Expression of differentiated functions in the developing porcine small intestine

Giuditta Perozzi, Daniela Barilà, Chiara Murgia, Denise Kelly, Robert Begbie, and Timothy P. King

Istituto Nazionale della Nutrizione, Via Ardeatina 546, 00178 Roma, Italy, and Rowett Research Institute, Bucksburn, Aberdeen, UK

Heterologous cDNA clones were used as hybridization probes to define the temporal expression of intestinal functions during fetal and postnatal development in the pig. Northern hybridization analysis revealed the presence of the mRNAs for the cellular retinol binding protein CRBP II, for the digestive enzyme aminopeptidase N, and for the microvillar proteins villin and ezrin in the small intestine of both weaned and 40-day fetal pigs. The presence of these mRNAs suggests that at the end of the first third of gestation the pig fetal intestine is already exhibiting some characteristics of a differentiated epithelium. The mRNAs for the two fatty acid-binding proteins I-FABP and L-FAPB, both involved in the metabolism of long chain fatty acids, were detected only in the intestinal mRNA extracted from weaned animals, while that for the cellular retinol-binding protein CRBP I was expressed only in the fetal tissue. The temporal limits of expression of intestinal genes in the pig epithelium seem therefore more easily defined than in other experimental animals with shorter times of fetal development. To isolate pig genes expressed at different developmental stages during intestinal epithelial cell differentiation, a cDNA library was constructed from poly(A) + RNA extracted from mature pig intestine. This library was employed in the isolation of clones encoding CRBP II and L-FABP. The nucleotide sequence of the two pig cDNA clones was determined, and the sequences of the deduced proteins compared with their homologues from other species. The results of this analysis showed that the two pig clones share a high level of homology with human and rat homologues both at the DNA and at the protein level. (J. Nutr. Biochem. 4:699–705, 1993.)

Keywords: absorptive epithelium; small intestine; pig cDNA clones; enterocyte maturation

Introduction

In the mammalian intestine, morphogenesis and cyto-differentiation are temporally highly organized processes. Developmental maturation of the gut epithelium leads to the establishment of four differentiated cell types that are continuously renewed in the adult. The processes of proliferation and differentiation are topologically highly organized, being restricted to the crypt and villus compartments, respectively. The intestinal epithelium is therefore an excellent model system for studying the intrinsic and extrinsic regulation of complex spatial patterns of cellular differentiation. Pronounced changes in gene expression during the fetal and postnatal periods are essential for the acquisition of specialized intestinal function. These temporal changes superimpose on the spatial diversity of gene expression found between upper and lower villus position and also between proximal and distal regions of the intestine.[1,2] Extensive structural and functional development of the intestinal epithelium occurs in utero. Variations in the timing and extent of intestinal development in different mammalian species generally reflect the length of the gestational period. For example, in the human fetus at 20 weeks gestation, intestinal morphology and many functional parameters resemble those of the newborn,[3] whereas in altricial species such as the rat, villi are not formed until just prior to birth,[4,5] and several aspects of functional development are initiated in the postnatal period.[6] Intestinal ontogenesis during the suckling and weaning periods involves extensive cryptogenesis and epithelial proliferation. At the

This work was supported by grants from the Scottish Office of Agriculture and Fisheries Department, and by the National Research Council of Italy, Special Project RAISA, sub-project 4, paper no. 914 and Target Project "Biotechnology and Bioinstrumentation". D.B. is supported by a grant from Nestlè. C.M. is supported by a CNR-RAISA fellowship. T.P.K. was the recipient of a CNR-RAISA exchange fellowship.

Address reprint requests to Dr. G. Perozzi at the Istituto Nazionale della Nutrizione, Via Ardeatina 546, 00178 Roma, Italy.

Received March 10, 1993; accepted June 28, 1993.

same time, major changes occur in gene expression for microvillar and intracellular enzymes, transport proteins, and receptors for circulating and luminal trophic factors and hormones.

In the current study, the pig intestinal epithelium has been chosen as a model system to study the molecular events involved in cellular differentiation. The use of a pig intestine as an experimental model has several advantages over its extensively used murine counterparts; in particular, an intestinal maturation program during fetal life that more closely resembles that in humans.[6,7] This paper describes the use of heterologous gene probes to monitor the temporal expression of structural and functional genes in the pig intestine, the preparation of a pig intestine cDNA library, and the isolation from this library of two clones representing proteins expressed in mature enterocytes.

Methods and materials

Animals

Two litters from a crossbred sow (Large White × Landrace) were studied. The first litter of 10 piglets was killed 2 weeks after weaning when the animals were 5 weeks of age. Jejunal tissue samples were taken from anesthetized animals at approximately the midpoint between the pylorus and the ileocaecal valve. A second litter of eight fetal pigs was surgically exposed at 40 days gestation. Small intestines were excised from the fetuses while under the influence of halothane anesthesia administered to the sow.

