TRPV5: A Ca^{2+} Channel for the Fine-Tuning of Ca^{2+} Reabsorption

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Contents

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Abstract

TRPV5 is one of the two channels in the TRPV family that exhibit high selectivity to Ca^{2+} ions. TRPV5 mediates Ca^{2+} influx into cells as the first step to transport Ca^{2+} across epithelia. The specialized distribution in the distal tubule of the kidney positions TRPV5 as a key player in Ca^{2+} reabsorption. The responsiveness in expression and/or activity of TRPV5 to hormones such as 1,25-dihydroxyvitamin D_3, parathyroid hormone, estrogen, and testosterone makes TRPV5 suitable for its role in the fine-tuning of Ca^{2+} reabsorption. This role is further optimized by the modulation of TRPV5 trafficking and activity via its binding partners; co-expressed proteins; tubular factors such as calbindin-D_{28k}, calmodulin, klotho, uromodulin, and plasmin; extracellular and intracellular factors such as proton, Mg^{2+}, Ca^{2+}, and phosphatidylinositol-4,5-bisphosphate; and fluid flow. These regulations allow TRPV5 to adjust its overall activity in response to the body's demand for Ca^{2+} and to prevent kidney stone formation. A point mutation in mouse *Trpv5* gene leads to hypercalciuria similar to *Trpv5* knockout mice, suggesting a possible role of TRPV5 in hypercalciuric disorders in humans. In addition, the single nucleotide polymorphisms in *Trpv5* gene prevalently present in African descents may contribute to the efficient renal Ca^{2+} reabsorption among African descendants. TRPV5 represents a potential therapeutic target for disorders with altered Ca^{2+} homeostasis.

Keywords

TRPV5 • TRPV6 • Gene duplication • Calcium channel • Calcium transport • Calcium reabsorption • Distal convoluted tubule • Hypercalciuria • Vitamin D • Protein–protein interaction • Single nucleotide polymorphisms • African American

1 Introduction

Ca^{2+} serves as an important intracellular and extracellular messenger, and it is indispensable for many physiological activities, such as muscle contraction, neuron excitability, and blood coagulation (Brown et al. 1995; Clapham 1995). For this reason, the plasma Ca^{2+} concentration is monitored by a Ca^{2+}-sensing receptor, which controls the secretion of parathyroid hormone (PTH) (Brown 1991). PTH regulates the synthesis of 1,25-dihydroxyvitamin D_3 [$1,25(OH)_2D_3$] (Henry 1985; Yang et al. 1999). PTH and $1,25(OH)_2D_3$ regulate Ca^{2+} homeostasis via intestinal

absorption and renal reabsorption, as well as deposition into and mobilization from the bone. In the intestine and kidney, Ca^{2+} is transported across epithelia through the tight junction between cells (paracellular pathway), or across the apical and basolateral membranes of epithelial cells (transcellular pathway) (Bronner 1998; Wasserman and Fullmer 1995). Only the transcellular pathway allows Ca^{2+} to be transported against an electrical and concentration gradient. The Ca^{2+}-transporting epithelial cells in the intestinal and the kidney express proteins critical in the transcellular Ca^{2+} transport pathway. Ca^{2+} channels TRPV5 and TRPV6 in the apical membrane mediate Ca^{2+} entering into the cell (Hoenderop et al. 1999; Peng et al. 1999; Zhuang et al. 2002). The steep Ca^{2+} concentration gradient (approximately 1 mM extracellular vs. 0.1 μM intracellular) and the transmembrane potential (typically −60 mV inside the cell) allow Ca^{2+} to enter the cells via channels. Ca^{2+} exits the cell via high-affinity Ca^{2+}–ATPase (PMCA) and/or Na^{+}–Ca^{2+} exchanger 1 (NCX1) at the expense of energy stored in ATP or in Na^{+} concentration gradient (Wasserman et al. 1992). Ca^{2+}-binding proteins (e.g., calbindins) are also expressed to keep the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) low so that the driving force for Ca^{2+} entry is maintained and $[Ca^{2+}]_i$ will not rise to a level which is toxic to cells (Christakos et al. 1992; Lambers et al. 2006; Zheng et al. 2004). Although TRPV5 and TRPV6 were identified at a much later date compared to calbindins, PMCA, and NCX, their specific roles in the transcellular Ca^{2+} transport pathway energized the studies of Ca^{2+} absorption and reabsorption at molecular level since the cloning of TRPV5 and TRPV6. Compared to the broader distribution of TRPV6, TRPV5 is relatively confined to the kidney, where it is responsive to physiological signals to regulate Ca^{2+}excretion into the urine.

2 Gene

TRPV5 was cloned as a result of searching for genes involved in Ca^{2+} reabsorption in kidney cells (Hoenderop et al. 1999). Using an expression cloning approach, Hoenderop and Bindels identified a cDNA from primary cells of rabbit connecting tubule (CNT) and cortical collecting duct (CCD) that stimulates $^{45}Ca^{2+}$ uptake in *Xenopus laevis* oocytes. The cDNA encodes a protein of 730 amino acid residues and was initially named as epithelial Ca^{2+} channel (ECaC) (Hoenderop et al. 1999). At the time of cloning, the only mammalian protein similar to TRPV5 was rat vanilloid receptor VR1 (TRPV1). Human, rat, and mouse TRPV5 share high levels of amino acid identity to rabbit TRPV5 (Peng et al. 2000a, 2001). The protein most similar to TRPV5 is TRPV6 (75 % amino acid identity), which was independently identified from rat duodenum using similar approach (Peng et al. 1999). TRPV5 shares approximately 40–45 % amino acid identity with other members of the TRPV family, including TRPV1 to TRPV4.

Trpv5 gene is located in human chromosome 7q35 side by side with *TRPV6* gene in 7q33–34 (Muller et al. 2000; Peng et al. 2001). The human *Trpv5* gene comprises 15 exons. Except for the first and last exons, exons 2–14 of *Trpv5* and *Trpv6* genes are identical in size (Peng et al. 2001). In fact, all genes of the TRPV family share a

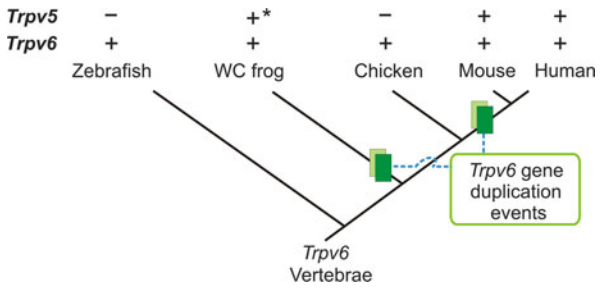

Fig. 1 *Trpv5* gene was likely produced by the duplication of *Trpv6* gene during the process of evolution. Gene duplication events are indicated on the respective branches. WC frog, Western clawed frog (*Xenopus tropicalis*). *Plus symbol* indicates present, *minus symbol* indicates absent, and *asterisk* indicates that there are five genes similar to TRPV6 in Western clawed frogs

conserved gene structure (Peng et al. 2001). In addition to *Trpv5* and *Trpv6* in 7q34–35, genes for *Trpv1*, *Trpv2*, and *Trpv3* are located in 17q11.2 to 17q13.2, suggesting recent gene duplication events in this subfamily. Fish and birds have only one *Trpv6*-like gene (Qiu and Hogstrand 2004; Yang et al. 2011), whereas mammalians have both *Trpv5* and *Trpv6* genes. Based on the Joint Genome Institute (JGI; http://genome.jgi-psf.org/) genome database for *Xenopus tropicalis* (Western clawed frog), five *Trpv6*-like genes are present. This is not restricted to *Trpv6*, as 6 *Trpv4*-like genes are present in *X. tropicalis* (Saito and Shingai 2006). Thus, it is likely that duplication events occurred in *Trpv6* gene when amphibians and mammals were evolved, respectively (Fig. 1). The physiological roles of the *Trpv6*-like genes in *X. tropicalis* are unclear. In mammals, the duplicated gene became *Trpv5* as it gained the ability for specialized expression in the distal tubule of kidney and properties for a finer regulation of channel function and trafficking.

3 Tissue Distribution

In contrast to the broader tissue distribution of TRPV6, TRPV5 is rather specifically distributed to the kidney. Due to the high sequence similarity between TRPV5 and TRPV6, initial studies showed strong signals in Northern blot analyses with TRPV5 cDNA probe in rabbit and rat duodenum (Peng et al. 2000a; Hoenderop et al. 1999), but this is likely due to cross hybridization with TRPV6 mRNA, which is highly expressed in the duodenum (Peng et al. 2000a). Similarly, although TRPV5 is also expressed in the placenta, the mRNA abundance of TRPV5 is much lower than that of TRPV6 in human placenta (Peng et al. 2001).

Based on Expressed Sequence Tag (EST) database from the National Center for Biotechnology Information (NCBI), among 45 human organs listed, TRPV5 is detected in only two of them (numbers in brackets are tag/million): blood [8] and lung [2]. In contrast, TRPV6 is present in 13 organs/tissues: bladder [33], blood [16], brain [4], cervix [20], eye [9], intestine [8], lung [2], mammary gland [6],

pancreas [4], placenta [215], prostate [47], testis [6], and trachea [19]. Both TRPV5 and TRPV6 are not detected in human kidney, indicating their number of EST is lower than 1/million. In mouse, among 47 organs/tissues listed, TRPV5 is present in 2, including embryonic tissue [1] and kidney [24], whereas TRPV6 is present in 9 of them including brain [10], embryonic tissue [2], extraembryonic tissue [120], lung [30], mammary gland [69], pancreas [37], salivary gland [103], skin [8], and thymus [8]. These EST profiles may not be accurate due to various reasons; however, they provide an unbiased overview of the gene expression. The expression profile of TRPV5 in mouse suggests a specific role of TRPV5 in the kidney.

