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Title Page

Review

Beyond voltage-gated ion channels: voltage-operated membrane proteins and cellular processes

Running title: Voltage-operated proteins and cellular processes

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Abstract: Voltage-gated ion channels were believed to be the only voltage-sensitive proteins in excitable (and some non-excitable) cells for a long time. Emerging evidence indicates that the voltage-operated model is shared by some other transmembrane proteins expressed in both excitable and non-excitable cells. In this

review, we summarize current knowledge about voltage-operated proteins, which are not classic voltage-gated ion channels as well as the voltage-dependent processes in cells for which single voltage-sensitive proteins have yet to be identified. Particularly, we will focus on the following. (1) Voltage-sensitive phosphoinositide phosphatases (VSP) with four transmembrane segments homologous to the voltage sensor domain (VSD) of voltage-gated ion channels; VSPs are the first family of proteins, other than the voltage-gated ion channels, for which there is sufficient evidence for the existence of the VSD domain. (2) Voltage-gated proton channels comprising of a single voltage-sensing domain and lacking an identified pore domain. (3) G protein coupled receptors (GPCRs) that mediate the depolarization-evoked potentiation of Ca^{2+} mobilization. (4) Plasma membrane (PM) depolarization-induced but Ca^{2+}-independent exocytosis in neurons. (5) Voltage-dependent metabolism of phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P_2, PIP_2) in the PM. These recent discoveries expand our understanding of voltage-operated processes within cellular membranes.

Keywords membrane potential, Ci-VSP, Hv1/VSOP, GPCRs, CIVDS, PtdIns(4,5)P_2 metabolism

Abbreviations

VSD, voltage sensor domain;
Ci-VSP, Ciona intestinalis voltage-sensor containing phosphatase;
PTEN, phosphatase and tensin homolog deleted on chromosome TEN;
PIP_2/PtdIns(4,5)P_2, phosphatidylinositol-4,5-bisphosphate;
PIP_3/PtdIns(3,4,5)P_3, phosphatidylinositol-3,4,5-trisphosphate;
TM, transmembrane;
PM, plasma membrane;
IP_3, inositol 1,4,5-trisphosphate;
DAG, diacylglycerol;
GPCR, G protein-coupled receptor;
TASK, TWIK-related acid sensitive potassium channels;
VSOP, voltage sensor only protein;
DRG, dorsal root ganglion;
CIVDS, Ca^{2+}-independent but voltage-dependent vesicular secretion;
CDS, Ca^{2+}-dependent secretion

Introduction

Although the understanding that voltage-gated ion channels might not be the only voltage-sensing proteins in cellular membranes has been around for a long time, until recently, the evidence has been mostly indirect. Recent work describing cloning of the first voltage sensitive phosphatase from Ciona intestinalis (Ci-VSP) (Murata et al., 2005) has stimulated substantial interest to this field. The Ci-VSP consists of a

transmembrane voltage sensor domain (VSD) homologous to the VSD of voltage-gated ion channels and a cytoplasmic phosphatidylinositol phosphate (PIP) phosphatase homologous to the PTEN (phosphatase and tensin homologue deleted on chromosome TEN) (Kalli et al., 2014; Murata et al., 2005; Okamura and Dixon, 2011). The voltage sensor of Ci-VSP controls its phosphatase activity rather than a channel pore. This discovery established that VSDs can control processes other than opening or closing of ion channels. The discovery of VSPs was soon followed by the cloning of voltage-gated proton channel Hv1/VSOP, which consists only of a VSD region and lacks a discernible pore domain that exists in all other voltage-gated ion channels (Ramsey et al., 2006; Sasaki et al., 2006). With VSD being its only TM domain, Hv1 exhibits proton-selective, voltage-dependent ion conductance, which places this protein outside all known VSD-containing voltage-gated ion channel families. These two examples significantly extended the original concept of VSDs being exclusively the regulators of voltage-gated ion channel pores.

Growing evidence suggests that some other cellular processes, particularly those within or near the plasma membrane of the cell, are also regulated by voltage. Thus, some G protein coupled receptors (GPCR) display sensitivity to membrane voltage, e.g., $P2Y_1$ receptor (Gurung et al., 2008; Martinez-Pinna et al., 2005b), muscarinic acetylcholine receptors (Ben-Chaim et al., 2006; Ben-Chaim et al., 2003; Navarro-Polanco et al., 2011), lysophosphatidic acid receptor (Martinez-Pinna et al., 2010) etc. Recently, new evidence indicated that two muscarinic receptors (M_1R and M_2R) display charge-movement-associated currents analogous to 'gating currents' of voltage-gated channels. In addition, the agonist binding of GPCRs is in itself a voltage-dependent event (Ben-Chaim et al., 2006; Navarro-Polanco et al., 2011).