To prepare rat intestinal RNA for comparison, the portion of small intestine between the pylorus and the ileocecal valve was dissected from 21-day-old rats (Sprague-Dawley) and inverted on a wooden stick. The exposed luminal surface was quickly rinsed in cold Hanks' buffered saline (Flow Laboratories, Irvine UK), and villi were dissected under a microscope using the blunt side of a scalpel.

Histology and electron microscopy

Excised intestinal tissues were fixed for 18 to 24 hours at 20° C in 4 % formaldehyde (freshly prepared from paraformaldehyde) in 0.1 M phosphate buffer, pH 7.3. Tissues were subsequently washed in phosphate buffer, dehydrated in an ethanol series, and embedded in Historesin (Leica, Milton Keynes, UK). One-μm thick sections were stained with toluidine blue and photographed on a Zeiss Axioscope (Zeiss Inc., Thornwood, NY, USA) light microscope. For electron microscopy, tissues were fixed for 18–24 hours at 20° C in 2.5% glutaraldehyde and 2% formaldehyde in 0.1 M phosphate buffer, pH 7.3. Tissues were subsequently washed in phosphate buffer, dehydrated in an ethanol series, and embedded in LR White resin (London Resin Company, Basingstoke, UK). Thin sections were contrasted with uranyl acetate and lead citrate and examined in a Jeol 1200 Ex B electron microscope (Jeol USA Inc., Peabody, MA USA).

RNA isolation and analysis

Excised intestinal tissues were frozen and stored in liquid nitrogen. Total RNA was extracted from frozen pulverized tissues as described by Chirgwin et al.[8] Poly(A)+ RNA was purified by affinity chromatography on oligo(dT) cellulose (Boehringer Mannheim, Lewes, East Sussex, UK). Northern hybridizations of total (20 μg/lane) or poly(A)+ RNA (5 μg/lane) following fractionation on formaldehyde-containing 1.2% agarose gels and blotting onto Hybond N membranes (Amersham International, Amersham, Buckinghamshire, UK) were performed according to standard protocols.[9]

cDNA probes and labelling procedures

The following cDNA fragments were used as hybridization probes: human CRBP I,[10] aminopeptidase N,[11] and villin[12]; rat CRBP II, L-FABP, ezrin,[13] and I-FABP.[14] The isolated fragments were labeled with [α-^{32}P] dCTP to a specific activity of 1–5 × 10^8 cpm/μg by nick translation.[9]

Construction and screening of the pig intestinal cDNA library

Double-stranded cDNA synthesis was performed according to the procedure of Gubler,[15] with oligo(dT) priming and M-MLV Reverse Transcriptase (Bethesda Research Laboratories, Gaithersburg, MD, USA). Synthetic adapter oligonucleotides containing the Eco RI, Sph I, Bam HI, and Sma I restriction sites were ligated to blunted cDNA ends,[16] and fragments were size fractionated by gel filtration chromatography on Sephacryl S-1000 (Pharmacia P-L Biochemicals, Milwaukee, WI, USA). Fractions containing cDNA fragments larger than 500 bp were pooled and used for ligation into EcoRI-cut λZAP II arms (Stratagene). Ligation mixtures were packaged in vitro with Gigapack Gold extracts (Stratagene, La Jolla, CA, USA) and the resulting phage library was titered on the *E. coli* host XL1 Blue. To screen for specific cDNA clones, recombinant plaques were plated at a density of 10,000 pfu/150 mm plate. Lifting of plaques onto Hybond N filters (Amersham, UK) was performed according to the manufacturer's protocols.

Filter hybridizations

Prehybridization of membrane-bound phage DNA for library screening was carried out at 65° C for a minimum of 4 hours in 6X SSC (1X SSC is 0.15 M NaCl, 0.015 M NaCitrate), 1% sodium dodecyl sulphate (SDS), 5X Denhardt's (1X Denhardt's is 0.02% ficoll, 0.02% bovine serum albumin, 0.02% polyvinylpirrolidone), 50 μg/ml heat denatured, sonicated salmon sperm DNA. Hybridization mixes contained 4 volumes prehybridization mix + 1 volume 50% Dextran sulphate + 1–5 × 10^6 cpm of the appropriate cDNA probe/ml of hybridization solution. Hybridized membranes were washed once in 2X SSC-0.1% SDS, followed by two washes each in 1X SSC-0.1% SDS and 0.5X SSC-0.1% SDS. All washes were at 60° C for 20 minutes. Northern hybridizations were performed at 42° C for 16 hours with the addition of 50% formamide to the hybridization mix described above. Hybridized membranes were washed at 50° C in 2X SSC-0.1% SDS and 1X SSC-0.1% SDS.

