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# Genomic Organization of the Human Adipocyte–Derived Leucine Aminopeptidase Gene and Its Relationship to the Placental Leucine Aminopeptidase/Oxytocinase Gene[1]

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**The genomic organization of the gene encoding the human adipocyte–derived leucine aminopeptidase (A-LAP) has been determined. The gene is composed of 20 exons and 19 introns and spans approximately 47 kilobases of chromosome 5q15. The gluzincin aminopeptidase motif, the HEXXH(X)$_{18}$E zinc-binding motif essential for enzymatic activity, is encoded by exons 6 and 7. A comparison of the exon/intron boundaries, together with phylogenetic analysis, shows the close relationship between A-LAP and placental leucine aminopeptidase (P-LAP)/oxytocinase, another gluzincin aminopeptidase considered to be important for the maintenance of normal pregnancy. Primer extension analysis revealed two transcriptional initiation sites. Analysis of the sequence immediately upstream of the transcriptional initiation sites revealed that the A-LAP promoter contains no canonical TATA- or CCAAT-box, but has a PyPyA$_{+1}$N(T/A)PyPy initiator consensus sequence and multiple putative regulatory elements. Finally, luciferase-reporter assays revealed a functional promoter activitiy of the 5′-flanking region of the gene, and suggested that the activity is regulated in a cell type–specific manner.**

**Key words: adipocyte-derived leucine aminopeptidase, exon, genomic organization, intron, placental leucine aminopeptidase/oxytocinase.**

Aminopeptidases are exopeptidases that catalyze the hydrolysis of amino acid residues from the N-terminus of peptide or protein substrates. It is generally thought that they play important roles in protein maturation, activation, modulation, and degradation of bioactive peptides, and the determination of protein stability (*1*).

Human adipocyte–derived leucine aminopeptidase (A-LAP), originally identified and cloned as a highly homologous protein to placental leucine aminopeptidase (P-LAP)/oxytocinase, is an aminopeptidase belonging to the M1 zinc-metallopeptidase (gluzincin) family (*2*). Subsequently, a rat homologue was cloned as puromycin-insensitive leucyl-specific aminopeptidase (PILS-AP) (*3*).

Gluzincin aminopeptidases share the consensus HEXXH(X)$_{18}$E zinc-binding motif essential for enzymatic activity (*4*). It is now clear that this growing family of mammalian zinc-containing aminopeptidases includes membrane-bound [P-LAP, aminopeptidase N (APN), aminopeptidase A (APA), and thyrotropin-releasing hormone degrading enzyme (TRHDE)], cytosolic [puromycin-sensitive aminopeptidase (PSA) and leukotriene A$_4$ hydrolase (LTA4H)], and secretory [A-LAP and aminopeptidase B (APB)] proteins (*2, 5–11*). Mutational analyses have revealed that the essential amino acid residues are well conserved among members of the family (*12–15*). In our previous work, we proposed that because of the close similarity between A-LAP and P-LAP, these two enzymes should be classified into an oxytocinase subfamily of zinc-metallopeptidases (*2*).

Biochemical and enzymatic characterization of the recombinant human A-LAP protein indicated that the enzyme is a monomeric protein that shows a preference for leucine when the enzyme activity was measured using synthetic substrates (*3, 16*). On the other hand, A-LAP revealed relatively broad substrate specificity toward naturally occuring peptide hormones Our initial characterization of the enzyme indicated that the enzyme cleaves angiotensin II and III and converts kallidin to bradykinin, suggesting a role in the regulation of blood pressure through the inactivation of angiotensins and/or the generation of bradykinin in the kidney (*16*).

In this study, we investigated the genomic organization of the human *A-LAP* gene and compared it especially with that of the closely related human *P-LAP* gene. Our results demonstrate that members of the M1 family of zinc-containing aminopeptidases can be classified into several subfamilies according to nucleotide sequence similarities and confirm the close relationship between A-LAP and P-LAP.

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Abbreviations: A-LAP, adipocyte-derived leucine aminopeptidase; APA, aminopeptidase A; APB, aminopeptidase B; APN, aminopeptidase N; LTA4H, leukotriene A$_4$ hydrolase; PSA, puromycin-sensitive aminopeptidase; P-LAP, placental leucine aminopeptidase; TRHDE, thyrotropin-releasing hormone degrading enzyme.

## EXPERIMENTAL PROCEDURES

*Identification of the Human A-LAP Gene*—We obtained a genomic sequence of approximately 178 kb from the GenBank™/EMBL/DDBJ Data Bank; this sequence encompasses a region on chromosome 5q15 where the known *P-LAP* gene is localized (*17*). The location of the exons of the *A-LAP* gene were determined using the BLAST 2 program.