In rabbit kidney, TRPV5 protein is expressed in the apical membrane of distal convoluted tubule (DCT), CNT, and CCD, where it co-localizes with calbindin-D_{28k} (Hoenderop et al. 1999). In rats, TRPV5 mRNA exhibits similar expression pattern as NCX1 and calbindin-D_{28k} (Peng et al. 2000a). In mouse kidney, TRPV5 is expressed in the late segment of DCT and CNT (Loffing et al. 2001).

In mice and rats, TRPV5 mRNA is much more abundant than that of TRPV6 in the kidney (Song et al. 2003; Van Cromphaut et al. 2007). TRPV5 mRNA level is approximately 10–20 times higher than that of TRPV6 in mouse kidney (Song et al. 2003). In contrast, we have observed much higher mRNA level of TRPV6 than TRPV5 in the human kidney (Peng et al. 2001). Our unpublished observations also indicated a higher mRNA level of TRPV6 in outer medulla than cortex of human kidney, opposite to that of TRPV5. Similarly, TRPV6 is also much abundant than TRPV5 in horse kidney (Rourke et al. 2010). Thus, TRPV6 may play more significant roles in some species such as humans and horses.

Even though TRPV5 is mainly expressed in the kidney, TRPV5 mRNA or protein has also been detected in other organs/tissues. TRPV5 is detected in mouse testis (Jang et al. 2012) and inner ear (Takumida et al. 2009) and rat testis, spermatogenic cells, sperm (Li et al. 2010), cochlea (Yamauchi et al. 2010), and osteoclast-like cells (Yan et al. 2011). In horse, TRPV5 mRNA is expressed in the duodenum and proximal jejunum (Rourke et al. 2010), and TRPV5 protein is detected in chondrocytes from the superficial zone of articular cartilage (Hdud et al. 2012). In human, TRPV5 protein and mRNA are present in placenta (Bernucci et al. 2006), osteoclasts (van der Eerden et al. 2005), retinal pigment epithelium (Kennedy et al. 2010), lymphocytes, Jurkat leukemia T cells (Vassilieva et al. 2013), and leukemia K562 cells (Semenova et al. 2009).

4 Structural Aspects

The mammalian TRPV5 proteins comprise around 730 amino acid residues (729, 731, 730, and 723 amino acids in human, horse, rabbit, rat, and mouse, respectively). Mammalian TRPV5 proteins consist of an intracellular N- and C-termini, six transmembrane domains, and a pore region between the last two transmembrane domains. In the intracellular portions of TRPV5, six ankyrin repeats are present in the N-terminal region, and a PDZ-binding motif is located in the C terminus (Phelps et al. 2008; de Groot et al. 2011b; Palmada et al. 2005;

Jing et al. 2011). An *N*-linked glycosylation site is located in the first extracellular loop, and asparagine 358 (N358) is indispensable for the *N*-linked glycosylation of TRPV5 (Chang et al. 2005; Jiang et al. 2008). Putative protein kinase A (PKA) and protein kinase C (PKC) phosphorylation sites and calmodulin-binding sites are present within the N- and C-terminal regions (Hoenderop et al. 1999; Kovalevskaya et al. 2012). Some of these sites have already been shown to be important in regulating TRPV5.

Four TRPV5 molecules form a homotetramer with a single pore in the middle (Nilius et al. 2001b; Hoenderop et al. 2003b). Two critical motifs at positions 64–77 (part of the first ankyrin repeat) in the N terminus and at 596–601 in the C terminus of TRPV5 are needed for the formation of the functional channel complex (Chang et al. 2004). The N-terminal ankyrin repeats appear to be important for channel assembly. The first ankyrin repeat does not appear to be critical to the formation of TRPV5 tetramer; however, it is likely essential for correct folding of TRPV5 into a functional channel complex (de Groot et al. 2011b). In contrast, the third ankyrin repeat initiates a zippering process through the fifth ankyrin repeat, and they form an anchor for channel assembly (Erler et al. 2004). The tetramerization of TRPV5 allows the four aspartate 542 (D542) residues in pore-forming loop between transmembrane domain 5 and 6 to form a Ca^{2+}-selective ring, which is also involved in Mg^{2+} blockade (Nilius et al. 2001b; Voets et al. 2004; Lee et al. 2005).

TRPV5 and TRPV6 appeared to form heterotetramers when four pieces of TRPV5 and TRPV6 were linked together and heterologously expressed in *X. laevis* oocytes (Hoenderop et al. 2003b). The more TRPV6 subunits presented in the heterotetramer, the more TRPV6 characteristics were exhibited (Hoenderop et al. 2003b). Other TRPV members preferentially form homomeric assembly; however, TRPV5 and TRPV6 can form heteromeric complex in HEK293 cells (Hellwig et al. 2005). This is likely due to the high degree of similarity between TRPV5 and TRPV6. Unlike in transfected cells, TRPV5 is likely outnumbered by TRPV6 in most cells. It is unclear whether heteromeric TRPV5 and TRPV6 complexes play a significant physiological role if they exist in vivo. In addition, TRPV5 and TRPV6 share certain structure–function similarities with TRPML3 (Grimm et al. 2007). They can form heteromers which display different features than the respective homomers (Guo et al. 2013). TRPML3 and TRPV5 are expressed in the kidney and inner ear (Takumida et al. 2009; Castiglioni et al. 2011); however, it is yet to be determined whether TRPML3 and TRPV5 form heteromeric complexes in native cells.

5 Biophysical Properties

5.1 Ca^{2+} Selectivity

TRPV5 is a Ca^{2+} permeant ion channel as it was identified as a result of increasing $^{45}Ca^{2+}$ uptake activity when expressed in *Xenopus* oocytes (Hoenderop et al. 1999). The most distinctive feature of TRPV5 is its Ca^{2+} selectivity. The permeability ratio

between Ca^{2+} and Na^+ (P_{Ca}:P_{Na}) is over 100 (Vennekens et al. 2000). The divalent cation selectivity profile of TRPV5 is $Ca^{2+} > Mn^{2+} > Ba^{2+} \approx Sr^{2+}$ (Vennekens et al. 2000). In the absence of divalent cation in the extracellular solution, TRPV5 allows monovalent cations to pass through. The permeation sequence for monovalent cations through TRPV5 is $Na^+ > Li^+ > K^+ > Cs^+ > NMDG^+$ (Nilius et al. 2000). The Na^+ current of TRPV5 correlates with the amplitude of Ca^{2+} uptake and is often used as a measure of TRPV5 channel activity. Some trivalent and divalent cations block current through TRPV5, and the sequence of block is $Pb^{2+} = Cu^{2+} = Gd^{3+} > Cd^{2+} > Zn^{2+} > La^{3+} > Co^{2+} > Fe^{2+} > Fe^{3+}$ (Nilius et al. 2001a). TRPV5 is also sensitive to ruthenium red and econazole (Nilius et al. 2001a). TRPV5 and TRPV6 show difference in ruthenium red sensitivity: the IC_{50} of ruthenium red for TRPV5 is around 121 nM, which is nearly100-fold lower than that for TRPV6 (Hoenderop et al. 2001b). Extracellular and intracellular Mg^{2+} blocks TRPV5 voltage dependently (Voets et al. 2001; Lee et al. 2005; Hoenderop et al. 2001b). D542 is the key residue to form the selectivity filter of TRPV5 and determines Ca^{2+} permeation and Mg^{2+} blockade (Nilius et al. 2001b; Lee et al. 2005; Dodier et al. 2007). The nonsynonymous single nucleotide polymorphism (SNP) A563T variation, close to D542, increases TRPV5 sensitivity to extracellular Mg^{2+}, resulting in suppressed Na^+ permeation through TRPV5 (Na et al. 2009). In clinical trials, urinary Ca^{2+} excretion is proportional to changes in magnesium excretion, and this is likely related to the blockade of TRPV5 by Mg^{2+} (Bonny et al. 2008).

TRPV5 currents exhibit strong inward rectification (Vennekens et al. 2000). Using TRPV6 as a model, Voets et al. demonstrated that intracellular Mg^{2+} acts as a permeant pore blocker and contributes to strong inward rectification of the channel (Voets et al. 2003). In addition, TRPV6 also exhibits intrinsic Mg^{2+}-independent inward rectification for which the mechanism is not fully understood (Voets et al. 2003). Similar mechanisms are likely applicable to TRPV5 due to the high degree of similarity in structure and function between TRPV5 and TRPV6. Unitary channel activity of TRPV5 could be detected in the absence of extracellular divalent cations (Nilius et al. 2000). The single-channel conductance is 77.5 pS for rabbit TRPV5 using Na^+ as a charge carrier (Nilius et al. 2000). Single-channel activity of TRPV5 was also detected using K^+ as a charge carrier (Vassilev et al. 2001). In addition, subconductance state (partial channel opening) of TRPV5 was also observed (subconductance at 29 pS vs. full conductance at 59 pS at intracellular pH 7.4) (Cha et al. 2007).

