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INVITED REVIEW

Evolutionary dynamics of metazoan TRP channels

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Abstract Transient receptor potential (TRP) channels are unusual among cation channels because of their diverse cation selectivities and activation mechanisms. TRP channels thus play major roles in various sensory perceptions by functioning as multimodal signal integrators. Some TRP subfamily members are also implicated in acute and chronic pain and inflammation. So far, most TRP channel studies have been targeted to human and model organisms within a limited evolutionary context. Classification of TRP channels in various animal genomes has revealed extensive gene gain and loss events across animal species. Furthermore, the chemical activation profiles of some orthologous TRP channels were different between species such as human and mouse. Amino acid substitutions must underlie such differences, and the crucial amino acid residues have been identified in some cases. These changes represent the evolution of TRP channels at the amino acid sequence level. There is also evidence that TRP channels have obtained species-diversity through alternative splicing and possibly *cis*-regulatory element mutations. All of the above demonstrate the dynamic and plastic evolutionary history of metazoan TRP channels at multiple levels, possibly in conjunction with the specific habitats and life histories of individual species.

Keywords TRP channel · Evolution · Animal genomes · Gene loss/gain · Amino acid substitution · Pre-mRNA splicing · *cis*-regulatory mutations

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Introduction

Transient receptor potential (TRP) superfamily channels share six common transmembrane segments (S1–S6), which form sensor and pore domains that are cation permeable (Fig. 1a). However, TRP channels are different from other ion channels because they have diverse cation selectivities and activation mechanisms [12, 27, 50, 72]. Therefore, TRP channels play major roles in various sensory signal transductions such as vision, thermosensation, olfaction, hearing, and mechanosensation by functioning as multimodal signal integrators and downstream signaling components [12]. TRP channels also enable individual cells to detect changes such as osmolarity and fluid flow in their local environment as well as inflammation, and thus, they play essential roles in several physiological processes. These include sensory functions, homeostatic functions, motile functions, and acute and chronic pain [12, 27, 50, 72].

The metazoan TRP superfamily is classified into seven subfamilies—TRPA, TRPC, TRPM, TRPML, TRPN, TRPP, and TRPV—based on their amino acid sequences and domains (Fig. 1b). Most functional TRP channels are thought to form homotetramers [12, 27, 50, 72]. Structural characterization of TRPV1 by electron cryo-microscopy has revealed that TRPV1 and voltage-gated ion channels (VGICs) share a similar overall structure. However, the opening of TRPV1 is associated with major structural changes in the outer pore as well as dilation of the lower gate. In contrast to VGICs, the S1–S4 voltage-sensor-like domain of TRPV1 does not appear to move [7, 41]. The N- and C-termini of TRP channels (both facing cytosol) are variable in length and contain different domains (Fig. 1b). The N-termini of TRPA, TRPC, TRPN, and TRPV channels contain ankyrin repeats (ARs), 33-residue motifs consisting of pairs of antiparallel α-helices connected by β-hairpin motifs. The numbers of ARs present in each channel are different: 14 to 15 in

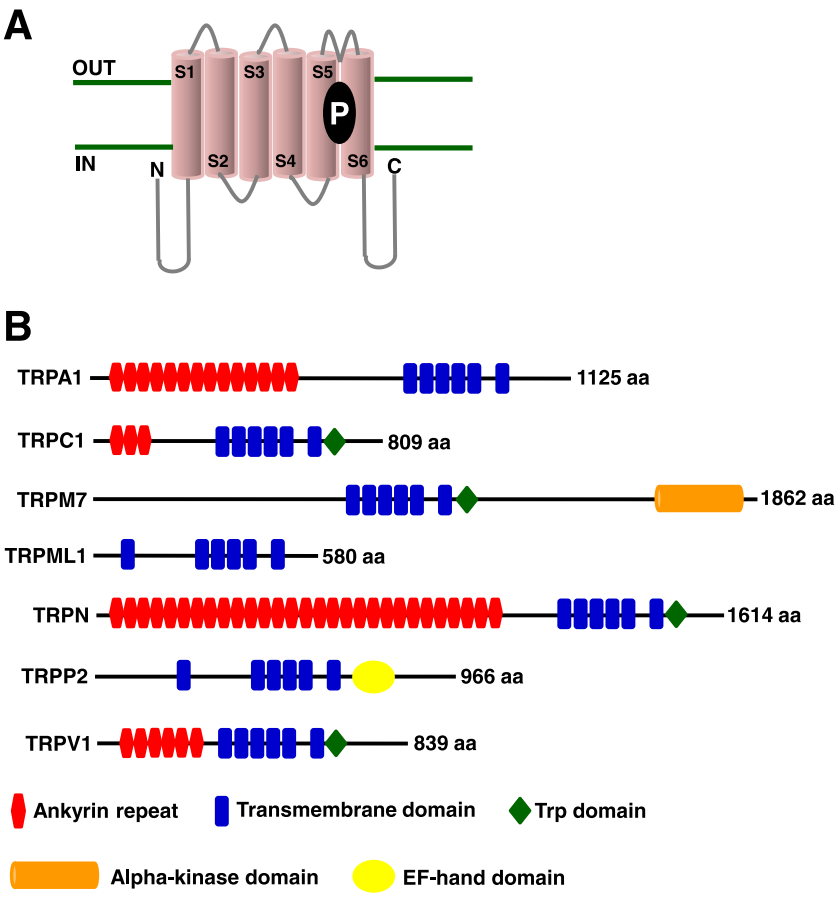

Fig. 1 Schematic representation of TRP channels. **a** All TRP channels contain six transmembrane segments (S1 to S6) with a pore domain (P) between S5 and S6. *Parallel green lines* represent, for example, plasma membrane separating the outside (*OUT*) and inside (*IN*) of a cell. Both amino (*N*) and carboxyl (*C*) termini with variable lengths are oriented toward the cytosol and contain different functional domains. TRPML and TRPP subfamily members have the longer extracellular loop between S1 and S2 than other subfamilies. **b** Functional domains in selected mouse TRP and zebrafish TRPN channels. The number and composition of functional domains are variable between different TRP channels and are only partially maintained within members of the same subfamily. The number of amino acids (*aa*) in each TRP channel is shown on the *right side*

TRPA, 3 to 4 in TRPC, 29 in TRPN, and 4 to 6 in TRPV channels. ARs appear to be necessary for interactions with ligands and protein partners and for temperature sensitivity [11, 17, 24]. A highly conserved domain of 23–25 amino acids (TRP domain) is present, which extends from the C-terminus to the transmembrane domains, in TRPC, TRPM, TRPN, and TRPV channels [41, 72]. Other functional domains are found in the C-terminal tails of TRP channels. For example, TRPM6 and TRPM7 contain an atypical α-kinase domain (Fig. 1b) involved in regulating channel function [46, 56]. Nevertheless, many functional domains are often not conserved, even between members of the same subfamily.

Many TRPA and TRPV channels are activated by temperature changes and various ligands and thus function in thermosensation (for cold and hot temperatures) and chemoreception [49, 74]. *Drosophila melanogaster* TRPV channels also have roles in hearing and hygrosensation, probably through mechanical activation [19, 31, 42]. *D. melanogaster* TRPN was shown to be a mechano-sensitive channel [83] and involved in hearing and mechanosensation [15, 63]. TRPC channels function in signal transduction in neurons and other cell types [12, 50, 72]; TRPM channels are involved in signal transduction, chemoreception, and thermosensation (cold and warm temperatures) [12, 50, 72, 73]. TRPML channels are important for endosomal/lysosomal function and autophagy [84], and most TRPP channels play important roles in cardiac, skeletal, and renal development as well as in spermatogenesis [50, 72]. These physiological functions have primarily been characterized in genetically tractable model organisms such as the mouse, the fruit fly, and the nematode.

