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The Pronatriodilatin Gene Is Located on the Distal Short Arm of Human Chromosome 1 and on Mouse Chromosome 4

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SUMMARY

Atrial natriuretic factors (ANF) are polypeptides having natriuretic, diuretic, and smooth muscle-relaxing activities that are synthesized from a single larger precursor: pronatriodilatin. Chromosomal assignment of the gene coding for human pronatriodilatin was accomplished by in situ hybridization of a [^{3}H]-labeled pronatriodilatin probe to human chromosome preparations and by Southern blot analysis of somatic cell hybrid DNAs with normal and rearranged chromosomes 1. The human pronatriodilatin gene was mapped to the distal short arm of chromosome 1, in band 1p36. Southern blot analysis of mouse × Chinese hamster somatic cell hybrids was used to assign the mouse pronatriodilatin gene to chromosome 4. This assignment adds another locus to the conserved syntenic group of homologous genes located on the distal half of the short arm of human chromosome 1 and on mouse chromosome 4.

INTRODUCTION

The atrium of the heart contains substances that are important in regulating extracellular fluid volume [1]. These substances increase the excretion of sodium from the kidney, most probably by increasing glomerular filtration rate [1, 2]. In addition to their natriuretic and diuretic effects on the kidney, they are

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able to relax precontracted vascular smooth muscle [3], raising the possibility that they have a role in regulating blood pressure as well [2].

These substances have been found to be a mixture of multiple peptides ranging from 3,000 to 13,000 mol. wt. [4], variously named atrial natriuretic factor (ANF) [5], cardionatrin [6], atriopeptins [7], and auriculins [4]. These atrial peptides from rat [4–10] and humans [11] have recently been purified and characterized. A high degree of amino acid sequence homology was found among the peptides. The sequence analyses of precursor cDNAs of rat and human atrial peptides suggested a common precursor, pronatriodilatin (PND), for the peptides [12–17]. The sequences, both cDNA and amino acid, appear to be unique.

Although multiple forms of ANFs are produced, there is strong evidence for the existence of a single gene, which is highly conserved, in human [12, 17], mouse, and rat genomes [17, 18]. The human [19, 20] and rat [18] PND genes have recently been isolated and sequenced. The human PND gene consists of three exons (~200 base pairs [bp], 327 bp, and 302 bp, respectively) and spans about 2 kilobase pairs (kb), including two introns (122 bp and ~1,050 bp, respectively) [19, 20]. We now report the chromosomal assignment of the PND gene in the human and the murine species. In situ hybridization of a radioactive PND-specific sequence to human chromosome preparations indicates that the PND gene is located at the distal short arm of chromosome 1, band 1p36. Southern blot analysis of human × Chinese hamster somatic cell hybrid DNAs with the PND probe has confirmed this assignment. The assignment of the murine PND gene to chromosome 4 was made by Southern blot analysis of DNA from a panel of mouse × Chinese hamster somatic cell hybrids.

MATERIALS AND METHODS

In Situ Hybridization

Human metaphase and prometaphase chromosomes were prepared from methotrexate-synchronized peripheral lymphocyte cultures of two normal individuals [21]. The recombinant plasmid M13 Mp9 · 10 *Xho* used in the hybridization contained a 1-kb genomic *Bam*HI – *Xho*II fragment that included about 500 bp of 5′ sequences, the first exon, and most of the second exon [19]. The probe was [^{3}H]-labeled to a specific activity of 2.7 × 10^7 cpm/μg by nick-translation using [^{3}H]dCTP, [^{3}H]dATP, and [^{3}H]dTTP.

