

A phosphorylation-dependent intramolecular interaction regulates the membrane association and activity of the tumor suppressor PTEN

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The PI 3-phosphatase PTEN (phosphatase and tensin homologue deleted on chromosome 10), one of the most important tumor suppressors, must associate with the plasma membrane to maintain appropriate steady-state levels of phosphatidylinositol 3,4,5-triphosphate. Yet the mechanism of membrane binding has received little attention and the key determinants that regulate localization, a phosphatidylinositol 4,5-bisphosphate (PIP₂) binding motif and a cluster of phosphorylated C-terminal residues, were not included in the crystal structure. We report that membrane binding requires PIP₂ and show that phosphorylation regulates an intramolecular interaction. A truncated version of the enzyme, PTEN_{1–351}, bound strongly to the membrane, an effect that was reversed by co-expression of the remainder of the molecule, PTEN_{352–403}. The separate fragments associated in vitro, an interaction dependent on phosphorylation of the C-terminal cluster, a portion of the PIP₂ binding motif, integrity of the phosphatase domain, and the CBR3 loop. Our investigation provides direct evidence for a model in which PTEN switches between open and closed states and phosphorylation favors the closed conformation, thereby regulating localization and function. Small molecules targeting these interactions could potentially serve as therapeutic agents in antagonizing Ras or PI3K-driven tumors. The study also stresses the importance of determining the structure of the native enzyme.

iRAP | phosphatase | PI3K | PIP₂ | PIP₃

PTEN (phosphatase and tensin homologue deleted on chromosome 10) is a lipid phosphatase that dephosphorylates phosphatidylinositol-3,4,5-triphosphate (PIP₃) and opposes PI3K signaling (1). This important tumor suppressor is frequently mutated in endometrium, prostate, and brain cancer, and alterations of PTEN activity leads to Cowden and Bannayan-Zonana syndromes in which patients develop benign hamartomas (2–4). Mice homozygous for a deletion of PTEN die in early embryogenesis whereas heterozygotes have a propensity to develop widespread neoplasms (5). Prostate-directed conditional deletions of PTEN result in metastatic prostate cancer (6–8). Neural and astrocyte-specific deletions lead to greatly enlarged brain size and abnormalities in astrocytes and neurons (9). Cultured mammalian cells lacking PTEN proliferate faster, resist apoptosis, and migrate aberrantly (10–12). *Dictyostelium discoideum* cells lacking PTEN have elevated PIP₃ and are severely defective in chemotaxis (13).

Regulation of PTEN activity, localization, and function are controlled by a variety of mechanisms. NOTCH1, by acting through transcription factors CBF-1 and MYC or HES-1, respectively, has been reported to either increase or decrease PTEN expression (14–16). Another level of regulation is post-translational modification. Acetylation by p300/CBP-associated factor blocks PTEN activity (17). PTEN catalytic activity is negatively regulated by reactive oxygen species under oxidative stress (18–20) by disulfide linkage of catalytic cysteine 124 with cysteine 71 (18). E3 ubiquitin ligase NEDD4–1 can mono- and poly-ubiquitinate PTEN (21). The

mono-ubiquitinated protein displays increased translocation to the nucleus and the poly-ubiquitinated form undergoes rapid degradation. Finally, phosphorylation of the C-terminal residues regulate protein stability and function in cells (22). In addition, PTEN is phosphorylated by a series of kinases, including RhoA-associated kinase (23), casein kinase 2, and glycogen synthase 3b (24–26).

