Another story of arginines in voltage sensing: the role of phosphoinositides in coupling voltage sensing to enzyme activity

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The sensing of transmembrane electrical potential has long been thought to be unique to voltage-gated ion channels. Recently, however, transmembrane voltage has been shown to regulate the enzymatic activity of a protein, called voltage-sensing phosphatase (VSP), that is conserved across diverse phyla (Murata et al., 2005; Murata and Okamura, 2007; Hossain et al., 2008). In VSP, the voltage sensor domain is linked not to a pore domain but to a phosphoinositide phosphatase with remarkable similarity to the tumor suppressor protein PTEN (phosphatase and tensin homologue deleted on chromosome 10). The PTEN-like domain of VSP dephosphorylates phosphoinositides, a species of negatively charged lipids that acts as signaling molecules for diverse cellular events. VSP shows asymmetrical capacitative currents derived from the motion of its voltage sensor domain, similar to the gating currents of voltage-gated ion channels (Murata et al., 2005; Murata and Okamura, 2007; Hossain et al., 2008). It has been established that the motion of the voltage sensor drives phosphatase activity as detected by the changes of activities of potassium channels that report the concentration of PI(4,5)P2 (Murata et al., 2005; Murata and Okamura, 2007). VSP most likely operates as a monomer, as revealed by single-molecule imaging (Kohout et al., 2008). Therefore, VSP potentially provides an excellent model to understand mechanisms of coupling between a voltage sensor and an effector (Okamura, 2007; Okamura et al., 2009).

Valuable clues to the coupling mechanisms of VSP come from extensive biochemical (Maehama et al., 2001) and x-ray crystallography (Lee et al., 1999) studies of PTEN. To function, phosphoinositide phosphatases need to come physically close to membranes to access their substrates. Proximity is achieved in several ways in different proteins: having an N-terminal transmembrane segment (Pagliarini et al., 2004), binding to other membrane-associated proteins, or association to membrane via a motif or domain with affinity for lipid (Begley and Dixon, 2005). PTEN contains two regions that mediate membrane targeting: a PI(4,5)P2-binding motif at the N terminus and a C2 domain (Fig. 1; Lee et al., 1999; Maehama et al., 2001; Iijima et al., 2004). With mutation or deletion of the PI(4,5)P2-binding motif, PTEN cannot associate with cell membranes (Iijima et al., 2004; Vazquez et al., 2006). PLIP (PTEN-like phosphatase), a 5′ phosphatase with significant sequence similarity to PTEN, has a transmembrane segment at the N terminus but does not contain the apparent phosphoinositide-binding motif (Merlot et al., 2003; Pagliarini et al., 2004). In VSP, four transmembrane segments of the voltage sensor are sufficient to bring the enzyme close to the membrane. VSP has a putative phosphoinositide-binding motif (PBM) containing three basic residues, KRR (K252, R253, and R254), which is similar to that of PTEN in the region between the voltage sensor domain and the enzyme catalytic domain (Fig. 1). In the linker region, N terminal to the KRR cluster, Ci-VSP also contains another pair of arginines (R246 and R247) that is not conserved in PTEN.

Villalba-Galea et al. (see p. 5 of this issue) have focused on the putative PBM of Ci-VSP and examined motions of the voltage sensor of Ci-VSP with mutations in this motif. KCNQ2/3 potassium channels were coexpressed with Ci-VSP as the readout of enzyme activity (Murata et al., 2005; Murata and Okamura, 2007). Mutagenesis of two arginines (R253 and R254) in the putative PBM eliminated coupling, as indicated by the loss of current decay of KCNQ2/3 channel currents. In addition, voltage-clamp recording showed that OFF-sensing currents of the R253A/R254A mutant returned more rapidly than the wild type. Villalba-Galea et al. (2008) have previously shown by voltage-clamp fluorometry that upon persistent depolarization, a voltage sensor enters into a third state distinct from the resting state and activated state, which is called the relaxed state. They took advantage of this phenomenon to address how the kinetics of voltage sensor changes with mutation of arginines in the PBM. Results showed that the voltage sensor of the R253A/R254A mutant returned more slowly from the relaxed state than in the wild-type protein. A potential criticism is that mutation of R253 and R254 may have

