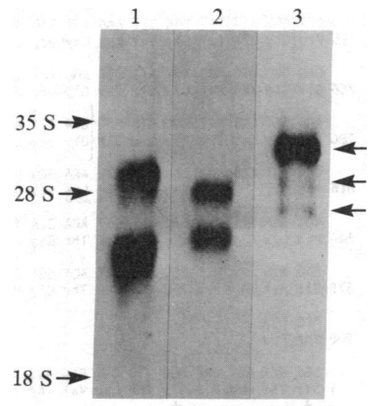

FIG. 5. Hybridization of human procollagen clones to fibroblast poly(A)$^+$ RNA. One-half microgram of GM3348 poly(A)$^+$ RNA per lane was electrophoresed in a 0.8% agarose/formaldehyde gel for 22 hr at 30 V, transferred to nitrocellulose, and hybridized to ^{32}P-labeled, nick-translated probes having a specific activity of 5–7 × 10^8 cpm/μg. Lane 1, the 2.4-kb α1(I) clone α12 (14), autoradiography for 6 hr; lane 2, the 2.4-kb α1(III) clone E6 (14), autoradiography for 30 hr; and lane 3, the 1.7-kb α1(IV) clone KK4, autoradiography for 80 hr. RNA markers indicated by arrows on the left are 35S poliovirus RNA and 28S and 18S human ribosomal RNAs. Arrows on the right point to the three α1(IV) procollagen transcripts that are estimated to be 6500, 5900, and 5300 nucleotides.

and avian (25) α2(I) mRNAs that differ in their 3′ untranslated region due to use of alternative polyadenylylation attachment sequences, and recently we have found two mRNAs hybridizing to a human α2(V) procollagen clone (unpublished data). Evidence for this feature being related to tissue specificity comes from studies of Focht and Adams (26) that show a difference in the avian α1(I) transcripts isolated from chondroblasts versus fibroblasts and smooth muscle cells. In addition, Barsh *et al.* (27) have recently reported a correlation between preferential synthesis of the smaller human α1(I) transcript in placenta and a change in DNase sensitivity in the 3′ part of the α1(I) gene.

DNA Sequencing of the Human α1(IV) Procollagen cDNA Clone. To determine if the cDNA clones encode part of the α1(IV) or α2(IV) procollagen chains, DNA sequencing was begun at the 5′ end of KK4. The 5′ *Pst* I/*Bam*HI and *Sph* I/*Pst* I fragments were cloned into M13 mp18 and mp19 vectors and sequenced by the Sanger dideoxy procedure (15). The 520-base-pair *Pst* I/*Bam*HI fragment contained entirely Gly-Xaa-Yaa triplets except for one four-amino acid interruption, Asp-Ile-Ile-Lys (Fig. 6). Comparison with the residues obtained by Edman degradation (10–12) showed the recombinant molecules to be α1 type IV procollagen clones. The only difference from the human protein data (10) was glutamic acid versus glutamine at position 825, and comparison with the mouse and bovine α1(IV) chains (11, 12) disclosed few substitutions, as reported (10). DNA sequencing of the adjacent *Bam*HI/*Hin*dIII fragment using the universal primer and five synthetic oligonucleotides revealed the end of the collagenous domain just 3′ to the *Bam*HI site, the 229-residue carboxyl-terminal peptide, and the beginning of a long 3′ untranslated region (Fig. 6).

DISCUSSION

Since the different members of the procollagen gene family appear to have arisen from duplication of a 54-base-pair ancestral sequence (28), we explored the possibility of homology between the uninterrupted triple helical regions of several human collagen chains and the unquestionably divergent type IV. Our recent studies of the α2(V) chain showed conservation of charge with α1(III), α1(I), and α2(I) but not nec-

