STRUCTURE AND EXPRESSION OF AMINOPEPTIDASE N

Jørgen Olsen, Klaus Kokholm, Ove Norén, and Hans Sjöström

Department of Medical Biochemistry and Genetics
Biochemistry Laboratory C
The Panum Institute
University of Copenhagen
Blegdamsvej 3. DK-2200 Copenhagen N. Denmark

1. STRUCTURE OF AMINOPEPTIDASE N

Aminopeptidase N (APN) is a very abundant membrane protein in the microvillar membrane of the small intestinal absorptive epithelial cell the enterocyte [1,2]. APN is an ectopeptidase and from its position in the brush border membrane it faces the small intestinal lumen. The enzyme is therefore readily supplied with substrate molecules in the form of oligopeptides derived from nutritional proteins following the actions of gastric and pancreatic proteases. It is generally accepted that the physiological function of APN (and other brush border peptidases) is to convert oligopeptides in the small intestinal lumen into amino acids which can subsequently be absorbed by amino acid carriers. Besides the small intestine APN is also found in a wide variety of other tissues such as the endometrium [3], the kidney, the spleen and the brain [2]. The physiological role of APN in these alternative locations is unknown but it has been suggested that APN might be involved in the degradation of regulatory peptides [2]. In the recent years the realisation that APN is expressed in cells of the immune system has lent support to the idea that APN might be involved in the processing of antigens [4].

Knowledge obtained from classical biochemical studies of APN [5,6], from electronmiscroscopic studies of liposomes containing reconstituted APN [7] and from the cloning of the human APN cDNA [8,9] permit a relatively detailed picture of APN to be drawn (fig. 1).

In the membrane APN is found as a dimer of two non covalently associated subunits with a relative molecular mass of 160 kDa. The APN monomer is a type II membrane protein and is anchored to the membrane via an uncleaved signal sequence. The membrane anchor is preceded by a short cytoplasmic tail of eight amino acids. A junctional segment of approximately 40 amino acids rich in serine and threonine residues separates the largest and catalytically active C-terminal part of the enzyme from the membrane by approximately 5 nm. Computer analysis of the amino acid sequence of the junctional segment show that it has a high probability of being glycosylated at one or more serine and threonine residues and recent

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experimental data using radioactively labelled sugars have confirmed that this part of the enzyme is indeed O-linked glycosylated (Kristina Norén et al., submitted). The cDNA sequence revealed 10 putative N glycosylation sites and previous analysis suggest that all of these sites are utilized. Two potential tyrosine sulfation sites were also predicted which is in agreement with the reported modification of tyrosine residues by sulphation in APN [10].

The cDNA sequence revealed the presence of the amino acid sequence His Glu x x His which is a Zn^{++} binding motif found in one class of metallo peptidases [11]. The APN HEXXH motif is located within a region displaying a relatively high homology to the E.coli aminopeptidase N (the product of the PEPN gene[12,13]). The bacterial enzyme has a cytosolic location which is in agreement with the enzyme lacking the region corresponding to the membrane anchor and the junctional segment. Thus these entities have been added or preserved during the evolution of the mammalian enzyme.

APN hydrolyses oligopeptides by successive cleavage of the N-terminal peptide bond. APN display highest activity towards oligopeptides with a aliphatic branched N-terminal amino acid, however, apart from oligopeptides with the structure NH_2 aminoacyl prolyl which can not be hydrolysed the enzyme is able to cleave a broad spectrum of oligopeptides [2].

The determination of the primary structure of APN led to two additional important discoveries. First it was shown that APN is identical to the myeloid surface marker CD13[9] which is a marker of haematopoietic cells committed to the myeloid lineage. Later it was discovered

