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REVIEW ARTICLE

Mechanotransduction by TRP Channels: General Concepts and Specific Role in the Vasculature

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Abstract Transient receptor potential (TRP) ion channel superfamily is involved in sensing and transmission of a broad variety of external or internal stimuli, including but not limited to mechanical stress. Based on homology analysis, genetic and molecular studies have recently identified TRP channels in different tissues, comprising blood vessels. In invertebrates, many TRP channels including five TRPV channels identified in *Caenorhabditis elegans* and two in *Drosophila* have been implicated in mechanosensory behaviors as molecular basis of volume regulation, hearing and touch sensitivity. Consistently, in mammals many TRP family members such as TRPC1, TRPC3, TRPC6, TRPM4, TRPM7, TRPN1, TRPA1, TRPY1, TRPP1, TRPP2, and notably, TRPV1, TPRV2 as well as TRPV4 have been reported to be involved in mechanotransduction. This review summarizes recent and at times controversial findings on the role and regulation of TRP channels in mechanotransduction. Specifically, we highlight the relevance of TRPV channels in vascular regulation and focus on TRPV4 in the vascular system of the lung, which is constantly exposed to a unique combination of circumferential and longitudinal strains. In light of our observation in intact pulmonary microvessels that mechanical stress induced Ca^{2+} signaling in endothelial cells is closely related to TRPV4 activity, we postulate that TRPV4 plays a critical role in lung vascular mechanotransduction. The progress in this rapidly expanding field may allow for the identification of new molecular targets and the development of new therapeutic approaches in a number of intractable diseases related to mechanical stress.

Keywords TRP channels · Vascular · Mechanotransduction

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Introduction

The continuous sensing, discrimination and transmission of small changes in mechanical forces, and the subsequent conversion of stimuli into adaptive biological signaling cascades, is of critical relevance in the regulation of vascular tone, local blood flow and vessel homeostasis [1–3]. Different cellular structures have been implicated into the sensing of mechanical forces by the endothelium including cytoskeletal components, cell–cell/cell–matrix interactions, caveolae, the endothelial surface layer, cilia, and in particular ion channels [4]. Yet, the molecular identity and regulation of the proposed mechanosensitive ion channels are still largely unknown. On the basis of homology analyses, growing evidence from extensive genetic and molecular investigations has recently identified members of the transient receptor potential (TRP) superfamily of ion channels as potential candidates for cellular mechanosensing and mechanotransduction. Of the 33 known mammalian TRP channels, research efforts have particularly focused on three

subfamilies: TRPC (canonical), TRPM (melastatin) and TRPV (vanilloid). Other recently identified families including the TRPP (polycystin), TRPML (mucolipin), TRPA (ankyrin) and TRPN (NOMPC) channels are yet less well characterized, particularly with respect to their expression and function in the vascular system. Several TRP channels, such as TRPA1, TRPC1, TPRC6, TRPV1, TRPV2, TRPV4, TRPM4, TRPM7 and TRPP2, have been implicated in mechanotransduction (elegantly reviewed in [5–13]). In vascular tissue, recent work has focused in particular on members of the TRP vanilloid (TRPV) subfamily of channels as promising candidates for mechanosensitive ion channels. This review summarizes current data characterizing the role of TRP channels in mechanotransduction, and specifically highlights the function of TRPV channels in the pulmonary vascular system.

Vascular Mechanotransduction

Vascular beds in the intact organism are constantly exposed to a complex combination of frequently changing and/or oscillating mechanical forces. Cardiovascular diseases are often associated with characteristic changes in mechanical stress, which may contribute pivotally to subsequent vascular pathology. Typically, blood vessels are subjected to two major types of mechanical stress, i.e. shear stress and stretch which differ considerably in amplitude, direction and oscillatory behavior in different vascular beds. Shear stress is the result of blood flow through the circulation which continuously exerts a viscous drag on the luminal endothelial surface. Pulsations of pressure and flow following the cardiac rhythm are not only propagated into arteries and arterioles, but also transmitted in part into the systemic capillary bed as well as the alveolar capillary network of the lung [14, 15]. Strikingly, in the alveolar capillary network, even under constant flow conditions and in the absence of respiratory movements, blood flow switches continuously between different perfusion pathways in a non-random pattern with a fractal dimension near 1.0 [16]. The constant recruitment and derecruitment of capillary segments results in a continuous switch between conditions of high and zero shear stress acting exclusively on the pulmonary capillary endothelium.

Stretch, on the other hand, typically results from the circumferential strain caused by radial distension of the vascular diameter due to vascular filling and rhythmic changes in vascular pressure over the cardiac cycle. While in the systemic circulation, small arteries and arterioles constrict in response to increasing vascular pressures by a mechanism known as myogenic response [3]; this intrinsic mechanism for stabilization of vascular strain is largely absent in the pulmonary vasculature [17, 18]. Pulmonary blood vessels are in addition exposed to oscillatory changes in longitudinal strain due to the axial elongation of lung tissue and blood vessels during the respiratory cycle [19]. Thus, the concert of mechanical forces acting upon microvessels differs considerably in between organs and vascular compartments, with a particularly unique role of the pulmonary microvascular bed.

Both vascular smooth muscle cells and the endothelium, acting separately or in combination, are involved in the process of vascular mechanotransduction. For instance, the pressure-dependent myogenic response is inherent to the vascular smooth muscle and largely independent of the endothelium [20]. In contrast, shear- or stretch-induced responses in systemic or pulmonary capillaries, where only endothelial cells constitute the vessel wall, are exclusively attributable to mechanosensation by the endothelial cell layer. In general, two seemingly conflicting theories exist how mechanical forces may elicit biological reactions in cells. According to the "decentralization" model, cellular responses to mechanical stress result from spatial-integrated molecular signaling, i.e. the mechanical stress is transmitted from a structural mechanosensor via a "mechanical continuum", which comprises cytoskeletal elements, distant molecules in the extracellular matrix, the cytoplasm and the nucleus, to eventually induce an adaptive cellular response at a site within the cell that is remote from the original location of mechanosensation [21, 22]. The contrasting notion of a "centralized" model argues that sensing of mechanical stress should ideally be localized to the site of action, i.e. the plasma membrane [23]. The latter theory is largely based on experimental evidence that membrane ion channels are functionally involved in vascular endothelial responses to mechanical stress. The elegant study by Lansman and colleagues marks the prelude in the recognition of vascular mechanosensory ion channels. By recording ion channel activity from aortic endothelial cell membrane patches, these authors identified a unitary inward current associated with the opening of a Ca^{2+}-permeable ion channel, which increased its opening frequency when the cell membrane was stretched [24]. These experiments demonstrated for the first time the existence of mechanosensitive ion channels in vascular cells. This theory was further substantiated and developed by the findings that shear stress can similarly activate a Ca^{2+}-permeable ion channel, inducing Ca^{2+} influx into the endothelial cytoplasm. This shear-activated mechanosensitive ion channel, significantly up-regulated in hypertensive rats, was shown to be Ca^{2+} selective (i.e. to be by far more permeable to Ca^{2+} than Na^{+} or Cs^{+}), indicating a critical role for Ca^{2+} influx as a second messenger signal in endothelial mechanotransduction [25–29]. The functional relevance of the resulting Ca^{2+} influx was shown in subsequent experiments demonstrating the activation of neighboring Ca^{2+} sensitive K^{+} channel [30–33], which could be inhibited by either removal of extracellular Ca^{2+} or administration of the trivalent lanthanide gadolinium, an unspecific

inhibitor of mechanosensitive cation channels [34]. Studies from our own group using real-time fluorescent imaging of the endothelial Ca^{2+} concentration ($[Ca^{2+}]_i$) in isolated perfused rat lungs likewise revealed an increase in endothelial Ca^{2+} in response to an elevation of vascular pressure. Similar to the observed Ca^{2+} influx in response to stretch or shear in vitro, the endothelial $[Ca^{2+}]_i$ increase in response to pressure was blocked by Ca^{2+} free perfusion or gadolinium, respectively (Fig. 1), thus confirming the functional presence of mechanosensitive cation channels in intact lung microvascular endothelial cells. However, the structural identity of these mechanosensitive ion channels and their regulation remained largely obscure. Only recently, our understanding of vascular mechanosensation has been revolutionized by the identification of the transient receptor potential (TRP) superfamily of ion channels, which comprises a group of channel proteins with multiple sensory functions.

General Features of TRP Channels

Marked by diversity in cell and tissue expression patterns and polymodal mechanisms of activation, TRP channels have been initially described in *Drosophila.* The critical observation of an abnormal electroretinogram from a *Drosophila* mutant [35] initiated a series of studies, which demonstrated an essential role for TRP channels in phototransduction [36–38]. Subsequent investigations identified some 70 TRP channels in both invertebrates and vertebrates. In sea squirts, nematodes and fruitflies, 30, 24 or 16 different TRP channels have been identified, respectively. TRP channels are also expressed in yeast, and zebrafish express as many as 60 TRP gene encoded channels. In mammals, 33 different TRP channels have thus far been identified [39, 40] (Fig. 2).

Typically, each TRP channel subunit consists of six putative transmembrane spanning segments (S1–6), implanted with a pore-forming loop between S5 and S6, and intracellularly located cytoplasmic NH_2 and COOH termini [41]. Depending on the TRP family, the N-terminus contains between zero and eight ankyrin repeats, a predicted coiled coil region, and a putative caveolin-binding domain. The C-terminus comprises a TRP signature motif (EWKFAR), a proline-rich motif, the calmodulin/inositol 1,4,5-trisphosphate (IP_3) receptor-binding (CIRB) domain, and a predicted coiled coil region (Fig. 3) [39, 42–46]. According to amino acid homologies, the TRP family is

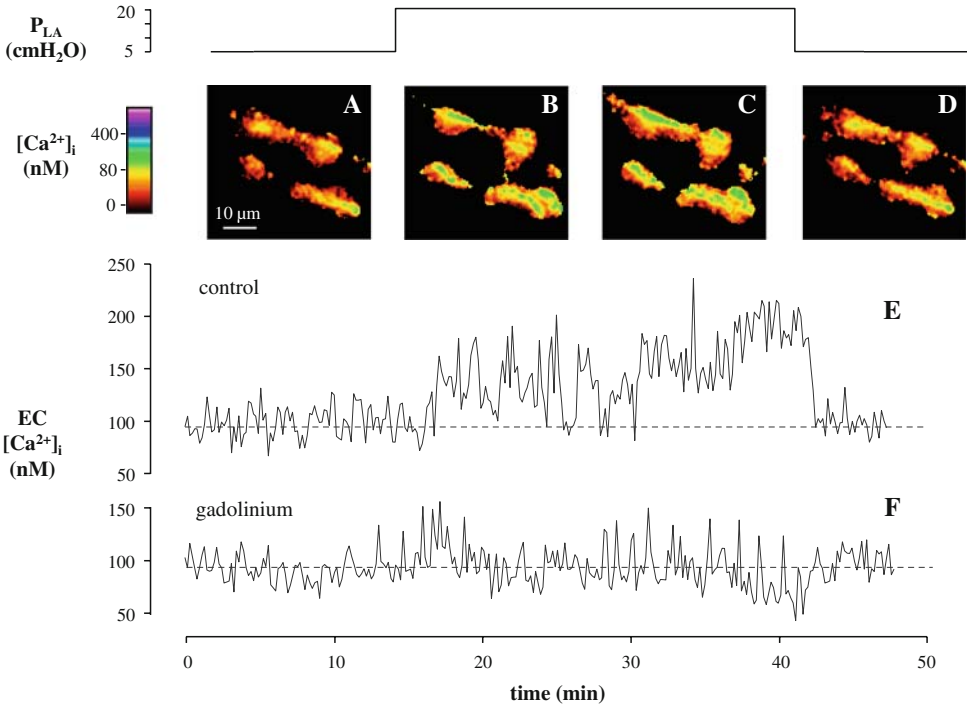

Fig. 1 Vascular endothelial $[Ca^{2+}]_i$ response to an increase in left atrial pressure (P_{LA}) from 5 to 20 cmH_2O and after return to 5 cmH_2O (*top*). (*A–D*) Sequential ratiometric images of fura-2 loaded endothelial cells in a lung venular capillary, color coded for endothelial $[Ca^{2+}]_i$. Images obtained in 10 min intervals at P_{LA} of 5 and 20 cmH_2O demonstrate a pressure-induced increase in mean endothelial $[Ca^{2+}]_i$. (*E*, *F*) Representative $[Ca^{2+}]_i$ profiles in single endothelial cells of lung venular capillaries in the absence (*E*) or presence (*F*) of gadolinium, an unspecific inhibitor of mechanosensitive cation channels. $[Ca^{2+}]_i$ is determined at baseline (P_{LA}, 5 cmH_2O), during 30 min of P_{LA} elevation to 20 cmH_2O, and for 5 min after return to baseline P_{LA}. Gadolinium blocks the pressure-induced increase in mean endothelial $[Ca^{2+}]_i$

