Journal of Cellular Biochemistry 39:327–337 (1989)
Cell Activation and Signal Initiation: Receptor and Phospholipase Control of Inositol Phosphate, PAF and Eicosanoid Production 23–33

Novel Gene Exon Homologous to Pancreatic Phospholipase A_2: Sequence and Chromosomal Mapping of Both Human Genes

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We described previously the cloning and DNA sequence of the human gene encoding pancreatic phospholipase A_2 [DNA 5, 519]. When pancreatic phospholipase A_2 (PLA_2) cDNA was used to screen a human genomic library, two classes of clones were obtained. One class encoded the pancreatic enzyme, and a second class encoded one exon of an apparently related PLA_2. No additional PLA_2 gene exons displayed sufficient homology to be detected by the probe. A homologous sequence in both rat and porcine genomic DNA was detected by DNA blot hybridization, and the corresponding gene fragments were cloned and sequenced. Within the deduced amino acid sequences, the presence of known functional residues along with the high degree of interspecies conservation suggests the genes encode a functional PLA_2 enzyme form. The encoded sequence lacks Cys_{11}, as do the "type II" viperid venom and other nonpancreatic mammalian PLA_2 enzymes. The sequence is distinct from porcine intestinal PLA_2 and appears not to be a direct homolog of the recently published rabbit ascites and rat platelet enzymes. Hybridization of DNA probes containing sequences from these genes to genomic DNA blots of mouse/human somatic cell hybrids permitted chromosomal assignment for both. The pancreatic gene mapped to human chromosome 12, and the homologous gene mapped to chromosome 1.

Key words: phospholipase A_2, human genes, pancreatic, human chromosome mapping

Over the past two decades, phospholipase A_2 (PLA_2) enzyme activities have been purified and characterized from various tissues, cultured cells, and exudates originating from several organisms. These isolated activities have been reported to vary in molecular weight, pH optima, Ca^{++} requirement, and solubility. From these data, it would appear that mammals contain several distinct forms of PLA_2, each

Received June 2, 1988; accepted August 29, 1988.

having its own unique properties and roles. Recently, partial sequences have been published for PLA_2 from porcine intestine [1], rat peritoneal fluid [2], platelets [3], and spleen [4], and rabbit ascites fluid [5]. As more PLA_2 amino acid sequences become available, it will be possible to classify the various enzyme forms in terms of the genes in which they are encoded.

In the course of our isolation of the gene encoding human pancreatic PLA_2 [6], another gene was obtained which appears to encode the amino-terminal portion of a different but related PLA_2. The encoded sequence is distinct from other published enzyme forms. Since the potential role(s) of its gene product are currently unknown, we have called the gene "nonpancreatic," or np PLA_2. We describe here its isolation, DNA sequence in three mammalian species, homology to other known PLA_2 sequences, and the chromosomal localization of both pancreatic and np genes. In a separate report, we have described two human genetic markers associated with the np PLA_2 gene [7].

MATERIALS AND METHODS

A human genomic library [8] was screened using the method described in Maniatis et al. [9]. First, 10^6 clones were plated onto 150-mm L-agar plates and lifted onto nitrocellulose filters. The filters were denatured, baked at 80°C for 2 h, washed at 65°C in 3× SSC (1× SSC is 0.16 M NaCl and 0.016 M sodium citrate), 0.1% sodium dodecyl sulfate (SDS), and prehybridized overnight at 37°C in prehybridization buffer (50% formamide, 1× Denhardt's, 2× SSC, and 10 mM Na_3PO_4; 1× Denhardt's is 0.02% Ficoll, 0.02% polyvinylpyrrolidone, and 0.02% bovine serum albumin). The hybridization probe, pig-1, was prepared by nick-translation of the insert from a clone encoding porcine pancreatic PLA_2 cDNA [see 6]. Hybridization was overnight at 37°C in prehybridization buffer plus 10% dextran sulfate. The filters were washed in 0.5% SSC, 0.1% SDS at 60°C and exposed to X-ray film. The rat genomic library, obtained from Clonetech Inc. (Mountain View, CA), was screened similarly using a probe derived from the human np PLA_2 gene. The porcine np PLA_2 gene was subcloned directly from BglII-digested genomic DNA by isolation of specific size fractions, cloning into bacteriophage lambda 1059, and screening the resulting plaques as above.