In situ hybridization was carried out according to the method of Harper and Saunders [22]. Chromosome preparations were treated with 100 μg/ml RNAase, 2 × SSC (0.3 M NaCl-0.03 M sodium citrate) for 1 hr at 37°C, denatured in 70% formamide, 2 × SSC at 70°C for 2 min, and followed by dehydration in ethanol. The probe mixture containing 10% dextran sulfate, 50% formamide, and a 250-fold excess of sonicated salmon sperm DNA as carrier was denatured at 70°C for 5 min and hybridized to the chromosome preparations at probe concentrations of 25 ng/ml and 50 ng/ml for 16 hrs at 37°C. Slides were rinsed well in three changes of 50% formamide, 2 × SSC and five changes of 2 × SSC at 39°C, followed by dehydration in ethanol. The slides were coated with Kodak NTB-2 emulsion, exposed for 10–14 days at 4°C, and developed in Kodak Dektol developer.

Chromosomes were stained with quinacrine mustard dihydrochloride and photographed under a fluorescence microscope. The chromosomes were then G-banded using Wright's stain, and a second photograph was taken of the previously selected cells. G-banded chromosomes were analyzed for silver-grain localization.

Hybrid Cell Lines

Somatic cell hybrids of series XII were generated by fusing leukocytes from a male donor with V79/380-6 Chinese hamster lung fibroblasts that lack the enzyme hypoxanthine phosphoribosyltransferase [23]. Series XXI hybrids were derived from a fusion between human donor cells, KG7, and Chinese hamster cells, Don/a-23 [24]. Series XV hybrids were derived from fusion of human fibroblasts, TH5, with V79/380-6 Chinese hamster cells [25]. Characterization of the chromosomal content and presence of isoenzymes in all hybrids has been described [25]. Hybrids of series I were generated by fusion of fibroblasts derived from a male mouse with Searle's t(X;16)16H translocation with V79/380-6 Chinese hamster lung fibroblasts [26]. The EAS, EBS, and EZS series of hybrids were produced in Dr. J. Minna's laboratory, National Cancer Institute, by fusing spleen cells from AKR(EAS), BALB/C(EBS), and NZB(EZS) mice, respectively, to Chinese hamster cell line E36. The hybrids were characterized as described [27]. When hybrids were expanded in cell culture for the purpose of preparing DNA extracts, their chromosome constitution was reanalyzed at the same time.

DNA Isolation and Southern Blotting

High molecular weight DNA was isolated from Chinese hamster V79/380-6 cells, human lymphoblastoid cells, mouse 3T3 cells, and somatic cell hybrids as described by de Martinville et al. [28] or Bass et al. [29]. Complete enzymatic digestion of 10 μg of genomic DNA by *Bam*HI or *Eco*RI was performed as recommended by the manufacturer (International Biotechnologies, New Haven, Conn.) using a four- to sevenfold excess of enzyme. DNA fragments were separated by electrophoresis on 0.8% agarose gels and transferred to nitrocellulose filters (Schleicher and Schuell, Keene, N.H., BA85) according to Southern [30].

The recombinant plasmid (M13 Mp9 · 10 *Xho*) described above for in situ hybridization was used to map the human PND gene and was labeled by nick-translation in the presence of [^{32}P]dCTP to a specific activity of 3.5×10^8 cpm/μg. Prehybridization at 67°C for 6 hrs was in 5 × SSC, 5 × Denhardt's solution, 50 mM sodium phosphate, pH 6.5, 100 μg/ml sheared salmon sperm DNA, 100 μg/ml yeast RNA, and 1% glycine. Hybridization (3×10^7 cpm/12 ml) was at 67°C for 18 hrs in 5 × SSC, 2 × Denhardt's solution, 20 mM sodium phosphate, pH 6.5, 100 μg/ml sheared salmon sperm DNA, 100 μg/ml yeast RNA, and 7% dextran sulfate. The filter was washed four times in 2 × SSC, 0.1% SDS, and four times in 0.1 × SSC, 0.1% SDS at 67°C. Autoradiography using Kodak X-Omat AR film was done in the presence of a Dupont Cronex intensifying screen at −70°C.