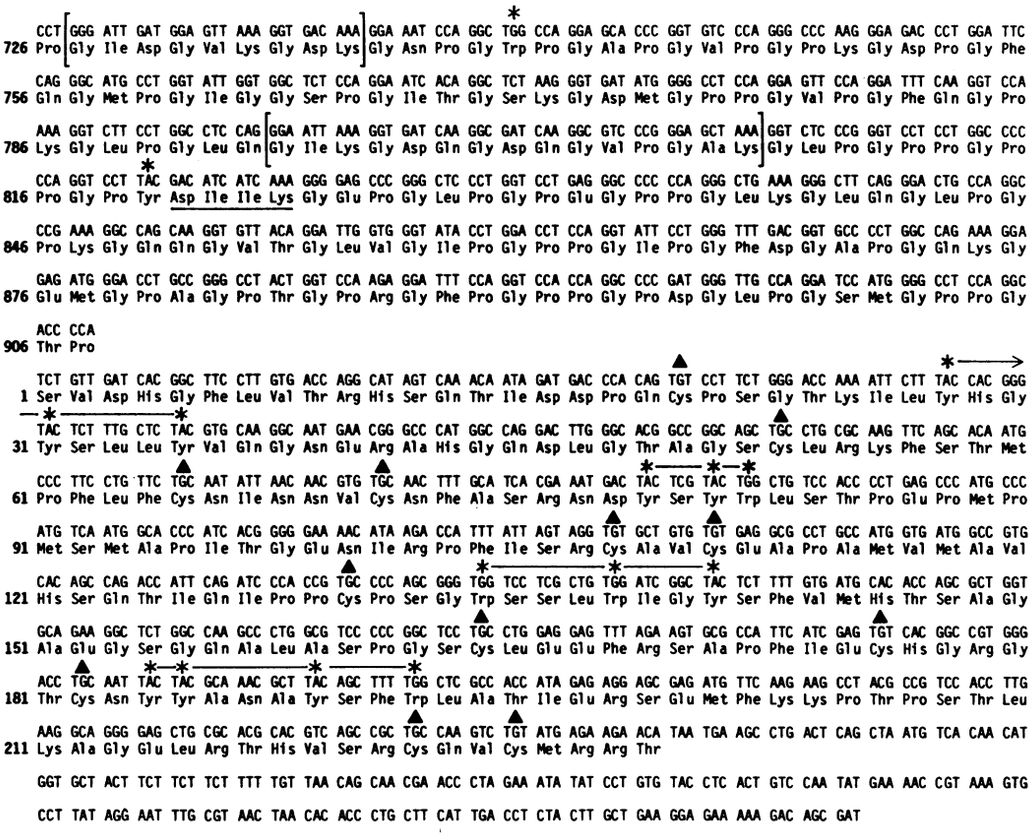

FIG. 6. Human procollagen α1(IV) DNA sequences and derived amino acids. The first seven lines show the DNA sequences and derived 182 amino acids of the 3′ end of the α1(IV) collagenous region numbered according to Babel and Glanville (10). Below is the 229-residue carboxyl-terminal propeptide and the first 198 nucleotides of the 3′ untranslated region. The two areas in α1(IV) homologous to other collagen chains and described in *Discussion* are in brackets, and the interruption in the α1(IV) collagenous domain is underlined. Tyrosine and tryptophan residues are indicated by asterisks and cysteines are indicated by triangles.

essarily conservation of the same amino acid (17). Therefore, we attempted to align α1(IV) with the four others by focusing on highly charged clusters containing glutamic acid, aspartic acid (−) and lysine, arginine (+). In the region between positions 919 and 933 in the four collagen chains are the five triplets G−+, G−y, G−+, G−+, and Gx+, where x and y are uncharged (17). Comparable in α1(IV), 101–115 amino acids 5′ to the carboxyl-terminal junction is the Gx+, G−y, G−y, Gxy, Gx+ sequence, in which four of eight charges are maintained (Fig. 6). The 3′ residue lysine has been shown in several collagen chains to be hydroxylated and to participate in crosslink formation (9, 29, 30). Perhaps also significant is that this seemingly conserved area lies within a long stretch lacking proline in α2(V) and α1(I). Consequently, in type I collagen a flexible site in the α chain was reported (31). Twelve amino acids 3′ in α2(V) and α1(I) constitute the end of the imino-free, less rigid region, whereas in α1(IV) an interruption of 4 amino acids is found, also designating a flexible site in the helix (31). One additional region of similarity was noted 57 amino acids 5′ in α1(IV), where the Gx−, Gx+, G−+ sequence (Fig. 6) is seen as G+−, Gxy, G−+ in α2(V) at an analogous position 60 amino acids 5′ to the first region (17). In neither case do the differences involve substitution by a residue of opposite charge.

The carboxyl-terminal noncollagenous region of α1(IV) displays an extremely different structure than the conserved and larger interstitial procollagen carboxyl propeptides (14, 32–37). Within the 229-residue α1(IV) peptide is a repeat symmetry of nearly equal-sized halves (Fig. 7). The six cysteines (C) and two tyrosine/tryptophan (T/T) groups (where T designates either tyrosine or tryptophan) in the 5′ half are paralleled in the 3′ half and are also arranged in a distinctive manner relative to each other (Figs. 6 and 7). C-1 is followed closely by the T-x-x-T-x-x-x-T sequence, C-2, C-3, and C-4 by T-x-T-T, whereas C-5 and C-6 terminate the 5′ half. In the 3′ half, C-1′ is followed by T-x-x-x-T-x-x-T, C-2′, C-3′, and C-4′ by T-T-x-x-x-T-x-x-T, whereas C-5′ and C-6′ terminate the peptide. C-1 and C-1′ stand alone, but C-2 and C-2′ are 11 residues from C-3 and C-3′, respectively, which, in turn, are 5 residues from C-4 and C-4′. C-5 and C-6 as well as C-5′ and C-6′ occur as C-x-x-C doublets. A number of amino acids surrounding the C and T/T groups are also identical in each half (Fig. 7).