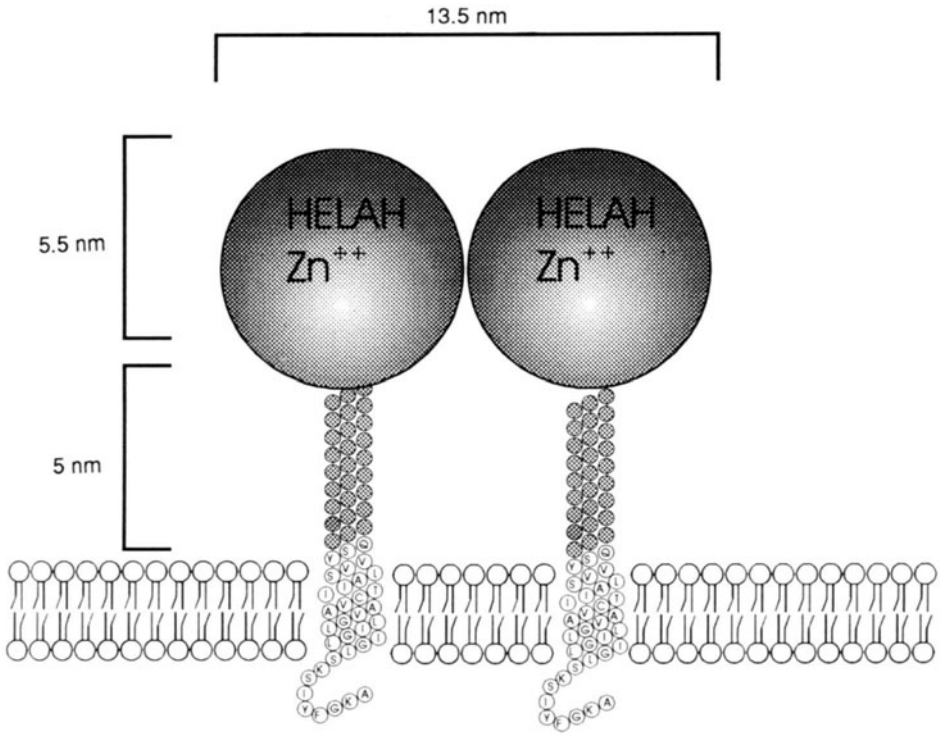

Figure 1. Structure of aminopeptidase N. APN is a type II membrane protein anchored to the membrane via an uncleaved signal sequence. The enzyme is found as a dimer of two non covalently associated monomers. The largest part of the enzyme has a globular shape and carries the active site which includes a HEXXH motif for Zn^{++} binding. The globular headgroup is separated from the membrane by a stalk which is O-linked glycosylated. In addition the APN amino acid sequence revealed 10 N linked glycosylation sites and 2 tyrosine sulphation sites.

that APN is used as a receptor for corona viridae. In pigs the transmissible gasteroenteridis virus (TGEV) which causes a severe gastroenteritis in piglets utilizes APN to enter the enterocytes [14] and likewise the pig respiratory corona virus (PRCV) make use of APN as receptor. In humans the 229E corona virus uses APN to enter alveolar cells and establish an upper respiratory tract infection [15].

2. AMINOPEPTIDASE N IN THE SMALL INTESTINE

Most of the knowledge obtained about APN is derived from studies on the enzyme in the small intestinal mucosa. The functional unit in the small intestinal epithelium is the crypt / villus entity. Immature stem cells located near the bottom of the crypts give rise to daughter cells which migrate out of the crypts and towards the tip of the villus where they undergo apoptosis and are eventually extruded to the intestinal lumen. The migration last from 2 to 3 days depending on the species and the epithelium is thus constantly renewed[16,17]. During the migration the enterocytes differentiate and start to express brush border enzymes. The expression of APN starts at the crypt / villus junction and this increase in APN expression is due to an increased level of APN mRNA [18,19]. Following its synthesis in the rough endoplasmic reticulum of the enterocyte APN is targeted to the apical microvillar membrane [20]. In the midvillar enterocyte which express the highest levels of APN the enzyme appears to be transported directly from the Golgi apparatus to the apical membrane [5,18] whereas in the more immature enterocytes in the upper crypt region some of the APN molecules may pass the basolateral membrane on their route to the apical membrane [18]. These findings have been extended by cell culture models. In the Madine Darby Canine Kidney (MDCK) cell line APN has been shown to be mainly directly transported to the apical membrane [21,22] whereas in the human colon carcinoma cell line Caco 2 which has the charasteristicts of an undifferentiated fetal enterocyte a substantial fraction of APN passes the basolateral membrane on the route to the apical microvillar membrane[23].

3. THE AMINOPEPTIDASE N GENE

The human and porcine APN genes have been cloned [24,25] (fig. 2) and mapped to chromosome 15 in man [26] and 7 in pigs [27]. The part of the human gene which encodes the

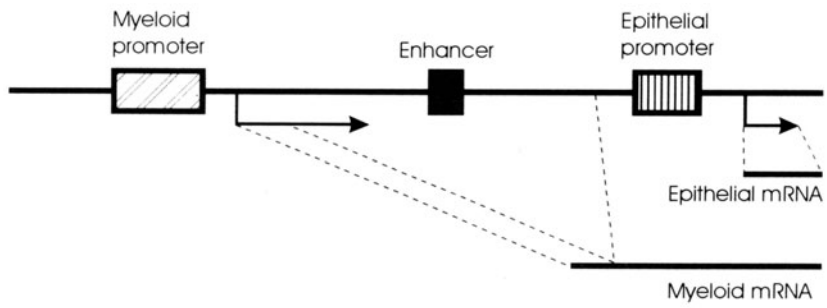

Figure 2. The APN gene is controlled by two promoters. An epithelial promoter close to the coding part of the gene drives transcription in epithelial cells. In cells of the hematopoetic system transcription occurs from an upstream myeloid promoter. The two promoters are separated by an intron which in the human gene measures approximately 8 kb. In this intron an enhancer with activity in both myeloid and epithelial cells is located. The mRNAs generated from the two promoters differ in their 5′ untranslated regions but encode the same enzyme as they share the same initiator methionine codon.

protein is divided by 20 exons [28]. Analysis of the transcriptional initiation sites in different tissues revealed the presence of two different APN promoters [29,30]. The promoters are arranged in tandem and in the human gene the distance between the two promoters have been mapped to approximately 8 kb. The epithelial promoter is closest to the coding part of the gene and is active in the enterocyte and other epithelial cells such as hepatocytes and endometrial cells [29,31]. The other myeloid promoter is placed upstream of the epithelial promoter and is active mainly in myeloid cells [30]. The transcripts from both promoters encode the same APN protein as they only differ in their 5′ untranslated regions. Sequence analysis shows that the epithelial promoter contains a TATA box whereas the myeloid promoter is GC rich and lacks a TATA box. In the large intron which separates the two promoters an enhancer with activity in both epithelial and myeloid cells is located [32].