The probe, pANF-44, used to assign the mouse PND gene, was constructed from a PND cDNA of 600 bp isolated from a rat atrial cDNA library and cloned into the *Pst*I site of pUC8 [13]. The cDNA insert was excised by *Pst*I cleavage, purified, and labeled in the presence of [^{32}P]dCTP to a specific activity of 2×10^8 cpm/μg [31]. Prehybridization at 42°C was for 6 hrs in 50% formamide, 5 × SSC, 5 × Denhardt's solution, 100 μg/ml denatured salmon sperm DNA. Hybridization at 42°C was for 18 hrs in 50% formamide, 5 × SSC, 1 × Denhardt's solution, 100 μg/ml denatured salmon sperm DNA, 7% dextran sulfate, and labeled pANF-44 (2×10^7 cpm/12 ml). The filter was rinsed twice in 2 × SSC at 25°C, then washed twice in 1 × SSC, 1% SDS, and twice in 0.1 × SSC, 0.1% SDS at 65°C for 30 min each.

RESULTS

PND Mapping in the Human

In situ hybridization of [^{3}H]-labeled PND probe to human chromosomes resulted in specific labeling at the distal short arm of chromosome 1, at band 1p36 (fig. 1). Fifty-six out of 157 metaphase cells (36%) exhibited silver grains

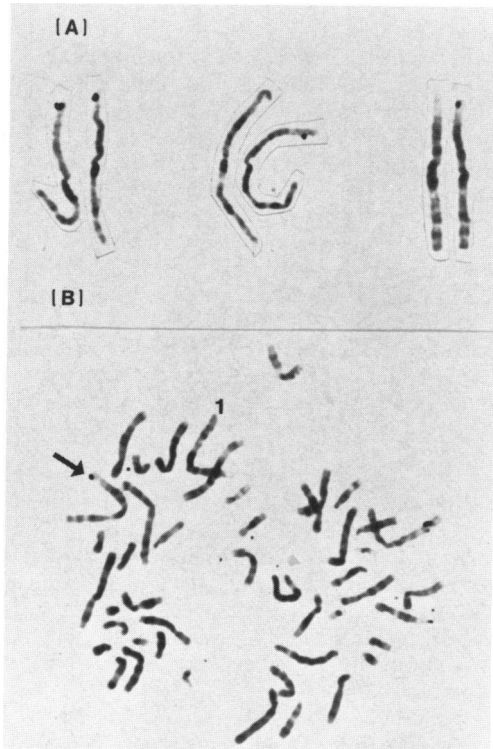

FIG. 1.—G-banded human chromosomes after in situ hybridization with [^{3}H]-labeled human PND probe. *A*, Partial karyotypes of three cells, illustrating typical labeling at the distal short arm (band p36) of one or both chromosomes 1. *B*, Representative normal male metaphase spread with a silver grain situated on the 1p36 region (*arrow*), illustrating low background label.

on a specific region of one or both chromosomes 1. Of 111 grains observed on chromosome 1, 70 (63%) were located on band p36, with a peak in subband 1p36.2 (fig. 2). Furthermore, grains over this region (1p36) represented 15% (70/468) of all chromosomal label and no other site was labeled above background (fig. 3).

Southern blot analysis of DNA from seven human × Chinese hamster somatic cell hybrids was used to confirm the chromosomal assignment made by in situ hybridization to metaphase chromosomes. An autoradiogram obtained following hybridization of *Bam*HI-cleaved genomic DNA to the [^{32}P]-labeled human PND probe is shown in figure 4. In the human control DNA (lane A), hybridization to a single fragment of 3.6 kb was observed. Weak hybridization to an 8-kb fragment was seen in Chinese hamster DNA and in Chinese hamster-derived somatic cell hybrids. The 3.6-kb fragment was present in hybrids containing a human chromosome 1, but not in hybrids that lack chromosome 1.

