

Commentary

Getting close. Lysosome-ER contact sites tailor Ca^{2+} signals

Sandip Patel

Department of Cell and Developmental Biology, University College London, Gower Street, London WC1E 6BT, United Kingdom



ARTICLE INFO

Keywords:

IP₃ receptors

Lysosomes

Endoplasmic reticulum

Membrane contact sites

 Ca^{2+} uptake

Inter-organelle communication is critical for the generation of complex Ca^{2+} signals. A recent study by Atakpa et al provides evidence that membrane contact sites between lysosomes and the ER facilitate lysosomal Ca^{2+} uptake to shape cytosolic Ca^{2+} signals derived from the ER.

Much work has highlighted the importance of lysosomes and other acidic organelles as mobilizable Ca^{2+} stores [1]. This role has contributed to the renaissance of lysosomes which are now recognised to regulate a number of cellular processes beyond their traditional function in macromolecule/organelle turnover. Whilst a bulk of effort has focussed on Ca^{2+} release from these organelles by second messengers, such as NAADP, and Ca^{2+} -permeable channels, such as two-pore channels (TPCs) [1,2], we know little concerning how these organelles take up Ca^{2+} . Previous work from the Taylor lab provided evidence that lysosomes sequestered Ca^{2+} upon mobilisation of ER Ca^{2+} stores thereby dampening Ca^{2+} signals evoked by an IP₃ forming agonist [3]. In the latest instalment, such communication is proposed to occur at membrane contact sites between the ER and lysosomes [4].

Membrane contact sites are regions of close apposition (~ 30 nm) between organelles that facilitate information flow. The ER forms extensive contacts with the plasma membrane and nearly all organelles [5]. These sites are no strangers to Ca^{2+} signalers. Contacts between the ER and mitochondria, for example, couple Ca^{2+} release from the ER (typically through IP₃ receptors) to mitochondrial Ca^{2+} uptake (through the mitochondrial uniporter). And ER-plasma membrane contact sites underpin store-operated Ca^{2+} entry - the process whereby depletion of ER Ca^{2+} stores stimulates Ca^{2+} influx. Contacts between the ER and lysosomes have also been described [6]. They are proposed to facilitate amplification of Ca^{2+} signals deriving from lysosomes by the ER [7]. But we know relatively little about their physiological roles during Ca^{2+} signalling.

In the new work [4], chemical or molecular inhibition of the V-type ATPase, which maintains lysosomal acidification, potentiated cytosolic Ca^{2+} signals evoked by the IP₃-forming agonist carbachol in HEK cells. These results corroborated previous findings [3] showing similar potentiation by the commonly used V-type ATPase inhibitor bafilomycin-A1, the lysosomotropic agent GPN and vacuolin, which promotes endo-lysosomal fusion. The enhancement of Ca^{2+} signals upon disrupting lysosomes was interpreted as lysosomes normally sequestering Ca^{2+} upon agonist stimulation, much like the buffering effect that mitochondrial Ca^{2+} uptake exerts on Ca^{2+} signals deriving from the ER. Importantly, compromising lysosome function/integrity did not affect store-operated Ca^{2+} entry pointing to a highly localised signalling event [3,4]. In a neat set of experiments, using a genetically-encoded Ca^{2+} indicator with a relatively low affinity for Ca^{2+} , Atakpa et al further showed that the signals evoked by agonist or direct IP₃ delivery were larger when the indicator was targeted to the cytosolic surface of lysosomes than when it was expressed in the cytosol. Signals recorded during store-operated Ca^{2+} entry however were similar using the two indicators. These data suggest lysosomes experience much larger Ca^{2+} fluctuations than the bulk cytosol when IP₃ receptors open. This is consistent with close apposition between the ER and lysosomes forming a micro-domain which in turn might selectively facilitate Ca^{2+} uptake into the lysosomes (Fig. 1).

Using proximity ligation assays, the authors provided physical evidence for a close association between IP₃ receptors and lysosomes. This immuno-technique allows visualisation of proteins within ~ 40 nm of each other. Such separation is about that between membranes at a contact site. The authors nicely demonstrated proximity between type 1 IP₃ receptors on the ER and the late endosome/lysosome markers, LAMP1 and Rab7. Coupled with impressive live cell imaging of lysosomes and IP₃ receptors showing a range of transient associations

E-mail address: patel.s@ucl.ac.uk.<https://doi.org/10.1016/j.ceca.2019.02.003>

Received 3 February 2019; Received in revised form 7 February 2019; Accepted 7 February 2019

Available online 08 February 2019

0143-4160/© 2019 Elsevier Ltd. All rights reserved.

