THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 280, No. 32, Issue of August 12, pp. 28936–28943, 2005
Printed in U.S.A.

Binding of PTEN to Specific PDZ Domains Contributes to PTEN Protein Stability and Phosphorylation by Microtubule-associated Serine/Threonine Kinases*

Received for publication, April 29, 2005, and in revised form, June 7, 2005
Published, JBC Papers in Press, June 10, 2005, DOI 10.1074/jbc.M504761200

Miguel Valiente‡, Amparo Andrés-Pons‡§, Beatriz Gomar‡¶, Josema Torres‡¶‖, Anabel Gil‡, Caroline Tapparel, Stylianos E. Antonarakis**, and Rafael Pulido‡ ‡‡**

*From the ‡Centro de Investigación Príncipe Felipe, Valencia 46013, Spain and **Division of Medical Genetics, Centre Medical Universitaire, University of Geneva Medical School, 1 Rue Michel Servet, Geneva CH-1211, Switzerland*

The tumor suppressor phosphatase PTEN is a key regulator of cell growth and apoptosis that interacts with PDZ domains from regulatory proteins, including MAGI-1/2/3, hDlg, and MAST205. Here we identified novel PTEN-binding PDZ domains within the MAST205-related proteins, syntrophin-associated serine/threonine kinase and MAST3, characterized the regions of PTEN involved in its interaction with distinctive PDZ domains, and analyzed the functional consequences on PTEN of PDZ domain binding. Using a panel of PTEN mutations, as well as PTEN chimeras containing distinct domains of the related protein TPTE, we found that the PTP and C2 domains of PTEN do not affect PDZ domain binding and that the C-terminal tail of PTEN (residues 350–403) provides selectivity to recognize specific PDZ domains from MAGI-2, hDlg, and MAST205. Binding of PTEN to the PDZ-2 domain from MAGI-2 increased PTEN protein stability. Furthermore, binding of PTEN to the PDZ domains from microtubule-associated serine/threonine kinases facilitated PTEN phosphorylation at its C terminus by these kinases. Our results suggest an important role for the C-terminal region of PTEN in the selective association with scaffolding and/or regulatory molecules and provide evidence that PDZ domain binding stabilizes PTEN and targets this tumor suppressor for phosphorylation by microtubule-associated serine/threonine kinases.

Alterations in the function of the PTEN phosphatase tumor suppressor protein are of major relevance for the incidence of a wide variety of human cancers, as well as for the occurrence of inherited growth disorders, grouped as *PTEN* hamartoma tumor syndromes (1). Structurally, PTEN protein is composed of an N-terminal phosphatase catalytic domain and a C-terminal phospholipid-binding C2 domain; the integrity of both domains is required for full PTEN phosphatase activity and binding to membranes (2). The analysis of tumor specimens, tumor cell lines, and model organisms defective in PTEN protein expression has shown that the 3-phosphoinositide phosphatase activity of PTEN toward the phospholipid phosphatidylinositol 3,4,5-trisphosphate is crucial for the control of cell growth, cell cycle, cell motility and migration, and apoptosis (3–6). In addition, some PTEN biological functions have been attributed to its protein phosphatase activity (7–10), and a PTEN phosphatase independent effect on the regulation of p53 stability and transcriptional activity has been reported (11). A major level of regulation of PTEN functions is related with its phosphorylation status, which has been involved in maintaining PTEN protein stability and in the control of PTEN subcellular location and/or its association with regulatory molecules (12–21). In this regard, PTEN possesses a C-terminal tail (last 54 amino acids; residues 350–403), which harbors at its far C terminus a functional PDZ domain-binding motif (residues Thr^{401}–Lys^{402}–Val^{403}–COOH). PDZ domains are modular protein interaction domains that in most cases recognize C-terminal motifs on their target proteins, playing important roles in protein targeting and/or protein complex assembly during cell signaling (22–24). The molecular basis of the protein-protein interactions mediated by PDZ domains has been extensively investigated by using peptides that mimic C-terminal binding motifs occurring in their cognate binding proteins (25, 26). However, very little is known about the involvement of other parts of the PDZ domain-binding proteins in conferring strength and/or specificity to the interaction with their counterpart PDZ domains. PDZ domains that bind to PTEN include the PDZ-2 domain from the scaffolding proteins MAGI-1/2/3, the PDZ-2 domain from the human homologue of the *Drosophila* Dlg, hDlg/SAP97, and the unique PDZ domain from the Ser/Thr kinase MAST205 (12, 27–29). The interaction of PTEN with MAGI-1/2 has been shown to favor PTEN recruitment into high molecular weight molecular complexes and to enhance the PTEN-mediated down-regulation of the cell survival and invasiveness activities of the protein kinase B/Akt oncogene (18, 27–30). However, the functional consequences of the interaction of PTEN with hDlg or MAST205 are unknown.

In the present study, using a panel of PTEN mutations and PTEN chimeras containing distinct domains of the related protein TPTE (31, 32), we have characterized the residues and regions of PTEN involved in the interaction with PDZ domains from MAGI-2, hDlg, and MAST205. In addition, we show that PTEN also binds to the PDZ domains from the MAST205-

* This work was supported in part by Grant SAF2002-00085 from Ministerio de Ciencia y Tecnología and Fondo Europeo de Desarrollo Regional and by a grant from Fundación Mutua Madrileña Automovilista (Spain). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

§ Recipient of a fellowship from Ministerio de Educación y Ciencia (Spain).

¶ Recipient of a fellowship from Generalitat Valenciana (Spain).

‖ Present address: Keratinocyte Laboratory, Cancer Research UK, 44 Lincoln's Inn Fields, London WCA 3PX, UK.

‡‡ To whom correspondence should be addressed: Centro de Investigación Príncipe Felipe, Avda. Autopista del Saler, 16-3, 46013 Valencia, Spain. Tel.: 34-96-3289680; Fax: 34-96-3289701; E-mail: rpulido@ochoa.fib.es.

related protein kinases, SAST[1] and MAST3. We further demonstrate that binding of PTEN to specific PDZ domains diminishes its degradation rate and facilitates its phosphorylation by MAST kinases. Our results suggest a regulatory role of PDZ domain binding on PTEN function by controlling its stability and phosphorylation status.

MATERIALS AND METHODS

Plasmids and Antibodies—pRK5 HA-PTEN (N-terminal tagging) and pGEX-4T PDZ-2/MAGI-2, pGEX-4T PTEN, and pCMV Myc-MAGI-2 (pCMV Myc-S-SCAM) have been described previously (16, 33). The plasmids pGEX-4T PDZ-2/MAGI-3 (residues 567–697), pGEX-4T PDZ-2/hDlg (residues 308–411), pGEX-4T PDZ-2/pDlg (residues 285–384), pGEX-4T PDZ/MAST205 (residues 1031–1138), pGEX-4T PDZ/MAST3 (residues 939–1046, DPS . . . VGP), pGEX-4T PDZ/MAST4 (residues 638–751, DSS . . . TGP), and pGEX-4T PDZ/SAST (residues 958–1065) (all encoding GST fusion proteins) were obtained by subcloning into pGEX-4T of the appropriate cDNA fragments, obtained by PCR amplification from the following plasmids: pC3-rMAGI-3 (rat sequence; provided by E. Peles; see Ref. 34), pGEX-2T hDlg (rat sequence; provided by A. Cuenda), Bluescript pDlg (human sequence; see Ref. 35; provided by Kazusa DNA Research Institute as KIAA0583), pUC-MAST205 (MAST2; mouse sequence; provided by P. D. Walden; see Ref. 36), Bluescript MAST3 (human sequence; provided by Kazusa DNA Research Institute as KIAA0561; GenBank™ accession number XM_038150), Bluescript MAST4 (human sequence; provided by Kazusa DNA Research Institute as KIAA0303; GenBank™ accession number AB002301), and pYX-Asc SAST (MAST1; mouse sequence; see Ref. 37; Mammalian Gene Collection, IMAGE ID 6400705). PTEN single amino acid substitutions, truncations, and deletions subcloned into pRK5 or pGEX were obtained by PCR oligonucleotide site-directed mutagenesis, and the mutations were confirmed by DNA sequencing. The plasmids pRK5 GST and pRK5 GST-PDZ-2/MAGI-2 were made by PCR amplification of GST and GST-PDZ-2/MAGI-2 from pGEX-4T and pGEX-4T PDZ-2/MAGI-2, respectively, followed by subcloning into pRK5. The plasmids pJG4-5 PDZ-2/MAGI-2, pJG4-5 PDZ-2/MAGI-3, pJG4-5 PDZ/MAST205, pJG4-5 PDZ/MAST3, pJG4-5 PDZ/MAST4, and pJG4-5 PDZ/SAST, encoding the corresponding PDZ domains fused at their N terminus to the B42 transcription activation domain, were obtained by direct subcloning from the pGEX constructs indicated above. The plasmids pEG202 PTEN, encoding PTEN fused at its N terminus to the LexA DNA-binding domain, were obtained by subcloning the entire PTEN coding sequence (residues 1–403), wild type or single amino acid substitutions, or PTEN truncation or deletion mutants into pEG202. To obtain the plasmid pRK5 HA-TPTE-(215–551), a TPTE fragment (residues 215–551) was amplified by PCR from pCDNA3 HA-TPTE encoding the full-length human TPTE (31) and subcloned into the pRK5 HA plasmid. The chimeras HA-PTEN-TPTE (PTEN-(1–185)/TPTE-(405–551)), HA-TPTE-PTEN (TPTE-(215–404)/PTEN-(186–403)), HA-TP2 (TPTE-(215–539)/PTEN-(350–403)), HA-TP3 (TPTE-(215–551)/PTEN-(369–403)), and HA-TP4 (TPTE-(215–551)/PTEN-(376–403)) were constructed by PCR using primers that were complementary to the fused regions of PTEN and TPTE. The chimeras HA-TPTE-ITKV (TPTE-(215–551)/PTEN-(400–403)) and TPTE-TKV (TPTE-(215–551)/PTEN-(401–403)) were constructed by PCR using primers that were complementary to the last TPTE residues plus the PTEN-ITKV or TKV N-terminal residues, followed by the PTEN stop codon. All the chimeras were tagged at their N terminus with the HA epitope, and their identity was verified by DNA sequencing. The plasmids pRK5 HA kinase/MAST205 (residues 430–741, ETD . . . AEF) and pRK5 HA kinase + PDZ/MAST205 (residues 444–1138, SQK . . . VGP) were obtained by subcloning into pRK5 HA plasmid of the appropriate cDNA fragments, obtained by PCR amplification from pUCMAST205. The plasmids pRK5 HA kinase/MAST3 (residues 366–581, FET . . . PFF) and pRK5 HA kinase/SAST (residues 337–590, FDT . . . PFF) were obtained by subcloning into pRK5 HA plasmid of the appropriate cDNA fragments, obtained by PCR amplification from Bluescript MAST3 and pYX-Asc SAST, respectively. The nucleotide sequences of all primers used in the construction of plasmids and mutagenesis are available upon request. The anti-HA monoclonal antibody (mAb) was 12CA5, and the anti-c-Myc (A-14) polyclonal antibody was from Santa Cruz Biotechnology.

