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Assignment of the human aminopeptidase N (peptidase E) gene to chromosome 15q13-qter

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The gene for aminopeptidase N (EC 3.4.11.2) has been located on the human chromosome 15q13-qter using *Hin*dIII-cleaved DNA from a panel of hybrids between rodent and human cells. The Southern blots were probed by the 5'-*Eco*RI fragment of the recently cloned human aminopeptidase N cDNA.

Aminopeptidase N; Gene mapping; Chromosome 15; (Human)

1. INTRODUCTION

Aminopeptidase N (EC 3.4.11.2) is located in the small-intestinal and renal microvillar membrane, and also in other plasma membranes (reviews [1,2]). (Aminopeptidase N is suggested to be identical with peptidase E [3] in the A,B,C,D, E,S nomenclature system [4].)

In the small intestine aminopeptidase N plays a role in the final digestion of peptides generated from hydrolysis of proteins by gastric and pancreatic proteases. Its function in proximal tubular epithelial cells and other cell types is still obscure although a role in the removal of biologically active peptides has been suggested [5].

The onset of expression of aminopeptidase N occurs late during intestinal development and is different from that of lactase-phlorizin hydrolase and sucrase-isomaltase [6]. Its expression is known to be influenced by glucocorticoids [7,8].

Whereas the genes of peptidase A–D and S have been localized [3,4], that of aminopeptidase N (i.e. peptidase 'E') has not. This paper reports on the chromosomal localisation of aminopeptidase N as part of a work aiming at an understanding of the regulation of the expression of aminopeptidase N at a molecular level. For this work restricted genomic DNA from rodent/human cell hybrids is probed with a DNA fragment from the recently cloned and sequenced aminopeptidase N cDNA [9].

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2. MATERIALS AND METHODS

2.1. *Parental cells*

The parental HPRT-deficient rodent cell lines were mouse RAG and A9 cells and Chinese hamster Wg3-h. The parental human cells were female fibroblasts with different balanced X-autosome translocations, except one case where 46,X, der(X) (Xqter-p22.3::?q11-qter) cells were used to produce the hybrid 445 × 392.K1.

2.2. *Hybrid cells*

The cell hybrids were produced by polyethylene glycol-mediated fusion of rodent cell lines and human fibroblasts, and human chromosomes in hybrid cell metaphases were analysed as described in [10,11] and table 1. About 12 metaphases were analysed for each cell hybrid.

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2.3. *DNA extraction, Southern transfer, and filter hybridization*

DNA was extracted from somatic cell hybrids according to Aldridge et al. [12]. DNA samples were digested with *Hin*dIII, separated electrophoretically on 0.7% agarose gels (50 V for 20 h) and transferred onto Hybond-N membranes (Amersham) as in [13]. The probe used was a 1.76 kb 5′ *Eco*RI fragment of a human intestinal aminopeptidase N cDNA clone [9]. The DNA probe was labelled with ^{32}P by nick translation to a specific activity of above 10^8 cpm/μg. Hybridization, stringent washes and autoradiography were performed by established methods [12].

3. RESULTS AND DISCUSSION

DNAs from 15 different human-rodent cell hybrids were analysed by Southern blotting after digestion with the restriction enzyme *Hin*dIII. Using the 5′ *Eco*RI fragment of human intestinal aminopeptidase N as the probe, 6 hybridizing bands are revealed in human genomic DNA whereas 3 cross-hybridizing bands are observed in mouse DNA and 2 bands in hamster DNA (fig.1). As the 5′ *Eco*RI fragment used as a probe has no *Hin*dIII cleavage sites, the 6 hybridizing bands in the human DNA indicate that this part of the coding sequence at the genomic level is divided into at least six exons, assuming that none of the bands are due to cross-hybridizing genes or pseudogenes. In lanes 2 and 3 an RFLP is observed in the two mouse lines, A9 and RAG. In table 1 the segregation of the human specific bands and the chromosomal contents of the hybrid cells is compared. Only chromosomes totally absent or present in at least 30% of the cells examined were taken into account, while those detected in less than 30%

Table 1

Analysis of human chromosomes and human aminopeptidase N gene in 15 rodent-human cell hybrids

