Proc. Natl. Acad. Sci. USA
Vol. 90, pp. 1804–1808, March 1993
Genetics

The putative phosphoinositide-specific phospholipase C gene, *PLC1*, of the yeast *Saccharomyces cerevisiae* is important for cell growth

(phospholipase C/signal transduction/polymerase chain reaction)

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Communicated by Norman H. Giles, November 16, 1992

ABSTRACT **Using the polymerase chain reaction technique, we have isolated a gene that encodes a putative phosphoinositide-specific phospholipase C (PLC) in the yeast *Saccharomyces cerevisiae*. The nucleotide sequence indicates that the gene encodes a polypeptide of 869 amino acid residues with a calculated molecular mass of 101 kDa. This polypeptide has both the X and Y regions conserved among mammalian PLC-β, -γ, and -δ, and the structure is most similar to that of mammalian PLC-δ. This putative yeast PLC gene has been designated *PLC1*. Disruption of *PLC1* results in slow growth or lethality for cells, depending on their genetic background and the medium, indicating that *PLC1* is important for cell growth. Expression of rat PLC-δ1 cDNA suppressed the growth defect of *plc1* disruptants, strongly suggesting that *PLC1* encodes PLC.**

In mammalian systems, hydrolysis of polyphosphoinositides by phospholipase C is one of the major pathways of signal transduction during response to extracellular signals, such as hormones, growth factors, and other regulatory ligands (reviewed in refs. 1–4). The phosphoinositide-specific phospholipase C (PLC) is responsible for the production of two second-messenger molecules, diacylglycerol, which is an activator of protein kinase C (5), and inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3], which causes the release of Ca^{2+} from endoplasmic reticulum (1). PLCs are categorized into four isoforms, PLC-α, -β, -γ, and -δ, on the basis of their enzymatic characteristics and comparison of their primary amino acid sequences (6, 7). Among PLC-β, -γ, and -δ there is significant similarity of amino acid sequences in two regions, designated the X and Y regions, which are essential for the PLC activity (8).

Purification and biochemical characterization of PLCs have been carried out with materials of mammalian origin. However, the functions of the enzyme *in vivo* remain unelucidated. To explore the *in vivo* functions of these enzymes, a genetic approach using a model organism is promising. In *Drosophila*, analysis of the *norpA* gene has revealed that it encodes a putative PLC (9), and this result, along with the fact that the *norpA* mutations render the fly blind (10), offers strong evidence that PLC is an essential component of the phototransduction pathway in *Drosophila*.

In the budding yeast *Saccharomyces cerevisiae* the gene of a putative protein kinase C, which is believed to play a role downstream from PLC, has been identified (11, 12), and existence of polyphosphoinositides has also been reported (13). In spite of these findings suggesting the presence of a phosphoinositide-mediated signal transduction pathway in *S. cerevisiae*, no PLC gene has been identified so far, to our knowledge. We report here the isolation of the putative yeast PLC gene,‖ whose function is important for cell growth. This finding suggests that the signal transduction systems mediated by phospholipid hydrolysis play an important role(s) for yeast cell growth.

MATERIALS AND METHODS

Microbial Techniques. Yeast transformations were performed by the method of Ito *et al.* (14), and other standard yeast genetic manipulations were performed as described by Sherman *et al.* (15). YPD and sporulation media have been described (ref. 15, pp. 163–169). SC medium contains 0.67% yeast nitrogen base without amino acids (Difco), 2% glucose, 20 ng each of adenine sulfate, uracil, L-histidine·hydrochloride, L-methionine, and L-tryptophan, and 30 ng each of L-leucine and L-lysine·hydrochloride per ml. SDCA medium contains 0.67% yeast nitrogen base without amino acids, 2% glucose, 0.5% casamino acids (Difco), and 20 ng each of adenine sulfate, uracil, and L-tryptophan per ml. YPGS was prepared by replacing the 2% glucose of YPD with 5% galactose and 0.3% sucrose. LB and 2× YT (ref. 16, pp. A.1–A.3) were used for bacterial culture. Bacterial cells were grown at 37°C. Yeast cells were grown at 30°C unless otherwise indicated.

DNA Manipulation. Plasmid DNA was propagated in *Escherichia coli* XL1-Blue or DH5α and prepared according to the alkaline lysis method described by Sambrook *et al.* (ref. 16, pp. 1.25–1.28). Yeast DNA was prepared as described by Sherman *et al.* (ref. 15, pp. 125–128). Restriction enzymes were purchased from Takara Shuzo (Kyoto), Toyobo (Osaka), and New England Biolabs, and were used according to the suppliers' directions. Probes for hybridization were prepared by the method described by Feinberg and Vogelstein (17, 18). Colony hybridization was performed as described by Grunstein and Hogness (19). Rapid hybridization buffer (Amersham) was used for hybridization, and the conditions of hybridization and washing were those recommended by the supplier. Deletion derivatives of plasmids for sequencing were constructed by the method of Henikoff (20), and the nucleotide sequences were determined by the method of McBride *et al.* (21) using an automated DNA sequencer, model 370A (Applied Biosystems).

