crosis within 24 hours. Eighty seedlings were harvested 0, 3, 6, 12, and 24 hours after injection. An equal mass of total RNA taken at each time point was pooled, and polyadenylated [poly(A)$^+$]RNA was prepared by oligo(dT) cellulose chromatography. An Amersham cDNA cloning kit was used to prepare and clone cDNA as described by the manufacturer. Eco RI adapters were ligated to double-stranded cDNA and subsequently ligated to Eco RI–digested arms of the phage vector λgt10. The phage was packaged and used to infect *E. coli* C600 cells. Approximately 5 × 10^5 recombinant plaques were transferred to Hybond N$^+$ membranes and screened with probe 1 (*9*). Five *Cf-9* cDNA clones were recovered, and the full-length inserts were cloned as Bam HI fragments into pUC119 and sequenced with primers derived from the genomic sequence of *Cf-9* and with primers flanking the polylinker cloning site of pUC119. The full-length insert of one of these clones was used as probe 2 (Fig. 2 and Fig. 4B).

11. G. von Heijne, *Nucleic Acids Res.* **14**, 4683 (1986).
12. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
13. F. M. Townsley and H. R. B. Pelham, *Eur. J. Cell Biol.* **64**, 221 (1994).
14. G. von Heijne and Y. Gavel, *Eur. J. Biochem.* **174**, 671 (1988).
15. T. Pryor, *Trends Genet.* **3**, 157 (1987); M. A. Sudupak, J. L. Bennetzen, S. H. Hulbert, *Genetics* **133**, 119 (1993).
16. G. B. Martin *et al.*, *Science* **262**, 1432 (1993).
17. The Brookhaven Protein Data Bank, SWISS-PROT, Protein Identification Resource, and GenPept protein databases were searched with the BlastP algorithm of S. F. Altschul, G. Warren, W. Miller, E. W. Myers, and D. J. Lipman [*J. Mol. Biol.* **215**, 403 (1990)].
18. J. C. Walker, *Plant J.* **3**, 451 (1993); C. Chang *et al.*, *Plant Cell* **4**, 1263 (1992); C. Valon, J. Smalle, H. M. Goodman, J. Giraudat, *Plant Mol. Biol.* **23**, 415 (1993).
19. H. U. Stotz, J. J. A. Contos, A. L. T. Powell, A. B. Bennett, J. M. Labavitch, *Plant Mol. Biol.* **25**, 607 (1994); H. U. Stotz *et al.*, *Plant Physiol.* **102**, 133 (1993); P. Toubart *et al.*, *Plant J.* **2**, 367 (1992). *Antirrhinum* FIL2 [M. Steinmayr, P. Motte, H. Sommer, H. Saedler, Z. Schwarz-Sommer, *ibid.* **5**, 459 (1994)] also showed homology to Cf-9 but is included among the PGIPs because of its extensive homology with them.
20. A. F. Bent *et al.*, *Science* **265**, 1856 (1994); M. Mindrinos, F. Katagiri, G. Yu, F. M. Ausubel, *Cell* **78**, 1089 (1994); S. Whitham *et al.*, *ibid.*, p. 1101.
21. T. Braun, P. R. Schofield, R. Sprengel, *EMBO J.* **10**, 1885 (1991); J. Ware *et al.*, *J. Clin. Invest.* **92**, 1213 (1993); N. Suzuki *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 8711 (1990); J. Field *et al.*, *Science* **247**, 464 (1990).
22. B. Tourvieille, S. D. Gorman, E. H. Field, T. Hunkapiller, J. R. Parnes, *Science* **234**, 610 (1986).
23. A. Veillette, M. A. Bookman. E. M. Horak, J. B. Bolen, *Cell* **55**, 301 (1988).
24. K. E. Hammond-Kosack, D. A. Jones, J. D. G. Jones, *Plant Cell* **6**, 361 (1994).
25. C. Hashimoto, K. L. Hudson, K. V. Anderson, *Cell* **52**, 269 (1988); S. Roth, *Curr. Biol.* **4**, 755 (1994).
26. R. K. Andrews and J. E. B. Fox, *J. Biol. Chem.* **267**, 18605 (1992); J. A. Lopez *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 5615 (1987).
27. H. Loosfelt *et al.*, *Science* **245**, 525 (1989); K. C. McFarland *et al.*, *ibid.*, p. 494.
28. We thank C. Rommens and J. Hille for making available the *Ds* stock used; S. Perkins, J. Darby, and M. Shailer for plant care; P. Bovill for assistance in seed processing; J. Maxon-Smith for producing some of the test cross seed under contract, and A. Cavill for carrying out some of the DNA sequencing under contract. Supported by AFRC PMB grants 522, 523, and PG83/570; by an EEC BRIDGE grant; and by a grant to the Sainsbury Laboratory from The Gatsby Charitable Foundation.

