J. Biochem. 125, 931-938 (1999)

Molecular Cloning of Adipocyte-Derived Leucine Aminopeptidase Highly Related to Placental Leucine Aminopeptidase/Oxytocinase[1]

Akira Hattori,* Hideko Matsumoto,* Shigehiko Mizutani,† and Masafumi Tsujimoto*,[2]

*Laboratory of Cellular Biochemistry, The Institute of Physical and Chemical Research (RIKEN), Wako-shi, Saitama 351-0198; and †Department of Obstetrics and Gynecology, Nagoya University School of Medicine, Tsurumai-cho, Showa-ku, Nagoya 466-8550

Received December 24, 1998; accepted February 8, 1999

In the current study, we report the cloning and initial characterization of a novel human cytosolic aminopeptidase named adipocyte-derived leucine aminopeptidase (A-LAP). The sequence encodes a 941-amino acid protein with significant homology (43%) to placental leucine aminopeptidase (P-LAP)/oxytocinase. The predicted A-LAP contains the HEXXH(X)$_{18}$E consensus sequence, which is characteristic of the M1 family of zinc-metallopeptidases. Although the deduced sequence contains a hydrophobic region near the N-terminus, the enzyme localized mainly in cytoplasm when expressed in COS-7 cells. Northern blot analysis revealed that A-LAP was expressed in all the tissues tested, some of which expressed at least three forms of mRNA, suggesting that the regulation of the gene expression is complex. When aminopeptidase activity of A-LAP was measured with various synthetic substrates, the enzyme revealed a preference for leucine, establishing that A-LAP is a novel leucine aminopeptidase with restricted substrate specificity. The identification of A-LAP, which reveals strong homology to P-LAP, might lead to the definition of a new subfamily of zinc-containing aminopeptidases belonging to the M1 family of metallopeptidases.

Key words: aminopeptidase, COS-7 cells, expressed sequence tags, metallopeptidase, molecular cloning.

Aminopeptidases hydrolyze N-terminal amino acids of protein or peptide substrates. They are distributed widely in animal and plant tissues as well as in bacteria and fungi, suggesting that they play important roles in various biological processes. They are essential for protein maturation, the activation, modulation, and degradation of bioactive peptides, and the determination of protein stability (*1*). In addition, some aminopeptidases function as differentiation antigens and control cell proliferation and/or differentiation (*2-5*).

In our previous work, we cloned human placental leucine aminopeptidase (P-LAP)/oxytocinase [EC 3.4.11.3], which is known to increase in maternal serum during pregnancy (*6*). Since P-LAP degraded such peptide hormones as oxytocin and vasopressin, whose increase might have a significant effect on the uterine tonus and uteroplacental blood flow, the enzyme is believed to play a crucial role in the maintenance of pregnancy (*7, 8*). Structural analysis revealed that the enzyme is a type II membrane-spanning protein belonging to the M1 family of zinc-metallopeptidases, which share the consensus HEXXH(X)$_{18}$E motif essential for the enzymatic activity. It is now clear that this growing family of mammalian zinc-containing aminopeptidases includes membrane-bound (*3, 9, 10*), cytosolic (*11-13*), and secretory proteins (*15, 16*). However, the biological significance of these aminopeptidases is still largely unclear.

To identify an additional enzyme belonging to this family, we have searched the expressed sequence tag (EST) database and cloned a novel protein that revealed significant homology to P-LAP. In this report, we describe the sequence and initial characterization of this novel protein termed A-LAP. Our results indicate that A-LAP is indeed an enzyme with aminopeptidase activity and preferentially cleaves the leucine residue of synthetic substrates. We also found by Northern blot analysis that transcripts of A-LAP were expressed in several tissues, some of which expressed at least three different sized mRNAs, suggesting that the regulation of the gene expression is complex.

EXPERIMENTAL PROCEDURES

Cloning and Sequencing of the Human A-LAP cDNA— The partial nucleotide sequences of cDNA encoding peptides with homology to P-LAP/oxytocinase were found in

[1] This work was supported in part by a grant for "Multibioprobe Research Program" from RIKEN. The nucleotide sequence reported in this paper has been submitted to the GenBank™/EMBL/DDBJ Data Bank with accession number AF106037.

[2] To whom correspondence should be addressed. Tel: +81-48-467-9370, Fax: +81-48-462-4670, E-mail: tsujimot@postman.riken.go.jp

Abbreviations: A-LAP, adipocyte-derived leucine aminopeptidase; AMC, 7-amino-4-methylcoumarin; AP-A, aminopeptidase A; AP-N, aminopeptidase N; (aminoacyl-)MCA, (aminoacyl-)4-methylcoumaryl-7-amide; EST, expressed sequence tags; LTA4H, leukotriene A$_4$ hydrolase; P-LAP, placental leucine aminopeptidase; PSA, puromycin-sensitive aminopeptidase.

the EST database of GenBank by use of the TBLASTN program. The 213-base pair fragment from the EST clone 77569 (GenBank accession number T27536) was used as a probe to screen a human white adipose tissue cDNA library (Clontech). λgt11 bacteriophages were plated at a density of approximately 2×10^4 plaque forming units/100-mm plate. They were transferred to a nylon membrane (Biodyne B, Pall), then hybridized using ^{32}P-labeled probe as described (*17*). DNA sequence was determined by the method of Sanger *et al.* (*18*) using a *Taq* dye terminator cycle sequencing kit and an Applied Biosystems model 377 DNA sequencer.

Expression of A-LAP in COS-7 Cells—The blunted *Eco*RI fragment of the human A-LAP1 cDNA was subcloned into the *Sma*I site of pSVL (Amersham-Pharmacia) (pSVL/A-LAP1). For transfection experiments, COS-7 cells were grown to confluency in Dulbecco's modified Eagle's medium containing 10% fetal calf serum. Then 2.5×10^6 cells per 100-mm dish were transfected with 20 μg of pSVL or pSVL/A-LAP1 mixed with 80 μg of DOSPER (Boehringer Mannheim). Following transfection, the cells were incubated in culture medium for 72 h and collected for the aminopeptidase assay.

