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Complete cDNA encoding a putative phospholipase C from transformed human lymphocytes

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Phosphoinositide-specific phospholipase C (PLC) is a crucial enzyme in transmembrane signaling. A cDNA encoding the putative phospholipase C was cloned from human lymphocytes that were transformed by infection with Epstein-Barr virus. The deduced amino acid sequence of the cDNA accounted for 146.1 kDa of the molecular mass of the complete enzyme and showed 50.2% sequence similarity to bovine brain PLC. This cDNA contained regions, the sequences of which were similar to those of some tyrosine kinase-related oncogene products. Northern blotting demonstrated that the mRNA for this PLC is expressed in human promyelocytic leukemia cells (HL-60). Since the other cloned cDNAs for PLCs could not hybridize with the RNA from this cell, it is strongly suggested that the gene obtained here encodes an additional isozyme of PLC in blood cells.

Phospholipase C; Molecular cloning; Lymphocyte; (Human)

1. INTRODUCTION

Phosphoinositide-specific phospholipase C (PLC) is a crucial enzyme in transmembrane signaling that works by hydrolyzing phosphatidylinositol 4,5-bisphosphate to generate two messengers: the calcium mobilizer, inositol 1,4,5-triphosphate and protein kinase C activator, 1,2-diacylglycerol [1,2]. There have been many publications on the purification and characterization of PLCs from various tissues: rat liver (68 kDa) [3], sheep seminal vesicular gland (65 kDa) [4], bovine brain (88, 145 and 154 kDa) [5–7], rat brain (85 kDa) [8], guinea pig uterus (62 kDa) [9], calf thymocytes (68 kDa) [10], bovine platelets (143 kDa) [11] and human platelets (61 kDa) [12]. The observed differences in molecular mass may indicate the presence of multiple forms of PLCs.

To assess the multiplicity or diversity of PLC, several laboratories have recently elucidated the primary structure of PLCs by cDNA cloning [13–18]. We report the molecular cloning and sequencing of a cDNA encoding the putative PLC in human lymphocytes transformed by Epstein-Barr virus.

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2. MATERIALS AND METHODS

2.1. *cDNA library from human lymphocytes*

The lymphocytes were transformed by infection with Epstein-Barr virus [19] and cultured in RPMI medium supplemented with 10% fetal bovine serum. Purification of poly(A^+) RNA and construction of the λgt10 library were performed as described [20,21]. From 10 μg of poly(A^+) RNA, 10^8 recombinants were obtained.

2.2. *Oligonucleotides*

Oligonucleotides for the cloning probes were synthesized in an automatic DNA synthesizer model 380B (Applied Biosystem, CA, USA). The oligonucleotides GTITGICCITGITAIATIACIGGCATICCITCIGGICCITCCCAGCAITC and TCITAITCIGCICCIGCIACITCIATITCIACIAAIGGACAIACIATICC were selected by chance to encode some regions of the complementary sequences of bovine brain PLC [13] (the amino acid numbers from 367 to 383 and from 1106

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to 1122, respectively). The 5′-ends were labeled with [γ-^{32}P]ATP and T_4 nucleotide kinase.

2.3. *Cloning and sequencing*

The cDNA library was screened with a mixture of the labeled oligonucleotides. Hybridization was performed at 45°C in 3 × SSC, 0.1% SDS, 10 × Denhart's solution for 12 h, followed by washing with 2 × SSC and 0.1% SDS for 3 h at room temperature. The positive clones were purified by successive plaque hybridization. The isolated insert DNA fragments from