The chromosome contents and discordancy analysis of this panel are summarized in table 1. In this table, + or − under PND indicates the presence or absence of hybridization to the human specific 3.6-kb *Bam*HI fragment. The

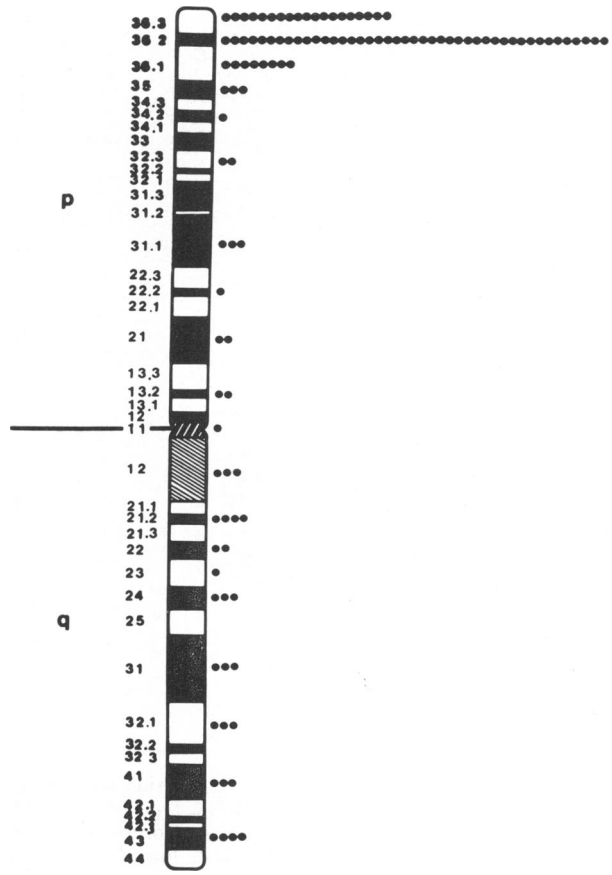

FIG. 2.—Silver-grain distribution along chromosome 1 (ideogram from ISCN 1981 [32]). Of 111 grains on chromosome 1, 70 (63%) were located at 1p36.

presence (+) or absence (−) of each human chromosome in the hybrids is below the chromosome number. Informative hybrids in which concordant segregation was observed (i.e., the human-specific PND gene was present together with each human chromosome [+/+] or absent together [−/−]) and in which discordant segregation was observed (i.e., the human-specific PND gene was present while the chromosome was absent [+/−] or the human PND gene was absent while the chromosome was present [−/+]) are indicated below each chromosome. No discordancies were found for chromosome 1, whereas all other chromosomes were excluded by at least one discordancy. Assignment of the human PND gene to chromosome 1 was therefore confirmed.

Regional localization to the short arm of chromosome 1 was also confirmed by Southern blot analysis. Somatic cell hybrids of series XV were generated by fusion of human cells containing a balanced reciprocal translocation between the short arms of chromosomes 1 and 6, t(1;6)(p32;p21) [25]. In the hybrid subclone, XV-18A-10b-D4 aza, the derivative chromosome 1, containing

