Actin dynamics is rapidly regulated by the PTEN and PIP2 signaling pathways leading to myocyte hypertrophy

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Li J, Tanhehco EJ, Russell B. Actin dynamics is rapidly regulated by the PTEN and PIP2 signaling pathways leading to myocyte hypertrophy. Am J Physiol Heart Circ Physiol 307: H1618–H1625, 2014. First published September 26, 2014; doi:10.1152/ajpheart.00393.2014.—Mature cardiac myocytes are terminally differentiated, and the heart has limited capacity to replace lost myocytes. Thus adaptation of myocyte size plays an important role in the determination of cardiac function. The hypothesis tested is that regulation of the dynamic exchange of actin leads to cardiac hypertrophy. ANG II was used as a hypertrophic stimulant in mouse heart and neonatal rat ventricular myocytes (NRVMs) in culture for assessment of a mechanism for regulation of actin dynamics by phosphatidylinositol 4,5-bisphosphate (PIP2). Actin dynamics in NRVMs rapidly increased in a PIP2-dependent manner, measured by imaging and fluorescence recovery after photobleaching (FRAP). A significant increase in PIP2 levels was found by immunoblotting in both adult mouse heart tissue and cultured NRVMs. Inhibition of phosphatase and tensin homolog (PTEN) in NRVMs markedly blunted ANG II-induced increases in actin dynamics, the PIP2 level, and cell size. Furthermore, PTEN activity was dramatically upregulated in ANG II-treated NRVMs but downregulated when PTEN inhibitors were used. The time course of the rise in the PIP2 level was inversely related to the fall in the PIP3 level, which was significant by 10 min, suggesting a crucial initial step for PTEN for the cellular responses to ANG II. In conclusion, PTEN and PIP2 signaling may play an important role in myocyte hypertrophy by the regulation of actin filament dynamics, which is induced by ANG II stimulation.

The addition of sarcomeric units to cardiomyocytes requires filaments to be added for cardiac hypertrophy. Live imaging of actin in striated muscle has revealed that actin subunits in the thin filaments are dynamically exchanged. One of the regulators for actin dynamics is the actin capping protein Z (CapZ), and malfunction of CapZ resulted in disorganization of myofibril structures or disease. Both enhancers and stabilizers of actin dynamics are important for myocyte hypertrophy. However, the mechanisms for actin filament addition are not yet well understood.

PIP2 is a lipid messenger in its own right, instead of simply being a precursor of messengers. Direct interaction of PIP2 with the actin accessory proteins profilin and gelsolin promoted or inhibited assembly of F-actin filaments. Recently, our group showed that PIP2 binding to CapZβ1 altered sarcomeric actin dynamics in cultured cardiomyocytes. Many PIP2 effector proteins function in other organelles, for example in focal adhesion formation, vesicle trafficking via integrin, or E-cadherin, and at sites of mRNA processing in the nucleus. PIP2 was localized to the Z-disc, which suggested its role in sarcomeric organization. Furthermore, PIP2 is implicated in ANG II-induced cardiac hypertrophy in an animal model.

The phosphatase and tensin homolog (PTEN) has numerous roles but was originally established as one of the most frequently mutated tumor suppressor genes in human cancer. PTEN is a 3′-lipid phosphatase, which comprises an NH2-terminal phosphatase domain, an NH2-terminal PIP2-binding polybasic tail, a C2 domain, and a COOH-terminal tail region that contains multiple phosphorylation sites. Much is known about PTEN chemistry and its action for the control of cell number. However, less is understood about cell size regulation even though PTEN is widely expressed in cardiomyocytes. The mechanisms involving PTEN in cardiac hypertrophy are controversial. The inactivation of PTEN increased cell size and growth by amplifying PI3K signaling, whereas overexpression of PTEN inhibited PI3K signaling, resulting in decreased cell size. However, a recent study identified that the loss of PTEN could prevent the development of maladaptive ventricular remodeling in response to pressure overload but not in response to ANG II, suggesting that additional complex regulatory pathways may exist.

