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Influence of membrane cholesterol in the molecular evolution and functional regulation of TRPV4

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ABSTRACT

TRPV4 is involved in several physiological and sensory functions as well as with several diseases and genetic disorders, though the molecular mechanisms for these are unclear. In this work we have analyzed molecular evolution and structure–function relationship of TRPV4 using sequences from different species. TRPV4 has evolved during early vertebrate origin (450 million years). Synteny analysis confirms that TRPV4 has coevolved with two enzymes involved in sterol biosynthesis, namely MVK and GLTP. Cholesterol-recognizing motifs are present within highly conserved TM4–Loop4–TM5 region of TRPV4. TRPV4 is present in lipid raft where it co-localizes with Caveolin1 and Filipin. TM4–Loop4–TM5 region as well as Loop4 alone can physically interact with cholesterol, its precursor mevalonate and derivatives such as stigmasterol and aldosterone. Mobility of TRPV4-GFP depends on membrane cholesterol level. Molecular evolution of TRPV4 shared striking parallelism with the cholesterol bio-synthesis pathways at the genetic, molecular and metabolic levels. We conclude that interaction with sterols and cholesterol-dependent membrane dynamics have influence on TRPV4 function. These results may have importance on TRPV4-medaited cellular functions and pathophysiology.

1. Introduction

TRPs represent a group of non-selective channels that are permeable to different cations. Among all, TRPV4 is unique as it is activated by several physical and chemical stimuli such as temperature, mechanical pressure, osmolarity, infrared, and compounds like vanilloids, 4αPDD, Apigenin, dimethylallyl pyrophosphate and PUFAs [1–4]. A common aspect of TRPV4-specific agonists is their high hydrophobicity, suggesting that these compounds primarily act on the transmembrane regions. Membrane deformation by stretch too causes rapid activation of TRPV4 [5]. All these suggest that TRPV4 function is dependent on the biochemical composition, structure and by the biophysical nature of the membrane. TRPV4 is specifically present in the cholesterol-enriched detergent-resistant membrane fraction (lipid raft) [6]. At the molecular level, TRPV4 forms signaling complex, which includes membranous components and sub-membranous cytoskeleton [7]. Such complexes are critical for proper function and regulation.

TRPV4 is present in several animals and is involved in detection of different physical and chemical stimuli. The primary function of TRPV4 remains conserved across different species. For example, hTRPV4 (but not hTRPV1) can rescue the defects in transduction of osmotic and mechanical stimuli in *osm-9* (but not *ocr-2*) mutants in *Caenorhabditis elegans* and low sequence similarities between hTRPV4 with *osm-9* suggests that smaller regions are sufficient to perform key tasks [8,9]. TRPV4-mediated sensory functions significantly contribute to the natural selection in specific habitats which favor organism's survival as fittest. Therefore, TRPV4 might have influence on the adaptation, speciation and evolution of different species, especially in response to certain selection pressure where the above mentioned sensory processes are involved. Indeed, TRPV4 can regulate certain behaviors that are linked with adaptation [8]. In this work we tested the molecular evolution of TRPV4 *per se*. TRPV4 physically interacts with sterols and some of its functions are dependent on availability of the cholesterol. We demonstrate that molecular evolution of TRPV4 has

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been influenced by the cholesterol-biosynthesis pathway, an unexpected finding that may also explain the molecular mechanism of TRPV4.

2. Materials and methods

2.1. Sequence retrieval and protein sequence analysis

All TRPV4 sequences were retrieved either from Ensembl or NCBI database (provided in Tab:S1). These protein sequences were aligned by using MUSCLE alignment tool with its default parameters within MEGA5 software suite. Histone H4 and Cytochrome C sequences were retrieved from ENSEMBL and NCBI databases as described previously [10].

2.2. Fragmentation of TRPV4 into different domains and motifs

Conservation of different domains and motifs of TRPV4 were analyzed separately (Table 1). In all cases, we used the hTRPV4 sequence (ENSP00000261740) as template. MUSCLE software was used to align and find out the respective regions present in other species. Distance Matrix generation and the statistical tests using "R" software was done as described before [10].

2.3. Phylogenetic analysis

MUSCLE alignment program was used to align the amino acid sequences for the purpose of phylogenetic analysis. The Bayesian phylogenetic tree was constructed by the Bayesian approach (5 runs, 7,500,000 generations, 25% burn-in-period, WAG-matrix-based model in the MrBayes 3.2 program.

2.4. Calculation of evolutionary time

The sequences among different classes were compared and number of changes of amino acids/100 amino acids was calculated by comparing birds with reptiles, fish with reptiles and reptiles with mammals for available TRPV1 and TRPV4 sequences. The hTRPV is considered as the most recent one (considered as 0 MY). The average changes were calculated and radiations of mammalian TRPV4 sequences were plotted against million years.

Table 1
Description of different domains and motifs.

Region	Location (amino acid number)	References
N-terminal	1–470	[9]
C-Terminal	732–871	[9]
Ank-1	149–189	[11,12]
Ank-2	190–236	[11,12]
Ank-3	237–283	[11,12]
Ank-4	284–319	[11,12]
Ank-5	320–368	[11,12]
Ank-6	369–396	[11,12]
TM-1	471–488	[9]
Loop-1	489–512	[9]
TM-2	513–530	[9]
Loop-2	531–550	[9]
TM-3	551–575	[9]
Loop-3	576–579	[9]
TM-4	580–597	[9]
Loop-4	598–614	[9]
Cholesterol binding domain	610–626	[13]
TM-5	615–632	[9]
Loop-5	633–665	[9]
Pore region	666–683	[9]
Loop-6	684–694	[9]
TM-6	695–731	[9]
TRP-box	732–737	[14]
Cam	812–831	[15]

2.5. Synteny analysis

We utilized Ensembl genome browser for building synteny of TRPV4 loci from selected vertebrate genomes. We examined *Xenopus tropicalis* genome using JGI genome browser.

2.6. Cell culture and cholesterol reduction/depletion

F11 cells were grown in Ham's F12 media supplemented with 10% FBS (HiMedia) as described before [7]. For cholesterol depletion/reduction, cells were maintained in serum-free media for 24 h and 1 μM pravastatin (Sigma–Aldrich) was added 12 h before cell fixing by 4% PFA. In certain cases βMCD (5 mM) (Sigma–Aldrich) was added to reduce membrane cholesterol 15 min before fixing or performing FRAP experiments.

