ASSIGNMENT OF THE GENE ENCODING TYPE 1 γ PROTEIN PHOSPHATASE CATALYTIC SUBUNIT (*PPP1CC*) ON HUMAN, RAT, AND MOUSE CHROMOSOMES

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Summary Using fluorescent *in situ* hybridization (FISH) method, a gene encoding the catalytic subunit of protein phosphatase type 1 γ (*PPP1CC*) was mapped to human 12q24.1-q24.2, rat 7q22, and mouse 10C. These results indicate that the *PPP1CC* is a member of conserved synteny group between rat chromosome 7, mouse chromosome 10 and human chromosome 12. These data and mapping data about other members of PP1 family show that in spite of the high identity of PP1 isoforms, each isoform is encoded by different genes which located on different chromosomes in human, rat, and mouse.

Key Words protein phosphatase type 1, PP1 γ , comparative gene mapping, FISH

INTRODUCTION

Various cellular functions are regulated phosphorylation-dephosphorylation of proteins. The physiological importance of protein phosphatase (PP), which functionally counteracts protein kinase, on cellular events has been extensively studied and many important aspects of this enzyme related to cell growth have been revealed (Cohen, 1989). At present, four major classes of PPs (PP1, PP2A, PP2B, and PP2C) have been identified (Cohen, 1989). In the past several years, by using cDNA cloning, there has been increasing knowledge about type 1 protein phosphatases (PP1). These findings demonstrate that the PP1 is a very highly conservative gene family with at least three different genes. PP1 γ is one of them

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that have been cloned in human (Barker *et al.*, 1993), rat (Sasaki *et al.*, 1990), and mouse (Ohkura *et al.*, 1989; mouse PP1 γ 2 is designated as dis2m1) which showed a high degree of homology with each other. Comparative study of nucleotide and deduced amino acid sequences of PP1 γ 1 and PP1 γ 2 suggest that they are derived from the same gene and produced by alternative splicing (Sasaki *et al.*, 1990; Barker *et al.*, 1993). Here, we report the comparative mapping of the gene encoding the catalytic subunit of PP1 γ (*PPP1CC*) to the human, rat, and mouse chromosomes which will provide evolutionary genomic organization in these species.

MATERIALS AND METHODS

The probe used for mapping was the *Apa*I-*Hind*III fragment (about 800 bp) of noncoding region of the rat PP1 γ 1 cDNA (Sasaki *et al.*, 1990). This fragment has no homology with other members of PP1 family but shows high level of conservation between human and rat, therefore, we used this cDNA fragment as a probe for gene assignment in human, rat, and mouse chromosomes. The rat cDNA probe detected cross-hybridization signals on human and mouse chromosomes which was critically dependent on the stringency condition, as described previously (Saadat *et al.*, 1995). Each chromosome preparation was made from a male human blood culture, primary cultures of fibroblasts of a male LEC rat and a DRC mouse. Fluorescent *in situ* hybridization (FISH) was performed using biotinylated cDNA fragment as previously described (Saadat *et al.*, 1995). The precise signal position was determined by the delineation of Q-banding patterns. We have applied the chromosome terminology for ISCN (1978); *i.e.* human, rat, and mouse chromosomes by HSA, RNO, and MMU, respectively.

Genomic DNAs were prepared from WKAH rat, C3H mouse, and the rat $\times$ mouse hybrid cell lines (Li *et al.*, 1989). Sense and antisense primers were; 5'-CCTCCCACTGCACACTTAAC-3' (nucleotide positions 1,623 to 1,642 of the rat PP1 γ 1 cDNA clone) and 5'-CTGTGCCAGCAGCCTTGTTG-3' (nucleotide positions 1,743 to 1,762 of the rat PP1 γ 1 cDNA clone), respectively. PCR was performed using the genomic DNA samples (200 ng/reaction), 25 pmol of each primer, 3 unit AmpliTaq polymerase, in 50 μ l of reaction mixture. PCR reactions were carried out as follows, 94°C for 3 min, 65°C for 30 sec, 72°C for 30 sec, 94°C for 1 min, for 35 cycles followed by 72°C for 10 min. The PCR products were subjected to 4% agarose gel electrophoresis and visualized with ethidium bromide.

RESULTS AND DISCUSSION

As shown in Fig. 1 a-c, the human *PPP1CC* was mapped to chromosome 12 at bands q24.1-q24.2. Analysis of 50 human metaphases showed that 4 metaphases had twin-spots on both homologs and 26 metaphases had a double-spot signal on sister chromatids at q24.1-q24.2 of HSA12, showing that the *PPP1CC* located on

Fig. 1. Chromosomal localization of the human (a-c), rat (d-f), and mouse (g-i) protein phosphatase type 1 γ gene (*PPP1CC*) by FISH. The chromosomes were stained with propidium iodide (a, d, g) and Q-banded (b, e, h). Arrows indicate the positions of hybridization signals. Arrowheads on each ideogram (c, f, i) showed the respective mapped site.

this region. These data confirm the previous assignment (Barker *et al.*, 1993).

For rat mapping, 60 rat metaphases were examined after FISH. Five metaphases had twin-spots on both homologous pairs and 34 metaphases had a twin-spot on one homolog of chromosome 7 at region q22, showing that the *Ppplcc* located on RNO7q22 (Fig. 1 d-f).

For mouse chromosome assignment, a total of 50 mouse metaphases was

examined. Of these 4 cells exhibited twin-spots on both homologs and 22 metaphases had a double-spot signal on one homolog of chromosome 10 at region C, indicating that the mouse *Ppp1cc* located on MMU10C (Fig. 1 g-i). It should be emphasized that such specific accumulation of double spot signals, which mentioned above, could not be detected on any other regions of human, rat, and mouse chromosomes.