DNA sequence determination

The DNA sequence was determined on double-stranded templates using the Sanger method of dideoxy chain termination[17] as modified by the use of Sequenase (United States Biochemicals, Cleveland, OH).[18]

Results

Histology of the fetal intestine

At 40 days gestation the luminal surface of the fetal pig small intestine was formed into nascent villi with a

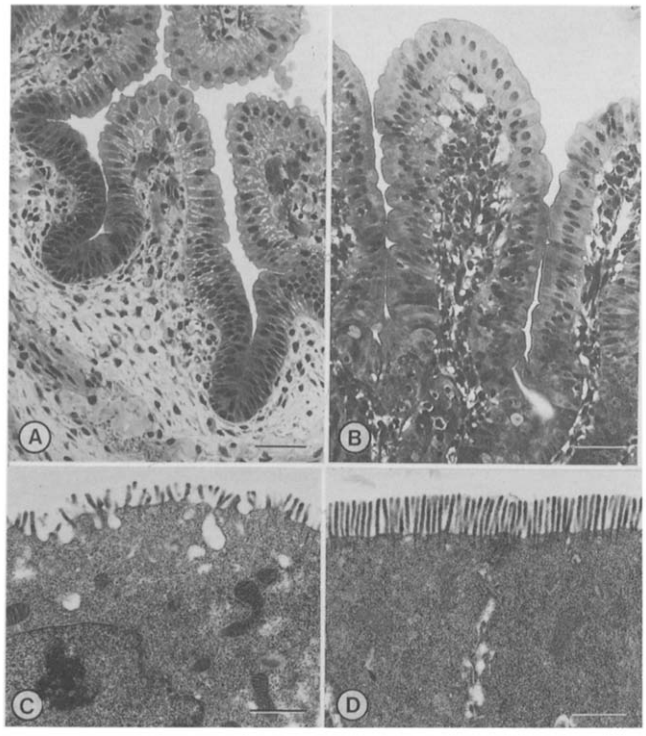

Figure 1 Histology of fetal (A) and weaned (B) pig jejunum showing morphological appearance of the villus epithelium. (C) Electron micrograph showing irregularly arranged brush border microvilli and apical endosomes characteristic of villus enterocytes in the 40-day fetal pig. (D) Electron micrograph showing regularly arranged and relatively longer microvilli typically found on villus enterocytes of the weaned pigs. Scale bars = 25 μm (A,B) 0.1 μm (C,D).

surface epithelium of irregularly shaped and differentiating columnar cells *(Figure 1A)*. The presence of a multilayered epithelium at the base of the nascent villi showed that the formation of crypt and villus compartments was not established at this stage of development. At the light microscopical level, the fetal epithelial cells possessed conspicuous apical brush borders that were similar in appearance to those seen in weaned animals *(Figure 1B)*. However, ultrastructurally the brush borders were markedly different in appearance. Those of the fetal enterocytes consisted of short, irregularly arranged microvilli associated with large apical endosomes *(Figure 1C)*, whereas in the weaned pigs the microvilli and associated terminal web structures were more highly organized, and endosomal activity was considerably less conspicuous *(Figure 1D)*.

Gene expression in pig intestine

Intestinal gene expression in the pig jejunum was studied by Northern hybridization. Poly(A)+ RNA was extracted from the intestine of 40-day fetuses and of 5-week-old weaned piglets and hybridized to several cDNA probes, representing genes expressed at different stages during enterocyte maturation. The RNA blots contained a sample of RNA extracted from a preparation of rat intestinal villi[13] to allow direct comparison of gene expression in the two species. The results in *Figure 2* show that all heterologous probes were found to hybridize with porcine poly(A)+ RNA at low stringencies. A slight difference in transcript size is observed for some of the genes between rat and pig, reflecting interspecies variation in the size of the transcriptional units or in the length of the poly(A) tails. The strong hybridization signals that are consistently displayed by the rat RNA sample are due to enrichment in epithelial cell mRNAs in the dissected villi, as well as to different degrees of enrichment in the poly(A)+ RNA preparations, as shown by control hybridization to the ferritin probe. The mRNAs for CRBP II and for the FABPs are among the most abundant transcripts in mature enterocytes; moreover, the corresponding cDNA probes used in this

