
Isolation and characterization of a human collagen $\alpha 1(I)$ -like gene from a cosmid library

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Received 4 January 1982; Revised and Accepted 5 March 1982

ABSTRACT

We have isolated a human collagen $\alpha 1(I)$ -like gene from a cosmid library. The clone which contains 37kb of human DNA has been shown to contain this gene by DNA sequencing, hybrid arrest and hybrid selection assays and Northern blot hybridizations. The collagen gene sequence extends through most of the cloned DNA and must, therefore, be at least 35kb in length.

INTRODUCTION

Collagen is one of the most important and abundant proteins of vertebrate connective tissue. At least 5 genetically distinct types of collagen have been identified and each tissue expresses one, or several, of these types. The types of collagen are assembled from 9 different chains encoded by distinct genes (1, 2). Nothing is known of the copy number or extent of polymorphism of these genes, and the number of genes in this family may indeed be much greater than 9. It is clear however that the regulation of the different collagen genes is basic to the development and differentiation of vertebrates.

The most abundant and best studied of the vertebrate collagens is Type I, a heteropolymer of two $\alpha 1(I)$ chains and one $\alpha 2(I)$ chain (1, 2). In vivo these molecules are synthesized as large precursor pro- α chains which associate to form procollagen. Genomic clones for the entire chick $\alpha 2(I)$ (3, 4) and part of the sheep $\alpha 2(I)$ gene (5) have been isolated and reveal several extraordinary features. First, 5kb of coding sequence are contained within a gene at least 38kb in length. Second, the coding sequence is interrupted by 50 intervening sequences, the largest number found in a gene to date. Third, the size of the coding regions is most frequently 54bp, or 18 amino acids, in length. Others are 108 (2 x 54), 99 ($[2 \times 54] - 9$), 45 (54-9). On the basis of these findings 54bp has been proposed as the

size of the primordial collagen gene (6, 7).

We report here the isolation and characterisation of a human collagen $\alpha 1$ (I)-like gene as a cosmid recombinant. This clone contains 37kb of human DNA and may contain the entire gene.

MATERIALS AND METHODS

The general procedures for the screening of cosmid libraries, growth and analysis of cosmid recombinants, nick translation and blot-hybridizations were performed as described previously (15). DNA sequences were determined as in Maxam and Gilbert(24).

Hybrid-arrested translation of procollagen mRNA

Total polyA⁺ RNA was prepared from approximately 10⁹ human skin fibroblasts as previously described (8). DNA (5 μ g) was dissolved in deionized formamide, heated to 90°C for 5 min., cooled and hybridized with 2 μ g polyA⁺ RNA in 80% formamide, 0.4M NaCl, 20mM PIPES pH6.4 for 3h at 48°C. The hybridization mixture was diluted 1 in 3 with ice-cold water and divided into 2 aliquots, one of which was heated at 90°C for 30 sec.

The RNA-DNA hybrids were precipitated with 2.5 vols. ethanol, redissolved and translated in a messenger-dependent reticulocyte cell-free system (9) under conditions optimal for procollagen mRNA (10). The translation products were analysed on SDS-polyacrylamide gels (10%) as described previously (10).

Hybrid-selected translation of mRNA

The EcoRI fragments of cosH col.I the chicken $\alpha 1$ (I) cDNA probe and a chicken actin cDNA probe (gift of D. Cleveland, Johns Hopkins University, Baltimore, USA) were each linked separately to diazobenzyloxymethyl (DBM) paper discs essentially as described by Alwine et al. (12) and Goldberg et al. (13). These fragments were prehybridized for 1-2h at 37°C in a mixture containing 50% formamide, 0.9M NaCl, 20mM PIPES pH6.4, 1mM EDTA, 0.2% SDS, 0.5 μ g/ μ l calf liver tRNA (Boehringer Mannheim) and 0.5 μ g/ μ l polyA (Sigma). Human fibroblast total polyA⁺ RNA (0.2 μ g/ μ l hybridization volume) was hybridized to these DNA fragments for 6h at 37°C in the same mixture, except polyA was excluded. At the end of the hybridization period the mRNA mixture was removed, diluted with ice-cold water and precipitated with 2.5 vols. ethanol. The DBM paper discs were washed twice for 10 min at 37°C in prehybridization buffer; 6 times with 1 x SSC (150mM NaCl, 15mM tri-sodium citrate) 0.2% SDS for 5 mins each at 37°C; twice in 10mM Tris-HCl pH7.4, 2mM EDTA for

5 min., each at 37°C. The mRNA hybridized to the DNA was eluted in 20mM PIPES pH6.4, 1mM EDTA, 0.2% SDS for 1-2h at 37°C, diluted 1 in 3 with ice-cold water and precipitated with ethanol. The bound and unbound mRNAs were translated in the reticulocyte lysate cell-free system and the translational products analyzed in SDS-polyacrylamide gels as before.