Northern Blot Analysis—Human Multiple Tissue Northern Blot was obtained from Clontech. The filter-bound mRNA was hybridized with the ^{32}P-labeled probes by use of ExpressHyb hybridization solution (Clontech) according to the instruction manual.

Preparation of Antibody—Synthetic peptide corresponding to the C-terminal 14 amino acids of A-LAP1 including cysteine linker (*i.e.* CKIRVWLQSEKLERM) was coupled to keyhole limpet hemocyanin according to the method described previously (*19*) and injected into rabbits for antibody production following standard procedures. Namely, the conjugate mixed with Freund's complete adjuvant was injected intraperitoneally (500 μg of peptide), followed by three every week injection of the conjugate (250 μg of peptide) with Freund's complete adjuvant.

Western Blot Analysis—The test samples were separated by SDS-PAGE on an 8% separating gel and transferred to polyvinylidene difluoride (PVDF) membranes (Fluorotrans, Pall). The membranes were blocked with Tris-HCl buffered saline (TBS), pH 7.4, containing 5% skim milk for 1 h at room temperature, then incubated in TBS containing 5% skim milk, 0.05% Tween 20 (T-TBS), and anti-A-LAP serum diluted to 1/1,000 for 2 h at room temperature. The filter was washed four times with T-TBS and incubated for 1 h with horseradish peroxidase-conjugated goat anti-rabbit IgG polyclonal antibody (Promega), diluted to 1/5,000 in TBS containing 5% skim milk. After washing the filter three times with T-TBS, the blots were detected by an enhanced chemiluminescence method using an ECL plus Western blotting detection kit obtained from Amersham-Pharmacia. The results were visualized by fluorography using RX-U Fuji medical X-ray film.

Aminopeptidase Activity Assay—Transfected COS-7 cells were washed twice with PBS, and then loosened by scraping with rubber policeman. Cells were collected by at centrifugation $500\times g$ for 5 min and suspended in 2 ml of 50 mM Tris-HCl, pH 7.5. To obtain cell-free extracts, the cells were lysed at 4°C in a Dounce-homogenizer and centrifuged for 10 min at $2,000\times g$ to remove nuclei and cell debris. The resulting lysate was centrifuged for 30 min at $100,000\times g$ at 4°C. Supernatant containing 1 mg of protein was collected and subjected to immunoprecipitation with C15 anti-A-LAP1 antibody. After mixing with C15 antibody bound to protein A-Sepharose (Amersham-Pharmacia) for 2 h at 4°C, the reaction mixture was centrifuged for 1 min at $15,000\times g$ at 4°C and washed five times in 50 mM Tris-HCl buffer pH 7.5. The precipitate was then suspended in 10 ml of the same buffer.

Aminopeptidase activity was determined with a fluorogenic substrate, aminoacyl-4-methylcoumaryl-7-amide (aminoacyl-MCA) as substrate (*20, 21*). In brief, the reaction mixture containing 25 μM aminoacyl-MCA and enzyme preparation (250 μl) in 0.5 ml of 25 mM Tris-HCl buffer (pH 7.5) was incubated at 37°C for 15 min. The reaction was terminated by adding 2.5 ml of 0.1 M sodium acetate buffer (pH 4.3) containing 0.1 M sodium monochloroacetate. The released 7-amino-4-methylcoumarin (AMC) was measured by use of a spectrofluorophotometer (Hitachi F-2000) at an excitation wavelength of 370 nm and an emission wavelength of 460 nm.

Materials—*S*-Benzyl-Cys-, Asp-, and Gly-MCAs were purchased from BACHEM AG (Switzerland). Arg-, Lys-, Ala-, Leu-, Phe-, and Met-MCAs were from Peptide Institute (Osaka). Inhibitors were obtained from Sigma, except for leupeptin and pepstatin, which were from Peptide Institute.

RESULTS AND DISCUSSION

Molecular Cloning and Sequencing of cDNA for A-LAP—To identify sequences similar to the human P-LAP/oxytocinase gene, we searched public databases of expressed sequence tags (ESTs). Three ESTs (76446, 77568, and 77569) derived from human white adipose tissue were shown to encode novel peptides with significant amino acid homology to P-LAP. A full-length clone was isolated from a human adipose tissue cDNA library. Sequence analysis of the cDNA demonstrated that the three clones represent fragments of the same gene. Since the protein encoded by the cDNA was first identified in ESTs derived from adipose tissue cDNA library, we have termed it adipocyte-derived leucine aminopeptidase (A-LAP).

Figure 1 shows the nucleotide sequence and the deduced amino acid sequence of the cDNA. The cDNA contains a 3′ poly(A) tail preceded by a poly(A) signal 16 nucleotides upstream and an open reading frame of 2,823 base pairs, ending with a TAA stop codon at nucleotides 2824-2826. The first ATG triplet (starting at nucleotide 1) was considered to be the initiation codon of the protein translation since the nucleotide sequence flanking it (AAGATGG) matches Kozak's rule for translational initiation consensus sequence (*22*) and the 5′-untranslated region contains an in-frame stop codon preceding this putative initiator methionine codon.

