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Evolution of TRP channels inferred by their classification in diverse animal species

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ABSTRACT

The functions of TRP channels have primarily been characterized in model organisms within a limited evolutionary context. We thus characterize the TRP channels in choanoflagellate, sponge, Cnidaria, Lophotrochozoa, and arthropods to understand how they emerged during early evolution of animals and have changed during diversification of various species. As previously reported, five metazoan TRP subfamily members (TRPA, TRPC, TRPM, TRPML, and TRPV) were identified in choanoflagellates, demonstrating that they evolved before the emergence of multicellular animals. TRPN was identified in *Hydra magnipapillata*, and therefore emerged in the last common ancestor of Cnidaria–Bilateria. A novel subfamily member (TRPVL) was identified in Cnidaria and *Capitella teleta*, indicating that it was present in the last common ancestor of Cnidaria–Bilateria but has since been lost in most bilaterians. The characterization of arthropod TRP channels revealed that *Daphnia pulex* and insects have specifically expanded the TRPA subfamily, which diverged from the ancient TRPA1 channel gene. The diversity of TRPA channels except TRPA1 was detectable even within a single insect family, namely the ant lineage. The present study demonstrates the evolutionary history of TRP channel genes, which may have diverged in conjunction with the specific habitats and life histories of individual species.

1. Introduction

Transient receptor potential (TRP) superfamily channels share six common transmembrane segments that form sensor and pore domains and confer cation permeability. However, TRP channels are unusual among various ion channels by displaying diverse cation selectivities and activation mechanisms (Venkatachalam and Montell, 2007). For this reason, TRP channels play major roles in a variety of sensory modalities such as vision, thermosensation, olfaction, hearing, and mechanosensation by functioning as primary signal integrators that allow animals to perceive the external environment (Damann et al., 2008). In fact, TRP channel gene was first identified in *Drosophila melanogaster* to be essential for the full visual activity (Montell and Rubin, 1989), and later it was proposed to encode a light-activated calcium channel in the photoreceptor cells (Hardie and Minke, 1992). TRP channels also enable individual cells to detect changes such as osmolarity and fluid flow in their local environment (Venkatachalam and Montell, 2007; Nilius and Owsianik, 2011). TRP channels thus play essential roles for several physiological processes; sensory functions, homeostatic functions, and motile functions such as muscle contraction.

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. Most of functional TRP channels are considered to form homotetramers, for example, TRPV1 (Moiseenkova-Bell et al., 2008). The recent structural characterization of TRPV1 by electron cryo-microscopy has revealed that TRPV1 and voltage-gated ion channels (VGICs) share similar overall structure; however, the opening of TRPV1 associates with the major structural changes in outer pore as well as the dilation of lower gate, and in contrast to VGICs, the S1–S4 voltage-sensor-like domain does not appear to move (Liao et al., 2013; Cao et al., 2013). N-and C-termini (both facing cytosol) are variable in length and contain different domains. 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: 14–19 in TRPA, 3–4 in TRPC, 29 in TRPN, and 6 in TRPV channels. ARs appear to be necessary for tetramerization of the channel and interactions with ligands and protein partners (Gaudet, 2008). Highly conserved domain of 23–25 amino acids (TRP domain) is present at C-terminal to the transmembrane segments in TRPC, TRPM, and TRPN channels. Other functional

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domains are found in the C-terminal tails of TRP channels, for example, TRPM6 and TRPM7 contain an atypical α-kinase domain involved in regulating the channel function (Runnels et al., 2001; Nadler et al., 2001). Nevertheless, many functional domains are often not conserved even between members in the same subfamily.

Many TRPA and TRPV channels are activated by temperature fluctuations and various ligands, and thus function in thermosensation (for cold and hot temperatures) and chemoreception (Nilius et al., 2012; Vriens et al., 2009). *D. melanogaster* TRPV channels also play roles in hearing and hygrosensation probably by mechanical activation (Kim et al., 2003; Gong et al., 2004; Liu et al., 2007). *D. melanogaster* TRPN has been recently shown to be a mechano-sensitive channel (Yan et al., 2013) and involved in hearing and mechanosensation (Sidi et al., 2003; Effertz et al., 2011). TRPC channels function in signal transduction in neurons and other cell types (Venkatachalam and Montell, 2007; Nilius and Owsianik, 2011); TRPM channels are involved in signal transduction, chemoreception, and the thermosensation of cold temperatures (Venkatachalam and Montell, 2007; Nilius and Owsianik, 2011; Voets et al., 2007). TRPML channels are important for endosomal/lysosomal function and autophagy (Zeevi et al., 2007), and TRPP channels play critical roles in cardiac, skeletal, and renal development as well as in spermatogenesis (Venkatachalam and Montell, 2007; Nilius and Owsianik, 2011). TRPP3 forms a complex with another TRPP channel, PKD_1L_3, and this complex is likely to function as a sour-sensor activated by acidic pH (Yu et al., 2012). Of course, these physiological functions have primarily been characterized in genetically tractable model organisms, mouse, fruit fly, and nematode.

Since TRP channels are involved in a wide range of physiological processes as mentioned above, the 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 the loss of function impairs endosomal/lysosomal function and autophagy (Colletti and Kiselyov, 2011). Polycystic kidney disease (PKD), the most common inherited kidney disease, is associated with a mutation in *TRPP2*. PKD is shown to develop the large epithelial-lined cysts filled with fluid and occupy most of the mass of abnormally enlarged kidneys resulting in the impaired functions (Köttgen, 2007).