Since TRP channels are involved in a wide range of physiological processes, as mentioned above, lesions in their genes are often associated with specific diseases. Mucolipidosis type IV disease, an autosomal-recessive neurodegenerative lysosomal storage disorder, is caused by mutations in *TRPML1*. TRPML1 is a calcium and iron permeable intracellular channel in lysosomes, and thus, loss-of-function impairs endosomal/lysosomal function and autophagy [10]. Polycystic kidney disease (PKD), the most common inherited kidney disease, is associated with a mutation in *TRPP2*. PKD causes large epithelial-lined cysts filled with fluid that occupy most of the mass of the abnormally enlarged kidneys, resulting in impaired kidney function [32]. Other examples include *TRPC6*, *TRPV4*, *TRPM1*, *TRPM4*, *TRPM6*, and *TRPA1*. *TRPC6* is involved in a human proteinuric kidney disease called focal and segmental glomerulosclerosis [55, 77]. *TRPV4* is associated with neurodegenerative disorders such as scapuloperoneal spinal muscular atrophy (SPSMA)

and Charcot-Marie-Tooth disease type 2C (CMT2C, also known as hereditary motor and sensory neuropathy type 2C) [2, 13, 37]. *TRPM1* is involved in autosomal-recessive congenital stationary night blindness (CSNB) [1, 40, 70], and *TRPM4* plays a role in autosomal-dominant progressive familial heart block type 1 (PFHB1) [35]. *TRPM6* is associated with hypomagnesemia with secondary hypocalcemia (HSH/HOMG) [75], and *TRPA1* is implicated in autosomal dominant familial episodic pain syndrome (FEPS) [34].

Although significant progress has been made toward understanding the activation mechanisms and physiological functions of TRP channels in human and model organisms, there have been relatively few studies or reviews focusing on their evolution [60]. Nevertheless, a number of examples of species-dependent activation of TRP channels by particular compounds have been reported. Although these are mediated by amino acid substitutions in particular TRP channels during evolution, the evolutionary impacts/aspects are often not completely discussed. In this review, I will explore the evolutionary plasticity and dynamics of metazoan TRP channels at different levels by explaining (1) the ancient origin of metazoan TRP channels, (2) evolutionary dynamics by gene gain and loss across animal species, (3) evolutionary dynamics at the amino acid sequence level, and (4) evolutionary dynamics at the pre-mRNA splicing and transcriptional levels.

Ancient origin of metazoan TRP channels

Because TRP channels have critical physiological and cellular functions, it is not surprising that they are highly conserved between yeast and mammals. Nevertheless, land plants do not appear to contain the TRP channel genes. *Saccharomyces cerevisiae* TrpY1 is a vacuolar membrane protein that functions as a mechano-sensor of vacuolar osmotic pressure [14, 53]. Because TrpY1 does not cluster with any of the metazoan TRP channels by phylogenetic analysis [6], it must have specifically emerged in fungi after the divergence of fungi and metazoans. A previous study showed that the apusozoan protist *Thecamonas trahens*, a sister species to the common ancestor of Holozoa and fungi, contained TRPP and TRPV [6], suggesting that they could be the most ancient metazoan TRP channels. Furthermore, two choanoflagellates (*Monosiga brevicollis* and *Salpingoeca rosetta*) have five TRP subfamilies (TRPA, TRPC, TRPM, TRPML, and TRPV [5, 54]) demonstrating that most of the current metazoan TRP channels emerged in the unicellular common ancestor of all Metazoa.

Primitive animals, such as sponges (*Amphimedon queenslandica*) and Placozoa (*Trichoplax adhaerens*), contain only a few cell types and no neurons [65, 66] so that all of their TRP channels must function in non-neuronal cells. Because choanoflagellates and sponges respond to environmental stimuli [4, 39], TRP channels may function to perceive such stimuli [43]. It should be of major interest to test the functions of TRP channels present in choanoflagellates, sponges, and Placozoa. The expression characteristics as well as the sensory functions of metazoan TRP channels in the neurons of higher animals must be the result of co-option during evolution. Meanwhile, TRPN is absent in apusozoan protists, choanoflagellates, sponges, and Placozoa, but it is present in *Hydra magnipapillata* (Cnidaria), suggesting that it first emerged in the common ancestor of Cnidaria and Bilateria [54]. The newly identified subfamily, TRPVL (TRPV-like), also emerged at the same time [54] (see below).

Evolutionary dynamics by gene gain and loss across animal species

The number of different TRP channel subfamily members is quite varied between the animal species we characterized (Table 1). For example, 12 *TRPA1* genes are present in *A. queenslandica*; however, *T. adhaerens*, *Tetranychus urticae* (spider mite), *Daphnia pulex* (water flea), and hymenopteran insects (bees, wasps, and ants) lack *TRPA1* genes in their genomes [45]. The 12 TRPA1 channels of *A. queenslandica* form a single cluster in the phylogenetic tree, suggesting that one ancestral *TRPA1* gene has expanded multiple times in *A. queenslandica* [54]. Similarly, *TRPA1* has expanded to four copies in a centipede, *Strigamia maritime* [54]. The absence of *TRPA1* in *T. adhaerens*, *T. urticae*, *D. pulex*, and hymenopteran insects demonstrates that it has been lost from their genomes. Thus, the loss of *TRPA1* has happened many times during metazoan evolution.

Hymenopteran insects contain *Hymenoptera-specific TRPA* (*HsTRPA*) which was generated by duplication of *Waterwitch* (*Wtrw*) in the genomes [45]. Honey bee HsTRPA, AmHsTRPA, functions as a noxious sensor to detect heat and irritants such as allyl isothiocyanate (AITC), cinnamaldehyde, and camphor to substitute the functions of TRPA1. Thus, neofunctionalization of HsTRPA following the duplication may have resulted in the loss of TRPA1 in Hymenoptera [33].

The TRPA subfamily in insects and *D. pulex* appears to have specifically expanded the members which diverged from the ancient *TRPA1* gene [29, 54]. Painless (Pain), Pyrexia (Pyx), Wtrw, and TRPA5 are found in insects and *Daphnia* but not in other Arthropod species, Cnidaria, Deuterostomia, or Lophotrochozoa [54]. This suggests that the above TRPA channels specifically arose in the common ancestor of insects and crustaceans (Altocrustacea). *D. melanogaster* Pyx was shown to have multiple physiological functions including high-temperature stress avoidance [38], negative geotaxis [67], and synchronization of the circadian clock [68, 79]. This suggests that Pyx can be directly activated by high temperature and mechanical stimuli and indirectly activated by unknown factors. *D. melanogaster* Pain has various functions in the avoidance of

Table 1 The number of TRP subfamily members in 21 metazoan and one holozoan species