Strains and Plasmids. The *S. cerevisiae* strains used in this study are S288C (*MATα CUP1 gal2 mal mel SUC2*), X2180-1A (*MATa CUP1 gal2 mal mel SUC2*), RAY-3A-D (*MATa/MATα his3/his3 leu2/leu2 trp1/trp1 ura3/ura3*) (22),

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Abbreviations: PLC, phosphoinositide-specific phospholipase C; Ins(1,4,5)P_3, inositol 1,4,5-trisphosphate; Plc1p, *PLC1* gene product.

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‖The sequence reported in this paper has been deposited in the GenBank data base (accession no. D12738).

and YPH501 (*MATa/MATα ade2/ade2 his3/his3 leu2/leu2 lys2/lys2 trp1/trp1 ura3/ura3*) (23). A cosmid library of *S. cerevisiae*, which contains ≈45-kb yeast genomic *Sau*3AI DNA fragments from strain X2180-1A inserted into the *Bam*HI site of cosmid pHC79 (24), was kindly provided by Y. Ohya (Univ. of Tokyo). Plasmid pSTS1-19 carrying *STT1* (*PKC1*) was from S. Yoshida (University of Tokyo) (12), and plasmid pRS314[*BCK1-20*], which carries *BCK1-20*, was from D. E. Levin (The Johns Hopkins University School of Hygiene and Public Health, Baltimore) (25). Plasmids pTY1–pTY6 were isolates from the yeast genomic cosmid library. Plasmids YEpUPLC1 and YCpUPLC1 carry the 3.4-kb *Bam*HI fragment of pTY4 containing the *PLC1* gene in YEp352 (26) and pRS316 (23), respectively. Plasmid pSδ1/TV119 carries the PLC-δ1 cDNA derived from a spontaneously hypertensive rat (27). The whole open reading frame of the PLC-δ1 cDNA in pSδ1/TV119 was inserted between the *Eco*RI and *Pvu* II sites of pKT10 (28), which contains *URA3*, the origin of replication of the 2-μm plasmid, and the *S. cerevisiae TDH3* promoter, to obtain pGAP-PLC-δ1. This construction placed the rat PLC-δ1 cDNA expression under the control of the *TDH3* promoter.

***In Vitro* DNA Amplification.** PCR was carried out essentially as described by Saiki *et al.* (29). The mixed oligonucleotide primers used for PCR were as follows: 5′-GGAATTCTCICAYAAYACITAYYT-3′ and 5′-CGGATC-CTYCCAICAITCYAAYTCIAIRCAICKRCAICC-3′, in which Y indicates C or T, I indicates inosine, R indicates A or G, and K indicates G or T. PCR was carried out with these primers at 2.5 μM and 20 ng of genomic DNA of *S. cerevisiae* strain S288C as a template in a 20-μl reaction mixture. The reactions were performed for 35 cycles as follows: the samples were heated at 94°C for 1 min, cooled at 37°C for 2 min, and heated at 72°C for 3 min.

RESULTS

Identification of the Yeast PLC Gene. Two conserved amino acid sequences, SHNTYL and GCRC(I/L/V)ELDCW(D/K/N), found in the X region of mammalian PLC-β, -γ, and -δ, were used as the basis for designing two primers for PCR. A 122-bp fragment was amplified by PCR using these primers and *S. cerevisiae* genomic DNA as a template. The fragment was cloned and its nucleotide sequence was determined. The deduced amino acid sequence was highly homologous to the corresponding sequence of the X region, suggesting that the amplified fragment was derived from a yeast PLC gene. An *S. cerevisiae* genomic cosmid library was screened, using this fragment as a probe, to obtain pTY1 and pTY2 (Fig. 1). The partial nucleotide sequences and the restriction maps of these cosmid clones indicated that neither of them contained the entire gene. Therefore, the genomic library was screened again, using the 1.5-kb *Mlu* I fragment of pTY1 as a probe. Four clones, designated pTY3–pTY6, were hybridized to the probe, and all of them carried an ≈4.7-kb *Hin*dIII fragment. This *Hin*dIII fragment from pTY4 was subcloned and the nucleotide sequence was determined on both strands. The sequence that was identical to that of the fragment amplified by PCR was found in this nucleotide sequence, and an open reading frame of 2607 bp was identified (Fig. 2). This encodes a polypeptide of 869 amino acids, and the calculated molecular weight is 100,546. The deduced amino acid sequence of the open reading frame is homologous to the sequences of mammalian and *Drosophila* PLCs, and the gene product possesses both the X and Y regions, which are conserved among PLC-β, -γ, and -δ. The gene product shows 24–25% amino acid sequence identity with mammalian PLC-γ1, -δ1, and -δ2. The regions corresponding to the X region and Y region are about 40% and about 30%, respectively, identical to those of mammalian PLCs. Therefore we designated this gene *PLC1*. The deduced structure of the gene products is diagramed in Fig. 3.

