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Review

Actin directly interacts with different membrane channel proteins and influences channel activities: AQP2 as a model☆

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ABSTRACT

The interplay between actin and 10 membrane channel proteins that have been shown to directly bind to actin are reviewed. The 10 membrane channel proteins covered in this review are aquaporin 2 (AQP2), cystic fibrosis transmembrane conductance regulator (CFTR), ClC2, short form of ClC3 (sClC3), chloride intracellular channel 1 (CLIC1), chloride intracellular channel 5 (CLIC5), epithelial sodium channel (ENaC), large-conductance calcium-activated potassium channel (Maxi-K), transient receptor potential vanilloid 4 (TRPV4), and voltage-dependent anion channel (VDAC), with particular attention to AQP2. In regard to AQP2, most reciprocal interactions between actin and AQP2 occur during intracellular trafficking, which are largely mediated through indirect binding. Actin and the actin cytoskeleton work as cables, barriers, stabilizers, and force generators for motility. However, as with ENaC, the effects of actin cytoskeleton on channel gating should be investigated further. This article is part of a Special Issue entitled: Reciprocal influences between cell cytoskeleton and membrane channels, receptors and transporters. Guest Editor: Jean Claude Hervé.

Contents

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1. Introduction

Actin is a major cytoskeleton protein found ubiquitously within the cells that is involved in almost all biological events, particularly events related to motility [1,2]. The production of membrane channel proteins involves the following sequential events: proteins are first produced in the endoplasmic reticulum (ER); then they are incorporated into the membrane of small vesicles, transported through the Golgi apparatus and trans-Golgi network, transferred to the sub-plasma membrane region of designated domains, introduced into a dense actin mesh beneath the plasma membrane, and lastly fused with the plasma membrane such that they appear on the surface. The membrane channel activities can be directly modulated while they are located at the surface, then the channels get endocytosed and pinched off from the plasma membrane, and finally transferred to a pool of recycling vesicles or lysosomes for degradation. In each of these sequential events, rapidly growing evidence indicates that actin and actin-based cytoskeleton complexes are involved. Molecular and proteomic studies have identified a large number of actin binding proteins, and expanded our understanding of functional interplay between membrane channel proteins and actin. The interplay can be by direct binding or indirect binding via actin binding proteins [1,3,4]. In this review, 10 membrane channel proteins that have been previously shown to directly bind to actin are selected and their binding characteristics are summarized (Table 1). We searched the PubMed database with the key words “actin and channel” and evaluated each report whether or not it concerned “direct binding”. Direct binding was judged by the presence of experiments with co-sedimentation, co-immunoprecipitation, gel overlay, surface plasmon resonance, fluorescence cross-correlation spectroscopy, FRET, and atomic force microscopy. Special attention is paid to AQP2, which serves as a model for direct and indirect binding of actin and membrane channel proteins.

Table 1
Membrane channel proteins directly bound to actin.

Channels	F- or G-actin	Detection methods	Physiological roles	References
AQP2	G-actin	Co-immunoprecipitation, co-immunoprecipitation, surface plasmon resonance, fluorescence cross-correlation spectroscopy	Exocytosis	Noda et al. [20,22], Moeller et al. [30]
CFTR	F-actin	Patch clump, atomic force microscopy	Channel activity	Cantiello et al. [42], Chasan et al. [43]
ClC2	F-actin	Gel overlay, co-sedimentation	Channel activity	Ahmed et al. [45]
sClC3	F-actin	Co-sedimentation	Channel activity?	McCloskey et al. [47]
CLIC1, CLIC5		In vitro reconstitution	Channel activity	Singh et al. [49]
ENaC	F-actin	In vitro reconstitution, co-immunoprecipitation, gel overlay, co-sedimentation	Channel activity	Berdiev et al. [57], Jovov et al. [58], Copeland et al. [59], Mazzochi et al. [60]
Maxi-K	F-actin?	Co-immunoprecipitation	Channel activity	Brainard et al. [64]
TRPV4	F-actin?	FRET, co-immunoprecipitation, co-sedimentation	Channel activity	Ramadass et al. [67], Goswami et al. [68], Shin et al. [69]
VDAC	G-actin	In vitro reconstitution, surface plasmon resonance	Channel activity	Xu et al. [70], Roman et al. [72]

F- or G-actin indicates the kind of binding actin; F for filamentatous and G for globular actin.

2. Actin and actin binding proteins

Actin is indispensably involved in cell shape and movement in all eukaryotic cells [5]. An actin monomer is a globular protein (G-actin) that contains one molecule of ATP or ADP. Its physiological function largely depends on the ability to polymerize and to form filamentous F-actin. F-actin consists of two strands characterized by a left-handed double helix. F-actin is polar in structure with a fast growing plus- or barbed-end and a minus- or pointed-end. The continuous occurrence of plus-end polymerization and minus-end depolymerization is called treadmilling. Actin binds a large number of actin-binding proteins, including proteins that initiate the nucleation of new actin filaments (Arp2/3 complex, WASP, formins); sequester monomeric actin (β-thymosins, profiling, cofilin); stabilize F-actin (tropomyosins); sever and cap F-actin (gelsolin, severin); crosslink F-actin (α-actinin, fimbrin), myosin filaments and motor proteins; and many others [1].

3. Direct or indirect binding

Membrane channel proteins can bind to actin directly or indirectly via actin-binding proteins. Given that the nature of actin is dynamic due to its continuous remodeling, the binding of actin to membrane channel proteins may not be static. This results in difficulty in examining the precise interaction between actin and membrane proteins. Nevertheless, many strategies have been employed to examine the binding of actin to membrane channel proteins, including co-sedimentation, co-immunoprecipitation, gel overlay, surface plasmon resonance, fluorescence cross-correlation spectroscopy, FRET, and atomic force microscopy. When the functional effects of bindings are considered, it is not a major issue whether binding of actin is direct or indirect via actin binding proteins. However, it is important when considering the precise mechanisms and the future development of drugs that interact the binding. The methodologies used to determine the direct binding of actin to each channel protein are summarized in Table 1. It is desirable that several methods are used to demonstrate direct binding, and indeed in most cases this has been done (Table 1).

