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Genetics

Human α1(III) and α2(V) procollagen genes are located on the long arm of chromosome 2

(*in situ* hybridization/gene family/connective tissue/collagen/cytological linkage)

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ABSTRACT **The multigene procollagen family encodes probably >20 genetically distinct but structurally related polypeptide chains. Recent characterization of human procollagen clones has allowed determination of functional domains within the proteins, genomic organization, and chromosomal location. Previously, we assigned the coordinately expressed type I genes (α1 and α2) to chromosomes 17 and 7, respectively, and now other investigators have mapped the type II gene to chromosome 12 [Strom, C. M., Eddy, R. L. & Shows, T. B. (1984) *Somatic Cell Genet.* 10, 651–655]. Recently, we isolated cDNA clones encoding the fourth interstitial procollagen, type III, and the α2 chain of the type V cytoskeletal components. To determine whether these genes were clustered with α1(I), α2(I), or α1(II) or were further dispersed in the genome, *in situ* hybridization of the α1(III) and α2(V) probes to metaphase chromosomes was carried out. Here we report a fourth autosome with procollagen gene loci but the first cytological evidence for linkage. By using normal and translocated cell lines, our results show that both the α1(III) and α2(V) procollagen genes map to the q24.3→q31 region of chromosome 2.**

The procollagens constitute a group of proteins responsible for the structural integrity of connective tissue (see refs. 1–4 for reviews). The polypeptide chains are generally composed of a large collagenous domain with the repeating Gly-X-Y sequence flanked by smaller amino- and carboxyl-extension peptides. More than five major and five minor types of collagen have been identified, which consist of one, two, or three different chains folded into a triple-helical molecule (1–9). While several types have a unique tissue distribution (II in cartilage and IV in basement membrane), others (I, III, and V) are usually coexpressed but at different levels depending on the cell type (1–4). We have been studying the structure and regulation of these latter three collagens normally found together in the proportion I > III > V. Whereas type I is the major collagen species in skin, bone, tendon, placenta, lung, and liver, type III appears to predominate in blood vessels and internal organs (1–4, 10, 11). Little is currently known about the minor component, type V, except for its distribution in pericellular regions (12).

Isolation of DNA clones encoding different procollagen chains has allowed determination of the amino acid sequence, genomic organization, and chromosomal assignment (see refs. 4 and 13 for reviews). For example, the sizes of the α1(I) and α2(I) genes (13–15) are inversely proportional to the 2:1 ratio of the mRNAs in cultured fibroblasts, reflecting the subunit composition of type I collagen (16). Interestingly, these two carefully regulated coding units, both interrupted by ≈50 introns, are located on the long arm of chromosomes 17 and 7, respectively, as determined from somatic cell and *in situ* hybridization (17–20). Since the collagens seem to have arisen by duplication of a 54-base-pair (bp) primordial unit (21), it had been speculated that these may be the two main chromosomal sites where different members of the procollagen gene family reside. However, recent chromosomal mapping of α1(II) genomic sequences (22) has identified chromosome 12 as a third procollagen locus (23), suggesting dispersion for at least the interstitial procollagen genes.

We have characterized cDNA clones encoding part of the α1(III) and α2(V) procollagen chains (24, 25) and compared the derived amino acid sequences with those reported for the human α1(I) and α2(I) polypeptides (26, 27). Immediately obvious is the maintenance of most charged positions in the uninterrupted Gly-Xaa-Yaa region and conserved functional domains within the carboxyl propeptides, indicating that these are closely related structural macromolecules. To determine then whether the α1(III) and α2(V) procollagen genes are clustered with previously assigned members of this gene family, chromosomal mapping studies were initiated. Here we report a fourth autosome with procollagen gene loci. *In situ* hybridization of the α1(III) and α2(V) probes to human metaphase chromosomes places them in the same region of chromosome 2, indicating the first linkage of two coordinately expressed procollagen genes.