Another example of voltage-sensitive membrane process is a Ca^{2+}-independent but voltage-dependent vesicular secretion (CIVDS) observed in some types of neurons (Abdrakhmanov et al., 2013; Liu et al., 2011; Silinsky et al., 1995; Yang et al., 2005; Zhang and Zhou, 2002; Zheng et al., 2009). This type of exocytosis is distinct from the regular Ca^{2+}-dependent vesicular secretion (CDS); CIVDC can amount to up to 20% of the total vesicular release (Yang et al., 2005). A novel form of Ca^{2+}-independent rapid endocytosis (RE) was found tightly coupled to CIVDS (RE-CIVDS) (Zhang et al., 2004). The molecular mechanism underlying CIVDS was proposed in a recent study (Chai et al., 2018).

Another novel voltage-operated membrane process has been described in xenopus oocytes: a depolarization-activated PtdIns(4,5)P_2 synthesis (Chen et al., 2011; Zhang et al., 2010). Thus, in contrast to the depolarization-activated Ci-VSP-mediated PtdIns(4,5)P_2 cleavage, depolarization of the oocyte membrane elevated plasma membrane PtdIns(4,5)P_2 levels. An underlying mechanism was proposed whereby a cell senses the voltage changes and in response elevates membrane PtdIns(4,5)P_2 levels via activation of PKC isozyme βII. This subsequently increases activity of PtdIns-4 kinase β. However, the mechanism underlying the voltage-sensitive step within this metabolic pathway is currently unknown. With increased recognition of the importance of PtdIns(4,5)P_2 in cell function (Choi et al., 2015; Sun et al., 2013), the effect of membrane depolarization on the PtdIns(4,5)P_2 metabolism is likely to

have important physiological implications.

The aim of this review is to discuss examples of voltage-operated membrane processes that are not directly mediated by voltage-gated ion channel activity. In the interest of focus we will not discuss here the mechanisms of neuronal activity-dependent transcription (excitation-transcription coupling) as this large field has attracted considerable attention in recent years and several excellent reviews on this topic are available (Dewenter et al., 2017; Hardingham et al., 2018; Kobrinsky, 2015).

Voltage-sensitive phosphatases

A typical voltage-gated ion channel (such as voltage-gated K^+ or TRP channels) consists of four subunits, each containing two major domains: S1 to S4 transmembrane domains constituting a voltage sensor, and S5 to S6 transmembrane domains (and the re-entrant loop connecting them) forming the ion-permeable pore (Fig. 1A) (Aggarwal and MacKinnon, 1996; Bezanilla, 2000; Bezanilla, 2005; Doyle et al., 1998; Jiang et al., 2003). The VSD could sense changes in the membrane voltage and regulate the opening and closing of channel pore (Jiang et al., 2003). Positively charged amino acid residues in the fourth transmembrane segment (S4) of the channel play key role in sensing changes in the electric field across the cell membrane and translating it into the conformational change that opens the channel gate (Aggarwal and MacKinnon, 1996; Seoh et al., 1996). Voltage-dependent movement of these charged residues of S4 can be recorded electrophysiologically as 'gating current' (Aggarwal and MacKinnon, 1996). For a long time, voltage-gated ion channels were the only proteins known to operate in a voltage-dependent manner, but this idea has recently been changed by discovery of a voltage sensor-containing phosphatase from ascidian Ciona intestinalis (Ci-VSP) (Murata et al., 2005).

The Ci-VSP consists a transmembrane voltage-sensing domain homologous to the S1–S4 segments of voltage-gated ion channels and a cytoplasmic domain homologous to an enzyme PTEN (phosphatase and tensin homologue deleted on chromosome TEN) (Fig. 1B, left panel) (Kalli et al., 2014; Murata et al., 2005; Okamura and Dixon, 2011). In contrast to the classic tetrameric voltage-gated ion channels, in which a common pore is cooperatively gated by four VSDs (Tombola et al., 2006), each Ci-VSP functions as a monomer (Fig. 1B, middle panel) (Kohout et al., 2008). The Ci-VSP's phosphatase activity is switched on by strong membrane depolarization sensed by its VSD. This was demonstrated by the robust PtdIns(4,5)P_2 depletion triggered by the depolarizing voltage step in a voltage-clamp experiments (Halaszovich et al., 2009; Murata and Okamura, 2007). The S4 transmembrane segment of the voltage sensor of Ci-VSP displays channel-like 'gating' currents similar to gating currents of voltage-gated channels (Hossain et al., 2008; Murata et al., 2005; Villalba-Galea et al., 2008). Thus, this enzyme directly translates changes in membrane potential into the turnover of phosphoinositides (Halaszovich et al., 2009; Murata and Okamura, 2007). Ci-VSP ortholog genes were identified from zebrafish, Xenopus, chick, mouse, rat, and human (Hossain et al., 2008). VSP ortholog gene

from zebrafish, Danio rerio (Dr-VSP), which shares most properties with Ci-VSP, showed much more robust gating currents and more steep voltage-dependence as compared to Ci-VSP (Hossain et al., 2008).