5.2 pH Sensitivity

Another feature of TRPV5 is its pH sensitivity. In the initial characterization, TRPV5-mediated Ca^{2+} uptake was significantly inhibited when the pH value in the extracellular medium was lowered to 5.9 (Hoenderop et al. 1999). At single-channel level, both full and subconductance and their open probabilities were reduced by lower intracellular pH (Cha et al. 2007). On the other hand, high pH

stimulates TRPV5-mediated Ca^{2+} uptake (Peng et al. 2000a). Low pH increases the blockage of monovalent cation current by extracellular Mg^{2+} (Vennekens et al. 2001). Glutamate 522 (E522) of rabbit TRPV5 acts as the extracellular pH sensor, and extracellular protons decrease the estimated diameter of channel pore to inhibit TRPV5 (Yeh et al. 2003, 2005). Glutamate 535 (E535) is also involved; however, its effect depends on E522 (Yeh et al. 2006). Meanwhile, intracellular protons also regulate TRPV5 (Yeh et al. 2005). Intracellular acidification promotes proton binding to lysine 607, intracellular pH sensor, and induces rotation of the pore helix, leading to decreases in the pore diameter, open probability, and single-channel conductance (Yeh et al. 2005; Cha et al. 2007).

In addition to channel property, pH may also affect the level of TRPV5 protein at the cell surface. Extracellular alkalization induces the recruitment of TRPV5 proteins to the cell surface from TRPV5-containing vesicles, and extracellular acidification leads to the retrieval of TRPV5 from plasma membrane (Lambers et al. 2007). Thus, TRPV5 could be regulated by pH in both channel activity and plasma membrane expression. Metabolic acidosis and alkalosis affect Ca^{2+} transport in the kidney (Sutton et al. 1979). Clinical trial data indicate that changes in urinary Ca^{2+} excretion and urine pH are inversely related (Bonny et al. 2008). The pH sensitivity of TRPV5 may contribute to the dysregulation of Ca^{2+} reabsorption under disturbance in acid–base balance.

5.3 Ca^{2+}-Dependent Inactivation

High level of free Ca^{2+} is toxic to cells. The Ca^{2+}-dependent inactivation limits the amount of Ca^{2+} ions that enter the cell through TRPV5 and prevents the toxic impact of Ca^{2+} overload in the cell. Calbindin-D_{28k} binds Ca^{2+} beneath the plasma membrane and therefore relieves the Ca^{2+}-dependent inactivation of TRPV5 to some extent; it may also buffer free Ca^{2+} to limit the toxic effect (Lambers et al. 2006). TRPV5 exhibits Ca^{2+}-dependent autoregulatory mechanisms, including fast inactivation and slow rundown (Vennekens et al. 2000). The C-terminal sequences in rabbit TRPV5 701–730 and 650–653 are critical determinants of Ca^{2+}-dependent inactivation (Nilius et al. 2003). Compared to TRPV6, TRPV5 exhibit a much slower initial inactivation phase (Nilius et al. 2002). The first intracellular loop is critical to the kinetics of the initial phase in the inactivation process; three residues at positions 409, 411, and 412 in TRPV5 are critical for the delayed inactivation (Nilius et al. 2002). In addition, Q579 following the last transmembrane domain of TRPV5 is also important to the fast Ca^{2+}-dependent inactivation kinetics of TRPV5 (Suzuki et al. 2002). The first intracellular loop of TRPV5 is capable of binding to CaM (Kovalevskaya et al. 2012), so is the C terminus of TRPV5 (de Groot et al. 2011a; Kovalevskaya et al. 2012). TRPV5 mutant deficient in interacting with CaM at C terminus (residues 696–729) exhibits diminished Ca^{2+}-dependent inactivation (de Groot et al. 2011a). However, it is yet to be clarified what roles the other CaM-binding sites of TRPV5 play in the Ca^{2+}-dependent inactivation mechanism. In addition, a helix-breaking mutation

(M490P) in transmembrane domain 5 reduces Ca^{2+}-dependent inactivation of TRPV5 and induces apoptosis due to Ca^{2+} overload in cells expressing $TRPV5^{M490P}$ (Lee et al. 2010). Corresponding mutation in TRPML3 (A419P) leads to constitutive channel activity, which likely results in hair cell death in the inner ear and profound deafness and other abnormalities in mice homozygous in $TRPML3^{A419P}$ (Grimm et al. 2007).

Although TRPV5 works constitutively in the kidney, its activity depends on the availability of phosphatidylinositol-4,5-bisphosphate (PIP_2). As the level of PIP_2 decreases, so does the activity of TRPV5 (Lee et al. 2005). PIP_2 activates TRPV5 in part by reducing conformational change-induced Mg^{2+} binding (Lee et al. 2005). Arginine 599 in mouse or rat TRPV5 (corresponding to R606 in rabbit and human TRPV5) in the "TRP domain" is likely the PIP_2-binding site (Rohacs et al. 2005). Elevation of $[Ca^{2+}]_i$ due to Ca^{2+} influx through TRPV5 may activate phospholipase C (PLC), which depletes PIP_2. Thus, removal of PIP_2 is an important Ca^{2+}-dependent inactivation mechanism for TRPV5 as well as TRPV6 (Thyagarajan et al. 2008).

5.4 Responsiveness to Fluid Flow

Flow-stimulated K^+ secretion in the distal nephron involves Ca^{2+}-activated K^+ channels. TRPV5 is expressed in the apical membrane in the distal nephron and therefore may provide a Ca^{2+} influx pathway to activate K^+ channels. Recent study indicates that both TRPV5 and TRPV6 expressed in HEK cells are activated by shear force generated by fluid flow in physiological range (Cha et al. 2013). Flow-induced surge in TRPV5 or TRPV6 activity leads to the activation of co-expressed Slo1 maxi-K^+ channel without affecting ROMK channel (Cha et al. 2013). Activation of maxi-K^+ channel may counteract the depolarization due to Ca^{2+} influx and cause hyperpolarization which in turn would improve Ca^{2+} influx through TRPV5. *N*-linked glycosylation of the channels is involved in the responsiveness to fluid flow (Cha et al. 2013). The responsiveness of TRPV5 and TRPV6 to flow may have implications not only in flow-stimulated K^+ secretion but also in thiazide-induced Ca^{2+} reabsorption.

6 Regulation by Interacting, Co-expressed, and Tubular Proteins

A number of proteins have been found to regulate TRPV5. These include TRPV5-interacting proteins, proteins co-expressed with TRPV5, and proteins in the tubular fluid (Table 1). Regulation of TRPV5 by some of the proteins is briefly summarized in the following.

Table 1 Proteins that regulate TRPV5

Protein name	Binding motif in TRPV5	Effect/mechanism	References
TRPV5-interacting proteins affecting trafficking/plasma membrane expression			
S100A10	^{598}VATTV^{602a}	Associates with annexin 2 to regulate the plasma membrane localization of TRPV5; upregulated by 1,25$(OH)_2D_3$	Lewit-Bentley et al. (2000)
Rab11a	^{603}MLERK^{607}	Plasma membrane localization of TRPV5	van de Graaf et al. (2006a)
NHERF2	^{726}VYHF^{729}	Trafficking and stability at plasma membrane	Embark et al. (2004), Palmada et al. (2005), Jing et al. (2011)
NHERF4	603–624	Unknown	van de Graaf et al. (2006b)
TRPV5-interacting proteins regulating channel function/activity			
Calbindin-D_{28k}	N- and C-termini	Buffers $[Ca^{2+}]_i$ and counteracts inactivation of TRPV5	Lambers et al., (2006)
Calmodulin	133–154, 310–330, 401–428, 591–612, 696–712	Regulates channel function such as in feedback inhibition by Ca^{2+}	Holakovska et al., (2011), de Groot et al. (2011a), Kovalevskaya et al. (2012)
80K-H	598–608	Intracellular Ca^{2+} sensor to regulate TRPV5 activity	Gkika et al. (2004)
BSPRY	C terminus	Inhibits TRPV5 activity. Inversely regulated by 1,25 $(OH)_2D_3$	van de Graaf et al. (2006c)
FKBP52	Full length	Enzymatic activity is involved in its inhibitory effect	Gkika et al. (2006c)
TRPML3	Unknown	Form heteromeric channel complex with TRPV5 in vitro	Guo et al. (2013)
Proteins in co-expressed cells or in tubular fluid that regulate TRPV5			
Klotho		Expressed in DCT and enhances the stability of TRPV5 on plasma membrane	Cha et al. (2008a), Chang et al. (2005), Lu et al. (2008), Leunissen et al. (2013)
Tissue transglutaminase		Promotes channel aggregation, leading to reduction in pore diameter	Boros et al. (2012)
Tissue kallikrein		Delays channel retrieval from plasma membrane	Gkika et al. (2006b)
Nedd4-2/Nedd4		Increases the degradation of TRPV5 protein	Zhang et al. (2010)
Ca^{2+}-sensing receptor		Stimulates TRPV5 via PKC	Topala et al. (2009)
SGK1/3		Enhances channel stability and trafficking	Embark et al. (2004), Sandulache et al. (2006), Palmada et al. (2005)

(continued)

Table 1 (continued)

Protein name	Binding motif in TRPV5	Effect/mechanism	References
WNK3		Increases TRPV5 delivery to the plasma membrane	Zhang et al. (2008)
WNK4		Increases forward trafficking or enhances endocytosis	Jiang et al. (2007, 2008, Jing et al. (2011), Cha and Huang (2010)
Plasmin		Suppresses TRPV5 by activating PAR-1	Tudpor et al. (2012)
Uromodulin		Secreted in the thick ascending limb and decreases endocytosis of TRPV5	Wolf et al. (2013)

[a]Human TRPV5 numbering throughout this table

6.1 Regulation by Interacting Proteins

Some TRPV5-interacting proteins, such as S100A10, Rab11a, and NHERF2, regulate the trafficking and plasma membrane abundance of TRPV5. On the other hand, some TRPV5-interacting proteins, such as calbindin-D_{28k}, CaM, 80H-K, BSPRY, and FKBP52, regulate TRPV5 activity. It is worth noting that the C terminus of TRPV5 interacts with most of its binding partners, and the region from 598 to 608 in TRPV5 within the conserved "TRP domain" binds to S100A10, Rab11a, 80K-H, NHERF4, and CaM (Fig. 2). This region also contains the PIP_2-binding site (R606) and a pH sensor (K607). It is also not far away from histidine 711 (corresponding to H712 in rabbit TRPV5), a critical residue involved in the constitutive internalization of TRPV5 (de Groot et al. 2010). Thus, it is plausible that TRPV5-binding partners may affect TRPV5 function, membrane stability, and trafficking. However, it is not likely that these TRPV5-binding proteins regulate TRPV5 at the same time. It is possible that some of the proteins only regulate TRPV5 at a certain cell type, at a specific developmental stage, or at a state being stimulated by a hormone or other physiological cues. Most TRPV5-interacting proteins also interact with TRPV6 due to the conserved binding sites in the two channels. Thus, it is likely some of the TRPV5-interacting proteins may be more involved in regulating TRPV6 than TRPV5. Lastly, although overlapping expression with TRPV5 was found for most of the TRPV5-interacting proteins, they do express in cells that do not express TRPV5; they all have functions other than regulating TRPV5.