Although the main substrate of PTEN is at the plasma membrane, the enzyme is mainly found in cytosol and the nucleus, but a small fraction is dynamically associated with the inner face of the plasma membrane (27). This interaction is critical, as mutations that do not affect catalytic activity against soluble substrates but impair membrane binding, such as deletion of an N-terminal “PIP₂ binding” motif, lead to a null phenotype in cells (27–29). Consistently, addition of PIP₂ to PIP₃-containing vesicles makes them more effective substrates (30). Furthermore, there are tumor-derived mutations that do not reduce catalytic activity in vitro but effectively inactivate PTEN by preventing membrane association (31). In addition to the PIP₂ binding motif, a globular phosphatase domain, and a C2 domain that binds lipid vesicles, human PTEN has a 51-aa C terminus that contains a cluster of phosphorylation sites. This cluster, thought to be the target of casein kinase 2, is an important regulatory region, as a version of PTEN with alanine substitutions of these phosphorylation sites, designated PTEN_{A4}, displays greatly increased membrane association (32). The PIP₂ binding motif and the entire C terminus were in the crystallized protein (33). Thus, the structures and conformations of the enzyme including the regions most critical for membrane association and function in cells are unknown.

Single-molecule imaging studies have shown that the increased steady-state levels of PTEN_{A4} on the membrane results from an increased association rate whereas the lifetimes of PTEN and PTEN_{A4} on the membrane were identical. This behavior is consistent with a model wherein PTEN exists in “closed” and “open” conformations, regulated by phosphorylation, and that the open conformation has a more favorable interaction with the membrane. Here we demonstrate that the C terminus of PTEN directly interacts with the remainder of the molecule in a phosphorylation-dependent manner, as well as plasma membrane binding and activity. Furthermore, we demonstrate that depletion of PIP₂ from the plasma membrane causes a concomitant loss of PTEN binding sites.

Results

As previously shown, the localization of PTEN_{WT}-YFP and PTEN_{A4}-YFP are dramatically different. In HEK293T cells, mem-

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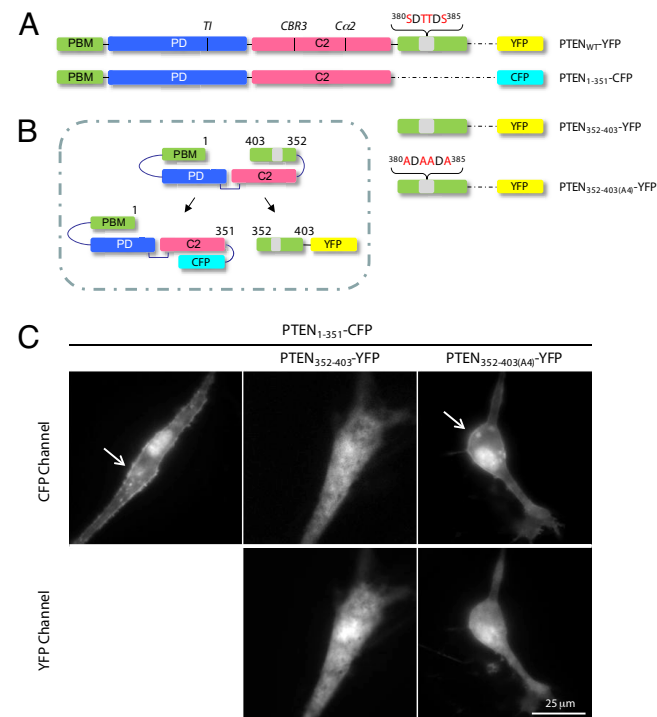


Fig. 3. PTEN₁₋₃₅₁ binds strongly to the plasma membrane and co-expression of the phosphorylated C-terminal domain, PTEN₃₅₂₋₄₀₃, blocks this interaction. (A) Schematic of PTEN_{WT}-YFP, PTEN₁₋₃₅₁-CFP, PTEN₃₅₂₋₄₀₃-YFP, and PTEN₃₅₂₋₄₀₃(A4)-YFP. (B) Depiction of the strategy used to assess the effects of PTEN₃₅₂₋₄₀₃ on membrane binding of PTEN₁₋₃₅₁. (C) U87MG cells were transiently transfected with PTEN₁₋₃₅₁-CFP alone or co-transfected with PTEN₃₅₂₋₄₀₃-YFP or PTEN₃₅₂₋₄₀₃(A4)-YFP for 48 h. Images of cells expressing both constructs were taken using CFP and YFP filter sets, and localization of the respective constructs was assessed.

