Assignment of the human tyrosine hydroxylase gene to chromosome 11

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Using a panel of human–mouse somatic cell hydrids and a cDNA probe for human tyrosine hydroxylase, we have assigned the structural gene for tyrosine hydroxylase to chromosome 11 by Southern blotting techniques.

Tyrosine hydroxylase *Chromosome* *Somatic cell* *Gene family*

1. INTRODUCTION

Tyrosine hydroxylase (EC 1.14.16.2, tyrosine 3-monooxygenase, TH) is the rate-limiting enzyme in the synthesis of the neurotransmitter dopamine, and is expressed in a few discrete neuronal systems in the brain. It has been intensively investigated because of the key role it plays in the physiology of adrenergic neurons and we are studying the regulation of TH gene expression using molecular biological technique [1,2]. As a complement to these studies of TH, we have assigned the human gene for this enzyme to chromosome 11 using Southern blotting techniques and a series of human-mouse hybrid cell lines.

2. MATERIALS AND METHODS

2.1. *Cell hybrids*

A panel of previously characterised human-mouse hybrid cell lines, having between them all or most human chromosomes, were used to follow the segregation of the human TH DNA fragment; 3 further hybrids with fewer human chromosomes were selected to confirm the assignment of the structural gene. The origin and karyotype of these hybrids are shown in tables 1 and 2. The karyotypic data was obtained by Giemsa 11 and trypsin banding of metaphase preparations. Some hybrid cell extracts were also analysed electrophoretically for the presence of various human enzymes using published procedures [3].

2.2. *Filter hybridisation*

The human tyrosine hydroxylase gene DNA fragment was detected by a filter hybridisation technique [4]. DNA isolation, restriction enzyme digestion, agarose gel electrophoresis and filter hybridisation were as described previously [4–6].

2.3. *Human tyrosine hydroxylase cDNA probe*

Northern blot analysis of human pheochromocytoma mRNA with the rat TH cDNA probe revealed a single 1.9 kb and identical to that found with rat adrenal mRNA [2]. The human TH cDNA probe was therefore isolated from a cDNA library made from this pheochromocytoma by cross-hybridisation with the rat TH probe at low stringency. Two clones were isolated from a library of 6000 colonies, and these clones had similar restriction patterns. The probe used in this study was 800 base pairs in length, and the sequence was 95% homologous to that of rat tyrosine hydroxylase (in preparation).

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Table 1

Karyotype and origin of human–mouse hybrids used in this study

Hybrid cell line	Human chromosomes																							Origin	Human TH DNA fragment
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X		
412-Z-12		+		+				(+)					(+)			(+)	(+)				+	+		(7)	–
DTP7.5		+		+								+	+	+	+		+			+	+		+	(8)	–
DUR5R					+					+	+	+	+	+			+	+		+	+	+		(9)	+ + +
LSR39	+	(+)	+	(+)	+	+	+	(+)	+	+	+	+	+	+	+	+	(+)	(+)	+		(+)	+	+	(10)	+ +
LSR40		+	+	(+)	+		(+)	+		+	+		(+)	(+)	+	+		+	(+)		+	+	+	(10)	+ +
LSR16									(+)	+	+	+	+	(+)	+					(+)			+	(10)	+
LSR18		+	+	+	+	+		(+)	(+)	+	+			(+)		+		+	+	+	+	+	+	(10)	+

Table 2

Karyotype and origin of human–mouse hybrids used in this study

Hybrid cell line	Human chromosomes																							Origin	Human TH DNA fragment
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X		
4W10								+			+										+	+	+	(11)	+/–
3W4							+			+	+	+		+	+		+				+		+	(11)	+
1W1LA4											+											+	+	(12)	+

