Regulation of TRPV5 and TRPV6 by associated proteins

Stan F. J. van de Graaf, Joost G. J. Hoenderop, and René J. M. Bindels

Department of Physiology, Nijmegen Centre for Molecular Life Sciences, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands

van de Graaf, Stan F. J., Joost G. J. Hoenderop, and René J. M. Bindels. Regulation of TRPV5 and TRPV6 by associated proteins. Am J Physiol Renal Physiol 290: F1295–F1302, 2006; doi:10.1152/ajprenal.00443.2005.—The epithelial Ca2+ channels TRPV5 and TRPV6 are the most Ca2+-selective members of the TRP channel superfamily. These channels are the prime target for hormonal control of the active Ca2+ flux from the urine space or intestinal lumen to the blood compartment. Insight into their regulation is, therefore, pivotal in our understanding of the (patho)physiology of Ca2+ homeostasis. The recent elucidation of TRPV5/6-associated proteins has provided new insight into the molecular mechanisms underlying the regulation of these channels. In this review, we describe the various means of TRPV5/6 regulation, the role of channel-associated proteins herein, and the relationship between both processes.

Rab11; calbindin; 80KH; BSPRY; Klotho; kidney

Transcellular Ca2+ (re)absorption is a pivotal process in the maintenance of extracellular Ca2+ balance. It allows the organism to respond to fluctuations in dietary Ca2+ and adapt to the body’s demand during processes such as growth, pregnancy, lactation, and aging. The first step in transcellular transport is the influx of Ca2+ across the apical membrane of the epithelial cell, which is a tightly controlled mechanism (Fig. 1). Next, Ca2+ enters the cell and is sequestered by specialized proteins called calbindins to maintain low cytosolic Ca2+ concentrations. Subsequently, bound Ca2+ diffuses to the basolateral side of the cell, where it is extruded into the bloodstream via the Na+/Ca2+ exchanger (NCX1) and plasma membrane Ca2+-ATPase (PMCA1b). The molecular identification of two members of the transient receptor potential (TRP) superfamily, TRPV5 (28) and TRPV6 (48), boosted the research addressing the molecular mechanism of epithelial Ca2+ transport. The physiological role of these channels has been substantiated in several mouse models of Ca2+ homeostasis. The recent elucidation of TRPV5/6-associated proteins has provided new insight into the molecular mechanisms underlying the regulation of these channels. Therefore, we describe the various means of TRPV5/6 regulation, the role of channel-associated proteins herein, and the relationship between both processes.

Structural Features of TRPV5 and TRPV6

TRPV5 and TRPV6 display the highest sequence homology with the vanilloid receptor subfamily (TRPV) of TRP channels. These channels share a predicated topology consisting of large intracellular NH2- and COOH-terminal tails flanking six transmembrane segments (TM) and an additional hydrophobic stretch between TM5 and TM6, forming the pore-forming region (Fig. 2). Several TRP channels contain ankyrin repeats in their NH2 terminus. The number of ankyrin repeats range from 0 to 14, and the members of the TRPV subfamily have 3–4 repeats (12), varying slightly depending on the sequence-similarity algorithm used. TRPV5 and TRPV6 display several properties that distinguish these Ca2+ channels from other TRP members. First, TRPV5 and TRPV6 are constitutively active at low intracellular Ca2+ concentrations and physiological membrane potentials (65). Other members of the TRP family are activated by various stimuli, including ligand binding, heat, and cold (12). Second, the current-voltage relationships show strong inward rectification of TRPV5/6, at both the level of single channels and whole cells (26, 64, 65). Third, TRPV5 and TRPV6 are 100 times more selective for Ca2+ than for Na+, making the epithelial Ca2+ channels the most Ca2+-selective members of the TRP superfamily (65). In a physiological context, this property renders these channels particularly suited to transport Ca2+ in the presence of Na+.