protein that prevents membrane binding. To test this hypothesis, we removed residues 352 through 403, the flexible region beyond the C2 domain, and fused the remainder of the protein to CFP (Fig. 3 *A* and *B*). Strikingly, when expressed in U87 cells, the association of PTEN₁₋₃₅₁-CFP with the plasma membrane was greatly increased, with a distribution similar to that observed for PTEN_{A4}-YFP (compare Figs. 1*A* and 3*C Upper*). Similar results were obtained in HEK293T cells (data not shown). To further demonstrate that the C-terminal region inhibited the interaction of PTEN_{WT} with the membrane, we co-expressed PTEN₃₅₂₋₄₀₃-YFP and found that it reversed the enhanced membrane binding of PTEN₁₋₃₅₁-CFP (Fig. 3*C* and Fig. S7). Importantly, the C-terminal portion of PTEN_{A4}, PTEN_{352-403(A4)}-YFP, did not reduce the increased PTEN₁₋₃₅₁-CFP membrane binding, indicating that phosphorylation of S380, T382, T383, and/or S385 are critical for the presumed intramolecular interaction. Whether co-expressed with PTEN₁₋₃₅₁-CFP or independently expressed, neither PTEN_{352-403(WT)}-YFP nor PTEN_{352-403(A4)}-YFP showed significant membrane association (Fig. 3*C Lower*). These observations suggest that a phosphorylation-dependent intramolecular interaction modulates PTEN membrane association and activity.

To directly test the phosphorylation-dependent interaction of the C- and N-terminal portions of the protein, we carried out a series of co-expression and co-immunoprecipitation experiments. We observed that PTEN₁₋₃₅₁-CFP-FLAG was able to effectively co-immunoprecipitate PTEN₃₅₂₋₄₀₃-YFP (Fig. 4A). However, it was not able to appreciably co-immunoprecipitate PTEN₃₅₂₋₄₀₃(A4)-YFP. The C-terminal fragment was invariably expressed at much higher levels, but approximately stoichiometric amounts of the N- and C-terminal fragments were bound in the immunoprecipitates

