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INVITED REVIEW

Bimal N. Desai · David E. Clapham

TRP channels and mice deficient in TRP channels

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Abstract Transient receptor potential (TRP) channels are a superfamily of functionally versatile cation-permeant ion channels present in almost all mammalian cell types. Although they were initially proposed as store-operated calcium channels, recent progress shows that they exhibit a variety of regulatory and functional themes. Here, we summarize the most salient features of TRP channels, the approaches that are providing meaningful discoveries, and the challenges ahead. We primarily emphasize the understanding gleaned from mouse models engineered to be deficient in various members of TRP superfamily and from the human patients that suffer clinically due to defects in TRP channels.

Introduction

Transient receptor potential (TRP) channels are a superfamily of functionally versatile cation-permeant ion channels that are present in most mammalian cell types. Initially proposed as store-operated calcium channels, recent progress now demonstrates that they exhibit a variety of regulatory and functional themes.

General properties of transient receptor potential channels

Higher organisms require rapid transmission of electrical and chemical signals to synchronize intracellular as well as intercellular processes. Electrical signals are vital for cellular physiology and are mediated primarily by ion channels, a specialized group of ion-permeating proteins embedded in the membranes of all living cells. In *Drosophila melanogaster*, the TRP channel plays a phototransductory role in the eye [1]. This channel was called the TRP channel because mutations in this gene result in a transient voltage response to continuous light. A large superfamily of related channels is present in the mammalian genome, and the members of this family were classified into three main subfamilies of closely related channels called canonical (TRPC), vanilloid (TRPV) and melastatin-related (TRPM) channels [2, 3]. There are four additional groups of more distantly related TRP subfamilies (TRPA, TRPP, TRPML, and TRPN). TRP channels have six transmembrane segments that anchor the channel to the lipid bilayer of the cellular membranes. A functional unit of TRP channel requires tetrameric assembly of homomeric or heteromeric TRP polypeptides. The mammalian TRP channels comprise a functionally versatile group of ion channels that are present in almost all cell types. From a biophysical standpoint, most share a theme of non-selective permeability to cations and low sensitivity to membrane voltage. Beyond this, TRP channels use a wide variety of regulatory themes and carry out functions as diverse as thermosensation, pheromone reception, magnesium homeostasis, and regulation of vascular tone. As a newly recognized and large group of ion channels, they have generated much interest in discovery and as targets for therapeutic strategies.

The discovery of the first mammalian TRP channel was based on close homology with the *Drosophila* TRP channel. Soon thereafter, presence of additional TRP homologues in the mammalian genome was recognized; the channels were identified, cloned, and characterized by heterologous expression in cell lines. Although much has been discovered, our understanding of TRP channels is still nascent, and many challenges lie ahead. In the case of some TRP channels, our understanding is limited to the knowledge of their amino acid sequence and their

B. N. Desai · D. E. Clapham (✉)
Department of Neurobiology, Harvard Medical School,
Pediatric Cardiology, Children's Hospital of Boston,
Howard Hughes Medical Institute, 4000 Jones Bridge Road,
Chevy Chase, MD 20815-6789, USA
E-mail: dclapham@enders.tch.harvard.edu

weak association with isolated physiological observations.

Because ion channels offer a direct readout of their activity via electrophysiological measurements, the study of their regulatory or gating mechanisms warrants a significant amount of attention. Early excitement about TRP channels was largely centered on their potential role as store-operated calcium channels (SOC). Store-operated calcium entry is a poorly understood phenomenon wherein depletion of intracellular stores stimulates a sustained influx of extracellular calcium [4]. The physiological significance of the sustained rise in calcium is well recognized, but the mechanisms that orchestrate this influx are a matter of continuing debate. TRPC channels were studied extensively as mediators of store-operated calcium entry [2]. There is general agreement that these channels are responsive to signals mediated by phosoholipase C, but their responsiveness to innate depletion of intracellular stores remains doubtful.