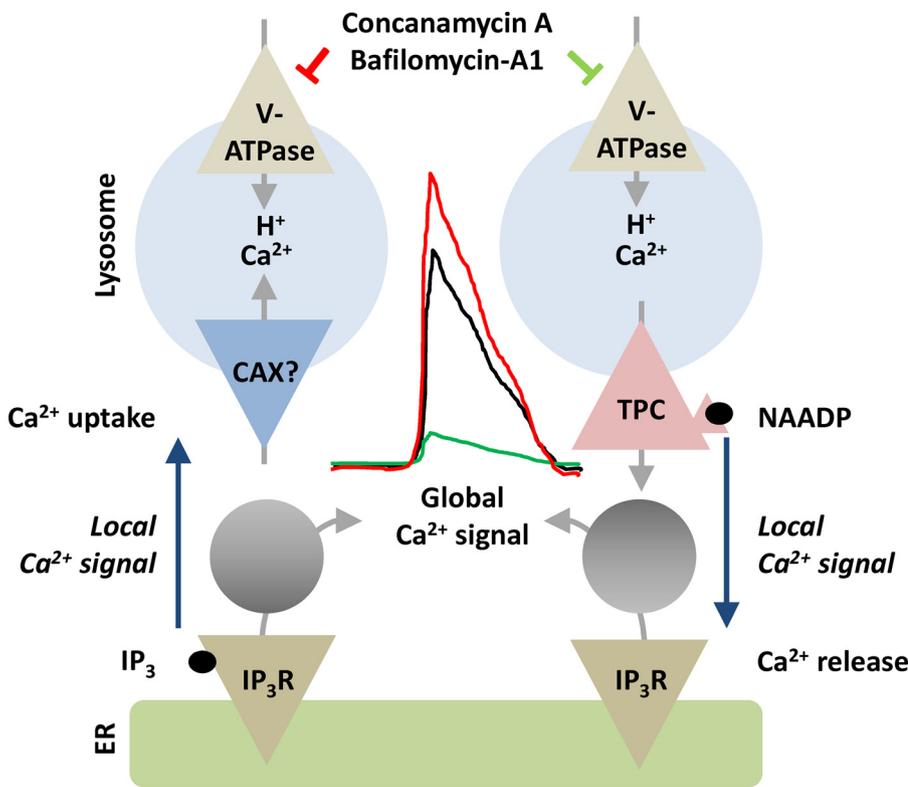


Fig. 1. Bidirectional Ca²⁺ fluxes at lysosome-ER membrane contact sites. Left, Activation of IP₃ receptors (IP₃R) by IP₃ induces a local Ca²⁺ signal (grey spheres) at contacts sites between the ER (rectangle) and lysosomes (large circles). This stimulates Ca²⁺ uptake into the lysosome during agonist stimulation (upward arrow) possibly through a Ca²⁺-H⁺ exchanger (CAX). Prior inhibition of the V-type ATPase by concanamycin A or bafilomycin-A₁ prevents Ca²⁺ uptake such that global Ca²⁺ signals are larger (red trace, centre). Right, Activation of TPCs by NAADP induces a local Ca²⁺ signal that is amplified by IP₃ receptors during agonist stimulation (downward arrow). Inhibition of the V-type ATPase in this scenario inhibits Ca²⁺ release from lysosomes such that global agonist-evoked Ca²⁺ signals are reduced (green trace, centre).

(albeit within the TIRF field i.e. ~ 200 nm of the plasma membrane), all point to the presence of IP₃ receptors at ER-lysosome contact sites. Supporting ultrastructural evidence however is currently lacking.