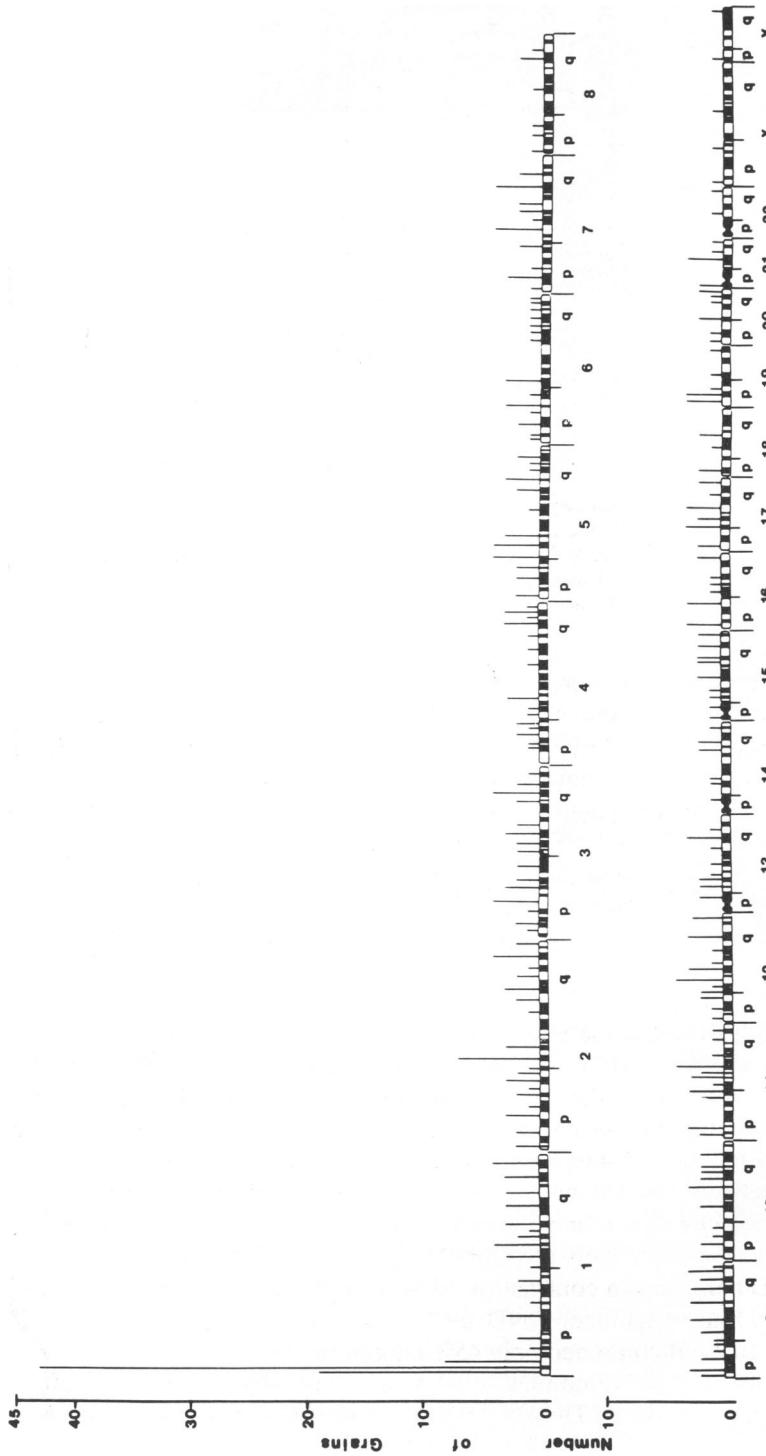

FIG. 3.—Histogram showing the distribution of silver grains over the human chromosome complement. Of 468 grains observed in 157 metaphase cells, 70 (15%) were located on 1p36.

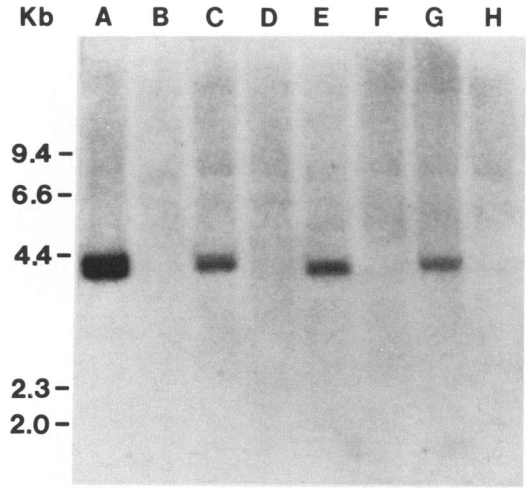

Fig. 4.—Hybridization of human PND probe (M13-Mp9 · 10 *Xho*) to *Bam*HI-digested DNA from human × Chinese hamster somatic cell hybrids. *Lane A*, human diploid cells; *lane H;* Chinese hamster cells; *lanes B–G*, human × Chinese hamster hybrids containing a normal chromosome 1 (*E, G*), no human chromosome 1 material (*D, F*), the long arm and proximal short arm of human chromosome 1 (1p32→1qter) (*B*), and the distal short arm of chromosome 1 (1pter→1p32) (*C*).

