

Chromosomal localization of the gene coding for the β -subunit of Na^+, K^+ -ATPase in the American mink (*Mustela vison*)

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The BATP gene coding for the β -subunit of Na^+, K^+ -ATPase has been localized on chromosome 13 of the American mink (*Mustela vison*) using mink-Chinese hamster somatic cell hybrids and pig cDNA clones as probes. The AATP gene for the α -subunit of Na^+, K^+ -ATPase is on mink chromosome 2 [(1987) FEBS Lett. 217, 42–44]. Consequently, the AATP and BATP genes for the Na^+, K^+ -ATPase occupy separate mink chromosomes.

Na^+, K^+ -ATPase; Chromosomal localization; Somatic cell hybrid; (American mink)

1. INTRODUCTION

Na^+, K^+ -ATPase is a universal membrane protein catalyzing transport of Na^+ and K^+ through the plasma membrane. The enzyme is composed of two subunits, α and β . The α -subunit is endowed with catalytic activity, and the β -subunit is a glycoprotein of unknown function. It has been suggested that the enzyme in an active complex has an $\alpha_2\beta_2$ subunit composition [1].

The primary structure of the Na^+, K^+ -ATPase α - and β -subunits has been determined by sequencing cDNA from various sources [2–8]. The data indicate a homology between the cDNA sequence encoding the Na^+, K^+ -ATPase α - and β -subunits in different animal species [2–8].

We have mapped the mink AATP gene for the α -subunit to mink chromosome 2 [9]. In mink, 8 other genes reside on it [10]; in man, homologous genes lie in two syntenic groups, of which one is localized on the short arm of chromosome 1, the

other being on chromosome 10 [11]. The existence of conserved syntenic gene groups in mammals [11] supports the idea [9] that the human AATP gene may be located on either chromosome 1 or 10. The subsequent mapping data demonstrate that the human AATP gene is indeed situated on the short arm of chromosome 1 [8].

The position of the BATP gene for the Na^+, K^+ -ATPase β -subunit remains unknown. It is of interest to determine whether the AATP and BATP genes coding for the α - and β -subunits, respectively, are located on the same or distinct chromosomes.

This paper concerns the chromosomal localization of the mink BATP gene, using mink-Chinese hamster cell hybrids and the pig Na^+, K^+ -ATPase β -subunit cDNA as probe [12].

2. MATERIALS AND METHODS

To map the mink BATP gene, we used a set of mink-Chinese hamster somatic cell hybrids [13,14].

Extraction of DNA from the cell hybrids and parental cell lines as well as conditions for digestion with restriction enzyme *EcoRI* have been described [9]. DNA was transferred to Zeta-probe blotting membrane in 0.4 M NaOH [15]. Prehybridization and

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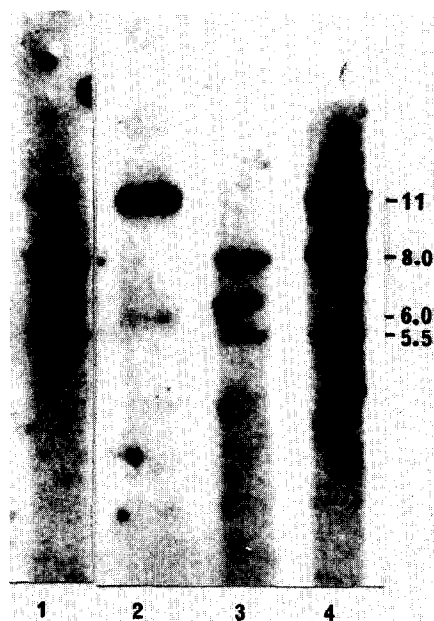


Fig.1. Hybridization of *Eco*RI DNA hydrolysates from mink, Chinese hamster, and some mink-Chinese hamster hybrid clones with a 355 bp fragment of pig β -subunit Na^+, K^+ -ATPase cDNA. 1, Chinese hamster cells, B14; 2, mink cells, MV; 3, clone F12B-1 negative for the mink BATP gene; 4, clone K02-1 positive for the mink BATP gene.

hybridization were carried out at 63°C in 4 × SSPE, 1% SDS, 0.5% nonfat powdered milk, 5% dextran sulphate in the presence of 250 µg/ml denaturated salmon sperm DNA. After hybridization, filters were washed at room temperature in 2 × SSC, 0.1% SDS and at 50°C in 0.4 × SSC, 1% SDS.