MATERIALS AND METHODS

Recombinant Clones and Restriction Analysis. Preparation and screening of the cDNA library have been described (24). The source of template mRNA was a normal human fibroblast cell line, GM3348, obtained from the Human Genetic Mutant Cell Repository (Camden, NJ). Endonuclease restriction analysis was carried out independently on both α2 type V clones. Enzymes were purchased from New England Biolabs and were used according to manufacturers specifications. DNA fragments were electrophoresed on agarose and/or polyacrylamide gels with appropriate size markers.

DNA and Protein Sequence Determination. All nucleotide sequencing was carried out by the Sanger M13 dideoxy procedure using the universal primer (28). Plasmid DNAs were cleaved with *Pst* I or *Hin*dIII/*Pst* I, and the fragments were directly ligated into M13 mp18 mp19 vectors. Protein sequences for the human α1(III) collagen chain, obtained by Edman degradation, have been reported (29). Isolation of the human α2(V) collagen chain from amnion/chorionic mem-

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Abbreviations: kb, kilobase(s); bp, base pair(s).

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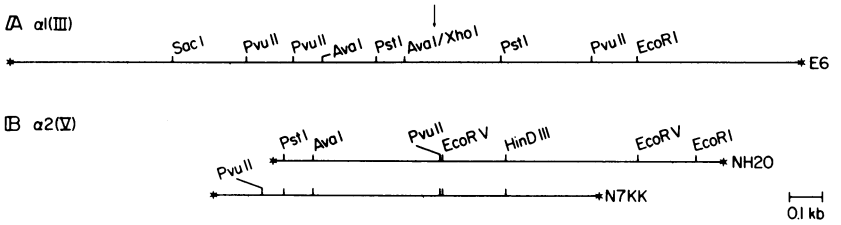

FIG. 1. Restriction endonuclease analysis of α1(III) and α2(V) procollagen cDNA clones. The three clones shown were inserted into the *Pst* I site (asterisks) of the plasmid vector pBR322. Clones used for *in situ* hybridization were α1(III) clone E6 containing a 2.4-kb insert (*A*), and α2(V) clone NH20 containing a 1.35-kb insert (*B*). Orientation is from left to right, 5→3. Arrow designates the junction of the triple-helical region and the COOH-terminal propeptide.

branes, cyanogen bromide cleavage, and partial amino acid determination of the COOH-terminal CB9 peptide have been detailed elsewhere (25).

Chromosome Preparations. Metaphase chromosome spreads were prepared from peripheral blood lymphocytes of normal males (46,XY) by standard techniques. Chromosome preparations were also made from a fibroblast line (GM1230) obtained from the Human Genetic Mutant cell repository (Camden, NJ). These cells carry a balanced translocation between chromosomes 1 and 2; 46,XY,t(1;2)(p36;q31). Air-dried slides were permitted to age in the cold (4°C) for at least 1 week prior to their use in mapping studies. The karyotype of the cells was confirmed by G-band analysis prior to their use for *in situ* hybridization. Slides for G banding were incubated at 95°C for 15 min, cooled, treated for 1–5 sec in 0.025% trypsin (Difco) in isotonic saline, rinsed, and stained for 6 min in a mixture of 0.3% Wright's stain (Fisher) prepared in methanol to Gurr's phosphate buffer (pH 6.8) (1:4) (Biomedical Specialties, Santa Monica, CA).

