Rendezvous with $PI(3,5)P_2$ - a rapalog gets caught opening TRPML1

Taufiq Rahman[a,*], Sandip Patel[b]

[a]Department of Pharmacology, University of Cambridge, Cambridge CB2 1PD, United Kingdom

[b]Department of Cell and Developmental Biology, University College London, London WC1E 6BT, United Kingdom

Abstract

TRPML1 is an endolysosomally-expressed cation channel, activated physiologically by $PI(3,5)P_2$ and by several synthetic agonists including rapamycin. New high resolution cryo-EM- structures of TRPML1 bound to both $PI(3,5)P_2$ and temsirolimus - a rapamycin analog provides molecular insight into how the channel integrates two agonists that bind to distal sites but act cooperatively.

Keywords

TRPML1, $PI(3,5)P_2$, rapamycin, allosteric, temsirolimus, lysosome, Ca^{2+} channels

Transient Receptor Potential Mucolipin (TRPML) channels are non-selective, Ca^{2+} permeable cation channels expressed mainly within the endo-lysosomal membranes of mammalian cells [1]. TRPML1, is the most ubiquitous and extensively studied TRPML isoform, given its importance in lysosomal homeostasis and mutation in type IV mucolipidosis [1]. TRPML channels are directly and selectively activated by $PI(3,5)P_2$ [2]. This low-abundance but endolysosome-specific phosphosphoinositide binds to a polybasic region (part of the 'mucolipin domain') within the cytosolic N terminus of TRPML1 [2]. Besides $PI(3,5)P_2$, there are synthetic small molecule agonists of TRPML1 that notably include ML-SA1 and its congeners [3, 4]. With the ongoing revolutionary progress in cryogenic electron microscopy (cryo-EM), we now have several 3D structures of mammalian TRPML1 [5-8]. To this growing repertoire, there has been an exciting recent addition by Jiang and colleagues [9] who solved mouse TRPML1 (mTRPML1) structures in apo and several ligand bound states using cryo-EM. The new structures are of higher resolution and include TRPML1 in its open state (PDB: 7SQ9) in presence of $PI(3,5)P_2$ and temsirolimus (Tem) - a very close analog of rapamycin (Rap) at 2.1Å.

Previously, using lysosomal patch-clamping, the Xu group showed that TRPML1 can be selectively and directly activated by Rap and several selected rapalogs, albeit with weak potencies, and this was independent of mTOR - the canonical biological target of these macrocycles [10]. The open state structure by Gan et al (2022) now clearly reveals the binding site for Tem (and thus presumably for Rap) which overlaps with sites on human TRPML1 (hTRPML1) where the agonist MLSA1 (PDBs: 5WJ9, 6E7Z) [6, 7] and the inhibitor MLSI3 (PDB:7MGL) [8] are known to bind. The dual agonist-bound open structures involving the physiological agonist $PI(3,5)P_2$ and a pharmacological agonist like Tem[9] or MLSA1 [6] provide insight into the synergy observed for these agonist combinations in lysosomal patch-clamp recordings of TRPML1 [9, 10]. This second site (Fig.1) which is largely hydrophobic and sits between S5 and S6 from two neighbouring subunits, appears to be a hotspot for fine tuning of TRPML1 activity by possible endogenous or synthetic modulators. Gan et al [9] show that $PI(3,5)P_2$ and Tem, despite binding to markedly distinct sites on mTRPML1, allosterically influence each other's recognition and mutually benefit each other's efficacy for channel activation. Comparative analyses of the apo and dual agonist-bound states of mTRPML1suggests that the allosteric communications between two agonists is largely mediated by the S4 helix: Tem binding expands S5, thereby imposing a thrust onto S4 which then moves towards the $PI(3,5)P_2$ binding site and facilitates its binding. Likewise, as S4 is pulled during $PI(3,5)P_2$ binding, S5 expands to accommodate Tem better at its site. Being directly-linked, the S4-S5 linker also undergoes the same outward motions with S4.