2.7. Caveolin1 and Filipin staining

Cells were seeded into a 24 well plate and TRPV4-GFP was expressed as described before [7]. Cells were fixed 36 h after transfection and immunostained with mouse monoclonal anti-Caveolin-1 antibody (Sigma–Aldrich; 1:250) and subsequently with anti-mouse Alexa-fluor-594-conjugated secondary antibody (1:500). For visualization of the cholesterol directly, fixed cells were probed by Flilipin (Sigma–Aldrich). Cells were imaged by confocal microscope (LSM780, Zeiss) with a 63× oil immersion objective (1.4 NA). Images were processed using LSM software (Zeiss) and Adobe Photoshop.

2.8. FRAP

F11 cells were grown on a glass coverslip and hTRPV4-GFP was expressed by transient transfection. Around 36 h after transfection, the cells were used for FRAP experiments. In each case, at least 50 ROI values are measured.

2.9. Cloning of hTRPV4 fragments, protein expression-purification and dot-blot assay

Different regions of hTRPV4 were cloned into the BamHI and SalI site of pGEX-6P1 vector, expressed in *Escherichia coli* by IPTG induction and purified further. The purified proteins were normalized for molar concentrations and used for blot-overlay experiments with mevalonate, cholesterol, stigmasterol and aldosterone (Sigma Aldrich and Avantipolar).

3. Results

3.1. TRPV4 has evolved during Silurian era

We reconstructed the phylogenetic history of vertebrate TRPV4 using Bayesian phylogenetic method (Fig. 1A). TRPV4-mediated functions in *C. elegans* can be rescued by hTRPV4, suggesting that certain functional features of TRPV4 are conserved throughout the evolution [16]. However, hTRPV4 protein shares less identity (~20%) and homology (~36%) with Osm9 (TRPV4 homologue in *C. elegans*). Similarly, NAN (homologue from *Drosophila*) also shares less homology with hTRPV4. Invertebrate homologues show several insertions and deletions (indels) (Fig:S1). In contrast, TRPV4 sequences are well conserved in vertebrates (Fig. 1A). TRPV4 protein from human and zebrafish share 68% identity and 79% homology. Notably, frog genome have six copies (named as TRPV4a–f) sharing more than 70% identity with each other and these paralogs are branched out separately from TRPV4 orthologs (TRPV4a) in Bayesian phylogenic tree (Fig. 1A). Our analysis suggests that