4. THE EPITHELIAL AMINOPEPTIDASE N PROMOTER

When various deletions of the APN epithelial promoter were placed in front of a reportergene and transfected into epithelial cell lines (Caco-2 and HepG2) it was found that the first 123 bp upstream of the transcriptional initiation site were sufficient to secure full activity of the promoter [29,33]. DNase I footprinting of the epithelial promoter using nuclear extract from the Caco 2 colon carcinoma cell line reveals three protected regions from position +44 to -307 (fig. 3 and [29]).

The region from position -53 to -30 binds the Sp1 transcription factor [29]. Sp1 is a ubiquitously expressed transcription factor which binds to its recognition element via a DNA binding region containing three Zn fingers [34–38]. Sp1 has been shown to be important for the transcription of a wide variety of genes including both the so-called housekeeping genes which have promoters without a TATA box but usually contains multiple Sp1 sites as well as genes which contains a TATA box and are expressed in a more restricted tissue specific manner. Sp1 is one of the members in a gene family with at least two other members [39,40] which all share the ability to bind to a GC rich sequence with the core consensus sequence 5′ GGCGGG 3′. When an electrophoretic mobility shift assay is carried out with the APN Sp1 element and nuclear extract from Caco 2 cells three retarded bands are observed (fig. 4). By the use of specific antisera it was demonstrated that the most slowly migrating band is due to the binding of Sp1 whereas the two faster migrating bands are due to the binding of another Sp1 family member Sp3 (fig. 4). Sp3 lacks a transactivation domain and its binding therefore leads to a competitive inhibition of Sp1 mediated transcription [41].

The region from position -85 to -58 binds members of the hepatocyte nuclear factor 1 family [29,31,33]. HNF1α is a homedomain protein [42–45] which binds to DNA as a dimer. Dimerization occurs prior to DNA binding and is mediated through an N terminally located dimerization domain [46]. HNF1β is the other HNF1 family member encoded by a seperate gene [47–49]. At least in humans additional forms of the HNF1 proteins are generated through alternative splicing [50]. The HNF1 proteins are expressed in a variety of epithelial cell types. High amounts are found in hepatocytes, kidney proximal tubular cells and enterocytes [48,49,51,52]. In some cell types such eg. endometrial cells [31] only HNF1β is expressed. Otherwise a mixture of HNF1α and HNF1β is expressed and both homo and heterodimers can be formed. Both HNF1α and HNF1β are able to activate the APN epithelial promoter (fig. 5).

The region from position -112 to -90 has been designated the UF region (the upstream footprint region). A heterogeneous population of proteins bind to this element in

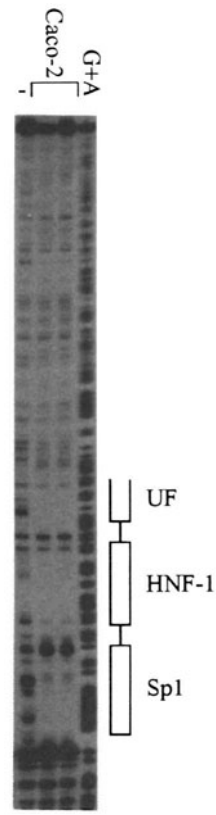

Figure 3. DNase I footprinting of the APN epithelial promoter. A DNA fragment (positions 307 to +44 of the porcine APN gene) was labelled on the upper strand, incubated without () or with 40μg nuclear proteins extracted from differentiated Caco 2 cells and digested with DNAse I. The resulting digestion products were eletrophoresed on a sequencing gel followed by autoradiography. The UF, the HNF1 and the Sp1 regions are indicated. A Maxam Gilbert G+A sequencing reaction is used as a marker.

different tissues. The sequence of the pig and the human UF element is an almost perfect inverted repeat of the sequence 5′ AGGTCA 3′ with a one bp spacing. This suggest that members of the steroid hormone nuclear receptor family bind to this element. Indeed competitive EMSA suggests that the COUP-tf factor which is a member of the steroid hormone nuclear receptor family [53] binds to the UF element [33]. Despite intensive investigations no function of the UF element could be demonstrated in neither Caco 2 nor in HepG2 cells [29,33]. However, it cannot be ruled out that the UF element under certain circumstances is important for stimulating transcription from the APN epithelial promoter.