6.1.1 S100A10

S100A10 binds to a conserved motif of five residues (^{598}VATTV^{602}) in the C terminus of TRPV5 (van de Graaf et al. 2003). S100A10 is a member of the EF-hand containing S100 protein family, and it forms heterotetramer with annexin 2, a Ca^{2+}, and phospholipid-binding protein in association with cytoskeleton underneath cell membrane (Gerke and Moss 2002). S100A10–annexin 2 complex is

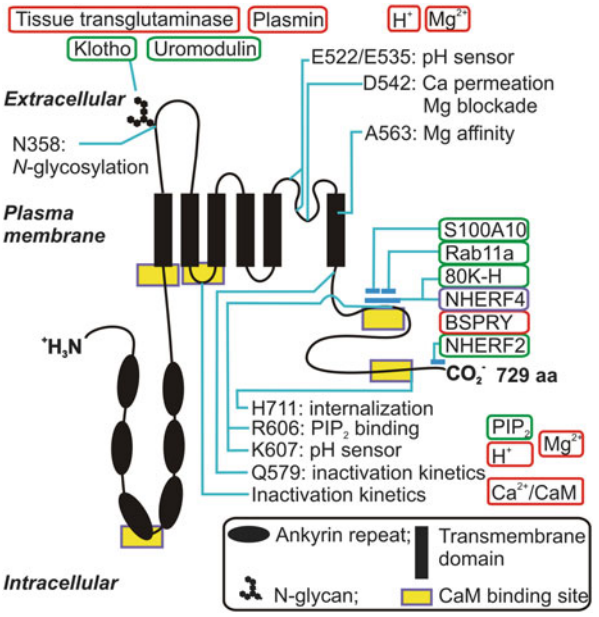

Fig. 2 Functional domains/sites in human TRPV5 (GenBank #: AF304464.1). Key residues, domains, and interacting motifs/sites for partners are indicated in the predicted structure of TRPV5. Factors with positive and negative effects on TRPV5 are labeled with *green* and *red rectangles*, respectively. See text for details

important for the plasma membrane localization of TRPV5 (van de Graaf et al. 2003). In addition, this complex may be involved in $1,25(OH)_2D_3$-induced Ca^{2+} reabsorption as S100A10 is upregulated by $1,25(OH)_2D_3$ (van de Graaf et al. 2003).

6.1.2 Rab11a

The small GTPase Rab11a is another TRPV5-interacting protein that regulates its trafficking to the plasma membrane (van de Graaf et al. 2006a). It binds to ^{596}MLERK^{600} of mouse TRPV5 (603–607 in human TRPV5), following the S100A10-binding motif (van de Graaf et al. 2006a). Glycine substitution in the MLERK motif disrupts the binding of Rab11a to TRPV5 and the plasma membrane expression of TRPV5 (van de Graaf et al. 2006a). TRPV5 is one of a few cargo proteins that bind directly to the GDP-bound Rab proteins.

6.1.3 PDZ Proteins

The last four amino acids of TRPV5 and TRPV6 are PDZ (postsynaptic density-95, Drosophila discs-large protein, zonula occludens protein 1)-binding motifs. TRPV5 interacts with Na^+/H^+ exchanger regulating factors 2 (NHERF2) (Palmada et al. 2005). Removal of the last three amino acids of TRPV5 abolishes the interaction (van de Graaf et al. 2006b). TRPV5 also interacts with NHERF4; however, the interacting motif is located between residues 596 and 617 of mouse TRPV5 (603–624 of human TRPV5) (van de Graaf et al. 2006b). In contrast, NHERF4 interact with the last three amino acid residues of TRPV6 (Kim et al. 2007), suggesting it is likely TRPV6 is the real binding partner of NHERF4.

The 4th PDZ domain of NHERF4 and the second PDZ domain of NHERF2 are essential for their interactions with TRPV5 (Kim et al. 2007; Palmada et al. 2005). NHERF2 is important to the effects of SGK1 and WNK4 on TRPV5 (Embark et al. 2004; Jing et al. 2011).

6.1.4 Calbindin-D_{28k}

Calbindins are Ca^{2+}-binding proteins that are well known for their responsiveness to vitamin D and possible roles in Ca^{2+} absorption and reabsorption (Christakos et al. 1992). Calbindin-D_{28k} is co-expressed with TRPV5 in DCT and CNT (Loffing et al. 2001). Both N- and C-termini of TRPV5 interact with calbindin-D_{28k} in the absence but not in the presence of Ca^{2+} ions (Lambers et al. 2006). When $[Ca^{2+}]_i$ is low, calbindin-D_{28k} translocates toward the plasma membrane and associates with TRPV5. Calbindin-D_{28k} buffers Ca^{2+} close to the vicinity of the channel opening, thereby reducing local accumulation of free Ca^{2+} ions. After binding to Ca^{2+}, calbindin-D_{28k} disassociates from TRPV5 and facilitates the diffusion of Ca^{2+} to the basolateral membrane (Lambers et al. 2006). Calbindin-D_{28k} expression in the kidney is greatly reduced in mice lacking TRPV5 (Hoenderop et al. 2003a). Mice lacking calbindin-D_{28k} do not show hypercalciuria, and mice lacking both TRPV5 and calbindin-D_{28k} do not exhibit more severe phenotype in Ca^{2+} homeostasis than mice lacking TRPV5 alone (Gkika et al. 2006a). These results indicate TRPV5 but not calbindin-D_{28k} is critical to Ca^{2+} reabsorption. Calbindin-D_{28k} acts as a dynamic Ca^{2+} buffer to avoid a sudden elevation of $[Ca^{2+}]_i$; however, it appears that its role could be compensated by other vitamin D-regulated Ca^{2+}-binding proteins. The role of calbindin-D_{28k} in Ca^{2+} homeostasis could only be observed in the absence of the effects of vitamin D: mice lacking vitamin D receptor (VDR) and calbindin-D_{28k} display more severe hypercalciuria and secondary hyperparathyroidism than mice lacking VDR alone (Zheng et al. 2004).

6.1.5 Calmodulin

Being an intracellular Ca^{2+} sensor, calmodulin (CaM) is expected to play a role in modulating TRPV5 function. However, CaM antagonists calmidazolium R24571 and trifluoperazine only modestly inhibit TRPV5 at high concentrations (Nilius et al. 2001a). In addition, Ca^{2+}-insensitive CaM mutants significantly reduced Na^+ and Ca^{2+} currents of TRPV6 but not those of TRPV5 (Lambers et al. 2004). Yet, CaM is capable of binding to TRPV5. So far five CaM-binding sites in TRPV5 have been identified, including residues 696–712 (de Groot et al. 2011a; Kovalevskaya et al. 2012) and 591–612 (Kovalevskaya et al. 2012; Holakovska et al. 2011) in the C terminus, 401–428 in the first intracellular loop (Kovalevskaya et al. 2012), and 310–330 and 133–154 in the N-terminal region (Kovalevskaya et al. 2012). These CaM-binding sites display diversity in binding mode, stoichiometry, and affinity in interaction with CaM in vitro (Kovalevskaya et al. 2012). The first intracellular loop of TRPV5/6 plays a critical role in the fast and slow inactivation kinetics (Nilius et al. 2002), but it is unclear to what extent Ca^{2+}/CaM is involved in this process. CaM negatively modulates TRPV5 activity by binding to the residues 696–729, and PTH-mediated PKA phosphorylation of the CaM-binding site reverses this action

(de Groot et al. 2009, 2011a). In addition, phosphorylation of TRPV5 by PKC at serine 144 (S144) in the N-terminal CaM-binding site (133–154) results in decreased pore size and open probability in TRPV5 (Tudpor et al. 2012). This regulation is important in mediating the action of plasmin on TRPV5 (Tudpor et al. 2012).

6.1.6 80K-H

80K-H is a Ca^{2+}-binding protein and a PKC substrate (Sakai et al. 1989). It was identified as a potential TRPV5-interacting protein because it was downregulated in $1,25(OH)_2D_3$-deficient mice and was upregulated by dietary Ca^{2+} (Gkika et al. 2004). 80K-H-binding site in TRPV5 is located between residues 598 and 608 (Gkika et al. 2004). Co-expression of 80K-H did not alter the level of TRPV5 in the cell surface, but increased the sensitivity of TRPV5 to $[Ca^{2+}]_i$ (Gkika et al. 2004). Thus, 80K-H acts as a Ca^{2+} sensor to control the activity of TRPV5 in addition to CaM.