Disruption of *PLC1* Results in a Growth Defect. To explore the functions of *PLC1*, we disrupted the chromosomal *PLC1* and studied the consequence of the disruption. First, we constructed plasmid pTY411ΔHIS3, which carries *plc1*::*HIS3*, where most of the *PLC1* coding sequence is replaced by *HIS3* (Fig. 1). pTY411ΔHIS3 was digested with *Stu* I and *Mlu* I and introduced into diploid yeast strains RAY-3A-D and YPH501 by replacement transformation. Histidine-independent transformants were isolated, and disruption of the genomic *PLC1* gene was confirmed by Southern hybridization analysis (data not shown). The resultant heterozygous *plc1*::*HIS3*/*PLC1* diploids, TY1, which was derived from RAY-3A-D, and TY4, which was derived from YPH501, were sporulated and dissected on YPD plates. In the TY1 strain, two large and two small colonies of haploid cells were obtained from each tetrad (Fig. 4*A*). All of the cells from large colonies were histidine auxotrophs, indicating that they carried the *PLC1* allele and implying that the cells in

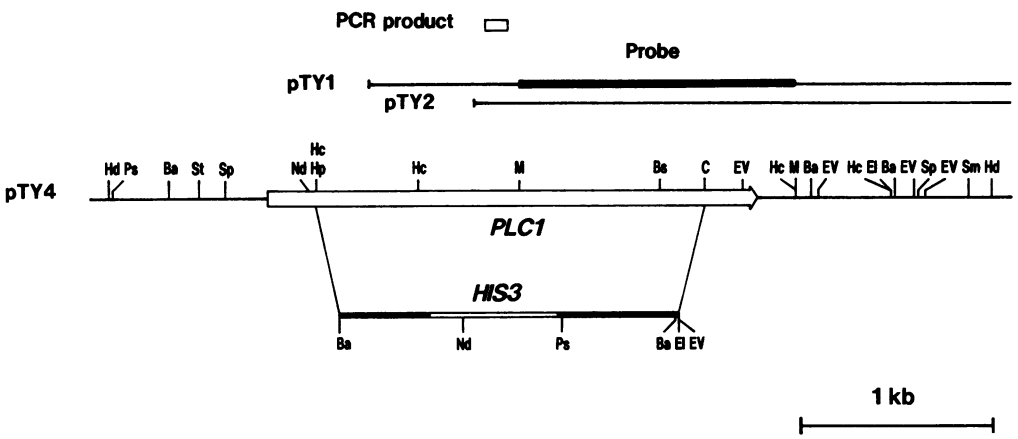

FIG. 1. Restriction map of the *PLC1* region and the construction of the *PLC1* deletion. The restriction map of the 4.7-kb *Hin*dIII–*Hin*dIII fragment of pTY4 is highlighted. pTY1 and pTY2 are primary isolates from the genomic DNA library. The 1.5-kb *Mlu* I fragment used for the secondary screening as a probe is indicated as a filled box in pTY1. An empty box indicates the DNA fragment amplified by PCR. The coding region of *PLC1* is indicated by an empty arrow. Plasmid pTY411ΔHIS3, which carries the *plc1*::*HIS3* allele, was constructed as follows: pTY411 carries the 4.2-kb *Pst* I–*Eco*RI fragment containing *PLC1* between the *Pst* I site and the *Eco*RI site of pUC19 (30). The 2.1-kb *Hpa* I–*Cla* I fragment in pTY411 was replaced with the 1.8-kb fragment carrying the *HIS3* gene to construct pTY411ΔHIS3. Restriction sites: Ba, *Bam*HI; Bs, *Bst*XI; C, *Cla* I; EI, *Eco*RI; EV, *Eco*RV; Hc, *Hin*cII; Hd, *Hin*dIII; Hp, *Hpa* I; M, *Mlu* I; Nd, *Nde* I; Ps, *Pst* I; Sm, *Sma* I; Sp, *Spe* I; St, *Stu* I.

