Am J Hum Genet 38:26–37, 1986

Further Evidence for the Dispersion of the Human Fibrillar Collagen Genes

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SUMMARY

Recombinant DNA probes specific for the human proα1(II) and proα1(III) collagen chains have been used for the chromosomal localization of the two genes. Restriction endonuclease analysis of DNA from human-rodent hybrid cell lines in conjunction with in situ hybridization of human metaphasic chromosomes have shown that the gene coding for the proα1 chain of type II collagen (COL2A1) is located on chromosome 12 in the segment 12q131→12q132. Likewise, the gene coding for the proα1 chain of type III collagen (COL3A1) was assigned to the segment 2q31→2q323 of chromosome 2.

INTRODUCTION

The collagen is a large and complex gene family that plays an essential role in the formation and maintenance of the extracellular framework of all vertebrates [1]. Because of their location and function outside the cell, the fibrillar collagens, types I, II, and III, represent a separate subgroup within this family of

Received April 18, 1985; revised May 13, 1985.

This work was supported by grant AM-32380 from the National Institutes of Health, The Human Growth Foundation, grant CRL81104 from INSERM, and grant ATP955194 from C.N.R.S.

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 0002-9297/86/3801-0004$02.00

proteins [2]. Four distinct genes code for the subunits of the fibrillar collagens: proα1(I), proα2(I), proα1(II), and proα1(III) chains [1]. The structure of the fibrillar collagen genes has been elucidated by a number of investigations. (For a review, see [3].) These studies have shown that the four genes are highly complex and, albeit of different sizes, they share a remarkable similarity in their exon-intron arrangement. This observation has led to the suggestion that the four fibrillar collagen genes diverged from each other after duplication from a common multiexon structure, concertedly maintained by the different genes under rigorous structural and functional requirements exerted upon their products [3]. To begin to understand the evolution of this subfamily of genes, the human proα1(I) (COL1A1) and proα2(I) (COL1A2) collagens have been mapped by molecular hybridization techniques on chromosomes 17 and 7, respectively [4, 5]. More precisely, the former was found to be located within segment 17q21→17q22 and the latter within segment 7q21→7q22 [5–8]. Here, we report the chromosomal assignment and regional mapping of the genes coding for the proα1(II) (COL2A1) and proα1(III) (COL3A1) chains. Our data clearly show that the four fibrillar collagen loci are dispersed on different human chromosomes.

MATERIALS AND METHODS

Parental and Hybrid Cell Lines

Somatic cell hybrids were independently derived from fusion between rodent cell lines and different human fibroblast or lymphocyte lines carrying various translocations as well as normal human lymphocytes. More precisely, the panel of hybrid cell lines used in these studies was the following: (1) hybrid cell lines L53: mouse C11D cells thymidine kinase-negative (TK^-) × human fibroblasts no. 53:46XX,t(2,17)(q14;q21); (2) hybrid cell line B82MS2: mouse B82 cells TK^- × human lymphocytes SU:47XY,+21,t(3;17) (p14;q23); (3) hybrid cell line RAG GM97: mouse RAG cells hypoxanthine phosphoribosyl transferase negative ($HPRT^-$) × human lymphocytes GM97: 46X,t(X,1)(q26;q12); (4) hybrid cell lines C34, C35, and C56: Chinese hamster CH cells ($HPRT^-$) × human fibroblasts no. 34: 46XY, t(X;2)(p22;q323), no. 35: 46XY,t(X;12)(q23;q12), and no. 56: 46XX,t(X;5)(q21;q11); (5) hybrid cell line V106: Chinese hamster V79-4 cells ($HPRT^-$) × human fibroblasts 106:46XY.

Cell lines were established and maintained as described [9–12]. Cell hybrids were characterized by isoenzyme and karyotype analysis. Chromosomes were identified by R-banding or Q-banding [13, 14].