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GAATTCGGCGCTGAGTGACCCGAGTCGGGACGCGGGCTGCGCGCGCGGGACCCCGGAGCCCAAACCCGGGGCAGGCGGGCAGCTGTGCCCGGGCGGCACGGCCAGCTTCCTGATTTCTCC  120
CGATTCCTTCCTTCTCCCTGGAGCGGCCGACAATGTCCACCACGGTCAATGTAGATTCCCTTGCGGAATATGAGAAGAGCCAGATCAAGAGAGCCCTGGAGCTGGGGACGGTGATGACTG  240
                                  M  S  T  T  V  N  V  D  S  L  A  E  Y  E  K  S  Q  I  K  R  A  L  E  L  G  T  V  M  T  V   30
TGTTCAGCTTCCGCAAGTCCACCCCCGAGCGGAGAACCGTCCAGGTGATCATGGAGACGCGGCAGGTGGCCTGGAGCAAGACCGCCGACAAGATCGAGGGCTTCTTGGATATCATGGAAA  360
  F  S  F  R  K  S  T  P  E  R  R  T  V  Q  V  I  M  E  T  R  Q  V  A  W  S  K  T  A  D  K  I  E  G  F  L  D  I  M  E  I   70
TAAAAGAAATCCGCCCAGGGAAGAACTCCAAAGATTTCGAGCGAGCAAAAGCAGTTCGCCAGAAAGAAGACTGCTGCTTCACCATCCTATATGGCACTCAGTTCGTCCTCAGCACGCTCA  480
  K  E  I  R  P  G  K  N  S  K  D  F  E  R  A  K  A  V  R  Q  K  E  D  C  C  F  T  I  L  Y  G  T  Q  F  V  L  S  T  L  S  110
GCTTGGCAGCTGACTCTAAAGAGGATGCAGTTAACTGGCTCTCTGGCTTGAAAATCTTACACCAGGAAGCGATGAATGCGTCCACGCCCACCATTATCGAGAGTTGGCTGAGAAAGCAGA  600
  L  A  A  D  S  K  E  D  A  V  N  W  L  S  G  L  K  I  L  H  Q  E  A  M  N  A  S  T  P  T  I  I  E  S  W  L  R  K  Q  I  150
TATATTCTGTGGATCAAACCAGAAGAAACAGCATCAGTCTCCGAGAGTTGAAGACCATCTTGCCCCTGATCAACTTTAAAGTGAGCAGTGCCAAGTTCCTTAAAGATAAGTTTGTGGAAA  720
  Y  S  V  D  Q  T  R  R  N  S  I  S  L  R  E  L  K  T  I  L  P  L  I  N  F  K  V  S  S  A  K  F  L  K  D  K  F  V  E  I  190
TAGGAGCACACAAAGATGAGCTCAGCTTTGAACAGTTCCATCTCTTCTATAAAAAACTTATGTTTGAACAGCAAAAATCGATTCTCGATGAATTCAAAAAGGATTCGTCCGTGTTCATCC  840
  G  A  H  K  D  E  L  S  F  E  Q  F  H  L  F  Y  K  K  L  M  F  E  Q  Q  K  S  I  L  D  E  F  K  K  D  S  S  V  F  I  L  230
TGGGGAACACTGACAGGCCGGATGCCTCTGCTGTTTACCTGCATGACTTCCAGAGGTTTCTCATACATGAACAGCAGGAGCATTGGGCTCAGGATCTGAACAAAGTCCGTGAGCGGATGA  960
  G  N  T  D  R  P  D  A  S  A  V  Y  L  H  D  F  Q  R  F  L  I  H  E  Q  Q  E  H  W  A  Q  D  L  N  K  V  R  E  R  M  T  270