4. AQP2: Intracellular trafficking is the main mechanism of regulation

AQP2 is a vasopressin-regulated water channel predominantly expressed in kidney collecting duct principal cells [6,7]. Upon vasopressin stimulation, water permeability of the collecting duct is dramatically increased, allowing significant water reabsorption and urine concentration. Gene mutations in AQP2 cause human nephrogenic diabetes insipidus, a disease characterized by the inability to concentrate urine, demonstrating the indispensable role of AQP2 in urine concentration [7]. Circulating vasopressin binds to vasopressin V2 receptor expressed on the basolateral membrane of collecting duct cells, stimulates adenylate cyclase, and increases intracellular cAMP levels. Increased cAMP then activates protein kinase A, which phosphorylates the AQP2 protein itself, and this phosphorylation in turn induces accumulation of AQP2 on the luminal surface via a balance between exocytosis and endocytosis [8,9]. Thus, the main regulatory mechanism of AQP2 is intracellular trafficking, where both actin and actin binding proteins are involved.

4.1. Transport to the subapical region of the luminal membrane

Newly produced AQP2 is transported from the ER to the subapical region of the apical membrane. AQP2, like other proteins, carries signals embedded within its structure, which specify its subcellular destinations and micro-domains [10]. AQP2 was thought to be delivered directly to the apical membrane; however, Yui et al. [11] recently showed that a considerable amount of AQP2 first travels to the basolateral membrane and then back to the apical membrane via a

process known as "transcytosis". The physiological significance of this needs to be further clarified.

Intracellular trafficking of AQP2 vesicles requires cables, motor proteins and other cargo associated (adapter) proteins. Findings from proteomic analysis of intracellular AQP2 vesicles provide useful information [12]. Immunoisolated AQP2-bearing intracellular vesicles from rat inner medullary collecting ducts show the presence of a large variety of proteins, including actin cytoskeletal proteins (i.e., β- and γ-actin, ARP2/3, destrin, gelsolin, β-spectrin-3, α-tropomyosin), myosin isoforms, tubulin, Rab GTPases, and SNARE proteins. The identified myosin isoforms include myosin regulatory light chain and conventional non-muscle myosin IIA and IIB as well as unconventional myosin 1C, VI, and IXB. All of these proteins together with actin fibers may participate in vesicle trafficking. It is important to note that the origin of vesicles could be heterogeneous, including vesicles during all stages of trafficking, and all these interacting proteins do not necessarily co-exist at the same time.

4.2. Exocytosis

Transported AQP2 vesicles are stored beneath the apical membrane and are characterized by Rab11 staining [13]. While these storage vesicles undergo exocytosis and fuse with the apical membrane after vasopressin stimulation, exocytosis occurs constitutively to a certain extent. The actin cytoskeleton has been thought of as a barrier for AQP2 exocytosis. Vasopressin-induced depolymerization of apical F-actin in rat inner medullary collecting duct supports this theory [14]. Tamma et al. [15] demonstrated that cytochalasin D, an F-actin depolymerizing agent, induces AQP2 plasma membrane accumulation in cultured collecting duct cells. RhoA and its downstream effecter Rho kinase are known to stimulate actin polymerization and indeed, the inhibition of this cascade by *Clostridium difficile* toxin B or by Y-27632 results in AQP2 accumulation in the plasma membrane [15,16], confirming the barrier function of F-actin during exocytosis.

Recent observations from experiments performed in adrenomedullary chromaffin cells (reviewed by Gutierrez [17]) seem highly relevant in examining the interplay between AQP2 exocytosis and actin. Advancements in microscopic technologies (i.e., use of high numerical aperture objectives in transmitted light scanning microscopy and three-dimensional reconstruction, and total internal reflection fluorescence microscopy) allow for the analysis of fine structures of living cells. Studies using novel techniques revealed that the cell cortex consists of a dense network of F-actin that blocks the access of vesicles to their secretory sites. After stimulation, dynamic changes occur in these dense cytoskeletal structures with the formation cortical disruption of F-actin mesh and channel-like structures, which allow vesicles to access their docking sites [18]. Thus, depolymerization of F-actin is necessary to clear the route toward the cell surface; however, positive participation of actin in the movement of secretory vesicles toward the cell surface is necessary. Actin forces vesicles to move toward the cell surface by the force generating mechanisms: 1) polymerization and creating new actin fibers, and 2) interactions with myosin motor proteins [19].

Similar mechanisms are expected to take place in the exocytosis of AQP2 vesicles. To clarify the molecular mechanisms involved in AQP2 trafficking, Noda et al. [20,21] isolated proteins that directly bind to AQP2. Isolation via immunoaffinity chromatography covalently coupled with anti-AQP2 antibody and subsequent analysis by MALDI-TOF MS identified 13 new AQP2-binding proteins; namely, actin, SPA-1, tropomyosin 5b (later classified as an isoform of tropomyosin 1), ionized calcium binding adapter molecule 2, myosin regulatory light chain smooth muscle isoforms 2-A and 2-B, annexins A2 and A6, scinderin, gelsolin, alpha-actinin 4, alpha-II spectrin, and myosin heavy chain nonmuscle type A. The region to which actin binds was determined to be at the C-terminus [20]. Since most identified proteins have actin binding ability, it was speculated that a part of these proteins may indirectly associate with AQP2 via actin, and that these proteins create AQP2-binding protein complexes. Thus, dynamic interactions of these proteins are expected to regulate AQP2 trafficking [10,19].

Studies have shown that both α- and γ-actin bind to the C-terminus of AQP2, and furthermore, that G-actin binds to AQP2, whereas F-actin does not [20]. Further studies by Noda et al. [22] showed that phosphorylation of AQP2 via cAMP dissociates G-actin from AQP2, and that phosphorylated AQP2 increases its affinity to tropomyosin 5b, resulting in reduction of tropomyosin 5b bound to F-actin, which subsequently induces F-actin destabilization and depolymerization. These findings indicate that the transport membrane protein, AQP2, per se works to reorganize its surrounding actin cytoskeleton to open up its way to the cell surface. Consistent with this idea, the presence of AQP2 is necessary for vasopressin-mediated burst of exocytosis and cortical F-actin depolymerization in cultured renal epithelial cells [23,24].