PTEN is a cytosolic enzyme in the inner leaflet of the plasma membrane that catalyzes the dephosphorylation of PtdIns(3,4,5)P_3 at the 3'-phosphate (Maehama and Dixon, 1998; Maehama et al., 2001). Because of the high sequence similarity of its catalytic domain to PTEN, Ci-VSP was originally proposed to dephosphorylate PtdIns(3,4,5)P_3 to PtdIns(4,5)P_2 in response to hyperpolarization (Murata et al., 2005). However, this hypothesis clashed with several experimental findings. First, in cells overexpressing Ci-VSP both membrane PtdIns(3,4,5)P_3 and PtdIns(4,5)P_2 levels were strongly decreased upon depolarization. PtdIns(4,5)P_2 level recovered upon hyperpolarization (as monitored by the optical PtdIns(4,5)P_2 reporter, PH-domain-GFP, using confocal microscopy) (Halaszovich et al., 2009; Iwasaki et al., 2008; Murata and Okamura, 2007). Secondly, the activity of two PtdIns(4,5)P_2-sensitive ion channels (GIRK2, KCNQ2/3) was severely reduced or even abolished upon depolarization when channels were co-expressed with Ci-VSP (Falkenburger et al., 2010; Klein et al., 2008; Murata et al., 2005; Murata and Okamura, 2007; Zhang et al., 2010). These observations suggested that not only PtdIns(3,4,5)P_3 but also PtdIns(4,5)P_2 is a substrate of Ci-VSP and the phosphatase activity of the latter is activated upon depolarization. Unlike PTEN, Ci-VSP dephosphorylate the 5' position of the inositol ring of both PtdIns(3,4,5)P_3 and PtdIns(4,5)P_2 upon voltage depolarization (Fig. 1B, right panel) (Halaszovich et al., 2009). Interestingly, at high voltages Ci-VSP and the orthologs do have significant 3' phosphatase activity toward PtdIns(3,4)P_2, but not PtdIns(3,4,5)P_3, which suggests that substrate specificity of the VSP changes with membrane potential (Kurokawa et al., 2012). Glutamic acid (Glu-411) in the "TI loop" in the active site of the Ci-VSP cytoplasmic region has been shown to contribute to the substrate specificity of Ci-VSP (Liu et al., 2012; Matsuda et al., 2011).

PtdIns(4,5)P_2 is localized mainly in the cytoplasmic leaflet of the plasma membrane and is required for activity of many membrane proteins, including ion channels and transporters (Gamper and Rohacs, 2012; Gamper and Shapiro, 2007; Hille et al., 2015). Yet, investigating ion channel regulation by PtdIns(4,5)P_2 is inherently difficult due to the scarcity of reliable tools for manipulations with PtdIns(4,5)P_2 concentrations in living cells. Since the identification of VSPs and characterization of their properties (i.e. voltage threshold, activation kinetics and substrate specificity), these fascinating enzymes became an invaluable research tool to study the dynamics and biological roles of phosphoinositides, especially PtdIns(4,5)P_2 (Falkenburger et al., 2010; Gamper and Rohacs, 2012; Hille et al., 2015; Zhang et al., 2010).

There are two features of VSPs that make them particularly attractive as tools for experimental manipulation with plasma membrane phosphoinositides. First, the drop in membrane phosphoinositide levels produced by VSPs is not accompanied by the release of other signaling molecules such as inositol 1,4,5-trisphosphate (IP_3), diacylglycerol (DAG) or Ca^{2+} (which is the case of the GPCR/PLC-induced

PtdIns(4,5)P_2 hydrolysis). Second, the levels of phosphoinositides can be acutely altered by VSPs through a simple jump of membrane potential. Thus, VSPs can be used in combination with the voltage-clamp techniques to alter the concentration of PtdIns(4,5)P_2 in living cells during real-time measurement of an ion channel activity. Using this approach, recent work has indicated that the activity of TRPM8 (Yudin et al., 2011), TRPV1 (Klein et al., 2008) and high-voltage activated Ca^{2+} channels (L-, N-, and P/Q-type) (Suh et al., 2010) is dependent of PtdIns(4,5)P_2 concentration, while the activity of low-voltage activated Ca^{2+} channels (T-type) (Suh et al., 2010) and two-pore K^+ channels TASK-1 and TASK-3 is independent of PtdIns(4,5)P_2 concentrations within the physiological range (Lindner et al., 2011). It is important to note though that different membrane proteins might be associated with distinct pools of PtdIns(4,5)P_2 that differ in their accessibility to VSPs (Rjasanow et al., 2015).

Voltage-gated proton channel

The VSD of VSPs controls the activity of a phosphatase rather than the opening or closing of a channel pore. This indicated that VSDs can work independently of an ion channel pore domain (PD). The expansion of the role of VSDs was followed by cloning of a unique voltage-gated proton channel that lacks a discernible classical PD (Fig. 1C, left panel) (Ramsey et al., 2006; Sasaki et al., 2006).

Voltage-gated proton channels are present in a variety of mammalian cell types including skeletal muscle cells, blood cells, granulocytes and monocytes (DeCoursey, 2013; Okamura, 2007). They regulate proton conductance in response to changes in the voltage across the cell membrane and, thus, contribute to regulation of cellular pH (Castillo et al., 2015; DeCoursey, 2013). The voltage-gated proton currents were originally discovered in snail neurons about 30 years ago, but the gene encoding the proton channel protein was not identified until recently when two groups successfully cloned and identified the Hv1 or voltage sensor only protein (VSOP) as the long-sought voltage-gated proton channel (Ramsey et al., 2006; Sasaki et al., 2006).