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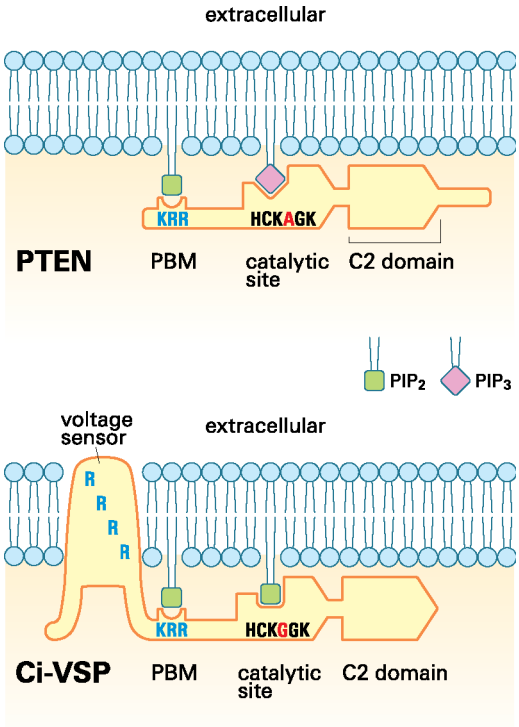

Figure 1. Scheme of the structure of PTEN and Ci-VSP. Ci-VSP has an active center for catalysis with one amino acid difference from PTEN (HCKGGK for Ci-VSP and HCKAGK for PTEN). Both proteins contain a phosphoinositide-binding motif (PBM) with KRR and C2 domains that are known to associate with membrane. Although it is not depicted in the figure, Ci-VSP contains another pair of arginines at residues 245 and 246 that is not conserved in PTEN.

altered the integrity of the enzyme. This is unlikely because the truncated protein synthesized in *Escherichia coli* exhibits normal enzymatic activity in vitro (Murata et al., 2005). Based on these experiments, it is suggested that phosphoinositide, possibly PI(4,5)P2, binds to R254 and/or R254 to regulate the catalytic action of the phosphatase, and this association with phospholipids slows the motions of the voltage sensor.

The altered function of the voltage sensor with mutation in the PBM in this study by Villalba-Galea et al. (2009) differs from that of teleost VSP studied by heterologous expression in a mammalian cell line (Hossain et al., 2008). Thus expressed, zebrafish VSP (Dr-VSP) exhibits robust sensing currents, allowing detailed analysis of the kinetics of the voltage sensor. Both ON- and OFF-sensing currents were significantly faster in the protein with mutation of the catalytic cysteine residue critical for phosphatase activity as compared with those of wild type (Hossain et al., 2008). Similar differences were seen when enzyme activity was suppressed by the phosphatase inhibitor pervanadate. In this study by Villalba-Galea et al. (2009), the acceleration of motions of the voltage sensor seen after mutation in the PBM were also observed in the background of mutation of the cysteine residue in the catalytic center.

Some results from mutation of arginines in the PBM of Ci-VSP were rather unexpected. In a previous study (Murata et al., 2005), deletion of eight amino acids, 248–255, including R253 and R254, did not suppress coupling, as reported by the activities of coexpressed KCNQ2/3 channels or K_{ir} channels. Instead, deletion of eight amino acids, 240–247, eliminated coupling. However, two arginines are included in each of these eight–amino acid segments at the same relative position (MKASSRRT vs. ISQNKRRY). Villalba-Galea et al. (2009) speculate that upon deletion of the 248–255 segment, two arginines, R245 and R246, in the 240–247 segment replaced the role of R253 and R254. This idea is supported by two lines of evidence. First, mutation of R245Q and R246Q partially reduced coupling as indicated by a milder reduction of KCNQ2/3 current at high depolarization. This is consistent with the idea that arginines at 245 and 246 can have similar roles as R253 and R254. Second, R to K mutation at 245 and 246 eliminated coupling more completely than R to A mutation at 253 and 254. Lysine has similar electrostatic properties as arginine. A more remarkable reduction of coupling efficiency in the R to K mutant suggests that arginine works not because of its electrostatic profile but in a side chain–specific manner. Why should lysine replacement of R245 and R246 have a more drastic effect than an R to A change? Lysine may attract PI(4,5)P2 with its positive charge but cannot fully mimic arginine, thereby blocking binding of the second R245/R246 site. However, R253A/R254A cannot even attract PI(4,5)P2 because of loss of positive charge, providing an opportunity for the second site, R245 and R245, to bind to PI(4,5)P2. Additional detailed studies are necessary to address the relationships between the two clusters of positively charged residues, R245/R246 and R253/R254.