Another study performed by Zwang et al. [25] isolated AQP2-binding proteins via proteomic analysis of pulled-down samples that were bound to a synthesized AQP2 C-terminus. Consequently, seven proteins were identified: heat shock protein 70 (isoforms 1, 2, 5, 8), annexin II, protein phosphatase 1 catalytic subunit (PP1c), GDP dissociation inhibitor 2 (GDI-2), and Ras-related nuclear protein (RAN). Although these proteins do not overlap with those found by Noda et al. [21], the difference may be explained by the use of different methodologies. Nonetheless, the important message from these proteomic analyses of AQP2-binding proteins is that membrane channel proteins have a large number of partners that directly or indirectly bind to them and are involved in their trafficking.

4.3. Endocytosis and recycling

The abundance of AQP2 at the surface of the plasma membrane depends on the balance between continuing endocytosis and exocytosis. These two opposing mechanisms occur constitutively at a significant rate even without vasopressin stimulation [26]. With respect to endocytosis, AQP2 accumulates in clathrin-coated pits and is internalized via a clathrin-mediated process. Indeed, accumulation of AQP2 is observed when actin-mediated endocytosis is inhibited by a GTPase-deficient dominant negative dynamin mutant [27] and a cholesterol-depleting agent, methyl-beta-cyclodextrin [28]. The heat shock protein, hsc70, an actin-binding protein that is important for uncoating clathrin-coated vesicles, has been shown to bind to the C-terminus of non-phosphorylated AQP2 and contribute to AQP2 endocytosis [29].

In cultured MDCK cells, Moeller et al. [30] demonstrated that internalization of a mutant AQP2, which mimics phosphorylated-AQP2 is slower than that of a wild-type AQP2. Further a co-immunoprecipitation study showed that the slower internalization corresponds to a reduced interaction of the mutant AQP2 with several proteins involved in endocytosis, namely Hsp70, Hsc70, dynamin, and clathrin heavy chain, indicating that these proteins participate in endocytosis of AQP2. Interestingly, in the same study, a strong binding of γ-actin to the non-phosphorylated mimicking AQP2 peptide was observed, whereas binding to the phosphorylated mimicking AQP2 was weak, indicating that γ-actin is released from AQP2 after it is phosphorylated [30]. This observation is consistent with the view of Noda et al. [22] described above in which the binding of actin works as stabilizers of AQP2 to keep AQP2 away from exocytic process.

Following endocytosis, AQP2 is retrieved to EEA1-positive early endosomes through a phosphatidylinositol-3-kinase-dependent mechanism and then is transferred to Rab11-positive storage vesicles [13]. This transfer is believed to be mediated by actin filaments because the disruption of actin filaments with cytochalasin D or latrunculin B induces the accumulation of AQP2 in EEA1-positive early endosomes [31]. Rab11-positive storage vesicles are specific to AQP2 and may exclude other membrane transport proteins. Furthermore, AQP2 in the storage vesicles reappears on the apical membrane upon vasopressin stimulation [13]. Ubiquitination is known to promote endocytosis and subsequent degradation by multivesicular bodies or proteasomes. Ubiquitination at

lysine 270 of AQP2 is important for AQP2 endocytosis and degradation [32].

4.4. Regulation of channel function

There is controversy concerning whether phosphorylation of AQP2 per se regulates the water permeability of the individual AQP2 molecule. An earlier study in a *Xenopus* oocyte expression system showed that phosphorylation of AQP2 increases the osmotic water permeability (Pf) of oocytes by 1.6-fold without a significant increase in AQP2 protein on the oocyte surface [33]. However, this finding was not supported by subsequent studies by other groups using a similar method [34,35]. Consistent with the negative results, Lande et al. [36] showed no significant differences in Pf values of phosphorylated and dephosphorylated AQP2-rich endosomes prepared from rat inner medullary collecting ducts. However, in a recent study, Eto et al. [37] reconstituted recombinant human AQP2 in proteoliposomes, and showed that the Pf of proteoliposomes was enhanced approximately 2-fold by phosphorylation at serine 256, indicating that AQP2 water channel activity (possibly open probability) is directly regulated by its phosphorylation. There is increasing evidence that aquaporins are gated [38]. X-ray crystal structure analysis of the spinach aquaporin SoPIP2;1 suggests that in the closed conformation, a cytosolic loop connecting α helices caps the pore of the channel from the cytoplasm, preventing water passage, and in the open conformation, the loop is displaced [39]. Interestingly, the presence of a layer of densities sandwiched between single-layered crystal sheets of AQP2 has been observed and has been interpreted to be the N- and/or C-termini of AQP2, suggesting the possibility that the N- and/or C-termini of AQP2 function as the gate of the channel [40]. Given that the C-terminus of AQP2 binds to many proteins, including actin as described above, regulation of the channel activity (the open probability) of AQP2 together with interacting proteins should be examined further.

As seen above, there are many mutual influences between AQP2 and the actin cytoskeleton through direct and indirect bindings. Most influences occur in the trafficking process, which may also apply to other membrane channel proteins.

5. Other membrane channel proteins directly bound to actin

Most interactions of channels with actin cytoskeleton are mediated by indirect bindings through acting-binding proteins including scaffolding proteins. However, several proteins in addition to AQP2 have been reported to directly bind to actin. Table 1 summarizes these proteins. Below is a brief description of how their bindings were determined and of their functional roles.

5.1. Cystic fibrosis transmembrane conductance regulator (CFTR)

CFTR (cystic fibrosis transmembrane conductance regulator) is localized in the apical plasma membrane of many epithelia, including the airway and sweat gland, and functions as a regulated chloride channel. Extensive studies have been performed to decipher its regulatory mechanisms, as genetic mutations of CFTR result in the most common channelopathy, cystic fibrosis [41]. Cantiello [42] showed that when actin is added to the cytoplasmic side of excised inside-out patches obtained from a CFTR-expressing cell line, the CFTR current is activated. In contrast, the same treatment on the patches from the parental cell line does not induce a chloride current. Additionally, when cells are exposed to cytochalasin D, protein kinase A-activated CFTR current does not occur; however, it can be reversed by the addition of actin [42].