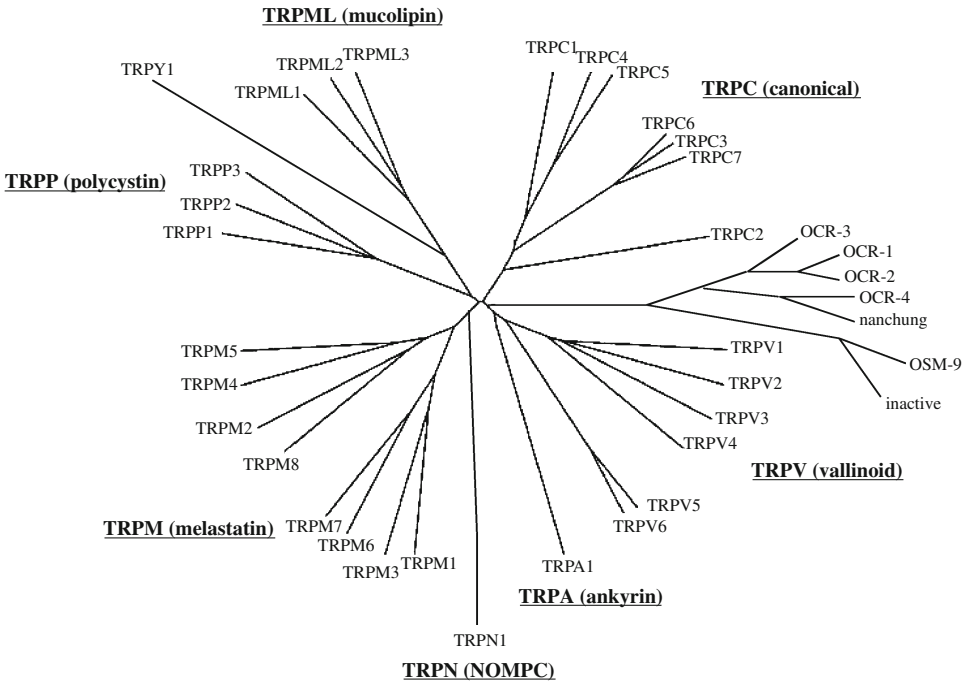

Fig. 2 Unrooted phylogenetic tree generated by aligning the transmembrane domains of the TRP channels. The seven main branches are denoted with *underline*, the letters and numbers following TRP indicate TRP subfamily and member, respectively

differentiated into seven subfamilies. Presently, there are seven (TRPC1-7) and eight (TRPM1-8) channels identified in the TRPC and TRPM subfamilies, respectively [40]. The TRPV subfamily comprises six members, while only a few members of the TRPA (TRPA1), TRPP (three members), and TRPML (three members) subfamilies have been identified in mammals so far. Channels of the TRPN subfamily have only been detected in worms, *Drosophila*, and zebrafish to date.

TRP channels are widely distributed throughout the body and frequently have numerous splice variants. They are expressed in a vast number of different cell types where they fulfill a plentitude of functions. While some TRP channels have been detected in central nervous system neurons, their expression is particularly abundant in sensory receptor cells, including cells mediating vision, pheromone sensation, and thermal sensation, taste, touch and cell volume regulation [47, 48]. The preferential distribution of TRP channels in sensory organs alludes to their critical and diverse role in signal sensation and transduction. Importantly, mechanosensory properties have been proposed for a number of TRP channels, and those expressed in the vasculature of vertebrates are summarized in Table 1.

Role of the TRPV Subfamily in Mechanotransduction

While sequence homology is not strikingly robust between mammalian and invertebrate TRPV proteins, *OSM-9* and *OCR-2* in *Caenorhabditis elegans* exhibit considerably functional orthology to their mammalian counterparts TRPV4 and TRPV2, respectively.

OSM-9 and OCRs

Colbert and Bargmann [49, 50] first identified the *osm-9* gene in a screen for mutants defective in olfaction in *C. elegans*, which heralded the recognition of the role of TRPV channels in mechanotransduction. *osm-9*-encoded protein was found to have structural similarity to the *Drosophila* phototransduction TRPs and is considered to constitute an invertebrate branch of the TRPV family. Expressed in the ASH, FLP and OLQ neurons in *C. elegans*, *osm-9* plays an important role for various sensory submodalities including chemosensation, osmosensation and mechanosensation as demonstrated by the identification of *osm-9* mutants in screens for mutant defective in volatile avoidance, osmotic avoidance, or response to nose touch [51].

Further studies identified four other genes in the nematode genome, *ocr-1* to *ocr-4*, of which at least *ocr-2* is related to osmosensing by *osm-9*. *osm-9* alone seems sufficient for olfactory adaptation in AWC neurons, but requires interaction with *ocr* proteins for the transmission of sensory signals in ASH and OLQ neurons [52]. *osm-9* and *ocr-2* may assemble into an integrated channel, or be components of a more heterogeneous mechanotransduction complex [47]. In ASH and OLQ neurons, which can be activated by mechanical stimuli, *osm-9* was found to be co-localized with *ocr-2* and *ocr-4*, respectively [52], suggesting a functional role of the TRPV superfamily in osmo-mechanotransduction.

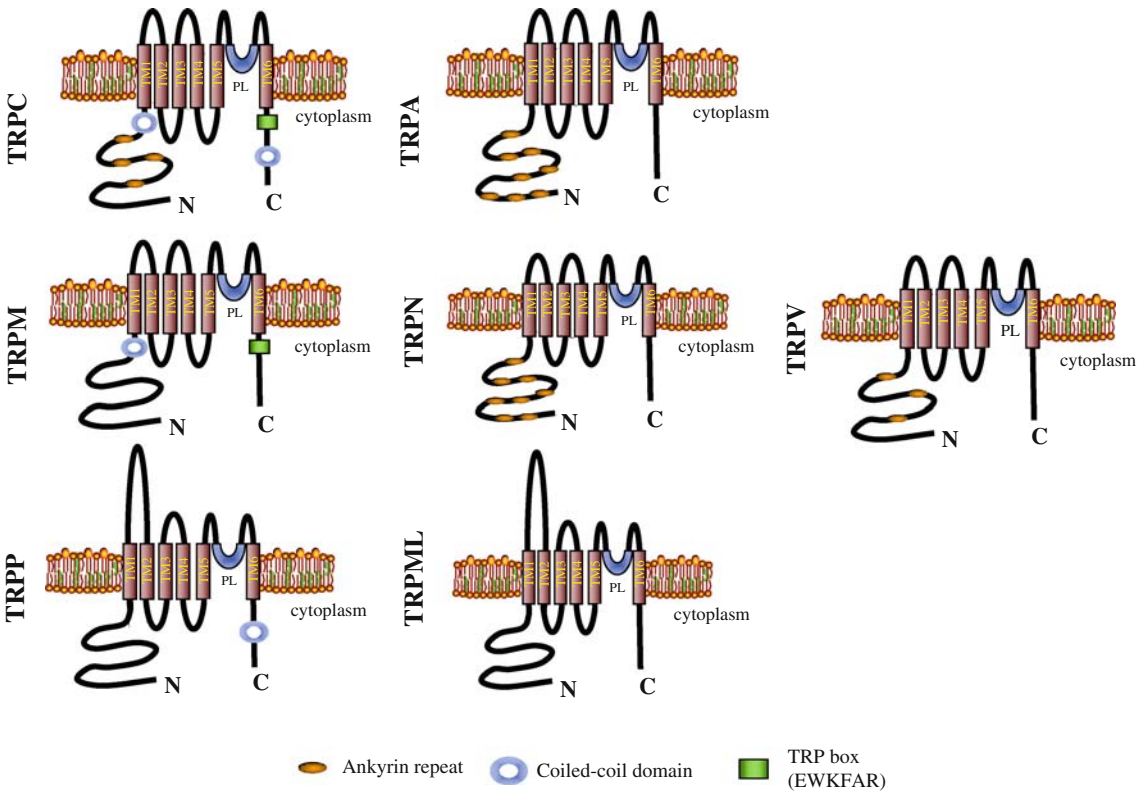

Fig. 3 Simplified schematic structure sketches of the seven subfamilies of TRP channels. The following domains are indicated as *TM* transmembrane segments, *PL* pore loop. Other domains as ankyrin repeats, coiled-coil domain, and TRP box are depicted as shown

Table 1 TRP channels with proposed mechanosensory or mechanotransducing function in vertebrates

TRP channel and ensemble gene identification no.	Modes of mechanical activation	Chromosome
TRPV1/ENSG00000043316	Hypotonicity [85, 168], stretch (mounted isolated airway rings) [68] (isolated rectal tissue attached to a cantilever pulley system) [169]	17p13.3
TRPV2/ENSG00000154039	Hypotonicity [78, 170], stretch (cell membrane stretch) [10, 89]	17p11.2
TRPV4/ENSG00000111199	Hypotonicity [55, 63, 171–177], shear (flow chamber) [61, 175, 178] (isolated perfused carotid artery) [65, 66], stretch (silicon chamber) [179] (uniaxial cyclic stretch or static stretch) [180] (cytometric experiments) [181] (overventilation [72] or elevated hydrostatic pressure [60] in isolated murine lungs)	12q24.1
polycystin-1/ENSG00000008710	Shear stress (flow chamber) [113]	16p13.3
TRPP1/ENSG00000118762	Shear stress (flow chamber) [113, 182]	4q21–23
TRPC1/ENSG00000144935	Stretch (pipette suction) [87]	3q22–3q24
TRPC3/ENSG00000138741	Shear stress/stretch (mounted isolated perfused aortic rings/cerebral arteries) [89, 91]	4q27
TRPC6/ENSG00000137672	Hypotonicity/shear stress (flow chamber)/stretch (pipette suction) [3, 89, 92]	11q21–22
TRPM4/ENSG00000130529	Shear stress (flow chamber)/stretch (mounted isolated arteries) [183, 184]	19q13.33
TRPM7/ENSG00000092439	Shear stress (flow chamber) [101, 103]/stretch (pipette suction) [102, 103]	15q21

Nanchung (NAN) and Inactive (IAV)

In *Drosophila* chordotonal organ, where the sound-induced antennal or tympanal mechanical vibrations are transmitted to ciliated sensory neurons and transduced to receptor potentials, Kim and colleagues identified the TRPV homologue Nanchung (NAN) protein. In mutants lacking NAN, the antennal sound-evoked potentials were completely absent, indicating that NAN was an essential component of the chordotonal mechanotransducer [53].

Gong et al. [54] subsequently identified that mutation of Inactive (IAV), the only other TRPV homologue in *Drosophila*, results in lack of NAN expression in proximal cilia, and vice versa, suggesting that both IAV and NAN may contribute to a heteromultimeric transduction channel in vivo. While these data clearly implicate a role of NAN in chordotonal mechanotransduction, it is important to note that they do not prove a direct sensory role of the channel, which may equally constitute an essential component of the early downstream signaling pathway.

TRPV4

Stimulated by the evidence that the *osm-9* gene encoded protein in *C. elegans* confers sensitivity in mechanosensation, vertebrate homologues were searched subsequently. Expressed-sequence tags encoding vertebrate proteins homologous to *osm-9* and vanilloid receptor VR1 were identified in GenBank and used as probes for high-stringency screening of mammalian libraries [55], which yielded the TRPV4 channel (also called OTRPC4, VR-OAC and VRL-2) [55–58]. TRPV4 is widely expressed in various tissues, including liver, heart, lung, trachea, testis, spleen, salivary glands, the stria vascularis of the cochlea, and vascular endothelial cells [55–60].

The structure of TRPV4 channels shares similarities with *osm-9*, thus conveying its mechanotransduction properties, as demonstrated by several lines of evidence. When a TRPV4 cDNA construct with a C-terminal V5 epitope was stably transfected into human embryonic kidney (HEK) 293 and Chinese hamster ovary cells, exposure of these TRPV4-transfected cells to shear stress (3–20 dyne/cm^2) induced channel activation, as evident by an increase in intracellular calcium that could be inhibited by ruthenium red. Of note, the ambient temperature is a critical modulator of this activation as characterized in detail in a series of elegant studies [55, 57, 61, 62]. In *C. elegans*, Liedtke et al. [63] found that expression of mammalian TRPV4 (VR-OAC) cDNA in sensory neurons of *C. elegans* directs behavioral responses to osmotic and mechanical stimuli, providing solid evidence that TRPV4 can function as a component of an osmotic/mechanical sensor in vivo. In the rat carotid artery and the *arteria gracilis*, Köhler and colleagues observed that pharmacological activation of endothelial TRPV4 by 4α-phorbol-12,13-didecanoate (4αPDD) triggered endothelial Ca^{2+} influx and subsequent nitric oxide (NO) mediated vasodilation. Importantly, elevated shear stress (3 dyne/cm^2) also elicited substantial vasodilation similar to the effects of 4αPDD, and the shear stress response could be reduced dramatically by the TRPV4 inhibitor ruthenium red. On this basis, the authors proposed that mechanical activation of TRPV4 by shear stress may play a critical role in endothelial mechanotransduction and shear stress-mediated vasodilation [64]. In a follow-up study, this notion was substantiated by data derived from TRPV4 genetic knockout mice. In carotid artery explants acutely derived from these mice, shear stress (3–7 dyne/cm^2) induced vasodilation was absent, whereas both acetylcholine-induced and endothelium-derived hyperpolarizing factor (EDHF)-mediated vasodilation remained intact [65]. The functional role of TRPV4 in flow-mediated vasodilation was recently confirmed in a study by Loot et al. [66] using a similar experimental setup. In addition, these authors identified that TRPV4 contributes to the EDHF-dependent component of the flow response in that TRPV4 activation by flow requires an active cytochrome P450 epoxygenase and the translocation of TRPV4 to the cell membrane. In line with this notion, Saliez et al. [67] showed in a recent study that Ca^{2+} entry via TRPV4 participates in EDHF-signaling initiation, which seems to require colocalization of TRPV4 and caveolin-1 in endothelial cells. TRPV4 is also expressed in airway smooth muscle cells of the lung where it contributes to bronchoconstriction upon hypotonic swelling, again in line with a stretch-induced activation of TRPV4 [68].

