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FAT FEEDING STIMULATES ONLY ONE OF THE TWO mRNAs ENCODING RAT INTESTINAL MEMBRANOUS AND SECRETED ALKALINE PHOSPHATASE

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We have identified two mRNAs in rat intestinal mucosa by Northern blot analysis, using cloned cDNAs encoding human placental alkaline phosphatase (PLAP). Probes from both the NH_2- and COOH-terminal ends of the human PLAP coding region identified, in rat intestine (especially duodenum), an mRNA of nearly identical size (3 kb) to that found in human placenta. A smaller mRNA (2.7 kb), detected only with the COOH-terminal probe, was more prevalent in jejunum. Following feeding of triacylglycerols, the prevalance of the 2.7 kb mRNA increased over 2-fold. The tissue distribution and response of the 2.7 kb mRNA to fat feeding corresponds exactly with the known behavior of the secreted alkaline phosphatase.

Alkaline phosphatase (EC 3.1,3.1) hydrolyzes a variety of organic phosphate esters. The intestinal isoenzyme is a relatively late phylogenic development (1), and has been shown to exist in a larger membranous (68 kD) and a smaller soluble, or secreted, (58 kD) form (2). The secreted form is especially abundant in the rat (3). Fat feeding increases the tissue levels of the secreted form three-fold, at the same time as it increases the blood level by a comparable amount (3). Translation of rat intestinal RNA has shown two products of differing size that precipitate with antiserum raised to intestinal alkaline phosphatase (4). The regional distribution of the two polypeptides within the intestine and the response of the smaller polypeptide to fat feeding led us to postulate that intestinal alkaline phosphatase is encoded by two separate mRNAs, the larger one encoding the membrane-bound enzyme; and the smaller one, the secreted enzyme. However, no direct demonstration of these mRNAs has been possible until the development of appropriate cDNA probes.

We have isolated a cloned cDNA encoding human placental alkaline phosphatase (PLAP)(5). In man, placental and intestinal alkaline phosphatases

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share antigenic determinants (6). In the rat, placental alkaline phosphatase is the same as the liver, or tissue unspecific, isoenzyme (1). From Northern blot analysis using probes from all regions of the human PLAP mRNA, we conclude that rat intestine does indeed contain two different mRNAs encoding alkaline phosphatase, that the smaller one encodes the secreted form of the enzyme, and that fat feeding leads to accumulation of only the smaller mRNA.

Materials and Methods

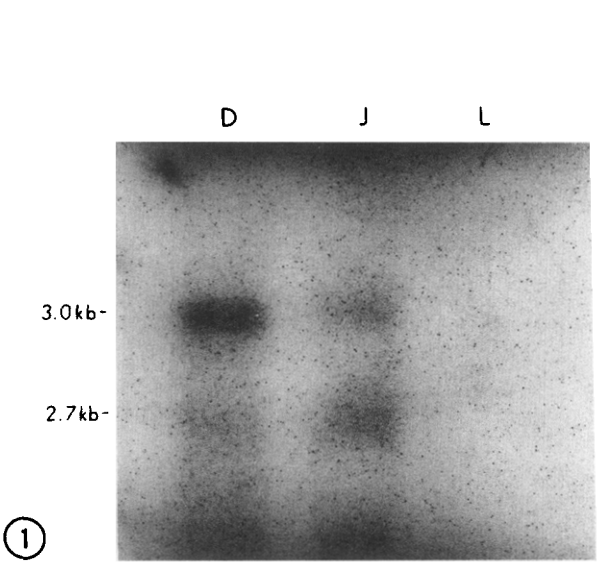
Rats were maintained for two weeks on standard lab chow, or on a 30% (W/V) triacyglycerol diet (modified from 45%, U. S. Biochemical Corp.). RNA was isolated from adult male rats after homogenization of scraped intestinal mucosa or liver in guanidine hydrochloride (7). After removal of the duodenum, jejunum refers to the proximate first quarter and ileum to the third quarter of the remaining intestine. Rat placentas were removed from pregnant dames on the 20th day of gestation. Electrophoresis of 15 µg of total RNA was performed in 1.5% agarose for 14 hours in 2.1 N formaldehyde and buffer containing 2.0 mM 3-[N-Morpholino] propanesulfonic acid, 5mM sodium acetate and 1 mM sodium EDTA, after denaturation at 65° for 15 min in 50% formamide, 2.1 N formaldehyde and buffer. RNA was transferred to nitrocellulose paper and baked at 80°C for 1.5 hours before hybridization.

