Cell Calcium 80 (2019) 25–28

Contents lists available at ScienceDirect

Cell Calcium

journal homepage: www.elsevier.com/locate/ceca

The structure of TRPC ion channels

Jian Li[a,b], Xu Zhang[c], Xiaojing Song[c], Rui Liu[c], Jin Zhang[c,*], Zongli Li[d,*]

[a] College of Pharmaceutical Sciences, Gannan Medical University, Ganzhou, Jiangxi, 341000, China
[b] Key Laboratory of Prevention and Treatment of Cardiovascular and Cerebrovascular Diseases of Ministry of Education, Ganan Medical University, Ganzhou, 341000, China
[c] School of Basic Medical Sciences, Nanchang University, Nanchang, Jiangxi, 330031, China
[d] Howard Hughes Medical Institute, Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA, 02115, USA

ARTICLE INFO

Keywords:
TRPC
Cryo-EM

ABSTRACT

Briefly review the recent structural work of transient receptor potential canonical (TRPC) ion channels by using electron cryo-microscopy (cryo-EM). The high resolution structures of TRPC3, TRPC4, TRPC5 and TRPC6 are discussed.

1. Introduction

Transient receptor potential canonical (TRPC) ion channels are calcium-permeable, nonselective cation (Na^+, K^+, Ca^{2+}) channels belonging to the TRP superfamily [1–3]. They are expressed in many cell types and tissues, including brain, placenta, adrenal gland, retina endothelia, testis, and kidney [4], and crucially involved in both the receptor-operated Ca^{2+} signaling and a store-operated calcium entry (SOCE) [5–7], a major mechanism regulating calcium homeostasis, which is triggered by the depletion of calcium stored in the endoplasmic reticulum (ER) [8]. TRPCs play important roles in many physiological processes and implicated in various pathogenesis and human diseases [9–13]. Among the seven members in TRPC family, they can be further divided into two subgroups based on the sequence similarity: TRPC1/4/5 and TRPC2/3/6/7 [14–18]. TRPC1/4, 1/5 heterotetramers and TRPC4 or 5 homotetramers can be activated by Gq protein-coupled receptors and increasing intracellular Ca^{2+}, as well as by PIP2 hydrolysis. TRPC3, TRPC6, and TRPC7 are unique in being activated by the lipid secondary messenger diacylglycerol (DAG), a degradation product of the signaling lipid phosphatidylinositol 4,5-bisphosphate (PIP2) [19–24]. There are many reviews on these topics [1,25–34].

Despite many investigations of TRPC ion channels, lack of high resolution structure information have long be a restraint for understanding the channels gating mechanism and structure-function relationship. In last few years electron cryo-microscopy (cryo-EM) have evolved rapidly and experienced the “resolution revolution”, becoming one of the major method for determining high resolution structure of protein/protein complex [35,36]. Compare to x-ray crystallography, cryo-EM method does not need protein to be crystallized and only use a few micron liter samples, and has thus greatly facilitate the structure determination of membrane proteins, especially the various ion channels. Transient receptor potential canonical (TRPC) channels, important to the cell calcium homeostasis, are among them. Here we summarize the recent development of the high resolution structure work of TRPC ion channels.

2. High resolution structure of TRPC ion channels

The structure of the full-length human TRPC3(hTRPC3) has been determined in a lipid-occupied apo state at various resolutions, ranging from 3.3 to 5.8 Å [37–39]. These structure revealed the atomic view of TRPC3 channel and its two lipid-binding sites. The cytoplasmic domain of human TRPC3 in the apo state was also determined at 4.0 Å [38]. The structure of full-length human TRPC6 (hTRPC6) in complex with inhibitor BTDM within the nanodiscs at 3.8 Å resolution, together with functional data elucidated the structural basis of high-affinity binding by a novel inhibitor [39]. The structure of cytoplasmic domain of murine TRPC6 at 3.8 Å resolution is also available [40]. The structure of the hTRPC3 shows a similar structure as hTRPC6, consistent with their high sequence homology (73%identity), the ion channel pore of hTRPC3 and hTRPC6 are all in the closed state. The structures of apo state TRPC4 in mouse and zebrafish have been reported at 3.3 Å and 3.6 Å, respectively [41,42]. The structure of mouse TRPC5 (mTRPC5) at pH 7.5 has been determined at an overall resolution of 2.8 Å [43], the highest resolution in all published TRP protein structures (Figs. 1 and 2).