1p32→1qter and 6p21→6pter, was present, while the normal chromosome 1 and the derivative chromosome 6 were absent. Likewise, the hybrid clone XV-16B-F4 aza retained the derivative chromosome 6 containing 1p32→1pter and 6p21→6qter, while the normal chromosome 1 and the derivative chromosome 1 were absent. As shown in figure 4, no hybridization was seen to DNA extracted from the clone XV-18A-10b-D4 aza (lane B), whereas hybridization to DNA from clone XV-16B-F4 aza (lane C) was clearly present. The gene coding for the atrial natriuretic factor was therefore localized to region 1p32→1pter, in good agreement with the localization determined by in situ hybridization.

PND Mapping in the Mouse

Assignment of the murine PND gene to chromosome 4 was made by Southern blot analysis of mouse × Chinese hamster somatic cell hybrids. Hybridization of pANF-44 to a 6.7-kb *Eco*RI fragment in mouse DNA and to a 16-kb *Eco*RI fragment in Chinese hamster DNA was observed. When pANF-44 was hybridized to mouse × Chinese hamster somatic cell hybrid DNAs, the 16-kb Chinese hamster fragment was observed in all hybrids whereas the mouse specific fragment was seen only in those hybrids that contained mouse chromosome 4 (fig. 5). The relatively strong hybridization of pANF-44 to the Chinese hamster PND gene was in contrast to the weak hybridization of pM13Mp9 · 10 *Xho* to the Chinese hamster PND gene. The human genomic DNA probe (pM13Mp9 · 10 *Xho*) contained only 45% exonic sequences and did not include the most conserved ANF-coding sequences. It was therefore not expected to hybridize as strongly to DNA from other species such as Chinese hamster [18, 33].

TABLE 1

DISCORDANCY ANALYSIS OF HUMAN × CHINESE HAMSTER HYBRIDS USING M13MP9 · 10 *Xho* (PND) AS A PROBE

		HUMAN CHROMOSOMES																							
HYBRID	PND	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	Y
XV-18A-10b-D4 aza	−	R	+	+	+	+	R	−	+	+	−	+	+	+	+	+	+	−	−	+	+	+	+	−	+
XV-16B-F4 aza	+	R	+	+	−	+	+	−	+	−	−	−	−	+	+	−	+	−	+	+	+	+	+	−	+
XXI-23A-2c	+	+	−	−	−	−	−	R	+	−	−	R	−	−	+	−	−	+	−	+	−	R	+	+	−
XXI-51B	−	−	−	+	+	+	−	+	+	+	+	+	−	−	+	+	+	+	+	+	+	−	+	R	+
XII-2D-1f HAT	+	+	+	+	+	−	+	−	+	+	−	−	+	+	+	+	+	−	+	+	−	+	+	R	−
XV-18B-7a-N4	+	+	+	+	R	+	R	−	+	+	−	R	+	−	+	+	+	+	+	+	+	+	+	+	+
XXI-22A-c-2c	−	−	−	+	−	+	−	−	+	−	−	R	−	+	+	+	+	+	−	−	L	+	+	+	−
	PND/Chr																								
Concordant	+/+	3	3	3	1	2	2	0	4	2	0	0	2	2	4	2	3	2	3	4	2	3	4	2	2
	−/−	2	2	0	1	0	2	2	0	1	2	0	2	1	0	0	0	1	2	1	0	1	0	1	1
Discordant	+/−	0	1	1	2	2	1	3	0	2	4	2	2	2	0	2	1	2	1	0	2	0	0	1	2
	−/+	0	1	3	2	3	0	1	3	2	1	2	1	2	3	3	3	2	1	2	2	2	3	1	2
No. discordant clones		0	2	4	4	5	1	4	3	4	5	4	3	4	3	5	4	4	2	2	4	2	3	2	4
No. informative clones*		5	7	7	6	7	5	6	7	7	7	4	7	7	7	7	7	7	7	7	6	6	7	5	7
Percent discordant		0	28	57	67	71	20	67	43	57	71	100	43	57	43	71	57	57	28	28	67	33	43	40	57

NOTE: L = frequency of .1 or less; R = chromosome involved in rearrangement.