RNA blotting (Northern) experiments

Fibroblast polyA⁺ RNA was denatured in 50% formamide, 6% formaldehyde, 20mM morpholinepropanesulphonic acid (MOPS) pH7.0, 5mM sodium acetate and 1mM EDTA, electrophoresed in a 0.8% agarose gel containing 2.2M formaldehyde and blotted onto nitrocellulose as described by Crain *et al.*, (14). The filters were prehybridized at 42°C for 3-4h in 50% formamide, 5 x SSC, 16.6mM phosphate buffer (pH7.2), 0.1% sodium pyrophosphate, 0.02% Ficoll (Pharmacia), 0.02% polyvinylpyrrolidone (Pharmacia), 20µg/ml nuclease-free bovine serum albumin, 100µg/ml denatured salmon sperm DNA (Sigma), 10% dextran sulphate (Pharmacia). Where the DNA probe was known to contain repetitive sequences, human DNA at 30µg/ml was included in the prehybridization and hybridization mixtures. The EcoRI fragments of cosH col.1, pCg54 and the actin cDNA clone were labelled with ³²P-dCTP (Amersham, U.K. specific activity 3000Ci/µmol) by nick-translation and hybridized to the RNA blots for 18-24h at 42°C in the same buffer as for prehybridization. After hybridization the filters were washed 4 times for 5 min. each in 2 x SSC 0.1% SDS at 20°C and twice for 15 min. each in 0.1 x SSC, 0.1% SDS at 50°C. The filters were dried and exposed to Kodak X-Omat S film at -70°C. The sizes of the mRNA molecules which hybridized to labelled probe were determined from a calibration curve made from the mobilities of fragments of ribosomal RNA of known molecular weight obtained from ppp(A2'p)₂A-treated mouse cells (gift of R.H.Silverman, ICRF, London).

RESULTS

Isolation of a human collagen gene cosmid

We have previously described the construction of a human cosmid library using the vector pJB8 (15). We screened this library using as probe the chicken procollagen α1(I) cDNA clone pCg54 (16). Several positive colonies were picked and after preliminary analysis, one clone, cosH col.1 was selected for further work.

The restriction map for this cosmid is shown in Fig. 1. The regions of the cosmid that contain the helical- and C terminal-coding segments of the

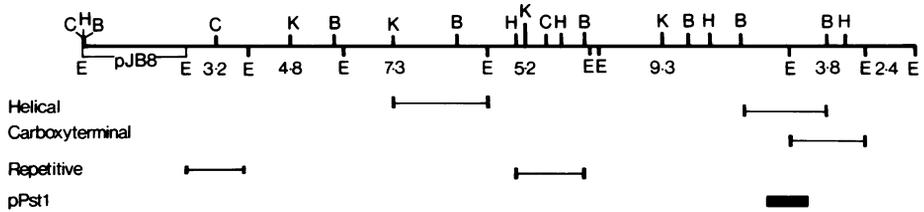


Fig. 1. Restriction map of cosH col.1.

The EcoRI sites at the ends of the insert were generated during the construction of the human cosmid library using the vector pJB8 (15). The 1.kb PstI fragment was isolated (pPst1) for DNA sequence analysis; its location is indicated. The location of sequences, coding for helical and C-terminal region or containing repetitive sequences are indicated. (B) BamHI; (C) ClaI; (E) EcoRI; (H) HindIII; (K) KpnI. The numbers refer to the size of the EcoRI fragments in kilobase pairs.

gene were localized by hybridization of the relevant portions of the chicken $\alpha 1(I)$ cDNA plasmid (see Fig. 2). In this way, the C terminal region was localized to a 3.8kb EcoRI fragment (E3.8) of the cloned DNA, while the helical segments were localized in E7.3, E9.3 and E3.8. These are placed on the map of Fig. 1; this shows that the C-terminal segment is near one end of the clone, while the helical segments extend through most of the remainder of the human DNA. Since the chicken cDNA probe used only covers the 3' terminal regions of the helical segments, the extent of the helical segments on the cosmid may well be an underestimate. Regions containing highly repetitive sequences were localized by hybridizing EcoRI digested cosH col.1 with ^{32}P nick-translated human placental DNA. Both the E5.2, which is located within the collagen gene, and the 5' terminal fragment E3.2 hybridize strongly to this probe (Fig. 2).

