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Brief Communication

Human Salivary Proline-Rich Protein Genes on Chromosome 12

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SUMMARY

A DNA probe (PRP1) for the proline-rich protein (PRP) genes was used to analyze the segregation of human *PRP* genes in human × mouse somatic cell hybrids. Endonuclease restriction analysis of 22 independent hybrid clones segregating human chromosomes demonstrated that *PRP* genes segregate with human chromosome 12 only and were therefore assigned to that chromosome. The PRP1 probe should prove useful for further mapping studies of human chromosome 12.

INTRODUCTION

The proline-rich proteins (PRPs) constitute about 70% of human salivary proteins and are unusual in their amino acid compositions, with proline comprising 25%–45% of the amino acid residues and proline, glycine, and glutamic acid (glutamine) constituting 70%–85% of the amino acid residues [1]. Some PRPs are probably important in normal tooth function: they bind avidly to hydroxyapatite, tooth enamel, and calcium. They also play a role in governing calcium concentrations in the saliva and in modulating calcium exchange at tooth sur-

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faces [1]. The PRPs may also have broader functions, since they were recently found in submucosal glands of the respiratory tract [2].

From extensive family and population studies of PRP polymorphisms, there are a number of closely linked genes for PRPs localized to a single autosome [3–8]. This gene family has been termed the SPC (salivary protein gene complex) [3]. Data from family linkage studies of PRP polymorphisms indicated that the *PRP* gene family might be localized to chromosome 6p, although the evidence was not conclusive [3, 7]. Chromosome 12 markers were not evaluated in these previous family linkage studies. To determine chromosomal localization of *PRP* genes, we have now used a direct molecular approach in which the endonuclease-digested DNAs of human × mouse somatic cell hybrids were Southern transferred and probed with a radiolabeled fragment from the coding region of a human PRP genomic clone. We present evidence for localization of PRP genes to human chromosome 12.

MATERIALS AND METHODS

Preparation of Probes

PRP1 probe. We previously screened a library of human genomic DNA fragments in bacteriophage lambda Charon 4A with a PRP cDNA synthesized and cloned from rat parotid gland mRNA [9]. Preliminary nucleotide sequence data of the human DNA from two of these clones (PRP1 and PRP2) include regions of nearly identical tandemly repeated sequences, each coding for about 21 amino acids [9]. The decoded consensus repeat sequence is homologous to the repeating amino acid units found by others in PRPs [10–12]. The two clones differ in several ways, indicating that they represent different members of the *PRP* gene family. We have prepared as a probe a 980-base pair (bp) *Hin*fI fragment from the coding region of the genomic clone PRP1 [9].

p12-16 probe. The p12-16 probe is a 1.1-kb cDNA (prepared from a HeLa cDNA library) that codes for a 12q restriction fragment length polymorphism (RFLP) [13]. The RFLP was mapped to chromosome 12 by using a panel of human × mouse somatic cell hybrids. In addition, by using in situ hybridization, the RFLP was sublocalized to the region 12q14–12q22, most probably 12q21 [13]. After Southern transfer of *Eco*RI-digested human DNAs, the probe detects two DNA fragments (9.7 and 8.5 kilobases [kb]) that are characteristic for the polymorphism. We used this probe as a confirmatory test to localize PRP genes to human chromosome 12.

Hybridization of Probes to Restricted DNAs

Each DNA sample (10 μg) was digested to completion with *Eco*RI, electrophoresed through a 0.8% agarose slab gel, and Southern transferred [14]. The probes were labeled with ^{32}P by nick-translation and hybridized to the filters as described [15].

Preparation and Characterization of Somatic Cell Hybrids

Human × mouse somatic cell hybrids. Human × mouse somatic cell hybrids segregating human chromosomes were formed by fusing human fibroblasts, leukocytes, or lymphoblastoid cells to either the rodent cell lines RAG ($HPRT^-$) or B82 (TK^-). Procedures for constructing and characterizing hybrid cell lines have been described [16, 17]. For these studies, a total of 22 independent cell hybrids derived from fusion experiments involving five unrelated human parental cells were utilized. Parallel cultures for each hybrid clone were used for chromosome, gene marker, and restriction analysis so that all the data were correlated.