Chasan et al. [43] used atomic force microscopy (AFM) to visualize a direct interaction between actin and CFTR. Purified functional CFTR that had been reconstituted into phospholipid vesicles was placed on freshly cleaved mica and observed by AFM. After adding actin to the sample, AFM images showed the formation of filamentous actin associating with the CFTR molecules, indicating that there is a direct interaction between F-actin and CFTR. Taken together, the results of electrophysiological studies (patch clump) and AFM studies suggest that direct binding of CFTR with actin modulates the channel activity of CFTR; however, two reservations remain: 1) the above studies used native cell-derived materials, and thus, the co-segregation of actin binding proteins cannot be denied; and 2) biochemical data supporting a direct interaction between the two proteins, such as co-immunoprecipitation assays, are lacking.

5.2. ClC2 and sClC3

Both ClC2 and sClC3 (short isoform of ClC3) belong to the family of voltage-regulated chloride channels and show characteristics of hypotonic cell swelling-sensitive channels. Activation of these channels has been shown to contribute to the maintenance of physiological cell volume and fluid secretion by airway and gastric mucosa epithelia [44]. These channels have different biophysical properties, namely ClC2 exhibits inward rectification, whereas sClC3 exhibits outward rectification. Remodeling of the actin cytoskeleton has been shown to modulate the activation of ClC2 in *Xenopus* oocytes [45] and sClC3 during hypotonic stress of pulmonary artery smooth muscle cells [46] by using actin-disrupting agents.

Ahmed et al. [45] showed that a GST fusion protein containing the N-terminus of ClC2, which is known as an inhibitory domain, is capable of binding F-actin in overlay and co-sedimentation assays. The binding of actin to this synthetic peptide is expected to be mediated through electrostatic interactions because binding is inhibited at high concentrations of NaCl. This work suggests that actin directly and electrostatically binds to the N-terminus of ClC-2 and contributes to the regulation of this channel [45].

McCloskey et al. [47] also used a GST-tagged C-terminus of sClC3 to co-sediment actin. These assays demonstrated strong binding between the C-terminus of sClC3 to F-actin, but not to G-actin. Using co-sedimentation assays, F-actin binding region was determined to be located between amino acids 690 and 760 of sClC3. Furthermore, synthetic peptides corresponding to the actin-binding region prevented the binding of F-actin in vitro and greatly reduced the maximal current density of hypotonic stress-induced currents. The authors interpreted these findings to indicate that sClC3 is the major source of the hypotonic stress-induced, volume-sensitive outwardly rectifying anion currents, and the channel is regulated by direct interaction with F-actin [47].

5.3. CLIC1 and CLIC5

The chloride intracellular channel (CLIC) proteins are anion channels located intracellularly that do not fit the characteristics of classical ion channel proteins, as they have soluble and integral membrane forms with ion channel function. While CLIC proteins are highly conserved in vertebrates and there are six distinct isoforms (CLIC1–CLIC6), their physiological functions are largely unknown and knockout mice of these isoforms do not produce apparent defects [48].

Singh et al. [49] purified recombinant mammalian CLIC1, CLIC4 and CLIC5, and incorporated them into planar lipid bilayers. CLIC-induced membrane conductance was observed after the incorporation of CLIC proteins. However, F-actin added to the cytosolic side reversibly inhibited the membrane conductance in CLIC1 and CLIC5 membranes, whereas there was no effect on conductance in the CLIC4 membrane. This inhibitory effect could be reversed by cytochalasins, indicating the involvement of F-actin. These results suggest that the membrane form of CLICs modifies solute transport through interaction with actin and contributes to many cellular activities, including swelling or division of the cell, endocytosis and exocytosis, intracellular vesicle fusion, and apoptosis [49].

5.4. ENaC

ENaC is an amiloride-sensitive epithelial sodium channel consisting of a heteromultimer composed of three homologous but distinct subunits (α, β, and γ) that are encoded by different genes [50]. ENaC is expressed in the epithelia of the distal nephron, distal colon, airway, and other tissues where aldosterone stimulates sodium reabsorption. Interestingly, in the distal nephron, ENaC is co-localized with AQP2 except for the inner medullary collecting duct, where ENaC is absent [51]. Aldosterone-regulated sodium reabsorption in the kidney plays a central role in the maintenance of body fluid and blood pressure. It is also known that vasopressin stimulates ENaC-mediated sodium reabsorption significantly, but to a lesser extent, in humans [52].

There are several mechanisms responsible for the hormone-induced stimulation of ENaC. Available evidence suggests that most of the effects are due to an increased abundance of ENaC proteins at the apical surface of distal nephrons, which are produced by both the stimulated transcription of the gene and dysregulated trafficking of ENaC to and from the apical membrane [53]. Regulation via channel gating (changing open probability) may not be dominant in the regulation of ENaC activity, although some controversy regarding this remains [54]. Endocytosis of ENaC determines the apical surface expression of ENaC, and this process is accelerated by binding to Nedd4-2, which results in ubiquitin-dependent internalization (clathrin-mediated endocytosis) and degradation. Thus, both aldosterone and vasopressin phosphorylate Nedd4-2, which in turn impairs the binding of ENaC to Nedd4-2, thereby increasing the protein abundance of ENaC and sodium reabsorption [53].

Similar to other channel proteins, ENaC proteins travel within the cell from its site of production to final degradation, and during this process, interact with actin (mostly indirect) [55,56]. However, there are several lines of evidence that ENaC also interacts directly with actin. Berdiev et al. [57] demonstrated that short F-actin causes a two-fold decrease in unitary conductance and a two-fold increase in the open probability of recombinant αβγ-ENaC subunits reconstituted into planar lipid bilayers. The same group further defined the region of the C-terminus participating in actin-mediated regulation of α-ENaC, which is a 14-amino acid residue region between E631 and F644 [58,59].