[1] The abbreviations used are: SAST, syntrophin-associated serine/threonine kinase; MAST, microtubule-associated serine/threonine kinase; GST, glutathione *S*-transferase; HA, hemagglutinin; mAb, monoclonal antibody.

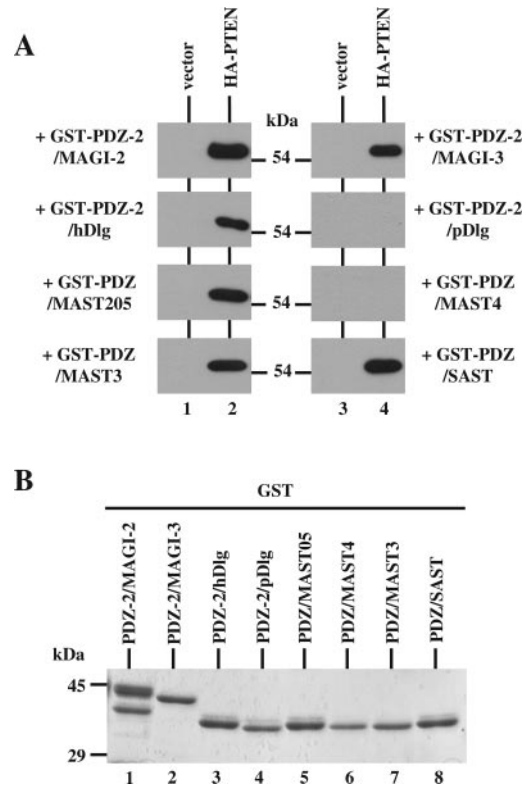

FIG. 1. **PTEN binds multiple PDZ domains.** *A*, COS-7 cells were transfected with pRK5 alone (*vector*) or pRK5 HA-PTEN, as indicated. Equal amounts of cellular lysates (0.3 mg) were subjected to pull-down with equivalent amounts (3 μg) of the following GST fusion proteins, as indicated: GST-PDZ-2/MAGI-2 (residues 591–732), GST-PDZ-2/MAGI-3 (residues 567–697), GST-PDZ-2/hDlg (residues 308–411), GST-PDZ-2/pDlg (residues 285–384), GST-PDZ/MAST205 (residues 1031–1138), GST-PDZ/MAST4 (residues 638–751), GST-PDZ/MAST3 (residues 939–1046), and GST-PDZ/SAST (residues 958–1065). The presence of HA-PTEN proteins in the pull-down pellets was analyzed by immunoblot using anti-HA mAb. All experiments were repeated at least three times with similar results, and a representative experiment is shown. *B*, visualization of the distinct purified GST fusion proteins (3 μg) used in *A*. In *lane 1*, a degradation band that runs faster than GST-PDZ-2/MAGI-2 (45 kDa) is observed. Samples were resolved by 10% SDS-PAGE under reducing conditions and stained with Coomassie.

Cell Culture, Transfection, Cell Lysis, and [^{35}S]Methionine Labeling of Proteins—COS-7 cells were grown in Dulbecco's modified Eagle's medium containing 5% heat-inactivated fetal calf serum and were transfected by the DEAE-dextran method. Cells were lysed in lysis buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% Igepal, 1 mM phenylmethylsulfonyl fluoride, 1 μg/ml aprotinin, 20 mM $Na_4P_2O_7$). The cell lysates were centrifuged (14,000 rpm, 10 min at 4 °C), and the supernatants were used for GST pull-down assays, co-immunoprecipitation, or immunoprecipitation as indicated. For [^{35}S]methionine pulse-chase experiments, transfected COS-7 cells were incubated for 1 h in methionine-free Dulbecco's modified Eagle's medium, and cells were then labeled for 4 h with [^{35}S]methionine (100 μCi/ml), followed by a pulse of different times in nonradioactive medium supplemented with an excess of cold methionine (1 mM).

GST Fusion Proteins, GST Pull-down, Immunoprecipitation, and Co-immunoprecipitation—The GST fusion proteins containing the different PDZ domains were expressed in bacteria and purified with glutathione-Sepharose using standard procedures. For GST pull-down experiments, COS-7 cells transfected with the expression plasmids encoding HA-tagged recombinant proteins were lysed as indicated above, and 0.3–0.5 mg of the cell lysates were incubated for 2 h on ice with 3 μg of the GST fusion proteins, followed by the addition of glutathione-Sepharose beads and further incubation for 2 h under constant shaking. Samples were washed four times with HNTG buffer (20 mM HEPES, pH 7.5, 150 mM NaCl, 0.1% Triton X-100, 1% glycerol), resolved on 10% SDS-polyacrylamide gels under reducing conditions, and analyzed by immunoblot with the anti-HA 12CA5 mAb. For co-immunoprecipitation experiments, COS-7 cells were separately trans-

TABLE I
Semi-quantitative yeast two-hybrid analysis of PTEN interaction with PDZ domains

	PDZ-2/MAGI-2	PDZ-2/MAGI-3	PDZ/MAST205	PDZ/MAST3	PDZ/MAST4	PDZ/SAST
			units[a]			
Vector	1.5 ± 0.9	1.7 ± 1.1	2.8 ± 1.7	3.2 ± 0.9	4.5 ± 0.5	3.9 ± 0.7
PTEN wild type	82.3 ± 14.4	106.7 ± 1.6	183.5 ± 15.3	23.5 ± 6.4	3.4 ± 2.1	123.8 ± 6.6
PTEN V403A	0.8 ± 0.7	1.7 ± 1.5	1.4 ± 1.0	4.1 ± 0.9	3.6 ± 1.4	4.0 ± 1.6

[a] Arbitrary units of β-galactosidase activity (×10^3) are shown as the mean ± S.D. of at least three separate experiments. Each experiment was performed in triplicate.