TRPV4 is also reported to mediate protease activated receptor 2 (PAR2) induced sensitization of colonic afferent nerves and visceral hyperalgesia. Activation of PAR2 can increase currents in the primary spinal afferent neurons innervating the colon, evoke discharge of action potentials from colonic afferent fibers, and induce mechanical hyperalgesia. These responses—although not directly linked to mechanotransudction *per se*—also seem to require the presence of TRPV4 [69]. Cenac et al. [70] detected that TRPV4 activation causes visceral hypersensitivity, and implicated the channel in the nociceptive response to colorectal distention under basal conditions and in PAR2 induced hypersensitivity, again supporting its functional role in mechanosensation.

The functional role of TRPV4 in mechanotransduction was further corroborated by studies in mammalian airways and pulmonary blood vessels, where it is abundantly expressed (Fig. 4). Already a decade ago, Parker et al. [71] had identified a stretch-activated, gadolinium-inhibitable cation channel in rodent lungs, which they proposed to mediate the increase in lung vascular permeability in response to ventilation with high peak inflation pressures (PIP) via Ca^{2+} influx into the endothelial cytoplasm. In a recent follow-up study, Hamanaka et al. [72] showed that high PIP induced endothelial Ca^{2+} influx and vascular permeability increase were blocked in mice in the presence of the TRPV4 inhibitor ruthenium red or in TRPV4 knockout mice, suggesting a critical role for TRPV4 in mechanosensing and or mechanotransduction in response to high PIP. Of note, PIP was shown to stimulate tyrosine

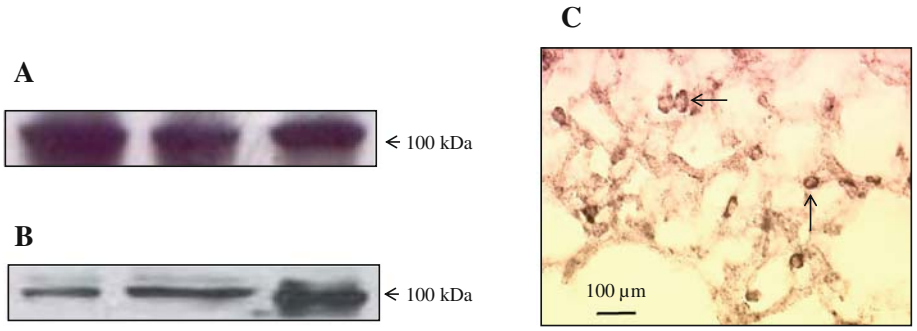

Fig. 4 TRPV4 expression in lung microvascular endothelial cells. Representative Western blots of TRPV4 are from whole lung homogenate (**a**) and freshly isolated lung vascular endothelial cells (**b**). Representative image of TRPV4 immunostaining in rat lungs (**c**). *Arrows* indicate TRPV4 positive endothelial cells in lung septal capillaries. Replicated in $n = 3$ each

phosphorylation of TRPV4 in lung homogenates, which may be relevant to the mechanosensing properties of the channel [72]. In line with those data, Alvarez et al. [59] revealed that activation of TRPV4 channels by stimuli other than mechanical stress, e.g. 4αPDD or 5,6- or 14,15-epoxyeicosatrienoic acids (EETs) can similarly increase lung vascular permeability and induce blebs or breaks in the endothelial and epithelial layers of the alveolar septal wall. Jian et al. demonstrated that the characteristic increase in lung vascular permeability in response to another mechanical stress, namely an increase in lung vascular pressure, was significantly attenuated by the TRPV4 antagonist ruthenium red, the phospholipase A_2 (PLA_2) inhibitor methyl arachidonyl fluorophosphonate, or the P450 epoxygenase inhibitor 6-(2-propargyloxyphenyl)hexanoic acid [73]. These findings indicate that an elevation in lung vascular pressure activates TRPV4 by a signaling cascade that sequentially induces activation of PLA_2, PLA_2-dependent release of arachidonic acid from the cell membrane phospholipids, cytochrome P450 epoxygenase-dependent synthesis of epoxyeicosatrienoic acids (EETs) from arachidonic acid, and the subsequent activation of TRPV4. From their observation that gadolinium, the unspecific blocker of mechanosensitive cation channels did not further attenuate the endothelial Ca^{2+} response in TRPV4$^{-/-}$ mice, the authors further concluded that TRPV4 is the exclusive mechanosensory cation channel mediating the endothelial response to elevated vascular pressure.

By use of in situ fluorescence imaging, our own group recently consolidated the notion that TRPV4 plays a major role in mechanotransduction in lung microvascular endothelial cells [60]. We found characteristic functional consequences of elevated lung hydrostatic pressure such as endothelial Ca^{2+} influx, vascular permeability increase and lung hydrostatic edema formation to be markedly attenuated by TRPV4 inhibitors or in lungs of TRPV4$^{-/-}$ mice (Fig. 5). Importantly, we could identify a cGMP-dependent negative feedback loop which attenuated TRPV4 activation by mechanical stress and limited downstream functional consequences. This finding not only provides novel insights into the regulation of TRPV4 in mechanotransduction (Fig. 6), but also identifies new targets to counteract TRPV4 activation in lung vascular pathologies associated with increased mechanical stress, such as hydrostatic lung edema or ventilator-induced lung injury. The inhibition of TRPV4 by cGMP may be translated into a therapeutic concept, e.g. by inhibition of PDE5 which degrades cGMP by hydrolysis to GMP. Clinically approved PDE5 inhibitors may thus present an attractive new strategy to inactivate TRPV4 and thus protect the lung vascular barrier in conditions of mechanical stress. In an experimental model of myocardial infarction induced acute lung edema, we provide proof-of-principle for this concept by demonstrating that the PDE5 inhibitor sildenafil effectively reduced protein and fluid extravasation and lung edema [60].

TRPV2

TRPV2 was first described as a component of the physiological response to noxious thermal stimuli [74, 75]. Yet, subsequent investigations indicated also a functional role in mechanotransduction for TRPV2. Kanzaki et al. observed that the growth-factor-regulated channel (GRC), a murine homologue of TRPV2, was rapidly recruited on or translocated to the plasma membrane surface in response to growth factors, and appeared to be activated by myocyte stretch [76]. For GRC, trafficking and activation mechanisms seem to intersect [77]. Furthermore, Muraki et al. [78] could demonstrate that in TRPV2 expressing cells, both hypotonic cell swelling and membrane stretch elicited calcium influx, which was inhibitable by ruthenium red or TRPV2 specific antisense oligonucleotides. At this stage, it seems reasonable to consider TRPV2 as a likely candidate

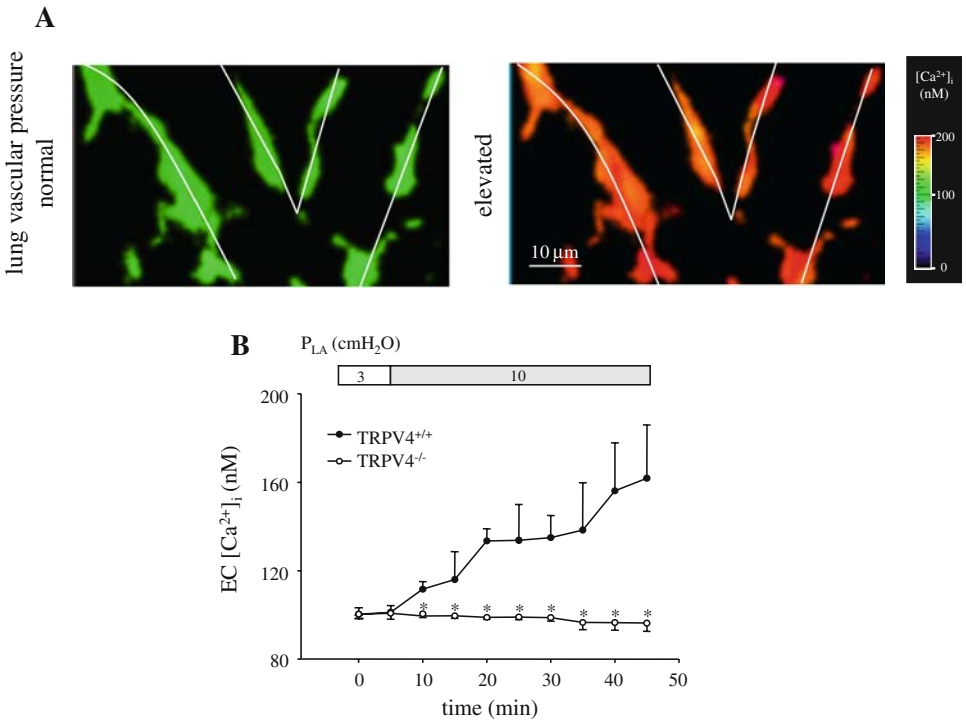

Fig. 5 **a** Fluorescence microscopic images of a murine lung venular capillary show fura-2 loaded endothelial cells color coded for $[Ca^{2+}]_i$ at baseline left atrial pressure (P_{LA}) of 3 cmH_2O (*left*) and 30 min after P_{LA} elevation to 10 cmH_2O (*right*). Note vessel distension at elevated P_{LA} indicating endothelial stretch, and the rise in fluorescence signal representing an increase in endothelial $[Ca^{2+}]_i$. **b** Group data of endothelial $[Ca^{2+}]_i$ (EC $[Ca^{2+}]_i$) in lungs of TRPV4$^{-/-}$ and wild type (TRPV4$^{+/+}$) mice are shown as 5-min averages at baseline left atrial pressure (P_{LA} = 3 cmH_2O) and over 40 min of P_{LA} elevation to 10 cmH_2O. The pressure-induced increase of EC $[Ca^{2+}]_i$ in TRPV4$^{+/+}$ is absent in TRPV4$^{-/-}$ mice. * $P < 0.05$ versus TRPV4$^{+/+}$

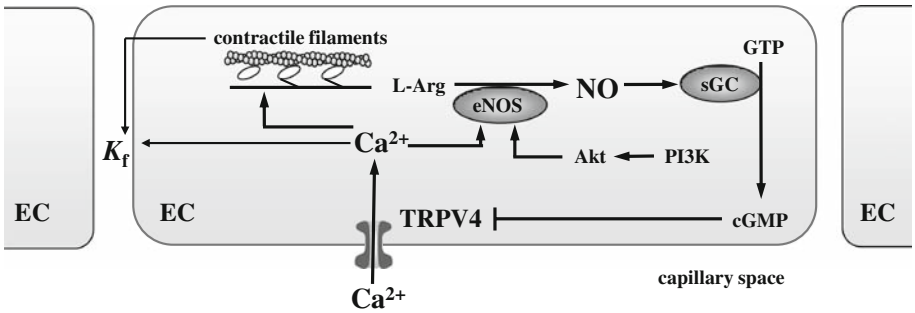

Fig. 6 Pulmonary vascular endothelial response to acute pressure stress. Schematic drawing illustrates pressure-induced Ca^{2+} entry into an endothelial cell via mechanosensitive TRPV4 channels and stimulation of the following downstream signalling cascades: (i) activation of contractile filaments with a subsequent increase in vascular filtration coefficient (K_f), (ii) Ca^{2+}- and PI3K-dependent activation of eNOS. The resulting formation of NO from L-arginine (L-Arg) limits the endothelial $[Ca^{2+}]_i$ response by blocking TRPV4 channels in a negative, cGMP regulated feedback loop. *sGC* soluble guanylate cyclase, *GTP* guanosine 5′-triphosphate

for mechanotransduction. Functional consequences and tissue and cell-type specific expression of TRPV2 are however less clear yet, and warrant further investigations.

TRPV1

Numerous studies have reported mechanotransduction effects of TRPV1 in heterologous organs or systems. TRPV1 was originally implicated with inflammatory thermal hyperalgesia, as well as with nociception and pain sensation [79, 80]. Birder et al. [81] observed that stretch-evoked ATP release and membrane capacitance changes were diminished in bladders excised from TRPV1$^{-/-}$ mice, which gave rise to the conclusion that TRPV1 is essential for normal mechanically evoked purinergic signaling by the urothelium. In agreement, absence of TRPV1 was found to result in a reduced afferent nerve response to mechanical stimulation in the jejunum [82]. In mesenteric arterioles, an increase in intraluminal pressure was shown to activate TRPV1 on C-fibers through generation of 20-hydroxyeicosatetraenoic acid (20-HETE) [83]. In patients suffering from fecal urgency, i.e. a pathological rectal hypersensitivity to mechanical distention, TRPV1 positive nerve fibers in the rectum were dramatically increased, which may provide the molecular correlation to a lower functional threshold to mechanical stretch in the rectum

tissue of these patients [84]. However, no electrical currents were detectable in urothelial cells in response to mechanical stimulation. This finding raises the question whether TRPV1 indeed acts as mechanosensor itself, or is not rather activated secondary by mediator molecules generated following tissue or cell stretch [6].