It is clear that TRP channels cannot be bundled along any single regulatory theme. The biophysical properties of TRP channels were relatively well studied by recording the activity of ectopically expressed channels and their mutants in common mammalian cell lines such as 293T cells. This approach has unraveled many mysteries about TRP channels, but proteins behave in accordance with the cellular context. Future studies on endogenous TRP channels will most certainly lead us to revise our nascent understanding of the regulatory mechanisms that govern this remarkable group of ion channels. However, for reasons outlined below, the study of endogenous TRP channels will constitute a formidable challenge.

In general, TRP channels show no particular preference for electrically excitable cells. Whereas some TRP channels clearly have a restricted tissue expression, most appear to exhibit wide tissue distribution. Determination of precise expression patterns of many TRP channels at a protein level was an obstacle because of their inherently low protein levels and unavailability of highly specific antibodies. Presence of multiple splice forms and heteromeric channels compound these difficulties for many, if not all, TRP channels. In the absence of definitive findings in this regard, the study of ectopically expressed TRP channels remains more tractable and provides the bulk of data currently available on functional as well as regulatory aspects of TRP channels. Despite these hurdles, research on TRP channel has made significant discoveries regarding their regulation and function.

Approaches to study the functional aspects of TRP channels, their merits, and limitations

Determination of protein function generally requires a way to perturb or neutralize that protein. For mammalian systems, common methods used for this purpose involve small molecule modulators, over-expression of dominant-negative variants, antisense oligonucleotides, RNAi, and targeted deletion of the gene using homologous recombination (in cells or in mice). Before we discuss the understanding gleaned from TRP-deficient mouse models, we outline some of the merits and difficulties associated with other methods in the context of TRP channels.

Ion channels have traditionally benefited from the availability of small-molecule or peptide-like agonists and antagonists of their activity. Despite the widespread enthusiasm around TRP channels as drug targets, studies on TRP channels have largely failed to report natural or synthetic modulators that are exquisitely specific for their target TRP channel. This handicap will continue to hamper the research on TRP channels for the near future because of the difficulties associated with high-throughput screening of TRP channel blockers (or activators). The rewards that await such findings are, however, well worth striving for, and there is a dire need for systematic and sustained efforts in this direction.

Studies on architecture of TRP channels yielded strong evidence that these channels function as tetramers; two transmembrane segments of each subunit are thought to assemble the permeation pore of the channel [5]. This finding suggested the use of over-expressed pore mutants as dominant-negative variants of TRP channels [6]. Such strategies were used to neutralize endogenous as well as ectopically expressed TRP channels, and this approach continues to yield reasonably good results. Many TRP channels were also shown to heteromultimerize to form functionally distinct channels. Although the physiological significance of such heteromerization is hard to study, the use of dominant-negative variants has the added feature (or the risk) of perturbing homomers as well as heteromers of the channel under investigation. When used appropriately, well-characterized dominant-negative variants hold considerable promise in cell biological as well as transgenic animal studies of TRP channels.

Anti-sense RNA was not used extensively in the area of TRP channels, because the era of TRP channels coincided to a significant degree with the advent of newer and better RNAi technologies that are capable of knocking down proteins in most cell lines. Different methods of RNAi introduction have found widespread use in the study of TRP channels, and there is widespread enthusiasm about the potential of this technique to shed light on the function of TRP channels in cultured cells. In this context, two issues should be noted. First, the data obtained using RNAi-mediated knockdown are susceptible to artifacts that result from non-specific effects of siRNA. This can often lead to ''false-positive'' results in terms of implicating a TRP channel in the studied phenomenon. Second, many TRP channels appear to have long turnover times, and effective knockdown can take many days after the introduction of RNAi in cells. This can often give the cells enough time to adapt and readjust their need for a particular

TRP channel by gradually up-regulating the activities of redundant machinery. There is, therefore, the risk of false-negative results and lost opportunities. The latter point is equally applicable to the use of dominant-negative variants, which are unlikely to affect the already assembled functional units at the cell surface.

The use of transgenic mice in the study of TRP channels

In light of the limitations of the approaches outlined above, definitive findings on the physiological functions of mammalian TRP channels will continue to depend heavily upon studies with genetically engineered mice. We will briefly summarize the current knowledge gained from such studies and highlight the challenges as well as the opportunities that lie ahead in this area.

