A well-known potassium channel plays a critical role in lysosomes

Michael X. Zhu

Department of Integrative Biology and Pharmacology, McGovern Medical School, The University of Texas Health Science Center at Houston, Houston, TX 77030

Whole-endolysosome patch clamping presents new opportunities to identify and characterize channels pivotal for these acidic organelles. In this issue (Wang et al., 2017. *J. Cell Biol.* https://doi.org/10.1083/jcb.201612123), the identification of a role for the large conductance calcium-activated potassium channel brings new thinking about regulation of lysosome membrane potential and function.

Ionic homeostasis and ion transport are essential for all life forms on Earth. Well defined concentrations of key ions, most commonly Na^+, K^+, Cl^-, Ca^{2+}, and H^+, are maintained not only in the cytoplasm and extracellular space but also inside subcellular organelles. Ionic gradients across membranes are created by ATPases (pumps) and ion exchangers. To some degree, these gradients mimic the ancient environment in which primordial living things thrived, but more importantly, they support diverse cellular functions ranging from substance transport to membrane potential generation and cell signaling. The latter two functions are carried by ion channels, proteinous structures embedded in the lipid bilayer that passively allow certain ions to pass through, giving rise to selective permeability. The uneven ionic concentrations across the membrane and selective permeability of the membrane to different ions then generate membrane potential.

Numerous studies over the past few decades have revealed rich information on the function, regulation, and structure of ion channels. However, much of our present knowledge about ion channels came from studying them on the plasma membrane (PM) of, especially, excitable cells such as neurons, muscles, and endocrine cells, which use electric signals to perform vital functions. Ion channels are also commonly found in nonexcitable cells and their importance in development, cell signaling, differentiation, proliferation, and cell survival/demise is increasingly being recognized. Although functional studies usually suggest ion channels to be at a much lower abundance in nonexcitable cells than in excitable cells, microarray and RNA sequencing data have often revealed ample expression of various ion channel genes in nonexcitable cells or their up- or down-regulation under conditions such as cancer and stress. However, efforts made to characterize these channels by electrophysiology, ion uptake, or fluorescence imaging often failed. This could be because of technical issues or low activity but quite possibly, the channels may not be expressed on the PM but rather in subcellular organelles. In this issue, Wang et al. demonstrate that the large conductance Ca^{2+}-activated K^+ (BK) channel actually plays an important role in lysosomes, highlighting intracellular organelles as the next front of ion channel research.

Similar to the PM, intracellular membranes also form barriers for ions, and ion passage has to be handled by pumps, exchangers, and channels. Some of the well-known players include inositol 1,4,5-trisphosphate receptors, ryanodine receptors, and sarco/endoplasmic reticulum Ca^{2+}-ATPases located on the endoplasmic reticulum membrane; mitochondrial Ca^{2+} uniporter; and permeability transition pore. More recently, endosomes and lysosomes (endolysosomes) have also been shown to express specific channels, e.g., transient receptor potential mucolipin (TRPML; Dong et al., 2008, 2010), two-pore channels (Calcraft et al., 2009), and CLC chloride transporters (Leisle et al., 2011). Thus, ion channels play pivotal roles in all cellular membranes. However, because of their importance, and perhaps also partly the relative ease of detection, Ca^{2+} and H^+ transports have been the most frequently studied. This raises the questions: Are the organellar channels specifically "designed" for particular organelles and are they mainly dedicated to Ca^{2+} and H^+ handling?

In recent years, there has been tremendous expansion on the knowledge about endolysosomal channels, owing to the development of whole-endolysosome patch clamp technique (Dong et al., 2008). This has helped reshape our understanding of the function and regulation of lysosomes, allowing not only a functional characterization of novel endolysosomal-specific channels, such as TRPMLs, two-pore channels, and TMEM175 (Dong et al., 2008; Wang et al., 2012; Cang et al., 2015), but also discovery of new functions of classical well-characterized channels previously thought to work mainly on PM. The two excellent examples for the latter include P2X4 purinergic receptors (Cao et al., 2015a) and BK channels (Cao et al., 2015b; Wang et al., 2017).