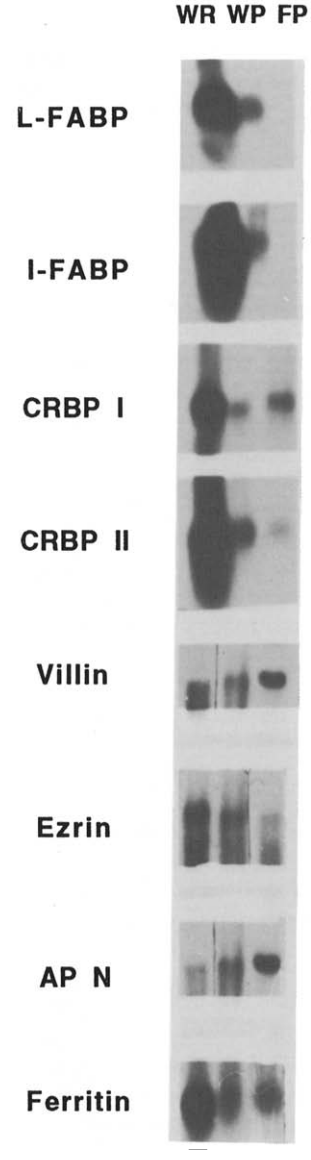

Figure 2 Northern hybridizations of the ^{32}P-labeled cDNA clones indicated to 5 μg/lane of poly (A) + RNA extracted from: weaned rat, 21 days postpartum, dissected intestinal villi (WR); weaned pig jejunum, 5 weeks postpartum (WP); fetal pig intestine, 40 days of gestation (FP). Hybridization to Ferritin (L subunit) mRNA is shown as internal control.

study were isolated from the rat, and therefore, as shown in *Figure 2*, hybridize better with their homologous mRNAs. The genes expressed only in the weaned pig intestine were those for the fatty acid-binding proteins, I-FABP and L-FABP. On the contrary, CRBP I transcription was restricted to the fetal pig RNA sample. Hybridization of this cDNA probe to the RNA from weaned gut is most likely due to cross-hybridization to the highly homologous CRBP II mRNA,[19] as evidenced by the smaller size of the hybridizing transcripts in the WR and WP lanes. Separate hybridization with the enterocyte-specific CRBP II cDNA confirms that this mRNA is very abundant in the intestine from weaned animals and has a low level of expression in the pig fetal gut. The mRNAs for the two proteins of the microvillar cytoskeleton, villin and ezrin,[20] and for the digestive enzyme aminopeptidase N[21] were all expressed in adult pig jejunum as well as in the fetal gut.

Isolation and molecular analysis of pig cDNA clones

With the aim of isolating porcine clones that could be helpful in a more detailed characterization of pig intestinal development and differentiation, using more sensitive molecular techniques such as in situ hybridization, we constructed a cDNA library in the bacteriophage vector λZAP II, using poly(A)+ RNA extracted from weaned pig intestine. The titer of the cDNA library was 3.1×10^6 pfu/ml, with a background of nonrecombinant phages lower than 10% and an average insert size of 1.5 Kb. Screening of 100,000 primary phage plaques with the rat CRBP II and L-FABP cDNA fragments resulted in the isolation of six and 20 homologous porcine clones, respectively. These numbers are likely to reflect the relative abundance of the two mRNAs in weaned pig intestine. The nucleotide sequences of porcine CRBP II and L-FABP are listed in *Figures 3 and 4*. Both clones contain the entire coding region, and 5′ and 3′ untranslated regions of different lengths. Nucleotide sequence comparison demonstrates a high degree of homology between the pig clones and the human and rat homologous sequences.[19,22] For the pig CRBP II clone, the overall level of sequence identity with its rat homologue was 83.5%, and the highest divergence was concentrated in the 3′ untranslated region. The same comparative analysis carried out between the pig L-FABP sequence and its human and rat counterparts showed 88% and 84.7% identity, respectively. *Figure 5* shows protein sequence comparison among the pig L-FABP and CRBP II gene products and the deduced protein sequences from other species. The pig-L-FABP protein of 127 aminoacids contains 12 and 23 aminoacid substitutions with respect to the human and rat sequences, respectively. In both cases, about 50% of these changes represent nonconservative aminoacid substitutions. The CRBP II protein sequence, on the other hand, displays 11 aminoacid substitutions in a sequence of 134 residues, eight of which are non conservative.

```
                                  CCCACCGTAACCAGAGGCCAC     21

      M   T   R   D   Q   N   G   T
CACC ATG ACG AGG GAC CAG AAT GGA ACC    49

W   E   M   E   S   N   D   N   F
TGG GAG ATG GAG AGT AAC GAC AAC TTT     76

E   G   Y   M   K   A   L   D   I
GAA GGC TAC ATG AAG GCC CTG GAT ATT    103

D   F   A   T   R   K   I   A   V
GAT TTT GCC ACC CGT AAG ATC GCT GTG    130

A   L   T   Q   T   K   I   I   E
GCT CTG ACT CAG ACG AAG ATC ATT GAG    157

Q   D   G   D   K   F   K   T   K
CAA GAC GGC GAT AAG TTC AAG ACA AAA    184

T   N   S   T   F   R   N   Y   D
ACC AAC AGC ACG TTC CGA AAC TAT GAC    211

L   D   F   T   V   G   V   E   F
TTG GAT TTC ACA GTC GGG GTG GAG TTT    238

D   E   Y   T   K   G   L   D   N
GAT GAG TAC ACA AAG GGC CTG GAC AAC    265

R   N   V   K   T   L   I   I   W
CGG AAT GTT AAG ACA CTG ATC ATC TGG    292

E   G   D   A   L   V   C   V   Q
GAA GGT GAT GCC CTC GTG TGC GTG CAG    319

K   G   E   K   E   N   R   G   W
AAA GGG GAA AAG GAG AAC CGA GGC TGG    356

K   Q   W   V   E   G   D   K   L
AAG CAG TGG GTC GAG GGA GAC AAG CTG    373

Y   L   E   L   T   C   G   D   Q
TAC CTG GAG CTG ACA TGC GGT GAC CAG    400

V   C   R   Q   V   F   K   K   K
GTG TGC CGT CAA GTG TTC AAG AAG AAG    427

TGATGCCGAGAGGGGGGGCCTAAGGAGCATGTGCT    462
CTACAGTCTCCAGGAAGAGTTTTCTGCTGGCTTTG    497
GGAGAACAGCCACAGGGACTCTCCCACTCCTGACA    532
GCCCAATGGGACATCTAGATGGGTTTTAAACAGAA    567
TGAGTGTGTAACAGTGACAGGATAATCTTCCATCC    602
TTGAAAGCTGGCATTAAATGAAAAA              627
```