The predicted translation product of the A-LAP cDNA encodes a protein of 941 amino acids (including the initiator methionine) with a calculated molecular weight (M_r) of 106,893.26, which corresponds to the M_r of recombinant human A-LAP protein estimated by SDS-PAGE (see below) and contains five potential *N*-glycosylation sites. Hydropathy analysis using the Kyte and Doolittle algorithm (*23*) showed that the enzyme carries a significantly hydrophobic region near the N-terminus (Pro^5-Ser^{27}),

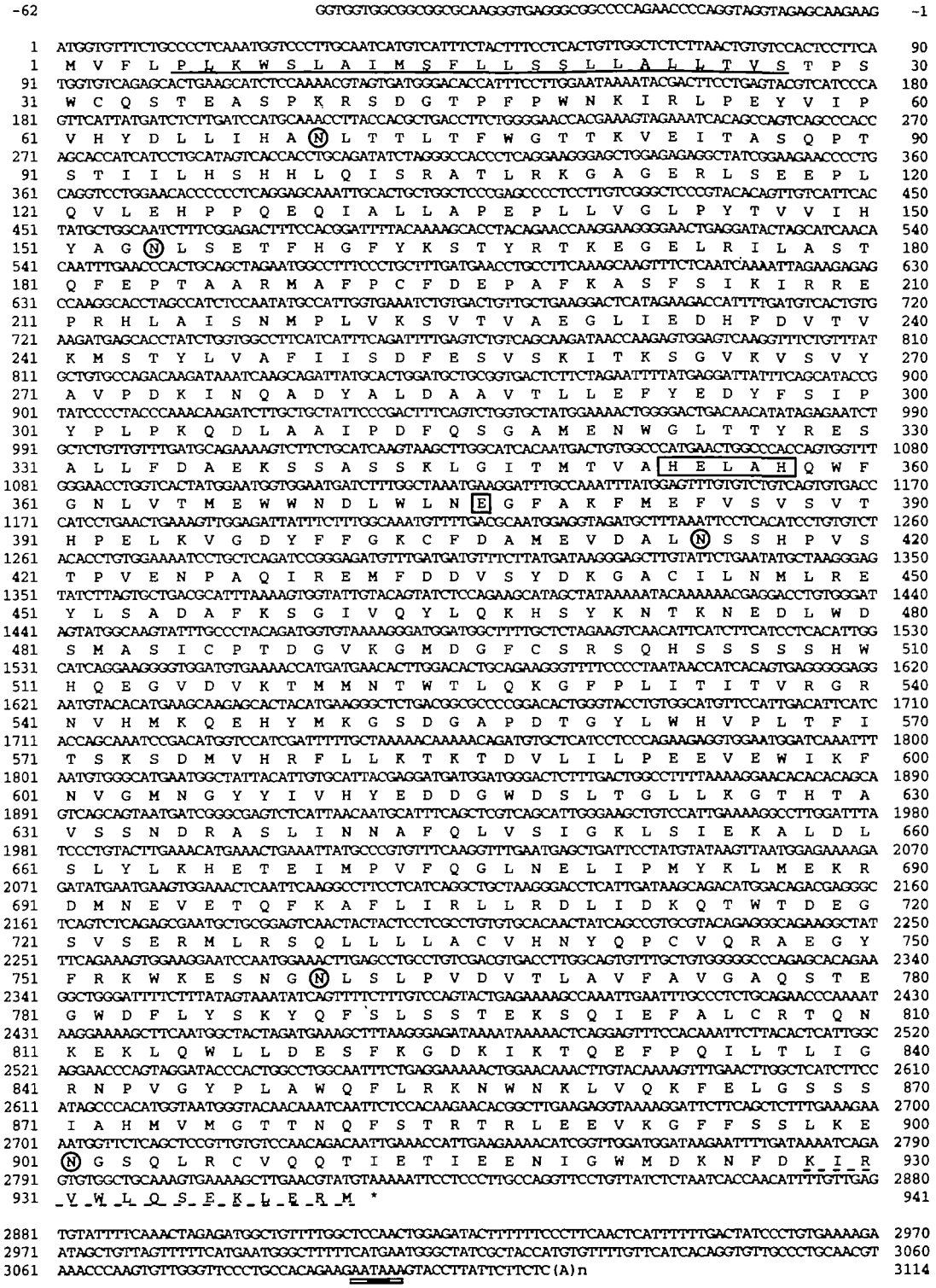

Fig. 1. **Nucleotide sequence of the cDNA and the deduced primary structure of A-LAP.** Nucleotide residues are numbered from 5′ to 3′ with the first residue of the ATG codon encoding the putative initiating methionine. The deduced amino acid sequence is displayed below the nucleotide sequence as a one letter code starting from the methionine. The N-terminal hydrophobic region is underlined, the potential glycosylation sites are circled, and the HEXXH consensus sequence and conserved glutamic acid are boxed. Dotted lines represent the sequences of peptides recognized by C15 anti-A-LAP1 antibody. In the 3′-nontranslated region, the putative polyadenylation signal is represented by a double line.

which could function as an internal signal peptide and a membrane-spanning domain. However, the role of this region is unclear at present since as shown below, recombinant A-LAP expressed in COS-7 cells was recovered