Hoenderop and co-workers (30) recently demonstrated that the TRPV5/6 channel has a tetrameric architecture, where the four subunits surround a single pore. It was shown that TRPV5/6 can form homo- and heterotetramers. The functional properties of these tetramers, including Ca2+-dependent inactivation, cation selectivity, and pharmacological blockade, were dependent on the subunit ratio of TRPV5 and TRPV6 (30). Erler and co-workers (18) identified the ankyrin repeat at positions 116–191 of the NH2 terminus as a stringent requirement for physical assembly of TRPV6 subunits. It was proposed that this repeat initiates a molecular zipping process that proceeds past the last ankyrin repeat and creates an intracellular anchor that is necessary for functional subunit assembly. Chang and co-workers (9) confirmed the important role of the NH2 terminus in channel assembly in general, and in particular demonstrated that a region near the first ankyrin repeat (positions 64–77) is critical in the assembly process of TRPV5. However, Chang et al. observed slightly increased binding efficiencies using constructs including the first 162
amino acids, suggesting that the ankyrin repeat identified for TRPV6 might be also involved in TRPV5 multimerization. Interestingly, a structural model of the outer pore of TRPV5 and TRPV6 was recently provided by Dodier et al. (14) and Voets et al. (67), respectively. Mutation of a single aspartic residue at position number 542 (D542), a residue crucial for high-affinity Ca\(^{2+}\) binding, altered the apparent pore diameter. This indicates that this residue lines the narrowest part of the

![Fig. 1. Integrated model of active epithelial Ca\(^{2+}\) transport. Ca\(^{2+}\) enters the cell from the luminal side via TRPV5 and/or TRPV6, subsequently binds to calbindin-D\(_{28k}\), and is extruded at the basolateral membrane by a Na\(^+/\)Ca\(^{2+}\) exchanger (NCX1) and/or a plasma membrane Ca\(^{2+}\)-ATPase (PMCA1b). In this way, there is net Ca\(^{2+}\) (re)absorption from the luminal space to the extracellular compartment. Ca\(^{2+}\) (re)absorption is fine-tuned by calciotropic hormones. The active form of vitamin D (1,25(OH)\(_2\)D\(_3\)) stimulates the individual steps of transcellular Ca\(^{2+}\) transport by increasing the expression level of TRPV5/6, calbindins, and the extrusion systems.](image1)

![Fig. 2. Predicted topology of TRPV5/6 including the various binding sites identified for each associated protein. The extracellular loop between transmembrane domain (TM) 1 and TM2 is glycosylated. In the COOH terminus, Rab11, 80K-H, and S100A10 bind within a ~30-amino acid-containing helical stretch juxtaposed to TM6. The S100A10-annexin 2 (Anx2) complex interacts with a conserved region within this stretch containing the VATTV amino acid sequence, whereas Rab11a and 80K-H share a binding site COOH terminally flanking this sequence. Na\(^+/\)H\(^+\) exchanger regulatory factor 2 (NHERF2) specifically interacts with TRPV5 at the extreme COOH terminus of this channel. Calmodulin (CaM) has multiple binding sites present in the NH\(_2\) terminus, COOH terminus, and transmembrane region. The region between TM2 and TM3 of TRPV6 (indicated by vertical lines) is functionally linked to the fast Ca\(^{2+}\)-dependent inactivation of this channel, suggesting the binding of an unidentified protein to this region.](image2)
REGULATION OF CHANNEL ABUNDANCE

Various hormones are involved in the maintenance of extracellular Ca\(^{2+}\) concentration, including vitamin D, parathyroid hormone (PTH), and estrogen. The physiological importance of vitamin D in Ca\(^{2+}\) homeostasis is reflected by severe disorders like VDDR-I and VDDR-II, linked to alterations in vitamin D synthesis or the vitamin D receptor (VDR), respectively. Genetically modified mouse models of these diseases including VDR (31) and 25-hydroxyvitamin D 1\(\alpha\)-hydroxylase (1\(\alpha\)-OHase) knockout mice (55), in which the vitamin D system has been inactivated, have been created to systematically dissect the genetic regulation of Ca\(^{2+}\) transport genes and their functional consequences with regard to transcellular Ca\(^{2+}\) transport. Using these animal models, it was demonstrated that TRPV5 and TRPV6 are positively regulated by vitamin D in the kidney and intestine (5, 25, 57, 58, 60). These results provided novel insight into the molecular mechanisms of vitamin D efficacy and shed light on the hormonal regulation of TRPV5 and TRPV6 expression levels. The regulation of channel abundance is elaborately discussed by Hoenderop et al. (26).