Figure 3 DNA sequence and translation product of the pig CRBP II clone. Numbering starts at the first nucleotide of the listed sequence.

Discussion

Many diseases that result in the impaired absorption of nutrients are associated with loss of the differentiated characteristics of the enterocyte, including cell polariza-

```
                M   N   F   S   G   K
        CCCGG ATG AAC TTC TCC GGC AAA  23

Y   Q   V   Q   S   Q   E   N   F
TAC CAA GTA CAG AGC CAG GAA AAC TTT  50

E   A   F   M   K   A   V   G   L
GAG GCC TTC ATG AAG GCA GTC GGT CTG  77

P   D   E   L   I   Q   K   G   K
CCC GAC GAA CTC ATC CAG AAG GGG AAG  104

D   I   K   G   T   S   E   I   V
GAC ATC AAG GGG ACA TCG GAA ATC GTG  131

Q   N   G   K   H   F   K   L   T
CAG AAT GGG AAG CAC TTC AAG TTG ACC  158

I   T   T   G   S   K   V   V   Q
ATC ACT ACC GGG TCC AAG GTC GTC CAG  185

N   E   F   T   L   G   E   E   C
AAT GAG TTC ACC TTG GGA GAG GAG TGT  212

E   M   E   T   L   T   G   E   K
GAG ATG GAG ACC CTG ACT GGG GAG AAG  239

V   K   T   V   V   Q   L   E   G
GTC AAG ACA GTG GTT CAG TTG GAA GGT  266

D   N   K   L   V   T   T   F   K
GAC AAT AAA CTG GTG ACA ACT TTC AAA  293

G   I   K   S   V   T   E   L   N
GGC ATC AAG TCT GTG ACT GAA CTC AAC  320

G   D   I   I   T   S   T   M   T
GGT GAC ATA ATC ACC AGT ACC ATG ACA  347

L   G   D   I   V   F   K   R   I
CTG GGT GAC ATT GTC TTC AAG AGA ATC  374

I   S   K   R   I   .
ATC AGC AAG AGA ATT TAG ACAAGTCCGCA  403
TTTCATAGTCTTATATCATGTAAAA            425
```

Figure 4 DNA sequence and translation product of the pig L-FABP clone. Numbering starts at the first nucleotide of the listed sequence.

tion.[23,24] The study of sequential gene expression during normal development and differentiation of the intestinal epithelium could therefore yield important clues to the understanding of the molecular basis of this process. The pig is becoming increasingly popular as a model system for the study of intestinal functions due to structural and functional similarities with human intestine, also during development.[6,25-27]