CosmidH col.1 specifically binds human procollagen $\alpha 1(I)$ mRNA

To determine which collagen gene we had isolated, we assayed for the ability of the cloned DNA to hybridize specifically to procollagen mRNA. To do this, we first subcloned a PstI fragment (pPst1) which hybridized strongly to the chicken pCg54 collagen $\alpha 1(I)$ cDNA plasmid. Total fibroblast polyA⁺ RNA was hybridized to pPst1 and the mRNA-DNA hybrids translated in a messenger dependent reticulocyte lysate cell-free system. The cell-free translation of procollagen $\alpha 1(I)$ mRNA was specifically arrested by hybridization of mRNA to pPst1, but when the mRNA-DNA hybrid was disrupted by heating to 90°C a polypeptide corresponding to procollagen $\alpha 1(I)$ chain was synthesized. The cell-free synthesis of the procollagen $\alpha 2(I)$ chain was unaffected by

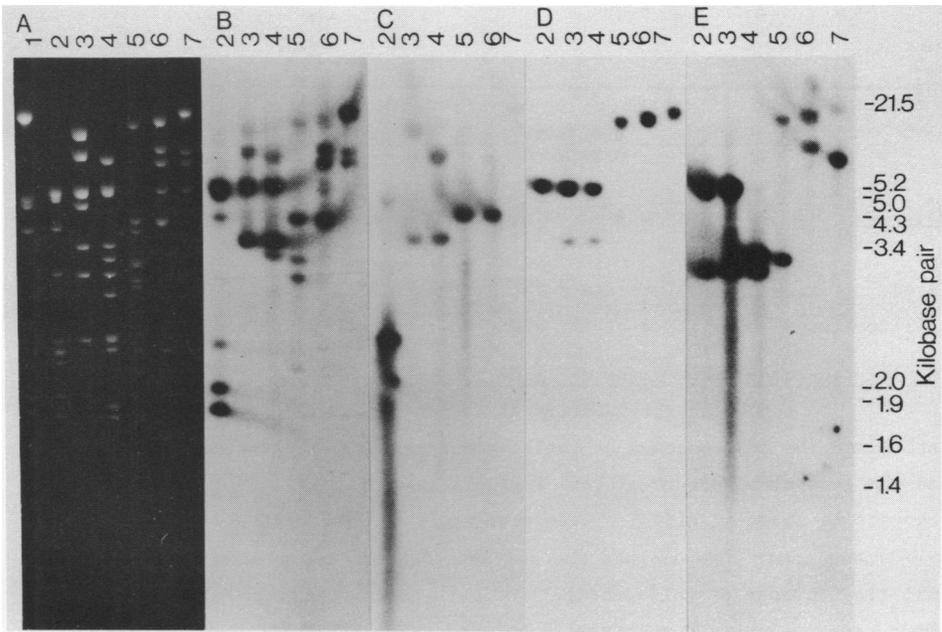


Fig. 2a. Analysis of *cosH col.1* by Southern blot hybridization.

- A Ethidium bromide staining
 B,C,D,E Autoradiographs
 B Hybridized with ^{32}P -labelled Kpn-HindIII fragment of pCg54 which corresponds to essentially the whole insert.
 C Hybridized with ^{32}P -labelled 630bp fragment of pCg54 coding for helical region (see Fig. 2b).
 D Hybridized with ^{32}P -labelled 360bp fragment of pCg54 coding for C-terminal region (see Fig. 2b).
 E Hybridized with ^{32}P -labelled human placental DNA.
- track 1: λ x HindIII/EcoRI
 2: *cosH col.1* digested with BamHI and EcoRI
 3: *cosH col.1* digested with EcoRI
 4: *cosH col.1* digested with KpnI and EcoRI
 5: *cosH col.1* digested with BamHI and KpnI
 6: *cosH col.1* digested with BamHI
 7: *cosH col.1* digested with KpnI

The 5.2kb band detected by the 360bp probe and the Kpn HindIII fragment of pCg54 is the cosmid vector pJB8 which hybridizes to traces of contaminating pBR322 from the pCg54 preparation.

hybridization to pPstI (data not shown). In addition, a hybrid selection experiment was performed; pPstI was bound to DBM paper and fibroblast polyA⁺ mRNA was hybridized to this immobilized DNA. As controls, pCg54 and a chicken actin cDNA clone were used. The hybridized RNA was then eluted and trans-

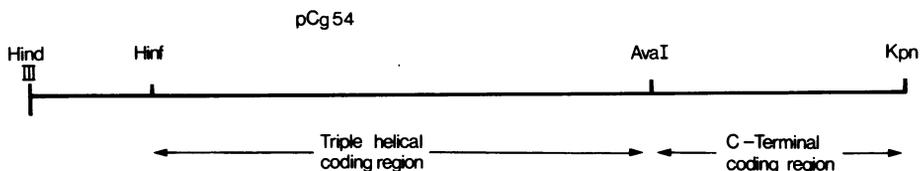


Fig. 2b. Schematic map of pCg54 showing the fragments used as probes.

lated. Fig. 3 shows that again the synthesis of pro $\alpha 1(I)$, but not pro $\alpha 2(I)$ collagen is directed by the mRNA bound to both pPst1 and pCg54.