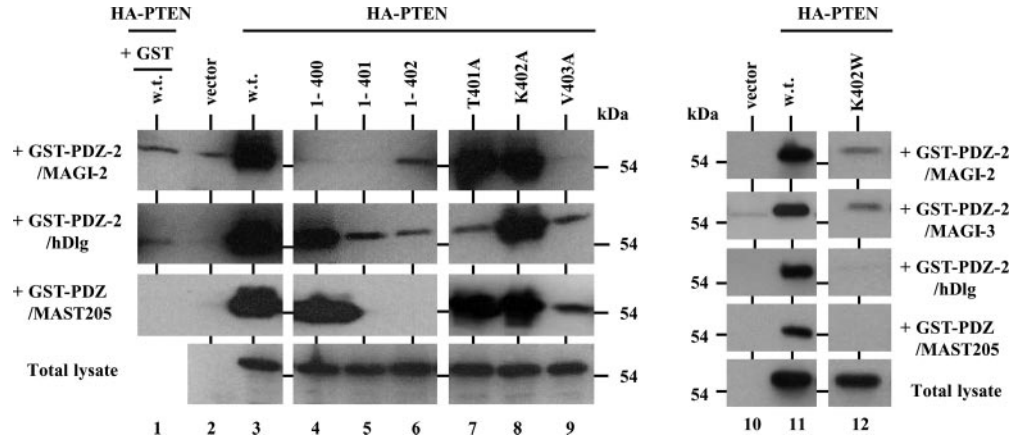

FIG. 2. **The C-terminal PDZ-binding motif (TKV-COOH) of PTEN is differentially recognized by distinct PDZ domains.** COS-7 cells were transfected with pRK5 alone (*vector*) or pRK5 HA-PTEN wild type (*w.t.*) or different mutations, as indicated. Equal amounts of cellular lysates (0.3 mg) were subjected to pull-down with equivalent amounts (3 μg) of GST-PDZ-2/MAGI-2, GST-PDZ-2/MAGI-3, GST-PDZ-2/hDlg, or GST-PDZ/MAST205, as indicated. The presence of HA-PTEN proteins in enriched pull-down pellets and in total lysates (40 μg) was analyzed by immunoblot using anti-HA mAb. All experiments were repeated at least three times with similar results, and a representative experiment is shown.

fected with pRK5 HA-PTEN, wild type or mutations, and with pCMV Myc-MAGI-2. 0.3–0.5 mg of cell lysates from each type of transfected cell was mixed in pairwise combinations and incubated for 2 h on ice, followed by further incubation (16 h, 4 °C) in the presence of the anti-Myc antibody. Protein A-Sepharose was then added and incubated for 2 h at 4 °C, under constant shaking. Samples were washed and processed for immunoblot as for the GST pull-down assays. For immunoprecipitation of ^{35}S-labeled HA-PTEN proteins, cell lysates were immunoprecipitated with the anti-HA 12CA5 mAb and protein A-Sepharose, followed by SDS-PAGE analysis, autoradiography, and quantitative analysis using a PhosphorImager.

Yeast Two-hybrid Analysis—The *Saccharomyces cerevisiae* strain EGY48 (*MATα*, *trp1*, *his3*, *ura3*, *LEU2*::*LexA-op6-LEU2*) was transformed with the *lacZ* reporter plasmid pMW107 (URA3 marker, LexA-op4-lacZ) together with pairwise combinations of the pEG202 PTEN (HIS3 marker) and the pJG4-5 PDZ (TRP1 marker) plasmids, and co-transformants were selected by plating on supplemental medium lacking Ura, His, and Trp (selective medium). Semi-quantitative yeast two-hybrid experiments were performed for each combination of pEG202 and pJG4-5 plasmids. As a control, the empty pEG202 vector was co-transformed with each pJG4-5 PDZ plasmid. Three independent co-transformant colonies were used to inoculate separately 3 ml of selective medium supplemented with 2% dextrose; after overnight growth, the cultures were diluted (1:25) in 5 ml of fresh selective medium supplemented with 2% galactose plus 1% raffinose and incubated for an additional 3–4 h to reach an A_{600} of 0.5–0.8. Cultures (0.1–1 ml) were collected by centrifugation, washed, and resuspended in 0.8 ml of buffer Z (60 mM Na_2HPO_4, 40 mM NaH_2PO_4, 10 mM KCl, 1 mM $MgSO_4$, 50 mM β-mercaptoethanol). Cells were lysed by adding 50 μl of $CHCl_3$ plus 50 μl of 0.1% SDS and incubated in the presence of the β-galactosidase substrate, *o*-nitrophenyl β-D-galactopyranoside (4 mg/ml in buffer Z; 160 μl per sample), for 15 min at 30 °C. Reactions were stopped by adding 0.4 ml of 1 M Na_2CO_3. Insoluble material was removed by centrifugation, and *o*-nitrophenyl β-D-galactopyranoside hydrolysis was measured by absorbance at 420 nm. The cell density of the cultures was measured, before cell lysis, from separate aliquots by absorbance at 600 nm. The arbitrary units of β-galactosidase activity were calculated from the formula A_{420}/A_{600} × volume of culture (ml) × time (min).

In Vitro Kinase Assays—COS-7 cells transfected with the expression plasmids pRK5 HA kinase/MAST3, HA kinase/SAST, HA kinase/MAST205, or HA kinase + PDZ/MAST205 were lysed in lysis buffer. The cell lysates were centrifuged (14,000 rpm, 10 min at 4 °C), and the supernatants were incubated for 4 h on ice in the presence of the anti-HA 12CA5 mAb. Protein A-Sepharose was then added and incubated for 2 h at 4 °C, under constant shaking. Samples were first washed three times with HNTG buffer and then with kinase reaction buffer (50 mM HEPES, pH 7.5, 20 mM $MgCl_2$, 2 mM dithiothreitol). The immune complexes were mixed with the GST-PTEN fusion proteins previously expressed in bacteria and purified with glutathione-Sepharose using standard procedures. The mixture was washed with kinase reaction buffer and then resuspended in 50 μl of the same buffer supplemented with 5 μM ATP and 5 μCi of [γ-^{32}P]ATP. Samples were incubated for 15 min at 30 °C, heated to 90 °C for 5 min in SDS sample buffer, and then analyzed by SDS-PAGE and autoradiography.

RESULTS

PTEN Binds to Multiple PDZ Domains—PTEN possesses at its C terminus a functional PDZ domain-binding motif (Thr^{401}-Lys^{402}-Val^{403}-COOH; TKV) that binds specific PDZ domains from MAGI-1/2/3, hDlg, and MAST205 (12, 27–29) (Fig. 1*A*). MAST205 belongs to the MAST kinase family, which also includes SAST, MAST3, and MAST4 kinases (36–38). Similarly, hDlg-related proteins include the pDlg protein (35). As shown by GST pull-down assays (Fig. 1*A*), the PDZ domains from SAST and MAST3 also bind to HA-tagged PTEN, whereas the PDZ domain from MAST4 or the PDZ-2 domain from pDlg do not. Yeast two-hybrid analysis was also performed to test the interaction of PTEN wild type and V403A mutation with the distinct PDZ domains. PTEN wild type bound to the PDZ domains of MAGI-2, MAGI-3, MAST205, MAST3, and SAST, but not to the PDZ domain of MAST4. The V403A mutation, within the PDZ-binding motif, did not bind to any PDZ domain (Table I). Thus, multiple PDZ domains specifically interact with the tumor suppressor PTEN. To compare the recognition of the TKV PTEN motif by some of its counterpart PDZ domains (PDZ-2/MAGI-2, PDZ-2/hDlg, and PDZ/MAST205), C-terminal truncations were generated on the background of the

HA-PTEN recombinant protein, and the binding of the truncated proteins was tested by pull-down assays using GST-PDZ-2/MAGI-2, GST-PDZ-2/hDlg, or GST-PDZ/MAST205 recombinant fusion proteins, as above (Fig. 2). Progressive amino acid deletions within the TKV motif differentially affected the recognition by the distinct PDZ domains. Thus, GST-PDZ-2/MAGI-2 did not bind to PTEN-(1–400), -(1–401), or -(1–402), whereas PDZ-2/hDlg and PDZ/MAST205 bound to PTEN-(1–400) but not to PTEN-(1–401) or -(1–402), indicating that certain C-terminal PTEN truncations are able to interact with some PDZ domains (Fig. 2, *lanes 4–6*). Moreover, individual alanine substitution of residues within the PTEN PDZ-binding motif affected differentially the binding of the distinct PDZ domains. The mutation T401A bound to PDZ-2/MAGI-2 and PDZ/MAST205 but not to the PDZ-2/hDlg; the mutation K402A had no effect on the association; and the mutation V403A inhibited the association of the three PDZ domains (Fig. 2, *lanes 7–9*). Most interestingly, the mutation K402W also abrogated binding to the PDZ domains from MAGI-2, MAGI-3, hDlg, and MAST205 (Fig. 2, *lane 12*). The inhibitory effect of the K402W mutation was also observed when the binding to the full-length Myc-MAGI-2 was tested by co-immunoprecipitation (data not shown). Together, these results indicate that the C-terminal motif of PTEN is important in the definition and specificity of the interaction with different PDZ domains, and these results suggest a distinct permissiveness of the PTEN PDZ-binding motif for recognition of specific PDZ domains from MAGI-2, hDlg, and MAST205.