To confirm these electrophysiological results, Mazzochi et al. [60] provided biochemical evidence for a direct interaction between F-actin and the C-terminus of α-ENaC. F-actin was found to co-immunoprecipitate with α-ENaC from whole cell lysate of MDCK cells stably transfected with three isoforms of ENaC. Gel overlay assays demonstrated that F-actin specifically binds to the C-terminus of α-ENaC. A direct interaction between F-actin and the C-terminus of α-ENaC was further corroborated by GST-tagged F-actin co-sedimentation studies. These data indicate that F-actin directly and specifically binds to ENaC and changes its channel activity, presumably the gating. Recently, even actin-binding proteins such as filamin and cortactin were shown to modulate the gating of ENaC [61,62]. Therefore, the importance of actin cytoskeleton binding either directly or indirectly in the channel gating of ENaC should be investigated further.

5.5. Maxi-K

The maxi-K channel, also called the big potassium (BK) channel or slo1, is large-conductance, voltage- and calcium-sensitive potassium channels. Maxi-K channels are formed by 2 subunits: the pore-forming α-subunit (KCNMA1) and the modulatory β-subunit. Intracellular calcium regulates the physical association between the α- and β-subunits. This channel creates a strong repolarizing current to counter-balance cell excitation in response to stimuli, and plays an essential role in the regulation of several key physiological processes, including smooth muscle tone and neuronal excitability [63].

Brainard et al. [64] found large amount of maxi-K channel proteins in the detergent-resistant membrane fractions (lipid raft microdomains) from human myometrium. Direct binding of actin to maxi-K was demonstrated by co-immunoprecipitation studies; both α- and γ-actin bind to it. Immunocytochemical and immunoelectron microscopy showed the proximity of both actin and the maxi-K channel within the same cell surface caveolar structures. Functionally, disruption of the actin cytoskeleton in cultured human myocardium smooth muscle cells by cytochalasins greatly increased the open-state probability of the channel, while stabilization of the actin cytoskeleton with jasplakinolide abolished this effect. These data indicate that direct interaction with actin cytoskeleton is important in the regulation of maxi-K channel function [64].

5.6. TRPV4

The transient receptor potential vanilloid 4 (TRPV4) cation channel, a member of the TRP vanilloid subfamily, is expressed in a wide range of tissues, where it contributes to the generation of a calcium signal and the depolarization of the membrane potential. The channel is activated by hypotonic cell swelling and other physical stimuli (shear stress and high temperature), and by many endogenous and synthetic ligands (arachidonic acids and phorbol esters) [65]. The importance of a functional interaction between TRPV4 and actin for the sensing of hypotonicity and the onset of regulatory volume decrease has been suggested in kidney collecting ducts where TRPV4 is located and may contribute to the concentration of hypotonic prourine [66].

Several proteins have been proposed to modulate TRPV4 subcellular localization and/or function. Ramadass et al. [67] used Forster resonance energy transfer (FRET) to examine the interaction of TRPV4 with actin. FRET is a nonradiative phenomenon between two fluorochromes requiring the emission spectra of the donor molecule overlapping with the absorption spectrum of the acceptor molecule. FRET only occurs when the interacting molecules are at a distance of 1–10 nm in living cells. In cultured CHO cells expressing both TRPV4-CFP and actin-YFP, excited donor molecules (CFP) exhibit an emission peak at 527 nm and a decreased lifetime between 460 and 490 nm, which corresponds to the resonance energy transfer to YFP. The observed difference in FRET efficiency indicates that the two fluorescent proteins are close in the range of 4 nm [67]. Thus, this study shows that TRPV4 and actin intimately associate with each other in living cells.

Goswami et al. [68] provided biological evidence for the direct binding of these two proteins by showing co-immunoprecipitation of TRPV4 and actin from cell lysate of CHO-TRPV4 stable cell line. They further showed that the C-terminus is the domain for actin binding. Similarly, using co-immunoprecipitation and co-sedimentation assays, Shin et al. [69] compared the bindings of actin with TRPV4 mutants and showed that phosphorylation on the Ser 824 residue of TRPV4 is required for its interaction with F-actin, which promotes channel activity, Ca^{2+} influx, and protein stability.

5.7. VDAC

The mitochondrial outer membrane is the interface between the mitochondria and the cytosol, and voltage-dependent anion channel (VDAC) is abundantly present in this membrane. There are three isoforms, specifically VDAC1–3, and almost all tissues express all three isoforms. VDAC is involved in the main pathways for ATP, ADP, and other mitochondrial metabolic substrates, and it performs multiple functions such as metabolite and energy exchange and apoptosis. Recent findings suggest that VDAC is regulated by cytosolic proteins, including actin [70,71].

Xu et al. [70] reconstituted purified yeast VDAC into membranes made with phosphatidylcholine. The addition of a very low concentration of actin (50 nM) to the bathing solution was enough to abolish most of the VDAC-mediated membrane conductance. The addition of α-actin had a stronger effect than β- and γ-actin had and the form of G-actin was effective, whereas F-actin was ineffective. Roman et al. [72] adopted another technology, surface plasmon resonance to examine

the interactions between VDAC and actin. They showed that rabbit muscle G-actin binds to immobilized yeast VDAC in a reversible and dose-dependent manner, confirming their direct binding; however, the role of this binding requires further investigation.

6. Concluding remarks

Herein, we reviewed the interplay between actin and membrane channel proteins, with a particular focus on their direct binding properties. As highlighted in AQP2, most reciprocal influences occur during intracellular trafficking of channel proteins, and the interactions are largely mediated through indirect binding. Although molecular and proteomic studies together with advancements in microscopic technologies have expanded our understanding of the interplay, further characterizations of the interplay with higher spatial and temporal resolutions are necessary. Further knowledge will allow for innovative new therapeutic options, including drugs that disrupt the bindings.