Recombinant Clones and DNA Analysis

The probes used for the analysis of the DNA from the human-rodent hybrid cell lines were derived from two genomic clones specific for the proα1(II) and proα1(III) human collagen genes. More precisely, from a proα1(II) genomic clone (Pis 2), a 3.7-kilobase (kb) *Eco*RI fragment (Pis 2/3.7 E) coding for the C-propeptide domain was isolated, subcloned into pBR322, and used for the localization of the corresponding gene (fig. 1*A*) [15]. Likewise, from a proα1(III) genomic clone (IdF17), a 1.6-kb *Eco*RI fragment (IdF17/1.6E), immediately adjacent to exon 1, was subcloned in pBR322 and used for the mapping of the proα1(III) gene (fig. 1*B*) [16]. Nuclear DNA purification and restriction endonucleases analysis were performed as described [5].

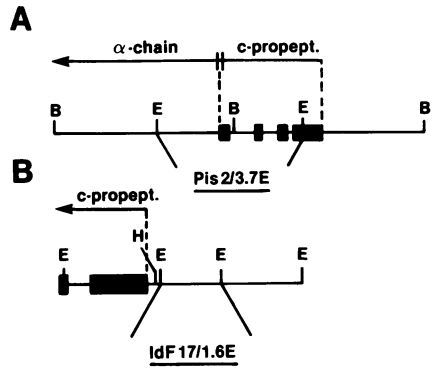

FIG. 1.—Schematic representation of the restriction endonuclease maps of the two genomic fragments used for the localization of the COL2A1 and COL3A1. *Panel A:* A section of the genomic clone Pis 2 with the relative position of the subclone Pis 2/3.7E. Indicated also are the locations of the four exons coding for the C-propeptide domain (*solid boxes*). The restriction sites of the enzymes *Eco*RI(E) and *Bam*HI(B) are shown. *Panel B:* A section of the genomic clone IdF17 with the relative position of the subclone IdF17/1.6E. Indicated also are the locations of the last two exons coding for the C-propeptide domain. The restriction site of the enzymes *Eco*RI(E) and *Hin*dIII(H) are shown. In the lower right corner is the scale of the two maps expressed in kb.

In Situ Hybridization on Metaphase Chromosomes

A phytohemagglutinin-stimulated culture of whole blood from normal man or woman was used to prepare air-dried metaphase chromosome spreads on clean glass slides, which were analyzed within 4 weeks. The cloned probes were nick-translated and used for in situ hybridizations according to the previously described protocol [17].

RESULTS

Chromosomal Localization of COL2A1 Using Somatic Cell Hybrids

DNA was prepared from parental human, mouse, and hamster cells and from eight independent mouse-human cell lines, as well as from 18 independent hamster-human cell lines. The DNA was digested by restriction endonuclease *Bam*HI (Chinese hamster-human hybrids) or *Eco*RI (mouse-human hybrids) and subjected to Southern blotting analysis using the proα1(II) genomic probe: Pis 2/3.7 E. In these experiments, a significant level of cross-hybridization was observed between the human probe and the rodent gene (fig. 2). However, the hybridizing bands of the Chinese hamster DNA (fig. 2) and mouse DNA (data not shown) were clearly different in size, and, therefore, they were easily distinguishable from the human pattern. Analysis of the human chromosome content of the different hybrid cell lines revealed that the presence of human proα1(II) collagen sequences correlated only with the presence of chromosome 12 (table 1). All othe chromosomes were excluded as demonsrated by a high score of discordant hybrids for chromosomes other than 12 (table 1). Analysis of the C35 hybrids panel allowed the regional mapping of COL2A1. In fact, C35 cells are derived from a human cell line carrying a balanced (X;12) translocation. Of the four independent C35 hybrids, only one, C35N, exhibited a positive hybridization with the proα1(II) collagen probe. Karyotyping of the C35 hy-

