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Assignment of the Gene Coding for the α-Subunit of Prolyl 4-Hydroxylase to Human Chromosome Region 10q21.3–23.1

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Summary

Prolyl 4-hydroxylase, an $\alpha_2\beta_2$ tetramer, catalyzes the formation of 4-hydroxyproline in collagens by the hydroxylation of proline residues in peptide linkages and plays a crucial role in the synthesis of these proteins. The gene for the β-subunit of prolyl 4-hydroxylase has recently been mapped to the long arm of human chromosome 17, at band 17q25. We report here chromosomal localization of the gene for the catalytically and regulatorily important α-subunit of human prolyl 4-hydroxylase. Analysis of 24 rodent × human cell hybrids by Southern blotting with cDNA probes for the human α-subunit indicated complete cosegregation of the gene for the α-subunit with human chromosome 10. A cell hybrid containing only part of chromosome 10 mapped the gene to 10q11→qter. In situ hybridization mapped the gene to 10q21.3-23.1. The gene for the α-subunit is thus not physically linked to that for the β-subunit of the enzyme.

Introduction

Prolyl 4-hydroxylase (E.C.1.14.11.2) catalyzes the formation of 4-hydroxyproline in collagens and in other proteins with collagen-like amino acid sequences, by the hydroxylation of proline residues in peptide linkages. This enzyme plays a crucial role in collagen synthesis, as the 4-hydroxyproline residues produced in the reaction are an absolute requirement for the folding of the newly synthesized procollagen polypeptide chains into triple-helical molecules. The active enzyme is a tetramer ($\alpha_2\beta_2$) consisting of two types of inactive subunit with molecular weights of about 64,000 (α-subunit) and 60,000 (β-subunit) (for reviews, see Kivirikko and Myllylä 1985; Kivirikko et al. 1989, and in press). The β-subunit has recently been found to be identical to the enzyme protein disulfide isomerase (Koivu et al. 1987; Pihlajaniemi et al. 1987) and to a major cellular thyroid hormone binding protein (Cheng et al. 1987; Yamauchi et al. 1987) and to be highly similar to a glycosylation site binding protein component of oligosaccharyl transferase (Geetha-Habib et al. 1988). The α-subunit appears to contribute a major part to the catalytic site of the enzyme and also to be regulated more efficiently than the β-subunit, with changes in the rate of collagen synthesis (see Kivirikko and Myllylä 1985; Kivirikko et al. 1989, and in press).

The gene coding for the multifunctional β-subunit of prolyl 4-hydroxylase (P4HB) has recently been mapped to the long arm of human chromosome 17 (Pajunen et al. 1988), at band 17q25 (Popescu et al. 1988). Chromosome 17 also contains the gene for the α1 chain of type I collagen, the most abundant collagen polypeptide chain in animals, which is located at band 17q21.31-q22.05 (Huerre et al. 1982; Solomon et al. 1984; Retief et al. 1985). We demonstrate here that the gene coding for the catalytically and regulatorily important α-subunit of prolyl 4-hydroxylase (P4HA) is located on chromosome 10, in region q21.3-23.1, and is thus not physically linked to the gene for P4HB.

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Material and Methods

The 24 rodent × human somatic cell hybrids used here were derived from multiple fusions between various mouse or hamster cell lines and human fibroblasts or lymphocytes. The human chromosome contents of hybrids 1αA9.498 (a gift from Dr. D. Cox, University of California, San Francisco), TRAXK2 (a gift from Dr. P. Goodfellow, University of Vancouver), DUR4R3, 2W1R70, DT1.2.4, Cl21, PCTBA1.8, HORL9D2R1, P7A2/7, HORL411B6P, HORL411B6N4, POTB2/B2,C10b, C4a, 2860H7, and CTP41A2 have been described in detail elsewhere (Bobrow and Cross 1974; Croce and Koprowski 1974; Jones and Moore 1976; Solomon et al. 1976; Swallow et al. 1977; Carritt 1980; Voss et al. 1980; Andrews et al. 1981; Bai et al. 1982; Goodfellow et al. 1982; Heisterkamp et al. 1982; Mulley and Callen 1986). Additional hybrid clones between 1R mouse cells (Nabholz et al. 1969) and various human diploid skin fibroblasts were obtained by polyethylene glycol–mediated fusion experiments followed by selection in a HAT medium. These hybrids have likewise been described elsewhere (Pajunen et al. 1988). Human skin fibroblasts and mouse 1R and hamster cells were used as the human and mouse controls, respectively. Karyotypic analysis of the hybrid cell lines was performed according to a modification of the trypsin-Giemsa method (Seabright 1971) combined with the G11 technique (Bobrow and Cross 1974).