***In Situ* Hybridization.** *In situ* hybridization studies were carried out by using a protocol modified from several in the literature (30, 31). Air-dried metaphase chromosome preparations on glass slides were used 1–3 weeks after preparation. Slides were treated with RNase to remove any chromosomally bound RNA, washed free of RNase, and then dehydrated through an alcohol series. Chromosomal DNA was denatured by immersing the slides in 2× NaCl/Cit/70% formamide (1× NaCl/Cit = 0.15 M NaCl/0.015 M Na citrate) at 70°C, followed by rapid transfer through an alcohol series for dehydration. Probe DNA was ^{3}H-labeled by nick-translation to a specific activity of 4×10^7 cpm/μg, according to the protocol described by Lai *et al.* (32) and separated from free-labeled nucleotides by G-50 Sephadex chromatography. Salmon sperm carrier DNA was added at an excess of 1000×, and the DNA was ethanol-precipitated. The DNA was resuspended in the hybridization mixture, which consisted of 25% or 50% formamide/2× NaCl/Cit/10% dextran sulfate, pH 7.0 (both formamide concentrations were used with each probe). Probe DNA was denatured for 5 min at 70°C, ice-chilled, added to the slides at a concentration of 0.07 μg/ml, and coverslips were placed on the slides. Slides were put in moist chambers and hybridized for 18 hr at 37°C. All slides were washed at stringent conditions (39°C in 50% formamide and 2× NaCl/Cit) to remove nonspecifically bound labeled DNA, and they were then dehydrated through an alcohol series. They were dipped in liquid nuclear track emulsion (Kodak NTB-2), stored in dark boxes, and developed at appropriate intervals. Chromosomes were banded by using a modified Wright's Giemsa protocol (33) and were analyzed under a microscope. Metaphase spreads were selected by the criteria of good chromosome morphology and limited background grains. Location of specific grains was noted.

A α1(III)

PROTEIN	PRO	ARG	GLY	ASN	ARG	GLY	GLU	ARG	GLY	SER	GLU	GLY	SER	HYP	GLY	HIS
	PRO	ARG	GLY	ASN	ARG	GLY	GLU	ARG	GLY	SER	GLU	GLY	SER	PRO	GLY	HIS
CLONE	CCT	CGA	GGT	AAC	AGA	GGT	GAA	AGA	GGA	TCT	GAG	GGC	TCC	CCA	GGC	CAC
	992															

PROTEIN	HYP	GLY	GLN	HYP	GLY	PRO	HYP	GLY	PRO	HYP	GLY	ALA	HYP	GLY	PRO	CYS
	PRO	GLY	GLN	PRO	GLY	PRO	PRO	GLY	PRO	PRO	GLY	ALA	PRO	GLY	PRO	CYS
CLONE	CCA	GGG	CAA	CCA	GGC	CCT	CCT	GGA	CCT	CCT	GGT	GCC	CCT	GGT	CCT	TGC
																1023

B α2(V)

PROTEIN	MET	HYP	GLY	LEU	HYP	GLY	PRO	ALA	GLY	THR	HYP	GLY	LYS	VAL	GLY	PRO
	MET	PRO	GLY	LEU	PRO	GLY	PRO	ALA	GLY	THR	PRO	GLY	LYS	VAL	GLY	PRO
CLONE	ATG	CCC	GGC	CTA	CCA	GGC	CCA	GCG	GGA	ACA	CCA	GGA	AAA	GTA	GGA	CCA
	797															

PROTEIN	THR	GLY	ALA	THR	GLY	ASP	LYS	GLY	PRO	HYP	GLY	PRO	VAL	GLY	PRO	HYP
	THR	GLY	ALA	THR	GLY	ASP	LYS	GLY	PRO	PRO	GLY	PRO	VAL	GLY	PRO	PRO
CLONE	ACT	GGT	GCA	ACA	GGA	GAT	AAA	GGT	CCA	CCT	GGA	CCT	GTG	GGG	CCC	CCA
																828

FIG. 2. Identification of cDNA clones encoding human α1(III) and α2(V) procollagen chains. (*A*) α1(III) collagen sequence of residues 992–1023 determined from Edman degradation of cyanogen bromide peptides (top line; see also ref. 29). Bottom lines are the amino acids derived from DNA sequencing of the internal *Pst* I fragment of the clone E6 (see Fig. 1*A*). (*B*) NH_2-terminal 31 amino acids of the α2(V) CB9 peptide determined by Edman degradation (top line), and corresponding amino acids obtained from the DNA sequence of the 5′ *Pst* I fragment of the clone N7KK (bottom lines) (see Fig. 1*B*). These include residues 797–828 of the human α2(V) collagen chain (25).