Perhaps most intriguing was reduced association between the ER and lysosomes upon V-type ATPase inhibition. Thus, proximity between Rab7 and VAP, both of which have been implicated in late endosome/ER contact sites [8], was reduced. And so too was proximity between IP₃ receptors and LAMP1/Rab7 although this reduction was more modest in comparison. These effects were associated with enlargement and redistribution, to the cell periphery, of a subset of lysosomes. Overall the distance between lysosomes and the IP₃ receptors was increased. Thus, inhibiting V-type ATPases may prevent Ca²⁺ uptake into lysosomes by separating lysosomes from the ER.

Whilst collectively the data presented certainly support the idea of localized Ca²⁺ uptake driven by IP₃ receptors at the ER-lysosome interface, much of the conclusions are inferred from changes in cytosolic Ca²⁺. Direct measurement of Ca²⁺ within the lysosome lumen is not trivial due to the low pH interfering with Ca²⁺ probes (see [9] for recent developments). In a previous study by the authors, use of an endocytosed dextran-conjugated Ca²⁺ indicator demonstrated an increase in luminal Ca²⁺ upon agonist stimulation [3]. However, the Ca²⁺ uptake was much slower than the rapid agonist-evoked Ca²⁺ signals in the cytosol which lysosomes appear capable of modulating. Might lysosomes negatively regulate IP₃ receptors in some way, so that when the lysosomes moves away, IP₃ causes a greater release of ER calcium?

The potentiating effects of V-type ATPase inhibition on agonist-evoked Ca²⁺ signals are modest (< 30% enhancement of amplitude). In many other cell types, V-type ATPase inhibition *inhibits* agonist-evoked Ca²⁺ signals, often selectively to cues that demonstrably elevate cellular NAADP levels (reviewed in [2]). The latter has been interpreted in the context of lysosomal Ca²⁺ depletion secondary to the increase in luminal pH preventing 'trigger' Ca²⁺ release by NAADP and subsequent amplification by the ER (Fig. 1). Thus, lysosomes may work to both temper and potentiate agonist-evoked signals. Might such bidirectional cross talk occur in the same cell in response to different agonists? If so, all lysosomes may not be created equal.

Another more general question relates to the molecular route for lysosomal Ca²⁺ uptake. A recent study has put forward the lysosomal P-type ATPase, ATP13A2, as a candidate [9]. Mutations in the gene encoding this protein (PARK9) induce a Parkinson disease-like disorder. And there is evidence for disrupted lysosomal Ca²⁺ signalling in other forms of familial Parkinson's [10,11]. However, a bulk of the current evidence suggests that a Ca²⁺-H⁺ exchange mechanism mediates lysosomal Ca²⁺ uptake. This is because of the reciprocal relationship between luminal pH and Ca²⁺, and the inhibitory effects of lysosome disruption on NAADP-evoked Ca²⁺ signals [1,2]. Vacuolar Ca²⁺-H⁺ exchangers (CAX proteins) are well characterised in yeast and plants and they have also recently been described in select animals [12]. Thus, as originally envisaged by the authors [3], inhibition of V-type ATPase might disrupt Ca²⁺-H⁺ exchange in addition to disrupting contact to reduce lysosomal Ca²⁺ uptake. It should be noted that Ca²⁺ ATPases also counter-transport H⁺. Interestingly, the potentiating effects of inhibitors on agonist-evoked signals are slow to develop and appear to manifest *after* stabilisation of luminal pH. This argues against a pH-dependent mechanism for Ca²⁺ uptake although lysosomes did not completely lose their ability to accumulate a fluorescent weak base.

So how does inhibiting the V-type ATPase reduce contact site formation? Recent evidence suggests that Ca²⁺ release by endo-lysosomal TPCs strengthens contact sites between the endo-lysosomal system and the ER [13]. Atakpa et al ruled out an analogous role for Ca²⁺ release from IP₃ receptors at the ER side of the contact. Thus, knockout of all three IP₃ receptor isoforms affected neither the proximity between lysosomes and the ER nor the ability of V-type ATPase blockade to potentiate 'leak' Ca²⁺ signals derived from the ER (evoked by inhibiting SERCA pumps). If basal lysosomal Ca²⁺ uptake requires a H⁺ gradient, then perhaps it is lysosomal Ca²⁺ depletion upon luminal alkalinisation that drives disrupted contact.

In sum, the present study further points to an intimate physical and functional link between acidic organelles and the ER in the control of Ca²⁺ dynamics. Molecular identification of the lysosomal Ca²⁺ uptake machinery and tethering complexes at the lysosome-ER interface is warranted.