The new structure of mTRPML1 bound to $PI(3,5)P_2$ alone [9] is also of higher resolution than previous ones [6]. But quite surprisingly, a π-cation interaction between Y355 and R403 was absent. This is in stark contrast with key suggestion from earlier work with hTRPML1 where this specific a π-cation interaction seemed

critical for the necessary movement of the S4-S5 linker en route to $PI(3,5)P_2$-evoked channel activation [6]. The precise explanation for such disparity is unclear, especially given this region is highly conserved across species [6] but the increased resolution might have allowed greater confidence in predicting the side chains of relevant residues for the recent mTRPML1 structures. Comparing the $PI(3,5)P_2$ bound closed and open states, Gan et al [9] report an extra salt bridge between Arg403 and $PI(3,5)P_2$ that relates specifically to the open state. Another consensus that seems to be emerging from the open state TRPML1 structures from this [9] and previous studies [6, 7] is that gating of TRPML1, unlike some members of the TRP channel family, involves subtle conformational changes at the selectivity filter region alongside dramatic widening at the lower gate. Last but not the least, this work [9] and the prior work from Xu and colleagues [10] supports an intriguing possibility that some of the biological actions of Rap and few rapalogs could be at least partly mediated through TRPML1 via facilitation of its opening at the basal low level of $PI(3,5)P_2$. These higher resolution structures will aid various structure- function and chemical biology studies in near future.

Funding

SP acknowledges funding from the BBSRC (BB/T015853/1).