5. THE MYELOID AMINOPEPTIDASE N PROMOTER

Little is known about functional elements present within the first 200 bp upstream of the major initiation site of the myeloid promoter. However, using nuclear extract prepared from the HUT 78 T cell line a footprint of potential importance have been identified (Kehlen et al., in preparation). The fact that the myeloid promoter is active in the HUT 78 cell line and that APN mRNA can be detected in these cells as well as in some other T cell

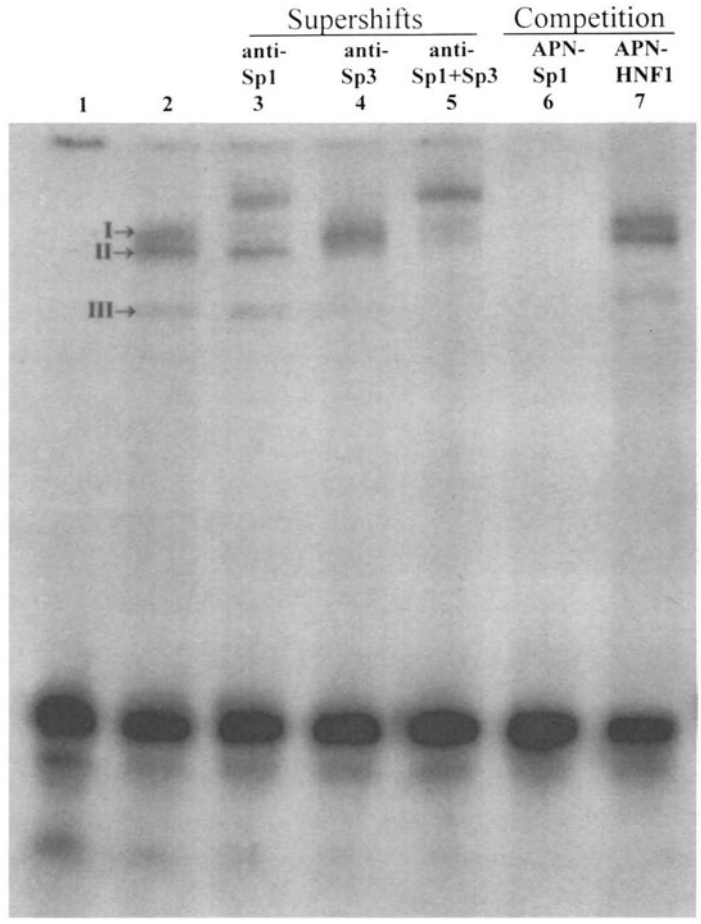

Figure 4. Sp1 and Sp3 bind to the APN promoter. Electrophoretic mobility shift assays (EMSA) with Caco-2 nuclear extracts (3 μg) was carried out with a ^{32}P labelled double-stranded oligonucleotide covering the Sp1 site of the APN promoter (APNSp1). Lane 3 shows a supershift with an antibody (1μl diluted 1/10) against Sp1 (Santa Cruz Biotechnologi): complex I is retarded. Addition of an antibody (1μl diluted 1/10) against Sp3 (Santa Cruz Biotechnologi) in lane 4 results in the disappearance of complexes II and III. The addition of both antibodies (lane 5) results in a supershift of complex I and the disappearance of complex II and III. Lanes 6 and 7 show the specificity of the three complexes. In lane 6 it is seen that all three complexes can be competed away with a 100 fold excess of unlabelled APNSp1 whereas a 100 fold excess of an unrelated probe (APNHNF1) does not affect any of the complexes.

lines [54–56] demonstrates that the CD13 marker is not entirely restricted to cells commited to the myeloid lineage although the highest levels of APN mRNA is found in myeloid cell types. With respect to putative enhancer elements present further upstream of the initiation sites utilized by the myeloid promoter, it has been elegantly shown that the Myb and Ets transcription factors cooperate to transactivate the myeloid APN promoter from sites present in the region from positions -291 to -411 [57]. Myb is a transcription factor which is known to be very important for hematopoesis as demonstrated by the severe disturbancies in the normal hematopoetic development observed in mice homozygous for a mutation in the myb gene [58]. Myb often cooperate with the Ets transcription factor [59–61] and this transcription factor was initially discovered as a fusion protein with a truncated Myb protein

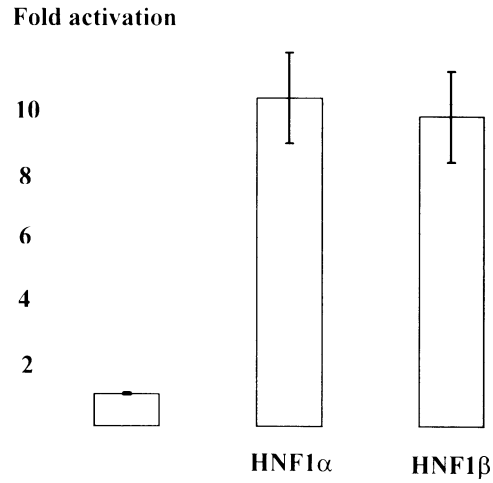

Figure 5. HNF1α and HNF1β activate the APN epithelial promoter. Transient transfections were carried out in C33-A cells which do not express HNF1 proteins. A plasmid (0.82 pmol) containing the CAT-gene under the control of a partial APN promoter (including the TATA-box, Sp1 site and the HNF1 site) was used together with a reference plasmid (0.25 pmol of pGL2 promoter; Promega Biotech, USA) containing the luciferase gene under the control of the constitutive SV40 promoter in all transfections. Co-transfections were performed as indicated with ekspression plasmids (0.67 pmol) for HNF1α (RSVHNF1α) and HNF1β (CMVHNF1β). This led to a ten fold activation from the APN promoter in either case.

in the E26 chicken leukemia virus [62,63]. Ets is the prototype in the large Ets transcription factor family which includes more than 20 members all sharing a common DNA binding motif the Ets domain [64]. Although Ets family members are also found in eg. epithelial cells many of the Ets domain factors are thought to be involved in the regulation of cell proliferation and differentiation in hematopoetic cells [64].