It is not yet clear whether phosphoinositide binds directly to the PBM of Ci-VSP as in PTEN. Nevertheless, the story of the involvement of phosphoinositide in the coupling of the voltage sensor and enzymatic activity will have significant impact in two directions. First, it may explain why VSP is the only enzyme to date that is directly coupled with a voltage sensor domain. From genomic searching, no kinase or protease regulated by a voltage sensor domain has been found. As one possibility, mechanisms of binding of phosphoinositide to the PBM might have evolved to be susceptible to a local stretch induced by the motions of the voltage sensor. In PTEN, it is still unknown how binding of PI(4,5)P2 to the PBM contributes to regulation of enzyme activity. Three models have been proposed that are not necessarily exclusive of each other. Iijima et al. (2004) and Rahdar et al. (2009) proposed that the PBM blocks the catalytic site, shielding it from substrate. In this model, binding of PI(4,5)P2 to the PBM causes the catalytic center to become accessible to the lipid bilayer, resulting in enzyme activity. Another

model is that PI(4,5)P2 binding to the PBM simply provides membrane tethering and stabilizes proper orientation or distance of the catalytic site from its substrate, PIP3 (Walker et al., 2004). A third model is that binding of PI(4,5)P2 to the PBM induces a conformational change in the phosphatase domain (Campbell et al., 2003). In support of this third model, it has recently been shown that binding of the PBM to PI(4,5)P2 increases the proportion of the α-helical structure of PTEN, suggesting that PI(4,5)P2 binding to PBM triggers structural change of the enzyme domain (Redfern et al., 2008). VSP may serve as a new platform to solve these controversies regarding regulatory mechanisms of PTEN and PTEN-related phosphatases as well as providing a clue to understanding mechanisms of voltage sensor–driven proteins.

A second direction of impact is that the regulation of enzymatic activity of the VSP by phosphoinositide will shed light on possible cellular functions of this enzyme, which is conserved from sea urchin to human. PI(4,5)P2 is dephosphorylated by VSP (Murata and Okamura, 2007; Iwasaki et al., 2008; Halaszovich et al., 2009). If binding of PI(4,5)P2 to PBM of VSP mediates coupling from membrane depolarization to enzyme, PI(4,5)P2 binding may provide a negative feedback mechanism for enzyme action. This contrasts with PTEN despite the sequence similarity of the PBM in the two proteins. The substrate specificity of PTEN is more rigid than that of VSP, and PTEN dephosphorylates only PIP3 into PI(4,5)P2 (Maehama and Dixon, 1998). Given that PI(4,5)P2 is the final catalytic product of PTEN, PI(4,5)P2-dependent activation of the enzyme activity through its binding to the PBM should operate as a positive feedback. A positive loop with phosphoinositide-dependent regulation is also an innate trait of myotubularin that produces PI(5)P by dephosphorylating PI(3,5)P and is up-regulated by PI(5)P (Begley and Dixon, 2005).

If PI(4,5)P2 binds to the PBM to regulate coupling to the enzyme activity of VSP, the kinetics of motion of the voltage sensor should be altered in an activity-dependent manner. For slightly different reasons, this has been examined in Dr-VSP (Hossain et al., 2008). The kinetics of sensing currents was unaltered when Dr-VSP was activated by long intervals of depolarization. However, the outcome of such experiments should depend on how the binding affinity of PI(4,5)P2 to the PBM of VSP and how PI(4,5)P2 can be depleted in cells. More rigorous studies with simultaneous analysis of the motions of the voltage sensor and PI(4,5)P2 level from the same cell will be needed to address this issue.

The work of Villalba-Galea et al. (2009) and that of other laboratories show that many more discoveries can be expected at the crossroads of the two streets of voltage-sensing and phosphoinositide phosphatase.

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