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References

[1] J. Saarikangas, H. Zhao, P. Lappalainen, Regulation of the actin cytoskeleton–plasma membrane interplay by phosphoinositides, Physiol. Rev. 90 (2010) 259–289.
[2] M. Anitei, B. Hoflack, Bridging membrane and cytoskeleton dynamics in the secretory and endocytic pathways, Nat. Cell Biol. 14 (2012) 11–19.
[3] S. Ono, Mechanism of depolymerization and severing of actin filaments and its significance in cytoskeletal dynamics, Int. Rev. Cytol. 258 (2007) 1–82.
[4] M.A. Chesarone, B.L. Goode, Actin nucleation and elongation factors: mechanisms and interplay, Curr. Opin. Cell Biol. 21 (2009) 28–37.
[5] T.D. Pollard, J.A. Cooper, Actin, a central player in cell shape and movement, Science 326 (2009) 1208–1212.
[6] K. Fushimi, S. Uchida, Y. Hara, Y. Hirata, F. Marumo, S. Sasaki, Cloning and expression of apical membrane water channel of rat kidney collecting tubule, Nature 361 (1993) 549–552.
[7] S. Sasaki, Aquaporin 2: from its discovery to molecular structure and medical implications, Mol. Aspects Med. 33 (2012) 535–546.
[8] D. Brown, U. Hasler, P. Nunes, R. Bouley, H.A. Lu, Phosphorylation events and the modulation of aquaporin 2 cell surface expression, Curr. Opin. Nephrol. Hypertens. 17 (2008) 491–498.
[9] H.B. Moeller, E.T. Olesen, R.A. Fenton, Regulation of the water channel aquaporin-2 by posttranslational modification, Am. J. Physiol. Renal Physiol. 300 (2011) F1062–F1073.
[10] S. Sasaki, Y. Noda, Aquaporin-2 protein dynamics within the cell, Curr. Opin. Nephrol. Hypertens. 16 (2007) 348–352.
[11] N. Yui, H.A. Lu, Y. Chen, N. Nomura, R. Bouley, D. Brown, Basolateral targeting and microtubule-dependent transcytosis of the aquaporin-2 water channel, Am. J. Physiol. Cell Physiol. 304 (2013) C38–C48.
[12] M. Barile, T. Pisitkun, M.J. Yu, C.L. Chou, M.J. Verbalis, R.F. Shen, M.A. Knepper, Large scale protein identification in intracellular aquaporin-2 vesicles from renal inner medullary collecting duct, Mol. Cell Proteomics 4 (2005) 1095–1106.
[13] Y. Tajika, T. Matsuzaki, T. Suzuki, T. Aoki, H. Hagiwara, M. Kuwahara, S. Sasaki, K. Takata, Aquaporin-2 is retrieved to the apical storage compartment via early endosomes and phosphatidylinositol 3-kinase-dependent pathway, Endocrinology 145 (2004) 4375–4383.
[14] H. Simon, Y. Gao, N. Franki, R.M. Hays, Vasopressin depolymerizes apical F-actin in rat inner medullary collecting duct, Am. J. Physiol. 265 (1993) C757–C762.
[15] G. Tamma, E. Klussmann, K. Maric, K. Aktories, M. Svelto, W. Rosenthal, G. Valenti, Rho inhibits cAMP-induced translocation of aquaporin-2 into the apical membrane of renal cells, Am. J. Physiol. Renal Physiol. 281 (2001) F1092–F1101.
[16] E. Klussmann, G. Tamma, D. Lorenz, B. Wiesner, K. Maric, F. Hofmann, K. Aktories, G. Valenti, W. Rosenthal, An inhibitory role of Rho in the vasopressin-mediated translocation of aquaporin-2 into cell membranes of renal principal cells, J. Biol. Chem. 276 (2001) 20451–20457.
[17] L.M. Gutierrez, New insights into the role of the cortical cytoskeleton in exocytosis from neuroendocrine cells, Int. Rev. Cell Mol. Biol. 295 (2012) 109–137.
[18] D. Giner, P. Neco, M.el.M. Frances, I. Lopez, S. Viniegra, L.M. Gutierrez, Real-time dynamics of the F-actin cytoskeleton during secretion from chromaffin cells, J. Cell Sci. 118 (2005) 2871–2880.
[19] Y. Noda, S. Sasaki, The role of actin remodeling in the trafficking of intracellular vesicles, transporters, and channels: focusing on aquaporin-2, Pflugers Arch. 456 (2008) 737–745.
[20] Y. Noda, S. Horikawa, Y. Katayama, S. Sasaki, Water channel aquaporin-2 directly binds to actin, Biochem. Biophys. Res. Commun. 322 (2004) 740–745.
[21] Y. Noda, S. Horikawa, Y. Katayama, S. Sasaki, Identification of a multiprotein "motor" complex binding to water channel aquaporin-2, Biochem. Biophys. Res. Commun. 330 (2005) 1041–1047.
[22] Y. Noda, S. Horikawa, E. Kanda, M. Yamashita, H. Meng, K. Eto, Y. Li, M. Kuwahara, K. Hirai, C. Pack, M. Kinjo, S. Okabe, S. Sasaki, Reciprocal interaction with G-actin and tropomyosin is essential for aquaporin-2 trafficking, J. Cell Biol. 182 (2008) 587–601.
[23] P. Nunes, U. Hasler, M. McKee, H.A. Lu, R. Bouley, D. Brown, A fluorimetry-based ssYFP secretion assay to monitor vasopressin-induced exocytosis in LLC-PK1 cells expressing aquaporin-2, Am. J. Physiol. Cell Physiol. 295 (2008) C1476–C1487.
[24] N. Yui, H.J. Lu, R. Bouley, D. Brown, AQP2 is necessary for vasopressin- and forskolin-mediated filamentous actin depolymerization in renal epithelial cells, Biol. Open 1 (2012) 101–108.
[25] N.A. Zwang, J.D. Hoffert, T. Pisitkun, H.B. Moeller, R.A. Fenton, M.A. Knepper, Identification of phosphorylation-dependent binding partners of aquaporin-2 using protein mass spectrometry, J. Proteome Res. 8 (2009) 1540–1554.