* Corresponding authors.
E-mail addresses: zhangxiaokong@hotmail.com (J. Zhang), zongli_li@hms.harvard.edu (Z. Li).

https://doi.org/10.1016/j.ceca.2019.03.005
Received 21 February 2019; Received in revised form 13 March 2019; Accepted 13 March 2019
Available online 14 March 2019
0143-4160/

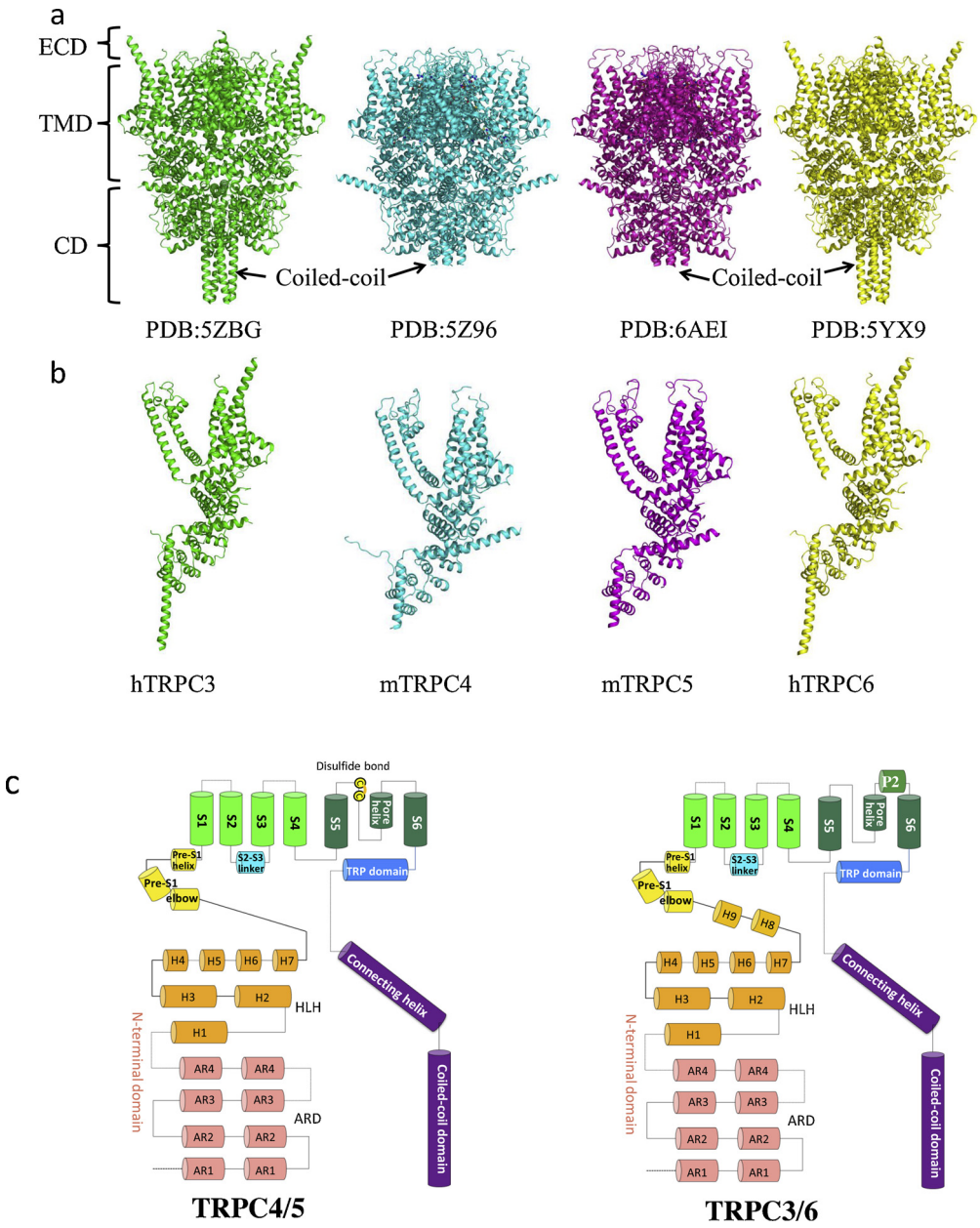

Fig. 1. Structures of TRPC channels. a, Ribbon representation of the overall atomic model of TRPC3 (PDB:5ZBG, green), TRPC4 (PDB:5Z96, cyan), TRPC5 (PDB:6AEI, magentas) and TRPC6 (PDB:5YX9, yellow); b, The Ribbon representation of monomeric of TRPC3 (PDB:6CUD, green), TRPC4 (PDB:5Z96, cyan), TRPC5 (PDB:6AEI, magentas) and TRPC6 (PDB:5YX9, yellow); c, Linear diagram depicting the major structural domains of the TRPC4/TRPC5 and TRPC3/TRPC6 monomer. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2.1. Overall architecture of the TRPC channel

Overall structure of these TRPCs are similar to each other, which are four-fold symmetric homotetramer and have solely alpha-helical composition [37–43]. Each monomer consists of a transmembrane domain (TMD) and a compact cytoplasmic domain (CD). The transmembrane domain comprises the voltage-sensor-like (VSL) domain and structurally conserved pore domain. Compared to the structures of mTRPC4/5, the long TMD alpha-helix S3 in TRPC3/6 elongate into extra cellular space, together with long linker between S3 and S4, form an “pseudo” extra cellular domain (ECD) [37,39]. The CD has an inverted bell shape and caps the lower end of the ion channel pore of TMD, which is composed of N- and C-terminal subdomains (NTD and CTD, respectively). The NTD consists of four ankyrin repeats (AR1-AR4) and several linker helices (LHs, seven in TRPC4/5 and nine in TRPC3/6), and the CTD contains a connecting helix (C-terminal helix 1, CH1) and a coiled-coil domain (C-terminal helix 2, CH2). Inter-subunit interactions mediate TRPC tetramer assembly, both the TMD and CD contribute to the tetrameric assembly of the TRPC channels. The CD of each subunit extensively interacts with the neighboring subunits. Moreover, CH1 inserts into the cavity between two neighboring subunits and glues them together. CH2 helices form a vertical four-helix bundle that further tightens the tetramer, reminiscent of TRPA1 [44] and TRPM [45–49] C-terminal four helix bundle structures. Uniquely, the four CH1-CH2 connection linkers pack tightly to form a knot-like structure on the top of CH2, indicating the essential role of the CH1-CH2 linker knot and the first half of the CH2 in tetramer assembly.

Compare the TRPCs’ structure with previously reported other TRP structures, the organization of six helices in each TMD is similar to that of other TRP channels, while the intracellular architecture is distinct.