	TRPA	TRPC	TRPM	TRPML	TRPN	TRPP	TRPV	TRPVL
M. brevicollis (Choanoflagellate, Holozoa)	2	1	3	1	0	0	1	0
A. queenslandica (Sponge, Metazoa)	12	0	0	2	0	0	0	0
T. adhaerens (Placozoan, Eumetazoa)	0	0	4	1	0	2	2	0
N. vectensis (Sea anemone, Cnidaria)	2	3	3	2	0	8	2	1
H. magnipapillata (Hydra, Cnidaria)	4	0	3	2	1	14	0	5
M. musculus (Mouse, Mammalia, Deuterostomia)	1	7	8	3	0	3	6	0
L. gigantea (Owl limpet, Lophotrochozoa)	1	4	4	1	1	7	2	0
C. teleta (Marine polychaete, Lophotrochozoa)	2	9	7	1	1	3	3	1
D. melanogaser (Fruit fly, Diptera, Insecta)	4	3	1	1	1	1	2	0
B. mori (Silk moth, Lepidoptera, Insecta)	6	3	1	1	1	0	2	0
T. castaneum (Red flour beetle, Coleoptera, Insecta)	5	3	1	1	1	1	2	0
A. mellifera (Honey bee, Hymenoptera, Insecta)	5	3	1	1	1	0	2	0
H. saltator (Jerdon's jumping ant, Hymenoptera, Insecta)	6	3	1	1	1	0	2	0
C. floridanus (Florida carpenter ant, Hymenoptera, Insecta)	16*	3	1	1	1	0	2	0
P. barbatus (Red harvester ant, Hymenoptera, Insecta)	5	3	1	1	1	0	2	0
S. invicta (Red imported fire ant, Hymenoptera, Insecta)	19*	3	1	1	1	0	2	0
A. pisum (Pea aphid, Hemiptera, Insecta)	4	2(3)	1	1	1	1	2	0
P. humanus (Human body louse, Phthiraptera, Insecta)	4	3	1	1	1	1	2	0
D. pulex (Water flea, Crustacea)	5	3	2	1	1	0	2	0
S. maritime (Centipede, Myriapoda)	4	1	2	1	1	2	2	0
T. urticae (Spider mite, Arachnida)	0	1(2)	1	3	2	2	0	0
M. occidentalis (Predatory mite, Arachnida)	1	2	1	1	0	2	0	0
I. scapularis (Deer tick, Arachnida)	0(1)	3	1(2)	2	1	2	0	0

Common name and order, class, or phylum are shown for each species. All species below *M. musculus*, *L. gigantea,* and *D. melanogaster* belong to Bilateria, Protostomia, and Arthropoda, respectively. *H. magnipapillata* may contain five additional TRP subfamily members. They were not phylogenetically characterized since the annotation of their transmembrane segments was incomplete. *C. floridanus* and *S. invicta* are predicted to contain 12 Pyx and 11 TRPA5 channels, respectively. More channels could be present due to the incomplete annotation of transmembrane segments. TRPA1 is absent in hymenopteran insects. *A. pisum*, *T. urticae*, and *I. scapularis* may contain additional TRP subfamily members as shown in parentheses. They were not phylogenetically characterized since the annotation of their transmembrane segments was incomplete [54]

noxious stimuli [69], negative geotaxis [67], mating behavior [61], and feeding behavior [82]. This channel can be directly activated by high temperatures [64] and possibly also by mechanical stimuli [69]. *D. melanogaster* Wtrw is essential for hygrosensation (detecting moist air) [42]. Some of these physiological functions might be shared with other insects and *Daphnia*. TRPA5 has not been characterized in any animals to date. Interestingly, the genes *Pain*, *Pyx*, and *TRPA5* emerged in Altocrustacea were not stable and went through multiple rounds of gene gain and loss later during evolution. *Pain* has duplicated twice in *Daphnia*, producing three genes in the genome. Meanwhile, *TRPA5* has been lost in the *D. melanogaster*, *Pediculus humanus*, and *Acyrthosiphon pisum* genomes [45, 54]. We also found that *Pain* and *TRPA5* have expanded in fire ant (*Solenopsis invicta*), and *Pyx* has expanded in Florida carpenter ant, *Camponotus floridanus* [54]. These results demonstrate that insect- and crustacean-specific TRPA subfamily members show extensive evolutionary plasticity through multiple gene gain and loss events. As a result, the number of TRPA subfamily members appears to be the most diverse within Arthropod species [45, 54].

During our classification of TRP channel genes in the genomes of cnidarians, *H. magnipapillata*, and *Nematostella vectensis*, we found a novel TRP subfamily, TRPVL (Table 1 and Fig. 2) [54]. TRPV and TRPVL share the same domain structures, 4–5 ARs and an ion transport domain containing six transmembrane segments. *H. magnipapillata* contains five TRPVL subfamily members (Table 1 and Fig. 2, *HmTRPVL1-5*) and lacks a TRPV channel, suggesting that evolution of TRPVL channels may have resulted in the loss of ancient TRPV channels. Meanwhile, *N. vectensis* has one TRPVL and two TRPV subfamily members (Table 1 and Fig. 2). Intriguingly, *Capitella teleta* (an annelid) also contains one TRPVL channel (Table 1 and Fig. 2); however, the other bilaterian species we analyzed do not have this channel. This suggests that TRPVL emerged in the last common ancestor of Cnidaria and Bilateria but was later lost in most bilaterians [54].

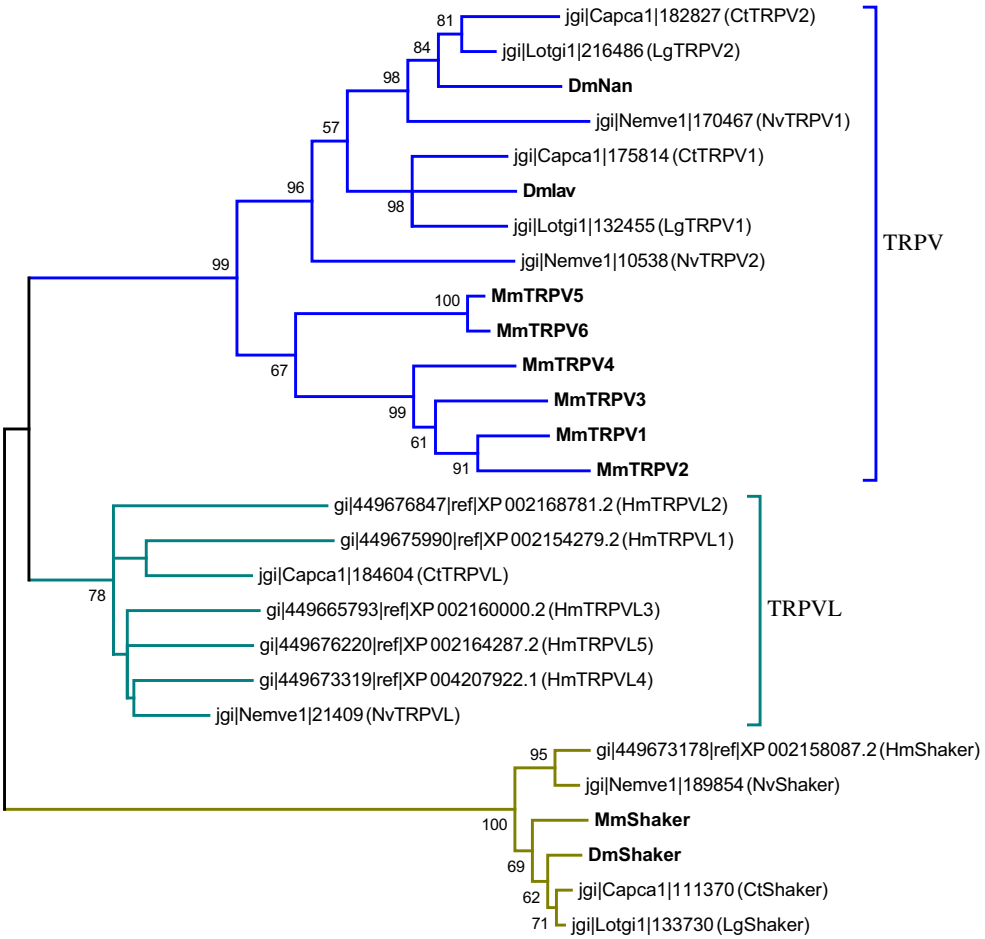

Fig. 2 Phylogeny of TRPV and TRPVL. The phylogenetic tree of the amino acid sequences encoding the channel-forming six transmembrane segments of TRPV and TRPVL channels from mouse (*m*), *D. melanogaster* (*Dm*), *N. vectensis* (*Nv*), *H. magnipapillata* (*Hm*), *L. gigantean* (*Lg*), and *C. teleta* (*Ct*). TRPV and TRPVL channels form separate clades. The Shaker K^+ channel sequences were used to root the tree [54]

Evolutionary dynamics at the amino acid sequence level

Even though the same TRP subfamily members are present in multiple species, amino acid substitutions took place during evolution. As a result, their channel properties could have become different. In fact, TRP channel genes appear to have evolved much faster than other cation channels such as potassium channels. For example, the amino acid sequence identity/similarity between frog (*Xenopus tropicalis*) and human TRPA1 is 53/71 %, whereas that between the frog and the human voltage-gated potassium channel subfamily KQT member is 78/86 %. There are many examples that indicate the species-specific activation or suppression of orthologous TRP channels, as described below.