The main physiological substrate of PTEN is membrane-bound PIP3, and PTEN is activated when recruited to the plasma membrane. PTEN converted the lipid second messenger phosphatidylinositol 3,4,5-trisphosphate (PIP3) to PIP2, as a negative regulator of phosphoinositide 3-kinase (PI3K) signaling. Thus the balance between PTEN and PI3K controls the basal levels of PIP3 in the plasma membrane, which in turn regulates cell survival and proliferation. Therefore, the activity and intracellular distribution of PTEN after treatment with the ANG II stimulus were measured in this study to test the hypothesis that PTEN plays a key role via PIP2 in regulating actin dynamics in cardiomyocytes. Our findings show that stimulation by ANG II increased the activity of PTEN, the level of PIP2, and dynamics of actin filament, resulting in increased cell size. Therefore, PTEN and PIP2 signaling may play an important role in myocyte hypertrophy by the regulation of actin filament dynamics, which is induced by ANG II stimulation.

MATERIALS AND METHODS

Ethical approval. All experiments were conducted following National Institutes of Health guidelines. All experiments were approved by the University Committee on the Use and Care of Animals at the University of Illinois at Chicago and the Biologic Resources Laboratory. All euthanasia was performed following the recommendations of the University Committee on the Use and Care of Animals and the
Biologic Resources Laboratory and the Committee for the Humane Use of Animals.

**Animals.** Eight 8-wk-old C57B6J mice were randomly divided into two groups of four animals. ANG II (Sigma-Aldrich) was administered with osmotic mini-pumps (Alzet model 1002, 1 μg·g⁻¹·day⁻¹) for 2 wk. In the control group, mice received vehicle (saline solution) for 2 wk. The dosage of ANG II was chosen from previous studies (20), which induced left ventricle hypertrophy in mice as confirmed by heart and weight measurements.

**NRVM culture.** Hearts were removed and cells isolated from 1- to 2-day-old Sprague-Dawley rats with collagenase type II (Worthington, Lakewood, NJ) as previously described (3). NRVMs were resuspended, filtered through a metal sieve to remove large material, and plated at high density (1,000 cells/mm²) in PC-1 medium (Lonza Group on fibronectin-coated (25 μg/ml) 6-well plates (200,000 cells/cm²). Cells were left undisturbed for 24 h in a 5% CO₂ incubator. Unattached cells were removed by aspiration, and PC-1 media was replenished. Myocytes were incubated for another 24 h for beating to be reestablished before use. ANG II (1 μM; Catalog No. 091M5065; Sigma-Aldrich), neomycin (500 μM; Catalog No. N6386; Sigma-Aldrich), and PTEN pharmacological inhibitor dipotassium bisperoxo oxovanadate V (bpV) (1 μM; Catalog No. ALX-270-206; Enzo Life Sciences, Farmingdale, NY) and SF1670 (200 nM; Catalog No. B-0350; Echelon Biosciences, Salt Lake City, UT) were used in NRVMs for all figures. A new ANG II control group was used in every experiment, and responses were tested simultaneously.

**Immunostaining and microscopy.** NRVMs were washed with PBS, fixed with 4% paraformaldehyde (Sigma-Aldrich) for 10 min, placed in cold 70% ethanol, and stored at −20°C until immunostaining. Primary anti-α-actinin antibody (Catalog No. ab9465; mouse IgG; Abcam, Cambridge, MA) was diluted (1:200) in 1% BSA in PBS (with 0.1% Triton X-100) and allowed to incubate on a shaker table at 4°C overnight. Secondary antibody (Catalog No. A-21202, Alexa Fluor 488 Goat anti-mouse IgG; Invitrogen, Grand Island, NY) was diluted at a ratio of 1:200 in 1% BSA in PBS and incubated for 1 h at 25°C. α-Actinin-positive mature cardiomyocytes were observed by Zeiss confocal microscopy. Cell surface areas were measured by ImageJ software. In each case, three independent experiments were performed, median values were calculated, and 20 cells from each condition were randomly chosen. These values were then used to calculate mean cell areas.