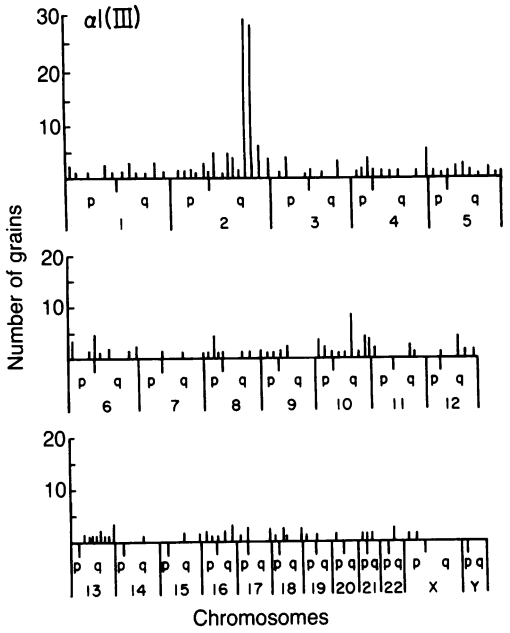

FIG. 3. *In situ* hybridization of the α1(III) clone to normal metaphase chromosomes. The histogram shows the grain distribution in 186 metaphase spreads hybridized with the ^{3}H-labeled α1(III) probe E6. Abscissa represents chromosomes in their relative size proportion; ordinate shows the number of silver grains.

RESULTS

Identification of α1(III) and α2(V) cDNA Clones. The two clones used for *in situ* hybridization were isolated from a normal fibroblast cDNA library (24). Restriction endonuclease analysis of the α1(III) and α2(V) cDNA clones is shown in Fig. 1. E6 contains a 2.4-kilobase (kb) insert coding for ≈430 amino acids of the α1(III) triple-helical region, the entire COOH-terminal propeptide, and several hundred nucleotides of the 3′ untranslated region (24). Nucleotide and derived amino acid sequences of the COOH end of the α chain were obtained from the internal 385-bp *Pst* I fragment (Fig. 2*A*). These are identical to the residues determined by Edman degradation of cyanogen bromide peptides of the human α1(III) collagen chain (29).

Fig. 1*B* shows restriction maps of the α2(V) cDNA clone, NH20, used for the chromosomal mapping studies and a 5′ overlapping later isolate, N7KK, from which positive identification of the clones coding for the α2(V) chain was established (25). The 5′ triplet in N7KK, Gly-Met-Pro (Fig. 2*B*), is the beginning of the COOH-terminal 223-residue CB9 peptide of the α2(V) collagen chain. Thirty-one amino acids adjacent to methionine were determined by Edman degradation and were found to be identical to those derived from N7KK (Fig. 2*B*; ref. 25). The 1.35-kb insert of NH20 encodes 153 amino acids of the α2(V) triple-helical region (25), the entire COOH-terminal propeptide, and part of the 3′ untranslated region (unpublished data). DNA fragments from N7KK that are also found in NH20 have been sequenced to completely verify the overlap between the two clones.