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-3                                                                                                                    TGA

 1 ATGACTGAAAGTGCTATAGATGACCAAAGGTTTAATCTTACAAAGGAGTTACAACGACACTCATGCAGAGATCAAGGGAAAATAACACAAAAGGATGATGCTTTAGATTTTATAAGTTAC
    M  T  E  S  A  I  D  D  Q  R  F  N  L  T  K  E  L  Q  R  H  S  C  R  D  Q  G  K  I  T  Q  K  D  D  A  L  D  F  I  S  Y   40

121 AGTTCTTTTCAATCCTCTTTTAATACTGACCAAAAAAGCGCGAACAATGGAAGTACTGTGAGAAGAAGCATAAGGTCGATTTTTAGAAGAGCAGCCGAATTGCCTAGAGTCCATATGGGG
    S  S  F  Q  S  S  F  N  T  D  Q  K  S  A  N  N  G  S  T  V  R  R  S  I  R  S  I  F  R  R  A  A  E  L  P  R  V  H  M  G   80

241 CCTCTTACTTATTCACATGGGATAAATGAGCTTGTTAACAAGAAATTAAGAAAAGACTGTGATCTCAGCACGCTATGTCGCGTATTGCAAAGAGGAATCAGGATGATTAGGATGACAAGA
    P  L  T  Y  S  H  G  I  N  E  L  V  N  K  K  L  R  K  D  C  D  L  S  T  L  C  R  V  L  Q  R  G  I  R  M  I  R  M  T  R  120

361 AGAAGAAGGAAGTTCTATGAATTTAAATTAATCAATAACAACGGGCAAATAATATGGAAAGATGGTTCGAAGTATCTGGAATTAGACTCCGTTAAAGACATCAGAATCGGTGATACGGCG
    R  R  R  K  F  Y  E  F  K  L  I  N  N  N  G  Q  I  I  W  K  D  G  S  K  Y  L  E  L  D  S  V  K  D  I  R  I  G  D  T  A  160

481 AGTACTTATCAAGAGGAAGTAGATCCGAAAAGATTGAGATCGGACAGTAAACTTTGGATTGCTATTATTTATAAGGTTTCCAACAAATTGAAAGCGTTACACGTAGTTGCTTTGAATGAA
    S  T  Y  Q  E  E  V  D  P  K  R  L  R  S  D  S  K  L  W  I  A  I  I  Y  K  V  S  N  K  L  K  A  L  H  V  V  A  L  N  E  200

601 TTGGACTTTAACACATTCTTAAGCTGCATTTGCGGTTTAGTGAAGTTAAGGAGGGAATTAATGGAAAGCATACTTTTGCCTGATAATTCGCAATTCGCTAGGATACATTGGCAAATTACT
    L  D  F  N  T  F  L  S  C  I  C  G  L  V  K  L  R  R  E  L  M  E  S  I  L  L  P  D  N  S  Q  F  A  R  I  H  W  Q  I  T  240

721 GTTTCTGAAAAAGAGGAAGATGAGAAAAAGGACACATTGAGCTTTGCAGATGTGAAGAAACTATGTGATAAATTTCATATTTATGTGTCAACTGGTCAATTGTTAGAGTTTTTCCAATTA
    V  S  E  K  E  E  D  E  K  K  D  T  L  S  F  A  D  V  K  K  L  C  D  K  F  H  I  Y  V  S  T  G  Q  L  L  E  F  F  Q  L  280

841 GCAGACATCAATCATAACGGACTATTAAATTATTTTGAATTTGAAAAGTTCATTAAAATCTTAAAGAATAGGAAAGAGGTAAATATGATTTGGTCTAAATTTACAAAACCTCCACACTCC
    A  D  I  N  H  N  G  L  L  N  Y  F  E  F  E  K  F  I  K  I  L  K  N  R  K  E  V  N  M  I  W  S  K  F  T  K  P  P  H  S  320

961 CACTTATCATTTGAAAATTTTTTTCAATTTTAATTACAGAGCAGCATGAGCAAGTGGATCGTCAAACTGCATGGTCATATTTTATTAAGTACAGAGAACCGACTCAGCTGACGATGGGG
    H  L  S  F  E  N  F  F  Q  F  L  I  T  E  Q  H  E  Q  V  D  R  Q  T  A  W  S  Y  F  I  K  Y  R  E  P  T  Q  L  T  M  G  360

