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

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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 IP_3 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 (~ 30nm) 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+} signallers. Contacts between the ER and mitochondria, for example, couple Ca^{2+} release from the ER (typically through IP_3 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 IP_3-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. Enhanced

Ca^{2+} signals upon disrupting lysosomes were 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 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 carbachol or direct IP_3 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 IP_3 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 IP_3 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 IP_3 receptors on the ER and the late endosome/lysosome markers, LAMP1 and Rab7. Coupled with impressive live cell imaging of lysosomes and IP_3 receptors showing a range of transient associations (albeit within the TIRF field ie ~ 200 nm of the plasma membrane), all point to the presence of IP_3 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 VAP and Rab7, both of which have been implicated in late endosome/ER contact sites [8], was reduced. And so too was proximity between IP_3 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 IP_3 receptors was increased. Thus, inhibiting V-type ATPases may prevent Ca^{2+} uptake into lysosomes by separating lysosomes from the ER.

Whilst collectively the data presented certainly support the idea of localized Ca^{2+} uptake driven by IP_3 receptors at the ER-lysosome interface, much of the conclusions are inferred from changes in cytosolic Ca^{2+}. Direct measurement of Ca^{2+} within the lysosome lumen is not trivial due to the low pH interfering with Ca^{2+} probes (see [9] for recent developments). In a previous study by the authors, use of an endocytosed dextran-conjugated Ca^{2+} indicator demonstrated an increase in luminal Ca^{2+} upon agonist stimulation [3]. However, the Ca^{2+} uptake was much slower than the rapid agonist-evoked Ca^{2+} signals in the cytosol which lysosomes appear capable of modulating. Might lysosomes negatively regulate IP_3 receptors in some way, so that when the lysosome moves away, IP_3 causes a greater release of ER calcium?

The potentiating effects of V-type ATPase inhibition on agonist-evoked Ca^{2+} signals are modest (<30% enhancement of amplitude). In many other cell types, V-type ATPase inhibition *inhibits* agonist-evoked Ca^{2+} signals, often selectively to cues that demonstrably elevate cellular NAADP levels (reviewed in [2]). The latter has been interpreted in the context of lysosomal Ca^{2+} depletion secondary to the increase in luminal pH preventing 'trigger' Ca^{2+} 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 Ca^{2+} 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 Ca^{2+} signalling in other forms of familial Parkinson's [10, 11]. However, a bulk of the current evidence suggests that a Ca^{2+}-H^+ exchange mechanism mediates lysosomal Ca^{2+} uptake. This is because of the reciprocal relationship between luminal pH and Ca^{2+}, and the inhibitory effects of lysosome disruption on NAADP-evoked Ca^{2+} signals [1, 2]. Vacuolar Ca^{2+}-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 Ca^{2+}-H^+ exchange in addition to disrupting contact to reduce lysosomal Ca^{2+} uptake. It should be noted that Ca^{2+} 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 Ca^{2+} 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 Ca^{2+} 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 Ca^{2+} release from IP_3 receptors at the ER side of the contact. Thus, knockout of all three IP_3 receptor isoforms affected neither the proximity between lysosomes and the ER nor the ability of V-type ATPase blockade to potentiate 'leak' Ca^{2+} signals derived from the ER (evoked by inhibiting SERCA pumps). If basal lysosomal Ca^{2+} uptake requires a H^+ gradient, then perhaps it is lysosomal Ca^{2+} depletion upon luminal alkalisation 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 Ca^{2+} dynamics. Molecular identification of the

lysosomal Ca^{2+} uptake machinery and tethering complexes at the lysosome-ER interface is warranted.