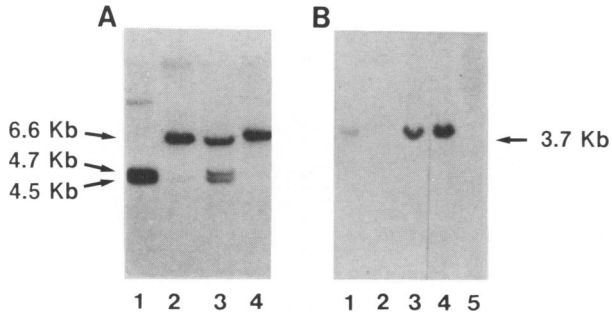

FIG. 2.—Chromosomal localization of COL2A1 and COL3A1 by Southern blotting hybridization analysis of DNA from human-rodent hybrid cell lines. *Panel A:* Autoradiogram pattern of nuclear DNA from parental human (*lane 1*) and hamster (*lane 2*) cell lines as well as from hybrid cell lines C56J (*lane 3*) and C56K (*lane 4*), after *Bam*HI digestion and hybridization to the genomic proα1(II) probe. *Panel B:* Autoradiogram pattern of nuclear DNA from parental human (*lane 4*) and mouse (*lane 5*) cell lines as well as from hybrid cell lines L53C (*lane 1*), L53F (*lane 2*), and L53G (*lane 3*) after *Hin*dIII digestion and hybridization to the genomic proα1(III) probe. The relative value in kb of the resulting positive bands is shown on the side of the two autoradiograms.

brids has shown that C35N is also the only cell line to contain both derivative chromosomes, whereas the other C35 hybrids carry only the derivative $12q^-$ chromosome. Finally, after counterselection in azaguanine [18], the C35N hybrid remained positive for COL2A1 sequences, therefore suggesting that the proα1(II) gene was most likely located within the segment 12q12→12qter of chromosome 12.

Regional Mapping of the Gene for COL2A1 by in Situ Hybridization

In situ experiments were carried out on metaphasic chromosomes in order to confirm the regional mapping of COL2A1 and to more precisely localize the gene within 12q12→12qter (fig. 3). Grain counts were done on 114 metaphasic chromosomes on which a total of 516 grains was found. Of these grains, 261 were cytoplasmic and 255 chromosomal. The distribution of the latter is shown in figure 4. Among the 255 chromosomal grains, 46 appeared on chromosome 12 with 20 in the region 12q131→12q132. Thus, 7.8% of the total number of grains were concentrated on 0.26% of the genome. This result was in agreement with the previous data obtained by DNA analysis of somatic cell hybrids and refined the regional mapping of COL1A2 to the chromosomal segment 12q131→12q132 (fig. 4).

Chromosomal Localization of COL3A1 Using Somatic Cell Hybrids

DNA from the parental human and rodent cells as well as the hybrid cell lines was digested with the restriction endonuclease *Hin*dIII and hybridized to the proα1(III) specific probe: IdF17/1.6E. In these experiments, no detectable hybridization of the homologous rodent sequences was observed, probably because of the noncoding nature of the probe used (fig. 2).

A computer analysis of the results obtained with the proα1(III) probe localized COL3A1 on chromosome 2 (table 2). The screening of two panels of

TABLE 1

CORRELATION OF HUMAN CHROMOSOME CONTENT AND PRESENCE OF TYPE II COLLAGEN-RELATED FRAGMENTS IN HUMAN × MOUSE AND HUMAN × CHINESE HAMSTER HYBRID CLONES