TRPC channels

TRPC1 was the mammalian TRP channel first reported based on strong homology to *Drosophila* TRP channel. TRPC1 was shown to form functionally distinct heteromers with its closest relatives TRPC4 and TRPC5 [7]. TRPC1 was initially proposed to function as a store-operated channel [8]. It is not entirely clear whether TRPC1 is activated by release of calcium stores or by other phospholipase C (PLC)-dependent pathways. Functionally, TRPC1 was shown to function as a mechanosensitive channel in *Xenopus* oocytes [9]. Introduction of mammalian TRPC1 in oocytes leads to a dramatic increase in the stretch-activated current density in oocytes, and introduction of TRPC1-antisense RNA produces an opposite effect. Whether TRPC1 functions as the stretch-activated channel in excitable and nonexcitable somatic cells in all vertebrates is unclear. A TRPC1-deficient mouse has not been reported.

TRPC2 was initially implicated in the sperm acrosome reaction by mediating the influx of calcium into mouse sperm during fertilization [10]. In mouse, TRPC2 is found to express predominantly in the vomeronasal organ (VNO), a specialized region of the vertebrate brain that is involved in sensing smell [11]. The potential role of TRPC2 in olfaction was confirmed when two groups generated TRPC2$^{-/-}$ mouse lines and demonstrated that these mice exhibit radically altered responses to pheromonal cues [12, 13]. TRPC2$^{-/-}$ males exhibit a loss of male–male aggression and instead, vigorously mount other males. The TRPC2$^{-/-}$ lactating females are docile and fail to challenge intruding males. At a cellular level, VNO neurons from TRPC2-deficient mice show a consistent phenotype by failing to respond electrically to the application of male urine pheromones. The TRPC2$^{-/-}$ mice, however, breed normally, and it is therefore clear that TRPC2 is not essential for successful culmination of the acrosomal reaction. However, it is still possible that a small number of sperm TRPC2 channels are moderately involved in this process, but other functionally redundant channels readily substitute for TRPC2. The human TRPC2 is unlikely to play a role in sensing pheromones or in fertilization because premature stop codons make it a pseudogene.

TRPC4 and TRPC5 are related genes that are capable of heteromerizing with TRPC1 [7, 14]. Endothelial cells use SOC to mediate sustained elevations in calcium that are necessary for activation of nitric oxide synthase and subsequent vasorelaxation. In endothelial cells isolated from TRPC4$^{-/-}$ mice, agonist-activated calcium entry is drastically reduced, markedly affecting vasorelaxation [15]. Because agonist-induced calcium influx is important for other physiological roles of endothelial cells, it was proposed that TRPC4 also plays a role in regulating the permeability of blood vessels. TRPC4 exhibits a wide tissue distribution, and it is surprising that a more drastic phenotype is not evident. Related TRP channels such as TRPC5 and TRPC1 may be playing compensating roles in TRPC4$^{-/-}$ mice to alleviate the physiological defects caused by TRPC4 deficiency. Mice that are doubly deficient in TRPC4 and TRPC5 are likely to clarify some of these possibilities. Because TRPC1 has been shown to form functionally distinct heteromers with TRPC4 and TRPC5, generation of a transgenic mouse that expresses a dominant-negative variant of TRPC1 may be a viable approach to answer questions related to specialized roles of heteromers formed by TRPC4 and TRPC5.

TRPC3, TRPC6, and TRPC7 channels are closely related TRP channels that share a tendency to heteromerize with each other when heterologously expressed in HEK cells [16]. All three of them were shown to be sensitive to diacyl glycerol (DAG) in a manner that is independent of protein kinase C activity [16]. TRPC6$^{-/-}$ mice appear to show a higher contractility in tracheal and aortic rings in response to methacholine and phenylephrine [17]. The DAG-induced currents in cells derived from these mice show paradoxically higher current density, and this phenotype was explained on the basis of overcompensation by upregulated TRPC3 and/or higher constitutive activity in the absence of TRPC6. The mRNA expression of TRPC3 in TRPC6$^{-/-}$ mice was shown to be threefold higher. Mice deficient in TRPC3 and TRPC7 have not been reported. Given the possibility that TRPC3, TRPC6, and TRPC7 share functional redundancies, it may be necessary to generate mice with double and triple deletions to extract meaningful information about their functions.