Clones corresponding to hybridization signals were purified, amplified, and DNA was extracted and prepared. Analysis of DNA restriction endonuclease patterns by agarose gel electrophoresis allowed identification of unique clones. DNA fragments to be further analyzed by DNA sequencing were isolated from agarose gels by elution onto and then from DE81 paper, followed by ethanol precipitation. The isolated fragment was then cloned into bacteriophage M13 vector DNA [10], previously cleaved with the appropriate enzyme. The resulting plaques were then used to prepare M13 templates. DNA sequences were determined using the dideoxy method [11], and extended using 17-mer oligonucleotides directed to the last reliable portion of the previous sequencing run. The sequence was assembled and analyzed using the GEL and SEQ programs from Intellicorp, Inc. (Mountain View, CA).

The region downstream of the np PLA_2 gene was expanded using the "chromosomal walking" technique. A segment of the sequence near the end of the original clone (clone 8) was selected which corresponded to single-copy DNA. From this sequence, a 30-mer oligonucleotide probe was synthesized on an automated DNA

synthesizer (Applied Biosystems Inc., Foster City, CA). Clones from the genomic library which hybridized to this probe were isolated and analyzed via agarose gel electrophoresis. An extended map of the np PLA_2 gene was then constructed by determination of the EcoRI-digestion products of each unique clone.

For human chromosomal gene localization, DNA probes made from both pancreatic and np PLA_2 gene coding regions were used to probe blots of genomic DNA from a panel of somatic cell hybrids derived from the fusion of thymidine kinase-deficient mouse cells and normal human male fibroblasts (IMR91). DNA isolated from the hybrid cells was digested with EcoRI (pancreatic) or PstI (np), electrophoresed through 1.2% agarose gels, and transferred to nylon membranes. The probes, labelled by random-primed synthesis, were hybridized to the filters under the conditions described above, followed by autoradiography, essentially as previously described [12,13]. By matching hybridizing bands with the human chromosomal contents of each hybrid cell line, the corresponding human chromosome containing the gene was deduced [12]. For the in situ mapping, chromosome squashes from metaphase cells were hybridized with similarly labelled unique DNA as described previously [13–15]. After autoradiography, individual silver grains were counted over each of the chromosomes and a histogram of the grain number found over each chromosome region was constructed.

RESULTS

When 10^6 plaques from a human genomic library were screened with porcine pancreatic PLA_2 cDNA, 13 hybridizing clones were detected. After purification and analysis by agarose gel electrophoresis, two clones represented unique clones encoding pancreatic PLA_2 [see 6] and four clones (8, 17, 20, and 21) represented unique clones distinct from the pancreatic PLA_2 gene. The latter four clones were found by restriction mapping to overlap in the same genomic region. Upon digestion of clone 8 with PstI, a single 3.3-kb band hybridized to the porcine pancreatic PLA_2 probe on DNA blots. This band was purified from the gel by DE81 elution and then was subcloned into bacteriophage M13. The DNA sequence of the entire 3,325-base fragment was determined and is shown in Figure 1. The deduced amino acid sequence beginning with the residue marked 1 encodes the amino-terminal portion of a PLA_2-like sequence, and represents the only region which hybridized to the porcine pancreatic cDNA probe.

As discussed below, this sequence displayed a remarkable resemblance to pancreatic PLA_2; however, it contained several interesting differences which made it worthy of further study. In an attempt to localize the sites of synthesis of np PLA_2 transcripts, total RNA from various tissues and cell lines was prepared. Blots of the various RNAs were hybridized with a nick-translated probe derived from a 700-bp PvuII fragment of the np PLA_2 gene constituting the coding region and flanking unique sequences. These sources of RNA included human placenta, heart, prostate, jejunum, lung, and phorbol ester-induced HL60 cells; rabbit alveolar macrophages and neutrophils; porcine liver, spleen, and intestine; rat liver, spleen, and testes; and mouse p388D1 cells. No hybridizing signals were detected with any of these sources. Better results were obtained, however, when we tested for the presence of an np PLA_2 homolog in DNA from other mammalian species. Single homologous bands were detected in blots of both porcine and rat genomic DNA using the same hybrid-