	Chromosomes																								Derivative chromosomes			
Hybrids	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	Y	Xq+	12q−	T	Gene
L53C	+	●	+		+	+	+	+			+		+	+		+	●	+	+	+	+						2q− 17q+	−
L53F		●						+			+						●				+						2q−	−
L53G		+●	+		+		+				+						●	+	+		+		+				17q+	−
L53K	+			+	+	+	+	+		+	+				/		+			+			+					−
L53O	/	/		+	+		+				/	+		/	/	/	+		+	+	+		/					+
B82MS2	+	+	+	+		+		+					+	+	+	+	+	+	+		+	+	+	+				−
A9SU		+	●			+							+	+			●	+			+						3p−	−
RAGGM97	●			+					+					+								+	●				1q−	−
C34FU			+	+				+	/					/		+			+	+				+				−
C34S						+	+		/	/	+	+		/		+				+	/							+
C34T			+		/	+					+	+				+												+
C34V	+		+	+	+	/		+	+		+	+	+	+	+	+		+	+	+									+
C34Z	+		+	+	+	+		+	+	+		+	+			+			/	/	+	/							+
V106					+					+				/	+				+	/	+	+	+	+					−
C56G				+				+					+	/	/					/	+		+						−
C56J			+	+	+●					/	+	+	+			+	+		+		/	/	/●				Xq+	+
C56K				+				+								+							/						−
C56L			+	+	+●			+	+		+	+		+		+		+	+		+		●				Xq+	+
C56V				+		+	/	+		+	+	+	/		+	+	/	/	+	/	/		+						+
C56W				+	/	+	/	+			+					+			+		/								−
C56X	+	+	+	+	+	+	/	+	+			+						/	+	+		+							+
C56Y						+		+		+	+	+	+						+		/								+
C35D1						+		+		+		●	+	/	/	/			+	+	+		●			+			−
C35D2			+	/	+	+						●										+	●			+			−
C35J						+						●											●			+			−
C35N	/				+	+		+	/			●							+			+	●		+	+			+
GENE:−/CHR:+	3	3	5	7	5	8	3	9	1	3	5	0	5	4	2	5	2	4	7	4	8	4	5	3	0	3		
GENE:+/CHR:−	6	9	5	4	3	3	7	4	5	6	3	0	6	7	8	3	8	7	2	5	4	7	7	11				
GENE:+/CHR:+	3	1	6	7	7	7	2	7	4	3	7	10	4	2	2	7	2	2	8	4	3	2	1	0				
GENE:−/CHR:−	11	12	9	7	9	7	11	6	13	12	10	15	10	7	10	9	12	11	8	9	6	11	8	12				

NOTE: +, The chromosome is present in at least 30% of mitoses; /, the chromosome is present in less than 30% of mitoses; "blank space," the chromosome is absent; ●, part of the chromosome is present as a derivative chromosome. The derivative chromosomes are: Xq+,12q−: t(X;12)(q23,q12); 2q−,17q+: t(2;17) (q14;q21); 3p−: t(3;17)(p14;q23); 1q−: t(X;1)(q26;q12); Xq+: t(X;5) (q21;q11). The evaluation of the extent of discordancy was based on the figures in the last four lines of the table. Chromosomes present in less than 30% of mitoses or present as a derivative chromosome were not taken into account for computerizing all data. Hybrids containing derivative chromosomes for chromosome 12 were used separately for estimation of discordancy in the right side column "derivative chromosomes."