The PTEN C-terminal Tail Contains Specific Recognition Determinants for Distinct PDZ Domains—The PTEN-related protein TPTE lacks a region similar to the PTEN C-terminal tail and does not possess a C-terminal PDZ domain-binding motif (31) (Fig. 3*A*). As a consequence, TPTE does not bind PDZ domains (Fig. 4*A*, *lane 3*). To further investigate the molecular requirements of PTEN for PDZ domain binding, deletion PTEN mutants and chimeras containing distinct domains of PTEN and TPTE were engineered with an N-terminal HA epitope (Fig. 3*A*) and tested for binding. The chimera containing the PTP domain of TPTE followed by the C2 domain and the C-terminal tail of PTEN (TPTE-(215–404)/PTEN-(186–403) (TPTE-PTEN)) bound to PDZ-2/MAGI-2, PDZ-2/hDlg, or PDZ/MAST205, whereas the chimera containing the PTP domain of PTEN followed by the C2 domain of TPTE (PTEN-(1–185)/TPTE-(405–551) (PTEN-TPTE)) did not bind to any of these three PDZ domains (Fig. 4*A*, *lanes 2* and *4*, respectively; and data not shown). Furthermore, a PTEN mutation lacking the C2 domain but retaining the C-terminal tail (PTEN-(Δ189–349) (PTEN ΔC2)) bound to PDZ-2/MAGI-2, PDZ-2/hDlg, and PDZ/MAST205 as PTEN wild type did (Fig. 4*B*, *lane 3*; and data not shown). Most interestingly, chimeras containing the PTP and C2 domains of TPTE, followed by the ITKV or the TKV C-terminal PTEN residues (TPTE-(215–551)/PTEN-(400–403) and TPTE-(215–551)/PTEN-(401–403)) only bound to PDZ/MAST205 (Fig. 4*C*, *lanes 3* and *4*). These results demonstrate that both the PTP and the C2 domains of PTEN are dispensable for PTEN-PDZ domain interaction, and these results suggest the existence of molecular determinants within the PTEN C-terminal tail, in addition to the ITKV C-terminal motif, necessary for recognition of specific PDZ domains. To explore this further, we tested the binding of chimeras containing the PTP and C2 domains of TPTE followed by distinct regions of the PTEN C-terminal tail (TPTE-(215–539)/PTEN-(350–403) (TP2), TPTE-(215–551)/PTEN-(369–403) (TP3), and TPTE-(215–551)/PTEN-(376–503) (TP4); see Fig. 3*A*). As shown, the chimera containing the entire PTEN C-terminal tail (TP2) bound to PDZ-2/MAGI-2, PDZ-2/hDlg, and PDZ/MAST205 (Fig. 4*C*, *lane 6*). However, the chimeras containing portions of the PTEN C-terminal tail (TP3 and TP4) bound to PDZ/MAST205 and PDZ-2/MAGI-2 but not to PDZ/hDlg (Fig. 4*C*, *lanes 7* and *8*). Finally, partial deletions of the PTEN C-terminal tail, which preserved the ITKV PDZ domain-binding motif, were constructed and tested for PDZ domain binding (Fig. 5). The deletion of the PTEN-phosphorylated region (PTEN-(Δ370–385)) did not affect the binding of any of the PDZ domains (Fig. 5*A*, *lane 6*), whereas deletions flanking this region (PTEN-(Δ350–368), PTEN-(Δ387–399), and PTEN-(Δ387–395)) differentially affected the binding of the distinct PDZ domains. PDZ-2/hDlg binding was lost on the PTEN-(Δ350–368) deletion, whereas PDZ-2/MAGI-2 and PDZ/MAST205 binding was maintained (Fig. 5*A*, *lane 5*). On the other hand, the PTEN-(Δ387–399) deletion abrogated the recognition of the three PDZ domains (Fig. 5*A*, *lane 7*). Most interestingly, the deletion PTEN-(Δ387–395) did not abrogate PDZ-2/MAGI-2 or PDZ/MAST205 binding (Fig. 5*A*, *lane 11*), suggesting that the Gln^{396}–His^{397}–Thr^{398}–Gln^{399} PTEN residues are important for specific recognition of these two PDZ domains. Similar results were obtained by co-immunoprecipitation assays using these PTEN deletion mutants and full-length Myc-MAGI-2 (Fig. 5*B*), as well as by two-hybrid protein-protein interaction analysis using the PTEN deletions and PDZ-2/MAGI-2 or PDZ/

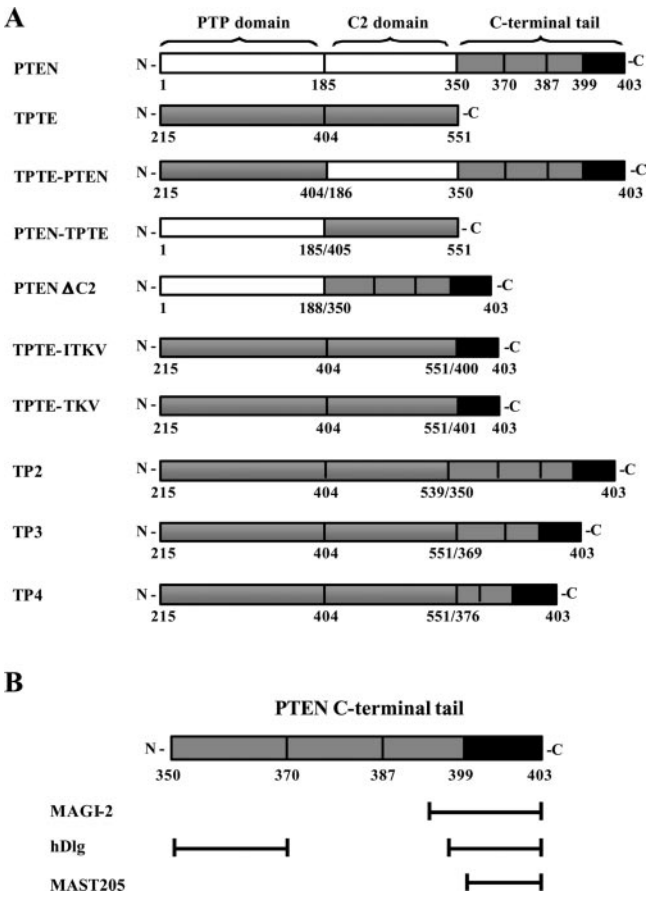

FIG. 3. **Depiction of the regions of PTEN that are important for PDZ domain binding.** *A*, schematic representation of the PTEN/TPTE chimeras and the PTEN ΔC2 deletion mutant used in the study. The distinct domains of PTEN and TPTE are indicated, as well as their amino acid boundaries (numbers below). The *black box* denotes the C-terminal PDZ domain-binding motif of PTEN (*TKV*). TPTE (residues 215–551) is represented without its transmembrane spanning region. *B*, schematic representation of the regions at the PTEN C-terminal tail that are important for binding to the PDZ domains from MAGI-2, hDlg, and MAST205. Amino acid numbering is indicated. The *lines below* the *numbers* indicate the regions that were found to be critical for binding to the distinct PDZ domains, from results in Fig. 4 and Fig. 5.