TRPV channels

TRPV1 is highly expressed in dorsal root ganglia (DRG) neurons and is activated in response to capsaicin, moderate heat, and lowering of pH [18]. The TRPV1$^{-/-}$ mice show deficiencies in their response to capsaicin and in detection of heat stimuli [19, 20]. This phenotype can be directly attributed to the loss of functions in the neurons of DRG. In contrast to DRG neurons from

wild-type mice, DRG neurons from TRPV1$^{-/-}$ mice show diminished electrophysiological responses to capsaicin, moderate heat, and acidic solutions. TRPV1-deficient mice also appear to have an attenuated hypersensitivity response during inflammation. This effect remains relatively less understood and may point toward a role for TRPV1 in the cells of the immune system. Additional effects of TRPV1 deficiency were recorded in the ability of the mouse to mount a fever response [21]. Most studies indicate that TRPV1 expression is restricted predominantly to the nervous system, but very low levels of TRPV1 in other cell types may still have a functional impact on mammalian physiology. In the epithelial cells of the urinary bladder, low levels of TRPV1 expression have a clearly discernible function. In wild-type mice, the urothelium responds to stretch stimuli by releasing ATP. The purinergic response is critical for normal bladder function. In TRPV1$^{-/-}$ mice, the stretch-evoked responses are diminished and the urothelial cells isolated from TRPV1$^{-/-}$ mice show a marked decrease in secretion of ATP in response to mechanical as well as hyposmolal conditions when compared to such responses produced by urothelial cells from wild-type littermates [22]. On a more general note, these findings suggest that regulatory as well as functional aspects of TRP channels may depend significantly on the cellular context. This could be accomplished either by utilizing different splice forms, functionally distinct heteromers, or by simply recruiting the TRP channel to an alternative signaling complex.

The presence of heat-sensitive channels, TRPV2 and TRPV3, was indicated by retention of some heat-induced responses in mice that are deficient in TRPV1. TRPV2 and TRPV3 share significant identity with TRPV1 and are found in genomic loci that are adjacent to TRPV1 on mouse chromosome 11. Functionally, TRPV2 and TRPV3 respond to temperature elevations, but in contrast to TRPV1, they are insensitive to capsaicin and have a wider tissue distribution [23]. Whereas TRPV1 is activated by temperature higher than 43°C, TRPV3 responds to a lower temperature range of 32–40°C [24, 25]. TRPV2 on the other hand was shown to respond to a noxious temperature of ≥52°C—the physiological significance of which is likely to be linked predominantly to nociception [26]. A recent report on TRPV3-deficient mice indicates that these mice have clearly detectable deficits in responses to innocuous as well as noxious heat [27]. The phenotype of TRPV2-deficient mice has not been reported.

Mammalian TRPV4 is the functional orthologue of OSM-9, a TRP channel used by *Caenorhabditis elegans* for sensing osmotic stimuli [28]. Osmolarity and mechanical responses in *osm-9* mutant worms can be restored by introduction of mammalian TRPV4 [29]. This complementation of behavioral responses is incomplete as the *osm-9*/TRPV-4 mutants retain their inability to avoid noxious odorants. The findings are consistent with the original identification of TRPV4 as a channel that is activated by hypotonicity and with the phenotype of TRPV4-deficient mice. One line of TRPV4$^{-/-}$ mice shows a tendency to drink less water and become more hyperosmolar when compared to wild-type littermates [30]. Another line of TRPV4-deficient mice, however, does not show a significant difference in water-intake behavior, and the serum osmolality remains essentially unaffected [31]. This study examined the secretion of arginine vasopressin (AVP) by brain slices exposed to hypertonic conditions and found that the secretion of AVP was extra-normal in brain slices obtained from TRPV4-deficient mice. TRPV4 may be involved in regulating the hyperosmolarity-induced secretion of AVP in the brain. Mechanosensory and nociceptive function for TRPV4 was also proposed based on the evidence that TRPV4$^{-/-}$ mice show abrogated tail responses to application of pressure and acid-induced pain [32]. Overall, TRPV4 appears to have retained the responsiveness of OSM-9 to pressure, but there is no evidence that implicates TRPV4 as an odorant receptor. TRPV4 is also activated by arachidonyl ethanolamide (anandamide) and its metabolite, arachidonic acid, suggesting a more complex role in the nervous system [33]. This activation is not direct and may require the formation of epoxyeicosatrienoic acid by cytochrome P450 epoxygenase. These findings raise the possibility that TRPV4 may have additional functions that remain to be identified.