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   1 CTGCAGCCCTCAGTGCGGCTCCAGGGACTGCTTGGGCAGCAGTGACCGCCCATGGGTTTCTTCCGCCACACCCCCGTTTA
  81 GACTGAACACGATAGGTAGATCGAAGGCCACCTGAGAAAACTCCCCCAAACTCTATTTCTGTTTCTCTTCTTCAAAGTTC
 161 ATGTCTTTGTTGTATTTTTATTGCAAATTTACTACATGCTTATAGTTAAAAAGTAAAATAAATGAGTATATAGCAACAAG
 241 GTAAAGCTCCTCCTCATCCTCCCCAGACCCCAGTTTTTTCCCTACATCCAGATGTGACCACTCTTAAGAGTTTGATATAC
 321 ATCCTCTATACAGCGTTTACCACACACACATTCAAAACACCATAATAGGAAGGGAACACATGCTGGGCCGGGCGCGGTTG
 401 TTCATGACTATAATCCCAGCACTTTGGGAGGCCGAGGCGGGCGGATCACCTGAGGTCAGGAGTTCGAGACCAGCCTGGCC
 481 AGCTGGCAAGATGGTGAAACCCGTCTCTATTAAAAATACAAAAAATTAGTCAAGCATGGCAGTTGGGCACCTGTAATCCC
 561 AGCTACTCAGGAGGCTGAGGCAGGAGAATTGCCTGAACCCGGGAGGCGGAGGTTGCAGTGAGCCGAGATCACACCATTGC
 641 ACTCCAGCCTGGGTAACAACAGCGAAACTCCGTCTCAAAAAAAAAAAAAAAAGAAGGAAAGGGACACACGCTTATTATGA
 721 AAGACATGAGACAGCGGAGACGTGTATAAATGATGTTGCCTGTTTTCTTTCTCTCTCTTCATCCATGCTAGAGATAGTGC
 801 TATCAAATGTAGTTATTTTGAGACACATATTTCGTATTATCCCTGTCGTGACATGTGGGTGGTTTCCAATTTTTTGATAT
 881 CACAGATAATGCTTCAGGAAACCATTTTGTGTATGCATTTGTGCCCACTCTCATAAGCATCTTGTAGAAGCAAAAACAGC
 961 TGAGTTCATGTGTACTTGTCATTTAAAAAAATAATAATTGAGGATACCTTTCCTGCCTCTTAAGTATTTTGTTTCTCCTG
1041 TGAGATAGTAAAGGCCTGATGACATCTGGAGGGACTGGCGTTTCTGGCTTTGAACTTTTGCCATTCATGTTGCATCAGAC
1121 CCGAGGGTGTTCTGCCTAGAACTGTGGTTTCTTGCTTTGAGGGGGAAGACTATGGTTGATGGGAAAGCCTTGTTCTGAAC
1201 CTCATGGAAACTGGGTATTCATCTGGGTTAGCAAAAAACTAGCTGTGTTACAGGGGCAAATCTGAACCTATTTTATTCCC
1281 CAGGAAAGAGGCTGGTGATTCCAGCCATGCCCCTTGCACTTCGCTTTGGGGATCTGGTGATATTTCGAATGCTCAGCACT
1361 CTAGTAAGGGGAGGGGACATCAAGGCAGCATCATGCTCATTGCAACTTCCTTCTTCCTTTTTTCTCATCGGTGGTGGCA
                                                                          ValValAla
                  *1*
1441 GCCCCCACCCACAGCAGTTTCTGGCAGTTTCAGAGGAGGGTCAAACACATCACGGGGCGAAGTGCCTTCTTCTCATATTA
     AlaProThrHisSerSerPheTrpGlnPheGlnArgArgValLysHisIleThrGlyArgSerAlaPhePheSerTyrTy
1521 CGGATATGGCTGCTACTGTGGGCTTGGGGATAAAGGGATCCCCGTGGATGACACTGACAGGTGGGTGCAGAGGCTCTAAG
     rGlyTyrGlyCysTyrCysGlyLeuGlyAspLysGlyIleProValAspAspThrAspArg
1601 GCCACTTATCATTTGTTTTGCATTAAAGTTCATGCTCAAAGCCAGAGAGAGGGTGTTAGGATTCTTGCCTGGCAAATAAC