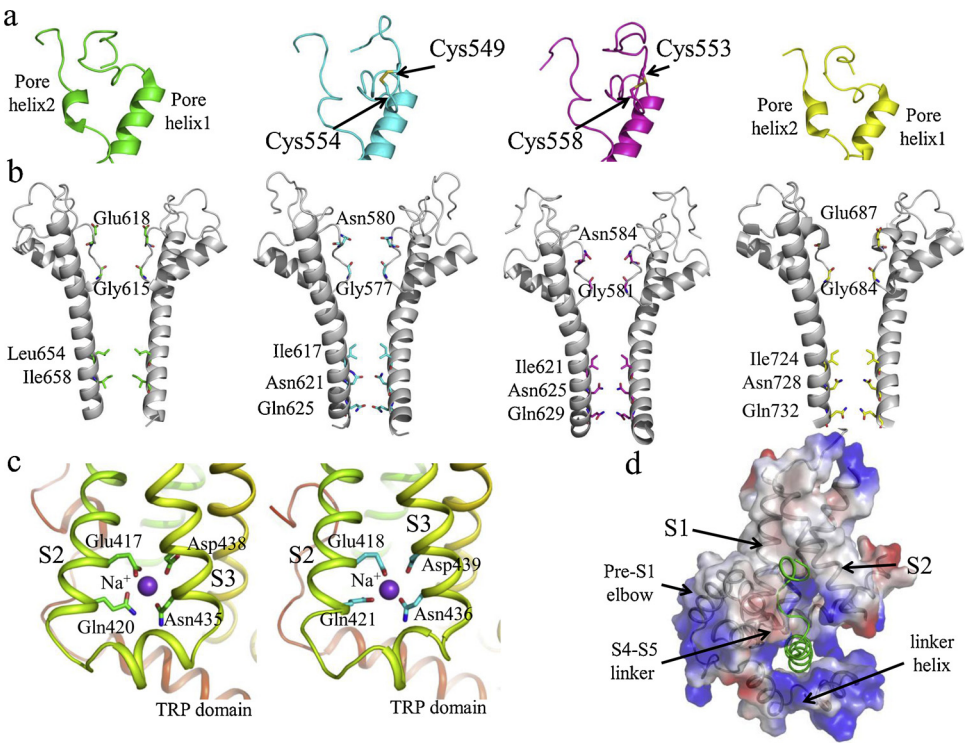

Fig. 2. a, Pore loop structures of TRPCs. Cysteine residues are shown in stick representation. b, Comparison of ion conducting pathways between TRPCs. The key residues involved are shown in stick representation in green (TRPC3), cyan (TRPC4), magentas (TRPC5) and yellow (TRPC6), respectively. c, The cation binding site in TRPCs. The key residues involved are shown in stick representation in green (TRPC4) and cyan (TRPC5), Sodium ion are shown in magentas spheres. d, The TRP domain locations in TRPCs. TRP domain is in green and other parts are in gray. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Distinctive features of TRPCs include: (1) Shorter S2-S3 linker limits its interactions with cytoplasmic regions [37–43]; (2) the disulfide bond in the TRPC4/5 structures lie in the loop linking S5 and the pore helix [41,43]; (3) a pre-S1 elbow helix connects different structures in different members.

2.2. Ion-conducting pathway

The ion-conducting pore of TRPCs are lined with an extracellular selectivity filter and an intracellular gate, with a wide central vestibule in the middle. The pore of all published TRPCs adopt a closed conformation, and thus preventing ion passage. The differences in the narrowest point of TRPCs structures may give some clue as to ion selectivity, the lower gate is too narrow to allow the passage of a fully or partially hydrated ion [37–43,50].

A key feature of TRPC ion channels is the conserved 'LFW' motif inside the pore domain, the pore helix and pore loop interact extensively with S5-S6 gating helices. For stabilizing the pore, a phenylalanine and tryptophan form part of the prominent hydrophobic contact between the pore helix of one protomer and helix S6 of the adjacent protomer. Different LFW motif, including 609–611 in hTRPC3, 571–573 in mTRPC4, 575–577 in mTRPC5 and 678–680 in hTRPC6 forms specific multiple hydrophobic interactions within the pore domain and therefore plays an important role in maintaining the proper structure of the pore domain. For instance, in the hTRPC3/6, motif 'EVTSV/EVKSV' forms a small helix in the middle of the intracellular loop connecting S6 and the pore helix and this unique helix is distinct from the corresponding motif of TRPC4/5. While the mTRPC5 motif contains 3 more amino acids, including one more negatively-charged residue (Asp548) and the hydrophobic residue(Ile547), thus potentially more attractive for cation entry into the pore.

In the mTRPC5, Arg593 interacts via a salt bridge with Glu598 and forms a hydrogen bond with the Val590 side chain. In addition, Arg593 is the only positively charged residue in the corresponding positions in TRPC channels (glycine in TRPC1, aspartic in TRPC3, glutamine in mTRPC4, asparagine in hTRPC6, and negatively-charged aspartic acid in hTRPC7). Therefore, Arg593 may serve as the molecular fulcrum, allowing the efficient transmission of the GPCR-G_q-PLC-powered gating force to mTRPC5′s pore helix-loop.

2.3. Disulfide bond

mTRPC4/5′s extracellular domains and long pore loops form intricate structures stabilized by a disulfide bond, this is different from TRPC3/6. In mTRPC4, C549 and C554 (C553 and C558 in mTRPC5) form a disulfide bridge and is not in close proximity to helix S6. The cysteines are likely involved in putting E555 (E559 in TRPC5) in place, which is located at the extracellular vestibule above the selectivity filter. Reducing the disulfide bond of cystines could thus lead to a destabilization of the upper region of the selectivity filter and the E555 could be released and orient towards the center of the pore, altering its properties. This conformational change could result in a negatively charged sink at the turret, thereby attracting cations, possibly explaining the effect of the increased Ca^{2+} conductance.

The two cysteines in the pore region are well conserved in TRPC1/4/5 subfamily, but not in TRPC2/3/6/7, implying that they have different mechanism. Interestingly, although TRPC4 and TRPC5 have similar functional properties, the loop preceding TRPC5′s disulfide bond is distinct from that of TRPC4, with 3 extra residues in TRPC5 [2,3,36–40].