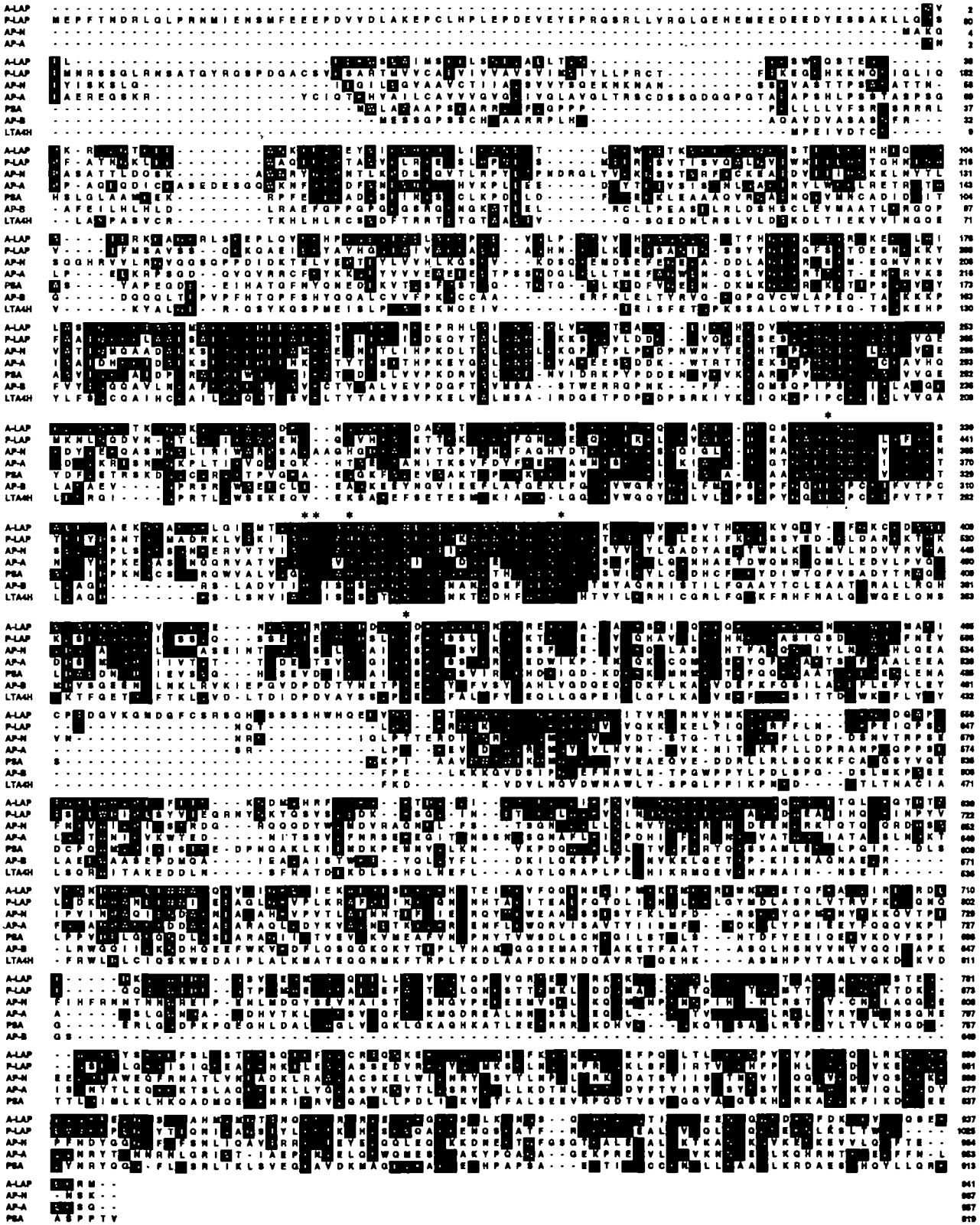

Fig. 2. **Alignment of A-LAP protein with other zinc-containing aminopeptidases.** The deduced amino acid sequence of A-LAP is aligned with the sequences of human P-LAP (*6*), AP-A (*4*), AP-N (*9*), PSA (*12*), LTA4H (*14*), and rat AP-B (*15*). Gaps are inserted into the sequences for optimal alignment. Residues identical with A-LAP are boxed. Asterisks indicate the essential residues for the hydrolytic activity of the enzymes so far reported.

mainly in the cytosolic fraction. It is possible that, under certain conditions, translation is initiated preferentially at the second methionine codon (Met^{13}), which is flanked by a Kozak consensus-like sequence. It was reported that puromycin-sensitive aminopeptidase (PSA) might be translated preferentially at the second methionine codon of the open reading frame, resulting in the loss of signal peptide and the cytosolic localization of the enzyme (*5*).

The deduced amino acid sequence contains a typical consensus sequence of the zinc-metallopeptidase family (*24*). Namely, there is an essential zinc binding site (HEXXH) of the peptidase at amino acid residues 353-357 with a second glutamic acid separated by 18 amino acids (*25, 26*). This motif is found in several reported aminopeptidases and allows the classification of the A-LAP in the M1 family of metallopeptidases (*27*).

Search for Homologous Proteins—A computer search revealed that the amino acid sequence of A-LAP has apparent homology to other members of the M1 family of zinc-containing aminopeptidases (Fig. 2). By using the Clustal W program, P-LAP was identified as the most closely related protein to A-LAP (43% identity at amino acid level). The identity between these two enzymes extends over their entire primary structures. Membrane-bound aminopeptidases such as aminopeptidase A (AP-A) and aminopeptidase N (AP-N) as well as PSA, a cytosolic enzyme, showed lower but significant (29-30%) homology to A-LAP. Other aminopeptidases such as leukotriene A_4 hydrolase (LTA4H) and aminopeptidase B (AP-B) had lower homology (13%) to A-LAP. The homology between P-LAP and these enzymes is higher in the region surrounding the consensus zinc-binding motif and lower in the N- and C-terminal regions of the enzymes. It should be noted that both Tyr and Glu residues, which play important roles in the hydrolytic activity of AP-A and AP-N, are conserved in all aminopeptidases listed (Fig. 2, Tyr^{436} and Glu^{318} in the A-LAP sequence) (*28-30*).