7 September 1994; accepted 4 October 1994

Interaction of a Protein Phosphatase with an *Arabidopsis* Serine-Threonine Receptor Kinase

Julie M. Stone, Margaret A. Collinge, Robert D. Smith, Mark A. Horn, John C. Walker*

A protein phosphatase was cloned that interacts with a serine-threonine receptor–like kinase, RLK5, from *Arabidopsis thaliana*. The phosphatase, designated KAPP (kinase-associated protein phosphatase), is composed of three domains: an amino-terminal signal anchor, a kinase interaction (KI) domain, and a type 2C protein phosphatase catalytic region. Association of RLK5 with the KI domain is dependent on phosphorylation of RLK5 and can be abolished by dephosphorylation. KAPP may function as a signaling component in a pathway involving RLK5.

Many signal transduction pathways involved in the control of cell proliferation and differentiation originate with transmembrane receptors containing cytoplasmic protein kinase domains. Although much of the research done has focused on receptor tyrosine kinases (RTKs) (*1*), receptor serine-threonine kinases have been identified as well. These include the transforming growth factor β and activin receptor superfamily (*2*) and all known receptor-like kinases from higher plants (*3*, *4*). We report here the identification of a protein phosphatase that interacts with the phosphorylated form of a putative plant receptor serine-threonine kinase. This interaction is reminiscent of the mechanisms by which RTKs activate cellular signaling events. The signaling process of RTKs includes recognition of a polypeptide ligand, dimerization of the receptor, and autophosphorylation of tyrosine residues in the cytoplasmic portion of the molecule. These phosphorylated tyrosines with their flanking amino acids serve as high-affinity binding sites for cellular proteins containing Src homology 2 (SH2) domains (*5*). Therefore, activation of RTKs leads to the formation of protein complexes at the plasma membrane that are capable of transmitting signals to the next molecule in the signal cascade.

The *RLK5* gene from *Arabidopsis thaliana* encodes a protein with features characteristic of the polypeptide growth factor receptor kinases: a large NH_2-terminal extracellular domain, a single transmembrane domain, and a COOH-terminal protein kinase catalytic domain (*3*). The protein kinase domain of *RLK5*, when expressed as a fusion protein in *Escherichia coli*, autophosphorylates exclusively on serine and threonine residues (*6*).

J. M. Stone, Department of Biochemistry, University of Missouri–Columbia, Columbia, MO 65211, USA.
M. A. Collinge, R. D. Smith, M. A. Horn, J. C. Walker, Division of Biological Sciences, University of Missouri–Columbia, Columbia, MO 65211, USA.
*To whom correspondence should be addressed.

To identify components of a signal transduction pathway involving RLK5, interaction cloning (*7*, *8*) was used. An *Arabidopsis* complementary DNA (cDNA) expression library was screened for proteins that interact with the protein kinase catalytic domain of RLK5 (RLK5CAT). The probe used in this filter-binding assay was a glutathione-S-transferase (GST)–RLK5CAT fusion protein labeled with ^{32}P at a protein kinase A recognition site at the junction of the fusion (*8*). A positive clone was purified, subcloned, and sequenced (*9*). The cDNA insert encodes a 239–amino acid domain referred to as the KI domain. Sequence comparison has not revealed any strong homologies with previously reported sequences (*10*).