Acknowledgements

Work in my lab is supported by the BBSRC, Parkinson's UK and the Wellcome Trust. I thank Stephen Bolsover, Bethan Kilpatrick and Yu (Cara) Yuan for comments on the manuscript.

References

- [1] S. Patel, S. Muallem, Acidic Ca^{2+} stores come to the fore, *Cell Calcium* 50 (2011) 109–112.
- [2] A. Galione, A.J. Morgan, A. Arredouani, L.C. Davis, K. Rietdorf, M. Ruas, J. Parrington, NAADP as an intracellular messenger regulating lysosomal calcium-release channels, *Biochem. Soc. Trans.* 38 (2010) 1424–1431.
- [3] C.I. Lopez-Sanjurjo, S.C. Tovey, D.L. Prole, C.W. Taylor, Lysosomes shape $\text{Ins}(1,4,5)\text{P}_3$ -evoked Ca^{2+} signals by selectively sequestering Ca^{2+} released from the endoplasmic reticulum, *J. Cell Sci.* 126 (2013) 289–300.
- [4] P. Atakpa, N.B. Thillaiappan, S. Mataragka, D.L. Prole, C.W. Taylor, IP_3 receptors preferentially associate with ER-Lysosome contact sites and selectively deliver Ca^{2+} to lysosomes, *Cell Rep.* 25 (2018) 3180–3193 e3187.
- [5] H. Wu, P. Carvalho, G.K. Voeltz, Here, there, and everywhere: the importance of ER membrane contact sites, *Science* 361 (2018).
- [6] B.S. Kilpatrick, E.R. Eden, A.H. Schapira, C.E. Futter, S. Patel, Direct mobilisation of lysosomal Ca^{2+} triggers complex Ca^{2+} signals, *J. Cell Sci.* 126 (2013) 60–66.
- [7] C.J. Penny, B.S. Kilpatrick, J.M. Han, J. Sneyd, S. Patel, A computational model of lysosome-ER Ca^{2+} microdomains, *J. Cell Sci.* 127 (2014) 2934–2943.
- [8] N. Rocha, C. Kuijl, R. van der Kant, L. Janssen, D. Houben, H. Janssen, W. Zwart, J. Neefjes, Cholesterol sensor ORP1L contacts the ER protein VAP to control Rab7-RILP-p150 glued and late endosome positioning, *J. Cell Biol.* 185 (2009) 1209–1225.
- [9] N. Narayanaswamy, K. Chakraborty, A. Saminathan, E. Zeichner, K. Leung, J. Devany, Y. Krishnan, A. pH-correctable, DNA-based fluorescent reporter for organellar calcium, *Nat. Methods* 16 (2019) 95–102.
- [10] L.N. Hockey, B.S. Kilpatrick, E.R. Eden, Y. Lin-Moshier, G.C. Brailoiu, E. Brailoiu, C. Futter, A.H. Schapira, J.S. Marchant, S. Patel, Dysregulation of lysosomal morphology by pathogenic LRRK2 is corrected by TPC2 inhibition, *J. Cell Sci.* 128 (2015) 232–238.
- [11] B.S. Kilpatrick, J. Magalhaes, M.S. Beavan, A. McNeill, M.E. Gegg, M.W. Cleeter, D. Bloor-Young, G.C. Churchill, M.R. Duchon, A.H. Schapira, S. Patel, Endoplasmic reticulum and lysosomal Ca^{2+} stores are remodelled in GBA1-linked Parkinson disease patient fibroblasts, *Cell Calcium* 59 (2016) 12–20.
- [12] M. Melchionda, J.K. Pittman, R. Mayor, S. Patel, $\text{Ca}^{2+}/\text{H}^+$ exchange by acidic organelles regulates cell migration in vivo, *J. Cell Biol.* 212 (2016) 803–813.
- [13] B.S. Kilpatrick, E.R. Eden, L.N. Hockey, E. Yates, C.E. Futter, S. Patel, An endosomal NAADP-Sensitive two-pore Ca^{2+} channel regulates ER-Endosome membrane contact sites to control growth factor signaling, *Cell Rep.* 18 (2017) 1636–1645.