Although comparative gene mapping demonstrates that there is a conserved segment between HSA12q, MMU10, and RNO7 (Davisson *et al.*, 1991; Levan *et al.*, 1991), the conserved segment among these three species is not defined, because the regional mapping is not made on the RNO7. The present direct comparative mapping demonstrates that the chromosome segment of HSA12q24, RNO7q22, and MMU10C is conserved among these three species. Phenylalanine hydroxylase gene (*PAH*) belongs to a syntenic group homologous to HSA12q and MMU10 (Davisson *et al.*, 1991) that was mapped on RNO7 (Fulchignoni-Lataud *et al.*, 1990). Although the *PAH* was not regionally mapped on RNO7, its location is predicted to be RNO7q22 from the present study. However, other genes on RNO7 syntenic to mouse and human share MMU15, HSA8, and HSA22 (Levan *et al.*, 1991), indicating that the chromosomal segment contained homologous genes has dispersed to different chromosomes during the karyotypic evolution among these species.

Our result of the rat gene mapping is conflicting to that reported by Muramatsu *et al.* (1994) who mapped the rat *Ppp1cc* on the RNO12 by analyzing somatic cell hybrid DNAs with the PCR. They also explained that the rat *Ppp1cc* is homologous to a gene for phosphoserine phosphatase (*PSP*) which has been mapped on HSA7 and MMU5 (Lalley and Diaz, 1984; Danesino *et al.*, 1987). However, it should be mentioned that PP1 γ belongs to the protein serine/threonine phosphatase type 1 family that removes phosphate from serine and/or threonine of phosphoproteins (Ohkura *et al.*, 1989; Sasaki *et al.*, 1990; Barker *et al.*, 1993), whereas PSP fills an important role in the biosynthesis of serine from carbohydrates by catalyzing the last step, hydrolysis of *O*-phosphoserine (Moro-Furlani *et al.*, 1980). Therefore, these two enzymes belong to a quite different family that is encoded by each different gene.

In order to confirm our results, the same strategy as reported by Muramatsu *et al.* (1994) was chosen. As shown in Fig. 2, PCR products (140 bp) were detected in rat, and two rat $\times$ mouse hybrid clones, ts4(4)-C6 and ts1(3)2-A1, which contained RNO7 and several other rat chromosomes without RNO12, whereas no corresponding PCR products were detected in mouse and two other hybrid clones, NA-3-2 and T1A-3, which contained RNO12 and several other rat chromosomes without RNO7 (Table 1). Thus, these results show that the *Ppp1cc* was not located on RNO12 but located on RNO7, confirming the present FISH mapping.

It is of interest to note that chromosomal abnormalities of HSA12, RNO7, and MMU10 are detected in tumor cells (Sasaki, 1982; Wiener *et al.*, 1982; Barbieri

Fig. 2. PCR amplification products were separated by 4% agarose gel electrophoresis, stained with ethidium bromide, and photographed under UV light. In panel, lanes 1-4 represent, DNA size marker, no DNA, C3H mouse DNA, and WKAH rat DNA. Lanes 5 and 6 represent rat x mouse hybrid clones NA-3-2 and T1A-3, respectively, which contained RNO12 and several other rat chromosomes without RNO7. Lanes 7 and 8 represent hybrid clones ts4(4)-C6 and ts1(3)2-A1, respectively, which contained RNO7 and several other rat chromosomes without RNO12.

Table 1. Segregation pattern of the protein phosphatase 1 γ gene (*Ppp1cc*) and rat chromosomes in mouse x rat somatic cell hybrids.

Mouse×rat cell hybrid clone	Rat chromosome ^a														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
NA-3-2	—	—	—	—	—	—	—	—	—	—	—	+	—	—	—
T1A-3	—	—	—	—	—	—	—	—	—	—	—	+	—	—	—
ts4(4)-C6	+	+	+	—	+	+	+	—	+	—	+	—	+	—	+
ts1(3)2-A1	+	—	+	—	—	+	+	+	+	+	+	—	+	+	—

Mouse×rat cell hybrid clone	Rat chromosome ^a							PCR product ^b
	16	17	18	19	20	X	Y	
NA-3-2	—	—	—	+	—	—	—	—
T1A-3	—	—	—	—	+	—	—	—
ts4(4)-C6	+	—	—	+	+	—	—	+
ts1(3)2-A1	—	+	—	+	+	—	—	+

^a +, Presence of a rat chromosome in a hybrid clone; -, absence.

^b Presence (+) or absence (-) of a 140 bp PCR-product of protein phosphatase 1 γ .

et al., 1986; Huret *et al.*, 1988; Kwong *et al.*, 1994) and that the gene encoding PP1 γ catalytic subunit located near the fragile sites in human, rat, and mouse chromosomes (Djalali *et al.*, 1987; Robinson and Elder, 1987; Hecht, 1988), rising the possibility that *PPP1CC* may be related to malignancy.

Recently we have mapped the PP1 α gene on HSA11q13, RNO1q43, and MMU7E3-F2 (Saadat *et al.*, 1995) and the PP1 β gene on HSA2p23, RNO6q21-q23, and MMU12D (Saadat *et al.*, 1994). The present data showed that PP1 γ gene

located on HSA12q24, RNO7q22, and MMU10C, indicating that in spite of the high identity of PP1 isoforms, each isoform was encoded by different genes which located on different chromosomes in human, rat, and mouse.

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