CAAAGTTCATTGATGACACCATGCGTGAAACTGCTGAGCCTTTCTTGTTTGTGGATGAGTTCCTCACGTACCTGTTTTCACGAGAAAACAGCATCTGGGATGAGAAGTATGACGCGGTGG 1080
  K  F  I  D  D  T  M  R  E  T  A  E  P  F  L  F  V  D  E  F  L  T  Y  L  F  S  R  E  N  S  I  W  D  E  K  Y  D  A  V  D  310
ACATGCAGGACATGAACAACCCCCTGTCTCATTACTGGATCTCCTCGTCACATAACACGTACCTTACAGGTGACCAGCTGCGGAGCGAGTCGTCCCCAGAAGCTTACATCCGCTGCCTGC 1200
  M  Q  D  M  N  N  P  L  S  H  Y  W  I  S  S  S  H  N  T  Y  L  T  G  D  Q  L  R  S  E  S  S  P  E  A  Y  I  R  C  L  R  350
GCATGGGCTGTCGCTGCATTGAACTGGACTGCTGGGACGGGCCCGATGGGAAGCCGGTCATCTACCATGGCTGGACGCGGACTACCAAGATCAAGTTTGATGACGTCGTGCAGGCCATCA 1320
  M  G  C  R  C  I  E  L  D  C  W  D  G  P  D  G  K  P  V  I  Y  H  G  W  T  R  T  T  K  I  K  F  D  D  V  V  Q  A  I  K  390
AAGACCACGCCTTTGTTACCTCGAGCTTCCCAGTGATCCTGTCCATCGAGGAGCACTGCAGCGTGGAGCAACAGCGTCACATGGCCAAGGCCTTCAAGGAAGTATTTGGCGACCTGCTGT 1440
  D  H  A  F  V  T  S  S  F  P  V  I  L  S  I  E  E  H  C  S  V  E  Q  Q  R  H  M  A  K  A  F  K  E  V  F  G  D  L  L  L  430
TGACGAAGCCCACGGAGGCCAGTGCTGAACCAGCTGCCCTCGCCCAGCCAGCTGCGGGAGAAGATCATCATCAAGCATAAGAAGCTGGGCCCCCGAGGCGATGTGGATGTCAACATGGAGG 1560
  T  K  P  T  E  A  S  A  D  Q  L  P  S  P  S  Q  L  R  E  K  I  I  I  K  H  K  K  L  G  P  R  G  D  V  D  V  N  M  E  D  470
ACAAGAAGGACGAACACAAGCAACAGGGGGAGCTGTACATGTGGGATTCCATTGACCAGAAATGGACTCGGCACTACTGCGCCATTGCTGATGCCAAGCTGTCCTTCAGTGATGACATTG 1680
  K  K  D  E  H  K  Q  Q  G  E  L  Y  M  W  D  S  I  D  Q  K  W  T  R  H  Y  C  A  I  A  D  A  K  L  S  F  S  D  D  I  E  510
AACAGACTATGGAGGAGGAAGTGCCCCAGGATATACCCCCTACAGAACTACATTTTGGGGAGAAATGGTTCCACAAGAAGGTGGAGAAGAGGACGAGTGCCGAGAAGTTGCTGCAGGAAT 1800
  Q  T  M  E  E  E  V  P  Q  D  I  P  P  T  E  L  H  F  G  E  K  W  F  H  K  K  V  E  K  R  T  S  A  E  K  L  L  Q  E  Y  550
ACTGCATGGAGACGGGGGGCAAGGATGGCACCTTCCTGGTTCGGGAGAGCGAGACCTTCCCCAATGACTACACCCTGTCCTTCTGGCGGTCAGGCCGGGTCCAGCACTGCCGGATCCGCT 1920