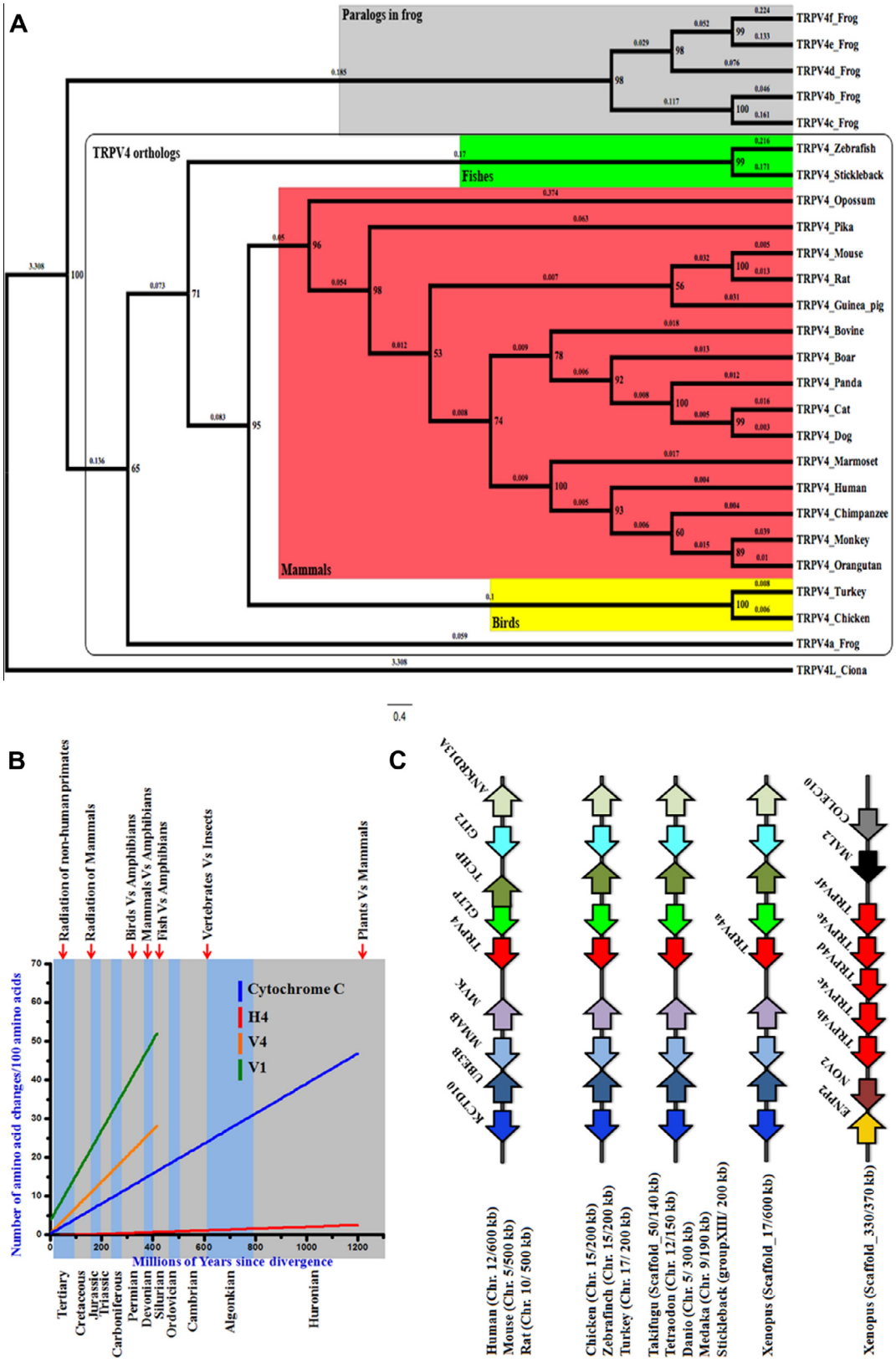


Fig. 1. Molecular evolution of TRPV4. (A) Bayesian phylogenetic tree illustrates six duplicates of TRPV4 in frogs (one ortholog: TRPV4a and five paralogs: TRPV4b–f, indicated in gray) while a single copy is maintained in other vertebrates (mammals: red; birds: yellow, fishes: green). This tree was generated using MrBayes 3.2 and the percentage posterior probabilities are marked at the node of the branches while mean branch length is marked in decimal on the respective branch. Putative TRPV4-like (TRPV4L) gene (gw1.02q.1264.1) from *Ciona intestinalis* served as "out-group-control". (B) Conservation analysis of TRPV4. Histone-H4, Cytochrome-C and TRPV1 were used as controls. (C) Two genes (MVK and GLTP) involved in cholesterol biosynthesis pathway have coevolved with TRPV4. This locus has maintained its organization since development of vertebrates (450 MYA). Another locus in *Xenopus* contains five paralogous TRPV4 originated by tandem duplication. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

TRPV4 share high homology and identity during vertebrate evolution.

Next we calculated the changes in the number of amino acids per 100 amino acids in full-length TRPV4 from different species [10]. We excluded the sequences from nematode and insects (~36% and ~33% homology with hTRPV4 respectively) due to low sequence homology. TRPV4 has originated at point of vertebrate emergence; ca 450 MYA (during the transition of Silurian from Devonian era) (Fig. 1B). TRPV4 is less conserved than histone-H4 (highly conserved) and Cytochrome-C (semi-conserved) [10]. Similar comparison indicates that TRPV1 and TRPV4 have been selected via different selection pressure during vertebrate evolution (Fig. 1B) [10].

3.2. TRPV4 and cholesterol biosynthesis pathway have coevolved

We analyzed syntenic organization in different vertebrate genomes. TRPV4 and mevalonate kinase (MVK) genes are clustered into head-to-head orientation flanked by triad of potassium channel tetramerization domain containing 10 (KCTD10), ubiquitin protein ligase E3B (UBE3B) and methylmalonic aciduria (cobalamin deficiency) cblB type (MMAB) on one side and a tetrad of glycolipid transfer protein (GLTP), trichoplein, keratin filament binding (TCHP), G-protein-coupled receptor kinase interacting ArfGAP 2 (GIT2) and ankyrin repeat domain 13A (ANKRD13A) in human (chr12/600 kb) (Fig. 1C). This genomic architecture is conserved in mammals (mouse: chr5/500 kb; rat: chr10/500 kb); in birds (chicken: chr15/200 kb; zebrafinch: chr15/200 kb; turkey: chr17/200 kb); in fishes (*Takifugu*: scaffold_50/140 kb, *Tetraodon*: chr12/150 kb, *Danio*: chr5/300 kb, Medaka: chr9/190 kb, Stickleback: group_XIII/200 kb), in amphibians (*Xenopus*: scaffold_17/600 kb with single TRPV4 i.e. TRPV4a). However, there is another cluster in frogs (scaffold_330/370 kb containing five paralogous (TRPV4b–f), flanked by a diad of ectonucleotide pyrophosphatase/phosphodiesterase 2 (ENPP2) and nephroblastoma overexpressed gene 2 (NOV2) in one side and a diad of T-cell differentiation protein 2 (MAL2) and C-type lectin, collectin sub-family member 10 (COLEC10) on the other side. Noteworthy point of this analysis is that TRPV4 is located between two genes (MVK and GLTP) involved in cholesterol biosynthesis and this synteny is maintained throughout vertebrate evolution (since 450 MY).

3.3. Different regions of TRPV4 have evolved through different selection pressure

To test the conservation of TRPV4 throughout the evolution, we have compared the sequences from different vertebrates and used statistical approach to quantify it. Analysis revealed that TRPV4 is conserved throughout vertebrate evolution ($p \leqslant 0.0001$; 17 species) (Fig. 2A). We compared separately the conservation of different domains, motifs and functional regions present in TRPV4 (Table 1) [9,11–15]. This reveals that functionally and structurally important regions of TRPV4 are conserved, though at different levels (Fig. 2A). Among all, Loop3, TM4, Loop4, TM5 and TRP-box reveal maximum conservation. Among all TM, the TM4 and TM5 are more conserved. The TM4 reveals highest conservation indicating the importance of this region in the channel function. The TM2 is the least conserved indicating that TM4 and TM5 regions are more important for the functional purpose. Among all ankyrin repeat domains, ARD3 is most and ARD1 is least conserved. Our results are in agreement with another report suggesting that the TM regions are conserved [17]. This result fits well with the identification of several deleterious mutations that are located in two regions, namely within the ARD3 and TM4–Loop4–TM5 of hTRPV4 [18]. In this regard, F592L, R594H, F596P, G600W, Y602C, I604M, R616Q, F617L, L618P, V620I, and M625I are important as these mutations cause pathophysiological disorders corroborating the importance of TM4–Loop–TM5 region (where all these mutations are clustered) of TRPV4 [19].

3.4. TM4–Loop4-TM5 of TRPV4 contains cholesterol recognition motifs

Since AA 576–632 is highly conserved in all vertebrates, we explored the significance of this region. As this region mainly represent the membrane-spanning helices and loops, we predicted that this region might be involved in interaction with lipids present in the membrane and/or involved in channel function such as channel gating. Indeed, this region contains sequence (KDLFRFLL, in hTRPV4) that represent a cholesterol-binding CRAC-like motif (L/V-$X_{(1-5)}$-Y-$X_{(1-5)}$-K/R) and spanning through Loop4–TM5 region (Y is replaced by F) [20]. This motif is conserved strictly in all other species including all 6 variants of *Xenopus* TRPV4 and thus suggest for a positive selection (Fig. 2B and C, Fig:S2). The motif present in the TM4-Loop4-TM5 of TRPV4 matches well with the reported CRAC-like motif present in TRPV1 in inverted order [13]. Similarly, there is an inverted CRAC-motif (LTGTYSIMIQK) present in the TM4-Loop4 (upstream of the CRAC-like motif) which is highly conserved in all vertebrates.