*Primer Extention Analysis*—Primer extension analysis was performed as described previously (*18*) using a $^{32}$P-labeled 24-mer primer, 5′-GGAAAGTAGAAATGACATGATTGC-3′, complementary to the sequence from 128 to 151 in the A-LAP cDNA previously numbered from 93 to 116 (*2*) The primer was hybridized with 10 or 20 μg of total RNA from HeLa S3 cells and then extended by SuperScript II reverse transcriptase (Life Technologies). The extended fragments were analyzed in a 6% polyacrylamide denaturing gel Since exon 1 is too short for analysis, we constructed a chimeric sequencing template plasmid containing the 5′-flanking sequence and the cDNA sequence from 1 to 151 prepared by deleting intron 1.

*Analysis of Promoter Activity*—The human A-LAP 5′-flanking region and its fragments, which include the transcriptional initiation site, were amplified by PCR from genomic DNA prepared from leukocytes obtained from healthy donors and the sequences were confirmed PCR products were initially subcloned into the pGEM-T Easy vector and the *Hin*dIII fragments were transferred to the 5′-end of the luciferase gene in the promoter-less plasmid, pGL3 basic (Promega). The nucleotide sequences of the primers used for PCR amplification were as follows (5′-3′): cccaagcttGAGATAGAAGGTAGGCAC: (sense strand of the sequence from −1305 to −1288) for phAPr1, cccaagcTTCTTGCGCAAAATCCAAG: (sense strand of the sequence from −944 to −926) for phAPr2, cccaagcttGGATCCGCGTTCAGAAAG: (sense strand of the from −248 to −231) for phAPr3, cccaagcttGTGCAGCGCTCATTTAC: (sense strand of the sequence from −176 to −160) for phAPr4, cccaagcttCCCCACTCCCGTTTAC· (sense strand of the sequence from −121 to −106) for phAPr5, cccaagcttGCCTGCCGCTAGGGCTC: (sense strand of the sequence from −68 to −52) for phAPr6, and cccaagcttAAAGTGAAAGTGGAGCCC: (antisense strand of the sequence from −13 to +5) for all constructs. We constructed phAPr7 by deleting a *Sma*I fragment from phAPr6.

HeLa S3 cells were seeded at 1 × $10^5$ cells per well in 12-well plates in Dulbecco's Modified Eagle's Medium containing 10% calf serum, and incubated for 24 h at 37°C. The cells were then washed two times with Opti-MEM, and the luciferase plasmids (2.4 μg) and a pCMVβ plasmid (0 8 μg) (Clontech), which served as an internal control of transfection efficiency, were transfected into cells with LipofectAMINE (Life Technologies). U937 cells were seeded at 2 × $10^6$ per well in 6-well plates in RPMI 1640 medium containing 10% calf serum, and transfected immediately with the luciferase plasmids (0.75 μg) and a pCMVβ plasmid (0.25 μg) using the Effectene Transfection Reagent (Qiagen). After 48 h of transfection, the cells were washed three times with PBS, and then lysed in reporter lysis buffer (Promega). Luciferase activity was then measured with a Luciferase Assay System (Promega) according to the manufacturer's instructions. Luciferase activity was measured in triplicate, the results were averaged, and then normalized to β-galactosidase activity to correct for transfection efficiency. β-Galactosidase activity was measured using *o*-nitrophenyl-β-D-galactopyranoside as the substrate.

## RESULTS AND DISCUSSION

*Isolation of the Human A-LAP Genome Clone*—To elucidate the structural features of the human *A-LAP* gene, we examined a human genome database prepared in BAC. This led to the identification of BAC clone RPCI-11 31O11 (Accession Number: AC009073), which contains all exons. All exons and intron–exon junctions were determined from the database. The sequences of the splice junctions of the gene obey the GT-AG rule (*19*) (Table I). As shown in Fig 1, the gene contains 20 exons ranging in size from 72 bp (exon 10) to 2,412 bp (exon 20) separated by introns ranging from 144 bp to 13,165 bp; the entire gene spans 47 kb The first exon (80 nucleotides) includes only the 5′-untranslated region. Exon 2 (541 nucleotides) includes the remaining 5′-untranslated region and the coding sequence for the first 175 amino acids of the protein, which includes a 23 amino acid signal sequence. The zinc-binding motif, HEXXH, which is conserved among many metallopeptidases (*4*), is encoded in exon 6, while the glutamic acid residue located 19 amino acids downstream, which together with the two histidine residues in the HEXXH motif binds the zinc ion, is encoded in exon 7. The GAMEN motif, which has been shown to be important for the enzymatic action of gluzincin aminopeptidases (*13, 15*), is also encoded in exon 6. Exon 19 contains the coding sequence for the last 51 amino acids of the A-LAP1 protein, the stop codon, and the 3′-untranslated region This 3′-untranslated region contains at least two consensus polyadenylation signals. Exon 20 contains the coding sequence for the last 9 amino acids of the A-LAP2 protein, the stop codon, and the portion of the 3′-untranslated region containing at last two consensus polyadenylation signals. A nucleotide sequence (GAAGAG*gt*aaaaaa) matching the GT-AG rule (*19*) in exon 19 functions as a splicing donor to generate A-LAP2 mRNAs. These results reflect the fact that four cDNAs encoding A-LAP have been cloned so far (*2, 3*).