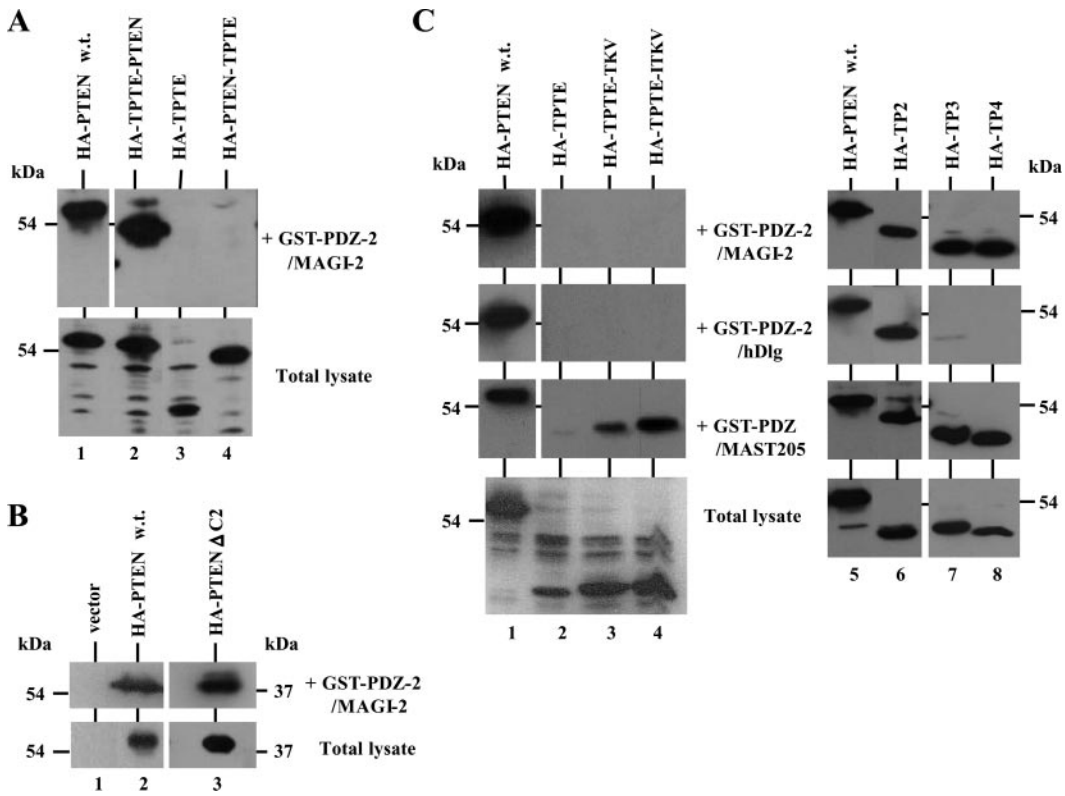

FIG. 4. **The PTP and C2 domains of PTEN are dispensable and the PTEN-C-terminal tail is sufficient for PDZ domain binding.** COS-7 cells were transfected with pRK5 alone (vector), pRK5 HA-PTEN wild type (*w.t.*), pRK5 HA-TPTE, pRK5 HA-TPTE/PTEN chimeras, or pRK5 HA-PTEN ΔC2, as indicated. Equal amounts of cellular lysates (0.3 mg) were subjected to pull-down with equivalent amounts (3 μg) of GST-PDZ-2/MAGI-2 (*A* and *B*) or of GST-PDZ-2/MAGI-2, GST-PDZ-2/hDlg, or GST-PDZ/MAST205 (*C*), as indicated. The presence of HA-PTEN proteins in total lysates (40 μg) and in pull-down pellets was analyzed by immunoblot using anti-HA mAb. The experiments were performed three times with similar results, and a representative experiment is shown.

MAST205 (data not shown). A scheme of the regions at the PTEN C-terminal tail that are necessary for binding to the distinct PDZ domains is shown in Fig. 3*B*. Together, our results support the notion that distinct regions at the C-terminal tail of PTEN differentially contribute to the specific recognition of distinct PDZ domains, and they outline the importance for PDZ domain binding of PTEN residues outside the C-terminal PDZ-binding motif.

Binding of PTEN to PDZ Domains Increases PTEN Protein Stability—The C-terminal region of PTEN is important to maintain PTEN protein stability (13–16, 39), suggesting a possible stabilization of PTEN by binding to PDZ domain-containing proteins. To test this hypothesis, pulse-chase experiments were performed using the single amino acid substitution mutant K402W, whose association with PDZ domains is diminished (see Fig. 2, *lane 12*). COS-7 cells were transfected with HA-PTEN or HA-PTEN K402W, pulsed with [^{35}S]methionine for 4 h, and then chased for 1 and 2 h. Samples were immunoprecipitated with an anti-HA mAb, and HA-PTEN protein bands were quantified in relation with the zero point chase time (Fig. 6*A*). As shown, PTEN wild type displayed a slower rate of degradation than the PTEN K402W mutation, suggesting that PDZ domains from endogenous proteins could bind and stabilize to PTEN. Next, the stability of PTEN was measured upon binding to a PDZ-2/MAGI-2 recombinant protein. For these experiments, HA-PTEN was co-expressed with GST alone or with GST-PDZ-2/MAGI-2, and cells were pulsed with [^{35}S]methionine for 4 h and chased for another 4 h. As shown in Fig. 6*B*, the presence of GST-PDZ-2/MAGI-2, but not of GST alone, diminished the degradation rate of PTEN after the 4-h pulse (79.9% of labeling *versus* 52.7% of labeling; compare *lanes 6* and *7* with *lanes 2* and *3*). Furthermore, the degradation of the HA-PTEN-(1–402) mutant, which does not associate with PDZ-2/MAGI-2 (see Fig. 2, *lane 6*), was not affected by the presence of GST-PDZ-2/MAGI-2 (32.5 *versus* 32.6%; compare *lanes 8* and *9* with *lanes 4* and *5*). Together, these results indicate that specific binding to cognate PDZ domains stabilizes PTEN.

PDZ Domain-mediated Binding to PTEN Facilitates Its Phosphorylation by MAST Kinases—The binding of PTEN to the PDZ domains of several MAST kinases raised the question of whether MAST kinases can phosphorylate PTEN. To explore this possibility, we first tested the ability of the kinase domain (kinase/MAST205) and the kinase plus the PDZ domains of MAST205 (kinase + PDZ/MAST205) to interact physically with PTEN (Fig. 7). COS-7 cells were transfected with plasmids encoding HA kinase/MAST205 or HA kinase + PDZ/MAST205 recombinant proteins, and pull-down assays were performed using GST-PTEN purified from bacteria (Fig. 7*B*). As expected, PTEN associated the kinase + PDZ/MAST205 protein (Fig. 7*B*, *lane 2*). Noticeably, PTEN also interacted, although at a lesser extent, with kinase/MAST205 (Fig. 7*B*, *lane 3*), suggesting that PTEN may serve as a substrate for MAST205. Next, the phosphorylation of PTEN by the kinase domains of MAST205 and related kinases was examined. GST-PTEN was incubated with purified HA kinase/SAST, HA kinase/MAST3, or HA kinase/MAST205, in the presence of [γ-^{32}P]ATP, and the phosphorylated proteins were resolved by SDS-polyacrylamide gels (Fig. 7*C*). GST-PTEN, but not GST alone, was phosphorylated by kinase/SAST, kinase/MAST3, and kinase/MAST205 (Fig. 7*C*, *lanes 2*, *4*, and *6*). To map the region of PTEN phosphorylated by MAST kinases, kinase assays were performed using kinase/MAST205 and GST-PTEN deletion and truncation mutants as the substrates. The trun-

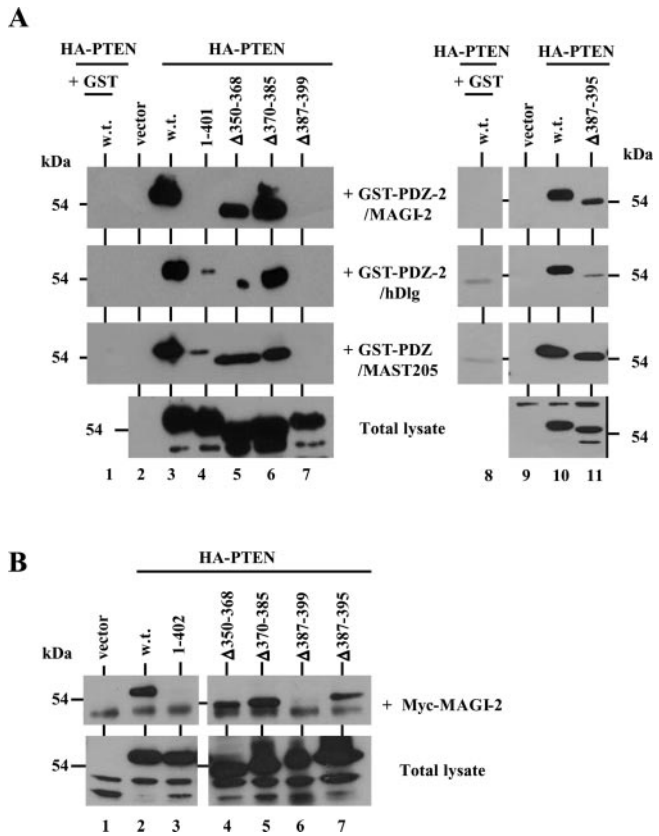

FIG. 5. **The PTEN C-terminal tail possesses specific recognition determinants for distinct PDZ domains.** *A*, COS-7 cells were separately transfected with pRK5 alone (*vector*), pRK5 HA-PTEN wild type (*w.t.*), or pRK5 HA-PTEN deletion mutants. Cell lysates (0.3–0.5 mg) were subjected to GST pull-down assays as in Fig. 1*A*. *B*, COS-7 cells were separately transfected with pRK5 alone (*vector*), pRK5 HA-PTEN wild type (*w.t.*), or HA-PTEN deletion mutations and with pCMV Myc-MAGI-2, as indicated. Equal amounts of pairwise combinations of cellular lysates (0.3 mg) were subjected to co-immunoprecipitation in the presence of the anti-Myc antibody. In all cases, the presence of HA-PTEN proteins in total lysates (40 μg) and in pull-down pellets or immunoprecipitates was analyzed by immunoblot using anti-HA mAb. All experiments were repeated at least three times with similar results, and a representative experiment is shown.

