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ASSIGNMENT OF THE VASCULAR SMOOTH MUSCLE ACTIN GENE *ACTSA* TO HUMAN CHROMOSOME 10

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Summary Human vascular smooth muscle actin gene (*ACTSA*) was cloned and its unique sequence was used as the hybridization probe for Southern blot analysis of DNAs from 18 rodent-human somatic cell hybrids; the gene was assigned to human chromosome 10. Regional mapping by *in situ* hybridization showed that the gene is located on the long arm (q22-q24) of the chromosome. Thus, the gene is on a different chromosome from the other four actin genes so far examined.

Key Words chromosomal assignment, vascular smooth muscle actin, gene, *ACTSA*, actin, chromosome

INTRODUCTION

There are at least six actin isoforms in higher vertebrates, *i.e.*, skeletal muscle, cardiac muscle, vascular and enteric smooth muscle, and β- and γ-cytoplasmic actins (Vandekerckhove and Weber, 1978). Their amino acid sequences are very similar; they have a difference of at most 25 amino acids in total of 374–375 amino acid residues (Vandekerckhove and Weber, 1979). Therefore, it is reasonable to postulate one progenitor gene from which genes for these actin species were evolved through multiple duplication. Nevertheless, it is already known that four actin genes so far examined are not linked to each other in the human genome (Gunning *et al.*, 1984; Ng *et al.*, 1985; Erba *et al.*, 1988). We reported the structure and nucleotide sequence of human vascular smooth actin gene (Ueyama *et al.*, 1984), but its chromosomal location was not determined yet. Here we show that the gene is mapped to human chromosome 10, which is also different from the location of the other four actin genes.

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MATERIALS AND METHODS

The human gene library for gene walking was kindly supplied by Dr. Takiguchi (Kumamoto University Medical School).

The cell fusion and isolation of rodent-human somatic cell hybrids has been described (Bruns *et al.*, 1979). High-molecular-weight DNAs isolated from 18 hybrid cells (10 μg each) were digested with appropriate enzymes, separated on a 0.7% agarose gel, and transferred to a nitrocellulose filter (Schleicher & Schuell, BA85) by the method of Southern (1975). A 2.7 kb *Eco*RI-*Hin*dIII fragment located around the first exon of human vascular smooth muscle actin gene (Nakano, 1988) was used as the hybridization probe, which was labeled with [α-^{32}P]dCTP by nick-translation to a specific activity of 5×10^8 cpm/μg of DNA. Hybridization and washing were carried out as described (Taga *et al.*, 1989), and the filter was autoradiographed for 7 days with intensifying screens on Fuji RX X-ray film at -80°C.

For *in situ* hybridization (Kanda *et al.*, 1983), air-dried slides of metaphase cells, stored at least one week in a vacuum dessicator, were denatured in a mixture containing 70% deionized formamide and $2\times$SSC ($1\times$SSC=0.15 M NaCl–0.015 M sodium citrate) at 65°C for 2 min and then dehydrated successively with 70, 80, and 95% ethanol. The 2.7 kb *Eco*RI-*Hin*dIII fragment was labeled by nick-translation to a specific activity of 2×10^7 cpm/μg of DNA with [^{3}H]dTTP, [^{3}H]dCTP and [^{3}H]dATP. The labeled probe corresponding to 2.5×10^5 cpm in 50 μl of hybridization mixture (50% formamide, $2\times$SSC, 10% dextran sulfate, and sheared single-strand DNA at 100 μg/ml) was added per slide, which was incubated in a humid atmosphere at 42°C for 20 hr. The slide was washed at 39°C with 50% deionized formamide in $2\times$SSC and then with $2\times$SSC, and dried with ethanol before being dipped in Kodak NTB-2 emulsion.

RESULTS AND DISCUSSION

Human vascular smooth muscle actin gene was already cloned (Ueyama *et al.*, 1984; Nakano, 1988; Kamada *et al.*, 1989), but we further picked up 5′- and 3′-flanking region-containing clones. The results are summarized in Fig. 1. We have walked from the initial gene (18 kb) 18 kb upstream and 13 kb downstream.

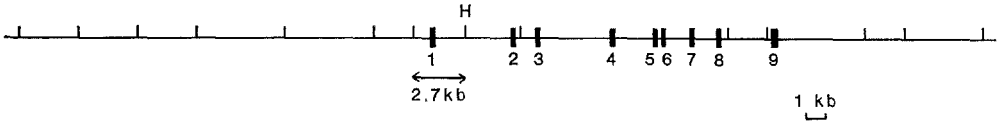

Fig. 1. Map of the human vascular smooth muscle actin gene. The 2.7 kb *Eco*RI-*Hin*dIII fragment indicated was used as the hybridization probe. Vertical bars, *Eco*RI sites; H, *Hin*dIII site. The exons are designated 1 through 9.

By using a fragment derived from the 3′-flanking region, we detected a *Taq*I RFLP (Ueyama and Ohsugi, manuscript in preparation). The region used as the hybridization probe in this study (the 2.7 kb *Eco*RI-*Hin*dIII fragment) is also shown in Fig. 1.

When the labeled probe for vascular smooth muscle actin gene was hybridized to an *Eco*RI-digest of human peripheral blood leukocyte DNA, a 5.5 kb band was detected. No hybridizing band was obtained in rodent DNAs. Southern blot analysis of the panel of 18 rodent-human hybrid DNAs digested with *Bgl*II, *Hin*dIII, or *Bam*HI showed that the signal for human vascular smooth muscle actin gene was in a good correlation with the presence of human chromosome 10 (Table 1).