1681 AGAAAACAACTCAGGCTAATGGAAGGAAGAACTGAACGGGATTTGGAGGATGGGTCTTGAGAAACCCAGGGTCGGGGCCA
1761 GCTTCTTGAGTGTGTGACCTGTGAAGTTTCACAGGGCCCAACACTCATAAGGGTCAGGGCCAGCTTCTTGAGCGTGTGAT
1841 CTGTAAAGTTTCACAGGGCCTGGCACTCATAAGGGCCTAAACATGGTTTACTGCTCTGCTGCCACATCTTGAAATTCTTA
1921 ATAAAGGGCCTCATGTTTTCATTTTGCTTTAGTCTCTGCAATTATGCCGTTGGTCCTGCCCAGAGCTCTAGAAGCTGTTT
2001 CATCCTCATAGTAAAAGTGCTGTGCTTTCAGCTCTCCAGCTTTTAGGACTATAGGCACAGCAACTGACTCACTAGTCC
2081 TAATTCCATATTCTGGAGAGGGCTCCAAAGTGGCCCAGTTTGGAGAAGTTGTCCATCTGGGTGAGGTTGCATGGCACAAA
2161 CCTGGCTTCAGGCCTACTCCAAAGGATGGGGGTGGGGGAGTGTGAGTTCCTAGAAAAAGTAGAGGTGGGTGTCATCTGGT
2241 GAATGTACGTGTGGGGAGGTAAGAAACGGGACAGTTTGCGTGTCAATTCATTTGAAGACATAAGAAAGCAAAATGTTCCT
2321 TGCCACATTTAACCTAGTATGGAGAAACATGTCCCACAGTGGCCTTAAATATCACTCTGAGCTCGAGTCTTGTGGTGGCT
2401 CATGAACCATGGAGGACCTAGAGCTTGGAAGGGCAATTGACGCTTATGAAATGCCCTTATGTGCCAAGCACTGGGACTGG
2481 CGATTGGCATACAAACCTAATTTAATTCTCGCAGGGAATGCACGAGACAGTTGATACCAGCCCATTTGACAGGTGAGGAC
2561 ATGTGAGTTGCTAAACCACCTCCTAAAGGCAATGCAGCTTCTAAGTGGCAGAGTTTAGGATTGAACGAGAATTTGCCTAT
2641 TTCAAAGTTTGTCCCCTCTCCTTGATGGTCTGTGCCTCCCCTGTCAAAGTCCAAAGGCTGATTAGAAATTGAACATCATT
2721 AGCCAAAGCTGATCAACACGCAGAGCCCCCACTTGCAGATGGGAATGGTGAGAGAGGGAGACTGAAACACTTTTTCTTGG
2801 CCTTTCAGGGTTTAGAATCCAAGCTTAAGTTTCTGCCTTCCTGTCCCTTGTGTAGTGGTTGAGGACATGGACTGAGCCCA
2881 TGCTCCAGATGGTATTTCTCCTCCAGTGCTCTCCCATCCAGCCCCCAGCCAACTCTGGGTGCCATGAATGGGACTACGTC
2961 GGCTTTTACAGACAGTTGTCTCCTCAGAGACCGTTACAGTGCCTGACTCACAGTAGGTGCTCAGTAAAAAGTGTTAAATG
3041 AATGAATGGGCCTAGGTTTGTGTCCTGGGTCTATCATTCTCCAGCTGCCTAAGTTTGGGAAATTGGCCTCTTGGAATCTC
3121 AGTCCCTCCCCTACAAAAGGGCAGCAATGATTGTACTTTATAGTTTCTATGAGTAGCTAATGAGATAGCAACAGATACTA
3201 CAGAGGGCTCAGGAAATGCTACTGGTTATTATTATTATTTTTTATTTTATTTATTTTTTGGGAGACGGGGTCTTGCTCTA
3281 TTATCCAGGCCTGGGGTGGAGAGGCTCAATCAGAGCTCACTGCAG 3325
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Fig. 1. DNA sequence of PstI 3,325-bp fragment containing np PLA_2 exon 2. The region encoding np PLA_2 exon 2 is shown with the corresponding amino acids. The beginning of the portion exhibiting homology to mature pancreatic PLA_2 is indicated (*1*).