6.1.7 BSPRY

BSPRY (B-box and SPRY-domain-containing protein), a protein with unknown function, interacts with TRPV5 C terminus (van de Graaf et al. 2006c). It inhibits Ca^{2+} transport activity of TRPV5 without altering its surface level. BSPRY co-localized with TRPV5 in the DCT and CNT, and it is inversely regulated by $1,25(OH)_2D_3$ (van de Graaf et al. 2006c). Thus, BSPRY adds a layer of regulation of TRPV5 by $1,25(OH)_2D_3$.

6.1.8 FKBP52

Immunophilin FKBP52, one of the downstream targets of FK-506, co-localizes with TRPV5 in the DCT and CNT and inhibits TRPV5 activity in vitro (Gkika et al. 2006c). The peptidyl-propyl *cis-trans* isomerase activity of FKBP52 is essential to its inhibitory effect on TRPV5, and the inhibitory effect is reversed by the administration of FK-506. FKBP52 interacts with full-length TRPV5, but not its N- or C terminus (Gkika et al. 2006c). FKBP52 expression is decreased in *Trpv5* KO mice, indicating a link between the two proteins (Gkika et al. 2006c). Since FK-506 induces hypercalciuria and causes a reduction in calbindin-D_{28k} and TRPV5 expression in the kidney (Nijenhuis et al. 2004; Lee et al. 2011; Aicher et al. 1997), further investigation is necessary to clarify the role of interaction between FKBP52 and TRPV5 in FK-506-induced hypercalciuria.

6.2 Regulation of TRPV5 by Co-expressed Proteins

In addition to the proteins that interact directly with TRPV5, proteins co-expressed with TRPV5 may also regulate TRPV5. These TRPV5-regulating proteins were studied because of physiological relevance, not as results of searching for TRPV5-binding partners. Transient protein interaction may or may not be involved in these regulations.

6.2.1 Klotho

Klotho functions to suppress aging process (Kuro-o et al. 1997). Klotho exists in membrane bound and circulating forms; it converts fibroblast growth factor (FGF) receptor FGFR1(IIIc) into a specific receptor for FGF 23 by binding with it (Urakawa et al. 2006). In the kidney, klotho is expressed in the DCT where it co-localizes with TRPV5 and increases TRPV5 activity in vitro (Chang et al. 2005; Lu et al. 2008; Cha et al. 2008a). The mechanism by which klotho regulates TRPV5 is not well understood. Because klotho exhibits β-glucuronidase activity (Tohyama et al. 2004), it was thought that klotho modifies TRPV5 glycan through this activity (Chang et al. 2005). Like klotho, β-glucuronidase also activates TRPV5 and TRPV6 but not related TRP channels TRPV4 and TRPM6 (Lu et al. 2008). Alternatively, klotho removes terminal sialic acids from their glycan chains of TRPV5 and exposes disaccharide galactose-*N*-acetylglucosamine, which binds to galactoside-binding lectin galectin-1 (Cha et al. 2008a). The galectin-1-linked TRPV5 proteins are likely resistant to endocytosis. However, complete removal of *N*-glycan by endoglycosidase-F also increases TRPV5 activity (Lu et al. 2008). However, galectin-3 but not galactin-1 co-localizes with TRPV5 in the DCT (Leunissen et al. 2013). Sialidase appears to increase TRPV5 activity by inhibiting lipid-raft-mediated endocytosis, and it does not discriminate *N*-glycan-deficient N358Q mutant and wild-type TRPV5 (Leunissen et al. 2013). Thus, klotho regulates TRPV5 in an *N*-glycan-dependent manner, whereas sialidase regulates TRPV5 in an *N*-glycan-independent and lipid-raft-mediated endocytosis-dependent manner (Leunissen et al. 2013). Although the mechanisms are still to be clarified, the regulation of TRPV5 by klotho represents a novel area of ion channel regulation. The dysregulation of TRPV5 may be responsible for the increased excretion of Ca^{2+} in *Kl* (klotho gene)-deficient mice, in which PTH-stimulated Ca^{2+} reabsorption in the CNT is impaired (Tsuruoka et al. 2006). Lower plasma klotho concentration is associated with older age and lower serum Ca^{2+} level and is an independent risk factor for mortality in community-dwelling adults of 65 years or older (Semba et al. 2011). Presumably reduced TRPV5 activity due to the lower klotho level likely contributes to the lower serum Ca^{2+} and may play a role in the aging process. Although klotho is considered a hormone, its action on TRPV5 from the luminal side is distinct from its hormonal action from interstitium. However, it could be considered as a factor in the tubular fluid similar to plasmin and uromodulin.

6.2.2 Tissue Transglutaminase

Tissue transglutaminase (tTG) catalyzes Ca^{2+}-dependent covalent cross-linking of specific lysine and glutamine residues of substrate proteins (Lorand and Graham 2003). Calbindin-D_{28K} and S100A10, which regulate TRPV5, are substrates of tTG (Vig et al. 2007; van de Graaf et al. 2003; Ruse et al. 2001; Lambers et al. 2006). In addition, activity of TRPV5 is inhibited by extracellular tTG treatment in HEK-293 and in rabbit CNT/CCD cells (Boros et al. 2012). This is caused by the reduction in channel pore diameter in aggregated TRPV5 in the plasma membrane as a result of tTG activity. *N*-glycosylation-deficient TRPV5 mutant is insensitive to tTG (Boros

et al. 2012). Klotho and tTG both require the presence of *N*-glycan of TRPV5 for their action, but they direct TRPV5 activity to opposite directions.

6.2.3 Tissue Kallikrein

Tissue kallikrein (TK) is a serine protease expressed in CNT (Figueroa et al. 1988). TK knockout (KO) mice exhibit hypercalciuria (Picard et al. 2005). Consistent with this, TRPV5 was shown to be regulated by TK via PLC/diacylglycerol/PKC pathway (Gkika et al. 2006b). TK enhances TRPV5-mediated Ca^{2+} influx by delaying its retrieval from the plasma membrane (Gkika et al. 2006b). This effect was abolished by S299A and S654A mutations, which may disrupt phosphorylation of TRPV5 by PKC (Gkika et al. 2006b), which increases TRPV5 activity by inhibiting its endocytosis from plasma membrane (Cha et al. 2008b).

6.2.4 SGK1/3

Co-expression of NHERF2 and serum/glucocorticoid regulated kinase 1 or 3 (SGK1/3) with TRPV5 enhances TRPV5 activity in *X. laevis* oocytes (Embark et al. 2004). However, NHERF2 or SGK1/3 alone does not alter TRPV5 activity (Embark et al. 2004). The second PDZ domain in NHERF2 is required for the stimulatory effect of SGK1/NHERF2 on TRPV5 (Palmada et al. 2005). In the SGK1 KO mice, TRPV5 protein abundance in the CNT was reduced; however, urinary Ca^{2+} excretion was also reduced (Sandulache et al. 2006). The increased Ca^{2+} reabsorption is likely due to a compensatory increase in the function of thick ascending limb in response to the salt loss in the aldosterone-sensitive distal nephron. The reduced expression of TRPV5 in the CNT of SGK1 KO mice is possibly a compensatory effect due to increased Ca^{2+} reabsorption in the thick ascending limb, not necessarily a result due to the lack of positive regulation of TRPV5 by SGK1 (Sandulache et al. 2006).

6.2.5 WNK Kinases

Point mutations in with-no-lysine (K) kinase 4 (WNK4) result in pseudohypoaldosteronism type II (PHAII, also known as familial hyperkalemia and hypertension or Gordon's syndrome) (Wilson et al. 2001). Hypercalciuria was observed in PHAII patients with $WNK4^{Q565E}$ mutation, leaving the possibility that WNK4 may regulate a Ca^{2+} channel or transporter in the kidney (Mayan et al. 2004). We found WNK4 increases the activity of TRPV5 channel by increasing the forward trafficking of the channel to the plasma membrane via the secretory pathway in *X. laevis* oocytes (Jiang et al. 2007, 2008). The positive effect of WNK4 on TRPV5 was greatly reduced when NCC was co-expressed (Jiang et al. 2007). Similar to WNK4, WNK3 also increases Ca^{2+} influx mediated by TRPV5 via a kinase-dependent pathway (Zhang et al. 2008). The effect of WNK4 on TRPV5 is enhanced and stabilized by NHERF2 (Jing et al. 2011). When the last two amino acid residues of TRPV5 were replaced by those of TRPV6, the effect of NHERF2 on TRPV5 was abolished (Jing et al. 2011). Thus, WNK4 and NHERF2 increase TRPV5 forward trafficking and membrane stability synergistically, leading to additive enhancement in TRPV5-mediated Ca^{2+} transport. On the other hand, Cha et al. found that WNK4

kinase enhances the endocytosis and decreases the plasma membrane abundance of TRPV5 in HEK293 cells (Cha and Huang 2010). The reason for the difference in WNK4-mediated regulation on TRPV5 is unclear.