The possibility that interaction between the KI domain and RLK5 is phosphorylation-dependent was explored by in vitro binding studies. Analyses of protein-protein interaction on membrane filters (*11*) demonstrated that the KI domain is capable of binding to RLK5CAT, which is autophosphorylated in *E. coli* (*6*). Treatment with a type 1 serine-threonine protein phosphatase, ZmPP1 (*12*), abolished the interaction, whereas phosphatase treatment in the presence of okadaic acid, an inhibitor of type 1 protein phosphatases, did not interfere with the interaction. Furthermore, the KI domain does not bind a mutant form of RLK5 that is incapable of autophosphorylation (Fig. 1). These results show that association of the KI domain with RLK5 requires phosphorylation. Furthermore, the KI domain does not indiscriminately bind phosphoproteins, including ^{32}P-GST, or an autophosphorylated receptor-like kinase from *Zea mays* (*13*).

Full-length cDNA clones were identified by screening of an *Arabidopsis* cDNA library with a nucleic acid probe corresponding to the KI domain. The full-length cDNA encodes a 582–amino acid protein with a predicted molecular weight of 65 kD (Fig. 2A). The NH_2-terminus

consists of a hydrophobic segment immediately followed by a number of positively charged residues, which indicates that it may function as a type I signal anchor (SAI) (Fig. 2B). An SAI topogenic signal acts as an uncleaved signal peptide, resulting in cytoplasmic orientation of a membrane-anchored protein (*14*). This localization is consistent with the protein's interaction with RLK5 and possibly with other membrane-localized phosphoproteins. The COOH-terminal region shows homology with type 2C serine-threonine protein phosphatases (*10*) (Fig. 2C). Type 2C protein phosphatases (PP2C) are characterized by an absolute requirement for divalent cations and by insensitivity to the phosphatase inhibitor okadaic acid (*15*). We have therefore named the protein KAPP, for kinase-associated protein phosphatase.

Protein phosphatase catalytic activity was assayed with a recombinant fusion protein containing the predicted protein phosphatase catalytic domain of KAPP (MBP-PrP) (Fig. 3). The dephosphorylation of ^{32}P-casein by MBP-PrP was linear with time in the presence of divalent cations. Addition of EDTA, a chelator of divalent cations, abolished phosphatase activity. MBP-PrP activity was unaffected by okadaic acid, a specific inhibitor of types 1 and 2A protein phosphatases (*13*). These observations are consistent with KAPP having a PP2C catalytic domain.

Genomic Southern (DNA) analysis indicated that *KAPP* is a single copy gene in *Arabidopsis* (Fig. 4A). Additional bands hybridized to the *KAPP* probe at low stringency (*13*), which suggests the presence of homologous genes. Low stringency hybridization of a *Z. mays* cDNA library revealed clones that hybridize to sequences encoding both the NH_2-terminal portion of KAPP and the PP2C catalytic domain (*16*). The existence of homologous proteins in maize and *Arabidopsis* suggests that KAPP may belong to a family of proteins involved in serine-threonine phosphorylation cascades in higher plants. RNA blot analysis indicated that *KAPP* is expressed in all tissues examined, with larger amounts seen in roots than in rosettes, whereas *RLK5* is expressed in the least amounts in roots (Fig. 4B). Therefore, KAPP may have an additional role in RLK5-independent signaling pathways.

The identification and preliminary characterization of a protein, KAPP, that interacts with the protein kinase catalytic domain of RLK5 may help elucidate the proximal events in signaling pathways originating from serine-threonine receptor kinases. The observation that the interaction is dependent on the phosphorylation status of RLK5 is of particular interest.