The DNA sequence of a segment of cosHG identifies an $\alpha(I)$ -like collagen gene

To establish the identity of the collagen gene in cosH col.1, we determined the DNA sequence of the subclone pPst1. The sequencing strategy and the general structure of this region is shown in Fig. 4 and the DNA sequence is shown in Fig. 5. The sequence shows the presence of 5 exons of a collagen gene. We aligned the polypeptide sequence predicted from this with the sequence of collagen $\alpha 1(I)$ of both chicken and calf (17, 18). The human sequence shows homology with both sequences from residues 838 to 937 (Fig. 6). There is no identifiable region of similarity with collagen $\alpha 2(I)$.

Comparison of amino acids 838-937 from the chick and calf $\alpha 1(I)$ chains reveals about 90% homology in the non-glycine residues. Our clone shows only 60-65% homology with these sequences. Greater conservation across species might be expected for a particular chain type and we therefore cannot exclude that we have cloned a collagen gene other than $\alpha 1(I)$. cosH col.1 cannot however contain the $\alpha 1(III)$ gene expressed by fibroblasts because our DNA sequence does not correspond with the published amino acid sequence of the human $\alpha 1(III)$ chain (25) cosH col.1 also cannot contain the $\alpha 1(II)$ gene because it does not hybridize with chick cartilage polyA⁺ mRNA, which includes $\alpha 1(II)$ mRNA (data not shown), nor does it show homology with this region of the calf $\alpha 1(II)$ chain (W. Butler, personal communication). We therefore propose that cosH col.1 is either the human $\alpha 1(I)$ gene, a closely related $\alpha 1(I)$ -like gene, or pseudogene (see Discussion). For the remainder of this article we shall refer to the gene cloned in cosH col.1 as the human collagen $\alpha 1(I)$ gene.

The gene structure of this region is very similar to that previously described for the chicken and sheep collagen $\alpha 2(I)$ genes, since the three complete exons are all multiples of 54 nucleotides (54, 108 and 54 nucleotides respectively (3, 4, 5)).

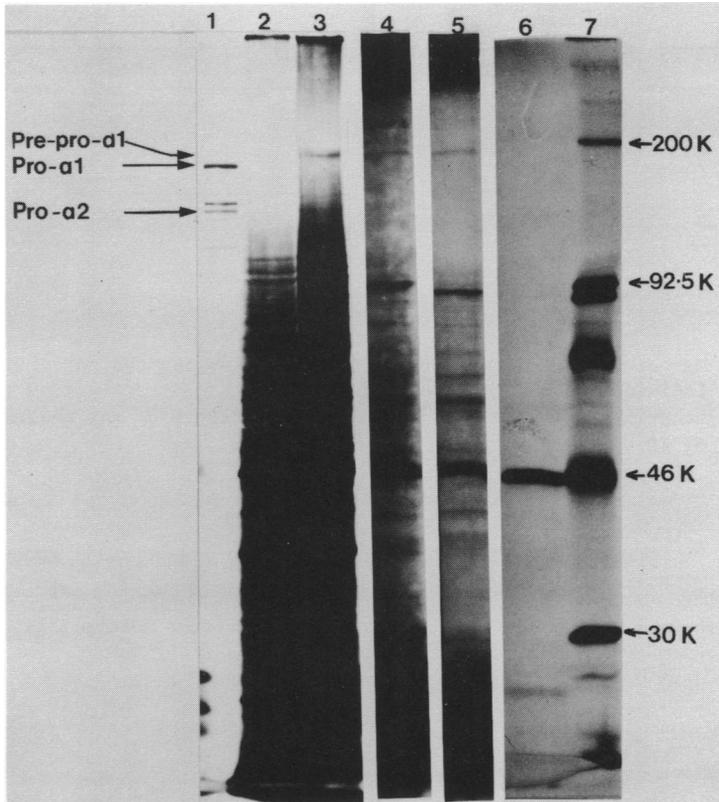


Fig. 3. Hybrid-selected translation of human fibroblast mRNA.

Fluorograms of SDS polyacrylamide gels of translation products labelled with ^{35}S -methionine. Tracks; 1, pro- α 1(I) and pro- α 2(I) standards; 2, translation products of residual mRNA not selected out by the different DNA fragments; 3, translation products of fibroblast mRNA; 4, products of pPstI selected mRNA; 5, products of pCg54 (chicken α 1(I) cDNA clone) - selected mRNA; 6, translation products of actin cDNA-selected mRNA; 7, polypeptide molecular weight markers (Amersham Radiochemicals U.K.).