ization probe. Subsequently, genomic libraries from both species were screened (see Materials and Methods) and the resulting clones were analyzed by DNA sequencing. The sequences of the coding regions of these clones were aligned with the human counterpart in Figure 2, and were compared with the corresponding region from human pancreatic PLA_2.

Since no other fragments from clone 8 hybridized to pancreatic cDNA probes, the other exons either were too divergent to be detected or were not contained within the clone. To investigate the latter possibility, we extended the analysis further in the 3′ direction by "chromosome walking." Restriction enzyme analysis of these "walk" clones allowed placement of the EcoRI fragments in the proper order to generate a restriction map for this region, shown in Figure 3. These clones extended the map another 18 kb downstream, creating a region of analysis which covered a total of 23 kb downstream from the coding exon. Hybridization of pancreatic PLA_2 cDNA probes to these "walk" clones failed to detect more hybridizing bands, even when hybridized (20% formamide, 37°C) and washed (55°C, 1× SSC) at lowered stringency and exposed to X-ray film for 2 weeks.

It was also possible that the np PLA_2 exon constituted an alternative exon to its corresponding pancreatic PLA_2 exon. If this was the case, the two genes should reside in the same chromosomal vicinity. Accordingly, DNA probes constituting unique sequence regions from the two genes were used to probe DNA blots from a panel of mouse-human somatic cell hybrids. Using a probe made from human pancreatic PLA_2 cDNA to screen blots of EcoRI-digested hybrid genomic DNA, human bands were seen at 8.2 and 11 kb, while a single mouse band of 10 kb was observed. Correlation of the presence of the human bands with the chromosomal content of the hybrid cells is summarized in Table I. As can be seen, only chromosome 12 shows no discordancies, permitting assignment of the pancreatic PLA_2 gene to human chromosome 12. For mapping of the np gene, PstI-digested hybrid chromosomal DNA was probed with a 700-bp PvuII fragment encompassing the exon and flanking unique DNA. A human band of 3.3 kb and a mouse band of 9.5 kb were observed. Correlation of the presence of the human band with the hybrid clones is also summarized in Table I. Only chromosome 1 shows no discordancies for human np PLA_2, consistent with the localization of gene on chromosome 1.

The pancreatic gene was further localized by in situ hybridization to human chromosomes using the human pancreatic PLA_2 probe. The results are summarized in histogram form in Figure 4. Although the background was unusually high with this probe, significantly higher numbers of grains occurred over the q 24 region of chromosome 12, suggesting that the gene lies within this region and confirming the results seen in Table I.

DISCUSSION

A sequence was found in human, porcine, rat, and mouse genomic DNA which appears to encode the amino-terminal portion of a unique PLA_2. The homologous exon of the pancreatic PLA_2 gene encodes the first third and most conserved portion of the molecule. We attempted to obtain the remainder of the gene by several methods, which would also help to demonstrate that the gene actually functions in vivo. First, a number of mammalian tissues were screened for transcripts originating from the np PLA_2 gene. None were detected; however, the possibility remains that the tissues

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GCCGCCGCCGACAGCGGCATCAGCCCTCGG GCCGTGTGGCAGTTCCGCAAAATGATCAAGTGCGTGATCCCGGGGAGTGACCCC   h panc

CCCTTTGCAGGGACAACCTCCACCCTCAGC AGCTTCTGGCAGTTCCAGAGGATGGTCAAA...CACATCACGGGGCGCAGCGCC   r np
CCTTGGGCAGTGCTGGCCCCCACCCAGAGC AGCTTCTGGCAGTTTCAAAGGATGGTCAAA...CACATCACAGGGTGGAGTGCC   p np
TCATCGGTGGTGGCAGCCCCCACCCACAGC AGTTTCTGGCAGTTTCAGAGGAGGGTCAAA...CACATCACGGGGCGAAGTGCC   h np
      ValValAlaAlaTrpThrHisSer SerPheTrpGlnPheGlnArgArgValLys...HisIleThrGlyArgSerAla   h np
X X XX XX X XXXX  X      XX      x          x  x    X                 x   X x  x


TTCTTGGAATACAACAACTACGGCTGCTACTGTGGCTTGGGGGGCTCAGGCACCCCCGTGGATGAACTGGACAA GTAAGTGATC   h panc

TTCTTCTCCTATTACGGATATGGCTGCTACTGTGGGCTTGGGGGCCGAGGGATCCCTGTGGACGCCACAGACAG GTGATGCTGA   r np
CTCTTCTCATATTACGGATATGGCTGCTACTGTGGGCTTGGGGGCAAAGGGACCCCCGTGGATGACACTGACAG GTGACTGCCA   p np
TTCTTCTCATATTACGGATATGGCTGCTACTGTGGGCTTGGGGATAAAGGGATCCCCGTGGATGACACTGACAG GTGGGTGCAG   h np
PhePheSerTyrTyrGlyTyrGlyCysTyrCysGlyLeuGlyAspLysGlyIleProValAspAspThrAspAr g            h np
X       X                                  xXX     X   x     x X   x        XXXXXXX
         <  ----------------------------->
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Fig. 2. Human, porcine, and rat np PLA_2. DNA sequence of the human (h), porcine (p), and rat (r) np PLA_2 exon was aligned with human pancreatic PLA_2 (h panc; 6). Conservative (x) and nonconservative (X) changes with respect to encoded amino acid are indicated below the sequences. The 100% conserved region containing the putative Ca^{++}-binding loop is indicated by arrows, and the intron donor consensus sequences are underlined.

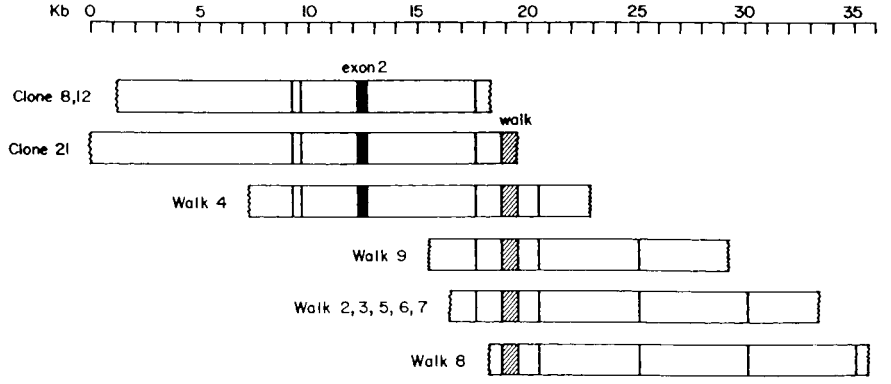

Fig. 3. Map of human np PLA_2 exon 2 and flanking regions. Eco RI sites in the region including and downstream from the np PLA_2 exon 2 are shown, along with the overlapping clones described in the text.