The isolation and characterization of cDNA clones for P4HA is described in detail elsewhere (Helaakoski et al. 1989). The identity of the cDNA clones was verified by the presence of several precise matches between the cDNA-derived amino acid sequences and the protein sequences and also by comparison between the cDNA-derived amino acid composition and that determined for the α-subunit. The Southern blot hybridization analyses were performed using either cDNA probe PA-11 or probe PA-15, both giving identical data. PA-11 contains 903 bp of coding sequences and 930 bp of 3′ noncoding sequences, while PA-15 contains 1,307 bp of coding sequences and 989 bp of 3′ noncoding sequences of the corresponding 3,000-nucleotide mRNA. The probes were ^{32}P-labeled by nick-translation to a specific activity of approximately 2×10^8 cpm/μg DNA by using standard techniques.

Human lymphocytes were cultured with phytohemagglutinin for 72 h at 37°C. Bromodeoxyuridine was then added to give a final concentration of 200 μg/ml. The cells were washed with fresh medium 16–17 h later and incubated in thymidine-rich (10^{-5}) medium for a further 6–7 h. Harvesting of the cells and all further procedures were carried out in subdued lighting.

In situ hybridization was performed essentially according to a method described by Harper and Saunders (1981) and Zabel et al. (1983). The probe used was a cDNA clone PA-49 which covers, 1,566 bp of coding sequences and 1,024 bp of 3′ noncoding sequences of the human α-subunit (Helaakoski et al. 1989). Labeling was carried out using ^{3}H deoxynucleotides and oligonucleotide primers (Feinberg and Vogelstein 1983) to a specific activity of 2.5×10^8 cpm/μg, and hybridization was carried out at 37°C using probe concentrations of 0.02 and 0.004 μg/ml. Slides were

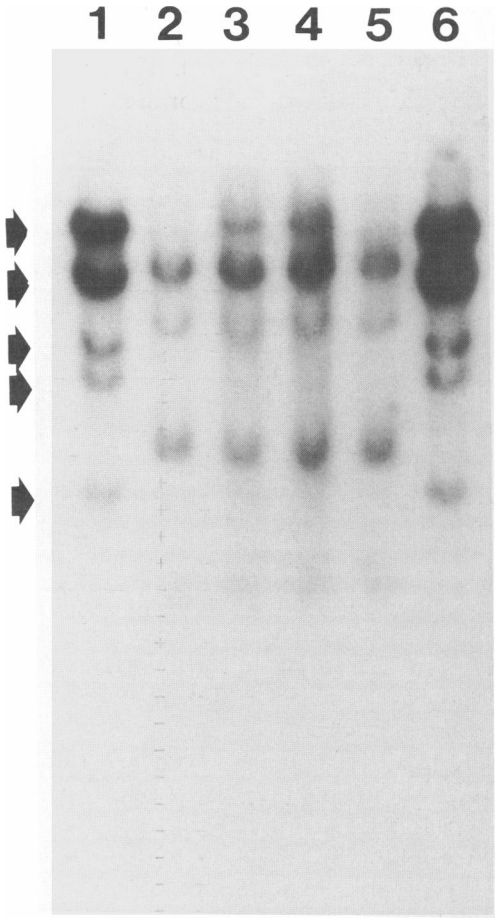

Figure 1 Southern blot analysis of mouse × human cell hybrid DNAs with human P4HA cDNA probes. Total DNAs from the cell lines indicated were digested with *Eco*RI, electrophoresed on agarose, blotted onto nitrocellulose, and hybridized with the cDNA probes. Cells were human skin fibroblasts (lanes 1 and 6), mouse 1R (lane 5), CTP41A2 (lane 2), LIM6A1 (lane 3), and DUR 4.3 (lane 4). The arrows indicate the positions of the major human restriction fragments (20.0, 8.4, 4.3, 3.6, and 2.1 kb).