6.2.6 Ubiquitin E3 Ligases

Nedd4-2 is an archetypal member of the ubiquitin E3 ligase family regulating cell surface stability of membrane proteins (Staub and Rotin 2006). Nedd4-2 is expressed in the DCT (Verrey et al. 2003) and CCD (Flores et al. 2005) in the kidney where TRPV5 is functionally expressed. When expressed in *Xenopus* oocytes, TRPV5/6-mediated Ca^{2+} uptake and Na^{+} current were decreased by Nedd4-2 and Nedd4 due to the reduction in TRPV5 protein level (Zhang et al. 2010). In all cases, Nedd4-2 exhibited stronger inhibitory effects than Nedd4 on both TRPV5 and TRPV6. WW1 and WW2 domains of Nedd4-2 may serve as a molecular switch to limit the ubiquitination of TRPV6/5 by the HECT domain (Zhang et al. 2010). Although Nedd4 and Nedd4-2 mediate TRPV5 degradation in vitro, it is unclear to what extent they do so in vivo. In addition, the degradation of TRPV5 is reduced by knocking down of Ubiquitin recoginition 4 (UBR4), another E3 ubiquitin ligase (Radhakrishnan et al. 2013). The physiological significance of these TRPV5 degradation pathways warrants further studies.

6.2.7 Ca^{2+}-Sensing Receptor

The mRNA and protein of Ca^{2+}-sensing receptor (CaR) are expressed in the DCT/CNT (Riccardi et al. 1996, 1998; Hoenderop et al. 1999). Activation of CaR increases TRPV5-mediated currents and elevates $[Ca^{2+}]_i$ in cells co-expressing TRPV5 and CaR (Topala et al. 2009). Phorbol-12-myristate-13-acetate (PMA)-insensitive PKC isoforms are likely involved in the signal pathway by which CaR stimulates TRPV5 (Topala et al. 2009). The stimulatory effect was abolished by mutation of two putative PKC phosphorylation sites, S299 and S654 in TRPV5, or by a dominant-negative CaR (R185Q) (Topala et al. 2009).

6.3 Regulation by Proteins in the Tubular Fluid

6.3.1 Plasmin

Plasminogen, which is elevated and can be filtered into the urine in nephrotic syndrome, is converted into active plasmin in the tubular fluid. When incubated with plasmin, either from commercial source or purified from nephrotic urine, TRPV5-mediated Ca^{2+} uptake in HEK-293 cells was inhibited (Tudpor et al. 2012). As a serine protease, plasmin does not cleave TRPV5 or alter TRPV5 surface abundance; instead, it affects TRPV5 by binding to protease-activated receptor-1 (PAR-1) (Tudpor et al. 2012). This activates the PAR-1/PLC/PKC pathway, which likely leads to phosphorylation of S144 within a CaM-binding site in the N terminus of TRPV5. The phosphorylation of S144 results in an alteration of CaM binding to TRPV5 and, in turn, a reduction in pore size and

open probability (Tudpor et al. 2012). Suppression of TRPV5 by plasmin likely takes effect in nephrotic patients.

6.3.2 Uromodulin

Uromodulin (also known as Tamm–Horsfall glycoprotein/THP) is a urinary glycoprotein secreted by the thick ascending loop of Henle (Bachmann et al. 1990). Co-expression with uromodulin increases TRPV5 current density and surface abundance in HEK293 cells (Wolf et al. 2013). Acting from the tubular luminal side, uromodulin decreases caveolin-mediated endocytosis of TRPV5, and the level of TRPV5 is lower in uromodulin KO mice (Wolf et al. 2013). This result suggests that uromodulin may act as a physiological regulator of TRPV5 to prevent kidney stone formation.

7 Regulation by Hormones

Trpv5 expression is regulated under physiological conditions. *Trpv5* expression in the kidney is upregulated under dietary Ca^{2+} restriction in mice (Song et al. 2003; Van Cromphaut et al. 2001); this regulation appears to be VDR dependent (Van Cromphaut et al. 2001). TRPV6 and TRPV5 in rat small intestine were decreased by immobilization and induced by endurance swimming, and a $1,25(OH)_2D_3$-dependent pathway is likely involved in these changes (Sato et al. 2006; Teerapornpuntakit et al. 2009). TRPV5 was upregulated by high-salt intake and downregulated by low-salt intake or dehydration in rats; this was likely caused by the increase or decrease in the delivery of Ca^{2+} to the distal tubule in the kidney (Lee et al. 2012). Aging is associated with alterations in Ca^{2+} homeostasis, such as decreased Ca^{2+} absorption and increased urinary Ca^{2+} excretion. Duodenal TRPV5/6 mRNA level in adult (12-month old) rats was less than half of that in young (2-month old) rats (Brown et al. 2005). *Trpv5* KO mice develop age-related hyperparathyroidism and osteoporotic characteristics earlier than wild-type mice, possibly due to the age-related vitamin D resistance and less robust compensatory expression of intestinal TRPV6 in older mice (van Abel et al. 2006). Thus, as *Trpv5* KO mice age, vitamin D resistance prevents the animals to compensate the renal loss of Ca^{2+} through intestinal absorption, leading to a negative Ca^{2+} balance.

Hormonal regulation is at least in part behind the adaptation of TRPV5 expression in response to physiological conditions. Indeed, a number of hormones, including $1,25(OH)_2D_3$, PTH, sex hormones, and vasopressin, have been found to regulate TRPV5 (Table 2). The effects of these hormones on TRPV5 are summarized briefly below.

7.1 Vitamin D

In 1991, Bindels and colleagues reported that $1,25(OH)_2D_3$ increases transcellular Ca^{2+} absorption in primary culture of rabbit kidney CCD (Bindels et al. 1991)

Table 2 Hormones that regulate TRPV5

Hormones	Effects	References
1,25 $(OH)_2D_3$	Increases *Trpv5* transcription	Hoenderop et al. (2001a, 2002), Song et al. (2003)
PTH	Increases TRPV5 expression and increases TRPV5 activity via PKA and/or PKC	van Abel et al. (2005), Cha et al. (2008b), de Groot et al. (2009)
Estrogen	Acutely increases TRPV5 activity and chronically increases TRPV5 expression	Oz et al. (2007), van Abel et al. (2002), Irnaten et al. (2009)
Testosterone	Decreases TRPV5 expression	Hsu et al. (2010)
Vasopressin	Increase transepithelial Ca^{2+} transport by the activation of cAMP/PKA pathway	Diepens et al. (2004), Hofmeister et al. (2009), van Baal et al. (1996)

where TRPV5 was later cloned (Hoenderop et al. 1999). After the identification, TRPV5 mRNA was shown to be upregulated by $1,25(OH)_2D_3$ in the kidney of vitamin D-depleted rats (Hoenderop et al. 2001a). Moreover, a single injection of $1,25(OH)_2D_3$ in vitamin D-deficient mice induced ~three- to fourfold increase in TRPV5 mRNA in the kidney that peaked at 12 h after injection following the peak of duodenal TRPV6 (6 h after injection) (Song et al. 2003). This suggests that the increase in TRPV5 in the kidney might be a secondary event due to the increased Ca^{2+} absorption. In mice deficient in 25-hydroxyvitamin D_3-1α-hydroxylase with undetectable level of $1,25(OH)_2D_3$, the reduction of renal TRPV5 and hypocalcemia were normalized by high Ca^{2+} diet, suggesting that TRPV5 is upregulated by increased Ca^{2+} load to the distal tubule as a result of $1,25(OH)_2D_3$-induced Ca^{2+} absorption (Hoenderop et al. 2002). Although potential vitamin D response elements (VDREs) have been identified in the promoter region of *Trpv5* gene (Muller et al. 2000; Weber et al. 2001), their function has not been examined as those bona fide VDREs in *Trpv6* gene promoter (Meyer et al. 2006). At least in mice duodenum, FGF-23 could reduce circulating $1,25(OH)_2D_3$ and diminish 1,25 $(OH)_2D_3$-induced surge of TRPV6 and TRPV5 (Khuituan et al. 2012).

7.2 Parathyroid Hormone

PTH regulates both the expression and activity of TRPV5. In parathyroidectomized rats and calcimimetic compound NPS R-467-infused mice that had a lower PTH level, renal TRPV5, calbindin-D_{28k}, and NCX1 levels were decreased; and they were restored by PTH supplementation (van Abel et al. 2005). The decrease in other Ca^{2+} transport proteins by PTH is likely secondary to the reduction of TRPV5-mediated Ca^{2+} influx (van Abel et al. 2005).

PTH is also capable of acutely increasing Ca^{2+} transport in the distal tubule (Bacskai and Friedman 1990). Both PKA and PKC are involved in this regulation (Friedman et al. 1996). Indeed, activation of TRPV5 by PTH via both PKA and PKC has been reported (de Groot et al. 2009; Cha et al. 2008b). PTH activates the cAMP–PKA signaling cascade and increases TRPV5 open probability via phosphorylation of threonine 709 (T709) in TRPV5 (de Groot et al. 2009). This

regulation requires a strong buffering of intracellular Ca^{2+}. However, the PKA phosphorylation site T709 is not conserved in human TRPV5. In addition to PKA, the increase of TRPV5 activity by heterogeneously expressed PTH receptor was prevented by PKC inhibitor (Cha et al. 2008b). Mutation of PKC phosphorylation sites S299/S654 in TRPV5 abolished the regulation. Caveolae-mediated endocytosis of TRPV5 appears to be inhibited by PTH via a PKC-dependent pathway (Cha et al. 2008b).

Besides vitamin D and PTH, calcitonin is a hormone that regulates Ca^{2+} homeostasis. However, calcitonin affects renal Ca^{2+} reabsorption mainly through the thick ascending limb (Elalouf et al. 1984; Di Stefano et al. 1990). TRPV5, which is mostly expressed in the DCT and CNT, is not involved in the effects of calcitonin on urinary excretion of Ca^{2+}, Na^+, and K^+ as no difference was observed between wild-type and *Trpv5* KO mice in this regard (Hsu et al. 2009).