Using heterologous probes in Northern hybridizations, we show in the present paper that the expression of some developmentally regulated intestinal genes in the pig differs markedly from their expression in the gut epithelium of the rat. Intestinal maturation in the rat occurs in a short time span at the end of pregnancy,[4] whereas in the pig, as well as in humans, this process appears to be initiated much earlier in fetal development. The present results provide molecular evidence of this earlier maturation process in the fetal porcine intestine. At 40 days of gestation (end of the first third of pregnancy), intestinal morphogenesis had already progressed to the point where recognizable villi were present. Ultrastructurally, the microvillar surfaces of the fetal villus enterocytes closely resembled those of cells in the crypt regions of postnatal pigs.[28] Expression of the genes for CRBP I, CRBP II, aminopeptidase N, ezrin, and villin in the fetal pig jejunum was compatible with the observed level of morphogenesis. The presence of villin mRNA in pig fetal gut, despite the presence of poorly developed microvilli, is not surprising, as the expression of this mRNA during mouse embryogenesis initiates as soon as the intestinal tube is formed.[29] However, in the undifferentiated cells, the protein is uniformly distributed throughout the cytoplasm, and it is recruited at the apical borders of the epithelial cells as differentiation proceeds.[30] Similarly, the synthesis of the integral membrane enzyme, aminopeptidase N, in the developing rat intestine is reported to occur during the last week of gestation and parallels the appearance of other polarized, differentiated features.[31] Of the two cellular retinol-binding proteins, CRBP I is expressed in murine gut throughout fetal development, while a switch to CRBP II gene expression occurs in mature enterocytes.[32] Coexpression of the two genes in the 40-day fetal pig intestine provides further evidence of an earlier maturation of gut epithelium in this species. The FABPs are among the most abundant proteins in mature enterocytes of most mammalian species, and it

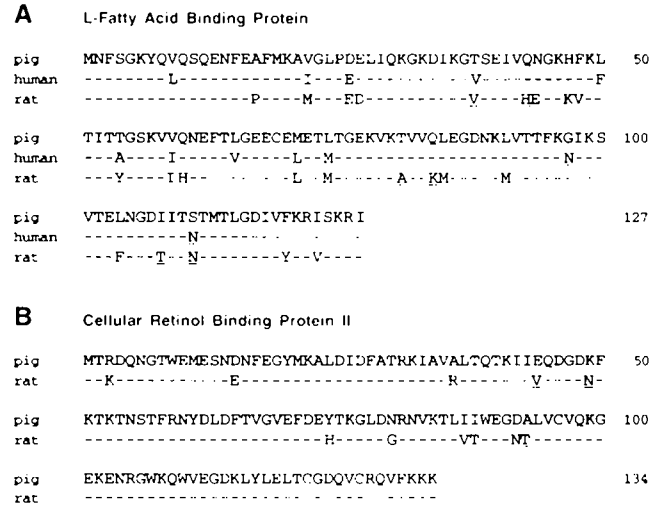

Figure 5 Protein sequence comparison among pig, human, and rat CRBP II and L-FABP gene products. Amino-acid substitutions only are shown for the human and rat proteins. The underlined residues indicate nonconservative amino-acid changes.

has been postulated that they play distinct roles in the intestinal metabolism of long chain fatty acids.[33] The sensitive molecular approach adopted in the present study demonstrated the expression of both L-FABP and I-FABP mRNAs in the small intestine of weaned piglets. Previous immunochemical studies employing antibodies against rat I-FABP failed to demonstrate the homologous protein in extracts of pig intestine,[34] and in recent biochemical studies FABP binding activity was presumed to represent the activity of both L-FABP and I-FABP.[35] The temporal expression of L-FABP and I-FABP mRNAs in porcine small intestine contrasts with that in murine small intestine, where the onset of expression of these two genes coincides with the appearance of nascent villi.[36,37] This study demonstrated differential gene expression in the developing porcine intestine: it is likely that with the relatively long gestational period of the pig (116 days) the windows of expression of different stage-specific intestinal genes may be wider and more readily distinguishable in this species than in the murine fetal intestine.

As yet, few intestinal cDNA clones have been isolated from the pig,[38-41] and this restricts the application of highly sensitive molecular techniques, such as in situ hybridization, for the study of developmental expression of differentiation-specific intestinal genes. With the final goal of isolating clones differentially expressed during enterocyte maturation, a cDNA library was constructed from poly(A)+ RNA extracted from mature pig intestine. Two pig cDNA clones, encoding the intestinal proteins CRBP II and L-FABP, were isolated and sequenced. The nucleotide and the deduced protein sequence of L-FABP were found to have a high degree of homology with both human and rat sequences. The peptide sequence of CRBP II was found to have a very strong conservation with its rat counterpart, consistent with the high level of homology reported to occur among the various members of the retinoid-binding protein family.[41] Further screening of the library is ongoing to identify other structural and functional genes expressed during intestinal differentiation.

Acknowledgments

We wish to thank M. Arpin, Institut Pasteur; N. Davidson, University of Chicago; and O. Norèn, University of Copenhagen for providing some of the cDNA clones used in this study.