2.4. S2-S3 binding pocket

In Duan's mTRPC4 structure, the Na^+ located at the cytoplasmic face is apparently coordinated by side chains of Glu417 and Gln420 from S2 and the Asp438 and Asn435 from S3. The negatively charged Glu417 and Asp438 are conserved within the TRPC subfamily. The corresponding residues in mTRPC5 are Glu418, Glu421, N436 and D439, respectively. Structural alignment of these residues from TRPC family members reveals that this is a well-preserved cation-binding site [6,7]. In addition, Tyr373 of S1 and the positively charged Arg491 of S4 are located above the cation binding site, forming a lid that may prevent the outward movement of cations.

3. Conclusions

The advances in cryo-EM enable the high resolution structure determination of TRP ion channels, including the TRPC sub-family. These structures shed light on the architecture of this receptor-activated TRPC channel and provide a structural basis in understanding how the ion conduction pathway is built and the gating mechanism. The findings have broad implications for understanding the structural basis underlying calcium homeostasis, epitope antibody selectivity, and the diverse functional and physiological roles of this ion channel family. Although TRPCs has wide pharmaceutical applications in treatment of various diseases, drug development specifically targeting TRPCs has been limited due to the lack of understanding of its molecular activation mechanisms and the relationships between structural properties and the physiological conditions. These structures provide the starting point for future investigation on these important molecules.

Acknowledgements

Z. L. was supported by Howard Hughes Medical Institute. J.L. was supported by the National Natural Science Foundation of China (Grant No. 81402850). J.Z. was supported by the Thousand Young Talents Program of China, National Natural Science Foundation of China (Grant No. 31770795) and Jiangxi Province Natural Science Foundation (Grant No. 20181ACB20014).