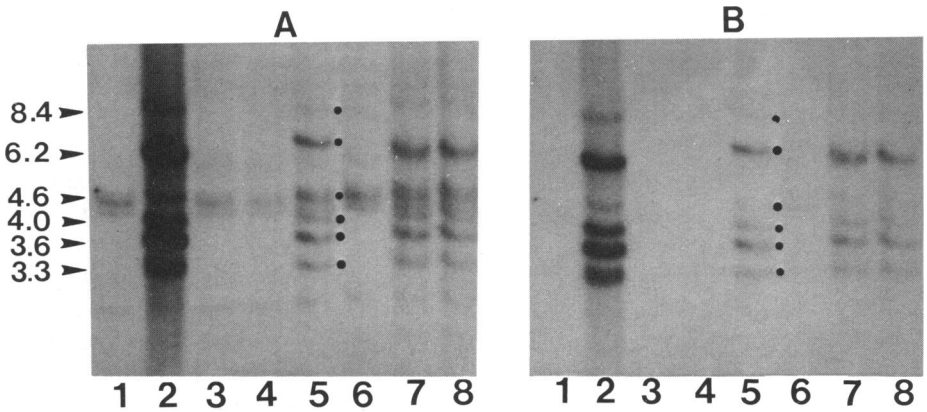

FIG. 1.—Hybridization of *Eco*RI restricted human, mouse, and somatic cell hybrid DNAs with ^{32}P-labeled PRP1. Molecular size in kb is shown *to the left*. *Blot A* is washed (68°C) with 3 × SSC, 0.5% SDS, and *blot B* is washed (68°C) with 0.3 × SSC, 0.5% SDS. *Sample 1*, total mouse DNA; *sample 2*, total human DNA; *samples 3–7*, DNAs from human × mouse somatic cell hybrids. *Hybrid samples 3, 4,* and *6* are negative, and *samples 5, 7,* and *8* are positive for human fragments hybridizing to PRP1. The major human bands are *spotted*.

RESULTS

Hybridization of DNA Fragments of Human × Mouse Somatic Cell Hybrids to the PRP1 Probe

Figure 1 shows hybridization of *Eco*RI-restricted DNAs of the human × mouse somatic cell hybrids, which were Southern transferred and probed with the radiolabeled PRP gene fragment (PRP1). The total human fibroblast DNA used in these studies (fig. 1*A*, sample 2) is not from cells employed in preparing the somatic cell hybrids. The digested total human DNA as well as the human DNA in the hybrids show no polymorphisms of the six major hybridizing bands [9]. Therefore, there is no confusion in comparing human hybridizing bands in total and hybrid DNA digests. After washing (68°C) in 3 × SSC with 0.5% SDS (3 × 0.15 M sodium chloride, 3 × 15 mM trisodium citrate, pH 7.0, with 0.5% sodium dodecyl sulfate), the total human DNA digest (fig. 1*A*, sample 2) shows six major hybridizing bands. These bands are 8.4, 6.2, 4.6, 4.0, 3.6, and 3.3 kb in size. There is a 2.5-kb weakly hybridizing band that overlaps a mouse band, but it does not persist after washing (68°C) at a higher stringency (0.3 × SSC with 0.5% SDS) (compare sample 2 in fig. 1*A* and *B*).

After washing (68°C) at a lower stringency (3 × SSC with 0.5% SDS), there is some overlap of mouse and human bands in the 4.4–5.0-kb and 8.1-kb regions (fig. 1*A*). However, after washing (68°C) at a higher stringency (0.3 × SSC with 0.5% SDS), the mouse hybridizing bands virtually disappear, but the six major human bands persist (fig. 1*B*). All the hybrid DNAs were studied under the above two conditions of washing. As shown in figure 1*A* and *B*, the human bands segregate coordinately in the hybrids and are either all present (samples 5, 7, and 8) or all absent (samples 3, 4, and 6). From these data, the human DNA fragments that hybridize must be on a single chromosome. The

finding of multiple human DNA fragments that hybridize to the PRP1 probe supports the presence of a multigene family.

PRP Genes Are Localized to Human Chromosome 12

The human × mouse somatic cell hybrids are characterized by a constant background of mouse chromosomes and random loss of the human chromosomes. When the 22 hybrids are scored for human chromosomal content (based on karyotype and enzymatic analysis) and hybridization of their human DNA fragments to the PRP1 probe, the only chromosome that segregates concordantly with the *PRP* genes is chromosome 12 (table 1). All other chromosomes show multiple discordancies varying from 23%–64%. From these results, *PRP* genes are localized to chromosome 12. Since five different human parental cells from unrelated individuals were used in constructing the hybrids, spurious results from an unrecognized chromosome rearrangement involving human chromosome 12 is extremely unlikely. The hybrid clone REW5 gives much lower activity for human peptidase B (a chromosome 12 marker) than the other clones and also shows the weakest signal with the PRP1 probe (not shown in fig. 1). This correspondence of peptidase B enzyme activity with the PRP1