It has been described that the VDDR-I and VDDR-II phenotype can be rescued in animal models of these diseases that include a high-Ca\(^{2+}\) diet (25, 37). Importantly, the reduced expression level of renal TRPV5, calbindin-D\(_{28K}\), and NCX1 in the 1\(\alpha\)-OHase knockout mice was restored by high dietary Ca\(^{2+}\) intake (25), showing a vitamin D-independent effect of dietary Ca\(^{2+}\) on the expression of renal Ca\(^{2+}\) transport proteins. Similarly, the expression of intestinal Ca\(^{2+}\) transport proteins, TRPV6, calbindin-D\(_{28K}\), and PMCA1b, was normalized by this Ca\(^{2+}\) diet (58). Importantly, the effects of this Ca\(^{2+}\) diet on the expression of renal and intestinal Ca\(^{2+}\) transport proteins were accompanied by normalization of plasma Ca\(^{2+}\) concentration (25, 58). Comparable observations were made in VDR knockout mice where duodenal TRPV5 and TRPV6 mRNA levels were upregulated by dietary Ca\(^{2+}\) (60). The molecular mechanism of this vitamin D-independent, Ca\(^{2+}\)-sensitive pathway remains elusive.

CHANNEL ACTIVITY AT THE PLASMA MEMBRANE

Electrophysiological studies in human embryonic kidney (HEK293) cells heterologously expressing TRPV5 or TRPV6 showed that these channels are constitutively open at physiological membrane potentials (65). Unlike several other members of the TRP superfamily (12), there are no indications that TRPV5 and TRPV6 require either a stimulus or ligand to become active. However, the activity of these channels is regulated by various means. First, TRPV5 and TRPV6 are rapidly inactivated on an increase in intracellular Ca\(^{2+}\) concentration, although the initial inactivation is faster in TRPV6 than in TRPV5 (42). The region between TM2 and TM3 was identified as a crucial domain in the fast inactivation of TRPV6 (42).

In addition, two domains in the COOH terminus of TRPV5 contribute to the Ca\(^{2+}\)-dependent inactivation. Deletion of the last 30 amino acids of the COOH terminus of TRPV5 (G701X) significantly decreased Ca\(^{2+}\) sensitivity (44). Detailed mutation analysis revealed that a region upstream in the COOH terminus (between E649 and C653) forms another critical stretch for Ca\(^{2+}\)-dependent inactivation of TRPV5 (44).

A second factor involved in the regulation of TRPV5/6 is extracellular pH. Early studies in excitable tissues indicated that a pH decrease inhibits voltage-gated Na\(^{+}\) channel activity (69). Similar findings were observed by Hess and collaborators (51) for L-type Ca\(^{2+}\) channels. Changes in pH have been found to regulate a number of TRP channels. External acidic pH increases TRPV1 (7) currents and decreases the activity of TRPP2 (23), a distant TRP family member of TRPV5/6.

Importantly, it was previously demonstrated that uptake in TRPV5-expressing Xenopus laevis oocytes is inhibited by acidification of the incubation medium (28, 47). Indeed, extracellular acidification reduced currents through TRPV5 carried by either monovalent or divalent cations (66).

Yeh et al. (71) demonstrated that mutation of the glutamate at position 522, preceding the pore region, to glutamine (E522Q) decreases the inhibition of the channel by extracellular protons. Therefore, this residue may act as the “pH sensor” of TRPV5 (71). Furthermore, they demonstrated that a rotation of the pore helix, mediated by intracellular protons, facilitates this inhibition of TRPV5 by extracellular acidification (70). It is well known that acidification of the apical medium inhibits transcellular Ca\(^{2+}\) absorption across primary cultures of rabbit connecting tubule and cortical collecting duct cells (4). Extrapolating the pH dependence of TRPV5 to the in vivo situation suggests that inhibition of TRPV5 by extracellular pH may provide, at least in part, the molecular basis of acidosis-induced calcireurin.