Northern Blot Analysis—The tissue distribution of A-LAP was determined by Northern blot analysis using the full length cDNA as a probe (Fig. 3). In spleen and ovary, three forms of transcripts with sizes of 3.6, 5.1, and 5.6 kb were clearly detected. The 3.6 kb transcript corresponded in size to the cDNA shown in Fig. 1 (termed A-LAP1). In other tissues except for brain, only the two smaller forms of mRNA (*i.e.*, 3.6 and 5.1 kb bands) were observed. In brain, only the 5.1 kb band was detected under our experimental

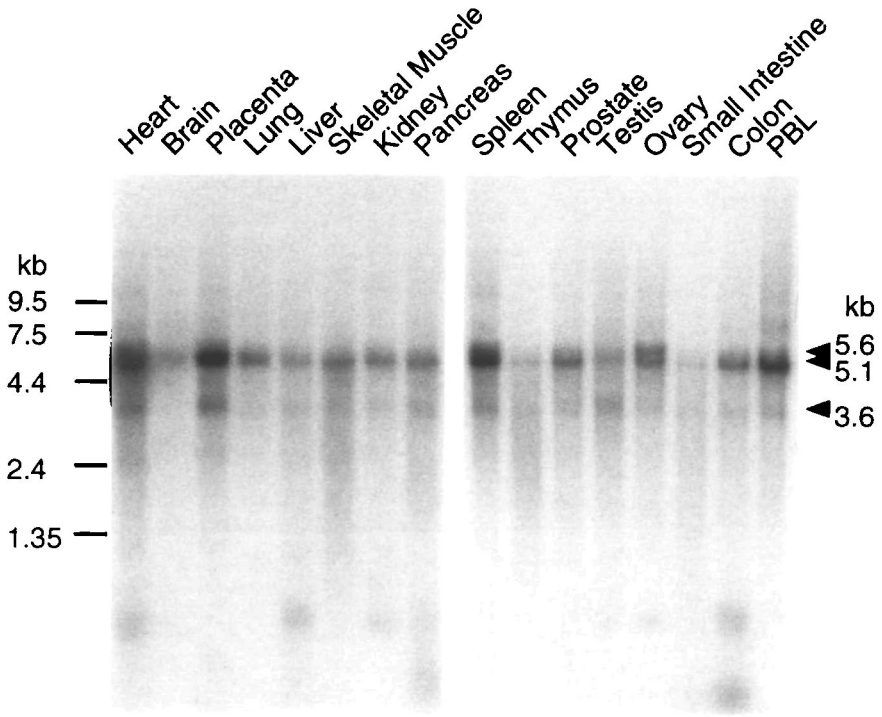

Fig. 3. **Northern blot analysis of poly-(A)$^+$ RNA from various human tissues.** A human adult tissue Northern blot (Clontech) was probed with ^{32}P-labeled cDNA as described in "EXRERIMENTAL PROCEDURES." Arrows indicate the positions of bands hybridized with the probe. Positions of molecular size markers are indicated at left. PBL: peripheral blood leukocyte.

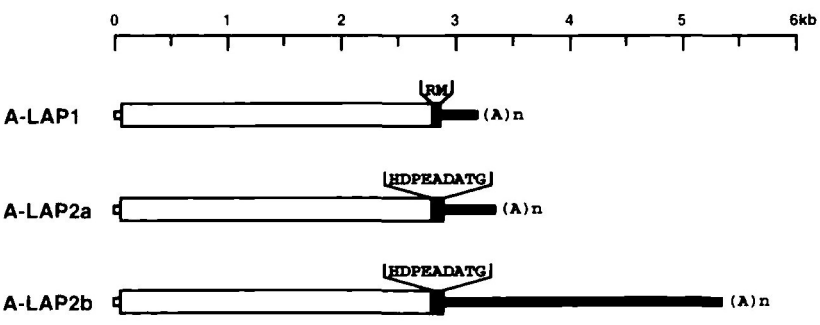

Fig. 4. **Schematic representation of three mRNAs encoding A-LAP.** The boxes denote the coding regions and thick lines represent untranslated regions. Amino acid sequences of the C-terminal ends different between A-LAP1 and A-LAP2 are shown in the figure.

conditions. Although the relative amount of mRNAs expressed in various tissues was not constant, their bands were reproducibly detected, indicating that there are at least three types of mRNA encoding A-LAP.

To obtain clones corresponding to 5.1 and 5.6 kb transcripts, we re-screened the human adipocyte cDNA library and isolated two new clones (termed A-LAP2a and A-LAP2b) encoding a different amino acid sequence from A-LAP1 (Fig. 4). Sequence analysis indicated that these two clones encoded the same protein of 948 amino acids, 7 amino acids longer than A-LAP1. As shown in the figure, the nucleotide sequences encoding the C-terminal amino acid and 3′-noncoding regions were entirely different between A-LAP1 and A-LAP2s, and the difference in 3′-noncoding region between A-LAP2a and A-LAP2b was due to the differential usage of polyadenylation signals. These results suggest that the A-LAP gene expression is regulated in a complicated manner. Since A-LAP2a differed in size from the 5.1 and 5.6 kb transcripts, there might be another transcript encoding A-LAP. For detailed analysis, we are now trying to determine the genomic sequence of the gene.

Expression and Subcellular Localization of A-LAP in COS-7 Cells—To determine the subcellular localization of A-LAP, COS-7 cells transiently transfected with the pSVL/A-LAP1 or the control vector (pSVL) were lysed in a Dounce-homogenizer and centrifuged for 1 h at 100,000 × *g* to prepare membrane and soluble protein fractions. A-LAP1 was then detected by Western blot analysis with an antibody against the C-terminal 15 amino acid sequence of the protein (termed C15). As expected from its calculated M_r, an immunoreactive protein of approximately 100 kDa was clearly detected in the lysate of COS-7 cells transfected with the expression vector but not in that of mock-transfected cells (Fig. 5, lanes 1 and 4). After centrifugation, the bulk of A-LAP1 was found in the soluble fraction with a minimal amount in the membrane fraction (Fig. 5, lanes 5 and 6). These results indicate that, in COS-7 cells, A-LAP1 is a soluble protein localized in cytosol. Because of the minimal structural difference between A-LAP1 and 2, it is probable that A-LAP2 would also localize in the cytosol if expressed in COS-7 cells. However, considering the minimal detection of the A-LAP1 protein in the membrane fraction, we could not completely rule out the possibility that, under certain conditions, A-LAP might localize in the membrane fraction due to the differential usage of translation start sites as discussed above. Unfortunately, we cannot test this possibility at present because of the low expression level of recombinant protein in COS-7 cells. It is noteworthy, however, that we recently found the production of A-LAP protein in Jurkat cells. After purification of these natural and/or recombinant proteins, we will analyze the structural features of A-LAP protein in detail, including its N-terminal end and oligomeric structure.

