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A Mutation in *PLC1*, a Candidate Phosphoinositide-Specific Phospholipase C Gene from *Saccharomyces cerevisiae*, Causes Aberrant Mitotic Chromosome Segregation

WILLIAM E. PAYNE† AND MOLLY FITZGERALD-HAYES*

Department of Biochemistry and Molecular Biology, Program in Molecular and Cellular Biology, University of Massachusetts, Amherst, Massachusetts 01003

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We identified a putative *Saccharomyces cerevisiae* homolog of a phosphoinositide-specific phospholipase C (PI-PLC) gene, *PLC1*, which encodes a protein most similar to the δ class of PI-PLC enzymes. The *PLC1* gene was isolated during a study of yeast strains that exhibit defects in chromosome segregation. *plc1-1* cells showed a 10-fold increase in aberrant chromosome segregation compared with the wild type. Molecular analysis revealed that *PLC1* encodes a predicted protein of 101 kDa with approximately 50 and 26% identity to the highly conserved X and Y domains of PI-PLC isozymes from humans, bovines, rats, and *Drosophila melanogaster*. The putative yeast protein also contains a consensus EF-hand domain that is predicted to bind calcium. Interestingly, the temperature-sensitive and chromosome missegregation phenotypes exhibited by *plc1-1* cells were partially suppressed by exogenous calcium.

Hydrolysis of inositol lipids by phospholipase C (PLC) to liberate the second messengers diacylglycerol and inositol 1,4,5-trisphosphate is a central reaction in one of the most widely used signal-generating mechanisms evolved by cells. Upon binding to their receptors, many growth factors and signaling molecules activate PLC. This initiates a signal transduction cascade, called the phosphoinositide (PI) pathway, which leads to activation of protein kinase C by diacylglycerol and release of calcium from intracellular stores by 1,4,5-trisphosphate (6, 43). Three classes of mammalian PI-specific PLC (PI-PLC) isozymes (β, γ, and δ) containing at least eight distinct isozyme subtypes (β1, β2, β3, γ1, γ2, δ1, δ2, and δ3) have been deduced by using biochemical, molecular, and immunological approaches (59, 60, 78).

PI-PLC-catalyzed hydrolysis of phospholipids to generate second-messenger molecules results from a wide range of stimuli and is implicated in many cellular processes, including mesoderm induction and axis determination in embryonic development (45), phototransduction (7, 53, 65, 66, 69, 83), secretion (58), growth and differentiation (4, 13), muscle contraction (33), and long-term potentiation of synaptic transmission in memory (11). Recent evidence indicates that a PI signaling pathway exists for communication between the nucleus and the cytoplasm (14, 54). In addition, PIs are involved in regulating the organization of the cytoskeleton (21, 22, 55, 67). Although many PI-PLC isozymes have been extensively characterized biochemically, the functions of the enzymes within any specific signal transduction pathway have not been elucidated, with the possible exception of the *Drosophila* PLC-β enzyme, which is known to be involved in phototransduction (7). Recently it was shown that mammalian PLC-β1 is regulated by a G-protein-dependent mechanism (72, 75, 80) and that PLC-γ1 is regulated by tyrosine phosphorylation (35, 47, 49, 79). It is likely that members of a particular PI-PLC subtype (e.g., β1, β2, and β3) are activated by G proteins or kinases that are specific for that subtype (59). The mechanism(s) whereby PLC-δ subtypes are activated is not known (59).

The nucleotide sequences of at least 14 PI-PLC cDNA molecules, representing members of all three classes of PI-PLC isozymes from mammalian systems, including humans, have been described (59, 78). Two genes that encode PI-PLC proteins which are homologous to the mammalian β class of PI-PLC isozymes have been isolated from *D. melanogaster* (7, 65, 69). In contrast, the PI-PLC enzymes isolated from bacteria show little structural similarity to the mammalian PI-PLC proteins. While bacterial PI-PLC does recognize PI, it does not hydrolyze the more highly phosphorylated derivatives of PI, such as phosphatidylinositol 4,5-biphosphate, which are involved in mammalian signal transduction (39). Until now, no PI-PLC genes or proteins have been isolated from any unicellular eucaryotic organism. We report here the cloning and molecular characterization of a putative PI-PLC gene, *PLC1*, from the budding yeast *Saccharomyces cerevisiae*. The putative protein encoded by *PLC1* has striking amino acid homology to mammalian PI-PLC proteins and has a structural organization that is most similar to the δ class of mammalian PI-PLC isozymes. We found that a mutant allele, *plc1-1*, slightly increased the frequency of chromosome segregation errors during mitosis and that the temperature-sensitive and chromosome missegregation phenotypes were partially suppressed by exogenous calcium. We also found that while *PLC1* was not an essential gene in *S. cerevisiae*, cells carrying a null allele, *plc1*Δ::*URA3*, exhibited extremely slow growth, even under ideal conditions.

MATERIALS AND METHODS

Strains, media, and genetic methods. The yeast strains used in this work are listed in Table 1. Yeast media were prepared, and genetic manipulations were performed as described by Sherman et al. (68). YPD medium is a rich medium (2% Bacto Peptone, 1% yeast extract, 2% glucose) which contains less than 200 μM calcium (1, 51) and was

* Corresponding author.
† Present address: Department of Biology, 56-534, Massachusetts Institute of Technology, Cambridge, MA 02139.

TABLE 1. Yeast strains used in this study

Strain	Relevant markers	Source, reference, or comment
41-14d	MATa/MATα cen3X69-URA3-SUP11/CEN3 ura3-52 trp1-Δ901 ade2-101 lys2-801	J. McGrew; 46; chromosome III disome
415F1X69	MATa ura3-52 trp1-Δ901 ade2-101 leu2-3,112 his3-11,15 cen3X69-URA3-SUP11	J. McGrew; 46
DBY3391	MATa ura3-52 leu2-3,112 his3-Δ200 cin2::LEU2	T. Stearns
TT44	MATa ura3-52 ade2-101 leu2-3,112 his3-Δ200 gal4::LEU2	T. Stearns
$\lambda^2$15.25	MATa/MATα cen3X69-URA3-SUP11/CEN3 ura3-52 trp1-Δ901 ade2-101 lys2-801 plc1-1	plc1-1 mutant disom
WPY252	MATα ura3-52 trp1-Δ901 ade2-101 his3-11,15	This study[a,b]
WPY253	MATa ura3-52 trp1-Δ901 ade2-101 his3-11,15	This study[a,b]
WPY254	MATa ura3-52 trp1-Δ901 ade2-101 leu2-3,112 his3-11,15 cen3X69-URA3-SUP11 plc1-1	This study[a,b]
WPY255	MATα ura3-52 trp1-Δ901 ade2-101 leu2-3,112 his3-11,15 cen3X69-URA3-SUP11 plc1-1	This study[a,b]
WPY263	MATα ura3-52 trp1-Δ901 ade2-101 leu2-3,112 his3-11,15 plc1-1	
WPY264	MATa/MATα cen3X69-URA3-SUP11/CEN3 ura3-52/ura3-52 trp1-Δ901/trp1-Δ901 ade2-101/ade2-101 leu2-3,112/LEU2 his3-11,15/his3-11,15 PLC1/PLC1	This study
WPY265	MATa/MATα cen3X69-URA3-SUP11/CEN3 ura3-52/ura3-52 trp1-Δ901/trp1-Δ901 ade2-101/ade2-101 leu2-3,112/LEU2 his3-11,15/his3-11,15 plc1-1/PLC1	This study
WPY267	MATa/MATα cen3X69-URA3-SUP11/CEN3 ura3-52/ura3-52 trp1-Δ901/trp1-Δ901 ade2-101/ade2-101 leu2-3,112/LEU2 his3-11,15/his3-11,15 plc1-1/plc1-1	This study
WPY290	MATa/MATα ura3-52/ura3-52 trp1-Δ901/trp1-Δ901 ade2-101/ade2-101 LEU2/leu2-3,112 his3-1,15/HIS3 LYS2/lys2-801	This study
WPY291	MATa/MATα ura3-52/ura3-52 trp1-Δ901/trp1-Δ901 ade2-101/ade2-101 LEU2/leu2-3,112 his3-11,15/HIS3 LYS2/lys2-801 PLC1/plc1::Tn10-LUK (URA3)	This study
WPY296	MATa/MATα ura3-52/ura3-52 trp1-Δ901/trp1-Δ901 ade2-101/ade2-101 LEU2/leu2-3,112 his3-11,15/HIS3 LYS2/lys2-801 PLC1/plc1Δ::URA3	This study
WPY297	MATa ura3-52 trp1-Δ901 ade2-101 leu2-3,112 his3-11,15 lys2-801	This study[b]
WPY298	MATa ura3-52 trp1-Δ901 ade2-101 plc1Δ::URA3	This study[b,c]
WPY299	MATα ura3-52 trp1-Δ901 ade2-101 leu2-3,112 his3-11,15	This study[b]
WPY300	MATa ura3-52 trp1-Δ901 ade2-101 plc1Δ::URA3	This study[b,c]
WPY308	MATα ura3-52 trp1-Δ901 ade2-101 leu2-3,112 his3-11,15 plc1-1 Tn10-LUK (URA3)	This study
WPY309	MATa/MATα cen3X69-URA3-SUP11/CEN3 ura3-52/ura3-52 trp1-Δ901/trp1-Δ901 ade2-101/ade2-101 leu2-3,112/leu2-3,112 his3-11,15/his3-11,15 plc1-1/plc1-1	This study
WPY309(pWP102)	MATa/MATα cen3X69-URA3-SUP11/CEN3 ura3-52/ura3-52 trp1-Δ901/trp1-Δ901 ade2-101/ade2-101 leu2-3,112/leu2-3,112 his3-11,15/his3-11,15 plc1-1/plc1-1 (pWP102)	This study
WPY517	MATa ura3-52 trp1-Δ901 ade2-101 his3-11,15	This study
WPY525	MATα ura3-52 trp1-Δ901 ade2-101 leu2-3,112 plc1-1	This study
WPY539	MATa/MATα ura3-52/ura3-52 TRP1/trp1-Δ901 ade2-101/ade2-101 leu2-3,112/leu2-3,112 his3-Δ200/HIS3 gal4::LEU2/GAL4 PLC1/plc1-1	This study
WPY541	MATa/MATα CEN3/cen3X69 URA3 SUP11 ura3-52/ura3-52 TRP1/trp1-Δ901 ADE2/ade2-101 leu2-3,112/leu2-3,112 his3-Δ200/his3-11,15 cin2::LEU2/CIN2 PLC1/plc1-1	This study