Given the critical physiological and cellular functions of TRP channels, it is not surprising that they are highly conserved between yeast and mammals. Nevertheless, TRP channel genes appear to be absent in plant genomes. *Saccharomyces cerevisiae* TrpY1 is a vacuolar membrane protein which functions as a mechano-sensor of vacuolar osmotic pressure (Palmer et al., 2001; Denis and Cyert, 2002). Because TrpY1 does not cluster with any of metazoan TRP channels by the phylogenetic analysis (Cai and Clapham, 2012), it specifically emerged in fungi after separating from metazoan ancestor. Previous study showed the apusozoan protist *Thecamonas trahens*, a sister species of common ancestor of Holozoa and fungi contained TRPP and TRPV (Cai and Clapham, 2012), suggesting that they could be the most ancient metazoan-type TRP channels. Furthermore, two choanoflagellates (*Monosiga brevicollis* and *Salpingoeca rosetta*) have five TRP subfamilies, TRPA, TRPC, TRPM, TRPML, and TRPV (Cai, 2008; Cai and Clapham, 2012), demonstrating that most of metazoan-type TRP channels emerged in the unicellular common ancestor of all Metazoa. However, comparative genomics and evolutionary studies of TRP channels are still limited. We therefore performed comparative genomics of metazoan TRP channels in choanoflagellate, sponge, Placozoan, Cnidarian (sea anemone and *Hydra magnipapillata*), Lophotrochozoan (mollusc and annelid), and Arthropod including insects (29 species in total). Analysis with choanoflagellate, sponge, Placozoan, and Cnidarian should give us insights into how TRP channels emerged during early evolution of animals. The data with Lophotrochozoan and Arthropod should reveal how TRP channels have changed during diversification of animal species. We will discuss about the evolutionary conservation and plasticity of metazoan TRP channels based on their identification and classification with above organisms.

2. Materials and methods

2.1. Identification and phylogenetic characterization of TRP channels

The amino acid sequences of 13 *D. melanogaster* and 28 mouse TRP channels were retrieved from FlyBase (http://flybase.org/) and NCBI protein database (http://www.ncbi.nlm.nih.gov/protein), respectively, and used as queries to identify TRP channel genes of all organisms shown in Tables 1–3 through a TBLASTN search with the cut-off E-value of 1E-10 against the genomic scaffold and gene model DNA sequences. The genome databases used were JGI (http://www.jgi.doe.gov/, for *M. brevicollis*, *Trichoplax adhaerens*, *Nematostella vectensis*, *Lottia gigantea*, *Capitella teleta*, and *Daphnia pulex*), NCBI (http://www.ncbi.nlm.nih.gov/, for *Amphimedon queenslandica*, *H. magnipapillata*, *Metaseiulus occidentalis*, *Bombus terrestris*, *Megachile rotundata*, *Tribolium castaneum*, *Nasonia vitripennis*, *Apis mellifera*, and *Acyrthosiphon pisum*), Silkworm Genome Research Program (http://sgp.dna.affrc.go.jp/index.html, for *Bombyx mori*), BCM (http://www.hgsc.bcm.tmc.edu/, for *T. castaneum*, *N. vitripennis*, and *Strigamia maritime*), BeeBase (Munoz-Torres et al., 2011, http://hymenopteragenome.org/beebase/, for *A. mellifera*), AphidBase (http://www.aphidbase.com/aphidbase/, for *A. pisum*), VectorBase (https://www.vectorbase.org/, for *Pediculus humanus* and *Ixodes scapularis*), Bioinformatics & Evolutionary Genomics (http://bioinformatics.psb.ugent.be/genomes/, for *Tetranychus urticae*), and Ant Genomes Portal (Munoz-Torres et al., 2011, http://hymenopteragenome.org/ant_genomes/, for *Harpegnathos saltator*, *Linepithema humile*, *Camponotus floridanus*, *Pogonomyrmex barbatus*, *Solenopsis invicta*, *Atta cephalotes*, and *Acromyrmex echinatior*).

In order to classify the identified TRP channels into different subfamilies, the amino acid sequences corresponding to six transmembrane ion transport segments of each TRP channel were phylogenetically characterized. These domains were identified by an InterProScan (http://www.ebi.ac.uk/Tools/InterProScan/) search. The lengths of retrieved amino acid sequences varied between 127 (*B. mori* TRPV, Nanchung) to 355 (Mouse TRPM4). The amino acid sequences of ion transport segments were aligned by the MUSCLE program (Edgar, 2004), and the aligned sequences were trimmed using the trimA1 tool (Capella-Gutierrez et al., 2009). MEGA5 allowed us to evaluate the fit of total 48 models for the amino acid substitutions (Tamura et al., 2011), and the goodness-of-fit of each model to the data was measured by the Bayesian information criterion (BIC). The evolutionary tree by the Neighbor-Joining (NJ) algorithm that uses a matrix of pairwise distances estimated under the Jones–Thornton–Taylor (JTT) model was used to evaluate the fit of substitution models to the data as a default setting. The model with the lowest BIC score was considered to describe the substitution pattern the best, and a WAG + F + G amino acid substitution model was selected for each phylogenetic tree (Figs. 1–5). Phylogenetic trees were inferred from the aligned sequences using the maximum likelihood method and a bootstrap value of 1,000 replicates with MEGA5 (Tamura et al., 2011). To classify TRPN subfamily members, *Caenorhabditis elegans* TRPN (CeTRP-4) and *Danio rerio* TRPN (DrTRPN1) were also included in the analysis. Each phylogenetic tree was rooted with the amino