Mammalian TRPV1 is a thermosensitive TRP channel activated by noxious high temperatures (>42 °C) as well as by various natural and synthetic compounds [27]. Although its high-temperature activation appears to be conserved [50], its chemical sensitivity is varied among vertebrates. For example, capsaicin and resiniferatoxin, vanilloids (present in chili peppers and *Euphorbia resinifera*, respectively) are potent activators of rat TRPV1 but not rabbit TRPV1 [18] or chicken TRPV1 [26]. Characterization of rat/chicken and rat/rabbit chimeric TRPV1 channels, as well as site-directed mutagenesis studies, demonstrated that four amino acid residues (Y511, S512, M547, and T550) in the third (S3) and fourth (S4) transmembrane segments of rat TRPV1 are responsible for the potent activation by vanilloids. Y511 and S512 are conserved between rat, rabbit, and chicken TRPV1 channels; however, T550 is substituted to I (isoleucine) and A (alanine) in rabbit and chicken, respectively. Moreover, M547 is substituted to L (leucine) in both rabbit and chicken. Thus, these two amino acid substitutions would be responsible for reduced activation of rabbit and chicken TRPV1 channels by vanilloids. Indeed, the reverse mutations (L547M, I550T, and L547M/I550T) made rabbit TRPV1 more sensitive to vanilloids [18, 26]. S512 and T550 are substituted to Y (tyrosine) and A (alanine) in *X. tropicalis* TRPV1, respectively, and the reverse mutations (Y512S, A550T, and Y512SA550T) increased the sensitivity to capsaicin [52].

As described above, TRPA1 is highly conserved between many animals and functions as a sensor for various noxious compounds and inflammatory agents [49]. Electrophilic compounds such as AITC and diallyl disulfide derived from mustard and allium, respectively, activate the channel by

reversibly adding thiol moieties to cysteines in the cytoplasmic N-terminus [22, 44]. A variety of non-electrophilic compounds also activates TRPA1 without causing covalent modifications. Among them, menthol, a cooling agent from mint leaves, activates human TRPA1 in a concentration-dependent manner. However, its action on mouse TRPA1 is bimodal; it activates at low concentrations but inhibits at high concentrations [30, 81]. *D. melanogaster* TRPA1 was reported to be insensitive to menthol at any concentration [81]. Characterization of human/mouse, human/fruit fly, and mouse/fruit fly chimeric channels demonstrated that the S5–S6 region of mouse TRPA1 is responsible for channel inhibition at high concentrations. The following site-directed mutagenesis experiments showed that S876 and T877 in S5 of mouse TRPA1 are critical for activation by menthol. These amino acids are conserved in human but not Fugu fish, fruit fly, or mosquito TRPA1 [81]. Meanwhile, G878 in S5 of mouse TRPA1 is critical for channel inhibition at high concentrations, and it is substituted to V (valine) in human TRPA1 [81].

Caffeine was shown to activate mouse TRPA1 but suppress the activation of human TRPA1 [48]. Characterization of human/mouse chimeric channels, as well as site-directed mutagenesis, indicated that M268 in the ARs of mouse TRPA1 has a major role in activation by caffeine. When M268 of mouse TRPA1 was substituted with proline, as is found in human TRPA1 (P267), caffeine did not activate the channel but instead suppressed it [47]. In addition, the reverse mutation (P267M) caused caffeine not to activate but instead suppress human TRPA1, suggesting that amino acids other than M268 are also important for activation of mouse TRPA1 by caffeine. M268 is conserved among rodents, whereas P267 is conserved among primates. Nevertheless, the TRPA1 channels of other species contain different amino acids at this position.

The electrophilic, thioaminal-containing compound, 4-methyl-*N*-[2,2,2-trichloro-1-(4-nitro-phenylsulfanyl)-ethyl]-benzamide (CMP1), covalently modifies a cysteine residue at the equivalent position in rat and human TRPA1 channels; however, it activates rat TRPA1 and suppresses human TRPA1 [9]. This is similar to the effect of caffeine on mouse and human TRPA1 channels as described above. Characterization of human/rat chimeric channels, as well as site-directed mutagenesis, demonstrated that A946 and M949 in S6 of rat TRPA1 are critical for channel activation by CMP1. In contrast, the equivalent residues in human TRPA1 (S943 and I946) determine channel block [9].

A bird repellent, methyl anthranilate (MA), has been shown to activate human, mouse, and chicken TRPA1 channels but not lizard (*Anolis carolinensis*) and weakly frog (*X. tropicalis*) TRPA1 channels [57]. Site-directed mutagenesis demonstrated that R596, T603, and P627 in the linker region between the ARs and S1 of chicken TRPA1 are crucial for activation by MA. These residues are conserved between chicken, mouse, and human TRPA1 channels except that R596 is substituted to K at the equivalent position in mouse and human. The above three amino acid residues are substituted to G594, A601, and A625 in a lizard TRPA1, and G597, V604, and A627 in a frog TRPA1, respectively. Because the reverse mutations in both lizard (G594R, A601T, and A625P) and frog (G597R, V604T, and A627P) TRPA1 channels did not alter their responses to MA, these three amino acids must not be the sole functional determinants [57].

Regarding the temperature sensitivity of TRPA1, the fruit fly, mosquito, silk moth, frog, lizard, snake, and chicken channels were shown to be heat sensitive [21, 28, 57, 59, 62]. However, whether mammalian TRPA1 is activated by heat or cold has been controversial [27, 49, 50]. The most recent report on this issue showed that rodent TRPA1 is cold-activated, whereas primate TRPA1 is insensitive to temperature fluctuation [8]. These results may suggest that the TRPA1 of the common ancestor of Bilateria was heat-sensitive and in some mammals it has become either cold sensitive or temperature-insensitive [57, 59]. G878 in the S5 of rodent TRPA1 (substituted to V875 in human TRPA1) is critical for cold activation since the G878V mutation abolished the cold sensitivity. The same amino acid is also required for inhibiting the channel in the presence of high concentrations of menthol, as mentioned above [81]. The reverse mutation (V875G in human TRPA1) failed to confer cold sensitivity, suggesting that G878 is necessary but not sufficient for cold activation.

Human and rodent TRPV1–TRPV4 are activated by warm and noxious high temperatures [50]; however, frog (*X. tropicalis*) TRPV3 was shown to respond to cold temperatures (<16 °C). Moreover, it is insensitive to some mammalian TRPV3 activators such as camphor, eucalyptol, menthol, vanillin, and eugenol [58]. Nevertheless, the amino acid residues responsible for above differences have not been identified.

Intriguingly, it was recently shown that three single-point mutations in the AR6 of mouse TRPA1 are individually sufficient to make the channel warm-activated without affecting the chemical sensitivity [24]. These results demonstrate that minimal changes in the protein sequence of a TRP channel can dramatically change its temperature sensitivity. This functional plasticity would explain why and how a single ancestral TRP channel has evolved into the current ones with the different chemical and temperature sensitivities in a species-specific manner.

Evolutionary dynamics at the pre-mRNA splicing and transcriptional levels

Many splicing isoforms have been identified for TRP channels [71]. Some of these, for example, function as dominant

negatives [76], and others have different ion permeability [51]. However, the presence of these splicing isoforms has never been systematically compared between different species. Given that species-specific alternative splicing is more common than previously thought [3], splice variants of TRP channels could also be present in a species-specific manner. Three such examples have been reported as discussed below.