3. RESULTS

The human TH probe can be used to detect human tyrosine hydroxylase DNA fragments after electrophoresis and transfer to nitrocellulose filters and hybridisation under stringent conditions. A fragment of 2.5 kb and sometimes a partial restriction enzyme fragment of 3.2 kb are found after digestion with Bam H1, while under the same conditions the mouse TH gene is not recognised (fig.1a); indeed the mouse Bam H1 restricted DNA fragments recognised by the rat TH cDNA clone have sizes of 2 and 10 kb. We have therefore used this probe to examine a panel of human-mouse somatic cell hybrids which contain between them most human chromosomes. From this initial screen (table 1, fig.1b) it appeared that the structural gene was on chromosome 10 or 11. In order to assign the gene unambiguously, a second series of hybrids having fewer human chromosomes was chosen which would distinguish between these chromosomes. These 3 hybrids were also analysed electrophoretically for the presence of human marker enzymes for chromosomes of interest.

The only human chromosomes present by karyotypic analysis in all of the second panel of hybrids were chromosomes 11 and X (table 2). This was confirmed by the presence of human lactate dehydrogenase A in extracts of all hybrids (fig.2); lactate dehydrogenase A is coded for by a gene on the short arm of chromosome 11 [13]. Furthermore, the retention of human chromosomes 11 and X in hybrid 1W1-LA4 was confirmed by karyotypic analysis of a contemporary culture. Hybridisation in situ with labelled total human genomic DNA indicated the absence of any critic human chromosome material in this hybrid. All the hybrids in the second panel also had the human TH DNA fragment. As the presence of the TH DNA fragment and the X chromosome were not associated in the first series of hybrids the data are consistent with the assignment of the tyrosine hydroxylase gene to chromosome 11.