[a] Segregant from the third backcross of $\lambda^2$15.25 to 415F1X69.
[b] Strains WPY252-WPY255 and WPY297-WPY300 are sets of haploids from one complete tetrad.
[c] Haploid strains carrying the *plc1Δ::URA3* allele do not grow on minimal medium; therefore, genotypes are either unknown or inferred.

supplemented with $CaCl_2$ where indicated. Color medium contains 0.6% yeast nitrogen base, 0.5% Casamino Acids, 2% glucose, 50 μg of tryptophan per ml, 30 μg of uracil per ml, and 8 μg of adenine per ml (25), but color medium without leucine does not contain Casamino Acids. Yeast transformations were performed by a modified lithium acetate procedure (20, 64). Yeast transformants were selected on minimal medium lacking uracil or leucine. *Escherichia coli* DH5α [F′ *endA1 hsdR17* (r_K^- m_K^+) *supE44 thi-1 recA1 gyrA96*(Nal^r) *relA1* Δ(*lacZYA-argF*)*U169* (φ80d*lac*Δ (*lacZ*)*M15*); BRL Life Technologies] was used for routine cloning. Standard recombinant DNA techniques were performed as previously described (44).

Genetic screen for chromosome segregation mutants. Disome yeast strain 41-14d, which contains one native chromosome III and one copy of chromosome III bearing the mutant centromere (*cen3X69*), *URA3*, and *SUP11* (the X69 chromosome) (46) was mutagenized with ethyl methanesulfonate to 10% survival and plated onto color medium. After 4 days at 24°C, yeast colonies exhibiting many red-white sectors were restreaked onto color medium plates. Strains showing a reproducible high-sectoring phenotype were then transferred to YPD plates and incubated at 38 or 15°C in secondary screens for heat- and cold-sensitive conditional phenotypes.

Mutant strains were backcrossed three times to demonstrate that the high-sectoring and conditional lethal phenotypes cosegregate and to remove other unlinked mutations. To eliminate possible additional *cis* mutations in the *cen3X69* centromere, haploid red colonies which had lost the X69 chromosome and were no longer disomic for chromosome III were backcrossed with 415F1X69, a haploid strain carrying the X69 chromosome. The diploid strains were sporulated, the tetrads were dissected, and the resulting haploids were tested for growth on YPD plates at 38°C. To test for cosegregation of the temperature-sensitive and high-sector-

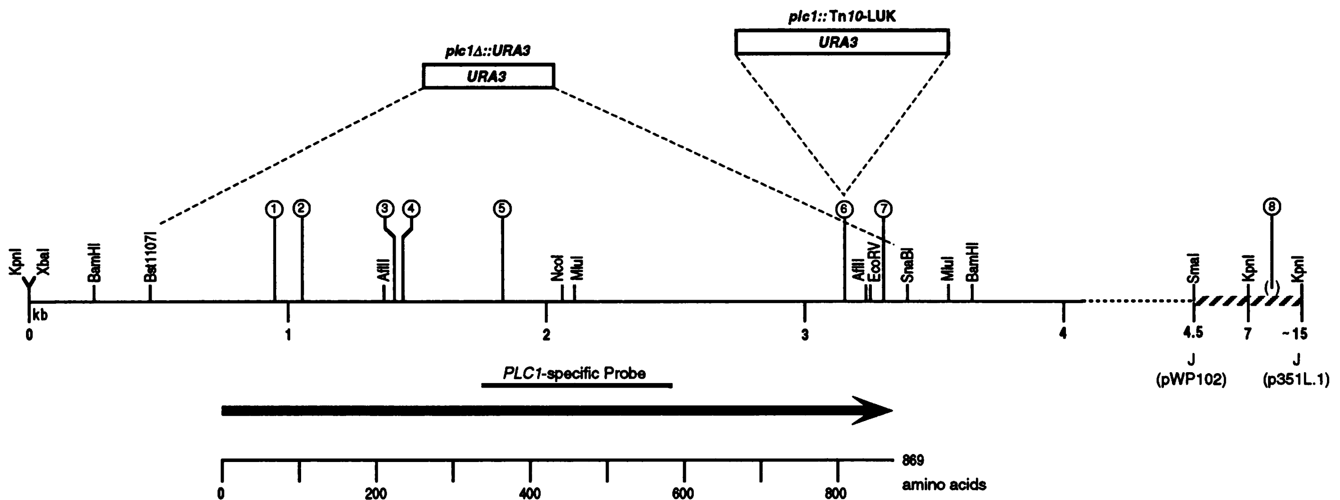

FIG. 1. Map of the *PLC1* locus. The coding region of *PLC1* is indicated by the large black arrow oriented in the direction of transcription. The numbered circles indicate sites of transposon insertions. The junctions between yeast DNA and vector DNA for plasmids pWP102 and p351L.1 are indicated by the letter J. The thin solid line represents the 4.1 kb of DNA encompassing the *PLC1* locus which was sequenced. The thin dashed line represents approximately 400 bp of DNA in pWP102 which was not sequenced. The thick hatched line represents approximately 10.5 kb of yeast DNA in the original p351L.1 isolate that contains Tn*10*-LUK no. 8. The structures of the disruption alleles are shown above the restriction map. The DNA between *Bst*1107I and *Sna*BI was deleted in *plc1*Δ::*URA3*. The insertion allele *plc1*::Tn*10*-LUK (*URA3*) was constructed by using Tn*10*-LUK no. 6.

ing phenotypes, diploid strains homozygous for *plc1-1* but heterozygous for *CEN3* (*CEN3/cen3X69-URA3-SUP11*) were constructed by using appropriate *MAT***a** and *MAT*α segregants from the backcrosses and streaked onto color medium. After 4 days at 24°C, the sectoring phenotypes of the diploids were scored.

Measurement of chromosome missegregation. Missegregation frequencies were determined by using a colony color assay that takes advantage of the dosage-dependent suppression by *SUP11* of an *ade2-101* ochre mutation to create a visual signal for chromosome ploidy (25; reviewed in reference 36). Diploid cells (*ade2-101/ade2-101*) with zero, one, two or more copies of *SUP11* are red, pink, and white, respectively. The *SUP11* gene is adjacent to the centromere on the X69 and *CEN314* chromosomes, thereby allowing us to detect aberrant segregation of the marked chromosomes and to distinguish between chromosome nondisjunction (2:0 segregation) and loss (1:0 segregation) events.

For each strain tested, one or two pink colonies were picked from color medium plates after 4 days at 24°C. The cells were suspended in water and sonicated, and 300 to 600 cells were plated onto each color medium plate (150 by 15 mm) with or without calcium, as indicated. Colonies were scored after 4 days at 24 or 30°C and overnight at 4°C. The number of white-red half-sectored colonies divided by the number of pink colonies plus the total number of half-sectored colonies represents the chromosome nondisjunction frequency. The number of pink-red half-sectored colonies divided by the number of pink colonies plus the total number of half-sectored colonies is the chromosome loss frequency (25). The data reported here (see Tables 2 and 4) represent averages of three independent experiments.

Cloning and sequence analysis of *PLC1*. Strain WPY254 was transformed with a YEp351 yeast genomic library (16). Leu$^+$ transformants were grown at 24°C for 36 to 40 h and then incubated at the nonpermissive temperature of 38°C to screen for rescue of the temperature-sensitive phenotype. Three transformants that required plasmids for growth at the nonpermissive temperature were obtained, and one plasmid, p351L.1, was subjected to Tn*10*-LUK mutagenesis (31). A 4.5-kb *Xba*I-*Sma*I fragment containing the region of p351L.1 defined by the Tn*10*-LUK insertions was subcloned into pRS315 (70) to construct pWP102.