It should be noted that exon 10 encodes a serine-rich sequence that may function as a hinge region This sequence is deleted in the amino acid sequence of P-LAP when aligned with A-LAP (*2*) Moreover, we have cloned another cDNA (termed L-NAP) (Accession Number: AY028805) encoding an amino acid sequence highly homologous to both A-LAP and P-LAP, and found that the stop codon is inserted into the corresponding sequence. Searching the database, these three genes are found to be located closely between versican and calpastatin at around chromosome 5q15 (Fig 2). Although we have not been able to detect the enzyme activity of L-NAP at present, we would like to speculate that the functional variation between these enzymes (if any) is mediated in part by the divergence of the corresponding sequence coded in exon 10 of the *A-LAP* gene.

*Phylogenetic Relationship of Aminopeptidases*—P-LAP and A-LAP are both classified into the M1 family of gluzincine aminopeptidases. The contiguous chromosomal location of A-LAP and P-LAP and their high sequence homology (43%) also suggest that they might have diverged by duplication of the ancestral gene. To gain better insight into

the relationships involved in the molecular evolution of this family, a phylogenetic tree was constructed by the method of Higgins and Sharp (*20*) based on the nucleotide sequence identities. According to this dendrogram, it is apparent that

TABLE I **Exon-intron boundaries of the human *A-LAP* gene.**

| Exon No | Size (bp) | 5'splice donor | Intron Size (bp) | 3'splice acceptor | Amino Acid at splice site |
|---|---|---|---|---|---|
| 1 | 80 | **CCCCAG***gtacagcg* | 3920 | *gccgcta**g*GTAGGT | --- |
| 2 | 541 | **ACTGAG***gtattttt* | 2403 | *aattttag***GATACT** | 175Arg$^{II}$ |
| 3 | 139 | **CCATTG***gtgagtct* | 3562 | *gattttag***GTGAAA** | 221Leu/Val$^{0}$ |
| 4 | 135 | **GTCAAG***gtgagcct* | 2012 | *ctctttag***GTTTCT** | 266Lys/Val$^{0}$ |
| 5 | 121 | **AACAAG***gtagagat* | 1087 | *ttcccaag***ATCTTG** | 307Asp$^{I}$ |
| 6 | 155 | **CACCAG***gtataagc* | 289 | *gcttttag***TGGTTT** | 358Gln/Trp$^{0}$ |
| 7 | 114 | **AAAGTT***gtaagtag* | 1207 | *ctcaatag***GGAGAT** | 396Val/Gly$^{0}$ |
| 8 | 132 | **GATAAG***gtaaaagt* | 1407 | *tcttttag***GGAGCT** | 440Lys/Gly$^{0}$ |
| 9 | 132 | **GCAAGT***gtgagtat* | 144 | *gtttttag***ATTTGC** | 484Ser/Ile$^{0}$ |
| 10 | 72 | **TCCTCA***gtaagttt* | 1611 | *cacaccag***CATTTG** | 508Ser/His$^{0}$ |
| 11 | 155 | **CACTGG***gtaatgct* | 1984 | *tcttttag***GTACCT** | 560Gly$^{II}$ |
| 12 | 80 | **AAACAG***gtaattta* | 498 | *tactctag***ATGTGC** | 587Asp$^{I}$ |
| 13 | 184 | **CGTCAG***gtaataca* | 1706 | *ttggtcag***CATTGG** | 648Ser$^{II}$ |
| 14 | 157 | **TTCAAG***gtaaaagc* | 682 | *cgccctag***GCCTTC** | 700Lys/Ala$^{0}$ |
| 15 | 185 | **CCTGAG***gtcagtcc* | 1194 | *ttctatag***CCTGCC** | 762Ser$^{II}$ |
| 16 | 162 | **TCAATG***gtgagtcc* | 494 | *ccttctag***GCTACT** | 886Trp$^{II}$ |
| 17 | 141 | **ACAAAA***gtaagtgg* | 552 | *ctctctag***GTTTGA** | 863Lys$^{II}$ |
| 18 | 82 | **GAAGAG***gtaaaaaa* | 3863 | *ctcacaag***GTAAAA** | 890Glu/Val$^{0}$ |
| 19$^{A\text{-}LAP1}$ | 428$^{1a}$ (2060$^{1b}$) | --- | --- | --- | --- |
| 19$^{A\text{-}LAP2}$ | 148 | **TTGAAC***gtatgtaa* | 13165 | *attttcag***ATGATC** | 940His$^{I}$ |
| 20 | 498$^{2a}$ (2412$^{2b}$) | --- | --- | --- | --- |

All exon sequences are represented by boldface uppercase letters, and intron sequences are represented by lowercase letters. The dinucleotide consensus sequence at the splice sites is depicted in italic lowercase letters. All nucleotide sequences are presented in the 5′ to 3′ direction. Superscripts following the amino acid at the splice junction denote codon phasing of the open reading frame. The size of exon 1 was determined by primer extension analysis. Superscripts following exons 19 and 20 denote the size of each exon of the four isoforms.