6. THE AMINOPEPTIDASE N ENHANCER

The screening of 5 kb upstream of the porcine epithelial APN promoter led to the identification of an enhancer fragment of 300 bp [32]. The enhancer is very well conserved between pig and man (Olsen et al., unpublished) and it is located between the two APN promoters 2.7 kb upstream of the epithelial promoter. In Caco 2 cells the addition of the enhancer increases transcription from the basal epithelial promoter almost 4 fold. The enhancer is a *bona fide* enhancer as it retains activity when it is transferred to a heterologous promoter (the SV40 early promoter) and the stimulation by the APN enhancer fragment is independent of its orientation and position relative to the heterologous promoter. Analysis of the promoter in 6 different cell lines showed that the activity of the enhancer is cell type dependent. Relatively high enhancer activity was found in both epithelial cell lines (Caco 2 and HepG2) and in the leukemia cell line K562 indicating that the enhancer might be able to cooperate with both APN promoters. The K562 cell line was established from a patient with chronic myelogeneous leukemia in blast chrisis [65] and has subsequently been shown to be able to differentiate into cells of both the erythroid and myeloid lineages [66]. Interestingly the enhancer was without effect in the adult T cell leukemia cell line Jurkat which expresses very little APN mRNA suggesting that the enhancer might be involved in

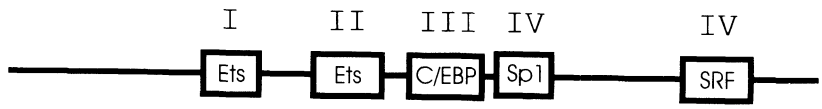

Figure 6. Structure of the APN enhancer. A schematical view of the 300 bp APN enhancer fragment from the porcine APN gene. The relative positions of regions protected by nuclear extract from Caco 2 cells are indcated as are the potential interacting transcription factors.

driving the transctription from the myeliod APN promoter preferentially in cells of the myeloid lienage. DNase I footprinting of the enhancer revealed 5 protected regions (fig. 6, I to V) using Caco 2 nuclear extracts. The footprints I to IV are all required for activity in Caco 2 cells as demonstrated by deletion analysis and by individual point mutations. The footprint V contains the consensus sequence for the serum response factor [67,68] and although the region containing this element was not required for activity in Caco 2 cells the possibility that the footprint V can confer responsiveness to growth factors still needs to be investigated. This is of particular importance as renal carcinoma cells have been shown to upregulate the expression of APN following stimulation with interleukin 4 [69]. Moreover epidermal growth factor (EGF) has also been shown to increase the intestinal expression of APN *in vivo* [70].

The regions covered by footprint I and II each contain a putative binding site for the PEA3 transcription factor. PEA3 is a member of the Ets transcription factor family and it is expressed in some epithelial cells [71]. As the Ets transcription factors recognise very similar DNA sequences [64] it is very probable that an Ets family member might bind to footprint I and II in myeloid cells and therefore have the posibility of cooperating with Myb bound upstream of the myeloid promoter. The footprint III contains a putative C/EBP binding site. C/EBP is a transcription factor which is mainly expressed in hepatocytes, enterocytes and adipocytes and it is involved in regulating transcription from several genes expressed in the liver [45]. Footprint IV contains a Sp1 element. From the mutational analysis it is clear that the most severe mutations were located to the middle of footprint I and in the 3′ end of footprint II. None of these mutations were directly in the Ets elements mentioned above and it is possible that other and yet unidentified factors bind to these regions.

7. TISSUE SPECIFIC TRANSCRIPTION FROM THE APN GENE

In a careful study carried out 30 years ago Rehfeld [72] could list 22 organs that expressed APN activity. The activity found in these organs varied by more than two orders of magnitude. We are now closer to understanding the molecular basis for the part of this remarkable regulation that occurs at the level of transcription. First of all two different promoters divide the task of mediating transcription of the common transcription unit. The epithelial promoter is a target for a ubiquitously expressed and a tissue specific transcription factor. The net result of this combination is a promoter with its activity limited to a subset of epithelial cells. The myeloid promoter responds to two well established haematopoietic transcription factors (Myb and Ets) which probably cooperates with other haematopoietic transcription factors binding to more proximal elements in the myeloid promoter. An enhancer with cell type dependent activity is located between the two promoters. The enhancer is active both in cell types which expresses APN as well as in cell

types that do not. However, the enhancer will only affect APN transcription in cell types where either the upstream or the downstream promoter is already active as the function of an enhancer is to modulate the activity of a corresponding promoter.

One aspect of APN expression that is still poorly understood is its regulation by cytokines and other growth factors. The enhancer display some properties suggesting that it may be involved in this process and the role of the enhancer as target for intracellular signal transduction molecules is a topic that will be important to illuminate in future experiments.