[26] D. Brown, R. Bouley, T.G. Paunescu, S. Breton, H.A. Lu, New insights into the dynamic regulation of water and acid–base balance by renal epithelial cells, Am. J. Physiol. Cell Physiol. 302 (2012) C1421–C1433.
[27] T.X. Sun, A. Van Hoek, Y. Huang, R. Bouley, M. McLaughlin, D. Brown, Aquaporin-2 localization in clathrin-coated pits: inhibition of endocytosis by dominant-negative dynamin, Am. J. Physiol. Renal Physiol. 282 (2002) F998–F1011.
[28] L.M. Russo, M. McKee, D. Brown, Methyl-beta-cyclodextrin induces vasopressin-independent apical accumulation of aquaporin-2 in the isolated, perfused rat kidney, Am. J. Physiol. Renal Physiol. 291 (2006) F246–F253.
[29] H.A. Lu, T.X. Sun, T. Matsuzaki, X.H. Yi, J. Eswara, R. Bouley, M. McKee, D. Brown, Heat shock protein 70 interacts with aquaporin-2 and regulates its trafficking, J. Biol. Chem. 282 (2007) 28721–28732.
[30] H.B. Moeller, J. Praetorius, M.R. Rutzler, R.A. Fenton, Phosphorylation of aquaporin-2 regulates its endocytosis and protein–protein interactions, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 424–429.
[31] Y. Tajika, T. Matsuzaki, T. Suzuki, A. Ablimit, T. Aoki, H. Hagiwara, M. Kuwahara, S. Sasaki, K. Takata, Differential regulation of AQP2 trafficking in endosomes by microtubules and actin filaments, Histochem. Cell Biol. 124 (2005) 1–12.
[32] E.J. Kamsteeg, G. Hendriks, M. Boone, I.B.M. Konings, V. Oorschot, P.D. Sluijs, J. Klumperman, P.M.T. Deen, Short-chain ubiquitination mediates the regulated endocytosis of the aquaporin-2 water channel, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 18344–18349.
[33] M. Kuwahara, K. Fushimi, Y. Terada, L. Bai, F. Marumo, S. Sasaki, cAMP-dependent phosphorylation stimulates water permeability of aquaporin-collecting duct water channel protein expressed in *Xenopus* oocytes, J. Biol. Chem. 270 (1995) 10384–10387.
[34] E.J. Kamsteeg, I. Heijnen, C.H. van Os, P.M. Deen, The subcellular localization of an aquaporin-2 tetramer depends on the stoichiometry of phosphorylated and nonphosphorylated monomers, J. Cell Biol. 151 (2000) 919–930.
[35] H.B. Moeller, N. MacAulay, M.A. Knepper, R.A. Fenton, Role of multiple phosphorylation sites in the COOH-terminal tail of aquaporin-2 for water transport: evidence against channel gating, Am. J. Physiol. Renal Physiol. 296 (2009) F649–F657.
[36] M.B. Lande, I. Jo, M.L. Zeidel, M. Somers, H.W. Harris, Phosphorylation of aquaporin-2 does not alter the membrane water permeability of rat papillary water channel-containing vesicles, J. Biol. Chem. 271 (1996) 5552–5557.
[37] K. Eto, Y. Noda, S. Horikawa, S. Uchida, S. Sasaki, Phosphorylation of aquaporin-2 regulates its water permeability, J. Biol. Chem. 285 (2010) 40777–40784.
[38] K. Hedfalk, S. Tornroth-Horsefield, M. Nyblom, U. Johanson, P. Kjellbom, R. Neutze, Aquaporin gating, Curr. Opin. Struct. Biol. 16 (2006) 447–456.
[39] S. Tornroth-Horsefield, Y. Wang, K. Hedfalk, U. Johanson, M. Karlsson, E. Tajkhorshid, R. Neutze, P. Kjellbom, Structural mechanism of plant aquaporin gating, Nature 439 (2006) 688–694.
[40] A.D. Schenk, P.J. Werten, S. Scheuring, B.L. de Groot, S.A. Muller, H. Stahlberg, A. Philippsen, A. Engel, The 4.5 Å structure of human AQP2, J. Mol. Biol. 350 (2005) 278–289.
[41] M.P. Rogan, D.A. Stoltz, D.B. Hornick, Cystic fibrosis transmembrane conductance regulator intracellular processing, trafficking, and opportunities for mutation-specific treatment, Chest 139 (2011) 1480–1490.
[42] H.F. Cantiello, Role of the actin cytoskeleton in the regulation of the cystic fibrosis transmembrane conductance regulator, Exp. Physiol. 81 (1996) 505–514.
[43] B. Chasan, N.A. Geisse, K. Pedatella, D.G. Wooster, M. Teintze, M.D. Carattino, W.H. Goldmann, H.F. Cantiello, Evidence for direct interaction between actin and the cystic fibrosis transmembrane conductance regulator, Eur. Biophys. J. 30 (2002) 617–624.
[44] T. Furukawa, T. Ogura, Y. Katayama, M. Hiraoka, Characteristics of rabbit ClC-2 current expressed in *Xenopus* oocytes and its contribution to volume regulation, Am. J. Physiol. 274 (1998) C500–C512.
[45] N. Ahmed, M. Ramjeesingh, S. Wong, A. Varga, E. Garami, C.E. Bear, Chloride channel activity of ClC-2 is modified by the actin cytoskeleton, Biochem. J. 352 (2000) 789–794.
[46] G.X. Wang, Y.P. Dai, S. Bongalon, W.J. Hatton, K. Murray, J.R. Hume, I.A. Yamboliev, Hypotonic activation of volume-sensitive outwardly rectifying anion channels (VSOACs) requires coordinated remodeling of subcortical and perinuclear actin filaments, J. Membr. Biol. 208 (2005) 15–26.
[47] D.T. McCloskey, L. Doherty, Y.P. Dai, L. Miller, J.R. Hume, I.A. Yamboliev, Hypotonic activation of short ClC3 isoform is modulated by direct interaction between its cytosolic C-terminal tail and subcortical actin filaments, J. Biol. Chem. 282 (2007) 16871–16877.
[48] D.R. Littler, S.J. Harrop, S.C. Goodchild, J.M. Phang, A.V. Mynott, L. Jiang, S.M. Valenzuela, M. Mazzanti, L.J. Brown, S.N. Breit, P.M. Curmi, The enigma of the