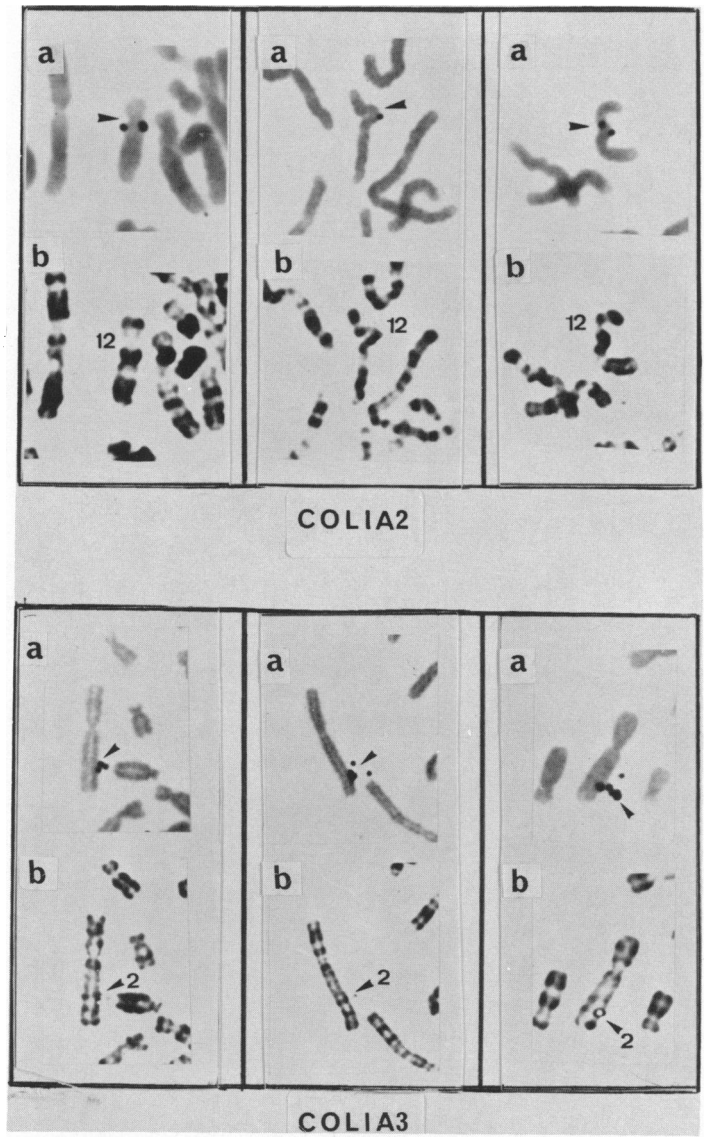

FIG. 3.—In situ hybridization of human metaphases with ^{3}H-labeled COL2A1 and COL3A1. Three partial metaphases show the specific site of hybridization for COL2A1 and COL3A1. *a*, Silver grain on the long arm of a chromosome after Giemsa staining. *b*, Identification of the chromosome with a silver grain by R-banding realized secondarily with the Fluorochrome-Photolysis Giemsa technique F.P.G.

FIG. 4.—Regional mapping of COL2A1 on chromosome 12 by in situ hybridization. *Panel A*: Diagrammatic representation of the distribution of 255 labeled sites in 114 methaphase cells. The no. grains per chromosomal unit is indicated on *the ordinate*, while each of the chromosomes and their centromeric subdivisions are shown on *the abscissa*. *Panel B*: Representation of chromosome 12 showing *on the left side* the extent of the region eliminated by the C35 hybrids *(a)*, which contain chromosomes derived from a (X;12) reciprocal translocation with a breakpoint at 12q12. *On the right side* is shown distribution of labeled sites on chromosome 12 identifying a significant clustering of grains on 12q131→12q132.

TABLE 2

CORRELATION OF HUMAN CHROMOSOME CONTENT AND PRESENCE OF TYPE III COLLAGEN-RELATED FRAGMENTS IN HUMAN × MOUSE AND HUMAN × CHINESE HAMSTER HYBRID CLONES