washed in 0.3 M sodium chloride/0.03 M sodium citrate pH 7.0 at 39°C, were dehydrated, and were immersed in Ilford K5 emulsion. The slides were developed after 1–3 wk and were G-banded using a variation of the method of Wolff and Perry (1974). Slides were stained for 30 min in 0.5 μg/ml Hoechst 33258, exposed to long wave UV light for 15 min, and stained with Wright's stain.

High-molecular-weight DNA was isolated from human, mouse, hamster, and hybrid cell lines according to a method described by Maniatis et al. (1982). The digestions with the restriction enzyme *Eco*RI or *Bam*HI were performed as specified by the enzyme supplier (Boehringer Mannheim). The digested DNA samples were size-fractionated on 0.8% agarose gels, blotted onto nitrocellulose filters (Schleicher & Schuell), and baked in vacuo (Southern 1975). The nitrocellulose filters were prehybridized and hybridized according to a method described by Maniatis et al. (1982).

Results

Hybridization of either *Eco*RI- or *Bam*HI-digested human genomic DNA to ^{32}P-labeled cDNA probes for the human α-subunit revealed DNA fragments that were clearly distinguishable from those of mouse and hamster DNA (not shown). The largest *Eco*RI fragment of human DNA (fig. 1) was particularly informative, and therefore only digestion with *Eco*RI was used in the subsequent experiments.

Analysis of 24 rodent × human cell hybrids demonstrated complete cosegregation of the human P4HA gene with human chromosome 10 (table 1). All the other human chromosomes showed at least 17% discordant segregation of this gene and the specific chromosome. Chromosome 17, which contains the P4HB gene, showed such discordant segregation in seven hybrids (30%). Cell hybrid TRAXK2, which contains only part of human chromosome 10, gave consistently positive data, thus localizing the gene to region 10q11→qter.

Table 1

Segregation of the Human Gene for P4HA with Human Chromosomes in Cell Hybrids

	Human Chromosomes																							
Hybrid Cell	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	x	P4HA
P7A2/7	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	+[a]	−	−	−	−	−	−	−
DUR4R3	−	−	+	−	+	−	−	−	−	−	+	+	+	+	−	−	+	+	−	+	+	+	−	−
LIM6B1	+	−	−	−	+	−	+	−	−	−	+	−	+	−	−	−	+	−	−	−	+	−	+	−
LIM6B2	+	−	+	+	+	−	+	−	−	−	+	−	−	−	−	−	+	−	+	−	+	−	−	−
LIM6B22	+	−	+[b]	+	+	−	+	−	−	+	+	−	−	−	−	−	+	−	+	−	+	−	−	+
LIM6A1	+	−	−	−	+	−	+	−	−	+	+	+	−	−	−	−	+	+	−	−	−	−	+	+
LIZMAI	−	−	−	−	+	−	+	−	−	−	+	+	+	−	−	+	+	+	−	−	−	−	+	−
PCTBA1.8	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−
LIM4D1	−	−	−	−	−	−	+	−	−	−	−	−	+	+	+	−	−	−	−	−	+	−	+	−
LIM6A2	+	+	+	−	+	−	+	−	−	+	−	+	−	−	−	−	+	+	−	−	+	−	−	+
Cl21	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
2W1R70	−	−	−	−	−	−	+	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−
DUR4.3	−	−	+	−	−	−	−	−	−	+	+	+	+	+	+	−	+	+	−	+	+	+	+	+
DT1.2.4	−	−	+	+	−	−	−	−	−	+	+	−	+	−	+	−	+	+	−	+	+	+	+	+
HORL9D2R1	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−
1αA9.498	−	−	−	−	−	+	−	−	−	−	−	+	−	−	−	−	−	−	−	−	+	−	+	−
HORL411B6P	+	−	+	−	−	−	−	−	−	−	+	−	+	+	−	−	−	+	−	−	−	+	+	−
HORL411B6N4	−	−	+	−	−	−	−	−	−	−	+	−	+	+	−	−	−	+	−	−	−	+	+	−
POTB2/B2	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	+	−	+	−	−	−	−	−
C10b	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	+	+	−
2860H7	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−
CTP41A2	−	+	+	−	−	+	+	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	+	−
C4a	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
TRAXK2	−	−	−	−	−	−	−	−	−	+[c]	+	−	−	−	+	+	−	−	−	−	+	+	+	+
% Discordant	20	25	33	20	29	33	42	29	29	0	33	29	46	42	17	29	30	25	29	20	25	29	42	

