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Article

Polycystin-2 (TRPP2) Regulation by Ca^{2+} Is Effected and Diversified by Actin-Binding Proteins

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ABSTRACT Calcium regulation of Ca^{2+}-permeable ion channels is an important mechanism in the control of cell function. Polycystin-2 (PC2, TRPP2), a member of the transient receptor potential superfamily, is a nonselective cation channel with Ca^{2+} permeability. The molecular mechanisms associated with PC2 regulation by Ca^{2+} remain ill-defined. We recently demonstrated that PC2 from human syncytiotrophoblast ($PC2_{hst}$) but not the in vitro translated protein ($PC2_{iv}$), functionally responds to changes in intracellular (*cis*) Ca^{2+}. In this study we determined the regulatory effect(s) of Ca^{2+}-sensitive and -insensitive actin-binding proteins (ABPs) on $PC2_{iv}$ channel function in a lipid bilayer system. The actin-bundling protein α-actinin increased $PC2_{iv}$ channel function in the presence of *cis* Ca^{2+}, although instead was inhibitory in its absence. Conversely, filamin that shares actin-binding domains with α-actinin had a strong inhibitory effect on $PC2_{iv}$ channel function in the presence, but no effect in the absence of *cis* Ca^{2+}. Gelsolin stimulated $PC2_{iv}$ channel function in the presence, but not the absence of *cis* Ca^{2+}. In contrast, profilin that shares actin-binding domains with gelsolin, significantly increased $PC2_{iv}$ channel function both in the presence and absence of Ca^{2+}. The distinct effect(s) of the ABPs on $PC2_{iv}$ channel function demonstrate that Ca^{2+} regulation of PC2 is actually mediated by direct interaction(s) with structural elements of the actin cytoskeleton. These data indicate that specific ABP-PC2 complexes would confer distinct Ca^{2+}-sensitive properties to the channel providing functional diversity to the cytoskeletal control of transient receptor potential channel regulation.

INTRODUCTION

Calcium is a universal second messenger that controls a wide number of cell functions. Particularly relevant are Ca^{2+}-induced self-inhibitory mechanisms of Ca^{2+}-permeable ion channels (1) linked to both Ca^{2+}-binding domains in the channel structure, as well as Ca^{2+} binding partners. The TRP (transient receptor potential) proteins are nonselective cation channels with permeability to Ca^{2+} that contribute to cell depolarization in nonexcitable tissues (1,2). TRP channels also contribute to cellular Ca^{2+} influx in Ca^{2+} handling epithelia, such as the intestine, kidney, and placenta (1). Among these TRP channels, PC2 is a protein with six transmembrane domains, and short intracellular N- and C-termini (3). PC2 shares homology with voltage-gated cation channels (4). Mutations in the *PKD2* gene account for 10–15% cases of autosomal dominant polycystic kidney disease (ADPKD), which is a common genetic disorder characterized by cyst formation in target organs, including kidneys, liver, and pancreas. Original reports of PC2 channel function established its Ca^{2+} permeability, although raising issues as to the potentially different Ca^{2+} regulation in different preparations (1). Recently, we reported that a local Ca^{2+} pool associated with putative cytoplasmic Ca^{2+}-binding regions linked to PC2 regulate its channel activity in the human syncytiotrophoblast (5). This regulation by Ca^{2+} is absent in the isolated protein, suggesting that Ca^{2+}-binding protein(s) would bind to, and control PC2 function.

There are important connections between PC2 and various components of the actin cytoskeleton that play important role(s) in PC2 channel function, and the anchoring of the PC2 channel complex to the plasma membrane (6). About half of the PC2 interacting partners identified thus far are cytoskeletal or cytoskeleton-associated proteins (6) including both actin binding partners (7–9) as well as microtubular components (10). PC2 directly interacts with α-actinin, an actin-bundling protein, which is implicated in important cell functions, including cytoskeletal organization, cell adhesion, proliferation, and migration (7). Both intracellular N- and C-termini of PC2 contribute to the binding with α-actinin. The structural interaction between these two proteins substantially increases PC2 channel function by increasing the channel open probability, but not its single-channel conductance (7). Interestingly, we recently reported that the actin-cross-linking protein filamin, with homology to α-actinin binds to, and substantially inhibits, $PC2_{hst}$ channel function (9). This is in agreement with the fact that dynamic changes in actin filament organization modulate the PC2 channel (8). The cytoskeletal regulation of PC2 function (7–10) suggests that PC2 acts as an integral part of a sensing complex for physical stimuli whose response is mediated by cytoskeletal changes. Interestingly,

Submitted September 10, 2014, and accepted for publication March 26, 2015.

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Editor: Miriam Goodman.

0006-3495/15/05/0001/10 $2.00

http://dx.doi.org/10.1016/j.bpj.2015.03.050

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although the previous evidence is actually elicited in the presence of high intracellular Ca^{2+} (7–9) the in vitro translated isolated protein, does not respond to changes in Ca^{2+} concentration (5), suggesting mediatory PC2 partners in this event. The interaction between PC2 and actin-binding proteins (ABPs) would confer the Ca^{2+}-regulation observed in the PC2 channel (5).

In this study, we demonstrated that Ca^{2+} regulation of PC2 is actually mediated by ABPs. $PC2_{iv}$ channel function was distinctly regulated by various Ca^{2+}-binding ABPs in a differential manner consistent with the Ca^{2+}-dependence of the ABP present. This suggests a pleiotropic Ca^{2+} response, including Ca^{2+} insensitivity of PC2, which may be physiologically relevant in the context of specific cellular responses and/or target tissues.

MATERIALS AND METHODS

Proteoliposome preparation and ion channel reconstitution

PC2-containing proteoliposomes were prepared with a 7:3 mixture of L-α-phosphatidylcholine (PC, 10 mg/ml, Avanti Polar Lipids, Birmingham, AL) and 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine (POPE, 25 mg/ml, Avanti) to get a lipid mixture in *n*-decane, which was dried with N_2. The lipid mixture was added to 4 ml of Na^+-cholate buffer (25 mM Na^+-cholate, 150 mM NaCl, 0.1 mM EDTA, 20 mM HEPES, pH 7.2) and sonicated in water for 45 min. To prepare PC2-containing proteoliposomes, isolated PC2 (1 mg/ml), prepared as previously reported (5), was diluted to 1:1000, added to 100 μl of the lipid solution, mixed well with 0.2 ml of Na^+-cholate, and dialyzed against dialysis buffer (150 mM NaCl, 0.1 mM EDTA, 20 mM HEPES, pH 7.2) for 3 days at 4°C (with three changes of buffer). The $PC2_{iv}$-containing vesicles were reconstituted in a lipid bilayer reconstitution system (BLM) as previously reported (11) in the presence of a KCl chemical gradient (*cis*/*trans*, 150:15 mM), and symmetrical 10 μM Ca^{2+}. The lipid mixture was a 7:3 ratio of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) and POPE (20–25 mg/mL; Avanti Polar Lipids) in *n*-decane. The *cis* chamber contained a solution of KCl 150 mM, $CaCl_2$ 10 μM, and HEPES 10 mM, at pH 7.40. The *trans* side contained a similar solution with lower KCl (15 mM), to create a KCl chemical gradient. Unless otherwise stated all ABP additions were carried out from the *cis* chamber, in experiments initiated in symmetrical Ca^{2+} (10 μM) and the absence of EGTA.

Actin-binding proteins

Profilin originally obtained from human platelets (MW 15 kDa/mol, Cytoskeleton (Denver, CO), PR01) was prepared by reconstituting 50 μg of the lyophilized product in 500 μl of distilled water (6.7 μM). 10 μl of a 1:10 dilution were added to the *cis* chamber to reach a concentration of 4.2 nM, which is the minimal value concentration at which there was a sizable regulatory effect. Gelsolin purified from *Escherichia coli* (MW = 82 kDa/mol, Cytoskeleton, HPG6) was prepared by reconstituting 20 μg of the lyophilized protein in 100 μl of distilled water (2.4 μM). A 1:10 dilution was prepared and 27 μl were added to the *cis* chamber to achieve a concentration of 4 nM, chosen with similar criteria as for profilin. The solution containing α-actinin obtained from chicken gizzard (MW 103 kDa/mol, Sigma-Aldrich (St. Louis, MO), CAS 11003-00-2) was prepared by reconstituting lyophilized 500 μg of protein in 500 μl of distilled water (9.7 μM), and added to the *cis* chamber, to reach a final concentration of 250 nM, previously reported effective to activate PC2 from hST (7). Filamin purified from chicken gizzard (MW = 250 kDa/mol, Cell Sciences (Canton, MA)) was prepared by reconstituting lyophilized 5 μg of protein in 500 μl of distilled water (80 μM) was added to the *cis* chamber, to obtain a final concentration of 250 nM.