Aminopeptidase Activity of A-LAP—Since A-LAP contains the catalytic zinc binding motif $HEXXH(X)_{18}E$ in the primary structure and shows homology to M1 family of aminopeptidases, it is reasonable to speculate that A-LAP is also an enzyme with aminopeptidase activity. However, our initial trial to detect aminopeptidase activity in the crude cell extracts of transfected COS-7 cells employing Leu-MCA as a substrate was unsuccessful because extracts prepared from the control cells also revealed substantial activity. Therefore, lysates from COS-7 cells transfected with pSVL/A-LAP1 were incubated with C15 anti-A-LAP1 antibody bound to protein A-Sepharose, and immunoprecipitates were assayed for the enzymatic activity. As shown in Fig. 6, aminopeptidase activity against Leu-MCA was detectable only in immunoprecipitates formed using C15 antibody from pSVL/A-LAP1 transfected COS-7 cells but not from mock-transfected cells, establishing that A-LAP is indeed an aminopeptidase belonging to the M1 family of zinc-metallopeptidases.

We then measured the relative hydrolytic activity of the enzyme toward various aminoacyl-MCAs. As shown in Fig. 7, depending on the nature of the aminoacyl residue, aminopeptidase activity was detected in the immunoprecipitated extracts of transfected cells. Of the substrates examined, Leu-MCA was most efficiently hydrolyzed,

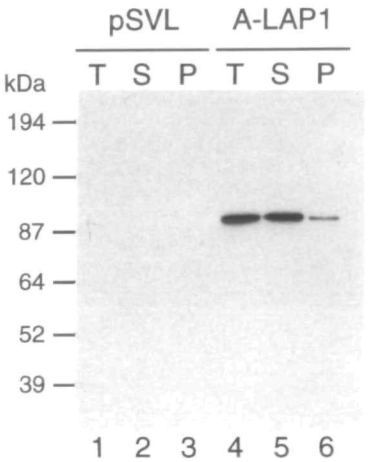

Fig. 5. **Localization of A-LAP expressed in COS-7 cells.** COS-7 cells transiently transfected with A-LAP and mock-transfected cells were lysed in a Dounce-homogenizer (T). The supernatant (S) and membrane (P) fractions were collected as described in "EXPERIMENTAL PROCEDURES," and samples were subjected to 8% SDS-PAGE followed by Western blot analysis using C15 anti A-LAP1 antibody. Positions of molecular size markers are indicated at left.

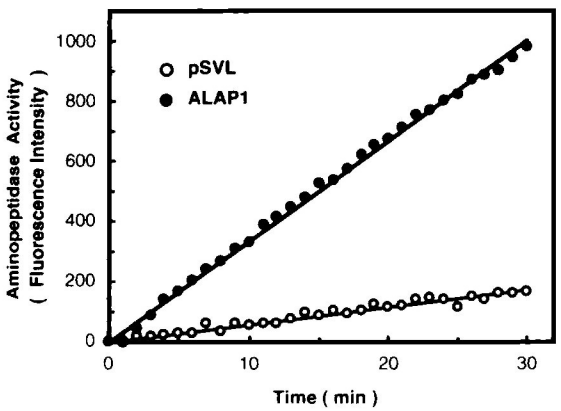

Fig. 6. **Aminopeptidase activity of A-LAP using Leu-MCA as a substrate.** Molecules immunoprecipitated by C15 anti A-LAP1 antibody from equal amounts of cell lysate preparations of COS-7 cells transiently transfected with A-LAP1 (●) or mock-transfected cells ( ) were assayed as described in "EXPERIMENTAL PROCEDURES."

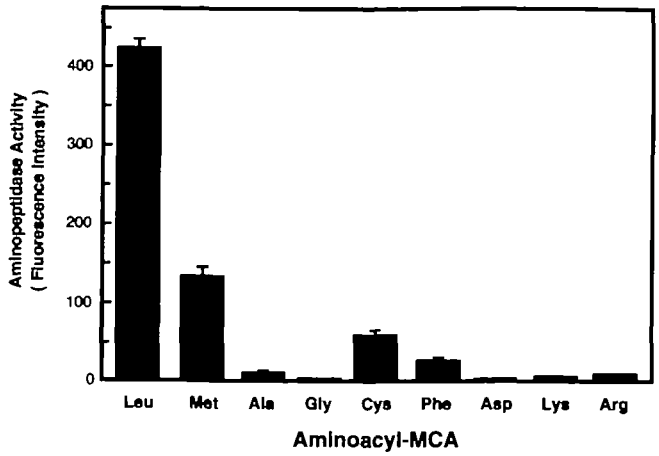

Fig. 7. **Substrate specificity of the aminopeptidase activity of A-LAP.** Immunoprecipitates of A-LAP-transfected or mock-transfected COS-7 cells were incubated with various aminoacyl-MCA substrates to assess peptidase activity. Differences between A-LAP- and mock-transfected COS-7 cell immunoprecipitates are presented in the figure. Error bars indicate SE values from four individual experiments.

followed by Met-MCA. Moderate activity was detected toward Phe- and *S*-benzyl-Cys-MCA, but little activity toward other aminoacyl-MCAs. These results clearly indicate that A-LAP preferentially hydrolyzed Leu-MCA and therefore should be considered a leucine aminopeptidase. Moreover, compared with P-LAP, which hydrolyzed Leu-, Arg-, and Lys-β-naphthylamide efficiently (*31*), A-LAP preferentially hydrolyzed Leu-MCA with little activity to Arg- and Lys-MCAs, also indicating its restricted substrate specificity.