P2X4 is a member of the P2X family of ionotropic purinergic receptors, which form Ca^{2+} permeable cation channels typically found on the PM. A previous study examined the subcellular distribution of P2X4 in several cell types and found it to be present in lysosomes (Cao et al., 2015a). Using whole-endolysosomal recording, they demonstrated P2X4 channel activation by ATP applied to the luminal side and this appeared to occur only when the pH of the acidic organelle was raised to near neutral. It turns out that the lysosomal lumen normally contains a high ATP content; however, this will not activate P2X4

Correspondence to Michael X. Zhu: michael.x.zhu@uth.tmc.edu

The Rockefeller University Press $30.00
J. Cell Biol. Vol. 216 No. 6 1513–1515
https://doi.org/10.1083/jcb.201704017

until the luminal pH goes up. Therefore, the lysosomal P2X4 receptor behaves more like a pH-regulated channel, rather than an ATP-regulated channel as on the PM. Importantly, as lysosomes represent the final destination of the degradation pathway, it is essential to show that proteins found in lysosomes are not there because they are destined for breakdown. One argument would be that things to be degraded should be trapped inside the lysosome and unlikely to exhibit activities on the lysosomal membrane. However, because the endolysosome patches have to be made on enlarged vacuoles, either drug-induced or naturally occurring (not common), it is not easy to rule out that somehow the supposedly trapped proteins got incorporated into the vacuole surface membrane and became functionally detectable. Therefore, another important criterion is to see if the channel plays unique lysosome-related roles. This is indeed the case for P2X4, which acts as a Ca^{2+} release channel that promotes the fusion of lysosomes among themselves or with endosomes. It was demonstrated that Ca^{2+} released from lysosomes via P2X4 is captured by calmodulin, which then drives membrane fusion between vesicles (Cao et al., 2015a).

A concurrent study from the same group also demonstrated BK channel function in lysosomes (Cao et al., 2015b). BK has been very well characterized in excitable cells as a Ca^{2+}- and voltage-gated K^+ channel important for membrane repolarization and hyperpolarization. Many of the biophysical properties of BK seem to be maintained in the lysosomal membrane such as the dual Ca^{2+} and voltage dependence. With respect to lysosome-related functions, Cao et al. (2015b) demonstrated a positive feedback mechanism in which lysosome Ca^{2+} release through TRPML1 causes BK activation and consequent K^+ entry into the lysosomal lumen. This may be viewed as either to provide a counter ion movement or to enhance the driving force for continued Ca^{2+} efflux. The net result is the increased Ca^{2+} release through TRPML1. Based on the premise that enhancing TRPML1 function has the potential to mitigate lysosomal dysfunction in lysosome storage disorders, such as Niemann-Pick type C1 (Shen et al., 2012), Cao et al. (2015b) tested whether up-regulating lysosomal BK function could also reduce cholesterol and lipofusion storage in human fibroblasts derived from Niemann-Pick type C1 patients and obtained encouraging results. They proposed that targeting BK could represent a viable strategy to treat lysosome storage disorders, a postulation similar to the one made for TRPML1 previously (Shen et al., 2012). However, both claims await support from studies in animal models and clinical settings.

In this issue, Wang et al. (2017) also report their independent findings on lysosomal BK channels. These authors tested many different cell types and concluded that BK-like currents are commonly present on lysosomes of, potentially, all cells. In nonexcitable cells, BK is preferentially present in lysosomes with hardly any current detectable on the PM. Notably, BK activities have been detected on the mitochondrial inner membrane and on the nucleus (as discussed in Wang et al., 2017). Therefore, BK may be generally an intracellular channel, with its PM function representing specialization that appeared later in evolution in some excitable cells. Becuase Wang et al. (2017) did not detect any other K^+ currents, including voltage-gated K^+ current and the recently reported lysosomal TMEM175 current (Cang et al., 2015), they concluded that BK mediates the main K^+ current across lysosomal membranes. Yet, this current is very small as compared with the relatively larger conductance to Na^+ and H^+, giving rise to an unexpected "positive" (luminal side negative, with the voltage at the cytoplasmic side defined as 0 mV) resting lysosomal membrane potential. Increasing the Ca^{2+} level near lysosomes, or juxta-lysosomal Ca^{2+}, triggers BK to open and thereby increases K^+ conductance, leading to lysosome hyperpolarization (luminal side positive). Because BK has a very large unitary conductance (>200 pS), Wang et al. (2017) believe that it may take the opening of just few BK channels to reverse polarization of lysosomal membrane.