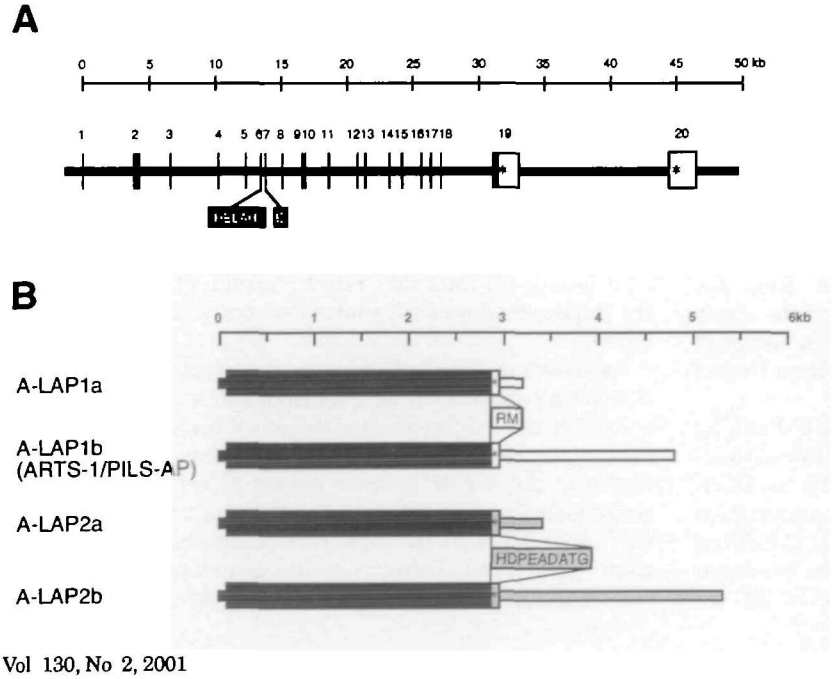


Fig 1 **Genomic structure of the human *A-LAP* gene.** A Schematic representation of the exon/intron structure of the gene. Exons are numbered and depicted as boxes. Asterisks indicate stop codons. B Structure of the four transcripts so far identified. The boxes denote the coding regions and thin lines represent untranslated regions. Amino acid sequences of the C-terminal ends that differ between A-LAP1 and A-LAP2 are shown in the figure. The human A-LAP1b cDNA sequence was depicted in the GenBank™/EMBL/DDBJ Data Bank as the aminopeptidase regulator of type I TNF receptor shedding (ARTS-1) (Accession Number AF183569) or PILS-AP (Accession Number AF222340). Accession numbers for the other sequences are as follows: A-LAP1a (AF106037), A-LAP2a (AY028806), and A-LAP2b (AY028807).

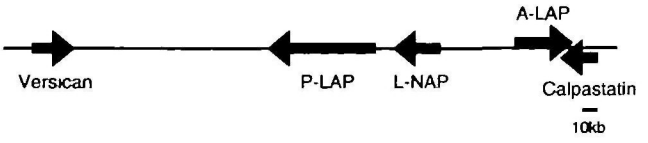


Fig 2. **Schematic representation of the contiguous genomic sequence around chromosome 5q15.** Genes are represented by arrows denoting the direction of the coding sequence.

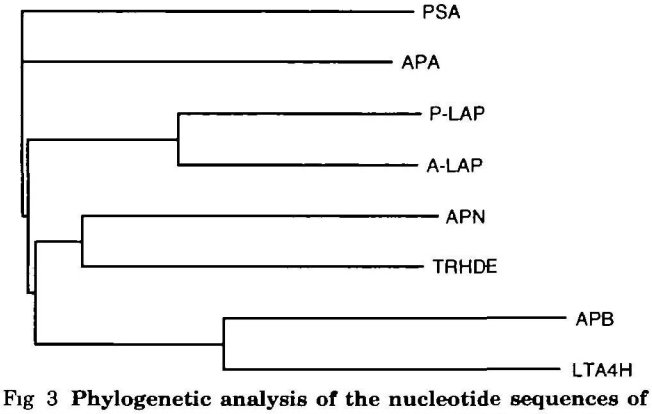


Fig 3 **Phylogenetic analysis of the nucleotide sequences of human aminopeptidases belonging to the M1 family of zinc-metallopeptidases.** This analysis was done with public software using ClustalW version 1.81 (http://www.ebi.ac.uk/clustalw/), the output was generated with TreeViewer. The following nucleotide sequences were used; A-LAP (AF106037), P-LAP (D50810), APA (NM_001977), APB (AJ242586), APN (X13276), PSA (NM_006310), TRHDE (NM_013381), and LTA4H (NM_000895) (GenBank™/DDBJ/EMBL databases). The distance between enzymes is proportional to the number of nucleotide differences and reflects evolutionary time since their divergence.