Tracks 1-3 and 6-7 represent shorter exposure times than tracks 4 and 5 because of the high incorporation of radioactivity in the former. Longer exposure of track 6 does not show any polypeptide of similar molecular size to pro- α 1(I) or pro- α 2(I) chains, but does show the products up to 92,000MW seen in tracks 4 and 5. These are also observed in the absence of added mRNA (not shown).

The products of cell-free translation of procollagen mRNA do not undergo the post-translational modification of glycosylation and hydroxylation which occur in vivo. Therefore unhydroxylated ^{14}C -proline labelled type I pro- α chains were used as standards. The translation products do not comigrate exactly with these standards because they are synthesized as pre-proforms but their collagenous identity has been established previously (10).

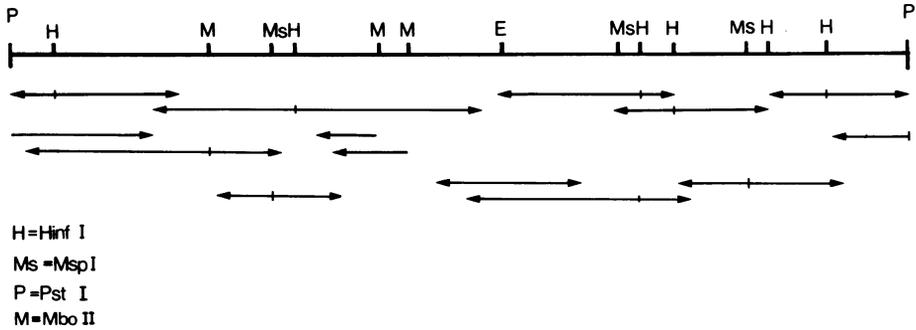


Fig.4 . Map of the 1.1kb PstI fragment (pPstI) showing the position of individual sequences obtained. The straight arrows give the 5'-3' direction and length of the fragment sequenced as in Maxam and Gilbert (24).

At least 35kb of cosH col.1 is collagen $\alpha 1(I)$ gene

The sizes of the sheep and chicken collagen $\alpha 2(I)$ genes are 30-38kb long (5, 3). Since cosH col.1 is 38kb and sequences corresponding to the carboxy terminus are located close to the vector moiety (Fig. 2), we

10	20	30	40	50	60	70	80	90	100
<u>CCTGAACCTC</u>	<u>CACGACAGCT</u>	GACCACTGAG	ACCCCTCGGC	CTGGCCCTGA	TTGGGCAGAC	GGCCCTCTGC	AGTCTCTGTC	CTGGCTCAGC	AAGCACAACC
110	120	130	140	150	160	170	180	190	200
CCCAGTCAGG	GCCTCCGAGA	AGGGGGCCGC	ACCCCTGGCC	GACAGCCGAA	AGCCTAGCTA	CAATGGGAAG	CTTGTCCGGC	AGAGAGACGG	GCATAGAGAC
210	220	230	240	250	260	270	280	290	300
CAAGGGCTGC	TTCTGGAAGG	AGGAGGGAAG	CTTGTGTGCG	AAACTTTGCG	TTCAAAGCTG	CAGTCACTGT	GGCAGAAGAG	GAGAGGCCCTG	GCCTTCTCAG
310	320	330	340	350	360	370	380	390	400
ACGGGCTGGG	GGACGACAGG	GGGAGCTGGA	CAGAGGACAG	CTCTAGCTGC	GTCTCTGTTT	CACCTTGTCC	<u>AGGGAAGGCC</u>	<u>CGGTGCTCAT</u>	<u>GGCCCCCCTC</u>
410	420	430	440	450	460	470	480	490	500
<u>GCACAGATGG</u>	<u>CCCTGCTCGA</u>	<u>CTCAAGGTGA</u>	GTGTCTGTGT	TCTGTGTGTG	CAGTGGCTTG	GGGAGGCACAT	TGCCCTCGGC	CTGACAGCTC	AGCTGGGGGT
510	520	530	540	550	560	570	580	590	600
GGCAGGTTGG	AACAAGTCTC	ATCTCAGCCT	AGAAGCACCT	TCTCTTCTG	TCTCTTCTGG	AACATTCTTC	TCTGAGCTCT	AGACCTCTCT	<u>CCTGACAGCC</u>
610	620	630	640	650	660	670	680	690	700
<u>TAATCTGTGT</u>	<u>GAAACCGGTG</u>	CTGTGGGAGC	TCCTGGAACC	CCTGGGCCCC	CTGGCTCCEC	TGGCCCGCCT	GGTCCAAGTC	GCAAGCAAGC	AGACAGAGGA
710	720	730	740	750	760	770	780	790	800
<u>CAAGCTCTAA</u>	GTATCTTGGA	ATTCAGTAAA	AGCCGCCCTC	CCCTGGCGGG	TGGGGCTGAG	GCACCTTCCTG	GGTTTTGGCA	GTCTCTGAGC	TAAGGAGCAC
810	820	830	840	850	860	870	880	890	900
TGGCCCCAGA	TCCAGAGGAG	GGCCCCACCT	CTCCTGCTTT	TCTCTAGCTT	GGCCTCACTC	TCTGCTCAGC	<u>GTGCACAAGG</u>	<u>CCCCATGGCA</u>	<u>CCCTCAGGAC</u>
910	920	930	940	950	960	970	980	990	1000
<u>CAGCTGGAGC</u>	<u>CCGGGGAATC</u>	<u>CAGGTGAGTA</u>	TCCAAGTGTG	CGGCACCGAG	TCCCGACAC	GGATAGGCTC	GGAGGGGAGC	CAGCCTGCAG	GTGGTTCCTC
1010	1020	1030	1040	1050	1060	1070	1080	1090	1100
GCCTCCAGCC	CTGTGCTTCC	GGGCAITCCT	CAGCTTGGCT	GGGACAGCAC	GGGGCTGCTC	TCCTGGCCCT	GACCTCACTC	AATGCTCTCT	TGTCTTGTTC
1110	1120	1130	1140	1150	1160	1170	1180	1190	
<u>CCAGGCTCTC</u>	<u>CAAGGCCCCA</u>	<u>GAGGTGACAA</u>	AGGAGAGCCT	GGAGAGCCTC	CCGACAGAGG	<u>CCTGAAGGGA</u>	<u>CACCCTGGCT</u>	<u>TCAGTGGCC</u>	