Reagents and Ca^{2+} chelation

EGTA was dissolved in NaOH and titrated with HCl to reach pH 7.1. The concentrated reagent (16 μL) was diluted in *cis* chamber (1600 mL) and buffered at pH 7.4 (10 mM HEPES), to reach final concentrations of 1 mM. The addition of the chelating agent did not affect the final pH. The Ca^{2+} concentration was calculated, as previously reported (5) by

$$\left[Ca^{2+}\right] = \frac{K_Q[CaQ]}{[Q]}, \quad (1)$$

where K_Q, is the dissociation constant of the Ca^{2+}-EGTA complex, $[Q]$ is the concentration of the free chelating agent, and $[CaQ]$ is the concentration of Ca^{2+} bound to Q. The final free Ca^{2+} concentration was estimated to be either 0.6 nM (pH ~7.4) in the presence of EGTA (1 mM). Calculations were corroborated by the free on-line site http://www.stanford.edu/~cpatton/CaEGTA-NIST.htm. This low Ca^{2+} condition is indicated as zero nominal Ca^{2+} (0Ca).

Electrophysiological data acquisition and analysis

$PC2_{iv}$ channel currents were obtained from reconstituted lipid bilayer membranes (BLM) with a PC501A patch-clamp amplifier (Warner Instruments, Hamden, CT) with a 10 GΩ feedback resistor (11). Output signals were acquired from the patch-clamp amplifier without internal filtering (5 kHz), and were lowpass-filtered at 700 Hz (3 dB) with an analog eight-pole, Bessel-type filter (Frequency Devices, Haverhill, MA). Signals were then digitized with a TL-1 with DMA A/D converter at 80 kHz (Axon Instruments, Foster City, CA). Digital signal acquisition with the pClamp 5.5 suite was set to 100 μsec. BLM current tracings were further filtered for display purposes only. Unless otherwise stated, the software pClamp 10.2 (Axon Instruments), was used for data analysis and the software Sigmaplot 11.0 (Jandel Scientific, Corte Madera, CA), was used for statistical analysis and graphics. Unless otherwise stated, all tracings were obtained at holding potentials between 40 and 60 mV.

Signal analysis and statistics

Experimental data were analyzed with Clampfit 10.2 (Axon Instruments). $PC2_{iv}$ channel currents were analyzed as follows: single sweep 12-s episodes of BLM currents were integrated for successive tracings under each of the experimental conditions. Each tracing was first computed for peak (I) and mean (I_m) currents, regardless of the channel number (N), which was calculated as the peak current I, divided by the single channel current (i) of the fully opened channel at the holding potential. Temporal changes in I_m were expressed relative to the same parameter under control condition (I_{ctrl}), as I_m/I_{ctrl}, which was then plotted versus real elapsed time in a given experiment. This method avoided any assumptions as to the actual N or any other kinetic parameters in the records, showing whether there were statistical differences between experimental conditions. To compare averaged data from different experimental conditions, I_m/I_{ctrl} values for each of the tracings under a given condition were further averaged and expressed as relative to the control condition, such that the relative NP_o were statistically analyzed in the bar graphs. Further analysis of the time records were approximated with a single exponential function, from which half-time ($t_{1/2}$) transition rates were obtained. Tracings were also analyzed for single channel current amplitude and dwell times, to obtain single channel

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conductance values and the open probability (P_o), from the fully opened state. Fitting was conducted by the expectation step-maximization step (EM) algorithm for initial parameter estimation, which computed a mixture of exponentials to fit the probability functions. The P_o of a single channel record was $P_o = t_o/T$, where t_o was the total open time of the channel, and T was the total recording time. For multiple, fully active channel BLMs, $P_o = t_o/NT_o$ was used instead, where N is the number of open channels, and $T_o = \Sigma\, Lt_o$, the total open time, where L is the level of the channel opening. This approach, however, biased the true N, whenever different current levels represented contributions from lower, subconductance states instead of fully open channels. The apparent N was assigned a value equal to or greater than the value obtained from dividing the I/i for the given holding potential. However, under conditions where low channel activity or few openings were observed, N was assumed to be that of the previous condition. For conditions where the apparent N levels did not reflect the actual N (low subconductance states in decaying channels), NP_o, was also computed as $T_o/(T_o + T_c)$, where T_c represented the total closed time in the tracing, although these numbers were usually not used in the averages. Averaged data were expressed as the mean ± SE (n) under each condition, where n represents the total number of experiments analyzed (12). Statistical significance was obtained by paired Student's t-test comparison of sample groups of the same size, and accepted at $p < 0.05$.

RESULTS

Effect of α-actinin on $PC2_{iv}$ channel function

$PC2_{iv}$ was reconstituted in a lipid bilayer system in the presence of KCl chemical gradient and symmetrical *cis* Ca^{2+} (10 μM). To evaluate the effect of Ca^{2+} on the regulation by α-actinin of $PC2_{iv}$, α-actinin (250 nM) was added after determining spontaneous $PC2_{iv}$ channel activity (Fig. 1 *a*). Addition of α-actinin increased $PC2_{iv}$ channel activity by 250 ± 14% ($n = 6$, see Figs. 1, *a* and *b*, and 2 *d*), by modifying both P_o and N active channels, but not the single channel conductance (Fig. 1 *c*), which is in agreement with previous reports (7). The average P_o calculated as the mean of individual values obtained from dwell time histograms (Fig. 1 *b*, see Materials and Methods) increased from 0.51 ± 0.11 to 0.77 ± 0.08, $n = 6$ ($p < 0.05$), for control and α-actinin, respectively, whereas the channel number N, increased from 0.96 ± 0.03 to 2.76 ± 0.38, $n = 6$ ($p < 0.001$), respectively. Subsequent addition of EGTA (1 mM), decreased the mean current to 39 ± 7.8% of control value ($p < 001$, $n = 6$), with averaged P_o and N of 0.16 ± 0.07 ($p < 0.001$) and 0.88 ± 0.03 ($p < 0.001$), $n = 6$, respectively, which reversed by further additions of Ca^{2+} (10–300 nM) that restored $PC2_{iv}$ channel activity (Fig. 1 *d*) to values of 0.62 ± 0.04 ($p < 0.001$) and 1.59 ± 0.22 ($p < 0.01$), $n = 6$, respectively. Thus, the stimulatory effect of α-actinin required the presence of *cis* Ca^{2+} (Fig. 1, *a* and *d*), consistent with previous observations on $PC2_{hst}$ (5).

To further explore the effect of α-actinin on $PC2_{iv}$ in the absence of Ca^{2+}, a second series of experiments was conducted. In these experiments, EGTA (1 mM) was added to the *cis* chamber after observing spontaneous channel activity, and before the addition of α-actinin (Fig. 2 *a*). As expected for $PC2_{iv}$ (5), there was no significant effect of Ca^{2+} removal on the PC2 channel currents (Fig. 2). Further