  C  M  E  T  G  G  K  D  G  T  F  L  V  R  E  S  E  T  F  P  N  D  Y  T  L  S  F  W  R  S  G  R  V  Q  H  C  R  I  R  S  590
CCACCATGGAGGGCGGGACCCTGAAATACTACTTGACTGACAACCTGAGGTTCAGGAGGATGTATGCCCTCATCCAGCACTACCGCGAGACGCACCTGCCGTGCGCCGAGTTCGAGCTGC 2040
  T  M  E  G  G  T  L  K  Y  Y  L  T  D  N  L  R  F  R  R  M  Y  A  L  I  Q  H  Y  R  E  T  H  L  P  C  A  E  F  E  L  R  630
GGCTCACGGACCCTGTGCCCAACCCCAACCCCCACGAGTCCAAGCCGTGGTACTATGACAGCCTGAGCCGCGGAGAGGCAGAGGACATGCTGATGAGGATTCCCCGGGACGGGGCCTTCC 2160
  L  T  D  P  V  P  N  P  N  P  H  E  S  K  P  W  Y  Y  D  S  L  S  R  G  E  A  E  D  M  L  M  R  I  P  R  D  G  A  F  L  670
TGATCCGGAAGCGAGAGGGGAGCGACTCCTATGCCATCACCTTCAGGGCTAGGGGCAAGGTAAAGCATTGTCGCATCAACCGGGACGGCCGGCACTTTGTGCTGGGGACCTCCGCCTATT 2280
  I  R  K  R  E  G  S  D  S  Y  A  I  T  F  R  A  R  G  K  V  K  H  C  R  I  N  R  D  G  R  H  F  V  L  G  T  S  A  Y  F  710
TTGAGAGTCTGGTGGAGCTCGTCAGTTACTACGAGAAGCATTCACTCTACCGAAAGATGAGACTGCGCTACCCCGTGACCCCCGAGCTCCTGGAGCGCTACAATACGGAAAGAGATATAA 2400
  E  S  L  V  E  L  V  S  Y  Y  E  K  H  S  L  Y  R  K  M  R  L  R  Y  P  V  T  P  E  L  L  E  R  Y  N  T  E  R  D  I  N  750
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  S  L  Y  D  V  S  R  M  Y  V  D  P  S  E  I  N  P  S  M  P  Q  R  T  V  K  A  L  Y  D  Y  K  A  K  R  S  D  E  L  S  F  790
TCTGCCGTGGTGCCCTCATCCACAATGTCTCCAAGGAGCCCGGGGGCTGGTGGAAAGGAGACTATGGAACCAGGATCCAGCAGTACTTCCCATCCAACTACGTCGAGGACATCTCAACTG 2640
  C  R  G  A  L  I  H  N  V  S  K  E  P  G  G  W  W  K  G  D  Y  G  T  R  I  Q  Q  Y  F  P  S  N  Y  V  E  D  I  S  T  A  830
CAGACTTCGAGGAGCTAGAAAAGCAGATTATTGAAGACAATCCCTTAGGGTCTCTTTGCAGAGGAATATTGGACCTCAATACCTATAACGTCGTGAAAGCCCCTCAGGGAAAAAACCAGA 2760
  D  F  E  E  L  E  K  Q  I  I  E  D  N  P  L  G  S  L  C  R  G  I  L  D  L  N  T  Y  N  V  V  K  A  P  Q  G  K  N  Q  K  870
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  S  F  V  F  I  L  E  P  K  E  Q  G  D  P  P  V  E  F  A  T  D  R  V  E  E  L  F  E  W  F  Q  S  I  R  E  I  T  W  K  I  910