The probes used were all derived from the sequence of the cloned human placental alkaline phosphatase cDNA previously reported (5). They included nucleotides from positions 1181 to 1650 (numbering corresponds to that in reference 8), encoding 154 amino acids near the COOH- terminus; nucleotides from 1839 - 2145, encoding part of the 3' nontranslated region; and a synthetic 39-mer, corresponding to the 13 amino acids at the NH_2-terminus of the mature protein (8,9). The cDNA probes were subcloned into pUC-13, and the plasmids were purified by the method of Holmes and Quigley (10), adding 2% LiCl to the DNA precipitation step. Inserts were isolated from the plasmids by digestion with appropriate restriction enzymes, electrophoresis in 5% polyacrylamide gels under nondenaturing conditions, recovery from the gel slices by electroelution and ethanol precipitation. Inserts were labeled with deoxycytidine 5'[α-^{32}P]triphosphate by the oligonucleotide primer method (11) and separated from free label by the spun column procedure (12). Filters were prehybridized at 42°C for 20 hrs in 50% formamide, 5 X SSC, 50 mM sodium phosphate, pH 7.4., 1 X Denhardt's solution, and 0.1 mg/ml denatured salmon sperm DNA. Hybridization was carried out for 20 hrs at 42°C in the same solution containing Dextran sulfate, 100 µg/ml and 4 X 10^5 cpm/ml of labelled probe. Filters were then washed 3 X 15 min and 1 X 45 min with 2 X SSC/0.2% SDS at room temperature. After exposure for 3 days to Kodak X-Omat AR film, the developed film was scanned using Zenith laser densitometry (Biomed Instruments, Inc., Fullerton, CA).

Results

When the cDNA probe encoding the COOH-terminal portion of human placental alkaline phosphatase (PLAP) was hybridized to Northern blots, two separate mRNA species were detected in rat intestinal mucosa (Figure 1). These RNAs were estimated at 3.0 and 2.7 kb in size. The 3 kb species predominated in duodenal RNA (left lane), whereas the 2.7 kb species was the major jejunal species (middle lane). No cross-hybridizing RNA species were identified by the COOH-terminal human PLAP probe in rat liver RNA (right lane).

The same nitrocellulose filter, after removal of the hybridizing cDNA, was probed with the NH_2-terminal oligonucleotide (39 bp) of human PLAP. This



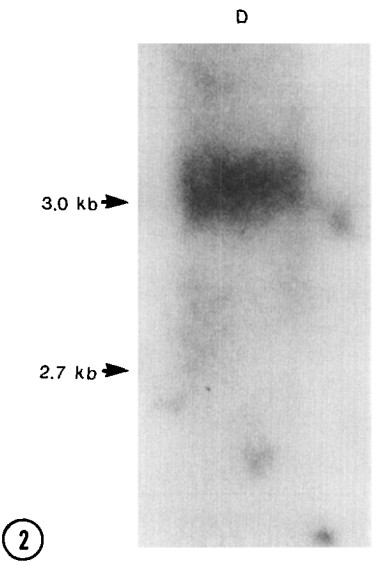


Figure 1. Northern blot of rat tissue RNAs using a COOH-terminal human PLAP probe.

Left lane, rat duodenal RNA; middle lane, rat jejunal RNA; right lane, rat liver RNA; Filters were hybridized to a probe corresponding to nucleotides 1181-1650 of human PLAP as described in Methods. The size of the RNAs was estimated using 28 S (4.7 kb) and 18 S (1.9 kb) ribosomal RNA as internal markers.

Figure 2. Northern blot of rat tissue RNA using an unambiguous oligonucleotide probe encoding the NH_2-terminal sequence of human PLAP. The duodenal lane of the same filter as in Figure 1 was reprobed with the synthetic 39-mer, as described in Methods.

probe identified the 3 kb RNA, but not the 2.7 kb species in duodenal mucosa (Figure 2). The cDNA probe encoding 306 bp of the 3' nontranslated region of the human PLAP cDNA hybridized to the 3 kb RNA in human placenta, but not to any species of RNA in rat tissues, including duodenum (data not shown).