7.3 Estrogen and Testosterone

As the most potent estrogen, 17β-estradiol increases TRPV5 mRNA and protein expression in the kidney (van Abel et al. 2002, 2003). Estrogen deficiency in aromatase-deficient mice results in decreased mRNA levels of renal TRPV5 and other Ca^{2+} transporters and Ca^{2+} wasting (Oz et al. 2007). In addition, 17β-estradiol (20–50 nM) rapidly increases TRPV5 current and $[Ca^{2+}]_i$ in rat CCD cells (Irnaten et al. 2009). Thus, estrogen acutely elevates TRPV5 activity and chronically increases its expression.

In contrast to the positive effects of estrogen, male hormone testosterone appears to have a negative effect on TRPV5. Male mice have higher urinary Ca^{2+} excretion than female mice (Hsu et al. 2010). Androgen deficiency increases renal TRPV5 mRNA and protein and decreases urinary Ca^{2+} excretion; these were normalized by testosterone treatment (Hsu et al. 2010). In addition, the negative effect of dihydrotestosterone on transcellular Ca^{2+} transport was demonstrated in primary rabbit CNT/CCD cells (Hsu et al. 2010).

7.4 Vasopressin

Arginine vasopressin stimulates transepithelial Ca^{2+} transport in primary cultures of rabbit CCD cells (van Baal et al. 1996). Vasopressin induces an increase in Ca^{2+} uptake and $[Ca^{2+}]_i$ in mpkDCT cells and freshly isolated late DCT and CNT cells, respectively, indicating that TRPV5 is a target of vasopressin (Diepens et al. 2004; Hofmeister et al. 2009).

8 Physiological Functions in Native Cells, Organs, Organ Systems

8.1 Ca^{2+} Reabsorption in the Kidney

TRPV5 is mainly distributed in the apical membrane of tubular cells in the DCT and CNT where it mediates the final reabsorption of Ca^{2+} via a transcellular pathway. TRPV5, calbindin-D_{28k}, NCX1, and PMCA are the major components of the pathway. Lines of evidence suggest that the expression of other Ca^{2+} transporters depends on TRPV5-mediated Ca^{2+} influx (van Abel et al. 2005; Hoenderop et al. 2003a). In this sense, TRPV5 is the rate-limiting component in the transcellular pathway of Ca^{2+} reabsorption.

TRPV5 is likely constitutively active in the apical membrane of tubular cells. At macroscopic level, TRPV5 acts as a facilitative transporter exhibiting saturable kinetics with apparent K_m values at sub-millimolar range, which are well suited for the luminal Ca^{2+} level in the distal tubule (Hoenderop et al. 1999; Peng et al. 1999, 2000a, b). The responsiveness of TRPV5 to vitamin D, PTH, Ca^{2+} load, pH, and tubular factors such as klotho, uromodulin, and plasmin makes TRPV5 well suited for the fine-tuning of Ca^{2+} reabsorption in the kidney.

8.2 Bone Resorption by Osteoclasts

TRPV5 also expresses in the ruffled border membrane of mouse osteoclasts (van der Eerden et al. 2005). Osteoclast numbers and area are increased, and paradoxically urinary bone resorption marker deoxypyridinoline was reduced in mice lacking TRPV5 (Hoenderop et al. 2003a). In vitro bone marrow culture system indicates that bone resorption by osteoclasts was impaired in *Trpv5* KO mice (van der Eerden et al. 2005). These results suggest a malfunction of osteoclast to some degree in the absence of TRPV5. However, bone resorption inhibitor alendronate normalizes the reduced bone thickness in *Trpv5* KO mice, even though it specifically increases bone TRPV5 expression (Nijenhuis et al. 2008). Furthermore, vitamin D analog ZK191784 partially restores the decreased bone matrix mineralization in *Trpv5* KO mice, suggesting the bone phenotype of *Trpv5* KO mice is secondary to the elevated $1,25(OH)_2D_3$ (van der Eerden et al. 2013). Thus, TRPV5 plays a role in osteoclast function, but it is not absolutely required for bone reabsorption. The role of TRPV5 in osteoclast function is not well understood, but TRPV5 is involved in receptor activator of NF-κB ligand (RANKL)-induced rise in $[Ca^{2+}]_i$ in human osteoclasts (Chamoux et al. 2010). This process is likely a part of the negative feedback loop to terminate RANKL-induced bone resorption (Chamoux et al. 2010).

8.3 Function in the Inner Ear

The low Ca^{2+} concentration of mammalian endolymph in the inner ear is required for normal hearing and balance. Marcus and colleagues showed that TRPV5 and TRPV6 may play roles in the function of inner ear (Yamauchi et al. 2005, 2010; Wangemann et al. 2007; Nakaya et al. 2007). TRPV5 was detected in native semicircular canal duct (SCCD) epithelial cells, cochlear lateral wall, and stria vascularis of adult rats along with other Ca^{2+} transport proteins (Yamauchi et al. 2010). TRPV5 protein was localized close to the apical membrane of strial marginal cells and in outer and inner sulcus cells of the cochlea (Yamauchi et al. 2010). TRPV5 transcript was responsive to $1,25(OH)_2D_3$ (Yamauchi et al. 2005); however, the protein level was not upregulated (Yamauchi et al. 2010). The levels of TRPV5 were decreased in the inner ear of older mice (Takumida et al. 2009). Mutations in pendrin (SLC26A4, an anion exchanger) cause the most common form of syndromic deafness. Reduced pH and utricular endolymphatic potential and increased Ca^{2+} concentration were found in pendrin KO mice (Wangemann et al. 2007; Nakaya et al. 2007). The reduced pH likely blocks the activity of TRPV5 and TRPV6, whose Ca^{2+} transport activity is reduced at low pH, similar to what was observed in primary SCCD cells (Nakaya et al. 2007). The elevation of endolymphatic Ca^{2+} level in pendrin KO mice may inhibit sensory transduction necessary for hearing and promote the degeneration of the sensory hair cells, which is necessary for the development of normal hearing (Wangemann et al. 2007).

8.4 Intestinal and Placental Ca^{2+} Transport

The levels of TRPV5 mRNA in intestine and placenta are much lower than TRPV6 (Peng et al. 2001). TRPV5 is regulated in the same direction with TRPV6 in the intestine in most cases, such as immobilization and exercise-induced alteration of gene expression (Teerapornpuntakit et al. 2009; Sato et al. 2006). In the placenta, Ca^{2+} transport in human syncytiotrophoblasts is insensitive to voltage and L-type Ca^{2+} channel modulators but is sensitive to TRPV5/6 blocker Mg^{2+} and ruthenium red (Moreau et al. 2002a). In cultured human trophoblasts isolated from term placenta, TRPV5 and TRPV6 expression correlated with the Ca^{2+} uptake potential along the differentiation of the trophoblasts (Moreau et al. 2002b). In contrast to the demonstrated role of TRPV6 in placental Ca^{2+} transport (Suzuki et al. 2008), the role of TRPV5 is unclear. TRPV5 likely plays a minor role in intestinal and placental Ca^{2+} transport due to its low levels of expression in these organs.

Table 3 Phenotype of *Trpv5* KO mice

Parameter	Alteration	Mechanism/explanation	References
Serum1,25 $(OH)_2D_3$	Elevated (2.9-fold)	Compensatory response	Hoenderop et al. (2003a)
Serum PTH	Elevated in older mice	Compensatory response	van Abel et al. (2006)
Ca^{2+} absorption	Increased (~30 % more). No increase in the absence of 25(OH) D_3 1α-hydroxylase	Due to the elevated 1,25$(OH)_2D_3$ and in turn increased intestinal TRPV6 and calbindin-D_{9k}	Hoenderop et al. (2003a), Renkema et al. (2005)
Ca^{2+} excretion	Increased (~sixfold)	TRPV5 plays a role in Ca^{2+} reabsorption	Hoenderop et al. (2003a)
Renal function	Increased urine volume and reduced urine pH	Activation of CaR in the collecting duct and subsequent upregulation of H^+–ATPase and reduction of aquaporin 2	Hoenderop et al. (2003a), Renkema et al. (2009b)
Bone	Reduced bone thickness	Prolonged elevation of 1,25 $(OH)_2D_3$	Hoenderop et al. (2003a), Nijenhuis et al. (2008), van der Eerden et al. (2013)

9 Lesson from *TRPV5* Knockout Mice

The most distinctive features of *Trpv5* KO mice include 2.9-fold elevation in serum 1,25$(OH)_2D_3$, sixfold increase in Ca^{2+} excretion, moderate reduced bone thickness in the femoral head, and normal level of plasma Ca^{2+} (Hoenderop et al. 2003a). In addition, polyuria and urine acidification were observed in *Trpv5* KO mice (Hoenderop et al. 2003a). These alterations in renal function are caused by the activation of CaR by the increased tubular Ca^{2+} in the collecting duct and subsequently upregulated proton secretion by the H^+–ATPase and reduced water reabsorption due to the downregulation of aquaporin 2 (Renkema et al. 2009b). These physiological adaptations reduce the risk of Ca^{2+} precipitations and stone formation. Intestinal Ca^{2+} absorption is increased in *Trpv5* KO mice as duodenal TRPV6 and calbindin-D_{9k} mRNAs are increased due to the elevated 1,25$(OH)_2D_3$ level (Hoenderop et al. 2003a). However, the serum Ca^{2+} levels are largely normal, even though the PTH level is elevated in older *Trpv5* KO mice (van Abel et al. 2006). The major phenotypes of *Trpv5* KO mice are listed in Table 3.