TTGACAGCAAGGAGAACAACATGAAGTACTGGGAGAAGAACCAGTCCATCGCCATCGAGCTCTCTGACCTGGTTGTCTACTGCAAACCAACCAGCAAAACCAAGGACAACTTAGAAAATC 3000
  D  S  K  E  N  N  M  K  Y  W  E  K  N  Q  S  I  A  I  E  L  S  D  L  V  V  Y  C  K  P  T  S  K  T  K  D  N  L  E  N  P  950
CTGACTTCCGAGAAATCCGCTCCTTTGTGGAGACGAAGGCTGACAGCATCATCAGACAGAAGCCCGTCGACCTCCTGAAGTACAATCAAAAGGGCCTGACCCGCGTCTACCCAAAGGGAC 3120
  D  F  R  E  I  R  S  F  V  E  T  K  A  D  S  I  I  R  Q  K  P  V  D  L  L  K  Y  N  Q  K  G  L  T  R  V  Y  P  K  G  Q  990
AAAGAGTTGACTCTTCAAACTACGACCCCTTCCGCCTCTGGCTGTGCGGTTCTCAGATGGTGGCACTCAATTTCCAGACGGCAGATAAGTACATGCAGATGAATCACGCATTGTTTTCTC 3240
  R  V  D  S  S  N  Y  D  P  F  R  L  W  L  C  G  S  Q  M  V  A  L  N  F  Q  T  A  D  K  Y  M  Q  M  N  H  A  L  F  S  L 1030
TCAACGGGCGCACGGGCTACGTTCTGCAGCCTGAGAGCATGAGGACAGAGAAATATGACCCGATGCCACCCGAGTCCCAGAGGAAGATCCTGATGACGCTGACAGTCAAGGTTCTCGGTG 3360
  N  G  R  T  G  Y  V  L  Q  P  E  S  M  R  T  E  K  Y  D  P  M  P  P  E  S  Q  R  K  I  L  M  T  L  T  V  K  V  L  G  A 1070
CTCGCCATCTCCCCAAACTTGGACGAAGTATTGCCTGTCCCTTTGTAGAAGTGGAGATCTGTGGAGCCGAGTATGGCAACAACAAGTTCAAGACGACGGTTGTGAATGATAATGGCCTCA 3480
  R  H  L  P  K  L  G  R  S  I  A  C  P  F  V  E  V  E  I  C  G  A  E  Y  G  N  N  K  F  K  T  T  V  V  N  D  N  G  L  S 1110
GCCCTATCTGGGCTCCAACACAGGAGAAGGTGACATTTGAAATTTATGACCCAAACCTGGCATTTCTGCGCTTTGTGGTTTATGAAGAAGATATGTTCAGCGATCCCAACTTTCTTGCTC 3600
  P  I  W  A  P  T  Q  E  K  V  T  F  E  I  Y  D  P  N  L  A  F  L  R  F  V  V  Y  E  E  D  M  F  S  D  P  N  F  L  A  H 1150
ATGCCACTTACCCCATTAAAGCAGTCAAATCAGGATTCAGGTCCGTTCCTCTGAAGAATGGGTACAGCGAGGACATAGAGCTGGCTTCCCTCCTGGTTTTCTGTGAGATGCGGCCAGTCC 3720
  A  T  Y  P  I  K  A  V  K  S  G  F  R  S  V  P  L  K  N  G  Y  S  E  D  I  E  L  A  S  L  L  V  F  C  E  M  R  P  V  L 1190
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  E  S  E  E  E  L  Y  S  S  C  R  Q  L  R  R  R  Q  E  E  L  N  N  Q  L  F  L  Y  D  T  H  Q  N  L  R  N  A  N  R  D  A 1230