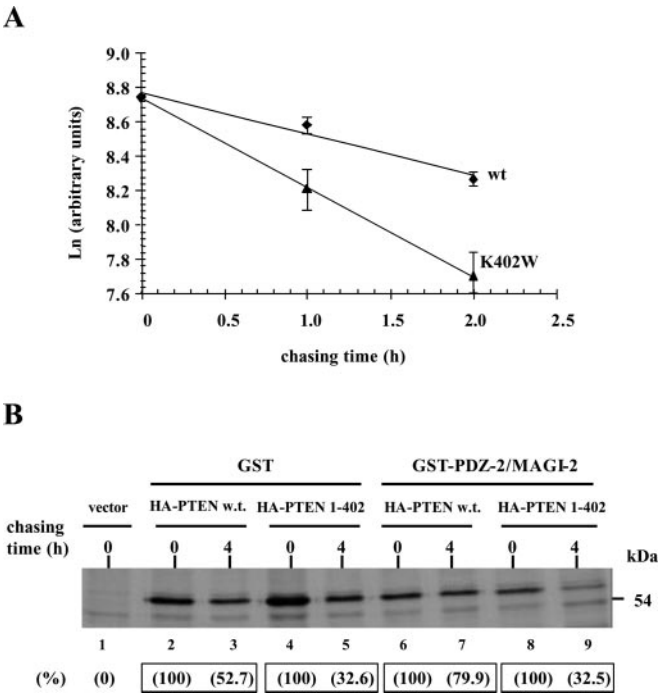

FIG. 6. **PDZ domain binding increases PTEN protein stability.** COS-7 cells were pulse-labeled with [^{35}S]methionine for 4 h and then chased at the indicated times. ^{35}S-Labeled HA-PTEN proteins were immunoprecipitated with the anti-HA mAb, resolved on 10% SDS-polyacrylamide gels, and visualized and/or quantified using a PhosphorImager or by autoradiography. *A*, COS-7 cells were transfected with pRK5 HA-PTEN wild type (*wt*) or K402W mutation, as indicated. Kinetic degradation plots of the mean ± S.D. of two separate experiments are shown. *B*, COS-7 cells were transfected with pRK5 alone (*vector*) (*lane 1*) or pRK5 HA-PTEN wild type (*w.t.*) or 1–402 truncation, together with pRK5 GST (*lanes 2–5*) or pRK5 GST-PDZ-2/MAGI-2 (*lanes 6–9*). Numbers in *parentheses* at the bottom indicate the intensity of the HA-PTEN bands, relative to the intensity at the chasing time 0 (100%). The experiment was performed twice with the same results, and a representative experiment is shown.

cation mutant lacking the PTEN C-terminal domain, GST-PTEN-(1–202), was not phosphorylated by kinase/MAST205, whereas a mutant lacking the C2 domain of PTEN, GST-PTEN ΔC2, was phosphorylated by this kinase (Fig. 7*C*, *lanes 7* and *8*, respectively). Furthermore, a GST fusion protein containing the PTEN C-terminal tail (GST-PTEN-(350–403)) was also phosphorylated by MAST205 (Fig. 7*C*, *lane 10*). Thus, MAST kinases phosphorylate *in vitro* the C-terminal portion of PTEN. We next tested whether binding through its PDZ domain could facilitate the phosphorylation of PTEN by MAST205. In these experiments, kinase/MAST205 or kinase + PDZ/MAST205 was incubated in the presence of GST-PTEN wild type or V403A mutation, and phosphorylation was measured at several time points by *in vitro* kinase assays, as above (Fig. 7*D*). As shown, phosphorylation of PTEN wild type by kinase + PDZ/MAST205 was facilitated, when compared with phosphorylation by kinase/MAST205 or to phosphorylation of the V403A mutation by kinase + PDZ/MAST205. Most interestingly, the kinase + PDZ/MAST205 was also phosphorylated under these conditions, and this phosphorylation was increased in the presence of PTEN wild type. Together, these results demonstrate that the binding of PTEN to the PDZ domain of MAST205 facilitates its phosphorylation by this kinase, and they suggest a regulatory role for PTEN on the phosphorylation of MAST205.

DISCUSSION

The regulation of the subcellular location and/or function of the tumor suppressor PTEN involves its interaction with distinct molecular components of the cell, including membrane lipids and recruiting proteins (13–21). The last three amino acids of the PTEN protein (residues Thr^{401}-Lys^{402}-Val^{403}-COOH; TKV) constitute a C-terminal binding motif for class I PDZ domains. Upon binding to specific PDZ domain containing proteins, PTEN is to be targeted to areas at the inner surface of the plasma membrane where cell signaling events take place (18, 27–30). In this study, we identify the PDZ domains from the MAST205-related kinases, SAST and MAST3, as novel PTEN PDZ-counterpart domains. In addition, by using different PTEN mutations within the PTEN PDZ-binding motif, deletions at the C-terminal tail, and PTEN/TPTE chimeras, we have compared the molecular requirements for the interaction of PTEN with several PDZ domains, namely PDZ-2/MAGI-2, PDZ-2/hDlg, and PDZ/MAST205. First, we have analyzed the importance of the PTEN PDZ-binding motif in the selective interaction with these various PDZ domains. Some mutations at the PTEN PDZ-binding motif, including PTEN-(1–400) and T401A, differentially affected the binding to the distinct PDZ domains. The recognition of the PTEN-(1–400) mutation by PDZ-2/hDlg and PDZ/MAST205 can be explained by the generation of a novel C-terminal PDZ domain-binding motif (Thr^{398}-Gln^{399}-Ile^{400}-COOH; TQI). Previous analysis using phage display and peptide binding revealed a preference for Trp at position −1 of the binding motif for binding to the