As exceptions to the rule, TRPV5 and TRPV6 are calcium-selective TRP channels (permeability ratio $P_{Ca}/P_{Na} > 100$) that are capable of forming functional heteromers [34, 35]. Because of their calcium selectivity and strongly inwardly rectifying currents, TRPV5 and TRPV6 were proposed as mediators of calcium uptake in many cell types [36]. Mice deficient in TRPV5 display variations in bone structure and appear to have reduced bone thickness. This phenotype is consistent with the finding that these mice show poor calcium reabsorption despite high levels of vitamin D [37]. It is possible that TRPV5$^{-/-}$ mice escape a more dramatic phenotype due to a compensating role of TRPV6. The relative contributions of TRPV5 and TRPV6 in calcium uptake pathways await the generation of mice deficient in TRPV6 as well as double mutants of TRPV5 and TRPV6.

TRPM channels

TRPM1, which is also known as melastatin, was originally identified as the protein whose expression is downregulated in highly metastatic melanoma cell lines [38]. The biophysical properties of TRPM1 have not been forthcoming because of the difficulties involved in recording TRPM1-mediated currents. From a functional standpoint, TRPM1 can be postulated to play a role in the regulation of cellular migration and invasiveness, but no definitive findings have been reported. Because of the prominent deficits in our understanding of TRPM1 channel properties, a reverse genetic approach in mouse may very well be the only immediately

applicable approach to divulge the functional significance of TRPM1. No such studies have been reported.

TRPM2 contains a carboxy-terminal NUDT9 homology regulatory domain that makes TRPM2 responsive to binding of ADP ribose (ADPR) and nicotinamide dinucleotide (NAD) [39]. Application of ADPR and NAD in cells expressing recombinant TRPM2 channels evokes a nonselective and linear whole-cell current. Functional significance of TRPM2 is not completely clear, but regulation of the channel by ADP ribose suggests that stress-response pathways that lead to the synthesis of ADP ribose may utilize TRPM2 for timely influx of cations. The finding that TRPM2 is activated by redox changes in cells and by application of hydrogen peroxide supports this view. Current models place TRPM2 in pathways that regulate cell death induced by disturbance in the redox state of the cells. Mice deficient in TRPM2 have not been reported.

TRPM3 is predominantly expressed in the kidney and nervous system. Electrophysiological measurements of TRPM3-mediated currents suggest a linear current–voltage relationship [40]. This current was found to be nonselective, constitutively active, and responsive to hypotonic conditions. A separate study reported calcium-imaging experiments suggesting that TRPM3 functions as a SOC [41]. Further work is necessary to clarify these findings, but the presence of numerous splice forms of TRPM3 is a significant hurdle in this regard. A TRPM3$^{-/-}$ mouse has not been reported.