A 4.1-kb region of p351L.1 was sequenced by using the linear amplification double-stranded DNA cycle sequencing system with *Taq* polymerase (Bethesda Research Laboratories). The nucleotide sequence of the entire coding region of *PLC1* was determined for both strands, beginning with primers homologous to the ends of the Tn*10*-LUK insertions (Fig. 1). Oligonucleotides used as sequencing primers were synthesized on an Applied Biosystems DNA synthesizer in the Molecular and Cellular Biology Core Facility of the University of Massachusetts, Amherst.

Nucleic acid techniques. Total yeast DNA was isolated as previously described (27). Plasmid DNA was purified from *E. coli* by boiling (28) and by alkaline lysis (Qiagen, Inc.). Total yeast RNA was isolated by hot phenol extraction (15). Poly(A)$^+$ RNA was purified with a Poly(A) Quick Kit (Stratagene) by following the manufacturer's instructions. RNA was separated on 1.2% agarose gels containing 1.1% formaldehyde (2). DNA and RNA were transferred to GeneScreen nylon membranes (Dupont), and high-stringency hybridizations were performed by standard techniques (44). Two different reduced-stringency hybridization conditions were used with the 700-bp *PLC1*-specific probe. The first used 3.3× SSC (90 mM sodium citrate, 0.9 M NaCl)–50 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid)–0.1% sodium dodecyl sulfate (SDS)–4× Denhardt's solution–250 μg of salmon sperm DNA (sonicated and denatured) per ml at 55°C for 15 h. After hybridization, the filters were washed twice in 3.3× SSC–0.5% SDS at 37°C for 10 min each time, twice in 3.3× SSC–0.5% SDS at 55°C for 30 min each time, and twice in 3.3× SSC without SDS at room temperature for 30 min each time. The second hybridization and wash conditions were 50% formamide–5× SSC–5× Denhardt's solution–0.5% SDS–30 μg of salmon

sperm DNA (sonicated and denatured) per ml at 25°C for 15 h. After hybridization, the filters were washed in 2× SSC–0.5% SDS at room temperature. DNA probes were radioactively labeled by random priming (17). The 700-bp *PLC1*-specific fragment was generated by polymerase chain reaction (for the primers used, see Fig. 4). Autoradiograms used Kodak XAR5 film with intensifying screens exposed at −70°C.

Genetic mapping of *PLC1* and construction of *plc1* alleles by homologous integration. Heterozygous strains (WPY541 and WPY539) were constructed by crossing WPY255 (*plc1-1*) with DBY3391 (*cin2*::*LEU2*) and WPY525 (*plc1-1*) with TT44 (*gal4*::*LEU2*). Diploids were sporulated, and the tetrads were analyzed by standard genetic methods (68). Meiotic map distances were calculated by using the Macintosh tetrad analysis program Tetrads (kindly provided by J. King).

Disrupted *PLC1* alleles were constructed by single-step gene transplacement (63). The p351L.1 plasmid derivative containing Tn*10*-LUK no. 6 (Fig. 1) was digested with *Kpn*I to release a 13-kb fragment containing the disrupted allele (*plc1*::Tn*10*-LUK). In a separate experiment, pWP102 DNA was cut with *Bst*1107I and *Sna*BI to delete the entire *PLC1* coding region, *Bgl*II linkers were added, and the vector portion was religated to form pWP107. A 1.1-kb *Bam*HI *URA3* fragment from YDp-Ura (5) was cloned into the *Bgl*II site in pWP107 to make pWP108. The disrupted allele (*plc1*Δ::*URA3*) was released from pWP108 by digestion with *Xba*I and *Sma*I. The DNA fragments containing the disrupted alleles were used separately to transform strain WPY290 to Ura^+.

Homologous recombination was used to integrate *URA3* into chromosome XVI adjacent to *plc1-1*. The p351L.1 plasmid derivative containing Tn*10*-LUK no. 8 (Fig. 1) was digested with *Kpn*I to release a 14-kb fragment containing the transposon and adjacent yeast sequences. The cut DNA was used to transform yeast strain WPY263 to Ura^+ to make WPY308. DNA from transformants was analyzed by Southern hybridization.

Computer analysis. Analysis of nucleic acid and protein sequences was performed with GCG software (version 7; Genetics Computer Group, Inc.) on a Sun Microsystems Sparcstation. The GenBank (release 72.0) and EMBL (release 31.0) nucleotide sequence data bases were searched by using the TFASTA algorithm of Pearson and Lipman (56). The similarities among the different PI-PLC proteins were determined by using BESTFIT. A multiple sequence alignment (see Fig. 5A) was created by the progressive alignment method of Feng and Doolittle (18) by using PILEUP. The predicted PLC1 amino acid sequence was searched for protein motifs in the PROSITE Dictionary of Protein Sites and Patterns (version 9.0; A. Bairoch) by using MOTIFS. Potential phosphorylation sites in the PLC1 protein were first located by using MOTIFS. However, putative protein kinase C phosphorylation sites were labelled only (see Fig. 4) if they met the more stringent consensus requirements ([R/K_{1-3}, X_{0-2}]-S/T-[X_{0-2}, R/K_{1-3}]) described by Kennelly and Krebs (34). DNA sequences were manipulated by using DNA Inspector IIe and Gene Construction Kit (Textco, Inc., Lebanon, N.H.) software on a Macintosh computer.

RESULTS

Isolation of a putative PI-PLC yeast mutant. We isolated a temperature-sensitive (Ts^-) allele of a putative PI-PLC gene by using a sensitive genetic screen designed to identify yeast strains that exhibit increases in aberrant mitotic chromosome segregation (46). This screen employs a yeast strain carrying a chromosome with a mutant centromere (the X69 chromosome) which makes it possible to use a colony color-sectoring method to identify mutations that have relatively small effects on chromosome segregation visually. We obtained 2,393 mutants that displayed increased frequencies of chromosome missegregation (high-sectoring phenotype) by screening approximately 100,000 colonies after ethyl methanesulfonate mutagenesis of strain 41-14d (Table 1) (Materials and Methods). Among these, we found 725 mutants that were temperature sensitive, cold sensitive, or both. Twenty-one of these strains which exhibited tight conditional phenotypes at the nonpermissive temperatures were subjected to genetic analysis as described previously (46).

One temperature-sensitive mutant, named $\lambda^2$15.25, exhibited cosegregation of the high-sectoring and conditional lethal phenotypes through three successive backcrosses and was chosen for further analysis. More than 50 tetrads were analyzed, and all of the Ts^- meiotic segregants, but none of the Ts^+ meiotic segregants, exhibited high-sectoring phenotypes when crossed to appropriate *MAT***a** and *MAT*α *plc1-1* segregants from the backcrosses. One of these haploid segregants, WPY254, was chosen for further analysis and was shown subsequently to contain a mutation in a gene that encodes a putative PI-PLC protein. The mutant gene was designated *plc1* (for phospholipase C), and the mutant allele in WPY254 was named *plc1-1*.

Isolation of the *PLC1* gene. The *PLC1* gene was cloned by rescuing the temperature-sensitive phenotype exhibited by WPY254 with a yeast genomic DNA library constructed in episomal plasmid YEp351 (16, 26) (gift from Jeanne Hirsch). Approximately 30,000 yeast transformants (three genome equivalents) which grew at the permissive temperature (24°C) were screened for the ability to grow at the nonpermissive temperature (38°C). Three Leu^+ transformants which showed wild-type growth at the nonpermissive temperature (Ts^+) were chosen for further study. Growth at 38°C was shown to be plasmid dependent since all three transformed strains coreverted to temperature sensitivity and leucine auxotrophy upon plasmid loss. Plasmid DNA was recovered from yeast colonies by transforming *E. coli* DB1328 (λ^r *leuB proA2 recA*) with total yeast DNA. We isolated one plasmid, p351L.1, that rescued both the temperature-sensitive and high-sectoring phenotypes of WPY254.

The region of DNA in p351L.1 required to rescue the Ts^- phenotype was localized by Tn*10*-LUK transposon mutagenesis (31). Transposon-containing plasmids that were unable to rescue the Ts^- phenotype were subjected to restriction enzyme mapping to locate the Tn*10*-LUK insertions. A 4.5-kb *Xba*I-to-*Sma*I fragment encompassing the functional region of p351L.1 (Fig. 1) was subcloned into the multiple cloning site of centromere plasmid pRS315 (70). The resulting low-copy-number plasmid, pWP102, was found to rescue both the high-sectoring (Fig. 2) and temperature-sensitive (Fig. 3) phenotypes of *plc1-1* strains.