which actually transcribe this gene were not analyzed. Indeed, RNA from many potentially appropriate tissues and cell types (e.g., various peripheral blood or precursor cell populations) is difficult or impossible to obtain. Alternately, transcripts from this gene could have been present at levels below detection by this method, or perhaps the cell type where synthesis occurs requires prior activation by an appropriate stimulus. Second, we tried to localize the other coding regions by extending the cloned region downstream from the known coding exon to a total of 23 kb and probing for other conserved regions. The fact that none were found indicates that either the intron is exceptionally large, or, more likely, that the other exons are sufficiently divergent at the nucleotide level as to preclude cross-hybridization. Finally, the possibility that the np gene represented an alternate pancreatic PLA_2 exon was found unlikely since the two genes reside on different chromosomes. Until a

TABLE I. Human Chromosomal Localization of Pancreatic and np PLA_2 Genes*

Hybrid clone	Panc	np	Human chromosomes*																						
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	Y
84-2	+	+	+	+	−	+	+	+	+	+	−	−	+	+	−	+	+	+	+	+	+	+	−	+	−	−
84-7	+	−	−	−	+	+	−	+	+	+	−	−	+	+	−	+	−	+	+	−	−	+	−	−	−	−
84-13	+	−	−	−	+	−	−	+	−	−	−	+	−	+	+	−	+	−	+	+	−	+	+	−	−	−
84-20	+	−	−	(+)	+	+	+	−	+	−	+	−	+	(+)	+	+	−	−	+	+	−	+	+	+	(+)	−
84-21	+	−	−	−	−	−	−	−	+	−	−	+	−	+	−	+	−	−	+	−	−	+	−	−	−	(+)
84-25	+	−	−	−	−	+	+	+	−	+	−	+	+	(+)	−	−	−	−	+	−	−	−	+	−	−	−
84-26	+	+	+	−	+	+	+	+	+	+	−	+	−	+	+	+	+	+	+	+	+	+	+	+	−	+
84-27	+	−	−	+	−	−	+	+	−	+	−	−	+	+	−	−	+	−	+	+	−	+	+	−	−	−
84-30	+	−	−	−	+	+	(+)	+	−	−	−	+	−	(+)	+	+	−	−	+	+	−	(+)	+	+	−	−
84-35	+	−	−	−	+	+	−	+	+	+	−	−	−	+	−	+	+	−	+	+	−	−	−	−	−	−
84-37	+	−	−	−	−	+	+	+	+	(+)	−	−	+	+	−	+	−	−	+	−	−	+	+	+	−	−
84-38	+	+	+	−	+	+	(+)	+	+	+	−	+	−	+	+	+	+	−	+	+	+	(+)	−	+	−	−
84-3	−	+	+	+	+	+	−	+	+	+	−	+	−	−	+	+	+	−	+	+	+	+	−	−	−	−
84-4	−	+	+	+	+	+	−	+	+	+	−	+	−	−	+	+	+	−	+	+	+	+	−	−	−	−
84-5	−	−	−	+	+	−	+	+	−	+	−	−	−	−	(+)	+	+	−	+	−	+	−	+	+	+	+
84-34	−	−	−	−	−	+	−	+	+	+	−	+	+	−	−	+	−	−	+	−	(+)	+	−	+	−	−
84-39	−	−	−	−	+	−	−	−	+	+	−	−	−	−	−	−	+	−	+	−	−	−	−	−	−	−
No. of discordant hybrids	Panc		11	12	9	6	5	6	8	8	12	9	7	0	10	5	10	9	5	6	13	5	5	8	12	12
	np		0	5	8	7	8	9	7	9	5	6	10	11	5	8	5	4	12	5	2	8	11	7	7	6

*Clones which contain the chromosome in 10–30% of cells surveyed are indicated by parenthesis.