When the same DNA probe but labeled with ^{3}H was used for *in situ* hybridization of human chromosomes, the autoradiographic grains were concentrated on the long arm of chromosome 10 (q22-q24, Fig. 2). Thus, the structural gene for vascular smooth muscle actin was found to be on a different chromosome from the other four actin genes so far examined.

The actin gene family has been suggested to be dispersed throughout the human genome (Soriano *et al.*, 1982); every chromosome was labeled *in situ* with an actin gene-specific probe. Among about 20 human actin genes (Humphries *et al.*, 1981), the skeletal actin gene was assigned to human chromosome 1 (p21-qter) (Gunning *et al.*, 1984), cardiac actin gene to chromosome 15 (q11-qter) (Gunning *et al.*, 1984), β-actin gene to chromosome 7 (pter-q22) (Hg *et al.*, 1985), γ-actin gene to chromosome 17 (Erba *et al.*, 1988), and some of actin pseudogenes to chromosomes 5, 7, 18, and X (Ng *et al.*, 1985). It is of interest that the genes for two very closely related cytoplasmic actins, which have only a 4-amino acid difference (Vadekerckhove and Weber, 1979) and are co-expressed in many tissues (Vandekerckhove and

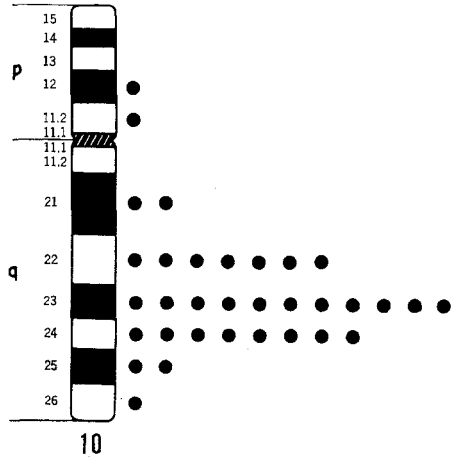

Fig. 2. *In situ* hybridization of human vascular smooth muscle actin gene probe to human chromosome 10. A clustering of autoradiographic grains over the long arm (q22-q24) is apparent.

Table 1. Distribution of vascular smooth muscle actin gene

Cell hybrids	*ACTSA*	Presence of										
		1	2	3	4	5	6	7	8	9	10	11
G35F5	−	±	−	+	+	−	+	−	−	+	−	+
G35C4	−	−	−	−	−	−	+	−	−	R	−	−
G35C1	+	−	−	R	−	−	R	+	−	−	+	+
G35D3	−	−	P	−	−	−	−	+	+	+	−	−
G35E4	−	−	−	−	+	−	−	−	−	+	−	−
G35A4	−	+	−	+	+	−	+	−	+	+	−	−
G35A2	−	−	−	+	+	−	+	−	−	−	−	+
G35F1	+	+	−	−	+	+	−	−	+	−	+	−
G56F3	−	−	−	−	−	−	+	−	−	−	−	−
G89E5	−	−	−	−	−	−	−	−	−	−	−	−
G95A4	−	−	−	−	−	−	−	−	−	−	−	−
G35B5	−	−	−	−	−	−	R	+	−	−	−	+
G35D5	+	+	+	+	−	−	+	+	−	−	+	−
G24A4	−	+	+	−	−	+	+	+	−	+	−	+
G24A9	+	−	+	−	+	−	+	−	+	+	+	−
G24B2	+	−	−	+	−	+	−	+	−	+	+	−
G17-11	+	−	−	−	−	+	+	+	−	−	+	−
G13C2	+	−	+	ND	+	+	−	+	−	+	+	+

P, short arm only; R, rearrangement; ND, not determined; *ACTSA*, vascular smooth muscle human-mouse cell hybrids.

Weber, 1981; Erba *et al.*, 1988), are on different chromosomes. Our data presented here indicate that the human vascular smooth muscle actin gene is not linked to these actin genes but on a different chromosome. Their chromosomal dispersion through transposition or translocation must have occurred after a tandem duplication and such a chromosomal dispersion of duplicated copies might be correlated with their different expression in a tissue-specific manner.

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and human chromosomes in human-rodent cell hybrids.

human chromosomes											
12	13	14	15	16	17	18	19	20	21	22	X
−	+	+	−	+	+	+	+	±	+	+	+
+	−	+	−	−	−	±	+	+	+	−	−
P	−	+	+	+	−	+	+	−	−	+	−
−	−	−	−	+	+	−	+	+	+	+	−
−	−	−	+	−	−	−	+	−	−	−	−
−	−	+	−	+	−	−	+	+	+	−	+
−	−	+	−	−	−	−	+	+	+	+	±
+	−	+	−	−	−	+	+	+	+	−	+
+	−	−	−	−	−	−	−	+	+	−	+
−	−	−	−	−	−	−	−	−	−	−	+
−	−	−	−	−	−	−	+	−	−	−	+
P	+	−	−	−	+	−	+	+	−	−	+
−	+	+	+	+	+	+	+	+	−	+	−
+	+	+	+	−	−	−	+	−	+	−	+
−	+	+	+	−	+	+	+	+	+	−	+
−	+	+	+	+	−	−	+	−	+	+	+
+	+	+	−	−	+	+	+	+	+	−	−
−	+	+	+	+	ND	+	−	+	+	ND	+

actin gene. G35F5 through G35D5 are human-Chinese hamster cell hybrids and the others are

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