References

[1] D.E. Clapham, TRP channels as cellular sensors, Nature 426 (2003) 517–524.
[2] S. Selvaraj, Y. Sun, B.B. Singh, TRPC channels and their implication in neurological diseases, CNS Neurol. Disord. Drug Targets 9 (2010) 94–104.
[3] M. Gees, B. Colsoul, B. Nilius, The role of transient receptor potential cation channels in Ca2+ signaling, Cold Spring Harb. Perspect. Biol. 2 (2010) a003962.
[4] M. Freichel, V. Tsvilovskyy, J.E. Camacho-London, TRPC4-and TRPC4-containing channels, Handb. Exp. Pharmacol. 222 (2014) 85–128.
[5] H.L. Ong, L.B. de Souza, I.S. Ambudkar, Role of TRPC channels in store-operated calcium entry, Adv. Exp. Med. Biol. 898 (2016) 87–109.
[6] J.T. Smyth, S.Y. Hwang, T. Tomita, W.I. DeHaven, J.C. Mercer, J.W. Putney, Activation and regulation of store-operated calcium entry, J. Cell. Mol. Med. 14 (2010) 2337–2349.
[7] M.J. Berridge, M.D. Bootman, H.L. Roderick, Calcium signalling: dynamics, homeostasis and remodelling, Nat. Rev. Mol. Cell Biol. 4 (2003) 517–529.
[8] Q. Xi, A. Adebiyi, G. Zhao, K.E. Chapman, C.M. Waters, A. Hassid, J.H. Jaggar, IP3 constricts cerebral arteries via IP3 receptor-mediated TRPC3 channel activation and independently of sarcoplasmic reticulum Ca2+ release, Circ. Res. 102 (2008) 1118–1126.
[9] E.B. Becker, B.L. Fogel, S. Rajakulendran, A. Dulneva, M.G. Hanna, S.L. Perlman, D.H. Geschwind, K.E. Davies, Candidate screening of the TRPC3 gene in cerebellar ataxia, Cerebellum 10 (2011) 296–299.
[10] N. Kitajima, T. Numaga-Tomita, M. Watanabe, T. Kuroda, A. Nishimura, K. Miyano, S. Yasuda, K. Kuwahara, Y. Sato, T. Ide, L. Birnbaumer, H. Sumimoto, Y. Mori, M. Nishida, TRPC3 positively regulates reactive oxygen species driving maladaptive cardiac remodeling, Sci. Rep. 6 (2016) 1–14.
[11] S.L. Yang, Q. Cao, K.C. Zhou, Y.J. Feng, Y.Z. Wang, Transient receptor potential channel C3 contributes to the progression of human ovarian cancer, Oncogene 28 (2009) 1320–1328.
[12] S. Selvaraj, Y. Sun, B.B. Singh, TRPC channels and their implication in neurological diseases, CNS Neurol. Disord. Drug Targets 9 (2010) 94–104.
[13] D.V. Ilatovskaya, A. Staruschenko, TRPC6 channel as an emerging terminant of the podocyte injury susceptibility in kidney diseases, Am. J. Physiol. Ren. Physiol. 309 (2015) 393–397.
[14] C. Strubing, G. Krapivinsky, L. Krapivinsky, D.E. Clapham, Formation of novel TRPC channels by complex subunit interactions in embryonic brain, J. Biol. Chem. 278 (2003) 39014–39019.
[15] C. Strubing, G. Krapivinsky, L. Krapivinsky, D.E. Clapham, TRPC1 and TRPC5 form a novel cation channel in mammalian brain, Neuron 29 (2001) 645–655.
[16] X. Zhu, M. Jiang, M. Peyton, G. Boulay, R. Hurst, E. Stefani, L. Birnbaumer, Trp, a novel mammalian gene family essential for agonistactivated capacitative Ca2+ entry, Cell 85 (1996) 661–671.