**Dot blots for PIP₂ and PIP₃ levels.** NRVMs were stimulated with ANG II or inhibited with neomycin or bpV or SF1670 for the indicated times from 5 to 60 min. Whole cell lysates were then extracted from NRVMs in each experimental condition, spotted onto nitrocellulose membranes (Bio-Rad Laboratories, Hercules, CA). These were probed with PIP₂ or PIP₃ antibody (Catalog No. ab2335, mouse IgG; Abcam, Cambridge, MA; PIP₃ antibody: Catalog No. Z-P345b, mouse IgG; Echelon Biosciences) at a 1:500 dilution and detected using a horseradish peroxidase conjugated secondary antibody (Catalog No. 7076, anti-mouse, HRP; Cell Signaling Technology, Boston, MA) and ECL. Experiments were repeated at least three times.

**Subcellular fractionation and localization of PTEN.** For subcellular fractionation of myocytes, the Calbiochem ProteoExtract Subcellular Proteome Extraction Kit was used (Catalog No. 539790; EMD Millipore, Billerica, MA), following a previously described detergent-based protocol (2). Cellular proteins were sequentially extracted into four compartments: cytosolic, membrane/organelles, nuclei, and cytoskeleton. In this study, only cytosol and membrane were needed. Digitonin-EDTA is used to remove the cytosol. Triton-EDTA is used to remove the membrane-organelle fraction. Cells were briefly washed three times in PBS between each extraction fraction to prevent cross-contamination. After each fraction, cells were observed by microscopy to ensure that they were still attached to the dish. Cell integrity is maintained throughout the fractionation process. The accuracy of the fractionation method was verified with antibodies to well-documented subcellular distribution markers [heat shock protein (Hsp)70 for cytosol, β-integrin for membrane; anti-Hsp70 from Catalog No. sc-24, Santa Cruz Biotechnology, Dallas, TX; anti-β-integrin from Catalog No. MAB1900, EMD Millipore]. Experiments were repeated at least three times.

**PTEN lipid phosphatase activity.** For the measurement of in vitro PTEN lipid phosphatase activity, the ELISA phosphatase assay kit (Echelon Biosciences) was used according to the manufacturer’s instructions. Briefly, 500 μg of cell lysate was subjected to PTEN immunoprecipitation by the addition of 4 μl anti-PTEN mAb (Catalog No. 04-035; EMD Millipore), and the immunocomplex formed was captured by incubation with 20 μl protein A/G beads with gentle rotation at 4°C overnight. The beads were then washed twice in lysis buffer and once in enzyme reaction buffer (ERB) containing (in mM) 50 Tris-HCl (pH 8.0), 50 NaCl, 10 DTT, and 10 MgCl₂ and distributed in triplicates of 30 μl in a 96-well flat-bottom plate (Echelon). The reaction was initiated by adding 30 μl of ERB containing the substrate dioctanoyl phosphatidylinositol3,4,5-trisphosphate (PIP₃-DiC₈) (P-3908; Echelon) to 8 μM final concentration. After 1 h at 37°C, the reaction was stopped by 60 μl ERB. An additional detector and stop solution were added for 30 min, and then the absorbance was read at 450 nm after 30 min. A PIP₃-only blank was used in parallel to correct for potential nonspecific phosphate release. The remaining beads were used for SDS-PAGE, Western blotting, and densitometric quantification to confirm that equivalent amounts of PTEN were immunoprecipitated from all samples. A standard curve was made by using the phosphate solution provided with the kit. The PTEN activity was expressed as of control group.

**Fluorescence recovery after photobleaching for actin dynamics.** Recently, several microscopic techniques, such as fluorescence recovery after photobleaching (FRAP) (31) have yielded qualitative and quantitative information about the processes that regulate actin polymerization in living myocytes. The methods and analysis for FRAP of actin-GFP were described by our laboratory (17). In the present study, five myocytes were analyzed per culture and at least three separate cultures were studied per experimental condition.