A third level of control of channel activity is the recovery from Ca\(^{2+}\)-dependent inactivation. This intriguing phenomenon may reflect a mechanism distinct from Ca\(^{2+}\)-dependent inactivation (43). Nilius et al. (43) showed that recovery from inhibition occurred on washout of extracellular Ca\(^{2+}\) (whole cell configuration) or by removal of Ca\(^{2+}\) from the inner side of the channel (inside-out patches). However, this process does not simply correlate with the removal of intracellular Ca\(^{2+}\), because full recovery occurs much later than restoration of the basal Ca\(^{2+}\) level in non-Ca\(^{2+}\)-buffered cells or after removal of Ca\(^{2+}\) from the inner side of excised membrane patches (43). It is currently unknown whether the
recovery reflects reopening of channels present in the plasma membrane or insertion of channels into the plasma membrane.

**TRAFFICKING OF TRPV5 AND TRPV6**

TRPV5 channels are located in or near the apical plasma membrane in distal convoluted tubules (DCT) of the kidney. However, in connecting tubules (CNT) of the kidney, a significant subset of TRPV5 channels is located subapically, hinting at a shuttling mechanism of these channels to the plasma membrane (38). Because TRPV5 and TRPV6 are constitutively active at physiological membrane potentials, control of the number of channels at the cell surface is essential for efficient and balanced Ca\(^{2+}\) transport. This is nicely illustrated by a recent elucidation of a novel mechanism of TRPV5 regulation by Chang and co-workers (10), Klotho, a novel hormone that has been linked to longevity (related to high levels of the protein) (34) and premature aging (associated with diminished Klotho levels) (33), was shown to hydrolyze extracellular sugar residues of TRPV5, resulting in cell surface-entrapped TRPV5 (10). This ultimately results in increased Ca\(^{2+}\) transport, which is in line with the observed effect of Klotho in Ca\(^{2+}\) homeostasis (10).

Despite these recent developments, information about the mechanisms underlying the trafficking of TRPV5/6 to and from the plasma membrane remains scarce. In general, our understanding of regulated TRP trafficking is still in its infancy. To date, channel trafficking to the plasma membrane has only been observed for TRPL (1), TRPV2 (32), TRPC5 (2), TRPC3 (53), and TRPC6 (8). However, these studies have initiated a discussion about the questions of whether TRP trafficking plays a general role in TRP regulation and about the relative contribution of changes in channel gating and channel trafficking (39).

**REGULATION OF TRPV5 AND TRPV6 BY CHANNEL-ASSOCIATED PROTEINS**

A number of regulatory proteins have recently been described that modify the biophysical, pharmacological, and expression properties of ion channels and transporters by direct interactions. These newly identified associated proteins have facilitated the elucidation of important molecular pathways modulating transport activity. Similarly, identification of the molecular players that associate with TRPV5 and TRPV6 could be pivotal in our understanding of the regulation of these channels. Until now, five regulatory proteins have been identified that associate with TRPV5 and TRPV6, i.e., S100A10-annexin 2, calmodulin (CaM), 80K-H, Na\(^{+}/\)H\(^{+}\) exchanger regulatory factor 2 (NHERF2), and Rab11a (17, 22, 35, 40, 46, 61, 62).

**S100A10/Annexin 2**

S100A10 (also known as p11 or annexin 2 light chain) was identified as an auxiliary protein for TRPV5 and TRPV6 using a yeast two-hybrid system (62). S100A10 is a 97-amino acid protein member of the S100 superfamily that is present in a large number of organisms, including vertebrates, insects, nematodes, and plants. S100A10 is a distinct member of this family, because its two EF hands carry deletions and substitutions that render it Ca\(^{2+}\) insensitive. S100A10 is predominantly present as a heterotetrameric complex with annexin 2, which has been implicated in several biological processes, including endocytosis, exocytosis, and membrane-cytoskeleton interactions (19, 20). Annexin 2 interacts with actin and is postulated to bind the cytoplasmic face of membrane rafts to stabilize these domains, thereby providing a link to the actin cytoskeleton (20). Van de Graaf et al. (62) provided the first evidence of a regulatory role for the S100A10-annexin 2 heterotetramer in vitamin D-mediated Ca\(^{2+}\) (re)absorption in general and in TRPV5 and TRPV6 functioning in particular. It was shown that S100A10, annexin 2, and TRPV5 or TRPV6 are coexpressed in Ca\(^{2+}\)-transporting cells of the kidney and small intestine (62). The association of S100A10 with TRPV5 and TRPV6 was restricted to a short peptide sequence, VATTV, located in the COOH termini of these channels (Fig. 2). This stretch is conserved among all identified species of TRPV5 and TRPV6. Interestingly, the TTV sequence in the S100A10-binding site resembles an internal type I PDZ consensus binding sequence, which is S/TXV (54). However, S100A10 does not contain PDZ domains, indicating that TRPV5-S100A10 interaction has a different nature. The first threonine of the S100A10 interaction motif is a crucial determinant for binding. Furthermore, the activity of TRPV5 and TRPV6 is abolished when this particular threonine is mutated, demonstrating that this motif is essential for channel function.