[17] M. Trebak, G. Vazquez, G.S. Bird, J.W. Putney, The TRPC3/6/7 subfamily of cation channels, Cell Calcium 33 (2003) 451–461.
[18] D.E. Clapham, D. Julius, C. Montell, G. Schultz, International Union of Pharmacology. XLIX. Nomenclature and structure–function relationships of transient receptor potential channels, Pharmacol. Rev. 57 (2005) 427–450.
[19] G. Boulay, Ca(2+)-calmodulin regulates receptor-operated Ca(2+) entry activity of TRPC6 in HEK-293 cells, Cell Calcium 32 (2002) 201–207.
[20] T. Hofmann, M. Schaefer, G. Schultz, T. Gudermann, Subunit composition of mammalian transient receptor potential channels in living cells, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 7461–7466.
[21] K. Venkatachalam, F. Zheng, D.L. Gill, Regulation of canonical transient receptor potential (TRPC) channel function by diacylglycerol and protein kinase C, J. Biol. Chem. 278 (2003) 29031–29040.
[22] K. Itsuki, Y. Imai, Y. Okamura, K. Abe, R. Inoue, M.X. Mori, Voltage-sensing phosphatase reveals temporal regulation of TRPC3/C6/C7 channels by membrane phosphoinositides, Channels 6 (2012) 206–209.
[23] J. Tang, Y. Lin, Z. Zhang, S. Tikunova, L. Birnbaumer, M.X. Zhu, Identification of common binding sites for calmodulin and inositol 1,4,5-trisphosphate receptors on the carboxyl termini of trp channels, J. Biol. Chem. 276 (2001) 21303–21310.
[24] B. Minke, TRP channels and Ca2+ signaling, Cell Calcium 40 (2006) 261–275.
[25] B. Nilius, V. Flockerzi, Mammalian transient receptor potential (TRP) cation channels. Part I: the TRPC subfamily, Handb. Exp. Pharmacol. 222 (2014) 15–188.
[26] S. Feng, TRPC channel structure and properties, Adv. Exp. Med. Biol. 976 (2017) 9–23.
[27] L.B. de Souza, I.S. Ambudkar, Trafficking mechanisms and regulation of TRPC channels, Cell Calcium 56 (2014) 43–50.
[28] H.L. Ong, L.B. de Souza, I.S. Ambudkar, Role of TRPC channels in store-operated calcium entry, Adv. Exp. Med. Biol. 898 (2016) 87–109.
[29] B. Constantin, Role of scaffolding proteins in the regulation of TRPC-dependent calcium entry, Adv. Exp. Med. Biol. 898 (2016) 379–403.
[30] H.L. Ong, L.B. de Souza, K.T. Cheng, I.S. Ambudkar, Physiological functions and regulation of TRPC channels, Handb. Exp. Pharmacol. 223 (2014) 1005–1034.
[31] I.S. Ambudkar, B.C. Bandyopadhyay, X. Liu, T.P. Lockwich, B. Paria, H.L. Ong, Functional organization of TRPC-Ca2+ channels and regulation of calcium microdomains, Cell Calcium 40 (2006) 495–504.
[32] J.C. Gonzalez-Cobos, M. Trebak, TRPC channels in smooth muscle cells, Front. Biosci. (Landmark Ed.) 15 (2010) 1023–1039.
[33] G.M. Salido, S.O. Sage, J.A. Rosado, TRPC channels and store-operated Ca(2+) entry, Biochim. Biophys. Acta 1793 (2009) 223–230.