To confirm that pWP102 contains complementing DNA and not a suppressor gene, we used integrative transformation to insert a copy of *URA3* adjacent to the temperature-sensitive *plc1-1* allele in the genome (Materials and Methods). The resulting haploid strain (WPY308 [*plc1-1* Ts^- Ura^+]) was mated to WPY517 (*PLC1* Ts^+ Ura^-), and the resulting diploid strain was subjected to meiotic analysis. The *URA3* gene was found to cosegregate with the *plc1-1*

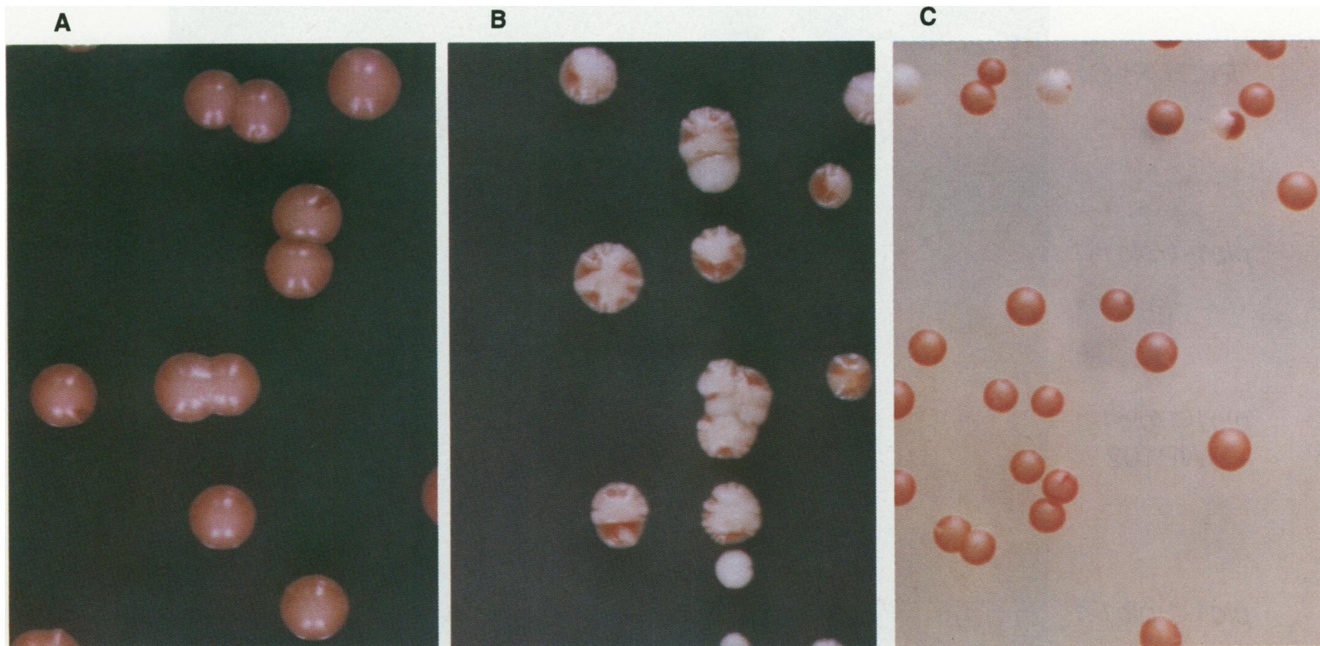

FIG. 2. Chromosome missegregation phenotype of *plc1-1* strains at 24°C. The levels of missegregation of the X69 chromosome are reflected by the frequencies of sectors in the colonies. Panels: A, *PLC1/PLC1* wild-type strain WPY264; B, *plc1-1/plc1-1* mutant strain WPY309; C, *plc1-1/plc1-1* mutant strain WPY309 carrying plasmid pWP102 (*PLC1*) and showing complementation of the sectoring phenotype.

(Ts^-) allele in all 32 of the tetrads examined, indicating that *URA3* and *plc1-1* are tightly linked in this strain (data not shown). These results demonstrate that pWP102 contains DNA from the authentic *PLC1* locus.

***PLC1* encodes a putative yeast PI-PLC.** The complete 4.1-kb DNA sequence located between *Xba*I and *Sma*I (Fig. 1) was determined and revealed an open reading frame that encodes a predicted protein of 869 amino acids (Fig. 4). The seven Tn*10*-LUK insertions that abolish the rescuing activity of p351L.1 map within this open reading frame (Fig. 1).

The predicted protein sequence shows significant homology to the PI-PLC isozymes from humans, bovines, rats, and *D. melanogaster* (Fig. 5A). The three classes of PI-PLC isozymes (β, γ, and δ) share two domains of significant amino acid sequence homology, designated X and Y (Fig. 5B) (59). Domains X and Y are approximately 60 and 40% identical, respectively, among the mammalian β, γ, and δ PLC isozymes. Domain X in the putative yeast PLC1 protein is 50% identical and 70% similar to domain X in the mammalian and fly isozymes (Fig. 5A). Even the less well-conserved Y domain in the yeast protein is 26% identical to the Y domains in the other PI-PLC isozymes. As found for the mammalian and fly isozymes, there is little amino acid similarity between the yeast PLC1 protein and the other PI-PLC isozymes in regions outside domains X and Y.

Comparison of the PLC1 amino acid sequence to the PROSITE protein pattern data base (release 9.0, 1992; A. Bairoch) revealed a consensus EF-hand calcium-binding domain (37) located at amino acid positions 273 to 301 in the yeast PLC1 protein (Fig. 4). Potential sites for phosphorylation by protein kinase C and cyclic AMP-dependent protein kinase (34) and a possible nuclear localization signal sequence (71) were also found in the yeast protein (Fig. 4).

Physical and genetic mapping of *PLC1*. *PLC1* was initially assigned to yeast chromosome XVI by hybridizing the ^{32}P-labeled 7-kb *Kpn*I fragment from p351L.1 (Fig. 1) to a Southern blot containing whole yeast chromosomes separated by electrophoresis (10) (data not shown). *PLC1* was further localized to the left arm of chromosome XVI by hybridizing the ^{32}P-labeled 700-bp *PLC1*-specific probe (Fig. 1) to filters containing a lambda library of mapped yeast genomic DNA fragments (61). The probe hybridized to two overlapping lambda clones that represent yeast DNA positioned 22 to 29 kb from the telomere on the left arm of chromosome XVI (data not shown).

The physical mapping results were confirmed by genetic mapping. Diploid strains WPY539 (*plc1-1/PLC1 GAL4/gal4*::*LEU2*) and WPY541 (*plc1-1/PLC1 CIN2/cin2*::*LEU2*) were sporulated, and the tetrads were dissected. In both crosses, the *plc1-1* allele was scored by testing the spore colonies for growth at 38°C and the *cin2* and *gal4* alleles were identified by leucine prototrophy. The meiotic mapping data obtained indicate that *PLC1* is linked to *CIN2* and *GAL4*. For the *plc1-gal4* interval in strain WPY539 and the *plc1-cin2* interval in strain WPY541, the parental ditype:nonparental ditype:tetratype phenotypes were 13:0:17 and 31:0:30, respectively, and the genetic distances were 28.3 ± 4.5 and 24.6 ± 3.2 centimorgans (48). Our physical and genetic mapping results, together with the published data on *CIN2* and *GAL4* (73), allow the genes to be ordered *cin2-gal4-plc1* (centromere proximal to centromere distal), making *PLC1* the new outermost marker on the left arm of chromosome XVI. No other known genes correspond to the map position of *PLC1*, thereby confirming that *PLC1* is a newly identified gene.

Disruption of the *PLC1* gene. To learn more about the function of the *PLC1* gene product in vivo, the genomic *PLC1* gene was disrupted in two ways. The first strategy utilized one of the transposon insertions into *PLC1* (Tn*10*-LUK no. 6; Fig. 1) that contained *URA3* for selection of the disrupted allele after transformation. We also constructed a null allele (*plc1*Δ::*URA3*) by deleting the coding region of *PLC1* and inserting *URA3* flanked by translation stop codons