REFERENCES

1. Norén O, Sjöström H, Danielsen EM, et al. Desnuelle P, Sjöström H, Norén O, editors.Molecular and cellular basis of digestion. Elsevier Science Publishers B.V. 1986; 19, The enzymes of the enterocyte plasma membrane. p. 335–65.
2. Kenny AJ, Stephenson LS, Turner AJ. ; Kenny AJ, Turner AJ, editors.Mammalian ectoenzymes. Amsterdam: Elsevier Science Publishers B.V. 1987; 7, Cell surface peptidases. p. 169–210.
3. Classen-Linke I, Denker H-W, Winterhager E. Apical plasma membrane-bound enzymes of rabbit uterine epithelium. Pattern changes during the periimplantation phase. Histochemistry 1987;87:517–29.
4. Larsen SL, Pedersen LO, Buus S, Stryhn A. T cell responses affected by aminopeptidase N (CD13)-mediated trimming of major histocompatibility complex class II-bound peptides. J Exp Med 1996;184:183–9.
5. Danielsen EM, Cowell GM, Norén O, et al. Kenny AJ, Turner AJ, editors.Mammalian ectoenzymes. Amsterdam: Elsevier Science Publishers B.V. 1987; 3, Biosynthesis. p. 47–85.
6. Danielsen EM, Cowell GM, Norén O, Sjöström H. Biosynthesis of microvillar proteins. Biochem J 1984;221:1–14.
7. Hussain MM, Tranum-Jensen J, Norén O, Sjöström H, Christiansen K. Reconstitution of purified amphiphilic pig intestinal microvillus aminopeptidase. Mode of membrane insertion and morphology. Biochem J 1981;199:179–86.
8. Olsen J, Cowel GM, K nigsh fer E, Danielsen EM, M ller J, Laustsen L, Hansen OC, Welinder KG, Engberg J, Hunziker W, Spies M, Sjöström H, Norén O. Complete amino acid sequence of human intestinal aminopeptidase N as deduced from cloned cDNA. FEBS Lett 1988;238:307–14.
9. Look AT, Ashmun RA, Shapiro LH, Peiper SC. Human myeloid plasma membrane glycoprotein CD13 (gp150) is identical to aminopeptidase N. J Clin Invest 1989;83:1299–307.
10. Danielsen EM. Tyrosine sulfation, a post-translational modification of microvillar enzymes in the small intestinal enterocyte. EMBO J 1987;6:2891–6.
11. Hooper NM. Families of zinc metalloproteases. [Review]. FEBS Letters 1994;354:1–6.
12. McCaman MT, Gabe JD. The nucleotide sequence of the pepN gene and its over-expression in Escherichia coli. Gene 1986;48:145–53.
13. Foglino M, Gharbi S, Lazdunski A. Nucleotide sequence of the pepN gene encoding aminopeptidase N of Escherichia coli. Gene 1986;49:303–9.
14. Delmas B, Gelfi J, L'Haridon R, Vogel LK, Sjöström H, Norén O, Laude, H. Aminopeptidase N is a major receptor for the entero-pathogenic coronavirus TGEV. Nature 1992;357:417–20.
15. Yeager CL, Ashmun RA, Williams RK, Cardellichio CB, Shapiro LH, Look, AT, Holmes KV. Human aminopeptidase N is a receptor for human coronavirus 229E. Nature 1992;357:420–2.
16. Gordon JI. Intestinal epithelial differentiation: new insights from chimeric and transgenic mice. J Cell Biol 1989;108:1187–94.
17. Simon TC, Gordon JI. Intestinal epithelial cell differentiation: new insights from mice, flies and nematodes. Curr Opin Genet Develop 1995;5:557–86.
18. Hansen GH, Niels-Christiansen LL, Poulsen MD, Norén O, Sjöström H. Distribution of three microvillar enzymes along the small intestinal crypt-villus axis. J Submicrosc Cytol Pathol 1994;26:453–60.
19. Norén O, Dabelsteen E, Høyer PE, Olsen J, Sjöström H, Hansen GH. Onset of transcription of the aminopeptidase N (leukemia antigen CD13) gene at the crypt/villus transition zone during rabbit enterocyte differentiation. FEBS Lett 1989;259:107–12.
20. Hansen GH, Sjöström H, Norén O, Dabelsteen E. Immunomiscroscopic localization of aminopeptidase N in pig enterocyte. Implications for the route of intercellular transport. Eur J Cell Biol 1987;43:253–9.
21. Wessels HP, Hansen GH, Fuhrer C, Look AT, Sjöström H, Norén O, Spiess M. Aminopeptidase N is directly sorted to the apical domain in MDCK cells. J Cell Biol 1990;111:2923–30.