3.5. TRPV4 is present in the cholesterol enriched lipid rafts

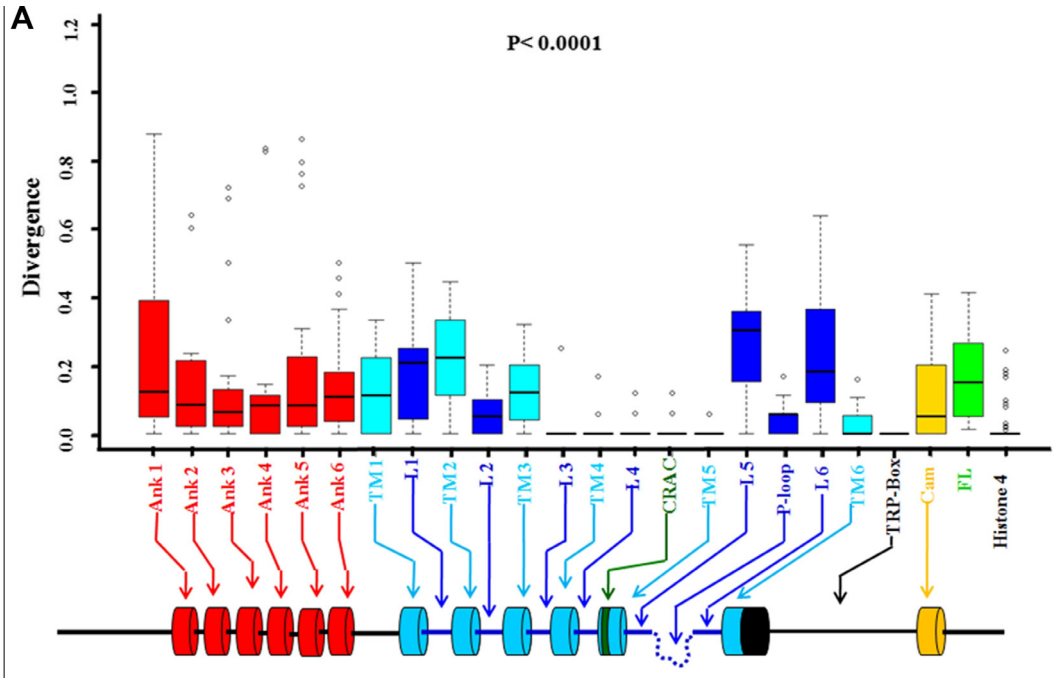
We expressed hTRPV4-GFP in F11 cells and stained for lipid raft markers. We noted that TRPV4 co-localizes with Caveolin-1 (Fig. 3A). However, after cholesterol depletion by βMCD, or by Pravastatin only (8 h) or by both, Caveolin-1 as well as TRPV4 reveal altered distribution, membrane clustering and much lesser colocalization (not shown). Still, some of the TRPV4 clusters remain intact and colocalized with Caveolin1. This indicates that TRPV4-enriched clusters can retain some cholesterol and resist complete cholesterol depletion in certain patches (data not shown). To confirm these results by another independent manner, we stained hTRPV4-GFP expressing cells with Flilipin, which directly detect cholesterol. We observed colocalization of TRPV4-GFP with Flilipin (Fig. 3B). Similarly, reduction of membrane cholesterol by βMCD and/or by Pravastatin (long-term treatment) results in low or no colocalization at all (Fig. 3B).

3.6. Loop4 alone or along with nearby helices is sufficient for physical interaction with cholesterol, its precursors and derivatives

To test, if the CRAC- and CRAC-like motifs present in the TM4–Loop4–TM5 of TRPV4 indeed interact with cholesterol, we expressed these sequences as different GST-tagged proteins and purified further (Fig. 4A and B). Using blot overlay, we explored if Loop4 alone or in combination with TM4 and/or TM5 can interact with cholesterol. We noted that Loop4 only is sufficient to interact with cholesterol, its precursor (mevalonate) and other cholesterol derivatives (like stigmasterol; steroid hormone aldosterone) (Fig. 4C). Similarly, TM4–Loop4, Loop4–TM5 and TM4–Loop4–TM5 also interact with cholesterol, its precursor, and derivatives though with variable extents. GST-only was used as a negative control which show no or very minimum interactions. This confirms that Loop4 in combination of TM4- and/or TM5 can directly interact with cholesterol, its precursors and derivatives and Loop4 is sufficient for these interactions.

3.7. Membrane mobility of TRPV4 is regulated by cholesterol

Next we performed FRAP in order to visualize the recovery of the hTRPV4-GFP (Fig. 4D). Mobility of TRPV4-GFP is significantly lower (recovers ~30% in 500 s) in control conditions (membrane with cholesterol). When cells were treated with cholesterol