Since the cysteine and tyrosine/tryptophan residues seem

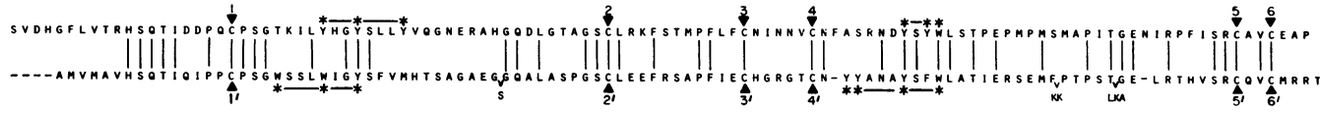

FIG. 7. Comparison of the 5′ and 3′ halves of the α1(IV) carboxyl-terminal peptide. The 229 amino acids in the α1(IV) carboxyl-terminal noncollagenous region are designated by the single-letter code. The 12 cysteines (C) are marked by triangles and numbered 1–6 in the 5′ half and 1′–6′ in the 3′ half. Tyrosine (Y) and tryptophan (W) residues are indicated by asterisks and connected by horizontal lines to show the four groups. Dashes represent no amino acid at that position. Vertical lines are drawn between the identical residues in each half.

to govern the repeating structure, we investigated whether a relationship exists between those in α1(IV) and in other human procollagen carboxyl propeptides (14, 33, 34). Five of the 12 cysteines in α1(IV) do correspond to 5 of the 8 in α1(III) and α1(I): 3 ~ 1, 4 ~ 2, 5 ~ 5, 3′ ~ 6, and 5′ ~ 7 (Fig. 7 and refs. 14 and 34). Since the last cysteine (no. 8 at residue 268) in α1(III) and α1(I) lies outside of the smaller 229-residue α1(IV) peptide, the comparison could well be limited to only the first 7 cysteines. In neither half of the α1(IV) structure does the homology pertain to the single cysteines C-1 and C-1′, the first of the triplets C-2 and C-2′, or the last of the doublets C-6 and C-6′. In addition, all four T/T groups in α1(IV) can be equated with most of those in the first 229 residues of α1(III), α1(I), and α2(I): T/T-1, positions 28, 31 versus 23, 24; T/T-2, positions 81, 82 versus 81, 82; T/T-3, positions 134, 141 versus 131, 144; and T/T-4, positions 184, 185 versus 182, respectively. [The numbering of interstitial procollagen residues is based on α1(III), ref. 14.] Lastly, the triplet Thr-Gly-Glu occurs only once in the α1(IV), α1(III), α1(I), and α2(I) carboxyl-terminal peptides. In α1(IV) it lies between residues 97 and 99 (Fig. 6) and in the latter three chains the sequence is found at a neighboring position, 102–104 (14).

Therefore, although α1(IV) presents a much more diverse structure than the interstitial and α2(V) procollagen chains, there is homology in apparently select areas. More definitive evidence that these analogous residues and regions have been purposefully maintained throughout evolution will be generated from isolation of possibly "intermediate" types linking α1(IV) to the group of highly conserved procollagen chains. Also of major interest is how the structural characteristics unique to type IV procollagen influence the morphology and function of basement membrane.