**Statistics.** Data are presented as means ± SE. Statistical significance was determined by one-way ANOVA. Significance was taken as P < 0.05.

**RESULTS**

**Increased actin dynamics and cardiomyocyte hypertrophy induced by ang II are dependent on the PIP₂ pathway.** The FRAP experiments revealed differences after ANG II treatment (Fig. 1). After 1 h of ANG II treatment, the actin-GFP had a faster dynamic protein exchange in ANG II-treated myocytes than the vehicle group (12.30 ± 1.62 vs. 7.70 ± 1.23, ×10⁻⁴ s⁻¹, P < 0.05; Fig. 1, A and B and Table 1). In NRVMs stimulated by ANG II and the PIP₂ scavenger neomycin, the increased dynamics of actin-GFP were markedly reduced compared with ANG II treatment alone (7.28 ± 1.19 vs. 12.30 ± 1.62, ×10⁻⁴ s⁻¹, P < 0.05; Table 1), demonstrating that dynamic exchange of actin-GFP is dependent on the PIP₂ pathway after ANG II treatment.

To determine whether PIP₂ is involved in cardiac hypertrophy, NRVMs were treated with neomycin and ANG II for 48 h. Neomycin alone had no effects on myocardial size or phenotype, indicating that the effect of neomycin was not secondary to a toxic cellular effect. ANG II induced approximately a 40% increase in myocyte size, which was inhibited by neomycin treatment (27%; Fig. 1, C and D). These results suggest that neomycin attenuates ANG II induced cardiac hypertrophy and PIP₂ plays an important role in it.

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**PIP2 increases in ang II-induced cardiac hypertrophy.** ANG II treatment in the mouse was used to test the hypothesis that PIP2 has a role in cardiac hypertrophy in vivo. The heart weight-to-body weight ratio (HW/BW) showed a significant increase in the ANG II-induced cardiac hypertrophy (Fig. 2C and Table 2). The PIP2 level increased significantly \( (P < 0.05) \) in ANG II-induced hypertrophic heart (Fig. 2, A and B). The PIP2 level increased in ANG II-stimulated NRVMs (Fig. 2, D and E), indicating that PIP2 may be involved in the ANG II-stimulated NRVM hypertrophic response.

**Increased PIP2 production by ANG II is dependent on the PTEN pathway.** The PIP2 level was significantly increased in NRVMs after a 1-h treatment of ANG II, and PIP3 level was markedly decreased (Fig. 3, A and B). By prevention of PTEN activity through pharmacological inhibition with bpV (1 μM), ANG II-induced PIP2 was markedly reduced. In contrast, the reduced PIP3 in ANG II group was strikingly increased with inhibition of activity of PTEN by bpV (Fig. 3, A and B). To confirm the PTEN inhibition study, a specific PTEN inhibitor SF1670 originally used in neutrophils (15) and was confirmed here in cardiomyocytes. ANG II increased PIP2, which was significantly reduced by SF1670 treatment while also dramatically increasing PIP3 (Fig. 3, A and B). Thus, these data strongly suggest the role for PTEN in regulating ANG II-induced PIP2 production in cardiac myocytes.

**Inhibition of PTEN activity attenuates ang II-induced increased actin dynamics.** To determine whether the effects of PIP2 on actin dynamics could be attenuated, NRVMs were pretreated with bpV or SF1670 for 30 min and then subjected to ANG II for 1 h. The marked increase in actin-GFP dynamics induced by ANG II was significantly reduced by bpV (7.45 ± 1.12 vs. 12.30 ± 1.62, \( P < 0.05 \)) or by SF1670. To confirm the PTEN inhibition study, a specific PTEN inhibitor SF1670 originally used in neutrophils (15) and was confirmed here in cardiomyocytes. ANG II increased PIP2, which was significantly reduced by SF1670 treatment while also dramatically increasing PIP3 (Fig. 3, A and B). Thus, these data strongly suggest the role for PTEN in regulating ANG II-induced PIP2 production in cardiac myocytes.