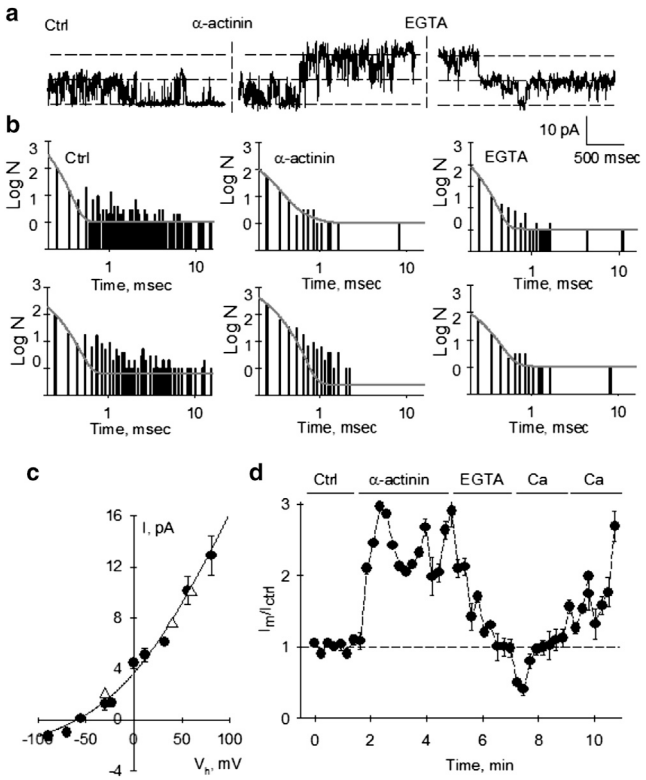

FIGURE 1 Effect of α-actinin on $PC2_{iv}$ channel function in the presence of Ca^{2+}. (*a*) Representative consecutive tracings of reconstituted $PC2_{iv}$ in the presence of Ca^{2+} under control (Ctrl) conditions, and after subsequent additions of both α-actinin (250 nM) and *cis* EGTA (1 mM). Horizontal dashed lines indicate closed state (*bottom*) and open channels in the preparation (*two* in this case after α-actinin). Vertical dashed lines represent additions of either α-actinin or EGTA. (*b*) Dwell times zero (*upper panels*) and one (*lower panels*) for tracings shown in (*a*). Data were fitted with single exponential, except for dwell 0 of α-actinin that best fitted with two exponentials. (*c*) Single channel current-to-voltage relationships in the presence of KCl chemical gradient obtained under control condition (*solid circles*), and after addition of α-actinin (*open triangles*). Solid line represents the best fitting of the Goldman-Hodgkin-Katz equation (11) to experimental data ($n = 6$). Data are expressed as the mean ± SE. (*d*) Relative current (I_m/I_{ctrl}) under various conditions, including control (Ctrl), and after subsequent additions of α-actinin (250 nM), EGTA (1 mM), and Ca^{2+} titration (10 and 300 nM, respectively). Experimental values (*solid circles*) are the mean ± SE, $n = 6$.

addition of α-actinin under these conditions, however, inhibited single channel currents (Fig. 2 *a*), from P_o and N of 0.63 ± 0.12 and 0.97 ± 0.09 ($p < 0.02$), respectively, to 0.22 ± 0.05 and 0.93 ± 0.06 ($p < 0.72$), $n = 4$, respectively. The relative I_m was reduced to values lower than control conditions (43 ± 1.5%, $n = 4$, $p < 0.05$, Fig. 2, *b* and *c*), indicating that α-actinin would act as an inhibitor of $PC2_{iv}$ channel function in the absence of *cis* Ca^{2+}. This inhibition reflected changes in the residence time of subconductance states (Fig. 2, *a* and *b*), but not the maximal conductance of the channel, as shown in the amplitude histograms (Fig. 2 *c*), in agreement with the previous results.

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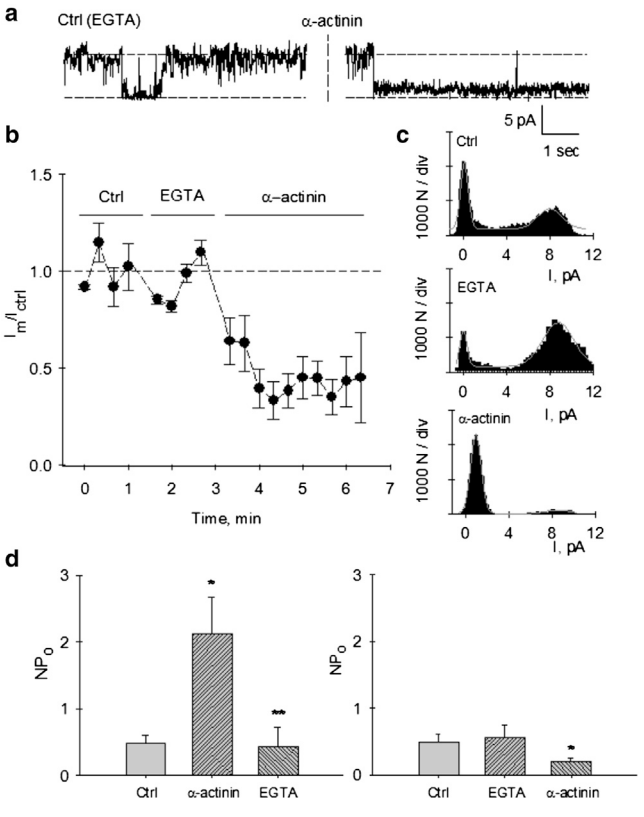

FIGURE 2 Effect of α-actinin on $PC2_{iv}$ channel function in the absence of Ca^{2+}. (*a*) Representative consecutive single channel currents of $PC2_{iv}$ in the absence of Ca^{2+} (EGTA, 1 mM), before and after addition of α-actinin (250 nM). Horizontal dashed lines indicate closed state (*bottom*) and open channels in the preparation. (*b*) Relative $PC2_{iv}$ currents (I_m/I_{ctrl}) under control condition (Ctrl) and after subsequent additions of EGTA and α-actinin. (*c*) Amplitude histograms of the three conditions shown in (*a*) as indicated. (*d*) (*Left*) Bar graph, showing NP_o for control (Ctrl) condition, after addition of α-actinin in the presence of Ca^{2+}, and subsequent Ca^{2+} chelating with EGTA. Please note this value, represents the P_o after chelating agent times *N* from the previous condition. Data are the mean ± SE for $n = 6$. (*Right*) Bar graph, showing NP_o for control (Ctrl) condition, after subsequent Ca^{2+} chelating with EGTA, and after addition of α-actinin. Data are the mean ± SE for $n = 4$. Asterisk indicates statistical significance.

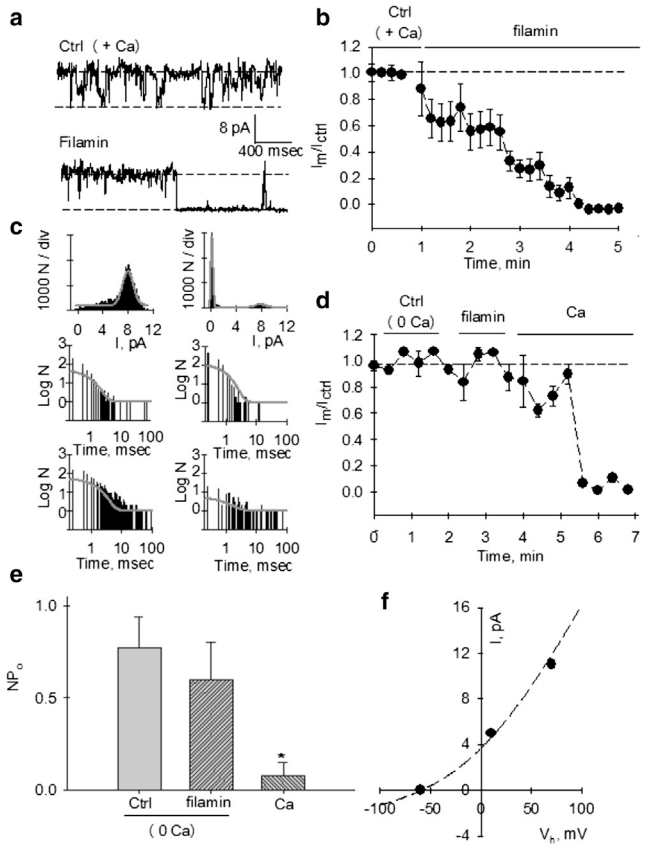

FIGURE 3 Effect of filamin on $PC2_{iv}$ function in the absence and presence of Ca^{2+}. (*a*) Representative consecutive single channel tracings of reconstituted $PC2_{iv}$ under control condition in the presence of Ca^{2+} (Ctrl (+ Ca)), and after addition of filamin (250 nM). Horizontal dashed lines indicate closed state (*bottom*) and open channels in the preparation. (*b*) Time response of relative I_m (I_m/I_{ctrl}) before and after filamin addition in the presence of Ca^{2+} (10 μM). Data are the mean ± SE, $n = 7$. (*c*) Amplitude histograms (*top*), dwells zero (*middle*), and one (*bottom*) for tracings shown in (*a*), control condition (*left*), and after filamin addition (*right*). (*d*) Time response of I_m/I_{ctrl} ratio before and after filamin addition in the absence of Ca^{2+}. Further addition of Ca^{2+} to the *cis* chamber (1 μM) elicited complete channel inhibition. Data are the mean ± SE, $n = 12$. (*e*) Bar graph showing NP_o for control condition (Ctrl), in the presence of filamin, and filamin plus Ca^{2+}. The asterisk indicates statistical significance. (*f*) Current-to-voltage relationship for single channel data in the presence of filamin (*solid circles*) superimposed on the dashed line, showing I/V curve under control condition.

