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

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The gene coding for the α-subunit of Na^+,K^+-ATPase has been localized on chromosome 2 of the American mink (*Mustela vison*) using the somatic cell hybrids mink-Chinese hamster and pig cDNA clones as hybridization probes.

($Na^+ + K^+$)-ATPase; Chromosomal localization; Somatic cell hybrid; (*Mustela vison*)

1. INTRODUCTION

Na^+,K^+-ATPase is an integral membrane protein of most animal cells. This enzyme catalyzes the transfer of Na^+ and K^+ through the plasma membrane. It is composed of two subunits: catalytic α and glycoprotein β of unknown function. At present, the amino acid sequence has been determined for the α-subunits of the Na^+,K^+-ATPase of ray [1], sheep [2], pig [3] and human HeLa cells [4].

The genes coding for the α-subunits of the ATPase have not yet been located in any mammalian species, including man. The gene for the α-subunit of the Na^+,K^+-ATPase has been mapped on mink chromosome 2 using the cDNA clones of the pig α-subunit as probes.

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2. MATERIALS AND METHODS

In order to map the gene coding for the mink α-subunit of Na^+,K^+-ATPase (AATP), a panel of American mink-Chinese hamster clones has been used. The indexed clones (1) have been described [5], and the rest of them were used for the first time. All the clones of this panel had been analysed cytogenetically, and those which had no visible rearrangements of mink chromosomes were employed in the further analysis. The distribution of mink chromosomes among the hybrid clones is shown in table 1.

Total DNA was isolated from the hybrid clones according to [6]. The cleavage of the DNAs with the *Eco*RI restriction endonuclease was carried out for 20 h with 10 units of the enzyme per μg of the DNA. The DNA fragments were transferred onto nitrocellulose as described [7]. Hybridization was performed at 65°C in 6×SSC containing 10× Denhardt, 0.1% SDS, 7% dextran sulfate, 0.1% sodium pyrophosphate, 4 mM EDTA and 250 μg/ml sonicated and denatured salmon sperm DNA.

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Nick-translated cDNA fragments coding for the pig ATPase served as probes. These fragments had a length of 1.2 kb (pB159) or 1.0 kb (pB29) [8]. Their specific activity was $1\text{–}5\times10^8$ cpm/μg of DNA. The concentration of the probe in the hybridization medium was about 10 ng/ml. After hybridization, the filters were washed at 55°C twice for 30 min in 2 × SSPE, 0.1% SDS, 0.1% pyrophosphate and then twice for 60 min in 0.2 × SSPE, 0.2 × SSC, 0.1% SDS.

3. RESULTS AND DISCUSSION

The hybridization results for the N-terminal cDNA fragment of the pig ATPase with the DNA from hybrid clones are presented in table 1 and fig.1. The restriction patterns of DNAs from mink and Chinese hamster show 2 major *EcoRI* fragments. The 8 kb mink fragment is obvious in the positively hybridizing clones. The segregation analysis for the AATP and the mink chromosomes in the hybrid clones has revealed the presence of the 8 kb mink fragment in the clones F12B-1, FD9M, and RO1-1. This fragment was not detected in the remaining clones of the panel (table 1).

The results reported here are in agreement with the data obtained in the case of the other cDNA fragment (pB29 clone) [8] coding for the C-terminal part of pig ATPase.

Thus the gene for the α-subunit of the mink ATPase has been localized on chromosome 2 (table 1).