TRPM4 and TRPM5 constitute a clearly distinct group of related TRP channels. These channels mediate calcium-activated, nonselective monovalent cationic currents that modulate the membrane potential of nonexcitable cells. They appear to have a wide tissue distribution and are likely to play a role in a large variety of calcium-sensitive physiological processes. One research group used differential screening methods to identify transcripts with restricted expression in taste receptor cells and identified TRPM5 as a candidate for a role in taste-sensing mechanisms [42]. Generation of TRPM5-deficient mice allowed Zhang et al. [43] to test this hypothesis and the findings indicated that TRPM5 is essential for sweet, umami, and bitter taste reception. This study also proposed that TRPM5 is regulated directly or indirectly by the activity of PLCβ2, a phospholipase that was also found to be essential for this particular sensory pathway. Authors compared the electrophysiological recordings for the taste-nerve in the tongues of wild-type, TRPM5$^{-/-}$, and PLCβ2$^{-/-}$ mice. The study demonstrated that in contrast to wild-type mice, a variety of bitter-tasting compounds, sugars, and amino acids failed to elicit action potentials in the TRPM5-deficient and PLCβ2-deficient mice. The response to salty and sour tasting agents was found to be intact, indicating that the mutant mice retained the general machinery of taste reception. Whether TRPM5$^{-/-}$ mice exhibit other physiological deficiencies is not clear.

TRPM6 and TRPM7 are TRPM channels possessing a carboxy-terminal kinase domain belonging to the α-kinase family [3, 44]. Both channels show nearly identical conduction properties of steeply outwardly rectifying current that is permeant to most divalent cations including calcium and magnesium [45, 46]. The distinct property of magnesium permeation was shown to have physiological relevance for TRPM6 as well as TRPM7. TRPM6 is expressed predominantly in the kidney and in gastrointestinal system [47]. Accordingly, humans with mutations in the *TRPM6* gene suffer from an autosomal recessive form of hypomagnesemia, the symptoms of which can be alleviated significantly by dietary supplements of high-dosage magnesium. TRPM7 appears to have nearly ubiquitous expression, but expression levels are often found to be very low. The chicken B-lymphocyte cell line DT-40 that is targeted for the deletion of TRPM7 is unable to survive in cell culture unless the growth medium is supplemented with relatively high concentrations of magnesium [48]. This result indicates that at least for chicken lymphocytes, TRPM7 plays a critical role in magnesium homeostasis. Whether this will hold true for cell types of mammalian origin remains to be seen. The significance of calcium permeation and the significance of the kinase domain in TRPM6 and TRPM7 is another question that remains unanswered. Mice deficient in TRPM6 and TRPM7 have not been reported. Our current understanding leads to legitimate concerns about the potential viability of mice that are deficient in TRPM7. Tissue specific or conditional knockouts of TRPM7 hold promise in shedding light on many of these questions.

TRPM8 was initially identified as a prostate-specific TRP channel that was upregulated in malignant tissue [49]. Subsequent work detected TRPM8 in DRG neurons, where it has been shown to be involved in a thermosensory role [50]. TRPM8 is activated by temperatures lower than 25°C and by cooling compounds such as menthol and icilin. The significance of TRPM8 expression in nonexcitable cells is not clear. TRPM8$^{-/-}$ mice have not been reported.

Distantly related TRP channels

TRPA1 or ANKTM1, a protein rich in ankyrin repeats, is a distal member of mammalian TRP superfamily that was shown to be present in the sensory neurons [51, 52]. ANKTM1 is activated by application of isothiocyanates, the active components of pungent substances such as horseradish and mustard [52]. A thermosensory role for ANKTM1 has also been reported with evidence indicating that low temperatures activate ANKTM1 [51]. The precise physiological role of ANKTM1 is still debated, and generations of ANKTM1-deficient mice will help clarify the reported findings.

TRPP1 and TRPP2 were implicated in autosomal dominant polycystic kidney disease (ADPKD) by the finding that patients suffering from this disease have

mutations in TRPP2 [53]. TRPP1 is a very large protein that is thought to require TRPP2 to form a calcium-permeable nonselective channel [54]. Mice deficient in TRPP1 and TRPP2 die in utero, with cysts in many organs and defects in development of the heart [55, 56]. Because TRPP1 and TRPP2 are expressed in a wide variety of tissues and ADKPD affects organs other than kidney, the precise functions of these channels may be of general importance to cell biology. In kidney, fluid flow is thought to regulate the gating of TRPP1 and TRPP2, which are targeted to the primary cilium of the kidney cells [57]. Whether this is a common regulatory theme for TRPP1 and TRPP2 expressed in other tissues is not clear.