[a] 17p11→qter (P7A2/7 omitted in calculating the % discordancy for chromosome 17).

[b] Chromosome present in only 10%–30% of the hybrid cells.

[c] 10q11→qter (TRAXK2 omitted in calculating the % discordancy for chromosome 10).

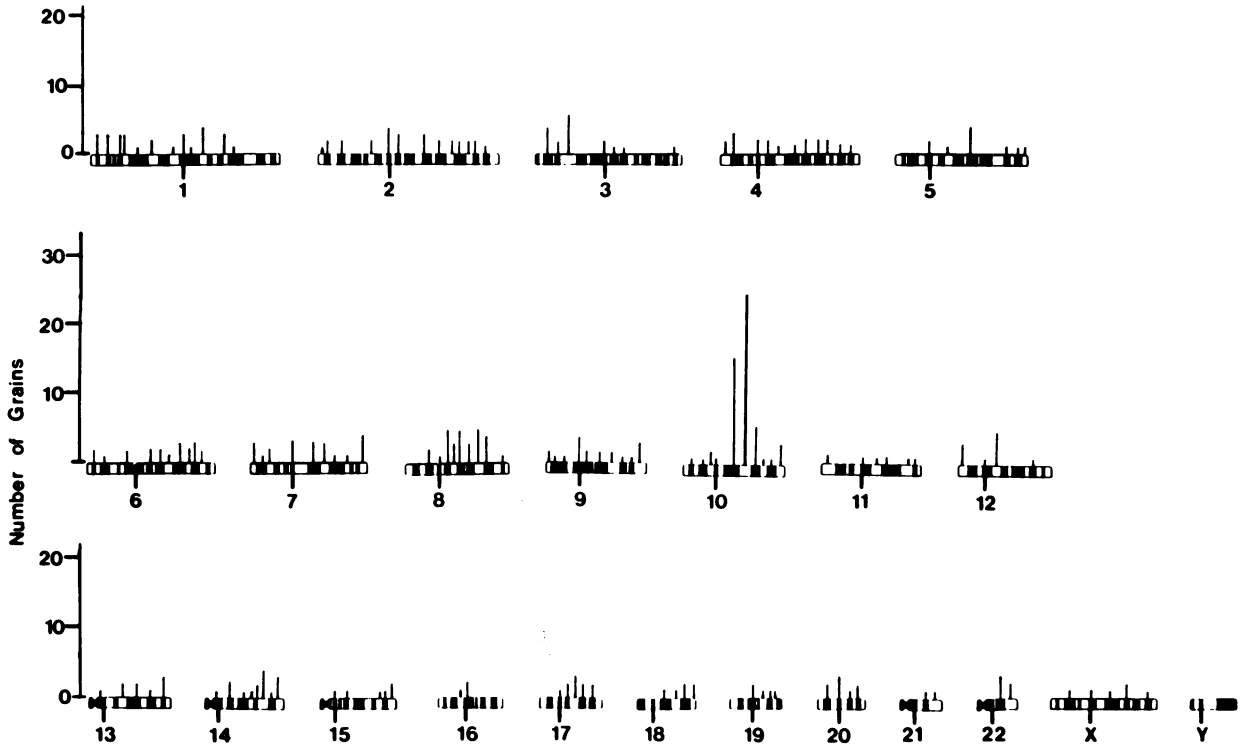

Figure 2 Distribution of grains in 71 cells after in situ hybridization with the P4HA probe (PA-49) to human lymphocytes. Specific labeling was observed on chromosome region 10q21.3-23.1.