Studies with *Trpv5* KO mice reveal some important points in Ca^{2+} transport physiology: (1) the transcellular Ca^{2+} transport in the DCT and CNT, as represented by TRPV5, plays an important role in Ca^{2+} reabsorption. This is supported by the fact that removal of TRPV5 results in significant hypercalciuria. (2) TRPV5 is not indispensable to maintain a Ca^{2+} balance as long as vitamin D and related systems work normally. The body reset Ca^{2+} homeostasis by elevating the level of 1,25 $(OH)_2D_3$, which increases intestinal Ca^{2+} absorption to compensate the renal loss of

Ca^{2+} (Renkema et al. 2005). A new Ca^{2+} balance is achieved at higher intestinal absorption and higher urinary Ca^{2+} excretion in the absence of TRPV5. (3) The TRPV5 KO model shows an example that polyuria and reduced urinary pH are natural responses to increased Ca^{2+} excretion in order to prevent kidney stone formation. In addition, *Trpv5* KO mouse is a useful model to clarify some basic physiological mechanisms. For example, with this model it has been shown that calcitonin or thiazide-induced hypocalciuria occurs independent of TRPV5; thus, the responsible mechanisms likely reside in more proximal segments of the nephron (Hsu et al. 2009; Nijenhuis et al. 2005). All the studies indicate that TRPV5 does not alter the bulk Ca^{2+} reabsorption; rather, it provides a key mechanism for the fine-tuning of Ca^{2+} reabsorption.

10 Role in Hereditary and Acquired Diseases

As we learned from *Trpv5* KO mice, the lack of TRPV5 could be compensated through an elevation of $1,25(OH)_2D_3$, which regulates other proteins (e.g., TRPV6) in maintaining Ca^{2+} homeostasis. Thus, humans with defect in TRPV5 may have altered Ca^{2+} homeostasis, but could be clinically asymptomatic except for hypercalciuria. However, no mutation in the exons of *Trpv5* gene was found to be associated with autosomal dominant hypercalciuria (Muller et al. 2002). In another study involving 20 renal hypercalciuria patients, nonsynonymous variation of TRPV5 (A8V, R154H, and A561T) and synonymous variations were identified; however, these variants apparently do not alter the property of TRPV5 (Renkema et al. 2009a). Recently, S682P mutation of TRPV5 causes autosomal dominant hypercalciuria in mouse model (Loh et al. 2013). Interestingly, no significant functional differences were found between S682P and wild-type TRPV5 when expressed in *Xenopus* oocytes or HEK293 cells; the only difference was that S682P produced a lower baseline $[Ca^{2+}]_i$ than wild-type TRPV5 in HEK293 cells (Loh et al. 2013). However, mice with homozygous $TRPV5^{S682P}$ did show reduced TRPV5 immunostaining in the kidney and phenotype resembling that of *Trpv5* KO mice (Loh et al. 2013). This study suggests that mutations in TRPV5 could result in hypercalciuria; however, the functional changes of the TRPV5 mutations may not be detected in vitro. Thus, it remains to be further examined that mutations in *Trpv5* gene cause hypercalciuric disorders in humans.

In addition to a possible role of TRPV5 in hypercalciuria, TRPV5 expression and potentially activity are altered in response to a number of diseases and therapeutic conditions (Table 4). Mechanisms for the change in TRPV5 expression are often not fully understood. TRPV5 expression could be regulated by hormones, chemicals, and likely intracellular or extracellular Ca^{2+}. A change in *Trpv5* expression likely leads to alteration in urinary Ca^{2+} excretion; on the other hand, the alteration of filtered Ca^{2+} load or reabsorption of Ca^{2+} elsewhere in the nephron leads to increased or decreased delivery of Ca^{2+} to the DCT and CNT and in turn causes an elevation or reduction in *Trpv5* expression. If specific modulators for TRPV5 and TRPV6 could be developed, they could be useful to correct

Table 4 Alteration of *Trpv5* expression under disease and therapeutic conditions

Disease/therapeutic conditions	Model	Alterations	References
Arterial calcifications	TIF1alpha-deficient kidneys	mRNA ↑	Ignat et al. (2008)
Familial hypomagnesemia with hypercalciuria and nephrocalcinosis	*Cldn16*$^{-/-}$ mice	mRNA ↑	Will et al. (2010)
Streptozotocin-induced diabetes mellitus	Rats	mRNA/ protein ↑	Lee et al. (2006)
Idiopathic hypercalciuria	Genetic hypercalciuric stone-forming rats	mRNA/ protein ↓	Wang et al. (2008)
Preeclampsia	Primary culture of syncytiotrophoblasts from patients	mRNA/ protein ↓	Hache et al. (2011)
Gitelman syndrome	*Slc12a3* S707X knockin mice	mRNA/ protein↑	Yang et al. (2010)
Hydrochlorothiazide (high dose)	Rats	mRNA/ protein↓	Nijenhuis et al. (2003)
Hydrochlorothiazide (medium dose)	Rats	Protein↑	Jang et al. (2009)
Chlorothiazide(acute, low dose)	Mice	mRNA↑	Lee et al. (2004)
Furosemide	Mice	mRNA↑	Lee et al. (2007)
Gentamicin	Mice	mRNA↑	Lee et al. (2007)
Dexamethasone	Rats/mice	mRNA/ protein↑	Nijenhuis et al. (2004), Kim et al. (2009a, b)
Tacrolimus (FK506)	Rats	mRNA/ protein↓	Nijenhuis et al. (2004)
Chronic exposure to uranium	Rats	mRNA↓	Wade-Gueye et al. (2012)

Expression data were from mouse or rat kidney except for preeclampsia (human syncytiotrophoblasts) and chronic to uranium (cortical and trabecular bone)

abnormality in Ca^{2+} homeostasis under disease or therapeutic conditions. For example, under disease conditions caused by vitamin D signaling defect, such as in vitamin D-dependent rickets, type 2a (Online Mendelian Inheritance in Man #277440), intestinal and renal TRPV5 and TRPV6 expressions are likely disrupted as demonstrated in the *Vdr* KO mice (Van Cromphaut et al. 2001). Enhancing TRPV5- and TRPV6-mediated Ca^{2+} absorption would be helpful to achieve a positive Ca^{2+} under this condition. Similarly, enhancers for TRPV5 and TRPV6 are desirable to prevent osteoporosis in women after the menopause due to reduced estrogen level (Oz et al. 2007; van Abel et al. 2002; Irnaten et al. 2009). On the other hand, TRPV6 inhibitors would be needed in preventing absorptive hypercalciuria and kidney stone disease, and TRPV5 stimulator would be useful in preventing kidney stone formation in patients with renal Ca^{2+} leak. Stimulators of TRPV5 would be also helpful in tacrolimus-induced hypercalciuria (Nijenhuis et al. 2004) or other conditions associated with reduced expression of TRPV5 in Table 4.

11 Single Nucleotide Polymorphisms of TRPV5 in African Populations

TRPV5 and TRPV6 have been shown to have high frequency of SNPs in African populations. By analyzing SNP data from 24 African Americans and 23 European Americans in genes sequenced in SeattleSNPs, a 115-kb region in chromosome 7q34-35 of 4 contiguous genes, including *EPHB6*, *TRPV6*, *TRPV5*, and *KEL4*, was identified with features of a recent demographic selection (Stajich and Hahn 2005; Akey et al. 2004). *TRPV6* SNPs defined by three nonsynonymous SNPs (C157R, M378V, and M681T) exhibit most striking footprint of positive selection (Akey et al. 2006; Hughes et al. 2008). In addition, four nonsynonymous SNPs in TRPV5 were identified by SeattleSNPs: three of them (A8V, A563T, and L712F) were only present in African Americans, not in European Americans; R154H is common in both populations. The nonsynonymous SNP variations in TRPV6 ancestral haplotype are conserved in other species; in contrast, the variations in TRPV5 are newly derived as they are not commonly present in other species surveyed including chimpanzee, dog, rat, and mouse, with the exceptions of 563T in dog and 8V in rat (Na et al. 2009). In addition, the nonsynonymous SNPs of TRPV5 are not associated with each other as are those in TRPV6. By expression in *Xenopus* oocytes, we found that two of the SNPs, A563T and L712F, significantly increased TRPV5-mediated Ca^{2+} uptake by approx. 50 % and 25 %, respectively (Na et al. 2009). For A563T variant, the increased Ca^{2+} uptake activity was not associated with increased protein abundance in the plasma membrane; rather it was associated with increased apparent K_m for Ca^{2+} and increased sensitivity to extracellular Mg^{2+}, suggesting increased permeation of Ca^{2+} in the cation translocation pathway of the channel (Na et al. 2009). The A563 residue in the last transmembrane domain is 20 residues away from D542 residue in the Ca^{2+} filter in the pore. It is likely in the cation translocation path of the channel (Fig. 2).

African Americans exhibit lower urinary Ca^{2+} excretion than Caucasians (Braun et al. 2007; Pratt et al. 1996; Taylor and Curhan 2007), and the risk of kidney stone in African Americans is lower than that in Caucasians (Sarmina et al. 1987; Stamatelou et al. 2003). In addition, African Americans have higher bone mass (Bell et al. 1991) and lower incidence of osteoporosis-related fractures than whites (Bohannon 1999). Because of the high allele frequencies of TRPV5 and TRPV6 SNPs in African populations, these SNPs may contribute to the Ca^{2+} conservation mechanisms in African populations. Further population studies are necessary to clarify the relationship of SNPs in TRPV5 and TRPV6 and Ca^{2+} homeostasis in African populations.

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