Surprisingly, the Hv1 gene codes for a protein containing a S1~S4 voltage sensor that is homologous to the VSDs of most other voltage-gated ion channels, yet, it lacks a canonical pore region (Fig. 1C, left panel). These findings indicated that the proton permeation pathway might be localized within the VSD region itself. Interestingly, mutations of the first S4 arginine (R362) of the VSD in Shaker potassium channels creates a proton permeability in the VSD when the cell is hyperpolarized. This gain-of-function gating pore (referred to as the “omega pore”) is distinct from the central K^+ pore of the tetrameric structure of Shaker (Starace and Bezanilla, 2004; Tombola et al., 2005). Similar phenomena were also discovered in Na_V channels (Sokolov et al., 2005; Sokolov et al., 2007).

Classical voltage-gated ion channels (like Kv channel superfamily members) are comprised of four subunits, and the S5-S6 of each subunit assembles to form a single ion pore (Fig. 1A, right panel) (Bezanilla, 2000; Capener et al., 2002). Considering the lack of pore domain, the Hv1/VSOP channels were assumed to be monomeric. However, there is solid evidence that Hv1/VSOP subunits are assembled into

homodimers, and each subunit contains its own pore. The cytoplasmic coiled-coil domain in the C-terminus (Fig. 1C) was reported to be necessary to induce dimerization of the subunits. Thus, deletion of the C terminus (Hv1-ΔC) resulted in depolymerization (Fujiwara et al., 2012; Koch et al., 2008; Lee et al., 2008; Li et al., 2010; Tombola et al., 2008). The channels with truncated C-terminus displayed large proton currents indicating that single Hv1/VSOP subunits could function independently as proton channels. The high proton selectivity of Hv1 channels is conferred by a single acidic residue Asp112 (Musset et al., 2011). The voltage dependence of H_V channels is regulated by the ΔpH across the plasma membrane; this facilitates the extrusion of excess acid in nearly all type of cells (except dinoflagellates) (Cherny et al., 1995; DeCoursey, 2013). The ΔpH dependence of channel gating suggests that VSD of H_V channels can also work as a proton sensor. But the underling mechanism of pH sensitivity remains mysterious. Several excellent reviews on the diverse function and regulation of H_V channels are available (Castillo et al., 2015; DeCoursey, 2013; DeCoursey et al., 2016).

GRCRs—emerging roles as voltage sensor

G protein-coupled receptors (GPCRs), also known as 7-TM domain receptors (Fig. 2A, middle panel), are important transmembrane signal transduction pathways. The GPCRs are sensors for extracellular chemical signals such as neurotransmitters, inflammatory mediators, odorants, etc. (Lefkowitz, 2007; Pierce et al., 2002). GPCRs were not thought to be directly modulated by the membrane potential until several recent studies challenged this belief (Ben-Chaim et al., 2006; Ben-Chaim et al., 2003; Mahaut-Smith et al., 2008; Martinez-Pinna et al., 2005a; Martinez-Pinna et al., 2010; Navarro-Polanco et al., 2011). The hypothesis that GPCRs can be modulated by the membrane potential originates from the evidence of potentiation of IP_3-dependent Ca^{2+} release by depolarization after activation of some GPCRs (Mahaut-Smith et al., 1999; Mason et al., 2000). Subsequently, direct sensitivity of the GPCRs to the membrane potential has been suggested by many observations on multiple receptors, e.g. $P2Y_1$ (Gurung et al., 2008; Martinez-Pinna et al., 2005b), muscarinic M_2 (Ben-Chaim et al., 2006; Ben-Chaim et al., 2003; Navarro-Polanco et al., 2011), lysophosphatidic acid receptor (Martinez-Pinna et al., 2010), etc. Therefore, building evidence indicate that voltage-sensitivity could be a common property amongst GPCRs (Mahaut-Smith et al., 2008). For example, the Mahaut-Smith lab offered a series of convincing studies on the voltage-sensitivity of $P2Y_1$ receptor-mediated GPCR signaling in rat megakaryocytes (Fig. 2A, left panel). The lack of voltage-gated Ca^{2+} channels in the plasma membrane and ryanodine receptors in the endoplasmic reticulum (ER) membrane of megakaryocytes helped to isolate $P2Y_1$ receptor-mediated IP_3-dependent Ca^{2+} mobilization in these cells (Mahaut-Smith et al., 1999; Mason et al., 2000; Mason and Mahaut-Smith, 2001). In these cells, inhibition of PLC or the IP_3 receptors abolished both the ADP-induced and the depolarization-induced Ca^{2+} release. This suggested that both events depend on the messenger system downstream of the GPCR activation. Furthermore, activation of IP_3

receptor by IP_3 flash photolysis induced Ca^{2+} increases but prevented further Ca^{2+} response to voltage, which indicated that the voltage-dependent Ca^{2+} mobilization is upstream of IP_3 production (Martinez-Pinna et al., 2005b). Unexpectedly, depolarization stimulated an Ca^{2+} increase in the absence of agonist during exposure to some competitive antagonists (A3P5PS and MRS2179, structurally related to ADP) (Gurung et al., 2008). In sum, these results supported the hypothesis that the voltage sensing mechanisms resides upstream of G protein activation and may be an intrinsic property of the $P2Y_1$ receptors themselves.