Fig.5 . The DNA sequence of the 1.1kb PstI fragment of cosH col.1. The underlined regions are the collagen exons. The position of introns and exons was deduced by comparison of the DNA sequence translated protein with the known sequence of other collagens and by aligning introns on the basis of the GT---AG rule.

	831		838						
pPst1	G E P G R E		G S P G A D G P P G R D G A A G V K						
chick $\alpha 1(I)$	A		A E A					P	
bovine $\alpha 1(I)$	S		A E S				S P	A	
	856								
pPst1	G N R G E T G A V G A P G T P G P P G S P G P A G P T								
chick $\alpha 1(I)$	D		P A P A A A					V A	
bovine $\alpha 1(I)$	D		P A P A A A					V A	
			892						
pPst1	G K Q G D R G E A		G A Q G P M G P S G P A G A R G J Q						
chick $\alpha 1(I)$	N		T P A A P					P A	
bovine $\alpha 1(I)$	S		T P A I V					P A	
	910								
pPst1	G P Q G P R G D K G E A G E P G E R G L K G H R G F T G								
chick $\alpha 1(I)$			T Q D M						
bovine $\alpha 1(I)$			Z T Z Z B I					S	

Fig.6 .

A comparison of the amino acid sequence encoded by the collagen exons in subclone pPst1 with the published sequences of chicken and bovine collagen $\alpha 1(I)$. The standard single letter amino acid code is used. The gaps represent the boundaries of the respective exons and the numbers in the sequence refer to the first amino acid of each exon. The sequence of the chicken and bovine collagens is shown only where they differ from the human sequence.

considered it possible that this clone contained the entire human collagen $\alpha 1(I)$ gene.

We have used the chicken cDNA clone pCg54, or the pPst1 subclone of the human collagen $\alpha 1(I)$ gene, described here as labelled probes in Northern blots of human fibroblast polyA⁺ mRNA. Both probes detect two mRNAs of 6.9kb and 5.7kb. We presume that one or both of these species is the human procollagen $\alpha 1(I)$ mRNA (Fig. 7; see refs. 19 and 20). We have used this Northern assay to determine the boundaries of the collagen $\alpha 1(I)$ gene on our cosmid clone. To do this, Northern blots of human fibroblast polyA⁺ mRNA were hybridized with each respective EcoRI fragment of cosH col.1(Fig. 7). All EcoRI fragments detect the 6.9kb and 5.7kb putative collagen $\alpha 1(I)$ mRNAs except the 5' terminal EcoRI + ClaI double digest fragment. Though the hybridization with E3.2 is weak (e.g. Fig. 7) we reproducibly observed positive hybridization to the putative procollagen mRNAs. It appears, therefore,