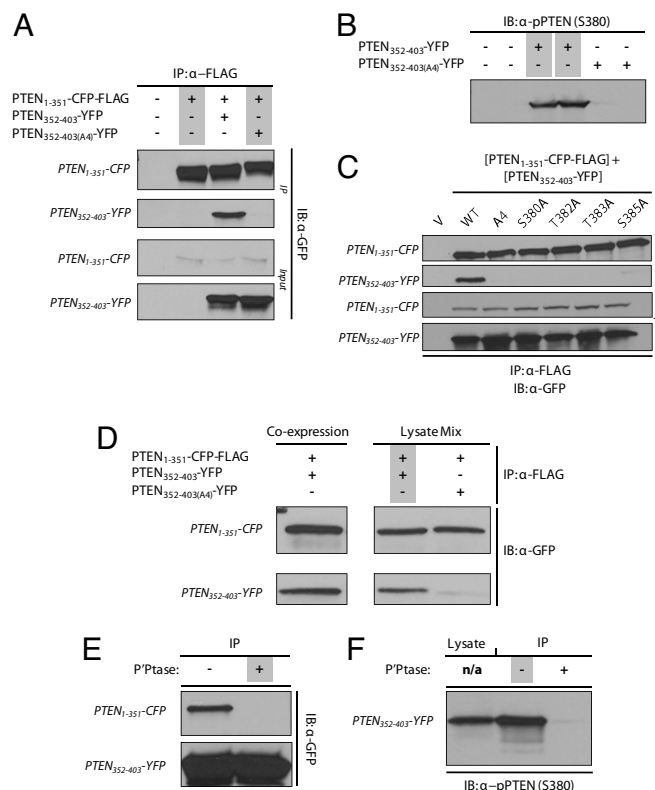


Fig. 4. The phosphorylated C-terminal domain, PTEN₃₅₂₋₄₀₃, interacts specifically with PTEN₁₋₃₅₁. (A) HEK293T cells were transiently co-transfected with PTEN₁₋₃₅₁-CFP-FLAG and either PTEN₃₅₂₋₄₀₃-YFP or PTEN₃₅₂₋₄₀₃(A4)-YFP. Cell lysates were immunoprecipitated and probed with anti-GFP antibody. (B) Lysates from HEK293T cells expressing PTEN₃₅₂₋₄₀₃-YFP or PTEN₃₅₂₋₄₀₃(A4)-YFP were analyzed by Western blot using anti-phosphorylated PTEN (i.e., S380) antibody. (C) Interaction of PTEN₁₋₃₅₁-CFP-FLAG with single alanine substituted PTEN₃₅₂₋₄₀₃-YFP (S380A, T382A, T383A, or S385A) was assessed. (D) Lysates from HEK293T cells expressing either PTEN₁₋₃₅₁-CFP-FLAG or PTEN₃₅₂₋₄₀₃-YFP were mixed in a 1:1 ratio. The mixture was subjected to previously described immunoprecipitation and Western blot analysis using anti-GFP antibody. The data were compared with co-expression experiments. (E) Cell lysate from HEK293T cells transfected with PTEN₃₅₂₋₄₀₃-YFP-FLAG were divided and immunoprecipitated. The immunoprecipitates were treated with and without protein phosphatase, washed, and mixed with cell lysates from HEK293T cells expressing PTEN₁₋₃₅₁-CFP. The mixture was immunoprecipitated and probed using anti-GFP antibody. (F) The cell lysate before first immunoprecipitation was analyzed by Western blot using anti-phospho-PTEN (i.e., S380) antibody and compared with immunoprecipitated fractions with and without phosphatase treatment.

(ratio of C-terminal to N-terminal, 0.79 ± 0.14 ; $n = 5$). To verify that PTEN_{352–403}-YFP was phosphorylated, we expressed it in HEK293T cells and performed immunoblotting with antibody specific for S380. As shown in Fig. 4B, PTEN_{352–403}-YFP was phosphorylated, whereas, as predicted, PTEN_{352–403}(A4)-YFP was not. These observations confirm a phosphorylation-dependent interaction between the N- and C-terminal regions of the protein. We then examined whether alanine substitutions of single phosphorylation sites would disrupt the interaction. PTEN_{1–351}-CFP-FLAG was co-expressed with PTEN_{352–403}-YFP containing S380A, T382A, T383A, or S385A substitution, and immunoprecipitated using anti-FLAG affinity beads. It was observed that substitution of any single phosphorylated residue in the cluster resulted in a loss of affinity for PTEN_{1–351}-CFP (Fig. 4C), suggesting all of the sites are required for optimal interaction.

We next tested whether the interaction of PTEN₁₋₃₅₁ with PTEN₃₅₂₋₄₀₃ required co-translation or whether the mature

proteins could associate. We performed a series of lysate mixing experiments to test the interaction and to determine whether it is phosphorylation-dependent. PTEN₁₋₃₅₁-CFP-FLAG, PTEN₃₅₂₋₄₀₃-YFP, or PTEN₃₅₂₋₄₀₃(A4)-YFP were separately expressed in HEK293T cells. The appropriate lysates were mixed and the samples were then immunoprecipitated using anti-FLAG affinity beads. Fig. 4D shows that PTEN₁₋₃₅₁-CFP interacted selectively with PTEN₃₅₂₋₄₀₃ but not with PTEN₃₅₂₋₄₀₃(A4). Furthermore, the ratios of PTEN₃₅₂₋₄₀₃-YFP co-immunoprecipitated with PTEN₁₋₃₅₁-CFP-FLAG were similar whether they were co-expressed or mixed after lysis. Thus, the interaction does not require co-translation and can occur at dilutions of more than 500 fold compared with the cellular volume, indicating a specific, high-affinity binding reaction.