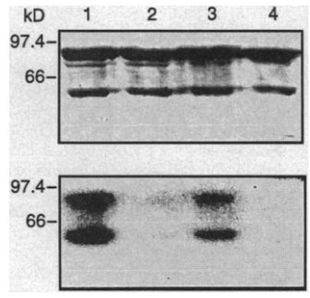

Fig. 1. The KI domain interacts with the autophosphorylated form of RLK5. Binding assays (*11*) demonstrate that the KI domain binds to the catalytic domain of RLK5. Maltose binding protein (MBP) fusions to RLK5CAT, which is active and autophosphorylated, and to RLK5CAT(Lys^{711} → Glu^{711}) (K711E) (*27*), which contains a point mutation at the conserved lysine required for phosphotransfer, were expressed in *E. coli*. The affinity-purified recombinant fusion proteins were subjected to SDS-PAGE, electrophoretically transferred to PVDF, and probed with ^{32}P-labeled fusion protein containing the KI domain (GST-KID) (*8*). Molecular weight markers are indicated on the left. The top panel shows a gel stained with Coomassie blue, and the bottom panel shows the corresponding autoradiogram after probing with GST-KID. The KI domain is capable of interacting with the catalytic domain of RLK5 (MBP-RLK5CAT) (lane 1). Pretreatment of MBP-RLK5CAT with a type 1 protein phosphatase, ZmPP1 (*12*), abolishes the interaction with the KI domain (lane 2). If ZmPP1 activity is blocked during pretreatment by okadaic acid, the KI domain still binds (lane 3). In addition, the KI domain cannot bind to the mutant form of RLK5 [MBP-RLK5CAT(K711E)] (lane 4), which is incapable of autophosphorylation (*6*). Control blots probed with ^{32}P-GST showed no binding, and the lower band, a proteolytic degradation product of MBP-RLK5CAT, is phosphorylated (*13*).