Malfunctioning of these mutant channels is accompanied by a major disturbance in their subcellular localization, indicating that the S100A10-annexin 2 heterotetramer facilitates the translocation of TRPV5 and TRPV6 channels to the plasma membrane. The importance of annexin 2 in this process was demonstrated by a small-interference RNA-based approach. Downregulation of annexin 2 that significantly inhibits the currents through TRPV5 and TRPV6 indicates that annexin 2, in conjunction with S100A10, is crucial for TRPV5 activity. Interestingly, an association of annexin 2 with TRPV5 was only shown in the presence of S100A10, demonstrating that annexin 2 binds indirectly to the channel, with S100A10 most likely operating as a molecular bridge between TRPV5 and annexin 2. In line with the submembranous localization of annexin 2 and its postulated function in organizing membrane domains, these findings provide the first functional evidence for a regulatory role of annexin 2 controlling Ca\(^{2+}\) channel trafficking and, therefore, Ca\(^{2+}\) homeostasis. Interestingly, previous studies indicated that the background K\(^{+}\) channel (TASK1) is associated with S100A10 via its COOH-terminal sequence SSV (21). The S100A10 interaction promotes the translocation of TASK1 to the plasma membrane, producing functional K\(^{+}\) channels (21). This sequence resembles the binding motif in TRPV5 and TRPV6 identified in the present study, suggesting a shared structural S100A10-binding pocket. However, this motif is absent in the tetrodotoxin-insensitive, voltage-gated Na\(^{+}\) channel (Nav1.8), which has been shown to bind S100A10 via its NH\(_{2}\) terminus (50). The binding of S100A10 is essential for plasma membrane trafficking of this Na\(^{+}\) channel (45). Finally, Donier et al. (15) have recently demonstrated the association of S100A10 with an acid-sensing ion channel (ASIC1) and confirmed this association in rat dorsal root ganglion neurons by coimmunoprecipitation. Taken together, these findings show that the S100A10-annexin 2 complex is a significant component for the trafficking of ion channels to the plasma membrane.
CaM

CaM is a ubiquitous protein, well known to be involved in Ca^{2+}-dependent feedback regulation of several ion channels (36). Niemeyer and co-workers (40) initially demonstrated that TRPV6 interacts with CaM in a Ca^{2+}-dependent manner. CaM was shown to bind the CaM-binding regions in the COOH and NH2 of TRPV5 and TRPV6 as well as the transmembrane domain of TRPV6 in a Ca^{2+}-dependent manner (35, 36). Removal of the CaM-binding site in the COOH terminus of TRPV6 resulted in a significant reduction of the slow component of channel inactivation, revealing a role of CaM in TRPV6 regulation (40). Furthermore, HEK293 cells heterologously coexpressing Ca^{2+}-insensitive CaM mutants along with TRPV5 or TRPV6 showed a significantly reduced Ca^{2+} current through TRPV6, whereas no effect was demonstrated on TRPV5 (35). This effect was localized to the high-Ca^{2+}-affinity EF-hand structures of CaM. These data demonstrated a regulatory role of CaM in TRPV6-mediated Ca^{2+} influx. It remains to be established whether CaM functions as a general Ca^{2+} sensor in TRPV5/6 channels or, alternatively, might explain the differences in Ca^{2+}-dependent inactivation between TRPV5 and TRPV6.