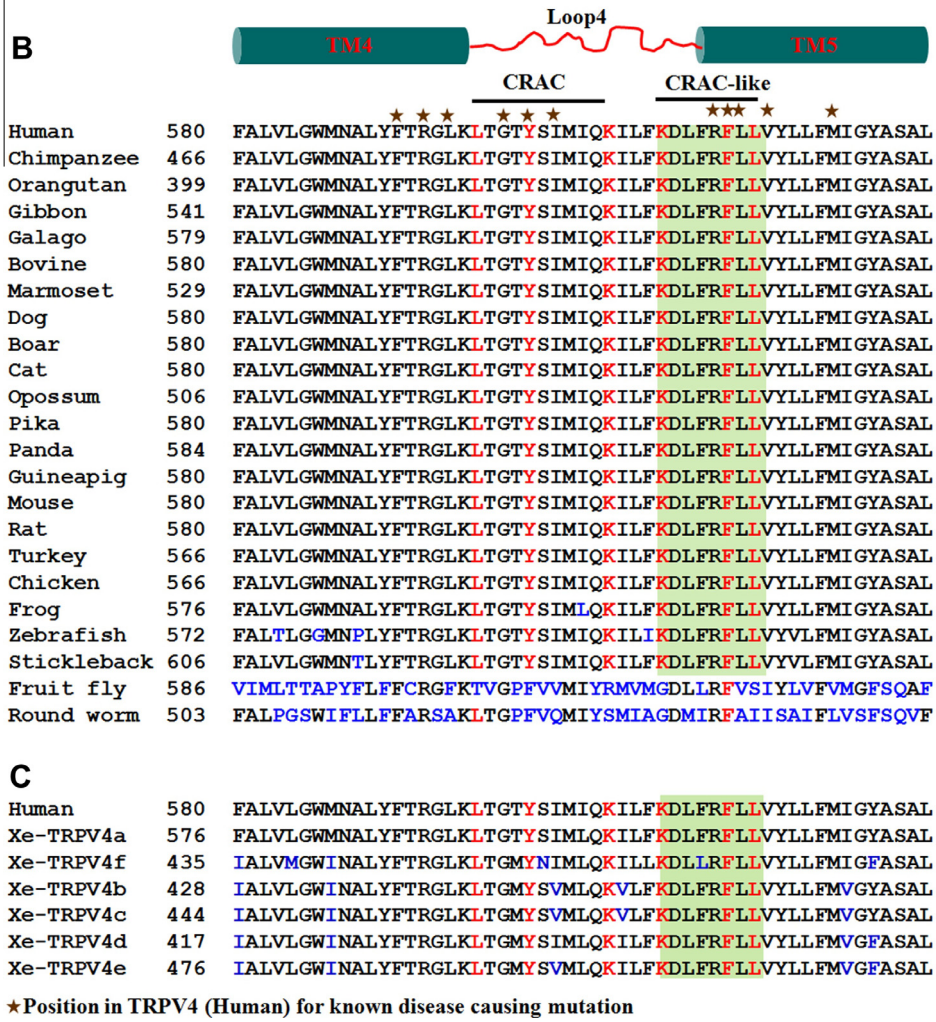


Fig. 2. Conserved domains and motifs including cholesterol interacting sites are present in TRPV4. (A) The lower and higher values indicate more and less conservation respectively. Different domains and motifs of TRPV4 are indicated by different colors. All values are significant ($p < 0.0001$, Kruskal–Wallis test). (B) The CRAC- and "CRAC-like (green shade) motifs present within the TM4-Loop4-TM5 are conserved throughout the vertebrate evolution. (i) The crucial amino acids that form the signature of these two motifs are labeled with red color. The positions of disease causing mutations (∗) and amino acids that differ from hTRPV4 (blue) are indicated. (ii) A similar comparison of *x*TRPV4 sequences with hTRPV4. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

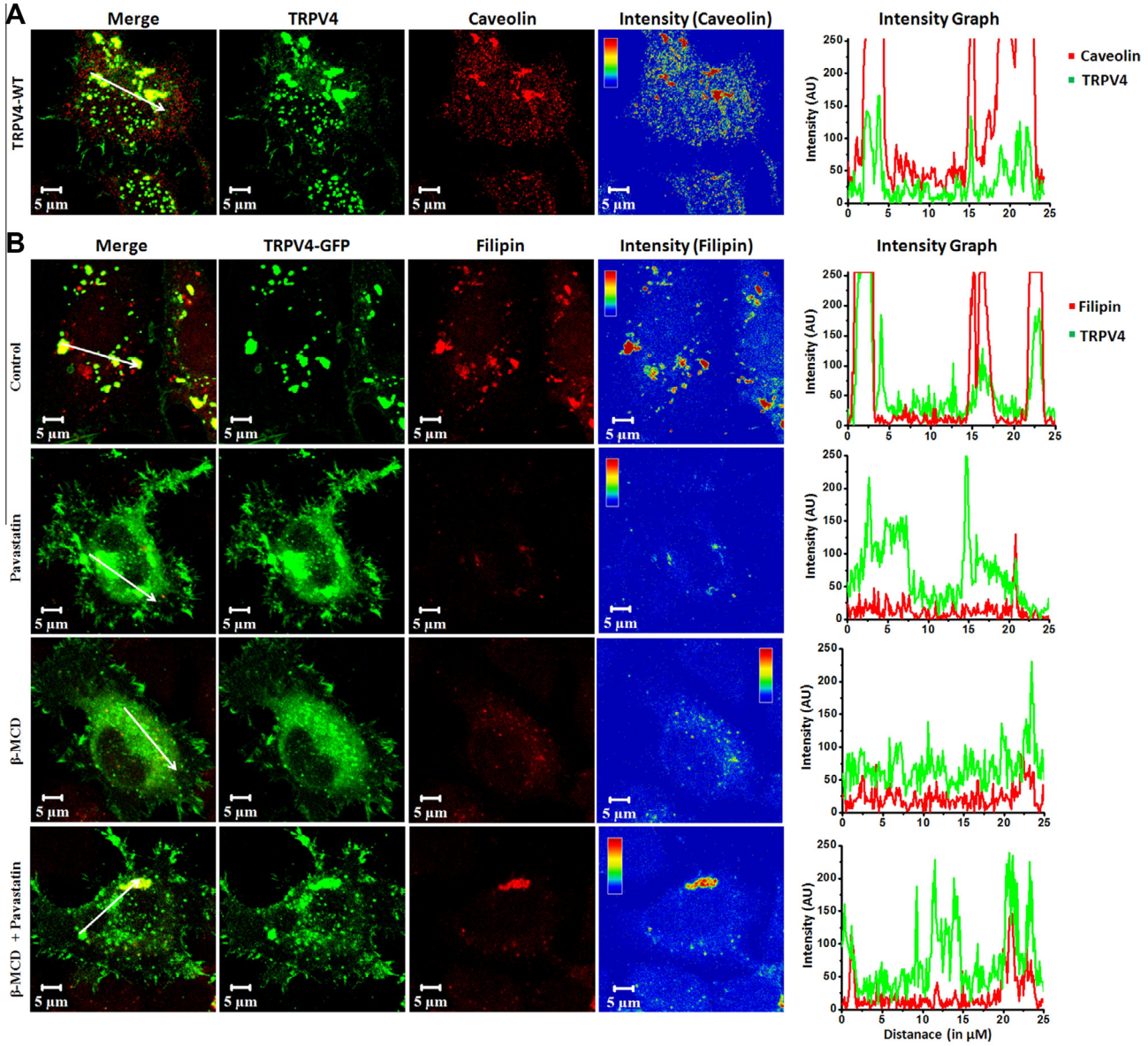


Fig. 3. TRPV4 localizes in cholesterol-enriched membrane microdomains. (A) Confocal images of F11 cells expressing GFP-TRPV4 (green) immunostained for endogenous Caveolin1 (Red). (B) Confocal images of F11 cell expressing GFP-TRPV4 (green) stained with Flilipin (Red). After treating the cells with cholesterol depleting agent βMCD (cholesterol biosynthesis blocker) Pravastatin, or with both. Cells were fixed in control conditions. In each case, the intensity plot is represented (right). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

depleting drug Pravastatin or βMCD; the mobility of TRPV4 increases further indicating that that mobility of TRPV4 is dependent on the membrane cholesterol.