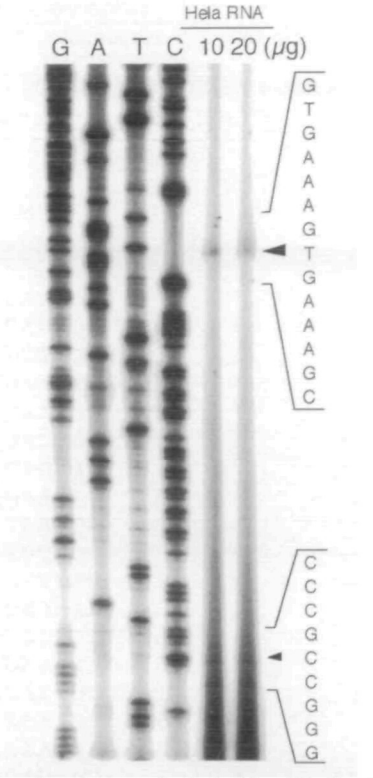


Fig 4 **Primer extension analysis to identify the transcription initiation sites.** A 24-mer $^{32}$P-labeled oligonucleotide complementary to the sequence from 128 to 151 in the A-LAP cDNA was hybridized with 10 or 20 μg of total RNA prepared from HeLa S3 cells. The primer-extended products were subjected to electrophoresis adjacent to a DNA sequencing ladder obtained using the chimeric sequencing template as described in "EXPERIMENTAL PROCEDURES." The sequences around the two initiation sites are shown in the figure

A-LAP and P-LAP belong to one distinct subfamily (Fig 3), suggesting the latest diversion from the common ancestral gene for this family. Indeed, alignment of the human *A-LAP* gene structure with the human *P-LAP* gene (*17*) shows that the exon–intron junctions are well conserved (not shown).

*Analysis of the Human A-LAP Gene Promoter*—Transcriptional initiation sites of the *A-LAP* gene were determined by primer extension analysis. Repeated experiments showed that although minor bands were observed by chance, at least two sites were constantly detected (Fig 4). The major site, which was designated as nucleotide position +1, matches a well-defined pyrimidine-rich initiator consensus sequence, $PyPyA_{+1}N(T/A)PyPy$, in which Py represents a pyrimidine residue (*21*). The minor site mapped at the guanosine locating 68 nucleotides downstream from the position +1 adenosine, and which does not match the above consensus sequence. Densitometric analysis that usage of the major site was, on average, 4.0-fold higher than that of the minor site.

Figure 5 shows the nucleotide sequence of the 5′-flanking promoter region of the human *A-LAP* gene. Computer analysis of the sequence revealed no canonical TATA- or CCAAT-box. On the other hand, several potential transcription factor binding motifs, such as MZF-1, IRF1/2, C/EBPα/β Sp1, and NF-κB (*22, 23*), were identified in the promoter region of the gene. Of note, there are unique 37 AC dinucleotide repeats from positions –322 to –249, which have been suggested to be important for calcium-dependent enkephalin gene transcription (*24*).

To characterize the regions regulating the transcriptional activity of the *A-LAP* gene, chimeric reporter plasmids encoding the luciferase gene and different lengths of the *A-LAP* gene were constructed. The resultant chimeric constructs were then transfected into either HeLa S3 or U937 cells, which express a natural A-LAP protein, in order to analyze the promoter activity. As a negative control, the promoter-less pGL3 basic plasmid was transfected into the cells. The luciferase activity due to each luciferase reporter plasmid was normalized for β-galactosidase activity by co-transfecting an internal control plasmid, pCMVβ, carrying the β-galactosidase gene under the control of the CMV promoter.

As shown in Fig. 6, all chimeric constructs containing the 5′-flanking sequence upstream from the $A_{+1}$ transcriptional initiation site exhibited some degree of functional promoter activity in HeLa S3 cells, indicating that the 5′-flanking region of the *A-LAP* gene is indeed able to support transcriptional initiation. The highest level of promoter activity was observed with the construct containing the sequence from –944 to +5. Deletion of the sequence from –248 to –177 significantly decreased the promoter activity (about