To further characterize the enzymatic activity of A-LAP, the aminopeptidase activity was measured in the presence of various known inhibitors of proteolytic enzymes (Table I). Aminopeptidase inhibitors such as bestatin and amastatin at 100 μM inhibited the enzymatic activity of A-LAP, but puromycin, a PSA inhibitor, had little effect. Of the chelating agents tested, 1,10-phenanthroline inhibited the enzyme at concentrations between 10 and 100 μM, but EDTA had no effect up to 500 μM. E64 (cysteine protease inhibitor), leupeptin (serine/cysteine protease inhibitor), and pepstatin (aspartic acid protease inhibitor) had no inhibitory effect at 100 μM. A-LAP was found to be highly sensitive to zinc ions: as little as 10 μM $ZnCl_2$ decreased the enzymatic activity to 45% and at higher concentrations no residual activity was detected. We concluded that the overall inhibitor profile of A-LAP is consistent with those of aminopeptidases hitherto characterized (*5, 11, 32, 33*).

In this study, by searching the EST database, we have identified a novel aminopeptidase belonging to the M1 family of metallopeptidases, which we termed A-LAP. When the amino acid sequences of A-LAP and P-LAP were aligned using a computer program to maximize matching, striking homology (45% at the nucleotide level and 43% at the amino acid level) extending over their entire primary structures was observed, suggesting a possible evolutionary relationship between these two enzymes in spite of their different subcellular localization. We suggest here that they should be classified into an oxytocinase subfamily of the zinc-metallopeptidases. In our preliminary results,

TABLE I. **Effects of various inhibitors on A-LAP activity.**

Inhibitor	Conc (μM)	Residual activity (%)
None		100.0
Bestatin	100	37.4±0.9
	10	85.7±2.0
Amastatin	100	15.9±0.3
	10	74.4±1.3
Puromycin	100	91.7±1.0
	10	107.4±2.8
1,10-Phenanthrolin	1,000	21.6±0.4
	100	72.6±0.1
EDTA	500	110.3±0.8
	50	109.3±1.5
$ZnCl_2$	10	45.7±1.1
E64	100	108.3±2.8
Leupeptin	100	105.1±0.3
Pepstatin	100	116.8±0.6

we have obtained another sequence that shows striking homology to both A-LAP and P-LAP.

Initial characterization revealed that A-LAP is a soluble protein localized in the cytosolic fraction and that it preferentially hydrolyzes Leu-MCA. Although elucidation of its biological significance must await the discovery of physiological substrates, it is noteworthy that Met-MCA was a second preferential substrate for the enzyme, implying that the enzyme might play a role in the maturation of proteins by removing the initiator methionine. In addition, we speculate that since A-LAP has significant homology (29-30%) to AP-A, AP-N, and PSA, it might share some biological activity with these three enzymes. It was reported that these three aminopeptidases participate in the control of cell proliferation under certain experimental conditions (*11, 34, 35*). The availability of cDNA and recombinant protein will make it possible to characterize this novel enzyme with respect to its biochemical properties and physiological and/or pathological functions in detail.

We are grateful for The Division of Laboratory Animal Research, RIKEN for the preparation of anti-A-LAP antibody.

REFERENCES

1. Taylor, A. (1993) Aminopeptidases: structure and function. *FASEB J.* **7**, 290-298
2. Shipp, M.A. and Look, T.A. (1993) Hematopoietic differentiation antigens that are membrane-associated enzymes: cuting is the key? *Blood* **82**, 1052-1070
3. Look, A.T., Ashmun, R.A., Shapiro, L.H., and Peiper, S.C. (1989) Human myeloid plasma membrane glycoprotein CD13 (gp150) is identical to aminopeptidase N. *J. Clin. Invest.* **83**, 1299-1307
4. Wu, Q., Lahti, J.M., Air, G.M., Burrows, P.D., and Cooper, M.D. (1990) Molecular cloning of the murine BP-1/6C6 antigen: a member of the zinc-dependent metallopeptidase family. *Proc. Natl. Acad. Sci. USA* **87**, 993-997
5. Nanus, D.M., Engelstein, D., Gastl, G.A., Gluck, L., Vidal, M.J., Morrison, M., Finstad, C.L., Bander, N.H., and Albino, A.P. (1993) Molecular cloning of the human kidney differentiation antigen gp-160: human aminopeptidase A. *Proc. Natl. Acad. Sci. USA* **90**, 7069-7073
6. Rogi, T., Tsujimoto, M., Nakazato, H., Mizutani, S., and Tomoda, Y. (1996) Human placental leucine aminopeptidase/oxytocinase: a new member of type II membrane-spanning zinc metallopeptidase family. *J. Biol. Chem.* **271**, 56-61
7. Tsujimoto, M., Mizutani, S., Adachi, H., Kimura, M., Nakazato,