CLIC proteins: ion channels, redox proteins, enzymes, scaffolding proteins? FEBS Lett. 584 (2010) 2093–2101.
[49] H. Singh, M.A. Cousin, R.H. Ashley, Functional reconstitution of mammalian 'chloride intracellular channels' CLIC1, CLIC4 and CLIC5 reveals differential regulation by cytoskeletal actin, FEBS J. 274 (2007) 6306–6316.
[50] C.M. Canessa, L. Schild, G. Buell, B. Thorens, I. Gautschi, J.D. Horisberger, B.C. Rossier, Amiloride-sensitive epithelial Na+ channel is made of three homologous subunits, Nature 367 (1994) 463–467.
[51] C. Duc, N. Farman, C.M. Canessa, J.P. Bonvalet, B.C. Rossier, Cell-specific expression of epithelial sodium channel alpha, beta, and gamma subunits in aldosterone-responsive epithelia from the rat: localization by in situ hybridization and immunocytochemistry, J. Cell Biol. 127 (1994) 1907–1921.
[52] L. Bankir, S. Fernandes, P. Bardoux, N. Bouby, D.G. Bichet, Vasopressin-V2 receptor stimulation reduces sodium excretion in healthy humans, J. Am. Soc. Nephrol. 16 (2005) 1920–1928.
[53] P.M. Snyder, Minireview: regulation of epithelial Na+ channel trafficking, Endocrinology 146 (2005) 5079–5085.
[54] B.C. Rossier, Hormonal regulation of the epithelial sodium channel ENaC: N or P(o)? J. Gen. Physiol. 120 (2002) 67–70.
[55] C. Mazzochi, D.J. Benos, P.R. Smith, Interaction of epithelial ion channels with the actin-based cytoskeleton, Am. J. Physiol. Renal Physiol. 291 (2006) F1113–F1122.
[56] M.B. Butterworth, R.S. Edinger, J.P. Johnson, R.A. Frizzell, Acute ENaC stimulation by cAMP in a kidney cell line is mediated by exocytic insertion from a recycling channel pool, J. Gen. Physiol. 125 (2005) 81–101.
[57] B.K. Berdiev, A.G. Prat, H.F. Cantiello, D.A. Ausiello, C.M. Fuller, B. Jovov, D.J. Benos, I.I. Ismailov, Regulation of epithelial sodium channels by short actin filaments, J. Biol. Chem. 271 (1996) 17704–17710.
[58] B. Jovov, A. Tousson, H.L. Ji, D. Keeton, V. Shlyonsky, P.J. Ripoll, C.M. Fuller, D.J. Benos, Regulation of epithelial Na(+) channels by actin in planar lipid bilayers and in the *Xenopus* oocyte expression system, J. Biol. Chem. 274 (1999) 37845–37854.
[59] S.J. Copeland, B.K. Berdiev, H.L. Ji, J. Lockhart, S. Parker, C.M. Fuller, D.J. Benos, Regions in the carboxy terminus of alpha-bENaC involved in gating and functional effects of actin, Am. J. Physiol. Cell Physiol. 281 (2001) C231–C240.
[60] C. Mazzochi, J.K. Bubien, P.R. Smith, D.J. Benos, The carboxyl terminus of the alpha-subunit of the amiloride-sensitive epithelial sodium channel binds to F-actin, J. Biol. Chem. 281 (2006) 6528–6538.
[61] D.V. Ilatovskaya, T.S. Pavlov, V. Levchenko, Y.A. Negulyaev, A. Staruschenko, Cortical actin binding protein cortactin mediates ENaC activity via Arp2/3 complex, FASEB J. 25 (2011) 2688–2699.
[62] Q. Wang, X.Q. Dai, Q. Li, J. Tuli, G. Liang, S.S. Li, X.Z. Chen, Filamin interacts with epithelial sodium channel and inhibits its channel function, J. Biol. Chem. 288 (2013) 264–273.
[63] C. Miller, An overview of the potassium channel family, Genome Biol. 1 (2000) 0004.1–0004.5, (reviews).
[64] A.M. Brainard, A.J. Miller, J.R. Martens, S.K. England, Maxi-K channels localize to caveolae in human myometrium: a role for an actin-channel–caveolin complex in the regulation of myometrial smooth muscle K+ current, Am. J. Physiol. Cell Physiol. 289 (2005) C49–C57.
[65] W. Everaerts, B. Nilius, G. Owsianik, The vanilloid transient receptor potential channel TRPV4: from structure to disease, Prog. Biophys. Mol. Biol. 103 (2010) 2–17.
[66] O. Pochynyuk, O. Zaika, R.G. O'Neil, M. Mamenko, Novel insights into TRPV4 function in the kidney, Pflugers Arch. 465 (2013) 177–186.
[67] R. Ramadass, D. Becker, M. Jendrach, J. Bereiter-Hahn, Spectrally and spatially resolved fluorescence lifetime imaging in living cells: TRPV4–microfilament interactions, Arch. Biochem. Biophys. 463 (2007) 27–36.
[68] C. Goswami, J. Kuhn, P.A. Heppenstall, T. Hucho, Importance of non-selective cation channel TRPV4 interaction with cytoskeleton and their reciprocal regulations in cultured cells, PLoS One 5 (2010) e11654.
[69] S.H. Shin, E.J. Lee, S. Hyun, J. Chun, Y. Kim, S.S. Kang, Phosphorylation on the Ser 824 residue of TRPV4 prefers to bind with F-actin than with microtubules to expand the cell surface area, Cell. Signal. 24 (2012) 641–651.
[70] X. Xu, J.G. Forbes, M. Colombini, Actin modulates the gating of *Neurospora crassa* VDAC, J. Membr. Biol. 180 (2001) 73–81.
[71] T.K. Rostovtseva, VDAC structure, function, and regulation of mitochondrial and cellular metabolism, Biochim. Biophys. Acta 1818 (2012) 1437.
[72] I. Roman, J. Figys, G. Steurs, M. Zizi, Direct measurement of VDAC–actin interaction by surface plasmon resonance, Biochim. Biophys. Acta 1758 (2006) 479–486.