In a recent study, Sharif-Naeini and colleagues identified an N-terminal splice variant of TRPV1 that is expressed in SON neurons and required for osmosensory transduction. SON neurons in TRPV1$^{-/-}$ mice could not generate the characteristic ruthenium red-sensitive increase in membrane conductance and depolarizing potentials in response to hyperosmotic stimulation, suggesting that the TRPV1 gene may encode a central component of the osmoreceptor [85]. Another study by the same group provided evidence that hypertonic conditions increase membrane cation conductance in neurons in the organum vasculosum lamina terminalis (OVLT), which is considered to present the primary locus of the brain for the detection of osmotic stimuli. The resulting inward currents depolarize the osmoreceptor and enhance the discharge of action potentials in the OVLT neurons. Importantly, this signaling cascade is absent in OVLT neurons from TRPV1$^{-/-}$ mice, suggesting that a product of the TPRV1 gene is required for osmosensory transduction in the OVLT [86].

Mechanotransduction by Miscellaneous TRP Channels

TRPC Channels

TRPC1

Heterologous expression of human TRPC1 or transfection of human TRPC1 into CHO-K1 cells resulted in a significant increase in mechanosensitive cation channel patch density, whereas injection of a TRPC1-specific antisense RNA abolished the mechanotransduction activity [87], suggesting that TRPC1 might be a mechanosensitive cation channel in vertebrates. However, a series of recent studies elegantly reviewed by Sharif-Naeini et al. [5] failed to confirm the mechanosensitivity of TRPC1 in native arterial myocytes or transfected cells. This view is in line with the fact that TRPC1$^{-/-}$ mice are viable, healthy, and fertile and show a physiological myogenic response, indicating that TRPC1 is not an obligatory component of stretch-activated channel complexes in vascular smooth muscle cells [88].

TRPC3 and TRPC6

Both TRPC3 and TRPC6 have been implicated in the regulation of vascular myogenic tone [3, 89–91]. Knockdown of TRPC6 by antisense oligodeoxynucleotides was shown to attenuate the myogenic response and inhibit the pressure induced depolarization in vascular smooth muscle cells [92]. Yet, the fact that TRPC6$^{-/-}$ mice have an amplified myogenic response in isolated arteries argues against a primary role of TRPC6 in vascular mechanotransduction [89]. Similarly to TRPC1, stretch activation of the homomultimeric TRPC6 channel could not be confirmed in transiently transfected African green monkey kidney (COS) cells [93]. Based on these data, Sharif-Naeini et al. concluded that TRPC6 *per se* cannot be opened by membrane stretch, and mechanical activation may either involve other interacting channel subunits or may be indirect [5] and secondary to other mechano-sensitive pathways such as diacylglycerol [94] or 20-hydroxyeicosatetraenoic acid [95–97] signaling.

TRPM Channels

TRPM4

TRPM4 but not TRPM5 is expressed in cerebral artery smooth muscle cells, and—like TRPC3 and TRPC6—has been considered to mediate membrane depolarization, thus participating in the regulation of myogenic vascular tone [98]. Knock-down of TRPM4 by antisense oligodeoxynucleotides attenuated the pressure-induced smooth muscle cell depolarization and myogenic vasoconstriction of intact cerebral arteries [99], suggesting a role for TRPM4 in vascular mechanosensation and/or mechanotransduction. However, recent experiments in TRPM4$^{-/-}$ mice did not reveal a role for TRPM4 in the pressure-induced myogenic response [5], indicating again that TRPM4 may not present the native mechanosensitive cation channel in arterial myocytes. Rather, its activation by stretch may be secondary to calcium and phosphorylation-dependent responses [98–100].

TRPM7

TRPM7 acts both as an ion channel and as a functional kinase. It has been linked to mechanotransduction in vascular smooth muscle cells because it was found to translocate to the plasma membrane in response to laminar flow. The resulting increase in membrane-localized TRPM7 correlated closely with increases in TRPM7 current [101]. In human HeLa cells, whole cell recordings showed TRPM7-like current to be augmented when cells were subjected to stretch. Both stretch-activated cation currents and swelling-activated, whole cell cation currents could be silenced by small interfering RNA (siRNA) targeting TRPM7, as well as by gadolinium or removal of extracellular Ca^{2+} [102]. The mechanism underlying

TRPM7-mediated mechano-transduction remains a matter of controversy and may involve both channel translocation and activation. While Oancea et al. [101] demonstrated the incorporation of TRPM7 into the plasma membrane following mechanical stress, Numata et al. [103] reported that stretching of the cell membrane may directly activate TRPM7 channels even when membrane trafficking from the Golgi is inhibited.

TRPP Channels

Members of the TRPP family have been reported to participate in mechanosensation in the primary cilium of kidney epithelial cells in the nephron [104, 105]. Polycystic kidney disease (PKD), an inherited disorder, is primarily caused by mutations of two genes, *PKD-1* and *PKD-2*. While *PKD-1* encodes for polycystin-1 (previously known as TRPP1), *PKD-2* and its homologues *PKD2L1* and *PKD2L2* encode for TRPP1 (previously known as TRPP2 or PKD2), TRPP2, and TRPP3 (previously known as TRPP5), respectively (nomenclature according to the International Union of Pharmacology) [106–108]. TRPP1 is prominently expressed in the single, non-motile renal cilia [109, 110] where it forms a non-selective cation channel by conformational interaction with polycystin-1 through coiled-coil domains [111, 112]. While bending of the wild type cell cilia by fluid flow has been shown to increase intracellular Ca^{2+} [104], Ca^{2+} influx in response to physiological fluid flow could not be detected in cells isolated from transgenic mice lacking functional polycystin-1. Moreover, blocking antibodies directed against TRPP1 similarly abolished the flow response in wild type cells, indicating both polycystin-1 and TRPP1 are required for mechanotransduction [113]. In addition, several lines of evidence suggest that the structural interaction of polycystin-1 with TRPP1 and the subsequent formation of protein complexes is critical for their functional role as cellular mechanosensors [113–116], while the homomeric TRPP1 proteins are not mechanosensitive [106]. Interestingly, the mechanosensitivity of TRPP1 has recently also been linked to TRPV4. Köttgen and coworkers found that TRPP1, which by itself lacks mechanosensitive properties, utilizes TRPV4 to form a mechano- and thermosensitive molecular sensor in primary cilia. Accordingly, the flow mediated Ca^{2+} transient in renal epithelial cells requires the presence of functional TRPP1 and TRPV4 [113, 117], demonstrating that both are essential components of the ciliary mechanosensor.

TRPN (NOMPC in *Drosophila*) Channels

In *Drosophila,* genetic screens have identified a mechanosensory channel termed no mechanoreceptor potential C (NOMPC). Loss-of-function mutations of the *NOMPC* gene virtually abrogate mechanotransduction in the bristle organ of the fruitfly [118]. While a NOMPC homologue (TRPN1) has been found in zebrafish larvae [119], no mammalian TRPN homologues have been identified thus far.

TRPA Channels

So far, only one mammalian TRPA family member, TRPA1, has been found. First cloned from mammalian fibroblasts [120], it was originally regarded as a thermosensitive and ligand-gated ion channel [121–123]. More recently, TRPA1 channels from mouse and zebrafish were considered as mechanically gated channels, in which the ankyrin repeats may constitute a gating spring to transduce mechanical forces [124, 125]. The mechanosensitive property of TRPA came to be recognized recently when the appearance of TRPA1 messenger RNA expression was found to coincide during development with the onset of mechanosensitivity in hair cell epithelia. Antibodies to TRPA1 stain hair bundles, while tip labeling disappears upon disrupture of the transduction apparatus by treatment with La^{3+} ions or the Ca^{2+} chelator BAPTA. Inhibition of TRPA1 protein expression in zebrafish and mouse inner ears was shown to inhibit receptor cell function, suggesting a critical role for TRPA1 in the mechanotransduction machinery in vertebrate hair cells [124]. However, this notion was largely refuted by two studies which independently demonstrated an unimpaired auditory function and a normal startle reflex to loud noise in TRPA1-deficient mice [126, 127]. Nevertheless, TRPA1-deficient mice displayed behavioral deficits in response to punctate mechanical stimuli [127]. Since it is currently unclear whether this phenotype relates to a direct mechanosensitivity of TRPA1 or rather to its general role in nociceptor signal transduction, the role of TRPA1 in mechanotransduction remains to be elucidated.

TRPML3

The opening of mechano-electrical transduction channels in the cochlear hair cell stereocilia is a critical and indispensable step for hearing. Due to their characteristic cochleovestibular disorders, the varitint-waddler (*Va*) mutant mice have been widely used for the study of hearing-loss diseases. Lately, two independent mutations at the *Va* locus have been identified and coined *Va* and Va^J alleles, respectively [128]. The allelic mutations were encoded by the gene identified as *TRPML3*. The *Va* allele was shown to have an alanine to proline substitution at amino acid position 419 of the TRPML3 protein (TRPML3-A419P), while the Va^J allele caused an

additional substitution at amino acid position 362 from isoleucine to threonine (TRPML3-I362T/A419P) [128]. While it is tempting to speculate that TRML3 itself may be a mechanotransduction channel, the fact that TRPML3 is primarily in cytoplasmic organelles but not the plasma-membrane suggests that deafness in varitint-waddler mutant mice arises not from mutation of a transduction channel but from indirect effects of abnormal organelle trafficking [129]. Recently, van Aken et al. [130] identified a critical role for TRPML3 at the ankle-link region during hair-bundle growth, and show that an adverse effect of mutant TRPML3 on bundle development and mechano-electrical transduction is the main cause of hearing loss in *Va*^{J}/+ mutant mice. While this finding stresses the notion that TRPML3 is not a component of the hair-cell transducer channel itself, it provides a general admonitory example on how TRP channels may contribute to a sensory organ's functional integrity without constituting a sensory channel themselves.

TRPY1 (Yvc1p in Yeast)

The only TRP-like channel gene in yeast, *yvc1p*, encodes a distant member of the TRP superfamily, which also shares mechanosensitive properties. In whole-vacuole patch clamp recordings as well as in excised cytoplasmic-side-out mode, direct mechanical pressure was shown to activate 400-pS Yvc1p conductance, and this activation was independent of the ambient Ca^{2+} concentration [131]. Analyses of TRPY1 illustrate that microbial TRPs are not only sequence homologues, but also functional counterparts of animal TRPs [132]. Hence, Yvc1p may provide a convenient and simple molecular model for further studies in the regulation of TRP channels.

Post-Translational Regulation of TRP Channels

Intense studies have related translocation of TRP channels to their mechanosensitive properties. Bezzerides and coworkers showed that growth factor stimulation initiates the rapid translocation of TRPC5 to the plasma membrane from vesicles which are held in juxtaposed reserve. The rapid vesicular insertion dramatically increases the number of membrane-associated TRPC5 channels and functional TRPC5 currents, and thus provides for a tight spatial–temporal control of these Ca^{2+}-permeant nonselective cation channels [133]. By using total internal reflection fluorescence (TIRF) microscopy, Oancea and coworkers revealed that exposure to shear stress induces a significant accumulation of functional TRPM7 channels, both heterologous GFP-TRPM7 and homologous endogenous TRPM7, at the cell membrane within <2 min. This flow-induced translocation of TRPM7 is not affected by inhibition of phosphatidylinositol 3-kinase (PI3 K) or phospholipase C [101], which differs from the growth factor induced translocation of TRPC5 that requires PI3K, the Rho GTPase Rac1 and phosphatidylinositol 4-phosphate 5-kinase [133]. In a mouse model of Duchenne muscular dystrophy (DMD) and in a rat model in which the dystrophin–glycoprotein complex is disrupted, Iwata et al. [134] demonstrated that cyclic stretch induces the translocation of TRPV2 protein to the sarcolemma, and causes Ca^{2+} influx into skeletal myotubes. This effect is reminiscent of the translocation of this protein in CHO cells expressing TRPV2 in the presence of insulin-like growth factor-1 [76]. In line with the notion that mechanical stress can cause rapid translocation of TRP channels, Loot et al. [66] identified the translocation of TRPV4 to the membrane of endothelial cells of mouse carotid arteries after stimulation with shear stress, an effect that may contribute to the EDHF-mediated vascular response to flow.

In the lung vasculatures, caveolae have recently received growing attention for their putative role in mechanotransduction [135–137]. Caveolae represent a specialized form of invaginated membrane microdomains enriched in proteins as well as lipids such as cholesterol and sphingolipids which play important roles in signal transduction by clustering ion channels and regulatory proteins. Based on the findings that caveolae are anchored to the cytoskeleton and frequently act as entry ports for extracellular Ca^{2+} [138–140], the notion of a potential role of caveolae in mechanotransduction emerged. This view is supported by the fact that disruption of caveolae by mild detergents or anti-caveolin 1 antibodies prevents the activation of ERK by shear stress [141]. Chronic shear stress has been shown to regulate the formation of caveolae, e.g. by recruiting or derecruiting caveolin-1 to the plasma membrane [142, 143], and this effect coincides with altered activation patterns of shear-sensitive signaling molecules such as ERK and Akt [144, 145].