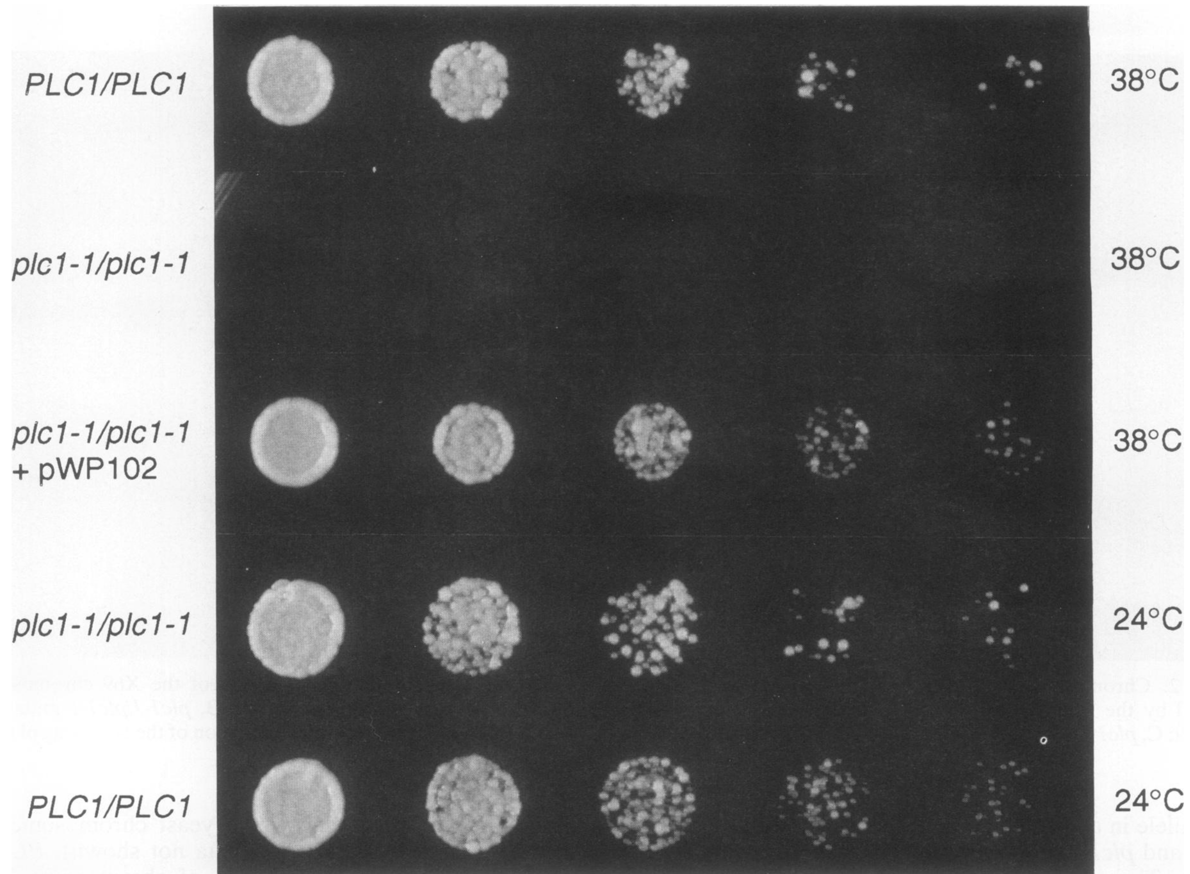

FIG. 3. Temperature-sensitive phenotype of *plc1-1* strains. Growth of *PLC1/PLC1* (WPY264), *plc1-1/plc1-1* (WPY309), and *plc1-1/plc1-1* (pWP102) [WPY309(pWP102)] cells at 24 and 38°C. Colonies grown on YPD plates at 24°C were picked and resuspended in water, and a dilution series for each strain was inoculated onto fresh YPD plates with a pronged device. Plates were incubated at 24 or 38°C for 3 days.

in all reading frames (Fig. 1; Materials and Methods). For each disruption, a linear DNA fragment containing either the insertion allele (transposon) or the deletion-insertion allele (null) was used separately to transform diploid yeast strain WPY290 (*PLC1/PLC1 ura3-52/ura3-52*) to Ura$^+$. The resulting diploid strains, WPY296 (*plc1*Δ::*URA3/PLC1*) and WPY291 [*plc1*::Tn*10*-LUK (*URA3*)/*PLC1*], were sporulated, and the tetrads were dissected. In both cases, more than 90% of the tetrads contained four haploid spores that germinated and grew at 30°C. However, two of the four spores in each complete tetrad produced Ura$^+$ colonies that exhibited extremely slow growth on rich media at 30°C (Fig. 6A). In fact, haploid cells with *PLC1* disrupted are temperature sensitive for growth at 38°C and do not grow on minimal medium at any temperature tested. In contrast, the two Ura$^-$ haploids from each tetrad showed wild-type growth rates on rich or minimal medium at 24 or 38°C. Southern hybridization analysis confirmed the structures of the wild-type and disrupted *PLC1* alleles (Fig. 6B). Introduction of the wild-type *PLC1* gene on a centromere plasmid (pWP102) into the null strain allowed the cells to grow like the wild type (data not shown). Our results demonstrate that although the *PLC1* gene product is not essential for mitotic growth under ideal conditions, yeast cells with *PLC1* disrupted (*plc1*Δ::*URA3*) grow about three times slower (doubling time, ~270 min) than wild-type cells (doubling time, ~95 min) in liquid cultures at 30°C (data not shown). The extremely poor growth and frequent occurrence of spontaneous growth-enhancing suppressors made subsequent chromosome segregation and cell morphology experiments with the null strain impossible.

***PLC1* is unique in the yeast genome.** The finding that *PLC1* is not essential for mitotic growth under some conditions suggested that there could be PLC isozymes in *S. cerevisiae* which substitute for PLC1 function in the *plc1*Δ::*URA3* null strain. However, we were unable to detect homologous sequences in the yeast genome by using the 700-bp *PLC1*-specific probe that encodes the highly conserved X domain (Fig. 1) and two different reduced-stringency hybridization conditions (Materials and Methods; data not shown). Still, the presence of related genes whose structures and functions are diverged from *PLC1* cannot be ruled out.

Expression of *PLC1*. The 700-bp *PLC1*-specific probe was hybridized to a filter containing yeast total RNA. As shown in Fig. 7, a single 2.9-kb *PLC1*-specific RNA was visualized in the lane containing total RNA isolated from wild-type haploid cells. Longer exposure of the autoradiogram failed to reveal additional transcripts. The *PLC1*-specific mRNA was not detected in total RNA isolated from a haploid *plc1*Δ::*URA3* strain (Fig. 7). A single 2.9-kb message was also observed when the *PLC1*-specific probe was hybridized to poly(A)$^+$ RNA isolated from wild-type haploid cells (data not shown).

***plc1-1* strains exhibit aberrant chromosome segregation.** We

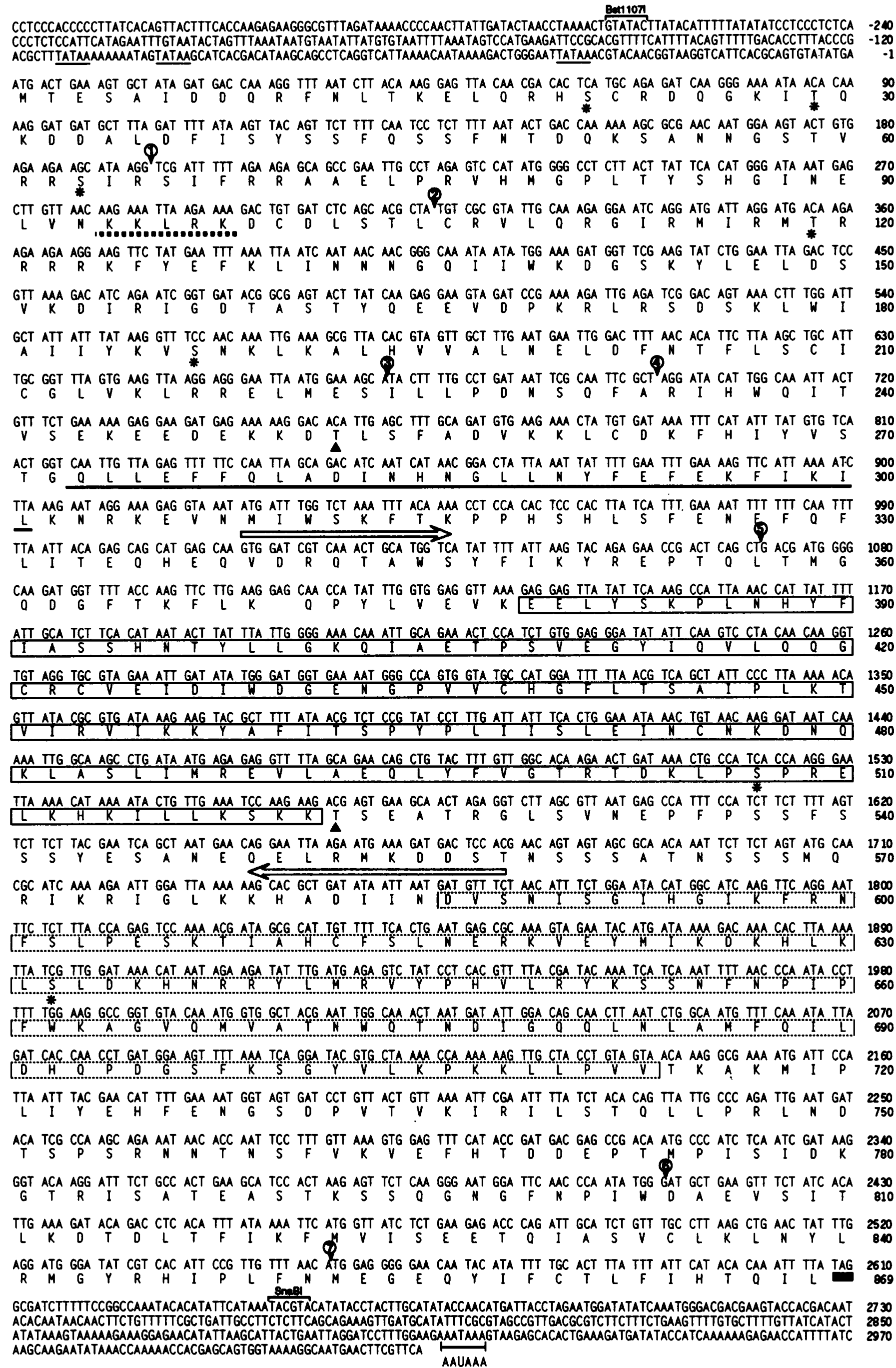

FIG. 4. Nucleotide sequence of *PLC1* and predicted amino acid sequence of the PLC1 protein. The DNA sequence encompassing the *PLC1* locus is shown numbered starting with the translation initiation codon. Numbers to the right of the sequence indicate nucleotide and amino acid positions. The translation termination codon is marked by the black box. The X domain is enclosed by a solid box. The Y domain is enclosed by a dotted box. The EF-hand calcium-binding domain (amino acids 273 to 301) is underlined with a thick solid line. The possible nuclear localization signal (amino acids 94 to 98) is underlined by a thick dotted line. Potential sites for phosphorylation by protein kinase C (*) and a cAMP-dependent protein kinase (▲) are indicated. The locations of Tn*10*-LUK insertions are indicated by numbered circles. The two open arrows above the sequence define the primers used to generate the 700-bp *PLC1*-specific probe by polymerase chain reaction. The *Bst*1107I and *Sna*BI sites are indicated. Possible TATA promoter elements are underlined by thin solid lines. A possible eucaryotic polyadenylation signal (AAUAAA) is indicated.