1081 CAAGATGGTTTTACCAAGTTCTTGAAGGAGCAACCATATTTGGTGGAGGTTAAAGAGGAGTTATATTCAAAGCCATTAAACCATTATTTTATTGCATCTTCACATAATACTTATTTATTG
     Q  D  G  F  T  K  F  L  K  E  Q  P  Y  L  V  E  V  K  E  E  L  Y  S  K  P  L  N  H  Y  F  I  A  S  S  H  N  T  Y  L  L  400

1201 GGGAAACAAATTGCAGAAACTCCATCTGTGGAGGGATATATTCAAGTCCTACAACAAGGTTGTAGGTGCGTAGAAATTGATATATGGGATGGTGAAAATGGGCCAGTGGTATGCCATGGA
     G  K  Q  I  A  E  T  P  S  V  E  G  Y  I  Q  V  L  Q  Q  G  C  R  C  V  E  I  D  I  W  D  G  E  N  G  P  V  V  C  H  G  440

1321 TTTTTAACGTCAGCTATTCCCTTAAAAACAGTTATACGCGTGATAAAGAAGTACGCTTTTATAACGTCTCCGTATCCTTTGATTATTTCACTGGAAATAAACTGTAACAAGGATAATCAA
     F  L  T  S  A  I  P  L  K  T  V  I  R  V  I  K  K  Y  A  F  I  T  S  P  Y  P  L  I  I  S  L  E  I  N  C  N  K  D  N  Q  480

1441 AAATTGGCAAGCCTGATAATGAGAGAGGTTTTAGCAGAACAGCTGTACTTTGTTGGCACAAGAACTGATAAACTGCCATCACCAAGGGAATTAAAACATAAAATACTGTTGAAATCCAAG
     K  L  A  S  L  I  M  R  E  V  L  A  E  Q  L  Y  F  V  G  T  R  T  D  K  L  P  S  P  R  E  L  K  H  K  I  L  L  K  S  K  520

1561 AAGACGAGTGAAGCAACTAGAGGTCTTAGCGTTAATGAGCCATTTCCATCTTCTTTTAGTTCTTCTTACGAATCAGCTAATGAACAGGAATTAAGAATGAAAGATGACTCCACGAACAGT
     K  T  S  E  A  T  R  G  L  S  V  N  E  P  F  P  S  S  F  S  S  S  Y  E  S  A  N  E  Q  E  L  R  M  K  D  D  S  T  N  S  560

1681 AGTAGCGCAACAAATTCTTCTAGTATGCAACGCATCAAAAGAATTGGATTAAAAAAGCACGCTGATATAATTAATGATGTTTCTAACATTTCTGGAATACATGGCATCAAGTTCAGGAAT
     S  S  A  T  N  S  S  S  M  Q  R  I  K  R  I  G  L  K  K  H  A  D  I  I  N  D  V  S  N  I  S  G  I  H  G  I  K  F  R  N  600

1801 TTCTCTTTACCAGAGTCCAAAACGATAGCGCATTGTTTTTCACTGAATGAGCGCAAAGTAGAATACATGATAAAAGACAAACACTTAAAATTATCGTTGGATAAACATAATAGAAGATAT
     F  S  L  P  E  S  K  T  I  A  H  C  F  S  L  N  E  R  K  V  E  Y  M  I  K  D  K  H  L  K  L  S  L  D  K  H  N  R  R  Y  640

1921 TTGATGAGAGTCTATCCTCACGTTTTACGATACAAATCATCAAATTTTAACCCAATACCTTTTTGGAAGGCCGGTGTACAAATGGTGGCTACGAATTGGCAAACTAATGATATTGGACAG
     L  M  R  V  Y  P  H  V  L  R  Y  K  S  S  N  F  N  P  I  P  F  W  K  A  G  V  Q  M  V  A  T  N  W  Q  T  N  D  I  G  Q  680

2041 CAACTTAATCTGGCAATGTTTCAAATATTAGATCACCAACCTGATGGAAGTTTTAAATCAGGATACGTGCTAAAACCAAAAAAGTTGCTACCTGTAGTAACAAAGGCGAAAATGATTCCA
     Q  L  N  L  A  M  F  Q  I  L  D  H  Q  P  D  G  S  F  K  S  G  Y  V  L  K  P  K  K  L  L  P  V  V  T  K  A  K  M  I  P  720

2161 TTAATTTACGAACATTTTGAAAATGGTAGTGATCCTGTTACTGTTAAAATTCGAATTTTATCTACACAGTTATTGCCCAGATTGAATGATACATCGCCAAGCAGAAATAACACCAATTCC
     L  I  Y  E  H  F  E  N  G  S  D  P  V  T  V  K  I  R  I  L  S  T  Q  L  L  P  R  L  N  D  T  S  P  S  R  N  N  T  N  S  760