[34] K. Kiselyov, R.L. Patterson, The integrative function of TRPC channels, Front. Biosci. (Landmark Ed.) 14 (2009) 45–58.
[35] E.H. Egelman, The current revolution in cryo-EM, Biophys. J. 110 (2016) 1008–1012.
[36] W. Kuhlbrandt, The resolution revolution, Science 343 (2014) 1443–1444.
[37] C. Fan, W. Choi, W. Sun, J. Du, W. Lu, Structure of the human lipid-gated cation channel TRPC3, Elife 7 (2018) 36852.
[38] F. Sierra-Valdez, C.M. Azujmaya, L.O. Romero, T. Nakagawa, J.F. Cordero-Morales, Structure–function analyses of the ion channel TRPC3 reveal that its cytoplasmic domain allosterically modulates channel, J. Biol. Chem. 293 (2018) 16102–16114.
[39] Q. Tang, W. Guo, L. Zheng, et al., Structure of the receptor-activated human TRPC6 and TRPC3 ion channels, Cell Res. 28 (2018) 746–755, https://doi.org/10.1038/s41422-018-0038-2.
[40] C.M. Azumaya, F. Sierra-Valdez, J.F. Cordero-Morales, T. Nakagawa, Cryo-EM structure of the cytoplasmic domain of murine transient receptor potential cation channel subfamily C member 6 (TRPC6), J. Biol. Chem. 293 (2018) 10381–10391, https://doi.org/10.1074/jbc.RA118.003183 Epub 2018 May 11.
[41] J. Duan, J. Li, B. Zeng, et al., Structure of the mouse TRPC4 ion channel, Nat. Commun. 9 (2018) 1–10, https://doi.org/10.1038/s41467-018-05247-9.
[42] D. Vinayagam, T. Mager, A. Apelbaum, et al., Electron cryo-microscopy structure of the canonical TRPC4 ion channel, Elife 7 (2018) 36615.
[43] J. Duan, J. Li, G.L. Chen, et al., Cryo-EM Structure of TRPC5 at 2.8 Å Resolution Reveals Unique and Conserved Structural Elements Essential for Channel Function, Sumitted, (BioRxiv: 467969 (2019), https://doi.org/10.1101/467969).
[44] C.E. Paulsen, J.P. Armache, Y. Gao, Y. Cheng, D. Julius, Structure of the TRPA1 ion channel suggests regulatory mechanisms, Nature 520 (2015) 511–517.
[45] J. Duan, Z. Li, J. Li, et al., Structure of full-length human TRPM4, Proc. Natl. Acad. Sci. U. S. A. 115 (2018) 2377–2382.
[46] Y. Yin, M. Wu, L. Zubcevic, W.F. Borschel, G.C. Lander, S.Y. Lee, Structure of the cold- and menthol-sensing ion channel TRPM8, Science 359 (2018) 237–241.
[47] H.E. Autzen, A.G. Myasnikov, M.G. Campbell, D. Asarnow, D. Julius, Y. Cheng, Structure of the human TRPM4 ion channel in a lipid nanodisc, Science 359 (2018) 228–232.
[48] J. Guo, J. She, W. Zeng, Q. Chen, X.C. Bai, Y. Jiang, Structures of the calcium-activated, non-selective cation channel TRPM4, Nature 552 (2017) 205–209.
[49] P.A. Winkler, Y. Huang, W. Sun, J. Du, W. Lu, Electron cryo-microscopy structure of a human TRPM4 channel, Nature 552 (2017) 200–204.
[50] M. Lichtenegger, T. Stockner, M. Poteser, H. Schleifer, D. Platzer, C. Romanin, K. Groschner, A novel homology model of TRPC3 reveals allosteric coupling between gate and selectivity filter, Cell Calcium 54 (2013) 175–185.