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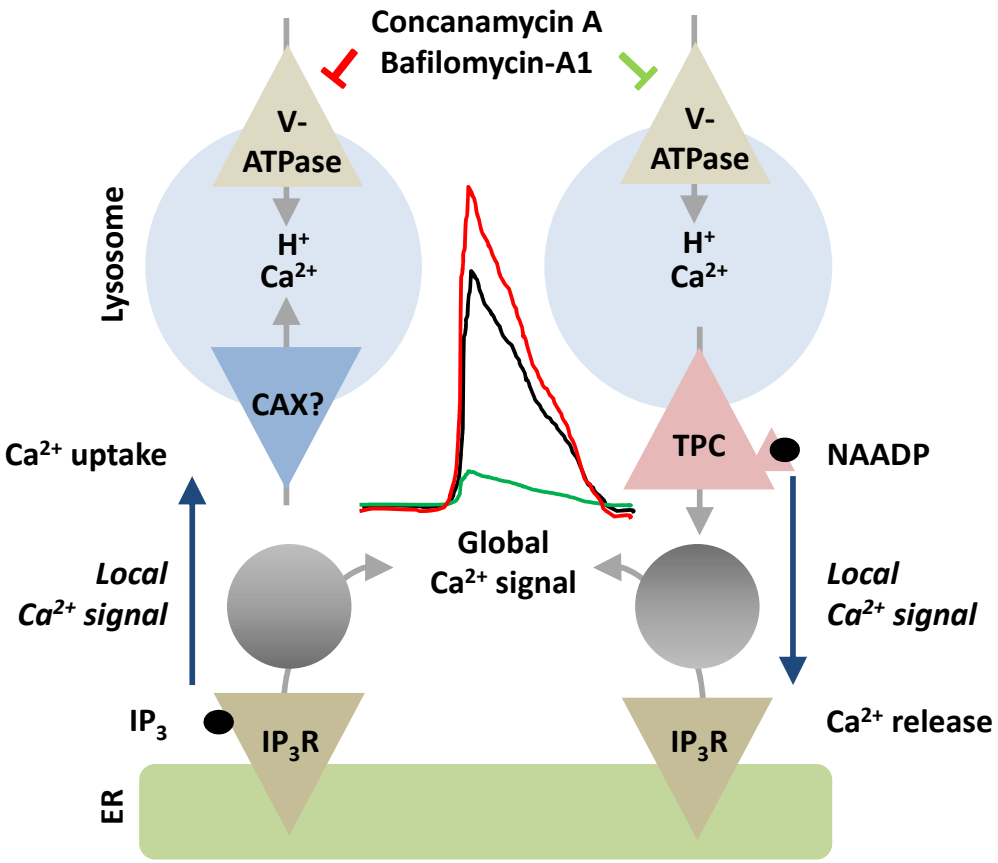
Figure 1. Bidirectional Ca^{2+} fluxes at lysosome-ER membrane contact sites. Left, Activation of IP_3 receptors (IP_3R) by IP_3 induces a local Ca^{2+} signal (grey spheres) at contacts sites between the ER (rectangle) and lysosomes (large circles). This stimulates Ca^{2+} uptake into the lysosome during agonist stimulation (upward arrow) possibly through a Ca^{2+}-H^+ exchanger (CAX). Prior Inhibition of the V-type ATPase by concanamycin A or bafilomycin-A_1 prevents Ca^{2+} uptake such that global Ca^{2+} signals are larger (red trace, centre). Right, Activation of TPCs by NAADP induces a local Ca^{2+} signal that is amplified by IP_3 receptors during agonist stimulation (downward arrow). Inhibition of the V-type ATPase in this scenario inhibits Ca^{2+} release from lysosomes such that global agonist-evoked Ca^{2+} signals are reduced (green trace, centre).


Concanamycin A
Bafilomycin-A1
V-ATPase
V-ATPase
Lysosome
H+
Ca2+
H+
Ca2+
CAX?
TPC
NAADP
Ca2+ uptake
Global
Ca2+ signal
Local
Ca2+ signal
Local
Ca2+ signal
IP3
IP3R
IP3R
Ca2+ release
ER