***In Situ* Hybridization to Normal Metaphase Chromosomes.** Three independent *in situ* hybridization experiments were performed with normal human metaphase chromosomes and the α1(III) probe E6 (Fig. 1*A*). The predominant site of hybridization in all experiments was the long arm of chromosome 2 (2q), with 25%, 30%, and 29% of the total grains. Chromosomal distribution of grains for the three experiments is shown in Fig. 3. The number of grains localized to 2q represented at least 6 times the number on any other chromosomal segment of similar length, suggesting that a single

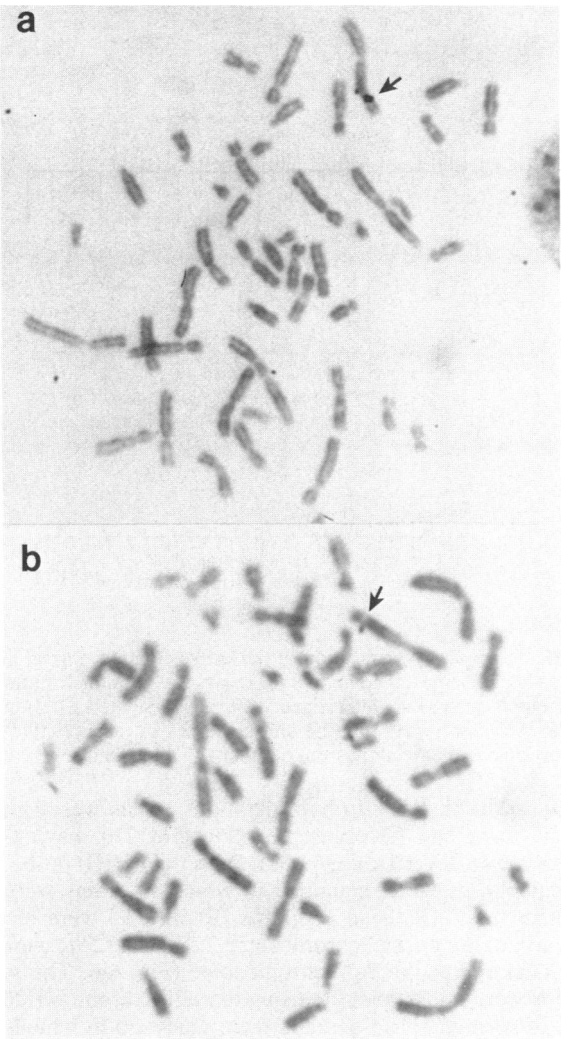

FIG. 4. Autoradiographs from *in situ* hybridization. ^{3}H-labeled procollagen probes were hybridized to metaphase chromosomes from a normal male (46,XY). Arrows indicate grains on the long arm of chromosome 2. Representative autoradiograph using the α1(III) probe E6 (*a*) and using the α2(V) probe NH20 (*b*).

α1(III) locus is present on the long arm of chromosome 2. The second most frequent site of hybridization was different in each of the three experiments and are as follows: (*i*) 10q, (*ii*) 5q, and (*iii*) 6q. A total of 186 normal metaphase spreads were analyzed in which 291 grains were on chromosomes. Of the 291 grains, 82 (28%) were located on 2q from q11→qter, and 59 (72%) of these grains were in the 2q24.3→q33 region (Fig. 4*a*). These results indicate regional mapping of the human α1(III) procollagen gene to the q24.3→q33 region.

Three separate *in situ* hybridization experiments were performed with the α2(V) probe NH20 (Fig. 1*B*) and normal human metaphase chromosomes. In all experiments, the predominant site of hybridization was 2q, with 22%, 19%, and 40% of the total grains. The second most frequent site of hybridization in the three experiments was (*i*) 4q and 10q, (*ii*) 15q, and (*iii*) 15q and the Y chromosome. A total of 174 metaphase spreads were analyzed in which 294 grains were chromosomally located. Of these 294 grains, 77 (26%) were located on 2q from q11→qter, and 62 (80.5%) of these grains were localized to the 2q24.3→q33 region (Fig. 4*b*). The chromosomal grain distribution is shown in Fig. 5. These results indicate regional mapping of the human α2(V) procollagen gene to the q24.3→q33 region of chromosome 2.