4. Discussion

We have combined protein sequence, genomic data, structural information, biochemical and cell-biological experimental results, which demonstrate that TRPV4 is a highly conserved protein and has evolved ~450 MY before when vertebrate evolution started. TRPV4 physically interacts with cholesterol, its precursor molecule mevalonate and some of its derivatives through the Loop4 and nearby helices, a region of TRPV4 which is 100% identical in all vertebrates. In agreement with this physical interaction, in all vertebrates TRPV4 gene is tightly linked with MVK and GLTP, two genes which are involved in the cholesterol-biosynthesis. We show that membrane mobility of TRPV4 depends on the availability of the cholesterol. In vertebrates, TRPV4 seem to be regulated by cholesterol as well as other metabolic components present in the cholesterol biosynthesis pathway. Such molecular interactions of TRPV4 with cholesterol, cholesterol-precursors/derivatives may have importance in the context of several sensory functions which are directly dependent on the TRPV4. Such physical and functional crosstalk may also be relevant for several physiological functions [21]. As TRPV4-mediated sensory and physiological functions can largely influence the adaptation, such functions are crucial determinants of natural selection, speciation and evolution. Therefore, molecular evolution of TRPV4 is guided by availability of membrane cholesterol and its intermediates/derivatives, i.e. metabolites of cholesterol biosynthesis pathway in general which is very much vertebrate-specific.

In this work we demonstrate that Loop3–TM4–Loop4–TM5 region (AA 576–632) of TRPV4 is highly conserved among

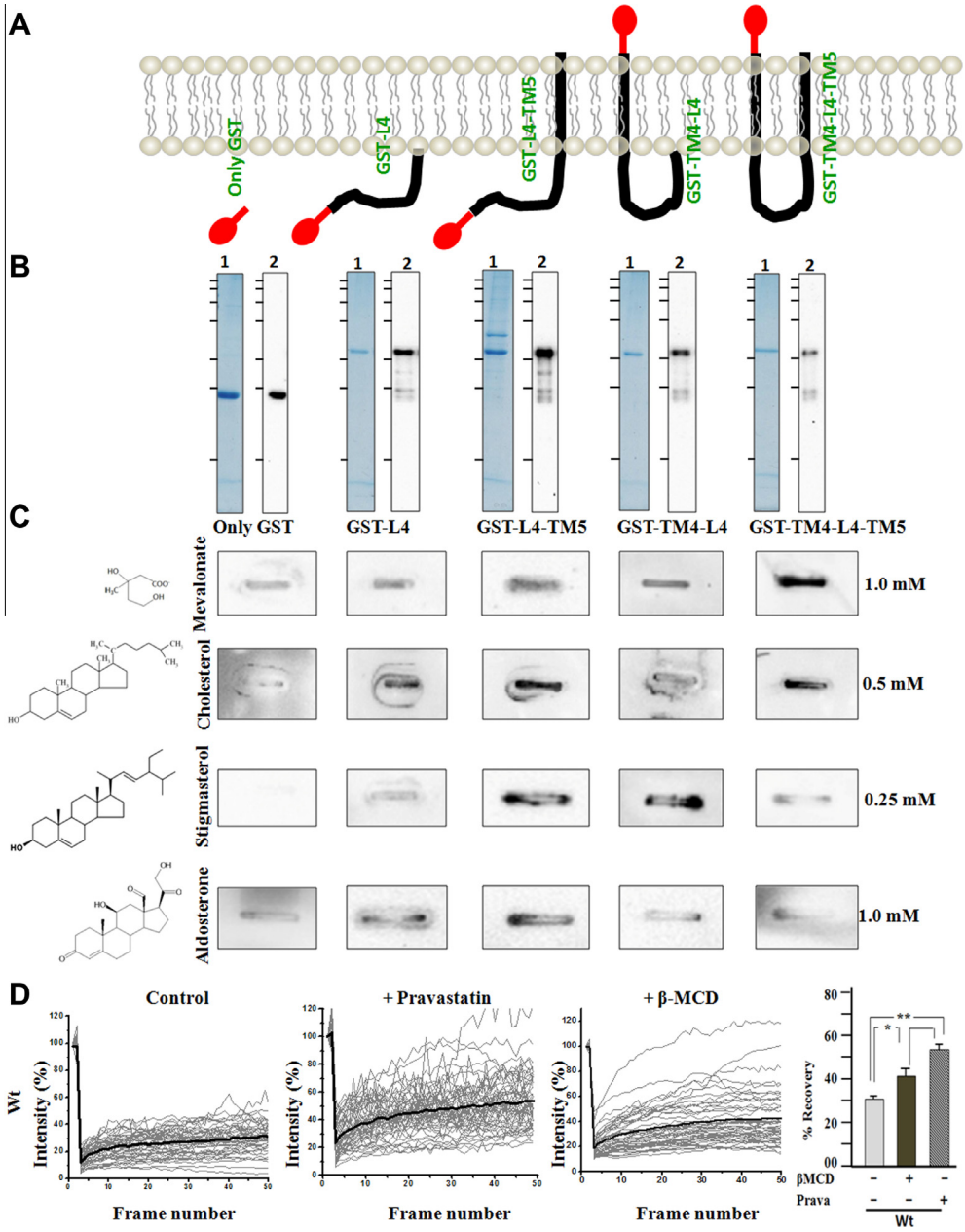


Fig. 4. Physical interaction and functional regulation of TRPV4 by cholesterol. (A) Loop4 alone or in combination with TM4 and/or TM5 is sufficient for physical interaction with cholesterol, its precursor and derivatives. (i) Schematic diagram of the GST-tagged TRPV4-fragments. (ii) SDS–PAGE (1) and Western Blot (2) analysis of purified GST-only, GST–Loop4, GST–Loop4–TM5, GST–TM4–Loop4 and GST–TM4–Loop4–TM5. (iii) The respective blot-overlay of these TRPV4 fragments against mevalonate, cholesterol, stigmasterol and aldosterone are shown. (B) Membrane mobility of TRPV4 depends on the cholesterol. FRAP pattern of cells expressing TRPV4-GFP in control (left), in Pravastatin-treated (middle) and in β-MCD treated conditions (right) are shown. In each case at least 50 ROIs were analyzed (gray lines) and the average value is indicated (bold black line). The fluorescence intensity (in % of the initial conditions) is plotted in *Y*-axis and frame numbers (time interval between each frame is 10 s) are plotted in *X*-axis. Average recovery at the end of 50th frame are shown in bar diagram ($^{*}p < 0.002$; $^{**}p < 0.0001$).