The number of genes which have been previously localized on mink chromosome 2 is eight. These genes code for the purine-nucleoside phosphorylase (NP), inorganic pyrophosphatase (PP), hexokinase-1 (HK1), adenosine kinase (ADK), glutamate-oxaloacetate transaminase-1 (GOT1), phosphogluconate dehydrogenase (PGD), enolase-1 (ENO1) and phosphoglucomutase-1 (PGM1) [9–11]. The homologous genes are members of the two synthetic groups in man. The PGD, ENO1, and PGM1 genes are members of the first group; they are located on human chromosome 1 [12]. The second group consists of the genes PP, HK1, ADK and GOT1, and these genes reside on human chromosome 10 [12]. Taking into consideration the extensive conservatism of the synthetic gene

Table 1

Segregation of the mink chromosomes and the mink AATP in American mink-Chinese hamster hybrid clones

Hybrid clones	AATP[a]	Mink chromosomes														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	X
F3M	−	+	−	+	−	+	+	+	−	+	−	−	−	+	+	+
F12B-1	+	−	+	+	+	+	−	−	+	+	+	−	+	−	+	+
FD9M	+	+	+	−	−	−	−	−	+	+	−	+	+	+	+	+
KO2-1	−	+	−	+	+	−	+	−	−	+	+	−	+	+	+	+
K12-1	−	+	−	+	−	+	+	+	−	−	+	+	+	−	+	+
L22-1	−	+	−	−	+	+	+	−	−	+	−	−	+	+	−	+
L15-1	−	−	−	−	+	−	−	−	+	−	+	+	−	+	+	+
L25-1	−	−	−	−	−	+	+	−	−	−	+	−	+	−	−	+
RO1-1	+	+	+	+	+	−	+	−	−	+	+	+	−	+	+	+
R14-1	−	−	−	−	−	−	+	−	−	−	+	−	+	+	−	+
D7B-1	−	−	−	+	+	+	−	−	+	+	+	+	+	−	−	+
D11B-1	−	−	−	−	−	−	+	+	−	+	−	−	−	−	+	+
D3M	−	+	−	+	−	−	−	+	−	−	+	−	+	−	−	+
D12M	−	+	−	−	+	−	+	−	+	+	+	−	+	−	−	+
D13M	−	+	−	+	−	−	+	−	+	+	−	−	−	−	+	+
Discordance	(%)	53	0	46	40	46	73	40	40	40	60	26	60	40	40	80

[a] Presence of the mink AATP gene was based on the presence (+) or absence (−) of the 8 kb *Eco*RI fragment

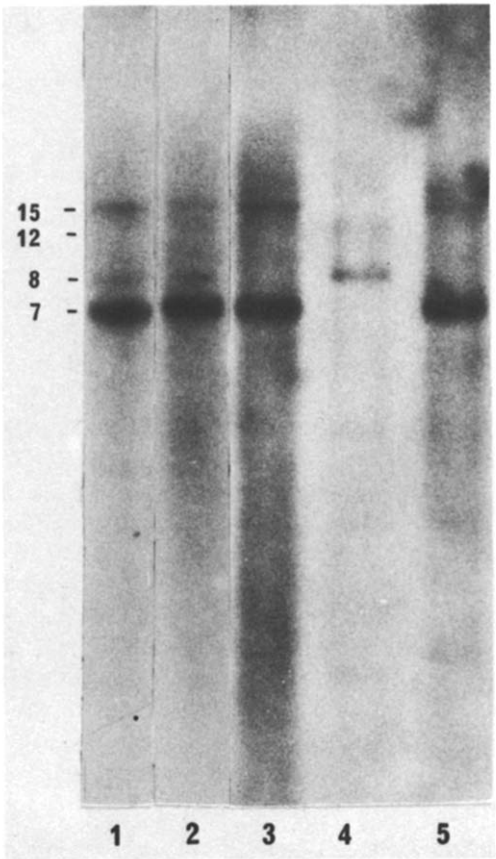

Fig.1. Hybridization of *Eco*RI hydrolysates of DNA from American mink-Chinese hamster clones with the *PstI* fragment of cDNA for the α-subunit of pig ATPase (pB159) [8]. Lanes: 1, clone F12B-1; 2, clone RO1-1; 3, clone K12 (negative for AATP of mink origin); 4, mink fibroblasts, MV cells; 5, Chinese hamster fibroblasts, V-79 cells.

associations in mammals [13], it may be suggested that the homologous human gene AATP may be located either in the short arm of chromosome 1 or chromosome 10.

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