80K-H

In a microarray screen designed to identify proteins that respond similarly to vitamin D and/or altered dietary Ca^{2+} intake as TRPV5, Gkika et al. (22) identified 80K-H. 80K-H was originally cloned as a PKC substrate of 80 kDa and was subsequently associated with intracellular signaling (52). However, these studies did not resolve the biological function of this protein. 80K-H contains two putative EF-hand structures, a highly negatively charged glutamate stretch, which might be a Ca^{2+}-interacting region, and a putative ER-targeting signal (HDEL). Using glutathione S-transferase (GST) pull-down assays and coimmunoprecipitations, a physical interaction between 80K-H and TRPV5 was demonstrated. Furthermore, both proteins colocalized in a subset of tubular segments in the kidney and show similar transcriptional regulation by vitamin D and dietary Ca^{2+} (22). Electrophysiological studies using 80K-H mutants showed that three domains of 80K-H (the 2 EF-hand structures, the glutamate stretch, and the HDEL sequence) are critical determinants of TRPV5 activity. 80K-H directly binds Ca^{2+}, which is abolished on inactivation of its two EF-hand structures. Importantly, inactivation of the EF-hand pair also reduces the TRPV5-mediated Ca^{2+} current and increased the TRPV5 sensitivity to intracellular Ca^{2+}, accelerating the feedback inhibition of the channel. Therefore, it is hypothesized that 80K-H acts a Ca^{2+} sensor to regulate TRPV5 activity at the plasma membrane.

NHERF2 and Serum- and Glucocorticoid-Indicible Kinase

Embark and co-workers (17) demonstrated that TRPV5 activity increases on coexpression with the PDZ domain containing protein NHERF2 and serum- and glucocorticoid-indicible kinase 1 (SGK1) or SGK3 in X. laevis oocytes. Coexpression of TRPV5 with NHERF2 or SGK only did not stimulate TRPV5-mediated currents, indicating that NHERF2 and SGK operate co dependently (17). Deletion of the second but not the first PDZ domain in NHERF2 abrogates the stimulating effect of SGK/NHERF2 on TRPV5 activity (46). Furthermore, it was demonstrated that TRPV5 activity could not be stimulated using a kinase-dead point mutant of SGK1 (K127N), suggesting a phosphorylation-mediated effect (17). Using GST pull-down and overlay assays, the specific interaction of NHERF2 with the last three amino acids (YHF) of the COOH terminus of TRPV5 was demonstrated (Ref. 46 and van de Graaf SF, Hoenderop JG, van der Kemp AW, Gisler SM, and Bindels RJ, unpublished observations). Furthermore, it was shown that TRPV6 does not bind NHERF2 (46). These findings suggest that regulation of the epithelial Ca^{2+} channels by NHERF2-SGK is limited to TRPV5 and operates via direct interaction with the channel. The coexpression of SGK1 with NHERF2 also stimulates the activity of ROMK1, a K^{+} channel involved in renal K^{+} handling. This results from a stabilization of ROMK1 in the plasma membrane (72). Therefore, it is postulated that NHERF2/SGK stimulates the activity of TRPV5 via a comparable mechanism.

Rab11a

Van de Graaf et al. (61) recently identified Rab11a as a novel TRPV5- and TRPV6-associated protein. Rab11a is a small GTPase involved in trafficking via recycling endosomes (6, 68). Although the role of Rab GTPases in protein trafficking has long been recognized, the underlying mechanism is far from understood. Rab11a colocalizes with TRPV5 and TRPV6 in Ca^{2+}-transporting epithelial cells of the kidney. Here, both TRPV5 and Rab11a are present in vesicular structures underlying the apical plasma membrane (61). Using a combination of GST pull-down and coimmunoprecipitation assays, a direct interaction between Rab11a and the epithelial Ca^{2+} channels was demonstrated, hinting at a novel mechanism of Rab11a-mediated trafficking (61). Other Rab GTPases, including Rab7 and Rab22a, did not bind TRPV5/6, indicating the specificity of the interaction. Association of cargo with Rab GTPases has recently received much attention with the identification of an association between Rab3 and the polymeric IgA receptor (63) and between Rab11a and the tromboxane A2 receptor (24). The binding of TRPV5/6 to Rab11a would provide the first evidence for an ion channel directly associating with a Rab GTPase. Furthermore, it was demonstrated that TRPV5 and TRPV6 preferentially interact with Rab11a in its GDP-bound conformation. Expression of a mutant Rab11a protein, locked in the GDP-bound state, results in a marked decrease in channels at the cell surface, indicating a direct role of Rab11a in the trafficking of TRPV5/6 toward the plasma membrane. Therefore, it is likely that TRPV5/6 channels, present on the (apical) plasma membrane, are being exchanged with TRPV5/6 channels from the intracellular (recycling) endosomes in a Rab11-dependent manner.