H., and Tomoda, Y. (1992) Identification of human placental leucine aminopeptidase as oxytocinase. *Arch. Biochem. Biophys.* **292**, 388-392
8. Mizutani, S. and Tomoda, Y. (1992) Oxytocinase: placental cystine aminopeptidase or placental leucine aminopeptidase (P-LAP). *Semin. Reprod. Endocrinol.* **10**, 146-153
9. Olsen, J., Cowell, G.M., Konigsofer, E., Danielsen, M., Moller, J., Laustsen, L., Hansen, O.C., Welinder, K., Engberg, J., Hunzilker, W., Spiess, M., Sjostrom, H., and Noren, O. (1988) Complete amino acid sequence of human intestinal aminopeptidase N as deduced from cloned cDNA. *FEBS Lett.* **238**, 307-314
10. Schauder, B., Schomburg, L., Kohler, J., and Bauer, K. (1994) Cloning of a cDNA encoding an ectoenzyme that degrades thyrotropin-releasing hormone. *Proc. Natl. Acad. Sci. USA* **91**, 9534-9538
11. Constam, D.B., Tobler, A.R., Resing-Ehl, A., Kemler, I., Hersh, L.B., and Fontana, A. (1995) Puromycin-sensitive aminopeptidase: sequence analysis, expression, and functional characterization. *J. Biol. Chem.* **270**, 26931-26939
12. Tobler, A.R., Constam, D.B., Schmitt-Graff, A., Malipiero, U., Schlapbach, R., and Fontana, A. (1997) Cloning of the human puromycin-sensitive aminopeptidase and evidence for expression in neurons. *J. Neurochem.* **68**, 889-897
13. Funk, C.D., Radmark, O., Fu, J.Y., Matsumoto, T., Jornvall, H., Shimizu, T., and Samuelsson, B. (1987) Molecular cloning and amino acid sequence of leukotriene A_4 hydrolase. *Proc. Natl. Acad. Sci. USA* **84**, 6677-6681
14. Minami, M., Ohno, S., Kawasaki, H., Radmark, O., Samuelsson, B., Jornvall, H., Shimizu, T., Seyama, Y., and Suzuki, K. (1987) Molecular cloning of a cDNA coding for human leukotriene A_4 hydrolase: Complete primary structure an enzyme involved in eicosanoid synthesis. *J. Biol. Chem.* **262**, 13873-13876
15. Fukasawa, K.Y., Fukasawa, K., Kanai, M., Fujii, S., and Harada, M. (1996) Molecular cloning and expression of rat liver aminopeptidase B. *J. Biol. Chem.* **271**, 30731-30735
16. Cadel, S., Foulon, T., Viron, A., Balogh, A., Midol-Monnet, S., Noel, N., and Cohen, P. (1997) Aminopeptidase B from the rat testis is a bifunctional enzyme structurally related to leucotriene-A_4 hydrolase. *Proc. Natl. Acad. Sci. USA* **94**, 2963-2968
17. Sambrook, J., Firitsch, E.F., and Maniatis, T. (1982) *Molecular Cloning: A Laboratory Manual*, pp. 2.108-2.111, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
18. Sanger, F., Nicklen, S., and Coulson, A.R. (1977) DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. USA* **74**, 5463-5467
19. Liu, F.-T., Zinnecker, M., Hamaoka, T., and Katz, D.H. (1979) New procedures for preparation and isolation of conjugates of proteins and a synthetic copolymer of D-amino acids and immunochemical characterization of such conjugates. *Biochemistry* **18**, 690-697
20. Kanaoka, Y., Takahashi, T., and Nakayama, H. (1977) A new fluorogenic substrate for aminopeptidase. *Chem. Pharm. Bull.* **25**, 362-363
21. Matsumoto, H. and Mori, T. (1998) Changes in cystine aminopeptidase (oxytocinase) activity in mouse serum, placenta, uterus and liver during pregnancy or after steroid hormone treatments *Zoolog. Sci.* **15**, 111-115
22. Kozak, M. (1984) Compilation and analysis of sequences upstream from the translational start site in eukaryotic mRNAs. *Nucleic Acids Res.* **12**, 857-872
23. Kyte, J. and Doolittle, R.F. (1982) A simple method for displaying the hydropathic character of a protein. *J. Mol. Biol.* **157**, 105-132
24. Hooper, N.M. (1994) Families of zinc metalloproteases. *FEBS Lett.* **354**, 1-6
25. Wang, J. and Cooper, M.D. (1993) Histidine residue in the zinc-binding motif of aminopeptidase A is critical for enzymatic activity. *Proc. Natl. Acad. Sci. USA* **90**, 1222-1226
26. Vazeux, G., Wang, J., Corvol, P., and Llorens-Cortes, C. (1996) Identification of glutamate residues essential for catalytic activity and zinc coordination in aminopeptidase A. *J. Biol. Chem.* **271**, 9069-9074
27. Rawlings, N.D. and Barrett, A. (1993) Evolutionary families of peptidases. *Biochem. J.* **290**, 205-218
28. Vazeux, G., Iturrioz, X., Corvol, P., and Llorens-Cortes, C. (1997) A tyrosine residue essential for catalytic activity in aminopeptidase A. *Biochem. J.* **327**, 883-889
29. Vazeux, G., Iturrioz, X., Corvol, P., and Llorens-Cortes, C. (1998) A glutamate residue contributes to the exopeptidase specificity in aminopeptidase A. *Biochem. J.* **334**, 407-413
30. Luciani, N., Marie-Claire, C., Ruffet, E., Beaumont, A., Roques, B.P., and Fournie-Zaluski, M.-C. (1998) Characterization of Glu^{350} as a critical residue involved in the N-terminal amine binding site of aminopeptidase N (EC 3.4.11.2): insights into its mechanism of action. *Biochemistry* **37**, 686-692
31. Sakura, H., Lin, T.Y., Doi, M., Mizutani, S., and Kawashima, Y. (1981) Purification and properties of oxytocinase, a metalloenzyme. *Biochem. Int.* **2**, 173-179
32. Yamada, R., Mizutani, S., Kurauchi, O., Okano, K., Imaizumi, H., Narita, O., and Tomoda, Y. (1988) Purification and characterization of human placental aminopeptidase A. *Enzyme* **40**, 223-230
33. Nagata, Y., Mizutani, S., Nomura, S., Kurauchi, O., Kasugai, M., and Tomoda, Y. (1991) Purification and properties of human placental aminopeptidase B. *Enzyme* **45**, 165-173
34. Ino, K., Goto, S., Okamoto, T., Nomura, S., Nawa, A., Isobe, K., Mizutani, S., and Tomoda, Y. (1994) Expression of aminopeptidase N on human choriocarcinoma cells and cell growth suppression by the inhibition of aminopeptidase N activity. *Jpn. J. Cancer Res.* **85**, 927-933
35. Welch, P.A. (1995) Regulation of B cell precursor proliferation by aminopeptidase A. *Int. Immunol.* **7**, 737-746