CCCTGGTTAAAGAGTTCAGTGTTAATGAGAACCACTCCAGCTGTACCAGGAGAAATGCAACAAGAGGTTAAGAGAGAAGAGAGTCAGCAACAGCAAGTTTTACTCATAGAAGCTGGGGTA 3960
  L  V  K  E  F  S  V  N  E  N  H  S  S  C  T  R  R  N  A  T  R  G  *
TGTGTGTAAGGGTATTGTGTGTGTGCGCATGTGTGTTTGCATGTAGGAGAACGTGCCCTATTCACACTCTGGGAAGACGCTAATCTGTGACATCTTTTCTTCAAGCCTGCCATCAAGGAC 4080
ATTTCTTAAGACCCAACTGGCATGAGTTGGGGTAATTTCCTATTATTTTCATCTTGGACAACTTCTAACTTATATCTTTATAGAGGATTCCCCAAAATGTGCTCCTCATTTTTGGCCTCT 4200
CATGTTCCAAACCTCATTGAATAAAAAGCAATGAAAACCTTGAAAAAAAAAAAAAAAAAAA
```

Fig.1. Nucleotide sequence of the putative phospholipase C and the deduced amino acid sequence. The first methionine in the open reading frame is proposed to the initiation codon and the numbers start at the methionine for the deduced sequence.

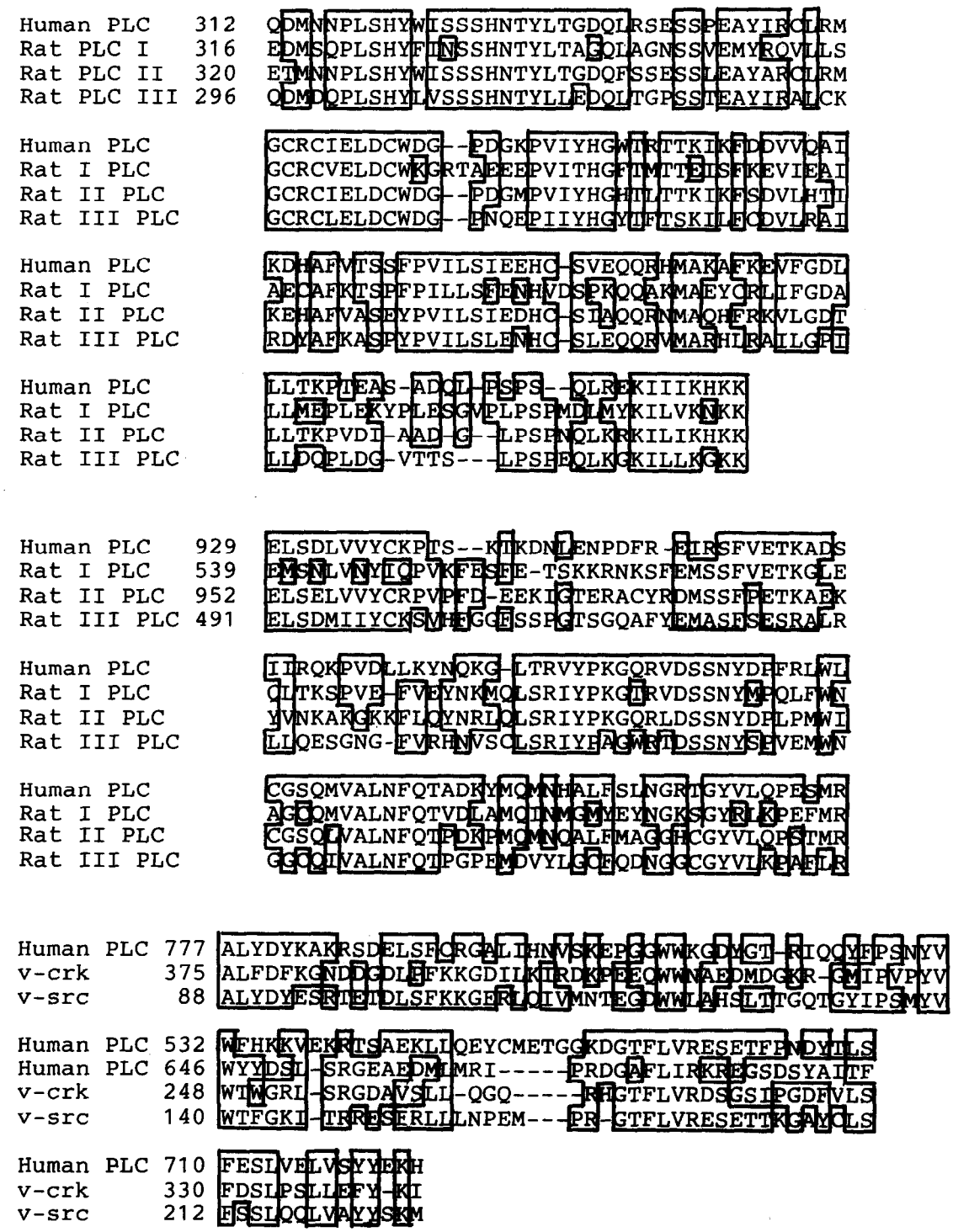

Fig.2. Comparison of part of the amino acid sequence with the sequences of other PLCs and tyrosine kinase-related oncogene products. The identical or conservatively changed amino acids are enclosed in boxes. See [13,14,16] for the published sequences.

the purified phage DNA (*Eco*RI and *Hin*dIII fragments) were directly analyzed by sequencing as described [22] (one of the *Hin*dIII sites is located in the λgt10 DNA and one of the *Eco*RI sites is located in the cloning site).

3.3. *Northern blotting*

Northern blotting was performed as described [23]. The *Eco*RI fragment of the cDNA was used as the hybridization probe.

3. RESULTS AND DISCUSSION

A human cDNA library of transformed lymphocytes was screened with oligonucleotide probes as described in section 2. Out of 1.2×10^6 recombinants, three positive clones were isolated and purified. Since the nucleotide sequence of the insert suggested that it did not contain the full length of the cDNA, the library was screened again with the cloned insert as the probe. The nucleotide sequence and the deduced amino acid sequence of the longest insert are shown in fig.1. The parts