**Table 1. Recovery kinetics \( (K_{frap}) \) for actin under experimental conditions**

<table>
<thead>
<tr>
<th>Experimental Conditions</th>
<th>Actin ( K_{frap} ) ( 10^{-4} ) s(^{-1} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.70 ± 2.23</td>
</tr>
<tr>
<td>ANG II</td>
<td>12.30 ± 2.62*</td>
</tr>
<tr>
<td>ANG II + neomycin</td>
<td>7.28 ± 2.19#</td>
</tr>
<tr>
<td>bpV</td>
<td>7.72 ± 2.19</td>
</tr>
<tr>
<td>ANG II + bpV</td>
<td>7.45 ± 2.12#</td>
</tr>
<tr>
<td>SF1670</td>
<td>7.81 ± 2.17</td>
</tr>
<tr>
<td>ANG II + SF1670</td>
<td>6.34 ± 0.53#</td>
</tr>
</tbody>
</table>

Values are means ± SE. bpV, bisperoxo oxovanadate V. *\( P < 0.05 \) vs. control neonatal rat ventricular myocytes (NRVMs); #\( P < 0.05 \) vs. ANG II-treated NRVMs.
Actin dynamics is rapidly regulated by the PTEN/PIP$_2$ pathway

**Table 2. Heart weight and body weight in ANG II-induced heart-hypertrophy mice**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Body Weight, g</th>
<th>Heart Weight, mg</th>
<th>Heart Weight-to-Body Weight Ratio, mg/g</th>
<th>Average</th>
<th>SE</th>
<th>t-test</th>
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<tbody>
<tr>
<td>Control</td>
<td>Before</td>
<td>After</td>
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<td></td>
</tr>
<tr>
<td>1</td>
<td>24.00</td>
<td>29.96</td>
<td>99.60</td>
<td>3.32</td>
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</tr>
<tr>
<td>2</td>
<td>24.00</td>
<td>28.72</td>
<td>95.20</td>
<td>3.31</td>
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<td></td>
</tr>
<tr>
<td>3</td>
<td>24.86</td>
<td>29.84</td>
<td>107.3</td>
<td>3.60</td>
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<td>0.095</td>
</tr>
<tr>
<td>ANG II</td>
<td>Before</td>
<td>After</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>28.00</td>
<td>32.83</td>
<td>127.40</td>
<td>4.00</td>
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<tr>
<td>2</td>
<td>25.00</td>
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<tr>
<td>3</td>
<td>24.00</td>
<td>29.62</td>
<td>116.70</td>
<td>3.94</td>
<td>4.11</td>
<td>0.141</td>
</tr>
</tbody>
</table>

Time course of PIP$_2$ and PIP$_3$ levels and the translocation of PTEN by ang II. The relative time courses of levels of PIP$_2$ and PIP$_3$ with ANG II are shown in Fig. 5. ANG II induced an increase of PIP$_3$, which was detected at 30 min and sustained at 60 min. In contrast, PIP$_3$ was decreased by ANG II, beginning at 30 min and staying low at 60 min.

The PTEN level in ANG II-induced cardiomyocytes was examined. The protein level of PTEN in ANG II-treated NRVMs was not significantly changed at different time points (Fig. 6, A and D). The plasma membrane fraction of PTEN was significantly increased by 10 min (Fig. 6, B and E), although the total amount of PTEN and cytosolic fraction was not altered (Fig. 6, C and F). Because PIP$_2$ production was increased at 30 min, which is later than the translocation of PTEN to plasma membrane by 10 min, these results suggest that the translocation of PTEN precedes the production of PIP$_2$. 