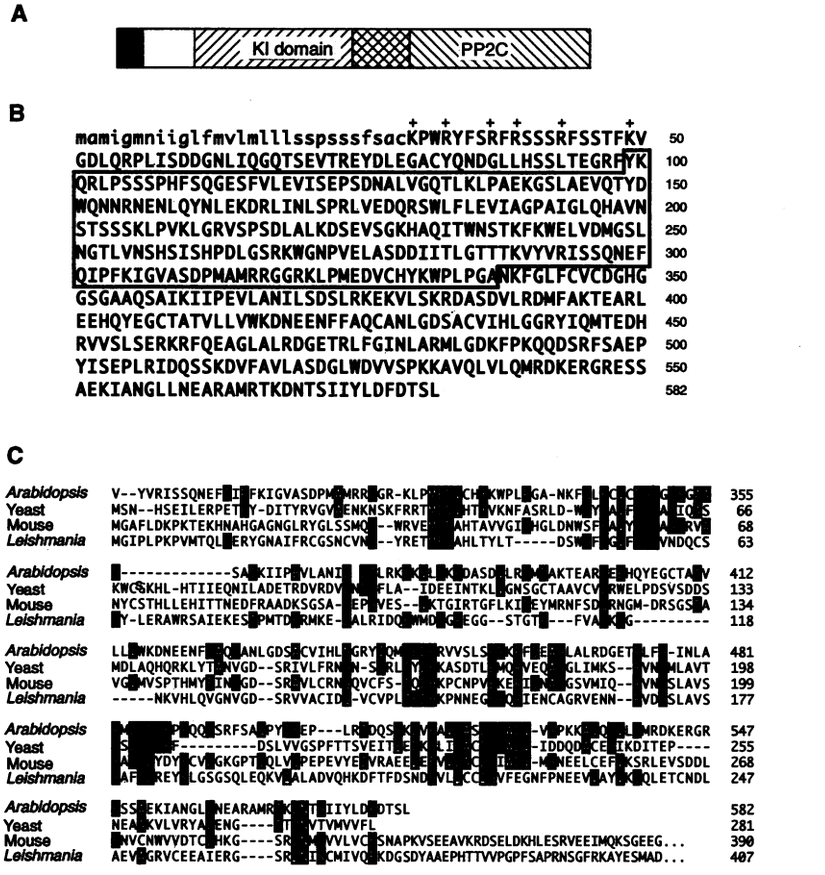

Fig. 2. Amino acid sequence of KAPP and comparison with type 2C serine-threonine protein phosphatases. (**A**) Schematic diagram of the protein encoded by full-length *KAPP* cDNA. The NH_2-terminal sequence characteristic of an SAI is shaded. The KI domain originally isolated by interaction cloning (*9*) and the COOH-terminal domain that shows homology to the type 2C serine-threonine protein phosphatases (PP2C) are indicated. (**B**) The single-letter amino acid sequence deduced from the *KAPP* cDNA (*27*). Amino acid numbers are indicated on the right. The putative hydrophobic NH_2-terminal signal anchor is shown in lowercase letters, and the basic residues adjacent to this region are designated by plus signs. The KI domain is outlined. The *KAPP* cDNA was cloned and sequenced as described (*28*). The nucleotide and protein sequences of *KAPP* have been deposited with GenBank (accession number U09505). (**C**) Alignment of the deduced KAPP protein (amino acids 290 to 582) with representative type 2C serine-threonine protein phosphatases. Alignments were made by the Clustal method with DNASTAR software (Madison, Wisconsin). The organisms from which the PP2Cs were isolated are indicated on the left and amino acid positions on the right. The identity between KAPP and PP2Cs from yeast, mouse, and *Leishmania* (*29*) is 19.2%, 15.0%, and 12.9%, respectively. Identical residues are signified by shading, and gaps introduced to optimize alignment are shown by dashes.

The KI domain may be a member of a group of protein domains (which includes the phosphotyrosine-binding SH2 domain subgroup) that interact specifically with phosphoamino acid–containing protein sequences (*5*, *17*).

In addition to its KI domain, KAPP also possesses PP2C enzymatic activity. PP2C activity has been found in a number of organisms (*18*), including higher plants (*19*), in which the product of *ABI1*, implicated in abscisic acid–mediated responses, has been identified as a PP2C (*20*). These and other protein phosphatases are important components of signal transduction pathways. For example, recent evidence suggests a role for SH2-containing protein tyrosine phosphatases in signaling from receptor tyrosine kinases (*21*). The fact that several of the tyrosine phosphatases are regulated by their NH_2-terminal domains (*22*) suggests the possibility that KAPP phosphatase activity is regulated by its NH_2-terminal KI domain by binding to autophosphorylated RLK5. Alternatively, as RLK5 has been shown to phosphorylate the KI domain (*13*), phosphorylation of KAPP might regulate its RLK5-binding ability or protein phosphatase activity or both. Continued study of the interaction of KAPP and RLK5 will undoubtedly expand our understanding of how signals are transduced from serine-threonine receptor kinases.

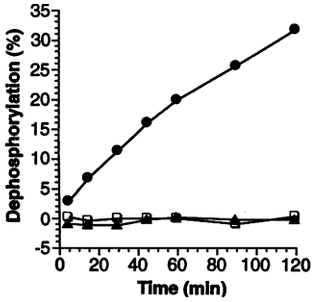

Fig. 3. KAPP has a protein phosphatase catalytic domain with activity characteristic of PP2Cs. Protein phosphatase activity is shown as percent of dephosphorylation with phosphorylated casein as a substrate (*30*). An MBP fusion protein containing the presumed protein phosphatase catalytic domain of KAPP (amino acids 162 to 582) (MBP-PrP) dephosphorylates casein in the presence of 10 mM Mg^{+2} and 10 mM Mn^{+2} (circles) but not 10 mM EDTA (triangles). Control protein (MBP) has no protein phosphatase activity in the presence of 10 mM Mg^{+2} and 10 mM Mn^{+2} (squares). The activity is also unaffected by 10 μM okadaic acid (*13*).

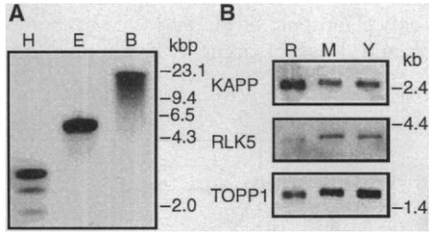

Fig. 4. *KAPP* is present in the *Arabidopsis* genome in a single copy and is expressed in the same tissues as *RLK5*. (**A**) Southern analysis. *Arabidopsis* genomic DNA was digested with Hind III (H), Bam HI (B), and Eco RI (E), separated in agarose, transferred to nitrocellulose, and probed with ^{32}P-labeled *KAPP* cDNA. DNA size markers are shown on the right in kilobase pairs. *KAPP* appears to be a single copy gene. (**B**) Northern (RNA) blot analysis. Polyadenylated RNA was isolated from cultured roots (R), mature rosettes (M), and young rosettes (Y), separated in a formaldehyde gel, blotted to a membrane, and sequentially probed with ^{32}P-cDNAs corresponding to *KAPP*, *RLK5* (*3*), and *TOPP1*, an *Arabidopsis* type 1 protein phosphatase that is constitutively expressed (*31*). Size markers are shown in kilobases.