The localization of the P4HA gene to human chromosome 10 was independently determined by in situ chromosomal hybridization with a ^{3}H-labeled human α-subunit cDNA probe. A total of 341 autoradiographic grains were counted over the chromosomes from 71 metaphases (figs. 2 and 3). Of these, 41 grains (12%) were present over chromosome bands 10q21.1-23.1. Extended banding techniques to about the 850-band stage were used to show that 10 of the 16 grains on chromosome band 10q21 could be more finely localized to 10q21.3. The grain distribution on chromosome 10 (fig. 3) suggests that the gene for P4HA is most likely located in the region q21.3-23.1 of chromosome 10. Grain counts were not significantly above background level on any other chromosomes.

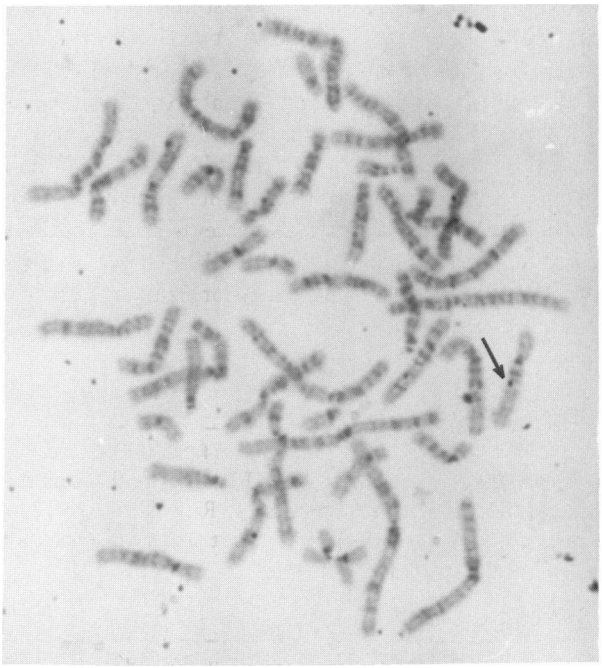

Figure 3 Representative human metaphase showing label (arrow) on chromosome 10 location 10q22.2 after hybridization with the PA-49 probe.

Discussion

Many heritable connective tissue disorders involve defects in collagens or in the posttranslational enzymes that process them (see Prockop and Kivirikko 1984; Byers and Bonadio 1985). The gene family in question includes more than 25 distinct genes for the different

collagen polypeptide chains (Gordon et al. 1987; Miller and Gay 1987; Pihlajaniemi et al. 1987), as well as at least 10 distinct genes coding for the various posttranslational enzymes of collagen synthesis (see Kivirikko and Myllylä 1985). Chromosomal locations have been reported for 12 of the collagen genes, which have been found to be dispersed among eight chromosomes (Myers and Emanuel 1987; Weil et al. 1988; Kimura et al. 1989; Pajunen et al. 1989; Shows et al., 1989). Only one of the enzyme genes has been mapped so far, namely, that coding for P4HB.

The present data identify the gene coding for P4HA as belonging to human chromosome 10, its region being q21.3-23.1. On the basis of this result it has already been possible to isolate genomic clones for the α-subunit from a human chromosome 10–specific human × rodent somatic cell hybrid genomic library (Helaakoski et al., in press). This supports the chromosomal assignment.

The gene coding for P4HA is clearly not physically linked to the P4HB gene, which is located on chromosome 17 (Pajunen et al. 1988; Popescu et al. 1988), or to any of the genes coding for the major collagen polypeptide chains (Myers and Emanuel 1987; Weil et al. 1988). Nevertheless, the gene coding for one of the minor collagen polypeptide chains, the α1 chain of type XIII collagen, has very recently been found to be located on the long arm of chromosome 10 (Pajunen et al. 1989), at region q22 (Shows et al. 1989). Furthermore, the genes coding for the M_r 35,000 pulmonary surfactant-associated proteins are also on chromosome 10, at region q21-23 (Bruns et al. 1987). These proteins contain a collagenous domain with many 4-hydroxyproline residues (Floros et al. 1986). Further studies are needed to determine the distances between the genes for P4HA, the α1 chain of type XIII collagen, and the M_r 35,000 surfactant-associated proteins in this chromosome region.

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