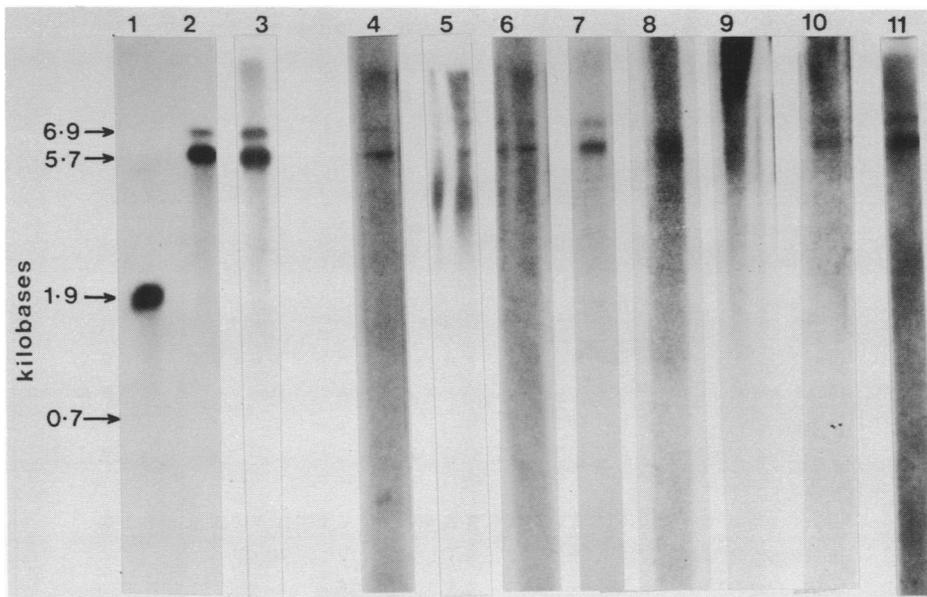


Fig.7. Hybridization of ^{32}P -labelled EcoRI fragments of *cosH col.1* to mRNA in a Northern blot.

The positions of the EcoRI fragment within *cosH col.1* are shown in Fig. 1. ^{32}P -labelled DNA fragments used as probes in tracks 1-11 were as follows: 1, actin cDNA clone; 2, pCg54; 3, E93; 4, E7.3; 5, E5.2; 6, E4.8; 7, E3.8; 8, E3.2; 9, 5' fragment of E3.2 digested with ClaI; 10, E2.4; 11, pPst1. The lengths of the mRNA species which hybridized to labelled probes are indicated and were determined from molecular weight markers of fragments of RNA (see Materials and Methods).

that the collagen $\alpha 1(\text{I})$ gene extends throughout essentially the entire human DNA insert in *cosH col.1*. The same experiment was performed using the hybrid selection assay. All the EcoRI fragments with the exception of the 5' terminal EcoRI + ClaI fragment select collagen $\alpha 1(\text{I})$ mRNA, as shown by this assay (not shown).

DISCUSSION

We describe here the isolation of the human collagen $\alpha(\text{I})$ gene and present a number of arguments for its identity. First, the cloned DNA sequence hybridizes specifically to human collagen $\alpha 1(\text{I})$ mRNA as shown by hybrid arrest and hybrid selection experiments. Second, the DNA sequence of a segment of this gene is homologous to the corresponding sequence of calf and chicken collagen $\alpha 1(\text{I})$. It cannot be excluded that this gene is a

collagen pseudogene, but this seems unlikely since we have used cosH col.1 and the pPst1 subclone in Southern blots of human DNA. These probes detect the DNA fragments predicted by the map of cosH col.1 (data not shown). In addition, the two terminal fragments of cosH col.1 are replaced by two new EcoRI fragments. Since the cosmid library was generated by partial digestion of human DNA with MboI, the 5' and 3' terminal EcoRI fragments are truncated (by MboI cleavage) in the cosmid clone. From the data described here, we conclude that the collagen $\alpha 1(I)$ gene that we have cloned is large; it must be at least 35kb as judged by our hybrid selection and Northern hybridization analyses. It is possible that the entire human collagen $\alpha 1(I)$ gene is present on this clone, since the 5' terminal fragment fails to hybridize to collagen $\alpha 1(I)$ mRNA. This fragment, however, may represent an intron and the remainder of the collagen $\alpha 1(I)$ gene could lie outside the DNA regions that we have cloned. Further work will elucidate this matter. Clearly the fact that most of this large gene is present on this clone re-emphasizes the advantage of cosmid cloning for large genes.