22. Vogel LK, Spiess M, Sjöström H, Norén O. Evidence for an apical sorting signal on the ectodomain of human aminopeptidase N. J Biol Chem 1992;267:2794–7.
23. Vogel LK, Norén O, Sjöström H. Transcytosis of aminopeptidase N in Caco-2 cells is mediated by a non-cytoplasmic signal. J Biol Chem 1995;270:22933–8.
24. Look AT, Peiper SC, Rebentisch MB, Ashmun RA, Roussel MF, Lemons RS, Le Beau MM, Rubin CM, Sherr CJ. Molecular cloning, expression, and chromosomal localization of the gene encoding a human myeloid membrane antigen (gp150). J Clin Invest 1986;78:914–21.
25. Olsen J, Sjöström H, Norén O. Cloning of the pig aminopeptidase N gene. Identification of possible regulatory elements and the exon distribution in relation to the membrane-spanning region. FEBS Letters 1989;251:275–81.
26. Kruse TA, Bolund L, Grzeschik KH, Ropers HH, Olsen J, Sjöström H, Norén O. Assignment of the human aminopeptidase N (peptidase E) gene to chromosome 15q13-qter. FEBS Letters 1988;239:305–8.
27. Poulsen PH, Thomsen PD, Olsen J. Assignment of the porcine aminopeptidase N (PEPN) gene to chromosome 7cenq21. Cytogenet Cell Genet 1991;57:44–6.
28. Lerche C, Vogel LK, Shapiro LH, Norén O, Sjöström H. Human aminopeptidase N is encoded by 20 exons. Mamm Genome 1996;7 In Press.
29. Olsen J, Laustsen L, Kärnström U, Sjöström H, Norén O. Tissue-specific interactions between nuclear proteins and the aminopeptidase N promoter. J Biol Chem 1991;266:18089–96.
30. Shapiro LH, Ashmun RA, Roberts WM, Look T. Separate promoters control transcription of the human aminopeptidase N gene in myeloid and intestinal epithelial cells. J Biol Chem 1991;266:11999–2007.
31. Olsen J, Classen-Linke I, Sjöström H, Norén O. Pseudopregnancy induces the expression of hepatocyte nuclear factor-1 beta and its target gene aminopeptidase N in rabbit endometrium via the epithelial promoter. Biochem J 1995;312:31–7.
32. Olsen J, Kokholm K, Troelsen JT, Laustsen L. An enhancer with cell-type specific activity is located between the myeloid and epithelial aminipeptidase N (CD13) promoters. Manuscript 1996.
33. Olsen J, Laustsen L, Troelsen J. HNF1a activates the aminopeptidase N promoter in intestinal (Caco-2) cells. FEBS Lett 1994;342:325–8.
34. Courey AJ, Tjian R. Analysis of Sp1 in vivo reveals multiple transcriptional domains, including a novel glutamine-rich activation motif. Cell 1988;55:887–98.
35. Kadonaga JT, Courey AJ, Ladika J, Tjian R. Distinct regions of Sp1 modulate DNA binding and transcriptional activation. Science 1988;242:1566–70.
36. Kadonaga JT, Carner KR, Masiarz FR, Tjian R. Isolation of cDNA encoding transcription factor Sp1 and functional analysis of the DNA binding domain. Cell 1987;51:1079–90.
37. Briggs MR, Kadonaga JT, Bell SP, Tjian R. Purification and biochemical characterization of the promoter-specific transcription factor, Sp1. Science 1986;234:47–52.
38. Kadonaga JT, Katherine A, Tjian R. Promoter-specific activation of RNA polymerase II transcription by Sp1. Trends Biochem Sci 1986;11:20–3.
39. Hagen G, Dennig J, Preiss A, Beato M, Suske G. Functional analyses of the transcription factor Sp4 reveal properties distinct from Sp1 and Sp3. J Biol Chem 1995;270:24989–94.
40. Hagen G, Muller S, Beato M, Suske G. Cloning by recognition site screening of two novel GT box binding proteins: a family of Sp1 related genes. Nucleic Acids Res 1992;20:5519–25.
41. Hagen G, Muller S, Beato M, Suske G. Sp1-mediated transcriptional activation is repressed by Sp3. EMBO J 1994;13:3843–51.
42. Tronche F, Yaniv M. HNF1, a homeoprotein member of the hepatic transcription regulatory network. BioEssays 1992;14:579–87.
43. Mendel DB, Crabtree GR. HNF-1, a member of a novel class of dimerizing homeodomain proteins. J Biol Chem 1991;266:677–80.
44. Lai E, Darnell JEJ. Transcriptional control in hepatocytes: a window on development. Trends Biochem Sci 1991;16:427–9.
45. De Simone V, Cortese R. Transcription factors and liver-specific genes. Biochim Biophys Acta 1992;1132:119–26.
46. Nicosia A, Monaci P, Tomei L, De Francesco R, Nuzzo M, Stunnenberg H, Cortese R. A myosin-like dimerization helix and an extra-large homeodomain are essential elements of the tripartite DNA binding structure of LFB1. Cell 1990;61:1225–36.
47. Mendel DB, Hansen LP, Graves MK, Conley PB, Crabtree GR. HNF-1α and HNF1 (vHNF-1) share dimerization and homeo domains, but not activation domains, and form heterodimers in vitro. Genes & Dev 1991;5:1042–56.
48. Rey-Campos J, Chouard T, Yaniv M, Cereghini S. vHNF1 is a homeoprotein that activates transcription and forms heterodimers with HNF1. EMBO J 1991;10:1445–57.