Effect of filamin on $PC2_{iv}$ channel function

We then tested the effect of filamin, an ABP that has strong homology with α-actinin in the ABP domains. In contrast to α-actinin, which induces actin bundling, filamin allows the formation of wide-angle cross-linked gels of F-actin, thus generating three-dimensional structures (13). The addition of filamin (250 nM) after $PC2_{iv}$ reconstitution in the presence of Ca^{2+} showed the expected inhibition (Fig. 3, *a* and *b*), as recently reported (9). The average P_o calculated from the mean of individual values obtained from dwell-time histograms (Fig. 3 *c*) decreased from 0.64 ± 0.16 to 0.08 ± 0.03, $n = 7$ ($p < 0.005$), for control and filamin addition, respectively, whereas the channel number *N*, remained constant from 0.98 ± 0.01 to 0.88 ± 0.06 ($p < 0.13$), respectively. Addition of filamin in the absence of *cis* Ca^{2+} (EGTA, 1 mM), however, had no effect on $PC2_{iv}$ channel function (Fig. 3, *d* and *e*), although further addition of *cis* Ca^{2+} (1 μM), completely inhibited $PC2_{iv}$ channel activity by 100 ± 5% ($n = 12$), a finding similar to the inhibitory effect of α-actinin (Fig. 2) in the absence of Ca^{2+}. The average P_o values were 0.79 ± 0.12 to 0.63 ± 0.18, $n = 12$ ($p < 0.22$), for control and filamin addition, respectively, whereas the channel number *N*, remained constant from 0.97 ± 0.06 to 0.98 ± 0.03 ($p < 0.88$), respectively. The values changed however, after addition of *cis* Ca^{2+} (1 μM) to 0.09 ± 0.06 ($p < 0.01$) and 0.92 ± 0.07 ($p < 0.60$) for P_o and *N*, respectively. The half-times ($t_{1/2}$) for either maneuver

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to the addition of filamin were similar, suggesting that the presence of Ca^{2+} before the addition of the ABP did not modify its binging to PC2, and conversely, filamin would not affect the ability of PC2 to bind Ca^{2+}.

Effect of gelsolin on $PC2_{iv}$ channel function

To further confirm the regulatory contribution of ABPs to the Ca^{2+} control of PC2 channel function, the effect of gelsolin was next explored. Gelsolin is a Ca^{2+}-dependent actin-severing protein that regulates endogenous $PC2_{hst}$ in the presence, but not the absence of Ca^{2+} (8). The effect of gelsolin (4 nM) was first tested in the absence of *cis* Ca^{2+} (EGTA, 1 mM, n = 7, Fig. 4 *a*, *top tracings*). Under these conditions, gelsolin had neither stimulatory nor inhibitory effects on $PC2_{iv}$ channel function, maintaining mean currents at control value (see Fig. 4 *d*, *open circles*). The average P_o was 0.59 ± 0.07 to 0.56 ± 0.08, $n = 7$ ($p < 0.78$), for control and gelsolin addition, respectively, whereas the channel number N, also remained constant from 1.01 ± 0.05 to 0.97 ± 0.01, respectively ($p < 0.45$). Addition of gelsolin in the presence of Ca^{2+} (10 μM), however, increased $PC2_{iv}$ channel function by 70 ± 5.0% ($n = 10$, $p < 0.05$, Fig. 4 *a*, *lower tracings*, Fig. 4 *d*, *solid circles*). The average P_o increased from 0.55 ± 0.03 to 0.86 ± 0.05, $n = 10$ ($p < 0.001$), for control and gelsolin addition, respectively. The channel number N increased from 0.89 ± 0.07 to 1.61 ± 0.14, $n = 10$ ($p < 0.001$). In agreement with stimulatory effects of other ABPs, the effect of gelsolin in the presence of Ca^{2+} depended on an increase in the open residence of the channel (increased P_o, Fig. 4 *c*), but not on changes in the single channel conductance (Fig. 4 *e*). Thus, gelsolin regulation of $PC2_{iv}$ channel function was strictly Ca^{2+}-dependent, as expected (8).

Effect of profilin on $PC2_{iv}$ channel function

We finally evaluated the effect of the Ca^{2+}-insensitive ABP, profilin, which shows structural homology with gelsolin (14). $PC2_{iv}$ was reconstituted under conditions similar to previous experiments, in the presence of symmetrical Ca^{2+} (10 μM). Addition of profilin (4 nM) increased $PC2_{iv}$ channel function by 50 ± 7.5% ($n = 7$, $p < 0.05$, Fig. 5, *a*, *d*, and *e*). The average P_o increased from 0.65 ± 0.03 to 0.80 ± 0.06, $n = 7$ ($p < 0.05$, for control and profilin addition, respectively, whereas the channel number N, changed from 1.01 ± 0.09 to 1.45 ± 0.13 ($p < 0.02$). To determine whether profilin stimulation depended on the presence of Ca^{2+}, $PC2_{iv}$ currents were also followed after addition of *cis* EGTA (1 mM), which showed a 47 ± 2.5% in current ($p < 0.05$, n = 5, Fig. 5, *d* and *e*). The average P_o increased from 0.63 ± 0.05 to 0.86 ± 0.03, $n = 7$ ($p < 0.005$), for control and profilin addition, respectively, whereas the channel number N, changed from 0.99 ± 0.03 to 1.51 ± 0.26 ($p < 0.08$). The presence

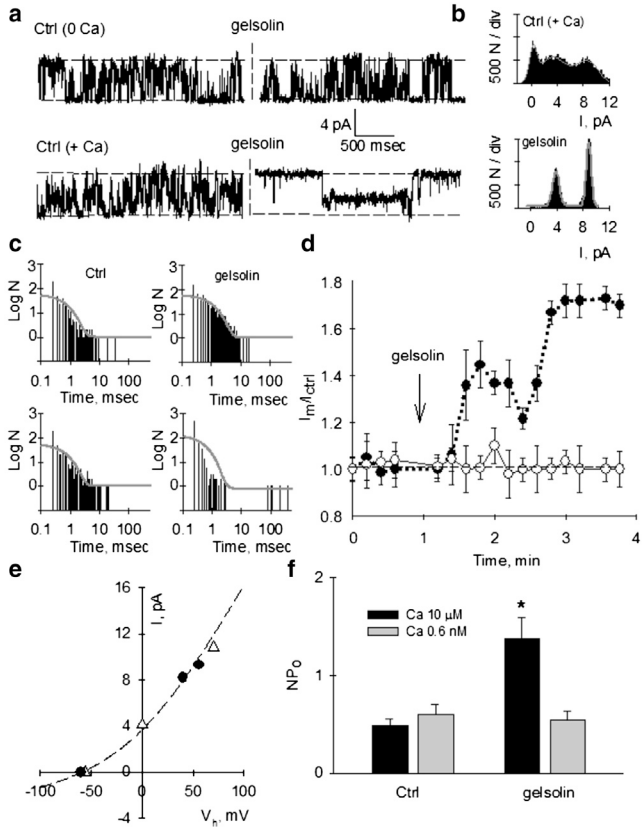

FIGURE 4 Effect of gelsolin on $PC2_{iv}$ channel function. (*a*) Representative consecutive single channel tracings of reconstituted $PC2_{iv}$ before and after *cis* gelsolin (4 nM) in the absence (Ctrl 0 Ca, *top*), or presence of Ca^{2+} (10 μM, *bottom*). Horizontal dashed lines indicate closed state (*bottom*) and open channels in the preparation. (*b*) Amplitude histograms in the presence of Ca^{2+} before and after gelsolin addition, as indicated. (*c*) Residence (*dwell*) times for states one and zero (*top* and *bottom*, respectively) before and after gelsolin addition, as indicated. (*d*) Time response of I_m/I_{ctrl} ratio after gelsolin addition either in the absence (*open symbols*) or presence of *cis* Ca^{2+} (10 μM, *solid symbols*). Data are the mean ± SE of $n = 7$ and 10, respectively. (*e*) Current-to-voltage relationship for single channel data in the presence of gelsolin with and without Ca^{2+} (*solid circles* and *open triangles*, respectively) superimposed on the dashed line, showing I/V curve under control condition. (*f*) Bar graphs showing NP_o under control conditions and after gelsolin addition (4 nM) either in the absence or presence of Ca^{2+}, as indicated. Data are the mean ± SE for $n = 7$ and 10, respectively. Asterisk indicates statistical significance.