The relative abundance of these mRNAs was examined after triacylglycerol feeding. This diet causes a three-fold increase in secreted intestinal alkaline phosphatase, but only a slight increase in membranous phosphatase (3). After such fat feeding the level of 3 kb RNA increased only 30% in the ileum, and changed very little in duodenum and jejunum (Table 1). However, the 2.7 kb RNA increased 40% in the duodenum but rose dramatically in both jejunum (2-fold) and ileum (3-fold).

Discussion

The human placenta contains two mRNA species that encode placental alkaline phosphatase (5). In rat intestine, using DNA probes from human PLAP, we have found two mRNA species (Figure 1). There is significant homology in

Table 1

Response of intestinal alkaline phosphatase mRNAs to triacylglyclerol feeding

Tissue	Diet	3 kb mRNA		2.7 kb mRNA	
		(Normalized Units)	(30% fat/ 5% fat)	(Normalized Units)	(30% fat/ 5% fat)
Duodenum	5% fat	1.00		0.42	
	30% fat	1.32	1.3	0.59	1.4
Jejunum	5% fat	0.37		0.62	
	30% fat	0.37	1.0	1.38	2.2
Ileum	5% fat	0.21		0.20	
	30% fat	0.3	1.4	0.64	3.2

The autoradiograph from a Northern blot probed with the COOH-terminal human PLAP sequence was scanned and the peaks above background cut out and weighed. The weights were normalized to the control diet (duodenal sample) for the 3 kb mRNA (value of 1.0).

the structure of this enzyme from E.coli (13) to human placenta (8,9). The structure of the alkaline phosphatase enzyme is highly conserved (8,9), and it would be expected that cDNA probes might identify sequences encoding similar proteins in different phyla or species. Furthermore, there is a high degree of homology between the 40 amino acids at the NH_2-terminus in human placental, human fetal and adult intestinal, and bovine intestinal phosphatases (14). Twenty-nine of these 40 residues are identical in human placental and bovine intestinal phosphatase. In addition, the calf intestinal enzyme differs from the adult one in only 3 positions, and the sequence of murine enzymes resemble those in the cow (14). These data suggest that the phosphatase isoenzymes arose from a common ancestral gene, and that mutations leading to the intestinal form are probably conservative ones.

The COOH-terminal probe that identified the 3 kb mRNA also identified the 2.7 kb mRNA. The identity of the larger RNA was confirmed by its detection with a probe from a different, highly conserved region (5'-coding) of the PLAP mRNA (14) (Figure 2). The lack of detection of the 2.7 kb mRNA may be related to the smaller size of this probe, coupled with the fact that it is probably not an exact match for the rat sequence. Alternatively, this may reflect a different NH_2-terminus for the rat intestinal phosphatase encoded by the 2.7 kb RNA. The 2.7 kb RNA is further identified as a phosphatase indirectly by its expected and proportionate response to fat feeding (Table 1).

There are many other examples of multiple mRNAs encoding one enzymatic activity. A single gene can encode multiple mRNAs by a variety of mechanisms: 1) the use of different promotors (15,16,18), 2) the presence of multiple

polyadenylation/termination signals (17-20), or 3) alternative splicing with inclusion or deletion of exons (18,21,22). From the data available, no conclusion can be drawn regarding the mechanism producing two mRNAs encoding intestinal alkaline phosphatase.

The unique feature of the multiple mRNAs reported here is their correlation with the divergent structure and physiological role of the membranous and secreted phosphatases in the rat enterocyte. The soluble tissue form is apparently identical with the secreted enzyme in the serum (2). The size of the membranous enzyme corresponds exactly with the larger enzyme identified in cell-free translation, and the mature secreted enzyme is nearly the same size as its smaller cell-free counterpart (4). Therefore, we tentatively identify the 3 kb mRNA as encoding the membranous enzyme, and the 2.7 kb mRNA as encoding the secreted phosphatase. The data in Table 1 are consistent with this conclusion. The proportion of secreted phosphatase in the duodenum, jejunum and ileum (2,10, and 20% respectively, ref 23) agrees with the greater abundance of the 2.7 kb RNA in the jejunum and ileum. Moreover, the relative increase in the abundance of the 2.7 kb mRNA (3-fold) after fat feeding corresponds well with the increase in secreted phosphatase previously described after the same stimulus (2,4). We conclude that fat feeding stimulates increased production of secreted alkaline phosphatase by increased mRNA concentration. Moreover, the data also show that the response is greatest in the jejunum and ileum, tissues with a much greater proportion of secreted enzyme, but much less total phosphatase activity than the duodenum. The final relationship between these two mRNAs will depend upon isolation of cDNA clones corresponding with each mRNA.