used the colony color sectoring assay to distinguish between the chromosome nondisjunction (2:0 segregation) and loss (1:0 segregation) events exhibited by the *plc1-1* strains (Materials and Methods) (25, 46). In these experiments, the X69 chromosome containing the mutant centromere (*cen3X69*) flanked by *SUP11* and *URA3* was used to monitor the frequency of missegregation events (46). Nondisjunction of this chromosome causes white-red sectors in the colonies,

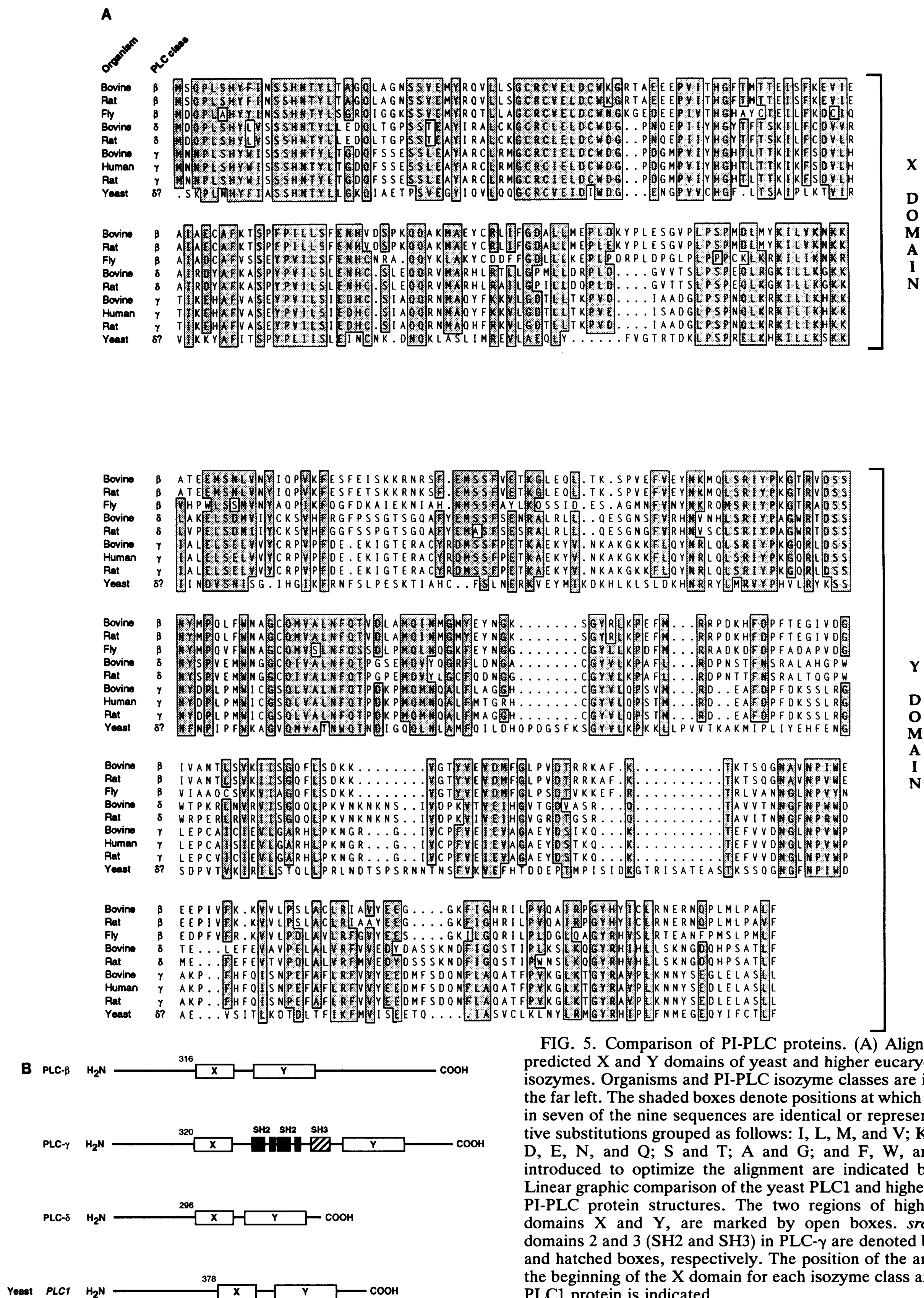

FIG. 5. Comparison of PI-PLC proteins. (A) Alignment of the predicted X and Y domains of yeast and higher eucaryotic PI-PLC isozymes. Organisms and PI-PLC isozyme classes are indicated on the far left. The shaded boxes denote positions at which amino acids in seven of the nine sequences are identical or represent conservative substitutions grouped as follows: I, L, M, and V; K, H, and R; D, E, N, and Q; S and T; A and G; and F, W, and Y. Gaps introduced to optimize the alignment are indicated by dots. (B) Linear graphic comparison of the yeast PLC1 and higher eucaryotic PI-PLC protein structures. The two regions of high homology, domains X and Y, are marked by open boxes. *src* homology domains 2 and 3 (SH2 and SH3) in PLC-γ are denoted by the black and hatched boxes, respectively. The position of the amino acid at the beginning of the X domain for each isozyme class and the yeast PLC1 protein is indicated.

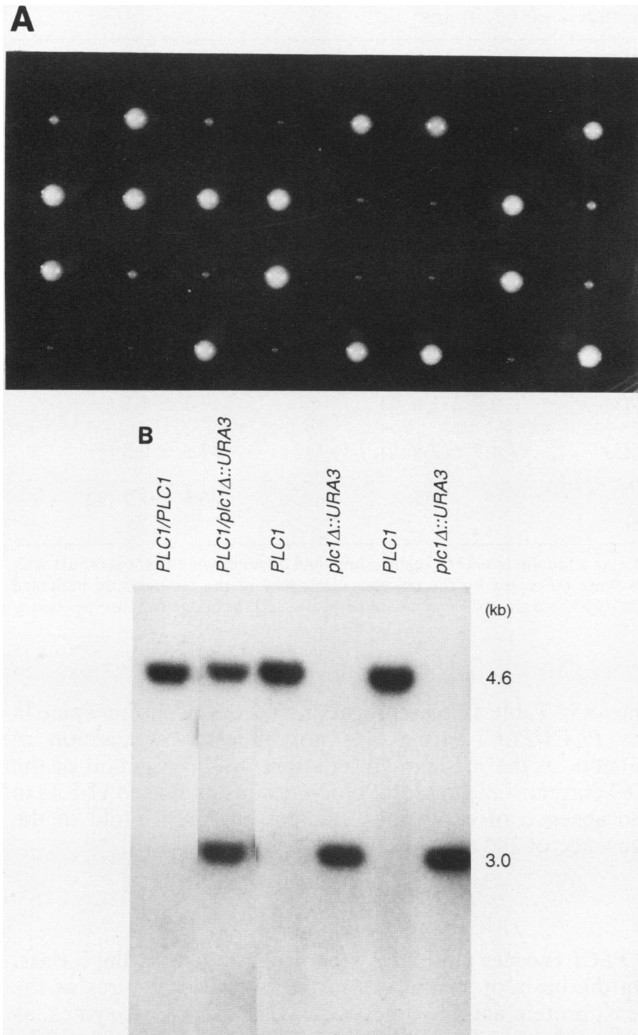

FIG. 6. Analysis of *plc1Δ::URA3* strains. (A) Heterozygous diploid strain WPY296 (*PLC1/plc1Δ::URA3*) was sporulated, tetrads were dissected, and the dissection plates were incubated at 30°C for 3 days. The four meiotic products from each tetrad are aligned in vertical rows; the 2:2 segregation pattern for slow growth is evident. Genetic analysis showed that the cells growing slowly at 30°C were all Ura^+ and the cells exhibiting wild-type growth were all Ura^-. (B) Southern hybridization analysis of *Hin*dIII-digested genomic DNA isolated from untransformed wild-type diploid parent cells (*PLC1/PLC1*; WPY290), transformed heterozygous diploid cells (*PLC1/plc1Δ::URA3*; WPY296), and haploid cells derived from a complete tetrad grown at 30°C (*plc1Δ::URA3*, WPY298 and WPY300; *PLC1*, WPY297 and WPY299). The blot was hybridized with the 700-bp *PLC1*-specific probe. The positions of the 4.6-kb *Hin*dIII fragment representing the wild-type allele and the 3.0-kb *Hin*dIII fragment containing the *plc1Δ::URA3* allele are marked at the right.