2281 TTTGTTAAAGTGGAGTTTCATACCGATGACGAGCCGACAATGCCCATCTCAATCGATAAGGGTACAAGGATTTCTGCCACTGAAGCATCCACTAAGAGTTCTCAAGGGAATGGATTCAAC
     F  V  K  V  E  F  H  T  D  D  E  P  T  M  P  I  S  I  D  K  G  T  R  I  S  A  T  E  A  S  T  K  S  S  Q  G  N  G  F  N  800

2401 CCAATATGGGATGCTGAAGTTTCTATCACATTGAAAGATACAGACCTCACATTTATAAAATTCATGGTTATCTCTGAAGAGACCCAGATTGCATCTGTTTGCCTTAAGCTGAACTATTTG
     P  I  W  D  A  E  V  S  I  T  L  K  D  T  D  L  T  F  I  K  F  M  V  I  S  E  E  T  Q  I  A  S  V  C  L  K  L  N  Y  L  840

2521 AGGATGGGATATCGTCACATTCCGTTGTTTAACATGGAGGGGGAACAATACATATTTTGCACTTTATTTATTCATACACAAATTTTATAGGCGATCTTTTTCCGGCCAAATACACATATT
     R  M  G  Y  R  H  I  P  L  F  N  M  E  G  E  Q  Y  I  F  C  T  L  F  I  H  T  Q  I  L  *                          869
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FIG. 2. Nucleotide sequence of *PLC1* and the deduced amino acid sequence. The numbers of the nucleotide sequence and the amino acid sequence are shown on the left and right, respectively. Nucleotides are numbered beginning with the A of the first ATG initiator codon that appears downstream from a nonsense codon found in-frame. The nucleotides with an underline indicate the region amplified by PCR.

small colonies carried the *plc1*::*HIS3* allele. When the segregants were transferred onto a fresh YPD plate and kept at 18°C, 30°C, or 37°C, the cells from the small colonies did grow, but slowly, irrespective of temperature, indicating that the cells lacking the *PLC1* gene product are viable. On the other hand, tetrads from TY4 strain yielded at most two visible colonies after 4 days of incubation (Fig. 4*B*), and also after 8 days of incubation. The cells from these colonies were all histidine auxotrophs. Microscopic observation of microcolonies that failed to become visible colonies revealed that the spores germinated but ceased to grow after five or six cell divisions. These results indicate that disruption of *PLC1* in the YPH501 background results in lethality for cells.

To examine the effect of the media on the *plc1* disruptant, asci from TY1 were dissected on plates of various media. When the asci were dissected on plates of SC, SDCA, YPD containing 7% glucose, YPGS, YPD containing 5% galactose, and YPD containing 5% sucrose, each tetrad yielded two or fewer visible colonies after 6 days of incubation (Fig. 4 *C–H*). Cells from visible colonies were all histidine auxotrophs, indicating that the *plc1* disruptants did not form colonies on these media. In contrast, addition of 5% sorbitol, which yeast cells cannot utilize as a carbon source, into YPD did not

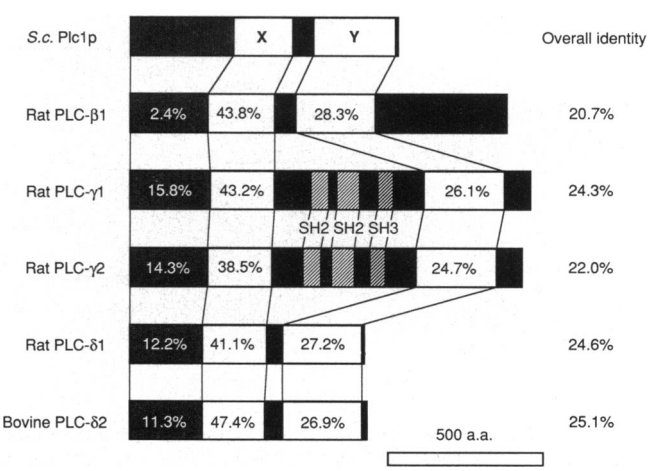

FIG. 3. Structure comparison between the putative *PLC1* gene product (Plc1p) and mammalian PLC-β, -γ, and -δ. Identities of each region are shown in the boxes by percent. The X region and Y region are indicated by open boxes. SH2 and SH3 indicate the regions homologous to noncatalytic regions of the *src* gene product (7).