* Data for chromosomes involved in rearrangements or present at a frequency of .1 or less were excluded.

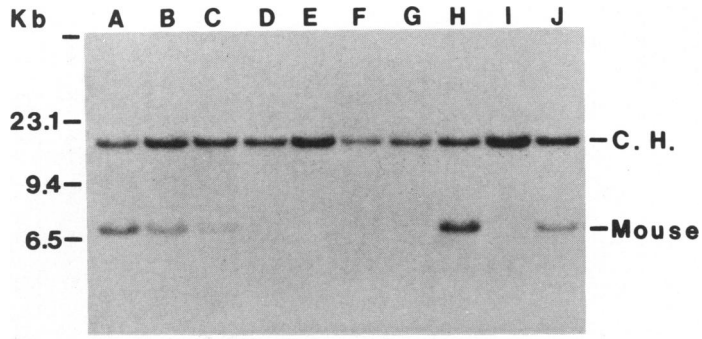

FIG. 5.—Hybridization of a rat PND cDNA probe (pANF-44) to *Eco*RI-digested DNA from mouse × Chinese hamster somatic cell hybrids. Chinese hamster (C.H.) and mouse hybridization bands are indicated. *Lane A*, I-13A-2a aza; *lane B*, I-18A; *lane C*, I-18A-2a aza; *lane D*, EBS 51; *lane E*, EAS2; *lane F*, EBS3; *lane G*, EBS10; *lane H*, EBS58; *lane I*, EBS82; *lane J*, EZS25.

The chromosome contents and the results of hybridization with the PND probe in 15 mouse × Chinese hamster somatic cell hybrids are summarized in table 2. The mouse PND gene cosegregated with the mouse chromosome 4 whereas all other chromosomes were excluded by two or more discordant hybrids. Weak hybridization to the mouse-specific fragment in hybrid clone I-18A-2a aza (fig. 5, lane C) correlates with a low frequency (.1) of chromosome 4 in this hybrid. The murine PND gene was therefore assigned to chromosome 4.

DISCUSSION

The results of in situ hybridization of [^{3}H]-labeled PND probe to human chromosome preparations allowed us to map the human pronatriodilatin gene to chromosome 1, band 1p36. Southern blot analysis of DNAs from somatic cell hybrids with normal and rearranged chromosomes 1 provided independent confirmation by assigning *PND* to the region 1p32→pter.

The chromosomal region 1p36 on which the PND gene is located is part of a known segment with genes that are conserved between human and mouse [34]. The *PGD, GDH, EN01, FUCA1, AK2,* and *PGM1* loci on human chromosome 1 (p22.1→pter) are functionally and evolutionarily related to loci *Pgd, Gpd-1, Eno-1, Fuca, Ak-2,* and *Pgm-2* on mouse chromosome 4 [34]. DNA analysis has already demonstrated that the PND gene is highly conserved among human, mouse, and rat [18, 19, 33]. Assignment of the murine *Pnd* locus to chromosome 4 adds a seventh locus to this conserved syntenic group.

Human and rodent ANFs are thought to play an important role in the regulation of extracellular fluid volume and blood pressure [2, 3]. The recent findings of ANF deficiency in BIO 14.6 hamsters with hereditary congestive heart failure [35] and in spontaneous hypertensive rats [36] is consistent with a role of ANF in cardiovascular regulation. The precise localization of *PND* should prove valuable in studying the genetic epidemiology of hypertension-related cardiovascular diseases. Essential hypertension is thought to be a multifactorial disorder, polygenically inherited, and environmentally influenced [37]. Mu-