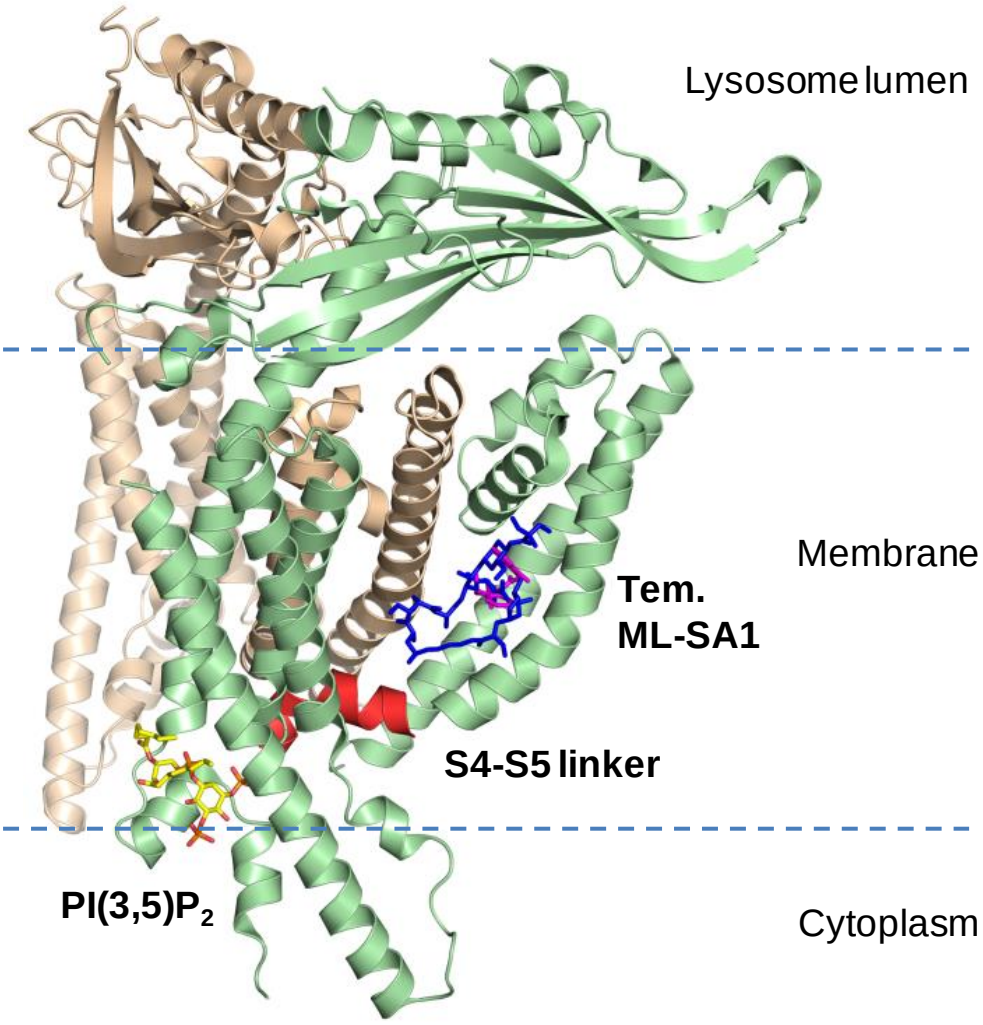

Figure 1. Structure of mouse TRPML1 in complex with agonists. Two neighbouring subunits of mouse TRPML1 (PDB id: 7SQ9) elucidated by Gan et al. (2022) are shown in cartoon representations and coloured in pale green (chain A) and wheat (chain C). Small molecule agonists are shown as sticks and coloured as yellow-red, blue and purple representing PI(3,5)P2, Temsirolimus (Tem) and MLSA1, respectively. The position of ML-SA1 was inferred from mouse TRPML1 bound to ML-SA1 (PDB id: 7SQ6). Unlike ML-SA1, Tem-interacting residues also extend to the S6 of the neighbouring subunit. The S4-S5 linker that seems critical for mediating the allosteric communication between PI(3,5)P2and Tem/MLSA1 is coloured in red. The image was created in PyMOL.

References

[1] S. Di Paola, A. Scotto-Rosato, D.L. Medina, TRPML1: The Ca((2+))retaker of the lysosome, Cell Calcium, 69 (2018) 112-121.
[2] X.P. Dong, D. Shen, X. Wang, T. Dawson, X. Li, Q. Zhang, X. Cheng, Y. Zhang, L.S. Weisman, M. Delling, H. Xu, PI(3,5)P(2) controls membrane trafficking by direct activation of mucolipin Ca(2+) release channels in the endolysosome, Nat Commun, 1 (2010) 38.
[3] D. Shen, X. Wang, X. Li, X. Zhang, Z. Yao, S. Dibble, X.P. Dong, T. Yu, A.P. Lieberman, H.D. Showalter, H. Xu, Lipid storage disorders block lysosomal trafficking by inhibiting a TRP channel and lysosomal calcium release, Nat Commun, 3 (2012) 731.
[4] L. Yu, X. Zhang, Y. Yang, D. Li, K. Tang, Z. Zhao, W. He, C. Wang, N. Sahoo, K. Converso-Baran, C.S. Davis, S.V. Brooks, A. Bigot, R. Calvo, N.J. Martinez, N. Southall, X. Hu, J. Marugan, M. Ferrer, H. Xu, Small-molecule activation of lysosomal TRP channels ameliorates Duchenne muscular dystrophy in mouse models, Sci Adv, 6 (2020) eaaz2736.
[5] Q. Chen, J. She, W. Zeng, J. Guo, H. Xu, X.C. Bai, Y. Jiang, Structure of mammalian endolysosomal TRPML1 channel in nanodiscs, Nature, 550 (2017) 415-418.
[6] M. Fine, P. Schmiege, X. Li, Structural basis for PtdInsP2-mediated human TRPML1 regulation, Nat Commun, 9 (2018) 4192.
[7] P. Schmiege, M. Fine, G. Blobel, X. Li, Human TRPML1 channel structures in open and closed conformations, Nature, 550 (2017) 366-370.
[8] P. Schmiege, M. Fine, X. Li, Atomic insights into ML-SI3 mediated human TRPML1 inhibition, Structure, 29 (2021) 1295-1302 e1293.
[9] N. Gan, Y. Han, W. Zeng, Y. Wang, J. Xue, Y. Jiang, Structural mechanism of allosteric activation of TRPML1 by PI(3,5)P2 and rapamycin, Proc Natl Acad Sci U S A, 119 (2022).
[10] X. Zhang, W. Chen, Q. Gao, J. Yang, X. Yan, H. Zhao, L. Su, M. Yang, C. Gao, Y. Yao, K. Inoki, D. Li, R. Shao, S. Wang, N. Sahoo, F. Kudo, T. Eguchi, B. Ruan, H. Xu, Rapamycin directly activates lysosomal mucolipin TRP channels independent of mTOR, PLoS Biol, 17 (2019) e3000252.