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1. Kefalides, N. A., Alper, R. & Clark, C. C. (1979) *Int. Rev. Cytol.* **61,** 167–228.
2. Cooper, A., Kurkinen, M., Taylor, A. & Hogan, B. L. M. (1981) *Eur. J. Biochem.* **119,** 189–197.
3. Kresina, T. F. & Miller, E. J. (1979) *Biochemistry* **18,** 3089–3097.
4. Crouch, E., Sage, H. & Bornstein, P. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 745–749.
5. Tryggvason, K., Robey, P. G. & Martin, G. R. (1980) *Biochemistry* **19,** 1284–1289.
6. Kefalides, N. A. (1971) *Int. Rev. Exp. Pathol.* **10,** 1–39.
7. Schuppan, D., Timpl, R. & Glanville, R. W. (1980) *FEBS Lett.* **115,** 297–300.
8. Minor, R. R., Clark, C. C., Strause, E. L., Koszalka, T. R., Brent, R. L. & Kefalides, N. A. (1976) *J. Biol. Chem.* **251,** 1789–1794.
9. Glanville, R. W., Voss, T. & Kuhn, K. (1982) in *New Trends in Basement Membrane Research*, eds. Kuhn, K., Schoene, H. & Timpl, R. (Raven, New York), pp. 69–77.
10. Babel, W. & Glanville, R. W. (1984) *Eur. J. Biochem.* **143,** 545–556.
11. Schuppan, D., Glanville, R. W. & Timpl, R. (1982) *Eur. J. Biochem.* **123,** 505–512.
12. Schuppan, D., Glanville, R. W., Timpl, R., Dixit, S. N. & Kang, A. H. (1984) *Biochem. J.* **220,** 227–233.
13. Wang, S. Y. & Gudas, L. J. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 5880–5884.
14. Loidl, H. R., Brinker, J. M., May, M., Pihlajaniemi, T., Morrow, S., Rosenbloom, J. & Myers, J. C. (1984) *Nucleic Acids Res.* **12,** 9383–9394.
15. Messing, J. (1983) *Methods Enzymol.* **101,** 20–78.
16. Matteucci, M. D. & Caruthers, M. H. (1981) *J. Am. Chem. Soc.* **103,** 3185–3191.
17. Myers, J. C., Loidl, H. R., Stolle, C. A. & Seyer, J. M. (1985) *J. Biol. Chem.* **260,** 5533–5541.
18. Krieg, T., Timpl, R., Alitalo, K., Kurkinen, M. & Vaheri, A. (1979) *FEBS Lett.* **104,** 405–410.
19. deWet, W. J., Pihlajaniemi, T., Myers, J. C., Kelly, T. E. & Prockop, D. J. (1983) *J. Biol. Chem.* **258,** 7721–7728.
20. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
21. Myers, J. C., Chu, M. L., Faro, S. H., Clark, W. J., Prockop, D. J. & Ramirez, F. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 3516–3520.
22. Strickland, S., Smith, K. K. & Marotti, K. R. (1980) *Cell* **21,** 347–355.
23. Kurkinen, M., Barlow, D. P., Helfman, D. M., Williams, J. G. & Hogan, B. L. M. (1983) *Nucleic Acids Res.* **11,** 6199–6209.
24. Myers, J. C., Dickson, L. A., deWet, W. J., Bernard, M. P., Chu, M. L., DiLiberto, M., Pepe, G., Sangiorgi, F. O. & Ramirez, F. (1983) *J. Biol. Chem.* **258,** 10128–10135.
25. Aho, S., Tate, V. & Boedtker, H. (1983) *Nucleic Acids Res.* **11,** 5443–5450.
26. Focht, R. J. & Adams, S. L. (1984) *Mol. Cell. Biol.* **4,** 1843–1852.
27. Barsh, G. S., Roush, C. L. & Gelinas, R. E. (1984) *J. Biol. Chem.* **259,** 14906–14913.
28. Yamada, Y., Avvedimento, V. E., Mudryj, M., Ohkubo, H., Vogeli, G., Irani, M., Pastan, I. & deCrombrugghe, B. (1980) *Cell* **22,** 877–892.
29. Fietzek, P. P. & Kuhn, K. (1976) *Int. Rev. Connect. Tissue Res.* **7,** 1–60.
30. Wu, J. J. & Eyre, D. R. (1984) *Biochemistry* **23,** 1850–1857.
31. Hofman, H., Voss, T., Kuhn, K. & Engel, J. (1984) *J. Mol. Biol.* **172,** 325–343.
32. Fuller, F. & Boedtker, H. (1981) *Biochemistry* **20,** 996–1006.
33. Bernard, M. P., Myers, J. C., Chu, M. L., Ramirez, F., Eikenberry, E. F. & Prockop, D. J. (1983) *Biochemistry* **22,** 1139–1145.
34. Bernard, M. P., Chu, M. L., Myers, J. C., Ramirez, F., Eikenberry, E. & Prockop, D. J. (1983) *Biochemistry* **22,** 5213–5223.
35. Yamada, Y., Kuhn, D. & deCrombrugghe, B. (1983) *Nucleic Acids Res.* **11,** 2733–2744.
36. Sandell, L. J., Prentice, H. L., Kravis, D. & Upholt, W. B. (1984) *J. Biol. Chem.* **259,** 7826–7834.
37. Ninomuja, Y., Showalter, A., van der Rest, M., Seidah, N. G., Chretien, M. & Olsen, B. R. (1984) *Biochemistry* **23,** 617–624.