Additionally, the voltage sensitivity of two muscarinic receptors (M_2R, Fig. 2A, middle panel, and M_1R) was also described (Ben-Chaim et al., 2003). Ben-Chaim and colleagues measured the activity of the M_1 and M_2 receptors by recording calcium-activated chloride current (CaCC) and the G-protein-activated inwardly rectifying potassium channel (GIRK) activity, respectively. They found that the apparent affinity of M_2R towards acetylcholine (ACh) was reduced upon membrane depolarization while that of M_1R increased (Ben-Chaim et al., 2003). These observations indicated that changes in membrane potential induced conformational changes in the ligand-binding site of these muscarinic receptors which, in turn, altered the ligand-receptor interactions in a ligand-specific manner (Navarro-Polanco et al., 2011). Importantly, the charge-movement-associated currents analogous to 'gating currents' of voltage-gated channels, were observed in M_2 muscarinic receptors (Fig. 2A, right panel)(Ben-Chaim et al., 2006), suggesting that, at least in this case, the voltage sensing mechanism may be located within the receptor itself. Using the ACh-activated potassium channels (I_{KACh}) as a probe, the authors showed that membrane depolarization decreased the potency of ACh and increased the potency of pilocarpine (muscarinic receptor agonist). Of note, ACh reduced while pilocarpine increased the gating charge displacement. Additionally, mutations in the putative ligand-binding site of M_2 receptor perturbed the gating charge displacement (Ben-Chaim et al., 2006). All of these observations supported the conclusion that GPCRs may serve a dual role as both voltage and chemical sensors at the PM.

Not all depolarization-induced neuronal vesicle release is Ca^{2+}-dependent.

In the traditional view, action potential arriving at the presynaptic terminal triggers vesicular exocytosis by engaging Ca^{2+}-sensitive release machinery via Ca^{2+} influx produced by the activation of voltage-gated Ca^{2+} channels in the plasma membrane (Bennett and Kearns, 2000; Zefirov et al., 2005). A series of studies from various laboratories pointed to a novel Ca^{2+}-independent but voltage-dependent vesicular secretion (CIVDS) in neurons (Fig. 2B) (Abdrakhmanov et al., 2013; Chai et al., 2018; Liu et al., 2011; Silinsky et al., 1995; Yang et al., 2005; Zhang and Zhou, 2002; Zheng et al., 2009). Thus, Zhuan Zhou's group reported that brief depolarization of plasma membranes in cultured dorsal root ganglion (DRG) neurons could induce vesicular secretion in the absence of extracellular Ca^{2+} and in the presence of an intracellular Ca^{2+} chelator BAPTA (Zhang and Zhou, 2002). The existence of CIVDS was shown via data obtained from a variety of independent methods including membrane

capacitance measurements, fluorescence Ca^{2+} imaging, radioimmunoassays of peptide secretion and electrochemical amperometric detection of transmitter release (Chai et al., 2018; Yang et al., 2005; Zhang and Zhou, 2002). The relative contributions of CIVDS and Ca^{2+}-dependent vesicular secretion (CDS) to the total depolarization-induced exocytosis in DRG neurons was estimated to be about 20% vs. 80%, respectively (Yang et al., 2005). These studies specifically demonstrated that both CDS and CIVDS components contributed the action potential (AP)-induced secretion, indicating that CIVDS is probably an essential physiological component of the activity-dependent secretion in the DRG somata (Zheng et al., 2009). It was also suggested that CIVDS and CDS access distinct vesicle pools in DRG neurons (Liu et al., 2011). The CIVDS are also found in frog motor nerve terminals (Abdrakhmanov et al., 2013; Silinsky et al., 1995). In a recent follow-up Zhou's group has shown that voltage sensing during CIVDS is provided by the N-type Ca^{2+} channels. $Ca_V2.2$ protein binds directly to the SNARE protein complex via its synprint region on the PM of DRG neurons. When PM is depolarized (e.g. with the arrival of an action potential), it drives a conformational change of the $Ca_V2.2$-SNARE complex and then triggers vesicle fusion and neurotransmitter release even without a rise in cytosolic Ca^{2+} (Chai et al., 2018).

In neurons, endocytosis is a critical step in synaptic vesicle recycling, which terminates signaling transduction and maintains the balance of cell surface membrane. Ca^{2+}-independent rapid endocytosis (RE) was found to be tightly coupled to CIVDS (RE-CIVDS) in DRG neurons (Zhang et al., 2004) and frog motor nerve terminals (Abdrakhmanov et al., 2013). Further study indicated that PKA-mediated phosphorylation is involved in depolarization-induced RE, and depolarization per se upregulated PKA activity in Ca^{2+}-free extracellular solutions (Zhang et al., 2004). These results uncovered a form of endocytosis with previously uncharacterized properties that might explain the mechanisms of synaptic vesicle recycling and how they are regulated.