DNA probes made from gene coding regions from both pancreatic and np PLA_2 genes were used to probe blots of genomic DNA from a panel of somatic cell hybrids derived from the fusion of thymidine kinase-deficient mouse cells and normal human male fibroblasts (IMR91). The columns, left to right, indicate the hybrid name, the hybridization results for both probes, and whether or not each human chromosome is present in the hybrid. The no. of discordant hybrids (wrong hybridization bands relative to each chromosome) is shown in the bottom rows.

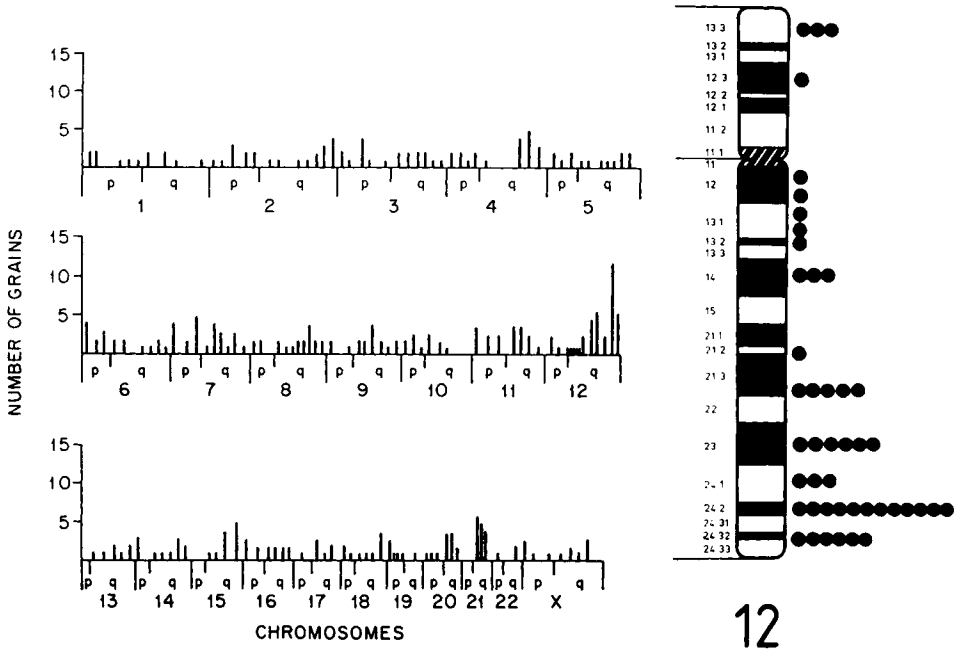

Fig. 4. In situ localization of human pancreatic PLA_2 gene. A DNA probe comprising a 537-bp stretch of pancreatic PLA_2 gene which contained exon 2 and nonrepetitive flanking DNA sequences was used to probe human chromosomal squashes. After autoradiography, individual silver grains were counted over each of the chromosomes and a histogram of the grain number found over each chromosome region was constructed. The tallied results from examination of 138 cells are shown in the histogram.

Enzyme	Pre	Pro	Mature
			1 10 20 30 40
A. pis			SLVELGKMIL-QETGKNAITSYGSYGCNCGWGHRGQPKDATDR
C. atr			SLVQFETLIM-KIAGRSGLLWYSAYGCYCGWGGHGLPQDATDR
r Asc			HLLDFRKMIR-YTTGKEAT?SYGAYGCSCGVGGR?APK?A
p Int			DLLNFRKMIK-LKTGKAPVPNYAFYGCYCGLGGKGSPKDATD?
r spl			?LLEFGQMIL-FKTGKRADVSYGFYGC?CGVGGRGSP
h np	VVAAPTHS		SFWQFQRRVK-HITGRSAFFSYYGYGCYCGLGDKGIPVDDTDR
p np	AVLAPTQS		AFWQFQRMVK-HITGWSALFSYYGYGCYCGLGGKGTPVDDTDR
r np	GTTSTLS		SFWQFQRMVK-HITGRSAFFSYYGYGCYCGLGGRGIPVDATDR
			*
h panc	VLLTVAAA	DSGISPR	AVWQFRKMIKCVIPGSDPFLEYNNYGCYCGLGGSGTPVDELDK
p panc	VGAA	DSGISSR	ALWQFRSMIKCAIPGSHPLMDFNNYGCYCGLGGSGTPVDELDR
r panc	AGVT	AHSISTR	AVWQFRNMIKCTIPGSDPFREYNNYGCYCGLGGSGTPVDDLDR

Fig. 5. Homology of known mammalian phospholipases A_2 (amino-terminal exon). A. pis = *Agkistrodon piscovorus* [19]; C. atr = *Crotalus atrox* [western diamondback rattlesnake; 16,17]; r Asc = rabbit ascites [5]; p int = porcine intestine [1]; r spl = rat platelet [3] and spleen [4]; np = human, porcine, and rat "nonpancreatic" PLA_2; panc = human [6,20], porcine [21], and rat [22] pancreatic PLA_2.

product for this gene is detected and the rest of the coding sequence is identified, the possibility remains that the segment constitutes a pseudogene.