This approach allowed us to directly show that the intramolecular interaction of PTEN₁₋₃₅₁ and PTEN₃₅₂₋₄₀₃ is phosphorylation-dependent. In a similar lysate mixing experiment, we separately expressed PTEN₃₅₂₋₄₀₃-YFP-FLAG and PTEN₁₋₃₅₁-CFP in HEK293T cells. The PTEN₃₅₂₋₄₀₃-FLAG was immunoprecipitated from the lysate and treated with λ -protein phosphatase, while a parallel sample was similarly treated in the absence of protein phosphatase. The samples were washed, lysate containing PTEN₁₋₃₅₁-CFP was added, the reaction was allowed to proceed, and the beads were collected again. As shown in Fig. 4E, the phosphatase treatment resulted in a nearly complete loss of the interaction. Consistently, Western blot analysis of PTEN₃₅₂₋₄₀₃-YFP-FLAG treated with λ -phosphatase verified the elimination of S380 phosphorylation (Fig. 4F). These observations confirm the findings from the alanine substitutions and definitively show that the interaction can be regulated by reversible phosphorylation.

To show that there is a phosphorylation-dependent intramolecular interaction within the native PTEN molecule, we designed a series of experiments illustrated in Fig. S3A. We envision that phosphorylation of the C-terminal region promotes a “closed” conformation that would be expected to prevent interaction of an exogenous phosphorylated C-terminal fragment. Conversely, PTEN_{A4} is expected to be in an “open” conformation in which the endogenous C-terminal portion would not interfere with its exogenous counterpart (Fig. S3A). These possibilities were assessed by comparing the interaction of PTEN₃₅₂₋₄₀₃-YFP-FLAG with either PTEN_{WT}-YFP or PTEN_{A4}-YFP. As shown in Fig. S3B, PTEN_{A4}-YFP showed significantly greater association with PTEN₃₅₂₋₄₀₃-YFP-FLAG than did PTEN_{WT}-YFP. Also, PTEN_{A4}-YFP association with PTEN₃₅₂₋₄₀₃-YFP was comparable to that of PTEN₁₋₃₅₁-CFP. All of these observations are consistent with a phosphorylation-dependent intramolecular interaction with the native PTEN molecule.

We next investigated the determinants involved in the binding of PTEN₃₅₂₋₄₀₃ to PTEN₁₋₃₅₁. As shown earlier, PTEN_{C124S,A4} binding to the membrane requires PIP₂, and previous studies had indicated that binding is mediated by the N-terminal PIP₂ binding motif. We set out to determine whether the C-terminal region interacted with this critical amino-terminal region. We removed the first 10 N-terminal amino acids from PTEN₁₋₃₅₁, which are required for membrane binding, and assessed the ability of PTEN₁₁₋₃₅₁-CFP-FLAG to effectively immunoprecipitate PTEN₃₅₂₋₄₀₃-YFP, as previously described. As shown in Fig. 5A, the N-terminal truncation had no effect on the ability of PTEN₁₁₋₃₅₁-CFP-FLAG to interact with PTEN₃₅₂₋₄₀₃-YFP. However, in separate experiments, deletion of the first 15 aa, the triple mutation K13R, R14A, and R15A, or the single substitution K13A, prevented the interaction of FLAG-PTEN₁₋₃₅₃ with PTEN₃₅₄₋₄₀₃-GFP or FLAG-PTEN_{A4} with PTEN₃₅₄₋₄₀₃-GFP (Fig. S4A and B). Thus, the entire N-terminal PIP₂-binding motif is necessary for membrane association, whereas only the most C-terminal-charged residues are needed for the intramolecular interaction.

We then explored whether the phosphorylated cluster might interact with the rest of the protein by docking near the catalytic