Fig. 2. Increased phosphatidylinositol 4,5-bisphosphate (PIP$_2$) production in mouse heart and in cultured NRVMs by ANG II treatment. A and B: dot blot analysis of PIP$_2$ was significantly increased in the heart after 2 wk of ANG II delivery by mini-pump. GAPDH was used to normalize the dot blot density. Means ± SE; *P < 0.05, n = 3. The increased heart weight (HW)-to-body weight (BW) ratio (C) in ANG II-treated mice showed significant cardiac hypertrophy. *P < 0.05, n = 3. D and E: after 1 h stimulation by ANG II, PIP$_2$ production dramatically increased in the ANG II-treated group. Means ± SE. *P < 0.05, n = 3. C, control.
DISCUSSION

The present study demonstrates that actin dynamics and cell size were increased in NRVMs by stimulation with ANG II, which were tightly correlated to changes in the PTEN and PIP2 pathways. Furthermore, the PIP2 and PIP3 production by ANG II treatment was dependent on the PTEN pathway. The model proposed is that PTEN redistributes to the plasma membrane where it converts PIP3 to PIP2 (Fig. 7). Our results suggest that a possible mechanism for thin filament assembly partly relies on this increased level of PIP2.

The regulation of organization of the actin cytoskeleton upon G protein-coupled receptors (GPCRs) signaling has rarely been addressed. The angiotensin receptor (ATR) is one of the GPCRs, but a mechanism for the direct regulation of sarcomeric actin dynamics by the ATR has not yet been reported. However, there are some complicated clues that suggest that the ATR might regulate actin assembly through inhibiting PI3K pathways via β-arrestin (6, 28, 37). Some other GPCRs have been shown to regulate nonsarcomeric actin dynamics through different mechanisms. For example, serotonin receptors affected F-actin reorganization through cAMP signaling (7), endothelin receptors activated and induced association of paxillin with a cytoskeleton-enriched membrane fraction in vascular tissue (26), and ACh receptors played a role in cytoskeletal remodeling during ACh-induced contraction of smooth muscle through tyrosine phosphorylation of paxillin (35). Therefore, a mechanism involving the angiotensin receptor regulation of sarcomeric

![Fig. 3. Phosphatase and tensin homolog (PTEN) inhibitors blunt PIP2 and PIP3 production and actin dynamics after ANG II treatment. NRVMs were treated with either the PTEN inhibitor bisperoxo oxovanadate V (bpV; 1 μM) or with SF1670 (200 nM) for 30 min and then stimulated with 1 μM ANG II for 1 h.](image)

A

B

C

![Fig. 4. PTEN activation increased with ANG II stimulation and decreased with PTEN inhibitors. NRVMs were treated with either the PTEN inhibitor bpV (1 μM) or with SF1670 (200 nM) for 30 min and then stimulated with 1 μM ANG II for 1 h. The fold change in PTEN phosphatase activity was increased by ANG II stimulation but decreased by bpV or SF1670 compared with the vehicle control group.](image)

![Fig. 5. Time course of production of PIP2 and PIP3 after ANG II treatment in NRVMs.](image)
actin dynamics in cardiomyocytes is a possibility. Hypertrophy, resulting from chronic demands, requires addition of new sarcomeres. In the present study, we focused on the initial changes to sarcomeric actin dynamics after 1 h of ANG II treatment. Cell sizes in NRVMs and the PIP2 level in ANG II-induced hypertrophic mouse heart were shown after 2 days and 1 wk, respectively. Thus the acute parameters of actin dynamics precede actual cardiac hypertrophy by an interval in which many additional steps could occur, rather than being a direct linkage.

PIP2 has a direct role in regulating actin assembly by interaction with partnering proteins in many cells. In platelets, the half-life for a capped filament was 28 min, whereas the half-life to remain uncapped was only 0.2 s. Interestingly, the addition of PIP2 in these platelets reduced the half-life of the capped filament to 46 s, suggesting PIP2 regulation of actin filament capping dynamics (33). Our previous study clearly showed that PIP2 localized to the Z-disc in cardiomyocytes and regulated actin filament dynamics (14). In the present study, the increased actin dynamics by ANG II was in a PIP2-dependent manner (Fig. 1), suggesting PIP2 may play a critical role in increased actin assembly by ANG II induction.