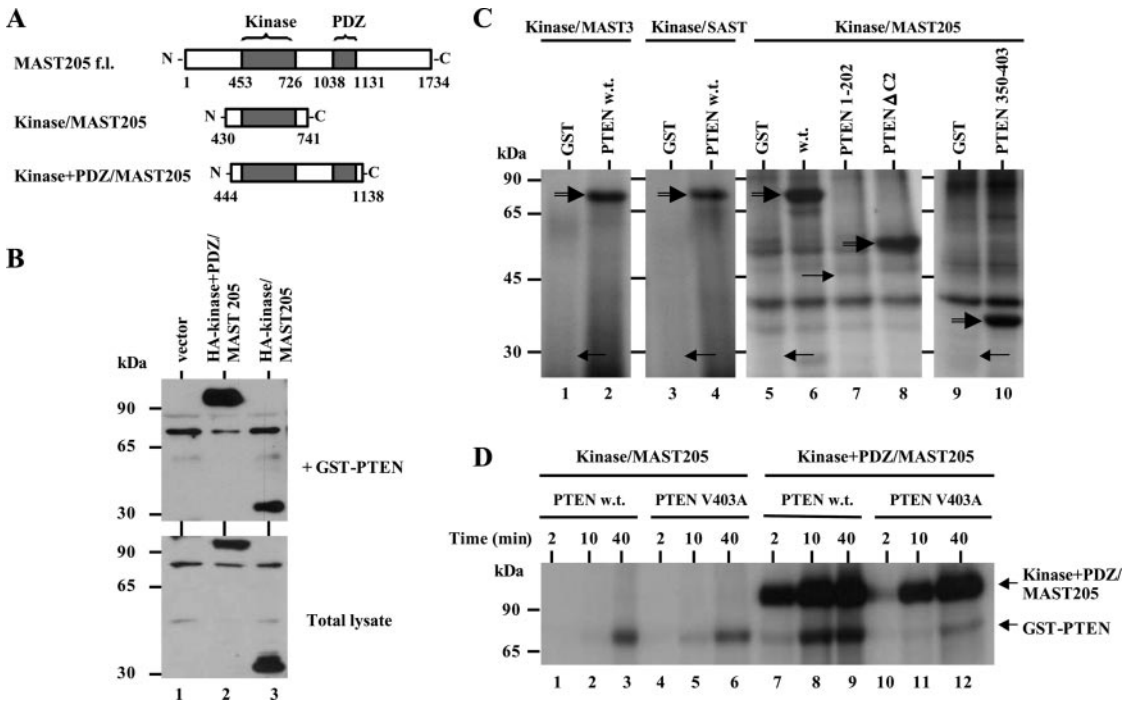

FIG. 7. **PTEN binds to and is phosphorylated by MAST kinases.** *A*, schematic representation of the HA-MAST205 recombinant proteins used. The kinase (residues 453–726) and the PDZ (residues 1038–1131) domains of MAST205 are indicated. *B*, binding of PTEN to the MAST205 kinase domain. COS-7 cells were transfected with pRK5 alone (*vector*), pRK5 HA kinase + PDZ/MAST205 (residues 444–1138), or pRK5 HA kinase/MAST205-(430–741), as indicated. Equal amounts of cellular lysates (0.3 mg) were subjected to pull-down with 3 μg of GST-PTEN. The presence of HA kinase/MAST205 or HA kinase + PDZ/MAST205 proteins in the pull-down pellets was analyzed by immunoblot using anti-HA mAb. *C*, phosphorylation of PTEN by MAST kinases. Lysates from COS-7 cells transfected with the expression plasmids pRK5 HA kinase/MAST3 (residues 366–581) (*lanes 1* and *2*), pRK5 HA kinase/SAST (residues 337–590) (*lanes 3* and *4*), or pRK5 HA kinase/MAST205 (*lanes 5–10*) were immunoprecipitated with the anti-HA mAb, and the immune complex pellets were mixed with GST alone or the distinct GST-PTEN fusion proteins (0.5 μg), as indicated, followed by *vitro* kinase assays in the presence of [γ-^{32}P]ATP. Samples were resolved by 10% SDS-PAGE, followed by autoradiography. *Double-line arrows* indicate the phosphorylated GST-PTEN proteins. *Single-line arrows* indicate the migration of the nonphosphorylated GST or GST-PTEN proteins. Equal amounts of all the protein substrates were used in the assay, as monitored by Coomassie staining (not shown). *D*, phosphorylation of PTEN by MAST205 is favored upon PDZ domain binding. Lysates from COS-7 cells transfected with the expression plasmids pRK5 HA kinase/MAST205 (*lanes 1–6*) or pRK5 HA kinase + PDZ/MAST205 (*lanes 7–12*) were immunoprecipitated with the anti-HA mAb, and the immune complex pellets were mixed with GST-PTEN wild type (*w.t.*) or GST-PTEN V403A (0.5 μg), for 2, 10, or 40 min, as indicated, followed by *vitro* kinase assays in the presence of [γ-^{32}P]ATP. Samples were resolved by 10% SDS-PAGE, followed by autoradiography. All experiments were repeated at least three times with similar results, and a representative experiment is shown.

PDZ-2/MAGI-3 domain (40). However, in our experiments, the substitution of Lys^{402} by Trp (K402W mutation; TKV motif substituted by TWV motif) abrogated binding of the full-length PTEN protein to PDZ-2/MAGI-2, PDZ-2/MAGI-3, PDZ-2/hDlg, and PDZ/MAST205. Thus, differences in PDZ domain recognition can be achieved when peptides and full-length PTEN protein binding are compared, suggesting that molecular requirements for this binding exist within the PTEN protein in addition to its canonical C-terminal PDZ domain-binding motif. Our experiments performed with PTEN/TPTE chimeras and PTEN deletion mutants demonstrate that both the PTP and the C2 domains of PTEN are dispensable for PDZ domain binding. On the other hand, several regions at the PTEN C-terminal tail, including regions flanked by the 387–399 and 350–368 residues, are important for specific recognition of the distinct PDZ domains. In addition, we have shown that binding of PTEN to PDZ domains can be blocked by anti-PTEN mAb that recognize epitopes at the PTEN C terminus (41). Together, these results outline the importance of the integrity of the PTEN C-terminal tail for the selective recognition of PTEN protein regulators.

Experimental evidence indicates that the tumor suppressor activity of PTEN mostly relies on its counteracting activity on the phosphatidylinositol 3-kinase/protein kinase B survival pathway, favoring apoptosis and/or cell cycle arrest (3). We have found that PTEN protein stability is increased upon binding to a cognate PDZ domain. This is in agreement with previous reports (27–30) demonstrating the positive role of PDZ domain binding in PTEN functional activity. In addition, mutations lacking the PTEN C-terminal PDZ domain-binding motif have been found to affect some PTEN activities, such as the inhibition of membrane ruffling and cell spreading, but not others, including the PTEN anti-oncogenic or anti-phosphati-

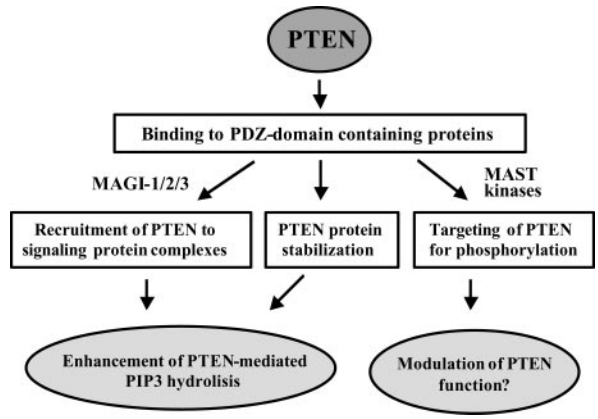

FIG. 8. **Regulation of PTEN function by binding to PDZ domains.** General schematic model that summarizes the involvement of PDZ domain binding in the regulation of PTEN function. The scheme is based on the results reported here and on findings reported by others (18, 27–30). The specific role of hDlg in PTEN function regulation remains unexplored. *PIP3*, phosphatidylinositol 3,4,5-trisphosphate.

dylinositol 3-kinase-dependent signaling activities (42–44). Together, these observations suggest that PDZ domain binding to PTEN may contribute to stabilize PTEN at particular subcellular locations where regulation of its function takes place. In this context, several of the PDZ domain-containing proteins that bind to PTEN, including MAGI-1/2/3 and hDlg, are multi-PDZ domain scaffolding proteins enriched at cell-cell junctions, which are involved in the assembling of multimolecular signaling complexes in the postsynaptic densities of neurons and in tight junctions from epithelial cells (27–30, 33, 34, 45). The Ser/Thr MAST kinases (MAST205, SAST, MAST3, and MAST4) are cytoskeletal associated kinases of unknown function that are also expressed at neuromuscular junctions and postsynaptic densities and associate through their PDZ domains to the PDZ domain of the syntrophins adapter proteins (36–38). Most interestingly, despite the strong sequence conservation between the PDZ domains from MAST3 and MAST4 (75% identity at the amino acid level), only PDZ/MAST3 was found to associate to PTEN, demonstrating that specific PTEN-binding determinants are present in the PTEN cognate PDZ domains. The autophosphorylation of MAST205 has been reported (36). Our results suggest that the MAST205 core region containing the kinase and PDZ domains includes the MAST205 autophosphorylation sites. MAST205 has also been involved in the regulation by the Fcγ receptor of the innate immune response in macrophages, although the identity of its protein substrates remains unknown (46). Our findings on the phosphorylation of PTEN by the kinase domains of MAST kinases suggest that PTEN could be a physiological substrate of MAST205 and related kinases. Moreover, the enhancement of PTEN phosphorylation by MAST205 upon binding through its PDZ domain suggests that PDZ domain recognition could be a specificity mechanism that targets PTEN for phosphorylation by these kinases. Remarkably, the PTEN region phosphorylated by MAST205 was mapped at its C-terminal tail, which has also been found to be phosphorylated by CK2 and to be involved in the binding to other proteins, including growth factor receptors and neutral endopeptidase (47, 48). The region of the PTEN C-terminal tail that is phosphorylated by CK2 (amino acids 370–386; see Ref. 14) was found in our study to be dispensable for binding to PDZ domains, whereas adjacent regions were important for this binding (see above). On the other hand, it has been reported that substitution of some of the PTEN CK2-phosphorylated residues to alanine enhances MAGI-2 binding (18, 49). Phosphorylation of PTEN by MAST205 could thus also affect PTEN binding to protein regulators. In summary, our studies suggest that distinct regions at the PTEN C-terminal tail may act as adjustment elements for specific and efficient PDZ domain recognition, which could be crucial in the control of PTEN protein phosphorylation, stability, and function. A schematic model that summarizes the role of PDZ domain binding in regulation of PTEN function is shown in Fig. 8. The molecular and functional dissection of the interaction of PTEN with its regulators will help to elucidate how the control of PTEN functions may coordinate the adaptive cell responses during cell growth and development.

Acknowledgments—We thank A. Cuenda, E. Peles, P. D. Walden, Y. Takai, Y. Hata, the Kazusa DNA Research Institute, and the UK Medical Research Council geneservice for providing plasmids. We also thank I. Roglá for expert technical assistance.