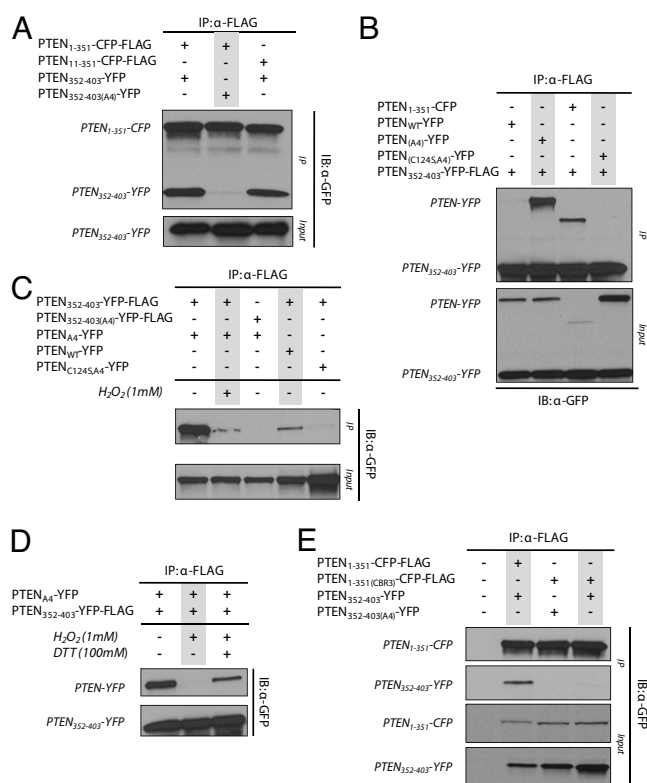


Fig. 5. Evaluation of binding determinants of PTEN₁₋₃₅₁ and PTEN₃₅₂₋₄₀₃. (A) Interaction of the C-terminal domain of PTEN with its N-terminal tail was assessed by co-expression and co-immunoprecipitation of PTEN₁₁₋₃₅₁-CFP-FLAG with PTEN₃₅₂₋₄₀₃-YFP. (B) The interaction of catalytically inactive PTEN with its C-terminal domain was assessed by co-expression of PTEN_{C124S,A4}-YFP with PTEN₃₅₂₋₄₀₃-YFP-FLAG. Lysates were immunoprecipitated and analyzed by Western blot. PTEN_{C124S,A4}-YFP association with PTEN₃₅₂₋₄₀₃-YFP-FLAG was compared with PTEN₃₅₂₋₄₀₃-YFP-FLAG affinity for PTEN_{WT}-YFP, PTEN_{A4}-YFP, and PTEN₁₋₃₅₁-CFP. (C) HEK293T cells were co-transfected with the indicated constructs. Cells expressing PTEN₃₅₂₋₄₀₃-YFP-FLAG and PTEN_{A4}-YFP were pre-treated with 1 mM H₂O₂ for 5 min and immunoprecipitated. Relative expression of full-length PTEN variants were also analyzed by Western blot using anti-GFP antibody. (D) HEK293T cells co-expressing PTEN_{A4}-YFP and PTEN₃₅₂₋₄₀₃-YFP-FLAG were treated with 1 mM H₂O₂ for 5 min before lysis. Equal volumes of the lysate were subjected to immunoprecipitation with anti-FLAG affinity beads with and without the presence of 100 mM DTT. (E) To evaluate the possible interaction of PTEN₃₅₂₋₄₀₃-YFP with the CBR3 loop of the C2 domain, lysines K260, K263, K266, K267, and K269 were alanine substituted to generate PTEN₁₁₋₃₅₁(CBR3)-CFP-FLAG, then co-expressed with PTEN₃₅₂₋₄₀₃-YFP in HEK293T cells and analyzed by Western blot using anti-GFP antibody. Results were compared with PTEN₁₁₋₃₅₁-CFP-FLAG co-expression with PTEN₃₅₂₋₄₀₃-YFP and PTEN₁₁₋₃₅₁(CBR3)-CFP-FLAG co-expression with PTEN₃₅₂₋₄₀₃(A4)-YFP.