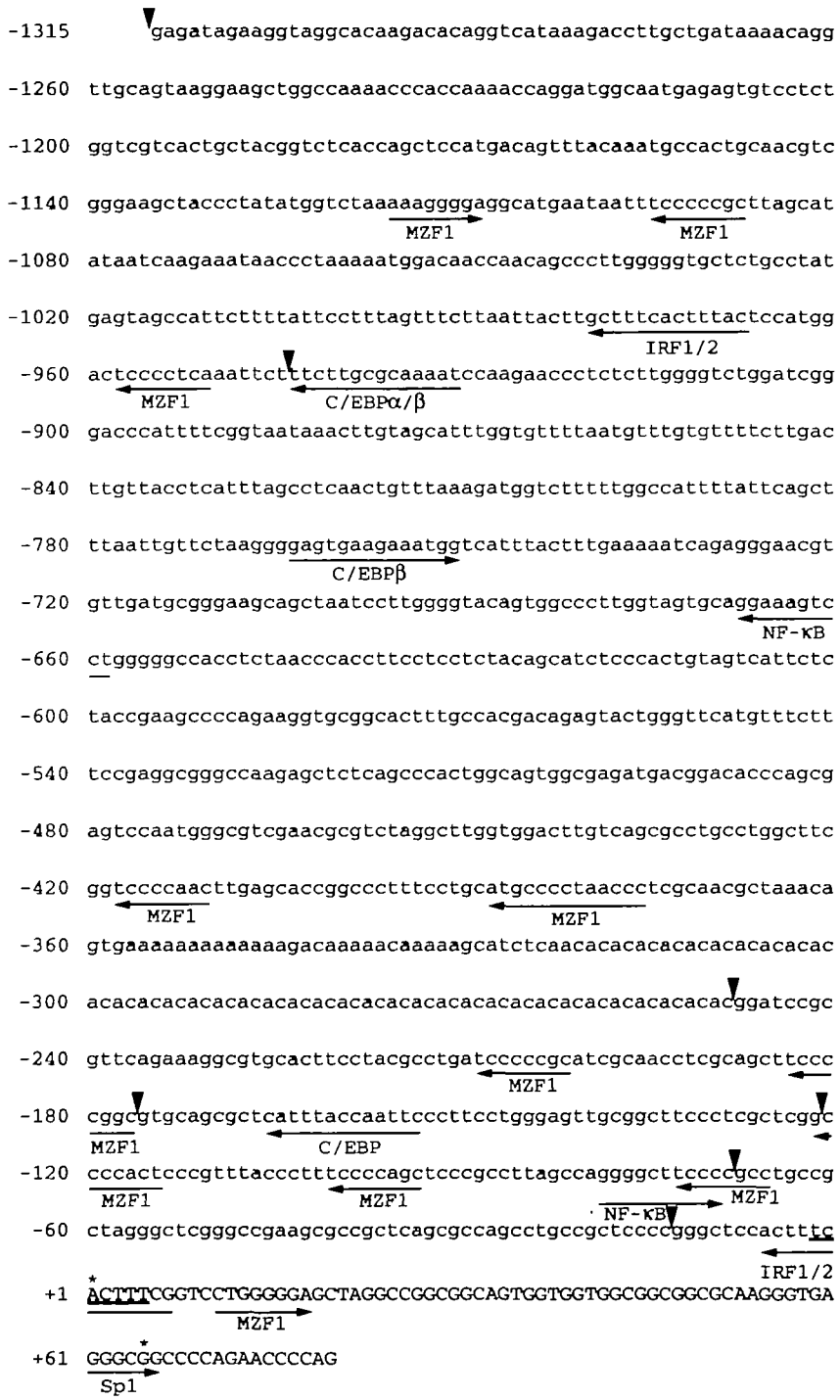


Fig 5 **Nucleotide sequence of the 5′-flanking region of the human *A-LAP* gene.** The 5′-flanking region was searched for transcription factor binding sites by TFSEARCH (http://mdap1 trc.rwcp.or.jp/research/db/TFSEARCH html) The exon sequence is shown in uppercase letters, while that of the untranscribed region is given in lowercase letters The transcriptional initiation sites determined by primer extension analysis are shown by asterisks. The numbering of the nucleotides begins with the adenosine matching the $PyPyA_{+1}N(T/A)PyPy$ consensus motif. The consensus binding sequences of some transcription factors are shown by arrows. For the detection of promoter activity, the start point of each construct is indicated by an arrowhead

50% decrease). A further decrease in the promoter activity was observed after the deletion of the sequence from −176 to −14. As expected, deletion of the initiation site caused the loss of promoter activity. On the other hand, chimeric constructs showing promoter activities were rather restricted in U937 cells. Deletion of the sequence from −1315 to −945 caused a decrease in the transcription activity of about 30%. A further 50% decrease in the activity was also observed after deletion of the sequence from −944 to −249. These results indicate that the 5′-flanking region of the *A-LAP* gene indeed has promoter activity and suggest that the transcriptional elements required for the full promoter activity of the *A-LAP* gene in HeLa S3 cells are different from those in U937 cells. Transcriptional *cis*-elements locating between −248 and −14 might act as co-operative activators in HeLa S3 cells but not in U937 cells. Further studies are required to characterize the element(s) responsible for the promoter activity of the *A-LAP* gene in detail.