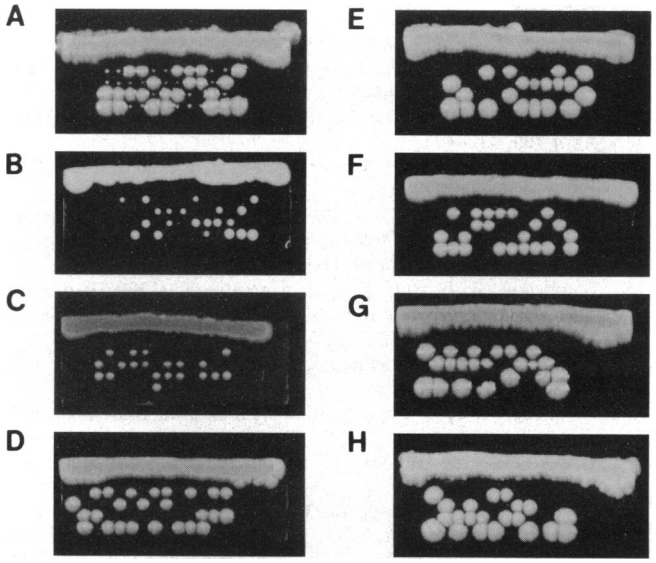

FIG. 4. Tetrad analysis of *plc1*::*HIS3*/*PLC1* heterozygous diploids TY1 (*A* and *C–H*) and TY4 (*B*). Asci were dissected on YPD (*A*, *B*), SC (*C*), SDCA (*D*), YPD containing 7% glucose (*E*), YPGS (*F*), YPD containing 5% galactose (*G*), and YPD containing 5% sucrose (*H*) plates. They were incubated at 30°C for 6 days (TY1) or at 30°C for 4 days (TY4).

abolish the colony formation of the *plc1* disruptant (data not shown), indicating that the inhibitory effect of a high concentration of sugar on colony formation of the *plc1* disruptant is not due to the high osmolarity of the media.

Expression of Rat PLC-δ1 Suppresses the Growth Defect of the *plc1* Disruptant. To investigate whether the growth defect of the *plc1* disruptant resulted from the reduced activity of PLC, the rat PLC-δ1 cDNA was expressed in the *plc1* disruptant. pGAP-PLC-δ1, a multicopy plasmid carrying rat PLC-δ1 cDNA under control of the yeast *TDH3* promoter and the selectable marker *URA3*, was introduced into the *PLC1*/*plc1*::*HIS3* strain TY1. When the uracil-independent transformants were sporulated and dissected on a YPD plate, more than three visible colonies were obtained from each tetrad after 4 days of incubation at 30°C, whereas the uracil-independent transformants with the plasmid without PLC-δ1 produced no more than 3 visible colonies, all of which were

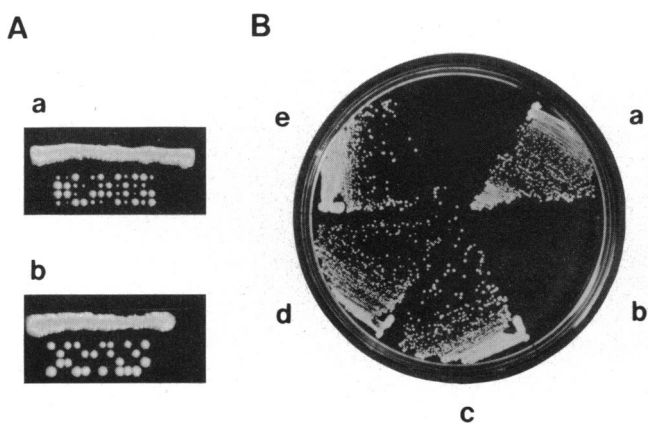

FIG. 5. Suppression of the growth defect of the *plc1* disruptant by expression of rat PLC-δ1 cDNA. (*A*) *plc1*::*HIS3*/*PLC1* heterozygous diploids, TY1, harboring the plasmid pGAP-PLC-δ1 (*a*) or the control plasmid, pKT10 (*b*), were sporulated, dissected on YPD plates, and incubated at 30°C for 4 days. (*B*) Haploid wild-type cells derived from RAY-3A-D (*a*) or *plc1*::*HIS3* cells from TY1 with no plasmid (*b*), with YEpUPLC1 (*c*), with YCpUPLC1 (*d*), or with pGAP-PLC-δ1 (*e*) were streaked on a YPD plate and incubated at 30°C for 2 days.

histidine auxotrophs, in each tetrad (Fig. 5*A*). Four days of incubation is not long enough for the *plc1*::*HIS3* cells to form a visible colony on YPD. In these segregants, all of the histidine-independent segregants were uracil-independent, indicating that a segregant carrying *plc1*::*HIS3* harbors pGAP-PLC-δ1 to form a visible colony. *plc1*::*HIS3* cells harboring pGAP-PLC-δ1 grew as well as wild-type cells (Fig. 5*B*). In the case of TY4, the result was essentially the same as with TY1 (data not shown). These results indicate that a lost *PLC1* gene is complemented by the PLC-δ1 activity.