HYBRIDS	CHROMOSOMES																								DERIVATIVE CHROMOSOMES					GENE
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	Y	2q−	17q+	Xp+	2q−	T	
L53C	+	●	+		+	+	+	+			+		+	+		+	●	+	+	+	+				+	+				+
L53F		●						+			+						●				+				+					−
L53G		+●	+		+		+				+						●	+	+		+		+			+				+
L53K	+			+	+	+	+	+		+	+				/		+			+			+		/	/				−
L53O	/	/		+	+		+				/	+		/	/	/	+		+	+	+		/							−
B82MS2	+	+	+	+		+		+					+	+	+	+	+	+	+		+	+	+	+						+
A9SU		+	●			+							+	+			●	+			+								3p−	+
RAGGM97	●			+					+					+								+	●						1q−	−
C34DW			+	/			/	+	+			+	/			/	/	/		+	+	/		/			+			−
C34FU			+	+				+	/					/		+			+	+				+			+			−
C34GT		/		+		+			+	+		+	+					+		/	+						+			−
C34S						+	+		/	/	+	+		/		+				+	/						+			−
C34T			+		/	+					+	+				+											+			−
C34U			+	/		+		+		+		+	+	/		+			/	+	+						+			−
C34V	+		+	+	+	/		+	+		+	+	+	+	+	+		+	+	+							+			−
C342	+		+	+	+	+		+	+	+		+	+			+			/	/	+	/					/			−
V106					+					+				/	+				+	/	+	+	+	+						−
C56G				+				+					+	/	/					/	+		+							−
C56J			+	+	+●					/	+	+	+			+	+		+		/	/	/●						Xq+	−
C56K				+				+								+							/							−
C56L			+	+	+●			+	+		+	+		+		+		+	+		+		●						Xq+	−
C56V				+		+	/	+		+	+	+	/		+	+	/	/	+	/	/		+							−
C56W				+	/	+	/	+			+					+			+		/									−
C56X	+	+	+	+	+	+	/	+	+			+						/	+	+		+								+
C56Y						+		+		+	+	+	+						+		/									−
GENE:−/CHR:+	3	0	8	13	7	9	3	13	6	7	10	12	7	3	3	11	3	3	9	7	9	2	4	2	1	0	7	0		
GENE:+/CHR:−	2	0	0	3	2	1	2	2	4	5	3	4	2	2	4	3	1	0	1	3	1	3	3	4	----------					
GENE:+/CHR:+	3	4	4	2	3	4	2	3	1	0	2	1	3	3	1	2	1	4	4	2	4	2	2	1	----------					
GENE:−/CHR:−	15	18	12	5	11	10	14	7	12	11	9	8	11	11	14	7	15	15	9	8	6	15	12	17	----------					

NOTE: +, The chromosome is present in at least 30% of mitoses; /, the chromosome is present in less than 30% of mitoses; "blank space," the chromosome is absent; ●, part of the chromosome is present as a derivative chromosome. The derivative chromosomes are: 2q−,17q+: t(2;17)(q14;q21); Xp+,2q−: t(X;2)(p22;q323); 3p−: t(3;17)(p14;q23); 1q−: t(X;1)(q26;q12); Xq+: t(X;5)(q21;q11). The evaluation of the extent of discordancy was based on the figures in the last four lines of the table. Chromosomes present in less than 30% of mitoses or present as a derivative chromosome were not taken into account for computerizing all data. Hybrids containing derivative chromosomes for chromosome 2 were used separately for estimation of discordancy in the right-side column "derivative chromosomes."

hybrid cell lines, L53 and C34, was highly suggestive for the regional localization of COL3A1. In fact, the finding that L53F hybrid was negative for COL3A1 sequences excluded the possibility that the proα1(III) gene was located within the segment 2pter→2q14. Likewise, exclusion of COL3A1 within the region 2q323→2qter was implied by the negative scoring for COL3A1 sequences of the C34 hybrid lines. Together, the two findings strongly suggested that the COL3A1 gene can be assigned to 2q14→2q323.

Regional Mapping of COL1A3 in Situ Hybridization

As for COL2A1, in situ hybridization experiments were performed to confirm results obtained by the DNA analysis of the hybrid cell lines (fig. 3). In 245 metaphases, 660 chromosomal and 523 cytoplasmic silver grains were observed. Nine percent of the chromosomal grains were on chromosome 2, and, among them, 31 appeared on segment 2q31→2q33 (fig. 5). The 31 grains represent 4.6% of the total number of grains on 0.84% of the genome. From the in situ and the Southern blotting hybridizations, we extrapolated that, since the breakpoint of the translocation in the C34 hybrid panel [11] lies in 2q323, the COL3A1 gene is located at 2q31→2q323 (fig. 5).