REFERENCES AND NOTES

1. W. J. Fantl, D. E. Johnson, L. T. Williams, *Annu. Rev. Biochem.* **62**, 453 (1993).
2. J. Massagué, *Cell* **69**, 1067 (1992); L. Attisano, J. L. Wrana, S. Cheifetz, J. Massagué, *ibid.* **68**, 97 (1992); H. Y. Lin and H. F. Lodish, *Trends Cell. Biol.* **3**, 14 (1993).
3. J. C. Walker, *Plant J.* **3**, 451 (1993).
4. C. Chang *et al.*, *Plant Cell* **4**, 1263 (1992); J. C. Stein, B. Howlett, D. C. Boyes, M. E. Nasrallah, J. B. Nasrallah, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 8816 (1991); C. M. Tobias, B. Howlett, J. B. Nasrallah, *Plant Physiol.* **99**, 284 (1992).
5. C. A. Koch, D. Anderson, M. F. Moran, C. Ellis, T. Pawson, *Science* **252**, 668 (1991); Z. Songyang *et al.*, *Mol. Cell. Biol.* **14**, 2777 (1994); Z. Songyang *et al.*, *Cell* **72**, 767 (1993); R. B. Birge and H. Hanafusa, *Science* **262**, 1522 (1993).
6. M. A. Horn and J. C. Walker, *Biochim. Biophys. Acta*, **1208**, 65 (1994).
7. D. W. Carr and J. D. Scott, *Trends Biochem. Sci.* **17**, 246 (1992); B. Margolis *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **89**, 8894 (1992); E. Y. Skolnik *et al.*, *Cell* **65**, 83 (1991).
8. M. A. Blanar and W. J. Rutter, *Science* **256**, 1014 (1992).
9. For interaction cloning, bacteriophages from an *Arabidopsis* λgt11 library were induced by being overlaid with isopropyl-β-D-thiogalactopyranoside–impregnated nitrocellulose filters (*23*). Filters were blocked for 4 hours at 4°C in 25 mM Hepes (pH 7.5), 5 mM $MgCl_2$, 1 mM KCl, and 5% nonfat dry milk, then probed overnight with ~2.5 × 10^5 cpm/ml of ^{32}P-GST-RLK5CAT (*8*) in 25 mM Hepes (pH 7.5), 75 mM KCl, 0.1 mM EDTA, 2.5 mM $MgCl_2$, and 1% nonfat dry milk (BB). Filters were washed in BB three times for 10 min each and exposed to film. The DNA sequence of the 732-bp cDNA insert was determined by the dideoxy chain termination method with the use of Sequenase (U.S. Biochemical).
10. Searches in the databases of GenBank, the European Molecular Biology Laboratory, Brookhaven Protein Data Bank, Protein Identification Resource, and SwissProt were done with the BLAST network service [S. F. Altschul, W. Gish, W. Miller, E. W. Myers, D. J. Lipman, *J. Mol. Biol.* **215**, 403 (1990)].
11. Binding assays were done as follows. One microgram of purified recombinant fusion proteins (*6*) was separated on 10% SDS–polyacrylamide gel electrophoresis (PAGE) gels and electrophoretically transferred to polyvinylidene fluoride (PVDF) membranes (*24*). Subsequent steps were as described (*9*). Dephosphorylation with ZmPP1 was done in 50 mM tris-HCl (pH 7.5), 1 mM $MnCl_2$, and 1 mM dithiothreitol at 37°C for 2 hours with or without 20 μM okadaic acid (LC Laboratories, Woburn, MA).
12. R. D. Smith and J. C. Walker, *Plant Physiol.* **97**, 677 (1991).
13. J. M. Stone, M. A. Collinge, R. D. Smith, M. A. Horn, J. C. Walker, unpublished results.
14. S. High and B. Dobberstein, *Curr. Opin. Cell Biol.* **4**, 581 (1992).
15. S. Shenolikar and A. C. Nairn, *Adv. Second Messenger Phosphoprotein Res.* **23**, 1 (1991).
16. D. M. Braun and J. C. Walker, unpublished results.
17. L. Buday and J. Downward, *Cell* **73**, 611 (1993); J. P. Olivier *et al.*, *ibid.*, p. 179; R. Nishimura *et al.*, *Mol. Cell. Biol.* **13**, 6889 (1993).