References

1 Gordon, J.I. (1989). Intestinal epithelial differentiation: new insights from chimeric and transgenic mice. *J. Cell Biol.* **108,** 1187–1194

2 Kelly, D., Begbie, R., and King, T.P. (1992). Post-natal intestinal development. *Br. Soc. Anim. Prod. Occasional Pub.* **15,** 63–79

3 Lebenthal, E. (1989). Concepts in gastrointestinal development. In *Human gastrointestinal development,* (E. Lebenthal, ed.), p. 3–18, Raven Press, New York, NY USA

4 Trier, J.S. and Moxey, P.C. (1979). Morphogenesis of the small intestine during fetal development. *Ciba Found. Symp.* **70,** 3–29

5 Montgomery, R.K., Sybicki, M.A., and Grand, R.J. (1981). Autonomous biochemical and morphological differentiation in fetal rat intestine transplanted at 17 and 20 days of gestation. *Dev. Biol.* **87,** 76–84

6 Henning, S.J. (1987). Functional development of the gastrointestinal tract. In *Physiology of the Gastrointestinal tract,* (L.R. Johnson, ed.), p. 285–300, Raven Press, New York, NY USA

7 Tumbleson, M.E. (1986). *Swine in biomedical research,* vols. 1–3, Plenum Press, New York, NY USA

8 Chirgwin, J.M., Przybyla, A.E., MacDonald, R.J., and Rutter, W.J. (1979). Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry* **18,** 5294–5299

9 Sambrook, J., Fritsch, E.F., and Maniatis, T. (1989). *Molecular cloning, a laboratory manual,* second edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY USA

10 Colantuoni, V., Cortese, R., Nilsson, M., Lundvall, J., Bavik, C.-O., Eriksson, U., Peterson, P.A., and Sundelin, J. (1985). Cloning and sequencing of a full-length cDNA corresponding to human cellular retinol-binding protein. *J. Biochem. Biophys. Res. Comm.* **130,** 431–439

11 Olsen, J., Cowell, G.M., Kønigshøher, E., Danielsen, E.M., Møller, J., Laustsen, L., Hansen, O.C., Welinder, K.G., Engberg, J., Hunziker, W., Spiess, M., Sjöström, H., and Norén, O. (1988). Complete amino acid sequence of human intestinal aminopeptidase N as deduced from cloned cDNA. *FEBS Lett.* **238,** 307–314

12 Arpin, M., Pringault, E., Finidori, J., Garcia, A., Jeltsch, J.M., Vandekerckove, J., and Louvard, D. (1988). Sequence of human villin: a large duplicated domain homologous with other actin-severing proteins and a unique small carboxy-terminal domain related to villin specificity. *J. Cell Biol.* **107,** 1759–1766

13 Barilà, D., Murgia, C., Nobili, F., Gaetani, S., and Perozzi, G. Subtractive hybridization cloning of novel genes differentially expressed during intestinal development. Submitted for publication.

14 Alpers, D.H., Strauss, A.W., Ockner, R.K., Bass, N.M., and Gordon, J. (1984). Cloning of a cDNA encoding rat intestinal fatty acid binding protein. *Proc. Natl. Acad. Sci. USA* **81,** 313–317

15 Gubler, U. (1988). A one tube reaction for the synthesis of blunt-ended double stranded cDNA. *Nucl. Ac. Res.* **16,** 2726

16 Wu, R., Wu, T., and Ray, A. (1987). Adaptors, linkers and methylation. *Methods in Enzymol.* **152,** 343–359

17 Sanger, R., Nicklen, S., and Coulson, A.R. (1977). DNA sequencing with chain-termination inhibitors. *Proc. Natl. Acad. Sci. USA* **74,** 5463–5467

18 Tabor, S. and Richardson C.C. (1987). DNA sequence analysis with a modified bacteriophage T7 DNA polymerase. *Proc. Natl. Acad. Sci. USA* **84,** 4767–4771

19 Li, E., Demmer, L.A., Sweetser, D.A., Ong, D.E., and Gordon, J.I. (1986). Rat cellular retinol-binding protein II: use of a cloned cDNA to define its primary structure, tissue-specific expression, and developmental regulation. *Proc. Natl. Acad. Sci. USA* **83,** 5779–5783

20 Mooseker, M.S. (1985). Organization, chemistry and assembly of the cytoskeletal apparatus of the intestinal brush border. *Ann. Rev. Cell Biol.* **1,** 209–241

21 Semenza, G. (1986). Anchoring and biosynthesis of stalked brush border membrane proteins: glycosidases and peptidases of enterocytes and renal tubuli. *Ann. Rev. Cell Biol.* **2,** 259–313

22 Sweetser, D.A., Birkenmeier, E.H., Klisak, I.J., Zollman, S., Sparkes, R.S., Mohandas, T., Lusis, A.J., and Gordon, J.I. (1987). The human and rodent intestinal fatty acid binding protein genes. *J. Biol. Chem.* **262,** 16060–16071