References

1. Komoda, T., Koyama, I., Nagata, A., Sakagishi, Y., DeSchryver -Kecskemeti, K. and Alpers, D. H. (1986) Gastroenterology 91,277-286.
2. Yedlin, S. T., Young, G. P., Seetharam, B., Seetharam, S. Alpers, D. H. (1981) J. Biol. Chem. **256**, 5620-5326.
3. Young, G. P., Yedlin, S. T., and Alpers, D. H. (1981) Am. J. Physiol. **241**, G461-G468.
4. Sussman, N. L., Seetharam, S., Blaufuss, M. C., Alpers, D. H. (1986) Biochem. J. **234**, 563-568.
5. Ovitt, C. E. Strauss, A. W., Alpers, D. H., Chou, J. Y., Boime, I. (1986) Proc. Natl. Acad. Sci. **83**. 3781-3785.
6. Harris, H. (1982) The Harvey Lectures, pp. 95-123, Academic Press, New York.
7. Gordon, J. I., Smith, D. P., Andy, R., Alpers, D. H., Schonfeld, G., Strauss, A. W. (1982) J. Biol. Chem. **257**, 971-978.
8. Millan, J. L. (1986) J. Biol. Chem. **261**, 3112-3115.
9. Kam, W., Clauser, E., Kim, Y. S., Kan, Y.W. and Rutter, W. J. (1985) Proc. Natl. Acad. Sci. U.S.A. **82**, 8715-8719.
10. Holmes, D. S. and Quigley, M. (1981) Anal. Biochem. 114, 193-197.
11. Feinberg, A. P. and Vogelstein, B. (1983) Anal. Biochem. 132, 6-13.

12. Maniatis, T., Fritsch, E. F. and Sambrook, J. (1982), pp. 466-467, Molecular Cloning, Cold Spring Harbor.
13. Bradshaw, R. A., Cencedda, F., Ericsson, L. H., Neumann, P. A., Piccoli, B. P., Schlesinger, M. J., Shreifer, K. and Welsh, K. A. (1981) Proc. Natl. Acad. Sci. U.S.A. **78**, 3473-3477.
14. Hua, J. C., Berger, J., Pan, Y. C. E., Hulmes, J.D., Udenfriend, S. (1986) Proc. Natl. Acad. Sci. U.S.A. **83**, 2368-2372.
15. Carlson, M. and Botstein, D. (1982) Cell **281**, 145-154.
16. Schibler. U., Hagenbuchl, O., Wellauer, K. and Pittet, A. C. (1983) Cell **33**, 501-508.
17. King, C. R. and Piatigorsky, J. (1983) Cell **32**, 707-712.
18. Reynolds, G. A., Goldstein, J. L., Brown, M. S.. (1985) J. Biol. Chem. **260**, 10369-10377.
19. Hickok, N. J., Seppanen, P. J., Kontula, K. K., Janne, P. A., Bardin, C. W. and Janne, O. A. (1986) Proc. Natl. Acad. Sci. U.S.A. **82**, 594-598.
20. Bilanchone, V., Duester, G., Edwards, Y., and Smith, M. (1986) Nuc. Acids Res. **14**, 3911-3926.
21. Milner, R. J., Lai, C., Nave, K. A., Lenoir, D., Ogata, J., and Sutcliffe, J. G. (1985) Cell **42**, 931-939.
22. Nawa, H., Katani, H. and Nakamishi, S. (1984) Nature (London) 312, 729-734.
23. Young, G. P., Yedlin, S. T. and Alpers, D. H. (1981) Biochem. Biophys. Acta **676**, 257-265.