Importantly, the functional role of a number of TRP channels may be linked to their localization to caveolae. In the presence of bradykinin, the canonical TRP channel TRPC6 was demonstrated to translocate to caveolin-1-rich domains of the endothelial cell membrane where it mediates Ca^{2+} influx [146]. Physical and functional interaction with caveolin-1 was also observed for TRPC1 [147], and isolation of lipid rafts indicates that all of the TRPC proteins are indeed associated with lipid rafts domains [148]. These findings substantiate a putative role of caveolae in mechano-regulated ion fluxes via TRP channels which certainly warrants further studies.

Moreover, accumulating evidence indicates that oligo- or multimerization of TRP channels may dramatically change their properties. The proposed intrinsic multimerization site

comprises the ankyrin repeat domain [149–152], coiled-coil domain [153–157] and the transmembrane domains [158, 159]. Splice variants of TRPV4 unable to oligomerize due to absence of parts of the ankyrin domains become retained in the endoplasmic reticulum and stay inactivated [160]. Similarly, inhibition of channel multimerization by various dominant negative N-terminal fragments of TRPV6 was shown to inhibit TRPV6 currents [152]. Deletion or single point mutation in the highly conserved C-terminal coiled-coil region (88–120 amino acids distal to the last transmembrane domain), another putative multimerization site, also largely attenuated TRPM8 currents [157], and TRPV1 function could be effectively abolished by a similar deletion [155]. The notion that TRP channels may have to oligo- or multimerize to serve as mechanosensors is in keeping with the fact that non-mechanosensitive TRPP1 homomeric channels contribute to mechanosensitivity in multimerized heteromeric polycystin-1/TRPP1 complexes [113] or in the presence of TRPV4 [117]. Notably, the endogenous endothelial store-operated current was recently shown to have a stoichiometry of one TRPC1 plus three TRPC4 subunits, demonstrating the presence of heteromeric TRP channel multimerization also in the vasculature [161].

Taken together, there is considerable evidence to suggest that post-translational regulation mechanisms such as translocation, interaction with membrane proteins, and multimerization directly regulate the function of TRP channels. It is thus conceivable that these regulatory mechanisms may be rapidly triggered by mechanical stress, as demonstrated e.g. for the shear stress induced translocation of TRPM7 and TRPV4, respectively [66, 101]. This notion stresses the possibility that at least in some cases of TRP-mediated mechanosensitivity, the involved TRP channels do not constitute the primary mechanosensor *per se*, but undergo rapid post-translational regulation in response to mechanical stress. More research in this rapidly evolving area is required to provide better insights into the molecular mechanisms underlying the post-translational regulation of TRP channels and its role in mechanotransduction.

Direct Versus Indirect Role of TRP Channels in Mechanosensation

Post-translational regulation provides a striking example that a critical role in the process of mechanotransduction does not necessarily establish an ion channel as mechanosensor. While a vast array of studies have implied a role for TRP channels in mechanotransduction, it often remains unclear whether TRP channels constitute the direct sensor of mechanical stimuli, or rather act as relay that is triggered by a yet unidentified upstream stimulus. For instance, TRPV4 has been implicated as mechanosensor in osmoregulation and mechanotransduction. Yet, several studies have shown that inhibition of phospholipase A_2 (PLA_2) and cytochrome P450 epoxygenase can inhibit the activation of TRPV4 by osmotic cell swelling [162, 163] and stretch [73], indicating that activation of TRPV4 by mechanical stress may be indirect and mediated via hydrolysis of membrane phospholipids by phospholipase A_2 (PLA_2) and subsequent arachidonic acid metabolism by cytochrome P450 epoxygenases to form epoxyeicosatrienoic acids [64, 73, 162, 163].

As discussed in the previous section of this review, translocation of TRP channels to the cell membrane may constitute another mechanism by which TRP-dependent mechanosensitivity is conferred. In line with this notion, shear stress was shown to induce translocation of TRPV4 to the endothelial cell membrane where it contributes to flow-mediated vasodilation [66]. Likewise, in vascular smooth muscle cells, fluid shear stress was shown to increase the membrane-oriented translocation of mechanosensitive TRPM7 channel, which closely correlated with a respective increase in TRPM7 current [101]. Whether TRPM7 may also still be directly activated by mechanical stimulation is still under debate, since osmotic swelling augmented whole-cell currents of the TRPM7 channel even under conditions where exocytotic events can hardly take place, i.e. in the cytosol dialyzed with ATP-free, Ca^{2+}-free and EGTA-containing pipette, or in the presence of brefeldin A, a blocker of vesicular protein trafficking [103].

Similarly a series of studies have identified a role for TRPC3 and TRPC6 in the myogenic response [3], yet their direct involvement in the process of mechanosensing remains questionable [5]. A series of recent reports suggest that the mechanosensitivity of TRPC channels is mediated by the activation of G_q-coupled receptors [164, 165], and thus indirect. This notion is in line with the classic observation that myogenic tone is coupled to G protein activation [166]. Recently, Mederos y Schnitzler and coworkers identified an agonist-independent activation of $G_{q/11}$-coupled receptors in response to membrane stretch. In the same expression system, TRPC6 *per se* did not confer mechanosensitivity, but became indirectly activated by membrane stretch when co-expressed with the angiotensin II type 1 receptor (AT1R) [164]. The $G_{q/11}$-coupled receptors are able to adapt to mechanical stimuli by conformation changes, thus allowing for productive G-protein coupling and recruitment of β-arrestin [164]. Activation of $G_{q/11}$-coupled receptors was proposed to subsequently activate TRPC channels in a G protein- and PLC-dependent manner. This study provides an intriguing new concept for vascular mechanotransduction. Yet, it should be noted that the mechanosensitivity of TRPC6 is still a matter of controversy, since stretch activation of TRPC6 channels

can, e.g., still be observed in the presence of the PLC inhibitor U73122, arguing against the theory of second-messenger activation [167].

Concluding Remarks

Vasculatures in the body are constantly exposed to mechanical stress of frequently rapidly varying degrees and directions. Although a series of putative mechanisms of vascular mechanosensation have been described in the past including a number of potential mechanosensitive cation channels, the notion of a family of mechanosensitive ion channels which share general structural features and a common origin was lacking until lately. From recent research, TRP channels have emerged as a group of ion channels with mechanosensation and/or mechanotransduction properties in the vasculature. While a rapidly increasing number of studies have identified the regulation of TRP channels by different modes of mechanical stimulation (summarized in Table 1), little is yet known on the differential expression patterns of these ion channels in different vascular cell types and/or vascular beds of different organs, developmental stages, or in disease conditions. The evolutionary conservation of TRP channels in mammals and non-mammalian organisms may facilitate future research on mechanosensation in that lower organisms serve as simplified models to address the characteristics of this superfamily of ion channels.

While it is fair to conclude based on the data available that many TRP channels fulfill essential roles in mechanotransduction, the question remains open whether they are directly activated by mechanical stimuli or rather serve as transducers that are functionally activated by upstream signaling cascades, translocation to the cell membrane or multimerization with other membrane proteins or channels. Better insights into the role of TRP channels in vascular mechanosensation and/or mechanotransduction will not only advance our basic knowledge of vascular regulation. TRP channels also present promising therapeutic targets in a variety of cardiovascular diseases. For instance, inhibition of TRPV4 channels in the pulmonary vasculature by cGMP or cGMP-elevating interventions such as phosphodiesterase inhibitors has proven effective to prevent hydrostatic lung edema secondary to acute myocardial infarction [60]. In light of the intense current research activities in this new and open field, the recognition of TRP channels as a new family of mechanotransducers in the vasculature has to be regarded as a milestone in our understanding of vascular physiology, disease and—hopefully in the near future—therapy.

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References

1. Davies, P. F., Barbee, K. A., Volin, M. V., Robotewskyj, A., Chen, J., Joseph, L., et al. (1997). Spatial relationships in early signaling events of flow-mediated endothelial mechanotransduction. *Annual Review of Physiology, 59*, 527–549.
2. Davies, P. F., Spaan, J. A., & Krams, R. (2005). Shear stress biology of the endothelium. *Annals of Biomedical Engineering, 33*, 1714–1718.
3. Davis, M. J., & Hill, M. A. (1999). Signaling mechanisms underlying the vascular myogenic response. *Physiological Reviews, 79*, 387–423.
4. Ali, M. H., & Schumacker, P. T. (2002). Endothelial responses to mechanical stress: Where is the mechanosensor? *Critical Care Medicine, 30*, S198–S206.
5. Sharif-Naeini, R., Dedman, A., Folgering, J. H., Duprat, F., Patel, A., Nilius, B., et al. (2008). TRP channels and mechanosensory transduction: Insights into the arterial myogenic response. *Pflugers Archiv, 456*, 529–540.
6. Christensen, A. P., & Corey, D. P. (2007). TRP channels in mechanosensation: Direct or indirect activation? *Nature Reviews. Neuroscience, 8*, 510–521.
7. Pedersen, S. F., & Nilius, B. (2007). Transient receptor potential channels in mechanosensing and cell volume regulation. *Methods in Enzymology, 428*, 183–207.
8. Liedtke, W., & Kim, C. (2005). Functionality of the TRPV subfamily of TRP ion channels: Add mechano-TRP and osmo-TRP to the lexicon! *Cellular and Molecular Life Sciences, 62*, 2985–3001.
9. Mutai, H., & Heller, S. (2003). Vertebrate and invertebrate TRPV-like mechanoreceptors. *Cell Calcium, 33*, 471–478.
10. O'Neil, R. G., & Heller, S. (2005). The mechanosensitive nature of TRPV channels. *Pflugers Archiv, 451*, 193–203.
11. Tobin, D. M., & Bargmann, C. I. (2004). Invertebrate nociception: Behaviors, neurons and molecules. *Journal of Neurobiology, 61*, 161–174.
12. Kahn-Kirby, A. H., & Bargmann, C. I. (2006). TRP channels in *C. elegans*. *Annual Review of Physiology, 68*, 719–736.
13. Liedtke, W. (2008). Molecular mechanisms of TRPV4-mediated neural signaling. *Annals of the New York Academy of Sciences, 1144*, 42–52.
14. Bergel, D. H., Caro, C. G., & Seed, W. A. (1967). Asymmetrical transmission of pressure waves in the pulmonary vascular system of the dog. *Journal of Physiology, 188*, 41P–42P.
15. Presson, R. G., Jr., Baumgartner, W. A., Jr., Peterson, A. J., Glenny, R. W., & Wagner, W. W., Jr. (2002). Pulmonary capillaries are recruited during pulsatile flow. *Journal of Applied Physiology, 92*, 1183–1190.
16. Wagner, W. W., Jr., Todoran, T. M., Tanabe, N., Wagner, T. M., Tanner, J. A., Glenny, R. W., et al. (1999). Pulmonary capillary perfusion: Intra-alveolar fractal patterns and interalveolar independence. *Journal of Applied Physiology, 86*, 825–831.
17. Kuebler, W. M., Ying, X., & Bhattacharya, J. (2002). Pressure-induced endothelial Ca^{2+} oscillations in lung capillaries. *American Journal of Physiology Lung Cellular and Molecular Physiology, 282*, L917–L923.