Regional Mapping by *in Situ* Hybridization to Translocated Chromosomes. To sublocalize these two procollagen genes

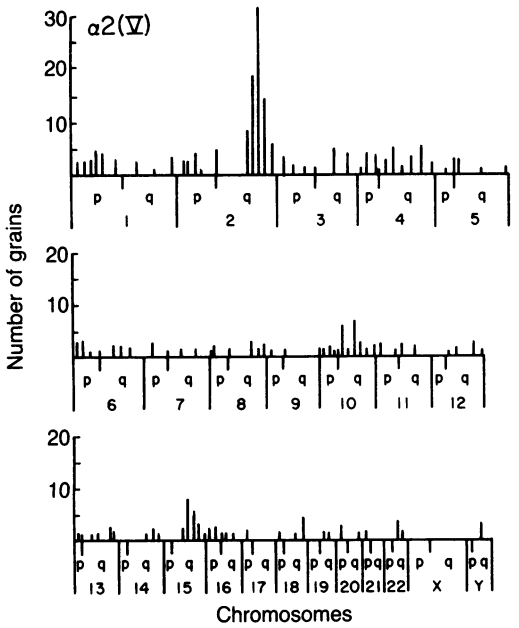

FIG. 5. *In situ* hybridization of the α2(V) clone to normal metaphase chromosomes. The histogram shows the grain distribution of 174 metaphase spreads hybridized with the ^{3}H-labeled α2(V) probe NH20. Abscissa represents the chromosomes in their relative size proportions; ordinate shows the number of silver grains.

on 2q, additional *in situ* hybridization studies were carried out by using the fibroblast cell line GM1230, having the karyotype 46,XY,t(1;2)(p36;q31). With the α1(III) probe E6, 27 metaphases were examined in which 44 grains were on chromosomes. Of these 44 grains, 10 (22.7%) were on the long arm of the normal chromosome 2 and 6 (13.5%) were on the distal end of the 2q− chromosome (Fig. 6*a*). The same studies were undertaken by using the α2(V) probe NH20. A total of 60 metaphase spreads were analyzed in which 117 grains were on chromosomes. Of these 117 grains, 27 (23%) were on the long arm of the normal chromosome 2 and 26 (22%) were on the 2q− chromosome at 2q24.3→2q31 (Fig. 6*b*). In neither study were grains observed on the relevant portion of the involved chromosome 1 and no other site had more than two grains. These results show that the α1(III) and α2(V) loci remain on the involved chromosome 2 and map in the q24.3→q31 region (Fig. 7).

DISCUSSION

We describe here the mapping of two procollagen genes, α1(III) and α2(V), to the q24.3→q31 region of chromosome 2. To our knowledge, these are the only members of this multigene family for which synteny has been demonstrated and the only procollagen genes to be assigned to chromosome 2. Previously, several other procollagen genes—α1(I), α2(I), and α1(II)—have been localized to chromosomes 17, 7, and 12, respectively (17–20, 23), which represent three additional autosomal loci. While the results suggest surprising dispersion of the genes coding for these structurally related polypeptide chains, our finding may indicate that there is in fact clustering of several procollagen members. This pattern, synteny for some and dispersion of others, is reminiscent of the α- and β-globin genes in which two separate multigene clusters are found on chromosomes 16 and 11 (34–36). In contrast, the large metallothionein gene family is located on chromosome 16 (37), but the three immunoglobulin genes are dispersed on chromosomes 14, 2, and 22 (38–40).

More than 20 procollagen genes encode the genetically distinct proteins in this family (1–4). Although the amino acids in the different interstitial procollagen chains of several species are highly conserved, human type III seems to have diverged more than α1(I) and α2(I) from their avian counter-