of the deduced amino acid sequence were compared with those of the published PLCs [14,16] as illustrated in fig.2. The similarity of the deduced amino acid sequence to the published sequences strongly suggest that the cDNA encodes a kind of PLC. Although 97% of the amino acids of the bovine brain 148-PLC [13] were identical with those of the rat brain (PLC II) [13], only 50.2% of the total amino acids were identical with PLC II (rat brain) or 148-PLC (bovine brain) by a maximum matching method. In addition, this cDNA contained regions, the sequences of which were similar to those of some tyrosine kinases-related oncogene products (fig.2).

In Northern blot experiments, three bands of poly(A^+) RNA prepared from human promyelocytic leukemia cells (HL-60) were hybridized with the cloned cDNA under weakly stringent conditions (indicated by arrows in fig.3). Only one band was hybridized under strongly stringent conditions (fig.3). Since the published clones [13,15] could be hybridized with human fibroblast RNA but not with HL-60 RNA, this putative PLC cDNA should be a different species of isozyme. In addition, the Northern blot experiment suggests that HL-60 cells may contain two additional isozymes of PLC. One of them may be a PLC of 61 kDa that was purified from human platelet membrane [12]. The interaction between PLC and GTP-binding proteins have been investigated in

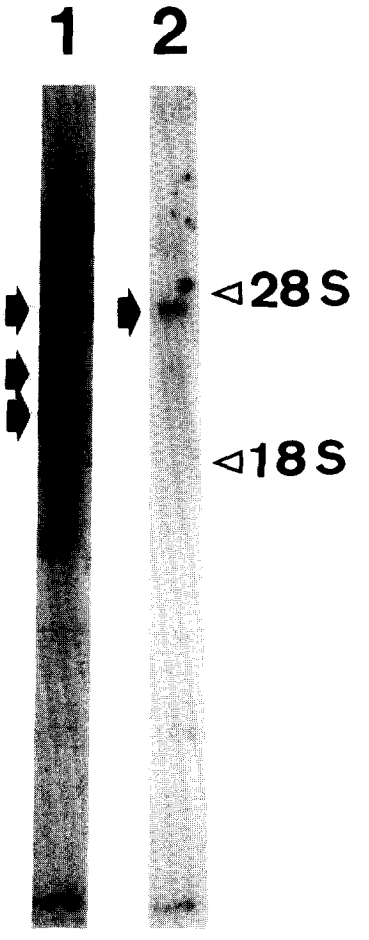

Fig.3. Northern blotting of mRNA from HL-60. Hybridization was performed using the 'Rapid hybridization system' (Amersham) at 55°C, and the membrane was washed with 2 × SSC and 0.1% SDS for 2 h at room temperature, then for 1 h at 55°C (lane 1) and for an additional 1.5 h at 65°C with 0.1% SDS and 0.1 × SSC (lane 2).

HL-60 [24]. Therefore, this clone will be potentially useful for various investigations on transmembrane signaling at the cellular level.

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