18. Sadurski, R., Tsukada, H., Ying, X., Bhattacharya, S., & Bhattacharya, J. (1994). Diameters of juxtacapillary venules determined by oil-drop method in rat lung. *Journal of Applied Physiology, 77*, 718–725.
19. Sun, R. Y., Nieman, G. F., Hakim, T. S., & Chang, H. K. (1987). Effects of lung volume and alveolar surface tension on pulmonary vascular resistance. *Journal of Applied Physiology, 62*, 1622–1626.
20. Folgering, J. H., Sharif-Naeini, R., Dedman, A., Patel, A., Delmas, P., & Honore, E. (2008). Molecular basis of the mammalian pressure-sensitive ion channels: Focus on vascular mechanotransduction. *Progress in Biophysics and Molecular Biology, 97*, 180–195.
21. Helmke, B. P., & Davies, P. F. (2002). The cytoskeleton under external fluid mechanical forces: Hemodynamic forces acting on the endothelium. *Annals of Biomedical Engineering, 30*, 284–296.
22. Ingber, D. E. (1997). Tensegrity: The architectural basis of cellular mechanotransduction. *Annual Review of Physiology, 59*, 575–599.
23. Tseng, H., Peterson, T. E., & Berk, B. C. (1995). Fluid shear stress stimulates mitogen-activated protein kinase in endothelial cells. *Circulation Research, 77*, 869–878.
24. Lansman, J. B., Hallam, T. J., & Rink, T. J. (1987). Single stretch-activated ion channels in vascular endothelial cells as mechanotransducers? *Nature, 325*, 811–813.
25. Schwarz, G., Droogmans, G., & Nilius, B. (1992). Shear stress induced membrane currents and calcium transients in human vascular endothelial cells. *Pflugers Archiv, 421*, 394–396.
26. Schwarz, G., Callewaert, G., Droogmans, G., & Nilius, B. (1992). Shear stress-induced calcium transients in endothelial cells from human umbilical cord veins. *Journal of Physiology, 458*, 527–538.
27. Hoyer, J., Kohler, R., Haase, W., & Distler, A. (1996). Up-regulation of pressure-activated Ca^{2+}-permeable cation channel in intact vascular endothelium of hypertensive rats. *Proceedings of the National Academy of Sciences of the United States of America, 93*, 11253–11258.
28. Hoyer, J., Kohler, R., & Distler, A. (1997). Mechanosensitive cation channels in aortic endothelium of normotensive and hypertensive rats. *Hypertension, 30*, 112–119.
29. Kohler, R., Schonfelder, G., Hopp, H., Distler, A., & Hoyer, J. (1998). Stretch-activated cation channel in human umbilical vein endothelium in normal pregnancy and in preeclampsia. *Journal of Hypertension, 16*, 1149–1156.
30. Hutcheson, I. R., & Griffith, T. M. (1994). Heterogeneous populations of K^+ channels mediate EDRF release to flow but not agonists in rabbit aorta. *American Journal of Physiology, 266*, H590–H596.
31. Alevriadou, B. R., Eskin, S. G., McIntire, L. V., & Schilling, W. P. (1993). Effect of shear stress on $^{86}Rb^+$ efflux from calf pulmonary artery endothelial cells. *Annals of Biomedical Engineering, 21*, 1–7.
32. Naruse, K., & Sokabe, M. (1993). Involvement of stretch-activated ion channels in Ca^{2+} mobilization to mechanical stretch in endothelial cells. *American Journal of Physiology, 264*, C1037–C1044.
33. Naruse, K., Yamada, T., & Sokabe, M. (1998). Involvement of SA channels in orienting response of cultured endothelial cells to cyclic stretch. *American Journal of Physiology, 274*, H1532–H1538.
34. Yang, X. C., & Sachs, F. (1989). Block of stretch-activated ion channels in *Xenopus oocytes* by gadolinium and calcium ions. *Science, 243*, 1068–1071.
35. Cosens, D. J., & Manning, A. (1969). Abnormal electroretinogram from a *Drosophila* mutant. *Nature, 224*, 285–287.
36. Montell, C., & Rubin, G. M. (1989). Molecular characterization of the *Drosophila* trp locus: A putative integral membrane protein required for phototransduction. *Neuron, 2*, 1313–1323.
37. Hardie, R. C., & Minke, B. (1992). The trp gene is essential for a light-activated Ca^{2+} channel in *Drosophila* photoreceptors. *Neuron, 8*, 643–651.
38. Wong, F., Schaefer, E. L., Roop, B. C., LaMendola, J. N., Johnson-Seaton, D., & Shao, D. (1989). Proper function of the *Drosophila* trp gene product during pupal development is important for normal visual transduction in the adult. *Neuron, 3*, 81–94.
39. Montell, C. (2001). Physiology, phylogeny, and functions of the TRP superfamily of cation channels. *Science's STKE, 2001*, RE1.
40. Clapham, D. E. (2003). TRP channels as cellular sensors. *Nature, 426*, 517–524.
41. Hoenderop, J. G., Voets, T., Hoefs, S., Weidema, F., Prenen, J., Nilius, B., et al. (2003). Homo- and heterotetrameric architecture of the epithelial Ca^{2+} channels TRPV5 and TRPV6. *EMBO Journal, 22*, 776–785.
42. Dohke, Y., Oh, Y. S., Ambudkar, I. S., & Turner, R. J. (2004). Biogenesis and topology of the transient receptor potential Ca^{2+} channel TRPC1. *Journal of Biological Chemistry, 279*, 12242–12248.
43. Montell, C. (2001). An end in sight to a long TRP. *Neuron, 30*, 3–5.
44. Vannier, B., Zhu, X., Brown, D., & Birnbaumer, L. (1998). The membrane topology of human transient receptor potential 3 as inferred from glycosylation-scanning mutagenesis and epitope immunocytochemistry. *Journal of Biological Chemistry, 273*, 8675–8679.
45. Vazquez, G., Wedel, B. J., Kawasaki, B. T., Bird, G. S., & Putney, J. W., Jr. (2004). Obligatory role of Src kinase in the signaling mechanism for TRPC3 cation channels. *Journal of Biological Chemistry, 279*, 40521–40528.
46. Vazquez, G., Wedel, B. J., Aziz, O., Trebak, M., & Putney, J. W., Jr. (2004). The mammalian TRPC cation channels. *Biochimica et Biophysica Acta, 1742*, 21–36.
47. Sukharev, S., & Corey, D. P. (2004). Mechanosensitive channels: Multiplicity of families and gating paradigms. *Science's STKE, 2004*, re4.
48. Kung, C. (2005). A possible unifying principle for mechanosensation. *Nature, 436*, 647–654.
49. Colbert, H. A., & Bargmann, C. I. (1995). Odorant-specific adaptation pathways generate olfactory plasticity in *C. elegans*. *Neuron, 14*, 803–812.
50. Colbert, H. A., & Bargmann, C. I. (1997). Environmental signals modulate olfactory acuity, discrimination, and memory in *Caenorhabditis elegans*. *Learning & Memory, 4*, 179–191.
51. Colbert, H. A., Smith, T. L., & Bargmann, C. I. (1997). OSM-9, a novel protein with structural similarity to channels, is required for olfaction, mechanosensation, and olfactory adaptation in *Caenorhabditis elegans*. *Journal of Neuroscience, 17*, 8259–8269.
52. Tobin, D., Madsen, D., Kahn-Kirby, A., Peckol, E., Moulder, G., Barstead, R., et al. (2002). Combinatorial expression of TRPV channel proteins defines their sensory functions and subcellular localization in *C. elegans* neurons. *Neuron, 35*, 307–318.
53. Kim, J., Chung, Y. D., Park, D. Y., Choi, S., Shin, D. W., Soh, H., et al. (2003). A TRPV family ion channel required for hearing in *Drosophila*. *Nature, 424*, 81–84.
54. Gong, Z., Son, W., Chung, Y. D., Kim, J., Shin, D. W., McClung, C. A., et al. (2004). Two interdependent TRPV channel subunits, inactive and Nanchung, mediate hearing in *Drosophila*. *Journal of Neuroscience, 24*, 9059–9066.
55. Liedtke, W., Choe, Y., Marti-Renom, M. A., Bell, A. M., Denis, C. S., Sali, A., et al. (2000). Vanilloid receptor-related osmotically activated channel (VR-OAC), a candidate vertebrate osmoreceptor. *Cell, 103*, 525–535.

56. Strotmann, R., Harteneck, C., Nunnenmacher, K., Schultz, G., & Plant, T. D. (2000). OTRPC4, a nonselective cation channel that confers sensitivity to extracellular osmolarity. *Nature Cell Biology, 2*, 695–702.
57. Wissenbach, U., Bodding, M., Freichel, M., & Flockerzi, V. (2000). Trp12, a novel Trp related protein from kidney. *FEBS Letters, 485*, 127–134.
58. Delany, N. S., Hurle, M., Facer, P., Alnadaf, T., Plumpton, C., Kinghorn, I., et al. (2001). Identification and characterization of a novel human vanilloid receptor-like protein, VRL-2. *Physiol Genomics, 4*, 165–174.
59. Alvarez, D. F., King, J. A., Weber, D., Addison, E., Liedtke, W., & Townsley, M. I. (2006). Transient receptor potential vanilloid 4-mediated disruption of the alveolar septal barrier: A novel mechanism of acute lung injury. *Circulation Research, 99*, 988–995.
60. Yin, J., Hoffmann, J., Kaestle, S. M., Neye, N., Wang, L., Baeurle, J., et al. (2008). Negative-feedback loop attenuates hydrostatic lung edema via a cGMP-dependent regulation of transient receptor potential vanilloid 4. *Circulation Research, 102*, 966–974.
61. Gao, X., Wu, L., & O'Neil, R. G. (2003). Temperature-modulated diversity of TRPV4 channel gating: Activation by physical stresses and phorbol ester derivatives through protein kinase C-dependent and -independent pathways. *Journal of Biological Chemistry, 278*, 27129–27137.
62. Guler, A. D., Lee, H., Iida, T., Shimizu, I., Tominaga, M., & Caterina, M. (2002). Heat-evoked activation of the ion channel, TRPV4. *Journal of Neuroscience, 22*, 6408–6414.
63. Liedtke, W., Tobin, D. M., Bargmann, C. I., & Friedman, J. M. (2003). Mammalian TRPV4 (VR-OAC) directs behavioral responses to osmotic and mechanical stimuli in *Caenorhabditis elegans*. *Proceedings of the National Academy of Sciences of the United States of America, 100*(Suppl 2), 14531–14536.
64. Kohler, R., Heyken, W. T., Heinau, P., Schubert, R., Si, H., Kacik, M., et al. (2006). Evidence for a functional role of endothelial transient receptor potential V4 in shear stress-induced vasodilatation. *Arteriosclerosis, Thrombosis, and Vascular Biology, 26*, 1495–1502.
65. Hartmannsgruber, V., Heyken, W. T., Kacik, M., Kaistha, A., Grgic, I., Harteneck, C., et al. (2007). Arterial response to shear stress critically depends on endothelial TRPV4 expression. *PLoS ONE, 2*, e827.
66. Loot, A. E., Popp, R., Fisslthaler, B., Vriens, J., Nilius, B., & Fleming, I. (2008). Role of cytochrome P450-dependent transient receptor potential V4 activation in flow-induced vasodilatation. *Cardiovascular Research, 80*, 445–452.
67. Saliez, J., Bouzin, C., Rath, G., Ghisdal, P., Desjardins, F., Rezzani, R., et al. (2008). Role of caveolar compartmentation in endothelium-derived hyperpolarizing factor-mediated relaxation: Ca^{2+} signals and gap junction function are regulated by caveolin in endothelial cells. *Circulation, 117*, 1065–1074.
68. Jia, Y., Wang, X., Varty, L., Rizzo, C. A., Yang, R., Correll, C. C., et al. (2004). Functional TRPV4 channels are expressed in human airway smooth muscle cells. *American Journal of Physiology Lung Cellular and Molecular Physiology, 287*, L272–L278.
69. Sipe, W. E., Brierley, S. M., Martin, C. M., Phillis, B. D., Cruz, F. B., Grady, E. F., et al. (2008). Transient receptor potential vanilloid 4 mediates protease activated receptor 2-induced sensitization of colonic afferent nerves and visceral hyperalgesia. *American Journal of Physiology. Gastrointestinal and Liver Physiology, 294*, G1288–G1298.
70. Cenac, N., Altier, C., Chapman, K., Liedtke, W., Zamponi, G., & Vergnolle, N. (2008). Transient receptor potential vanilloid-4 has a major role in visceral hypersensitivity symptoms. *Gastroenterology, 135*, 937–946, 946.
71. Parker, J. C., Ivey, C. L., & Tucker, J. A. (1998). Gadolinium prevents high airway pressure-induced permeability increases in isolated rat lungs. *Journal of Applied Physiology, 84*, 1113–1118.
72. Hamanaka, K., Jian, M. Y., Weber, D. S., Alvarez, D. F., Townsley, M. I., Al-Mehdi, A. B., et al. (2007). TRPV4 initiates the acute calcium-dependent permeability increase during ventilator-induced lung injury in isolated mouse lungs. *American Journal of Physiology Lung Cellular and Molecular Physiology, 293*, L923–L932.
73. Jian, M. Y., King, J. A., Al-Mehdi, A. B., Liedtke, W., & Townsley, M. I. (2008). High vascular pressure-induced lung injury requires P450 epoxygenase-dependent activation of TRPV4. *American Journal of Respiratory Cell and Molecular Biology, 38*, 386–392.
74. Caterina, M. J., & Julius, D. (1999). Sense and specificity: A molecular identity for nociceptors. *Current Opinion in Neurobiology, 9*, 525–530.
75. Minke, B., & Cook, B. (2002). TRP channel proteins and signal transduction. *Physiological Reviews, 82*, 429–472.
76. Kanzaki, M., Zhang, Y. Q., Mashima, H., Li, L., Shibata, H., & Kojima, I. (1999). Translocation of a calcium-permeable cation channel induced by insulin-like growth factor-I. *Nature Cell Biology, 1*, 165–170.
77. Stokes, A. J., Wakano, C., Del Carmen, K. A., Koblan-Huberson, M., & Turner, H. (2005). Formation of a physiological complex between TRPV2 and RGA protein promotes cell surface expression of TRPV2. *Journal of Cellular Biochemistry, 94*, 669–683.
78. Muraki, K., Iwata, Y., Katanosaka, Y., Ito, T., Ohya, S., Shigekawa, M., et al. (2003). TRPV2 is a component of osmotically sensitive cation channels in murine aortic myocytes. *Circulation Research, 93*, 829–838.
79. Caterina, M. J., Leffler, A., Malmberg, A. B., Martin, W. J., Trafton, J., Petersen-Zeitz, K. R., et al. (2000). Impaired nociception and pain sensation in mice lacking the capsaicin receptor. *Science, 288*, 306–313.
80. Davis, J. B., Gray, J., Gunthorpe, M. J., Hatcher, J. P., Davey, P. T., Overend, P., et al. (2000). Vanilloid receptor-1 is essential for inflammatory thermal hyperalgesia. *Nature, 405*, 183–187.
81. Birder, L. A., Nakamura, Y., Kiss, S., Nealen, M. L., Barrick, S., Kanai, A. J., et al. (2002). Altered urinary bladder function in mice lacking the vanilloid receptor TRPV1. *Nature Neuroscience, 5*, 856–860.
82. Rong, W., Hillsley, K., Davis, J. B., Hicks, G., Winchester, W. J., & Grundy, D. (2004). Jejunal afferent nerve sensitivity in wild-type and TRPV1 knockout mice. *Journal of Physiology, 560*, 867–881.
83. Scotland, R. S., Chauhan, S., Davis, C., De, F. C., Hunt, S., Kabir, J., et al. (2004). Vanilloid receptor TRPV1, sensory C-fibers, and vascular autoregulation: A novel mechanism involved in myogenic constriction. *Circulation Research, 95*, 1027–1034.
84. Chan, C. L., Facer, P., Davis, J. B., Smith, G. D., Egerton, J., Bountra, C., et al. (2003). Sensory fibres expressing capsaicin receptor TRPV1 in patients with rectal hypersensitivity and faecal urgency. *Lancet, 361*, 385–391.
85. Sharif-Naeini, R., Witty, M. F., Seguela, P., & Bourque, C. W. (2006). An N-terminal variant of Trpv1 channel is required for osmosensory transduction. *Nature Neuroscience, 9*, 93–98.
86. Ciura, S., & Bourque, C. W. (2006). Transient receptor potential vanilloid 1 is required for intrinsic osmoreception in organum vasculosum lamina terminalis neurons and for normal thirst responses to systemic hyperosmolality. *Journal of Neuroscience, 26*, 9069–9075.
87. Maroto, R., Raso, A., Wood, T. G., Kurosky, A., Martinac, B., & Hamill, O. P. (2005). TRPC1 forms the stretch-activated cation channel in vertebrate cells. *Nature Cell Biology, 7*, 179–185.