Mouse TRPML1 has two isoforms containing different C-terminal cytoplasmic tails (either 55 or 86 amino acids) by alternative splicing, whereas human TRPML1 has only one isoform with a 55 amino acid C-terminal cytoplasmic tail. The unique 86 amino acid C-terminal tail of mouse TRPML1 lacks the lysosomal targeting signal. Therefore, it was suggested that this isoform may not localize to the lysosome [16]. However, the physiological relevance of this mouse-specific isoform is not known.

Mouse *TRPA1* was shown to generate two isoforms: *TRPA1a* and *TRPA1b,* in which the 90-base pair exon 20 is excluded. As a result, TRPA1b lacks 30 amino acids present in the S2 domain and the first intracellular loop of TRPA1a. In contrast to TRPA1a, TRPA1b is insensitive to chemical compounds such as AITC. TRPA1b directly interacts with TRPA1a and increases the expression level of TRPA1a at the plasma membrane, suggesting that it may have a role as a chaperone. Interestingly, rat and human *TRPA1* do not appear to have the equivalent *TRPA1b* isoform [86]. The question of why TRPA1b is specific to mouse remains to be answered.

Vampire bats, but not closely related fruit bats, have pit organs surrounding the nose that are capable of detecting infrared radiation through trigeminal nerve fibers to locate hotspots on warm-blooded prey. Vampire bat trigeminal ganglia (TG) express substantial amounts of a novel short isoform of TRPV1 (TRPV1-S) that lacks 62 amino acids from the carboxyl terminus of the ubiquitous TRPV1 (TRPV1-L). The ratio of TRPV1-S to TRPV1-L in the vampire bat TG is about 1:1, and this ratio is much less in both the vampire bat dorsal root ganglia and the fruit bat TG. Intriguingly, TRPV1-S is activated at lower threshold temperatures than TRPV1-L (30.5±0.7 versus 39.6±0.4 °C in HEK293 cells), and the TRPV1-S/TRPV1-L mixed complex responds to an intermediate threshold temperature of 33.9±1.2 °C. Thus, the specific presence of TRPV1-S in the vampire bat TG allows the bat to detect infrared radiation to locate warm-blooded prey. TRPV1-S synthesis involves the inclusion of a 23-base pair exon (exon 14a) present between exon 14 and exon 15 by alternative splicing. Although the exons 14 and 15 are ubiquitously present in mammals, the exon 14a sequence is specifically present in bats, and in closely related species such as cows, moles, and dogs, but it is absent in rodents or humans. Furthermore, the inclusion of exon 14a in cow and mole TRPV1 is rare (<6 % of total TRPV1), suggesting that its inclusion is highly specific to the vampire bat TG. Thus, the efficient synthesis of TRPV1-S is species- and tissue-specific, and it depends on both *cis*-elements and *trans*-acting factors to regulate the alternative splicing event [20].

TRP channels are expressed in a wide variety of cell types and tissues including neurons [25, 36], and thus, the upstream regulatory sequences (URSs) and promoters must have important roles in determining such specific expression patterns. However, none of the URSs/promoters have been characterized in detail to date. Furthermore, there has been no systematic comparison of the expression patterns of orthologous TRP channels between different species. Considering the physiological roles of TRP, the expression profiles are expected to be similar between closely related species. Nevertheless, mutations in the URSs (*cis*-regulatory elements) are capable of changing the expression patterns of TRP channels without altering the amino acid sequence and, thus, the channel properties. For example, *cis*-regulatory element mutations have been shown to play important roles in the evolution of novel morphological phenotypes [78]. This could also be the case for TRP channels. As mentioned above, TRPA, TRPC, TRPM, TRPML, TRPP, and TRPV are present in unicellular eukaryotes and the non-neuronal cells of primitive animals without nervous systems. This suggests that some of these TRP channels were recruited to neurons to function in sensory perception and other roles upon emergence of nervous systems (co-option). Thus, these TRP channel genes must have gained *cis*-regulatory elements that drive expression in neurons during evolution. One example of evolutionary change at the transcriptional level is that of the silk moth TRPA1, which appears to be ubiquitously expressed in the epidermal cells of various embryonic tissues [62]. In contrast, fruit fly TRPA1 is primarily expressed in the peripheral sensory neurons and brain of the embryo and larva [85]. These observations suggest that silk moth and fruit fly *TRPA1* genes must be under control of different URSs that drive differential expression profiles.

Concluding remarks

It is evident that evolution of metazoan TRP channels is plastic and dynamic in terms of both gene number and amino acid sequence. However, a major question remains: What are the physiological consequences of such changes? Another important question is whether these changes are driven by adaptive evolution. To answer above questions, it is necessary to characterize the channel properties as well as the physiological functions of particular TRP channels. Regarding the plasticity of the amino acid sequences, it is also essential to prove that TRP channel genes are under positive selection through evolutionary analysis. The evolutionary plasticity of TRP channels with respect to alternative splicing or transcriptional control has not yet been fully examined. We still need to

accumulate more data by systematically comparing the alternatively spliced variants and expression patterns of orthologous TRP channels between different species. Given the example of species- and tissue-specific alternative splicing of TRPV1 in vampire bat TG, the evolutionary plasticity of TRP channels at this level could also be quite common.

The key to understanding the evolutionary dynamics of metazoan TRP channels is to learn more about the channels in a variety of animals. Research on TRP has been primarily limited to human and major model organisms so far; however, it will be essential to expand the research to other species. The channel properties of TRP can be studied using a heterologous expression system in HEK293 cells or *Xenopus* oocytes. Additionally, studying the physiological functions in non-model organisms is now feasible using the TALEN [80] or CRISPR [23] systems. Finally, the number of genome sequences available is expected to increase every year so that identification and classification of TRP channels in diverse genomes should also become possible. The above tools would give us an opportunity to answer an important question: How have metazoan TRP channels evolved in association with the specific animal habitat and life history?

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Compliance with ethical standards