4. DISCUSSION

In this paper we describe the localisation of the

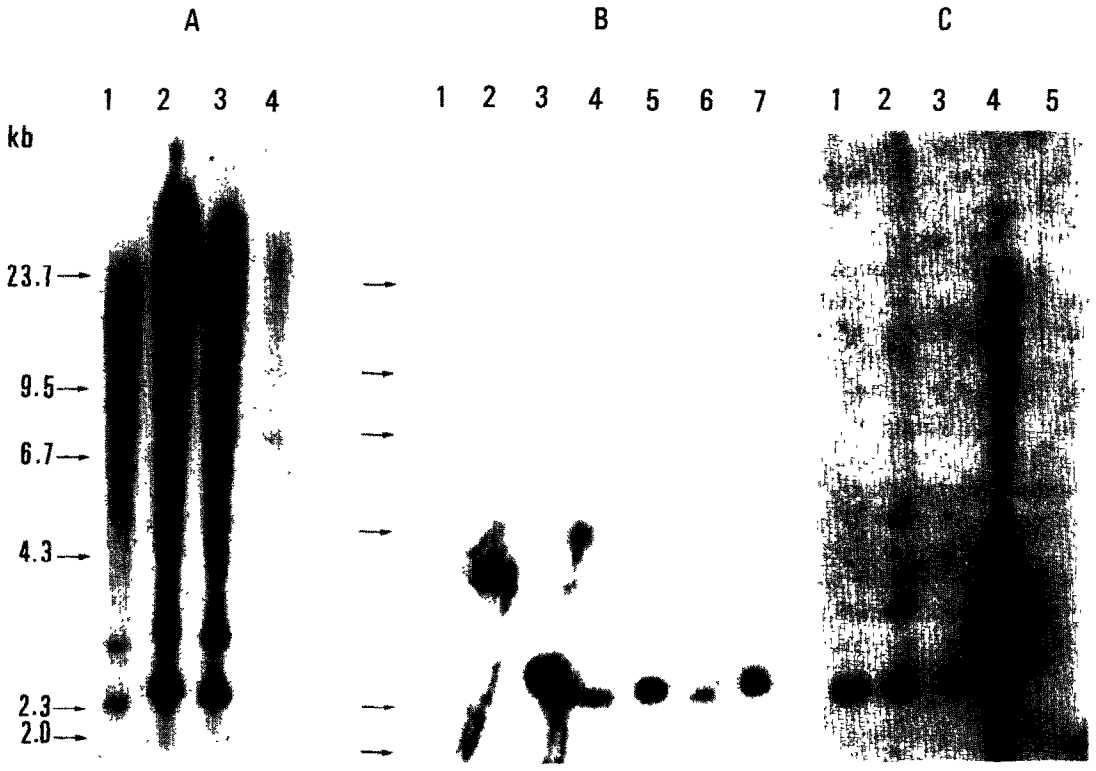

Fig.1. Southern blot analysis of human-mouse hybrids. Digested DNAs were electrophoresed on a 0.8% agarose slab gel. Hybridisation and washing procedures were as described [4,5] except that no dextran sulphate was added in the hybridisation step. Labelled sequences homologous to the human cDNA TH probe were detected by exposing the filters to Fuji films for 2 weeks at −70°C. The different tracks on this figure represent DNA isolated from the following cell lines: (A) 1,2,3: 3 different human lymphocytes; 4: rat lymphocytes. (B) 1, hybrid 412-Z-12; 2, hybrid DTP7.5; 3, hybrid DUR5R; 4, hybrid LSR 18; 5, hybrid LSR 39; 6, hybrid LSR 16; 7, hybrid LSR 40. (C) 1, hybrid 1W1-LA4; 2, hybrid 3W4; 3, hybrid 4W10; 4, human HeLa; 5, mouse 1R. 20 μg DNA were loaded on the gel except for hybrid 4W10 and human lymphocyte (A1) where only 10 μg of DNA were loaded. The molecular mass markers are given by *Hin*dIII restricted phage lambda DNA.

human gene for tyrosine hydroxylase to chromosome 11. As the human cDNA probe was strongly species specific and did not recognise the mouse TH gene under stringent conditions, this facilitated our analysis.

This localisation also raises some important evolutionary considerations. Tyrosine hydroxylase may represent one member of a larger gene family. It has been suggested that dopamine β-

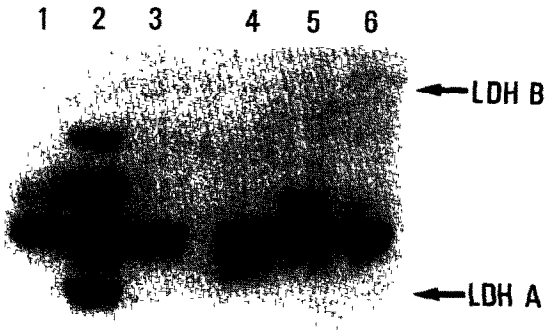

Fig.2. Electrophoretic analysis of LDH in human–mouse somatic cell hybrids. Hybrid cell extracts were electrophoresed and stained as described previously [3]. Cell extracts were: 1, mouse 1R; 2, human HeLa; 3, hybrid 4W10; 4, hybrid 1W1-LA4; 5, hybrid 3W4; 6, mouse 3T3. The positions of human LDH A and B are indicated.

hydroxylase, phenylethanolamine *N*-methyl transferase and tyrosine hydroxylase share common sequences, inferred from cross-reactivity of their respective antisera and similarity in peptide maps [14]. It is therefore possible that they may have arisen by gene duplication events. Although linkage is not always the case, a close chromosomal linkage has been reported for members of other gene families [15].

Other enzymes such as phenylalanine and tryptophan hydroxylase may be related to tyrosine hydroxylase. They catalyse very similar reactions and utilise the same co-factors, indeed tyrosine hydroxylase will catalyse the conversion of phenylalanine to tyrosine [16]. Deficiency of phenylalanine hydroxylase is characterised by the disease phenylketonuria (PKU [17]), and a molecular probe is now available for the diagnosis of PKU [18]. Therefore, it will be important to compare the chromosomal location of these putative members of the tyrosine hydroxylase gene family.

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