88. Dietrich, A., Kalwa, H., Storch, U., Schnitzler, M., Salanova, B., Pinkenburg, O., et al. (2007). Pressure-induced and store-operated cation influx in vascular smooth muscle cells is independent of TRPC1. *Pflugers Archiv, 455*, 465–477.
89. Inoue, R., Jensen, L. J., Shi, J., Morita, H., Nishida, M., Honda, A., et al. (2006). Transient receptor potential channels in cardiovascular function and disease. *Circulation Research, 99*, 119–131.
90. Inoue, R., Jensen, L. J., Jian, Z., Shi, J., Hai, L., Lurie, A. I., et al. (2009). Synergistic activation of vascular TRPC6 channel by receptor and mechanical stimulation via phospholipase C/diacylglycerol and phospholipase A_2/omega-hydroxylase/20-HETE pathways. *Circulation Research, 104*, 1399–1409.
91. Dietrich, A., Mederos, Y. S., Gollasch, M., Gross, V., Storch, U., Dubrovska, G., et al. (2005). Increased vascular smooth muscle contractility in TRPC6$^{-/-}$ mice. *Molecular and Cellular Biology, 25*, 6980–6989.
92. Welsh, D. G., Morielli, A. D., Nelson, M. T., & Brayden, J. E. (2002). Transient receptor potential channels regulate myogenic tone of resistance arteries. *Circulation Research, 90*, 248–250.
93. Gottlieb, P., Folgering, J., Maroto, R., Raso, A., Wood, T. G., Kurosky, A., et al. (2008). Revisiting TRPC1 and TRPC6 mechanosensitivity. *Pflugers Archiv, 455*, 1097–1103.
94. Narayanan, J., Imig, M., Roman, R. J., & Harder, D. R. (1994). Pressurization of isolated renal arteries increases inositol trisphosphate and diacylglycerol. *American Journal of Physiology, 266*, H1840–H1845.
95. Harder, D. R., Lange, A. R., Gebremedhin, D., Birks, E. K., & Roman, R. J. (1997). Cytochrome P450 metabolites of arachidonic acid as intracellular signaling molecules in vascular tissue. *Journal of Vascular Research, 34*, 237–243.
96. Roman, R. J. (2002). P-450 metabolites of arachidonic acid in the control of cardiovascular function. *Physiological Reviews, 82*, 131–185.
97. Basora, N., Boulay, G., Bilodeau, L., Rousseau, E., & Payet, M. D. (2003). 20-Hydroxyeicosatetraenoic acid (20-HETE) activates mouse TRPC6 channels expressed in HEK293 cells. *Journal of Biological Chemistry, 278*, 31709–31716.
98. Earley, S., Straub, S. V., & Brayden, J. E. (2007). Protein kinase C regulates vascular myogenic tone through activation of TRPM4. *American Journal of Physiology. Heart and Circulatory Physiology, 292*, H2613–H2622.
99. Earley, S., Waldron, B. J., & Brayden, J. E. (2004). Critical role for transient receptor potential channel TRPM4 in myogenic constriction of cerebral arteries. *Circulation Research, 95*, 922–929.
100. Morita, H., Honda, A., Inoue, R., Ito, Y., Abe, K., Nelson, M. T., et al. (2007). Membrane stretch-induced activation of a TRPM4-like nonselective cation channel in cerebral artery myocytes. *Journal of Pharmacological Sciences, 103*, 417–426.
101. Oancea, E., Wolfe, J. T., & Clapham, D. E. (2006). Functional TRPM7 channels accumulate at the plasma membrane in response to fluid flow. *Circulation Research, 98*, 245–253.
102. Numata, T., Shimizu, T., & Okada, Y. (2007). TRPM7 is a stretch- and swelling-activated cation channel involved in volume regulation in human epithelial cells. *American Journal of Physiology. Cell Physiology, 292*, C460–C467.
103. Numata, T., Shimizu, T., & Okada, Y. (2007). Direct mechano-stress sensitivity of TRPM7 channel. *Cellular Physiology and Biochemistry, 19*, 1–8.
104. Praetorius, H. A., & Spring, K. R. (2001). Bending the MDCK cell primary cilium increases intracellular calcium. *Journal of Membrane Biology, 184*, 71–79.
105. Praetorius, H. A., & Spring, K. R. (2005). A physiological view of the primary cilium. *Annual Review of Physiology, 67*, 515–529.
106. Giamarchi, A., Padilla, F., Coste, B., Raoux, M., Crest, M., Honore, E., et al. (2006). The versatile nature of the calcium-permeable cation channel TRPP2. *EMBO Reports, 7*, 787–793.
107. Clapham, D. E., Julius, D., Montell, C., & Schultz, G. (2005). International Union of Pharmacology. XLIX. Nomenclature and structure-function relationships of transient receptor potential channels. *Pharmacological Reviews, 57*, 427–450.
108. Arnaout, M. A. (2001). Molecular genetics and pathogenesis of autosomal dominant polycystic kidney disease. *Annual Review of Medicine, 52*, 93–123.
109. Pazour, G. J., San Agustin, J. T., Follit, J. A., Rosenbaum, J. L., & Witman, G. B. (2002). Polycystin-2 localizes to kidney cilia and the ciliary level is elevated in orpk mice with polycystic kidney disease. *Current Biology, 12*, R378–R380.
110. Yoder, B. K., Hou, X., & Guay-Woodford, L. M. (2002). The polycystic kidney disease proteins, polycystin-1, polycystin-2, polaris, and cystin, are co-localized in renal cilia. *Journal of the American Society of Nephrology, 13*, 2508–2516.
111. Qian, F., Germino, F. J., Cai, Y., Zhang, X., Somlo, S., & Germino, G. G. (1997). PKD1 interacts with PKD2 through a probable coiled-coil domain. *Nature Genetics, 16*, 179–183.
112. Hanaoka, K., Qian, F., Boletta, A., Bhunia, A. K., Piontek, K., Tsiokas, L., et al. (2000). Co-assembly of polycystin-1 and -2 produces unique cation-permeable currents. *Nature, 408*, 990–994.
113. Nauli, S. M., Alenghat, F. J., Luo, Y., Williams, E., Vassilev, P., Li, X., et al. (2003). Polycystins 1 and 2 mediate mechanosensation in the primary cilium of kidney cells. *Nature Genetics, 33*, 129–137.
114. Hughes, J., Ward, C. J., Peral, B., Aspinwall, R., Clark, K., San Millan, J. L., et al. (1995). The polycystic kidney disease 1 (PKD1) gene encodes a novel protein with multiple cell recognition domains. *Nature Genetics, 10*, 151–160.
115. Sandford, R., Sgotto, B., Aparicio, S., Brenner, S., Vaudin, M., Wilson, R. K., et al. (1997). Comparative analysis of the polycystic kidney disease 1 (PKD1) gene reveals an integral membrane glycoprotein with multiple evolutionary conserved domains. *Human Molecular Genetics, 6*, 1483–1489.
116. Koulen, P., Cai, Y., Geng, L., Maeda, Y., Nishimura, S., Witzgall, R., et al. (2002). Polycystin-2 is an intracellular calcium release channel. *Nature Cell Biology, 4*, 191–197.
117. Kottgen, M., Buchholz, B., Garcia-Gonzalez, M. A., Kotsis, F., Fu, X., Doerken, M., et al. (2008). TRPP2 and TRPV4 form a polymodal sensory channel complex. *Journal of Cell Biology, 182*, 437–447.
118. Walker, R. G., Willingham, A. T., & Zuker, C. S. (2000). A *Drosophila* mechanosensory transduction channel. *Science, 287*, 2229–2234.
119. Sidi, S., Friedrich, R. W., & Nicolson, T. (2003). NompC TRP channel required for vertebrate sensory hair cell mechanotransduction. *Science, 301*, 96–99.
120. Jaquemar, D., Schenker, T., & Trueb, B. (1999). An ankyrin-like protein with transmembrane domains is specifically lost after oncogenic transformation of human fibroblasts. *Journal of Biological Chemistry, 274*, 7325–7333.
121. Bandell, M., Story, G. M., Hwang, S. W., Viswanath, V., Eid, S. R., Petrus, M. J., et al. (2004). Noxious cold ion channel TRPA1 is activated by pungent compounds and bradykinin. *Neuron, 41*, 849–857.
122. Jordt, S. E., Bautista, D. M., Chuang, H. H., McKemy, D. D., Zygmunt, P. M., Hogestatt, E. D., et al. (2004). Mustard oils and cannabinoids excite sensory nerve fibres through the TRP channel ANKTM1. *Nature, 427*, 260–265.
123. Story, G. M., Peier, A. M., Reeve, A. J., Eid, S. R., Mosbacher, J., Hricik, T. R., et al. (2003). ANKTM1, a TRP-like channel