REFERENCES

1. Eng, C. (2003) *Hum. Mutat.* **22,** 183–198
2. Lee, J.-O., Yang, H., Georgescu, M.-M., Di Cristofano, A., Maehama, T., Shi, Y., Dixon, J. E., Pandolfi, P., and Pavletich, N. P. (1999) *Cell* **99,** 323–334
3. Maehama, T., Taylor, G. S., and Dixon, J. E. (2001) *Annu. Rev. Biochem.* **70,** 247–279
4. Yamada, K. M., and Araki, M. (2001) *J. Cell Sci.* **114,** 2375–2382
5. Leslie, N. R., and Downes, C. P. (2002) *Cell. Signal.* **14,** 285–295
6. Goberdhan, D. C., and Wilson, C. (2003) *Hum. Mol. Genet.* **12,** Suppl. 2, R239–R248
7. Tamura, M., Gu, J., Matsumoto, K., Aota, S., Parsons, R., and Yamada, K. M. (1998) *Science* **280,** 1614–1617
8. Hlobilkova, A., Guldberg, P., Thullberg, M., Zeuthen, J., Lukas, J., and Bartek, J. (2000) *Exp. Cell Res.* **256,** 571–577
9. Weng, L. P., Brown, J. L., and Eng, C. (2001) *Hum. Mol. Genet.* **10,** 599–604
10. Park, M.-J., Kim, M.-S., Park, I.-C., Kang, H.-S., Yoo, H., Park, S. H., Rhee, C. H., Hong, S.-I., and Lee, S.-H. (2002) *Cancer Res.* **62,** 6318–6322
11. Freeman, D. J., Li, A. G., Wei, G., Li, H. H., Kertesz, N., Lesche, R., Whale, A. D., Martinez-Diaz, H., Rozengurt, H., Cardiff, R. D., Liu, X., and Wu, H. (2003) *Cancer Cells* **3,** 117–130
12. Adey, N. B., Huang, L., Ormonde, P. A., Baumgard, M. L., Pero, R., Byreddy, D. V., Tavtigian S. V., and Bartel, P. L. (2000) *Cancer Res.* **60,** 35–37
13. Vazquez, F., Ramaswamy, S., Nakamura, N., and Sellers, W. R. (2000) *Mol. Cell. Biol.* **20,** 8969–8982
14. Torres, J., and Pulido, R. (2001) *J. Biol. Chem.* **276,** 993–998
15. Birle, D., Bottini, N., Williams, S., Huynh, H., deBelle, I., Adamson, E., and Mustelin, T. (2002) *J. Immunol.* **169,** 286–291
16. Torres, J., Rodriguez, J., Myers, M. P., Valiente, M., Graves, J. D., Tonks, N. K., and Pulido, R. (2003) *J. Biol. Chem.* **278,** 30652–30660
17. Okahara, F., Ikawa, H., Kanaho, Y., and Maehama, T. (2004) *J. Biol. Chem.* **279,** 45300–45303
18. Vazquez, F., Grossman, S. R., Takahashi, Y., Rokas, M. V., Nakamura, N., and Sellers, W. R. (2001) *J. Biol. Chem.* **276,** 48627–48630
19. Das, S., Dixon, J. E., and Cho, W. (2003) *Proc. Natl. Acad. Sci. U. S. A.* **100,** 7491–7496
20. Raftopoulou, M., Etienne-Manneville, S., Self, A., Nicholls, S., and Hall, A. (2004) *Science* **303,** 1179–1181
21. Li, Z., Dong, X., Wang, Z., Liu, W., Deng, N., Ding, Y., Tang, L., Hla, T., Zeng, R., Li, L., and Wu, D. (2005) *Nat. Cell Biol.* **7,** 399–404
22. Sheng, M., and Sala, C. (2001) *Annu. Rev. Neurosci.* **24,** 1–29
23. Fan, J.-S., and Zhang, M. (2002) *Neurosignals* **11,** 315–321
24. Hung, A. Y., and Sheng, M. (2002) *J. Biol. Chem.* **277,** 5699–5702
25. Doyle, D. A., Lee, A., Lewis, J., Kim, E., Sheng, M., and MacKinnon, R. (1996) *Cell* **85,** 1067–1076
26. Songyang, Z., Fanning, A. S., Fu, C., Xu, J., Marfatia, S. M., Chisti, A. H., Crompton, A., Chan, A. C., Anderson, J. M., and Cantley, L. C. (1997) *Science* **275,** 73–77
27. Wu, X., Hepner, K., Castelino-Prabhu, S., Do, D., Kaye, M. B., Yuan, X.-J., Wood, J., Ross, C., Sawyers, C. L., and Whang, Y. E. (2000) *Proc. Natl. Acad. Sci. U. S. A.* **97,** 4233–4238
28. Wu, Y., Dowbenko, D., Spencer, S., Laura, R., Lee, J., Gu, Q., and Lasky L. A. (2000) *J. Biol. Chem.* **275,** 21477–21485
29. Kotelevets, L., van Hengel, J., Bruyneel, E., Mareel, M., van Roy, F., and Chastre, E. (2005) *FASEB J.* **19,** 115–117
30. Subauste, M. C., Nalban, P., Adamson, E. D., and Hahn, K. M. (2005) *J. Biol. Chem.* **280,** 5676–5681
31. Chen, H., Rossier, C., Morris, M. A., Scott, H. S., Gos, A., Bairoch, A., and Antonarakis, S. E. (1999) *Hum. Genet.* **105,** 399–409
32. Tapparel, C., Reymond, A., Girardet, C., Guillou, L., Lyle, R., Lamon, C., Hutter, P., and Antonarakis, S. E. (2003) *Gene* (*Amst.*) **24,** 189–199
33. Hirao, K., Hata, Y., Ide, N., Takeuchi, M., Irie, M., Yao, I., Deguchi, M., Toyoda, A., Sudhof, T. C., and Takai, Y. (1998) *J. Biol. Chem.* **273,** 21105–21110
34. Adamsky, K., Arnold, K., Sabanay, H., and Peles, E. (2003) *J. Cell Sci.* **116,** 1279–1289
35. Nakamura, H., Sudo, T., Tsuiki, H., Miyake, H., Morisaki, T., Sasaki, J., Masuko, N., Koci, M., Ushio, Y., and Saya, H. (1998) *FEBS Lett.* **433,** 63–67
36. Walden, P. D., and Cowan, N. J. (1993) *Mol. Cell. Biol.* **13,** 7625–7635
37. Lumeng, C., Phelps, S., Crawford, G. E., Walden, P. D., Barald, K., and Chamberlain, J. S. (1999) *Nat. Neurosci.* **2,** 611–617
38. Caenepeel, S., Charydczak, G., Sudarsanam, S., Hunter, T., and Manning, G. (2004) *Proc. Natl. Acad. Sci. U. S. A.* **101,** 11707–11712
39. Georgescu, M.-M., Kirsch, K. H., Akagi, T., Shishido, T., and Hanafusa, H. (1999) *Proc. Natl. Acad. Sci. U. S. A.* **96,** 10182–10187
40. Fuh, G., Pisabarro, M. T., Li, Y., Quan, C., Lasky, L. A., and Sidhu, S. S. (2000) *J. Biol. Chem.* **275,** 21486–21491
41. Andrés-Pons, A., Valiente, M., Torres, J., Gil, A., Roglá, I., Ripoll, F., Cervera, J., and Pulido, R. (2005) *Cancer Lett.* **223,** 303–312
42. Tolkacheva, T., and Chan, A. M. (2000) *Oncogene* **19,** 680–689
43. Leslie, N. R., Gray, A., Pass, I., Orchiston, E. A., and Downes, C. P. (2000) *Biochem. J.* **346,** 827–833
44. Leslie, N. R., Bennett, D., Gray, A., Pass, I., Hoang-Xuan, K., and Downes, C. P. (2001) *Biochem. J.* **357,** 427–435
45. Marfatia, S. M., Morais Cabral, J. H., Lin, L., Hough, C., Bryant, P. J., Stolz, L., and Chishti, A. H. (1996) *J. Cell Biol.* **135,** 753–766
46. Zhou, H., Xiong, H., Li, H., Plevy, S. E., Walden, P. D., Sassaroli, M., Prestwich, G. D., and Unkeless, J. C. (2004) *J. Immunol.* **172,** 2559–2568
47. Mahimainathan, L., and Choudhury, G. G. (2004) *J. Biol. Chem.* **279,** 15258–15268
48. Sumitomo, M., Iwase, A., Zheng, R., Navarro, D., Kaminetzky, D., Shen, R., Georgescu, M.-M., and Nanus, D. M. (2004) *Cancer Cells* **5,** 67–78
49. Tolkacheva, T., Boddapati, M., Sanfiz, A., Tsuchida, K., Kimmelman, A. C., and Chan, A. M. (2001) *Cancer Res.* **61,** 4985–4989

Binding of PTEN to Specific PDZ Domains Contributes to PTEN Protein Stability and Phosphorylation by Microtubule-associated Serine/Threonine Kinases

Miguel Valiente, Amparo Andrés-Pons, Beatriz Gomar, Josema Torres, Anabel Gil, Caroline Tapparel, Stylianos E. Antonarakis and Rafael Pulido

J. Biol. Chem. 2005, 280:28936-28943.
doi: 10.1074/jbc.M504761200 originally published online June 10, 2005