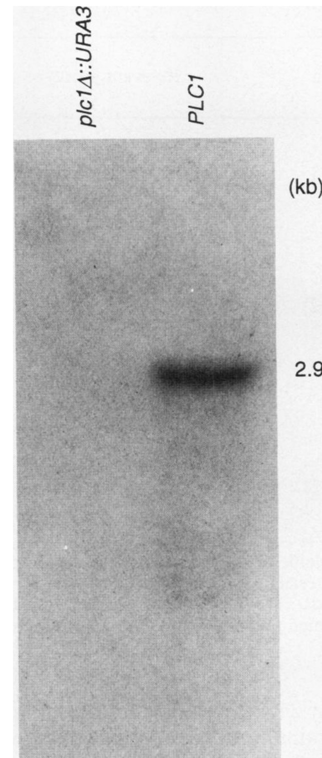

FIG. 7. Northern analysis. Total RNAs from *PLC1* (WPY299) and *plc1Δ::URA3* (WPY298) strains were separated electrophoretically and hybridized with the 700-bp *PLC1*-specific probe (Materials and Methods).

while loss of this chromosome causes pink-red sectoring. A single chromosome nondisjunction or loss event in the first division after a cell is plated results in a white-red or pink-red half-sectored colony. The number of half-sectored colonies is a measure of the frequency of chromosome nondisjunction or chromosome loss events (25).

The homozygous *plc1-1* diploid strain produced sectored colonies at 24°C (Fig. 2B) that reflect a 10-fold increase in missegregation of the X69 chromosome, primarily because of an increase in chromosome nondisjunction events (Table 2). Introduction of a centromere plasmid carrying *PLC1* (pWP102) into these cells decreased sectoring to the same level as observed for *PLC1/PLC1* strains (Fig. 2C). A strain heterozygous for *plc1-1* showed a threefold increase in missegregation of the X69 chromosome (Table 2).

We also tested whether the *plc1-1* allele affects the segregation of a chromosome III derivative containing a wild-type centromere by using 5-fluoroorotic acid (8) to select for Ura^- cells that have lost the *CEN314* chromosome (46). By using this method to detect infrequent missegregation events, we observed an eightfold increase in missegregation of the *CEN314* chromosome in *plc1-1/plc1-1* strains compared with the wild type (data not shown). We also found that *plc1-1* did not cause a detectable increase in the frequency of mitotic recombination (data not shown).

The chromosome segregation defect of *plc1-1* cells increases at a semipermissive temperature. To test the effect of temperature on the *plc1-1* chromosome segregation defect, we performed the colony color sectoring assay at the semipermissive temperature of 30°C. This is the maximum temperature at which homozygous *plc1-1* strains grow at nearly wild-type rates on color medium. At temperatures above 30°C, *plc1-1/plc1-1* strains grow very slowly and form mottled colonies that are not appropriate for analysis with the sectoring assay. As shown in Table 2, incubation at the semipermissive temperature caused a significant, 32-fold, increase in missegregation events in the homozygous *plc1-1* diploid strain compared with the wild-type control. A strain which is heterozygous for *plc1-1* showed a frequency of chromosome segregation errors similar to that observed for

TABLE 2. Chromosome segregation in *plc1-1* strains[a]

Temp (°C) and strain	Relevant genotype	(2:0) segregation nondisjunction (10^{-3}) (mean ± SD)	(1:0) segregation loss (10^{-3}) (mean ± SD)	Total events (nondisjunction + loss) (10^{-3}) (mean ± SD)
24				
WPY264	*PLC1 CEN3* / *PLC1 cen3X69-URA3-SUP11*	4.7 ± 2.8 (1)	2.3 ± 1.4 (1)	7.0 ± 4.2 (1)
WPY265	*PLC1 CEN3* / *plc1-1 cen3X69-URA3-SUP11*	19.6 ± 3.5 (4)	3.3 ± 0.7 (1.4)	22.9 ± 4.2 (3)
WPY267	*plc1-1 CEN3* / *plc1-1 cen3X69-URA3-SUP11*	65.4 ± 25.0 (14)	3.6 ± 5.1 (1.6)	69.0 ± 30.0 (10)
WPY267(pWP102)	*plc1-1 CEN3*(pWP102) / *plc1-1 cen3X69-URA3-SUP11*	3.0 ± 1.4 (0.6)	1.0 ± 0.8 (0.4)	4.0 ± 2.3 (0.6)
30				
WPY264	*PLC1 CEN3* / *PLC1 cen3X69-URA3-SUP11*	6.3 ± 0.5 (1)	0.7 ± 1.0 (1)	7.1 ± 1.6 (1)
WPY265	*PLC1 CEN3* / *plc1-1 cen3X69-URA3-SUP11*	32.7 ± 2.1 (5)	4.7 ± 2.4 (7)	37.5 ± 0.3 (5)
WPY267	*plc1-1 CEN3* / *plc1-1 cen3X69-URA3-SUP11*	ND	ND	226.6 ± 1.7 (32)

[a] All strains are diploids containing one copy of *SUP11*-marked chromosome III bearing a mutant (*cen3X69*) centromere and one copy of chromosome III with wild-type *CEN3* (Materials and Methods). Chromosome missegregation frequencies were measured by the colony color assay at the temperature indicated (Materials and Methods). The numbers in parentheses are fold increases relative to wild-type backgrounds at each temperature. ND, not done because frequency of sectoring was too high to distinguish white-red from pink-red sectors.

the same strain at 24°C. Missegregation of the X69 chromosome in *PLC1* strains was essentially the same at 24 and 30°C (Table 2).

Calcium partially suppresses the chromosome segregation and growth defects of *plc1-1* strains. The finding that the PLC1 protein contains an EF hand (Fig. 8) prompted us to test whether calcium affects the temperature-sensitive growth and chromosome missegregation phenotypes of *plc1-1* strains (Tables 3 and 4). We found that addition of 100 mM calcium to the medium allowed *plc1-1* cells to grow at 38°C, indicating partial suppression of the Ts⁻ growth defect by calcium. This is especially significant since *plc1-1* cells showed no growth at 38°C in the absence of calcium (Fig. 3). Addition of calcium did not permit growth of *plc1Δ::URA3* cells at 38°C.

We also found that calcium partially suppressed the chromosome segregation defect exhibited by *plc1-1* cells. As

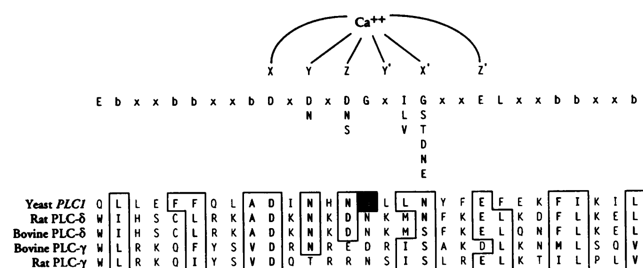

FIG. 8. Putative EF-hand calcium-binding sequence in PLC1. At the top is the consensus EF-hand calcium-binding motif, with the calcium-coordinating amino acid residues labelled (X, Y, Z, Y′, X′, and Z′). The letter b in the consensus sequence represents any hydrophobic amino acid, and x represents any amino acid. The central glycine residue, present in 90% of known calcium-binding proteins, is highlighted by the black box. Boxed residues represent adherence to the consensus. The position of the EF-hand domain in each amino acid sequence is as follows: yeast PLC1, 273 to 301; rat PLC-δ, 144 to 172; bovine PLC-δ, 83 to 111; bovine PLC-γ1, 156 to 184; rat PLC-γ2, 145 to 173.

shown in Table 4, missegregation of the X69 chromosome in the *PLC1/PLC1* strain was not affected by addition of calcium to the medium. In contrast, missegregation of the X69 chromosome in *plc1-1/plc1-1* strains increased 11-fold in the absence of exogenous calcium but only 5-fold in the presence of 100 mM calcium.

DISCUSSION

***PLC1* encodes a putative yeast PI-PLC gene of the δ class.** On the basis of similarities in the overall structures of the yeast protein and the other eucaryotic PI-PLC isozymes, as well as the significant homologies among domains X and Y, we conclude that *PLC1* encodes a yeast PI-PLC protein. The arrangement of the X and Y domains, the lack of *src* homology domains, and the short carboxy-terminal region in the yeast PI-PLC protein indicate that the structure of the yeast protein is most similar to that of the δ class of PI-PLC enzymes.

In mammalian cells, the X and Y domains are thought to be involved in the catalytic activity of the PI-PLC enzymes, and indeed, domain X has been shown to be required for the PLC-γ1 enzyme to cleave its substrate (9). The nonhomolo-

TABLE 3. Calcium suppression of the Ts⁻ defect of *plc1* strains[a]

Strain	Relevant genotype	No Ca^{2+} 24°C	No Ca^{2+} 38°C	100 mM Ca^{2+} 24°C	100 mM Ca^{2+} 38°C
WPY299	*PLC1*	+++	+++	+++	+++
WPY263	*plc1-1*	+++	−	+++	++
WPY298	*plc1Δ::URA3*	+	−	+	−

Growth under the following conditions shown in columns 3–6.