Voltage-dependent increase of membrane PtdIns(4,5)P_2

Phosphoinositide levels in the biological membranes are dynamically modulated and such modulation represents an important signaling mechanism for controlling many membrane-localized cellular processes, including migration, intracellular trafficking, ion channel activity etc. (Balla, 2013; Gamper and Rohacs, 2012; Gamper and Shapiro, 2007; Hille et al., 2015). Recently we reported mechanism of voltage-dependent regulation of membrane PtdIns(4,5)P_2 metabolism: a depolarization-induced increase in plasma membrane levels of PtdIns(4,5)P_2 (Fig. 2C) (Chen et al., 2011; Zhang et al., 2010). Similarly to studies of Ci-VSP, we used PtdIns(4,5)P_2-sensitive KCNQ voltage-gated K^+ channels as a biosensor for plasma membrane PtdIns(4,5)P_2 levels. When expressed in Xenopus oocytes, a 30-min depolarization (+20 mV) led to a continuous increase (>2 folds) of KCNQ2/3 current amplitude, and the increase was gradually reversed when the membrane was repolarized to -80 mV. The depolarization-induced potentiation of KCNQ2/3 currents

was clearly voltage-dependent with a half-maximum effect ($V_{1/2}$) observed at -26.1 ± 0.5 mV. Similar results were obtained when depolarization was induced by elevating the extracellular K^+ concentration (Chen et al., 2011; Zhang et al., 2010). The depolarization-induced 'run-up' of KCNQ2/3 activity was produced by the increase of plasma membrane PtdIns(4,5)P_2, as verified by Ci-VSP and direct measurement of phosphoinositide levels by thin-layer chromatography (Zhang et al., 2010). Elevated PtdIns(4,5)P_2 was most likely the result of increased activity of PtdIns-4 kinase β because its knockdown greatly reduced the depolarization-induced enhancement of KCNQ2/3 currents (Chen et al., 2011). This depolarization-induced PtdIns-4 kinase β activation was presumably mediated by protein kinase C (PKC) since PKC activator, phorbol-12-myristate, 13-acetate (PMA) mimicked the effect of depolarization on KCNQ2/3 current amplitude with a similar time-course (Chen et al., 2011; Zhang et al., 2010). Yet, the mechanism linking depolarization with PKC activation remains to be elucidated. With increased recognition of the importance of PtdIns(4,5)P_2 in cell function, the effect of membrane depolarization in PtdIns(4,5)P_2 metabolism may have important physiological implications.

Conclusions and Future Directions

Apart from the well-known effects of membrane potentials on the functions of voltage-dependent ion channels, accumulating evidence suggests that membrane voltage is an important regulator of non-conventional voltage sensing proteins like Ci-VSP, Hv1/VSOP and GPCRs, voltage-operated cellular processes like voltage-dependent vesicular secretion (CIVDS), or voltage-dependent increase of membrane PtdIns(4,5)P_2. In addition to discussed above, there are other examples of regulation of cellular function by membrane voltage. Thus, membrane depolarization was also found to influence mitogenic signaling in cell proliferation through nanoscale organization of the membrane phosphatidylserine and PtdIns(4,5)P_2 and activation of K-Ras signaling (Zhou et al., 2015). In addition, there are a number of reports suggesting that VSDs of some ion channels, such as EAG (Hegle et al., 2006), EAG1 (Downie et al., 2008), Kv1.3 and Kv1.5 (Cidad et al., 2012; Jimenez-Perez et al., 2016) can regulate cell proliferation via a non-canonical, ion-flux independent mechanisms (Zhou et al., 2015). Moreover, several novel ion channel-enzyme fusion proteins were reported, and their enzymatic and ion channel activity were interdependent (Runnels et al., 2001; Scharenberg, 2005; Schultz et al., 1992; Weber et al., 2004; Wu et al., 2010). All these examples, as well as the well-recognized phenomenon of excitation-transcription coupling, broaden our understanding of the role of membrane potential in intracellular signaling and general regulation of cellular functions. Yet, the exact voltage sensing mechanisms in many of these phenomena remain to be elucidated.