18. T. Maeda, A. Y. M. Tsai, H. Saito, *Mol. Cell. Biol.* **13**, 5408 (1993); D. J. Mann, D. G. Campbell, C. H. McGowan, P. T. W. Cohen, *Biochim. Biophys. Acta* **1130**, 100 (1992).
19. C. MacKintosh, J. Coggins, P. Cohen, *Biochem. J.* **273**, 733 (1991).
20. J. Leung *et al.*, *Science* **264**, 1448 (1994); K. Meyer, M. P. Leube, E. Grill, *ibid.*, p. 1452.
21. G.-S. Feng and T. Pawson, *Trends Genet.* **10**, 54 (1994); W. Li *et al.*, *Mol. Cell. Biol.* **14**, 509 (1994); L. A. Perkins, I. Larsen, N. Perrimon, *Cell* **70**, 225 (1992).
22. U. Dechert, M. Adam, K. W. Harder, I. Clark-Lewis, F. Jirik, *J. Biol. Chem.* **269**, 5602 (1994); R. J. Leichleider *et al.*, *ibid.* **268**, 21478 (1993); R. Townley, S.-H. Shen, D. Banville, C. Ramachandran, *Biochemistry* **32**, 13414 (1993).
23. T. V. Huynh, R. A. Young, R. W. Davis, in *DNA Cloning: A Practical Approach*, D. M. Glover, Ed. (IRL, Oxford, 1985), vol. 1, pp. 49–78.
24. U. K. Laemmli, *Nature* **227**, 680 (1970); E. Harlow and D. Lane, *Antibodies: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1988).
25. J. C. Walker and R. Zhang, *Nature* **345**, 743 (1990).
26. C. H. McGowan and P. Cohen, *Methods Enzymol.* **159**, 416 (1987).
27. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
28. An *Arabidopsis* λZipLox cDNA library was screened by plaque hybridization with the use of a ^{32}P-labeled nucleic acid probe corresponding to the KI domain cDNA insert. Hybridization of filters (*25*) and subcloning and sequencing were as described (*9*).
29. The GenBank accession numbers of the PP2Cs from yeast, mouse, and *Leishmania* are L14593, D17412, and L15559, respectively.
30. Bovine milk casein (Sigma) was labeled with [γ-^{32}P]ATP as described (*8*). Radiolabeled casein was precipitated with trichloroacetic acid (TCA), washed, dried, and resuspended in 0.2 M tris-HCl (pH 8) at a concentration of 4.5 mg/ml (3 × 10^5 cpm/mg of protein). Phosphatase activity was determined by standard procedures (*26*). Briefly, 90-μl reactions containing 50 mM tris-HCl (pH 7), 0.1% (v/v) 2-mercaptoethanol, ~3 × 10^6 cpm ^{32}P-casein, 10 μg of recombinant protein, and either 10 mM EDTA or Mg^{+2} and Mn^{+2} (10 mM each) were incubated at 25°C. Ten-microliter aliquots were removed at each time point and added to 100 μl of 20% TCA. Radioactivity in 90 μl of supernatant was measured by scintillation counting.
31. R. D. Smith and J. C. Walker, *Plant Mol. Biol.* **21**, 307 (1993).

32. We thank K. Cone for critical review of the manuscript, C. LaBarbera for technical assistance, the Ohio State University Arabidopsis Biological Resource Center for the λZipLox cDNA library, A. DeLisle (University of Nevada) for the λgt11 cDNA library, and M. Blanar (Princeton, NJ) for the protein kinase A tagging vectors. Supported by NSF grants MCB-9105388 and MCB-9219075 and by the University of Missouri Food for the 21st Century Program (J.C.W.). J.M.S. is a U.S. Department of Agriculture National Needs predoctoral fellow and M.A.C. was supported by a University of Missouri Molecular Biology Postdoctoral Fellowship.

13 June 1994; accepted 20 September 1994