The very nature of the np PLA_2 gene exon sequences, however, makes it difficult to imagine that the exon is merely a pseudogene or otherwise nonfunctional genetic element. First, the np PLA_2 gene is very highly conserved across species, much more than between the two PLA_2 gene segments within the same species. Only 79 of 126 nucleotides and 22 of 43 amino acids of the human np gene are conserved in the same coding region of the pancreatic gene. In contrast, 110 of 123 nucleotides and 39 of 42 amino acids of the human np sequence are conserved in a species as evolutionarily distant as rat. This pattern implies a strong selective pressure must be maintaining the integrity of the np PLA_2 gene sequence, which would be atypical of nonessential genetic elements such as pseudogenes. Moreover, the evolution of the two PLA_2 genes exhibits a clear pattern in which key structural elements are conserved. Note in Figure 2 that the the DNA sequence of the np PLA_2 gene region corresponding to the Ca^{++}-binding region of pancreatic PLA_2 (arrow) [16,17] was 100% conserved across the three species. Also, the DNA sequences surrounding the encoding exon contain typical intron acceptor, $[^{C}/_{T}]_nN[^{C}/_{T}]AG\hat{}G$, and donor, $[^{C}/_{A}]AG\hat{}GT[^{A}/_{G}]AGT$, consensus sequences [18] in all three species, and the positions of the introns are identical at the carboxy end of all four sequences. As would be expected, the homology disappears abruptly at both ends of the exon.

In Figure 5, sequences from other PLA_2 enzymes published to date were aligned with the deduced amino acid sequences of np PLA_2 from the three mammalian species. The np PLA_2 sequences contain most of the key conserved residues found in all known PLA_2 sequences (underlined in figure; see [16,17]). An interesting exception is the subtle but conserved substitution of Val_9 for Ile_9, a residue reported to be part of a wall of nonpolar side chains which partially encloses the active site (* in Figure 5) [23,24]. In addition, the human sequence (but not the porcine or rat) has substituted Arg for the highly conserved Met_8. Most of the np sequences from the three species resemble the pancreatic enzyme, particularly residues 2–5, 37–43, and the Ca^{++}-binding loop, 25–32. However, most of the more variable residues such as 6, 7, and 12–21 differ from pancreatic PLA_2.

The human np sequence contains the same number of basic residues in similar positions near the amino-terminus as the rabbit ascites and porcine intestine sequence. These basic residues have been proposed [5,25] to be determinants of responsiveness to the "bactericidal/permeability-increasing" (BPI) protein synthesized by neutrophils. Notably absent from the np genes is Cys_{11}, meaning it is of the "type II" or viperid venom class of enzymes, as are all other known mammalian PLA_2 enzymes other than those of pancreatic origin. Both of these characteristics are typical of those PLA_2 enzyme forms which exhibit the highest degree of proinflammatory character.

The start of the homologous region in the human np PLA_2 gene is immediately preceded by a short peptide resembling the carboxy-terminal portion of a hydrophobic signal sequence core, VVAAPTH [26]. Notably absent from the sequence is a "propeptide" region. This suggests the product of this gene probably would not be a zymogen, and could potentially be secreted directly as an active enzyme in vivo. This pattern also occurs in the porcine gene; however, the rat gene appears to have a different structure in this region, perhaps implying a different role for the rat gene product.

In summary, the strict conservation of key amino acid residues and exon/intron splice junctions across three divergent mammalian species of this np PLA_2 gene exon

suggests that its gene product is both synthesized and utilized in vivo. Further, its sequence would suggest that np PLA_2 is a relatively proinflammatory, BPI-responsive, Ca^{++}-requiring, nonlysosomal PLA_2 enzyme (or PLA_2-related protein) of relatively critical function. As the sequences of more of the various PLA_2 isolates become available, the identity and role as well as the rest of the coding region of this gene may become known. In the meantime, knowledge of the chromosomal localization of these two genes may facilitate direct correlations with other human genetic markers as they arise.

NOTE ADDED IN PROOF

This gene is distinct from the recently sequenced PLA_2 from human synovial fluid [27].

ACKNOWLEDGMENTS

We thank K. Hjerrild, T. Crisp, and T. Mikita for assistance in DNA sequencing. This work was supported by Biotechnology Research Partnership, Ltd., and NIH grant HL28481 (to AJL). A.J.L. is an Established Investigator of the American Heart Association.

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