TABLE 2

SOUTHERN BLOT ANALYSIS OF MOUSE × CHINESE HAMSTER SOMATIC CELL HYBRIDS USING pANF-44 AS A PROBE

HYBRID	PND	CHROMOSOME 4 FREQUENCY*	MOUSE CHROMOSOMES																				
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	X	Y
I-3-2D-1d aza	+	0.4	+	+	−	+	−	+	+	+	+	+	−	+	L	+	+	R	+	+	+	R	−
I-4A1-2a aza	+	0.9	+	+	+	+	+	+	+	+	+	−	−	+	+	+	+	+	+	+	+	+	+
I-10C	+	0.7	+	+	+	+	−	+	+	+	−	+	L	+	−	+	+	+	+	+	+	+	−
I-13A-1a aza	+	0.7	+	+	+	+	−	+	+	+	+	−	−	+	+	+	+	+	+	+	+	R	+
I-13A-2a aza	+	0.9	+	+	+	+	L	+	+	+	−	−	−	+	+	+	+	−	+	+	+	−	−
I-18A	+	0.6	+	+	−	+	−	+	−	−	−	−	−	+	−	−	−	+	+	L	+	+	+
I-18A-2a aza	+	0.1	+	+	−	L	+	L	−	−	−	−	−	+	−	−	−	−	+	L	+	−	+
EAS2	−	0	L	−	−	−	R	+	−	−	+	−	−	+	−	−	+	+	+	−	L	+	
EBS2 GTG	−	0	L	L	+	−	−	−	+	+	−	+	−	+	+	−	+	−	+	−	+	−	
EBS3	−	0	−	+	−	−	−	−	+	L	−	−	−	+	L	−	+	L	+	−	+	−	
EBS10	−	0	+	+	+	−	−	L	+	−	−	+	−	+	+	L	+	+	−	+	−	+	−
EBS51	−	0	−	+	+	−	−	+	+	+	+	−	−	+	+	−	+	L	+	+	+	+	−
EBS58	+	0.8	+	+	+	+	−	+	+	−	−	−	−	+	+	−	+	−	+	−	+	+	−
EBS82	−	0	−	+	−	−	−	−	+	−	+	−	−	+	−	−	+	−	+	R	+	+	−
EZS25	+	0.6	−	+	−	+	−	−	+	+	−	−	−	+	+	−	+	+	+	L	+	+	−
	PND/Chr																						
Concordant	+/+		8	9	5	8	2	7	7	6	3	2	0	9	5	5	7	5	9	5	9	5	3
	−/−		3	1	3	6	5	3	1	3	3	4	6	0	2	5	0	2	1	3	1	2	3
Discordant	+/−		1	0	4	0	6	1	2	3	6	7	8	0	3	4	2	3	0	1	0	2	5
	−/+		1	4	3	0	0	2	5	2	3	2	0	6	3	0	6	2	5	2	4	4	0
No. discordant clones			2	4	7	0	6	3	7	5	9	9	8	6	6	4	8	5	5	3	4	6	5
No. informative clones†			13	14	15	14	13	13	15	14	15	15	14	15	13	14	15	12	15	11	14	13	11
Percent discordant			15	29	47	0	46	23	47	36	60	60	57	40	46	29	53	42	33	27	29	46	45

NOTE: L = frequency of .1 or less; R = chromosome involved in rearrangement.
* Average copy no./cell.
† L and R data were excluded.

tations in one of these genes (such as *PND*) could influence mechanisms of homeostasis. The cluster of genes for small nuclear U1 RNA, *ENO*, and *PGD* also mapped to 1p36 [38, 39] are known to be polymorphic [40–42] and could be used in linkage studies of families with essential hypertension because of their physical proximity to the PND gene. In addition, a two-allele restriction fragment length polymorphism has been discovered with the human PND probe (M. N. and J. D., unpublished data, 1985). Knowledge of the genetic map surrounding the PND gene may prove useful in detecting individuals with genetic predisposition to hypertension secondary to a mutation in the PND gene. Furthermore, knowledge of the chromosomal position of the homologous locus *Pnd* in the mouse will facilitate the identification of mouse mutants. Such animal models will be valuable for experimental studies of hypertension related disorders.

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