Functionally, Wang et al. (2017) did not find BK to be particularly important for lysosomal Ca^{2+} release as shown by Cao et al. (2015b). Rather, they showed the channel to be pivotal for Ca^{2+} refilling into lysosomes following a prior depletion using two complementary methods. First, they assessed lysosomal luminal Ca^{2+} levels directly using a Ca^{2+}-sensitive fluorescence probe conjugated to dextran, which can be taken up by lysosomes; second, they compared Ca^{2+} rises near lysosomes in response to two consecutive stimulations of TRPML1, monitored using a genetically encoded Ca^{2+} indicator fused to the cytoplasmic side of TRPML1. Because of the different roles assigned to BK, the two studies reported opposite effects of BK inhibition on lysosomal Ca^{2+} contents: a reduction, at least shortly after store emptying (Wang et al., 2017), versus a marked increase at rest (Cao et al., 2015b). Nonetheless, both studies agreed on that the loss of BK caused lysosomal dysfunction in degradation and trafficking and an increase in lysosome biogenesis.

Thus, ion channels are actively involved in the functional regulation of endolysosomes, including membrane potentials, trafficking, substance degradation, and signaling. It is inspiring to learn that some of the well-characterized channels previously known to work mainly on PM may exert specific roles in lysosomal function and to a large extent, the biophysical properties of the channel do not differ from when it is on the PM. Thus, the endolysosomes should be a gold mine for exploration by channel biophysicists and cell biologists. Not only are there exciting opportunities to uncover new functions of known channels but one may also reveal activities of novel channel-like proteins (e.g., TMEM175 [Cang et al., 2015]), splice variants or auxiliary subunits of existing channels, or channels shown to be expressed but without a detectable function at PM. However, many challenges lie ahead. First, the whole-endolysosome recording technique may be harder to master than whole-cell recording. Compared with cells, the enlarged vacuoles are small and hard to keep in shape because of the lack of cytoskeleton. Second, vacuoles that are suitable for recording, either made artificially or naturally, are products of fusion of multiple lysosomes and other subcellular vesicles, e,g., endosomes and autophagosomes. They are not cleanly just lysosomes and the sizes are much bigger than the majority of normal lysosomes, which are smaller than 200 nm in diameter (Cao et al., 2015a). The small size means that ionic gradient can dissipate rapidly when a channel opens, a concept that rarely applies to PM channels. Third, lysosomes are diverse in composition and function, representing different stages of biogenesis, regeneration or dismantle, or specific involvement in various pathways. However, little is known about lysosome subpopulations. Better probes are needed to distinguish and assay the channel function (mostly via fluorescence imaging) in defined subpopulations. Because of the small size and fast dynamics, these detailed studies also need microscopes capable of superresolution imaging at high speed. Moreover, most fluorescence probes are pH sensitive, especially in the broad pH range (~4–7) of the lysosome lumen; therefore, how to calibrate and interpret the fluorescence signals

becomes a big challenge. Finally, because of the overwhelming number of lysosomes in a cell, tracing individual lysosomes for activity and/or trafficking is not trivial. Selectively labeling a small number of vesicles (e.g., via photoactivation) may represent a good strategy, but many technical difficulties still exist.