DISCUSSION

Using a combination of Southern blotting analysis of DNA from human-rodent hybrid cell lines and in situ hybridization of human metaphasic chromosomes, we have determined both the chromosomal localization and the regional mapping of the proα1(II) (COL2A1) and proα1(III) (COL3A1) collagen genes. These results, together with those previously published for the other two human fibrillar collagens [4–8], conclusively demonstrate that this subgroup of the collagen multigene family is dispersed on different chromosomes. Based on the example of the globin gene family, where syntenic members are structurally more related to each other than those located on nonhomologous chromosomes [19], we have previously argued that the length differences among the fibrillar collagen genes could be a reflection of their divergence on separate chromosomes [20]. The data presented here strongly support this notion. Interestingly, preliminary data indicate that the proα2(V) collagen gene (COL5A2) is closely linked to COL3A1 and that the fibronectin gene is located in the contiguous region 2q232→2qter, thus suggesting that the distal region of the long arm of chromosome 2 may indeed contain a cluster of genes coding for some of the extracellular matrix components. The regional mapping of COL2A1 confirms and refines the localization of this gene reported by Strom et al. [21] while this work was already in progress. Our data also refute the immunological assignment of COL3A1 to chromosome 7 [22], where COL1A2 is located [4, 6–8]. Using similar immunological techniques, human type IV collagen expression was detected in hybrid cell lines that had retained chromosome 17 [23], where COL1A1 is located [5]. The future availability of molecular probes specific for the proα1(IV) and proα2(IV) collagen chains will make it possible to test the validity of the assignment of one or both of the type IV genes to chromosome 17.

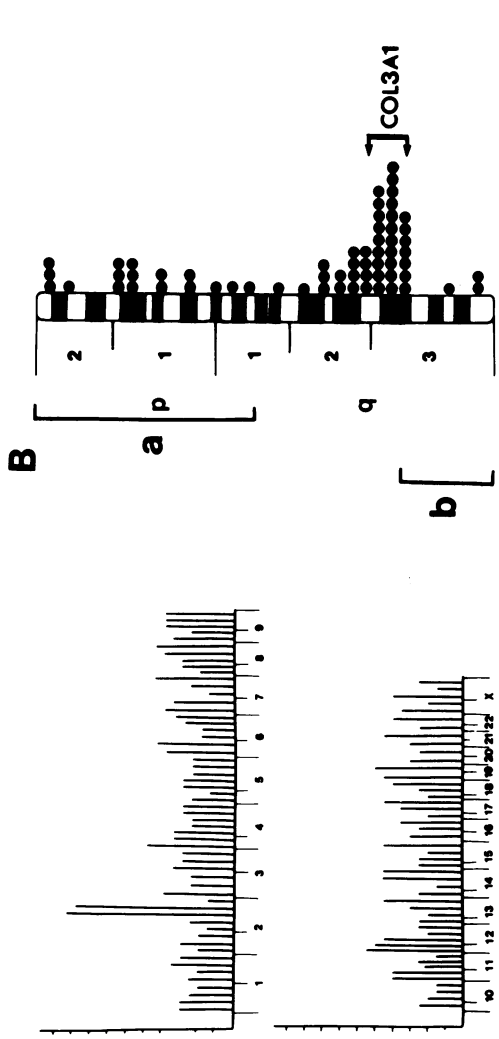

FIG. 5.—Regional mapping COL3A1 on chromosome 2 by in situ hybridization. *Panel A*: Diagrammatic representation of the distribution of 660 labeled sites in 245 metaphase cells. The no. grains per chromosomal unit is indicated on *the ordinate*, while each of the human chromosomes and their centromeric subdivisions are shown on *the abscissa*. *Panel B*: Representation of chromosome 2 showing *on the left side* the extent of the regions eliminated by the hybrids C34 (*a*) and L53 (*b*), which were derived from human parental cell lines carrying chromosomal translocations with breakpoints at q323 and q14, respectively. *On the right side* is shown distribution of labeled sites on chromosome 2 identifying a significant clustering of grains on 2q31→2q33.

In conclusion, the molecular mapping of the various collagen genes in conjunction with the structural analysis of their coding elements is generating new insights into the evolution and the controlled expression of this important multigene family.

ACKNOWLEDGMENTS

We thank Dr. P. Tsipouras for many critical suggestions, Dr. M. Jeanpierre for the computer analysis of the data, and Ms. K. Valentine for typing the manuscript.

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