The closely related TRPML1, TRPML2, and TRPML3 are intracellular channels that were initially called mucolipins, because mutations in TRPML1 (then referred to as mucolipin 1) were first shown to cause a developmental neurodegenerative disorder called mucolipidosis type IV [58]. This autosomal-recessive disease is detectable early in life, progresses slowly, and has clinical manifestations that include psychomotor retardation, ophthalmologic complications, and blood iron deficiency [59]. At a cellular level, the disease is typified by the accumulation of mucopolysaccharides, gangliosides, and lipids in abnormally large lysosome-like vacuoles. Although the pH is often observed to be slightly elevated, this particular form of lysosomal storage disease is not accompanied by prominent deficiencies in the catalytic activity of lysosomal hydrolases [59]. The *C. elegans* orthologue CUP-5, which resides primarily in the endosomal compartments, yields meaningful mechanistic clues about similarly localized TRPML1. Normal endocytic traffic in most cells involves ''fusion'' events that form a large hybrid endosome–lysosome compartment and subsequent ''fission'' of this hybrid vacuole toward reformation of discrete lysosomes and endosomes. Involvement of *C. elegans* CUP-5 in the fission of the hybrid vacuole is indicated by the persistence of this large vacuole in *cup-5* mutants [60]. The role of mammalian TRPML1 and TRPML-3 is likely to be analogous to that of CUP-5 because introduction of TRPML-1 or TRPML-3 complements *cup-5* mutants adequately [61, 62]. Because calcium plays a critical role in regulation of such events involving cellular membranes, and because the lysosomal TRPML channels are permeant to calcium, the prevailing model places TRPML channels as important regulators of calcium-sensitive mechanisms during lysosomal biogenesis. This model is largely consistent with clinical manifestations of mucolipidosis type IV, but further involvement of TRPML channels in mechanistically similar processes such as synaptic transmission cannot be ruled out. Although mutations in human TRPML channels have helped us to understand these channels to a certain degree, generation of mouse models deficient in these proteins is likely to offer the best chance of teasing apart their functional complexity. Such mice have not been reported yet but interestingly, ''varitint-waddler'' mice, which suffer from spontaneous deafness, pigmentation abnormalities, and perinatal lethality, were shown to have mutations in TRPML3 [63]. From a functional standpoint, TRPML channels appear to share an evolutionary link with the only TRP channel found in unicellular organisms: the vacuolar TRP channel of *Saccharomyces cerevisiae*.

Concluding remarks

Although reverse genetics in mouse is proving to be a powerful approach in illuminating the functions of many TRP channels, the propensity of TRP channels to execute redundant roles will delay definitive conclusions in many cases. Generation of double and triple knockouts by crossbreeding mice deficient in related TRP channels is likely to attract a great deal of attention in this regard. Functional significance of TRP heteromers can also be addressed using mice where dominant-negative variants of TRP channels were ''knocked in.'' This approach is likely to yield more sweeping phenotypes and stimulate meaningful hypotheses pertaining to regulation and function of functionally distinct TRP heteromers. In the case of ''chanzymes'' (TRPM2, TRPM6, and TRPM7), the significance of their enzymatic domains remains an enduring mystery in this field. Whereas studies carried out in heterologous expression systems and cell lines are providing interesting clues about their function, results obtained using mice that were knocked-in with chanzyme variants without enzymatic activity will prove to be most meaningful. Long after all the TRP channels are deleted in mice, transgenic approaches will thus continue to have a telling impact on research pertaining to TRP channels. The challenge that lies over the horizon is the mechanistic delineation of signaling pathways used by TRP channels to have such a powerful impact on cellular and organismal physiology. These challenges will require identification of natural or synthetic small molecules that are capable of serving as agonists or antagonists of TRP channels with a very high selectivity, if not exquisite specificity. Such tools will enable a mechanistic dissection of TRP channels and complement the reverse-genetic efforts in mouse models. Together, these tools will help unravel the potential of TRP channels in therapeutic settings.

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