vertebrates and have limited substitutions. Accordingly, different point mutations which are clustered in this region of hTRPV4 are linked with the development of several diseases and pathophysiological situations commonly known as channelopathy [18]. We correlate this conservation as a prerequisite for interaction with cholesterol, its precursors and derivatives. There are several CRAC-motifs present within the N- and C-termini of hTRPV4 and few of these are conserved in most vertebrates (not shown). However, except the TM4–Loop4–TM5 region, we could not find any other CRAC/CRAC-like motif(s) in other TM regions (in hTRPV4). In TRPV1 and TRPV4, the CRAC-motif is 8AA long. Notably, AA 576–632 of TRPV4 is more conserved than that of the cholesterol-binding region of TRPV1 [10]. Even this region is not highly conserved when compared to other TRPVs where this motif is absent (Fig:S3).

The cholesterol interaction seems to be important for "activation/inhibition" of TRPV1/TRPV4 by specific hydrophobic compounds and for "thermo-gating". Indeed, experiments confirmed that TRPV1 function is altered in cholesterol-depleted/saturated membranes [22–23]. Also, cholesterol interaction is needed for temperature-induced activation of TRPV4. For example, when rTRPV4 is expressed in yeast (which cannot synthesize cholesterol); it can be activated by changes in osmolarity but not by temperature [24].

The cross-talk between TRPV4 with sterol compounds is also evident from genomic organization, genetic interaction and

pharmacological evidences. Synteny analysis suggests that TRPV4 share tight linkage with MVK and GLTP, genes involved in cholesterol biosynthesis pathway. Notably this linkage is conserved for 450 MY. In human too, MVK (a key enzyme catalyzing sterol synthesis from mevalonate) gene is tightly linked with TRPV4 (located very closely at 12q24.1) suggesting the dependency and fine regulation of TRPV4 by metabolites of the cholesterol biosynthesis pathway [25]. Common involvement of TRPV4 and sterols in same functions is also prominent from genetic interaction studies. Indeed, mutations in 3β-hydroxysterol Δ14-reductase, (involved in cholesterol biosynthesis pathway) give rise to Greenberg skeletal dysplasia [26]. Similarly, point mutations present in the CRAC-motif region of hTRPV4 give rise to "Skeletal dysplasia" [18].

Non-genomic regulation of TRPs by the metabolites present in the sterol/steroid biosynthesis pathway is important for several physiological, developmental, and endocrinological point of view [21]. For example, while dimethylallyl pyrophosphate activate TRPV4; isopentenyl pyrophosphate inhibits TRPV3 and TRPA1 [3,27,28]. Farnesyl pyrophosphate (FPP; precursor of sterol biosynthesis pathway) activate TRPV3 [27]. While cholesterol inhibits TRPM3, Pregnenolone (cholesterol precursor) can activate it [29,30]. Progesterone (sex hormone) also inhibits TRPM3 [31]. Taken together, results suggest that TRPVs are the molecular targets of cholesterol, its precursors and derivatives, such as steroid hormones. Though the effect of different steroids on TRPV4 has not been fully characterized yet, the crosstalk between TRPV4 and steroid hormones has been established to some extent. For example, progesterone can regulate TRPV4 expression [32]. TRPV4 is essential for mechanosensitivity in the aldosterone-sensitive distal nephron [33]. Steroid hormones also alter the mechanical hyperalgesia where TRPV4 is involved [34–36]. Such aspects may be relevant for vesicular recycling regulated by TRPV channels [37].

Taken together, our results reveal a strong dependency of TRPV4 on sterol molecules indicating that sterol biosynthesis pathway and TRPV4 function may have shared co-evolution. We conclude that TRPV4 structure and function has been determined by the physical interaction with sterol compounds through highly conserved motif sequences and such interactions may have diverse and important physiological functions.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2014.11.077.