DISCUSSION

We have isolated a putative PLC gene, designated *PLC1*, from *S. cerevisiae*. The *PLC1* gene product (Plc1p) possesses the X region and Y region, which are hallmarks of PLC-β, -γ, and -δ. Plc1p does not have either the SH2 or the SH3 region, both of which are found in the PLC-γ family, or an extended carboxyl-terminal sequence after the Y region, which is common among the PLC-β family. The overall amino acid identity between Plc1p and bovine PLC-δ2 is 25.1%, which is the highest identity between Plc1p and other PLCs reported. From the structural point of view, we propose that Plc1p be classified into the PLC-δ family.

The molecular mass of mammalian PLC-δs (about 85–87 kDa; refs. 31–34) is smaller than that of Plc1p (about 101 kDa) when the longest open reading frame of *PLC1* is adopted. Expression of the open reading frame started from the second methionine of the 79th codon under the control of the *GAL7* promoter and complemented the lost *PLC1* (unpublished data), suggesting that the deduced N-terminal sequence of 78 residues shown in Fig. 2 is not essential for the Plc1p activity. This result is consistent with the fact that the amino-terminal amino acid sequence is variable among PLCs. However, more work is necessary to reach the conclusion. It is still unclear whether Plc1p has PLC activity. In this context, it should be emphasized that the lost *PLC1* was complemented with the mammalian PLC-δ1 activity *in vivo*. This result strongly suggests that *PLC1* encodes PLC. Purification and characterization of Plc1p should clarify this matter.

The disruption of *PLC1* resulted in a severe growth defect: slow growth or lethality for cells, depending on the strain background and medium. Viability of the *plc1* disruptant was tested by staining with methylene blue (35), and we found that few cells in the slowly growing *plc1* disruptant culture were inviable. When the *plc1* disruptant cells grown on YPD or those in microcolonies formed on an SC plate were inspected by microscopy, these cells showed no morphological aberration and their growth was not arrested at any specific stage of the cell cycle. This observation suggests that *PLC1* may be required at the various stages of cell cycle. The reason why the phenotype of the *plc1* disruptant in the RAY-3A-D background was different from that in the YPH501 background is obscure at this moment. There may be a factor(s) bypassing the function(s) of *PLC1* in the RAY-3A-D background. We do not know the reason for the lethality of *plc1* disruption on synthetic media and the media containing a high concentration of fermentable carbon source. Isolating conditional mutants of *PLC1* should allow us to address these questions.

It remains unclear whether Plc1p is involved in any signal transduction system. Like growth factors in mammalian systems, nutrients, especially fermentable carbon sources, are one of major factors that promote yeast cells to transit G_1 phase to S phase. The loss of *PLC1* caused sensitivity to a high concentration of fermentable carbon sources and to the synthetic media, suggesting that Plc1p might mediate the signal transduction functioning in nutrient response. Assuming that Plc1p possesses the catalytic function of PLC, it is likely that the growth defect of the *plc1* disruptant resulted

from decreased diacylglycerol and Ins(1,4,5)P_3, which are believed to activate the downstream pathways. The *PKC1* gene product, a putative protein kinase C in yeast, is a candidate that plays a role downstream from the *PLC1* function(s). In *S. cerevisiae, BCK1-20*, a dominant mutation of the *BCK1* gene, has been isolated as an extragenic suppressor of a *pkc1* deletion mutant (25). However, we have no evidence supporting the possibility that these genes interact with *PLC1*, since the suppressors of the defect of the *PKC1* pathway, such as multicopies of *PKC1, BCK1-20*, and addition of osmotic stabilizing agents into media, did not suppress the growth defect of the *plc1* disruptant on YPD (data not shown). But it is premature to exclude the possibility that *PKC1* acts downstream from *PLC1*, since it is possible that the pathway mediated by Ins(1,4,5)P_3 is also important for cell growth, and this pathway may be independent of the protein kinase C pathway. Genetic analysis of the *PLC1* function(s) should shed some light on understanding the PLC functions.

We thank David E. Levin (The Johns Hopkins University School of Hygiene and Public Health, Baltimore) and Satoshi Yoshida (Univ. of Tokyo) for providing plasmids. We thank Yoshikazu Ohya (Univ. of Tokyo) for providing a yeast genomic DNA library. We thank Tomoko Ando (Univ. of Tokyo) for constructing the plasmid YCpUPLC1. This work was supported in part by a Grant-in-Aid for Scientific Research from the Mombusho.

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