of profilin in either the absence of Ca^{2+} did not affect the current amplitude of the channel (see Fig. 5 *b*). This is further confirmed by analysis of the single channel conductance (Fig. 5 *f*), which was identical to that under control conditions. Thus, profilin stimulates $PC2_{iv}$ channel function by modifying its NP_o properties, in a Ca^{2+} independent manner, which is consistent with its ABP properties.

DISCUSSION

PC2 is a TRP-type channel that forms structural and functional tetramers (15), and behaves as a Ca^{2+}-permeable

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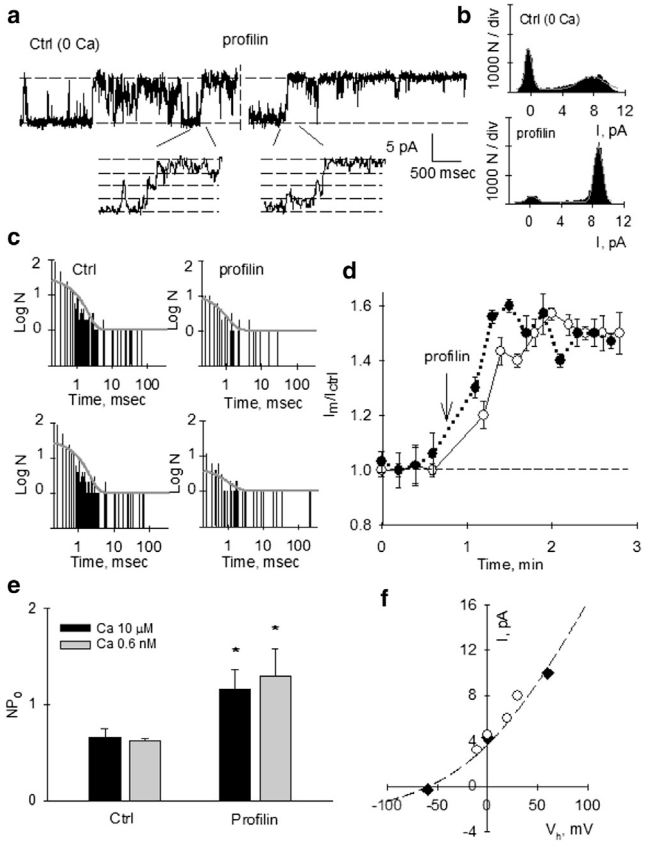

FIGURE 5 Effect of profilin on $PC2_{iv}$ channel function. (*a*) Representative consecutive $PC2_{iv}$ single channel currents under control conditions in the absence of Ca^{2+} (Ctrl (0 Ca), *left*), and after addition of profilin (4 nM, *right*). Horizontal dashed lines indicate closed state (*bottom*) and open channels in the preparation. Expanded tracings at the bottom show stepwise transitions from fully closed to fully open channel current in subconductance states, as reported (15). (*b*) Amplitude histograms for tracings shown in panel *a*, control condition (*upper*), and after profilin addition (*lower*). (*c*) Residence (*dwell*) times zero and one (*top* and *bottom*, respectively) before and after profilin addition, as indicated. (*d*) Time response of I_m/I_{ctrl} ratio before and after profilin addition either in the absence (*open circles*) or presence of *cis* Ca^{2+} (10 μM, *solid circles*). Data are the mean ± SE of five and seven experiments, respectively. (*e*) Bar graphs showing NP_o before and after profilin addition (4 nM) either in the absence or presence of Ca^{2+}. Data are the mean ± SE of $n = 5$ or 7, respectively. Asterisks indicate statistical significance for both conditions. (*f*) Current-to-voltage relationship for single channel data in the presence of profilin with and without Ca^{2+} (*open* and *solid circles*, respectively) superimposed on the dashed line, showing I/V curve under control conditions.

nonselective cation channel that is implicated in Ca^{2+} transport in epithelial tissues, including the kidney (16) and placenta (11). Previous studies showed that PC2 channel activity is regulated by Ca^{2+}. Vassilev et al. (17) observed PC2 activation by low external Ca^{2+} (1 μM) and inhibition by higher (mM) concentrations of the cation. Other publications reported that Ca^{2+} concentrations up to 1.26 mM increase PC2's open probability (18), whereas higher concentrations are inhibitory (11,18). It was also found that truncation mutants of PC2, including R742X and 1–703 PC2 (19), missing most of the C-terminus did not respond to changes in Ca^{2+}. To further explore these issues, we recently tested the effect of Ca^{2+} on reconstituted PC2 from human syncytiotrophoblast ($PC2_{hst}$) before and after cytoplasmic exposure to the Ca^{2+} chelating agents EGTA or BAPTA (5). Lowering Ca^{2+} to ~0.6–0.8 nM inhibited spontaneous $PC2_{hst}$ channel activity, with time responses dependent on the chelating agent used. Reversal of this inhibition by Ca^{2+} followed a Hill-type function most consistent with four putative, highly cooperative, Ca^{2+} binding sites with apparent dissociation constants in the range of 1–5 nM. Reversal of PC2 channel inhibition by lowering *cis* Ca^{2+} was also achieved by increasing *trans* (extracellular) Ca^{2+}, consistent with Ca^{2+} transport through the channel feeding the regulatory sites. This finding suggested the location of the Ca^{2+} binding sites at, or close to, the PC2 channel. Interestingly, similar experiments conducted with the reconstituted in vitro translated PC2 protein, showed no response to the various Ca^{2+} changing maneuvers. Thus, one plausible explanation for this finding was that the regulatory Ca^{2+} binding sites of PC2 were not intrinsic to the channel protein, but instead present in PC2-associated proteins.

This study was undertaken to explore potential PC2 partners that confer Ca^{2+} regulation to the channel. Several lines of evidence strongly suggested that the most likely candidates would be ABPs. PC2 is known to interact with a number of cytoskeleton-associated or cytoskeletal proteins including Hax-1 (20), the kinesin-2 motor subunits KIF3A and KIF3B (10,21), tropomyosin-1 (22) and troponin-I (23), α-actinin (7), gelsolin (8), and more recently, filamin (9). In most cases, these interactions were not only structural to anchor the channel to cytoskeletal networks, but also regulatory of PC2 channel function. The actin bundling protein α-actinin binds to PC2 and increases its channel function (7). This is a phenomenon observed with other channel proteins, including the Kv1.5 K^+ channel (24), L-type Ca^{2+} channels (25), and NMDA receptors (26,27). Interestingly, the α-actinin highly homologous filamin, an actin cross-linking protein, was also found to bind directly to PC2, although in this case, the regulatory function was inhibitory (9). Both α-actinin and filamin interacting domains on PC2 seem to respond to a common theme that engage the N- and C-terminal ends of the channel protein.