expressed in nociceptive neurons, is activated by cold temperatures. *Cell, 112*, 819–829.
124. Corey, D. P., Garcia-Anoveros, J., Holt, J. R., Kwan, K. Y., Lin, S. Y., Vollrath, M. A., et al. (2004). TRPA1 is a candidate for the mechanosensitive transduction channel of vertebrate hair cells. *Nature, 432*, 723–730.
125. Howard, J., & Bechstedt, S. (2004). Hypothesis: A helix of ankyrin repeats of the NOMPC-TRP ion channel is the gating spring of mechanoreceptors. *Current Biology, 14*, R224–R226.
126. Bautista, D. M., Jordt, S. E., Nikai, T., Tsuruda, P. R., Read, A. J., Poblete, J., et al. (2006). TRPA1 mediates the inflammatory actions of environmental irritants and proalgesic agents. *Cell, 124*, 1269–1282.
127. Kwan, K. Y., Allchorne, A. J., Vollrath, M. A., Christensen, A. P., Zhang, D. S., Woolf, C. J., et al. (2006). TRPA1 contributes to cold, mechanical, and chemical nociception but is not essential for hair-cell transduction. *Neuron, 50*, 277–289.
128. Di, P. F., Belyantseva, I. A., Kim, H. J., Vogt, T. F., Kachar, B., & Noben-Trauth, K. (2002). Mutations in Mcoln3 associated with deafness and pigmentation defects in varitint-waddler (Va) mice. *Proceedings of the National Academy of Sciences of the United States of America, 99*, 14994–14999.
129. Corey, D. P. (2006). What is the hair cell transduction channel? *Journal of Physiology, 576*, 23–28.
130. van Aken, A. F., tiba-Davies, M., Marcotti, W., Goodyear, R. J., Bryant, J. E., Richardson, G. P., et al. (2008). TRPML3 mutations cause impaired mechano-electrical transduction and depolarization by an inward-rectifier cation current in auditory hair cells of varitint-waddler mice. *Journal of Physiology, 586*, 5403–5418.
131. Zhou, X. L., Batiza, A. F., Loukin, S. H., Palmer, C. P., Kung, C., & Saimi, Y. (2003). The transient receptor potential channel on the yeast vacuole is mechanosensitive. *Proceedings of the National Academy of Sciences of the United States of America, 100*, 7105–7110.
132. Myers, B. R., Saimi, Y., Julius, D., & Kung, C. (2008). Multiple unbiased prospective screens identify TRP channels and their conserved gating elements. *Journal of General Physiology, 132*, 481–486.
133. Bezzerides, V. J., Ramsey, I. S., Kotecha, S., Greka, A., & Clapham, D. E. (2004). Rapid vesicular translocation and insertion of TRP channels. *Nature Cell Biology, 6*, 709–720.
134. Iwata, Y., Katanosaka, Y., Arai, Y., Komamura, K., Miyatake, K., & Shigekawa, M. (2003). A novel mechanism of myocyte degeneration involving the Ca^{2+}-permeable growth factor-regulated channel. *Journal of Cell Biology, 161*, 957–967.
135. Rizzo, V., Sung, A., Oh, P., & Schnitzer, J. E. (1998). Rapid mechanotransduction in situ at the luminal cell surface of vascular endothelium and its caveolae. *Journal of Biological Chemistry, 273*, 26323–26329.
136. Yu, J., Bergaya, S., Murata, T., Alp, I. F., Bauer, M. P., Lin, M. I., et al. (2006). Direct evidence for the role of caveolin-1 and caveolae in mechanotransduction and remodeling of blood vessels. *Journal of Clinical Investigation, 116*, 1284–1291.
137. Radel, C., Carlile-Klusacek, M., & Rizzo, V. (2007). Participation of caveolae in β1 integrin-mediated mechanotransduction. *Biochemical and Biophysical Research Communications, 358*, 626–631.
138. van Deurs, B., Roepstorff, K., Hommelgaard, A. M., & Sandvig, K. (2003). Caveolae: Anchored, multifunctional platforms in the lipid ocean. *Trends in Cell Biology, 13*, 92–100.
139. Isshiki, M., & Anderson, R. G. (2003). Function of caveolae in Ca^{2+} entry and Ca^{2+}-dependent signal transduction. *Traffic, 4*, 717–723.
140. Murata, T., Lin, M. I., Stan, R. V., Bauer, P. M., Yu, J., & Sessa, W. C. (2007). Genetic evidence supporting caveolae microdomain regulation of calcium entry in endothelial cells. *Journal of Biological Chemistry, 282*, 16631–16643.
141. Park, H., Go, Y. M., Darji, R., Choi, J. W., Lisanti, M. P., Maland, M. C., et al. (2000). Caveolin-1 regulates shear stress-dependent activation of extracellular signal-regulated kinase. *American Journal of Physiology. Heart and Circulatory Physiology, 278*, H1285–H1293.
142. Conrad, P. A., Smart, E. J., Ying, Y. S., Anderson, R. G., & Bloom, G. S. (1995). Caveolin cycles between plasma membrane caveolae and the Golgi complex by microtubule-dependent and microtubule-independent steps. *Journal of Cell Biology, 131*, 1421–1433.
143. Sun, R. J., Muller, S., Zhuang, F. Y., Stoltz, J. F., & Wang, X. (2003). Caveolin-1 redistribution in human endothelial cells induced by laminar flow and cytokine. *Biorheology, 40*, 31–39.
144. Rizzo, V., Morton, C., dePaola, N., Schnitzer, J. E., & Davies, P. F. (2003). Recruitment of endothelial caveolae into mechanotransduction pathways by flow conditioning in vitro. *American Journal of Physiology. Heart and Circulatory Physiology, 285*, H1720–H1729.
145. Boyd, N. L., Park, H., Yi, H., Boo, Y. C., Sorescu, G. P., Sykes, M., et al. (2003). Chronic shear induces caveolae formation and alters ERK and Akt responses in endothelial cells. *American Journal of Physiology. Heart and Circulatory Physiology, 285*, H1113–H1122.
146. Fleming, I., Rueben, A., Popp, R., Fisslthaler, B., Schrodt, S., Sander, A., et al. (2007). Epoxyeicosatrienoic acids regulate Trp channel dependent Ca^{2+} signaling and hyperpolarization in endothelial cells. *Arteriosclerosis, Thrombosis, and Vascular Biology, 27*, 2612–2618.
147. Kwiatek, A. M., Minshall, R. D., Cool, D. R., Skidgel, R. A., Malik, A. B., & Tiruppathi, C. (2006). Caveolin-1 regulates store-operated Ca^{2+} influx by binding of its scaffolding domain to transient receptor potential channel-1 in endothelial cells. *Molecular Pharmacology, 70*, 1174–1183.
148. Pani, B., & Singh, B. B. (2009). Lipid rafts/caveolae as microdomains of calcium signaling. *Cell Calcium, 45*, 625–633.
149. Jin, X., Touhey, J., & Gaudet, R. (2006). Structure of the N-terminal ankyrin repeat domain of the TRPV2 ion channel. *Journal of Biological Chemistry, 281*, 25006–25010.
150. Chang, Q., Gyftogianni, E., van de Graaf, S. F., Hoefs, S., Weidema, F. A., Bindels, R. J., et al. (2004). Molecular determinants in TRPV5 channel assembly. *Journal of Biological Chemistry, 279*, 54304–54311.
151. Erler, I., Hirnet, D., Wissenbach, U., Flockerzi, V., & Niemeyer, B. A. (2004). Ca^{2+}-selective transient receptor potential V channel architecture and function require a specific ankyrin repeat. *Journal of Biological Chemistry, 279*, 34456–34463.
152. Kahr, H., Schindl, R., Fritsch, R., Heinze, B., Hofbauer, M., Hack, M. E., et al. (2004). CaT1 knock-down strategies fail to affect CRAC channels in mucosal-type mast cells. *Journal of Physiology, 557*, 121–132.
153. Lussier, M. P., Cayouette, S., Lepage, P. K., Bernier, C. L., Francoeur, N., St-Hilaire, M., et al. (2005). MxA, a member of the dynamin superfamily, interacts with the ankyrin-like repeat domain of TRPC. *Journal of Biological Chemistry, 280*, 19393–19400.
154. Xu, X. Z., Chien, F., Butler, A., Salkoff, L., & Montell, C. (2000). TRPγ, a drosophila TRP-related subunit, forms a regulated cation channel with TRPL. *Neuron, 26*, 647–657.
155. Garcia-Sanz, N., Fernandez-Carvajal, A., Morenilla-Palao, C., Planells-Cases, R., Fajardo-Sanchez, E., Fernandez-Ballester, G., et al. (2004). Identification of a tetramerization domain in the C terminus of the vanilloid receptor. *Journal of Neuroscience, 24*, 5307–5314.

156. Engelke, M., Friedrich, O., Budde, P., Schafer, C., Niemann, U., Zitt, C., et al. (2002). Structural domains required for channel function of the mouse transient receptor potential protein homologue TRP1beta. *FEBS Letters, 523*, 193–199.
157. Tsuruda, P. R., Julius, D., & Minor, D. L., Jr. (2006). Coiled coils direct assembly of a cold-activated TRP channel. *Neuron, 51*, 201–212.
158. Hellwig, N., Albrecht, N., Harteneck, C., Schultz, G., & Schaefer, M. (2005). Homo- and heteromeric assembly of TRPV channel subunits. *Journal of Cell Science, 118*, 917–928.
159. Schindl, R., & Romanin, C. (2007). Assembly domains in TRP channels. *Biochemical Society Transactions, 35*, 84–85.
160. Arniges, M., Fernandez-Fernandez, J. M., Albrecht, N., Schaefer, M., & Valverde, M. A. (2006). Human TRPV4 channel splice variants revealed a key role of ankyrin domains in multimerization and trafficking. *Journal of Biological Chemistry, 281*, 1580–1586.
161. Cioffi, D. L., Uhlig, S., & Stevens, T. (2009). Subunit stoichiometry of the endogenous endothelial ISOC channel in the pulmonary microcirculation [Abstract]. *FASEB Journal, 23*, 964.12.
162. Vriens, J., Watanabe, H., Janssens, A., Droogmans, G., Voets, T., & Nilius, B. (2004). Cell swelling, heat, and chemical agonists use distinct pathways for the activation of the cation channel TRPV4. *Proceedings of the National Academy of Sciences of the United States of America, 101*, 396–401.
163. Vriens, J., Owsianik, G., Fisslthaler, B., Suzuki, M., Janssens, A., Voets, T., et al. (2005). Modulation of the Ca^{2+} permeable cation channel TRPV4 by cytochrome P450 epoxygenases in vascular endothelium. *Circulation Research, 97*, 908–915.
164. Mederos y Schnitzler, M., Storch, U., Meibers, S., Nurwakagari, P., Breit, A., Essin, K., et al. (2008). Gq-coupled receptors as mechanosensors mediating myogenic vasoconstriction. *EMBO Journal, 27*, 3092–3103.
165. Sharif-Naeini, R., Folgering, J. H., Bichet, D., Duprat, F., Delmas, P., Patel, A., et al. (2009). Sensing pressure in the cardiovascular system: G_q-coupled mechanoreceptors and TRP channels. *Journal of Molecular and Cellular Cardiology* [Epub ahead of print].
166. Osol, G., Laher, I., & Kelley, M. (1993). Myogenic tone is coupled to phospholipase C and G protein activation in small cerebral arteries. *American Journal of Physiology, 265*, H415–H420.
167. Spassova, M. A., Hewavitharana, T., Xu, W., Soboloff, J., & Gill, D. L. (2006). A common mechanism underlies stretch activation and receptor activation of TRPC6 channels. *Proceedings of the National Academy of Sciences of the United States of America, 103*, 16586–16591.
168. Zhang, Z., & Bourque, C. W. (2003). Osmometry in osmosensory neurons. *Nature Neuroscience, 6*, 1021–1022.
169. Spencer, N. J., Kerrin, A., Singer, C. A., Hennig, G. W., Gerthoffer, W. T., & McDonnell, O. (2008). Identification of capsaicin-sensitive rectal mechanoreceptors activated by rectal distension in mice. *Neuroscience, 153*, 518–534.
170. Shimohira, D., Kido, M. A., Danjo, A., Takao, T., Wang, B., Zhang, J. Q., et al. (2009). TRPV2 expression in rat oral mucosa. *Histochemistry and Cell Biology*, *132*, 423–433.
171. Chen, L., Liu, C., & Liu, L. (2009). Osmolality-induced tuning of action potentials in trigeminal ganglion neurons. *Neuroscience Letters, 452*, 79–83.
172. Chen, L., Liu, C., Liu, L., & Cao, X. (2009). Changes in osmolality modulate voltage-gated sodium channels in trigeminal ganglion neurons. *Neuroscience Research, 64*, 199–207.
173. Becker, D., Bereiter-Hahn, J., & Jendrach, M. (2009). Functional interaction of the cation channel transient receptor potential vanilloid 4 (TRPV4) and actin in volume regulation. *European Journal of Cell Biology, 88*, 141–152.
174. Garcia-Elias, A., Lorenzo, I. M., Vicente, R., & Valverde, M. A. (2008). IP_3 receptor binds to and sensitizes TRPV4 channel to osmotic stimuli via a calmodulin-binding site. *Journal of Biological Chemistry, 283*, 31284–31288.
175. Wegierski, T., Lewandrowski, U., Muller, B., Sickmann, A., & Walz, G. (2009). Tyrosine phosphorylation modulates the activity of TRPV4 in response to defined stimuli. *Journal of Biological Chemistry, 284*, 2923–2933.
176. Mizuno, A., Matsumoto, N., Imai, M., & Suzuki, M. (2003). Impaired osmotic sensation in mice lacking TRPV4. *American Journal of Physiology. Cell Physiology, 285*, C96–C101.
177. Liedtke, W., & Friedman, J. M. (2003). Abnormal osmotic regulation in $trpv4^{-/-}$ mice. *Proceedings of the National Academy of Sciences of the United States of America, 100*, 13698–13703.
178. Troidl, C., Troidl, K., Schierling, W., Cai, W. J., Nef, H., Mollmann, H., et al. (2008). Trpv4 induces collateral vessel growth during regeneration of the arterial circulation. *Journal of Cellular and Molecular Medicine* [Epub ahead of print].
179. Mochizuki, T., Sokabe, T., Araki, I., Fujishita, K., Shibasaki, K., Uchida, K., et al. (2009). The TRPV4 cation channel mediates stretch-evoked Ca^{2+} influx and ATP release in primary urothelial cell cultures. *Journal of Biological Chemistry*, *284*, 21257–21264.
180. Thodeti, C. K., Matthews, B., Ravi, A., Mammoto, A., Ghosh, K., Bracha, A. L., et al. (2009). TRPV4 channels mediate cyclic strain-induced endothelial cell reorientation through integrin-to-integrin signaling. *Circulation Research, 104*, 1123–1130.
181. Gevaert, T., Vriens, J., Segal, A., Everaerts, W., Roskams, T., Talavera, K., et al. (2007). Deletion of the transient receptor potential cation channel TRPV4 impairs murine bladder voiding. *Journal of Clinical Investigation, 117*, 3453–3462.
182. Tsiokas, L. (2009). Function and regulation of TRPP2 at the plasma membrane. *American Journal of Physiology. Renal Physiology, 297*, F1–F9.
183. Nilius, B., Prenen, J., Tang, J., Wang, C., Owsianik, G., Janssens, A., et al. (2005). Regulation of the Ca^{2+} sensitivity of the nonselective cation channel TRPM4. *Journal of Biological Chemistry, 280*, 6423–6433.
184. Kwan, H. Y., Huang, Y., & Yao, X. (2007). TRP channels in endothelial function and dysfunction. *Biochimica et Biophysica Acta, 1772*, 907–914.