After all, lysosomes are membrane enclosures that require their own sets of ion homeostatic control and use it to regulate the functions of not just the organelles themselves but also the entire cell. Lysosome ion channels deserve our attention and their importance in health and disease has been clearly established. An important lesson here is that lysosome ion channels do not have to be new channels dedicated uniquely on lysosomes. Some well-established PM channels may find their use in these acidic organelles or perhaps they might have evolved from lysosomes first. There is no question that intriguing new information about life and human health will be learned from studying lysosomal channels.

Acknowledgments

Research conducted in the author's laboratory is supported by grants from National Institutes of Health (GM092759 and NS092377) and American Heart Association (15GRNT23040032).

The author declares no competing financial interests.

References

Calcraft, P.J., M. Ruas, Z. Pan, X. Cheng, A. Arredouani, X. Hao, J. Tang, K. Rietdorf, L. Teboul, K.T. Chuang, et al. 2009. NAADP mobilizes calcium from acidic organelles through two-pore channels. *Nature.* 459:596–600. http://dx.doi.org/10.1038/nature08030

Cang, C., K. Aranda, Y.J. Seo, B. Gasnier, and D. Ren. 2015. TMEM175 is an organelle K^+ channel regulating lysosomal function. *Cell.* 162:1101–1112. http://dx.doi.org/10.1016/j.cell.2015.08.002

Cao, Q., X.Z. Zhong, Y. Zou, R. Murrell-Lagnado, M.X. Zhu, and X.P. Dong. 2015a. Calcium release through P2X4 activates calmodulin to promote endolysosomal membrane fusion. *J. Cell Biol.* 209:879–894. http://dx.doi.org/10.1083/jcb.201409071

Cao, Q., X.Z. Zhong, Y. Zou, Z. Zhang, L. Toro, and X.P. Dong. 2015b. BK channels alleviate lysosomal storage diseases by providing positive feedback regulation of lysosomal Ca^{2+} release. *Dev. Cell.* 33:427–441. http://dx.doi.org/10.1016/j.devcel.2015.04.010

Dong, X.P., X. Cheng, E. Mills, M. Delling, F. Wang, T. Kurz, and H. Xu. 2008. The type IV mucolipidosis-associated protein TRPML1 is an endolysosomal iron release channel. *Nature.* 455:992–996. http://dx.doi.org/10.1038/nature07311

Dong, X.P., D. Shen, X. Wang, T. Dawson, X. Li, Q. Zhang, X. Cheng, Y. Zhang, L.S. Weisman, M. Delling, and H. Xu. 2010. $PI(3,5)P_2$ controls membrane trafficking by direct activation of mucolipin Ca^{2+} release channels in the endolysosome. *Nat. Commun.* 1:38. http://dx.doi.org/10.1038/ncomms1037

Leisle, L., C.F. Ludwig, F.A. Wagner, T.J. Jentsch, and T. Stauber. 2011. ClC-7 is a slowly voltage-gated $2Cl^-/1H^+$-exchanger and requires Ostm1 for transport activity. *EMBO J.* 30:2140–2152. http://dx.doi.org/10.1038/emboj.2011.137

Shen, D., X. Wang, X. Li, X. Zhang, Z. Yao, S. Dibble, X.P. Dong, T. Yu, A.P. Lieberman, H.D. Showalter, and H. Xu. 2012. Lipid storage disorders block lysosomal trafficking by inhibiting a TRP channel and lysosomal calcium release. *Nat. Commun.* 3:731. http://dx.doi.org/10.1038/ncomms1735

Wang, W., X. Zhang, Q. Gao, M. Lawas, L. Yu, X. Cheng, M. Gu, N. Sahoo, X. Li, P. Li, et al. 2017. A voltage-dependent K^+ channel in the lysosome is required for refilling lysosomal Ca^{2+} stores. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201612123

Wang, X., X. Zhang, X.P. Dong, M. Samie, X. Li, X. Cheng, A. Goschka, D. Shen, Y. Zhou, J. Harlow, et al. 2012. TPC proteins are phosphoinositide-activated sodium-selective ion channels in endosomes and lysosomes. *Cell.* 151:372–383. http://dx.doi.org/10.1016/j.cell.2012.08.036