pocket of the enzyme. Initial evidence of this possibility arose serendipitously when we used the catalytically dead version of PTEN, PTEN_{C124S,A4}. We observed that PTEN_{C124S,A4}-YFP did not interact with PTEN₃₅₂₋₄₀₃-YFP-FLAG (Fig. 5B). To further explore this phenomenon, we exploited the observations of Lee *et al.* (18, 20). In studies investigating the effects of radical oxygen species on PTEN activity, they discovered that H₂O₂ can reversibly inhibit PTEN activity by covalently linking Cys-71 to Cys-124. Cells co-expressing PTEN₃₅₂₋₄₀₃-CFP-FLAG and PTEN_{A4}-YFP were treated with 1 mM H₂O₂. We found that, following H₂O₂ pre-treatment, PTEN_{A4} displayed notably lower binding to the exogenous C-terminal region (Fig. 5C). This inhibition could be partially reversed by inclusion of 100 mM DTT in the immunoprecipitation buffer (Fig. 5D). Furthermore, in separate experiments, mutation of charged residues at the start of the TI loop, R161A, K163A, and K164A prevented the interaction of

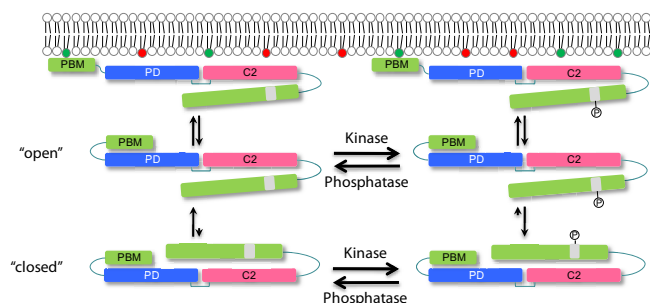


Fig. 6. Phosphorylation-dependent switch between open and closed conformations regulates rate of association of PTEN with the membrane. PTEN molecules can exist in open or closed conformations, and open molecules bind to the membrane. Membrane shows scattered molecules of PIP₂ (green), which are required for binding, and PIP₃ (red), which are PTEN substrates. Phosphorylation of the C-terminal cluster tilts the equilibrium toward the closed conformation, whereas most dephosphorylated molecules are in the open conformation. Alanine substitution of the C-terminal cluster or removal of the C-terminal region locks all of the molecules in an open conformation, promoting membrane association.

FLAG-PTEN_{1–353} with PTEN_{354–403}-GFP (Fig. S4A). These observations indicate that the phosphatase domain, particularly access to the catalytic pocket, is required for the interaction with the C-terminal region.

We next examined the possible role of the CBR3 loop and the Ca2 region and its adjacent loop of the C2 domain in binding of PTEN_{1–351} to PTEN_{352–403}. We reasoned that, as the CBR3 is required for PTEN membrane association, the C-terminal region might regulate CBR3 membrane accessibility. We disrupted the CBR3 loop by alanine substitution of lysine residues K260, K263, K266, K267, and K269. PTEN_{1–351}(CBR3)-CFP-FLAG was co-expressed with PTEN_{352–403}-YFP and immunoprecipitated with anti-FLAG affinity beads. Following Western blot analysis, we observed that these mutations of the CBR3 loop completely disrupted the association of the C-terminal fragment with the rest of the protein (Fig. 5E). In a similar experiment, substitutions of CBR3 loop residues K263A, M264A, L265G, K266A, K267A, and K269A also disrupted the association. Conversely, alanine substitution of Ca2 and its adjacent loop had no effect (Fig. S4A). The observations suggest that the interaction of the C-terminal region with the remainder of the protein is in part dependent on intramolecular interactions with its C2 domain.

Discussion

The interaction of PTEN with the membrane requires a short N-terminal PIP₂ binding motif and is negatively regulated by phosphorylation of a cluster of residues in the C-terminal region of the protein. In this report we showed that indeed PIP₂ is part of the binding site and that PTEN activity in cells is correlated with the level of membrane association. Furthermore, we have found that the negative regulation is caused by an intramolecular interaction between the phosphorylated C-terminal region and the phosphatase and the C2 domains. Our observations are consistent with a model in which PTEN switches between open and closed states and phosphorylation of the C-terminal cluster favors the closed conformation (Fig. 6). First, we demonstrated that removal of the C-terminal region enhances PTEN membrane localization in a variety of cell types, and that co-expression of a phosphorylatable C-terminal fragment blocks membrane binding. Second, we confirmed the suggested intramolecular interaction between the N- and C-terminal regions by showing that two separate fragments, PTEN_{1–351} and PTEN_{352–403}, respectively, could be co-immunoprecipitated whether they are co-expressed or expressed separately and mixed. Eliminating phosphorylation by alanine substitution or phosphatase treatment of the C-terminal fragment prevented the