Human-rodent hybrids	Human chromosomes																					Human aminopeptidase N
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	
445×393 K1	+	+	+	+	+	+	+	−	−	−	+	−	−	/	−	−	−	−	+	−	/	−	−
697×175 K36	−	/	−	+	−	+	−	−	−	−	−	−	−	+	/	/	−	−	−	−	/	/	−
617×347 K6	−	−	−	−	−	/	−	−	−	−	/	+	−	−	/	/	/	/	−	/	+	−	−
422 K2	−	−	+	−	+	+	−	+	+	−	+	−	+	/	−	/	−	−	−	+	+	+	−
749	+	−	+	+	+	+	+	−	−	−	/	+	−	+	−	/	−	+	−	−	+	/	−
494×393 K6	−	+	−	−	−	−	−	−	−	+	−	−	/	−	−	−	−	−	−	−	−	−	−
790×175 K6	−	−	−	−	−	−	−	−	−	−	−	−	+	−	/	−	−	−	−	−	+	−	−
750	/	−	−	/	/	−	−	−	−	−	+	−	−	+	/	−	+	−	−	/	−	/	−
PI-RAG-72	+	−	+	−	+	+	+	+	+	−	−	+	−	+	+[d]	−	−	+	−	−	−	+	+
GO-RAG-4	−	−	+	/	−	−	/	+	−	−	−	−	−	+	+	−	−	+	−	−	−	+	+
GM194-RAG-7	−	−	+[b]	+	+	−	−	−	−	/	−	−	−	−	−	−	−	/	+	−	+	−	−
GM194-RAG-5-5	−	−	+[b]	+	+	+	+	−	−	+	−	+	+	+	−	+	−	−	−	+	+	+	−
GM97-RAG-8-13	+[a]	−	−	+	−	−	−	−	+	−	−	−	−	+	−	−	−	−	−	−	+	+	−
GM89-A9-9c-7	−	−	+	+	−	−	+	−	−	−	+	−	−	+	+	/	−	+	/	+	+	+	+
MS58-A9-26	−	−	−	−	−	−	−	−	−	−	+	−	+	+[c]	+	−	−	−	−	−	−	−	+
+/+	1	0	3	1	1	1	2	2	1	0	2	1	1	2	3	0	0	3	0	1	1	3	
−/−	7	8	6	4	5	5	8	10	9	8	6	8	7	4	7	6	9	8	9	7	3	5	
+/−	2	2	3	6	5	5	3	1	2	2	3	3	3	5	0	1	1	1	2	2	6	3	
−/+	3	4	1	2	3	3	1	2	3	4	2	3	3	1	0	3	4	1	3	3	3	7	
Concordant	8	8	9	5	6	6	10	12	10	8	8	9	8	6	10	6	9	11	9	8	4	8	
Discordant	5	6	4	8	8	8	4	3	5	6	5	6	6	6	0	4	5	2	5	5	9	4	

[a] GM97-RAG-8-13 only contains the region pter-q12 of chromosome 1

[b] GM194-RAG-7 and GM194-RAG-5-5 only contain the regions q21-qter and pter-q21 of chromosome 3, respectively

[c] MS58-A9-26 only contains the region pter-q32 of chromosome 14

[d] PI-RAG-72 only contains the region q13-qter of chromosome 15

(+) Chromosome detected in at least 30% of hybrid cells; (/) chromosome detected in less than 30% of hybrid cells; (−) chromosomes not detected

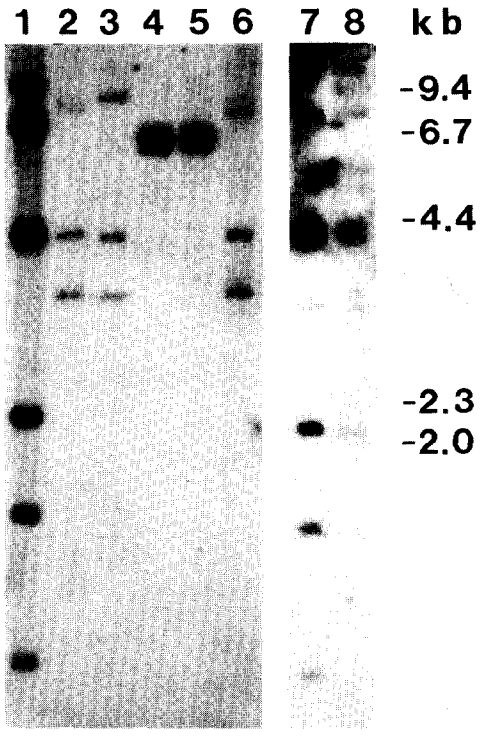

Fig.1. Detection of human aminopeptidase N gene sequences by Soutern blot analysis. Lanes: 1, human DNA; 2, RAG (mouse); 3, A9 (mouse); 4, Wg3-h (hamster); 5, hybrid 422 K2; 6, hybrid 750; 7, hybrid MS58-A9-26; 8, hybrid GM89-A9-9c-7.

of the cells examined were considered uninformative. The presence of the aminopeptidase N gene and human chromosome 15 in the hybrids shows 100% concordance. All other chromosomes showed at least 15% discordant segregation of the specific hybridization signal and a specific chromosome. One of the hybrids, PI-RAG-7-2, contains only the chromosome 15 fragment 15q13-qter. The aminopeptidase N gene probe also hybridizes to this hybrid leading to the conclusion that the aminopeptidase N (peptidase E) gene is located on chromosome 15q13-qter.

The amino acid sequence of brush border aminopeptidase N (including the active site) shows extensive homology with the *E. coli* enzyme (see [9], particularly fig.3), but also with other metallopeptidases, like thermolysin, human endopeptidase 24.11 and human collagenase [9]. This suggests that the metallopeptidases might have arisen from a common primordial gene by gene duplication. As collagenase is located on chromosome 11q11-q23 [14,15], this might indicate that the duplication event occurred early in evolution. Other human peptidases (peptidases A–D, S) are all located on different chromosomes [3,4].

The brush border of the small intestine contains a characteristic set of enzymes like the peptidases and glucosidases [1,2]. Of these sucrase-isomaltase has been located on chromosome 3 [16], lactase-phlorizin hydrolase on chromosome 2 [17] and γ-glutamyl transpeptidase on chromosome 22 [18,19]. It is clear that the genes of enzymes belonging to the same metabolic pathway (proteolytic/catabolic pathway) and the same organelle (brush border) are not located on the same chromosome which is in line with data from other systems [20]. The genes of the brush border enzymes are then far from each other and not under a common regulatory DNA element. This is also reflected by a difference in appearance during development [6].

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