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Figure Legend

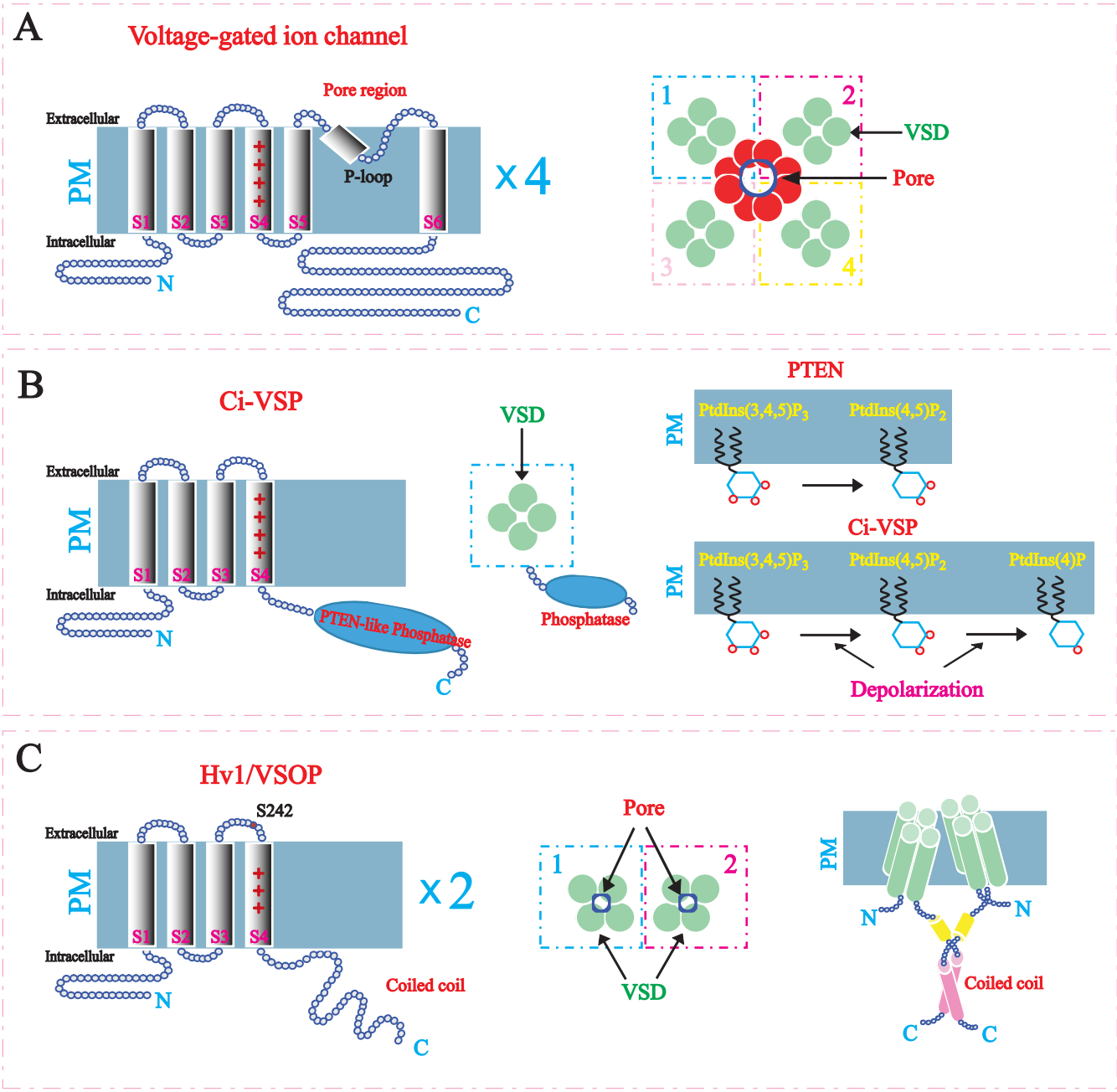
Figure 1. Membrane proteins that contain S1-S4 VSDs.

(A) Left: Topology of one voltage-gated ion channel subunit. Right: simplified

schematic of tetrameric voltage-gated ion channel viewed from the extracellular side. The four pore domains (S5-S6) form one single central pore, and the four individual VSDs control the gate of the pore. (B) Left: Topology of Ci-VSP. Middle: simplified schematic of monomeric Ci-VSP viewed from the extracellular side. Right: Cartoon of VSP's enzyme reaction with phosphoinositides compared with that of PTEN. Phosphates on the inositol ring are shown by the red circles. (C) Left: Topology of one Hv1/VOSP subunit. Middle: Model of dimeric Hv1/VOSP channel viewed from the extracellular side. Right: A cartoon representation of the Hv1 dimer.

Figure 2. Voltage-regulated GPCRs, CIVDS, and phosphoinositide metabolism.
(A) Left: Depolarization transiently and repeatedly enhances $P2Y_1$ receptor-evoked Ca^{2+} responses after exposure to the physiological agonist ADP (using data taken from (Gurung et al., 2008)). Middle: Topology of M_2 muscarinic receptor. Right: Gating current of M_2R, control and M_2R(D120N, R121N) (using data taken from (Ben-Chaim et al., 2006)). (B) Depolarization-induced capacitance changes (indicative of exocytosis) in DRG neurons in Ca^{2+}-containing and Ca^{2+}-free baths (using data taken from (Zhang and Zhou, 2002)). (C) Signaling transduction of voltage-dependent phosphoinositide metabolism.