TABLE 1

A Comparative Analysis of Human × Mouse Somatic Cell Hybrids for Human Chromosome Content and Hybridization of Human DNA Fragments with the PRP1 Probe

Chromosome	+/+	+/−	−/+	−/−	% discordancies
1	10	3	6	3	41
2	9	4	6	3	45
3	10	3	4	5	32
4	7	6	2	7	36
5	8	5	4	5	41
6	10	3	3	6	27
7	8	1	5	1	40
8	4	2	5	0	63
9	5	5	3	3	50
10	12	1	4	5	23
11	10	3	6	3	41
12	13	0	0	9	0
13	11	2	7	2	41
14	12	1	9	0	45
15	10	3	5	4	36
16	4	8	3	5	55
17	10	0	7	0	41
18	11	2	7	2	41
19	9	4	3	6	32
20	4	9	2	7	50
21	10	2	8	1	48
22	0	7	0	4	64
X	11	2	6	3	36

Note: The +/+ and −/− categories show concordancies, whereas +/− and −/+ categories show discordancies. The result of the PRP1 probe is shown first; thus +/− indicates hybridization with the PRP1 probe but the absence of a particular chromosome.

signal intensity further substantiates localization of *PRP* genes to chromosome 12.

We confirmed localization of *PRP* genes to human chromosome 12 by probing *Eco*RI-digested and Southern-transferred DNAs of 14 of the above hybrids with a ^{32}P-labeled 1.1-kb cDNA(p12-16) that codes for a 12q RFLP [13]. There is a 100% concordance of the PRP1 and p12-16 probe results ($P < .001$).

DISCUSSION

From our previous data, the PRP1 probe used in this study hybridizes to genomic DNA fragments that code for *PRP* genes, although pseudogenes cannot be completely excluded until complete DNA sequencing is done [9]. The genomic clone PRP1 (which is the source of the PRP1 probe) as well as another genomic clone, PRP2, show features that are expected for *PRP* genes [9]: they code for characteristic 21 amino acid repetitive units whose decoded amino acid sequences closely resemble the repetitive amino acid sequences previously reported for several known human PRPs [10–12]. Also, using the PRP1 probe, we screened a human cDNA library prepared from mRNA of parotid salivary gland origin. The probe strongly hybridized to many cDNAs, and one of these cDNAs apparently codes for a known acidic PRP: protein C ([10] and N. Maeda, E. A. Azen, and O. Smithies, unpublished data, 1984).

It is possible that our probe, PRP1, does not detect all of the human *PRP* genes; however, from our somatic cell hybrid data, the *PRP* genes detectable by hybridization to the PRP1 probe are localized to human chromosome 12 without apparent dispersal to other chromosomes. This result agrees with previous linkage data (derived from family studies of proline-rich protein genetic polymorphisms) indicating that *PRP* genes are linked on a single autosome. The mouse *PRP* gene complex is also localized to a single chromosome (chromosome 8) [18]. The reason for this apparent evolutionary conservation of a gene family on a single chromosome is unknown. Some gene families such as the globin [19] and immunoglobulin [20] gene families are dispersed among the chromosomes, whereas others, such as the genes for the major urinary proteins of the mouse [21] and the PRP proteins, appear to be localized to a single chromosome.

The mouse *PRP* genes are located on chromosome 8 [18]. Therefore, it might be expected from previous studies of "homology mapping" (where certain linkage groups are conserved during evolution [22–24] that human *PRP* genes of man might be located on either chromosome 8 or 16 [17, 25]. Thus, *Gr-1, Got-2, Prt-2,* and *Aprt* are found on mouse chromosome 8 with a homolog for *Gr-1* on human chromosome 8 and homologs for *Got-2, Prt-2,* and *Aprt* on human chromosome 16. Although groups of closely linked genes tend to be conserved [25], homology mapping is not accurate over long genetic distances.

The PRP1 gene probe, which detects polymorphic differences in digested human DNAs, should prove useful for genetic linkage studies with other human chromosome 12 markers [26]. In a preliminary study of DNAs from 24 human subjects, we observed frequent polymorphic differences (seen as doublets cor-

responding to several of the bands) after probing Southern-transferred digests of total DNA with the PRP1 probe [9]. It follows that the proline-rich protein polymorphisms will also be useful chromosome 12 markers because of their extensive genetic variations.

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