ABP-mediated, Ca^{2+}-controlled PC2 channel regulation

Herein, we observed that the isolated, Ca^{2+} insensitive PC2 channel acquires Ca^{2+} sensitivity by acting as a ligand-activated unit, where the intracellular Ca^{2+} regulatory mechanism lies in the associated ABP. Interestingly, ABPs (i.e., α-actinin, profilin), were also able to modulate PC2 channel activity in the absence of Ca^{2+}. The dynamic nature of this interaction was further disclosed by the fact

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that α-actinin was stimulatory in the presence of micromolar Ca^{2+}, and inhibitory in its absence. Thus, the Ca^{2+} sensitivity of PC2 would not be a universal phenomenon, but instead a selective mechanism that depends on the presence and/or competition with specific ABPs conveying wider variability of physiological significance to the Ca^{2+} regulation. Our previous findings on the Ca^{2+} regulation of the PC2 from the human syncytiotrophoblast indicate nanomolar affinities for the putative Ca^{2+} binding sites (5). Thus, it is unclear as to whether a given ABP conveys conformational changes to the channel protein based on its own ability to interact with Ca^{2+} or whether the ABP modifies the affinity of the channel protein to Ca^{2+}. It is possible that direct binding of Ca^{2+} to the channel modulates its affinity to the different ABPs thereby altering the PC2-ABP association; which results in either channel activation or inhibition. In this case, the direct binding of Ca^{2+} to PC2 would play an important physiological role in the regulation of the channel's activity. The C-terminus of PC2 contains both a coiled-coil domain and a Ca^{2+}-binding EF-hand that is apparently involved either directly or indirectly on PC2 Ca^{2+} regulation. Previous findings on the regulation by Ca^{2+} of PC2 have been interpreted to claim that binding of Ca^{2+} to the C-terminus of PC2 has a regulatory effect on the channel. This region of PC2 is particularly relevant because of its capacity to interact with a number of regulatory proteins, including the PC2 partner PC1 (28,29), other ion channels such as the IP3 receptor, and TRPC1, and cytoskeletal proteins, including α-actinin, filamin, CD2AP, mDia1, troponin I, and tropomyosin-1, intracellular trafficking proteins (PACS-1, PACS-2, and PIGEA-14), and even a transcription factor (Id2) (6). Assuming, as expected, that the Ca^{2+} dependence of the PC2 channel is a physiological mode of channel regulation, it appears that PC2 requires an intact C-terminus. PC2 channel activity can be altered by the C-terminus of PC1 (29), a PC2 partner that causes ADPKD, and many ADPKD causing mutations in PKD2 result in truncations of the C-terminal tail. Furthermore, phosphorylation of the C-terminus acidic linker at Ser-812 modulates the threshold concentration of Ca^{2+} required for PC2 channel activation (3). Together, these findings suggest that the C-terminus of PC2 may play a role in its regulation. Ehrlich's group and collaborators have studied the Ca^{2+} binding and conformational changes in the EF hand and coiled-coil isolated domain of PC2 (30–32). In those studies, structural analysis and modeling of the C-terminal domains of PC2 suggested functional roles in Ca^{2+}-dependent channel activation (via the EF-hand domain) and oligomerization (via the coiled coil domain). These findings showed, however, that Ca^{2+} binding to the C-terminus of PC2 has a very low affinity. The apparent K_D value of the first binding site was 179 μM, and that of the second site was 55 μM (30). These Ca^{2+} binding sites have much lower affinities than the expected physiological cytoplasmic concentrations where the C-terminus is located. It is entirely possible that conformational changes in the entire channel structure, of which the C-terminus is physically a small part, may help to make accessible and physiologically relevant these cooperative sites. These changes may take place driven by interactions with ABPs, based on the fact that both α-actinins, and filamins bind both the N- and C-termini of PC2, it is also possible changes in low affinity Ca^{2+} binding site(s) in PC2 may actually take place by posttranslational events such as phosphorylation of the C-terminus in PC2, which may convey structural changes to the channel, and its interaction with relevant partners such as PC1 (28,33).

Spectrin family members regulate PC2 channel function

The α-actinins are structural dimers that act as actin bundling proteins that belong to the spectrin superfamily and form filament bundles such as stress fibers and mediate the Ca^{2+}-dependent membrane attachments. The α-actinin monomer has a C-terminal head region consisting of a calmodulin-homology (CaM) domain that contains a pair of Ca^{2+}-binding EF-hands. Previously, we had observed that PC2 binds to both Ca^{2+}-sensitive and -insensitive α-actinins (7), in an interaction that involves the N- and two-thirds of the C-terminal ends of PC2. Interestingly, the interaction between the different α-actinins and PC2 were similarly observed either in the presence (1 mM) or absence of Ca^{2+} (1 mM EGTA), indicating a Ca^{2+}-independent association between the two proteins, which is in agreement with the regulatory effect(s) of α-actinin observed in this study.

The α-actinin highly homologous filamin, which cross-links and gelates F-actin instead of bundling (13,34) was functionally inhibitory only in the presence of Ca^{2+}, in contrast to the stimulatory effect of α-actinin. Furthermore, this inhibitory effect was not observed in the absence of Ca^{2+}, in which filamin showed no apparent effect (no apparent kinetic differences were observed in PC2 channel behavior in the presence or absence of filamin). To note is the fact, however, that no information is yet available on Ca^{2+}-dependent conformational changes in filamin. Previous studies explored the Ca^{2+}-calmodulin interaction with filamin on the ability to gelate actin (35). It was observed that although the presence of Ca^{2+} alone (in the absence of calmodulin) in the filamin-F-actin interaction showed a slightly lower gelation, the phenomenon was attributed to an effect of Ca^{2+} on the formation of shorter actin filaments as opposed to Mg^{2+}, and not to Ca^{2+}-induced conformational changes in the filamin structure. Similar to the case of α-actinins, filamins bind to numerous cellular components other than F-actin. Filamin A has been implicated in the trafficking of Kv4.2 K^+ channels in neurons, Kir2.1 in smooth muscle cells, and SK2 in cardiac myocytes, as well as the modulation of cystic fibrosis transmembrane conductance regulator, and the large-conductance

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Ca^{2+}-activated K^+ (BK) channel (see 36). Interestingly, the Ca^{2+}-dependent filamin A effect on SK2 channel trafficking also relied on the presence of α-actinin-2, revealing a highly sophisticated assembly of cytoskeletal proteins that coordinate membrane expression of ion channels. The combined results reinforce the hypothesis that ABPs directly interact with PC2, thus forming ABP-channel complexes that exhibit distinct Ca^{2+}-dependent interactions depending on the presence of local Ca^{2+} ions, and the specific interacting ABP.

Functional homology between α-actinin and gelsolin

A large number of ABPs, including α-actinin, filamin, and fimbrin, interact with filamentous (F) but not monomeric (G) actin (37). In contrast, proteins like gelsolin or severin that sever actin filaments and cap their barbed ends, require both F- and G-actin binding sites for their activity (38). Previously, we had observed the Ca^{2+}-dependent regulation of PC2 channel function by gelsolin (8). Those findings were originally evaluated in the context of disruption of cytoskeletal structures, because similar effect(s) were observed by cytochalasin D, an F-actin disrupter. However, this study showed that addition of gelsolin to the isolated PC2 mimicked the Ca^{2+}-dependent stimulatory effect of gelsolin on $PC2_{hst}$ (8). The results suggest that gelsolin also interacts directly with PC2, and further supports the contention that the ABP bound to PC2 conveys sensitivity to the divalent cation. Although gelsolin does not belong to the spectrin superfamily it shares functionally equivalent sequences with α-actinin on F-actin (39). Way et al. (39) proposed that the actin-binding domains of gelsolin and α-actinin might share common structural features based on their similar sizes, tandem sequence repeats, and Ca^{2+} independent F-actin binding properties. This has been confirmed by hybrid replacement of the F-actin binding domain of α-actinin to that of gelsolin (39). Thus, the most likely scenario for the gelsolin effect on PC2 is that the protein is actually able to physically interact with PC2 in a manner similar to that of α-actinin.