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References

1. Audo I, Kohl S, Leroy BP, Munier FL, Guillonneau X, Mohand-Said S, Bujakowska K, Nandrot EF, Lorenz B, Preising M, Kellner U, Renner AB, Bernd A, Antonio A, Moskova-Doumanova V, Lancelot M-E, Poloschek CM, Drumare I, Defoort-Dhellemmes S, Wissinger B, Leveillard T, Hamel CP, Schorderet DF, De Baere E, Berger W, Jacobson SG, Zrenner E, Sahel J-A, Bhattacharya SS, Zeitz C (2009) TRPM1 is mutated in patients with autosomal-recessive complete congenital stationary night blindness. Am J Hum Genet 85:720–729. doi:10.1016/j.ajhg.2009.10.013
2. Auer-Grumbach M, Olschewski A, Papic L, Kremer H, McEntagart ME, Uhrig S, Fischer C, Froehlich E, Balint Z, Tang B, Strohmaier H, Lochmueller H, Schlotter-Weigel B, Senderek J, Krebs A, Dick KJ, Petty R, Longman C, Anderson NE, Padberg GW, Schelhaas HJ, van Ravenswaaij-Arts CMA, Pieber TR, Crosby AH, Guelly C (2010) Alterations in the ankyrin domain of TRPV4 cause congenital distal SMA, scapuloperoneal SMA and HMSN2C. Nat Genet 42:160–U196. doi:10.1038/ng.508
3. Barbosa-Morais NL, Irimia M, Pan Q, Xiong HY, Gueroussov S, Lee LJ, Slobodeniuc V, Kutter C, Watt S, Colak R, Kim T, Misquitta-Ali CM, Wilson MD, Kim PM, Odom DT, Frey BJ, Blencowe BJ (2012) The evolutionary landscape of alternative splicing in vertebrate species. Science 338:1587–1593. doi:10.1126/science.1230612
4. Boenigk J, Arndt H (2002) Bacterivory by heterotrophic flagellates: community structure and feeding strategies. Anton Leeuw Int J Gen Mol Microbiol 81:465–480. doi:10.1023/a:1020509305868
5. Cai X (2008) Unicellular Ca(2+) signaling 'toolkit' at the origin of Metazoa. Mol Biol Evol 25:1357–1361. doi:10.1093/molbev/msn077
6. Cai X, Clapham DE (2012) Ancestral Ca2+ signaling machinery in early animal and fungal evolution. Mol Biol Evol 29:91–100. doi:10.1093/molbev/msr149
7. Cao E, Liao M, Cheng Y, Julius D (2013) TRPV1 structures in distinct conformations reveal activation mechanisms. Nature 504:113. doi:10.1038/nature12823
8. Chen J, Kang D, Xu J, Lake M, Hogan JO, Sun C, Walter K, Yao B, Kim D (2013) Species differences and molecular determinant of TRPA1 cold sensitivity. Nat Commun 4. doi:10.1038/ncomms3501
9. Chen J, Zhang X-F, Kort ME, Huth JR, Sun C, Miesbauer LJ, Cassar SC, Neelands T, Scott VE, Moreland RB, Reilly RM, Hajduk PJ, Kym PR, Hutchins CW, Faltynek CR (2008) Molecular determinants of species-specific activation or blockade of TRPA1 channels. J Neurosci 28:5063–5071. doi:10.1523/jneurosci. 0047-08.2008
10. Colletti GA, Kiselyov K (2011) TRPML1. Transient receptor potential channels. Adv Exp Med Biol 704:209–219. doi:10.1007/978-94-007-0265-3_11
11. Cordero-Morales JF, Gracheva EO, Julius D (2011) Cytoplasmic ankyrin repeats of transient receptor potential A1 (TRPA1) dictate sensitivity to thermal and chemical stimuli. Proc Natl Acad Sci U S A 108:E1184–E1191. doi:10.1073/pnas.1114124108
12. Damann N, Voets T, Nilius B (2008) TRPs in our senses. Curr Biol 18:R880–R889. doi:10.1016/j.cub.2008.07.063
13. Deng H-X, Klein CJ, Yan J, Shi Y, Wu Y, Fecto F, Yau H-J, Yang Y, Zhai H, Siddique N, Hedley-Whyte ET, DeLong R, Martina M, Dyck PJ, Siddique T (2010) Scapuloperoneal spinal muscular atrophy and CMT2C are allelic disorders caused by alterations in TRPV4. Nat Genet 42:165–U102. doi:10.1038/ng.509
14. Denis V, Cyert MS (2002) Internal Ca2+ release in yeast is triggered by hypertonic shock and mediated by a TRP channel homologue. J Cell Biol 156:29–34. doi:10.1083/jcb.200111004
15. Effertz T, Wiek R, Goepfert MC (2011) NompC TRP channel is essential for drosophila sound receptor function. Curr Biol 21:592–597. doi:10.1016/j.cub.2011.02.048
16. Falardeau JL, Kennedy JC, Acierno JS, Sun M, Stahl S, Goldin E, Slaugenhaupt SA (2002) Cloning and characterization of the mouse McolnI gene reveals an alternatively spliced transcript not seen in humans. BMC Genomics 3. doi:10.1186/1471-2164-3-3
17. Gaudet R (2008) A primer on ankyrin repeat function in TRP channels and beyond. Mol BioSyst 4:372–379. doi:10.1039/b801481g
18. Gavva NR, Klionsky L, Qu YS, Shi LC, Tamir R, Edenson S, Zhang TJ, Viswanadhan VN, Toth A, Pearce LV, Vanderah TW, Porreca F, Blumberg PM, Lile J, Sun Y, Wildt K, Louis JC, Treanor JJS (2004) Molecular determinants of vanilloid sensitivity in TRPV1. J Biol Chem 279:20283–20295. doi:10.1074/jbc.M312577200
19. Gong ZF, Son WS, Chung YD, Kim JW, Shin DW, McClung CA, Lee Y, Lee HW, Chang DJ, Kaang BK, Cho HW, Oh U, Hirsh J, Kernan MJ, Kim CS (2004) Two interdependent TRPV channel subunits, inactive and Nanchung, mediate hearing in Drosophila. J Neurosci 24:9059–9066. doi:10.1523/jneurosci. 1645-04.2004
20. Gracheva EO, Cordero-Morales JF, Gonzalez-Carcacia JA, Ingolia NT, Manno C, Aranguren CI, Weissman JS, Julius D (2011)