Five exons of the human collagen $\alpha 1(I)$ gene have been sequenced, three of which are complete, and the general arrangement of this gene appears similar to that previously described for the sheep and chicken $\alpha 2(I)$ genes (3-5). Of the three complete exons, two are 54bp separated by one of 108bp. Of the remaining exons not completely sequenced, one is larger than 54bp. A unit of 54bp has been postulated as the size of the primordial collagen gene and is based on the structure of the chicken $\alpha 2(I)$ gene (6). The exon sizes so far determined in the human $\alpha 1(I)$ gene correlate well with this hypothesis, as does the mouse $\alpha 1(I)$ gene (21). The 108bp exon may have evolved by the elimination of an intron and the subsequent fusion of two exons of 54bp.

Comparison of the structure of the human $\alpha 1(I)$ gene with the chicken $\alpha 2(I)$ and mouse $\alpha 1(I)$ genes reveals similarities. Particularly interesting is the fact that in the human gene the length of the exons and their position with respect to the protein sequence are the same, not only in the mouse $\alpha 1(I)$ gene (21), but also in the chicken $\alpha 2(I)$ gene (4). Thus, in the chicken $\alpha 2(I)$, mouse $\alpha 1(I)$ and human $\alpha 1(I)$ genes, amino acids 892-909 and 838-855 (exons no. 8 and 10 in ref 4) occur as two separate 54bp exons (Figs. 5 and 6 and refs. 4, 6 and 21). Similarly, amino acids 856-891 in the chicken $\alpha 2(I)$ is thought to occur either as two 54bp exons or in one exon of 108 because this region has not been completely sequenced (4, 6). In both the mouse and human $\alpha 1(I)$ genes these amino acids occur as an exon of 108bp.

Amino acids 910-945 are encoded in a 108bp exon in the chicken $\alpha 2(I)$ (6). This exon is incompletely sequenced in the human $\alpha 1(I)$, but is larger than 54bp and could, therefore, be also 108bp. The fact that the position and sizes of these exons is the same in the $\alpha 1(I)$ and $\alpha 2(I)$ genes (at least for those regions examined) is interesting, since these genes are believed to have diverged some 700 million years ago (see ref. 7). This result is reminiscent of a similar conservation in the α - and β -globin genes which diverged in a similar time period. It is noteworthy that, in the region sequenced, the human collagen $\alpha 1(I)$ gene seems to have shorter introns than the chicken collagen $\alpha 2(I)$ gene. A similar relatively compressed structure has also been found in this region of the mouse $\alpha 1(I)$ gene. Since approximately 5% of the gene is contained in 1kb of DNA, it may be expected that if the rest of the gene were organised likewise the human $\alpha 1(I)$ gene would be approximately 20kb in length. However, the DBM selection and Northern blot analyses (Fig. 7) suggest that the gene is at least 35kb long. Since the exons sequenced in the human gene lie in the 3' half of the gene and the density of exons in this region of the $\alpha 2(I)$ gene is higher than in the 5' half (4), this apparent difference may not be so exaggerated.

Studies on the cDNA clones for the chicken pro $\alpha(I)$ chain reveal a clear predominance of the codons CGC for glycine, CCC for proline and GCC for alanine (23). In the mouse, collagen $\alpha 1(I)$ gene, however, T is preferred in the third position for these amino acids (21). In the human $\alpha 1(I)$ gene, T is also preferred in the third position for alanine (8/11) but C(13/34) and A(13/34) are equally preferred for glycine; and T(7/15) and C(6/15) for proline. In the chicken $\alpha 2(I)$ gene T is preferred for glycine, T and C for alanine and there was little preference for proline (22). It would appear, therefore, that there is no clear evidence for a common interspecies or inter-chain codon usage for the collagen $\alpha 1(I)$ and $\alpha 2(I)$ genes.

The isolation of a clone containing a substantial, if not complete, portion of the human collagen $\alpha 1(I)$ gene has made possible the first determination of some human collagen amino acid sequences. In addition, this clone makes possible the study of inherited diseases in man where the functioning of this collagen gene is disturbed, as well as detailed studies of collagen gene linkage. The $\alpha 1(I)$ collagen gene has previously been assigned to chromosome 7 on the basis of phenotypic analysis (22). Using our DNA probes, we have recently confirmed this location.

ACKNOWLEDGEMENTS

We are especially indebted to Dr. H. Boedtker for the gift of the collagen $\alpha 1(I)$ cDNA clone and for helpful discussions. We would like to thank Bill Butler (The University of Alabama Medical Center, Birmingham, Alabama) for sharing with us his unpublished $\alpha 1(II)$ amino acid sequence data. We also wish to thank Dr. D. Cleveland for the actin cDNA clone and Dr. R.H. Silverman for rRNA markers. E.W. was supported by a Deutsche Forschungsgemeinschaft fellowship, H.H.M.D. by an EMBO long term fellowship and F.G.G. by a Royal Society European Exchange fellowship. This work was supported in part by the British Medical Research Council.

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