Integrating the data into a phenomenological model

Taken together, the effect of both Ca^{2+}-dependent effects of α-actinin and gelsolin on PC2 function, and the Ca^{2+}-dependent gelsolin-like activity of α-actinin hybrids (39) allow us to propose a potential scenario where PC2 interacts with moieties in various ABPs, such that the Ca^{2+} requirement for PC2 regulation would occur through binding of the ABP to PC2 in the absence of Ca^{2+}. Under these conditions, the position or orientation of the ABP interacting moiety would not produce optimal binding in the ABP-PC2 complex (i.e., filamin), hence, depending on the size and affinity of the ABP to PC2, either no PC2 gating or Ca^{2+}-independent gating may occur. Ca^{2+} would then considerably enhance the affinity of the ABP moiety for PC2, facilitating further conformational changes in the channel. Similar to the case of F-actin capping by gelsolin, which can occur in the absence of Ca^{2+}, PC2 gating could also occur by Ca^{2+}-insensitive ABPs in the absence of Ca^{2+}. The Ca^{2+}-dependent dual effect(s) of α-actinin (stimulation-inhibition) as opposed to gelsolin would support an allosteric inhibition in the absence (low affinity interaction with PC2) or presence (high affinity interaction with PC2) of Ca^{2+}. Further hypothesizing this dual interaction by Ca^{2+} depending on the ability of the ABP to bind the ion and induce conformational changes in PC2, a Ca^{2+} independent ABP would have a Ca^{2+} independent effect on PC2 function. The strong stimulatory, Ca^{2+} insensitive, effect of profilin on PC2 function is an example of this property. This finding is actually consistent with the hypothesis that the topological features of the cytoplasmic domain of the channel mimic those expected for the actin binding site in ABPs. Profilin is a small (<17 kDa) ABP, which has a unique molecular mechanism of action (40). Profilin causes F-actin depolymerization in vitro by binding to actin monomers (41), which in turn, accelerates ADP-ATP exchange, and would promote, F-actin formation. Consistent with our findings and proposed model, the regulatory function of profilin on PC2 was insensitive to Ca^{2+} (14).

The findings in this report allow us to propose a ligand-dependent channel behavior of PC2, in which different modes of Ca^{2+} regulation depend on whether a particular ABP binds to the channel, and responds to the presence or absence of local Ca^{2+}. The fact that both α-actinin and profilin elicit conformational changes to the channel, whereas in the absence of Ca^{2+} suggests that it is the ABP that conveys the particular gating properties of PC2. This is further supported by the fact that the ineffective filamin interaction in the absence of Ca^{2+} renders similar kinetic results upon repletion of the cation as the converse experiment (see Fig. 3, *b* and *d*). Also in agreement with those findings, no apparent kinetic changes (dwell time residency) were observed with either filamin or gelsolin under Ca^{2+}-free conditions, as compared with control conditions (data not shown). The ABP regulation of PC2 helps to drive Ca^{2+} into the cell, which would then feed back into the particular ABP-PC2 interaction. This phenomenon was actually demonstrated for the gelsolin regulation of PC2 in the human syncytiotrophoblast (8). Because of the availability of various ABPs within the cell's environment, and the dynamic (often Ca^{2+}-dependent) interactions between ABPs and the actin cytoskeleton, it is expected that, the feedback mechanisms linking cytoskeletal dynamics and channel function, may rely, in part, on the ABP's effects on both, the particular channel, and the F-actin networks as well. In the context of this particular type of regulation, the final outcome may rely on whether the activation (and/or

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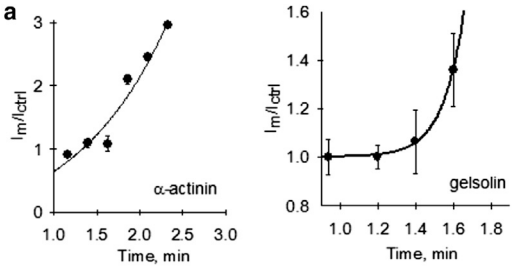
inhibition) is slow or fast, which is, in turn, reflected on the amount of Ca^{2+} being delivered through the open channel. Thus, this Ca^{2+}-dependent regulatory mechanism may play a substitute role for the Ca^{2+}-induced channel inhibition of voltage-gated Ca^{2+} channels (42). Surprisingly, the slow rate of transition ($t_{1/2}$) between the control condition and the ABP-induced effect on the PC2 channel functional mode, correlated with the size of the ABP (Fig. 6), following a logistic function (43,44). This finding suggests that the larger the protein, the slower the Ca^{2+}-ABP-induced conformational change of the channel. This would be an intelligent way to effect conformational changes induced by the ion (mediated by channel function) on cytoskeletal structures, which is the other part of this functional interface. In the absence of local free Ca^{2+} (e.g., available local calcium bound to other moieties), only Ca^{2+}-independent ABPs will play a role in helping the faster delivery of Ca^{2+} through the channel, whereas the larger molecules (i.e., filamin) would have longer, Ca^{2+}-dependent rates of channel gating.

a



b

ABP	MW	$t_{1/2}$, min 0 Ca^{2+}	+ Ca^{2+}
profilin	15	0.63	0.40
gelsolin	90	-	0.55
α-actinin	115	0.68	0.65
filamin	250	-	1.6

c

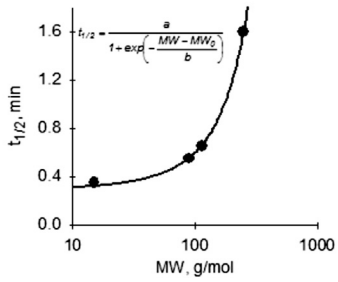


FIGURE 6 Model of $PC2_{iv}$ regulation by ABPs. (*a*) Experimental data during time (*t*) response to transitions between control and a given ABP condition were replotted in Linear-Linear scale, and fitted to the function $y = A + B \times \exp(t/t_{1/2})$ (*solid line*), to obtain $t_{1/2}$ as reported in (*b*). (*b*) Half-time transition rates ($t_{1/2}$) obtained for the various ABPs (indicated with molecular weight), either in the absence or presence of Ca^{2+}. The $t_{1/2}$ values indicate the transition rate, regardless of whether it follows activation (*positive slope*) or inhibition (*negative slope*). (*c*) Relationship between $t_{1/2}$ and ABP molecular weight followed a logistic function, suggesting a correlation between the size of the molecule, and the rate of transition (see main text).

CONCLUSIONS

Here, we determined molecular aspects of the regulation by Ca^{2+} of PC2. We showed that PC2 channel function is indeed regulated by Ca^{2+} in the presence, but not the absence of interacting ABPs. The fact that ABP regulation of PC2 may also be achieved in a Ca^{2+}-independent manner adds physiological diversity to this regulatory phenomenon. Our findings further suggest that the size of the ligand (ABP and/or ABP-Ca^{2+}) also modulates the kinetics of the Ca^{2+}-dependent channel gating, the larger the ABP, the slower the gating mechanism, and thus the longer onset of Ca^{2+} delivery into the cell. Regulatory Ca^{2+} binding to PC2 cannot be ruled out in this study. This interaction may allosterically contribute to changing the binding affinity, and thus the functional effect of an ABP on the channel.

AUTHOR CONTRIBUTIONS

R.C. performed research and analyzed data; H.C. designed research; H.C. and R.C. wrote the article.

ACKNOWLEDGMENTS

This study was funded by FonCyT-MinCyT, PICT 2012 No. 1559, Argentina. The authors are members of the National Research Council of Argentina (CONICET).

REFERENCES

1. Hoenderop, J. G. J., B. Nilius, and R. J. M. Bindels. 2005. Calcium absorption across epithelia. *Physiol. Rev.* 85:373–422.
2. Hoenderop, J. G., R. Vennekens, …, B. Nilius. 2001. Function and expression of the epithelial Ca($^{2+}$) channel family: comparison of mammalian ECaC1 and 2. *J. Physiol.* 537:747–761.
3. Cai, Y., Y. Maeda, …, S. Somlo. 1999. Identification and characterization of polycystin-2, the *PKD2* gene product. *J. Biol. Chem.* 274: 28557–28565.
4. Mochizuki, T., G. Wu, …, S. Somlo. 1996. *PKD2*, a gene for polycystic kidney disease that encodes an integral membrane protein. *Science.* 272:1339–1342.
5. Cantero, Mdel. R., and H. F. Cantiello. 2013. Calcium transport and local pool regulate polycystin-2 (TRPP2) function in human syncytiotrophoblast. *Biophys. J.* 105:365–375.
6. Chen, X. Z., Q. Li, …, H. F. Cantiello. 2008. Submembraneous microtubule cytoskeleton: interaction of TRPP2 with the cell cytoskeleton. *FEBS J.* 275:4675–4683.
7. Li, Q., N. Montalbetti, …, X. Z. Chen. 2005. Alpha-actinin associates with polycystin-2 and regulates its channel activity. *Hum. Mol. Genet.* 14:1587–1603.
8. Montalbetti, N., Q. Li, …, H. F. Cantiello. 2005. Cytoskeletal regulation of calcium-permeable cation channels in the human syncytiotrophoblast: role of gelsolin. *J. Physiol.* 566:309–325.
9. Wang, Q., X.-Q. Dai, …, X.-Z. Chen. 2012. Regulation of the function of polycystin-2 by filamin. *PLoS ONE.* 7:e40448.