association. Furthermore, perturbations of the catalytic pocket or mutation of the CBR3 loop in the C2 domain prevented the interaction of the two fragments. Removal of the N-terminal PIP₂ binding motif did not. Third, substitution of the phosphorylated cluster, in context of the full-length protein, greatly increased access for exogenous phosphorylated C-terminal fragment.

We propose that there is a steady-state distribution between open and closed conformations of PTEN (Fig. 6). Phosphorylated molecules are found predominantly in the closed conformation, whereas un-phosphorylated molecules are found predominantly in the open conformation. Open molecules encountering the membrane bind transiently, whereas the closed molecules fail to associate. Therefore, only a small fraction of phosphorylated WT PTEN is associated with membrane at steady state. Substitution of the phosphorylated cluster would be expected to shift the distribution toward the open conformation and thereby increase the rate of association with the membrane. Consistently, previous studies have shown that non-phosphorylatable PTEN has a more rapid rate of association with the membrane but its lifetime on the membrane is identical to WT (27). Similarly, removal of the C-terminal region would be expected to render PTEN in the open conformation. As we have shown here, this manipulation greatly increases association with the membrane and co-expression of the phosphorylated C-terminal region closes the molecule and reverses this effect. We think it is unlikely that the phosphorylation/dephosphorylation events are directly linked to each cycle of binding. Both PTEN_{WT} and PTEN_{A4} dissociate at identical rates, yet clearly PTEN_{A4} cannot receive a phosphate as it dissociates. We assume that a similar situation holds for PTEN_{1–351}, although we have not directly measured its lifetime. Nevertheless, future work is needed to determine the rates of phosphate turnover and compare them with binding lifetime.

Our studies extend previous work showing that the conserved PIP₂ binding motif on the N terminal of PTEN is required for membrane association and that, in mixed micelles, inclusion of PIP₂ enhances phosphatase activity against PIP₃ (30). The latter effect was abolished by the tumor-derived mutation K13E within the PIP₂ binding motif. We show here that rapid depletion of PIP₂ from the plasma membrane triggered by forced recruitment of Inp54p causes PTEN_{C124S,A4} to immediately re-localize to the cytosol. This shows that PIP₂ comprises one element of the binding site for PTEN. Taken together, these observations suggest an association of the PIP₂ binding motif with PIP₂. Interestingly, only a portion of the PIP₂ binding motif was required for the phosphorylation-dependent intramolecular interaction between the C-terminal region and the remainder of the protein.

The transition between open and closed states favored by phosphorylation depends on direct association of C-terminal region with the phosphatase and C2 domains. Within the phosphatase domain, substitution of the catalytic cysteine by serine (i.e., C124S) completely prevented the interaction. Cross-linking of the C124 to C71 by oxidation with H₂O₂ also blocked the intramolecular interaction, and this effect was reversed by reduction with DTT. Based on our model (Fig. 6), these perturbations would be expected to open the molecule and increase membrane binding. Indeed, both human and *Dictyostelium* versions of PTEN_{C124S} show greater membrane association than their WT counterparts. In the human version, this effect appears to be partially additive, as the combined mutation PTEN_{C124S,A4} displays higher membrane association compared with PTEN_{C124S} or PTEN_{A4}. Further studies are needed to determine if the perturbations of the catalytic pocket inhibit C-terminal interaction by conformational changes within the phosphatase domain or loss of protein phosphatase activity. In addition to the phosphatase domain, the integrity of the C2 domain is required as mutations of the CBR3 loop also prevented the intramolecular interaction. Our studies open the way for determination of the exact docking regions by biophysical methods.

During the course of our studies, Odorizola *et al.* presented evidence for an intramolecular interaction within the PTEN mol-