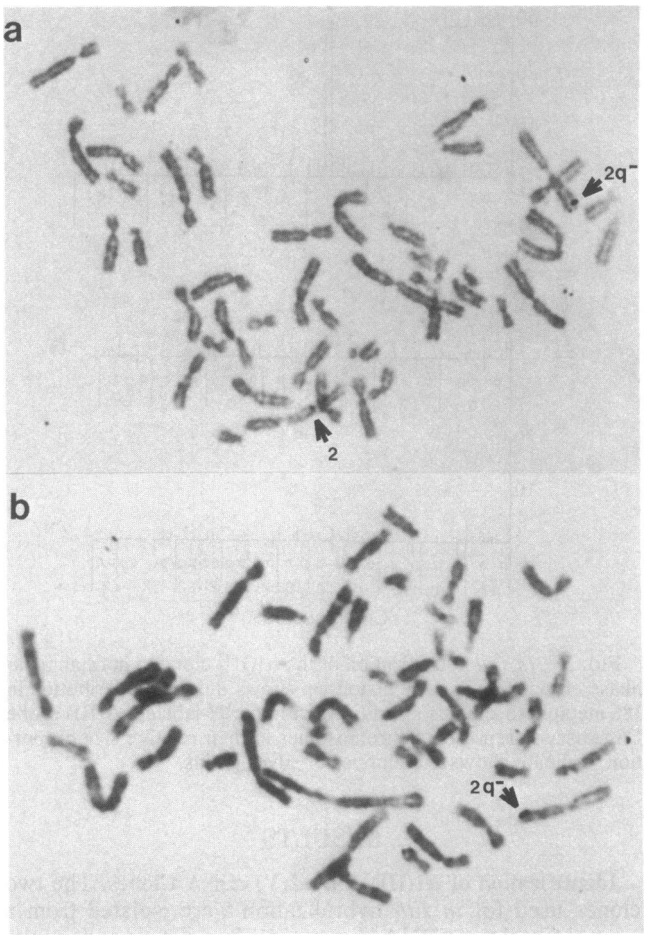

FIG. 6. Autoradiographs from *in situ* hybridization of the cDNA clones to translocated chromosomes. ^{3}H-labeled procollagen probes were hybridized to metaphase chromosomes from the GM1230 cell line having the karyotype 46,XY,t(1;2)(p36;q31). (*A*) Arrows indicate grains on normal chromosome 2 and 2q− after hybridization with E6. (*b*) Arrow indicates grain on 2q− chromosome hybridized with NH20.

FIG. 7. Idiogram of chromosome 2. Arrow at 2q31 indicates translocation breakpoint in chromosome 2 from the GM1230 cell line. Bracket shows the region of chromosome 2 (q24.3→q31 proximal) where the α1(III) and α2(V) procollagen genes are located.

parts (24). In turn, our recent data on the COOH terminus of the cytoskeletal α2(V) chain shows residues unique from those common to the human α1(I), α2(I), and α1(III) chains (25). Therefore, α2(V) may be a more ancestral gene and/or part of a collagen subfamily of genes (5) coding for the different type V chains [α1(V), α2(V), and α3(V)] and structurally related cartilage components (1α, 2α, and 3α).

With the little that is currently known about the role of type III and even less about type V, it is difficult as yet to envision the effect gene linkage may have on their expression. Some information about type III procollagen has been generated from studies of individuals affected by an inherited disease of connective tissue (Ehlers Danlos type IV). Rupture of organs and large arteries, in which smooth muscle cells rich in type III predominate, appears to be caused by decreased levels and/or structurally abnormal type III procollagen (4, 41). Type V has a specific pericellular distribution, and it is not considered an interstitial procollagen (see ref. 12 for review). Type V may also be involved in changes in blood vessels, because during atherosclerosis types I and V are increased and type III is decreased (42–45). While it is certainly premature to speculate that the synteny location of these two coding units is related to their coordinate regulation, a report showing reciprocal relationship of types III and V collagens in response to platelet-derived growth factor is intriguing (46).

The actual molecular distance between these two cytologically linked genes may in fact be considerable. Determination of the size of the intervening region by "walking on the chromosome" will reveal whether polymorphisms detected at either locus can serve as markers for the involvement of these procollagens in inherited and genetically linked connective tissue diseases, thereby elucidating their function.

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