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10. Li, Q., N. Montalbetti, …, H. F. Cantiello. 2006. Polycystin-2 cation channel function is under the control of microtubular structures in primary cilia of renal epithelial cells. *J. Biol. Chem.* 281:37566–37575.
11. González-Perrett, S., K. Kim, …, H. F. Cantiello. 2001. Polycystin-2, the protein mutated in autosomal dominant polycystic kidney disease (ADPKD), is a Ca^{2+}-permeable nonselective cation channel. *Proc. Natl. Acad. Sci. USA.* 98:1182–1187.
12. Snedecor, G. W., and W. G. Cochran. 1973. Statistical Methods, 6th ed. Iowa State University Press, Ames, IA.
13. Nakamura, F., T. P. Stossel, and J. H. Hartwig. 2011. The filamins: organizers of cell structure and function. *Cell Adhes. Migr.* 5:160–169.
14. Barron-Casella, E. A., M. A. Torres, …, J. F. Casella. 1995. Sequence analysis and chromosomal localization of human Cap Z. Conserved residues within the actin-binding domain may link Cap Z to gelsolin/severin and profilin protein families. *J. Biol. Chem.* 270:21472–21479.
15. Zhang, P., Y. Luo, …, H. F. Cantiello. 2009. The multimeric structure of polycystin-2 (TRPP2): structural-functional correlates of homo- and hetero-multimers with TRPC1. *Hum. Mol. Genet.* 18:1238–1251.
16. Luo, Y., P. M. Vassilev, …, J. Zhou. 2003. Native polycystin 2 functions as a plasma membrane Ca^{2+}-permeable cation channel in renal epithelia. *Mol. Cell. Biol.* 23:2600–2607.
17. Vassilev, P. M., L. Guo, …, J. Zhou. 2001. Polycystin-2 is a novel cation channel implicated in defective intracellular $Ca(^{2+})$ homeostasis in polycystic kidney disease. *Biochem. Biophys. Res. Commun.* 282: 341–350.
18. Koulen, P., Y. Cai, …, S. Somlo. 2002. Polycystin-2 is an intracellular calcium release channel. *Nat. Cell Biol.* 4:191–197.
19. Gallagher, A. R., S. Hoffmann, …, R. Witzgall. 2006. A truncated polycystin-2 protein causes polycystic kidney disease and retinal degeneration in transgenic rats. *J. Am. Soc. Nephrol.* 17:2719–2730.
20. Gallagher, A. R., A. Cedzich, …, R. Witzgall. 2000. The polycystic kidney disease protein *PKD2* interacts with Hax-1, a protein associated with the actin cytoskeleton. *Proc. Natl. Acad. Sci. USA.* 97:4017–4022.
21. Wu, Y., X. Q. Dai, …, X. Z. Chen. 2006. Kinesin-2 mediates physical and functional interactions between polycystin-2 and fibrocystin. *Hum. Mol. Genet.* 15:3280–3292.
22. Li, Q., Y. Dai, …, X.-Z. Chen. 2003. Polycystin-2 associates with tropomyosin-1, an actin microfilament component. *J. Mol. Biol.* 325: 949–962.
23. Li, Q., P. Y. Shen, …, X. Z. Chen. 2003. Polycystin-2 interacts with troponin I, an angiogenesis inhibitor. *Biochemistry.* 42:450–457.
24. Maruoka, N. D., D. F. Steele, …, D. Fedida. 2000. alpha-actinin-2 couples to cardiac Kv1.5 channels, regulating current density and channel localization in HEK cells. *FEBS Lett.* 473:188–194.
25. Sadeghi, A., A. D. Doyle, and B. D. Johnson. 2002. Regulation of the cardiac L-type Ca^{2+} channel by the actin-binding proteins alpha-actinin and dystrophin. *Am. J. Physiol. Cell Physiol.* 282:C1502–C1511.
26. Rycroft, B. K., and A. J. Gibb. 2004. Regulation of single NMDA receptor channel activity by alpha-actinin and calmodulin in rat hippocampal granule cells. *J. Physiol.* 557:795–808.
27. Wyszynski, M., J. Lin, …, M. Sheng. 1997. Competitive binding of alpha-actinin and calmodulin to the NMDA receptor. *Nature.* 385: 439–442.
28. Streets, A. J., O. Wessely, …, A. C. Ong. 2013. Hyperphosphorylation of polycystin-2 at a critical residue in disease reveals an essential role for polycystin-1-regulated dephosphorylation. *Hum. Mol. Genet.* 22:1924–1939.
29. Xu, G. M., S. González-Perrett, …, H. F. Cantiello. 2003. Polycystin-1 activates and stabilizes the polycystin-2 channel. *J. Biol. Chem.* 278:1457–1462.
30. Celić, A., E. T. Petri, …, T. J. Boggon. 2008. Domain mapping of the polycystin-2 C-terminal tail using de novo molecular modeling and biophysical analysis. *J. Biol. Chem.* 283:28305–28312.
31. Petri, E. T., A. Celic, …, M. E. Hodsdon. 2010. Structure of the EF-hand domain of polycystin-2 suggests a mechanism for Ca^{2+}-dependent regulation of polycystin-2 channel activity. *Proc. Natl. Acad. Sci. USA.* 107:9176–9181.
32. Ćelić, A. S., E. T. Petri, …, T. J. Boggon. 2012. Calcium-induced conformational changes in C-terminal tail of polycystin-2 are necessary for channel gating. *J. Biol. Chem.* 287:17232–17240.
33. Streets, A. J., A. J. Needham, …, A. C. Ong. 2010. Protein kinase D-mediated phosphorylation of polycystin-2 (TRPP2) is essential for its effects on cell growth and calcium channel activity. *Mol. Biol. Cell.* 21:3853–3865.
34. Hartwig, J. H., and T. P. Stossel. 1975. Isolation and properties of actin, myosin, and a new actinbinding protein in rabbit alveolar macrophages. *J. Biol. Chem.* 250:5696–5705.
35. Nakamura, F., J. H. Hartwig, …, P. T. Szymanski. 2005. Ca^{2+} and calmodulin regulate the binding of filamin A to actin filaments. *J. Biol. Chem.* 280:32426–32433.
36. Rafizadeh, S., Z. Zhang, …, N. Chiamvimonvat. 2014. Functional interaction with filamin A and intracellular Ca^{2+} enhance the surface membrane expression of a small-conductance Ca^{2+}-activated K^+ (SK2) channel. *Proc. Natl. Acad. Sci. USA.* 111:9989–9994.
37. Matsudaira, P. 1991. Modular organization of actin cross-linking proteins. *Trends Biochem. Sci.* 16:87–92.
38. Pope, B., S. Maciver, and A. Weeds. 1995. Localization of the calcium-sensitive actin monomer binding site in gelsolin to segment 4 and identification of calcium binding sites. *Biochemistry.* 34:1583–1588.
39. Way, M., B. Pope, and A. G. Weeds. 1992. Evidence for functional homology in the F-actin binding domains of gelsolin and alpha-actinin: implications for the requirements of severing and capping. *J. Cell Biol.* 119:835–842.
40. Yarmola, E. G., and M. R. Bubb. 2009. How depolymerization can promote polymerization: the case of actin and profilin. *BioEssays.* 31:1150–1160.
41. Carlsson, L., L. E. Nyström, …, S. Lövgren. 1976. Crystallization of a non-muscle actin. *J. Mol. Biol.* 105:353–366.
42. Zühlke, R. D., and H. Reuter. 1998. Ca^{2+}-sensitive inactivation of L-type Ca^{2+} channels depends on multiple cytoplasmic amino acid sequences of the α1C subunit. *Proc. Natl. Acad. Sci. USA.* 95:3287–3294.
43. Yin, X., J. Goudriaan, …, H. J. Spiertz. 2003. A flexible sigmoid function of determinate growth. *Ann. Bot. (Lond.).* 91:361–371.
44. Tjørve, E. 2003. Shapes and functions of species-area curves: a review of possible models. *J. Biogeogr.* 30:827–835.