49. De Simone V, De Magistris L, Lazzaro D, Gerstner J, Monaci P, Nicossia A, Cortese R. LFB3, a heterodimer-forming homeoprotein of the LFB1 family, is expressed in specialized epithelia. EMBO J 1991;10:1435–43.
50. Bach I, Yaniv M. More potent transcriptional activators or a transdominant inhibitor of the HNF1 homeoprotein family are generated by alternative RNA processing. EMBO J 1993;12:4229–42.
51. Cereghini S, Ott M-O, Power S, Maury M. Expression patterns of vHNF1 and HNF1 homeoproteins in early postimplantation embryos suggest distinct and sequential developmental roles. Development 1992;116:783–97.
52. Blumenfeld M, Maury M, Chouard T, Yaniv M, Condamine H. Hepatic nuclear factor 1 (HNF1) shows a wider distribution than products of its known target genes in developing mouse. Development 1991;113:589–99.
53. Wang LH, Tsai SY, Cook RG, Beattie WG, Tsai MJ, O'Malley BW. COUP transcription factor is a member of the steroid receptor superfamily. Nature 1989;340:163–6.
54. Riemann D, Gohring B, Langner J. Expression of aminopeptidase N/CD13 in tumour-infiltrating lymphocytes from human renal cell carcinoma. Immunol Lett 1994;42:19–23.
55. Wex T, Lendeckel U, Wex H, Frank K, Ansorge S. Quantification of aminopeptidase N mRNA in T cells by competitive PCR. FEBS Lett 1995;374:341–4.
56. Lendeckel U, Wex T, Kahne T, Frank K, Reinhold D, Ansorge S. Expression of the aminopeptidase N (CD13) gene in the human T cell lines HuT78 and H9. Cell Immunol 1994;153:214–26.
57. Shapiro LH. Myb and Ets proteins cooperate to transactivate an early myeloid gene. J Biol Chem 1995;270:8763–71.
58. Mucenski ML, McLain K, Kier AB, Swerdlow SH, Schreiner CM, Miller TA, Pietryga DW, Scott WJ, Jr., Potter SS. A functional c-myb gene is required for normal murine fetal hepatic hematopoiesis. Cell 1991;65:677–89.
59. Metz T, Graf T. v-myb and v-ets transform chicken erythroid cells and cooperate both in trans and in cis to induce distinct differentiation phenotypes. Genes & Dev 1991;5:369–80.
60. Melotti P, Calabretta B. Ets-2 and c-Myb act independently in regulating expression of the hematopoietic stem cell antigen CD34. J Biol Chem 1994;269:25303–9.
61. Dudek H, Tantravahi RV, Rao VN, Reddy ES, Reddy EP. Myb and Ets proteins cooperate in transcriptional activation of the mim-1 promoter. Proc Natl Acad Sci USA 1992;89:1291–5.
62. Nunn MF, Seeburg PH, Moscovici C, Duesberg PH. Tripartite structure of the avian erythroblastosis virus E26 transforming gene. Nature 1983;306:391–5.
63. Leprince D, Gegonne A, Coll J, de Taisne C, Schneeberger A, Lagrou C, Stehelin D. A putative second cell-derived oncogene of the avian leukaemia retrovirus E26. Nature 1983;306:395–7.
64. Wasylyk B, Hahn SL, Giovane A. The Ets family of transcription factors [published erratum appears in Eur J Biochem 1993 Aug 1;215(3):907]. [Review]. Eur J Biochem 1993;211:7–18.
65. Lozzio CB, Lozzio BB. Human chronic myelogenous leukemia cell-line with positive Philadelphia chromosome. Blood 1975;45:321–34.
66. Hay R, Caputo J, Chen TR, et al.American Type Culture Collection. Catalogue of Cell Lines and Hybridomas. 7th ed. Rockville, Maryland: American Type Culture Collection; 1992.
67. Treisman R. Ternary complex factors: growth factor regulated transcriptional activators. Curr Opin Genet Develop 1994;4:96–101.
68. Treisman R. The serum response element. Trends Biochem Sci 1992;17:423–6.
69. Riemann D, Kehlen A, Langner J. Stimulation of the expression and the enzyme activity of aminopeptidase N/CD13 and dipeptidylpeptidase IV/CD26 on human renal cell carcinoma cells and renal tubular epithelial cells by T cell-derived cytokines, such as IL-4 and IL-13. Clin Exp Immunol 1995;100:277–83.
70. Foltzer-Jourdainne C, Raul F. Effect of epidermal growth factor on the expression of digestive hydrolases in the jejunum and colon of newborn rats. Endocrinology 1990;127:1763–9.
71. Xin JH, Cowie A, Lachance P, Hassell JA. Molecular cloning and characterization of PEA3, a new member of the Ets oncogene family that is differentially expressed in mouse embryonic cells. Genes & Dev 1992;6:481–96.
72. Rehfeld N, Peters JE, Giesecke H, Haschen RJ. Untersuchungen über aminosaüre-arylamidasen. I Verteilung und isoenzyme der aryl-amidase im menschlichen organismus. Acta Biologica et Medica Germanica 1967;19:819–30.