Ganglion-specific splicing of TRPV1 underlies infrared sensation in vampire bats. Nature 476:88. doi:10.1038/nature10245
21. Gracheva EO, Ingolia NT, Kelly YM, Cordero-Morales JF, Hollopeter G, Chesler AT, Sanchez EE, Perez JC, Weissman JS, Julius D (2010) Molecular basis of infrared detection by snakes. Nature 464:1006–U1066. doi:10.1038/nature08943
22. Hinman A, H-h C, Bautista DM, Julius D (2006) TRP channel activation by reversible covalent modification. Proc Natl Acad Sci U S A 103:19564–19568. doi:10.1073/pnas.0609598103
23. Hsu PD, Lander ES, Zhang F (2014) Development and applications of CRISPR-Cas9 for genome engineering. Cell 157:1262–1278. doi:10.1016/j.cell.2014.05.010
24. Jabba S, Goyal R, Sosa-Pagan JO, Moldenhauer H, Wu J, Kalmeta B, Bandell M, Latorre R, Patapoutian A, Grandl J (2014) Directionality of temperature activation in mouse TRPA1 ion channel can be inverted by single-point mutations in ankyrin repeat six. Neuron 82:1017–1031. doi:10.1016/j.neuron.2014.04.016
25. Jang Y, Lee Y, Kim SM, Yang YD, Jung J, Oh U (2012) Quantitative analysis of TRP channel genes in mouse organs. Arch Pharm Res 35:1823–1830. doi:10.1007/s12272-012-1016-8
26. Jordt SE, Julius D (2002) Molecular basis for species-specific sensitivity to "hot" chili peppers. Cell 108:421–430. doi:10.1016/s0092-8674(02)00637-2
27. Julius D (2013) TRP channels and pain. Annu Rev Cell Dev Biol 29(29):355–384. doi:10.1146/annurev-cellbio-101011-155833
28. Kang K, Panzano VC, Chang EC, Ni L, Dainis AM, Jenkins AM, Regna K, Muskavitch MAT, Garrity PA (2012) Modulation of TRPA1 thermal sensitivity enables sensory discrimination in Drosophila. Nature 481:76–U82. doi:10.1038/nature10715
29. Kang K, Pulver SR, Panzano VC, Chang EC, Griffith LC, Theobald DL, Garrity PA (2010) Analysis of Drosophila TRPA1 reveals an ancient origin for human chemical nociception. Nature 464:597–U155. doi:10.1038/nature08848
30. Karashima Y, Damann N, Prenen J, Talavera K, Segal A, Voets T, Nilius B (2007) Bimodal action of menthol on the transient receptor potential channel TRPA1. J Neurosci 27:9874–9884. doi:10.1523/jneurosci. 2221-07.2007
31. Kim J, Chung YD, Park DY, Choi SK, Shin DW, Soh H, Lee HW, Son W, Yim J, Park CS, Kernan MJ, Kim C (2003) A TRPV family ion channel required for hearing in Drosophila. Nature 424:81–84. doi:10.1038/nature01733
32. Koettgen M (2007) TRPP2 and autosomal dominant polycystic kidney disease. Biochim Biophys Acta Mol basis Dis 1772:836–850. doi:10.1016/j.bbadis.2007.01.003
33. Kohno K, Sokabe T, Tominaga M, Kadowaki T (2010) Honey bee thermal/chemical sensor, AmHsTRPA, reveals neofunctionalization and loss of transient receptor potential channel genes. J Neurosci 30:12219–12229. doi:10.1523/jneurosci. 2001-10.2010
34. Kremeyer B, Lopera F, Cox JJ, Momin A, Rugiero F, Marsh S, Woods CG, Jones NG, Paterson KJ, Fricker FR, Villegas A, Acosta N, Pineda-Trujillo NG, Diego Ramirez J, Zea J, Burley M-W, Bedoya G, Bennett DLH, Wood JN, Ruiz-Linares A (2010) A gain-of-function mutation in TRPA1 causes familial episodic pain syndrome. Neuron 66:671–680. doi:10.1016/j.neuron.2010.04.030
35. Kruse M, Schulze-Bahr E, Corfield V, Beckmann A, Stallmeyer B, Kurtbay G, Ohmert I, Schulze-Bahr E, Brink P, Pongs O (2009) Impaired endocytosis of the ion channel TRPM4 is associated with human progressive familial heart block type I. J Clin Investig 119:2737–2744. doi:10.1172/jci38292
36. Kunert-Keil C, Bisping F, Krueger J, Brinkmeier H (2006) Tissue-specific expression of TRP channel genes in the mouse and its variation in three different mouse strains. BMC Genomics 7. doi:10.1186/1471-2164-7-159
37. Landoure G, Zdebik AA, Martinez TL, Burnett BG, Stanescu HC, Inada H, Shi Y, Taye AA, Kong L, Munns CH, Choo SS, Phelps CB, Paudel R, Houlden H, Ludlow CL, Caterina MJ, Gaudet R, Kleta R, Fischbeck KH, Sumner CJ (2010) Mutations in TRPV4 cause Charcot-Marie-Tooth disease type 2C. Nat Genet 42:170–U109. doi:10.1038/ng.512
38. Lee Y, Lee J, Bang S, Hyun S, Kang J, Hong ST, Bae E, Kaang BK, Kim J (2005) Pyrexia is a new thermal transient receptor potential channel endowing tolerance to high temperatures in Drosophila melanogaster. Nat Genet 37:305–310. doi:10.1038/ng1513
39. Leys SP, Degnan BM (2001) Cytological basis of photoresponsive behavior in a sponge larva. Biol Bull 201:323–338. doi:10.2307/1543611
40. Li Z, Sergouniotis PI, Michaelides M, Mackay DS, Wright GA, Devery S, Moore AT, Holder GE, Robson AG, Webster AR (2009) Recessive mutations of the gene TRPM1 abrogate ON bipolar cell function and cause complete congenital stationary night blindness in humans. Am J Hum Genet 85:711–719. doi:10.1016/j.ajhg.2009.10.003
41. Liao M, Cao E, Julius D, Cheng Y (2013) Structure of the TRPV1 ion channel determined by electron cryo-microscopy. Nature 504:107. doi:10.1038/nature12822
42. Liu L, Li Y, Wang R, Yin C, Dong Q, Hing H, Kim C, Welsh MJ (2007) Drosophila hygrosensation requires the TRP channels water witch and nanchung. Nature 450:294–U214. doi:10.1038/nature06223
43. Ludeman DA, Farrar N, Riesgo A, Paps J, Leys SP (2014) Evolutionary origins of sensation in metazoans: functional evidence for a new sensory organ in sponges. BMC Evol Biol 14:3. doi:10.1186/1471-2148-14-3
44. Macpherson LJ, Dubin AE, Evans MJ, Marr F, Schultz PG, Cravatt BF, Patapoutian A (2007) Noxious compounds activate TRPA1 ion channels through covalent modification of cysteines. Nature 445:541–545. doi:10.1038/nature05544
45. Matsuura H, Sokabe T, Kohno K, Tominaga M, Kadowaki T (2009) Evolutionary conservation and changes in insect TRP channels. BMC Evol Biol 9:228. doi:10.1186/1471-2148-9-228
46. Nadler MJS, Hermosura MC, Inabe K, Perraud AL, Zhu QQ, Stokes AJ, Kurosaki T, Kinet JP, Penner R, Scharenberg AM, Fleig A (2001) LTRPC7 is a Mg center dot ATP-regulated divalent cation channel required for cell viability. Nature 411:590–595. doi:10.1038/35079092
47. Nagatomo K, Ishii H, Yamamoto T, Nakajo K, Kubo Y (2010) The Met268Pro mutation of mouse TRPA1 changes the effect of caffeine from activation to suppression. Biophys J 99:3609–3618. doi:10.1016/j.bpj.2010.10.014
48. Nagatomo K, Kubol Y (2008) Caffeine activates mouse TRPA1 channels but suppresses human TRPA1 channels. Proc Natl Acad Sci U S A 105:17373–17378. doi:10.1073/pnas.0809769105
49. Nilius B, Appendino G, Owsianik G (2012) The transient receptor potential channel TRPA1: from gene to pathophysiology. Arch Eur J Physiol 464:425–458. doi:10.1007/s00424-012-1158-z
50. Nilius B, Owsianik G (2011) The transient receptor potential family of ion channels. Genome Biol 12. doi:10.1186/gb-2011-12-3-218
51. Oberwinkler J, Lis A, Giehl KM, Flockerzi V, Philipp SE (2005) Alternative splicing switches the divalent cation selectivity of TRPM3 channels. J Biol Chem 280:22540–22548. doi:10.1074/jbc.M503092200
52. Ohkita M, Saito S, Imagawa T, Takahashi K, Tominaga M, Ohta T (2012) Molecular cloning and functional characterization of Xenopus tropicalis frog transient receptor potential vanilloid 1 reveal its functional evolution for heat, acid, and capsaicin sensitivities in terrestrial vertebrates. J Biol Chem 287:2388–2397. doi:10.1074/jbc.M111.305698
53. Palmer CP, Zhou XL, Lin JY, Loukin SH, Kung C, Saimi Y (2001) A TRP homolog in Saccharomyces cerevisiae forms an intracellular