Figure 1


A
Voltage-gated ion channel
Pore region
Extracellular
PM
Intracellular
P-loop
S1
S2
S3
S4
S5
S6
N
C
x 4
1
2
3
4
VSD
Pore
B
Ci-VSP
Extracellular
PM
Intracellular
S1
S2
S3
S4
N
PTEN-like Phosphatase
C
VSD
Phosphatase
PTEN
PM
PtdIns(3,4,5)P3
PtdIns(4,5)P2
Ci-VSP
PM
PtdIns(3,4,5)P3
PtdIns(4,5)P2
PtdIns(4)P
Depolarization
C
Hv1/VSOP
S242
Extracellular
PM
Intracellular
S1
S2
S3
S4
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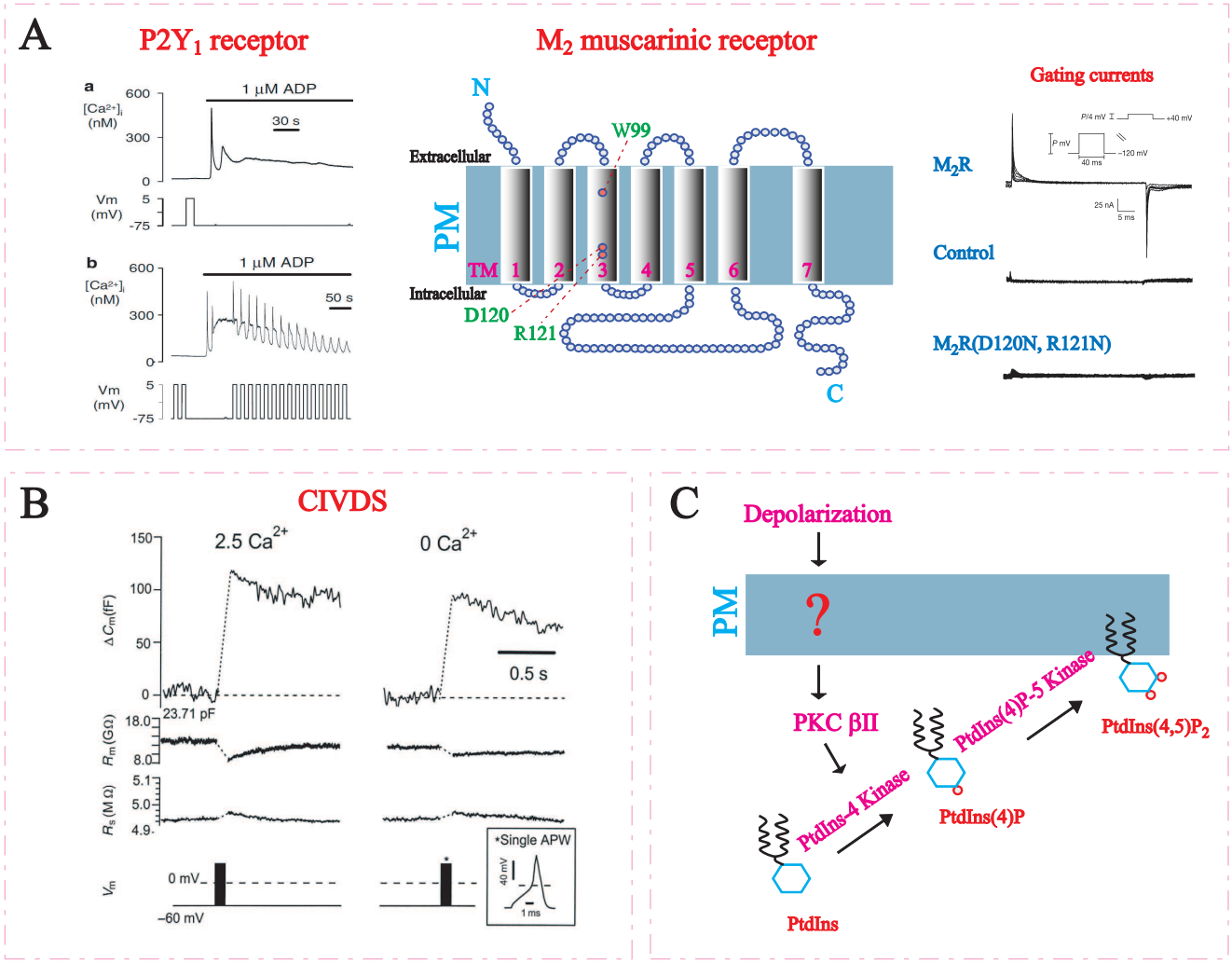
Coiled coil
C
x 2
Pore
1
2
VSD
PM
N
N
Coiled coil
C
C

Figure 2


A
P2Y1 receptor
M2 muscarinic receptor
Gating currents
a
1 μM ADP
30 s
[Ca2+]i (nM)
600
300
0
Vm (mV)
5
-75
b
1 μM ADP
50 s
N
W99
Extracellular
PM
TM 1 2 3 4 5 6 7
Intracellular
D120
R121
C
M2R
Control
M2R(D120N, R121N)
B
CIVDS
2.5 Ca2+
0 Ca2+
0.5 s
ΔCm(fF)
23.71 pF
Rm(GΩ)
Rs(MΩ)
Vm
0 mV
−60 mV
*Single APW
40 mV
1 ms
C
Depolarization
PM
?
PKC βII
PtdIns-4 Kinase
PtdIns(4)P-5 Kinase
PtdIns
PtdIns(4)P
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