Plasmid pNβ31 carrying the coding sequence of the β -subunit of pig [12] was digested with restrictase *Pst*I and subjected to electrophoresis in 0.8% agarose gels and electroelution to obtain two pig ATPase β -subunit cDNA fragments (one 627 bp N-end; the other 355 bp C-end) used as probes. The DNA fragments were labeled with ^{32}P to a specific activity of 1–2 × 10⁹ cpm/µg by the random-priming technique [16]. The probe was hybridized with the fixed DNA on nylon filters at a concentration of 10 ng/ml.

3. RESULTS AND DISCUSSION

Fig.1 presents the results of hybridization of *Eco*RI-digested DNA from mink, Chinese hamster, and hybrid cells with a 355 bp fragment of pig ATPase cDNA. The BATP gene of Chinese hamster is represented by two fragments (5, 7 kb) and that of mink also includes two fragments (11, 5.5 kb). Mink and Chinese hamster BATPs have additional fragments of 4.0 kb and 2.4 kb, respectively, detectable after the longer exposure. The hybrid clones containing the 11 kb fragment were identified as positive for BATP gene of mink origin (fig.1).

Table 1

Segregation of mink chromosomes and mink BATP gene in 15 independent mink-Chinese hamster hybrid clones

Hybrid clones	BATP ^a	Mink chromosomes														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	X
F3M	+	+	–	+	–	+	+	+	–	+	–	–	–	+	+	+
K02-1	+	+	–	+	+	–	+	–	–	+	+	–	+	+	+	+
L22-1	+	+	–	–	+	+	+	–	–	+	–	–	+	+	–	+
L15-1	+	–	–	–	+	–	–	–	+	–	+	+	–	+	+	+
D7B-1	+	–	–	+	+	+	–	–	+	+	+	+	+	–	–	+
FD9M	+	+	+	–	–	–	–	–	+	+	–	+	+	+	+	+
R14-1	+	–	–	–	–	–	+	–	–	–	+	–	+	+	–	+
R01-1	+	+	+	+	+	–	+	–	–	+	+	+	–	+	+	+
D3M	–	+	–	+	–	–	–	+	–	–	+	–	+	–	–	+
F12B-1	–	–	+	+	+	+	–	–	+	+	+	–	+	–	+	+
K12-1	–	+	–	+	–	+	+	+	–	–	+	+	+	–	+	+
L25-1	–	–	–	–	–	+	+	–	–	–	+	–	+	–	–	+
D11B-1	–	–	–	–	–	–	+	+	–	+	–	–	–	–	+	+
D12M	–	+	–	–	+	–	+	–	+	+	+	–	+	–	–	+
D13M	–	+	–	+	–	–	+	–	+	+	–	–	–	–	+	+
Discordance (%)		46	46	53	33	53	53	66	53	40	53	33	53	7	46	46

^a BATP gene was scored as the presence (+) or absence (–) of the 11 kb mink *Eco*RI fragment

Table 1 shows that the presence of the 11 kb fragment correlates with the appearance of mink chromosome 13. An exception is clone D7B-1. This lacks mink chromosome 13, yet it has the 11 kb mink BATP fragment. Hybrid clone D7B-1 contains a chromosome similar to mink chromosome in G-banding pattern, but cytogenetic analysis does not assign it reliably to mink chromosome 13 [14]. It either contains a fragment of mink chromosome 13 or rearranges in such a way that it becomes like chromosome 13 [14]. It should be noted that the D7B-1 clone contains mink peptidase C (PEPC), a marker of mink chromosome 13 [17].

Thus, according to hybridization, the BATP gene encoding the Na^+, K^+ -ATPase β -subunit is assigned to chromosome 13 in mink. The results obtained with the other pig BATP cDNA fragment (see section 2) support this conclusion (not shown).

To reiterate, the AATP gene for the Na^+, K^+ -ATPase α -subunit is located on chromosome 2 in mink [9]. Consequently, the AATP and BATP genes for the α - and β -subunits, respectively, occupy distinct mink chromosomes.

Chromosome 13 is one of the smallest chromosomes in mink, and we have positioned on it a single gene for PEPS [17]. The PEPS gene is located on the long arm on chromosome 1 in man and, hence, BATP may lie together with PEPS in the same linkage group in man.

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