In this study, we determined the genomic structure of the human *A-LAP* gene and characterized its promoter region employing a luciferase-reporter system. Our results also clarify the close relationship between A-LAP and P-LAP

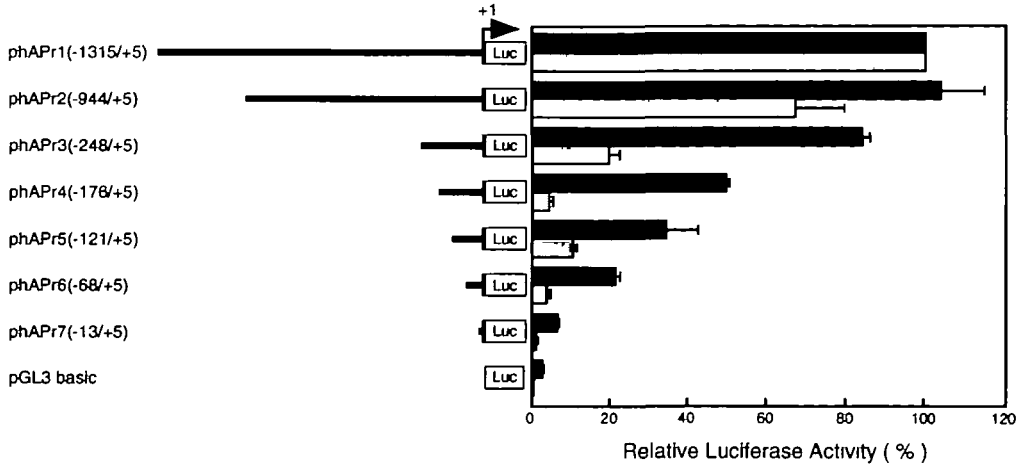


Fig 6 **Promoter activity of the human *A-LAP* gene.** Luciferase expression clones containing sequentially deleted fragments of the A-LAP chimera promoter constructs were transfected into either HeLa S3 cells (closed bars) or U937 cells (dotted bars) Luciferase activity was measured as described in "EXPERIMENTAL PROCEDURES" and normalized to the β-galactosidase activity of a co-transfected internal control plasmid The luciferase activity obtained from cells transfected with phAPr1 was taken as 100%

with respect to their genomic organization and chromosomal localization. Therefore we would like to propose that these two enzymes, together with the L-NAP protein, be classified into the oxytocinase subfamily of M1 zinc-metallopeptidases

Growing evidence indicates the biological significance of aminopeptidases belonging to the M1 family of zinc-metallopeptidases. Several physiological and/or pathological brain functions including blood pressure regulation and apoptosis are reported to be modulated by aminopeptidases such as PSA, APN, and APA (*9, 25–27*). In addition, it is believed that P-LAP plays a role in controlling the concentration of utero-tonic and vasoactive hormones, such as oxytocin and vasopressin, possibly derived from the fetus at the interface between fetus and mother, and thus preventing premature delivery and preeclampsia (*28, 29*). As for A-LAP, we found that the expression of the A-LAP protein increases transiently during the differentiation of murine 3T3-L1 preadipocytes into adipocytes (unpublished observation). In the database, A-LAP is described as a VEGF-induced aminopeptidase (Accession Number: AB047552), suggesting that the enzyme plays some roles during angiogenesis. Since we also observed an increase in the expression of the P-LAP protein during the NGF-induced differentiation of PC 12 cells into neuronal cells (*30*), it is tempting to speculate that aminopeptidases belonging to the oxytocinase subfamily play some roles during cell growth and differentiation. We are now analyzing the promoter activity of the murine *A-LAP* gene during the differentiation of 3T3-L1 preadipocytes into adipocytes. Taken together, our results provide the basis for further studies to elucidate the gene regulation and biological significance of A-LAP.

## REFERENCES


1 Taylar, A. (1993) Aminopeptidases: structure and function. *FASEB J* **7**, 290–298
2. Hattori, A., Matsumoto, H., Mizutani, S., and Tsujimoto, M. (1999) Molecular cloning of adipocyte-derived leucine aminopeptidase highly related to placental leucine aminopeptidase/oxytocinase. *J. Biochem.* **125**, 931–938
3 Schomburg, L., Kollmus, H., Friedrichsen, S., and Bauer, K. (2000) Molecular characterization of a puromycin-insensitive leucyl-specific aminopeptidase, PILS-AP *Eur J Biochem* **267**, 3198–3207
4. Hooper, N M (1994) Families of zinc metalloproteases. *FEBS Lett* **354**, 1–6
5. 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
6 Olsen, J, Cowell, G M, Konigsofer, E, Danielsen, M, Moller, J, Lausten, L, Hansen, O C, Welinder, K., Engberg, J, Hunziker, 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
7 Wu, Q, Lahti, J M, Air, G M, Burrows, P D, and Cooper, M D. (1990) Molecular cloning of the murine BP-1/6C3 antigen a member of the zinc-dependent metallopeptidase family. *Proc. Natl Acad. Sci. USA* **87**, 993–997
8. 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
9. Costam, D B., Togler, A.R., Resing-Ehl, A, Kemler, I, Hersh, L B, and Fantana, A. (1995) Puromycin-sensitive aminopeptidase sequence analysis, expression, and functional characterization *J. Biol Chem* **270**, 26931–26939
10 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
11. Fukasawa, K.Y, Fukasawa, K., Kanai, M, Fujii, S, and Harada, M. (1997) Molecular cloning and expression of rat liver aminopeptidase B. *J Biol Chem* **271**, 30731–30735
12 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
13 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
14. Iturrioz, X., Vazeux, G, Celerier, J., Corvol, P., and Llorens-Cortes, C (2000) Histidine 450 plays a crucial role in catalysis and, with $Ca^{2+}$, contributes to the substrate specificity of aminopeptidase A. *Biochemistry* **39**, 3061–3068
15 Laustsen, P G., Vang, S., and Kristensen, T (2001) Mutational analysis of the active site of human insulin-regulated amino-