[a] To determine the effect of calcium on the Ts phenotype, colonies grown on YPD plates at 24°C were picked and suspended in water and a dilution series for each strain was inoculated onto fresh YPD plates with a pronged device. The plates were incubated at 24 or 38°C. After 4 days, growth was scored as follows: +++, wild type; ++, moderate; +, slow; −, none.

TABLE 4. Calcium suppression of the chromosome segregation defect of *plc1-1* strains[a]

Strain	Relevant genotype	Mean ± SD missegregation frequency (10^{-3})					
		No Ca^{2+}			100 mM Ca^{2+}		
		2:0	1:0	Total	2:0	1:0	Total
WPY264	*PLC1 CEN3* / *PLC1 cen3X69-URA3-SUP11*	6.0 ± 0.5 (1)	1.1 ± 0.5 (1)	7.1 ± 1.0 (1)	7.5 ± 0.6 (1)	2.1 ± 1.0 (1)	9.6 ± 0.3 (1)
WPY265	*PLC1 CEN3* / *plc1-1 cen3X69-URA3-SUP11*	36.1 ± 1.4 (6)	2.5 ± 0.5 (2)	38.6 ± 1.9 (5)	21.4 ± 0.5 (3)	1.5 ± 0.1 (0.7)	22.9 ± 0.5 (2)
WPY267	*plc1-1 CEN3* / *plc1-1 cen3X69-URA3-SUP11*	70.6 ± 1.4 (12)	5.3 ± 3.1 (5)	75.9 ± 17.3 (11)	38.6 ± 3.1 (5)	5.7 ± 7.3 (3)	44.1 ± 10.1 (5)

[a] Strains WPY264, WPY265, and WPY267 are diploids containing one copy of *SUP11*-marked chromosome III bearing a mutant (*cen3X69*) centromere and one copy of chromosome III with wild-type *CEN3* (Materials and Methods). Chromosome missegregation frequencies were measured by the colony color assay at 24°C at the calcium concentration indicated (Materials and Methods). The numbers in parentheses are fold increases relative to the wild type.

gous regions among the PI-PLC enzyme classes are postulated to be involved in the differential regulation of each isozyme. Recently, PI-PLC isozymes of the β and γ classes were found to be regulated by two distinct mechanisms. PLC-β1, which contains a long carboxy-terminal region, is regulated by a G-protein-dependent mechanism (72, 75, 80). PLC-γ1, which contains *src* homology domains located between domains X and Y, is regulated by tyrosine phosphorylation (35, 47, 49, 79). Nothing is known about the regulation or activation of PLC-δ enzymes (59).

The predicted yeast PLC1 protein amino acid sequence differs from the other PI-PLC enzymes in that it contains 378 instead of approximately 300 amino acids preceding the X domain (Fig. 5B). Although the signals that activate the known PI signal transduction pathways are all extracellular, recently reported biochemical evidence suggests that there is a nucleus-specific PI signaling mechanism (14, 54). It is intriguing that the amino-terminal end of the yeast PLC1 protein contains a possible nuclear localization signal, as well as five potential sites for phosphorylation by protein kinase C. The importance of this region for the function and/or regulation of the yeast protein awaits further study.

The cDNA molecules for three subtypes (δ1, δ2, and δ3) of the PLC-δ class of PI-PLC enzymes have been isolated from higher eucaryotes (38, 60). The δ1, δ2, and δ3 isozymes are approximately 70% identical in domain X, 50% identical in domain Y, and 40% identical in the 300-amino-acid N-terminal region (12). The regions between domains X and Y in PLC-δ3 and in the β class of PI-PLC enzymes are highly acidic (38). However, the putative PLC1 yeast protein most resembles the PLC-δ1 enzyme, since both of these proteins lack an acidic region between domains X and Y.

Immunohistochemical and in situ mRNA hybridization experiments indicate that PLC-δ1 is widely distributed in rat tissues, is found in low abundance in brain tissue (62), is seen at higher levels in spleen tissue and seminal vesicles, and is most highly expressed in skeletal muscle tissue (29). It has been proposed that the widespread expression of PLC-δ mRNA suggests that PLC1-δ1 has a role in some fundamental cellular process, such as intracellular calcium regulation (29).

In support of this idea, we found that the yeast PLC1 protein contains a consensus EF-hand calcium-binding motif as defined by comparison of over 100 putative and known calcium-binding proteins and by X-ray crystallographic studies (Fig. 8) (37). On the basis of this information, the EF hand in PLC1 is predicted to bind calcium. Interestingly, the only higher eucaryotic PI-PLC isozyme which contains a consensus EF hand is PLC-δ (3). An ancient EF hand is found in the PLC-γ isozyme, but this sequence is probably a result of divergent evolution and is predicted not to bind calcium because its sequence differs considerably from the EF hand consensus (3).

It is known that the PI-PLC isozymes exhibit differential, calcium-dependent substrate specificities for PIs (60). The presence of an EF-hand calcium-binding motif supports the proposal that mammalian PLC-δ could play a unique role by catalyzing PI hydrolysis in response to calcium changes in vivo (3). Calcium is also required for the activity of some mammalian protein kinase C subtypes (α, β, and γ) (50). The yeast protein kinase C gene (*PKC1*) encodes a homolog of the Ca^{2+}-dependent mammalian isozymes which contain moderately conserved calcium-binding domains (41). One yeast mutant carrying a conditional allele of *PKC1* (*sst1*; 82) exhibits an arrest phenotype similar to that observed for wild-type yeast cells depleted of calcium (32). Other independent isolates carrying mutant alleles of *PKC1* exhibited Ca^{2+} dependency for growth or temperature-sensitive phenotypes that were suppressed by exogenous calcium (40). One possible mechanism proposed to explain this suppression involves the stimulation of PLC by calcium to generate high levels of diacylglycerol (40). Our finding that the temperature-sensitive and chromosome missegregation phenotypes of *plc1-1* cells can be partially suppressed by calcium is consistent with the idea that calcium plays an important role in the function of the yeast PI-PLC enzyme. The inability of calcium to suppress the growth defect of *plc1*Δ::*URA3* cells suggests that suppression requires expression of the temperature-sensitive mutant protein and is not the result of bypassing the requirement for PLC1.

Relationship between *plc1-1* and chromosome segregation. The fact that we isolated an allele of *PLC1* that affects the fidelity of chromosome transmission is intriguing, but the implications of this finding are not clear. In support of the idea that *PLC1* has a direct role in chromosome transmission in *S. cerevisiae*, we found that growth of the Ts^- *plc1-1* mutant strain at 30°C significantly increased chromosome missegregation and that calcium partially suppressed both the growth and segregation defects. However, since *plc1-1* cells appear to have normal nuclei and spindle morphologies and are not supersensitive to the microtubule-destabilizing drug benomyl (data not shown), it seems very unlikely that the *plc1-1* chromosome missegregation phenotype results from a defect in the components of the mitotic segregation apparatus. It seems more likely that *plc1-1* affects chromosome segregation indirectly, perhaps by changing the timing of the cell cycle or by altering intracellular calcium concentrations. In fact, some mutations in *S. cerevisiae* which delay

progression through the cell cycle (19, 23) or affect the cell cycle feedback control pathways (30, 42, 81) also increase aberrant chromosome segregation.

By analogy with mammalian systems, it is likely that *PLC1* functions in the same pathway as protein kinase C (*PKC1*) in *S. cerevisiae*. Yeast cells depleted of protein kinase C exhibit a cell division cycle-specific osmotic stability defect, suggesting that *PKC1* could function at a cell cycle checkpoint or be involved in regulating independent pathways that control cell growth and division (40, 41). In addition, analyses of *pkc1* mutants indicate that the protein product of the *PKC1* gene may play an important role in bud formation (52). We observed rapid loss of viability and lysis of *plc1-1* cells following a shift to the nonpermissive temperature (data not shown). Similar phenotypes have also been observed in *pkc1* mutants (41). In fact, we found that lysis in *plc1-1* cells always occurred in the bud (data not shown). Paravicini et al. (52) have shown by electron microscopy that the cell wall composition of budding *pkc1* mutant cells is altered such that the plasma membrane is partially detached from the cell wall. Apparently, yeast cells are most sensitive to modifications of the cell wall at the time of bud emergence, causing cell cycle arrest. Therefore, if the protein products of the *PLC1* and *PKC1* genes do function in the same pathway, the chromosome segregation defect could be an indirect result of cell structure defects.

Changes in intracellular calcium concentrations have long been implicated in controlling mitosis in higher eucaryotic cells (24, 57, 74, 76). In fact, increases in intracellular calcium induced by the second messenger inositol 1,4,5-trisphosphate, a product of the reaction of PLC with its substrate, have been shown to cause premature chromosome condensation in sea urchin embryos (77). Calcium is also required for growth of yeast cells (32), and at least one protein involved in the yeast cell cycle feedback control pathway is a potential calcium-binding protein (42). Here we report the isolation of a yeast homolog of PI-PLC, the mammalian enzyme known to function in signal transduction pathways involving calcium.

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ADDENDUM IN PROOF

After this paper was submitted, we found that *PLC1* had been independently cloned by Yoko-O et al. (Proc. Natl. Acad. Sci. USA **90**:1804–1808, 1993) and by Flick and Thorner (submitted for publication).

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