References

[1] X. Gao, L. Wu, R.G. O'Neil, Temperature-modulated diversity of TRPV4 channel gating: activation by physical stresses and phorbol ester derivatives through protein kinase C-dependent and -independent pathways, J. Biol. Chem. 278 (2003) 27129–27137.
[2] X. Ma, D. He, X. Ru, et al., Apigenin, a plant-derived flavone, activates transient receptor potential TRPV4 cation channel, Br. J. Pharmacol. 166 (2011) 349–358.
[3] S. Bang, S. Yoo, T. Yang, et al., Nociceptive and proinflammatory effects of dimethylallyl pyrophosphate via TRPV4 activation, Br. J. Pharmacol. 166 (2012) 1433–1443.
[4] H. Watanabe, J. Vriens, J. Prenen, et al., Anandamide and arachidonic acid use epoxyeicosatrienoic acids to activate TRPV4 channels, Nature 424 (2003) 434–438.
[5] B.D. Matthews, C.K. Thodeti, J.D. Tytell, et al., Ultra-rapid activation of TRPV4 ion channels by mechanical forces applied to cell surface beta1 integrins, Integr. Biol. (Camb.) 2 (2010) 435–442.
[6] F.R. Carreño, L.L. Ji, J.T. Cunningham, Altered central TRPV4 expression and lipid raft association related to inappropriate vasopressin secretion in cirrhotic rats, Am. J. Physiol. Regul. Integr. Comp. Physiol. 296 (2009) R454–R466.
[7] C. Goswami, J. Kuhn, P.A. Heppenstall, et al., Importance of non-selective cation channel TRPV4 interaction with cytoskeleton and their reciprocal regulations in cultured cells, PLoS One 5 (2010) e11654.
[8] W. Liedtke, TRPV4 plays an evolutionary conserved role in the transduction of osmotic and mechanical stimuli in live animals, J. Physiol. 567 (2005) 53–58.
[9] W. Liedtke, Y. Choe, M.A. Martí-Renom, et al., Vanilloid receptor-related osmotically activated channel (VR-OAC), a candidate vertebrate osmoreceptor, Cell 103 (2000) 525–535.
[10] P. Sardar, A. Kumar, A. Bhandari, et al., Conservation of tubulin-binding sequences in TRPV1 throughout evolution, PLoS One 7 (2012) e31448.
[11] C.B. Phelps, R.J. Huang, P.V. Lishko, et al., Structural analyses of the ankyrin repeat domain of TRPV6 and related TRPV ion channels, Biochemistry 47 (2008) 2476–2484.
[12] P.V. Lishko, E. Procko, X. Jin, et al., The ankyrin repeats of TRPV1 bind multiple ligands and modulate channel sensitivity, Neuron 54 (2007) 905–918.
[13] G. Picazo-Juárez, S. Romero-Suárez, A. Nieto-Posadas, et al., Identification of a binding motif in the S5 helix that confers cholesterol sensitivity to the TRPV1 ion channel, J. Biol. Chem. 286 (2011) 24966–24976.
[14] P. Valente, N. García-Sanz, A. Gomis, et al., Identification of molecular determinants of channel gating in the transient receptor potential box of vanilloid receptor I, FASEB J. 22 (2008) 3298–3309.
[15] R. Strotmann, G. Schultz, T.D. Plant, Ca^{2+}-dependent potentiation of the nonselective cation channel TRPV4 is mediated by a C-terminal calmodulin binding site, J. Biol. Chem. 278 (2003) 26541–26549.
[16] W. Liedtke, D.M. Tobin, C.I. Bargmann, et al., Mammalian TRPV4 (VR-OAC) directs behavioural responses to osmotic and mechanical stimuli in *Caenorhabditis elegans*, Proc. Natl. Acad. Sci. U.S.A. 100 (2003) 14531–14536.
[17] P. Doñate-Macián, A. Perálvarez-Marín, Dissecting domain-specific evolutionary pressure profiles of transient receptor potential vanilloid subfamily members 1 to 4, PLoS One 9 (2014) e110715.
[18] P. Verma, A. Kumar, C. Goswami, TRPV4-mediated channelopathies, Channels (Austin) 4 (2010) 319–328.
[19] S.R. Lamandé, Y. Yuan, I.L. Gresshoff, et al., Mutations in TRPV4 cause an inherited arthropathy of hands and feet, Nat. Genet. 43 (2011) 1142–1146.
[20] C.J. Baier, J. Fantini, F.J. Barrantes, Disclosure of cholesterol recognition motifs in transmembrane domains of the human nicotinic acetylcholine receptor, Sci. Rep. 1 (2011) 69.
[21] A. Kumar, S. Kumari, R.K. Majhi, et al., Regulation of TRP channels by steroids: implications in physiology and diseases, Gen. Comp. Endocrinol. (2014), http://dx.doi.org/10.1016/j.ygcen.2014.10.004.
[22] M. Liu, W. Huang, D. Wu, et al., TRPV1, but not P2X, requires cholesterol for its function and membrane expression in rat nociceptors, Eur. J. Neurosci. 24 (2006) 1–6.
[23] E. Szoke, R. Börzsei, D.M. Tóth, et al., Effect of lipid raft disruption on TRPV1 receptor activation of trigeminal sensory neurons and transfected cell line, Eur. J. Pharmacol. 628 (2010) 67–74.
[24] S.H. Loukin, Z. Su, C. Kung, Hypotonic shocks activate rat TRPV4 in yeast in the absence of polyunsaturated fatty acids, FEBS Lett. 583 (2009) 754–758.
[25] I. Buhaescu, H. Izzedine, Mevalonate pathway: a review of clinical and therapeutical implications, Clin. Biochem. 40 (2007) 575–584.
[26] A.M. Bennati, M. Castelli, M.A.D. Fazia, et al., Sterol dependent regulation of human TM7SF2 gene expression: role of the encoded 3beta-hydroxysterol Delta14-reductase in human cholesterol biosynthesis, Biochim. Biophys. Acta 1761 (2006) 677–685.
[27] S. Bang, S. Yoo, T.J. Yang, et al., Farnesyl pyrophosphate is a novel pain-producing molecule via specific activation of TRPV3, J. Biol. Chem. 285 (2010) 19362–19371.
[28] S. Bang, S. Yoo, T.J. Yang, et al., Isopentenyl pyrophosphate is a novel antinociceptive substance that inhibits TRPV3 and TRPA1 ion channels, Pain 152 (2011) 1156–1164.
[29] J. Naylor, J. Li, C.J. Milligan, et al., Pregnenolone sulphate- and cholesterol-regulated TRPM3 channels coupled to vascular smooth muscle secretion and contraction, Circ. Res. 106 (2010) 1507–1515.
[30] T.F. Wagner, S. Loch, S. Lambert, et al., Transient receptor potential M3 channels are ionotropic steroid receptors in pancreatic beta cells, Nat. Cell Biol. 10 (2008) 1421–1430.
[31] Y. Majeed, S. Tumova, B.L. Green, et al., Pregnenolone sulphate-independent inhibition of TRPM3 channels by progesterone, Cell Calcium 51 (2012) 1–11.
[32] C. Jung, C. Fandos, I.M. Lorenzo, et al., The progesterone receptor regulates the expression of TRPV4 channel, Pflugers Arch. 459 (2009) 105–113.
[33] M. Mamenko, O. Zaika, M. Jin, et al., Purinergic activation of Ca^{2+}-permeable TRPV4 channels is essential for mechano-sensitivity in the aldosterone-sensitive distal nephron, PLoS One 6 (2011) e22824.
[34] O.A. Dina, K.O. Aley, W. Isenberg, et al., Sex hormones regulate the contribution of PKCepsilon and PKA signalling in inflammatory pain in the rat, Eur. J. Neurosci. 13 (2001) 2227–2233.
[35] Y. Ji, B. Tang, R.J. Traub, Modulatory effects of estrogen and progesterone on colorectal hyperalgesia in the rat, Pain 117 (2005) 433–442.
[36] W.J. Kowalczyk, M.A. Sullivan, S.M. Evans, et al., Sex differences and hormonal influences on response to mechanical pressure pain in humans, J. Pain 11 (2010) 330–342.
[37] C. Goswami, N. Rademacher, K.H. Smalla, et al., TRPV1 acts as a synaptic protein and regulates vesicle recycling, J. Cell Sci. 123 (2010) 2045–2057.