peptidases. *Eur J Biochem.* **268**, 98–104
16 Hattori, A., Kitatani, K., Matsumoto, H , Miyazawa, S , Rogi, T., Tsuruoka, N , Mizutani, S , Natori, Y , and Tsujimoto, M (2000) Characterization of recombinant human adipocyte-derived leucine aminopeptidase expressed in Chinese hamster ovary cells. *J. Biochem.* **128**, 755–762
17. Rasmussen, T E , Pedraza-Diaz, S , Hardre, R , Laustsen, P.G , Carrion, A.G , and Kristensen, T (2000) Structure of the human oxytocinase/insulin-regulated aminopeptidase gene and localization to chromosome 5q21. *Eur J Biochem* **267**, 2297–2306
18 Matsumoto, K., Okuwaki, M , Kawase, H , Handa, H , Hanaoka, F , and Nagata, K. (1995) Stimulation of DNA transcription by the replication factor from the adenovirus genome in a chromatin-like structure. *J. Biol. Chem* **270**, 9645–9650
19. Shapiro, M B and Senapathy, P (1987) RNA splice junctions of different classes of eukaryotes sequence statistics and functional implications in gene expression *Nucleic Acids Res.* **15**, 7155–7174
20 Higgins, D G and Sharp, P H. (1988) Clustal a package for performing multiple sequence alignment on a micro computer. *Gene* **73**, 237–244
21. Javahery, R , Khachi, A , Lo, K., Zenzie-Gregory, B , and Smale, S T (1994) DNA sequence requirememts for transcriptional initiator activity in mammalian cells. *Mol Cell Biol* **14**, 116–127
22 Morris, J F , Hromas, R , and Rauscher, F J (1994) Characterization of the DNA-binding properties of the myeloid zinc finger protein MZF1 two independent DNA-binding domains recognize two DNA consensus sequence with a common G-rich core *Mol Cell Biol.* **14**, 1786–1795
23 Faisst, S and Meyer, S (1992) Compilation of vertebrate-encoded transcription factors. *Nucleic Acids Res.* **20**, 3–26
24. Dobi, A. and Agoston, D V. (1998) Submillimolar levels of calcium regulates DNA structure at the dinucleotide repeat $(TG/AC)_n$ *Proc. Natl Acad Sci USA* **95**, 5981–5986
25 Zini, S , Fournie-Zaluski, M -C , Chauvel, E., Roques, B P., Corvol, P , and Llorens-Cortes, C (1999) Identification of metabolic pathways of brain angiotensin II and III using specific aminopeptidase inhibitors predominant role of angiotensin III in the control of vasopressin release. *Proc. Natl Acad. Sci. USA* **93**, 11968–11973
26 Reaux, A., Fournie-Zaluski, M.-C , David, C , Zini, S , Roques, B , Corvol, P , and Llorens-Cortes, C (1999) Aminopeptidase A inhibitors as potential central antihypertensive agents. *Proc. Natl Acad. Sci. USA* **96**, 13415–13420
27 Osada, T , Ikegami, S , Takiguchi-Hayashi, K., Yamazaki, Y , Katoh-Fukui, Higashinakagawa, T , Sakai, Y , and Takeuchi, T (1999) Increased anxiety and impaired pain response in puromycin-sensitive aminopeptidase gene-deficient mice obtained by a mouse gene-trap method *J Neurosci* **19**, 6068–6078
28 Mizutani, S and Tomoda, Y (1992) Oxytocinase cystine aminopeptidase or placental leucine aminopeptidase (P-LAP) *Semin Reprod Endocrinol* **10**, 146–153
29 Matsumoto, H , Rogi, T , Yamashiro, K., Kodama, S , Tsuruoka, N , Hattori, A., Takio, K., Mizutani, S , and Tsujimoto, M (2000) Characterization of a recombinant soluble form of human placental leucine aminopeptidase/oxytocinase expressed in Chinese hamster ovary cells. *Eur J Biochem.* **267**, 46–52
30 Matsumoto, H , Nagasaka, T , Hattori, A., Rogi, T , Tsuruoka, N , Mizutani, S , and Tsujimoto, M (2001) Expression of placental leucine aminopeptidase/oxytocinase in neuronal cells and its action on neuronal peptides. *Eur J Biochem* **268**, 3259–3266