23 Cutz, E., Rhoads, J.M., Drumm, B., Sherman, P.M., Durie, P.R., and Forstner, G.G. (1989). Microvillus inclusion disease: an inherited defect of brush border assembly and differentiation. *New Engl. J. Med.* **320,** 646–651

24 Losowsky, M.S. (1989). Coeliac disease. *Curr. Op. Gastroenterology* **5,** 258–263

25 Miller, E.R. and Ullrey, D.E. (1987). The pig as a model for human nutrition. *Ann. Rev. Nutr.* **7,** 361–382

26 Moughan, P.J., Birtles, M.J., Cranwell, P.D., Smith, W.C., and Pedraza, M. (1992). The piglet as a model system for study-

ing aspects of digestion and absorption in mild-fed human infants. In *Nutritional triggers for health and disease. World Rev. Nutr. Diet.* (A.P. Simopolous, ed.) vol. 67, p. 40–113, Karger, Basel, Switzerland

27 Puchal, A.A. and Buddington, R.K. (1992). Postnatal development of monosaccharide transport in pig intestine. *Am. J. Physiol.* **262,** G895–G902

28 Smith, M.W. and Peacock, M.A. (1989). Comparative aspects of microvillus development in avian and mammalian enterocytes. *Comp. Biochem. Physiol.* **93A,** 617–622

29 Louvard, D. (1989). The function of the major cytoskeletal components of the brush border. *Curr. Op. Cell Biol.* **1,** 51–57

30 Shibayama, T., Carboni, J.M., and Mooseker, M.S. (1987). Assembly of the intestinal brush border: appearance and redistribution of microvillar core proteins in developing chick enterocytes. *J. Cell Biol.* **105,** 335–344

31 Quaroni, A. (1985). Pre- and postnatal development of differentiated functions in rat intestinal epithelial cells. *Dev. Biol.* **111,** 280–292

32 Levin, M.S., Li, E., Ong, D.E., and Gordon, J.I. (1987). Comparison of the tissue-specific expression and developmental regulation of two closely linked rodent genes encoding cytosolic retinol-binding proteins. *J. Biol Chem.* **262,** 7118–7124

33 Sweetser, D.A., Heuckeroth, R.O., and Gordon, J.I. (1987). The metabolic significance of mammalian fatty-acid-binding proteins: abundant proteins in search of a function. *Ann. Rev. Nutr.* **7,** 337–359

34 Reinhart, G.A., Simmen, F.A., Mahan, D.C., Simmen, R.C.M., and White, M.E. (1990). Isolation, characterization, and developmental expression of pig intestinal fatty acid-binding proteins. *J. Nutr. Biochem.* **1,** 592–598

35 Reinhart, G.A., Simmen, F.A., Mahan, D.C., Simmen, R.C.M., White, M.E., and Trulzsch, M.D. (1992). Perinatal ontogeny of fatty acid binding protein activity in porcine small intestine. *Nutr. Res.* **12,** 1345–1356

36 Gordon, J.I., Elshourbagy, N., Lowe, J.B., Liao, W.S., Alpers, D.H., and Taylor, J.M. (1985). Tissue specific expression and developmental regulation of two genes coding for rat fatty acid binding proteins. *J. Biol. Chem.* **260,** 1995–1998

37 Rubin, D.C., Ong, D.E., and Gordon, J.I. (1989). Cellular differentiation in the emerging fetal rat small intestinal epithelium: mosaic patterns of gene expression. *Proc. Natl. Acad. Sci. USA* **86,** 1278–1282

38 Olsen, J., Sjöström, H., and Norén, O. (1989) Cloning of the pig aminopeptidase N gene. *FEBS Lett.* **251,** 275–281

39 Teng, B., Black, D.D., and Davidson, N.O. (1990). Apolipoprotein B messenger RNA editing is developmentally regulated in pig small intestine: nucleotide comparison of apolipoprotein B editing regions in five species. *Biochem. Biophys. Res. Comm.* **173,** 74–80

40 Weiler-Güttler, H., Sommerfeldt, M., Papandrikopoulou, A., Mischek, U., Bonitz, D., Frey, A., Grupe, M., Scheerer, J., and Gassen, H.G. (1990). Synthesis of apolipoprotein A1 in pig brain microvascular endothelial cells. *J. Neurochem.* **54,** 444–450

41 Eckhardt, A.E., Timpte, S.C., Abernethy, J.L., Zhao, Y., and Hill, R.L. (1991). Porcine submaxillary mucin contains a cystine-rich, carboxyl-terminal domain in addition to a highly repetitive, glycosylated domain. *J. Biol. Chem.* **266,** 9678–9686

42 Chytil, F. and Ong, D.E. (1987). Intracellular vitamin A-binding proteins. *Ann. Rev. Nutr.* **7,** 321–335