Ca2+-permeable channel in the yeast vacuolar membrane. Proc Natl Acad Sci U S A 98:7801–7805. doi:10.1073/pnas.141036198
54. Peng G, Shi X, Kadowaki T (2015) Evolution of TRP channels inferred by their classification in diverse animal species. Mol Phylogenet Evol 84:145–157. doi:10.1016/j.ympev.2014.06.016
55. Reiser J, Polu KR, Moller CC, Kenlan P, Altintas MM, Wei CL, Faul C, Herbert S, Villegas I, Avila-Casado C, McGee M, Sugimoto H, Brown D, Kalluri R, Mundel P, Smith PL, Clapham DE, Pollak MR (2005) TRPC6 is a glomerular slit diaphragm-associated channel required for normal renal function. Nat Genet 37:739–744. doi:10.1038/ng1592
56. Runnels LW, Yue LX, Capham DE (2001) TRP-PLIK, a bifunctional protein with kinase and ion channel activities. Science 291:1043–1047. doi:10.1126/science.1058519
57. Saito S, Banzawa N, Fukuta N, Saito CT, Takahashi K, Imagawa T, Ohta T, Tominaga M (2014) Heat and noxious chemical sensor, chicken TRPA1, as a target of bird repellents and identification of its structural determinants by multispecies functional comparison. Mol Biol Evol 31:708–722. doi:10.1093/molbev/msu001
58. Saito S, Fukuta N, Shingai R, Tominaga M (2011) Evolution of vertebrate transient receptor potential vanilloid 3 channels: opposite temperature sensitivity between mammals and western clawed frogs. PLoS Genet 7. doi:10.1371/journal.pgen.1002041
59. Saito S, Nakatsuka K, Takahashi K, Fukuta N, Imagawa T, Ohta T, Tominaga M (2012) Analysis of Transient Receptor Potential Ankyrin 1 (TRPA1) in frogs and lizards illuminates both nociceptive heat and chemical sensitivities and coexpression with TRP Vanilloid 1 (TRPV1) in ancestral vertebrates. J Biol Chem 287:30743–30754. doi:10.1074/jbc.M112.362194
60. Saito S, Tominaga M (2015) Functional diversity and evolutionary dynamics of thermoTRP channels. Cell Calcium 57:214–221. doi:10.1016/j.ceca.2014.12.001
61. Sakai T, Kasuya J, Kitamoto T, Aigaki T (2009) The Drosophila TRPA channel, painless, regulates sexual receptivity in virgin females. Genes Brain Behav 8:546–557. doi:10.1111/j.1601-183X.2009.00503.x
62. Sato A, Sokabe T, Kashio M, Yasukochi Y, Tominaga M, Shiomi K (2014) Embryonic thermosensitive TRPA1 determines transgenerational diapause phenotype of the silkworm, Bombyx mori. Proc Natl Acad Sci U S A 111:E1249–E1255. doi:10.1073/pnas.1322134111
63. Sidi S, Friedrich RW, Nicolson T (2003) NompC TRP channel required for vertebrate sensory hair cell mechanotransduction. Science 301:96–99. doi:10.1126/science.1084370
64. Sokabe T, Tsujiuchi S, Kadowaki T, Tominaga M (2008) Drosophila Painless is a Ca2+ -requiring channel activated by noxious heat. J Neurosci 28:9929–9938. doi:10.1523/jneurosci. 2757-08.2008
65. Srivastava M, Begovic E, Chapman J, Putnam NH, Hellsten U, Kawashima T, Kuo A, Mitros T, Salamov A, Carpenter ML, Signorovitch AY, Moreno MA, Kamm K, Grimwood J, Schmutz J, Shapiro H, Grigoriev IV, Buss LW, Schierwater B, Dellaporta SL, Rokhsar DS (2008) The Trichoplax genome and the nature of placozoans. Nature 454:955–U919. doi:10.1038/nature07191
66. Srivastava M, Simakov O, Chapman J, Fahey B, Gauthier MEA, Mitros T, Richards GS, Conaco C, Dacre M, Hellsten U, Larroux C, Putnam NH, Stanke M, Adamska M, Darling A, Degnan SM, Oakley TH, Plachetzki DC, Zhai Y, Adamski M, Calcino A, Cummins SF, Goodstein DM, Harris C, Jackson DJ, Leys SP, Shu S, Woodcroft BJ, Vervoort M, Kosik KS, Manning G, Degnan BM, Rokhsar DS (2010) The Amphimedon queenslandica genome and the evolution of animal complexity. Nature 466:720–U723. doi:10.1038/nature09201
67. Sun Y, Liu L, Ben-Shahar Y, Jacobs JS, Eberl DF, Welsh MJ (2009) TRPA channels distinguish gravity sensing from hearing in Johnston's organ. Proc Natl Acad Sci U S A 106:13606–13611. doi:10.1073/pnas.0906377106
68. Tang X, Platt MD, Lagnese CM, Leslie JR, Hamada FN (2013) Temperature integration at the AC thermosensory neurons in Drosophila. J Neurosci 33:894–901. doi:10.1523/jneurosci. 1894-12.2013
69. Tracey WD, Wilson RI, Laurent G, Benzer S (2003) Painless, a Drosophila gene essential for nociception. Cell 113:261–273. doi:10.1016/s0092-8674(03)00272-1
70. van Genderen MM, Bijveld MMC, Claassen YB, Florijn RJ, Pearring JN, Meire FM, McCall MA, Riemslag FCC, Gregg RG, Bergen AAB, Kamermans M (2009) Mutations in TRPM1 are a common cause of complete congenital stationary night blindness. Am J Hum Genet 85:730–736. doi:10.1016/j.ajhg.2009.10.012
71. Vazquez E, Valverde MA (2006) A review of TRP channels splicing. Semin Cell Dev Biol 17:607–617. doi:10.1016/j.semcdb.2006.11.004
72. Venkatachalam K, Montell C (2007) TRP channels. Annu Rev Biochem 76:387–417. doi:10.1146/annurev.biochem.75.103004.142819
73. Voets T, Owsianik G, Janssens A, Talavera K, Nilius B (2007) TRPM8 voltage sensor mutants reveal a mechanism for integrating thermal and chemical stimuli. Nat Chem Biol 3:174–182. doi:10.1038/nchembio862
74. Vriens J, Appendino G, Nilius B (2009) Pharmacology of vanilloid transient receptor potential cation channels. Mol Pharmacol 75:1262–1279. doi:10.1124/mol.109.055624
75. Walder RY, Landau D, Meyer P, Shalev H, Tsolia M, Borochowitz Z, Boettger MB, Beck GE, Englehardt RK, Carmi R, Sheffield VC (2002) Mutation of TRPM6 causes familial hypomagnesemia with secondary hypocalcemia. Nat Genet 31:171–174. doi:10.1038/ng901
76. Wang CB, Hu HZ, Colton CK, Wood JD, Zhu MX (2004) An alternative splicing product of the murine trpv1 gene dominant negatively modulates the activity of TRPV1 channels. J Biol Chem 279:37423–37430. doi:10.1074/jbc.M407205200
77. Winn MP, Conlon PJ, Lynn KL, Farrington MK, Creazzo T, Hawkins AF, Daskalakis N, Kwan SY, Ebersviller S, Burchette JL, Pericak-Vance MA, Howel DN, Vance JM, Rosenberg PB (2005) A mutation in the TRPC6 cation channel causes familial focal segmental glomerulosclerosis. Science 308:1801–1804. doi:10.1126/science.1106215
78. Wittkopp PJ, Kalay G (2012) Cis-regulatory elements: molecular mechanisms and evolutionary processes underlying divergence. Nat Rev Genet 13:59–69. doi:10.1038/nrg3095
79. Wolfgang W, Simoni A, Gentile C, Stanewsky R (2013) The Pyrexia transient receptor potential channel mediates circadian clock synchronization to low temperature cycles in Drosophila melanogaster. Proc R Soc B Biol Sci 280. doi:10.1098/rspb.2013.0959
80. Wright DA, Li T, Yang B, Spalding MH (2014) TALEN-mediated genome editing: prospects and perspectives. Biochem J 462:15–24. doi:10.1042/bj20140295
81. Xiao B, Dubin AE, Bursulaya B, Viswanath V, Jegla TJ, Patapoutian A (2008) Identification of transmembrane domain 5 as a critical molecular determinant of menthol sensitivity in mammalian TRPA1 channels. J Neurosci 28:9640–9651. doi:10.1523/jneurosci. 2772-08.2008
82. Xu J, Sornborger AT, Lee JK, Shen P (2008) Drosophila TRPA channel modulates sugar-stimulated neural excitation, avoidance and social response. Nat Neurosci 11:676–682. doi:10.1038/nn.2119
83. Yan Z, Zhang W, He Y, Gorczyca D, Xiang Y, Cheng LE, Meltzer S, Jan LY, Jan YN (2013) Drosophila NOMPC is a mechanotransduction channel subunit for gentle-touch sensation. Nature 493:221–225. doi:10.1038/nature11685

84. Zeevi DA, Frumkin A, Bach G (2007) TRPML and lysosomal function. Biochim Biophys Acta Mol basis Dis 1772:851–858. doi:10.1016/j.bbadis.2007.01.004
85. Zhong L, Bellemer A, Yan H, Honjo K, Robertson J, Hwang RY, Pitt GS, Tracey WD (2012) Thermosensory and nonthermosensory isoforms of Drosophila melanogaster TRPA1 Reveal heat-sensor domains of a ThermoTRP channel. Cell Rep 1:43–55. doi:10.1016/j.celrep.2011.11.002
86. Zhou Y, Suzuki Y, Uchida K, Tominaga M (2013) Identification of a splice variant of mouse TRPA1 that regulates TRPA1 activity. Nat Commun 4. doi:10.1038/ncomms3399