The next question is how PIP2 is produced by ANG II. Some signaling pathways, such as PKC and PI4K, were involved in producing PIP2 in myocytes isolated from adult mice treated with ANG II (40). In addition, the PIP2 level could be converted at plasma membrane to PIP3 by PTEN, which is one of the regulators of PIP2 located there. In our results, the PTEN was activated by ANG II in NRVMs (Fig. 4), and PIP2 and PIP3 levels induced by ANG II were conversely changed by PTEN inhibition, suggesting that PTEN plays a role in PIP2 production by ANG II.

Fig. 6. PTEN translocates to the plasma membrane in response to ANG II stimulation. Cytosol and plasma membrane levels of PTEN in NRVM at 0, 5, 10, 30, and 60 min after treatment with 1 μM ANG II. The amount of cytosol or whole cell PTEN was not altered (A and D, C and F); membrane PTEN was significantly increased with ANG II treatment at 10, 30, and 60 min (B and E). Means ± SE. *P < 0.05, n = 3. Hsp70, heat shock protein 70.
There are two main explanations of how PTEN binds to the plasma membrane. The binding of PTEN to PIP2 might be critical for membrane localization (9). In contrast, PTEN might interact with the nonspecific electrostatic charges at the plasma membrane arising from lipids, such as phosphatidylserine (25). In our time course results, PTEN significantly increased at plasma membrane after only 10 min of ANG II stimulation (Fig. 6, B and E), whereas increased PIP2/decreased PIP3 did not change significantly until 30 min (Fig. 5). This time sequence suggests that PTEN interacted with the plasma membrane first and then converted PIP3 to PIP2 at plasma membrane.

Since the majority of cellular PTEN is found in the cytosol (30), how is PTEN able to execute different cellular functions that require its membranous lipid phosphatase activity? The lipid phosphatase activity of PTEN is at the plasma membrane. The plasma membrane PTEN increased in a time-dependent manner with ANG II treatment (Fig. 6, B and E). A decrease of cytosolic PTEN was expected but not found (Fig. 6, C and F) perhaps because most of the PTEN remains in this compartment. The level of PTEN in unstimulated myocytes was over 50-fold higher in the cytosol than in the membrane detected by Western blotting (data not shown).

The phosphatase activity of PTEN was significantly increased in ANG II-treated cardiomyocytes, whereas it was blunted by PTEN inhibitors bpV or SF1670 (Fig. 4). The subcellular localization of PTEN and its enzymatic activity are regulated by its various posttranslational modifications. PTEN contains multiple domains, including an NH2-terminal phosphatase domain, a central C2 domain, and a COOH terminal tail. The phosphorylation sites mapped on PTEN could be serine and threonine residues (39), Ser370 and Ser385 (38) and Ser362 and Thr366 (1) in its COOH-terminal tail. Furthermore, PTEN was phosphorylated by RhoA-associated kinase at Ser229, Thr232, Thr319, and Thr321 in the C2 domain (16). In contrast, Thr366 phosphorylation promoted PTEN degradation (21). In this study, the focus was on the membrane translocation of PTEN that may contribute to its lipid phosphatase activity, since there are so many phosphorylation sites in inactive PTEN in cytosol. Our results were consistent with changes seen in cultured cardiomyocytes whereby ANG II-induced increased actin dynamics was blocked by the PTEN inhibitor bpV or SF1670 (Fig. 3C).

In conclusion, PIP2 plays a major role in regulating actin cytoskeleton dynamics. Furthermore, our results provide a crucial link between PTEN and the cellular responses to ANG II in which the translocation of PTEN was associated. We speculate that similar mechanisms may be responsible for ventricular remodeling and progression to heart failure.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: J.L. conception and design of research; J.L. and E.J.T. performed experiments; J.L. analyzed data; J.L. and B.R. interpreted results of experiments; J.L. prepared figures; J.L. drafted manuscript; B.R. edited and revised manuscript; B.R. approved final version of manuscript.
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