COORDINATED REGULATION OF CHANNEL EXPRESSION, TRAFFICKING, AND ACTIVITY

As described above, TRPV5/6 channels are controlled by mechanisms that target their expression, trafficking, or activity. The identification and characterization of channel-associated proteins reveal two interesting relationships between TRPV5/6-regulatory mechanisms. First, various observations suggest that S100A10/annexin 2 and Rab11a employ identical pathways to regulate TRPV5/6. For instance, Zobiack and co-
workers (73) have demonstrated that the annexin 2-S100A10 complex controls the distribution of Rab11-positive endosomes. These endosomes have previously been identified as recycling endosomes, involved in the transport of internalized membrane proteins back to the cell surface (6). Furthermore, annexin 2 was shown to be enriched in these Rab11-containing structures (56). Therefore, impaired transport of TRPV5 from recycling endosomes to the plasma membrane could explain the inhibitory effect of annexin 2 downregulation on TRPV5/6-mediated currents. This suggests that two crucial pathways controlling the cell surface expression of TRPV5 and TRPV6 intersect.

Second, it was recently demonstrated that S100A10 and annexin 2 are coregulated by the calciotropic hormone vitamin D in a similar fashion as TRPV5 (62). This indicates that vitamin D exerts its physiological function by concertedly regulating the expression of the apical Ca$^{2+}$ influx proteins and stimulation of the insertion of these proteins into the plasma membrane. Similarly, Chen and co-workers (11) recently demonstrated that Rab11 is regulated in an estrogen-dependent manner. Finally, 80K-H responds like TRPV5 to alterations in vitamin D levels and dietary Ca$^{2+}$ intake (22). Together, this suggests that changes in channel expression, trafficking to the plasma membrane, and channel activity are modulated in a well-orchestrated manner to ultimately control the activity of TRPV5/6.

In summary, the identification of TRPV5/6 binding partners and characterization of their functional importance is an emerging field that has significantly contributed to our understanding of the regulation of apical Ca$^{2+}$ influx in epithelial cells. Some of the newly identified proteins operate at the plasma membrane, whereas others are involved in intracellular trafficking pathways leading to and from this membrane. These findings provide new information about the physiological function of the channel-interacting proteins and point to novel mechanisms regulating apical Ca$^{2+}$ influx in Ca$^{2+}$-transporting epithelia. Identification of the proteins that interact with TRPV5/6 is only a first step in a long endeavor. One of the major challenges of the future is to establish a comprehensive view of TRPV5/6-interacting proteins and their relative contribution to channel regulation. Most importantly, to fully understand the regulation of TRPV5/6, the relationship between protein interactions and signaling processes involved in epithelial Ca$^{2+}$ transport has to be revealed.

GRANTS

This work was supported by the Dutch Organization of Scientific Research (Zon-Mw 016.006.001, Zon-Mw 902.18.298, NWO-ALW 810.38.004, NWO-ALW 805–09.042, NWO-ALW 814–02.001, NWO 812–08.002, NWO Talent stipendium 591–282), the Stomach, Liver, Intestine Foundation (MWO 805–09.042, NWO-ALW 814–02.001, NWO 812–08.002, NWO Talent stipendium 591–282), the Stomach, Liver, Intestine Foundation (MWO 805–09.042, NWO-ALW 814–02.001, NWO 812–08.002, NWO Talent stipendium 591–282), the Stomach, Liver, Intestine Foundation (MWO 805–09.042, NWO-ALW 814–02.001, NWO 812–08.002, NWO Talen

REFERENCES


doi:10.1152/ajpregu.00719.2016


